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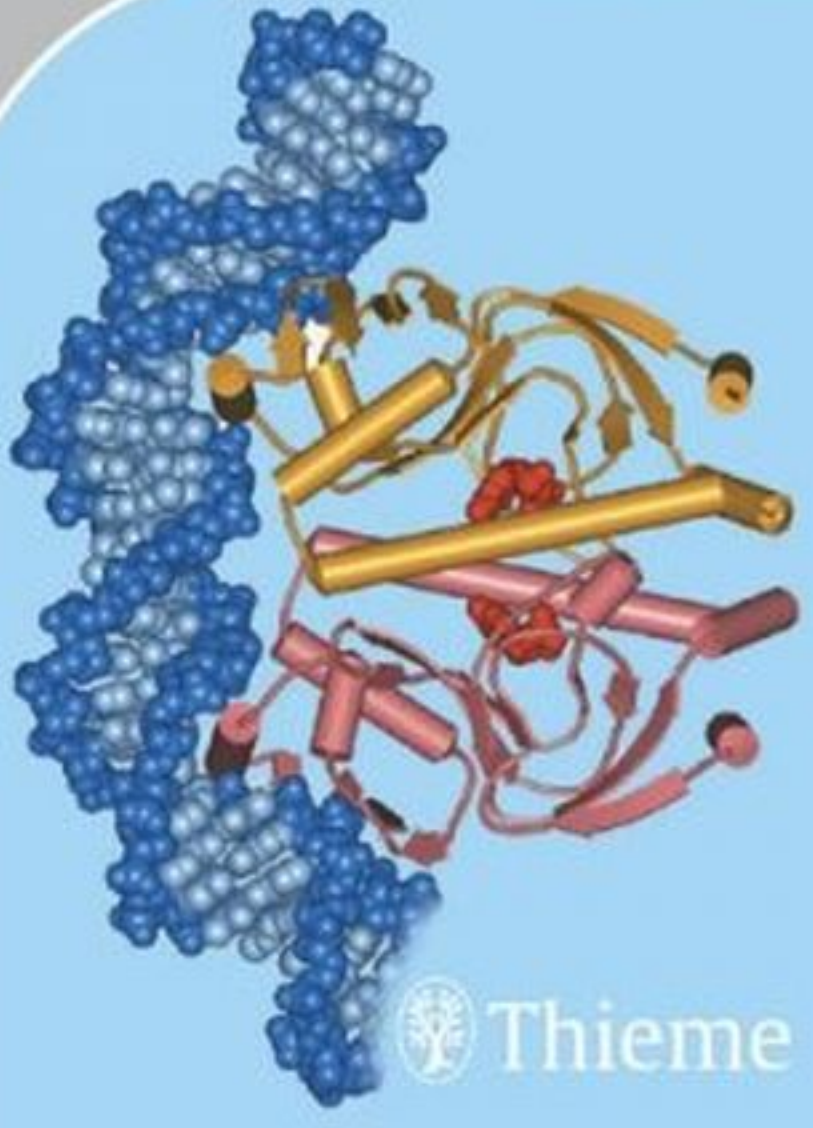
Color Atlas of Biochemistry

J. Koolman
K. H. Roehm

Second edition, revised and enlarged

basic sciences



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Jan Koolman
Professor
Philipps University Marburg
Institute of Physiologic Chemistry
Marburg, Germany

Klaus-Heinrich Roehm
Professor
Philipps University Marburg
Institute of Physiologic Chemistry
Marburg, Germany

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About the Authors

Jan Koolman (left) was born in Lübeck, Germany, and grew up with the sea wind blowing off the Baltic. The high school he attended in the Hanseatic city of Lübeck was one that focused on providing a classical education, which left its mark on him. From 1963 to 1969, he studied biochemistry at the University of Tübingen. He then took his doctorate (in the discipline of chemistry) at the University of Marburg, under the supervision of biochemist Peter Karlson. In Marburg, he began to study the biochemistry of insects and other invertebrates. He took his postdoctoral degree in 1977 in the field of human medicine, and was appointed Honorary Professor in 1984. His field of study today is biochemical endocrinology. His other interests include educational methods in biochemistry. He is currently Dean of Studies in the Department of Medicine in Marburg; he is married to an art teacher.

Klaus-Heinrich Röhm (right) comes from Stuttgart, Germany. After graduating from the School of Protestant Theology in Urach —another institution specializing in classical studies—and following a period working in the field of physics, he took a diploma in biochemistry at the University of Tübingen, where the two authors first met. Since 1970, he has also worked in the Department of Medicine at the University of Marburg. He took his doctorate under the supervision of Friedhelm Schneider, and his postdoctoral degree in 1980 was in the Department of Chemistry. He has been an Honorary Professor since 1986. His research group is concerned with the structure and function of enzymes involved in amino acid metabolism. He is married to a biologist and has two children.

Jürgen Wirth (center) studied in Berlin and at the College of Design in Offenbach, Germany. His studies focused on free graphics and illustration, and his diploma topic was "The development and function of scientific illustration." From 1963 to 1977, Jürgen Wirth was involved in designing the exhibition space in the Senckenberg Museum of Natural History in Frankfurt am Main, while at the same time working as a freelance associate with several publishing companies, providing illustrations for schoolbooks, non-fiction titles, and scientific publications. He has received several awards for book illustration and design. In 1978, he was appointed to a professorship at the College of Design in Schwäbisch Gmünd, Germany, and in 1986 he became Professor of Design at the Academy of Design in Darmstadt, Germany. His specialist fields include scientific graphics/information graphics and illustration methods. He is married and has three children.

Preface

Biochemistry is a dynamic, rapidly growing field, and the goal of this color atlas is to illustrate this fact visually. The precise boundaries between biochemistry and related fields, such as cell biology, anatomy, physiology, genetics, and pharmacology, are dif cult to define and, in many cases, arbitrary. This overlap is not coincidental. The object being studied is often the same—a nerve cell or a mitochondrion, for example—and only the point of view differs.

For a considerable period of its history, biochemistry was strongly influenced by chemistry and concentrated on investigating metabolic conversions and energy transfers. Explaining the composition, structure, and metabolism of biologically important molecules has always been in the foreground. However, new aspects inherited from biochemistry's other parent, the biological sciences, are now increasingly being added: the relationship between chemical structure and biological function, the pathways of information transfer, observance of the ways in which biomolecules are spatially and temporally distributed in cells and organisms, and an awareness of evolution as a biochemical process. These new aspects of biochemistry are bound to become more and more important.

Owing to space limitations, we have concentrated here on the biochemistry of humans and mammals, although the biochemistry of other animals, plants, and microorganisms is no less interesting. In selecting the material for this book, we have put the emphasis on subjects relevant to students of human medicine. The main purpose of the atlas is to serve as an overview and to provide visual information quickly and ef ciently. Referring to textbooks can easily fill any gaps. For readers encountering biochemistry for the first time, some of the plates may look rather complex. It must be emphasized, therefore, that the atlas is not intended as a substitute for a comprehensive textbook of biochemistry.

As the subject matter is often dif cult to visualize, symbols, models, and other graphic elements had to be found that make complicated phenomena appear tangible. The graphics were designed conservatively, the aim being to avoid illustrations that might look too spectacular or exaggerated. Our goal was to achieve a visual and aesthetic way of representing scientific facts that would be simple and at the same time effective for teaching purposes. Use of graphics software helped to maintain consistency in the use of shapes, colors, dimensions, and labels, in particular. Formulae and other repetitive elements and structures could be handled easily and precisely with the assistance of the computer.

Color-coding has been used throughout to aid the reader, and the key to this is given in two special color plates on the front and rear inside covers. For example, in molecular models each of the more important atoms has a particular color: gray for carbon, white for hydrogen, blue for nitrogen, red for oxygen, and so on. The different classes of biomolecules are also distinguished by color: proteins are always shown in brown tones, carbohydrates in violet, lipids in yellow, DNA in blue, and RNA in green. In addition, specific symbols are used for the important coenzymes, such as ATP and NAD^+. The compartments in which biochemical processes take place are color-coded as well. For example, the cytoplasm is shown in yellow, while the extracellular space is shaded in blue. Arrows indicating a chemical reaction are always black and those representing a transport process are gray.

In terms of the visual clarity of its presentation, biochemistry has still to catch up with anatomy and physiology. In this book, we sometimes use simplified ball-and-stick models instead of the classical chemical formulae. In addition, a number of compounds are represented by space-filling models. In these cases, we have tried to be as realistic as possible. The models of small molecules are based on conformations calculated by computer-based molecular modeling. In illustrating macromolecules, we used structural infor-

mation obtained by X-ray crystallography that is stored in the Protein Data Bank. In naming enzymes, we have followed the of - cial nomenclature recommended by the IUBMB. For quick identification, EC numbers (in italics) are included with enzyme names.
To help students assess the relevance of the material (while preparing for an examination, for example), we have included symbols on the text pages next to the section headings to indicate how important each topic is. A filled circle stands for "basic knowledge," a half-filled circle indicates "standard knowledge," and an empty circle stands for "in-depth knowledge." Of course, this classification only reflects our subjective views.
This second edition was carefully revised and a significant number of new plates were added to cover new developments.

We are grateful to many readers for their comments and valuable criticisms during the preparation of this book. Of course, we would also welcome further comments and suggestions from our readers.

August 2004

Jan Koolman,
Klaus-Heinrich Röhm
Marburg

Jürgen Wirth
Darmstadt

Contents

Key to color-coding:
see front and rear inside covers

Introduction

This paperback atlas is intended for students of medicine and the biological sciences. It provides an introduction to biochemistry, but with its modular structure it can also be used as a reference book for more detailed information. The 216 color plates provide knowledge in the field of biochemistry, accompanied by detailed information in the text on the facing page. The degree of dif - culty of the subject-matter is indicated by symbols in the text:

- ● stands for "basic biochemical knowledge"
- ◑ indicates "standard biochemical knowledge"
- ○ means "specialist biochemical knowledge."

Some general rules used in the structure of the illustrations are summed up in two *explanatory plates* inside the front and back covers. Keywords, definitions, explanations of unfamiliar concepts and chemical formulas can be found using the *index*. The book starts with a few **basics** in biochemistry (pp. 2–33). There is a brief explanation of the concepts and principles of chemistry (pp. 2–15). These include the periodic table of the elements, chemical bonds, the general rules governing molecular structure, and the structures of important classes of compounds. Several basic concepts of *physical chemistry* are also essential for an understanding of biochemical processes. Pages 16–33 therefore discuss the various forms of energy and their interconversion, reaction kinetics and catalysis, the properties of water, acids and bases, and redox processes.

These basic concepts are followed by a section on the structure of the important biomolecules (pp. 34–87). This part of the book is arranged according to the different classes of metabolites. It discusses carbohydrates, lipids, amino acids, peptides and proteins, nucleotides, and nucleic acids.

The next part presents the reactions involved in the interconversion of these compounds—the part of biochemistry that is commonly referred to as **metabolism** (pp. 88–195). The section starts with a discussion of the enzymes and coenzymes, and discusses the mechanisms of metabolic regulation and the so-called *energy metabolism*. After this, the central metabolic pathways are presented, once again arranged according to the class of metabolite (pp. 150–195).

The second half of the book begins with a discussion of the functional compartments within the cell, the **cellular organelles** (pp. 196–235). This is followed on pp. 236–265 by the current field of **molecular genetics** (*molecular biology*). A further extensive section is devoted to the biochemistry of individual **tissues and organs** (pp. 266–359). Here, it has only been possible to focus on the most important organs and organ systems—the digestive system, blood, liver, kidneys, muscles, connective and supportive tissues, and the brain.

Other topics include the biochemistry of **nutrition** (pp. 360–369), the structure and function of important **hormones** (pp. 370–393), and **growth and development** (pp. 394–405).

The paperback atlas concludes with a series of schematic **metabolic "charts"** (pp. 407–419). These plates, which are not accompanied by explanatory text apart from a brief introduction on p. 406, show simplified versions of the most important synthetic and degradative pathways. The charts are mainly intended for reference, but they can also be used to review previously learned material. The enzymes catalyzing the various reactions are only indicated by their EC numbers. Their names can be found in the systematically arranged and annotated enzyme list (pp. 420–430).

Periodic table

A. Biologically important elements ◑

There are 81 stable elements in nature. Fifteen of these are present in all living things, and a further 8–10 are only found in particular organisms. The illustration shows the first half of the **periodic table**, containing all of the biologically important elements. In addition to physical and chemical data, it also provides information about the distribution of the elements in the living world and their abundance in the human body. The laws of atomic structure underlying the periodic table are discussed in chemistry textbooks.

More than 99% of the atoms in animals' bodies are accounted for by just four elements—hydrogen (H), oxygen (O), carbon (C) and nitrogen (N). Hydrogen and oxygen are the constituents of water, which alone makes up 60–70% of cell mass (see p. 196). Together with carbon and nitrogen, hydrogen and oxygen are also the major constituents of the **organic compounds** on which most living processes depend. Many biomolecules also contain sulfur (S) or phosphorus (P). The above **macroelements** are essential for all organisms.

A second biologically important group of elements, which together represent only about 0.5% of the body mass, are present almost exclusively in the form of **inorganic ions**. This group includes the *alkali metals* sodium (Na) and potassium (K), and the *alkaline earth metals* magnesium (Mg) and calcium (Ca). The halogen *chlorine* (Cl) is also always ionized in the cell. All other elements important for life are present in such small quantities that they are referred to as **trace elements**. These include transition metals such as iron (Fe), zinc (Zn), copper (Cu), cobalt (Co) and manganese (Mn). A few *nonmetals,* such as iodine (I) and selenium (Se), can also be classed as essential trace elements.

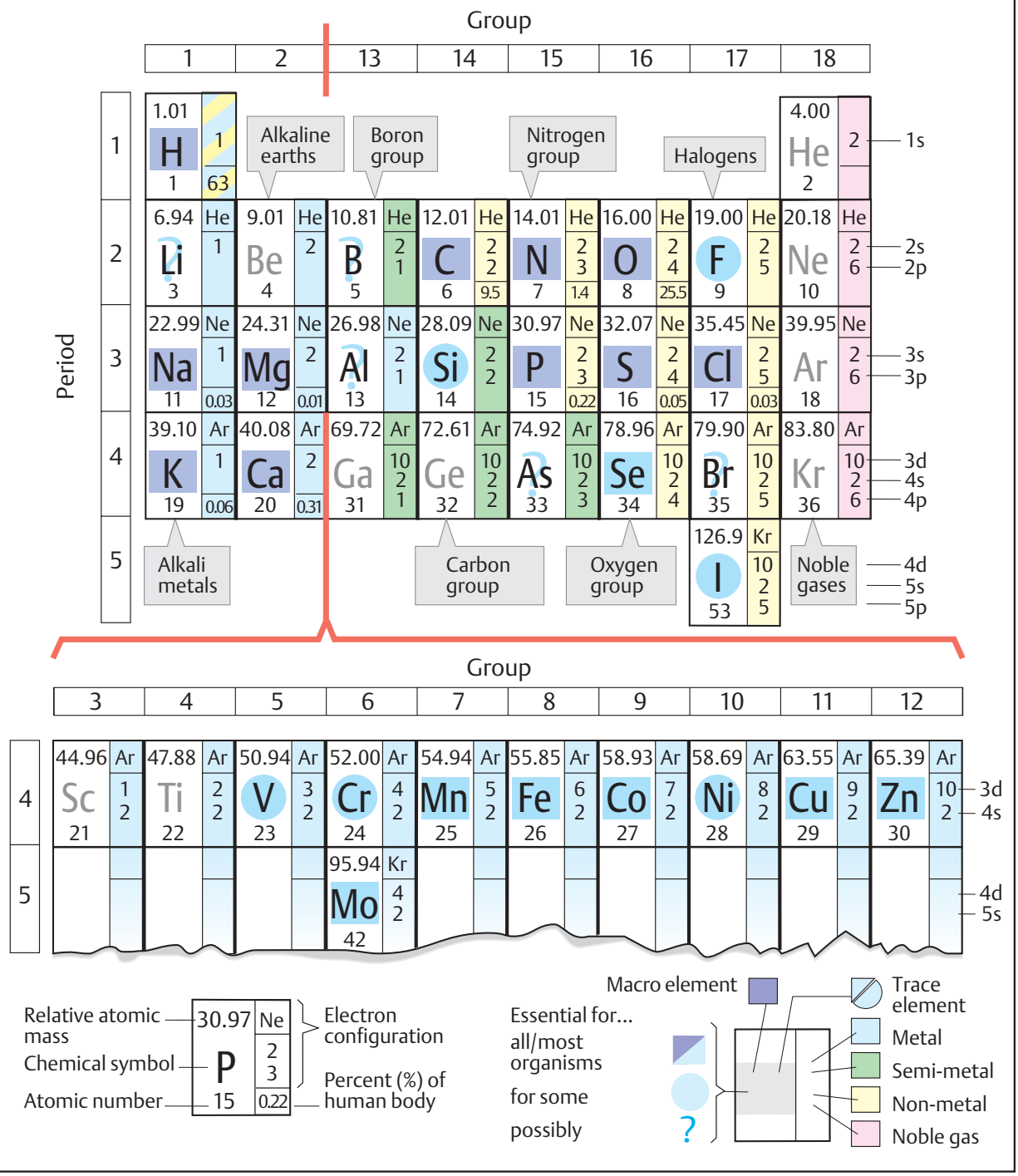
B. Electron configurations: examples ○

The chemical properties of atoms and the types of bond they form with each other are determined by their electron shells. The **electron configurations** of the elements are therefore also shown in Fig. **A**. Fig. **B** explains the symbols and abbreviations used. More detailed discussions of the subject are available in chemistry textbooks.

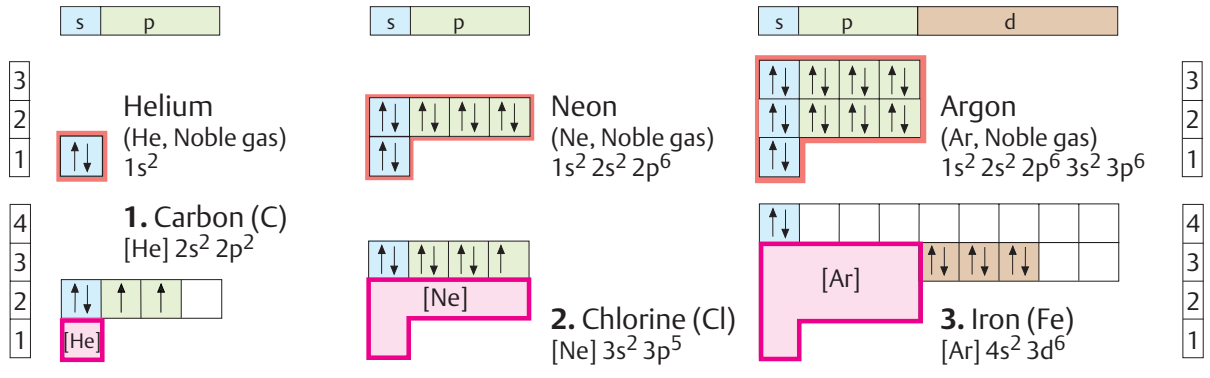
The possible states of electrons are called **orbitals**. These are indicated by what is known as the principal quantum number and by a letter—s, p, or d. The orbitals are filled one by one as the number of electrons increases. Each orbital can hold a maximum of two electrons, which must have oppositely directed "spins." Fig. **A** shows the distribution of the electrons among the orbitals for each of the elements. For example, the six electrons of carbon (**B1**) occupy the 1s orbital, the 2s orbital, and two 2p orbitals. A filled 1s orbital has the same electron configuration as the noble gas helium (He). This region of the electron shell of carbon is therefore abbreviated as "He" in Fig. **A**. Below this, the numbers of electrons in each of the other filled orbitals (2s and 2p in the case of carbon) are shown on the right margin. For example, the electron shell of chlorine (**B2**) consists of that of neon (Ne) and seven additional electrons in 3s and 3p orbitals. In iron (**B3**), a transition metal of the first series, electrons occupy the 4s orbital even though the 3d orbitals are still partly empty. Many reactions of the transition metals involve empty d orbitals—e. g., redox reactions or the formation of complexes with bases.

Particularly stable electron arrangements arise when the outermost shell is fully occupied with eight electrons (the "**octet rule**"). This applies, for example, to the noble gases, as well as to ions such as Cl^- ($3s^2 3p^6$) and Na^+ ($2s^2 2p^6$). It is only in the cases of hydrogen and helium that two electrons are already suf cient to fill the outermost 1s orbital.

A. Biologically important elements



B. Electron configurations: examples



Bonds

A. Orbital hybridization and chemical bonding ○

Stable, covalent bonds between nonmetal atoms are produced when orbitals (see p. 2) of the two atoms form **molecular orbitals** that are occupied by one electron from each of the atoms. Thus, the four bonding electrons of the carbon atom occupy 2s and 2p atomic orbitals (**1a**). The 2s orbital is spherical in shape, while the three 2p orbitals are shaped like dumbbells arranged along the x, y, and z axes. It might therefore be assumed that carbon atoms should form at least *two different* types of molecular orbital. However, this is not normally the case. The reason is an effect known as **orbital hybridization**. Combination of the s orbital and the three p orbitals of carbon gives rise to four equivalent, tetrahedrally arranged sp^3 atomic orbitals (**sp^3 hybridization**). When these overlap with the 1s orbitals of H atoms, four equivalent σ-molecular orbitals (**1b**) are formed. For this reason, carbon is capable of forming four bonds—i. e., it has a valency of four. Single bonds between nonmetal atoms arise in the same way as the four σ or **single bonds** in methane (CH_4). For example, the hydrogen phosphate ion (HPO_4^{2-}) and the ammonium ion (NH_4^+) are also tetrahedral in structure (**1c**).

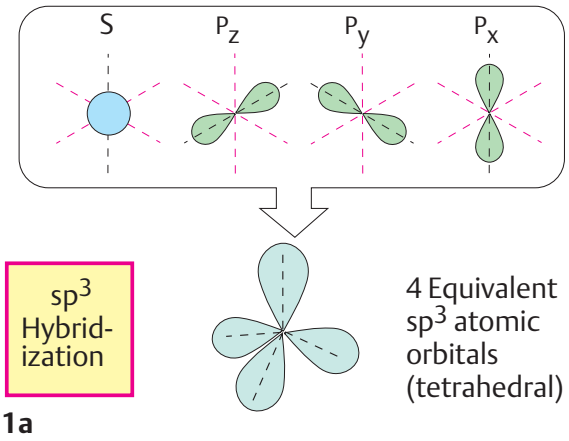
A second common type of orbital hybridization involves the 2s orbital and only *two* of the three 2p orbitals (2a). This process is therefore referred to as **sp^2 hybridization**. The result is three equivalent sp^2 hybrid orbitals lying in one plane at an angle of 120° to one another. The remaining $2p_x$ orbital is oriented perpendicular to this plane. In contrast to their sp^3 counterparts, sp^2-hybridized atoms form two *different* types of bond when they combine into molecular orbitals (**2b**). The three sp^2 orbitals enter into σ bonds, as described above. In addition, the electrons in the two $2p_x$ orbitals, known as **π electrons**, combine to give an additional, elongated π molecular orbital, which is located above and below the plane of the σ bonds. Bonds of this type are called **double bonds**. They consist of a σ bond and a π bond, and arise only when both of the atoms involved are capable of sp^2 hybridization. In contrast to single bonds, double bonds are not freely rotatable, since rotation would distort the π-molecular orbital. This is why all of the atoms lie in one plane (**2c**); in addition, *cis–trans* isomerism arises in such cases (see p. 8). Double bonds that are common in biomolecules are C=C and C=O. C=N double bonds are found in aldimines (Schiff bases, see p. 178).

B. Resonance ◑

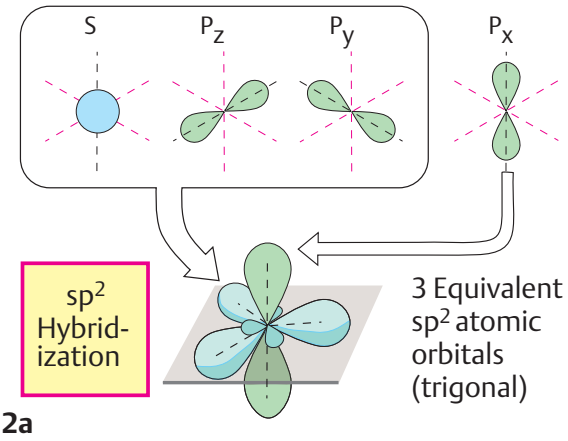
Many molecules that have several double bonds are much less reactive than might be expected. The reason for this is that the double bonds in these structures cannot be localized unequivocally. Their π orbitals are not confined to the space between the double-bonded atoms, but form a shared, extended **π-molecular orbital**. Structures with this property are referred to as **resonance hybrids**, because it is impossible to describe their actual bonding structure using standard formulas. One can either use what are known as **resonance structures**—i. e., idealized configurations in which π electrons are assigned to specific atoms (cf. pp. 32 and 66, for example)—or one can use dashed lines as in Fig. **B** to suggest the extent of the delocalized orbitals. (Details are discussed in chemistry textbooks.)

Resonance-stabilized systems include carboxylate groups, as in *formate*; aliphatic hydrocarbons with conjugated double bonds, such as *1,3-butadiene*; and the systems known as **aromatic ring systems**. The best-known aromatic compound is *benzene,* which has six delocalized π electrons in its ring. Extended resonance systems with 10 or more π electrons absorb light within the visible spectrum and are therefore *colored.* This group includes the aliphatic carotenoids (see p. 132), for example, as well as the heme group, in which 18 π electrons occupy an extended molecular orbital (see p. 106).

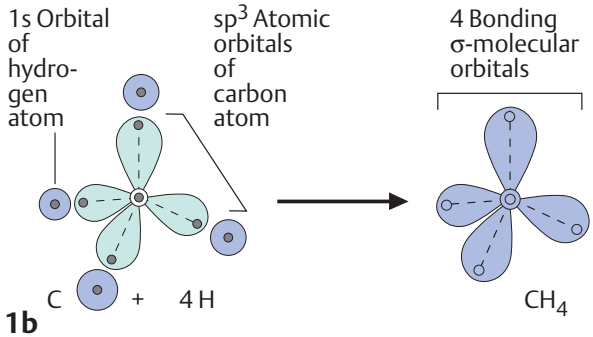
A. Orbital hybridization and chemical bonding



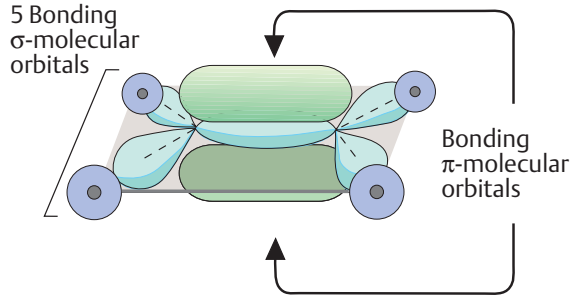
1a



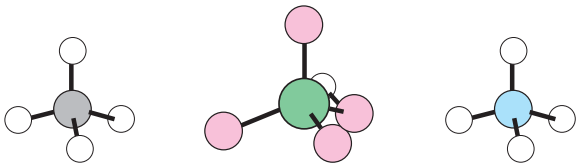
2a



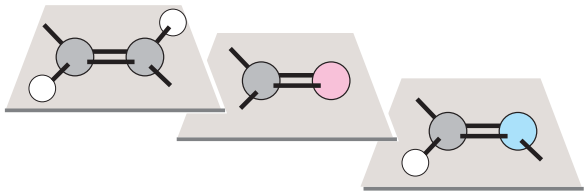
1b



2b

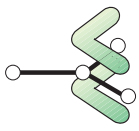
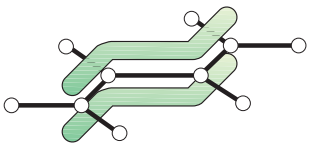
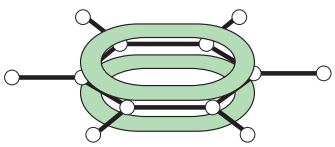
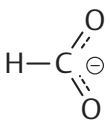
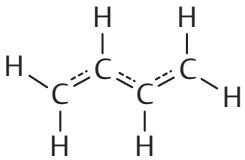
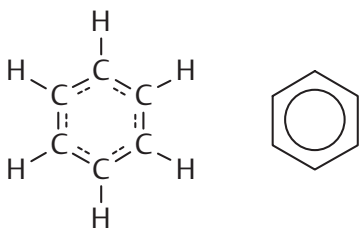


1c



2c

B. Resonance

	Formate	1,3-Butadiene	Benzene
π-Molecular orbitals			
Formula			

Molecular structure

The physical and chemical behavior of molecules is largely determined by their **constitution** (the type and number of the atoms they contain and their bonding). Structural formulas can therefore be used to predict not only the chemical reactivity of a molecule, but also its size and shape, and to some extent its conformation (the spatial arrangement of the atoms). Some data providing the basis for such predictions are summarized here and on the facing page. In addition, L-dihydroxyphenylalanine (L-dopa; see p. 352), is used as an example to show the way in which molecules are illustrated in this book.

A. Molecule illustrations ◑

In traditional two-dimensional **structural formulas** (**A1**), atoms are represented as letter symbols and electron *pairs* are shown as lines. Lines between two atomic symbols symbolize two **bonding electrons** (see p. 4), and all of the other lines represent **free electron pairs**, such as those that occur in O and N atoms. Free electrons are usually not represented explicitly (and this is the convention used in this book as well). Dashed or continuous circles or arcs are used to emphasize delocalized electrons.

Ball-and-stick models (**A2**) are used to illustrate the spatial structure of molecules. Atoms are represented as colored balls (for the color coding, see the inside front cover) and bonds (including multiple bonds) as gray cylinders. Although the relative bond lengths and angles correspond to actual conditions, the size at which the atoms are represented is too small to make the model more comprehensible.

Space-filling **van der Waals models** (**A3**) are useful for illustrating the actual shape and size of molecules. These models represent atoms as truncated balls. Their effective extent is determined by what is known as the van der Waals radius. This is calculated from the energetically most favorable distance between atoms that are not chemically bonded to one another.

B. Bond lengths and angles ○

Atomic radii and distances are now usually expressed in picometers (pm; 1 pm = 10^{-12} m). The old angstrom unit (Å, Å = 100 pm) is now obsolete. The length of single bonds approximately corresponds to the sum of what are known as the **covalent radii** of the atoms involved (see inside front cover). Double bonds are around 10–20% shorter than single bonds. In sp^3-hybridized atoms, the angle between the individual bonds is approx. 110°; in sp^2-hybridized atoms it is approx. 120°.

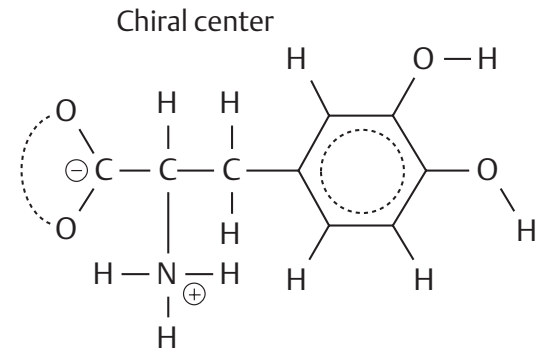
C. Bond polarity ○

Depending on the position of the element in the periodic table (see p. 2), atoms have different **electronegativity**—i. e., a different tendency to take up extra electrons. The values given in **C2** are on a scale between 2 and 4. The higher the value, the more electronegative the atom. When two atoms with very different electronegativities are bound to one another, the bonding electrons are drawn toward the more electronegative atom, and the **bond** is **polarized**. The atoms involved then carry positive or negative partial charges. In **C1**, the van der Waals surface is colored according to the different charge conditions (red = negative, blue = positive). Oxygen is the most strongly electronegative of the biochemically important elements, with C=O double bonds being especially highly polar.

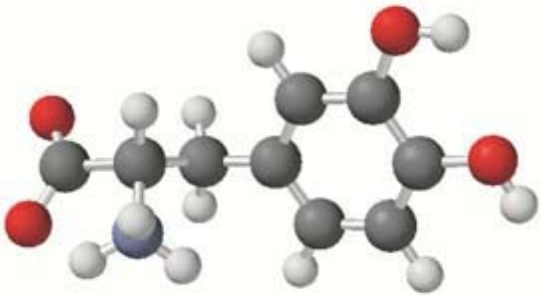
D. Hydrogen bonds ◑

The **hydrogen bond**, a special type of noncovalent bond, is extremely important in biochemistry. In this type of bond, hydrogen atoms of OH, NH, or SH groups (known as hydrogen bond **donors**) interact with free electrons of **acceptor** atoms (for example, O, N, or S). The bonding energies of hydrogen bonds (10–40 kJ mol^{-1}) are much lower than those of covalent bonds (approx. 400 kJ mol^{-1}). However, as hydrogen bonds can be very numerous in proteins and DNA, they play a key role in the stabilization of these molecules (see pp. 68, 84). The importance of hydrogen bonds for the properties of water is discussed on p. 26.

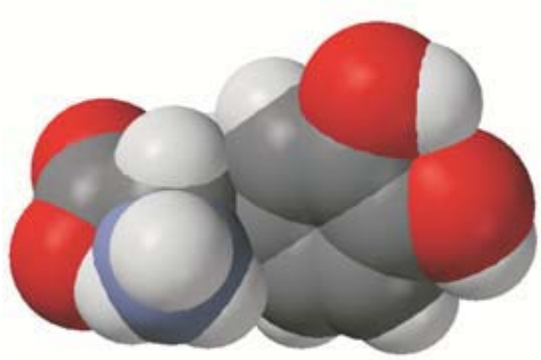
A. Molecule illustrations



1. Formula illustration

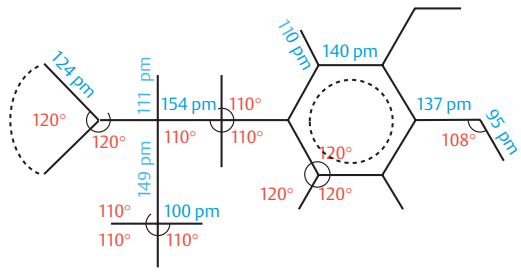


2. Ball- and-stick model

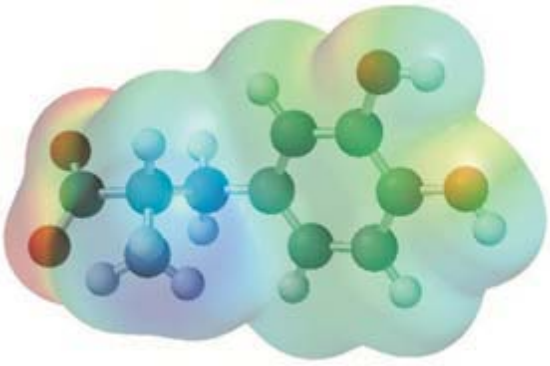


3. Van der Waals model

B. Bond lengths and angles

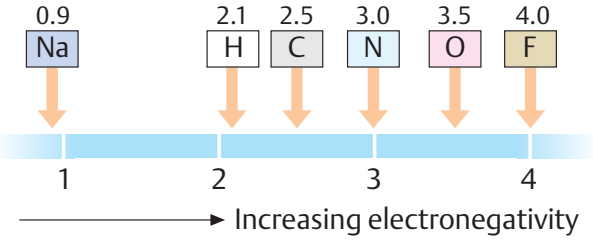


C. Bond polarity



Positive Neutral Negative

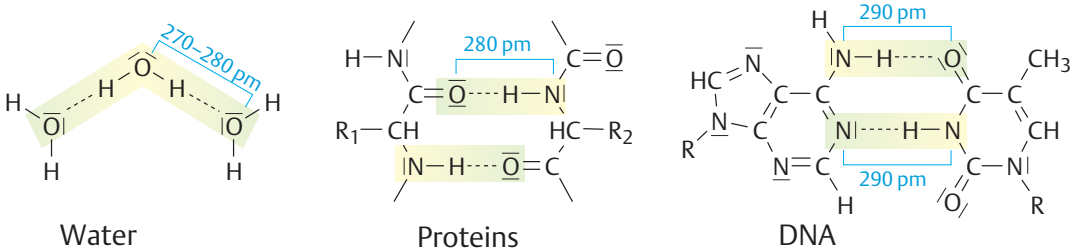
1. Partial charges in L-dopa



2. Electronegativities

D. Hydrogen bonds

1. Principle



2. Examples

Isomerism

Isomers are molecules with the same composition (i.e. the same molecular formula), but with different chemical and physical properties. If isomers differ in the way in which their atoms are bonded in the molecule, they are described as **structural isomers** (cf. citric acid and isocitric acid, **D**). Other forms of isomerism are based on different arrangements of the substituents of bonds (**A**, **B**) or on the presence of chiral centers in the molecule (**C**).

A. cis–trans isomers ◑

Double bonds *are not freely rotatable* (see p. 4). If double-bonded atoms have different substituents, there are two possible orientations for these groups. In **fumaric acid**, an intermediate of the tricarboxylic acid cycle (see p. 136), the carboxy groups lie on *different* sides of the double bond (*trans* or *E* position). In its isomer **maleic acid**, which is not produced in metabolic processes, the carboxy groups lie on the *same* side of the bond (*cis* or *Z* position). *Cis–trans* isomers (**geometric isomers**) have different chemical and physical properties—e.g., their melting points (Fp.) and pK_a values. They can only be interconverted by chemical reactions.

In lipid metabolism, *cis–trans* isomerism is particularly important. For example, double bonds in natural fatty acids (see p. 48) usually have a *cis* configuration. By contrast, unsaturated intermediates of β oxidation have a *trans* configuration. This makes the breakdown of unsaturated fatty acids more complicated (see p. 166). Light-induced *cis–trans* isomerization of retinal is of central importance in the visual cycle (see p. 358).

B. Conformation ◑

Molecular forms that arise as a result of rotation around freely rotatable bonds are known as **conformers**. Even small molecules can have different conformations in solution. In the two conformations of **succinic acid** illustrated opposite, the atoms are arranged in a similar way to fumaric acid and maleic acid. Both forms are possible, although conformation 1 is more favorable due to the greater distance between the COOH groups and therefore occurs more frequently. Biologically active macromolecules such as proteins or nucleic acids usually have well-defined ("native") conformations, which are stabilized by interactions in the molecule (see p. 74).

C. Optical isomers ◑

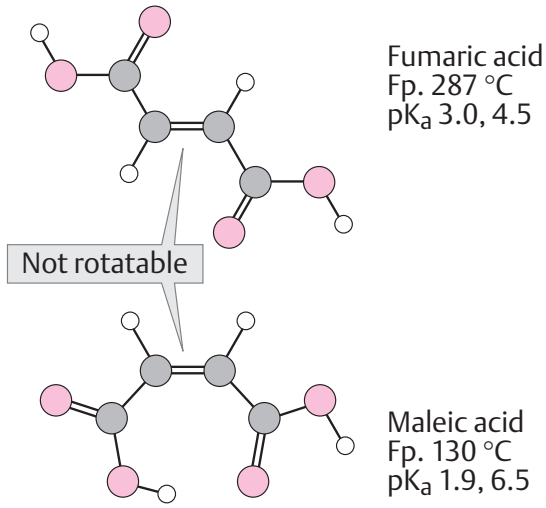
Another type of isomerism arises when a molecule contains a **chiral center** or is chiral as a whole. Chirality (from the Greek *cheir,* hand) leads to the appearance of structures that behave like image and mirror-image and that cannot be superimposed ("mirror" isomers). The most frequent cause of chiral behavior is the presence of an asymmetric C atom—i.e., an atom with four *different* substituents. Then there are two forms **(enantiomers)** with different **configurations.** Usually, the two enantiomers of a molecule are designated as L and D forms. Clear classification of the configuration is made possible by the *R/S system* (see chemistry textbooks).

Enantiomers have very similar chemical properties, but they rotate polarized light in opposite directions (**optical activity**, see pp. 36, 58). The same applies to the enantiomers of **lactic acid**. The dextrorotatory L-lactic acid occurs in animal muscle and blood, while the D form produced by microorganisms is found in milk products, for example (see p. 148). The Fischer projection is often used to represent the formulas for chiral centers (cf. p. 58).

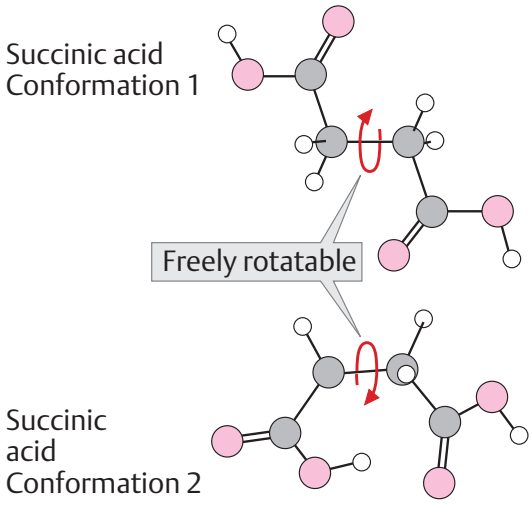
D. The aconitase reaction ○

Enzymes usually function *stereospecifically.* In chiral substrates, they only accept one of the enantiomers, and the reaction products are usually also sterically uniform. *Aconitate hydratase* (aconitase) catalyzes the conversion of citric acid into the constitution isomer isocitric acid (see p. 136). Although citric acid is not chiral, aconitase only forms one of the four possible isomeric forms of isocitric acid (*2R,3S*-isocitric acid). The intermediate of the reaction, the unsaturated tricarboxylic acid *aconitate,* only occurs in the *cis* form in the reaction. The *trans* form of aconitate is found as a constituent of certain plants.

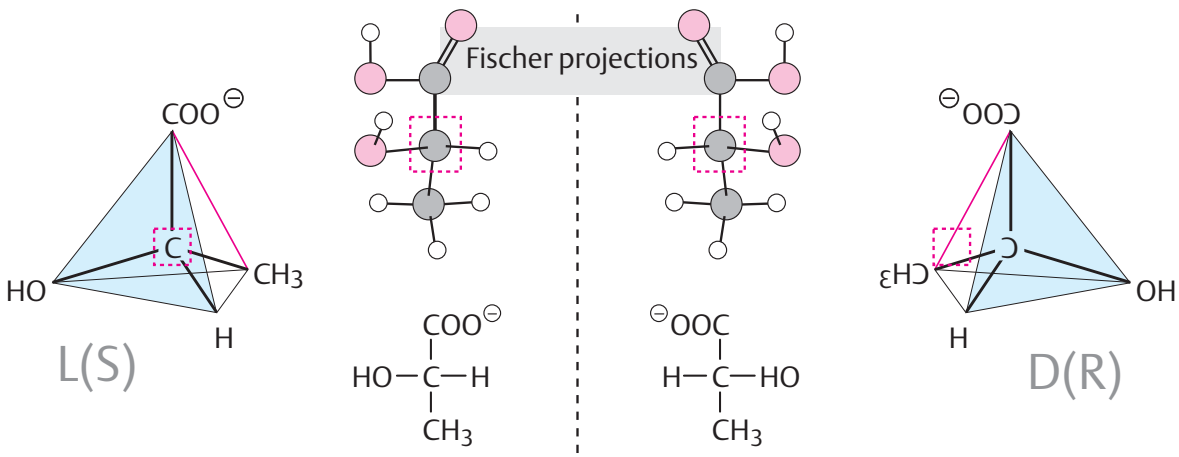
A. *cis–trans* isomers



B. Conformers



C. Optical isomers



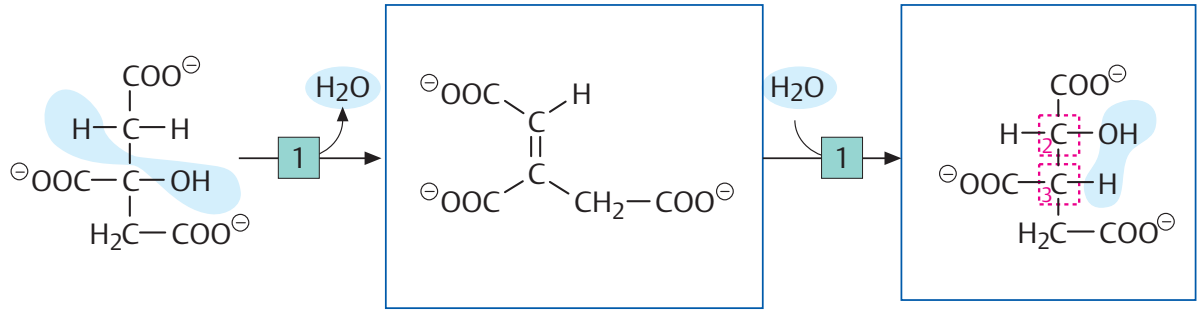
	L-lactic acid
Fp.	53 °C
pKa value	3.7
Specific rotation	+ 2.5°

In muscle, blood

In milk products

D-lactic acid	
53 °C	Fp.
3.7	pKa value
-2.5°	Specific rotation

D. The aconitase reaction



Citrate (prochiral)

cis-Aconitate (intermediate product)

(*2R,3S*)-Isocitrate

trans-Aconitate occurs in plants

1 Aconitase *4.2.1.3*

Biomolecules I

A. Important classes of compounds ●

Most biomolecules are derivatives of simple compounds of the non-metals oxygen (O), hydrogen (H), nitrogen (N), sulfur (S), and phosphorus (P). The biochemically important oxygen, nitrogen, and sulfur compounds can be formally derived from their compounds with hydrogen (i.e., H_2O, NH_3, and H_2S). In biological systems, phosphorus is found almost exclusively in derivatives of phosphoric acid, H_3PO_4.

If one or more of the hydrogen atoms of a non-metal hydride are replaced formally with another group, R—e.g., alkyl residues—then derived compounds of the type R-XH_{n-1}, R-XH_{n-2}-R, etc., are obtained. In this way, **alcohols** (R-OH) and **ethers** (R-O-R) are derived from water (H_2O); primary **amines** (R-NH_2), secondary amines (R-NH-R) and tertiary amines (R-N-R′R″) amines are obtained from ammonia (NH_3); and **thiols** (R-SH) and **thioethers** (R-S-R′) arise from hydrogen sulfide (H_2S). Polar groups such as -OH and -NH_2 are found as substituents in many organic compounds. As such groups are much more reactive than the hydrocarbon structures to which they are attached, they are referred to as **functional groups**.

New functional groups can arise as a result of **oxidation** of the compounds mentioned above. For example, the oxidation of a thiol yields a **disulfide** (R-S-S-R). Double oxidation of a primary alcohol (R-CH_2-OH) gives rise initially to an **aldehyde** (R-C(O)-H), and then to a **carboxylic acid** (R-C(O)-OH). In contrast, the oxidation of a secondary alcohol yields a **ketone** (R-C(O)-R). The carbonyl group (C=O) is characteristic of aldehydes and ketones.

The addition of an amine to the carbonyl group of an aldehyde yields—after removal of water—an **aldimine** (not shown; see p. 178). Aldimines are intermediates in amino acid metabolism (see p. 178) and serve to bond aldehydes to amino groups in proteins (see p. 62, for example). The addition of an alcohol to the carbonyl group of an aldehyde yields a **hemiacetal** (R-O-C(H)OH-R). The cyclic forms of sugars are well-known examples of hemiacetals (see p. 36). The oxidation of hemiacetals produces carboxylic acid esters.

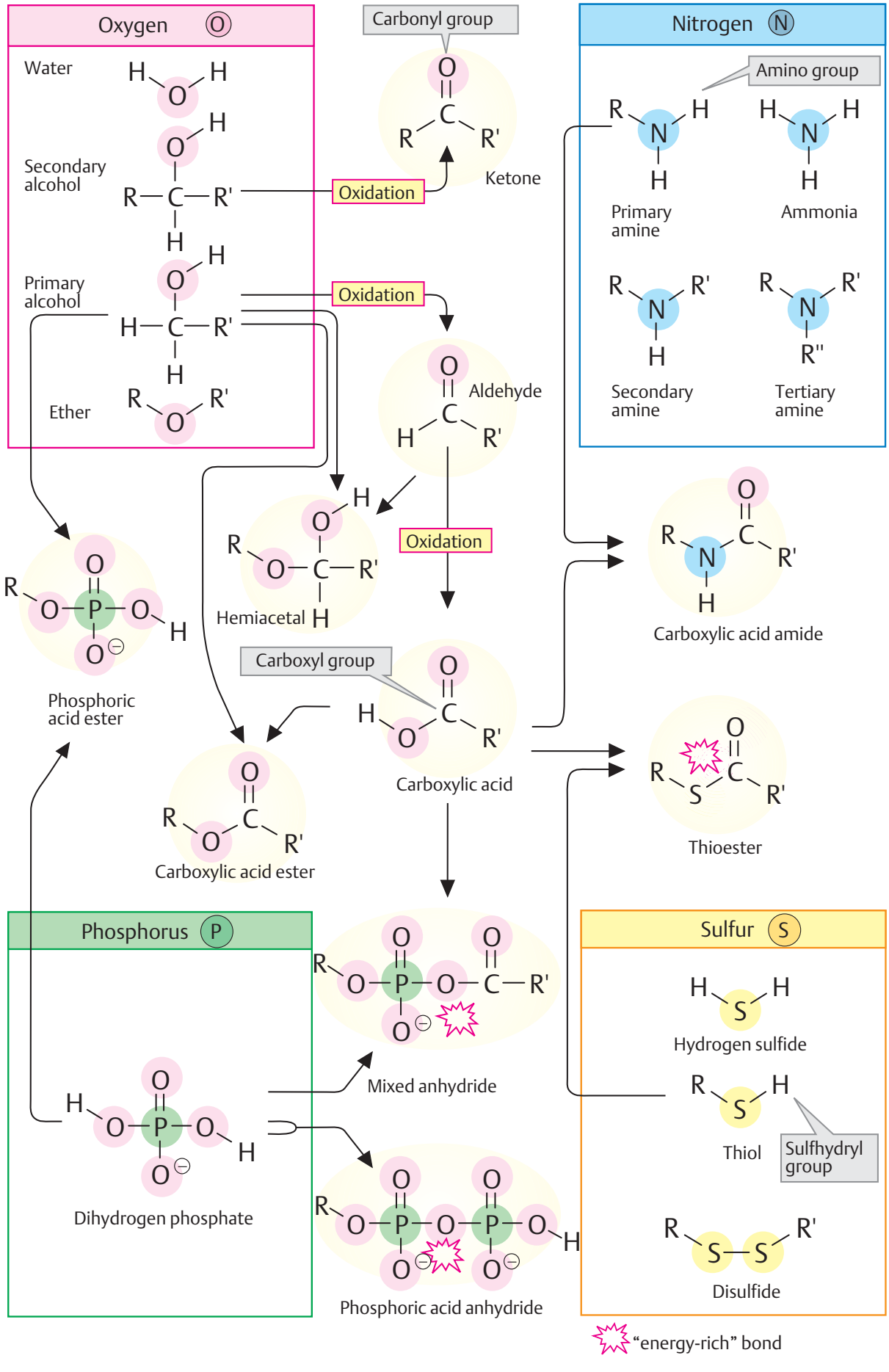
Very important compounds are the **carboxylic acids** and their derivatives, which can be formally obtained by exchanging the OH group for another group. In fact, derivatives of this type are formed by nucleophilic substitutions of activated intermediate compounds and the release of water (see p. 14). **Carboxylic acid esters** (R-O-CO-R′) arise from carboxylic acids and alcohols. This group includes the fats, for example (see p. 48). Similarly, a carboxylic acid and a thiol yield a **thioester** (R-S-CO-R′). Thioesters play an extremely important role in carboxylic acid metabolism. The best-known compound of this type is acetyl-coenzyme A (see p. 12).

Carboxylic acids and primary amines react to form **carboxylic acid amides** (R-NH-CO-R′). The amino acid constituents of peptides and proteins are linked by carboxylic acid amide bonds, which are therefore also known as peptide bonds (see p. 66).

Phosphoric acid, H_3PO_4, is a tribasic (triprotic) acid—i.e., it contains three hydroxyl groups able to donate H^+ ions. At least one of these three groups is fully dissociated under normal physiological conditions, while the other two can react with alcohols. The resulting products are phosphoric acid monoesters (R-O-P(O)O-OH) and diesters (R-O-P(O)O-O-R′). **Phosphoric acid monoesters** are found in carbohydrate metabolism, for example (see p. 36), whereas **phosphoric acid diester** bonds occur in phospholipids (see p. 50) and nucleic acids (see p. 82).

Compounds of one acid with another are referred to as **acid anhydrides**. A particularly large amount of energy is required for the formation of an acid—anhydride bond. Phosphoric anhydride bonds therefore play a central role in the storage and release of chemical energy in the cell (see p. 122). Mixed anhydrides between carboxylic acids and phosphoric acid are also very important "energy-rich metabolites" in cellular metabolism.

A. Important classes of compounds



Biomolecules II

Many biomolecules are made up of smaller units in a modular fashion, and they can be broken down into these units again. The construction of these molecules usually takes place through condensation reactions involving the removal of water. Conversely, their breakdown functions in a hydrolytic fashion—i.e., as a result of water uptake. The page opposite illustrates this modular principle using the example of an important coenzyme.

A. Acetyl CoA ◑

Coenzyme A (see also p. 106) is a nucleotide with a complex structure (see p. 80). It serves to activate residues of carboxylic acids (acyl residues). Bonding of the carboxy group of the carboxylic acid with the thiol group of the coenzyme creates a **thioester bond** (-S-CO-R; see p. 10) in which the **acyl residue** has a **high chemical potential**. It can therefore be transferred to other molecules in exergonic reactions. This fact plays an important role in lipid metabolism in particular (see pp. 162 ff.), as well as in two reactions of the tricarboxylic acid cycle (see p. 136).

As discussed on p. 16, the **group transfer potential** can be expressed quantitatively as the change in free enthalpy (ΔG) during hydrolysis of the compound concerned. This is an arbitrary determination, but it provides important indications of the chemical energy stored in such a group. In the case of acetyl-CoA, the reaction to be considered is:

$$\text{Acetyl CoA} + H_2O \rightarrow \text{acetate} + \text{CoA}$$

In standard conditions and at pH 7, the change in the chemical potential G (ΔG^0, see p. 18) in this reaction amounts to −32 kJ mol^{-1} and it is therefore as high as the ΔG^0 of ATP hydrolysis (see p. 18). In addition to the "energy-rich" **thioester bond**, acetyl-CoA also has seven other hydrolyzable bonds with different degrees of stability. These bonds, and the fragments that arise when they are hydrolyzed, will be discussed here in sequence.

(1) The reactive thiol group of coenzyme A is located in the part of the molecule that is derived from **cysteamine**. Cysteamine is a *biogenic amine* (see p. 62) formed by decarboxylation of the amino acid cysteine.

(2) The amino group of cysteamine is bound to the carboxy group of another biogenic amine via an **acid amide bond** (-CO-NH-). β-**Alanine** arises through decarboxylation of the amino acid aspartate, but it can also be formed by breakdown of pyrimidine bases (see p. 186).

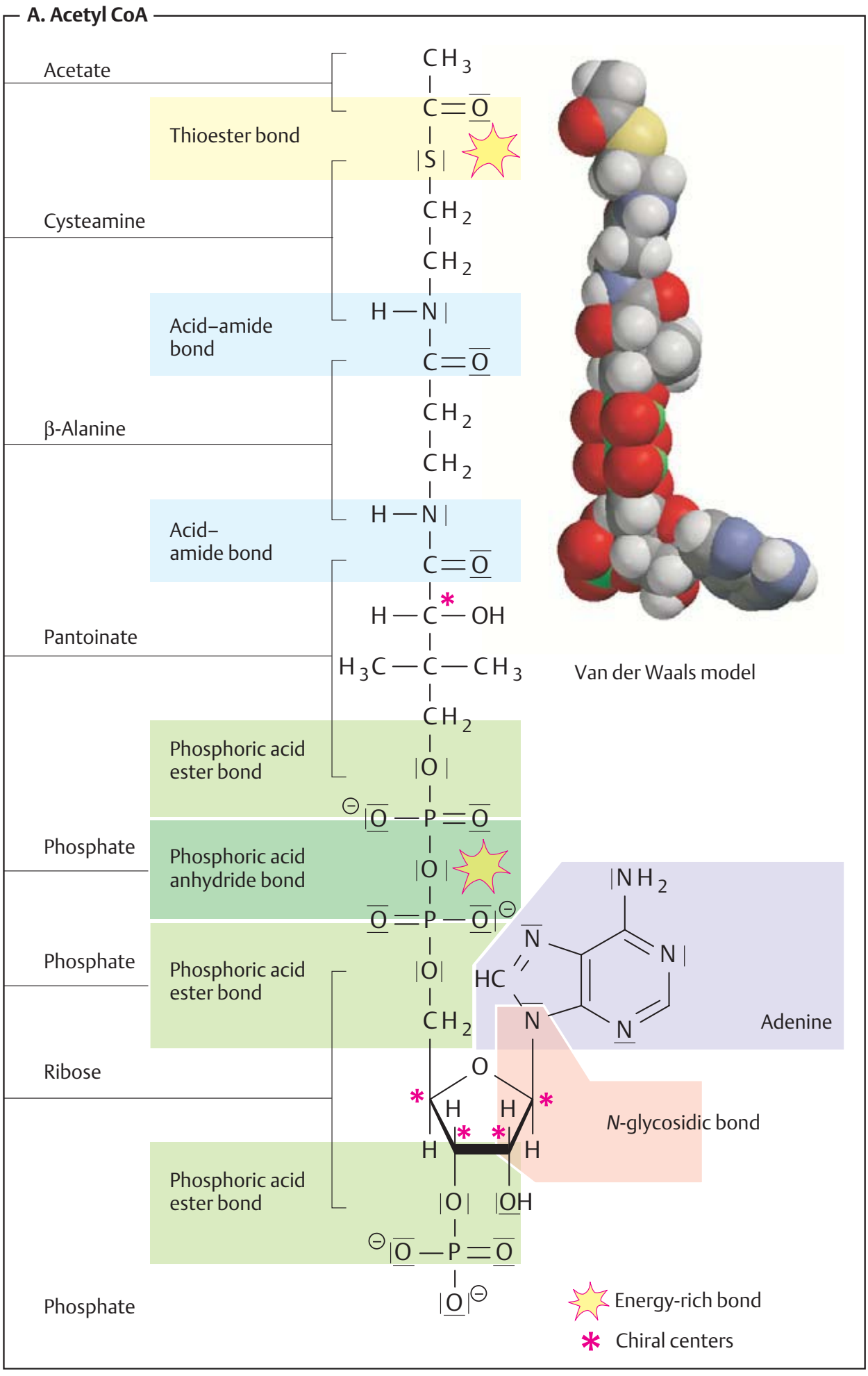
(3) Another **acid amide bond** (-CO-NH-) creates the compound for the next constituent, **pantoinate**. This compound contains a *chiral center* and can therefore appear in two enantiomeric forms (see p. 8). In natural coenzyme A, only one of the two forms is found, the (*R*)-pantoinate. Human metabolism is not capable of producing pantoinate itself, and it therefore has to take up a compound of β-alanine and pantoinate—**pantothenate** ("pantothenic acid")—in the form of a vitamin in food (see p. 366).

(4) The hydroxy group at C-4 of pantoinate is bound to a **phosphate** residue by an **ester bond**.

The section of the molecule discussed so far represents a functional unit. In the cell, it is produced from pantothenate. The molecule also occurs in a protein-bound form as **4′-phosphopantetheine** in the enzyme *fatty acid synthase* (see p. 168). In coenzyme A, however, it is bound to 3′,5′-adenosine diphosphate.

(5) When two phosphate residues bond, they do not form an ester, but an "energy-rich" **phosphoric acid anhydride bond**, as also occurs in other nucleoside phosphates. By contrast, (6) and (7) are ester bonds again.

(8) The base **adenine** is bound to C-1 of **ribose** by an **N-glycosidic** bond (see p. 36). In addition to C-2 to C-4, C-1 of ribose also represents a *chiral* center. The *β-configuration* is usually found in nucleotides.



Chemical reactions

Chemical reactions are processes in which electrons or groups of atoms are taken up into molecules, exchanged between molecules, or shifted within molecules. Illustrated here are the most important types of reaction in organic chemistry, using simple examples. Electron shifts are indicated by red arrows.

A. Redox reactions ◑

In redox reactions (see also p. 32), **electrons** are **transferred** from one molecule (the reducing agent) to another (the oxidizing agent). One or two protons are often also transferred in the process, but the decisive criterion for the presence of a redox reaction is the electron transfer. The reducing agent is oxidized during the reaction, and the oxidizing agent is reduced.

Fig. **A** shows the oxidation of an alcohol into an aldehyde (**1**) and the reduction of the aldehyde to alcohol (**2**). In the process, one *hydride ion* is transferred (two electrons and one proton; see p. 32), which moves to the oxidizing agent A in reaction **1**. The superfluous proton is bound by the catalytic effect of a base B. In the reduction of the aldehyde (**2**), A-H serves as the reducing agent and the acid H-B is involved as the catalyst.

B. Acid–base reactions ◑

In contrast to redox reactions, only **proton transfer** takes place in acid–base reactions (see also p. 30). When an acid dissociates (**1**), water serves as a proton acceptor (i. e., as a base). Conversely, water has the function of an acid in the protonation of a carboxylate anion (**2**).

C. Additions/eliminations ◑

A reaction in which atoms or molecules are taken up by a multiple bond is described as **addition**. The converse of addition—i. e., the removal of groups with the formation of a double bond, is termed **elimination**. When water is added to an alkene (**1a**), a proton is first transferred to the alkene. The unstable carbenium cation that occurs as an intermediate initially takes up water (not shown), before the separation of a proton produces alcohol (**1b**). The elimination of water from the alcohol (**2**, dehydration) is also catalyzed by an acid and passes via the same intermediate as the addition reaction.

D. Nucleophilic substitutions ◑

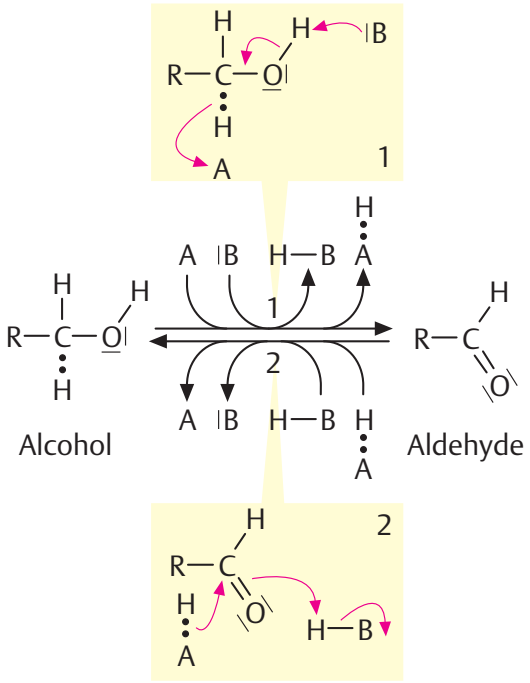
A reaction in which one functional group (see p. 10) is replaced by another is termed **substitution**. Depending on the process involved, a distinction is made between nucleophilic and electrophilic substitution reactions (see chemistry textbooks). Nucleophilic substitutions start with the addition of one molecule to another, followed by elimination of the so-called *leaving group*.

The hydrolysis of an ester to alcohol and acid (**1**) and the esterification of a carboxylic acid with an alcohol (**2**) are shown here as an example of the S_N2 mechanism. Both reactions are made easier by the marked polarity of the C=O double bond. In the form of ester hydrolysis shown here, a proton is removed from a water molecule by the catalytic effect of the base B. The resulting strongly nucleophilic OH^- ion attacks the positively charged carbonyl C of the ester (**1a**), and an unstable sp^3-hybridized transition state is produced. From this, either water is eliminated (**2b**) and the ester re-forms, or the alcohol ROH is eliminated (**1b**) and the free acid results. In esterification (**2**), the same steps take place in reverse.

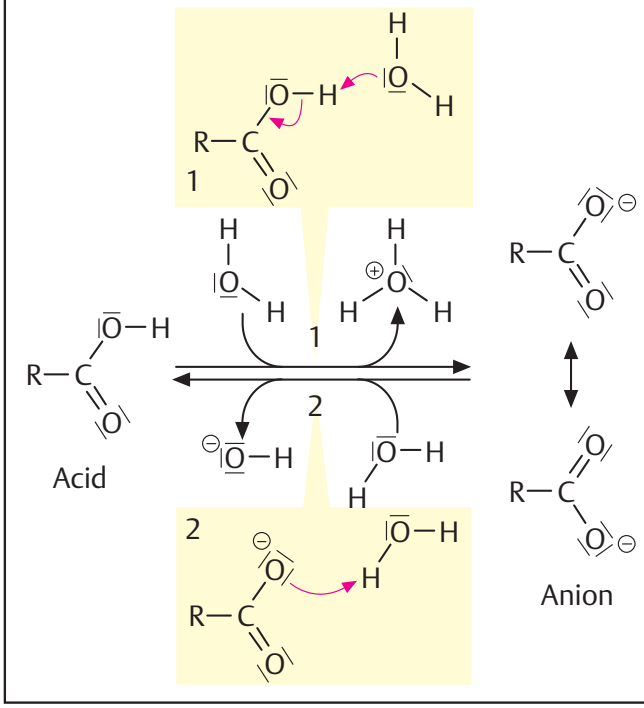
Further information

In **rearrangements** (isomerizations, not shown), groups are shifted within one and the same molecule. Examples of this in biochemistry include the isomerization of sugar phosphates (see p. 36) and of methylmalonyl-CoA to succinyl CoA (see p. 166).

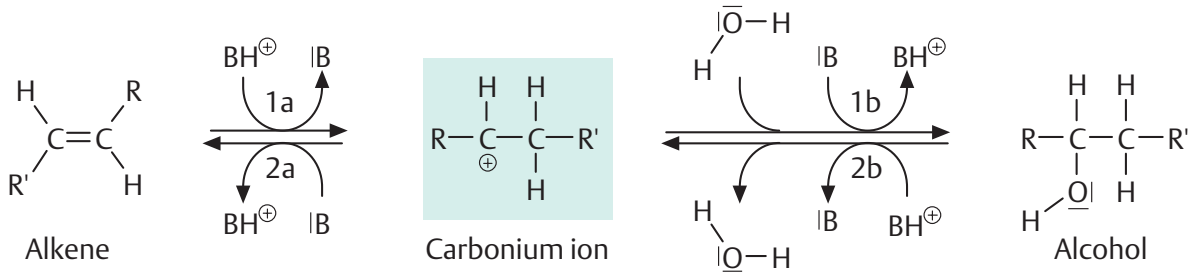
A. Redox reactions



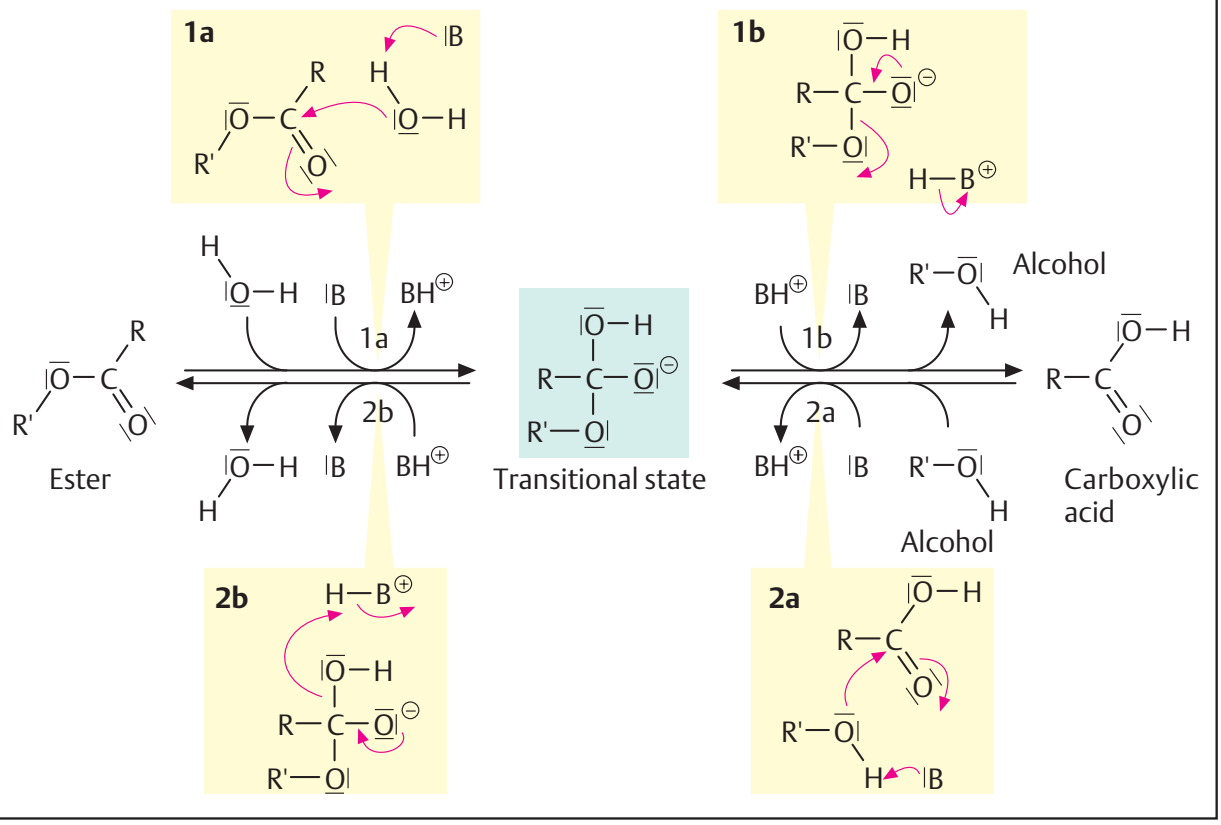
B. Acid–base reactions



C. Additions/eliminations



D. Nucleophilic substitutions



Energetics

To obtain a better understanding of the processes involved in energy storage and conversion in living cells, it may be useful first to recall the physical basis for these processes.

A. Forms of work ●

There is essentially no difference between work and energy. Both are measured in **joule** (J = 1 N m). An outdated unit is the **calorie** (1 cal = 4.187 J). **Energy is defined as the ability of a system to perform work.** There are many different forms of energy—e.g., mechanical, chemical, and radiation energy.

A system is capable of performing work when matter is moving along a potential gradient. This abstract definition is best understood by an example involving mechanical work (**A1**). Due to the earth's gravitational pull, the mechanical potential energy of an object is the greater the further the object is away from the center of the earth. A **potential difference** (ΔP) therefore exists between a higher location and a lower one. In a waterfall, the water spontaneously follows this potential gradient and, in doing so, is able to perform work—e.g., turning a mill.

Work and energy consist of two quantities: an **intensity** factor, which is a measure of the potential difference—i.e., the "driving force" of the process—(here it is the height difference) and a **capacity factor**, which is a measure of the quantity of the substance being transported (here it is the weight of the water). In the case of electrical work (**A2**), the intensity factor is the voltage—i.e., the electrical potential difference between the source of the electrical current and the "ground," while the capacity factor is the amount of charge that is flowing.

Chemical work and chemical energy are defined in an analogous way. The intensity factor here is the **chemical potential** of a molecule or combination of molecules. This is stated as **free enthalpy** *G* (also known as "Gibbs free energy"). When molecules spontaneously react with one another, the result is products at lower potential. The difference in the chemical potentials of the educts and products (the **change in free enthalpy**, ΔG) is a measure of the "driving force" of the reaction. The capacity factor in chemical work is the amount of matter reacting (in mol). Although absolute values for free enthalpy G cannot be determined, ΔG can be calculated from the equilibrium constant of the reaction (see p. 18).

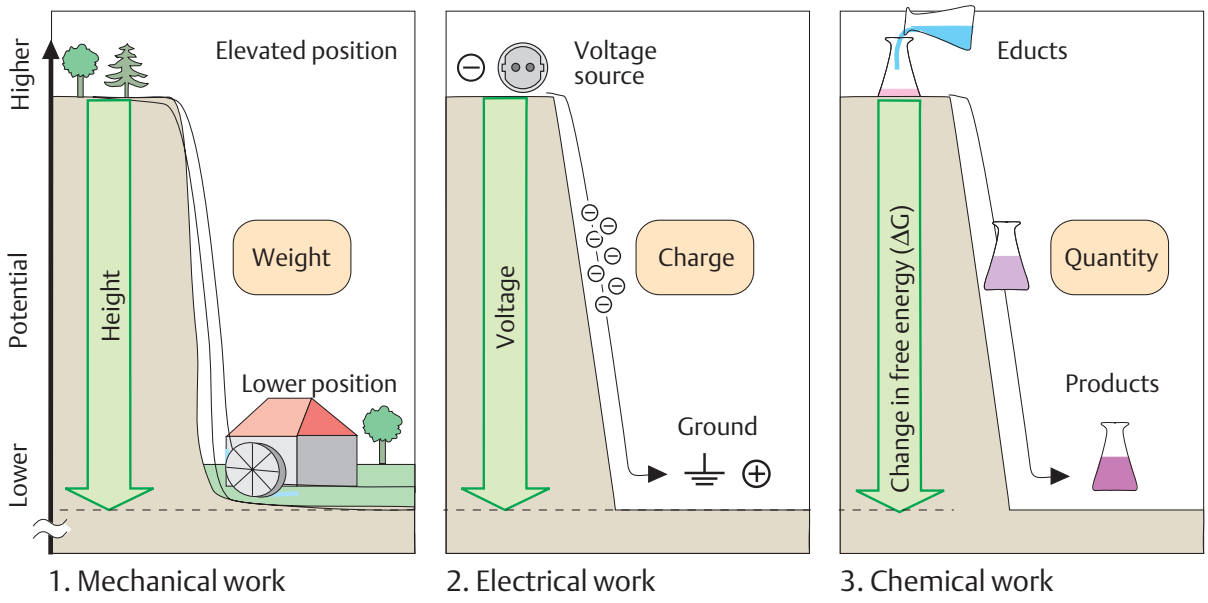
B. Energetics and the course of processes ●

Everyday experience shows that water never flows uphill *spontaneously*. Whether a particular process can occur spontaneously or not depends on whether the potential difference between the final and the initial state, $\Delta P = P_2 - P_1$, is positive or negative. If P_2 is smaller than P_1, then ΔP will be negative, and the process will take place and perform work. Processes of this type are called **exergonic** (**B1**). If there is no potential difference, then the system is in **equilibrium** (**B2**). In the case of **endergonic** processes, ΔP is positive (**B3**). Processes of this type do *not* proceed spontaneously.

Forcing endergonic processes to take place requires the use of the principle of **energetic coupling**. This effect can be illustrated by a mechanical analogy (**B4**). When two masses M_1 and M_2 are connected by a rope, M_1 will move upward even though this part of the process is endergonic. The *sum* of the two potential differences ($\Delta P_{eff} = \Delta P_1 + \Delta P_2$) is the determining factor in coupled processes. When ΔP_{eff} is negative, the entire process can proceed.

Energetic coupling makes it possible to convert different forms of work and energy into one another. For example, in a flashlight, an exergonic chemical reaction provides an electrical voltage that can then be used for the endergonic generation of light energy. In the luminescent organs of various animals, it is a chemical reaction that produces the light. In the musculature (see p. 336), chemical energy is converted into mechanical work and heat energy. A form of storage for chemical energy that is used in all forms of life is **adenosine triphosphate** (ATP; see p. 122). Endergonic processes are usually driven by coupling to the strongly exergonic breakdown of ATP (see p. 122).

A. Forms of work

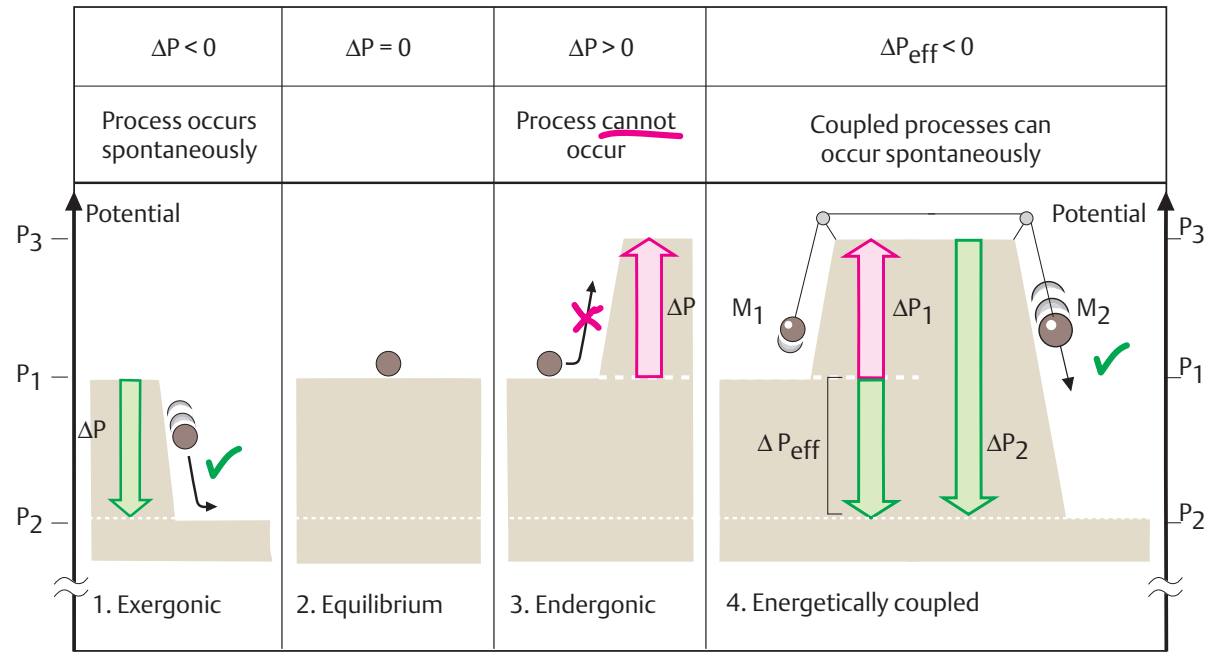


1. Mechanical work 2. Electrical work 3. Chemical work

J = Joule = N · m = 1 kg · m^2 · s^{-2}, 1 cal = 4.187 J

Form of work	Intensity factor	Unit	Capacity factor	Unit	Work = ⇨ · ▭	Unit
Mechanical	Height	m	Weight	$J \cdot m^{-1}$	Height · Weight	J
Electrical	Voltage	$V = J \cdot C^{-1}$	Charge	C	Voltage · Charge	J
Chemical	Free-enthalpy change ΔG	$J \cdot mol^{-1}$	Quantity	mol	ΔG · Quantity	J

B. Energetics and the course of processes



Equilibriums

A. Group transfer reactions ◑

Every chemical reaction reaches after a time a **state of equilibrium** in which the forward and back reactions proceed at the same speed. The **law of mass action** describes the concentrations of the educts (A, B) and products (C, D) *in equilibrium.* The **equilibrium constant K** is directly related to ΔG^0, the change in free enthalpy *G* involved in the reaction (see p. 16) under standard conditions ($\Delta G^0 = -$ R T ln K). For any given concentrations, the lower equation applies. At $\Delta G < 0$, the reaction proceeds spontaneously for as long as it takes for equilibrium to be reached (i. e., until $\Delta G = 0$). At $\Delta G > 0$, a *spontaneous* reaction is no longer possible (endergonic case; see p. 16). In biochemistry, ΔG is usually related to pH 7, and this is indicated by the "prime" symbol ($\Delta G^{0\prime}$ or $\Delta G'$).

As examples, we can look at two group transfer reactions (on the right). In ATP (see p. 122), the terminal phosphate residue is at a high chemical potential. Its transfer to water (reaction **a**, below) is therefore strongly **exergonic**. The equilibrium of the reaction ($\Delta G = 0$; see p. 122) is only reached when more than 99.9% of the originally available ATP has been hydrolyzed. ATP and similar compounds have a high **group transfer potential** for phosphate residues. Quantitatively, this is expressed as the **ΔG of hydrolysis** ($\Delta G^{0\prime} = -32$ kJ mol^{-1}; see p. 122).

In contrast, the **endergonic** transfer of ammonia (NH_3) to glutamate (Glu, reaction **b**, $\Delta G^{0\prime} = +14$ kJ mol^{-1}) reaches equilibrium so quickly that only minimal amounts of the product glutamine (Gln) can be formed in this way. The synthesis of glutamine from these preliminary stages is only possible through **energetic coupling** (see pp. 16, 124).

B. Redox reactions ◑

The course of electron transfer reactions (redox reactions, see p. 14) also follows the law of mass action. For a single redox system (see p. 32), the Nernst equation applies (top). The **electron transfer potential** of a redox system (i. e., its tendency to give off or take up electrons) is given by its **redox potential E** (in standard conditions, E^0 or $E^{0\prime}$). The *lower* the redox potential of a system is, the *higher* the chemical potential of the transferred electrons. To describe reactions between two redox systems, ΔE—the difference between the two systems' redox potentials—is usually used instead of ΔG. ΔG and ΔE have a simple relationship, but opposite signs (below). A redox reaction proceeds spontaneously when $\Delta E > 0$, i. e. $\Delta G < 0$.

The right side of the illustration shows the way in which the redox potential E is dependent on the composition (the proportion of the reduced form as a %) in two biochemically important redox systems (pyruvate/lactate and $NAD^+/NADH+H^+$; see pp. 98, 104). In the standard state (both systems reduced to 50%), electron transfer from lactate to NAD^+ is *not* possible, because ΔE is negative ($\Delta E = -0.13$ V, red arrow). By contrast, transfer can proceed successfully if the pyruvate/lactate system is reduced to 98% and NAD^+/NADH is 98% oxidized (green arrow, $\Delta E = +0.08$ V).

C. Acid–base reactions ◑

Pairs of *conjugated* acids and bases are always involved in proton exchange reactions (see p. 30). The dissociation state of an acid–base pair depends on the H^+ concentration. Usually, it is not this concentration itself that is expressed, but its negative decadic logarithm, the **pH value**. The connection between the pH value and the dissociation state is described by the *Henderson–Hasselbalch equation* (below). As a measure of the **proton transfer potential** of an acid–base pair, its **pK_a value** is used—the negative logarithm of the acid constant K_a (where "a" stands for acid).

The *stronger* an acid is, the *lower* its pK_a value. The acid of the pair with the lower pK_a value (the stronger acid—in this case acetic acid, CH_3COOH) can protonate (green arrow) the base of the pair with the higher pK_a (in this case NH_3), while ammonium acetate (NH_4^+ and CH_3COO^-) only forms very little CH_3COOH and NH_3.

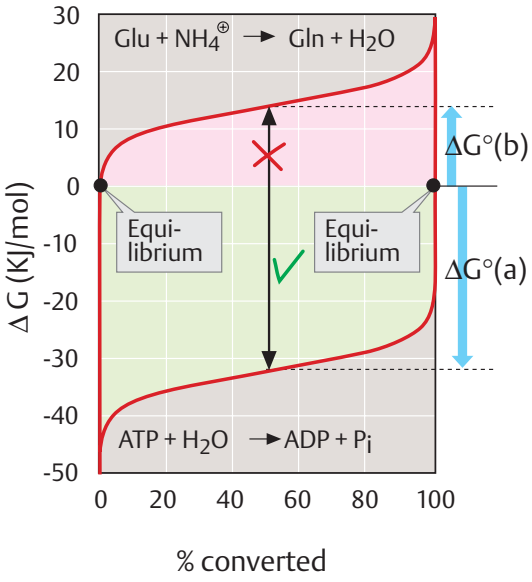
A. Group transfer reactions

Reaction: $A + B \rightleftharpoons C + D$

Law of mass action: $K = \frac{[C] \cdot [D]}{[A] \cdot [B]}$ (Only applies in chemical equilibrium; Equilibrium constant)

Relationship between ΔG^0 and K: $\Delta G^\circ = -R \cdot T \cdot \ln K$, $R = 8.314\ J \cdot mol^{-1} \cdot K^{-1}$

In any conditions: $\Delta G = \Delta G^\circ + R \cdot T \cdot \ln \frac{[C] \cdot [D]}{[A] \cdot [B]}$ (Measure of group transfer potential)



B. Redox reactions

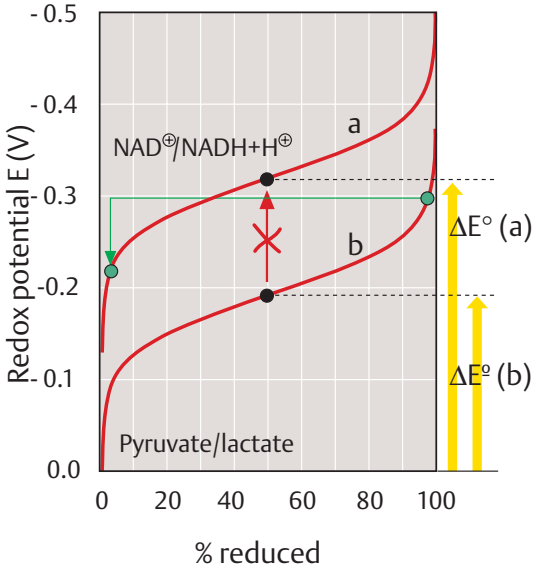
$A_{red} \rightleftharpoons A_{ox}$

For a redox system: $E = E^\circ + \frac{R \cdot T}{n \cdot F} \cdot \ln \frac{[A_{ox}]}{[A_{red}]}$ (Measure of electron transfer potential)

For any redox reaction: $\Delta E = \Delta E^\circ + \frac{R \cdot T}{n \cdot F} \cdot \ln \frac{[B_{ox}] \cdot [A_{red}]}{[B_{red}] \cdot [A_{ox}]}$

Definition and sizes: $\Delta E = E_{Acceptor} - E_{Donor}$, $\Delta G = -n \cdot F \cdot \Delta E$

n = No. of electrons transferred
F = Faraday constant



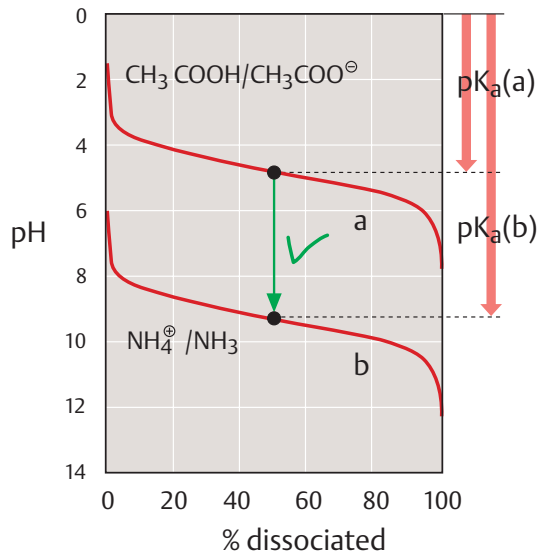
C. Acid–base reactions

Standard reaction: $HA + H_2O \rightleftharpoons A^\ominus + H_3O^\oplus$

Law of mass action: $K = \frac{[A^\ominus] \cdot [H_3O^\oplus]}{[HA] \cdot [H_2O]}$

Simplified: $K_a = \frac{[A^\ominus] \cdot [H^\oplus]}{[HA]}$

Henderson–Hasselbalch equation: $pH = pK_a + \log \frac{[A^\ominus]}{[HA]}$ (Measure of proton transfer potential)



Enthalpy and entropy

The change in the free enthalpy of a chemical reaction (i.e., its ΔG) depends on a number of factors—e.g., the concentrations of the reactants and the temperature (see p. 18). Two further factors associated with molecular changes occurring during the reaction are discussed here.

A. Heat of reaction and calorimetry ◑

All chemical reactions involve heat exchange. Reactions that release heat are called **exothermic**, and those that consume heat are called **endothermic**. Heat exchange is measured as the enthalpy change ΔH (the heat of reaction). This corresponds to the heat exchange at constant pressure. In exothermic reactions, the system *loses* heat, and ΔH is negative. When the reaction is endothermic, the system gains heat, and ΔH becomes positive.

In many reactions, ΔH and ΔG are similar in magnitude (see **B1**, for example). This fact is used to estimate the caloric content of foods. In living organisms, nutrients are usually oxidized by oxygen to CO_2 and H_2O (see p. 112). The maximum amount of chemical work supplied by a particular foodstuff (i.e., the ΔG for the oxidation of the utilizable constituents) can be estimated by burning a weighed amount in a **calorimeter** in an oxygen atmosphere. The heat of the reaction increases the water temperature in the calorimeter. The reaction heat can then be calculated from the temperature difference ΔT.

B. Enthalpy and entropy ◑

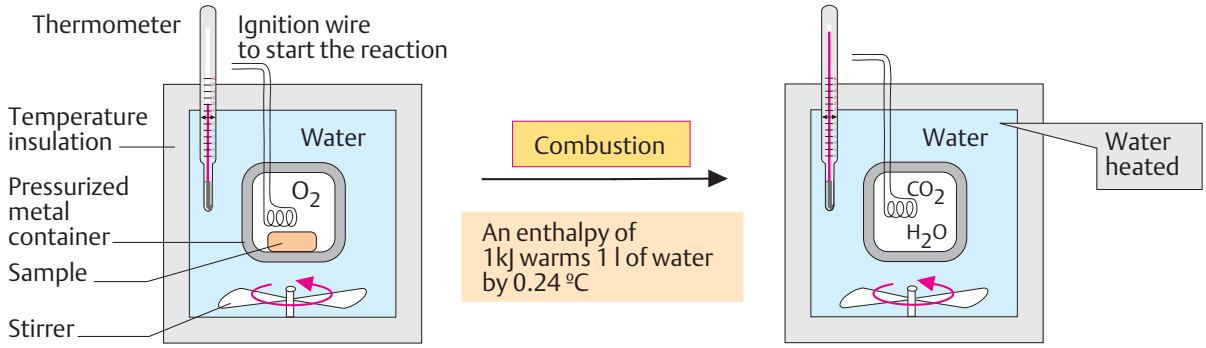
The reaction enthalpy ΔH and the change in free enthalpy ΔG are not always of the same magnitude. There are even reactions that occur spontaneously ($\Delta G < 0$) even though they are endothermic ($\Delta H > 0$). The reason for this is that changes in the degree of order of the system also strongly affect the progress of a reaction. This change is measured as the **entropy change (ΔS)**.

Entropy is a physical value that describes the **degree of order of a system**. The *lower* the degree of order, the larger the entropy. Thus, when a process leads to increase in disorder—and everyday experience shows that this is the normal state of affairs—ΔS is positive for this process. An increase in the order in a system ($\Delta S < 0$) always requires an input of energy. Both of these statements are consequences of an important natural law, the Second Law of Thermodynamics. The connection between changes in enthalpy and entropy is described quantitatively by the **Gibbs–Helmholtz equation** ($\Delta G = \Delta H - T\ \Delta S$). The following examples will help explain these relationships.

In the *knall-gas* **(oxyhydrogen) reaction** (**1**), gaseous oxygen and gaseous hydrogen react to form liquid water. Like many redox reactions, this reaction is strongly exothermic (i.e., $\Delta H < 0$). However, during the reaction, the degree of order increases. The total number of molecules is reduced by one-third, and a more highly ordered liquid is formed from freely moving gas molecules. As a result of the increase in the degree of order ($\Delta S < 0$), the term $-T\ \Delta S$ becomes positive. However, this is more than compensated for by the decrease in enthalpy, and the reaction is still strongly exergonic ($\Delta G < 0$).

The **dissolution of salt in water** (**2**) is endothermic ($\Delta H > 0$)—i.e., the liquid cools. Nevertheless, the process still occurs spontaneously, since the degree of order in the system *decreases*. The Na^+ and Cl^- ions are initially rigidly fixed in a crystal lattice. In solution, they move about independently and in random directions through the fluid. The decrease in order ($\Delta S > 0$) leads to a negative $-T\ \Delta S$ term, which compensates for the positive ΔH term and results in a negative ΔG term overall. Processes of this type are described as being **entropy-driven**. The folding of proteins (see p. 74) and the formation of ordered lipid structures in water (see p. 28) are also mainly entropy-driven.

A. Heat of reaction and calorimetry



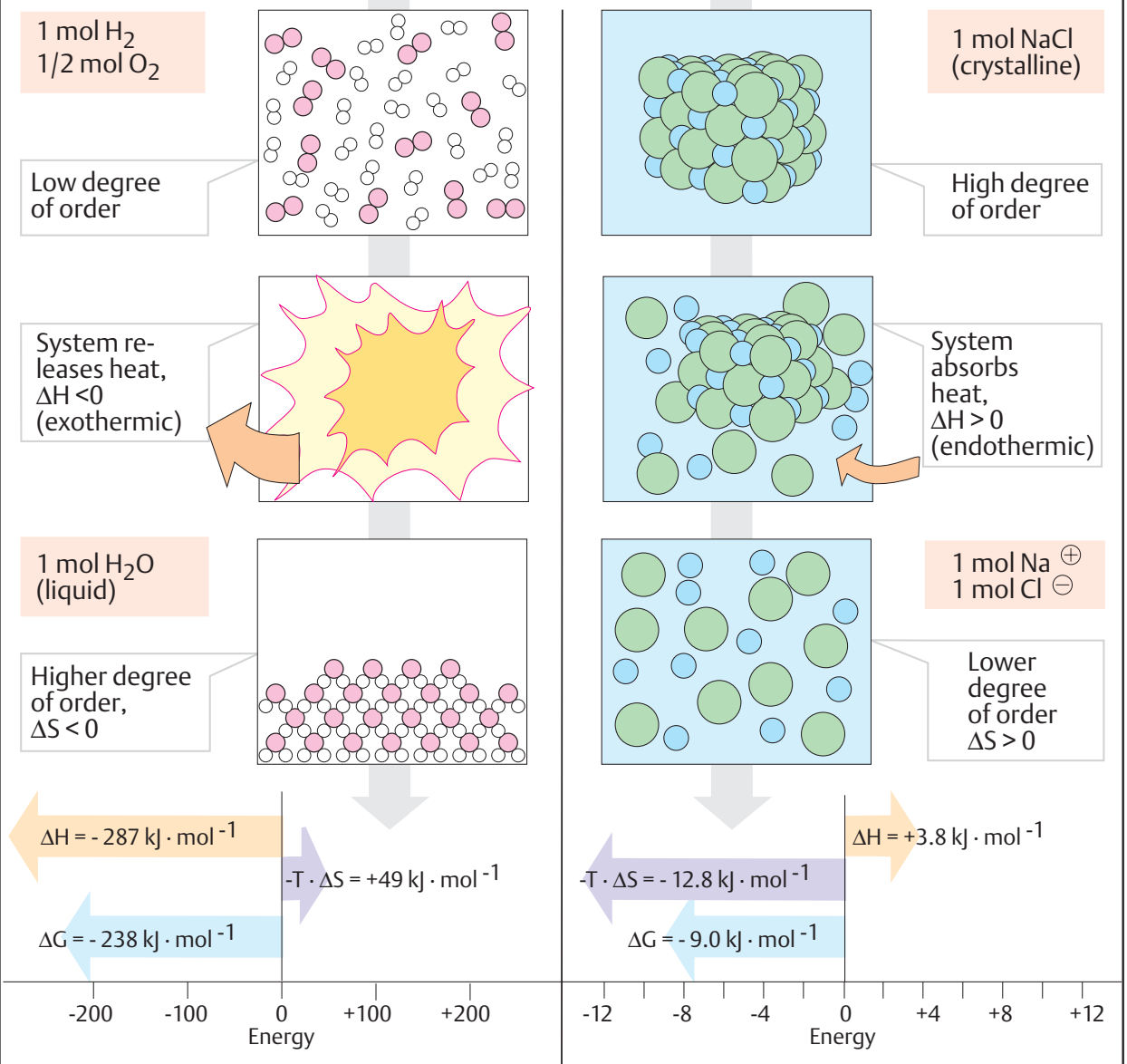
B. Enthalpy and entropy

$\Delta G = \Delta H - T \cdot \Delta S$

Gibbs-Helmholtz equation

ΔH: change of enthalpy, heat exchange

ΔS: change of entropy, i.e. degree of order



Reaction kinetics

The change in free enthalpy ΔG in a reaction indicates whether or not the reaction can take place spontaneously in given conditions and how much work it can perform (see p. 18). However, it does not tell us anything about the *rate* of the reaction—i. e., its **kinetics**.

A. Activation energy ◑

Most organic chemical reactions (with the exception of acid–base reactions) proceed only very slowly, regardless of the value of ΔG. The reason for the slow reaction rate is that the molecules that react—the educts—have to have a certain minimum energy before they can enter the reaction. This is best understood with the help of an energy diagram (**1**) of the simplest possible reaction $A \rightarrow B$. The educt A and the product B are each at a specific **chemical potential** (G_e and G_p, respectively). The change in the free enthalpy of the reaction, ΔG, corresponds to the difference between these two potentials. To be converted into B, A first has to overcome a potential energy barrier, the peak of which, G_a, lies well above G_e. The potential difference $G_a - G_e$ is the **activation energy E_a** of the reaction (in kJ mol^{-1}).

The fact that A can be converted into B at all is because the potential G_e only represents the average potential of all the molecules. Individual molecules may occasionally reach much higher potentials—e. g., due to collisions with other molecules. When the increase in energy thus gained is greater than E_a, these molecules can overcome the barrier and be converted into B. The energy distribution for a group of molecules of this type, as calculated from a simple model, is shown in (**2**) and (**3**). $\Delta n/n$ is the fraction of molecules that have reached or exceeded energy E (in kJ per mol). At 27 °C, for example, approximately 10% of the molecules have energies > 6 kJ mol^{-1}. The typical activation energies of chemical reactions are much higher. The course of the energy function at energies of around 50 kJ mol^{-1} is shown in (**3**). Statistically, at 27 °C only two out of 10^9 molecules reach this energy. At 37 °C, the figure is already four. This is the basis for the long-familiar "Q_{10} law"—a rule of thumb that states that the speed of biological processes approximately doubles with an increase in temperature of 10 °C.

B. Reaction rate ◑

The velocity *v* of a chemical reaction is determined experimentally by observing the change in the concentration of an educt or product over time. In the example shown (again a reaction of the $A \rightarrow B$ type), 3 mmol of the educt A is converted per second and 3 mmol of the product B is formed per second in one liter of the solution. This corresponds to a rate of

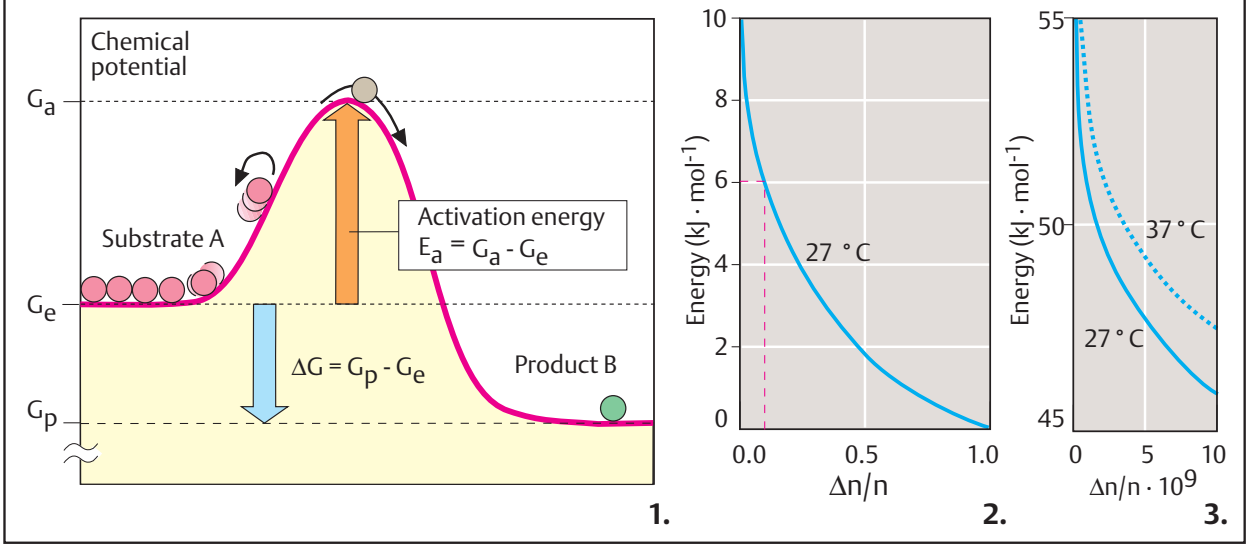
$$v = 3 \text{ mM } s^{-1} = 3 \text{ } 10^{-3} \text{ mol } L^{-1} \text{ } s^{-1}$$

C. Reaction order ◑

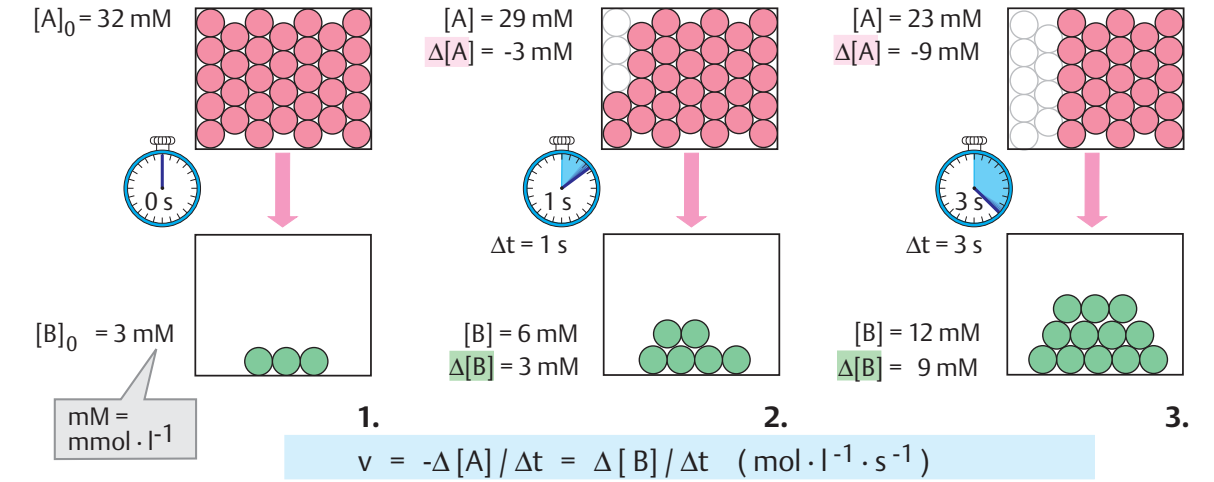
Reaction rates are influenced not only by the activation energy and the temperature, but also by the concentrations of the reactants. When there is only one educt, A (**1**), *v* is proportional to the concentration [A] of this substance, and a **first-order reaction** is involved. When *two* educts, A and B, react with one another (**2**), it is a **second order reaction** (shown on the right). In this case, the rate *v* is proportional to the *product* of the educt concentrations (12 mM^2 at the top, 24 mM^2 in the middle, and 36 mM^2 at the bottom). The proportionality factors k and k′ are the **rate constants** of the reaction. They are *not* dependent on the reaction concentrations, but depend on the external conditions for the reaction, such as temperature.

In **B**, only the kinetics of simple irreversible reactions is shown. More complicated cases, such as reaction with three or more reversible steps, can usually be broken down into first-order or second-order partial reactions and described using the corresponding equations (for an example, see the Michaelis–Menten reaction, p. 92).

A. Activation energy

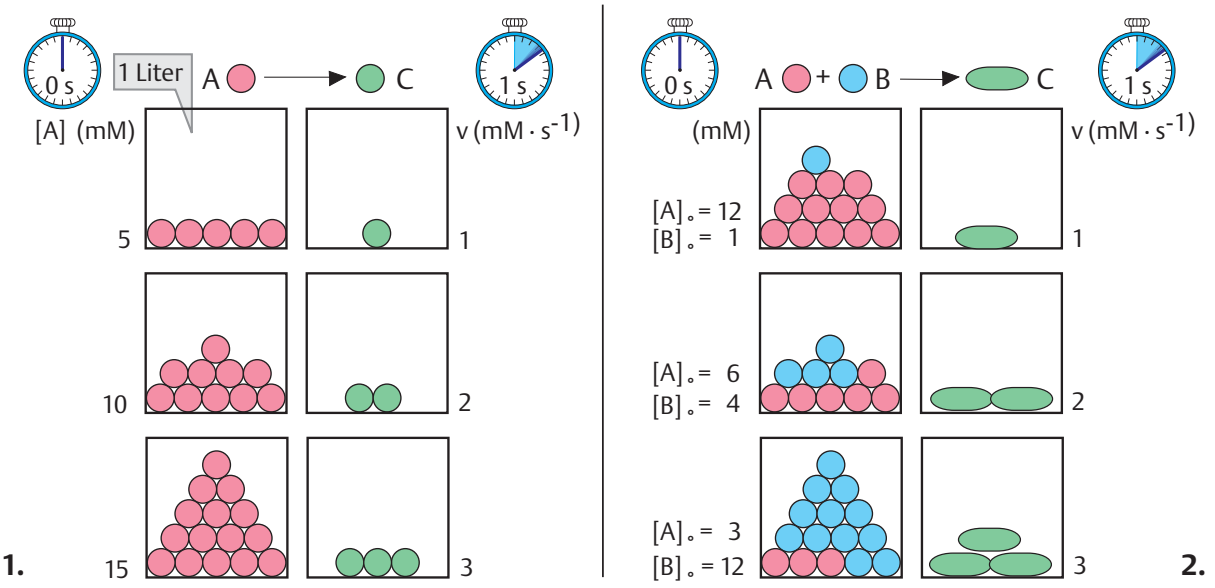


B. Reaction rate



$$v = -\Delta[A]/\Delta t = \Delta[B]/\Delta t \quad (\text{mol} \cdot \text{l}^{-1} \cdot \text{s}^{-1})$$

C. Reaction order



$v = k \cdot [A]$ | k, k' : Rate constants | $v = k' \cdot [A] \cdot [B]$

First-order reaction: $k = 1/5\ s^{-1}$

Second-order reaction: $k' = 1/12\ l \cdot mmol^{-1} \cdot s^{-1}$

Catalysis

Catalysts are substances that accelerate chemical reactions without themselves being consumed in the process. Since catalysts emerge from the catalyzed reaction without being changed, even small amounts are usually suf cient to cause a powerful acceleration of the reaction. In the cell, **enzymes** (see p. 88) generally serve as catalysts. A few chemical changes are catalyzed by special RNA molecules, known as *ribozymes* (see p. 246).

A. Catalysis: principle ●

The reason for the slow rates of most reactions involving organic substances is the high **activation energy** (see p. 22) that the reacting molecules have to reach before they can react. In aqueous solution, a large proportion of the activation energy is required to remove the hydration shells surrounding the educts. During the course of a reaction, resonance-stabilized structures (see p. 4) are often temporarily suspended; this also requires energy. The highest point on the reaction coordinates corresponds to an energetically unfavorable **transition state** of this type (**1**).

A catalyst creates a new pathway for the reaction (**2**). When all of the transition states arising have a lower activation energy than that of the uncatalyzed reaction, the reaction will proceed more rapidly along the alternative pathway, even when the number of intermediates is greater. Since the starting points and end points are the same in both routes, the change in the enthalpy ΔG of the reaction is not influenced by the catalyst. Catalysts—including enzymes—are in principle *not* capable of altering the equilibrium state of the catalyzed reaction.

The often-heard statement that "a catalyst reduces the activation energy of a reaction" is not strictly correct, since a *completely different* reaction takes place in the presence of a catalyst than in uncatalyzed conditions. However, its activation energy is lower than in the uncatalyzed reaction.

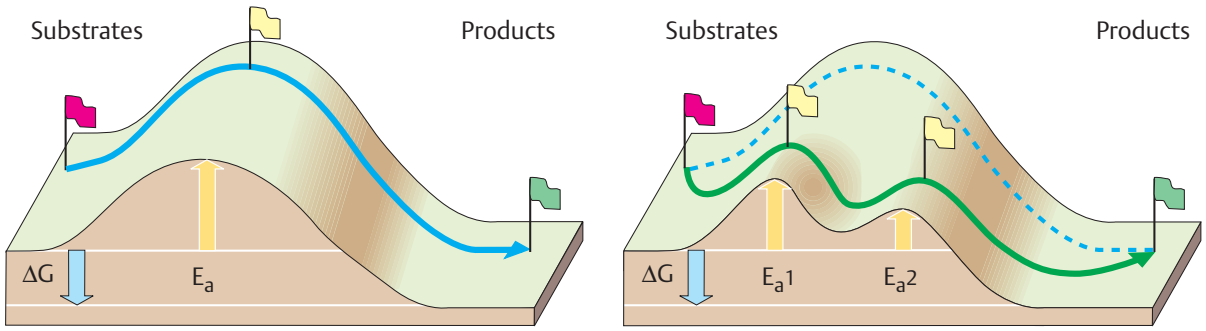
B. Catalysis of H_2O_2 – breakdown by iodide ○

As a simple example of a catalyzed reaction, we can look at the disproportionation of hydrogen peroxide (H_2O_2) into oxygen and water. In the uncatalyzed reaction (at the top), an H_2O_2 molecule initially decays into H_2O and atomic oxygen (O), which then reacts with a second H_2O_2 molecule to form water and molecular oxygen (O_2). The activation energy E_a required for this reaction is relatively high, at 75 kJ mol^{-1}. In the presence of **iodide** (I^-) as a catalyst, the reaction takes a different course (bottom). The intermediate arising in this case is hypoiodide (OI^-), which also forms H_2O and O_2 with another H_2O_2 molecule. In this step, the I^- ion is released and can once again take part in the reaction. The lower activation energy of the reaction catalyzed by iodide (E_a = 56 kJ mol^{-1}) causes acceleration of the reaction by a factor of 2000, as the reaction rate depends exponentially on E_a ($v \sim e^{-Ea/R\,T}$).

Free metal ions such as iron (Fe) and platinum (Pt) are also effective catalysts for the breakdown of H_2O_2. **Catalase** (see p. 284), an enzyme that protects cells against the toxic effects of hydrogen peroxide (see p. 284), is much more catalytically effective still. In the enzyme-catalyzed disproportionation, H_2O_2 is bound to the enzyme's heme group, where it is quickly converted to atomic oxygen and water, supported by amino acid residues of the enzyme protein. The oxygen atom is temporarily bound to the central iron atom of the heme group, and then transferred from there to the second H_2O_2 molecule. The activation energy of the enzyme-catalyzed reaction is only 23 kJ mol^{-1}, which in comparison with the uncatalyzed reaction leads to acceleration by a factor of 1.3 10^9.

Catalase is one of the most ef cient enzymes there are. A single molecule can convert up to 10^8 (a hundred million) H_2O_2 molecules per second.

A. Catalysis: principle

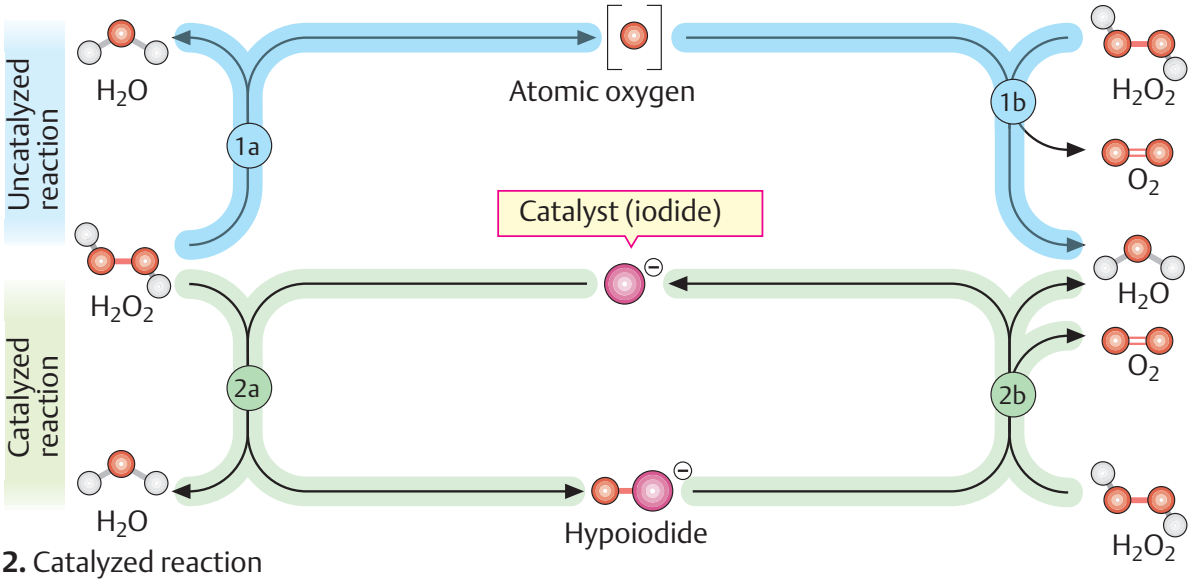


1. Energy profile without catalyst

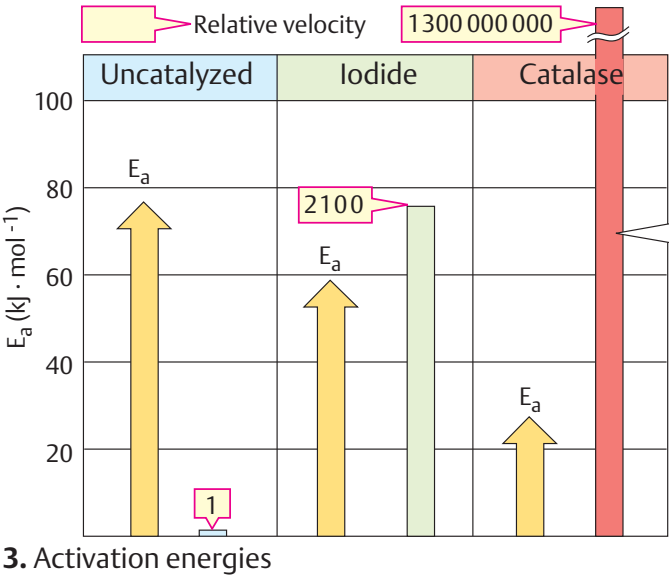
2. Energy profile with catalyst

B. Catalysis of H_2O_2 – breakdown by iodide

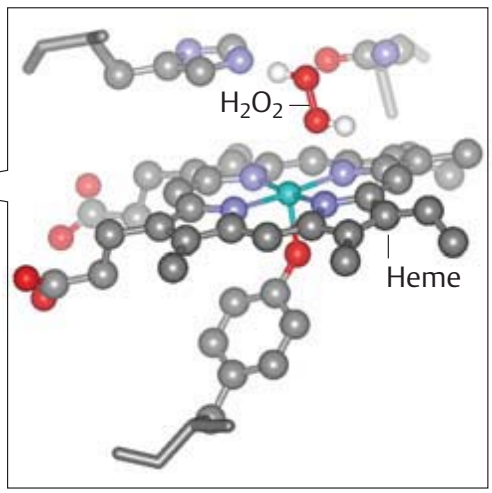
1. Breakdown of hydrogen peroxide



2. Catalyzed reaction



3. Activation energies



Active center of catalase

Water as a solvent

Life as we know it evolved in water and is still absolutely dependent on it. The properties of water are therefore of fundamental importance to all living things.

A. Water and methane ◑

The special properties of **water (H_2O)** become apparent when it is compared with **methane (CH_4)**. The two molecules have a similar mass and size. Nevertheless, the boiling point of water is more than 250 °C above that of methane. At temperatures on the earth's surface, water is liquid, whereas methane is gaseous. The high boiling point of water results from its high vaporization enthalpy, which in turn is due to the fact that the density of the electrons within the molecule is unevenly distributed. Two corners of the tetrahedrally-shaped water molecule are occupied by unshared electrons (green), and the other two by hydrogen atoms. As a result, the H–O–H bond has an angled shape. In addition, the O–H bonds are polarized due to the high electronegativity of oxygen (see p. 6). One side of the molecule carries a partial charge (δ) of about –0.6 units, whereas the other is correspondingly positively charged. The spatial separation of the positive and negative charges gives the molecule the properties of an **electrical dipole**. Water molecules are therefore attracted to one another like tiny magnets, and are also connected by hydrogen bonds (**B**) (see p. 6). When liquid water vaporizes, a large amount of energy has to be expended to disrupt these interactions. By contrast, methane molecules are not dipolar, and therefore interact with one another only weakly. This is why liquid methane vaporizes at very low temperatures.

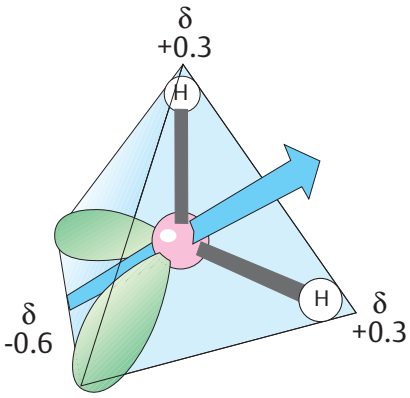
B. Structure of water and ice ◑

The dipolar nature of water molecules favors the formation of **hydrogen bonds** (see p. 6). Each molecule can act either as a donor or an acceptor of H bonds, and many molecules in liquid water are therefore connected by H bonds (**1**). The bonds are in a state of constant fluctuation. Tetrahedral networks of molecules, known as water "clusters," often arise. As the temperature decreases, the proportion of water clusters increases until the water begins to crystallize. Under normal atmospheric pressure, this occurs at 0 °C. In **ice**, most of the water molecules are fixed in a **hexagonal lattice** (**3**). Since the distance between the individual molecules in the frozen state is on average greater than in the liquid state, the density of ice is lower than that of liquid water. This fact is of immense biological importance—it means, for example, that in winter, ice forms on the surface of open stretches of water first, and the water rarely freezes to the bottom.

C. Hydration ◑

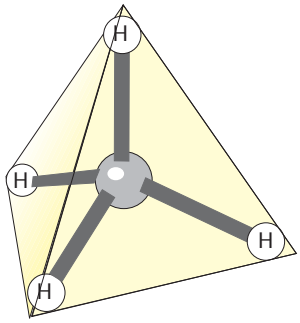
In contrast to most other liquids, water is an excellent **solvent for ions**. In the electrical field of cations and anions, the dipolar water molecules arrange themselves in a regular fashion corresponding to the charge of the ion. They form **hydration shells** and shield the central ion from oppositely charged ions. Metal ions are therefore often present as hexahydrates ($[Me(H_2O)_6^{2+}]$, on the right). In the inner hydration sphere of this type of ion, the water molecules are practically immobilized and follow the central ion. Water has a high dielectric constant of 78—i.e., the electrostatic attraction force between ions is reduced to 1/78 by the solvent. Electrically charged groups in organic molecules (e.g., carboxylate, phosphate, and ammonium groups) are also well hydrated and contribute to water solubility. Neutral molecules with several hydroxy groups, such as glycerol (on the left) or sugars, are also easily soluble, because they can form H bonds with water molecules. The higher the proportion of polar functional groups there is in a molecule, the more water-soluble (**hydrophilic**) it is. By contrast, molecules that consist exclusively or mainly of hydrocarbons are poorly soluble or insoluble in water. These compounds are called **hydrophobic** (see p. 28).

A. Water and methane



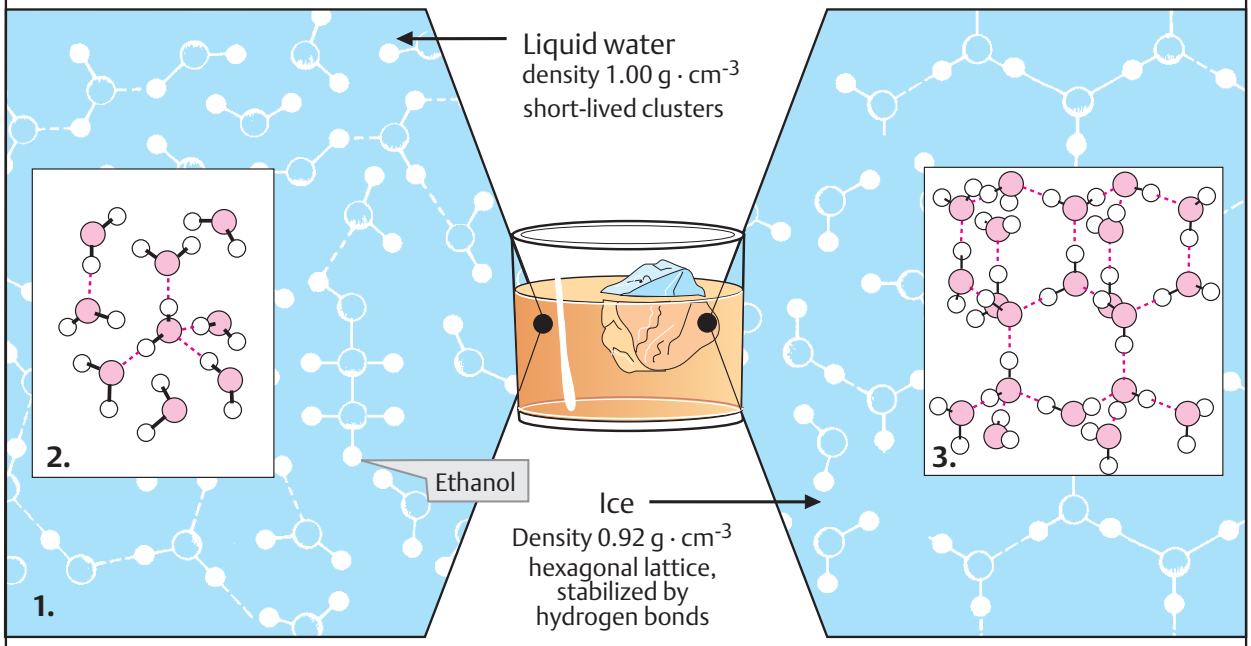
Water (H_2O)

H_2O		CH_4
18 Da	Molecular mass	16 Da
+100 °C	Boiling point	-162 °C
41	Heat of vaporization ($kJ \cdot mol^{-1}$)	8
6.2	Dipole moment (10^{-30} C · m)	0

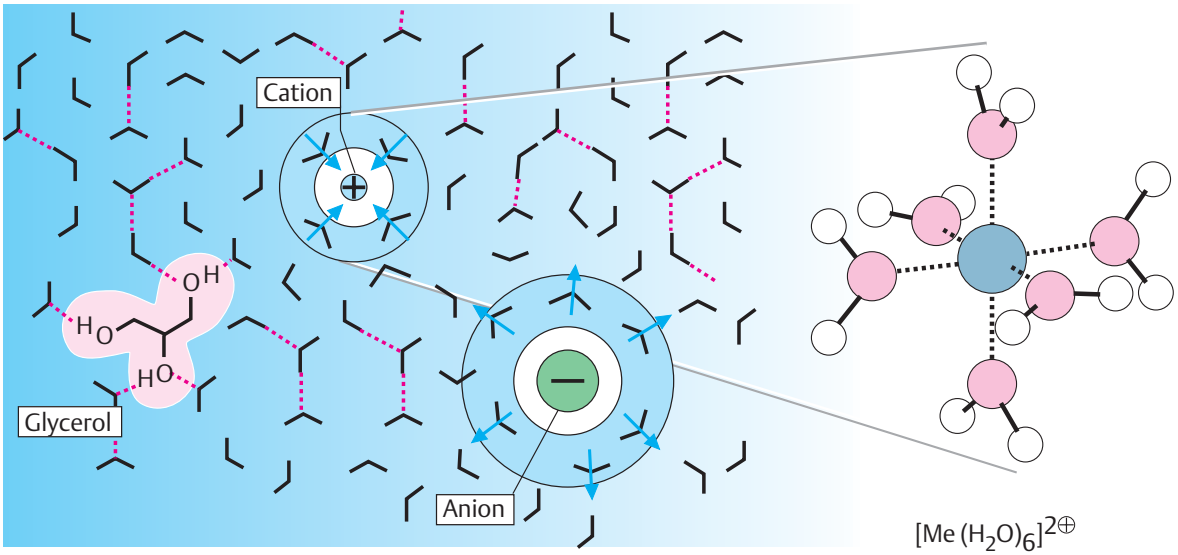


Methane (CH_4)

B. Structure of water and ice



C. Hydration



Hydrophobic interactions

Water is an excellent solvent for ions and for substances that contain polarized bonds (see p. 20). Substances of this type are referred to as **polar** or **hydrophilic** ("water-loving"). In contrast, substances that consist mainly of hydrocarbon structures dissolve only poorly in water. Such substances are said to be **apolar** or **hydrophobic**.

A. Solubility of methane ○

To understand the reasons for the poor water solubility of hydrocarbons, it is useful first to examine the energetics (see p. 16) of the processes involved. In (**1**), the individual terms of the Gibbs–Helmholtz equation (see p. 20) for the simplest compound of this type, **methane**, are shown (see p. 4). As can be seen, the transition from gaseous methane to water is actually exothermic ($\Delta H^0 < 0$). Nevertheless, the change in the free enthalpy ΔG^0 is positive (the process is endergonic), because the entropy term $T\ \Delta S^0$ has a strongly positive value. The entropy change in the process (ΔS^0) is evidently negative—i. e., a solution of methane in water has a *higher* degree of order than either water or gaseous methane. One reason for this is that the methane molecules are less mobile when surrounded by water. More importantly, however, the water around the apolar molecules forms cage-like **"clathrate" structures**, which—as in ice—are stabilized by H bonds. This strongly increases the degree of order in the water—and the more so the larger the area of surface contact between the water and the apolar phase.

B. The "oil drop effect" ◑

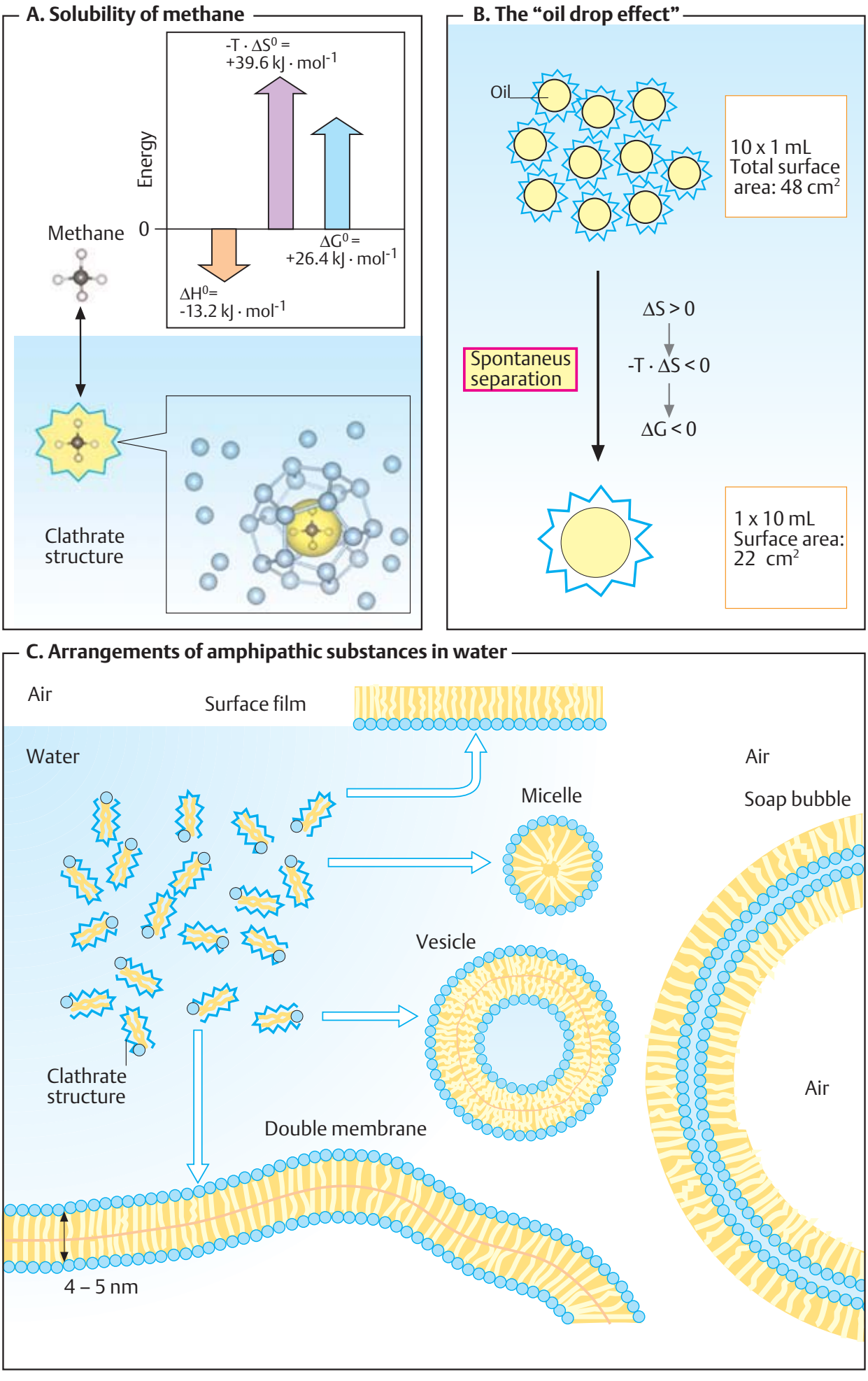
The spontaneous separation of oil and water, a familiar observation in everyday life, is due to the energetically unfavorable formation of clathrate structures. When a mixture of water and oil is firmly shaken, lots of tiny oil drops form to begin with, but these quickly coalesce spontaneously to form larger drops—the two phases separate. A larger drop has a smaller surface area than several small drops with the same volume. Separation therefore reduces the area of surface contact between the water and the oil, and consequently also the extent of clathrate formation. The ΔS for this process is therefore positive (the *dis*order in the water increases), and the negative term $-T\ \Delta S$ makes the separation process exergonic ($\Delta G < 0$), so that it proceeds spontaneously.

C. Arrangements of amphipathic substances in water ◑

Molecules that contain both polar *and* apolar groups are called **amphipathic** or amphiphilic. This group includes soaps (see p. 48), phospholipids (see p. 50), and bile acids (see p. 56).

As a result of the "oil drop effect" amphipathic substances in water tend to arrange themselves in such a way as to minimize the area of surface contact between the apolar regions of the molecule and water. On water surfaces, they usually form single-layer **films** (top) in which the polar "head groups" face toward the water. **Soap bubbles** (right) consist of double films, with a thin layer of water enclosed between them. In water, depending on their concentration, amphipathic compounds form **micelles**—i. e., spherical aggregates with their head groups facing toward the outside, or extended bilayered **double membranes**. Most biological membranes are assembled according to this principle (see p. 214). Closed hollow membrane sacs are known as **vesicles**. This type of structure serves to transport substances within cells and in the blood (see p. 278).

The separation of oil and water (**B**) can be prevented by adding a strongly amphipathic substance. During shaking, a more or less stable **emulsion** then forms, in which the surface of the oil drops is occupied by amphipathic molecules that provide it with polar properties externally. The emulsification of fats in food by bile acids and phospholipids is a vital precondition for the digestion of fats (see p. 314).



Acids and bases

A. Acids and bases ●

In general, **acids** are defined as substances that can donate hydrogen ions (protons), while **bases** are compounds that accept protons.

Water enhances the acidic or basic properties of dissolved substances, as water itself can act as either an acid or a base. For example, when **hydrogen chloride** (HCl) is in aqueous solution, it donates protons to the solvent (**1**). This results in the formation of chloride ions (Cl^-) and protonated water molecules (**hydronium ions, H_3O^+**, usually simply referred to as H^+). The proton exchange between HCl and water is virtually quantitative: in water, HCl behaves as a *very strong acid* with a negative pK_a value (see p. 18).

Bases such as **ammonia** (NH_3) take over protons from water molecules. As a result of this, **hydroxyl ions** (OH^-) and positively charged ammonium ions (NH_4^+, **3**) form. Hydronium and hydroxyl ions, like other ions, exist in water in hydrated rather than free form (see p. 26).

Acid–base reactions always involve *pairs* of **acids** and the associated **conjugated bases** (see p. 18). The stronger the acid or base, the *weaker* the conjugate base or acid, respectively. For example, the very strongly acidic hydrogen chloride belongs to the very weakly basic chloride ion (**1**). The weakly acidic ammonium ion is conjugated with the moderately strong base ammonia (**3**).

The equilibrium constant K for the acid—base reaction between H_2O molecules (**2**) is very small. At 25 °C,

$$K = [H^+]\ [OH^-] / [H_2O] = 2\ \ 10^{-16}\ \text{mol}\ \ L^{-1}$$

In pure water, the concentration $[H_2O]$ is practically constant at 55 mol L^{-1}. Substituting this value into the equation, it gives:

$$K_w = [H^+]\ [OH^-] = 1\ \ 10^{-14}\ \text{mol}\ \ L^{-1}$$

The product $[H^+]\ [OH^-]$—the **ion product** of water—is constant even when additional acid–base pairs are dissolved in the water. At 25 °C, pure water contains H^+ and OH^- at concentrations of $1\ \ 10^{-7}$ mol L^{-1} each; it is **neutral** and has a pH value of exactly 7.

B. pH values in the organism ◑

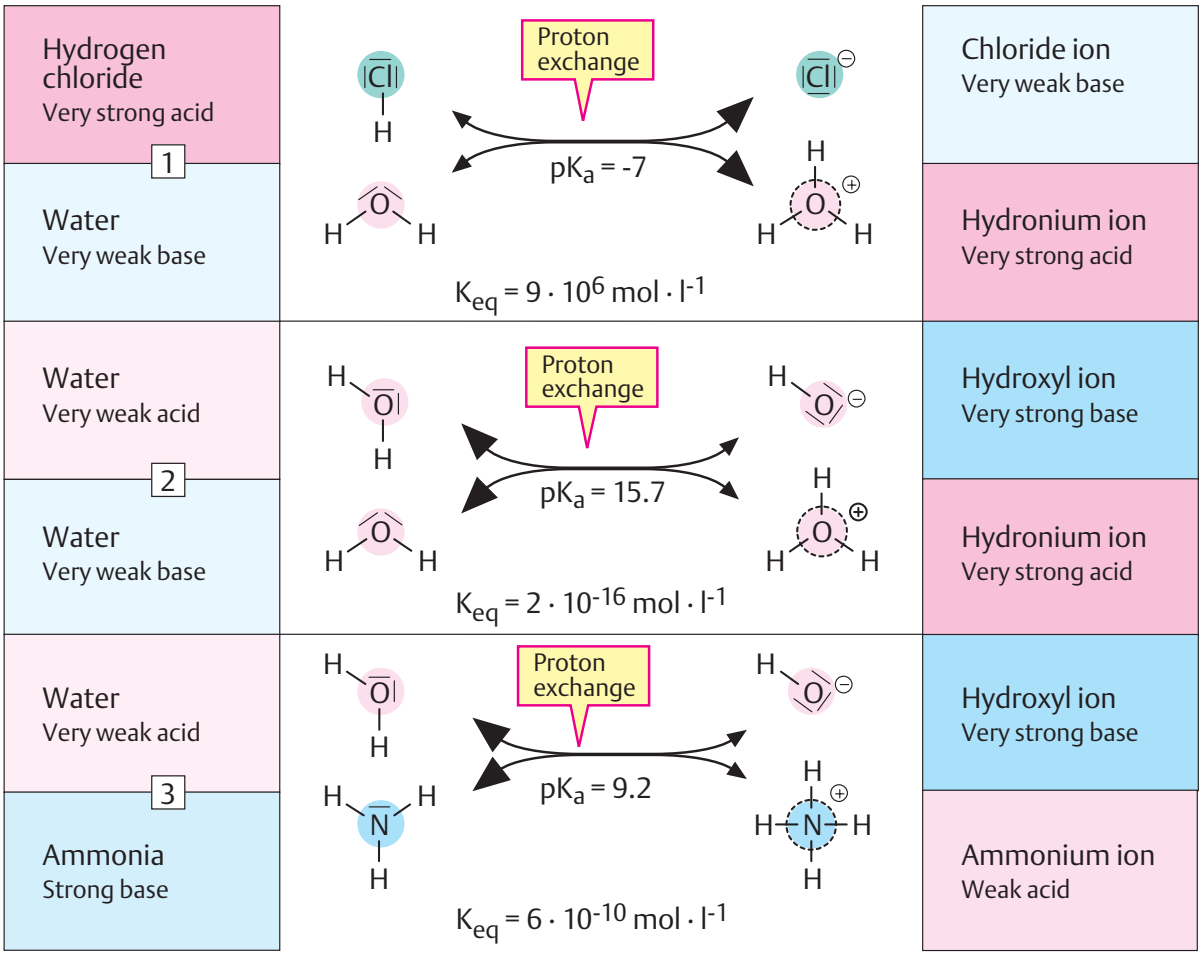
pH values in the cell and in the extracellular fluid are kept constant within narrow limits. In the blood, the pH value normally ranges only between 7.35 and 7.45 (see p. 288). This corresponds to a maximum change in the H^+ concentration of ca. 30%. The pH value of cytoplasm is slightly lower than that of blood, at 7.0–7.3. In lysosomes (see p. 234; pH 4.5–5.5), the H^+ concentration is several hundred times higher than in the cytoplasm. In the lumen of the gastrointestinal tract, which forms part of the outside world relative to the organism, and in the body's excretion products, the pH values are more variable. Extreme values are found in the stomach (ca. 2) and in the small bowel (> 8). Since the kidney can excrete either acids or bases, depending on the state of the metabolism, the pH of urine has a particularly wide range of variation (4.8–7.5).

C. Buffers ●

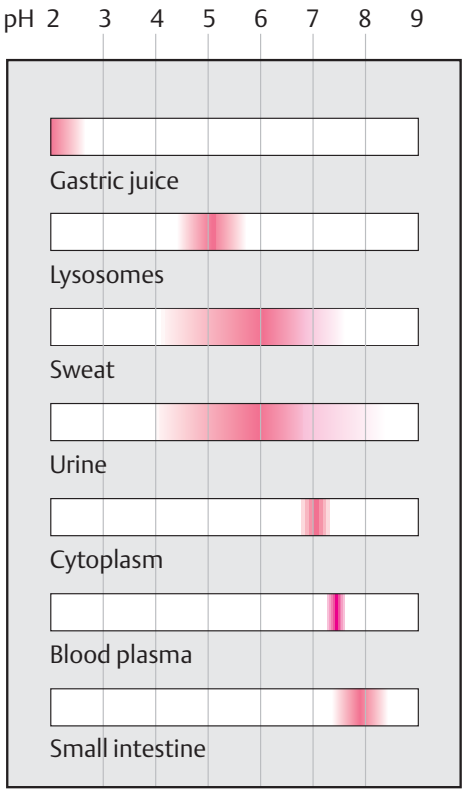
Short–term pH changes in the organism are cushioned by **buffer systems**. These are mixtures of a weak acid, HB, with its conjugate base, B^-, or of a weak base with its conjugate acid. This type of system can neutralize both hydronium ions and hydroxyl ions.

In the first case (left), the base (B^-) binds a large proportion of the added protons (H^+) and HB and water are formed. If hydroxyl ions (OH^-) are added, they react with HB to give B^- and water (right). In both cases, it is primarily the $[HB]/[B^-]$ ratio that shifts, while the pH value only changes slightly. The **titration curve** (top) shows that buffer systems are most effective at the pH values that correspond to the pK_a value of the acid. This is where the curve is at its steepest, so that the pH change, ΔpH, is at its smallest with a given increase Δc in $[H^+]$ or $[OH^-]$. In other words, the **buffer capacity** $\Delta c / \Delta$pH is highest at the pK_a value.

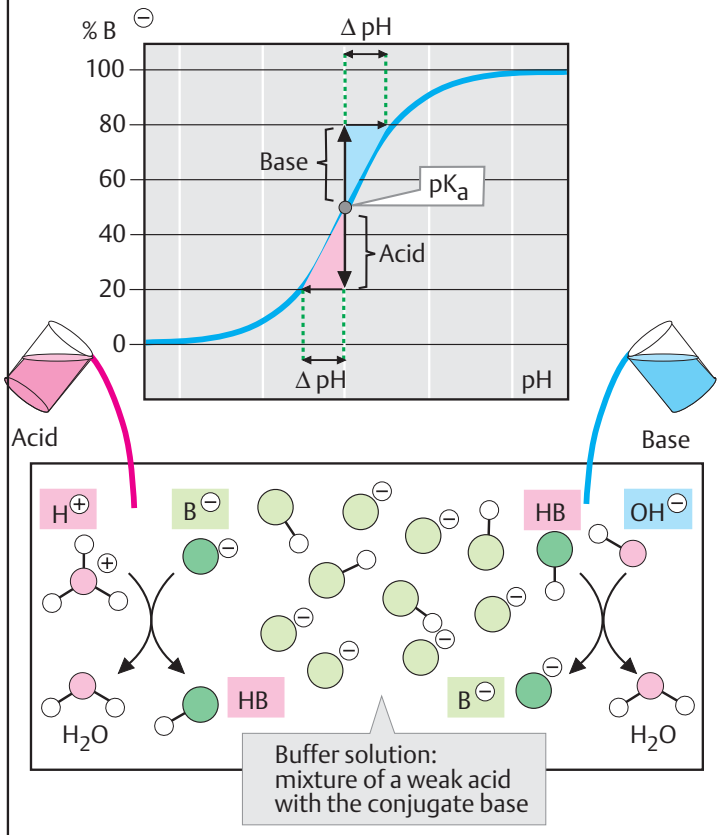
A. Acids and bases



B. pH values in the body



C. Buffers



Redox processes

A. Redox reactions ●

Redox reactions are chemical changes in which electrons are transferred from one reaction partner to another (**1**; see also p. 18). Like acid–base reactions (see p. 30), redox reactions always involve *pairs* of compounds. A pair of this type is referred to as a **redox system** (**2**). The essential difference between the two components of a redox system is the number of electrons they contain. The more electronrich component is called the **reduced form** of the compound concerned, while the other one is referred to as the **oxidized form**. The reduced form of one system (the **reducing agent**) donates electrons to the oxidized form of another one (the **oxidizing agent**). In the process, the reducing agent becomes oxidized and the oxidizing agent is reduced (**3**). Any given reducing agent can reduce only certain other redox systems. On the basis of this type of observation, redox systems can be arranged to form what are known as **redox series** (**4**).

The position of a system within one of these series is established by its **redox potential E** (see p. 18). The redox potential has a sign; it can be more negative or more positive than a reference potential arbitrarily set at zero (the normal potential of the system [2 H^+/H_2]). In addition, E depends on the concentrations of the reactants and on the reaction conditions (see p. 18). In redox series (**4**), the systems are arranged according to their increasing redox potentials. Spontaneous electron transfers are only possible if the redox potential of the donor is *more negative* than that of the acceptor (see p. 18).

B. Reduction equivalents ◑

In redox reactions, protons (H^+) are often transferred along with electrons (e^-), or protons may be released. The combinations of electrons and protons that occur in redox processes are summed up in the term **reduction equivalents**. For example, the combination 1 e^-/1 H^+ corresponds to a hydrogen *atom,* while 2 e^- and 2 H^+ together produce a hydrogen *molecule.* However, this does not mean that atomic or molecular hydrogen is actually transferred from one molecule to the other (see below). Only the combination 2 e^-/1 H^+, the **hydride ion**, is transferred as a unit.

C. Biological redox systems ◑

In the cell, redox reactions are catalyzed by enzymes, which work together with soluble or bound redox cofactors.

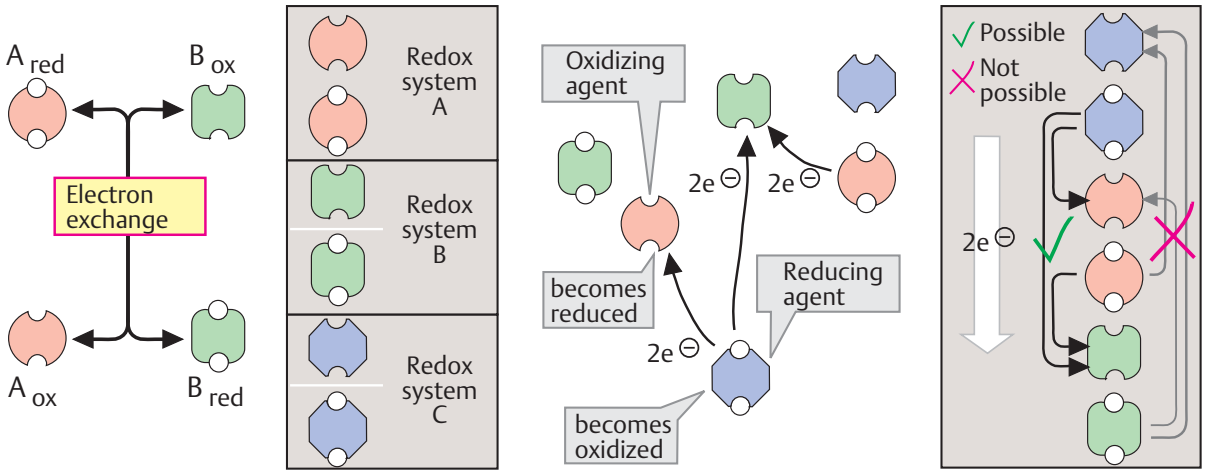
Some of these factors contain **metal ions** as redox-active components. In these cases, it is usually single electrons that are transferred, with the metal ion changing its valency. Unpaired electrons often occur in this process, but these are located in d orbitals (see p. 2) and are therefore less dangerous than single electrons in non-metal atoms ("free radicals"; see below).

We can only show here a few examples from the many organic redox systems that are found. In the complete reduction of the **flavin coenzymes** FMN and FAD (see p. 104), 2 e^- and 2 H^+ are transferred. This occurs in two separate steps, with a *semiquinone radical* appearing as an intermediate. Since organic radicals of this type can cause damage to biomolecules, flavin coenzymes never occur freely in solution, but remain firmly bound in the interior of proteins.

In the reduction or oxidation of **quinone/quinol systems**, free radicals also appear as intermediate steps, but these are less reactive than flavin radicals. Vitamin E, another quinone-type redox system (see p. 104), even functions as a radical scavenger, by delocalizing unpaired electrons so effectively that they can no longer react with other molecules.

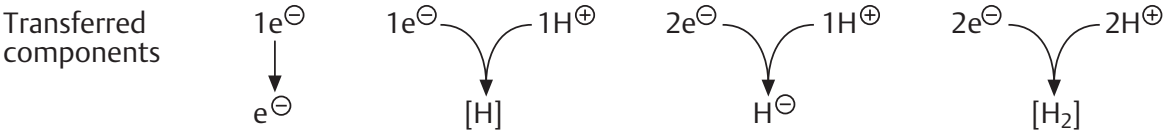
The **pyridine nucleotides** NAD^+ and $NADP^+$ always function in unbound form. The oxidized forms contain an aromatic nicotinamide ring in which the positive charge is delocalized. The right-hand example of the two *resonance structures* shown contains an electron-poor, positively charged C atom at the *para* position to nitrogen. If a **hydride ion** is added at this point (see above), the reduced forms NADH or NADPH arise. No radical intermediate steps occur. Because a proton is released at the same time, the reduced pyridine nucleotide coenzymes are correctly expressed as NAD(P)H+H^+.

A. Redox reactions

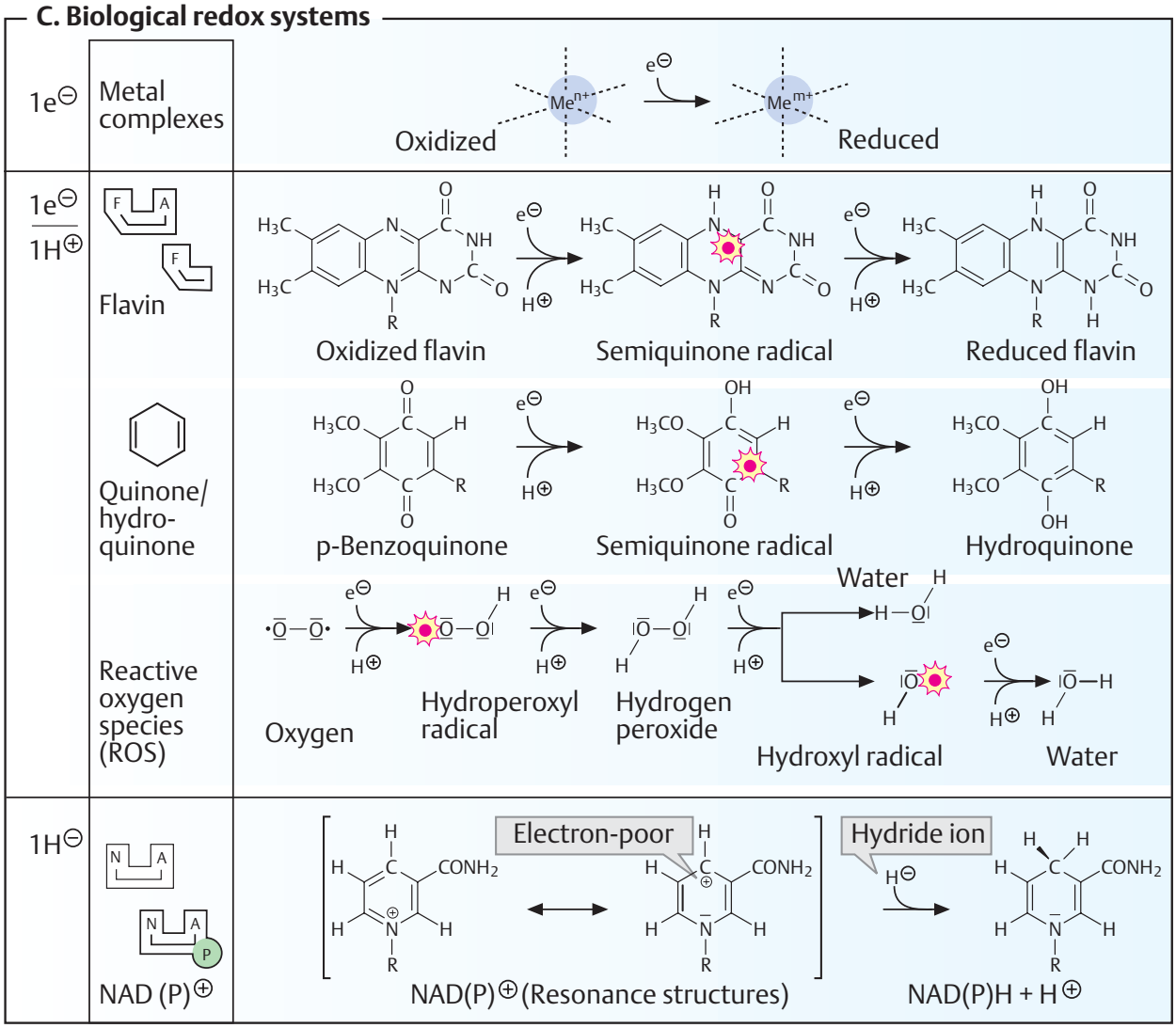


1. Principle **2.** Redox systems **3.** Possible electron transfers **4.** Redox series

B. Reducing equivalents



C. Biological redox systems



Overview

The **carbohydrates** are a group of naturally occurring carbonyl compounds (aldehydes or ketones) that also contain several hydroxyl groups. The carbohydrates include **single sugars (monosaccharides)** and their polymers, the **oligosaccharides** and **polysaccharides**.

A. Carbohydrates: overview ●

Polymeric carbohydrates–above all starch, as well as some disaccharides–are important (but not essential) **components of food** (see p. 360). In the gut, they are broken down into monosaccharides and resorbed in this form (see p. 272). The form in which carbohydrates are distributed by the blood of vertebrates is *glucose* ("blood sugar"). This is taken up by the cells and either broken down to obtain energy (glycolysis) or converted into other metabolites (see pp. 150–159). Several organs (particularly the liver and muscles) store *glycogen* as a polymeric **reserve carbohydrate** (right; see p. 156). The glycogen molecules are covalently bound to a protein, *glycogenin*. Polysaccharides are used by many organisms as **building materials**. For example, the cell walls of bacteria contain *murein* as a stabilizing component (see p. 40), while in plants *cellulose* and other polysaccharides fulfill this role (see p. 42). Oligomeric or polymeric carbohydrates are often covalently bound to lipids or proteins. The **glycolipids** and **glycoproteins** formed in this way are found, for example, in cell membranes (center). Glycoproteins also occur in the blood in solute form (plasma proteins; see p. 276) and, as components of *proteoglycans,* form important constituents of the intercellular substance (see p. 346).

B. Monosaccharides: structure ◑

The most important natural monosaccharide, **D-glucose**, is an aliphatic aldehyde with six C atoms, five of which carry a hydroxyl group (**1**). Since C atoms 2 to 5 represent chiral centers (see p. 8), there are 15 further isomeric *aldohexoses* in addition to D-glucose, although only a few of these are important in nature (see p. 38). Most natural monosaccharides have the same configuration at C-5 as D-glyceraldehyde–they belong to the D **series**.

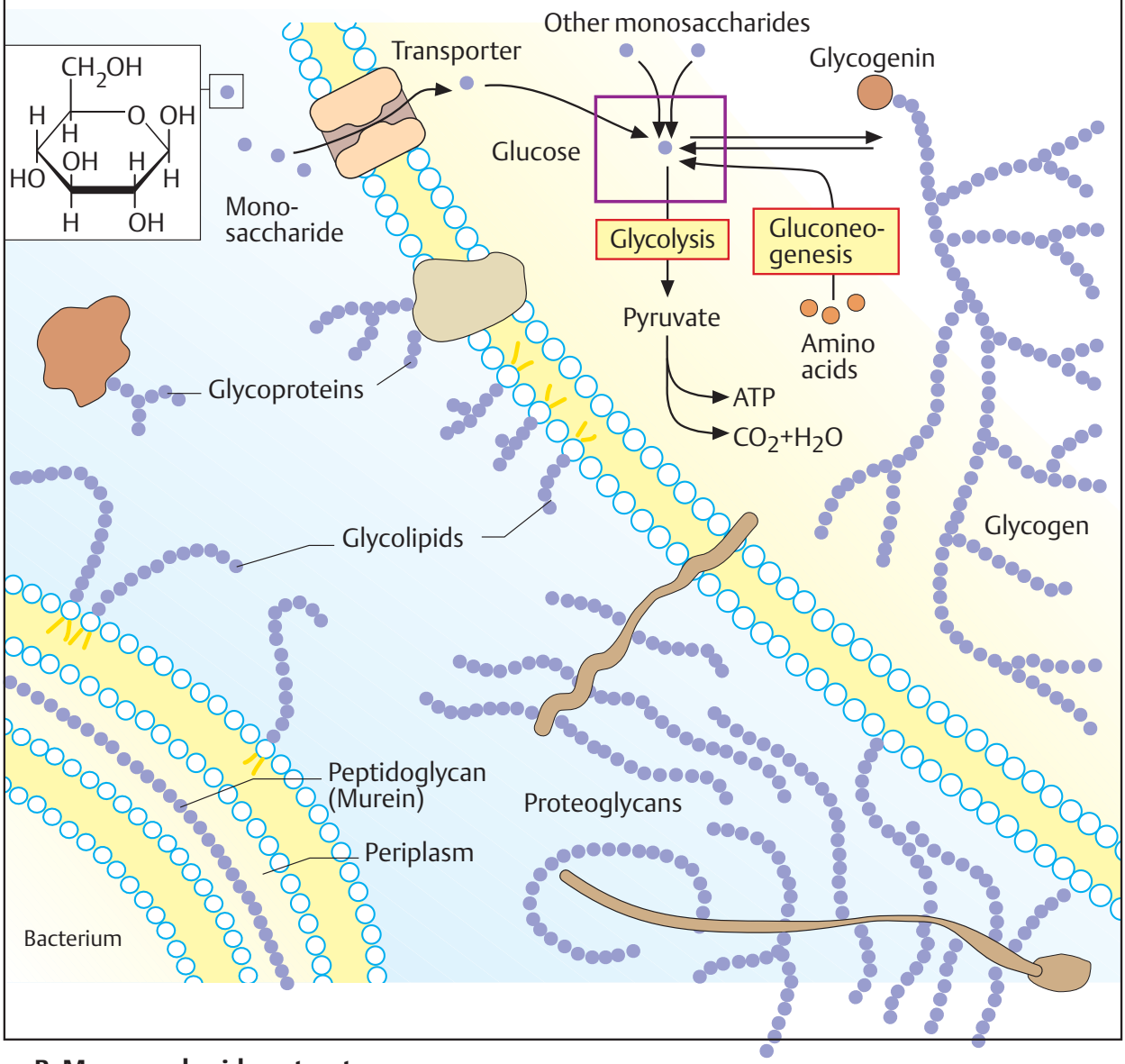
The open-chained form of glucose shown in (**1**) is found in neutral solution in less than 0.1% of the molecules. The reason for this is an intramolecular reaction in which one of the OH groups of the sugar is added to the aldehyde group of the *same* molecule (**2**). This gives rise to a cyclic **hemiacetal** (see p. 10). In aldohexoses, the hydroxy group at C-5 reacts preferentially, and a six-membered pyran ring is formed. Sugars that contain this ring are called **pyranoses**. By contrast, if the OH group at C-4 reacts, a five-part furan ring is formed. In solution, *pyranose* forms and *furanose* forms are present in equilibrium with each other and with the open-chained form, while in glucose polymers only the pyranose form occurs.

The **Haworth projection** (**2**) is usually used to depict sugars in the cyclic form, with the ring being shown in perspective as viewed from above. Depending on the configuration, the substituents of the chiral C atoms are then found above or below the ring. OH groups that lie on the *right* in the Fischer projection (**1**) appear *under* the ring level in the Haworth projection, while those on the *left* appear *above* it.

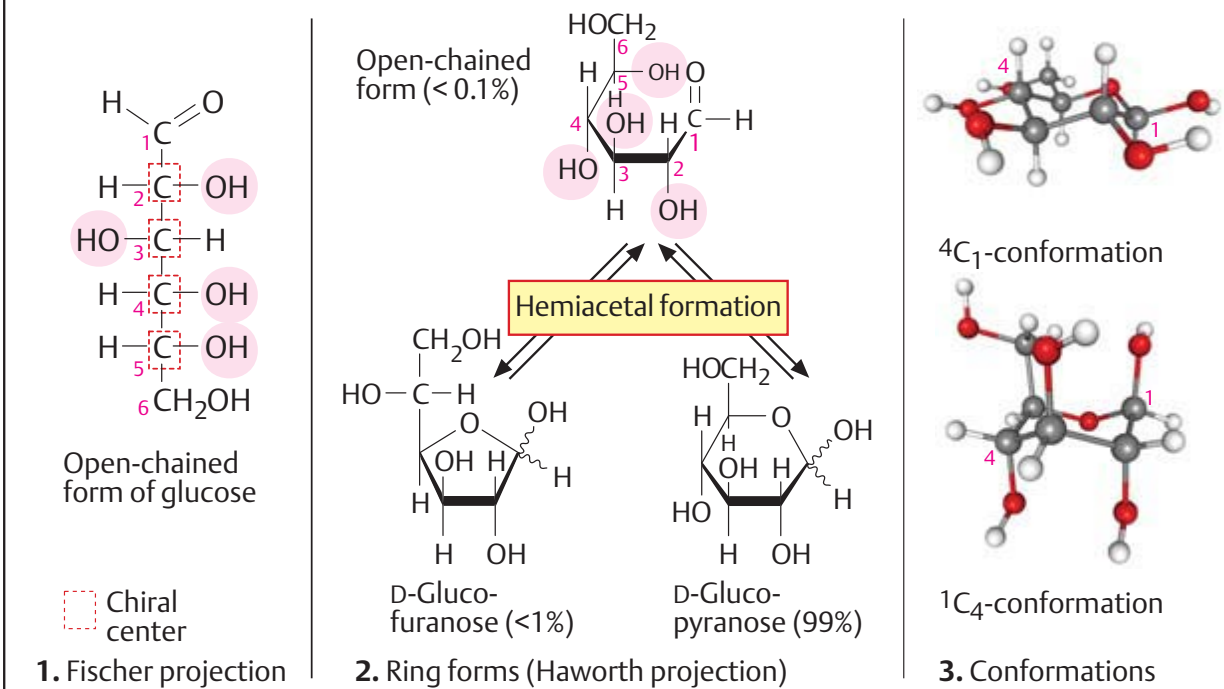
As a result of hemiacetal formation, an additional chiral center arises at C-1, which can be present in both possible configurations (anomers) (see p. 8). To emphasize this, the corresponding bonds are shown here using wavy lines.

The Haworth formula does not take account of the fact that the pyran ring is not plain, but usually has a *chair conformation*. In **B3**, two frequent conformations of D-glucopyranose are shown as ball-and-stick models. In the $^1C_4$ conformation (bottom), most of the OH groups appear vertical to the ring level, as in the Haworth projection (**axial** or **a** position). In the slightly more stable $^4C_1$ conformation (top), the OH groups take the **equatorial** or **e** position. At room temperature, each form can change into the other, as well as into other conformations.

A. Carbohydrates: overview



B. Monosaccharides: structure



Chemistry of sugars

A. Reactions of the monosaccharides ◑

The sugars (monosaccharides) occur in the metabolism in many forms (derivatives). Only a few important conversion reactions are discussed here, using **D**-glucose as an example.

1. Mutarotation. In the cyclic form, as opposed to the open-chain form, aldoses have a chiral center at C-1 (see p. 34). The corresponding isomeric forms are called **anomers**. In the β-anomer (center left), the OH group at C-1 (the anomeric OH group) and the CH_2OH group lie on the *same* side of the ring. In the α-anomer (right), they are on different sides. The reaction that interconverts anomers into each other is known as *mutarotation* (**B**).

2. Glycoside formation. When the anomeric OH group of a sugar reacts with an alcohol, with elimination of water, it yields an *O–glycoside* (in the case shown, α–methylglucoside). The glycosidic bond is not a normal ether bond, because the OH group at C-1 has a hemiacetal quality. Oligosaccharides and polysaccharides also contain *O*-glycosidic bonds. Reaction of the anomeric OH group with an NH_2 or NH group yields an *N-glycoside* (not shown). *N*-glycosidic bonds occur in nucleotides (see p. 80) and in glycoproteins (see p. 44), for example.

3. Reduction and oxidation. Reduction of the anomeric center at C-1 of glucose (2) produces the sugar alcohol *sorbitol*. Oxidation of the aldehyde group at C-1 gives the intramolecular ester (lactone) of *gluconic acid* (a glyconic acid). Phosphorylated gluconolactone is an intermediate of the pentose phosphate pathway (see p. 152). When glucose is oxidized at C-6, *glucuronic acid* (a glycuronic acid) is formed. The strongly polar glucuronic acid plays an important role in biotransformations in the liver (see pp. 194, 316).

4. Epimerization. In weakly alkaline solutions, glucose is in equilibrium with the ketohexose D-*fructose* and the aldohexose D-*mannose,* via an enediol intermediate (not shown). The only difference between glucose and mannose is the configuration at C-2. Pairs of sugars of this type are referred to as *epimers*, and their interconversion is called *epimerization*.

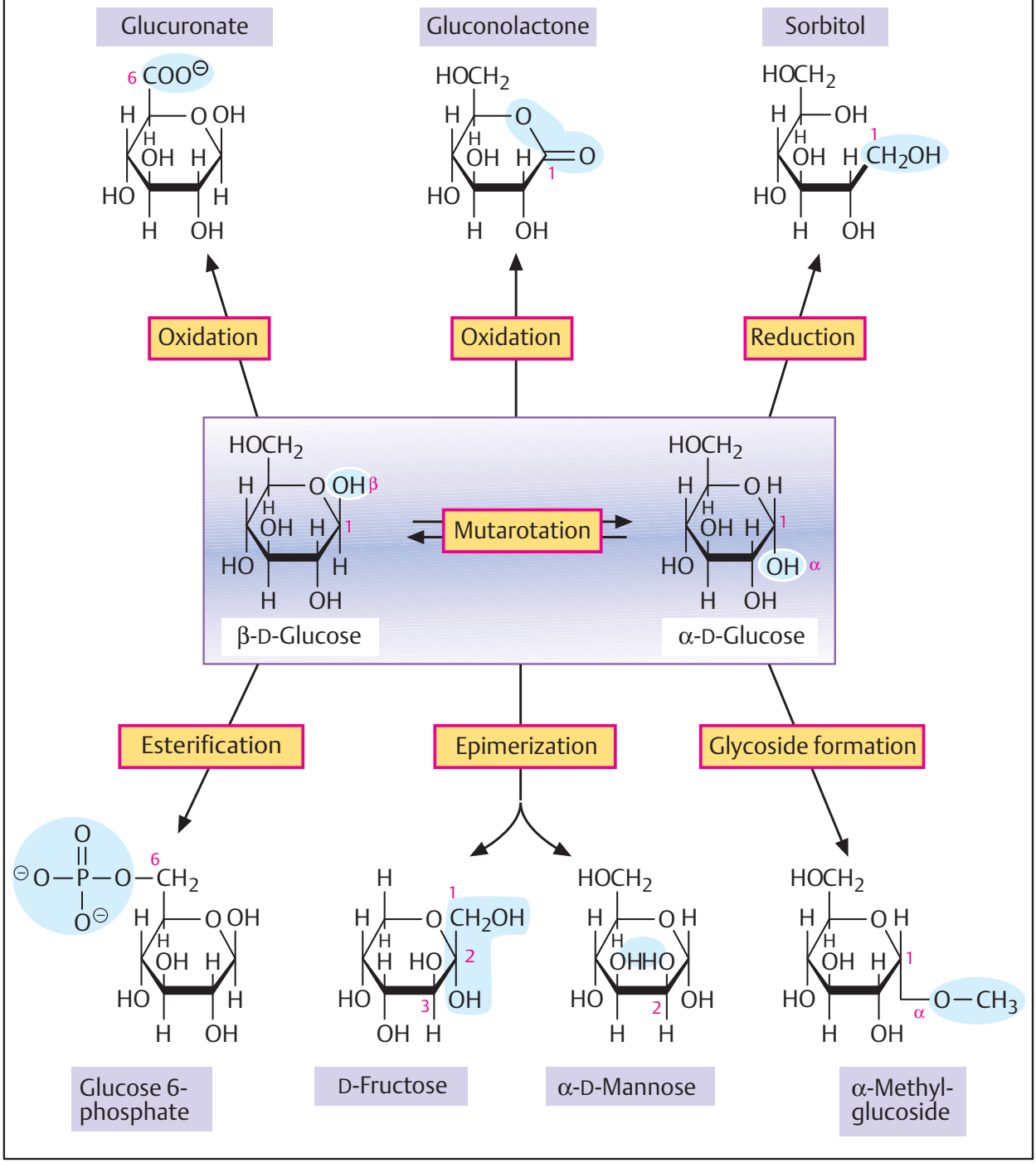
5. Esterification. The hydroxyl groups of monosaccharides can form *esters* with acids. In metabolism, phosphoric acid esters such as *glucose 6-phosphate* and *glucose 1-phosphate* (6) are particularly important.

B. Polarimetry, mutarotation ○

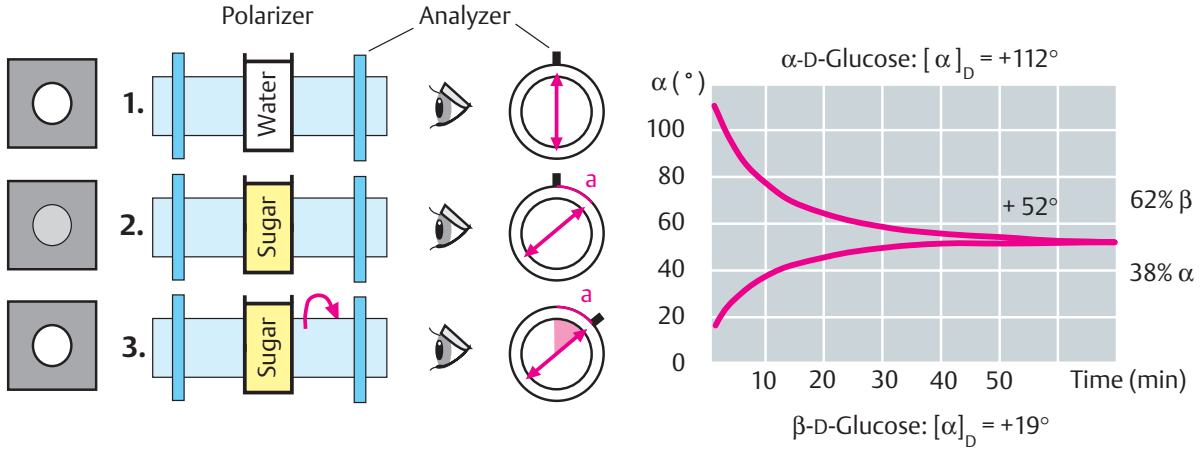
Sugar solutions can be analyzed by **polarimetry**, a method based on the interaction between chiral centers and linearly polarized light—i. e., light that oscillates in only *one* plane. It can be produced by passing normal light through a special filter (a **polarizer**). A second polarizing filter of the same type (the **analyzer**), placed behind the first, only lets the polarized light pass through when the polarizer and the analyzer are in alignment. In this case, the field of view appears bright when one looks through the analyzer (**1**). Solutions of chiral substances rotate the plane of polarized light by an angle α either to the left or to the right. When a solution of this type is placed between the polarizer and the analyzer, the field of view appears darker (**2**). The angle of rotation, α, is determined by turning the analyzer until the field of view becomes bright again (**3**). A solution's **optical rotation** depends on the type of chiral compound, its concentration, and the thickness of the layer of the solution. This method makes it possible to determine the sugar content of wines, for example.

Certain procedures make it possible to obtain the α and β anomers of glucose in pure form. A 1-molar solution of α-D-glucose has a rotation value $[\alpha]_D$ of +112°, while a corresponding solution of β-D-glucose has a value of +19°. These values change spontaneously, however, and after a certain time reach the same end point of +52°. The reason for this is that, in solution, **mutarotation** leads to an equilibrium between the α and β forms in which, independently of the starting conditions, 62% of the molecules are present in the β form and 38% in the α form.

A. Reactions of the monosaccharides



B. Polarimetry, mutarotation



Monosaccharides and disaccharides

A. Important monosaccharides ◑

Only the most important of the large number of naturally occurring **monosaccharides** are mentioned here. They are classified according to the number of C atoms (into pentoses, hexoses, etc.) and according to the chemical nature of the carbonyl function into aldoses and ketoses.

The best-known **aldopentose** (**1**), D-*ribose*, is a component of RNA and of nucleotide coenzymes and is widely distributed. In these compounds, ribose always exists in the furanose form (see p. 34). Like ribose, D-*xylose* and L-*arabinose* are rarely found in free form. However, large amounts of both sugars are found as constituents of polysaccharides in the walls of plant cells (see p. 42).

The most important of the **aldohexoses** (**1**) is D-glucose. A substantial proportion of the biomass is accounted for by glucose polymers, above all cellulose and starch. Free D-glucose is found in plant juices ("grape sugar") and as "blood sugar" in the blood of higher animals. As a constituent of lactose (milk sugar), D-galactose is part of the human diet. Together with D-mannose, galactose is also found in glycolipids and glycoproteins (see p. 44).

Phosphoric acid esters of the **ketopentose** D-*ribulose* (**2**) are intermediates in the pentose phosphate pathway (see p. 152) and in photosynthesis (see p. 128). The most widely distributed of the **ketohexoses** is D-*fructose*. In free form, it is present in fruit juices and in honey. Bound fructose is found in sucrose (**B**) and plant polysaccharides (e. g., inulin).

In the **deoxyaldoses** (**3**), an OH group is replaced by a hydrogen atom. In addition to 2-*deoxy-D-ribose*, a component of DNA (see p. 84) that is reduced at C-2, L-fucose is shown as another example of these. Fucose, a sugar in the λ series (see p. 34) is reduced at C-6.

The **acetylated amino sugars** *N-acetyl-D-glucosamine* and *N-acetyl-D-Galactosamine* (**4**) are often encountered as components of glycoproteins.

N-acetylneuraminic acid (sialic acid, **5**), is a characteristic component of glycoproteins. Other **acidic monosaccharides** such as D-*glucuronic acid*, D-*galacturonic acid*, and *liduronic acid*, are typical constituents of the glycosaminoglycans found in connective tissue.

Sugar alcohols (**6**) such as *sorbitol* and *mannitol* do not play an important role in animal metabolism.

B. Disaccharides ◑

When the anomeric hydroxyl group of one monosaccharide is bound glycosidically with one of the OH groups of another, a **disaccharide** is formed. As in all glycosides, the glycosidic bond does *not* allow mutarotation. Since this type of bond is formed stereospecifically by enzymes in natural disaccharides, they are only found in *one* of the possible configurations (α or β).

Maltose (**1**) occurs as a breakdown product of the starches contained in malt ("malt sugar"; see p. 148) and as an intermediate in intestinal digestion. In maltose, the anomeric OH group of one glucose molecule has an α-glycosidic bond with C-4 in a second glucose residue.

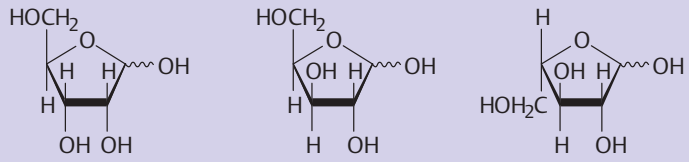
Lactose ("milk sugar," **2**) is the most important carbohydrate in the milk of mammals. Cow's milk contains 4.5% lactose, while human milk contains up to 7.5%. In lactose, the anomeric OH group of galactose forms a β-glycosidic bond with C-4 of a glucose. The lactose molecule is consequently elongated, and both of its pyran rings lie in the same plane.

Sucrose (**3**) serves in plants as the form in which carbohydrates are transported, and as a soluble carbohydrate reserve. Humans value it because of its intensely sweet taste. Sources used for sucrose are plants that contain particularly high amounts of it, such as sugar cane and sugar beet (*cane sugar, beet sugar*). Enzymatic hydrolysis of sucrose-containing flower nectar in the digestive tract of bees—catalyzed by the enzyme *invertase*—produces **honey**, a mixture of glucose and fructose. In sucrose, the two anomeric OH groups of glucose and fructose have a glycosidic bond; sucrose is therefore one of the non-reducing sugars.

A. Important monosaccharides

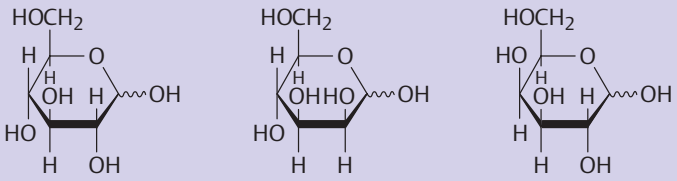
① Aldoses

D-Ribose (Rib) | D-Xylose (Xyl) | L-Arabinose (Ara)



Pentoses

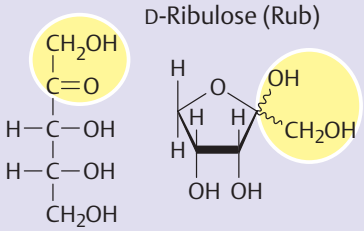
D-Glucose (Glc) | D-Mannose (Man) | D-Galactose (Gal)



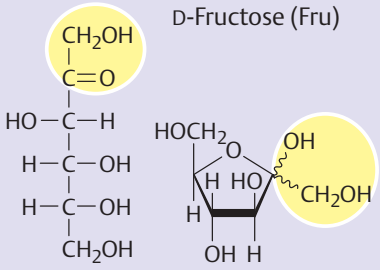
Hexoses

② Ketoses

D-Ribulose (Rub)

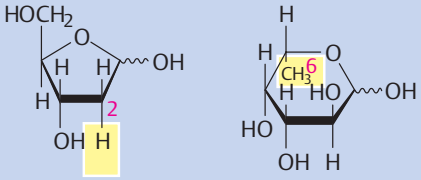


D-Fructose (Fru)



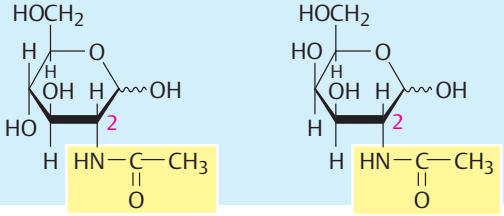
③ Deoxyaldoses

2-Deoxy-D-ribose (dRib) | L-Fucose (Fuc)



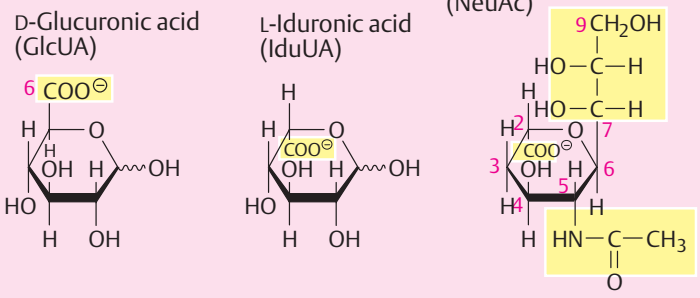
④ Acetylated amino sugars

N-Acetyl-D-glucosamine (GlcNAc) | *N*-Acetyl-D-galactosamine (GalNAc)



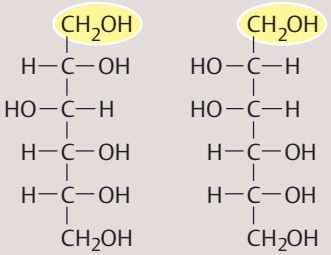
⑤ Acidic monosaccharides

D-Glucuronic acid (GlcUA) | L-Iduronic acid (IduUA) | *N*-Acetylneuraminic acid (NeuAc)

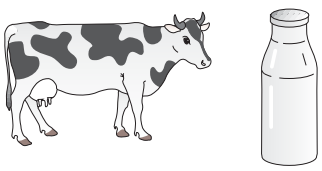


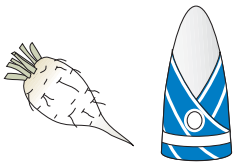
⑥ Sugar alcohols (alditoles)

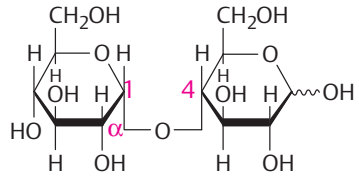
D-Sorbitol | D-Mannitol



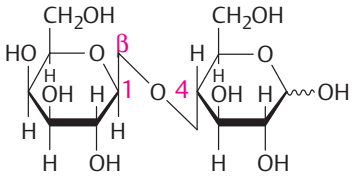
B. Disaccharides



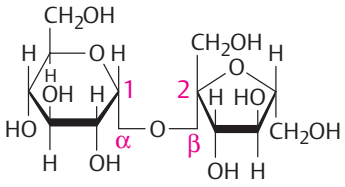




1. Maltose
α-D-Glucopyranosyl-(1→4)-D-glucopyranose



2. Lactose
β-D-Galactopyranosyl-(1→4)-D-glucopyranose



3. Sucrose
α-D-Glucopyranosyl-(1↔2)-β-D-fructofuranoside

Polysaccharides: overview

Polysaccharides are ubiquitous in nature. They can be classified into three separate groups, based on their different *functions*. **Structural polysaccharides** provide mechanical stability to cells, organs, and organisms. **Waterbinding polysaccharides** are strongly hydrated and prevent cells and tissues from drying out. Finally, **reserve polysaccharides** serve as carbohydrate stores that release monosaccharides as required. Due to their polymeric nature, reserve carbohydrates are osmotically less active, and they can therefore be stored in large quantities within the cell.

A. Polysaccharides: structure ◑

Polysaccharides that are formed from only *one* type of monosaccharide are called **homoglycans**, while those formed from different sugar constituents are called **heteroglycans**. Both forms can exist as either linear or branched chains.

A section of a **glycogen** molecule is shown here as an example of a branched homoglycan. Amylopectin, the branched component of vegetable starch (see p. 42), has a very similar structure. Both molecules mainly consist of α1→4-linked glucose residues. In glycogen, on average every 8th to 10th residue carries —via an α1→6 bond—another 1,4-linked chain of glucose residues. This gives rise to branched, tree-like structures, which in animal glycogen are covalently bound to a protein, *glycogenin* (see p. 156).

The linear heteroglycan **murein**, a structural polysaccharide that stabilizes the cell walls of bacteria, has a more complex structure. Only a short segment of this thread-like molecule is shown here. In murein, two different components, both β1→4-linked, alternate: *N-acetylglucosamine* (GlcNAc) and *N-acetylmuraminic acid* (MurNAc), a lactic acid ether of *N*-acetylglucosamine. *Peptides* are bound to the carboxyl group of the lactyl groups, and attach the individual strands of murein to each other to form a three-dimensional network (not shown). Synthesis of the network-forming peptides in murein is inhibited by *penicillin* (see p. 254).

B. Important polysaccharides ◑

The table gives an overview of the composition and make-up both of the glycans mentioned above and of several more.

In addition to murein, bacterial polysaccharides include **dextrans**—glucose polymers that are mostly α1→6-linked and α1→3-branched. In water, dextrans form viscous slimes or gels that are used for chromatographic separation of macromolecules after chemical treatment (see p. 78). Dextrans are also used as components of blood plasma substitutes (plasma expanders) and foodstuffs.

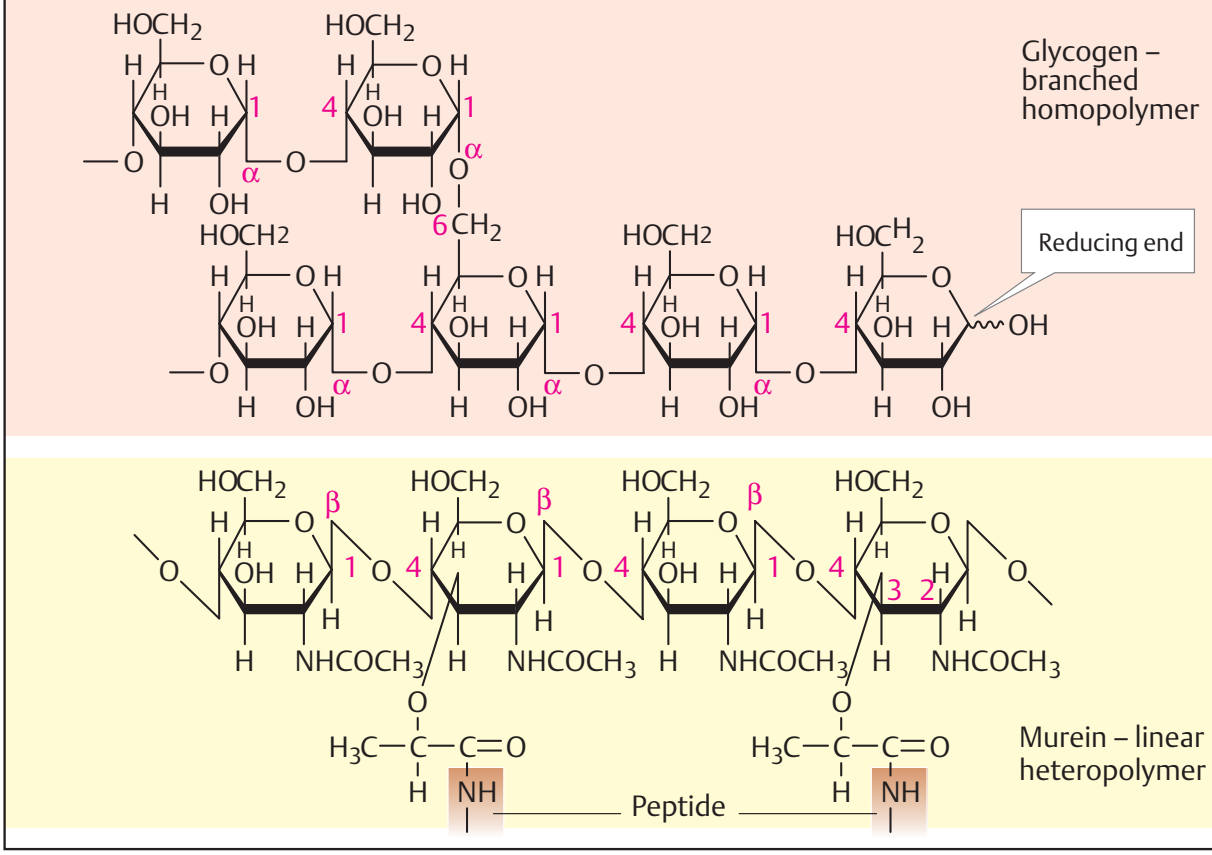
Carbohydrates from algae (e.g., **agarose** and **carrageenan**) can also be used to produce gels. Agarose has been used in microbiology for more than 100 years to reinforce culture media ("agar-agar"). Algal polysaccharides are also added to cosmetics and ready-made foods to modify the consistency of these products.

The **starches**, the most important vegetable reserve carbohydrate and polysaccharides from plant cell walls, are discussed in greater detail on the following page. **Inulin**, a fructose polymer, is used as a starch substitute in diabetics' dietary products (see p. 160). In addition, it serves as a test substance for measuring renal clearance (see p. 322).

Chitin, a homopolymer from β1→4-linked *N*-acetylglucosamine, is the most important structural substance in insect and crustacean shells, and is thus the most common animal polysaccharide. It also occurs in the cell wall of fungi.

Glycogen, the reserve carbohydrate of higher animals, is stored in the liver and musculature in particular (**A**, see pp. 156, 336). The formation and breakdown of glycogen are subject to complex regulation by hormones and other factors (see p. 120).

A. Polysaccharides: structure



B. Important polysaccharides

Poly-saccharide	Mono-saccharide 1	Mono-saccharide 2	Linkage	Branch-ing	Occurrence	Function
Bacteria						
Murein	D-GlcNAc	D-MurNAc[1)]	β 1 →4	—	Cell wall	SC
Dextran	D-Glc	—	α 1 →6	α 1→3	Slime	WB
Plants						
Agarose	D-Gal	L-aGal[2)]	β 1 →4	β 1→3	Red algae (agar)	WB
Carrageenan	D-Gal	—	β 1 →3	α 1→4	Red algae	WB
Cellulose	D-Glc	—	β 1 →4	—	Cell wall	SC
Xyloglucan	D-Glc	D-Xyl (D-Gal, L-Fuc)	β 1 →4	β 1→6 (β 1→2)	Cell wall (Hemicellulose)	SC
Arabinan	L-Ara	—	α 1 →5	α 1→3	Cell wall (pectin)	SC
Amylose	D-Glc	—	α 1 →4	—	Amyloplasts	RC
Amylopectin	D-Glc	—	α 1 →4	α 1→6	Amyloplasts	RC
Inulin	D-Fru		β 2 →1	—	Storage cells	RC
Animals						
Chitin	D-GlcNAc	—	β 1 →4	—	Insects, crabs	SK
Glycogen	D-Glc	—	α 1 →4	α 1 →6	Liver, muscle	RK
Hyaluronic acid	D-GlcUA	D-GlcNAc	β 1 →4 β 1 →3	—	Connective tissue	SK,WB

SC= structural carbohydrate, RC= reserve carbohydrate,
WB = water-binding carbohydrate; [1)] *N*-acetylmuramic acid, [2)] 3,6-anhydrogalactose

Plant polysaccharides

Two glucose polymers of plant origin are of special importance among the polysaccharides: β1→4-linked polymer **cellulose** and **starch**, which is mostly α1→4-linked.

A. Cellulose ◑

Cellulose, a linear homoglycan of β1→4-linked glucose residues, is the *most abundant organic substance* in nature. Almost half of the total biomass consists of cellulose. Some 40–50% of plant *cell walls* are formed by cellulose. The proportion of cellulose in *cotton fibers,* an important raw material, is 98%. Cellulose molecules can contain more than 10^4 glucose residues (mass 1–2 10^6 Da) and can reach lengths of 6–8 μm.

Naturally occurring cellulose is *extremely mechanically stable* and is highly *resistant* to chemical and enzymatic hydrolysis. These properties are due to the conformation of the molecules and their supramolecular organization. The unbranched β1→4 linkage results in linear chains that are stabilized by hydrogen bonds within the chain and between neighboring chains (**1**). Already during biosynthesis, 50–100 cellulose molecules associate to form an **elementary fibril** with a diameter of 4 nm. About 20 such elementary fibrils then form a **microfibril** (**2**), which is readily visible with the electron microscope.

Cellulose microfibrils make up the basic framework of the **primary wall** of young plant cells (**3**), where they form a complex network with other polysaccharides. The linking polysaccharides include **hemicellulose**, which is a mixture of predominantly neutral heteroglycans (xylans, xyloglucans, arabinogalactans, etc.). Hemicellulose associates with the cellulose fibrils via noncovalent interactions. These complexes are connected by neutral and acidic **pectins**, which typically contain galacturonic acid. Finally, a collagen-related protein, **extensin**, is also involved in the formation of primary walls.

In the higher animals, including humans, cellulose is **indigestible**, but important as **roughage** (see p. 273). Many herbivores (e. g., the ruminants) have symbiotic unicellular organisms in their digestive tracts that break down cellulose and make it digestible by the host.

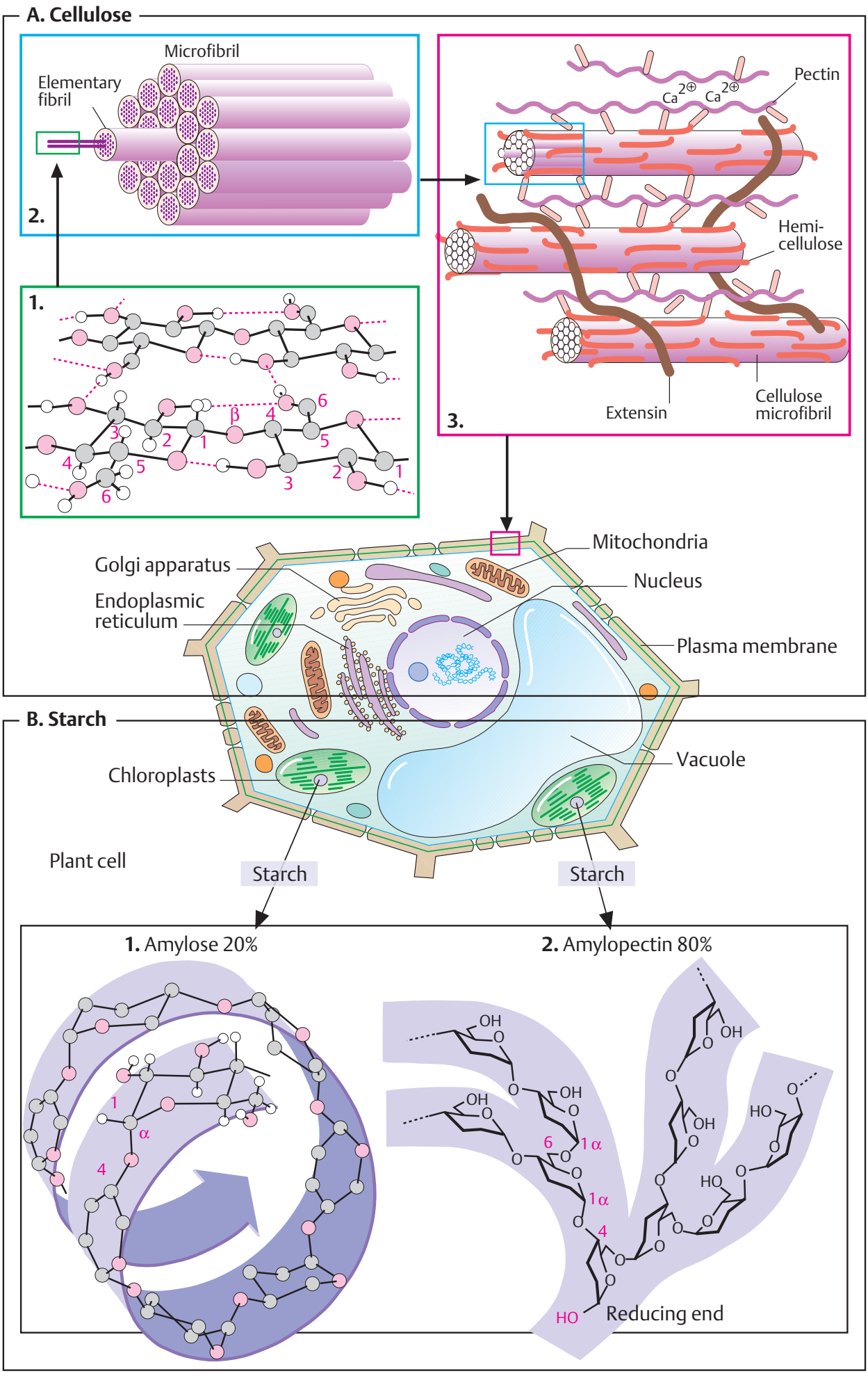
B. Starch ◑

Starch, a **reserve polysaccharide** widely distributed in plants, is the *most important carbohydrate in the human diet.* In plants, starch is present in the chloroplasts in leaves, as well as in fruits, seeds, and tubers. The starch content is especially high in cereal grains (up to 75% of the dry weight), potato tubers (approximately 65%), and in other plant storage organs.

In these plant organs, starch is present in the form of microscopically small granules in special organelles known as **amyloplasts**. *Starch granules* are virtually insoluble in cold water, but swell dramatically when the water is heated. Some 15–25% of the starch goes into solution in colloidal form when the mixture is subjected to prolonged boiling. This proportion is called amylose ("soluble starch").

Amylose consists of *unbranched* α1→4-linked chains of 200–300 glucose residues. Due the α configuration at C-1, these chains form a *helix* with 6–8 residues per turn (**1**). The blue coloring that soluble starch takes on when iodine is added (the "iodine–starch reaction") is caused by the presence of these helices—the iodine atoms form chains inside the amylose helix, and in this largely nonaqueous environment take on a deep blue color. Highly branched polysaccharides turn brown or reddishbrown in the presence of iodine.

Unlike amylose, **amylopectin**, which is practically insoluble, is *branched*. On average, one in 20–25 glucose residues is linked to another chain via an α1→6 bond. This leads to an extended tree-like structure, which—like amylose—contains only *one* anomeric OH group (a "reducing end"). Amylopectin molecules can contain hundreds of thousands of glucose residues; their mass can be more than 10^8 Da.



Glycosaminoglycans and glycoproteins

A. Hyaluronic acid ○

As constituents of proteoglycans (see p. 346), the glycosaminoglycans—a group of acidic heteropolysaccharides—are important structural elements of the extracellular matrix.

Glycosaminoglycans contain *amino sugars* as well as *glucuronic acid* and *iduronic acid* as characteristic components (see p. 38). In addition, most polysaccharides in this group are esterified to varying extents by sulfuric acid, increasing their acidic quality. Glycosaminoglycans can be found in free form, or as components of proteoglycans throughout the organism.

Hyaluronic acid, an unesterified glycosaminoglycan with a relatively simple structure, consists of disaccharide units in which *N-acetylglucosamine* and *glucuronic acid* are alternately β1→4-linked and β1→3-linked. Due to the unusual β1→3 linkage, hyaluronic acid molecules–which may contain several thousand monosaccharide residues—are coiled like a helix. Three disaccharide units form each turn of the helix. The outwardfacing hydrophilic carboxylate groups of the glucuronic acid residues are able to bind Ca^{2+} ions. The **strong hydration** of these groups enables hyaluronic acid and other glycosaminoglycans to bind water up to 10 000 times their own volume in gel form. This is the function which hyaluronic acid has in the *vitreous body* of the eye, which contains approximately 1% hyaluronic acid and 98% water.

B. Oligosaccharide in immunoglobulin G (IgG) ○

Many proteins on the surface of the plasma membrane, and the majority of secreted proteins, contain oligosaccharide residues that are post-translationally added to the endoplasmic reticulum and in the Golgi apparatus (see p. 230). By contrast, cytoplasmic proteins are rarely glycosylated. **Glycoproteins** can contain more than 50% carbohydrate; however, the proportion of protein is generally much greater.

As an example of the carbohydrate component of a glycoprotein, the structure of one of the oligosaccharide chains of immunoglobulin G (IgG; see p. 300) is shown here. The oligosaccharide has an *N-glycosidic* link to the amide group of an asparagine residue in the F_c part of the protein. Its function is not known.

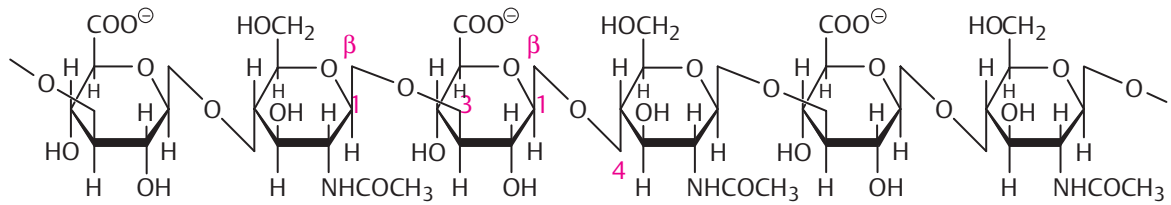
Like all *N*-linked carbohydrates, the oligosaccharide in IgG contains a T-shaped **core structure** consisting of two *N-acetylglucosamines* and three *mannose* residues (shown in violet). In addition, in this case the structure contains two further *N-acetylglucosamine* residues, as well as a *fucose* residue and a *galactose* residue. Glycoproteins show many different types of branching. In this case, we not only have β1→4 linkage, but also β1→2, α1→3, and α1→6 bonds.

C. Glycoproteins: forms ◑

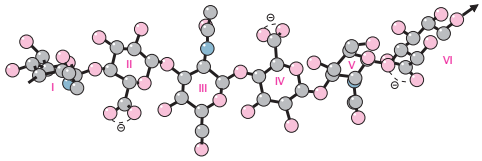
On the cell surface of certain glycoproteins, **O-glycosidic** links are found between the carbohydrate part and a serine or threonine residue, instead of *N*-glycosidic links to asparagine residues. This type of link is less common than the ***N*-glycosidic** one.

There are two types of oligosaccharide structure with *N*-glycosidic links, which arise through two different biosynthetic pathways. During glycosylation in the ER, the protein is initially linked to an oligosaccharide, which in addition to the core structure contains six further mannose residues and three terminal glucose residues (see p. 230). The simpler from of oligosaccharide (the **mannose-rich** type) is produced when only the glucose residues are cleaved from the primary product, and no additional residues are added. In other cases, the mannose residues that are located outside the core structure are also removed and replaced by other sugars. This produces oligosaccharides such as those shown on the right (the **complex type**). At the external end of the structure, glycoproteins of the complex type often contain *N*-acetylneuraminic acid residues, which give the oligosaccharide components negative charges.

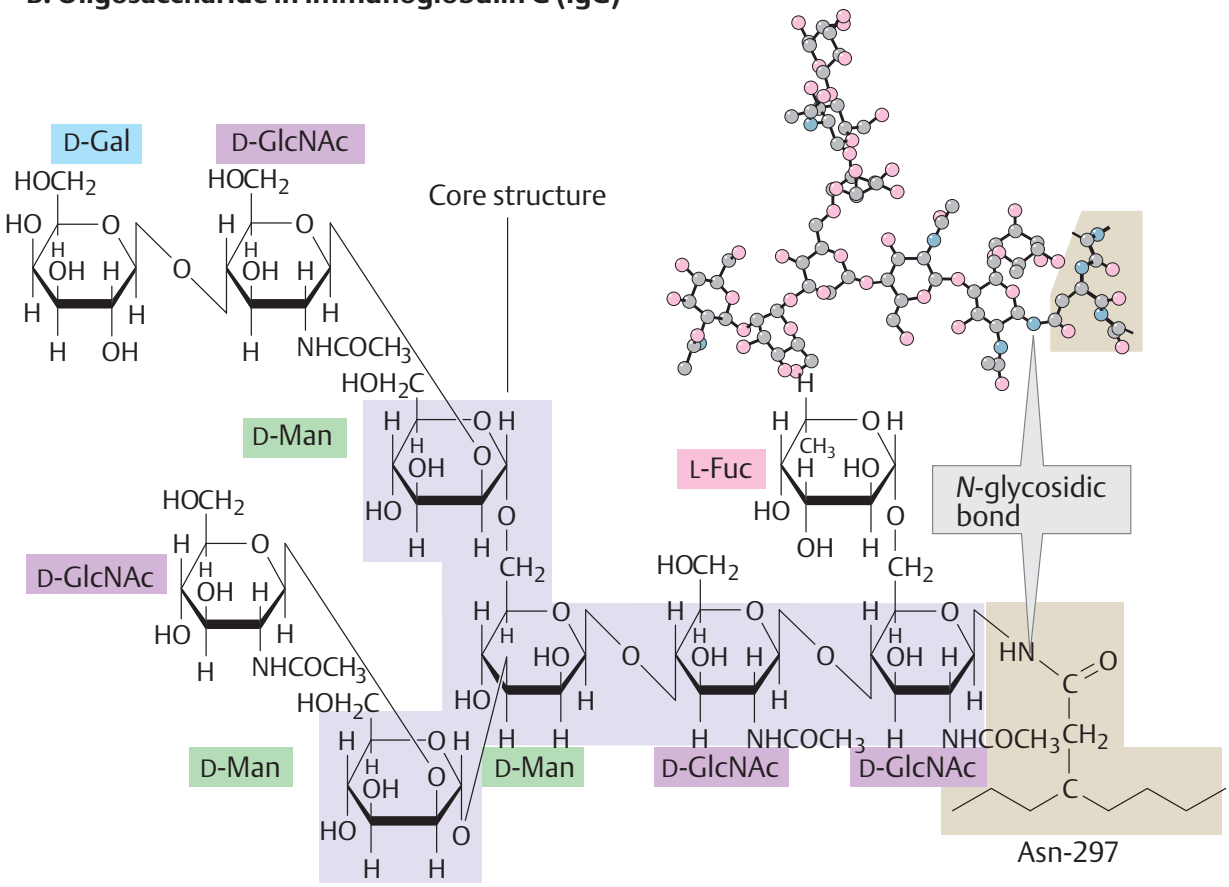
A. Hyaluronic acid



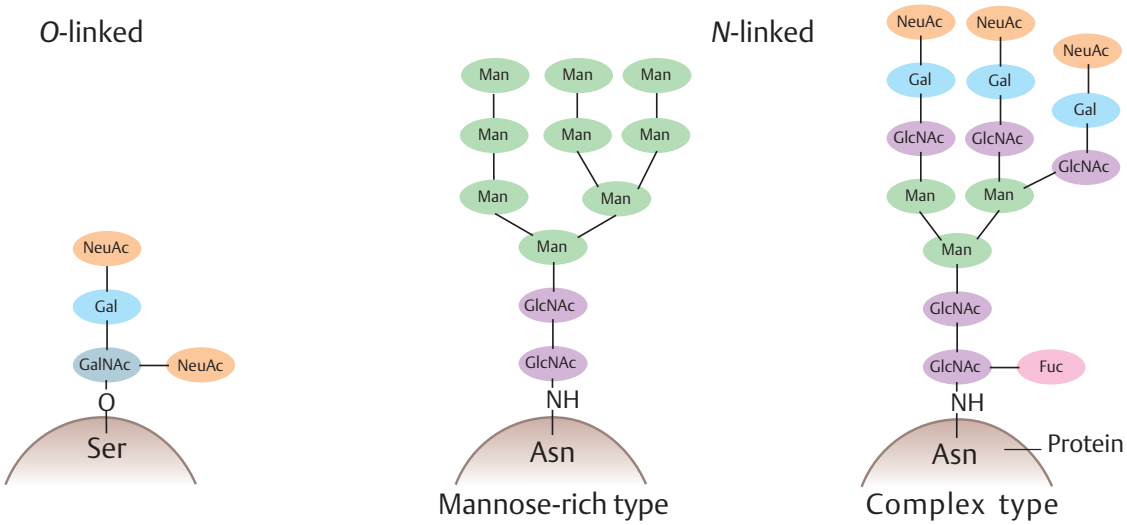
[→3)-β-D-GlcNAc-(1 →4)-β-D-GlcUA-(1 →4]$_n$



B. Oligosaccharide in immunoglobulin G (IgG)



C. Glycoproteins: forms



Overview

A. Classification ●

The **lipids** are a large and heterogeneous group of substances of biological origin that are easily dissolved in organic solvents such as methanol, acetone, chloroform, and benzene. By contrast, they are either insoluble or only poorly soluble in water. Their low water solubility is due to a lack of polarizing atoms such as O, N, S, and P (see p. 6).

Lipids can be classified into substances that are either *hydrolyzable*— i. e., able to undergo hydrolytic cleavage—or *nonhydrolyzable*. Only a few examples of the many lipids known can be mentioned here. The individual classes of lipids are discussed in more detail in the following pages.

Hydrolyzable lipids (components shown in brackets). The simple **esters** include the *fats* (triacylglycerol; one glycerol + three acyl residues); the *waxes* (one fatty alcohol + one acyl residue); and the *sterol esters* (one sterol + one acyl residue). The **phospholipids** are esters with more complex structures. Their characteristic component is a phosphate residue. The phospholipids include the *phosphatidic acids* (one glycerol + two acyl residues + one phosphate) and the *phosphatides* (one glycerol + two acyl residues + one phosphate + one amino alcohol). In the **sphingolipids**, glycerol and one acyl residue are replaced by sphingosine. Particularly important in this group are the sugar-containing **glycolipids** (one sphingosine + one fatty acid + sugar). The *cerebrosides* (one sphingosine + one fatty acid + one sugar) and *gangliosides* (one sphingosine + one fatty acid + several different sugars, including neuraminic acid) are representatives of this group.

The components of the hydrolyzable lipids are linked to one another by **ester bonds**. They are easily broken down either enzymatically or chemically.

Non-hydrolyzable lipids. The **hydrocarbons** include the *alkanes* and *carotenoids*. The **lipid alcohols** are also not hydrolyzable. They include long-chained *alkanols* and cyclic *sterols* such as cholesterol, and *steroids* such as estradiol and testosterone. The most important **acids** among the lipids are *fatty acids*. The *eicosanoids* also belong to this group; these are derivatives of the polyunsaturated fatty acid arachidonic acid (see p. 390).

B. Biological roles ●

1. Fuel. Lipids are an important source of energy in the diet. In quantitative terms, they represent the principal energy reserve in animals. Neutral fats in particular are stored in specialized cells, known as *adipocytes*. Fatty acids are released from these again as needed, and these are then oxidized in the mitochondria to form water and carbon dioxide, with oxygen being consumed. This process also gives rise to reduced coenzymes, which are used for ATP production in the respiratory chain (see p. 140).

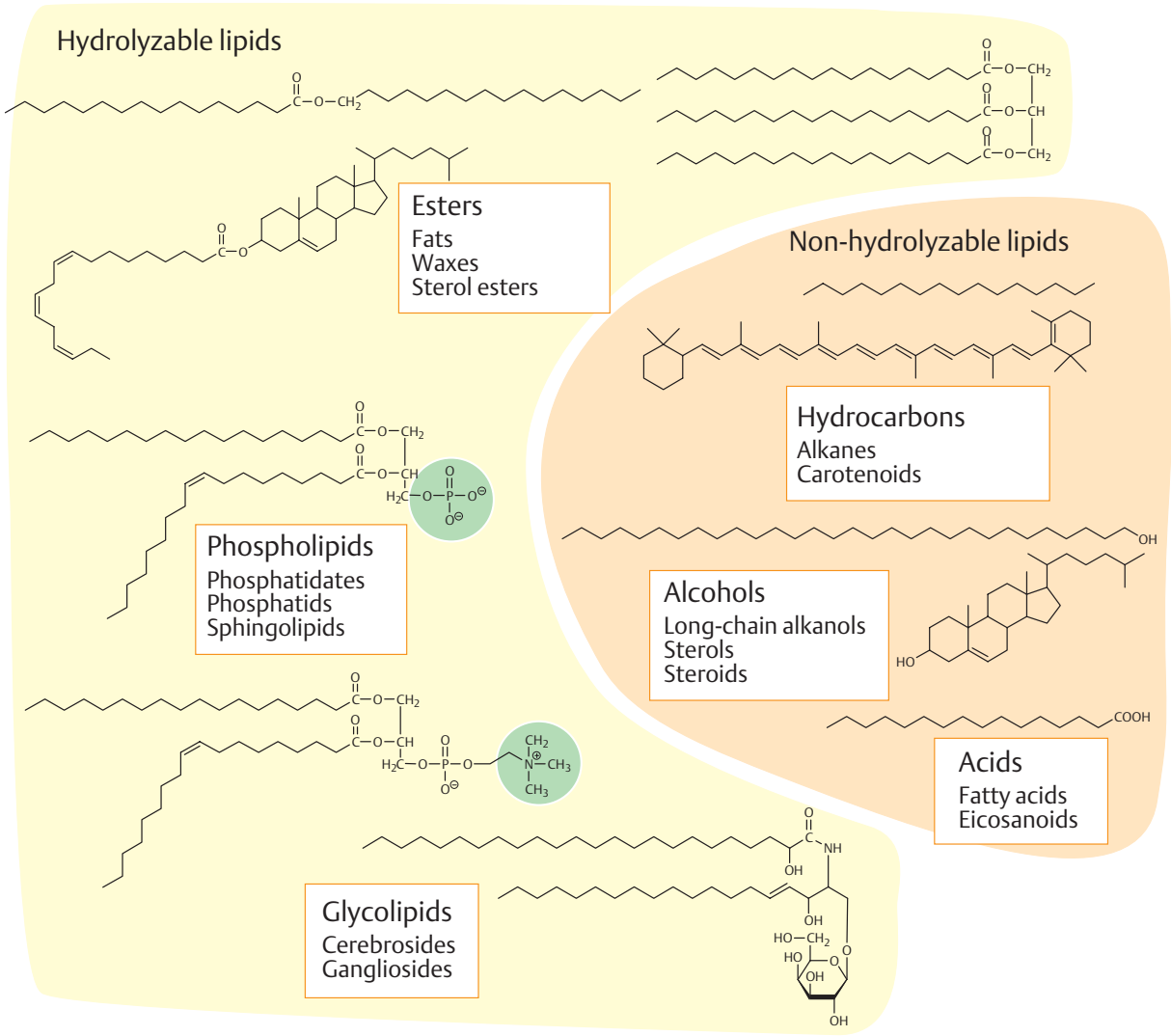
2. Nutrients. Amphipathic lipids are used by cells to build membranes (see p. 214). Typical membrane lipids include phospholipids, glycolipids, and cholesterol. Fats are only weakly amphiphilic and are therefore not suitable as membrane components.

3. Insulation. Lipids are excellent insulators. In the higher animals, neutral fats are found in the subcutaneous tissue and around various organs, where they serve as mechanical and thermal insulators. As the principal constituent of cell membranes, lipids also insulate cells from their environment mechanically and electrically. The impermeability of lipid membranes to ions allows the formation of the membrane potential (see p. 126).

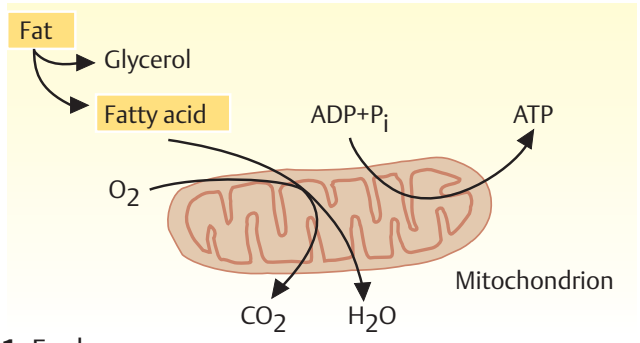
4. Special tasks. Some lipids have adopted special roles in the body. Steroids, eicosanoids, and some metabolites of phospholipids have *signaling functions*. They serve as hormones, mediators, and second messengers (see p. 370). Other lipids form *anchors* to attach proteins to membranes (see p. 214). The lipids also produce *cofactors for enzymatic reactions*—e. g., vitamin K (see p. 52) and ubiquinone (see p. 104). The carotenoid retinal, a light-sensitive lipid, is of central importance in the *process of vision* (see p. 358).

Several lipids are not formed independently in the human body. These substances, as **essential fatty acids** and **fat-soluble vitamins**, are indispensable components of nutrition (see pp. 364 ff.)

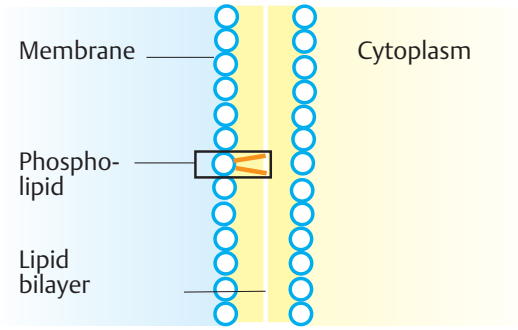
A. Classification



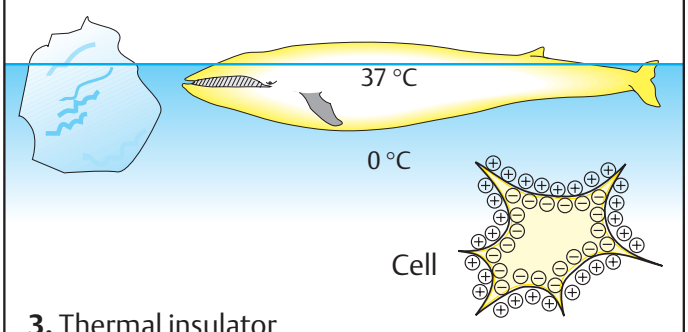
B. Biological roles



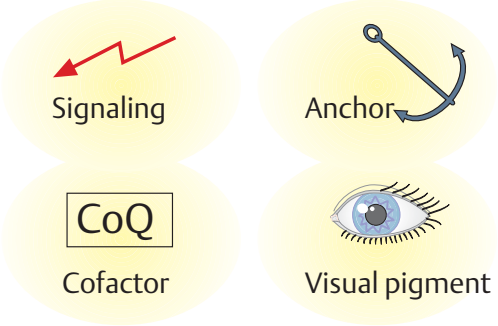
1. Fuel



2. Building block



3. Thermal insulator



4. Special tasks

Fatty acids and fats

A. Carboxylic acids ◑

The naturally occurring **fatty acids** are carboxylic acids with unbranched hydrocarbon chains of 4–24 carbon atoms. They are present in all organisms as components of fats and membrane lipids. In these compounds, they are esterified with alcohols (glycerol, sphingosine, or cholesterol). However, fatty acids are also found in small amounts in unesterified form. In this case, they are known as *free fatty acids* (FFAs). As free fatty acids have strongly amphipathic properties (see p. 28), they are usually present in protein-bound forms.

The table lists the full series of aliphatic carboxylic acids that are found in plants and animals. In higher plants and animals, unbranched, longchain fatty acids with either 16 or 18 carbon atoms are the most common—e. g., palmitic and stearic acid. The number of carbon atoms in the longer, natural fatty acids is always even. This is because they are biosynthesized from C_2 building blocks (see p. 168).

Some fatty acids contain one or more isolated *double bonds*, and are therefore *"unsaturated."* Common **unsaturated fatty acids** include oleic acid and linoleic acid. Of the two possible *cis–trans* isomers (see p. 8), usually only the *cis* forms are found in natural lipids. Branched fatty acids only occur in bacteria. A shorthand notation with several numbers is used for precise characterization of the structure of fatty acids—e g., 18:2;9,12 for linoleic acid. The first figure stands for the number of C atoms, while the second gives the number of double bonds. The positions of the double bonds follow after the semicolon. As usual, numbering starts at the carbon with the highest oxidation state (i. e., the carboxyl group corresponds to C-1). Greek letters are also commonly used (α = C-2; β = C-3; ω = the last carbon, ω-3 = the third last carbon).

Essential fatty acids are fatty acids that have to be supplied in the diet. Without exception, these are all polyunsaturated fatty acids: the C_{20} fatty acid *arachidonic acid* (20:4;5,8,11,14) and the two C_{18} acids *linoleic acid* (18:2;9,12) and *linolenic acid* (18:3;9,12,15). The animal organism requires arachidonic acid to synthesize eicosanoids (see p. 390). As the organism is capable of elongating fatty acids by adding C_2 units, but is not able to introduce double bonds into the end sections of fatty acids (after C-9), arachidonic acid has to be supplied with the diet. Linoleic and linolenic acid can be converted into arachidonic acid by elongation, and they can therefore replace arachidonic acid in the diet.

B. Structure of fats ◑

Fats are esters of the trivalent alcohol *glycerol* with three fatty acids. When a single fatty acid is esterified with glycerol, the product is referred to as a *monoacylglycerol* (fatty acid residue = acyl residue).

Formally, esterification with additional fatty acids leads to *diacylglycerol* and ultimately to *triacylglycerol,* the actual fat (formerly termed "triglyceride"). As triacylglycerols are uncharged, they are also referred to as *neutral fats*. The carbon atoms of glycerol are not usually equivalent in fats. They are distinguished by their *"sn"* number, where sn stands for "stereospecific numbering."

The three acyl residues of a fat molecule may differ in terms of their chain length and the number of double bonds they contain. This results in a large number of possible combinations of individual fat molecules. When extracted from biological materials, fats always represent mixtures of very similar compounds, which differ in their fatty acid residues. A chiral center can arise at the middle C atom (*sn* -C-2) of a triacylglycerol if the two external fatty acids are different. The monoacylglycerols and diacylglycerols shown here are also chiral compounds. Nutritional fats contain palmitic, stearic, oleic acid, and linoleic acid particularly often. Unsaturated fatty acids are usually found at the central C atom of glycerol.

The length of the fatty acid residues and the number of their double bonds affect the melting point of the fats. The shorter the fatty acid residues and the more double bonds they contain, the lower their melting points.

A. Carboxylic acids

Name	Number of carbons : Number of double bonds; Position of double bonds
Formic acid	1 : 0
Acetic acid	2 : 0
Propionic acid	3 : 0
Butyric acid	4 : 0
Valerianic acid	5 : 0
Caproic acid	6 : 0
Caprylic acid	8 : 0
Capric acid	10 : 0
Lauric acid	12 : 0
Myristic acid	14 : 0
Palmitic acid	16 : 0
Stearic acid	18 : 0
Oleic acid	18 : 1; 9
Linoleic acid	18 : 2; 9,12
Linolenic acid	18 : 3; 9,12,15
Arachidic acid	20 : 0
Arachidonic acid	20 : 4; 5,8,11,14
Behenic acid	22 : 0
Erucic acid	22 : 1; 13
Lignoceric acid	24 : 0
Nervonic acid	24 : 1; 15

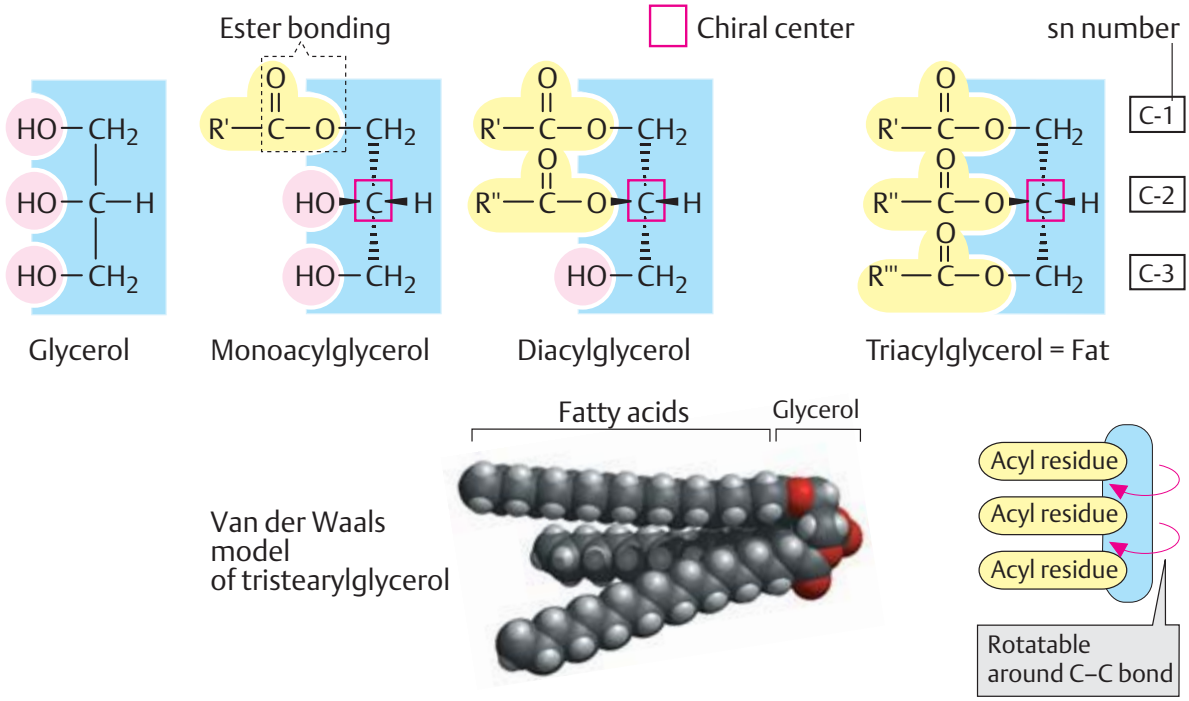
Not contained in lipids

$HOOC-CH_2-CH_2-CH_2-CH_2-CH_3$

Caproic acid

Essential in human nutrition

B. Structure of fats



Phospholipids and glycolipids

A. Structure of phospholipids and glycolipids ◑

Fats (triacylglycerol, **1**) are esters of glycerol with three fatty acids (see p. 48). Within the cell, they mainly occur as fat droplets. In the blood, they are transported in the hydrophobic interior of lipoproteins (see p. 278).

Phospholipids (**2**) are the main constituents of biological membranes (see pp. 214–217). Their common feature is a phosphate residue that is esterified with the hydroxyl group at C-3 of glycerol. Due to this residue, phospholipids have at least one negative charge at a neutral pH.

Phosphatidates (anions of the phosphatidic acids), the simplest phospholipids, are phosphate esters of diacylglycerol. They are important intermediates in the biosynthesis of fats and phospholipids (see p. 170). Phosphatidates can also be released from phospholipids by phospholipases.

The other phospholipids can be derived from phosphatidates (residue = phosphatidyl). Their phosphate residues are esterified with the hydroxyl group of an amino alcohol (*choline*, *ethanolamine*, or *serine*) or with the cyclohexane derivative *myo-inositol*. *Phosphatidylcholine* is shown here as an example of this type of compound. When two phosphatidyl residues are linked with one glycerol, the result is *cardiolipin* (not shown), a phospholipid that is characteristic of the inner mitochondrial membrane. *Lysophospholipids* arise from phospholipids by enzymatic cleavage of an acyl residue. The hemolytic effect of bee and snake venoms is due in part to this reaction.

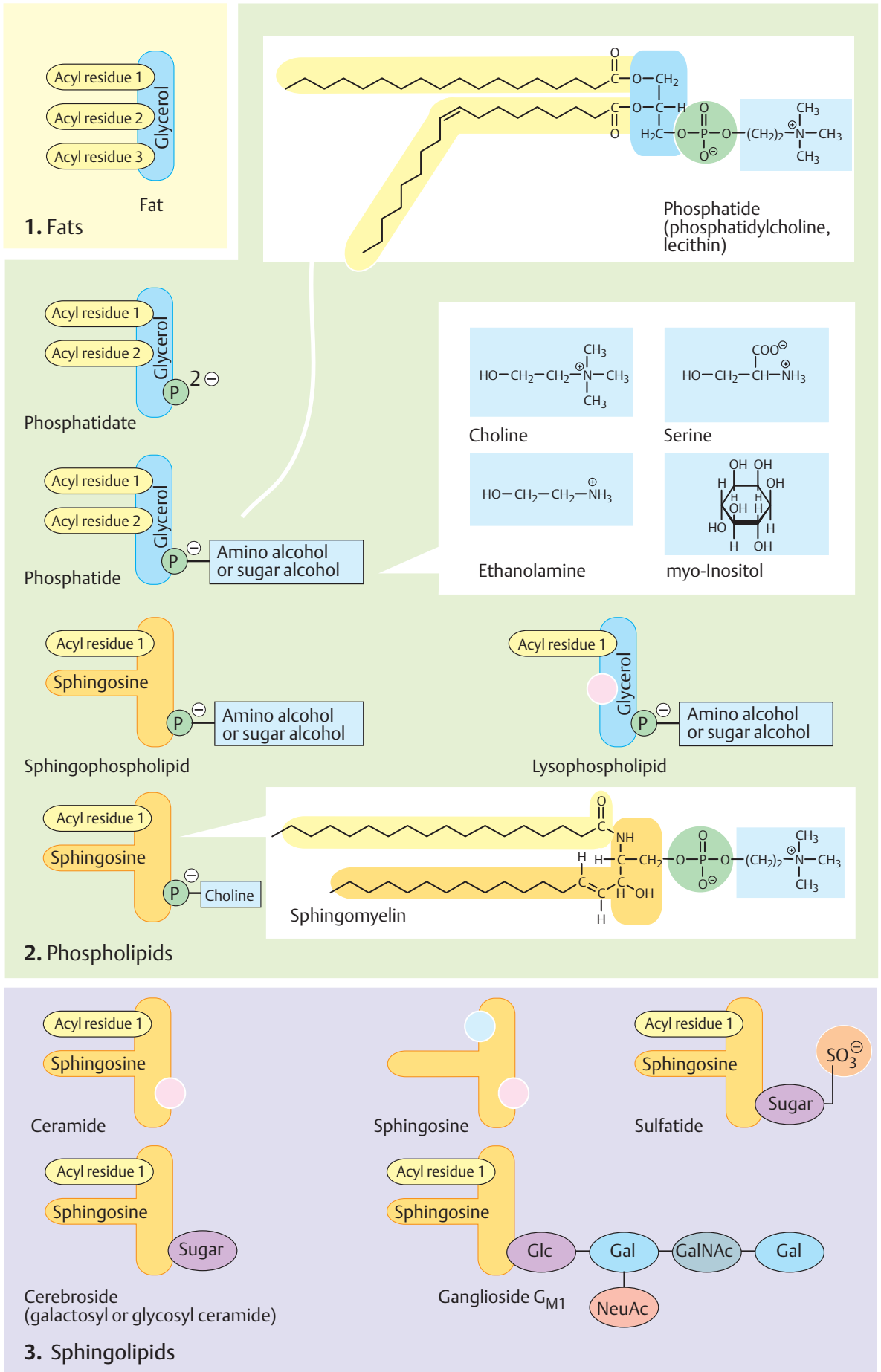
Phosphatidylcholine (lecithin) is the most abundant phospholipid in membranes. *Phosphatidylethanolamine* (cephalin) has an ethanolamine residue instead of choline, and *phosphatidylserine* has a serine residue. In *phosphatidylinositol,* phosphatidate is esterified with the sugarlike cyclic polyalcohol *myo*-inositol. A doubly phosphorylated derivative of this phospholipid, phosphatidylinositol 4,5-bisphosphate, is a special component of membranes, which, by enzymatic cleavage, can give rise to two *second messengers*, diacylglycerol (DAG) and inositol 1,4,5trisphosphate ($InsP_3$; see p. 386).

Some phospholipids carry additional charges, in addition to the negative charge at the phosphate residue. In phosphatidylcholine and phosphatidylethanolamine, the *N*-atom of the amino alcohol is positively charged. As a whole, these two phosphatides therefore appear to be neutral. In contrast, phosphatidylserine—with one additional positive charge and one additional negative charge in the serine residue—and phosphatidylinositol (with no additional charge) have a negative net charge, due to the phosphate residue.

Sphingolipids (**3**), which are found in large quantities in the membranes of nerve cells in the brain and in neural tissues, have a slightly different structure from the other membrane lipids discussed so far. In sphingolipids, *sphingosine*, an amino alcohol with an unsaturated alkyl side chain, replaces glycerol and one of the acyl residues. When sphingosine forms an amide bond to a fatty acid, the compound is called *ceramide* (**3**). This is the precursor of the sphingolipids. *Sphingomyelin* (2)—the most important sphingolipid—has an additional phosphate residue with a choline group attached to it on the sphingosine, in addition to the fatty acid.

Glycolipids (**3**) are present in all tissues on the outer surface of the plasma membrane. They consist of sphingosine, a fatty acid, and an oligosaccharide residue, which can sometimes be quite large. The phosphate residue typical of phospholipids is absent. *Galactosylceramide* and *glucosylceramide* (known as cerebroside) are simple representatives of this group. Cerebrosides in which the sugar is esterified with sulfuric acid are known as *sulfatides*. *Gangliosides* are the most complex glycolipids. They constitute a large family of membrane lipids with receptor functions that are as yet largely unknown. A characteristic component of many gangliosides is *N*-acetylneuraminic acid (sialic acid; see p. 38).

A. Structure of fats, phospholipids, and glycolipids



Isoprenoids

A. Activated acetic acid as a component of lipids ◑

Although the lipids found in plant and animal organisms occur in many different forms, they are all closely related biogenetically; they are all derived from **acetyl-CoA**, the "activated acetic acid" (see pp. 12, 110).

1. One major pathway leads from acetyl-CoA to the activated fatty acids (**acyl-CoA**; for details, see p. 168). *Fats, phospholipids,* and *glycolipids* are synthesized from these, and fatty acid derivatives in particular are formed. Quantitatively, this is the most important pathway in animals and most plants.

2. The second pathway leads from acetyl-CoA to isopentenyl diphosphate (*"active isoprene"*), the basic component for the **isoprenoids**. Its biosynthesis is discussed in connection with biosynthesis of the isoprenoid, cholesterol (see p. 172).

B. Isoprenoids ◑

Formally, isoprenoids are derived from a single common building block, isoprene (2-methyl-1,3-butadiene), a methyl-branched compound with five C atoms. Activated isoprene, *isopentenyl diphosphate,* is used by plants and animals to biosynthesize linear and cyclic oligomers and polymers. For the isoprenoids listed here—which only represent a small selection—the number of isoprene units (I) is shown.

From activated isoprene, the metabolic pathway leads via dimerization to activated *geraniol* (I = 2) and then to activated *farnesol* (I = 3). At this point, the pathway divides into two. Further extension of farnesol leads to chains with increasing numbers of isoprene units—e. g., *phytol* (I = 4), *dolichol* (I = 14–24), and *rubber* (I = 700–5000). The other pathway involves a "head-to-head" linkage between two farnesol residues, giving rise to *squalene* (I = 6), which, in turn, is converted to *cholesterol* (I = 6) and the other *steroids*.

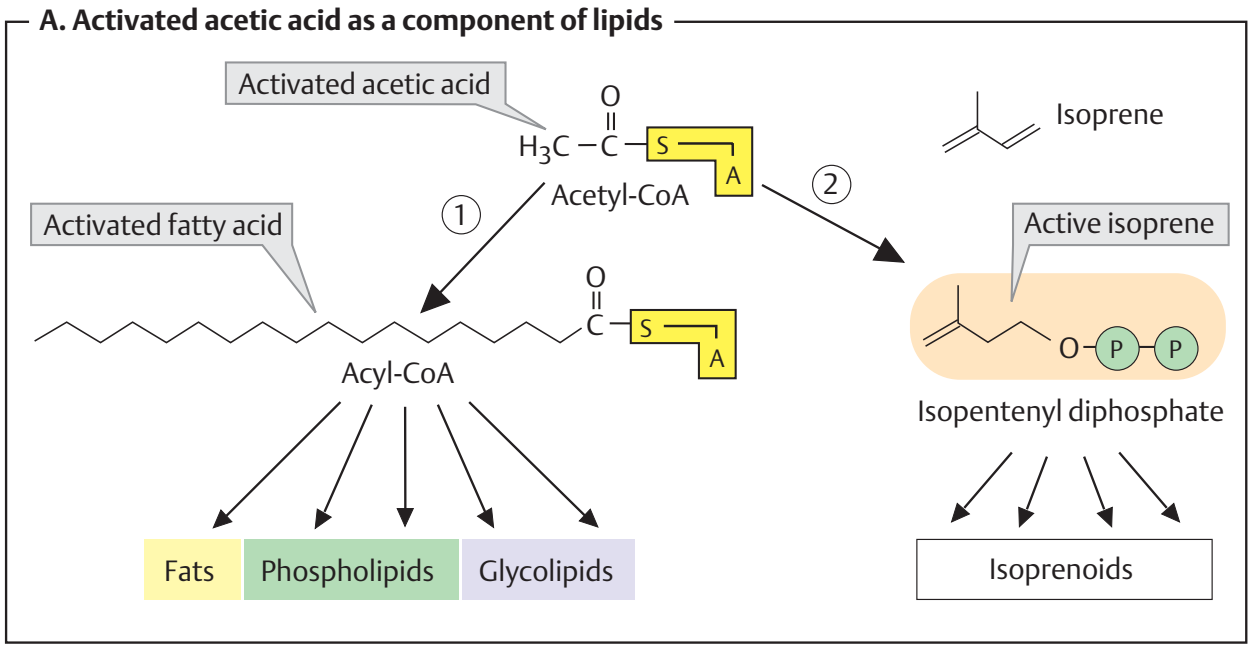
The ability to synthesize particular isoprenoids is limited to a few species of plants and animals. For example, rubber is only formed by a few plant species, including the rubber tree (*Hevea brasiliensis*). Several isoprenoids that are required by animals for metabolism, but cannot be produced by them independently, are vitamins; this group includes *vitamins A, D, E,* and *K*. Due to its structure and function, vitamin D is now usually classified as a steroid hormone (see pp. 56, 330).

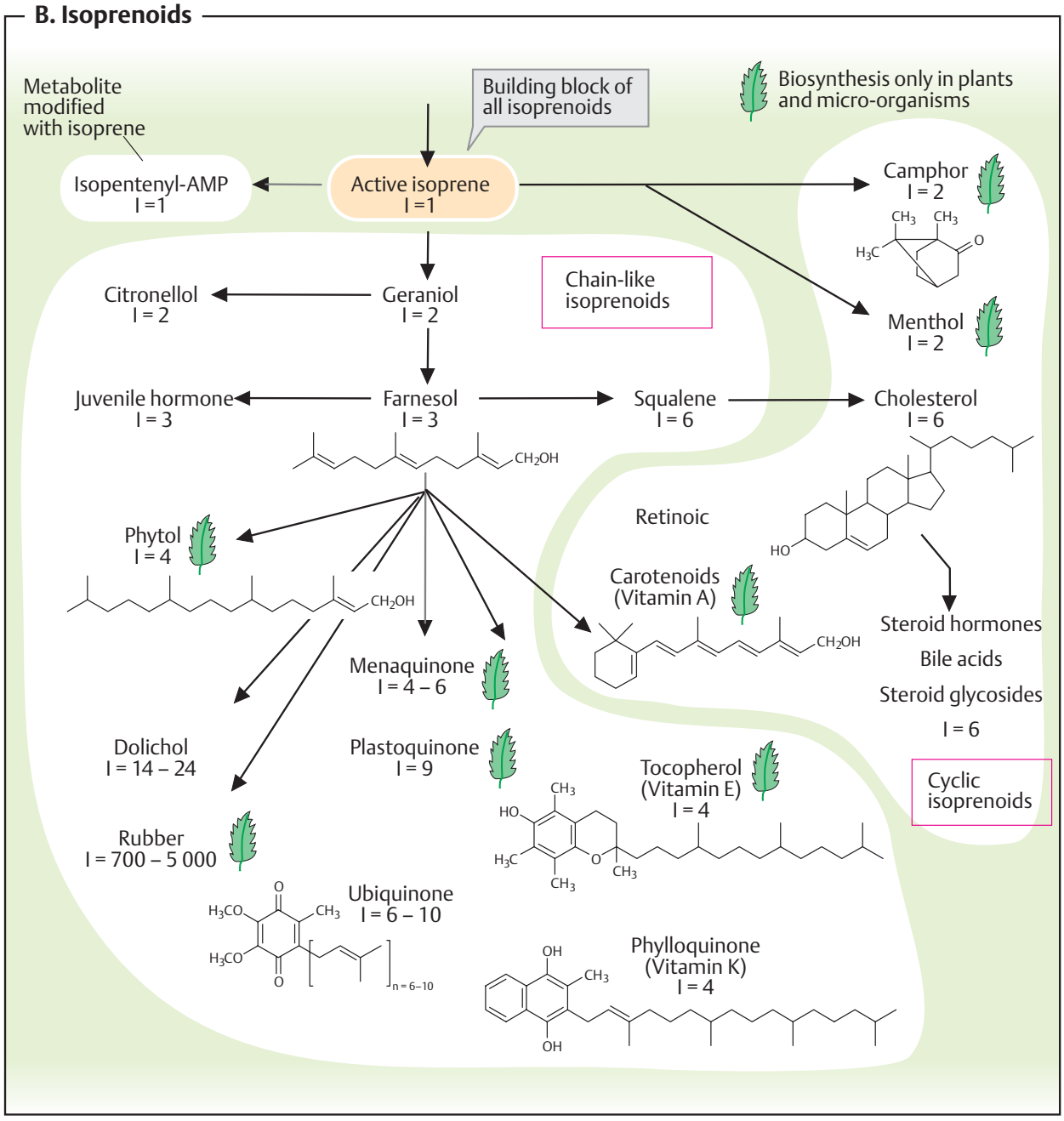
Isoprene metabolism in plants is very complex. Plants can synthesize many types of aromatic substances and volatile oils from isoprenoids. Examples include *menthol* (I= 2), *camphor* (I = 2), and *citronellal* (I = 2). These C_{10} compounds are also called *monoterpenes*. Similarly, compounds consisting of three isoprene units (I = 3) are termed *sesquiterpenes*, and the steroids (I = 6) are called *triterpenes*.

Isoprenoids that have hormonal and signaling functions form an important group. These include *steroid hormones* (I = 6) and *retinoate* (the anion of retinoic acid; I = 3) in vertebrates, and *juvenile hormone* (I = 3) in arthropods. Some plant hormones also belong to the isoprenoids—e. g., the cytokinins, abscisic acid, and brassinosteroids.

Isoprene chains are sometimes used as lipid anchors to fix molecules to membranes (see p. 214). Chlorophyll has a *phytyl* residue (I = 4) as a lipid anchor. Coenzymes with isoprenoid anchors of various lengths include *ubiquinone* (coenzyme Q; I = 6–10), *plastoquinone* (I = 9), and *menaquinone* (vitamin K; I = 4–6). Proteins can also be anchored to membranes by *isoprenylation*.

In some cases, an isoprene residue is used as an element to modify molecules chemically. One example of this is *N'*-isopentenyl-AMP, which occurs as a modified component in tRNA.





Steroid structure

A. Steroid building blocks ◑

Common to all of the steroids is a molecular core structure consisting of four saturated rings, known as *gonane*. At the end of the steroid core, many steroids also carry a side chain, as seen in *cholestane*, the basic component of the *sterols* (steroid alcohols).

B. Spatial structure ○

The four rings of the steroids are distinguished using the letters A, B, C, and D. Due to the tetrahedral arrangement of the single carbon bonds, the rings are not flat, but puckered. Various *ring conformations* are known by the terms "chair," "boat," and "twisted" (not shown). The *chair* and *boat* conformations are common. Fivemembered rings frequently adopt a conformation referred to as an "envelope". Some rings can be converted from one conformation to another at room temperature, but with steroids this is dif cult.

Substituents of the steroid core lie either approximately in the same plane as the ring (e = *equatorial*) or nearly perpendicular to it (a = *axial*). In threedimensional representations, substituents pointing toward the observer are indicated by an unbroken line (β position), while bonds pointing into the plane of the page are indicated by a dashed line (α position). The so-called *angular* methyl groups at C-10 and C-13 of the steroids always adopt the β position.

Neighboring rings can lie in the same plane (*trans*; **2**) or at an angle to one another (*cis*; **1**). This depends on the positions of the substituents of the shared ring carbons, which can be arranged either *cis* or *trans* to the angular methyl group at C-10. The substituents of steroid that lie at the points of intersection of the individual rings are usually in *trans* position. As a whole, the core of most steroids is more or less planar, and looks like a flat disk. The only exceptions to this are the ecdysteroids, bile acids (in which A:B is *cis*), cardiac glycosides, and toad toxins.

A more realistic impression of the threedimensional structure of steroids is provided by the space-filling model of *cholesterol* (**3**). The four rings form a fairly rigid scaffolding, onto which the much more mobile side chain is attached.

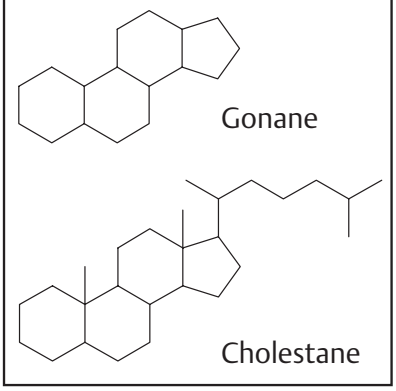
Steroids are relatively apolar (hydrophobic). Some polar groups—e. g., hydroxyl and oxo groups—give them amphipathic properties. This characteristic is especially pronounced with the bile acids (see p. 314).

C. Thin-layer chromatography ○

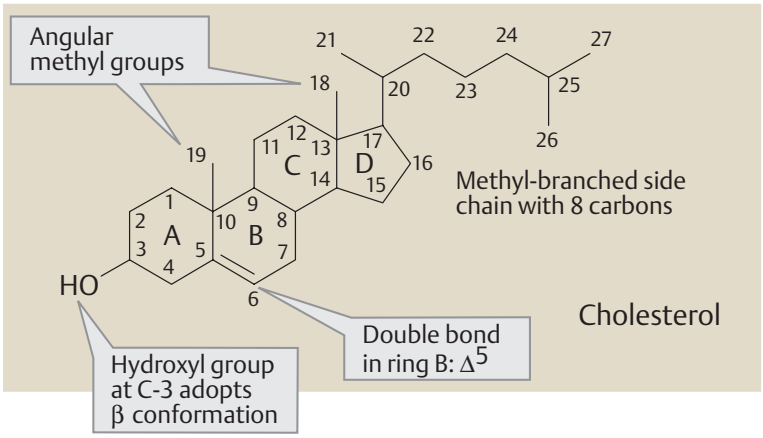
Thin-layer chromatography (TLC) is a powerful, mainly analytic, technique for rapidly separating lipids and other small molecules such as amino acids, nucleotides, vitamins, and drugs. The *sample* being analyzed is applied to a *plate* made of glass, aluminum, or plastic, which is covered with a thin layer of silica gel or other material (**1**). The plate is then placed in a chromatography chamber that contains some *solvent*. Drawn by capillary forces, the solvent moves up the plate (**2**). The substances in the sample move with the solvent. The speed at which they move is determined by their distribution between the *stationary phase* (the hydrophilic silica), and the *mobile phase* (the hydrophobic solvent). When the solvent reaches the top edge of the plate, the chromatography is stopped. After evaporation of the solvent, the separated substances can be made visible using appropriate staining methods or with physical processes (e. g., ultraviolet light) (**3**). The movement of a substance in a given TLC system is expressed as its R_f value. In this way, compounds that are not known can be identified by comparison with reference substances.

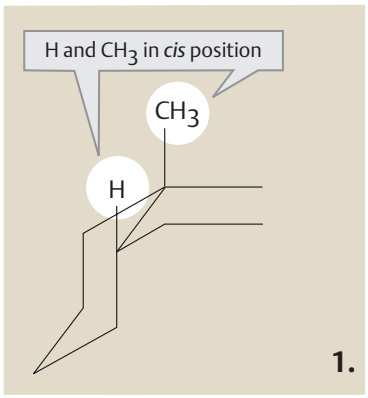
A process in which the polarity of the stationary and mobile phases is reversed—i. e., the stationary phase is apolar and the solvent is polar—is known as "reversed-phase thin-layer chromatography" (RP-TLC).

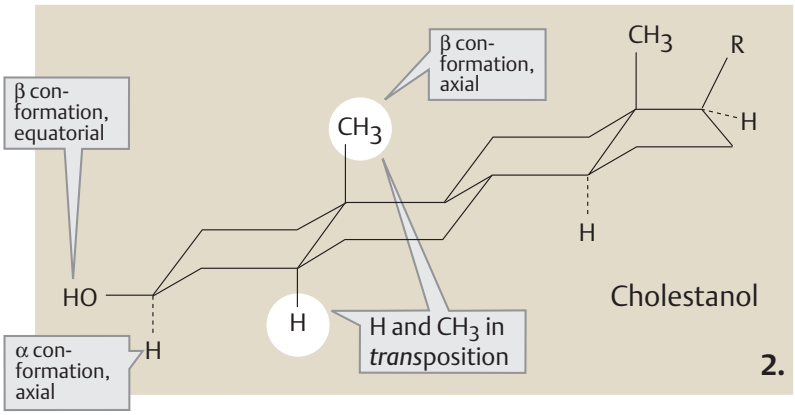
A. Steroid building blocks

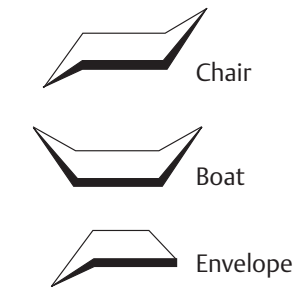


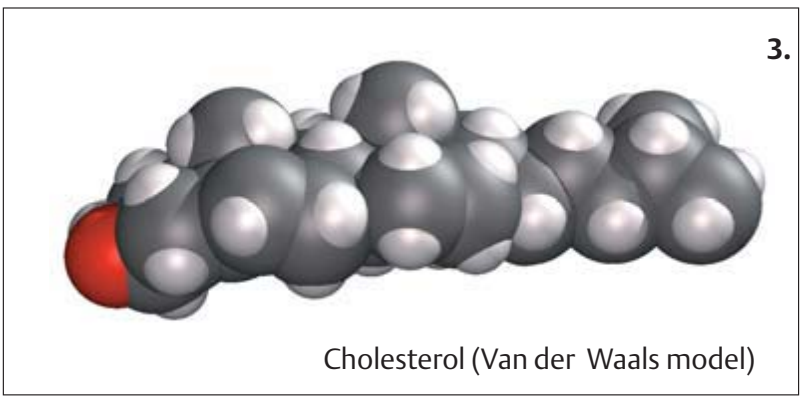
B. 3D structure



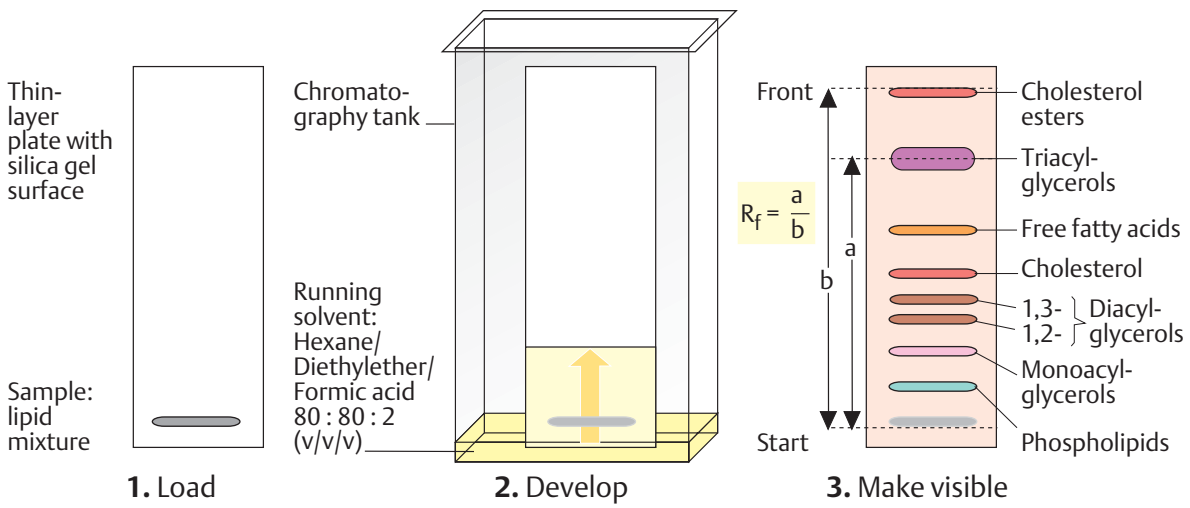








C. Thin-layer chromatography



Steroids: overview

The three most important groups of steroids are the *sterols*, *bile acids*, and *steroid hormones*. Particularly in plants, compounds with steroid structures are also found that are notable for their pharmacological effects—steroid alkaloids, digitalis glycosides, and saponins.

A. Sterols ◑

Sterols are *steroid alcohols*. They have a β-positioned hydroxyl group at C-3 and one or more double bonds in ring B and in the side chain. There are no further oxygen functions, as in the carbonyl and carboxyl groups.

The most important sterol in animals is **cholesterol**. Plants and microorganisms have a wide variety of closely related sterols instead of cholesterol—e.g., **ergosterol**, β-**sitosterol**, and **stigmasterol**.

Cholesterol is present in all animal tissues, and particularly in neural tissue. It is a major constituent of cellular membranes, in which it regulates fluidity (see p. 216). The storage and transport forms of cholesterol are its esters with fatty acids. In lipoproteins, cholesterol and its fatty acid esters are associated with other lipids (see p. 278). Cholesterol is a constituent of the bile and is therefore found in many gallstones. Its biosynthesis, metabolism, and transport are discussed elsewhere (see pp. 172, 312).

Cholesterol-rich lipoproteins of the LDL type are particularly important in the development of arteriosclerosis, in which the arterial walls are altered in connection with an excess plasma cholesterol level. In terms of dietary physiology, it is important that plant foodstuffs are low in cholesterol. By contrast, animal foods can contain large amounts of cholesterol—particularly butter, egg yolk, meat, liver, and brain.

B. Bile acids ◑

Bile acids are synthesized from cholesterol in the liver (see p. 314). Their structures can therefore be derived from that of cholesterol. Characteristic for the bile acids is a side chain shortened by three C atoms in which the last carbon atom is oxidized to a carboxyl group. The double bond in ring B is reduced and rings A and B are in *cis* position relative to each other (see p. 54). One to three hydroxyl groups (in α position) are found in the steroid core at positions 3, 7, and 12. Bile acids keep bile cholesterol in a soluble state as micelles and promote the digestion of lipids in the intestine (see p. 270). **Cholic acid** and **chenodeoxycholic acid** are *primary bile acids* that are formed by the liver. Their dehydroxylation at C-7 by microorganisms from the intestinal flora gives rise to the *secondary bile acids* **lithocholic acid** and **deoxycholic acid**.

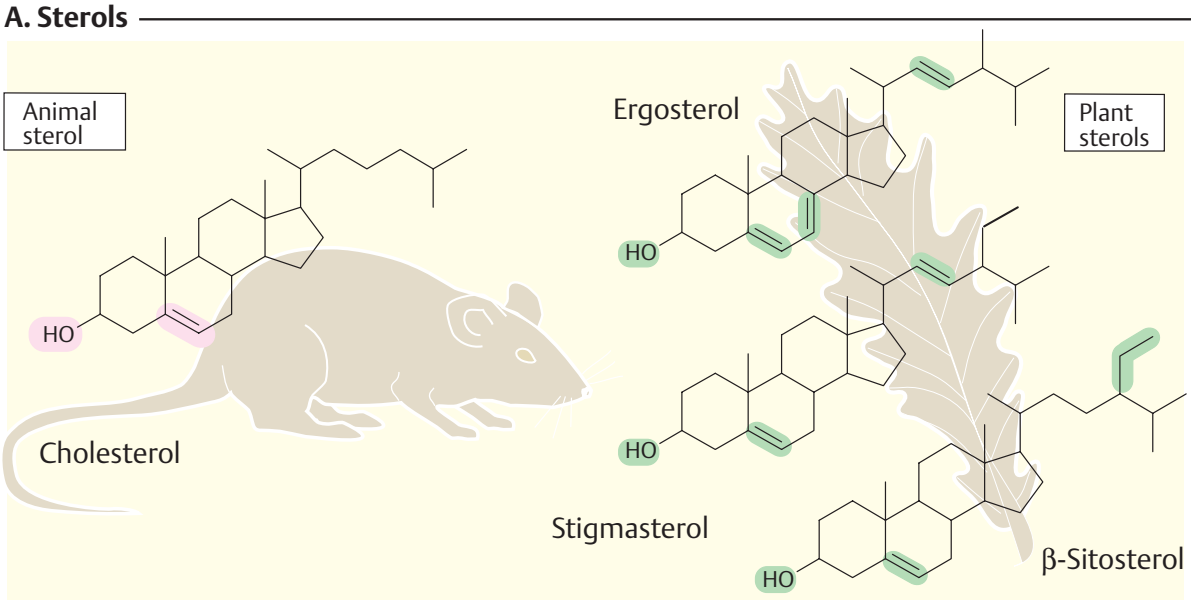
C. Steroid hormones ◑

The conversion of cholesterol to *steroid hormones* (see p. 376) is of minor importance quantitatively, but of major importance in terms of physiology. The steroid hormones are a group of lipophilic signal substances that regulate metabolism, growth, and reproduction (see p. 374).

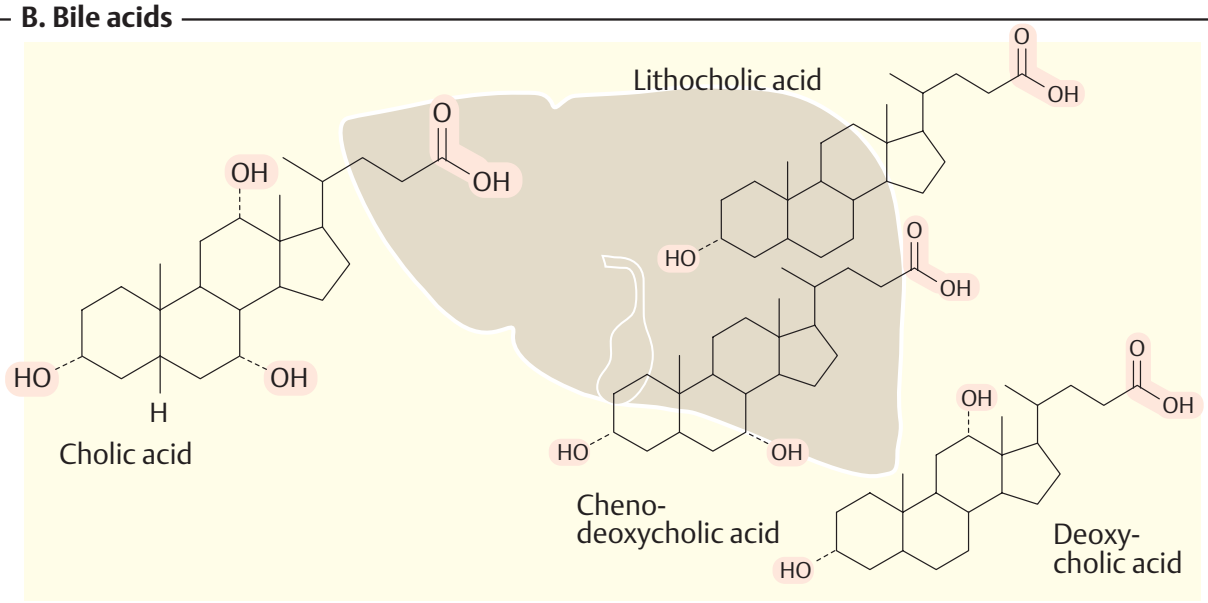
Humans have six steroid hormones: **progesterone**, **cortisol**, **aldosterone**, **testosterone**, **estradiol**, and **calcitriol**. With the exception of calcitriol, these steroids have either no side chain or only a short side one consisting of two carbons. Characteristic for most of them is an oxo group at C-3, conjugated with a double bond between C-4 and C-5 of ring A. Differences occur in rings C and D. Estradiol is aromatic in ring A, and its hydroxyl group at C-3 is therefore phenolic. Calcitriol differs from other vertebrate steroid hormones; it still contains the complete carbon framework of cholesterol, but lightdependent opening of ring B turns it into what is termed a "secosteroid" (a steroid with an open ring).

Ecdysone is the steroid hormone of the arthropods. It can be regarded as an early form of the steroid hormones. Steroid hormones with signaling functions also occur in plants.

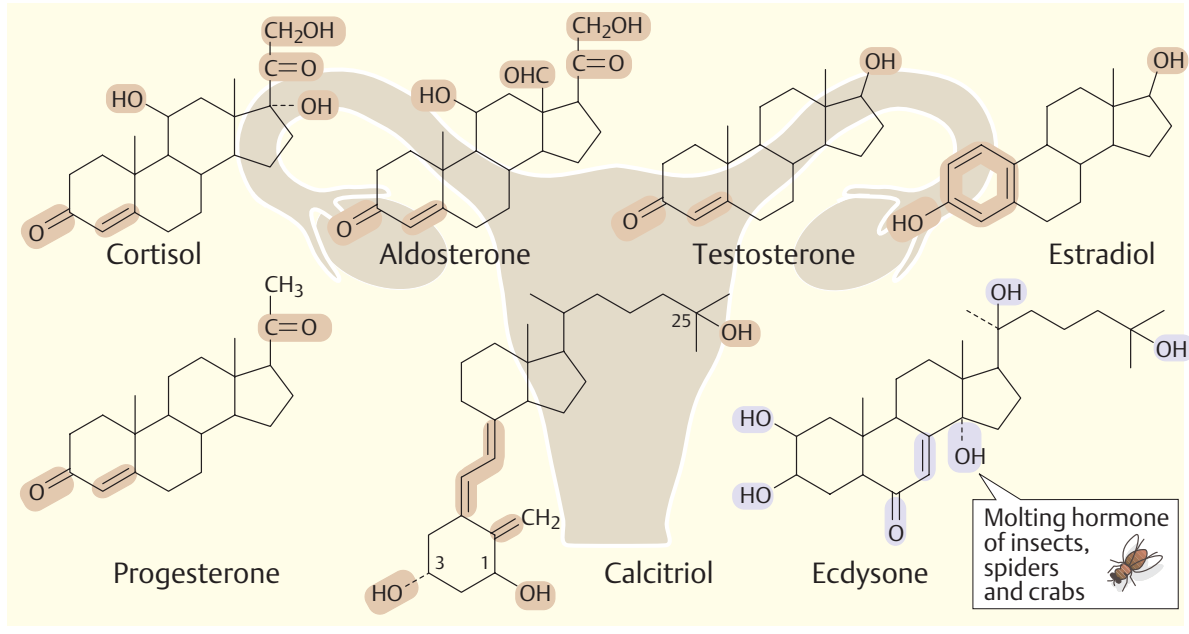
A. Sterols



B. Bile acids



C. Steroid hormones



Amino acids: chemistry and properties

A. Amino acids: functions ●

The amino acids (2-aminocarboxylic acids) fulfill various functions in the organism. Above all, they serve as the **components of peptides and proteins**. Only the 20 *proteinogenic amino acids* (see p. 60) are included in the genetic code and therefore regularly found in proteins. Some of these amino acids undergo further (post-translational) change following their incorporation into proteins (see p. 62). Amino acids or their derivatives are also form components of **lipids**—e. g., serine in phospholipids and glycine in bile salts. Several amino acids function as **neurotransmitters** themselves (see p. 352), while others are precursors of neurotransmitters, mediators, or hormones (see p. 380). Amino acids are important (and sometimes essential) components of food (see p. 360). Specific amino acids form **precursors** for other metabolites—e. g., for glucose in gluconeogenesis, for purine and pyrimidine bases, for heme, and for other molecules. Several nonproteinogenic amino acids function as intermediates in the synthesis and breakdown of proteinogenic amino acids (see p. 412) and in the urea cycle (see p. 182).

B. Optical activity ◑

The natural amino acids are mainly α-amino acids, in contrast to β-amino acids such as β-alanine and taurine. Most α-amino acids have four different substituents at C-2 (Cα). The α atom therefore represents a *chiral center*—i. e., there are two different **enantiomers** (L- and D-amino acids; see p. 8). Among the proteinogenic amino acids, only glycine is *not* chiral (R = H). In nature, it is almost exclusively **L-amino acids** that are found. **D**-Amino acids occur in bacteria—e. g., in murein (see p. 40)—and in peptide antibiotics. In animal metabolism, D-Amino acids would disturb the enzymatic reactions of L-amino acids and they are therefore broken down in the liver by the enzyme *D-amino acid oxidase*.

The **Fischer projection** (center) is used to present the formulas for chiral centers in biomolecules. It is derived from their three-dimensional structure as follows: firstly, the tetrahedron is rotated in such a way that the most oxidized group (the carboxylate group) is at the top. Rotation is then continued until the line connecting line COO^- and R (red) is level with the page. In L-amino acids, the NH_3^+ group is then on the left, while in **D**-amino acids it is on the right.

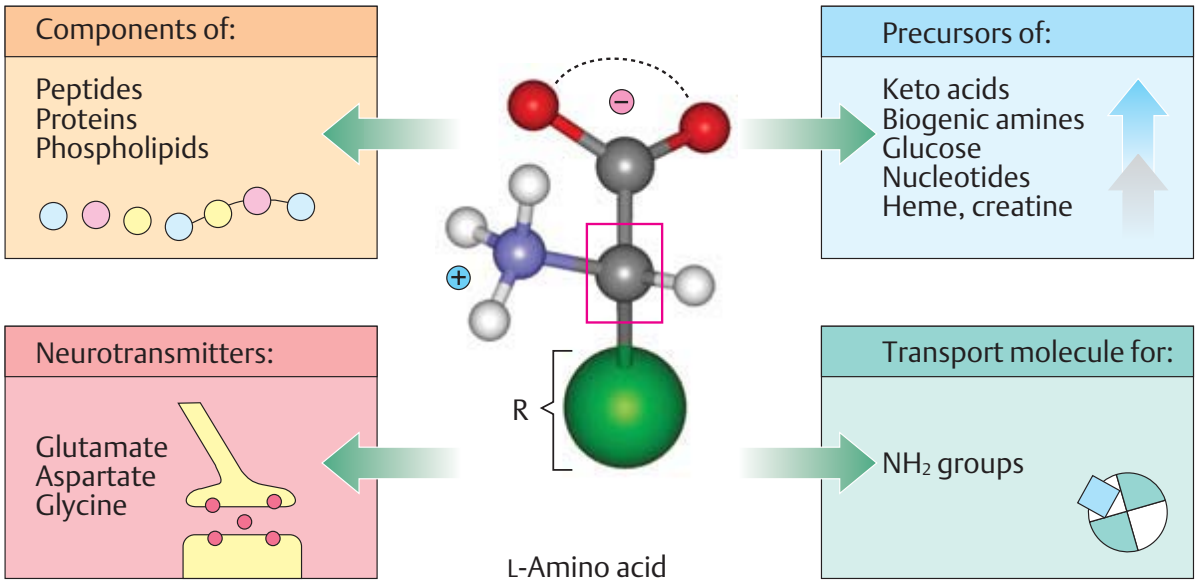
C. Dissociation curve of histidine ◑

All amino acids have at least two ionizable groups, and their net charge therefore depends on the pH value. The COOH groups at the α-C atom have pK_a values of between 1.8 and 2.8 and are therefore more acidic than simple monocarboxylic acids. The basicity of the α-amino function also varies, with pK_a values of between 8.8 and 10.6, depending on the amino acid. Acidic and basic amino acids have additional ionizable groups in their side chain. The pK_a values of these side chains are listed on p. 60. The electrical charges of peptides and proteins are mainly determined by groups in the side chains, as most α-carboxyl and α-amino functions are linked to peptide bonds (see p. 66).

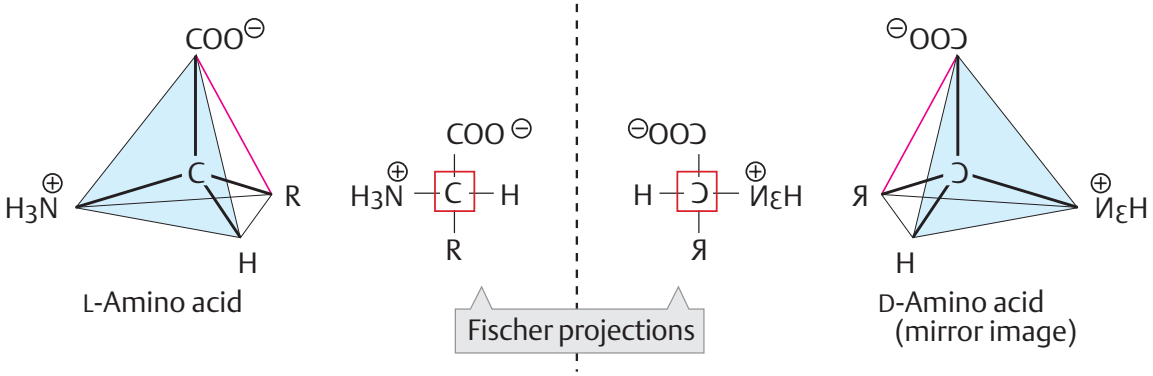
Histidine can be used here as an example of the pH-dependence of the net charge of an amino acid. In addition to the carboxyl group and the amino group at the α-C atom with pK_a values of 1.8 and 9.2, respectively, histidine also has an imidazole residue in its side chain with a pK_a value of 6.0. As the pH increases, the net charge (the sum of the positive and negative charges) therefore changes from +2 to –1. At pH 7.6, the net charge is zero, even though the molecule contains two almost completely ionized groups in these conditions. This pH value is called the **isoelectric point**.

At its isoelectric point, histidine is said to be **zwitterionic**, as it has both anionic *and* cationic properties. Most other amino acids are also zwitterionic at neutral pH. Peptides and proteins also have isoelectric points, which can vary widely depending on the composition of the amino acids.

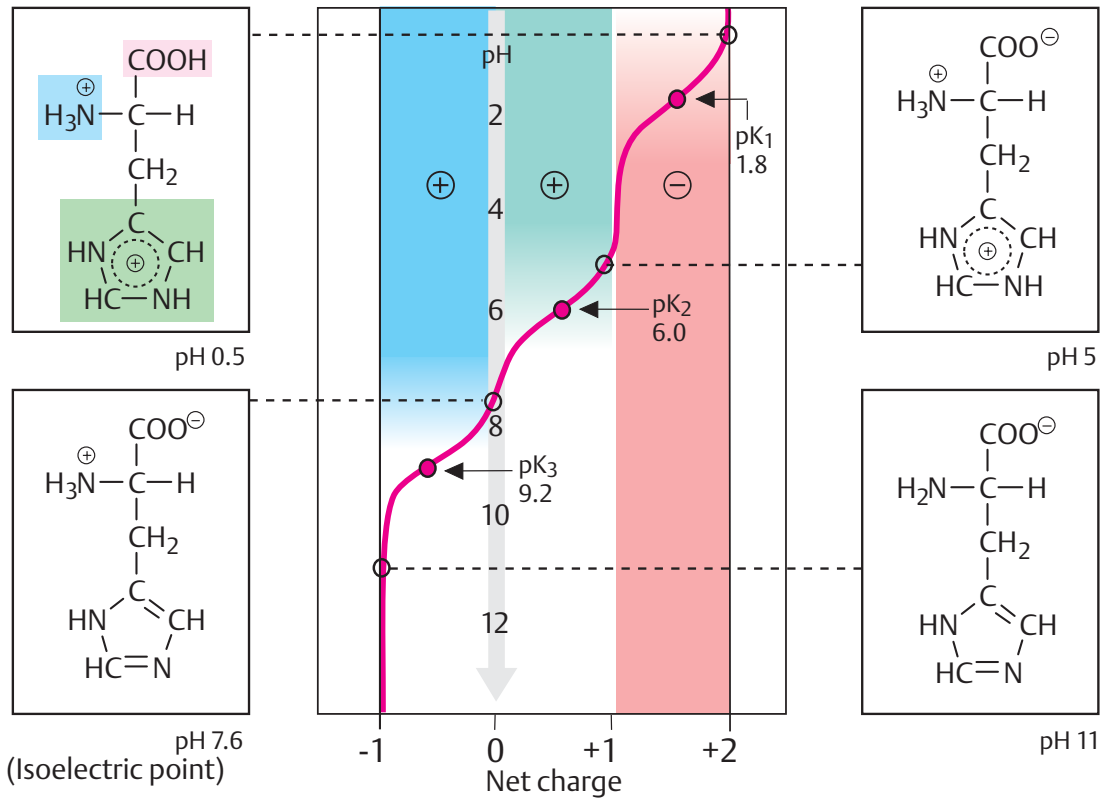
A. Amino acids: functions



B. Optical activity



C. Dissociation curve of histidine



Proteinogenic amino acids

A. The proteinogenic amino acids ◑

The amino acids that are included in the genetic code (see p. 248) are described as "proteinogenic." With a few exceptions (see p. 58), only these amino acids can be incorporated into proteins through *translation*. Only the side chains of the 20 proteinogenic amino acids are shown here. Their classification is based on the chemical structure of the side chains, on the one hand, and on their polarity on the other (see p. 6). The literature includes several slightly different systems for classifying amino acids, and details may differ from those in the system used here.

For each amino acid, the illustration names:
- *Membership of structural classes* I–VII (see below; e. g., III and VI for histidine)
- Name and abbreviation, formed from the first three letters of the name (e. g., histidine, His)
- The *one-letter symbol* introduced to save space in the electronic processing of sequence data (H for histidine)
- A quantitative *value for the polarity* of the side chain (bottom left; 10.3 for histidine). The more positive this value is, the *more polar* the amino acid is.

In addition, the polarity of the side chains is indicated by color. It increases from yellow, through light and dark green, to bluish green. For ionizing side chains, the corresponding pK_a values are also given (red numbers).

The **aliphatic** amino acids (class I) include *glycine, alanine, valine, leucine,* and *isoleucine*. These amino acids do not contain heteroatoms (N, O, or S) in their side chains and do not contain a ring system. Their side chains are markedly apolar. Together with threonine (see below), valine, leucine, and isoleucine form the group of *branched-chain amino acids*. The **sulfurcontaining amino acids** *cysteine* and *methionine* (class II), are also apolar. However, in the case of cysteine, this only applies to the undissociated state. Due to its ability to form disulfide bonds, cysteine plays an important role in the stabilization of proteins (see p. 72). Two cysteine residues linked by a disulfide bridge are referred to as *cystine* (not shown).

The **aromatic amino acids** (class III) contain resonancestabilized rings. In this group, only *phenylalanine* has strongly apolar properties. *Tyrosine* and *tryptophan* are moderately polar, and *histidine* is even strongly polar. The imidazole ring of histidine is already protonated at weakly acidic pH values. Histidine, which is only aromatic in protonated form (see p. 58), can therefore also be classified as a basic amino acid. Tyrosine and tryptophan show strong light absorption at wavelengths of 250–300 nm.

The **neutral** amino acids (class IV) have hydroxyl groups (*serine, threonine*) or amide groups (*asparagine, glutamine*). Despite their nonionic nature, the amide groups of asparagine and glutamine are markedly polar.

The carboxyl groups in the side chains of the **acidic** amino acids *aspartic acid* and *glutamic acid* (class V) are almost completely ionized at physiological pH values. The side chains of the **basic** amino acids *lysine* and *arginine* are also fully ionized—i. e., positively charged—at neutral pH. Arginine, with its positively charge guanidinium group, is particularly strongly basic, and therefore extremely polar.

Proline (VII) is a special case. Together with the α-C atom and the α-NH_2 group, its side chain forms a fivemembered ring. Its nitrogen atom is only weakly basic and is not protonated at physiological pH. Due to its ring structure, proline causes *bending of the peptide chain* in proteins (this is important in collagen, for example; see p. 70).

Several proteinogenic amino acids cannot be synthesized by the human organism, and therefore have to be supplied from the diet. These **essential amino acids** (see p. 360) are marked with a star in the illustration. Histidine and possibly also arginine are essential for infants and small children.

A. The proteinogenic amino acids

Aliphatic

Glycine (Gly, G)	Alanine (Ala, A)	Valine (Val, V) ☆	Leucine (Leu, L) ☆	Isoleucine (Ile, I) ☆
H	CH_3	$H_3C-CH(CH_3)$	$CH_2(CH(CH_3)_2)$	$H_3C-C^*H(CH_2CH_3)$
Polarity				
−2.4	−1.9	−2.0	−2.3	−2.2

Sulfur-containing

Cysteine (Cys, C)	Methionine (Met, M) ☆
CH_2SH	$CH_2CH_2SCH_3$
8.3 (pK_a value)	
−1.2	−1.5

Aromatic

Phenylalanine (Phe, F) ☆	Tyrosine (Tyr, Y)	Tryptophan (Trp, W) ☆
CH_2-phenyl	CH_2-phenyl-OH	CH_2-indole
	OH 10.1	Indole ring
+0.8	+6.1	+5.9

Cyclic

Proline (Pro, P)
Pyrrolidine ring
+6.0

Neutral

Serine (Ser, S)	Threonine (Thr, T) ☆
CH_2OH	$H_3C-C^*H(OH)$
+5.1	+4.9

☆ Essential amino acids

□ Chiral center

Neutral

Asparagine (Asn,N)	Glutamine (Gln, Q)
CH_2CONH_2	$CH_2CH_2CONH_2$
+9.7	+9.4

Acidic

Aspartic acid (Asp, D)	Glutamic acid (Glu, E)
CH_2COO^- 4.0	$CH_2CH_2COO^-$ 4.3
+11.0	+10.2

Basic

Histidine (His, H)	Lysine (Lys,K) ☆	Arginine (Arg, R)
Imidazole ring 6.0	$CH_2CH_2CH_2CH_2NH_3^+$ 10.8	$CH_2CH_2CH_2NHC(NH_2)_2^+$ 12.5
+10.3	+15.0	+20.0

Non-proteinogenic amino acids

In addition to the 20 proteinogenic amino acids (see p. 60), there are also many more compounds of the same type in nature. These arise during metabolic reactions (**A**) or as a result of enzymatic modifications of amino acid residues in peptides or proteins (**B**). The "biogenic amines" (**C**) are synthesized from α-amino acids by decarboxylation.

A. Rare amino acids ○

Only a few important representatives of the non-proteinogenic amino acids are mentioned here. The basic amino acid **ornithine** is an analogue of lysine with a shortened side chain. Transfer of a carbamoyl residue to ornithine yields **citrulline**. Both of these amino acids are intermediates in the urea cycle (see p. 182). **Dopa** (an acronym of 3,4-**d**ihydr**o**xy-**p**henyl**a**lanine) is synthesized by hydroxylation of tyrosine. It is an intermediate in the biosynthesis of catecholamines (see p. 352) and of melanin. It is in clinical use in the treatment of *Parkinson's disease*. **Selenocysteine**, a cysteine analogue, occurs as a component of a few proteins—e. g., in the enzyme glutathione peroxidase (see p. 284).

B. Post-translational protein modification ◑

Subsequent alteration of amino acid residues in finished peptides and proteins is referred to as *post-translational modification.* These reactions usually only involve polar amino acid residues, and they serve various purposes.

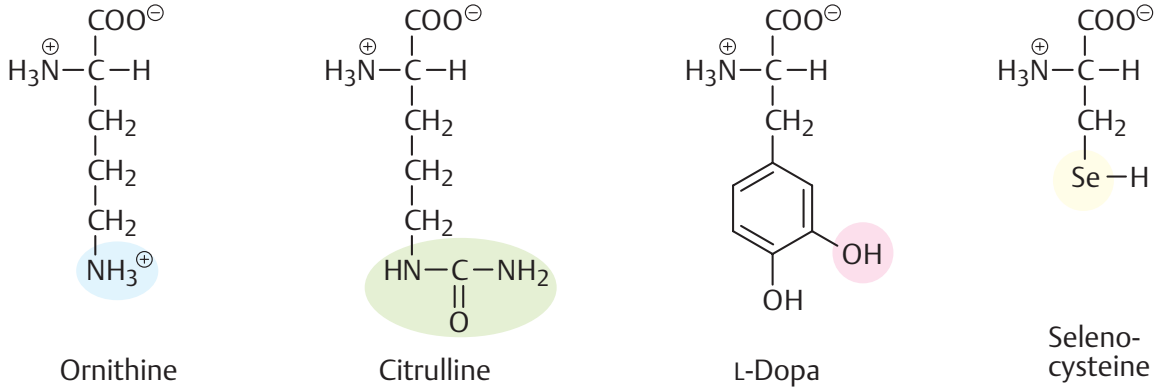
The free α-amino group at the *N*-terminus is blocked in many proteins by an acetyl residue or a longer acyl residue (**acylation**). *N*-terminal glutamate can cyclize into a pyroglutamate residue, while the *C*-terminal carboxylate group can be present in an amidated form (see TSH, p. 380). The side chains of serine and asparagine residues are often linked to oligosaccharides (**glycosylation**, see p. 230). **Phosphorylation** of proteins mainly affects serine and tyrosine residues. These reactions have mainly regulatory functions (see p. 114). Aspartate and histidine residues of enzymes are sometimes phosphorylated, too. A special modification of glutamate residues, **γ-carboxylation**, is found in coagulation factors. It is essential for blood coagulation (see p. 290). The ε-amino group of lysine residues is subject to a particularly large number of modifications. Its **acetylation** (or deacetylation) is an important mechanism for controlling genetic activity (see p. 244). Many coenzymes and cofactors are covalently linked to lysine residues. These include biotin (see p. 108), lipoic acid (see p. 106), and pyridoxal phosphate (see p. 108), as well as retinal (see p. 358). Covalent modification with **ubiquitin** marks proteins for breakdown (see p. 176). In collagen, lysine and proline residues are modified by **hydroxylation** to prepare for the formation of stable fibrils (see p. 70). Cysteine residues form **disulfide bonds** with one another (see p. 72). Cysteine **prenylation** serves to anchor proteins in membranes (see p. 214). Covalent bonding of a cysteine residue with heme occurs in cytochrome *c*. Flavins are sometimes covalently bound to cysteine or histidine residues of enzymes. Among the modifications of tyrosine residues, conversion into iodinated **thyroxine** (see p. 374) is particularly interesting.

C. Biogenic amines ◑

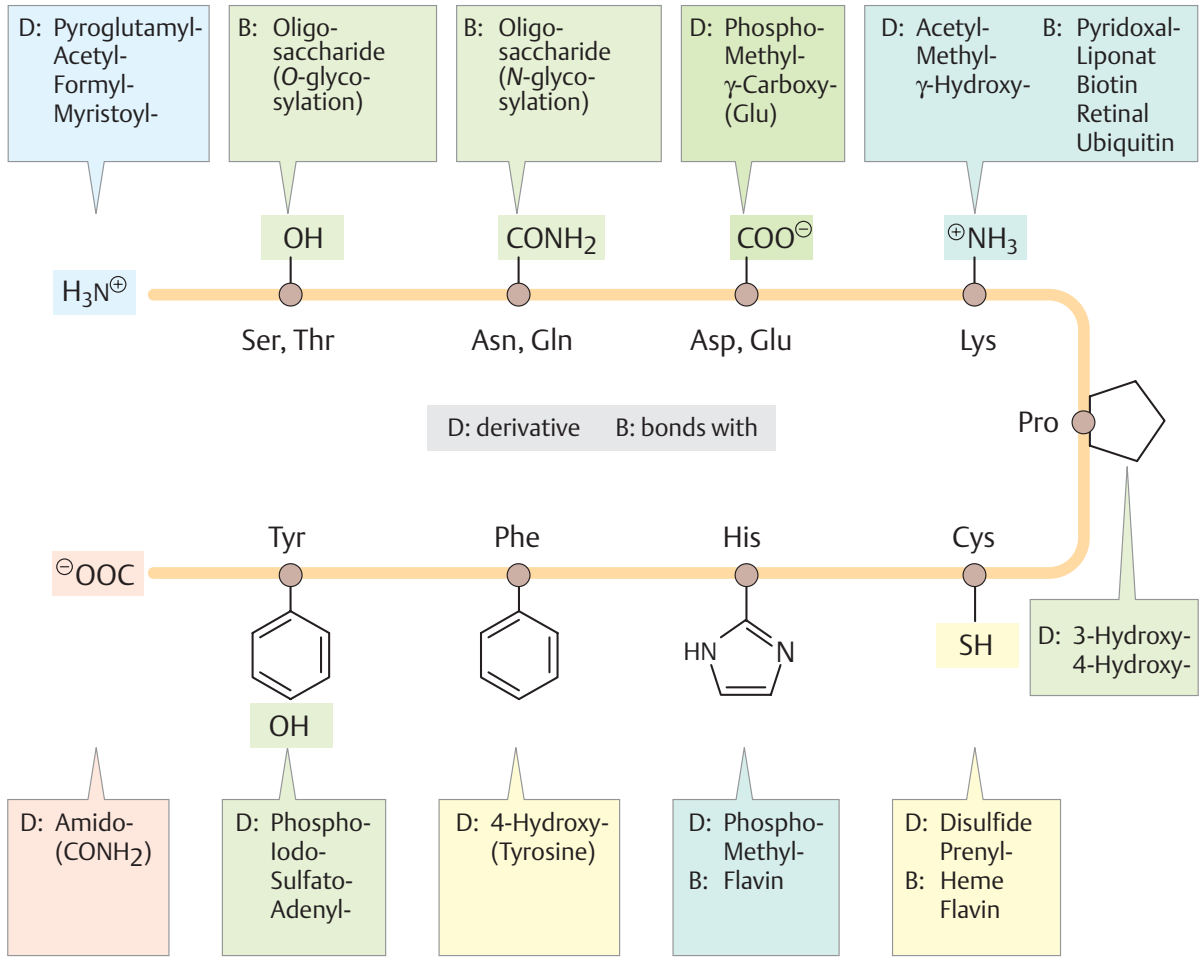
Several amino acids are broken down by *decarboxylation.* This reaction gives rise to what are known as biogenic amines, which have various functions. Some of them are **components of biomolecules**, such as *ethanolamine* in phospholipids (see p. 50). *Cysteamine* and *γ-alanine* are components of coenzyme A (see p. 12) and of pantetheine (see pp. 108, 168). Other amines function as signaling substances. An important **neurotransmitter** derived from glutamate is γ-aminobutyrate (GABA, see p. 356). The transmitter *dopamine* is also a precursor for the catecholamines epinephrine and norepinephrine (see p. 352). The biogenic amine *serotonin*, a substance that has many effects, is synthesized from tryptophan via the intermediate 5-hydroxytryptophan.

Monamines are inactivated into aldehydes by *amine oxidase* (monoamine oxidase, "MAO") with deamination and simultaneous oxidation. MAO inhibitors therefore play an important role in pharmacological interventions in neurotransmitter metabolism.

A. Rare amino acids



B. Post-translational protein modification



C. Biogenic amines

Amino acid	Amine	Function
Serine	Ethanol-amine	Glutamate
Cysteine	Cysteamine	Component of coenzyme A
Threonine	Amino-propanol	Component of vitamin B_{12}
Aspartate	β-Alanine	Component of coenzyme A

Amino acid	Amine	Function
Glutamate	γ-Amino-butyrate	Neurotrans-mitter (GABA)
Histidine	Histamine	Mediator, neuro-transmitter
Dopa	Dopamine	Neurotransmitter
5-Hydroxy-tryptophan	Serotonin	Mediator, neuro-transmitter

Peptides and proteins: overview

A. Proteins ◑

When amino acids are linked together by acid–amide bonds, linear macromolecules (peptides) are produced. Those containing more than ca. 100 amino acid residues are described as **proteins** (polypeptides). Every organism contains thousands of different proteins, which have a variety of functions. At a magnification of ca. 1.5 million, the semischematic illustration shows the structures of a few intra and extracellular proteins, giving an impression of their variety. The functions of proteins can be classified as follows.

Establishment and maintenance of structure. Structural proteins are responsible for the *shape and stability* of cells and tissues. A small part of a **collagen** molecule is shown as an example (right; see p. 70). The complete molecule is 1.5 300 nm in size, and at the magnification used here it would be as long as three pages of the book. **Histones** are also structural proteins. They organize the arrangement of DNA in chromatin. The basic components of chromatin, the *nucleosomes* (top right; see p. 218) consist of an octameric complex of histones, around which the DNA is coiled.

Transport. A wellknown transport protein is **hemoglobin** in the erythrocytes (bottom left). It is responsible for the transport of oxygen and carbon dioxide between the lungs and tissues (see p. 282). The blood plasma also contains many other proteins with transport functions. **Prealbumin** (transthyretin; middle), for example, transports the thyroid hormones thyroxin and triiodothyronine. **Ion channels** and other integral membrane proteins (see p. 220) facilitate the transport of ions and metabolites across biological membranes.

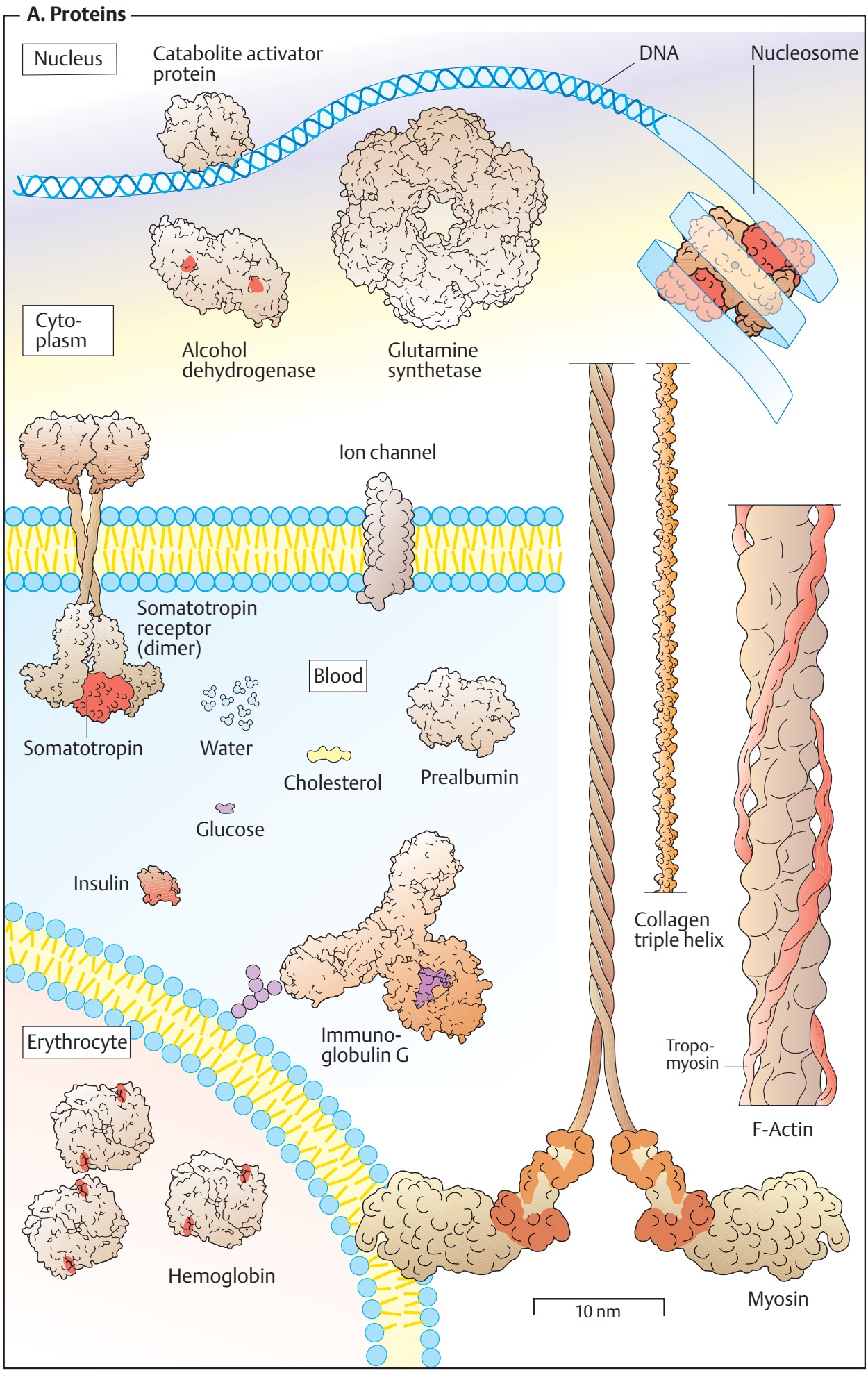
Protection and defense. The immune system protects the body from pathogens and foreign substances. An important component of this system is **immunoglobulin G** (bottom left; see p. 300). The molecule shown here is bound to an erythrocyte by complex formation with surface glycolipids (see p. 292).

Control and regulation. In biochemical signal chains, proteins function as signaling substances (hormones) and as hormone receptors. The complex between the growth hormone **somatotropin** and its **receptor** is shown here as an example (middle). Here, the extracellular domains of two receptor molecules here bind one molecule of the hormone. This binding activates the cytoplasmic domains of the complex, leading to further conduction of the signal to the interior of the cell (see p. 384). The small peptide hormone **insulin** is discussed in detail elsewhere (see pp. 76, 160). DNA-binding proteins (*transcription factors;* see p. 118) are decisively involved in regulating the metabolism and in differentiation processes. The structure and function of the **catabolite activator protein** (top left) and similar bacterial transcription factors have been particularly well investigated.

Catalysis. *Enzymes,* with more than 2000 known representatives, are the largest group of proteins in terms of numbers (see p. 88). The smallest enzymes have molecular masses of 10–15 kDa. Intermediatesized enzymes, such as **alcohol dehydrogenase** (top left) are around 100–200 kDa, and the largest—including **glutamine synthetase** with its 12 monomers (top right)—can reach more than 500 kDa.

Movement. The interaction between actin and myosin is responsible for muscle contraction and cell movement (see p. 332). **Myosin** (right), with a length of over 150 nm, is among the largest proteins there are. Actin filaments (**F-actin**) arise due to the polymerization of relatively small protein subunits (G-actin). Along with other proteins, **tropomyosin,** which is associated with F-actin, controls contraction.

Storage. Plants contain special **storage proteins**, which are also important for human nutrition (not shown). In animals, *muscle proteins* constitute a nutrient reserve that can be mobilized in emergencies.



Peptide bonds

A. Peptide bond ◑

The amino acid components of peptides and proteins are linked together by *amide* bonds (see p. 60) between α-carboxyl and α-amino groups. This type of bonding is therefore also known as **peptide bonding**. In the **dipeptide** shown here, the serine residue has a free ammonium group, while the carboxylate group in alanine is free. Since the amino acid with the free NH_3^+ group is named first, the peptide is known as **seryl alanine**, or in abbreviated form Ser-Ala or SA.

B. Resonance ◑

Like all acid–amide bonds, the peptide bond is **stabilized by resonance** (see p. 4). In the conventional notation (top right) it is represented as a combination of a C=O double bond with a C–N single bond. However, a C=N double bond with charges at O and N could also be written (middle). Both of these are only extreme cases of electron distribution, known as *resonance structures*. In reality, the π electrons are *delocalized* throughout all the atoms (bottom). As a mesomeric system, the peptide bond is *planar*. Rotation around the C–N bond would only be possible at the expense of large amounts of energy, and the bond is therefore *not freely rotatable*. Rotations are only possible around the single bonds marked with arrows. The state of these is expressed using the angles ϕ and ψ (see **D**). The plane in which the atoms of the peptide bond lie is highlighted in light blue here and on the following pages.

C. Peptide nomenclature ◑

Peptide chains have a *direction* and therefore two different ends. The amino terminus (**N terminus**) of a peptide has a free ammonium group, while the carboxy terminus (**C terminus**) is formed by the carboxylate group of the last amino acid. In peptides and proteins, the amino acid components are usually linked in linear fashion. To express the **sequence** of a peptide, it is therefore suf cient to combine the three-letter or single-letter abbreviations for the amino acid residues (see p. 60). This sequence always starts at the N terminus. For example, the peptide hormone *angiotensin II* (see p. 330) has the sequence Asp-Arg-Val-Tyr-Ile-His-Pro-Phe, or DRVYIHPF.

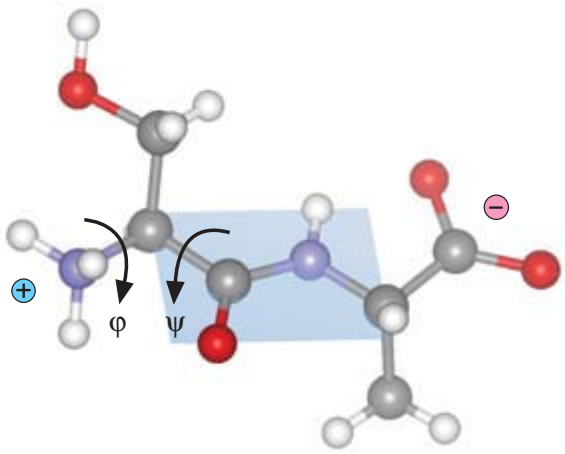
D. Conformational space of the peptide chain ○

With the exception of the terminal residues, every amino acid in a peptide is involved in *two* peptide bonds (one with the preceding residue and one with the following one). Due to the restricted rotation around the C–N bond, rotations are only possible around the N–C_α and C_α–C bonds (**2**). As mentioned above, these rotations are described by the dihedral angles ϕ (phi) and ψ (psi). The angle describes rotation around the N–C_α bond; ψ describes rotation around C_α–C—i. e., the position of the subsequent bond.

For steric reasons, only specific combinations of the dihedral angles are possible. These relationships can be illustrated clearly by a so-called ϕ/ψ *diagram* (**1**). Most combinations of ϕ and ψ are sterically "forbidden" (red areas). For example, the combination ϕ = 0° and ψ = 180° (**4**) would place the two carbonyl oxygen atoms less than 115 pm apart—i. e., at a distance much smaller than the sum of their van der Waals radii (see p. 6). Similarly, in the case of ϕ = 180° and ψ = 0° (**5**), the two NH hydrogen atoms would collide. The combinations located within the green areas are the only ones that are sterically feasible (e. g., **2** and **3**). The important secondary structures that are discussed in the following pages are also located in these areas. The conformations located in the yellow areas are energetically less favorable, but still possible.

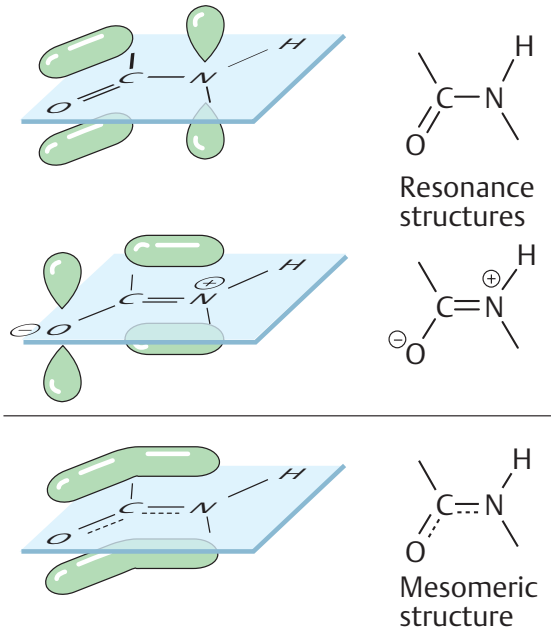
The ϕ/ψ diagram (also known as a **Ramachandran plot**) was developed from modeling studies of small peptides. However, the conformations of most of the amino acids in proteins are also located in the permitted areas. The corresponding data for the small protein, insulin (see p. 76), are represented by black dots in **1**.

A. Peptide bonds

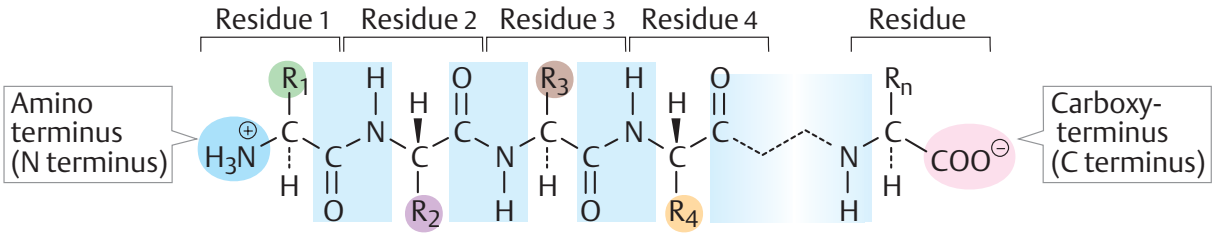


Seryl alanine
(Ser-Ala, $^{\oplus}H_3N$-Ser-Ala-$COO^{\ominus}$, SA)

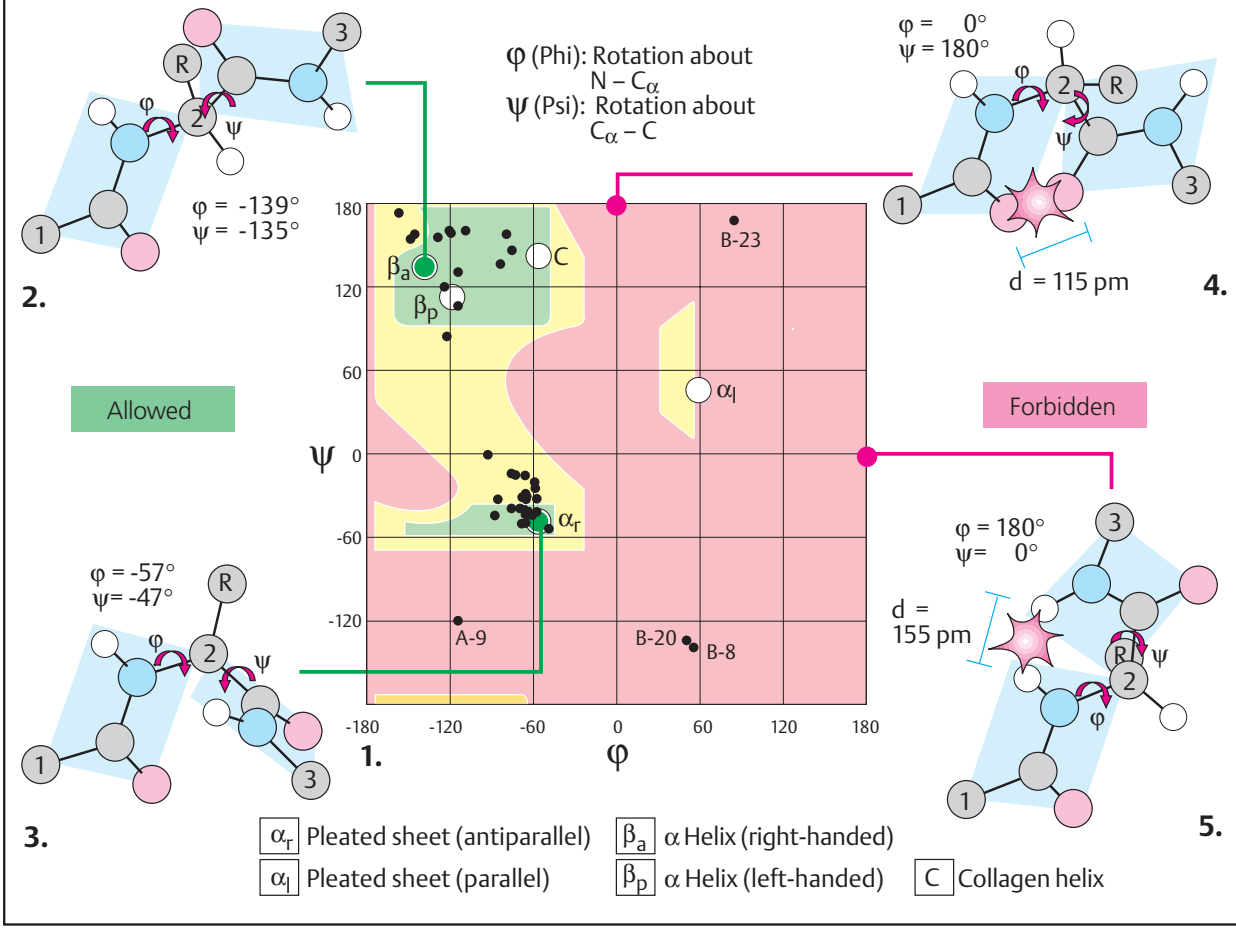
B. Resonance



C. Peptide nomenclature



D. Conformation space of the peptide chain



Secondary structures

In proteins, specific combinations of the dihedral angles ϕ and ψ (see p. 66) are much more common than others. When several successive residues adopt one of these conformations, defined **secondary structures** arise, which are stabilized by hydrogen bonds either within the peptide chain or between neighboring chains. When a large part of a protein takes on a defined secondary structure, the protein often forms mechanically stable filaments or fibers. **Structural proteins** of this type (see p. 70) usually have characteristic amino acid compositions.

The most important secondary structural elements of proteins are discussed here first. The illustrations only show the course of the peptide chain; the side chains are omitted. To make the course of the chains clearer, the levels of the peptide bonds are shown as blue planes. The dihedral angles of the structures shown here are also marked in diagram D1 on p. 67.

A. α-Helix ◑

The **right-handed** α-helix (α_R) is one of the most common secondary structures. In this conformation, the peptide chain is wound like a screw. Each turn of the screw (the screw axis in shown in orange) covers approximately 3.6 amino acid residues. The *pitch* of the screw (i. e., the smallest distance between two equivalent points) is 0.54 nm. α-Helices are stabilized by almost linear *hydrogen bonds* between the NH and CO groups of residues, which are four positions apart from each another in the sequence (indicated by red dots; see p. 6). In longer helices, most amino acid residues thus enter into *two* H bonds. Apolar or amphipathic α-helices with five to seven turns often serve to anchor proteins in biological membranes (*transmembrane helices;* see p. 214).

The mirror image of the α_R helix, the **left-handed α-helix** (α_L), is rarely found in nature, although it would be energetically "permissible."

B. Collagen helix ◑

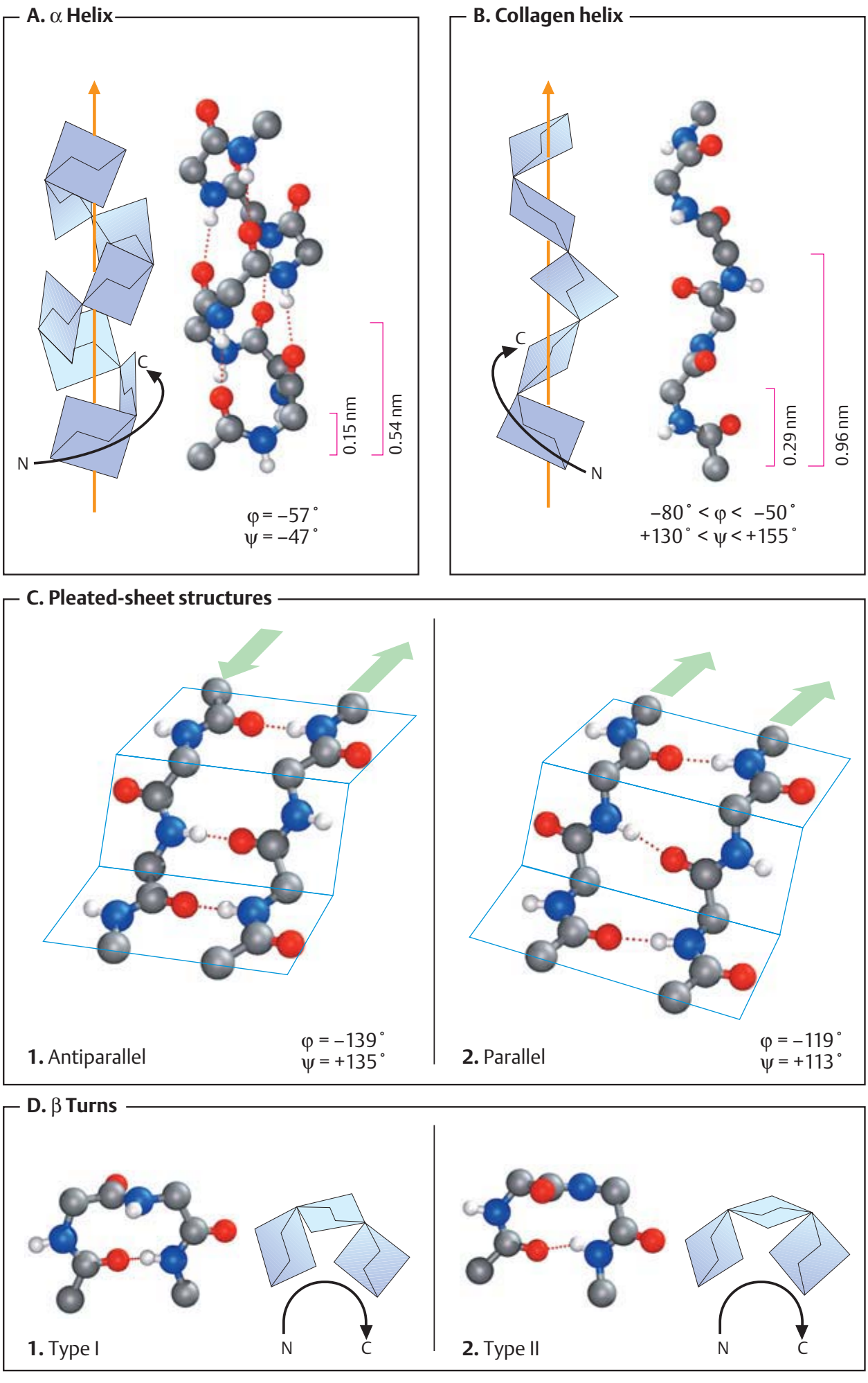
Another type of helix occurs in the collagens, which are important constituents of the connectivetissue matrix (see pp. 70, 344). The **collagen helix** is **left-handed**, and with a pitch of 0.96 nm and 3.3 residues per turn, it is steeper than the α-helix. In contrast to the α-helix, H bonds are not possible *within* the collagen helix. However, the conformation is stabilized by the association of three helices to form a righthanded **collagen triple helix** (see p. 70).

C. Pleated-sheet structures ◑

Two additional, almost stretched, conformations of the peptide chain are known as **β pleated sheets**, as the peptide planes are arranged like a regularly folded sheet of paper. Again, H bonds can only form between *neighboring chains* ("strands") in pleated sheets. When the two strands run in opposite directions (**1**), the structure is referred to as an **antiparallel pleated sheet** (β_a). When they run in the same direction (**2**), it is a **parallel pleated sheet** (β_p). In both cases, the α-C atoms occupy the highest and lowest points in the structure, and the side chains point alternately straight up or straight down (see p. 71 C). The β_a structure, with its almost linear H bonds, is energetically more favorable. In extended pleated sheets, the individual strands of the sheet are usually not parallel, but twisted relative to one another (see p. 74).

D. β Turns ◑

β Turns are often found at sites where the peptide chain changes direction. These are sections in which four amino acid residues are arranged in such a way that the course of the chain reverses by about 180° into the opposite direction. The two turns shown (types I and II) are particularly frequent. Both are stabilized by hydrogen bonds between residues 1 and 4. β Turns are often located between the individual strands of antiparallel pleated sheets, or between strands of pleated sheets and α helices.



Structural proteins

The **structural proteins** give extracellular structures mechanical stability, and are involved in the structure of the cytoskeleton (see p. 204). Most of these proteins contain a high percentage of specific secondary structures (see p. 68). For this reason, the amino acid composition of many structural proteins is also characteristic (see below).

A. α Keratin ○

α-Keratin is a structural protein that predominantly consists of α helices. Hair (wool), feathers, nails, claws and the hooves of animals consist largely of keratin. It is also an important component of the cytoskeleton (cytokeratin), where it appears in intermediate filaments (see p. 204).

In the keratins, large parts of the peptide chain show right-handed α-helical coiling. Two chains each form a left-handed **superhelix**, as is also seen in myosin (see p. 65). The superhelical keratin dimers join to form tetramers, and these aggregate further to form **protofilaments**, with a diameter of 3 nm. Finally, eight protofilaments then form an **intermediate filament**, with a diameter of 10 nm (see p. 204).

Similar keratin filaments are found in **hair**. In a single wool fiber with a diameter of about 20 μm, millions of filaments are bundled together within dead cells. The individual keratin helices are cross-linked and stabilized by numerous disulfide bonds (see p. 72). This fact is exploited in the *perming* of hair. Initially, the disulfide bonds of hair keratin are disrupted by reduction with thiol compounds (see p. 8). The hair is then styled in the desired shape and heat-dried. In the process, new disulfide bonds are formed by oxidation, which maintain the hairstyle for some time.

B. Collagen ◑

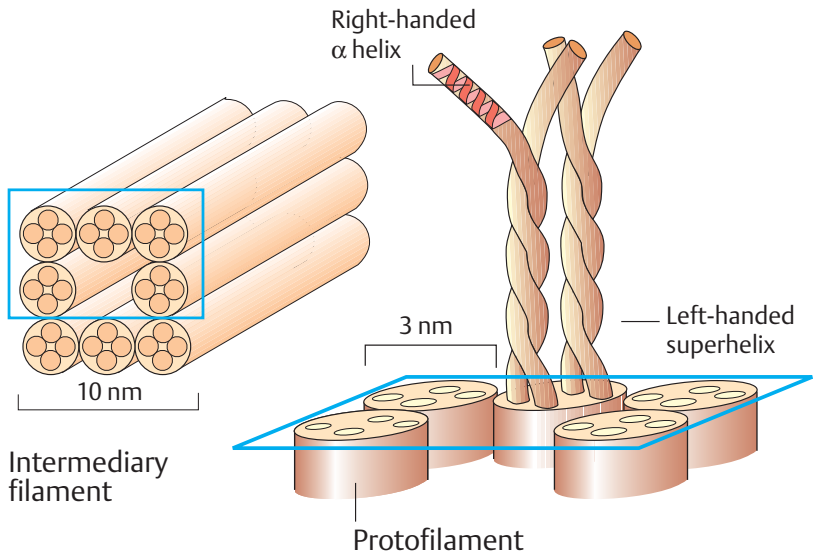
Collagen is the quantitatively most important protein in mammals, making up about 25% of the total protein. There are many different types of collagen, particularly in connective tissue. Collagen has an unusual amino acid composition. Approximately one-third of the amino acids are *glycine* (Gly), about 10% *proline* (Pro), and 10% *hydroxyproline* (Hyp). The two latter amino acids are only formed during collagen biosynthesis as a result of *posttranslational modification* (see p. 344).

The triplet Gly-X-Y (**2**) is constantly repeated in the sequence of collagen, with the X position often being occupied by Pro and the Y position by Hyp. The reason for this is that collagen is largely present as a **triple helix** made up of three individual collagen helices (**1**). In triple helices, every third residue lies on the inside of the molecule, where for steric reasons there is only room for glycine residues (**3**; the glycine residues are shown in yellow). Only a small section of a triple helix is illustrated here. The complete collagen molecule is approximately 300 nm long.

C. Silk fibroin ○

Silk is produced from the spun threads from silkworms (the larvae of the moth *Bombyx mori* and related species). The main protein in silk, **fibroin**, consists of antiparallel *pleated sheet structures* arranged one on top of the other in numerous layers (**1**). Since the amino acid side chains in pleated sheets point either straight up or straight down (see p. 68), only compact side chains fit between the layers. In fact, more than 80% of fibroin consists of glycine, alanine, and serine, the three amino acids with the shortest side chains. A typical repetitive amino acid sequence is *(Gly-Ala-Gly-Ala-Gly-Ser).* The individual pleated sheet layers in fibroin are found to lie alternately 0.35 nm and 0.57 nm apart. In the first case, only glycine residues (R = H) are opposed to one another. The slightly greater distance of 0.57 nm results from repulsion forces between the side chains of alanine and serine residues (**2**).

A. α-Keratin

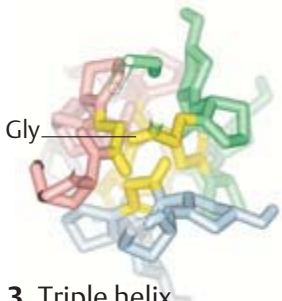


B. Collagen

1. Triple helix (section)

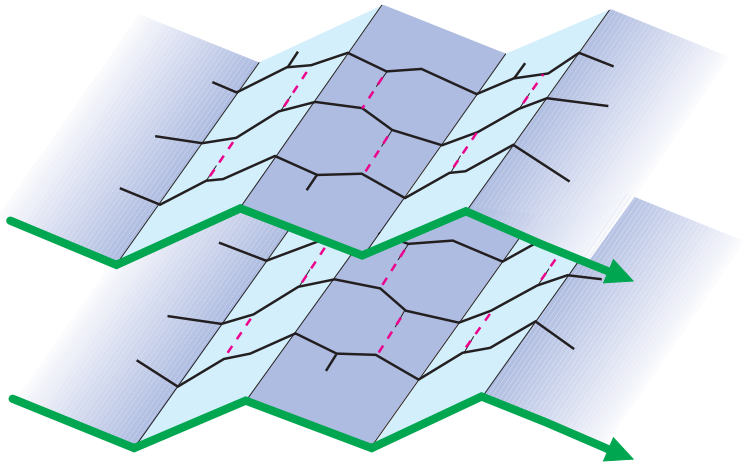
Gly	Arg	Hyp
Gly	Gln	Arg
Gly	Pro	Hyp
Gly	Pro	Gln
Gly	Ala	Arg
Gly	X	Y

2. Typical sequence

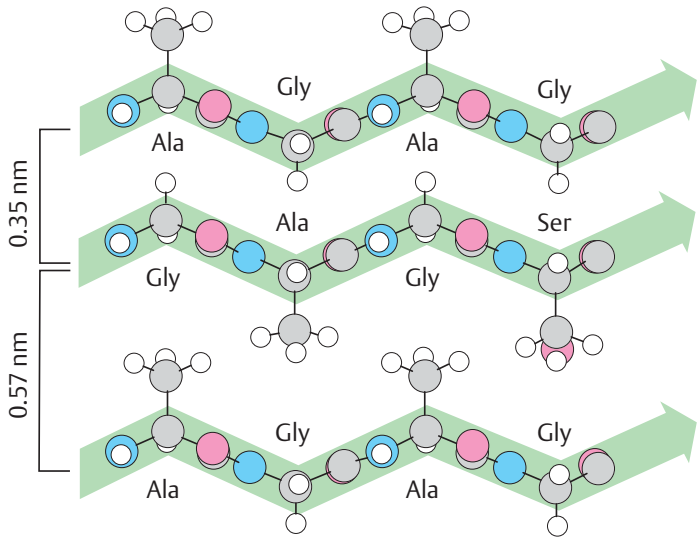


3. Triple helix (view from above)

C. Silk fibroin



1. Spatial illustration



2. Front view

Globular proteins

Soluble proteins have a more complex structure than the fibrous, completely insoluble structural proteins. The shape of soluble proteins is more or less spherical (globular). In their biologically active form, **globular proteins** have a defined spatial structure (the **native conformation**). If this structure is destroyed (**denaturation**; see p. 74), not only does the biological effect disappear, but the protein also usually precipitates in insoluble form. This happens, for example, when eggs are boiled; the proteins dissolved in the egg white are denatured by the heat and produce the solid egg white.

To illustrate protein conformations in a clear (but extremely simplified) way, *Richardson diagrams* are often used. In these diagrams, α-helices are symbolized by red cylinders or spirals and strands of pleated sheets by green arrows. Less structured areas of the chain, including the β-turns, are shown as sections of gray tubing.

A. Conformation-stabilizing interactions ◑

The native conformation of proteins is stabilized by a number of different interactions. Among these, only the **disulfide bonds** (**B**) represent covalent bonds. **Hydrogen bonds**, which can form inside secondary structures, as well as between more distant residues, are involved in all proteins (see p. 6). Many proteins are also stabilized by **complex formation** with metal ions (see pp. 76, 342, and 378, for example). The **hydrophobic effect** is particularly important for protein stability. In globular proteins, most hydrophobic amino acid residues are arranged in the interior of the structure in the native conformation, while the polar amino acids are mainly found on the surface (see pp. 28, 76).

B. Disulfide bonds ◑

Disulfide bonds arise when the SH groups of two cysteine residues are covalently linked as a dithiol by oxidation. Bonds of this type are only found (with a few exceptions) in extracellular proteins, because in the interior of the cell *glutathione* (see p. 284) and other reducing compounds are present in such high concentrations that disulfides would be reductively cleaved again. The small plant protein *crambin* (46 amino acids) contains three disulfide bonds and is therefore very stable. The high degree of stability of insulin (see p. 76) has a similar reason.

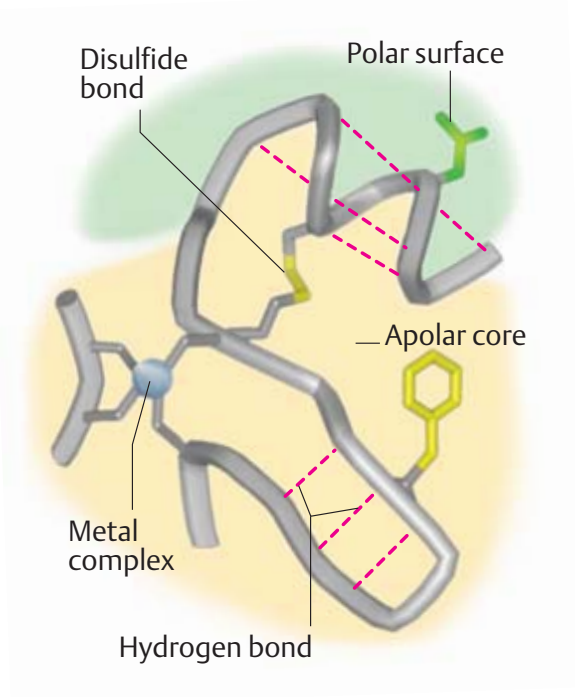
C. Protein dynamics ◑

The conformations of globular proteins are not rigid, but can change dramatically on binding of ligands or in contact with other proteins. For example, the enzyme *adenylate kinase* (see p. 336) has a mobile domain (domain = independently folded partial structure), which folds shut after binding of the substrate (yellow). The larger domain (bottom) also markedly alters its conformation. There are large numbers of **allosteric proteins** of this type. This group includes, for example, *hemoglobin* (see p. 280), *calmodulin* (see p. 386), and many allosteric enzymes such as *aspartate carbamoyltransferase* (see p. 116).

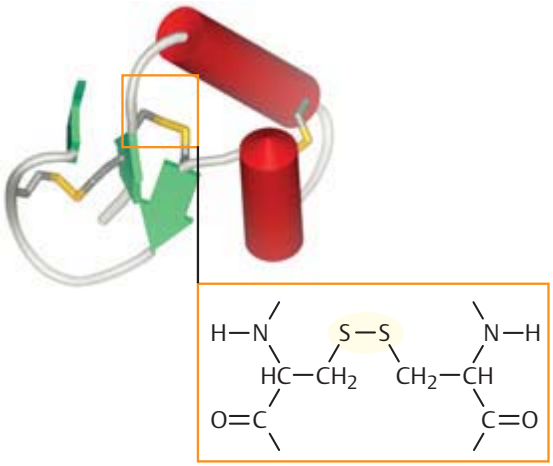
D. Folding patterns ○

The globular proteins show a high degree of variability in folding of their peptide chains. Only a few examples are shown here. Purely helically folded proteins such as *myoglobin* (**1**; see p. 74, heme yellow) are rare. In general, pleated sheet and helical elements exist alongside each other. In the hormone-binding domain of the *estrogen receptor* (**2**; see p. 378), a small, two-stranded pleated sheet functions as a "cover" for the hormone binding site (estradiol yellow). In *flavodoxin,* a small flavoprotein with a redox function (**3**; FMN yellow), a fan-shaped, pleated sheet made up of five parallel strands forms the core of the molecule. The conformation of the β subunit of the G-protein *transducin* (**4**; see pp. 224, 358) is very unusual. Seven pleated sheets form a large, symmetrical "β propeller." The *N*-terminal section of the protein contains one long and one short helix.

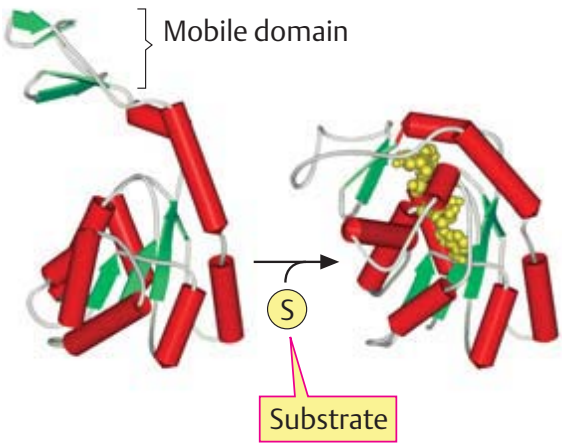
A. Conformation-stabilizing interactions



B. Disulfide bonds



C. Protein dynamics

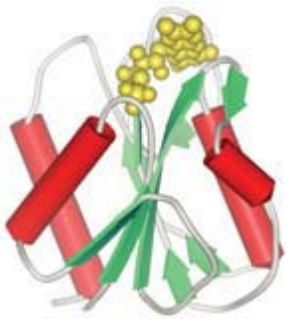


Adenylate kinase

D. Folding patterns

1. Myoglobin

2. Estrogen receptor (domain)



3. Flavodoxin

4. Transducin (β subunit)

Protein folding

Information about the biologically active (*native*) conformation of proteins is already encoded in their amino acid sequences. The native forms of many proteins arise spontaneously in the test tube and within a few minutes. Nevertheless, there are special auxiliary proteins (chaperonines) that support the folding of other proteins in the conditions present within the cell (see p. 232). An important goal of biochemistry is to understand the laws governing **protein folding**. This would make it possible to predict the conformation of a protein from the easily accessible DNA sequence (see p. 260).

A. Folding and denaturation of ribonuclease A ◑

The **folding** of proteins to the native form is favored under physiological conditions. The native conformation is lost, as the result of **denaturation**, at extreme pH values, at high temperatures, and in the presence of organic solvents, detergents, and other denaturing substances, such as urea.

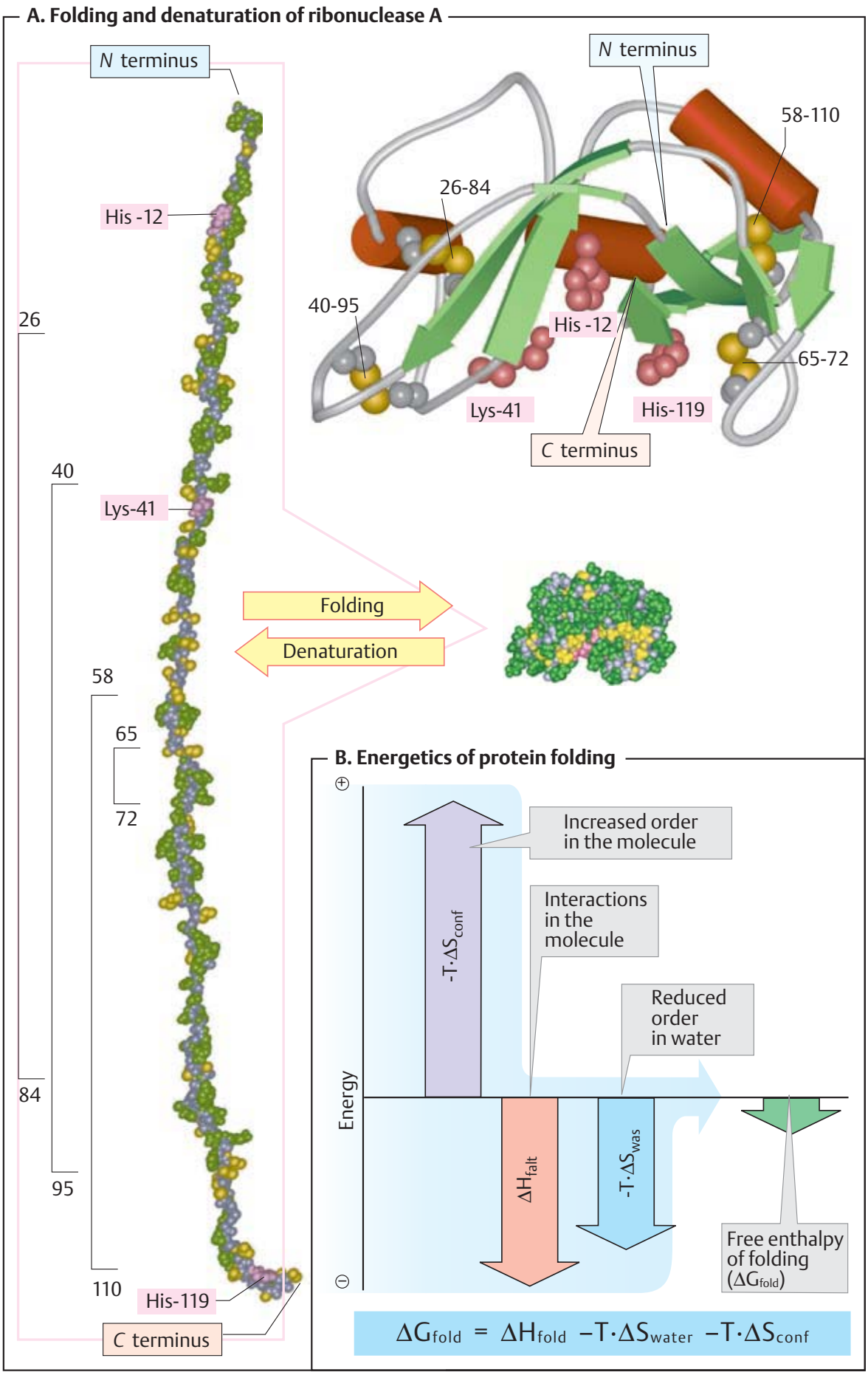
The fact that a denatured protein can spontaneously return to its native conformation was demonstrated for the first time with **ribonuclease**, a digestive enzyme (see p. 266) consisting of 124 amino acids. In the native form (top right), there are extensive pleated sheet structures and three α helices. The eight cysteine residues of the protein are forming four disulfide bonds. Residues His-12, Lys-41 and His-119 (pink) are particularly important for catalysis. Together with additional amino acids, they form the enzyme's *active center.*

The disulfide bonds can be reductively cleaved by *thiols* (e.g., mercaptoethanol, $HO\text{-}CH_2\text{-}CH_2\text{-}SH$). If *urea* at a high concentration is also added, the protein unfolds completely. In this form (left), it is up to 35 nm long. Polar (green) and apolar (yellow) side chains are distributed randomly. The denatured enzyme is completely inactive, because the catalytically important amino acids (pink) are too far away from each other to be able to interact with each other and with the substrate.

When the urea and thiol are removed by dialysis (see p. 78), secondary and tertiary structures develop again spontaneously. The cysteine residues thus return to a suf ciently close spatial vicinity that disulfide bonds can once again form under the oxidative effect of atmospheric oxygen. The active center also reestablishes itself. In comparison with the denatured protein, the native form is astonishingly compact, at 4.5 2.5 nm. In this state, the apolar side chains (yellow) predominate in the interior of the protein, while the polar residues are mainly found on the surface. This distribution is due to the "hydrophobic effect" (see p. 28), and it makes a vital contribution to the stability of the native conformation (**B**).

B. Energetics of protein folding ○

The **energetics** of protein folding are not at present satisfactorily understood. Only a simplified model is discussed here. The conformation of a molecule is stable in any given conditions if the change in its free enthalpy during folding (ΔG_{fold}) is negative (see p. 16). The magnitude of the folding enthalpy is affected by several factors. The main factor working *against* folding is the strong increase in the ordering of the molecule involved. As discussed on p. 20, this leads to a negative change in entropy of ΔS_{conf} and therefore to a strongly positive entropy term $-T\ \Delta S$ (violet arrow). By contrast, the covalent and noncovalent bonds in the interior of the protein have a *stabilizing* influence. For this reason, the change in folding enthalpy ΔH_{fold} is negative (red arrow). A third factor is the change in the system's entropy due to the hydrophobic effect. During folding, the degree of order in the *surrounding water* decreases—i.e., ΔS_{water} is positive and therefore $-T\ \Delta S$ is negative (blue arrow). When the sum of these effects is negative (green arrow), the protein folds spontaneously into its native conformation.



Molecular models: insulin

The opposite page presents models of insulin, a small protein. The biosynthesis and function of this important hormone are discussed elsewhere in this book (pp. 160, 388). Monomeric insulin consists of 51 amino acids, and with a molecular mass of 5.5 kDa it is only half the size of the smallest enzymes. Nevertheless, it has the typical properties of a globular protein.

Large quantities of pure insulin are required for the treatment of *diabetes mellitus* (see p. 160). The annual requirement for insulin is over 500 kg in a country the size of Germany. Formerly, the hormone had to be obtained from the pancreas of slaughtered animals in a complicated and expensive procedure. **Human insulin**, which is produced by *overexpression* in genetically engineered bacteria, is now mainly used (see p. 262).

A. Structure of insulin ○

There are various different structural levels in proteins, and these can be briefly discussed again here using the example of insulin.

The **primary structure** of a protein is its amino acid sequence. During the biosynthesis of insulin in the pancreas, a continuous peptide chain with 84 residues is first synthesized—*proinsulin* (see p. 160). After folding of the molecule, the three disulfide bonds are first formed, and residues 31 to 63 are then proteolytically cleaved releasing the so-called *C peptide*. The molecule that is left over (**1**) now consists of two peptide chains, the *A chain* (21 residues, shown in yellow) and the *B chain* (30 residues, orange). One of the disulfide bonds is located inside the A chain, and the two others link the two chains together.

Secondary structures are regions of the peptide chain with a defined conformation (see p. 68) that are stabilized by H-bonds. In insulin (**2**), the α-helical areas are predominant, making up 57% of the molecule; 6% consists of β-pleated-sheet structures, and 10% of β-turns, while the remainder (27%) cannot be assigned to any of the secondary structures.

The three-dimensional conformation of a protein, made up of secondary structural elements and unordered sections, is referred to as the **tertiary structure**. In insulin, it is compact and wedge-shaped (**B**). The tip of the wedge is formed by the B chain, which changes its direction at this point.

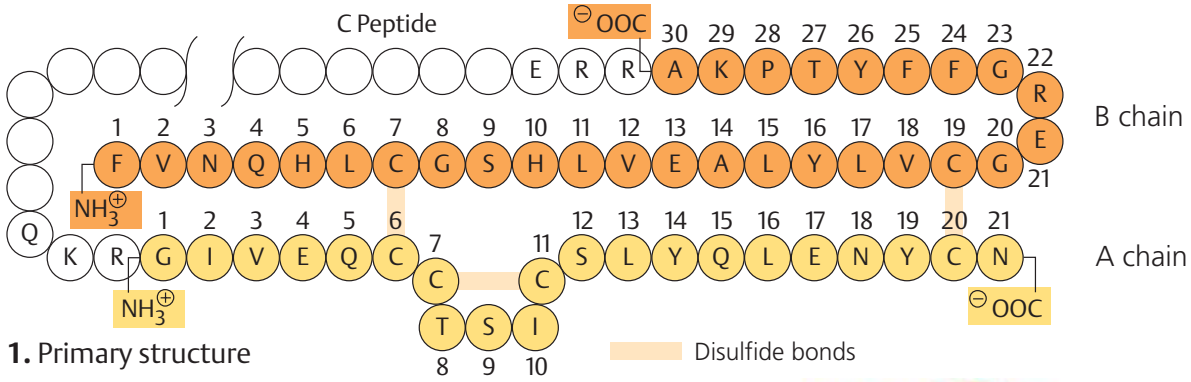
Quaternary structure. Due to non-covalent interactions, many proteins assemble to form symmetrical complexes (oligomers). The individual components of oligomeric proteins (usually 2–12) are termed *subunits* or *monomers*. Insulin also forms quaternary structures. In the blood, it is partly present as a dimer. In addition, there are also hexamers stabilized by Zn^{2+} ions (light blue) (**3**), which represent the form in which insulin is stored in the pancreas (see p. 160).

B. Insulin (monomer) ○

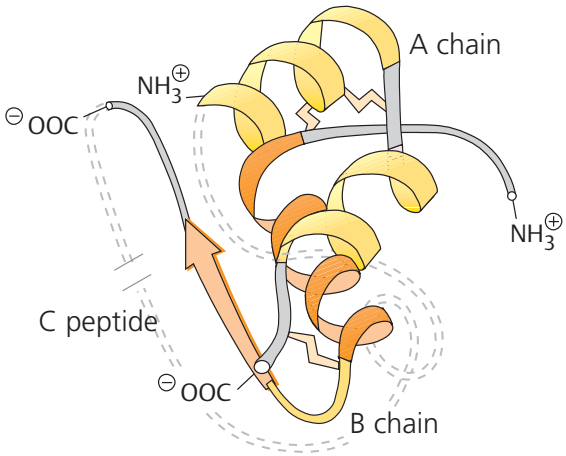
The van der Waals model of monomeric insulin (**1**) once again shows the wedge-shaped tertiary structure formed by the two chains together. In the second model (**3**, bottom), the side chains of polar amino acids are shown in blue, while apolar residues are yellow or pink. This model emphasizes the importance of the "hydrophobic effect" for protein folding (see p. 74). In insulin as well, most hydrophobic side chains are located on the inside of the molecule, while the hydrophilic residues are located on the surface. Apparently in contradiction to this rule, several apolar side chains (pink) are found on the surface. However, all of these residues are involved in hydrophobic interactions that stabilize the dimeric and hexameric forms of insulin.

In the third model (**2**, right), the colored residues are those that are located on the surface and occur *invariably* (red) or *almost invariably* (orange) in all known insulins. It is assumed that amino acid residues that are not replaced by other residues during the course of evolution are essential for the protein's function. In the case of insulin, almost all of these residues are located on one side of the molecule. They are probably involved in the binding of the hormone to its receptor (see p. 224).

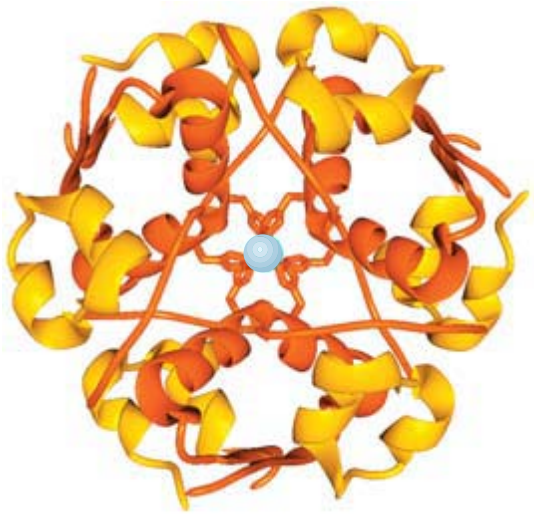
A. Structure of insulin



1. Primary structure

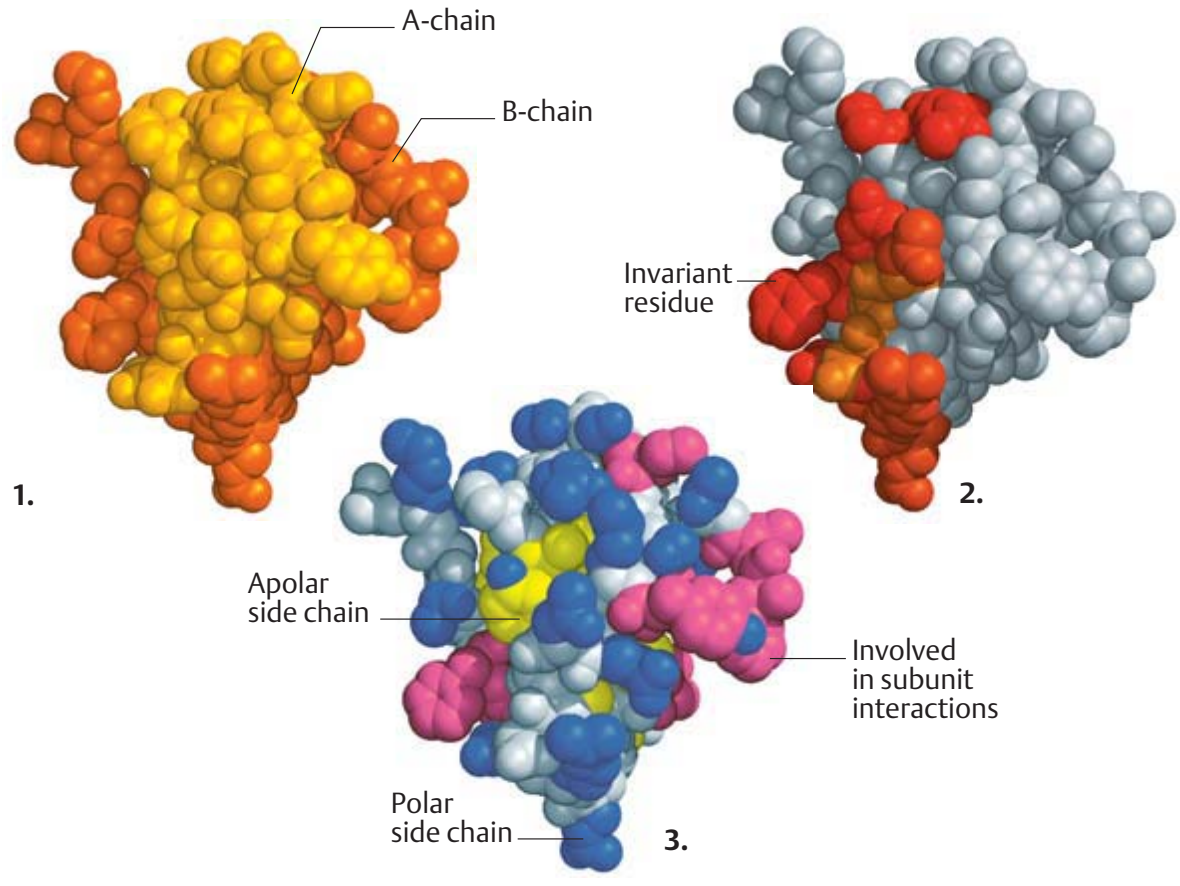


2. Secondary and tertiary structure



3. Quaternary structure

B. Insulin (monomer)



Isolation and analysis of proteins

Purified proteins are nowadays required for a wide variety of applications in research, medicine, and biotechnology. Since the globular proteins in particular are very unstable (see p. 72), purification is carried out at low temperatures (0–5 °C) and particularly gentle separation processes are used. A few of the methods of purifying and characterizing proteins are discussed on this page.

A. Salt precipitation ○

The solubility of proteins is strongly dependent on the salt concentration (*ionic strength*) of the medium. Proteins are usually poorly soluble in pure water. Their solubility increases as the ionic strength increases, because more and more of the well-hydrated anorganic ions (blue circles) are bound to the protein's surface, preventing aggregation of the molecules (**salting in**). At very high ionic strengths, the salt withdraws the hydrate water from the proteins and thus leads to aggregation and precipitation of the molecules (**salting out**). For this reason, adding salts such as ammonium sulfate $(NH_4)_2SO_4$ makes it possible to separate proteins from a mixture according to their degree of solubility (fractionation).

B. Dialysis ○

Dialysis is used to remove lower-molecular components from protein solutions, or to exchange the medium. Dialysis is based on the fact that due to their size, protein molecules are unable to pass through the pores of a **semipermeable membrane**, while lower-molecular substances distribute themselves evenly between the inner and outer spaces over time. After repeated exchanging of the external solution, the conditions inside the *dialysis tube* (salt concentration, pH, etc.) will be the same as in the surrounding solution.

C. Gel filtration ○

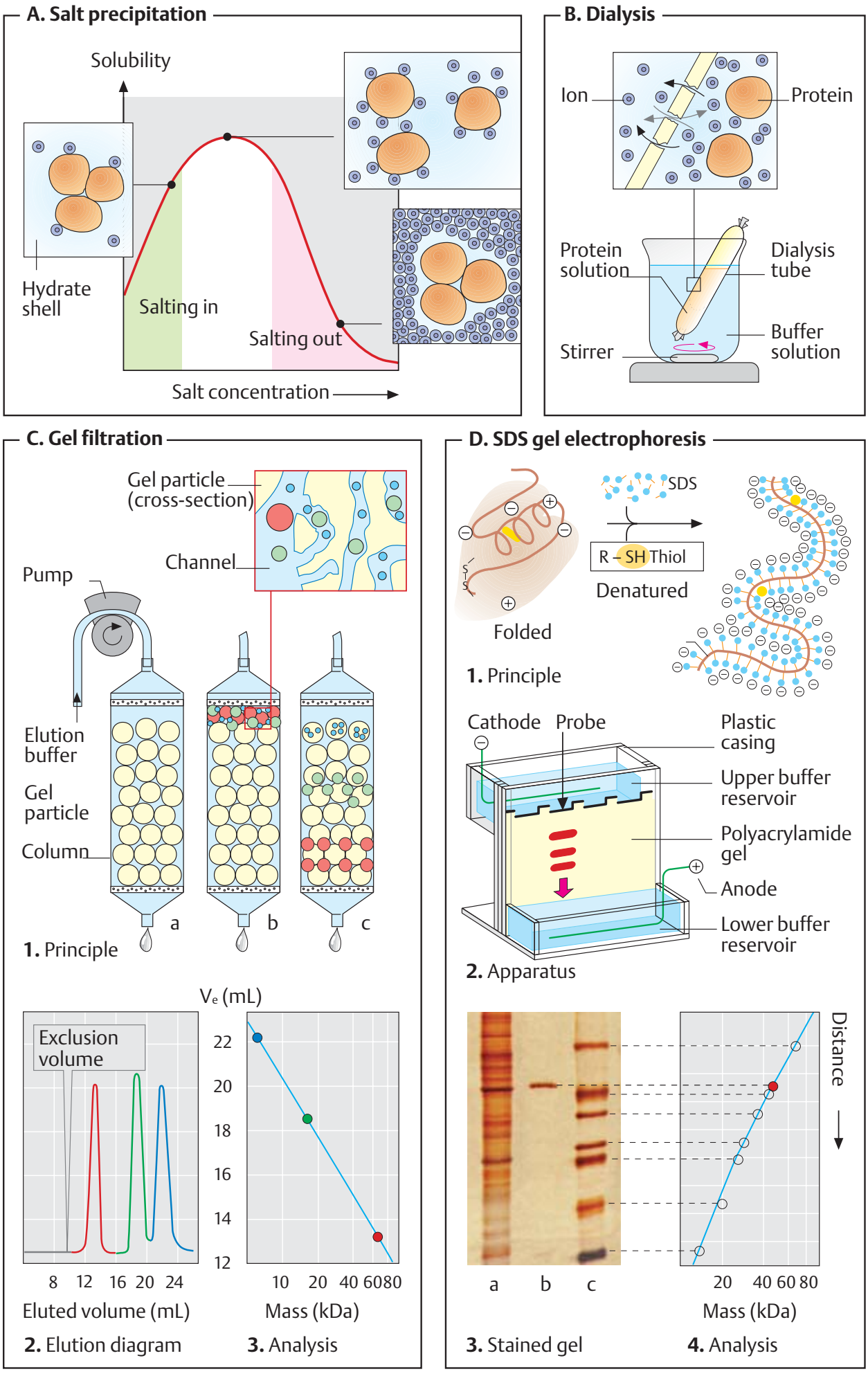
Gel permeation chromatography ("gel filtration") separates proteins according to their size and shape. This is done using a *chromatography column,* which is filled with spherical *gel particles* (diameter 10–500 μm) of polymeric material (shown schematically in **1a**). The insides of the particles are traversed by channels that have defined diameters. A protein mixture is then introduced at the upper end of the column (**1b**) and *elution* is carried out by passing a buffer solution through the column. Large protein molecules (red) are unable to penetrate the particles, and therefore pass through the column quickly. Medium-sized (green) and small particles (blue) are delayed for longer or shorter periods (**1c**). The proteins can be collected separately from the ef uent (*eluate*) (**2**). Their elution volume V_e depends mainly on their molecular mass (**3**).

D. SDS gel electrophoresis ○

The most commonly used procedure for checking the purity of proteins is sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). In electrophoresis, molecules move in an electrical field (see p. 276). Normally, the speed of their movement depends on three factors—their size, their shape, and their electrical charge.

In SDS-PAGE, the protein mixture is treated in such a way that only the molecules' mass affects their movement. This is achieved by adding *sodium dodecyl sulfate* ($C_{12}H_{25}$-OSO_3Na), the sulfuric acid ester of lauryl alcohol (dodecyl alcohol). SDS is a *detergent* with strongly amphipathic properties (see p. 28). It separates oligomeric proteins into their subunits and denatures them. SDS molecules bind to the unfolded peptide chains (ca. 0.4 g SDS / g protein) and give them a strongly negative charge. To achieve complete denaturation, thiols are also added in order to cleave the disulfide bonds (**1**).

Following electrophoresis, which is carried out in a vertically arranged gel of polymeric acrylamide (**2**), the separated proteins are made visible by staining. In example (**3**), the following were separated: **a**) a cell extract with hundreds of different proteins, **b**) a protein purified from this, and **c**) a mixture of proteins with known masses.



Bases and nucleotides

The nucleic acids play a central role in the storage and expression of genetic information (see p. 236). They are divided into two major classes: **deoxyribonucleic acid (DNA)** functions solely in information storage, while **ribonucleic acids (RNAs)** are involved in most steps of gene expression and protein biosynthesis. All nucleic acids are made up from **nucleotide components**, which in turn consist of a *base*, a *sugar*, and a *phosphate residue*. DNA and RNA differ from one another in the type of the sugar and in one of the bases that they contain.

A. Nucleic acid bases ◑

The bases that occur in nucleic acids are *aromatic* heterocyclic compounds derived from either **pyrimidine** or **purine**. Five of these bases are the main components of nucleic acids in all living creatures. The purine bases **adenine** (abbreviation Ade, *not* "A"!) and **guanine** (Gua) and the pyrimidine base **cytosine** (Cyt) are present in both RNA *and* DNA. In contrast, **uracil** (Ura) is only found in RNA. In DNA, **uracil** is replaced by thymine (Thy), the 5-methyl derivative of uracil. 5-methylcytosine also occurs in small amounts in the DNA of the higher animals. A large number of other modified bases occur in tRNA (see p. 82) and in other types of RNA.

B. Nucleosides, nucleotides ◑

When a nucleic acid base is N-glycosidically linked to ribose or 2-deoxyribose (see p. 38), it yields a **nucleoside**. The nucleoside **adenosine** (abbreviation: A) is formed in this way from adenine and ribose, for example. The corresponding derivatives of the other bases are called *guanosine* (G), *uridine* (U), *thymidine* (T) and *cytidine* (C). When the sugar component is 2-deoxyribose, the product is a **deoxyribonucleoside**—e. g., 2′-deoxyadenosine (dA, not shown). In the cell, the 5′OH group of the sugar component of the nucleoside is usually esterified with phosphoric acid. 2′-Deoxythymidine (dT) therefore gives rise to **2′-deoxythymidine-5′-monophosphate (dTMP)**, one of the components of DNA (**2**). If the 5′phosphate residue is linked via an acid–anhydride bond to additional phosphate residues, it yields nucleoside diphosphates and triphosphates—e. g., ADP and ATP, which are important coenzymes in energy metabolism (see p. 106). All of these nucleoside phosphates are classified as **nucleotides**.

In nucleosides and nucleotides, the pentose residues are present in the furanose form (see p. 34). The sugars and bases are linked by an *N*-**glycosidic bond** between the C-1 of the sugar and either the N-9 of the purine ring or N-1 of the pyrimidine ring. This bond always adopts the β-configuration.

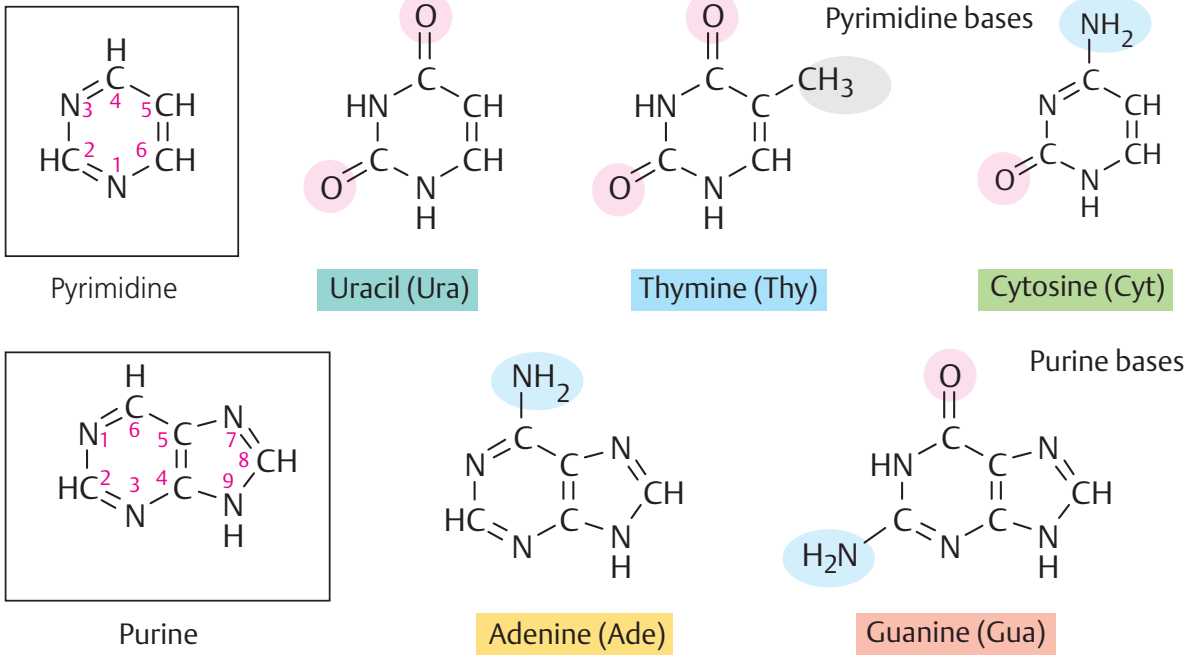
C. Oligonucleotides, polynucleotides ◑

Phosphoric acid molecules can form acid–anhydride bonds with each other. It is therefore possible for two nucleotides to be linked via the phosphate residues. This gives rise to *dinucleotides with a phosphoric acid–anhydride* structure. This group includes the coenzymes $NAD(P)^+$ and CoA, as well as the flavin derivative **FAD** (**1**; see p. 104).

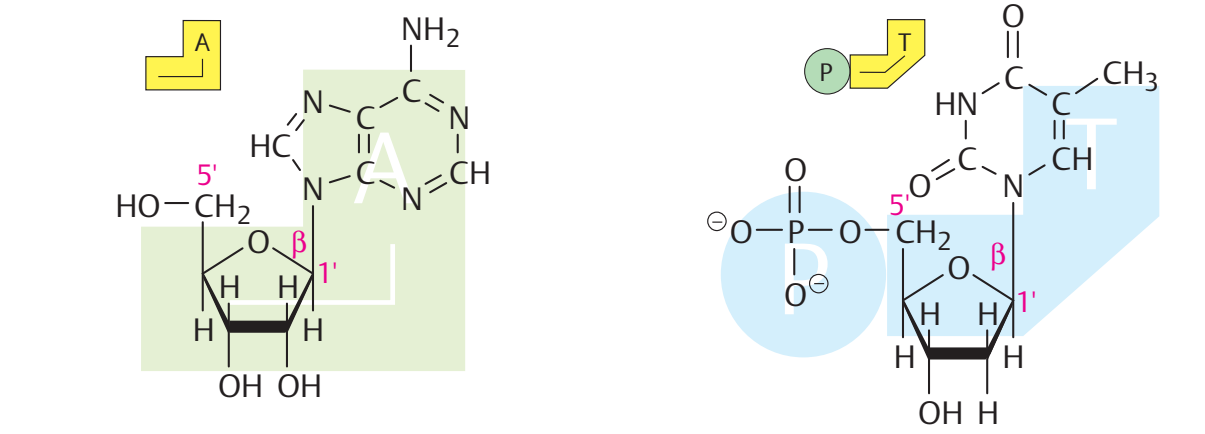
If the phosphate residue of a nucleotide reacts with the 3′-OH group of a second nucleotide, the result is a *dinucleotide with a phosphoric acid diester structure*. Dinucleotides of this type have a free phosphate residue at the *5′end* and a free OH group at the *3′end*. They can therefore be extended with additional mononucleotides by adding further phosphoric acid diester bonds. This is the way in which **oligonucleotides**, and ultimately **polynucleotides**, are synthesized.

Polynucleotides consisting of ribonucleotide components are called **ribonucleic acid (RNA)**, while those consisting of deoxyribonucleotide monomers are called **deoxyribonucleic acid (DNA**; see p. 84). To describe the structure of polynucleotides, the abbreviations for the *nucleoside* components are written from left to right *in the 5′→3′* direction. The position of the phosphate residue is also sometimes indicated by a "p". In this way, the structure of the RNA segment shown Fig. **2** can be abbreviated as ..pUpG.. or simply as $..^{UG}..$.

A. Nucleic acid bases



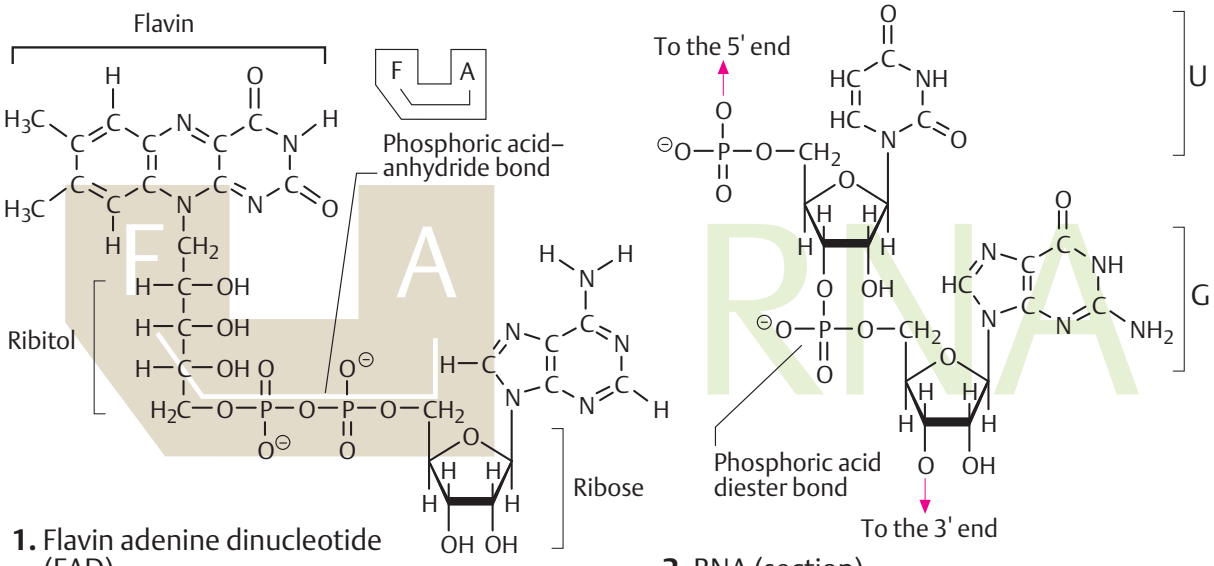
B. Nucleosides, nucleotides



1. Adenosine (Ado)

2. 2'-Deoxythymidine 5'-monophosphate (dtMP)

C. Oligonucleotides, polynucleotides



1. Flavin adenine dinucleotide (FAD)

2. RNA (section)

RNA

Ribonucleic acids (RNAs) are polymers consisting of nucleoside phosphate components that are linked by phosphoric acid diester bonds (see p. 80). The bases the contain are mainly uracil, cytosine, adenine, and guanine, but many unusual and modified bases are also found in RNAs (**B**).

A. Ribonucleic acids (RNAs) ◑

RNAs are involved in all the individual steps of gene expression and protein biosynthesis (see pp. 242–253). The properties of the most important forms of RNA are summarized in the table. The schematic diagram also gives an idea of the secondary structure of these molecules.

In contrast to DNA, RNAs do not form extended double helices. In RNAs, the base pairs (see p. 84) usually only extend over a few residues. For this reason, substructures often arise that have a finger shape or clover-leaf shape in two-dimensional representations. In these, the paired stem regions are linked by loops. Large RNAs such as ribosomal 16S-rRNA (center) contain numerous "stem and loop" regions of this type. These sections are again folded three-dimensionally—i. e., like proteins, RNAs have a tertiary structure (see p. 86). However, tertiary structures are only known of small RNAs, mainly tRNAs. The diagrams in Fig. **B** and on p. 86 show that the "clover-leaf" structure is not recognizable in a three-dimensional representation.

Cellular RNAs vary widely in their size, structure, and lifespan. The great majority of them are ribosomal RNA **(rRNA)**, which in several forms is a structural and functional component of *ribosomes* (see p. 250). Ribosomal RNA is produced from DNA by transcription in the nucleolus, and it is processed there and assembled with proteins to form ribosome subunits (see pp. 208, 242). The bacterial 16S-rRNA shown in Fig. **A**, with 1542 nucleotides (nt), is a component of the small ribosomae subunit, while the much smaller 5S-rRNA (118 nt) is located in the large subunit.

Messenger RNAs **(mRNAs)** transfer genetic information from the cell nucleus to the cytoplasm. The primary transcripts are substantially modified while still in the nucleus (mRNA maturation; see p. 246). Since mRNAs have to be read codon by codon in the ribosome, they must not form a stable tertiary structure. This is ensured in part by the attachment of *RNA-binding proteins,* which prevent base pairing. Due to the varying amounts of information that they carry, the lengths of mRNAs also vary widely. Their lifespan is usually short, as they are quickly broken down after translation.

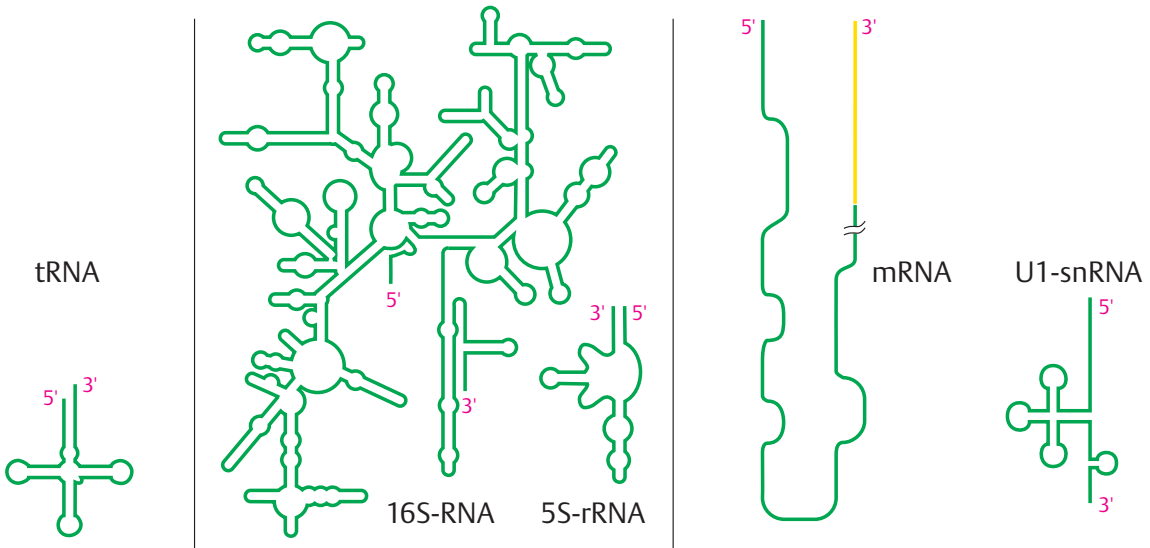
Small nuclear RNAs **(snRNAs)** are involved in the splicing of mRNA precursors (see p. 246). They associate with numerous proteins to form "spliceosomes."

B. Transfer RNA ($tRNA^{Phe}$) ◑

The transfer RNAs **(tRNAs)** function during translation (see p. 250) as links between the nucleic acids and proteins. They are small RNA molecules consisting of 70–90 nucleotides, which "recognize" specific mRNA codons with their *anticodons* through base pairing. At the same time, at their 3′ end (sequence .. CCA-3′) they carry the amino acid that is assigned to the relevant mRNA codon according to the genetic code (see p. 248).

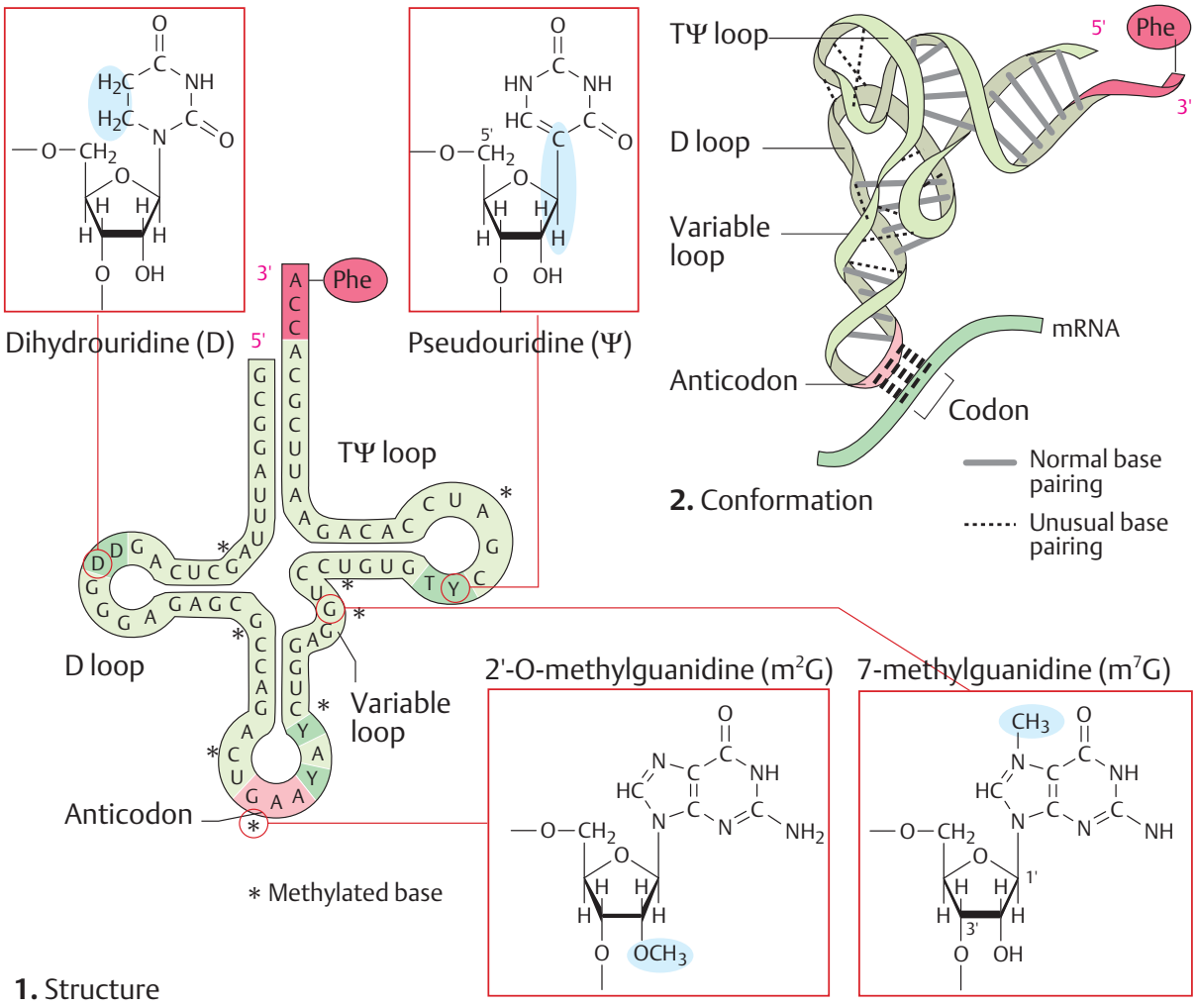
The base sequence and the tertiary structure of the yeast tRNA specific for phenylalanine ($tRNA^{Phe}$) is typical of all tRNAs. The molecule (see also p. 86) contains a high proportion of unusual and modified components (shaded in dark green in Fig. **1**). These include *pseudouridine* (Ψ), *dihydrouridine* (D), *thymidine* (T), which otherwise only occurs in DNA, and many methylated nucleotides such as *7-methylguanidine* (m^7G) and—in the anticodon—*2′-O-methylguanidine* (m^2G). Numerous base pairs, sometimes deviating from the usual pattern, stabilize the molecule's conformation (**2**).

A. Ribonucleic acids (RNAs)



tRNA	rRNA	Type	mRNA	snRNA
>50	4	Species per cell	> 1000	~ 10
74 - 95	120 - 5000	Length (b)	400 - 6000	100 - 300
10-20%	80%	Proportion	5%	< 1%
Long	Long	Lifespan	Short	Long
Translation	Translation	Function	Translation	Splicing

B. Transfer RNA (tRNA^{Phe})



DNA

A. DNA: structure ●

Like RNAs (see p. 82), deoxyribonucleic acids (DNAs) are polymeric molecules consisting of nucleotide building blocks. Instead of ribose, however, DNA contains 2′-deoxyribose, and the *uracil* base in RNA is replaced by *thymine*. The spatial structure of the two molecules also differs (see p. 86).

The first evidence of the special structure of DNA was the observation that the amounts of adenine and thymine are almost equal in every type of DNA. The same applies to guanine and cytosine. The model of DNA structure formulated in 1953 explains these *constant base ratios:* intact DNA consists of *two* polydeoxynucleotide molecules ("strands"). Each base in one strand is linked to a *complementary* base in the other strand by H-bonds. Adenine is complementary to thymine, and guanine is complementary to cytosine. One purine base and one pyrimidine base are thus involved in each **base pair**.

The complementarity of A with T and of G with C can be understood by considering the H bonds that are possible between the different bases. Potential donors (see p. 6) are amino groups (Ade, Cyt, Gua) and ring NH groups. Possible acceptors are carbonyl oxygen atoms (Thy, Cyt, Gua) and ring nitrogen atoms. *Two* linear and therefore highly stable bonds can thus be formed in A–T pairs, and *three* in G–C pairs.

Base pairings of this type are only possible, however, when the *polarity* of the two strands differs—i. e., when they run in opposite directions (see p. 80). In addition, the two strands have to be intertwined to form a **double helix**. Due to steric hindrance by the 2′-OH groups of the ribose residues, RNA is unable to form a double helix. The structure of RNA is therefore less regular than that of DNA (see p. 82).

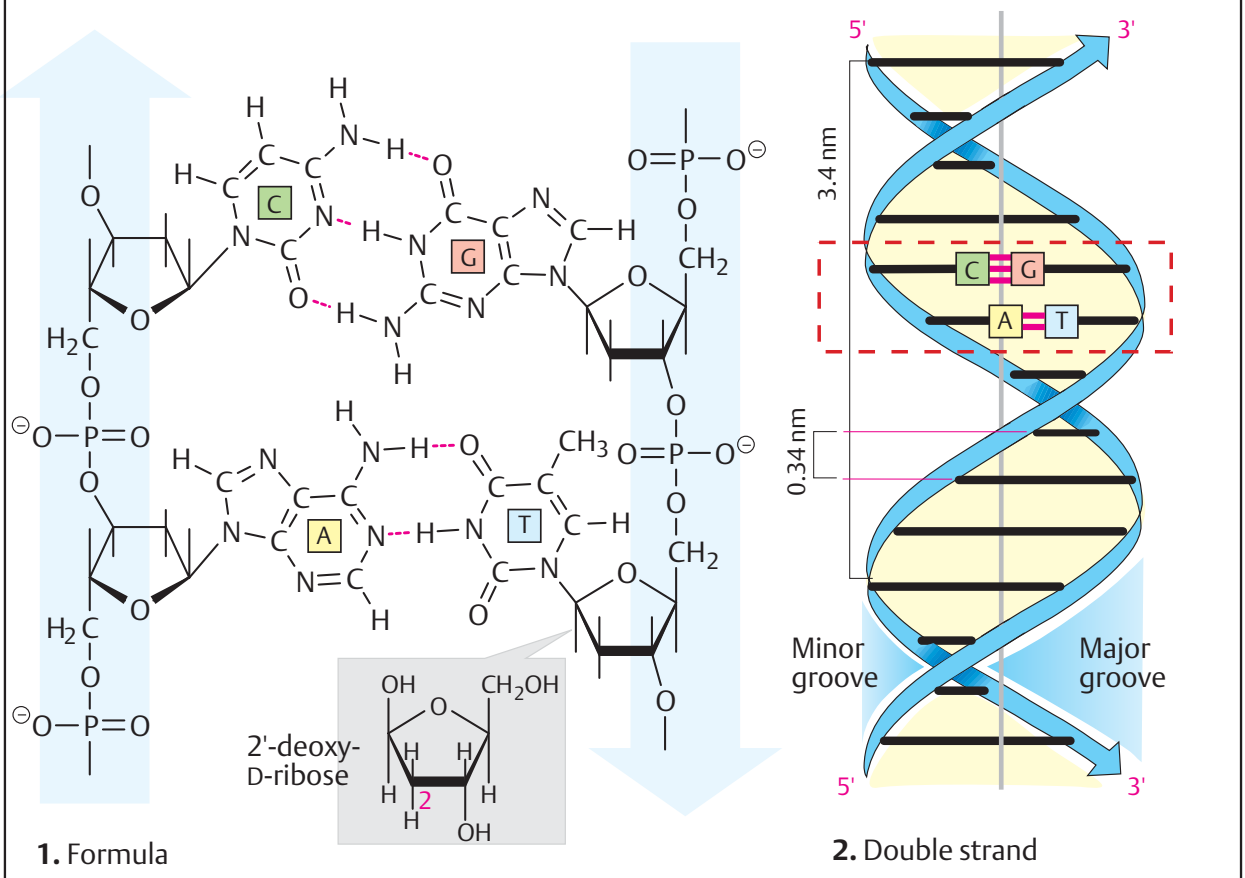
The conformation of DNA that predominates within the cell (known as **B-DNA**) is shown schematically in Fig. **A2** and as a van der Waals model in Fig. **B1**. In the schematic diagram (**A2**), the deoxyribose–phosphate "backbone" is shown as a ribbon. The bases (indicated by lines) are located on the inside of the **double helix**. This area of DNA is therefore apolar. By contrast, the molecule's surface is polar and negatively charged, due to the sugar and phosphate residues in the backbone. Along the whole length of the DNA molecule, there are two depressions—referred to as the "minor groove" and the "major groove"—that lie between the strands.

B. Coding of genetic information ●

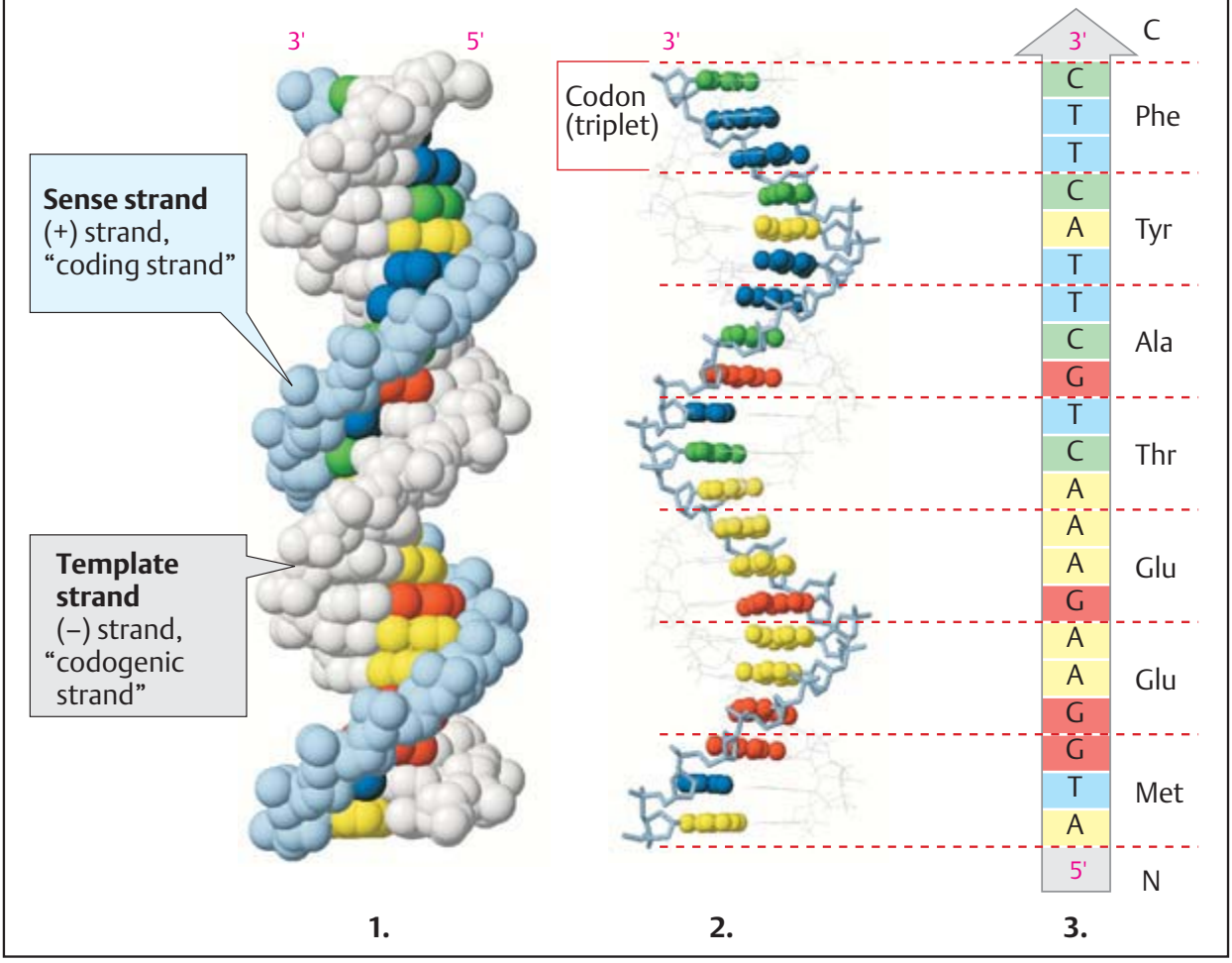
In all living cells, DNA serves to **store genetic information**. Specific segments of DNA ("genes") are transcribed as needed into RNAs, which either carry out structural or catalytic tasks themselves or provide the basis for synthesizing proteins (see p. 82). In the latter case, the DNA codes for the primary structure of proteins. The "language" used in this process has four letters (A, G, C, and T). All of the words ("codons") contain three letters ("triplets"), and each triplet stands for one of the 20 proteinogenic amino acids.

The two strands of DNA are not functionally equivalent. The **template strand** (the (–) strand or "codogenic strand," shown in light gray in Fig. **1**) is the one that is read during the synthesis of RNA (transcription; see p. 242). Its sequence is complementary to the RNA formed. The **sense strand** (the (+) strand or "coding strand," shown in color in Figs. **1** and **2**) has the *same sequence as the RNA,* except that T is exchanged for U. By convention, it is agreed that gene sequences are expressed by reading the sequence of the sense strand in the 5′→3′ direction. Using the genetic code (see p. 248), in this case the protein sequence (**3**) is obtained directly in the reading direction usual for proteins—i. e., from the *N* terminus to the *C* terminus.

A. DNA: structure



B. Coding of genetic information



Molecular models: DNA and RNA

The illustration opposite shows selected nucleic acid molecules. Fig. **A** shows various conformations of DNA, and Fig. **B** shows the spatial structures of two small RNA molecules. In both, the van der Waals models (see p. 6) are accompanied by ribbon diagrams that make the course of the chains clear. In all of the models, the polynucleotide "backbone" of the molecule is shown in a darker color, while the bases are lighter.

A. DNA: conformation ○

Investigations of synthetic DNA molecules have shown that DNA can adopt several different conformations. All of the DNA segments shown consist of 21 base pairs (bp) and have the same sequence.

By far the most common form is **B-DNA** (**2**). As discussed on p. 84, this consists of two antiparallel polydeoxynucleotide strands intertwined with one another to form a **right-handed double helix**. The "backbone" of these strands is formed by deoxyribose and phosphate residues linked by phosphoric acid diester bonds.

In the B conformation, the aromatic rings of the nucleobases are stacked at a distance of 0.34 nm almost at right angles to the axis of the helix. Each base is rotated relative to the preceding one by an angle of 35°. A complete turn of the double helix (360°) therefore contains around 10 base pairs (abbreviation: bp), i. e., the *pitch* of the helix is 3.4 nm. Between the backbones of the two individual strands there are two grooves with different widths. The *major groove* is visible at the top and bottom, while the narrower *minor groove* is seen in the middle. DNA-binding proteins and transcription factors (see pp. 118, 244) usually enter into interactions in the area of the major groove, with its more easily accessible bases.

In certain conditions, DNA can adopt the **A conformation** (**1**). In this arrangement, the double helix is still right-handed, but the bases are no longer arranged at right angles to the axis of the helix, as in the B form. As can be seen, the A conformation is more compact than the other two conformations. The minor groove almost completely disappears, and the major groove is narrower than in the B form. A-DNA arises when B-DNA is dehydrated. It probably does not occur in the cell.

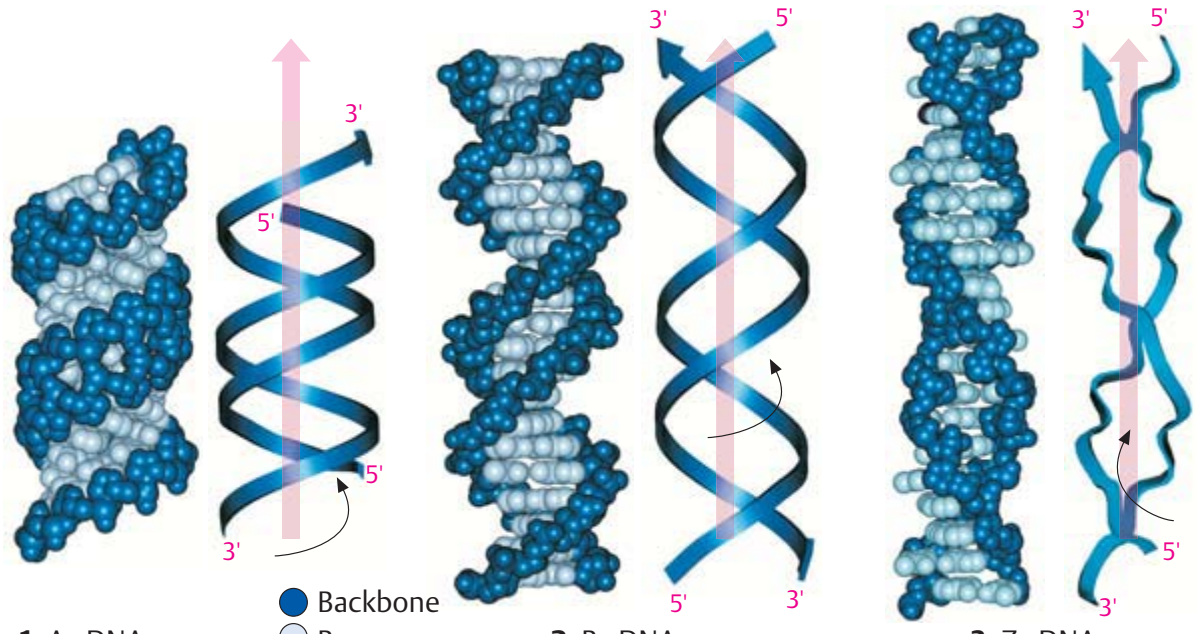
In the **Z-conformation** (**3**), which can occur within GC-rich regions of B-DNA, the organization of the nucleotides is completely different. In this case, the helix is *left-handed,* and the backbone adopts a characteristic *zig-zag* conformation (hence "Z-DNA"). The Z double helix has a smaller pitch than B-DNA. DNA segments in the Z conformation probably have physiological significance, but details are not yet known.

B. RNA ○

RNA molecules are unable to form extended double helices, and are therefore less highly ordered than DNA molecules. Nevertheless, they have defined secondary and tertiary structures, and a large proportion of the nucleotide components enter into base pairings with other nucleotides. The examples shown here are **5S-rRNA** (see p. 242), which occurs as a structural component in ribosomes, and a **tRNA** molecule from yeast (see p. 82) that is specific for phenylalanine.

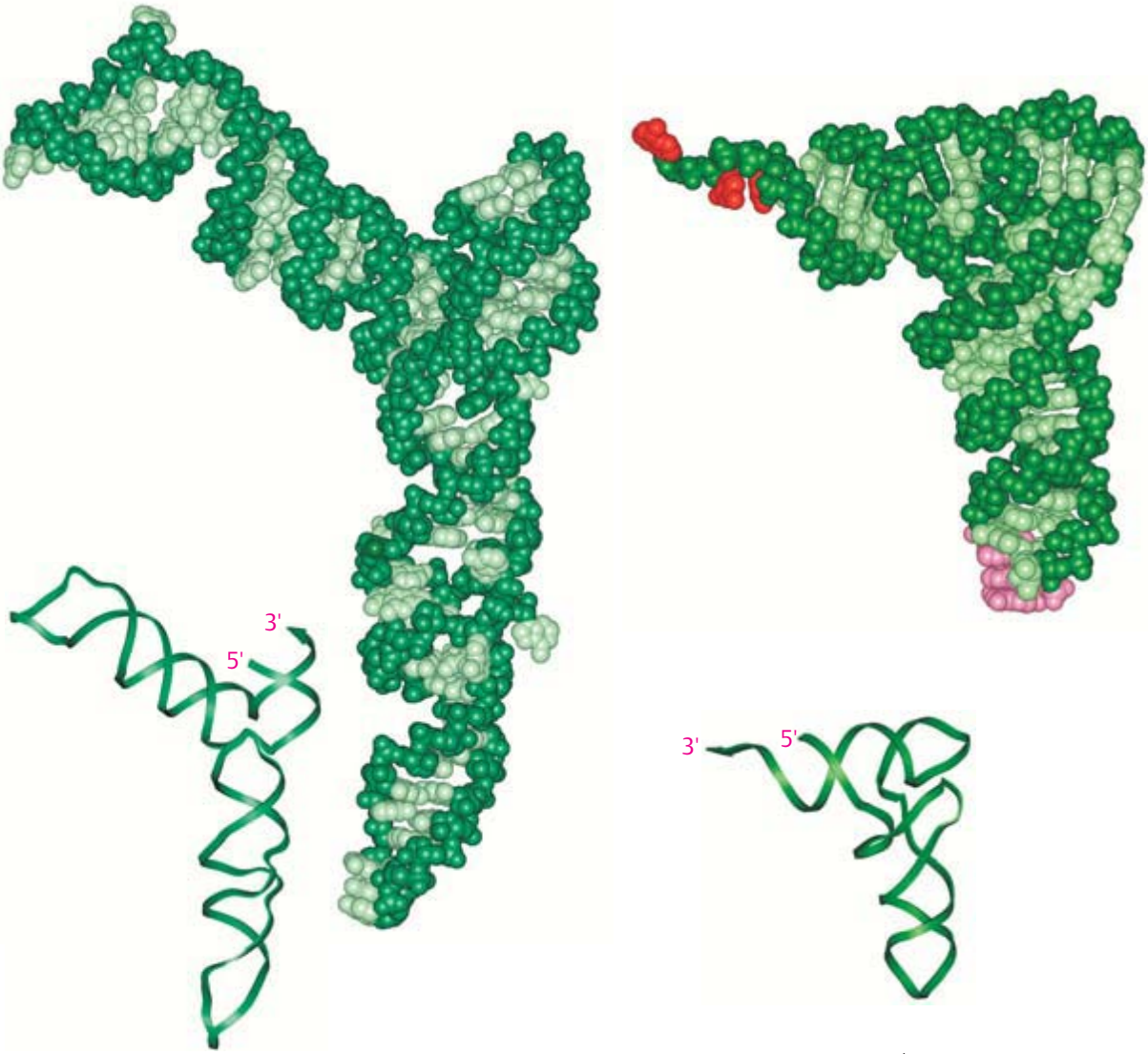
Both molecules are folded in such a way that the 3′ end and the 5′ end are close together. As in DNA, most of the bases are located in the inside of the structures, while the much more polar "backbone" is turned outwards. An exception to this is seen in the three bases of the *anticodon* of the tRNA (pink), which have to interact with mRNA and therefore lie on the surface of the molecule. The bases of the conserved CCA triplet at the 3′ end (red) also jut outward. During amino acid activation (see p. 248), they are recognized and bound by the ligases.

A. DNA: conformation



1. A - DNA **2.** B - DNA **3.** Z - DNA

B. RNA



1. 5S-rRNA (118 nucleotides)

2. Phe-tRNA^{Phe} (77 nucleotides)

Enzymes: basics

Enzymes are **biological catalysts**—i.e., substances of biological origin that accelerate chemical reactions (see p. 24). The orderly course of metabolic processes is only possible because each cell is equipped with its own genetically determined set of enzymes. It is only this that allows coordinated sequences of reactions (**metabolic pathways**; see p. 112). Enzymes are also involved in many regulatory mechanisms that allow the metabolism to adapt to changing conditions (see p. 114). Almost all enzymes are **proteins**. However, there are also catalytically active ribonucleic acids, the *"ribozymes"* (see pp. 246, 252).

A. Enzymatic activity ●

The catalytic action of an enzyme, its **activity**, is measured by determining the **increase in the reaction rate** under precisely defined conditions—i.e., the difference between the turnover (violet) of the catalyzed reaction (orange) and uncatalyzed reaction (yellow) in a specific time interval. Normally, reaction rates are expressed as the *change in concentration per unit of time* (mol 1^{-1} s^{-1}; see p. 22). Since the catalytic activity of an enzyme is independent of the volume, the unit used for enzymes is usually *turnover per unit time*, expressed in **katal** (kat, mol s^{-1}). However, the **international unit U** is still more commonly used (μmol turnover min^{-1}; 1 U = 16.7 nkat).

B. Reaction and substrate specificity ●

The action of enzymes is usually very *specific*. This applies not only to the type of reaction being catalyzed (**reaction specificity**), but also to the nature of the reactants ("substrates") that are involved (**substrate specificity**; see p. 94). In Fig. **B**, this is illustrated schematically using a bond-breaking enzyme as an example. Highly specific enzymes (type A, top) catalyze the cleavage of only *one* type of bond, and only when the structure of the substrate is the correct one. Other enzymes (type B, middle) have narrow reaction specificity, but broad substrate specificity. Type C enzymes (with low reaction specificity *and* low substrate specificity, bottom) are very rare.

C. Enzyme classes ◑

More than 2000 different enzymes are currently known. A system of *classification* has been developed that takes into account both their *reaction specificity* and their *substrate specificity*. Each enzyme is entered in the *Enzyme Catalogue* with a four-digit Enzyme Commission number (**EC number**). The first digit indicates membership of one of the six **major classes**. The next two indicate subclasses and subsubclasses. The last digit indicates where the enzyme belongs in the subsubclass. For example, lactate dehydrogenase (see pp. 98–101) has the EC number *1.1.1.27* (class 1, oxidoreductases; subclass 1.1, CH–OH group as electron *donor*; sub-subclass 1.1.1, $NAD(P)^+$ as electron *acceptor*).

Enzymes with similar reaction specificities are grouped into each of the six major classes:

The **oxidoreductases** (*class 1*) catalyze the transfer of reducing equivalents from one redox system to another.

The **transferases** (*class 2*) catalyze the transfer of other groups from one molecule to another. Oxidoreductases and transferases generally require coenzymes (see pp. 104 ff.).

The **hydrolases** (*class 3*) are also involved in group transfer, but the acceptor is always a *water molecule.*

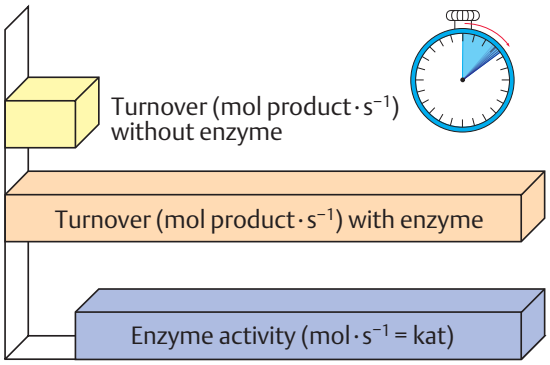
Lyases (*class 4*, often also referred to as "synthases") catalyze reactions involving either the cleavage or formation of chemical bonds, with double bonds either arising or disappearing.

The **isomerases** (*class 5*) move groups within a molecule, without changing the gross composition of the substrate.

The ligation reactions catalyzed by **ligases** ("synthetases," *class 6*) are energy-dependent and are therefore always coupled to the hydrolysis of nucleoside triphosphates.

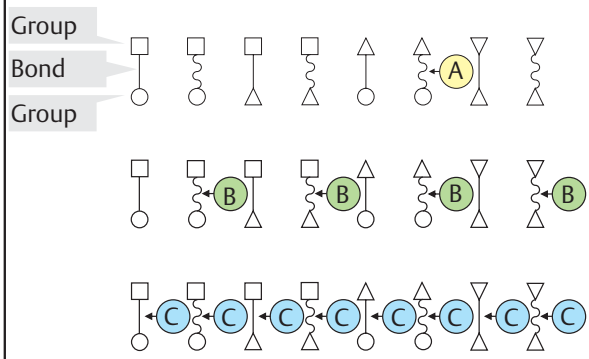
In addition to the enzyme name, we also usually give its EC number. The *annotated enzyme list* (pp. 420 ff.) includes all of the enzymes mentioned in this book, classified according to the Enzyme Catalog system.

A. Enzymatic activity



1 Katal (kat): Amount of enzyme which increases turnover by 1 mol · s^{-1}

B. Reaction and substrate specificity



	Reaction specificity	Substrate specificity
A	High	High
B	High	Low
C	Low	Low

C. The enzyme classes

Class	Reaction type	Important subclasses
1 Oxidoreductases	○ = Reduction equivalent $A_{red} + B_{ox} \rightleftharpoons A_{ox} + B_{red}$	Dehydrogenases Oxidases, peroxidases Reductases Monooxygenases Dioxygenases
2 Transferases	A–B + C ⇌ A + B–C	C_1-Transferases Glycosyltransferases Aminotransferases Phosphotransferases
3 Hydrolases	A–B + H_2O ⇌ A–H + B–OH	Esterases Glycosidases Peptidases Amidases
4 Lyases ("synthases")	A + B ⇌ A–B	C-C-Lyases C-O-Lyases C-N-Lyases C-S-Lyases
5 Isomerases	A ⇌ Iso-A	Epimerases *cis trans* Isomerases Intramolecular transferases
6 Ligases ("synthetases")	B + A + XTP (X = A, G, U, C) ⇌ A–B + XDP + P	C-C-Ligases C-O-Ligases C-N-Ligases C-S-Ligases

Enzyme catalysis

Enzymes are extremely effective **catalysts**. They can increase the rate of a catalyzed reaction by a factor of 10^{12} or more. To grasp the mechanisms involved in enzyme catalysis, we can start by looking at the course of an uncatalyzed reaction more closely.

A. Uncatalyzed reaction ○

The reaction A + B → C + D is used as an example. In solution, **reactants A and B** are surrounded by a shell of water molecules (the *hydration shell*), and they move in random directions due to thermal agitation. They can only react with each other if they collide in a favorable orientation. This is not very probable, and therefore only occurs rarely. Before conversion into the products C + D, the **collision complex A-B** has to pass through a **transition state**, the formation of which usually requires a large amount of **activation energy, E_a** (see p. 22). Since only a few A–B complexes can produce this amount of energy, a productive transition state arises even less often than a collision complex. In solution, a large proportion of the activation energy is required for the *removal of the hydration shells* between A and B. However, charge displacements and other *chemical processes* within the reactants also play a role. As a result of these limitations, conversion only happens occasionally in the absence of a catalyst, and the reaction rate v is low, even when the reaction is thermodynamically possible—i. e., when $\Delta G < 0$ (see p. 18).

B. Enzyme-catalyzed reaction ◑

Shown here is a *sequential mechanism* in which substrates A and B are bound and products C and D are released, in that order. Another possible reaction sequence, known as the *"ping-pong mechanism,"* is discussed on p. 94.

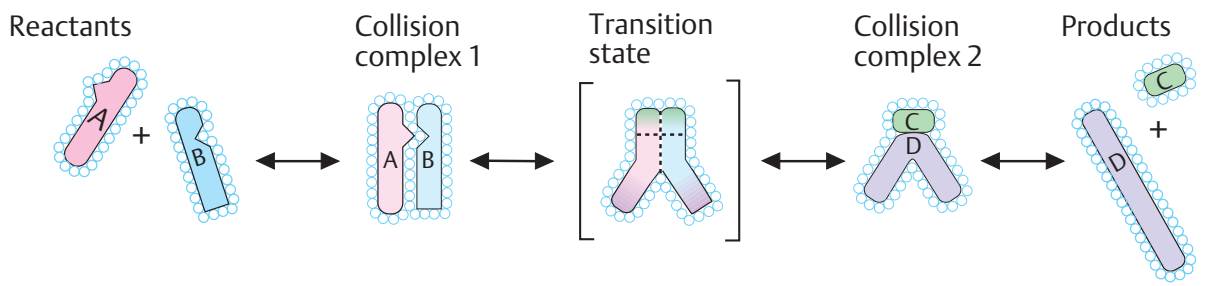
Enzymes are able to bind the reactants (their *substrates*) specifically at the **active center**. In the process, the substrates are oriented in relation to each other in such a way that they take on the *optimal orientation* for the formation of the transition state (**1**–**3**). The **proximity and orientation of the substrates** therefore strongly increase the likelihood that *productive* A–B complexes will arise. In addition, binding of the substrates results in removal of their hydration shells. As a result of the **exclusion of water**, very different conditions apply in the active center of the enzyme during catalysis than in solution (**3**–**5**). A third important factor is the **stabilization of the transition state** as a result of interactions between the amino acid residues of the protein and the substrate (**4**). This further reduces the activation energy needed to create the transition state. Many enzymes also take up groups from the substrates or transfer them to the substrates during catalysis.

Proton transfers are particularly common. This **acid–base catalysis** by enzymes is much more effective than the exchange of protons between acids and bases in solution. In many cases, chemical groups are temporarily bound covalently to the amino acid residues of the enzyme or to coenzymes during the catalytic cycle. This effect is referred to as **covalent catalysis** (see the transaminases, for example; p. 178). The principles of enzyme catalysis sketched out here are discussed in greater detail on p. 100 using the example of lactate dehydrogenase.

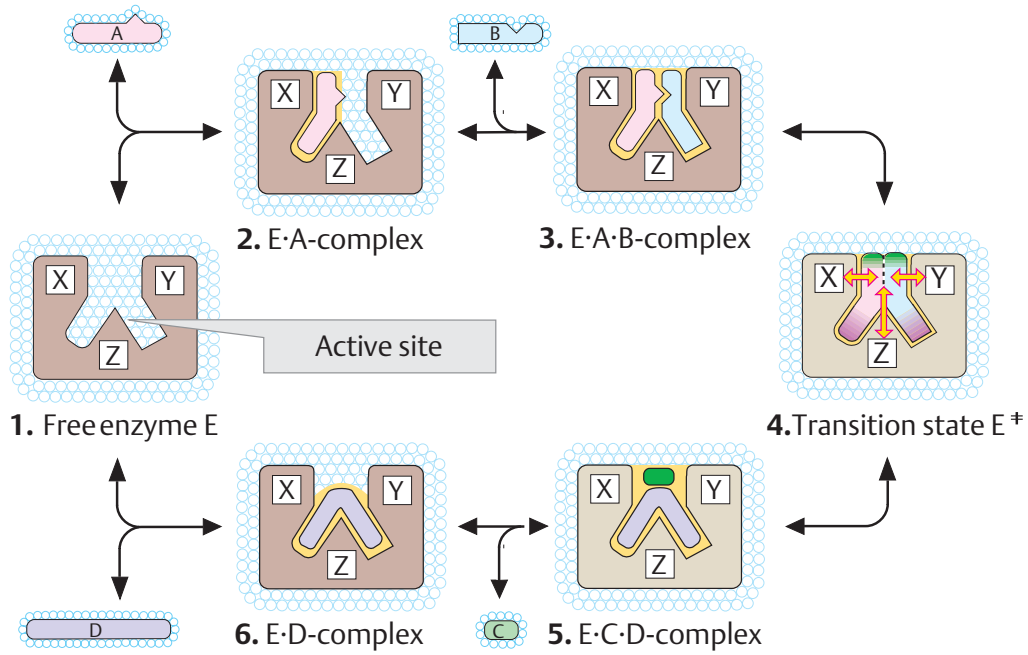
C. Principles of enzyme catalysis ◑

Although it is dif cult to provide quantitative estimates of the contributions made by individual catalytic effects, it is now thought that the enzyme's **stabilization of the transition state** is the most important factor. It is not tight binding of the *substrate* that is important, therefore—this would increase the activation energy required by the reaction, rather than reducing it—but rather the binding of the transition state. This conclusion is supported by the very high af nity of many enzymes for analogues of the transition state (see p. 96). A simple mechanical analogy may help clarify this (right). To transfer the metal balls (the reactants) from location EA (the substrate state) via the higher-energy transition state to EP (the product state), the magnet (the catalyst) has to be orientated in such a way that its attractive force acts on the transition state (bottom) rather than on EA (top).

A. Uncatalyzed reaction



B. Enzyme-catalyzed reaction



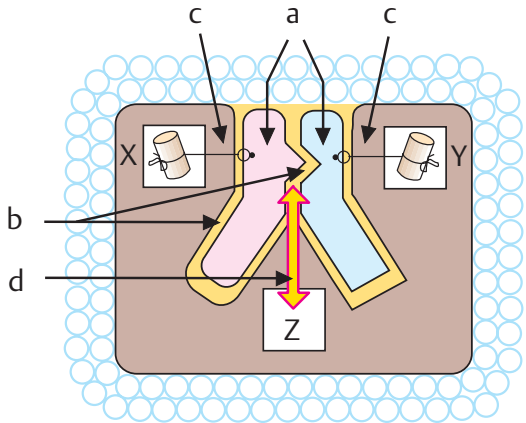
C. Principles of enzyme catalysis

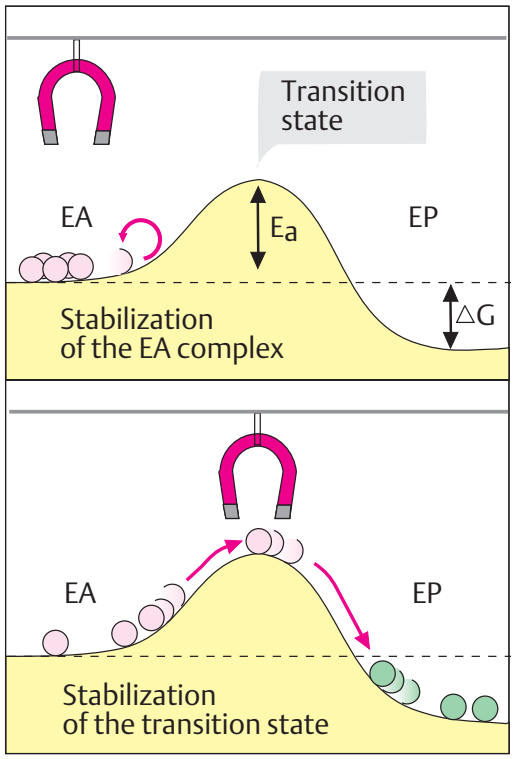
a Approximation and orientation of the substrates

b Exclusion of water

c Stabilization of the transition state

d Group transfer





Enzyme kinetics I

The **kinetics** of enzyme-catalyzed reactions (i. e., the dependence of the reaction rate on the reaction conditions) is mainly determined by the *properties of the catalyst.* It is therefore more complex than the kinetics of an uncatalyzed reaction (see p. 22). Here we discuss these issues using the example of a simple first-order reaction (see p. 22)

A. Michaelis–Menten kinetics ◑

In the absence of an enzyme, the reaction rate *v* is proportional to the concentration of substance A (top). The constant k is the *rate constant* of the uncatalyzed reaction. Like all catalysts, the enzyme E (total concentration $[E]_t$) creates a new reaction pathway. Initially, A is bound to E (partial reaction 1, left). If this reaction is in chemical equilibrium, then with the help of the law of mass action—and taking into account the fact that $[E]_t = [E] + [EA]$—one can express the concentration [EA] of the *enzyme–substrate* complex as a function of [A] (left). The **Michaelis constant** K_m thus describes the state of equilibrium of the reaction. In addition, we know that $k_{cat} > k$—in other words, enzyme-bound substrate reacts to B much faster than A alone (partial reaction 2, right). k_{cat}, the enzyme's **turnover number**, corresponds to the number of substrate molecules converted by one enzyme molecule per second. Like the conversion $A \rightarrow B$, the formation of B from EA is a first-order reaction—i. e., $v = k\ [EA]$ applies. When this equation is combined with the expression already derived for EA, the result is the **Michaelis–Menten equation.**

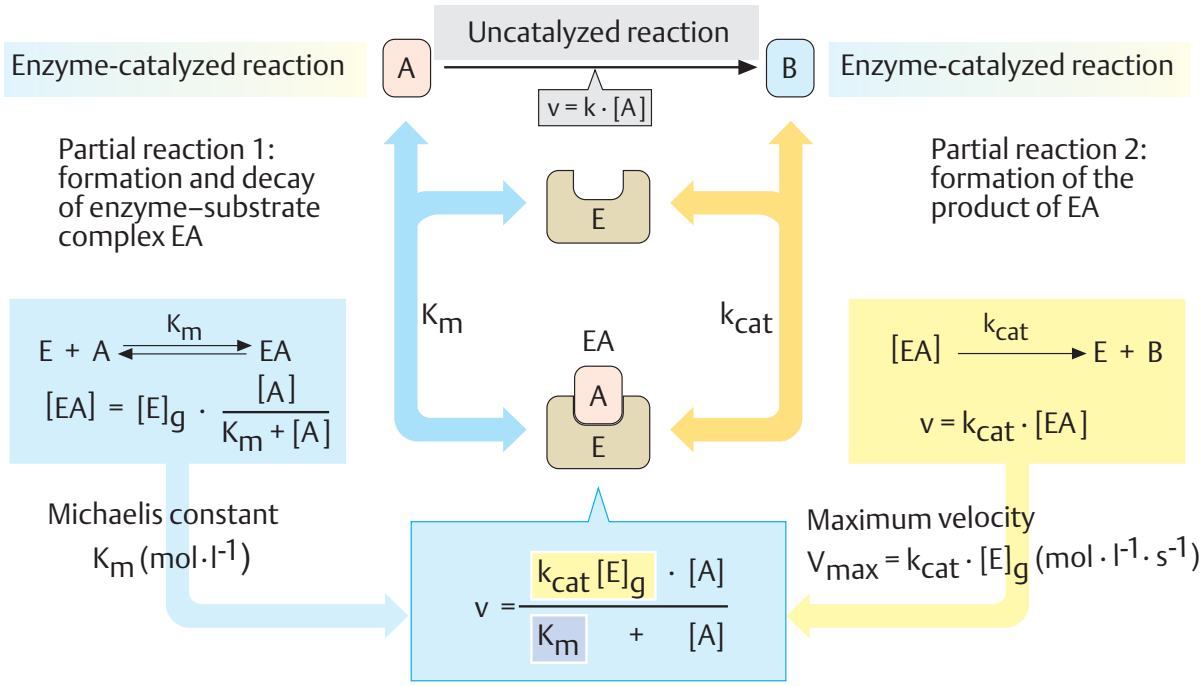
In addition to the *variables* v and [A], the equation also contains two *parameters* that do not depend on the substrate concentration [A], but describe properties of the enzyme itself: the product $k_{cat}\ [E]_g$ is the limiting value for the reaction rate at a very high [A], the **maximum velocity V_{max}** of the reaction (recommended abbreviation: V). The **Michaelis constant** K_m characterizes the *af nity* of the enzyme for a substrate. It corresponds to the substrate concentration at which *v* reaches half of V_{max} (if $v = V_{max}/2$, then $[A]/(K_m + [A]) = 1/2$, i. e. [A] is then $= K_m$). A *high* af nity of the enzyme for a substrate therefore leads to a *low* K_m value, and vice versa. Of the two enzymes whose *substrate saturation curves* are shown in diagram 1, enzyme 2 has the higher af nity for A [$K_m = 1$ mmol l^{-1}); V_{max}, by contrast, is much lower than with enzyme 1.

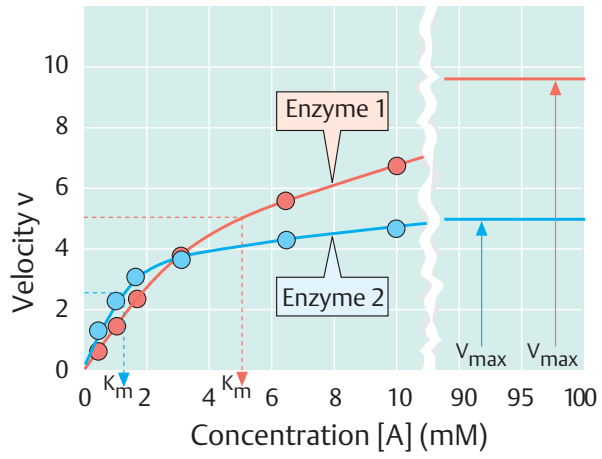
Since v approaches V *asymptotically* with increasing values of [A], it is dif cult to obtain reliable values for V_{max}—and thus for K_m as well—from diagrams plotting v against [A]. To get around this, the Michaelis–Menten equation can be arranged in such a way that the measured points lie on a *straight line.* In the **Lineweaver–Burk plot (2)**, 1/v is plotted against 1/[A]. The intersections of the line of best fit with the axes then produce $1/V_{max}$ and $-1/K_m$. This type of diagram is very clear, but for practical purposes it is less suitable for determining V_{max} and K_m. Calculation methods using personal computers are faster and more objective.

B. Isosteric and allosteric enzymes ◑

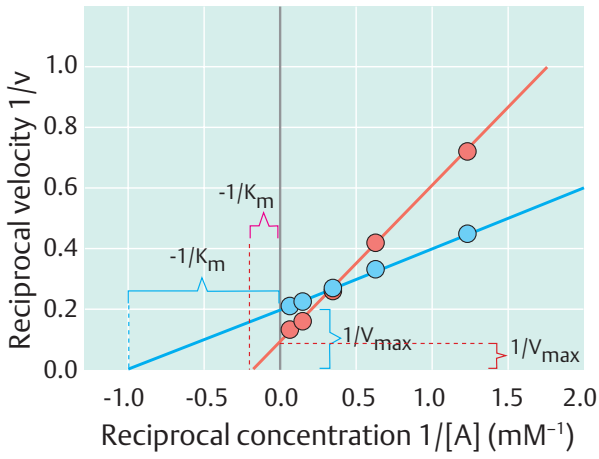
Many enzymes can occur in various *conformations* (see p. 72), which have different catalytic properties and whose proportion of the total number of enzyme molecules is influenced by substrates and other ligands (see pp. 116 and 280, for example). **Allosteric enzymes** of this type, which are usually present in oligomeric form, can be recognized by their S-shaped (*sigmoidal*) saturation curves, which cannot be described using the Michaelis model. In the case of isosteric enzymes (with only *one* enzyme conformation, **1**), the ef ciency of substrate binding (dashed curve) declines constantly with increasing [A], because the number of free binding sites is constantly decreasing. In most allosteric enzymes (**2**), the binding ef ciency initially rises with increasing [A], because the free enzyme is present in a low-af nity conformation (square symbols), which is gradually converted into a higher-af nity form (round symbols) as a result of binding with A. It is only at high [A] values that a lack of free binding sites becomes noticeable and the binding strength decreases again. In other words, the af nity of allosteric enzymes is not constant, but depends on the type and concentration of the ligand.

A. Michaelis Menten kinetics



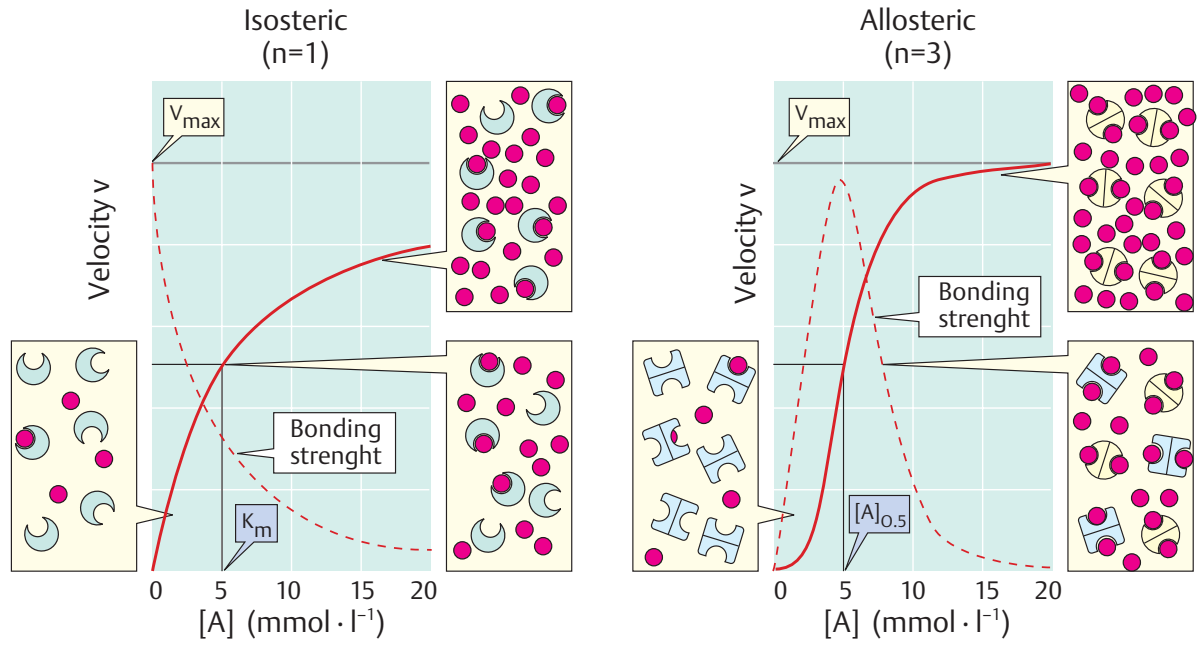


1. Hyperbolic plot



2. Lineweaver–Burk plot

B. Isosteric and allosteric enzymes



Enzyme kinetics II

The catalytic properties of enzymes, and consequently their activity (see p. 90), are influenced by numerous factors, which all have to be optimized and controlled if activity measurements are to be carried out in a useful and reproducible fashion. These factors include physical quantities (temperature, pressure), the chemical properties of the solution (pH value, ionic strength), and the concentrations of the relevant substrates, cofactors, and inhibitors.

A. pH and temperature dependency of enzyme activity ◑

The effect of enzymes is strongly dependent on the pH value (see p. 30). When the activity is plotted against pH, a *bell-shaped curve* is usually obtained (**1**). With animal enzymes, the **pH optimum**—i. e., the pH value at which enzyme activity is at its maximum—is often close to the pH value of the cells (i. e., pH 7). However, there are also exceptions to this. For example, the proteinase *pepsin* (see p. 270), which is active in the acidic gastric lumen, has a pH optimum of 2, while other enzymes (at least in the test tube) are at their most active at pH values higher than 9. The bell shape of the activity–pH profile results from the fact that amino acid residues with ionizable groups in the side chain are essential for catalysis. In example (**1**), these are a basic group B ($pK_a = 8$), which has to be protonated in order to become active, and a second acidic amino acid AH ($pK_a = 6$), which is only active in a dissociated state. At the optimum pH of 7, around 90% of both groups are present in the active form; at higher and lower values, one or the other of the groups increasingly passes into the inactive state.

The **temperature dependency** of enzymatic activity is usually asymmetric. With increasing temperature, the increased thermal movement of the molecules initially leads to a rate acceleration (see p. 22). At a certain temperature, the enzyme then becomes unstable, and its activity is lost within a narrow temperature difference as a result of denaturation (see p. 74). The optimal temperatures of the enzymes in higher organisms rarely exceed 50 °C, while enzymes from thermophilic bacteria found in hot springs, for instance, may still be active at 100 °C.

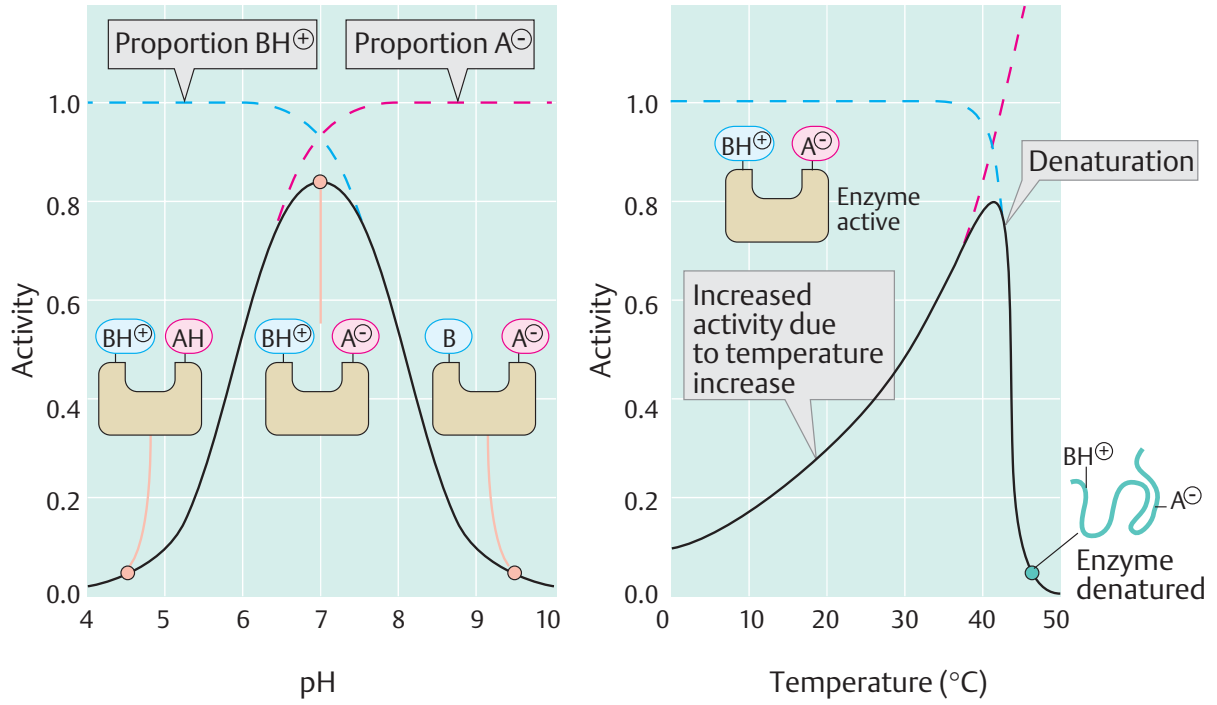
B. Substrate specificity ◑

Enzymes "recognize" their substrates in a highly specific way (see p. 88). It is only the marked **substrate specificity** of the enzymes that makes a regulated metabolism possible. This principle can be illustrated using the example of the two closely related proteinases *trypsin* and *chymotrypsin*. Both belong to the group of serine proteinases and contain the same "triad" of catalytically active residues (Asp–His–Ser, shown here in green; see p. 176). Trypsin selectively cleaves peptide bonds on the C-terminal side of basic amino acids (lysine and arginine), while chymotrypsin is specific for hydrophobic residues. The substrate binding "pockets" of both enzymes have a similar structure, but their amino acid sequences differ slightly. In trypsin, a negatively charged aspartate residue (Asp-189, red) is arranged in such a way that it can bind and fix the basic group in the side chain of the substrate. In chymotrypsin, the "binding pocket" is slightly narrower, and it is lined with neutral and hydrophobic residues that stabilize the side chains of apolar substrate amino acids through hydrophobic interactions (see p. 28).

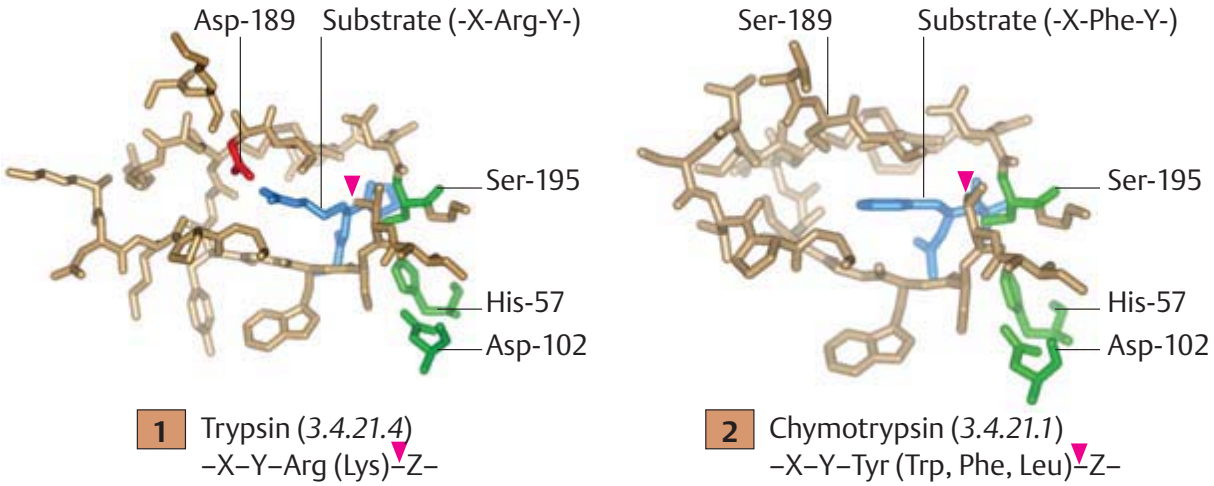
C. Bisubstrate kinetics ○

Almost all enzymes—in contrast to the simplified description given on p. 92—have more than one substrate or product. On the other hand, it is rare for more than two substrates to be bound *simultaneously*. In bisubstrate reactions of the type $A + B \rightarrow C + D$, a number of reaction sequences are possible. In addition to the *sequential mechanisms* (see p. 90), in which all substrates are bound in a specific sequence before the product is released, there are also mechanisms in which the first substrate A is bound and immediately cleaved. A part of this substrate remains bound to the enzyme, and is then transferred to the second substrate B after the first product C has been released. This is known as the **ping-pong mechanism**, and it is used by *transaminases*, for example (see p. 178). In the Lineweaver–Burk plot (right; see p. 92), it can be recognized in the parallel shifting of the lines when [B] is varied.

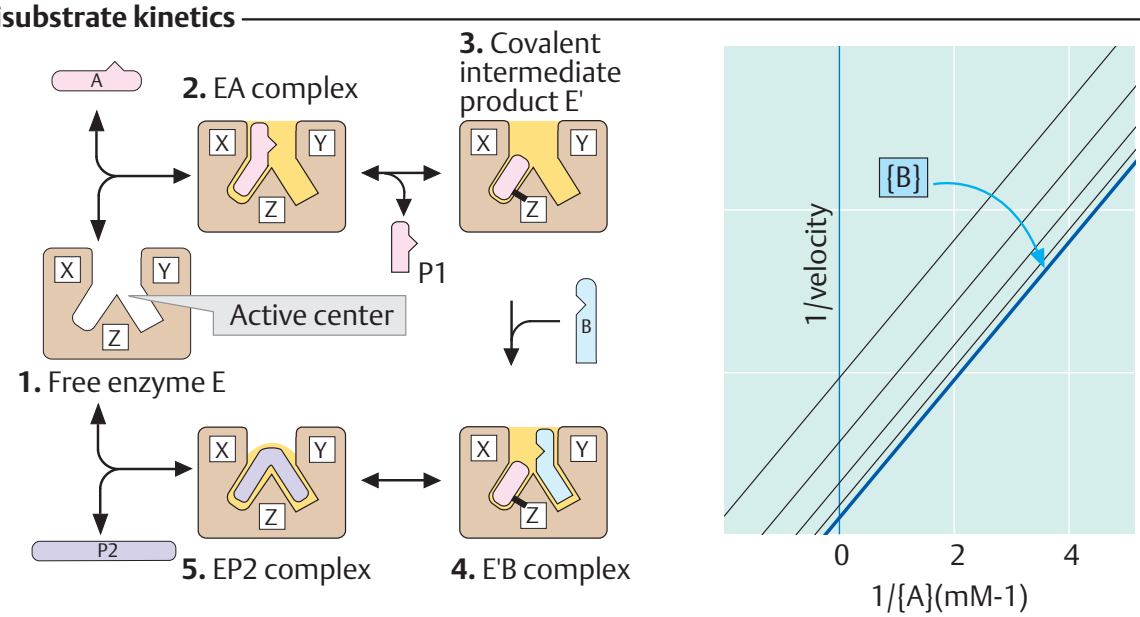
A. pH and temperature dependency of enzyme activity



B. Substrate specificity



C. Bisubstrate kinetics



Inhibitors

Many substances can affect metabolic processes by influencing the activity of enzymes. **Enzyme inhibitors** are particularly important here. A large proportion of **medicines** act as enzyme inhibitors. Enzyme-kinetic experiments are therefore an important aspect of drug development and testing procedures. Natural *metabolites* are also involved in regulatory processes as inhibitors (see p. 114).

A. Types of inhibitor ◑

Most enzyme inhibitors act **reversibly**—i. e., they do not cause any permanent changes in the enzyme. However, there are also **irreversible** inhibitors that permanently modify the target enzyme. The mechanism of action of an inhibitor—its **inhibition type**—can be determined by comparing the kinetics (see p. 92) of the inhibited and uninhibited reactions (**B**). This makes it possible to distinguish *competitive inhibitors* (left) from *noncompetitive inhibitors* (right), for example. *Allosteric inhibition* is particularly important for metabolic regulation (see below).

Substrate analogs (**2**) have properties similar to those of one of the substrates of the target enzyme. They are bound by the enzyme, but cannot be converted further and therefore *reversibly* block some of the enzyme molecules present. A *higher* substrate concentration is therefore needed to achieve a half-maximum rate; the Michaelis constant K_m increases (**B**). High concentrations of the substrate displace the inhibitor again. The maximum rate V_{max} is therefore not influenced by this type of inhibition. Because the substrate and the inhibitor compete with one another for the *same* binding site on the enzyme, this type of inhibition is referred to as **competitive**. **Analogs of the transition state** (**3**) usually also act competitively.

When an inhibitor interacts with a group that is important for enzyme activity, but does not affect binding of the substrate, the inhibition is **non-competitive** (right). In this case, K_m remains unchanged, but the concentration of functional enzyme $[E]_t$, and thus V_{max}, decrease. Non-competitive inhibitors generally act irreversibly, by modifying functional groups of the target enzyme (**4**).

"Suicide substrates" (**5**) are substrate analogs that also contain a reactive group. Initially, they bind reversibly, and then they form a covalent bond with the active center of the enzyme. Their effect is therefore also non-competitive. A well-known example of this is the antibiotic *penicillin* (see p. 254).

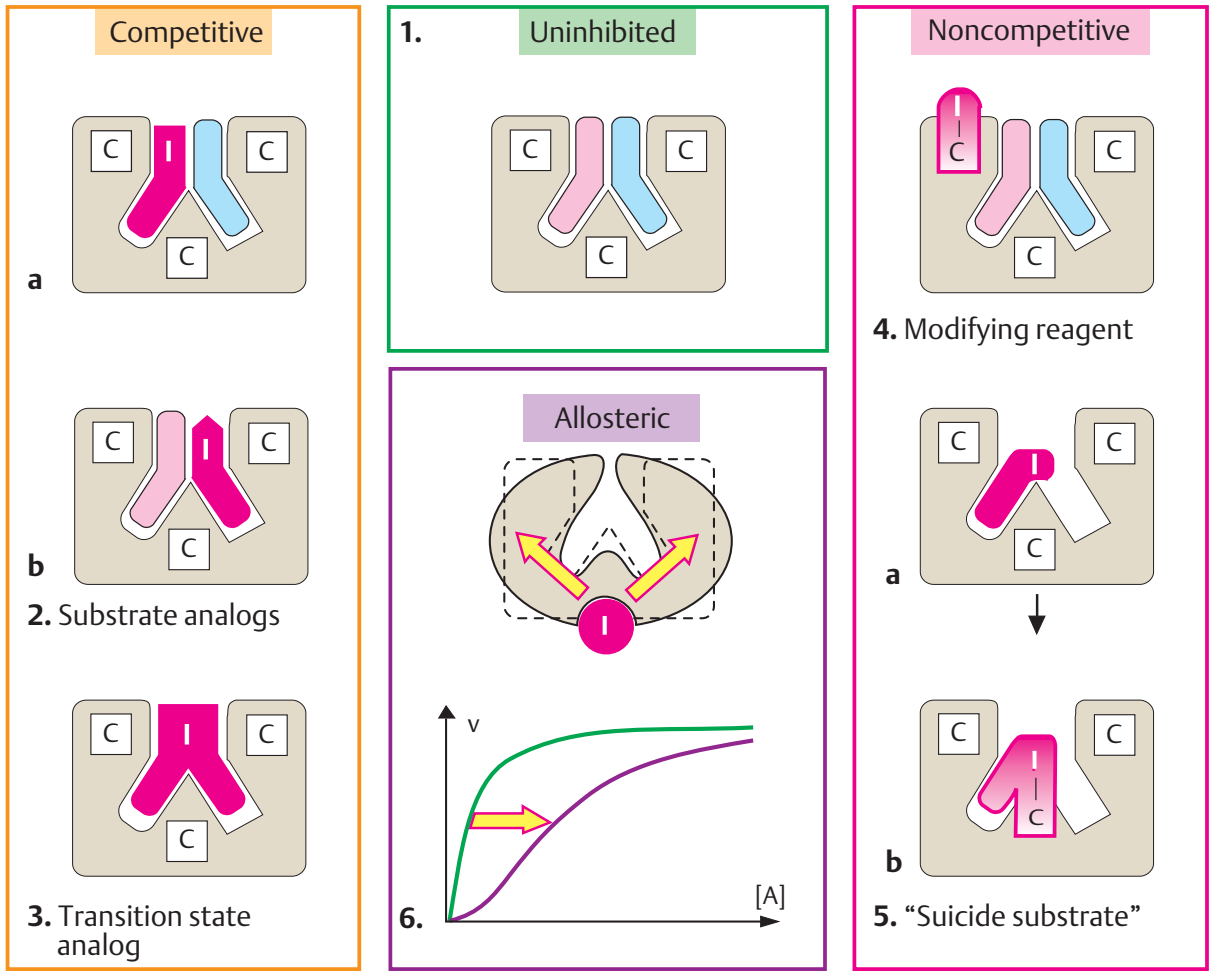
Allosteric inhibitors bind to a separate binding site outside the active center (**6**). This results in a *conformational change* in the enzyme protein that indirectly reduces its activity (see p. 116). Allosteric effects practically only occur in *oligomeric enzymes.* The kinetics of this type of system can no longer be described using the simple Michaelis–Menten model.

B. Inhibition kinetics ◑

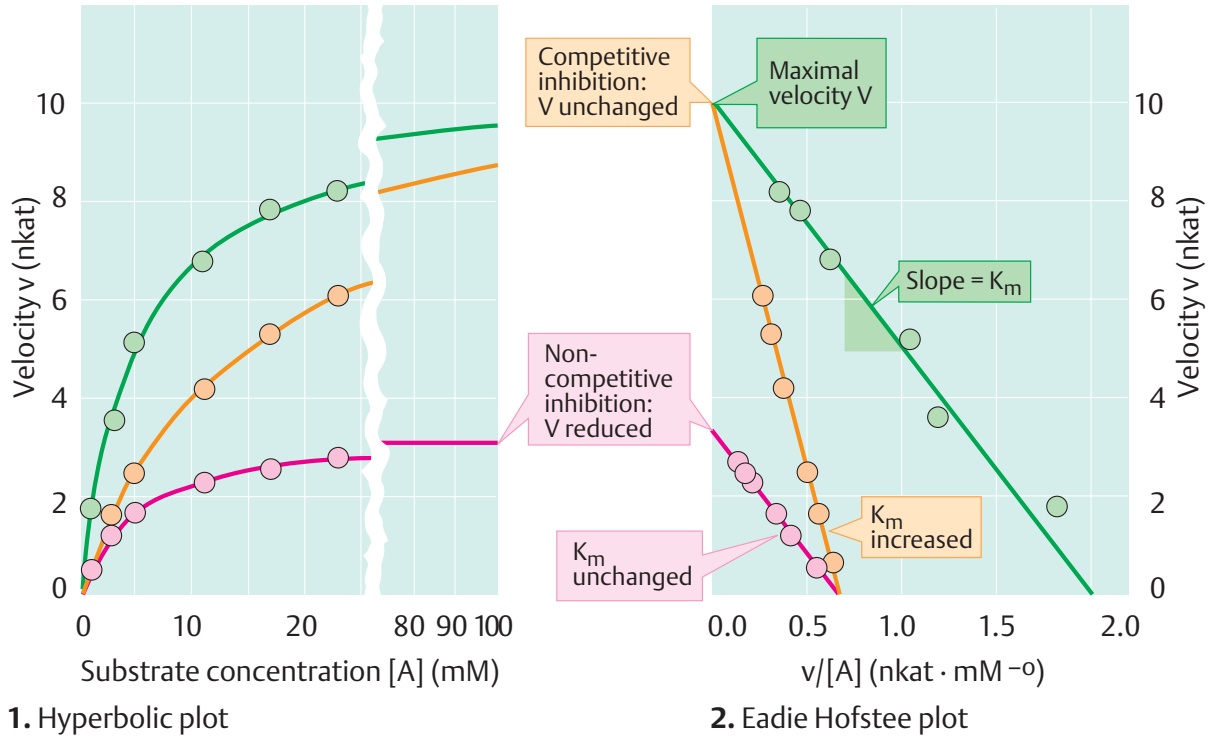
In addition to the Lineweaver–Burk plot (see p. 92), the *Eadie–Hofstee plot* is also commonly used. In this case, the velocity v is plotted against v /[A]. In this type of plot, V_{max} corresponds to the intersection of the approximation lines with the v axis, while K_m is derived from the gradient of the lines. Competitive and non-competitive inhibitors are also easily distinguishable in the Eadie—Hofstee plot. As mentioned earlier, **competitive** inhibitors only influence K_m, and not V_{max}. The lines obtained in the absence and presence of an inhibitor therefore intersect on the ordinate. **Non-competitive inhibitors** produce lines that have the same slope (K_m unchanged) but intersect with the ordinate at a lower level. Another type of inhibitor, not shown here, in which V_{max} and K_m are reduced by the same factor, is referred to as **uncompetitive**. Inhibitors with purely uncompetitive effects are rare. A possible explanation for this type of inhibition is selective binding of the inhibitor to the EA complex.

Allosteric enzymes shift the target enzyme's saturation curve to the left (see p. 92). In Eadie–Hofstee and Lineweaver–Burk plots (see p. 92), allosteric enzymes are recognizable because they produce curved lines (not shown).

A. Types of inhibitor



B. Kinetics of inhibition



Lactate dehydrogenase: structure

Lactate dehydrogenase (LDH, *EC 1.1.1.27*) is discussed in some detail here and on the next page as an example of the structure and function of an enzyme.

A. Lactate dehydrogenase: structure ○

The active form of lactate dehydrogenase (mass 144 kDa) is a **tetramer** consisting of four subunits (**1**). Each monomer is formed by a peptide chain of 334 amino acids (36 kDa). In the tetramer, the subunits occupy *equivalent positions* (**1**); each monomer has an active center. Depending on metabolic conditions, LDH catalyzes NADH-dependent reduction of pyruvate to lactate, or NAD^+-dependent oxidation of lactate to pyruvate (see p. 18).

The active center of an LDH subunit is shown schematically in Fig. **2**. The peptide backbone is shown as a light blue tube. Also shown are the substrate *lactate* (red), the coenzyme *NAD^+* (yellow), and three amino acid side chains (Arg-109, Arg-171, and His-195; green), which are directly involved in the catalysis. A *peptide loop* (pink) formed by amino acid residues 98–111 is also shown. In the absence of substrate and coenzyme, this partial structure is open and allows access to the substrate binding site (not shown). In the enzyme lactate NAD^+ complex shown, the peptide loop closes the active center. The catalytic cycle of lactate dehydrogenase is discussed on the next page.

B. Isoenzymes ◑

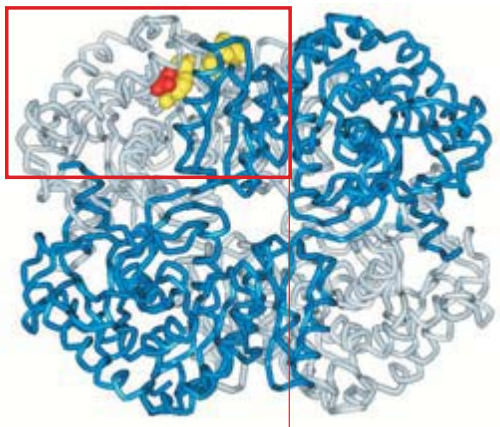
There are two *different* LDH subunits in the organism—M and H—which have a slightly different amino acid sequence and consequently different catalytic properties. As these two subunits can associate to form tetramers randomly, a total of five different **isoenzymes** of LDH are found in the body.

Fig. **1** shows sections from the amino acid sequences of the two subunits, using the single-letter notation (see p. 60). A common precursor gene was probably duplicated at some point in evolution. The two genes then continued to develop further independently of each other through mutation and selection.

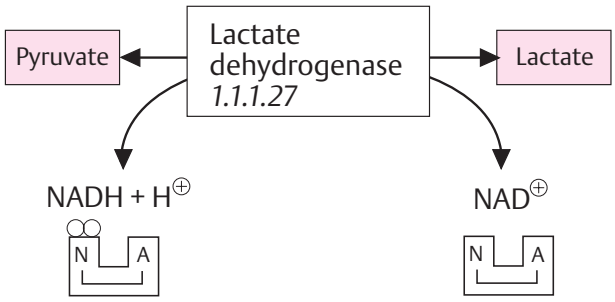
The differences in sequence between the M and H subunits are mainly *conservative*—i. e., both residues are of the same type, e. g. glycine (G) and alanine (A), or arginine (R) and lysine (K). Non-conservative exchanges are less frequent—e. g., lysine (K) for glutamine (Q), or threonine (T) for glutamic acid (E). Overall, the H subunit contains more acidic and fewer basic residues than the M form, and it therefore has a more strongly negative charge. This fact is exploited to separate the isoenzymes using electrophoresis (**2**; see pp. 78, 276). The isoenzyme LDH-1, consisting of four H subunits, migrates fastest, and the M_4 isoenzyme is slowest.

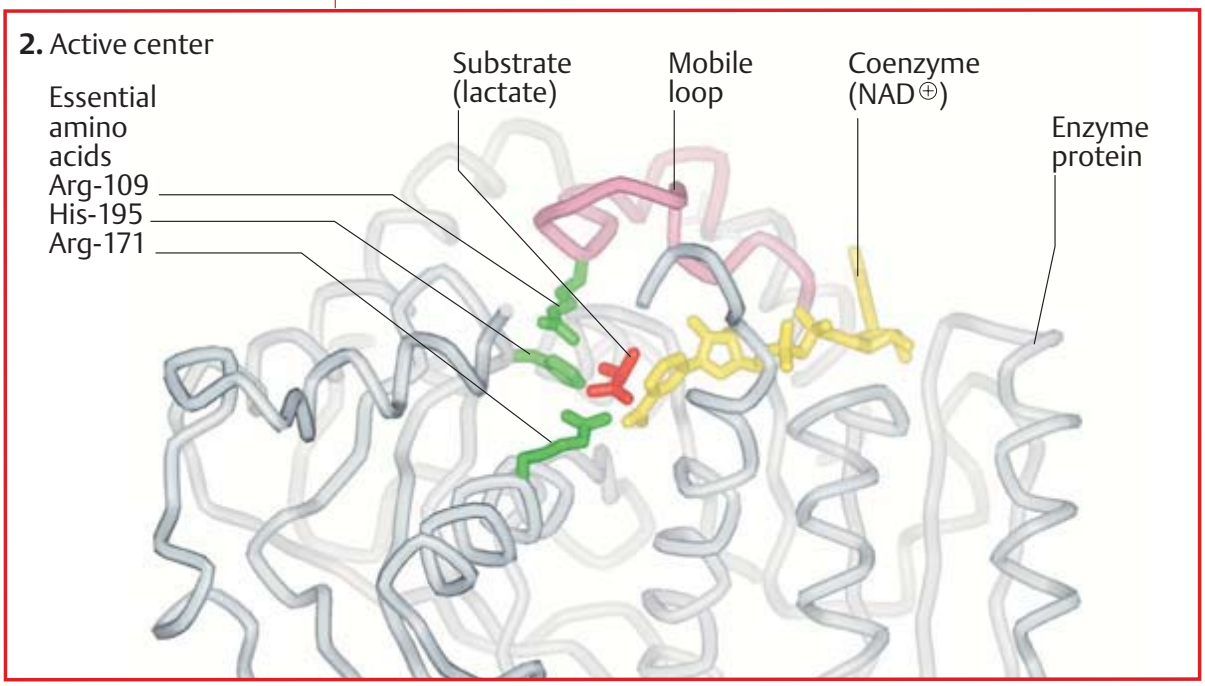
The separation and analysis of isoenzymes in blood samples is important in the diagnosis of certain diseases. Normally, only small amounts of enzyme activity are found in serum. When an organ is damaged, intracellular enzymes enter the blood and can be demonstrated in it (**serum enzyme diagnosis**). The total activity of an enzyme reflects the severity of the damage, while the type of isoenzyme found in the blood provides evidence of the site of cellular injury, since each of the genes is expressed in the various organs at different levels. For example, the liver and skeletal muscles mainly produce M subunits of lactate dehydrogenase (M for muscle), while the brain and cardiac muscle mainly express H subunits (H for heart). In consequence, each organ has a characteristic *isoenzyme pattern* (**3**). Following cardiac infarction, for example, there is a strong increase in the amount of LDH-1 in the blood, while the concentration of LDH-5 hardly changes. The isoenzymes of *creatine kinase* (see p. 336) are also of diagnostic importance.

A. Lactate dehydrogenase: structure

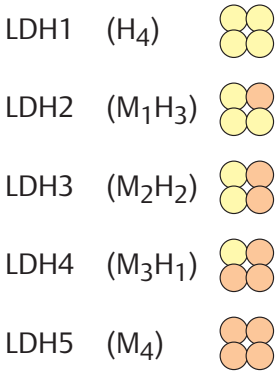


1. Tetramer 144 kDa

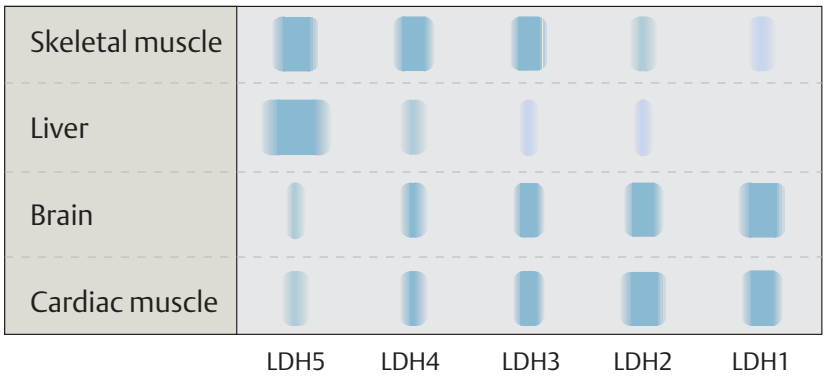




B. Isoenzymes



2. Forms



3. Separation by gel electrophoresis

Lactate dehydrogenase: mechanism

The principles of enzyme catalysis discussed on p. 90 can be illustrated using the reaction mechanism of lactate dehydrogenase (LDH) as an example.

A. Lactate dehydrogenase: catalytic cycle ○

LDH catalyzes the transfer of hydride ions (see p. 32) from lactate to NAD^+ or from NADH to pyruvate.

$$\text{L-lactate} + NAD^+ \leftrightarrows \text{pyruvate} + NADH + H^+$$

The equilibrium of the reaction strongly favors lactate *formation*. At high concentrations of lactate and NAD^+, however, oxidation of lactate to pyruvate is also possible (see p. 18). LDH catalyzes the reaction in *both* directions, but—like all enzymes—it has *no* effect on chemical equilibrium.

As the reaction is reversible, the catalytic process can be represented as a closed loop. The **catalytic cycle** of LDH is reduced to six "snapshots" here. Intermediate steps in catalysis such as those shown here are extremely short-lived and therefore dif cult to detect. Their existence was deduced indirectly from a large number of experimental findings—e. g., kinetic and binding measurements.

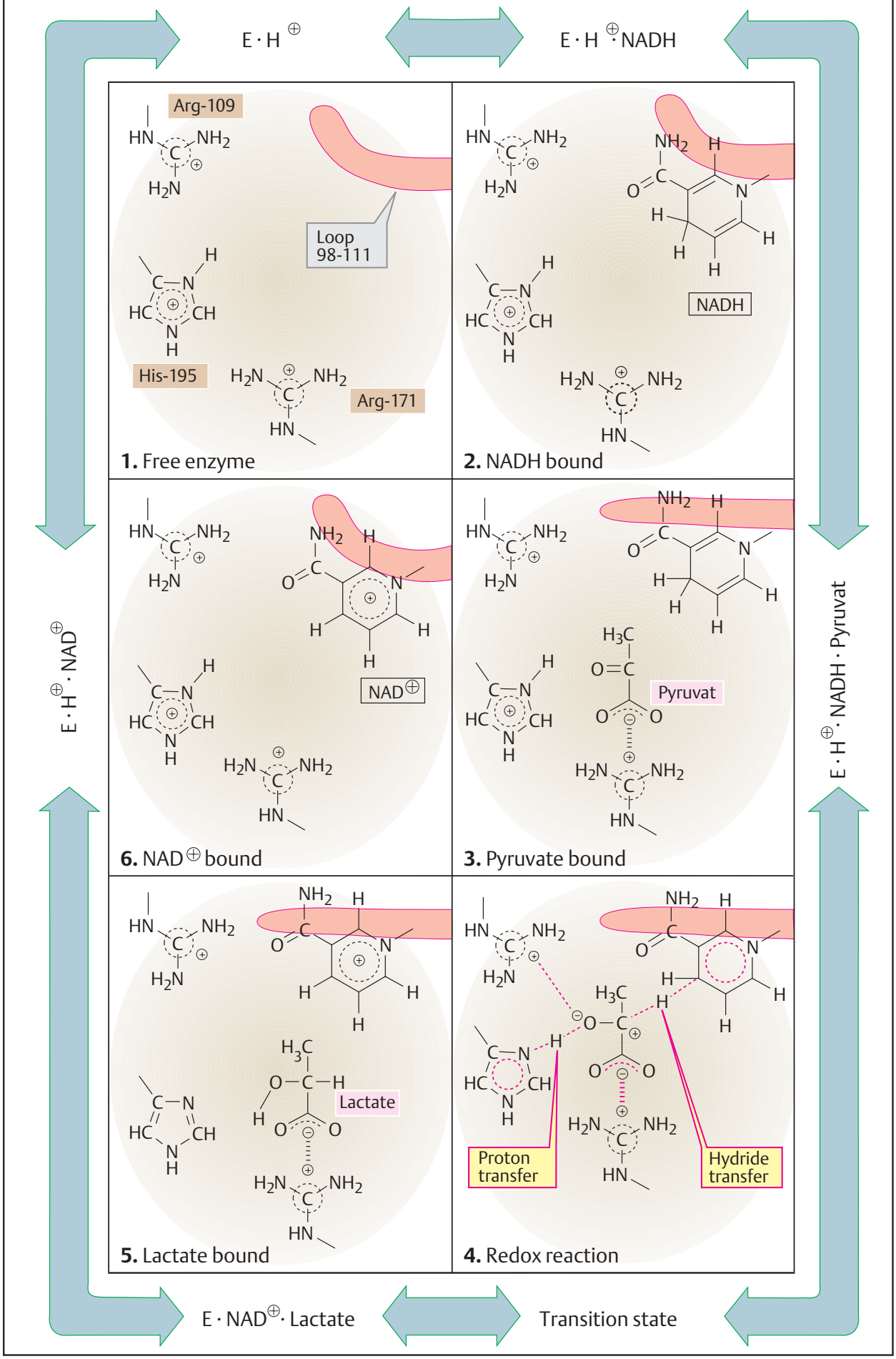
Many amino acid residues play a role in the **active center** of LDH. They can mediate the binding of the substrate and coenzyme, or take part in one of the steps in the catalytic cycle directly. Only the side chains of three particularly important residues are shown here. The positively charged guanidinium group of **arginine-171** binds the carboxylate group of the substrate by electrostatic interaction. The imidazole group of **histidine-195** is involved in acid–base catalysis, and the side chain of **arginine-109** is important for the stabilization of the transition state. In contrast to His-195, which changes its charge during catalysis, the two essential arginine residues are constantly protonated. In addition to these three residues, the **peptide loop 98–111** mentioned on p. 98 is also shown here schematically (red). Its function consists of closing the active center after binding of the substrate and coenzyme, so that water molecules are largely excluded during the electron transfer.

We can now look at the **partial reactions** involved in LDH-catalyzed pyruvate reduction.

In the free enzyme, His195 is protonated (**1**). This form of the enzyme is therefore described as E H^+. The coenzyme NADH is bound first (**2**), followed by pyruvate (**3**). It is important that the carbonyl group of the pyruvate in the enzyme and the active site in the nicotinamide ring of the coenzyme should have a fairly optimal position in relation to each other, and that this orientation should become fixed (*proximity and orientation of the substrates*). The 98–111 loop now closes over the active center. This produces a marked decrease in polarity, which makes it easier to achieve the **transition state** (**4**; *water exclusion*). In the transition state, a hydride ion, H^- (see p. 32), is transferred from the coenzyme to the carbonyl carbon (*group transfer*). The transient—and energetically unfavorable—negative charge on the oxygen that occurs here is stabilized by electrostatic interaction with Arg-109 (*stabilization of the transition state*). At the same time, a proton from His-195 is transferred to this oxygen atom (*group transfer*), giving rise to the enzyme-bound products lactate and NAD^+ (**5**). After the loop opens, lactate dissociates from the enzyme, and the temporarily uncharged imidazole group in His-195 again binds a proton from the surrounding water (**6**). Finally, the oxidized coenzyme NAD^+ is released, and the initial state (**1**) is restored. As the diagram shows, the proton that appears in the reaction equation ($NADH + H^+$) is not bound together with NADH, but after release of the lactate—i. e., between steps (**5**) and (**6**) of the *previous* cycle.

Exactly the same steps occur during the oxidation of lactate to pyruvate, but in the opposite direction. As mentioned earlier, the direction which the reaction takes depends not on the enzyme, but on the equilibrium state—i. e., on the concentrations of all the reactants and the pH value (see p. 18).

A. Lactate dehydrogenase: catalytic cycle



Enzymatic analysis

Enzymes play an important role in *biochemical analysis*. In biological material—e. g., in body fluids—even tiny quantities of an enzyme can be detected by measuring its catalytic activity. However, enzymes are also used as *reagents* to determine the concentrations of metabolites—e. g., the blood glucose level (**C**). Most enzymatic analysis procedures use the method of spectrophotometry (**A**).

A. Principle of spectrophotometry ○

Many substances *absorb* light in the visible or ultraviolet region of the spectrum. This property can be used to determine the concentration of such a substance. The extent of light absorption depends on the type and concentration of the substance and on the wavelength of the light used. **Monochromatic light**—i. e., light with a defined wavelength isolated from white light using a monochromator—is therefore used. Monochromatic light with an intensity of I_0 is passed through a rectangular vessel made of glass or quartz (a *cuvet*), which contains a solution of the absorbing substance. The **absorption A** of the solution (often also referred to as its *extinction*) is defined as the *negative decadic logarithm of the quotient* I/I_0. The **Beer–Lambert law** states that A is proportional to the concentration c of the absorbing substance and the thickness d of the solution it passes through. As mentioned earlier, the **absorption coef•cient** ε depends on the type of substance and the wavelength.

B. Measurement of lactate dehydrogenase activity ○

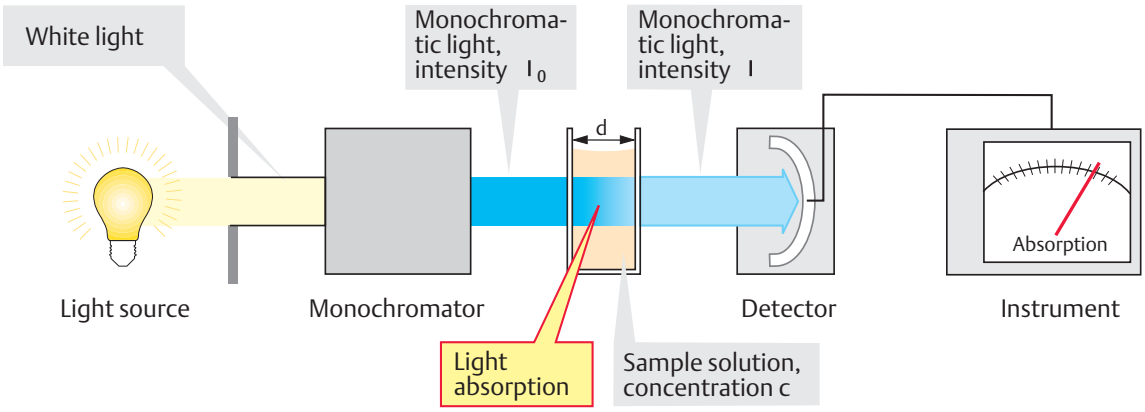
Measurement of lactate dehydrogenase (LDH) activity takes advantage of the fact that while the reduced coenzyme NADH + H^+ absorbs light at 340 nm, oxidized NAD^+ does not. *Absorption spectra* (i. e., plots of A against the wavelength) for the substrates and the coenzymes of the LDH reaction are shown in Fig. **1**. Differences in absorption behavior between NAD^+ and NADH between 300 and 400 nm result from changes in the nicotinamide ring during oxidation or reduction (see p. 32). To measure the activity, a solution containing lactate and NAD^+ is placed in a cuvet, and absorption is recorded at a *constant wavelength* of 340 nm. The uncatalyzed LDH reaction is very slow. It is only after addition of the enzyme that measurable quantities of NADH are formed and absorption increases. Since according to the Beer–Lambert law the rate of the increase in absorption $\Delta A/\Delta t$ is proportional to the reaction rate $\Delta c/\Delta t$. The absorption coef cient ε at 340 nm or comparison with a standard solution can be used to calculate LDH activity.

C. Enzymatic determination of glucose ○

Most biomolecules do not show any absorption in the visible or ultraviolet spectrum. In addition, they are usually present in the form of mixtures with other—similar—compounds that would also react to a chemical test procedure. These two problems can be avoided by using an appropriate enzyme to produce a colored dye selectively from the metabolite that is being analyzed. The absorption of the dye can then be measured.

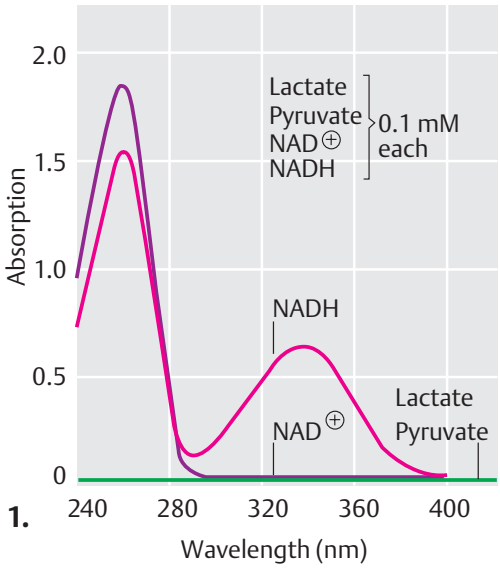
A procedure (**1**) that is often used to measure glucose when monitoring blood glucose levels (see p. 160) involves two successive reactions. The glucose-specific enzyme *glucose oxidase* (obtained from fungi) first produces hydrogen peroxide, H_2O_2, which in the second step—catalyzed by a *peroxidase*—oxidizes a colorless precursor into a green dye (**2**). When all of the glucose in the sample has been used up, the amount of dye formed—which can be measured on the basis of its light absorption—is equivalent to the quantity of glucose originally present.

A. Principle of spectrophotometry



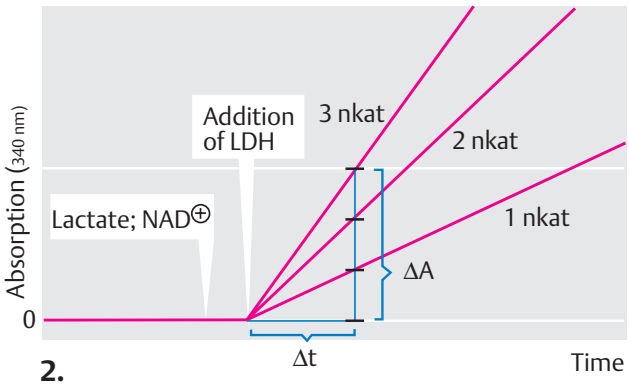
Absorption $A = -\log \frac{I}{I_0} = \varepsilon \cdot c \cdot d$ Beer Lambert law

B. Assay of lactate dehydrogenase activity



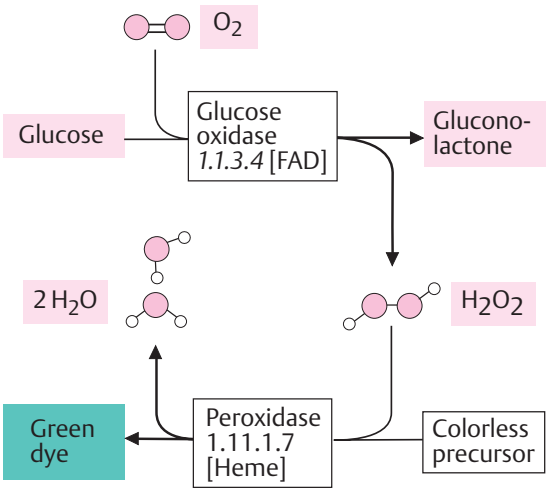
1.

$\frac{\Delta A}{\varepsilon} = \Delta c$; $\frac{\Delta A}{\Delta t \cdot \varepsilon} = \frac{\Delta c}{\Delta t} = v$; $v \approx$ Activity

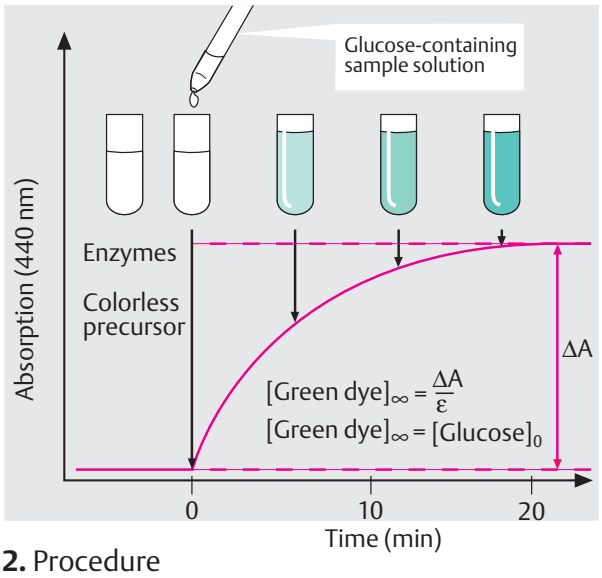


2.

C. Enzymatic determination of glucose



1. Reaction



2. Procedure

Coenzymes 1

A. Coenzymes: definitions ●

In many enzyme-catalyzed reactions, electrons or groups of atoms are transferred from one substrate to another. This type of reaction always also involves additional molecules, which temporarily accept the group being transferred. Helper molecules of this type are called **coenzymes**. As they are not catalytically active themselves, the less frequently used term *"cosubstrate"* would be more appropriate. In contrast to substrates for which a given enzyme is usually specific (see p. 88), coenzymes cooperate with many enzymes of varying substrate specificity. We have rather arbitrarily divided the coenzymes here into group-transferring and redox coenzymes. Strictly speaking, redox coenzymes also transfer groups—namely, reducing equivalents (see p. 32).

Depending on the type of interaction with the enzyme, a distinction is made between soluble coenzymes and prosthetic groups. **Soluble coenzymes** (**1**) are *bound like substrates* during a reaction, undergo a chemical change, and are then *released again*. The original form of the coenzyme is regenerated by a second, independent reaction. **Prosthetic groups** (**2**), on the other hand, are coenzymes that are *tightly bound to the enzyme* and remain associated with it during the reaction. The part of the substrate bound by the coenzyme is later transferred to another substrate or coenzyme of the *same* enzyme (not shown in Fig. **2**).

B. Redox coenzymes 1 ◑

All oxidoreductases (see p. 88) require coenzymes. The most important of these redox coenzymes are shown here. They can act in soluble form (S) or prosthetically (P). Their normal potentials $E^{0'}$ are shown in addition to the type of reducing equivalent that they transfer (see p. 18).

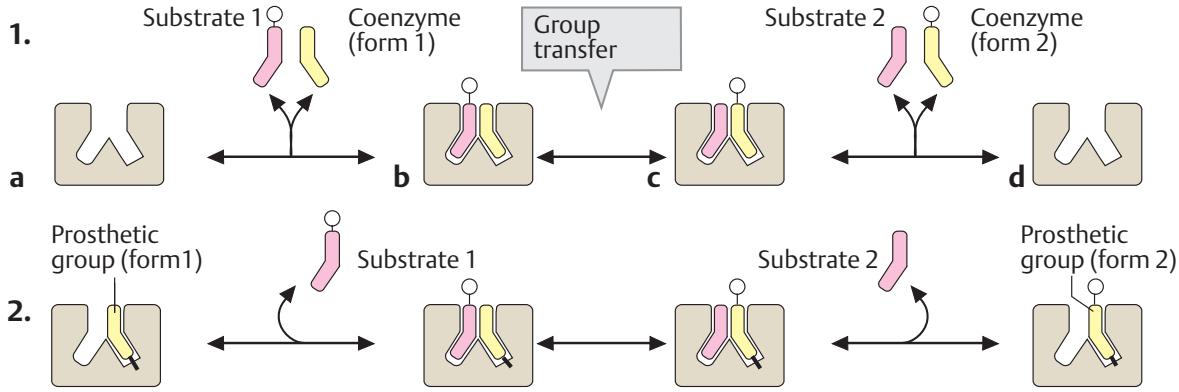
The pyridine nucleotides **NAD^+** and **$NADP^+$** (**1**) are widely distributed as coenzymes of dehydrogenases. They transport *hydride ions* ($2e^-$ and 1 H^+; see p. 32) and *always* act in soluble form. NAD^+ transfers reducing equivalents from catabolic pathways to the respiratory chain and thus contributes to energy metabolism. In contrast, reduced $NADP^+$ is the most important *reductant* involved in biosynthesis (see p. 112).

The flavin coenzymes **FMN** and **FAD** (**2**, **3**) contain *flavin* (isoalloxazine) as a redox-active group. This is a three-membered, *N*-containing ring system that can accept a maximum of two electrons and two protons during reduction. FMN carries the phosphorylated sugar alcohol *ribitol* at the flavin ring. FAD arises from FMN through bonding with AMP. The two coenzymes are functionally similar. They are found in *dehydrogenases, oxidases,* and *monooxygenases*. In contrast to the pyridine nucleotides, flavin reactions give rise to *radical intermediates* (see p. 32). To prevent damage to cell components, the flavins always remain bound as prosthetic groups in the enzyme protein.

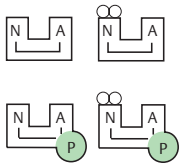
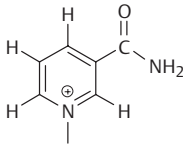
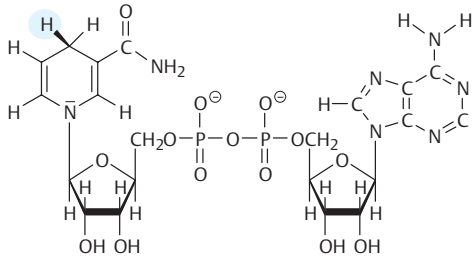
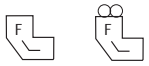
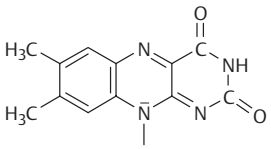
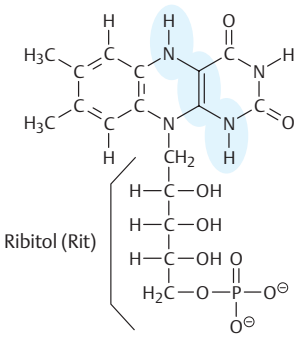
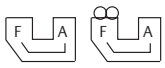
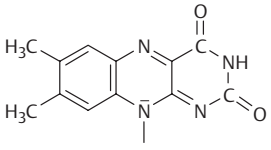
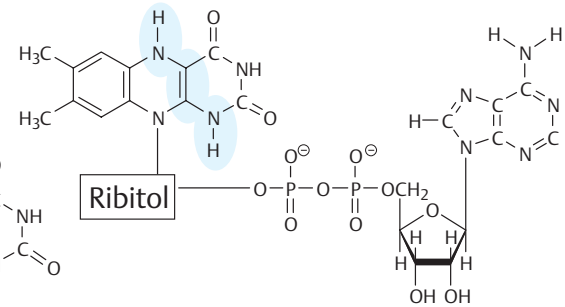
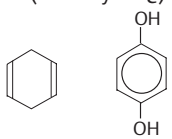
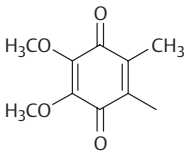
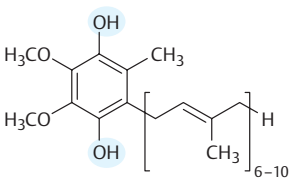
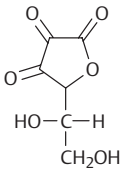
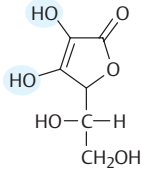
The role of **ubiquinone** (coenzyme Q, **4**) in transferring reducing equivalents in the respiratory chain is discussed on p. 140. During reduction, the *quinone* is converted into the *hydroquinone* (ubiquinol). The isoprenoid side chain of ubiquinone can have various lengths. It holds the molecule in the membrane, where it is freely mobile. Similar coenzymes are also found in photosynthesis (plastoquinone; see p. 132). *Vitamins E and K* (see p. 52) also belong to the quinone/hydroquinone systems.

L-Ascorbic acid (vitamin C, **5**) is a powerful reducing agent. As an **antioxidant**, it provides nonspecific protection against oxidative damage (see p. 284), but it is also an essential **cofactor** for various monooxygenases and dioxygenases. Ascorbic acid is involved in the hydroxylation of proline and lysine residues during the biosynthesis of collagen (see p. 344), in the synthesis of catecholamines (see p. 352) and bile acids (see p. 314), as well as in the breakdown of tyrosine (see p. 415). The reduced form of the coenzyme is a relatively strong acid and forms salts, the **ascorbates**. The oxidized form is known as **dehydroascorbic acid**. The stimulation of the immune system caused by ascorbic acid has not yet been fully explained.

A. Coenzymes: definitions



B. Redox coenzymes

Coenzyme	Oxidized form	Reduced form	Type	Trans-ferred	$E^{0\prime}$ (V)
1. NAD(P)$^{\oplus}$ 			L	$H^{\ominus}$	−0.32
2. Flavin mononucleotide (FMN) 			P	2[H]	−0.3 to +0.2
3. Flavin adenine dinucleotide (FAD) 			P	2[H]	−0.3 to +0.2
4. Ubiquinone (coenzym Q) 			L	2[H]	−0 to +0.2
5. Ascorbic acid			L	2[H]	+0.1

Coenzymes 2

A. Redox coenzymes 2 ◑

In **lipoic acid** (**6**), an intramolecular *disulfide bond* functions as a redox-active structure. As a result of reduction, it is converted into the corresponding *dithiol*. As a prosthetic group, lipoic acid is usually covalently bound to a lysine residue (R) of the enzyme, and it is then referred to as **lipoamide**. Lipoamide is mainly involved in oxidative decarboxylation of 2-oxo acids (see p. 134). The peptide coenzyme **glutathione** is a similar disulfide/dithiol system (not shown; see p. 284).

Iron–sulfur clusters (**7**) occur as prosthetic groups in oxidoreductases, but they are also found in lyases—e.g., *aconitase* (see p. 136) and other enzymes. Iron–sulfur clusters consist of 2–4 iron ions that are coordinated with cysteine residues of the protein (–SR) and with anorganic sulfide ions (S). Structures of this type are only stable in the interior of proteins. Depending on the number of iron and sulfide ions, distinctions are made between $[Fe_2S_2]$, $[Fe_3S_4]$, and $[Fe_4S_4]$ clusters. These structures are particularly numerous in the respiratory chain (see p. 140), and they are found in all complexes except complex IV.

Heme coenzymes (**8**) with redox functions exist in the *respiratory chain* (see p. 140), in *photosynthesis* (see p. 128), and in *monooxygenases* and *peroxidases* (see p. 24). Heme-containing proteins with redox functions are also referred to as **cytochromes**. In cytochromes, in contrast to hemoglobin and myoglobin, the iron changes its valence (usually between +2 and +3). There are several classes of heme (a, b, and c), which have different types of substituent $-R_1$ to $-R_3$. Hemoglobin, myoglobin, and the heme enzymes contain heme b. Two types of heme a are found in cytochrome *c* oxidase (see p. 132), while heme c mainly occurs in cytochrome *c*, where it is covalently bound with cysteine residues of the protein part via thioester bonds.

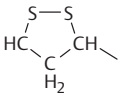
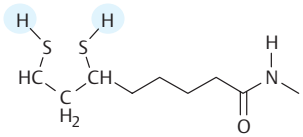
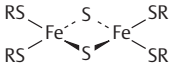
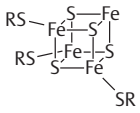
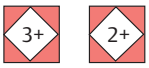
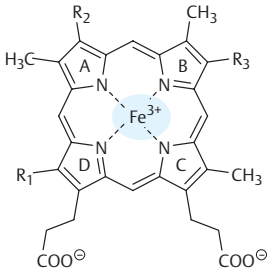
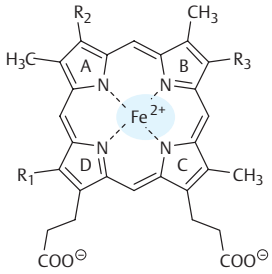
B. Group-transferring coenzymes 1 ◑

The **nucleoside phosphates** (**1**) are not only *precursors* for nucleic acid biosynthesis; many of them also have coenzyme functions. They serve for *energy conservation,* and as a result of *energetic coupling* (see p. 124) also allow endergonic processes to proceed. Metabolites are often made more reactive ("activated") as a result of the transfer of phosphate residues (*phosphorylation*). Bonding with nucleoside diphosphate residues (mainly UDP and CDP) provides activated precursors for polysaccharides and lipids (see p. 110). Endergonic formation of bonds by *ligases* (enzyme class 6) also depends on nucleoside triphosphates.

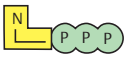
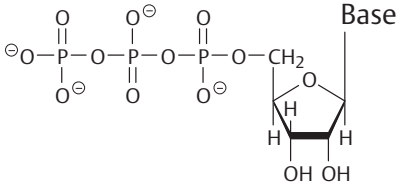
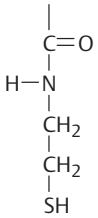
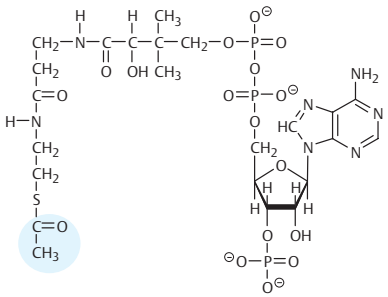
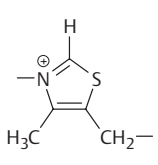
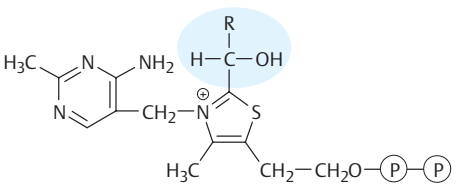
Acyl residues are usually activated by transfer to **coenzyme A** (**2**). In coenzyme A (see p. 12), *pantetheine* is linked to 3′-phospho-ADP by a phosphoric acid anhydride bond. Pantetheine consists of three components connected by amide bonds—*pantoic acid*, *β-alanine*, and *cysteamine*. The latter two components are biogenic amines formed by the decarboxylation of aspartate and cysteine, respectively. The compound formed from pantoic acid and β–alanine (*pantothenic acid*) has vitamin-like characteristics for humans (see p. 368). Reactions between the thiol group of the cysteamine residue and carboxylic acids give rise to **thioesters**, such as acetyl CoA. This reaction is strongly endergonic, and it is therefore coupled to exergonic processes. Thioesters represent the *activated form of carboxylic acids,* because acyl residues of this type have a high chemical potential and are easily transferred to other molecules. This property is often exploited in metabolism.

Thiamine diphosphate (TPP, **3**), in cooperation with enzymes, is able to activate aldehydes or ketones as *hydroxyalkyl groups* and then to pass them on to other molecules. This type of transfer is important in the transketolase reaction, for example (see p. 152). Hydroxyalkyl residues also arise in the decarboxylation of oxo acids. In this case, they are released as aldehydes or transferred to lipoamide residues of 2-oxoacid dehydrogenases (see p. 134). The functional component of TPP is the sulfur- and nitrogen-containing *thiazole ring*.

A. Redox coenzymes 2

Coenzyme	Oxidized form	Reduced form	Type	Trans-ferred	$E^{0\prime}$
6. Lipoamide 			P	2[H]	−0.29
7. Iron–sulfur cluster	$[Fe_2S_2]^{n+}$ 	$[Fe_4S_4]^{m+}$ 	P	$1e^{\ominus}$	−0.6 to +0.5
8. Heme 			P	$1e^{\ominus}$	0 to +0.5

B. Group-transferring coenzymes 1

Coenzyme (symbol)	Free form	Charged form	Group(s) trans-ferred	Important enzymes
1. Nucleoside phosphates 			P B-Rib B-Rib-P B-Rib-PP	Phospho-transferases Nucleotidyl-transferases (2.7.n.n) Ligases (6.n.n.n)
2. Coenzyme A 			Acyl residues	Acyltrans-ferases (2.3.n.n) CoA trans-ferases (2.8.3.n)
3. Thiamine diphosphate 			Hydroxy-alkyl residues	Decarboxy-lases (4.1.1.n) Oxoacid de-hydrogenases (1.2.4. n) Transketolase (2.2.1.1)

Coenzymes 3

A. Group-transferring coenzymes 2 ◑

Pyridoxal phosphate (**4**) is the most important coenzyme in amino acid metabolism. Its role in *transamination* reactions is discussed in detail on p. 178. Pyridoxal phosphate is also involved in other reactions involving amino acids, such as *decarboxylations* and *dehydrations*. The aldehyde form of pyridoxal phosphate shown here (left) is not generally found in free form. In the absence of substrates, the aldehyde group is covalently bound to the ε-amino group of a lysine residue as *aldimine* ("Schiff's base"). **Pyridoxamine phosphate** (right) is an intermediate of transamination reactions. It reverts to the aldehyde form by reacting with 2-oxoacids (see p. 178).

Biotin (**5**) is the coenzyme of the *carboxylases*. Like pyridoxal phosphate, it has an amide-type bond via the carboxyl group with a lysine residue of the carboxylase. This bond is catalyzed by a specific enzyme. Using ATP, biotin reacts with hydrogen carbonate (HCO_3^-) to form ***N*-carboxybiotin** . From this activated form, *carbon dioxide* (CO_2) is then transferred to other molecules, into which a carboxyl group is introduced in this way. Examples of biotindependent reactions of this type include the formation of oxaloacetic acid from pyruvate (see p. 154) and the synthesis of malonyl-CoA from acetyl-CoA (see p. 162).

Tetrahydrofolate (THF, **6**) is a coenzyme that can transfer *C_1 residues* in different oxidation states. THF arises from the vitamin *folic acid* (see p. 366) by double hydrogenation of the heterocyclic pterin ring. The C_1 units being transferred are bound to N-5, N-10, or both nitrogen atoms. The most important derivatives are:

a) N^5**-formyl-THF** and N^{10}**-formyl-THF**, in which the formyl residue has the oxidation state of a carboxylic acid;

b) N^5**-methylene-THF**, with a C_1 residue in the oxidation state of an aldehyde; and

c) N^5**-methyl-THF**, in which the methyl group has the oxidation state of an alcohol.

C_1 units transferred by THF play a role in the synthesis of methionine (see p. 412), purine nucleotides (see p. 188), and dTMP (see p. 190), for example. Due to the central role of THF derivatives in the biosynthesis of DNA precursors, the enzymes involved in THF metabolism are primary targets for cytostatic drugs (see p. 402).

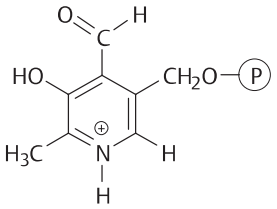
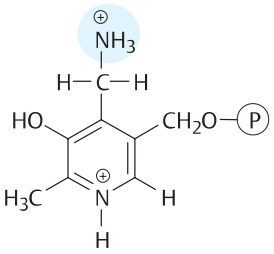
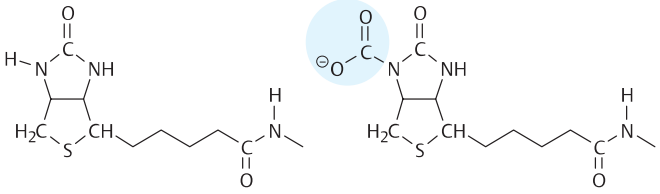
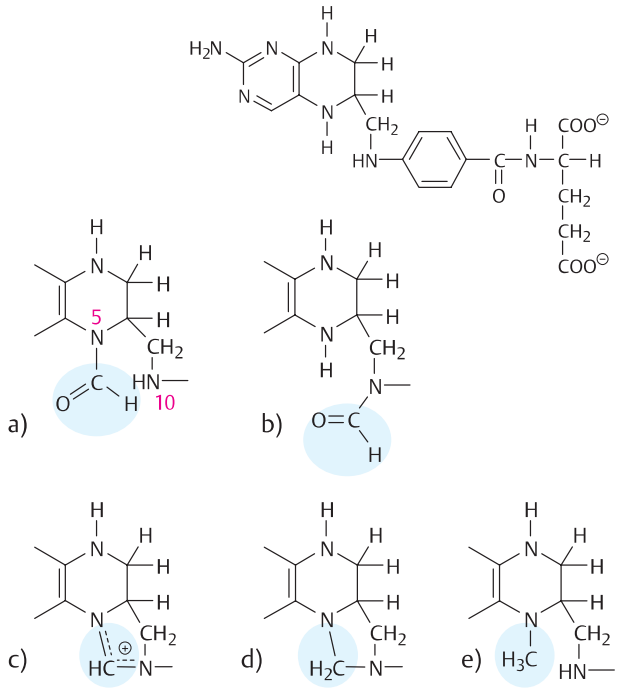
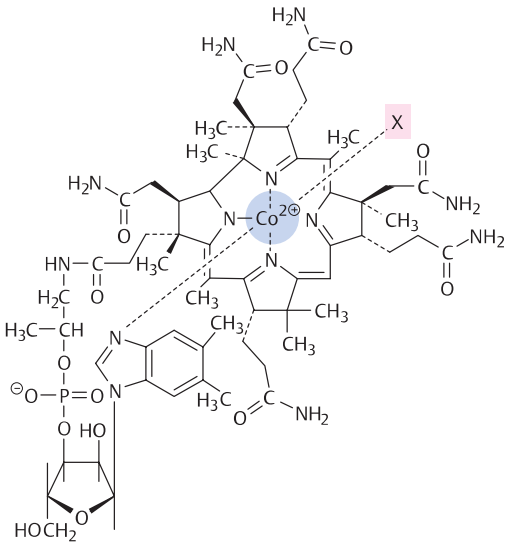
The **cobalamins** (**7**) are the chemically most complex form of coenzyme. They also represent the only natural substances that contain the transition metal *cobalt* (Co) as an essential component. Higher organisms are unable to synthesize cobalamins themselves, and are therefore dependent on a supply of **vitamin B_{12}** synthesized by bacteria (see p. 368).

The central component of the cobalamins is the **corrin** ring, a member of the tetrapyrroles, at the center of which the cobalt ion is located. The end of one of the side chains of the ring carries a nucleotide with the unusual base *dimethylbenzimidazole.* The ligands for the metal ion are the four N atoms of the pyrrole ring, a nitrogen from dimethylbenzimidazole, and a **group X**, which is organometallically bound—i. e., *mainly covalently.*

In **methylcobalamin**, X is a methyl group. This compound functions as a coenzyme for several *methyltransferases,* and among other things is involved in the synthesis of methionine from homocysteine (see p. 418). However, in human metabolism, in which methionine is an essential amino acid, this reaction does not occur.

Adenosylcobalamin (coenzyme B_{12}) carries a covalently bound adenosyl residue at the metal atom. This is a coenzyme of various *isomerases,* which catalyze rearrangements following a radical mechanism. The radical arises here through *homolytic cleavage* of the bond between the metal and the adenosyl group. The most important reaction of this type in animal metabolism is the rearrangement of methylmalonyl-CoA to form succinyl-CoA, which completes the breakdown of odd-numbered fatty acids and of the branched amino acids valine and isoleucine (see pp. 166 and 414).

A. Group-transferring coenzymes 2

Coenzyme	Free form	Charged form	Group(s) transferred	Important enzymes
4. Pyridoxal phosphate PLP			Amino group Amino acid residues	Transaminases (2.6.1.n) Many lyases (4.n.n.n)
5. Biotin B			$[CO_2]$	Carboxylases (6.4.1.n)
4. Pyridoxal phosphate THF			C_1 groups a) N^5-Formyl b) N^{10}-Formyl c) N^5N^{10}-Methenyl d) N^5N^{10}-Methylene e) N^5N^{10}-Methyl	C_1 transferases (2.1.n.n)
7. Cobalamin coenzymes			X = Adenosyl- X = Methyl-	Mutases *(5.4.n.n)* Methyltransferases *(2.1.1.n.)*

Activated metabolites

Many coenzymes (see pp. 104ff.) serve to *activate* molecules or groups that are poorly reactive. Activation consists of the formation of reactive intermediate compounds in which the group concerned is located at a higher chemical potential and can therefore be transferred to other molecules in an exergonic reaction (see p. 124). Acetyl-CoA is an example of this type of compound (see p. 12).

ATP and the other **nucleoside triphosphate coenzymes** not only transfer phosphate residues, but also provide the nucleotide components for this type of activation reaction. On this page, we discuss metabolites or groups that are activated in the metabolism by bonding with nucleosides or nucleotides. Intermediates of this type are mainly found in the metabolism of complex carbohydrates and lipids.

A. Activated metabolites ◑

1. Uridine diphosphate glucose (UDPglucose)

The inclusion of glucose residues into polymers such as glycogen or starches is an endergonic process. The activation of the **glucose** building blocks that is required for this takes places in several steps, in which two ATPs are used per glucose. After the phosphorylation of free glucose, glucose 6-phosphate is isomerized to glucose 1-phosphate (**a**), reaction with UTP (**b**) then gives rise to UDPglucose, in which the anomeric OH group at C-1 of the sugar is bound with phosphate. This "energy-rich" compound (an acetal phosphate) allows exergonic transfer of glucose residues to glycogen (**c**; see pp. 156, 408) or other acceptors.

2. Cytidine diphosphate choline (CDPcholine)

The amino alcohol **choline** is activated for inclusion in phospholipids following a similar principle (see p. 170). Choline is first phosphorylated by ATP to form choline phosphate (**a**), which by reaction with CTP and cleavage of diphosphate, then becomes CDPcholine. In contrast to (**1**), it is not choline that is transferred from CDPcholine, but rather choline phosphate, which with diacylglycerol yields phosphatidylcholine (lecithin).

3. Phosphoadenosine phosphosulfate (PAPS)

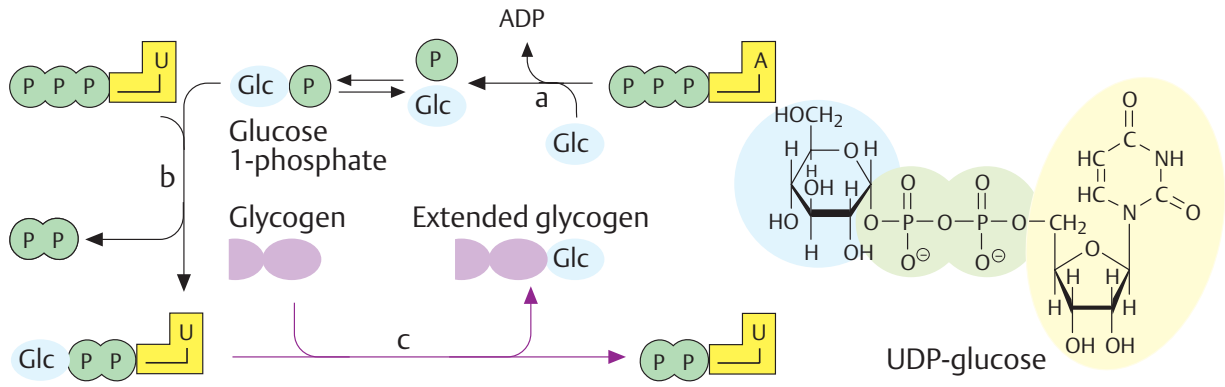
Sulfate residues occur as strongly polar groups in various biomolecules—e. g., in *glycosaminoglycans* (see p. 346) and *conjugates* of steroid hormones and xenobiotics (see p. 316). In the synthesis of the "activated sulfate" PAPS, ATP first reacts with anorganic sulfate to form adenosine phosphosulfate (APS, **a**). This intermediate already contains the "energy-rich" mixed anhydride bond between phosphoric acid and sulfuric acid. In the second step, the 3′-OH group of APS is phosphorylated, with ATP being used again. After transfer of the sulfate residue to OH groups (**c**), adenosine-3′,5′-bisphosphate remains.

4. *S*-adenosyl methionine (SAM)

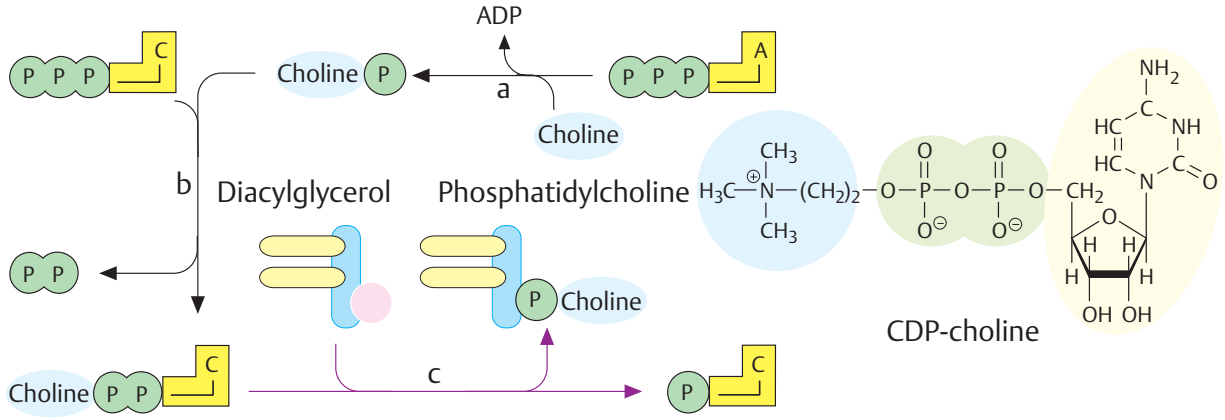
The coenzyme *tetrahydrofolate* (THF) is the main agent by which C_1 fragments are transferred in the metabolism. THF can bind this type of group in various oxidation states and pass it on (see p. 108). In addition, there is "activated methyl," in the form of *S*-adenosyl methionine (SAM). SAM is involved in many **methylation reactions**—e. g., in creatine synthesis (see p. 336), the conversion of norepinephrine into epinephrine (see p. 352), the inactivation of norepinephrine by methylation of a phenolic OH group (see p. 316), and in the formation of the active form of the cytostatic drug 6-mercaptopurine (see p. 402).

SAM is derived from degradation of the proteinogenic amino acid **methionine**, to which the adenosyl residue of an ATP molecule is transferred. After release of the activated methyl group, *S*-adenosyl homocysteine (SAH) is left over. This can be converted back into methionine in two further steps. Firstly, cleavage of the adenosine residue gives rise to the non-proteinogenic amino acid **homocysteine**, to which a methyl group is transferred once again with the help of N^5-methyl-THF (see p. 418). Alternatively, homocysteine can also be broken down into propionyl-CoA.

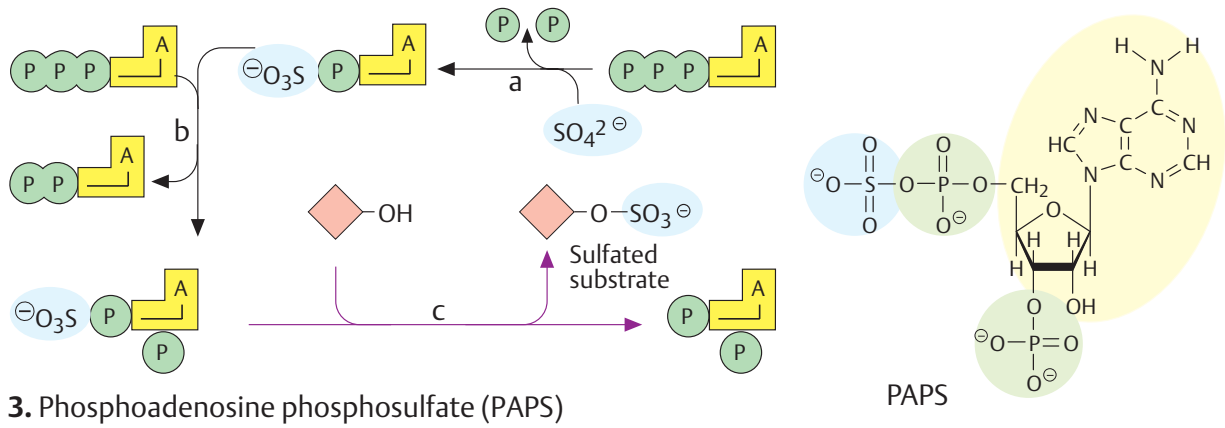
A. Activated metabolites



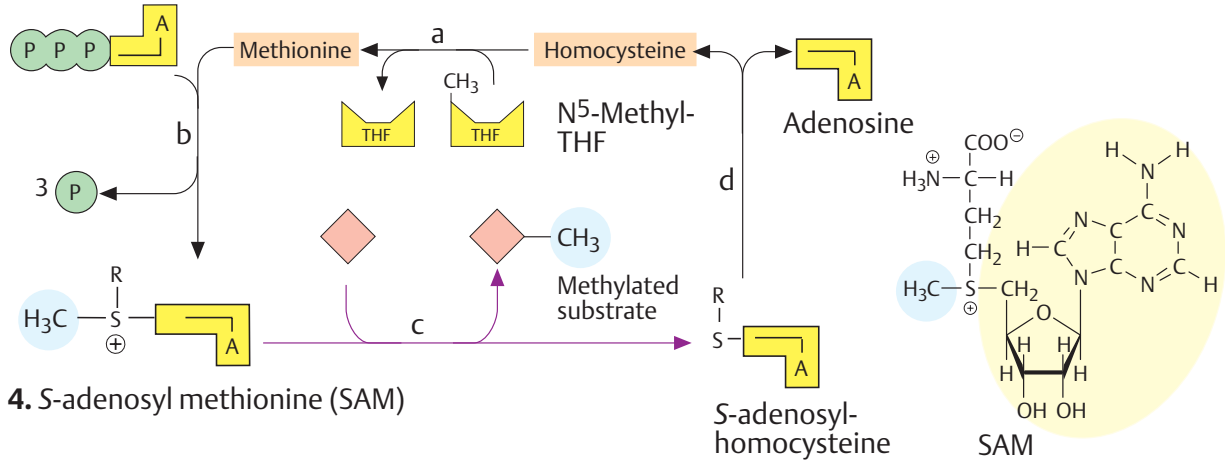
1. Uridine diphosphate glucose (UDP-glucose)



2. Cytidine diphosphate choline (CDPcholine)



3. Phosphoadenosine phosphosulfate (PAPS)



4. *S*-adenosyl methionine (SAM)

Intermediary metabolism

Hundreds of chemical reactions are constantly taking place in every cell, and taken together these are referred to as the **metabolism**. The chemical compounds involved in this are known as **metabolites**. Outside of the cell, almost all of the chemical changes in metabolites would only take place very slowly and without any specific direction. By contrast, organized sequences of chemical reactions with a high rate of throughput, known as **metabolic pathways**, become possible through the existence of specific **enzymes** (see p. 88).

A. Intermediary metabolism: overview ●

A number of central metabolic pathways are common to most cells and organisms. These pathways, which serve for synthesis, degradation, and interconversion of important metabolites, and also for energy conservation, are referred to as the **intermediary metabolism**.

In order to survive, all cells constantly require organic and inorganic *nutrients*, as well as *chemical energy,* which is mainly derived from ATP (see below). Depending on the way in which these needs are satisfied, organisms can be classified into autotrophic and heterotrophic groups. The **autotrophs**, which include plants and many microorganisms, can synthesize organic molecules from inorganic precursors (CO_2). An autotrophic lifestyle is possible through **photosynthesis**, for example (see p. 128). The **heterotrophs**—e. g., animals and fungi—depend on organic substances supplied in their diet. The schema shown on this page provides an overview of animal metabolism.

The polymeric substances contained in the diet (proteins, carbohydrates, and nucleic acids—top) cannot be used by the organism directly. Digestive processes first have to degrade them to monomers (amino acids, sugars, nucleotides). These are then mostly broken down by **catabolic pathways** (pink arrows) into smaller fragments. The metabolites produced in this way (generally referred to as the "metabolite pool") are then either used to obtain energy through further catabolic conversion, or are built up again into more complex molecules by **anabolic pathways** (blue arrows). Of the numerous metabolites in the pool, only three particularly important representatives—pyruvate, acetyl-CoA, and glycerol—are shown here. These molecules represent connecting links between the metabolism of proteins, carbohydrates, and lipids. The metabolite pool also includes the intermediates of the tricarboxylic acid cycle (**6**). This cyclic pathway has both catabolic and anabolic functions—i. e., it is **amphibolic** (violet; see p. 138).

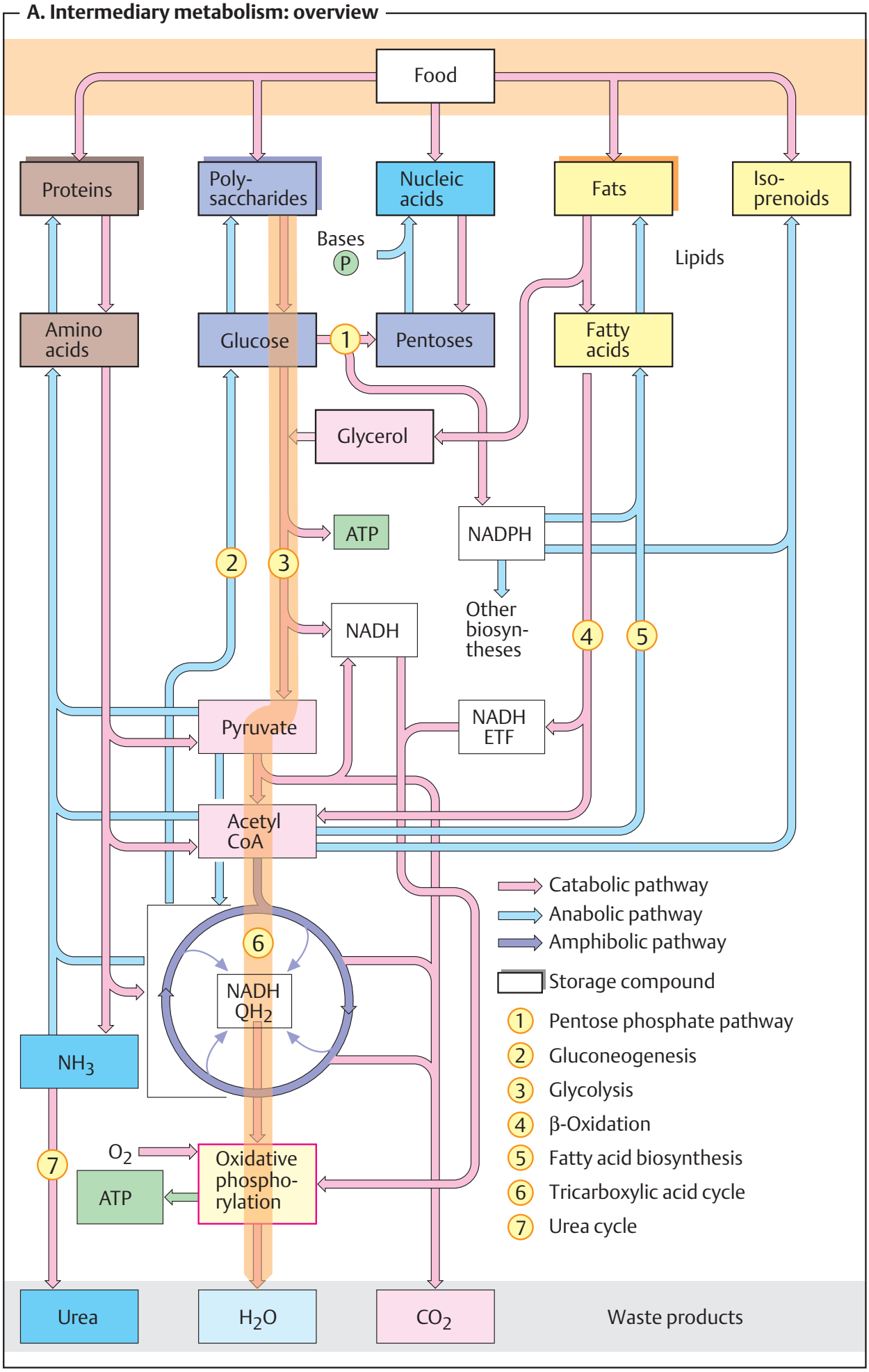
Waste products from the degradation of organic substances in animal metabolism include *carbon dioxide* (CO_2), *water* (H_2O), and *ammonia* (NH_3). In mammals, the toxic substance ammonia is incorporated into *urea* and excreted in this form (see p. 182).

The most important form of storage for chemical energy in all cells is **adenosine triphosphate** (ATP, see p. 122). ATP *synthesis* requires energy—i. e., the reaction is *endergonic*. Conversely, cleavage of ATP into ADP and phosphate releases energy. *Exergonic* hydrolysis of ATP, as a result of **energetic coupling** (see p. 16), makes energy-dependent (*endergonic*) processes possible. For example, most anabolic pathways, as well as movement and transport processes, are energy-dependent.

The most important pathway for the synthesis of ATP is **oxidative phosphorylation** (see p. 122). In this process, catabolic pathways first form reduced cofactors ($NADH+H^+$, QH_2, $ETFH_2$). Electrons are then transferred from these compounds to oxygen. This strongly exergonic process is catalyzed by the **respiratory chain** and used indirectly for the ATP synthesis (see p. 140). In *anaerobic conditions*—i. e., in the absence of oxygen—most organisms can fall back on ATP that arises in glycolysis (**3**). This less ef cient type of ATP synthesis is referred to as **fermentation** (see p. 146).

While NADH exclusively supplies oxidative phosphorylation, $NADPH+H^+$—a very similar coenzyme—is the reducing agent for anabolic pathways. $NADPH + H^+$ is mainly formed in the pentose phosphate pathway (PPP, **1**; see p. 152).

A. Intermediary metabolism: overview



Regulatory mechanisms

A. Fundamental mechanisms of metabolic regulation ◑

The activities of all metabolic pathways are subject to precise regulation in order to adjust the synthesis and degradation of metabolites to physiological requirements. An overview of the regulatory mechanisms is presented here. Further details are shown on pp. 116ff.

Metabolite flow along a metabolic pathway is mainly determined by the activities of the **enzymes** involved (see p. 88). To regulate the pathway, it is suf cient to change the activity of the enzyme that catalyzes the *slowest* step in the reaction chain. Most metabolic pathways have **key enzymes** of this type on which the regulatory mechanisms operate. The activity of key enzymes is regulated at three independent levels:

Transcriptional control. Here, Biosynthesis of the enzyme protein is influenced at the genetic level (**1**). Interventions in enzyme synthesis mainly affect synthesis of the corresponding mRNA—i. e., *transcription* of the gene coding for the enzyme. The term "transcriptional control" is therefore used (see pp. 118, 244). This mechanism is mediated by *regulatory proteins* (transcription factors) that act directly on DNA. The genes have a special regulatory segment for this purpose, known as the *promoter* region, which contains binding sites (control elements) for regulatory proteins. The activity of these proteins is, in turn, affected by metabolites or hormones. When synthesis of a protein is increased by transcriptional control, the process is referred to as **induction**; when it is reduced or suppressed, it is referred to as **repression**. Induction and repression processes take some time and are therefore not immediately effective.

Interconversion of key enzymes (**2**) takes effect considerably faster than transcriptional control. In this case, the enzyme is already present at its site of effect, but it is initially still inactive. It is only when needed that it is converted into the catalytically active form, after signaling and mediation from second messengers (see p. 120) through an *activating enzyme* (E_1). If the metabolic pathway is no longer required, an *inactivating enzyme* (E_2) returns the key enzyme to its inactive resting state.

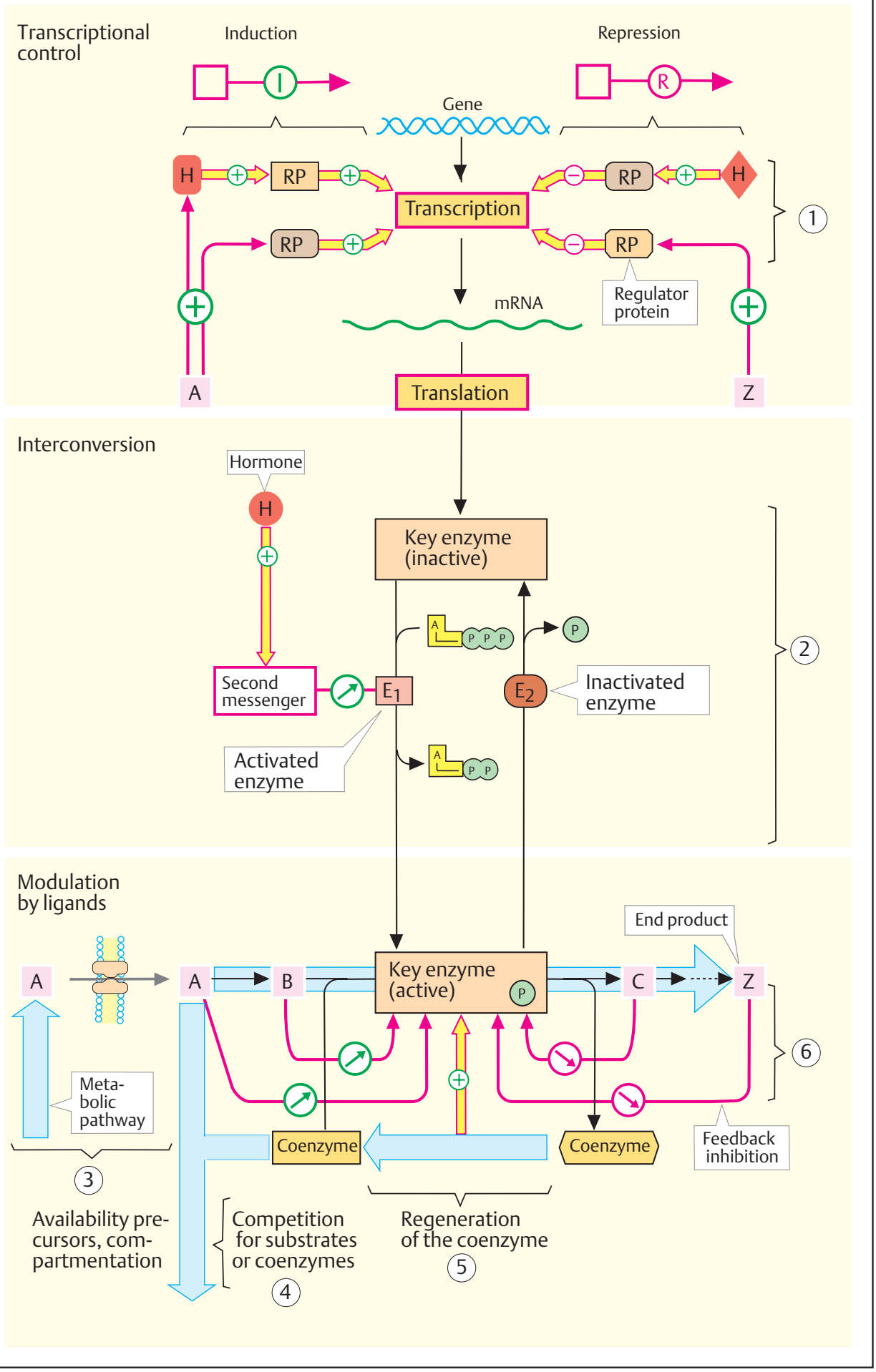
Interconversion processes in most cases involve **ATP-dependent phosphorylation** of the enzyme protein by a *protein* kinase or **dephosphorylation** of it by a *protein phosphatase* (see p. 120). The phosphorylated form of the key enzyme is usually the more active one, but the reverse may also occur.

Modulation by ligands. An important variable that regulates flow through a metabolic pathway is **precursor availability** (metabolite A in the case shown here). The availability of precursor A increases along with the activity of the metabolic pathways that form A (**3**) and it decreases with increasing activity of other pathways that also consume A (**4**). Transport from one cell compartment to another can also restrict the availability of A.

Coenzyme availability can also often have a limiting effect (**5**). If the coenzyme is regenerated by a second, independent metabolic pathway, the speed of the second pathway can limit that of the first one. For example, glycolysis and the tricarboxylic acid cycle are mainly regulated by the availability of NAD^+ (see p. 146). Since NAD^+ is regenerated by the respiratory chain, the latter indirectly controls the breakdown of glucose and fatty acids (respiratory control, see p. 144).

Finally, the activity of key enzymes can be regulated by *ligands* (substrates, products, coenzymes, or other effectors), which as *allosteric effectors* do not bind at the active center itself, but at another site in the enzyme, thereby modulating enzyme activity (**6**; see p. 116). Key enzymes are often inhibited by immediate reaction products, by end products of the reaction chain concerned (*"feedback" inhibition*), or by metabolites from completely different metabolic pathways. The precursors for a reaction chain can stimulate their own utilization through enzyme activation.

A. Fundamental mechanisms of metabolic regulation



Allosteric regulation

The regulation of **aspartate carbamoyltransferase** (ACTase), a key enzyme of pyrimidine biosynthesis (see p. 188) is discussed here as an example of allosteric regulation of enzyme activity. Allosteric effects are mediated by the substrate itself or by inhibitors and activators (*allosteric effectors,* see p. 114). The latter bind at special sites outside the active center, producing a conformational change in the enzyme protein and thus indirectly lead to an alteration in its activity.

A. Aspartate carbamoyltransferase: reaction ○

ACTase catalyzes the transfer of a carbamoyl residue from carbamoyl phosphate to the amino group of L-aspartate. The *N*-carbamoyl L-aspartate formed in this way already contains all of the atoms of the later pyrimidine ring (see p. 188). The ACTase of the bacterium *Escherichia coli* is inhibited by cytidine triphosphate (CTP), an end product of the anabolic metabolism of pyrimidines, and is activated by the precursor ATP.

B. Kinetics ◑

In contrast to the kinetics of isosteric (normal) enzymes, allosteric enzymes such as ACTase have **sigmoidal** (S-shaped) **substrate saturation curves** (see p. 92). In allosteric systems, the enzyme's af nity to the substrate is not constant, but depends on the substrate concentration [A]. Instead of the Michaelis constant K_m (see p. 92), the *substrate concentration at half-maximal rate* ($[A]_{0.5}$) is given. The sigmoidal character of the curve is described by the **Hill coef•cient h**. In isosteric systems, h = 1, and h increases with increasing sigmoidicity.

Depending on the enzyme, *allosteric effectors* can influence the maximum rate V_{max}, the semi-saturation concentration $[A]_{0.5}$, and the Hill coef cient h. If it is mainly V_{max} that is changed, the term "**V system**" is used. Much more common are "**K systems**", in which allosteric effects only influence $[A]_{0.5}$ and h.

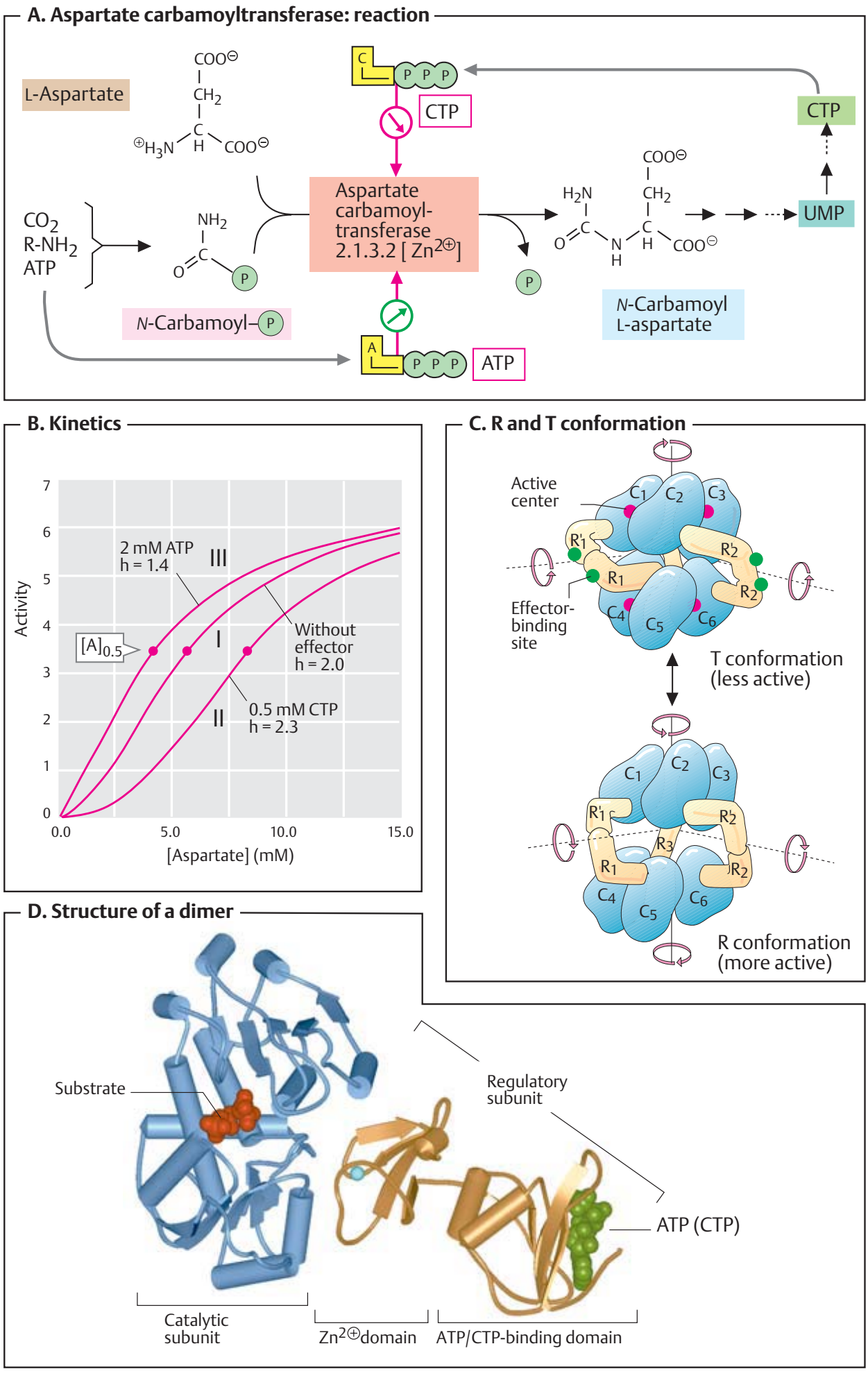
The K type also includes ACTase. The inhibitor CTP in this case leads to *right-shifting* of the curve, with an increase in $[A]_{0.5}$ and h (curve II). By contrast, the activator ATP causes a *left shift;* it reduces both $[A]_{0.5}$ and h (curve III). This type of allosteric effect was first observed in *hemoglobin* (see p. 280), which can be regarded as an "honorary" enzyme.

C. R and T states ○

Allosteric enzymes are almost always *oligomers* with 2–12 subunits. ACTase consists of six catalytic subunits (blue) and six regulatory subunits (yellow). The latter bind the allosteric effectors CTP and ATP. Like hemoglobin, ACTase can also be present in two conformations—the less active **T state** (for "tense") and the more active **R state** (for "relaxed"). Substrates and effectors influence the equilibrium between the two states, and thereby give rise to sigmoidal saturation behavior. With increasing aspartate concentration, the equilibrium is shifted more and more toward the R form. ATP also stabilizes the R conformation by binding to the regulatory subunits. By contrast, binding of CTP to the same sites promotes a transition to the T state. In the case of ACTase, the structural differences between the R and T conformations are particularly dramatic. In T → R conversion, the catalytic subunits separate from one another by 1.2 nm, and the subunits also rotate around the axis of symmetry. The conformations of the subunits themselves change only slightly, however.

D. Structure of a dimer ○

The subunits of ACTase each consist of two *domains*—i. e., independently folded partial structures. The *N*-terminal domain of the regulatory subunit (right) mediates interaction with CTP or ATP (green). A second, Zn^{2+}-containing domain (Zn^{2+} shown in light blue) establishes contact with the neighboring catalytic subunit. Between the two domains of the catalytic subunit lies the active center, which is occupied here by two substrate analogs (red).



Transcription control

A. Functioning of regulatory proteins ◑

Regulatory proteins (transcription factors) are involved in controlling gene expression in all cells. These regulatory proteins bind to specific DNA sequences and thereby activate or inhibit the transcription of genes **(Transcription control)**. The effects of transcription factors are usually reversible and are often controlled by *ligands* or by *interconversion.*

The nomenclature for transcription factors is confusing. Depending on their mode of action, various terms are in use both for the proteins themselves and for the DNA sequences to which they bind. If a factor blocks transcription, it is referred to as a **repressor**; otherwise, it is called an **inducer.** DNA sequences to which regulatory proteins bind are referred to as **control elements.** In prokaryotes, control elements that serve as binding sites for RNA polymerases are called **promoters,** whereas repressor-binding sequences are usually called **operators**. Control elements that bind activating factors are termed **enhancers**, while elements that bind inhibiting factors are known as **silencers**.

The numerous regulatory proteins that are known can be classified into four different groups (**1**–**4**), based on their mechanisms of action. **Negative gene regulation**—i. e., switching off of the gene concerned—is carried out by **repressors**. Some repressors only bind to DNA (**1a**) in the absence of specific ligands (L). In this case, the complex between the repressor and the ligand loses its ability to bind to the DNA, and the promoter region becomes accesible for binding of RNA polymerase (**1b**). It is often the free repressor that does not bind to the DNA, so that transcription is only blocked in the presence of the ligand (**2a**, **2b**). A distinction between two different types of **positive gene regulation** can be made in the same way. If it is only the free inducer that binds, then transcription is inhibited by the appropriate ligand (**3**). Conversely, many **inducers** only become active when they have bound a ligand (**4**). This group includes the receptors for steroid hormones, for example (see p. 378).

B. Lactose operon ○

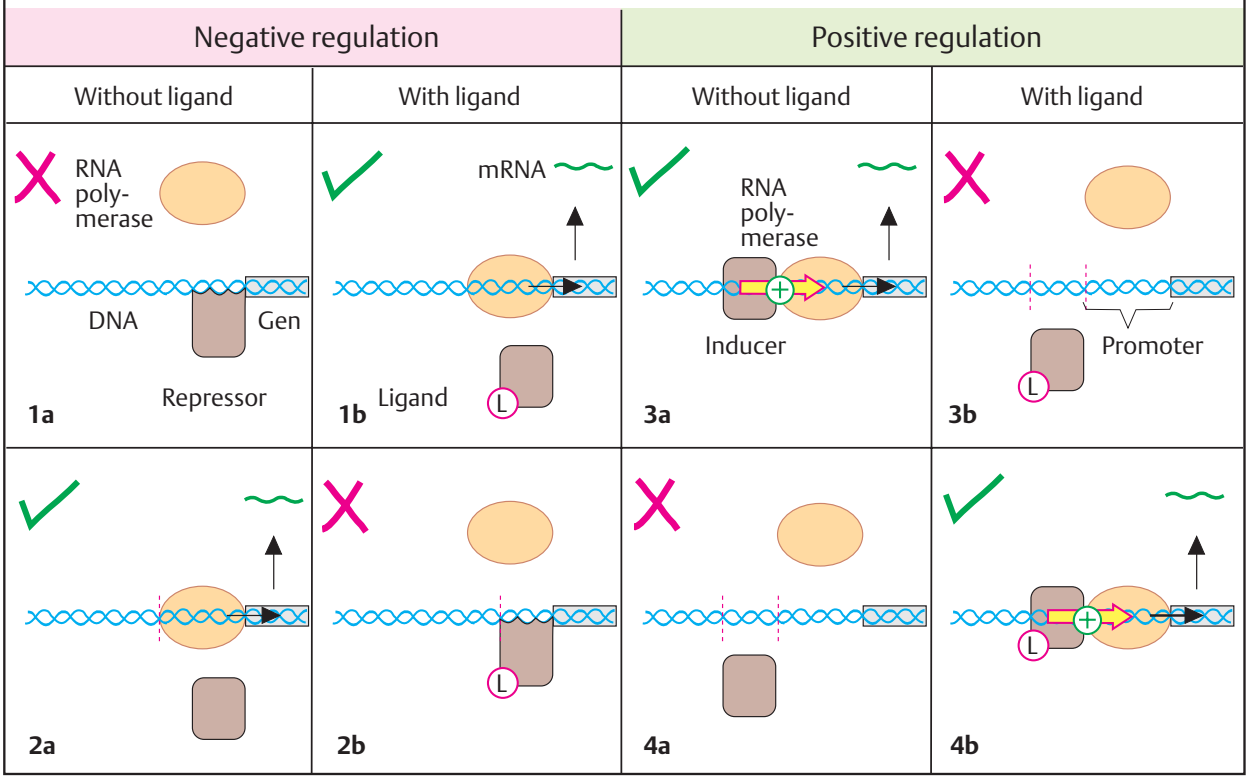
The well-investigated **lactose operon** of the bacterium *Escherichial coli* can be used here as an example of transcriptional control. The *lac* operon is a DNA sequence that is simultaneously subject to negative and positive control. The operon contains the *structural genes* for three proteins that are required for the utilization of lactose (one transporter and two enzymes), as well as *control elements* that serve to regulate the operon.

Since lactose is converted to glucose in the cell, there is no point in expressing the genes if glucose is already available. And indeed, the genes are in fact only transcribed when *glucose is absent* and *lactose is present* (**3**). This is achieved by interaction between two regulatory proteins. In the absence of lactose, the ***lac repressor*** blocks the promoter region (**2**). When lactose is available, it is converted into *allolactose*, which binds to the repressor and thereby detaches it from the operator (**3**). However, this is still not suf cient for the transcription of the structural genes. For binding of the RNA polymerase to take place, an *inducer*—the **catabolite activator protein (CAP)**—is required, which only binds to the DNA when it is present as a complex with 3,5′-cyclo-AMP (cAMP; see p. 386). cAMP, a signal for nutrient deficiency, is only formed by *E. coli* in the *absence* of glucose.

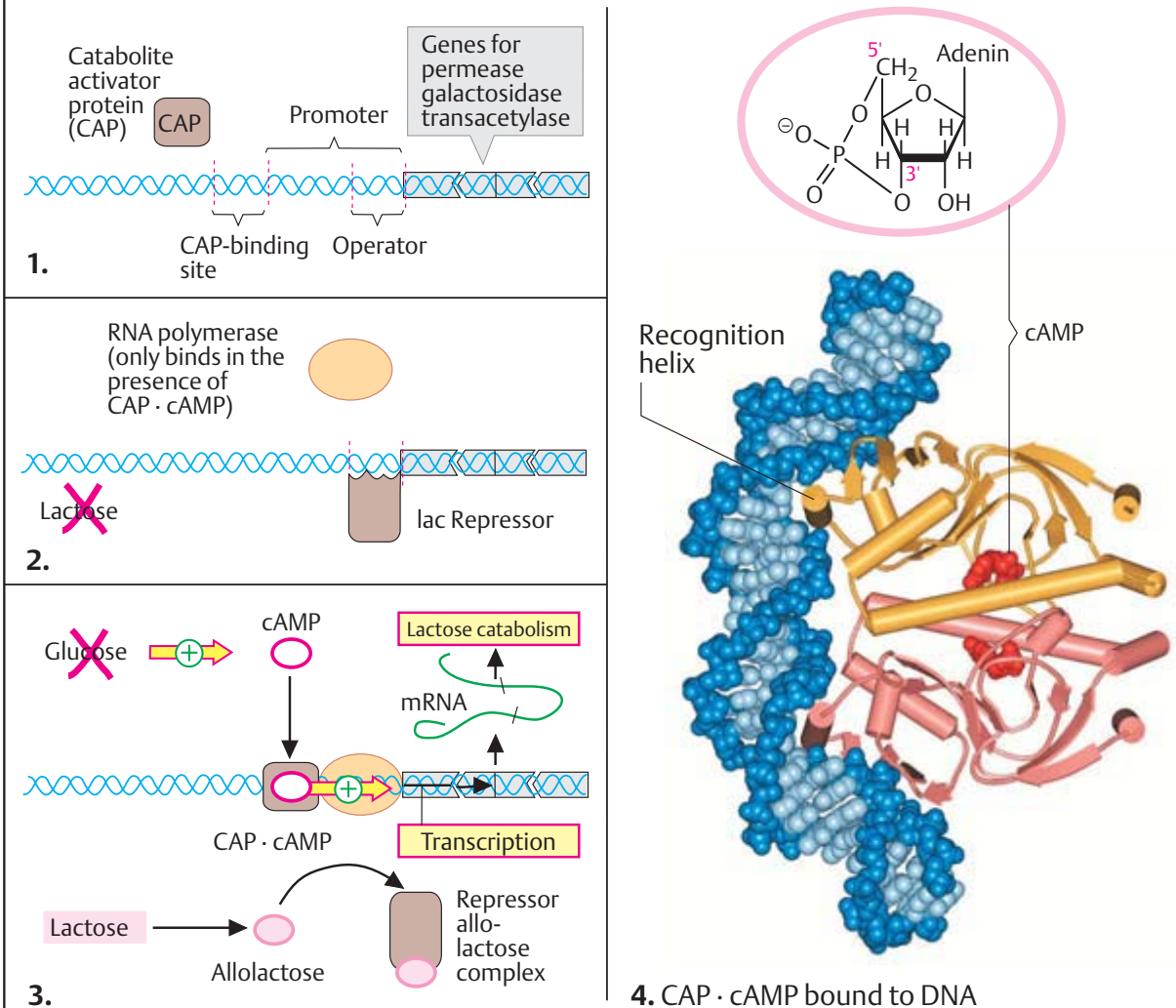
The interaction between the CAP–cAMP complex and DNA is shown in Fig. **4**. Each subunit of the dimeric inducer (yellow or orange) binds one molecule of cAMP (red). Contact with the DNA (blue) is mediated by two "recognition helices" that interact with the major groove of the DNA. The bending of the DNA strand caused by CAP has functional significance.

Transcription control is much more complex in eukaryotes (see p. 244). The number of transcription factors involved is larger, and in addition the gene activity is influenced by the state of the chromatin (see p. 238).

A. Functions of regulatory proteins



B. Lactose operon



Hormonal control

In higher organisms, metabolic and other processes (growth, differentiation, control of the internal environment) are controlled by **hormones** (see pp. 370ff.)

A. Principles of hormone action ●

Depending on the type of hormone, hormone signals are transmitted to the target cells in different ways. Apolar (lipophilic) hormones penetrate the cell and act in the cell nucleus, while polar (hydrophilic) hormones act on the external cell membrane.

Lipophilic hormones, which include the steroid hormones, thyroxine, and retinoic acid, bind to a specific *receptor protein* inside their target cells. The complex formed by the hormone and the receptor then influences *transcription* of specific genes in the cell nucleus (see pp. 118, 244). The group of **hydrophilic hormones** (see p. 380) consists of hormones derived from amino acids, as well as peptide hormones and proteohormones. Their *receptors* are located in the plasma membrane. Binding of the hormone to this type of receptor triggers a signal that is transmitted to the interior of the cell, where it controls the processes that allow the hormone signal to take effect (**signal transduction**; see pp. 384ff.)

B. Hormonal regulation of glucose metabolism in the liver ◑

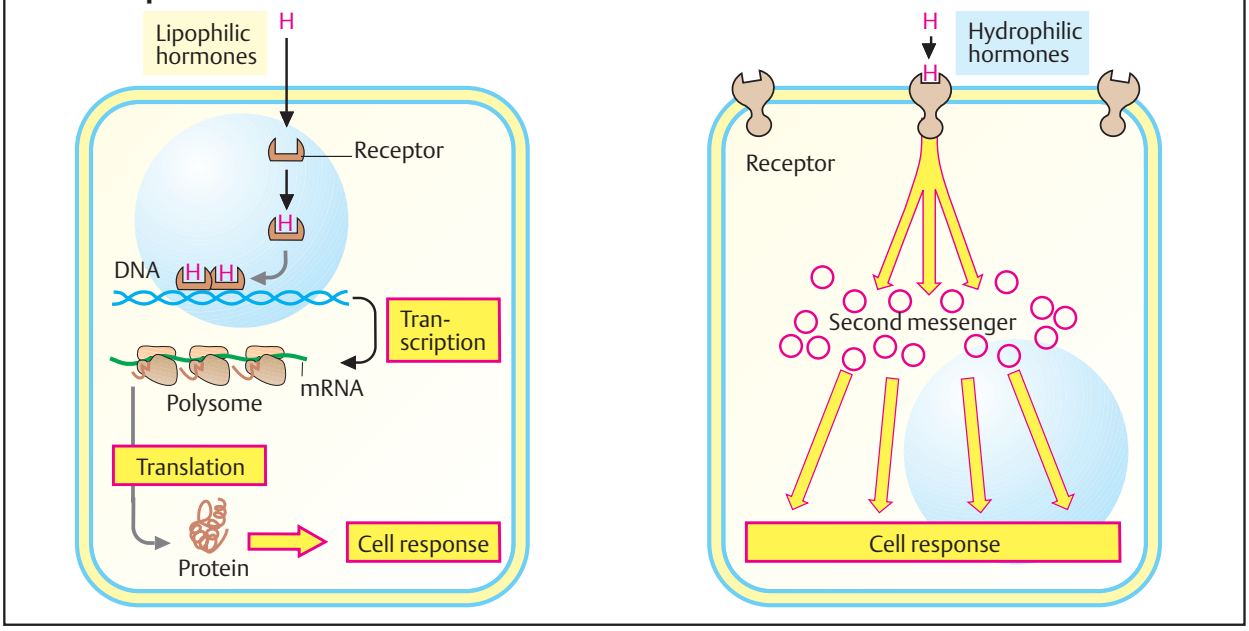
The liver plays a major role in glucose homeostasis in the organism (see p. 310). If glucose deficiency arises, the liver releases glucose into the blood, and when blood sugar levels are high, it takes glucose up from the blood and converts it into different metabolites. Several hormones from both groups are involved in controlling these processes. A very simplified version of the way in which they work is presented here. **Glycogen** is the form in which glucose is stored in the liver and muscles. The rate of glycogen synthesis is determined by *glycogen synthase* (bottom right), while its breakdown is catalyzed by *glycogen phosphorylase* (bottom left).

Regulation by interconversion (bottom). If the blood glucose level falls, the peptide hormone **glucagon** is released. This activates glycogen breakdown, releasing glucose, and at the same time inhibits glycogen synthesis. Glucagon binds to receptors in the plasma membrane (bottom left) and, with mediation by a G-protein (see p. 386), activates the enzyme *adenylate cyclase*, which forms the *second messenger* 3,5′-cyclo-AMP (**cAMP**) from ATP. cAMP binds to another enzyme, *protein kinase A* (PK-A), and activates it. PK-A has several points of attack. Through *phosphorylation,* it converts the active form of *glycogen synthase* into the inactive form, thereby terminating the synthesis of glycogen. Secondly, it activates another protein kinase (not shown), which ultimately converts the inactive form of *glycogen phosphorylase* into the active form through phosphorylation. The active phosphorylase releases glucose 1-phosphate from glycogen, which after conversion into glucose 6-phosphate supplies free glucose. In addition, via an inhibitor (I) of protein phosphatase (PP), active PK-A inhibits inactivation of glycogen phosphorylase. When the cAMP level falls again, *phosphoprotein phosphatases* become active, which dephosphorylate the various phosphoproteins in the cascade described, and thereby arrest glycogen breakdown and re-start glycogen synthesis. Activation and inactivation of proteins through phosphorylation or dephosphorylation is referred to as **interconversion**.

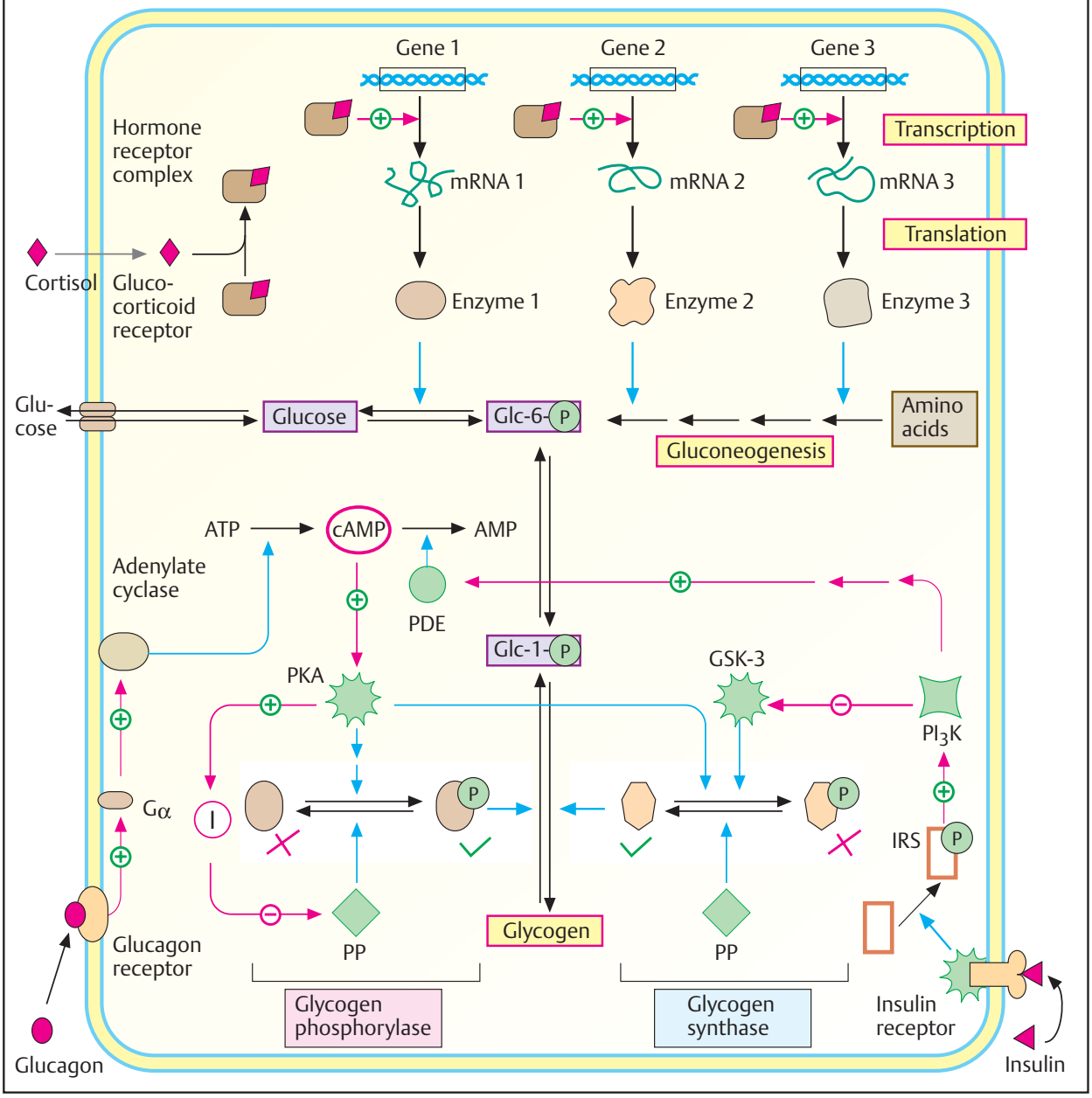
In contrast to glucagon, the peptide hormone **insulin** (see p. 76) increases glycogen synthesis and inhibits glycogen breakdown. Via several intermediates, it inhibits protein kinase GSK-3 (bottom right; for details, see p. 388) and thereby prevents inactivation of glycogen synthase. In addition, insulin reduces the cAMP level by activating *cAMP phosphodiesterase* (PDE).

Regulation by transcriptional control (top). If the liver's glycogen reserves have been exhausted, the steroid hormone **cortisol** maintains glucose release by initiating the conversion of amino acids into glucose (*gluconeogenesis;* see p. 154). In the cell nucleus, the complex of cortisol and its receptor (see p. 378) binds to the promoter regions of various key enzymes of gluconeogenesis and leads to their transcription. The active enzymes are produced through translation of the mRNA formed. Control of the transcription of the gluconeogenesis enzyme *PEP carboxykinase* is discussed on p. 244.

A. Principles of hormone action



B. Hormonal regulation of glucose metabolism in the liver



ATP

The nucleotide coenzyme **adenosine triphosphate** (ATP) is the most important **form of chemical energy** in all cells. Cleavage of ATP is strongly exergonic. The energy this provides (ΔG; see p. 16) is used to drive endergonic processes (such as biosynthesis and movement and transport processes) through *energetic coupling* (see p. 124). The other *nucleoside triphosphate coenzymes* (GTP, CTP, and UTP) have similar chemical properties to ATP, but they are used for different tasks in metabolism (see p. 110).

A. ATP: structure ◑

In ATP, a chain of three phosphate residues is linked to the 5′-OH group of the nucleoside adenosine (see p. 80). These phosphate residues are termed α, β, and γ. The α phosphate is bound to ribose by a *phosphoric acid ester bond*. The linkages *between* the three phosphate residues, on the other hand, involve much more unstable *phosphoric acid anhydride bonds*. The active coenzyme is in fact generally a complex of ATP with an Mg^{2+} ion, which is coordinatively bound to the α and β phosphates (Mg^{2+} ATP^{4-}). However, the term "ATP" is usually used for the sake of simplicity.

B. Hydrolysis energies ◑

The formula for phosphate residues shown in Fig. **A**, with single and double bonds, is not an accurate representation of the actual charge distribution. In ATP, the oxygen atoms of all three phosphate residues have similarly strong negative charges (orange), while the phosphorus atoms represent centers of positive charge. One of the reasons for the instability of phosphoric anhydride bonds is the *repulsion between these negatively charged oxygen atoms*, which is partly relieved by cleavage of a phosphate residue. In addition, the free phosphate anion formed by hydrolysis of ATP is *better hydrated* and *more strongly resonance-stabilized* than the corresponding residue in ATP. This also contributes to the strongly exergonic character of ATP hydrolysis.

In *standard conditions*, the change in free enthalpy $\Delta G^{0'}$ (see p. 18) that occurs in the hydrolysis of phosphoric acid anhydride bonds amounts to –30 to –35 kJ mol^{-1} at pH 7. The particular anhydride bond of ATP that is cleaved only has a minor influence on $\Delta G^{0'}$ (**1**–**2**). Even the hydrolysis of diphosphate (also known as pyrophosphate; **4**) still yields more than –30 kJ mol^{-1}. By contrast, cleavage of the ester bond between ribose and phosphate only provides –9 kJ mol^{-1} (**3**).

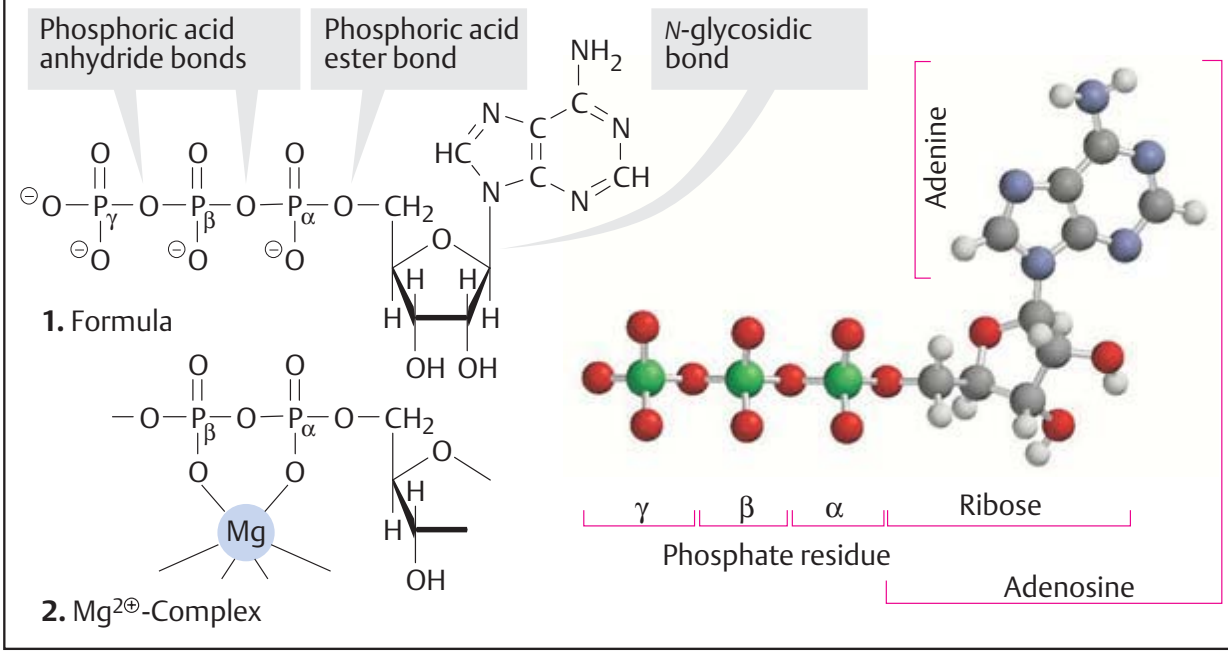
In the cell, the ΔG of ATP hydrolysis is substantially larger, because the concentrations of ATP, ADP and P_i are much lower than in standard conditions and there is an excess of ATP over ADP (see p. 18). The pH value and Mg^{2+} concentration also affect the value of ΔG. The *physiological energy yield* of ATP hydrolysis to ADP and anorganic phosphate (P_i) is probably around –50 kJ mol^{-1}.

C. Types of ATP formation ●

Only a few compounds contain phosphate residues with a group transfer potential (see p. 18) that is high enough to transfer them to ADP and thus allow **ATP synthesis**. Processes that raise anorganic phosphate to this type of high potential are called **substrate level phosphorylations** (see p. 124). Reactions of this type take place in glycolysis (see p. 150) and in the tricarboxylic acid cycle (see p. 136). Another "energy-rich" phosphate compound is *creatine phosphate*, which is formed from ATP in muscle and can regenerate ATP as needed (see p. 336).

Most cellular ATP does not arise in the way described above (i. e., by transfer of phosphate residues from organic molecules to ADP), but rather by **oxidative phosphorylation**. This process takes place in mitochondria (or as light-driven phosphorylation in chloroplasts) and is energetically coupled to a proton gradient over a membrane. These H^+ gradients are established by electron transport chains and are used by the enzyme *ATP synthase* as a source of energy for direct linking of anorganic phosphate to ADP. In contrast to substrate level phosphorylation, oxidative phosphorylation requires the presence of oxygen (i. e., *aerobic* conditions).

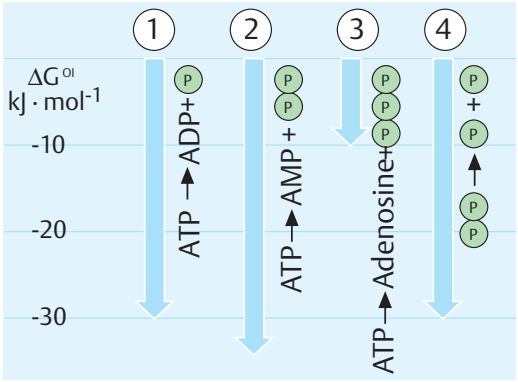
A. ATP: structure



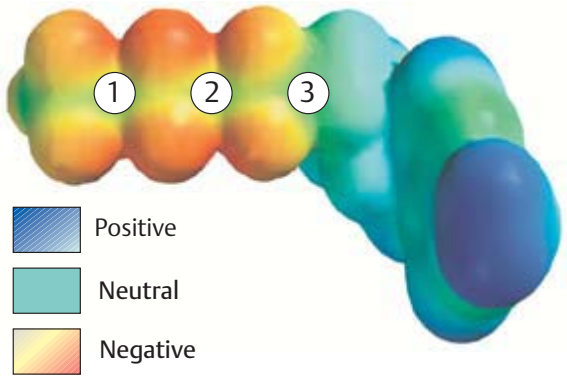
1. Formula

2. $Mg^{2\oplus}$-Complex

B. Hydrolysis energies

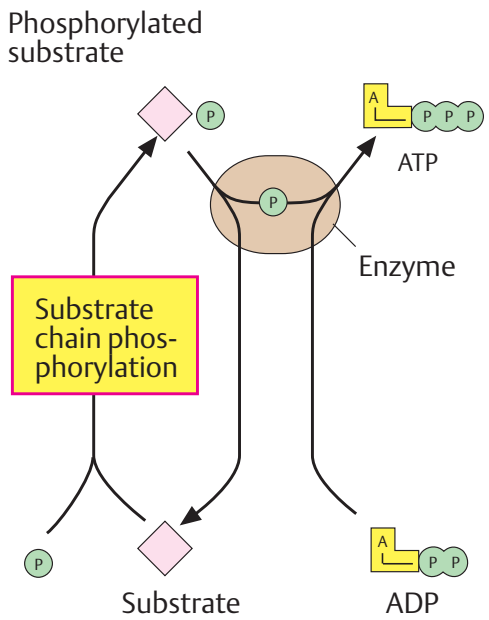


1. Hydrolysis energies

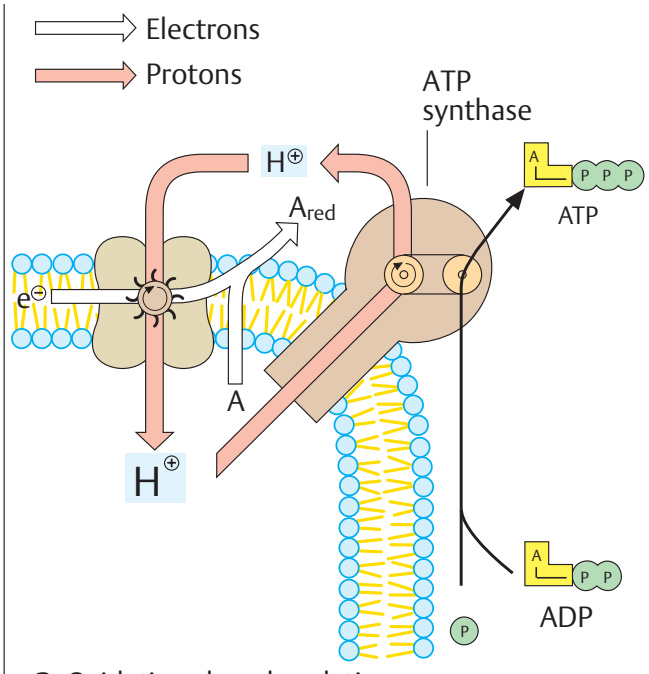


2. ATP: charge density

C. Types of ATP formation



1. Phosphate transfer



2. Oxidative phosphorylation

Energetic coupling

The cell stores chemical energy in the form of "energy-rich" metabolites. The most important metabolite of this type is adenosine triphosphate (**ATP**), which drives a large number of energy-dependent reactions via **energetic coupling** (see p. 16).

A. Energetic coupling ◑

The change in free enthalpy ΔG^0 during hydrolysis (see p. 18) has been arbitrarily selected as a measure of the group transfer potential of "energy-rich" compounds. However, this does not mean that ATP is in fact hydrolyzed in energetically coupled reactions. If ATP hydrolysis and an endergonic process were simply allowed to run alongside each other, the hydrolysis would only produce heat, without influencing the endergonic process. For coupling, the two reactions have to be linked in such a way that a *common intermediate* arises. This connection is illustrated here using the example of the **glutamine synthetase reaction**.

Direct transfer of NH_3 to glutamate is endergonic ($\Delta G^{0'}$ = +14 kJ mol^{-1}; see p. 18), and can therefore not take place. In the cell, the reaction is divided into two exergonic steps. First, the γ-phophate residue is transferred from ATP to glutamate. This gives rise to an "energy-rich" *mixed acid anhydride*. In the second step, the phosphate residue from the intermediate is substituted by NH_3, and glutamine and free phosphate are produced. The energy balance of the reaction as a whole ($\Delta G^{0'}$ = –17 kJ mol^{-1}) is the sum of the changes in free enthalpy of direct glutamine synthesis ($\Delta G^{0'}$ = 14 kJ mol^{-1}) plus ATP hydrolysis ($\Delta G^{0'}$ = –31 kJ mol^{-1}), although ATP has not been hydrolyzed at all.

B. Substrate-level phosphorylation ◑

As mentioned earlier (see p. 122), there are a few metabolites that transfer phosphate to ADP in an exergonic reaction and can therefore form ATP. In ATP synthesis, anorganic phosphate or phosphate bound in an ester-like fashion is transferred to bonds with a high phosphate transfer potential. Reactions of this type are termed *"substrate-level phosphorylations,"* as they represent individual steps within metabolic pathways.

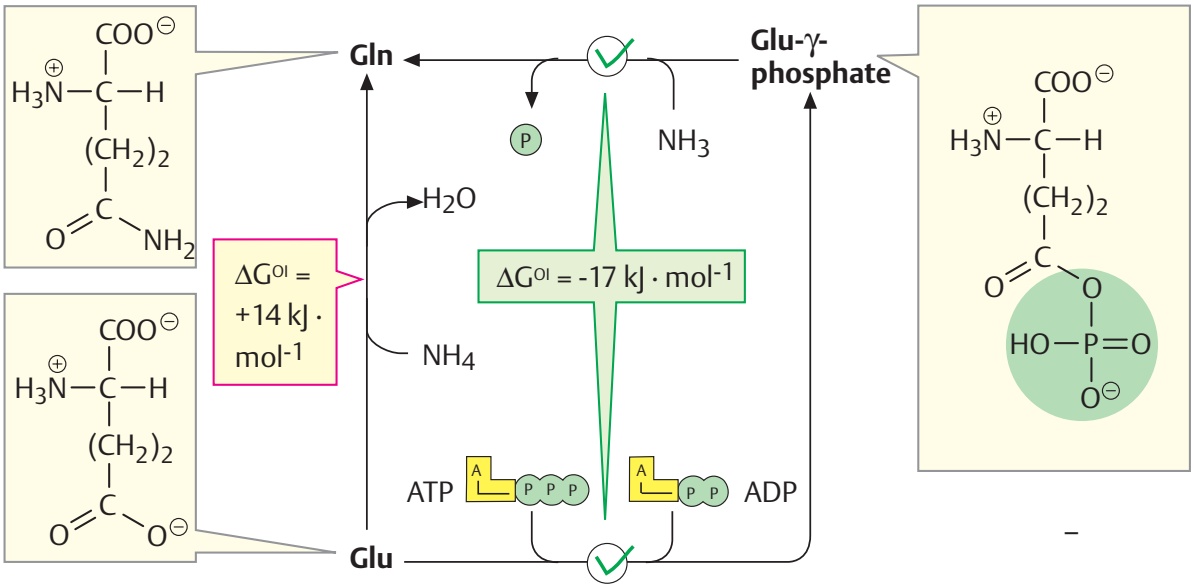
In the **glyceraldehyde 3-phosphate dehydrogenation** reaction, a step involved in glycolysis (**1**; see also **C**), the aldehyde group in glyceraldehyde 3-phosphate is oxidized into a carboxyl group. During the reaction, an anorganic phosphate is also introduced into the product, producing a mixed acid anhydride—1,3-bisphosphoglycerate. **Phosphopyruvate hydratase** ("enolase", **2**) catalyzes the elimination of water from 2-phosphoglycerate. In the *enol phosphate* formed (phospho*enol* pyruvate), the phosphate residue—in contrast to 2-phosphoglycerate—is at an extremely high potential ($\Delta G^{0'}$ of hydrolysis: –62 kJ mol^{-1}). A third reaction of this type is the formation of succinyl phosphate, which occurs in the tricarboxylic acid cycle as an individual step in the **succinyl CoA ligase** reaction. Here again, anorganic phosphate is introduced into a mixed acid anhydride bond to be transferred from there to GDP. Succinyl phosphate is only an intermediate here, and is not released by the enzyme.

In the literature, the term "substrate level phosphorylation" is used inconsistently. Some authors use it to refer to reactions in which *anorganic* phosphate is raised to a high potential, while others use it for the subsequent reactions, in which ATP or GTP is formed from the energy-rich intermediates.

C. Glyceraldehyde-3-phosphate dehydrogenase ○

The reaction catalyzed during glycolysis by *glyceraldehyde-3-phosphate dehydrogenase* (GADPH) is shown here in detail. Initially, the SH group of a cysteine residue of the enzyme is added to the carbonyl group of glyceraldehyde 3-phosphate (**a**). This intermediate is oxidized by NAD^+ into an "energy-rich" thioester (**b**). In the third step (**c**), anorganic phosphate displaces the thiol, and the mixed anhydride *1,3-bisphosphoglycerate* arises. In this bond, the phosphate residue is at a high enough potential for it to be transferred to ADP in the next step (not shown; see p. 150).

A. Energetic coupling

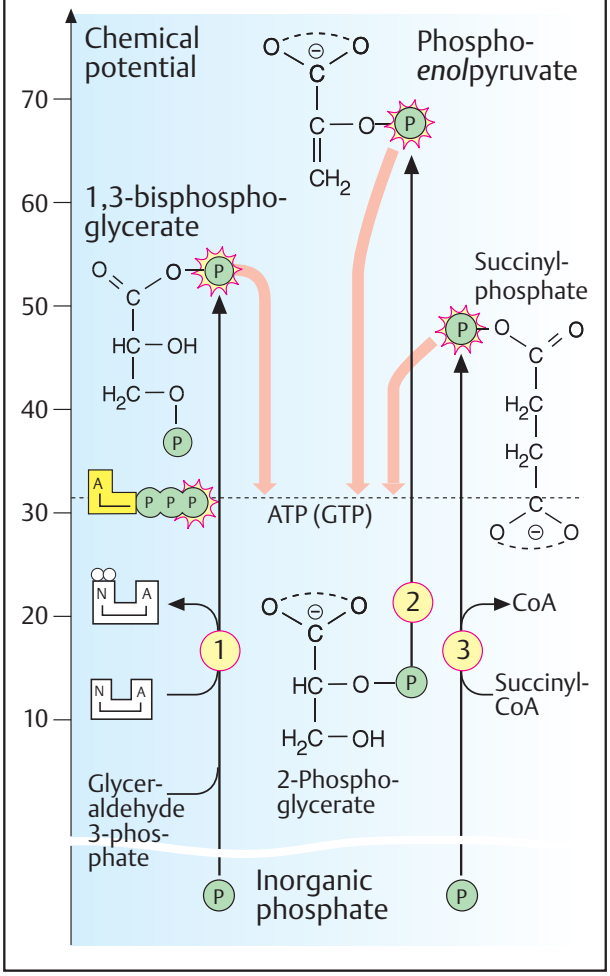


1. Glutamine synthetase reaction

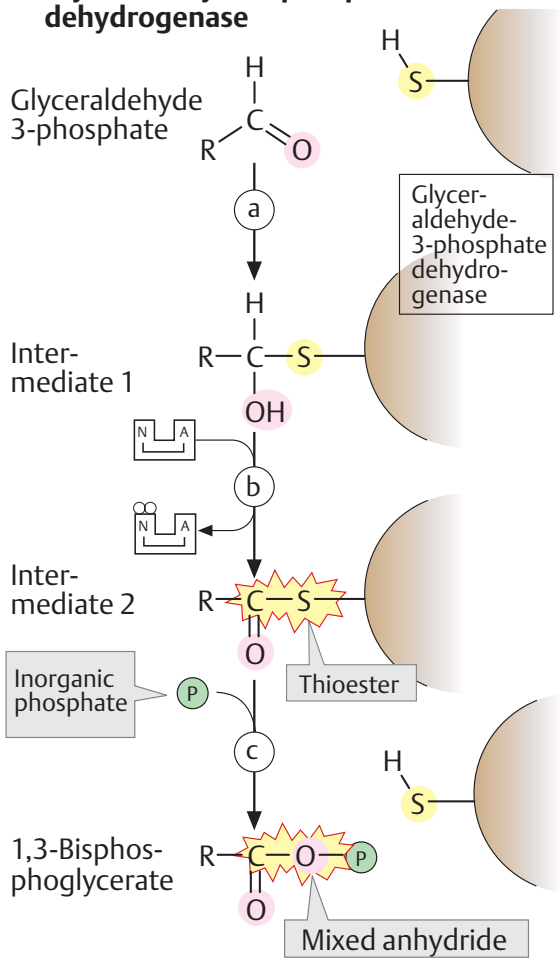
Reaction 1:	Glutamate	+	NH_3	+14 kJ · mol^{-1}	Glutamine	+	H_2O	
Reaction 2:	ATP	+	H_2O	− 31 kJ · mol^{-1}	ADP	+	P	
Total:	Glutamate	+	NH_3 + ATP	− 17 kJ · mol^{-1}	Glutamine	+	ADP	+ P

2. Energy balance

B. Substrate level phosphorylation



C. Glyceraldehyde 3-phosphate dehydrogenase



Energy conservation at membranes

Metabolic energy can be stored not only in the form of "energy-rich" bonds (see p. 122), but also by separating electric charges from each other using an insulating layer to prevent them from redistributing. In the field of technology, this type of system would be called a *condenser*. Using the same principle, energy is also stored ("conserved") at cell membranes. The membrane functions as an insulator; electrically charged atoms and molecules (*ions*) function as charges.

A. Electrochemical gradient ◑

Although artificial lipid membranes are almost impermeable to ions, biological membranes contain **ion channels** that selectively allow individual ion types to pass through (see p. 222). Whether an ion can cross this type of membrane, and if so in which direction, depends on the **electrochemical gradient**—i. e., on the concentrations of the ion on each side of the membrane (the *concentration gradient*) and on the *difference* in the electrical potential between the interior and exterior, the **membrane potential**.

The membrane potential of resting cells (**resting potential**; see p. 350) is –0.05 to –0.09 V—i. e., there is an excess negative charge on the inner side of the plasma membrane. The main contributors to the resting potential are the two cations Na^+ and K^+, as well as Cl^- and organic anions (**1**). Data on the concentrations of these ions outside and inside animal cells, and permeability coef - cients, are shown in the table (**2**).

The behavior of an ion type is described quantitatively by the **Nernst equation** (**3**). $\Delta\psi_G$ is the membrane potential (in volts, V) at which there is *no* net transport of the ion concerned across the membrane (**equilibrium potential**). The factor R T/F n has a value of 0.026 V for monovalent ions at 25 °C. Thus, for K^+, the table (**2**) gives an equilibrium potential of ca. –0.09 V—i. e., a value more or less the same as that of the resting potential. By contrast, for Na^+ ions, $\Delta\psi_G$ is much higher than the resting potential, at +0.07 V. Na^+ ions therefore immediately flow into the cell when Na^+ channels open (see p. 350). The disequilibrium between Na^+ and K^+ ions is constantly maintained by the enzyme *Na^+/K^+-ATPase*, which consumes ATP.

B. Proton motive force ◑

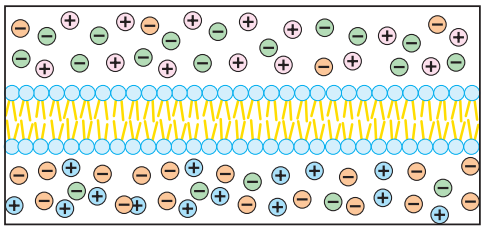
Hydronium ions ("H^+ ions") can also develop electrochemical gradients. Such a **proton gradient** plays a decisive part in cellular ATP synthesis (see p. 142). As usual, the energy content of the gradient depends on the concentration gradients—i. e., on the **pH difference** эpH between the two sides of the membrane. In addition, the **membrane potential** $\Delta\psi$ also makes a contribution. Together, these two values give the **proton motive force** Δp, a measure for the work that the H^+ gradient can do. The proton gradient across the inner mitochondrial membrane thus delivers approximately 24 kJ per mol H^+.

C. Energy conservation in proton gradients ◑

Proton gradients can be built up in various ways. A very unusual type is represented by **bacteriorhodopsin** (**1**), a *light-driven proton pump* that various bacteria use to produce energy. As with rhodopsin in the eye, the light-sensitive component used here is covalently bound retinal (see p. 358). In photosynthesis (see p. 130), reduced *plastoquinone* (QH_2) transports protons, as well as electrons, through the membrane (**Q cycle**, **2**). The formation of the proton gradient by the **respiratory chain** is also coupled to redox processes (see p. 140). In complex III, a Q cycle is responsible for proton translocation (not shown). In *cytochrome c oxidase* (complex IV, **3**), H^+ transport is coupled to electron flow from cytochrome *c* to O_2.

In each of these cases, the H^+ gradient is utilized by an **ATP synthase** (**4**) to form ATP. ATP synthases consist of two components—a proton channel (F_0) and an inwardly directed protein complex (F_1), which conserves the energy of back-flowing protons through ATP synthesis (see p. 142).

A. Electrochemical gradient



⊕ Na ⊕ K ⊖ Cl ⊖ Organic anions

1. Cause

Ion	Concentrations		Permeability coefficient
	Cyto-plasm (mM)	Extracelluar space (mM)	($cm \cdot s^{-1} \cdot 10^9$)
$K^{\oplus}$	100	5	500
$Na^{\oplus}$	15	150	5
$Ca^{2\oplus}$	0.0002	2	
$Cl^{\ominus}$	13	150	10
Organic anions	138	34	0

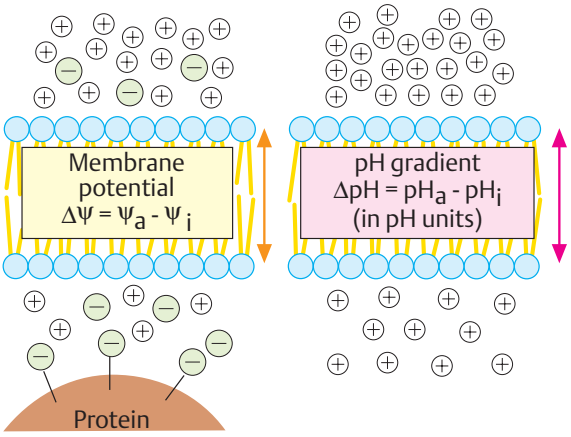
2. Concentrations

$$\Delta\Psi_G = \frac{R \cdot T}{F \cdot n} \cdot \ln \frac{C_{outside}}{C_{inside}}$$

R = gas constant
T = temperature (K)
n = Ion charge
F = Faraday constant

3. Nernst equation

B. Proton motive force

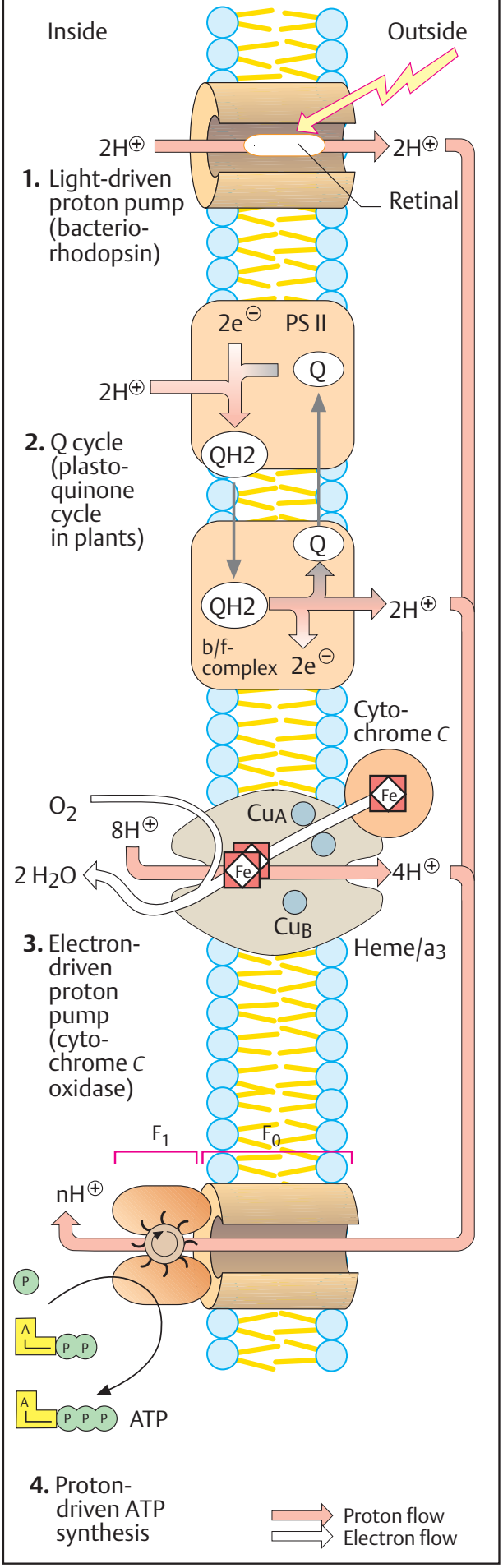


Proton motive force ≈ 0.06 V

$$\Delta p = \Delta\psi - \frac{2.3 \cdot R \cdot T}{F} \cdot \Delta pH$$

$$\Delta G_{H_a^{\oplus} \leftrightarrow H_i^{\oplus}} = -F \cdot \Delta p$$

C. Energy conservation in proton gradients



Photosynthesis: light reactions

Sunlight is the most important source of energy for nearly all living organisms. With the help of **photosynthesis,** light energy is used to produce organic substances from CO_2 and water. This property of *phototrophic organisms* (plants, algae, and some bacteria) is exploited by *heterotrophic* organisms (e.g., animals), which are dependent on a supply of organic substances in their diet (see p. 112). The atmospheric *oxygen* that is vital to higher organisms is also derived from photosynthesis.

A. Photosynthesis: overview ◑

The chemical balance of photosynthesis is simple. Six molecules of CO_2 are used to form one hexose molecule (right). The hydrogen required for this reduction process is taken from water, and molecular oxygen is formed as a by-product (left). Light energy is required, since water is a very poor reducing agent and is therefore not capable of reducing CO_2.

In the light-dependent part of photosynthesis—the "**light reactions** "—H_2O molecules are split into protons, electrons, and oxygen atoms. The electrons undergo *excitation* by light energy and are raised to an energy level that is high enough to reduce $NADP^+$. The $NADPH+H^+$ formed in this way, in contrast to H_2O, is capable of "fixing" CO_2 reductively—i.e., of incorporating it into organic bonds. Another product of the light reactions is ATP, which is also required for CO_2 fixation. If $NADPH+H^+$, ATP, and the appropriate enzymes are available, CO_2 fixation can also take place in darkness. This process is therefore known as the "**dark reaction**."

The excitation of electrons to form NADPH is a complex photochemical process that involves **chlorophyll**, a tetrapyrrole dye containing Mg^{2+} that bears an extra phytol *residue* (see p. 132).

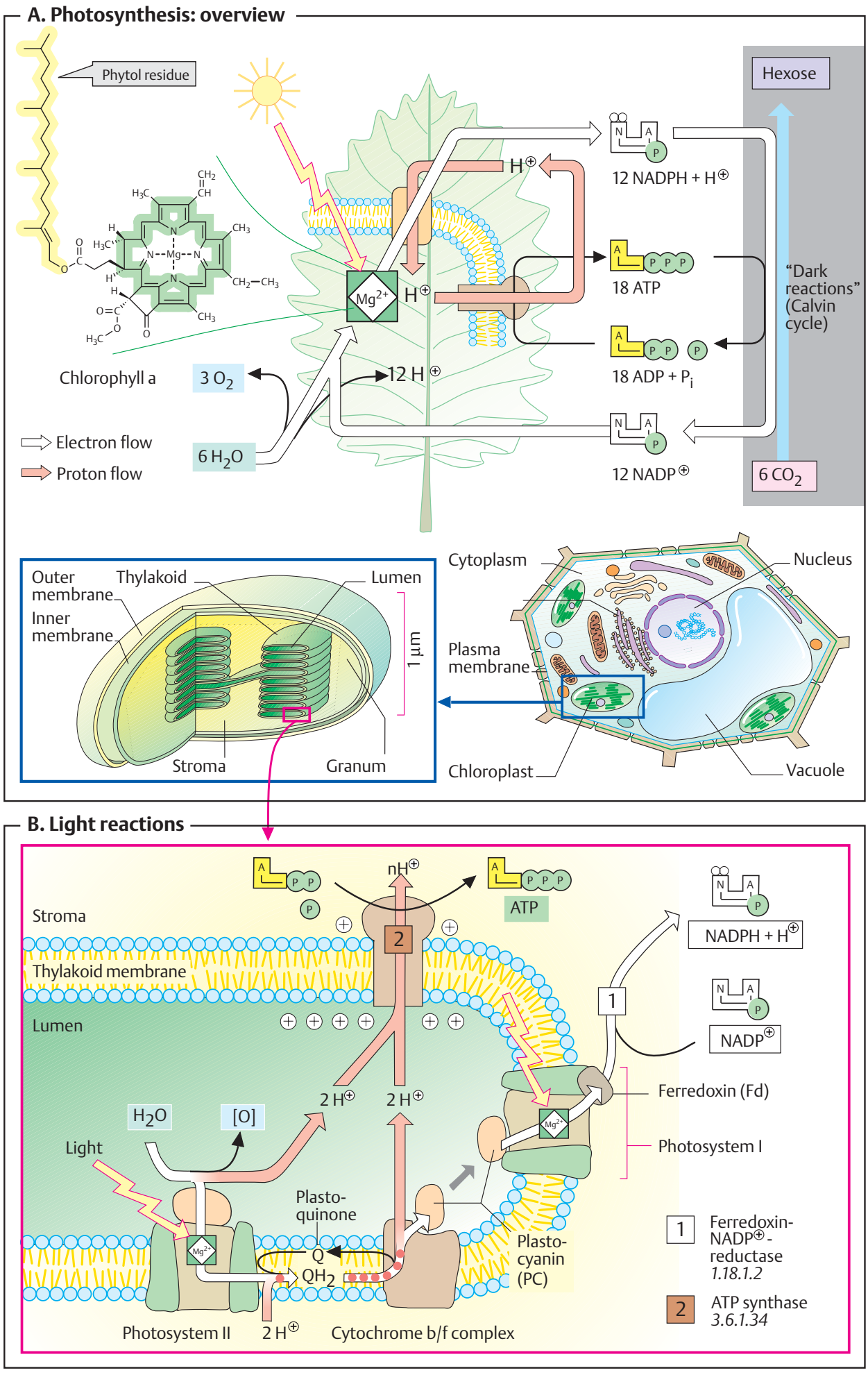
B. Light reactions ○

In green algae and higher plants, photosynthesis occurs in **chloroplasts.** These are organelles, which—like mitochondria—are surrounded by two membranes and contain their own DNA. In their interior, the *stroma*, *thylakoids* or flattened membrane sacs are stacked on top of each other to form *grana*. The inside of the thylakoid is referred to as the *lumen*. The light reactions are catalyzed by enzymes located in the thylakoid membrane, whereas the dark reactions take place in the stroma.

As in the respiratory chain (see p. 140), the light reactions cause electrons to pass from one redox system to the next in an **electron transport chain.** However, the *direction of transport* is opposite to that found in the respiratory chain. In the respiratory chain, electrons flow from $NADH+H^+$ to O_2, with the production of water and energy.

In photosynthesis, electrons are taken up from water and transferred to $NADP^+$, with an *expenditure of energy.* Photosynthetic electron transport is therefore energetically "uphill work." To make this possible, the transport is stimulated at two points by the *absorption of light energy.* This occurs through two **photosystems**—protein complexes that contain large numbers of chlorophyll molecules and other pigments (see p. 132). Another component of the transport chain is the **cytochrome b/f complex,** an aggregate of integral membrane proteins that includes two cytochromes (b_{563} and f). **Plastoquinone**, which is comparable to ubiquinone, and two soluble proteins, the coppercontaining **plastocyanin** and **ferredoxin**, function as mobile electron carriers. At the end of the chain, there is an enzyme that transfers the electrons to $NADP^+$.

Because photosystem II and the cytochrome b/f complex release protons from reduced plastoquinone into the lumen (via a Q cycle), photosynthetic electron transport establishes an **electrochemical gradient** across the thylakoid membrane (see p. 126), which is used for ATP synthesis by an *ATP synthase.* ATP and $NADPH+H^+$, which are both needed for the dark reactions, are formed in the stroma.



Photosynthesis: dark reactions

The "light reactions" in photosynthesis bring about two strongly endergonic reactions—the reduction of $NADP^+$ to $NADPH+H^+$ and ATP synthesis (see p. 122). The chemical energy needed for this is produced from radiant energy by two photosystems.

A. Photosystem II ○

The photosynthetic electron transport chain in plants starts in **photosystem II** (PS II; see p. 128). PS II consists of numerous protein subunits (brown) that contain bound **pigments**—i. e., dye molecules that are involved in the absorption and transfer of light energy.

The schematic overview of PS II presented here (**1**) only shows the important pigments. These include a special chlorophyll molecule, the *reaction center* P_{680}; a neighboring Mg^{2+} free chlorophyll (*pheophytin*); and two bound *plastoquinones* (Q_A and Q_B). A third quinone (Q_P) is not linked to PS II, but belongs to the plastoquinone pool. The white arrows indicate the direction of electron flow from water to Q_P. Only about 1% of the chlorophyll molecules in PS II are *directly* involved in photochemical excitation (see p. 128). Most of them are found, along with other pigments, in what are known as light-harvesting or antenna complexes (green). The energy of light quanta striking these can be passed on to the reaction center, where it can be utilized.

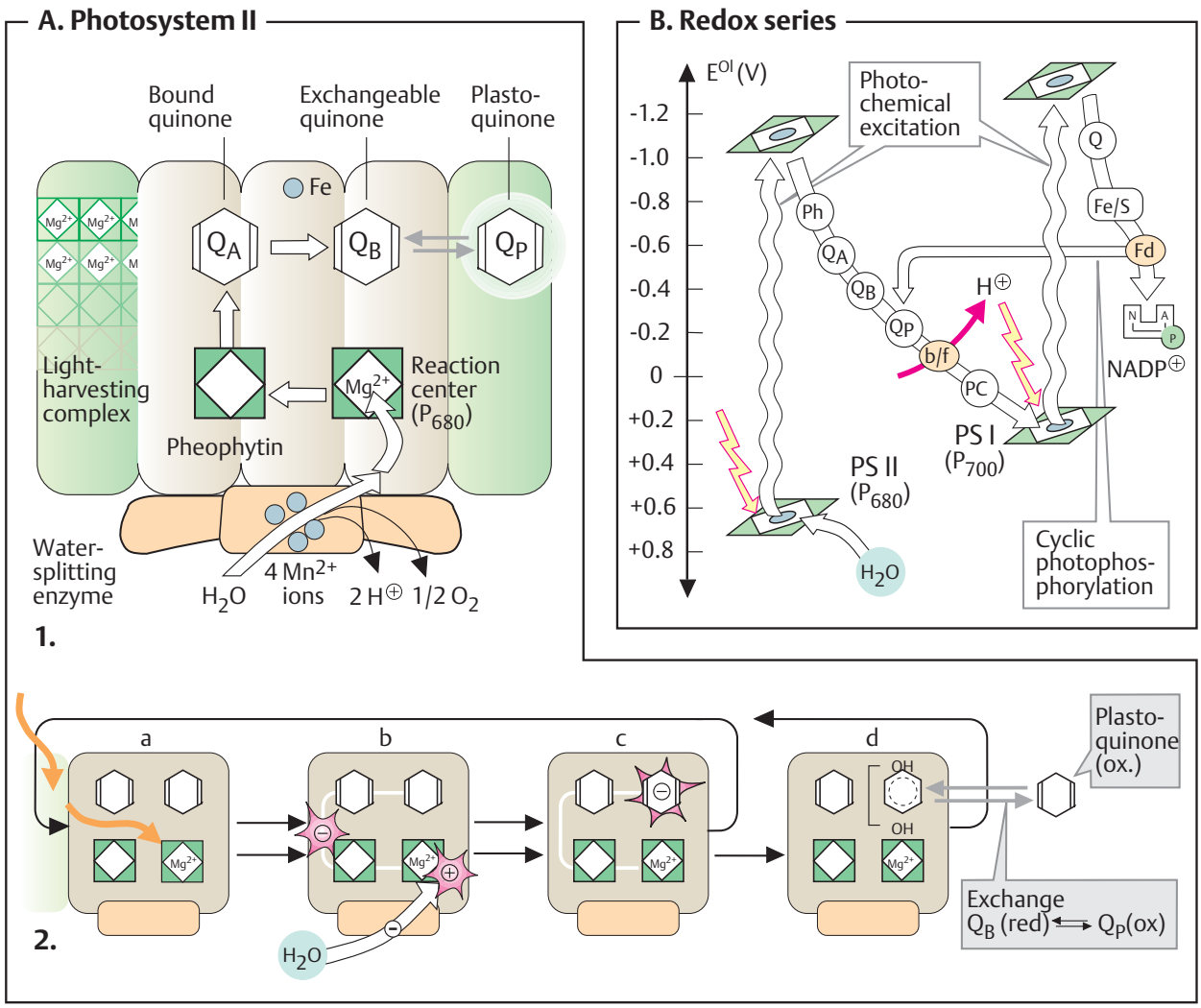
In Fig. **2**, photosynthetic electron transport in PS II is separated into the individual steps involved. Light energy from the light-harvesting complexes (**a**) raises an electron of the chlorophyll in the reaction center to an excited *"singlet state."* The excited electron is immediately passed on to the neighboring pheophytin. This leaves behind an "electron gap" in the reaction center—i. e., a positively charged P_{680} radical (**b**). This gap is now filled by an electron removed from an H_2O molecule by the *water-splitting enzyme* (b). The excited electron passes on from the pheophytin via Q_A to Q_B, converting the latter into a *semiquinone radical* (**c**). Q_B is then reduced to *hydroquinone* by a second excited electron, and is then exchanged for an oxidized quinone (Q_P) from the plastoquinone pool. Further transport of electrons from the plastoquinone pool takes place as described on the preceding page and shown in **B**.

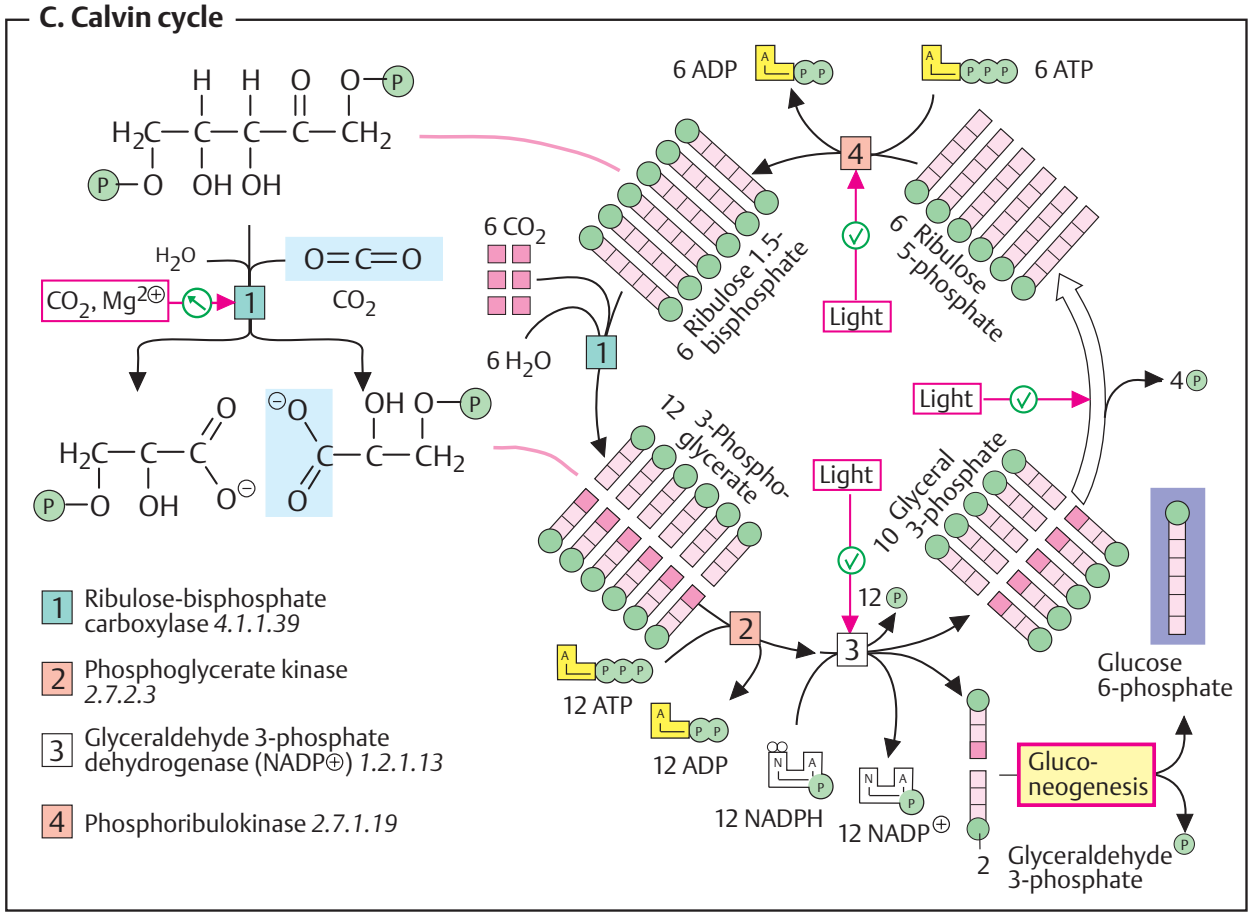
B. Redox series ○

It can be seen from the *normal potentials* E^0 (see p. 18) of the most important redox systems involved in the light reactions why two excitation processes are needed in order to transfer electrons from H_2O to $NADP^+$. After excitation in PS II, E^0 *rises* from around –1 V back to positive values in plastocyanin (PC)—i. e., the energy of the electrons has to be increased again in PS I. If there is no $NADP^+$ available, photosynthetic electron transport can still be used for ATP synthesis. During *cyclic photophosphorylation,* electrons return from ferredoxin (Fd) via the plastoquinone pool to the b/f complex. This type of electron transport does not produce any NADPH, but does lead to the formation of an H^+ gradient and thus to ATP synthesis.

C. Calvin cycle ○

The synthesis of hexoses from CO_2 is only shown in a very simplified form here; a complete reaction scheme is given on p. 407. The actual **CO_2 fixation**—i. e., the incorporation of CO_2 into an organic compound—is catalyzed by *ribulose bisphosphate carboxylase/oxygenase* ("rubisco"). Rubisco, the most abundant enzyme on Earth, converts ribulose 1,5-bisphosphate, CO_2 and water into *two molecules* of 3-phosphoglycerate. These are then converted, via 1,3-bisphosphoglycerate and 3-phosphoglycerate, into glyceraldehyde 3-phosphate (glyceral 3-phosphate). In this way, 12 glyceraldehyde 3-phosphates are synthesized from six CO_2. Two molecules of this intermediate are used by gluconeogenesis reactions to synthesize *glucose 6-phosphate* (bottom right). From the remaining 10 molecules, six molecules of *ribulose 1,5-bisphosphate* are regenerated, and the cycle then starts over again. In the Calvin cycle, ATP is required for phosphorylation of 3-phosphoglycerate and ribulose 5-phosphate. $NADPH+H^+$, the second product of the light reaction, is consumed in the reduction of 1,3-bisphosphoglycerate to glyceraldehyde 3-phosphate.





Molecular models: membrane proteins

The plates show, in simplified form, the structures of **cytochrome *c* oxidase** (**A**; complex IV of the respiratory chain) and of **photosystem I** of a cyanobacterium (**B**). These two molecules are among the few integral membrane proteins for which the structure is known in detail. Both structures were determined by X-ray crystallography.

A. Cytochrome *c* oxidase ○

The enzyme cytochrome *c* oxidase ("COX," *EC 1.9.3.1*) catalyzes the final step of the respiratory chain. It receives electrons from the small heme protein cytochrome *c* and transfers them to molecular oxygen, which is thereby reduced to water (see p. 140). At the same time, 2–4 protons per water molecule formed are pumped from the matrix into the intermembrane space.

Mammalian COX (the illustration shows the enzyme from bovine heart) is a dimer that has two identical subunits with masses of 204 kDa each. Only one subunit is shown in detail here; the other is indicated by gray lines. Each subunit consists of 13 different polypeptides, which all span the inner mitochondrial membrane. Only polypeptides I (light blue) and II (dark blue) and the linked cofactors are involved in electron transport. The other chains, which are differently expressed in the different organs, probably have regulatory functions. The two heme groups, heme a (orange) and heme a_1 (red) are bound in polypeptide 1. The copper center Cu_A consists of two copper ions (green), which are coordinated by amino acid residues in polypeptide II. The second copper (Cu_B) is located in polypeptide I near heme a_3.

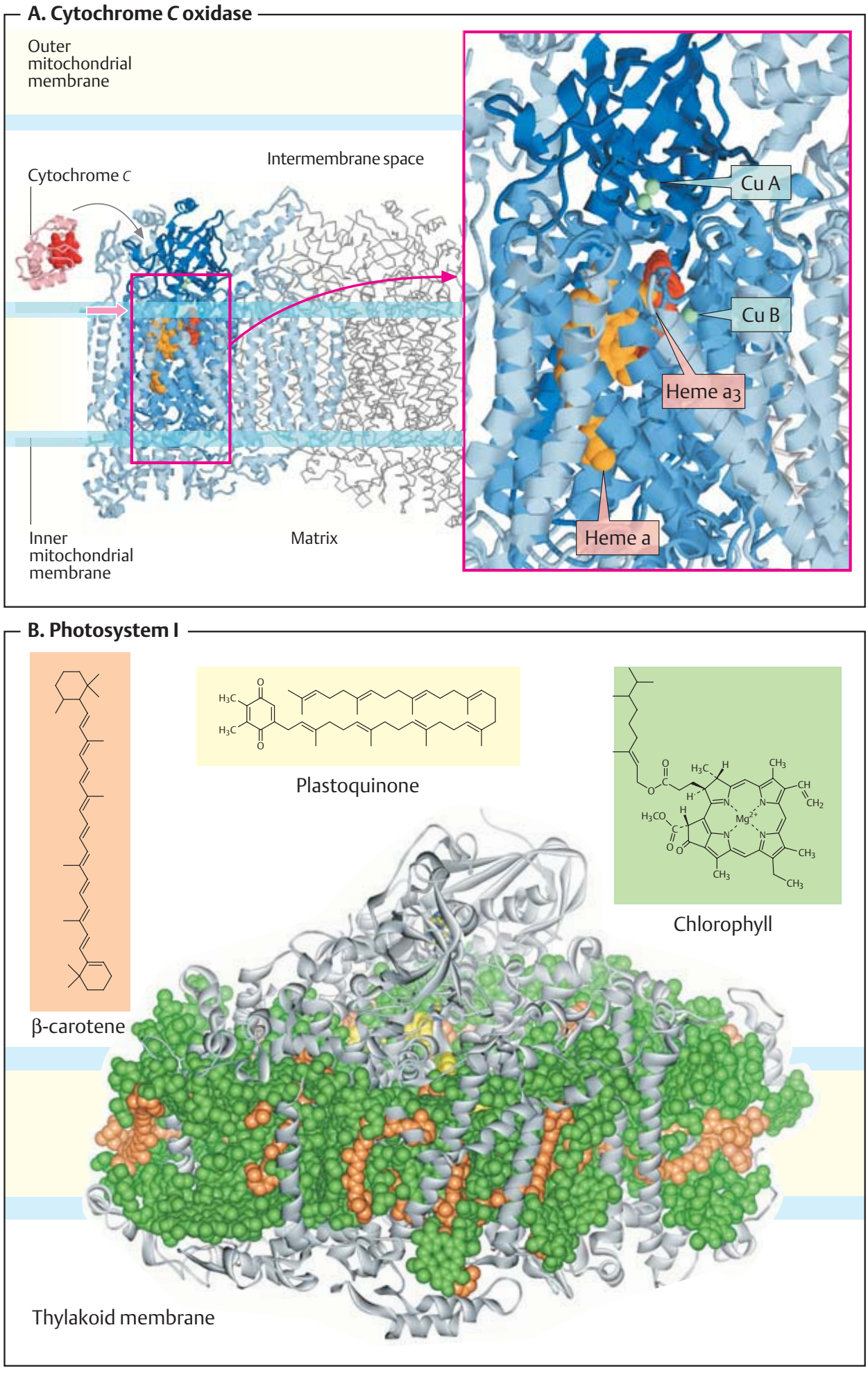
To reduce an O_2 molecule to two molecules of H_2O, a total of four electrons are needed, which are supplied by cytochrome *c* (pink, top left) and initially given off to Cu_A. From there, they are passed on via heme a and heme a_3 to the enzyme's reaction center, which is located between heme a_3 and Cu_B. The reduction of the oxygen takes place in several steps, without any intermediate being released. The four protons needed to produce water and the H^+ ions pumped into the intermembrane space are taken up by two channels (D and K, not shown). The mechanism that links proton transport to electron transfer is still being investigated.

B. Reaction center of *Synechococcus elongatus* ○

Photosystem I (PS I) in the cyanobacterium *Synechococcus elongatus* is the first system of this type for which the structure has been solved in atomic detail. Although the bacterial photosystem differs slightly from the systems in higher plants, the structure provides valuable hints about the course of the light reactions in photosynthesis (see p. 128). The functioning of the photosystem is discussed in greater detail on p. 130.

The functional form of PS I in *S. elongatus* consists of a trimer with a mass of more than 10^6 Da that is integrated into the membrane. Only one of the three subunits is shown here. This consists of 12 different polypeptides (gray-blue), 96 chlorophyll molecules (green), 22 carotenoids (orange), several phylloquinones (yellow), and other components. Most of the chlorophyll molecules are so-called **antenna pigments**. These collect light energy and conduct it to the **reaction center**, which is located in the center of the structure and therefore not visible. In the reaction center, an electron is excited and transferred via various intermediate steps to a ferredoxin molecule (see p. 128). The **chlorophylls** (see formula) are heme-like pigments with a highly modified tetrapyrrole ring, a central Mg^{2+} ion, and an apolar phytol side chain. Shown here is chlorophyll a, which is also found in the reaction center of the *S. elongatus* photosystem.

The yellow and orange-colored **carotenoids**—e. g., *β-carotene* (see formula)—are auxiliary pigments that serve to protect the chloroplasts from oxidative damage. Dangerous radicals can be produced during the light reaction—particularly *singlet oxygen*. Carotenoids prevent compounds of this type from arising, or render them inactive. Carotenoids are also responsible for the coloring of leaves seen during fall. They are left behind when plants break down chlorophyll in order to recover the nitrogen it contains.



Oxoacid dehydrogenases

The intermediary metabolism has *multienzyme complexes* which, in a complex reaction, catalyze the **oxidative decarboxylation** of 2-oxoacids and the transfer to coenzyme A of the acyl residue produced. NAD^+ acts as the electron acceptor. In addition, thiamine diphosphate, lipoamide, and FAD are also involved in the reaction. The *oxoacid dehydrogenases* include a) the *pyruvate dehydrogenase complex* (PDH, pyruvate → acetyl CoA), b) the *2-oxoglutarate dehydrogenase complex* of the tricarboxylic acid cycle (ODH, 2-oxoglutarate → succinyl CoA), and c) the *branched chain dehydrogenase complex,* which is involved in the catabolism of valine, leucine, and isoleucine (see p. 414).

A. Pyruvate dehydrogenase: reactions ◑

The pyruvate dehydrogenase reaction takes place in the mitochondrial matrix (see p. 210). Three different enzymes [E1–E3] form the PDH multienzyme complex (see **B**).

[1] Initially, *pyruvate dehydrogenase* [E1] catalyzes the decarboxylation of pyruvate and the transfer of the resulting hydroxyethyl residue to **thiamine diphosphate** (TPP, **1a**). The same enzyme then catalyzes oxidation of the TPP-bound hydroxyethyl group to yield an acetyl residue. This residue and the reducing equivalents obtained are then transferred to **lipoamide** (**1b**).

[2] The second enzyme, *dihydrolipoamide acetyltransferase* [E2], shifts the acetyl residue from lipoamide to **coenzyme A** (**2**), with dihydrolipoamide being left over.

[3] The third enzyme, *dihydrolipoamide dehydrogenase* [E3], reoxidizes dihydrolipoamide, with **$NADH+H^+$** being formed. The electrons are first taken over by enzyme-bound **FAD** (**3a**) and then transferred via a catalytically active disulfide bond in the E3 subunit (not shown) to soluble NAD^+ (**3b**).

The five different **coenzymes** involved are associated with the enzyme components in different ways. Thiamine diphosphate is non-covalently bound to E1, whereas lipoamide is covalently bound to a lysine residue of E2 and FAD is bound as a *prosthetic group* to E3. NAD^+ and coenzyme A, being soluble coenzymes, are only temporarily associated with the complex.

An important aspect of PDH catalysis is the spatial relationship between the components of the complex. The covalently bound lipoamide coenzyme is part of a mobile domain of E2, and is therefore highly mobile. This structure is known as the *lipoamide arm,* and swings back and forth between E1 and E3 during catalysis. In this way, lipoamide can interact with the TPP bound at E1, with solute coenzyme A, and also with the FAD that serves as the electron acceptor in E3.

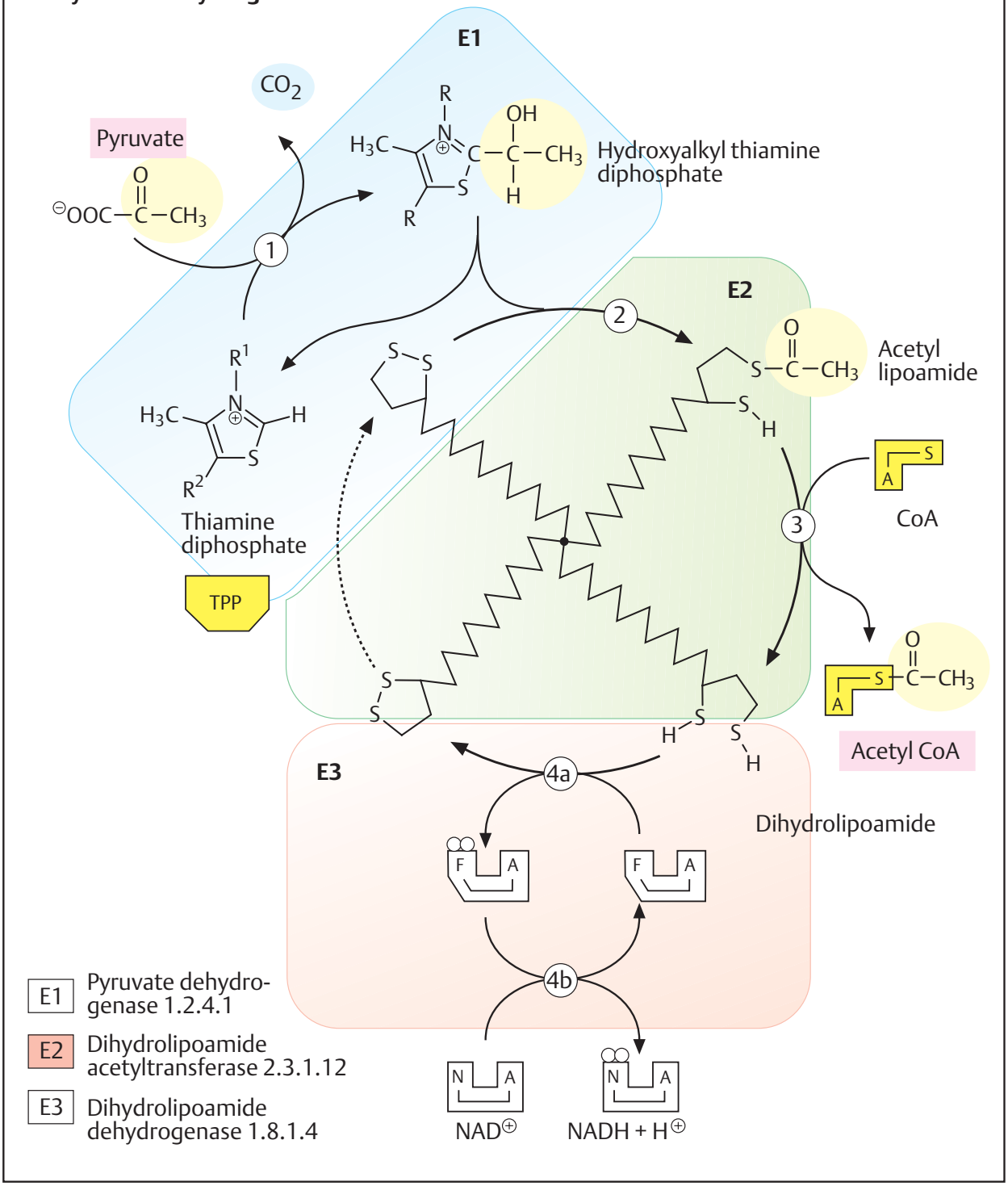
B. PDH complex of *Escherichia coli* ○

The PDH complex of the bacterium *Escherichia coli* has been particularly well studied. It has a molecular mass of 5.3 10^6, and with a diameter of more than 30 nm it is larger than a ribosome. The complex consists of a total of 60 polypeptides (**1**, **2**): 24 molecules of E2 (eight trimers) form the almost cube-shaped core of the complex. Each of the six surfaces of the cube is occupied by a dimer of E3 components, while each of the twelve edges of the cube is occupied by dimers of E1 molecules. Animal oxoacid dehydrogenases have similar structures, but differ in the numbers of subunits and their molecular masses.

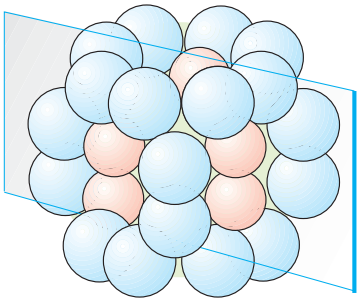
Further information

The PDH reaction, which is practically irreversible, occupies a strategic position at the interface between carbohydrate and fatty acid metabolism, and also supplies acetyl residues to the tricarboxylic acid cycle. PDH activity is therefore strictly regulated (see p. 144). **Interconversion** is particularly important in animal cells (see p. 120). Several PDH-specific *protein kinases* inactivate the E1 components through phosphorylation, while equally specific *protein phosphatases* reactivate it again. The binding of the kinases and phosphatases to the complex is in turn regulated by metabolites. For example, high concentrations of acetyl CoA promote binding of kinases and thereby inhibit the reaction, while Ca^{2+} increases the activity of the phosphatase. Insulin activates PDH via inhibition of phosphorylation.

A. Pyruvate dehydrogenase: reactions

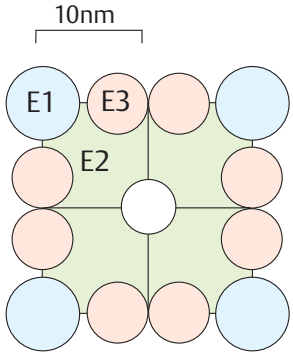


B. PDH complex of *Escherichia coli*



1.

E1	12 · 2 = 24
E2	8 · 3 = 24
E3	6 · 2 =12
E1+ E2 + E3 = 60	



2.

Tricarboxylic acid cycle: reactions

The **tricarboxylic acid cycle** (TCA cycle, also known as the citric acid cycle or Krebs cycle) is a cyclic metabolic pathway in the mitochondrial matrix (see p. 210). In eight steps, it oxidizes acetyl residues (CH_3-CO-) to carbon dioxide (CO_2). The reducing equivalents obtained in this process are transferred to NAD^+ or ubiquinone, and from there to the respiratory chain (see p. 140). Additional metabolic functions of the cycle are discussed on p. 138.

A. Tricarboxylic acid cycle ◑

The acetyl-CoA that supplies the cycle with acetyl residues is mainly derived from *β-oxidation* of fatty acids (see p. 164) and from the *pyruvate dehydrogenase reaction.* Both of these processes take place in the mitochondrial matrix.

[1] In the first step of the cycle, *citrate synthase* catalyzes the transfer of an acetyl residue from **acetyl CoA** to a carrier molecule, oxaloacetic acid. The product of this reaction, **tricarboxylic acid,** gives the cycle its name.

[2] In the next step, tricarboxylic acid undergoes isomerization to yield **isocitrate.** In the process, only the hydroxyl group is shifted within the molecule. The corresponding enzyme is called *aconitate hydratase* ("aconitase"), because unsaturated *aconitate* arises as an enzyme-bound intermediate during the reaction (not shown; see p. 8). Due to the properties of aconitase, the isomerization is absolutely *stereospecific*. Although citrate is not chiral, isocitrate has two chiral centers, so that it could potentially appear in *four* isomeric forms. However, in the tricarboxylic acid cycle, only one of these stereoisomers, (*2R,3S*)-isocitrate, is produced.

[3] The first oxidative step now follows. *Isocitrate dehydrogenase* oxidizes the hydroxyl group of isocitrate into an oxo group. At the same time, a carboxyl group is released as CO_2, and **2-oxoglutarate** (also known as α-ketoglutarate) and $NADH+H^+$ are formed.

[4] The next step, the formation of **succinyl CoA,** also involves one oxidation and one decarboxylation. It is catalyzed by *2-oxoglutarate dehydrogenase,* a multienzyme complex closely resembling the PDH complex (see p. 134). $NADH+H^+$ is once again formed in this reaction.

[5] The subsequent cleavage of the thioester succinylCoA into **succinate** and coenzyme A by *succinic acid-CoA ligase* (succinyl CoA synthetase, succinic thiokinase) is strongly *exergonic* and is used to synthesize a phosphoric acid anhydride bond ("*substrate level phosphorylation*", see p. 124). However, it is not ATP that is produced here as is otherwise usually the case, but instead **guanosine triphosphate (GTP)**. However, GTP can be converted into ATP by a *nucleoside diphosphate kinase* (not shown).

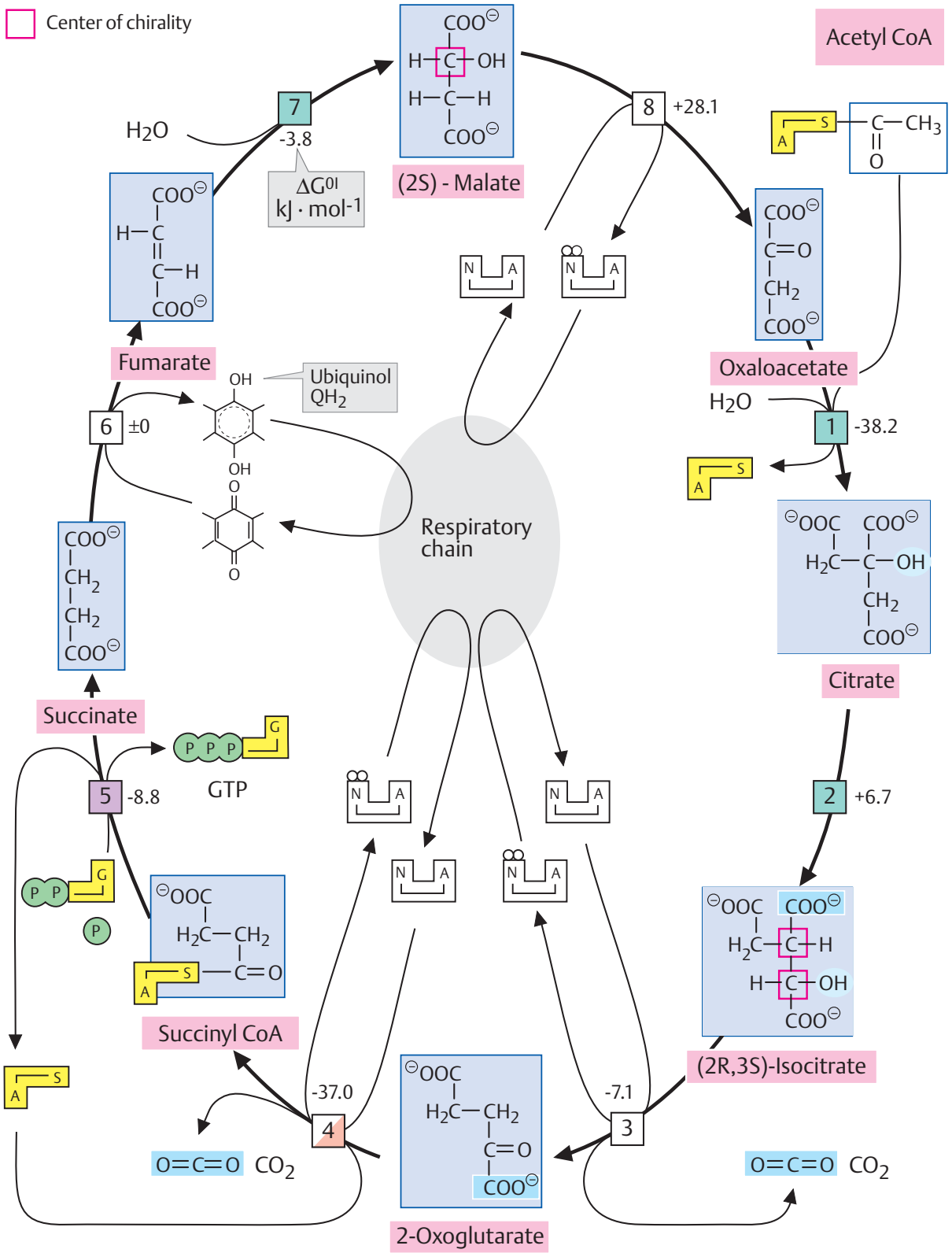
[6] Via the reactions described so far, the acetyl residue has been completely oxidized to CO_2. At the same time, however, the carrier molecule oxaloacetate has been reduced to succinate. Three further reactions in the cycle now regenerate oxaloacetate from succinate. Initially, *succinate dehydrogenase* oxidizes succinate to **fumarate**. In contrast to the other enzymes in the cycle, succinate dehydrogenase is an integral protein of the inner mitochondrial membrane. It is therefore also assigned to the respiratory chain as complex II. Although succinate dehydrogenase contains FAD as a prosthetic group, **ubiquinone** is the real electron acceptor of the reaction.

[7] Water is now added to the double bond of fumarate by *fumarate hydratase* (*"fumarase"*), and chiral *(2S)*-**malate** is produced.

[8] In the last step of the cycle, malate is again oxidized by *malate dehydrogenase* into **oxaloacetate**, with $NADH+H^+$ again being produced. With this reaction, the cycle is complete and can start again from the beginning. As the equilibrium of the reaction lies well on the side of malate, the formation of oxaloacetic acid by reaction [8] depends on the strongly exergonic reaction [1], which immediately removes it from the equilibrium.

The **net outcome** is that each rotation of the tricarboxylic acid cycle converts one acetyl residue and two molecules of H_2O into two molecules of CO_2. At the same time, one GTP, three $NADH+H^+$ and one reduced ubiquinone (QH_2) are produced. By oxidative phosphorylation (see p. 122), the cell obtains around nine molecules of ATP from these reduced coenzymes (see p. 146). Together with the directly formed GTP, this yields a total of 10 ATP per acetyl group.

A. Tricarboxylic acid cycle



1	Citrate synthase 4.1.3.7	4	2-Oxoglutarate DH complex 1.2.4.2, 1.8.1.4, 2.3.1.61	6	Succinate DH 1.3.5.1 [FAD, Fe_2S_2, Fe_4S_4]
2	Aconitase 4.2.1.3 [Fe_4S_4]	5	Succinate-CoA ligase 6.2.1.4	7	Fumarate hydratase 4.2.1.2
3	Isocitrate DH 1.1.1.41		DH = dehydrogenase	8	Malate DH 1.1.1.37

Tricarboxylic acid cycle: functions

A. Tricarboxylic acid cycle: functions ◑

The tricarboxylic acid cycle (see p. 136) is often described as the "hub of intermediary metabolism." It has both catabolic and anabolic functions—it is **amphibolic.**

As a **catabolic pathway**, it initiates the **"terminal oxidation"** of energy substrates. Many catabolic pathways lead to intermediates of the tricarboxylic acid cycle, or supply metabolites such as pyruvate and acetyl-CoA that can enter the cycle, where their C atoms are oxidized to CO_2. The reducing equivalents (see p. 14) obtained in this way are then used for *oxidative phosphorylation*—i. e., to aerobically synthesize ATP (see p. 122).

The tricarboxylic acid cycle also supplies important **precursors for anabolic pathways.** Intermediates in the cycle are converted into:

- Glucose (gluconeogenesis; precursors: oxaloacetate and malate—see p. 154)
- Porphyrins (precursor: succinyl-CoA—see p. 192)
- Amino acids (precursors: 2-oxoglutarate, oxaloacetate—see p. 184)
- Fatty acids and isoprenoids (precursor: citrate—see below)

The intermediates of the tricarboxylic acid cycle are present in the mitochondria only in very small quantities. After the oxidation of acetyl-CoA to CO_2, they are constantly regenerated, and their concentrations therefore remain constant, averaged over time. Anabolic pathways, which remove intermediates of the cycle (e. g., gluconeogenesis) would quickly use up the small quantities present in the mitochondria if metabolites did not reenter the cycle at other sites to replace the compounds consumed. Processes that replenish the cycle in this way are called **anaplerotic reactions**.

The degradation of most amino acids is anaplerotic, because it produces either intermediates of the cycle or pyruvate (*glucogenic amino acids;* see p. 180). Gluconeogenesis is in fact largely sustained by the degradation of amino acids. A particularly important anaplerotic step in animal metabolism leads from pyruvate to oxaloacetic acid. This ATP-dependent reaction is catalyzed by *pyruvate carboxylase* [1]. It allows pyruvate yielding amino acids and lactate to be used for gluconeogenesis.

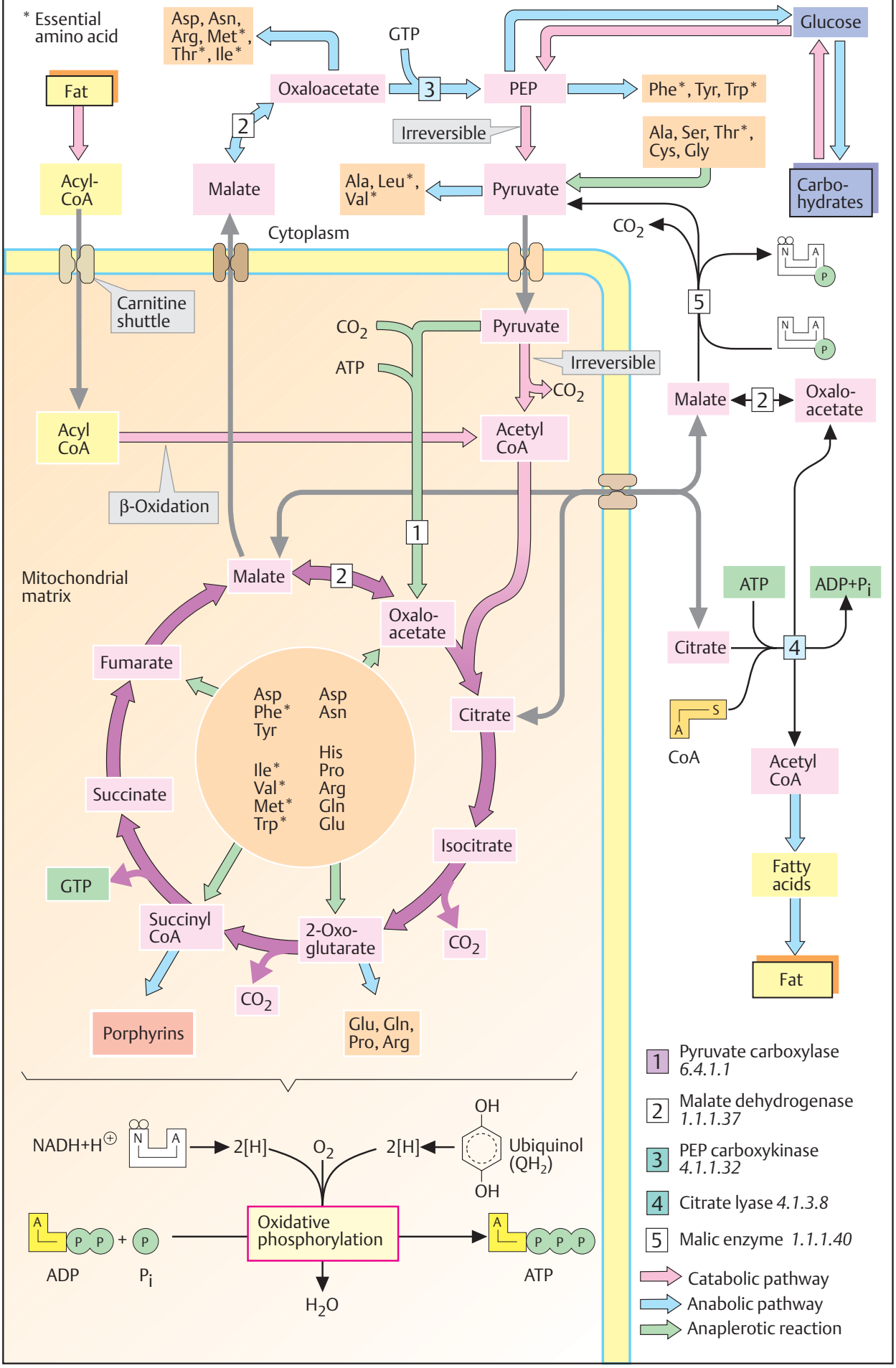
By contrast, *acetyl CoA does not have anaplerotic effects* in animal metabolism. Its carbon skeleton is completely oxidized to CO_2 and is therefore no longer available for biosynthesis. Since fatty acid degradation only supplies acetyl CoA, animals are unable to convert fatty acids into glucose. During periods of hunger, it is therefore not the fat reserves that are initially drawn on, but proteins. In contrast to fatty acids, the amino acids released are able to maintain the blood glucose level (see p. 308).

The tricarboxylic acid cycle not only takes up acetyl CoA from fatty acid degradation, but also supplies the material for the *biosynthesis of fatty acids* and isoprenoids. Acetyl CoA, which is formed in the matrix space of mitochondria by pyruvate dehydrogenase (see p. 134), is not capable of passing through the inner mitochondrial membrane. The acetyl residue is therefore condensed with oxaloacetate by mitochondrial *citrate synthase* to form citrate. This then leaves the mitochondria by antiport with malate (right; see p. 212). In the cytoplasm, it is cleaved again by ATP-dependent *citrate lyase* [4] into acetyl-CoA and oxaloacetate. The oxaloacetate formed is reduced by a cytoplasmic *malate dehydrogenase* to malate [2], which then returns to the mitochondrion via the antiport already mentioned. Alternatively, the malate can be oxidized by *"malic enzyme"* [5], with decarboxylation, to pyruvate. The $NADPH+H^+$ formed in this process is also used for fatty acid biosynthesis.

Additional information

Using the so-called **glyoxylic acid cycle**, plants and bacteria are able to convert acetyl-CoA into succinate, which then enters the tricarboxylic acid cycle. For these organisms, fat degradation therefore functions as an anaplerotic process. In plants, this pathway is located in special organelles, the *glyoxysomes.*

A. Tricarboxylic acid cycle: functions



Respiratory chain

The **respiratory chain** is one of the pathways involved in *oxidative phosphorylation* (see p. 122). It catalyzes the steps by which electrons are transported from $NADH+H^+$ or reduced ubiquinone (QH_2) to molecular oxygen. Due to the wide difference between the redox potentials of the donor ($NADH+H^+$ or QH_2) and the acceptor (O_2), this reaction is strongly exergonic (see p. 18). Most of the energy released is used to establish a proton gradient across the inner mitochondrial membrane (see p. 126), which is then ultimately used to synthesize ATP with the help of *ATP synthase.*

A. Components of the respiratory chain ◑

The **electron transport chain** consists of three protein complexes (**complexes I, III, and IV**), which are integrated into the inner mitochondrial membrane, and two mobile carrier molecules—**ubiquinone** (coenzyme Q) and **cytochrome *c***. *Succinate dehydrogenase,* which actually belongs to the tricarboxylic acid cycle, is also assigned to the respiratory chain as **complex II**. *ATP synthase* (see p. 142) is sometimes referred to as **complex V**, although it is not involved in electron transport. With the exception of complex I, detailed structural information is now available for every complex of the respiratory chain.

All of the complexes in the respiratory chain are made up of numerous polypeptides and contain a series of different protein bound **redox coenzymes** (see pp. 104, 106). These include *flavins* (FMN or FAD in complexes I and II), *iron–sulfur clusters* (in I, II, and III), and *heme groups* (in II, III, and IV). Of the more than 80 polypeptides in the respiratory chain, only 13 are coded by the mitochondrial genome (see p. 210). The remainder are encoded by nuclear genes, and have to be imported into the mitochondria after being synthesized in the cytoplasm (see p. 228).

Electrons enter the respiratory chain in various different ways. In the oxidation of $NADH+H^+$ by *complex I,* electrons pass via FMN and Fe/S clusters to ubiquinone (Q). Electrons arising during the oxidation of succinate, acyl CoA, and other substrates are passed to ubiquinone by *succinate dehydrogenase* or other *mitochondrial dehydrogenases* via enzyme-bound $FADH_2$ and the electron-transporting flavoprotein (ETF; see p. 164). Ubiquinol passes electrons on to *complex III,* which transfers them via two b-type heme groups, one Fe/S cluster, and heme c_1 to the small heme protein *cytochrome c*. Cytochrome *c* then transports the electrons to complex IV—*cytochrome c oxidase*. Cytochrome *c* oxidase contains redox-active components in the form of two copper centers (Cu_A and Cu_B) and hemes a and a_3, through which the electrons finally reach *oxygen* (see p. 132). As the result of the two-electron reduction of O_2, the strongly basic O^{2-} anion is produced (at least formally), and this is converted into water by binding of two protons. The electron transfer is coupled to the **formation of a proton gradient** by complexes I, III, and IV (see p. 126).

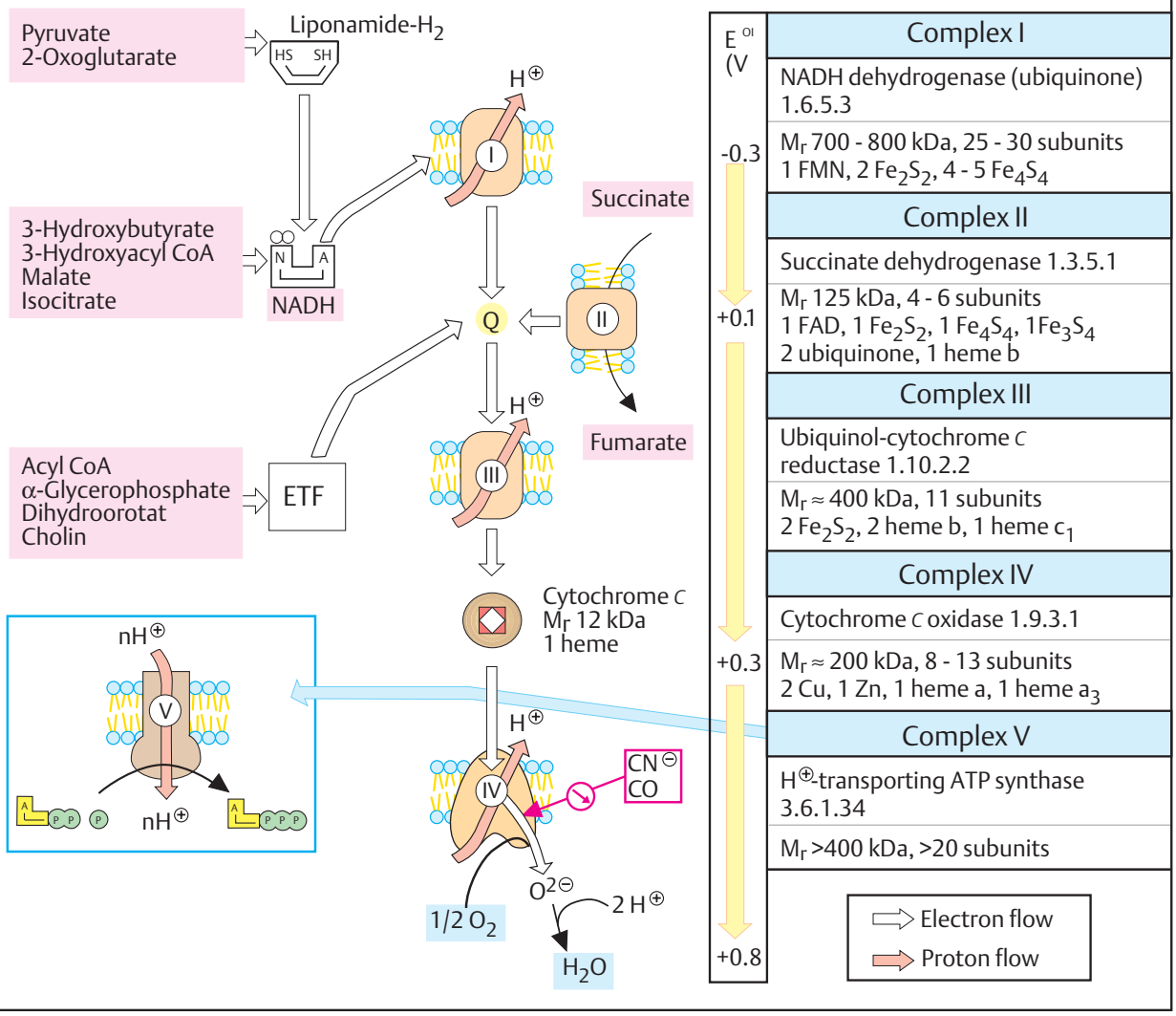
B. Organization ○

Proton transport via complexes I, III, and IV takes place *vectorially* from the matrix into the intermembrane space. When electrons are being transported through the respiratory chain, the H^+ concentration in this space increases—i. e., the pH value there is reduced by about one pH unit. For each H_2O molecule formed, around 10 H^+ ions are pumped into the intermembrane space. If the inner membrane is intact, then generally only *ATP synthase* (see p. 142) can allow protons to flow back into the matrix. This is the basis for the coupling of electron transport to ATP synthesis, which is important for regulation purposes (see p. 144).

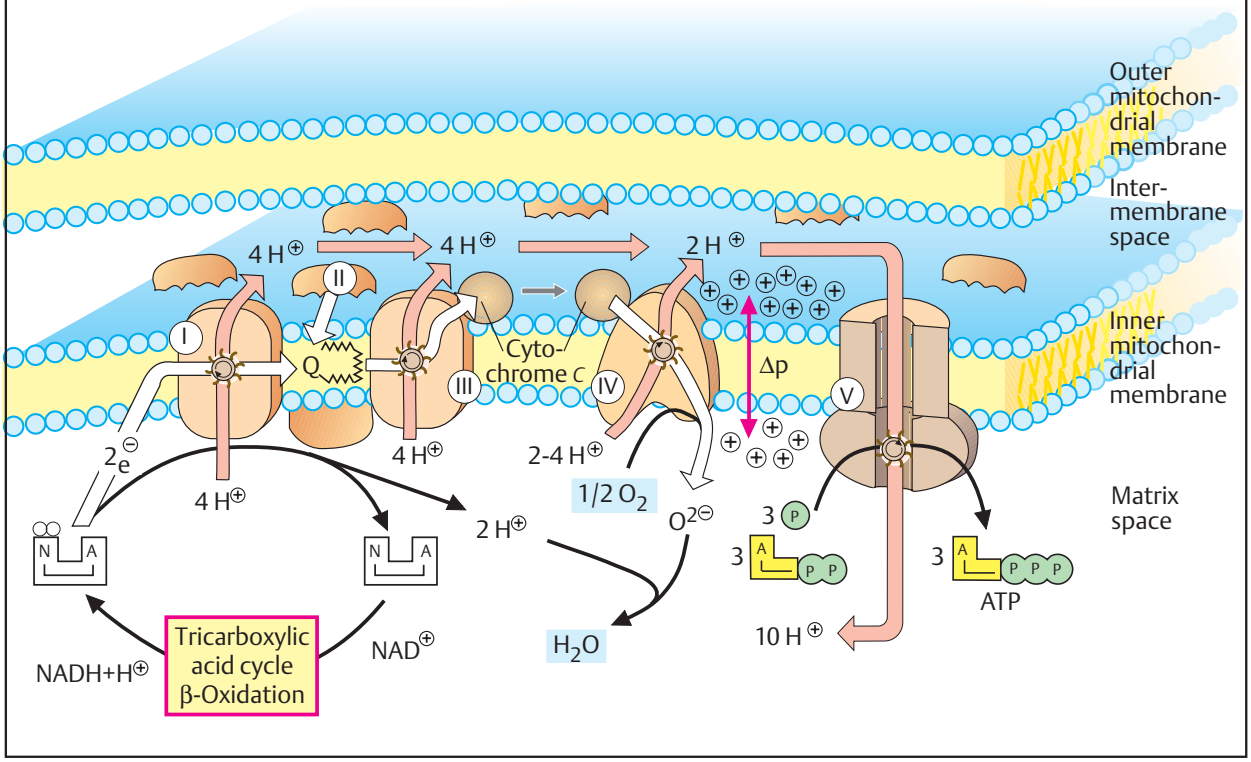
As mentioned, although complexes I through V are all integrated into the inner membrane of the mitochondrion, they are not usually in contact with one another, since the electrons are transferred by ubiquinone and cytochrome *c*. With its long apolar side chain, ubiquinone is freely mobile within the membrane. Cytochrome *c* is water-soluble and is located on the *outside* of the inner membrane.

NADH oxidation via complex I takes place on the *inside* of the membrane—i. e., in the matrix space, where the tricarboxylic acid cycle and β-oxidation (the most important sources of NADH) are also located. O_2 reduction and ATP formation also take place in the matrix.

A. Components of the respiratory chain



B. Organization



ATP synthesis

In the **respiratory chain** (see p. 140), electrons are transferred from NADH or ubiquinol (QH_2) to O_2. The energy obtained in this process is used to establish a proton gradient across the inner mitochondrial membrane. ATP synthesis is ultimately coupled to the return of protons from the intermembrane space into the matrix.

A. Redox systems of the respiratory chain ◑

The electrons provided by NADH do not reach oxygen directly, but instead are transferred to it in various steps. They pass through at least 10 intermediate redox systems, most of which are bound as **prosthetic groups** in complexes I, III, and IV. The large number of coenzymes involved in electron transport may initially appear surprising. However, as discussed on p. 18, in redox reactions, the *change in free enthalpy* ΔG—i. e., the chemical work that is done—depends only on the difference in redox potentials ΔE between the donor and the acceptor. Introducing additional redox systems does not alter the reaction's overall energy yield. In the case of the respiratory chain, the difference between the normal potential of the donor ($NAD^+/NADH+H^+$, $E^{0'} = -0.32$ V) and that of the acceptor (O_2/H_2O, $E^{0'} = +0.82$ V) corresponds to an energy difference $\Delta G^{0'}$ of more than 200 kJ mol^{-1}. This large amount is divided into smaller, more manageable "packages," the size of which is determined by the difference in redox potentials between the respective *intermediates*. It is assumed that this division is responsible for the astonishingly high energy yield (about 60%) achieved by the respiratory chain.

The illustration shows the important redox systems involved in mitochondrial electron transport and their approximate redox potentials. These potentials determine the path followed by the electrons, as the members of a **redox series** have to be arranged in order of increasing redox potential if transport is to occur spontaneously (see p. 32).

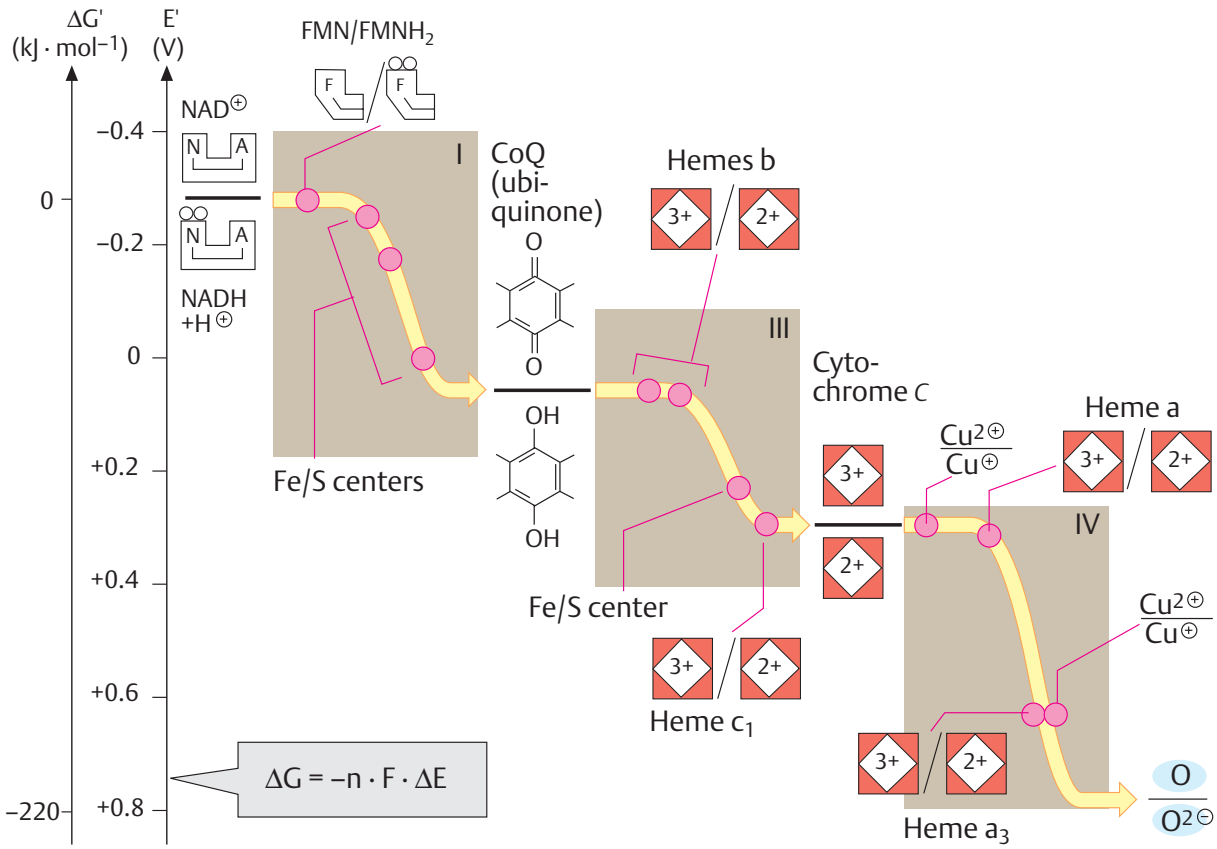
In complex 1, the electrons are passed from *$NADH+H^+$* first to *FMN* (see p. 104) and then on to several *iron–sulfur (Fe/S) clusters*. These redox systems are only stable in the interior of proteins. Depending on the type, Fe/S clusters may contain two to six iron ions, which form complexes with inorganic sulfide and the SH groups of cysteine residues (see p. 286). *Ubiquinone* (coenzyme Q; see p. 104) is a mobile carrier that takes up electrons from complexes I and II and from reduced ETF and passes them on to complex III. *Heme groups* are also involved in electron transport in a variety of ways. Type b hemes correspond to that found in hemoglobin (see p. 280). Heme c in cytochrome *c* is covalently bound to the protein, while the tetrapyrrole ring of heme a is isoprenylated and carries a formyl group. In complex IV, a *copper ion* (Cu_B) and heme a_3 react directly with oxygen.

B. ATP synthase ◑

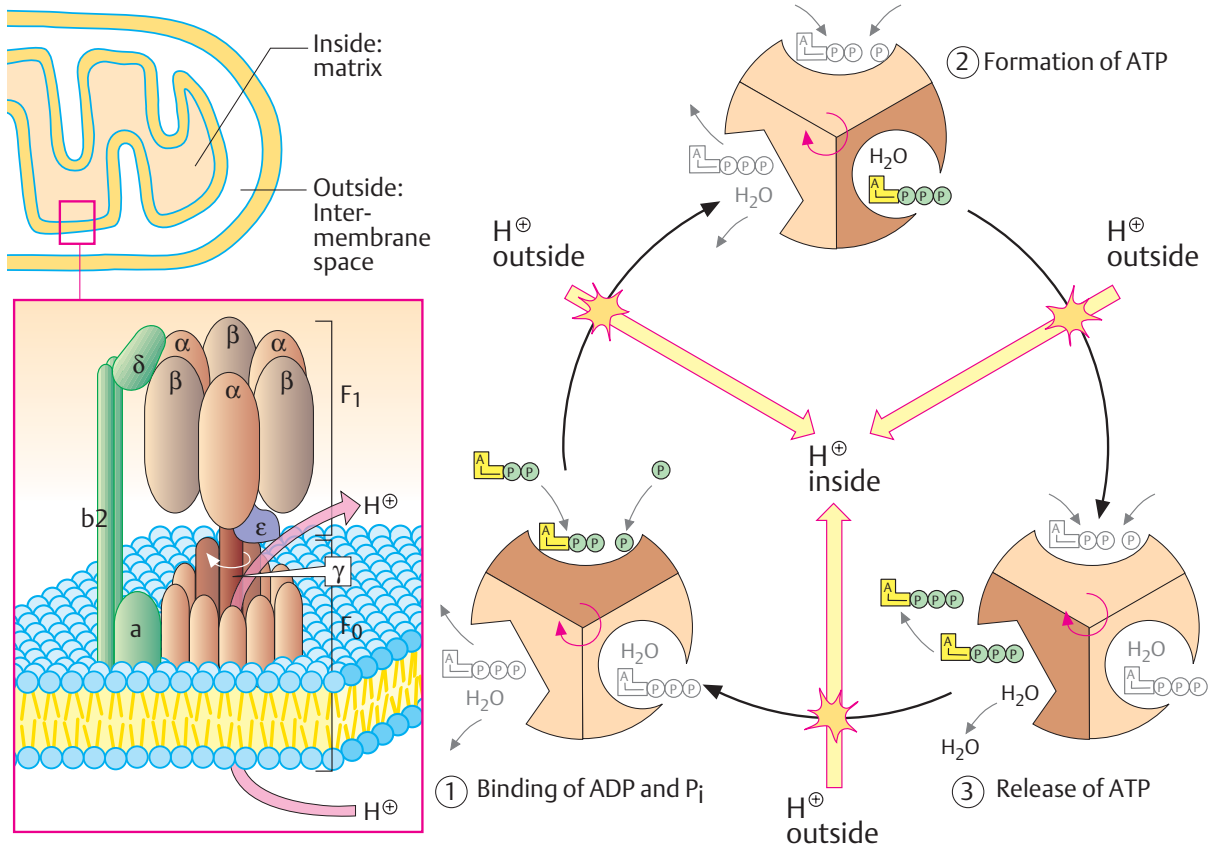
The ATP synthase (*EC 3.6.1.34,* complex V) that transports H^+ is a complex molecular machine. The enzyme consists of two parts—a *proton channel* (F_o, for "oligomycin-sensitive") that is integrated into the membrane; and a *catalytic unit* (F_1) that protrudes into the matrix. The F_o part consists of 12 membrane-spanning c-peptides and one a-subunit. The "head" of the F_1 part is composed of three α and three β subunits, between which there are three active centers. The "stem" between F_o and F_1 consists of one γ and one ε subunit. Two more polypeptides, b and δ, form a kind of "stator," fixing the α and β subunits relative to the F_o part.

The catalytic cycle can be divided into three phases, through each of which the three active sites pass in sequence. First, ADP and P_i are bound (**1**), then the anhydride bond forms (**2**), and finally the product is released (**3**). Each time protons pass through the F_o channel protein into the matrix, all three active sites change from their current state to the next. It has been shown that the energy for proton transport is initially converted into a rotation of the γ subunit, which in turn cyclically alters the conformation of the α and β subunits, which are stationary relative to the F_o part, and thereby drives ATP synthesis.

A. Redox systems of the respiratory chain



B. ATP synthase



1. Structure and location **2.** Catalytic cycle

Regulation

The amount of nutrient degradation and ATP synthesis have to be continually adjusted to the body's changing energy requirements. The need to coordinate the production and consumption of ATP is already evident from the fact that the *total amounts* of coenzymes in the organism are low. The human body forms about 65 kg ATP per day, but only contains 3–4 g of adenine nucleotides (AMP, ADP, and ATP). Each ADP molecule therefore has to be phosphorylated to ATP and dephosphorylated again many thousand times a day.

A. Respiratory control ◑

The simple regulatory mechanism which ensures that ATP synthesis is "automatically" coordinated with ATP consumption is known as **respiratory control**. It is based on the fact that the different parts of the oxidative phosphorylation process are *coupled* via shared coenzymes and other factors (left).

If a cell is not using any ATP, hardly any ADP will be available in the mitochondria. Without ADP, *ATP synthase* (**3**) is unable to break down the proton gradient across the inner mitochondrial membrane. This in turn inhibits electron transport in the respiratory chain (**2**), which means that $NADH+H^+$ can no longer be reoxidized to NAD^+. Finally, the resulting high $NADH/NAD^+$ ratio inhibits the tricarboxylic acid cycle (**C**), and thus slows down the degradation of the substrate SH_2 (**1**). Conversely, high rates of ATP utilization stimulate nutrient degradation and the respiratory chain via the same mechanism.

If the formation of a proton gradient is prevented (right), substrate oxidation (**1**) and electron transport (**2**) proceed much more rapidly. However, instead of ATP, only heat is produced.

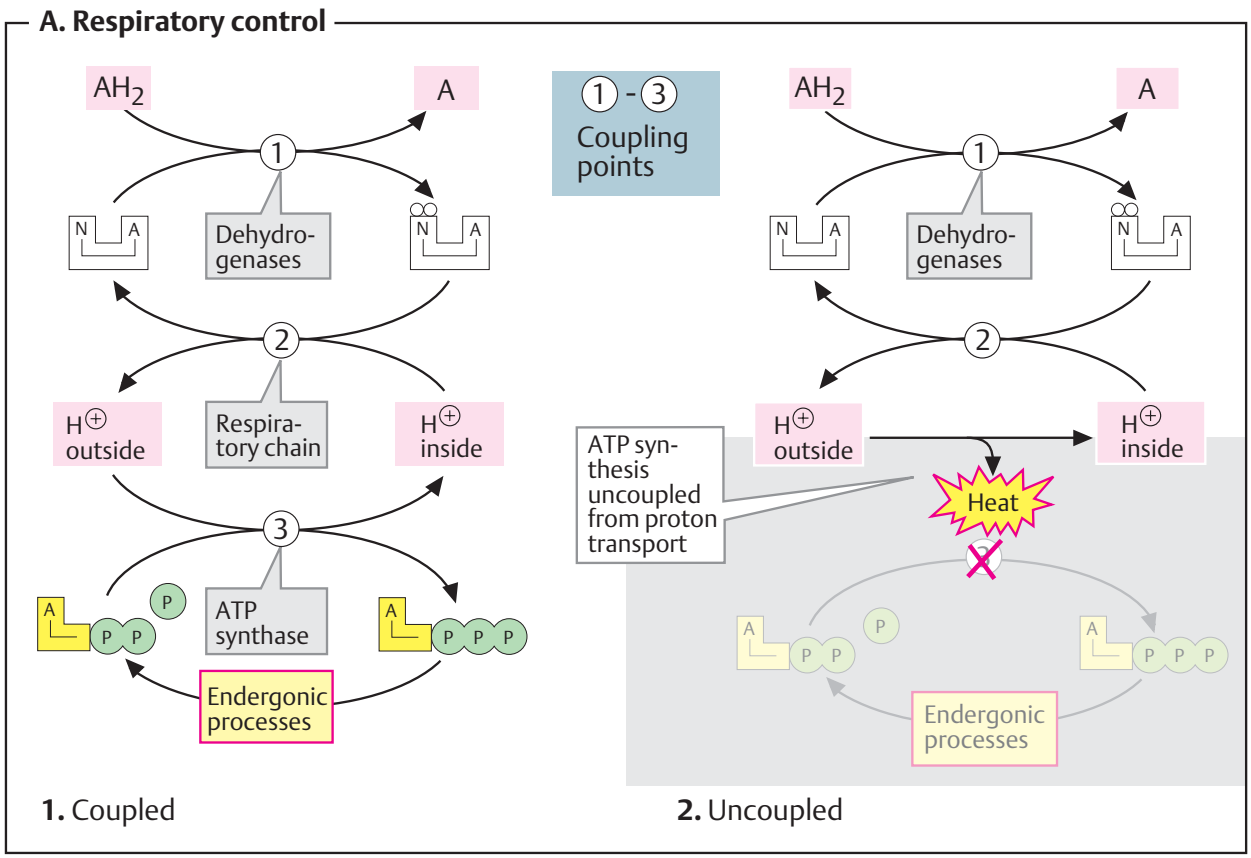
B. Uncouplers ◑

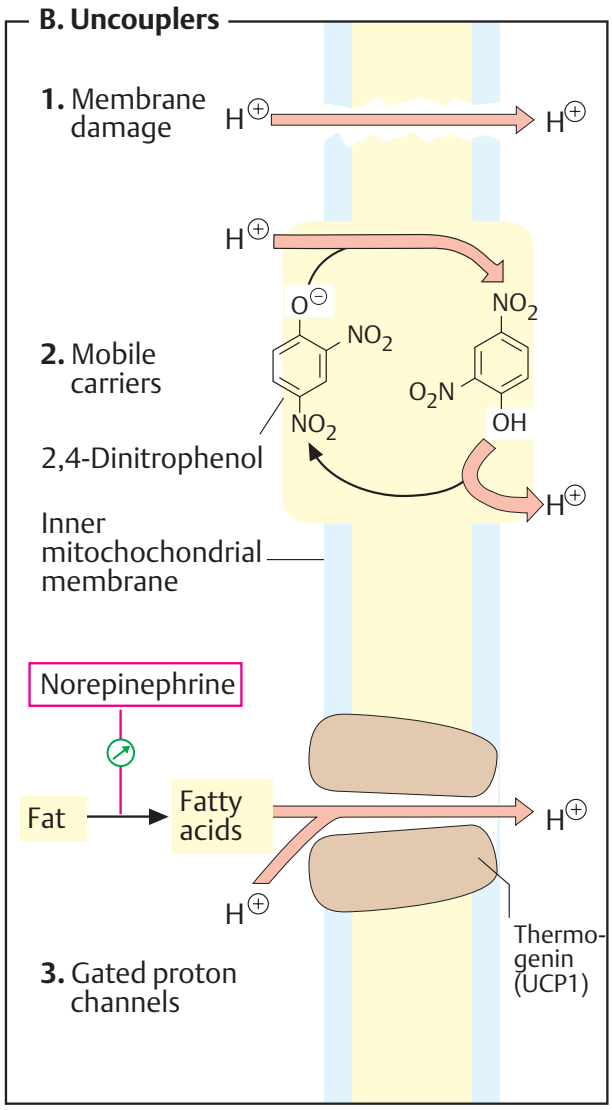
Substances that functionally separate oxidation and phosphorylation from one another are referred to as uncouplers. They break down the proton gradient by allowing H^+ ions to pass from the intermembrane space back into the mitochondrial matrix without the involvement of ATP synthase. Uncoupling effects are produced by **mechanical damage** to the inner membrane (**1**) or by lipid-soluble substances that can transport protons through the membrane, such as **2,4-dinitrophenol** (DNP, **2**). **Thermogenin** (uncoupling protein-1, UCP-1, **3**)—an ion channel (see p. 222) in mitochondria of *brown fat* tissue—is a naturally occurring uncoupler. Brown fat is found, for example, in newborns and in hibernating animals, and serves exclusively to generate heat. In cold periods, norepinephrine activates the *hormone-sensitive lipase* (see p. 162). Increased lipolysis leads to the production of large quantities of free fatty acids. Like DNP, these bind H^+ ions in the intermembrane space, pass the UCP in this form, and then release the protons in the matrix again. This makes fatty acid degradation independent of ADP availability—i.e., it takes place at maximum velocity and only produces heat (**A**). It is becoming increasingly clear that there are also UCPs in other cells, which are controlled by hormones such as thyroxine (see p. 374). This regulates the ATP yield and what is known as the basal metabolic rate.

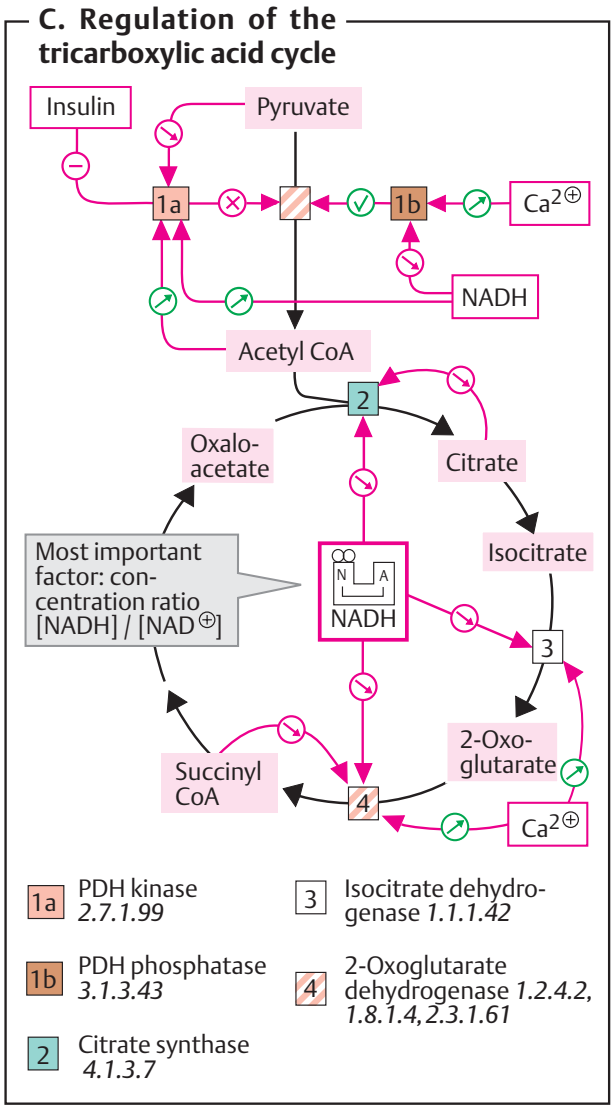
C. Regulation of the tricarboxylic acid cycle ◑

The most important factor in the regulation of the cycle is the **$NADH/NAD^+$ ratio**. In addition to *pyruvate dehydrogenase* (PDH) and *oxoglutarate dehydrogenase* (ODH; see p. 134), *citrate synthase* and *isocitrate dehydrogenase* are also inhibited by NAD^+ deficiency or an excess of $NADH+H^+$. With the exception of isocitrate dehydrogenase, these enzymes are also subject to **product inhibition** by acetyl-CoA, succinyl-CoA, or citrate.

Interconversion processes (see p. 120) also play an important role. They are shown here in detail using the example of the PDH complex (see p. 134). The *inactivating protein kinase* [1a] is inhibited by the substrate pyruvate and is activated by the products acetyl-CoA and $NADH+H^+$. The p*rotein phosphatase* [1b]—like *isocitrate dehydrogenase* [3] and the *ODH complex* [4]—is activated by Ca^{2+}. This is particularly important during muscle contraction, when large amounts of ATP are needed. *Insulin* also activates the PDH complex (through inhibition of phosphorylation) and thereby promotes the breakdown of glucose and its conversion into fatty acids.







Respiration and fermentation

A. Aerobic and anaerobic oxidation of glucose ◑

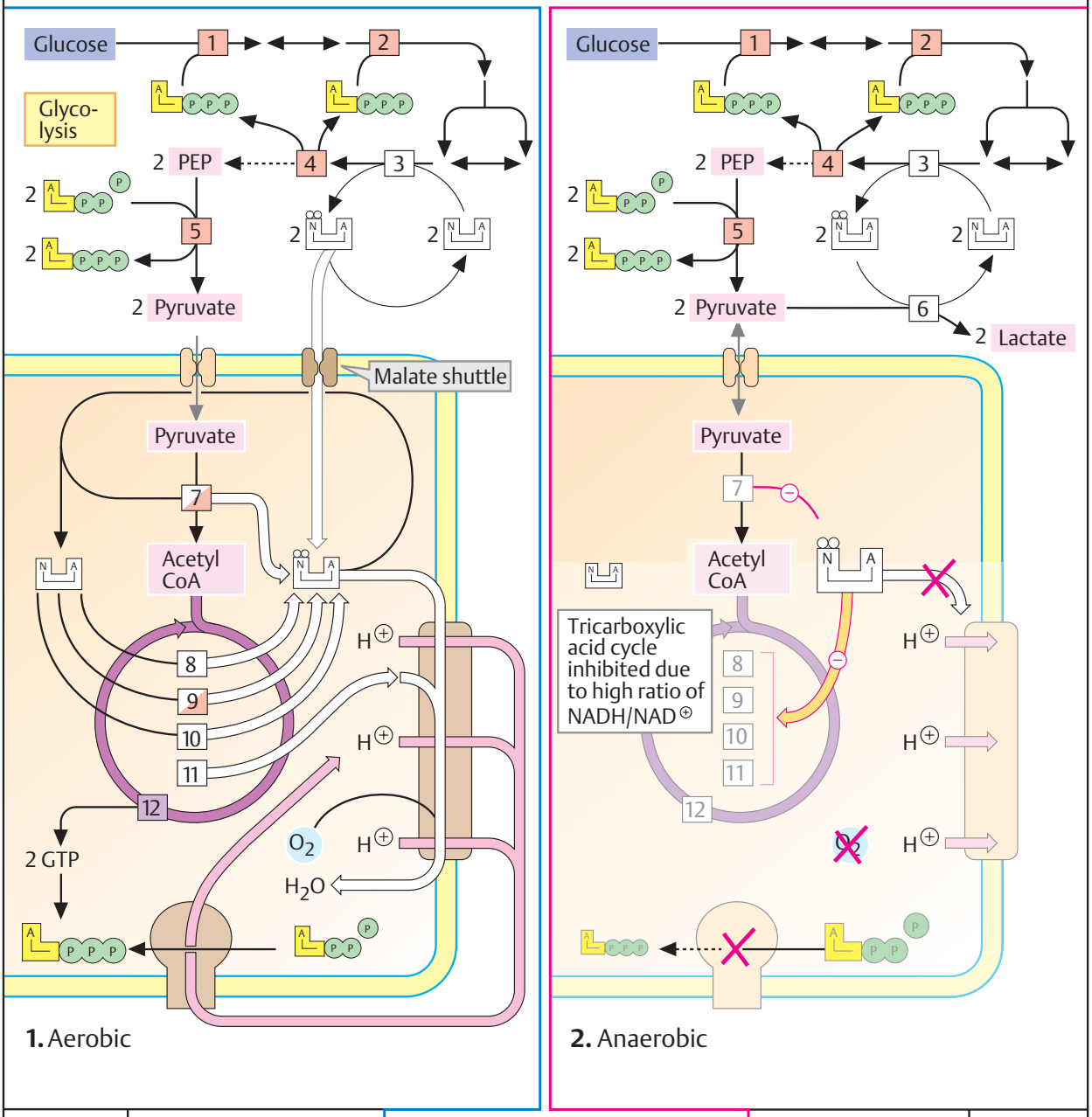
In the presence of oxygen (i.e., in *aerobic* conditions), most animal cells are capable of "respiring" various types of nutrient (lipids, amino acids, and carbohydrates)—i.e., using oxidative processes to break them down completely. If oxygen is lacking (i.e., in *anaerobic* conditions), only glucose can be used for ATP synthesis. Although in these conditions glucose breakdown in animals already ends in lactate and only produces small quantities of ATP, it is decisively important for the survival of cells at times of oxygen deficiency.

In **aerobic conditions** (left), ATP is derived almost exclusively from oxidative phosphorylation (see p. 140). **Fatty acids** enter the mitochondria with the help of carnitine (see p. 164), and are broken down there into CoA-bound acetyl residues. **Glucose** is converted into pyruvate by glycolysis (see p. 150) in the cytoplasm. Pyruvate is then also transported into the mitochondrial matrix, where it is oxidatively decarboxylated by the pyruvate dehydrogenase complex (see p. 134) to yield acetyl-CoA. The reducing equivalents (2 $NADH+H^+$ per glucose) that arise in glycolysis enter the mitochondrial matrix via the malate shuttle (see p. 212). The acetyl residues that are formed are oxidized to CO_2 in the tricarboxylic acid cycle (see p. 136). Breakdown of **amino acids** also produces acetyl residues or products that can directly enter the tricarboxylic acid cycle (see p. 180). The reducing equivalents that are obtained are transferred to oxygen via the respiratory chain as required. In the process, chemical energy is released, which is used (via a proton gradient) to synthesize ATP (see p. 140).

In the absence of oxygen—i.e., in **anaerobic conditions**—the picture changes completely. Since O_2 is missing as the electron acceptor for the respiratory chain, $NADH+H^+$ and QH_2 can no longer be reoxidized. Consequently, not only is mitochondrial ATP synthesis halted, but also almost the whole metabolism in the mitochondrial matrix. The main reason for this is the high $NADH+H^+$ concentration and lack of NAD^+, which inhibit the tricarboxylic acid cycle and the pyruvate dehydrogenase reaction (see p. 144). β-Oxidation and the malate shuttle, which are dependent on free NAD^+, also come to a standstill. Since amino acid degradation is also no longer able to contribute to energy production, the cell becomes totally dependent on ATP synthesized via the degradation of glucose by **glycolysis.** For this process to proceed continuously, the $NADH+H^+$ formed in the cytoplasm has to be constantly reoxidized. Since this can no longer occur in the mitochondria, in anaerobic conditions animal cells reduce pyruvate to lactate and pass it into the blood. This type of process is called **fermentation** (see p. 148). The ATP yield is low, with only two ATPs per glucose arising during lactate synthesis.

To estimate the number of ATP molecules formed in an aerobic state, it is necessary to know the **P/O quotient**—i.e., the molar ratio between synthesized ATP ("P") and the water formed ("O"). During transport of two electrons from $NADH+H^+$ to oxygen, about 10 protons are transported into the intermembrane space, while from ubiquinol (QH_2), the number is only six. ATP synthase (see p. 142) probably requires three H^+ to synthesize one ATP, so that maximum P/O quotients of around **3 or 2** are possible. This implies a yield of up to 38 ATP per mol of glucose. However, the actual value is much lower. It needs to be taken into account that the transport of specific metabolites into the mitochondrial matrix and the exchange of ATP^{4-} for ADP^{3-} are also driven by the proton gradient (see p. 212). The P/O quotients for the oxidation of $NADH+H^+$ and QH_2 are therefore more in the range of **2.5 and 1.5**. If the energy balance of aerobic glycolysis is calculated on this basis, the result is a yield of around **32 ATP per glucose**. However, this value is also not constant, and can be adjusted as required by the cell's own uncouplers (UCPs; see p. 144) and other mechanisms.

A. Aerobic and anaerobic oxidation of glucose



ATP	Coenzymes		Enzymes	Coenzymes	ATP
−1		−1 ATP	1 Hexokinase	−1 ATP	−1
−2		−1 ATP	2 6-Phosphofructokinase	−1 ATP	−2
+3	+5 ATP ←	+2 NADH	3 Glyceraldehyde-3(P)DH	+2 NADH ←	−2
+5		+2 ATP	4 Phosphoglycerate kinase	+2 ATP NAD⊕	0
+7		+2 ATP	5 Pyruvate kinase	+2 ATP recycled	+2
			6 Lactate dehydrogenase	−2 NADH ←	
+12	+5 ATP ←	+2 NADH	7 Pyruvate dehydrogenase		
+17	+5 ATP ←	+2 NADH	8 Isocitrate dehydrogenase		
+22	+5 ATP ←	+2 NADH	9 Oxoglutarate dehydrogenase		
+27	+5 ATP ←	+2 NADH	10 Malate dehydrogenase		
+30	+3 ATP ←	+2 QH_2	11 Succinate dehydrogenase		
+32	+2 ATP ←	+2 GTP	12 Succinate-CoA ligase		

Sum: 32 ATP/glucose

DH = dehydrogenase

Sum: 2 ATP/glucose

Fermentations

As discussed on p. 146, degradation of glucose to pyruvate is the only way for most organisms to synthesize ATP in the *absence of oxygen*. The $NADH+H^+$ that is also formed in this process has to be constantly reoxidized to NAD^+ in order to maintain glycolysis and thus ATP synthesis. In the animal organism, this is achieved by the reduction of pyruvate to lactate. In microorganisms, there are many other forms of NAD^+ regeneration. Processes of this type are referred to as **fermentations.** Microbial fermentation processes are often used to produce foodstuffs and alcoholic beverages, or to preserve food. Features common to all fermentation processes are that they start with pyruvate and only occur under *anaerobic conditions.*

A. Lactic acid and propionic acid fermentation ○

Many milk products, such as sour milk, yogurt, and cheese are made by *bacterial lactic acid fermentation* (**1**). The reaction is the same as in animals. Pyruvate, which is mainly derived from degradation of the disaccharide *lactose* (see p. 38), is reduced to lactate by *lactate dehydrogenase* [1]. Lactic acid fermentation also plays an important role in the production of sauerkraut and silage. These products usually keep for a long time, because the *pH reduction* that occurs during fermentation inhibits the growth of putrefying bacteria.

Bacteria from the genera *Lactobacillus* and *Streptococcus* are involved in the first steps of dairy production (**3**). The raw materials produced by their effects usually only acquire their final properties after additional fermentation processes. For example, the characteristic taste of Swiss cheese develops during a subsequent propionic acid fermentation. In this process, bacteria from the genus *Propionibacterium* convert pyruvate to propionate in a complex series of reactions (**2**).

B. Alcoholic fermentation ○

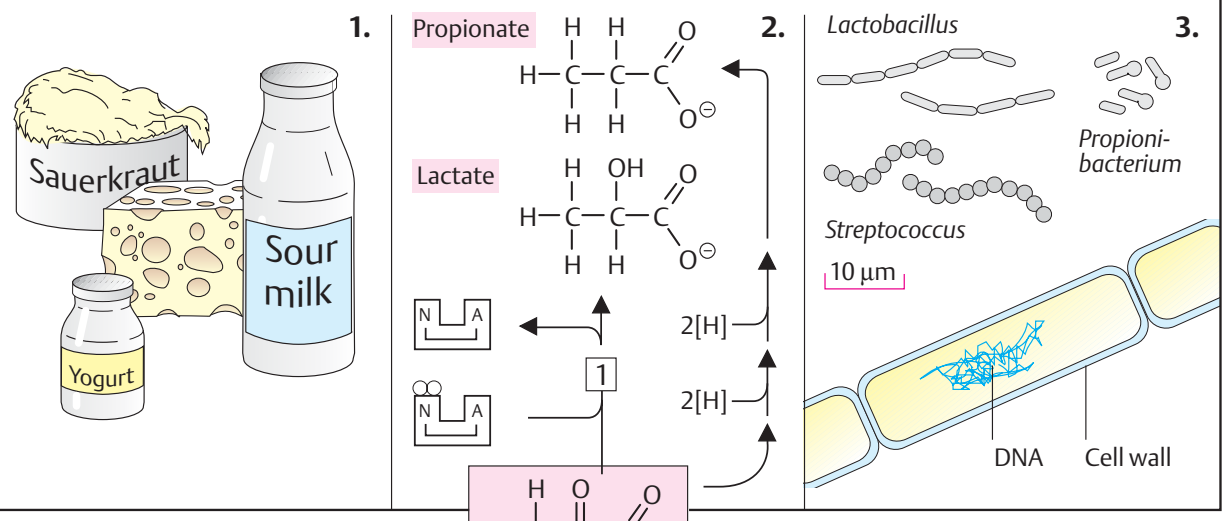
Alcoholic beverages are produced by the fermentation of plant products that have a high carbohydrate content. Pyruvate, which is formed from glucose, is initially decarboxylated by *pyruvate decarboxylase* [2], which does not occur in animal metabolism, to produce acetaldehyde (ethanal). When this is reduced by alcohol dehydrogenase [3], with NADH being consumed, *ethanol* [3] is formed.

Yeasts, unicellular fungi that belong to the eukaryotes (**3**), rather than bacteria, are responsible for this type of fermentation. Yeasts are also often used in baking. They produce CO_2 and ethanol, which raise the dough. Brewers' and bakers' yeasts *(Saccharomyces cerevisiae)* are usually haploid and reproduce asexually by budding (**3**). They can live both aerobically and anaerobically. Wine is produced by other types of yeast, some of which already live on the grapes. To promote the formation of ethanol, efforts are made to generally exclude oxygen during alcoholic fermentation—for example, by covering dough with a cloth when it is rising and by fermenting liquids in barrels that exclude air.

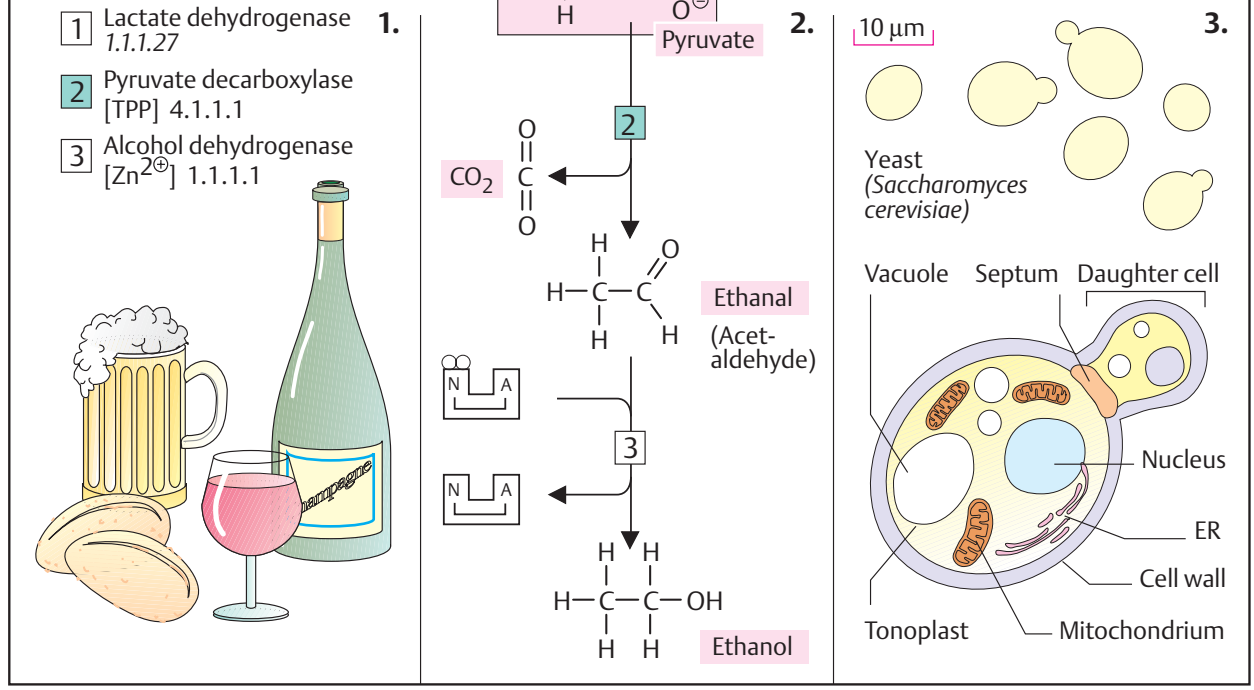
C. Beer brewing ○

Barley is the traditional starting material for the brewing of beer. Although cereal grains contain starch, they hardly have any *free* sugars. The barley grains are therefore first allowed to germinate so that starch-cleaving *amylases* are formed. Careful warming of the sprouting grain produces **malt.** This is then ground, soaked in water, and kept warm for a certain time. In the process, a substantial proportion of the starch is broken down into the disaccharide *maltose* (see p. 38). The product (the wort) is then boiled, **yeast** and **hops** are added, and the mixture is allowed to ferment for several days. The addition of hops makes the beer less perishable and gives it its slightly bitter taste. Other substances contained in hops act as sedatives and diuretics.

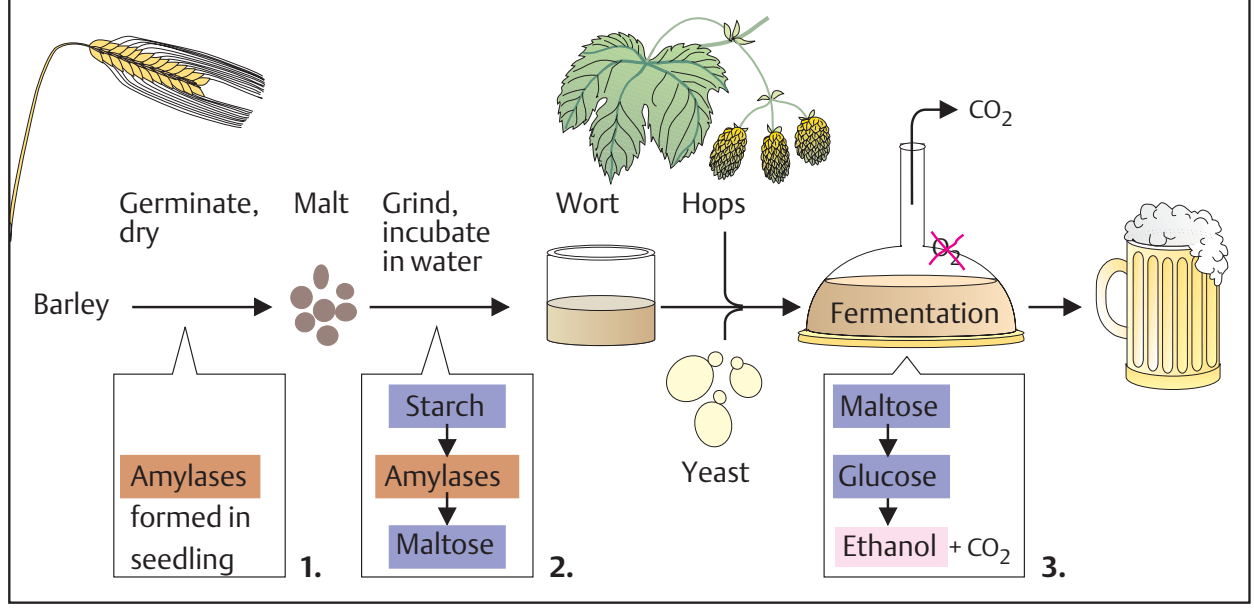
A. Lactic acid and propionic acid fermentation



B. Alcoholic fermentation



C. Beer brewing



Glycolysis

A. Balance ●

Glycolysis is a catabolic pathway in the cytoplasm that is found in almost all organisms—irrespective of whether they live aerobically or anaerobically. The balance of glycolysis is simple: glucose is broken down into two molecules of pyruvate, and in addition two molecules of ATP and two of $NADH+H^+$ are formed.

In the presence of oxygen, pyruvate and $NADH+H^+$ reach the mitochondria, where they undergo further transformation (**aerobic glycolysis**; see p. 146). In anaerobic conditions, *fermentation products* such as lactate or ethanol have to be formed in the cytoplasm from pyruvate and $NADH+H^+$, in order to regenerate NAD^+ so that glycolysis can continue (**anaerobic glycolysis**; see p. 146). In the anaerobic state, glycolysis is the only means of obtaining ATP that animal cells have.

B. Reactions ◑

Glycolysis involves ten individual steps, including three isomerizations and four phosphate transfers. The only redox reaction takes place in step [6].

[1] Glucose, which is taken up by animal cells from the blood and other sources, is first phosphorylated to **glucose 6-phosphate**, with ATP being consumed. The glucose 6-phosphate is not capable of leaving the cell.

[2] In the next step, glucose 6-phosphate is isomerized into **fructose 6-phosphate**.

[3] Using ATP again, another phosphorylation takes place, giving rise to **fructose 1,6-bisphosphate.** *Phosphofructokinase* is the most important key enzyme in glycolysis (see p. 144).

[4] Fructose 1,6-bisphosphate is broken down by *aldolase* into the C_3 compounds **glyceraldehyde 3-phosphate** (also known as glyceral 3-phosphate) and **glycerone 3-phosphate** (dihydroxyacetone 3-phosphate).

[5] The latter two products are placed in fast equilibrium by *triosephosphate isomerase.*

[6] Glyceraldehyde 3-phosphate is now oxidized by *glyceraldehyde-3-phosphate dehydrogenase,* with $NADH+H^+$ being formed. In this reaction, *inorganic phosphate* is taken up into the molecule (*substrate-level phosphorylation*; see p. 124), and **1,3-bisphosphoglycerate** is produced. This intermediate contains a *mixed acid–anhydride bond,* the phosphate part of which is at a high chemical potential.

[7] Catalyzed by *phosphoglycerate kinase,* this phosphate residue is transferred to ADP, producing **3-phosphoglycerate** and ATP. The ATP balance is thus once again in equilibrium.

[8] As a result of shifting of the remaining phosphate residue within the molecule, the isomer **2-phosphoglycerate** is formed.

[9] Elimination of water from 2-phosphoglycerate produces the phosphate ester of the *enol form* of pyruvate—**phospho*enol*pyruvate** (PEP). This reaction also raises the second phosphate residue to a high potential.

[10] In the last step, *pyruvate kinase* transfers this residue to ADP. The remaining enol pyruvate is immediately rearranged into **pyruvate**, which is much more stable. Along with step [7] and the thiokinase reaction in the tricarboxylic acid cycle (see p. 136), the pyruvate kinase reaction is one of the three reactions in animal metabolism that are able to produce ATP independently of the respiratory chain.

In glycolysis, two molecules of ATP are initially used for activation ([1], [3]). Later, two ATPs are formed *per C_3 fragment.* Overall, therefore, there is a small net gain of 2 mol ATP per mol of glucose.

C. Energy profile ○

The energy balance of metabolic pathways depends not only on the standard changes in enthalpy ΔG^0, but also on the concentrations of the metabolites (see p. 18). Fig. **C** shows the *actual* enthalpy changes ΔG for the individual steps of glycolysis in erythrocytes.

As can be seen, only three reactions ([1], [3], and [10]), are associated with large changes in free enthalpy. In these cases, the equilibrium lies well on the side of the products (see p. 18). All of the other steps are freely reversible. The same steps are also followed—in the reverse direction—in gluconeogenesis (see p. 154), with the same enzymes being activated as in glucose degradation. The non-reversible steps [1], [3], and [10] are bypassed in glucose biosynthesis (see p. 154).