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Biochemistry

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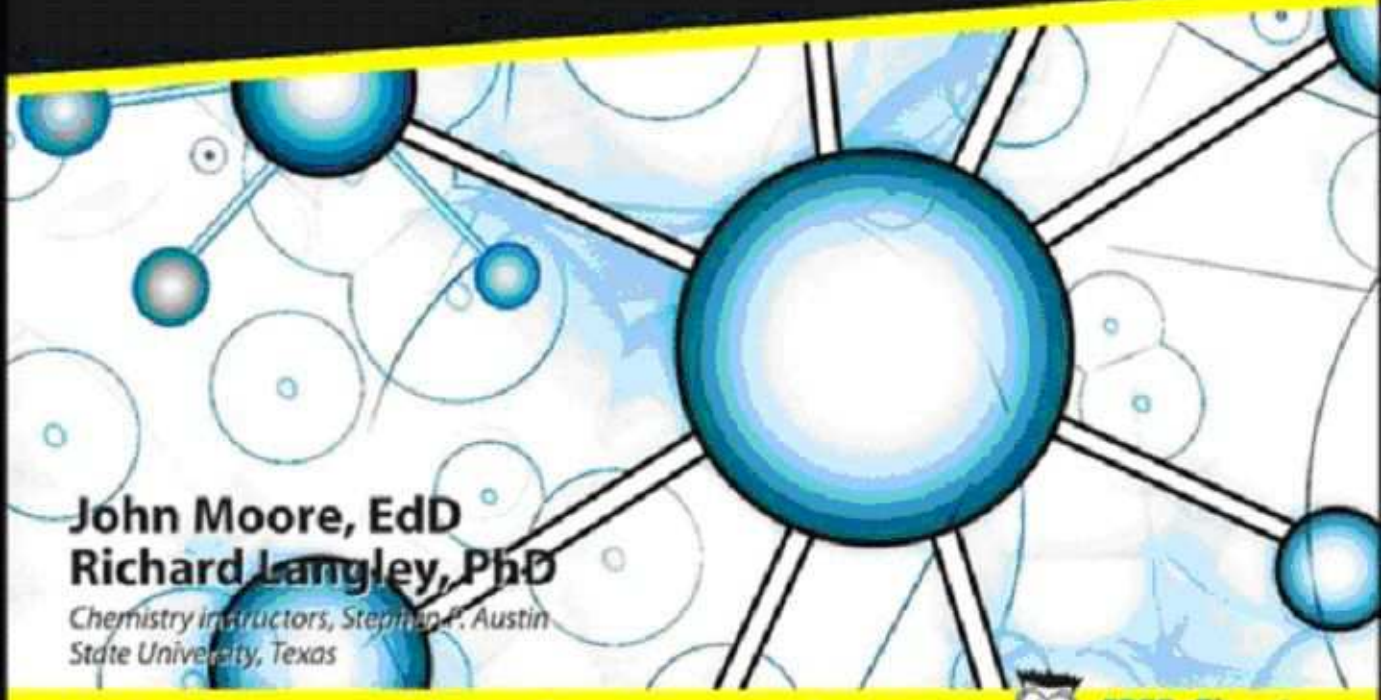

John Moore, EdD
Richard Langley, PhD
Chemistry instructors, Stephen F. Austin State University, Texas

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Biochemistry FOR DUMMIES®

by John T. Moore, EdD and Richard Langley, PhD

Wiley Publishing, Inc.

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About the Authors

John Moore grew up in the foothills of Western North Carolina. He attended the University of North Carolina at Asheville, where he received his bachelor's degree in chemistry. He earned his master's degree in chemistry from Furman University in Greenville, South Carolina. After a stint in the United States Army, he decided to try his hand at teaching. In 1971, he joined the chemistry faculty of Stephen F. Austin State University in Nacogdoches, Texas, where he still teaches chemistry. In 1985 he started back to school part time and in 1991 received his doctorate in education from Texas A&M University. For the last five years has been the co-editor (along with one of his former students) of the "Chemistry for Kids" feature of *The Journal of Chemical Education*. In 2003, his first book, *Chemistry For Dummies*, was published, soon to be followed by *Chemistry Made Simple*. John enjoys cooking and making custom knife handles from exotic woods.

Richard Langley grew up in southwestern Ohio. He attended Miami University in Oxford, Ohio, where he received bachelor's degrees in chemistry and mineralogy and then a master's degree in chemistry. His next stop was the University of Nebraska, where he received his doctorate in chemistry. Afterwards, he took a postdoctoral position at Arizona State University in Tempe, Arizona, followed by a visiting assistant professor position at the University of Wisconsin at River Falls. In 1982, he moved to Stephen F. Austin State University. For the past several years, he and John have been graders for the Free Response portion of the AP Chemistry Exam. He and John have collaborated on several writing projects, including *5 Steps To A 5 on the AP: Chemistry* and *Chemistry for the Utterly Confused*. Rich enjoys jewelry making and science fiction.

Dedication

To my wife, Robin; sons, Matthew and Jason; my wonderful daughter-in-law, Sara; and the two most wonderful grandkids in the world, Zane and Sadie. I love you guys. — John

To my mother. — Rich

Authors' Acknowledgments

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We're proud of this book; please send us your comments through our Dummies online registration form located at www.dummies.com/register/.

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Contents at a Glance

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Introduction

Welcome to *Biochemistry For Dummies!*

We are certainly happy you have decided to delve into the fascinating world of biochemistry. Biochemistry is a complex area of chemistry, but understanding biochemistry isn't really complex. It takes hard work, attention to detail, and the desire to know and to imagine. Biochemistry, like any area of chemistry, is not a spectator sport. You must interact with the material, try different explanations, and ask yourself why things happen the way they do.

Work hard and you will get through your biochem course. More importantly, you might grow to appreciate the symphony of chemical reactions that take place within a living organism, whether it be a one-celled organism, a tree, or a person. As each individual instrument contributes to an orchestra, each chemical reaction is necessary, and sometimes its part is quite complex. However, when you combine all the instruments, and each instrument functions well, the result can be a wonder to behold and hear. If one or two instruments are a little out of tune or aren't being played well, the orchestra still functions — but things are a little off. The sound isn't quite as beautiful, or there is a nagging sensation of something being wrong. The same is true of an organism. If all the reactions occur correctly at the right time, the organism functions well. If a reaction or a few reactions are off in some way, the organism may not function nearly as well. Genetic diseases, electrolyte imbalance, and other problems may cause the organism to falter. And what happens then? Biochemistry is often where ways of restoring the organism to health are found.

About This Book

Biochemistry For Dummies is an overview of the material covered in a typical college-level biochemistry course. We have made every attempt to keep the material as current as possible, but the field is changing ever so quickly. The basics, however, stay the same, and that is where we concentrate our efforts. We also include information on some of the applications of biochemistry that you read about in your everyday life, such as forensics, cloning, gene therapy, genetic testing, genetically modified foods, and so on.

As you flip through this book you will see a lot of chemical structures and reactions. Much of the biochemistry revolves around knowing the structures of the molecules involved in biochemical reactions. If you are in a biochemistry course, you probably have had at least one semester of organic chemistry. You will recognize many of the structures, or at least the functional groups, from your study of organic chem. You will see many of those mechanisms that you loved/hated here in biochemistry.

If you bought this book just to gain general knowledge about a fascinating subject, try not to get bogged down in the details. Skim the chapters. If you find a topic that interests you, stop and dive in. Have fun learning something new.

Conventions Used in This Book

We have organized this text in a logical progression of topics that might be used in a biochemistry course. We have made extensive use of structures and reactions. While reading, try to follow along in the associated figures, whether they be structures or reactions. The icons point out things to which you should pay particular attention, for various reasons. If you are taking a biochemistry course, use this rather inexpensive book to supplement that very expensive biochemistry textbook.

Icons Used in This Book

If you have ever read other *For Dummies* books (such as the wonderful *Chemistry For Dummies*) you will recognize the icons used in this book, but here are their meanings anyway:

This icon is a flag for those really important things that you shouldn't forget as you go deeper into the world of biochemistry.

We use this icon to alert you to a tip on the easiest or quickest way to learn a concept. Between the two of us, we have almost 70 years of teaching experience. We've learned a few tricks along the way and we don't mind sharing.

The Real World icon points out information that has direct application in the everyday world.

The Warning icon points to a procedure or potential outcome that can be dangerous. We call it our Don't-Try-This-At-Home icon.

What You're Not to Read

Don't read what you don't need. Concentrate on the area(s) in which you need help. If you are interested in real-world applications of biochemistry, by all means read those sections (indicated by the Real World icon). However, if you just need help on the straight biochemistry, feel free to skip the applications. You don't have a whole lot of money invested in this book, so don't feel obligated to read everything. When you're done, you can put it in your bookshelf alongside *Chemistry For Dummies, The Doctor Who Error Finder,* and *A Brief History of Time* as a conversation piece.

Foolish Assumptions

We assume — and we all know about the perils of assumptions — that you are one of the following:

- A student taking a college-level biochemistry course.
- A student reviewing your biochemistry for some type of standardized exam (the MCAT, for example).
- An individual who just wants to know something about biochemistry.
- A person who has been watching way too many forensic TV shows.

If you fall into a different category, we hope you enjoy this book anyway.

How This Book Is Organized

Here is a very brief overview of the topics we cover in the various parts of this book. Use these descriptions and the Table of Contents to map out your strategy of study.

Part I: Setting the Stage: Basic Biochemistry Concepts

This part deals with basic aspects of chemistry and biochemistry. In the first chapter you find out about the field of biochemistry and its relationship to other fields within chemistry and biology. You also get a lot of info about the different types of cells and their parts. In Chapter 2 we review some aspects of water chemistry that have direct applications to the field of biochemistry, including pH and buffers. Finally, you end up with a one-chapter review of organic chemistry, from functional groups to isomers.

Part II: The Meat of Biochemistry: Proteins

In this part we concentrate on proteins. You are introduced to amino acids, the building blocks of proteins. Having the building blocks in hand, in the next chapter we show you the basics of amino acid sequencing and the different types of protein structure. Finally, we will finish this part with a discussion of enzyme kinetics, both catalysts (speeding up reactions) and inhibitors (slowing them down).

Part III: Carbohydrates, Lipids, Nucleic Acids, and More

In this part we show you a number of biochemical species. You'll see that carbohydrates are far more complex than that doughnut you just ate might lead you to believe, but we do show you some biochemistry that is just sweet! Then we jump over to lipids and steroids. Next are nucleic acids and the genetic code (Da Vinci, eat your heart out!) of life with DNA and RNA. Then it's on to vitamins (they are involved more than once a day) and hormones (no humor here — it would be just too easy).

Part IV: Bioenergetics and Pathways

It all comes down to energy, one way or another. In these chapters we look at energy requirement and where that energy goes. This is where you meet our friend ATP and battle the formidable Citric Acid Cycle. Finally, since you will be hot and sweaty anyway, we throw you into the really smelly bog of nitrogen chemistry.

Part V: Genetics: Why We Are What We Are

In this part we tell you all about making more DNA, the processes of replication, and several of the applications related to DNA sequencing. Then it's off to RNA and protein synthesis. We also spend some time talking about the Human Genome Project.

Part VI: The Part of Tens

In this final part of the book we discuss ten great applications of biochemistry to the everyday world and reveal ten not-so-typical biochemical careers.

Where to Go from Here

The answer to this question really depends of your prior knowledge and goals. As with all *For Dummies* books, this one attempts to make all the chapters independent, so that you can pick a chapter containing material you are having difficulty with and get after it, without having to have read other chapters first. If you feel comfortable with the topics covered in general and organic chemistry, feel free to skip Part I. If you want a general overview of biochemistry, skim the remainder of the book. Dive deeper into the gene pool when you find a topic that interests you.

And for all of you, no matter who you are or why you are reading this book, we hope that you have fun reading it and that it helps you to learn biochemistry.

Part I

Setting the Stage: Basic Biochemistry Concepts

The 5th Wave By Rich Tennant

"Look – I'm only going to do this once, but it should help you remember the nonpolar hydrophobic principle and its effect on the surface tension of water."

In this part . . .

We go over some basic aspects of chemistry, organic chemistry, and biochemistry. First we survey the field of biochemistry and its relationship to other disciplines within chemistry and biology. We cover several different types of cells and their parts. Then we look at some features of water chemistry that apply to biochemistry, paying attention to pH and buffers. In the end, you get a brush-up on your organic chemistry, which sets the stage for Part II.

Chapter 1

Biochemistry: What You Need to Know and Why

In This Chapter

- Considering biochemistry
- Finding out about the types of cells
- Seeing the differences between plant and animal cells

If you are enrolled in a biochemistry course, you may want to skip this chapter and go right to the chapter(s) where we discuss the material you are having trouble with. But if you are *thinking* about taking a course in biochemistry or just want to explore an area that you know little about, keep reading. This chapter gives you basic information about cell types and the parts of the cell — which are extremely important in biochemistry.

Sometimes it's easy to get lost in the technical stuff and forget about the big picture. This chapter sets the stage for the details.

Why Biochemistry?

We suppose the flippant answer would be "Why not?" or "Because it is required."

That first response is not too bad an answer, actually. Look around. See all the living or once living things around you? The processes that allow them to grow, multiply, age, and die are all biochemical in nature. Sometimes we sit back and marvel at the complexity of life, the myriad of chemical reactions that are taking place right now within our own bodies, how all these biochemical reactions are working together so that we can sit and contemplate them. When John learned about the minor structural difference between starch and cellulose he remembers thinking: "Just that little difference in the one linkage

between those units is basically the difference between a potato and a tree?" It made him want to learn more, to delve into the complexity of the chemistry of living things, to try to understand. We encourage you to step back from the details occasionally and marvel at the complexity and beauty of life.

What Is Biochemistry and Where Does It Take Place?

Biochemistry is the chemistry of living organisms. Biochemists study the chemical reactions that occur at the molecular level of organisms. Normally it is listed as a separate field of chemistry. However, in some schools it is part of biology, and in others it is separate from both chemistry and biology.

Biochemistry really reaches out and combines aspects of all the fields of chemistry. Because carbon is the element of life, *organic chemistry* plays a large part in biochemistry. Many times biochemists study how fast reactions occur — that's *physical chemistry.* Often metals are incorporated into biochemical structures (such as iron in hemoglobin) — that's *inorganic chemistry.* Biochemists use sophisticated instrumentation to determine amounts and structures — that's *analytical chemistry.* Biochemistry is similar to *molecular biology;* both study living systems at the molecular level, but biochemists concentrate on the chemical reactions that are occurring.

Biochemists may study individual electron transport within the cell, or they may study the processes involved in digestion. If it's alive, biochemists will study it.

Types of Living Cells

All living organisms contain cells. A *cell* is a prison of sorts. The working apparatus of the cell is imprisoned within the "bars" — known as the *cell membrane*. Just as a prison inmate can still communicate with the outside world, so can the cell contents. The prisoner must be fed, so nutrients must be able to enter every living cell. There is a sanitary system for the elimination of waste. And, just as inmates may work to provide materials for society outside the prison, a cell may produce materials for life outside the cell.

There are two types of cells: prokaryotes and eukaryotes. (Viruses also bear some similarities to cells, but these are limited.) Prokaryotic cells are the simplest type of cells. Many one-celled organisms are prokaryotes.

The simplest way to distinguish these two types is that a *prokaryotic cell* contains no well-defined nucleus, whereas the opposite is true for a *eukaryotic cell.*

Prokaryotes

Prokaryotes are mostly bacteria. Besides the lack of a nucleus, there are few well-defined structures inside a prokaryotic cell. The prison wall has three components: a cell wall, an outer membrane, and a plasma membrane. This wall allows a controlled passage of material into or out of the cell. The materials necessary for proper functioning of the cell float about inside it, in a soup known as the *cytoplasm.* Figure 1-1 depicts a simplified version of a prokaryotic cell.

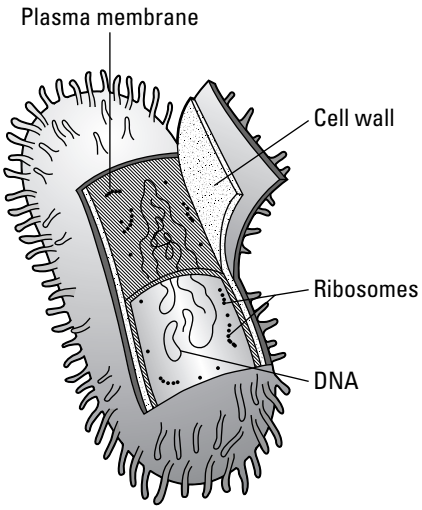


Figure 1-1: Simplified prokaryotic cell.

Eukaryotes

Eukaryotes are animals, plants, fungi, and protists. *You* are a eukaryote. In addition to having a nucleus, eukaryotic cells have a number of membrane-enclosed components known as *organelles*. Eukaryotic organisms may be either unicellular or multicellular. In general, eukaryotic cells contain much more genetic material than prokaryotic cells.

Animal Cells and How They Work

All animal cells (which are, as you now know, eukaryotic cells) have a number of components, most of which are considered to be organelles. The primary components of animal cells are listed in Table 1-1. (These components, and a few others, are also present in plant cells.) Figure 1-2 illustrates a simplified animal cell.

Table 1-1 Parts of an Animal Cell

Cell membrane	Centrioles
Endoplasmic reticulum	Golgi apparatus
Lysosomes	Mitochondria
Nucleus and nucleolus	Ribosomes
Small vacuoles	

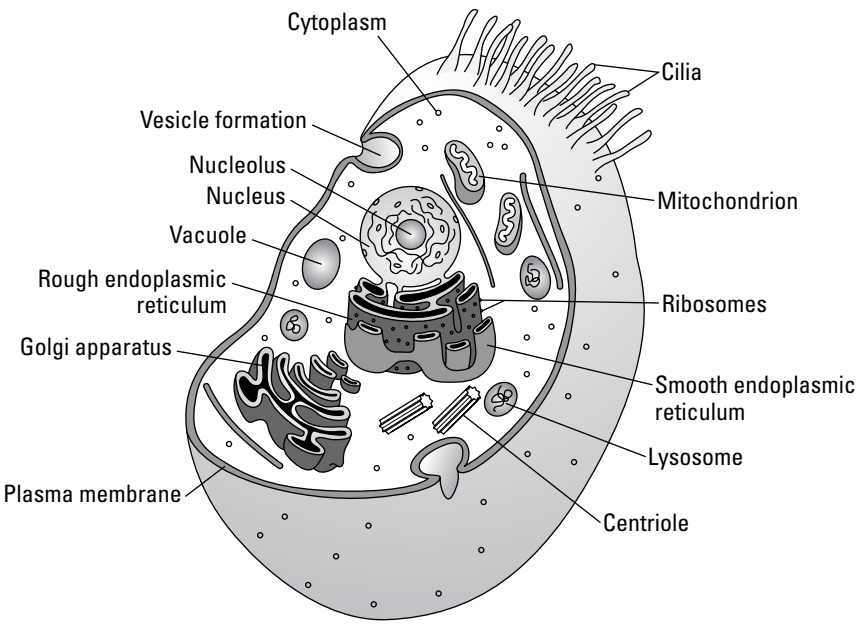


Figure 1-2: Simplified illustration of an animal cell.

The plasma membrane separates the material inside the cell from everything outside the cell. The *plasma* or cytoplasm is the fluid inside the cell. It is important for the health of the cell to prevent this fluid from leaking out. However, necessary materials must be able to enter through the membrane, and other materials, including waste, must be able to exit through the membrane.

Transport through the membrane may be active or passive. *Active transport* requires that a price be paid for a ticket to enter (or leave) the cell. The cost of the ticket is energy. *Passive transport* does not require a ticket. Passive transport methods include diffusion, osmosis, and filtration.

Centrioles behave as the "train conductors" of the cell. They organize microtubules, which help move the parts of the cell during cell division.

The cell can be thought of as a smoothly running factory. The *endoplasmic reticulum* is the main part of the cell factory. There are two basic regions to this structure, known as the *rough* endoplasmic reticulum and the *smooth* endoplasmic reticulum. The rough endoplasmic reticulum contains ribosomes, and the smooth endoplasmic reticulum contains no ribosomes (more about ribosomes and their function is coming up in this chapter). The rough endoplasmic reticulum, through the ribosomes, is the assembly line of the factory. The smooth endoplasmic reticulum is more like the shipping department, which ships the products of the reactions that occur within the cell, to the Golgi apparatus.

The *Golgi apparatus* serves as the postal system of the cell. It looks a bit like a maze, and within it, materials produced by the cell are packaged in vesicles, small membrane-enclosed sacs. The vesicles are then mailed to other organelles or to the cell membrane for export. The cell membrane contains "customs officers" (called *channels*), who allow secretion of the contents from the cell. Secreted substances are then available for other cells or organs.

Lysosomes are the landfills of the cell. They contain digestive enzymes that break down substances that may harm the cell (Chapter 6 has a lot more about enzymes). The products of this digestion may then safely reenter the cell. Lysosomes also digest "dead" organelles. This slightly disturbing process, called *autodigestion,* is really part of the cell digesting itself.

The *mitochondria* (singular mitochondrion) are the cell's power plants, where the cell produces energy. Mitochondria use food, primarily the carbohydrate *glucose,* to produce energy, which comes mainly in the form of *adenosine triphosphate* (ATP — to which Chapter 13 is dedicated).

Each cell has a *nucleus* and, inside it, a *nucleolus.* These serve as the control center of the cell and are the root from which all future generations originate. A double layer known as the *nuclear membrane* surrounds the nucleus. Usually the nucleus contains a mass of material called *chromatin.* If the cell is entering a stage leading to reproducing itself through cell division, the chromatin separates into *chromosomes.*

In addition to conveying genetic information to future generations, the nucleus produces two important molecules for the interpretation of this information. These molecules are *messenger ribonucleic acid* (mRNA) and *transfer ribonucleic acid* (tRNA). The nucleolus produces a third type of ribonucleic acid known as *ribosomal ribonucleic acid* (rRNA). (Chapter 9 is all about nucleic acids.)

Ribosomes contain protein and ribonucleic acid subunits. It is in the ribosomes where the amino acids are assembled into *proteins.* Many of these proteins are enzymes, which are part of nearly every process occurring in the organism. (Part II of this book is devoted to amino acids, proteins, and enzymes.)

The *small vacuoles,* or simply *vacuoles,* serve a variety of functions, including storage and transport of materials. The stored materials may be for later use or may be waste material no longer needed by the cell.

A Brief Look at Plant Cells

Plant cells contain the same components as animal cells — plus a cell wall, a large vacuole, and, in the case of green plants, chloroplasts. Figure 1-3 illustrates a typical plant cell.

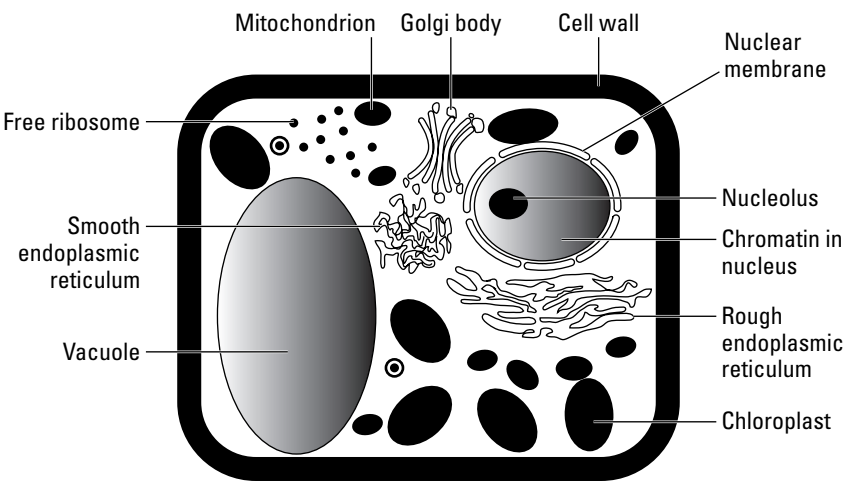


Figure 1-3: Simplified illustration of a plant cell.

The *cell wall* is composed of cellulose. Cellulose, like starch, is a polymer of glucose. The cell wall provides structure and rigidity.

The *large vacuole* serves as a warehouse for large starch molecules. Glucose, which is produced by photosynthesis, is converted to *starch,* a polymer of glucose. At some later time, this starch is available as an energy source. (Chapter 7 talks a lot more about glucose and other carbohydrates.)

Chloroplasts, present in green plants, are specialized chemical factories. These are the sites of photosynthesis, in which *chlorophyll* absorbs sunlight and uses this energy to combine carbon dioxide and water to produce glucose and release oxygen gas.

The green color of many plant leaves is due to the magnesium-containing compound chlorophyll.

Now that you know a little about cells, press on and let's do some biochemistry!

Chapter 2

Dive In: Water Chemistry

In This Chapter

- Understanding the roles and properties of water
- Exploring the differences between acids and bases
- Examining acid-base equilibria with the Brønsted-Lowry theory
- Controlling pH with buffers

Water is one of the most important substances on earth. We swim, bathe, boat, and fish in it. It carries our waste from our homes and is used in the generation of electrical power. We drink it in a variety of forms: pure water, soft drinks, tea, coffee, margaritas, and so on. Water, in one form or another, moderates the temperature of the earth and of our bodies.

In the area of biochemistry, water is also one of the lead actors. Our bodies are about 70 percent water. Water plays a role in the transport of material to and from cells. And many, many aqueous solutions take part in the biochemical reactions in the body.

In this chapter, we examine the structure and properties of the water molecule. We explain how water behaves as a solvent. We look at the properties of acids and bases and the equilibria that they may undergo. Finally, we discuss the pH scale and buffers, including the infamous Henderson-Hasselbalch equation. Sit back, grab a glass of water, and dive in!

The Fundamentals of H_2O

Water is essential to life; in fact, human beings are essentially big sacks of water. Water accounts for 60–95 percent of our living cells, and 55 percent of the water in the human body is in intracellular fluids. The remaining 45 percent (extracellular) is divided between the following:

- Plasma (8 percent)
- Interstitial and lymph (22 percent)
- Connective tissue, cartilage, and bone (15 percent)

Water also is necessary as a solvent for the multitude of biochemical reactions that occur in the body:

- Water acts as a transport medium across membranes, carrying substances into and out of cells.
- Water helps maintain the temperature of the body.
- Water acts as a solvent (carrying dissolved chemicals) in the digestive and waste excretion systems.

Healthy humans have an intake/loss of about two liters of water per day. The intake is about 45 percent from liquids and 40 percent from food, with the remainder coming from the oxidation of food. The loss is about 50 percent from urine and 5 percent from feces, with the remainder leaving through evaporation from the skin and lungs. A water balance must be maintained within the body. If the loss of water significantly exceeds the intake, the body experiences dehydration. If the water loss is significantly less than the intake, water builds up in the body and causes *edema* (fluid retention in tissues).

Let's get wet! Physical properties of water

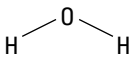
The medium in which biological systems operate is water, and physical properties of water influence the biological systems. Therefore, it is important to review some properties of water that you learned in general chemistry.

Water is a polar molecule

Because it's *polar,* water has a tendency to "wet" substances, like grandma's fine dining-room table or a baby's diaper. It's also a *bent* molecule , not linear (see Figure 2-1). The hydrogen atoms have a partially positive charge ($\delta+$); the oxygen atom has a partially negative charge ($\delta-$). This charge distribution is due to the *electronegativity* difference between hydrogen and oxygen atoms (the attraction that an atom has for a bonding pair of electrons). The water molecule in Figure 2-1 is shown in its bent shape with a bond angle of about 105°.

Normally, such partial charges result in an intermolecular force known as a *dipole-dipole force,* in which the positive end of one molecule attracts the negative end of another molecule. The very high electronegativity of oxygen combined with the fact that a hydrogen atom has only one electron results in a charge difference significantly greater than you'd normally expect. This leads to stronger-than-expected intermolecular forces. These unexpectedly strong intermolecular forces have a special name: *hydrogen bonds.*

Figure 2-1: Structure of a water molecule.



The term *hydrogen bond* doesn't refer to an actual bond to a hydrogen atom, but to the overall *interaction* of a hydrogen atom bonded to either oxygen, nitrogen, or fluorine atoms with an oxygen, nitrogen or fluorine on another molecule (intermolecular) or the same molecule (intramolecular). Hence the term *intermolecular force.* (Note that although hydrogen bonds occur when hydrogen bonds to fluorine, you don't normally find such combinations in biological systems.)

Water has strong intermolecular forces

Hydrogen bonds in oxygen- and nitrogen-containing molecules are very important in biochemistry because they influence reactions between such molecules and the structures of these biological molecules. The interaction between water and other molecules in which there may be an opportunity for hydrogen bonding explains such properties as solubility in water and reactions that occur with water as a solvent.

The term *hydrogen bond* doesn't refer to an actual bond to a hydrogen atom, but to an overall interaction.

One environmentally important consequence of hydrogen bonding is that, upon freezing, water molecules are held in a solid form that's less dense than the liquid form. The hydrogen bonds lock the water molecules into a crystalline lattice that contains large holes, which decreases the density of the ice. The less-dense ice — whether in the form of an ice cube or an iceberg — floats on liquid water. In nearly all other cases where a solid interacts with water, the reverse is true: The solid sinks in the liquid. So, why is the buoyancy of ice important? Ask ice fishermen! The layer of ice that forms on the surface of cold bodies of water insulates the liquid from the cold air, protecting the organisms still living under the ice.

Water has a high specific heat

Specific heat is the amount of heat required to change the temperature of a gram of water 1° Celsius. A high specific heat means it isn't easy to change the temperature of water. Water also has a high *heat of vaporization.* Humans can rid their bodies of a great deal of heat when their sweat evaporates from their skin, making sweat a very effective cooling method. We're sure you'll notice this cooling effect during your biochem exams.

As a result of water's high specific heat and heat of vaporization, lakes and oceans can absorb and release a large amount of heat without a dramatic change in temperature. This give and take helps moderate the earth's temperature and makes it easier for an organism to control its body temperature. Warm-blooded animals can maintain a constant temperature, and cold-blooded animals — including lawyers and some chemistry teachers — can absorb enough heat during the day to last them through the night.

Water's most important biochemical role: The solvent

The polar nature of water means that it attracts (soaks up) other polar materials. Water is often called *the universal solvent* because it dissolves so many types of substances. Many ionic substances dissolve in water, because the negative ends of the water molecules attracts the *cations* (positively charged ions) from the *ionic* compound (compound resulting from the reaction of a metal with a non-metal) and the positive ends attract the *anions* (negatively charged ions). Covalently bonded (resulting from the reactions between non-metals) polar substances, such as alcohols and sugars, also are soluble in water because of the dipole-dipole (or hydrogen-bonding) interactions. However, covalently bonded nonpolar substances, such as fats and oils are *not* soluble in water.

Polar molecules, because of their ability to interact with water molecules, are classified as *hydrophilic* (water-loving). Nonpolar molecules, which don't appreciably interact with (dissolve in) water, are classified as *hydrophobic* (water-hating). Some molecules are *amphipathic* because they have both hydrophilic and hydrophobic regions.

Figure 2-2 shows the structure of a typical amphipathic molecule. The molecule appears on the left, with its hydrophilic and hydrophobic regions shown. The alternate portion of the figure is a symbolic way of representing the molecule. The round "head" is the hydrophilic portion, and the long "tail" is the hydrophobic portion.

Certain amphipathic molecules, such as soap molecules, can form *micelles,* or very tiny droplets that surround insoluble materials. This characteristic is the basis of the cleaning power of soaps and detergents. The hydrophobic portion of the molecule (a long hydrocarbon chain) dissolves in a nonpolar substance, such as normally insoluble grease and oil, leaving the hydrophilic portion (commonly an ionic end) out in the water. Soap or detergent breaks up the grease or oil and keeps it in solution so it can go down the drain.

A micelle behaves as a large polar molecule (see Figure 2-3). The structure of a micelle is closely related to the structure of cell membranes.

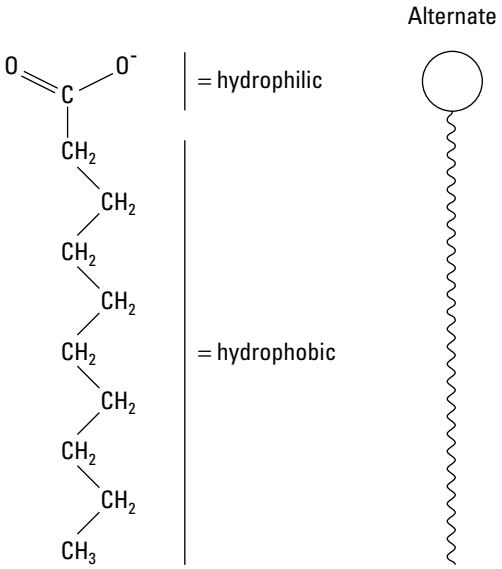


Figure 2-2: Structure of a typical amphipathic (both water-loving and water-hating) molecule.

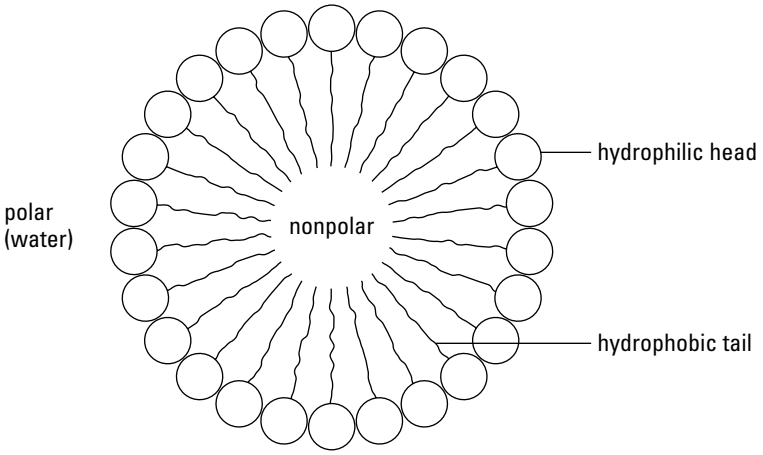


Figure 2-3: Structure of a micelle, composed of amphipathic molecules, with their hydrophilic "heads" pointing "out."

Hydrogen Ion Concentration: Acids and Bases

In aqueous solutions — especially in biological systems — the concentration of hydrogen ions (H^+) is very important. Biological systems often take great pains to make sure that their hydrogen ion concentration — represented as $[H^+]$ or by the measurement of pH (the measure of acidity in a solution) — doesn't change.

Even minor changes in hydrogen ion concentration can have dire consequences to a living organism. For example, in our blood, only a very small range of hydrogen ion allows the body to function properly. Hydrogen ion concentrations higher or lower than this range can cause death.

Because living organisms are so dependent on pH, let's take a few moments to review the concepts of acids, bases, and pH.

Achieving equilibrium

When the concentrations of hydrogen ion (H^+) and hydroxide ion (OH^-) are the same, the solution is *neutral.* If the hydrogen ion concentration exceeds the hydroxide ion concentration, the solution is *acidic.* If the hydroxide ion concentration is greater, the solution is *basic.* These chemical species are related through a chemical equilibrium.

Acidic solutions, such as lemon juice, taste sour. Basic solutions, such as tonic water, taste bitter. (The addition of gin doesn't change the bitter taste!)

The equilibrium of hydrogen ions is present in all aqueous solutions. It may or may not be the major hydrogen ion source (usually it isn't). Water is a contributor to the hydrogen ion concentration because it undergoes *autoionization*, as shown by the following equation:

$$H_2O(l) \quad H^+(aq) + OH^-(aq)$$

You often see $H^+(aq)$ represented as H_3O^+.

The double arrow ( ) indicates that this is an equilibrium; as such, there must be an associated *equilibrium constant* (K). The equilibrium constant in the preceding equation is K_w. The value of K_w is the product of the concentrations of the hydrogen ion and the hydroxide ion:

$$K_w = [H^+]\,[OH^-] = 1.0 \times 10^{-14} \text{ (at } 25^\circ C)$$

The value of the constant K_w, like all Ks, is only constant if the temperature is constant. In the human body, where $T = 37^\circ C$, $K_w = 2.4 \times 10^{-14}$.

In pure water, at $25^\circ C$, $[H^+] = 1.0 \times 10^{-7}$ M (1.6×10^{-7} M at $37^\circ C$). The hydroxide ion concentration is the same as the hydrogen ion concentration, because they are formed in equal amounts during the autoionization reaction.

M is a concentration term, the molarity. *Molarity* is the number of moles of solute per liter of solution.

Sour and bitter numbers: The pH scale

It isn't always convenient to report hydrogen ion concentrations in an exponential form, such as 1.0×10^{-7}. Thankfully, you have a way of simplifying the representation of the hydrogen ion concentration: the pH. You can calculate the pH for any solution by using the following equation:

$$pH = -\log [H^+]$$

For instance, in the case of a solution with a hydrogen ion concentration of 1.0×10^{-7} M, the pH would be

$$pH = -\log (1.0 \times 10^{-7}) = 7.0$$

Table 2-1 gives similar calculations for my hydrogen ion concentrations.

Table 2-1 The pH Scale and the Associated Hydrogen Ion Concentration

$[H^+]$	*pH*	*Solution Property*
1.0×10^{0} M	0	Acidic
1.0×10^{-1} M	1	Acidic
1.0×10^{-2} M	2	Acidic
1.0×10^{-3} M	3	Acidic
1.0×10^{-4} M	4	Acidic
1.0×10^{-5} M	5	Acidic
1.0×10^{-6} M	6	Acidic
1.0×10^{-7} M	7	**Neutral**
1.0×10^{-8} M	8	Basic
1.0×10^{-9} M	9	Basic
1.0×10^{-10} M	10	Basic
1.0×10^{-11} M	11	Basic
1.0×10^{-12} M	12	Basic
1.0×10^{-13} M	13	Basic
1.0×10^{-14} M	14	Basic

If a solution has a pH less than 7, it's acidic. Solutions with a pH greater than 7 are basic. Solutions whose pH is 7 are neutral. The pH of pure water is 7. Be careful, though: Not every solution that has a pH of 7 is pure water! For example, if you add table salt to water, the pH will remain at 7, but the resulting solution is certainly not pure water.

The pH scale is an open-ended scale, meaning you can have a pH greater than 14 or less than 0. For example, the pH of a 1.0×10^{1} M solution of hydrochloric acid is –1. John loves to ask questions based on this topic to his advanced chemistry students! The 0–14 scale is a convenient part of the pH scale for most real-world solutions — especially ones found in biochemistry. Most biological systems have a pH near 7, although significant deviations may exist (the pH in your stomach is close to 1).

Calculating pOH

You can calculate pOH in a similar manner to the pH calculation. That is, you can use the equation pOH = –log $[OH^-]$. You can calculate the hydroxide ion concentration from the hydrogen ion concentration and the K_w (equilibrium constant) relationship:

$$[OH^-] = K_w \div [H^+]$$

A useful shortcut to get from pH to pOH is the following relationship: pH + pOH = 14.00 for any aqueous solution ($14.00 = pK_w = -\log K_w = -\log 1.0 \times 10^{-14}$).

For example, if a solution has a $[H^+] = 6.2 \times 10^{-6}$, its pH would be

$$pH = -\log [H^+]$$

$$pH = -\log [6.2 \times 10^{-6}]$$

$$pH = 5.21$$

The calculation for the pOH of that solution becomes pretty simple: 14.00 – pH = 14.00 – 5.21 = 8.79.

Now, if you have the pH or pOH, getting the corresponding $[H^+]$ or $[OH^-]$ becomes a pretty simple task:

$$[H^+] = 10^{-pH} \text{ and } [OH^-] = 10^{-pOH}$$

For example, a solution with a pH of 7.35 has a $[H^+] = 10^{-7.35} = 2.2 \times 10^{-7}$.

Strong and weak: Brønsted-Lowry theory

Since the acidity (pH) of the biological medium is so very important, let's take a look at one of the most accepted theories concerning acids and bases – the Brønsted-Lowry theory. According to this theory, acids are proton (H^+) donors, and bases are proton acceptors.

Strong and weak acids

Acids increase the hydrogen ion concentration of a solution (they lower the pH, in other words). Some acids, known as *strong acids,* are very efficient at changing hydrogen ion concentration; they essentially completely ionize in water. Most acids — particularly biologically important acids — aren't very efficient at generating hydrogen ions; they only partially ionize in water. These acids are known as *weak acids.*

Bases accept (react with) rather than donate hydrogen ions in solutions. Bases decrease the hydrogen ion concentration in solutions because they react with these ions. Strong bases, although they can accept hydrogen ions very well, aren't too important in biological systems. The majority of biologically important bases are weak bases.

The Brønsted-Lowry theory helps to explain the behavior of acids and bases with respect to equilibrium. A Brønsted-Lowry acid is a hydrogen ion (H^+) donor, and a Brønsted-Lowry base is a hydrogen ion acceptor. Acetic acid, a weak acid found in vinegar, partially ionizes in solution, evidenced by the following equation:

$$CH_3COOH \quad H^+ + CH_3COO^-$$

The double arrow indicates that the acetic acid doesn't completely ionize. (For a strong acid, complete ionization would occur, and a single arrow would be present.) The equilibrium arrow ( ) indicates that all three chemical species are present in the solution: the acetic acid, the acetate ion, and the hydrogen ion, along with the water solvent.

In the Brønsted-Lowry theory, you consider the acetate ion to be a base because it can accept a hydrogen ion to become acetic acid. According to this theory, two substances differing by only one hydrogen ion — such as acetic acid and the acetate ion — are members of a *conjugate acid-base pair.* The species with one additional hydrogen ion is the *conjugate acid* (CA), and the species with one less hydrogen ion is the *conjugate base* (CB).

You can express the equilibrium from the acetate example, like all equilibria, by using a *mass-action expression* — as long as a balance among the species is present. This expression is also known as a *reaction quotient* and as an *equilibrium constant*. For acetic acid, this expression is as follows:

$$K_a = \frac{[H^+][CH_3COO^-]}{[CH_3COOH]}$$

The *a* subscript means that this expression represents an acid. The square brackets refer to the molar equilibrium concentrations of the species present. You can express the K_a as a pK_a. The calculation of pK_a is similar to the calculation of pH:

$$pK_a = -\log K_a$$

In terms of conjugate acids and bases, every K_a expression appears as

$$K_a = \frac{[H^+][CB]}{[CA]}$$

No variations are allowed in this equation other than the actual formulas of the conjugate acid and base.

Like an acid, a base has a K_b value (the subscript *b* meaning base). A weak base, like ammonia, is part of the following equilibrium:

$$NH_3 + H_2O \quad OH^- + NH_4^+$$

The equilibrium constant expression for this equilibrium is

$$K_b = \frac{[OH^-][NH_4^+]}{[NH_3]}$$

The generic form of a K_b expression is

$$K_b = \frac{[OH^-][CA]}{[CB]}$$

As with a K_a expression, a K_b expression has no variations other than the actual formulas of the conjugate acid and base.

Every conjugate acid has a K_a, and its corresponding conjugate base has a K_b. The K_a and the K_b of a conjugate acid-base pair are related by the K_w — the ionization constant for water. For a conjugate acid-base pair, $K_aK_b = K_w = 1.0 \times 10^{-14}$. In addition, you can use the following shortcut: $pK_a + pK_b = 14.00$.

The K_b for the acetate ion, the conjugate base of acetic acid, is

$$CH_3COO^- + H_2O \quad OH^- + CH_3COOH$$

The K_a for the ammonium ion, the conjugate acid of ammonia, is

$$NH_4^+ \quad H^+ + NH_3$$

An acid may be capable of donating more than one hydrogen ion. A biologically important example of this type of acid is phosphoric acid (H_3PO_4), which is a triprotic acid (meaning that it can donate three hydrogen ions). This acid is capable of donating three hydrogen ions, one at a time. The equilibria for this acid are

K_{a1}: $H_3PO_4 \quad H^+ + H_2PO_4^-$

K_{a2}: $H_2PO_4^- \quad H^+ + HPO_4^{2-}$

K_{a3}: $HPO_4^{2-} \quad H^+ + PO_4^{3-}$

The subscripts are modified to indicate the loss of hydrogen 1, hydrogen 2, or hydrogen 3. The associated K_a expressions are all of the form

$$K_a = \frac{[H^+][CB]}{[CA]}$$

Here's the breakdown for each K_a:

$$K_{a_1} = \frac{[H^+][H_2PO_4^-]}{[H_3PO_4]}$$

$$K_{a_2} = \frac{[H^+][HPO_4^{2-}]}{[H_2PO_4^-]}$$

$$K_{a_3} = \frac{[H^+][PO_4^{3-}]}{[HPO_4^{2-}]}$$

The value for each successive equilibrium constant often is significantly lower than the preceding value. Table 2-2 runs through some biologically important acids. You can refer to this table when working buffer problems or determining which acid is stronger.

Table 2-2 The K_a Values for Biologically Important Acids

Acid	K_{a1}	K_{a2}	K_{a3}
Acetic acid (CH_3COOH)	1.7×10^{-5}		
Pyruvic acid ($CH_3COCOOH$)	3.2×10^{-3}		
Lactic acid ($CH_3CHOHCOOH$)	1.4×10^{-4}		
Succinic acid ($HOOCCH_2CH_2COOH$)	6.2×10^{-5}	2.3×10^{-6}	
Carbonic acid (H_2CO_3)	4.5×10^{-7}	5.0×10^{-11}	
Citric acid ($HOOCCH_2C(OH)(COOH)CH_2COOH$)	8.1×10^{-4}	1.8×10^{-5}	3.9×10^{-6}
Phosphoric acid (H_3PO_4)	7.6×10^{-3}	6.2×10^{-8}	2.2×10^{-13}

Acid or base? They just can't decide

Some substances can't make up their minds about what they are; they can act as either an acid or a base. Chemists classify these substances as *amphiprotic* or *amphoteric* substances. For example, the bicarbonate ion (HCO_3^-) can act as either an acid or a base:

$$HCO_3^- \quad H^+ + CO_3^{2-}$$

$$HCO_3^- + H_2O \quad OH^- + H_2CO_3$$

Biochemically important molecules may also exhibit amphiprotic behavior. Amino acids contain both a basic amine ($-NH_2$) group and an acidic carboxyl ($-COOH$) group. Therefore, they can act as either acids or bases. For example, glycine (H_2N-CH_2-COOH) may undergo the following reactions:

$$H_2N-CH_2-COOH \quad H^+ + H_2N-CH_2-COO^-$$

$$H_2N-CH_2-COOH + H_2O \quad OH^- + {}^+H_3N-CH_2-COOH$$

In fact, amino acids may undergo proton transfer from the carboxyl end to the amine end, forming an overall neutral species that has a positive and negative end. Species such as these are called *zwitterions:*

$H_2N–CH_2–COOH \quad {}^{+}H_3N–CH_2–COO^{-}$

Buffers and pH Control

A solution that contains the conjugate acid-base pair of any weak acid or base is a *buffer solution.* A buffer solution resists changes in pH when either an acid or a base is added. Therefore, buffers control the pH of the solution. Buffer solutions are important in most biological systems. Many biological processes proceed effectively only within a limited pH range. The presence of buffer systems keeps the pH within this limited range.

Identifying common physiological buffers

In the human body, the pH of various body fluids is important. The pH of blood is 7.4, the pH of stomach acid is 1–2, and the pH in the intestinal tract is 8–9. If the pH of blood is more than 0.2 pH units lower than normal, a condition known as *acidosis* results; a corresponding increase in pH is *alkalosis.* Acidosis and alkalosis may lead to serious health problems. There are two general causes of acidosis and of alkalosis:

- Respiratory acidosis is the result of many diseases that impair respiration. These diseases include pneumonia, emphysema, and asthma. These diseases lead to inefficient expulsion of carbon dioxide. This leads to an increase in the concentration of the acid H_2CO_3.
- Metabolic acidosis is due to a decrease in the concentration of HCO_3^-. This may be the results of certain kidney diseases, uncontrolled diabetes, and cases of vomiting involving nonacid fluids. Poisoning by an acid salt may also lead to metabolic acidosis.
- Respiratory alkalosis may be the result of hyperventilation, as there is an excessive removal of carbon dioxide, which leads to a decrease in the H_2CO_3 concentration. Immediate treatment may include breathing into a paper bag, thus increasing the carbon dioxide concentration in the inhaled air and therefore in the blood.
- Metabolic alkalosis may result from excessive vomiting of stomach acid.

To resist these pH problems, the blood has a number of buffer systems. These include several proteins in blood plasma and the bicarbonate buffer system.

The *bicarbonate buffer system* is the main extracellular buffer system. This system also provides a means of eliminating carbon dioxide. The dissolution of carbon dioxide in aqueous systems sets up the following equilibria:

$$CO_2 + H_2O \quad H_2CO_3 + H^+ + HCO_3^-$$

The presence of the conjugate acid-base pair (H_2CO_3 and HCO_3^-) means this is a buffer system. The conjugate acid-base ratio is about 20:1 at a pH of 7.4 in the bloodstream. This buffer system is coupled with the following equilibrium (nstrumental in the removal of carbon dioxide in the lungs):

$$CO_2(blood) \quad CO_2(g)$$

The second ionization of phosphoric acid, K_{a2}, is the primary intracellular buffer system. The pH of this conjugate acid-base pair ($H_2PO_4^-$ and HPO_4^{2-}) is 7.21 for a solution with equal concentrations of these two species.

Calculating a buffer's pH

To determine a buffer's pH, you may use a K_a or K_b calculation, discussed previously, or the Henderson-Hasselbalch equation, which gives a shortcut.

There are two forms of the Henderson-Hasselbalch equation:

$$pH = pK_a + \log\frac{[CB]}{[CA]}$$

and

$$pOH = pK_b + \log\frac{[CA]}{[CB]}$$

The terms in either form are the same as defined earlier. For example, suppose we wanted to calculate the pH of a buffer composed of 0.15 M pyruvic acid and 0.25 M sodium pyruvate. Looking at Table 2-2, you see that the K_a of pyruvic acid is 3.2×10^{-3}.

The pK_a would be 2.50. Therefore:

$$pH = pK_a + \log\frac{[CB]}{[CA]}$$

$$pH = -\log 3.2 \times 10^{-3} + \log\frac{[CH_3COCOO^-]}{[CH_3COCOOH]}$$

$$pH = 2.50 + \log\frac{[.25]}{[.15]}$$

$$pH = 2.50 + \log(1.67)$$

$$pH = 2.50 + 0.22 = 2.72$$

The greater the values of [CA] and [CB], the greater the buffer capacity of the solution is. The buffer capacity indicates how much acid or base may be added to a buffer before the buffer ceases to function.

Chapter 3

Fun with Carbon: Organic Chemistry

In This Chapter

- Understanding why carbon is fundamental to biochemistry
- Examining the nature of weak interactions
- Finding out about functional groups
- Checking out isomerism

Most biologically important molecules are composed of *organic compounds,* which means compounds of carbon. Therefore, the student of biochemistry must have a general knowledge of organic chemistry, which is the study of carbon compounds, in order to understand the function and reactions of biochemical molecules. In this chapter we review the basics of organic chemistry, including the various functional groups and isomers that are important in the field of biochemistry.

The Role of Carbon in the Study of Life

Long ago, scientists believed that all carbon compounds were the result of biological processes, which meant organic chemistry was synonymous with biochemistry under this Vital Force theory. In the mid-1800s, though, researchers debunked that long-held notion — the synthesis of urea from inorganic materials showed that there were other paths to the production of carbon compounds. Organic chemists now synthesize many important organic chemicals without the use of living organisms; however, biosynthesis is still an important source of many organic compounds.

Why are there so many carbon compounds? The answer lies primarily in two reasons, both tied to carbon's versatility in creating stable bonds:

- **Carbon bonds to itself.** Carbon atoms are capable of forming stable bonds to other carbon atoms. The process of one type of atom bonding to identical atoms is *catenation.* Many other elements can catenate, but carbon is the most effective at it. There appears to be no limit to how many carbon atoms can link together. These linkages may be in chains, branched chains, or rings, as shown in Figure 3-1.
- **Carbon bonds to other elements.** Carbon is capable of forming stable bonds to a number of other elements. These include the biochemically important elements hydrogen, nitrogen, oxygen, and sulfur. The latter three elements form the foundation of most of the *functional groups* (reactive groups of a molecule) necessary for life. Bonds between carbon and hydrogen are usually unreactive under biochemical conditions; thus, hydrogen often serves as an "inert" substituent.

It's All in the Numbers: Carbon Bonds

Carbon is capable of forming four bonds. In bonding to itself and other elements, carbon uses a variety of types of *hybridization* — when it bonds to another carbon molecule, for example, these may have four single bonds, a double and two single bonds, two double bonds, or a triple and a single bond. Double bonds to oxygen atoms are particularly important in many biochemicals. Table 3-1 shows the number of bonds carbon may have with some selected non-metals, along with the hybridization of those bonds.

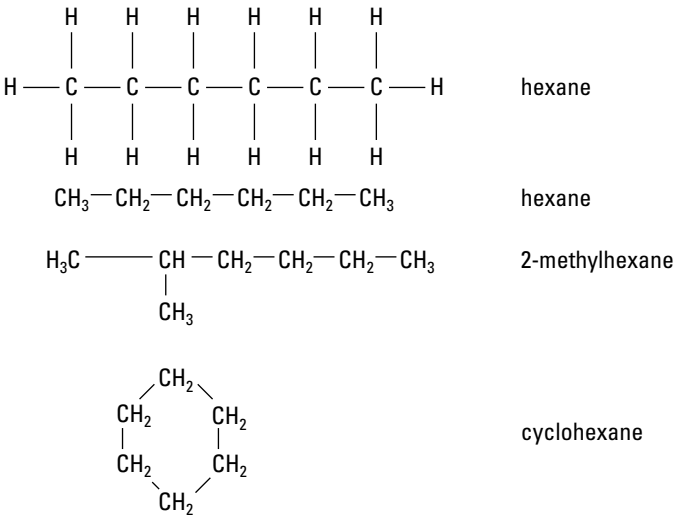


Figure 3-1: Top: straight chain hydrocarbon, expanded and condensed. Middle: branched chain hydrocarbon. Bottom: ring hydrocarbon.

Table 3-1 Possible Bonds of Carbon and Selected Non-metals

Element	*Number of Possible Bonds with Carbon*	*Some Possible Hybridizations for Second Period Elements*
Carbon (C)	4	4 single (sp^3) 2 single and one double (sp^2) 1 single and one triple (sp) 2 doubles (sp)
Nitrogen (N)	3	3 single (sp^3) 1 single and 1 double (sp^2) 1 triple (sp)
Oxygen (O)	2	2 single (sp^3) 1 double (sp^2)
Sulfur (S)	2	2 single (sp^3) 1 double (sp^2)
Hydrogen (H)	1	1 single
Fluorine (F)	1	1 single
Chlorine (Cl)	1	1 single
Bromine (Br)	1	1 single
Iodine (I)	1	1 single

Sticky Chemistry: Bond Strengths

Covalent bonds are important *intramolecular forces* (forces within the same molecule) in biochemistry. *Intermolecular forces* (forces between chemical species) are also extremely important. Among other things, intermolecular forces are important to hydrophilic (water-loving) and hydrophobic (water-hating) interactions.

Everybody has 'em: Intermolecular forces

All intermolecular forces are *van der Waals forces,* that is they are not true bonds in the sense of sharing or transferring electrons, but are weaker attractive forces. These forces include dipole-dipole forces, hydrogen bonding, and ionic interactions.

Dipole-dipole forces

Dipole-dipole forces exist between polar regions of different molecules. The presence of a dipole means that the molecule has a partially positive ($\delta+$) end and a partially negative ($\delta-$) end. Opposite partial charges will attract each other, whereas like partial charges will repel. In most cases, biological systems utilize a special type of dipole-dipole force known as *hydrogen bonding* (see next section).

Hydrogen bonding

Hydrogen bonding, as the name implies, involves hydrogen. The hydrogen atom must be bonded to either an oxygen atom or a nitrogen atom. (In non-biological situations, hydrogen bonding also occurs when a hydrogen atom bonds to a fluorine atom.) Hydrogen bonding is significantly stronger than a "normal" dipole-dipole force and is very much stronger than London dispersion forces (very weak and short-lived attractions between molecules that arise due to the nucleus of one atom attracting the electron cloud of another atom). The hydrogen that is bonded to either a nitrogen or oxygen atom is strongly attracted to a different nitrogen or oxygen atom. Hydrogen bonding may be either intramolecular or intermolecular.

Ionic interactions

In biological systems, ionic interactions may serve as intermolecular or intramolecular forces. In some cases, these may involve metal cations, such as Na^+, or anions, such as Cl^-. In many cases, the cation is an ammonium ion from an amino group, such as RNH_3^+; the anion may be from a carboxylic acid, such as $RCOO^-$. Oppositely charged ions attract each other strongly.

Water-related interactions: Both the lovers and the haters

The predominant factor leading to hydrophobic (water-hating) interactions is the presence of portions of a molecule containing only carbon and hydrogen. Hydrocarbon regions are nonpolar and are attracted to other nonpolar regions by London dispersion forces.

In general, the presence of any atom other than carbon and hydrogen makes a region polar. Oxygen and nitrogen are the most effective elements in biochemistry for making a region of a molecule polar. Sulfur is least effective of the common biologically important elements at imparting polar character. Dipole-dipole, hydrogen bonding, and ionic interactions are all hydrophilic interactions. London dispersion forces are hydrophobic interactions.

The more carbon and hydrogen atoms, without other atoms, the more important the hydrophobic nature of a region becomes in defining the properties of the molecule. Note that a molecule may have both a hydrophilic and a hydrophobic region, and both regions are important to the behavior of the molecule. The formation of a micelle is an example of using molecules with both hydrophilic and hydrophobic regions.

How bond strengths affect physical properties of substances

The physical properties of biological substances depend on the intermolecular forces present. The sequence of strength is: ions > hydrogen bonding > dipole-dipole > London. The strongest types of intermolecular forces involve ions. Next strongest is hydrogen bonding. Polar substances interact through dipole-dipole forces, which are weaker than hydrogen bonds. All biological substances containing oxygen, nitrogen, sulfur, or phosphorus are polar. London forces, the weakest intermolecular forces, are important in nonpolar situations. The hydrocarbon portion of biological molecules is nonpolar.

Melting points, boiling points, and solubility

As the strength of forces decreases, so do the melting points, boiling points, and solubility in water. Besides that, the vapor pressure and the solubility in nonpolar solvents also increases.

Substances that have a high solubility in water are hydrophilic, and substances with a low solubility in water are hydrophobic.

A molecule may have both hydrophilic and hydrophobic regions. The region that represents a greater portion of the molecule will predominate. For this reason, for example, CH_3COOH is more hydrophilic than $CH_3CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2CH_2COOH$. In addition, $HOCH_2CH_2CH_2CH_2CH_2CH_2OH$ is more hydrophilic than $CH_3CH_2CH_2CH_2CH_2CH_2OH$.

Odors

Many functional groups have distinctive odors. Small carboxylic acids smell like acetic acid (vinegar), while larger ones have unpleasant odors. Most esters, if volatile, have pleasant odors — that is why esters are used extensively in the flavor and perfume industry. Most sulfur-containing compounds have strong unpleasant odors. Small amines have an ammonia odor, whereas larger amines have a fishy odor or worse.

Defining a Molecule's Reactivity: Functional Groups

Most carbon compounds have one or more reactive sites composed of a specific grouping of atoms in their structure. It is at these sites that chemical reactions occur. These specific grouping of atoms that react are called *functional groups.* These functional groups contain atoms other than carbon and hydrogen and/or double or triple bonds and define the reactivity of the organic molecule.

Hydrocarbons

Alkanes are hydrocarbons — compounds containing only carbon and hydrogen, with no traditional functional groups. For this reason, they are not very reactive. *Alkenes* and *alkynes* are also hydrocarbons. They contain a carbon-carbon double and triple bond, respectively. The presence of more than one bond makes them more reactive. *Aromatic hydrocarbons,* normally ring structures with alternating single and double carbon-to-carbon bonds, contain one or more *aromatic systems*, which are much less reactive than other systems containing double bonds. Alkynes are not very common in biological systems. Figure 3-2 shows the structure of these compounds.

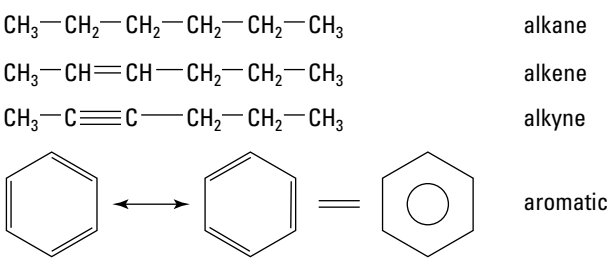


Figure 3-2: Examples of alkanes, alkenes, alkynes, and aromatic hydrocarbons.

Functional groups with oxygen and sulfur

Many functional groups contain oxygen, including alcohols, ethers, aldehydes, and ketones, which appear in carbohydrates. In carbohydrates, many ether groups are known as *glycoside linkages*. In addition, carboxylic acids and esters are important functional groups that appear as fatty acids and in fats and oils.

Alcohols and ethers contain only singly bonded oxygen atoms. An alcohol group attached to an aromatic ring is a phenol. Aldehydes and ketones contain only doubly bonded oxygen atoms. Carboxylic acids and esters contain both singly and doubly bonded oxygen atoms. The combination of a carbon atom connected to an oxygen atom by a double bond is a carbonyl group.

Sulfur, the element immediately below oxygen on the periodic table, may replace oxygen in both alcohols and ethers to give *thiols* (mercaptans) and *thioethers.* Many of these sulfur-containing compounds really stink! Sulfur may also form a disulfide where there is a bond between two sulfur atoms. Figure 3-3 illustrates these compounds.

R = any organic (hydrocarbon) group

R′ = any organic group, which may or may not = R

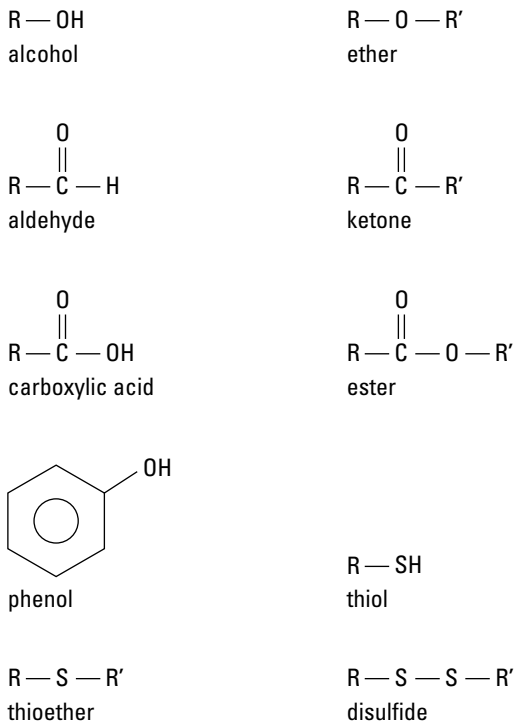


Figure 3-3: Oxygen- and sulfur-containing functional groups.

Functional groups containing nitrogen

Amines and amides are two important functional groups containing nitrogen. *Amines* are present in amino acids and alkaloids. *Amides* are present in proteins, where they are known as *peptide bonds.*

The difference between an amine and an amide is that amides have a carbonyl group adjacent to the nitrogen atom. Amines are derivatives of ammonia, NH_3, where one or more organic groups replace hydrogen atoms. In a *primary amine,* an organic group replaces one hydrogen atom. In *secondary* and *tertiary amines,* two and three organic groups, respectively, replace two or three hydrogen atoms. Figure 3-4 shows these compounds.

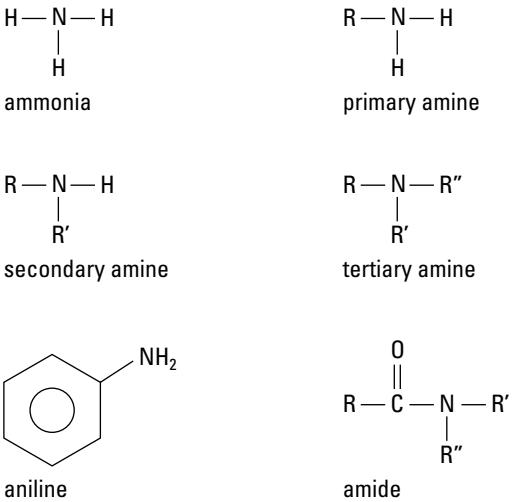


Figure 3-4: Some nitrogen-containing functional groups.

Alkaloids are basic compounds produced by plants. Examples include nicotine, caffeine, and morphine.

Functional groups containing phosphorus

Phosphorus is also an important element in biological systems and is normally present as part of a phosphate group. Phosphate groups come from phosphoric acid, H_3PO_4. The phosphate groups may be alone, part of a diphosphate, part of a triphosphate, or part of a phosphate ester.

Phosphates appear in teeth and bone and are a part of the energy transport molecules ATP and ADP (see Chapter 12 for more on these). Figure 3-5 illustrates phosphorous-containing functional groups.

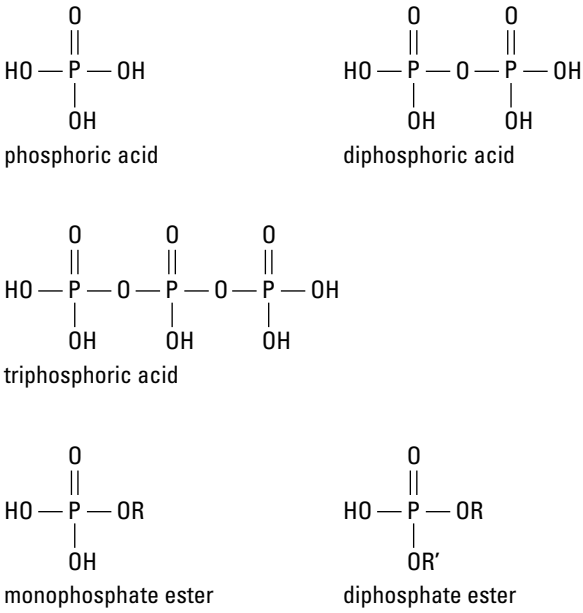


Figure 3-5: Phosphate-containing functional groups.

Reactions of functional groups

As you study the different biochemical molecules and their functions within the living organism, you see that the way a certain molecule reacts is primarily determined by the functional groups in the molecule's structure. Take a few minutes and refresh your organic chemistry knowledge about the typical reactions of the various functional groups.

Alcohols

Alcohols are subject to oxidation. Mild oxidation of a primary alcohol (where the –OH is attached to an end carbon) produces an aldehyde, which may undergo further oxidation to a carboxylic acid. Under similar conditions, a secondary alcohol (–OH is attached to a carbon bonded to two other carbons) will yield a ketone, and a tertiary alcohol (–OH attached to a carbon bonded to three other carbons) will not react. This behavior is important in the chemistry of many carbohydrates.

The presence of the OH leads people mistakenly to assume that alcohols are bases. Nothing could be further from the truth! Alcohols, under biological conditions, are neutral compounds. Phenols, though, are weak acids.

Aldehydes and ketones

Aldehydes easily undergo oxidation to carboxylic acids, but ketones do not undergo mild oxidation. With difficulty, it is possible to reduce aldehydes and ketones back to the appropriate alcohols.

Reducing sugars behave as such due to mild oxidation of the carbonyl groups present. Tollen's test uses silver nitrate, in which a reducing sugar generates a silver mirror on the walls of the container. Both Benedict's and Fehling's tests use copper compounds, and a reducing sugar produces a red precipitate with either of these tests. These simple organic qualitative tests find some use in biochemical tests described later in this book.

The carbonyl group of an aldehyde or ketone may interact with an alcohol to form acetals and hemiacetals. (Modern terminology only uses the terms acetals and hemiacetals — you may sometime see the terms *hemiketal,* which is a type of hemiacetal, and *ketal,* a type of acetal.) See Figure 3-6 for an illustration of these.

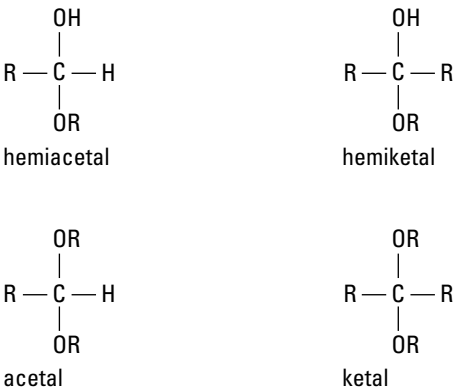


Figure 3-6: Acetals, hemiacetals, hemiketals, and ketals.

Carboxylic acids

Carboxylic acids, along with phosphoric acid, are the most important biological acids. Carboxylic acids react with bases such as the amines to produce salts. The salts contain an ammonium ion from the amine and a carboxylate ion from the acid.

Carboxylic acids combine with alcohols to form esters and can indirectly combine with amines to form amides. Hydrolysis of an ester or an amide breaks the bond and inserts water. An acid, base, or enzyme is needed to

catalyze it. Under acidic conditions, it is possible to isolate the acid and either the alcohol or the ammonium ion from the amine. Under basic conditions, you can isolate the carboxylate ion and either the alcohol or the amine.

Thiols and amines

Under mild oxidation, two thiols join to form a disulfide. Mild reducing conditions, catalyzed by enzymes, reverse this process. Such formation of disulfide linkages is important in the chemistry of many proteins, such as insulin.

Amines are the most important biological bases. As bases, they can react with acids. The behavior is related to the behavior of ammonia.

$NH_3 + H^+$ (from an acid) $\rightarrow NH_4^+$ (ammonium ion)

$NRH_2 + H^+$ (from an acid) $\rightarrow NRH_3^+$ (ammonium ion)

$NR_2H + H^+$ (from an acid) $\rightarrow NR_2H_2^+$ (ammonium ion)

$NR_3 + H^+$ (from an acid) $\rightarrow NR_3H^+$ (ammonium ion)

Many medications have amine groups. In order to make them more readily soluble, it is necessary to convert many of these amines to ammonium ions. For example, the reaction of the medication with hydrochloric acid forms the chloride, which often appears on the label as the hydrochloride.

It is possible to replace all the hydrogen atoms from an ammonium ion, NH_4^+, to produce a quaternary ammonium ion, NR_4^+.

Phosphoric acid

Phosphoric acid, H_3PO_4, may behave like a carboxylic acid and form esters. The esters will have an organic group, R, replacing one, two, or three of the hydrogen atoms. The resultant compounds are monoesters, diesters, and triesters. The hydrogen atoms remaining in the mono- and diesters are acidic.

pH and functional groups

Many of the biological functions of substances are pH dependent. For this reason, it is important to know which functional groups are acidic, basic, or neutral. Neutral functional groups behave the same no matter what the pH is. Table 3-3 lists the functional groups and whether or not they are acidic, neutral, or basic. In addition, we list whether they are of medium weakness, weak, or very weak. The weaker a substance in terms of pH, the less likely it will be affected by its solution pH.

Table 3-3 Acid-Base Properties of Biologically Important Functional Groups

Acids:		
	Monophosphate esters and diphosphate esters (medium)	
	Carboxylic acids (weak)	
	Phenols (very weak)	
	Thiols (very weak)	
	Amine salts (very weak)	
Bases:		
	Amines (weak)	
	Carboxylate ions (very weak)	
Neutral:		
	Alcohols	Carboxylic esters
	Ethers	Triphosphate esters
	Thioethers	Disulfides
	Amides	Ketones
	Aldehydes	

Same Content, Different Structure: Isomerism

Isomers are compounds that have the same molecular formula, but different structural formulas. Some organic and biochemical compounds may exist in different isomeric forms. Many times, especially in biological systems, these different isomers have different properties. The two most common types of isomers in biological systems are cis-trans isomers and isomerism due to the presence of a chiral carbon.

Cis-trans isomers

The presence of carbon-carbon double bonds leads to the possibility of having isomers present. Double bonds are rather restrictive and limit molecular movement. Groups on the same side of the double bond tend to remain in that position (cis), whereas groups on opposite sides tend to remain across the bond from each other (trans). See Figure 3-7 for an illustration.

Figure 3-7: Cis and trans isomers.

If the two groups attached to either of the carbon atoms of the double bond are the same, cis-trans isomers are not possible. Cis isomers are the normal form of fatty acids, whereas food processing tends to convert some of the cis isomers to the trans isomers.

Cis-trans isomers are also possible in cyclic systems. The cis form has similar groups on the same side of the ring, whereas the trans form has similar groups above and below the ring.

Chiral carbons

Trying to put your gloves on the wrong hands is kind of like another property of biological systems: handedness. There are *left-handed* molecules and *right-handed* molecules.

Identifying chiral molecules

The presence of an asymmetric, or *chiral,* carbon atom is sufficient to produce a "handed" molecule.

A chiral carbon atom has four different groups attached to it. The majority of biological molecules have one or more chiral carbon atoms and, for this reason, they are chiral. Figure 3-8 shows the chiral nature of glucose.

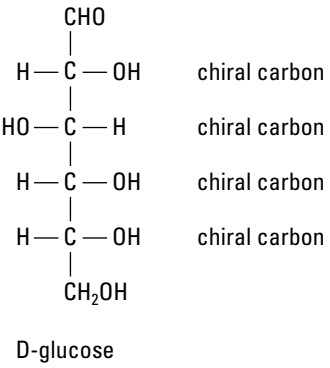


Figure 3-8: The structure of glucose, a sugar with four chiral carbon atoms.

Determining the chiral form: Enantiomer or sterioisomer?

All substances have a mirror image (okay, except vampires); however, if there is a chiral carbon atom present, the mirror images are nonsuperimposable. Hold out your left and right hands, palms up — they are nonsuperimposable mirror images. These two mirror images are called *enantiomers.* The different chiral forms differ from each other in two aspects:

- How they affect light
- How they interact with other chiral substances (usually only one chiral form will be biologically active)

To determine how a particular form affects light, it is necessary to use *plane polarized light,* in which all the light waves vibrate in the same plane. When you use this kind of light, a chiral substance rotates the vibrational plane of the light — one form (the dextrorotatory, *d*, (+) isomer) rotates the plane to the right, while the other (the levorotatory, *l*, (–) isomer) rotates the plane to the left. The *d* and *l* forms are *stereoisomers* and are optically active.

Illustrating the chiral compound: Fisher projection formulas

A chemist named Emil Fischer developed a method of drawing a compound to illustrate which stereoisomer was present. These Fischer projection formulas are very useful in biochemistry. In a projection formula, a chiral carbon is placed in the center of a + pattern. The vertical lines (bonds) are pointing away from the viewer, and the horizontal lines are pointing toward the viewer. Fischer used the D designation if the most important group (the group whose central atom had the largest atomic number) was to the right of the carbon, and the L designation if the most important group (lowest atomic number) was to the left of the carbon. Figure 3-9 shows two Fischer projection formulas.

Figure 3-9: Fischer projection formulas distinguish stereoisomers.

The *d* and *l* symbols are not necessarily the D and L forms respectively; thus, confusion may occur and lead to incorrect predictions. For this reason, the use of *d* and *l* is diminishing. The use of D and L is gradually being replaced by the R and S system of designating isomers. This system is particularly useful when more than one chiral carbon atom is present. For a description of this system, see *Organic Chemistry For Dummies* by Arthur Winter (Wiley).

Part II

The Meat of Biochemistry: Proteins

The 5th Wave By Rich Tennant

"Who wants to help Grandma make her famous gingerbread man cookies? You kids get the flour, eggs, and sugar, and I'll get the amino acids and enzymes."

In this part . . .

We focus, not surprisingly, on proteins, starting with amino acids, protein's building blocks. After that we detail the processes of amino acid sequencing and the various kinds of protein structure. We finish up this part by discussing enzyme kinetics, covering catalysts (which speed up reactions) and inhibitors (which — can you guess? That's right — slow them down).

Chapter 4

Amino Acids: The Building Blocks of Protein

In This Chapter

- Looking at the structure and properties of amino acids
- Examining the common amino acids
- Finding out about the interactions of amino acids
- Seeing how amino acids combine

All cells contain thousands of types of proteins, and amino acids are the building blocks of these proteins. The sequential order, number, and chemical identity of the amino acids in the protein determine the structure of the protein as well as how the protein functions. That's why it's important to understand the chemical properties of amino acids before you can understand the behavior of proteins.

Amino acids are relatively simple molecules containing both an amine group and an acid group. The biologically important amino acids are the α-amino acids that have the amine and acid groups attached to the same carbon atom. There are more than 100 known natural amino acids; however, only 20 of them are used in protein synthesis. Francis Crick (who with James Watson determined the structure of DNA) labeled this set of amino acids the *magic 20*. Other amino acids are found in certain proteins, but in almost all cases these additional amino acids result from the modification of one of the magic 20 after the protein formed.

In this chapter, we examine the structure and properties of amino acids, especially the more common ones, and show how they interact and combine.

General Properties of Amino Acids

Like any organic compound, the properties of the molecules are largely determined by the functional groups present. In biological systems, the important properties of the amino acids include the following:

- **They can join to form proteins.** The average molecular weight of an amino acid is about 135. Proteins have molecular weights ranging from about 6,000 to several million. Thus, a large number of amino acids must be joined together to produce a protein.
- **They all have both an acid and a base.** The α-carbon (end carbon) not only has an amine group ($-NH_2$) and a carboxylic acid group (–COOH), but also two additional groups: a hydrogen atom and an R– group. The side chain, R group, identifies the amino acid.
- **They all have variations in what part of the structure is protonated depending on the pH of the solution and the structure of the rest of the molecule.**
- **They all, except glycine, have a chiral nature, influencing the reactions that the compound will undergo.**

Amino acids are positive and negative: The zwitterion formation

The presence of both an acid and a base (amine) in the same molecule leads to an interaction between the two. This interaction results in a transfer of a hydrogen ion from the acid portion to the base portion of the molecule. An amino acid with both positive and negative regions is a called a *zwitterion.* The net charge of the zwitterion is 0. This leaves the acid end of the amino acid with a negative charge ($-COO^-$) and a positive charge at the base end ($-NH_3^+$). The deprotonated portion (portion that has lost a hydrogen ion) is a carboxylate group, and the protonated group (group that has gained a hydrogen ion) is an ammonium group. The presence of a charge on the amino acid makes them water-soluble. Figure 4-1 shows zwitterion formation.

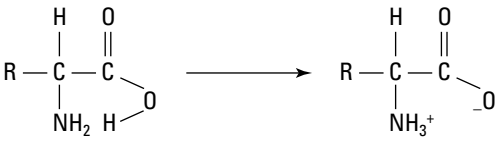


Figure 4-1: Zwitterion formation.

The unionized amino acid molecule shown in Figure 4-1 does not actually exist. However, many books and instructors draw the unionized form as a simplification, as if the ionization did not occur.

Protonated? pH and the isoelectric point

How amino acids react, because of their acid-base nature, is dependent on the pH of the solution in which they are found. Here we look at some of the implications of this pH dependency. The zwitterion is the predominant form at a particular pH, which is designated the *isoelectric point* (pI). The isoelectric point is midway between the two different pK_a values. Under most physiological conditions, isolated amino acids exist in their zwitterion form (Figure 4-2 (a)). Pure amino acids are also in the zwitterion form — and, for this reason, are *ionic solids.*

- **At a pH below the isoelectric point, some of the carboxylate groups will be protonated.** (See Figure 4-2 (b).) The pH required to cause this protonation of the carboxylate group depends on the K_a of the acid. For this reason the pK_a of the carboxylic acid group is important. Typical values are between 1 and 3. If, for example, the pK_a is 2.5, at a pH of 2.5, 50 percent of the carboxylate groups will be protonated. The net charge of the protonated form is +1.
- **At a pH above the isoelectric point, some of the ammonium groups will be deprotonated.** (See Figure 4-2 (c).) The pH required to cause this deprotonation of the ammonium group depends on the K_a of the ammonium group. For this reason, the pK_a of the ammonium group is important. Typical values are between 8 and 11. If, for example, the pK_a is 10, at a pH of 10, 50 percent of the ammonium groups will be deprotonated. The net charge of the protonated form is –1.

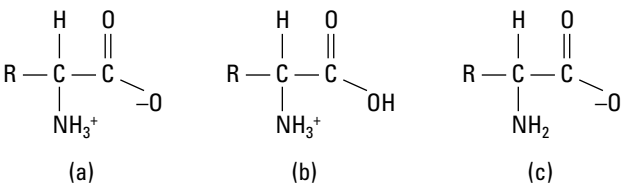


Figure 4-2: (a) Zwitterion form, (b) protonated form, and (c) deprotonated form.

Some of the side chains are also acidic or basic. In these cases, an additional pK_a becomes significant in the reactions of these molecules and will obviously complicate the pH behavior of the amino acid.

Asymmetry: Chiral amino acids

In a typical α-amino acid, four different groups are attached to the α-carbon (–COOH, $–NH_2$, –R, and –H). This makes the α-carbon asymmetric or *chiral*. The only exception is the amino acid glycine, where the R– group is a hydrogen atom. The presence of two hydrogen atoms on the α-carbon means that, in the case of glycine, the carbon atom is achiral. Chiral materials are optically active; the different forms affect light in different ways. (See Chapter 3 for more on what makes a molecule chiral.)

The arrangement of the groups around a chiral carbon atom is important. Just as your left hand only fits into your left glove, only certain arrangements of the groups will fit (because of what is called *handedness*).

There are two different forms of the chiral amino acids: the D- and the L- forms. Only the L- forms are constituents of proteins. The D- forms appear in some antibiotics and in the cell walls of certain bacteria. Fischer projections, as we explain in Chapter 3, are commonly used to represent the arrangement about the chiral carbon. Figure 4-3 illustrates some different ways to draw the Fischer projections of the structure of amino acids.

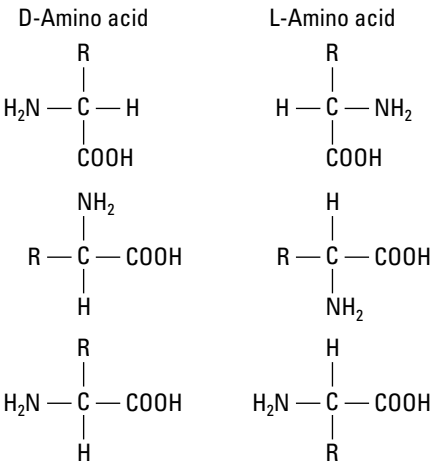


Figure 4-3: Different ways of drawing the Fischer projections of the amino acids.

A few amino acids contain two asymmetric carbon atoms. In these cases, there are four possible isomers. Biological activity is usually limited to only one of these four isomers.

The Magic 20 Amino Acids

Amino acids are subdivided into four subgroups based on the nature of the side chain (groups attached to the the α-carbon) and the general behavior of the amino acid:

- Nonpolar (hydrophobic) and uncharged
- Polar (hydrophilic) and uncharged
- Acidic (polar and charged)
- Basic (polar and charged)

The properties of the side chains are not only important to the behavior of the individual amino acids but also to the properties of the proteins resulting from the combination of certain amino acids.

In the following section we examine the structures of the individual amino acids. It is possible to represent each of the amino acids by either a three-letter or a one-letter abbreviation. Like the chemical symbols for the elements, these are fixed abbreviations. The three-letter abbreviations are easier to relate to the name of the specific amino acid. For example, we use glu for glutamine. The one-letter abbreviations are shorter, but not always related to the name. For example, we use Q for glutamine.

Nonpolar (hydrophobic) amino acids

The nonpolar amino acids are as follows:

- Alanine (ala, A)
- Valine (val, V)
- Leucine (leu, L)
- Isoleucine (ile, I)
- Proline (pro, P)
- Methionine (met, M)
- Phenylalanine (phe, F)
- Tryptophan (trp, W)

Figure 4-4 shows these amino acids.

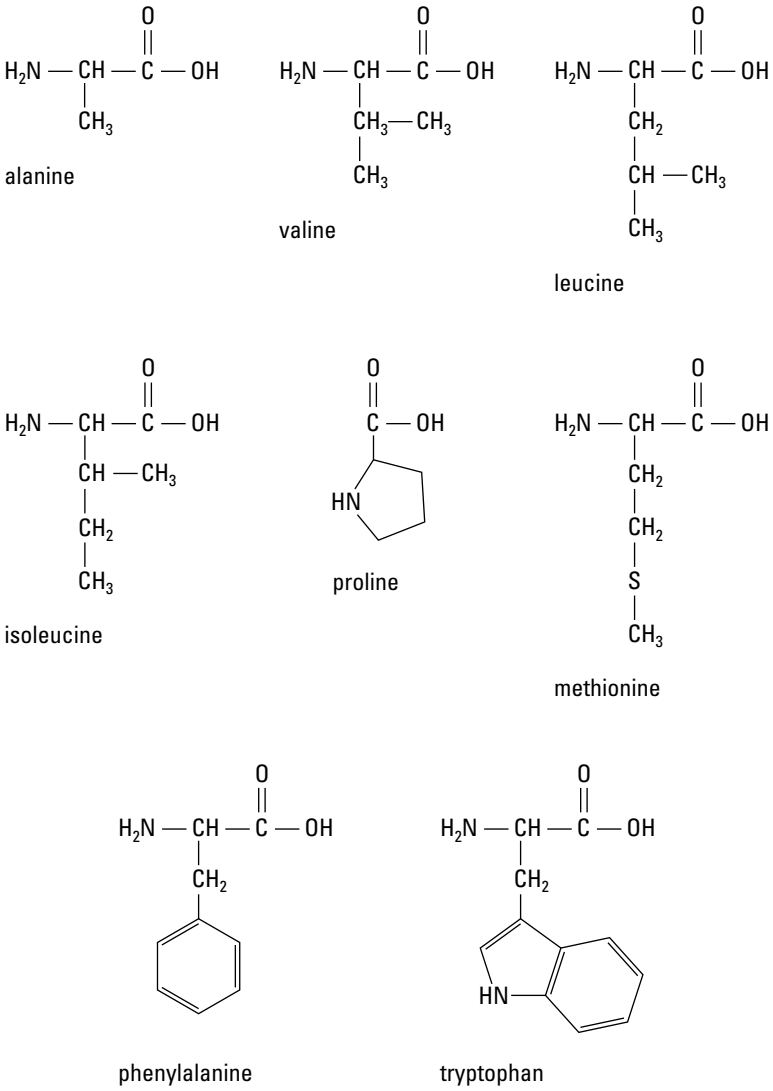


Figure 4-4: Nonpolar amino acids.

Proline has an unusual cyclic structure, which has a significant influence on protein structure. Tryptophan is a borderline case because the –NH from the ring system can interact with water to a limited extent.

Polar and uncharged (hydrophilic) amino acids

The polar and uncharged amino acids, other than glycine, can hydrogen bond to water. For this reason, they are usually more soluble than the nonpolar amino acids. The amino acids in this group are as follows:

- Glycine (gly, G)
- Serine (ser, S)
- Asparagine (asn, N)
- Glutamine (gln, Q)
- Threonine (thr, T)
- Tyrosine (tyr, Y)
- Cysteine (cys, C)

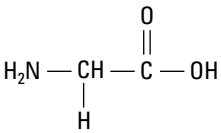
Glycine seems to be an unexpected member of this group. The small size of the R group in the case of glycine leads to the predominance of the amino and carboxylate functional groups, giving glycine's similarity to other amino acids in this group. The amide, alcohol, and sulfhydryl (–SH) groups of the remaining members of this group are very polar and neutral. At very high pH values, the phenolic group on tyrosine ionizes to yield a polar charged group. Figure 4-5 shows these amino acids.

Acidic amino acids

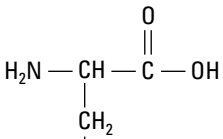
The acidic amino acids are as follows:

- Aspartic acid (asp, D)
- Glutamic acid (glu, E)

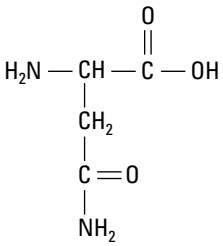
In both of these amino acids, the side group contains a carboxylic acid group. This secondary carboxylic acid group is a weaker acid (higher pK_a) than the primary carboxylic acid group. This additional carboxylate group leads to a net –1 charge at a pH where the "normal" zwitterion has a 0 net charge. The carboxylate side chain is important in the interaction of many proteins with metal ions, as *nucleophiles* (an electron-rich group replacing some group attached to a carbon) in many enzymes, and in ionic interactions. Figure 4-6 shows these amino acids.



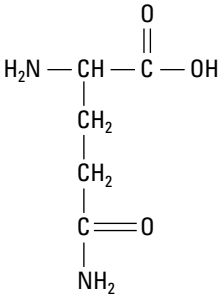
glycine



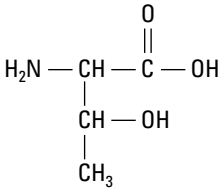
serine



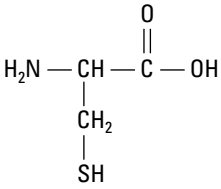
asparagine



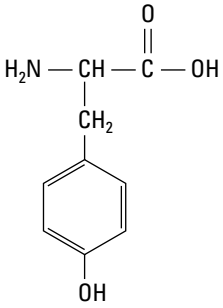
glutamine



threonine



cysteine



tyrosine

Figure 4-5: Polar and uncharged amino acids.

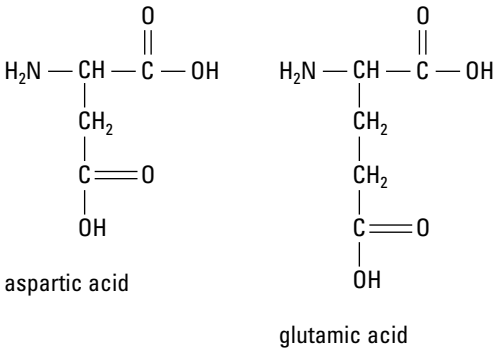


Figure 4-6: Acidic amino acids.

Basic amino acids

The basic amino acids are as follows:

- Histidine (his, H)
- Arginine (arg, R)
- Lysine (lys, K)

All of these are classified as basic amino acids, but dramatic changes in pH can affect their reactivities. This is especially true of histidine.

In all three of these amino acids, there is a basic group capable of accepting a hydrogen ion. In the case of lysine, this is a simple ammonium ion. Arginine forms the guanidinium group. Histidine forms an imidazolium group. As in the case of the acidic side chains, these side chains have a pK_a value. Both arginine and lysine are usually protonated at physiological pH values. In these cases, there is a net +1 charge present. In proteins, this net charge may be part of an ionic interaction. The pK_a of the side chain of histidine is lower than other basic groups. Protonation of histidine becomes significant at much lower pH values. In many proteins, histidine is not protonated, but is important in many enzymes in hydrogen ion transfer processes. Figure 4-7 shows these basic amino acids.

Lest We Forget: Rarer Amino Acids

In a few cases, an amino acid may undergo modification once it is incorporated into a protein. Collagen and gelatin, for example — proteins present in higher vertebrates — contain hydroxylysine and hydroxyproline. These two amino acids contain an additional –OH group on the side chain.

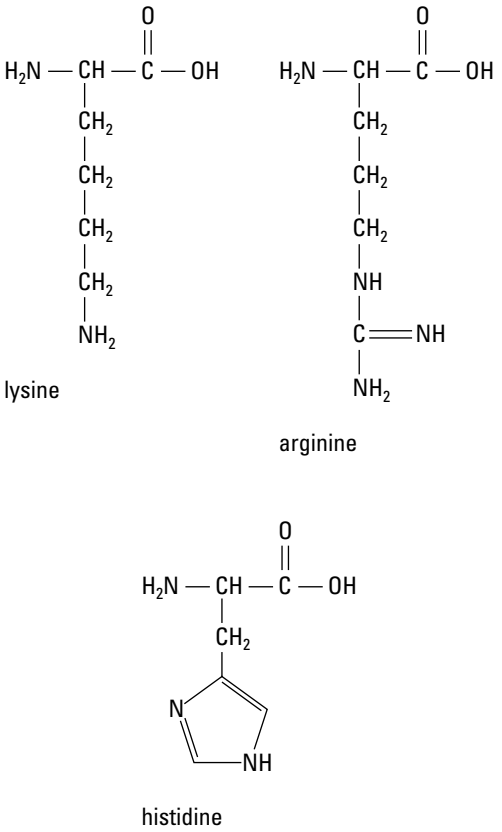


Figure 4-7: Basic amino acids.

Certain amino acids do not occur in proteins. The neurotransmitter γ-aminobutyric acid — GABA — is one example. Citrulline is the amino acid that serves as a precursor of arginine. Ornithine, homocysteine, and homoserine are important as metabolic intermediates. Figure 4-8 shows a couple of these amino acids.

Rudiments of Amino Acid Interactions

Amino acids are the ingredients used in the recipe in making a protein. Just as the individual ingredients in a recipe lead to distinct characteristics of what eventually shows up on the dinner table, the amino acids present contribute properties to proteins. And just as you cannot replace the flour in a recipe with pepper, you generally cannot replace one amino acid in a protein with another. In both cases, the final product will be different. In the next section, we show you some of the ways that amino acids interact. These interactions set the stage for our discussion of bonding among the amino acids.

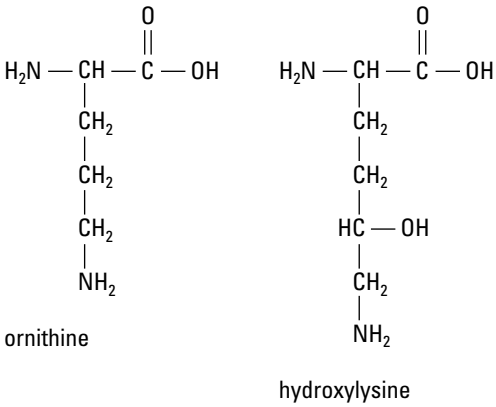


Figure 4-8: Two of the less common amino acids.

Intermolecular forces: How an amino acid reacts with other molecules

Amino acids can interact with other molecules — and we mean *any* other molecules, including fluids, other amino acids, and other biological molecules — in a variety of ways. We cover intermolecular forces in general in Chapter 3, but in this section we show you how they play out when amino acids are involved. The carboxylic acid and amine parts of the amino acids define much of the reactivity of the molecule, but the side chains can also interact with other molecules. There are three general ways in which they can interact.

- **Hydrophobic interactions:** The nonpolar side groups are hydrophobic and are attracted to each other through London dispersion forces. Nonpolar groups tend to clump together and exclude not only water but also all other types of side chains.
- **Hydrophilic reactions:** The polar and uncharged side groups are hydrophilic. The presence of a number of these groups increases the solubility of a protein. These groups hydrogen bond not only to water but also to each other. Polar groups tend to interact strongly and "push" the nonpolar groups out.
- **Ionic interactions:** The presence of acidic or basic side chains leads to ionic charges — opposite charges attract. A carboxylate group from one side chain is attracted to the ammonium ion of another side chain through an ionic interaction. This ionic bond is very strong.

The amino acid cysteine can interact with a second cysteine molecule through a different type of interaction (Figure 4-9). The mild oxidation of two cysteine sulfhydryl groups leads to the formation of cystine. A disulfide linkage joins the two amino acids with a covalent bond. Mild reduction can reverse this process.

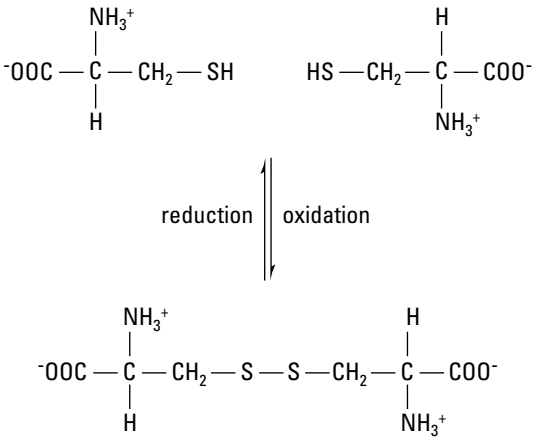


Figure 4-9: Joining two cysteine molecules to form cystine.

A hair perm utilizes an oxidation reduction reaction creating disulfide linkages. The greater the number of disulfide linkages, the curlier the hair!

Altering interactions by changing an amino acid's pH

As we discuss in Chapter 3, the function of many substances, especially biochemical ones, is dependent on pH. If you change the pH, you change some of the interactions. In this section we show how those changes affect interactions involving amino acids.

Just like any other molecule, an amino acid has two or three functional groups, depending on the amino acid. Those functional groups include those with oxygen and sulfur, those with nitrogen, and those with phosphorus. A change in pH affects one to three of those functional groups in terms of interactions. So if an amino acid has a functional group that changes from a dipole-dipole interaction to an ionic interaction.

One example of the dipole-dipole to ionic interaction change is the process of milk curdling. If you add an acid to milk, it coagulates. Casein has an isoelectric point at 4.6 pH, so that adding an acid causes the formation of ionic bonds among the molecules. This works against the dipole-dipole interactions with water, so that the protein precipitates.

The pK_a values for the various groups present in the different amino acids are shown in Table 4-1. If the pH of the solution matches one of these values, then half the species is in the protonated form and half is in the deprotonated

form. At a lower pH, more than half is protonated, whereas at a higher pH more than half is deprotonated.

The pH dependence of the protonation of amino acids aids in their separation and identification. Because the amino acids use the carboxylic acid and amine ends when they join to form a protein, only the pK_a values of the side chains are important in additional interactions and reactions.

Table 4-1 pK_a Values for the Amino Acids

Amino acid	*pK_a –COOH*	*pK_a –NH_3^+*	*pK_a R group*
Alanine	2.35	9.69	
Arginine	2.17	9.04	12.48
Asparagine	2.02	8.8	
Aspartic acid	2.09	9.82	3.86
Cysteine	1.71	10.78	8.33
Glutamic acid	2.19	9.67	4.25
Glutamine	2.17	9.13	
Glycine	2.34	9.6	
Histidine	1.82	9.17	6.0
Isoleucine	2.36	6.68	
Leucine	2.36	9.60	
Lysine	2.18	8.95	10.53
Methionine	2.28	9.21	
Phenylalanine	1.83	9.13	
Proline	1.99	10.60	
Serine	2.21	9.15	
Threonine	2.63	10.43	
Tryptophan	2.38	9.39	
Tyrosine	2.20	9.11	10.07
Valine	2.32	9.62	

Combining Amino Acids: How It Works

A *protein* is a string of at least 150 amino acids (residues) joined. We cover the fundamentals about protein creation in Chapter 5, but before you dive into that topic, this section gives you a solid understanding of how two amino acids join together in the first place, and how additional amino acids link onto the chain gang. The process is reversible (as in digestion).

When drawing the chemical structures of amino acids and their bonds, the standard convention is to first draw the structures from the ammonium group of the first amino acid (the N-terminal residue), starting at the left, and continuing the drawing to the right, ending with the carboxylate group (C-terminal residue) of the last amino acid.

The peptide bond and the dipeptide

One of the most important types of bonds in all of biochemistry is the *peptide bond.* As you will see, it is this type of bond that will be used in the synthesis of proteins. The interaction of two amino acids at the body's pH results in the formation of a peptide bond as illustrated in Figure 4-10.

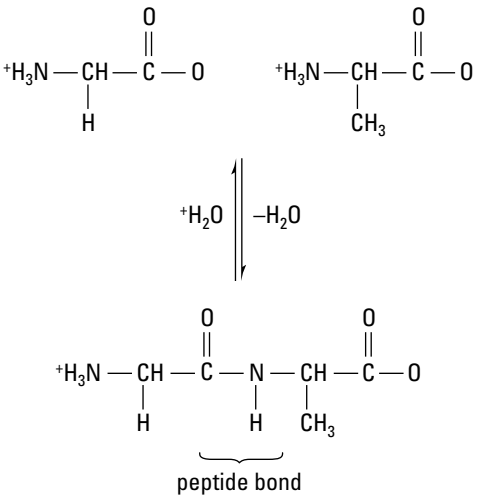


Figure 4-10: The formation of a peptide bond.

The two residues react to expel a water molecule, the same dehydration you used so much in organic chemistry. The reverse of this condensation reaction is hydrolysis. The resultant amide group is a peptide bond. The presence of two amino acid residues means the product is a dipeptide.

The peptide bond is a *flat* (planar) structure. It is stabilized by our old organic friend, *resonance.* Figure 4-11 illustrates the stabilization. The resonance increases the polarity of the nitrogen and oxygen. This increase in polarity leads to hydrogen bonds that are much stronger than most other hydrogen bonds. The double bond character between the carbon and the nitrogen restricts rotation about this bond. That's why the peptide bond is planar.

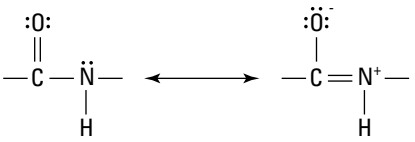


Figure 4-11: Resonance stabilization of a peptide bond.

Tripeptide: adding an amino acid to a dipeptide

A repetition of the process illustrated in Figure 4-10 joins a third amino acid to produce a *tripeptide.* For example, combining glycine, alanine, and serine yields the illustration in Figure 4-12. Notice that everything begins with the N-terminal residue and ends with the C-terminal residue. (You could designate this tripeptide as *gly-ala-ser* using the three letter abbreviations.)

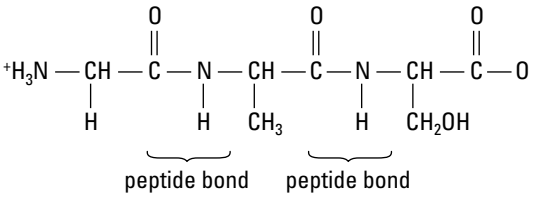


Figure 4-12: A tripeptide.

The repetition of the process of linking amino acids hundreds or thousands of times produces a protein. In the next chapter, we cover that topic in full.

Chapter 5

Protein Structure and Function

In This Chapter

- Finding out about the structure of proteins
- Understanding amino acid sequencing in proteins
- Learning about applications of protein sequencing

In Chapter 4, we show you how amino acids combine through the use of a peptide bond, and we mention there that if at least 150 or so amino acids join hands, they rise to the rank of a protein. However, distinguishing an amino acid chain as a protein isn't exactly simple — just as written English is an extremely diverse set of words made by combining letters from an alphabet of just 26 letters, proteins are an extremely diverse set of biochemicals made by combining 20 different amino acids.

In this chapter, we show you more about these proteins, including the four types of protein structure that determine a protein's function and the sequence of amino acids in a particular protein.

There are two general categories of proteins:

- **Fibrous proteins** are found only in animals. They usually serve as structural entities — for example, connective tissue, tendons, and muscle fiber. They are normally insoluble in water.
- **Globular proteins** usually do not serve a structural function — they act as transporters, like hemoglobin, and are often enzymes. They are usually water-soluble.

Proteins are utilized in living organisms in a number of ways, such as:

- **Structure:** Skin and bone contain collagen, a fibrous protein.
- **Catalysis:** These proteins, called enzymes, allow reactions to occur in the organism under mild conditions and with great specificity.
- **Movement:** Proteins make up a large protein of muscle fiber and help in the movement of various parts of our bodies.

- **Transport:** These proteins transport small molecules through the organism. Hemoglobin, the protein that transports oxygen to the cells, is a transport protein.
- **Hormones:** Proteins called hormones help regulate cell growth.
- **Protection:** Proteins called antibodies help rid the body of foreign proteins.
- **Storage:** These protein help store other substance in the organism. For example, iron is stored in the liver in a complex with the protein ferritin.
- **Regulation:** These proteins help mediate cell responses, such as the protein rhodopsin, found in the eye and involved in the vision process.

The function that a particular protein assumes is, in many cases, directly related to the structure of that protein. Proteins may have as many as four levels of structure (key word being *levels,* not different structures), each of which places the components into a position where these intermolecular forces can interact most advantageously. The levels are simply labeled primary, secondary, tertiary, and quaternary. Primary is the most fundamental level that all proteins have, and quaternary is the most specific level that only some proteins have. Intermolecular forces themselves are important to the function of a protein, of course, but the arrangement of the molecules is even more significant.

If present, the secondary, tertiary, and quaternary structures of a protein may be destroyed — in a number of ways:

- Heating (cooking) can break hydrogen bonds.
- Changing the pH can protonate or deprotonate the molecule and interrupt ionic interactions.
- Reducing agents can break disulfide linkages.

In some cases, the process may be reversible.

Primary Structure: The Structure Level All Proteins Have

The primary structure of a protein is simply the sequence of amino acids comprising the molecule. The primary structure of a protein is the amino acid sequence within the molecule. All proteins have a primary structure, because all proteins by definition consist of a sequence of amino acids. The primary structure serves as the foundation upon which all higher levels of protein structure build.

Next we take a look at how a protein is assembled from its building blocks, the amino acids.

Building a protein: Outlining the process

During the synthesis of a protein, the chain of amino acids is built one link at a time, roughly as follows:

1. **The transfer RNA (tRNA) molecule transfers specific amino acids to the mitochondria of the cell to connect to the growing chain.**
2. **Each amino acid joins to the chain through the formation of a peptide bond.** (See Chapter 4 for more on peptide bonds.)
3. **The first peptide bond joins two amino acids to form a dipeptide.**
4. **The second peptide bond joins three amino acids to produce a tripeptide.**
5. **This process continues hundreds, if not thousands, of times to produce a polypeptide — a protein.**

When two or more amino acids combine, a molecule of water is removed. What remains of each amino acid is called a *residue.* They lack a hydrogen atom on the amino group, or an –OH on the carboxyl group, or both.

The cell's DNA ultimately controls the sequence of amino acids. This information goes from the DNA to the messenger RNA (mRNA), which serves as the template for the creation of the primary structure of the protein. It is necessary to supply energy, as we will see later, to synthesize the protein.

Organizing the amino acids

One end of the primary structure has an amino group, and the other end has a carboxylate group. By convention, the end with the amino group is considered the "beginning" of the protein. Drawing, naming, numbering, and other treatments of the primary structure always begin with the amino end (called the *N-terminal*) and stop with the carboxylate end (the *C-terminal*). For example, in the hexapeptide Met-Thr-Ser-Val-Asp-Lys (see Chapter 4 for a list of the amino acids and their abbreviations), methionine (Met) is the N-terminal amino acid, and lysine (Lys) is the C-terminal amino acid. Note that reversing the sequence to Lys-Asp-Val-Ser-Thr-Met also gives a hexapeptide with the same composition but with different chemical properties because you initially started with a different amino acid. Therefore an amino acid that lost a hydrogen in one sequence will lose an –OH in the other.

The polypeptide chain has a backbone consisting of the same, rather simple, repeating unit. Variations take place in the form of side chains — the R groups of the amino acids. You can see this repeating sequence in Figure 5-1. Notice that the repeating unit (indicated by the brackets) is the amino-carbon-carbonyl sequence and that there can be different R groups attached to the carbon unit of this backbone.

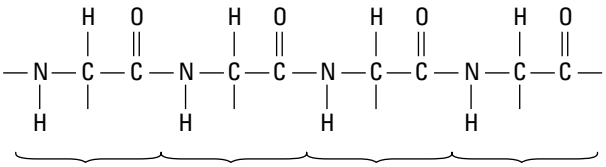


Figure 5-1: Repeating sequence of the protein backbone.

The protein backbone has many places where hydrogen bonds may form. Every residue — other than the amino acid proline — has an NH, which may serve as a *donor* to a hydrogen bond. And every residue has a carbonyl group, which can serve as the *acceptor* of a hydrogen bond. The presence of donors and acceptors leads to the possibility of forming numerous hydrogen bonds.

Each of the peptide bonds exhibits no free rotation about the carbon-nitrogen bond because of the contribution of the resonance form, which has a double bond. Thus, there is a planar unit of four atoms, and in almost all cases, the oxygen atom is trans to the hydrogen atom. The remainder of the backbone can rotate. The ability to rotate or not influences how the three-dimensional structure of the protein is established. There are restrictions to this rotation because the side-chains can "bump" into each other — called *steric* hindrance. The rigidity of the peptide bond and rotation restrictions lower the entropy of the three-dimensional structure of a protein relative to a random chain of amino acids. Lowering the entropy helps stabilize the structure.

Example: The primary structure of insulin

The first determination of the primary structure of a protein was that of bovine insulin, the structure of which appears in Figure 5-2. Since this landmark determination, the primary structures of more than 100,000 proteins have been determined. In all cases, the protein has a unique primary structure.

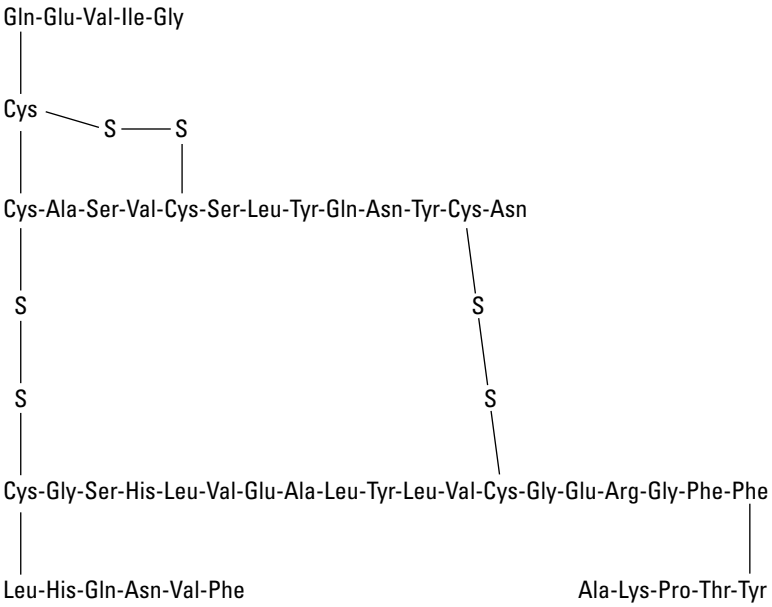
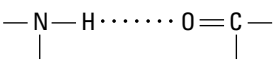


Figure 5-2: Structure of bovine insulin.

Secondary Structure: A Structure Level Most Proteins Have

It is possible for one peptide bond to form a hydrogen bond to another peptide bond. In general, the formation of these hydrogen bonds leads to the secondary structure of a protein. The secondary structure is the result of many hydrogen bonds, not just one. The hydrogen bonds are intramolecular, that is between segments of the same molecule, as shown in Figure 5–3:

Figure 5-3: Hydrogen bonding between two peptide bonds.



The α-helix and β-pleated sheet are the secondary structures that result from this hydrogen bonding. Secondary structures may be only a small portion of the structure of a protein or can make up 75 percent or more.

The α-helix

In the α-helix, the primary structure twists into a tightly wound, spring or rod-like structure. Each turn consists of 3.6 amino acid residues. These turns allow hydrogen bonding between residues spaced four apart. Every peptide bond participates in two hydrogen bonds: one from an NH to a neighboring carbonyl, and one from a neighboring NH to the carbonyl (Figure 5-4).

Structurally, the helices may be either right-handed or left-handed (see Chapter 3 for more on handedness). Essentially all known polypeptides are right-handed. Slightly more steric hindrance is present in a left-handed helix, and the additional steric hindrance makes the structure less stable. Keratin — the protein of fur, hair, and nails — consists of three right-handed α-helices wrapped around each other in a left-handed coil.

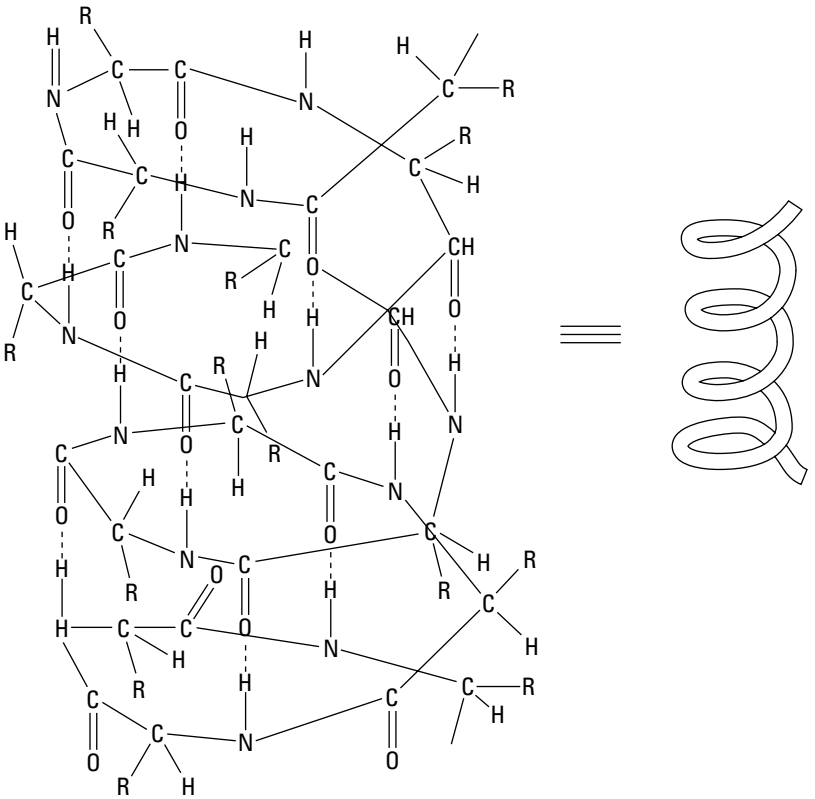


Figure 5-4: The α-helix.

Certain amino acids destabilize the α-helix. Proline, for example, creates bends or "kinks" in the primary structure, which inhibit the formation of a regular pattern of hydrogen bonds. A group of isoleucine residues disrupts the secondary structure because of the steric hindrance caused by their bulky R groups. The small R group of glycine, only an H, allows too much freedom of movement, which leads to a destabilization of the helix. A concentration of aspartic acid and/or glutamic acid residues also destabilizes the structure because the negative charges on the side chains repel each other. Other residues that destabilize the helix, for similar reasons, are lysine, arginine, serine, and threonine.

The β-pleated sheet

The β-pleated sheet, or simply the β sheet, is the other major secondary protein structure. Here, the primary structure is extended instead of tightly winding into a helix. There are two forms of this structure, known as the *parallel* β-pleated sheet and the *anti-parallel* β-pleated sheet. Again, hydrogen bonds are the source of these structures. A β-pleated sheet forms when two or more strands link by hydrogen bonds. The strands are different parts of the same primary structure.

In the parallel structure, the adjacent polypeptide strands align along the same direction from N-terminal end to C-terminal end. In the anti-parallel structure, the alignment is such that one strand goes from N-terminal end to C-terminal end, while the adjacent strand goes from C-terminal end to N-terminal end (Figure 5-5).

In the β-pleated sheet structures, the side chains of adjacent amino acids point in opposite directions. The hydrogen bonding pattern in the parallel structure is the more complicated. Here, the NH group of one residue links to a CO on the adjacent strand, whereas the CO of the first residue links to the NH on the adjacent strand that is two residues down the strand. In the anti-parallel structure, the NH and CO groups of one residue link to the respective CO and NH groups of one residue on the adjacent strand.

Schematically, broad arrows indicate the presence of β-pleated sheets. If the arrows point in the same direction, it is the parallel structure, and if they point in opposite directions, it is the anti-parallel structure. The sheets are typically 4 or 5 strands wide, but 10 or more strands are possible. The arrangements may be purely parallel, purely anti-parallel, or mixed (refer to Figure 5-5).

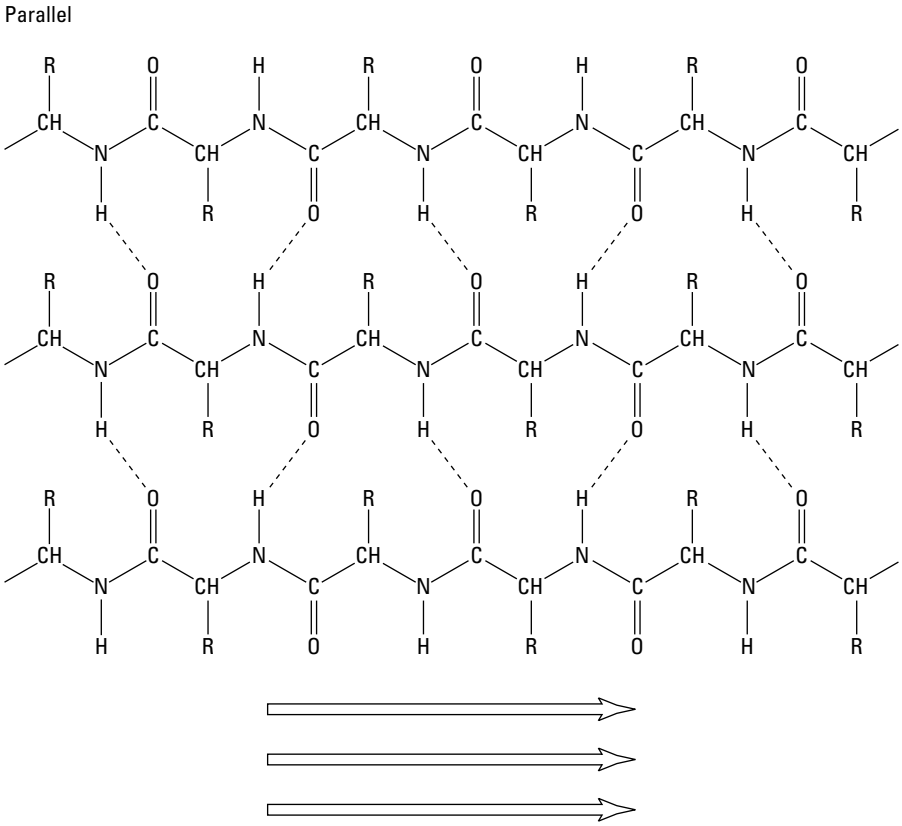


Figure 5-5: Parallel and anti-parallel β-pleated sheet structures.

β-turns and the Ω-loops

There are additional secondary structures involving hydrogen bonding between peptide bonds; these are much smaller units. The best known are the β-turn — or *hairpin bend* — and the Ω-loop. The hairpin bend is simply a bend in the primary structure held in place by a hydrogen bond. The Ω-loop gets its name because of the loose similarity of its shape to the Greek letter. Both are found on the exterior of proteins.

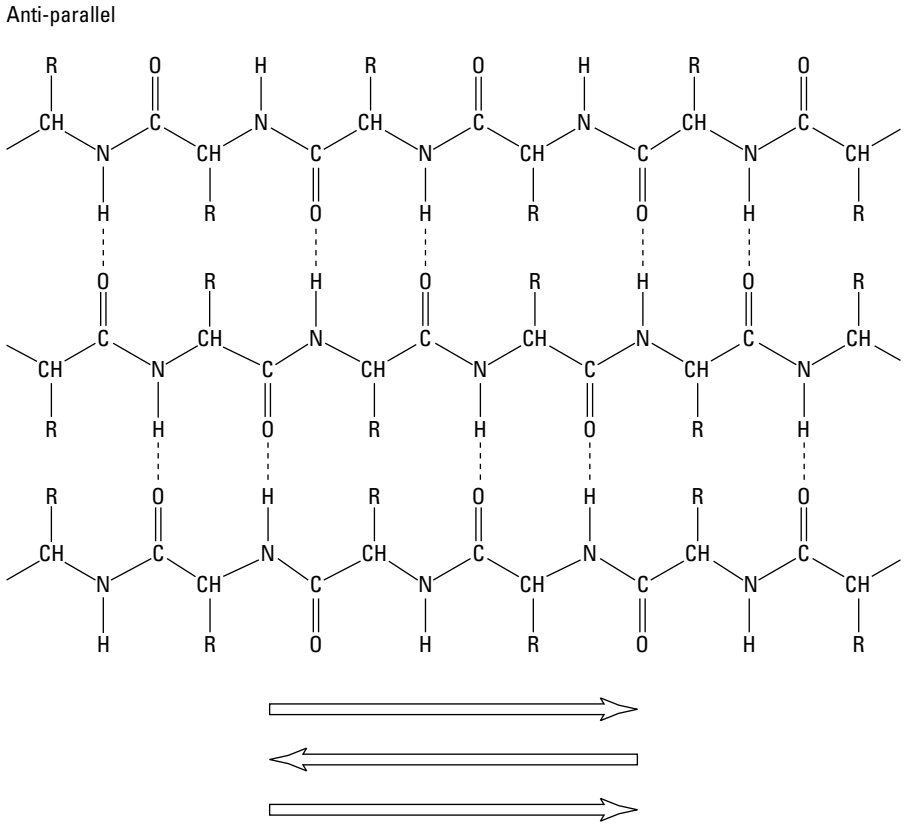


Figure 5-5: (continued)

Tertiary Structure: A Structure Level Many Proteins Have

The overall shape of a protein is determined by its primary and secondary structures along with interactions between the side chains. This gives rise to what is called the protein's *tertiary structure.* Nonpolar side chains are hydrophobic and, although repelled by water, are attracted to each other. Polar side chains attract other polar side chains through either dipole-dipole forces or hydrogen bonds.

For example, both aspartic acid and glutamic acid yield side chains with a negative charge that are strongly attracted to the positive charges in the side chains of lysine and arginine. Two cysteine residues can connect by forming a disulfide linkage — a covalent bond (Figure 5-6).

What induces a protein to adopt a very specific tertiary structure? Examination of the structures of many proteins shows a preponderance of nonpolar side chains in the interior with a large number of polar or ionic side chains on the exterior. In an aqueous environment, the hydrophobic (nonpolar) groups induce the protein to fold upon itself, burying the hydrophobic groups away from the water and leaving the hydrophilic groups adjacent to water. The result is similar in structure to a micelle.

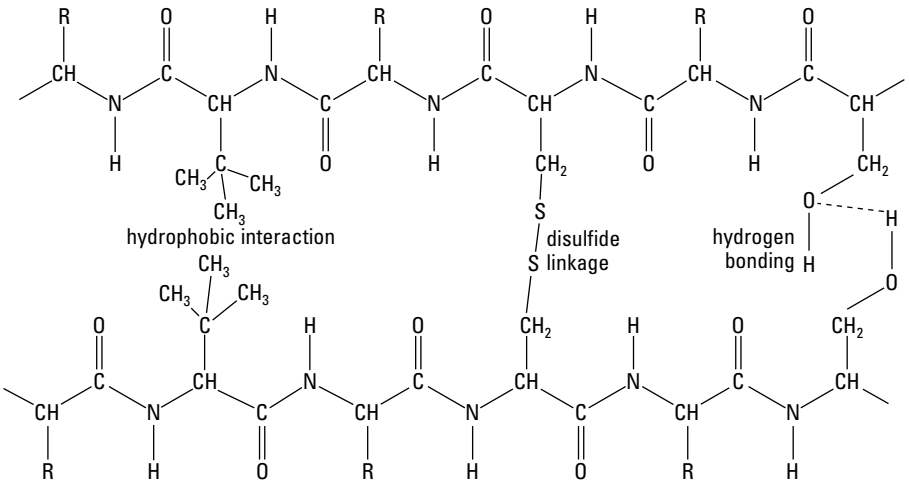


Figure 5-6: Some tertiary structures appearing in proteins.

Quaternary Structure: A Structure Level Some Proteins Have

The quaternary structure found in some proteins results from interactions between two or more polypeptide chains — interactions that are usually the same as those that give rise to the tertiary structure. These interactions include hydrogen bonding and disulfide bonds. This quaternary structure locks the complex of proteins into a specific geometry. An example is hemoglobin, which has four polypeptide chains. There are two identical α-chains and two identical β-chains. (The designations α and β simply refer to two different proteins and not to secondary structures.)

Dissecting a Protein for Study

The previous sections have discussed the different types of protein structure. Now it is time to see how a biochemist goes about determining the structure(s) of a particular protein.

Additional information about the structure of a protein comes from immunology. An animal generates an antibody in response to a foreign substance known as an *antigen*. *Antibodies* are proteins found in the blood serum. Exposure to diseases, certain chemicals, and allergies induce the formation of specific antibodies. These antigens collect on the surface of red blood cells. Every antigen has a specific antibody.

Antibodies are very specific and have a strong affinity for their specific antigens, recognizing specific amino acid sequences on the antigens. Animals have a large number of antibodies present in their bodies, based on their environmental history. One application of antibodies and antigens is in the analysis of bloods, specifically in the field of forensics investigations (see nearby "Forensics: Analysis of bloodstains" sidebar).

Separating proteins within a cell and purifying them

There are thousands of different proteins in each cell. In order to examine and study one of them, you need to separate it from all the others. The methods of separating proteins are, in general, applicable to all other types of biochemicals. Initially, simple filtration and solubility can remove gross impurities, but much more needs to be done before the sample is pure. The key separation and purification methods depend on two physical properties of the proteins: size and charge.

Separating proteins by size

Methods relying on separation by protein size and mass include ultrafiltration, ultracentrifugation, and size exclusion chromatography. *Ultrafiltration* is a modification of dialysis in which molecules smaller than a certain size diffuse through a semipermeable membrane, and larger ones don't. Ultrafiltration can separate smaller molecules from larger impurities or larger molecules from smaller impurities.

In *ultracentrifugation,* a powerful centrifuge causes heavier molecules to sink faster and, which allows their separation — much as the lighter water is separated from the heavier lettuce in a salad spinner. Ultracentrifugation also gives the molar mass of the protein.

In *size exclusion chromatography,* also known as *molecular sieve chromatography* or *gel filtration chromatography,* a solution passes through a chromatography column filled with porous beads. Molecules that are too large for the pores pass straight through. Molecules that may enter the pores are slowed. The molecules that may enter the pores undergo separation depending on how easily they can enter.

Forensics: Analysis of bloodstains

The study of proteins has many applications to forensics. One of them is the examination of bloodstains, blood being the most common form of evidence examined by a forensic serologist. The presence of blood can link a suspect to both a victim and a crime scene. Bloodstain patterns can also give evidence of how a violent attack took place. Criminals recognize the significance of this evidence and often try to conceal it.

Blood is mostly water, but it also contains a number of additional materials including cells, proteins, and enzymes. The fluid portion, or *plasma,* is mostly water. The *serum* is yellowish and contains platelets and white blood cells. The *platelets,* or red blood cells, outnumber the white blood cells by about 500 to 1. White blood cells are medically important, whereas red blood cells and, to a lesser extent, serum are important to the forensic serologist. Because blood quickly clots when exposed to air, serologists must separate the serum from the clotted material. The serum contains antibodies that have forensic applications, and red blood cells have substances such as antigens on their surfaces that also have forensic applications. Antibodies and antigens are the keys to forensic serology: Even identical twins with identical DNA have different antibodies. As you know from this chapter, antibodies, and some antigens, are proteins, and this is why methods of studying proteins are important to their analysis.

Analysis of bloodstains initially attempts to answer five questions.

- **Is this a blood sample?** To answer this question, the investigator can use a number of tests. The generic term for a test of this type is a *presumptive* test. The Kastle-Meyer test uses phenolphthalein, which, when it comes into contact with hemoglobin, or a few other substances, forms a bright pink color from the release of peroxidase enzymes. The luminol test is useful in detecting invisible bloodstains because, in contact with blood, or a few other chemicals, luminol emits light, which can be seen in a darkened room. The Wagenhaar, Takayama, and Teichman tests take advantage of the fact that long-dried blood will crystallize or can be induced to crystallize.
- **Is the blood from a human or an animal?** The forensic investigator answers this question (and the next one, if applicable) by means of an *antiserum* test. It is important to know whether the blood came from a human or an animal such as a pet. The standard test is the precipitin test. Injecting human blood into an animal results in the production of antibodies in the animal's bloodstream, and isolating these antibodies from the animal's blood yields an antiserum. If human antiserum creates clotting in a blood sample, the sample must be human.
- **If the sample is from an animal, what is the species?** It is possible to create animal antiserums in an analogous manner, and test for each type of animal.
- **If the blood is from a human, what is the blood type?** The procedure for answering this depends on the quantity and quality of the sample. If the quality is good, *direct* typing is done — otherwise, *indirect* typing is used. (Direct typing, to classify blood in the A-B-O system, is discussed in this chapter's other sidebar.) A dried bloodstain normally requires indirect typing. The most common indirect typing method is the absorption-elution test. Treatment of a sample with antiserum antibodies gives a solution which, upon addition to a known sample, causes coagulation.

- **Is it possible to determine the sex, race, and age of the source of the blood?** Here the answers become less precise. Clotting and crystallization indicate age. Testing for testosterone levels and chromosome testing can determine sex. And certain controversial, racial genetic markers based on protein and enzyme tests may indicate race.

  Other body fluids may contain the same antibodies and antigens found in blood. Therefore, similar tests work on these fluids as well.

Separating proteins by charge

Methods of separating proteins relying on the charge of the protein include solubility, ion exchange chromatography, and electrophoresis. Each of these methods is pH dependent.

Proteins are least soluble at their isoelectric point. (The *isoelectric point* is the pH where the net charge on the protein is 0.) At the isoelectric point, many proteins precipitate from solution. At a pH below the isoelectric point, the protein has a net positive charge, whereas a pH above the isoelectric point imparts a net negative charge. The magnitude of the charge depends on the pH and the identity of the protein. Therefore, two proteins coincidently having the same isoelectric point will not necessarily have the same net charge at a pH that is one unit lower than the isoelectric point.

Both ion exchange chromatography and electrophoresis take advantage of the net charge. In *ion exchange chromatography,* the greater the magnitude of the charge, the slower a protein moves through a column — this is similar to the ion-exchange process that occurs in water-softening units.

In *electrophoresis,* the sample solution is placed in an electrostatic field. Molecules with no net charge do not move, but species with a net positive charge move toward the negative end, and those with a net negative charge move toward the positive end. The magnitude of the net charge determines how fast the species moves. Other factors influence the rate of movement, but the charge is the key. There are numerous modifications of electrophoresis.

In protein analysis, rarely do biochemists use only one single technique. They commonly use several in order to confirm their findings.

Digging into the details: Uncovering a protein's amino acid sequence

Once a pure sample of protein is available, it is possible to begin determining its amino acid sequence, in order to identify the specific protein. The general

procedure for doing so, with slight modification, works for other biochemicals as well:

Step 1: Separating and purifying the polypeptide chains

If you determine that more than one polypeptide chain is present in the protein, you need to separate and purify the chains so you can sequence them individually. (Because many proteins only have one polypeptide chain, this step is not always necessary.) Denaturing the protein, disrupting its three-dimensional structure without breaking the peptide bonds, using pH extremes will normally suffice. If disulfide linkages are present between the chains, apply the procedure outlined in Step 2 to separate the chains for isolation.

Step 2: Slashing intrachain disulfide linkages

Step 2 requires breaking (cleaving) the disulfide linkages. A simple reduction accomplishes this. However, the linkages may reform later, so it is necessary to cleave the linkages and prevent their reformation via reductive cleavage followed by alkylation. Oxidative cleavage, where oxidation of the sulfur to $-SO_3^-$ occurs, also prevents a reversal of the process.

Step 3: Determining amino acid concentration of the chain

Step 3 is easily accomplished using an *amino acid analyzer*, an automated instrument that can determine the amino acid composition of a protein in less than an hour. The instrument requires less than a nanomole of protein. The analyzer's output is the percentages of each of the amino acids present.

Step 4: Identifying the terminal amino acids

Step 4 not only identifies the terminal amino acids but also indicates whether more than one chain is present. A polypeptide chain only has one N-terminal and one C-terminal amino acid. Therefore, if more than one N- or C-terminal amino acid is present, there must be more than one polypeptide chain.

It is possible to identify the N-terminal residue in a number of ways. In general, procedures begin by adding a reagent that reacts with the N-terminal amino acid and tags it. Subsequent hydrolysis destroys the polypeptide, allowing separation of the tagged residue and its identification. Such methods use Sanger's reagent, dansyl chloride, and leucine aminopeptidase. The method of choice nowadays is called the Edman degradation. This method, as do other methods, tags the N-terminal residue; however, only the terminal amino acid is cleaved from the chain, so the remainder of the chain is not destroyed as in other methods. It is possible to repeat the procedure on the shortened chain to determine the next residue. In principle, repetition of the Edman degradation can yield the entire sequence, but, in most cases, determination of the first 30 to 60 residues is the limit.

It is also possible to determine the C-terminal residue by tagging. The akabori reaction (hydrazinolysis) and reduction with lithium aluminum hydride tag the C-terminal residue. It is also possible to selectively cleave the C-terminal residue using the enzyme carboxypeptidase, a variety of which are available. Unfortunately, the enzyme doesn't stop with one cleavage — given sufficient time, it proceeds down the entire polypeptide chain.

Steps 5 and 6: Breaking the chain into smaller pieces

In Step 5, you cleave the polypeptide into smaller fragments and determine the amino acid composition and sequence of each fragment. Step 6 repeats Step 5 using a different cleavage procedure to give a different set of fragments. Steps 5 and 6 break the chain into smaller pieces to ease identification.

Most of the methods here employ enzymes; however, other less-specific methods are useful in some cases. Partial acid hydrolysis randomly cleaves the protein chain into a number of fragments. Trypsin, a digestive enzyme, specifically cleaves on the C-side of arginine or lysine. Using trypsin gives additional information that the total number of arginine and lysine residues present is one less than the number of fragments generated. The digestive enzyme chymotrypsin preferentially cleaves residues containing aromatic rings (tyrosine, phenylalanine, and tryptophan). It slowly cleaves other residues especially leucine. Clostripain cleaves positively charged amino acids, especially arginine. It cleaves lysine more slowly. Fragments with a C-terminal aspartic acid or glutamic acid form from the interaction of staphylococcal protease on a protein in a phosphate buffer. In the presence of bicarbonate or acetate buffer, only C-terminal glutamic acid fragments result. A number of less specific enzymes can complete the breakdown of the fragments, including elastase, subtilisin, thermolysin, pepsin, and papain.

Chemical methods of breaking up the fragments include treatment with cyanogen bromide, hydroxylamine, and heating an acidic solution. Cyanogen bromide specifically attacks methionine. Hydroxylamine specifically attacks asparagine-glycine bonds. If a solution at pH = 2.5 is heated to 40°C, selective cleavage of aspartic acid-proline bonds occurs.

It is possible to apply the Edman degradation on each of the fragments. This can simplify the determination of the sequence of a large protein.

Step 7: Combining information to get the total sequence

Step 7 is where the information from the various procedures comes together. For example, look at a simple octapeptide fragment from a protein. This fragment gave, upon complete hydrolysis, one molecule each of alanine (Ala), aspartic acid (Asp), glycine (Gly), lysine (Lys), phenylalanine (Phe), and valine (Val), and two molecules of cysteine (Cys). The following fragments were

Basics of blood typing

The determination of blood type in the A-B-O system, first begun in 1901, is based on antigen-antibody reactions. Over the years, additional reactions have been discovered. More than 256 antigens are known, leading to 23 different blood groups. Each blood group is defined by the antibodies present in the serum and the antigens present on the red blood cells.

In basic blood typing, one needs two antiserums, labeled *anti-A* and *anti-B*. Adding a drop of one of these to a blood sample causes coagulation if the appropriate antigens are present. Anti-A interacts with both A and AB blood. Anti-B interacts with both B and AB blood. Neither interacts with type O blood. The approximate distribution of the different blood types is: 43–45 percent type O; 40–42 percent type A; 10–12 percent B; and 3–5 percent AB. Subgrouping is also possible with designations such as O1 and O2. There are other very rare types as well.

The Rh factor provides an additional means of subdividing blood. The *Rh factor* (the name comes from the rhesus monkey) is an antigen on the surface of red blood cells. A person with a positive Rh factor contains a protein (antibody) that is also present in the bloodstream of the rhesus monkey. About 85 percent humans are Rh positive. A person lacking this protein is, naturally, Rh negative. Assigning a blood sample as Rh positive or Rh negative is a useful simplification. There are about 30 possible combinations of factors.

Additional factors can determine whether blood belongs to a specific individual: the identification of other proteins and enzymes present in the blood. A forensic serologist (see this chapter's other sidebar for more) does this level of testing in every case where the quality of the sample allows. One of the characteristics of proteins or enzymes in the blood is *polymorphism*, or the ability to be present as isoenzymes. Polymorphism means that the protein may exist in different forms or variants. One well-known example is the polymorphism of hemoglobin into the form causing sickle cell anemia. Some well-recognized polymorphisms are:

Adenyl kinase	AK
Adenosine deaminase	ADA
Erythrocyte acid phosphatase	EAP
Esterase D	EsD
Glucose-6-phosphate dehydrogenase	G-6-PD
Glutamic pyruvate transaminase	GPT
Phosphoglucomutase	PGM 2-1
6-phosphogluconate dehydrogenase	6-PGD
Transferrin	Tf

The distribution of each of these *polymorphs* in the population is well established. The determination of each of these additional factors narrows down the number of possible individuals.

isolated after partial hydrolysis: Gly-Cys, Phe-Val-Gly, Cys-Asp, Cys-Ala, Lys-Cys, and Cys-Asp-Lys. Now we match the fragments, deduce the amino acid sequence in the octapeptide, and write a primary structure for the peptide:

Cys-Asp Lys-Cys

Cys-Asp-Lys Cys-Ala

Gly-Cys

Phe-Val-Gly

Phe-Val-Gly-Cys-Asp-Lys-Cys-Ala

Step 8: Locating the disulfide linkages

Step 8 does not specifically deal with the primary structure of the protein, but it is related. If the disulfide linkages are left intact by skipping Step 2, different fragments result. x-ray diffraction analysis can locate each amino acid residue. This can be used to determine the overall shape of a protein. In some cases, more detailed structural information can be determined by sophisticated instrumental analysis techniques.

Chapter 6

Enzymes Kinetics: Getting There Faster

In This Chapter

- Understanding enzymes classification
- Examining kinetics
- Studying the Michaelis-Menten equation
- Comprehending enzyme inhibition and regulation

Enzymes are complex biological molecules, primarily or entirely protein, which behave as biological catalysts. As *catalysts,* they alter the rate of a chemical reaction without themselves being consumed in the reaction. Enzymes are normally very specific in their action, often targeting only one specific reacting species, known as the *substrate.*

This specificity includes *stereospecificity,* the arrangement of the substrate atoms in three-dimensional space. Stereospecificity is illustrated by the fact that if the D-glucose in your diet were replaced by its enantiomer, L-glucose, you would not be able to metabolize this otherwise identical enantiomer.

Enzymes occur in many forms. Some enzymes consist entirely of proteins, whereas others have non-protein portions known as *cofactors.* The cofactor may be a metal ion, such as magnesium, or an organic substance. We call an organic cofactor a *coenzyme* (there is no specific term for a metallic cofactor). An enzyme lacking its cofactor is an *apoenzyme,* and the combination of an apoenzyme and its cofactor is a *holoenzyme.* A metalloenzyme contains an apoenzyme and a metal ion cofactor. A tightly bound coenzyme is a prosthetic group. (Wow! We know that this is a lot of terminology, but hang in there. The key is the enzyme.)

One region on the enzyme, the *active site,* is directly responsible for interacting with the reacting molecule(s). When a reacting molecule, the substrate, binds to this active site, a reaction may occur. Other materials besides the enzyme and substrate, may be necessary for the reaction to occur.

In many cases, the cell initially produces the enzyme in an inactive form called a *proenzyme* or *zymogen,* which must undergo activation for it to function. The enzyme trypsin illustrates why it is sometimes necessary to generate an inactive form of an enzyme. Trypsin is one of the enzymes present in the stomach that is responsible for the digestion of proteins. Its production, as an inactive form, occurs in the cells of the stomach walls, and activation occurs after its release into the stomach. If trypsin were produced in the active form, it would immediately proceed to begin digesting the cell that produced it. Eating yourself is not a good thing.

The activation of the inactive form of an enzyme serves as one form of enzyme control. Inhibition is another method of enzyme control. The two general types of inhibition are competitive inhibition and noncompetitive inhibition. In *competitive* inhibition, another species competes with the substrate to interact with the active site on the enzyme. In *noncompetitive* inhibition, the other species binds to some site other than the active site. This binding alters the overall structure of the enzyme so that it no longer functions as a catalyst.

Enzyme Classification: The Best Catalyst for the Job

Ever wonder who gets to name chemicals? Well, the answer varies, but for enzymes it's the Enzyme Commission of the International Union of Biochemistry that's responsible. Common names for enzymes begin with some description of its action plus an *-ase* suffix. (Enzymes that were named before the implementation of the -ase system, such as trypsin, do not follow this convention.) The Enzyme Commission has also developed a numerical system for classifying enzymes. The names begin with EC, for Enzyme Commission, and end with four numbers, separated by decimal points, describing the enzyme. An example of this nomenclature is EC 2.7.4.4.

The first number in the EC name refers to the major enzyme *class*, and there are six major enzyme classes, summarized in Table 6-1. To continue with our example, the 2 in EC 2.7.4.4 designates the enzyme as a transferase. The second number, the 7, indicates what *group* the enzyme transfers. The third number, the first 4, indicates the *destination* of the transferred group. And the last number, the second 4, refines the information given by the third number.

Table 6-1 Six Basic Types of Enzymes

Class of Enzymes	*What They Catalyze*
Oxidoreductases	Redox reactions
Transferases	The transfer groups of atoms
Hydrolases	Hydrolysis
Lyases	Additions to a double bond, or the formation of a double bond
Isomerases	The isomerization of molecules
Ligases or synthetases	The joining of two molecules

Up one, down one: Oxidoreductases

Oxidoreductases catalyze a simultaneous oxidation and a reduction. An *oxidation* involves the increase in the oxidation state of an element, whereas a *reduction* involves the decrease in the oxidation state of an element. It is impossible to have one without the other. Examples of the types of reactions that qualify as oxidation and reduction reactions are in Table 6-2. In general, the substrate undergoes either oxidation or reduction, while the enzyme temporarily does the opposite but eventually returns to its original form.

Table 6-2 Some Possible Types of Oxidation and Reduction Reactions

Oxidation	*Reduction*
Loss of one or more electrons	Gain of one or more electrons
Addition of oxygen	Loss of oxygen
Loss of hydrogen	Gain of hydrogen

An example: Succinate dehydrogenase catalyzes the oxidation of the succinate ion. In this case, the oxidation involves the loss of two hydrogen atoms with the formation of a trans double bond. The enzyme alcohol dehydrogenase

removes two hydrogen atoms from an alcohol to produce an aldehyde. The general form, unbalanced, of these reactions is as follows:

$$^{-}OOCCH_2CH_2COO^{-} \xrightarrow{\text{succinate dehydrogenase}} {}^{-}OOCCH{=}CHCOO^{-}$$

$$CH_3CH_2OH \xrightarrow{\text{alcohol dehydrogenase}} CH_3CHO$$

You don't belong here: Transferases

The purpose of a transferase is to catalyze the transfer of a group from one molecule to another. Aminotransferase transfers an amino group, and phosphotransferase transfers a phosphoryl group. The general form, unbalanced, of these reactions appears in Figure 6-1.

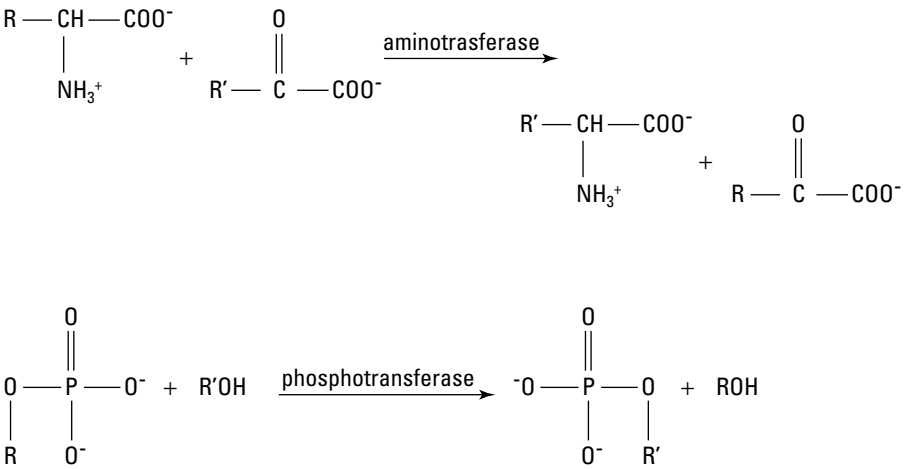
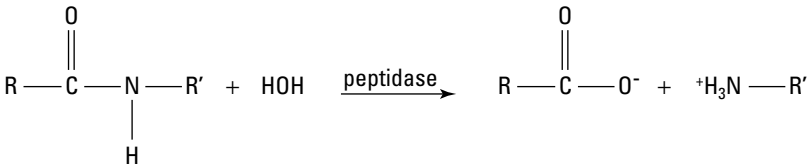


Figure 6-1: General form, unbalanced, of two transferase catalyzed reactions.

Water does it again: Hydrolases

Hydrolases catalyze the cleavage of a bond through the insertion of a water molecule (as an H and an OH). There may be a pH dependence, which results in the subsequent loss of a hydrogen ion. A *phosphatase* catalyzes the hydrolysis of a monophosphate ester, and a *peptidase* catalyzes the hydrolysis of a peptide bond. The general form of these reactions appears in Figure 6-2.



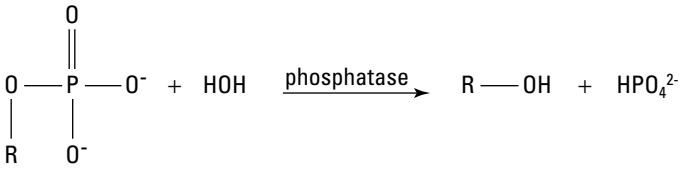
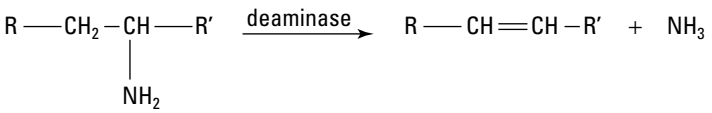


Figure 6-2: General form of two hydrolase catalyzed reactions.

Taking it apart: Lyases

Lyases catalyze the removal of a group. This process is accompanied by the formation of a double bond or the addition of a group to a double bond. A *deaminase* aids in the removal of ammonia, and a *decarboxylase* catalyzes the loss of CO_2. The general form of these reactions appears in Figure 6-3.



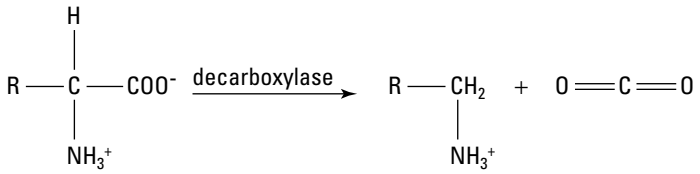


Figure 6-3: General form of two lyase catalyzed reactions.

Shuffling the deck: Isomerases

Racemase and epimerase are isomerases. *Isomerase* enzymes catalyze the conversion of one isomer to another. The *racemase* illustrated at the top of Figure 6-4 catalyzes the racemization of enantiomers. An *epimerase,* like the one at the bottom of Figure 6-4, catalizes the change of one epimer to another. Like all catalyzed reactions, these are equilibrium processes.

Putting it together: Ligases

Ligase enzymes catalyze reactions leading to the joining of two molecules in which a covalent bond forms between the two molecules. The process often utilizes high-energy bonds such as in ATP. Figure 6-5 illustrates the action of two ligases, pyruvate carboxylase and acetyl-CoA synthetase. *Pyruvate carboxylase* catalyzes the formation of a C-C bond. *Acetyl-CoA synthetase* catalyzes the formation of a C-S bond.

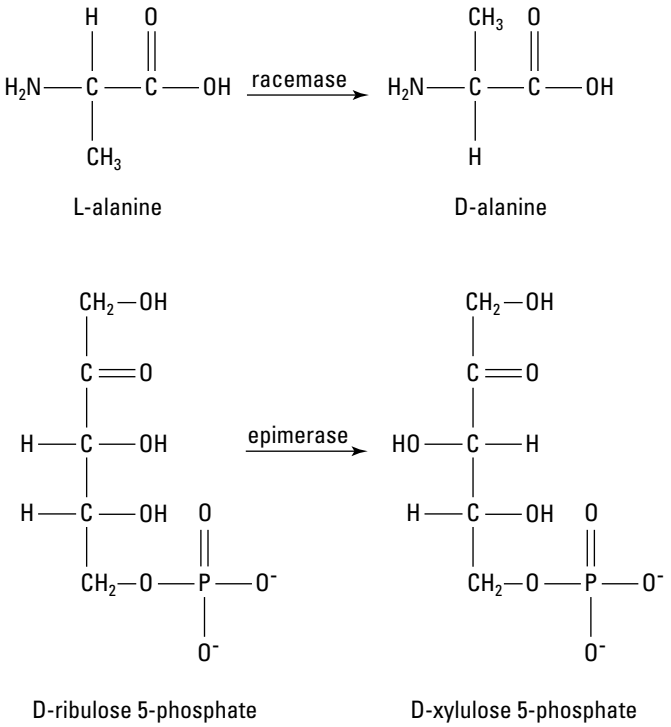


Figure 6-4: Examples of isomerase reactions catalyzed by a racemase and an epimerase.

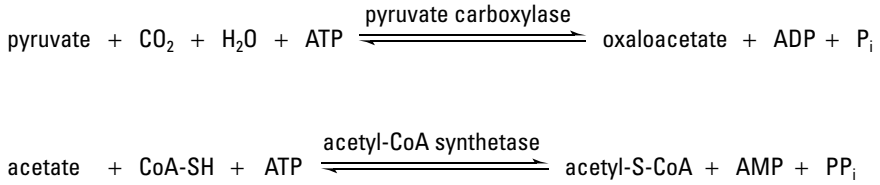


Figure 6-5: Reactions illustrating the action of the ligases pyruvate carboxylase and acetyl-CoA synthetase.

Enzymes as Catalysts: When Fast Is Not Fast Enough

The action of an enzyme begins with the formation of an enzyme-substrate complex. In this formation, the substrate in some way binds to the active site of the enzyme. The interaction between the enzyme and the substrate must, in some way, facilitate the reaction, and it opens a new reaction pathway.

The active site is typically a very small part of the overall enzyme structure. The amino acid residues comprising the active site may come from widely separated regions of the protein (primary structure), and it is only through interactions leading to higher structure levels that they are brought close together. Amino acid residues not in the active site serve many different functions that aid the function of the enzyme.

Models of catalysis: Lock and key versus induced-fit

The first attempt at explaining this process led to the *Lock and Key Model,* in which the substrate behaves as a key that fits into a lock, the enzyme (Figure 6-6). The Lock and Key Model, to a certain degree, explains the specificity of enzymes. Just as only the right key will fit into a lock, only the right substrate fits into the enzyme.

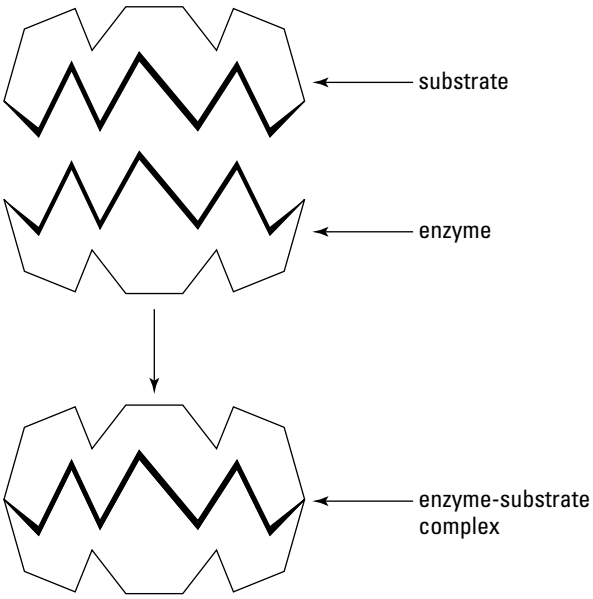


Figure 6-6: The Lock and Key Model of enzyme catalysis.

One limitation of the Lock and Key Model is that it does not explain why the reaction actually occurs, and another is that enzymes are flexible and not rigid as this theory implies.

The *Induced-Fit Model* overcomes some of the limitations of the Lock and Key Model. In this model, the substrate still needs to fit into the enzyme like a key, but instead of simply fitting into the "keyhole," some type of modification is induced in the substrate, enzyme, or both. The modification begins the process of the reaction. Figure 6-7 illustrates how the Induced-Fit Model applies to the formation of the same enzyme-substrate in Figure 6-6.

All About Kinetics

As you know, all reactions involve energy. The reactants begin with a certain level of energy, an additional quantity of energy is absorbed to reach the transition state (ΔG^*, where the asterisk indicates the transition state), and then energy is released to reach the products. The difference in the energy between the reactants and products is ΔG.

If the energy level of the products is greater than that of the reactants (energy is absorbed), the reaction is *endergonic,* and nonspontaneous. If the energy level of the products is less than the reactants (energy is released), the process is *exergonic,* and spontaneous.

But just because a reaction is spontaneous does not mean it will occur at an appreciable rate. The rate depends on the value of ΔG^*. The greater the value of ΔG^*, the slower the reaction is. An enzyme, like any catalyst, lowers the value of ΔG^* and consequently increases the rate of the reaction. The difference between the reactants and products remains unchanged, as does the equilibrium distribution of the reactants and products. The enzyme facilitates the formation of the transition state (Figure 6-8).

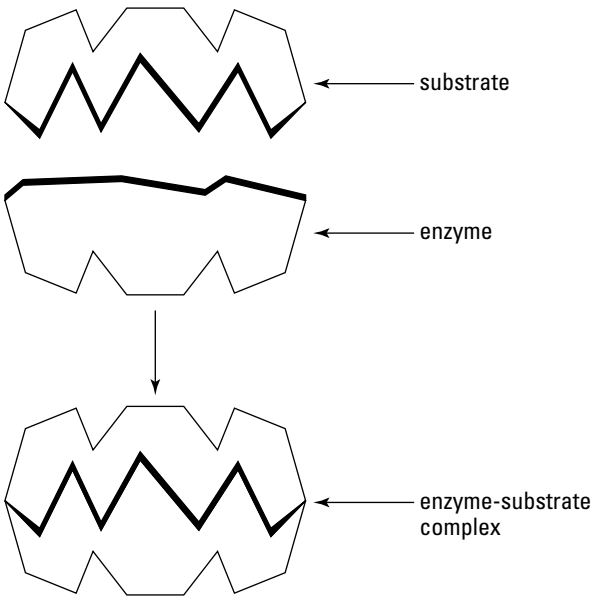


Figure 6-7: The Induced-Fit Model of enzyme catalysis.

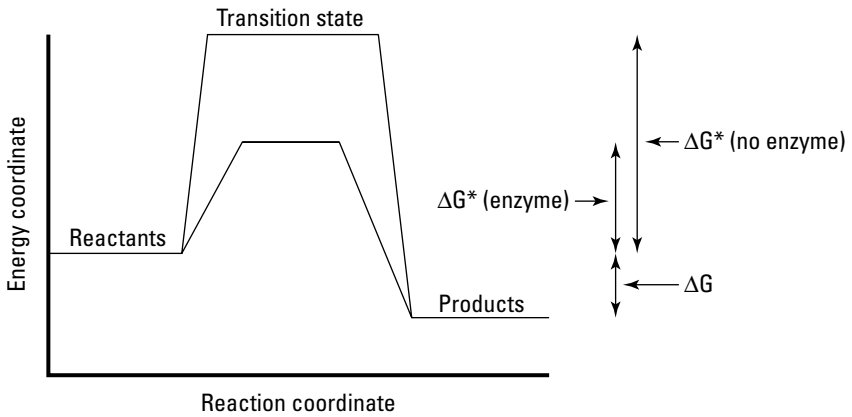


Figure 6-8: Effect of an enzyme on a reaction.

A species has two possible fates in the transition state: It may lose energy and return to the reactant form, or it may lose energy and move to the product form. These two fates lead to two equilibria. One of the equilibria involves the reactant (substrate) and the transition state, and the other involves the product(s) and the transition state. Rapid removal of the product(s) does not allow establishment of the reverse process that leads to the equilibrium. Removal of the product simplifies the analysis of the kinetic data.

Enzymes, like all catalysts, catalyze both the forward and the reverse reaction. The lowering of ΔG^* accelerates both reactions. The ultimate equilibrium concentrations of substrate and products will be the same whether an enzyme is present or not — the enzyme merely changes the amount of time necessary to reach this state.

Enzyme assays: Fixed time and kinetic

An *enzyme assay* is an experiment to determine the catalytic activity of an enzyme. It is possible to measure either the rate of disappearance of the substrate or the rate of appearance of a product. The experimental mode of detection depends on the particular chemical and physical properties of the substrate or the product, and the rate is the change in concentration per change in time. In *fixed time assay,* you simply measure the amount of reaction in a fixed amount of time. In *kinetic assay,* you monitor the progress of a reaction continuously. Once you determine the rate of change in concentration of any reactant or product, it is possible to determine the rate of change of for any other reactant or product of the reaction

It is important to control the conditions precisely. Minor changes in variables such as the temperature or the pH can drastically alter the catalytic activity of an enzyme. For example, the study of enzymes important to humans should be carried out at 37°C, because this is normal body temperature.

Rate determination: How fast is fast?

It is important to control kinetic experiments closely. Once you determine the basic conditions, you can run a series of experiments using a fixed enzyme concentration and varying concentrations of substrate. Up to a point, an increase in substrate concentration results in an increase in rate. The rate increases until the enzyme is saturated. This *saturation point* is where all the enzyme molecules are part of an enzyme-substrate complex. When this occurs, an increase in the substrate concentration yields no increase in the rate, because there are no enzymes available to interact with the

Enzymes in medical diagnosis and treatment

Enzyme levels may indicate medical problems, and that makes enzyme assays useful for both the diagnosis and treatment of medical problems. For examine, creatine kinese (CK) is an enzyme that aids in the synthesis and degradation of creatine phosphate.

CK exists as three different isoenzymes. Each is composed of two polypeptide chains. In the case of muscle CK, the chains are identical, and it's labeled CK-MM. CK found in the brain also has identical polypeptide chains, but they are different from the ones associated with muscle CK and are labeled CK-BB. Finally, the CK found in the heart is a hybrid of the two with one M chain and one B chain: CK-MB.

Normal blood serum contains a little CK-MM and almost no CK-BB and CK-MB. When tissue undergoes injury, though, some of the intracellular enzymes leak into the blood where they can be measured. Elevated levels of total CK (all three isoenzymes) may be indicative of sketal-muscle trauma or myocardial infarction (MI, or heart attack). Analysis of the individual isoenzymes may give additional clues.

For example, an individual falls off a ladder and suffers several broken bones. He is taken to the hospital, where his blood serum CK is measured. It is elevated as expected, but the physician also orders a CK-MB level determination. It turns out to also be highly elevated, indicating that the reason the man fell off the latter to begin with was that he was suffering a heart attack (CK-MB). This knowledge allows the doctor to start a regime of treatment that helps to minimize permanent heart damage.

additional substrate molecules. For most reactions, the rate of the reaction approaches the saturation level along a hyperbolic curve. Theoretically, the reaction rate will only reach saturation at infinite substrate concentration.

A plot of the reaction rate, V, versus the substrate concentration, [substrate], supplies several bits of useful data (see Figure 6-9). The experiment is at constant enzyme concentration. One piece of useful data is the maximum reaction rate, V_{max}. The rate approaches V_{max} asymptotically. At low substrate concentrations, the reaction approaches first-order kinetics, where the rate of reaction depends only on the concentration of one reactant. At high concentrations, the reaction approaches zero-order kinetics, where the rate of reaction is independent of reactant concentration. (Later in this chapter you will see that this graph varies with less simple enzyme-substrate interactions.) In the region between the zero-order region and the first-order region, the kinetics are mixed and difficult to interpret. Important values in the low-concentration region (first-order region) are $\frac{1}{2} V_{max}$ and K_M. The value $\frac{1}{2} V_{max}$ is one-half the V_{max} value. K_M is the Michaelis constant, which corresponds to the substrate concentration producing a rate of $\frac{1}{2} V_{max}$. The Michaelis constant, measured in terms of molarity, is a rough measure of the enzyme-substrate affinity. K_M values vary widely.

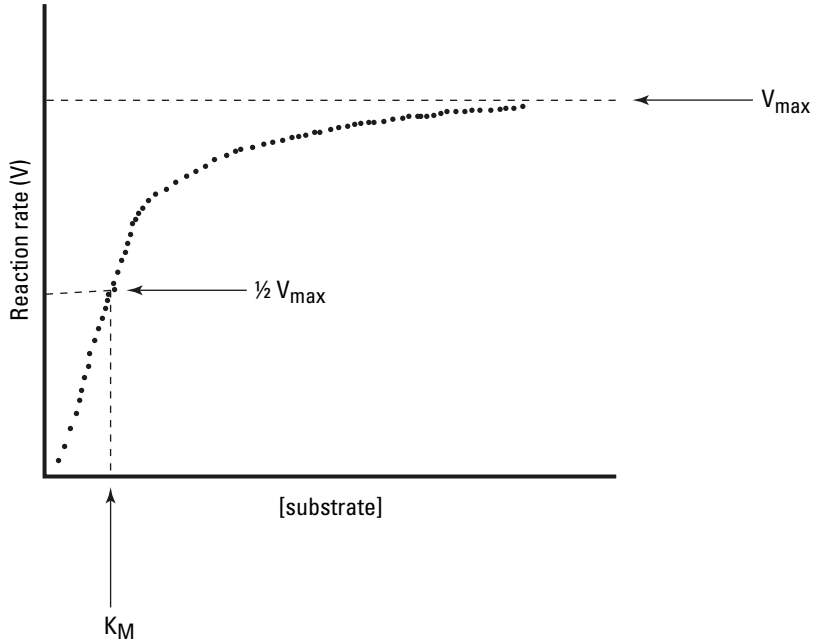


Figure 6-9: Plot of reaction rate, V, versus substrate concentration, [substrate].

At low substrate concentrations, there is an approximately linear relationship between [substrate] and V. At high substrate concentrations, though, V is nearly independent of [substrate]. The low substrate region is useful in the application of the Michaelis-Menten equation (see the next section).

In an uncatalyzed reaction, increasing the substrate concentration does not lead to a limiting V_{max}. The rate continues to increase with increasing substrate concentration. This indirect evidence leads to the conclusion that there is an *enzyme-substrate complex,* a tightly-bound grouping of the enzyme and the substrate. The limit occurs when all the enzyme molecules are part of a complex so that there are no free enzyme molecules available to accommodate the additional substrate molecules. Various x-ray and spectroscopic techniques provide direct evidence to confirm the formation of an enzyme-substrate complex.

Measuring Enzyme Behavior: The Michaelis-Menten Equation

One of the breakthroughs in the study of enzyme kinetics was the development of the Michaelis-Menten equation. It is possible to interpret the behavior of many enzymes by applying the equation to kinetic data. (There are exceptions, and they do not give a graph similar to the one appearing back in

Figure 6-9.) In general, the results of the kinetics experiments are for allosteric enzymes. The *Michaelis-Menten equation* is as follows:

$$V = \frac{V_{max}[S]}{[S] + K_M}$$

In this equation, V is the rate of the reaction, [S] is the substrate concentration, V_{max} is the maximum reaction rate, and K_M is the Michaelis constant. As seen in Figure 6-9, the rate of catalysis, V, increases linearly at low substrate concentration, but begins to level off at higher concentrations. Interpretation begins with examining the following general reaction pathway:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

In this pathway, E refers to the enzyme, S is the substrate, ES is the enzyme-substrate complex, and P is the product. The various instances of k refer to the rate constants of the various steps — a negative rate constant is for the reverse process. In the first step, the separate enzyme and substrate combine to form the enzyme-substrate complex (transition state). The rate of formation of ES is k_1. After ES forms, it may break down to E and S (k_{-1}) or it may proceed to product (k_2). (Note: Some texts refer to k_2 as k_{cat}.)

Because the enzyme will catalyze the reverse process, E and P may combine to reform the complex (k_{-2}). Ignoring the reverse reaction (k_{-2}) simplifies the interpretation of the data. This is not an unreasonable assumption if data collection is near the beginning of the reaction, where the concentration of P is low. The assumption that k_{-2} is negligible leads to a simplification of the preceding equation to:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Through this simplification, the chemists Leonor Michaelis and Maud Menten were able to propose a model that explains the kinetics of many different enzymes. Through their work, an expression relating the catalytic rate to the concentrations of the enzyme and substrate and to the individual rates was developed. The starting point for this expression is the relationship between the rate of the reaction and the concentration of the enzyme-substrate complex:

$$V = k_2[ES]$$

Similarly, the rate of formation of ES is $k_1[E]\,[S]$, and the rate for the breakdown of ES is $(k_{-1} + k_2)\,[ES]$. Throughout most of the reaction, the concentration of ES remains nearly constant. This is the steady-state assumption, which assumes that during a reaction the concentrations of any intermediates remain nearly constant. This assumption means that the rate of formation of ES must be equal to the rate of breakdown of ES, or:

$$k_1[E][S] = (k_{-1} + k_2)[ES]$$

This equation rearranges to:

$$\frac{[E][S]}{[ES]} = \frac{(k_{-1} + k_2)}{k_1} = K_M$$

The combination of the three rate constants yields a new constant: the Michaelis constant, K_M, which is independent of the enzyme and substrate concentrations and is an important characteristic of enzyme-substrate interactions. Using the Michaelis constant, the concentration of ES is:

$$[ES] = \frac{[E][S]}{K_M}$$

When the enzyme concentration is much lower than the substrate concentration, the value of [S] is very close to the total substrate concentration. The enzyme concentration, [E], is equal to the total enzyme concentration, $[E]_T$, minus the concentration of the enzyme-substrate complex, or $[E] = [E]_T - [ES]$. If we enter this relationship into the preceding equation, we get:

$$[ES] = \frac{([E]_T - [ES])[S]}{K_M}$$

Rearranging this equation gives:

$$[ES] = \frac{[E]_T / K_M}{1 + [S]/K_M} = \frac{[E]_T[S]}{[S] + K_M}$$

Substituting this relationship into $V = k_2[ES]$ or ($V = k_{cat}[ES]$) gives:

$$V = k_2[E]_T \frac{[S]}{[S] + K_M}$$

The maximum rate, V_{max}, occurs when all the enzyme molecules are associated with substrate. That is, $[ES] = [E]_T$. This changes $V = k_2[ES]$ to $V_{max} = k_2[ES]_T$. This relationship changes the preceding equation to the Michaelis-Menten equation:

$$V = \frac{V_{max}[S]}{[S] + K_M}$$

This equation accounts for the information depicted in Figure 6-9. At very low concentrations, $[S] << K_M$, we see $V = (V_{max} / K_M) / [S]$, and when [S] is greater than K_M (high [S]), $V = V_{max}$. When $[S] = K_M$ it leads to $V = V_{max} / 2$.

Ideal applications

The Michaelis-Menten equation explains the behavior of many enzymes. It is relatively easy to determine both the K_M and V_{max} values, and this is normally done graphically using computer programs that generate the best-fit curve.

The K_M values vary widely. The value depends on the identity of the substrate and on a variety of environmental factors such as temperature, ionic strength, and pH. Because K_M indicates the substrate concentration required

to fill half of the active sites on the enzyme, it gives an indication of the minimum substrate concentration for significant catalytic activity to occur. It is possible to determine the fraction of sites filled, f_{ES}, from the value of K_M:

$$f_{ES} = \frac{V}{V_{max}} = \frac{[S]}{[S] + K_M}$$

K_M also gives information about the rate constants for the reaction.

$$\frac{(k_{-1} + k_2)}{k_1} = K_M$$

In the special case where k_{-1} is significantly greater than k_2, $K_M = k_{-1} / k_1$, which relates to the equilibrium constant for the dissociation of the enzyme-substrate complex:

$$K_{ES} = \frac{[E][S]}{[ES]} = \frac{k_{-1}}{k_1}$$

Under these special conditions, K_M is a measure of the binding in the enzyme-substrate complex. A high K_M value indicates that the binding is weak, whereas a low value indicates that the binding is strong.

Don't forget: These conclusions only apply under the special conditions of $k_{-1} >> k_2$.

The value of V_{max} supplies the turnover number of the enzyme. The *turnover number* gives the number of substrate molecules transforming to products per unit of time for a fully saturated enzyme. You can determine k_2 from this value. (The constant k_2 is also known as the catalytic constant, k_{cat}.) If the concentration of active sites, $[E]_T$, is known, this relationship applies:

$$V_{max} = k_2[E]_T$$

And:

$$k_2 = V_{max} / [E]_T$$

Realistic applications

The ideas in the preceding section provide useful information about the behavior of many enzymes. In cells, however, the enzymes are seldom saturated with substrate. Under typical conditions [S] / K_M is usually between 1.0 and 0.01. If K_M is much greater than [S], the catalytic rate k_{cat} (or k_2) is significantly less than the ideal value because only a small portion of the active sites contain substrate. The ratio k_{cat} / K_M allows you to compare the substrate preferences of an enzyme.

The maximum rate of catalytic activity is limited by the rate of diffusion to bring the enzyme and substrate together. Some enzymes can exceed this limit by forming *assemblages.* In these groups, the product of one enzyme is the substrate for a closely associated enzyme. This allows a substrate to enter the group and pass from enzyme to enzyme as if it were in an assembly line.

Another complication is that many enzymes require more than one substrate. It is possible to utilize these multiple substrates through sequential displacement or through double displacement. In *sequential displacement,* all substrates must simultaneously bind to the enzyme before the release of the product. In this type of displacement, the order in which the substrates bind is unimportant. In *double displacement,* or *ping-pong,* situations, one or more products leave before all the substrates bind. Double displacement mechanisms temporarily modify the enzyme.

Here we go again: Lineweaver-Burk plots

Once upon a time, before the invention of computers, the determination of K_M and V_{max} was a tedious process. Today curve-fitting programs allow rapid analysis of the data to determine these values. However, a relatively simple method allows a relatively accurate determination of these two constants. This method is to construct a *Lineweaver-Burk plot,* also known as a *double-reciprocal plot.* The basis of a Lineweaver-Burk plot comes from the manipulation of the Michaelis-Menten equation to the form:

$$\frac{1}{V} = \frac{K_M}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

This equation has the form y = mx + b, and describes a straight line. A plot of the reciprocal of the rate, 1 / V, versus the reciprocal of the substrate concentration, 1 / [S], gives a line with a y-intercept equal to 1 / V_{max} and an x-intercept of –1 / K_M. An example of this type of plot appears in Figure 6-10.

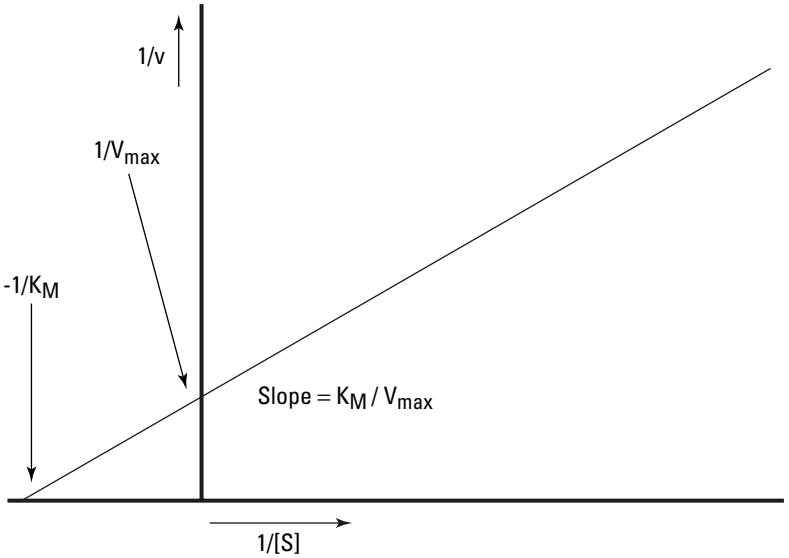


Figure 6-10: A Lineweaver-Burk plot.

The Lineweaver-Burk plot is the most widely used graphical technique for the determination of K_M and V_{max}. However, there are other methods. The Woolf plot, Figure 6-11, uses the equation:

$$\frac{[S]}{V} = \frac{1}{V_{max}} \times [S] + \frac{K_M}{V_{max}}$$

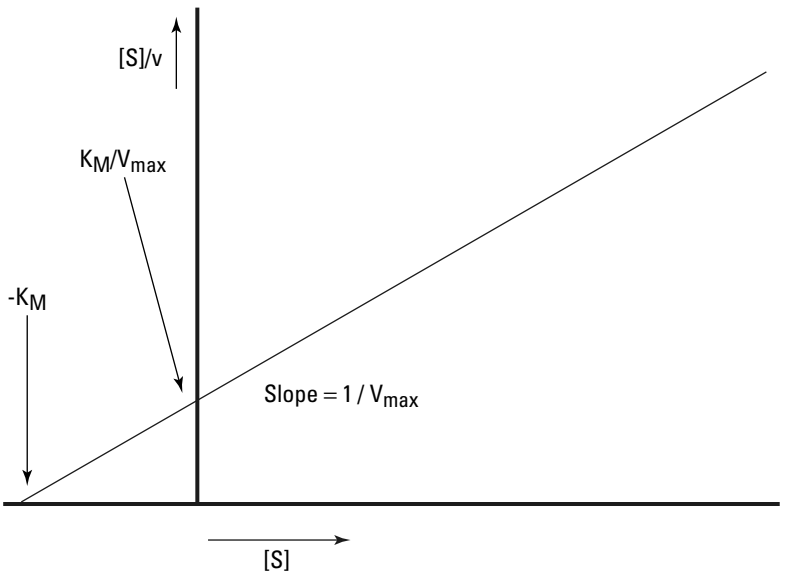


Figure 6-11: A Woolf plot.

Plotting [S] / V versus [S] gives a straight line. An Eadie-Hofstee plot, shown in Figure 6-12, uses the equation:

$$V = -K_M \times \frac{V}{[S]} + V_{max}$$

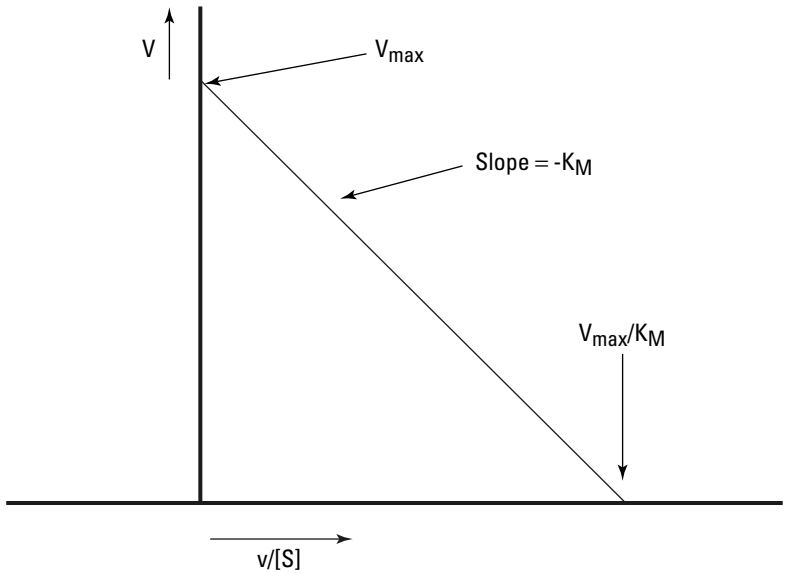


Figure 6-12: An Eadie-Hofstee plot.

Plotting V versus V / [S] gives a straight-line.

Enzyme Inhibition: Slowing It Down

Inibitors are substances that decrease the activity of an enzyme, and they come in two general classes: *competitive* inhibitors, which compete with the substrate, and *noncompetitive* inhibitors, which do not compete. (Mixed inhibition has characteristics of both competitive and noncompetitive inhibition.) In general, these processes are reversible, but there are also irreversible inhibiters that permanently alter the enzyme or bind very strongly to the enzyme. All inhibition may serve as a method of regulating enzymatic activity. There are also many medical applications of this form of inhibition. Examples include anti-epileptic and chemotherapy drugs, along with the ever-popular Viagra. The action of many poisons is also through inhibition.

Competititive inhibition

A competitive inhibitor enters the active site of an enzyme and, thus, prevents the substrate from entering. This prevention results in a decrease in the number of enzyme-substrate complexes that form, and, hence, a decrease in the rate of catalysis. In most cases, a portion of the inhibitor mimics a portion of the substrate. An increase in the substrate concentration overcomes this inhibition because of the increased probability of a substrate molecule entering the active site than an inhibitor molecule.

Noncompetitive inhibition

Noncompetitive inhibitors do not enter the active site but instead bind to some other region of the enzyme. These species usually do not mimic the substrate. This type of inhibitor reduces the turnover number of the enzyme. Unlike competitive inhibition, an increase in the substrate does not overcome noncompetitive inhibition. This type of inhibition takes many different forms, so there is no simple model.

Graphing inhibition

Lineweaver-Burk plots are useful in the study of enzyme inhibition. Figures 6-13 and 6-14 illustrate how the graph changes in the presence of a noncompetitive and a competitive inhibitor. The plot of enzyme inhibition allows us to quickly determine the type of inhibition. In noncompetitive enzyme inhibition, the value of K_M remains unchanged. In competitive inhibition, however, it is $1/V_{max}$ that remains unchanged.

Enzyme Regulation

In general, an increase in the concentration of a substrate, if unregulated, will induce an increase in the rate of reaction. An increase in the concentration of a product will, in general, have the reverse effect. Product regulation is a type of feedback control. In many cases, it is necessary to regulate the activity of enzymes more precisely. There are four general types of enzyme regulation:

- Allosteric control
- Multiple enzyme forms
- Covalent modification
- Proteolytic activation

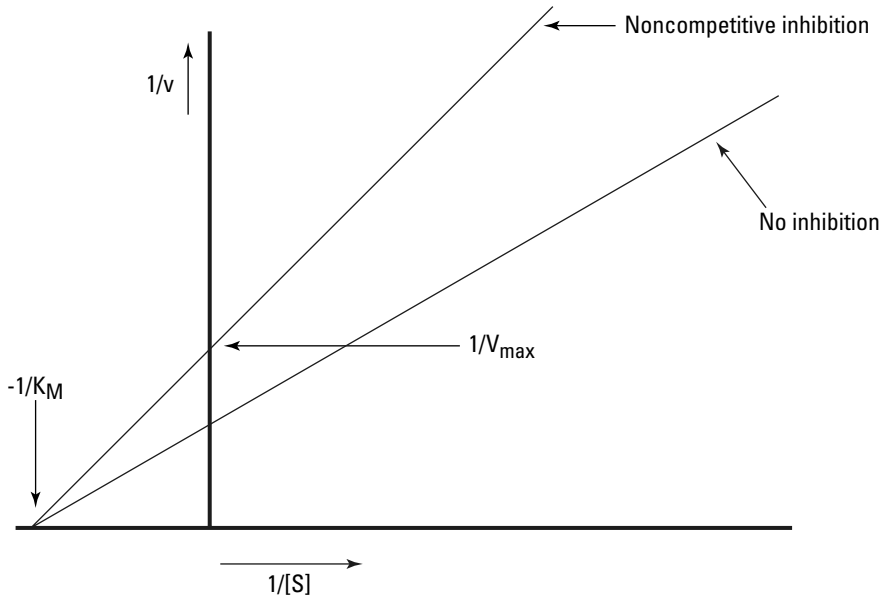


Figure 6-13: A Lineweaver-Burk plot indicating non-competitive inhibition.

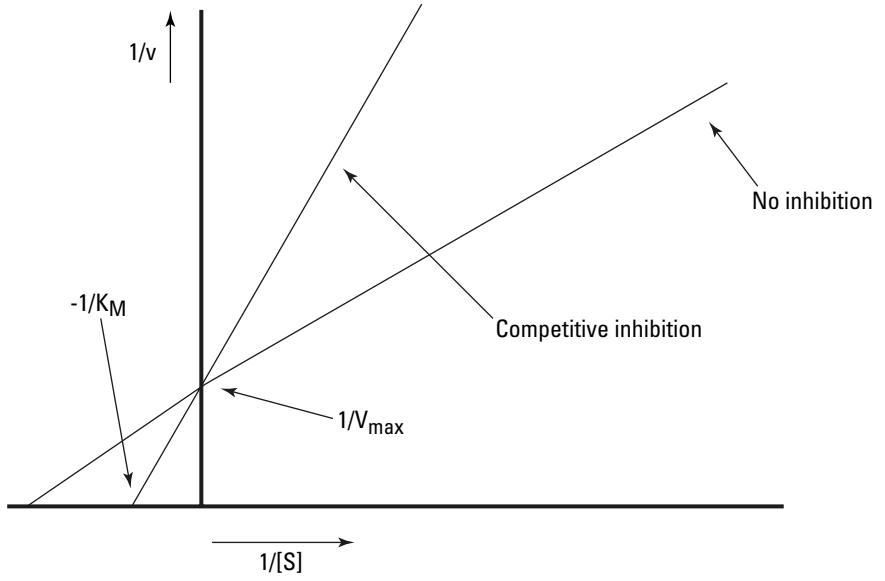


Figure 6-14: A Lineweaver-Burk plot indicating competitive inhibition.

Allosteric control

An allosterically regulated enzyme has a regulatory site. When a small molecule, called a *regulator,* binds to the regulatory site, it induces a conformational change in the enzyme, making it into its active form.

Multiple enzyme forms

Some enzymes have multiple forms known as *isozymes* or *isoenzymes.* There are slight differences in the structures of the forms. These differences lead to differences in the K_M and V_{max} values, and, therefore, in the general activity.

Covalent modification

In this form of regulation, the attachment of a group, often a phosphoryl group, alters the activity of the enzyme. This process is a reversible form of control. Protein kinases catalyze this type of activation, whereas other enzymes catalyze deactivation.

Proteolytic activation

In this form of regulation, an inactive form of an enzyme — a proenzyme or a zymogen — often undergoes irreversible conversion to the active form, often through the hydrolysis of one or more peptide bonds.

Where the money is: Enzymes and industry

The industrial implementation of enzymes originated from studies in the food, wine, and beer industries. Scientists, such as Louis Pasteur, laid much of the groundwork for these applications.

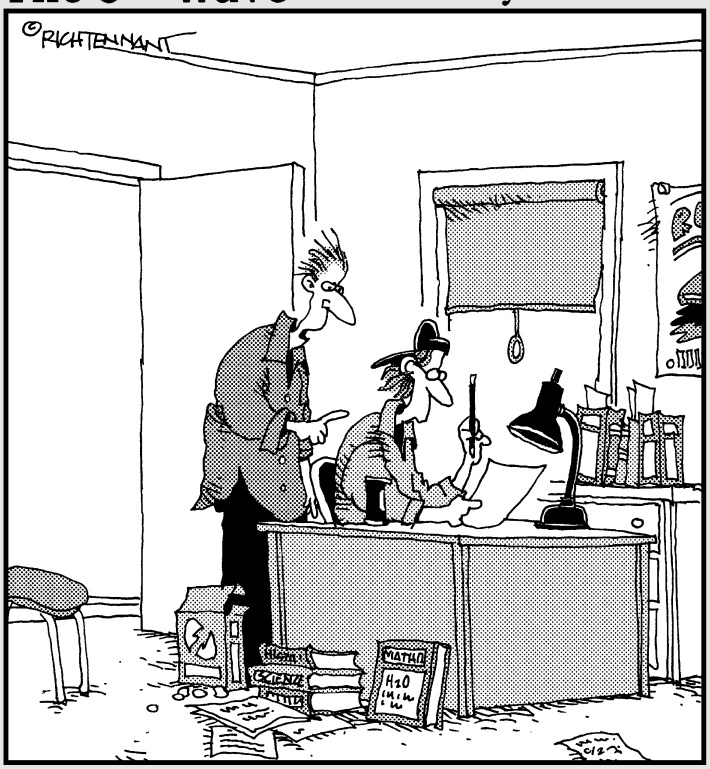
Many of the applications of enzymes to industry involve immobilized enzymes. An *immobilized enzyme* is covalently bonded to an insoluble matrix such as cellulose or glass beads. The immobilization of an enzyme stabilizes it and allows prolonged use. Some useful commercial enzymes are as follows:

Carbohydrases
Amylase: Digestive aid for precooked food
Amyloglucosidase: Converts starch to dextrose
Cellulase and hemicellulase: Conversion of sawdust to sugar and production of liquid coffee concentrates
Glucose isomerase: Production of fructose from cornstarch
Glucose oxidase: Removes glucose from egg solids
Invertase: Stabilizes sugars in soft-centered candy
Lactase: Prevents the crystallization of lactose in ice cream
Pectinase: Clarifies wine and fruit juice
Catalase
Removes H_2O_2 used in the "cold pasteurization" of milk
Proteases
Rennin: Used in cheesemaking
Ficin, Streptodornase, and Trypsin: Debridement of wounds
Pepsin: Digestive aid for precooked food
Papain: Meat tenderizer and beer stabilizer
Bromelain: Meat tenderizer
Alcalase: Additive to detergent for removal of protein stains
Lipoxygenase: Whitening of bread
Lipase: Produces flavor in cheese

Part III

Carbohydrates, Lipids, Nucleic Acids, and More

The 5th Wave By Rich Tennant



"Your formula for a carbohydrate is close, but not entirely accurate. I'm pretty sure carbohydrates consist of carbon, hydrogen, oxygen, sour cream, and bacon bits."

In this part . . .

We go over many biochemical species. Beginning with carbohydrates, we move on to perhaps less tasty-sounding fare: lipids and steroids. Next up: nucleic acids and that amazing encyclopedia about you that sits on the shelf inside every one of your cells: the genetic code of life, guest starring DNA and RNA. After that we end up talking about vitamins and hormones.

Chapter 7

What We Crave: Carbohydrates

In This Chapter

- Finding out about carbohydrates
- Checking out monosaccharides
- Reviewing oligosaccharides

Admit it: You love your carbohydrates. From simple sugars to complex carbohydrates, a day without carbs is a boring day. And carbs are plentiful: In terms of mass, carbohydrates are the most abundant biochemical.

Carbohydrates are a product of photosynthesis, where inorganic carbon dioxide becomes organic carbon with the utilization of solar energy, accompanied by the release of oxygen gas. The conversion of solar energy to chemical energy produces carbohydrates, which are the primary energy source for metabolic processes. Carbs are not only an important energy source but also are the raw materials for the synthesis of other biochemicals. They have structural uses and are a component of nucleic acids.

The term carbohydrate originally referred to "hydrates of carbon" because the general formula of these compounds was $C_nH_{2n}O_n$ or $C_n(H_2O)_n$. However, some materials with this general formula are *not* carbohydrates, and some carbohydrates do not have this general formula. It is better (though not much more conversational) to define carbohydrates as *polyhydroxyaldehydes and polyhydroxyketones and their derivatives*.

Natural carbohydrates are subdivided into *monosaccharides,* or simple sugars containing three to nine carbon atoms, *polysaccharides,* or polymers of monosaccharides, and an intermediate category of *oligosaccharides,* with two to ten monosaccharide units joined. The most important oligosaccharides to humans economically and biologically are the *disaccharrides.*

Properties of Carbohydrates

In general, the names of most carbohydrates are recognizable by an *-ose* suffix. An *aldose,* for example, is a monosaccharide where the carbonyl group is an aldehyde, whereas in a *ketose* the carbonyl group is a ketone. Chemists also use roots referring to the number of carbon atoms. *Pentoses,* five-carbon atoms, and hexoses, six-carbon atoms, are very important. *Trioses, tetroses,* and so on are also found in nature. It is possible to combine these generic names to give terms such as *aldohexose* and *ketopentose.*

They contain one or more chiral carbons

Chiral carbons are those that have four different groups, atoms or groups of atoms, attached to them. Most carbohydrates contain one or more chiral carbons. For this reason, they are *optically active,* rotating polarized light in different directions and many times having different activity in biological systems. Fischer projections are useful in indicating the asymmetry around each of the chiral carbon atoms. Figure 7-1 illustrates the construction of a Fischer projection. In the Fischer projection, the vertical lines project back, and the horizontal lines project forward. There are two arrangements of groups around a chiral center: These arrangements are called *enantiomers* and represent nonsuperimposable mirror images, like left and right gloves. The enantiomers comprise a D/L pair, where the D form rotates polarized light to the right, and the L form rotates polarized light to the left.

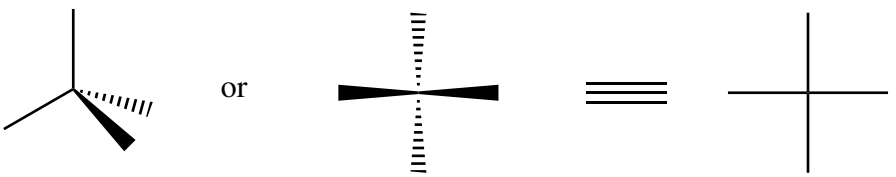


Figure 7-1: The relationship between the three-dimensional structure and the Fischer projection.

Fischer projections are not only useful for representing chiral carbons, but they are useful in identifying which enantiomeric form is present in a sample. To determine whether two projections are enantiomers or just simply two representations of the same molecule, it is necessary to compare the

two drawings. During this comparison, rotate one of the projections by 180° about an axis perpendicular to the plane of the paper (in other words, turn the paper while it's lying on a table). If the diagrams are identical after this rotation, then they are simply two representations of the same molecule. If the diagrams are not identical, they represent a pair of enantiomers.

They have multiple chiral centers

Because many carbohydrates have more than one chiral center (more than one chiral carbon), there can be more than two stereoisomers. The number of stereoisomers is 2^n, where n is the number of chiral carbons. For example, if the compound has two chiral carbons, there are a total of four stereoisomers — two pairs of enantiomers. Although the members of each pair are enantiomers, members of the different pairs are referred to as *diastereomers*.

The structure for D-glucose, a typical monosaccharide, appears in Figure 7-2. In this figure (a Fischer projection), all the carbon atoms except the ones at the top and bottom are chiral — a common way of representing monosaccharides. The carbon atoms appear as a vertical chain with the carbonyl carbon as near the top as possible (it is at the top for an aldose). Numbering the carbon atoms begins at the top, as indicated with the top carbon labeled C_1. The highest-numbered chiral carbon in this case is number five. By convention, if the –OH on this carbon atom appears on the right, it's the D form of the monosaccharide; if it is on the left, it's the L form.

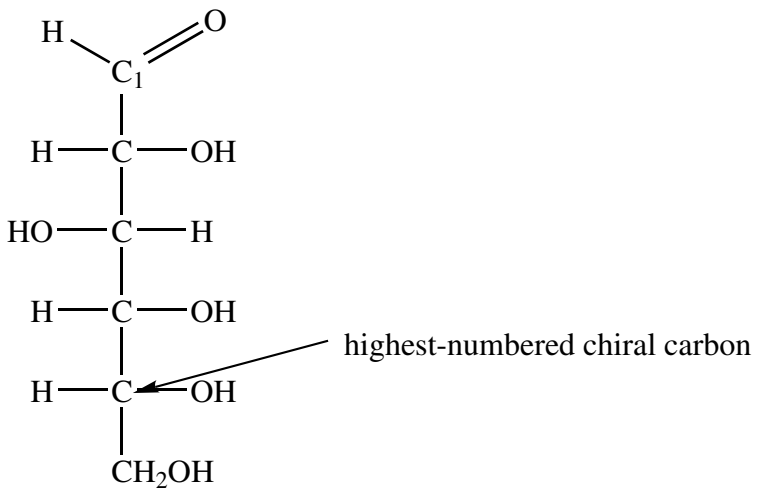


Figure 7-2: Structure of D-glucose.

Any change in the relative positions of the groups attached about any of the chiral carbon atoms in a Fischer projection produces either a different enantiomer or a diastereomer (assuming that the result is not simply a different way of drawing the original structure). In the case of D-glucose, with 4 chiral centers, there are 16 structures. One is D-glucose, and another is its enantiomer: L-glucose. The remaining 14 structures are diastereomers consisting of 7 enantiomeric pairs. Each of the enantiomeric pairs consists of a different monosaccharide. In the case of glucose, you have glucose, allose, altrose, mannose, gulose, idose, galactose, and talose, shown in Figure 7-3. The different D-ketohexoses are in Figure 7-4.

A Sweet Topic: Monosaccharides

The *monosaccharides,* or simple sugars, are an important class of biochemicals. For example, glucose, one of the most common monosaccharides, is the primary form of energy storage in the body. Most monosaccharides taste sweet. The relatively large number of hydroxyl groups and the polar carbonyl group mean that most of these compounds are water-soluble. And, as mentioned earlier, most are optically active.

The most stable monosaccharide structures: Pyranose and furanose forms

The most important monosaccharide is D-glucose (one form of D-glucose appears back in Figure 7-2). This form exists in equilibrium with two slightly different ring forms. The ring form results from an internal cyclization reaction, where a two groups on the same molecule join forming a ring. (The rings appear as planar structures even though the actual structures are not planar.) This cyclization involves a reaction between the carbonyl group and the highest-numbered chiral carbon, producing one of the following structures: a hemiacetal, an acetal, a hemiketal, or a ketal. In the case of D-glucose a pyranose ring forms. Haworth projection formulas are useful when representing the ring forms of a monosaccharide (Figure 7-5).

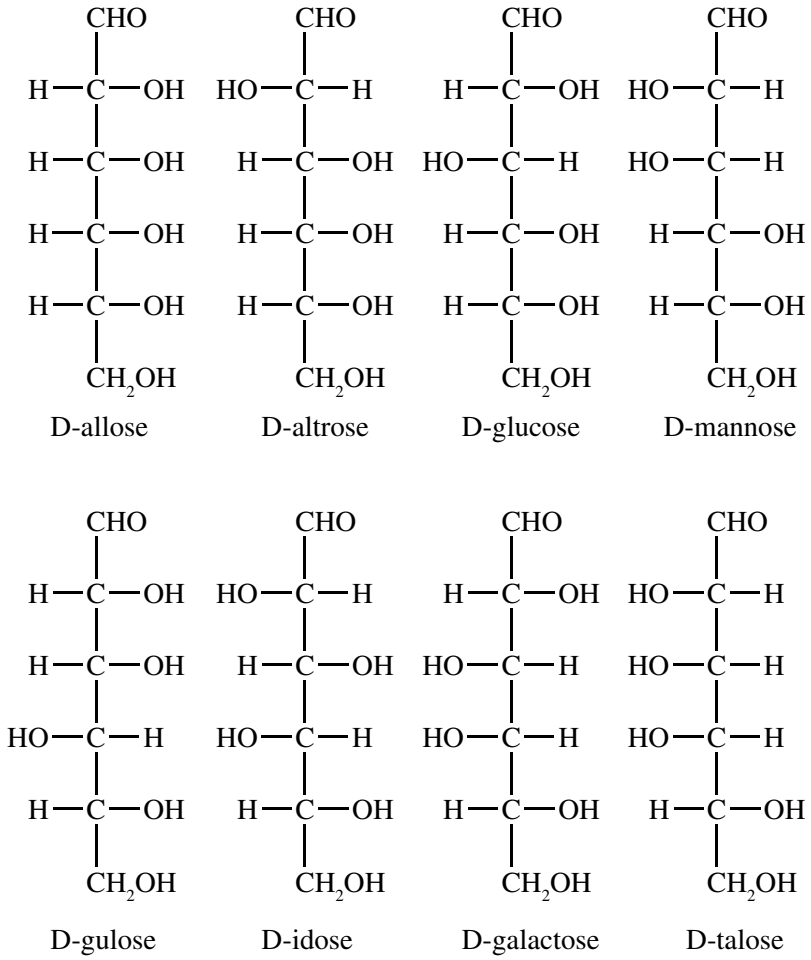


Figure 7-3: Structures of the D-aldohexoses.

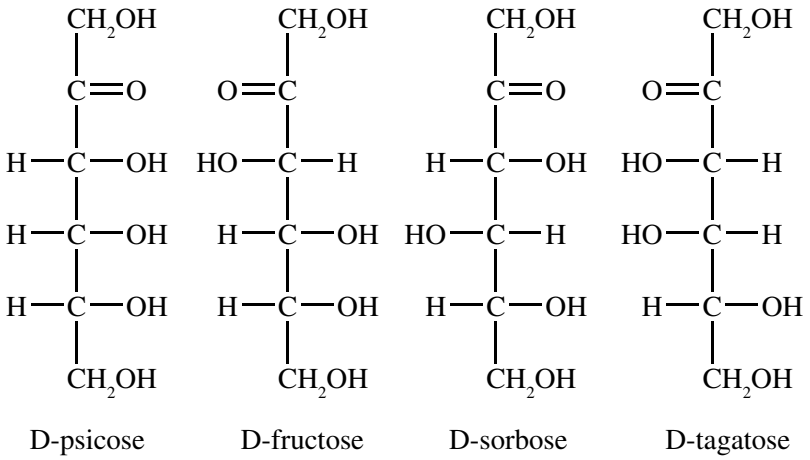


Figure 7-4: Structures of the D-ketohexoses.

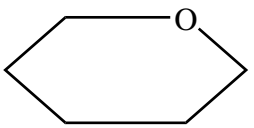


Figure 7-5: A pyranose ring.

There are two possible structures for the pyranose structure of D-glucose (see Figure 7-6), and other monosaccharides. If we examine the Fischer projection for D-glucose, we can see why:

- **Structure 1:** Hydroxyl group on one carbon in the up position.
- **Structure 2:** Hydroxyl group on the corresponding carbon in the down position.

If you "bend" the carbonyl group around and then allow a reaction with the highest numbered chiral carbon, you have two choices: right or left. This gives two forms known as *anomers.* The anomers are labeled α and β. The carbonyl carbon — C_1, in this case – is the anomeric carbon, which should be on the right side of a Haworth projection. The relative positions of –H and –OH about the anomeric carbon determine whether it is the α or β form. The hydroxyl group points down in the α form, and the hydroxyl group points up in the β form. (Reversing the drawing of the rings may give a structure with the opposite orientation of the groups about the anomeric carbon.) In solution, each of the anomers is in equilibrium with the open chain form represented by the Fischer projection. Therefore, there is an interconversion between the α and β forms known as *mutarotation.*

It is also possible to form a five-membered ring, called a *furanose* ring. A simplified furanose structure appears in Figure 7-7. Ribose is an example of a monosaccharide that may form a furanose ring.

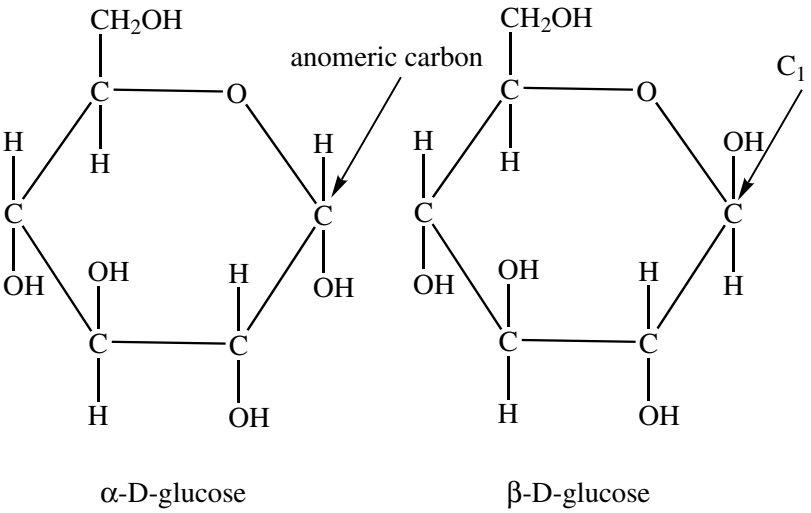


Figure 7-6: The Haworth projections for the pyranose structures of D-glucose.

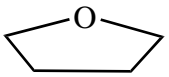


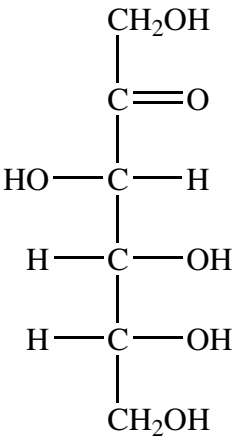
Figure 7-7: A furanose ring.

The pyranose and furanose forms are the thermodynamically more stable forms of the monosaccharides. In general, in the equilibria involving ring and open forms, less than ten percent of the molecules are in the open form. Fructose is a ketose that may form a furanose ring. Structures of D-fructose are shown in Figure 7-8.

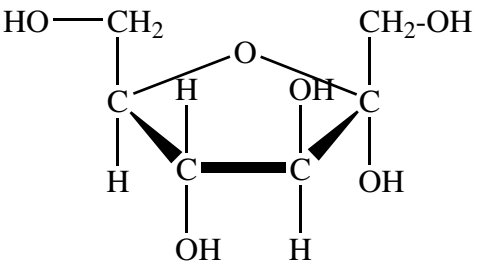
Chemical properties of monosaccharides

Many aldoses, because of the aldehyde group, are reducing sugars — that is, they are reducing agents in certain redox reactions. A number of tests for reducing sugars, include using Fehling's solution or Benedict's solution. These tests are useful to check for glucose in the urine of a diabetic.

The reaction of a monosaccharide with methanol, CH_3OH, in the presence of hydrochloric acid, HCl, replaces the hydrogen atom of the hydroxyl group on C_1 with a methyl group, forming a *glycosidic bond.* (Nitrogen may also be part of a glycosidic bond.) Once the glycoside forms, the ring is "locked," meaning it will not reopen; therefore, mutarotation will no longer take place. A formerly reducing sugar will no longer be a reducing sugar.



D-fructose



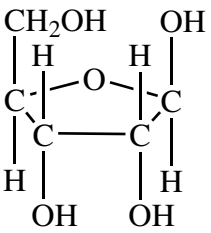
α-D-fructose

Figure 7-8: Two forms of D-fructose.

Derivatives of the monosaccharides

A variety of derivatives of the monosaccharides are formed through the alteration of one or more of the functional groups present. In this section we examine some of these derivatives using D-ribose as the parent monosaccharide. Two forms of the structure of D-ribose appear in Figure 7-9.

The reduction of the carbonyl group to an alcohol yields a reduced sugar (polyhydric alcohol). The reduction of D-ribose forms D-ribitol (Figure 7-10).



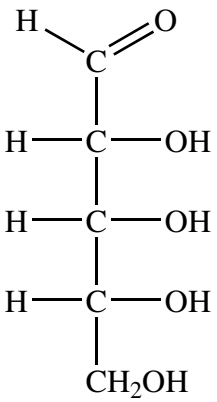


Figure 7-9: Two representations of the structure of D-ribose.

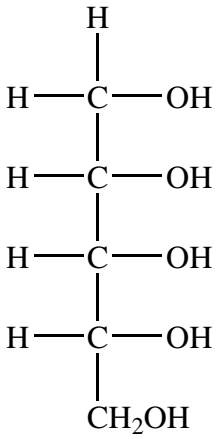


Figure 7-10: D-ribitol.

It is also possible to oxidize a monosaccharide to a carboxylic acid. There are two important oxidations: oxidation of an aldehyde (aldose) to an aldonic acid, and oxidation of the alcohol on the highest-numbered carbon atom to a uronic acid. In the case of D-ribose, it is possible to form D-ribonic acid (Figure 7-11) or D-ribouronic acid (Figure 7-12).

Monosaccharides, like all alcohols, may react with acids to form esters. The combination with phosphoric acid (phosphate sugar) is a biologically important reaction. Any of the alcohol groups may react. Figure 7-13 shows one example: D-ribose-1-phosphate. (The "1" refers to the attachment of the phosphate group to C_1.)

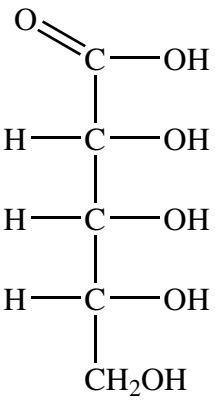


Figure 7-11: D-ribonic acid, an aldonic acid.

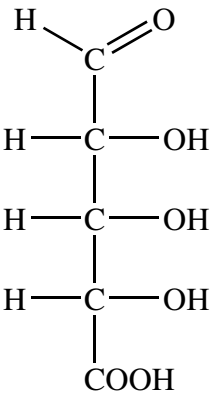


Figure 7-12: D-ribouronic acid, a uronic acid.

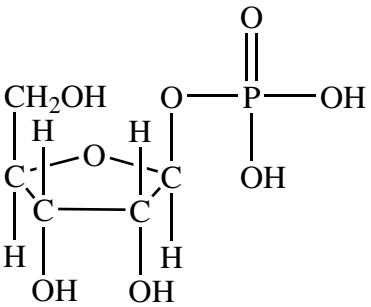


Figure 7-13: D-ribose-1-phosphate.

The most common monosaccharides

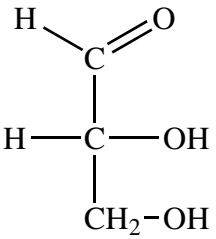
Glucose, or blood sugar, is also known as dextrose. The anomeric carbon is part of a hemiacetal, and the name of the pyranose structure is *glucopyranose.*

Blood is commonly tested for blood glucose levels, which are controlled by the hormone *insulin,* produced within the body in the pancreas. In a healthy human, blood glucose levels rise slightly after eating. The pancreas then releases insulin in order to keep the levels from rising too high. A healthy individual has a fasting blood sugar of 70–99 milligrams of glucose per deciliter of blood and 70–145 mg/dL two hours after eating. The American Diabetes Association associates blood glucose levels of 126 mg/dL (fasting) or 200 mg/dL (two hours after eating) with *diabetes* — the inability of the pancreas to produce enough insulin.

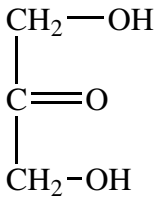
The simplest aldose is glyceraldehyde, and the simplest ketose is dihydroxy acetone. Figure 7-14 shows the structures of these two compounds.

The beginning of life: Ribose and deoxyribose

The monosaccharides D-ribose and D-deoxyribose are important components of the nucleic acids. They are present in these complex molecules in the form of a furanose ring. In addition, they are present as the β anomer. The difference between these two monosaccharides is that there is one less oxygen atom present in deoxyribose, hence the "deoxy." The "missing" oxygen atom is at C_2. The structures of these two sugars appear in Figure 7-15.

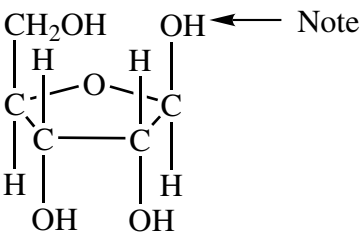


D-glyceraldehyde

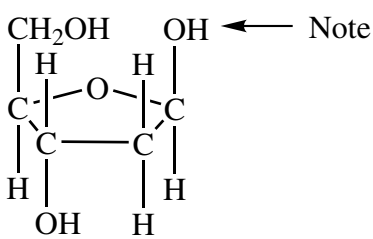


Dihydroxyacetone

Figure 7-14: Glyceraldehyde and dihydroxyacetone.



ribose



deoxyribose

Figure 7-15: The arrows point to the positions of the alcohol groups leading to these becoming the β anomers.

Sugars Joining Hands: Oligosaccharides

The joining of two or more monosaccharides forms an *oligosaccharide,* with two to ten monosaccharide units, or a *polysaccharide,* a polymer having many more monosaccharide units. One or more glycoside linkages hold the monosaccharides together. The simplest, and most common, oligosaccharides are the disaccharides.

Keeping it simple: Disaccharides

A *disaccharide* is an oligosaccharide composed of two monosaccharide units. The best-known disaccharide (and surely the most well liked) is probably sucrose, which you know as table sugar or cane sugar. Each molecule of this sugar is a combination of a glucose molecule and a fructose molecule. There are many other important disaccharides — among them, maltose, malt sugar, and lactose, milk sugar, each of which contains two molecules of glucose. Due to its simplicity,where two identical monosaccharides are joined, we will use maltose to illustrate several points concerning disaccharides, and, by implication, other oligosaccharides and polysaccharides. The structure of maltose appears in Figure 7-16.

The oxygen atom joining the two glucose rings of the maltose molecule in Figure 7-16 is a glycoside linkage — an α(1-4) linkage. The α refers to the anomeric form of the ring on the left. If β-D-glucose were present instead, then lactose would result (see Figure 7-17). The 1-4 indicates that C_1 of the left ring links to C_4 of the right ring. The loss of a water molecule accompanies the formation of the linkage, which locks the left ring so that mutarotation is no longer possible. The locked ring is also no longer a reducing sugar. But mutarotation can still occur on the right ring.

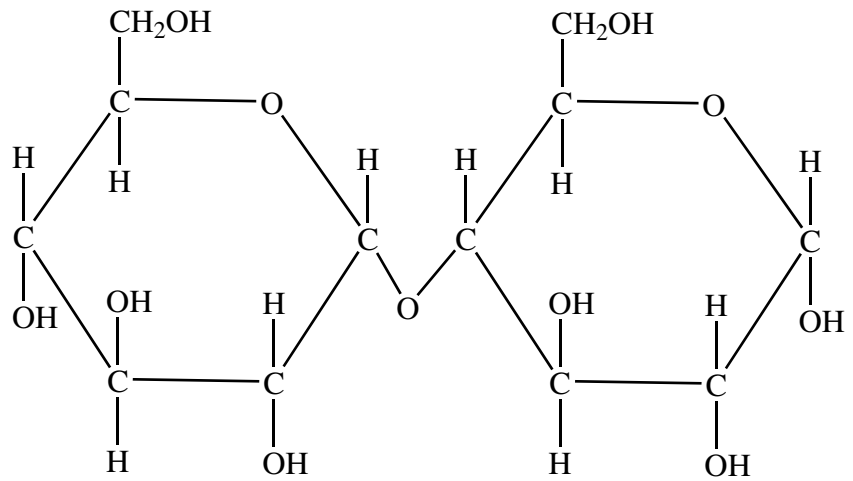


Figure 7-16: The structure of maltose with a α(1-4) linkage present.

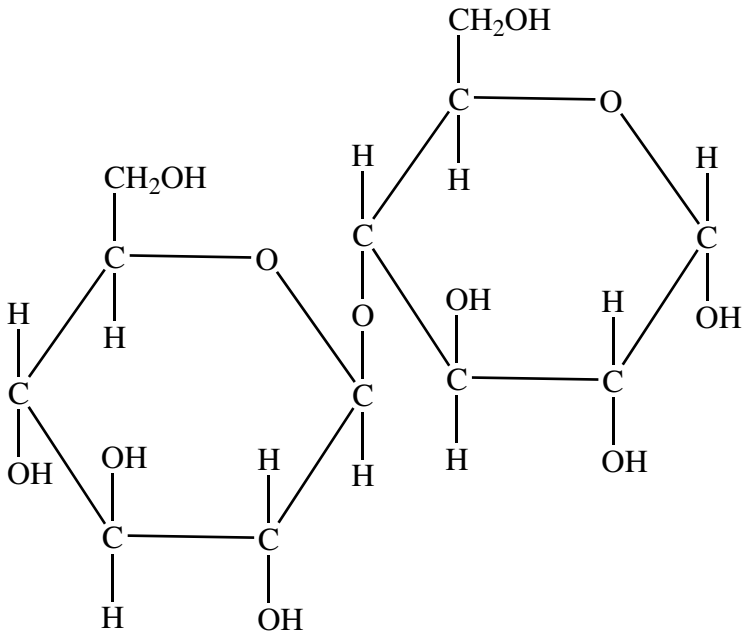


Figure 7-17: Lactose showing its β(1-4) linkage.

Sucrose, table sugar, is a disaccharide like maltose. It forms when D-glucose links to a D-fructose by a α(1-2) linkage. This situation locks both rings so that mutarotation of neither ring can occur. The formation of sucrose appears in Figure 7-18.

If the sweetness of sucrose is 100, then the sweetness level of glucose is 74, and that of fructose, 173. Fructose, found in corn syrup, is the sweetest common sugar — meaning you need less of it to make foods taste sweet. Less sugar translates to fewer calories. There are also naturally occurring, sweet-tasting proteins, some of which are hundreds of times sweeter than sugar.

Quite a few artificial sweeteners are used in commercial products. The best known are *saccharin* (about 500 times as sweet as sucrose, *aspartame* (200 times as sweet as sucrose), and *sucralose* (marketed as Splenda) — which is a whopping 600 times as sweet as sucrose. Sucralose is created by replacing three of the hydroxyl groups of sucrose with chlorines.

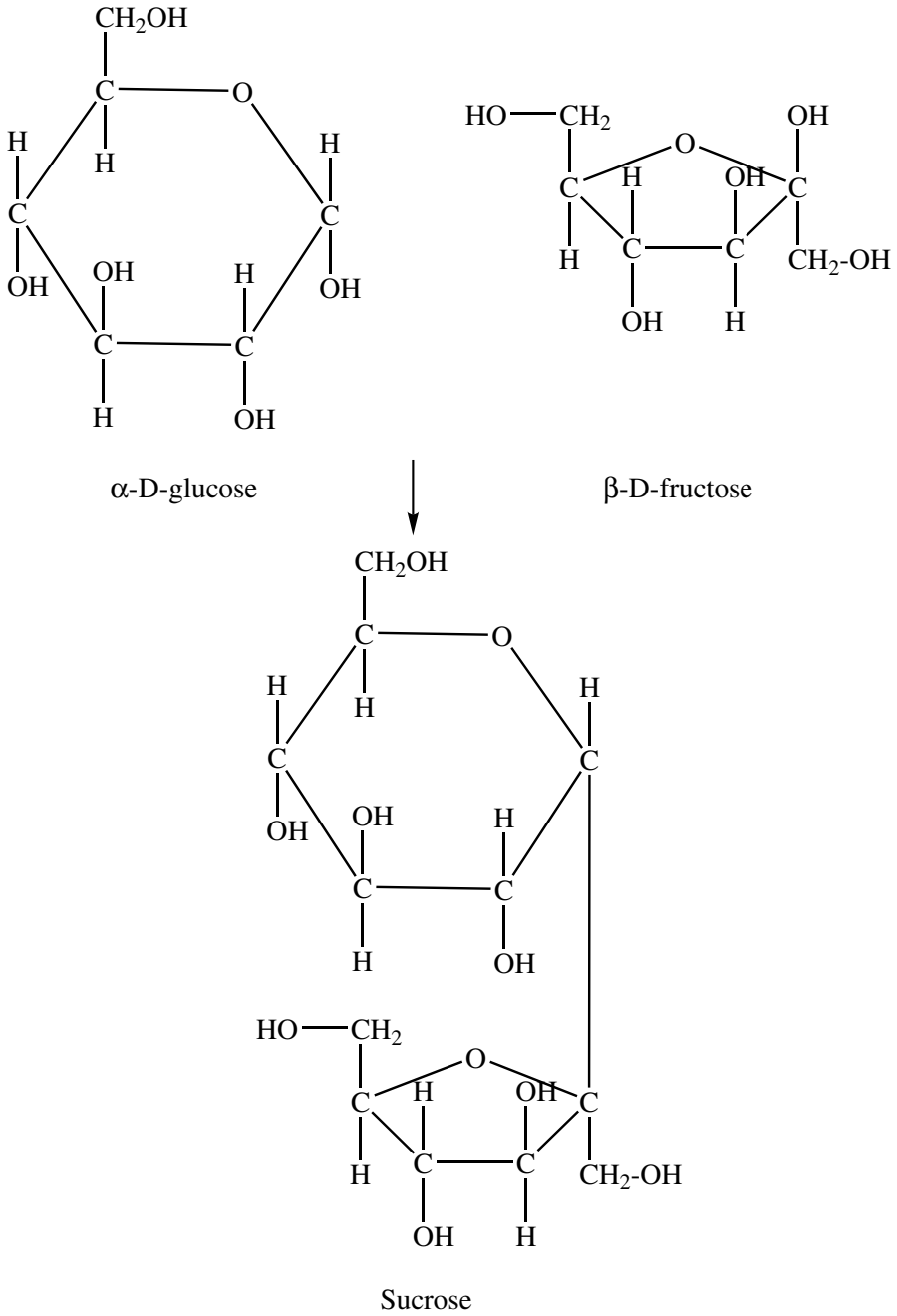


Figure 7-18: Structure of sucrose, formed by joining α-D-glucose and β-D-fructose.

Starch and cellulose: Polysaccharides

The two most important polysaccharides are starch and cellulose. Both of these are polymers of D-glucose. The basic difference between these two polymers is the linkages between the glucose units. Starch is related to maltose and cellulose is related to lactose.

Bread, pasta, and potatoes: Starches

Of all the carbohydrates, we think starches are our favorite. Bring on the potatoes and pastas! The different types of these lovely, delicious polysaccharides are very closely related by the linkages between their monomer units. *Starch* is a polymer of α-D-glucose. There are three common types of starch: amylase, amylopectin, and glycogen. Amylase is the combination of α(1-4) glucose groups. Amylopectin, like amylase, has α((1-4) glucose linkages, but, in addition, it has α((1-6) branches. Glycogen, animal starch, is similar to amylopectin except that it has more branches. All three are useful in storing glucose, and all three give an intense dark blue color in the presence of iodine — a simple and useful test.

Keeping the termites happy: Cellulose

Ever wonder why you can eat a potato but not a tree? *Cellulose* is similar to starch except that the linkages are β(1-4) glucose. The primary use of cellulose in nature is structure. Cleavage of the linkages is only possible with enzymes produced by certain bacteria or fungi. For this reason, only certain creatures, such as termites, and ruminants like cows, who have these bacteria in their GI tracts, can digest and utilize cellulose as an energy source. Cellulose is one of the most abundant biochemicals on earth.

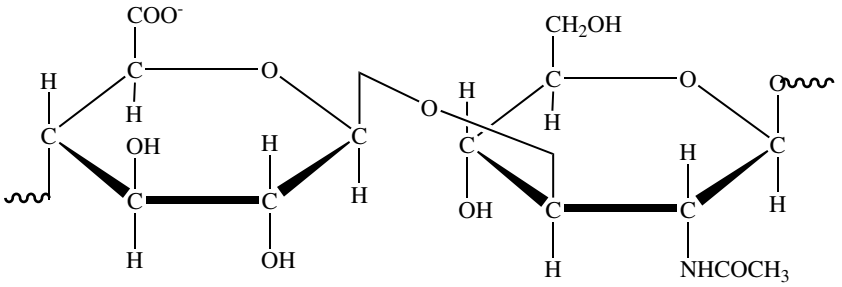
Biological connective tissue: Acidic polysaccharides

One of the major uses of polysaccharies in the body is the area of connective tissues, the compounds that hold our parts together. This group of tissue includes tendons, ligaments, and collagen. (Fuller lips, anyone?) Acidic polysaccharides are important to the structure and function of connective tissue. The repeating units of these polysaccharide derivatives are disaccharides. One of the components of the disaccharide is an *amino sugar* (where an amino group substitutes for an alcohol group). One or both of the components of the disaccharide unit have a negatively charged group (either a sulfate or a carboxylate). Examples are hyaluronic acid and heparin. The hyaluronate and heparin repeating units appear in Figure 7-19.

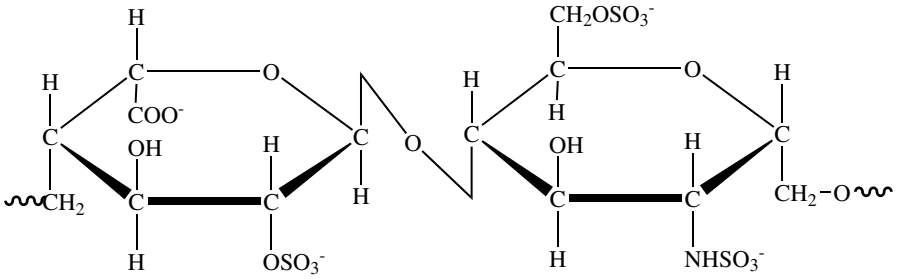
Heparin is used to treat and prevent blood clots from forming, especially in the lungs and legs. It is commonly used after dialysis, after surgery or when the patient has been unable to move for extended periods of time. It acts as an anticoagulant by binding to one of the anti-clotting proteins, increasing its efficiency up to a thousand-fold.

Glycoproteins

Most of the proteins occurring in blood serum are *glycoproteins,* which are proteins with carbohydrates attached. The presence of the carbohydrate tends to increase the hydrophilic nature of the protein. In general, the linkage is by attachment to an asparagine, serine, or threonine residue. Some soluble proteins and some membrane proteins are glycoproteins. We will see glycoproteins again at various times in later chapters.



Hyaluronate



Heparin

Figure 7-19: Disaccharide repeating units in hyaluronate and heparin.

Chapter 8

Lipids and Membranes

In This Chapter

- Living with lipids
- Examining triglycerides
- Finding out about membranes
- Seeing how steroids and other lipids operate

Along with cholesterol, lipids tend to have a bad reputation in today's world, even though they are absolutely necessary to good health. The *lipids* are an exceedingly diverse group of biologically important materials that are distinguished by solubility. A lipid is a member of a group of compounds that are not soluble (or only sparingly soluble) in water but that are soluble in nonpolar solvents or solvents of low polarity. The nonpolar nature of lipids is due to the fact that a large portion of the molecule contains only carbon and hydrogen. If there were significant amounts of oxygen or nitrogen in the structure, the substance would be more polar and hence more soluble in water.

Lovely Lipids: An Overview

Lipids have many important biological roles, including being highly concentrated energy sources, membrane components, and molecular signals. There are lots of kinds of lipids. Figure 8-1 provides a diagram showing the relationship between many of the different categories of lipids. Arachidonic acid, a fatty acid, appears in Figure 8-1 twice — once as the precursor (compound leading) to leukotrienes and prostaglandins and again as a member of the fatty acid group. We double-listed arachidonic acid this way because of its very different roles in these two chemical pathways.

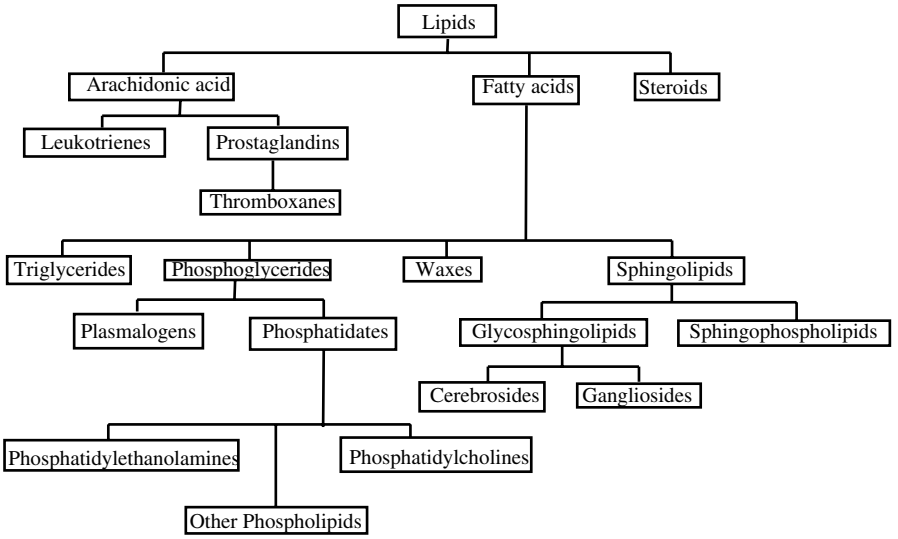


Figure 8-1: The relationships among the many types of lipids.

In the body, lipids provide energy storage and structure (cell membranes) and regulate bodily functions. Many of the lipids work like soap and detergents. Like soaps, lipids have a nonpolar region — usually a fatty acid — and a polar region. Figure 8-2 shows a representation of the structure of a soap.

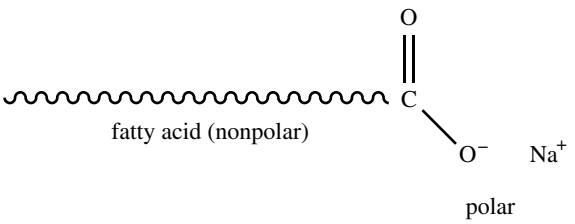


Figure 8-2: Representation of a soap.

In water, soap forms a *micelle,* (see Figure 2-3 in Chapter 2) in which the nonpolar portions of the different molecules coalesce and leave the polar portions on the outside next to the water. If there is any other nonpolar material present, such as grease from dirty dishes, it tends to migrate to the interior of the micelle. With the polar portions of the soap molecules on the outside, the micelle appears as one large polar molecule instead of a number of smaller molecules that have polar and nonpolar regions.

The *dual solubility* nature of soap is why it removes grease or oil from your skin or clothes. The grease or oil is nonpolar and, therefore, is not soluble in water. The soap forms a micelle that surrounds the grease/oil in the nonpolar portion of the micelle. The polar end of the soap micelle is soluble in water,

allowing the grease and oil to be removed during rinsing. Although many different types of lipids exist, our discussion in this chapter focuses on the following four types of lipids:

- **Fatty acids and derivatives (esters):** Fats, oils, and waxes
- **Complex lipids:** Phosphoglycerides and sphingolipids
- **Steroids**
- **Arachidonic acid devivatives:** Prostaglandins, thromboxanes, and eukotrienes

Lipids are important not only as individual molecules but also in terms of their interactions with other lipids and non-lipids in the formation of lipid bilayers or cell membranes. These interactions occur both at the cell boundary and around some interior structures. The fatty acids portions of the lipids are especially important in their physical and chemical properties. The naturally occurring fatty acids have a few key features:

- They are all straight-chained with generally 10–20 (but sometimes more) carbon atoms.
- They have an even number of carbon atoms.
- If carbon-carbon double bonds are present, only the cis-isomer is present.

Table 8-1 lists a few of the common fatty acids.

Table 8-1 Common Fatty Acids

Lauric acid	$CH_3(CH_2)_{10}COOH$
Myristic acid	$CH_3(CH_2)_{12}COOH$
Palmitic acid	$CH_3(CH_2)_{14}COOH$
Palmitoleic acid	$CH_3(CH_2)_5CH{=}CH(CH_2)_7COOH$
Stearic acid	$CH_3(CH_2)_{16}COOH$
Oleic acid	$CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$
Linoleic acid	$CH_3(CH_2)_3(CH_2CH{=}CH)_2(CH_2)_7COOH$
Linolinic acid	$CH_3(CH_2CH{=}CH)_3(CH_2)_7COOH$
Arachidonic acid	$CH_3(CH_2)_4(CH{=}CHCH_2)_4(CH_2)_2COOH$

A *wax* is a simple ester of a fatty acid and a long-chain alcohol. The fatty acid typically contains at least 10 carbon atoms, whereas the alcohol portion is typically 16–30 carbon atoms. In general, a wax, such as the wax in your ears, serves as a protective coating. Because they tend to be somewhat unreactive, we do not discuss waxes in much detail in this book.

A Fatty Subject: Triglycerides

Fats (and oils) are *triglycerides* or *triacylglycerols.* That is, they are triesters of fatty acids with glycerol. *Glycerol* is a trihydroxy alcohol (see Figure 8-3). In a fat, each of the three alcohol groups becomes part of an ester through the reaction with a fatty acid. The fatty acids may or may not be the same.

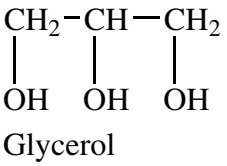


Figure 8-3: Structure of glycerol.

Properties and structures of fats

The basic difference between a fat and an oil is that a fat is a solid at room temperature and an oil is a liquid. That said, two important structural factors distinguish a fat from an oil. One is the size of the fatty acids, and the other is the presence or absence of double bonds. The longer the fatty acid chain, the higher the melting point. The greater the number of carbon-carbon double bonds, the lower the melting point.

A *saturated* fat consists of fatty acids with no carbon-carbon double bonds. An *unsaturated* fat has a double bond while a *polyunsaturated* fat has multiple double bonds.

There are more than 70 known naturally occurring fatty acids. In most natural fats, there is a cis geometry about the double bonds. The presence of double bonds puts "kinks" in the carbon chain, which prevent the fatty acid chains from stacking together as roughly parallel chains. The inability of unsaturated fatty acid chains to stack together inhibits the fat's ability to solidify.

The treatment of an unsaturated fat or oil with hydrogen in the presence of a catalyst such as nickel will lead to hydrogenation of some or all of the carbon-carbon double bonds, forming carbon-to-carbon single bonds. This procedure changes an unsaturated fat into a saturated fat. In most cases, only

partial hydrogenation takes place, and the hydrogenation raises the melting point of the compound. By this procedure, it is possible to convert an oil (liquid) into a fat (solid). Incomplete hydrogenation may change some of the cis arrangements into trans arrangements, producing a *transfat.*

Figure 8-4 shows the structure of a typical fat. Note that that the two upper fatty acid chains (saturated) "stack" next to each other, but the lower chain (unsaturated) does not.

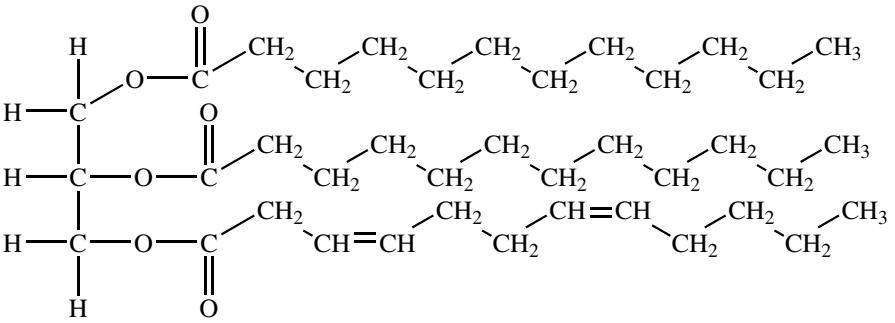


Figure 8-4: Structure of a typical fat: Upper chains are saturated, bottom chain is unsaturated.

Cleaning up: Breaking down a triglyceride

For centuries, the treatment of a fat (commonly animal fat) with a strong base catalyst (generally lye — sodium hydroxide) has been used to produce soap. John's grandmother made soap by boiling hog fat with wood ashes — which contain potassium and sodium hydroxides. She then skimmed off the soap and pressed it into cakes. Unfortunately, Granny wasn't very good at getting all the proportions just right and tended to use too much base, making the soap very alkaline. In this kind of reaction, called a *saponification reaction,* hydrolysis of the ester groups in the presence of a base yields glycerol and the carboxylate ions of the three fatty acids. A soap is really a sodium or potassium salt of a fatty acid. The calcium and magnesium analogues, on the other hand, are insoluble. If the soap is used with *hard* water (containing calcium or magnesium ions), it precipitates as a greasy scum: bathtub ring.

Acids also catalyze the hydrolysis of a fat to produce glycerol and a fatty acid. Acid hydrolysis is reversible, whereas the presence of excess base inhibits the reverse of saponification. During digestion, lipases break down triglycerides, and bile salts make the fatty acid portions soluble. A *lipase* is an enzyme that catalyses the decomposition of a fat. *Bile salts* are oxidation products of cholesterol that act as detergents to make the products of the breakdown soluble. In humans, absorption of the products occurs in the small intestine.

No Simpletons Here: Complex Lipids

So far, we have been discussing simple lipids. However, some lipids are somewhat more complex. In general, complex lipids are esters of glycerol or some other alcohol. The two major categories of complex lipids are the phosphoglycerides and the sphingolipids. The *phosphoglycerides* are the plasmalogens and the phosphatidates. The *sphingolipids* are the glycosphingolipids and the sphingophospholipids. (Further subdivision is shown back in Figure 8-1.) A phospholipid is either a phosphoglyceride or a sphingophospholipid. Phospholipids are major components of membranes. Any carbohydrate-containing lipid is a glycolipid. The classifications of lipids overlap. (As you may have noticed, nothing in biochemistry is ever truly simple.) For this reason, a lipid may fall into more than one subcategory.

Phosphoglycerides

The alcohol here is glycerol, to which two fatty acids and a phosphoric acid are attached as esters. This basic structure is a phosphatidate. *Phosphatidate* is an important intermediate in the synthesis of many phosphoglycerides. The presence of an additional group attached to the phosphate allows for many different phosphoglycerides.

By convention, structures of these compounds show the three glycerol carbon atoms vertically with the phosphate attached to carbon atom number three (at the bottom). The occurrence of phosphoglycerides is almost exclusive to plant and animal cell membranes. Plasmalogens and phosphatidates are examples. These are also known as glycerophospholipids.

Plasmalogens

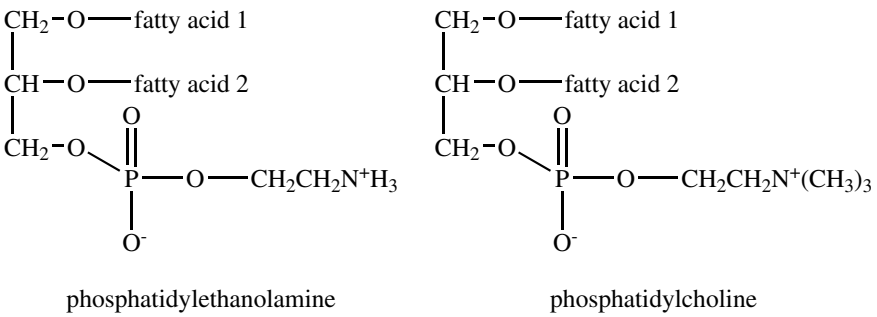
Plasmalogens are a type of phosphoglyceride. The first carbon of glycerol has a hydrocarbon chain attached via an ether, not ester, linkage. Ether linkages are more resistant to chemical attack than ester linkages are. The second (central) carbon atom has a fatty acid linked by an ester. The third carbon links to an ethanolamine or choline by means of a phosphate ester. These compounds are key components of the membranes of muscles and nerves.

Phosphatidates

Phosphatidates are lipids in which the first two carbon atoms of the glycerol are fatty acid esters, and the third is a phosphate ester. The phosphate serves as a link to another alcohol — usually ethanolamine, choline, serine, or a carbohydrate. The identity of the alcohol determines the subcategory of the phosphatidate. There is a negative charge on the phosphate and, in the case of choline or serine, a positive quaternary ammonium ion. (Serine also has a negative carboxylate group.) The presence of charges gives a “head” with an

overall charge. The phosphate ester portion ("head") is hydrophilic, whereas the remainder of the molecule, the fatty acid "tail", is hydrophobic. These are important components for the formation of lipid bilayers.

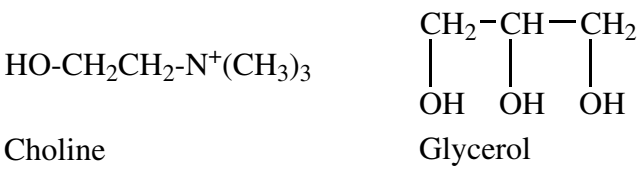
Phosphatidylethanolamines, phosphatidylcholines, and other phospholipids are examples of phosphatidates. Figure 8-5 illustrates examples of a phosphatidylethanolamine and a phosphatidylcholine.



phosphatidylethanolamine phosphatidylcholine

Figure 8-5: Examples of the general formulas of a phosphatidylethanolamine and a phosphatidylcholine.

The structures of some of the alcohols present in lipids appear in Figure 8-6.



Choline Glycerol

Ethanolamine Serine

Figure 8-6: Alcohol components of lipids.

Phosphatidylethanolamines

These are the most common phosphoglycerides in animals and plants. In animals, many of these are the *cephalins,* which are present in nerves and brain tissue. They are also factors involved in blood clotting. Recall that the phosphate has a negative charge and that the nitrogen of the enthanolamine is a quaternary ammonium ion with a positive charge.

Phosphatidylcholines

These are the lecithins. Choline is the alcohol, with a positively charged quaternary ammonium, bound to the phosphate, with a negative charge. Lecithins are present in all living organisms. An egg yolk has a high concentration of lecithins — which are commercially important as an emulsifying agent in products such as mayonnaise. Lecithins are also present in brain and nerve tissue.

Other phospholipids

There are many other phospholipids, some of which are glycolipids. The glycolipids include phosphatidyl sugars where the alcohol functional group is part of a carbohydrate. Phosphatidyl sugars are present in plants and certain microorganisms. A carbohydrate is very hydrophilic due to the large number of hydroxyl groups present.

Sphingolipids

Sphingolipids occur in plants and animals, and are especially abundant in brain and nerve tissue. In these lipids, sphingosine (Figure 8-7) replaces glycerol. The alcohol groups in the sphingosine may form esters just like the similar groups on glycerol. The amino group can form an amide. The combination of a fatty acid and sphingosine, via an amide linkage, is a *ceramide,* which is an intermediate in the formation of other sphingolipids.

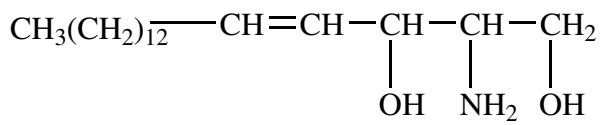


Figure 8-7: Structure of sphingosine.

Glycosphingolipids

A *glycosphingolipid* is an important membrane lipid containing a carbohydrate attached to a ceramide. The carbohydrate serves as a polar (hydrophilic) head. The carbohydrate may be either a monosaccharide or an oligosaccharide. The carbohydrate sequence in the oligosaccharide is important in helping these compounds recognize other compounds in biochemical reactions sequences. The carbohydrate portion is always on the outside of the membrane.

Cerebrosides

A *cerebroside* consists of a monosaccharide attached to a ceramide. The carbohydrate is either glucose or galactose. Cerebrosides are present in nerve and brain cells, though most animal cells contain some of these compounds.

Gangliosides

Gangliosides are sphingolipids with complex structures. The ceramide has an oligosaccharide, containing three to eight monosaccharide units, attached. The monosaccharide units may or may not be substituted. They are very common as part of the outer membranes of nerve cells, where the sugar sequence leads to cell recognition and communication. Small quantities of gangliosides are part of the outer membranes of other cells. When present in a membrane, the carbohydrate portion is always extracellular.

Sphingophospholipids

Sphingophospholipids contain sphingosine, a fatty acid, phosphate, and choline. An example is sphingomyelin, which is an important constituent of the myelin sheath surrounding the axon of all nerve cells. Multiple sclerosis, among other diseases, is a consequence of a fault with the myelin sheath. Sphingomyelin is the most common of the sphingolipids, and it is the only sphingosine phospholipid found in membranes.

Membranes: The Bipolar and the Bilayer

One use of lipids is in the construction of membranes. *Membranes* are used to separate regions both in and around cells — a typical membrane, as shown in Figure 8-8, is a lipid bilayer or bimolecular sheet. The polar portions of the lipids, the heads, are on the outside edges of the bilayer, whereas the nonpolar portions, the tails, are in the interior. The heads of the lipids appear as circles in our illustrations, and the tails appear as strings. The tails are usually long fatty acid chains. The hydrophilic heads, often with a charge, are in contact with aqueous material, and the hydrophobic tails are away from the aqueous material. Interactions between the hydrophobic tails are the key factors leading to the formation of lipid bilayers. *Lipid bilayers* tend to form closed structures or compartments to avoid having exposed hydrophobic edges. The membranes tend to be self-sealing.

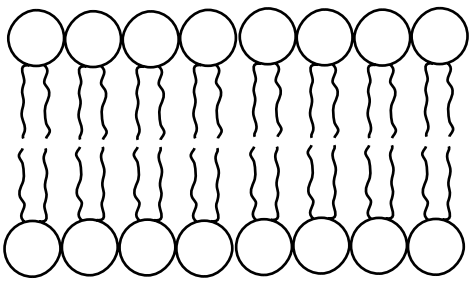


Figure 8-8: A simplified representation of a lipid bilayer.

Actual cell membranes are not as symmetrical as the one shown in Figure 8-8. This asymmetry is due in part to the presence of other components, and in part to differences between the intracellular and extracellular surfaces. If the fatty acid portions are not saturated, the tails will not form parallel structures, and there will be "holes" present within the bilayer. These holes are an essential feature leading to membrane fluidity. Other components include proteins and cholesterol. The carbohydrate portion of glycolipids is on the extracellular side of the bilayer instead of the intracellular side.

Polar materials cannot readily pass through the hydrophobic region of membranes, and nonpolar materials cannot readily pass through the hydrophilic outer region. Water, due to its small size and high concentration, can transverse the bilayer faster than ions and most other polar molecules. In actual cells, certain mechanisms allow material to cross the bilayer but require other components to be present in the bilayer. These components, mostly proteins, give selective permeability of the membranes. In addition, other materials, such as cholesterol, are necessary to serve other functions, such as stiffening the membrane.

Membranes may contain roughly from 20 to 80 percent protein, which may be *peripheral* (on the surface of the membrane) or *integral* (extending into or through the membrane). Integral proteins interact extensively with the hydrophobic portion of the bilayer, as illustrated in Figures 8-9 and 8-10.

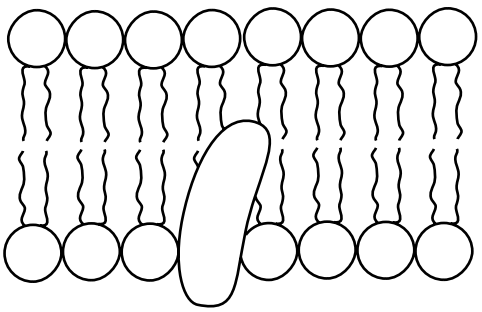


Figure 8-9: An integral protein that does not pass through the membrane.

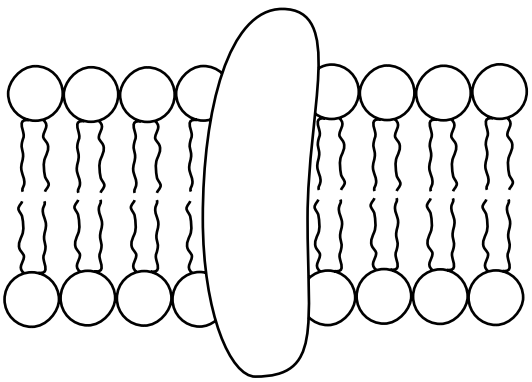


Figure 8-10: An integral protein passing through the membrane.

Peripheral proteins typically bind to the surface through electrostatic or hydrogen bonding, although covalent interactions are possible. Proteins are important for most membrane processes. If the protein is a glycoprotein, the carbohydrate portion lies on the external side of the membrane and is important to intercellular recognition.

Crossing the wall: Membrane transport

A lipid bilayer is, by its nature, impermeable to polar molecules and ions (hydrophilic species). Nevertheless, cells need to be able to bypass this feature and get hydrophilic materials in and out. There are two ways to circumvent impermeability: A *pump* involves active transport using energy to work against a concentration gradient, and a *channel* involves passive transport or facilitated diffusion using a concentration gradient.

Nonpolar molecules are lipophilic and dissolve in the lipid bilayer. In general, lipophilic materials pass through the membrane by simple diffusion along a concentration gradient. Channels and pumps are mainly to allow hydrophilic species to transverse the hydrophobic region of the bilayer.

Pumps

Pumps require energy to function. In many cases, the hydrolysis of ATP provides the needed energy. The generic name for this type of pump is a P-type ATPase. The name derives from the transfer of a phosphate from an ATP to an intermediate, a step that is essential to the action of the pump. Pumps can transfer other species than ions.

When is a solid a liquid? The fluid mosaic model

The lipid bilayer structure gives much insight into the structure of membranes but little information about their function. Many functions of the membrane depend upon its fluidity, best described by using the *fluid mosaic model.* In this model, the membrane serves as a permeability barrier and as a solvent for the integral proteins. Diffusion along the plane of the membrane — *lateral diffusion* — of the membrane components is often rapid. In general, lipids move more rapidly than proteins, with some proteins being essentially immobile. Diffusion of membrane components across the membrane — *transverse diffusion* — is usually slow.

The fluidity of the membrane depends on a number of factors. Bacteria adjust the fluidity by utilizing fatty acid chains — longer chains are less fluid than are shorter chains. The presence of double bonds makes the membrane more fluid. In animals, cholesterol controls the fluidity: The greater the cholesterol concentration, the less fluid the membrane. The transition from the rigid to the fluid state occurs at a temperature known as the *melting temperature*, T_m.

Most animal cells have a high potassium ion and a low sodium ion concentration relative to the extracellular environment. It requires energy to generate and maintain this gradient. The transport system is the Na^+-K^+ pump, also referred to as Na^+-K^+ ATPase. Hydrolysis of ATP provides the energy to transport potassium ions into the cell and sodium ions out of the cell. Both the sodium and potassium ions must be simultaneously bound to the pump. The pump simultaneously transports three sodium ions out of the cell as it transports two potassium ions in.

Not all pumps require the hydrolysis of ATP to supply energy. Some utilize the transport on one species to facilitate the transport of another. The transport of one species with the concentration gradient can pump another against the concentration gradient. The responsible membrane proteins are the cotransporters or secondary transporters. *Cotransporters* may be either symporters or antiporters. In a symporter, both transported species move in the same direction, whereas in an antiporter, the species move in opposite directions. The sodium-calcium exchanger is an example of an antiporter, which pumps three sodium ions into a cell for every two calcium ions pumped out. Some animal cells use a symporter to pump glucose coupled with sodium ions into the cells.

Channels

A channel provides a means of passively transporting a species across a membrane. It is possible to transport a species through a channel more than 1,000 times as fast as a pump's. A channel is technically a tube running through the membrane, but its behavior is significantly more complicated.

Channels are highly selective. Some select on size — sodium is smaller than potassium — whereas others differentiate between anions and cations. A channel exists in an open state to allow transport and a closed state to inhibit it. Some type of regulation is required to convert a channel between an open and a closed state. When a chemical potential regulates the channel, it is a voltage-regulated gate. The regulation may be due to specific chemicals. Chemically controlled regulation is ligand-gated. After the appropriate regulator is removed, the open channels will spontaneously close.

The best-known ligand-gated channel is the acetylcholine receptor. This channel is important for the transmission of nerve impulses. When a nerve impulse reaches the junction between one nerve and the next — the synapse — it triggers the release of acetylcholine, which transverses the small gap to the next nerve and attaches to acetylcholine receptors. This attachment opens the channel, leading to inward sodium ion diffusion and outward potassium ion diffusion. The change in the ion concentrations transmits the nerve impulse into the second nerve cell.

The increase in the sodium ion concentration in the second nerve cell triggers a mechanism to remove sodium ions from the nerve cell. Later another gate brings potassium ions back into the cell.

Steroids: Pumping up

Steroids are another class of lipids. All steroids have the basic core shown in Figure 8-11. A, B, C, and D are common labels for the rings. Different steroids have additions to this basic structure; these may include side chains, other functional groups, and unsaturation or aromaticity of the rings.

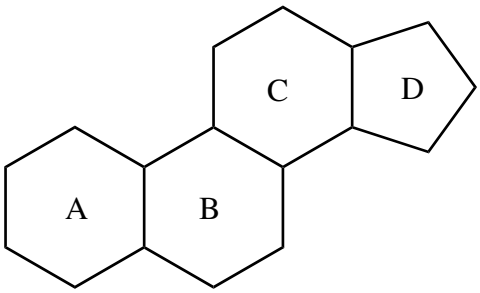


Figure 8-11: Basic structure of a steriod.

Cholesterol is the most abundant steroid. It is a membrane component and serves as the source of other steroids and related materials. Cholesterol comes from the diet, but if insufficient cholesterol is available there, it is synthesized in the liver. The steroid hormones are regulators produced from cholesterol.

Bile salts (mentioned earlier) are a group of materials produced by the oxidation of cholesterol. Unlike cholesterol and the other lipids, bile salts are soluble in water. They are useful as "detergents" to aid in digestion.

The steroids you hear about in the news being used by athletes and bodybuilders are *anabolic steroids,* which increase the body's ability to prevent muscle breakdown and to actually increase the ability to produce muscle. They have structures similar to testosterone, whose function is to enhance male characteristics such as facial hair and muscle mass. However, steroids in large doses have serious side effects: impotence, reduced testicle size, liver tumors, enlargement of the heart, enlargement of the breasts in men, aggressive behavior, and so on. (Sounds great, doesn't it?) Their use without a valid prescription has been illegal since 1991.

Prostaglandins, Thromboxanes, and Leukotrienes: Mopping Up

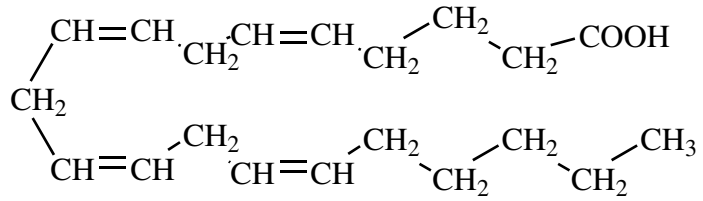
Arachidonic acid, a 20-carbon, polyunsaturated fatty acid, serves as the direct or indirect starting material for the formation of prostaglandins, thromboxanes, and leukotrienes. Cells synthesize both leukotrienes and prostaglandins from arachidonic acid. Additional prostaglandins and thromboxanes come from the prostaglandin derived from arachidonic acid. All three classes of compounds are local hormones. Unlike other hormones, they are not transported via the bloodstream. They are short-lived molecules that alter the activity of the cell producing them and neighboring cells.

All of these compounds are extremely potent chemicals that serve as hormone mediators. They also have many other medical applications and can cause medical problems. They are also known as *eicosanoids* — from the Greek for *twenty,* which alludes to the presence of 20 carbon atoms (Figure 8-12).

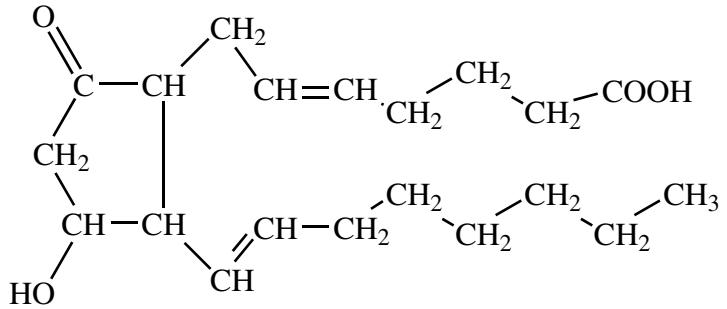
The name *prostaglandin* came from the belief that the prostate gland was its source because they were first isolated from seminal fluid in 1935. Now we know that they are produced in a very wide variety of cells. Prostaglandins differ slightly from each other, but they all contain a five-carbon ring. These minor difference lead to distinct behaviors, although all prostaglandins lower blood pressure, induce contractions in smooth muscles, and are part of the inflammatory response system.

A number of medications are synthetic prostaglandins. For example, derivatives of the prostaglandin PGE_2 are useful in inducing labor. Prostaglandins associated with inflammation are the main cause of the associated redness, pain, and swelling. The half-life of many prostaglandins is only a few minutes or less. Platelets in the blood generate thromboxanes to serve as vasoconstrictors and to induce aggregation of the platelets, two steps leading to the formation of a blood clot. Thromboxane A_2 is an example of one of these agents that induces blood clotting. White blood cells, leukocytes, and other tissues produce leukotrienes, whose name refers to where they were first discovered (leukocytes) and to the presence of three conjugated double bonds (triene). Leukotrienes are associated with allergy attacks.

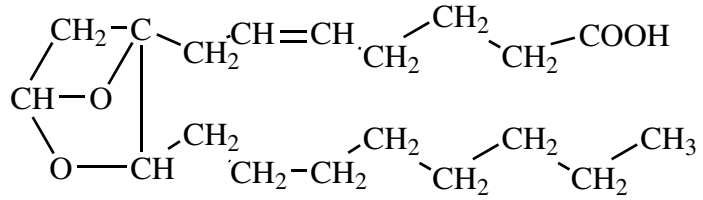
Aspirin interferes with the synthesis of prostaglandins and thromboxanes. Aspirin is an anti-inflammatory agent because it counters the inflammation induced by prostaglandins. The interference with the formation of thromboxanes may be part of the reason why low doses of aspirin help prevent heart attacks and strokes. Low thromboxane levels would inhibit blood clotting. Another anti-inflammatory drug, cortisone, inhibits the release of arachidonic acid from cell membranes, which, in turn, inhibits the formation of the eicosanoids. The fatty acids in fish oils inhibit the formation of the more potent leukotrienes and thromboxanes.



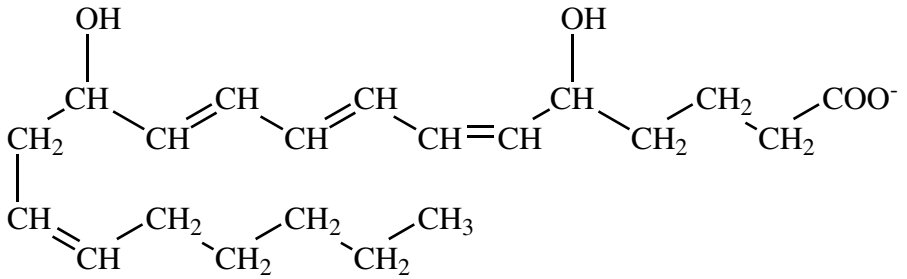
arachidonic acid



a prostaglandin (PGE_2)



a thromboxane (A_2)



a leukotriene (B_4)

Figure 8-12: Structures of arachidonic acid, a typical prostaglandin, thromboxane, and leukotriene.

Chapter 9

Nucleic Acids and the Code of Life

In This Chapter

- Finding out about the structure of proteins
- Understanding amino acid sequencing in proteins
- Going over applications of protein sequencing

Nucleic acids get their name because they were first found in the nuclei of cells. DNA (*deoxyribonucleic acid*) — the most famous nucleic acid — is part of the *chromosomes,* which contain the genes. And the *genes* are ultimately responsible for the synthesis of proteins. Most, if not all, of these proteins are enzymes, each catalyzing a specific chemical reaction occurring in the organism. Indeed, there is a one-gene-one-enzyme hypothesis, where each gene is responsible for the synthesis of one enzyme.

DNA has two direct purposes: It must generate new DNA (replication) so that new generations of cells will have the information necessary to their survival. And it must generate RNA (*ribonucleic acid*). The RNA is involved in the direct synthesis of proteins, called *translation.* These proteins are essential for the maintenance of life.

Nucleotides: The Guts of DNA and RNA

Both DNA and RNA are polymers of nucleotides. A *nucleotide* is a combination of a nitrogen base, a 5-carbon sugar, and a phosphoric acid. There are five different bases present in a nucleotide, and two different sugars. We take a closer look at the components of these nucleotides and then show you how they all fit together.

Reservoir of genetic info: Nitrogen bases

The bases fall into two categories, the general defining structures of which appear in Figure 9-1.

- The purines (adenine and guanine), composed of two fused rings incorporating two nitrogen atoms in each ring and
- The pyramidines (cytosine, thymine, and uracil), composed of a single ring with two nitrogen atoms in the ring structure

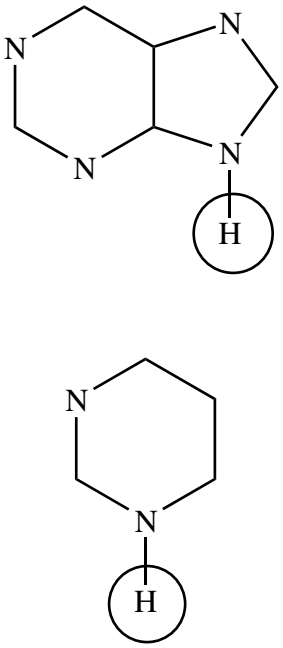
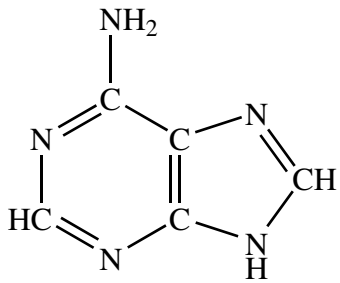


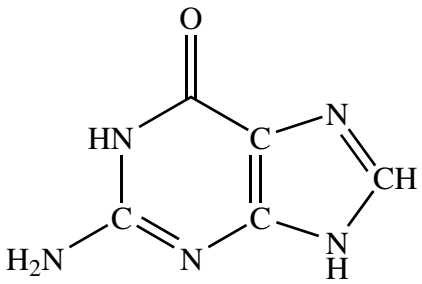
Figure 9-1: Basic purine structure (top) and basic pyramidine structure (bottom).

Adenine (A), guanine (G), and cytosine (C) occur in both DNA and RNA. Thymine (T) is only found in DNA, whereas uracil (U) only occurs in RNA. There are modified forms of some of these bases present in some nucleic acid molecules. The circled hydrogen atoms shown in Figure 9-1 are lost when combining with other components to produce a nucleic acid. The complete structures of the five bases are shown in Figure 9-2. It is the sequence of these bases that stores the genetic information.

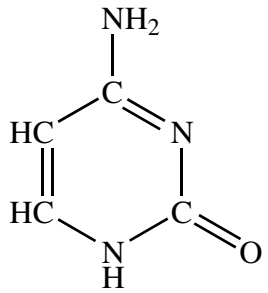
The nitrogen and oxygen atoms present on the nitrogen bases provide a number of sites where hydrogen bonding is possible. Hydrogen bonding is most effective and easily formed between certain combinations of nitrogen bases. Because of this, certain combinations will form, and it is this pattern that is responsible for the transmission of information. The atoms on the nitrogen bases normally use a regular numbering system, whereas the atoms in the sugar component use primed numbers.



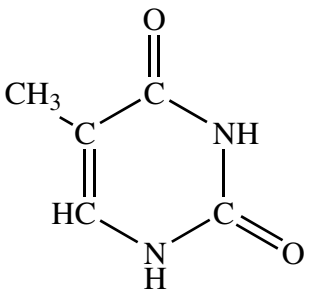
Adenine (A)



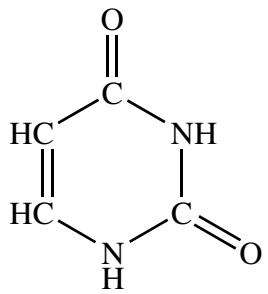
Guanine (G)



Cytosine (C)



Thymine (T)



Uracil (U)

Figure 9-2: Adenine (A), guanine (G), cytosine (C), thymine (T), uracil (U).

The sweet side of life: The sugars

The 5-carbon sugars found in the nucleic acids are D-ribose and D-deoxyribose. The difference between these two sugars is that deoxyribose is missing an oxygen atom on carbon atom number 2'. The structures for these two sugars appear in Figure 9-3. The arrows in the figure point to the alcohol group on carbon atom number 1', the *anomeric* carbon. This is where the nitrogen base will attach. Both sugars adopt the β form of the furanose ring. Numbering of the sugar begins with the anomeric carbon (1') and proceeds clockwise with the $-CH_2OH$ carbon being 5'.

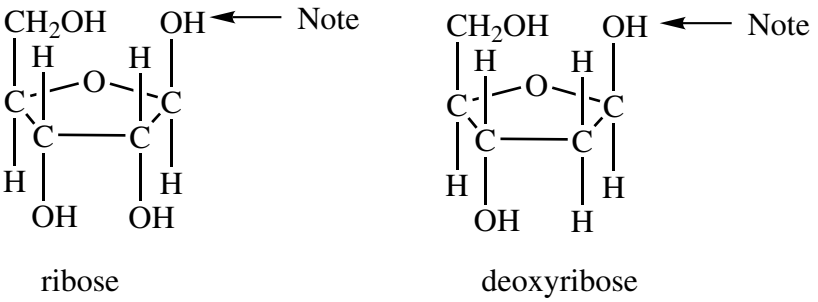


Figure 9-3: Structures of the 5-carbon sugars present in nucleic acids.

The sour side of life: Phosphoric acid

The third component of a nucleotide is a phosphoric acid (Figure 9-4). At physiological pH it does not exist in the fully protonated form shown in the figure. It is responsible for the "acid" in nucleic acid.

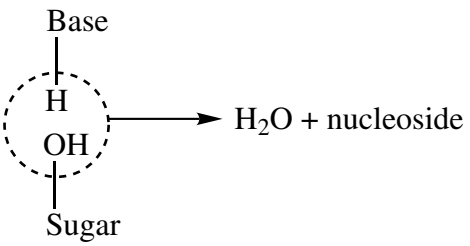


Figure 9-4: Structure of phosphoric acid.

Tracing the Process: From Nucleoside to Nucleotide to Nucleic Acid

Remember Legos and Tinker Toys? Putting together the pieces to get something new? That's what goes on in the construction of nucleic acids. Nature first joins a nitrogen base and a 5-carbon sugar to form a nucleoside; then that nucleoside joins with phosphoric acid to form a nucleotide; finally, the combination of these nucleotides produces a nucleic acid.

First reaction: Nitrogen base + 5-carbon sugar = nucleoside

The combination of a nitrogen base with a 5-carbon sugar is a *nucleoside.* The general reaction appears in Figure 9-5. It is a *condensation* reaction. Remember the condensation reactions you studied in ester formation in organic chemistry? This is exactly the same type. Here a compound containing hydrogen (the nitrogen base) approaches another molecule containing an –OH group (a sugar). The hydrogen combines with the –OH to form water, which is expelled. A bond forms in the remaining fragments.

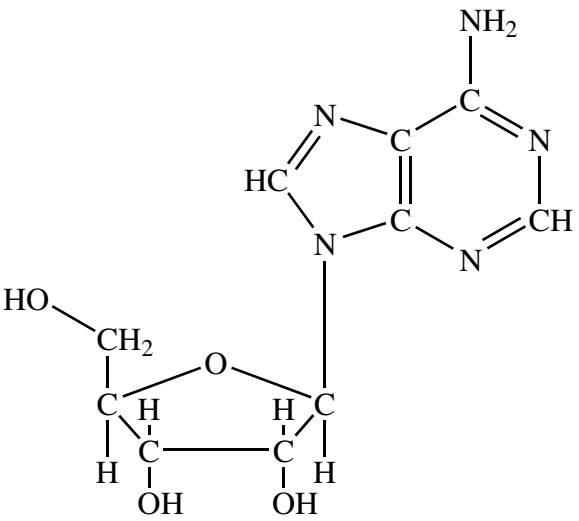


Figure 9-5: General reaction for the formation of a nucleoside.

The name of the nucleoside comes from the nitrogen base if the sugar is ribose; it has a prefix if the sugar is deoxyribose. For example, adenine combines with ribose to form adenosine and combines with deoxyribose to form deoxyadenosine. The structure for the nucleoside adenosine is in Figure 9-6. The hydrogen atom lost from the base was the one circled in Figure 9-1.

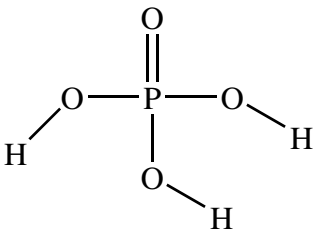


Figure 9-6: Structure of the nucleoside adenosine.

Second reaction: Phosphoric acid + nucleoside = nucleotide

The combination of a phosphoric acid with a nucleoside produces a nucleotide, which is a phosphate ester, as seen in Chapter 3, of a nucleoside. The formation involves a condensation reaction between the phosphoric acid and the alcohol group on carbon number 5, the $-CH_2OH$ (Figure 9-7).

Adenosine monophosphate (AMP) is an example of a nucleotide (Figure 9-8). Nucleotides are the monomers from which nucleic acids form. AMP is not only one of the "Legos" that makes RNA but is also very much involved in the energy transfer process in the cells (much more on AMP in Part IV).

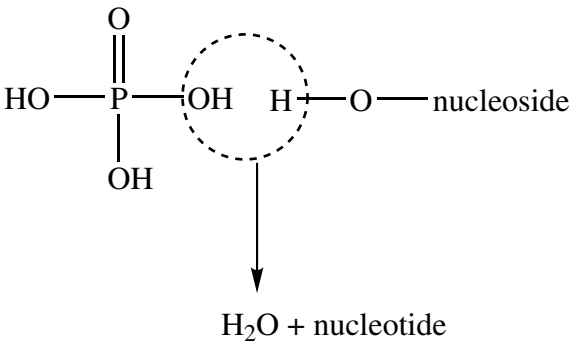


Figure 9-7: Simplified representation of the formation of a nucleotide.

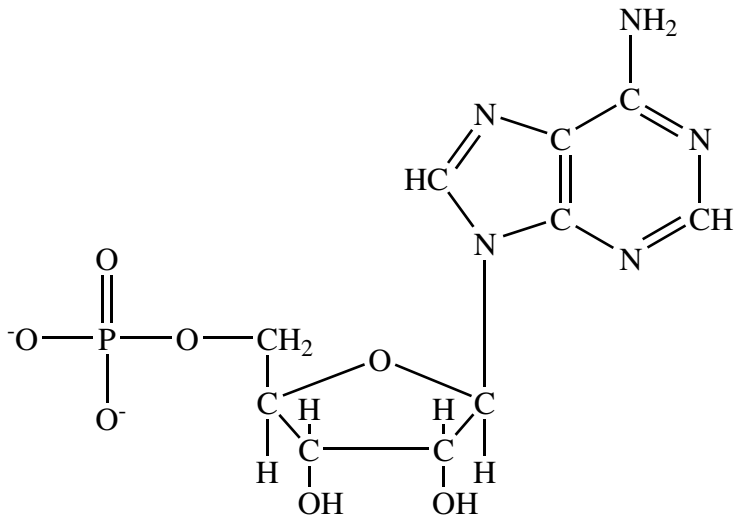


Figure 9-8: Structure of adenosine monophosphate (AMP).

If the sugar is ribose, then the result is one of four ribonucleotides. If the sugar is deoxyribose, the result is one of the four deoxyribonucleotides.

Third reaction: Nucleotide becomes nucleic acid

Nucleic acids form by joining nucleotides using the same condensation reactions we've mentioned. This condensation reaction involves the phosphate of one nucleotide reacting with the alcohol group on carbon atom number 3' of another nucleotide. Figure 9-9 illustrates. Note that the lower –OH, in the circle, is from the phosphoric acid, attached to carbon-5'. The upper –H in the circle is from the alcohol on carbon-3'.

The starting end of the polymer is 5', whereas the terminal end is 3'. Figure 9-10 illustrates the 5' and 3' carbon atoms on adenosine monophosphate.

A Primer on Nucleic Acids

Nucleic acids are responsible for storing and directing the information our cells use for reproduction and growth. They are large molecules found in the cell's nucleus. The genetic information is contained in the DNA, in terms of its primary and secondary structure. As a cell divides and reproduces, the genetic information in the cell is *replicated* to the new cells, which must be done accurately and precisely — no mistakes must be made. RNA's role is to

transfer the genetic information found in the DNA to the ribosomes, where protein synthesis occurs. DNA and RNA allow us to live and function.

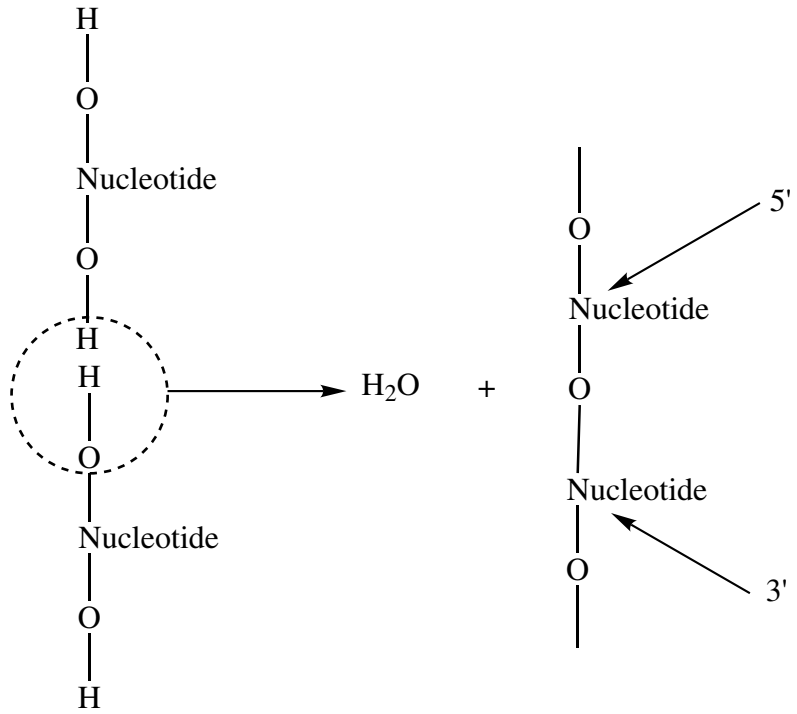


Figure 9-9: Simplified representation of the joining of two nucleotides.

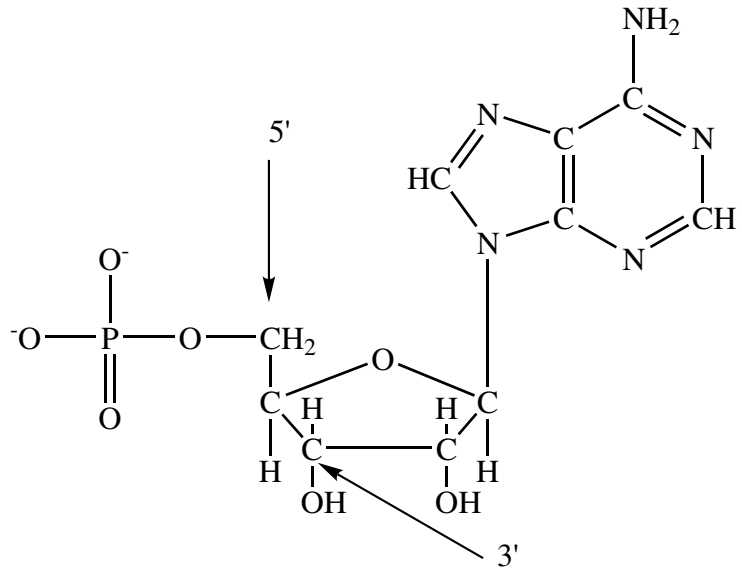


Figure 9-10: 5' and 3' carbon atoms on adenosine monophosphate.

DNA and RNA in the grand scheme of life

Both DNA and RNA are polymers composed of nucleotide subunits. However, DNA is a much larger molecule than RNA. DNA molecules typically have molecular weights in the billions. The human genome contains about 3 billion nucleotides.

As a simplification, the structure of a particular nucleic acid may be represented as 5'-C-G-T-A-3'. This abbreviation indicates that we begin at the 5' end and end at the 3' end (as always), and the nitrogen bases on the nucleotides are, in order, cytosine (C), guanine (G), thymine (T) and adenine (A). There are three different types of RNA, and each one has a specific use:

- Ribosomal RNA (rRNA) is the most common: 75–80 percent occurs within the ribosomes of the cell.
- Transfer RNA (tRNA) accounts for 10–15 percent.
- Messenger RNA (mRNA) makes up the remainder.

All three types are important to protein synthesis — which occurs in the ribosomes, home of ribosomal RNA (rRNA). The amino acids necessary for protein synthesis are transferred to the ribosomes by transfer RNA (tRNA). The message instructing the ribosomes how to assemble the protein travels from the DNA to the ribosome via messenger RNA (mRNA). This message tells the ribosome the sequence of amino acids to make a specific protein.

Transfer RNA contains the fewest nucleotides: 70–90. The average mRNA has about 1,200 nucleotides. There are three subcategories of rRNA ranging from about 120 to over 3,700 nucleotides. (DNA typically has between 1 million and 100 million nucleotides, though viral DNA tends to be smaller.) *Ribonucleotides* have other uses in addition to building RNA. They are present in energy molecules (ATP), in intracellular hormone mediator (cyclic AMP), and in certain coenzymes (FAD and NAD^+). Plants and animals contain both DNA and RNA. Viruses can contain either DNA or RNA.

Nucleic acid structure

The structure of a particular nucleic acid controls its function within the organism. For example, the structure of a particular tRNA determines which specific amino acid it will transfer to the ribosome for protein synthesis. In fact, the difference between DNA and RNA resides in the structure of the molecules. Because of the complexity of these types of molecules, there may be more than one key type of structure present.

The primary structure of the nucleic acids is the sequence of nucleotides, the order in which the individual nucleotides have been joined. This sequencing determines which hydrogen bonds form, and this, in turn, controls much of the function of the nucleic acid. DNA also has an important secondary structure, a consequence of hydrogen bonding between the nitrogen bases on the DNA strands. The result is that DNA consists of a *double helix* — which looks like a ladder twisted lengthwise — where hydrogen bonds (the rungs in the ladder) hold the two primary structures together.

The hydrogen bonds between the two stands of DNA make the two strands *complementary (*paired). Every A is complementary to a T, and every G is complementary to a C in *base pairing.* Base pairing is essential for the function of the nucleic acids.

The two DNA strands are *antiparallel,* which means that the 5' end of one strand connects to the 3' end of its complementary strand. This pairing also places the more polar (more hydrophilic) sugar and phosphate groups on the outside and the less polar (more hydrophobic) nitrogen bases on the inside. (Note that *hydrophilic* and *hydrophobic* as used here are relative terms.) The antiparallel nature affects how DNA produces new DNA (the replication process) and new RNA (the transcription process).

Although each of the nitrogen bases is very efficient at forming hydrogen bonds, certain combinations are extremely effective. In DNA, an adenine is capable of forming two hydrogen bonds to thymine (Figure 9-11), and guanine can form three hydrogen bonds to cytosine (Figure 9-12).

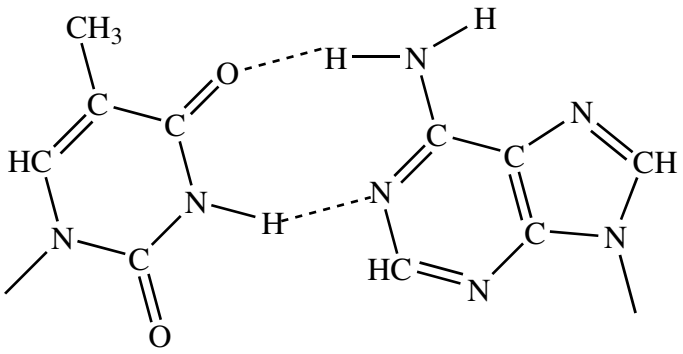


Figure 9-11: Hydrogen bonds (dotted lines) form between adenine (right) and thymine (left).

Adenine is also able to form hydrogen bonds with uracil when DNA interacts with RNA or when two RNA molecules interact. The interaction between adenine and uracil is shown in Figure 9-13.

The ability to form these specific combinations is important in real life — this is the *genetic code* we all have heard so much about. The sequencing of nucleotides in the nucleic acids and the sequencing of amino acids in the proteins all depend on these hydrogen bonds. Without them, the appropriate information would not be transferred precisely, and you might produce kittens instead of kids. The result? DNA, the structure of life (Figure 9-14).

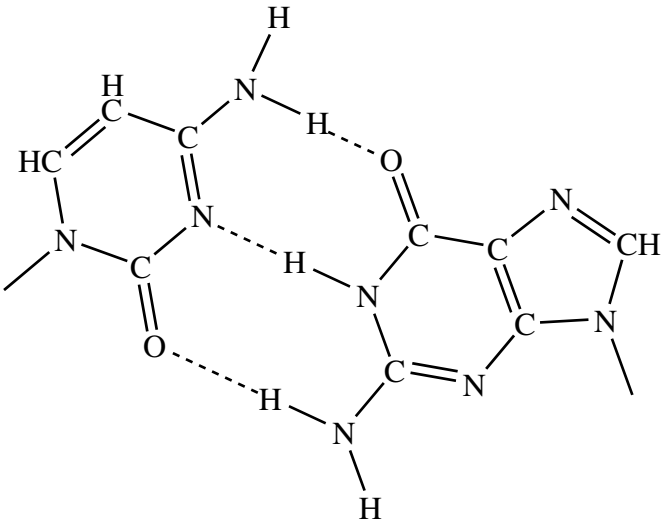


Figure 9-12: Hydrogen bonds (dotted lines) form between guanine (right) and cytosine (left).

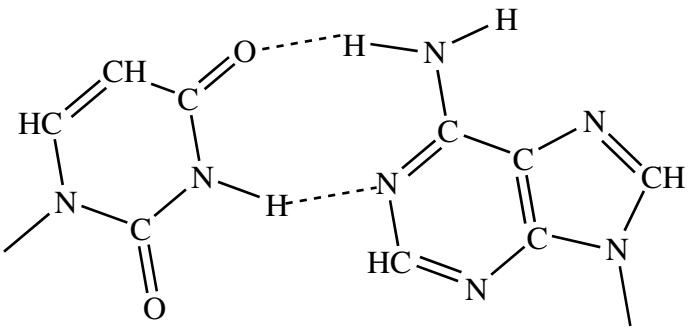


Figure 9-13: Hydrogen bonds (dotted lines) form between adenine (right) and uracil (left).

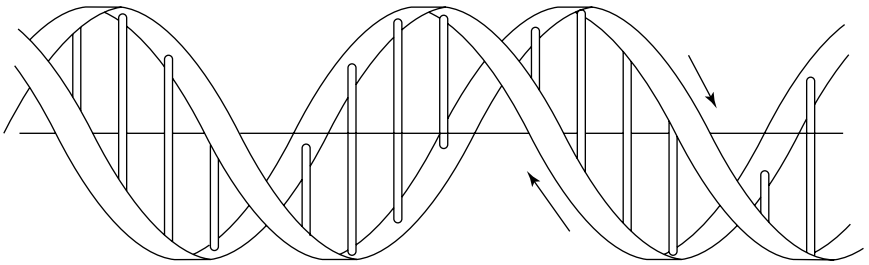


Figure 9-14: The secondary structure of DNA.

Chapter 10

Vitamins and Nutrients

In This Chapter

- Taking a look at the purpose of vitamins
- Understanding B vitamins
- Assessing other vitamins and nutrients

An organism must absorb a variety of materials to live, many of which fall into the category of food, certainly one of *our* favorite categories, especially John's. These foodstuffs required by an organism for life and growth are classified as nutrients. *Nutrients* are the substances in the diet necessary for growth, replacement, and energy. Here are the six general classes of nutrients:

- Carbohydrates
- Lipids
- Proteins
- Vitamins
- Minerals
- Water

Digestion converts large molecules in food into smaller molecules that can be absorbed. During digestion, carbohydrates (with the exception of the monosaccharides), lipids, and proteins are broken down into their components. These components are often used by the organism directly for growth and replacement. For animals, energy comes primarily from carbohydrates and lipids, but proteins can also serve as an energy source.

Vitamins are other organic materials required by an organism, and minerals are inorganic materials required by an organism. In addition, all living organisms require water to survive. Water is a wonderful substance. For more about the unusual properties of water, check out Chapter 2 in this book or *Chemistry For Dummies* by John T. Moore (Wiley).

More than One-a-Day: Basics of Vitamins

Vitamins are organic compounds that are required, in small quantities, for normal metabolism. The term *active form* is used to describe the structural form of the molecule, in this case vitamins, that performs its function (exhibits *activity*) within the organism. In general, humans cannot synthesize sufficient quantities of vitamins; thus, vitamins must come from other sources — through the diet and/or in pill form. A deficiency of a vitamin in the diet leads to a health problem. The general symptoms for any vitamin deficiency include frequent illness, slow healing of wounds, and tiredness. It was not until the early 1900s that the need for trace nutrients such as vitamins and minerals was first established.

There are two categories of vitamins: water-soluble and fat-soluble. *Water-soluble* vitamins include vitamin C and the B vitamins. Vitamins A, D, E, and K comprise the other category, the *fat-soluble* vitamins. Water-soluble vitamins tend to have more oxygen and nitrogen in their structure than fat-soluble vitamins, which have significant hydrocarbon portions in their structure. The majority of water-soluble vitamins either act as coenzymes or are important in the synthesis of coenzymes. Fat-soluble vitamins serve a variety of biochemical functions.

The body can easily eliminate an excess of the water-soluble vitamins, normally in the urine. The bright yellow of the urine of a person taking large doses of vitamin C attests to that fact. Because the body does not store water-soluble vitamins, continual replacement is necessary. The body can store excess amounts of a fat-soluble vitamin in the body's fatty tissue, and therefore elimination is not very easy. Unfortunately, this can lead to an accumulation of these vitamins, sometimes to toxic levels. One should consider this before consuming mega quantities of the fat-soluble vitamins.

To B or Not to B: B Complex Vitamins

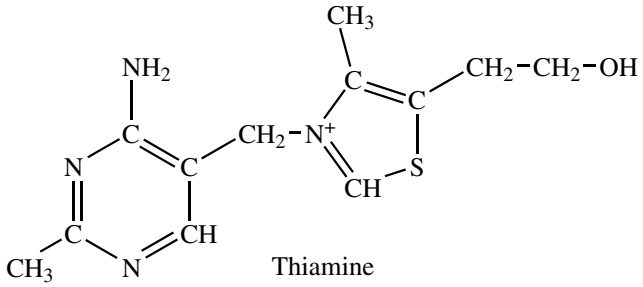
The B vitamins — or B complex — comprise a number of water-soluble vitamins that are found together in a number of sources. Originally, this mixture was thought to be only one vitamin (vitamin B). With the possible exception of vitamin B_6, these appear to be relatively nontoxic. In general, the B complex is important for healthy skin and nervous systems.

Vitamin B_1 (thiamine)

Thiamine is important to carbohydrate metabolism. Like the other B vitamins, the body does not store it. In addition, prolonged cooking of food can destroy it. Once absorbed in the body, thiamine is converted to a form that is biologically active through the attachment of a pyrophosphate (diphosphate) group to give thiamine pyrophosphate (TPP). The structures of vitamin B_1 and thiamine pyrophosphate are shown in Figure 10-1.

TPP is a coenzyme used in decarboxylating pyruvate to acetyl-CoA and α-ketoglutarate to succinyl-CoA. In addition, TPP is necessary for the synthesis of ribose.

A deficiency in thiamine leads to beriberi, which causes deterioration in the nervous system. Beriberi was prevalent in regions where rice was a major food source. Rice, particularly polished rice, is low in thiamine. Using brown rice, which has more thiamine, alleviates this problem. Nursing infants are particularly at risk when their mothers have a thiamine deficiency. Many alcoholics also suffer from this condition because many "foods" high in alcohol are particularly low in vitamins.



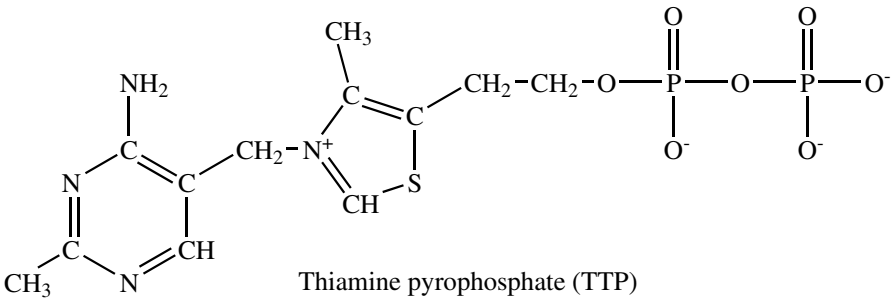


Figure 10-1: Structures of vitamin B^1 (thiamine) and thiamine pyrophosphate (TPP).

Good dietary sources of thiamine include liver, spinach, green peas, navy and pinto beans, whole-grain cereals, and most legumes.

Vitamin B_2 (riboflavin)

Riboflavin is essential to the synthesis of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The structures of these materials are shown in Figure 10-2. FMN and FAD are important coenzymes involved in a number of biochemical redox processes. The name *riboflavin* alludes to the presence of *ribitol,* an alcohol derived from ribose. The other part of riboflavin is the ring system isoalloazine, a flavin derivative.

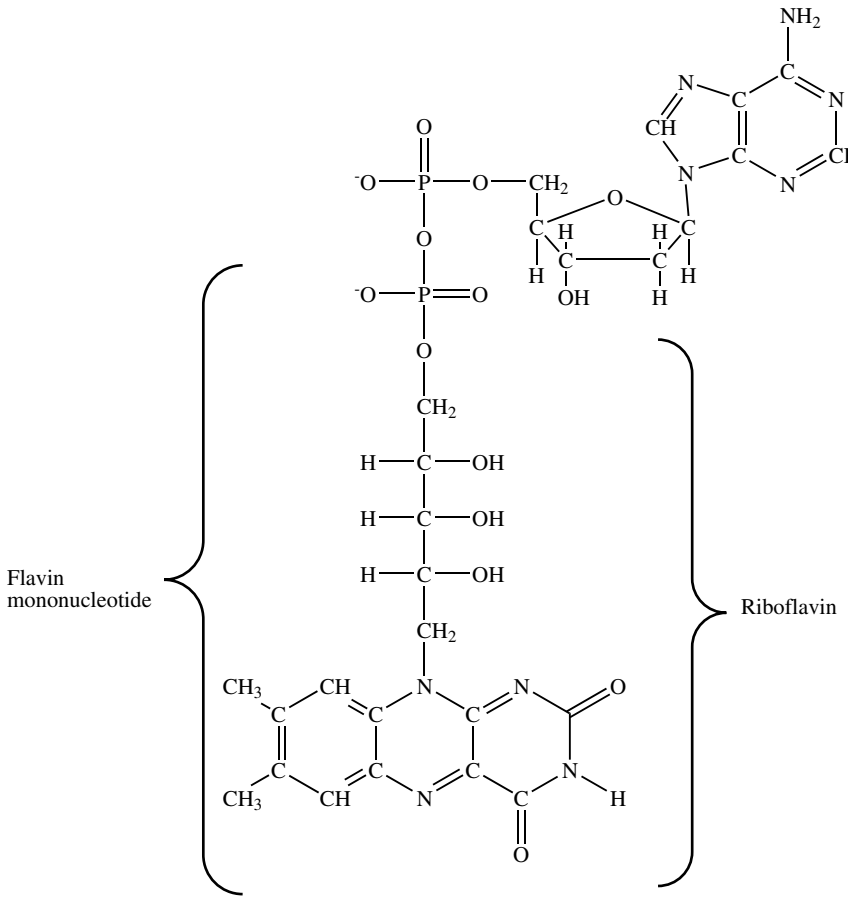


Figure 10-2 : Structure of flavin adenine dinucleotide (the entire structure) and the component materials flavin mononucleotide and riboflavin.

No deficiency diseases are associated with riboflavin; however, a deficiency does lead to burning and itchy eyes, dermatitis, and anemia. Dietary sources of this vitamin include soybeans, liver, milk, cheese and green leafy vegetables. Riboflavin is stable during cooking, but is broken down by light.

Vitamin B_3 (niacin)

The term *niacin* applies to two compounds: nicotinic acid and nicotinamide. These two compounds along with nicotinamide adenine dinucleotide (NAD^+) appear in Figure 10-3. Nicotinamide is part of the coenzymes NAD^+ and nicotinamide dinucleotide phosphate ($NADP^+$). These coenzymes work with a number of enzymes in catalyzing a number of redox processes in the body.

Niacin is one of the few vitamins that the body *can* synthesize. The synthesis utilizes tryptophan and is not very efficient.

Pellagra is a niacin-deficiency disease. Symptoms include loss of appetite, dermatitis, mental disorders, diarrhea, and possibly death. It was common in the southern United States in the early 1900s because many people had a diet of corn, which is neither a good source of niacin or tryptophan.

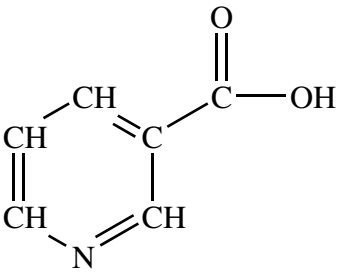
There are many dietary sources for niacin, including most meats and vegetables, milk, cheese, and grains.

Vitamin B_6 (pyridoxine)

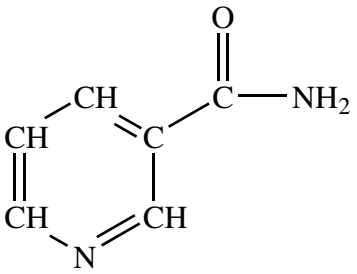
This vitamin consists of three components: pyridoxine, pyridoxal, and pyridoxamine. All three need to be converted to pyridoxal phosphate, a form that is biologically active in the organism. The structures for these compounds appear in Figure 10-4. Pyridoxal phosphate serves as a coenzyme in a variety of processes, including the interconversion of α-keto acids and amino acids.

Avocados, chicken, fish nuts, liver, and bananas are especially good food sources of vitamin B_6. Heating decreases its concentration in food.

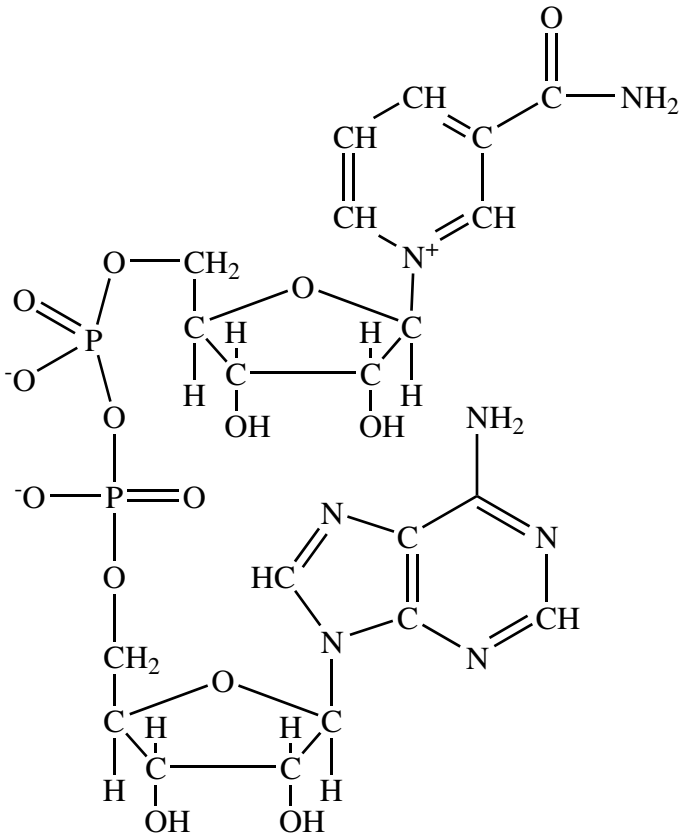
There is no pyridoxine-deficiency disease known; however, low levels can lead to irritability, depression, and confusion. Unlike the other water-soluble vitamins, there is evidence that large doses of vitamin B_6 may lead to health problems. The symptoms of excess vitamin B_6 consumption include irreversible nerve damage.



Nicotinic acid

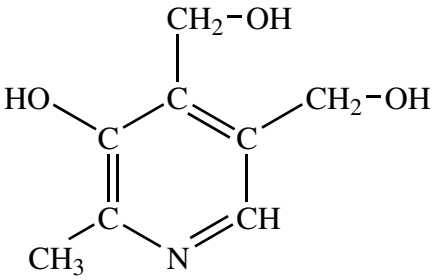


Nicotinamide

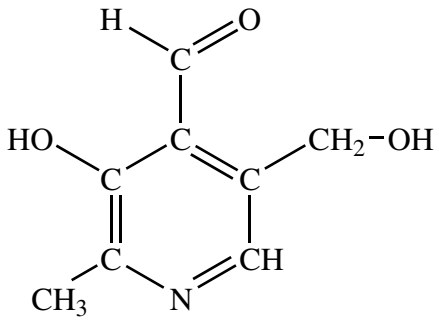


Nicotinamide adenine dinucleotide (NAD^+)

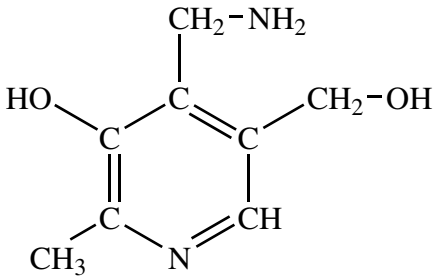
Figure 10-3: Structures of nicotinic acid, nicotinamide, and nicotinamide adenine dinucleotide (NAD^+).



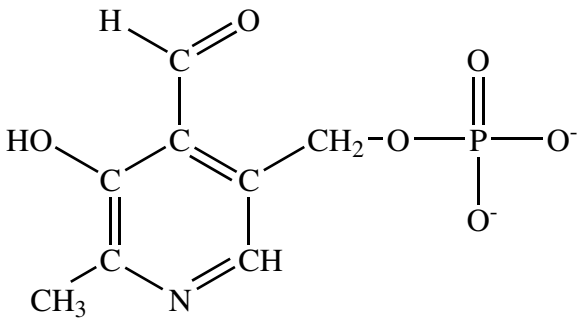
Pyridoxine



Pyridoxal



Pyridoxamine



Pyridoxal phosphate

Figure 10-4: Structures of pyridoxine, pyridoxal, pyridoxamine, and pyridoxal phosphate.

Biotin

Biotin is a coenzyme important to many carboxylation reactions. Biotin is the carbon transporter in both lipid and carbohydrate metabolism.

Bacteria in the intestinal track synthesize biotin in sufficient quantities to minimize the chances for a deficiency. However, antibiotics can inhibit the growth of these bacteria and induce a deficiency. In these circumstances, the symptoms include nausea, dermatitis, depression, and anorexia. Biotin is stable to cooking. Its structure is shown in Figure 10-5.

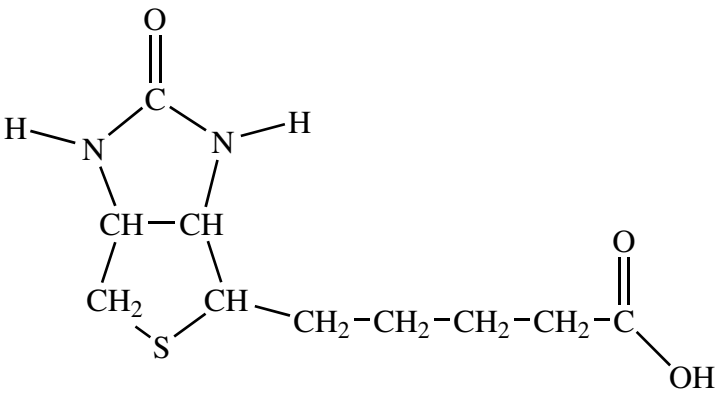


Figure 10-5: Structure of biotin.

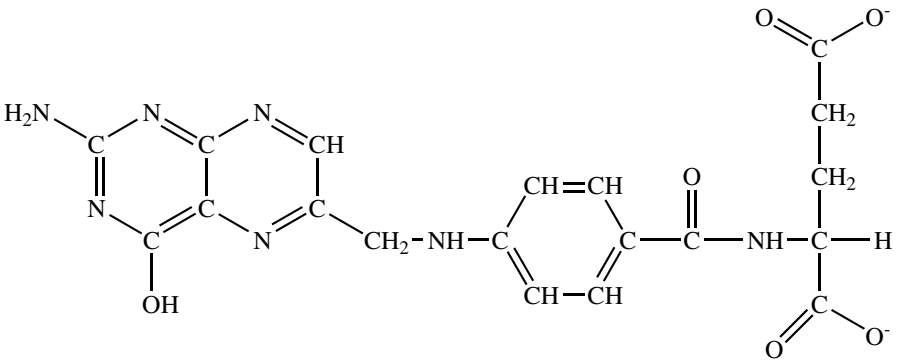
Folic acid

Bacteria in the intestinal track also produce *folic acid;* however, green leafy vegetables, dried beans, and liver are also sources. Reduction of folic acid yields tetrahydrofolic acid, the active form. Both structures are shown in Figure 10-6. The coenzyme transports a carbon, usually as a methyl or formyl, in the synthesis of heme, nucleic acids, choline, and several other compounds. Although cooking easily destroys the compound, intestinal bacteria normally produce sufficient quantities.

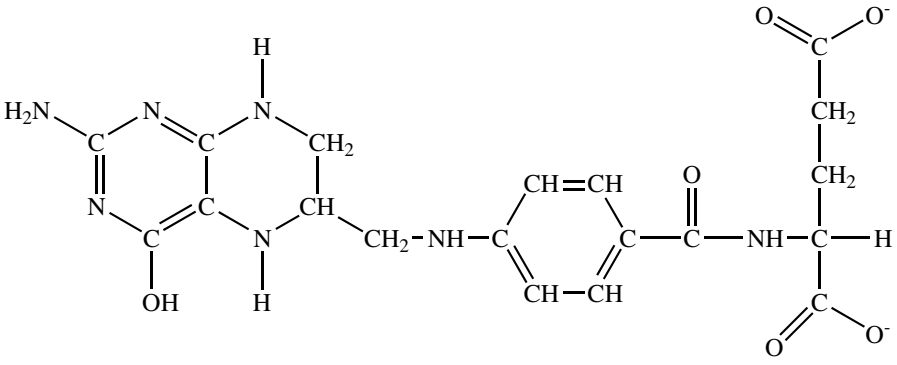
Folic acid is critical to the prevention of malformations of the brain (*anencephaly*) and spine (*spina bifida*). A deficiency of folic acid affects the synthesis of purines — symptoms include gastrointestinal disturbances and anemia. Pregnant women are normally advised to take a vitamin high in folic acid to help in the normal development of the fetus, especially the spine and brain. Sulfa drugs interfere with the formation of folic acid by some pathogens via a form of competitive inhibition.

Pantothenic acid

Pantothenic acid's name derives from a Greek word meaning "from everywhere." As you might expect, then, it has numerous sources, including whole grains, eggs, and meat. Deficiency is virtually unknown. The vitamin is not destroyed by moderate cooking temperatures, but it is not stable at high cooking temperatures. Its structure appears in Figure 10-7.



Folic acid



Tetrahydrofolic acid

Figure 10-6: Structures of folic acid and tetrahydrofolic acid.

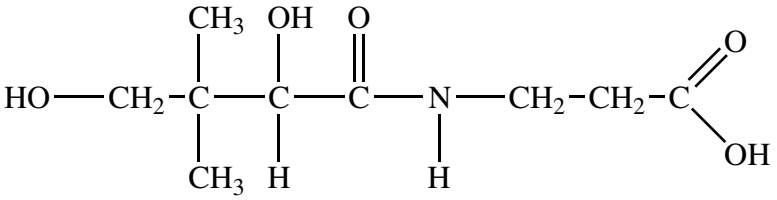


Figure 10-7: Structure of pantothenic acid.