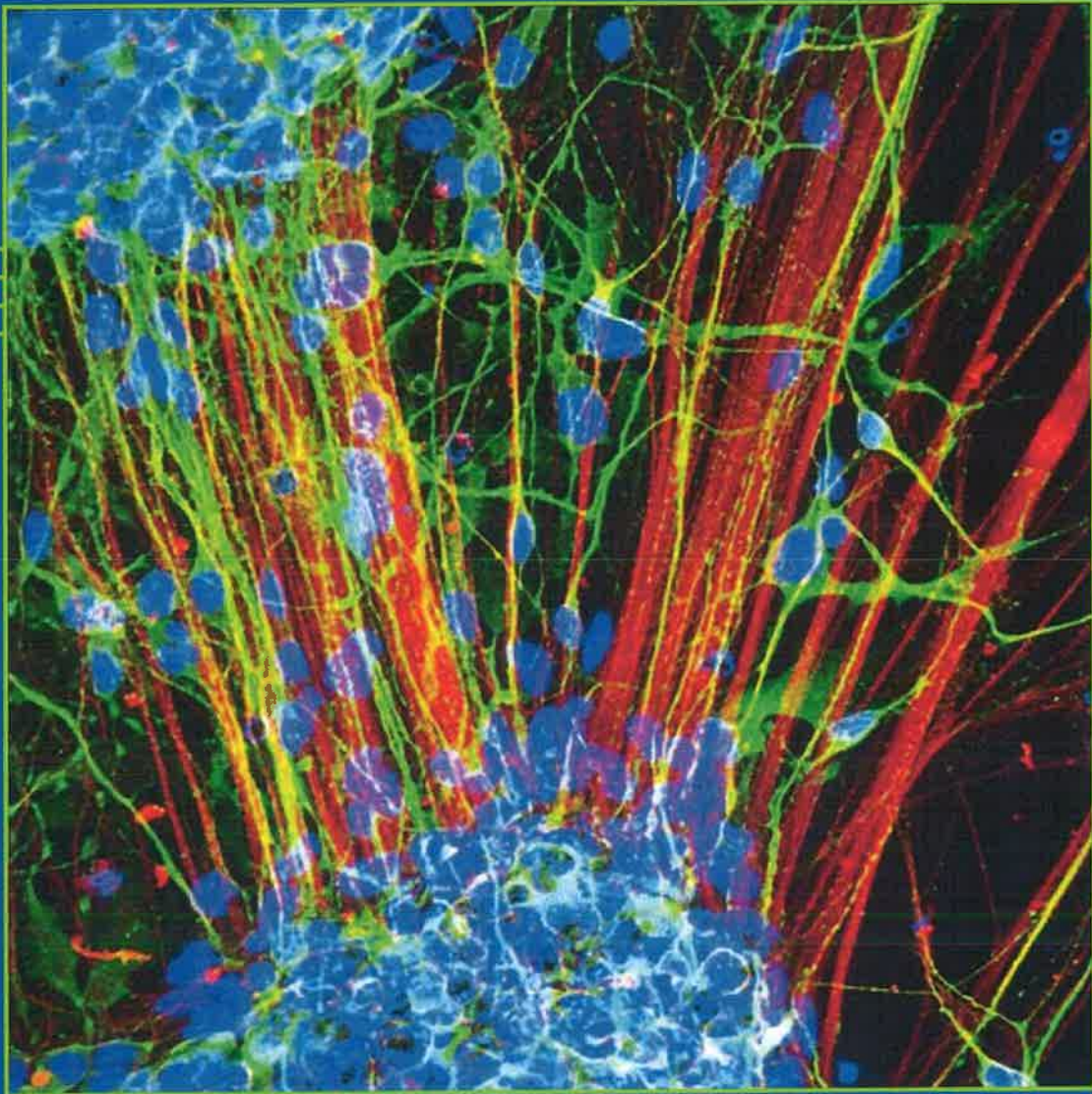




CELL AND MOLECULAR BIOLOGY

CONCEPTS AND EXPERIMENTS

Gerald Karp

7th Edition

Nobel Prizes Awarded for Research in Cell and Molecular Biology Since 1958

<i>Year</i>	<i>Recipient*</i>	<i>Prize</i>	<i>Area of Research</i>	<i>Pages in Text</i>
2012	John B. Gurdon	M & P**	Animal cloning, nuclear reprogramming	513
	Shinya Yamanaka		Cell reprogramming	22, 519
	Brian K. Kobilka	Chemistry	G protein-coupled receptors	621
2011	Robert J. Lefkowitz	M & P	Innate immunity	700
	Bruce A. Beutler			
	Jules A. Hoffmann			
	Ralph M. Steinman			
2009	Venkatraman Ramakrishnan	Chemistry	Ribosome structure and function	479
	Thomas A. Steitz			
	Ada E. Yonath			
	Eliazabeth H. Blackburn	M & P	Telomeres and telomerase	505
	Carol W. Greider			
	Jack W. Szostak			
2008	Francoise Barré-Sinoussi	M & P	Discovery of HIV	24
	Luc Montagnier			
	Harald zur Hausen		Role of HPV in cancer	668
	Martin Chalfie	Chemistry	Discovery and development of GFP	273, 737
	Osamu Shimomura			
	Roger Tsien			
2007	Mario R. Capecchi	M & P	Development of techniques for knockout mice	778
	Martin J. Evans			
	Oliver Smithies			
2006	Andrew Z. Fire	M & P	RNA Interference	455, 780
	Craig C. Mello			
	Roger D. Kornberg	Chemistry	Transcription in eukaryotes	433, 494
2004	Richard Axel	M & P	Olfactory receptors	634
	Linda B. Buck			
	Aaron Ciechanover	Chemistry	Ubiquitin and proteasomes	541
	Avram Hershko			
	Irwin Rose			
2003	Peter Agre	Chemistry	Structure of membrane channels	150, 152
	Roderick MacKinnon			
2002	Sydney Brenner	M & P	Introduction of <i>C. elegans</i> as a model organism	18
	John Sulston			
	H. Robert Horvitz		Apoptosis in <i>C. elegans</i>	657
	John B. Fenn	Chemistry	Electrospray ionization in MS	758
	Koichi Tanaka		MALDI in MS	758
	Kurt Wüthrich		NMR analysis of proteins	57
2001	Leland H. Hartwell	M & P	Control of the cell cycle	576, 611
	Tim Hunt			
	Paul Nurse			
2000	Arvid Carlsson	M & P	Synaptic transmission and signal transduction	168
	Paul Greengard			617
	Eric Kandel			
1999	Günter Blobel	M & P	Protein trafficking	281
1998	Robert Furchgott	M & P	NO as intercellular messenger	655
	Louis Ignarro			
	Ferid Murad			

<i>Year</i>	<i>Recipient*</i>	<i>Prize</i>	<i>Area of Research</i>	<i>Pages in Text</i>
1997	Jens C. Skou	Chemistry	Na ⁺ /K ⁺ -ATPase	157
	Paul Boyer		Mechanism of ATP synthesis	201
	John Walker			
	Stanley B. Prusiner	M & P	Protein nature of prions	66
1996	Rolf M. Zinkernagel	M & P	Recognition of virus-infected cells by the immune system	727
	Peter C. Doherty			
1995	Edward B. Lewis	M & P	Genetic control of embryonic development	EP12
	Christiane Nüsslein-Volhard			
	Eric Wieschaus			
1994	Alfred Gilman	M & P	Structure and function of GTP-binding (G) proteins	624
	Martin Rodbell			
1993	Kary Mullis	Chemistry	Polymerase chain reaction (PCR)	769
	Michael Smith		Site-directed mutagenesis (SDM)	778
	Richard J. Roberts	M & P	Intervening sequences	444
	Phillip A. Sharp			
1992	Edmond Fischer	M & P	Alteration of enzyme activity by phosphorylation/dephosphorylation	115, 627
	Edwin Krebs			
1991	Erwin Neher	M & P	Measurement of ion flux by patch-clamp recording	152
	Bert Sakmann			
1990	Joseph E. Murray	M & P	Organ and cell transplantation in human disease	716, 20
	E. Donnall Thomas			
1989	J. Michael Bishop	M & P	Cellular genes capable of causing malignant transformation	695
	Harold Varmus			
	Thomas R. Cech	Chemistry	Ability of RNA to catalyze reactions	477
	Sidney Altman			
1988	Johann Deisenhofer	Chemistry	Bacterial photosynthetic reaction center	218
	Robert Huber			
	Hartmut Michel			
1987	Susumu Tonegawa	M & P	DNA rearrangements responsible for antibody diversity	713
1986	Rita Levi-Montalcini	M & P	Factors that affect nerve outgrowth	379
	Stanley Cohen			
1985	Michael S. Brown	M & P	Regulation of cholesterol metabolism and endocytosis	319
	Joseph L. Goldstein			
1984	Georges Köhler	M & P	Monoclonal antibodies	782
	Cesar Milstein			
	Niels K. Jerne		Antibody formation	704
1983	Barbara McClintock	M & P	Mobile elements in the genome	408
1982	Aaron Klug	Chemistry	Structure of nucleic acid-protein complexes	79
1980	Paul Berg	Chemistry	Recombinant DNA technology	764
	Walter Gilbert		DNA sequencing technology	771
	Frederick Sanger			
	Baruj Bennacerraf	M & P	Major histocompatibility complex	716
	Jean Dausset			
	George D. Snell			
1978	Werner Arber	M & P	Restriction endonuclease technology	764
	Daniel Nathans			
	Hamilton O. Smith			
	Peter Mitchell	Chemistry	Chemiosmotic mechanism of oxidative phosphorylation	187
1976	D. Carleton Gajdusek	M & P	Prion-based diseases	66
1975	David Baltimore	M & P	Reverse transcriptase and tumor virus activity	694
	Renato Dulbecco			
	Howasrd M. Temin			

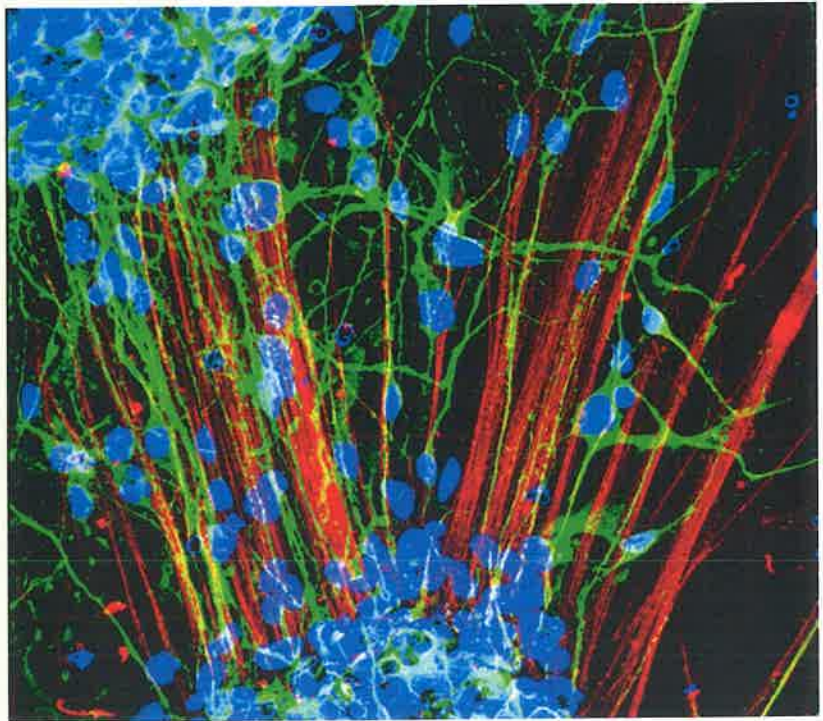
<i>Year</i>	<i>Recipient*</i>	<i>Prize</i>	<i>Area of Research</i>	<i>Pages in Text</i>
1974	Albert Claude Christian de Duve George E. Palade	M & P	Structure and function of internal components of cells	275
1972	Gerald Edelman Rodney R. Porter Christian B. Anfinsen	M & P Chemistry	Immunoglobulin structure Relationship between primary and tertiary structure of proteins	711 63
1971	Earl W. Sutherland	M & P	Mechanism of hormone action and cyclic AMP	627
1970	Bernard Katz Ulf von Euler Luis F. Leloir	M & P Chemistry	Nerve impulse propagation and transmission Role of sugar nucleotides in carbohydrate synthesis	165 285
1969	Max Delbrück Alfred D. Hershey Salvador E. Luria	M & P	Genetic structure of viruses	23, 422
1968	H. Gobind Khorana Marshall W. Nirenberg Robert W. Holley	M & P	Genetic code Transfer RNA structure	462 465
1966	Peyton Rous	M & P	Tumor viruses	694
1965	Francois Jacob Andre M. Lwoff Jacques L. Monod	M & P	Bacterial operons and messenger RNA	484, 428
1964	Dorothy C. Hodgkin	Chemistry	X-ray structure of complex biological molecules	758
1963	John C. Eccles Alan L. Hodgkin Andrew F. Huxley	M & P	Ionic basis of nerve membrane potentials	164
1962	Francis H. C. Crick James D. Watson Maurice H. F. Wilkins John C. Kendrew Max F. Perutz	M & P Chemistry	Three-dimensional structure of DNA Three-dimensional structure of globular proteins	393 58
1961	Melvin Calvin	Chemistry	Biochemistry of CO ₂ assimilation during photosynthesis	226
1960	F. MacFarlane Burnet Peter B. Medawar	M & P	Clonal selection theory of antibody formation	704
1959	Arthur Kornberg Severo Ochoa	M & P	Synthesis of DNA and RNA	550, 463
1958	George W. Beadle Joshua Lederberg Edward L. Tatum Frederick Sanger	M & P Chemistry	Gene expression Primary structure of proteins	427 55

*In a few cases, corecipients whose research was in an area outside of cell and molecular biology have been omitted from this list.

**Medicine and Physiology

This page is intentionally left blank

7th Edition



Cell and Molecular Biology

Concepts and Experiments

Gerald Karp

Chapter 12 was revised in collaboration with

James G. Patton
DEPARTMENT OF BIOLOGICAL SCIENCES
VANDERBILT UNIVERSITY

WILEY

VICE PRESIDENT & PUBLISHER	Kaye Pace
ACQUISITIONS EDITOR	Kevin Witt
MARKETING MANAGER	Clay Stone
ASSOCIATE DIRECTOR OF MARKETING	Amy Scholz
CONTENT MANAGER	Juanita Thompson
ASSISTANT EDITOR	Lauren Stauber
ASSOCIATE CONTENT EDITOR	Lauren Morris
SENIOR PRODUCT DESIGNER	Bonnie Roth
PRODUCTION EDITOR	Sandra Dumas
DESIGN DIRECTOR	Harry Nolan
SENIOR DESIGNER	Madelyn Lesure
PHOTO EDITOR	Jennifer Atkins
PRODUCTION MANAGEMENT SERVICES	Furino Production

COVER PHOTO CREDIT: Courtesy Fred H. Gage and Kristen Brennand

Stethoscope icon repeated throughout text: ©Alan Crawford/istockphoto

This book was typeset in 10.5/12 Adobe Caslon at Aptara and printed and bound by QuadGraphics, Inc. The cover was printed by QuadGraphics, Inc.

Founded in 1807, John Wiley & Sons, Inc. has been a valued source of knowledge and understanding for more than 200 years, helping people around the world meet their needs and fulfill their aspirations. Our company is built on a foundation of principles that include responsibility to the communities we serve and where we live and work. In 2008, we launched a Corporate Citizenship Initiative, a global effort to address the environmental, social, economic, and ethical challenges we face in our business. Among the issues we are addressing are carbon impact, paper specifications and procurement, ethical conduct within our business and among our vendors, and community and charitable support. For more information, please visit our website: www.wiley.com/go/citizenship.

The paper in this book was manufactured by a mill whose forest management programs include sustained yield-harvesting of its timberlands. Sustained yield harvesting principles ensure that the number of trees cut each year does not exceed the amount of new growth.

This book is printed on acid-free paper.

Copyright © 2013, 2010, 2008, 2005, 2002 John Wiley and Sons, Inc.. All rights reserved.

No part of this publication may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying recording, scanning or otherwise, except as permitted under Sections 107 or 108 of the 1976 United States Copyright Act, without either the prior written permission of the Publisher or authorization through payment of the appropriate per-copy fee to the Copyright Clearance Center, 222 Rosewood Drive, Danvers, MA 01923, (978) 750-8400, fax (978) 646-8600. Requests to the Publisher for permission should be addressed to the Permissions Department, John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030-5774, (201) 748-6011, fax (201) 748-6008.

Evaluation copies are provided to qualified academics and professionals for review purposes only, for use in their courses during the next academic year. These copies are licensed and may not be sold or transferred to a third party. Upon completion of the review period, please return the evaluation copy to Wiley. Return instructions and a free of charge return shipping label are available at www.wiley.com/go/returnlabel. Outside of the United States, please contact your local representative.

ISBN 13 978-1118-20673-7
ISBN 13 978-1118-30179-1

Printed in the United States of America.
10 9 8 7 6 5 4 3 2 1

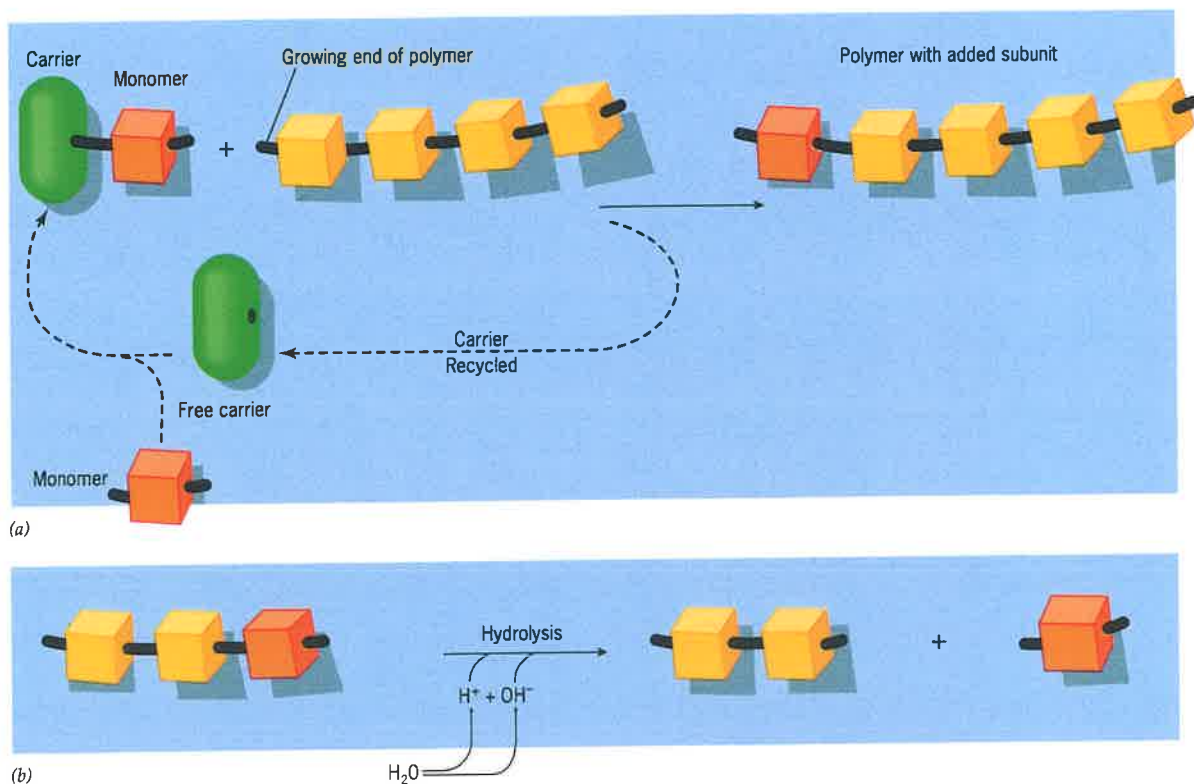


Figure 2.10 Monomers and polymers; polymerization and hydrolysis. (a) Polysaccharides, proteins, and nucleic acids consist of monomers (subunits) linked together by covalent bonds. Free monomers do not simply react with each other to become macromolecules. Rather, each monomer is first activated by attachment

to a carrier molecule that subsequently transfers the monomer to the end of the growing macromolecule. (b) A macromolecule is disassembled by hydrolysis of the bonds that join the monomers together. Hydrolysis is the splitting of a bond by water. All of these reactions are catalyzed by specific enzymes.

include sugars, which are the precursors of polysaccharides; amino acids, which are the precursors of proteins; nucleotides, which are the precursors of nucleic acids; and fatty acids, which are incorporated into lipids.

3. **Metabolic intermediates (metabolites).** The molecules in a cell have complex chemical structures and must be synthesized in a step-by-step sequence beginning with specific starting materials. In the cell, each series of chemical reactions is termed a **metabolic pathway**. The cell starts with compound A and converts it to compound B, then to compound C, and so on, until some functional end product (such as an amino acid building block of a protein) is produced. The compounds formed along the pathways leading to the end products might have no function per se and are called **metabolic intermediates**.
4. **Molecules of miscellaneous function.** This is obviously a broad category of molecules but not as large as you might expect; the vast bulk of the dry weight of a cell is made up of macromolecules and their direct precursors. The molecules of miscellaneous function include such substances as vitamins, which function primarily as adjuncts to proteins; certain steroid or amino acid hormones; molecules

involved in energy storage, such as ATP; regulatory molecules such as cyclic AMP; and metabolic waste products such as urea.

REVIEW

1. What properties of a carbon atom are critical to life?
2. Draw the structures of four different functional groups. How would each of these groups alter the solubility of a molecule in water?

2.5 | Four Types of Biological Molecules

The macromolecules just described can be divided into four types of organic molecules: carbohydrates, lipids, proteins, and nucleic acids. The localization of these molecules in a number of cellular structures is shown in an overview in Figure 2.11.



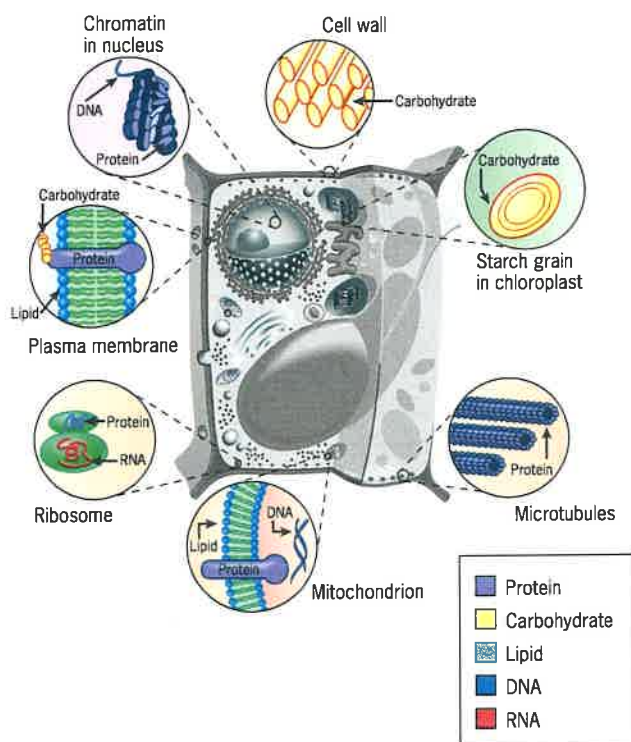


Figure 2.11 An overview of the types of biological molecules that make up various cellular structures.

Carbohydrates

Carbohydrates (or **glycans** as they are often called) include simple sugars (or *monosaccharides*) and all larger molecules constructed of sugar building blocks. Carbohydrates function primarily as stores of chemical energy and as durable building materials for biological construction. Most sugars have the general formula $(\text{CH}_2\text{O})_n$. The sugars of importance in cellular metabolism have values of n that range from 3 to 7. Sugars containing three carbons are known as *trioses*, those with four carbons as *tetroses*, those with five carbons as *pentoses*, those with six carbons as *hexoses*, and those with seven carbons as *heptoses*.

The Structure of Simple Sugars Each sugar molecule consists of a backbone of carbon atoms linked together in a linear array by single bonds. Each of the carbon atoms of the backbone is linked to a single hydroxyl group, except for one that bears a *carbonyl* ($\text{C}=\text{O}$) group. If the carbonyl group is located at an internal position (to form a ketone group), the sugar is a *ketose*, such as fructose, which is shown in Figure 2.12a. If the carbonyl is located at one end of the sugar, it forms an aldehyde group and the molecule is known as an *aldose*, as exemplified by glucose, which is shown in Figure 2.12b–f. Because of their large numbers of hydroxyl groups, sugars tend to be highly water soluble.

Although the straight-chained formulas shown in Figure 2.12a,b are useful for comparing the structures of various sugars, they do not reflect the fact that sugars with five or more carbon atoms undergo a self-reaction (Figure 2.12c) that converts them into a closed, or ring-containing, molecule. The ring forms of sugars are usually depicted as flat (*planar*) structures (Figure 2.12d) lying perpendicular to the plane of the paper with the thickened line situated closest to the reader. The H and OH groups lie parallel to the plane of the paper,

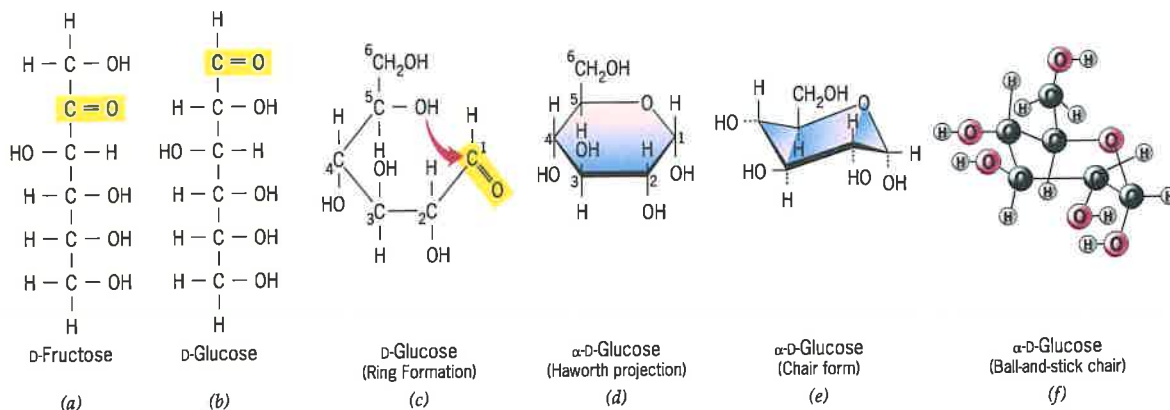


Figure 2.12 The structures of sugars. (a) Straight-chain formula of fructose, a ketohexose [keto, indicating the carbonyl (yellow), is located internally, and hexose because it consists of six carbons]. (b) Straight-chain formula of glucose, an aldohexose (aldo because the carbonyl is located at the end of the molecule). (c) Self-reaction in which glucose is converted from an open chain to a closed ring (a pyranose ring). (d) Glucose is commonly depicted in the form of a flat (planar) ring

lying perpendicular to the page with the thickened line situated closest to the reader and the H and OH groups projecting either above or below the ring. The basis for the designation α -D-glucose is discussed in the following section. (e) The chair conformation of glucose, which depicts its three-dimensional structure more accurately than the flattened ring of part d. (f) A ball-and-stick model of the chair conformation of glucose.

projecting either above or below the ring of the sugar. In actual fact, the sugar ring is not a planar structure, but usually exists in a three-dimensional conformation resembling a chair (Figure 2.12e,f).

Stereoisomerism As noted earlier, a carbon atom can bond with four other atoms. The arrangement of the groups around a carbon atom can be depicted as in Figure 2.13a with the carbon placed in the center of a tetrahedron and the bonded groups projecting into its four corners. Figure 2.13b depicts a molecule of glyceraldehyde, which is the only aldotriose. The second carbon atom of glyceraldehyde is linked to four different groups ($-\text{H}$, $-\text{OH}$, $-\text{CHO}$, and $-\text{CH}_2\text{OH}$). If the four groups bonded to a carbon atom are all different, as in glyceraldehyde, then two possible configura-

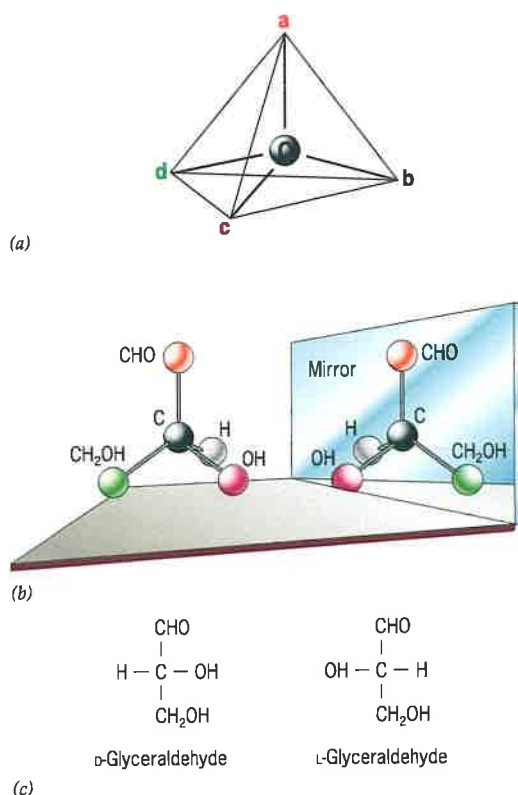


Figure 2.13 Stereoisomerism of glyceraldehyde. (a) The four groups bonded to a carbon atom (labeled a, b, c, and d) occupy the four corners of a tetrahedron with the carbon atom at its center. (b) Glyceraldehyde is the only three-carbon aldose; its second carbon atom is bonded to four different groups ($-\text{H}$, $-\text{OH}$, $-\text{CHO}$, and $-\text{CH}_2\text{OH}$). As a result, glyceraldehyde can exist in two possible configurations that are not superimposable, but instead are mirror images of each other as indicated. These two stereoisomers (or enantiomers) can be distinguished by the configuration of the four groups around the asymmetric (or *chiral*) carbon atom. Solutions of these two isomers rotate plane-polarized light in opposite directions and, thus, are said to be optically active. (c) Straight-chain formulas of glyceraldehyde. By convention, the D-isomer is shown with the OH group on the right.

tions exist that cannot be superimposed on one another. These two molecules (termed *stereoisomers* or *enantiomers*) have essentially the same chemical reactivities, but their structures are mirror images (not unlike a pair of right and left human hands). By convention, the molecule is called D-glyceraldehyde if the hydroxyl group of carbon 2 projects to the right, and L-glyceraldehyde if it projects to the left (Figure 2.13c). Because it acts as a site of stereoisomerism, carbon 2 is referred to as an *asymmetric* carbon atom.

As the backbone of sugar molecules increases in length, so too does the number of asymmetric carbon atoms and, consequently, the number of stereoisomers. Aldotetroses have two asymmetric carbons and thus can exist in four different configurations (Figure 2.14). Similarly, there are 8 different aldopentoses and 16 different aldohexoses. The designation of each of these sugars as D or L is based by convention on the arrangement of groups attached to the asymmetric carbon atom farthest from the aldehyde (the carbon associated with the aldehyde is designated C1). If the hydroxyl group of this carbon projects to the right, the aldose is a D-sugar; if it projects to the left, it is an L-sugar. The enzymes present in living cells can distinguish between the D and L forms of a sugar. Typically, only one of the stereoisomers (such as D-glucose and L-fucose) is used by cells.

The self-reaction in which a straight-chain glucose molecule is converted into a six-membered (*pyranose*) ring was shown in Figure 2.12c. Unlike its precursor in the open chain, the C1 of the ring bears four different groups and thus becomes a new center of asymmetry within the sugar molecule. Because of this extra asymmetric carbon atom, each type of pyranose exists as α and β stereoisomers (Figure 2.15). By convention, the molecule is an α -pyranose when the OH group of the first carbon projects below the plane of the ring, and a β -pyranose when the hydroxyl projects upward. The difference between the two forms has important biological consequences, resulting, for example, in the compact shape of glycogen and starch molecules and the extended conformation of cellulose (discussed later).

Linking Sugars Together Sugars can be joined to one another by covalent **glycosidic bonds** to form larger molecules. Glycosidic bonds form by reaction between carbon atom C1 of one sugar and the hydroxyl group of another sugar, generating a $-\text{C}-\text{O}-\text{C}-$ linkage between the two sugars. As discussed below (and indicated in Figures 2.16 and 2.17), sugars can be joined by quite a variety of different glycosidic

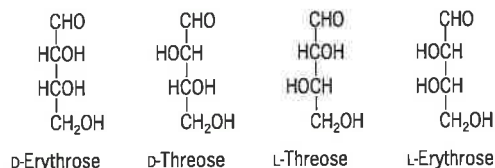


Figure 2.14 Aldotetroses. Because they have two asymmetric carbon atoms, aldotetroses can exist in four configurations.

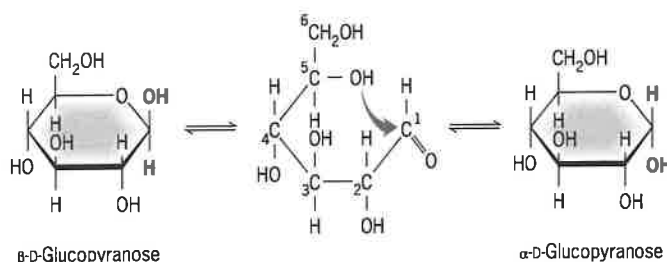


Figure 2.15 Formation of an α - and β -pyranose. When a molecule of glucose undergoes self-reaction to form a pyranose ring (i.e., a six-membered ring), two stereoisomers are generated. The two isomers are in equilibrium with each other through the open-chain form of the

molecule. By convention, the molecule is an α -pyranose when the OH group of the first carbon projects below the plane of the ring, and a β -pyranose when the hydroxyl group projects upward.

bonds. Molecules composed of only two sugar units are *disaccharides* (Figure 2.16). Disaccharides serve primarily as readily available energy stores. Sucrose, or table sugar, is a major component of plant sap, which carries chemical energy from one part of the plant to another. Lactose, present in the milk of most mammals, supplies newborn mammals with fuel for early growth and development. Lactose in the diet is hydrolyzed by the enzyme lactase, which is present in the plasma membranes of the cells that line the intestine. Many people lose this enzyme after childhood and find that eating dairy products causes digestive discomfort.

Sugars may also be linked together to form small chains called **oligosaccharides** (*oligo* = few). Most often such chains are found covalently attached to lipids and proteins, converting them into glycolipids and glycoproteins, respectively. Oligosaccharides are particularly important on the glycolipids and glycoproteins of the plasma membrane, where they project from the cell surface (see Figure 4.4c). Because oligosaccharides may

be composed of many different combinations of sugar units, these carbohydrates can play an informational role; that is, they can serve to distinguish one type of cell from another and help mediate specific interactions of a cell with its surroundings.

Polysaccharides By the middle of the nineteenth century, it was known that the blood of people suffering from diabetes had a sweet taste due to an elevated level of glucose, the key sugar in energy metabolism. Claude Bernard, a prominent French physiologist of the period, was looking for the cause of diabetes by investigating the source of blood sugar. It was assumed at the time that any sugar present in a human or an animal had to have been previously consumed in the diet. Working with dogs, Bernard found that, even if the animals were placed on a diet totally lacking carbohydrates, their blood still contained a normal amount of glucose. Clearly, glucose could be formed in the body from other types of compounds.

After further investigation, Bernard found that glucose enters the blood from the liver. Liver tissue, he found, contains an insoluble polymer of glucose he named **glycogen**. Bernard concluded that various food materials (such as proteins) were carried to the liver where they were chemically converted to glucose and stored as glycogen. Then, as the body needed sugar for fuel, the glycogen in the liver was transformed to glucose, which was released into the bloodstream to satisfy glucose-depleted tissues. In Bernard's hypothesis, the balance between glycogen formation and glycogen breakdown in the liver was the prime determinant in maintaining the relatively constant (*homeostatic*) level of glucose in the blood.

Bernard's hypothesis proved to be correct. The molecule he named glycogen is a type of **polysaccharide**—a polymer of sugar units joined by glycosidic bonds.

Glycogen and Starch: Nutritional Polysaccharides Glycogen is a branched polymer containing only one type of monomer: glucose (Figure 2.17a). Most of the sugar units of a glycogen molecule are joined to one another by $\alpha(1 \rightarrow 4)$ glycosidic bonds (type 2 bond in Figure 2.17a). Branch points contain a sugar joined to three neighboring units rather than to two, as in the unbranched segments of the polymer. The extra neighbor, which forms the branch, is linked by an $\alpha(1 \rightarrow 6)$ glycosidic bond (type 1 bond in Figure 2.17a).

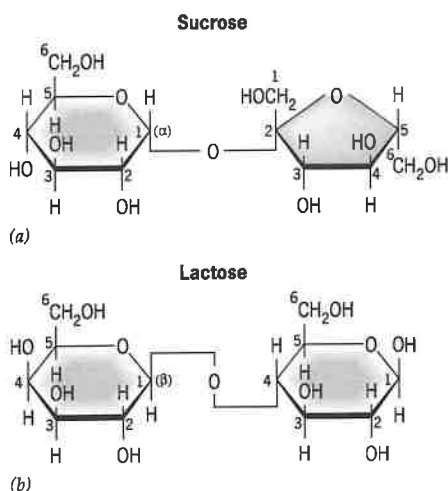


Figure 2.16 Disaccharides. Sucrose and lactose are two of the most common disaccharides. Sucrose is composed of glucose and fructose joined by an $\alpha(1 \rightarrow 2)$ linkage, whereas lactose is composed of glucose and galactose joined by a $\beta(1 \rightarrow 4)$ linkage.

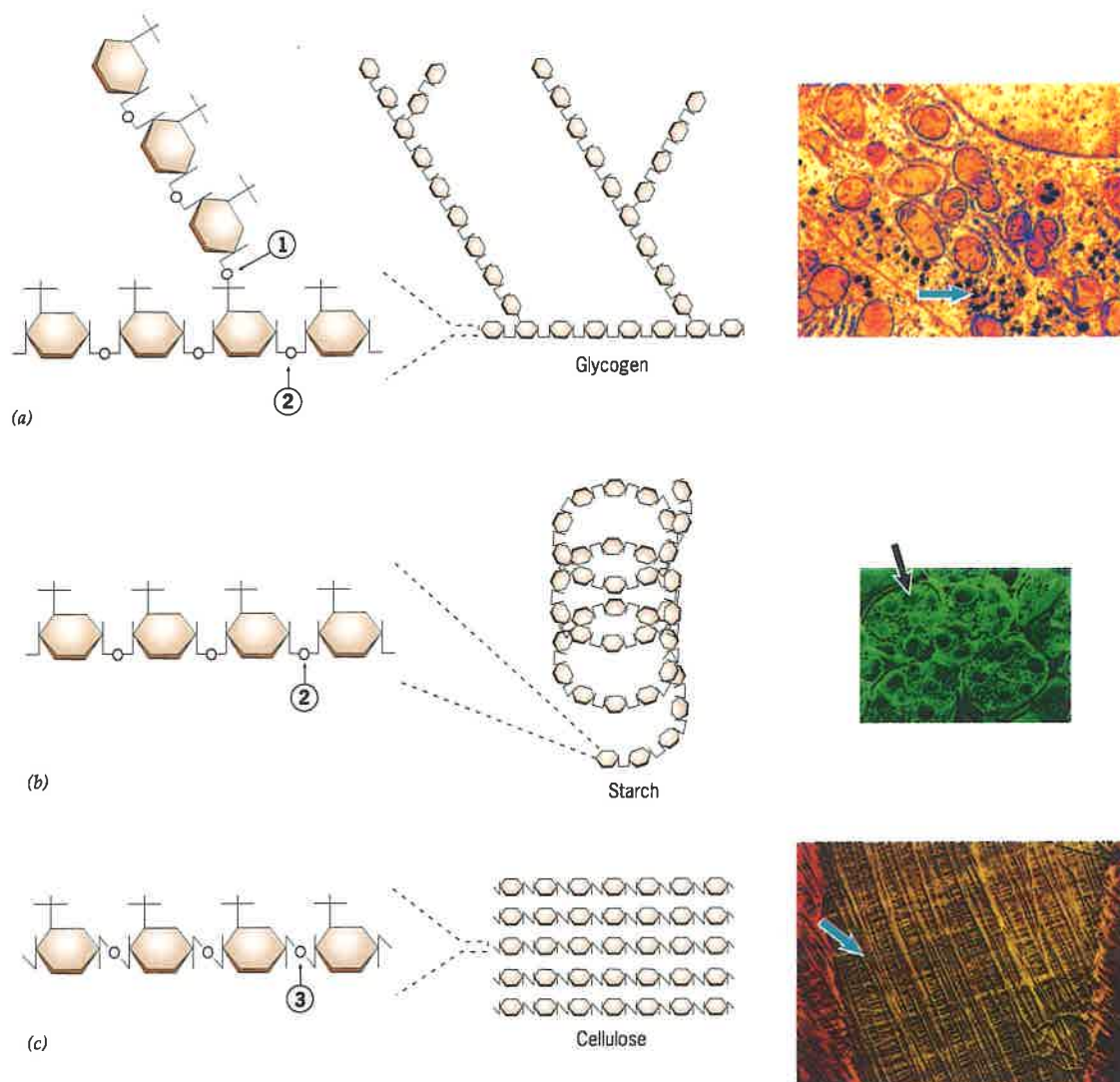


Figure 2.17 Three polysaccharides with identical sugar monomers but dramatically different properties. Glycogen (a), starch (b), and cellulose (c) are each composed entirely of glucose subunits, yet their chemical and physical properties are very different due to the distinct ways that the monomers are linked together (three different types of linkages are indicated by the circled numbers). Glycogen molecules are the most highly branched, starch molecules assume a helical arrangement, and cellulose molecules are unbranched and highly

extended. Whereas glycogen and starch are energy stores, cellulose molecules are bundled together into tough fibers that are suited for their structural role. Colorized electron micrographs show glycogen granules in a liver cell, starch grains (amyloplasts) in a plant seed, and cellulose fibers in a plant cell wall; each is indicated by an arrow. [PHOTO INSETS: (TOP) DON FAWCETT/PHOTO RESEARCHERS, INC.; (CENTER) JEREMY BURGESS/PHOTO RESEARCHERS, INC.; (BOTTOM) BIOPHOTO ASSOCIATES/PHOTO RESEARCHERS, INC.]

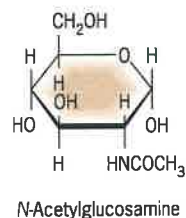
Glycogen serves as a storehouse of surplus chemical energy in most animals. Human skeletal muscles, for example, typically contain enough glycogen to fuel about 30 minutes of moderate activity. Depending on various factors, glycogen typically ranges in molecular weight from about one to four million daltons. When stored in cells, glycogen is highly concentrated in what appears as dark-staining, irregular granules in electron micrographs (Figure 2.17a, right).

Most plants bank their surplus chemical energy in the form of **starch**, which like glycogen is also a polymer of glucose. Potatoes and cereals, for example, consist primarily of starch. Starch is actually a mixture of two different polymers, amylose and amylopectin. Amylose is an unbranched, helical molecule whose sugars are joined by $\alpha(1 \rightarrow 4)$ linkages (Figure 2.17b), whereas amylopectin is branched. Amylopectin differs from glycogen in being much less branched and having

an irregular branching pattern. Starch is stored as densely packed granules, or *starch grains*, which are enclosed in membrane-bound organelles (*plastids*) within the plant cell (Figure 2.17*b*, right). Although animals don't synthesize starch, they possess an enzyme (*amylase*) that readily hydrolyzes it.

Cellulose, Chitin, and Glycosaminoglycans: Structural Polysaccharides Whereas some polysaccharides constitute easily digested energy stores, others form tough, durable structural materials. Cotton and linen, for example, consist largely of **cellulose**, which is the major component of plant cell walls. Cotton textiles owe their durability to the long, unbranched cellulose molecules, which are ordered into side-by-side aggregates to form molecular cables (Figure 2.17*c*, right panel) that are ideally constructed to resist pulling (tensile) forces. Like glycogen and starch, cellulose consists solely of glucose monomers; its properties differ dramatically from these other polysaccharides because the glucose units are joined by $\beta(1 \rightarrow 4)$ linkages (bond 3 in Figure 2.17*c*) rather than $\alpha(1 \rightarrow 4)$ linkages. Ironically, multicellular animals (with rare exception) lack the enzyme needed to degrade cellulose, which happens to be the most abundant organic material on Earth and rich in chemical energy. Animals that “make a living” by digesting cellulose, such as termites and sheep, do so by harboring bacteria and protozoa that synthesize the necessary enzyme, cellulase.

Not all biological polysaccharides consist of glucose monomers. **Chitin** is an unbranched polymer of the sugar *N*-acetylglucosamine, which is similar in structure to glucose but has an acetyl amino group instead of a hydroxyl group bonded to the second carbon atom of the ring.



Chitin occurs widely as a structural material among invertebrates, particularly in the outer covering of insects, spiders, and crustaceans. Chitin is a tough, resilient, yet flexible material not unlike certain plastics. Insects owe much of their success to this highly adaptive polysaccharide (Figure 2.18).

Another group of polysaccharides that has a more complex structure is the **glycosaminoglycans** (or **GAGs**). Unlike other polysaccharides, they have the structure —A—B—A—B—, where A and B represent two different sugars. The best-studied GAG is heparin, which is secreted by cells in the lungs and other tissues in response to tissue injury. Heparin inhibits blood coagulation, thereby preventing the formation of clots that can block the flow of blood to the heart or lungs. Heparin accomplishes this feat by activating an inhibitor (antithrombin) of a key enzyme (thrombin) that is required for blood coagulation. Heparin, which is normally extracted from pig tissue, has been used for decades to prevent blood clots in patients following major surgery. Unlike heparin, most GAGs



Figure 2.18 Chitin is the primary component of the outer skeleton of this grasshopper. (ANTHONY BANNISTER/GALLO IMAGES/© CORBIS)

are found in the spaces that surround cells, and their structure and function are discussed in Section 7.1. The most complex polysaccharides are found in plant cell walls (Section 7.6).

Lipids

Lipids are a diverse group of nonpolar biological molecules whose common properties are their ability to dissolve in organic solvents, such as chloroform or benzene, and their inability to dissolve in water—a property that explains many of their varied biological functions. Lipids of importance in cellular function include fats, steroids, and phospholipids.

Fats Fats consist of a glycerol molecule linked by ester bonds to three fatty acids; the composite molecule is termed a **triacylglycerol** (Figure 2.19*a*). We will begin by considering the structure of **fatty acids**. Fatty acids are long, unbranched hydrocarbon chains with a single carboxyl group at one end (Figure 2.19*b*). Because the two ends of a fatty acid molecule have a very different structure, they also have different properties. The hydrocarbon chain is hydrophobic, whereas the carboxyl group ($-\text{COOH}$), which bears a negative charge at physiological pH, is hydrophilic. Molecules having both hydrophobic and hydrophilic regions are said to be **amphipathic**; such molecules have unusual and biologically important properties. The properties of fatty acids can be appreciated by considering the use of a familiar product: soap, which consists of fatty acids. In past centuries, soaps were made by heating animal fat in strong alkali (NaOH or KOH) to break the bonds between the fatty acids and the glycerol.

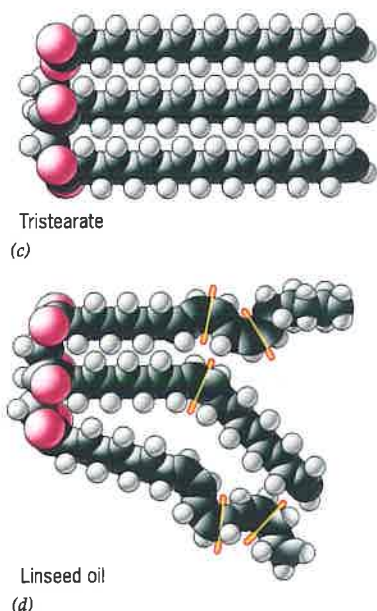
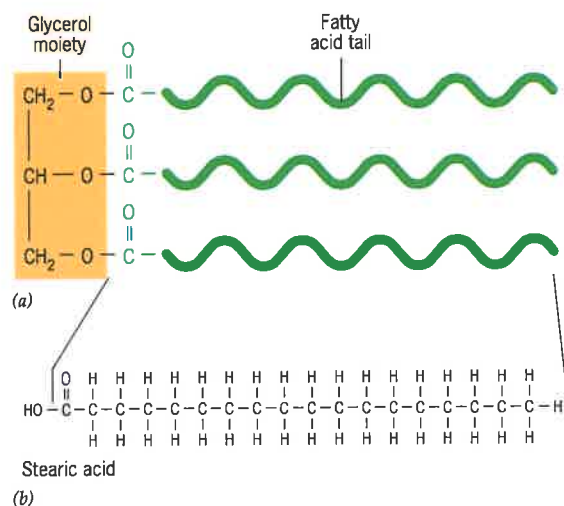


Figure 2.19 Fats and fatty acids. (a) The basic structure of a triacylglycerol (also called a triglyceride or a neutral fat). The glycerol moiety, indicated in orange, is linked by three ester bonds to the carboxyl groups of three fatty acids whose tails are indicated in green. (b) Stearic acid, an 18-carbon saturated fatty acid that is common in animal fats. (c) Space-filling model of tristearate, a triacylglycerol containing three identical stearic acid chains. (d) Space-filling model of linseed oil, a triacylglycerol derived from flax seeds that contains three unsaturated fatty acids (linoleic, oleic, and linolenic acids). The sites of unsaturation, which produce kinks in the molecule, are indicated by the yellow-orange bars.

Today, most soaps are made synthetically. Soaps owe their grease-dissolving capability to the fact that the hydrophobic end of each fatty acid can embed itself in the grease, whereas the hydrophilic end can interact with the surrounding water.

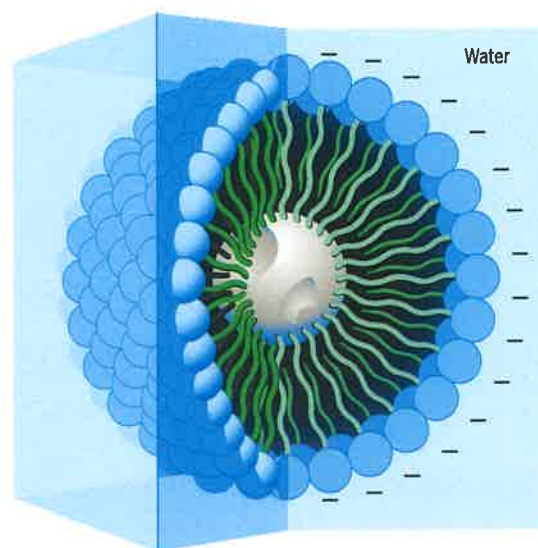
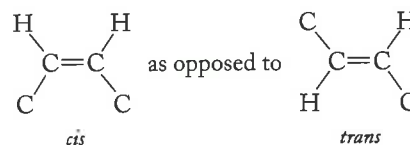


Figure 2.20 Soaps consist of fatty acids. In this schematic drawing of a soap micelle, the nonpolar tails of the fatty acids are directed inward, where they interact with the greasy matter to be dissolved. The negatively charged heads are located at the surface of the micelle, where they interact with the surrounding water. Membrane proteins, which also tend to be insoluble in water, can also be solubilized in this way by extraction of membranes with detergents.

As a result, greasy materials are converted into complexes (*micelles*) that can be dispersed by water (Figure 2.20).

Fatty acids differ from one another in the length of their hydrocarbon chain and the presence or absence of double bonds. Fatty acids present in cells typically vary in length from 14 to 20 carbons. Fatty acids that lack double bonds, such as stearic acid (Figure 2.19b), are described as **saturated**; those possessing double bonds are **unsaturated**. Naturally occurring fatty acids have double bonds in the *cis* configuration. Double bonds (of the *cis* configuration)



produce kinks in a fatty acid chain. Consequently, the more double bonds that fatty acid chains possess, the less effectively these long chains can be packed together. This lowers the temperature at which a fatty acid-containing lipid melts. Tristearate, whose fatty acids lack double bonds (Figure 2.19c), is a common component of animal fats and remains in a solid state well above room temperature. In contrast, the profusion of double bonds in vegetable fats accounts for their liquid state—both in the plant cell and on the grocery shelf—and for their being labeled as “polyunsaturated.” Fats that are liquid at room temperature are described as **oils**. Figure 2.19d shows

the structure of linseed oil, a highly volatile lipid extracted from flax seeds, that remains a liquid at a much lower temperature than does tristearate. Solid shortenings, such as margarine, are formed from unsaturated vegetable oils by chemically reducing the double bonds with hydrogen atoms (a process termed *hydrogenation*). The hydrogenation process also converts some of the *cis* double bonds into *trans* double bonds, which are straight rather than kinked. This process generates partially hydrogenated or *trans*-fats.

A molecule of fat can contain three identical fatty acids (as in Figure 2.19c), or it can be a *mixed fat*, containing more than one fatty acid species (as in Figure 2.19d). Most natural fats, such as olive oil or butterfat, are mixtures of molecules having different fatty acid species.

Fats are very rich in chemical energy; a gram of fat contains over twice the energy content of a gram of carbohydrate (for reasons discussed in Section 3.1). Carbohydrates function primarily as a short-term, rapidly available energy source, whereas fat reserves store energy on a long-term basis. It is estimated that a person of average size contains about 0.5 kilograms (kg) of carbohydrate, primarily in the form of glycogen. This amount of carbohydrate provides approximately 2000 kcal of total energy. During the course of a strenuous day's exercise, a person can virtually deplete his or her body's entire store of carbohydrate. In contrast, the average person contains approximately 16 kg of fat (equivalent to 144,000 kcal of energy), and as we all know, it can take a very long time to deplete our store of this material.

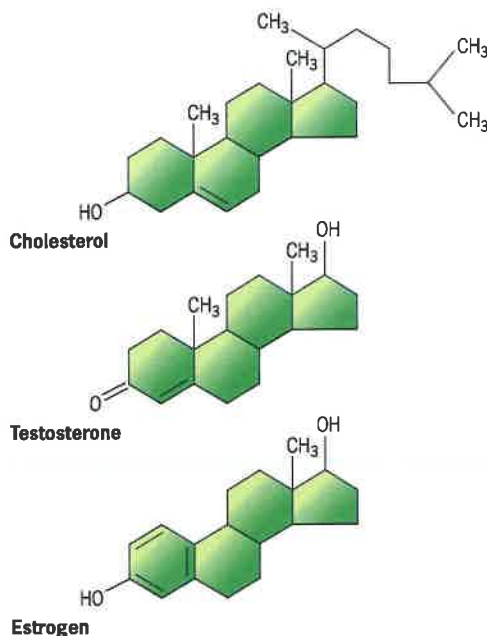


Figure 2.21 The structure of steroids. All steroids share the basic four-ring skeleton. The seemingly minor differences in chemical structure between cholesterol, testosterone, and estrogen generate profound biological differences.

Because they lack polar groups, fats are extremely insoluble in water and are stored in cells in the form of dry lipid droplets. Since lipid droplets do not contain water as do glycogen granules, they represent an extremely concentrated storage fuel. In many animals, fats are stored in special cells (*adipocytes*) whose cytoplasm is filled with one or a few large lipid droplets. Adipocytes exhibit a remarkable ability to change their volume to accommodate varying quantities of fat.

Steroids Steroids are built around a characteristic four-ringed hydrocarbon skeleton. One of the most important steroids is *cholesterol*, a component of animal cell membranes and a precursor for the synthesis of a number of steroid hormones, such as testosterone, progesterone, and estrogen (Figure 2.21). Cholesterol is largely absent from plant cells, which is why vegetable oils are considered “cholesterol-free,” but plant cells may contain large quantities of related compounds.

Phospholipids The chemical structure of a common phospholipid is shown in Figure 2.22. The molecule resembles a fat (triacylglycerol), but has only two fatty acid chains rather than three; it is a *diacylglycerol*. The third hydroxyl of the glycerol backbone is covalently bonded to a phosphate group, which in turn is covalently bonded to a small polar group, such as choline, as shown in Figure 2.22. Thus, unlike fat molecules, phospholipids contain two ends that have very different properties: the end containing the phosphate group has a distinctly hydrophilic character; the other end composed of the two fatty acid tails has a distinctly hydrophobic character. Because phospholipids function primarily in cell membranes, and because the properties of cell membranes depend on their phospholipid components, they will be discussed further in Sections 4.3 and 15.2 in connection with cell membranes.

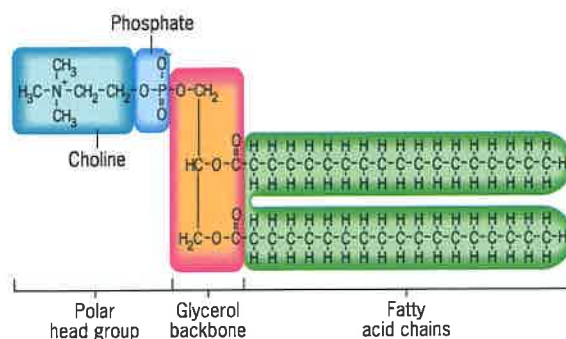


Figure 2.22 The phospholipid phosphatidylcholine. The molecule consists of a glycerol backbone whose hydroxyl groups are covalently bonded to two fatty acids and a phosphate group. The negatively charged phosphate is also bonded to a small, positively charged choline group. The end of the molecule that contains the phosphorylcholine is hydrophilic, whereas the opposite end, consisting of the fatty acid tail, is hydrophobic. The structure and function of phosphatidylcholine and other phospholipids are discussed at length in Section 4.3.

Proteins

Proteins are the macromolecules that carry out virtually all of a cell's activities; they are the molecular tools and machines that make things happen. As enzymes, proteins vastly accelerate the rate of metabolic reactions; as structural cables, proteins provide mechanical support both within cells and outside their perimeters (Figure 2.23*a*); as hormones, growth factors, and gene activators, proteins perform a wide variety of regulatory functions; as membrane receptors and transporters, proteins determine what a cell reacts to and what types of substances enter or leave the cell; as contractile filaments and molecular motors, proteins constitute the machinery for biological movements. Among their many other functions, proteins act as antibodies, serve as toxins, form blood clots, absorb or refract light (Figure 2.23*b*), and transport substances from one part of the body to another.

How can one type of molecule have so many varied functions? The explanation resides in the virtually unlimited molecular structures that proteins, *as a group*, can assume. Each protein, however, has a unique and defined structure that enables it to carry out a particular function. Most importantly, proteins have shapes and surfaces that allow them to interact *selectively* with other molecules. Proteins, in other words, exhibit a high degree of **specificity**. It is possible, for example, for a particular DNA-cutting enzyme to recognize a segment of DNA containing one specific sequence of eight nucleotides, while ignoring all the other 65,535 possible sequences composed of this number of nucleotides.

The Building Blocks of Proteins Proteins are polymers made of amino acid monomers. Each protein has a unique se-

quence of amino acids that gives the molecule its unique properties. Many of the capabilities of a protein can be understood by examining the chemical properties of its constituent amino acids. Twenty different amino acids are commonly used in the construction of proteins, whether from a virus or a human. There are two aspects of amino acid structure to consider: that which is common to all of them and that which is unique to each. We will begin with the shared properties.

The Structures of Amino Acids All amino acids have a carboxyl group and an amino group, which are separated from each other by a single carbon atom, the α -carbon (Figure 2.24*a,b*). In a neutral aqueous solution, the α -carboxyl group loses its proton and exists in a negatively charged state ($-\text{COO}^-$), and the α -amino group accepts a proton and exists in a positively charged state (NH_3^+) (Figure 2.24*b*). We saw on page 44 that carbon atoms bonded to four different groups can exist in two configurations (*stereoisomers*) that cannot be superimposed on one another. Amino acids also have asymmetric carbon atoms. With the exception of glycine, the α -carbon of amino acids bonds to four different groups so that each amino acid can exist in either a D or an L form (Figure 2.25). Amino acids used in the synthesis of a protein on a ribosome are always L-amino acids. The “selection” of L-amino acids must have occurred very early in cellular evolution and has been conserved for billions of years. Microorganisms, however, use D-amino acids in the synthesis of certain small peptides, including those of the cell wall and several antibiotics (e.g., gramicidin A).

During the process of protein synthesis, each amino acid becomes joined to two other amino acids, forming a long, continuous, unbranched polymer called a **polypeptide chain**.



(a)

Figure 2.23 Two examples of the thousands of biological structures composed predominantly of protein. These include (a) feathers, which are adaptations in birds for thermal insulation, flight, and sex



(b)

recognition; and (b) the lenses of eyes, as in this spider, which focus light rays. (A: DARRELL GULIN/GETTY IMAGES; B: THOMAS SHAHAN/PHOTO RESEARCHERS, INC.)

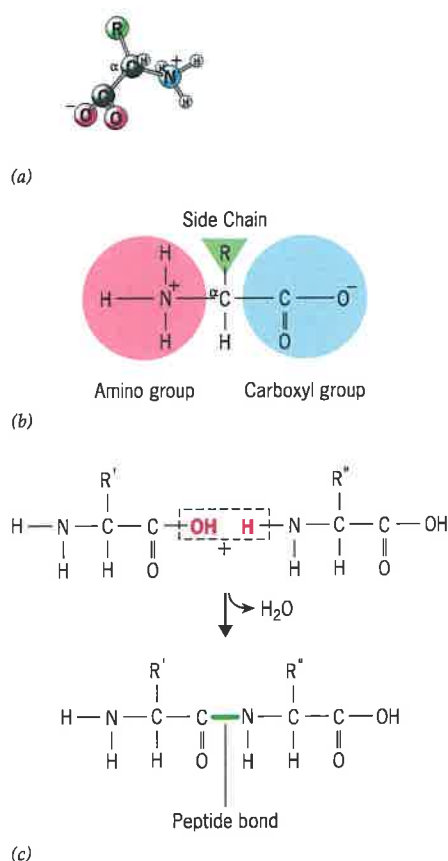


Figure 2.24 Amino acid structure. Ball-and-stick model (a) and chemical formula (b) of a generalized amino acid in which R can be any of a number of chemical groups (see Figure 2.26). (c) The formation of a peptide bond occurs by the condensation of two amino acids, drawn here in the uncharged state. In the cell, this reaction occurs on a ribosome as an amino acid is transferred from a carrier (a tRNA molecule) onto the end of the growing polypeptide chain (see Figure 11.49).

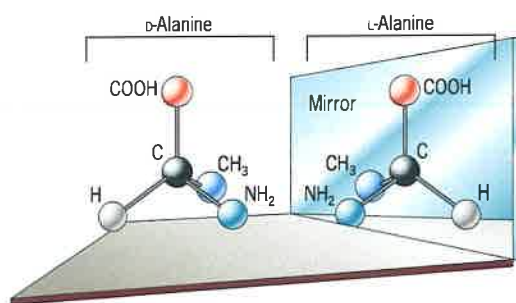
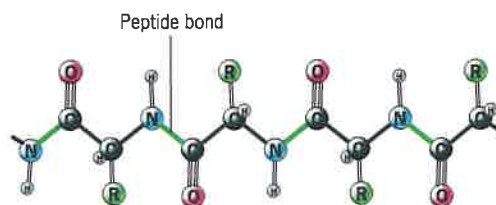


Figure 2.25 Amino acid stereoisomerism. Because the α -carbon of all amino acids except glycine is bonded to four different groups, two stereoisomers can exist. The D and L forms of alanine are shown.

The amino acids that make up a polypeptide chain are joined by **peptide bonds** that result from the linkage of the carboxyl group of one amino acid to the amino group of its neighbor, with the elimination of a molecule of water (Figure 2.24c). A polypeptide chain composed of a string of amino acids joined by peptide bonds has the following backbone:



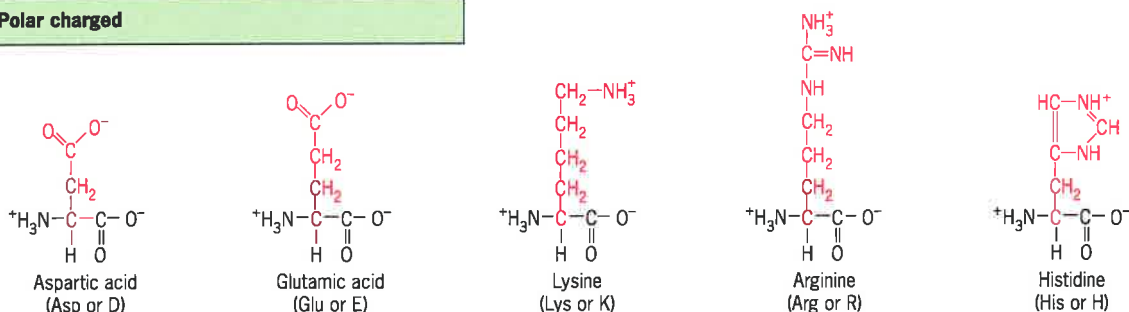
The “average” polypeptide chain contains about 450 amino acids. The longest known polypeptide, found in the muscle protein titin, contains more than 30,000 amino acids. Once incorporated into a polypeptide chain, amino acids are termed **residues**. The residue on one end of the chain, the *N-terminus*, contains an amino acid with a free (unbonded) α -amino group, whereas the residue at the opposite end, the *C-terminus*, has a free α -carboxyl group. In addition to amino acids, many proteins contain other types of components that are added after the polypeptide is synthesized. These include carbohydrates (to form glycoproteins), metal-containing groups (to form metalloproteins) and organic groups (e.g., flavoproteins).

The Properties of the Side Chains The backbone, or main chain, of the polypeptide is composed of that part of each amino acid that is common to all of them. The **side chain** or **R group** (Figure 2.24), bonded to the α -carbon, is highly variable among the 20 building blocks, and it is this variability that ultimately gives proteins their diverse structures and activities. If the various amino acid side chains are considered together, they exhibit a large variety of structural features, ranging from fully charged to hydrophobic, and they can participate in a wide variety of covalent and noncovalent bonds. As discussed in the following chapter, the side chains of the “active sites” of enzymes can facilitate (catalyze) many different organic reactions. The assorted characteristics of the side chains of the amino acids are important in both *intramolecular* interactions, which determine the structure and activity of the molecule, and *intermolecular* interactions, which determine the relationship of a polypeptide with other molecules, including other polypeptides (page 61).

Amino acids are classified on the character of their side chains. They fall roughly into four categories: polar and charged, polar and uncharged, nonpolar, and those with unique properties (Figure 2.26).

1. **Polar, charged.** Amino acids of this group include aspartic acid, glutamic acid, lysine, and arginine. These four amino acids contain side chains that become fully charged; that is, the side chains contain relatively strong organic acids and bases. The ionization reactions of glutamic acid and

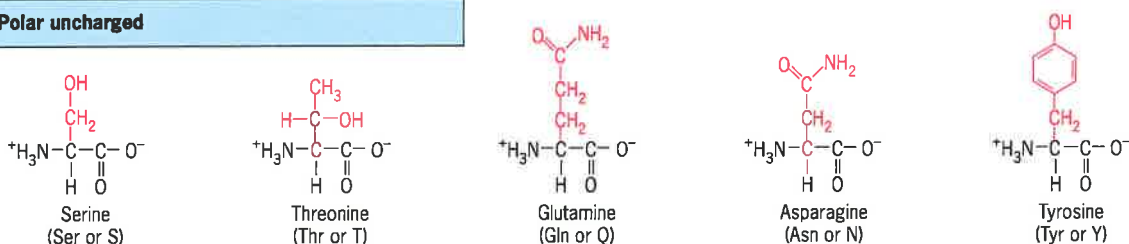
Polar charged



Properties of side chains (R groups):

Hydrophilic side chains act as acids or bases which tend to be fully charged (+ or -) under physiologic conditions. Side chains form ionic bonds and are often involved in chemical reactions.

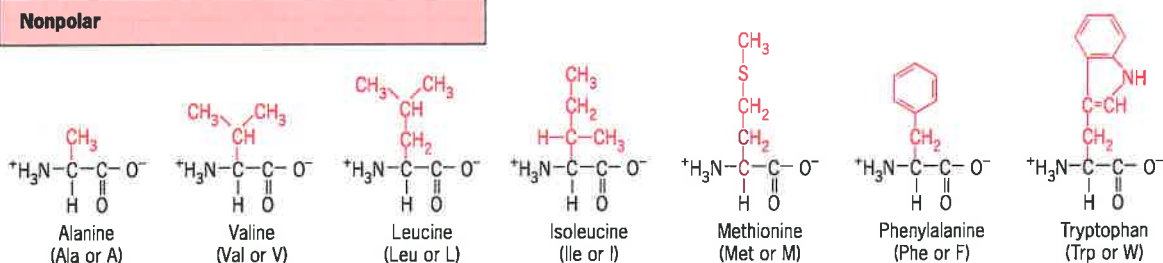
Polar uncharged



Properties of side chains:

Hydrophilic side chains tend to have partial + or - charge allowing them to participate in chemical reactions, form H-bonds, and associate with water.

Nonpolar



Properties of side chains:

Hydrophobic side chain consists almost entirely of C and H atoms. These amino acids tend to form the inner core of soluble proteins, buried away from the aqueous medium. They play an important role in membranes by associating with the lipid bilayer.

Side chains with unique properties



Side chain consists only of hydrogen atom and can fit into either a hydrophilic or hydrophobic environment. Glycine often resides at sites where two polypeptides come into close contact.

Though side chain has polar, uncharged character, it has the unique property of forming a covalent bond with another cysteine to form a disulfide link.

Though side chain has hydrophobic character, it has the unique property of creating kinks in polypeptide chains and disrupting ordered secondary structure.

Figure 2.26 The chemical structure of amino acids. These 20 amino acids represent those most commonly found in proteins and, more specifically, those encoded by DNA. Other amino acids occur as the result of a modification to one of those shown here. The amino acids

are arranged into four groups based on the character of their side chains, as described in the text. All molecules are depicted as free amino acids in their ionized state as they would exist in solution at neutral pH.

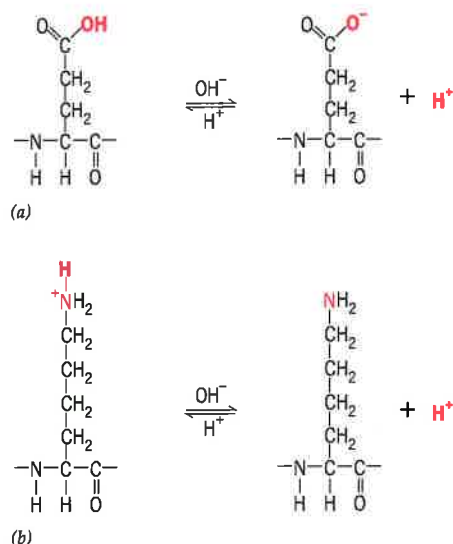


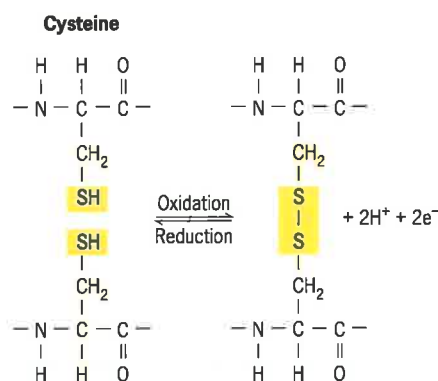
Figure 2.27 The ionization of charged, polar amino acids. (a) The side chain of glutamic acid loses a proton when its carboxylic acid group ionizes. The degree of ionization of the carboxyl group depends on the pH of the medium: the greater the hydrogen ion concentration (the lower the pH), the smaller the percentage of carboxyl groups that are present in the ionized state. Conversely, a rise in pH leads to an increased ionization of the proton from the carboxyl group, increasing the percentage of negatively charged glutamic acid side chains. The pH at which 50 percent of the side chains are ionized and 50 percent are unionized is called the pK , which is 4.4 for the side chain of free glutamic acid. At physiologic pH, virtually all of the glutamic acid residues of a polypeptide are negatively charged. (b) The side chain of lysine becomes ionized when its amino group gains a proton. The greater the hydroxyl ion concentration (the higher the pH), the smaller the percentage of amino groups that are positively charged. The pH at which 50 percent of the side chains of lysine are charged and 50 percent are uncharged is 10.0, which is the pK for the side chain of free lysine. At physiologic pH, virtually all of the lysine residues of a polypeptide are positively charged. Once incorporated into a polypeptide, the pK of a charged group can be greatly influenced by the surrounding environment.

lysine are shown in Figure 2.27. At physiologic pH, the side chains of these amino acids are almost always present in the fully charged state. Consequently, they are able to form ionic bonds with other charged species in the cell. For example, the positively charged arginine residues of histone proteins are linked by ionic bonds to the negatively charged phosphate groups of DNA (see Figure 2.3). Histidine is also considered a polar, charged amino acid, though in most cases it is only partially charged at physiologic pH. In fact, because of its ability to gain or lose a proton in physiologic pH ranges, histidine is a particularly important residue in the active site of many proteins (as in Figure 3.13).

- Polar, uncharged.** The side chains of these amino acids have a partial negative or positive charge and thus can

form hydrogen bonds with other molecules including water. These amino acids are often quite reactive. Included in this category are asparagine and glutamine (the amides of aspartic acid and glutamic acid), threonine, serine, and tyrosine.

- Nonpolar.** The side chains of these amino acids are hydrophobic and are unable to form electrostatic bonds or interact with water. The amino acids of this category are alanine, valine, leucine, isoleucine, tryptophan, phenylalanine, and methionine. The side chains of the nonpolar amino acids generally lack oxygen and nitrogen. They vary primarily in size and shape, which allows one or another of them to pack tightly into a particular space within the core of a protein, associating with one another as the result of van der Waals forces and hydrophobic interactions.
- The other three amino acids**—glycine, proline, and cysteine—have unique properties that separate them from the others. The side chain of glycine consists of only a hydrogen atom, and glycine is a very important amino acid for just this reason. Owing to its lack of a side chain, glycine residues provide a site where the backbones of two polypeptides (or two segments of the same polypeptide) can approach one another very closely. In addition, glycine is more flexible than other amino acids and allows parts of the backbone to move or form a hinge. Proline is unique in having its α -amino group as part of a ring (making it an imino acid). Proline is a hydrophobic amino acid that does not readily fit into an ordered secondary structure, such as an α helix (page 55), often producing kinks or hinges. Cysteine contains a reactive sulfhydryl ($-SH$) group and is often covalently linked to another cysteine residue, as a **disulfide** ($-SS-$) **bridge**.



Disulfide bridges often form between two cysteines that are distant from one another in the polypeptide backbone or even in two separate polypeptides. Disulfide bridges help stabilize the intricate shapes of proteins, particularly those present outside of cells where they are subjected to added physical and chemical stress.

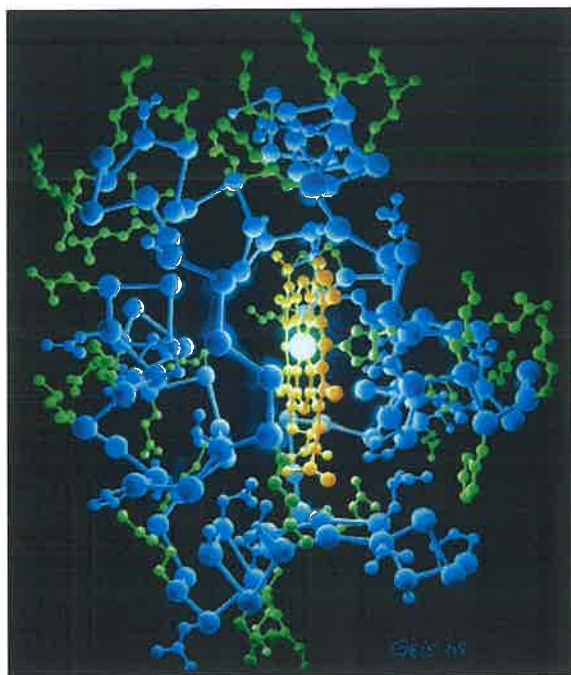
Not all of the amino acids described in this section are found in all proteins, nor are the various amino acids distributed in an equivalent manner. A number of other amino acids

are also found in proteins, but they arise by alterations to the side chains of the 20 basic amino acids *after* their incorporation into a polypeptide chain. For this reason they are called **posttranslational modifications (PTMs)**. Dozens of different types of PTMs have been documented. The most widespread and important PTM is the reversible addition of a phosphate group to a serine, threonine, or tyrosine residue. Lysine acetylation is another widespread and important PTM affecting thousands of proteins in a mammalian cell. PTMs can generate dramatic changes in the properties and function of a protein, most notably by modifying its three-dimensional structure, level of activity, localization within the cell, life span, and/or its interactions with other molecules. The presence or absence of a single phosphate group on a key regulatory protein has the potential to determine whether or not a cell will behave as a cancer cell or a normal cell. Because of PTMs, a single polypeptide can exist as a number of distinct biological molecules.

The ionic, polar, or nonpolar character of amino acid side chains is very important in protein structure and function. Most soluble (i.e., nonmembrane) proteins are constructed so that the polar residues are situated at the surface of the molecule where they can associate with the surrounding water and contribute to the protein's solubility in aqueous solution

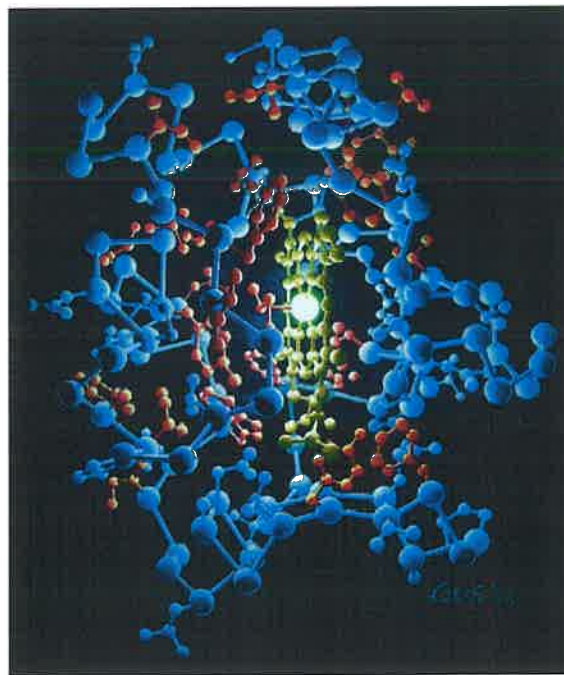
(Figure 2.28a). In contrast, the nonpolar residues are situated predominantly in the core of the molecule (Figure 2.28b). The hydrophobic residues of the protein interior are often tightly packed together, creating a type of three-dimensional jigsaw puzzle in which water molecules are generally excluded. Hydrophobic interactions among the nonpolar side chains of these residues are a driving force during protein folding (page 64) and contribute substantially to the overall stability of the protein. In many enzymes, reactive polar groups project into the nonpolar interior, giving the protein its catalytic activity. For example, a nonpolar environment can enhance ionic interactions between charged groups that would be lessened by competition with water in an aqueous environment. Some reactions that might proceed at an imperceptibly slow rate in water can occur in millionths of a second within the protein.

The Structure of Proteins Nowhere in biology is the intimate relationship between form and function better illustrated than with proteins. The structure of most proteins is completely defined and predictable. Each amino acid in one of these giant macromolecules is located at a specific site within the structure, giving the protein the precise shape and reactivity required for the job at hand. Protein structure can be described at several levels of organization, each emphasizing a



(a)

Figure 2.28 Disposition of hydrophilic and hydrophobic amino acid residues in the soluble protein cytochrome c. (a) The hydrophilic side chains, which are shown in green, are located primarily at the surface of the protein where they contact the surrounding aqueous medium. (b) The hydrophobic residues, which are shown in red, are located



(b)

primarily within the center of the protein, particularly in the vicinity of the central heme group. (ILLUSTRATION, IRVING GEIS. IMAGE FROM IRVING GEIS COLLECTION/HOWARD HUGHES MEDICAL INSTITUTE. RIGHTS OWNED BY HHMI. REPRODUCED BY PERMISSION ONLY.)