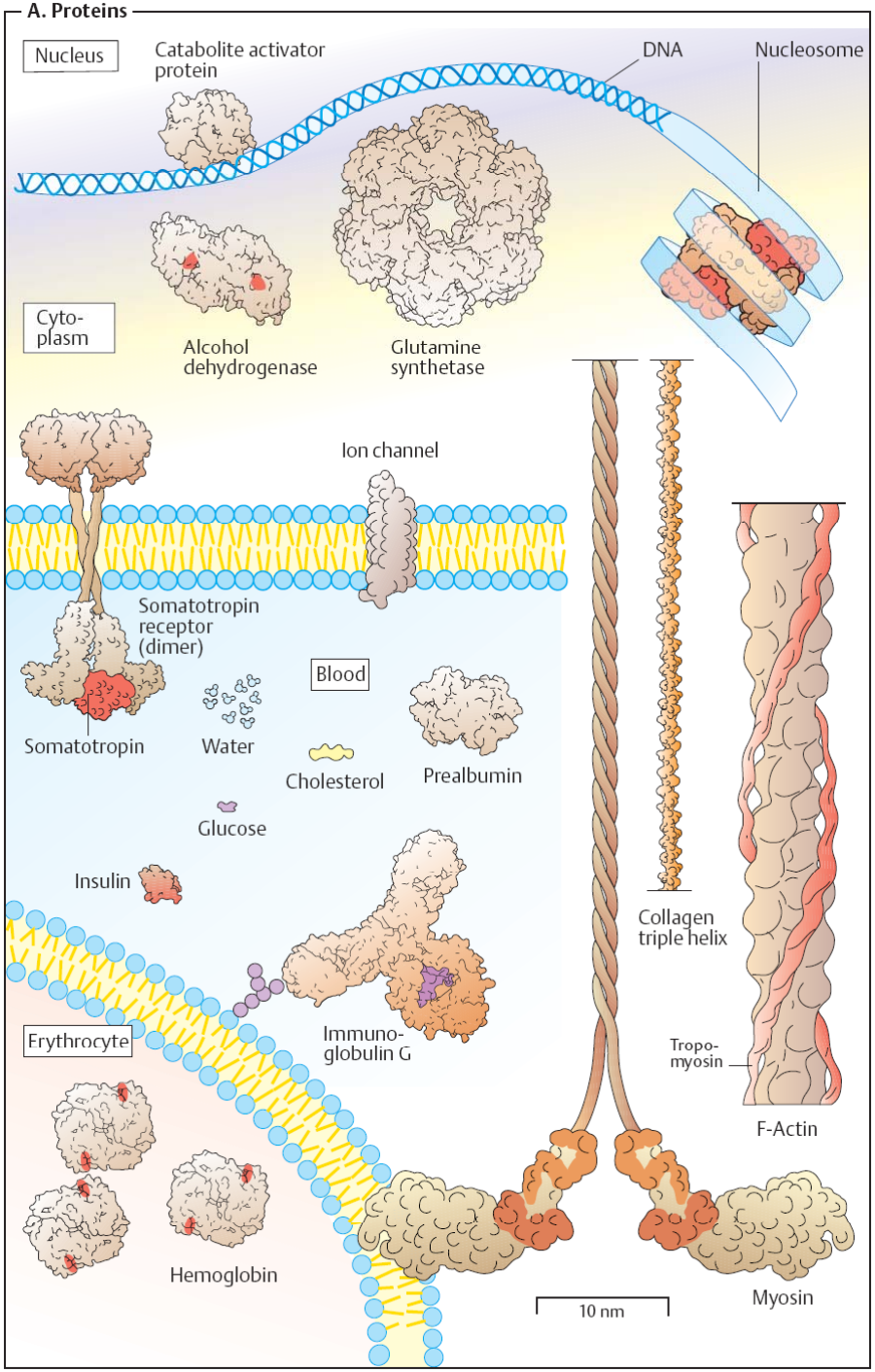
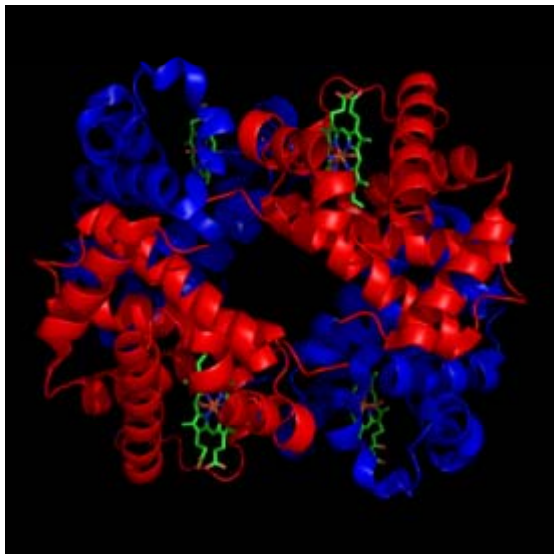
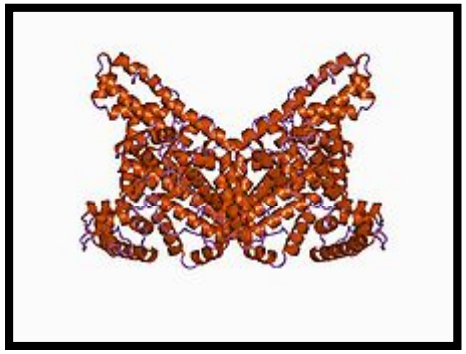
AMINO ACIDS, PEPTIDES AND PROTEINS





Hemoglobin



Albumin

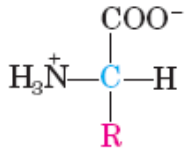


FIGURE 3–2 General structure of an amino acid. This structure is common to all but one of the α-amino acids. (Proline, a cyclic amino acid, is the exception.) The R group or side chain (red) attached to the α carbon (blue) is different in each amino acid.

The Amino Acid Residues in Proteins Are L Stereoisomers

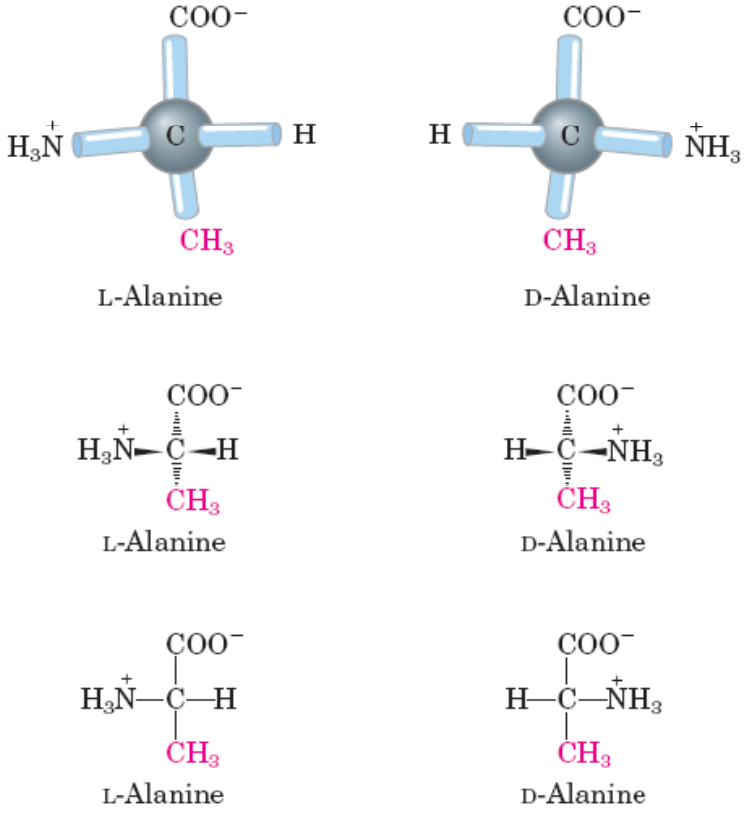


FIGURE 3–3 Stereoisomerism in α-amino acids. (a) The two stereoisomers of alanine, L- and D-alanine, are nonsuperimposable mirror images of each other (enantiomers). (b, c) Two different conventions for showing the configurations in space of stereoisomers. In perspective formulas (b) the solid wedge-shaped bonds project out of the plane of the paper, the dashed bonds behind it. In projection formulas (c) the horizontal bonds are assumed to project out of the plane of the paper, the vertical bonds behind. However, projection formulas are often used casually and are not always intended to portray a specific stereochemical configuration.

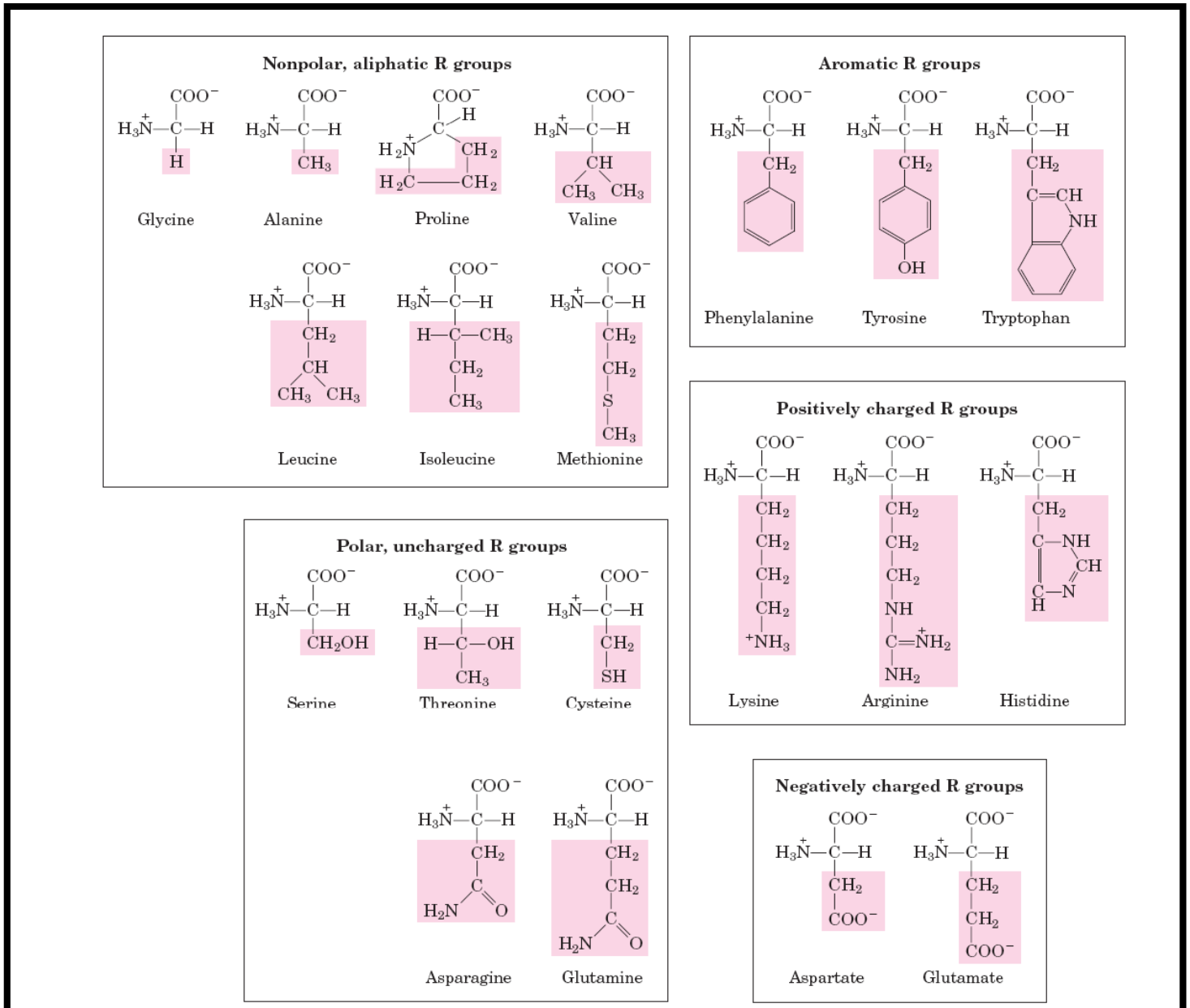
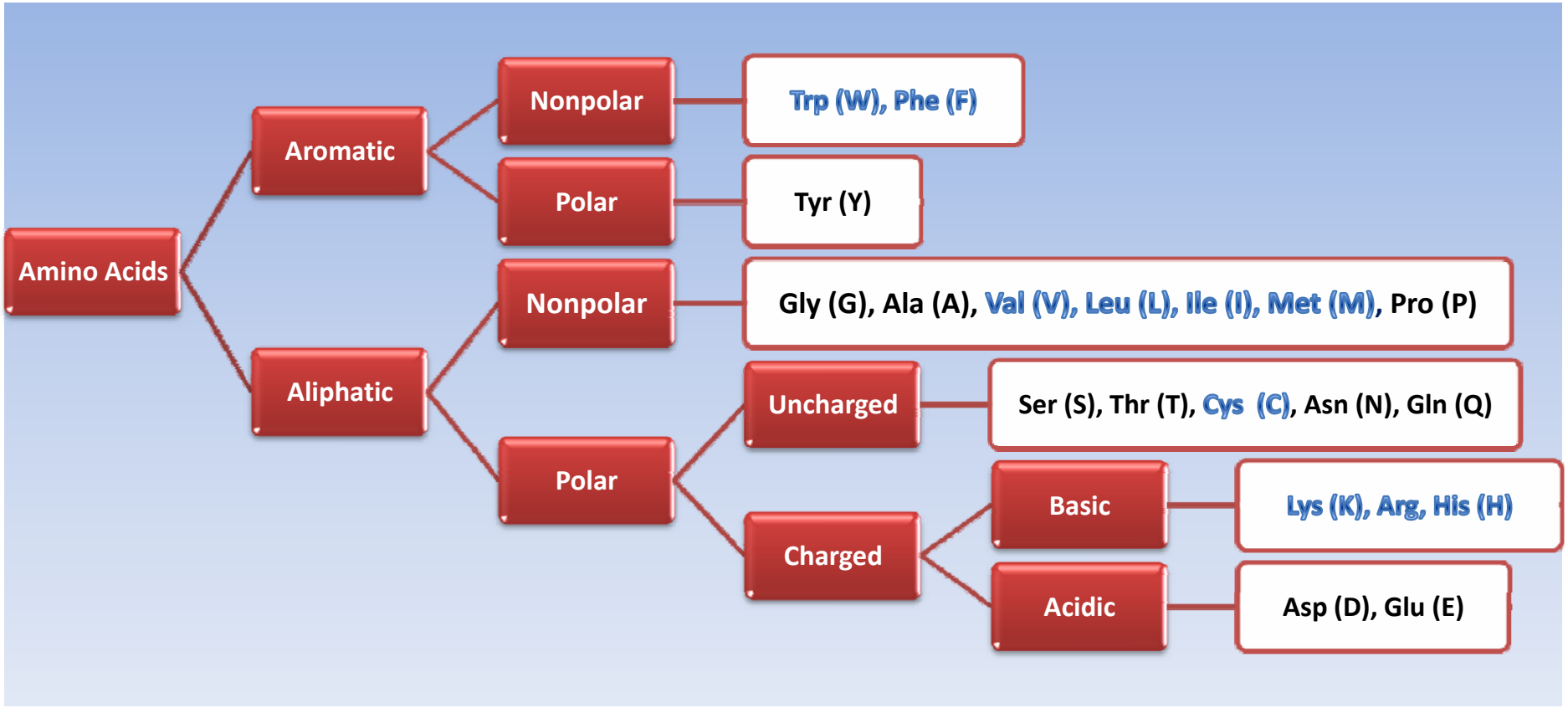
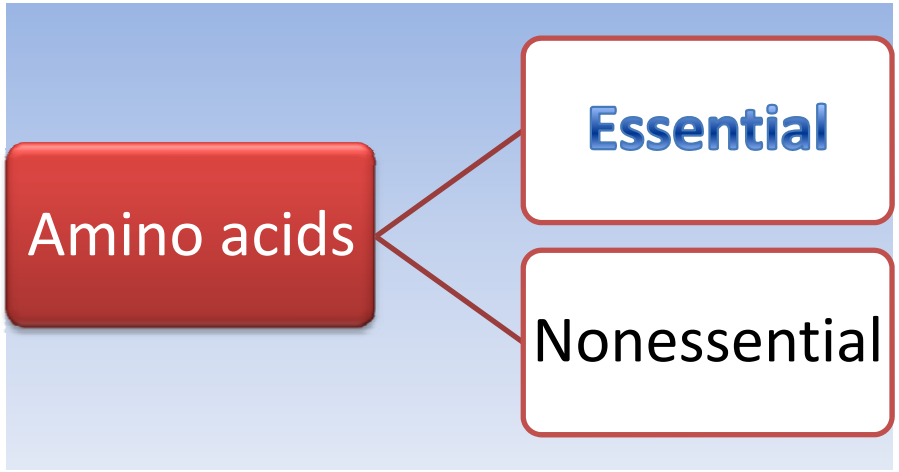


FIGURE 3–5 **The 20 common amino acids of proteins.** The structural formulas show the state of ionization that would predominate at pH 7.0. The unshaded portions are those common to all the amino acids; the portions shaded in red are the R groups. Although the R group of histidine is shown uncharged, its pK_a (see Table 3–1) is such that a small but significant fraction of these groups are positively charged at pH 7.0.





- Standard amino acids (20)
- Prolin (P) → imino acid
- Ile and Thr → Two chiral center
- Gly → without chiral center

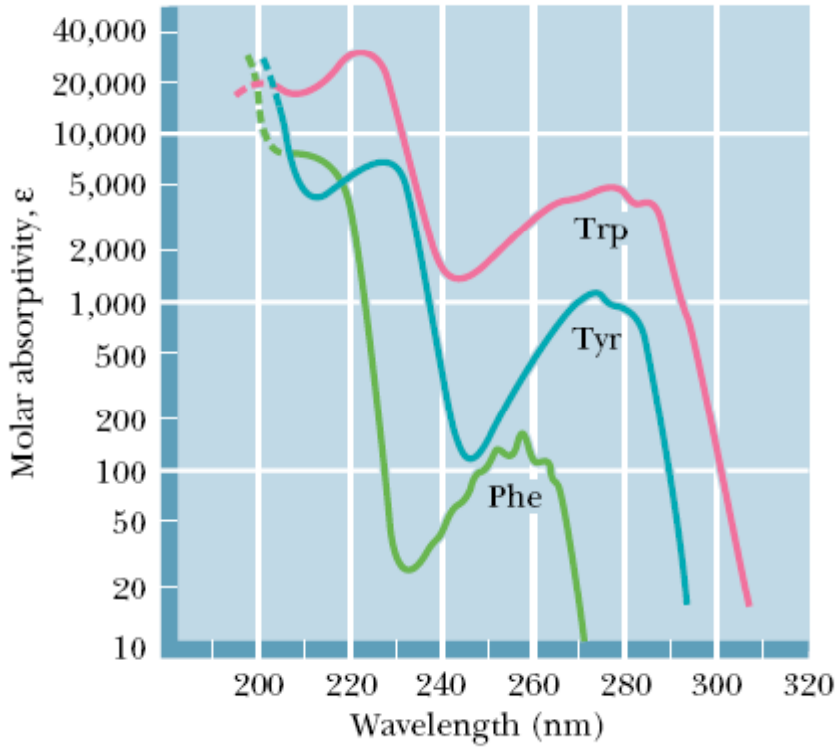
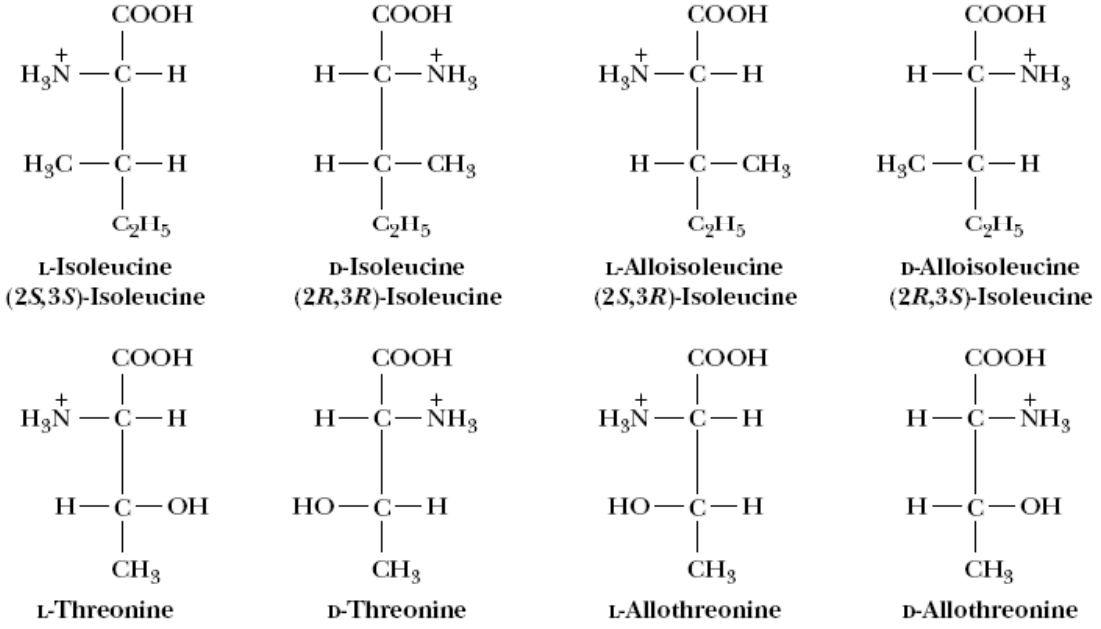
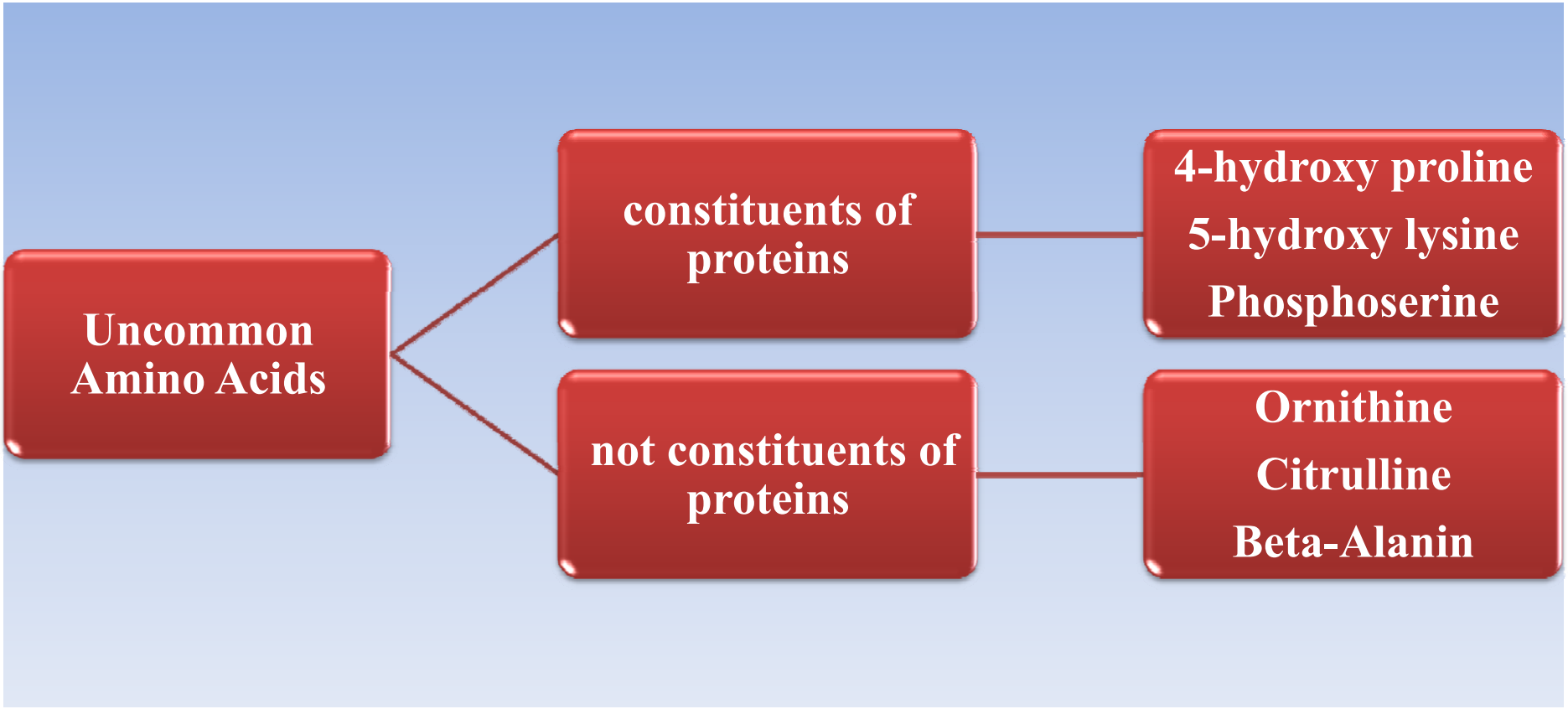


FIGURE 4.15 • The ultraviolet absorption spectra of the aromatic amino acids at pH 6. *(From Wetlaufer, D.B., 1962. Ultraviolet spectra of proteins and amino acids.* Advances in Protein Chemistry *17:303–390.)*

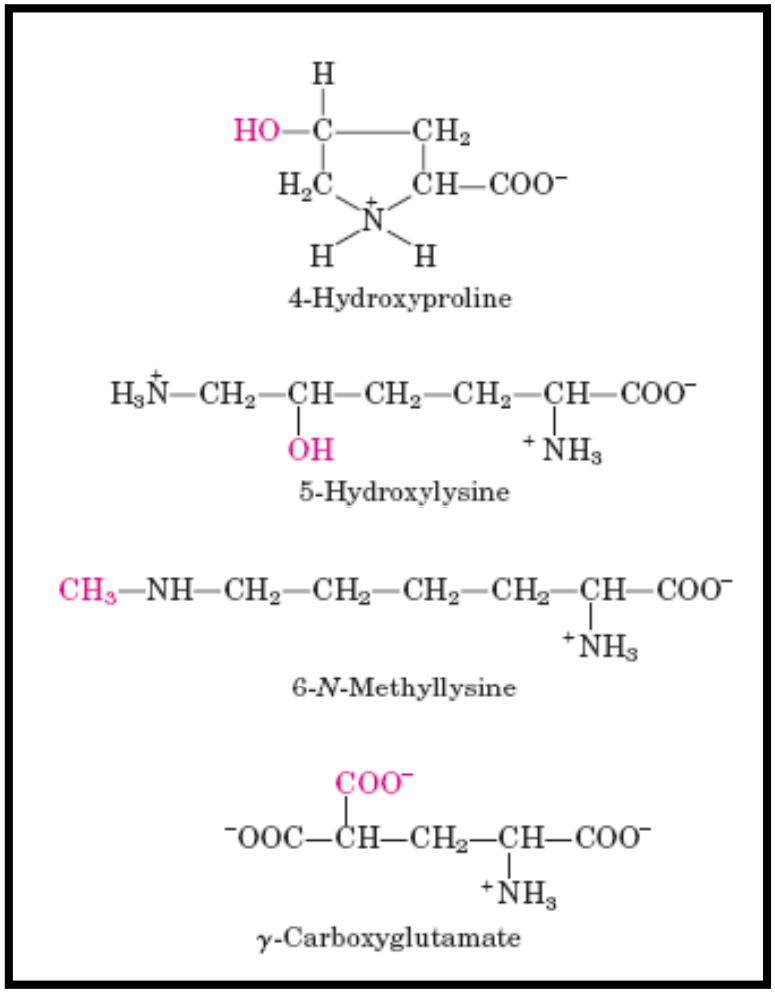
Tyr, Trp → Abs (280 nm)
Phe → Abs (260 nm)

FIGURE 4.14 • The stereoisomers of isoleucine and threonine. The structures at the far left are the naturally occurring isomers.





Uncommon amino acids created by modification of common residues already incorporated into a polypeptide.



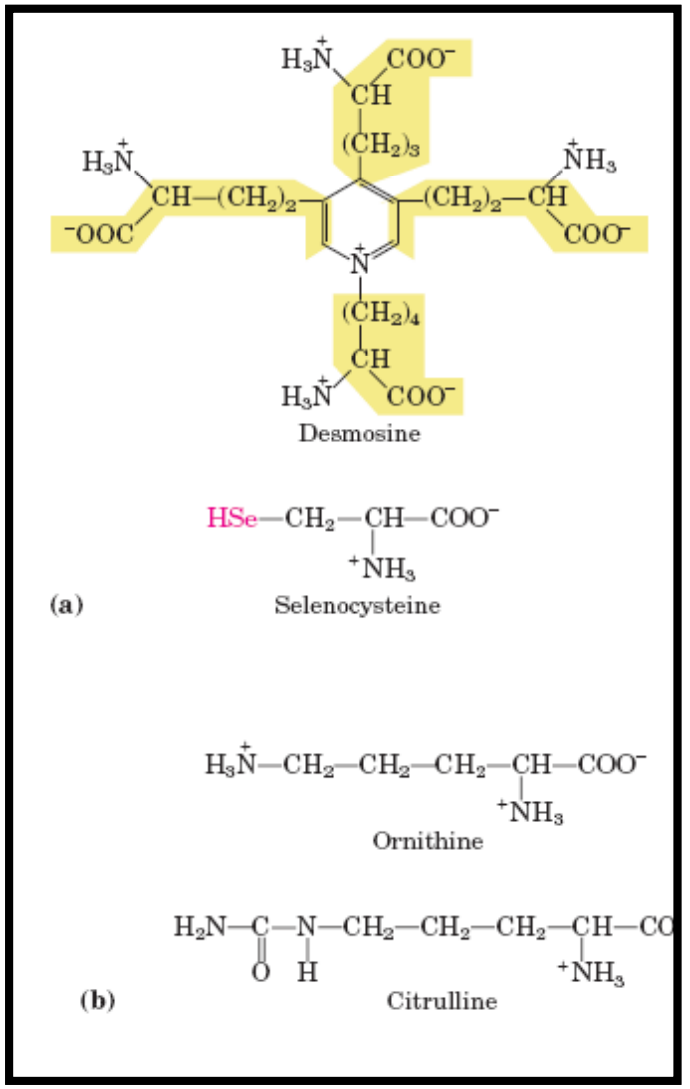


FIGURE 3–8 **Uncommon amino acids.** (a) Some uncommon amino acids found in proteins. All are derived from common amino acids. Extra functional groups added by modification reactions are shown in red. Desmosine is formed from four Lys residues (the four carbon backbones are shaded in yellow). Note the use of either numbers or Greek letters to identify the carbon atoms in these structures. (b) Ornithine and citrulline, which are not found in proteins, are intermediates in the biosynthesis of arginine and in the urea cycle.

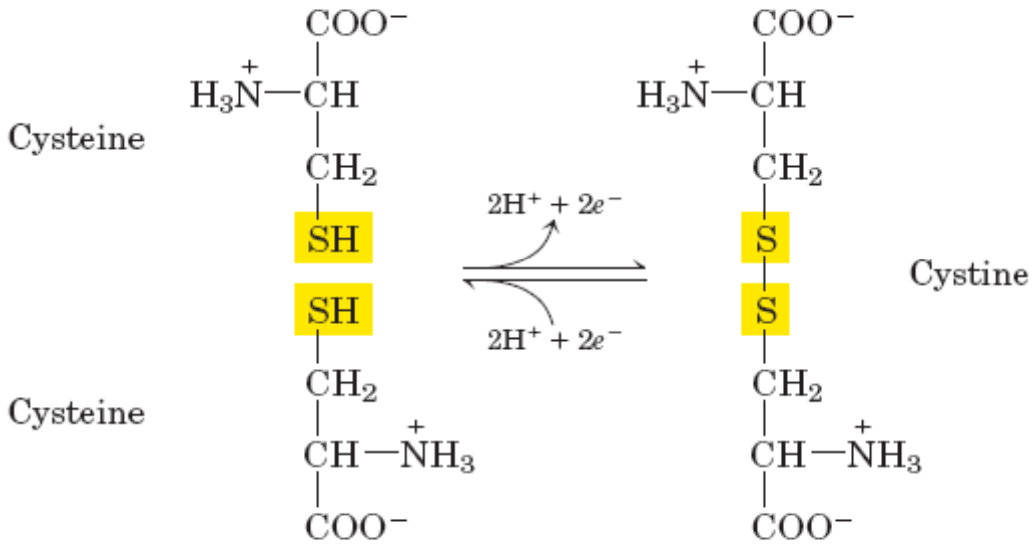
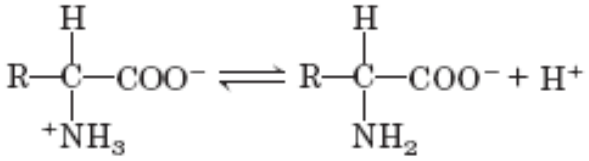
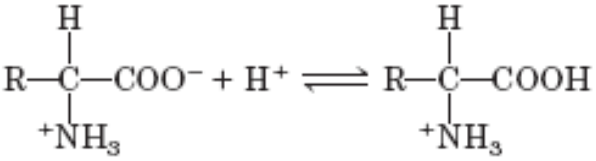


FIGURE 3–7 Reversible formation of a disulfide bond by the oxidation of two molecules of cysteine. Disulfide bonds between Cys residues stabilize the structures of many proteins.



Zwitterion

or a base (proton acceptor):



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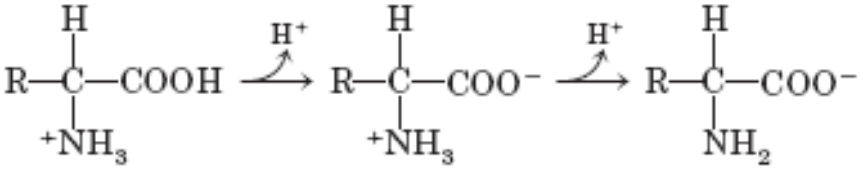


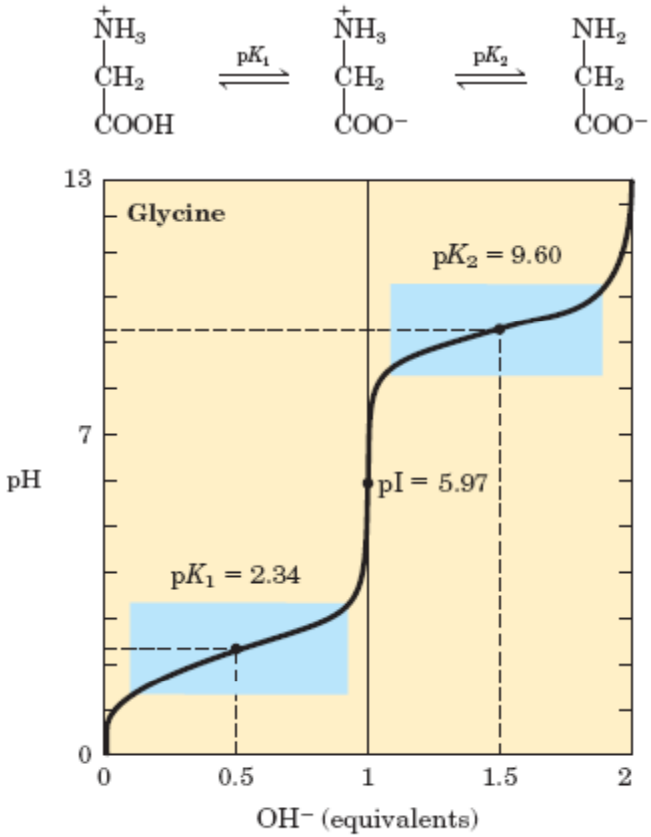
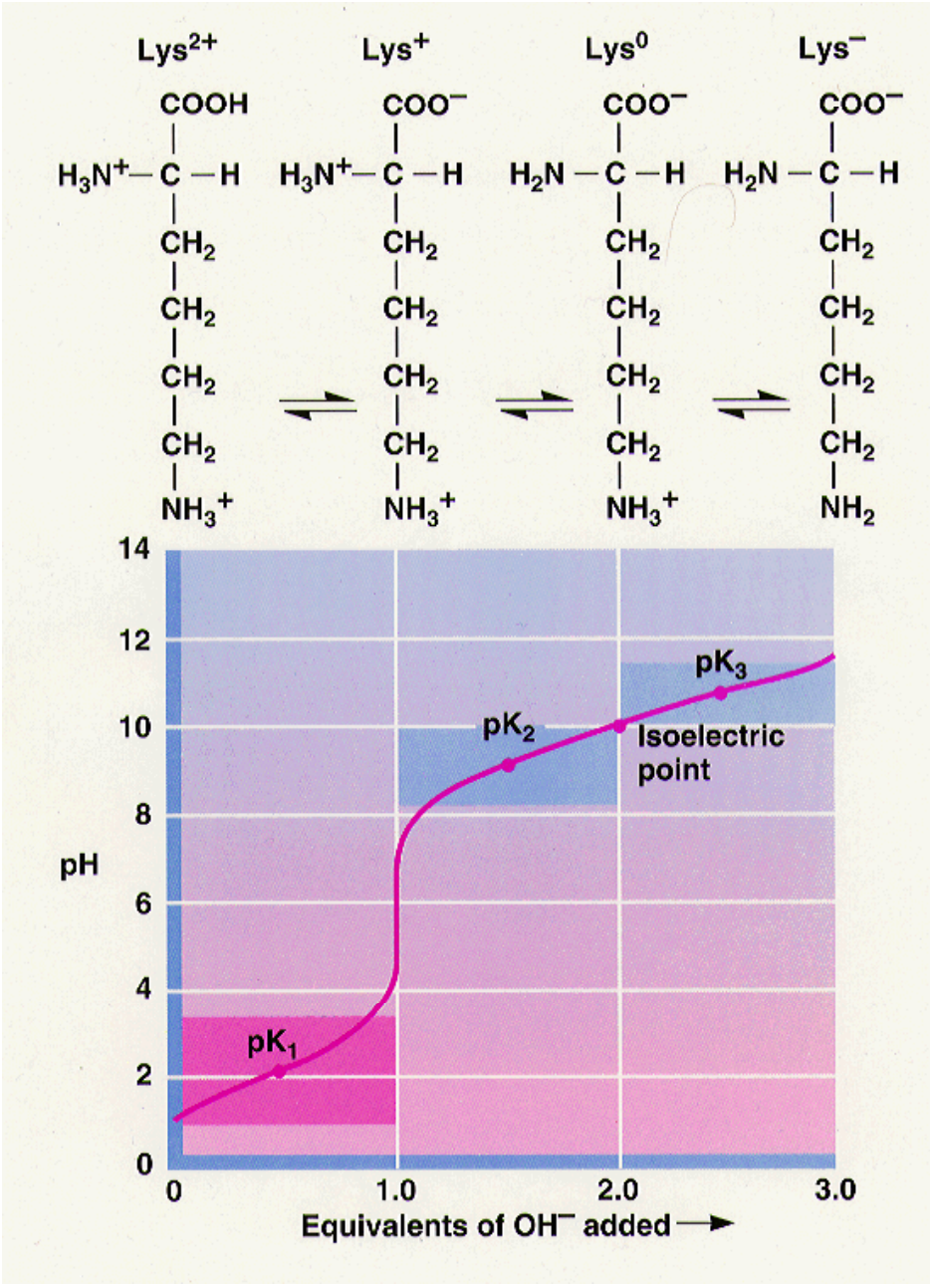


FIGURE 3–10 **Titration of an amino acid.** Shown here is the titration curve of 0.1 M glycine at 25 °C. The ionic species predominating at key points in the titration are shown above the graph. The shaded boxes, centered at about $pK_1 = 2.34$ and $pK_2 = 9.60$, indicate the regions of greatest buffering power.


Lys2+
Lys+
Lys0
Lys−
COOH
COO−
H3N+—C—H
H2N—C—H
CH2
NH3+
NH2
14
12
10
8
6
4
2
0
pH
pK1
pK2
pK3
Isoelectric point
0
1.0
2.0
3.0
Equivalents of OH− added

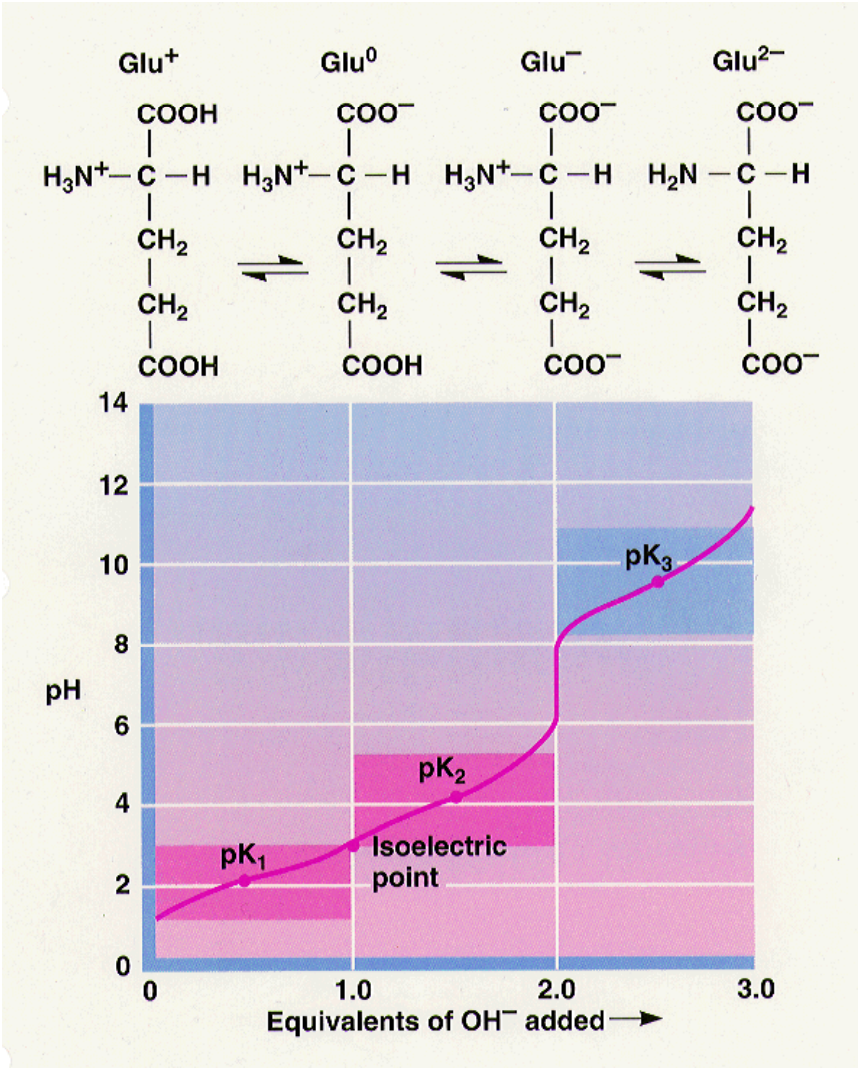

Glu+
Glu0
Glu−
Glu2−
COOH
COO−
H3N+—C—H
H2N—C—H
CH2
14
12
10
8
6
4
2
0
pH
pK1
pK2
pK3
Isoelectric point
0
1.0
2.0
3.0
Equivalents of OH− added

TABLE 3–1 Properties and Conventions Associated with the Common Amino Acids Found in Proteins

Amino acid	Abbreviation/ symbol	M_r	pK_a values: pK_1 (—COOH)	pK_2 ($—NH_3^+$)	pK_R (R group)	pI	Hydropathy index*	Occurrence in proteins (%)†
Nonpolar, aliphatic R groups								
Glycine	Gly G	75	2.34	9.60		5.97	−0.4	7.2
Alanine	Ala A	89	2.34	9.69		6.01	1.8	7.8
Proline	Pro P	115	1.99	10.96		6.48	1.6	5.2
Valine	Val V	117	2.32	9.62		5.97	4.2	6.6
Leucine	Leu L	131	2.36	9.60		5.98	3.8	9.1
Isoleucine	Ile I	131	2.36	9.68		6.02	4.5	5.3
Methionine	Met M	149	2.28	9.21		5.74	1.9	2.3
Aromatic R groups								
Phenylalanine	Phe F	165	1.83	9.13		5.48	2.8	3.9
Tyrosine	Tyr Y	181	2.20	9.11	10.07	5.66	−1.3	3.2
Tryptophan	Trp W	204	2.38	9.39		5.89	−0.9	1.4
Polar, uncharged R groups								
Serine	Ser S	105	2.21	9.15		5.68	−0.8	6.8
Threonine	Thr T	119	2.11	9.62		5.87	−0.7	5.9
Cysteine	Cys C	121	1.96	10.28	8.18	5.07	2.5	1.9
Asparagine	Asn N	132	2.02	8.80		5.41	−3.5	4.3
Glutamine	Gln Q	146	2.17	9.13		5.65	−3.5	4.2
Positively charged R groups								
Lysine	Lys K	146	2.18	8.95	10.53	9.74	−3.9	5.9
Histidine	His H	155	1.82	9.17	6.00	7.59	−3.2	2.3
Arginine	Arg R	174	2.17	9.04	12.48	10.76	−4.5	5.1
Negatively charged R groups								
Aspartate	Asp D	133	1.88	9.60	3.65	2.77	−3.5	5.3
Glutamate	Glu E	147	2.19	9.67	4.25	3.22	−3.5	6.3

*A scale combining hydrophobicity and hydrophilicity of R groups; it can be used to measure the tendency of an amino acid to seek an aqueous environment (− values) or a hydrophobic environment (+ values). See Chapter 11. From Kyte, J. & Doolittle, R.F. (1982) A simple method for displaying the hydropathic character of a protein. *J. Mol. Biol.* **157**, 105–132.

†Average occurrence in more than 1,150 proteins. From Doolittle, R.F. (1989) Redundancies in protein sequences. In *Prediction of Protein Structure and the Principles of Protein Conformation* (Fasman, G.D., ed.), pp. 599–623, Plenum Press, New York.

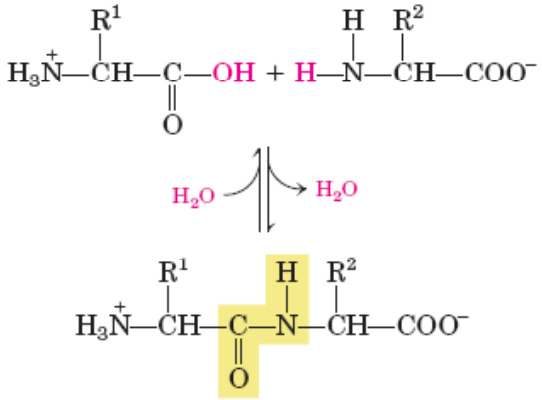


FIGURE 3–13 Formation of a peptide bond by condensation. The α-amino group of one amino acid (with R^2 group) acts as a nucleophile to displace the hydroxyl group of another amino acid (with R^1 group), forming a peptide bond (shaded in yellow). Amino groups are good nucleophiles, but the hydroxyl group is a poor leaving group and is not readily displaced. At physiological pH, the reaction shown does not occur to any appreciable extent.

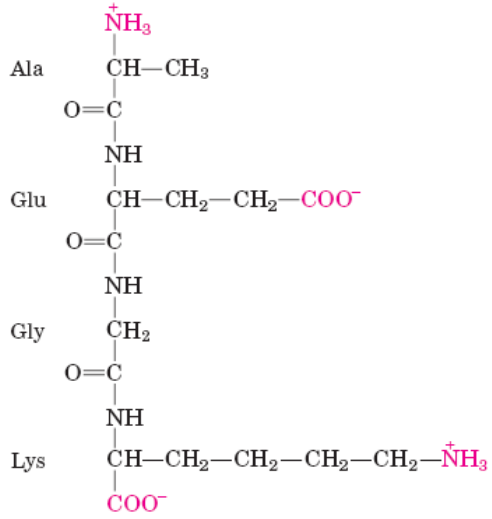


FIGURE 3–15 Alanylglutamylglycyllysine. This tetrapeptide has one free α-amino group, one free α-carboxyl group, and two ionizable R groups. The groups ionized at pH 7.0 are in red.

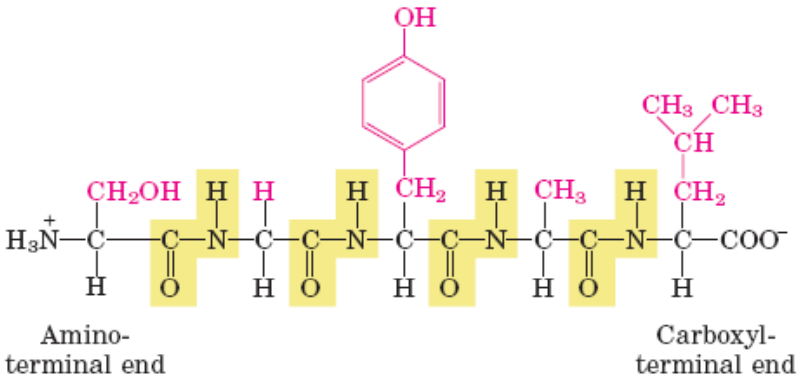


FIGURE 3–14 The pentapeptide serylglycyltyrosylalanylleucine, or Ser–Gly–Tyr–Ala–Leu. Peptides are named beginning with the amino-terminal residue, which by convention is placed at the left. The peptide bonds are shaded in yellow; the R groups are in red.

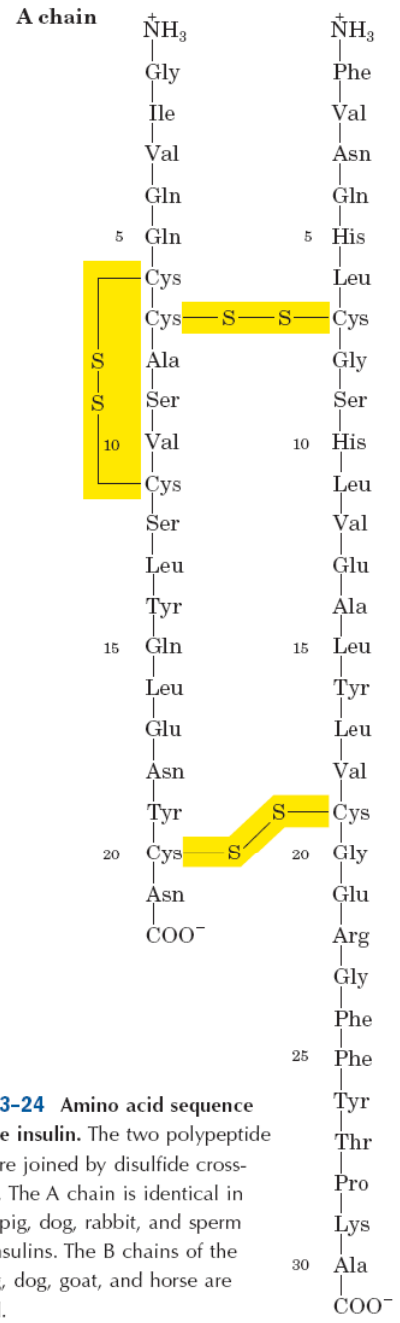
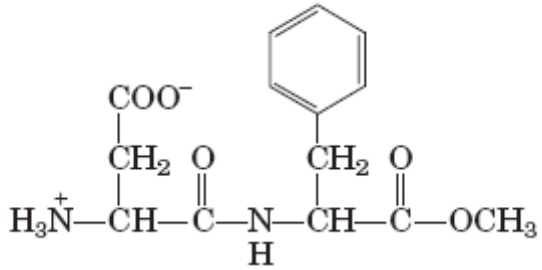


FIGURE 3–24 Amino acid sequence of bovine insulin. The two polypeptide chains are joined by disulfide cross-linkages. The A chain is identical in human, pig, dog, rabbit, and sperm whale insulins. The B chains of the cow, pig, dog, goat, and horse are identical.

Biologically Active Peptides and Polypeptides Occur in a Vast Range of Sizes



L-Aspartyl-L-phenylalanine methyl ester (aspartame)

TABLE 3–4 Conjugated Proteins

Class	*Prosthetic group*	*Example*
Lipoproteins	Lipids	β_1-Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron	Ferritin
	Zinc	Alcohol dehydrogenase
	Calcium	Calmodulin
	Molybdenum	Dinitrogenase
	Copper	Plastocyanin

TABLE 3–2 Molecular Data on Some Proteins

	Molecular weight	*Number of residues*	*Number of polypeptide chains*
Cytochrome *c* (human)	13,000	104	1
Ribonuclease A (bovine pancreas)	13,700	124	1
Lysozyme (chicken egg white)	13,930	129	1
Myoglobin (equine heart)	16,890	153	1
Chymotrypsin (bovine pancreas)	21,600	241	3
Chymotrypsinogen (bovine)	22,000	245	1
Hemoglobin (human)	64,500	574	4
Serum albumin (human)	68,500	609	1
Hexokinase (yeast)	102,000	972	2
RNA polymerase (*E. coli*)	450,000	4,158	5
Apolipoprotein B (human)	513,000	4,536	1
Glutamine synthetase (*E. coli*)	619,000	5,628	12
Titin (human)	2,993,000	26,926	1

THE THREE-DIMENSIONAL STRUCTURE OF PROTEINS

The Function of a Protein Depends on Its Amino Acid Sequence

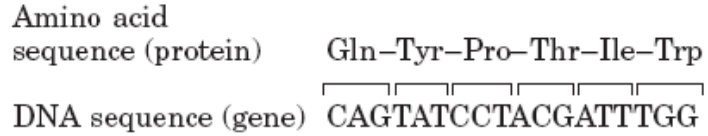


FIGURE 3–28 Correspondence of DNA and amino acid sequences. Each amino acid is encoded by a specific sequence of three nucleotides in DNA. The genetic code is described in detail in Chapter 27.

A Protein's Conformation Is Stabilized Largely by Weak Interactions

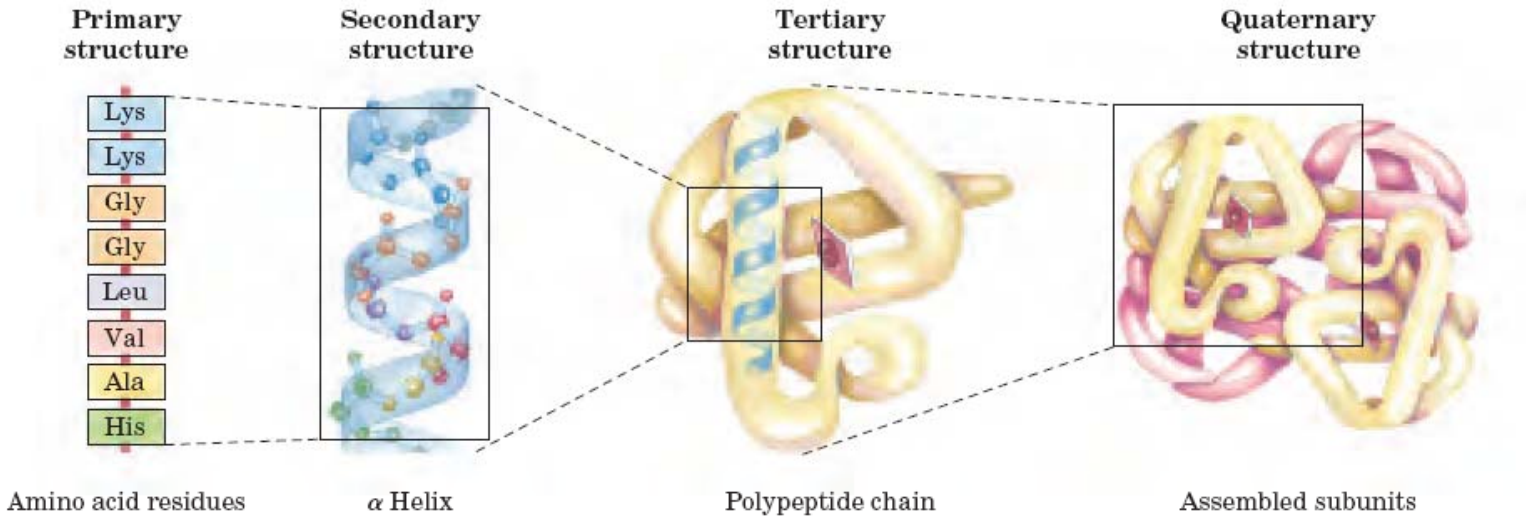
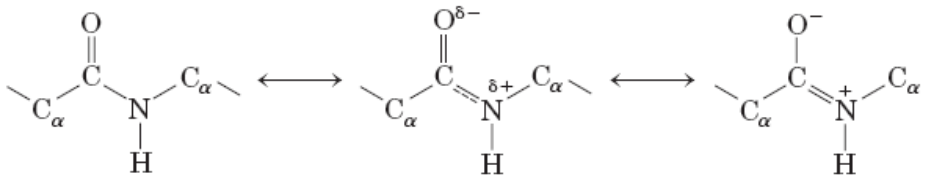
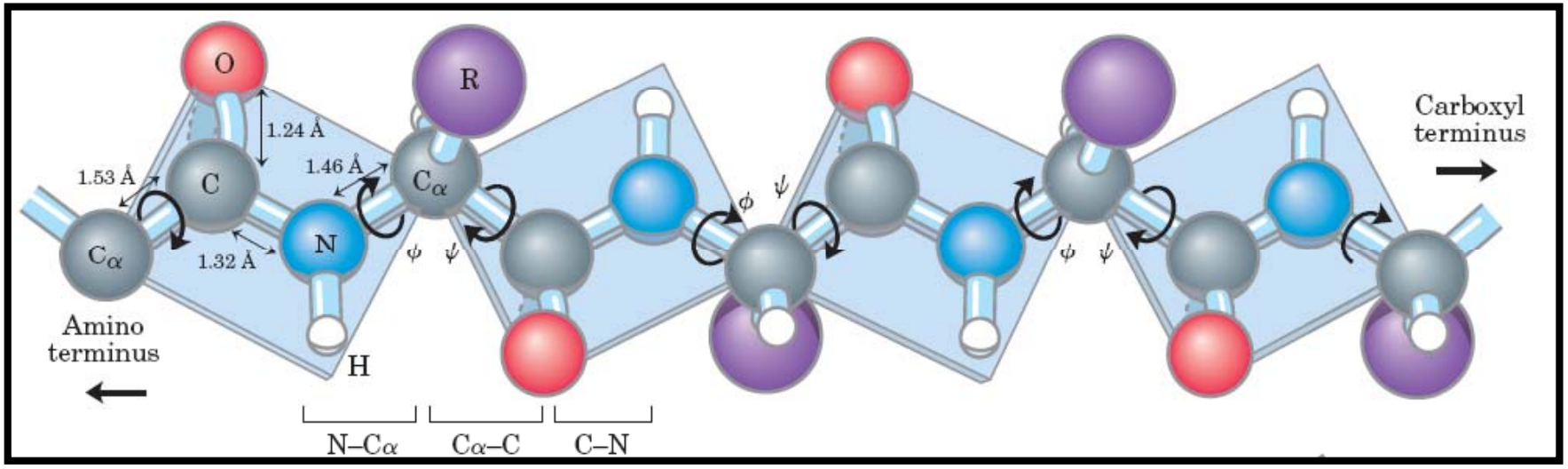


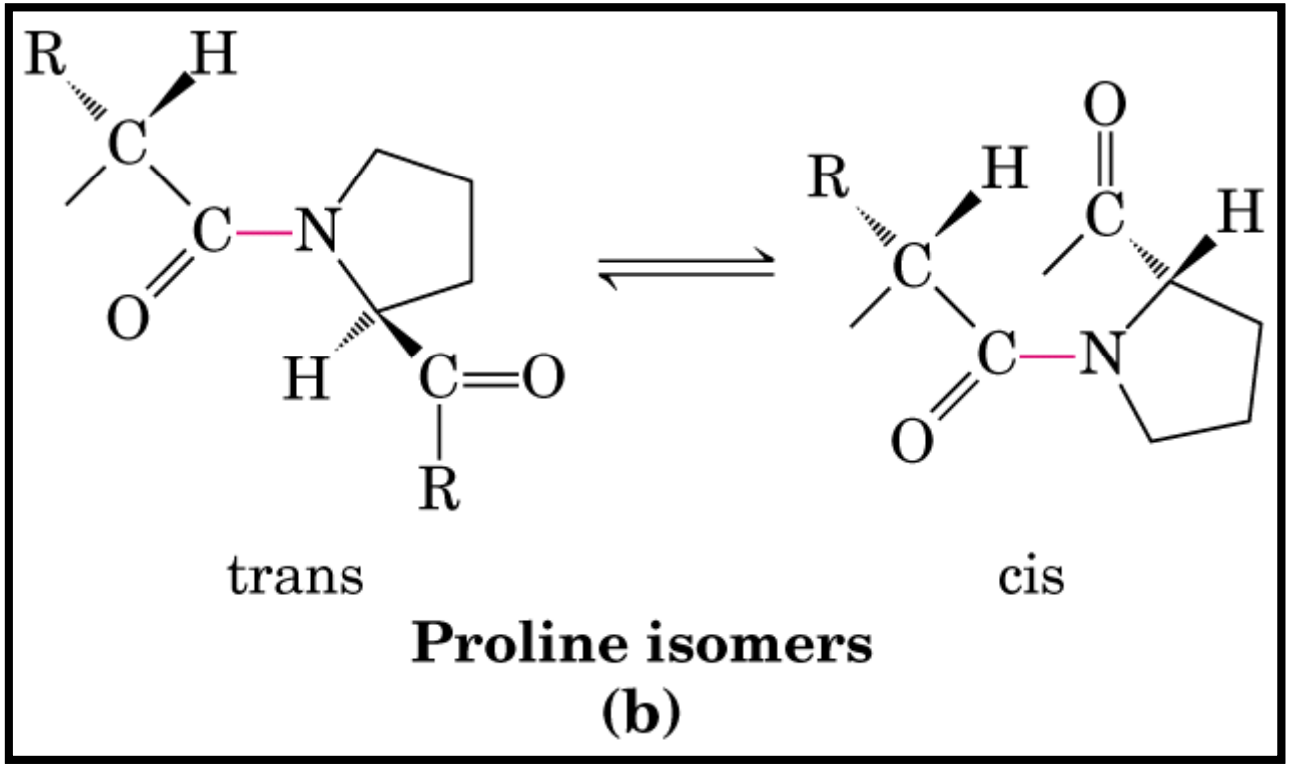
FIGURE 3–16 Levels of structure in proteins. The *primary structure* consists of a sequence of amino acids linked together by peptide bonds and includes any disulfide bonds. The resulting polypeptide can be coiled into units of *secondary structure,* such as an α helix. The helix is a part of the *tertiary structure* of the folded polypeptide, which is itself one of the subunits that make up the *quaternary structure* of the multisubunit protein, in this case hemoglobin.

The Peptide Bond Is Rigid



The carbonyl oxygen has a partial negative charge and the amide nitrogen a partial positive charge, setting up a small electric dipole. Virtually all peptide bonds in proteins occur in this trans configuration; an exception is noted in Figure 4–8b.

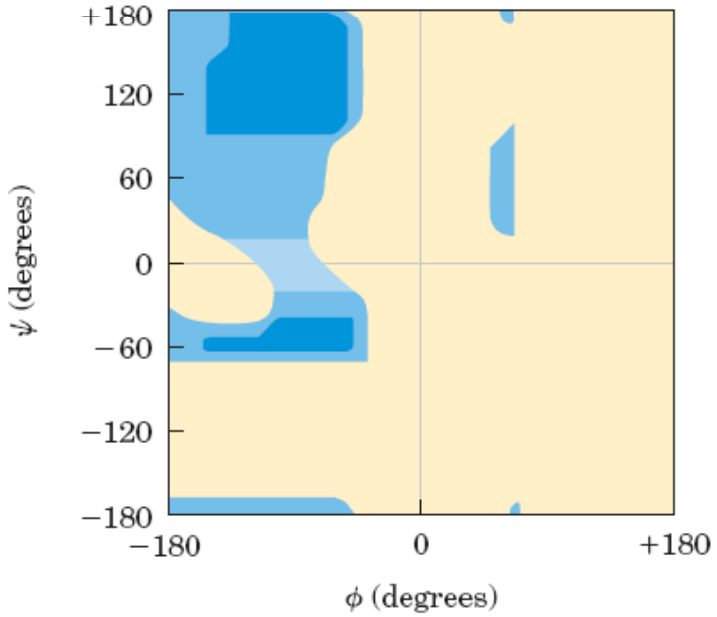


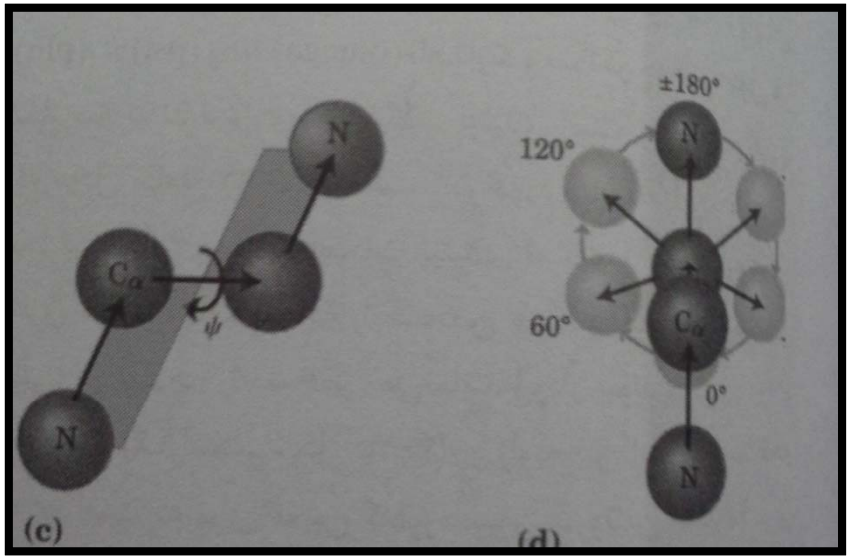


Proline isomers

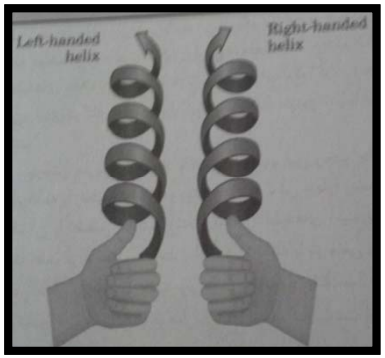
(b)

FIGURE 4–3 Ramachandran plot for L-Ala residues. The conformations of peptides are defined by the values of ϕ and ψ. Conformations deemed possible are those that involve little or no steric interference, based on calculations using known van der Waals radii and bond angles. The areas shaded dark blue reflect conformations that involve no steric overlap and thus are fully allowed; medium blue indicates conformations allowed at the extreme limits for unfavorable atomic contacts; the lightest blue area reflects conformations that are permissible if a little flexibility is allowed in the bond angles. The asymmetry of the plot results from the L stereochemistry of the amino acid residues. The plots for other L-amino acid residues with unbranched side chains are nearly identical. The allowed ranges for branched amino acid residues such as Val, Ile, and Thr are somewhat smaller than for Ala. The Gly residue, which is less sterically hindered, exhibits a much broader range of allowed conformations. The range for Pro residues is greatly restricted because ϕ is limited by the cyclic side chain to the range of $-35°$ to $-85°$.





The α Helix Is a Common Protein Secondary Structure



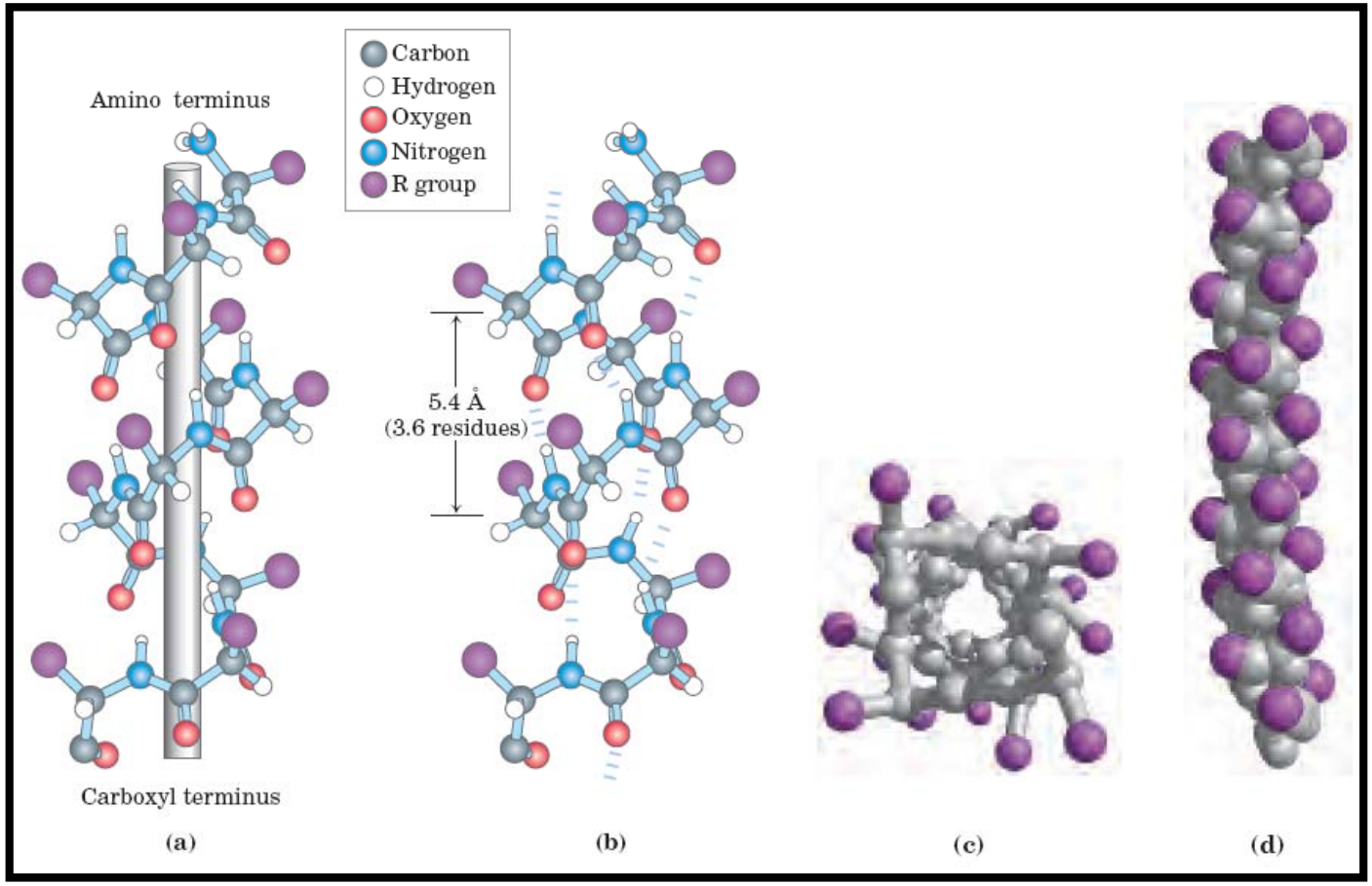
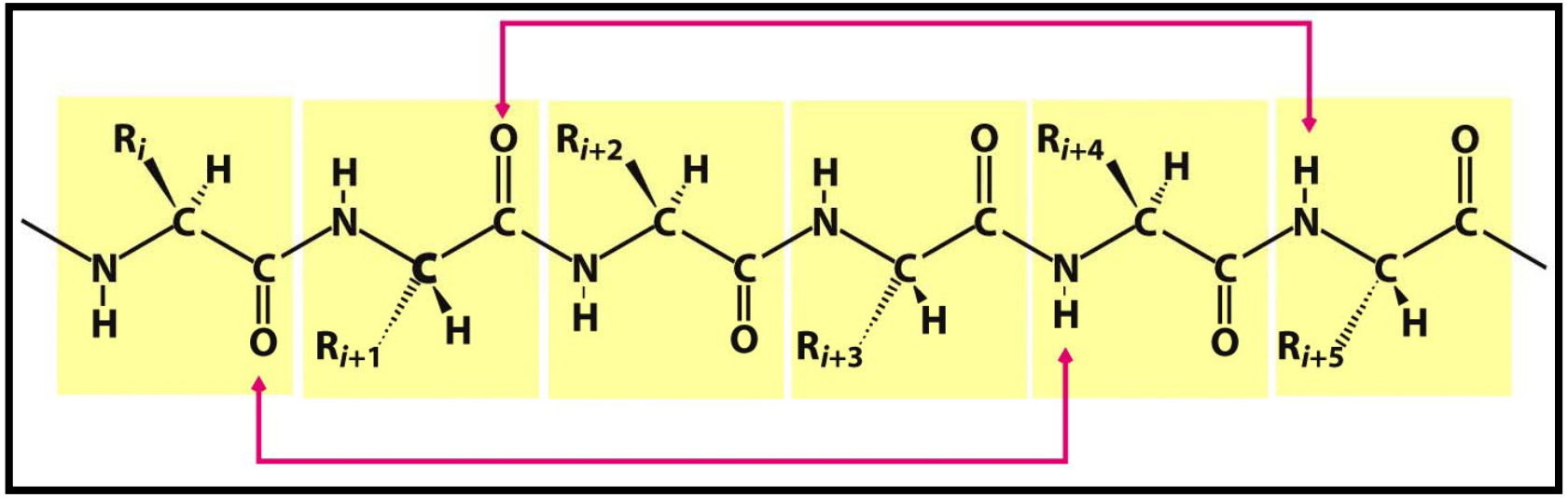
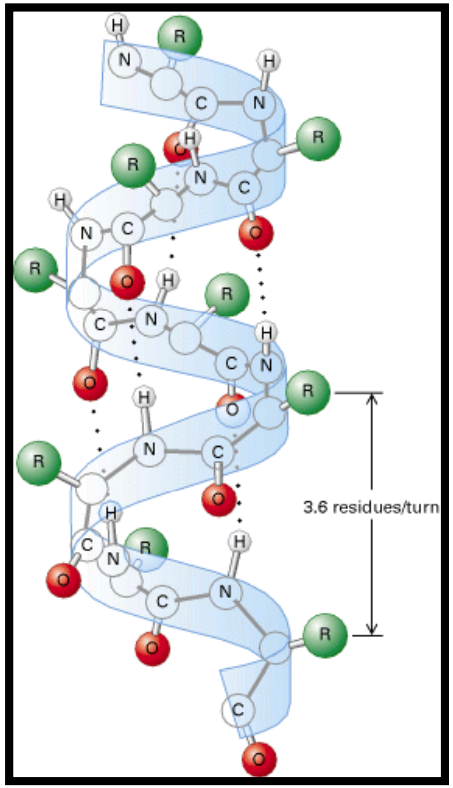
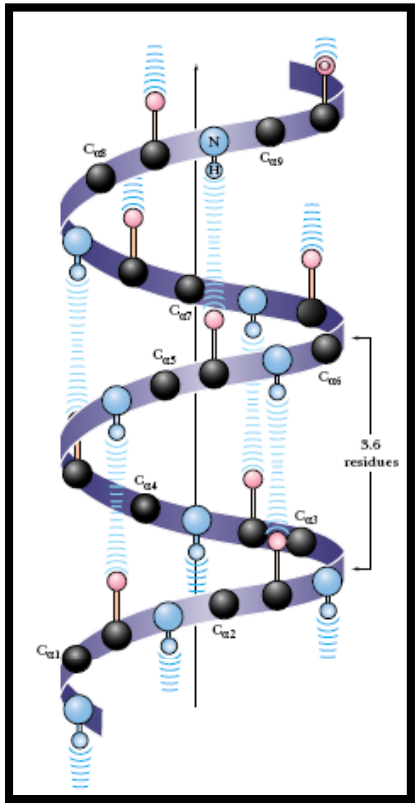


FIGURE 4–4 Four models of the α helix, showing different aspects of its structure. **(a)** Formation of a right-handed α helix. The planes of the rigid peptide bonds are parallel to the long axis of the helix, depicted here as a vertical rod. **(b)** Ball-and-stick model of a right-handed α helix, showing the intrachain hydrogen bonds. The repeat unit is a single turn of the helix, 3.6 residues. **(c)** The α helix as viewed from one end, looking down the longitudinal axis (derived from PDB ID 4TNC). Note the positions of the R groups, represented by purple spheres. This ball-and-stick model, used to emphasize the helical arrangement, gives the false impression that the helix is hollow, because the balls do not represent the van der Waals radii of the individual atoms. As the space-filling model **(d)** shows, the atoms in the center of the α helix are in very close contact.


R_i
R_{i+1}
R_{i+2}
R_{i+3}
R_{i+4}
R_{i+5}


3.6 residues/turn


3.6
residues

The β Conformation Organizes Polypeptide Chains into Sheets

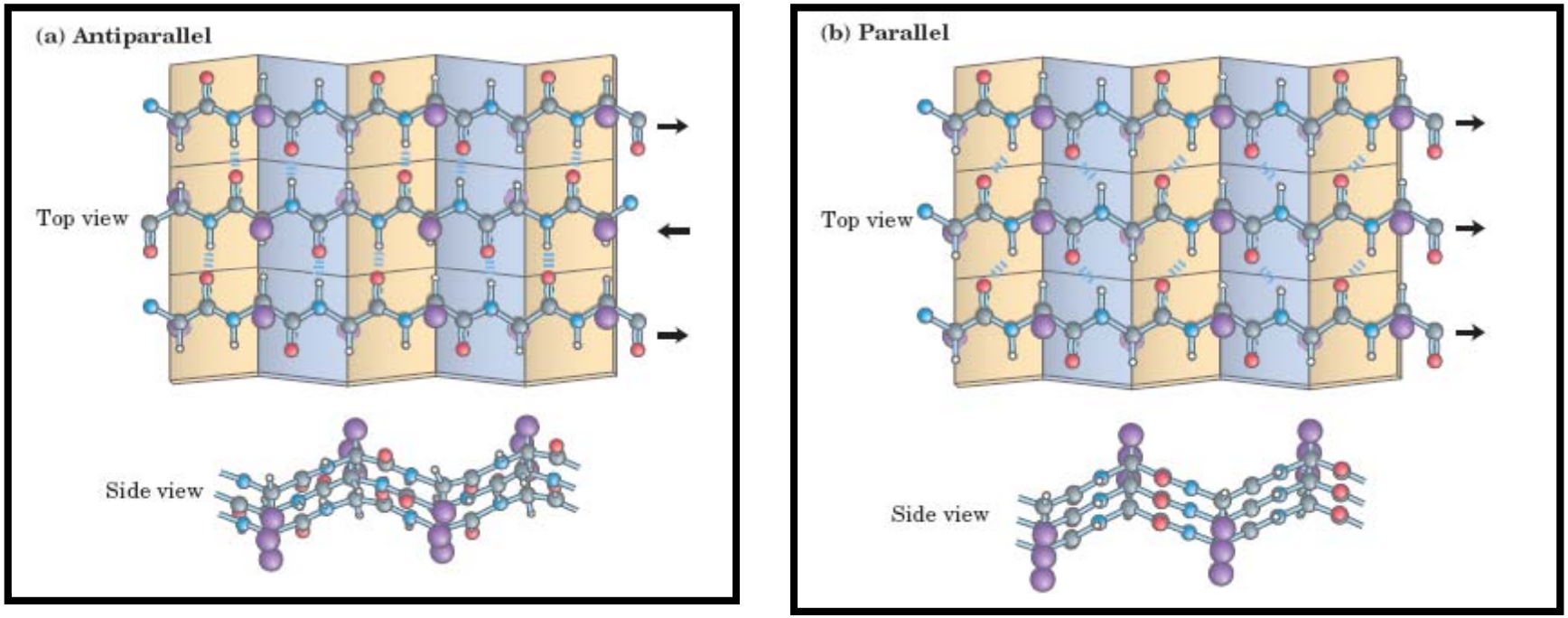


FIGURE 4–7 The β conformation of polypeptide chains. These top and side views reveal the R groups extending out from the β sheet and emphasize the pleated shape described by the planes of the peptide bonds. (An alternative name for this structure is β-pleated sheet.) Hydrogen-bond cross-links between adjacent chains are also shown. **(a)** Antiparallel β sheet, in which the amino-terminal to carboxyl-terminal orientation of adjacent chains (arrows) is inverse. **(b)** Parallel β sheet.

β Turns Are Common in Proteins

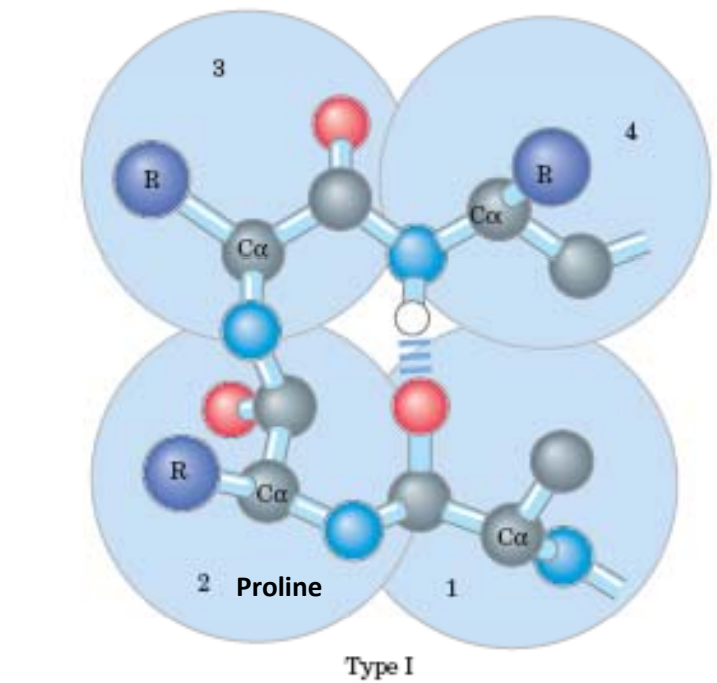


FIGURE 4–8 Structures of β turns. (a) Type I and type II β turns are most common; type I turns occur more than twice as frequently as type II. Type II β turns always have Gly as the third residue. Note the hydrogen bond between the peptide groups of the first and fourth residues of the bends. (Individual amino acid residues are framed by large blue circles.) (b) The trans and cis isomers of a peptide bond involving the imino nitrogen of proline. Of the peptide bonds between amino acid residues other than Pro, over 99.95% are in the trans configuration. For peptide bonds involving the imino nitrogen of proline, however, about 6% are in the cis configuration; many of these occur at β turns.

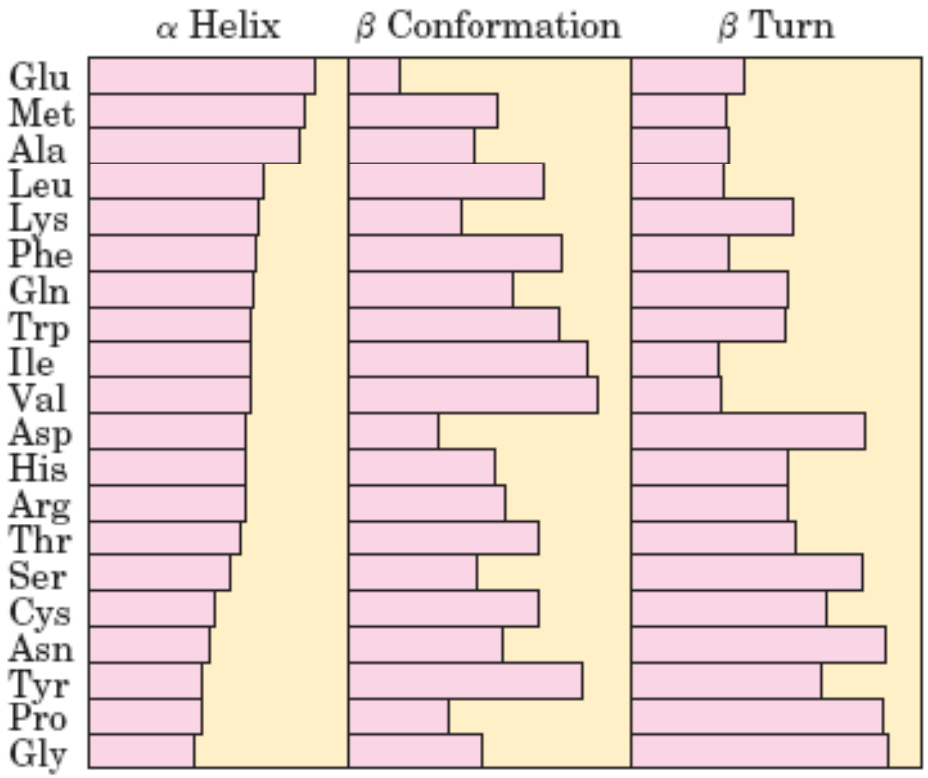


FIGURE 4–10 Relative probabilities that a given amino acid will occur in the three common types of secondary structure.

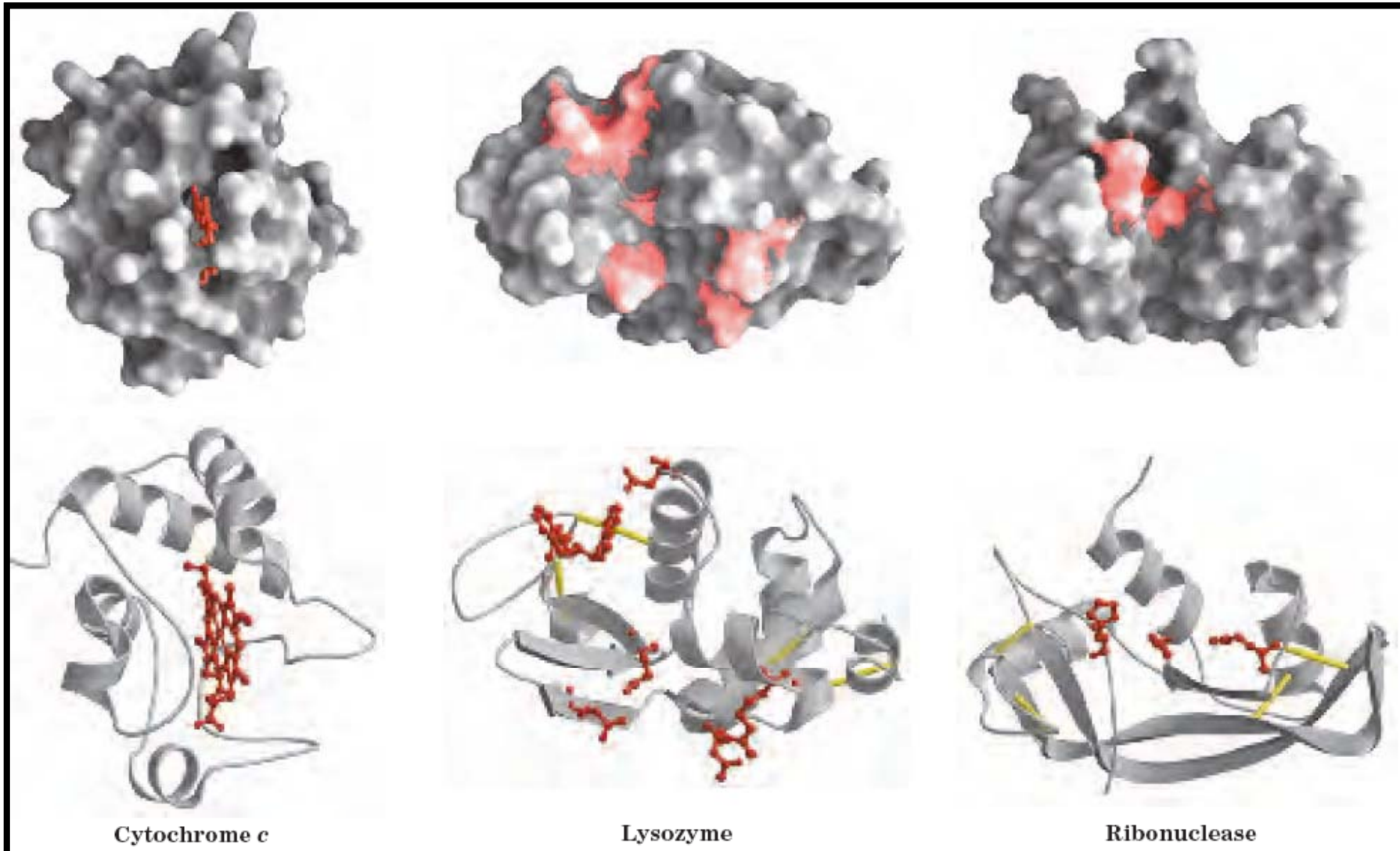


FIGURE 4–18 Three-dimensional structures of some small proteins. Shown here are cytochrome *c* (PDB ID 1CCR), lysozyme (PDB ID 3LYM), and ribonuclease (PDB ID 3RN3). Each protein is shown in surface contour and in a ribbon representation, in the same orientation. In the ribbon depictions, regions in the β conformation are represented by flat arrows and the α helices are represented by spiral ribbons. Key functional groups (the heme in cytochrome *c;* amino acid side chains in the active site of lysozyme and ribonuclease) are shown in red. Disulfide bonds are shown (in the ribbon representations) in yellow.

Protein Denaturation and Folding

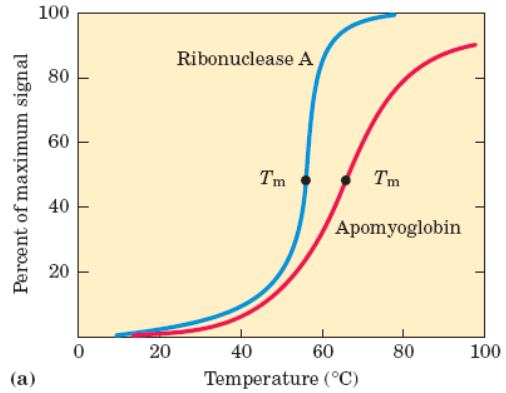


FIGURE 4–26 Protein denaturation. Results are shown for proteins denatured by two different environmental changes. In each case, the transition from the folded to unfolded state is fairly abrupt, suggesting cooperativity in the unfolding process. **(a)** Thermal denaturation of horse apomyoglobin (myoglobin without the heme prosthetic group) and ribonuclease A (with its disulfide bonds intact; see Fig. 4–27). The midpoint of the temperature range over which denaturation occurs is called the melting temperature, or T_m. The denaturation of apomyoglobin was monitored by circular dichroism, a technique that measures the amount of helical structure in a macromolecule. Denaturation of ribonuclease A was tracked by monitoring changes in the intrinsic fluorescence of the protein, which is affected by changes in the environment of Trp residues. **(b)** Denaturation of disulfide-intact ribonuclease A by guanidine hydrochloride (GdnHCl), monitored by circular dichroism.

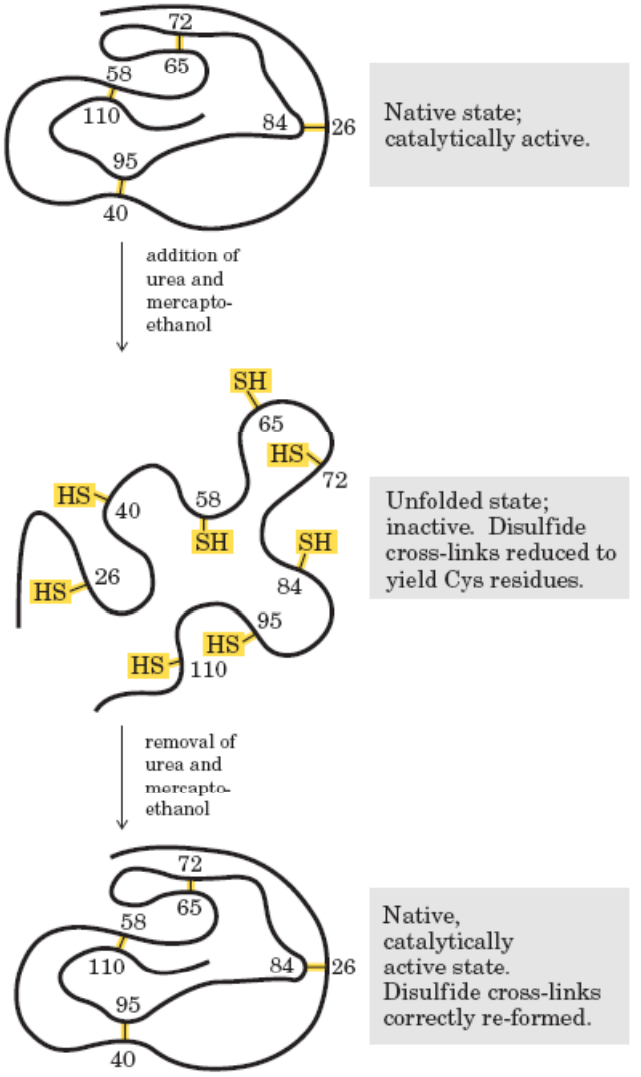
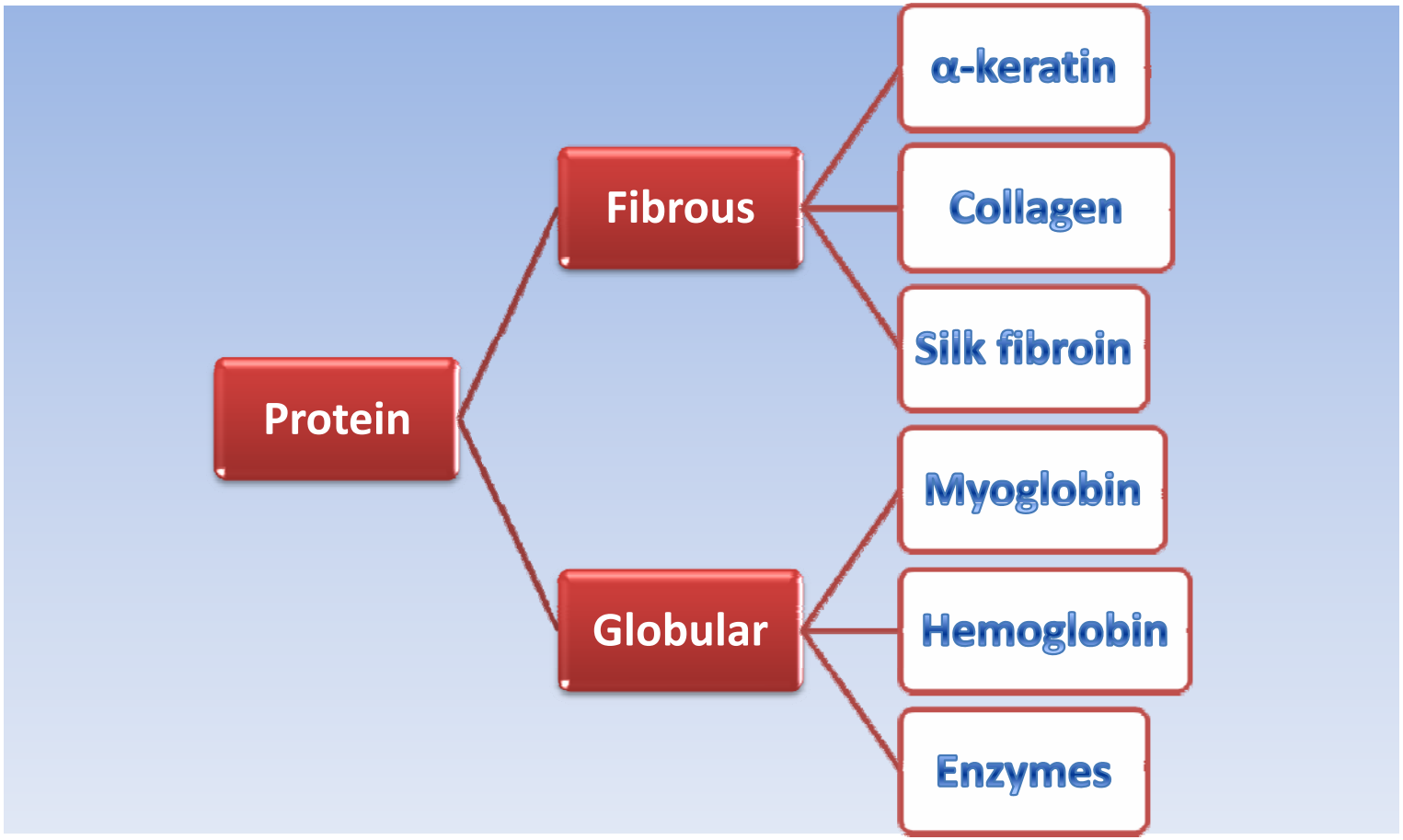


FIGURE 4–27 Renaturation of unfolded, denatured ribonuclease. Urea is used to denature ribonuclease, and mercaptoethanol ($HOCH_2CH_2SH$) to reduce and thus cleave the disulfide bonds to yield eight Cys residues. Renaturation involves reestablishment of the correct disulfide cross-links.


Protein
Fibrous
Globular
α-keratin
Collagen
Silk fibroin
Myoglobin
Hemoglobin
Enzymes

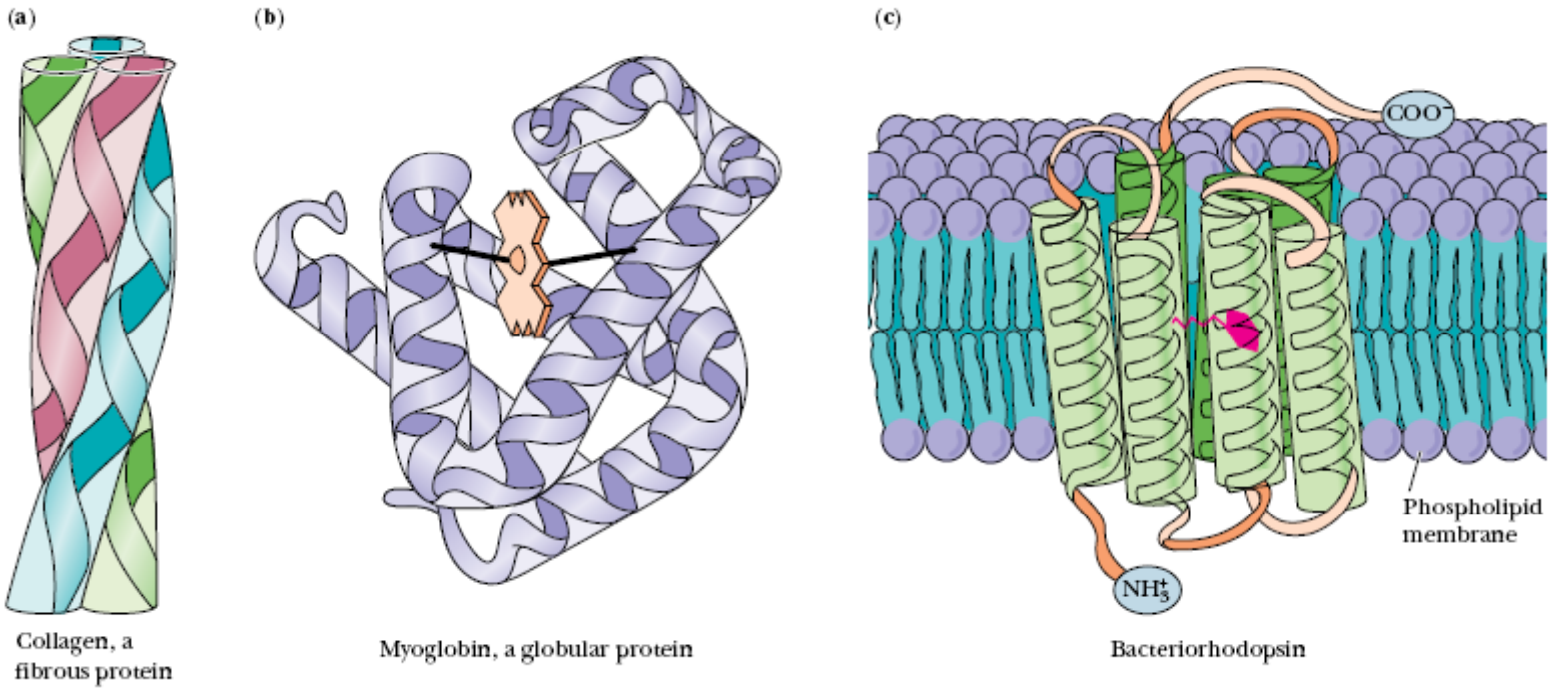
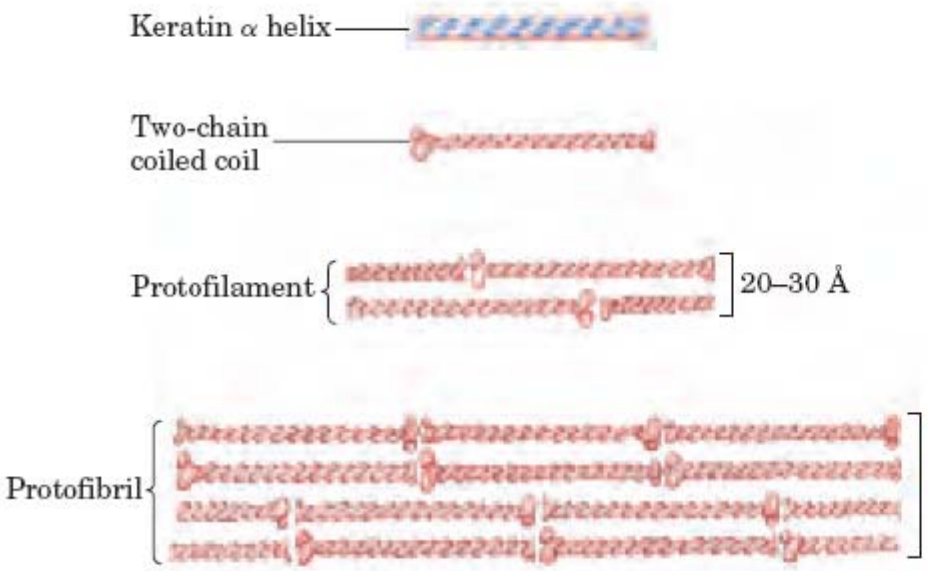


FIGURE 5.7 • (a) Proteins having structural roles in cells are typically fibrous and often water insoluble. Collagen is a good example. Collagen is composed of three polypeptide chains that intertwine. (b) Soluble proteins serving metabolic functions can be characterized as compactly folded globular molecules, such as myoglobin. The folding pattern puts hydrophilic amino acid side chains on the outside and buries hydrophobic side chains in the interior, making the protein highly water soluble. (c) Membrane proteins fold so that hydrophobic amino acid side chains are exposed in their membrane-associated regions. The portions of membrane proteins extending into or exposed at the aqueous environments are hydrophilic in character, like soluble proteins. Bacteriorhodopsin is a typical membrane protein; it binds the light-absorbing pigment, *cis*-retinal, shown here in red. (*a, b, Irving Geis*)



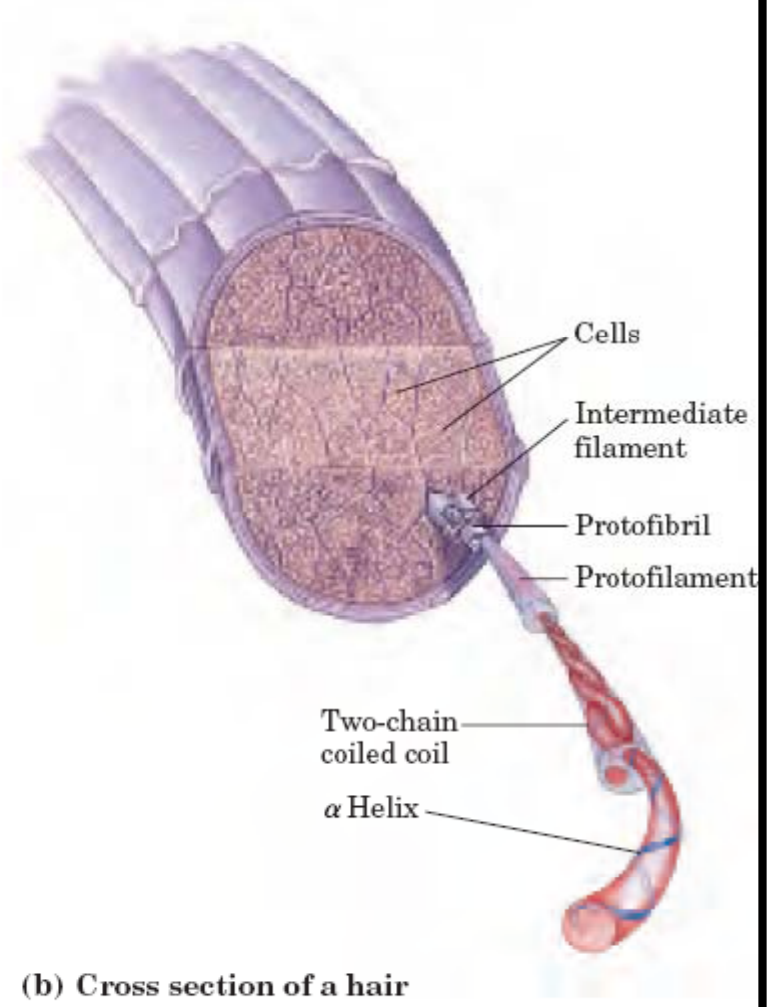
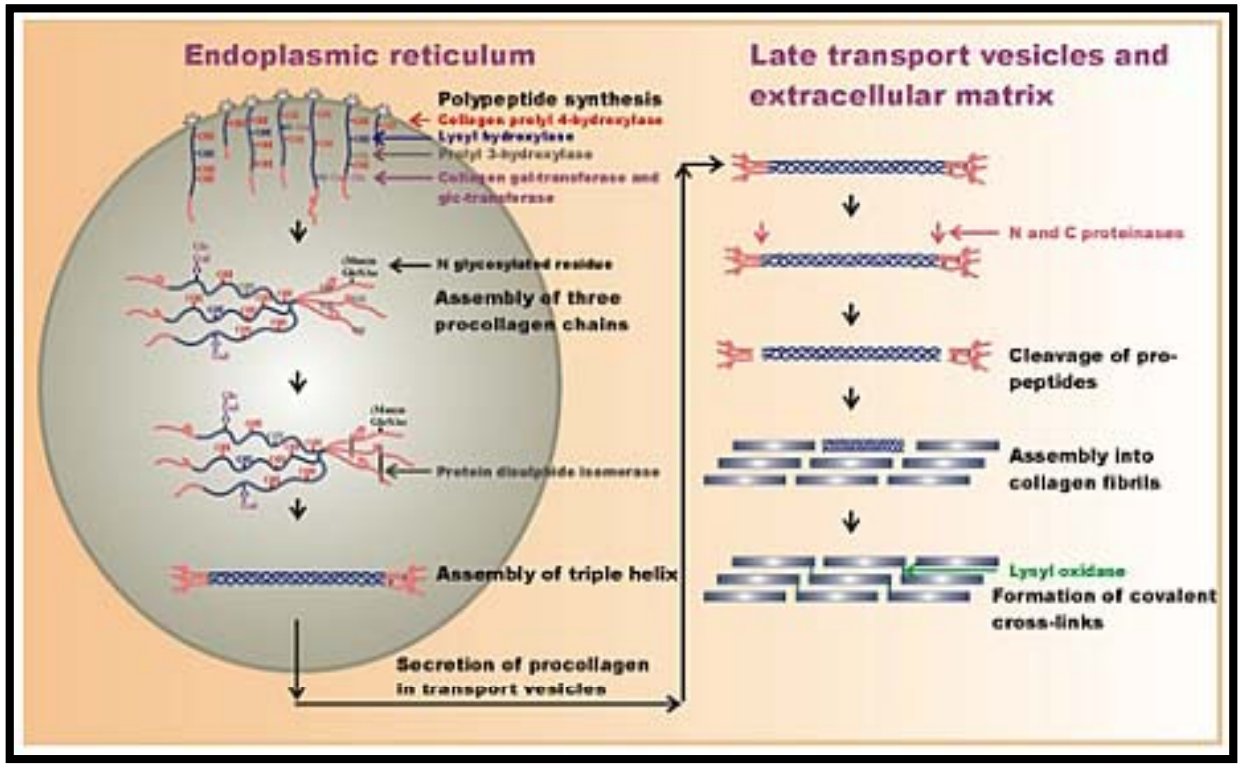
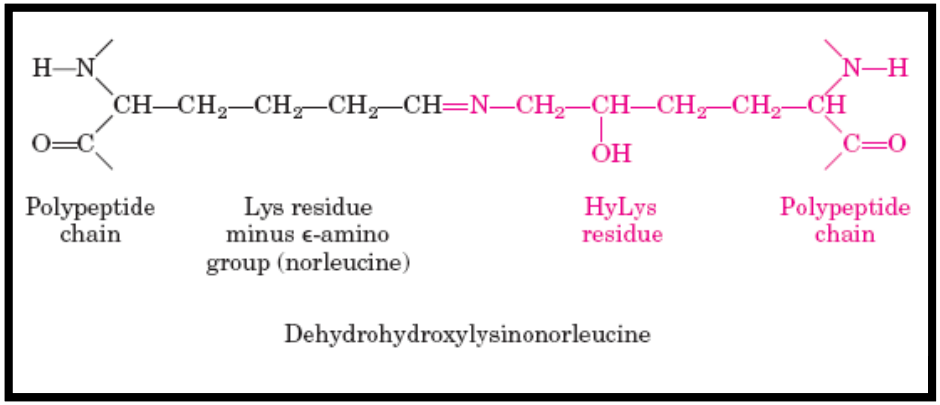
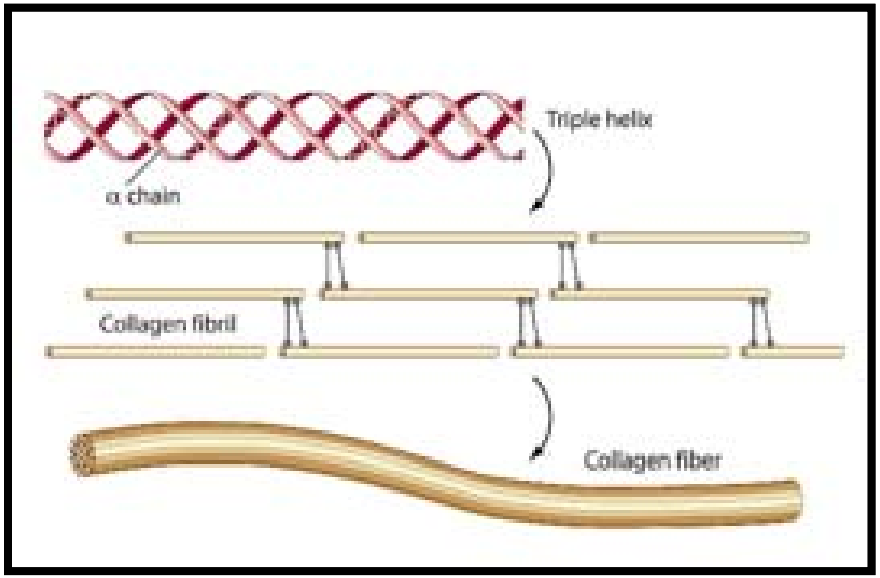


FIGURE 4–11 **Structure of hair.** (a) Hair α-keratin is an elongated α helix with somewhat thicker elements near the amino and carboxyl termini. Pairs of these helices are interwound in a left-handed sense to form two-chain coiled coils. These then combine in higher-order structures called protofilaments and protofibrils. About four protofibrils—32 strands of α-keratin altogether—combine to form an intermediate filament. The individual two-chain coiled coils in the various substructures also appear to be interwound, but the handedness of the interwinding and other structural details are unknown. (b) A hair is an array of many α-keratin filaments, made up of the substructures shown in (a).


Endoplasmic reticulum
Late transport vesicles and extracellular matrix
Polypeptide synthesis
Collagen prolyl 4-hydroxylase
Lysyl hydroxylase
Prolyl 3-hydroxylase
Collagen galtransferase and glc-transferase
N glycosylated residue
Assembly of three procollagen chains
Protein disulphide isomerase
Assembly of triple helix
Secretion of procollagen in transport vesicles
N and C proteinases
Cleavage of pro-peptides
Assembly into collagen fibrils
Lysyl oxidase
Formation of covalent cross-links

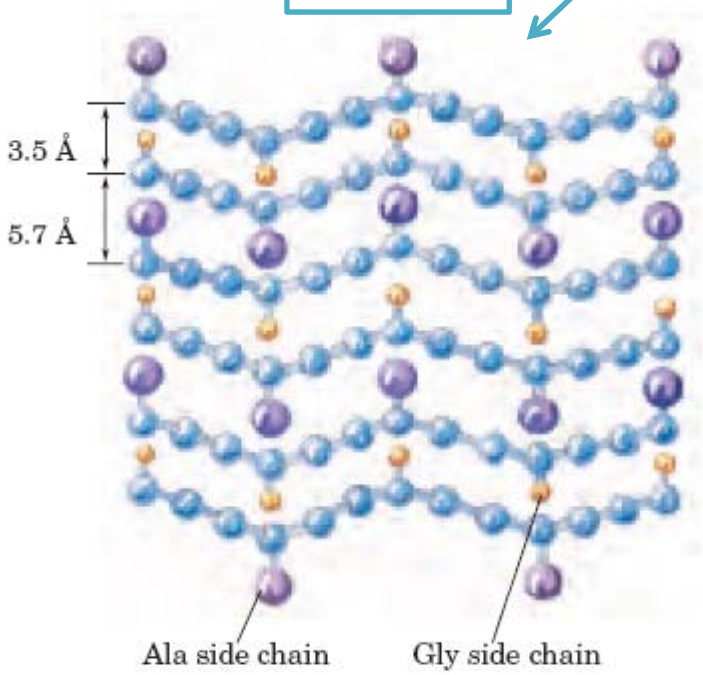
Gly-Pro-HPro


Polypeptide chain
Lys residue minus ϵ-amino group (norleucine)
HyLys residue
Polypeptide chain
Dehydrohydroxylysinonorleucine


Triple helix
α chain
Collagen fibril
Collagen fiber

Fibroin strands

Structure



(a)

(b)

FIGURE 4–14 **Structure of silk.** The fibers used to make silk cloth or a spider web are made up of the protein fibroin. **(a)** Fibroin consists of layers of antiparallel β sheets rich in Ala (purple) and Gly (yellow) residues. The small side chains interdigitate and allow close packing of each layered sheet, as shown in this side view. **(b)** Strands of fibroin (blue) emerge from the spinnerets of a spider in this colorized electron micrograph.

Oxygen Can Be Bound to a Heme Prosthetic Group

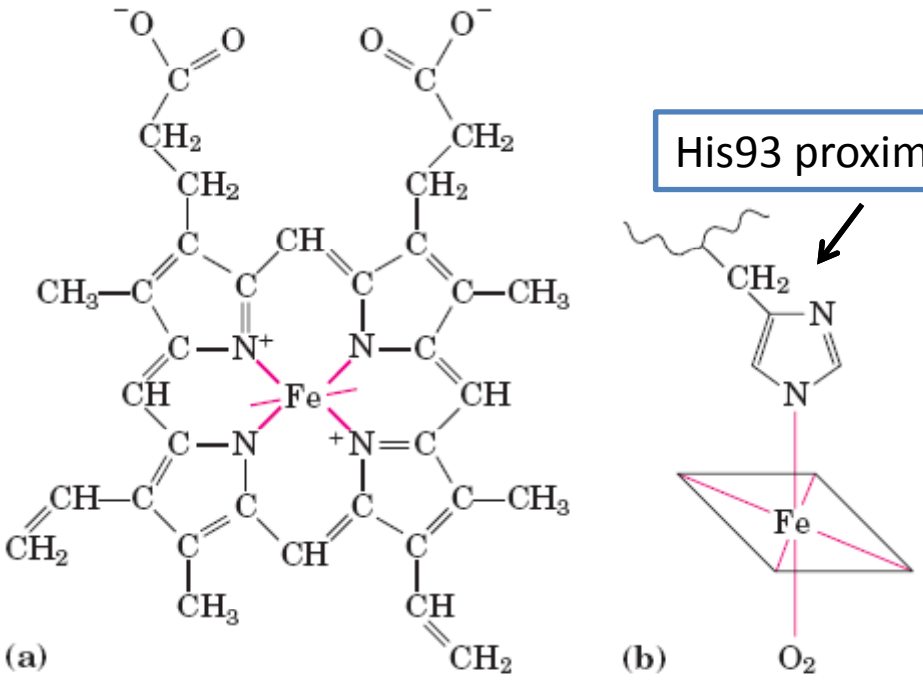


FIGURE 4–17 The heme group. This group is present in myoglobin, hemoglobin, cytochromes, and many other heme proteins. **(a)** Heme consists of a complex organic ring structure, protoporphyrin, to which is bound an iron atom in its ferrous (Fe^{2+}) state. The iron atom has six coordination bonds, four in the plane of, and bonded to, the flat porphyrin molecule and two perpendicular to it. **(b)** In myoglobin and hemoglobin, one of the perpendicular coordination bonds is bound to a nitrogen atom of a His residue. The other is "open" and serves as the binding site for an O_2 molecule.

Myoglobin Has a Single Binding Site for Oxygen

His93 proximal (F8)

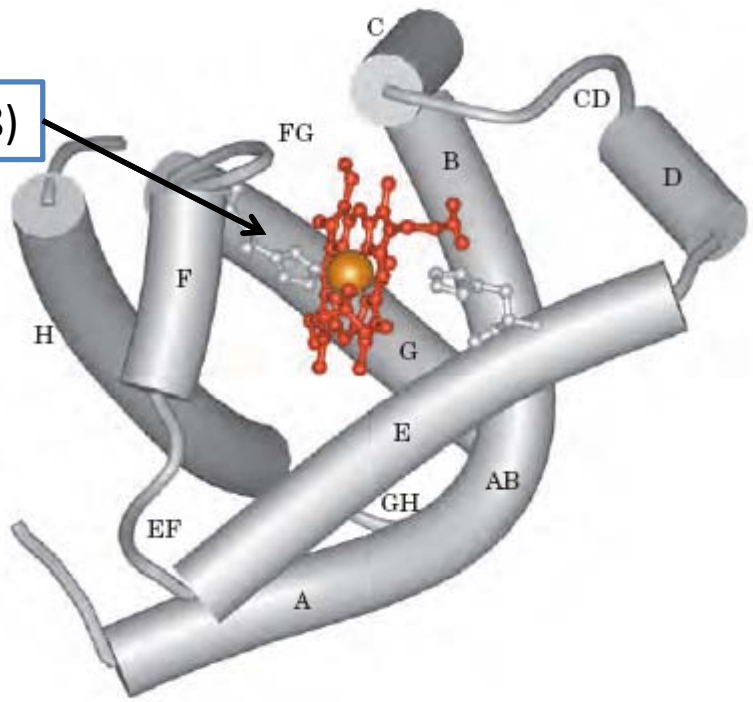


FIGURE 5–3 The structure of myoglobin. (PDB ID 1MBO) The eight α-helical segments (shown here as cylinders) are labeled A through H. Nonhelical residues in the bends that connect them are labeled AB, CD, EF, and so forth, indicating the segments they interconnect. A few bends, including BC and DE, are abrupt and do not contain any residues; these are not normally labeled. (The short segment visible between D and E is an artifact of the computer representation.) The heme is bound in a pocket made up largely of the E and F helices, although amino acid residues from other segments of the protein also participate.

- Heme = protoporphyrin IX + (ferrous) Fe^{2+}
- Mb: 153 a.a.

Protein-Ligand Interactions Can Be Described Quantitatively

In general, the reversible binding of a protein (P) to a ligand (L) can be described by a simple **equilibrium expression:**

$$P + L \rightleftharpoons PL \qquad (5\text{–}1)$$

The reaction is characterized by an equilibrium constant, K_a, such that

$$K_a = \frac{[PL]}{[P][L]} \qquad (5\text{–}2)$$

The term K_a is an **association constant** (not to be confused with the K_a that denotes an acid dissociation constant; p. 63).

$$K_a[L] = \frac{[PL]}{[P]}$$

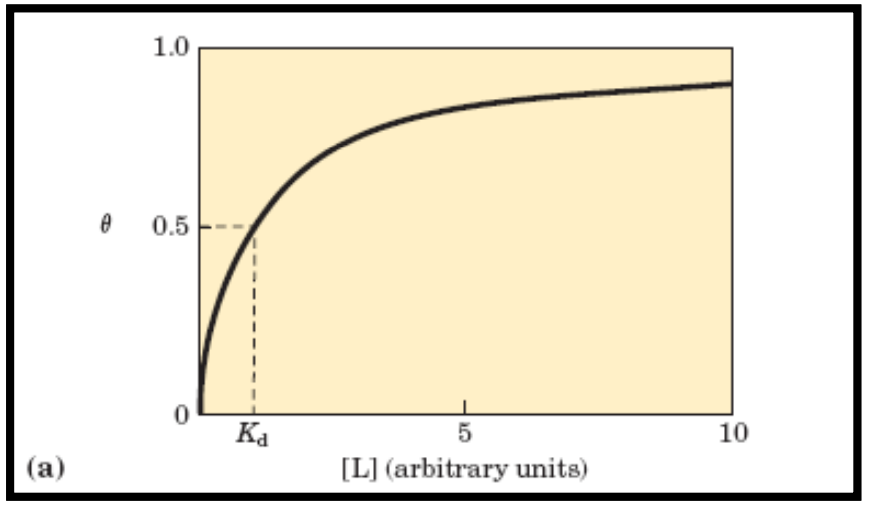
$$\theta = \frac{\text{binding sites occupied}}{\text{total binding sites}} = \frac{[PL]}{[PL] + [P]} \qquad (5\text{–}4)$$

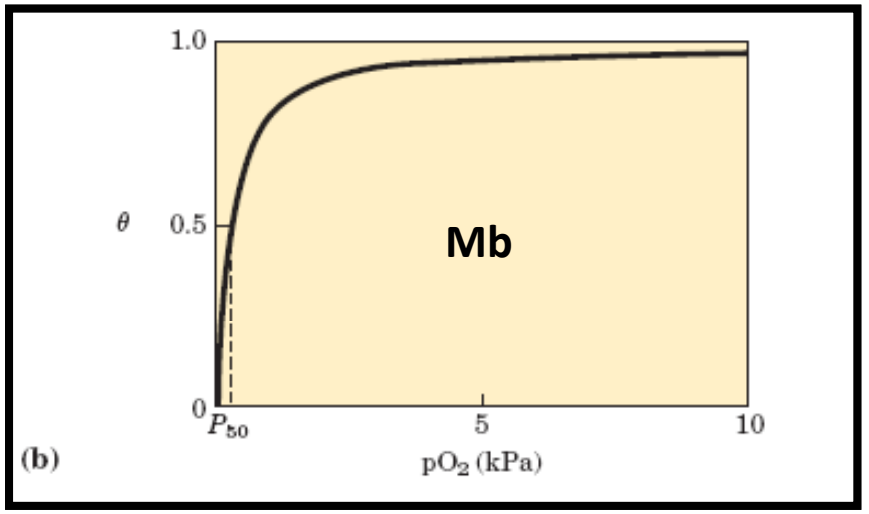
$$\theta = \frac{K_a[L][P]}{K_a[L][P] + [P]} = \frac{K_a[L]}{K_a[L] + 1} = \frac{[L]}{[L] + \frac{1}{K_a}}$$

$K_d = \dfrac{[P][L]}{[PL]}$ **dissociation constant, K_d;** $(K_d = 1/K_a)$

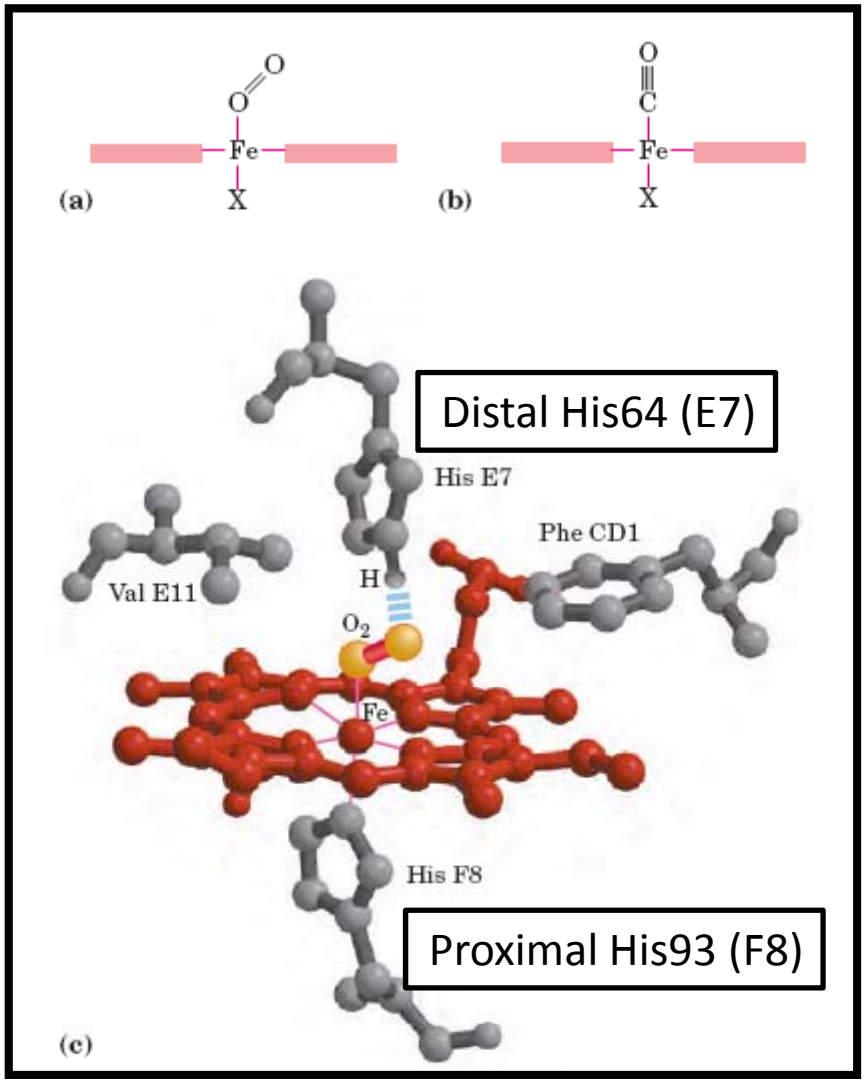
$$[PL] = \frac{[P][L]}{K_d}$$

$$\theta = \frac{[L]}{[L] + K_d}$$





$$\theta = \frac{pO_2}{pO_2 + P_{50}}$$



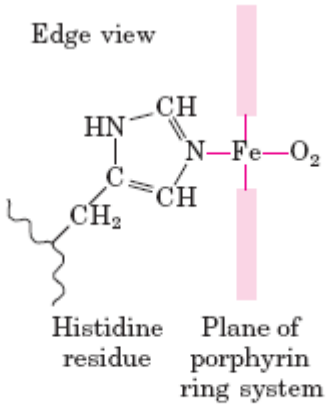


FIGURE 5–2 **The heme group viewed from the side.** This view shows the two coordination bonds to Fe^{2+} perpendicular to the porphyrin ring system. One of these two bonds is occupied by a His residue, sometimes called the proximal His. The other bond is the binding site for oxygen. The remaining four coordination bonds are in the plane of, and bonded to, the flat porphyrin ring system.

FIGURE 5–5 **Steric effects on the binding of ligands to the heme of myoglobin.** (a) Oxygen binds to heme with the O_2 axis at an angle, a binding conformation readily accommodated by myoglobin. (b) Carbon monoxide binds to free heme with the CO axis perpendicular to the plane of the porphyrin ring. When binding to the heme in myoglobin, CO is forced to adopt a slight angle because the perpendicular arrangement is sterically blocked by His E7, the distal His. This effect weakens the binding of CO to myoglobin. (c) Another view (derived from PDB ID 1MBO), showing the arrangement of key amino acid residues around the heme of myoglobin. The bound O_2 is hydrogen-bonded to the distal His, His E7 (His^{64}), further facilitating the binding of O_2.

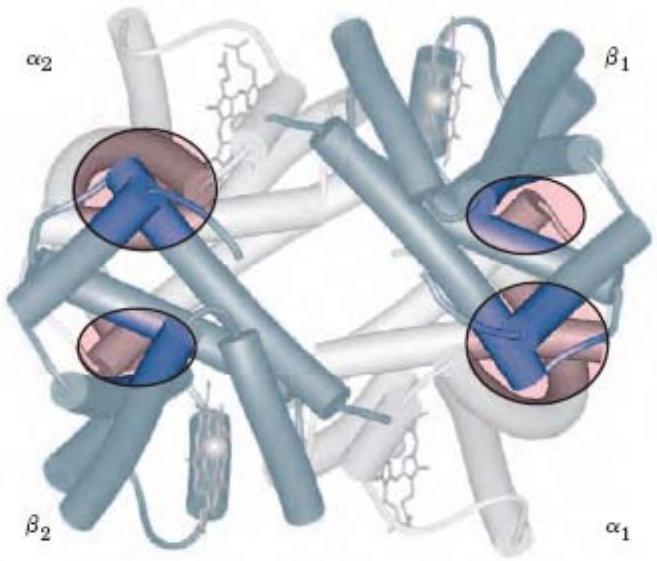


FIGURE 5–8 Dominant interactions between hemoglobin subunits. In this representation, α subunits are light and β subunits are dark. The strongest subunit interactions (highlighted) occur between unlike subunits. When oxygen binds, the $\alpha_1\beta_1$ contact changes little, but there is a large change at the $\alpha_1\beta_2$ contact, with several ion pairs broken (PDB ID 1HGA).

T state → $\alpha_1\beta_2$ (and $\alpha_2\beta_1$) interface

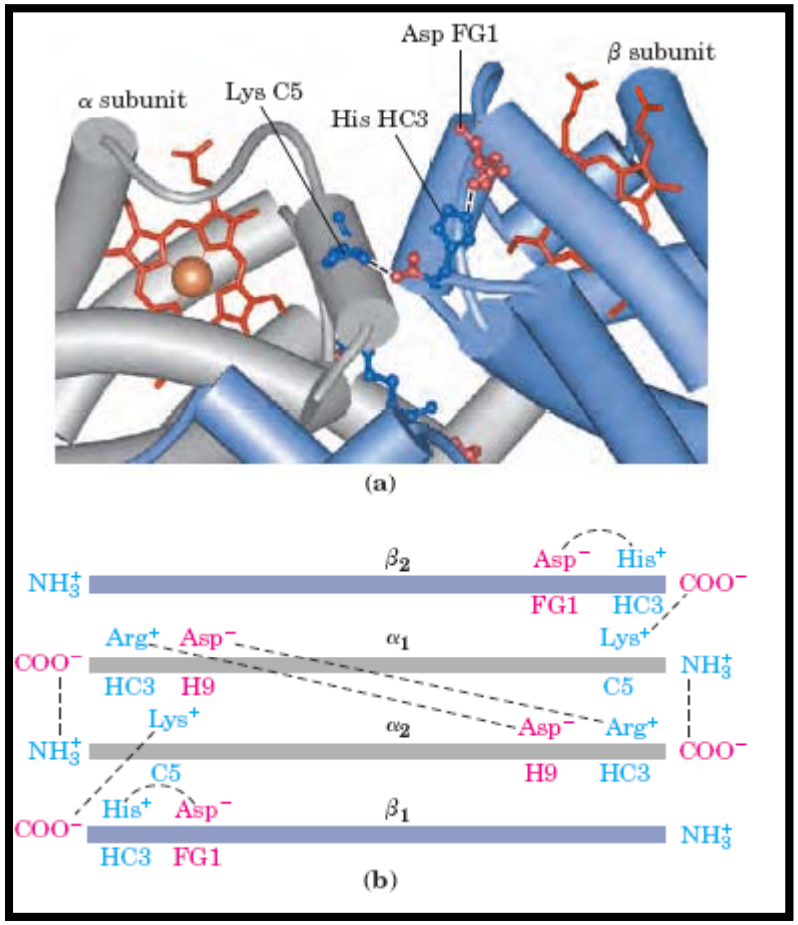


FIGURE 5–9 Some ion pairs that stabilize the T state of deoxyhemoglobin. **(a)** A close-up view of a portion of a deoxyhemoglobin molecule in the T state (PDB ID 1HGA). Interactions between the ion pairs His HC3 and Asp FG1 of the β subunit (blue) and between Lys C5 of the α subunit (gray) and His HC3 (its α-carboxyl group) of the β subunit are shown with dashed lines. (Recall that HC3 is the carboxyl-terminal residue of the β subunit.) **(b)** The interactions between these ion pairs, and between others not shown in **(a)**, are schematized in this representation of the extended polypeptide chains of hemoglobin.

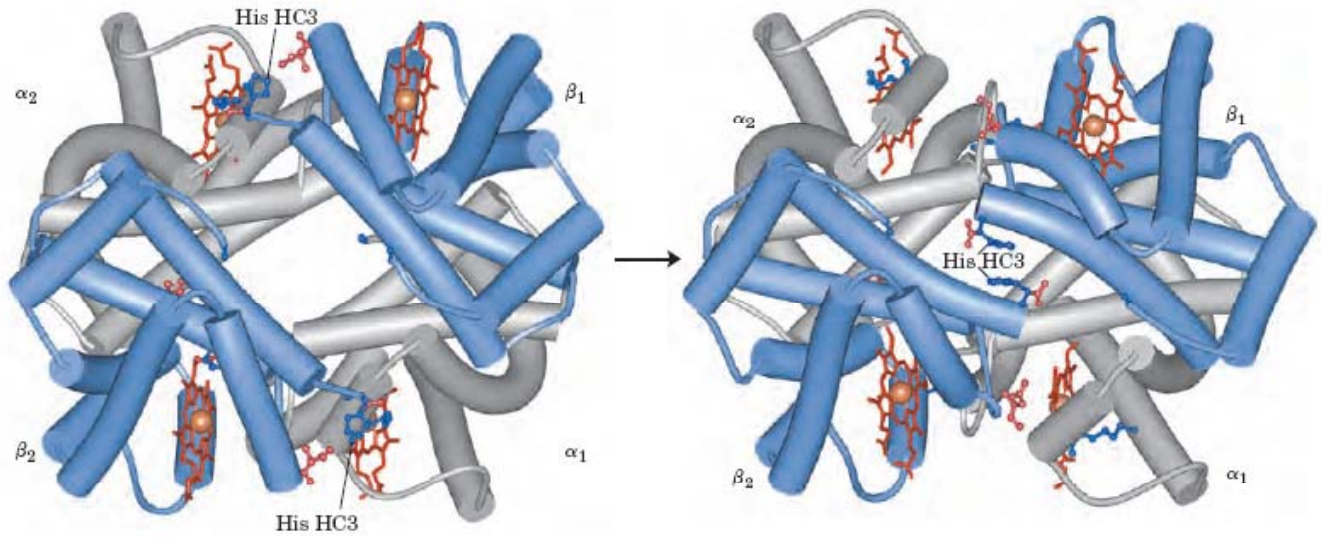


FIGURE 5–10 The T ⟶ R transition. (PDB ID 1HGA and 1BBB) In these depictions of deoxyhemoglobin, as in Figure 5–9, the β subunits are blue and the α subunits are gray. Positively charged side chains and chain termini involved in ion pairs are shown in blue, their negatively charged partners in red. The Lys C5 of each α subunit and Asp FG1 of each β subunit are visible but not labeled (compare Fig. 5–9a). Note that the molecule is oriented slightly differently than in Figure 5–9. The transition from the T state to the R state shifts the subunit pairs substantially, affecting certain ion pairs. Most noticeably, the His HC3 residues at the carboxyl termini of the β subunits, which are involved in ion pairs in the T state, rotate in the R state toward the center of the molecule, where they are no longer in ion pairs. Another dramatic result of the T ⟶ R transition is a narrowing of the pocket between the β subunits.

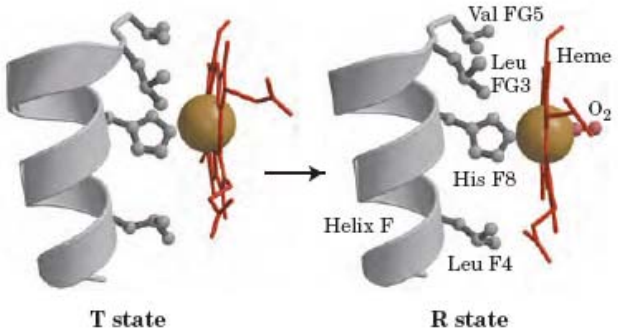


FIGURE 5–11 Changes in conformation near heme on O_2 binding to deoxyhemoglobin. (Derived from PDB ID 1HGA and 1BBB.) The shift in the position of the F helix when heme binds O_2 is thought to be one of the adjustments that triggers the T ⟶ R transition.

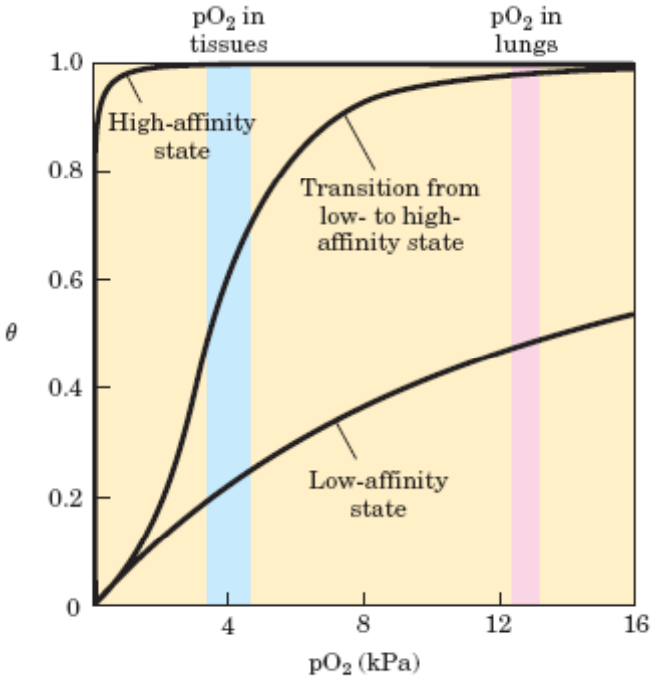


FIGURE 5–12 **A sigmoid (cooperative) binding curve.** A sigmoid binding curve can be viewed as a hybrid curve reflecting a transition from a low-affinity to a high-affinity state. Cooperative binding, as manifested by a sigmoid binding curve, renders hemoglobin more sensitive to the small differences in O_2 concentration between the tissues and the lungs, allowing hemoglobin to bind oxygen in the lungs (where pO_2 is high) and release it in the tissues (where pO_2 is low).

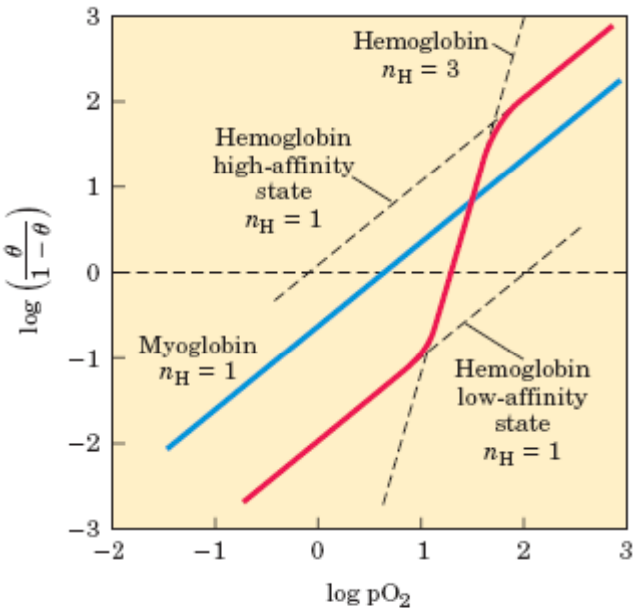


FIGURE 5–14 **Hill plots for the binding of oxygen to myoglobin and hemoglobin.** When $n_H = 1$, there is no evident cooperativity. The maximum degree of cooperativity observed for hemoglobin corresponds approximately to $n_H = 3$. Note that while this indicates a high level of cooperativity, n_H is less than n, the number of O_2-binding sites in hemoglobin. This is normal for a protein that exhibits allosteric binding behavior.

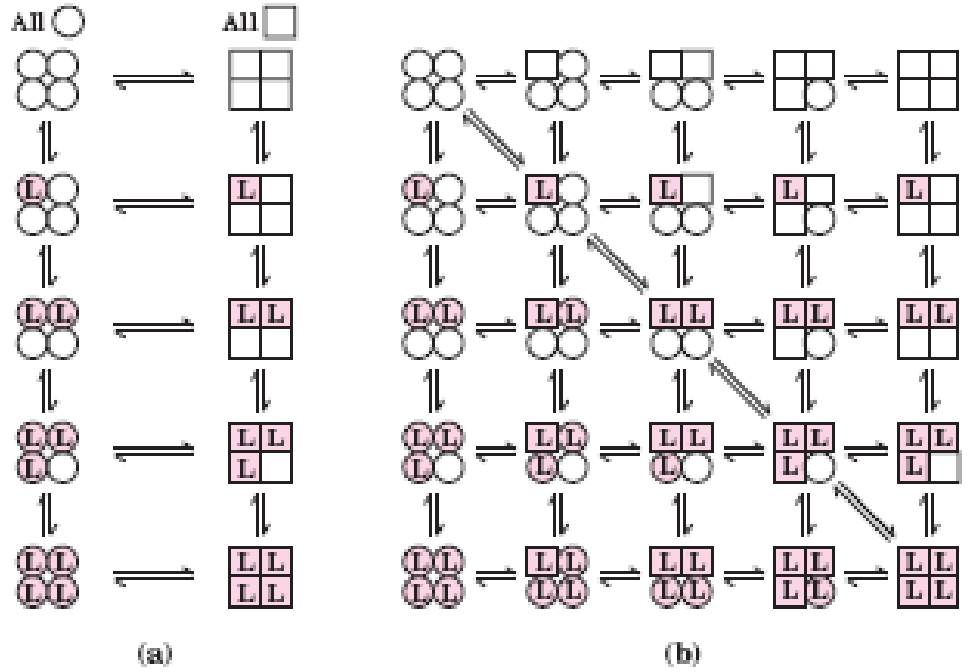


FIGURE 5–15 Two general models for the interconversion of inactive and active forms of cooperative ligand-binding proteins. Although the models may be applied to any protein—including any enzyme (Chapter 6)—that exhibits cooperative binding, we show here four subunits because the model was originally proposed for hemoglobin. In the concerted, or all-or-none, model (MWC model) (a) all the subunits are postulated to be in the same conformation, either all ○ (low affinity or inactive) or all □ (high affinity or active). Depending on the equilibrium, K_1, between ○ and □ forms, the binding of one or more ligand molecules (L) will pull the equilibrium toward the □ form. Subunits with bound L are shaded. In the sequential model (b), each individual subunit can be in either the ○ or □ form. A very large number of conformations is thus possible.

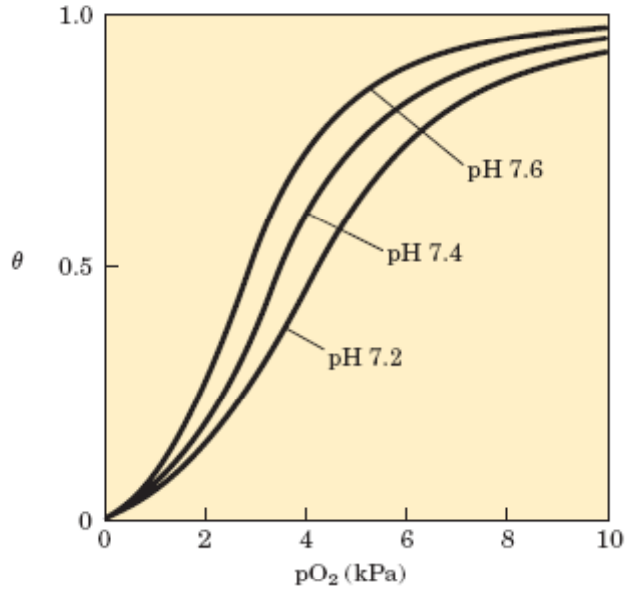
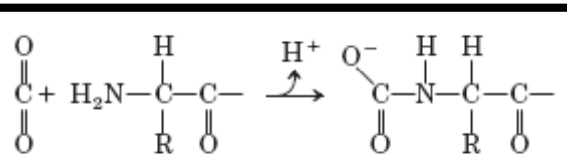


FIGURE 5–16 **Effect of pH on the binding of oxygen to hemoglobin.** The pH of blood is 7.6 in the lungs and 7.2 in the tissues. Experimental measurements on hemoglobin binding are often performed at pH 7.4.



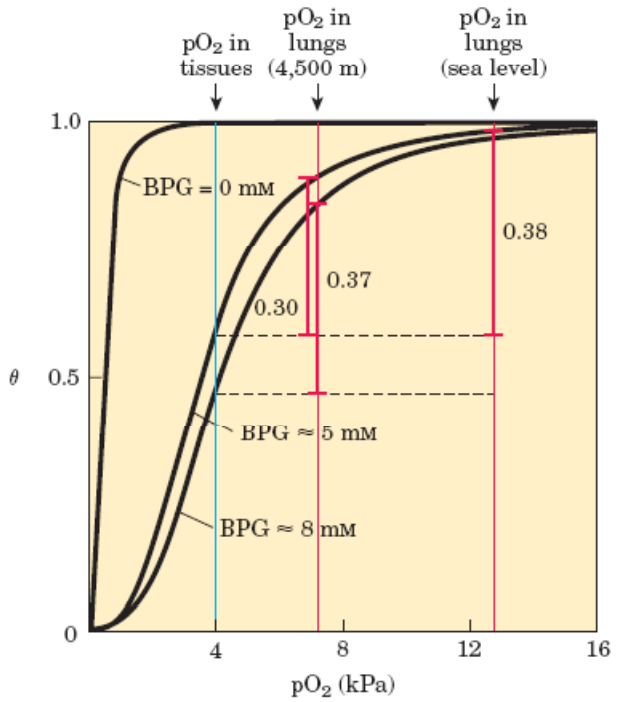


FIGURE 5–17 **Effect of BPG on the binding of oxygen to hemoglobin.** The BPG concentration in normal human blood is about 5 mM at sea level and about 8 mM at high altitudes. Note that hemoglobin binds to oxygen quite tightly when BPG is entirely absent, and the binding curve appears to be hyperbolic. In reality, the measured Hill coefficient for O_2-binding cooperativity decreases only slightly (from 3 to about 2.5) when BPG is removed from hemoglobin, but the rising part of the sigmoid curve is confined to a very small region close to the origin. At sea level, hemoglobin is nearly saturated with O_2 in the lungs, but only 60% saturated in the tissues, so the amount of oxygen released in the tissues is close to 40% of the maximum that can be carried in the blood. At high altitudes, O_2 delivery declines by about one-fourth, to 30% of maximum. An increase in BPG concentration, however, decreases the affinity of hemoglobin for O_2, so nearly 40% of what can be carried is again delivered to the tissues.