4 Protein Conformation

Proteins consist of polypeptides folded into specific three-dimensional configurations. This chapter considers the interactions that determine these configurations and examines some representative protein structures.

Concepts

4.1 The amino acid sequence of a polypeptide determines its conformation in solution.

Many proteins, after being unfolded artificially (denatured), will refold spontaneously to their original (native) conformation when incubated at an appropriate temperature, pH, and ionic strength. Thus the information in the amino acid sequence of a polypeptide is sufficient to direct the correct three-dimensional folding under physiological conditions.

4.2 Noncovalent interactions are primarily responsible for maintaining protein conformations.

The forces that determine and maintain protein conformations result primarily from noncovalent interactions of amino acid residues with each other and the surrounding medium. Three principal kinds of noncovalent interactions are involved:

1. Hydrophobic interactions, which are quantitatively the most important of the three, lead to a decrease in free energy when hydrophobic side chains are removed from the aqueous environment by folding to the protein interior (Concept 4.3). Van der Waals bonds between hydrophobic groups also contribute to protein stability in molecules where there are many such contacts.

2. H-bonds can be formed by the C=O and N—H groups of each peptide bond and the electronegative atoms of polar side chains (Concept 1.2). These groups can H-bond with each other or with water molecules on the exterior of the protein, as shown in Figure 4.1. H-bonds are strongest when the three participating atoms lie in a straight line (Figure 4.1).

3. Ionic bonds can form between ionized side chains of opposite charge, such as Asp and Lys (Concept 1.2).

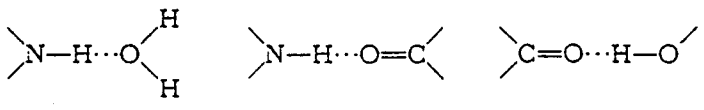


Figure 4.1
Three H-bonds found in proteins. The atoms involved in each H-bond are shaded.

4.3 Polypeptides in solution fold so as to minimize free energy.

A. The interactions that maintain protein conformation can be explained by considering the thermodynamics of the folding process. A randomly oriented polypeptide will fold to minimize the free energy, G, of the molecule and its immediate surroundings. The resulting free-energy decrease, ΔG, expressed in kilocalories per mole (kcal/mol), is defined by the equation

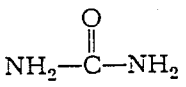
$$\Delta G = \Delta H - T\Delta S \tag{4.1}$$

in which H is the enthalpy or heat content (kcal/mol), T is the absolute temperature (degrees Kelvin), and S is the entropy or degree of disorder in the system (kcal/mol deg). [*Note:* Most standard references list entropy values in entropy units (e.u.): 1 e.u. = 1 cal/mol deg.] Interactions leading to a decrease in free energy (negative value of ΔG) are thermodynamically favored. Such interactions decrease the enthalpy (negative ΔH) or increase the entropy (positive ΔS) of the system or do both. (For a more detailed explanation of free energy see Concept 9.2.)

B. Hydrophobic interactions are primarily entropy driven. The currently favored explanation is that water molecules surrounding exposed hydrophobic side chains somehow are restricted to ordered configurations. Consequently, folding of hydrophobic groups out of the aqueous phase into the protein interior increases the entropy of the surrounding water. Although the ΔH of this process usually is slightly positive and therefore unfavorable, the large positive ΔS greatly favors folding. Hydrophobic associations, unlike true chemical bonds, increase in stability with increasing temperature as $T\Delta S$ increases. They are relatively nonspecific because they result from solvent properties, rather than from bonding between specific side chains.

C. Formation of internal H-bonds and ionic bonds does not contribute substantially to the ΔG of folding, but does ensure that correct folding occurs. Neutral and charged polar groups inside the protein interact with water molecules before folding and with each other afterward, with little net change in free energy. However, if polar group–water bonds are broken and *not* replaced by internal polar group–polar group bonds, there is a large positive ΔG, which makes incorrect folding highly unfavorable. Structures maintained by H-bonds and ionic bonds decrease in stability as temperature increases.

D. If the environment is changed so that the native configuration is no longer the minimum-free-energy state, proteins will tend to denature. Denaturing reagents such as urea,



and nonpolar solvents stabilize exposed hydrophobic side chains, thereby lowering the free energy of the unfolded state. Extremes of pH and temperature also denature most proteins. The fully denatured state of a polypeptide is referred to as a **random coil**, implying a lack of ordered structure.

E. The energies of the noncovalent bonds involved in maintaining protein conformation are listed in Table 4.1, together with the energies of two covalent bonds in proteins for comparison. (Bond energy is the energy required to break a bond.)

Table 4.1
Bond Energies of Some Bonds Found in Proteins

Bond	Bond energy[a] (kcal/mol)
Covalent	
C—C (ethane)	83
S—S	50
Noncovalent	
H-bond	3–7
Ionic bond	3–7
Hydrophobic association	(3–5)[b]
Van der Waals bond	1–2

[a] The free energy needed to break the bond.
[b] Here the value represents the free energy that must be supplied to unfold a nonpolar side chain from the protein interior into the aqueous surroundings at 25°C. This energy increases with temperature, unlike other values in the table, and is not really a bond energy, because most of it is not used to break bonds in the unfolding process.

4.4 Native proteins in aqueous surroundings have most nonpolar side chains inside and most polar side chains outside.

To attain minimum-free-energy conformations, proteins must fold so as to shield hydrophobic side chains from the aqueous surroundings while exposing hydrophilic side chains. The few internal polar side chains are in contact with each other through H-bonds or ionic bonds. These important generalizations follow directly from the thermodynamic considerations in Concept 4.3. However, although gross folding is predictable, the interactions determining protein conformation are not yet understood well enough to allow prediction of detailed three-dimensional structure from amino acid sequence.

4.5 Many proteins are stabilized by intramolecular disulfide bonds.

Disulfide bonds can form between Cys residues that become juxtaposed by folding of the polypeptide into a minimum-free-energy configuration. Some proteins undergo internal cleavages following —S—S— bond formation, so that a portion of the polypeptide is removed (e.g., insulin is formed by cleavage of an internal peptide from proinsulin). The three-dimensional structures of such proteins often are no longer minimum-free-energy configurations; they are maintained only by the stability of the disulfide bonds.

4.6 There are four levels of organization in protein structures.

In describing the three-dimensional structure of proteins it is customary to consider four levels of organization. **Primary structure** is the linear sequence of amino acids in a polypeptide (Chapter 3). **Secondary structure** refers to certain repeating conformational patterns, the most common of which are described in Concept 4.7. **Tertiary structure** refers to the overall polypeptide conformation. No clear distinction can be made between secondary and tertiary structure. **Quaternary structure** refers to the spatial relationships between subunits in proteins that consist of two or more polypeptides (**multimeric proteins**; see Chapter 5).

4.7 The α helix and the β sheet are common repeating structural patterns in proteins.

When a polypeptide folds in solution, the backbone polar groups in the interior must interact with each other (Concept 4.3, part C). Two repeating conformations called the **α helix** and the **β sheet** satisfy this condition in many proteins.

1. The α helix, shown in Figures 4.2 and 4.3, is a regular coiled configuration of the polypeptide chain. There are 3.6 amino acids per helical turn, spanning an axial distance of 0.54 nanometers (nm). The side chains point to the outside of the helix. Each peptide nitrogen is H-bonded to the oxygen of a peptide carbonyl group four residues down the chain. These H-bonds are linear and therefore maximally stable. The α helix is prevented from forming by Pro residues, by two or more consecutive residues with side chains that branch at the β carbon (Val, Ilu, and Thr), or by two or more consecutive residues with ionized side chains of like charge. The α helix is flexible and elastic.

2. The β sheet, shown in Figures 4.4 and 4.5, is a pleated structure composed of side-by-side polypeptides connected by H-bonds. The two-residue repeat distance is 0.70 nm. The adjacent polypeptides generally run antiparallel to each other, as shown in Figure 4.6. The β sheet is formed most readily by polypeptides with repeating sequences of amino acids with compact side chains, such as (Gly-Ser-Gly-Ala-Gly-Ala)$_n$. The β sheet is flexible, but inelastic.

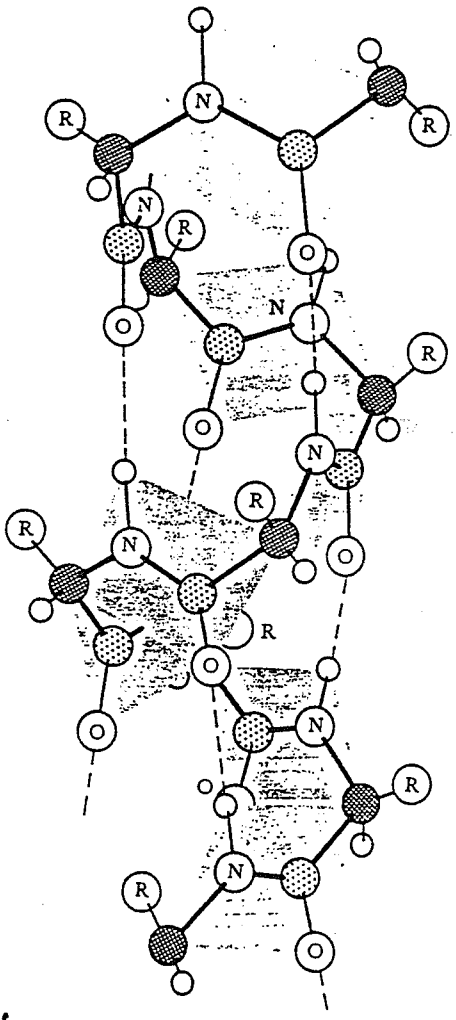


Figure 4.2
The α helix. Only the right-handed form of the helix, shown here, is found in proteins; O atoms, N atoms, and side-chain groups (R) are lettered, and C_α atoms are represented by darker shaded spheres. H-bonds are shown as dashed lines. (Adapted from R. E. Dickerson and I. Geis, *The Structure and Action of Proteins,* Benjamin/Cummings, Menlo Park, Calif. © 1969 Dickerson and Geis.)

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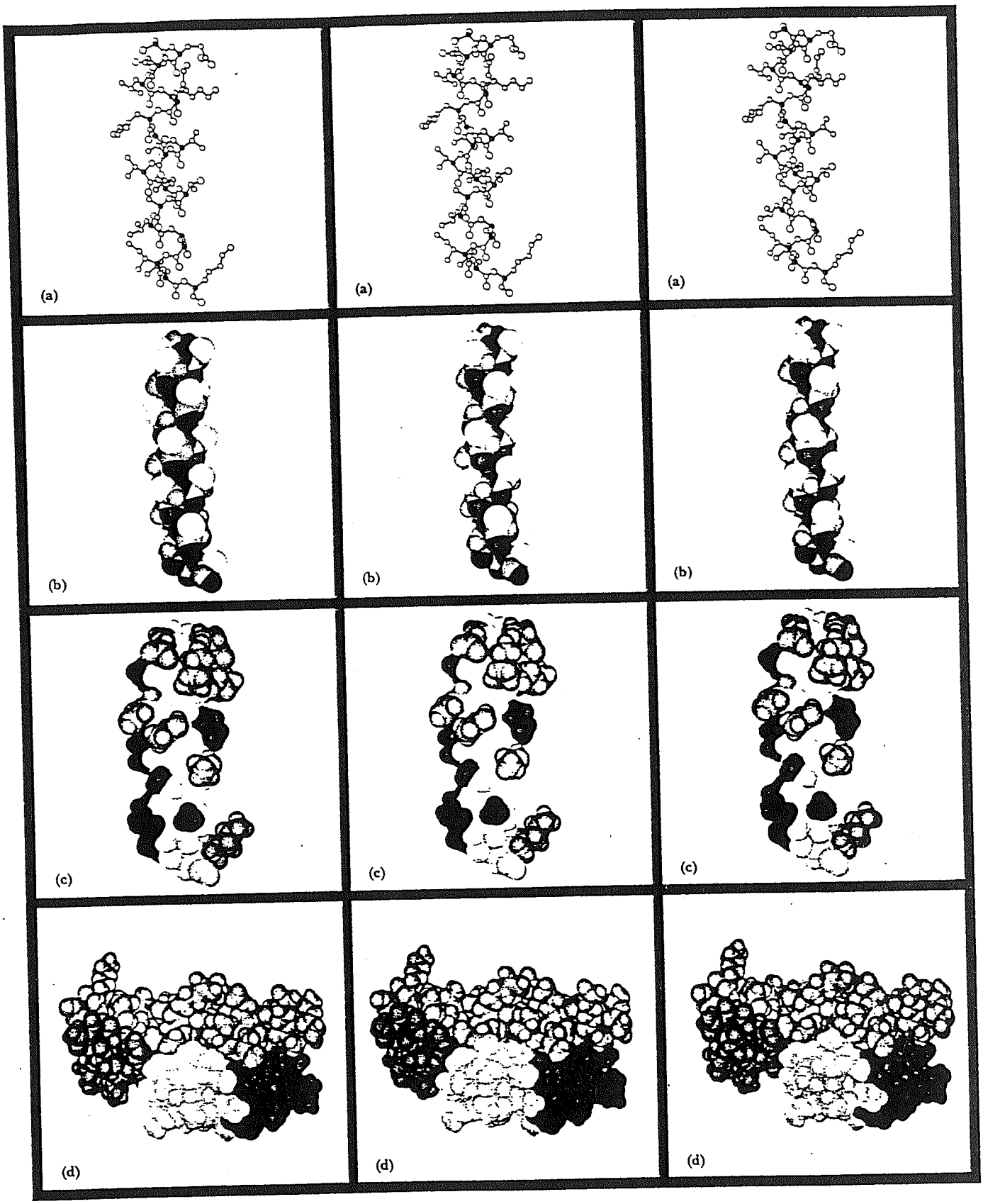

Figure 4.3
Stereo triptychs of α-helices from sperm whale myoglobin. (a) Skeletal model of helix E. C_α-atoms are shown in black; H-atoms are not shown. (b) Space-filling model of helix E. Atoms involved in H bonds are shown in black. R groups are shown as light balls. (c) Space-filling model of helix E with R groups included. Black R groups are hydrophobic; gray R groups are hydrophilic. (d) Packing of four α-helices. For stereo viewing, see instructions in the Appendix to Chapter 4. (Stereo figures courtesy of Richard J. Feldmann, NIH.)

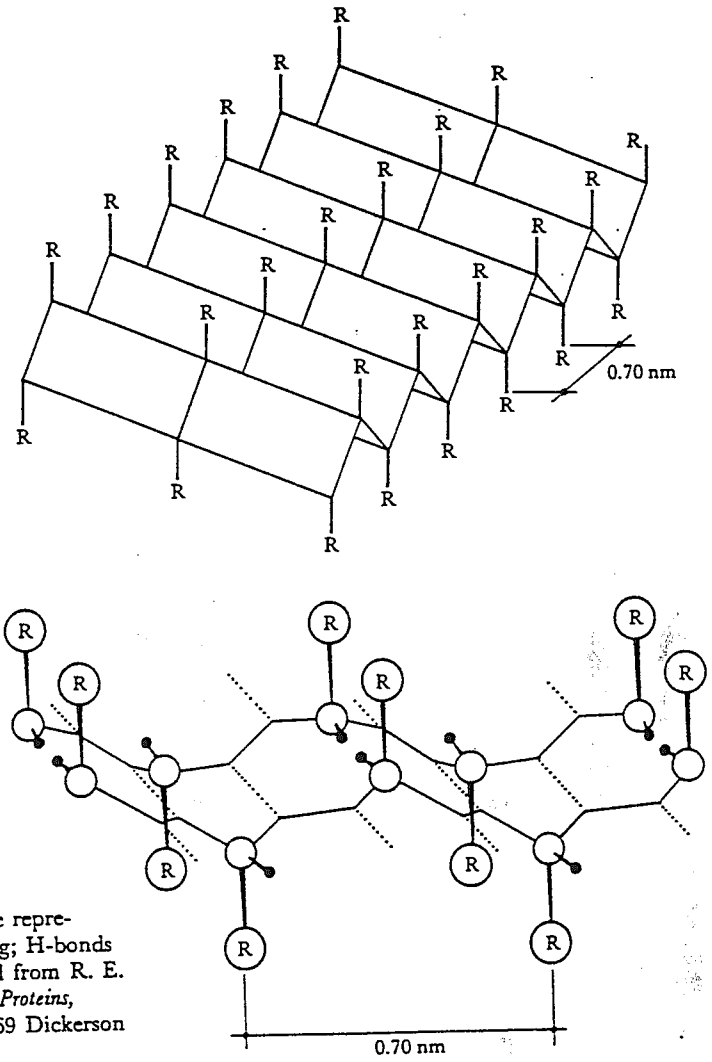

Figure 4.4
Two representations of the β sheet. C_α atoms are represented as unlettered spheres in the lower drawing; H-bonds are shown as dotted lines. (Lower figure adapted from R. E. Dickerson and I. Geis, *The Structure and Action of Proteins*, Benjamin/Cummings, Menlo Park, Calif. © 1969 Dickerson and Geis.)

4.8 The three-dimensional structures of several proteins have been established by x-ray diffraction.

When x-rays pass through a protein crystal, they are diffracted by the atoms of the protein molecules. From the diffraction pattern it is possible to determine the relative positions of these atoms. There is substantial evidence that the native conformations of proteins in crystals and in solution are the same.

The three-dimensional conformations of three proteins are shown in Figures 4.7, 4.9, and 4.10 as computer-generated, stereo triptych diagrams. Viewing these diagrams in three dimensions is a striking and instructive experience, worth the effort it may take to learn how to do so (see viewing instructions in the appendix to this chapter).

1. **Myoglobin** (Figure 4.7) is a single polypeptide of 153 amino acid residues with a molecular weight of 17,500. It functions as an oxygen-storage protein in muscle tissues. Almost all the hydrophobic residues are on the interior of the protein. Within the myoglobin molecule is a heme group, which has the structure shown in Figure 4.8. The heme group also is found in hemoglobin, the oxygen-carrier protein of blood; O_2 is carried on the iron atom of the heme.

2. **Lysozyme** (Figure 4.9) is a single polypeptide of 129 amino acids with a molecular weight of 14,600. It catalyzes the cleavage of certain bacterial polysaccharides.

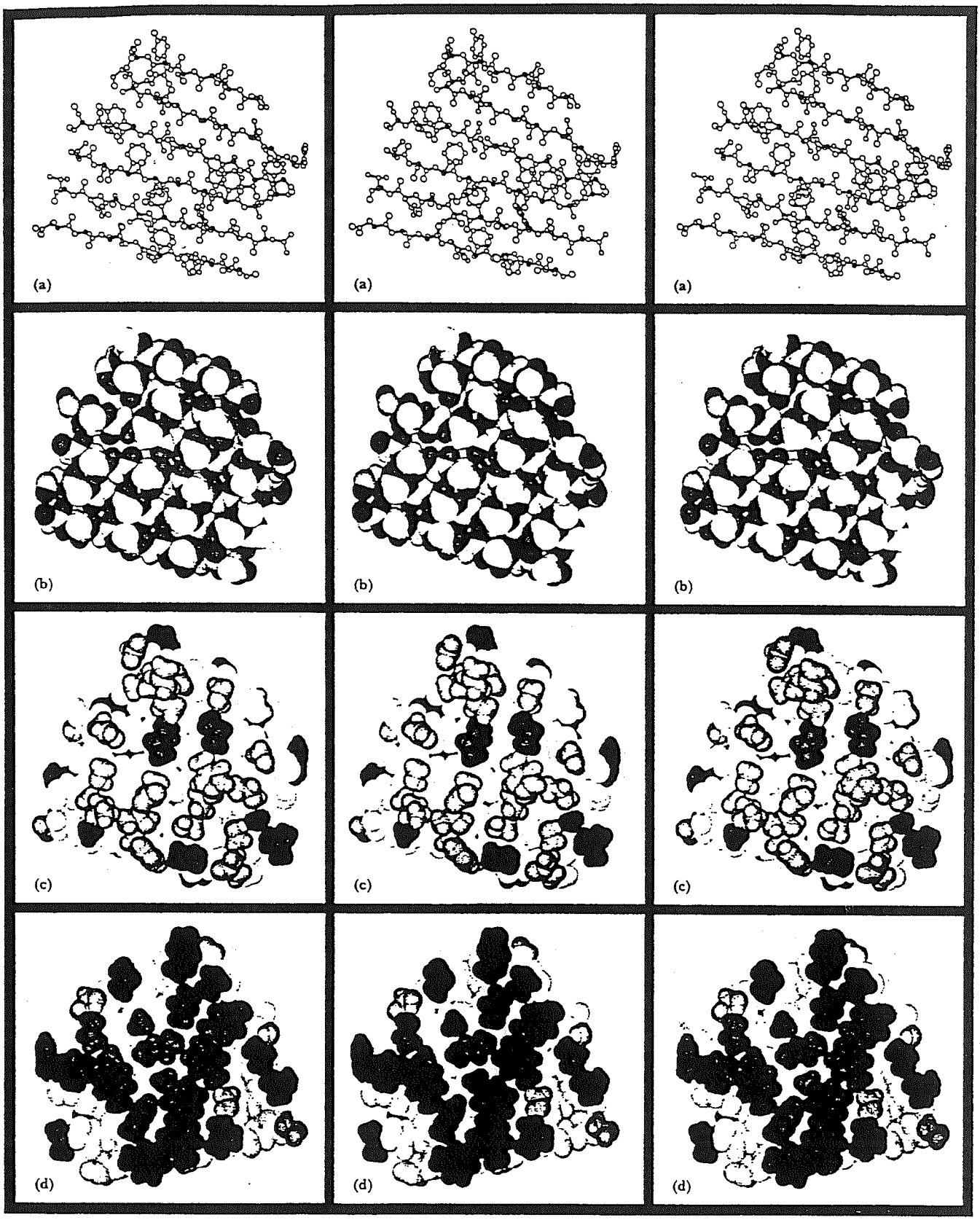

Figure 4.5
Stereo triptychs of a β-sheet structure from Jack bean concanavalin A. (a) Skeletal model. C_α-atoms are shown in black; H atoms are not shown. (b) Space-filling model. Atoms involved in H bonds are shown in black. R groups are shown as light balls. (c) Space-filling model with R groups included. Black R groups are hydrophobic; gray R groups are hydrophilic. (d) 180° rotation of view C about a vertical axis to show the other side of the β-sheet. Color coding as in (c). The hydrophobic side of the β-sheet faces the interior of the protein; the hydrophilic side faces the solvent. For stereo viewing, see instructions in the Appendix to Chapter 4. (Stereo figures courtesy of Richard J. Feldmann, NIH.)

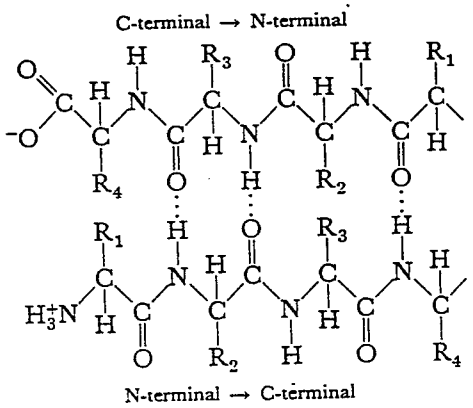

Figure 4.6
Antiparallel polypeptides in a β sheet.

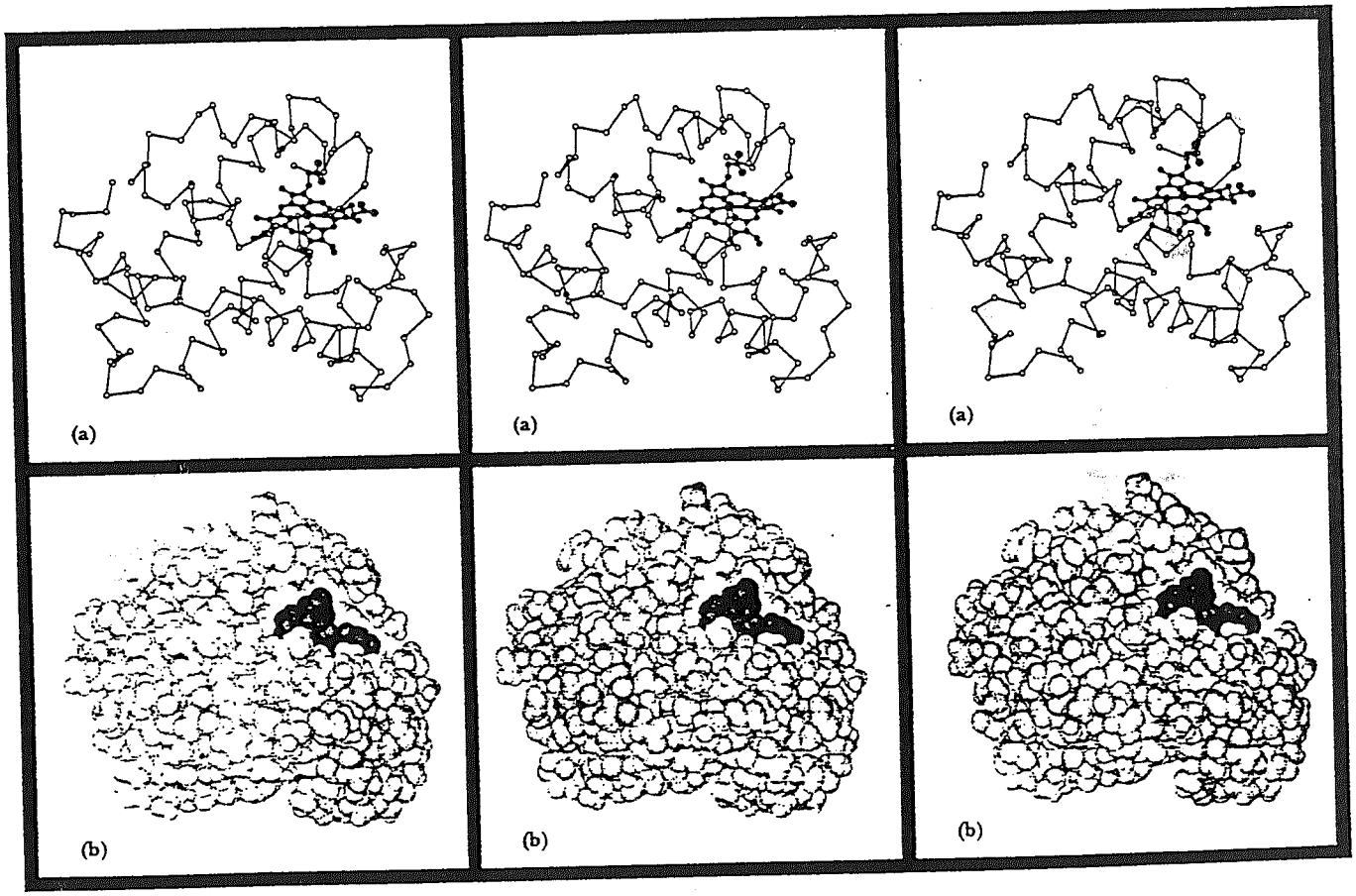

Figure 4.7
Stereo triptychs of sperm whale myoglobin. (a) Skeletal model showing only the C_α-atoms of the protein. The atoms in the heme group are shown in black. (b) Space-filling model. The atoms in the heme group are shown in black. For stereo viewing, see instructions in the Appendix to Chapter 4. (Stereo figures courtesy of Richard J. Feldmann, NIH.)

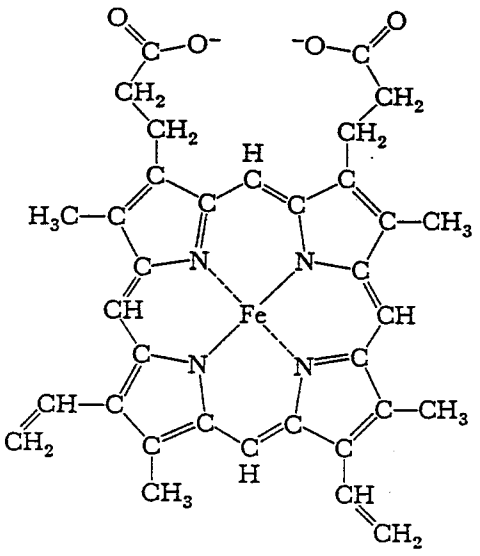

Figure 4.8
Structure of the heme group in myoglobin and hemoglobin.

3. Carboxypeptidase A (Figure 4.10) is a single polypeptide of 307 amino acids with a molecular weight of 34,600. It catalyzes the cleavage of amino acids from the C termini of polypeptides (Chapter 3).

4.9 Additional concepts and techniques are presented in the Problems section.

A. Structure of collagen. Problem 4.15.
B. Optical rotation of polypeptides. Problem 4.18.
C. Ramachandran diagrams. Problems 4.19 and 4.20.
D. Helical pitch. Problem 4.21.

Appendix: Viewing Stereo Triptychs

The stereo triptych (*trip'tik*) is a convenient viewing format suitable for a standard stereoscope or for stereo viewing with unaided eyes using proximal or distal convergence. The most flexible stereo viewing system is a trained pair of eyes, and the training required for stereo viewing is relatively easy. This appendix considers some relevant aspects of normal vision and describes a step-by-step training method for learning to see stereo pictures with unaided eyes.

4A.1 During normal binocular vision, convergence and focus are interdependent.

A. Convergence describes the coordinated orienting of the two eyes. The orientation of each eye defines a line of sight from the object being looked at through the center of the lens to the small differentiated spot on the retina, the fovea, which is the point of maximum visual acuity (Figure 4A.1). The lines of sight from the eyes intersect (converge) at the object. This convergence defines your point of attention—the point in visual space at which you look. (The degree to which the point of attention behaves as a point is remarkable, as you can observe by scrutinizing a single letter on this page.)

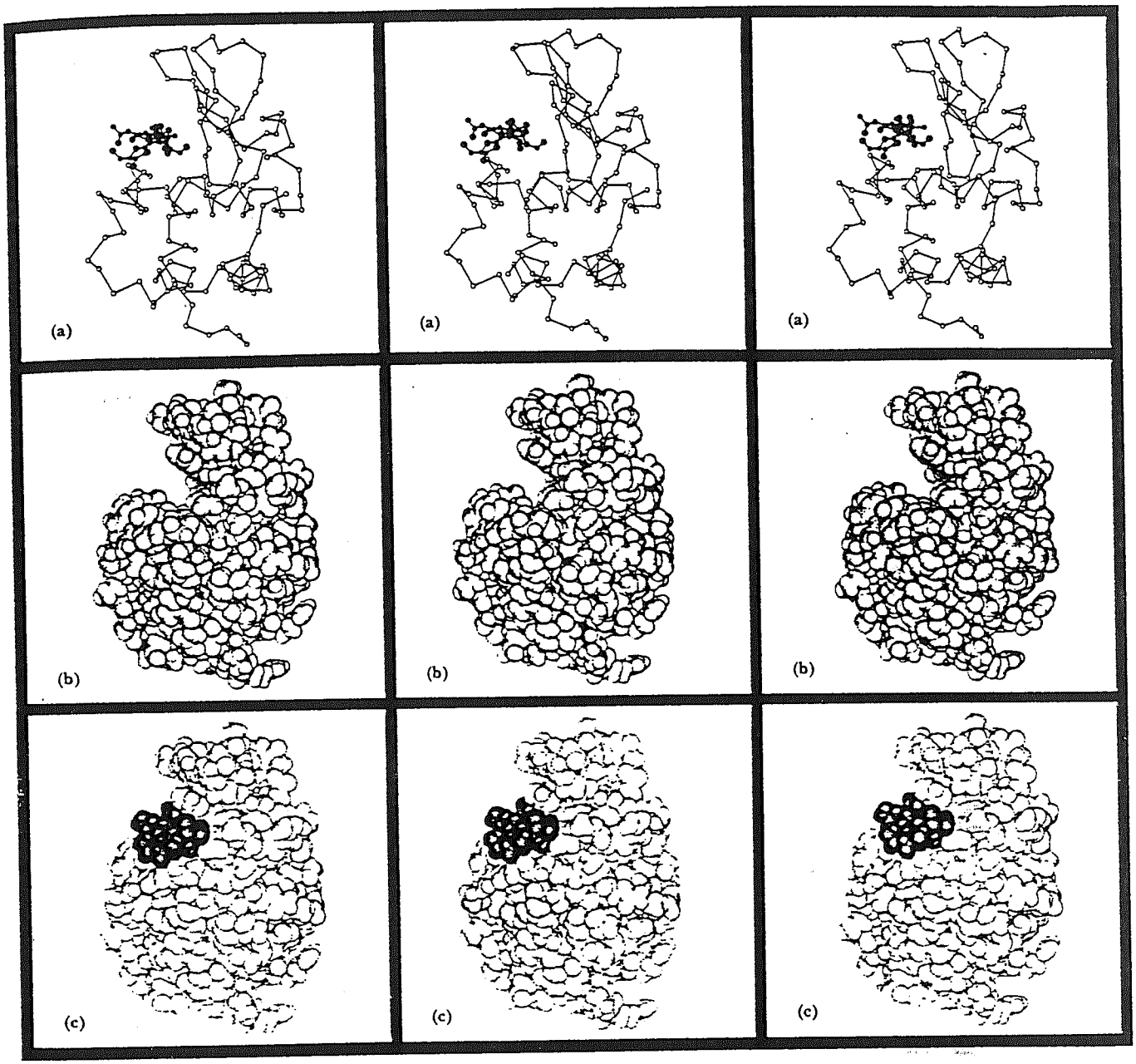

Figure 4.9
Stereo triptychs of chicken lysozyme. (a) Skeletal model showing only the C_α-atoms of the protein. Black spheres represent the atoms of a trimeric analogue of the polymeric substrate, positioned in the active site. (b) Space-filling model with the active site open. (c) Space-filling model with the trimeric substrate analogue (black atoms) positioned in the active site. For stereo viewing, see instructions in the Appendix to Chapter 4. (Stereo figures courtesy of Richard J. Feldmann, NIH.)

B. Variations in tension on the lens of the eye permit objects at different distances to be brought into focus. A particular tension on the lens defines a particular plane of focus. During normal vision, tension automatically adjusts so that the plane of focus always includes the point of attention (Figure 4A.1). Consequently, the object you are looking at is seen clearly. (Most prescription glasses shift the focal plane so that aligning it with the point of attention—that is, focusing—is within the range of the wearer's natural abilities.)

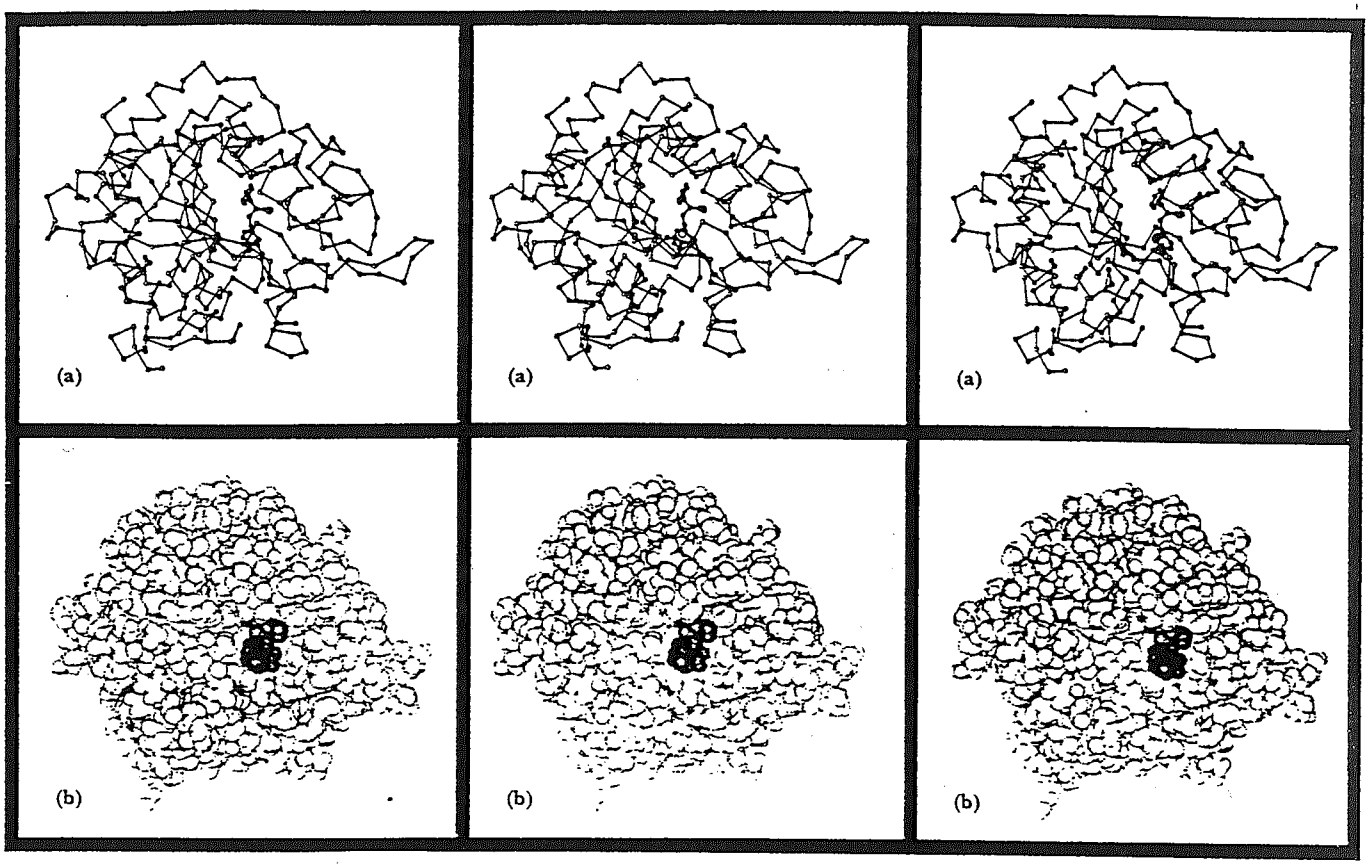

Figure 4.10
Stereo triptychs of bovine carboxypeptidase A. (a) Skeletal model showing only the C_α-atoms of the protein. Black spheres represent the atoms of a substrate analogue (carbobenzoxy-Ala-Ala-Tyr) positioned for catalysis in the active site. (b) Space-filling model. The atoms of the substrate analogue are shown in black. For stereo viewing, see instructions in the Appendix to Chapter 4. (Stereo figures courtesy of Richard J. Feldmann, NIH.)

C. The three-dimensionality of normal binocular visual perception depends on the slight disparity in the views from each eye. The two slightly different images received by the eyes are fused by the brain into a single percept, which is three-dimensional. This natural fusion mechanism can be defeated by applying gentle pressure below one eye. Do you see a doubling of images? Viewing stereo pictures with unaided eyes uses this natural fusion mechanism in a new way.

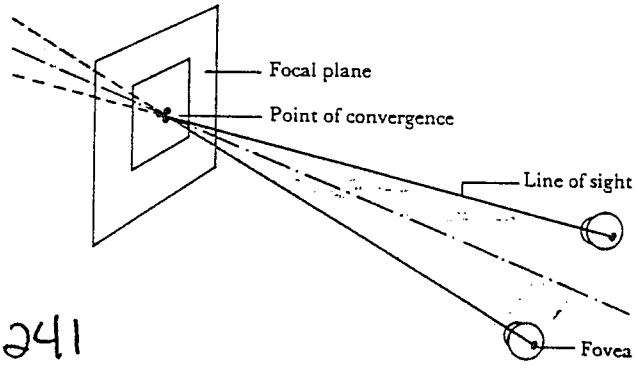

Figure 4A.1
Normal coordination of focus and convergence.

4A.2 Stereo viewing with unaided eyes requires independence of convergence and focus.

A. An artificial three-dimensional image can be produced from two pictures taken from slightly different viewpoints. For a correct three-dimensional image the picture taken from the "right" viewpoint must be seen by the right eye, and that from the "left" viewpoint must be seen by the left eye. There are two ways of viewing such stereo-pair pictures, as illustrated in Figure 4A.2. For viewing with distal convergence, the pictures are arranged in the standard manner used with a stereoscope. For viewing with proximal convergence, the pictures are switched. In the stereo triptych these two arrangements are combined into a format that can be viewed with a stereoscope or with unaided eyes using proximal or distal convergence (Figure 4A.3). The format shown is standard throughout this book.

B. Stereo viewing with unaided eyes is unusual in that you must look two places at once. To see both pictures simultaneously, your lines of sight must converge either proximally or distally to the plane of the pictures (Figure 4A.2). However, for the pictures to be in focus, the focal plane must coincide with the plane of the pictures, and not, as normally, with the convergence point. Therefore, you must be able to control focus and convergence independently. Although perhaps difficult to imagine, this skill is relatively easy to learn.

4A.3 Stereo viewing with unaided eyes is a learned skill.

A. The first step in learning to view stereo pictures is to become aware of the visual field beyond your point of attention. Hold a pencil (or finger) about 10 in. in front of your eyes and look fixedly at the tip. Are you aware of one or two images of objects beyond your point of attention? It may help to pick out some relatively isolated and conspicuous object in order to decide. If you see two images, you will be able to learn this viewing method and are ready for the next step. If you are aware of only one image,

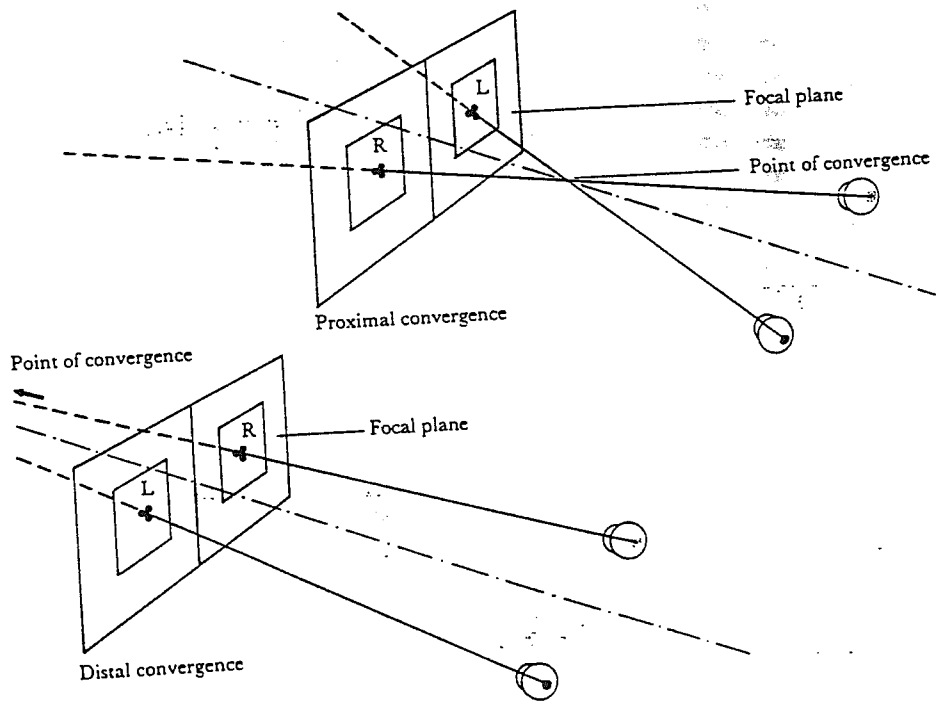

Figure 4A.2
Two arrangements for viewing stereo-pair pictures.

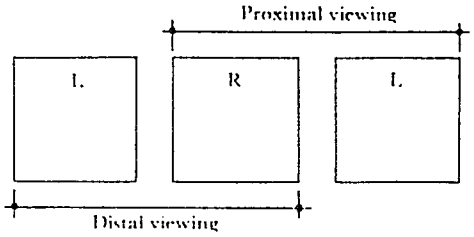

Figure 4A.3
Standard format for stereo triptychs.

line up your pencil tip with that image and, by blinking your eyes alternately, decide with which eye you are seeing that image. Most of you in this category are suppressing the visual information from your weaker eye. It may help you to become aware of the second image by alternately covering and uncovering your stronger eye while gazing at the pencil tip, or by dimming the image from your stronger eye by covering it with a piece of tinted glass that will reduce the entering light without changing its color, such as a lens from a pair of gray sunglasses (neutral density filter).

B. The second step is to fuse two images using either proximal or distal convergence. Practice on the stereo pair of images in Figure 4A.4.

For proximal convergence adjust your point of attention to between you and the stereo pair until you are aware of three separate but out-of-focus images in the background. (It may help to use a pencil tip as a guide or simply cross your eyes slightly.) Three images will appear when you are looking at a point approximately halfway between you and the page. (If you see four separate images, your point of attention is too close to your eyes.) When you see three images, keep your attention on the center image, disregarding the outer two. The center image, which is a combination of the two original images, contains the information necessary for a three-dimensional percept. When the two images are properly overlaid your fusion mechanism will tend to "lock" onto it. You may need to tilt your head slightly toward one shoulder to bring the overlaid images to the same level. In any case note the effect of head tilting; proper head position is essential for comfortable viewing.

For distal convergence, your point of attention must be beyond the stereo pictures. You may find it helpful to look at an object in the distance and then move the stereo pictures into your field of view. The goal, as before, is to see three images.

When you have obtained three images, you can verify whether you are converging proximally or distally to the page by blinking one eye and observing which of the two outer images disappears. If your point of attention is proximal to the page, the right-hand outside image will disappear when you blink your right eye.

C. The third and final step is to focus on the fused image. The image is out of focus because your eyes are focused proximally or distally, at your point of convergence. You must now learn to adjust your focus to the plane of the page while maintaining the three images. The first few times you try this there will be a natural tendency for the central image to split apart. That is because you have trained yourself since

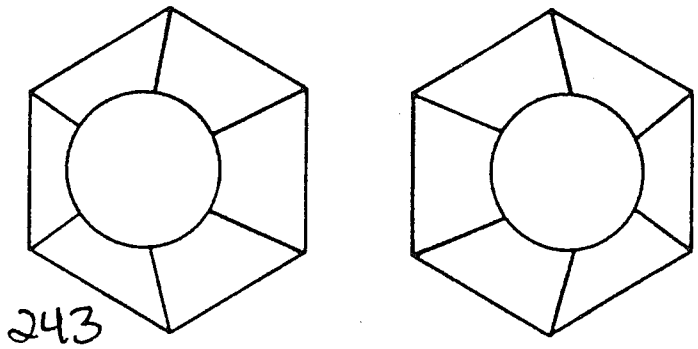

Figure 4A.4
A stereo-pair drawing.

infancy to converge and focus at the same point in space. You may find it useful to move the picture toward or away from your eyes. Once you see the central image in focus you should become aware of a three-dimensional percept within a few seconds. If you are converging proximally, the circle will appear in front of the hexagon; if you are converging distally, the circle will appear behind the hexagon.

It may take you some time to become aware of your ability to control convergence and focus independently. This step is rate-limiting in the overall learning process, and may require considerable effort at first. However, you will find that it becomes easier with practice and that the results will be worth the initial effort.

D. To view a stereo triptych choose the appropriate pair of pictures, depending on whether you prefer proximal or distal convergence, and view them as before. Initially it may help to cover the third picture in the triptych. When the third picture is not covered, note that once you have obtained a three-dimensional image by fusing one pair of adjacent pictures in the triptych, you can shift your gaze to give a three-dimensional image of the other pair without losing fusion or focus. (The surrounding border is designed to aid you in maintaining fusion as you shift your attention, especially in groups of three-dimensional pictures.) The second three-dimensional image is reversed back to front. For wire models this reversal produces a three-dimensional mirror image. For space-filling figures the reversal produces a bizarre effect that has been termed **pseudoscopic.**

4A.4 Stereo viewing differs with proximal and distal convergence.

A. Eye physiology restricts the possible size formats for proximal and distal stereo viewing. The geometry of viewing with proximal convergence can accommodate a large

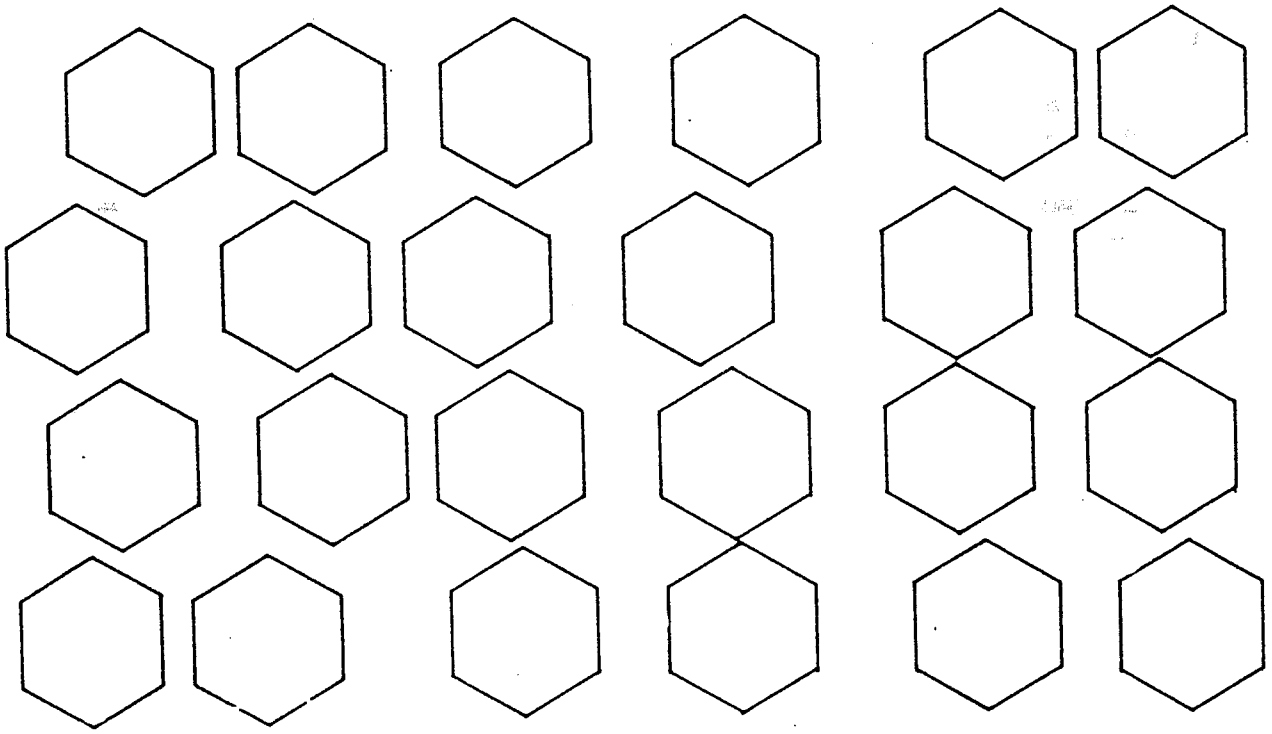

Figure 1A.5
Perceived size as a function of perceived distance. Fuse any adjacent pair of hexagons in the same row. When you have fused one pair you will be able to look around the entire grid and see hexagons at different apparent distances. Compare the sizes of near and distant hexagons.

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variety of picture sizes and a corresponding wide range of viewing distances. (Viewing large pictures at a distance represents an additional learning step.) Stereo viewing with distal convergence is more restricted. Because your eyes cannot turn outward, the width of pictures that you can view is limited; their centers cannot be separated by more than the distance between your pupils. This restriction on picture size imposes a corresponding restriction on viewing distance. Fortunately, the size format for standard stereoscopes usually permits them to be viewed with distal convergence. However, the absolute size limit varies with the individual, so you may experience difficulty with some stereo figures.

B. You may notice that the three-dimensional image you perceive is noticeably different in size than the picture on the page. With proximal convergence the image appears smaller, whereas with distal convergence it appears larger. This illusion results from a systematic distortion that is a normal component of visual perception. It may make you curious about how you perceive size (see Figure 4A.5). However, your ability to resolve detail in the picture on the page and in the three-dimensional percept will be the same, because the size of the retinal image remains constant at a given viewing distance, regardless of changes in the point of convergence.

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Problems

4.1 Answer the following with true or false. If false, explain why.

a. H-bonding occurs between hydrogen atoms on the surfaces of proteins in solution.
b. The thermodynamically most stable conformation of a protein is the structure of lowest free energy.
c. Formation of internal H-bonds is the major interaction that drives protein folding.
d. Organic solvents denature proteins primarily by preventing ionic interactions.
e. Folding of a hydrophobic protein is accompanied by an increase in entropy of the polypeptide.
f. The term *quaternary structure* refers to protein configuration in the fourth dimension, that is, as a function of time.
g. Disulfide bonds covalently link Cys residues whose proximity is determined by previous noncovalent interactions.
h. The amide hydrogen of every peptide bond in an α helix is H-bonded.
i. From the complete primary structure of a protein, it is possible to calculate its three-dimensional configuration.

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4.2 a. Minimum-free-energy configurations of proteins often are reinforced by covalent