Protein Function: Myoglobin and Hemoglobin, Muscle Contraction, and Antibodies

The nearly colorless icefish is the only adult vertebrate that lacks hemoglobin. It can survive without this otherwise essential oxygen-binding protein because in the cold (−1.9°C) Antarctic waters where it lives, the fish consumes little oxygen and the solubility of oxygen is relatively high. [© Doug Allan/Peter Arnold, Inc.]

Chapter Contents

The preceding two chapters have painted a broad picture of the chemical and physical properties of proteins but have not delved deeply into their physiological functions. Nevertheless, it should come as no surprise that the structural complexity and variety of proteins allow them to carry out an enormous array of specialized biological tasks. For example, the enzyme catalysts of virtually all metabolic reactions are proteins (we consider enzymes in detail in Chapters 11 and 12). Genetic information would remain locked in DNA were it not for the proteins that participate in decoding and transmitting that information. Remarkably, the thousands of proteins that participate in building, supporting, recognizing, transporting, and transforming cellular components act with incredible speed and accuracy and in many cases are subject to multiple regulatory mechanisms.

The specialized functions of proteins, from the fibrous proteins we examined in Section 6-1C to the precisely regulated metabolic enzymes we discuss in later chapters, can all be understood in terms of how proteins bind to and interact with other components of living systems. In this chapter, we focus on three sets of proteins: the oxygen-binding proteins myoglobin and hemoglobin, the actin and myosin proteins responsible for muscle contraction, and antibody molecules. The molecular structures and physiological roles of these proteins are known in detail, and their proper functioning is vital for human health. In addition, these proteins serve as models for many of the proteins we will examine later when we discuss metabolism and the management of genetic information.

1 Oxygen Binding to Myoglobin and Hemoglobin

KEY CONCEPTS

- Myoglobin, with its single heme prosthetic group, exhibits a hyperbolic O_2-binding curve.
- Hemoglobin can adopt the deoxy (T) or oxy (R) conformation, which differ in O_2-binding affinity.
- Oxygen binding triggers conformational changes in hemoglobin so that oxygen binds to the protein cooperatively, yielding a sigmoidal binding curve.
- The Bohr effect and BPG alter hemoglobin's O_2-binding affinity.
- Mutations can change hemoglobin's O_2-binding properties and cause disease.

We begin our study of protein function with two proteins that reversibly bind molecular oxygen (O_2). **Myoglobin,** the first protein whose structure was determined by X-ray crystallography, is a small protein with relatively simple oxygen-binding behavior. **Hemoglobin,** a tetramer of myoglobin-like polypeptides, is a more complicated protein that functions as a sophisticated system for delivering oxygen to tissues throughout the body. The efficiency with which hemoglobin binds and releases O_2 is reminiscent of the specificity and efficiency of metabolic enzymes. It is worthwhile to study hemoglobin's structure and function because many of the theories formulated to explain O_2 binding to hemoglobin also explain the control of enzyme activity.

A Myoglobin Is a Monomeric Oxygen-Binding Protein

Myoglobin is a small intracellular protein in vertebrate muscle. Its X-ray structure, determined by John Kendrew in 1959, revealed that most of myoglobin's 153 residues are members of eight α helices (traditionally labeled A through H) that are arranged to form a globular protein with approximate dimensions 44 × 44 × 25 Å (Fig. 7-1).

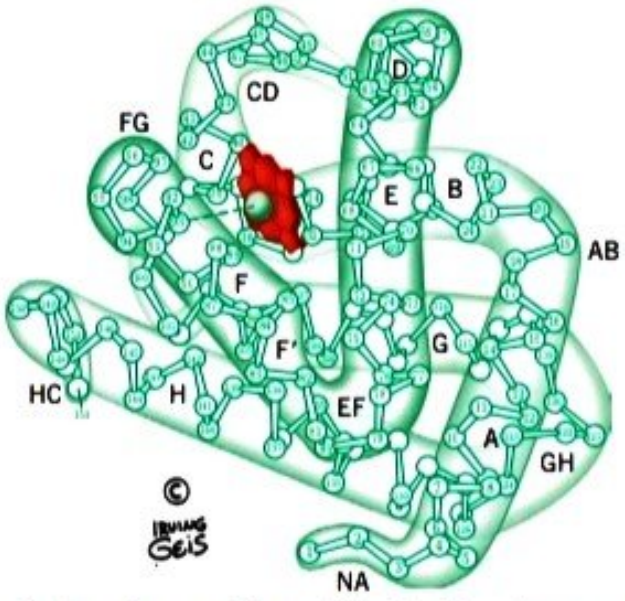


FIG. 7-1 Structure of sperm whale myoglobin. This 153-residue monomeric protein consists of eight α helices, labeled A through H, that are connected by short polypeptide links (the last half of what was originally thought to be the EF corner has been shown to form a short helix that is designated the F' helix). The heme group is shown in red. [Illustration, Irving Geis. Image from the Irving Geis Collection/Howard Hughes Medical Institute. Rights owned by HHMI. Reproduction by permission only.] **See Kinemage Exercise 6-1.**

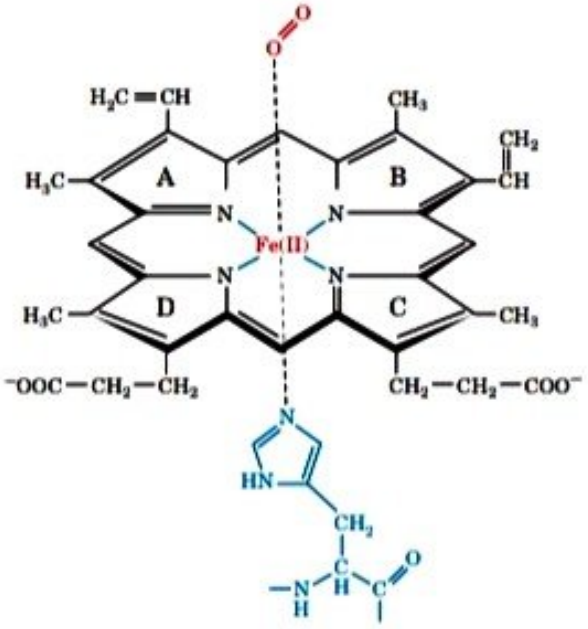
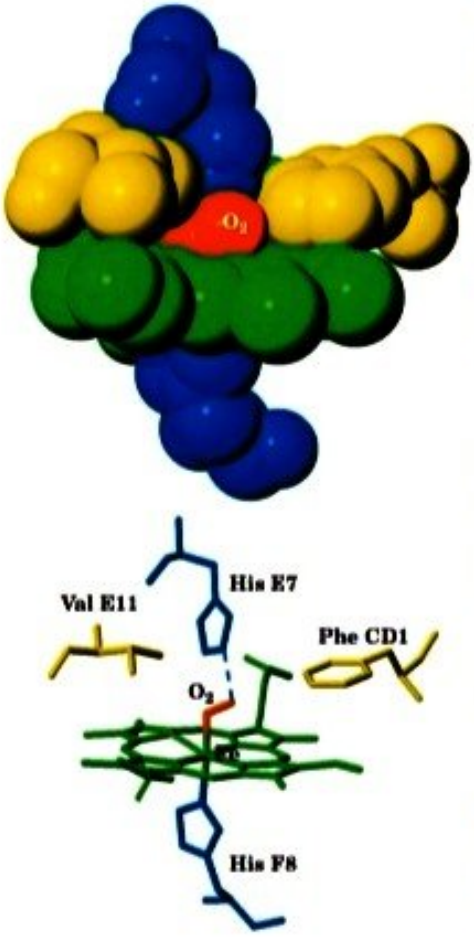


FIG. 7-2 The heme group. The central Fe(II) atom is shown liganded to the four N atoms of the porphyrin ring, whose pyrrole groups are labeled A–D. The heme is a conjugated system, so all the Fe—N bonds are equivalent. The Fe(II) is also liganded to a His side chain and, when it is present, to O_2. The six ligands are arranged at the corners of an octahedron centered on the Fe ion (octahedral geometry).



Myoglobin Contains a Heme Prosthetic Group. Myoglobin, other members of the globin family of proteins (Section 5-4B), and a variety of other proteins such as cytochrome *c* (Sections 5-4A and 6-2D) all contain a single **heme** group (Fig. 7-2). The heme is tightly wedged in a hydrophobic pocket between the E and F helices in myoglobin. The heterocyclic ring system of heme is a **porphyrin** derivative containing four **pyrrole** groups (labeled A–D) linked by methene bridges (other porphyrins vary in the substituents attached to rings A–D). The Fe(II) atom at the center of heme is coordinated by the four porphyrin N atoms and one N from a His side chain (called, in a nomenclature peculiar to myoglobin and hemoglobin, His F8 because it is the eighth residue of the F helix). A molecule of oxygen (O_2) can act as a sixth ligand to the iron atom. His E7 (the seventh residue of helix E) hydrogen bonds to the O_2 with the geometry shown in Fig. 7-3. Two hydrophobic side chains on the O_2-binding side of the heme, Val E11 and Phe CD1 (the first residue in the segment between helices C and D), help hold the heme in place. These side chains presumably swing aside as the protein "breathes" (Section 6-4A), allowing O_2 to enter and exit.

When exposed to oxygen, the Fe(II) atom of isolated heme is irreversibly oxidized to Fe(III), a form that cannot bind O_2. The protein portion of myoglobin (and of hemoglobin, which contains four heme groups in four globin chains) prevents this oxidation and makes it possible for O_2 to bind reversibly to the heme group. **Oxygenation** alters the electronic state of the Fe(II)–heme complex, as indicated by its color change from dark purple (the color of hemoglobin in venous blood) to brilliant scarlet (the color of hemoglobin in arterial blood). Under some conditions, the Fe(II) of myoglobin or hemoglobin becomes oxidized to Fe(III) to form **metmyoglobin** or **methemoglobin,** respectively; these proteins are responsible for the brown color of old meat and dried blood.

In addition to O_2, certain other small molecules such as CO, NO, and H_2S can bind to heme groups in proteins. These other compounds bind with

FIG. 7-3 The heme complex in myoglobin. In the upper drawing, atoms are represented in space-filling form (H atoms are not shown). The lower drawing shows the corresponding skeletal model with a dashed line representing the hydrogen bond between His E7 and the bound O_2. [Based on an X-ray structure by Simon Phillips, MRC Laboratory of Molecular Biology, Cambridge, U.K. PDBid 1MBO.] **See Kinemage Exercise 6-1.**

much higher affinity than O_2, which accounts for their toxicity. CO, for example, has 200-fold greater affinity for hemoglobin than does O_2.

Myoglobin Binds O_2 to Facilitate Its Diffusion. Although myoglobin was originally thought to be only an oxygen-storage protein, it is now apparent that *its major physiological role is to facilitate oxygen diffusion in muscle* (the most rapidly respiring tissue under conditions of high exertion). The rate at which O_2 can diffuse from the capillaries to the tissues is limited by its low solubility in aqueous solution ($\sim 10^{-4}$ M in blood). Myoglobin increases the effective solubility of O_2 in muscle cells, acting as a kind of molecular bucket brigade to boost the O_2 diffusion rate. The oxygen-storage function of myoglobin is probably significant only in aquatic mammals such as seals and whales, whose muscle myoglobin concentrations are around 10-fold greater than those in terrestrial mammals (which is one reason why Kendrew chose the sperm whale as a source of myoglobin for his X-ray crystallographic studies). Nevertheless, mice in which the gene for myoglobin has been "knocked out" appear to be normal, although their muscles are lighter in color than those of wild-type mice. However, closer scrutiny revealed several compensatory adaptations in these mice, including a greater concentration of red blood cells and increased capillary density in their muscles. Moreover, many of the mutant embryos died *in utero* due to cardiovascular defects. Vertebrates also express two recently discovered globins: **neuroglobin,** which is present mainly in brain, retina, and endocrine tissues, and **cytoglobin,** which occurs in most tissues. Neuroglobin protects neurons (nerve cells) from damage under conditions of **ischemia** (inadequate blood flow, such as in a stroke), most likely by preventing **reperfusion injury** (the damage caused by the oxygen radicals generated when blood flow is restored). Cytoglobin may have similar functions.

Myoglobin's Oxygen-Binding Curve Is Hyperbolic. The reversible binding of O_2 to myoglobin **(Mb)** is described by a simple equilibrium reaction:

$$Mb + O_2 \rightleftharpoons MbO_2$$

The dissociation constant, K, for the reaction is

$$K = \frac{[Mb][O_2]}{[MbO_2]} \qquad [7\text{-}1]$$

Note that biochemists usually express equilibria in terms of dissociation constants, the reciprocal of the association constants favored by chemists. The O_2 dissociation of myoglobin can be characterized by its **fractional saturation,** Y_{O_2}, which is defined as the fraction of O_2-binding sites occupied by O_2:

$$Y_{O_2} = \frac{[MbO_2]}{[Mb] + [MbO_2]} \qquad [7\text{-}2]$$

Y_{O_2} ranges from zero (when no O_2 is bound to the myoglobin molecules) to one (when the binding sites of all the myoglobin molecules are occupied). Equation 7-1 can be rearranged to

$$[MbO_2] = \frac{[Mb][O_2]}{K} \qquad [7\text{-}3]$$

When this expression for $[MbO_2]$ is substituted into Eq. 7-2, the fractional saturation becomes

$$Y_{O_2} = \frac{\dfrac{[Mb][O_2]}{K}}{[Mb] + \dfrac{[Mb][O_2]}{K}} \qquad [7\text{-}4]$$

Factoring out the [Mb]/K term in the numerator and denominator gives

$$Y_{O_2} = \frac{[O_2]}{K + [O_2]} \qquad [7\text{-}5]$$

Since O_2 is a gas, its concentration is conveniently expressed by its **partial pressure, pO_2** (also called the oxygen tension). Equation 7-5 can therefore be expressed as

$$Y_{O_2} = \frac{pO_2}{K + pO_2} \qquad [7\text{-}6]$$

This equation describes a rectangular ***hyperbola*** *and is identical in form to the equations that describe a hormone binding to its cell-surface receptor or a small molecular substrate binding to the active site of an enzyme.* This hyperbolic function can be represented graphically as shown in Fig. 7-4. At low pO_2, very little O_2 binds to myoglobin (Y_{O_2} is very small). As the pO_2 increases, more O_2 binds to myoglobin. At very high pO_2, virtually all the O_2-binding sites are occupied and myoglobin is said to be **saturated** with O_2.

The steepness of the hyperbola for a simple binding event, such as O_2 binding to myoglobin, increases as the value of K decreases. This means that *the lower the value of K, the tighter is the binding.* K is equivalent to the concentration of ligand at which half of the binding sites are occupied. In other words, when $pO_2 = K$, myoglobin is half-saturated with oxygen. This can be shown algebraically by substituting pO_2 for K in Eq. 7-6:

$$Y_{O_2} = \frac{pO_2}{K + pO_2} = \frac{pO_2}{2pO_2} = 0.5 \qquad [7\text{-}7]$$

Thus, K can be operationally defined as the value of pO_2 at which $Y = 0.5$ (Fig. 7-4).

It is convenient to define K as $\boldsymbol{p_{50}}$, that is, the oxygen pressure at which myoglobin is 50% saturated. The p_{50} for myoglobin is 2.8 torr (760 torr = 1 atm). Over the physiological range of pO_2 in the blood (100 torr in arterial blood and 30 torr in venous blood), myoglobin is almost fully saturated with oxygen; for example, $Y_{O_2} = 0.97$ at $pO_2 = 100$ torr and 0.91 at 30 torr (see Sample Calculation 7-1). Consequently, *myoglobin efficiently relays oxygen from the capillaries to muscle cells.*

Myoglobin, a single polypeptide chain with one heme group and hence one oxygen-binding site, is a useful model for other binding proteins. Even proteins with multiple binding sites for the same small molecule, or **ligand,** may generate hyperbolic binding curves like myoglobin's. *A hyperbolic binding curve occurs when ligands interact independently with their binding sites.* In practice, the affinity of a ligand for its binding protein may not be known. Constructing a binding curve such as the one shown in Fig. 7-4 may provide this information.

SAMPLE CALCULATION 7-1

At what oxygen concentration will myoglobin be 75% saturated with oxygen?

Rearrange Eq. 7-6 and let $p_{50} = 2.8$ torr.

$$Y_{O_2} = \frac{pO_2}{p_{50} + pO_2}$$
$$pO_2 = Y_{O_2}(p_{50} + pO_2)$$
$$pO_2 = Y_{O_2}p_{50} + Y_{O_2}pO_2$$
$$pO_2 - Y_{O_2}pO_2 = Y_{O_2}p_{50}$$
$$pO_2 = \frac{Y_{O_2}p_{50}}{(1 - Y_{O_2})}$$
$$pO_2 = \frac{(0.75)(2.8\text{ torr})}{(1 - 0.75)} = 8.4\text{ torr}$$

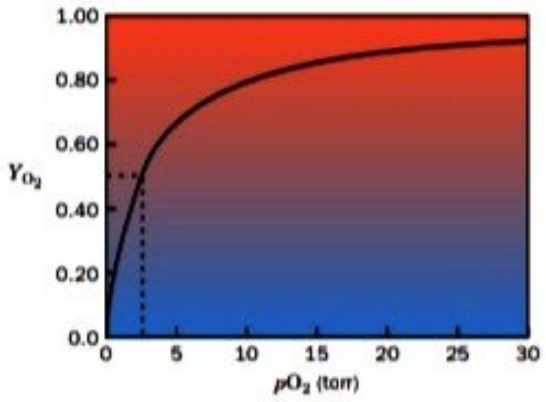


FIG. 7-4 Oxygen-binding curve of myoglobin. Myoglobin is half-saturated with O_2 ($Y_{O_2} = 0.5$) at an oxygen partial pressure (pO_2) of 2.8 torr (*dashed lines*). The hyperbolic shape of myoglobin's binding curve is typical of the simple binding of a small molecule to a protein. The background is shaded to indicate the color change that myoglobin undergoes as it binds O_2.

? How much does fractional saturation change between pO_2 values of 5 and 15, and 15 and 25 torr?

B Hemoglobin Is a Tetramer with Two Conformations

Hemoglobin, the intracellular protein that gives red blood cells their color, is one of the best-characterized proteins and was one of the first proteins to be associated with a specific physiological function (oxygen transport). Animals that are too large (>1 mm thick) for simple diffusion to deliver sufficient oxygen to their tissues have circulatory systems containing hemoglobin or a protein of similar function that does so (Box 7-1).

Mammalian hemoglobin, as we saw in Fig. 6-33, is an $\alpha_2\beta_2$ tetramer (a dimer of $\alpha\beta$ protomers). The α and β subunits are structurally and evolutionarily related to each other and to myoglobin. The structure of hemoglobin was determined by Max Perutz (Box 7-2). Only about 18% of the residues are identical in myoglobin and in the α and β subunits of hemoglobin, but the three polypeptides have remarkably similar tertiary structures (hemoglobin subunits follow the myoglobin helix-labeling system, although the α chain has no D helix). The $\alpha\beta$ protomers of hemoglobin are symmetrically related by a 2-fold rotation (i.e., a rotation of 180° brings the protomers into coincidence). In addition, hemoglobin's structurally similar α and β subunits are related by an approximate 2-fold rotation (pseudosymmetry) whose axis is perpendicular to that of the exact 2-fold rotation. Thus, hemoglobin has exact C_2 symmetry and pseudo-D_2 symmetry (Section 6-3; objects with D_2 symmetry have

Box 7-1 Perspectives in Biochemistry

Other Oxygen-Transport Proteins

The presence of O_2 in the earth's atmosphere and its utility in the oxidation of metabolic fuels have driven the evolution of various mechanisms for storing and transporting oxygen. Small organisms rely on diffusion to supply their respiratory oxygen needs. However, since the rate at which a substance diffuses varies inversely with the square of the distance it must diffuse, organisms of >1-mm thickness overcome the constraints of diffusion with circulatory systems and boost the limited solubility of O_2 in water with specific O_2-transport proteins.

Many invertebrates, and even some plants and bacteria, contain heme-based O_2-binding proteins. Single-subunit and multimeric hemoglobins are found both as intracellular proteins and as extracellular components of blood and other body fluids. The existence of hemoglobin-like proteins in some species of bacteria is evidence of gene transfer from animals to bacteria at one or more points during evolution. In bacteria, these proteins may function as sensors of environmental conditions such as local O_2 concentration. In some leguminous plants, the so-called **leghemoglobins** bind O_2 that would otherwise interfere with nitrogen fixation carried out by bacteria that colonize plant root nodules (Section 21-7). The **chlorocruorins,** which occur in some annelids (e.g., earthworms), contain a somewhat differently derivatized porphyrin than that in hemoglobin, which accounts for the green color of chlorocruorins.

The two other types of O_2-binding proteins, **hemerythrin** and **hemocyanin** (neither of which contains heme groups), occur only in invertebrate animals. Hemerythrin, which occurs in only a few species of marine worms, is an intracellular protein with a subunit mass of ~13 kD. It contains two Fe atoms liganded by His and acidic residues. It is violet-pink when oxygenated and colorless when deoxygenated.

Hemocyanins, which are exclusively extracellular, transport O_2 in mollusks and arthropods. The molluscan and arthropod hemocyanins are large multimeric proteins that differ in their primary through quaternary structures. However, their oxygen-binding sites are highly similar, consisting of a pair of copper atoms, each liganded by three His residues.

In this model of the O_2-binding site of hemocyanin from the horseshoe crab *Limulus polyphemus*, atoms are colored according to type with C gray, N blue, O red, and Cu purple. The otherwise colorless complex turns blue when it binds O_2.

Hemocyanins must be present at high concentrations in order to function efficiently as oxygen carriers. For example, octopus **hemolymph** (its equivalent of blood) contains about 100 mg/mL hemocyanin. In order to minimize the osmotic pressure of so much protein, hemocyanins form multimeric structures with masses as great as 9×10^6 D in some species. Hemocyanins are often the predominant extracellular protein and may therefore have additional functions as buffers against pH changes and osmotic fluctuations. In some invertebrates, hemocyanins may serve as a nutritional reserve, for example, during metamorphosis or molting.

[Figure based on an X-ray structure by Wim Hol, University of Washington School of Medicine. PDBid 1OXY.]

Box 7-2 Pathways of Discovery

Max Perutz and the Structure and Function of Hemoglobin

Max Perutz (1914–2002) The determination of the three-dimensional structures of proteins has become so commonplace that it is difficult to appreciate the challenges that faced the first protein crystallographers. Max Perutz was a pioneer in this area, spending many years determining the structure of hemoglobin at atomic resolution and then using this information to explain the physiological function of the protein.

In 1934, two years before Perutz began his doctoral studies in Cambridge, J.D. Bernal and Dorothy Crowfoot Hodgkin had placed a crystal of the protein pepsin in an X-ray beam and obtained a diffraction pattern. Perutz tried the same experiment with hemoglobin, chosen because of its abundance, ease of crystallization, and obvious physiological importance. Hemoglobin crystals yielded diffraction patterns with thousands of diffraction maxima (called reflections), the result of X-ray scattering by the thousands of atoms in each protein molecule. At the time, X-ray crystallography had been used to determine the structures of molecules containing no more than around 40 atoms, so the prospect of using the technique to determine the atomic structure of hemoglobin seemed impossible. Nevertheless, Perutz took on the challenge and spent the rest of his long career working with hemoglobin.

In X-ray crystallography, the intensities and the positions of the reflections can be readily determined, but the values of their phases (the relative positions of the wave peaks, the knowledge of which is as important as wave amplitude for image reconstruction) cannot be directly measured. Although computational techniques for determining the values of the phases had been developed for small molecules, methods for solving this so-called phase problem for such complex entities as proteins seemed hopelessly out of reach. In 1952, Perutz realized that the method of isomorphous replacement might suffice to solve the phase problem for hemoglobin. In this method, a heavy atom such as a Hg^{2+} ion, which is rich in electrons (the particles that scatter X-rays), must bind to specific sites on the protein without significantly disturbing its structure (which would change the positions of the reflections). If this causes measurable changes in the intensities of the reflections, these differences would provide the information to determine their phases. With trepidation followed by jubilation, Perutz observed that Hg-doped hemoglobin crystals indeed yielded reflections with measurable changes in intensity but no changes in position. Still, it took another 5 years to obtain the three-dimensional structure of hemoglobin at low (5.5-Å) resolution and it was not until 1968, some 30 years after he began the project, that he determined the structure of hemoglobin at near atomic (2.8-Å) resolution. In the meantime, Perutz's colleague John Kendrew used the method of isomorphous replacement to solve the structure of myoglobin, a smaller and simpler relative of hemoglobin. For their groundbreaking work, Perutz and Kendrew were awarded the 1962 Nobel Prize in Chemistry.

For Perutz, obtaining the structure of hemoglobin was only part of his goal of understanding hemoglobin. For example, functional studies indicated that the four oxygen-binding sites of hemoglobin interacted, as if they were in close contact, but Perutz's structure showed that the binding sites lay in deep and widely separated pockets. Perutz was also intrigued by the fact that crystals of hemoglobin prepared in the absence of oxygen would crack when they were exposed to air (the result, it turns out, of a dramatic conformational change). Although many other researchers also turned their attention to hemoglobin, Perutz was foremost among them in ascribing oxygen-binding behavior to protein structural features. He also devoted considerable effort to relating functional abnormalities in mutant hemoglobins to structural changes.

Perutz's groundbreaking work on the X-ray crystallography of proteins paved the way for other studies. For example, the first X-ray structure of an enzyme, lysozyme, was determined in 1965. The nearly 80,000 macromolecular structures that have been obtained since then owe a debt to Perutz and his decision to pursue an "impossible" task and to follow through on his structural work to the point where he could use his results to explain biological phenomena.

Perutz, M.F., Rossmann, M.G., Cullis, A.F., Muirhead, H., Will, G., and North, A.C.T., Structure of haemoglobin: A three-dimensional Fourier synthesis at 5.5 Å resolution, obtained by X-ray analysis. *Nature* **185,** 416–422 (1960).

the rotational symmetry of a tetrahedron). The hemoglobin molecule has overall dimensions of about 64 × 55 × 50 Å.

Oxygen binding alters the structure of the entire hemoglobin tetramer, so the structures of **deoxyhemoglobin** (Fig. 7-5*a*) and **oxyhemoglobin** (Fig. 7-5*b*) are noticeably different. In both forms of hemoglobin, the α and β subunits form extensive contacts: Those at the α_1–β_1 interface (and its α_2–β_2 symmetry equivalent) involve 35 residues, and those at the α_1–β_2 (and α_2–β_1) interface involve 19 residues. These associations are predominantly hydrophobic, although numerous hydrogen bonds and several ion pairs are also involved. Note, however, that the α_1–α_2 and β_1–β_2 interactions are tenuous at best because these subunit pairs are separated by an ~20-Å-diameter solvent-filled channel that parallels the 50-Å length of hemoglobin's exact 2-fold axis (Fig. 7-5).

When oxygen binds to hemoglobin, the α_1–β_2 (and α_2–β_1) contacts shift, producing a change in quaternary structure. Oxygenation rotates one αβ

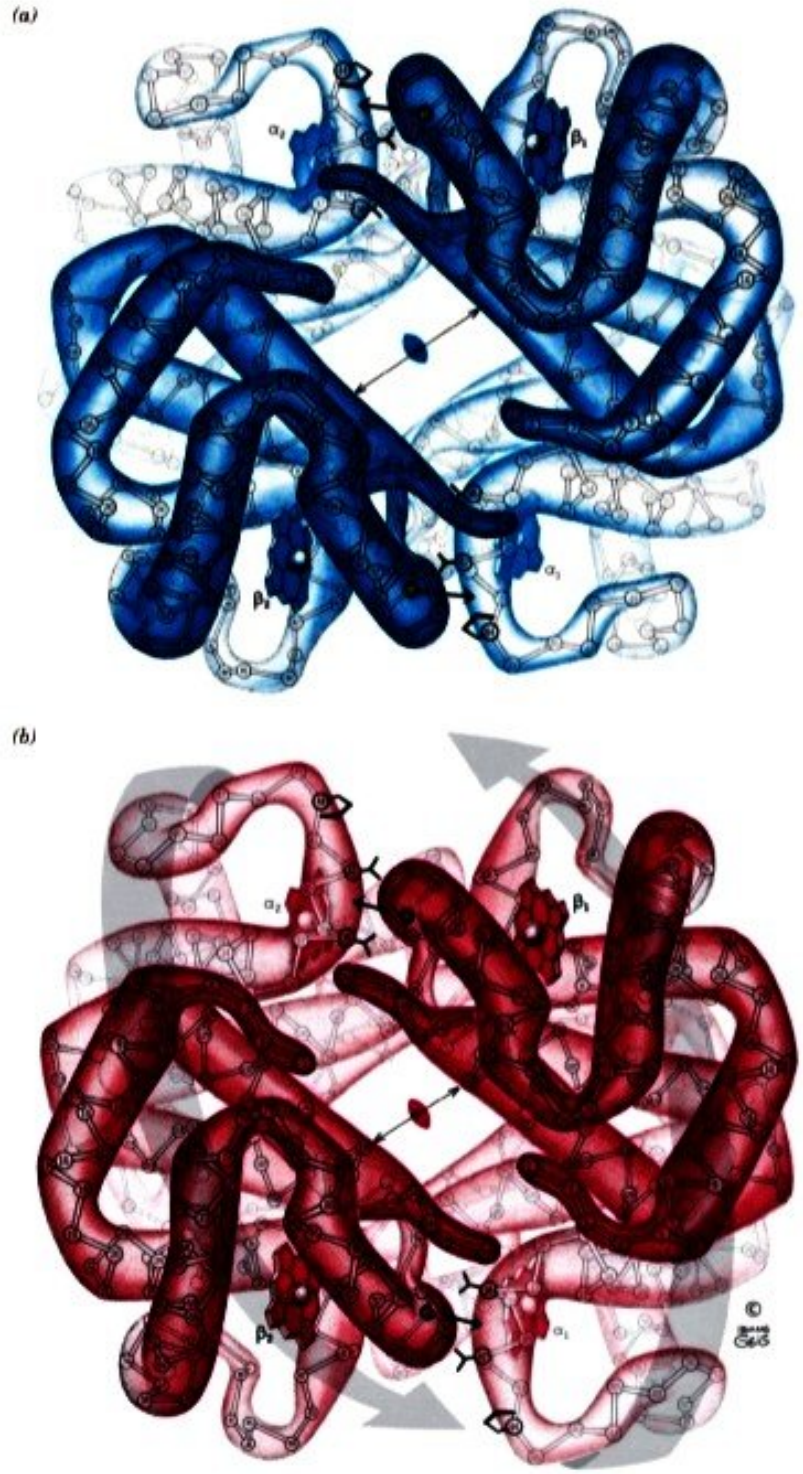


FIG. 7-5 Hemoglobin structure. (*a*) Deoxyhemoglobin and (*b*) oxyhemoglobin. The $\alpha_1\beta_1$ protomer is related to the $\alpha_2\beta_2$ protomer by a 2-fold axis of symmetry (*lenticular symbol*), which is perpendicular to the page. Oxygenation causes one protomer to rotate ~15° relative to the other, bringing the β chains closer together (compare the lengths of the double-headed arrows) and shifting the contacts between subunits at the α_1–β_2 and α_2–β_1 interfaces (some of the relevant side chains are drawn in black). The large gray arrows in Part *b* indicate the molecular movements that accompany oxygenation. [Illustration, Irving Geis. Image from the Irving Geis Collection/Howard Hughes Medical Institute. Rights owned by HHMI. Reproduction by permission only.] **See Kinemage Exercises 6-2 and 6-3.**

dimer ~15° with respect to the other αβ dimer (gray arrows in Fig. 7-5*b*), which brings the β subunits closer together and narrows the solvent-filled central channel (Fig. 7-5). Some atoms in the α_1–β_2 and α_2–β_1 interfaces shift by as much as 6 Å (oxygenation causes such extensive quaternary structural changes that crystals of deoxyhemoglobin shatter on exposure to O_2). This structural rearrangement is a crucial element of hemoglobin's oxygen-binding behavior.

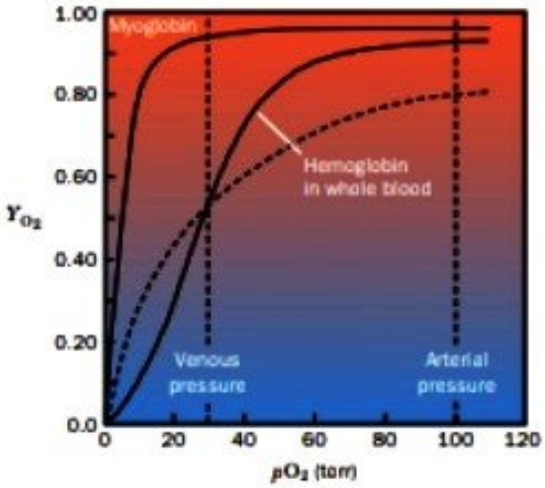


FIG. 7-6 Oxygen-binding curve of hemoglobin. In whole blood, hemoglobin is half-saturated at an oxygen pressure of 26 torr. The normal sea level values of human arterial and venous pO_2 are indicated (atmospheric pO_2 is 160 torr at sea level). The O_2-binding curve for myoglobin is included for comparison. The dashed line is a hyperbolic O_2-binding curve with the same p_{50} as hemoglobin. The background is shaded to indicate the color change that hemoglobin undergoes as it binds O_2. **See the Animated Figures.**

C Oxygen Binds Cooperatively to Hemoglobin

Hemoglobin has a p_{50} of 26 torr (i.e., hemoglobin is half-saturated with O_2 at an oxygen partial pressure of 26 torr), which is nearly 10 times greater than the p_{50} of myoglobin. Moreover, hemoglobin does not exhibit a myoglobin-like hyperbolic oxygen-binding curve. Instead, O_2 binding to hemoglobin is described by a **sigmoidal** (S-shaped) **curve** (Fig. 7-6). *This permits the blood to deliver much more O_2 to the tissues than if hemoglobin had a hyperbolic curve with the same p_{50}* (dashed curve in Fig. 7-6). For example, hemoglobin is nearly fully saturated with O_2 at arterial oxygen pressures (Y_{O_2} = 0.95 at 100 torr) but only about half-saturated at venous oxygen pressures (Y_{O_2} = 0.55 at 30 torr). This 0.40 difference in oxygen saturation, a measure of hemoglobin's ability to deliver O_2 from the lungs to the tissues, would be only 0.25 if hemoglobin exhibited hyperbolic binding behavior.

*In any binding system, a sigmoidal curve is diagnostic of a **cooperative** interaction between binding sites.* This means that the binding of a ligand to one site affects the binding of additional ligands to the other sites. In the case of hemoglobin, O_2 binding to one subunit increases the O_2 affinity of the remaining subunits. The initial slope of the oxygen-binding curve (Fig. 7-6) is low, as hemoglobin subunits independently compete for the first O_2. However, an O_2 molecule bound to one of hemoglobin's subunits increases the O_2-binding affinity of its other subunits, thereby accounting for the increasing slope of the middle portion of the sigmoidal curve.

The Hill Equation Describes Hemoglobin's O_2-Binding Curve. The earliest attempt to analyze hemoglobin's sigmoidal O_2 dissociation curve was formulated by Archibald Hill in 1910. Hill assumed that hemoglobin **(Hb)** bound n molecules of O_2 in a single step,

$$Hb + nO_2 \rightarrow Hb(O_2)_n$$

that is, with infinite cooperativity. Thus, in analogy with the derivation of Eq. 7-6,

$$Y_{O_2} = \frac{(pO_2)^n}{(p_{50})^n + (pO_2)^n} \qquad [7\text{-}8]$$

which is known as the **Hill equation.** Like Eq. 7-6, it describes the degree of saturation of hemoglobin as a function of pO_2 (see Sample Calculation 7-2).

Infinite O_2 binding cooperativity, as Hill assumed, is a physical impossibility. Nevertheless, n may be taken to be a nonintegral parameter related to the degree of cooperativity among interacting hemoglobin subunits rather than the number of subunits that bind O_2 in one step. The Hill equation can then be taken as a useful empirical curve-fitting relationship rather than as an indicator of a particular model of ligand binding.

SAMPLE CALCULATION 7-2

Calculate the fractional saturation of hemoglobin at pO_2 = 50 torr and n = 3.

Use Eq. 7-8 and let p_{50} = 26 torr.

$$Y_{O_2} = \frac{(pO_2)^n}{(p_{50})^n + (pO_2)^n}$$

$$= \frac{(50)^3}{(26)^3 + (50)^3} = \frac{125{,}000}{17{,}576 + 125{,}000} = 0.88$$

The quantity n, the ***Hill constant,*** *increases with the degree of cooperativity of a reaction and therefore provides a convenient although simplistic characterization of a ligand-binding reaction.* If $n = 1$, Eq. 7-8 describes a hyperbola as does Eq. 7-6 for myoglobin, and the O_2-binding reaction is said to be **noncooperative.** If $n > 1$, the reaction is described as being **positively cooperative,** because O_2 binding increases the affinity of hemoglobin for further O_2 binding (cooperativity is infinite in the limit that $n = 4$, the number of O_2 binding sites in hemoglobin). Conversely, if $n < 1$, the reaction is said to be **negatively cooperative,** because O_2 binding would then reduce the affinity of hemoglobin for subsequent O_2 binding.

The Hill coefficient, n, and the value of p_{50} that best describe hemoglobin's saturation curve can be graphically determined by rearranging Eq. 7-8. First, divide both sides by $1 - Y_{O_2}$:

$$\frac{Y_{O_2}}{1 - Y_{O_2}} = \frac{\dfrac{(pO_2)^n}{(p_{50})^n + (pO_2)^n}}{1 - Y_{O_2}} = \frac{\dfrac{(pO_2)^n}{(p_{50})^n + (pO_2)^n}}{1 - \dfrac{(pO_2)^n}{(p_{50})^n + (pO_2)^n}} \qquad [7\text{-}9]$$

Factoring out the $[(p_{50})^n + (pO_2)^n]$ term gives

$$\frac{Y_{O_2}}{1 - Y_{O_2}} = \frac{(pO_2)^n}{[(p_{50})^n + (pO_2)^n] - (pO_2)^n} = \frac{(pO_2)^n}{(p_{50})^n} \qquad [7\text{-}10]$$

Taking the log of both sides yields a linear equation:

$$\log\left(\frac{Y_{O_2}}{1 - Y_{O_2}}\right) = n\log pO_2 - n\log p_{50} \qquad [7\text{-}11]$$

The linear plot of $\log[Y_{O_2}/(1 - Y_{O_2})]$ versus $\log pO_2$, the **Hill plot,** has a slope of n and an intercept on the $\log pO_2$ axis of $\log p_{50}$ (recall that the linear equation $y = mx + b$ describes a line with a slope of m and an x intercept of $-b/m$).

Figure 7-7 shows the Hill plots for myoglobin and purified hemoglobin. For myoglobin, the plot is linear with a slope of 1, as expected. Although all subunits of hemoglobin do not bind O_2 in a single step as was assumed in

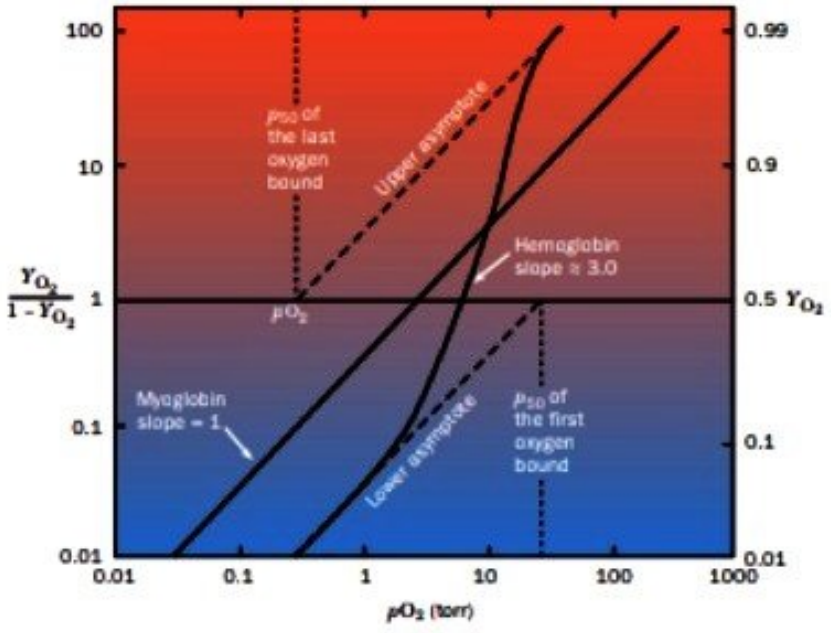


FIG. 7-7 Hill plots for myoglobin and purified hemoglobin. Note that this is a log–log plot. At $pO_2 = p_{50}$, $Y_{O_2}/(1 - Y_{O_2}) = 1$. [The p_{50} for hemoglobin *in vivo* is higher than the p_{50} of purified hemoglobin due to its binding of certain substances present in the red cell (see below).]

deriving the Hill equation, its Hill plot is essentially linear for values of Y_{O_2} between 0.1 and 0.9. When $pO_2 = p_{50}$, $Y_{O_2} = 0.5$, and

$$\frac{Y_{O_2}}{1 - Y_{O_2}} = \frac{0.5}{1 - 0.5} = 1.0 \qquad [7\text{-}12]$$

As can be seen in Fig. 7-7, this is the region of maximum slope, whose value is customarily taken to be the Hill coefficient, n. For normal human hemoglobin, the Hill coefficient is between 2.8 and 3.0; that is, hemoglobin's oxygen binding is highly, but not infinitely, cooperative. Many abnormal hemoglobins exhibit smaller Hill coefficients (Section 7-1E), indicating that they have a less than normal degree of cooperativity.

At Y_{O_2} values near zero, when few hemoglobin molecules have bound even one O_2 molecule, the Hill plot for hemoglobin assumes a slope of 1 (Fig. 7-7, lower asymptote) because the hemoglobin subunits independently compete for O_2 as do molecules of myoglobin. At Y_{O_2} values near 1, when at least three of hemoglobin's four O_2-binding sites are occupied, the Hill plot also assumes a slope of 1 (Fig. 7-7, upper asymptote) because the few remaining unoccupied sites are on different molecules and therefore bind O_2 independently.

Extrapolating the lower asymptote in Fig. 7-7 to the horizontal axis indicates, according to Eq. 7-11, that $p_{50} = 30$ torr for binding the first O_2 to purified hemoglobin. Likewise, extrapolating the upper asymptote yields $p_{50} = 0.3$ torr for binding hemoglobin's fourth O_2. Thus, *the fourth O_2 binds to hemoglobin with 100-fold greater affinity than the first.* This difference, as we will see below, is entirely due to the influence of the globin chain on the O_2 affinity of heme.

D Hemoglobin's Two Conformations Exhibit Different Affinities for Oxygen

The cooperativity of oxygen binding to hemoglobin arises from the effect of the ligand-binding state of one heme group on the ligand-binding affinity of another. Yet the hemes are 25 to 37 Å apart—too far to interact electronically. Instead, information about the O_2-binding status of a heme group is mechanically transmitted to the other heme groups by motions of the protein. These movements are responsible for the different quaternary structures of oxy- and deoxyhemoglobin depicted in Fig. 7-5.

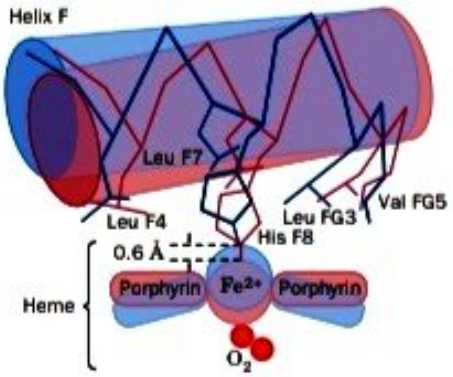
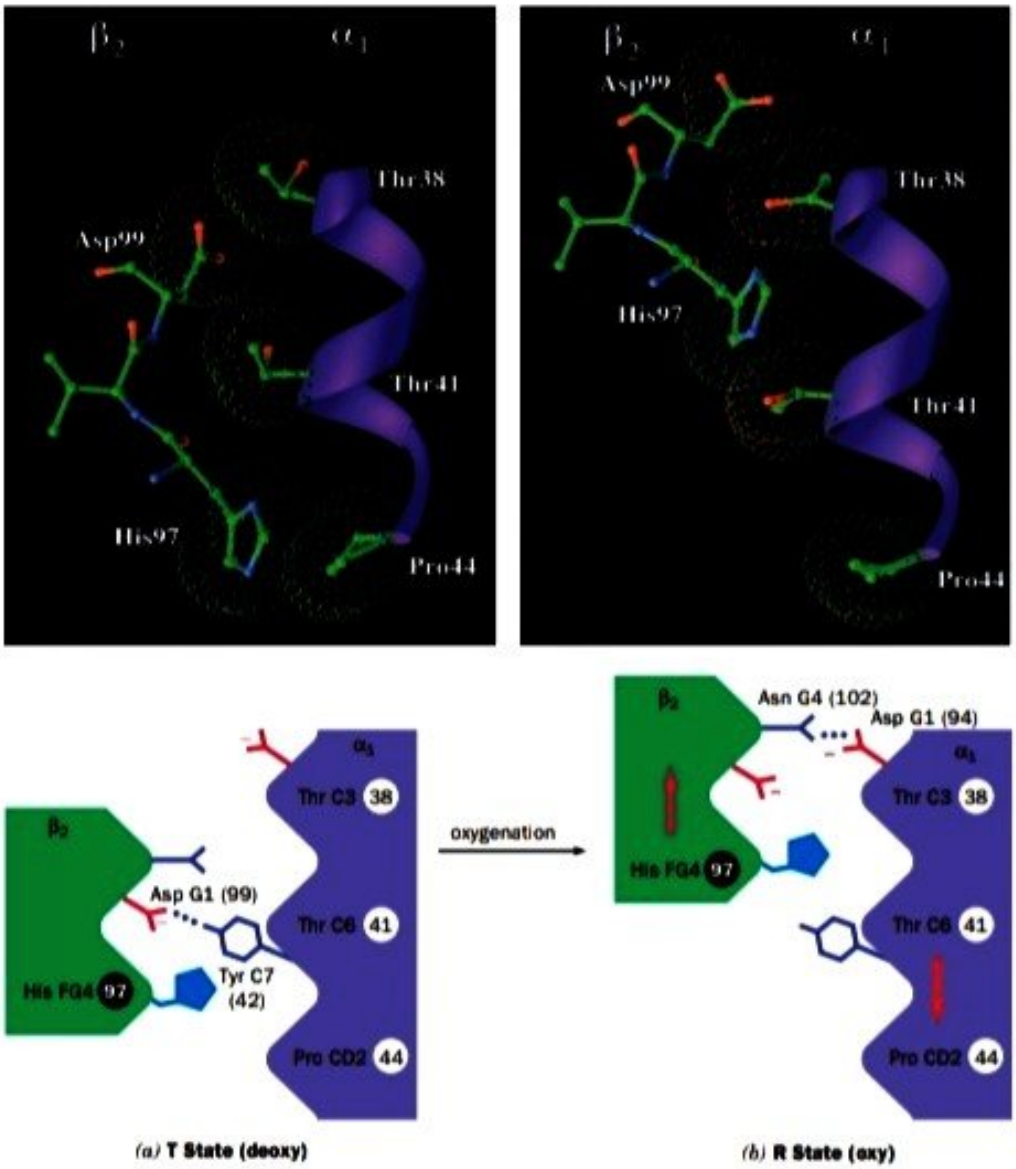


FIG. 7-8 Movements of the heme and F helix during the T → R transition in hemoglobin. In the T form (*blue*), the Fe is 0.6 Å above the center of the domed porphyrin ring. On assuming the R form (*red*), the Fe moves into the plane of the now undomed porphyrin, where it can more tightly bind O_2, and, in doing so, pulls His F8 and its attached F helix with it. **See Kinemage Exercise 6-4 and the Animated Figures.**

Oxygen Binding to Hemoglobin Triggers a Conformational Change from T to R. On the basis of the X-ray structures of oxy- and deoxyhemoglobin, Perutz formulated a model for hemoglobin oxygenation. *In the **Perutz mechanism,** hemoglobin has two stable conformational states, the **T state** (the conformation of deoxyhemoglobin) and the **R state** (the conformation of oxyhemoglobin).* The conformations of all four subunits in T-state hemoglobin differ from those in the R state. Oxygen binding initiates a series of coordinated movements that result in a shift from the T state to the R state within a few microseconds:

1. In the T state, the Fe(II) in each of the four hemes is situated ~0.6 Å out of the heme plane because of a pyramidal doming of the porphyrin group toward His F8 and because the Fe—$N_{porphyrin}$ bonds are too long to allow the Fe to lie in the porphyrin plane (Fig. 7-8). O_2 binding changes the heme's electronic state, which shortens the Fe—$N_{porphyrin}$ bonds by ~0.1 Å and causes the porphyrin doming to subside. Consequently, during the T → R transition, the Fe(II) moves into the center of the heme plane.
2. The Fe(II) drags the covalently linked His F8 along with it. However, the direct movement of His F8 by 0.6 Å toward the heme plane would cause it to collide with the heme. To avoid this steric clash, the attached F helix tilts and translates by ~1 Å across the heme plane.

3. The changes in tertiary structure are coupled to a shift in the arrangement of hemoglobin's four subunits. The largest change produced by the T → R transition is the result of movements of residues at the α_1–β_2 and α_2–β_1 interfaces, that is, at the interface between the two protomeric units of hemoglobin. In the T state, His 97 in the β chain contacts Thr 41 in the α chain (Fig. 7-9*a*). In the R state, His 97 contacts Thr 38, which is positioned one turn back along the C helix (Fig. 7-9*b*). In both conformations, the "knobs" on one subunit mesh nicely with the "grooves" on the other. An intermediate position would



(a) **T State (deoxy)** *(b)* **R State (oxy)**

FIG. 7-9 Changes at the α_1–β_2 interface during the T → R transition in hemoglobin. (*a*) The T state and (*b*) the R state. In the upper drawings, the C helix is represented by a purple ribbon, the contacting residues forming the α_1C–β_2FG contact are shown in ball-and-stick form colored by atom type (C green, N blue, and O red), and their van der Waals surfaces are outlined by like-colored dots. The lower drawings are the corresponding schematic diagrams of the α_1C–β_2FG contact. Upon a T → R transformation, the β_2FG region shifts by one turn along the α_1C helix with no stable intermediate (note how in both conformations, the knobs formed by the side chains of His 97β and Asp 99β fit between the grooves on the C helix formed by the side chains of Thr 38α, Thr 41α, and Pro 44α). The subunits are joined by different hydrogen bonds in the two quaternary states. Figure 7-5 provides another view of these interactions. [Based on X-ray structures by Giulio Fermi, Max Perutz, and Boaz Shaanan, MRC Laboratory of Molecular Biology, Cambridge, U.K. PDBids (*a*) 2HHB and (*b*) 1HHO.] **See Kinemage Exercise 6-5.**

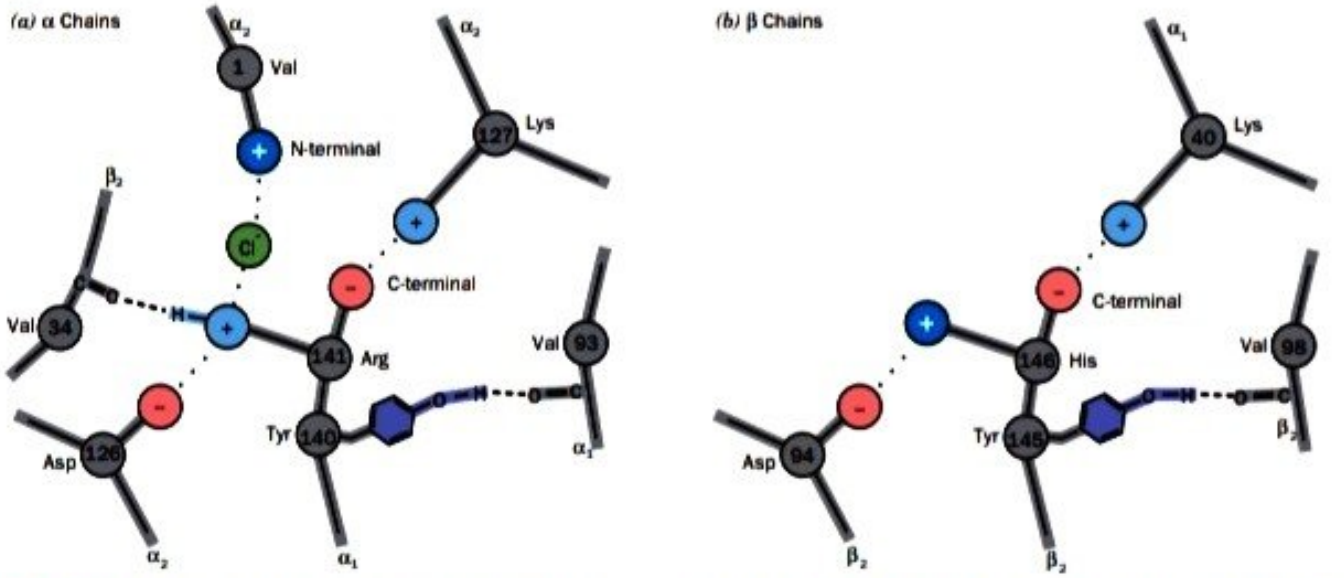


FIG. 7-10 Networks of ion pairs and hydrogen bonds in deoxyhemoglobin. These bonds, which involve the last two residues of (*a*) the α chains and (*b*) the β chains, are ruptured in the T → R transition. Two groups that become partially deprotonated in the R state (part of the Bohr effect) are indicated by white plus signs. [Illustration, Irving Geis. Image from the Irving Geis Collection/Howard Hughes Medical Institute. Rights owned by HHMI. Reproduction by permission only.]

be severely strained because it would bring His 97 and Thr 41 too close together (i.e., knobs on knobs).

4. The C-terminal residues of each subunit (Arg 141α and His 146β) in T-state hemoglobin each participate in a network of intra- and intersubunit ion pairs (Fig. 7-10) that stabilize the T state. However, the conformational shift in the T → R transition tears away these ion pairs in a process that is driven by the energy of formation of the Fe—O_2 bonds.

The essential feature of hemoglobin's T → R transition is that *its subunits are so tightly coupled that large tertiary structural changes within one subunit cannot occur without quaternary structural changes in the entire tetrameric protein.* Hemoglobin is limited to only two quaternary forms, T and R, because the intersubunit contacts shown in Fig. 7-9 act as a binary switch that permits only two stable positions of the subunits relative to each other. The inflexibility of the α_1–β_1 and α_2–β_2 interfaces requires that the T → R shift occur simultaneously at both the α_1–β_2 and α_2–β_1 interfaces. No one subunit or dimer can greatly change its conformation independently of the others.

We are now in a position to structurally rationalize the cooperativity of oxygen binding to hemoglobin. The T state of hemoglobin has low O_2 affinity, mostly because of the 0.1 Å greater length of its Fe—O_2 bond relative to that of the R state (see the blue structure shown in Fig. 7-8). Experimental evidence indicates that when at least one O_2 has bound to each αβ dimer, the strain in the T-state hemoglobin molecule is sufficient to tear away the C-terminal ion pairs, thereby snapping the protein into the R state. All the subunits are thereby simultaneously converted to the R-state conformation whether or not they have bound O_2. Unliganded subunits in the R-state conformation have increased oxygen affinity because they are already in the O_2-binding conformation. This accounts for the high O_2 affinity of nearly saturated hemoglobin.

The Bohr Effect Enhances Oxygen Transport. The conformational changes in hemoglobin that occur on oxygen binding decrease the p*K*'s of several groups. Recall that the tendency for a group to ionize depends on its microenvironment, which may include other ionizable groups. For example, in T-state hemoglobin, the N-terminal amino groups of the α subunits and the

C-terminal His of the β subunits are positively charged and participate in ion pairs (see Fig. 7-10). The formation of ion pairs increases the pK values of these groups (makes them less acidic and therefore less likely to give up their protons). In R-state hemoglobin, these ion pairings are absent, and the pK's of the groups decrease (making them more acidic and more likely to give up protons). Consequently, under physiological conditions, hemoglobin releases ~0.6 protons for each O_2 it binds. Conversely, increasing the pH, that is, removing protons, stimulates hemoglobin to bind more O_2 (Fig. 7-11). This phenomenon is known as the **Bohr effect** after Christian Bohr (father of the physicist Niels Bohr), who first reported it in 1904.

The Bohr effect has important physiological functions in transporting O_2 from the lungs to respiring tissue and in transporting the CO_2 produced by respiration back to the lungs (Fig. 7-12). The CO_2 produced by respiring tissues diffuses from the tissues to the capillaries. This dissolved CO_2 forms bicarbonate (HCO_3^-) only very slowly, by the reaction

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$$

However, in the **erythrocyte** (red blood cell; Greek: *erythros*, red + *kytos*, a hollow vessel), the enzyme **carbonic anhydrase** greatly accelerates this reaction. Accordingly, most of the CO_2 in the blood is carried in the form of bicarbonate (in the absence of carbonic anhydrase, bubbles of CO_2 would form in the blood).

In the capillaries, where pO_2 is low, the H^+ generated by bicarbonate formation is taken up by hemoglobin in forming the ion pairs of the T state, thereby inducing hemoglobin to unload its bound O_2. This H^+ uptake, moreover, facilitates CO_2 transport by stimulating bicarbonate formation. Conversely, in the lungs, where pO_2 is high, O_2 binding by hemoglobin disrupts the T-state ion pairs to form the R state, thereby releasing the Bohr protons, which recombine with bicarbonate to drive off CO_2. These reactions are closely matched, so they cause very little change in blood pH (see Box 2-2).

The Bohr effect provides a mechanism whereby additional oxygen can be supplied to highly active muscles, where the pO_2 may be <20 torr. Such muscles generate lactic acid (Section 15-3A) so fast that they lower the pH of the blood passing through them from 7.4 to 7.2. At a pO_2 of 20 torr, hemoglobin releases ~10% more O_2 at pH 7.2 than it does at pH 7.4 (Fig. 7-11).

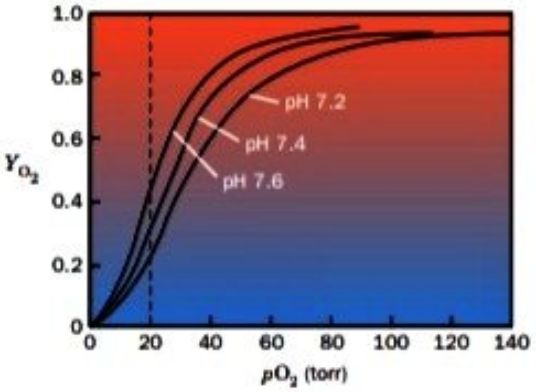


FIG. 7-11 The Bohr effect. The O_2 affinity of hemoglobin increases with increasing pH. The dashed line indicates the pO_2 in actively respiring muscle. [After Benesch, R.E. and Benesch, R., *Adv. Protein Chem.* **28,** 212 (1974).] **See the Animated Figures.**

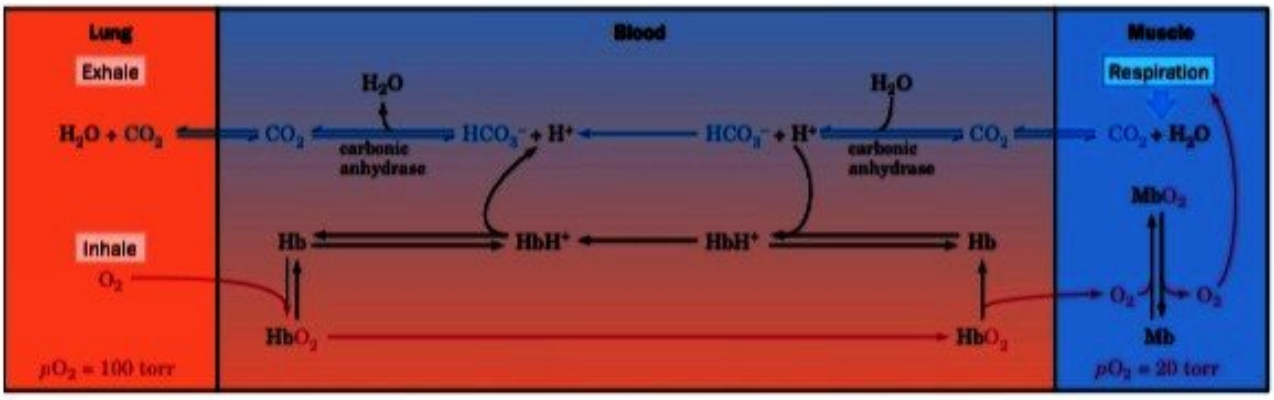


FIG. 7-12 The roles of hemoglobin and myoglobin in O_2 and CO_2 transport. Oxygen is inhaled into the lungs at high pO_2, where it binds to hemoglobin in the blood. The O_2 is then transported to respiring tissue, where the pO_2 is low. The O_2 therefore dissociates from the Hb and diffuses into the tissues, where it is used to oxidize metabolic fuels to CO_2 and H_2O. In rapidly respiring muscle tissue, the O_2 first binds to myoglobin (whose oxygen affinity is higher than that of hemoglobin). This increases the rate at which O_2 can diffuse from the capillaries to the tissues by, in effect, increasing its solubility. The Hb and CO_2 (mostly as HCO_3^-) are then returned to the lungs, where the CO_2 is exhaled.

? Explain how carbonic anhydrase affects CO_2 transport by hemoglobin.

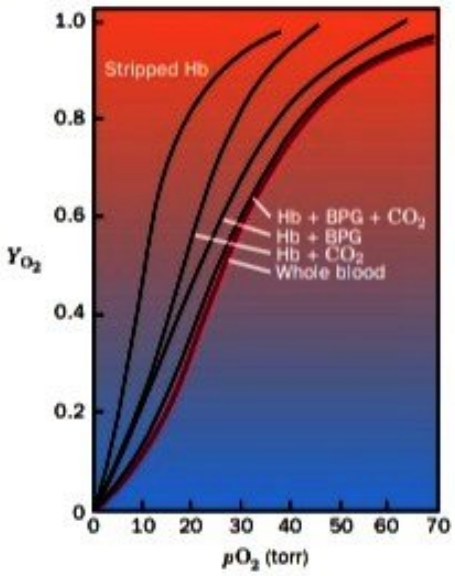


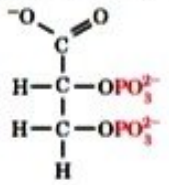
FIG. 7-13 The effects of BPG and CO_2 on hemoglobin's O_2 dissociation curve. Stripped hemoglobin (*left*) has higher O_2 affinity than whole blood (*red curve*). Adding BPG or CO_2 or both to hemoglobin shifts the dissociation curve back to the right (lowers hemoglobin's O_2 affinity). [After Kilmartin, J.V. and Rossi-Bernardi, L., *Physiol. Rev.* **53,** 884 (1973).] **See the Animated Figures.**

CO_2 also modulates O_2 binding to hemoglobin by combining reversibly with the N-terminal amino groups of blood proteins to form **carbamates:**

$$R{-}NH_2 + CO_2 \rightleftharpoons R{-}NH{-}COO^- + H^+$$

The T (deoxy) form of hemoglobin binds more CO_2 as carbamate than does the R (oxy) form. When the CO_2 concentration is high, as it is in the capillaries, the T state is favored, stimulating hemoglobin to release its bound O_2. The protons released by carbamate formation further promote O_2 release through the Bohr effect. Although the difference in CO_2 binding between the oxy and deoxy states of hemoglobin accounts for only ~5% of the total blood CO_2, it is nevertheless responsible for around half the CO_2 transported by the blood. This is because only ~10% of the total blood CO_2 is lost through the lungs in each circulatory cycle.

Bisphosphoglycerate Binds to Deoxyhemoglobin. Highly purified ("stripped") hemoglobin has a much greater oxygen affinity than hemoglobin in whole blood (Fig. 7-13). This observation led Joseph Barcroft, in 1921, to speculate that blood contains some other substance besides CO_2 that affects oxygen binding to hemoglobin. This compound is **D-2,3-bisphosphoglycerate (BPG).**



D-2,3-Bisphosphoglycerate (BPG)

BPG binds tightly to deoxyhemoglobin but only weakly to oxyhemoglobin. *The presence of BPG in mammalian erythrocytes therefore decreases hemoglobin's oxygen affinity by keeping it in the deoxy conformation.* In other vertebrates, different phosphorylated compounds elicit the same effect.

BPG has an indispensable physiological function: In arterial blood, where pO_2 is ~100 torr, hemoglobin is ~95% saturated with O_2, but in venous blood, where pO_2 is ~30 torr, it is only 55% saturated (Fig. 7-6). Consequently, in passing through the capillaries, hemoglobin unloads ~40% of its bound O_2. In the absence of BPG, little of this bound O_2 would be released since hemoglobin's O_2 affinity is increased, thus shifting its O_2 dissociation curve significantly toward lower pO_2 (Fig. 7-13, *left*). BPG also plays an important role in adaptation to high altitudes (Box 7-3).

The X-ray structure of a BPG–deoxyhemoglobin complex shows that BPG binds in the central cavity of deoxyhemoglobin (Fig. 7-14). The anionic groups of BPG are within hydrogen-bonding and ion-pairing distances of the N-terminal amino groups of both β subunits. The T → R transformation brings the two βH helices together, which narrows the central cavity (compare Figs. 7-5*a* and 7-5*b*) and expels the BPG. It also widens the

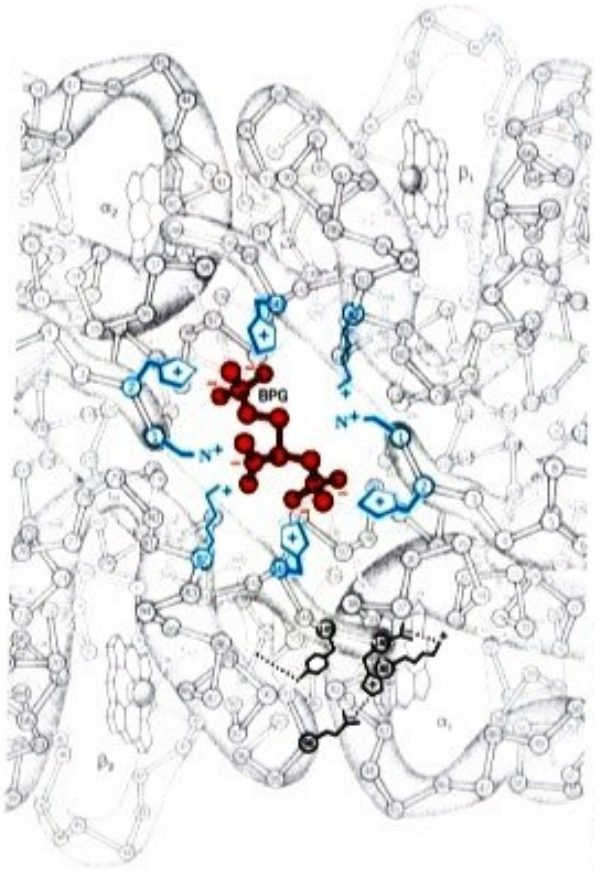


FIG. 7-14 Binding of BPG to deoxyhemoglobin. BPG (*red*) binds in hemoglobin's central cavity. The BPG, which has a charge of −5 under physiological conditions, is surrounded by eight cationic groups (*blue*) extending from the two β subunits. In the R state, the central cavity is too narrow to contain BPG. Some of the ion pairs and hydrogen bonds that help stabilize the T state (Fig. 7-10*b*) are indicated at the lower right. [Illustration, Irving Geis. Image from the Irving Geis Collection/Howard Hughes Medical Institute. Rights owned by HHMI. Reproduction by permission only.] **See Kinemage Exercise 6-3.**

Box 7-3 Biochemistry in Health and Disease

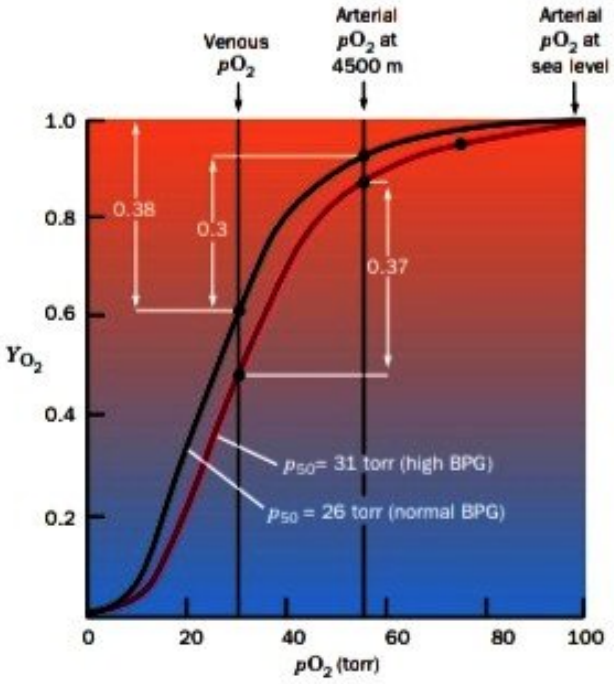
High-Altitude Adaptation

Atmospheric pressure decreases with altitude, so that the oxygen pressure at 3000 m (10,000 feet) is only ~110 torr, 70% of its sea-level pressure. A variety of physiological responses are required to maintain normal oxygen delivery (without adaptation, pO_2 levels of 85 torr or less result in mental impairment).

High-altitude adaptation is a complex process that involves increases in the number of erythrocytes and the amount of hemoglobin per erythrocyte. It normally requires several weeks to complete. Yet, as is clear to anyone who has climbed to high altitude, even a 1-day stay there results in a noticeable degree of adaptation. This effect results from a rapid increase in the amount of BPG synthesized in erythrocytes (from ~4 mM to ~8 mM; BPG cannot cross the erythrocyte membrane). As illustrated by plots of Y_{O_2} versus pO_2, the high altitude–induced increase in BPG causes the O_2-binding curve of hemoglobin to shift from its sea-level position (*black line*) to a lower affinity position (*red line*).

At sea level, the difference between arterial and venous pO_2 is 70 torr (100 torr − 30 torr), and hemoglobin unloads 38% of its bound O_2. However, when the arterial pO_2 drops to 55 torr, as it does at an altitude of 4500 m, hemoglobin would be able to unload only 30% of its O_2. High-altitude adaptation (which decreases the amount of O_2 that hemoglobin can bind in the lungs but, to a greater extent, increases the amount of O_2 it releases at the tissues) allows hemoglobin to deliver a near-normal 37% of its bound O_2. BPG concentrations also increase in individuals suffering from disorders that limit the oxygenation of the blood **(hypoxia)**, such as various anemias and cardiopulmonary insufficiency.

The BPG concentration in erythrocytes can be adjusted more rapidly than hemoglobin can be synthesized (Box 15-2; erythrocytes lack nuclei and therefore cannot synthesize proteins). An altered BPG level is also a more sensitive regulator of oxygen delivery than an altered respiratory rate. Hyperventilation, another early response to high altitude, may lead to respiratory alkalosis (Box 2-2). Interestingly, individuals in long-established Andean and Himalayan populations exhibit high lung capacity, along with high hemoglobin levels and, often, enlarged right ventricles (reflecting increased cardiac output), compared to individuals from low-altitude populations.



In contrast to the mechanism of human adaptation to high altitude, most mammals that normally live at high altitudes (e.g., the llama) have genetically altered hemoglobins that have higher O_2-binding affinities than do their sea-level cousins. Thus, both raising and lowering hemoglobin's p_{50} can provide high-altitude adaptation.

distance between the β N-terminal amino groups from 16 to 20 Å, which prevents their simultaneous hydrogen bonding with BPG's phosphate groups. BPG therefore binds to and stabilizes only the T conformation of hemoglobin by cross-linking its β subunits. This shifts the T ⇌ R equilibrium toward the T state, which lowers hemoglobin's O_2 affinity.

Fetal Hemoglobin Has Low BPG Affinity. The effects of BPG also help supply the fetus with oxygen. A fetus obtains its O_2 from the maternal circulation via the placenta. The concentration of BPG is the same in adult and fetal erythrocytes, but BPG binds more tightly to adult hemoglobin than to fetal hemoglobin. The higher oxygen affinity of fetal hemoglobin facilitates the transfer of O_2 to the fetus.

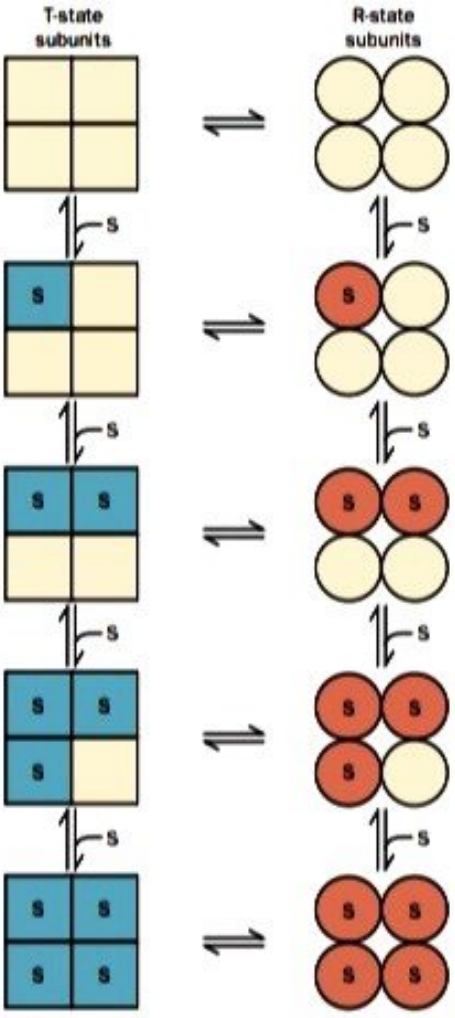
Fetal hemoglobin has the subunit composition $\alpha_2\gamma_2$ in which the γ subunit is a variant of the β chain (Section 5-4B). Residue 143 of the β chain of adult hemoglobin has a cationic His residue, whereas the γ chain has an uncharged Ser residue. The absence of this His eliminates a pair of interactions that stabilize the BPG–deoxyhemoglobin complex (Fig. 7-14).

Hemoglobin Is a Model Allosteric Protein. The cooperativity of oxygen binding to hemoglobin is a classic model for the behavior of many other

multisubunit proteins (including many enzymes) that bind small molecules. In some cases, binding of a ligand to one site increases the affinity of other binding sites on the same protein (as in O_2 binding to hemoglobin). In other cases, a ligand decreases the affinity of other binding sites (as when BPG binding decreases the O_2 affinity of hemoglobin). All these effects are the result of **allosteric interactions** (Greek: *allos*, other + *stereos*, solid or space). *Allosteric effects, in which the binding of a ligand at one site affects the binding of another ligand at another site, generally require interactions among subunits of oligomeric proteins.* The T → R transition in hemoglobin subunits explains the difference in the oxygen affinities of oxy- and deoxyhemoglobin. Other proteins exhibit similar conformational shifts, although the molecular mechanisms that underlie these phenomena are not completely understood.

Two models that account for cooperative ligand binding have received the most attention. One of them, the **symmetry model** of allosterism, formulated in 1965 by Jacques Monod, Jeffries Wyman, and Jean-Pierre Changeux, is defined by the following rules:

1. An allosteric protein is an oligomer of symmetrically related subunits (although the α and β subunits of hemoglobin are only pseudosymmetrically related).
2. Each oligomer can exist in two conformational states, designated R and T; these states are in equilibrium.
3. The ligand can bind to a subunit in either conformation. *Only the conformational change alters the affinity for the ligand.*
4. *The molecular symmetry of the protein is conserved during the conformational change.* The subunits must therefore change conformation in a concerted manner; in other words, there are no oligomers that simultaneously contain R- and T-state subunits.



The symmetry model is diagrammed for a tetrameric binding protein in Fig. 7-15. If a ligand binds more tightly to the R state than to the T state, ligand binding will promote the T → R shift, thereby increasing the affinity of the unliganded subunits for the ligand.

One major objection to the symmetry model is that it is difficult to believe that oligomeric symmetry is perfectly preserved in all proteins, that is, that the T → R shift occurs simultaneously in all subunits regardless of the number of ligands bound. In addition, the symmetry model can account only for positive cooperativity, although some proteins exhibit negative cooperativity.

An alternative to the symmetry model is the **sequential model** of allosterism, proposed by Daniel Koshland. According to this model, ligand binding induces a conformational change in the subunit to which it binds, and cooperative interactions arise through the influence of those conformational changes on neighboring subunits. The conformational changes occur sequentially as more ligand-binding sites are occupied (Fig. 7-16). The ligand-binding affinity of a subunit varies with its conformation and may be higher or lower than that of the subunits in the ligand-free protein. Thus, proteins that follow the sequential model of allosterism may be positively or negatively cooperative.

If the mechanical coupling between subunits in the sequential model is particularly strong, the conformational changes occur simultaneously and the oligomer retains its symmetry, as in the symmetry model. Thus, the symmetry model of allosterism may be considered to be an extreme case of the more general sequential model.

FIG. 7-15 The symmetry model of allosterism. Squares and circles represent T- and R-state subunits, respectively, of a tetrameric protein. The T and R states are in equilibrium regardless of the number of ligands (represented by S) that have bound to the protein. All the subunits must be in either the T or the R form; the model does not allow combinations of T- and R-state subunits in the same protein.

FIG. 7-16 The sequential model of allosterism. Ligand binding progressively induces conformational changes in the subunits, with the greatest changes occurring in those subunits that have bound ligand. The symmetry of the oligomeric protein is not preserved in this process as it is in the symmetry model.

Oxygen binding to hemoglobin exhibits features of both models. The quaternary T → R conformational change is concerted, as the symmetry model requires. Yet ligand binding to the T state does cause small tertiary structural changes, as the sequential model predicts. These minor conformational shifts are undoubtedly responsible for the buildup of strain that eventually triggers the T → R transition. It therefore appears that the complexity of ligand–protein interactions in hemoglobin and other proteins allows binding processes to be fine-tuned to the needs of the organism under changing internal and external conditions. We will revisit allosteric effects when we discuss enzymes in Chapter 12.

E Mutations May Alter Hemoglobin's Structure and Function

Before the advent of recombinant DNA techniques, mutant hemoglobins provided what was an almost unique opportunity to study structure–function relationships in proteins. This is because, for many years, hemoglobin was the only protein of known structure that had a large number of well-characterized naturally occurring **variants.** The examination of individuals with physiological disabilities, together with the routine electrophoretic screening of human blood samples, has led to the discovery of over 1000 variant hemoglobins, >90% of which result from single amino acid substitutions in a globin polypeptide chain. Indeed, about 5% of the world's human population are carriers of an inherited variant hemoglobin.

Not all hemoglobin variants produce clinical symptoms, but some abnormal hemoglobin molecules do cause debilitating diseases (~300,000 individuals with serious hemoglobin disorders are born every year; naturally occurring hemoglobin variants that are lethal are, of course, never observed). Table 7-1 lists several hemoglobin variants. Mutations that destabilize hemoglobin's tertiary or quaternary structure alter hemoglobin's oxygen-binding affinity (p_{50}) and

TABLE 7-1 Some Hemoglobin Variants

Name[a]	Mutation	Effect
Hammersmith	Phe CD1(42)β → Ser	Weakens heme binding
Bristol	Val E11(67)β → Asp	Weakens heme binding
Bibba	Leu H19(136)α → Pro	Disrupts the H helix
Savannah	Gly B6(24)β → Val	Disrupts the B–E helix interface
Philly	Tyr C1(35)β → Phe	Disrupts hydrogen bonding at the α_1–β_1 interface
Boston	His E7(58)α → Tyr	Promotes methemoglobin formation
Milwaukee	Val E11(67)β → Glu	Promotes methemoglobin formation
Iwate	His F8(87)α → Tyr	Promotes methemoglobin formation
Yakima	Asp G1(99)β → His	Disrupts a hydrogen bond that stabilizes the T conformation
Kansas	Asn G4(102)β → Thr	Disrupts a hydrogen bond that stabilizes the R conformation

[a]Hemoglobin variants are usually named after the place where they were discovered (e.g., hemoglobin Boston).

reduce its cooperativity (Hill coefficient). Moreover, the unstable hemoglobins are degraded by the erythrocytes, and their degradation products often cause the erythrocytes to **lyse** (break open). The resulting **hemolytic anemia** (anemia is a deficiency of red blood cells) compromises O_2 delivery to tissues.

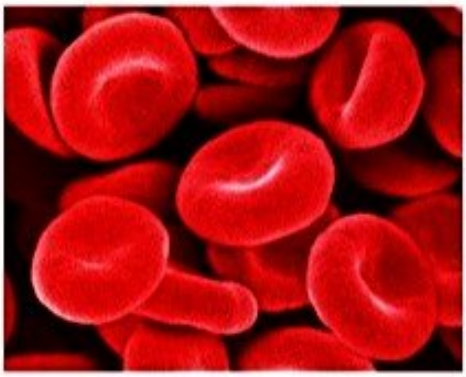
Certain mutations at the O_2-binding site of either the α or β chain favor the oxidation of Fe(II) to Fe(III). Individuals carrying the resulting methemoglobin subunit exhibit **cyanosis,** a bluish skin color, due to the presence of methemoglobin in their arterial blood. These hemoglobins have reduced cooperativity (Hill coefficient ~1.2 compared to a maximum value of 2, since only two subunits in each of these methemoglobins can bind oxygen).

Mutations that increase hemoglobin's oxygen affinity lead to increased numbers of erythrocytes in order to compensate for the less than normal amount of oxygen released in the tissues. Individuals with this condition, which is named **polycythemia,** often have a ruddy complexion.

A Single Amino Acid Change Causes Sickle-Cell Anemia. Most harmful hemoglobin variants occur in only a few individuals, in many of whom the mutation apparently originated. However, ~10% of African-Americans and as many as 25% of black Africans carry a single copy of (are **heterozygous** for) the gene for **sickle-cell hemoglobin (hemoglobin S).** Individuals who carry two copies of (are **homozygous** for) the gene for hemoglobin S suffer from **sickle-cell anemia,** in which deoxyhemoglobin S forms insoluble filaments that deform erythrocytes (Fig. 7-17). In this painful, debilitating, and often fatal disease, the rigid, sickle-shaped cells cannot easily pass through the capillaries. Consequently, in a sickle-cell "crisis," the blood flow to some tissues may be completely blocked, resulting in tissue death. In addition, the mechanical fragility of the misshapen cells results in hemolytic anemia. Heterozygotes, whose hemoglobin is ~40% hemoglobin S, usually lead a normal life, although their erythrocytes have a shorter than normal lifetime.

In 1945, Linus Pauling hypothesized that sickle-cell anemia was the result of a mutant hemoglobin, and in 1949 he showed that the mutant hemoglobin had a less negative ionic charge than normal adult hemoglobin. This was the first evidence that a disease could result from an alteration in the molecular structure of a protein. Furthermore, since sickle-cell anemia is an inherited disease, a defective gene must be responsible for the abnormal protein. Nevertheless, the molecular defect in sickle-cell hemoglobin was not identified until 1956, when Vernon Ingram showed that hemoglobin S contains Val rather than Glu at the sixth position of each β chain. This was the first time an inherited disease was shown to arise from a specific amino acid change in a protein.

The X-ray structure of deoxyhemoglobin S has revealed that one mutant Val side chain in each hemoglobin S tetramer nestles into a hydrophobic pocket on the surface of a β subunit in another hemoglobin tetramer (Fig. 7-18). This intermolecular contact allows hemoglobin S tetramers to form linear polymers. Aggregates of 14 strands that wind around each other form fibers extending throughout the length of the erythrocyte (Fig. 7-19). The hydrophobic pocket on the β subunit cannot accommodate the normally occurring Glu side chain, and the pocket is absent in oxyhemoglobin. Consequently, neither normal hemoglobin nor oxyhemoglobin S can polymerize. In fact, hemoglobin S fibers dissolve essentially instantaneously on oxygenation, so none are present in arterial blood. The danger of sickling is greatest when erythrocytes pass through



(a)

(b)

FIG. 7-17 Scanning electron micrographs of human erythrocytes. (a) Normal erythrocytes are flexible, biconcave disks that can tolerate slight distortions as they pass through the capillaries (many of which have smaller diameters than erythrocytes). (b) Sickled erythrocytes from an individual with sickle-cell anemia are elongated and rigid and cannot easily pass through capillaries. [(a) © Susumu Nishinaga/Photo Researchers, Inc.; (b) Bill Longcore/Photo Researchers, Inc.]

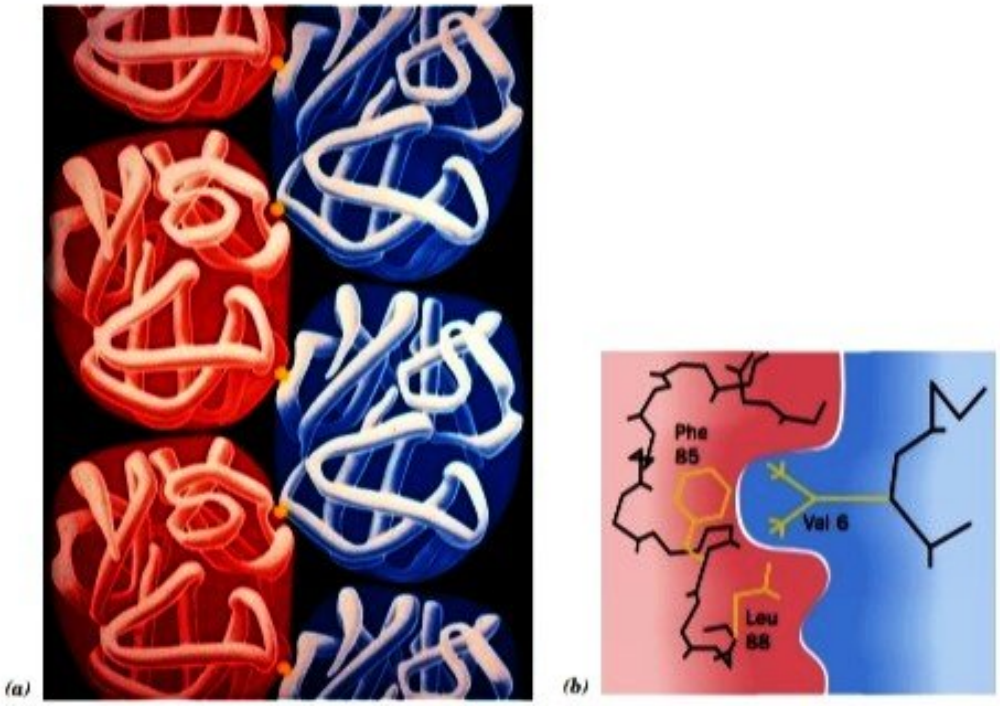
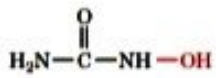


FIG. 7-18 Structure of a deoxyhemoglobin S fiber. (*a*) The arrangement of deoxyhemaglobin S molecules in the fiber. Only three subunits of each deoxyhemaglobin S molecule are shown. (*b*) The side chain of the mutant Val 6 in the β_2 chain of one hemaglobin S molecule (yellow knob in Part a) binds to a hydrophobic pocket on the β_1 subunit of a neighboring deoxyhemaglobin S molecule. [Illustration, Irving Geis. Image from the Irving Geis Collection/Howard Hughes Medical Institute. Rights owned by HHMI. Reproduction by permission only.]

the capillaries, where deoxygenation occurs. The polymerization of hemoglobin S molecules is time and concentration dependent, which explains why blood flow blockage occurs only sporadically (in a sickle-cell "crisis").

Interestingly, many hemoglobin S homozygotes have only a mild form of sickle-cell anemia because they express relatively high levels of fetal hemoglobin, which contains γ chains rather than the defective β chains. The fetal hemoglobin dilutes the hemoglobin S, making it more difficult for hemoglobin S to aggregate during the 10–20 s it takes for an erythrocyte to travel from the tissues to the lungs for reoxygenation. The administration of **hydroxyurea,**



Hydroxyurea

the first and as yet the only effective treatment for sickle-cell anemia, ameliorates the symptoms of sickle-cell anemia by increasing the fraction of cells containing fetal hemoglobin (although the mechanism whereby hydroxyurea acts is unknown).

FIG. 7-19 Electron micrograph of deoxyhemoglobin S fibers spilling out of a ruptured erythrocyte. [Courtesy of Robert Josephs, University of Chicago.]

Hemoglobin S Protects Against Malaria. Before the advent of modern palliative therapies, individuals with sickle-cell anemia rarely survived to maturity. Natural selection has not minimized the prevalence of the hemoglobin S variant, however, because heterozygotes are more resistant to **malaria,** an often lethal infectious disease. Of the 2.5 billion people living within malaria-endemic areas, 100 million are clinically ill with the disease at any given time and around

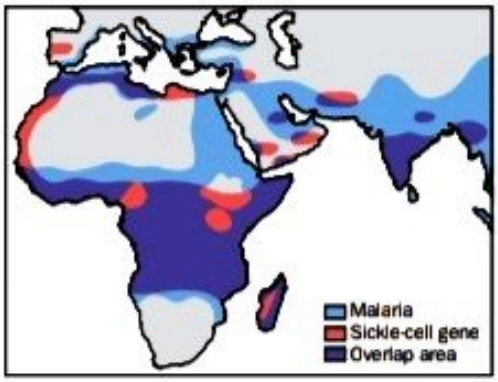


FIG. 7-20 Correspondence between malaria and the sickle-cell gene. The blue areas of the map indicate regions where malaria is or was prevalent. The pink areas represent the distribution of the gene for hemoglobin S. Note the overlap (*purple*) of the distributions.

CHECKPOINT

- Describe the O_2-binding behavior of myoglobin in terms of pO_2 and K. How is K defined?
- Explain the structural basis for cooperative oxygen binding to hemoglobin.
- Sketch a binding curve (% bound ligand versus ligand concentration) for cooperative and noncooperative binding.
- Explain why the O_2-binding behavior of myoglobin and hemoglobin can be summed up by a single number (the p_{50}).
- Could a binding protein have a Hill constant of zero?
- Describe how myoglobin and hemoglobin function in delivering O_2 from the lungs to respiring tissues.
- What is the physiological relevance of the Bohr effect and BPG?
- Explain why mutations can increase or decrease the oxygen affinity and cooperativity of hemoglobin. How can the body compensate for these changes?

1 million, mostly very young children, die from it each year. Malaria is caused by the mosquito-borne protozoan *Plasmodium falciparum*, which resides within an erythrocyte during much of its 48-h life cycle. Infected erythrocytes adhere to capillary walls, causing death when cells impede blood flow to a vital organ.

The regions of equatorial Africa where malaria is a major cause of death coincide closely with those areas where the sickle-cell gene is prevalent (Fig. 7-20), thereby suggesting that the sickle-cell gene confers resistance to malaria. How does it do so? Plasmodia increase the acidity of infected erythrocytes by ~0.4 pH units. The lower pH favors the formation of deoxyhemoglobin via the Bohr effect, thereby increasing the likelihood of sickling in erythrocytes that contain hemoglobin S. Erythrocytes damaged by sickling are normally removed from the circulation by the spleen. During the early stages of a malarial infection, parasite-enhanced sickling probably allows the spleen to preferentially remove infected erythrocytes. In the later stages of infection, when the parasitized erythrocytes attach to the capillary walls (presumably to prevent the spleen from removing them from the circulation), sickling may mechanically disrupt the parasite. Consequently, heterozygous carriers of hemoglobin S in a malarial region have an adaptive advantage: They are more likely to survive to maturity than individuals who are homozygous for normal hemoglobin. Thus, in malarial regions, the fraction of the population who are heterozygotes for the sickle-cell gene increases until their reproductive advantage is balanced by the correspondingly increased proportion of homozygotes (who, without modern medical treatment, die in childhood).

2 Muscle Contraction

KEY CONCEPTS

- Myosin is a motor protein that undergoes conformational changes as it hydrolyzes ATP.
- The sliding filament model of muscle contraction describes the movement of thick filaments relative to thin filaments.
- The globular protein actin can form structures such as microfilaments and the thin filaments of muscle.

One of the most striking characteristics of living things is their capacity for organized movement. Such phenomena occur at all structural levels and include such diverse vectorial processes as the separation of replicated chromosomes during cell division, the beating of flagella and cilia, and, most obviously, muscle contraction. In this section, we consider the structural and chemical basis of movement in **striated muscle,** one of the best understood mobility systems.

A Muscle Consists of Interdigitated Thick and Thin Filaments

The voluntary muscles, which include the skeletal muscles, have a striated (striped) appearance when viewed by light microscopy (Fig. 7-21). Such muscles consist of long multinucleated cells (the muscle fibers) that contain parallel bundles of **myofibrils** (Greek: *myos,* muscle; Fig. 7-22). Electron micrographs show that muscle striations arise from the banded structure of multiple in-register myofibrils. The bands are formed by alternating regions of greater and lesser electron density called **A bands** and **I bands,** respectively (Fig. 7-23). The myofibril's repeating unit, the **sarcomere** (Greek: *sarkos,* flesh), is bounded by **Z disks** at the center of each I band. The A band is centered on the **H zone,** which in turn is centered on the **M disk.** The A band contains 150-Å-diameter **thick filaments,** and the I band contains 70-Å-diameter **thin filaments.** The two sets of filaments are linked by cross-bridges where they overlap.

A contracted muscle can be as much as one-third shorter than its fully extended length. The contraction results from a decrease in the length of the sarcomere, caused by reductions in the lengths of the I band and the H zone

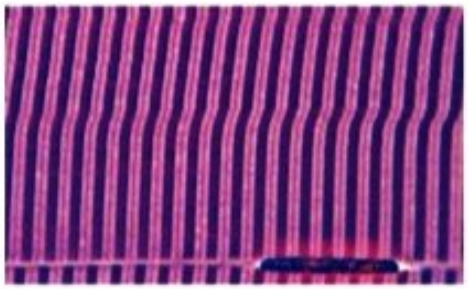


FIG. 7-21 Photomicrograph of a muscle fiber. The longitudinal axis of the fiber is horizontal (perpendicular to the striations). The alternating pattern of dark A bands and light I bands from multiple in-register myofibrils is clearly visible. [J.C. Revy, CNRI/Photo Researchers.]

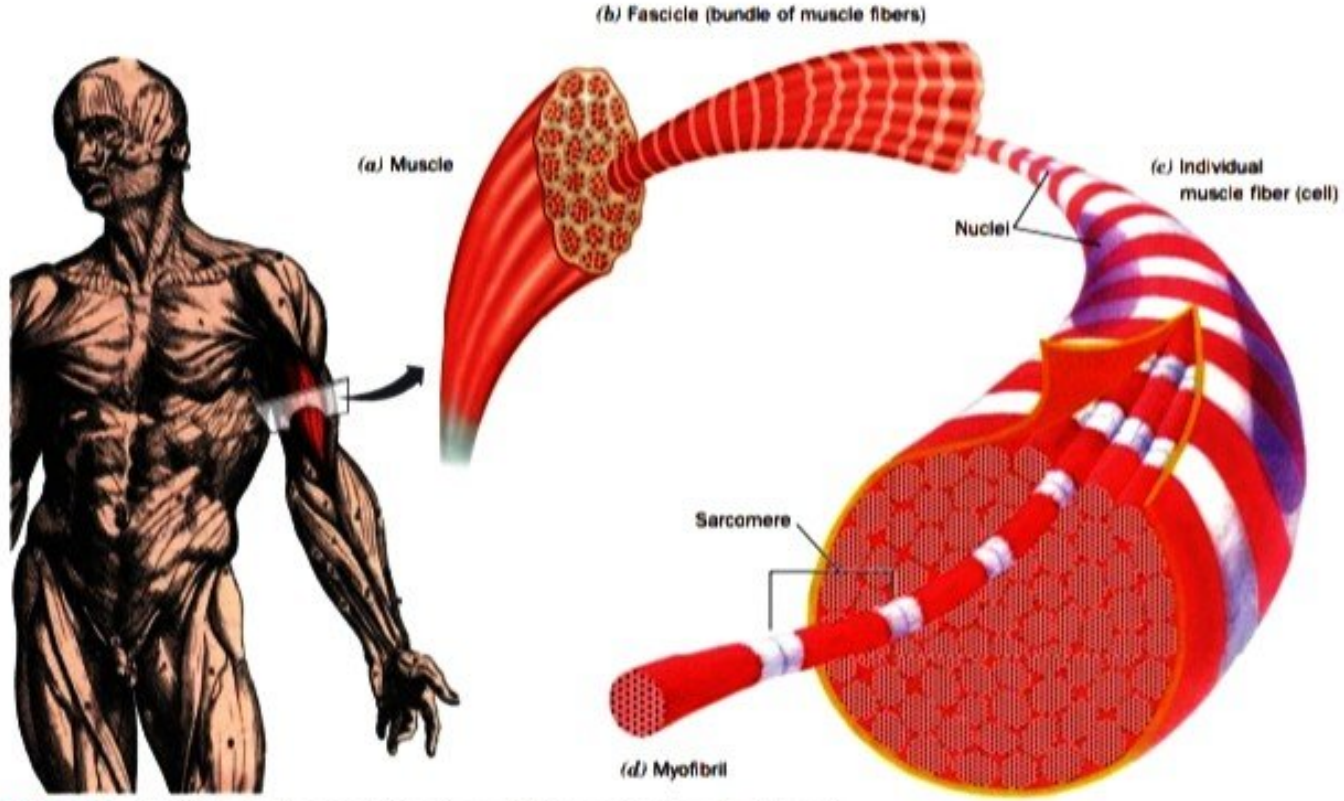


FIG. 7-22 Skeletal muscle organization. A muscle (*a*) consists of bundles of muscle fibers (*b*), each of which is a long, thin, multinucleated cell (*c*) that may run the length of the muscle. Muscle fibers contain bundles of laterally aligned myofibrils (*d*), which in turn consist of bundles of alternating thick and thin filaments.

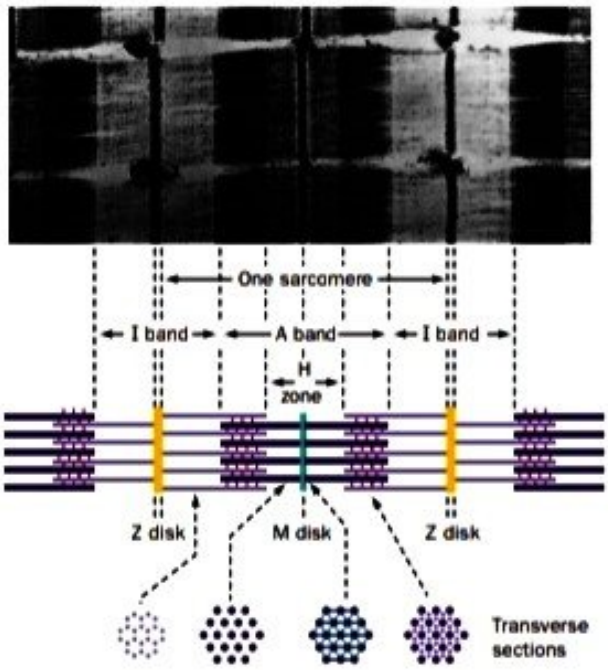


FIG. 7-23 Anatomy of the myofibril. The electron micrograph shows parts of three myofibrils, which are separated by horizontal gaps. The accompanying interpretive drawing shows the major features of the myofibril: the light I band, which contains only thin filaments; the A band, whose dark H zone contains only thick filaments and whose darker outer segments contain overlapping thick and thin filaments; the Z disk, to which the thin filaments are anchored; and the M disk, which arises from a bulge at the center of each thick filament. The myofibril's functional unit, the sarcomere, is the region between two successive Z disks. [Courtesy of Hugh Huxley, Brandeis University.]

(Fig. 7-24*a*). These observations, made by Hugh Huxley in 1954 (Box 7-4), are explained by the **sliding filament model** in which interdigitated thick and thin filaments slide past each other (Fig. 7-24*b*). Thus, during a contraction, a muscle becomes shorter, and because its total volume does not change, it also becomes thicker.

Thick Filaments Consist Mainly of Myosin. Vertebrate thick filaments are composed almost entirely of a single type of protein, **myosin,** which consists of six polypeptide chains: two 220-kD **heavy chains** and two pairs of different **light chains,** the so-called **essential** and **regulatory light chains (ELC** and **RLC)** that vary in size between 15 and 22 kD, depending on their source. X-Ray structure determinations by Ivan Rayment and Hazel Holden of the N-terminal half of the myosin heavy chain, the so-called **myosin head,** reveals

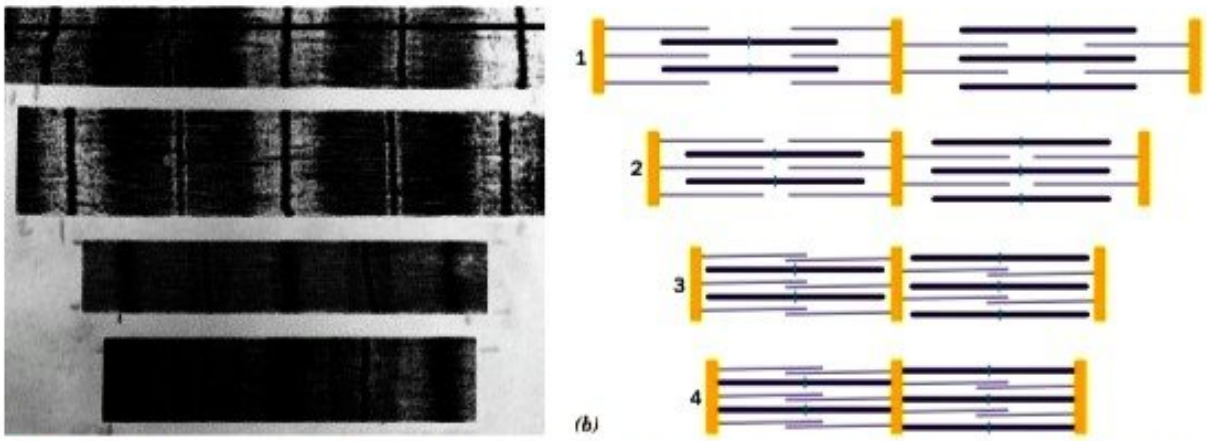


FIG. 7-24 Myofibril contraction. (*a*) Electron micrographs showing myofibrils in progressively more contracted states. The lengths of the I band and H zone decrease on contraction, whereas the lengths of the thick and thin filaments remain constant. (*b*) Interpretive drawings showing interpenetrating sets of thick and thin filaments sliding past each other. [Courtesy of Hugh Huxley, Brandeis University.]

Box 7-4 Pathways of Discovery

Hugh Huxley and the Sliding Filament Model

Hugh Huxley (1924–) The mechanism of muscle action has fascinated scientists for hundreds if not thousands of years. The first close look at muscle fibers came in 1682, when Antoni van Leeuwenhoek's early microscope revealed a pattern of thin longitudinal fibers. In the modern era, research on muscle has followed one of two approaches. First, it is possible to study muscle as an energy-transducing system, in which metabolic energy is generated and consumed. This line of research received a tremendous boost in the 1930s with the discovery that ATP is the energy source for muscle contraction. The second approach involves treating muscle as a mechanical system, that is, sorting out its rods and levers. Ultimately, a molecular approach united the mechanical and energetic aspects of muscle research. The insights of Hugh Huxley made this possible.

The molecular characterization of muscle did not occur overnight. In 1859, Willi Kühne isolated a proteinaceous substance from muscle tissue that he named "myosin" (almost certainly a mixture of many proteins), but it tended to aggregate and was therefore not as popular a study subject as the more soluble proteins such as hemoglobin. A major breakthrough in muscle protein chemistry came in 1941, when the Hungarian biochemist Albert Szent-Györgyi showed that two types of protein could be extracted from ground muscle by a solution with high salt concentration (Szent-Györgyi also contributed to the elucidation of the citric acid cycle; Box 17-1). Extraction for 20 minutes yielded a protein he named myosin A but which is now called myosin. However, extraction overnight yielded a second protein which he named myosin B but is now called actomyosin. It soon became apparent that myosin B was a mixture of two proteins, myosin and a new protein which was named actin. Further work showed that threads of actomyosin contracted to ~10% of their original length in the presence of ATP. Since actin and myosin alone do not contract in the presence of ATP, the contraction must have resulted from their interaction. However, it took another decade to develop a realistic model of how myosin and actin interact.

A number of theories had been advanced to explain muscle contraction. According to one theory, the cytoplasm of muscle cells moved like that of an amoeba. Other theories proposed that muscle fibers took up and gave off water or repelled and attracted other fibers electrostatically. Linus Pauling, who had recently discovered the structures of the α helix and β sheet (Box 6-1), ventured that myosin could change its length by shifting between the two protein conformations. Huxley formulated an elegant—and correct—explanation in his sliding filament model for muscle contraction.

In 1948, Huxley began his doctoral research at Cambridge University in the United Kingdom, in the laboratory of John Kendrew (who 10 years later determined the first X-ray structure of a protein, that of myoglobin; Section 7-1A). There, through X-ray studies on frog muscle fibers, Huxley established that the X-ray diffraction pattern changes with the muscle's physiological state. Furthermore, he showed that muscle contained two sets of parallel fibers, rather than one, and that these fibers were linked together by multiple cross-links. These observations became the germ for further research, which he carried out at MIT in 1953 and 1954. He teamed up with Jean Hanson, a Briton who was also working at MIT. Hanson made good use of her knowledge of muscle physiology and her expertise in phase-contrast microscopy, a technique that could visualize the banded patterns of muscle fibers. Huxley and Hanson observed rabbit muscle fibers under different experimental conditions, making precise measurements of the width of the A and I bands in sarcomeres (Fig. 7-23). In one experiment, they extracted myosin from the muscle fiber, noted the loss of the dark A band, and concluded that the A band consists of myosin. When they extracted both actin and myosin, all identifiable structure was lost, and they concluded that actin is present throughout the sarcomere.

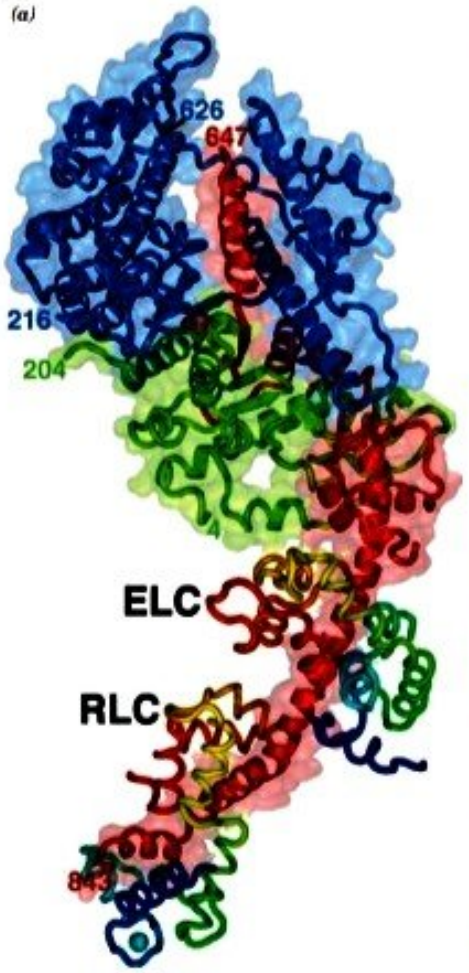
When ATP was added, the muscle slowly contracted, and Huxley and Hanson were able to measure the shortening of the I band. The A band maintained a constant length but became darker. A muscle fiber under the microscope could also be stretched by pulling on the coverslip. As the muscle "relaxed," the I band increased in width and the A band became less dense. Measurements were made for muscle fibers contracted to 60% of their original length and stretched to 120% of their original length.

The key to the sliding filament model that Huxley described and subsequently refined is that the individual molecules (that is, their observable fibrous forms) do not shrink or extend but instead slide past each other. During contraction, actin filaments (thin filaments) in the I band are drawn into the A band, which consists of stationary myosin-containing filaments (thick filaments). During stretching, the actin filaments withdraw from the A band. Similar conclusions were reached by the team of Andrew Huxley (no relation to Hugh) and Rolf Niedergerke, who examined the contraction of living frog muscle fibers. Both groups published their work in back-to-back papers in *Nature* in 1954.

Hugh Huxley went on to supply additional details to his sliding filament model. For example, he showed that myosin forms cross-bridges with actin fibers. However, these bridges are asymmetric, pointing in opposite directions in the two halves of the sarcomere. This arrangement allows myosin to pull thin filaments in opposite directions toward the center of the sarcomere (Fig. 7-24).

While Huxley was describing the mechanism of muscle contraction, Watson and Crick discovered the structure of DNA, and Max Perutz made a decisive breakthrough in the use of heavy metal atoms to solve the phase problem in his X-ray studies of hemoglobin (Box 7-2). Collectively, these discoveries indicated the tremendous potential for describing biological phenomena in molecular terms. Subsequent studies of muscle contraction have used electron microscopy, X-ray crystallography, and enzymology to probe the fine details of the sliding filament model, including the structure of myosin's lever arm, the composition of the thin filament, and the exact role of ATP in triggering conformational changes that generate mechanical force.

Huxley, H.E. and Hanson, J., Changes in the cross-striations of muscle during contraction and stretch and their structural interpretation, *Nature* **173**, 973–976 (1954).



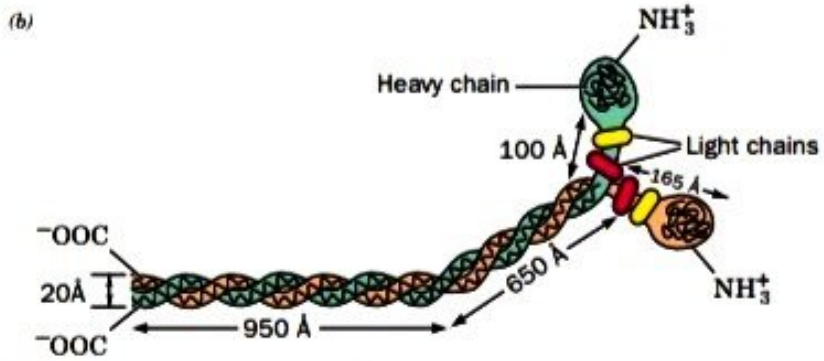
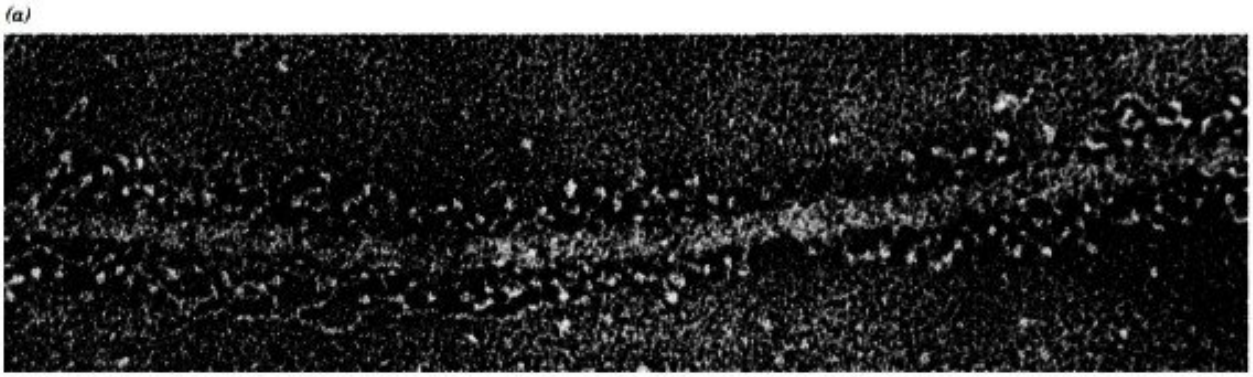


FIG. 7-25 Structure of myosin. (*a*) A ribbon diagram of the myosin head from chicken muscle. The heavy chain is represented by a ribbon diagram embedded in its semi-transparent molecular surface with different portions green, blue, and red. The essential and regulatory light chains, RLC and ELC, are drawn in worm form with each colored in rainbow order from its N-terminus (*blue*) to its C-terminus (*red*). A sulfate ion, shown in space-filling form (C red and S yellow), occupies the binding site of ATP's β-phosphate group. An RLC-bound Ca^{2+} ion (*lower left*) is represented by a cyan sphere. [Based on an X-ray structure by Ivan Rayment and Hazel Holden, University of Wisconsin. PDBid 2MYS.] (*b*) Diagram of the myosin molecule. Its two identical heavy chains (*green and orange*) each have an N-terminal globular head and an α-helical tail. Between the head and tail is an α helix, the lever arm, that associates with the two kinds of light chains (*magenta and yellow*). The tails wind around each other to form a 1600-Å-long parallel coiled coil.

that it forms an elongated (55 × 165 Å) globular head to which one subunit each of ELC and RLC bind (Fig. 7-25*a*). The C-terminal half of the heavy chain forms a long fibrous α-helical tail, two of which associate to form a left-handed coiled coil. Thus, *myosin consists of a 1600-Å-long rodlike segment with two globular heads* (Fig. 7-25*b*). The amino acid sequence of myosin's α-helical tail is characteristic of coils such as those in keratin (Section 6-1C): It has a seven-residue pseudorepeat, *a-b-c-d-e-f-g*, with nonpolar residues predominating at positions *a* and *d*.



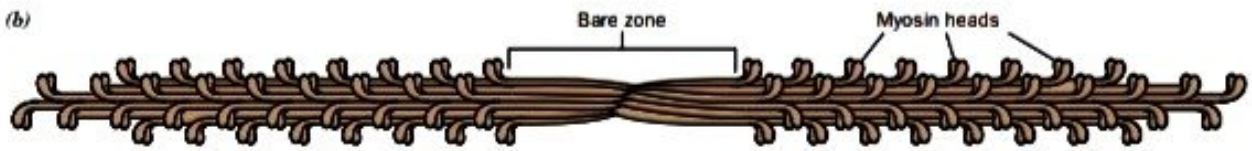


FIG. 7-26 Structure of the thick filament. (*a*) Electron micrograph showing the myosin heads projecting from the thick filament. [From Trinick, J. and Elliott, A., *J. Mol. Biol.* **131,** 135 (1977).] (*b*) Drawing of a thick filament, in which several hundred myosin molecules form a staggered array with their globular heads pointing away from the filament.

Under physiological conditions, several hundred myosin molecules aggregate to form a thick filament. The rodlike tails pack end to end in a regular staggered array, leaving the globular heads projecting to the sides on both ends (Fig. 7-26). These myosin heads form the cross-bridges to thin filaments in intact myofibrils. The myosin head, which is an **ATPase** (ATP-hydrolyzing enzyme), has its ATP-binding site located in a 13-Å-deep V-shaped pocket.

Thin Filaments Consist Mainly of Actin. Thin filaments consist mainly of polymers of **actin,** the most abundant cytosolic protein in eukaryotes. In its monomeric form, this ~375-residue protein is known as **G-actin** (G for globular); when polymerized, it is called **F-actin** (F for fibrous). Each actin subunit has binding sites for ATP and a Ca^{2+} or Mg^{2+} ion that are located in a deep cleft (Fig. 7-27). ATP hydrolysis to ADP + P_i is not required for actin polymerization but occurs afterward (Section 7-2C).

The fibrous nature of F-actin and its variable fiber lengths have thwarted its crystallization in a manner suitable for X-ray crystallographic analysis. Consequently, our current understanding of the atomic structure of F-actin is based on electron micrographs (Fig. 7-28*a*) together with low resolution models based on X-ray studies of oriented gels of F-actin into which high resolution atomic models of G-actin have been fitted (Fig. 7-28*b*). These models indicate that the actin polymer is a double-stranded helix of subunits in which each subunit contacts four others. Each actin subunit has the same head-to-

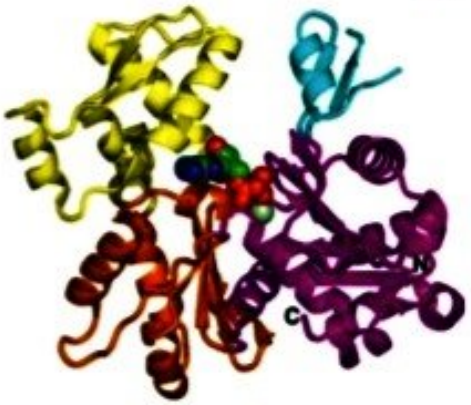


FIG. 7-27 X-Ray structure of rabbit muscle G-actin in complex with ATP and a Ca^{2+} ion. The four domains of the protein are colored cyan, magenta, orange, and yellow, and the N- and C-termini are labeled. The ATP, which is drawn in space-filling form with C green, N blue, O red, and P orange, binds at the bottom of a deep cleft between the domains. The Ca^{2+} ion is represented by a light green sphere. [Based on an X-ray structure by Leslie Burtnik, University of British Columbia, Vancouver, British Columbia. PDBid 3HBT.]

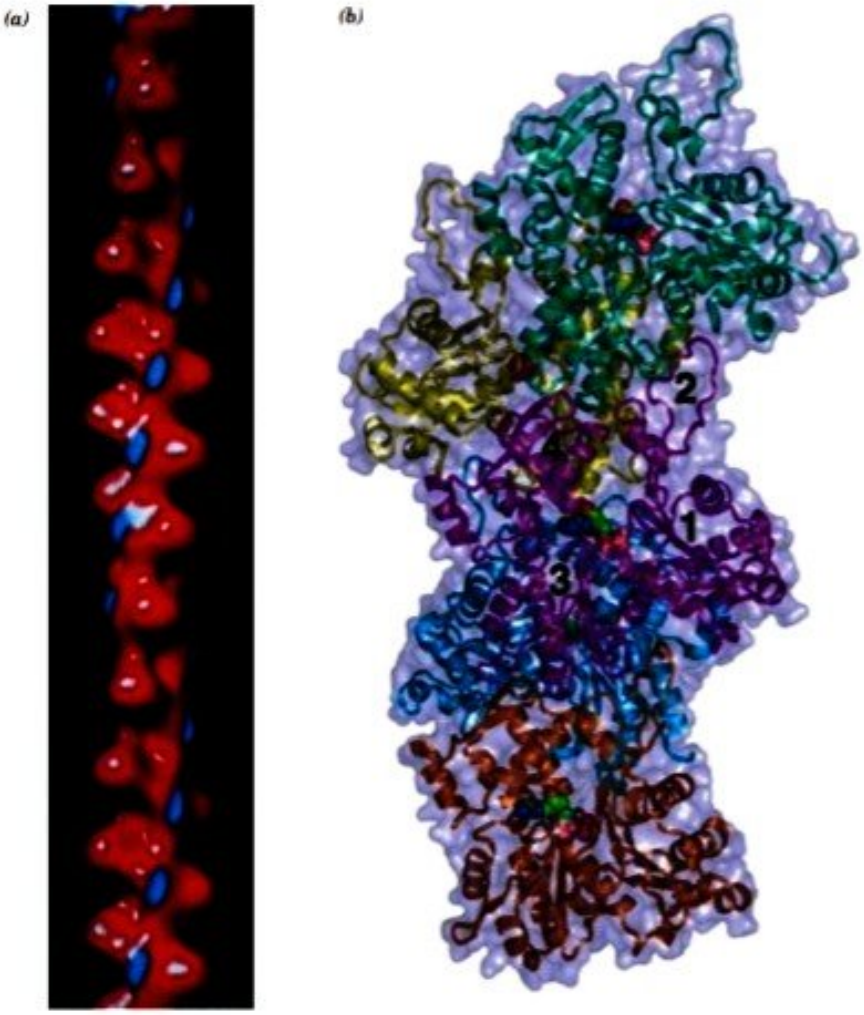


FIG. 7-28 Structure of the actin filament. (*a*) Cryoelectron microscopy–based image. The tropomyosin binding sites (see below) are blue. [Courtesy of Daniel Safer, University of Pennsylvania, and Ronald Milligan, The Scripps Research Institute, La Jolla, California.] (*b*) Model based on fitting a known X-ray structure of G-actin to a cryoEM-based image of F-actin, represented by five consecutive actin · ADP subunits drawn in ribbon form, each with a different color, embedded in their semitransparent molecular surface. The ADPs are drawn in space-filling form with C green, N blue, O red, and P orange. The central subunit (*magenta*) is oriented as is the G-actin in Fig. 7-27. [Based on a structure by Keiichi Namba, Osaka University, Japan. PDBid 3MFP.]

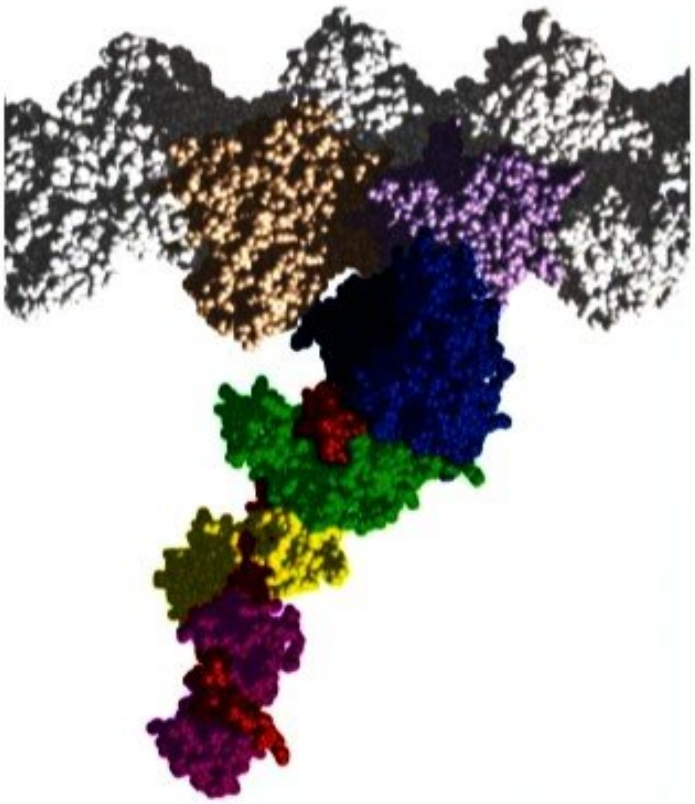
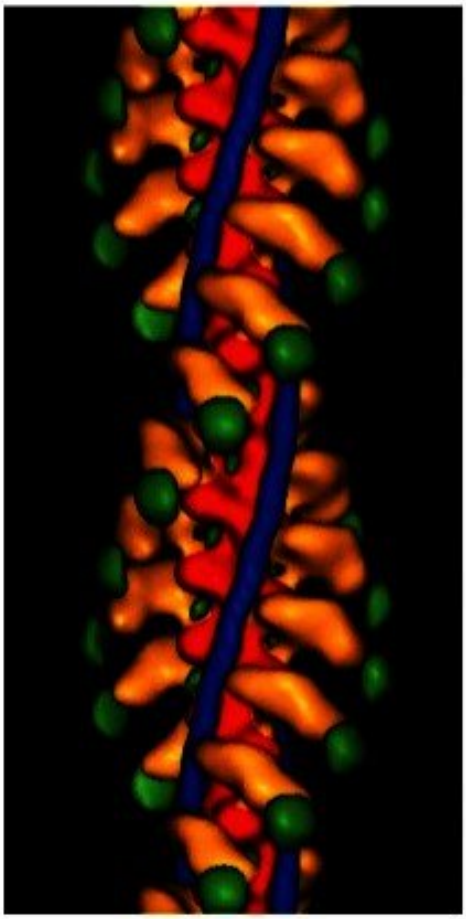


FIG. 7-29 Model of the myosin–actin interaction. This space-filling model was constructed from the X-ray structures of actin and the myosin head and electron micrographs of their complex. The actin filament is at the top. The myosin heavy chain is colored as in Fig. 7-25 and the light chains are yellow and magenta. The coiled-coil tail is not shown. An ATP-binding site is located in a cleft in the blue domain of the myosin head. In a myofibril, every actin monomer has the potential to bind a myosin head, and the thick filament has many myosin heads projecting from it. [Modified from a drawing by Ivan Rayment and Hazel Holden, University of Wisconsin.]



tail orientation (e.g., all the nucleotide-binding clefts open upward in Fig. 7-28*b*), so the assembled fiber has a distinct polarity. The end of the fiber toward which the nucleotide-binding sites open is known as the **(−) end,** and its opposite end is the **(+) end.** The (+) ends of the thin filaments bind to the Z disk (Fig. 7-23).

Each of muscle F-actin's monomeric units can bind a single myosin head (Fig. 7-29), probably by ion pairing and by the association of hydrophobic patches on each protein. Electron micrographs indicate that the myosin heads bound to an F-actin filament all have the same orientation (Fig. 7-30) and that in thin filaments that are still attached to the Z disk, the myosin heads all point away from the Z disk.

Tropomyosin and Troponin Are Thin Filament Components. Myosin and actin, the major components of muscle, account for 60 to 70% and 20 to 25% of total muscle protein, respectively. Of the remainder, two proteins that are associated with the thin filaments are particularly prominent:

1. **Tropomyosin,** a homodimer whose two 284-residue α-helical subunits wrap around each other to form a parallel coiled coil that extends nearly the entire 400-Å length of the molecule (a portion of which is shown in Fig. 6-15*b*). Multiple copies of these rod-shaped proteins are joined head-to-tail to form cables wound in the grooves of the F-actin helix such that each tropomyosin molecule contacts seven consecutive actin subunits in a quasi-equivalent manner (Fig. 7-30).

FIG. 7-30 Cryoelectron microscopy–based image at <25-Å resolution of a thin filament decorated with myosin heads. F-actin is red, tropomyosin is blue, the myosin motor domain is yellow, and the essential light chain is green. The helical filament has a pitch (rise per turn) of 370 Å. [Courtesy of Ronald Milligan, The Scripps Research Institute, La Jolla, California.]

2. **Troponin,** which consists of three subunits: **TnC,** a Ca^{2+}-binding protein; **TnI,** which binds to actin; and **TnT,** an elongated molecule, which binds to tropomyosin at its head-to-tail junctions. The X-ray structure of troponin in complex with four Ca^{2+} ions (Fig. 7-31), determined by Robert Fletterick, reveals that TnI closely resembles the myosin light chains and that the inhibitory segment of TnI binds to TnC's rigid central helix in this Ca^{2+}-activated state.

The tropomyosin–troponin complex, as we will see below, regulates muscle contraction by controlling the access of the myosin heads to their binding sites on actin.

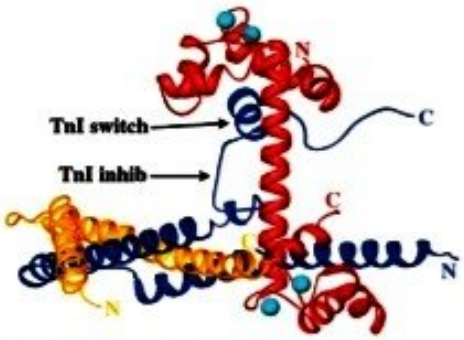


FIG. 7-31 X-Ray structure of chicken skeletal muscle troponin. TnC is red, TnI is blue, and TnT is gold. The four Ca^{2+} ions bound by TnC are represented by cyan spheres. [Based on an X-ray structure by Robert Fletterick, University of California at San Francisco. PDBid 1YTZ.]

Muscle Contains Numerous Minor Proteins That Organize Its Structure. Other proteins serve to form the Z disk and the M disk and to organize the arrays of thick and thin filaments. For instance, **α-actinin,** a rodlike homodimeric protein that cross-links F-actin filaments, is localized in the Z disk's interior and is therefore thought to attach oppositely oriented thin filaments to the Z disk.

One of the more unusual muscle proteins, **titin,** the longest known polypeptide chain (34,350 residues), is composed of ~300 repeating globular domains. Three to six titin molecules associate with each thick filament, spanning the 1-μm distance between the M and Z disks. Titin is believed to function as a molecular bungee cord to keep the thick filament centered on the sarcomere: During muscle contraction, it compresses as the sarcomere shortens, but when the muscle relaxes, titin resists sarcomere extension past the starting point.

Nebulin, which is also extremely large (6669 residues), is a mainly α-helical protein that is associated with the thin filament. It is thought to set the length of the thin filament by acting as a template for actin polymerization. This length is held constant by **tropomodulin,** which caps the (−) end of the thin filament (the end not attached to the Z disk), thereby preventing further actin polymerization and depolymerization. **CapZ** (also called **β-actinin**) is an α-actinin-associated heterodimer that similarly caps the (+) end of F-actin.

The M disk (Fig. 7-23) arises from the local enlargement of in-register thick filaments. Two proteins that are associated with this structure, **myomensin** and **M-protein,** bind to titin and are therefore likely to participate in thick filament assembly, as does the thick filament–associated **myosin-binding protein C.**

Duchenne muscular dystrophy (DMD) and the less severe **Becker muscular dystrophy (BMD)** are both sex-linked muscle-wasting diseases. In DMD, which has an onset age of 2 to 5 years, muscle degeneration exceeds muscle regeneration, causing progressive muscle weakness and ultimately death, typically due to respiratory disorders or heart failure, usually by age 25. In BMD, the onset age is 5 to 10 years and there is an overall less progressive course of muscle degeneration and a longer (sometimes normal) life span than in individuals with DMD.

The gene responsible for DMD/BMD encodes a 3685-residue protein named **dystrophin,** which has a normal abundance in muscle tissue of 0.002%. Individuals with DMD usually have no detectable dystrophin in their muscles, whereas those with BMD mostly have dystrophins of altered sizes. Evidently, the dystrophins of individuals with DMD are rapidly degraded, whereas those of individuals with BMD are semifunctional.

Dystrophin is a member of a family of flexible rod-shaped proteins that includes other actin-binding cytoskeletal components. Dystrophin associates on the inner surface of the muscle plasma membrane with a transmembrane glycoprotein complex, where it helps anchor F-actin to the extracellular matrix and thereby protects the plasma membrane from being torn by the mechanical stress of muscle contraction. Although such small tears are common in muscle cells, they occur much more frequently in dystrophic cells, leading to a greatly increased rate of cell death.

B Muscle Contraction Occurs when Myosin Heads Walk up Thin Filaments

In order to complete our description of muscle contraction we must determine how ATP hydrolysis is coupled to the sliding filament model. If the sliding filament model is correct then it would be impossible for a myosin cross-bridge to remain attached to the same point on a thin filament during muscle contraction. Rather, it must repeatedly detach and then reattach itself at a new site further along the thin filament toward the Z disk. This, in turn, suggests that *muscular tension is generated through the interaction of myosin cross-bridges with thin filaments.* The actual contractile force is provided by ATP hydrolysis. Thus, myosin is a **motor protein** that converts the chemical energy of ATP hydrolysis to the mechanical energy of movement. Edwin Taylor formulated a model for myosin-mediated ATP hydrolysis, which has been refined by the structural studies of Rayment, Holden, and Ronald Milligan as follows (Fig. 7-32):

1. ATP binds to a myosin head in a manner that causes myosin's actin-binding site to open up and release its bound actin.
2. Myosin's active site (distinct from its actin-binding site) closes around the ATP. The resulting hydrolysis of the ATP to ADP + P_i "cocks" the myosin head, that is, puts it into its "high energy" conformation in which it is approximately perpendicular to the thick filament.
3. The myosin head binds weakly to an actin monomer that is closer to the Z disk than the one to which it had been bound previously.
4. Myosin releases P_i, which causes its actin-binding site to close, thereby increasing its affinity for actin.
5. The resulting transient state is immediately followed by the power stroke, a conformational shift that sweeps the myosin head's C-terminal tail by an estimated ~100 Å toward the Z disk relative to the actin-binding site on its head, thus translating the attached thin filament by this distance toward the M disk.
6. ADP is released, thereby completing the cycle.

Because the reaction cycle involves several steps, some of which are irreversible (e.g., ATP hydrolysis and P_i release), the entire cycle is unidirectional. The ~500 myosin heads on every thick filament asynchronously cycle through this reaction sequence about five times each per second during a strong muscular contraction. *The myosin heads thereby "walk" or "row" up adjacent thin filaments toward the Z disk with the concomitant contraction of the muscle.* Although myosin is dimeric, its two heads function independently.

Calcium Triggers Muscle Contraction. Highly purified actin and myosin can contract regardless of the Ca^{2+} concentration, but preparations containing intact thin filaments contract only in the presence of Ca^{2+}, due to the regulatory action of troponin C (Fig. 7-31). Stimulation of a myofibril by a nerve impulse results in the release of Ca^{2+} from the **sarcoplasmic reticulum** (a system of flattened vesicles derived from the endoplasmic reticulum). As a result, the intracellular [Ca^{2+}] increases from ~10^{-7} to ~10^{-5} M. The higher

FIG. 7-32 Mechanism of force generation in muscle. *(Opposite)* The myosin head "walks" up the actin thin filament through a unidirectional cyclic process that is driven by ATP hydrolysis to ADP and P_i. Only one myosin head is shown. The actin monomer to which the myosin head is bound at the beginning of the cycle is more darkly colored for reference. [After Rayment, I. and Holden, H., *Curr. Opin. Struct. Biol.* **3,** 949 (1993).] **See the Animated Figures.**

? Why doesn't "ATP hydrolysis" fully describe the role of ATP in muscle contraction?

PROCESS DIAGRAM

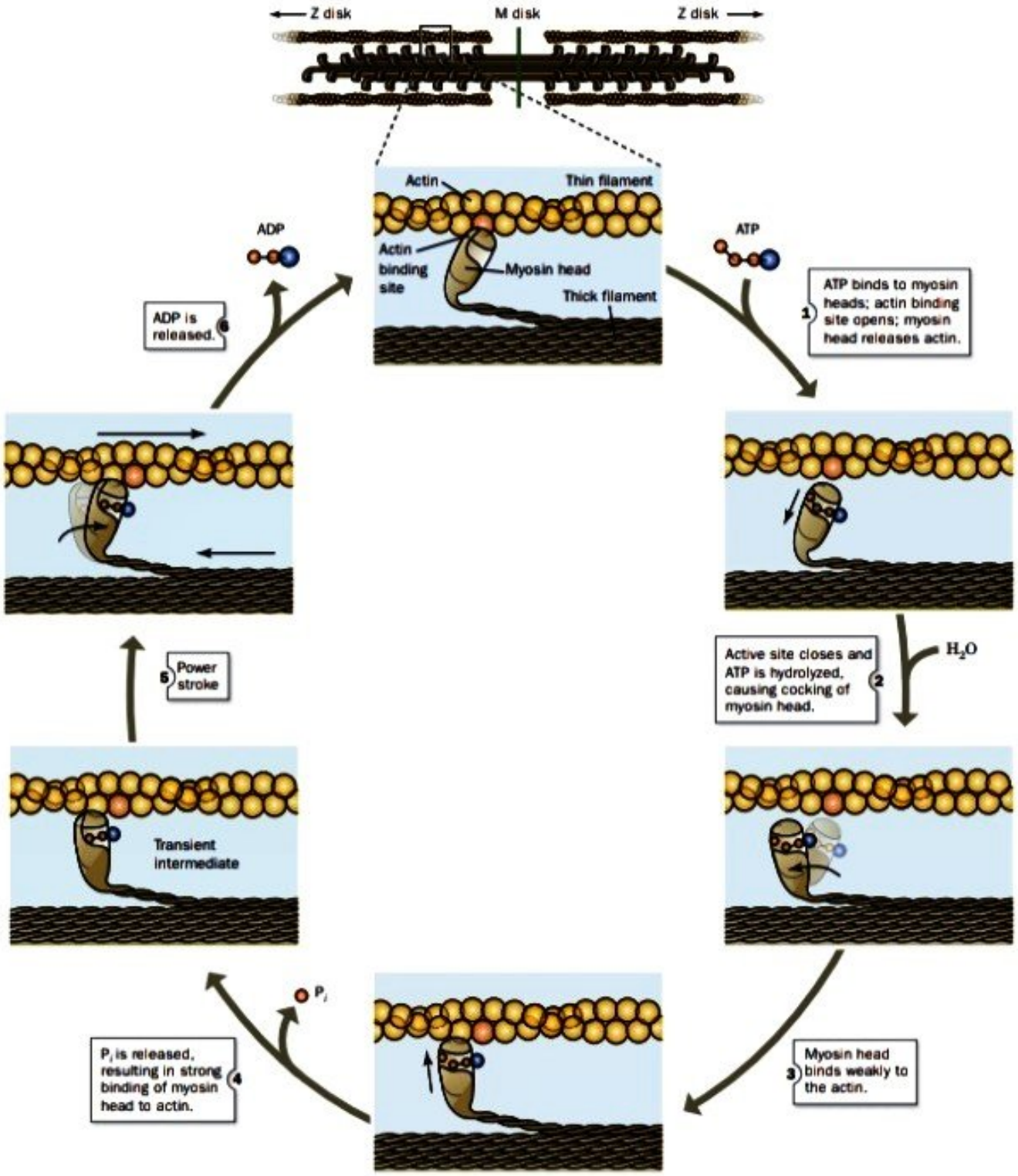


FIG. 7-33 Comparison of the positions of tropomyosin on the thin filament in the absence and presence of Ca^{2+}. In this superposition of cryoelectron microscopy–based images, the F-actin filament is gold, the tropomyosin in the absence of Ca^{2+} is red, and that in the presence of Ca^{2+} is green. [Courtesy of William Lehman, Boston University School of Medicine.]

calcium concentration triggers the conformational change in the troponin–tropomyosin complex that exposes the site on actin where the myosin head binds (Fig. 7-33). When the myofibril [Ca^{2+}] is low (Ca^{2+} is rapidly pumped back into the sarcoplasmic reticulum by ATP-requiring Ca^{2+} pumps; Section 10-3B), the troponin–tropomyosin complex assumes its resting conformation, blocking myosin binding to actin and causing the muscle to relax.

C Actin Forms Microfilaments in Nonmuscle Cells

Although actin and myosin are most prominent in muscle, they also occur in other tissues. In fact, actin is ubiquitous and is usually the most abundant cytoplasmic protein in eukaryotic cells, typically accounting for 5 to 10% of their total protein. Nonmuscle actin forms ~70-Å-diameter fibers known as **microfilaments** that can be visualized by **immunofluorescence microscopy** (in which a fluorescent-tagged antibody is used to "stain" the actin to which it binds; Fig. 7-34). In nonmuscle cells, actin plays an essential role in many processes, including changes in cell shape, cell division, endocytosis, and organelle transport.

Microfilament Treadmilling Can Mediate Locomotion. ATP–G-actin binds to both ends of an F-actin filament but with a greater affinity for its (+) end (hence its name). This polymerization activates F-actin subunits to hydrolyze their bound ATP to ADP + P_i with the subsequent dissociation of P_i. The resulting conformation change reduces the affinity of an ADP–F-actin subunit for its neighboring subunits relative to that of ATP–F-actin. Since F-actin–catalyzed ATP hydrolysis occurs more slowly than actin polymerization and F-actin's bound nucleotide does not exchange with those in solution (its nucleotide-binding site is blocked by its associated subunits), F-actin's more recently polymerized and hence predominantly ATP-containing subunits occur largely at its (+) end, whereas its (−) end consists mainly of less recently polymerized and hence predominantly ADP-containing subunits. The lesser affinity of ADP-containing actin subunits for F-actin results in their net dissociation from the (−) end of the polymer.

The steady state (when the microfilament maintains a constant length) occurs when the net rate of addition of subunits at the (+) end matches the net rate of dissociation of subunits at the (−) end. Then, *subunits that have*

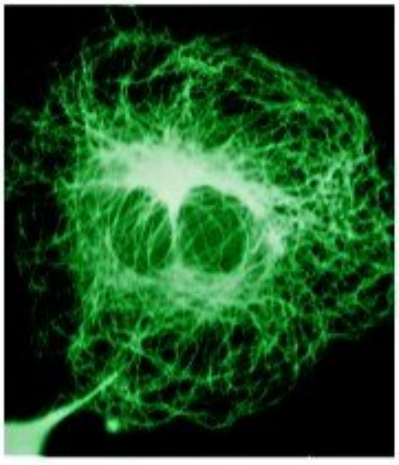


FIG. 7-34 Actin microfilaments. The microfilaments in a fibroblast resting on the surface of a culture dish are revealed by immunofluorescence microscopy using a fluorescently labeled antibody to actin. When the cell begins to move, the filaments disassemble. [Courtesy of John Victor Small, Austrian Academy of Sciences, Salzburg.]

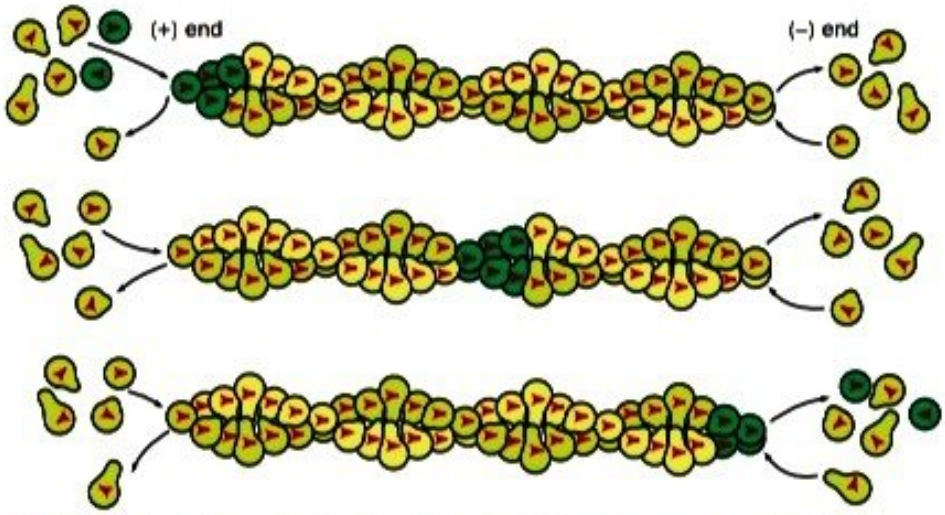


FIG. 7-35 Microfilament treadmilling. In the steady state, actin monomers continually add to the (+) end of the filament (*left*) but dissociate at the same rate from the (−) end (*right*). The filament thereby maintains a constant length while its component monomers translocate from left to right.

FIG. 7-36 Scanning electron micrograph of a crawling macrophage. The leading edge of this white blood cell (*top*) is ruffled where it has become detached from the surface and is in the process of extending. The cell's trailing edge or tail (*bottom*), which is still attached to the surface, is gradually being pulled toward the leading edge. The rate of actin polymerization is greatest at the leading edge. The macrophage is in the course of engulfing *Staphyloccus sp.* bacteria (*orange globules*) by a process known as **phagocytosis.** [© Eye of Science/Photo Researchers, Inc.]

added to the (+) end move toward the (−) end where they dissociate, a process called ***treadmilling*** (Fig. 7-35). Thus, a fluorescently labeled actin monomer is seen to move from the (+) end of the microfilament toward its (−) end. Treadmilling is driven by the free energy of ATP hydrolysis and hence is not at equilibrium.

The directional growth of actin filaments exerts force against the plasma membrane, allowing a cell to extend its cytoplasm in one direction. If the cytoplasmic protrusion anchors itself to the underlying surface, then the cell can use the adhesion point for traction to advance further. In order for the cell to crawl, however, the trailing edge of the cell must release its contacts with the surface while newer contacts are being made at the leading edge (Fig. 7-36). In addition, as microfilament polymerization proceeds at the leading edge, depolymerization must occur elsewhere in the cell, since the pool of G-actin is limited. A variety of actin-binding proteins modulate the rate of actin depolymerization and repolymerization *in vivo*.

Actin-mediated cell locomotion, that is, amoeboid motion, is the most primitive mechanism of cell movement. Nevertheless, virtually all eukaryotic cells undertake some version of it, even if it involves just a small patch of actin near the cell surface. More extensive microfilament rearrangements are essential for cells such as neutrophils (a type of white blood cell) that travel relatively long distances to sites of infection or inflammation.

CHECKPOINT

- Explain how myosin structure relates to its function as a motor protein.
- Draw a diagram of the components of a sarcomere, including the locations of all the proteins mentioned in the text. Which components are globular and which are fibrous?
- Explain the molecular basis of the sliding filament model of muscle contraction.
- What are the roles of Ca^{2+} and ATP in muscle contraction?
- Is it possible to construct a fibrous protein from globular subunits?
- Describe the process of treadmilling in a microfilament.
- How does a microfilament differ from the thin filament in a myofibril?
- Describe the process of actin polymerization and depolymerization during cell crawling.

3 Antibodies

KEY CONCEPTS

- The humoral immune response is mediated by antibodies, which include constant domains as well as variable domains that recognize specific antigens.
- Antibody gene rearrangement and hypermutation contribute to antibody diversity.

All organisms are continually subject to attack by other organisms, including disease-causing microorganisms and viruses. In higher animals, these **pathogens** may penetrate the physical barrier presented by the skin and mucous membranes (a first line of defense) only to be identified as foreign invaders and destroyed by the **immune system.** Two types of immunity have been distinguished:

1. **Cellular immunity,** which plays a role in fighting most pathogens and is particularly effective at eliminating virally infected cells, is mediated by **T lymphocytes** or **T cells,** so called because they mature in the thymus.
2. **Humoral immunity** (*humor* is an archaic term for fluid), which is most effective against bacterial infections and the extracellular phases of viral infections, is mediated by an enormously diverse collection of related proteins known as **antibodies** or **immunoglobulins.** Antibodies are produced by **B lymphocytes** or **B cells,** which in mammals mature in the bone marrow.

In this section we focus on the structure, function, and generation of antibodies.

The immune response is triggered by the presence of a foreign macromolecule, often a protein or carbohydrate, known as an ***antigen.*** B cells display immunoglobulins on their surfaces. If a B cell encounters an antigen that binds to its particular immunoglobulin, it engulfs the antigen–antibody complex, degrades it, and displays the antigen fragments on the cell surface. T cells then stimulate the B cell to proliferate. Most of the B cell progeny are circulating cells that secrete large amounts of the antigen-specific antibody. These antibodies can bind to additional antigen molecules, thereby marking them for destruction by other components of the immune system. Although most B cells live only a few weeks unless stimulated by their corresponding antigen, a few long-lived **memory B cells** can recognize antigen several months or even many years later and can mount a more rapid and massive immune response (called a secondary response) than B cells that have not yet encountered their antigen (Fig. 7-37).

A Antibodies Have Constant and Variable Regions

The immunoglobulins form a related but enormously diverse group of proteins. All immunoglobulins contain at least four subunits: two identical

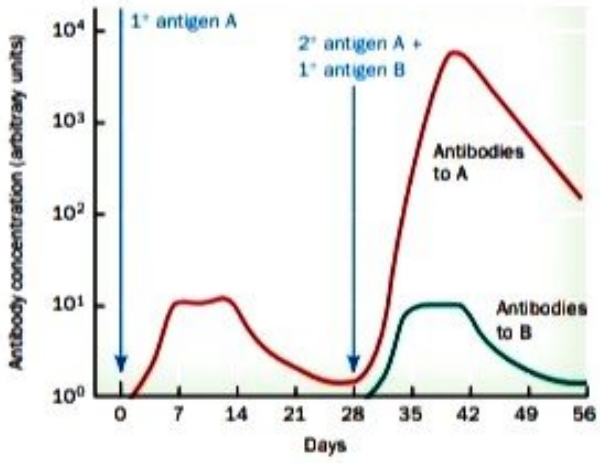


FIG. 7-37 Primary and secondary immune responses. Antibodies to antigen A appear in the blood following primary immunization on day 0 and secondary immunization on day 28. Antigen B is included in the secondary immunization to demonstrate the specificity of immunological memory for antigen A. The secondary response to antigen A is both faster and greater than the primary response.

~23-kD **light chains (L)** and two identical 53- to 75-kD **heavy chains (H).** These subunits associate by disulfide bonds and by noncovalent interactions to form a roughly Y-shaped symmetric molecule with the formula $(LH)_2$ (Fig. 7-38).

The five classes of immunoglobulin **(Ig)** differ in the type of heavy chain they contain and, in some cases, in their subunit structure (Table 7-2). For example, **IgM** consists of five Y-shaped molecules arranged around a central **J subunit; IgA** occurs as monomers, dimers, trimers, and tetramers. The various immunoglobulin classes also have different physiological functions. IgM is most effective against microorganisms and is the first immunoglobulin to be secreted in response to an antigen. **IgG,** the most common immunoglobulin, is equally distributed between the blood and the extravascular fluid. IgA occurs predominantly in the intestinal tract and defends against pathogens by adhering to their antigenic sites so as to block their attachment to epithelial (outer) surfaces. **IgE,** which is normally present in the blood in minute concentrations, protects against parasites and has been implicated in allergic reactions. **IgD,** which is also present in small amounts, has no clearly known function. Our discussion of antibody structure will focus on IgG.

IgG can be cleaved through limited proteolysis with the enzyme **papain** into three ~50-kD fragments: two identical **Fab fragments** and one **Fc fragment.** The Fab fragments are the "arms" of the Y-shaped antibody and contain an en-

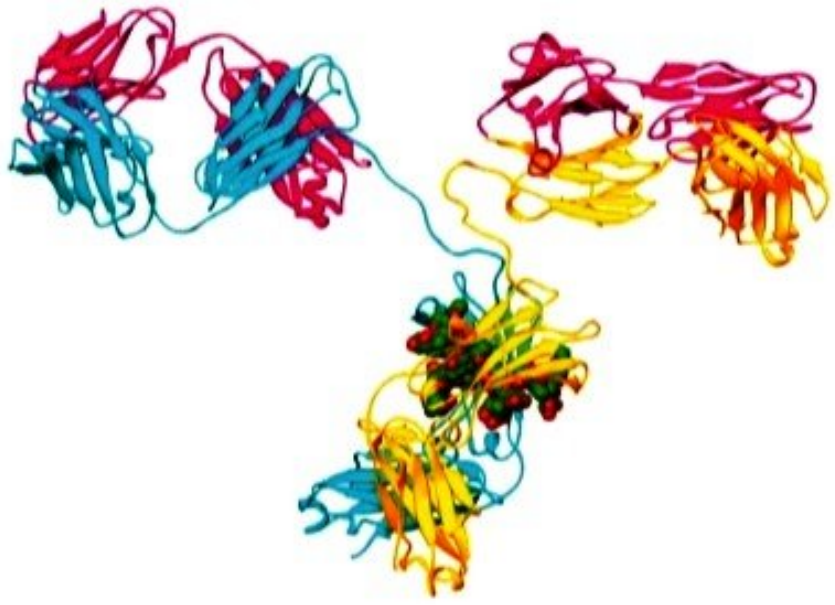


FIG. 7-38 X-Ray structure of an antibody. The protein is shown in ribbon form with its two heavy chains gold and cyan and its two light chains both magenta. Its two identical carbohydrate chains are drawn in space-filling form with C green, N blue, and O red. The antigen-binding sites are located at the ends of the two approximately horizontal Fab arms formed by the association of the light chains with the heavy chains. This particular antibody recognizes canine lymphoma (a type of cancer) and is therapeutically useful against it. [Based on an X-ray structure by Alexander McPherson, University of California at Irvine. PDBid 1IGT.] **See Interactive Exercise 3.**

? How many discrete protein domains are visible in this model?

TABLE 7-2 Classes of Human Immunoglobulins

Class	Heavy Chain	Light Chain	Subunit Structure	Molecular Mass (kD)
IgA	α	κ or λ	$(\alpha_2\kappa_2)_n]^a$ or $(\alpha_2\lambda_2)_n]^a$	180–720
IgD	δ	κ or λ	$\delta_2\kappa_2$ or $\delta_2\lambda_2$	160
IgE	ε	κ or λ	$\varepsilon_2\kappa_2$ or $\varepsilon_2\lambda_2$	190
IgG[b]	γ	κ or λ	$\gamma_2\kappa_2$ or $\gamma_2\lambda_2$	150
IgM	μ	κ or λ	$(\mu_2\kappa_2)_5$J or $(\mu_2\lambda_2)_5$J	950

[a] n = 1, 2, 3, or 4.
[b] IgG has four subclasses, IgG1, IgG2, IgG3, and IgG4, which differ in their γ chains.

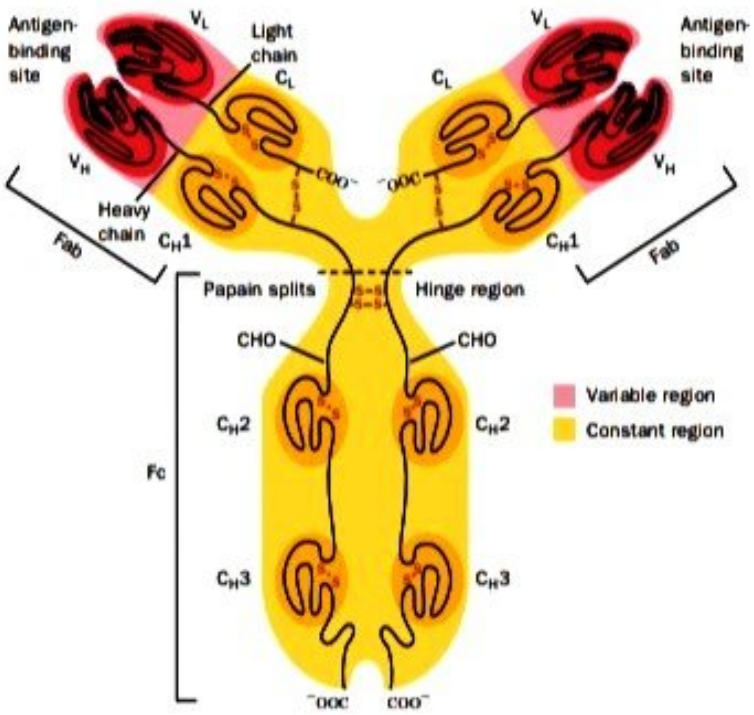


FIG. 7-39 Diagram of human immunoglobulin G (IgG). Each light chain contains a variable (V_L) and a constant (C_L) region, and each heavy chain contains one variable (V_H) and three constant (C_H1, C_H2, and C_H3) regions. Each of the variable and constant domains contains a disulfide bond, and the four polypeptide chains are linked by disulfide bonds. Hypervariable loops, three in each variable domain (*fuzzy lines*), determine antigen specificity. The proteolytic enzyme papain cleaves IgG at the hinge region to yield two Fab fragments and one Fc fragment. CHO represents carbohydrate chains. [Illustration, Irving Geis. Image from Irving Geis Collection/Howard Hughes Medical Institute. Rights owned by HHMI. Reproduction by permission only.]

tire L chain and the N-terminal half of an H chain (Fig. 7-39). These fragments contain IgG's antigen-binding sites (the "ab" in Fab stands for *a*ntigen *b*inding). The Fc portion ("c" because it *c*rystallizes easily) derives from the "stem" of the antibody and consists of the C-terminal halves of two H chains. The arms of the Y are connected to the stem by a flexible hinge region. The hinge angles may vary, so an antibody molecule may not be perfectly symmetrical (e.g., Fig. 7-38).

Although all IgG molecules have the same overall structure, IgGs that recognize different antigens have different amino acid sequences. The light chains of different antibodies differ mostly in their N-terminal halves. These polypeptides are therefore said to have a **variable region, V_L** (residues 1 to ~108), and a **constant region, C_L** (residues 109 to 214). Comparisons of H chains, which have ~446 residues, reveal that H chains also have a variable region, **V_H,** and a constant region, **C_H.** As indicated in Fig. 7-39, the C_H region consists of three ~110-residue segments, **C_H1, C_H2,** and **C_H3,** which are homologous to each other and to C_L. In fact, all the constant and variable regions resemble each other in sequence and in disulfide-bonding pattern. These similarities suggest that the six different homology units of an IgG evolved through the duplication of a primordial gene encoding an ~110-residue protein.

B Antibodies Recognize a Huge Variety of Antigens

The immunoglobulin homology units all have the same characteristic **immunoglobulin fold:** a sandwich composed of three- and four-stranded antiparallel β sheets that are linked by a disulfide bond (Fig. 6-29*b*). Nevertheless, the basic immunoglobulin structure must accommodate an enormous variety of antigens. The ability to recognize antigens resides in three loops in each variable domain (Fig. 7-40). Most of the amino acid variation among antibodies is concentrated in these three short segments, called **hypervariable** sequences. As hypothesized by Elvin Kabat, the hypervariable sequences line an immunoglobulin's antigen-binding site, so that their amino acids determine its binding specificity.

Scientists have determined the X-ray structures of Fab fragments from **monoclonal antibodies** (Box 7-5) and monospecific antibodies isolated from

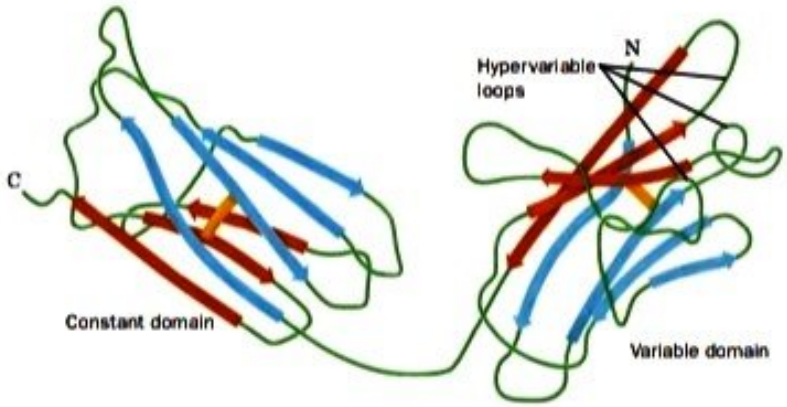


FIG. 7-40 Immunoglobulin folds in a light chain. Both the constant and variable domains consist of a sandwich of a four-stranded antiparallel β sheet (*blue*) and a three-stranded antiparallel β sheet (*orange*) that are linked by a disulfide bond (*yellow*). The positions of the three hypervariable sequences in the variable domain are indicated. [After Schiffer, M., Girling, R.L., Ely, K.R., and Edmundson, A.B., *Biochemistry* **12,** 4628 (1973).]

patients with **multiple myeloma** (a disease in which a cancerous B cell proliferates and produces massive amounts of a single immunoglobulin; immunoglobulins purified from ordinary blood are heterogeneous and hence cannot be used for detailed structural studies). As predicted by the positions of the hypervariable sequences, the antigen-binding site is located at the tip of each Fab fragment in a crevice between its V_L and V_H domains.

The association between antibodies and their antigens involves van der Waals, hydrophobic, hydrogen bonding, and ionic interactions. Their dissociation constants range from 10^{-4} to 10^{-10} M, comparable (or even greater) in strength to the associations between enzymes and their substrates. The specificity and strength of an antigen–antibody complex are a function of the exquisite structural complementarity between the antigen and the antibody (e.g., Fig. 7-41). These are also the features that make antibodies such useful laboratory reagents (Fig. 5-3, for example).

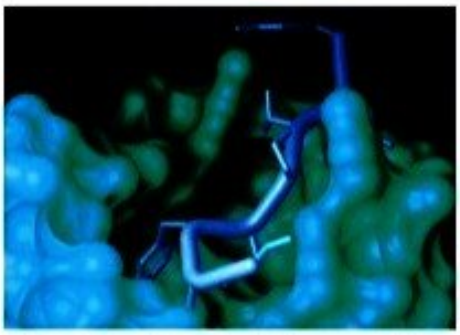


FIG. 7-41 Interaction between an antigen and an antibody. This X-ray structure shows a portion of the solvent-accessible surface of a monoclonal antibody Fab fragment (*green*) with a stick model of a bound nine-residue fragment of its peptide antigen (*lavender*). [Courtesy of Ian Wilson, The Scripps Research Institute, La Jolla, California. PDBid 1HMM.]

Most immunoglobulins are divalent molecules; that is, they can bind two identical antigens simultaneously (IgM and IgA are multivalent). A foreign substance or organism usually has multiple antigenic regions, and a typical immune response generates a mixture of antibodies with different specificities. Divalent binding allows antibodies to cross-link antigens to form an extended lattice (Fig. 7-42), which hastens the removal of the antigen and triggers B cell proliferation.

Antibody Diversity Results from Gene Rearrangement and Mutation. A novel antigen does not direct a B cell to begin manufacturing a new immunoglobulin to which it can bind. Rather, *an antigen stimulates the proliferation of a preexisting B cell whose antibodies happen to recognize the antigen.* The immune system has the potential to produce an enormous number of different antibodies, probably $>10^{18}$. Even though this number is so large that an individual can synthesize only a small fraction of its potential immunoglobulin repertoire during its lifetime, this fraction is still sufficient to react with almost any antigen the individual might encounter. Yet the number of immunoglobulin genes is far too small to account for the observed level of antibody diversity. The diversity in antibody sequences arises instead from genetic changes during B lymphocyte development.

Each chain of an immunoglobulin molecule is encoded by multiple DNA segments: V, J, and C segments for the light chains, and V, D, J, and C segments for heavy chains. These segments are joined together by **somatic**

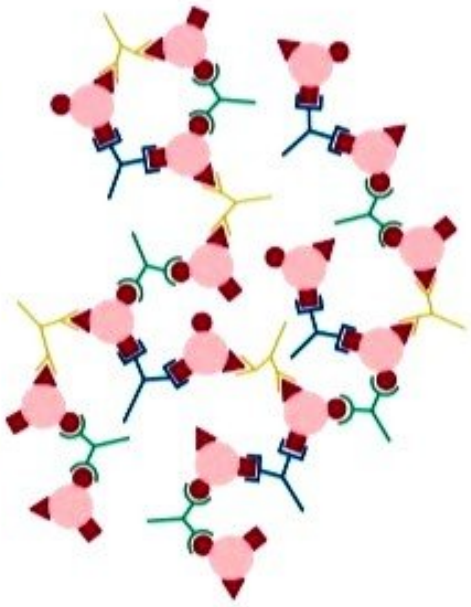


FIG. 7-42 Antigen cross-linking by antibodies. A mixture of divalent antibodies that recognizes the several different antigenic regions of an intruding particle such as a toxin molecule or a bacterium can form an extensive lattice of antigen and antibody molecules.

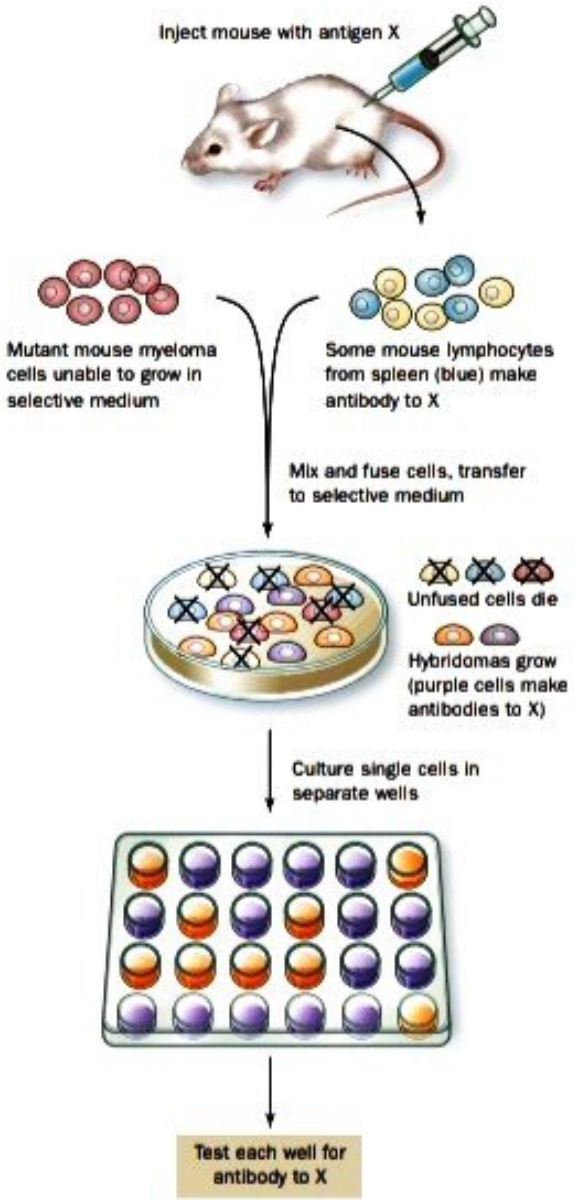
Box 7-5 Perspectives in Biochemistry

Monoclonal Antibodies

Introducing a foreign molecule into an animal induces the synthesis of large amounts of antigen-specific but heterogeneous antibodies. One might expect that a single lymphocyte from such an animal could be cloned (allowed to reproduce) to yield a harvest of homogeneous immunoglobulin molecules. Unfortunately, lymphocytes do not grow continuously in culture. In the late 1970s, however, César Milstein and Georges Köhler developed a technique for immortalizing such cells so that they can grow continuously and secrete virtually unlimited quantities of a specific antibody. Typically, lymphocytes from a mouse that has been immunized with a particular antigen are harvested and fused with mouse myeloma cells (a type of blood system cancer), which can multiply indefinitely (see figure). The cells are then incubated in a selective medium that inhibits the synthesis of purines, which are essential for myeloma growth [the myeloma cells lack the enzyme **hypoxanthine phosphoribosyl transferase (HPRT),** which could otherwise participate in a purine nucleotide salvage pathway; Section 23-1D]. The only cells that can grow in the selective medium are fused cells, known as **hybridoma cells,** that combine the missing HPRT (it is supplied by the lymphocyte) with the immortal attributes of the myeloma cells. Clones derived from single fused cells are then screened for the presence of antibodies to the original antigen. Antibody-producing cells can be grown in large quantities in tissue culture or as semisolid tumors in mouse hosts.

Monoclonal antibodies are used to purify macromolecules (Section 5-2), to identify infectious diseases, and to test for the presence of drugs and other substances in body tissues. Because of their purity and specificity and, to some extent, their biocompatibility, monoclonal antibodies also hold considerable promise as therapeutic agents against cancer and other diseases. In fact, the monoclonal antibody known as **Herceptin** binds specifically to the growth factor receptor **HER2** that is overexpressed in about one-quarter of breast cancers. Herceptin binding to HER2 blocks its growth-signaling activity, thereby causing the tumor to stop growing or even regress.

Other monoclonal antibodies have been developed to bind to and interfere with components of the inflammatory response in order to treat the symptoms of diseases such as rheumatoid arthritis. In some cases, these "biologic" drugs have been genetically engineered to make them less mouselike and more humanlike and therefore less likely to be recognized and rejected as foreign by the patient's own immune system.



recombination during B cell development before being transcribed and translated into protein. The process is called somatic (Greek: *soma*, body) to distinguish it from the recombination that occurs in reproductive cells. Because there are multiple versions of the V, D, J, and C segments in the genome, the combinatorial possibilities are enormous. In addition, the recombination process sometimes adds or deletes nucleotides at the junctions between gene segments, further contributing to the diversity of the encoded protein. The generation of antibody diversity is further discussed in Section 28-3D.

Additional changes can occur after a B cell has encountered its antigen and begun secreting antibody molecules. As the antibody-producing B cells divide, their rate of immunoglobulin gene mutation increases dramatically, favoring the substitution of one nucleotide for another and leading to an average of one

TABLE 7-3 Some Antibody-Mediated Autoimmune Diseases

Disease	Target Tissue	Major Symptoms
Addison's disease	Adrenal cortex	Low blood glucose, muscle weakness, Na^+ loss, K^+ retention, increased susceptibility to stress
Graves' disease	Thyroid gland	Oversecretion of thyroid hormone resulting in increased appetite accompanied by weight loss
Insulin-dependent diabetes mellitus	Pancreatic β cells	Loss of ability to make insulin
Myasthenia gravis	Acetylcholine receptors at nerve–muscle synapses	Progressive muscle weakness
Rheumatoid arthritis	Connective tissue	Inflammation and degeneration of the joints
Systemic lupus erythematosus	DNA, phospholipids, other tissue components	Rash, joint and muscle pain, anemia, kidney damage, mental dysfunction

amino acid change for every cell generation. This process, which is called **somatic hypermutation,** permits the antigen specificity of the antibody to be fine-tuned over many cell generations, because the rate of B cell proliferation increases with the antigen-binding affinity of the antibody it produces.

The Immune System Loses Its Tolerance in Autoimmune Diseases. Another remarkable property of the immune system is that its power is unleashed only against foreign substances and not against any of the tens of thousands of endogenous (self) molecules of various sorts. Virtually all macromolecules are potentially antigenic, as can be demonstrated by transplanting tissues from one individual to another, even within a species. This incompatibility presents obvious challenges for therapies ranging from routine blood transfusions to organ transplants.

The mechanism whereby an individual's immune system distinguishes self from non-self is not completely understood but includes the elimination of self-reactive B and T cells before they are fully mature. Self-tolerance begins to develop around the time of birth and must be ongoing, since new lymphocytes arise throughout an individual's lifetime. Occasionally, the immune system loses tolerance to some of its self-antigens, resulting in an **autoimmune disease.**

All the body's organ systems are theoretically susceptible to attack by an immune system that has lost its self-tolerance, but some tissues are attacked more often than others. Some of the most common antibody-mediated autoimmune diseases are listed in Table 7-3 (other autoimmune diseases result mainly from inappropriate T cell activation). The symptoms of a particular disease reflect the type of tissue with which the autoantibodies react or, in the case of systemic diseases, the accumulation of antigen–antibody complexes in multiple locations. In general, autoimmune diseases are chronic, often with periods of remission, and their clinical severity may differ among individuals.

The loss of tolerance to one's own antigens may result from an innate malfunctioning of the mechanism by which the immune system distinguishes self from non-self, possibly precipitated by an event, such as trauma or infection, in which tissues that are normally sequestered from the immune system are exposed to lymphocytes. For example, breaching the blood–brain barrier may allow lymphocytes access to the brain or spinal cord, and injury may allow access to the spaces at joints, which are not normally served by blood vessels. There is also evidence that some autoimmune diseases are caused by antibodies to certain viral or bacterial antigens that cross-react with endogenous substances because of chance antigenic similarities. Some diseases, such as systemic lupus erythematosus, represent a more generalized breakdown of the immune system, so that antibodies to many endogenous substances (e.g., DNA and phospholipids) may be generated.

CHECKPOINT

- Without looking at Fig. 7-39, draw a diagram of an IgG molecule and identify the heavy and light chains, the constant and variable domains, and the antigen-binding site(s).
- Explain how a single human can potentially generate billions of different antibody molecules.
- What are some factors that might contribute to the development of an autoimmune disease?
- Why do the symptoms of an autoimmune disease often differ between individuals?

Summary

1 Oxygen Binding to Myoglobin and Hemoglobin

- Myoglobin, a monomeric heme-containing muscle protein, reversibly binds a single O_2 molecule.
- Hemoglobin, a tetramer with pseudo-D_2 symmetry, has distinctly different conformations in its oxy and deoxy states.
- Oxygen binds to hemoglobin in a sigmoidal fashion, indicating cooperative binding.
- O_2 binding to a heme group induces a conformational change in the entire hemoglobin molecule that includes movements at the subunit interfaces and the disruption of ion pairs. The result is a shift from the T to the R state.
- CO_2 promotes O_2 dissociation from hemoglobin through the Bohr effect. BPG decreases hemoglobin's O_2 affinity by binding to deoxyhemoglobin.
- The symmetry and sequential models of allosterism explain how binding of a ligand at one site affects binding of another ligand at a different site.
- Hemoglobin variants have revealed structure–function relationships. Hemoglobin S produces the symptoms of sickle-cell anemia by forming rigid fibers in its deoxy form.

2 Muscle Contraction

- The thick filaments of a sarcomere are composed of the motor protein myosin and the thin filaments are composed mainly of actin.
- The heads of myosin molecules in thick filaments form bridges to actin in thin filaments such that the detachment and reattachment of the myosin heads cause the thick and thin filaments to slide past each other during muscle contraction. The contractile force derives from conformational changes in myosin that are triggered by ATP hydrolysis.
- In nonmuscle cells, actin forms microfilaments, which are components of the cytoskeleton. Microfilaments are dynamic structures whose growth and regression are responsible for certain types of cell movement.

3 Antibodies

- The immune system responds to foreign macromolecules through the production of antibodies (immunoglobulins).
- The Y-shaped IgG molecule consists of two heavy and two light chains. The two antigen-binding sites are formed by the hypervariable sequences in the variable domains at the ends of a heavy and a light chain.
- Antibody diversity results from somatic recombination during B cell development and from somatic hypermutation.

Key Terms

heme 178
oxygenation 178
Y_{O_2} 179
pO_2 180
hyperbola 180
saturation 180
p_{50} 180
ligand 180
sigmoidal curve 184
cooperative binding 184
Hill equation 184
Hill constant 185
noncooperative binding 185
positive cooperativity 185
negative cooperativity 185
Perutz mechanism 186
T state 186
R state 186
Bohr effect 189
erythrocyte 189
allosteric interaction 192
symmetry model 192
sequential model 192
variant 193
lyse 194
anemia 194
cyanosis 194
polycythemia 194
heterozygote 194
homozygote 194
sickle-cell anemia 194
striated muscle 196
myofibril 197
sarcomere 197
thick filament 197
thin filament 197
sliding filament model 198
(−) end 202
(+) end 202
motor protein 204
microfilament 206
treadmilling 207
pathogen 208
immune system 208
cellular immunity 208
lymphocyte 208
humoral immunity 208
immunoglobulin (Ig) 208
antigen 208
memory B cell 208
Fab fragment 209
Fc fragment 209
variable region 210
constant region 210
immunoglobulin fold 210
hypervariability 210
monoclonal antibody 210
multiple myeloma 211
somatic recombination 211
somatic hypermutation 213
autoimmune disease 213

Problems

1. Estimate K from the following data describing ligand binding to a protein.

[Ligand] (mM)	Y
0.25	0.30
0.5	0.45
0.8	0.56
1.4	0.66
2.2	0.80
3.0	0.83
4.5	0.86
6.0	0.93

2. Which set of binding data is likely to represent cooperative ligand binding to an oligomeric protein?

(a) [Ligand] (mM)	Y	(b) [Ligand] (mM)	Y
0.1	0.3	0.2	0.1
0.2	0.5	0.3	0.3
0.4	0.7	0.4	0.6
0.7	0.9	0.6	0.8

3. In active muscles, the pO_2 may be 10 torr at the cell surface and 1 torr at the mitochondria (the organelles where oxidative metabolism occurs). Use Eq. 7-6 to show how myoglobin (p_{50} = 2.8 torr) facilitates the diffusion of O_2 through these cells.
4. If myoglobin had the same p_{50} value as hemoglobin, how well would it facilitate O_2 diffusion under the conditions described in Problem 3?
5. Use Eq. 7-8 to estimate the fractional saturation of hemoglobin when pO_2 is (a) 20 torr, (b) 40 torr, and (c) 60 torr.
6. Calculate the p_{50} value for hemoglobin if Y_{O_2} = 0.82 when pO_2 = 25 torr.
7. Drinking a few drops of a commercial preparation called "vitamin O," which consists of oxygen and sodium chloride dissolved in water, is claimed to increase the concentration of oxygen in the body. (a) Use your knowledge of oxygen transport to evaluate this claim. (b) Would vitamin O be more or less effective if it were infused directly into the bloodstream?
8. Explain why long-distance runners prefer to train at high altitude even when the race is to be held at sea level. Why must the runners spend more than a day or two at the higher elevation?
9. Some primitive animals have a hemoglobin that consists of two identical subunits. Sketch an oxygen-binding curve for this protein.
10. What is the likely range of the Hill constant for the hemoglobin described in Problem 9?
11. In humans, the urge to breathe results from high concentrations of CO_2 in the blood; there are no direct physiological sensors of blood pO_2. Skindivers often hyperventilate (breathe rapidly and deeply for several minutes) just before making a dive in the belief that this will increase the O_2 content of their blood. (a) Does it do so? (b) Use your knowledge of hemoglobin function to evaluate whether this practice is useful.
12. The crocodile, which can remain under water without breathing for up to 1 h, drowns its air-breathing prey and then dines at its leisure. An adaptation that aids the crocodile in doing so is that it can utilize virtually 100% of the O_2 in its blood whereas humans, for example, can extract only ~65% of the O_2 in their blood. Crocodile Hb does not bind BPG. However, crocodile deoxyHb preferentially binds HCO_3^-. How does this help the crocodile obtain its dinner?
13. Is the p_{50} higher or lower than normal in (a) hemoglobin Yakima and (b) hemoglobin Kansas? Explain.
14. In hemoglobin Rainier, Tyr 145β is replaced by Cys, which forms a disulfide bond with another Cys residue in the same subunit. This prevents the formation of ion pairs that normally stabilize the T state. How does hemoglobin Rainier differ from normal hemoglobin with respect to (a) oxygen affinity, (b) the Bohr effect, and (c) the Hill constant?
15. Hemoglobin S homozygotes who are severely anemic often have elevated levels of BPG in their erythrocytes. Is this a beneficial effect?
16. In the variant hemoglobin C, glutamate at position 6 of the β chain has been replaced with lysine. (a) Would you expect this mutant hemoglobin to polymerize as hemoglobin S does? (b) Red blood cells containing hemoglobin C have a shorter lifespan than red blood cells containing normal hemoglobin. How might this affect a person's resistance to malaria?
17. Is myosin a fibrous protein or a globular protein? Explain.
18. In striated muscle, cells undergo mitosis (nuclear division) without cytokinesis (cellular division), giving rise to large multinucleate cells. Explain why muscle cells would be less effective if cytokinesis occurred with every round of mitosis.
19. A myosin head can undergo five ATP hydrolysis cycles per second, each of which moves an actin monomer by ~100 Å. How is it possible for an entire sarcomere to shorten by 1000 Å in this same period?
20. **Rigor mortis,** the stiffening of muscles after death, is caused by depletion of cellular ATP. Describe the molecular basis of rigor.
21. Explain why a microfilament is polar whereas a filament of keratin is not.
22. Cells contain an assortment of proteins that promote microfilament disassembly during cell shape changes. How can such proteins distinguish newly synthesized microfilaments from older microfilaments?
23. Give the approximate molecular masses of an immunoglobulin G molecule analyzed by (a) gel filtration chromatography, (b) SDS-PAGE, and (c) SDS-PAGE in the presence of 2-mercaptoethanol.
24. Many fish produce a tetrameric IgM. If each H chain has a mass of 70 kD, each light chain has a mass of 25 kD, and each J chain has a mass of 15 kD, what is the approximate mass of the IgM molecule?
25. Explain why the variation in V_L and V_H domains of immunoglobulins is largely confined to the hypervariable loops.
26. How many hypervariable loops are present in (a) IgG and (b) IgM?
27. Why do antibodies raised against a native protein sometimes fail to bind to the corresponding denatured protein?
28. Antibodies raised against a macromolecular antigen usually produce an antigen–antibody precipitate when mixed with that antigen. Explain why no precipitate forms when (a) Fab fragments from

those antibodies are mixed with the antigen; (b) antibodies raised against a small antigen are mixed with that small antigen; and (c) the antibody is in great excess over the antigen and vice versa.

29. Some bacteria produce proteases that can cleave the hinge region of IgA molecules without affecting antigen binding. Explain why these proteases would give the bacteria a better chance of starting an infection.

30. Individuals with systemic lupus erythematosus (SLE) produce antibodies to DNA and phospholipids. (a) Explain why normal individuals do not make antibodies to these substances. (b) During a normal response to a viral or bacterial infection, the immune system produces large amounts of antigen-specific antibodies, and the resulting antigen–antibody complexes are subsequently removed from the circulation and degraded. Explain why antigen–antibody complexes accumulate in the tissues of individuals with SLE.

CASE STUDIES

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Case 8 Hemoglobin, the Oxygen Carrier

Focus concept: A mutation in the gene for hemoglobin results in an altered protein responsible for the disease sickle-cell anemia. An understanding of the biochemistry of the disease may suggest possible treatments.

Prerequisite: Chapter 7

- Hemoglobin structure and function

Case 9 Allosteric Interactions in Crocodile Hemoglobin

Focus concept: The effect of allosteric modulators on oxygen affinity for crocodile hemoglobin differs from that of other species.

Prerequisite: Chapter 7

- Hemoglobin structure and function

Case 10 The Biological Roles of Nitric Oxide

Focus concept: Nitric oxide, a small lipophilic molecule, acts as a second messenger in blood vessels.

Prerequisite: Chapter 7

- Hemoglobin structure and function

MORE TO EXPLORE (a) Look up information about hemoglobin variants. Why don't they generate the same symptoms? How do the symptoms of thalassemias differ? Which hemoglobin variants appear to offer a selective advantage under certain conditions? (b) In addition to myosin, which interacts with actin filaments, cells contain several other motor protein systems. Describe the structure and activity of the motor proteins kinesin and dynein. Against what fibrous proteins do they exert force? How do these systems differ from the actin–myosin system? (c) Explain how immunological memory is exploited in the development of vaccines for viral and bacterial infections. What factors make a vaccine most effective in preventing disease?

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