

Protein Function

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Knowing the three-dimensional structure of a protein is an important part of understanding how the protein functions. However, the structure shown in two dimensions on a page is deceptively static. Proteins are dynamic molecules whose functions almost invariably depend on interactions with other molecules, and these interactions are affected in physiologically important ways by sometimes subtle, sometimes striking changes in protein conformation. In this chapter, we explore how proteins interact with other molecules and how their interactions are related to dynamic protein structure. The importance of molecular interactions to a protein's function can hardly be overemphasized. In Chapter 4, we saw that the function of fibrous proteins as structural elements of cells and tissues depends on stable, long-term quaternary interactions between identical polypeptide chains. As we shall see in this chapter, the functions of many other proteins involve interactions with a variety of different molecules. Most of these interactions are fleeting, though they may be the basis of complex physiological processes such as oxygen transport, immune function, and muscle contraction—the topics we examine in this chapter. The proteins that carry out these processes illustrate the following key principles of protein function, some of which will be familiar from the previous chapter:

The functions of many proteins involve the reversible binding of other molecules. A molecule bound reversibly by a protein is called a **ligand**. A ligand may be any kind of molecule, including another protein. The transient nature of protein-ligand interactions is critical to life, allowing an organism

to respond rapidly and reversibly to changing environmental and metabolic circumstances.

A ligand binds at a site on the protein called the **binding site**, which is complementary to the ligand in size, shape, charge, and hydrophobic or hydrophilic character. Furthermore, the interaction is specific: the protein can discriminate among the thousands of different molecules in its environment and selectively bind only one or a few. A given protein may have separate binding sites for several different ligands. These specific molecular interactions are crucial in maintaining the high degree of order in a living system. (This discussion excludes the binding of water, which may interact weakly and nonspecifically with many parts of a protein. In Chapter 6, we consider water as a specific ligand for many enzymes.)

Proteins are flexible. Changes in conformation may be subtle, reflecting molecular vibrations and small movements of amino acid residues throughout the protein. A protein flexing in this way is sometimes said to “breathe.” Changes in conformation may also be quite dramatic, with major segments of the protein structure moving as much as several nanometers. Specific conformational changes are frequently essential to a protein's function.

The binding of a protein and ligand is often coupled to a conformational change in the protein that makes the binding site more complementary to the ligand, permitting tighter binding. The structural adaptation that occurs between protein and ligand is called **induced fit**.

In a multisubunit protein, a conformational change in one subunit often affects the conformation of other subunits.

Interactions between ligands and proteins may be regulated, usually through specific interactions with one or more additional ligands. These other ligands may cause conformational changes in the protein that affect the binding of the first ligand.

Enzymes represent a special case of protein function. Enzymes bind and chemically transform other molecules—they catalyze reactions. The molecules acted upon by enzymes are called reaction **substrates** rather than ligands, and the ligand-binding site is called the **catalytic site** or **active site**. In this chapter we emphasize the noncatalytic functions of proteins. In Chapter 6 we consider catalysis by enzymes, a central topic in biochemistry. You will see that the themes of this chapter—binding, specificity, and conformational change—are continued in the next chapter, with the added element of proteins participating in chemical transformations.

5.1 Reversible Binding of a Protein to a Ligand: Oxygen-Binding Proteins

Myoglobin and hemoglobin may be the most-studied and best-understood proteins. They were the first proteins for which three-dimensional structures were determined, and these two molecules illustrate almost every aspect of that most central of biochemical processes: the reversible binding of a ligand to a protein. This classic model of protein function tells us a great deal about how proteins work.  **Oxygen-Binding Proteins—Myoglobin: Oxygen Storage**

Oxygen Can Bind to a Heme Prosthetic Group

Oxygen is poorly soluble in aqueous solutions (see Table 2–3) and cannot be carried to tissues in sufficient quantity if it is simply dissolved in blood serum. Diffusion of oxygen through tissues is also ineffective over distances greater than a few millimeters. The evolution of larger, multicellular animals depended on the evolution of proteins that could transport and store oxygen. However, none of the amino acid side chains in proteins

are suited for the reversible binding of oxygen molecules. This role is filled by certain transition metals, among them iron and copper, that have a strong tendency to bind oxygen. Multicellular organisms exploit the properties of metals, most commonly iron, for oxygen transport. However, free iron promotes the formation of highly reactive oxygen species such as hydroxyl radicals that can damage DNA and other macromolecules. Iron used in cells is therefore bound in forms that sequester it and/or make it less reactive. In multicellular organisms—especially those in which iron, in its oxygen-carrying capacity, must be transported over large distances—iron is often incorporated into a protein-bound prosthetic group called **heme** (or haem). (Recall from Chapter 3 that a prosthetic group is a compound permanently associated with a protein that contributes to the protein's function.)

Heme consists of a complex organic ring structure, **protoporphyrin**, to which is bound a single iron atom in its ferrous (Fe^{2+}) state (**Fig. 5–1**). The iron atom has six coordination bonds, four to nitrogen atoms that are part of the flat **porphyrin ring** system and two perpendicular to the porphyrin. The coordinated nitrogen atoms (which have an electron-donating character) help prevent conversion of the heme iron to the ferric (Fe^{3+}) state. Iron in the Fe^{2+} state binds oxygen reversibly; in the Fe^{3+} state it does not bind oxygen. Heme is found in many oxygen-transporting proteins, as well as in some proteins, such as the cytochromes, that participate in oxidation-reduction (electron-transfer) reactions (Chapter 19).

Free heme molecules (heme not bound to protein) leave Fe^{2+} with two “open” coordination bonds. Simultaneous reaction of one O_2 molecule with two free heme molecules (or two free Fe^{2+}) can result in irreversible conversion of Fe^{2+} to Fe^{3+} . In heme-containing proteins, this reaction is prevented by sequestering each

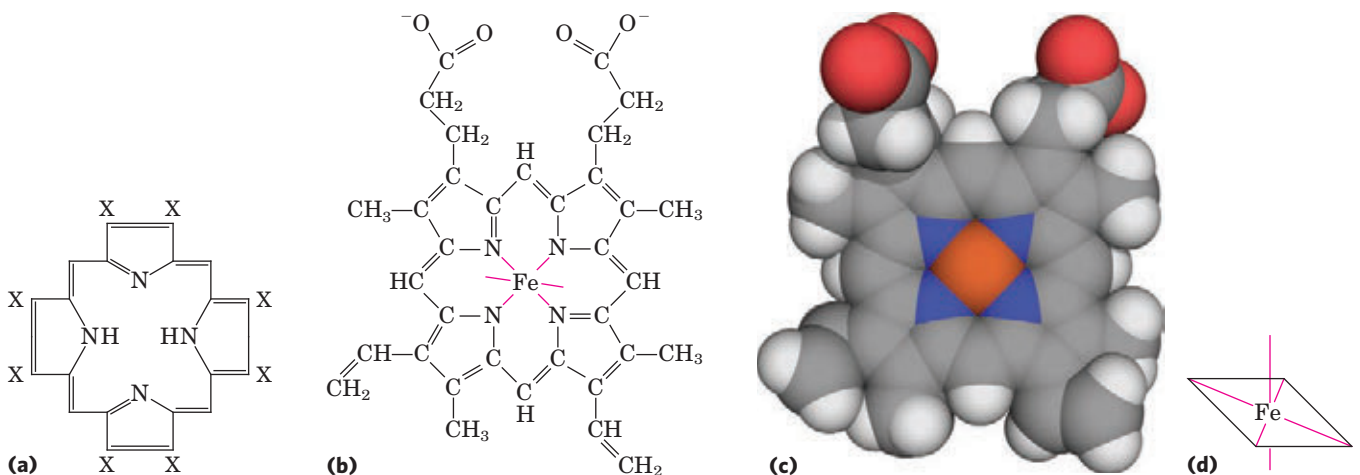


FIGURE 5–1 Heme. The heme group is present in myoglobin, hemoglobin, and many other proteins, designated heme proteins. Heme consists of a complex organic ring structure, protoporphyrin IX, with a bound iron atom in its ferrous (Fe^{2+}) state. **(a)** Porphyrins, of which protoporphyrin IX is only one example, consist of four pyrrole rings linked by

methine bridges, with substitutions at one or more of the positions denoted X. **(b, c)** Two representations of heme (derived from PDB ID 1CCR). The iron atom of heme has six coordination bonds: four in the plane of, and bonded to, the flat porphyrin ring system, and **(d)** two perpendicular to it.

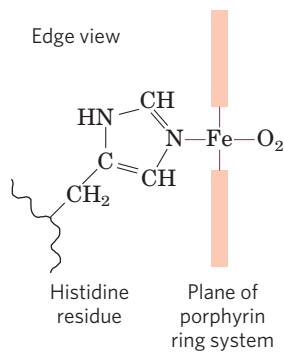


FIGURE 5-2 The heme group viewed from the side. This view shows the two coordination bonds to Fe^{2+} that are perpendicular to the porphyrin ring system. One is occupied by a His residue, sometimes called the proximal His; the other is the binding site for oxygen. The remaining four coordination bonds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

heme deep within the protein structure. Thus, access to the two open coordination bonds is restricted. One of these two coordination bonds is occupied by a side-chain nitrogen of a His residue. The other is the binding site for molecular oxygen (O_2) (**Fig. 5-2**). When oxygen binds, the electronic properties of heme iron change; this accounts for the change in color from the dark purple of oxygen-depleted venous blood to the bright red of oxygen-rich arterial blood. Some small molecules, such as carbon monoxide (CO) and nitric oxide (NO), coordinate to heme iron with greater affinity than does O_2 . When a molecule of CO is bound to heme, O_2 is excluded, which is why CO is highly toxic to aerobic organisms (a topic explored later, in Box 5-1). By surrounding and sequestering heme, oxygen-binding proteins regulate the access of CO and other small molecules to the heme iron.

Globins Are a Family of Oxygen-Binding Proteins

The **globins** are a widespread family of proteins, all having similar primary and tertiary structures. Globins are commonly found in eukaryotes of all classes and even in some bacteria. Most function in oxygen transport or storage, although some play a role in the sensing of oxygen, nitric oxide, or carbon monoxide. The simple nematode worm *Caenorhabditis elegans* has genes encoding 33 different globins. In humans and other mammals, there are at least four kinds of globins. The monomeric myoglobin facilitates oxygen diffusion in muscle tissue. Myoglobin is particularly abundant in the muscles of diving marine mammals such as seals and whales, where it also has an oxygen-storage function for prolonged excursions undersea. The tetrameric hemoglobin is responsible for oxygen transport in the blood stream. The monomeric neuroglobin is expressed largely in neurons and helps to protect the brain from hypoxia (low oxygen) or ischemia (restricted blood supply). Cytochrome, another monomeric globin, is found at high levels in a range of tissues, but its function is unknown.

Myoglobin Has a Single Binding Site for Oxygen

Myoglobin (M_r 16,700; abbreviated Mb) is a single polypeptide of 153 amino acid residues with one molecule of heme. As is typical for a globin polypeptide, myoglobin is made up of eight α -helical segments connected by bends (**Fig. 5-3**). About 78% of the amino acid residues in the protein are found in these α helices.

Any detailed discussion of protein function inevitably involves protein structure. In the case of myoglobin, we first introduce some structural conventions peculiar to globins. As seen in Figure 5-3, the helical segments are named A through H. An individual amino acid residue is designated either by its position in the amino acid sequence or by its location in the sequence of a particular α -helical segment. For example, the His residue coordinated to the heme in myoglobin, His⁹³ (the 93rd residue from the amino-terminal end of the myoglobin polypeptide sequence), is also called His F8 (the 8th residue in α helix F). The bends in the structure are designated AB, CD, EF, FG, and so forth, reflecting the α -helical segments they connect.

Protein-Ligand Interactions Can Be Described Quantitatively

The function of myoglobin depends on the protein's ability not only to bind oxygen but also to release it when and where it is needed. Function in biochemistry

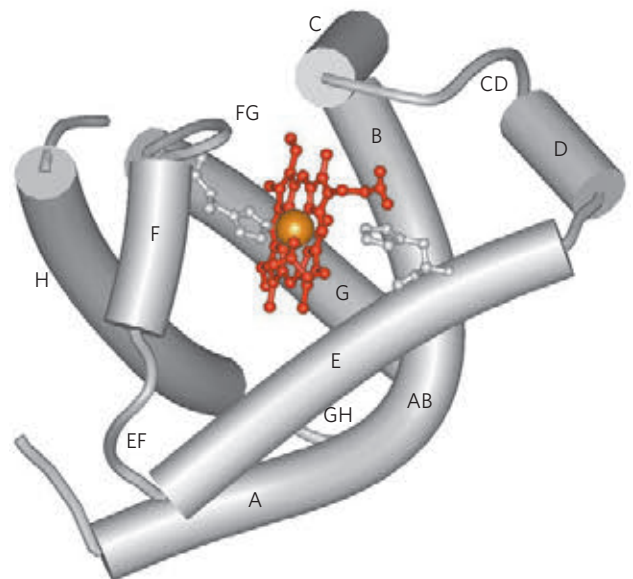


FIGURE 5-3 Myoglobin. (PDB ID 1MBO) The eight α -helical segments (shown here as cylinders) are labeled A through H. Nonhelical residues in the bends that connect them are labeled AB, CD, EF, and so forth, indicating the segments they interconnect. A few bends, including BC and DE, are abrupt and do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

often revolves around a reversible protein-ligand interaction of this type. A quantitative description of this interaction is therefore a central part of many biochemical investigations.

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



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

$$K_a = \frac{[PL]}{[P][L]} = \frac{k_a}{k_d} \quad (5-2)$$

where k_a and k_d are rate constants (more on these below). The term K_a is an **association constant** (not to be confused with the K_a that denotes an acid dissociation constant; p. 62) that describes the equilibrium between the complex and the unbound components of the complex. The association constant provides a measure of the affinity of the ligand L for the protein. K_a has units of M^{-1} ; a higher value of K_a corresponds to a higher affinity of the ligand for the protein.

The equilibrium term K_a is also equivalent to the ratio of the rates of the forward (association) and reverse (dissociation) reactions that form the PL complex. The association rate is described by a rate constant k_a , and dissociation by the rate constant k_d . As discussed further in the next chapter, rate constants are proportionality constants, describing the fraction of a pool of reactant that reacts in a given amount of time. When the reaction involves one molecule, such as the dissociation reaction $PL \rightarrow P + L$, the reaction is *first order* and the rate constant (k_d) has units of reciprocal time (s^{-1}). When the reaction involves two molecules, such as the association reaction $P + L \rightarrow PL$, it is called *second order*, and its rate constant (k_a) has units of $M^{-1} s^{-1}$.

KEY CONVENTION: Equilibrium constants are denoted with a capital K and rate constants with a lower case k . ■

A rearrangement of the first part of Equation 5-2 shows that the ratio of bound to free protein is directly proportional to the concentration of free ligand:

$$K_a[L] = \frac{[PL]}{[P]} \quad (5-3)$$

When the concentration of the ligand is much greater than the concentration of ligand-binding sites, the binding of the ligand by the protein does not appreciably change the concentration of free (unbound) ligand—that is, $[L]$ remains constant. This condition is broadly applicable to most ligands that bind to proteins in cells and simplifies our description of the binding equilibrium.

We can now consider the binding equilibrium from the standpoint of the fraction, θ (theta), of ligand-

binding sites on the protein that are occupied by ligand:

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

Substituting $K_a[L][P]$ for $[PL]$ (see Eqn 5-3) and rearranging terms gives

$$\theta = \frac{K_a[L][P]}{K_a[L][P] + [P]} = \frac{K_a[L]}{K_a[L] + 1} = \frac{[L]}{[L] + \frac{1}{K_a}} \quad (5-5)$$

The value of K_a can be determined from a plot of θ versus the concentration of free ligand, $[L]$ (**Fig. 5-4a**). Any equation of the form $x = y/(y + z)$ describes a hyperbola, and θ is thus found to be a hyperbolic function of $[L]$. The fraction of ligand-binding sites occupied approaches saturation asymptotically as $[L]$ increases. The $[L]$ at which half of the available ligand-binding sites are occupied (that is, $\theta = 0.5$) corresponds to $1/K_a$.

It is more common (and intuitively simpler), however, to consider the **dissociation constant**, K_d , which is the reciprocal of K_a ($K_d = 1/K_a$) and is given in units of molar concentration (M). K_d is the equilibrium constant

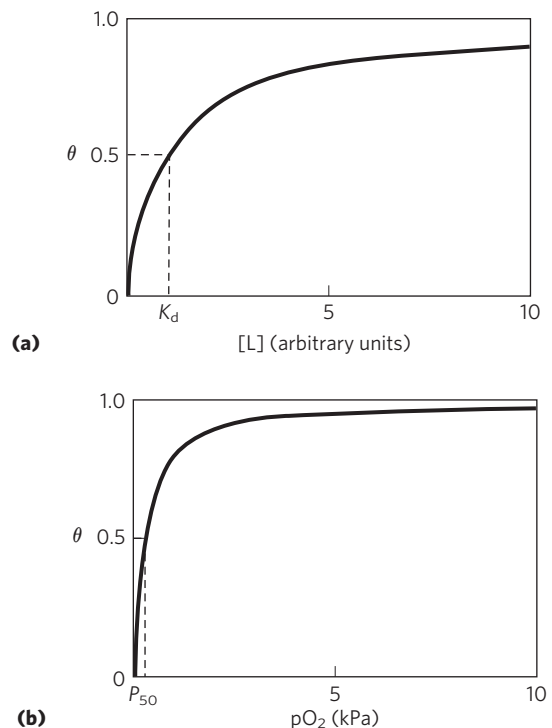
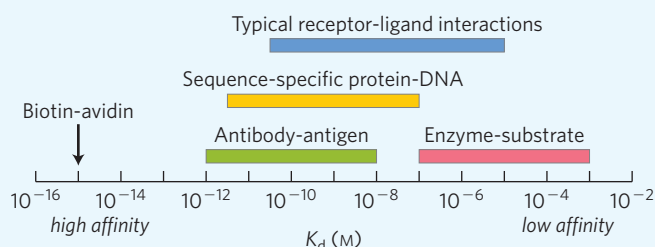


FIGURE 5-4 Graphical representations of ligand binding. The fraction of ligand-binding sites occupied, θ , is plotted against the concentration of free ligand. Both curves are rectangular hyperbolas. **(a)** A hypothetical binding curve for a ligand L. The $[L]$ at which half of the available ligand-binding sites are occupied is equivalent to $1/K_a$, or K_d . The curve has a horizontal asymptote at $\theta = 1$ and a vertical asymptote (not shown) at $[L] = -1/K_a$. **(b)** A curve describing the binding of oxygen to myoglobin. The partial pressure of O_2 in the air above the solution is expressed in kilopascals (kPa). Oxygen binds tightly to myoglobin, with a P_{50} of only 0.26 kPa.

TABLE 5-1 Some Protein Dissociation Constants

Protein	Ligand	K_d (M)*
Avidin (egg white)	Biotin	1×10^{-15}
Insulin receptor (human)	Insulin	1×10^{-10}
Anti-HIV immunoglobulin (human) [†]	gp41 (HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (<i>E. coli</i>)	Ni^{2+}	1×10^{-7}
Calmodulin (rat) [‡]	Ca^{2+}	3×10^{-6}
		2×10^{-5}



Color bars indicate the range of dissociation constants typical of various classes of interactions in biological systems. A few interactions, such as that between the protein avidin and the enzyme cofactor biotin, fall outside the normal ranges. The avidin-biotin interaction is so tight it may be considered irreversible. Sequence-specific protein-DNA interactions reflect proteins that bind to a particular sequence of nucleotides in DNA, as opposed to general binding to any DNA site.

*A reported dissociation constant is valid only for the particular solution conditions under which it was measured. K_d values for a protein-ligand interaction can be altered, sometimes by several orders of magnitude, by changes in the solution's salt concentration, pH, or other variables.

[†]This immunoglobulin was isolated as part of an effort to develop a vaccine against HIV. Immunoglobulins (described later in the chapter) are highly variable, and the K_d reported here should not be considered characteristic of all immunoglobulins.

[‡]Calmodulin has four binding sites for calcium. The values shown reflect the highest- and lowest-affinity binding sites observed in one set of measurements.

for the release of ligand. The relevant expressions change to

$$K_d = \frac{[P][L]}{[PL]} = \frac{k_d}{k_a} \quad (5-6)$$

$$[PL] = \frac{[P][L]}{K_d} \quad (5-7)$$

$$\theta = \frac{[L]}{[L] + K_d} \quad (5-8)$$

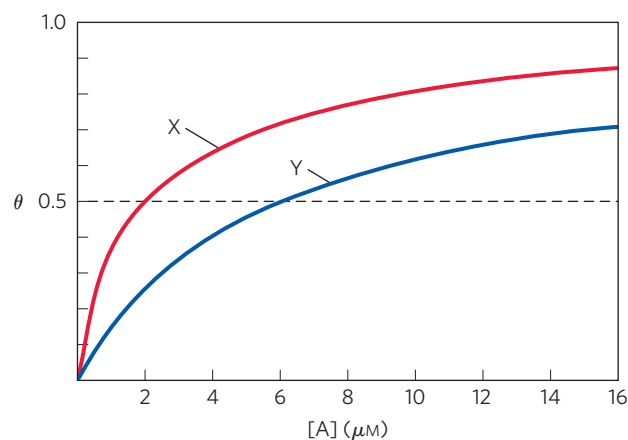
When $[L]$ equals K_d , half of the ligand-binding sites are occupied. As $[L]$ falls below K_d , progressively less of the protein has ligand bound to it. In order for 90% of the available ligand-binding sites to be occupied, $[L]$ must be nine times greater than K_d .

In practice, K_d is used much more often than K_a to express the affinity of a protein for a ligand. Note that a lower value of K_d corresponds to a higher affinity of ligand for the protein. The mathematics can be reduced to simple statements: K_d is equivalent to the molar concentration of ligand at which half of the available ligand-binding sites are occupied. At this point, the protein is said to have reached half-saturation with respect to ligand binding. The more tightly a protein binds a ligand, the lower the concentration of ligand required for half

the binding sites to be occupied, and thus the lower the value of K_d . Some representative dissociation constants are given in Table 5-1; the scale shows typical ranges for dissociation constants found in biological systems.

WORKED EXAMPLE 5-1 Receptor-Ligand Dissociation Constants

Two proteins, X and Y, bind to the same ligand, A, with the binding curves shown below.



What is the dissociation constant, K_d , for each protein? Which protein (X or Y) has a greater affinity for ligand A?

Solution: We can determine the dissociation constants by inspecting the graph. Since θ represents the fraction of binding sites occupied by ligand, the concentration of ligand at which half the binding sites are occupied—that is, the point where the binding curve crosses the line where $\theta = 0.5$ —is the dissociation constant. For X, $K_d = 2 \mu\text{M}$; for Y, $K_d = 6 \mu\text{M}$. Because X is half-saturated at a lower $[A]$, it has a higher affinity for the ligand.

The binding of oxygen to myoglobin follows the patterns discussed above. However, because oxygen is a gas, we must make some minor adjustments to the equations so that laboratory experiments can be carried out more conveniently. We first substitute the concentration of dissolved oxygen for $[L]$ in Equation 5–8 to give

$$\theta = \frac{[\text{O}_2]}{[\text{O}_2] + K_d} \quad (5-9)$$

As for any ligand, K_d equals the $[\text{O}_2]$ at which half of the available ligand-binding sites are occupied, or $[\text{O}_2]_{0.5}$. Equation 5–9 thus becomes

$$\theta = \frac{[\text{O}_2]}{[\text{O}_2] + [\text{O}_2]_{0.5}} \quad (5-10)$$

In experiments using oxygen as a ligand, it is the partial pressure of oxygen ($p\text{O}_2$) in the gas phase above the solution that is varied, because this is easier to measure than the concentration of oxygen dissolved in the solution. The concentration of a volatile substance in solution is always proportional to the local partial pressure of the gas. So, if we define the partial pressure of oxygen at $[\text{O}_2]_{0.5}$ as P_{50} , substitution in Equation 5–10 gives

$$\theta = \frac{p\text{O}_2}{p\text{O}_2 + P_{50}} \quad (5-11)$$

A binding curve for myoglobin that relates θ to $p\text{O}_2$ is shown in Figure 5–4b.

Protein Structure Affects How Ligands Bind

The binding of a ligand to a protein is rarely as simple as the above equations would suggest. The interaction is greatly affected by protein structure and is often accompanied by conformational changes. For example, the specificity with which heme binds its various ligands is altered when the heme is a component of myoglobin. Carbon monoxide binds to free heme molecules more than 20,000 times better than does O_2 (that is, the K_d or P_{50} for CO binding to free heme is more than 20,000 times lower than that for O_2), but it binds only about 200 times better than O_2 when the heme is bound in myoglobin. The difference may be partly explained by steric hindrance. When O_2 binds to free heme, the axis of the oxygen molecule is positioned at an angle to the Fe—O bond (Fig. 5–5a). In contrast, when CO binds to free heme, the Fe, C, and O atoms lie in a straight line

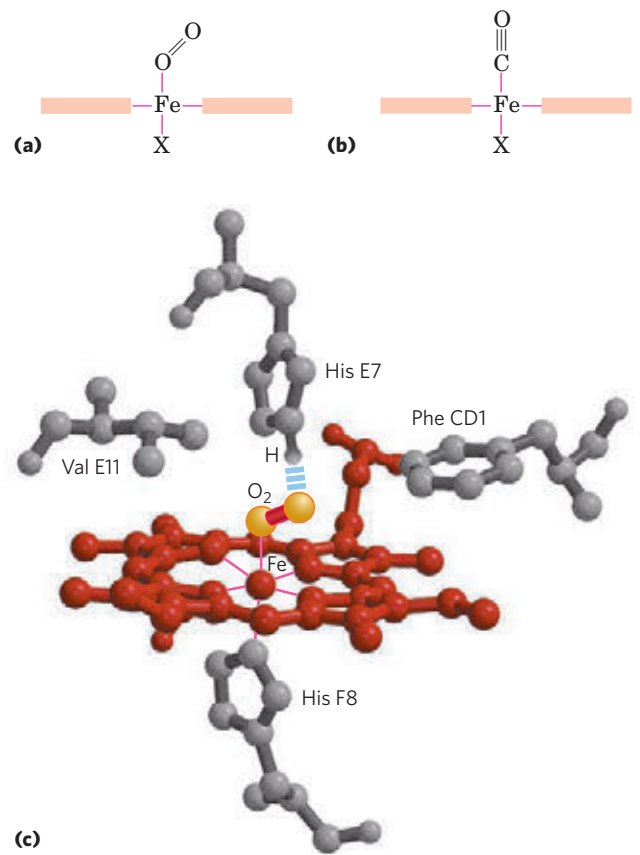


FIGURE 5-5 Steric effects caused by ligand binding to the heme of myoglobin. (a) Oxygen binds to heme with the O_2 axis at an angle, a binding conformation readily accommodated by myoglobin. (b) Carbon monoxide binds to free heme with the CO axis perpendicular to the plane of the porphyrin ring. When binding to the heme in myoglobin, CO is forced to adopt a slight angle because the perpendicular arrangement is sterically blocked by His E7, the distal His. This effect weakens the binding of CO to myoglobin. (c) Another view of the heme of myoglobin (derived from PDB ID 1MBO), showing the arrangement of key amino acid residues around the heme. The bound O_2 is hydrogen-bonded to the distal His, His E7 (His⁶⁴), further facilitating the binding of O_2 .

(Fig. 5–5b). In both cases, the binding reflects the geometry of hybrid orbitals in each ligand. In myoglobin, His⁶⁴ (His E7), on the O_2 -binding side of the heme, is too far away to coordinate with the heme iron, but it does interact with a ligand bound to heme. This residue, called the *distal His* (as distinct from the *proximal His*, His F8), forms a hydrogen bond with O_2 (Fig. 5–5c) but may help preclude the linear binding of CO, providing one explanation for the selectively diminished binding of CO to heme in myoglobin (and hemoglobin). A reduction in CO binding is physiologically important, because CO is a low-level byproduct of cellular metabolism. Other factors, not yet well-defined, also may modulate the interaction of heme with CO in these proteins.

The binding of O_2 to the heme in myoglobin also depends on molecular motions, or “breathing,” in the protein structure. The heme molecule is deeply buried in the folded polypeptide, with no direct path for oxygen to move from the surrounding solution to the ligand-binding site. If the protein were rigid, O_2 could

not enter or leave the heme pocket at a measurable rate. However, rapid molecular flexing of the amino acid side chains produces transient cavities in the protein structure, and O_2 makes its way in and out by moving through these cavities. Computer simulations of rapid structural fluctuations in myoglobin suggest that there are many such pathways. One major route is provided by rotation of the side chain of the distal His (His⁶⁴), which occurs on a nanosecond (10^{-9} s) time scale. Even subtle conformational changes can be critical for protein activity.

In neuroglobin, cytoglobin, and some globins found in plants and invertebrates, the distal His (His E7) is directly coordinated with the heme iron. In these globins, the oxygen or other ligand must displace the distal His in the process of binding.

Hemoglobin Transports Oxygen in Blood

Oxygen-Binding Proteins—Hemoglobin: Oxygen Transport Nearly all the oxygen carried by whole blood in animals is bound and transported by hemoglobin in erythrocytes (red blood cells). Normal human erythrocytes are small (6 to 9 μm in diameter), biconcave disks. They are formed from precursor stem cells called **hemocytoblasts**. In the maturation process, the stem cell produces daughter cells that form large amounts of hemoglobin and then lose their intracellular organelles—nucleus, mitochondria, and endoplasmic reticulum. Erythrocytes are thus incomplete, vestigial cells, unable to reproduce and, in humans, destined to survive for only about 120 days. Their main function is to carry hemoglobin, which is dissolved in the cytosol at a very high concentration (~34% by weight).

In arterial blood passing from the lungs through the heart to the peripheral tissues, hemoglobin is about 96% saturated with oxygen. In the venous blood returning to the heart, hemoglobin is only about 64% saturated. Thus, each 100 mL of blood passing through a tissue releases about one-third of the oxygen it carries, or 6.5 mL of O_2 gas at atmospheric pressure and body temperature.

Myoglobin, with its hyperbolic binding curve for oxygen (Fig. 5-4b), is relatively insensitive to small changes in the concentration of dissolved oxygen and so functions well as an oxygen-storage protein. Hemoglobin, with its multiple subunits and O_2 -binding sites, is better suited to oxygen transport. As we shall see, interactions between the subunits of a multimeric protein can permit a highly sensitive response to small changes in ligand concentration. Interactions among the subunits in hemoglobin cause conformational changes that alter the affinity of the protein for oxygen. The modulation of oxygen binding allows the O_2 -transport protein to respond to changes in oxygen demand by tissues.

Hemoglobin Subunits Are Structurally Similar to Myoglobin

Hemoglobin (M_r 64,500; abbreviated Hb) is roughly spherical, with a diameter of nearly 5.5 nm. It is a tetrameric protein containing four heme prosthetic groups,

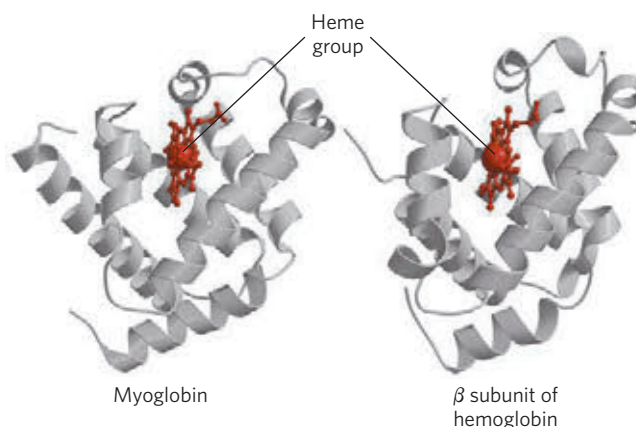


FIGURE 5-6 Comparison of the structures of myoglobin (PDB ID 1MBO) and the β subunit of hemoglobin (derived from PDB ID 1HGA).

one associated with each polypeptide chain. Adult hemoglobin contains two types of globin, two α chains (141 residues each) and two β chains (146 residues each). Although fewer than half of the amino acid residues are identical in the polypeptide sequences of the α and β subunits, the three-dimensional structures of the two types of subunits are very similar. Furthermore, their structures are very similar to that of myoglobin (Fig. 5-6), even though the amino acid sequences of the three polypeptides are identical at only 27 positions (Fig. 5-7). All three polypeptides are members of the globin family of proteins. The helix-naming convention described for myoglobin is also applied to the hemoglobin polypeptides, except that the α subunit lacks the short D helix. The heme-binding pocket is made up largely of the E and F helices in each of the subunits.

The quaternary structure of hemoglobin features strong interactions between unlike subunits. The $\alpha_1\beta_1$ interface (and its $\alpha_2\beta_2$ counterpart) involves more than 30 residues, and its interaction is sufficiently strong that although mild treatment of hemoglobin with urea tends to disassemble the tetramer into $\alpha\beta$ dimers, these dimers remain intact. The $\alpha_1\beta_2$ (and $\alpha_2\beta_1$) interface involves 19 residues (Fig. 5-8). Hydrophobic interactions predominate at all the interfaces, but there are also many hydrogen bonds and a few ion pairs (or salt bridges), whose importance is discussed below.

Hemoglobin Undergoes a Structural Change on Binding Oxygen

X-ray analysis has revealed two major conformations of hemoglobin: the **R state** and the **T state**. Although oxygen binds to hemoglobin in either state, it has a significantly higher affinity for hemoglobin in the R state. Oxygen binding stabilizes the R state. When oxygen is absent experimentally, the T state is more stable and is thus the predominant conformation of **deoxyhemoglobin**. T and R originally denoted “tense” and “relaxed,” respectively, because the T state is stabilized by a greater number of ion pairs, many of which lie at the $\alpha_1\beta_2$ (and $\alpha_2\beta_1$) interface (Fig. 5-9). The binding of O_2

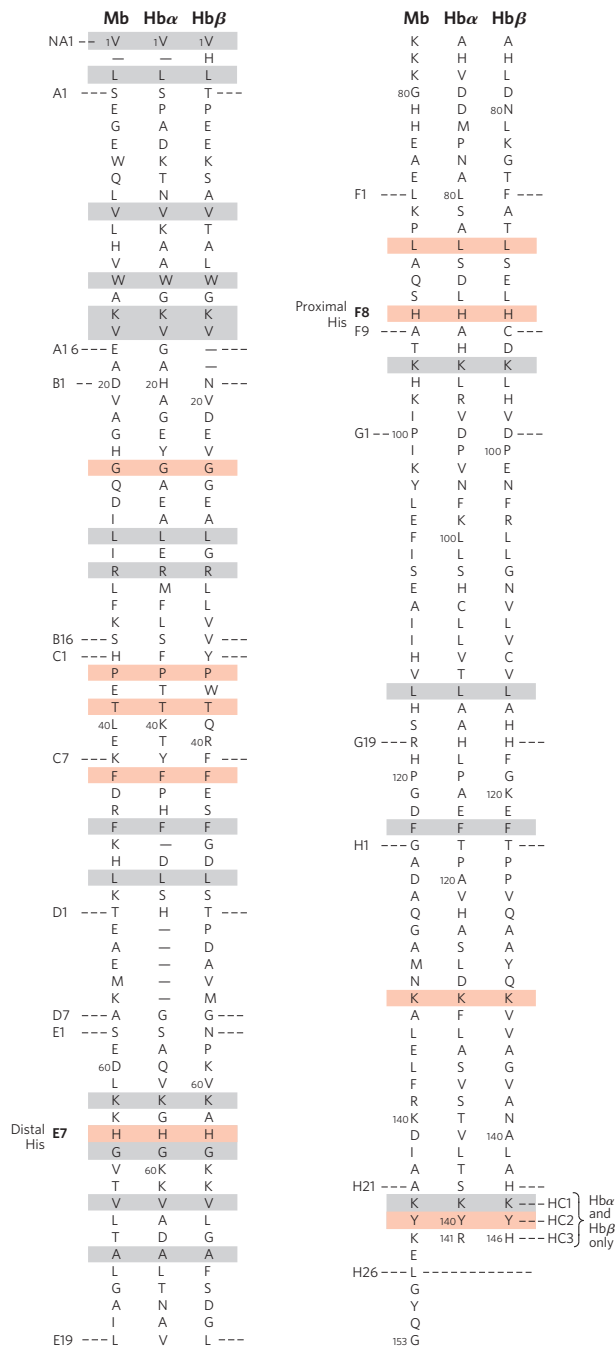


FIGURE 5-7 The amino acid sequences of whale myoglobin and the α and β chains of human hemoglobin. Dashed lines mark helix boundaries. To align the sequences optimally, short gaps must be introduced into both Hb sequences where a few amino acids are present in the other, compared sequences. With the exception of the missing D helix in Hb α , this alignment permits the use of the helix lettering convention that emphasizes the common positioning of amino acid residues that are identical in all three structures (shaded). Residues shaded in light red are conserved in all known globins. Note that the common helix-letter-and-number designation for amino acids does not necessarily correspond to a common position in the linear sequence of amino acids in the polypeptides. For example, the distal His residue is His E7 in all three structures, but corresponds to His⁶⁴, His⁵⁸, and His⁶³ in the linear sequences of Mb, Hb α , and Hb β , respectively. Nonhelical residues at the amino and carboxyl termini, beyond the first (A) and last (H) α -helical segments, are labeled NA and HC, respectively.

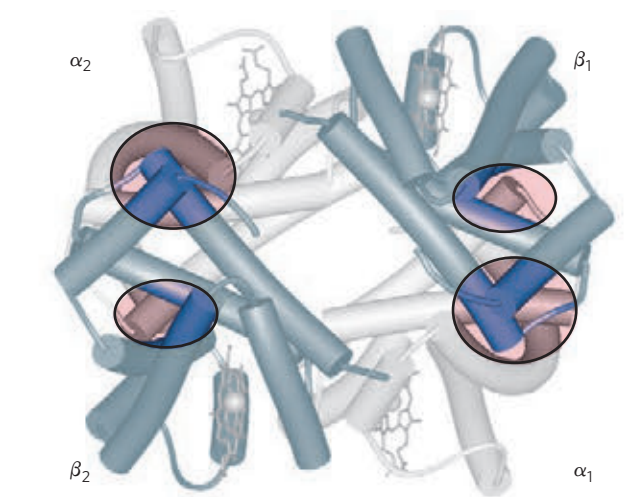


FIGURE 5-8 Dominant interactions between hemoglobin subunits. (PDB ID 1HGA) In this representation, α subunits are light and β subunits are dark. The strongest subunit interactions (highlighted) occur between unlike subunits. When oxygen binds, the $\alpha_1\beta_1$ contact changes little, but there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

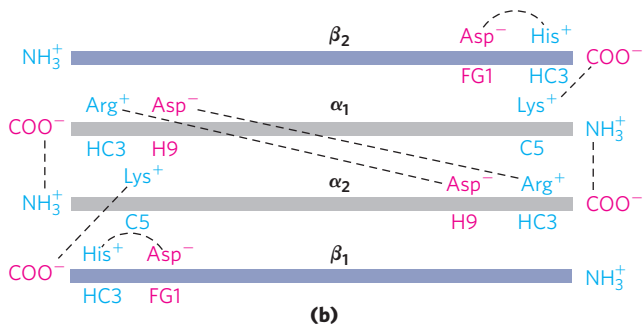
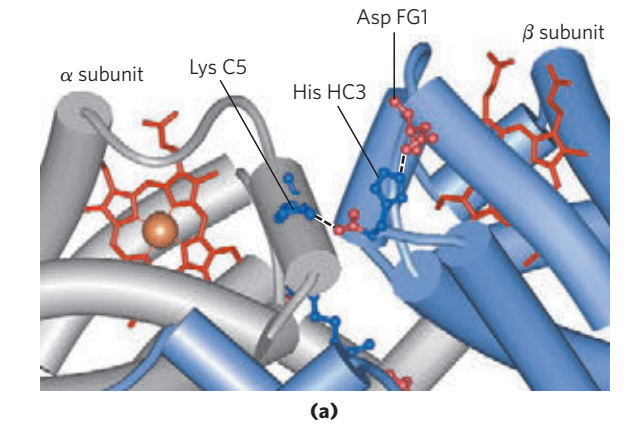


FIGURE 5-9 Some ion pairs that stabilize the T state of deoxyhemoglobin. (a) Close-up view of a portion of a deoxyhemoglobin molecule in the T state (PDB ID 1HGA). Interactions between the ion pairs His HC3 and Asp FG1 of the β subunit (blue) and between Lys C5 of the α subunit (gray) and His HC3 (its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

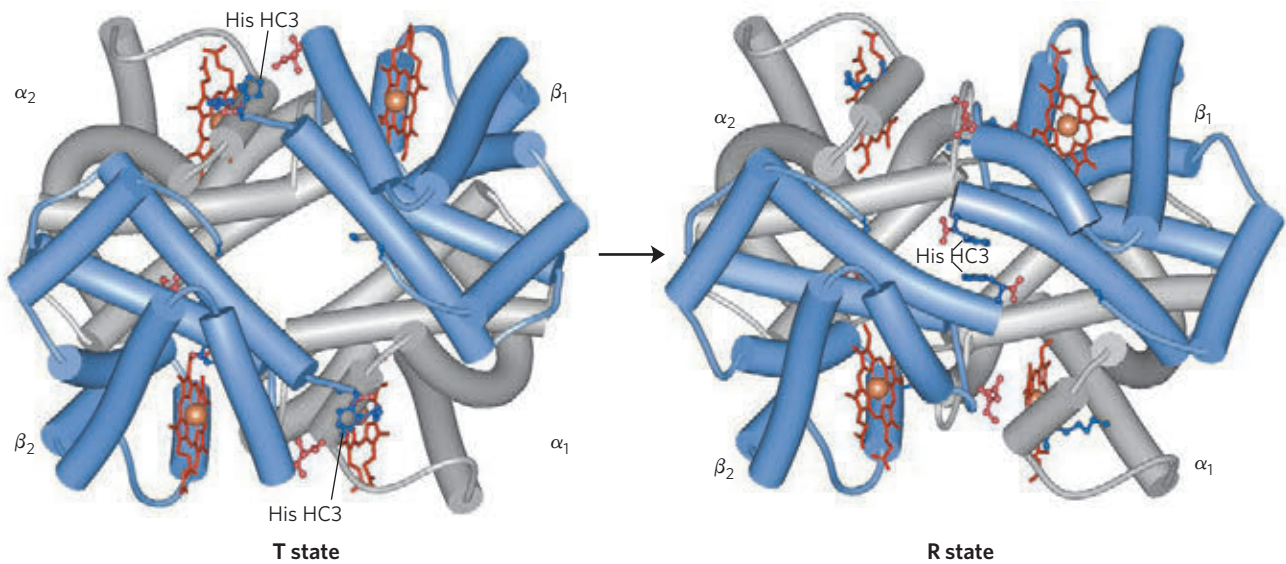


FIGURE 5-10 The T $\rightarrow$ R transition. (PDB ID 1HGA and 1BBB) In these depictions of deoxyhemoglobin, as in Figure 5-9, the β subunits are blue and the α subunits are gray. Positively charged side chains and chain termini involved in ion pairs are shown in blue, their negatively charged partners in red. The Lys C5 of each α subunit and Asp FG1 of each β subunit are visible but not labeled (compare Fig. 5-9a). Note that the molecule is oriented slightly differently than in Figure 5-9. The

transition from the T state to the R state shifts the subunit pairs substantially, affecting certain ion pairs. Most noticeably, the His HC3 residues at the carboxyl termini of the β subunits, which are involved in ion pairs in the T state, rotate in the R state toward the center of the molecule, where they are no longer in ion pairs. Another dramatic result of the T $\rightarrow$ R transition is a narrowing of the pocket between the β subunits.

to a hemoglobin subunit in the T state triggers a change in conformation to the R state. When the entire protein undergoes this transition, the structures of the individual subunits change little, but the $\alpha\beta$ subunit pairs slide past each other and rotate, narrowing the pocket between the β subunits (**Fig. 5-10**). In this process, some of the ion pairs that stabilize the T state are broken and some new ones are formed.

Max Perutz proposed that the T $\rightarrow$ R transition is triggered by changes in the positions of key amino acid side chains surrounding the heme. In the T state, the porphyrin is slightly puckered, causing the heme iron to protrude somewhat on the proximal His (His F8) side. The binding of O_2 causes the heme to assume a more planar conformation, shifting the position of the proximal His and the attached F helix (**Fig. 5-11**). These changes lead to adjustments in the ion pairs at the $\alpha_1\beta_2$ interface.

Hemoglobin Binds Oxygen Cooperatively

Hemoglobin must bind oxygen efficiently in the lungs, where the pO_2 is about 13.3 kPa, and release oxygen in the tissues, where the pO_2 is about 4 kPa. Myoglobin, or any protein that binds oxygen with a hyperbolic binding curve, would be ill-suited to this function, for the reason illustrated in **Figure 5-12**. A protein that bound O_2 with high affinity would bind it efficiently in the lungs but would not release much of it in the tissues. If the protein bound oxygen with a sufficiently low affinity to release it in the tissues, it would not pick up much oxygen in the lungs.

Hemoglobin solves the problem by undergoing a transition from a low-affinity state (the T state) to a high-affinity state (the R state) as more O_2 molecules are bound. As a result, hemoglobin has a hybrid S-shaped, or sigmoid, binding curve for oxygen (**Fig. 5-12**). A single-subunit protein with a single ligand-binding site cannot produce a sigmoid binding curve—even if binding elicits a conformational change—because each molecule of ligand binds independently and cannot affect ligand binding to another molecule. In contrast, O_2 binding to individual subunits of hemoglobin can alter the affinity for O_2 in adjacent subunits. The first molecule of O_2 that interacts with deoxyhemoglobin binds weakly, because it binds to a subunit in the T state. Its binding, however,

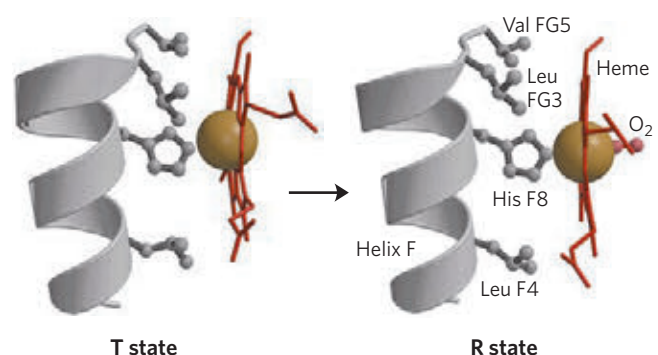


FIGURE 5-11 Changes in conformation near heme on O_2 binding to deoxyhemoglobin. (Derived from PDB ID 1HGA and 1BBB) The shift in the position of helix F when heme binds O_2 is thought to be one of the adjustments that triggers the T $\rightarrow$ R transition.

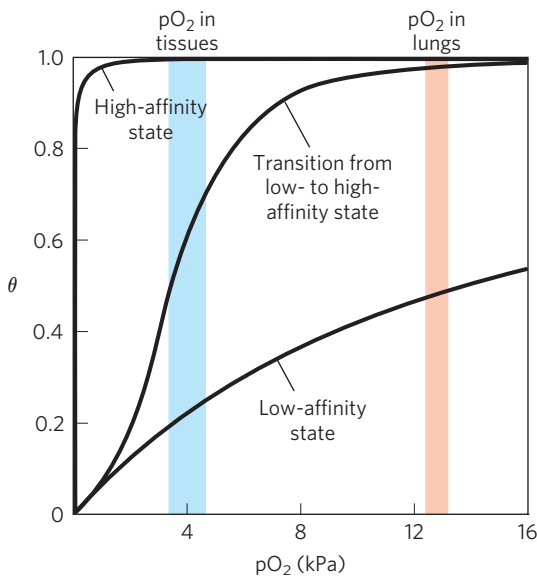


FIGURE 5-12 A sigmoid (cooperative) binding curve. A sigmoid binding curve can be viewed as a hybrid curve reflecting a transition from a low-affinity to a high-affinity state. Because of its cooperative binding, as manifested by a sigmoid binding curve, hemoglobin is more sensitive to the small differences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

leads to conformational changes that are communicated to adjacent subunits, making it easier for additional molecules of O_2 to bind. In effect, the $T \rightarrow R$ transition occurs more readily in the second subunit once O_2 is bound to the first subunit. The last (fourth) O_2 molecule binds to a heme in a subunit that is already in the R state, and hence it binds with much higher affinity than the first molecule.

An **allosteric protein** is one in which the binding of a ligand to one site affects the binding properties of another site on the same protein. The term “allosteric” derives from the Greek *allos*, “other,” and *stereos*, “solid” or “shape.” Allosteric proteins are those having “other shapes,” or conformations, induced by the binding of ligands referred to as modulators. The conformational changes induced by the modulator(s) interconvert more-active and less-active forms of the protein. The modulators for allosteric proteins may be either inhibitors or activators. When the normal ligand and modulator are identical, the interaction is termed **homotropic**. When the modulator is a molecule other than the normal ligand, the interaction is **heterotropic**. Some proteins have two or more modulators and therefore can have both homotropic and heterotropic interactions.

Cooperative binding of a ligand to a multimeric protein, such as we observe with the binding of O_2 to hemoglobin, is a form of allosteric binding. The binding of one ligand affects the affinities of any remaining unfilled binding sites, and O_2 can be considered as both a ligand and an activating homotropic modulator. There is only one binding site for O_2 on each subunit, so the allosteric

effects giving rise to cooperativity are mediated by conformational changes transmitted from one subunit to another by subunit-subunit interactions. A sigmoid binding curve is diagnostic of cooperative binding. It permits a much more sensitive response to ligand concentration and is important to the function of many multisubunit proteins. The principle of allostery extends readily to regulatory enzymes, as we shall see in Chapter 6.

Cooperative conformational changes depend on variations in the structural stability of different parts of a protein, as described in Chapter 4. The binding sites of an allosteric protein typically consist of stable segments in proximity to relatively unstable segments, with the latter capable of frequent changes in conformation or intrinsic disorder (**Fig. 5-13**). When a ligand binds, the moving parts of the protein’s binding site may be stabilized in a particular conformation, affecting the conformation of adjacent polypeptide

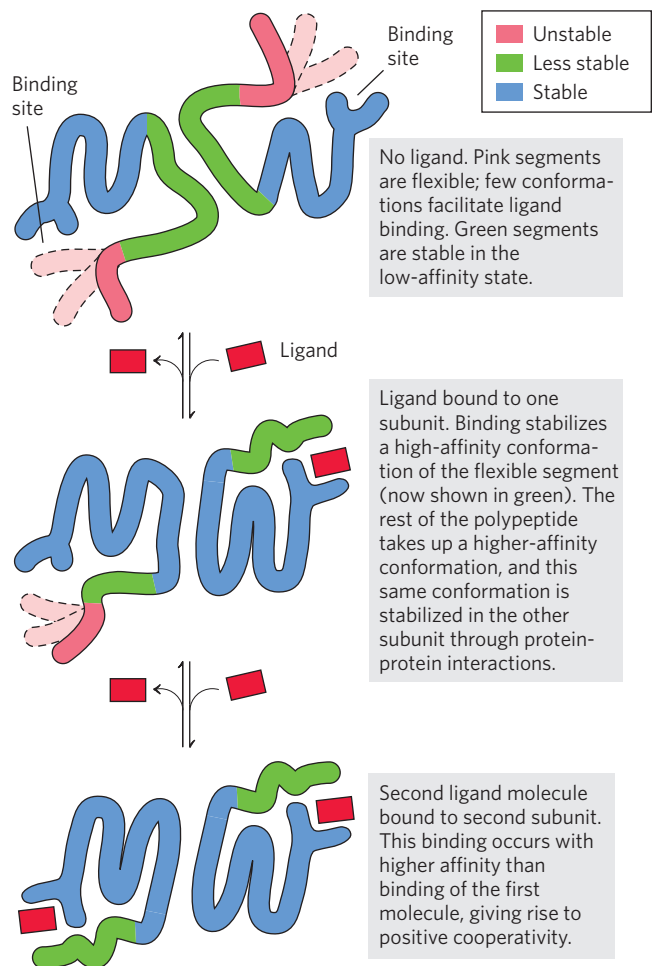


FIGURE 5-13 Structural changes in a multisubunit protein undergoing cooperative binding to ligand. Structural stability is not uniform throughout a protein molecule. Shown here is a hypothetical dimeric protein, with regions of high (blue), medium (green), and low (red) stability. The ligand-binding sites are composed of both high- and low-stability segments, so affinity for ligand is relatively low. The conformational changes that occur as ligand binds convert the protein from a low- to a high-affinity state, a form of induced fit.

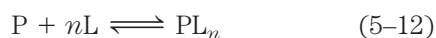
subunits. If the entire binding site were highly stable, then few structural changes could occur in this site or be propagated to other parts of the protein when a ligand binds.

As is the case with myoglobin, ligands other than oxygen can bind to hemoglobin. An important example is carbon monoxide, which binds to hemoglobin about 250 times better than does oxygen. Human exposure to CO can have tragic consequences (Box 5-1).

Cooperative Ligand Binding Can Be Described Quantitatively

Cooperative binding of oxygen by hemoglobin was first analyzed by Archibald Hill in 1910. From this work came a general approach to the study of cooperative ligand binding to multisubunit proteins.

For a protein with n binding sites, the equilibrium of Equation 5-1 becomes



and the expression for the association constant becomes

$$K_a = \frac{[PL_n]}{[P][L]^n} \quad (5-13)$$

The expression for θ (see Eqn 5-8) is

$$\theta = \frac{[L]^n}{[L]^n + K_d} \quad (5-14)$$

Rearranging, then taking the log of both sides, yields

$$\frac{\theta}{1 - \theta} = \frac{[L]^n}{K_d} \quad (5-15)$$

$$\log\left(\frac{\theta}{1 - \theta}\right) = n \log [L] - \log K_d \quad (5-16)$$

where $K_d = [L]_{0.5}^n$.

Equation 5-16 is the **Hill equation**, and a plot of $\log [\theta/(1 - \theta)]$ versus $\log [L]$ is called a **Hill plot**. Based on the equation, the Hill plot should have a slope of n . However, the experimentally determined slope actually reflects not the number of binding sites but the degree of interaction between them. The slope of a Hill plot is therefore denoted by n_H , the **Hill coefficient**, which is a measure of the degree of cooperativity. If n_H equals 1, ligand binding is not cooperative, a situation that can arise even in a multisubunit protein if the subunits do not communicate. An n_H of greater than 1 indicates positive cooperativity in ligand binding. This is the situation observed in hemoglobin, in which the binding of one molecule of ligand facilitates the binding of others. The theoretical upper limit for n_H is reached when $n_H = n$. In this case the

binding would be completely cooperative: all binding sites on the protein would bind ligand simultaneously, and no protein molecules partially saturated with ligand would be present under any conditions. This limit is never reached in practice, and the measured value of n_H is always less than the actual number of ligand-binding sites in the protein.

An n_H of less than 1 indicates negative cooperativity, in which the binding of one molecule of ligand *impedes* the binding of others. Well-documented cases of negative cooperativity are rare.

To adapt the Hill equation to the binding of oxygen to hemoglobin we must again substitute pO_2 for $[L]$ and P_{50}^n for K_d :

$$\log\left(\frac{\theta}{1 - \theta}\right) = n \log pO_2 - n \log P_{50} \quad (5-17)$$

Hill plots for myoglobin and hemoglobin are given in **Figure 5-14**.

Two Models Suggest Mechanisms for Cooperative Binding

Biochemists now know a great deal about the T and R states of hemoglobin, but much remains to be learned about how the $T \rightarrow R$ transition occurs. Two models for the cooperative binding of ligands to proteins with multiple binding sites have greatly influenced thinking about this problem.

The first model was proposed by Jacques Monod, Jeffries Wyman, and Jean-Pierre Changeux in 1965, and is called the **MWC model** or the **concerted**

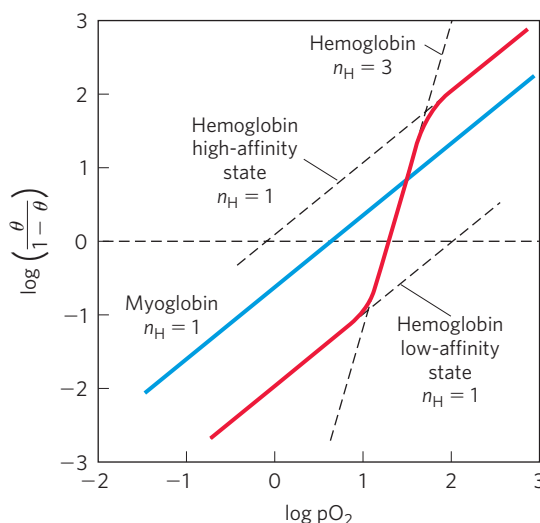


FIGURE 5-14 Hill plots for oxygen binding to myoglobin and hemoglobin.

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

BOX 5-1



MEDICINE

Carbon Monoxide: A Stealthy Killer

Lake Powell, Arizona, August 2000. A family was vacationing in a rented houseboat. They turned on the electrical generator to power an air conditioner and a television. About 15 minutes later, two brothers, aged 8 and 11, jumped off the swim deck at the stern. Situated immediately below the deck was the exhaust port for the generator. Within two minutes, both boys were overcome by the carbon monoxide in the exhaust, which had become concentrated in the space under the deck. Both drowned. These deaths, along with a series of deaths in the 1990s that were linked to houseboats of similar design, eventually led to the recall and redesign of the generator exhaust assembly.

Carbon monoxide (CO), a colorless, odorless gas, is responsible for more than half of yearly deaths due to poisoning worldwide. CO has an approximately 250-fold greater affinity for hemoglobin than does oxygen. Consequently, relatively low levels of CO can have substantial and tragic effects. When CO combines with hemoglobin, the complex is referred to as carboxyhemoglobin, or COHb.

Some CO is produced by natural processes, but locally high levels generally result only from human activities. Engine and furnace exhausts are important sources, as CO is a byproduct of the incomplete combustion of fossil fuels. In the United States alone, nearly 4,000 people succumb to CO poisoning each year, both accidentally and intentionally. Many of the accidental deaths involve undetected CO buildup in enclosed spaces, such as when a household furnace malfunctions or leaks, venting CO into a home. However, CO poisoning can also occur in open spaces, as unsuspecting people at work or play inhale the exhaust from generators, outboard motors, tractor engines, recreational vehicles, or lawn mowers.

Carbon monoxide levels in the atmosphere are rarely dangerous, ranging from less than 0.05 part per million (ppm) in remote and uninhabited areas to 3 to 4 ppm in some cities of the northern hemisphere. In the United States, the government-mandated (Occupational Safety and Health Administration, OSHA) limit for CO at worksites is 50 ppm for people working an eight-hour shift. The tight binding of CO to hemoglobin

means that COHb can accumulate over time as people are exposed to a constant low-level source of CO.

In an average, healthy individual, 1% or less of the total hemoglobin is complexed as COHb. Since CO is a product of tobacco smoke, many smokers have COHb levels in the range of 3% to 8% of total hemoglobin, and the levels can rise to 15% for chain-smokers. COHb levels equilibrate at 50% in people who breathe air containing 570 ppm of CO for several hours. Reliable methods have been developed that relate CO content in the atmosphere to COHb levels in the blood (Fig. 1). In tests of houseboats with a generator exhaust like the one responsible for the Lake Powell deaths, CO levels reached 6,000 to 30,000 ppm under the swim deck, and atmospheric O₂ levels under the deck declined from 21% to 12%. Even above the swim deck, CO levels of up to 7,200 ppm were detected, high enough to cause death within a few minutes.

How is a human affected by COHb? At levels of less than 10% of total hemoglobin, symptoms are rarely observed. At 15%, the individual experiences mild headaches. At 20% to 30%, the headache is severe and is generally accompanied by nausea, dizziness, confusion,

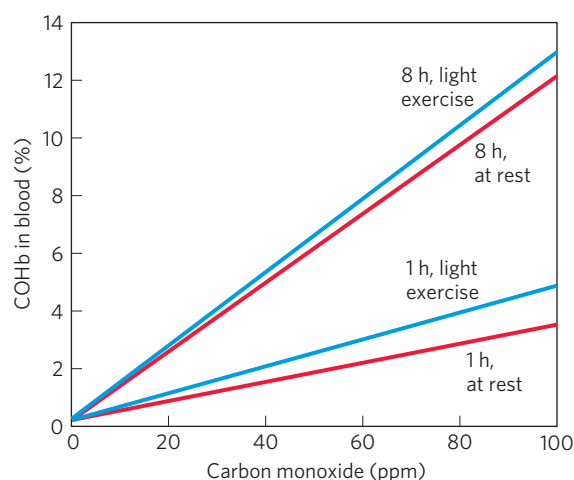


FIGURE 1 Relationship between levels of COHb in blood and concentration of CO in the surrounding air. Four different conditions of exposure are shown, comparing the effects of short versus extended exposure, and exposure at rest versus exposure during light exercise.

model (Fig. 5-15a). The concerted model assumes that the subunits of a cooperatively binding protein are functionally identical, that each subunit can exist in (at least) two conformations, and that all subunits undergo the transition from one conformation to the other simultaneously. In this model, no protein has individual subunits in different conformations. The two conformations are in equilibrium. The ligand can bind to either

conformation, but binds each with different affinity. Successive binding of ligand molecules to the low-affinity conformation (which is more stable in the absence of ligand) makes a transition to the high-affinity conformation more likely.

In the second model, the **sequential model** (Fig. 5-15b), proposed in 1966 by Daniel Koshland and colleagues, ligand binding can induce a change of

disorientation, and some visual disturbances; these symptoms are generally reversed if the individual is treated with oxygen. At COHb levels of 30% to 50%, the neurological symptoms become more severe, and at levels near 50%, the individual loses consciousness and can sink into coma. Respiratory failure may follow. With prolonged exposure, some damage becomes permanent. Death normally occurs when COHb levels rise above 60%. Autopsy on the boys who died at Lake Powell revealed COHb levels of 59% and 52%.

Binding of CO to hemoglobin is affected by many factors, including exercise (Fig. 1) and changes in air pressure related to altitude. Because of their higher base levels of COHb, smokers exposed to a source of CO often develop symptoms faster than nonsmokers. Individuals with heart, lung, or blood diseases that reduce the availability of oxygen to tissues may also experience symptoms at lower levels of CO exposure. Fetuses are at particular risk for CO poisoning, because fetal hemoglobin has a somewhat higher affinity for CO than adult hemoglobin. Cases of CO exposure have been recorded in which the fetus died but the mother recovered.

It may seem surprising that the loss of half of one's hemoglobin to COHb can prove fatal—we know that people with any of several anemic conditions manage to function reasonably well with half the usual complement of active hemoglobin. However, the binding of CO to hemoglobin does more than remove protein from the pool available to bind oxygen. It also affects the affinity of the remaining hemoglobin subunits for oxygen. As CO binds to one or two subunits of a hemoglobin tetramer, the affinity for O_2 is increased substantially in the remaining subunits (Fig. 2). Thus, a hemoglobin tetramer with two bound CO molecules can efficiently bind O_2 in the lungs—but it releases very little of it in the tissues. Oxygen deprivation in the tissues rapidly becomes severe. To add to the problem, the effects of CO are not limited to interference with hemoglobin function. CO binds to other heme proteins and a variety of metalloproteins. The effects of these interactions are not yet well understood, but they may be responsible for some of the longer-term effects of acute but nonfatal CO poisoning.

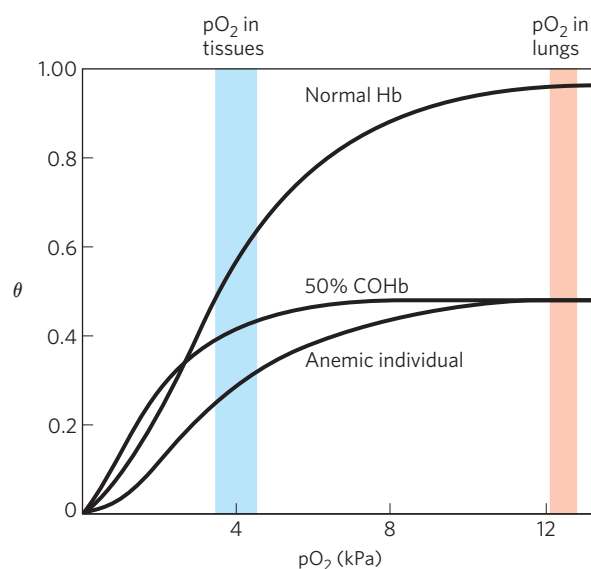


FIGURE 2 Several oxygen-binding curves: for normal hemoglobin, hemoglobin from an anemic individual with only 50% of her hemoglobin functional, and hemoglobin from an individual with 50% of his hemoglobin subunits complexed with CO. The pO_2 in human lungs and tissues is indicated.

When CO poisoning is suspected, rapid evacuation of the person away from the CO source is essential, but this does not always result in rapid recovery. When an individual is moved from the CO-polluted site to a normal, outdoor atmosphere, O_2 begins to replace the CO in hemoglobin—but the COHb levels drop only slowly. The half-time is 2 to 6.5 hours, depending on individual and environmental factors. If 100% oxygen is administered with a mask, the rate of exchange can be increased about fourfold; the half-time for O_2 -CO exchange can be reduced to tens of minutes if 100% oxygen at a pressure of 3 atm (303 kPa) is supplied. Thus, rapid treatment by a properly equipped medical team is critical.

Carbon monoxide detectors in all homes are highly recommended. This is a simple and inexpensive measure to avoid possible tragedy. After completing the research for this box, we immediately purchased several new CO detectors for our homes.

conformation in an individual subunit. A conformational change in one subunit makes a similar change in an adjacent subunit, as well as the binding of a second ligand molecule, more likely. There are more potential intermediate states in this model than in the concerted model. The two models are not mutually exclusive; the concerted model may be viewed as the “all-or-none” limiting case of the sequential model. In

Chapter 6 we use these models to investigate allosteric enzymes.

Hemoglobin Also Transports H^+ and CO_2

In addition to carrying nearly all the oxygen required by cells from the lungs to the tissues, hemoglobin carries two end products of cellular respiration— H^+ and CO_2 —from the tissues to the lungs and the kidneys, where

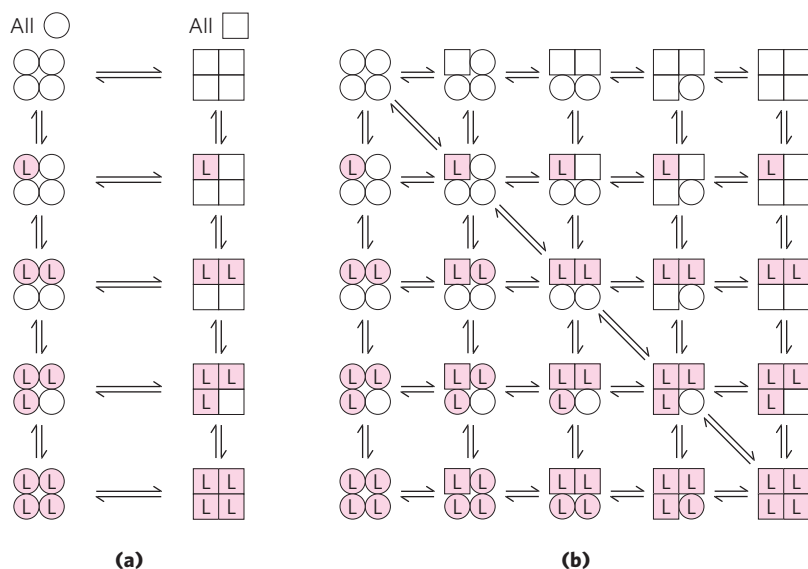
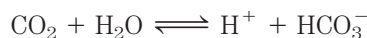


FIGURE 5-15 Two general models for the interconversion of inactive and active forms of a protein during cooperative ligand binding. Although the models may be applied to any protein—including any enzyme (Chapter 6)—that exhibits cooperative binding, we show here four subunits because the model was originally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

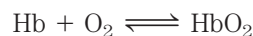
they are excreted. The CO_2 , produced by oxidation of organic fuels in mitochondria, is hydrated to form bicarbonate:



This reaction is catalyzed by **carbonic anhydrase**, an enzyme particularly abundant in erythrocytes. Carbon dioxide is not very soluble in aqueous solution, and bubbles of CO_2 would form in the tissues and blood if it were not converted to bicarbonate. As you can see from the reaction catalyzed by carbonic anhydrase, the hydration of CO_2 results in an increase in the H^+ concentration (a decrease in pH) in the tissues. The binding of oxygen by hemoglobin is profoundly influenced by pH and CO_2 concentration, so the interconversion of CO_2 and bicarbonate is of great importance to the regulation of oxygen binding and release in the blood.

Hemoglobin transports about 40% of the total H^+ and 15% to 20% of the CO_2 formed in the tissues to the lungs and kidneys. (The remainder of the H^+ is absorbed by the plasma's bicarbonate buffer; the remainder of the CO_2 is transported as dissolved HCO_3^- and CO_2 .) The binding of H^+ and CO_2 is inversely related to the binding of oxygen. At the relatively low pH and high CO_2 concentration of peripheral tissues, the affinity of hemoglobin for oxygen decreases as H^+ and CO_2 are bound, and O_2 is released to the tissues. Conversely, in the capillaries of the lung, as CO_2 is excreted and the blood pH consequently rises, the affinity of hemoglobin for oxygen increases and the protein binds more O_2 for transport to the peripheral tissues. This effect of pH and CO_2 concentration on the binding and release of oxygen by hemoglobin is called the **Bohr effect**, after Christian Bohr, the Danish physiologist (and father of physicist Niels Bohr) who discovered it in 1904.

The binding equilibrium for hemoglobin and one molecule of oxygen can be designated by the reaction



but this is not a complete statement. To account for the effect of H^+ concentration on this binding equilibrium, we rewrite the reaction as



where HHb^+ denotes a protonated form of hemoglobin. This equation tells us that the O_2 -saturation curve of hemoglobin is influenced by the H^+ concentration (**Fig. 5-16**). Both O_2 and H^+ are bound by hemoglobin, but with inverse affinity. When the oxygen concentration is high, as in the lungs, hemoglobin binds O_2 and releases protons. When the oxygen concentration is

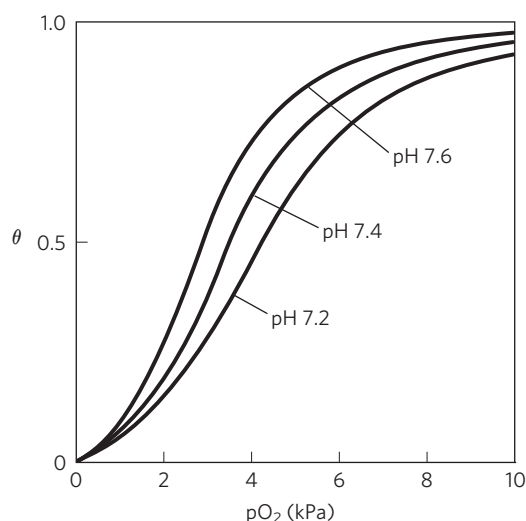
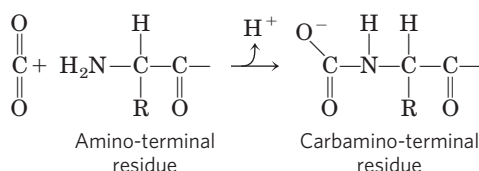


FIGURE 5-16 Effect of pH on oxygen binding to hemoglobin. The pH of blood is 7.6 in the lungs and 7.2 in the tissues. Experimental measurements on hemoglobin binding are often performed at pH 7.4.

low, as in the peripheral tissues, H^+ is bound and O_2 is released.

Oxygen and H^+ are not bound at the same sites in hemoglobin. Oxygen binds to the iron atoms of the hemes, whereas H^+ binds to any of several amino acid residues in the protein. A major contribution to the Bohr effect is made by His¹⁴⁶ (His HC3) of the β subunits. When protonated, this residue forms one of the ion pairs—to Asp⁹⁴ (Asp FG1)—that helps stabilize deoxyhemoglobin in the T state (Fig. 5–9). The ion pair stabilizes the protonated form of His HC3, giving this residue an abnormally high pK_a in the T state. The pK_a falls to its normal value of 6.0 in the R state because the ion pair cannot form, and this residue is largely unprotonated in oxyhemoglobin at pH 7.6, the blood pH in the lungs. As the concentration of H^+ rises, protonation of His HC3 promotes release of oxygen by favoring a transition to the T state. Protonation of the amino-terminal residues of the α subunits, certain other His residues, and perhaps other groups has a similar effect.

Thus we see that the four polypeptide chains of hemoglobin communicate with each other not only about O_2 binding to their heme groups but also about H^+ binding to specific amino acid residues. And there is still more to the story. Hemoglobin also binds CO_2 , again in a manner inversely related to the binding of oxygen. Carbon dioxide binds as a carbamate group to the α -amino group at the amino-terminal end of each globin chain, forming carbaminohemoglobin:

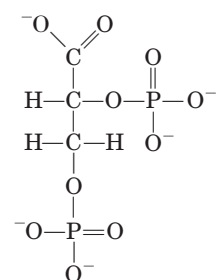


This reaction produces H^+ , contributing to the Bohr effect. The bound carbamates also form additional salt bridges (not shown in Fig. 5–9) that help to stabilize the T state and promote the release of oxygen.

When the concentration of carbon dioxide is high, as in peripheral tissues, some CO_2 binds to hemoglobin and the affinity for O_2 decreases, causing its release. Conversely, when hemoglobin reaches the lungs, the high oxygen concentration promotes binding of O_2 and release of CO_2 . It is the capacity to communicate ligand-binding information from one polypeptide subunit to the others that makes the hemoglobin molecule so beautifully adapted to integrating the transport of O_2 , CO_2 , and H^+ by erythrocytes.

Oxygen Binding to Hemoglobin Is Regulated by 2,3-Bisphosphoglycerate

The interaction of **2,3-bisphosphoglycerate (BPG)** with hemoglobin molecules further refines the function of hemoglobin, and provides an example of heterotropic allosteric modulation.



2,3-Bisphosphoglycerate

BPG is present in relatively high concentrations in erythrocytes. When hemoglobin is isolated, it contains substantial amounts of bound BPG, which can be difficult to remove completely. In fact, the O_2 -binding curves for hemoglobin that we have examined to this point were obtained in the presence of bound BPG. 2,3-Bisphosphoglycerate is known to greatly reduce the affinity of hemoglobin for oxygen—there is an inverse relationship between the binding of O_2 and the binding of BPG. We can therefore describe another binding process for hemoglobin:



BPG binds at a site distant from the oxygen-binding site and regulates the O_2 -binding affinity of hemoglobin in relation to the pO_2 in the lungs. BPG is important in the physiological adaptation to the lower pO_2 at high altitudes. For a healthy human at sea level, the binding of O_2 to hemoglobin is regulated such that the amount of O_2 delivered to the tissues is nearly 40% of the maximum that could be carried by the blood (**Fig. 5–17**). Imagine that such a person is suddenly transported from sea level to an altitude of 4,500 meters, where the pO_2 is considerably lower. The delivery of O_2 to the tissues is now reduced. However, after just a few hours at the higher altitude, the BPG concentration in the blood has begun to rise, leading to a decrease in the affinity of hemoglobin for oxygen. This adjustment in the BPG level has only a small effect on the binding of O_2 in the lungs but a considerable effect on the release of O_2 in the tissues. As a result, the delivery of oxygen to the tissues is restored to nearly 40% of the O_2 that can be transported by the blood. The situation is reversed when the person returns to sea level. The BPG concentration in erythrocytes also increases in people suffering from **hypoxia**, lowered oxygenation of peripheral tissues due to inadequate functioning of the lungs or circulatory system.

The site of BPG binding to hemoglobin is the cavity between the β subunits in the T state (**Fig. 5–18**). This cavity is lined with positively charged amino acid residues that interact with the negatively charged groups of BPG. Unlike O_2 , only one molecule of BPG is bound to each hemoglobin tetramer. BPG lowers hemoglobin's affinity for oxygen by stabilizing the T state. The transition to the R state narrows the binding pocket for BPG, precluding BPG binding. In the absence

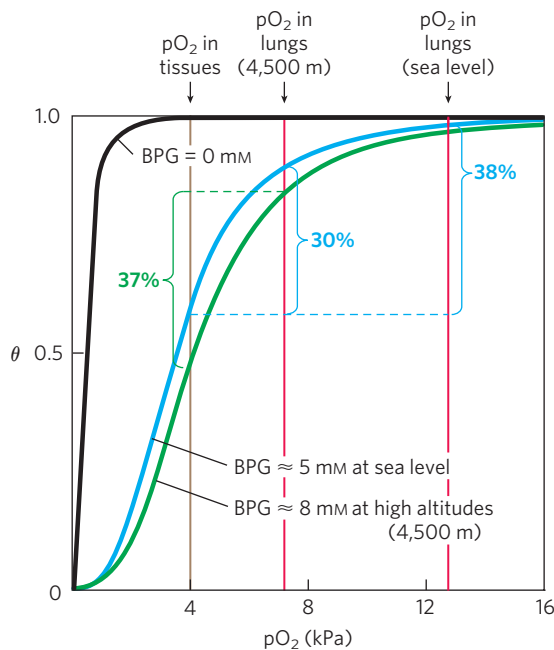


FIGURE 5-17 Effect of BPG on oxygen binding to hemoglobin. The BPG concentration in normal human blood is about 5 mM at sea level and about 8 mM at high altitudes. Note that hemoglobin binds to oxygen quite tightly when BPG is entirely absent, and the binding curve seems to be hyperbolic. In reality, the measured Hill coefficient for O_2 -binding cooperativity decreases only slightly (from 3 to about 2.5) when BPG is removed from hemoglobin, but the rising part of the sigmoid curve is confined to a very small region close to the origin. At sea level, hemoglobin is nearly saturated with O_2 in the lungs, but just over 60% saturated in the tissues, so the amount of O_2 released in the tissues is about 38% of the maximum that can be carried in the blood. At high altitudes, O_2 delivery declines by about one-fourth, to 30% of maximum. An increase in BPG concentration, however, decreases the affinity of hemoglobin for O_2 , so approximately 37% of what can be carried is again delivered to the tissues.

of BPG, hemoglobin is converted to the R state more easily.

Regulation of oxygen binding to hemoglobin by BPG has an important role in fetal development. Because a fetus must extract oxygen from its mother's blood, fetal hemoglobin must have greater affinity than the maternal hemoglobin for O_2 . The fetus synthesizes γ subunits rather than β subunits, forming $\alpha_2\gamma_2$ hemoglobin. This tetramer has a much lower affinity for BPG than normal adult hemoglobin, and a correspondingly higher affinity for O_2 . **Oxygen-Binding Proteins—Hemoglobin Is Susceptible to Allosteric Regulation**

Sickle-Cell Anemia Is a Molecular Disease of Hemoglobin



The hereditary human disease sickle-cell anemia demonstrates strikingly the importance of amino

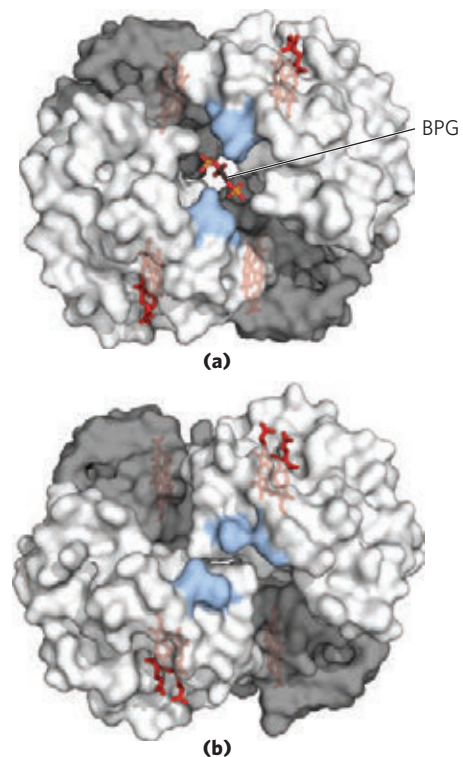


FIGURE 5-18 Binding of BPG to deoxyhemoglobin. (a) BPG binding stabilizes the T state of deoxyhemoglobin (PDB ID 1B86). The negative charges of BPG interact with several positively charged groups (shown in blue in this surface contour image) that surround the pocket between the β subunits on the surface of deoxyhemoglobin in the T state. (b) The binding pocket for BPG disappears on oxygenation, following transition to the R state (PDB ID 1BBB). (Compare with Fig. 5-10.)

acid sequence in determining the secondary, tertiary, and quaternary structures of globular proteins, and thus their biological functions. Almost 500 genetic variants of hemoglobin are known to occur in the human population; all but a few are quite rare. Most variations consist of differences in a single amino acid residue. The effects on hemoglobin structure and function are often minor but can sometimes be extraordinary. Each hemoglobin variation is the product of an altered gene. The variant genes are called alleles. Because humans generally have two copies of each gene, an individual may have two copies of one allele (thus being homozygous for that gene) or one copy of each of two different alleles (thus heterozygous).

Sickle-cell anemia occurs in individuals who inherit the allele for sickle-cell hemoglobin from both parents. The erythrocytes of these individuals are fewer and also abnormal. In addition to an unusually large number of immature cells, the blood contains many long, thin, sickle-shaped erythrocytes (**Fig. 5-19**). When hemoglobin from sickle cells (called hemoglobin S) is deoxygenated, it becomes insoluble and forms polymers that

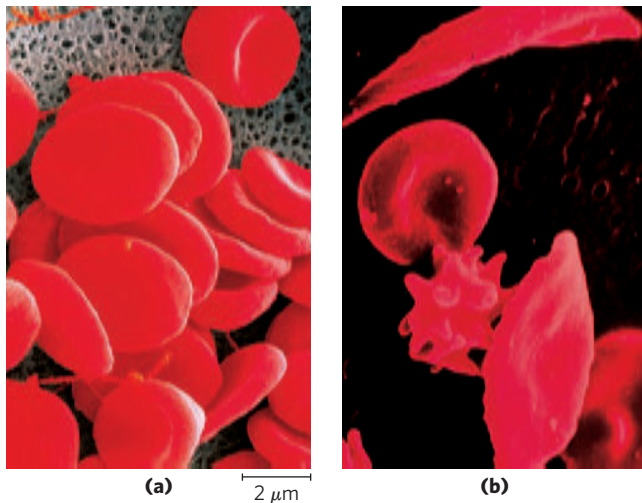


FIGURE 5-19 A comparison of (a) uniform, cup-shaped, normal erythrocytes with (b) the variably shaped erythrocytes seen in sickle-cell anemia, which range from normal to spiny or sickle-shaped.

aggregate into tubular fibers (**Fig. 5-20**). Normal hemoglobin (hemoglobin A) remains soluble on deoxygenation. The insoluble fibers of deoxygenated hemoglobin S cause the deformed, sickle shape of the erythrocytes, and the proportion of sickled cells increases greatly as blood is deoxygenated.

The altered properties of hemoglobin S result from a single amino acid substitution, a Val instead of a Glu residue at position 6 in the two β chains. The R group of valine has no electric charge, whereas glutamate has a negative charge at pH 7.4. Hemoglobin S therefore has two fewer negative charges than hemoglobin A (one fewer on each β chain). Replacement of the Glu residue by Val creates a “sticky” hydrophobic contact point at position 6 of the β chain, which is on the outer surface of the molecule. These sticky spots cause deoxyhemoglobin S molecules to associate abnormally with each other, forming the long, fibrous aggregates characteristic of this disorder. **Oxygen-Binding Proteins—Defects in Hb Lead to Serious Genetic Disease**

Sickle-cell anemia, as we have noted, occurs in individuals homozygous for the sickle-cell allele of the gene encoding the β subunit of hemoglobin. Individuals who receive the sickle-cell allele from only one parent and are thus heterozygous experience a milder condition called sickle-cell trait; only about 1% of their erythrocytes become sickled on deoxygenation. These individuals may live completely normal lives if they avoid vigorous exercise and other stresses on the circulatory system.

Sickle-cell anemia is life-threatening and painful. People with this disease suffer repeated crises brought on by physical exertion. They become weak, dizzy, and short of breath, and they also experience heart murmurs and an increased pulse rate. The hemoglobin content of their blood is only about half the normal value of 15 to 16 g/100 mL, because sickled cells are very fragile and

rupture easily; this results in anemia (“lack of blood”). An even more serious consequence is that capillaries become blocked by the long, abnormally shaped cells, causing severe pain and interfering with normal organ function—a major factor in the early death of many people with the disease.

Without medical treatment, people with sickle-cell anemia usually die in childhood. Curiously, the frequency of the sickle-cell allele in populations is unusually high in

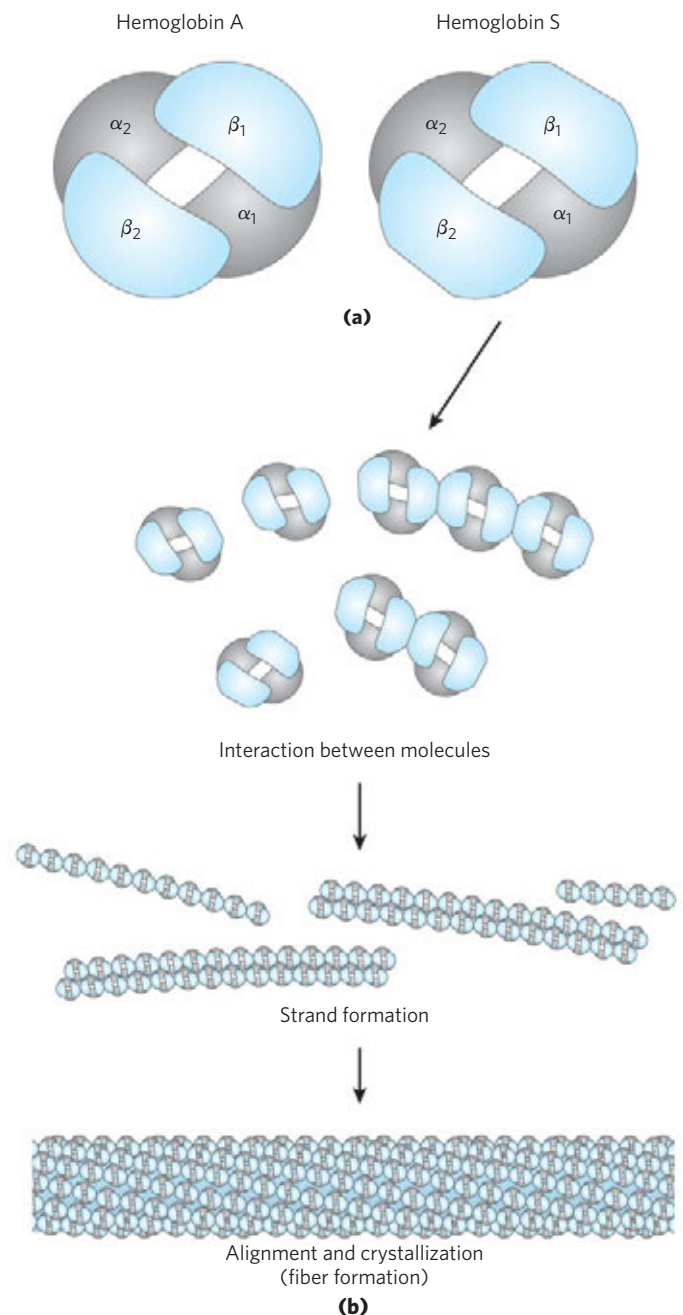


FIGURE 5-20 Normal and sickle-cell hemoglobin. (a) Subtle differences between the conformations of hemoglobin A and hemoglobin S result from a single amino acid change in the β chains. (b) As a result of this change, deoxyhemoglobin S has a hydrophobic patch on its surface, which causes the molecules to aggregate into strands that align into insoluble fibers.

certain parts of Africa. Investigation into this matter led to the finding that in heterozygous individuals, the allele confers a small but significant resistance to lethal forms of malaria. Natural selection has resulted in an allele population that balances the deleterious effects of the homozygous condition against the resistance to malaria afforded by the heterozygous condition. ■

SUMMARY 5.1 Reversible Binding of a Protein to a Ligand: Oxygen-Binding Proteins

- ▶ Protein function often entails interactions with other molecules. A protein binds a molecule, known as a ligand, at its binding site. Proteins may undergo conformational changes when a ligand binds, a process called induced fit. In a multisubunit protein, the binding of a ligand to one subunit may affect ligand binding to other subunits. Ligand binding can be regulated.
- ▶ Myoglobin contains a heme prosthetic group, which binds oxygen. Heme consists of a single atom of Fe^{2+} coordinated within a porphyrin. Oxygen binds to myoglobin reversibly; this simple reversible binding can be described by an association constant K_a or a dissociation constant K_d . For a monomeric protein such as myoglobin, the fraction of binding sites occupied by a ligand is a hyperbolic function of ligand concentration.
- ▶ Normal adult hemoglobin has four heme-containing subunits, two α and two β , similar in structure to each other and to myoglobin. Hemoglobin exists in two interchangeable structural states, T and R. The T state is most stable when oxygen is not bound. Oxygen binding promotes transition to the R state.
- ▶ Oxygen binding to hemoglobin is both allosteric and cooperative. As O_2 binds to one binding site, the hemoglobin undergoes conformational changes that affect the other binding sites—an example of allosteric behavior. Conformational changes between the T and R states, mediated by subunit-subunit interactions, result in cooperative binding; this is described by a sigmoid binding curve and can be analyzed by a Hill plot.
- ▶ Two major models have been proposed to explain the cooperative binding of ligands to multisubunit proteins: the concerted model and the sequential model.
- ▶ Hemoglobin also binds H^+ and CO_2 , resulting in the formation of ion pairs that stabilize the T state and lessen the protein's affinity for O_2 (the Bohr effect). Oxygen binding to hemoglobin is also modulated by 2,3-bisphosphoglycerate, which binds to and stabilizes the T state.
- ▶ Sick-cell anemia is a genetic disease caused by a single amino acid substitution (Glu^6 to Val^6) in

each β chain of hemoglobin. The change produces a hydrophobic patch on the surface of the hemoglobin that causes the molecules to aggregate into bundles of fibers. This homozygous condition results in serious medical complications.

5.2 Complementary Interactions between Proteins and Ligands: The Immune System and Immunoglobulins

We have seen how the conformations of oxygen-binding proteins affect and are affected by the binding of small ligands (O_2 or CO) to the heme group. However, most protein-ligand interactions do not involve a prosthetic group. Instead, the binding site for a ligand is more often like the hemoglobin binding site for BPG—a cleft in the protein lined with amino acid residues, arranged to make the binding interaction highly specific. Effective discrimination between ligands is the norm at binding sites, even when the ligands have only minor structural differences.

All vertebrates have an immune system capable of distinguishing molecular “self” from “nonself” and then destroying what is identified as nonself. In this way, the immune system eliminates viruses, bacteria, and other pathogens and molecules that may pose a threat to the organism. On a physiological level, the immune response is an intricate and coordinated set of interactions among many classes of proteins, molecules, and cell types. At the level of individual proteins, the immune response demonstrates how an acutely sensitive and specific biochemical system is built upon the reversible binding of ligands to proteins.

The Immune Response Features a Specialized Array of Cells and Proteins

Immunity is brought about by a variety of **leukocytes** (white blood cells), including **macrophages** and **lymphocytes**, all of which develop from undifferentiated stem cells in the bone marrow. Leukocytes can leave the bloodstream and patrol the tissues, each cell producing one or more proteins capable of recognizing and binding to molecules that might signal an infection.

The immune response consists of two complementary systems, the humoral and cellular immune systems. The **humoral immune system** (Latin *humor*, “fluid”) is directed at bacterial infections and extracellular viruses (those found in the body fluids), but can also respond to individual foreign proteins. The **cellular immune system** destroys host cells infected by viruses and also destroys some parasites and foreign tissues.

At the heart of the humoral immune response are soluble proteins called **antibodies** or **immunoglobulins**, often abbreviated **Ig**. Immunoglobulins bind bacteria, viruses, or large molecules identified as foreign and target them for destruction. Making up 20% of blood

a series of conformational changes in the myosin head, leading to dissociation of myosin from one F-actin subunit and its eventual reassociation with another, farther along the thin filament. The myosin thus slides along the actin filaments.

- Muscle contraction is stimulated by the release of Ca^{2+} from the sarcoplasmic reticulum. The Ca^{2+} binds to the protein troponin, leading to a conformational change in a troponin-tropomyosin complex that triggers the cycle of actin-myosin interactions.

Key Terms

Terms in bold are defined in the glossary.

ligand 157	T lymphocyte or
binding site 157	T cell 175
induced fit 157	antigen 175
heme 158	epitope 175
porphyrin 158	hapten 175
globins 159	immunoglobulin fold 175
equilibrium expression 160	polyclonal
association constant, K_a 160	antibodies 178
dissociation constant,	monoclonal
K_d 160	antibodies 178
allosteric protein 166	ELISA 179
Hill equation 167	immunoblotting 179
Bohr effect 170	Western blotting 179
lymphocytes 174	myosin 179
antibody 174	actin 181
immunoglobulin 174	sarcomere 181
B lymphocyte or	
B cell 175	

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Problems

1. Relationship between Affinity and Dissociation Constant Protein A has a binding site for ligand X with a K_d of 10^{-6} M. Protein B has a binding site for ligand X with a K_d of 10^{-9} M. Which protein has a higher affinity for ligand X? Explain your reasoning. Convert the K_d to K_a for both proteins.

2. Negative Cooperativity Which of the following situations would produce a Hill plot with $n_H < 1.0$? Explain your reasoning in each case.

(a) The protein has multiple subunits, each with a single ligand-binding site. Binding of ligand to one site decreases the binding affinity of other sites for the ligand.

(b) The protein is a single polypeptide with two ligand-binding sites, each having a different affinity for the ligand.

(c) The protein is a single polypeptide with a single ligand-binding site. As purified, the protein preparation is heterogeneous, containing some protein molecules that are partially denatured and thus have a lower binding affinity for the ligand.

3. Hemoglobin's Affinity for Oxygen What is the effect of the following changes on the O_2 affinity of hemoglobin? (a) A drop in the pH of blood plasma from 7.4 to 7.2. (b) A decrease

in the partial pressure of CO_2 in the lungs from 6 kPa (holding one's breath) to 2 kPa (normal). (c) An increase in the BPG level from 5 mM (normal altitudes) to 8 mM (high altitudes). (d) An increase in CO from 1.0 part per million (ppm) in a normal indoor atmosphere to 30 ppm in a home that has a malfunctioning or leaking furnace.

4. Reversible Ligand Binding I The protein calcineurin binds to the protein calmodulin with an association rate of $8.9 \times 10^3 \text{ M}^{-1}\text{s}^{-1}$ and an overall dissociation constant, K_d , of 10 nM. Calculate the dissociation rate, k_d , including appropriate units.

5. Reversible Ligand Binding II Three membrane receptor proteins bind tightly to a hormone. Based on the data in the table below, (a) what is the K_d for hormone binding by protein 2? (Include appropriate units.) (b) Which of these proteins binds *most* tightly to this hormone?

Hormone concentration (nM)	θ		
	Protein 1	Protein 2	Protein 3
0.2	0.048	0.29	0.17
0.5	0.11	0.5	0.33
1	0.2	0.67	0.5
4	0.5	0.89	0.8
10	0.71	0.95	0.91
20	0.83	0.97	0.95
50	0.93	0.99	0.98

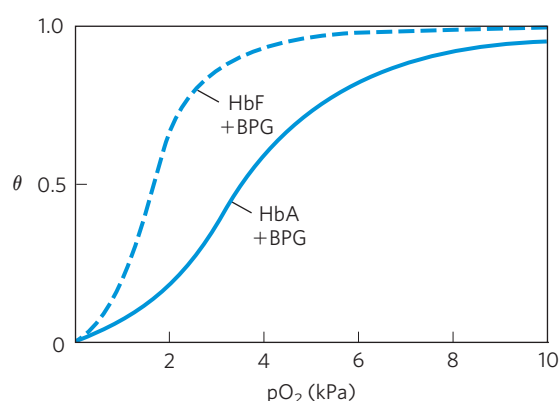
6. Cooperativity in Hemoglobin Under appropriate conditions, hemoglobin dissociates into its four subunits. The isolated α subunit binds oxygen, but the O_2 -saturation curve is hyperbolic rather than sigmoid. In addition, the binding of oxygen to the isolated α subunit is not affected by the presence of H^+ , CO_2 , or BPG. What do these observations indicate about the source of the cooperativity in hemoglobin?

7. Comparison of Fetal and Maternal Hemoglobins Studies of oxygen transport in pregnant mammals show that the O_2 -saturation curves of fetal and maternal blood are markedly different when measured under the same conditions. Fetal erythrocytes contain a structural variant of hemoglobin, HbF, consisting of two α and two γ subunits ($\alpha_2\gamma_2$), whereas maternal erythrocytes contain HbA ($\alpha_2\beta_2$).

(a) Which hemoglobin has a higher affinity for oxygen under physiological conditions, HbA or HbF? Explain.

(b) What is the physiological significance of the different O_2 affinities?

(c) When all the BPG is carefully removed from samples of HbA and HbF, the measured O_2 -saturation curves (and consequently the O_2 affinities) are displaced to the left. However, HbA now has a greater affinity for oxygen than does HbF. When BPG is reintroduced, the O_2 -saturation curves return to normal, as shown in the graph. What is the effect of BPG on the O_2 affinity of hemoglobin? How can the above information be used to explain the different O_2 affinities of fetal and maternal hemoglobin?



8. Hemoglobin Variants There are almost 500 naturally occurring variants of hemoglobin. Most are the result of a single amino acid substitution in a globin polypeptide chain. Some variants produce clinical illness, though not all variants have deleterious effects. A brief sample follows.

- HbS (sickle-cell Hb): substitutes a Val for a Glu on the surface
- Hb Cowtown: eliminates an ion pair involved in T-state stabilization
- Hb Memphis: substitutes one uncharged polar residue for another of similar size on the surface
- Hb Bibba: substitutes a Pro for a Leu involved in an α helix
- Hb Milwaukee: substitutes a Glu for a Val
- Hb Providence: substitutes an Asn for a Lys that normally projects into the central cavity of the tetramer
- Hb Philly: substitutes a Phe for a Tyr, disrupting hydrogen bonding at the $\alpha_1\beta_1$ interface

Explain your choices for each of the following:

- (a) The Hb variant *least* likely to cause pathological symptoms.
- (b) The variant(s) most likely to show pI values different from that of HbA on an isoelectric focusing gel.
- (c) The variant(s) most likely to show a decrease in BPG binding and an increase in the overall affinity of the hemoglobin for oxygen.

9. Oxygen Binding and Hemoglobin Structure A team of biochemists uses genetic engineering to modify the interface region between hemoglobin subunits. The resulting hemoglobin variants exist in solution primarily as $\alpha\beta$ dimers (few, if any, $\alpha_2\beta_2$ tetramers form). Are these variants likely to bind oxygen more weakly or more tightly? Explain your answer.

10. Reversible (but Tight) Binding to an Antibody An antibody binds to an antigen with a K_d of $5 \times 10^{-8} \text{ M}$. At what concentration of antigen will θ be (a) 0.2, (b) 0.5, (c) 0.6, (d) 0.8?

11. Using Antibodies to Probe Structure-Function Relationships in Proteins A monoclonal antibody binds to G-actin but not to F-actin. What does this tell you about the epitope recognized by the antibody?



12. The Immune System and Vaccines A host organism needs time, often days, to mount an immune response against a new antigen, but memory cells permit a rapid response to pathogens previously encountered. A vaccine to