3: Globular Proteins

Overview

The previous chapter described the types of secondary and tertiary structures that are the bricks and mortar of protein architecture. By arranging these fundamental structural elements in different combinations, widely diverse proteins can be constructed that are capable of various specialized functions. This chapter examines the relationship between structure and function for the clinically important globular hemeproteins. Fibrous structural proteins are discussed in **Chapter 4**.

Globular Hemeproteins

Hemeproteins are a group of specialized proteins that contain heme as a tightly bound prosthetic group. (See p. 54 for a discussion of prosthetic groups.) The role of the heme group is dictated by the environment created by the three-dimensional structure of the protein. For example, the heme group of a cytochrome functions as an electron carrier that is alternately oxidized and reduced (see p. 75). In contrast, the heme group of the enzyme catalase is part of the active site of the enzyme that catalyzes the breakdown of hydrogen peroxide (see p. 148). In hemoglobin and myoglobin, the two most abundant hemeproteins in humans, the heme group serves to reversibly bind oxygen (O_2).

Heme structure

Heme is a complex of protoporphyrin IX and ferrous iron (Fe^{2+}), as shown in **Figure 3.1**. The iron is held in the center of the heme molecule by bonds to the four nitrogens of the porphyrin ring. The heme Fe^{2+} can form two additional bonds, one on each side of the planar porphyrin ring. In myoglobin and hemoglobin, one of these positions is coordinated to the side chain of a histidine residue of the globin molecule, whereas the other position is available to bind O_2 (**Fig. 3.2**). (See pp. 278 and 282, respectively, for a discussion of heme synthesis and degradation.)

FIGURE 3.1

A. Hemeprotein (cytochrome c). **B.** Structure of heme.

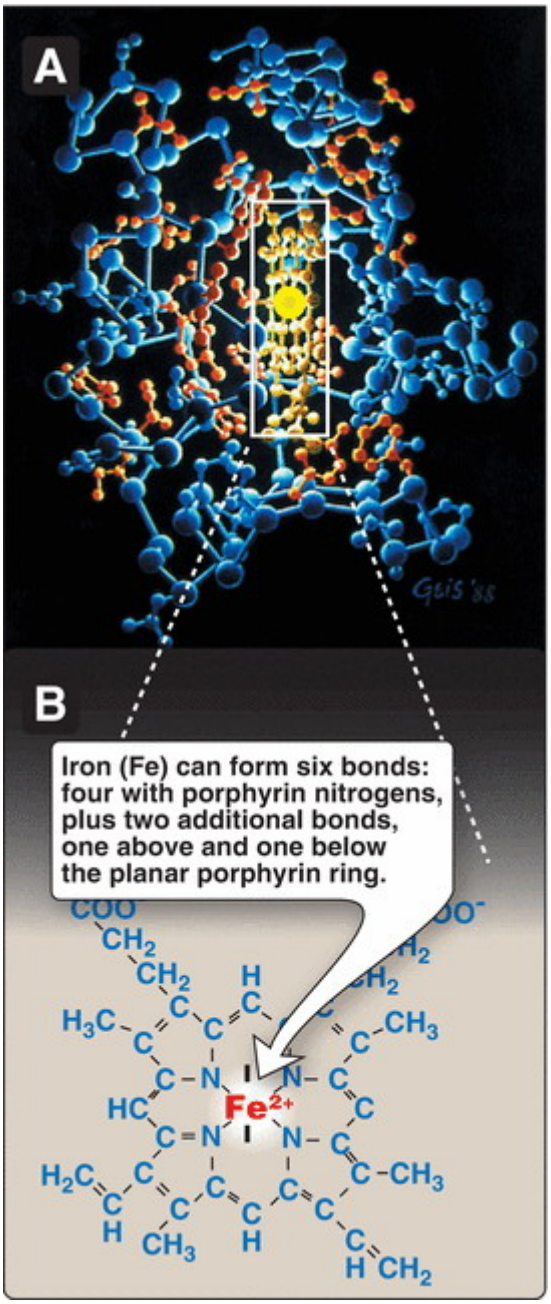

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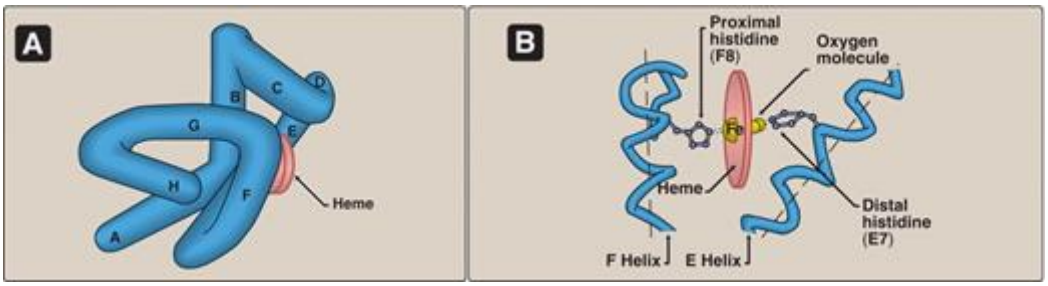
FIGURE 3.2

A. Model of myoglobin showing α-helices A to H. **B.** Schematic diagram of the oxygen-binding site of myoglobin.



Myoglobin structure and function

Myoglobin, a hemeprotein present in heart and skeletal muscle, functions both as an oxygen reservoir and as an oxygen carrier that increases the rate of oxygen transport within the muscle cell. [Note: Surprisingly, mouse myoglobin double knockouts (see p. 502) have an apparently normal phenotype.] Myoglobin consists of a single polypeptide chain that is structurally similar to the individual polypeptide chains of the tetrameric hemoglobin molecule. This homology makes myoglobin a useful model for interpreting some of the more complex properties of hemoglobin.

α-Helical content

Myoglobin is a compact molecule, with ~80% of its polypeptide chain folded into eight stretches of α-helix. These α-helical regions, labeled A to H in **Figure 3.2A**, are terminated either by the presence of proline, whose five-membered ring cannot be accommodated in an α-helix (see p. 16) or by β-bends and loops stabilized by hydrogen bonds and ionic bonds (see p. 19). [Note: Ionic bonds are also termed electrostatic interactions or salt bridges.]

Location of polar and nonpolar amino acid residues

The interior of the globular myoglobin molecule is composed almost entirely of nonpolar amino acids. They are packed closely together, forming a structure stabilized by hydrophobic interactions between these clustered residues (see p. 19). In contrast, polar amino acids are located almost exclusively on the surface, where they can form hydrogen bonds, both with each other and with water.

Binding of the heme group

The heme group of the myoglobin molecule sits in a crevice, which is lined with nonpolar amino acids. Notable exceptions are two histidine residues (see **Fig. 3.2B**). One, the proximal histidine (F8), binds directly to the Fe^{2+} of heme. The second, or distal histidine (E7), does not directly interact with the heme group but helps stabilize the binding of O_2 to Fe^{2+}. Thus, the protein, or globin, portion of myoglobin creates a special microenvironment for the heme that permits the reversible binding of one oxygen molecule (oxygenation). The simultaneous loss of electrons by Fe^{2+} (oxidation to the ferric [Fe^{3+}] form) occurs only rarely.

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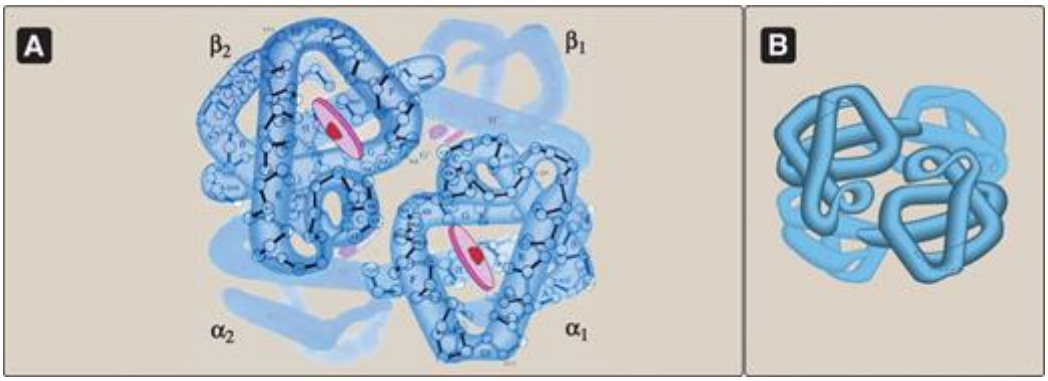
Hemoglobin structure and function

Hemoglobin is found exclusively in red blood cells (RBC), where its main function is to transport O_2 from the lungs to the capillaries of the tissues. Hemoglobin A, the major hemoglobin in adults, is composed of four polypeptide chains (two α chains and two β chains) held together by noncovalent interactions (**Fig. 3.3**). Each chain (subunit) has stretches of α-helical structure and a hydrophobic heme-binding pocket similar to that described for myoglobin. However, the tetrameric hemoglobin molecule is structurally and functionally more complex than myoglobin. For example, hemoglobin can transport protons (H^+) and carbon dioxide (CO_2) from the tissues to the lungs and can carry four molecules of O_2 from the lungs to the cells of the body. Furthermore, the oxygen-binding properties of hemoglobin are regulated by interaction with allosteric effectors (see p. 29).

FIGURE 3.3

A. Structure of hemoglobin showing the polypeptide backbones. **B.** Simplified drawing showing the α-helices.



Obtaining O_2 from the atmosphere solely by diffusion greatly limits the size of organisms. Circulatory systems overcome this, but transport molecules such as hemoglobin are also required because O_2 is only slightly soluble in aqueous solutions such as blood.

Quaternary structure

The hemoglobin tetramer can be envisioned as composed of two identical dimers, $(\alpha\beta)_1$ and $(\alpha\beta)_2$. The two polypeptide chains within each dimer are held tightly together primarily by hydrophobic interactions (**Fig. 3.4**). [Note: In this instance, hydrophobic amino acid residues are localized not only in the interior of the molecule but also in a region on the surface of each subunit. Multiple interchain hydrophobic interactions form strong associations between α-subunits and β-subunits in the dimers.] In contrast, the two dimers are held together primarily by polar bonds. The weaker interactions between the dimers allow them to move with respect to one other. This movement results in the two dimers occupying different relative positions in deoxyhemoglobin as compared with oxyhemoglobin (see **Fig. 3.4**).

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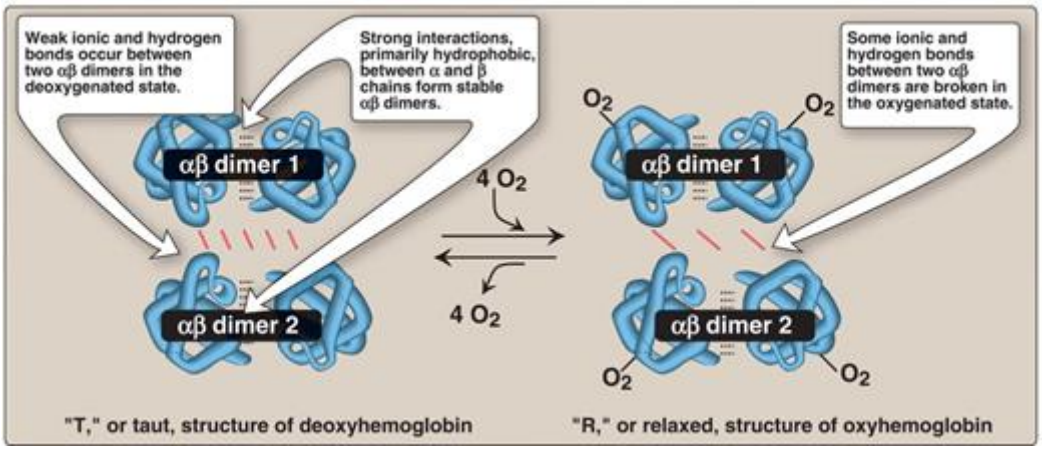
FIGURE 3.4

Schematic diagram showing structural changes resulting from oxygenation and deoxygenation of hemoglobin.



T form

The deoxy form of hemoglobin is called the “T,” or taut (tense) form. In the T form, the two αβ dimers interact through a network of ionic bonds and hydrogen bonds that constrain the movement of the polypeptide chains. The T conformation is the low-oxygen-affinity form of hemoglobin.

R form

The binding of O_2 to hemoglobin causes the rupture of some of the polar bonds between the two αβ dimers, allowing movement. Specifically, the binding of O_2 to the heme Fe^{2+} pulls the iron into the plane of the heme (**Fig. 3.5**). Because the iron is also linked to the proximal histidine (F8), the resulting movement of the globin chains alters the interface between the αβ dimers. This leads to a structure called the “R,” or relaxed form (see **Fig. 3.4**). The R conformation is the high-oxygen-affinity form of hemoglobin.

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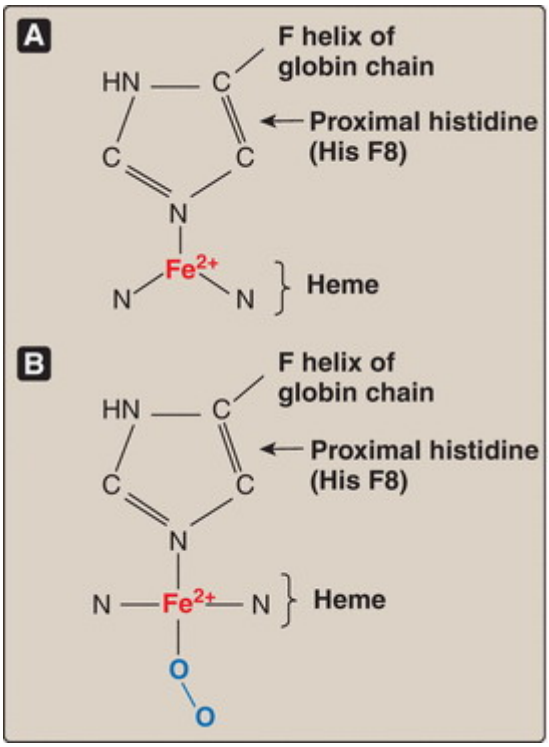
FIGURE 3.5

Movement of heme iron (Fe).

A. Out of the plane of the heme when oxygen (O_2) is not bound. **B.** Into the plane of the heme upon O_2 binding.



Oxygen binding to myoglobin and hemoglobin

Myoglobin can bind only one molecule of O_2, because it contains only one heme group. In contrast, hemoglobin can bind four molecules of O_2, one at each of its four heme groups. The degree of saturation (Y) of these oxygen-binding sites on all myoglobin or hemoglobin molecules can vary between zero (all sites are empty) and 100% (all sites are full), as shown in **Figure 3.6**. [Note: Pulse oximetry is a noninvasive, indirect method of measuring the oxygen saturation of arterial blood based on differences in light absorption by oxyhemoglobin and deoxyhemoglobin.]

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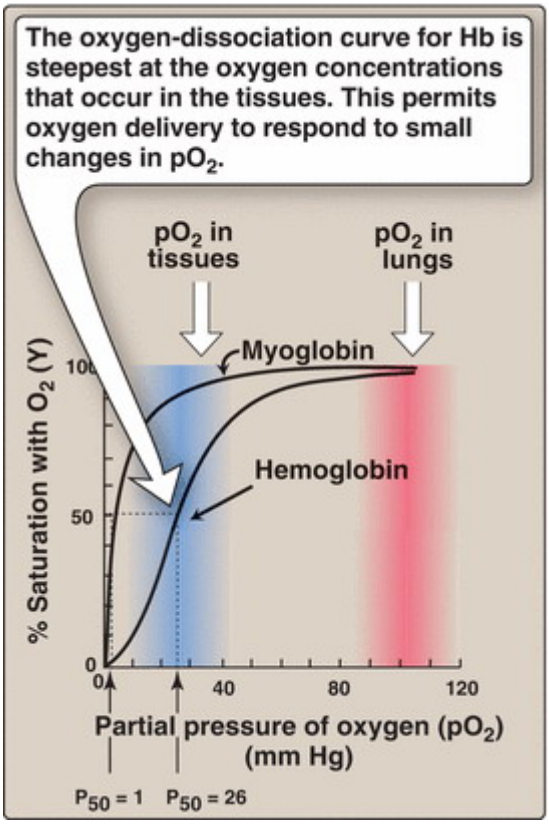
FIGURE 3.6

Oxygen-dissociation curves for myoglobin and hemoglobin (Hb).



Oxygen-dissociation curve

A plot of Y measured at different partial pressures of oxygen (pO_2) is called the oxygen-dissociation curve. [Note: pO_2 may also be represented as PO_2.] The curves for myoglobin and hemoglobin show important differences (see **Fig. 3.6**). This graph illustrates that myoglobin has a higher oxygen affinity at all pO_2 values than does hemoglobin. The partial pressure of oxygen needed to achieve half saturation of the binding sites (P_{50}) is ~1 mm Hg for myoglobin and 26 mm Hg for hemoglobin. The higher the oxygen affinity (that is, the more tightly O_2 binds), the lower the P_{50}.

Myoglobin

The oxygen-dissociation curve for myoglobin has a hyperbolic shape (see **Fig. 3.6**). This reflects the fact that myoglobin reversibly binds a single molecule of O_2. Thus, oxygenated (MbO_2) and deoxygenated (Mb) myoglobin exist in a simple equilibrium:

$$Mb + O_2 \rightleftarrows MbO_2$$

The equilibrium is shifted to the right or to the left as O_2 is added to or removed from the system. [Note: Myoglobin is designed to bind O_2 released by hemoglobin at the low pO_2 found in muscle. Myoglobin, in turn, releases O_2 within the muscle cell in response to oxygen demand.]

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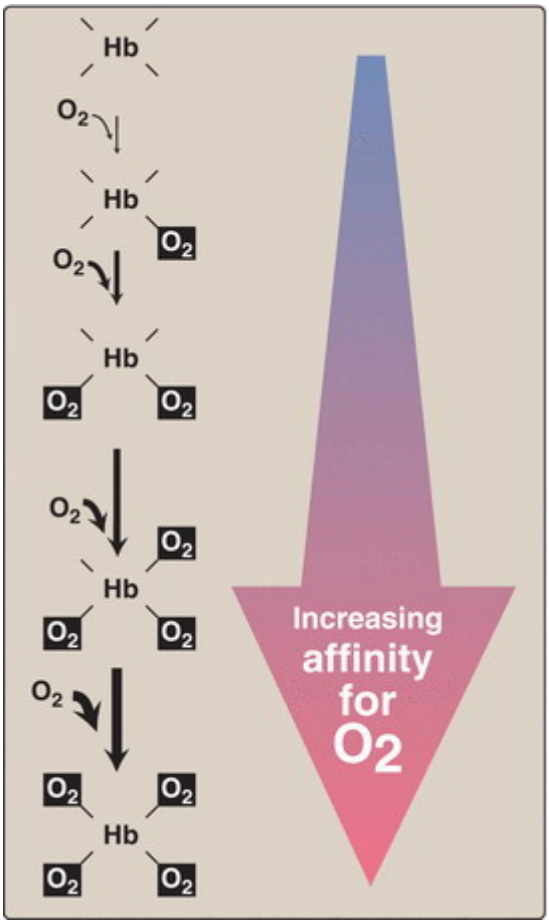
Hemoglobin

The oxygen-dissociation curve for hemoglobin is sigmoidal in shape (see **Fig. 3.6**), indicating that the subunits cooperate in binding O_2. Cooperative binding of O_2 by the four subunits of hemoglobin means that the binding of an oxygen molecule at one subunit increases the oxygen affinity of the remaining subunits in the same hemoglobin tetramer (**Fig. 3.7**). Although it is more difficult for the first oxygen molecule to bind to hemoglobin, the subsequent binding of oxygen molecules occurs with high affinity, as shown by the steep upward curve in the region near 20–30 mm Hg (see **Fig. 3.6**).

FIGURE 3.7

Hemoglobin (Hb) binds successive molecules of oxygen (O_2) with increasing affinity.



Allosteric effectors

The ability of hemoglobin to reversibly bind O_2 is affected by the pO_2, the pH of the environment, the partial pressure of carbon dioxide (pCO_2), and the availability of 2,3-bisphosphoglycerate (2,3-BPG). These are collectively called allosteric ("other site") effectors, because their interaction at one site on the tetrameric hemoglobin molecule causes structural changes that affect the binding of O_2 to the heme iron at other sites on the molecule. [Note: The binding of O_2 to monomeric myoglobin is not influenced by allosteric effectors.]

Oxygen

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The sigmoidal oxygen-dissociation curve reflects specific structural changes that are initiated at one subunit and transmitted to other subunits in the hemoglobin tetramer. The net effect of this cooperativity is that the affinity of hemoglobin for the last oxygen molecule bound is ~300 times greater than its affinity for the first oxygen molecule bound. Oxygen, then, is an allosteric effector of hemoglobin. It stabilizes the R form.

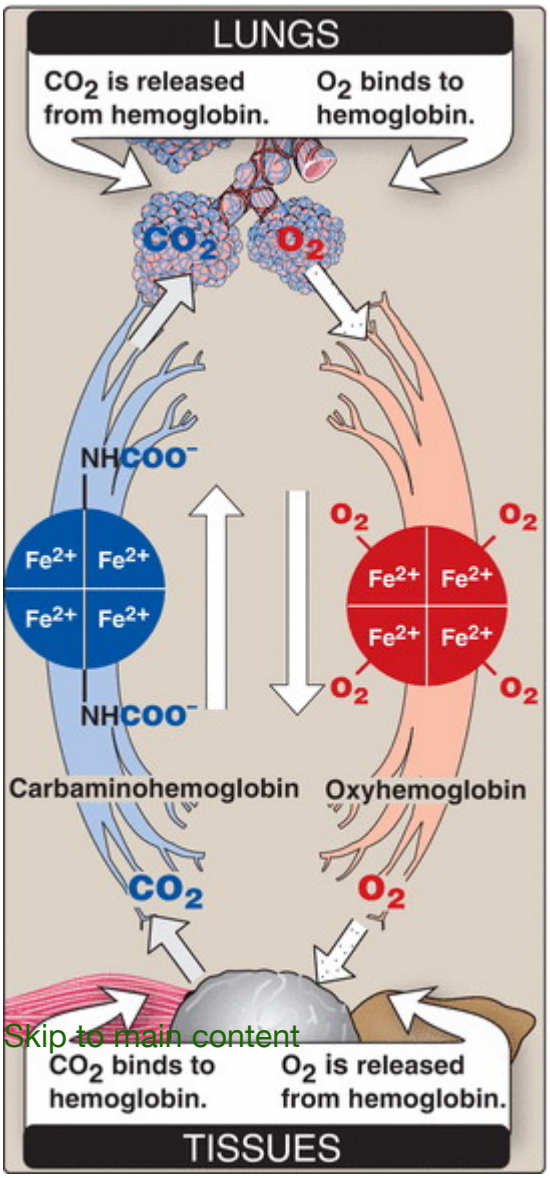
Loading and unloading oxygen

The cooperative binding of O_2 allows hemoglobin to deliver more O_2 to the tissues in response to relatively small changes in the pO_2. This can be seen in **Figure 3.6**, which indicates pO_2 in the alveoli of the lung and the capillaries of the tissues. For example, in the lung, oxygen concentration is high, and hemoglobin becomes virtually saturated (or "loaded") with O_2. In contrast, in the peripheral tissues, oxyhemoglobin releases (or "unloads") much of its O_2 for use in the oxidative metabolism of the tissues (**Fig. 3.8**).

FIGURE 3.8

Transport of oxygen and carbon dioxide by hemoglobin.

Fe = iron.



Significance of the sigmoidal oxygen-dissociation curve

The steep slope of the oxygen-dissociation curve over the range of oxygen concentrations that occur between the lungs and the tissues permits hemoglobin to carry and deliver O_2 efficiently from sites of high to sites of low pO_2. A molecule with a hyperbolic oxygen-dissociation curve, such as myoglobin, could not achieve the same degree of O_2 release within this range of pO_2. Instead, it would have maximum affinity for O_2 throughout this oxygen pressure range and, therefore, would deliver no O_2 to the tissues.

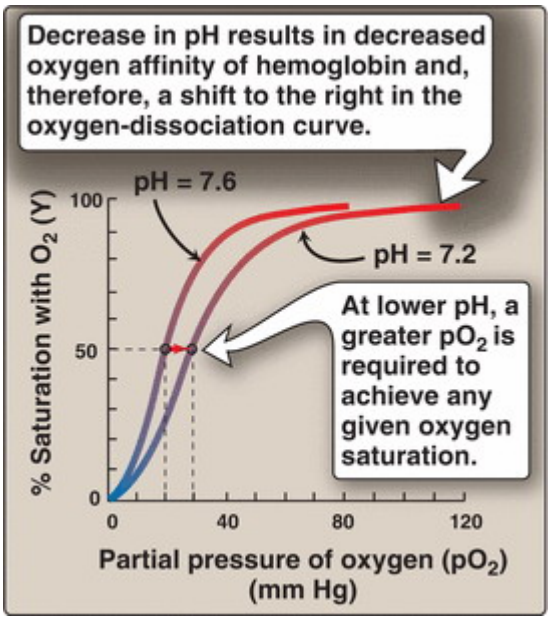
Bohr effect

The release of O_2 from hemoglobin is enhanced when the pH is lowered (proton concentration $[H^+]$ is increased) or when the hemoglobin is in the presence of an increased pCO_2. Both result in decreased oxygen affinity of hemoglobin and, therefore, a shift to the right in the oxygen-dissociation curve (**Fig. 3.9**). Both, then, stabilize the T (deoxy) form. This change in oxygen binding is called the Bohr effect. Conversely, raising the pH or lowering the concentration of CO_2 results in a greater oxygen affinity, a shift to the left in the oxygen-dissociation curve, and stabilization of the R (oxy) form.

FIGURE 3.9

Effect of pH on the oxygen affinity of hemoglobin.

Protons are allosteric effectors of hemoglobin.



Source of the protons that lower pH

The concentration of both H^+ and CO_2 in the capillaries of metabolically active tissues is higher than that observed in alveolar capillaries of the lungs, where CO_2 is released into the expired air. In the tissues, CO_2 is converted by zinc-containing carbonic anhydrase to carbonic acid:

$$CO_2 + H_2O \rightleftarrows H_2CO_3$$

which spontaneously loses a H^+, becoming bicarbonate (the major blood buffer):

$$H_2CO_3 \rightleftarrows HCO_3^- + H^+$$

The H^+ produced by this pair of reactions contributes to the lowering of pH. This differential pH gradient (that is, lungs having a higher pH and tissues a lower pH) favors the unloading of O_2 in the peripheral tissues and the loading of O_2 in the lung. Thus, the oxygen affinity of the hemoglobin molecule responds to small shifts in pH between the lungs and oxygen-consuming tissues, making hemoglobin a more efficient transporter of O_2.

Mechanism of the Bohr effect

The Bohr effect reflects the fact that the deoxy form of hemoglobin has a greater affinity for H^+ than does oxyhemoglobin. This is caused by ionizable groups such as specific histidine side chains that have a higher pK_a (see p. 6) in deoxyhemoglobin than in oxyhemoglobin. Therefore, an increase in the concentration of H^+ (resulting in a decrease in pH) causes these groups to become protonated (charged) and able to form ionic bonds (salt bridges). These bonds preferentially stabilize the deoxy form of hemoglobin, producing a decrease in oxygen affinity. [Note: Hemoglobin, then, is an important blood buffer.]

The Bohr effect can be represented schematically as:

$$\underset{\text{Oxyhemoglobin}}{HbO_2} + \text{2,3-BPG} \rightleftarrows \underset{\text{Deoxyhemoglobin}}{Hb - \text{2,3-BPG}} + O_2$$

where an increase in H^+ (or a lower pO_2) shifts the equilibrium to the right (favoring deoxyhemoglobin), whereas an increase in pO_2 (or a decrease in H^+) shifts the equilibrium to the left.

2,3-BPG effect on oxygen affinity

2,3-BPG is an important regulator of the binding of O_2 to hemoglobin. It is the most abundant organic phosphate in the RBC, where its concentration is approximately that of hemoglobin. 2,3-BPG is synthesized from an intermediate of the glycolytic pathway (**Fig. 3.10**; see p. 101 for a discussion of 2,3-BPG synthesis in glycolysis).

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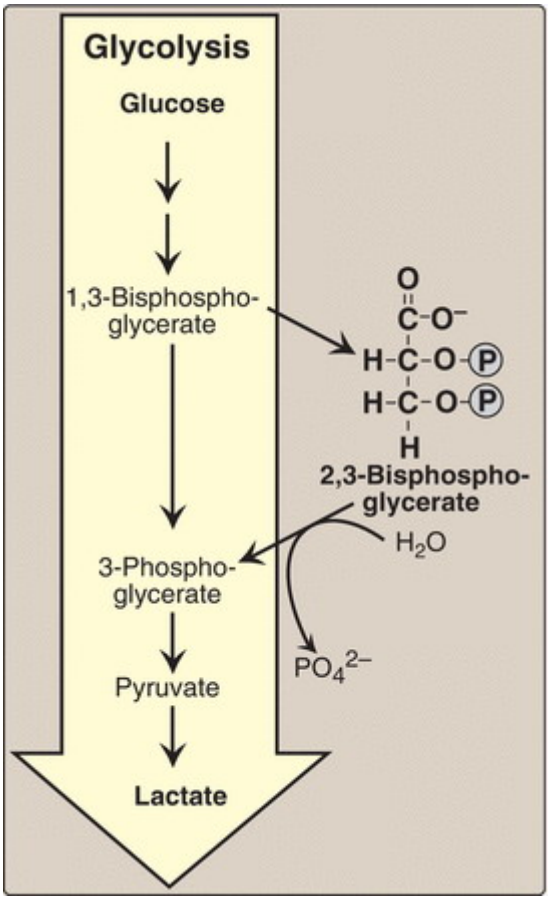
FIGURE 3.10

Synthesis of 2,3-bisphosphoglycerate.

[Note: P is a phosphoryl group, PO_3^{2-}.] In older literature, 2, 3-bisphosphoglycerate (2,3-BPG) may be referred to as 2,3-diphosphoglycerate (2,3-DPG).



2,3-BPG binding to deoxyhemoglobin

2,3-BPG decreases the oxygen affinity of hemoglobin by binding to deoxyhemoglobin but not to oxyhemoglobin. This preferential binding stabilizes the T conformation of deoxyhemoglobin. The effect of binding 2,3-BPG can be represented schematically as:

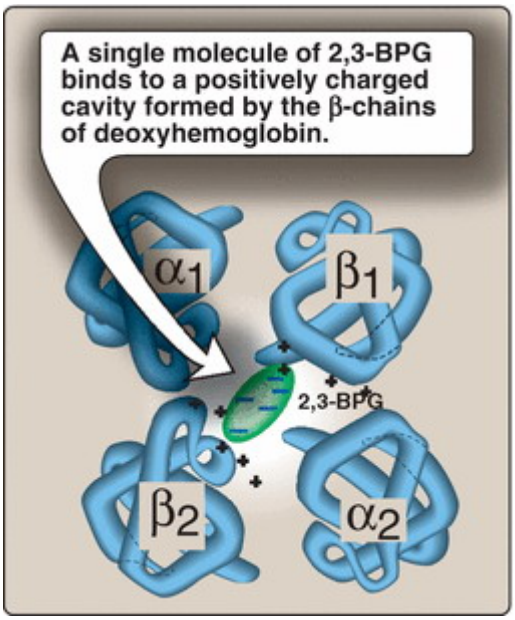
$$\underset{\text{Oxyhemoglobin}}{HbO_2} + \text{2,3-BPG} \rightleftarrows \underset{\text{Deoxyhemoglobin}}{\text{Hb} - \text{2,3-BPG}} + O_2$$

2,3-BPG binding site

One molecule of 2,3-BPG binds to a pocket, formed by the two β-globin chains, in the center of the deoxyhemoglobin tetramer (**Fig. 3.11**). This pocket contains several positively charged amino acids that form ionic bonds with the negatively charged phosphate groups of 2,3-BPG. [Note: Replacement of one of these amino acids can result in hemoglobin variants with abnormally high oxygen affinity that may be compensated for by increased RBC production (erythrocytosis).] Oxygenation of hemoglobin narrows the pocket and causes 2,3-BPG to be released.

FIGURE 3.11

Binding of 2,3-bisphosphoglycerate (2,3-BPG) by deoxyhemoglobin.

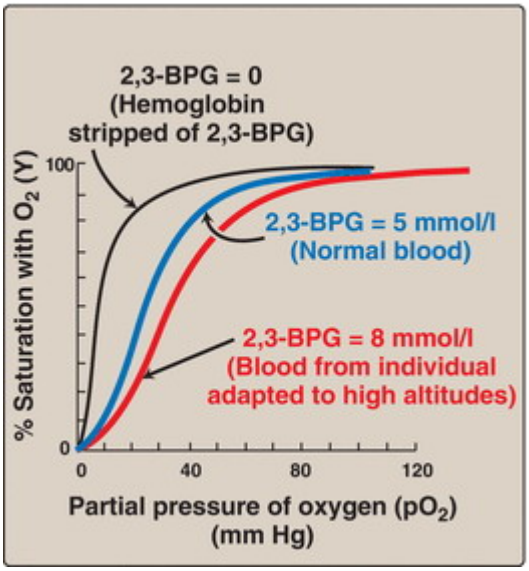


Oxygen-dissociation curve shift

Hemoglobin from which 2,3-BPG has been removed has high oxygen affinity. However, as seen in the RBC, the presence of 2,3-BPG significantly reduces the oxygen affinity of hemoglobin, shifting the oxygen-dissociation curve to the right (**Fig. 3.12**). This reduced affinity enables hemoglobin to release O_2 efficiently at the partial pressures found in the tissues.

FIGURE 3.12

Allosteric effect of 2,3-bisphosphoglycerate (2,3-BPG) on the oxygen affinity of hemoglobin.



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2,3-BPG levels in chronic hypoxia or anemia

The concentration of 2,3-BPG in the RBC increases in response to chronic hypoxia, such as that observed in chronic obstructive pulmonary disease (COPD) like emphysema, or at high altitudes, where circulating hemoglobin may have difficulty receiving sufficient O_2. Intracellular levels of 2,3-BPG are also elevated in chronic anemia, in which fewer than normal RBC are available to supply the body's oxygen needs. Elevated 2,3-BPG levels lower the oxygen affinity of hemoglobin, permitting greater unloading of O_2 in the capillaries of tissues (see **Fig. 3.12**).

2,3-BPG in transfused blood

2,3-BPG is essential for the normal oxygen transport function of hemoglobin. However, storing blood in the currently available media results in the gradual depletion of 2,3-BPG. Consequently, stored blood displays an abnormally high oxygen affinity and fails to unload its bound O_2 properly in the tissues. Thus, hemoglobin deficient in 2,3-BPG acts as an oxygen "trap" rather than as an oxygen delivery system. Transfused RBC are able to restore their depleted supplies of 2,3-BPG in 6–24 hours. However, severely ill patients may be compromised if transfused with large quantities of such 2,3-BPG–depleted blood. Stored blood, therefore, is treated with a "rejuvenation" solution that rapidly restores 2,3-BPG. [Note: Rejuvenation also restores ATP lost during storage.]

CO2 binding

Most of the CO_2 produced in metabolism is hydrated and transported as bicarbonate ion (see **Fig. 1.12** on p. 9). However, some CO_2 is carried as carbamate bound to the terminal amino groups of hemoglobin (forming carbaminohemoglobin as shown in **Fig. 3.8**), which can be represented schematically as follows:

$$Hb-NH_2 + CO_2 \rightleftarrows Hb-NH-COO^- + H^+$$

The binding of CO_2 stabilizes the T, or deoxy, form of hemoglobin, resulting in a decrease in its oxygen affinity (see p. 28) and a right shift in the oxygen-dissociation curve. In the lungs, CO_2 dissociates from the hemoglobin and is released in the breath.

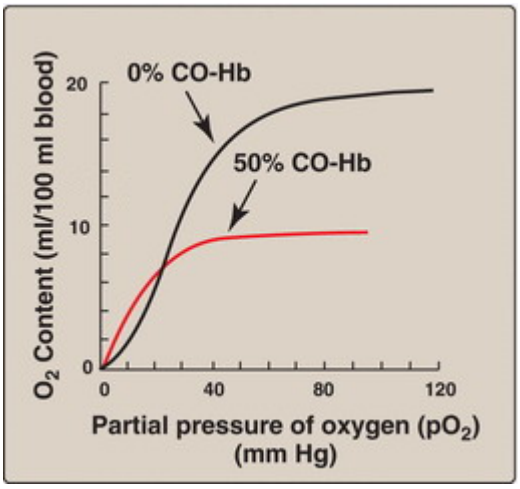
CO binding

Carbon monoxide (CO) binds tightly (but reversibly) to the hemoglobin iron, forming carboxyhemoglobin. When CO binds to one or more of the four heme sites, hemoglobin shifts to the R conformation, causing the remaining heme sites to bind O_2 with high affinity. This shifts the oxygen-dissociation curve to the left and changes the normal sigmoidal shape toward a hyperbola. As a result, the affected hemoglobin is unable to release O_2 to the tissues (**Fig. 3.13**). [Note: The affinity of hemoglobin for CO is 220 times greater than for O_2. Consequently, even minute concentrations of CO in the environment can produce toxic concentrations of carboxyhemoglobin in the blood. For example, increased levels of CO are found in the blood of tobacco smokers. CO toxicity appears to result from a combination of tissue hypoxia and direct CO-mediated damage at the cellular level.] CO poisoning is treated with 100% O_2 at high pressure (hyperbaric oxygen therapy), which facilitates the dissociation of CO from the hemoglobin. [Note: CO inhibits Complex IV of the electron transport chain (see p. 76).] In addition to O_2, CO_2, and CO, nitric oxide gas (NO) also is carried by hemoglobin. NO is a potent vasodilator (see p. 151). It can be taken up (salvaged) or released from RBC, thereby modulating NO availability and influencing vessel diameter.

FIGURE 3.13

Effect of carbon monoxide (CO) on the oxygen affinity of hemoglobin.

CO competes with O_2 for binding the heme iron. CO-Hb = carboxyhemoglobin (carbon monoxyhemoglobin).



Minor hemoglobins

It is important to remember that human hemoglobin A (HbA) is just one member of a functionally and structurally related family of proteins, the hemoglobins (**Fig. 3.14**). Each of these oxygen-carrying proteins is a tetramer, composed of two α-globin (or α-like) polypeptides and two β-globin (or β-like) polypeptides. Certain hemoglobins, such as HbF, are normally synthesized only during fetal development, whereas others, such as HbA_2, are synthesized in the adult, although at low levels compared with HbA. HbA can also become modified by the covalent addition of a hexose (see 3. below).

FIGURE 3.14

Normal adult human hemoglobins.

HbA_{1c} is a subtype of HbA (or, HbA_1). [Note: The α chains in these hemoglobins are identical.] Hb = hemoglobin.

Form	Chain composition	Fraction of total hemoglobin
HbA	$\alpha_2\beta_2$	90%
HbA_2	$\alpha_2\delta_2$	2%–3%
HbF	$\alpha_2\gamma_2$	<2%
HbA_{1c}	$\alpha_2\beta_2$-glucose	4%–6%

Fetal hemoglobin

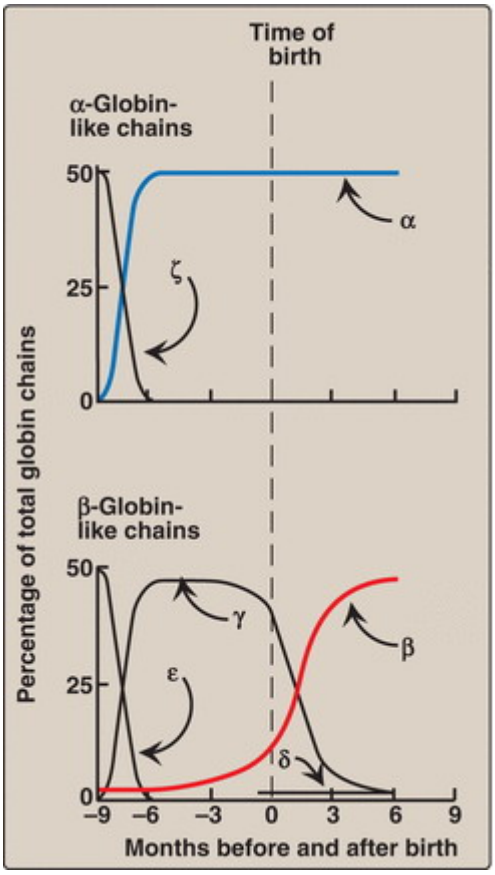
HbF is a tetramer consisting of two α chains identical to those found in HbA, plus two γ chains ($\alpha_2\gamma_2$; see **Fig. 3.14**). The γ chains are members of the β-globin gene family (see p. 34).

HbF synthesis during development

In the first month after conception, embryonic hemoglobins such as Hb Gower 1, composed of two α-like zeta (ζ) chains and two β-like epsilon (ε) chains ($\zeta_2\varepsilon_2$), are synthesized by the embryonic yolk sac. In the fifth week of gestation, the site of globin synthesis shifts, first to the liver and then to the marrow, and the primary product is HbF. HbF is the major hemoglobin found in the fetus and newborn, accounting for ~60% of the total hemoglobin in the RBC during the last months of fetal life (**Fig. 3.15**). HbA synthesis starts in the bone marrow at about the eighth month of pregnancy and gradually replaces HbF. **Figure 3.15** shows the relative production of each type of hemoglobin chain during fetal and postnatal life. [Note: HbF represents <2% of the hemoglobin in most adults and is concentrated in RBC known as F cells.]

FIGURE 3.15

Developmental changes in globin production.



2,3-BPG binding to HbF

Under physiologic conditions, HbF has a higher oxygen affinity than does HbA as a result of HbF only weakly binding 2,3-BPG. [Note: The γ-globin chains of HbF lack some of the positively charged amino acids that are responsible for binding 2,3-BPG in the β-globin chains.] Because 2,3-BPG serves to reduce the oxygen affinity of hemoglobin, the weaker interaction between 2,3-BPG and HbF results in a higher oxygen affinity for HbF relative to HbA. In contrast, if both HbA and HbF are stripped of their 2,3-BPG, they then have a similar oxygen affinity. The higher oxygen affinity of HbF facilitates the transfer of O_2 from the maternal circulation across the placenta to the RBC of the fetus.

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Hemoglobin A2

HbA_2 is a minor component of normal adult hemoglobin, first appearing shortly before birth and, ultimately, constituting ~2% of the total hemoglobin. It is composed of two α-globin chains and two δ-globin chains ($\alpha_2\delta_2$; see **Fig. 3.14**).

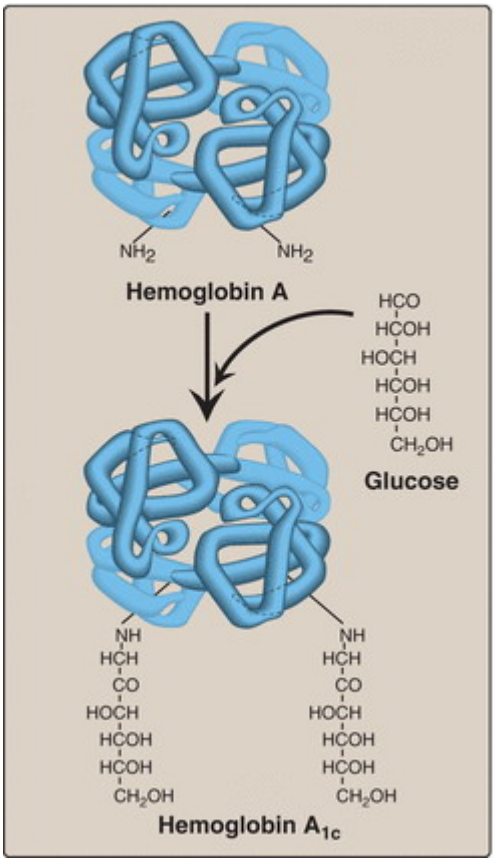
Hemoglobin A1c

Under physiologic conditions, HbA is slowly glycated (nonenzymically condensed with a hexose), the extent of glycation being dependent on the plasma concentration of the hexose. The most abundant form of glycated hemoglobin is HbA_{1c}. It has glucose residues attached predominantly to the amino groups of the N-terminal valines of the β-globin chains (**Fig. 3.16**). Increased amounts of HbA_{1c} are found in RBC of patients with diabetes mellitus, because their HbA has contact with higher glucose concentrations during the 120-day lifetime of these cells. (See p. 340 for a discussion of the use of HbA_{1c} levels in assessing average blood glucose levels in patients with diabetes.)

FIGURE 3.16

Nonenzymatic addition of glucose to hemoglobin.

The nonenzymatic addition of a sugar to a protein is referred to as glycation.



Globin Gene Organization

To understand diseases resulting from genetic alterations in the structure or synthesis of hemoglobin, it is necessary to grasp how the hemoglobin genes, which direct the synthesis of the different globin chains, are structurally organized into gene families and also how they are expressed.

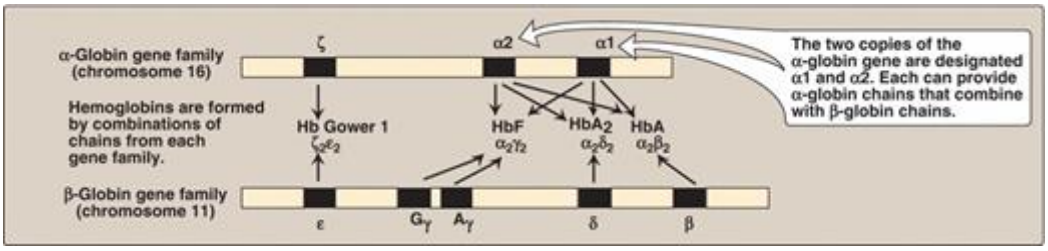
α-Gene family

The genes coding for the α-globin and β-globin subunits of the hemoglobin chains occur in two separate gene clusters (or families) located on two different chromosomes (**Fig. 3.17**). The α-gene cluster on chromosome 16 contains two genes for the α-globin chains. It also contains the ζ gene that is expressed early in development as an α-globin-like component of embryonic hemoglobin. [Note: Globin gene families also contain globin-like genes that are not expressed, that is, their genetic information is not used to produce globin chains. These are called pseudogenes.]

FIGURE 3.17

Organization of the globin gene families.

Hb = hemoglobin.



β-Gene family

A single gene for the β-globin chain is located on chromosome 11 (see **Fig. 3.17**). There are an additional four β-globin-like genes: the ε gene (which, like the ζ gene, is expressed early in embryonic development), two γ genes (G_γ and A_γ that are expressed in HbF), and the δ gene that codes for the globin chain found in the minor adult hemoglobin HbA_2.

Steps in globin chain synthesis

Expression of a globin gene begins in the nucleus of RBC precursors, where the DNA sequence encoding the gene is transcribed. The ribonucleic acid (RNA) produced by transcription is actually a precursor of the messenger RNA (mRNA) that is used as a template for the synthesis of a globin chain. Before it can serve this function, two noncoding stretches of RNA (introns) must be removed from the mRNA precursor sequence and the remaining three fragments (exons) joined in a linear manner. The resulting mature mRNA enters the cytosol, where its genetic information is translated, producing a globin chain. (A summary of this process is shown in **Figure 3.18**. A more detailed description of gene expression is presented in Unit VII, **Chapters 30**, **31**, **32**.)

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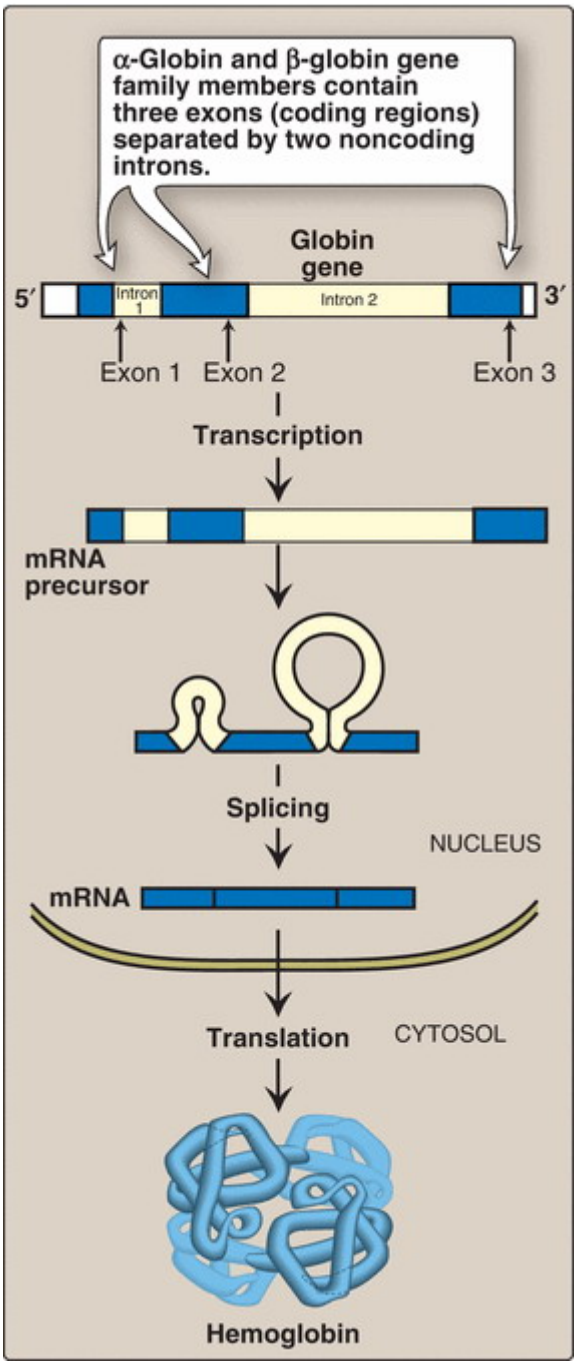
FIGURE 3.18

Synthesis of globin chains.

mRNA = messenger ribonucleic acid.



Hemoglobinopathies

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Hemoglobinopathies are defined as a group of genetic disorders caused by production of a structurally abnormal hemoglobin molecule, synthesis of insufficient quantities of normal hemoglobin, or, rarely, both. Sickle cell anemia (HbS), hemoglobin C disease (HbC), hemoglobin SC disease (HbS + HbC = HbSC), and the thalassemias are representative hemoglobinopathies that can have severe clinical consequences. The first three conditions result from production of hemoglobin with an altered amino acid sequence (qualitative hemoglobinopathy), whereas the thalassemias are caused by decreased production of normal hemoglobin (quantitative hemoglobinopathy).

Sickle cell anemia (hemoglobin S disease)

Sickle cell anemia, the most common of the RBC sickling diseases, is a genetic disorder caused by a single nucleotide substitution (a point mutation, see p. 449) in the gene for β-globin. It is the most common inherited blood disorder in the United States, affecting 50,000 Americans. It occurs primarily in the African American population, affecting 1 in 500 newborn African American infants. Sickle cell anemia is an autosomal-recessive disorder. It occurs in individuals who have inherited two mutant genes (one from each parent) that code for synthesis of the β chains of the globin molecules. [Note: The mutant β-globin chain is designated β^S, and the resulting hemoglobin, $\alpha_2\beta^S_2$, is referred to as HbS.] An infant does not begin showing symptoms of the disease until sufficient HbF has been replaced by HbS so that sickling can occur (see p. 36). Sickle cell anemia is characterized by lifelong episodes of pain (“crises”), chronic hemolytic anemia with associated hyperbilirubinemia (see p. 284), and increased susceptibility to infections, usually beginning in infancy. [Note: The lifetime of a RBC in sickle cell anemia is <20 days, compared with 120 days for normal RBC, hence, the anemia.] Other symptoms include acute chest syndrome, stroke, splenic and renal dysfunction, and bone changes due to marrow hyperplasia. Life expectancy is reduced. Heterozygotes, representing 1 in 12 African Americans, have one normal and one sickle cell gene. The blood cells of such heterozygotes contain both HbS and HbA, and these individuals have sickle cell trait. They usually do not show clinical symptoms (but may under conditions of extreme physical exertion with dehydration) and can have a normal life span.

Amino acid substitution in HbS β chains:

A molecule of HbS contains two normal α-globin chains and two mutant β-globin chains (β^S), in which glutamate at position six has been replaced with valine (**Fig. 3.19**). Therefore, during electrophoresis at alkaline pH, HbS migrates more slowly toward the anode (positive electrode) than does HbA (**Fig. 3.20**). This altered mobility of HbS is a result of the absence of the negatively charged glutamate residues in the two β chains, thereby rendering HbS less negative than HbA. [Note: Electrophoresis of hemoglobin obtained from lysed RBC is routinely used in the diagnosis of sickle cell trait and sickle cell anemia (or, sickle cell disease). DNA analysis also is used (see p. 493).]

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FIGURE 3.19

Amino acid substitutions in hemoglobin S (HbS) and hemoglobin C (HbC).

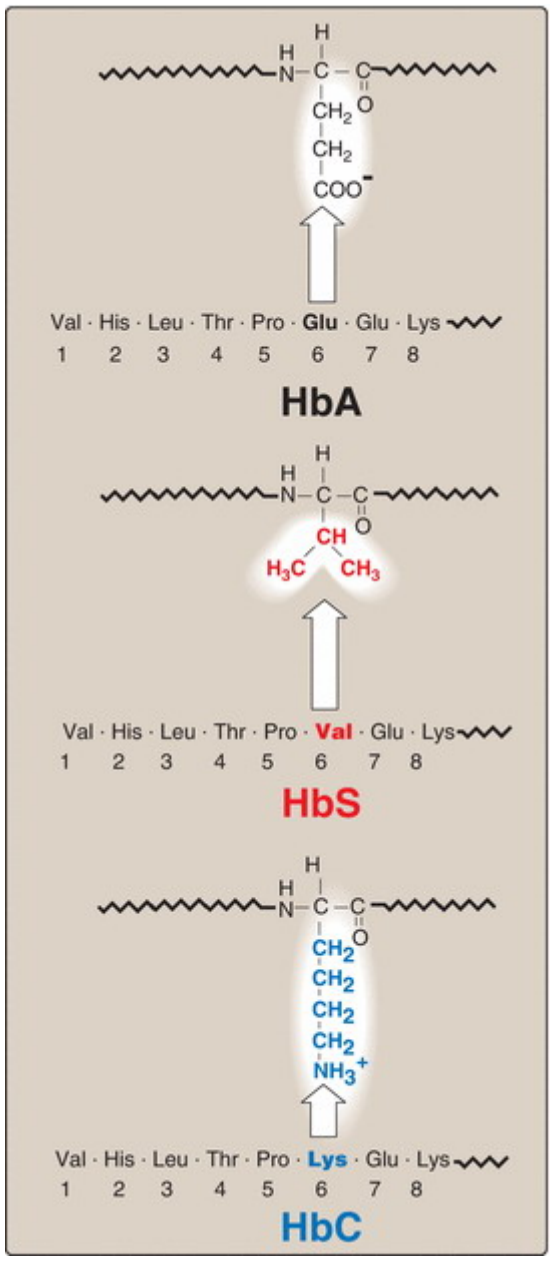

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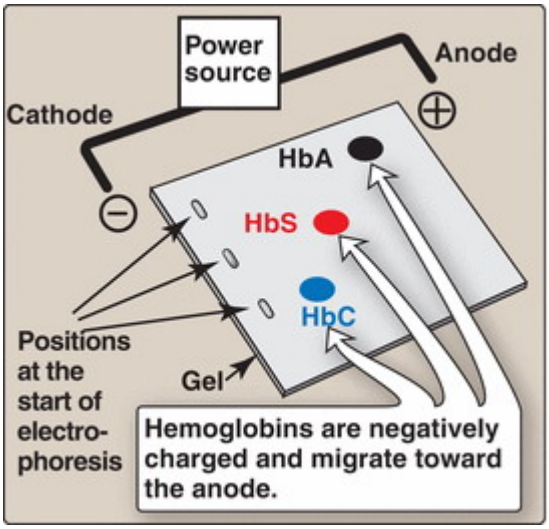
FIGURE 3.20

Diagram of hemoglobins (HbA), (HbS), and (HbC) after electrophoresis.



Sickling and tissue anoxia

The replacement of the charged glutamate with the nonpolar valine forms a protrusion on the β chain that fits into a complementary site on the β chain of another hemoglobin molecule in the cell (**Fig. 3.21**). At low oxygen tension, deoxyhemoglobin S polymerizes inside the RBC, forming a network of insoluble fibrous polymers that stiffen and distort the cell, producing rigid, misshapen RBC. Such sickled cells frequently block the flow of blood in the narrow capillaries. This interruption in the supply of O_2 leads to localized anoxia (oxygen deprivation) in the tissue, causing pain and eventually ischemic death (infarction) of cells in the vicinity of the blockage. The anoxia also leads to an increase in deoxygenated HbS. [Note: The mean diameter of RBC is 7.5 μm, whereas that of the microvasculature is 3–4 μm. Compared to normal RBC, sickled cells have a decreased ability to deform and an increased tendency to adhere to vessel walls. This makes moving through small vessels difficult, thereby causing microvascular occlusion.]

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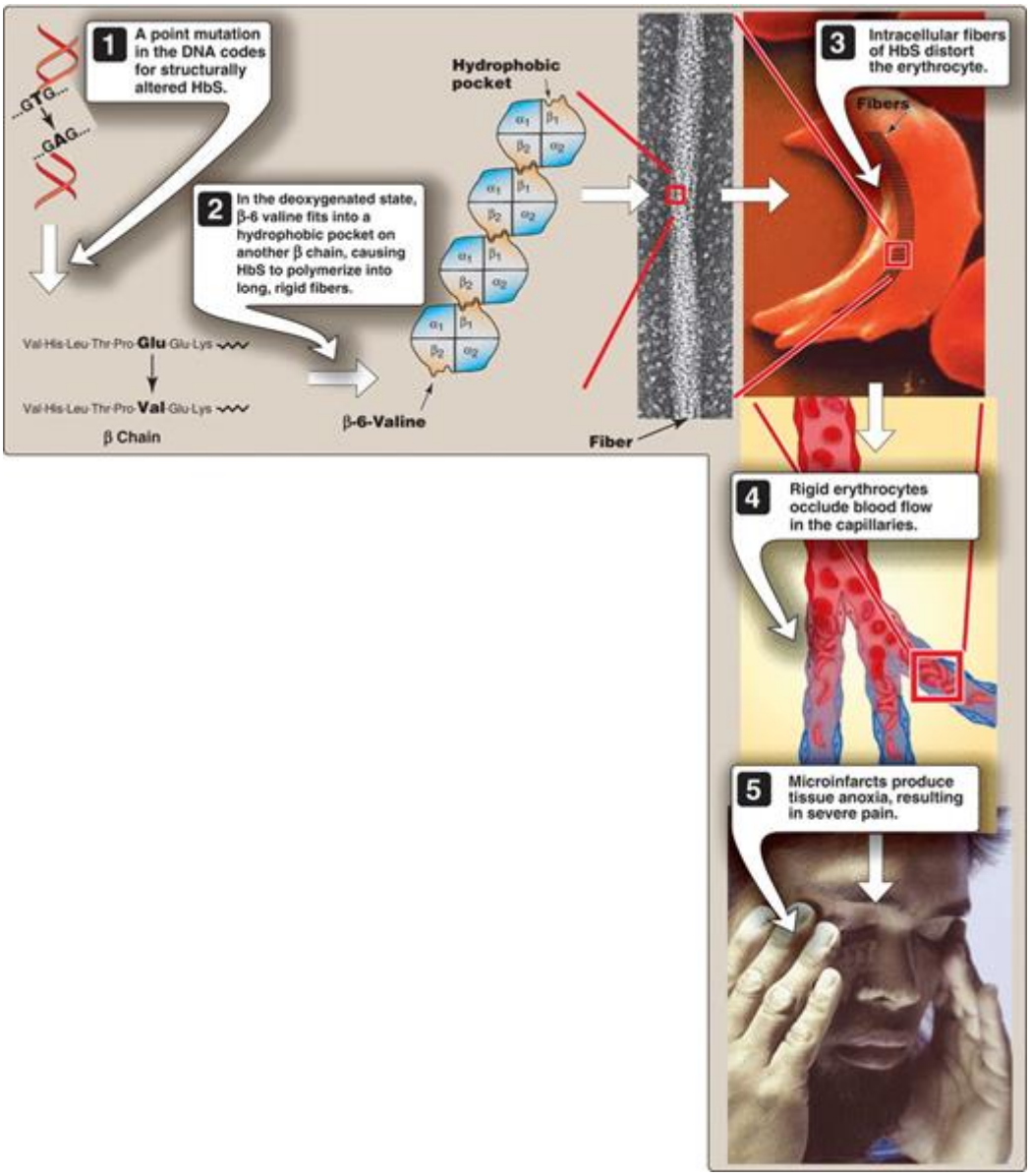
FIGURE 3.21

Molecular and cellular events leading to sickle cell crisis.

HbS = hemoglobin S.

Photo courtesy of Photodyne Incorporated, Hartland, WI.



Variables that increase sickling

The extent of sickling and, therefore, the severity of disease are enhanced by any variable that increases the proportion of HbS in the deoxy state (that is, reduces the oxygen affinity of HbS). These variables include decreased pO_2, increased pCO_2, decreased pH, dehydration, and an increased concentration of 2,3-BPG in RBC.

Treatment

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Therapy involves adequate hydration, analgesics, aggressive antibiotic therapy if infection is present, and transfusions in patients at high risk for fatal occlusion of blood vessels. Intermittent transfusions with packed RBC reduce the risk of stroke, but the benefits must be weighed against the complications of transfusion, which include iron overload that can result in hemosiderosis (see p. 404), bloodborne infections, and immunologic complications. Hydroxyurea (hydroxycarbamide), an antitumor drug, is therapeutically useful because it increases circulating levels of HbF, which decreases RBC sickling. This leads to decreased frequency of painful crises and reduces mortality. Stem cell transplantation is possible. [Note: The morbidity and mortality associated with sickle cell anemia has led to its inclusion in newborn screening panels to allow prophylactic antibiotic therapy to begin soon after the birth of an affected child.]

Possible selective advantage of the heterozygous state

The high frequency of the β^S mutation among black Africans, despite its damaging effects in the homozygous state, suggests that a selective advantage exists for heterozygous individuals. For example, heterozygotes for the sickle cell gene are less susceptible to the severe malaria caused by the parasite Plasmodium falciparum. This organism spends an obligatory part of its life cycle in the RBC. One theory is that because these cells in individuals heterozygous for HbS, like those in homozygotes, have a shorter life span than normal, the parasite cannot complete the intracellular stage of its development. This may provide a selective advantage to heterozygotes living in regions where malaria is a major cause of death. For example, in Africa, the geographic distribution of sickle cell anemia is similar to that of malaria.

Hemoglobin C disease

Like HbS, HbC is a hemoglobin variant that has a single amino acid substitution in the sixth position of the β-globin chain (see **Fig. 3.19**). In HbC, however, a lysine is substituted for the glutamate (as compared with a valine substitution in HbS). [Note: This substitution causes HbC to move more slowly toward the anode than HbA or HbS does (see **Fig. 3.20**).] Rare patients homozygous for HbC generally have a relatively mild, chronic hemolytic anemia. They do not suffer from infarctive crises, and no specific therapy is required.

Hemoglobin SC disease

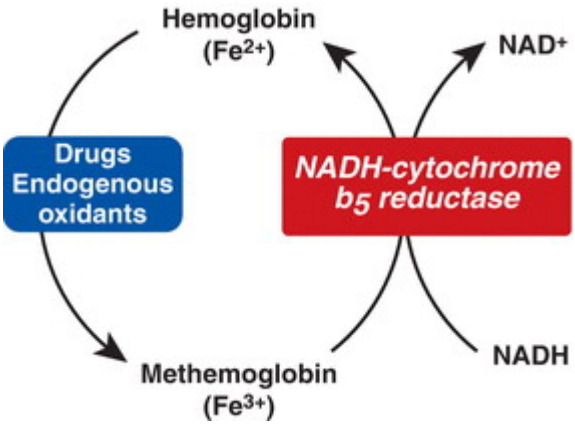
HbSC disease is another of the RBC sickling diseases. In this disease, some β-globin chains have the sickle cell mutation, whereas other β-globin chains carry the mutation found in HbC disease. [Note: Patients with HbSC disease are doubly heterozygous. They are called compound heterozygotes because both of their β-globin genes are abnormal, although different from each other.] Hemoglobin levels tend to be higher in HbSC disease than in sickle cell anemia and may even be at the low end of the normal range. The clinical course of adults with HbSC anemia differs from that of sickle cell anemia in that symptoms such as painful crises are less frequent and less severe. However, there is significant clinical variability.

Methemoglobinemias

Oxidation of the heme iron in hemoglobin from Fe^{2+} to Fe^{3+} produces methemoglobin, which cannot bind O_2. This oxidation may be acquired and caused by the action of certain drugs, such as nitrates, or endogenous products such as reactive oxygen species (see p. 148). The oxidation may also result from congenital defects, for example, a deficiency of NADH-cytochrome b_5 reductase (also called NADH-methemoglobin reductase), the enzyme responsible for the conversion of methemoglobin (Fe^{3+}) to hemoglobin (Fe^{2+}), leads to the accumulation of methemoglobin (**Fig. 3.22**). [Note: The RBC of newborns have approximately half the capacity of those of adults to reduce methemoglobin.] Additionally, rare mutations in the α- or β-globin chain can cause the production of HbM, an abnormal hemoglobin that is resistant to the reductase. The methemoglobinemias are characterized by "chocolate cyanosis" (a blue coloration of the skin and mucous membranes and brown-colored blood) as a result of the dark-colored methemoglobin. Symptoms are related to the degree of tissue hypoxia and include anxiety, headache, and dyspnea. In rare cases, coma and death can occur. Treatment is with methylene blue, which is oxidized as Fe^{3+} is reduced.

FIGURE 3.22

Formation of methemoglobin and its reduction to hemoglobin by *NADH-cytochrome b_5 reductase*.



Thalassemias

The thalassemias are hereditary hemolytic diseases in which an imbalance occurs in the synthesis of globin chains. As a group, they are the most common single-gene disorders in humans. Normally, synthesis of the α- and β-globin chains is coordinated, so that each α-globin chain has a β-globin chain partner. This leads to the formation of $\alpha_2\beta_2$ (HbA). In the thalassemias, the synthesis of either the α- or the β-globin chain is defective, and hemoglobin concentration is reduced. A thalassemia can be caused by a variety of mutations, including entire gene deletions, or substitutions or deletions of one of many nucleotides in the DNA. [Note: Each thalassemia can be classified as either a disorder in which no globin chains are produced (α^0- or β^0-thalassemia), or one in which some chains are synthesized but at a reduced level (α^+- or β^+-thalassemia).]

β-Thalassemias

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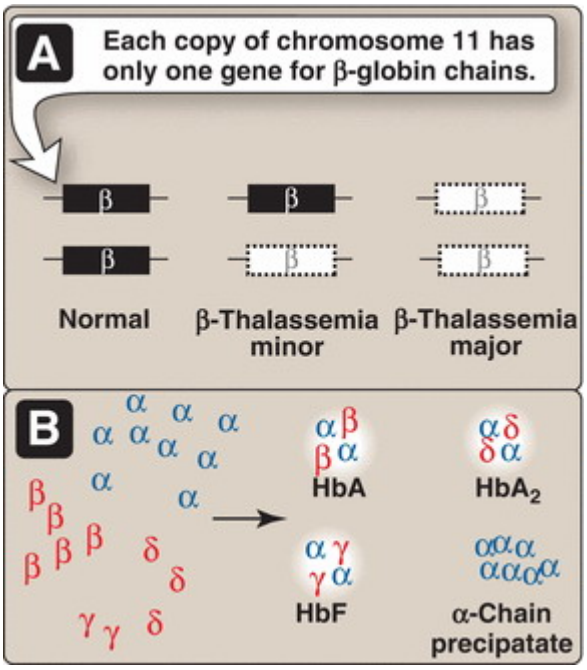
In these disorders, synthesis of β-globin chains is decreased or absent, typically as a result of point mutations that affect the production of functional mRNA. However, α-globin chain synthesis is normal. Excess α-globin chains cannot form stable tetramers and so precipitate, causing the premature death of cells initially destined to become mature RBC. Increase in $\alpha_2\delta_2$ (HbA_2) and $\alpha_2\gamma_2$ (HbF) also occurs. There are only two copies of the β-globin gene in each cell (one on each chromosome 11). Therefore, individuals with β-globin gene defects have either β-thalassemia trait (β-thalassemia minor) if they have only one defective β-globin gene or β-thalassemia major (Cooley anemia) if both genes are defective (**Fig. 3.23**). Because the β-globin gene is not expressed until late in prenatal development, the physical manifestations of β-thalassemias appear only several months after birth. Those individuals with β-thalassemia minor make some β chains and usually do not require specific treatment. However, those infants born with β-thalassemia major are seemingly healthy at birth but become severely anemic, usually during the first or second year of life, due to ineffective erythropoiesis. Skeletal changes as a result of extramedullary hematopoiesis also are seen. These patients require regular transfusions of blood. [Note: Although this treatment is lifesaving, the cumulative effect of the transfusions is iron overload. Use of iron chelation therapy has improved morbidity and mortality.] The only curative option available is hematopoietic stem cell transplantation.

FIGURE 3.23

A. β-Globin gene mutations in the β-thalassemias. **B.** Hemoglobin (Hb) tetramers formed in β-thalassemias.



α-Thalassemias

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In these disorders, synthesis of α-globin chains is decreased or absent, typically as a result of deletional mutations. Because each individual's genome contains four copies of the α-globin gene (two on each chromosome 16), there are several levels of α-globin chain deficiencies (**Fig. 3.24**). If one of the four genes is defective, the individual is termed a "silent" carrier of α-thalassemia, because no physical manifestations of the disease occur. If two α-globin genes are defective, the individual is designated as having α-thalassemia trait. If three α-globin genes are defective, the individual has hemoglobin H (β_4) disease, a hemolytic anemia of variable severity. If all four α-globin genes are defective, hemoglobin Bart (γ_4) disease with hydrops fetalis and fetal death results, because α-globin chains are required for the synthesis of HbF. [Note: Heterozygote advantage against malaria is seen in both α- and β-thalassemias.]

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FIGURE 3.24

A. α-Globin gene deletions in the α-thalassemias. **B.** Hemoglobin (Hb) tetramers formed in α-thalassemias.

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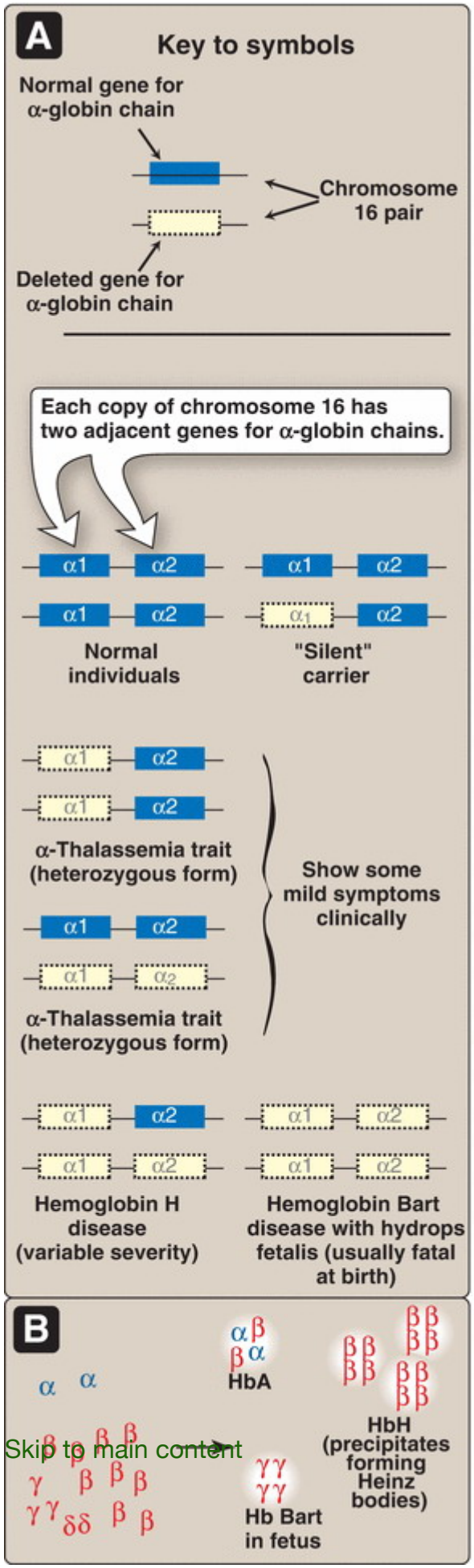

Chapter Summary

Hemoglobin A (**HbA**), the major hemoglobin in adults, is composed of four polypeptide chains (two α chains and two β chains, $\alpha_2\beta_2$) held together by noncovalent interactions (**Fig. 3.25**). The subunits occupy different relative positions in deoxyhemoglobin compared with oxyhemoglobin. The **deoxy form** of Hb is called the "**T**," or **taut** (**tense**), **conformation**. It has a constrained structure that limits the movement of the polypeptide chains. The T form is the **low-oxygen-affinity form** of Hb. The binding of oxygen (O_2) to the heme iron causes rupture of some of the ionic and hydrogen bonds and movement of the dimers. This leads to a structure called the "**R**," or **relaxed**, **conformation**. The R form is the **high-oxygen-affinity form** of Hb. The **oxygen-dissociation curve** for Hb is **sigmoidal** in shape (in contrast to that of **myoglobin**, which is **hyperbolic**), indicating that the subunits cooperate in binding O_2. The binding of an oxygen molecule at one heme group increases the oxygen affinity of the remaining heme groups in the same Hb molecule (**cooperativity**). Hb's ability to bind O_2 reversibly is affected by the partial pressure of oxygen (**pO_2**), the **pH** of the environment, the partial pressure of carbon dioxide (**pCO_2**), and the availability of **2,3-bisphosphoglycerate** (**2,3-BPG**). For example, the release of O_2 from Hb is enhanced when the pH is lowered or the pCO_2 is increased (the **Bohr effect**), such as in **exercising muscle**, and the oxygen-dissociation curve of Hb is shifted to the right. To cope long-term with the effects of **chronic hypoxia** or **anemia**, the concentration of **2,3-BPG** in **red blood cells** increases. **2,3-BPG** binds to the Hb and decreases its oxygen affinity. It therefore also shifts the oxygen-dissociation curve to the right. **Fetal hemoglobin (HbF)** binds 2,3-BPG less tightly than does HbA and has a higher oxygen affinity. **Carbon monoxide** (**CO**) binds tightly (but reversibly) to the Hb iron, forming **carboxyhemoglobin**. **Hemoglobinopathies** are disorders primarily caused either by production of a structurally abnormal Hb molecule as in **sickle cell anemia** or synthesis of insufficient quantities of normal Hb subunits as in the **thalassemias** (**Fig. 3.26**).

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FIGURE 3.25

Key concept map for hemoglobin structure and function.

Fe^{2+} = ferrous iron.

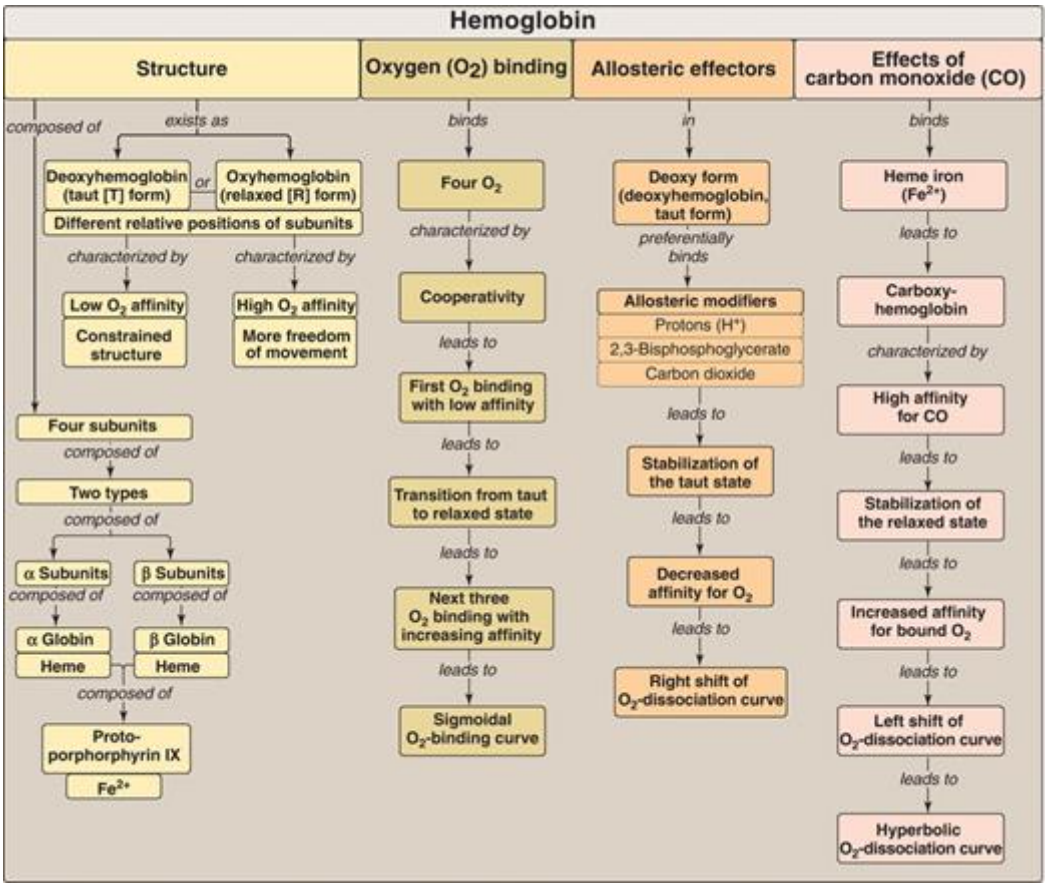

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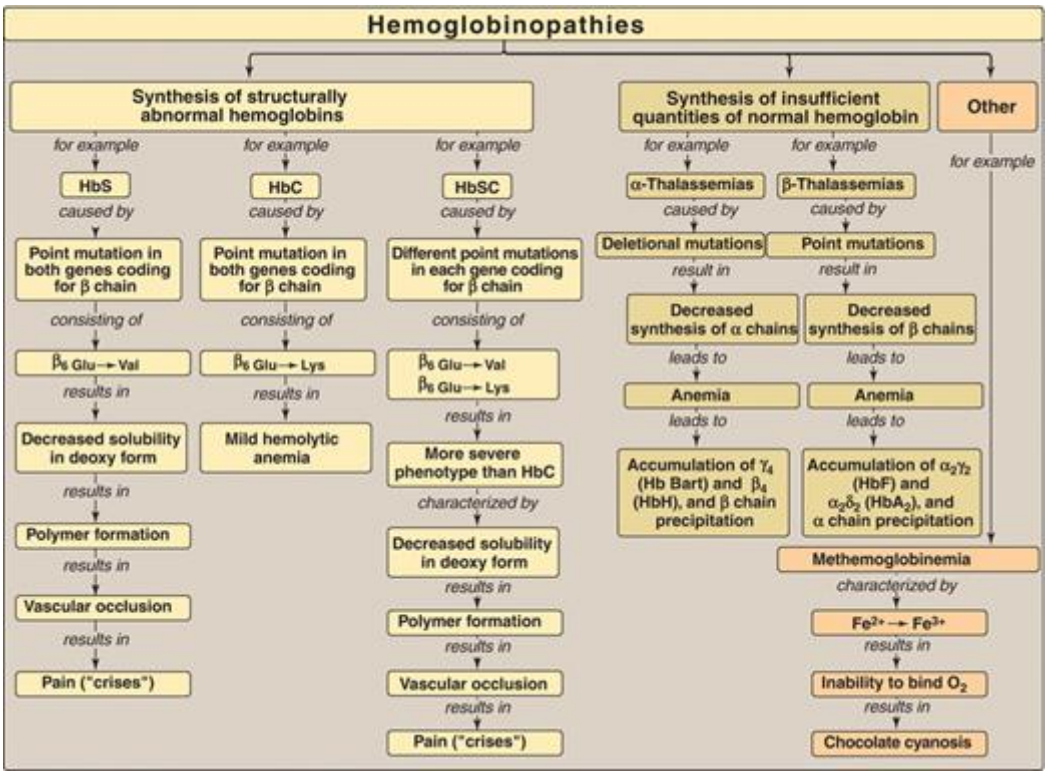
FIGURE 3.26

Key concept map for hemoglobinopathies.

Hb = hemoglobin; Fe = iron; O_2 = oxygen.



Study Questions

Choose the ONE best answer.

3.1. Which one of the following statements concerning the hemoglobins is correct?

A. HbA is the most abundant hemoglobin in normal adults.

B. Fetal blood has a lower affinity for oxygen than does adult blood because HbF has an increased affinity for 2,3-bisphosphoglycerate.

C. The globin chain composition of HbF is $\alpha_2\delta_2$.

D. HbA_{1c} differs from HbA by a single, genetically determined amino acid substitution.

E. HbA_2 appears early in fetal life.

Correct answer = A. HbA accounts for over 90% of the hemoglobin in a normal adult. If HbA_{1c} is included, the percentage rises to ~97%. Because 2,3-bisphosphoglycerate (2,3-BPG) reduces the affinity of hemoglobin for oxygen, the weaker interaction between 2,3-BPG and HbF results in a higher oxygen affinity for HbF relative to HbA. HbF consists of $\alpha_2\gamma_2$. HbA_{1c} is a glycated form of HbA, formed nonenzymically in red blood cells. HbA_2 is a minor component of normal adult hemoglobin, first appearing shortly before birth and rising to adult levels (~2% of the total hemoglobin) by age 6 months.

3.2. Which one of the following statements concerning the ability of acidosis to precipitate a crisis in sickle cell anemia is correct?

A. Acidosis decreases the solubility of HbS.

B. Acidosis increases the oxygen affinity of hemoglobin.

C. Acidosis favors the conversion of hemoglobin from the taut to the relaxed conformation.

D. Acidosis shifts the oxygen-dissociation curve to the left.

E. Acidosis decreases the ability of 2,3-bisphosphoglycerate to bind to hemoglobin.

Correct answer = A. HbS is significantly less soluble in the deoxygenated form, compared with oxyhemoglobin S. Decreased pH (acidosis) causes the oxygen-dissociation curve to shift to the right, indicating decreased oxygen affinity (increased delivery). This favors the formation of the deoxy, or taut, form of hemoglobin and can precipitate a sickle cell crisis. The binding of 2,3-bisphosphoglycerate is increased, because it binds only to the deoxy form of hemoglobin.

3.3. Which one of the following statements concerning the binding of oxygen by hemoglobin is correct?

A. The Bohr effect results in a lower oxygen affinity at higher pH values.

B. Carbon dioxide increases the oxygen affinity of hemoglobin by binding to the C-terminal groups of the polypeptide chains.

C. The oxygen affinity of hemoglobin increases as the percentage saturation increases.

D. The hemoglobin tetramer binds four molecules of 2,3-bisphosphoglycerate.

E. Oxyhemoglobin and deoxyhemoglobin have the same affinity for protons.

Correct answer = C. The binding of oxygen at one heme group increases the oxygen affinity of the remaining heme groups in the same molecule. A rise in pH results in increased oxygen affinity. Carbon dioxide decreases oxygen affinity because it lowers the pH. Moreover, binding of carbon dioxide to the N-termini stabilizes the taut, deoxy form. Hemoglobin binds one molecule of 2,3-bisphosphoglycerate. Deoxyhemoglobin has a greater affinity for protons than does oxyhemoglobin.

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3.4. β-Lysine 82 in HbA is important for the binding of 2,3-bisphosphoglycerate. In Hb Helsinki, this amino acid has been replaced by methionine. Which of the following should be true concerning Hb Helsinki?

A. It should be stabilized in the taut, rather than the relaxed, form.

B. It should have increased oxygen affinity and, consequently, decreased oxygen delivery to tissues.

C. Its oxygen-dissociation curve should be shifted to the right relative to HbA.

D. It results in anemia.

Correct answer = B. Substitution of lysine by methionine decreases the ability of negatively charged phosphate groups in 2,3-bisphosphoglycerate (2,3-BPG) to bind the β subunits of hemoglobin. Because 2,3-BPG decreases the oxygen affinity of hemoglobin, a reduction in 2,3-BPG should result in increased oxygen affinity and decreased oxygen (O2) delivery to tissues. The relaxed form is the high-oxygen-affinity form of hemoglobin. Increased oxygen affinity (decreased delivery) results in a left shift in the oxygen-dissociation curve. Decreased delivery of O_2 is compensated for by increased RBC production.

3.5. A 67-year-old man presented to the emergency department with a 1-week history of angina and shortness of breath. He complained that his face and extremities had taken on a blue color. His medical history included chronic stable angina treated with isosorbide dinitrate and nitroglycerin. Blood obtained for analysis was brown. Which one of the following is the most likely diagnosis?

A. Carboxyhemoglobinemia

B. Hemoglobin SC disease

C. Methemoglobinemia

D. Sickle cell anemia

E. β-Thalassemia

Correct answer = C. Oxidation of the ferrous (Fe^{2+}) iron to the ferric (Fe^{3+}) state in the heme prosthetic group of hemoglobin forms methemoglobin. This may be caused by the action of certain drugs such as nitrates. The methemoglobinemias are characterized by chocolate cyanosis (a blue coloration of the skin and mucous membranes and chocolate-colored blood) as a result of the dark-colored methemoglobin. Symptoms are related to tissue hypoxia and include anxiety, headache, and dyspnea. In rare cases, coma and death can occur. [Note: Benzocaine, an aromatic amine used as a topical anesthetic, is a cause of acquired methemoglobinemia.]

3.6. Why is hemoglobin C disease a nonsickling disease?

In HbC, the polar glutamate is replaced by polar lysine rather than by nonpolar valine as in HbS.

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3.7. What would be true about the extent of red blood cell sickling in individuals with HbS and hereditary persistence of HbF?

It would be decreased because HbF reduces HbS concentration. It also inhibits polymerization of deoxy HbS.