Chapter 7 Protein Function

The proteins responsible for oxygen binding and transport, muscle contraction, and the immune response are discussed in this chapter. These examples are chosen because of the depth of information known about them as well as their importance to human health. The function of a protein is dictated by its amino acid sequence and is revealed in its secondary, tertiary, and quaternary structure. Myoglobin and hemoglobin are globular soluble proteins that bind oxygen. Muscle proteins, on the other hand, form long filamentous bundles that contract upon the hydrolysis of ATP. Antibodies are proteins that have common overall structures but variable sequences, which thereby form billions of possible proteins, many of which specifically bind foreign macromolecules. Many amino acid residues are critical for these functions, so as you read and study this chapter, try to glean the importance of structure–function relationships in each example.

Myoglobin and hemoglobin are involved in oxygen storage and transport. Myoglobin, whose primary role is to facilitate oxygen transport in muscle (and, in aquatic mammals, to store oxygen), binds oxygen with a simple equilibrium constant. Hemoglobin transports oxygen, first binding oxygen in the capillaries of the lungs (or gills or skin), where oxygen concentrations are high, and then releasing the oxygen in the tissues, where the oxygen concentrations are lower. The binding of oxygen to hemoglobin is not as simple as that for myoglobin. Several factors influence oxygen binding to hemoglobin, including the partial pressure of oxygen itself, pH, the concentration of CO_2, and 2,3-bisphosphoglycerate (BPG). The study of mutant hemoglobin molecules with amino acid substitutions that affect hemoglobin function provides important insights into the roles of individual amino acids.

Muscle fibers consist of bundles of myofibrils, which are striated due to the presence of repeating protein assemblies. The myofibrils consist of interdigitated thick and thin filaments. The thick filaments are made of myosin, whose two heavy chains and four light chains form a long rodlike segment with two globular heads. Hundreds of myosin molecules aggregate in a thick bundle. Thin filaments contain three proteins: actin, tropomyosin, and troponin. Interactions between the thick and thin filaments allow the proteins to move past each other in a process that is driven by the hydrolysis of ATP.

Antibodies are the first line of defense against disease-causing pathogens such as microorganisms and viruses. Cellular immunity is mediated by *T* lymphocytes, which are formed in the thymus. Humoral immunity is mediated by antibodies (immunoglobulins) that are produced by *B* lymphocytes, which mature in the bone marrow. A *B* cell makes only one type of antibody. Antigen binding to a specific antibody located on the surface of a *B* cell triggers an immune response in which the *B* cells secreting that antibody proliferate. *B* cells usually live for a few days; however, memory *B* cells that recognize specific antigens remain and proliferate when more antigen is present. The specificity of an antibody–antigen interaction arises from variable amino sequences in the immunoglobulin, which create a unique antigen-binding site.

Essential Concepts

Myoglobin

1. Myoglobin is a single polypeptide containing a heme group, which consists of a porphyrin ring whose coordinated Fe(II) atom binds molecular oxygen. The protein prevents oxidation of the heme iron to Fe(III), which does not bind oxygen. Oxidized myoglobin is called metmyoglobin.

2. Myoglobin facilitates oxygen transport in muscle, where the solubility of oxygen is low, and acts as an oxygen-storage protein in aquatic mammals. A simple equilibrium equation describes O_2 binding to myoglobin (MB): $Mb + O_2 \rightleftharpoons MbO_2$. The fractional saturation of myoglobin is defined as $Y_{O2} = pO_2/(K + pO_2)$, where pO_2 is the partial pressure of oxygen and K is the dissociation constant. A plot of Y_{O2} versus pO_2 is a simple hyperbolic binding curve. It is convenient to define K as p_{50}, the partial pressure of O_2 at which 50% of myoglobin has bound oxygen. The p_{50} for myoglobin is 2.8 torr, which is much lower than the pO_2 in venous blood (30 torr), so that myoglobin is nearly saturated with oxygen under physiological conditions.

Hemoglobin

3. Hemoglobin binds oxygen in the lungs (pO_2 = 100 torr) and releases it in the capillaries (pO_2 = 30 torr). The efficiency of oxygen transport is greater than expected if oxygen binding were hyperbolic, as in myoglobin. Hemoglobin, which is an $\alpha_2\beta_2$ tetramer, each of whose subunits contains a heme group, binds O_2 cooperatively and thus has a sigmoidal oxygen binding curve. Deoxyhemoglobin is bluish (the color of venous blood), whereas oxyhemoglobin is bright red (the color of arterial blood).

4. The p_{50} of hemoglobin is about 26 torr, which is nearly 10 times greater than that of myoglobin. Because hemoglobin exhibits a sigmoidal oxygen-binding curve, it releases a much greater fraction of its bound O_2 in passing from the lungs to the tissues than would myoglobin. The Hill equation describes the cooperative nature of oxygen binding, and the Hill constant, n, describes the degree of cooperativity. The Hill constant, which is not necessarily an integer, is obtained experimentally. The binding of O_2 to hemoglobin is said to be cooperative because the binding of O_2 to one subunit increases the O_2 affinity of the other subunits. The fourth oxygen to bind to hemoglobin does so with a 100-fold greater affinity than the first.

5. Hemoglobin has only two stable conformational states, the T state (the conformation of deoxyhemoglobin) and the R state (the conformation of oxyhemoglobin). Oxygen binding causes the T state to shift to the R state, which has greater affinity for oxygen. The T to R shift is triggered by oxygen binding to the heme iron, which pulls the heme iron atom into the heme plane. This movement is transmitted to the F helix through His F8, which ligands the iron atom. Conformational changes in one subunit are transmitted across the α_1–β_2 and α_2–β_1 interfaces. Due to the conformational constraints at these interfaces, the conformational shift

of one subunit must be accompanied by the conformational shift of all subunits, thereby increasing the oxygen affinity of the unoccupied subunits.

6. Decreases in pH promote the release of oxygen from hemoglobin. This effect, called the Bohr effect, is driven by dissolved carbon dioxide, which forms bicarbonate ion and a hydrogen ion. The hydrogen ion protonates hemoglobin, thereby stabilizing its T (deoxy) state. In the lungs, the reaction is reversed: Oxygen binding causes a switch to the R (oxy) state. The released hydrogen ions shift the equilibrium between bicarbonate and carbon dioxide, thereby forming carbon dioxide for expulsion from the lungs. Due to the Bohr effect, the low pH in highly active muscles causes the amount of oxygen delivered by hemoglobin to increase by nearly 10%. Carbon dioxide also binds preferentially to the N-terminal amine groups of T-state hemoglobin as carbamates and hence is released by R-state hemoglobin. This accounts for about half of the carbon dioxide released from the blood in the lungs.

7. Hemoglobin stripped of 2,3-bisphosphoglycerate (BPG) binds oxygen more tightly than hemoglobin in the blood. BPG binds to the T state but not the R state, thereby decreasing hemoglobin's oxygen affinity. This allows nearly 40% of the oxygen to be unloaded in venous blood. Fetal hemoglobin does not bind BPG as tightly and therefore has a higher affinity for oxygen than adult hemoglobin.

8. The analysis of mutant hemoglobins with altered functions has conveyed considerable knowledge about protein structure–function relationships. One variant, hemoglobin S, has a substitution of valine for glutamic acid in the β subunit. In the deoxy state, hemoglobin S aggregates, causing erythrocytes to sickle and block the capillaries. Individuals who are homozygous for the gene specifying hemoglobin S have sickle cell anemia, a debilitating and often fatal disease.

9. Allosteric proteins are oligomers with multiple ligand-binding sites, in which ligand binding at one site alters the protein's binding affinity for a ligand at another site. In the symmetry model of allosterism, the oligomer has only two binding states, T and R, and ligands bind preferentially to one state. In the sequential model, ligand binding progressively induces conformational changes in the subunits of an oligomer.

Myosin and Actin

10. Striated muscle is made of parallel bundles called myofibrils. As seen in the electron microscope, I bands of lesser electron density alternate with A bands of greater density. The repeating unit, the sarcomere, is bounded by Z disks at the centers of adjacent I bands and includes the A band, which is centered on the M disk. Within the sarcomere, thick filaments are linked to thin filaments by cross-bridges. Muscle contraction occurs when the filaments slide past each other, bringing the Z disks closer together.

11. Thick filaments are made of myosin, which consists of six subunits: two heavy chains, two essential light chains (ELC), and two regulatory light chains (RLC). A heavy chain consists of a globular head and a long α-helical tail. Two such tails associate in a 1600-Å-long left-

handed coiled coil, yielding a rodlike molecule that has two globular heads. One subunit each of the RLC and ELC bind to each globular head. Thin filaments are composed of actin, tropomyosin, and troponin. Actin, which has two globular domain, polymerizes toformthe core of the thin filament. Tropomyosin is a coiled coil that winds in the actin polymer's helical grove so as to contact seven successive actin monomers. Each troponin molecule binds to a tropomyosin molecule.

12. The myosin heads of the thick filaments bind to the thin filaments. When ATP binds to the myosin head, myosin releases the actin. Subsequent ATP hydrolysis cocks the head of myosin and allows it to rebind weakly to actin. The release of inorganic phosphate increases the strength of binding and causes the head of myosin to snap back in the power stroke. This pulls the filaments past each other. Each myosin head acts in this manner to cause muscle contraction.

13. Ca^{2+}, which binds to troponin C, triggers muscle contraction. Nerve impulses induce the release of Ca^{2+}, which binds to troponin and causes a conformational change that exposes additional myosin binding sites on the thin filament. At lower Ca^{2+} concentrations, the myosin head is blocked from binding actin and the muscle is relaxed.

14. Actin also occurs in nonmuscle cells, where its assembly and disassembly motivates such cellular processes as ameboid locomotion, cytokinesis, cytoplasmic streaming, and the extension and retraction of various cellular protuberances.

Antibodies

15. Antibodies (immunoglobulins) are proteins produced by the immune system of higher organisms to protect them against pathogens such as viruses and bacteria. Antibodies are produced by *B* lymphocytes, which recognize foreign macromolecules (antigens). The primary response to an antigen requires several days for *B* cells to generate an the required antibodies. If the organism subsequently encounters the same antigen, a secondary response results, in which large amounts of the antibody are produced more rapidly.

16. Antibodies contain at least four subunits, two identical light chains and two identical heavy chains, which are held together in part by interchain disulfide bonds to form a Y-shaped molecule. Of the five classes of antibodies, IgG is the most abundant. The classes are distinguished by the type of heavy chain (α, δ, ε, γ, and μ). There are two types of light chain (κ and λ). Heavy chains each consist of three domains of constant sequence, designated C_H, and one variable domain, designated V_H. The light chains each consist of one constant domain, C_L, and one variable domain, V_L. The V_H and V_L are located at the two ends of the Y-shaped protein.

17. The variable regions contain the antigen-binding sites in which hypervariable sequences determine the exquisite specificity of antibody–antigen interactions.

18. A *B* cell produces one kind of antibody. An antibody-producing *B* cell can be immortalized by fusing it with a myeloma cell. Cloning of the resulting hybridoma cells yields a colony of cells that produce a single type of antibody, called a monoclonal antibody.

19. The binding of an antibody to its antigen is highly specific and has a dissociation constant ranging form 10^{-4} to 10^{-10} M. Because antibodies have two antigen-binding sites, a population of antibodies can form large antigen–antibody aggregates that hasten the removal of the antigen and induce *B* cell proliferation.

20. Antigens stimulate the proliferation of a population of pre-existing *B* cells whose diversity arises from genetic changes that occur in *B* cell development. Sequence variation allows the synthesis of potentially billions of immunoglobulins with different antigen-binding specificities.

21. In autoimmune diseases, the organism loses its self-tolerance and produces antibodies against its own tissues. This process is sometimes triggered by trauma or infection and may result from the resemblance of a self-antigen to some foreign antigen. Autoimmune diseases have variable symptoms ranging from mild to lethal.

Key Equations

$$Y_{O2} = \frac{pO_2}{K + pO_2}$$

$$Y_{O2} = \frac{(pO_2)^n}{(p_{50})^n + (pO_2)^n}$$

Guide to Study Exercises (text p. 194)

1. The structures of myoglobin and hemoglobin differ in two ways: (1) Myoglobin contains a single globin polypeptide, whereas hemoglobin is an $\alpha_2\beta_2$ tetramer (a dimer of $\alpha\beta$ dimers) with four globin chains. (2) Myoglobin does not undergo a significant conformational change on binding O_2, whereas hemoglobin undergoes a dramatic change in conformation when O_2 is bound to at least one subunit in each $\alpha\beta$ dimer.

   Myoglobin facilitates O_2 transport in muscle under conditions of high exertion, whereas hemoglobin functions to transport O_2 in blood. These different functions of myoglobin and hemoglobin are reflected in their structural differences. Myoglobin, which has a relatively high affinity for O_2 (p_{50} = 2.8 torr), has a simple O_2-binding behavior so that its binding curve is hyperbolic. Hemoglobin has a more modest affinity for O_2 (p_{50} = 26 torr) and its four subunits bind O_2 cooperatively so that its binding curve is sigmoidal. (Sections 7-1 and 7-2A and B).

2. The keys to the hemoglobin–myoglobin O_2-delivery system are the high affinity of myoglobin for O_2 and the cooperative binding of O_2 to hemoglobin. The p_{50} of hemoglobin is 26 torr, so that, in the lungs, hemoglobin becomes almost fully saturated. (arterial pO_2 is ~100 torr). At low pO_2, the affinity of hemoglobin for O_2 falls off rapidly because of its sigmoidal (cooperative) binding behavior, so at venous pO_2 (~30 torr), hemoglobin is only about half-saturated with O_2. The O_2 released from hemoglobin is efficiently bound by myoglobin. Even in the venous circulation, the pO_2 is still much greater than the p_{50} of myoglobin (2.8 torr), so the myoglobin is essentially saturated with O_2. This, in effect, increases the solubility of O_2 in the tissues and thereby increases the rate at which O_2 can diffuse from the capillaries to the tissues, where it is consumed. (Section 7-2B)

3. Hemoglobin exhibits positive cooperativity in O_2 binding; that is, O_2 binding increases the affinity of the hemoglobin for additional O_2 molecules. This is accomplished through conformational changes in the globin subunits. The four globin chains of deoxyhemoglobin are in the T state, in which the heme is domed and the Fe is out of the plane of the heme toward His F8, to which it is liganded. O_2 binding to Fe causes the Fe to move into the plane of the heme and the heme to become more planar, thereby pulling His F8, and the F helix to which it is linked, toward it. The resulting change in tertiary structure is communicated to the other subunits primarily at the α_1–β_2 and α_2–β_1 interfaces. For example, when O_2 binds to a β subunit, its His FG4 (which is located in a loop at the end of the F helix), which contacts residue Thr C6 in the adjacent α chain, moves to contact Thr C3. This forces the α chain to assume the R conformation. This conformational shift also disrupts ion pairs that stabilize the T state. When at least one O_2 has bound to each αβ dimer of hemoglobin, the entire tetramer snaps into the R state. The affinity of hemoglobin for O_2 increases because all the subunits are now in the R state, which is the O_2-binding conformation. (Section 7-2C)

4. When hemoglobin binds O_2, the T→R conformational changes in the globin subunits disrupt networks of ion pairs that involve the C-terminal residues of each subunit. Several groups, including the N-terminal amino group of the α chains and the C-terminal His of the β chains, thereby become more acidic (deprotonated). For this reason, increasing the pH (decreasing $[H^+]$) promotes the T→R transition in hemoglobin (which favors O_2 binding). Decreasing the pH (increasing $[H^+]$) promotes the R→T transition (which favors the dissociation of O_2). This behavior (called the Bohr effect) is important for delivering O_2 to the tissues. Respiring tissues produce CO_2, which is converted to bicarbonate + H^+. This H^+ induces the hemoglobin to unload its bound O_2 to the tissues, where it is needed. Furthermore, when hemoglobin takes up H^+, more bicarbonate is formed, thereby drawing CO_2 from the tissues. The opposite reactions occur in the lungs: O_2 binding to hemoglobin causes the T→R transition, releasing the Bohr protons so that they can recombine with bicarbonate to drive off CO_2. (Section 7-2C)

5. BPG binds preferentially to deoxyhemoglobin, where it occupies the central cavity between the globin subunits. In the R (oxy) state, the central cavity is too narrow to accommodate BPG, and the N-terminal amino groups of the β subunits to which BPG binds have moved apart so that BPG cannot simultaneously bind both of them as it does in T-state

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hemoglobin.. Hence BPG binding stabilizes the T (deoxy) state relative to the R state. BPG binding thereby induces the unloading of O_2 from hemoglobin. (Section 7-2C)

6. In the symmetry model of allosterism, all the subunits of the oligomer change conformation in a concerted manner in response to ligand binding. Only two conformational states for the oligomer are permitted, and ligand binding necessarily increases the affinity of the other subunits for the ligand. In the sequential model, ligand binding induces a conformational change in the subunit to which it binds, which affects neighboring subunits more than more distant subunits. As more ligand-binding sites in the oligomer are occupied, more conformational changes occur, until the entire oligomer has shifted conformation. In the sequential model, ligand binding to one subunit can either increase or decrease the affinity of the other subunits for the ligand. (Section 7-2E)

7. See Figure 7-23.

8. According to the sliding filament model of muscle contraction, each myosin molecule must repeatedly detach and reattach itself to new sites on the actin thin filament. This activity causes the thin and thick filaments to slide past one another, thereby causing the muscle to contract. The molecular events occur in a cycle: ATP binding to a myosin head causes it to release its bound actin. Subsequent ATP hydrolysis provides the free energy for myosin to assume a "cocked" conformation that allows it to bind weakly to an actin monomer further along the thin filament toward the Z disk. Myosin then releases P_i, which increase its affinity for actin. The ensuing conformational change causes the myosin head to return to its original position, thereby moving the thin filament toward the M disk. ADP release from myosin completes the reaction cycle. (Section 7-3C)

9. See Figure 7-34.

Questions

Myoglobin and Hemoglobin

1. Match the descriptions on the left with the terms on the right.

____ A component of cytochromes	A. methemoglobin
____ Binds O_2	B. myoglobin
____ Contains iron in the Fe(III) state	C. hemoglobin
____ Found in muscle only	D. hemoglobin S
____Forms filaments in the deoxy state	E. heme

2. The heme moiety by itself can bind oxygen. What physiological function does the globin serve?

3. How do tissues with high metabolic activity facilitate oxygen delivery?

4. How would a lower p_{50} affect hemoglobin's oxygen acquisition in the lungs and oxygen delivery to the peripheral tissues?

5. Describe, on the molecular level, the role of myoglobin in O_2 transport in rapidly respiring muscle tissue.

6. What function(s) does carbamate formation in hemoglobin serve?

7. Which of the following modulators of O_2 binding to hemoglobin counteract each other? CO_2, H^+, BPG.

8. Explain why individuals with severe carbon monoxide poisoning are often given transfusions instead of oxygen-rich gas.

9. Match each of the structural elements of myoglobin or hemoglobin with its function below.

A.	His F8	F.	α_1–β_1 interface/α_2–β_2 interface
B.	His E7	G.	oxymyoglobin/deoxymyoglobin
C.	E and F helices	H.	oxyhemoglobin/deoxyhemoglobin
D.	O_2 - Fe(II)	I.	α_1–β_2 interface/α_2–β_1 interface
E.	Val E11	J.	C-terminal salt bridges

____ Forms a coordination bond with Fe(II)
____ Involved in the binding of heme
____ Partially occludes the O_2-binding site
____ Conformations are nearly superimposable
____ Forms a hydrogen bond with O_2
____ Is associated with a rotational shift of the hemoglobin $\alpha_1\beta_1$ dimer with respect to the $\alpha_2\beta_2$ dimer.
____ Disrupts the N- and C-terminal salt bridges in hemoglobin
____ Stabilizes the T state
____ Involved in the interactions of hemoglobin subunits

10. Oxygen binding to hemoglobin decreases the pK of the imidazole group of His 146β from 8.0 to 7.1. How does this contribute to the Bohr effect?

11. Describe how BPG decreases the O_2-binding affinity of hemoglobin in terms of the T ⇌ R equilibrium.

12. What physiological condition leads to hemoglobin S fiber formation in the capillaries?

13. Some sickle-cell individuals have significant levels of fetal hemoglobin in their erythrocytes. Why is this an advantage?

14. What kind of allosteric effect is inconsistent with the symmetry model?

15. How many conformational states are possible for a trimeric binding protein, whose three subunits each contain a ligand-binding site, when binding follows the (a) symmetry model or (b) the sequential model of allosterism?

Myosin and Actin

16. Describe how ATP hydrolysis is involved in muscle contraction.

17. How does calcium regulate muscle contraction?

Antibodies

18. In a typical protocol for preparing antibodies for laboratory use, an animal is injected with an antigen several times over a period of weeks or months. Why are multiple injections useful?

19. Indicate which class of immunoglobulin (IgA, IgD, IgE, IgG, or IgM) best corresponds to each characteristic listed below (more than one may be implicated by each description).

    (a) First to be secreted in response to an antigen
    (b) Implicated in allergic reactions
    (c) Occurs in the intestinal tract
    (d) Contains J chains
    (e) The most abundant antibody

20. The following questions refer to the production of monoclonal antibodies.
    (a) Why are myeloma cells used?
    (b) Do all cells growing in the selective medium produce antibodies to antigen X?
    (c) Why do only hybrid cells grow in the selective medium?

21. You wish to isolate a large amount of Fab fragments in order to examine their binding to protein X by X-ray crystallography. At first, you inject a large rabbit with protein X to obtain antibody. On further reflection, you decide to inject the antigen into a mouse in order to produce monoclonal antibodies.
    (a) How can you purify protein X–specific antibodies from rabbit serum or the hybridoma medium?
    (b) How do the rabbit and mouse antibody preparations differ?
    (c) Would the rabbit or mouse antibodies be more suitable for X-ray crystallography?

22. Why are the hypervariable sequences of immunoglobulins located in loops?

Answers to Questions

1. E A component of cytochromes
   B, C, D Binds O_2
   A Contains iron in the Fe(III) state
   B Found in muscle only
   D Forms filaments in the deoxy state

2. The globin prevents the oxidation of its bound heme to the Fe(III) state, and in the case of hemoglobin, permits cooperative O_2 binding, which is responsible for the efficient transport of O_2 from the lungs to the tissues.

3. High metabolic activity generates CO_2, which reacts with water to form H_2CO_3, which in turn ionizes to yield $HCO_3^- + H^+$. The protons preferentially bind to T-state hemoglobin (the Bohr effect) and thereby cause O_2 to be released.

4. A lower p_{50}, or higher affinity of hemoglobin for O_2, would have almost no effect on O_2 uptake in the lungs, since hemoglobin is nearly saturated at arterial pO_2. However, the lower p_{50} would result in less O_2 released at the peripheral tissues, where the pO_2 is ~30 torr. The p_{50} of normal hemoglobin (26 torr) is an evolutionary compromise that allows hemoglobin to become saturated with O_2 in the lungs but to be deoxygenated in the oxygen-poor peripheral tissues.

5. The O_2 that is released in the capillaries diffuses to the mitochondria, where it is reduced to water. However, the solubility of O_2 in aqueous solution is too low to support its required rate of diffusion in rapidly respiring muscle. The presence of myoglobin, in effect, increases the solubility of O_2 in muscle tissue. Thus, the O_2 released by hemoglobin is passed from myoglobin molecule to myoglobin molecule in a kind of molecular bucket brigade until it is taken up by the mitochondria. In this way, myoglobin increases the rate that O_2 can diffuse through the tissues.

6. CO_2 reacts preferentially with amino groups in deoxyhemoglobin to form carbamates. This helps transport CO_2 and facilitates the release of O_2 by stabilizing the deoxy state.

7. None; CO_2, H^+, and BPG all bind preferentially to deoxyhemoglobin to reduce the affinity of hemoglobin for O_2.

8. CO binds to hemoglobin with so much higher affinity than O_2 that CO cannot be displaced by high concentrations of oxygen. For practical purposes, CO binding to hemoglobin is therefore irreversible (at least in the short term). Therefore, a fresh blood transfusion is required to counteract the effects of this poison.

9.

A	Forms a coordination bond with Fe(II)
A, C	Involved in the binding of heme
E	Partially occludes the O_2-binding site
G	Conformations are nearly superimposable
B	Forms a hydrogen bond with O_2
I	Is associated with a rotational shift of the hemoglobin $\alpha_1\beta_1$ dimer with respect to the $\alpha_2\beta_2$ dimer.
D	Disrupts the N- and C-terminal salt bridges in hemoglobin
J	Stabilizes the T state
F, I	Involved in the interactions of hemoglobin subunits

10. The decrease in p*K* promotes deprotonation of the imidazole group at pH 7.4. The release of H^+ contributes to the Bohr effect.

11. BPG binds preferentially to the T state and stabilizes it through the formation of salt bridges between BPG and the β subunits. This makes the shift to the R state less energetically favorable, thereby decreasing hemoglobin's ability to bind O_2.

12. The low pO_2 of the capillaries, which leads to an increase in the concentration of deoxyhemoglobin, promotes polymerization of hemoglobin S.

13. Fetal hemoglobin (which contains γ globin chains rather than β chains) dilutes the hemoglobin S in erythrocytes, so that the deoxyhemoglobin S in the venous blood is less likely to achieve the critical concentration for fiber formation.

14. The symmetry model cannot account for negative cooperativity, in which the binding of a ligand to one subunit decreases the binding affinity of the other subunits.

15. (a) Only two conformational states are possible, either R or T, since all subunits change conformation simultaneously.
    (b) Four conformations are possible, corresponding to a trimer in which 0, 1, 2, or 3 subunits have bound ligand.

16. Myosin moves along the actin filament via repeated cycles of conformational changes in the head region of the myosin molecule. This cycle of conformational changes is unidirectional because it is coupled to the irreversible hydrolysis of ATP. When the myosin head binds ATP, it releases the actin filament to which it is bound. ATP hydrolysis follows, resulting in a change in the conformation of the myosin head to the "cocked" or high-energy state. The myosin head then binds weakly to the actin filament at a new position. The strength of this binding interaction increases upon the release of P_i. The myosin head then undergoes a second major conformational change, producing the power stroke that causes the translocation of the actin filament relative to the myosin filament. Upon completion of the power stroke, the myosin head releases ADP but remains bound to the actin filament. It can then bind another ATP molecule to begin the cycle anew. The thick filament's multiple

myosin heads, each undergoing multiple ATP-driven reaction cycles, cause the thick filament to "walk" along the thin filament. As the thick and thin filaments slide past each other, the sarcomere decreases in length and the muscle thereby contracts.

17. The thin filaments of striated muscle contain actin in complex with tropomyosin and troponin. At resting Ca^{2+} concentrations, the muscle is relaxed because tropomyosin blocks the myosin binding sites on the actin filament. When the intracellular Ca^{2+} concentration increases in response to a nerve impulse, troponin C (subunit of troponin) binds Ca^{2+}, causing a conformational change in troponin. This results in the movement of tropomyosin, which uncovers the myosin binding sites, and thus permits the myosin heads to interact with the thin filament.

18. A secondary immune response is greater than the first (see Figure 7-32), so more antibody can be recovered.

19. (a) IgM (b) IgE (c) IgA (d) IgA, IgM (e) IgG

20. (a) Antibody-producing lymphocytes have a limited proliferative capacity and are therefore unsuitable for growing in large numbers in culture to produce large amounts of antibody. Myeloma cells, like all cancer cells, have an unlimited proliferative capacity and impart their immortal phenotype to the hybrid cells.
    (b) All the lymphocytes harvested from the immunized animal can potentially fuse with the myeloma cells. The resulting hybridomas will therefore secrete a variety of different antibodies, only a small fraction of which are specific for protein X.
    (c) Unfused lymphocytes have no metabolic deficiency but grow for only a limited time, and then die out. In the selective medium, unfused myeloma cells cannot synthesize purines, which are necessary for DNA replication and cell division. Only the fused cells can both synthesize purines and proliferate without limit.

21. (a) Affinity chromatography using immobilized protein X would be the best procedure.
    (b) It is likely that protein X contains several antigenic features that are recognized by *B* cells and elicit antibody production. The anti-protein X antibodies isolated from the rabbit are therefore a mixture of different IgG molecules that recognize different antigenic features on protein X. Even antibodies that recognize the same portion of the antigen may have different amino acid sequences and different antigen-binding affinities. In contrast, each mouse monoclonal antibody preparation is homogeneous. Because monoclonal antibodies are all the products of identical *B* cells, they all have the same unique sequence and antigen-binding specificity.
    (c) One of the mouse monoclonal antibodies would be most suitable, since it would yield identical Fab fragments that would be more likely to form a regular crystal than the Fab fragments isolated from a heterogeneous population of rabbit antibodies.

22. The loops can accommodate a wide variety of amino acid sequences because they are on the surface of the protein. Such sequence variation in the β sheet core of the protein would likely disrupt its structure.