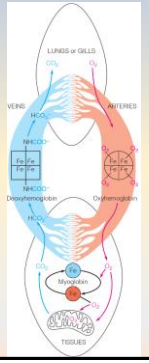
Protein Function and Evolution

Outline

- Oxygen Transport: The Roles of Hemoglobin and Myoglobin
- The Mechanism of Oxygen Binding by Heme Proteins
- Oxygen Transport: Hemoglobin
- Allosteric Effectors of Hemoglobin
- Protein Evolution: Myoglobin and Hemoglobin as Examples
- Hemoglobin Variants: Evolution in Progress
- Immunoglobulins: Variability in Structure Yields Versatility in Binding

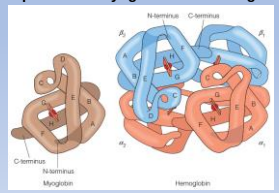
Oxygen Transport: The Roles of Hemoglobin and Myoglobin



- Vertebrate animals use hemoglobin and myoglobin to provide their tissues with a continuous O_2 supply.
- Hemoglobin transports O_2 from the lungs or gills to the respiring tissues, where it is used for aerobic metabolism in the mitochondria.
- Inside cells, dissolved O_2 diffuses freely or is bound to myoglobin, which aids transport of O_2 to the mitochondria.
- Myoglobin can also store O_2 for later use (as in deep-diving mammals).
- CO_2 produced by oxidative processes in the tissues is carried back to the lungs or gills by hemoglobin and released.

Oxygen Transport: The Roles of Hemoglobin and Myoglobin

Comparison of myoglobin and hemoglobin:



- Each of the four chains in hemoglobin has a folded structure similar to that of myoglobin, and each carries a heme.
- Hemoglobin contains two identical *a* chains and two identical *b* chains. The letters A–H indicate *a*-helical regions.
- The *a* and *b* chains are very similar but have distinct primary structures and folds (note that the *a* chain does not have a "D" helix).

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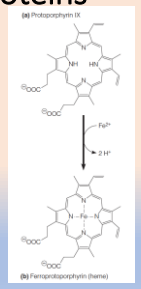
The Mechanism of Oxygen Binding by Heme Proteins



The structures of protoporphyrin IX and heme:

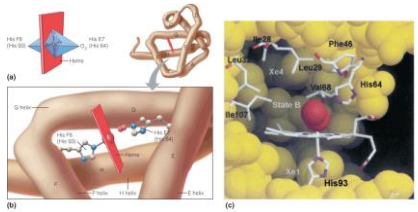
•The protoporphyrin IX is the tetrapyrrole portion of the heme molecule.

•Heme, which is protoporphyrin IX complexed with Fe(II), is the prosthetic group of hemoglobin and myoglobin.

•Because of resonance delocalization of the electrons in the porphyrin ring, all N–Fe bonds within the heme are equivalent.

The Mechanism of Oxygen Binding by Heme Proteins

The geometry of iron coordination in oxymyoglobin:

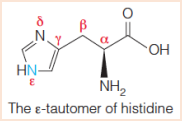


•The octahedral coordination of the iron ion. The iron and the four nitrogens from protoporphyrin IX lie nearly in a plane.

•A histidine (F8, or His 93) occupies one of the axial positions, and O_2 the other.

•Schematic drawing of the heme pocket, showing the proximal (F8; His93) and distal (E7; His64) histidine side chains.

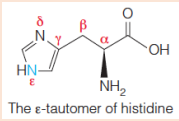
The Mechanism of Oxygen Binding by Heme Proteins



The ε-tautomer of histidine

- Coordination of Fe(II) in a porphyrin (heme) within a hydrophobic globin pocket allows reversible O_2 binding without iron oxidation.
- The H-bond between His E7 (the distil histidine) and O_2 selectively increases the affinity of Mb for vs. CO, which doesn't make a similar bond to His E7.
- Even so, CO binds ~200 times more tightly to Mb than does however, without the E7 H-bond, that ratio would be ~6,000:1 in favor of CO.

The Mechanism of Oxygen Binding by Heme Proteins



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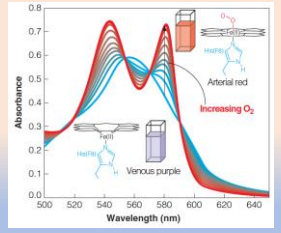


Changes in the visible spectrum of hemoglobin.

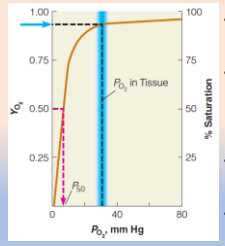
Spectra for hemoglobin in the deoxygenated state (blue trace) and the O_2-bound state (red trace) are shown.

Hemoglobin in the deoxygenated state is a venous purple, whereas completely oxy-Hb is bright red.

As more O_2 binds to Hb, the visible spectrum shifts from the blue to the red trace

The Mechanism of Oxygen Binding by Heme Proteins

Oxygen-binding curve for myoglobin:



- The free oxygen concentration is expressed as P_{O2}, the partial pressure of oxygen.
- The proportion of myoglobin binding sites that are occupied is expressed as a fraction (Y_{O2}, on the left) or as percent saturation (on the right).
- As P_{O2} becomes large, 100% saturation is approached asymptotically.
- The value of P_{50}, the partial pressure of oxygen at 50% saturation, is indicated on the graph (black arrow). The dashed blue lines show that at P_{O2}, of 30 mm Hg, Mb would be 90% saturated with O_2.

The Mechanism of Oxygen Binding by Heme Proteins

- The P_{50} is an indicator of the relative binding affinity of a globin for a ligand:
  - For a globin with higher O_2-binding affinity, the value of P_{50} is *lower*.
  - For a globin with lower O_2-binding affinity, the value of P_{50} is *higher*.
- Binding of a ligand (like O_2) to a single site on a protein (like Mb) is described by a *hyperbolic* binding curve.

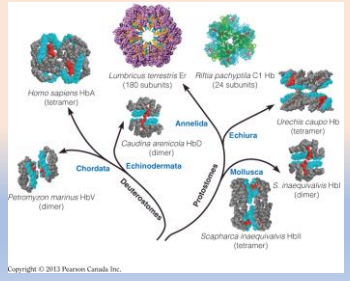
The Mechanism of Oxygen Binding by Heme Proteins

- Dynamic motions of myoglobin facilitate ligand binding and release.
- Myoglobin has evolved to bind and release O_2 under conditions of relatively low oxygen concentration.

Oxygen Transport: Hemoglobin

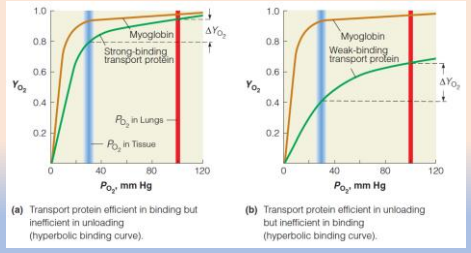
Diversity in hemoglobin structures:

Space-filling models of Hb dimers and tetramers are shown with heme groups in red, E and F helices in cyan, and the rest of the main chain in gray.



Oxygen Transport: Hemoglobin

Cooperative vs. noncooperative O_2-binding curves:



(a) Transport protein efficient in binding but inefficient in unloading (hyperbolic binding curve).

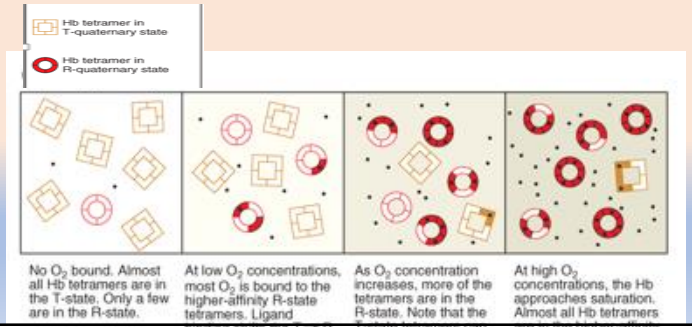
(b) Transport protein efficient in unloading but inefficient in binding (hyperbolic binding curve).

Oxygen Transport: Hemoglobin

- The cooperative binding of oxygen by hemoglobin is one example of what is referred to as an ***allosteric effect***.
- In allosteric binding, the uptake of one ligand by a protein influences the affinities of remaining unfilled binding sites.
- The ligands may be of the same kind, as in the case of binding to hemoglobin, or they may be different.
- Allostery is also an important mechanism for regulating the activity of enzymes.

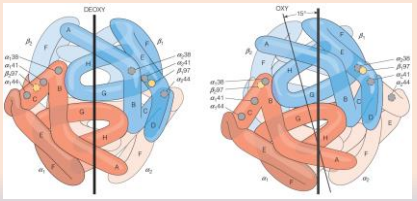
Oxygen Transport: Hemoglobin

- Hemoglobin switches between conformational states with lower and higher O_2-binding affinities.
- In the O_2-rich environment of the lungs or gills the higher affinity state is favored, and oxygen binds to hemoglobin.
- In the O_2-poor environment of respiring tissues the lower affinity state is favored, and oxygen is released from hemoglobin.
  - *R*-state hemoglobin has a higher O_2-binding affinity (lower P_{50}).
  - *T*-state hemoglobin has a lower O_2-binding affinity (higher P_{50}).
- Vertebrate hemoglobins are tetramers ($\alpha_2\beta_2$) made up of two kinds of myoglobin-like chains.
- Oxygenation causes hemoglobin quaternary structure to change:
  - One $\alpha\beta$ dimer rotates and slides with respect to the other.



Oxygen Transport: Hemoglobin

The change in hemoglobin quaternary structure during oxygenation:



•The transition viewed along an axis perpendicular to the two-fold axis, with the dimer (darker blue and red areas) in front of the dimer.

•Deoxyhemoglobin is shown on the left, and oxyhemoglobin on the right.

•The rotation of about 15˚ is accompanied by sliding because the center of rotation is not centrally located.

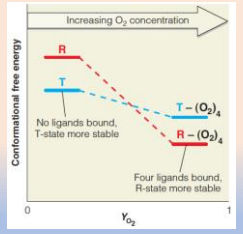
Oxygen Transport: Hemoglobin

A simplified view of ligand binding and conformation energies in hemoglobin:

•The deoxy (*T*) conformation is favored when no ligands are bound, due to the increased number of noncovalent interactions in the *T* state.

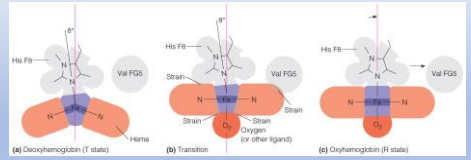
•As Y_{O2} increases (i.e., more ligands are bound) the energy provided by formation of the Fe-O_2 bond stabilizes the *R* conformation relative to the *T* conformation.

•The energetic cost of breaking stabilizing interactions in the deoxy state is paid by the formation of Fe-O_2 bonds in the oxy state.



•The binding of oxygen to deoxyhemoglobin causes conformational changes in the heme.

•Binding of the O_2 ligand pulls the iron into the heme plane, flattening the heme and causing strain.



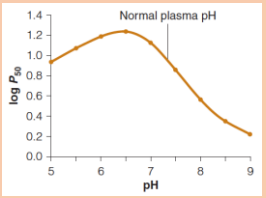
Allosteric Effectors of Hemoglobin

- Cooperative binding and transport of oxygen are only part of the allosteric behavior of hemoglobin.
- As oxygen is utilized in tissues, carbon dioxide is produced and must be transported back to the lungs or gills.
- Accumulation of CO_2 also lowers the pH in erythrocytes through the bicarbonate reaction catalyzed carbonic anhydrase:

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

- At the same time, the high demand for oxygen, especially in muscle involved in vigorous activity, can result in oxygen deficit, or hypoxia, which lowers the pH by the production of lactic acid.
- The falling pH in tissue and venous blood signals a demand for more oxygen delivery. Hemoglobin does so through its allosteric transition between high-affinity oxy (*R*) and low-affinity deoxy (*T*) states.

Allosteric Effectors of Hemoglobin



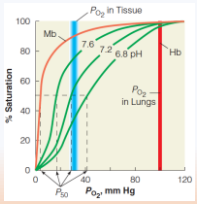
Oxygen affinity of hemoglobin as a function of pH.

A decrease in blood pH results in stabilization of the deoxy state and thereby favors greater O_2 released from hemoglobin.

This response of hemoglobin to pH change is called the ***Bohr effect.***

$$Hb{\cdot}4O_2 + 4H^+ \rightarrow Hb{\cdot}4H^+ + 4O_2$$

Allosteric Effectors of Hemoglobin



The Bohr effect in hemoglobin:

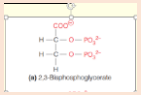
•O_2-binding curves for hemoglobin are shown for pH 7.6, 7.2, and 6.8.

•Note that the efficiency of O_2 unloading, as measured by the difference 30 mm curves at a P_{O2} of 30 mm Hg increases greatly as the pH drops.

•As the hemoglobin circulates from lungs to tissues, the lower pH favors the lower-affinity conformation.

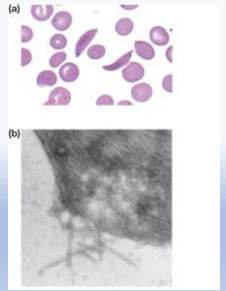
•Myoglobin displays little Bohr effect, so its O_2-binding curve is approximately the same at all three pH values.

Allosteric Effectors of Hemoglobin



- 2,3-Bisphosphoglycerate (2,3-BPG), found in mammals.
- 2,3-BPG is found inside red blood cells and is a potent allosteric effector that lowers the affinity of hemoglobin.

Hemoglobin Variants: Evolution in Progress

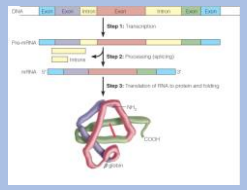


Erythrocytes in sickle-cell disease:

a) Typical sickled cells, together with some normal, rounded red blood cells.

b) Scanning electron micrograph of a sickled cell that has ruptured, with hemoglobin fibers spilling out.

Hemoglobin Variants: Evolution in Progress



Sickle-cell hemoglobin:

a) Molecules of sickle-cell Hb tend to aggregate, forming long fibers.

b) An electron micrograph of one sickle-cell fiber.

c) A computer-graphic depiction of one fiber.

- A schematic model of fiber formation. DeoxyHb S molecules lock together to form a two-stranded cluster because Val 6 in the β chain of one Hb molecule fits into a pocket in an adjacent molecule.
- Interaction of these two-stranded structures with one another produces the multistrand fibers shown in (a) and (b).

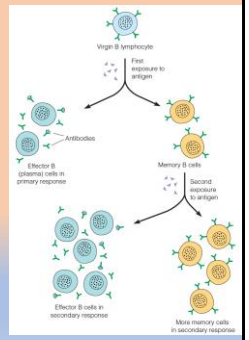
Immunoglobulins: Variability in Structure Yields Versatility in Binding

- The immune response involves the defense of the body against foreign substances or pathogens and operates via many different cellular mechanisms.
- In the ***humoral*** immune response, ***B lymphocytes*** secrete ***antibodies (immunoglobulins)*** that react with specific antigens.
- These ***immunoglobulin proteins***, whose primary function is the specific, and essentially irreversible, binding of substances that appear to be of nonself origin, such as bacterial or viral pathogens.

Immunoglobulins: Variability in Structure Yields Versatility in Binding

Two developmental paths for stimulated B lymphocytes:

- Exposure to antigen causes two kinds of cells to develop from B lymphocytes.
  - Cells of one type (effector B cells, or plasma cells) synthesize soluble antibody.
  - Cells of the second class (memory cells) carry membrane-bound antibody to allow a rapid and enhanced response to a second exposure of the same antigen.



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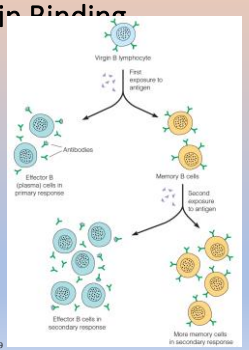

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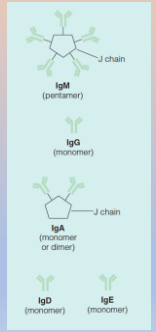


- Each immunoglobulin monomer (e.g., IgG) consists of four chains:
  - Two identical heavy chains (M = 53,000 Da each)
  - Two identical light chains (M = 23,000 Da each)
  - Held together by disulfide bonds.
- Immunoglobulin molecules contain both ***constant*** and ***variable*** regions.
- The ***variable regions*** are the ***antigen binding sites.***

Immunoglobulins: Variability in Structure Yields Versatility in Binding

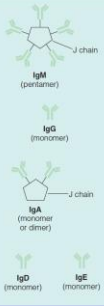
TABLE 7.2 The five classes of immunoglobulins

IgM is produced during the early response to an invading microorganism. It is the largest immunoglobulin, containing five Y-shaped units of two light and two heavy chains each. The units are held together by a component called a J chain. The relatively large size of IgM restricts it to the bloodstream. It is also effective in triggering an important mechanism for foreign cell destruction, called the complement system.

IgG molecules, also known as *γ-globulin*, are the most abundant of circulating antibodies. A variant is attached to B-cell surfaces. IgG molecules consist of a single Y-shaped unit and can traverse blood vessel walls rather readily; they also cross the placenta to carry some of the mother's immune protection to the developing fetus. Specific receptors allow such passage. IgG also triggers the complement system.

IgA is found in body secretions, including saliva, sweat, and tears, and along the walls of the intestines. It is the major antibody of colostrum, the initial secretion from a mother's breasts after birth, and of milk. IgA occurs as a monomer or as double-unit aggregates of the Y-shaped protein molecule. IgA molecules tend to be arranged along the surface of body cells and to combine there with antigens, such as those on a bacterium, thus preventing the foreign substance from directly attaching to the body cell. The invading substance can then be swept out of the body together with the IgA molecule.

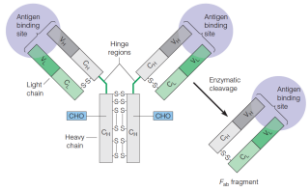
Less is known about the IgD and IgE immunoglobulins. IgD molecules are found on the surface of B cells, though little is known about their function. IgE is associated with some of the body's allergic responses, and its levels are elevated in individuals who have allergies. The constant regions of IgE molecules can bind tightly to mast cells, a type of epithelial and connective tissue cell that releases histamines as part of the allergic response. Both IgD and IgE consist of single Y-shaped units.



Immunoglobulins: Variability in Structure Yields Versatility in Binding

Schematic models of an IgG antibody molecule and an F_{ab} fragment:

- The IgG is made from two identical heavy chains and two identical light chains, all held together by disulfide bonds. Each chain contains both constant domains (C) and variable domains (V).

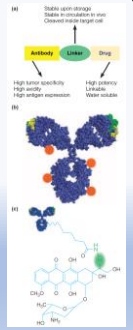


- Constant domains are the same in all antibody molecules of a given class, whereas variable domains confer specificity to a given antigenic determinant.
- Cleavage by certain proteolytic enzymes such as papain at the hinge and one Fc fragment regions allows production of two identical monovalent F_{ab} fragments and one F_c fragment.

Immunoglobulins: Variability in Structure Yields Versatility in Binding

- The diversity as well as the exquisite specificity of antigen binding sites is determined by the ***hypervariable complementarity determining regions (CDR)*** from both the light and the heavy chains.

Immunoglobulins: Variability in Structure Yields Versatility in Binding



Immunoconjugate drugs for targeted chemotherapy:

a) The desirable features for each component of the immunoconjugate: targeting antibody, linker, and cytotoxic drug.

b) Common sites of attachment of drugs to the antibody constant regions are shown as orange spheres. The tumor-specific antigen binding sites are shown in green and yellow.

c) A schematic of the immunoconjugate. The acid-labile hydrazone linker is highlighted in green, but linker is stable in circulation in blood (pH 7.4); but, is cleaved in the acidic environment of the endosome following endocytosis into the tumor cell.