UNIT IV: Specialized proteins

CLASS 1

Proteins, Sem II, B.Sc (H) Biochemistry

Dr. Jayita Thakur

- Transport protein: Haemoglobin - Oxygen binding curves
- Influence of 2,3-BPG, CO2 and H+, Hill plot, Cooperativity between subunits and models to explain the phenomena - concerted and sequential models.
- Haemoglobin disorders-sickle cell anemia, thalassemias.
- Motor proteins- Actin and myosin.
- Defense proteins- Antibodies,
- Membrane proteins- Integral and membrane associated proteins.
- Hydropathy plots to predict transmembrane domains.

E resources

Textbook/PDF

- Nelson, D.L., Cox, M.M. (2013). *Lehninger: Principles of Biochemistry* (6th ed.). New York, WH: Freeman and Company, pg 157-174
- https://www.rsb.org.uk/images/13_Transport_of_oxygen_in_the_blood.pdf
- https://pdb101.rcsb.org/motm/41
- http://www.csun.edu/~jm77307/Hemoglobin.pdf
- https://biochem.web.utah.edu/iwasa/projects/hemoglobin.html

Videos

- https://www.youtube.com/watch?v=b2hKDxX-KjE
- https://www.youtube.com/watch?v=GVU_zANtroE

- Nearly all the oxygen carried by whole blood in animals is bound and transported by hemoglobin in erythrocytes (red blood cells).

- Normal human erythrocytes are small (6-9 μm in diameter), biconcave disks. They are formed from precursor stem cells called **hemocytoblasts**. In the maturation process, the stem cell produces daughter cells that form large amounts of hemoglobin and then lose their intracellular organelles—nucleus, mitochondria, and endoplasmic reticulum.

- Erythrocytes are thus incomplete, vestigial cells, unable to reproduce and, in humans, destined to survive for only about 120 days.

- The main function of erythrocytes is to carry hemoglobin, which is dissolved in the cytosol at a very high concentration (~34% by weight).

- In arterial blood passing from the lungs through the heart to the peripheral tissues, hemoglobin is about 96% saturated with oxygen. In the venous blood returning to the heart, hemoglobin is only about 64% saturated.

- Thus, each 100 mL of blood passing through a tissue releases about one-third of the oxygen it carries, or 6.5 mL of O_2 gas at atmospheric pressure and body temperature.

Hemoglobin

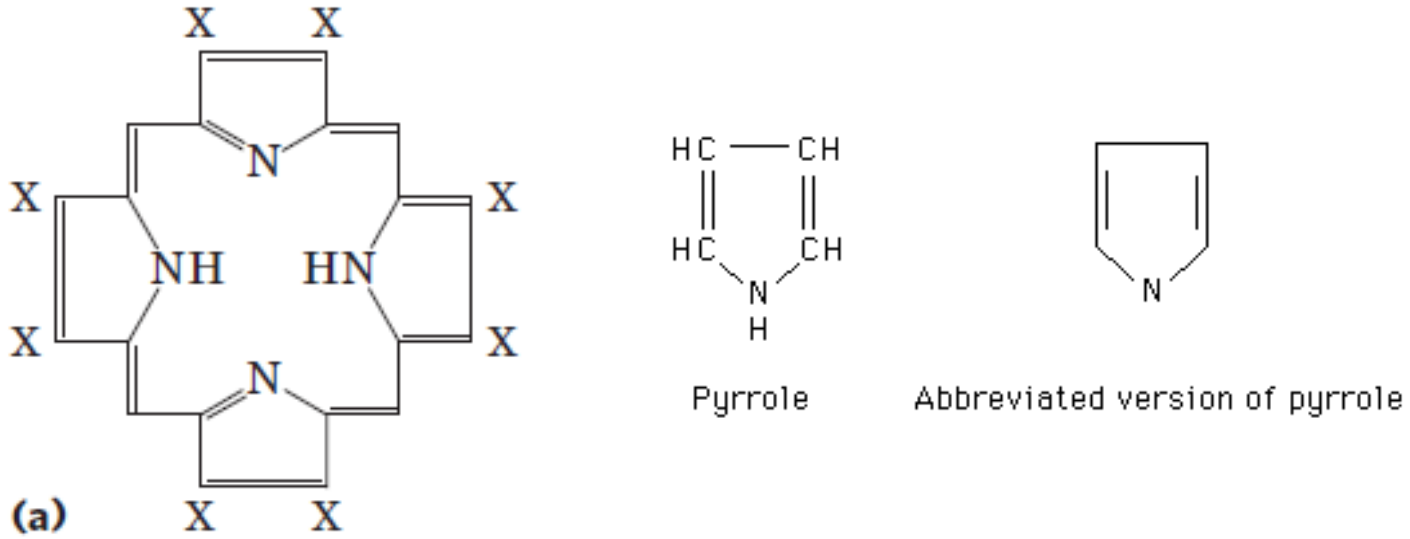
- The early history of protein chemistry is essentially that of hemoglobin.

- Hemoglobin has many firsts:

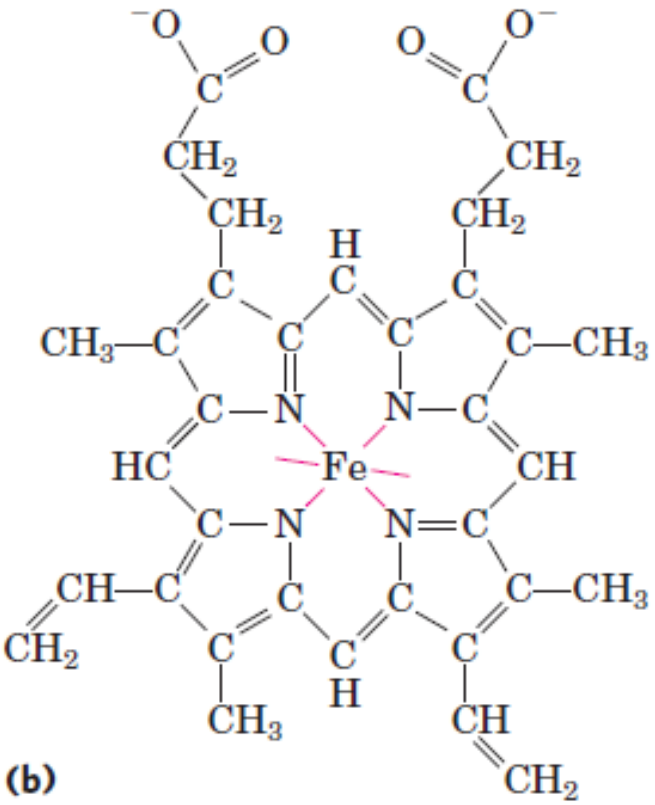
- First proteins to have its molecular mass accurately determined
- First protein to be characterized by ultracentrifugation
- First to be associated with a specific physiological function (that of oxygen transport)
- In sickle-cell anemia, the first in which a point mutation was demonstrated to cause a single amino acid change.
- The first protein X-ray structures to be elucidated were those of hemoglobin and myoglobin.

Hemoglobin (*Mr* 64,500; abbreviated **Hb**)

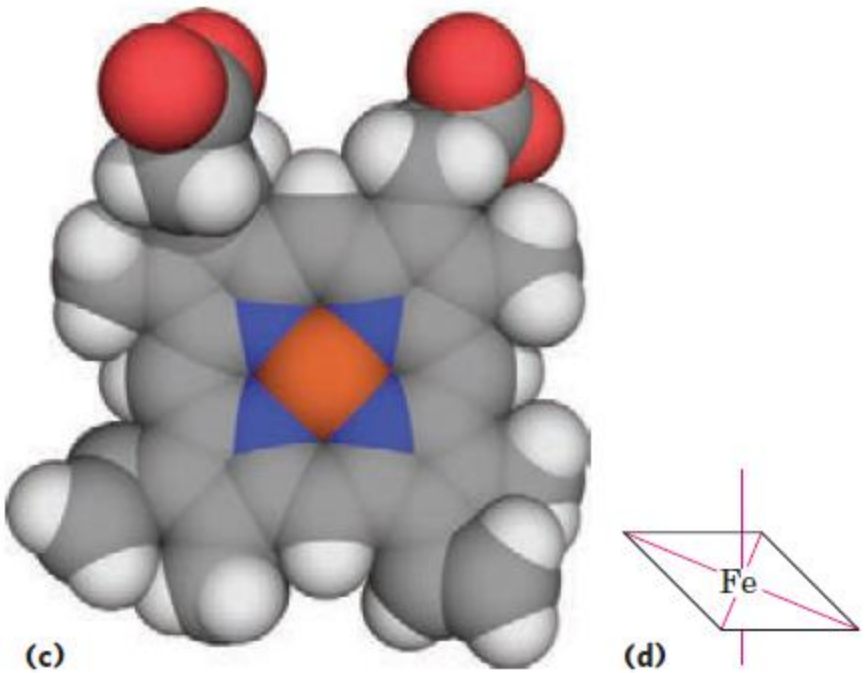
- Roughly spherical, with a diameter of nearly 5.5 nm.
- Tetrameric protein containing four heme prosthetic groups, one associated with each polypeptide chain.



Porphyrins, of which protoporphyrin IX is only one example, consist of four pyrrole rings linked by methene bridges, with substitutions at one or more of the positions denoted X.

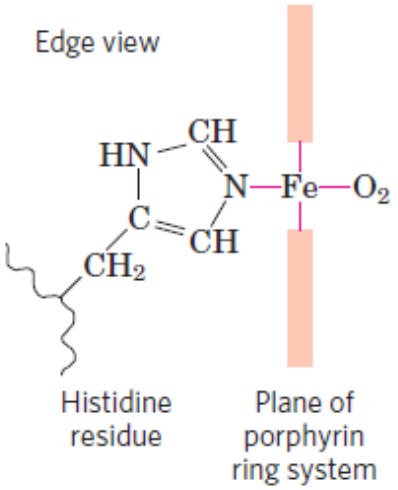


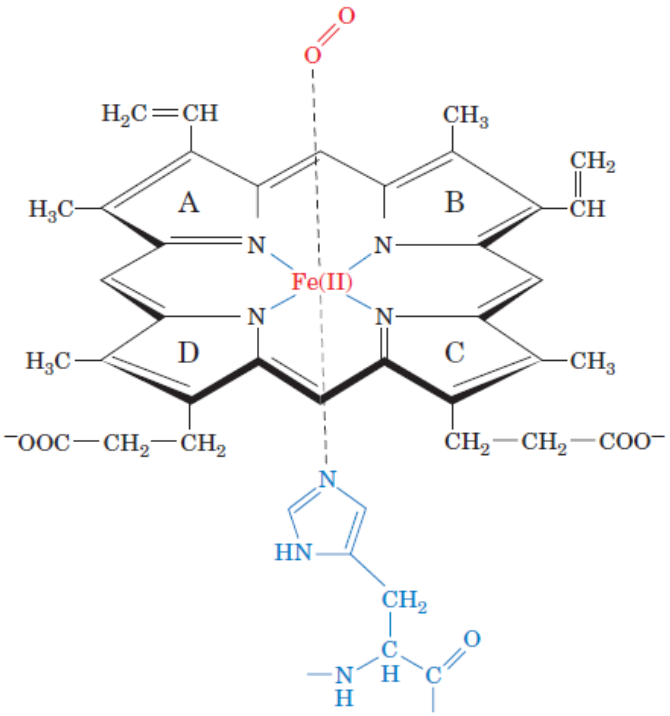
The heme group is present in myoglobin, hemoglobin, and many other proteins, designated heme proteins. Heme consists of a complex organic ring structure, protoporphyrin IX, with a bound iron atom in its ferrous (Fe^{2+}) state.



Free heme molecules (heme not bound to protein) leave Fe^{2+} with two "open" coordination bonds.

Simultaneous reaction of one O2 molecule with two free heme molecules (or two free Fe^{2+}) can result in irreversible conversion of Fe^{2+} to Fe^{3+}.





- In heme-containing proteins, this reaction is prevented by sequestering each heme deep within the protein structure.
- Thus, access to the two open coordination bonds is restricted.
- One of these two coordination bonds is occupied by a side chain nitrogen of a His residue.
- The other is the binding site for molecular oxygen (O2)

- Adult hemoglobin contains two types of globin, two chains (141 residues each) and two chains (146 residues each).

- Although fewer than half of the amino acid residues are identical in the polypeptide sequences of the α and β subunits, the three-dimensional structures of the two types of subunits are very similar.

- Also, their structures are very similar to that of myoglobin, even though the amino acid sequences of the three polypeptides are identical at only 27 positions

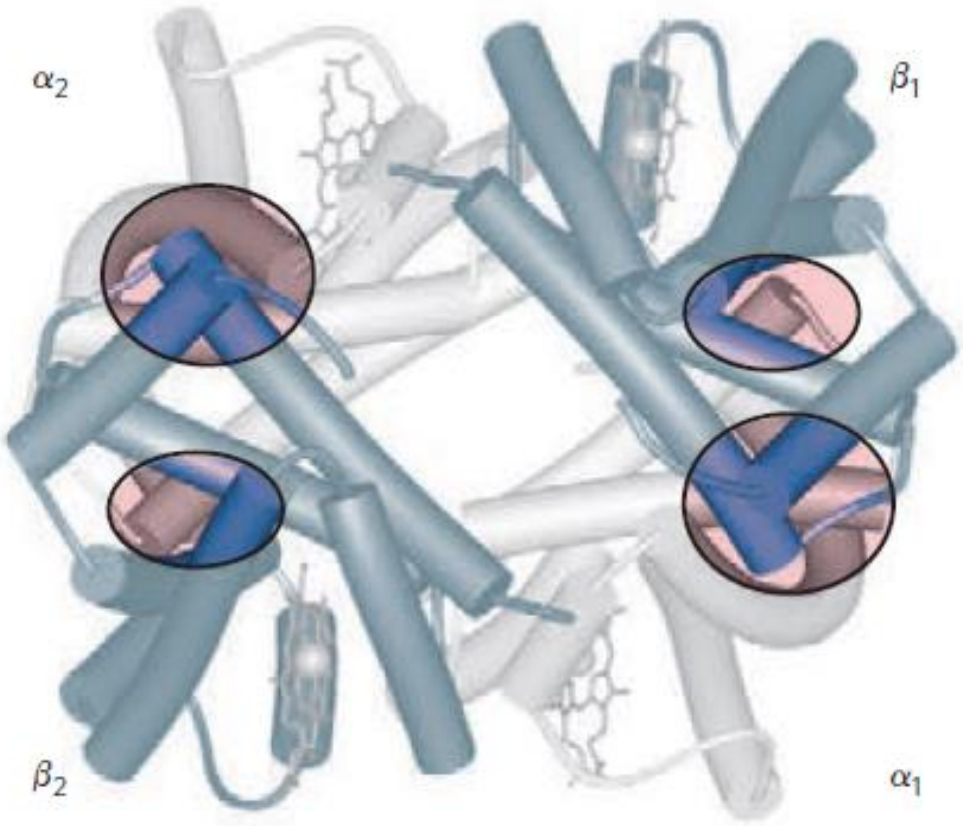
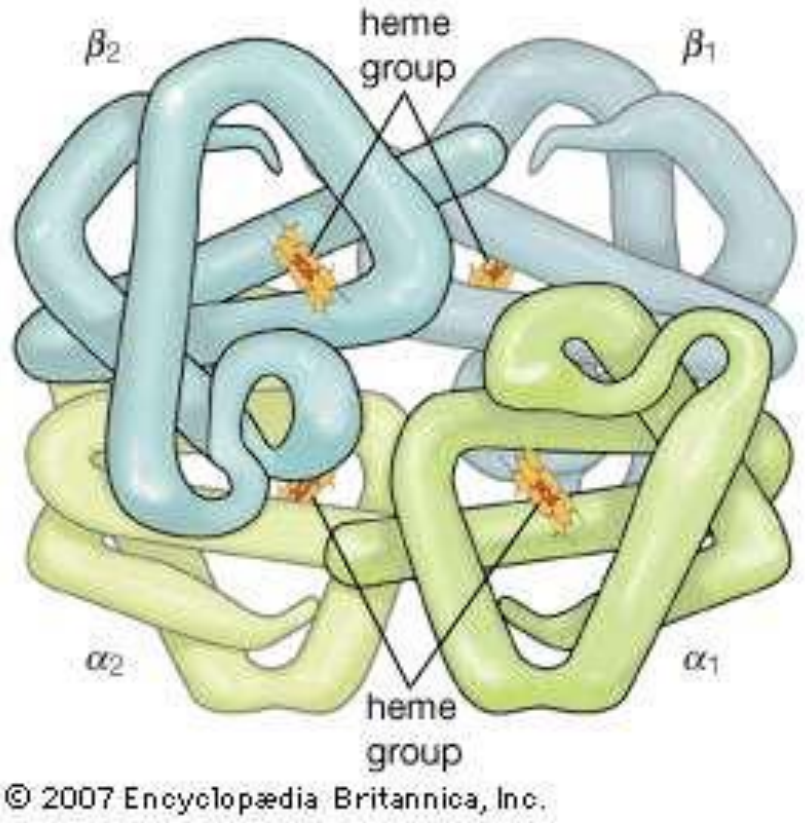


FIGURE 5–8 Dominant interactions between hemoglobin subunits. (PDB ID 1HGA) In this representation, α subunits are light and β subunits are dark. The strongest subunit interactions (highlighted) occur between unlike subunits. When oxygen binds, the $\alpha_1\beta_1$ contact changes little, but there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

- Hemoglobin is an oligomeric protein made up of 2 αβ dimers, a total of 4 polypeptide chains: $\alpha_1\beta_1\alpha_2\ \beta_2$.
- Each subunit has a heme-binding pocket.
- Hydrophobic interactions predominate at all the interfaces, but there are also many hydrogen bonds and a few ion pairs
  (or salt bridges)



- The 3D- structures of α (141 aa) and β (146 aa) subunits of hemoglobin and the single polypeptide of myoglobin are very similar; all three are members of the globin family.

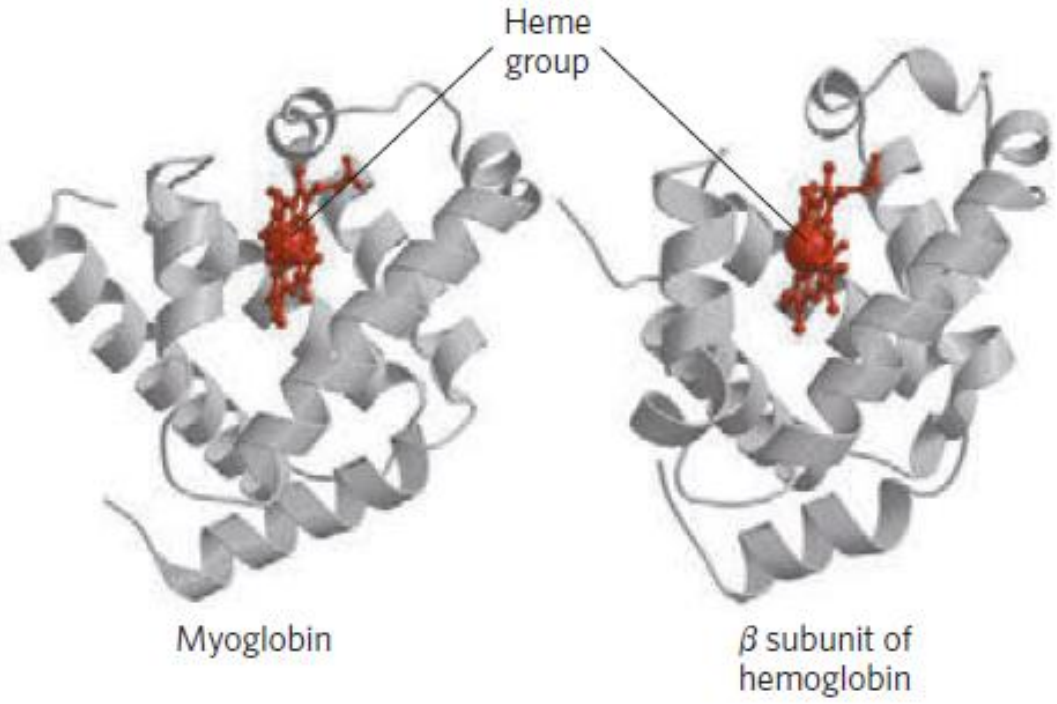


FIGURE 5–6 Comparison of the structures of myoglobin (PDB ID 1MBO) and the β subunit of hemoglobin (derived from PDB ID 1HGA).

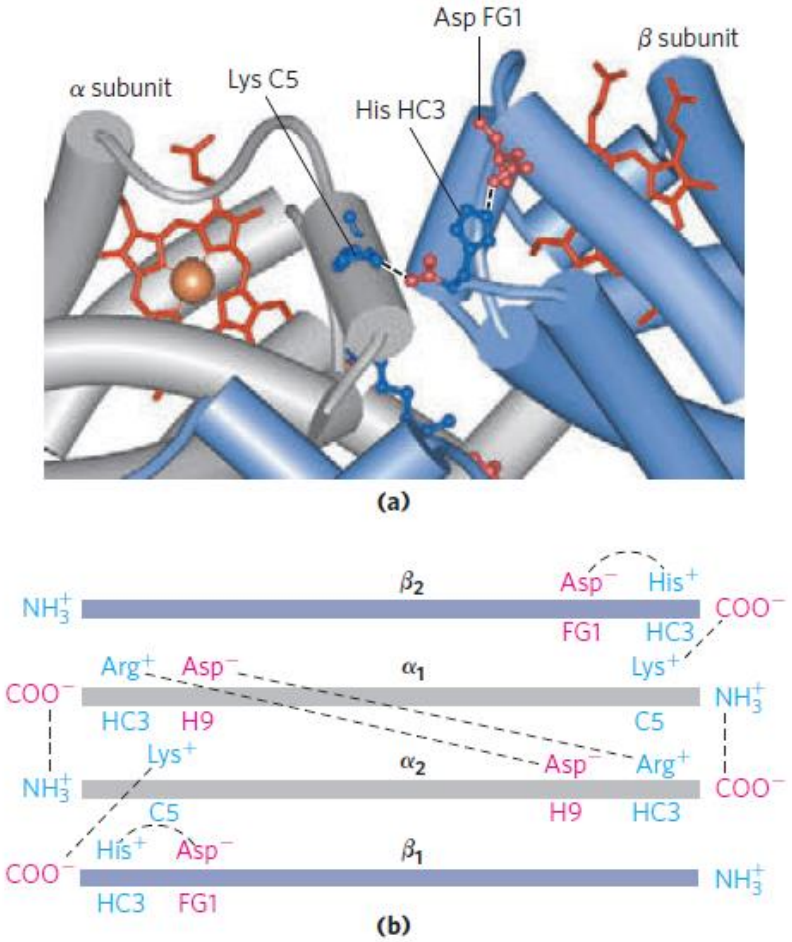


FIGURE 5–9 Some ion pairs that stabilize the T state of deoxyhemoglobin. (a) Close-up view of a portion of a deoxyhemoglobin molecule in the T state (PDB ID 1HGA). Interactions between the ion pairs His HC3 and Asp FG1 of the β subunit (blue) and between Lys C5 of the α subunit (gray) and His HC3 (its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

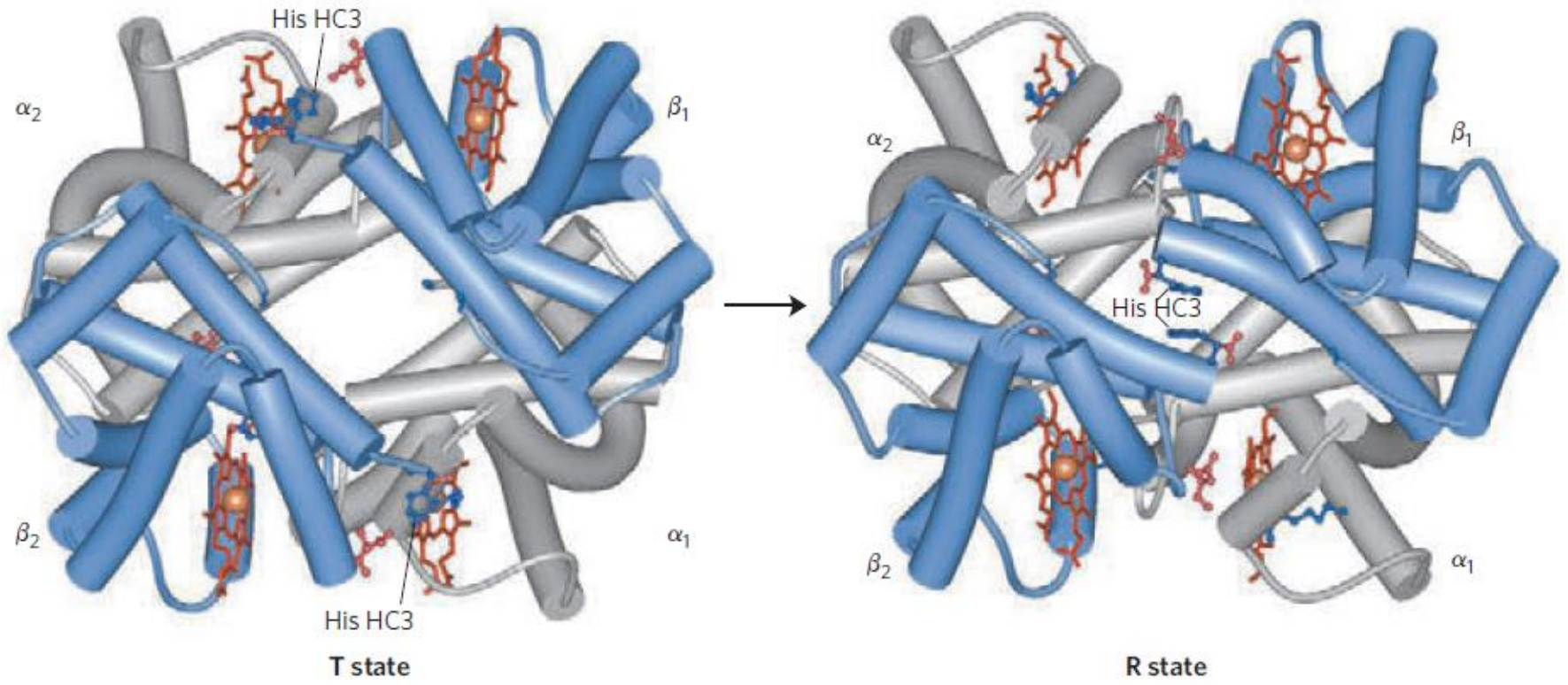


FIGURE 5–10 The T → R transition. (PDB ID 1HGA and 1BBB) In these depictions of deoxyhemoglobin, as in Figure 5–9, the β subunits are blue and the α subunits are gray. Positively charged side chains and chain termini involved in ion pairs are shown in blue, their negatively charged partners in red. The Lys C5 of each α subunit and Asp FG1 of each β subunit are visible but not labeled (compare Fig. 5–9a). Note that the molecule is oriented slightly differently than in Figure 5–9. The transition from the T state to the R state shifts the subunit pairs substantially, affecting certain ion pairs. Most noticeably, the His HC3 residues at the carboxyl termini of the β subunits, which are involved in ion pairs in the T state, rotate in the R state toward the center of the molecule, where they are no longer in ion pairs. Another dramatic result of the T → R transition is a narrowing of the pocket between the β subunits.

T and R states of Hemoglobin

Hemoglobin exists in two major conformational states: Relaxed (R) and Tense (T)

- Although oxygen binds to hemoglobin in either state, it has a significantly higher affinity for hemoglobin in the R state.

• In the absence of O_2, T state is more stable; when O_2 binds, R state is more stable, so hemoglobin undergoes a conformational change to the R state.

• The structural change involves readjustment of interactions between subunits.

- T and R originally denoted as such because the T state is stabilized by a greater number of ion pairs, many of which lie at the $\alpha_1\beta_2$ (and $\alpha_2\beta_1$) interface

- When the protein undergoes this transition, the structures of the individual subunits change little, but the subunit pairs slide past each other and rotate, narrowing the pocket between the subunits.

- In this process, some of the ion pairs that stabilize the T state are broken and some new ones are formed.

- In the T state, the porphyrin is slightly puckered, causing the heme iron to protrude somewhat on the proximal His (His F8) side.

- The binding of O2 causes the heme to assume a more planar conformation, shifting the position of the proximal His and the attached F helix. These changes lead to adjustments in the ion pairs at the $\alpha_1\beta_2$ interface.

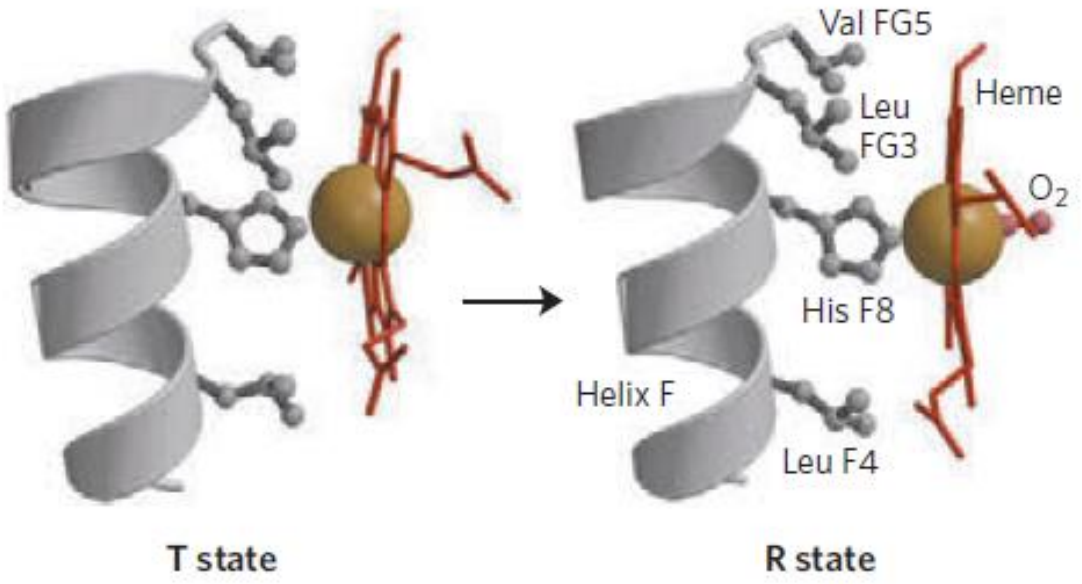


FIGURE 5–11 Changes in conformation near heme on O_2 binding to deoxyhemoglobin. (Derived from PDB ID 1HGA and 1BBB) The shift in the position of helix F when heme binds O_2 is thought to be one of the adjustments that triggers the T → R transition.

When oxygen binds, the electronic properties of heme iron change; this accounts for the **change in color** from the **dark purple of oxygen-depleted venous blood** to the **bright red of oxygen-rich arterial blood**.

Some small molecules, such as carbon monoxide (CO) and nitric oxide (NO), coordinate to heme iron with greater affinity than does O_2. When a molecule of CO is bound to heme, O_2 is excluded, which is why **CO is highly toxic to aerobic organisms.**

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

$$P + L \rightleftharpoons PL \qquad (5\text{–}1)$$

The reaction is characterized by an equilibrium constant, *K*a, such that

$$K_a = \frac{[PL]}{[P][L]} = \frac{k_a}{k_d} \qquad (5\text{–}2)$$

where

- k_a and k_d are rate constants
- K_a is an **association constant** that describes the equilibrium between the complex and the unbound components of the complex. The association constant provides a measure of the affinity of the **ligand L** for the protein. K_a has units of M^{-1};

A higher value of K_a corresponds to a higher affinity of the ligand for the protein.

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

$$K_a[\mathrm{L}] = \frac{[\mathrm{PL}]}{[\mathrm{P}]} \qquad (5\text{–}3)$$

We can now consider the binding equilibrium from the standpoint of the fraction, (θ), of ligand binding sites on the protein that are occupied by ligand:

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

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

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

Any equation of the form $x = y/(y + z)$ describes a hyperbola, and θ is thus found to be a hyperbolic function of [L].

The fraction of ligand-binding sites occupied approaches saturation asymptotically as [L] increases.

The [L] at which half of the available ligand binding sites are occupied (that is, θ= 0.5) corresponds to $1/K_a$.

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

K_d is the equilibrium constant for the release of ligand. The relevant expressions change to:

$$K_d = \frac{[P][L]}{[PL]} = \frac{k_d}{k_a} \qquad (5\text{–}6)$$

$$[PL] = \frac{[P][L]}{K_d} \qquad (5\text{–}7)$$

$$\theta = \frac{[L]}{[L] + K_d} \qquad (5\text{–}8)$$

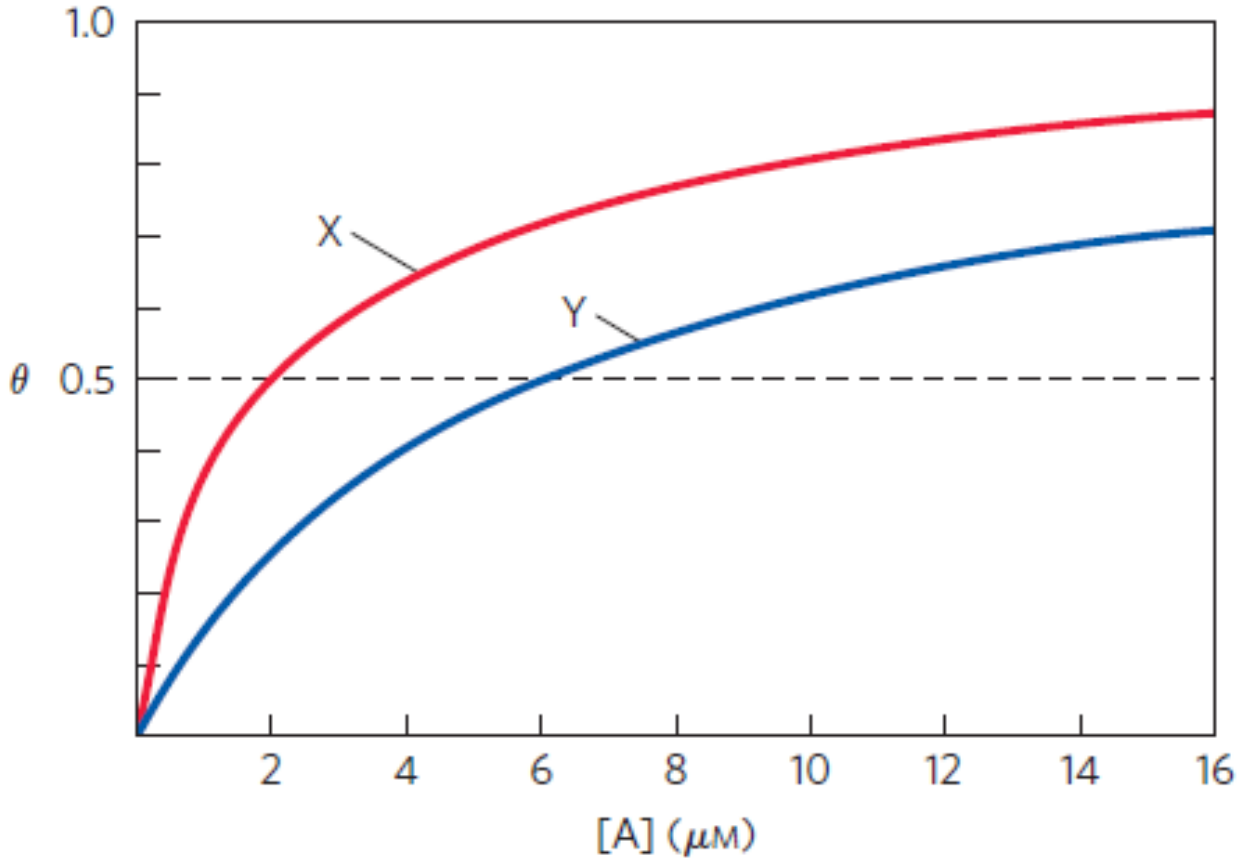
- ***K*d is equivalent to the molar concentration of ligand at which half of the available ligand binding sites are occupied.**

- At this point, the protein is said to have reached half-saturation with respect to ligand binding.

- The more tightly a protein binds a ligand, the lower the concentration of ligand required for half the binding sites to be occupied, and thus the lower the value of *K*d.

WORKED EXAMPLE 5–1 Receptor-Ligand Dissociation Constants

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



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

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