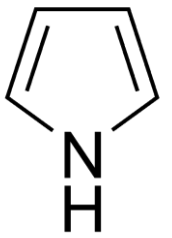
Myoglobin and Hemoglobin

Myoglobin (Mb)

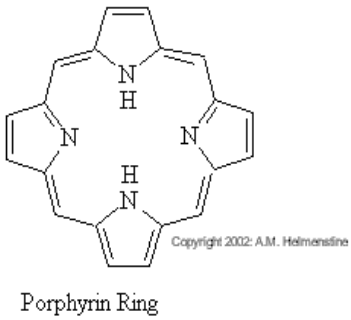
Myoglobin, an intracellular heme protein found in most cells that stores O_2 especially in heart and skeletal muscle. Its physiological function is to store O_2 in muscles and facilitate oxygen diffusion because O_2 solubility in aqueous solution is low.
Myoglobin binds the O_2 released by hemoglobin which it stores to meet the demands of muscle contraction. As O_2 is used in the muscle cell for generation of ATP during contraction, it is released from myoglobin and picked up by cytochrome oxidase, a heme-containing enzyme in the electron transport chain that has an even higher affinity for oxygen than myoglobin.
Myoglobin consists of a single polypeptide chain of 153 amino acids attached to a single heme group. About 80% of myoglobin proteins are α helix. It consists of eight α helical segments; these are termed helices A–H. Each helical segment is terminated either by the presence of proline (whose five-membered ring cannot be accommodated in an α-helix) or by β-bends and loops.
The eight α helical segments are folded into a globular structure, creating a cradle (box) and within this cradle lay a single heme group and the binding site of O_2. The heme of myoglobin lies between helices E and F.
The polypeptide of myoglobin may be viewed as serving three critical functions: 1- it hold the heme group, 2- it protects the heme iron atom from oxidation, and 3- it provides a pocket into which the O_2 can fit.

Structure of heme in myoglobin

Both myoglobin and hemoglobin have heme. Heme is a complex of porphyrin and ferrous iron (Fe^{2+}). Porphyrins are the pigment in red blood cells they are a group of organic compound that have four pyrrole subunits interconnected via α-methylene bridges (=CH-) and are readily bind metals (Fe). A pyrrole ring is a group of four carbon atoms and a nitrogen atom bonded together in a ring (see figure).

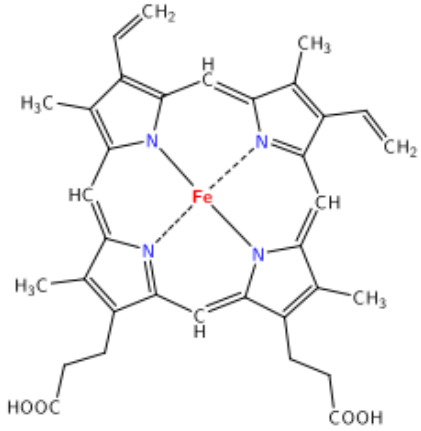


Pyrrole ring



Porphyrin Ring

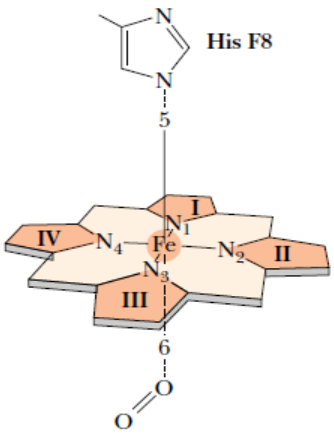
Porphyrins



Heme

The iron is held in the centre of the porphyrin ring. Iron ions prefer to interact with six ligands

A- Four of the ligands to this iron ion are provided by nitrogen atoms in the pyrrole ring system.
B- The fifth ligand, on one side of the heme, is provided by a nitrogen atom from the imidazole group of His 93 (proximal histidine) (also known as His F8 the eighth residue of the 'F helix' of myoglobin).



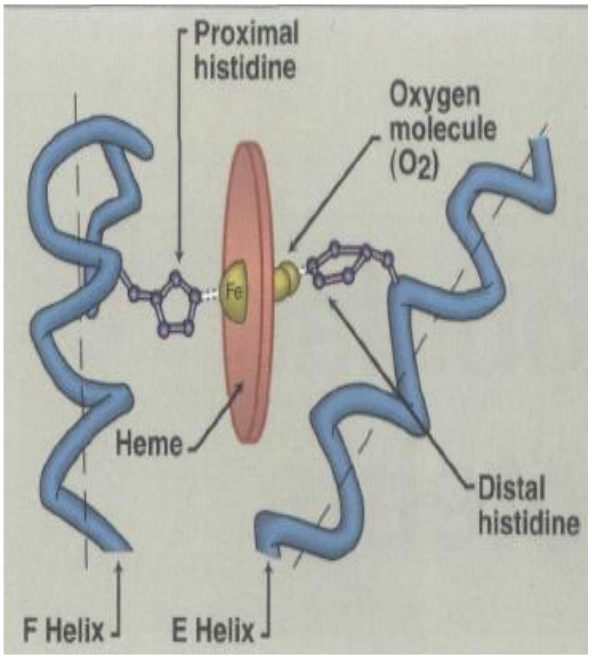
C- The sixth ligand to iron is provided by molecular oxygen, which binds to the heme group (see figure).

D- On the oxygen-binding side of the heme lays another histidine residue, His E7 (also known as distal histidine and His 64). While its imidazole function lies too far away to interact with the Fe atom, it is close enough to contact the O_2. His E7 hydrogen bond with O_2. Two hydrophobic side chains on the O_2-binding side of the heme, valline E11 and Phenylalanine CD1 (the first residue in the segment between helices C and D), help hold the heme in place and they are flexible allowing O_2 to enter and exit. Therefore, the O_2- binding site is a sterically hindered region. It helps stabilize the binding of oxygen to the ferrous iron through creating a special microenvironment for the heme.

As said above at the centre of the heme group is the iron +2 metal ion. The nitrogen atoms and oxygen bind to the iron ion through what are called coordinate covalent bonds (dipolar bond). This means that, unlike normal covalent bonds where each atom contributes one electron for the bond, the nitrogen contributes both electrons for the coordinate covalent bond. The oxygen molecule will ultimately bind to this iron ion also using a coordinate covalent bond.

The folding pattern puts hydrophilic amino acid side chains on the outside and buries hydrophobic side chains in the interior, making the protein highly water soluble. The hydrophobic environment in the interior of myoglobin or hemoglobin prevents the oxidation of iron.

The interior contains only nonpolar residues such as Leu, Val, Phe, and Met. The exceptions are His E7 and His F8 that found in the interior which has a function in O2 binding.



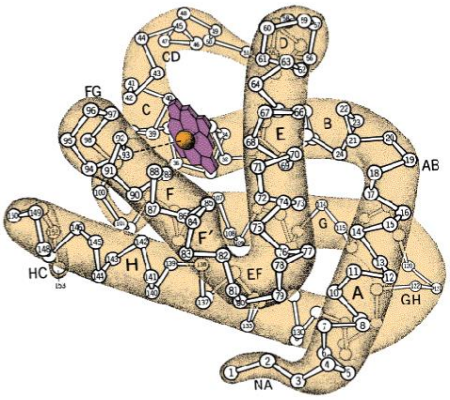
Hemoglobin (Hb)

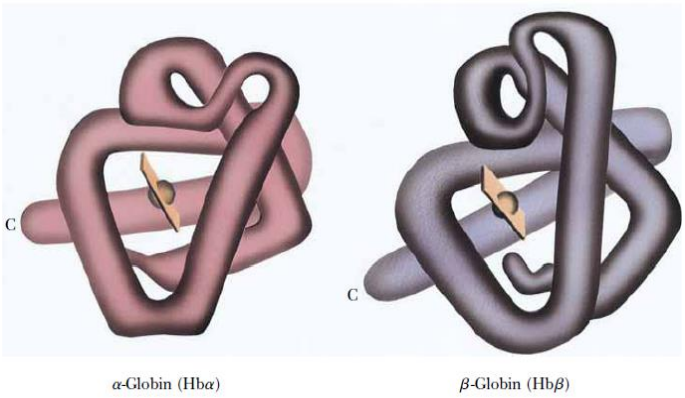
Hemoglobin, the intracellular protein that gives red blood cells their colour. Hemoglobin is responsible for binding oxygen in the lung and transporting the bound oxygen throughout the body where it is used in aerobic metabolic pathways. When the Hemoglobin is carrying an oxygen molecule it is call oxyhemoglobin, when it is not carrying an oxygen molecule it is called deoxyhemoglobin.

Hemoglobin (Hb) is a compact globular protein. It consists of four polypeptide chains, 2 α chains and 2 of β chains, each of which is very similar structurally to the myoglobin polypeptide chain, and each bears a heme group. The α and β subunits differ in primary structure (i.e., they have different sequences of amino acids and are encoded by different genes) and are related to myoglobin. Only about 18% of the residues are identical in myoglobin and in α and β subunit in Hb but the three polypeptides have similar tertiary structures.

The β chain at 146 amino acid residues is shorter than the myoglobin chain (153 residues), mainly because its final helical segment, the H helix, is shorter.

The α-chain (141 residues) also has a shortened H helix and lacks the D helix (see figure below).



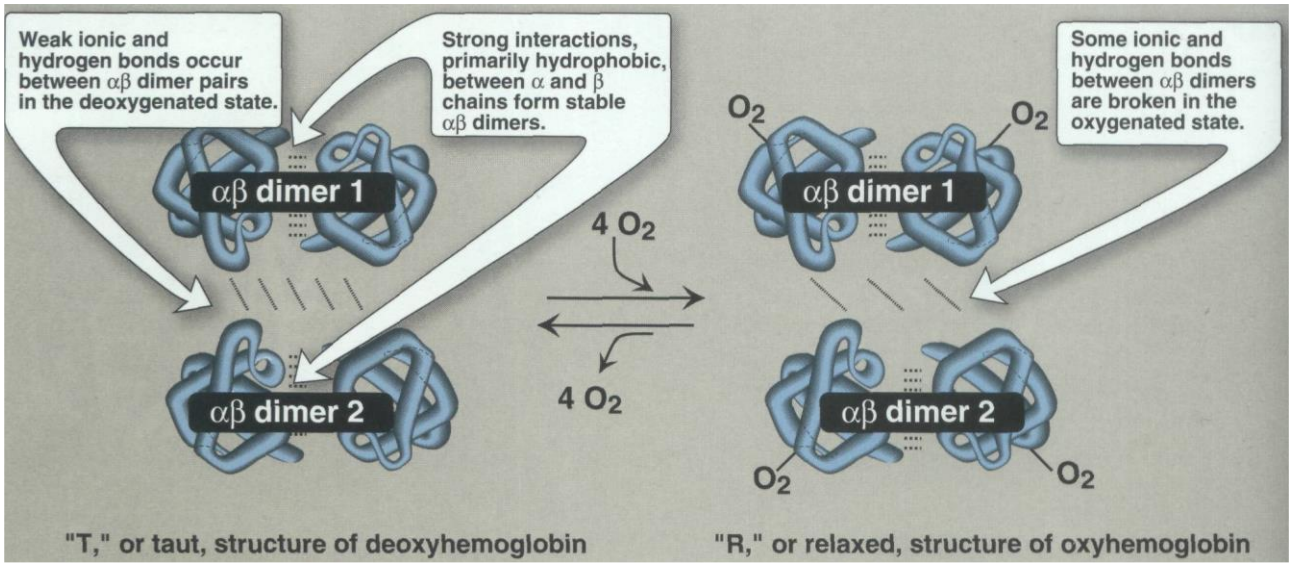


Myoglobin α- globin in Hb β-globin in Hb

Quaternary structure of hemoglobin: The subunit interactions are between dissimilar chains; α1 associate with β1 subunit (α1β1) and α2 associate with β2 (α2β2). The hemoglobin tetramer can be visualized as being composed of two identical dimmers, dimmer one (α1β1) and dimmer two (α2β2). The two polypeptide chains within each dimmer are held tightly together, primarily by hydrophobic interactions although hydrogen bonds and ion bond are also involved. (the ionic bond is weak ionic bond and is called salt bridge (salt bond)) [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]. The α1α2 and β1β2 interactions are weak because these subunits are separated by solvent filled channels.
Interchain hydrophobic interactions form strong associations between α-subunits and β subunits in the dimmers.

Hemoglobin exists in to form T & R forms:
a) T form: The deoxy form of hemoglobin is called the "T" or taut **(tense)** form. In the T form, the two αβ dimmers interact through a network of ionic bonds and hydrogen bonds that constrain the movement of the polypeptide chains. The T form is the **low oxygen-affinity form** of hemoglobin.

b) R form: The binding of oxygen to hemoglobin causes the rupture of some of the ionic bonds and hydrogen bonds between the αβ dimmers. This leads to a structure called the **"R,"** or **relaxed** form, in which the polypeptide chains have more freedom of movement. The R form is the **high oxygen-affinity form** of hemoglobin.



RBCs are typically shaped as biconcave disks: flattened and depressed in the centre. Biconcave shape gives them a much greater surface area with which to exchange oxygen and carbon dioxide in the lungs and with body cells besides giving them flexibility to squeeze through tiny capillaries. Red blood cells are able to carry oxygen so efficiently because of haemoglobin.

Carbon monoxide: the heme group has a much greater bonding affinity for carbon monoxide than for oxygen. The affinity between hemoglobin and carbon monoxide is approximately 230 times stronger than the affinity between hemoglobin and oxygen. However, the distal E7 histidine hinders bonding of CO at the preferred angle to the plane of the heme ring and thus create a hindered environment for CO binding. If the distal histidine was absent, even low levels of CO would compete oxygen for the iron binding site, resulting in suffocation.

Methemoglobin

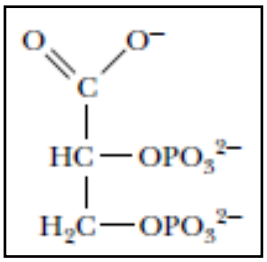
To bind oxygen, the iron of hemoglobin must be in the ferrous (Fe^{2+}) state. Reactive oxygen species can oxidize the iron to the ferric (Fe^{3+}) state, producing methemoglobin. Methemoglobin is useless in transporting oxygen. Only a very small amount of methemoglobin is present in normal blood, as the red blood cell possesses an effective system for reducing heme Fe^{3+} back to the Fe^{2+} state. This system consists of NADH (generated by glycolysis), cytochrome b5 reductase (also known as methemoglobin reductase), and cytochrome b5.
Cytochrome *b5* reduces the Fe^{3+} of methemoglobin. The oxidized cytochrome *b5* is then reduced by cytochrome *b5* reductase, using NADH as the reducing agent.

Binding of oxygen to myoglobin and hemoglobin: Allosteric effects

The oxygen-binding properties of hemoglobin are regulated by interaction with **allosteric effectors.** The ability of hemoglobin to reversibly bind oxygen is affected by the pO_2, the pH of the environment, the pCO_2 and the availability of **2,3-bisphosphoglycerate.** These are collectively called allosteric ("other site") effectors, because their interaction at one site on the hemoglobin molecule affects the binding of oxygen to heme groups at other locations on the molecule. [Note: The binding of oxygen to myoglobin is not influenced by the allosteric effectors of hemoglobin.]

2,3-Bisphosphoglycerate (2,3-BPG) is an Important Allosteric Effector for Hemoglobin

BPG is an intermediary product in the glycolytic pathway for the oxidation of glucose. BPG is an important regulator of the binding of oxygen to hemoglobin. It is the most abundant organic phosphate in the red blood cell. A low PO_2 in peripheral tissues promotes the synthesis in RBC of 2,3-bisphosphoglycerate (2,3-BPG). The binding of 2,3-BPG to Hb promotes the release of O_2 by stabilizing the quaternary structure of deoxyhemoglobin. The Hb molecule has one binding site for 2,3-BPG. This site is situated within the central cavity formed by the association of the four amino acids; the strongly negative 2,3-BPG molecule (see Fig) binds to these positively charged amino acids. Once bounded, 2,3-BPG cross-links the two β-subunits. The ionic bonds between 2,3-BPG and the two β-chains aid in stabilizing the conformation of Hb in its deoxy form, thereby favouring the dissociation of oxygen. Hence, if humans did not have the BPG effect on hemoglobin, then hemoglobin would not release O_2 as effectively as it does as it passes through the capillaries.

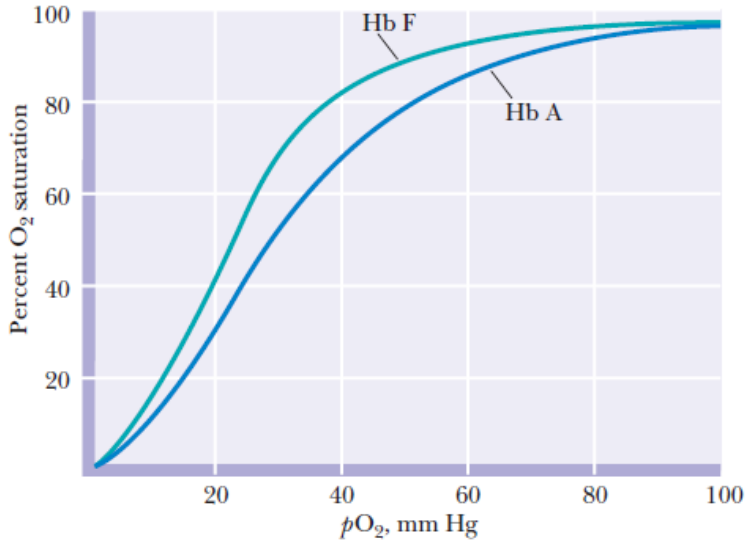


Thus, 2,3-BPG and O_2 are mutually exclusive allosteric effectors for Hb, even though their binding sites are physically distinct.

$$\underset{\text{Oxyhemoglobin}}{\mathbf{HbO}} + \mathbf{2,3\text{-}BPG} \leftrightarrow \underset{\text{deoxyhemoglobin}}{\mathbf{Hb\text{-}2,3\text{-}BPG}} + \mathbf{O_2}$$

Fetal Hemoglobin Has a Higher Affinity for O_2 Because It Has a Lower Affinity for BPG

The fetus depends on its mother for an adequate supply of oxygen, but its circulatory system is entirely independent. Gas exchange takes place across the placenta. Ideally fetal Hb should be able to absorb O_2 better than maternal Hb so that an effective transfer of oxygen can occur. Fetal Hb differs from adult Hb in that the β-chains are replaced by very similar, but not identical, 146-residue subunits called γ chains (gamma chains). Fetal Hb is thus $\alpha_2\gamma_2$. 2,3-BPG binds less effectively with the γ chains of fetal Hb (also called Hb F). (Fetal γ chains have Serine (uncharged) instead of Histidine at position 143, and thus lack two of the positive charges in the central BPG-binding cavity). Figure below compares the relative affinities of adult Hb (also known as Hb A) and Hb F for O_2 under similar conditions of pH and [2,3-BPG]. Note that Hb F binds O_2 at pO_2 values where most of the oxygen has dissociated from Hb A. Much of the difference can be attributed to the low capacity of Hb F to bind 2,3-BPG; Hb F thus has an intrinsically greater affinity for O_2, and oxygen transfer from mother to fetus is ensured.



Comparison of the oxygen saturation curves of Hb A and Hb F under similar conditions of pH and 2,3-BPG