4. Metal Ions in Biology
   Hemoglobin and Myoglobin
Application of coordination chemistry in biology

**Hemoglobin and Myoglobin**

The roles of inorganic elements in general and transition metal ions in particular in many biological processes are well recognized. In recent years, the field of bio-inorganic chemistry has grown very vast as a result of many recent findings. In this course, the discussion will be limited to the subtle role of a metal ion in an important biological process viz., oxygen transport in living systems.

There are two metalloproteins in vertebrates known as **Hemoglobin and Myoglobin**, which are responsible for oxygen uptake and transport. These are large polymeric entities. Hemoglobin picks up oxygen from lungs (or gills), transports it and delivers it to myoglobin, which is present in tissues. The role of myoglobin is to store the oxygen and release it for metabolic process. Also it may serve as an oxygen reserve from which the organism can draw during increased metabolism or oxygen deprivation.

Both hemoglobin and myoglobin contain an **active center or site** (where the function of the metalloprotein is carried out-in this case uptake and release of oxygen). This active site is wrapped up by the folding of the protein attached to it.

**Hemoglobin (Hb)**
- MW= 64,500 g
- \(\alpha_2\beta_2\)
- \(\alpha= 141\) residues
- \(\beta= 146\) residues
- active site: Fe(por)
- function: cooperatively binds and transports \(O_2\)

**Myoglobin (Mb)**
- MW= 17,800 g
- \(\alpha\)
- \(\alpha= 161\) residues

Notice that the hemoglobin is essentially a tetramer of myoglobin, viz., there are four myoglobin like units in hemoglobin.
Active site of Hemoglobin and Myoglobin

The active center of hemoglobin and myoglobin is made of the heme unit. This consists of a macrocyclic ligand known as porphyrin, in the cavity of which is bound the iron atom. The porphyrin macrocycle is made up of four pyrrole rings with conjugated double bonds. The periphery of the macrocyclic ring can have several substituents. However, it may be noted that these substituents on the periphery can be used to tune the stereoelectronic properties of the complex. The inside of the porphyrin ring has four nitrogen atoms, two of which have hydrogens attached to them. It can be anticipated that these hydrogens can be quite readily deprotonated to generate a dianionic ligand (with a –2 charge). In this form the ligand can readily bind to a number of divalent metal ions. The distance from the centre of the porphyrin ring and the nitrogen atoms is about 2.0 Å, which corresponds to a normal M-N distance involving the first row transition metal ions. Another feature of the porphyrin ring needs to be mentioned. Because of this versatility in terms of ligand properties the porphyrin ligand is present in a variety of biologically important systems.

The role of the protein. If free heme in an aqueous solution is exposed to oxygen it is converted immediately into a µ-oxo dimer in a stepwise reaction sequence as shown below. Obviously it is important to be able to stop reactions 2,3 and 4 if oxygen transport is to be successfully carried out in a continuous manner. The fact that the heme unit is wrapped around by the huge polymeric
protein sheath has suggested to researchers that it may be functioning as a steric block to prevent the reaction 2 from occurring. In order to test this hypothesis a model compound called picket-fence porphyrin was synthesized. Its design incorporated sterically hindered substituents on one side of the porphyrin. The Fe(II) complex of this porphyrin undergoes oxygenation reversibly without undergoing the irreversible reactions 2, 3 and 4. This means that the sterically hindered substituents prevent two such units to come together to form a µ-oxo complex. If we extend this logic to the naturally occurring system, the role of the protein (at least one of the important roles) would be also to prevent the irreversible µ-oxo complex formation.
Coordination environment of Fe (II) in deoxy myoglobin and oxy myoglobin. Iron (II) in deoxy myoglobin is in a high spin state. The coordination environment comprises of four nitrogens from the porphyrin ring, one imidazole nitrogen arising out of the amino acid residue, histidine, of the protein chain, and possibly a second distant imidazole nitrogen or a water molecule. (Notice that the coordination environment is very similar to the model picket-fence porphyrin). Note also that the Fe (II) is not present in the plane of the porphyrin ring but is about 0.4 Å above the plane. Upon oxygenation the coordination number of iron goes up unambiguously to six. The oxygen ligand is found to bond to the iron atom in a bent manner. The Fe-O-O bond angle is about 150° and the Fe-O bond distance is about 1.8 Å. Secondly the iron atom now sits in the plane of the four porphyrin nitrogens. This aspect will be commented on latter in the discussion on the mechanism of oxygen transport. Another important change is that the molecule becomes diamagnetic upon oxygenation.
Diamagnetic behavior of the oxygenated complex. One possibility is that the Fe (II) is oxidized to Fe (III), which now becomes low spin and consequently $t_{2g}^5$ configuration. Oxygen, which picks up the electron, is now $O_2^-$. This would contain an odd electron. The odd electron in Fe (III) and in $O_2^-$ is coupled anti-ferromagnetically to afford a diamagnetic compound. In the model picket fence iron complex upon oxygenation the O-O stretching frequency has been measured and is found to be 1105 cm$^{-1}$. This value is much closer to that found for KO$_2$ (1145 cm$^{-1}$) than for O$_2$ (1560 cm$^{-1}$). Some recent vibrational studies on the oxyhemoglobin also suggest that a similar situation is present. In short, the experimental fact is that oxy hemoglobin and oxy myoglobin become
diamagnetic and the iron centers occupy the same plane as that of the porphyrin nitrogens. This has a great bearing on the mechanism of oxygen transport, as we will see below.

**Deoxy-Hb**

Electronic spectrum: \( \lambda_{\text{max}} \approx 400\text{nm} \)  
Soret band \((\pi \rightarrow \pi^*)\)

Magnetism: \( S = 2 \) (isolated paramagnet)

**Oxy-Hb**

Magnetism:  
- \( S = 0 \) (low T)  
- Increases towards \( S = 1 \) (higher T)  

\[ \rightarrow \text{magnetic coupling} \]

Resonance Raman:  
\( \nu_{O-O} = 1105 \text{ cm}^{-1} \)

| \( \nu_{O-O} \text{ (cm}^{-1}) \) | \( O_2 \) | \( O_2^- \) | \( O_2^{2-} \) |
|---|---|---|
| ~1560 | ~1100 | 850–740 |

\( S = 1 \quad S = 1/2 \quad S = 0 \)

**Conclude:**

\[ O_{2^-} + Fe^{3+} \rightarrow Fe^{3+} \quad \text{superoxide radical} \quad S = 1/2 \]

\[ \text{low-spin} \quad Fe^{3+} \quad (S = 1/2) \]

\[ \text{Coupled spins} \]
Conclude: The proximal base increases O₂ affinity.
The mechanism of oxygen transport and cooperativity

Oxygen affinities of hemoglobin and myoglobin

The function of hemoglobin (Hb) is to bind oxygen in the lungs (at high oxygen partial pressures) and to carry it without any loss and release it to myoglobin (Mb) in the cellular tissues (at low oxygen partial pressures). This implies that Mb should have a greater affinity for oxygen at low partial pressures. Experimentally, the oxygenation saturation curves for Hb and Mb are as shown in the figure to the right. Another feature of the oxygen binding is that $O_2$ is released more readily at lower
**pH.** Thus, in cells where metabolism is active the released CO$_2$ causes the pH to be lowered. It is here that O$_2$ is released.

**Cooperativity in Hemoglobin.** Myoglobin is a monomer (one heme group) whereas hemoglobin is a tetramer (four heme groups). This difference is important, since it allows the four heme units of Hb to bind O$_2$ cooperatively; once one oxygen is bound to Hb the affinity for subsequent oxygen molecules increases. M. F. Perutz initially proposed this mechanism. He argued that when a high spin Fe (II) that is about 0.4 Å above the plane of the porphyrin ring is bound to oxygen it shrinks in size (because it has been oxidized to Fe$^{3+}$) and is brought down into the plane. This causes a pulling of the histidine residue (imidazole group) along with the protein attached to it. As a result the shape of the protein is adjusted and the binding characteristics of other sites are modified. This leads to greater oxygen affinity for the second iron center and so on. During the release of oxygen from Hb to Mb also such cooperativity behavior exists.