Department of Chemistry
L.S COLLEGE MUZAFFARPUR
B. R. A. BIHAR UNIVERSITY
Dr. Priyanka

Subject: Bio-Inorganic chemistry.

Topic: Biological oxygen carriers.

Hill equation.

Bohr effect and their implications.

Introduction.

- The process of respiration requires oxygen to produce ATP.
- ATP provides energy to the body.
- The oxygen which is needed by our body is provided by biological oxygen carriers.

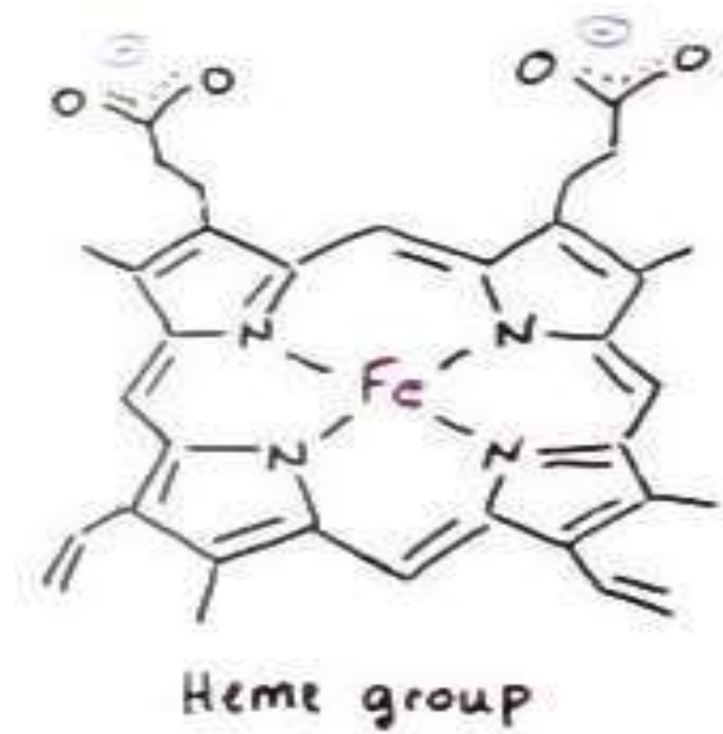
Biological oxygen carriers.

- Hemoglobin. (Hb) (humans).
- Myoglobin. (Mb) (humans) (stores)
- Hemerythrene .(He) (marine invertebrates)
- Hemocyanin. (Hc) (marine animals)

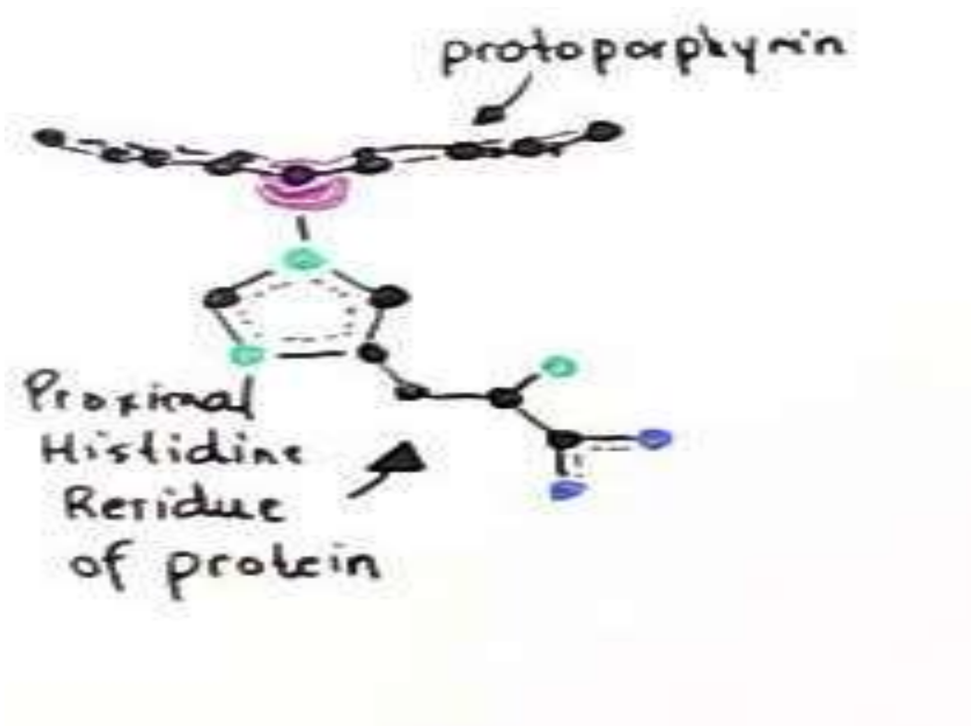
Heme group.

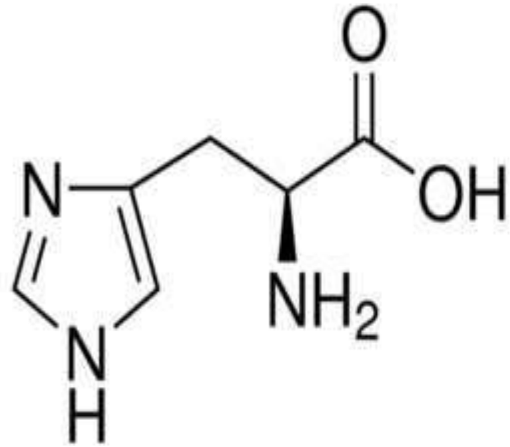
- The group consist of an organic component called PROTOPORPHYRIN.
- It consist of an inorganic component that consist of an IRON atom.
- The Fe atom is at the center of the ring and is bounded to 4 nitrogen atom .
- At this stage the Fe is in its ferrous state and has a +2 oxidation state (Fe has +6 o.s. this means it can form two more bonds).

- Myoglobin and hemoglobin are used to store and carry oxygen in our body .both of these proteins contain the HEME GROUP which is responsible for binding oxygen.



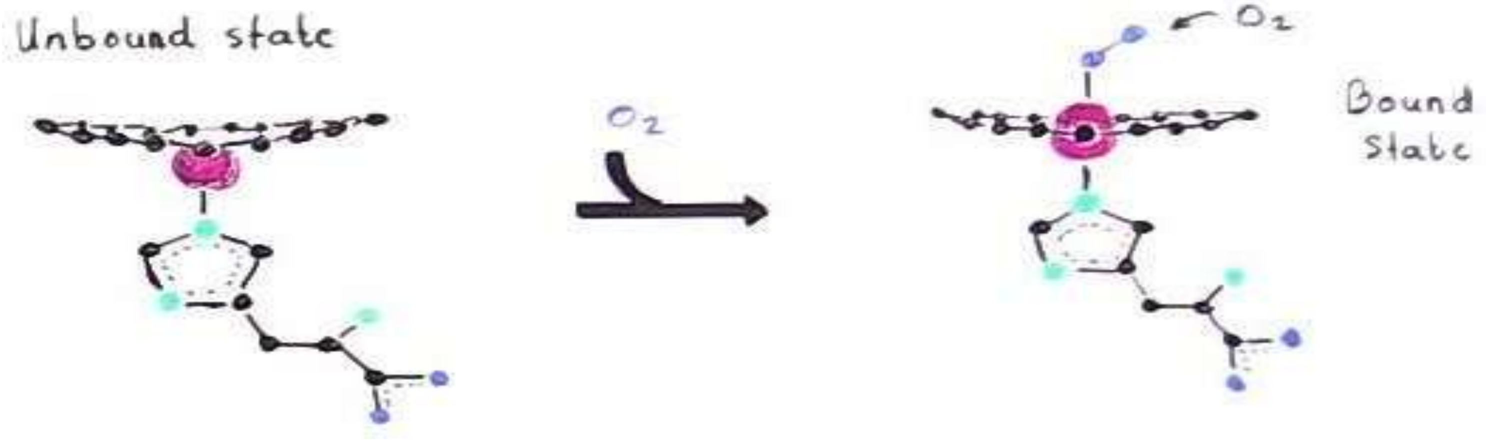
One side of the protorphyrin plane the Fe is bonded to the histidine (α-amino acid) residue of the protein .This is known as the proximal histidine .



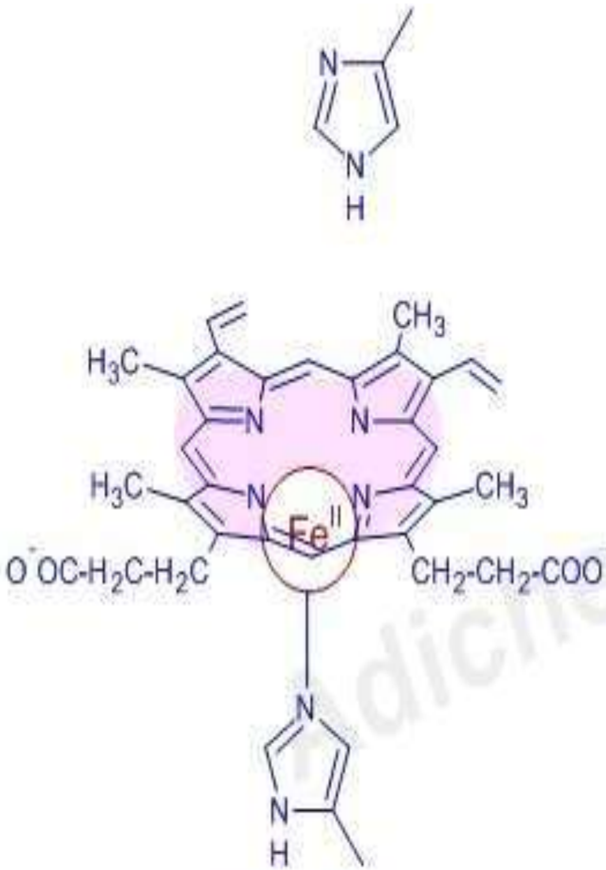


Pink = iron.
Green =nitrogen .
Blue =oxygen.

•In deoxy-hemoglobin or deoxy-myoglobin, the Fe atom remains unbounded to oxygen . In this state ,the Fe atom is too large to fit into the center of the protoporphyrin ring and so the iron remains below the plane of the protoporphyrin.

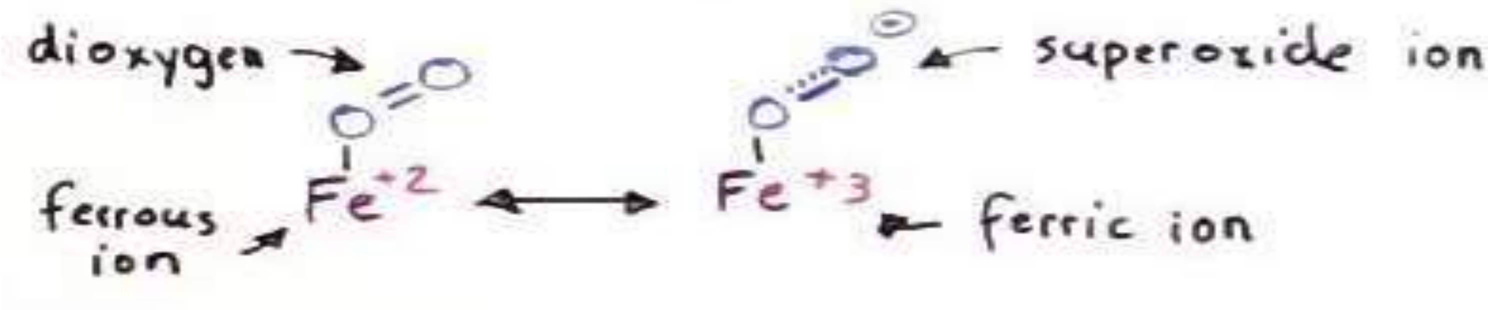


The binding of the oxygen atom to the iron pulls away the electron density from the iron making it smaller. This allows it to fit into the center of the protoporphyrin plane.

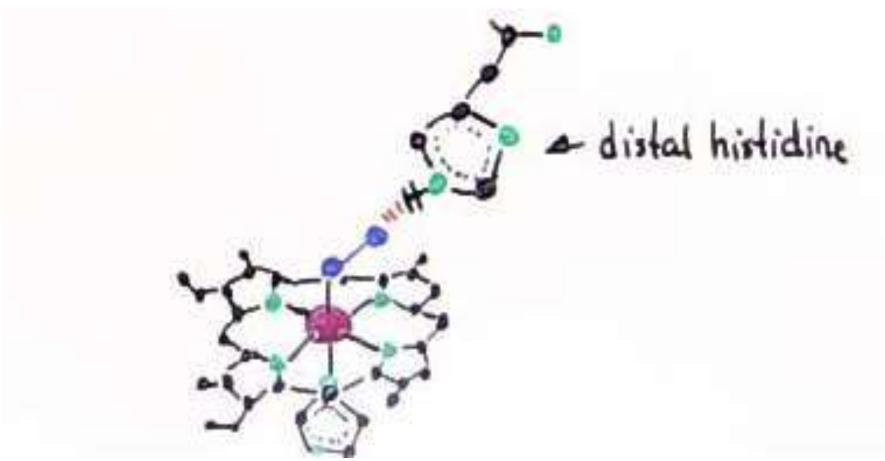


Deoxy Hb
Domed porphyrin
High spin Fe(II) - out of ring

Oxy Hb
Planar porphyrin
Fe ion moves into the ring
Bent O_2

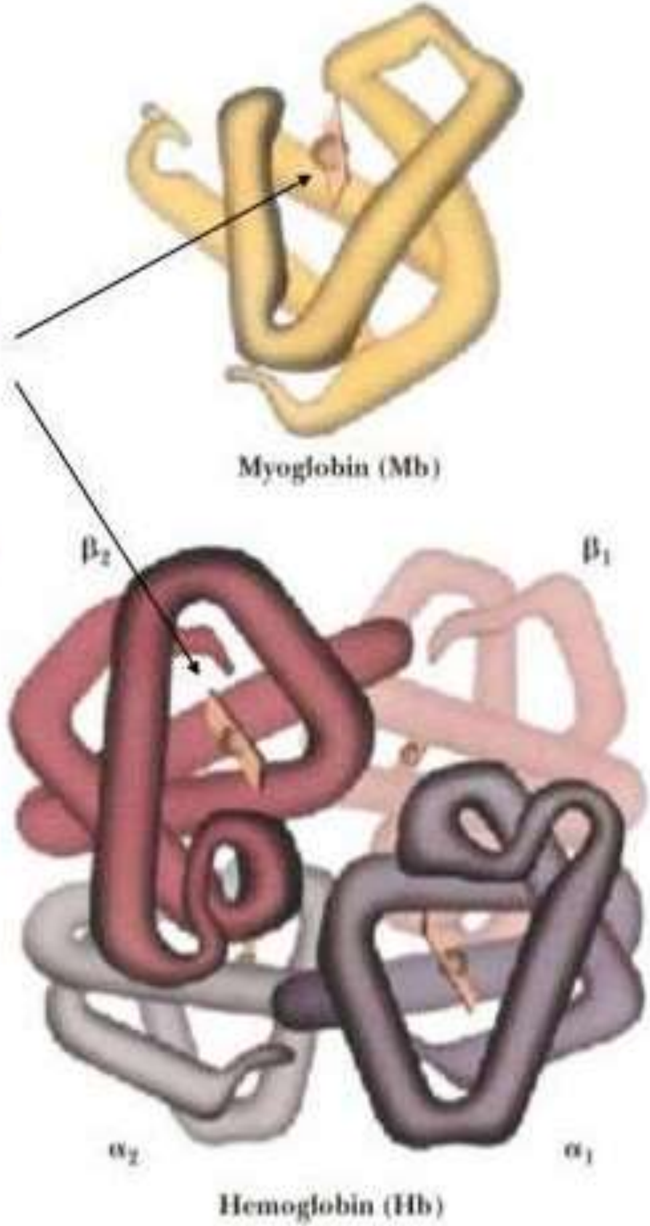


The actual structure of the iron oxygen is resonance stabilized as shown above. The superoxide form has a negative charge . The region is stabilized by another histidine residue called DISTAL HISTEDINE of the protein.



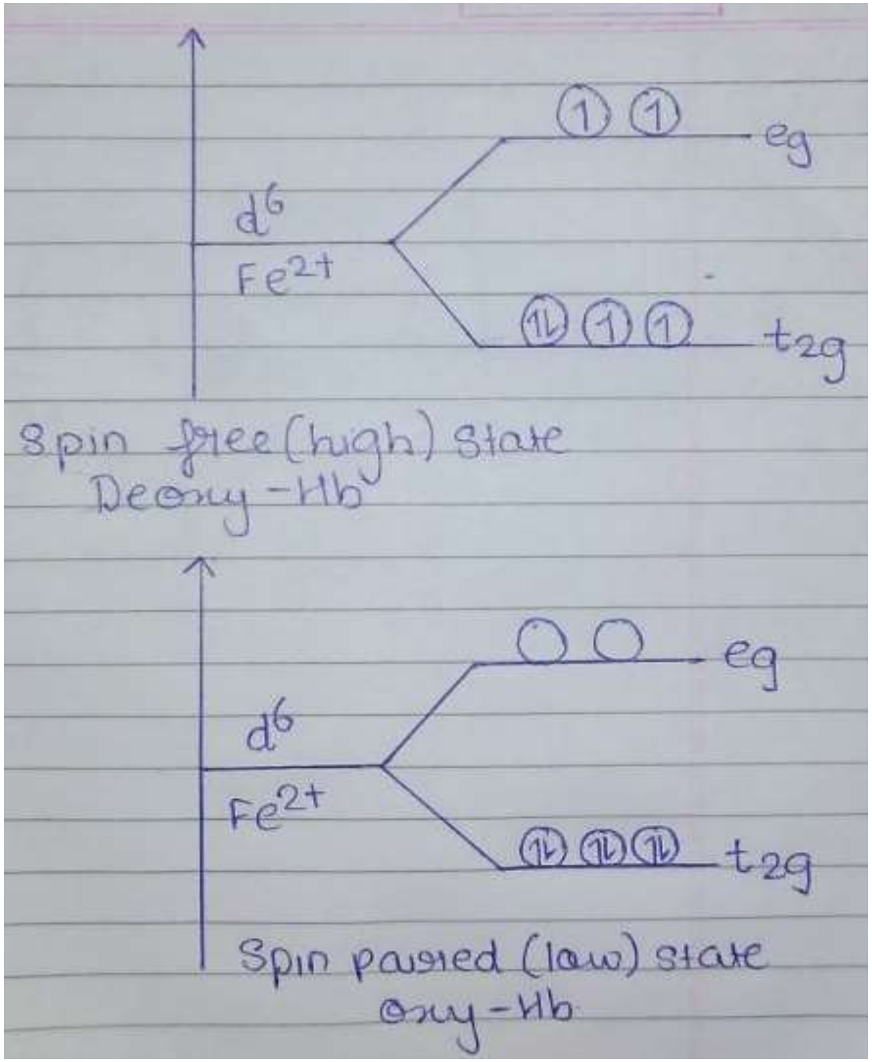
Heme Prosthetic Group

Prosthetic Group = non-polypeptide unit of a protein that can function without the protein



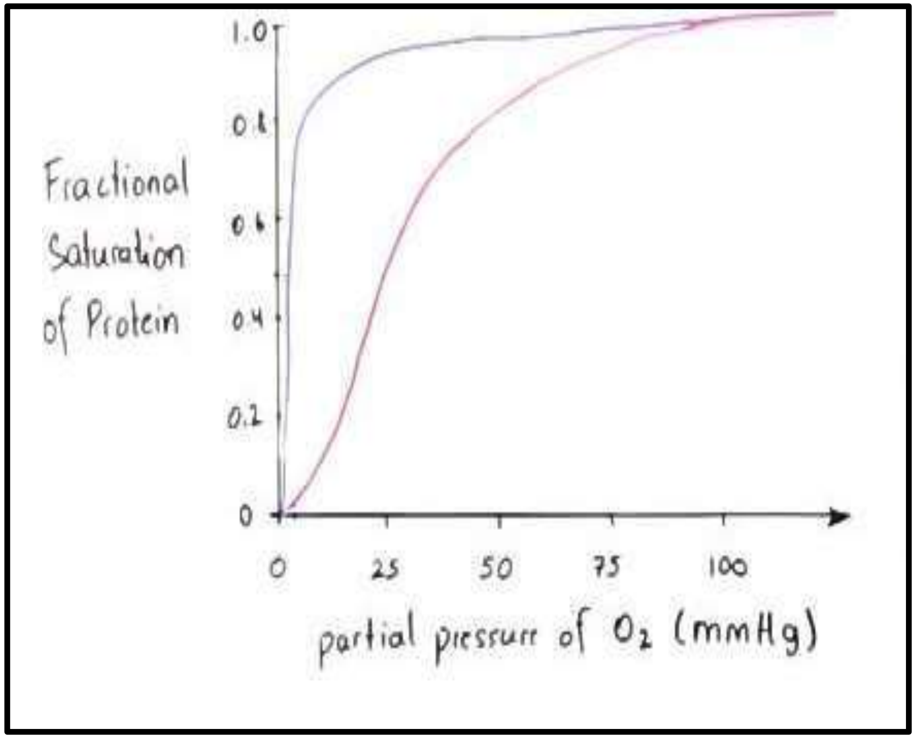
The spin pairing of Fe (II) in Hb.

- In Deoxy-Hb the Fe^{2+} is spin free (high) spin state which there are unpaired electrons & is paramagnetic in nature.
- In this the size of Fe^{2+} is 92 picometer which is larger and does not fit into the porphyrin cavity & lie 0.8 A^0 below the plane because of which it is a less stable complex.
- In Oxy-Hb the Fe^{2+} is spin paired (low) spin state which have no unpaired electrons & is diamagnetic in nature.
- In this the size of Fe^{2+} is 75 picometer which is smaller and can fit into the porphyrin cavity & hence it is a stable complex.



Oxygen binding curve .

- The oxygen binding curve can be used to describe the oxygen binding properties of Hb and Mb.
- The y-axis describes the relative fraction of proteins that are saturated with O_2 .
- The x-axis describes the concentration of O2 (mmHg).
- Conclusion :Mb dose not bind oxygen co-operatively, which makes it great at storing oxygen in the muscle cell.
- Hb co-operative behavior makes it great O2 carrier .it can readily pick up O2 in the lungs and drop it off at the tissue cells.



Hb= red.

Mb= blue

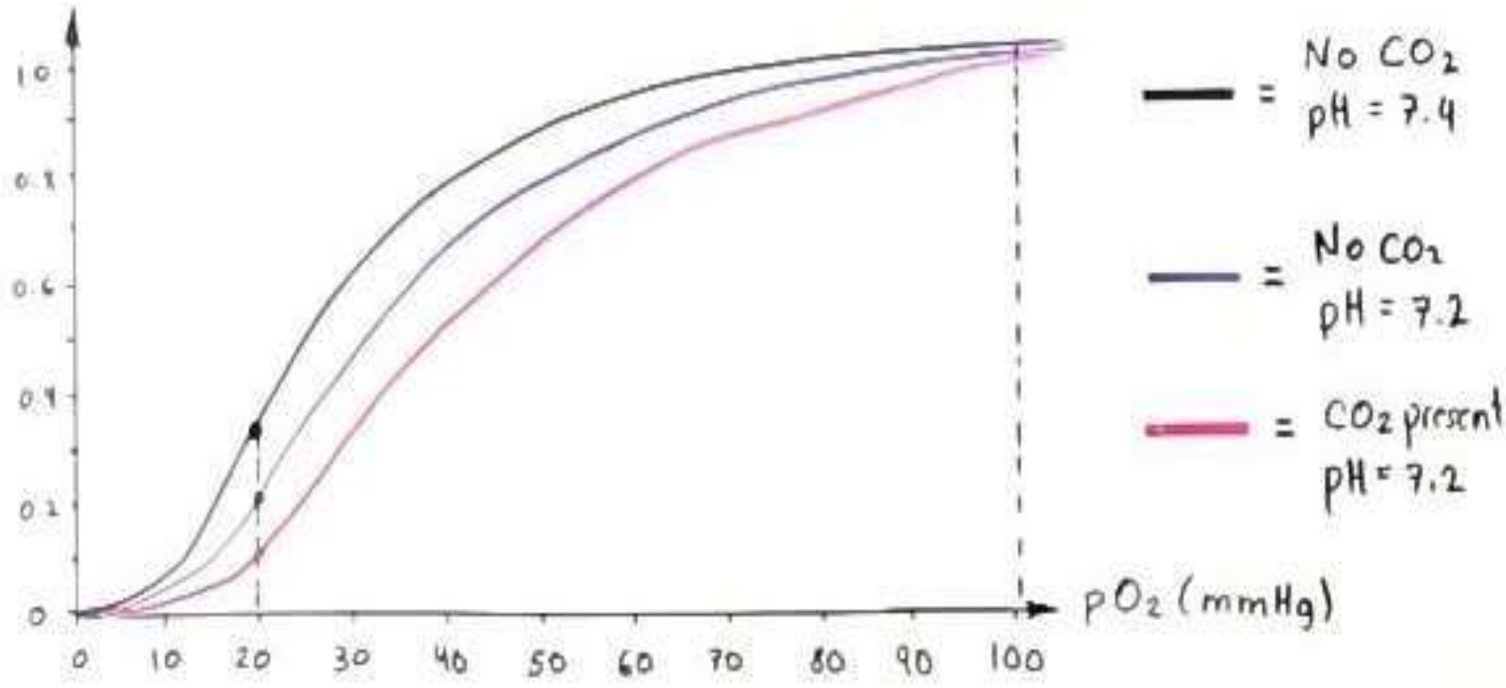
Comparison between Hb and Mb

Hb	**Mb**
• It is oxygen carrier. • It is present in R.B.C. • It consist of 4 polypeptide chain i.e. it is a tetramer.(1 and 2. 1 and 2) • Its MW=5400. • It shows bohr effect. • It shows sigmoid curve	• It stores oxygen. • It is present in muscles and bone marrow. • It consist of single polypeptide chain. i.e. it is a monomer. • Its MW=1600. • It does not show bohr effect • It shows hyperbolic curve .

Bohr effect.

- The Bohr effect is a change in oxygen affinity of hemoglobin with a change in pH.

•This effect is beneficial at the tissue level where the lower pH decreases oxygen affinity and promotes oxygen release .



- Based on this graph:
  - 98% - 32% = 66% is released to the tissues in the absence of CO_2 and pH = 7.4.
  - 98% - 21% = 77% is released in the absence of CO_2 and a lower pH of 7.2.
  - 98% - 10% = 88% is released in the presence of CO_2 and a lower pH of 7.2.
- A higher concentration of CO_2 and a lower pH (higher amount of H^+) shifts the curve to the right, thereby decreasing the affinity of hemoglobin for oxygen and allowing it to unload more oxygen to the tissues.

Hill equation.

The affinity of Hemoglobin towards oxygen binding can be explained on the basis of Hill equation.

For Oxy-Hb

$$Hb + nO_2 \rightarrow Hb(O_2)_n$$

$$K = \frac{[Hb(O_2)_n]}{[Hb]+[O_2]^n}$$

If f represents a fraction of hemoglobin oxygenated and p is the partial pressure of O_2 then K is,

$$K = \frac{f}{(1-f)P}$$

Hemoglobin contains four heme units show more complex behavior & the equation is given as:

$$f = \frac{KP^n O_2}{1+KP^n O_2} \qquad \text{(where n=2.8)}$$

The value of n depends upon the pH

n = constant or hill coefficient
Conditions

1.If n>1 there is positive cooperativity.

2.If n<1 there is negative cooperativity.

3.It n=1 there is no cooperativity.

The value of n can be obtained from a graph which is a
Plot of log[oxy-Hb]/[deoxy-Hb] versus log PO_2 or log f/1-f.

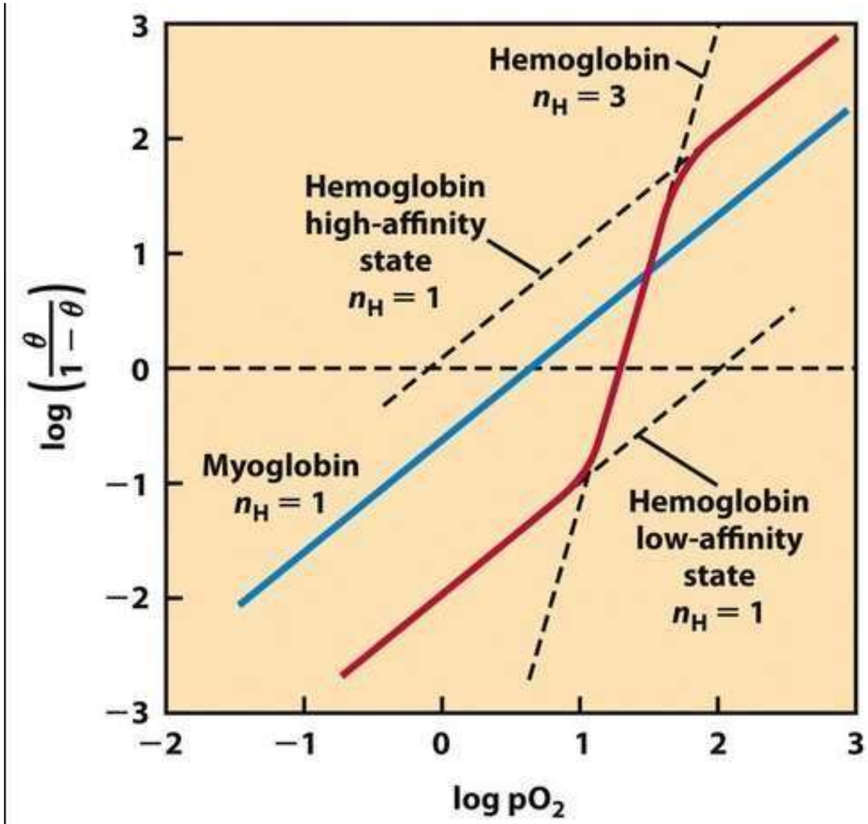
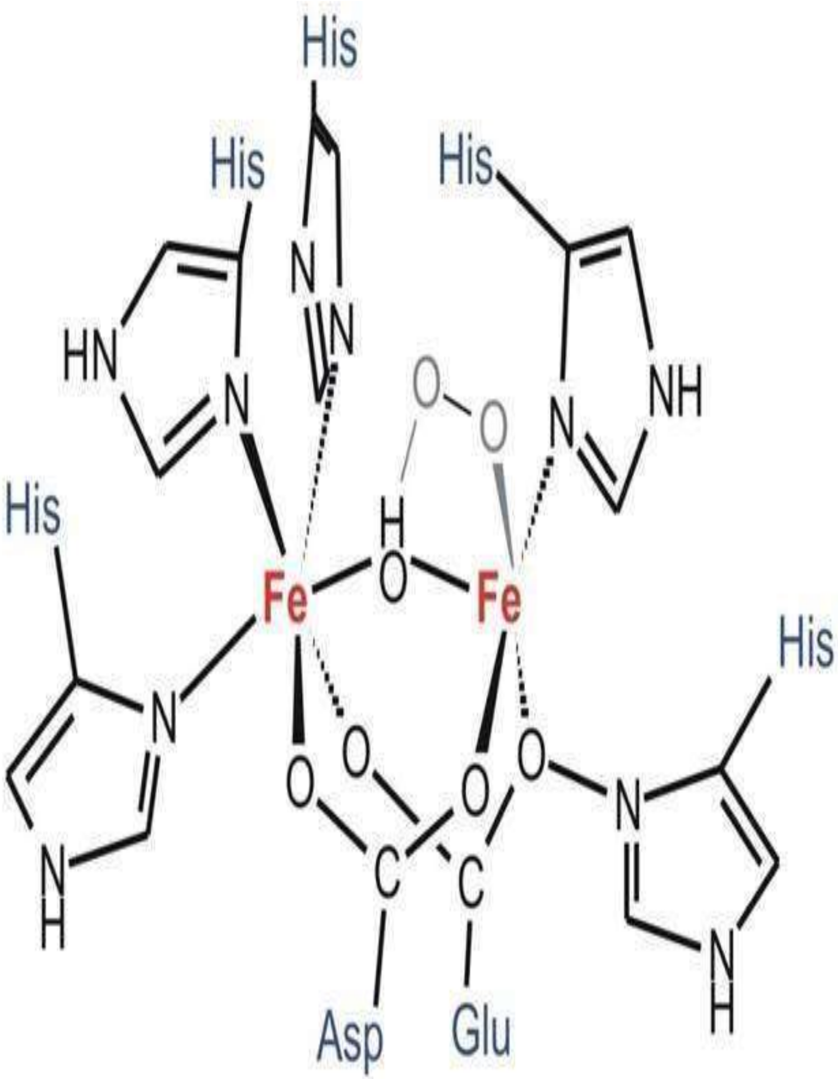


FIGURE 5-14 Hill plots for oxygen binding to myoglobin and hemoglobin. When $n_H = 1$, there is no evident cooperativity. The maximum degree of cooperativity observed for hemoglobin corresponds approximately to $n_H = 3$. Note that while this indicates a high level of cooperativity, n_H is less than n, the number of O_2-binding sites in hemoglobin. This is normal for a protein that exhibits allosteric binding behavior.

Hemerythrene

- It is a non-heme protein and it has no porphyrin skeleton .
- It is found in large number of marine invertebrates.
- It does not shows Bohr effect because it is a non heme protein.

Hemerythrene



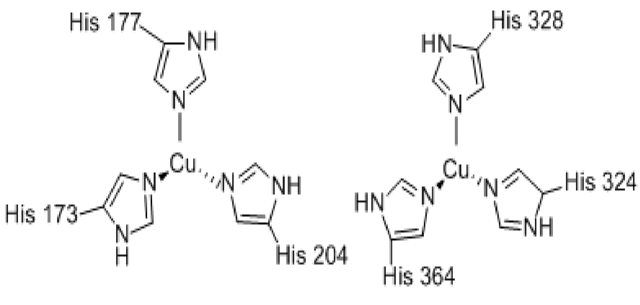
- Each subunit contains 2 Fe(Π) centers. The Oxy-Hr is reddish violet in color & is diamagnetic in nature & the Deoxy is paramagnetic in nature.
- The primary structure contains a long polypeptide chain having 113 amino acid residue. Hence the subunit have 2 Fe centers attached to 3 nitrogen atoms of histidine.
- The Fe have distorted octahedral environment. It binds with the peroxo & convert into the Oxy form.
- Each Fe pair binds with 1 O_2 molecule hence Fe:O is 2:1.
- The 2 Fe(Π) centers are joined by two bridging carboxylates (Glu-58,Asp-106) of the protein chain there is also oxo or hydroxo to act as a 3rd bridging ligand

Hemocyanin

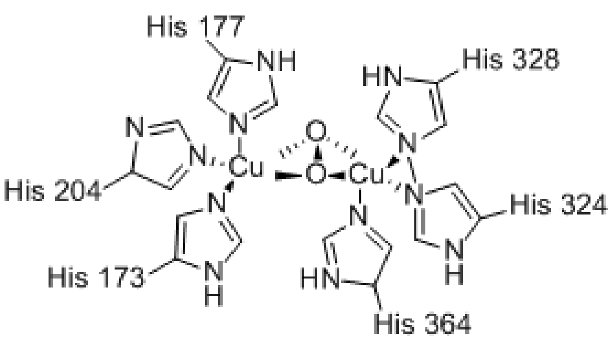
- Hemocyanin are copper containing complexes which is present in many marine species
- It occurs freely in the blood stream. It exist as polymeric form & composed of 6,12,24 & 48

  units no monomeric form exists

Hemocyanin structure .



Deoxyhemocyanin



Oxyhemocyanin

- The oxygen binding centre is composed of a pair of copper atoms. Each Cu atom is bounded by 3 histidine ligands.An empty cavity exist between the Cu atoms.
- The Cu is in +1 oxidation state in the deoxy form & it is diamagnetic in nature & so it is colourless.
- The Cu is in +2 oxidation state in the oxy form & it is paramagnetic in nature & so it is blue in color.
- The polypeptide chain must have a molecular weight between 50000-75000
- The O_2 is bridged between the two copper centers. It means oxyhemocyanine binds with oxygen because of which the Cu gets oxidized from +1 to +2

If the hemocyanine contains n number of Cu centers then it will contain n/2 of O_2 molecules. It is also called as oxo species

Questions .

1. Comment ‘oxygen binds co-orperatively to the hemoglobin molecule’.
2. With respect to the hemoglobin molecule explain the “bhor effect”.
3. Discuss the role of hemerythrene and hemocyanin in biological systems.
4. Discuss the T-R transition in hemoglobin molecule.
5. Discuss the different interactions among globin protien chain in T-form and R-form of hemoglobin.
6. What do you mean by co-orperative interaction in oxygen affinity of hemoglobin and explain the phenomenon by hill equation and hill plot.