OXYGEN UPTAKE PROTEIN

The four Oxygen- uptake 0proteins for transport and storage of oxygen in biological systems. These are Hemoglobin, Myoglobin, Hemerythrin and Hemocyanin. Hemoglobin and Myoglobin are fe (II)-heme proteins but Hemerythrin is a non-heme fe(II) protein, Hemocyanin contain Copper at its Oxygen binding site.

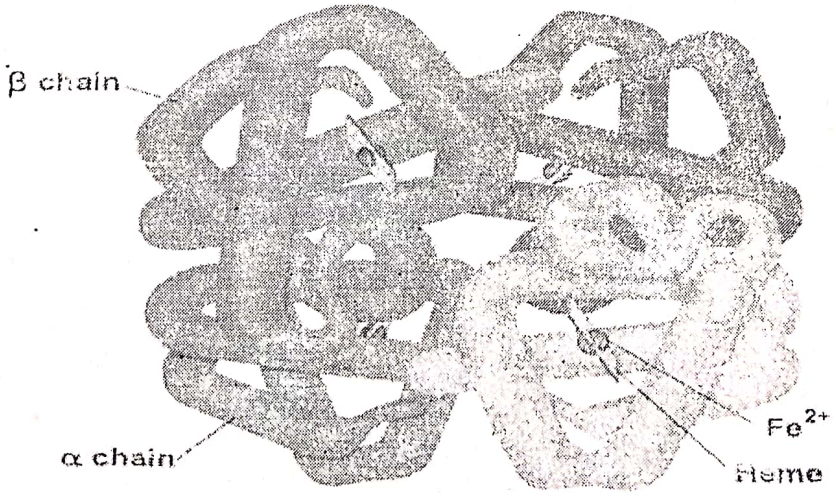
The important functions of the heme-proteins are

(i) Transport and Storage of dioxygen (Hb & Mb)
(ii) Electron transport(Cyt-B_5)
(iii) Catalysis in redox reactions (Catalase, Peroxidase etc.)

Hemoglobin:

Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates with the exception of the fish family Channichthyidae as well as the tissues of some invertebrates. Hemoglobin in the blood carries oxygen from the respiratory organs (lungs or gills) to the rest of the body (i.e., the tissues) where it releases the oxygen to burn nutrients to provide energy to power the functions of the organism, and collects the resultant carbon dioxide to bring it back to the respiratory organs to be dispensed from the organism.

Hemoglobin is a tetrameric protein, Hb_4, (MW=64500daltons,), consisting of two α and two β peptide chains, interlinked through hydrogen bonded $C00^- \ldots N^+H_3$ interaction. In Hb due to these salt-bridge interactions, the peptide chain in deoxy Hb4 is constrained.



Hemoglobin has an oxygen binding capacity of 1.34 ml O_2 per gram of hemoglobin, which increases the total blood oxygen capacity seventy-fold compared to dissolved oxygen in

blood. The mammalian hemoglobin molecule can bind (carry) up to four oxygen molecules. . Our blood stream contains about 150 g/L of the protein known as **hemoglobin** (Hb), which is so effective as an oxygen-carrier that the concentration of O_2 in the blood stream reaches 0.01 *M* — the same concentration as air. Once the Hb-O_2 complex reaches the tissue that consumes oxygen, the O_2 molecules are transferred to another protein — **myoglobin** (Mb) — which transports oxygen through the muscle tissue.

Hemoglobin is involved in the transport of other gases: it carries some of the body's respiratory carbon dioxide (about 10% of the total) as carbaminohemoglobin, in which CO_2 is bound to the globin protein. The molecule also carries the important regulatory molecule nitric oxide bound to a globin protein thiol group, releasing it at the same time as oxygen.

Myoglobin:

Myoglobin and hemoglobin are hemeproteins whose physiological importance is principally related to their ability to bind molecular oxygen. Myoglobin is a monomeric heme protein found mainly in muscle tissue where it serves as an intracellular storage site for oxygen. During periods of oxygen deprivation **oxymyoglobin** releases its bound oxygen which is then used for metabolic purposes. Mb is a monomeric pritein (MW = 17100 daltones) having a single polypeptide chain that is not conductive of self association.

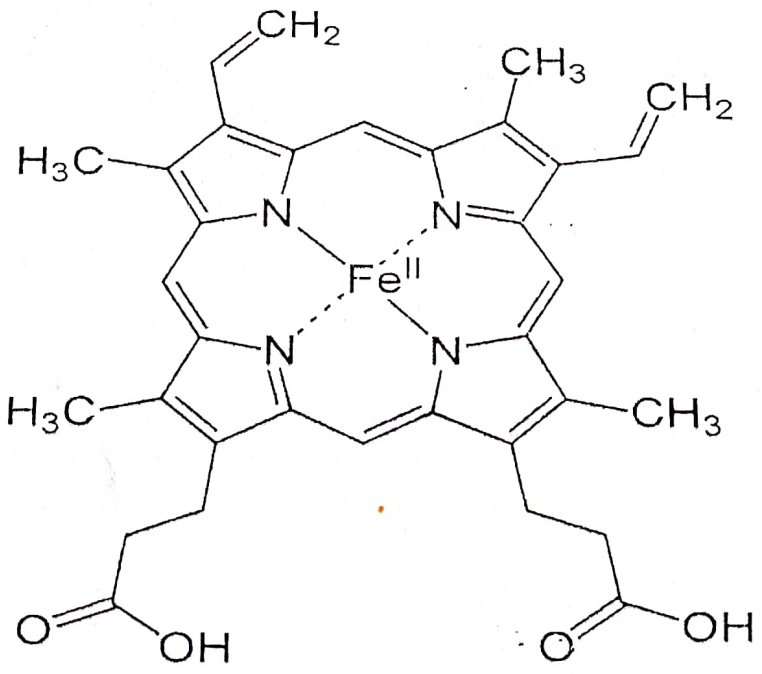


Fig: Protoporphyrin IX (PIX)

STRUCTURE OF Hb AND Mb:

Hemoglobin has a quaternary structure characteristic of many multi-subunit globular proteins. Most of the amino acids in hemoglobin form alpha helices, connected by short non-helical segments. Hydrogen bonds stabilize the helical sections inside this protein, causing attractions within the molecule, folding each polypeptide chain into a specific shape. Hemoglobin's quaternary structure comes from its four subunits in roughly a tetrahedral arrangement.

The active sites of both Hb_4 and Mb contain the heme group in which Fe(II) is equatorially coordinated by the four purrole nitrogen atom of protoporphyrin IX (PIX). [The porphyrin ring consists of four pyrrole molecules cyclically linked together (by methene bridges) with the iron ion bound in the center].The fifth position is coordinated by imidazole nitrogen atom of a Histidine residue of the protein chain I,e, globin. The sixth position in deoxy-Hb_4 or deoxy-Mb is vacant but hydrophobically shielded by the protein chain. As a result, only non-polar neutral molecules such as O2, CO etc can bind to the sixth position by a coordinate covalent bond, completing the octahedral group of six ligands. In absence of the protein (globin) the sixth position is readily coordinated by polar water molecules and Fe(ii)-heme is irreversibly oxidised by oxygen of the air to Fe(iii)-heme, Hematin. The later, because of its residual positive charge, is reluctant to bind uncharged ligand such as O_2 but readily binds charged ligands such as CN^-, S^{-2}, OH^- etc, which inhibit oxygenation.

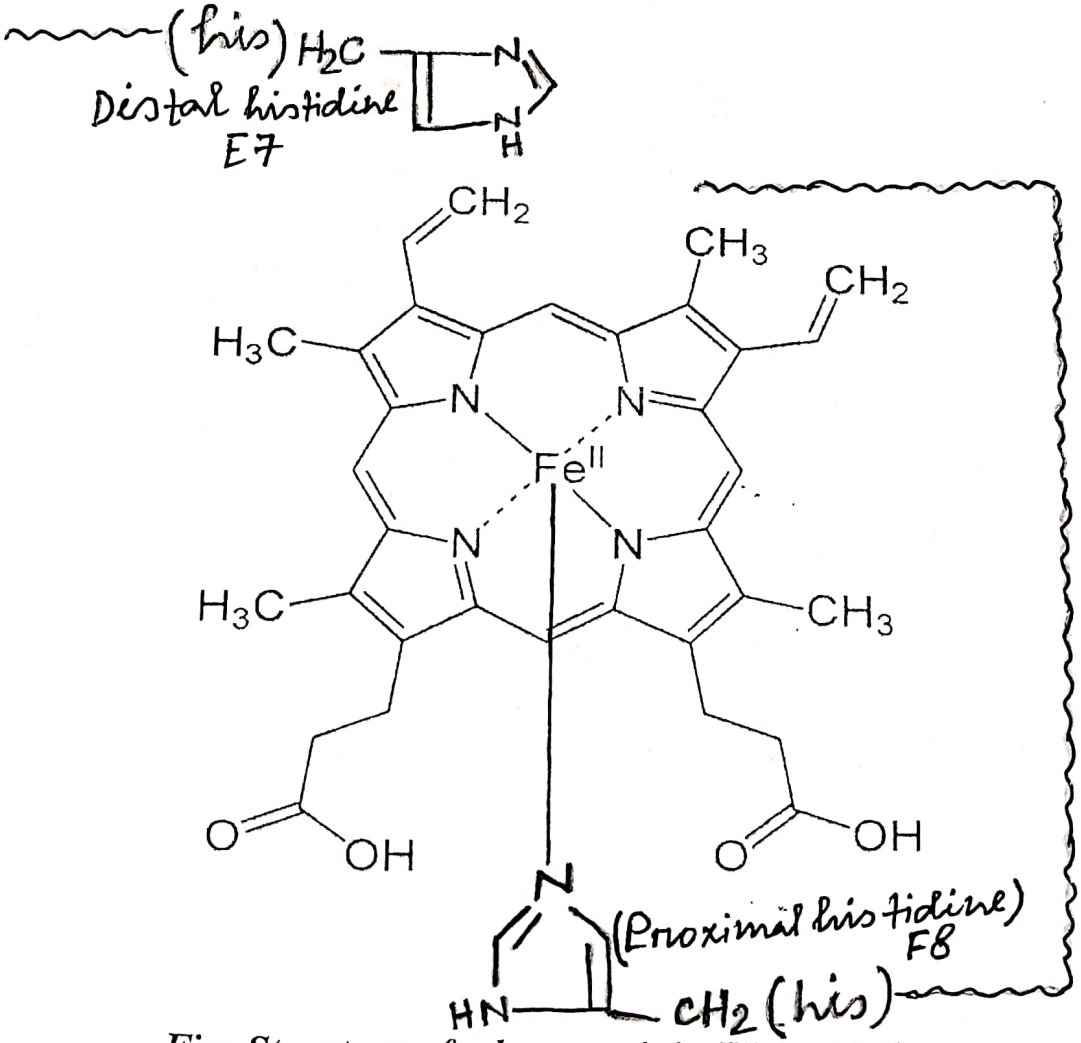
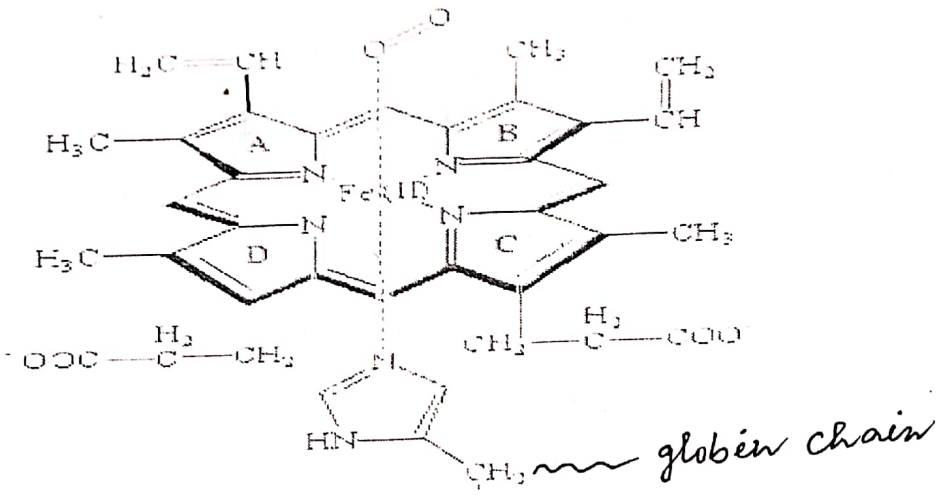


Fig: Structure of a heme unit in Hb and Mb

Hemoglobin (Hb) carris O_2 from lungs to tissues where it is transferred to Myoglobin (Mb) and stored therein for metabolic requirements. To make this process thermodynamically possible, the oxygen affinity of Hb in lungs where oxygen concentration is high should be greater than that of Mb and reverse condition should arise in the tissuses where oxygen concentration is less. Nature has designed Hb and Mb in such a fashion that this condition is attained automatically. These are evident from the characteristics of O_2-binding interaction with Hb and Mb.



(Proximal Histidine F8)

Fig: Oxy- Hb and Oxy-Mb

Functions of globin Protein: The Crucial role of globin Protein as -(i) Protection of Hb and Mb from irreversible oxidation by oxygen.

(ii) Maintain the biological pH and CO_2 transport.

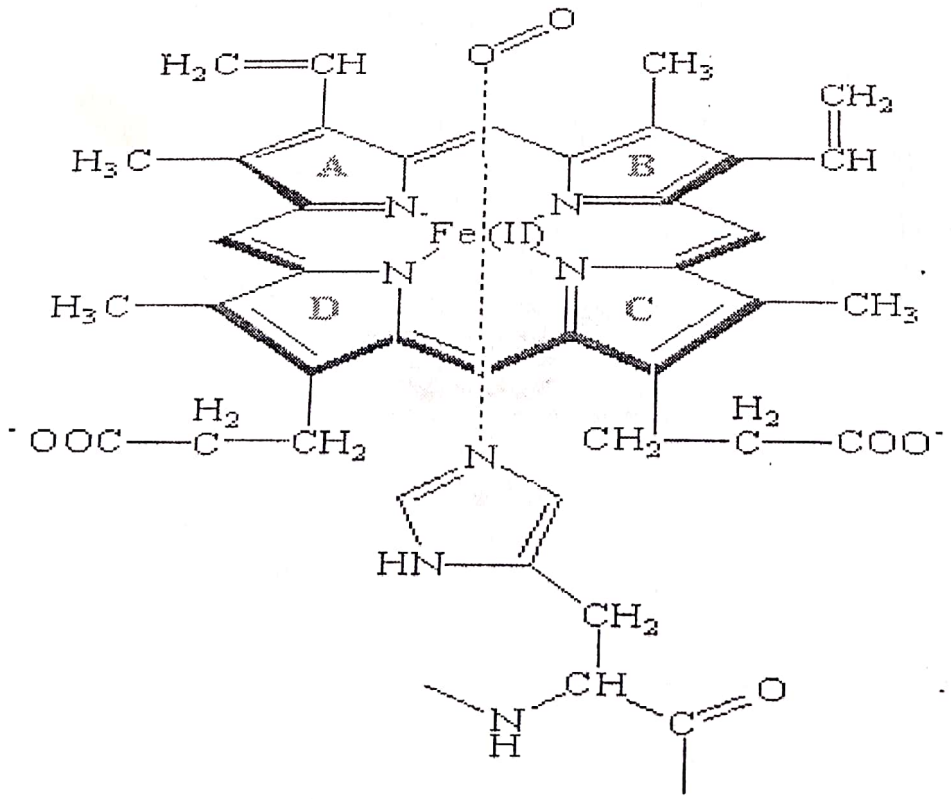
(iii) Weakening the interaction of CO with heme and stabilizing the binding of O_2 by ~~Proximal~~ the distal histidine residue (E7).

(iv) Allosteric effects of O_2, CO_2, H and Cl^- on O_2 affinity of Hb.

Function of Himoglobin:

The primary function of hemoglobin (Hb) is to transport oxygen. Since oxygen is not very soluble in water (the major constituent of blood), an oxygen transport protein must be used t allow oxygen to be 'soluble'. Hemoglobin (Hb) is the oxygen transport protein used in the blood of vertebrates. It is composed of 4 polypeptide chains (represented in this diagram, Each chain contains one heme group (colored orange), each of which contains one iron ion (not shown). The iron is the site of oxygen binding; each iron can bind one O_2 molecule thu each hemoglobin molecule is capable of binding a total to four (4) O_2 molecules.

Hemoglobin exists in two forms, a *taut form* (T) and a *relaxed form* (R). Various factors su as low pH, high CO_2 and high 2,3 BPG at the level of the tissues favor the taut form, which has low oxygen affinity and releases oxygen in the tissues. The opposite of these abovementioned factors at the level of the lung capillaries favors the relaxed form which c better bind oxygen.



Hill equation:

As one subunit of tetrameric hemoglobin (Hb_4) is oxygenated, cooparative interactions predispose another subunits to take up oxygen.

$$Hb_4 + O_2 \underset{}{\overset{K_1}{\rightleftharpoons}} (HbO_2)Hb_3 \qquad K_1 \approx 5 \text{ to } 60$$

$$(HbO_2)Hb_3 + O_2 \overset{K_2}{\rightleftharpoons} (HbO_2)_2 Hb_2$$

$$(HbO_2)_2 Hb_2 + O_2 \overset{K_3}{\rightleftharpoons} (HbO_2)_3 Hb$$

$$(HbO_2)_3 Hb + O_2 \overset{K_4}{\rightleftharpoons} (HbO_2)_4 \qquad K_4 \sim (3-6) \times 10^3$$

As a result, the successive oxygen binding constants of Hb_4 gradually increases ($K_1 < K_2 < K_3 < K_4$) instead of a statistically expected decreases ($K_1 > K_2 > K_3 > K_4$). The constant, K_4 corresponds to oxygenation of the relaxed Hb_4 tetramer and it is quite close to the oxygen binding constant of Mb, in which cooparative interaction is absent.

To effect the transfer of O_2 from oxy-Hb_4 to Mb in the cell, O_2 affinity of Mb must be higher than that of Hb_4. Due to monomeric nature and the absence of cooparative interaction, Mb takes up O_2 in 1:1 molar ratio

$$Mb + O_2 \rightleftharpoons Mb(O_2)$$

$$K_{Mb} = [Mb(O_2)] / [Mb] \cdot pO_2$$

On the other hand, due to tetrameric nature and cooparative interaction, oxygenation of Hb_4 may be expressed according to

$$Hb_4 + nO_2 \rightleftharpoons Hb_4(O_2)_n$$

$$K_{Hb_4} = \frac{[Hb_4(O_2)_n]}{[Hb_4] \cdot [pO_2]^n}$$

fraction (f) of Mb and Hb_4 oxygenated could be expressed by

$$(f)_{MbO_2} = \frac{[Mb(O_2)]}{[Mb] + [Mb(O_2)]} = \frac{K_{Mb} \cdot pO_2}{1 + K_{Mb} \cdot pO_2}$$

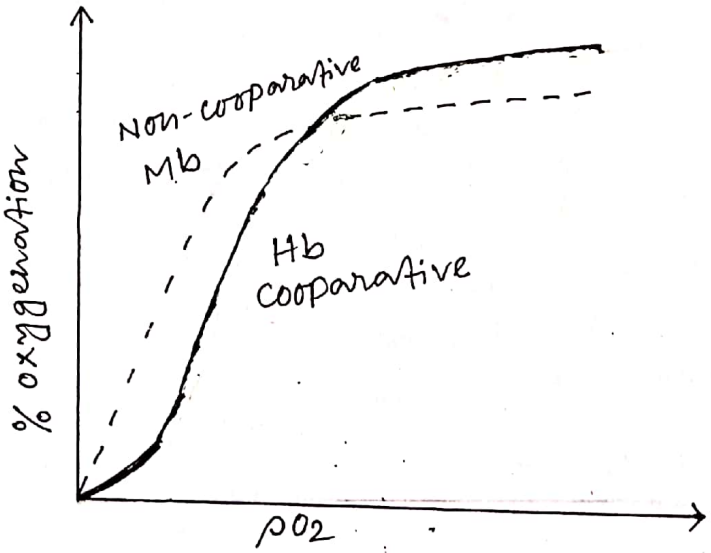
$$(f)_{Hb_4O_2} = \frac{[Hb_4(O_2)_n]}{[Hb_4] + [Hb_4(O_2)_n]} = \frac{K_{Hb_4} \cdot (pO_2)^n}{1 + K_{Hb_4}(pO_2)^n}$$

which is Hill equation for oxygenated of Mb and Hb_4 respectively.

$$\log\left(\frac{f}{1-f}\right)_{Mb} = \log K_{Mb} + \log pO_2$$

$$\log\left(\frac{f}{1-f}\right)_{Hb_4} = \log K_{Hb_4} + n \log pO_2$$

The plot of $\log\left(\frac{f}{1-f}\right)$ against pO_2 is popularly called Hill plot. This plot of $\log(f/1-f)$ against pO_2 gives Hill constant (n), which is approximately 3 for Hb_4 oxygenation indicating that the cooparative interaction between the subunits are so strong as to produce the effect of almost three molecules of O_2 binding simultaneously. This means that the presence of one or more bound O_2, instead of favouring dissociation of oxy-Hb_4, favours further oxygenation. For this reason, Hb_4 is more oxygenated than Mb ($n = 1$) at higher oxygen pressure as are available in the lungs, skins and gills.

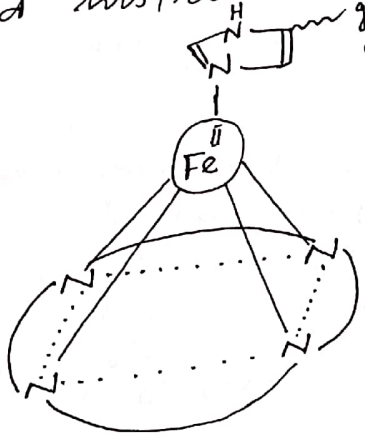


oxygen saturation curve of Hb & Mb

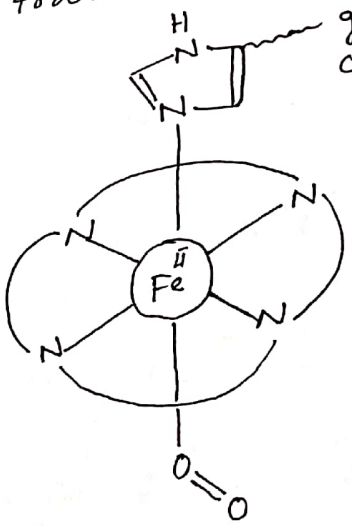
On the otherhand, Hb_4 is less oxygenated at lower oxygen pressure Mb is largly converted to $Mb(O_2)$ even at low O_2 pressure which makes possible the transfer of O_2 from oxy-Hb_4 to Mb in the tissues.

$$Hb_4(O_2)_n + Mb \rightleftharpoons MbO_2 + Hb_4(O_2)_{n-1}$$

Cooparative interaction: In deoxy-Hb4 and deoxy-Mb the 5-coordinate Fe(II) is present in high spin state. Fe(II)-N bond lengths in high spin model Fe(II)-N compounds are ~ 2.18 Å, which is much greater than the mean radius ~ 2.05 Å of the porphyrin cavity. Pentacoordinated iron(II) in deoxy-Hb4 & deoxy-Mb has a square pyramidal geometry and it is situated about 0.8 Å out of the porphyrin plane, being shifted towards the apically coordinated histidine.



Deoxy-Hb or Mb



OXY-Hb or Mb

oxygen binds to the Fe(II)-heme at the vacent sixth position and the resulting octahedral field is sufficiently strong to transform high spin Fe(II) [radius ~ 0.92 Å] to low spin Fe(II) [radius ~ 0.75 Å]. As a result of that, Fe(II) radius is contracted by about 0.17 Å and Fe(II) in the active sites of oxy-Hb4 and oxy-Mb moves towards the porphyrin plane and ultimately sits in the porphyrin cavity. This movement of Fe(II) causes the coordinated histidine to move towards the porphyrin plane. This brings about a conformational change throughout the peptide chain amounting to rupture of some or all the $COO^- \cdots {}^+NH_3$ salt-bridge interactions. The constrained Hb tetramer then relaxes by exposing the sixth positions of the remaining heme groups to oxygenation. This phenomenon is known as cooparative interaction. oxygenation of Hb4 is autocatalytic due to this cooparative interaction but such effects are absent in Mb due to its monomeric nature.

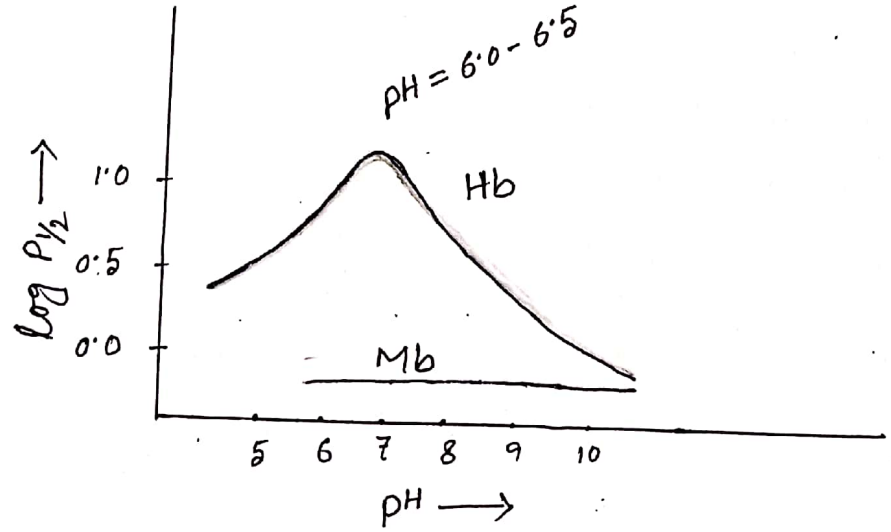
Bohr effect: As the oxygenation of Hb_4 is subject to cooparative effect involving the break-down of $COO^- \cdots\cdots {}^+NH_3$ salt-bridge bonds between and within the subunits, oxygenation of Hb_4 is pH dependent. This phenomenon is known as Bohr effect. Due to the absence of cooparative interaction, Mb oxygenation does not show Bohr effect. oxygenation is favoured in basic condition due to the elimination of $COO^- \cdots {}^+NH_3$ salt-bridge bonds. on the other hand deoxygenation is favoured in acidic condition.

$$\text{Deoxy-Hb} + O_2 \underset{\text{acid}}{\overset{\text{base}}{\rightleftharpoons}} \text{oxy-Hb}$$

The maxima between pH 6.0 - 6.5 corresponds to the pH range of lowest O_2 affinity of Hb_4. Under such weakly acidic condition, the transfer of O_2 from oxy-Hb_4 to Mb is greatly favoured. Tissues consume O_2 to produce lactic acid, CO_2 and carbonic acid, which help release of O_2 from oxy-Hb_4. Thus Bohr effect explains the release of O_2 increases the total concentration of dissolved CO_2 & facilitates its transport from tissues to lungs. As the concentration of CO_2 increases, pH is lowered due to the formation of bicarbonate ion according to the reaction,

$$CO_2 + 2H_2O \rightleftharpoons HCO_3^- + H_3O^+$$

This increased acidity favours the release of O_2 from oxyhemoglobin, resulting in the Bohr effect.



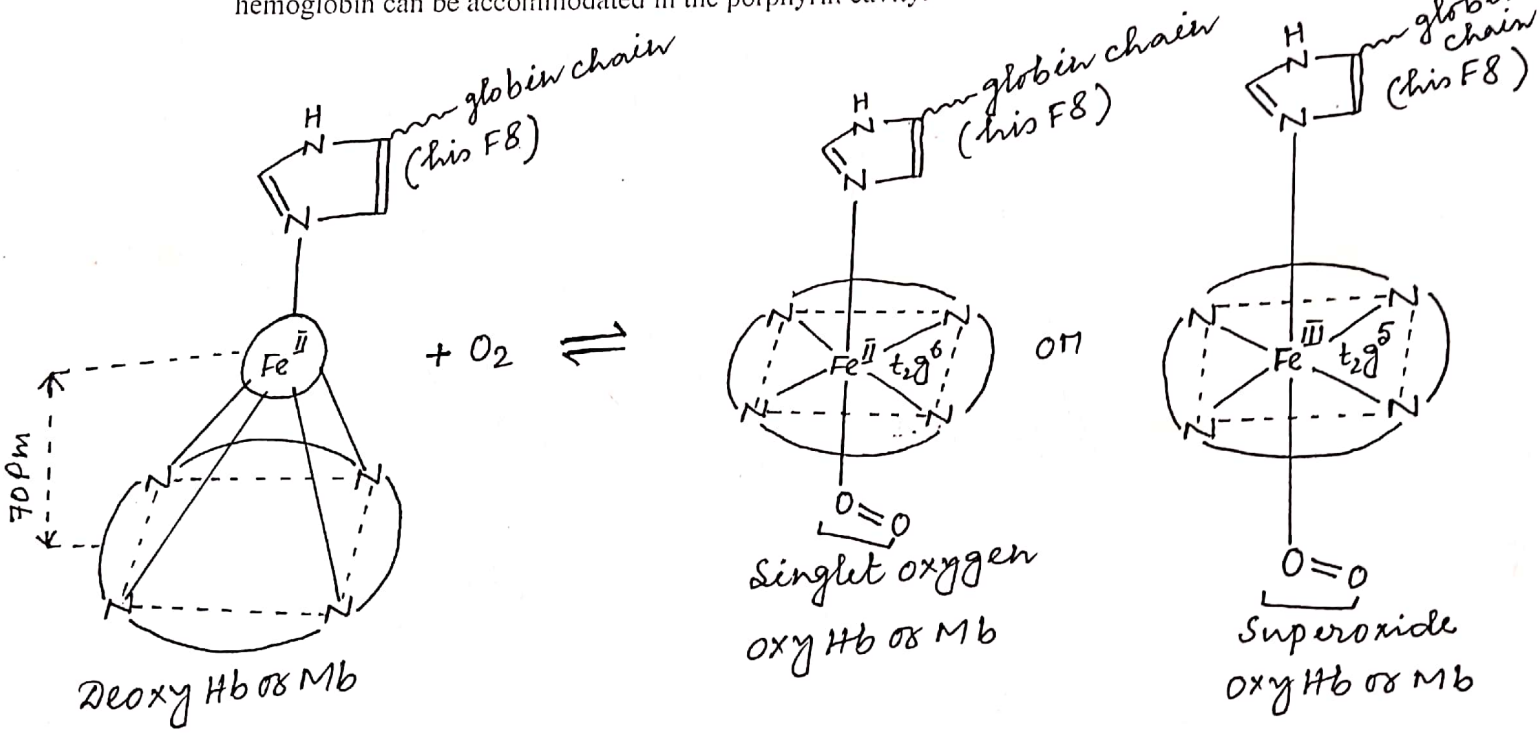
PH dependence of oxygenation of Hb (Bohr effect)

$P_{1/2}$ = oxygen pressure required to half saturate Hb/Mb.

Mechanism:

In Hb, the four polypeptide chains are coiled to experience hydrogen bonding interaction, hydrophobic interaction and salt-bridge interaction to attain the quaternary structure (i,e, tense or ***T-form***). On oxygenation, the above interactions are weakened and it attain the ***R-form*** (relaxed form).

On oxygenation, a tigger mechanism proposed by Perutz operates through the heme-heme interaction to carry out the change, ***T-form*** to ***R-form***. To understand the key steps leading to this change, it has been suggested by some workers that in deoxy-Hb, Fe (II) remain in high-spin state ($t_2g^4\ e_g^2$ assuming octahedral geometry),but on oxygenation fe(II) attain the low spin state ($t_{2g}6\ e_go$). This spin state change acts as the trigger. The Fe(II) ---N bond length (H.S) is **218** pm. The eg electrons directly interact with the ligands and thus the removal of eg electrons in attaining the low-spin state reduces the bond length. The size of the porphyrin cavity allows the sitting of the metal having the corresponding M---N bond length about **200-205** pm. Thus the high-spin Fe (II) in deoxy-Hb (Fe---N bond length **-218** pm) cannot sit in the porphyrin cavity. In fact, Fe (II) in deoxy Hb, lies above the porphyrin plane by **70** pm in the direction of proximal histidine (F8). But on oxygenation, due to the change of spin state (high spin to low spin) the radius of Fe (II) decreases by about **17** pm. Thus Fe (II) in oxy-hemoglobin can be accommodated in the porphyrin cavity.



The evidence to support this fact in the oxygenated form of Hb, iron exist as Fe(III) and O2 as O2- (superoxide). The observed O-O stretching frequency -1106 cm- is closed to that of O_2^- (1097cm-). The Fe-O-O bond angle is closed to 120o. in fact fe(ii) is reversibly oxidised to Fe(III) in this O_2- uptake process and the site of delivery of O_2, it again attaind the fe(II) state through deoxygenetion. The radius of Fe (III) in oxy-Hb is less than that of fe(II) in deoxy-Hb.Thus the model also accounts for the shrinkage in size of iron due to oxygenation.