Mb - globin + heme gp

Hb - property of Hb is the co-operative (auto catalytic) nature of its O_2 binding. — The effect of this cooperativity can be seen in the oxygen-binding curves.

At high conc. — Hb & Mb bind O_2 approximately equally strong.

At low conc. such as in muscle tissue during or immediately after muscular activity Hb is a poorer oxygen binder & therefore can pass its ~~O_2~~ oxygen on to Mb.

The difference in O_2-binding ~~activity~~ ability b/w Mb & Hb is accentuated at low pH values: hence the transfer from Hb to Mb has a ~~the~~ greater driving force where it is most needed viz. in tissue where O_2 has been converted to CO_2

- The oxygen-binding curves of Hb indicates that the 4 subunits of Hb donot act independently. They somehow communicate with each other. When one heme gp reacts with an O_2 molecule, the other three show an increased ability to react with O_2 molecules. Thus Hb behaves somewhat as if it could combine either with four oxygen molecules or none at all.

$$Hb + 4O_2 \rightleftharpoons Hb(O_2)_4$$

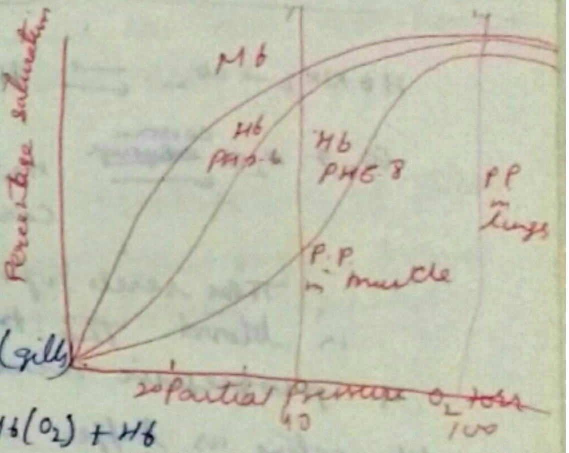
It is generally believed that coordination of an oxygen molecule to the 5-coordinate iron(II) in a deoxy heme causes conformational changes within the protein, which affect the ability of the other heme gps to combine with oxygen. This is the basis of the allosteric theory, which explains heme-heme interaction without postulating any direct communication b/w the heme gp.

- So Hb two alternative structure T for Tense & R for relaxed, which favoured the deoxygenated & oxygenated states respectively. These structures are very similar but differ significantly in the bonding both b/w subunits

& within subunits. These structural changes are closely integrated & occur in a concerted action.

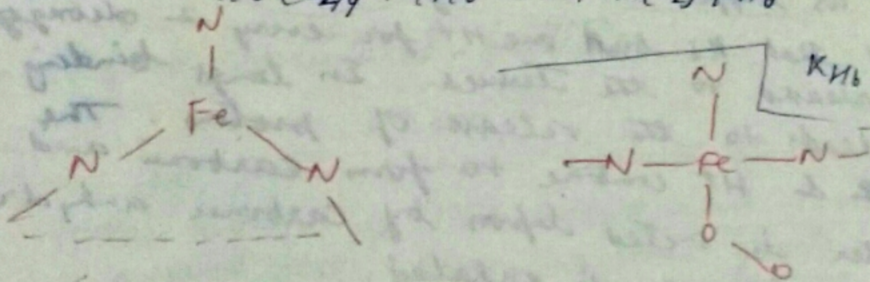
In both stns the four subunits are held together in essentially a tetrahedral arrangement by H-bonds & salt bridges { an electrostatic bond b/w a quaternary N atom with a positive formal charge and an oxygen atom with a negative formal charge.}

Oxygen binding curve for Mb is hyperbolic to show a single equilibrium constant for the binding of O_2. However the equilibrium constant for the formation of oxyhemoglobin is more complicated & upto four molecules



$Hb + 4O_2 \longrightarrow Hb(O_2)_4$

Tissue :- $Hb(O_2)_4 + 4Mb \longrightarrow 4Mb(O_2) + Hb$



$K_{Hb} = \dfrac{[Hb(O_2)_4]}{[Hb][O_2]^{2.8}}$

The 2.8 exponent for dioxygen shows that the binding of 4 dioxygen molecules is not independent

deoxy-Hb (T form salt bridges)

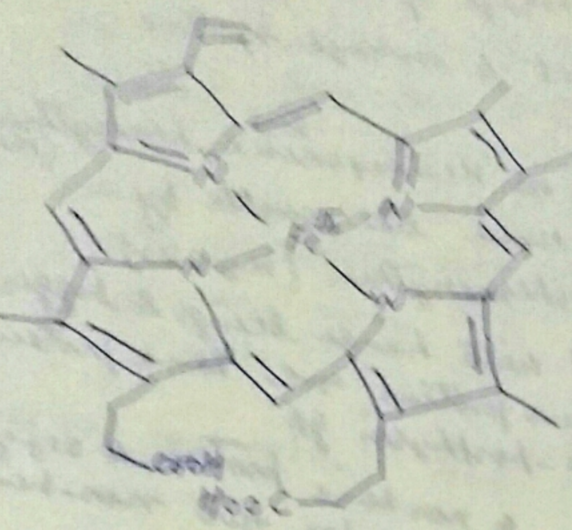
Oxy Hb

R

The rxn of Hb with protons when it liberates O_2 explain how Hb aids the transport of CO_2 to the lungs. About two protons are taken up for every four molecules of O_2 released & two protons are released again when four molecules of O_2 are absorbed. The CO_2 released in tissue is too insoluble to be transported entirely as such, but it can be solubilized by conversion of bicarbonate. Thus the rxn occurring during the release of O_2 by Hb can be roughly represented as

$Hb(O_2)_4 + 2CO_2 + 2H_2O \longrightarrow H_2Hb + 2HCO_3^- + 4O_2$

CO_2 solubilized by conversion to bicarbonate



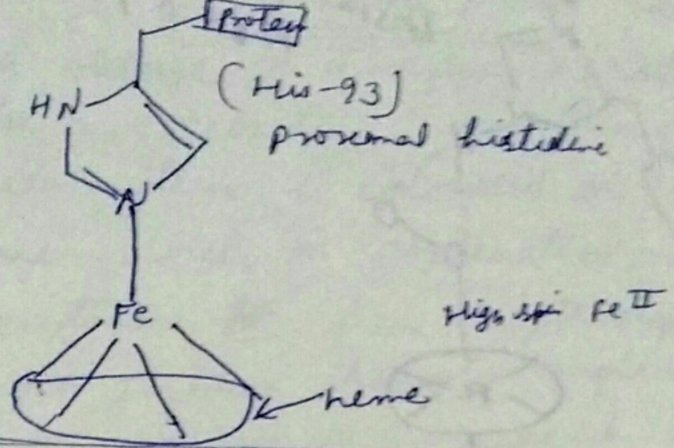
Fe^{II} protoporphyrin IX (heme b)

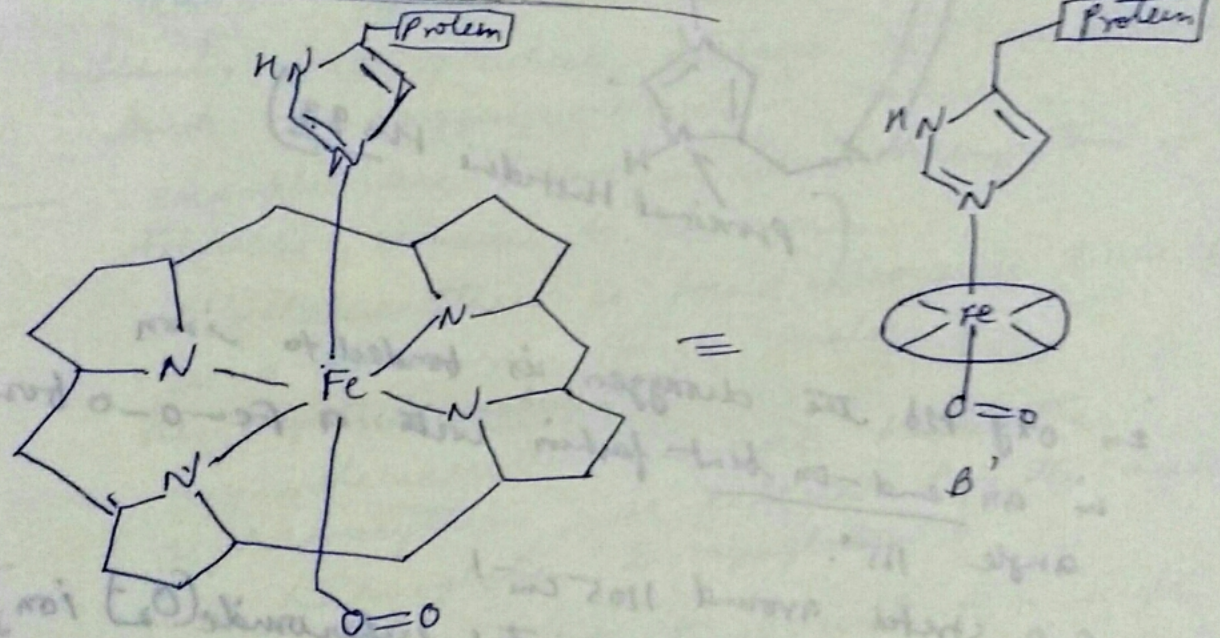
Myoglobin

In all ferroporphyrin-protein complexes the iron atom has an octahedral structure of chemical type d^2sp^3 (double pyramid with square base formed by 4 pyrrolic nitrogen atoms). The fifth coordination position of ~~the~~ iron is occupied by the imidazole nitrogen ~~from~~ from His-93 of the proximal histidine this is F8 or 8th residue of the helix F in the Perutz nomenclature.

The heme gp is attached to the protein in both Mb & Hb through a coordinate histidine nitrogen atom (F8)

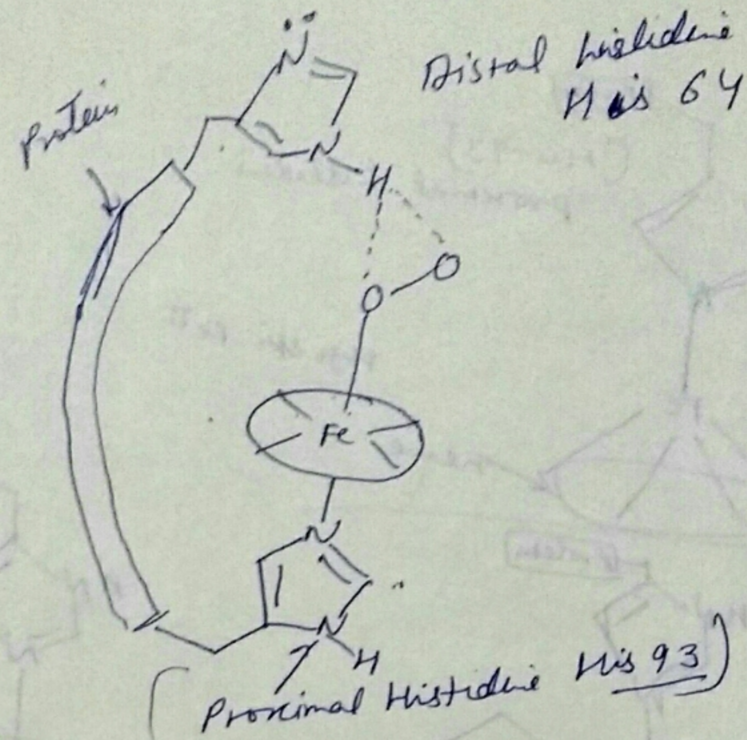
— deoxymyoglobin is has a pentacoordinate iron(II) center in the high spin state [iron II is d6] with high spin it has a radius of around 92 pm in a pseudo-octahedral environment.
square pyramidal





low spin Fe^{II}

on binding of dioxygen the iron atom moves towar the plane of heme gp, dragging the proxim histidine ligand along with it.



In oxy Mb the dioxygen is bonded to iron in an end-on bent fashion with a Fe–O–O bond angle 115°.

O–O stretch around 1105 cm^{-1}

(It is characteristic of coordinated superoxide (O_2^-) ion) suggests that MbO_2 & HbO_2 adducts are probably best represented by iron (III) complexes of the ~~ … ~~ ligand.

┌ respiratory metalloproteins

Hemeerythrin — Non heme protein used in the transport and storage of dioxygen by several phyla of marine invertebrates.

— Deoxyhaemerythrin is colourless or pale pink & becomes violet on oxygenation.

— Haemerythrin from erythrocytes of the worm Golfingia gouldii has a molecular weight of about 108,000 & is made up of eight identical subunits, each of which contains two iron atoms & binds one oxygen molecule.

— examples are known in which haemerythrin is trimeric, dimeric & tetrameric.

— Myohaemerythrin is found in muscle tissue & is a monomeric species analogus to the hemerythrin subunit.

— Oxymyohaemerythrin is more stable than oxyhaemerythrin, as expected from the analogy with haemoglobin & myoglobin.

— The essential structural unit is a single chain protein of ~113 amino acid residue which contains two atoms of iron.

— Deoxyhemerythrin has a magnetic susceptibility & a mossbauer spectrum indicating that both iron atoms in each pair are high spin iron(II) ions.

— It has also been established by chemical & spectroscopic means that when hemerythrin binds O_2 it form an $Fe_2^{III}(O_2^{2-})$ type of complex in which there is antiferromagnetic coupling between nonequivalent ferric ions.

A hydroxyl group serves as bridging ligand but also function as a proton donar to the O_2 substrate. This proton transfer result in the formation of a single oxygen atom (μ-oxo) bridge in oxy- & methemerythrin.

- α, β subunits 13-14 KDa each
  Each subunit has a four-α-helix fold bind a binuclear iron centre.

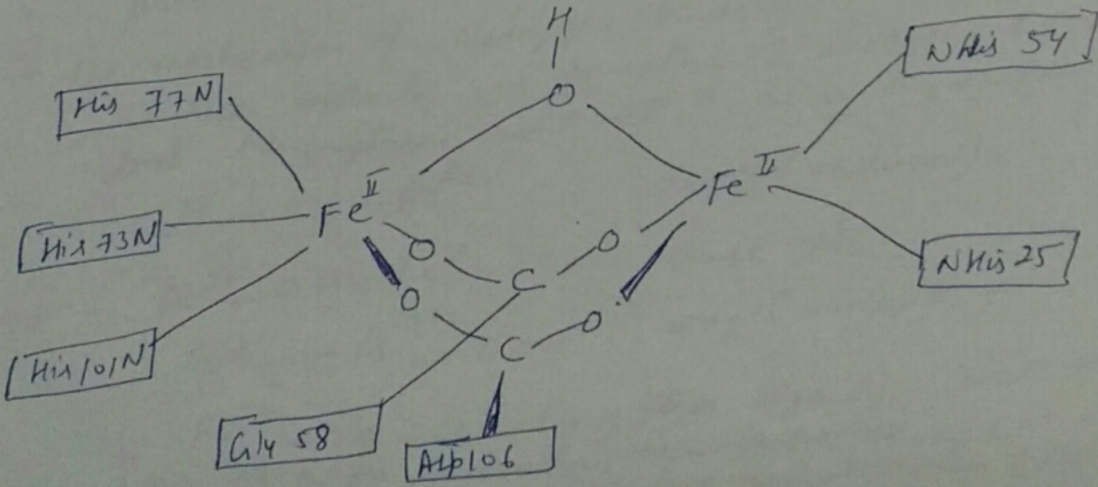
- Most often the in vivo operating systems consist of eight such units associated to form a tertiary structure with four fold symmetry, but there are also a no. of species containing trimers of the basic unit

The peroxo state of the bound O_2 is very clearly shown by the resonance Raman spectrum where $\nu(O-O)$ is found at $844\ cm^{-1}$, a value ~~of~~ typical for the O–O single bond of peroxides. when the oxygenated hemerythrin is treated with anionic ligands (L^-) such as NCS^-, N_3^-, CN^- or F^- one of the so called methemerythrin is formed

$$Fe_2^{III}(O_2)^{2-} + L^- + H_2O \longrightarrow Fe_2^{III}(L^-) + OH^- + O_2H^-$$

⇒ structurally the two Fe(II) atoms in deoxyhemerythrin in each subunit are placed close to each other and have nearly octahedral coordination. These are bridged by an oxygen atom (from water hydroxo or oxo):

- one of the Fe(II) atom is bound to three histidine residues & the other to two.
- Moreover there are two bridges b/w these ~~iron~~ iron (II) atoms made of carboxylate gps of glutamate (Glu 58) & aspartate (Asp 106) residues of the protein.



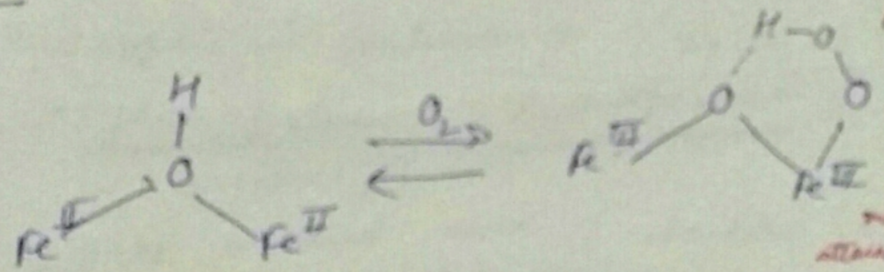
Mechanism of oxygen binding:-

It has been proved That dioxygen binds asymmetrically in oxyhemerythrin. The end on coordination of dioxygen to only one of the iron center in oxyhemerythrin has been revealed.

— The two Fe(II) centers combine with the one O_2 molecule & get oxidised to Fe(III) while O_2 is reduced to Peroxide form.

— O_2 binding in hemerythrin is a redox r^n.

—



O_2 binds to pentacoordinate Fe(II) center & (2 e) gets transferred from two Fe(II) centers. This gives a binuclear Fe(III) moiety with peroxide attached to one Fe(III) center

Dioxygen attaches to only one Fe center. Hydrogen atom of OH bridge of Hemerythrin has not been located but is shown here To stablise the peroxide ion via H-bond.

- The mechanism of dioxygen binding is unusual. Most O_2 carriers operate via formation of dioxygen complexes, but hemerythrin holds the O_2 as a hydroperoxide.

$Fe^{2+}—OH—Fe^{2+}$ deoxy (reduced)

$Fe^{2+}—OH—Fe^{3+}$ semi met

$Fe^{3+}—O—Fe^{3+}—OOH$ oxy (oxidised)

$Fe^{3+}—OH—Fe^{3+}$ (any other ligand) met oxidised

The uptake of O_2 by hemerythrin is accompanied by $2e^-$ oxidation of the diferrous centre to produce a hydroperoxide (OOH^-) complex.

Hemocyanin ~ respiratory protein
— A non heme – non cyanide containing dioxygen carrier – copper protein ; →

Hc — are found in many species in the mollusca & Arthopoda. On oxidation the colorless protein becomes blue. (cyanin from cyanos Greek for blue)

— In HC the oxygen binding centres are similar & are composed of a pair of Cu ~~at~~ atoms c̄ accommodates dioxygen molecule

— The copper ~~is~~ is in +1 oxidation state in the deoxy form & +2 in the oxy form

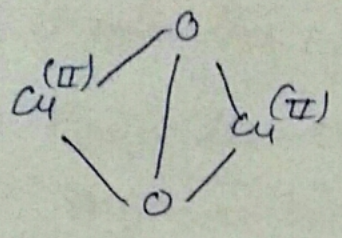
one may recall, that the geometry of dioxygen is superoxo (M–O–O) in oxyhemoglobin & hydroperoxo M–O–M OOH in Oxyhemerythrin.

— Here is third mode of binding b/w oxygen carrying metal atoms & dioxygen.

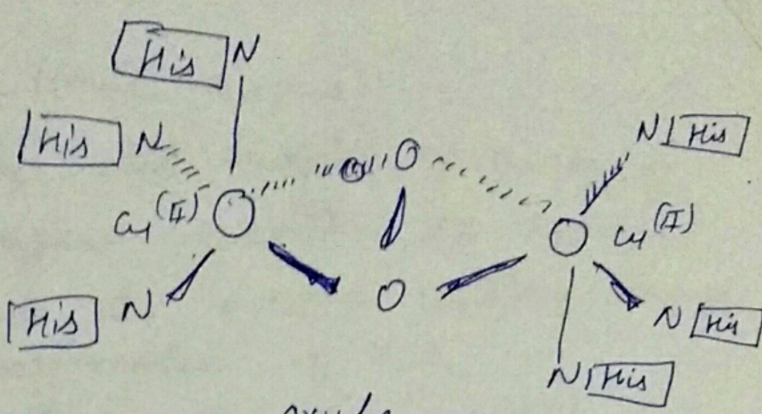
— The dioxygen molecule oxidizes each copper I to copper (II) & it itself gets reduced to the peroxide ion O_2^{2-}. ~~The~~ !

— The two copper atoms are bridged by the peroxide ion (each oxygen atom is bonded to both copper atom). That the binding ligand O_2^{2-} has geometry A & not B in the agreement with spectral features.

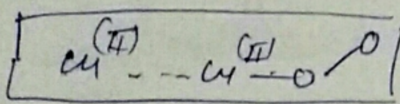
— They are very large with molecular weights ~~of~~ b/w 4.5×10^5 & 9×10^6



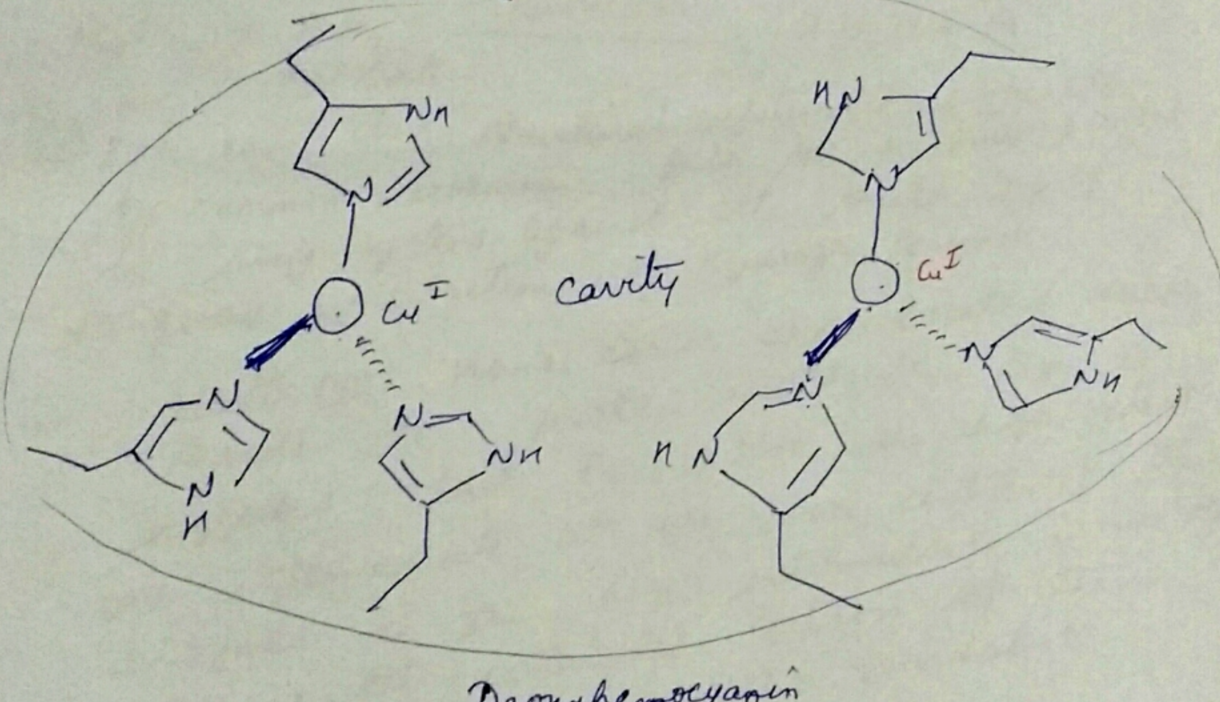
A



Oxyhemocyanin



B



Deoxyhemocyanin