The exponent 'n' is called the **Hill constant**.

$$\frac{f}{1-f} = k\,pO_2^{\,n}$$ is the Hill eqn.

for Hb, $n = 2.8$ in the pH range of physiological importance in muscle tissues.

There are two points

⇒ for $n > 1$, it is indicative of that the attachment of O_2 to one Heme group of Hb ↑es the binding constant for next O_2 which intern ↑es the binding constant for next one and so on.

⇒ In contrast to the hyperbolic curve obtained for Mb, the data for Hb shows "sigmoidal" behaviour which indicates interaction between the subunits. (The data obtained from Hb b/w 10 & 90% oxygenation can be fitted into Hill eqn to give values of $n \approx 3$ for normal Hb.

These two points indicate that there is co-operative effect/interactn b/w the subunits of Hb. That is, the addition of oxygen to a subunit affects the oxygen affinities of other subunits,

this is an example of Allosteric effect which literally means "a stressed state or state of being under tension".

for Mb:- The hyperbolic curve shows that the myoglobin binds oxygen more strongly than the first O_2 of Hb.

BOHR'S EFFECT:-

The cooperative effect is pH dependent. The affinity of Hb for O_2 ↓es with ↓ in pH. This is called Bohr Effect.

The CO_2 released in muscle tissue is the end product of breakdown of glucose. CO_2 being acidic, it ↓es the pH in muscle tissue & lowers the pH. ∴ in muscles pO_2 is low, low pH & high pCO_2.

The CO_2 produced in muscle tissue, is transported in form of soluble HCO_3^- ions

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

(carbonic anhydrase).

The formatn of HCO_3^- is facilitated by the protein chains of deoxyhemoglobin which acts as buffer by picking up the accompanying protons.

The HCO_3^- in the sol? in the serum of venous blood back to the lungs. so release of H^+ from Hb on oxygenatn produces. H_2CO_3 from HCO_3^- ion. Then carbonic anhydrase converts H_2CO_3 into CO_2 which is exhaled out.

$$HCO_3^- + H^+ \rightleftharpoons H_2CO_3$$

$$H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

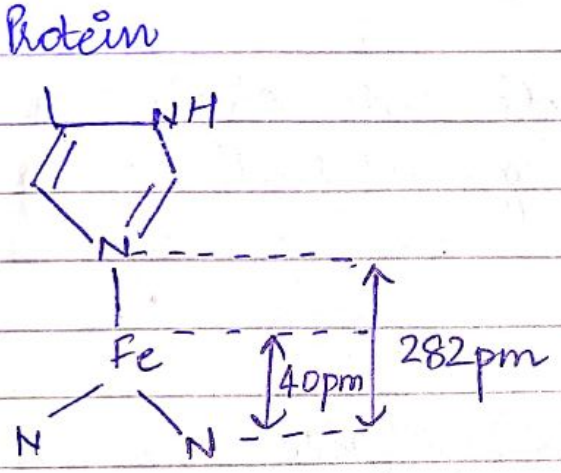
$$HbH_x^+ + 4O_2 \rightleftharpoons Hb(O_2)_4 + xH^+$$ where x is about 1

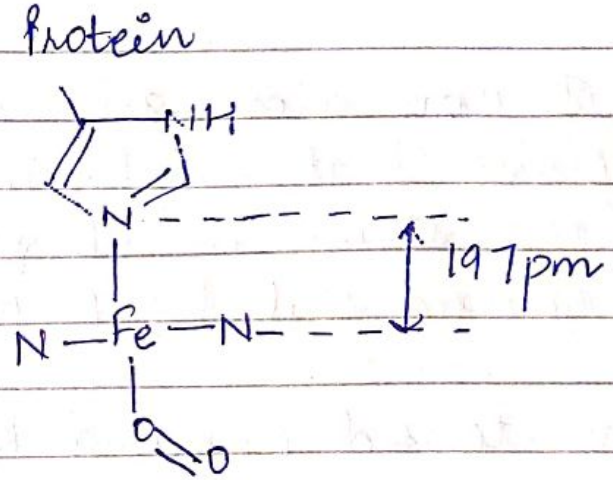
Mechanism of oxygenation in Hb and Mb.

Acc. to Perutz mech. for the cooperativity of the four heme groups in hemoglobin. In deoxyhemoglobin, iron is coordinated to 4 nitrogens of the planar protoporphyrin IX and the fifth coordination site is occupied by the nitrogen atom on Imidazole of a proximal histidine of globin protein. The sixth vacant site trans to the imidazole nitrogen is vacant and reserved for dioxygen.

The deoxyhemoglobin, Iron is Fe(II) H.Spin with one electron occupying the dx^2-y^2 orbitals that points directly toward

the nitrogen atoms of protoporphyrin IX. The presence of this electron increases the size of Fe(II) in these directions by repelling the lone pair of electrons on N atom. As a consequence, Fe(II) becomes too large to fit easily within the hole of the planar ring. ∴ The Fe lies about 40 pm out of the plane in the direction of the histidine grp. The Fe atom in deoxyHb has sq. pyramidal coordination.





The changes in Heme of Hb upon oxygenation.

Although O_2 is not a strong ligand, the cordinatn of O_2 trans to histidine grp as 6th ligand alters the strength of the ligand field and causes the pairing of e^- on Fe without affecting the O.S. of ion. So Fe(II) becomes Low spin and diamagnetic. In low spin Fe(II) it becomes 17 pm smaller than high spin Fe(II).

so the Fe(II) slips into the hole of planar ring and now the complex has an octahedral geometry.

The electrostatic interactions b/w $-NH_3^+$ and $-COO^-$ grps present on all 4 polypeptide chains of Hb. These interactions (salt bridges) introduce strain in the molecule ∴ the deoxy-Hb is called Tensed (or T state)

The movement of iron atom and imidazole side chain towards the porphyrin plane that results in breaking the salt bridges which reduces the strain in Hb molecule ∴ The oxy-Hb form is called the relaxed state (ie R State)

→ These two forms - tensed (T) and Relaxed (R) of Hb are present as an Eqlbm mixture.

→ With no O_2, T is more stable than R ∴ Hb is found in 'T' state

→ The initial O_2 affinity of Hb is lower than observed for the individual subunits. Addition of O_2 to deoxy Hb changes the Eqlbm b/w T & R state. As the Hb picks up oxygen, the Eqlbm shifts towards the R state. Thus more O_2 molecules bound to Hb, the higher the probability that Hb is in R state.

It is the structural change which occurs in the binding of the O_2 to the Heme that results in the decrease in the stability of T conformation relative to R form and causes the observed cooperativi

1 (*) Imp points to consider

In the planar porphyrin ring of heme unit of Hb and Mb, due to the presence of low lying π^* and π orbitals and conjugated double bonds in the porphyrin they allow the characteristic charge transfer electronic transitions to give the red colour to the blood. These transition occur in the range 400 - 600 nm giving rise to Soret (400 - 500 nm) & α, β-Bands (500 - 600 nm)

Soret bands: - The high energy $\pi - \pi^*$ (near to UV) transistion of the iron porphyrin ring is Soret band. (also called γ) I an intense $\pi \rightarrow \pi^*$ abs^ band in blue region in the optical absorptn spectrum of a heme protein.