

Dioxygen (O_2): Uptake, storage & transport

As high concentration of O_2 is there in earth's atmosphere (21 vol%), is the result of continuous and efficient photosynthesis of higher organism is possible.

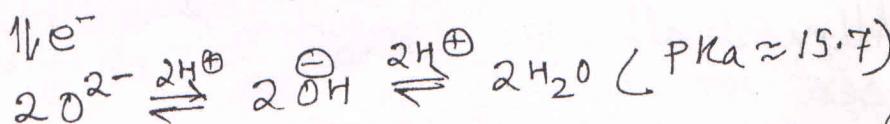
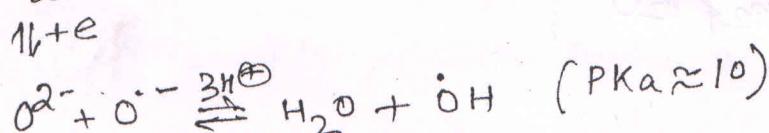
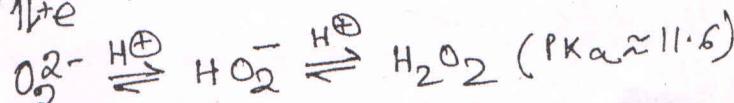
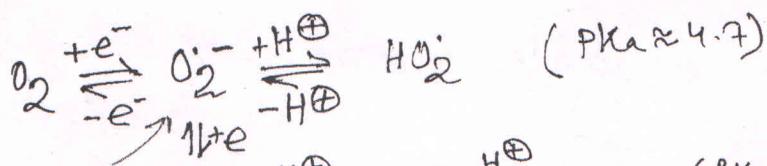
Initially it has been classified as a toxic byproducts. Studies of atmospheres of planets & moon shows O_2 content of 1 vol%.

2.5 billion years ago \rightarrow earth 1 vol%.

2 " " " $[O_2]$ started to increase

400 million " " \rightarrow stable equilibrium between CO_2/O_2 .

O_2 considered toxic oxidant
Therefore, only those aerobic organisms have survived in contact with the atmosphere that are able to develop protective mechⁿs against O_2 and the very toxic intermediates resulting from its partial redⁿ (see Antioxidants)



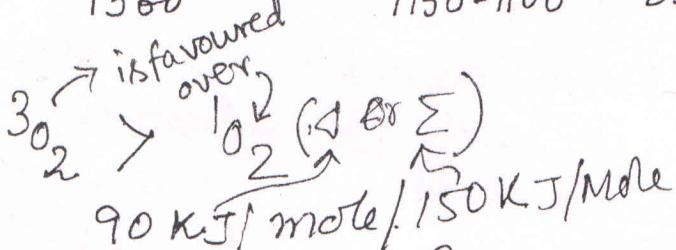
As a consequence of O_2 production the ozone layer in the stratosphere has formed that protects the organisms from high-energy components of solar radiation.

for organisms

Why the presence of free O_2 is necessary requires the discussion on molecular properties and coordination behaviour of O_2 .

2nd higher electronegative atom. Thus O_2 is a strong oxidant.

| | $3O_2$ | $2O_2^-$ | $1O_2^-$ | $1O_2(^1\Delta)$ | $1O_2(^1\Sigma)$ |
|-----------------------|--|---|---|---|---|
| orbital energy | $\uparrow \pi_{2p}^* \uparrow \pi_{2p}^* \uparrow \pi_{2p}^* \uparrow$ | $\uparrow \uparrow \uparrow \uparrow \uparrow \uparrow$ |
| B.O | 2 | 1.5 | 1 | | |
| B.length | 1.21 \AA | 1.28 | 1.49 | | |
| $\nu(\text{cm}^{-1})$ | 1560 | 1150-1100 | 850-740 | | |



The reactions of $3O_2$ with most normal singlet molecules are thus inhibited as it requires flipping of spins.

presence of combustibles (wood, fossil fuel, carbohydrates etc.) and an O_2 rich atmosphere thus possible without instant formation of the lowest energy products $CO_2 + H_2O$.

$1O_2$ is another toxic form of oxygen.

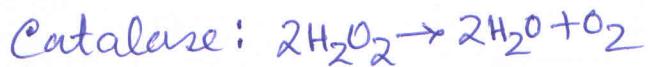
Almost all reacⁿs between O_2 and metal complexes proceed irreversibly.

The redox potential for the redⁿ of O_2 decrease in the presence of electrophiles such as M^\oplus / H^\oplus .

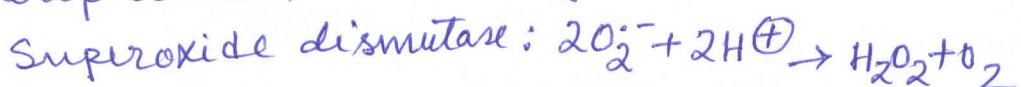
The toxicity of peroxide (O_2^{2-}), superoxide radical ($O_2^{\cdot-}$) and hydroxyl radical ($\dot{O}H$) can be tackled by the antioxidant activity of following enzymes/processes.

Antioxidant

H_2O_2

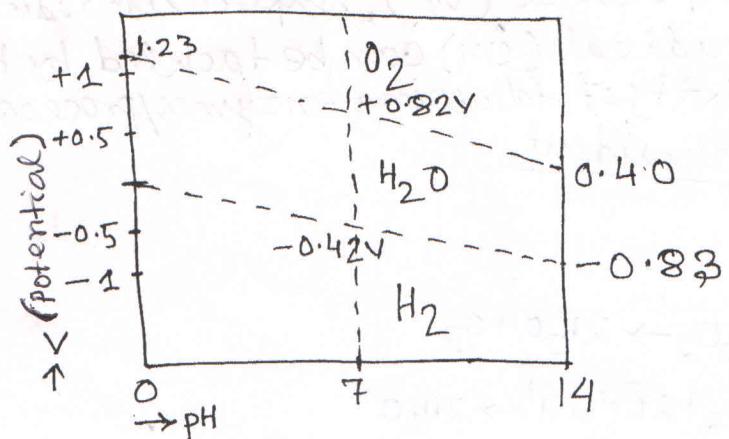


$O_2^{\cdot-}$ radical



$\dot{O}H$ radical

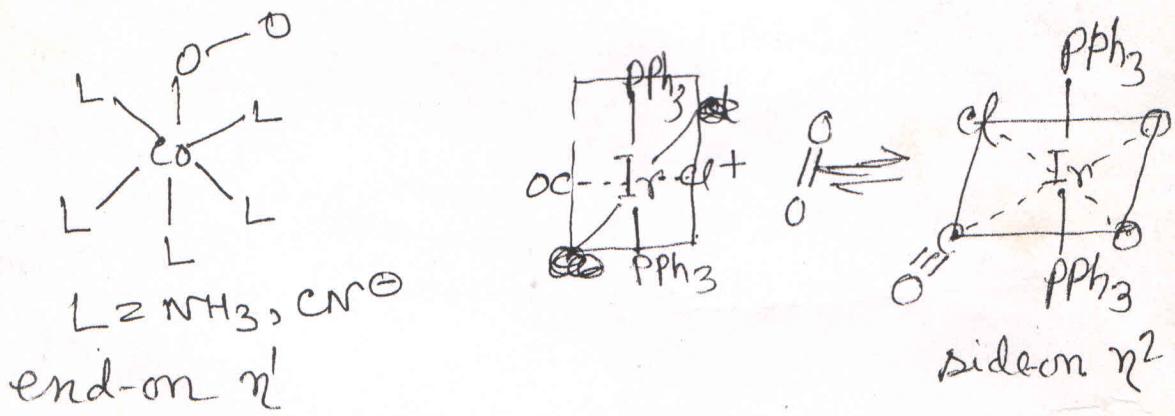




Stability diagram of H_2O .

Inorganic and organometallic complexes of the group 9 metals like Co, Rh, Ir. reversibly bind O_2 .

Vasua's compd shows reversible uptake and release of side-on η^2 coordinated O_2 .

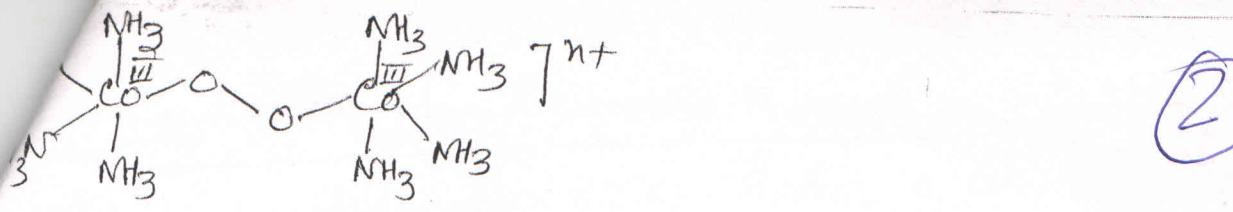


Structural and EPR studies reveals that redox active non-innocent O_2 ligand mainly bound in their singly or doubly reduced form (O_2^-/O_2^{2-}). The ability of $O_2^-/O_2^{2-}/OH^-/O^{2-}$ acting as bridging ligand between metal centers contributes to the irreversibility of O_2 coordination.

O_2 is σ or π donor/ π acceptor ligand.

free e pair or double bond \rightarrow donor.

π^* orbital as acceptor.



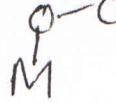
$$n=5, \quad d(O-O) = 1.31 \text{ \AA}$$

$$n=4, \quad d(O-O) = 1.47 \text{ \AA}$$

O_2^- ligand

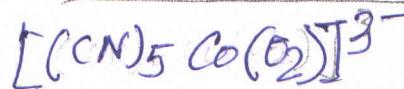
O_2^2- ligand

Possibilities of O_2 coordination in biological O_2 utilization



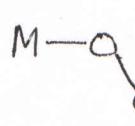
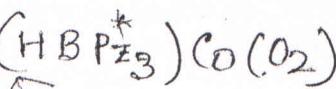
η^1

end-on



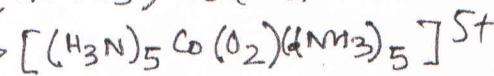
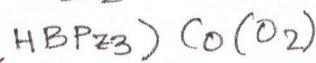
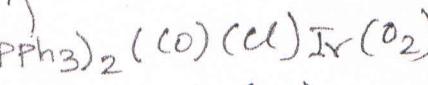
η^2

side-on



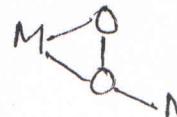
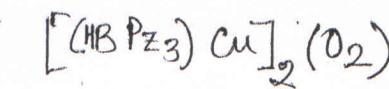
$\mu-\eta^1: \eta^1$

end-on
bridging



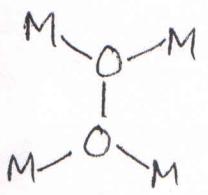
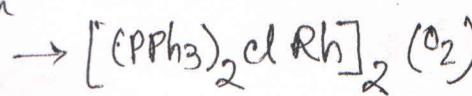
$\mu-\eta^2: \eta^2$

side-on
bridging



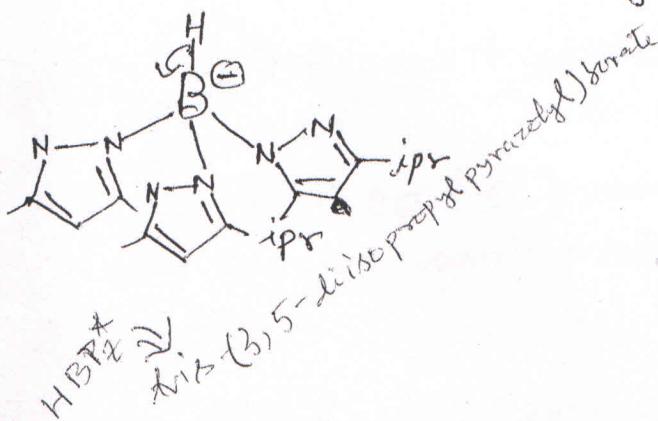
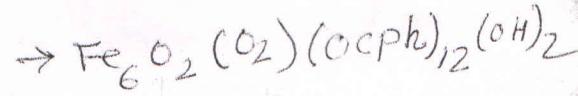
$\mu-\eta^1: \eta^2$

end-on/ side-on
bridging



μ_4^-

end-on
four-fold
bridging



Hb

Mb

trans

ferrti

hemoc

cata

pere

O₂ Transport and storage through Hb & Mb

(3)

controlled utilization of O₂. Before O₂ can be metabolized it has to be taken up from the atmosphere and transported to the O₂ depleted tissue where it must be stored until actual use.

certain groups of molluscs, crustaceans, spiders & worms / higher organisms → differ through their strategies in O₂ coordination.

Invertebrates → Hc & Hr.
↓ ↓
Cu Fe

vertebrates → heme system.

corresponding proteins are tetramer Hb (Hb, O₂ uptake in the lungs and transport in the blood stream) monomer Mb (Mb, O₂ storage and transport within muscle tissue).

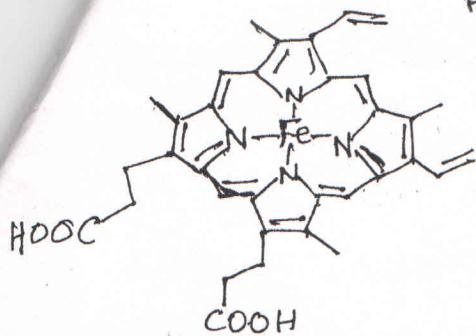
O₂ transport is not catalytic but a stoichiometric function.

65% Fe present in human body is confined to the transport protein Hb alone.

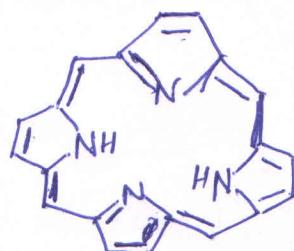
6% Fe in Mb (O₂ storage protein).

Blood has 30% higher solubility of O₂ than H₂O.

| | | |
|---|------|-----------------------------------|
| Hb | Fe% | - function |
| → | 65 | O ₂ transport in blood |
| Mb | 6% | O ₂ storage in muscle |
| transferrin | 0.2% | Fe transport |
| ferritin | 13% | Fe storage in cells. |
| hemosiderin | 12% | Fe storage |
| Catalase peroxidase, cytochrome c oxidase, flavoprotein, RNA | | |

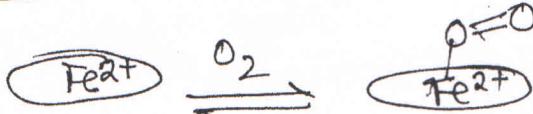


Many catalytically active ^{iron} enzymes contain the heme groups; peroxidases, cytochrome cyt. C oxidases and P-450 system belong to the group of heme proteins.

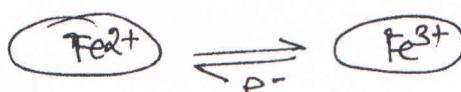


Fe-protoporphyrin IX = heme

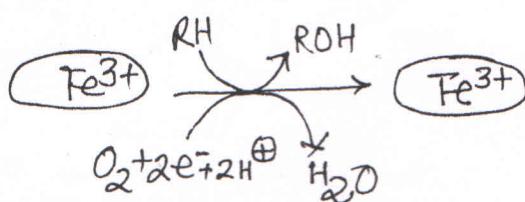
Hb
Mb



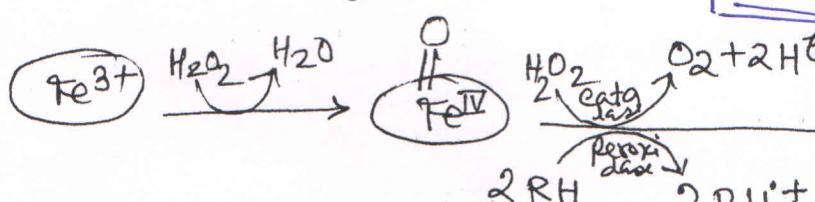
cytochromes



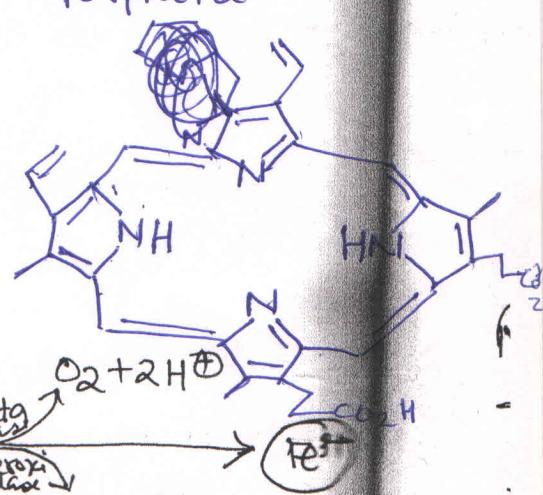
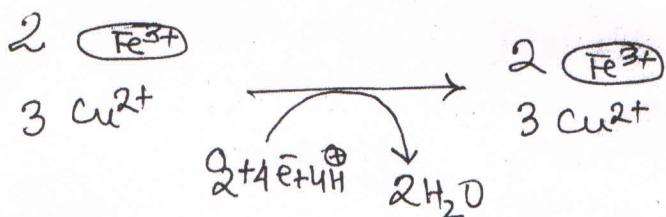
cytochrome
P-450



Catalase and
peroxidase



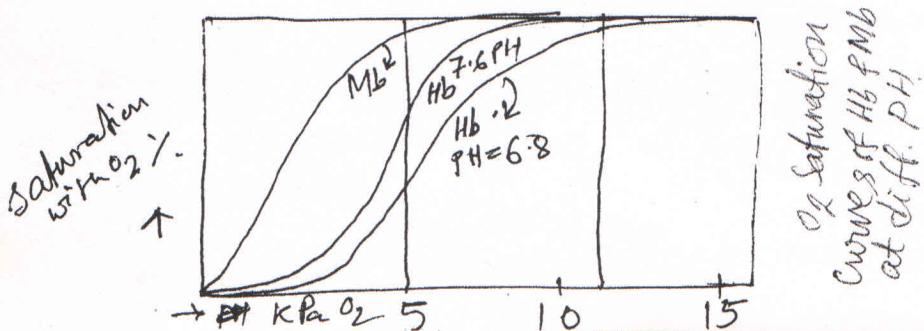
cytochrome
C oxidase



The transport system for O_2 has to take up this molecule as effectively as possible in its ground state form $3\text{O}_2 \rightarrow$ intermediate storage system

In the case of Hb with 4 hemes \rightarrow efficiency guaranteed.

1 ml O_2 per 1 gm Hb.

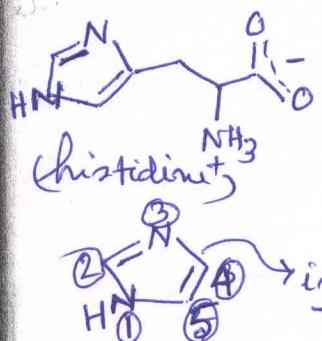


* COO
O2
NO
TW
Y.
is
line
**

According to the fig. the cooperative effect guarantees an efficient transfer of O_2 to the storage site. (5)

Hb \rightarrow Crystal Structure J. C. Kendrew / M. F. Perutz (Nobel 1962).

\hookrightarrow Tetramer



\Rightarrow Free for O_2 coord.
with a distal histidine
that form H bond with O_2 .

imidazole ring (proximal histidine)

Regarding oxd^n and spin-state of metal center before and after O_2 coordination i.e. the deoxy and oxy form.

high-spin Fe(II) \rightarrow deoxy form of Hb/Mb confirmed.

$S^{\ddagger} = 2$ ground state.
with 4 unpaired e^- .

* diamagnetic oxy form ($S=0$) with end-on coordinated O_2 ($F-\overset{\leftrightarrow}{O}-O \approx 120^\circ$), the oxd^n state assignment is less ambiguous because of the non-innocent nature of coordinated dioxygen.

Two alternatives

The presence of an even no. of unpaired e^- is favourable for a rapid, not spin-inhibited binding of $3O_2$ with its $S=1$ ground state.

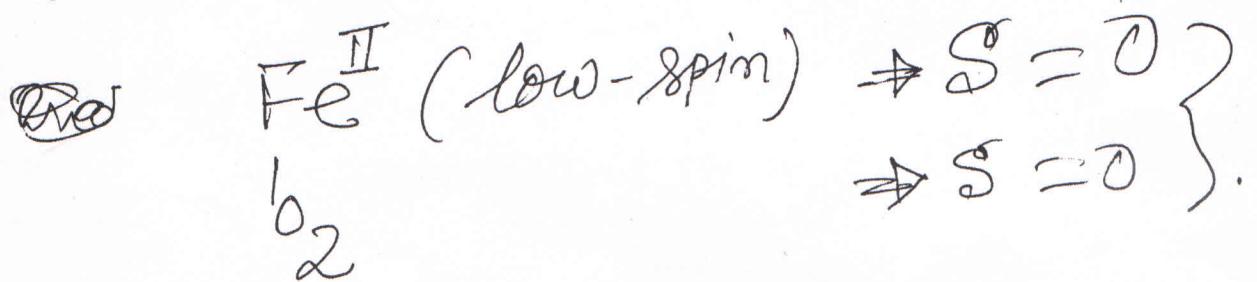
Oxdⁿ state in oxy form

(6)

Two ↓ alternatives.

1936

Pauling and Coryell explained the observed diamag. netism involving: low-spin Fe^{II} and singlet($\frac{1}{2}$) dioxygen. Both components would each be diamagnetic and binding would be achieved through the σ acceptor/ π donor character of the reduced metal and the σ donor/ π acceptor characters of the unsaturated ligand.



~~2nd~~ Alternative was formulated by Weiss. Following a single-e transfer from the metal to the approaching ligand in the ground state, low-spin Fe^{III} with $S = \frac{1}{2}$ and a superoxide radical anion O_2^- , also with $S = \frac{1}{2}$.

Diamagnetism is due to strong antiferro magnetic coupling.

Some experimental results such as vibrational frequency of the O-O bond at about 1100 cm^{-1} (characteristic of O_2^-), data from

(7)

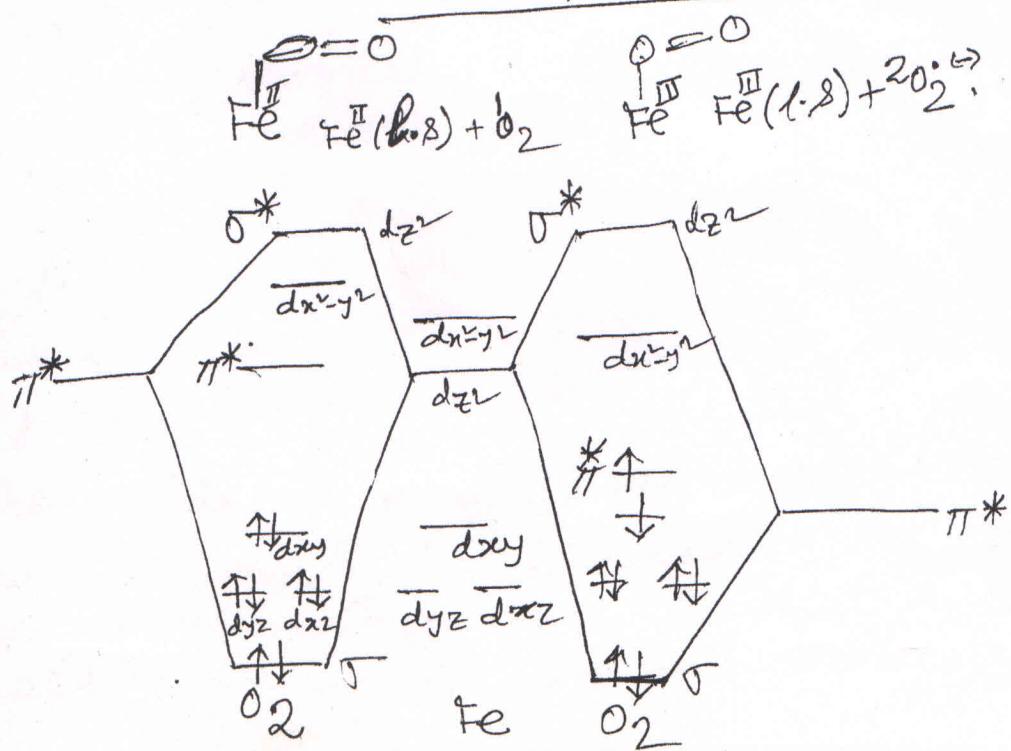
Mössbauer spectra and some aspects of chemical reactivity seem to favour the formulation of Weiss.

In substitution reaction O_2^- behaves as a pseudohalide thus Mb bound O_2 can be easily displaced by Cl^- .

When Fe is substituted by Co (hemo-globin \rightarrow coboglobin), its neighbour in the periodic table with one additional e⁻, EPR spectroscopy shows that the additional e⁻ resides mainly at the dioxygen ligand corresponding to a formulation O_2^- ($S=1/2$) low-spin Co(III) ($S=0$).

These all are indirect evidence in favour of the Weiss formulation.

MO representation

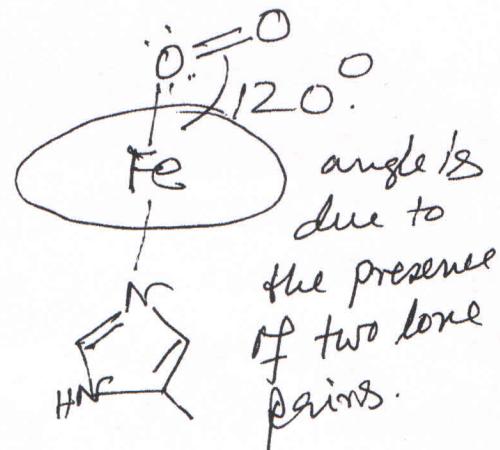
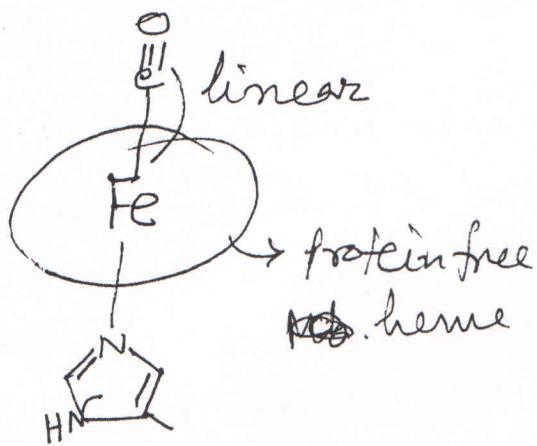


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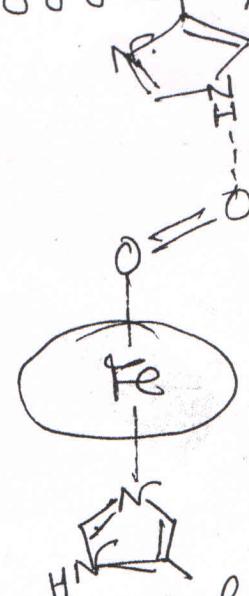
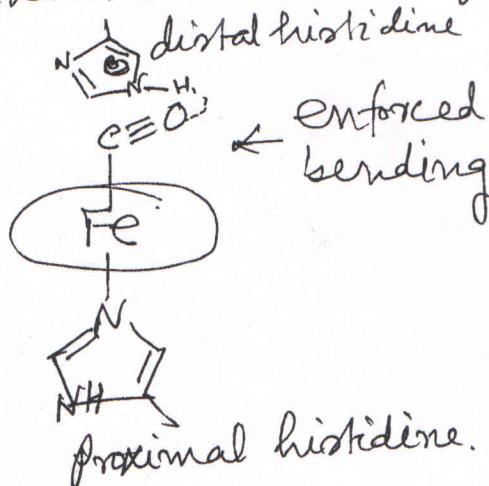
in favour of Pauling's model



CO, NO and other π -acceptor ligands predominantly coordinate to the lowervalent metal ion and can replace the dioxygen.



In Mb spatial restrictions through the protein environment as well as the possibility of H bond formation with distal histidine cause a more favorable equilibrium for binding of the weaker π -acceptor O_2 ($K_{CO}/K_{O_2} = 200/25000$ in protein-free heme model system).



The imidazole ring of the distal histidine blocks an unhindered access to the sixth free coordination site so that a controlled rapid binding of small molecule may

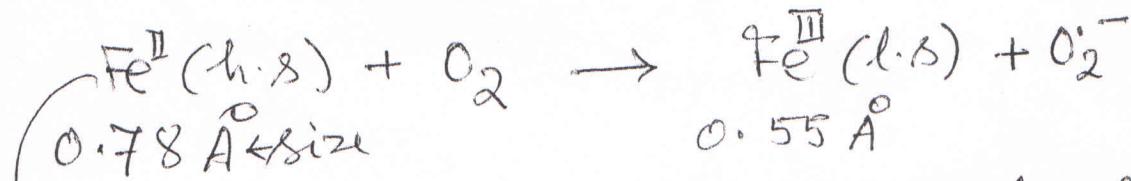
(9)

result only as a consequence of side-chain dynamics of the globin protein.

Modifications of the distal histidine led to a less favorable binding ratio O_2/CO .

~~Distal~~ histidine is valuable as the distal amino acid residue is basic in nature thus keeping protons away from the coordinated O_2^- . Protons act as electrophilic competitors in relation to the coordinating Fe, weakening its bond to O_2 and thus favor deleterious autoxidation processes.

Cooperative effect



is not fit properly in the cavity of porphyrin ring. Domed macrocycle result.

~20 pm contraction of metal size probably is the essential factor for cooperative effect.

Hb \rightarrow Tetramer

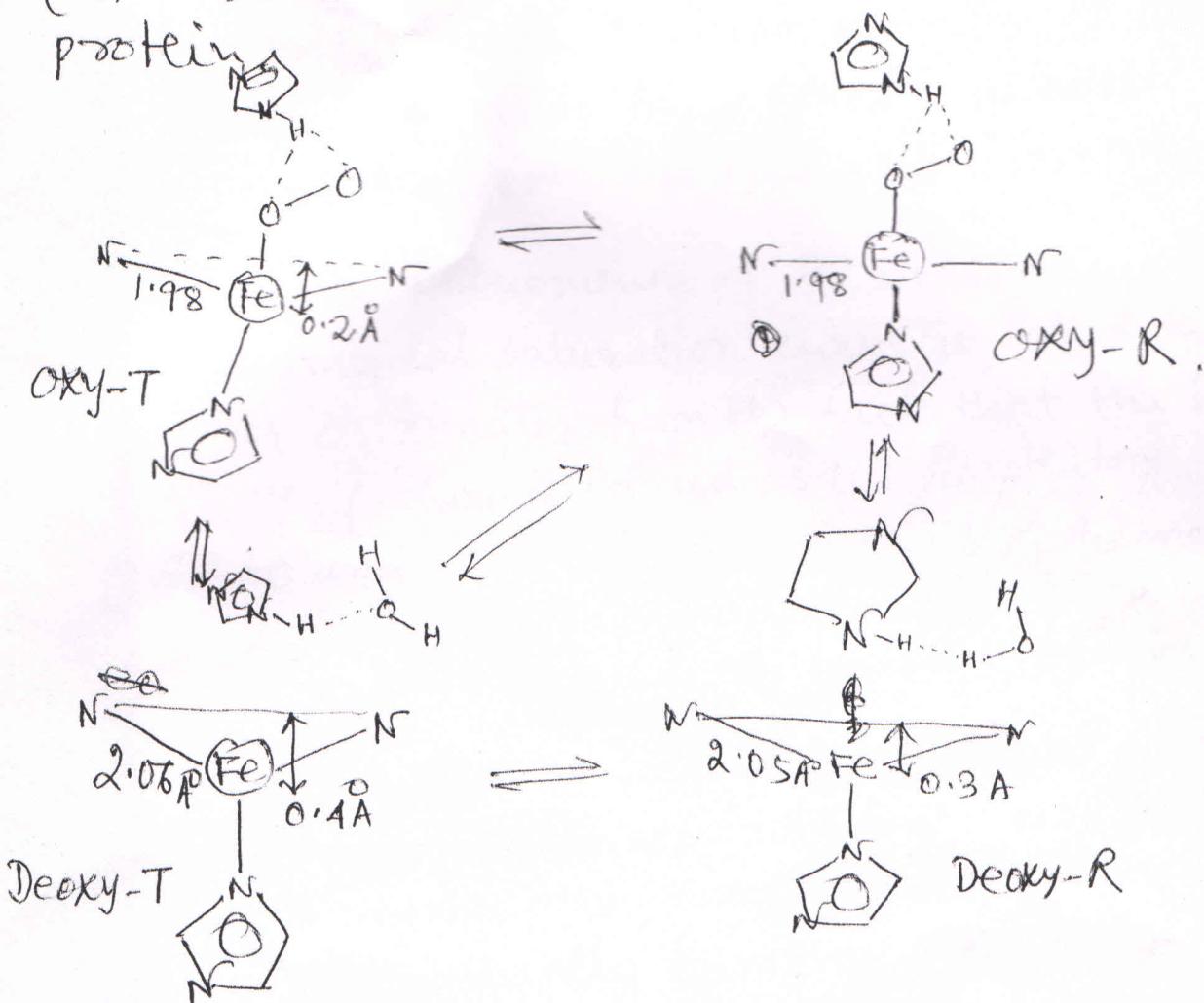
one monomer when binds O_2 the interaction of 4 hemes will change and triggered to the other heme systems.

Each of the 4 protein chains are linked to each other by electrostatic interactions (salt bridges) shows a geometrical changes upon O_2 coordination. This change is transferred to the other units by a process called

alized like a spring-tension model.

(10)

A two state model has been formulated
a tense (T, low O₂ affinity) and a relaxed
(R, high O₂ affinity) state of the tetrameric
protein



According to this scheme there are four different states of the individual heme system (OXY, deoxy, T or R state).

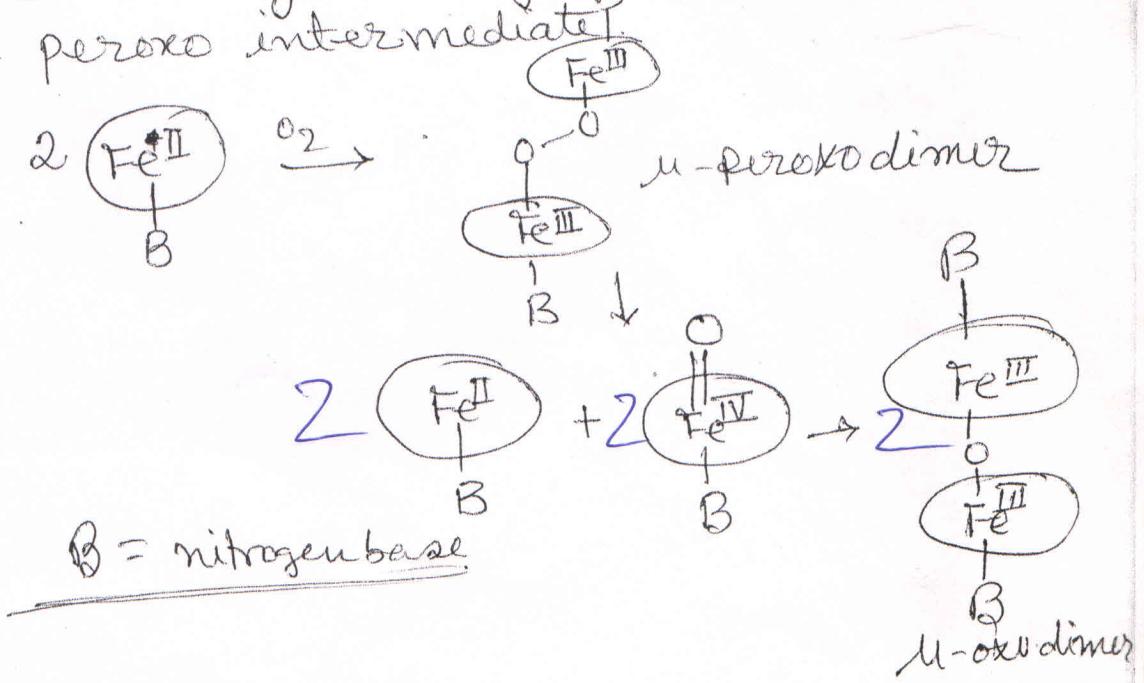
Initial binding of O₂ to the significantly domed T form leads to the not yet completely planar oxy T form with a decrease of the Fe-N(porphyrin) distance before a relaxation takes place to a planar oxy R form with a straightened Fe-N bond.

Release of O_2 from the oxy-R form results in doming of the porphyrin system (deoxy R state).

The distal amino acid residues are influenced by these geometrical changes thus being able to effect allosteric interprotein interaction in the Hb tetramer.

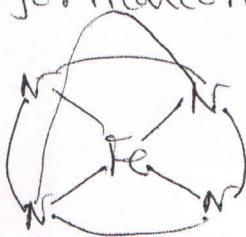
The phenomenon of pH dependence of the sigmoidal saturation curve is called the Bohr effect. It originates from the fact that the binding of CO_2 (which is formed as the respiratory end product) to a terminal amino group of hemoglobin reduces its capability to bind oxygen. Hb thus act as a O_2 and CO_2 transport protein.

In contrast to Hb and Mb, most simple Fe^{II} -porphyrin complexes which are not protected by a protein environment react irreversibly with O_2 eventually forming μ -oxo bridged dimer via peroxo intermediate.

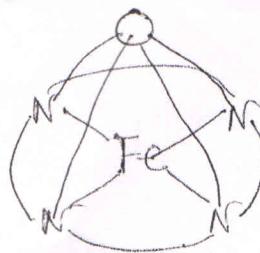


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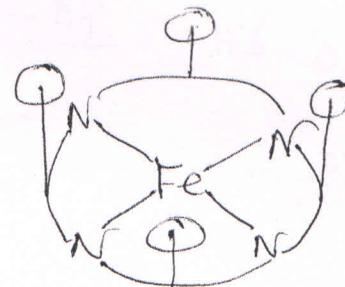
Di
merization can be avoided using different strategies. Among the available approaches Collman's 'picket fence porphyrins' leave a cavity for O_2 binding but prohibit monomer formation.



Strapped

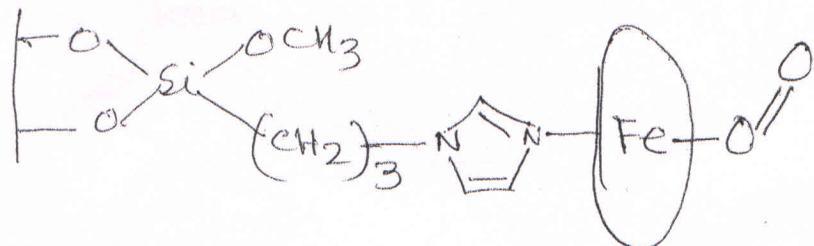


Capped



Picket fence.

Immobilization of the heme analogue e.g. on the surface of a silica support, this prevent dimerisation.



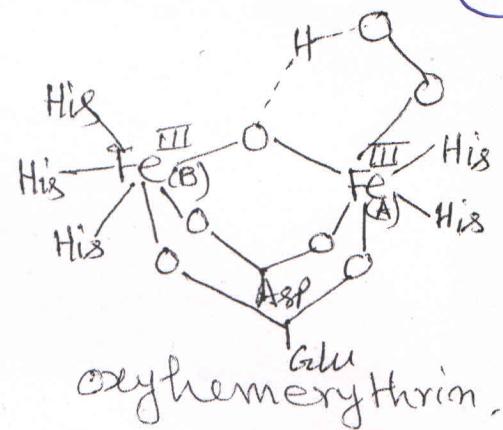
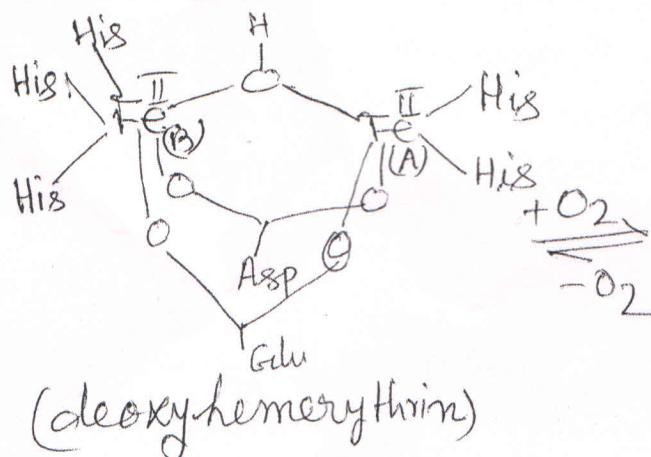
Alternative O_2 transport in lower animals:
Hemerythrin and Hemocyanin.

Several groups of invertebrates like crustaceans, molluscs (snail), arthropods (spider) or marine worms possess nonporphyrinoid metalloproteins for reversible O_2 uptake.

Hemerythrin
Fe containing } not heme
present
Hr

Hemocyanin
Cu containing } non heme
Hc

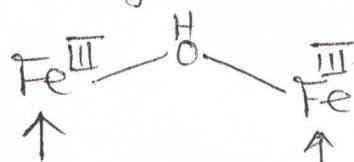
(13)



Magnetism

Magnetic measurement of the colorless deoxy form indicate h.s. $\text{Fe}(\text{II}) \rightarrow 4 \bar{e}$ unpaired weak antiferromagnetic coupling observed.

Red violet oxy form \rightarrow indicate two strongly antiferromagnetically coupled ($S = \frac{1}{2}$)



Light Absorption

Absence of strong light absorption in the deoxy form suggest protein bound metal center with no porphyrin π system.

Oxy form colour is due to the charge transfer transition, from electron rich peroxide ligand with a doubly occupied $\pi^*(\text{2P})$ orbital to electron deficient, oxidized $\text{Fe}(\text{III})$ with only partly filled d orbitals. $2\text{Fe}^{\text{II}} + \text{O}_2 \rightleftharpoons 2\text{Fe}^{\text{III}} + \text{O}_2^{2-}$

Vibrational Spectroscopy

Resonance Raman experiment \rightarrow a range of LMCT absorption band of oxyhemerythrin a resonance enhanced vibrational band at 848 cm^{-1} is observed which is characteristic of peroxide.

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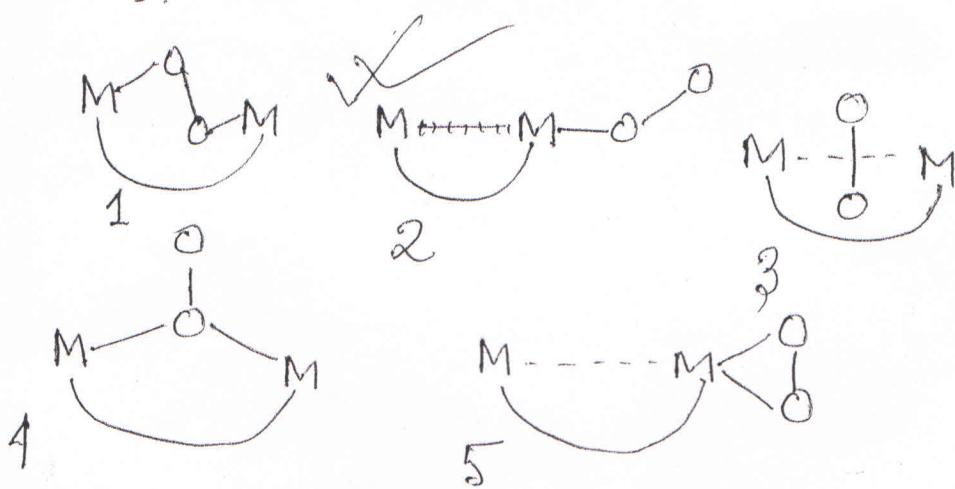
in the isotopic combination $^{16}\text{O}-^{18}\text{O}$ is used for the bound dioxygen two signals are obtained for the O-O stretching vibration suggesting a strongly asymmetric coordination e.g end-on.

Mössbauer

Mössbauer spectroscopy of oxyhemerythrin shows two distinctly different Fe^{III} resonance signals whereas the Fe^{II} centers of the deoxy form cannot be distinguished.

Structure

Conceivable of the alternatives for the $\text{O}_2^{\cdot-}$ coordination by the dinuclear metal arrangements only the situation 2 or strongly distorted 4 or 5 remain considering the above-mentioned results.

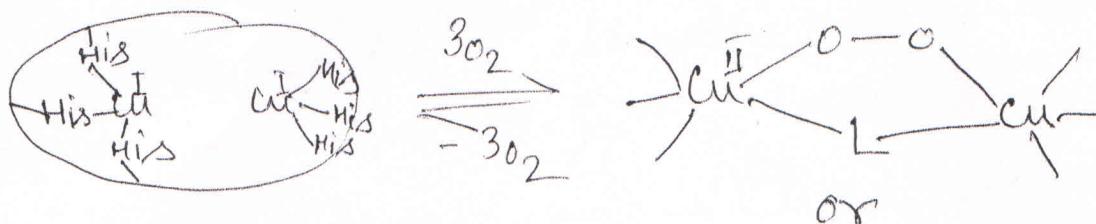


The doubly $\eta^1:\eta^1$ carboxylate and ~~slightly~~ singly hydroxide bridged high-spin Fe^{II} centers of the deoxy forms are coordinatively saturated with 5 His ligands except for one position at Fe_A . When dioxygen is bound there both Fe^{II} will get oxidized to Fe^{III} and form an oxo bridge ($\text{OH} \rightarrow \text{O}^{\cdot-}$). Thus O_2 taken presumably exist as hydroperoxo ligand $\text{HO}^{\cdot-}$.

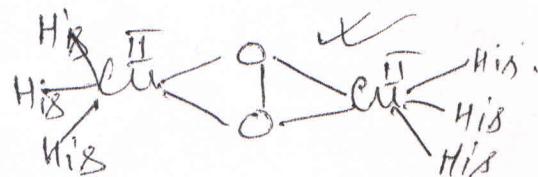
(15)

allowing a H-send to the O₂ group.
It is astonishing that O₂ binding in the protein
is reversible.

Hemocyanin



or



UV-VIS: 350 (20,000), 600 ($1000 \text{ M}^{-1} \text{ cm}^{-1}$)

EPR - inactive.

Similarities and differences in O₂ coordination
by Heme and ^(Hb, Mb) ~~Heme~~-non-heme metal dimers
(Hc, Hr).

(II)
very
one

d
and
O₂ taken up
400.