Name of the Teacher: SUTAPA CHAKRABARTY
Subject: CHEMISTRY
Class for which the note is prepared: Semester-6
Paper: C13T (Inorganic Chemistry)
Topic: Bio-Chemistry

Hemoglobine (Hb) and Myoglobin (Mb)

Hemoglobin and Myoglobin are responsible for the transport and storage of oxygen in higher animals ie mammals. Hemoglobin transports oxygen from its source (eg; lungs, skin and gills, etc) to the muscle cell where oxygen is transformed to myoglobin for use in mitrochondrial oxidation. (ie, respiration)

The complexes defineing the heme-group are those of the iron (II) complexes of protoporphyrin -IX (P_{IX}). The protoporphyrins are the class of nitrogen macrocyclic ligand, derived from porphin.

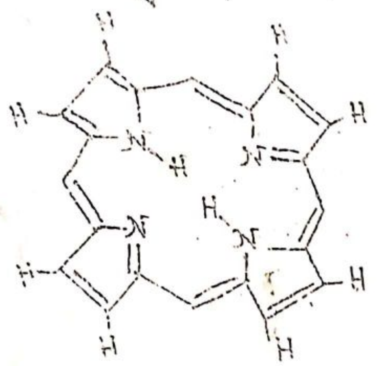


fig: Structure of porphin.

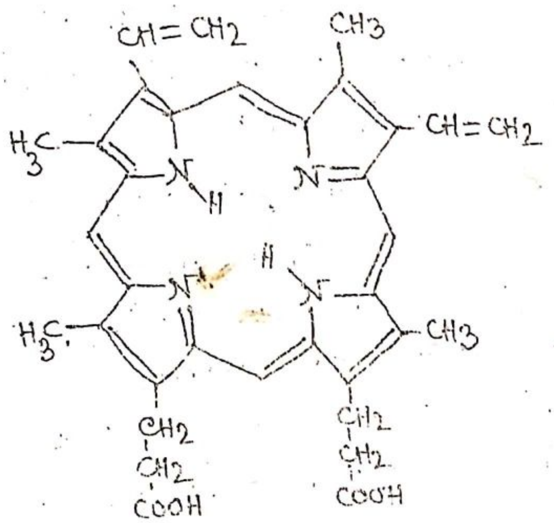


fig: Protoporphyrin IX (P_{IX})

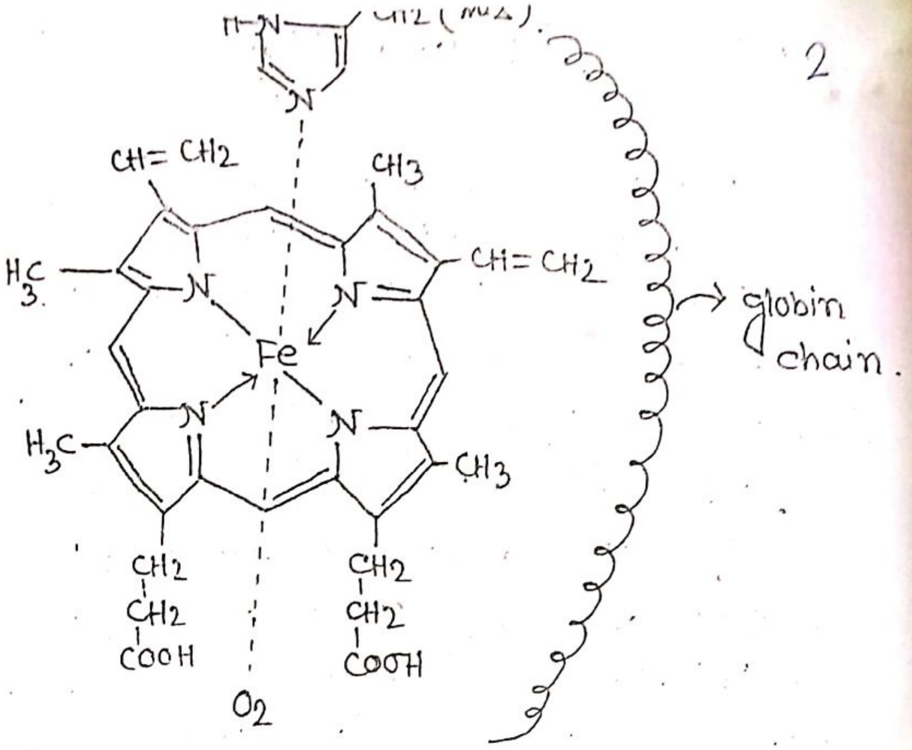


fig: The Heme-group in Hb and Mb (oxygenated)

Myoglobine is a monomeric protein (molecular weight 17000) having a single polypeptide chain which is not conductive of self association. But hemoglobin is a tetrameric protein (mol.wt 64500) consisting of 2α and 2β peptide chain interlinked through H-bonded $(-\overset{O}{\overset{\|}{C}}-O^{\ominus}\text{----}\overset{\oplus}{N}H_3-)$ intaractions.

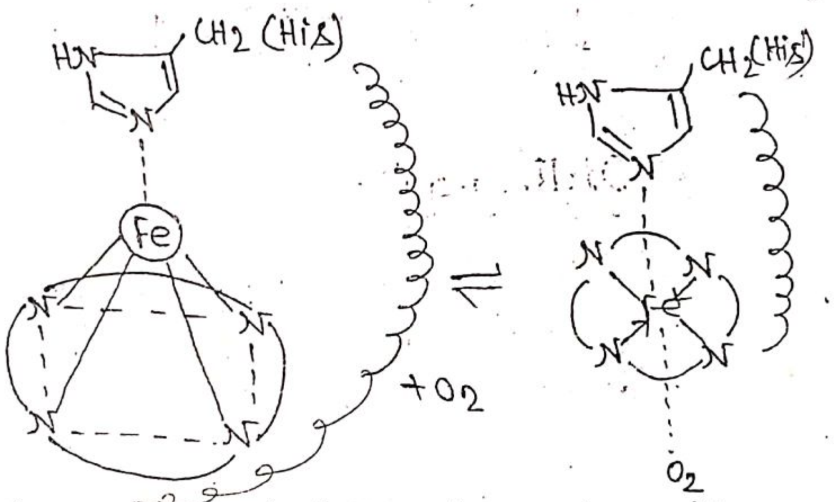
Active sites of Hb & Mb :

The active sites of both Hb and Mb contain the Heme-group in which iron (II) is equitorially co-ordinated by the four pyrolle N-atoms of P_{IX}. The fifth position is co-ordinated by the N-atom of a histidine of the protein chain (ie, globin). The sixth position in de-oxyhemoglobine or deoxyMb is vacant, but hydrofobically shielded by the protein chain. Therefore only nonpolar neutral molecule such as O_2, CO etc. can bind to the sixth position.

In the absence of protein (globin) the sixthposition is readily co-ordinated by polar water molecule and iron-(II) heme is irreversibly oxidised by O_2 of the air to Fe(III)-heme i.e, 'Hematine'. formation of hematin is a kind of desease. Because of its residual positive charge, ie reluctant to bind uncharged ligands like O_2 but readily binds charge ligand such as $CN^{\ominus}$, $OH^{\ominus}$, $S^{=}$ etc which inhibit oxygenetion-

$$\text{(globin) Fe(II) - heme} + O_2 \rightleftharpoons \text{globin Fe(II) - heme} - O_2$$

$$\text{Fe(II)-heme} + O_2 \longrightarrow \text{Fe(III)-heme (hematin)}$$



Being five-coordinated iron (II) in deoxyHb or deoxy Mb is present in high spin configuration, penta co-ordinated Fe(II) deoxy Hb or deoxy Mb has a square pyramidal geometry and iron (II) is situated about 0.8 A° out of the porphyrin plane. When O_2 binds to the Fe(II) heme at the vacant sixth position, it results an octahedral geometry where the HS Fe(II) (radius = 0.92 A°) is converted to LS Fe(II) (radius = 0.75 A°). As a result Fe(II) radius is contracted by about 0.17 A°, thus ultimately ~~by~~ about ~~0.17 A°~~ results the fitting of Fe(II) in the porphyrin cavity. This movement of Fe(III) causes the co-ordinated histidine to move towards the porphyrin plane. This brings about a conformational change through out the peptide chain amounting to ~~raughture~~ rupture of some or all the $-COO^{\ominus}---\overset{\oplus}{N}H_3$ salt bridge interaction. The constrained [illegible] Hb tetramer then reluxes by exposing the sixth position of the remaining heme group to oxygenation. This phenomenan is known as co-operative interaction. Oxygenotion of Hb is autocatalytic due to this co-operative interaction. But such effects are absent in Mb due to its monomeric nature. Due to constrained in the stucture, the overall rate of binding of ligands (O_2) to Hb is much slower than that of Mb.

Hill's equ^n:

As the subunit of tetrameric Hb is oxygenated, co-operative interaction helps another subunits to take up oxygen

$$Hb + O_2 \xrightleftharpoons{K_1} HbO_2$$

$$Hb(O_2) + O_2 \xrightleftharpoons{K_2} Hb(O_2)_2$$

$$Hb(O_2)_2 + O_2 \xrightleftharpoons{K_3} Hb(O_2)_3$$

$$Hb(O_2)_3 + O_2 \xrightleftharpoons{K_4} Hb(O_2)_4$$

As a result, the succesive oxygen binding constants of Hb gradually increases $K_1 < K_2 < K_3 < K_4$. The constant K_4 corresponds to oxygenation of reluxed Hb tetramer and it is quite close to the oxygen binding constant of Mb in which co-operative interaction is absent. Due to monomeric nature and the absence of cooperative interaction Mb takes up O_2 in 1:1 molar ratio.

$$Mb + O_2 \underset{}{\overset{K_{Mb}}{\rightleftharpoons}} MbO_2$$

$$\therefore K_{Mb} = \frac{[Mb(O_2)]}{[Mb]\,P_{O_2}} \quad ----①$$

On the other hand due to tetrameric nature and co-operative interaction oxygenation of Hb may be expressed as

$$Hb + nO_2 \rightleftharpoons Hb(O_2)n$$

$$K_{Hb} = \frac{[Hb(O_2)n]}{[Hb]\,P_{O_2}^{n}} \quad ----②$$

Fraction (f) of Mb & Hb oxygenated could be expressed as follows;

$$f_{Mb} = \frac{\text{Oxygenated Mb}}{\text{Total Mb}}$$

$$= \frac{[MbO_2]}{[Mb]+[MbO_2]}$$

$$= \frac{K_{Mb}\cdot P_{O_2}}{1+K_{Mb}P_{O_2}} \quad -----③$$

$$\text{Similarly } (f)_{Hb} = \frac{[Hb(O_2)n]}{[Hb]+[Hb(O_2)n]}$$

$$= \frac{K_{Hb}P_{O_2}^{n}}{1+K_{Hb}P_{O_2}^{n}}$$

From equ

From equⁿ ③ and ④ we can write

$$\log\left(\frac{f}{1-f}\right)_{Mb} = \log K_{Mb} + \log P_{O_2} \quad ----⑤$$

$$\log\left(\frac{f}{1-f}\right)_{Hb} = \log K_{Hb} + n\log P_{O_2} \quad ---⑥$$

The equⁿ ⑤ and ⑥ are known as Hill's equⁿ.

Significance of Hill's eqn :

The plot of $(\log \frac{f}{1-f})$ vs P_{O_2} is popularly called Hill's plot. This plot of $\log \frac{f}{1-f}$ against P_{O_2} gives $n = 2.95 \pm 0.5 \approx 3$ for Hb oxygenation, indicating that the co-operative interaction b/w the subunits of Hb is such as to produce the effect of 3 molecules of O_2 binding :- simultaneously. This means the oxygenation and deoxygenation of Hb takes place simultaneously.

Hb is more oxygenated than Mb at higher oxygen pressure as are available in lungs, skins and gills. This means that at higher oxygen pressure ~~the pressure of~~ one or more bound oxygen instead of favouring dissociation of oxy Hb, favours further oxygenation.

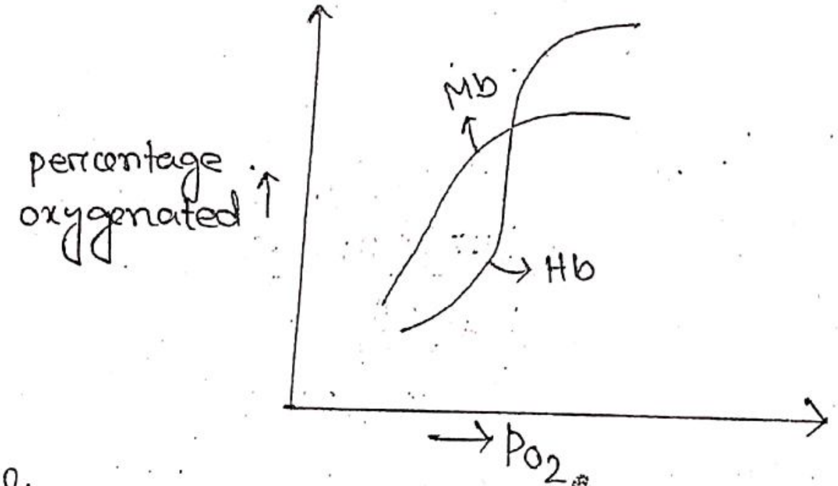


fig: Oxygen saturation curves for Hb and Mb.

that is why it is possible that the transfer of O_2 from oxy Hb to Mb in tissues.

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

Bohr effect :

As the oxygenation of Hb is subjected to co-operative interaction involving the break-down of $-COO^{\ominus}---\overset{\oplus}{N}H_3$ salt bridge bonds b/w and with in the subunits, oxygenation of Hb is pH dependent. This phenomenan is known as Bohr effect. Due to absence of co-operative interaction Mb oxygenation does not show Bohr effect.

Oxygenetion is favour in basic condition due to the elemination of $COO^{\ominus}---\overset{\oplus}{N}H_3$ salt bridge bonds. On the other hand deoxygenation is favours in acidic medium.

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

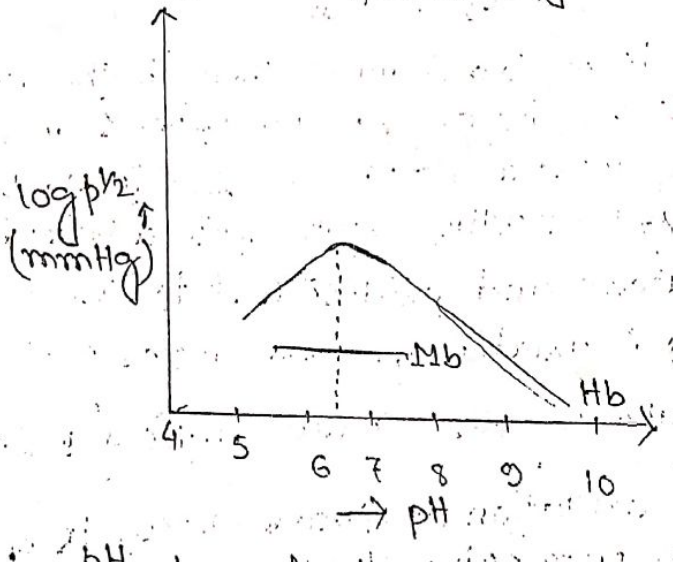


fig: pH dependence of oxygenation of Hb (Bohr effect) [p½ = oxygen pressure require to half saturate Hb or Mb]

The maxima b/w pH 6–6.5 corresponds to the pH range of lowest oxygen affinity of Hb. Under such weakly acidic condition the transfer of O_2 from oxy Hb to Mb is very much favoured.

Tissues consuming O_2 produce CO_2, H_2CO_3 and lactic acid which help release of O_2 from oxyHb. Thus Bohr effect explains release of O_2, increase the total concentration of resolved CO_2 and facilitates its transport from tissues to lungs.

① V.V.I

Role of Hb in Biological System

Hb is very sensitive to oxygen and hence readily combines with it. Thus when we breathe in O_2, Hb ~~release~~ present in our blood combines reversibly with O_2 in the lungs to form oxyhemoglobin.

$$Hb + O_2 \rightleftharpoons HbO_2$$

As the blood runs through the arteries (ধমনী) to the tissues, the oxygen pressures decreases and O_2 bound with Hb is set free. This free O_2 diffuses into the body cells where it combines with glucose and oxidises it to CO_2 and H_2O. Thus O_2 is used in the combusions of food.

$$C_6H_{12}O_6 + 6O_2 \longrightarrow 6CO_2 + 6H_2O + E\ (ATP)$$

The oxidation of glucose to CO_2 is an energy releasing process in which energy in the form of ATP is produced. This energy is utilised by the living organisms to perform their various metabollic activities and for mentaining their body tempʳ.

Tissues that have already consumed oxygen releases CO_2 which in turn lowers the pH of blood and hence induces Hb to release more oxygen to Mb (Bohr effect).

CO_2, a product of the citric acid cycle and other de-carboxylation rea^n, deffuses out of the tissues into the blood plasma. The Hb carries back CO_2 to the lungs. The CO_2 gets bound to α-amino groups of Hb to form a carbamate ($-NH_2 + CO_2 \rightarrow -NH-COO^{\ominus} + H^+$) which releases CO_2 in the lungs. CO_2 reacts with H_2O to give H_2CO_3.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3.$$

The H_2CO_3 radily dissociates into H^+ and HCO_3^- ions. The H^+ ion promotes loss of O_2 from oxyhemoglobin.

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

$$H^+ + HbO_2 \rightleftharpoons [HHb]^+ + O_2$$

By the time the blood returns to the lungs. It is ~~realeas~~ reached in CO_2. In the lungs O_2 intake leads to the formation of oxy Hb which causes release of H^+. This H^+ then combines with $HCO_3^{\ominus}$ to give H_2CO_3 which then breaks up into H_2O and CO_2.

$$HHb^+ + O_2 \rightleftharpoons H^+ + HbO_2$$

$$H^+ + HCO_3^{\ominus} \rightleftharpoons H_2CO_3$$

$$H_2CO_3 \rightleftharpoons H_2O + CO_2$$

The CO_2 is then exhaled into our atmosphere.

① Hemerythrin :

It is a non Heme respiratory (শ্বসন) metalloprotein found in lower animals like marine-worms. This metalloprotein has Fe(II) as the metal ion but there is no porphyrin macrocyclic ie it is a non-Heme metalloprotein. The basic protein chain has arround 110 amino acid residues. One difference from Hb is that while one Fe(II) binds one O_2 molecule in Hb, two Fe(II) binds one O_2 in Hemerythin.

Crystal structure studies of Hemerythrin shows two Fe(II) being bridged by two oxygen atoms from the glutamate and by two oxygen atoms from the aspertate carboxylate groups of the protein chain and a bridging -OH group. The Fe(II) are further co-ordinated unevenly (nonequivalent) to histidine ~~molecule~~ N's, one Fe(II) to two and the other to three histidines. Thus the two Fe(II) are non equivalent. Both the Fe(II) are high spin

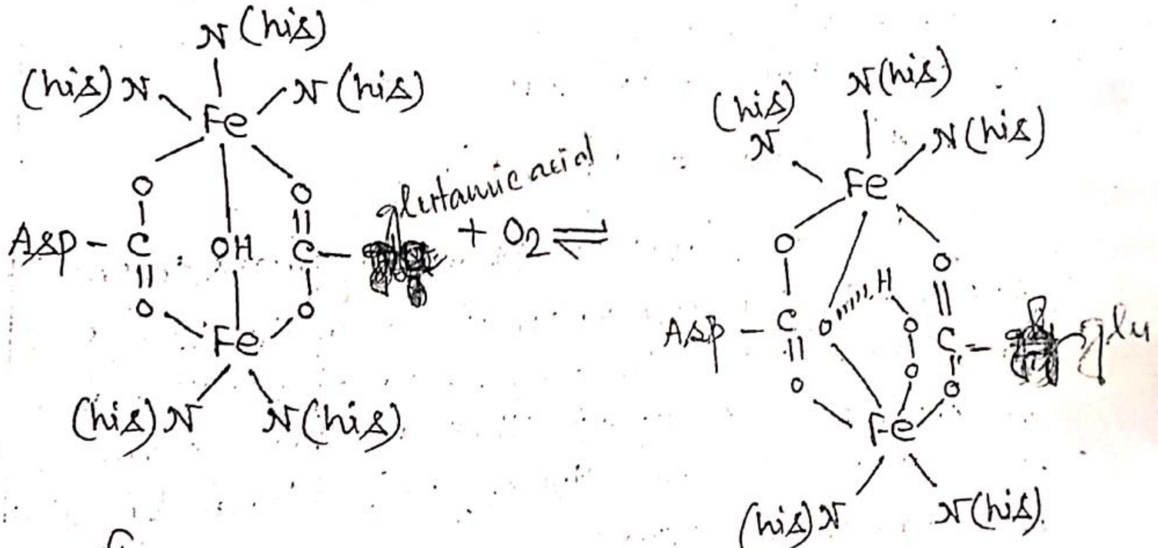


Fig: Binding of O_2 to Hemerythrin.

On binding an oxygen molecule the Fe(II)'s are oxidised to Fe(III). Each Fe(II) transfers one electron [$Fe^{+2} - e \rightarrow Fe^{+3}$] to giving peroxo gr. ($O_2^{2-}$). For converting oxyhemerythrin to active hemerythrin the two Fe(III) have to undergo reduction to Fe(II) and the μ-oxo gr. protonated to μ-OH gr.

Write the difference between the Hb & Mb.

⇒ The main difference between Hb and Mb are shown in table below:

Hb.		Mb.
i) It acts as a dioxygen transport.	i)	It acts as dioxygen storage.
ii) It is tetramer.	ii)	It is monomer
iii) It is found in blood.	iii)	It is found in mucle cell.
iv) It contains four polypeptide chain. (2α & 2β)	iv)	It contains one polypeptide chain.
v) Its molecular weight is 64500.	v)	Its molecular weight is 17000
vi) Oxygenation of Hb is pH dependent ie, it shows Bohr effect.	vi)	Oxygenation of Mb is pH independent.

OXYGEN CARRING PROTIENS

Nature has designed four oxygen carrying protems for transport and storage of oxygen in biological systems. These are Hb, Mb hemerythrin and hemocyanin. Hb and Mb are Fe(II) – heme proteins while hemerythrin is a non heme

Fe (II) protein. On the other hand hemocyanin contains copper at its oxygen binding sites.

Differentiat between oxigenation and oxidation :

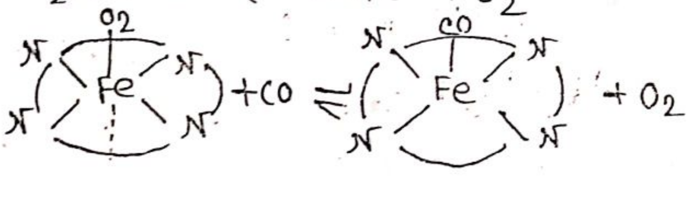
The differences between oxigenation and oxidation are shown in table below ;

Oxygenation	Oxidation
i) In this process dioxygen molecule acts as a ligand and co-ordinated to the metal centre.	i) This process involves loss of one or more electrons by atoms or ions either free or in the combine state.
ii) Oxygen must be involved in this process	ii) Oxygen may be involved or not.
iii) The identity of the dioxygen molecule does not change.	iii) The identity of the dioxygen molecule must be change.
iv) $Hb + O_2 \rightleftharpoons HbO_2$	iv) $Mg + Cl_2 = MgCl_2$ (Oxygen absent) $2Mg + O_2 = 2MgO$ (Identity changed)

State how 'co' affects biological system :

⇒ 'CO' attacks Hb and displaces oxygen from oxyHb to form carboxyHb.

$$O_2Hb + CO \rightleftharpoons COHb + O_2$$



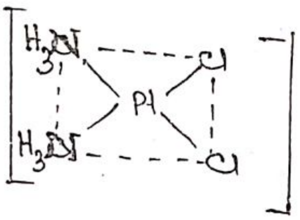
The carboxy Hb is stabler complex than oxy Hb so that the net result is the reduction in the bloods capasity for carrying O_2.

In initial effect of co poisoning is loss of awareness and judgement which are responsible for many automobile accident. Which increasing exposure to higher levels ~~to~~ of 'co', various metabolic disorders will occur, ending in death.

'co' poisoning can be cured by exposing the affected person to fresh O_2 whereby the reverse reaⁿ occurs.

$$COHb + O_2 \rightleftharpoons HbO_2 + CO.$$

① Cis-Platin: (SSP)



SSP is the best known example of the application of co-ordination compounds in medicine. Rosenberg and his collaboralers started working with this along back. After much investigation it was established that cis platin was responsible for the inhibition of cell division. It acts as an anticancer agent. It is not active against all type of cancers but is particularly effective against testicular cancer and active against cancers of lungs, ovary, bladder, head, neck and cervix.

Cis platin is administered as injection every few weeks; solⁿ are usually given in physiological saline. It is highly toxic agent. Chemoprotector like sodium diethyl dithiocarbamate is used to reduces the toxicity.

The mode of action of cis-platin based on its capasity to bind to DNA and block replication. Since the $Cl^{\ominus}$ ion concentration in extra cellular fluids is large, the $Cl^{\ominus}$ ligands in cis platin are not significantly substituted by water molecules outsides the cell. The $Cl^{\ominus}$ conc. being much less inside the cell, so the $Cl^{\ominus}$ ligands in cisplatin are replaced by H_2O, forming products like $[Pt(NH_3)_2(H_2O)_2]^{2+}$ and $[Pt(NH_3)_2(OH)_2]$. The Pt (II) now binds to the two nitrogen atoms (N-7) of two adjucent guanine bases - mostly in the same strained, to form a chelated interstained crosslink and upsets the normal replication of the DNA and thus inhibits groth of the cancer cells.

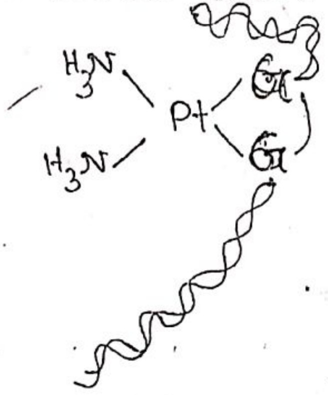


fig: ~~Intra trans~~ Interstained crosslink by cis-platin in DNA.

VU

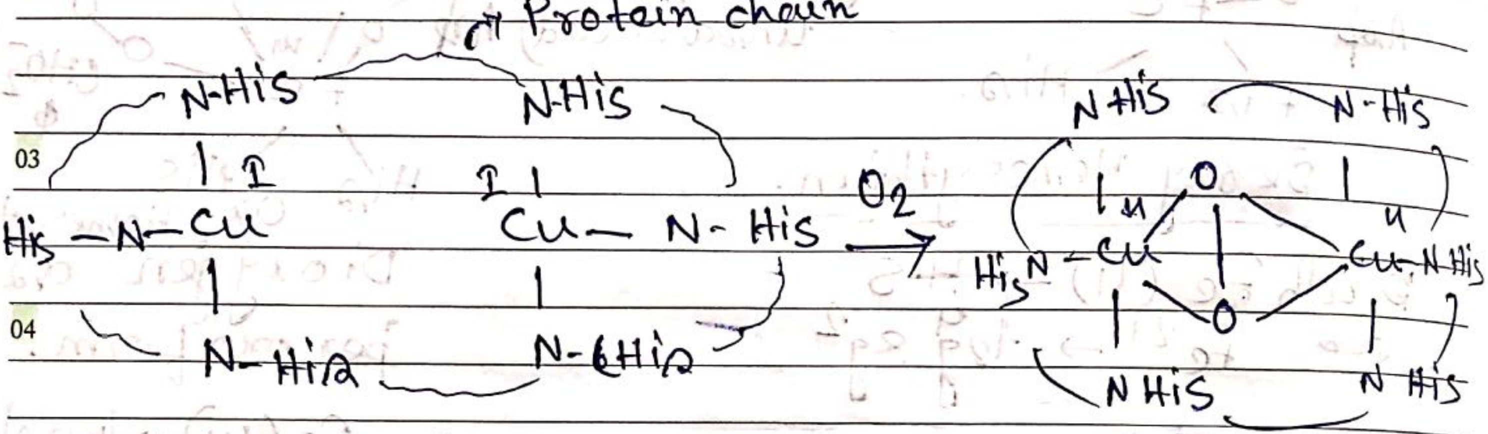
2001 → 11 (a)
2002 → 12(a),(b),(c)
2003 → 12 (a),(b).
2004 → 12 (b)
2005 → 8(f); 10(c)
2008 →
(old) → 9 (v), 13
(New → 14 (d)
(3T) 2009 → 12(a), 13(b), 9(b)
2010 (N) → 3 (d), 7 (c).
2011 → 9 (e), 13(b) i, 14 (b)
2012 → 7,(d),(e), 11 (c), 13 (a)(c), 14 (d)

Hemocyanin → In some invertibrates. e.g.- snails, crabs.

→ Hemocyanin → blue blood.

→ polymeric form. ⇒ 6, 12, 18, 24 subunit. but not monomeric.

→ Active side → Cu.



⇒ Deoxy form.	⇒ Oxy form.
⇒ Cu(I)	⇒ Cu(II)
⇒ d^{10} ~~→ H.S.~~	⇒ d^9
⇒ Diamagnetic.	⇒ antiferromagnetic coupling.
⇒ epr inactive.	⇒ dia-magnetic
⇒ colourless.	⇒ blue colour due to ⇒ $O_2^{2-} \rightarrow Cu^{II}$ LMCT

NOTE → In some cases Hemocyanin show co-operativity effect).

• Photosynthesis :

Photosynthesis involves a series of complex reactions in the chloroplasts of plants with the overall conversion of water and Carbon di-oxide in presence of sunlight and Chlorophil to glucose and ~~and~~ dioxygen. Glucose is further converted into polysacharides like starch. In a simplified way, the process of photosyn-thesis can be shown as :

$$6CO_2 + 6H_2O + \text{solar energy} \xrightarrow{\text{chlorophyl}} \underset{\text{(glucose)}}{C_6H_{12}O_6} + 6O_2$$

[Tracer ~~sty~~ studies have shown that all di-oxygen evolved in the above reaction comes from oxidation of H_2O and not from CO_2.

$$6CO_2 + 6H_2\overset{*}{O}\ (O^* = {}_{8}O^{18}) + \underset{\text{energy}}{\text{solar}} \xrightarrow{\text{chlorophyl}} \underset{\text{(glucose)}}{C_6H_{12}O_6} + 6\overset{*}{O}_2$$
]

The reaction is endothermic to the extent of about 469 KJ/mol of CO_2 is initiated by visible solar radiation in the range 600-700 nm. A part of the series of reactions may proceed in dark as well.

Plants absorbe light with the help of chlorophyls which are Mg-Porphyrin complex. The molecules are capable of absorbing photons around 700 nm and transmit the energy on to other species in the reaction sequence. Some chlorophyls act as antenna for solar radiation—also called light Harvesting centros (LHC) which pass the photon energy through the reaction site through excitation transfer within picoseconds. The overall photosynthetic conversion is a series of complex reactions and involves light absorbtion at two different regieons of wavelength— Photosystem-I (PS-I) and photosystem-II (PS-II). PS-I is based on a special form of chlorophyl-a having absorption maxima at 700 nm. (P-700) while PS II utilised another form of chlorophyl-a having maxima at 680 nm (P-680).

- **Photosystem-II :-**

A Mn clusture [Mn(IV)] Catalyses the oxidation of water to di-oxygen.

$$2H_2O \longrightarrow O_2 + 4H^+ + 4e$$

The electrons are excited photo-chemically by P-680 to the primary electron acceptor Pheophytin. Therefore, it is transferred to photosystem-I via the intermediacy of

(i) cytochrome b_3, (ii) Plastoquinone
(iii) Fe-S protein, (iv) cytochrome-f
(v) plastocyanin.

Photosystem-I :

The electron received ~~for~~ from PS-II is further excited by P-700 and is ultimately used up in the reduction of CO_2 via (i) Fe-S centre. (ii) Ferredoxin NADP reductase and (iii) NADPH

The electron flow also results in the formation of ADP from ADP. The main reactions may be written as

- PS-II :

$$[2H_2O \longrightarrow O_2 + 4H^+ + 4e] \times 6$$

- PS-I :

$$[2NADP^+ + 2H^+ + 4e \longrightarrow 2NADPH] \times 6$$

Dark reaction :

$$12NADPH + 12H^+ + 6CO_2 \longrightarrow 12NADP^+ + \underset{(glucose)}{C_6H_{12}O_6} + 6H_2O$$

Net reaction $6H_2O + 6CO_2 = C_6H_{12}O_6 + 6O_2$

Representation :

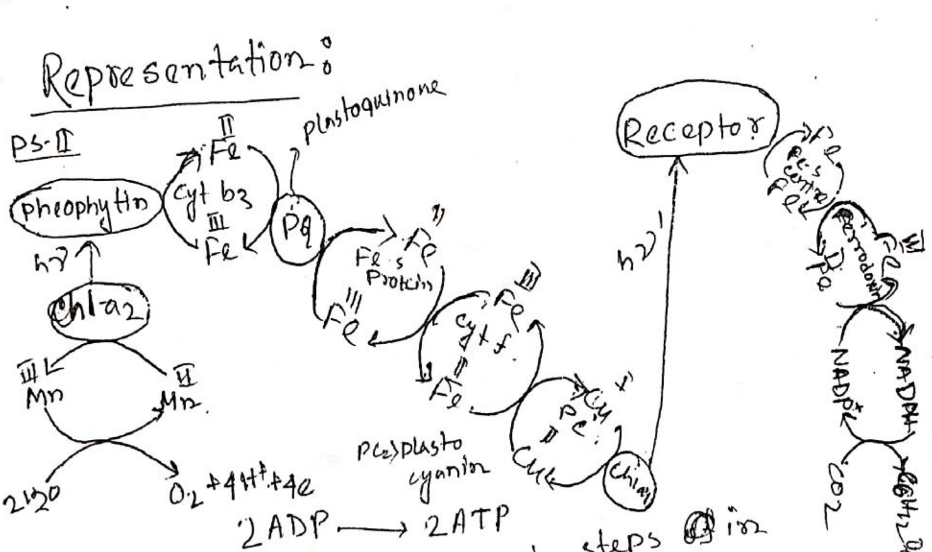


Fig: Representation of the main steps in photosynthesis.

Carbonic Anhydrase :

Carbonic anhydrase is a Zn-containing metalo enzyme present in animals, plants and in certain micro organisms. It catalyses the hydration of CO_2.

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^{\oplus} + HCO_3^{\ominus}$$

Such hydration-dehydration reactions of CO_2 are of great rate physiological significant in the context of transport of CO_2 formed due to metabollic oxidation of food nutrients in the tissues, to the lungs and its release to the atmosphere.

Nonenzymic rates of hydration of CO_2 by H_2O is nearly 10^7 times slower than the enzyme catalysed reaction. In addition to hydration of CO_2, the enzyme also catalyzes the hydration of Carbonyl compounds and hydrolysis of esters.

Carbonic anhydrases-A, B and C are slightly different and occur in different organisms. The most well characterised is the ~~human~~ Carbonic anhydrase-B which is a monomeric protein containing one atom of tightly bound Zn^{2+} in a molecular weight of about 30,000. It consists of 256-265 amino acid residues. It is an enzyme containing Zn^{2+} ion. These Zn^{2+} ion is bound to three histidine residues (his-93, 95 and 117) in a distorted tetrahedral geometry. ~~situated at the bottom of a~~ The fourth co-ordination site of tetrahedral Zn^{2+} ion is occupied by a water molecule.

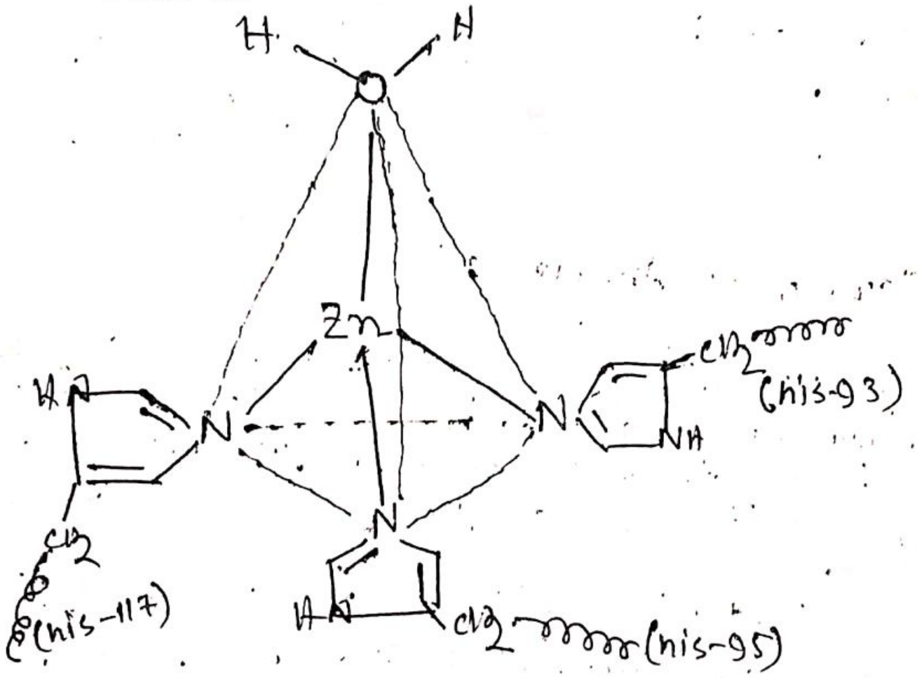


fig: active site structure of carbonic anhydrase-B

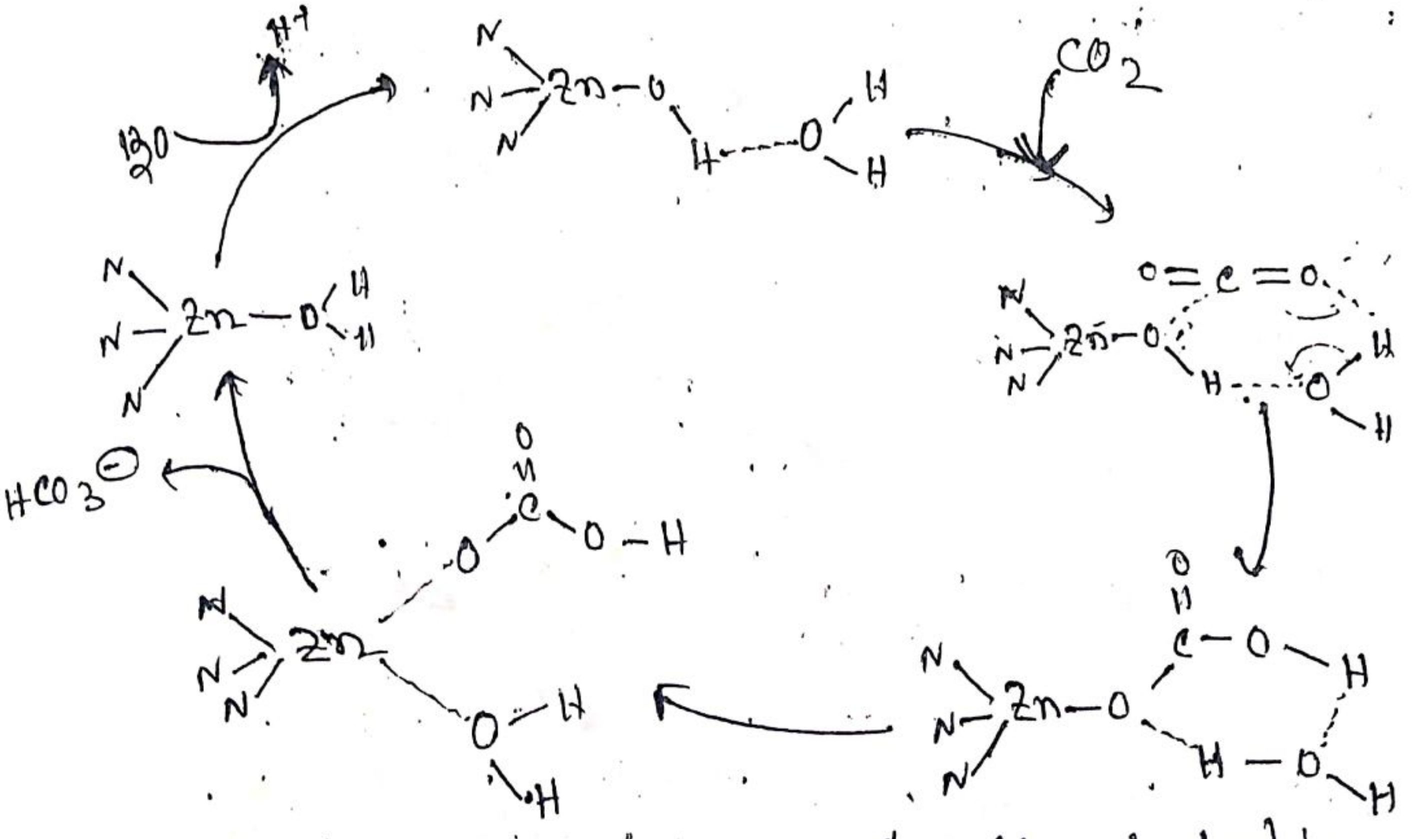


Fig:- catalytic cycle for the hydration of CO_2 by Carbonic anhydrase-B.