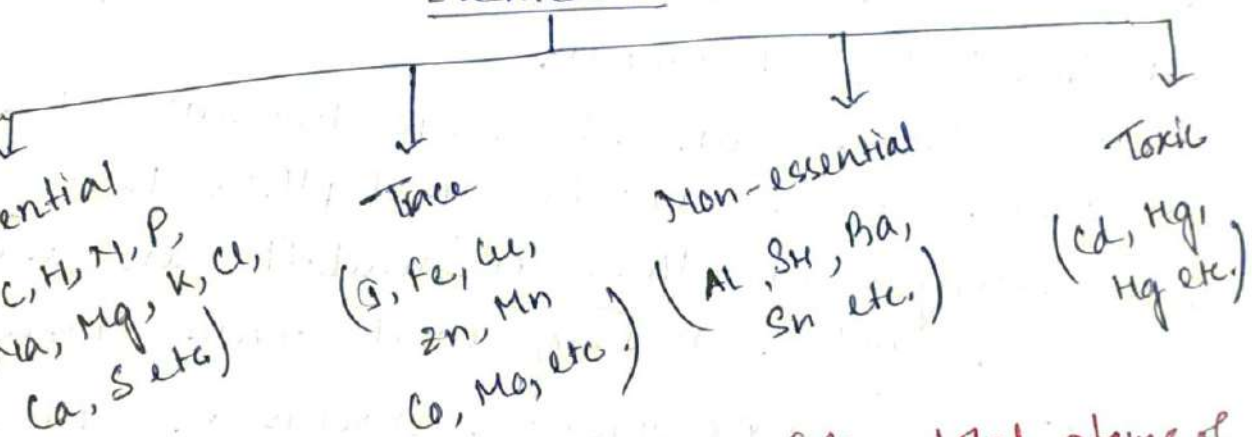
BIOINORGANIC CHEMISTRY

↳ A field that studies the role of metals and non-metals in biological systems.

— Study of the structures and biological functions of inorganic substances.

Classification of elements according to their action in the biological system —



(H, O, C & N constitute about 99% of the total atoms of a living body.

↳ Essential elements are absolutely ~~ess~~ essential for life process.

↳ Trace elements are also necessary for life process.

↳ Non-essential elements are not essential, If they are absent other elements may serve the same function

↳ Toxic elements disturb the natural functions of the biological system.

Geochemical effect on the distribution of metals :—

↳ Except for Mo & P (non-metal), all the biologically abundant elements are also abundant in earth's crust.

↳ But the elements abundant in earth's crust are not always biologically important. eg:- Si, Al, Ti & 2H are abundant in earth's crust but these are not accepted in the biological process.

— These four elements remains almost insoluble oxides at biological pH. & this insolubility made them unavailable in the biological system as they donot form stable complexes with complexing agents of biological significance.

↳ Some elements occur trace quantities terrestrially, but they play vital roles in the life process. eg:- Cu, Se.

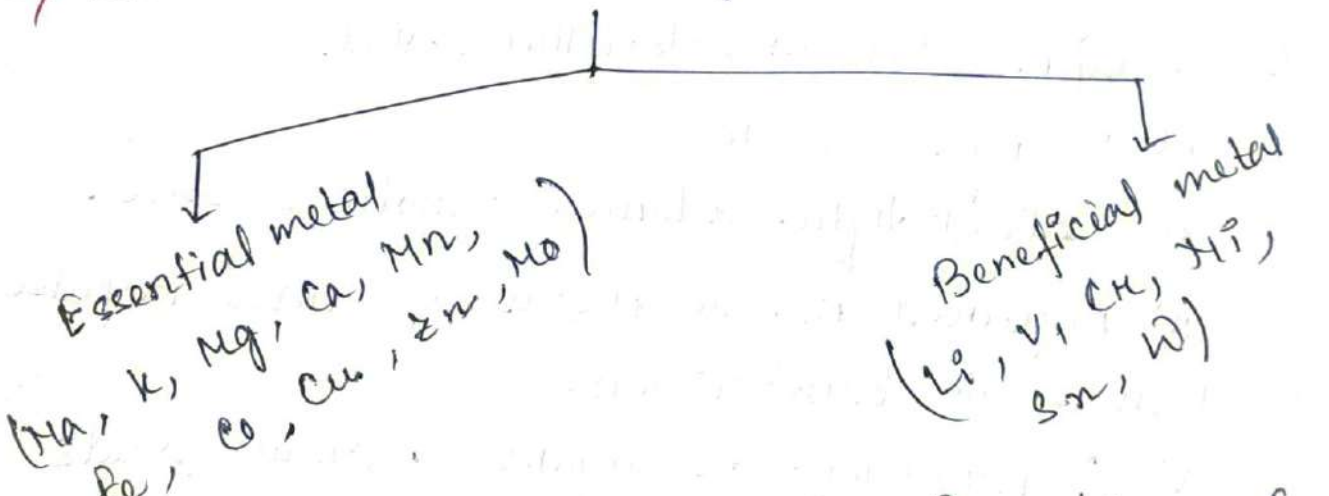
↳ Some elements of low terrestrial abundance eg:- As, Pd, Cd, Be, Ti, Hg, U etc. are extremely toxic. These are extremely toxic even at trace quantities & their no beneficial role is yet known.

↳ Almost any metal & element can be harmful at very high concentration. eg:- NaCl at very high concentration is toxic as it creats an imbalance in electrolyte distribution.

— For all essential & beneficial elements, there is a specific optimum concentration range for biological requirement & both deficiencies & excesses cause unwanted effects.

Metal ions present in biological systems —

↳ Biometals are classified as —



↳ Living system can't survive without essential metal one eventually dies

↳ In absence of beneficial metals, the life process gets hampered but it can't lead to death.

↳ At biological pH, all these biometals (except Na, K & Ca to some extent) cannot exist as free ions.

↳ Form insoluble hydroxides & phosphates.

↳ With biligands form soluble complexes.
eg:- amino acids, lactate, citrate, crown ethers, cryptands etc.

Biological functions of biometals/bioelements—

Li — Antipsychosis activity.
eg:- Li_2CO_3 → used in treatment of mental health.

Na — i) Major cation in extracellular fluid.
ii) Charge carrier
iii) electrolytic balance, osmotic balance.
iv) required in the process of nerve impulse creation & its transmission.
v) Co-transport of sugars & amino acids into cells.
vi) High level of Na cause → Hypertension

K — i) Intracellular cation.
ii) Helps in nerves to function
iii) Muscles to contract.
iv) Heartbeat stay regular.

v) move nutrients into cells & waste products out of cells.

vi) A diet rich in potassium helps to offset some of sodium's harmful effects on blood pressure.

vii) Low level of K cause hypokalemia

viii) High level of K cause heart attacks.

Mg —

— vital to both plant & animal life.

— Chlorophyll pigment in plants is a Mg-posphyrin complex.

— All anzymatic reaction in men & animal that are catalyzed by ATP require Mg as a co-factor.

— RNA function, protein synthesis, DNA transcription, oxidative phosphorylation etc. require Mg.

Ca — — Body needs Ca to maintain strong bones.

— Helps muscles to move.

— Helps nerves to carry messages between the brain & every body part.

— Many enzymes require Ca ~~is~~ as acts

as a co-factor.
— Excess of Ca leads to the formation of stones, hardening of arteries & cataracts in the eye.
— Helps in blood clotting

Al — — Activation of anzymes like succinic dehydrogenase.
— May cause "Alzheimer's disease".
— High Al^{3+} along with low Mg^{2+} & Ca^{2+} concentrations induce neurologic disorders.

V — — Required chicks & rats for growth, development of feather.

Cr — — Required as a glucose tolerance factor (GTF) in glucose metabolism
— Required in lipid & protein metabolism.

Mn — — Required in photosynthesis (PS-II)
— In structure formation
— In synthesis of cholesterol, glycoproteins etc.

— Enzyme activators in RNA & DNA polymerases & for most Mg(II)-containing enzymes.

Fe —
- Required in O_2-uptake proteins, eg:- hemoglobin, myoglobin & hemerythrein.
- Required in different e⁻-transport proteins like. R-S protein, cytochromes,
— In storage protein (eg:- ferritin)
— In different oxygenase enzymes.
— Deficiency causes anemia (because red cells of blood containing less hemoglobin than in normal condition.
— Deposition of iron in tissues & organs of the body may cause siderosis.

Co —
— Required in Vitamin B_{12} co-enzyme.
— Deficiency may cause pernicious anaemia.

Ni — — Required in the metallo enzymes ureases (in some plants)

Cu —

— Required in enzymes like cytochrome c oxidase, ascorbic acid oxidase etc.

— In e⁻ transport proteins (eg:- plastocyanin, azurin etc.)

— In O_2 transport proteins (eg:- hemocyanin)

— In storage protein (eg:- ceruloplasmin)

— Deficiency may cause Menke's syndrome.

— Accumulation of Cu can cause Wilson's disease.

Zn —

— Required in structure formation.

— to stabilize the coiled ribosomes.

— In DNA, RNA polymerases, regulatory Zn-finger protein etc.

— Deficiency may cause hair-loss, skin rash etc.

Mo — Required in many oxidoreductase enzymes (eg:- nitrogenase, nitrate reductase xanthan oxidase ~~eg~~ etc.

— Excess Mo can cause Cu-deficiency.

Toxicity —

Toxicity of metals may result from —

i) Blocking the essential biological functional groups of biomolecules such as enzymes.

A toxic metal ion may bind with the active sites of the enzymes & block the activity of the enzyme.

iii) Displacing the essential metal ions from biomolecules. A biomolecule with a foreign metal ion losses its activity.

iii) Modifying the active conformation of biomolecules.

– Biomolecules are having specific active conformations & if this active conformation is lost due to the co-ordination of a metal ion, the activity of the biomolecule is lost.

Toxic metals –

Mercury:–

– Can be toxic by ingestion or ~~inhalation~~ inhalation.

– Can cause "Minamata disease"

– Hg(II) binds strongly with the thiol (SH) group of proteins & enzymes & this binding changes the conformation of protein. Hg is a soft acid & –S of –SH group is a soft base, so strong interaction between Hg & –SH group takes place.

Cadmium (Cd) —

— Leads to nausea, salivation, diarrhea, abdominal pain & vomiting.

— Cd deposition tends to be cumulative in the kidney with lower concentrations in the liver.

— Cd is similar to Zn. Therefore, Cd(II) can displace Zn(II) in many zinc enzymes eg:- Carbonic anhydrase, Carboxy-peptidase etc. Cd(II) effects the active site due to the strong binding.

Lead:— (Pb)

— Affect on brain, peripheral nervous system causing cramps, paralysis etc.

— Also causes anemia.

— Like Hg(II) & Cd(II), lead Pb inhibits SH-enzyme but less strongly.

— Major biochemical effect of Pb is its interference with heme synthesis by inhibiting several of key enzymes involved in the overall process of heme synthesis.

Arsenic (As)—

— Contaminated waters used for drinking, food preparation & irrigation of food crops posses the greatest threat to public health.

— Long time exposes can cause cancer & skin lesions.

— Also can cause cardiovascular disease & diabetes.

— 'As' causes toxicity by combining with thiol (-SH) group present on several enzymes & thereby blocking their action.

— Pentavalent or As (V) can imitate phosphorous & replace it in the backbone of DNA, resulting in conformational changes & strand breakage.

— Pure metallic As is not that poisonous but its salt & oxides are very poisonous.

eg:- White arsenic (As_2O_3)

Na/K-Pump :—

— Active and Passive transport across the membrane —

— Movement of a solute across the membrane from its higher concentration region to its lower concentration region is associated with a

free energy chang (ΔG) negative & this process is called passive transport.

— Movement of a solute species against its concentration gradient is called active transpost where the free energy change change(ΔG) is positive

— Thus passive transpost is thermodynamically favoured and the active-transpost needs to be coupled with anothen thermodynamically favoured process to make the resultant ΔG negative.

↳ Ion transpost —

— The charge transfer is mainly done by H^+, Na^+, K^+, Mg^{2+} and Ca^{2+}. All the required free energy for life process comes from the e⁻ transfer process.

— In our body, it occurs through ionic conduction. The electrical conduction is done by the ions.

↳ Cell membranes (thickness about 70 Å) are composed of double layers of protein separated by lipids. cations cannot pass through the lipid bilayer membranes and these are carried by the specially designed carriers whose outer surface is hydrophobic & lipid soluble.

↳ The ions distributed accross the membrane

in such a way that electroneutrality is maintained on both sides.

↳ Mainly Ca^{2+} & Na^+ are concentrated in the body fluids outside the cell, while Mg^{2+} & K^+ are concentrated inside the cell.

↳ Ionophores —

— It is a chemical species that reversibly binds ions. Many ionophores are lipid soluble & can transport ions across the cell membrane.

eg:- Dianemycin, monensin, valinomycin, nonactin, actinomycin etc.

↳ Valinomycin & nonactin are very much selective towards K^+.

↳ Actinomycin binds Na^+ preferably compared to K^+.

(Valinomycin → Carrier ionophore)

(Gramicidin A → Channel forming ionophores)

↳ Carrier ionophore — This type of ionophores produces metal complexes & escort the metal through hydrophobic environment of the cell membrane.

↳ Channel forming ionophore — This type of ionophore spans the membrane providing a hydrophillic channel through which the cation pass.

The Na/K Pump

— The concentration gradients for Na^+ & K^+ ions are maintained by the $Na^+ - K^+$ pump driven by an integral enzyme, known as $Na^+ - K^+ - ATP$-ase (Mol wt. = 280 kDA)

— The energy is obtained from the hydrolysis of ATP to run the active transport process.

$Na^+ - K^+ - ATPase$ —— ($\alpha_2 \beta_2$ tetramer)

— Contains two α- subunits & two β-sub-units.

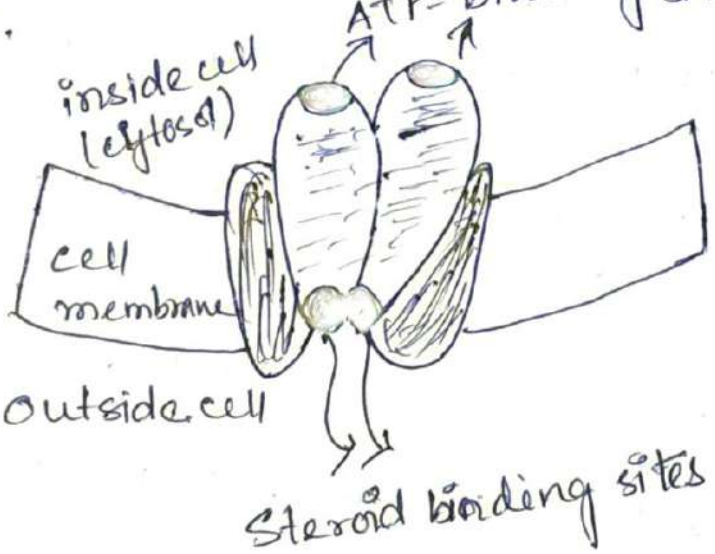


fig:- Schematic representation of the sub-units ($\alpha_2 \beta_2$) of $Na^+ - K^+ - ATP$-ase

— α_2-unit actually acts as the revolving door.

— α-chains contain the selective metal binding sites & phosphorylation sites. And traverse the plasma membrane.

— β-chains mainly contain the carbohydrate.

4) Role of Mg^{2+} — Plays two crucial role
— Catalysis in ATP hydrolysis
— structures forming effect to change the protein conformation.

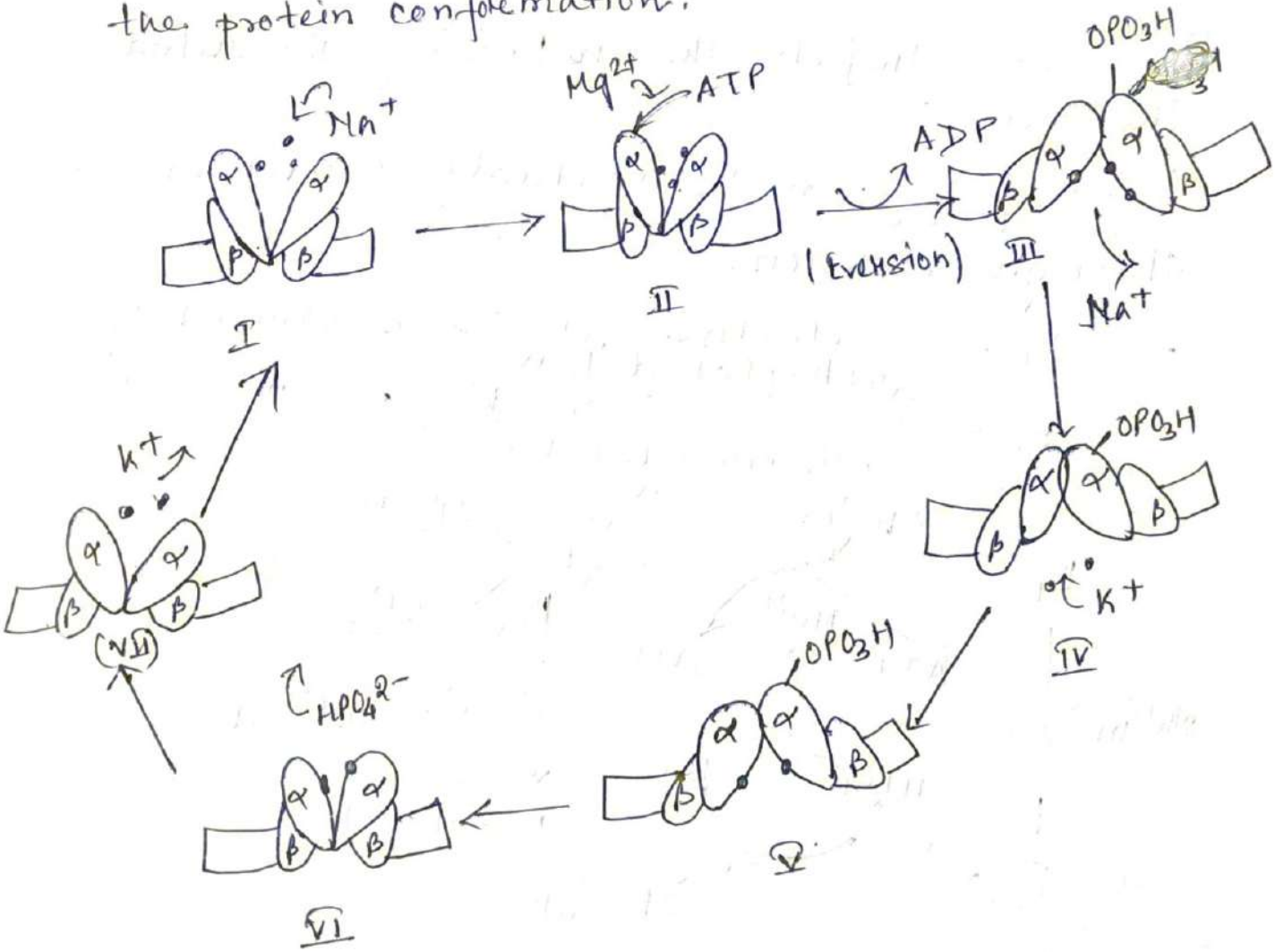


fig:- Schematic representation of the functioning of $Na^+ - K^+$ - pump.

The overall process is —

$$3(Na^+)_{in} + 2(K^+)_{out} + MgATP^{2-} + H_2O \longrightarrow 3(Na^+)_{out} + 2(K^+)_{in} + MgADP^- + HPO_4^{2-} + H^+$$

If we consider —

Two different conformations E_1 & E_2

where —

E_1 = Projects the ion binding sites towards the cytosol site

E_2 = Projects the ion binding sites outside the cell

— E_1 & E_2 can be mutually converted through eversion.

— E_1 → selective for Na^+ & stabilized by dephosphorylation

E_2 → " " K^+ & " "
phosphorylation.

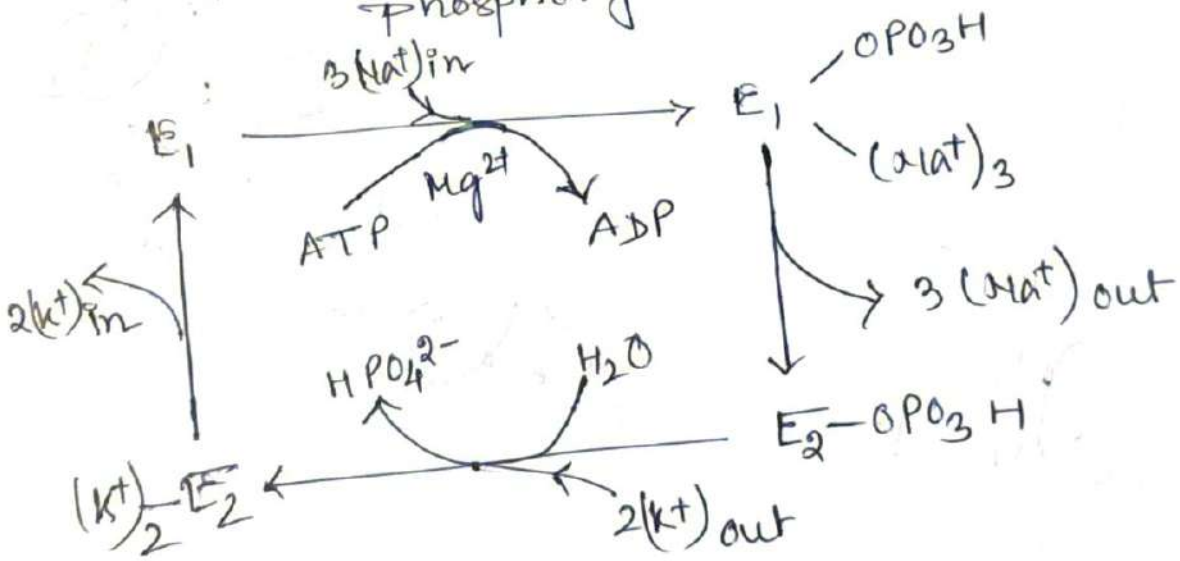


fig:- Schematic representation of the functioning of Na^+–K^+ pump in terms of two different conformations (E_1 & E_2)

↳ Vanadate (VO_4^{3-}) can inhibit the function of Na^+–K^+ pump.

— VO_4^{3-} & PO_4^{3-} ⟶ structurally similar

so, VO_4^{3-} can compete with PO_4^{3-}

Removal of PO_4^{3-} through hydrolysis is possible & this diphosphorylation (from an aspartate moiety) causes an eversion to change the conformation. But, if vanade is bound with the aspartate moiety, then its removal through hydrolysis cannot occur to carry the eversion, & consequently the bound K^+ cannot be released.

→ Selectivity of Na^+–K^+ Pump —

In one conformation(E_1) —

— E_1 selective towards Na^+

In the conformation (E_2) —

— E_2 selective towards K^+

Na^+ → Hard cations ← K^+ → Large in size

- Smaller in size
- Harder than K^+
- Complexing power is greater than K^+
- More strongly hydrated ($-302\ kJ\ mol^{-1}$) → Hydration energy.

(For K^+ — $-230\ kJ\ mol^{-}$

Bases stronger than H_2O will bind with Na^+ preferably.

— Bases slightly weaker than H_2O can also dis-place K^+ from its hydration sphere.

—— In case of Na^+ binding—

Basicity of the ligating sites should be better, otherwise-

ΔH becomes highly +ve

⇓

Due to high hydration energy

⇓

enthalpic disfavour can not be compensated by ~~complexation~~ complexation with a macrocycle.

[$\Delta G = \Delta H - T\Delta S$, $\Delta S = +ve$]

— At the same time, the preference is also decided by the required metal–ligand distance.

(Metal - ligand distance is longer for K^+)

⇓

Macrocyclic cavity size to bind K^+ must be larger than that required to bind Na^+

Carbonic Anhydrase (CA)—

↳ Contain Zn(II)

↳ Catalyses reversible hydration of CO_2 in blood

↳ CA obtained from different sources are found to have the homogeneous str., known as isoenzymes.

$$CO_2 + H_2 \rightleftharpoons HCO_3^- + H^+ \quad (k = 10^{-1}\ s^{-1})$$

(at physiological pH)

$$CO_2 + H_2 \rightleftharpoons HCO_3^- \quad (k = 10^4\ M^{-1} s^{-1})$$

$(pH > 9.5)$

$$CO_2 + H_2O \xrightleftharpoons{CA} HCO_3^- + H^+ \quad (k_{cat.} = 10^8\ s^{-1})$$

(at physiological pH)

↳ turnover no. $= 10^6\ s^{-1}$

(↳ No. of substrate molecules transformed per unit time per each molecule of the enzyme, when the enzyme is fully saturated with the substrate)

↳ CA ~~also~~ also catalyses the hydration of carbonyl compounds and ~~hydration~~ hydrolysis of esters.

Characteristics of Enzymatic Activity —

Prosthetic group – A tightly bound non-peptide (poly) unit required for the biological

function of some proteins.

— With the ~~removal~~ removal Zn^{2+} from CA, the remaining apoenzyme (inactive enzyme) become completly inactive. The activity can be restored by ading Zn^{2+} ion in 1:1 molar ratio to the apoenzyme.

Formation of Zn(II) – OH —

— It offers a bettere nucleophile.

nucleophilicity order —

$OH > H_2O$

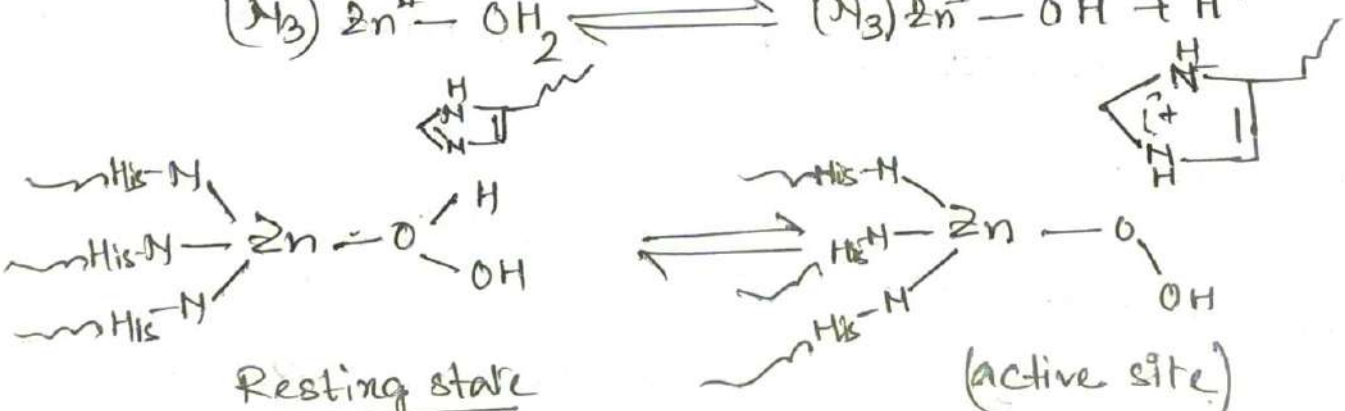
∴ Hydration of CO_2 by OH^- is faster than by H_2O

— But in physiological pH —

— $-OH$ is unavailable

— CA enzyme $(N_3)Zn-OH_2$ genereates the metal bound OH group through depro-tonation —

$$(N_3)Zn^{II}-OH_2 \rightleftharpoons (N_3)Zn^{II}-OH + H^+$$



Resting state (active site)

fig:- Generation of Zn-OH moiety at the active site of CA.

Structure of CA —

— Co-ordinated to 3- Histidine residues via imidazole moieties. (His 94, His 96, His 119) & H_2O molecule OH hydroxide molecule.

— Imidazole (Im) ligands can act as π-acid ligand —

$$M(d) \longrightarrow Im(\pi^*)$$

$$d \longrightarrow \pi^* \text{ (backbonding)}$$

↙ facilitates Lewis acidity of Zn(II) in CA.

— Im moiety present as suitable position facilitates the H^+ transfer to generate active Zn(II) — OH^- centre.

Plausible catalytic cycle of CA —

— The relative bonding power of the zinc ion toward halides is —

$$I^- > Br^- > Cl^- > F^-$$

while for free Zn^{2+} ion —

$$F^- > Cl^- > Br^- > I^-$$

— This indicates that in the enzyme, the apoenzyme softens the Zn(II) centre ~~Due to imidazole moieties of the ligand to the metal centre.~~

→ Due to the presence of imidazole moieties.

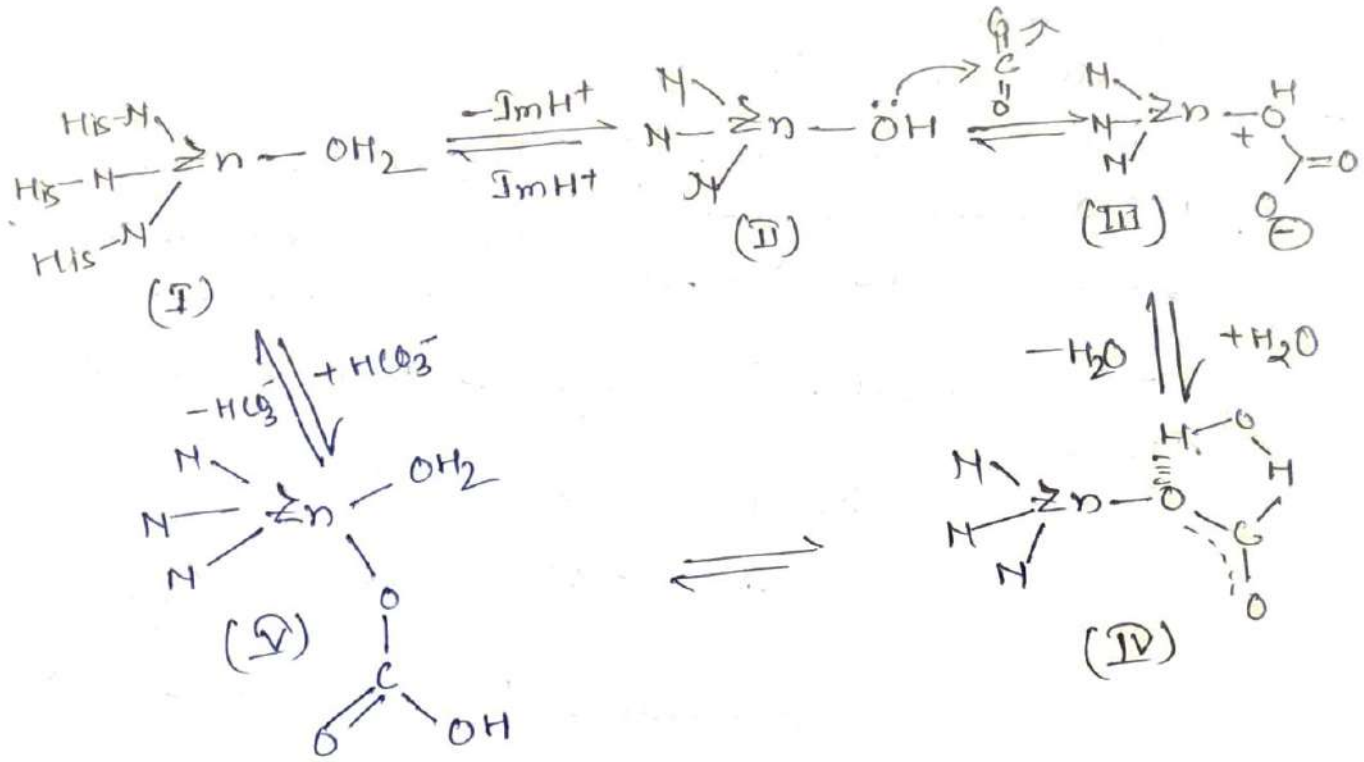


fig: ~~Schematic~~ Enzymatic activity of CA

Inhibition of enzymatic activity—

— Can be inhibited by anions like — $I^{(-)}$, $SH^{(-)}$, $CN^{(-)}$, $N_3^{(-)}$ etc. & neutral substances like — sulfonamides (RSO_2NH_2) & imidazole.

Since Zn(II) is soft ~~base~~ acid (lewis acid) ⟶ So replace of hardere base $^{\oplus}OH$ from Zn(II) with relatively softere bases is quite thermodynamically favourable.

1⟩ Anions like NO_3^-, CNO^- & N_3^- are isoelectronic & isostructural with reactants & products of the enzymatic reaction —

eg:- NO_3^-, CO_3^{2-} & HCO_3^- = 32 e⁻ system

CO_2, CNO^- & N_3^- = 22 e⁻ "

- The attachment of such anions blocks the active site of the enzyme.

— Sulfonamide binds through NH or O to Zn(II) & blocks the catalytic site. It is used in the treatment of glucoma to reduce intraocular pressure (IOL) through the inhibition of CA.

Peptide hydrolysis & peptidases —

$$R-CO-NH-R' + H_2O \rightleftharpoons RCO_2^{(-)} + R'NH_3^{(+)}$$

- Hydrolysis of Peptide catalyzed by carboxypeptidase or thermolycin.

i) Polarization of the C=O bond through Metal-oxygen co-ordination (Lewis acid character of the metal centre)

ii) Generation of metal-hydroxo (M–OH) species at the biological pH.

Acts as a powerful nucleophile

↳ Peptidases are the biological catalysts involved in degradation of proteins into its primary constituents

↓

Amino acids

— Breaks the peptide linkage

— Contain Zn(II) in the active sites

↳ Depending on the position of peptide linkage to be attacked, the peptidases are classified ——————

1) Endopeptidases

↓

Catalyse the hydrolysis of non-terminal peptide bonds

2) Exopeptidases

↓

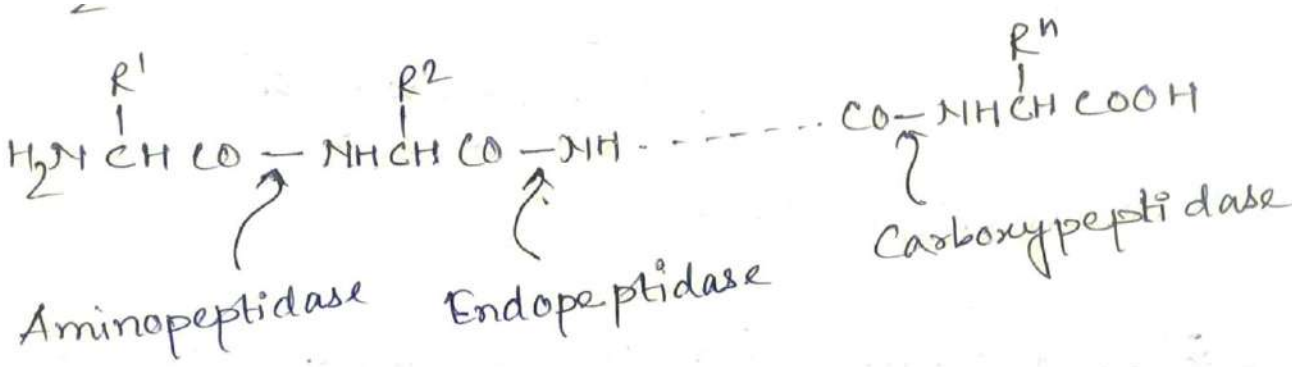
Catalyse the hydrolysis of terminal peptide bonds.

a) Carboxypeptidase

→ hydrolysis of C-terminal peptide bond

b) Aminopeptidase

→ hydrolysis of N-terminal peptide bond.



Carboxypeptidase —

→ These enzymes are generally activated by metal ions like Zn(II), Mn(II), Co(II) etc.

eg:- Carboxypeptidase A & Carboxypeptidase B } Zn(II)

~~Carboxypeptidase C~~ →

Carboxypeptidase A — (CPA)

→ Pancreatic enzyme (helps in the digestion of protein)

→ Specific to hydrolysis of teremnal peptide linkage at carboxy end.

↓

Preferably towards the side chain of the teremnal residue contains some aromatic moiety or branched aliphatic ~~moiety~~ chain with L-configuration.

→ Can also show esterase activity
⇓
ester hydrolysis.

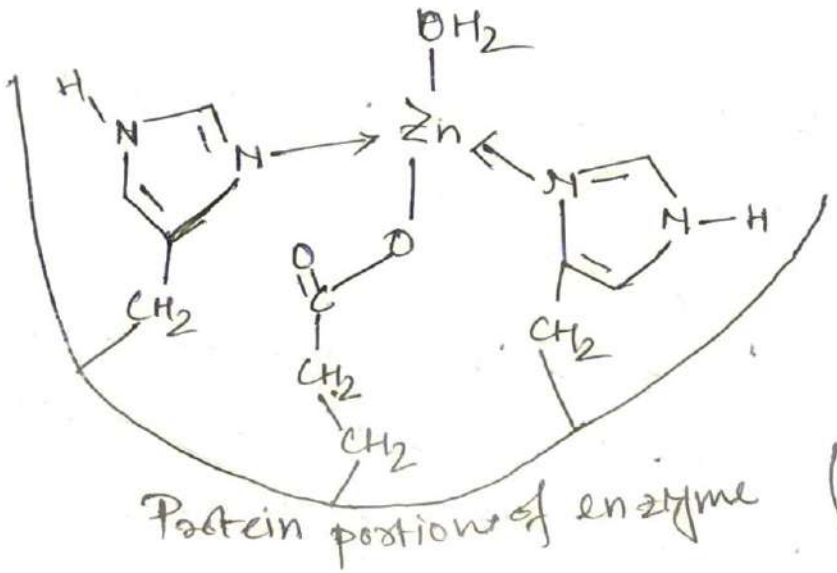
Structures of CPA —

↳ The protein chain or prosthetic group of the enzyme chain bears about 307 amino acid residues & one Zn^{2+} ion

Mol. wt. ≅ 34,600

↳ Roughly egg-shaped & active site situated in cleft in the protein structure.

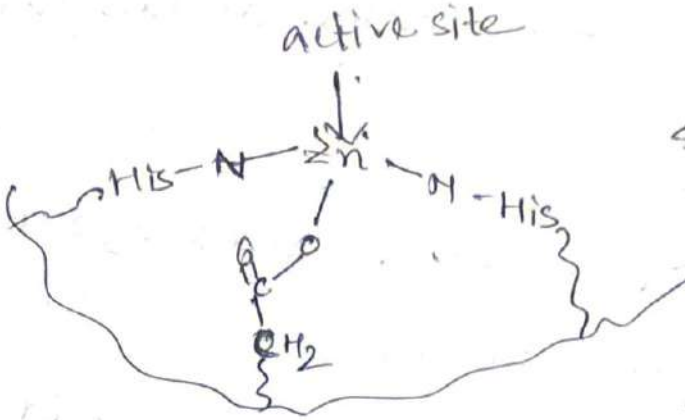
↳ Zn(II) Co-ordinated approximately tetrahedrally to two N-sites (His-69, His-196), one carboxylate oxygen of the glutamate (Glu-72) & a water molecule.



Resting State

(Distorted Td)

1→ Active site —



4th co-ordination site is free to accept a pair of e-s from a donor atom in the substrate to be cleaved.

Active state

In the active site —

Three amino acid residues —

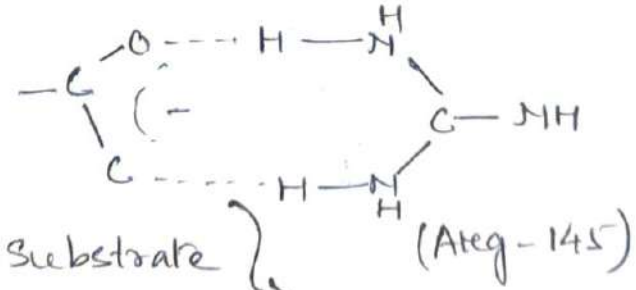
i) Protonated guanidyl moiety of Arg-145

ii) Phenolic OH of Tyr-248

iii) Carboxylate end of Glu-270 are present & play some important roles.

Role of Arg-145 —

— Terminal carboxyl group of substrate forms a salt-bridge with protonated Arg-145



i) It keeps a substrate in proper position & orientation required for hydrolysis & helps to recognise the substrate.

ii) Helps the rupture of the N-C bond of peptide linkage

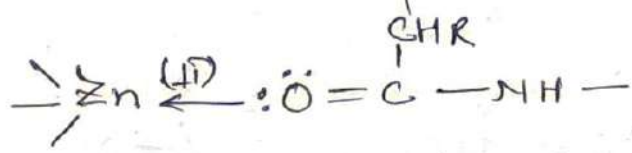
This is why, the enzyme is specific for the terminal peptide linkage at the carbonyl end.

Role of Tyr-248 —

The carbonyl oxygen replaces H_2O molecule at the active site of Zn(II)

↓

Increases the Lewis acidic character of Zn(II) & polarizes the 'C=O' bond & develop a carbocationic character on the carbonyl carbon centre.



This cationic character is enhanced by hydrogen bonding interaction between the -NH gp (of the peptide linkage) and the phenolic -OH gp of Tyr-248. This helps cleavage of N-C bond.

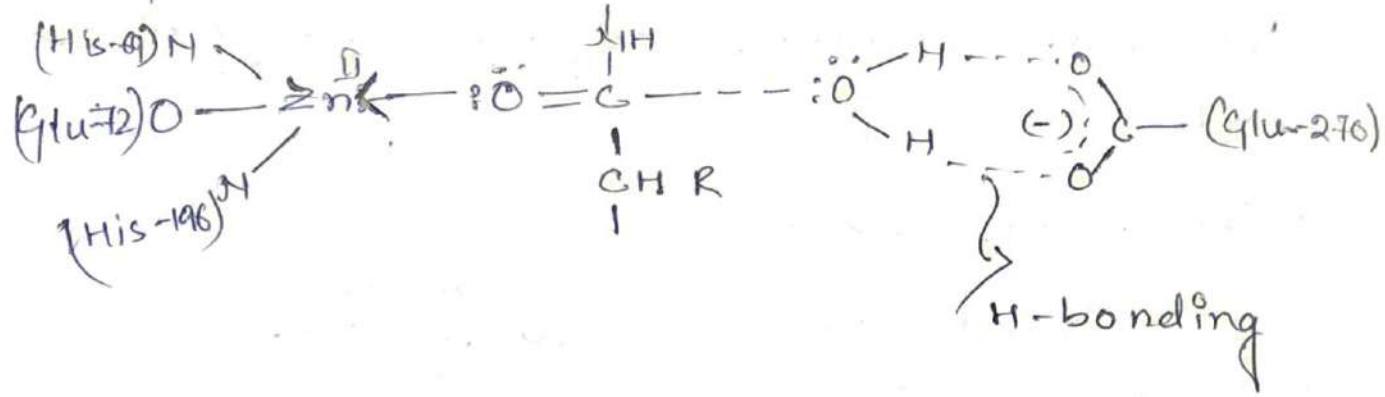
↓

nucleophilic attack is facilitated due to +ve charge on the carbonyl carbon centre.

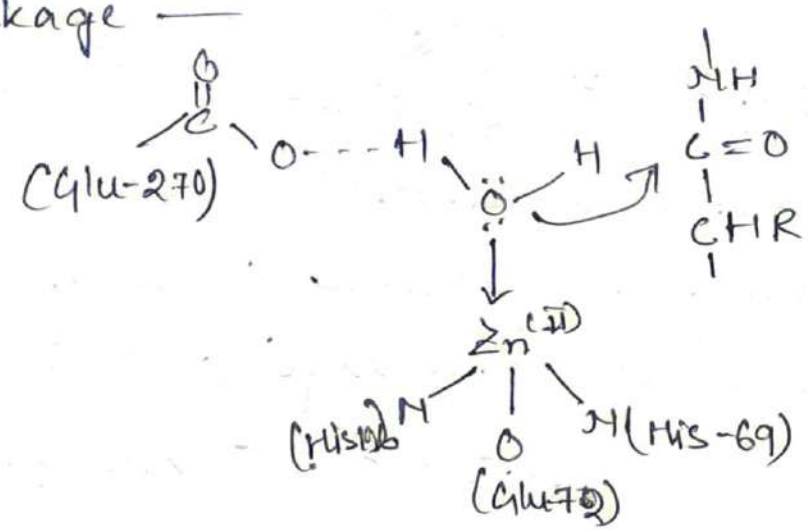
Role of Glu-270 —

— keeps the nucleophile (i.e., H_2O) in a proper position through H-bonding.

— Helps the attack on the carbonyl carbon



— The carboxylate group may interact with H_2O molecule bound with Zn (II) to generate metal bound hydroxide group which is a powerful nucleophile to attack the peptide linkage —



— Carboxylate group of Glu-270 can itself act as a good nucleophile & produce an acid anhydride.

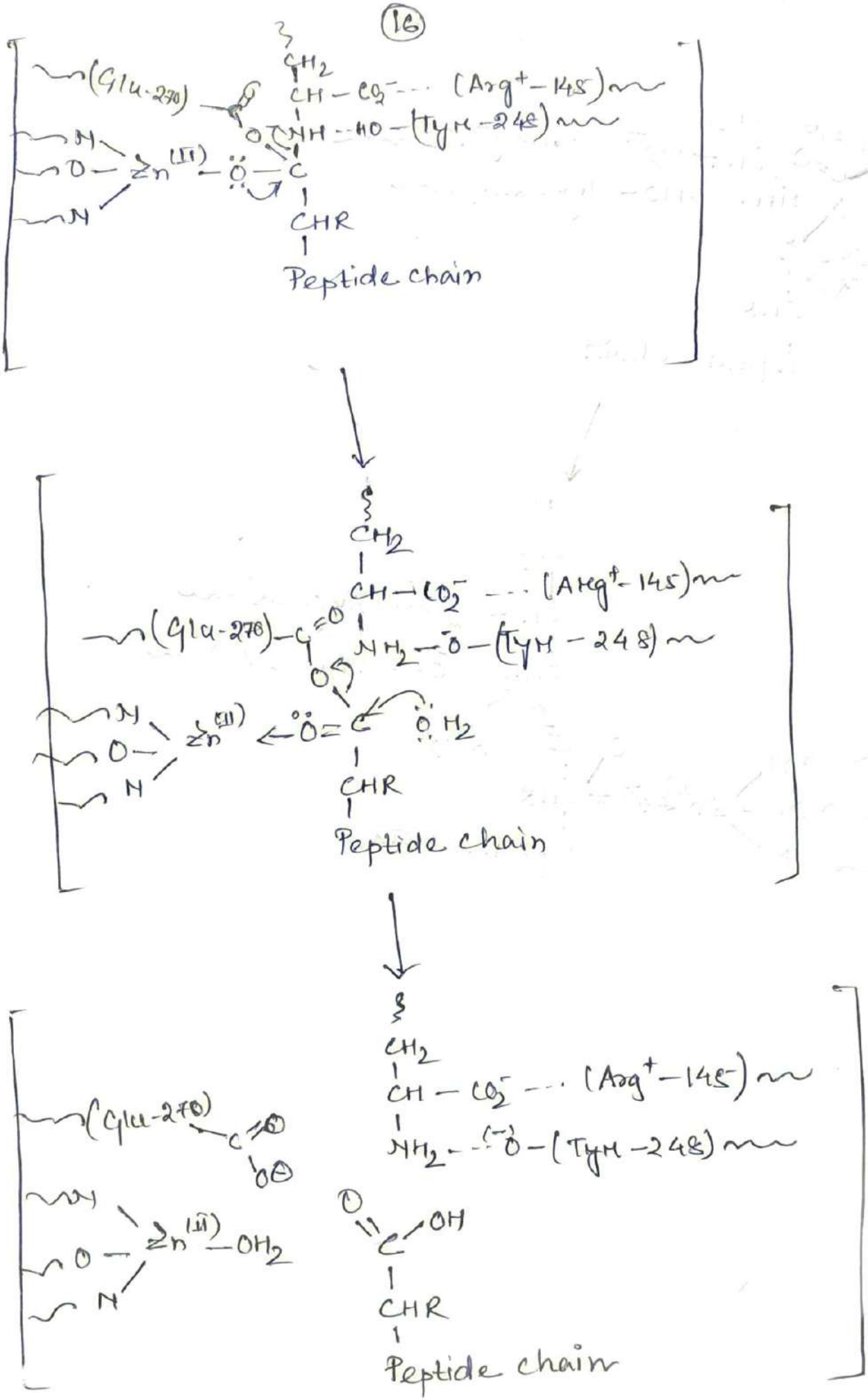


Fig:- Enzymatic activity of CPA.

Iron & its application in bio-system —

↳ It is an essential element for blood-production. About 70% of the body's iron is found in red blood cells & responsible for O_2-storage & transport eg:- Hemoglobin, Myoglobin etc.

↳ About 6% of body iron is a component of certain proteins, which are responsible for respiration & energy metabolism, eg:- cytochromes, Fe-S proteins etc.

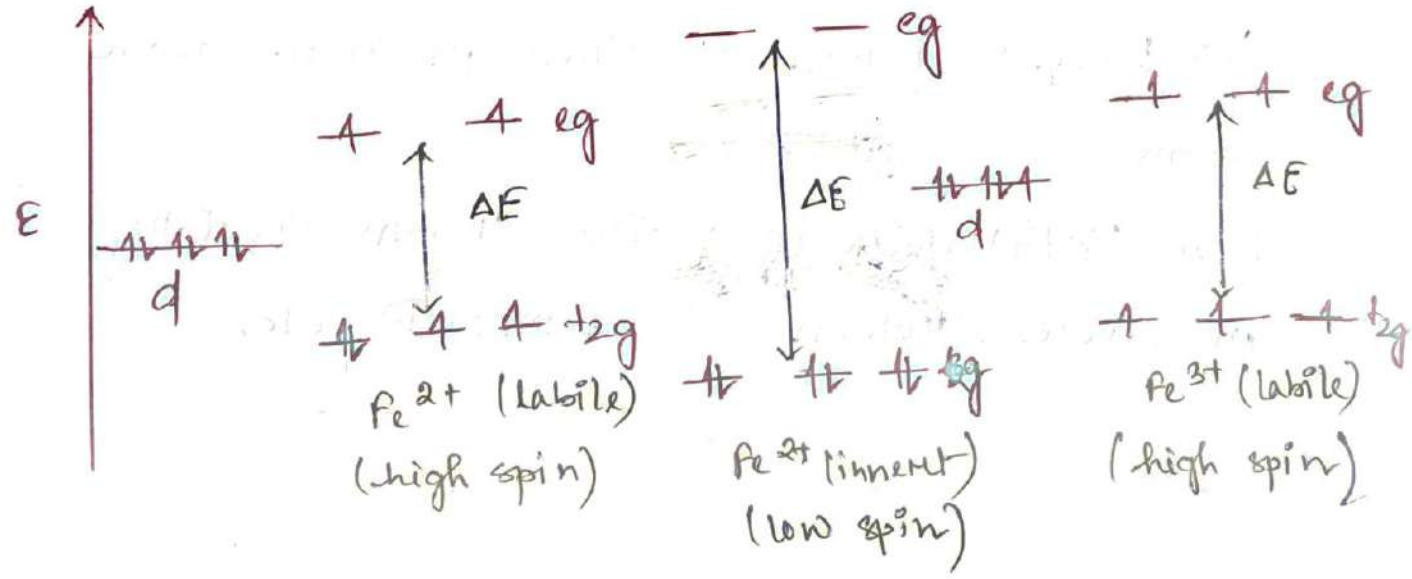
↳ About 25% of iron in the body is stored as ferritin, found in cells & circulates in the blood.

↳ i) Fe(II) & Fe(III) → oxidation states

↳ readily interconvertible

Both Fe(II) & Fe(III) high spin states are quite labile & their complexation occurs rapidly.

$Fe^{2+} = 3d^6$ $Fe^{3+} = 3d^5$



ii) Depending on the environment, Fe(II)/Fe(III) complexes can adopt both octahedral & tetrahedral complexes.

↳ Because of these properties, iron finds uses in —

O_2 - transport & storage system —

eg:- Hemoglobin, myoglobin etc.

e^- transport system —

eg:- cytochromes, ferredoxins etc.

Redox - metallo-enzymes —

eg:- Hydrogenase, reductases, nitrogenases etc.

In some basic biological reactions —

eg:- ribonucleotide reduction (DNA synthesis, energy production (respiration) solar energy conversion (photosynthesis) etc.

Storage of iron — (Ferritin, Hemosiderin)

Ferritin —

↳ Excess of iron is stored in non-toxic forms.

↳ Distributed in various organs specially in liver, spleen, bone-marrow etc.

Structure of Ferritin:-

↳ It consists of a core of ferric hydroxy phosphate surrounded by a protein sheath called apoferritin.

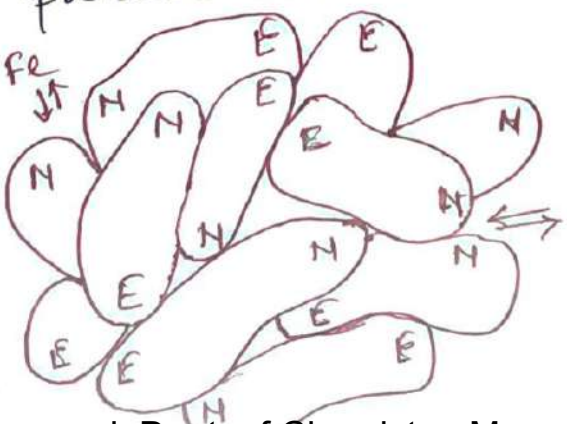
↳ The lipophillic sheath makes the Fe(III)-complex soluble in biological fluid.

↳ Ferritin can be considered as a micelle.

↳ Ferritin contains about 12-20% of iron (i.e., 2000 - 4000 Fe atoms per molecule)

In ferritin core —

$Fe_2O_3(H_2O)_x$ is present with various amounts of phosphate. This ferric hydroxyphosphate can be compared as minerals & the formation of ferritin core is also known as biomineralisation process (formation of minerals by organisms). The soluble Fe(III)-centres can reversibly bind with the insoluble biominerals inside the protein sheath.



Where —
N = polar N-terminal end
E = nonpolar helical segment

Fig: Arrangement of protein subunits to produce the protein sheath of ferritin

Structure of Ferritin

- The protein coat (sheath)
- iron-protein interface
- iron core (contains Fe(III))

↳ Apoferritin sheath consists of 24 subunits of coiled polypeptide chain.

Each subunit (Mol. wt. = 20,000 Daltons) → cylindrical shaped
→ two ends are different

→ Arranged in outer surface (H & E)

→ So ferritin micelle looks like spherical

↳ Core consists of a sheet str. of Fe(III)-oxide.

→ The hydroxide & phosphate groups present helps to balance the charge & binding at the protein surface.

→ Fe is octahedrally (surrounded by oxygen)

Function of Ferritin —

↳ It stores iron.

↳ When it is required, releases iron to transferrin for biochemical purposes.

// achived

Iron release ~~achied~~ by reduction of Fe(III) to Fe(II) & ote by chelating ligand.

//

24 Subunits of ferritin arranged in such a way that —

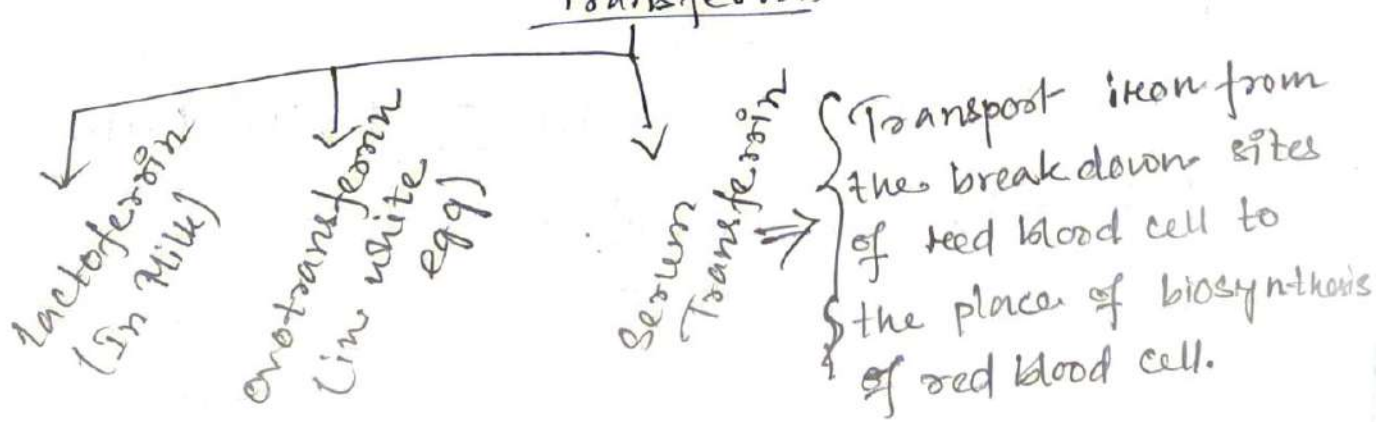
3-subunits meet with their N-ends to form a polar channel through which iron can be ~~transfered~~ in & out.

Transfer of iron: — (Transferrin)

Transferrin —

↳ Carriers of iron.

Depending upon the distribution of this protein can be divided —



Structure of Transferrin —

↳ Mol. wt. = 8×10^4 Daltons

↳ In transferrin Fe(III) is octahedrally co-ordinated in which CO_3^{2-} or HCO_3^- remains co-ordinated as a synergistic anion ligand.

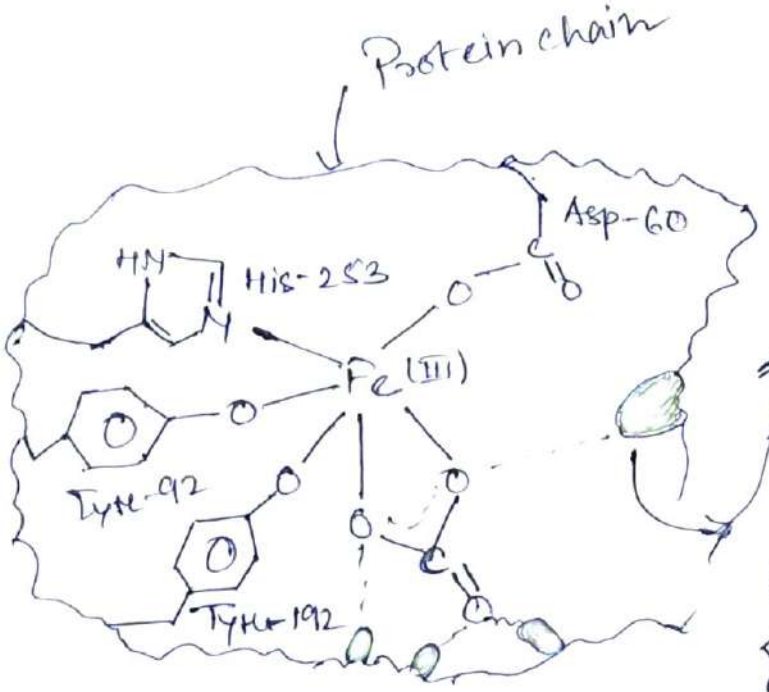


Fig:- Co-ordination sphere of Fe(III) in transferrin & role of synergic anion CO_3^{2-}.

Role of CO_3^{2-} or HCO_3^- —

↳ plays a crucial role for binding of Fe(III) with the apotransferrin.

↳ without CO_3^{2-} or HCO_3^-, Fe(III) fails to retain as Fe(III).

↳ Forms bridges betn Fe(III) & cationic sites of encircling protein

⇓

Minimized the electrostatic repulsion betn Fe (III) & the cationic sites of the protein chain.

↳ Also forms H-bonding with amino-acid residues of protein

⇓

helps to fold the protein chain to facilitate the interaction between the Fe(III) centre & co-ordinating sites coming from the protein chain.

Stability of chelate —

At pH = 7, Fe (III) - chelate is stable

⇙

No dissociation of iron from chelate occurs.

At pH < 7 or pH ≃ 5, iron readily dissociates from the chelate.

⇓

Due to acid catalyzed dissociation of CO_3^{2-} or HCO_3^-.

↳ At lower pH, the $Fe(III)$ – chalate becomes unstable

⇓

The reduction of $Fe(III)$ to $Fe(II)$ facilitates the release of iron from transferrin.

⇓

Since the binding sites in apoprotein are hard bases & prefer $Fe(III)$ to $Fe(II)$.

↳ The uptake of iron by transferrin needs oxidation of $Fe(II)$ by O_2 to $Fe(III)$, this process is catalyzed by Cu-containing protein, ceruloplasmin.

⇓

When iron passes from stomach (acidic range to blood (pH = 7·4), this oxidation occurs favourably.

⇓

Participation of ceruloplasmin in the path of iron metabolism explaines how copper deficiency causes anemia.

↙

Due to impaired function of ~~the~~ cerulo-plasmin, in spite of abundant storage of ion, iron metabolism is constrained.

Mechanism of Transferrin —

Apotransferrin binds Fe(III) very tightly so that other bioligands cannot successfully compete with apotransferrin to snatch the iron.

↳ Iron release from transferrin at the site of requirement (to reticulocyte i.e, immature red blood cells in the bone marrow), Fe(III) is reduced to Fe(II). But, E_0 of transferrin = -0.5 V)

↳ reduction pot.

↳ can't be achieved by biological reducing agents.

Since CO_3^{2-} or HCO_3^- bound in transferrin stabilizes the apotransferrin-Fe(III) interaction.

↳ A slight fall in pH can drastically diminish this interaction, as removal of CO_3^{2-} or HCO_3^- is high acid catalyzed.

Transferrin releases iron by binding to the cell surface & forming a vesicle inside

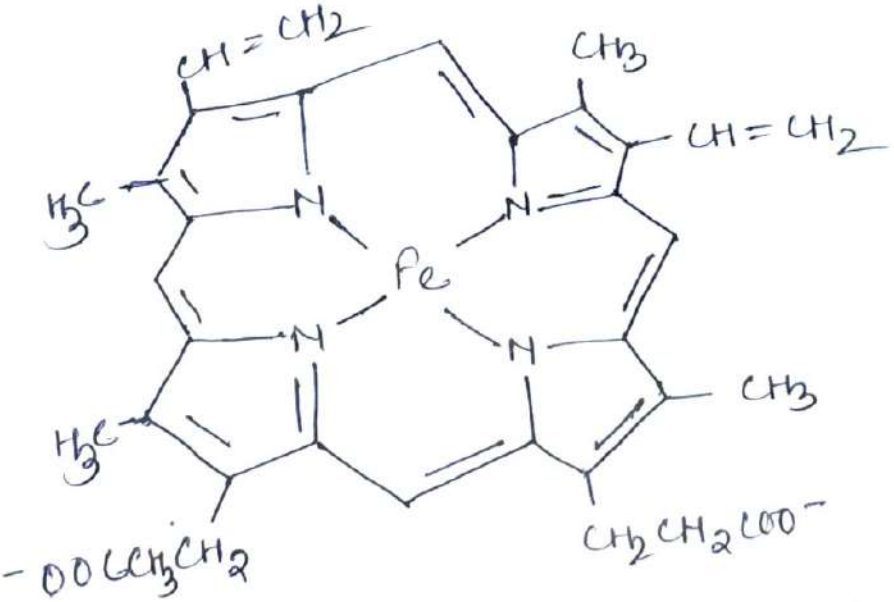
the cell (where pH is relatively low). After releasing iron it again comes back to plasma to capture iron.

↳ About 30 mg of Fe is required per day for the biosynthesis of red blood cells in an adult ⟶ Transferrin is solely responsible for carrying & recycling this 30 mg of Fe.

Hemoglobin (Hb) And Myoglobin (Mb) —

↳ Heme is the prosthetic group of Hb & Mb

↳ Fe-porphyrin ring.



Fe(II) - porphyrin complex (heme-b)

↳ Transport & storage of O_2

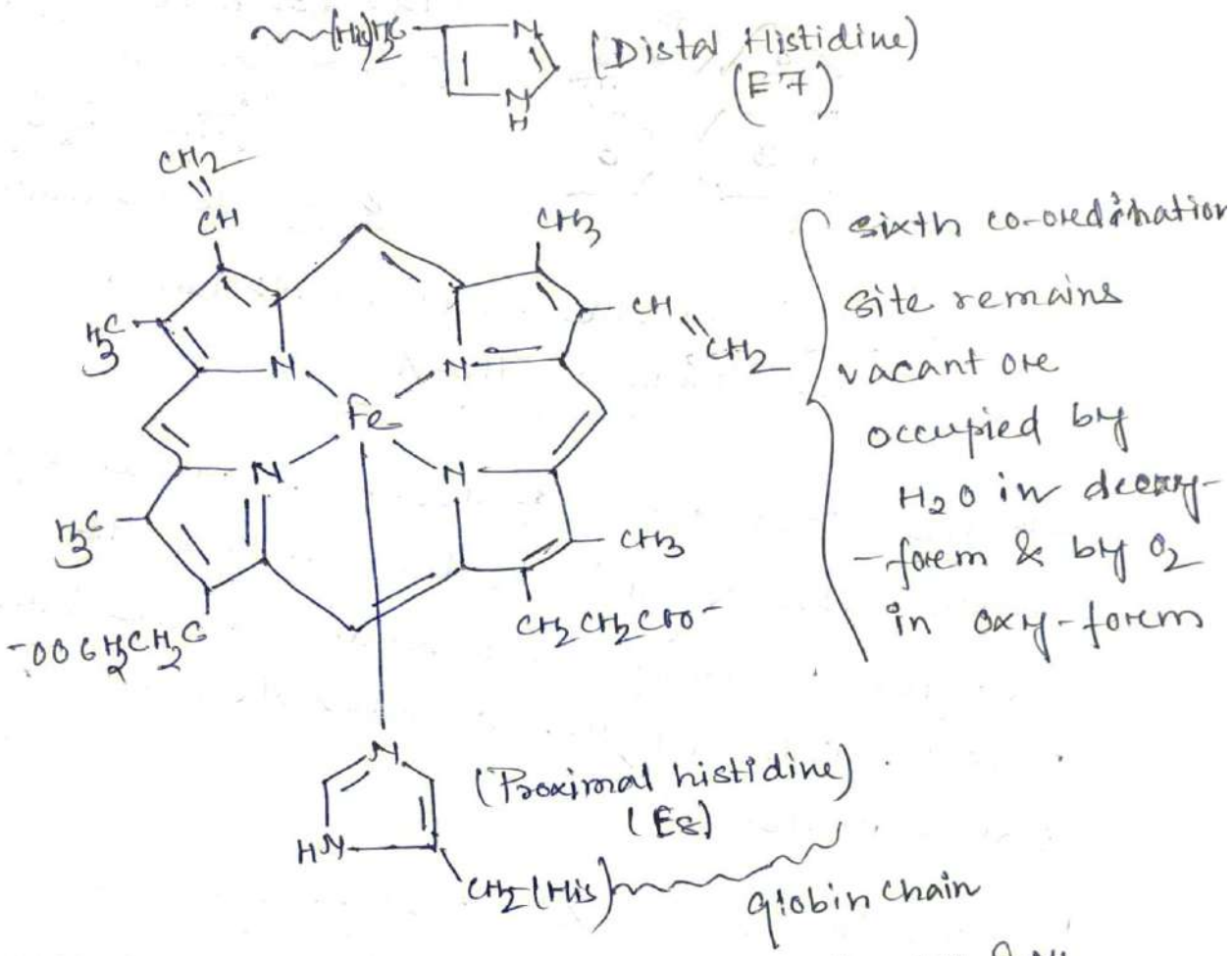


fig: Structure of a heme unit in Hb & Mb.

↳ A heme unit including the globin protein chain is called Mb (Mol. wt. = 16,000 Daltons)

↳ Hb (Mol. wt. = 64,000 Daltons) is a tetramer of myoglobinic subunits.

Hb-A ($\alpha_2\beta_2$) ← 4 units are similar but not identical. (2 = β units & 2 = α units)

In case of adult

The protein chains bear $-COO^-$ & $-NH_2^+$ gps & the chains are coiled to bring about salt-

-bridge - interactions (i.e., $-CO_2^- \cdots {}^+H_3N-$)

DPG = 2,3-diphosphoglycerate

⊡ → salt-bridge ($\overset{\wedge}{}NH^+ \cdots {}^-O_2C$)

● → Heme unit

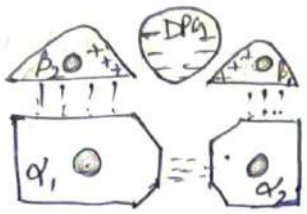


fig:- schematic representation of tetrameric Hb-A

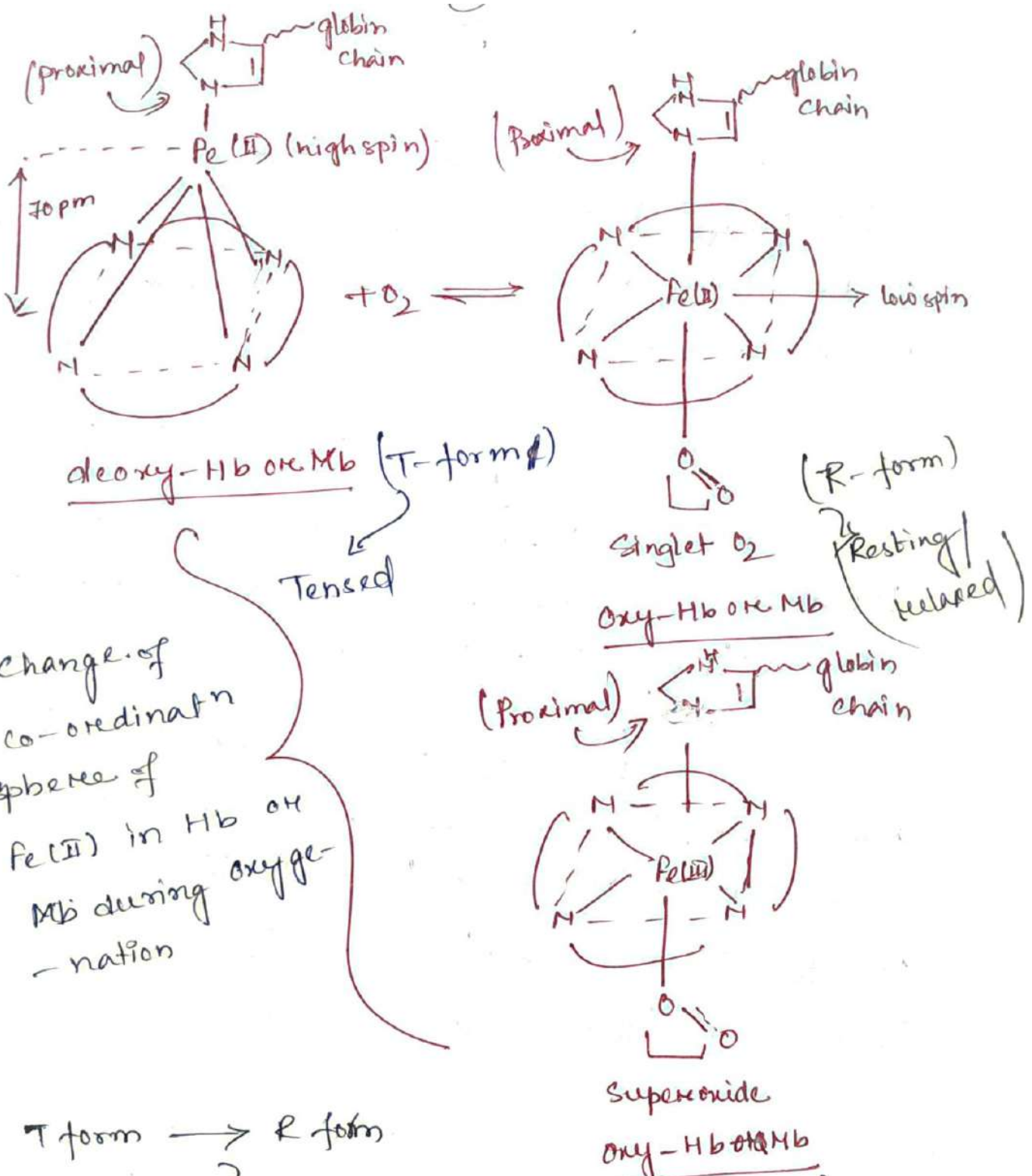
↳ Hb - transports O_2 from its source (eg:- lungs, skin & gills) to the site of its biological use (eg= respiration) inside the muscle cells where O_2 is transferred & stored in Mb.

↳ Due to $\pi \longrightarrow \pi^*$ charge transfer electronic transitions give the red colour of blood.

↳ described generally as Soret band.

An intense ($\pi \rightarrow \pi^*$) absorption band in the blue region of the optical absorption spectrum of a heme protein (eg:- Hb, Mb etc.) is called Soret band.

Appears near UV-region.



Due to oxygenation change of spin state occurs (high spin Fe(II) $(t_{2g}^4 e^2)$ to low spin Fe(II) $(t_{2g}^6 e_g^0)$ → the radius of Fe(II) decreases by 17 pm & hence Fe(II) in oxy-hemoglobin or myoglobin can sit in the porphyrin cavity.

Role of Distal & Proximal Histidine —

↳ Proximal histidine binds to the fifth co-ordination site of heme unit via its imidazole moiety.

↳ acts as a good σ-donor

↓

Fe-ion acts as a better π-donor towards the π-acid ligand O_2 at the trans-position (i.e., sixth position)

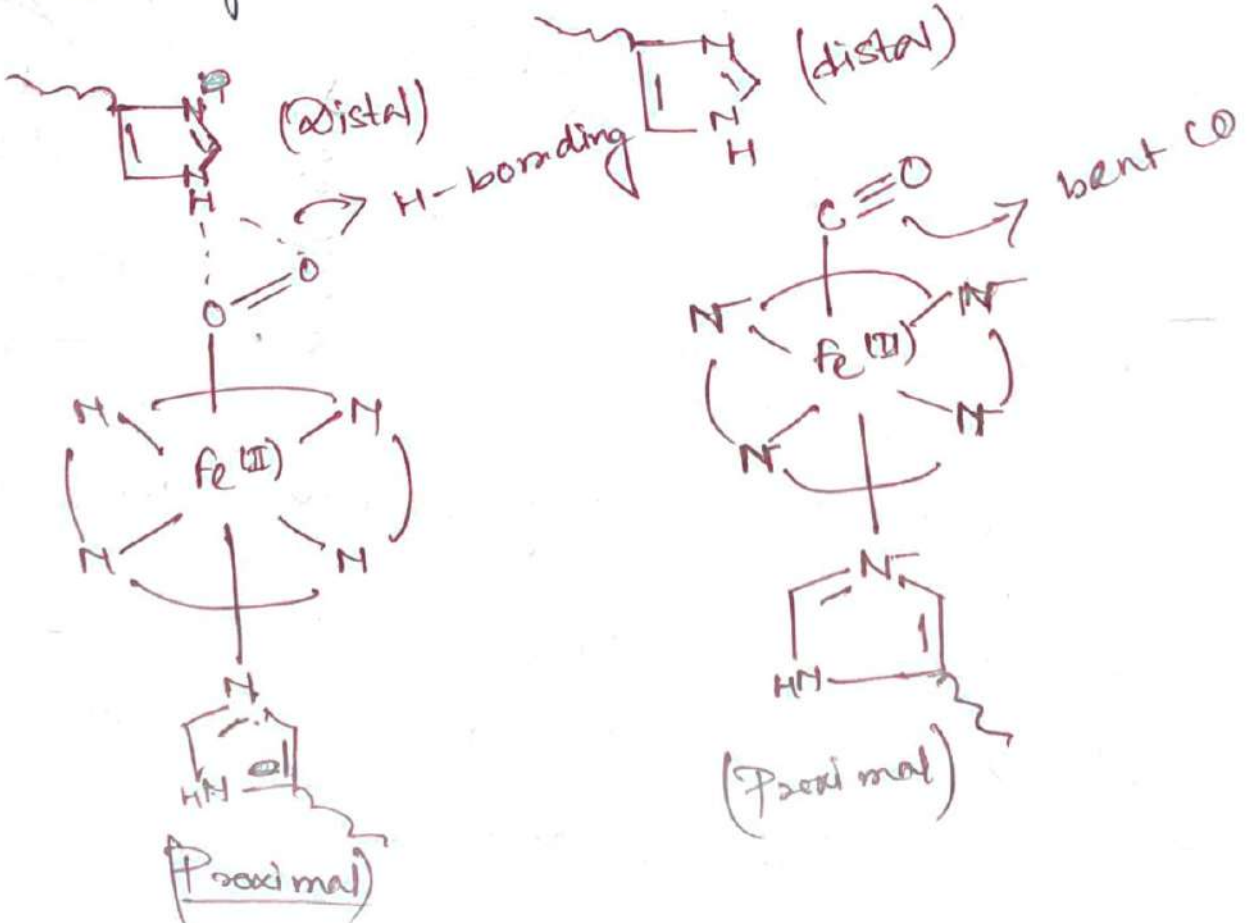
↳ helps O_2 to act as a better π-acid ligand (π-acceptance) to induce the spin pairing at iron (i.e., Fe(III) high spin to Fe(II) low spin. (Since, O_2 = strong field ligand / low spin ligand)

↳ Distal histidine residue resides in the region of 6th co-ordination site, but doesnot co-ordinate with iron in both oxy- & deoxy forms.

CO is very powerful poison to Mb & Hb, as the heme group has a very high affinity for the π-acid ligand like

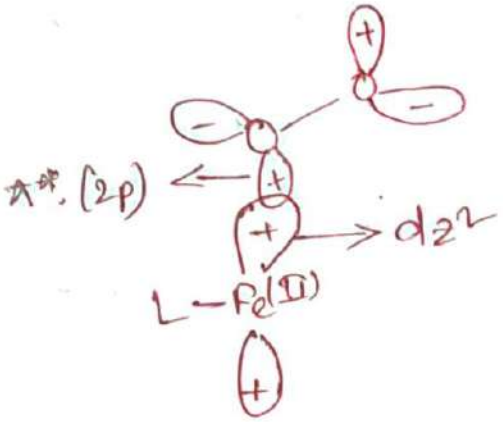
CO_2, O_2 etc., Because of the presence of distal histidine residue in the region of sixth co-ordinatn site, it does not allow CO to form linear Fe—C≡O bond & CO is forced to make a bent bond.

The affinity of CO in Hb & Mb is drastically diminished because of this weak bent bond formation. Thus the distal potein weakens the interaction with CO & optimises the binding of O_2 in Hb & Mb. Also the imidazole moiety of distal-histidine stabilises the oxygenated compound through H-bonding ——



Nature of Heme–dioxygen bonding –

↳ During oxygenation, O_2 makes a bent bond with the metal centre.



σ- bond = d_{z^2}, π*

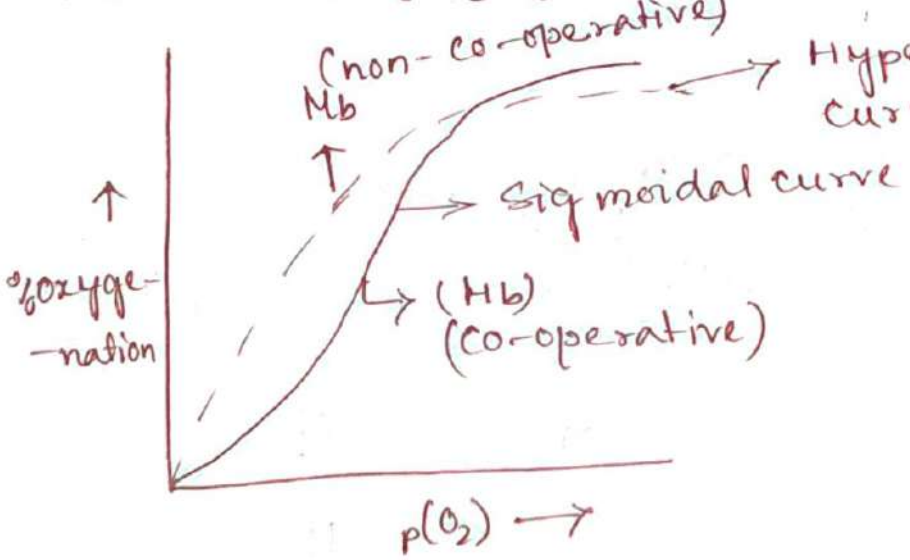
π - bond = d_{yz}, π*

M (d_{yz}) $\xrightarrow{e^-}$ O_2 (π*) (back bonding)

. In oxy-Hb, O_2 binds as bent superoxo complex of Fe[III] with Fe–O–O moiety

Supports — Fe(III) – O_2^- (Model)

Function of Hb & Mb —



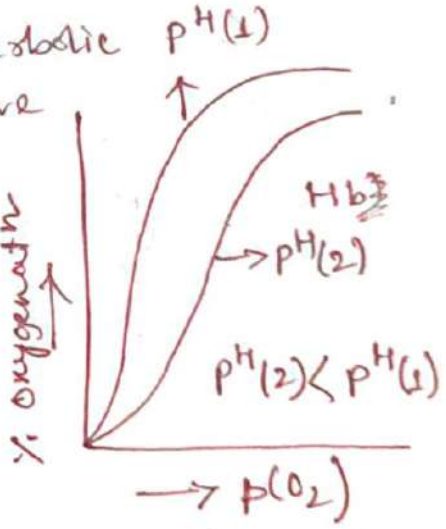


Fig: Effect of pH on oxygenation. (Bohr effect)

If P_{50} = Partial pressure [$p(O_2)$] of O_2 at which 50% of oxygenation is attained

Then, for Mb, $P_{50} \sim 1$ Torr

for Hb, $P_{50} \sim 26$ Torr

In case of Mb —
Mb has only one O_2-binding site —

$$Mb + O_2 \rightleftharpoons MbO_2, \quad K_M = \frac{[MbO_2]}{[Mb]\,[p(O_2)]} \longrightarrow (1)$$

(K_M: equilibrium const.)

If f_M = fraction of total Mb bearing O_2

Then, $$f_M = \frac{[MbO_2]}{[Mb] + [MbO_2]} \longrightarrow (2)$$

~~$K_M = f_M / [\,]$~~

$$\therefore K_M = \frac{f_M}{\{(1 - f_M)\}\{p(O_2)\}} = \frac{1}{P_{50}}$$

Again from eqn (2) —

$$f_M = \frac{K_M\, p(O_2)}{[1 + K_M\, p(O_2)]}$$ [Substituting the value of $[MbO_2]$ from eqn (1)] $\longrightarrow (3)$

At value of $f_M = 0.5$ (i.e., 50% of total Mb is oxygenated), the corresponding $p(O_2)$ is denoted by P_{50} & it leads to —

$$1/K_M = P_{50}$$

∴ Hill eqn —

$$\log\left(\frac{f_M}{1 - f_M}\right) = \log\{p(O_2)\} + \log K_M$$
$$= \log\{p(O_2)\} - \log(P_{50}) \longrightarrow (4)$$

In case of Hb ——

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

$$K_H = \frac{[Hb(O_2)n]}{[Hb]\{P(O_2)\}^n} = \frac{f_H}{(1-f_H)\{P(O_2)\}^n} = \frac{1}{(P_{50})^n}$$

$$f_H = \frac{K_H\{P(O_2)\}^n}{\left[1 + K_H\{P(O_2)\}^n\right]}$$

∴ Hill eqn ——

$$\log\left(\frac{f_H}{1-f_H}\right) = n \log\{P(O_2)\} + \log K_H$$

$$= n \log\{P(O_2)\} - n \log(P_{50}) \rightarrow (5)$$

For, Hb, the exponent n (= 2·8) ⇒ Hill co-efficient.

For Mb, $n = 1$

∵ For Hb, $n > 1$, ⇒ O_2 - binding in the subunits of Hb is interdependent & it suggests +ve co-operativity among the heme units due to heme - heme interaction.

Co-operativity ⟶ The binding of O_2 molecule with one heme facilitates the binding of additional O_2 molecules to the other heme sites. (homotropic

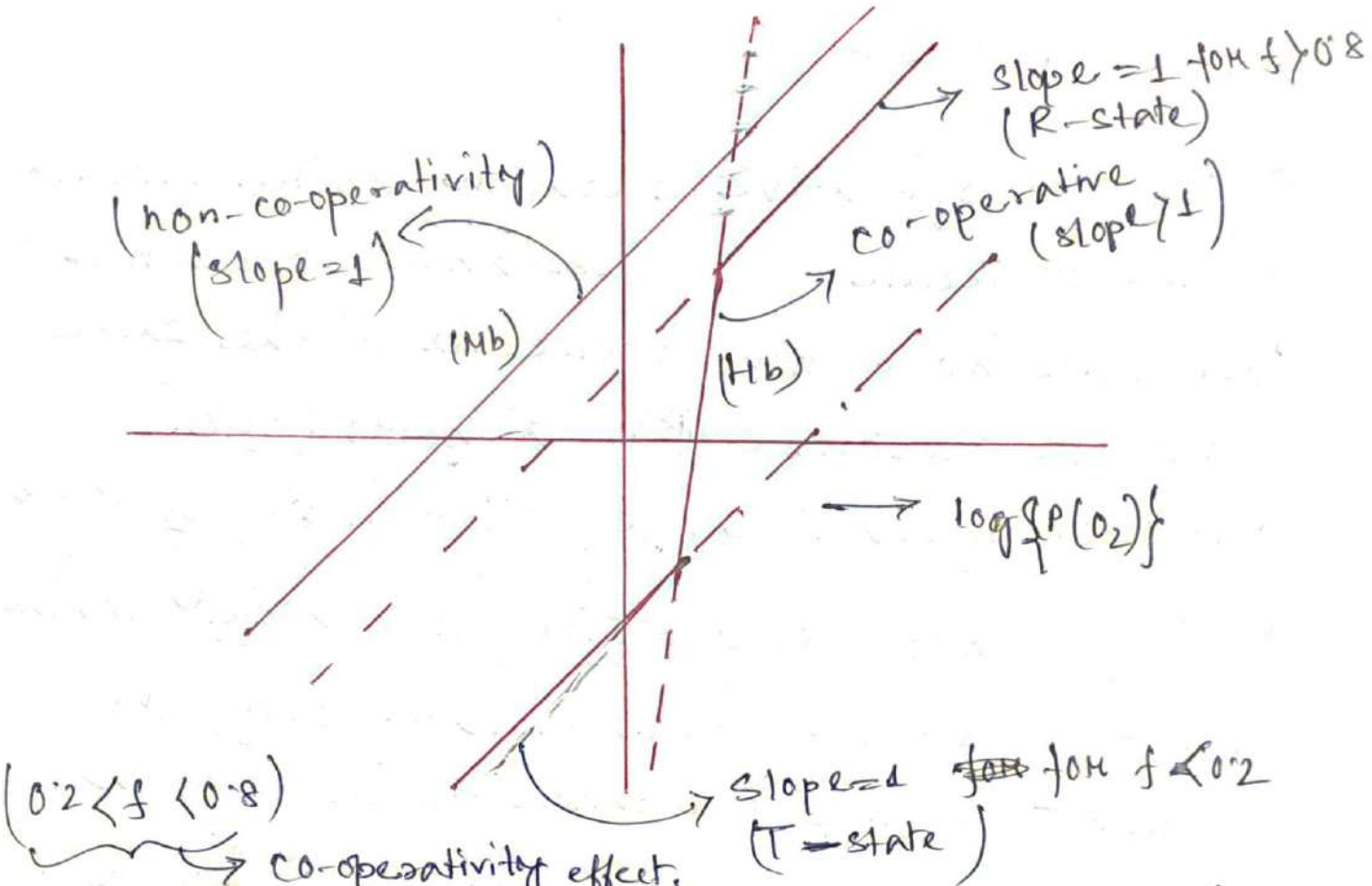


fig:- Hill plot (effect of co-operativity in oxygenation of Mb & Hb)

$$Hb + O_2 \rightleftharpoons Hb(O_2), \quad k_1(H)$$

$$Hb(O_2) + O_2 \rightleftharpoons Hb(O_2)_2, \quad k_2(H)$$

$$Hb(O_2)_2 + O_2 \rightleftharpoons Hb(O_2)_3, \quad k_3(H)$$

$$Hb(O_2)_4 + O_2 \rightleftharpoons Hb(O_3)_4, \quad k_4(H)$$

Due to +ve co-operativity ——

$$K_1(H) < K_2(H) < K_3(H) < K_4(H)$$

[K = eqm or O_2 binding const.]

allosteric interation)

* The co-operative interation where binding of one molecule of a substance influences the binding of next molecules of the same kind is described as a homotropic allosteric interaction. eg:- binding of O_2 in Hb.

* A heterotropic allosteric interaction involves the co-operative interaction among the different types of substances binding with the target protein. eg:- effect of H^+, CO_2, & cl⁻ on binding of O_2 with Hb.

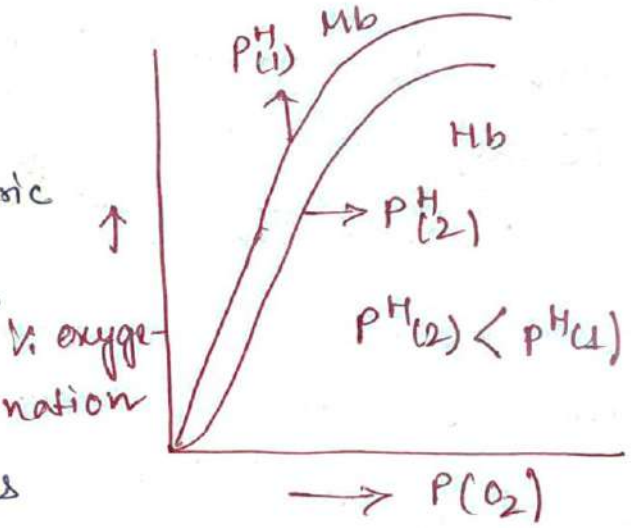
↳ All these allosteric interactions are absent in Mb.

↳ When Hb releases the O_2 to muscle tissues, Mb picks it up & keeps it until it is required. In lungs, where $p(O_2)$ is high, binding efficiency & affinity of Hb equalize with Mb. But in muscle where $p(O_2)$ is low, Mb shows better efficiency & affinity to bind O_2.

So, Mb acts as storage of O_2 & Hb transfers O_2.

Bohr effect — The removal of O_2 is favoured by pH change. The decreased in pH favours the release of O_2 from Hb. This effect is called as Bohr effect. Due to co-operative effect, as one O_2 get removed from Hb, it triggers the release of remaining O_2 molecules.

↳ Both H^+ & CO_2 shows a heterotropic allosteric effects. O_2-affinity of Hb decreases in working tissues, the lower pH stimulates the release of O_2 from oxy-Hb. In working tissues, CO_2 & lactic acid are produced & hence lowers the pH. Lactic acid is produced from the incomplete oxidation of glucose due to insufficient supply of O_2.



$$HbO_2 + H^+ + CO_2 \rightleftharpoons \underset{\backslash CO_2}{\overset{H^+}{Hb}} + O_2$$

Role of DPG (2, 3-diphosphoglycerate) —

↳ DPG shows a heterotropic allosteric effect due to which O_2 affinity of Hb decreases with the increase of DPG concentration.

In human red cells, DPG is present & due to its present Hb - releases O_2 in red cells.

Role of Globin Protein —

At biological pH (~7.0) free heme groups (without globin protein) gets irreversibly oxidised by air (i.e., O_2) in aqueous media to give hemin or hematin consisting of Fe(III) —

Heme-[Fe(II)] $\xrightarrow{O_2, \text{water}}$ Hemin-[Fe(III)]

Hemin-[Fe(III)] ⇓

Oxidized forms containing Fe(III) of Hb & Mb ⇐ methemoglobin (Met-Hb) & metmyoglobin (Met-Hb)

⇓

These are no use from the standpoint of O_2 - transport.

To act as an O_2-carrier, in oxy-Hb, O_2 must be able to reversibly bind.

Without globin protein, Fe(II) will be irreversibly oxidised.

Mechanism of irreversible oxidation of Fe(II) —L ——

$$L-Fe(II)+O_2 \rightleftharpoons L-Fe(III)-O_2^-$$

$$L-Fe(III)-O_2^- + Fe(III)-L \rightleftharpoons L-Fe(III)-O_2^{2-}-Fe(III)-L$$

$$L-Fe(III)-O_2^{2-}-Fe(III)-L \xrightarrow{fast} 2\,L-Fe(IV)=O^{2-}$$

$$L-Fe(IV)=O^{2-} + Fe(II)-L \xrightarrow{fast} L-Fe(III)-O-Fe(III)-L$$
(hematin)

→ i) Thus irreversible oxidation passes through the formation of peroxo & oxo-bridged binuclear complexes & formation of these binuclear complexes are sterically hindered in case of Hb & Mb due to presence of bulky globin protein chain.

ii) During oxidation of Fe(II), in the transition state, an ionic charge separation will occur & such charge separation is highly disfavoured in presence of hydrophobic environment provided by globin protein.

iii) Hydrophic environment denies the solvation of the ions ~~present~~ produced in the irreversible oxidation.

iv) In irreversible oxidation, the simultaneous presence of O_2 & H_2O required, but globin protein protects Hb & Mb by preventing the simultaneous presence of O_2 & H_2O.

Use of chelating ligands in medicine :—

↳ If a toxic metal entered in the food chain, remain unchanged for ever. It can poinson the food chain at any stage, the poisoning activity will continue throughout the food chain, regardless the length of time.

↳ To detoxify these toxic metals from the living system, it requires the chelation therapy which utilises the administration of some suitable chelating agents to remove the toxic metals from the living body.

Requirements of a chelating ligand/agent/ antidote in metal ion detoxification —

↳ i) Conditional stability constant —

↳ It gives the measure of stability of a complex (i.e., metal-ligand interaction) under the actual conditions, i.e., biological conditions the present case.

↳ The toxic-metal-specils remains bound with the biogenic ligands & consequently the selected chelating ligand should successfully compete with the biogenic ligands to snatch ~~it~~ away the bound metal.

↳ Therefore, the condition stability constant between the toxic metal & chelating drug must be greater than that of the competing bioligand involved such as proteins.

↳ ii) Lipophilicity of the chelating drug —

↳ The chelating ligand / drug should be sufficiently lipophilic to penetrate the lipid cellular membranes to reach the body compartment where the toxic metal is accumulated.

↳ In such cases, by introducing a lipophilic moiety in the chelating drug, the activity may be remarkebly improved.

↳ iii) HSAB (Hard & soft acid & bases) theory & Selection of chelating ligand —

↳ According to this theory, to remove a heavy

Toxic metal ion, a chelating drug with the hard donor sites is preferred & to detoxify a soft metal, the chelating drug should have the soft binding sites.

Eg:-

Metals to be removed	Chelating drug (binding sites)	HSAB matching
Fe(III)	Desferrioxamine B (Several O)	Hard-Hard
Hg (II)	Unithiol (2S)	Soft - Soft
As (III)	British Antilewisite (2S)	Soft - Soft
Pb(II)	EDTA (4O, 2N)	Borederline-Borederline

→ iv) Designing of antidotes with the binding sites mimicking the endogenous binding sites —

→ If the binding sites of the chelating drug are similar to those of the endogeneous binding sites trapping the target toxic metal, then the drug can yield bettere results.

→ v) Toxic effects of the chelating drug —

↳ The administered chelating drugs should

not be toxic & the drugs should not be metabolised in preforming the scavenging action. Thus drugs with higher LD50 (Lethal Dose, 50%) values are preferred. Since sometimes, the toxic metal-drug chelate may enhance the toxicity due to translo-cation of the chelate.

(vi) Urinary & biliary excretion —

↳ Urinary excretion is favoured for the water soluble complexes of low Mol. wt., while the biliary excretion is favoured for the high Mol. wt. complexes of a very limited water solubility.

↳ To remove the toxic metal from an interior compartment, the drug must form a lipophilic complex in the body compartment then it must change to a hydrophilic complex upon reaching the blood plasma so that elimination of the metal complex is possible through the urinary excretion rather than its redistribution back into the tissue.

eg:- i) Unithiol —

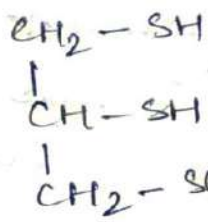
↳ Chelating drug having -SH groups.

↳ Water soluble

↳ Used to detoxify the soft metals like— As, Hg etc.

In detoxification of CH_3Hg^+ —

The corresponding CH_3Hg – unithol complex being charged can not pass through the biological membrane.



Unithiol (2,3-dimercapto-1-propane sulphonic acid, dmps)

Limitations of chelation therapy in metal ion detoxification —

→ May produce undesirable symptoms like diarrhoea, skin rashes etc.

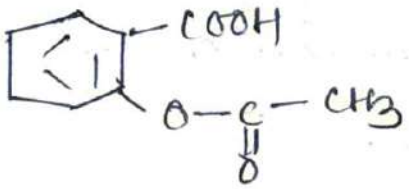
→ In urinary excretion, the chelating antidotes increase the concentration of the toxic metals in the kidneys & place a variety of burdens on the kidneys.

→ Prolonged chelation therapy may lead

to depletion of essential metal ions specially Zn^{2+} & Ca^{2+}.

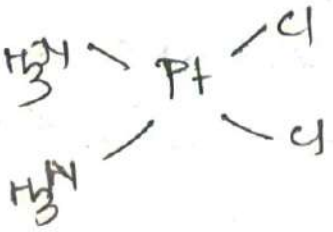
Example of some chelating drugs —

Aspirin — used in treatment of high copper level in blood. Due to depletion of essential coppere from the metalloproteins & metalloenzymes, the Cu-Level in blood increases. Aspirin picks up the Cu from blood through chelation & gives it back to the cells to ~~eat~~ ~~repeat~~ repair the biochemical process. Thus, the aspirin reduces the flood of Cu in blood as well as reactivate the cells by melting their Cu-deficiency.



Aspirin

Cis-platin — (Anti cancer drug)



cis-diamminedichloroplatinum (II)

→ Pt binds to DNA, with the chloride ligands first being replaced by water molecules & then by a DNA base such as guanine of a fast growing tumor.

From NMR study, it indicates that N7 position of ~~ga~~ guanine is favoured site for Pt-co-ordination.

$$cis\text{-}[Pt(NH_3)_2Cl_2] + H_2O \rightleftharpoons cis\text{-}[Pt(NH_3)_2Cl(H_2O)]^+ + Cl^-$$

$$\downarrow DNA$$

$$cis\text{-}[Pt(NH_3)_2\text{-}Cl]\text{-}DNA$$

$$\downarrow\uparrow {}^{+H_2O}_{-Cl}$$

two adjacent guanine bases of DNA are bound

$$cis\text{-}[Pt(NH_3)_2]\text{=}DNA \xleftarrow{DNA} cis\text{-}[Pt(NH_3)_2(H_2O)]\text{-}DNA$$

Binding of Pt-distorts the local DNA str. & therefore inhibits the cell division.

(Watson-Crick base pairing)

Table 12.1.2

Effects of some elements on human health

Metal	Disease due to deficiency	Disease due to excessive accumulation
Li	Maniac depressive psychosis.	CNS disorder; nephrotoxicity.
F	Poor bones (*i.e. osteoporosis*) and dental caries.	*Fluorosis*; mottled teeth; bone sclerosis.
I	Hypothyroidism; goiter.	Hyperthyroidism
Na	Addison's disease; hyponatremia (reduced blood pressure); Stoker's cramps.	Hypernatremia (increase in blood pressure).
K	—	Addison's disease; cardiac failure.
Mg	Neuromuscular problem like convulsion.	Anaesthesia; cardio-vascular problems.
Ca	Abnormalities in bone (*e.g. rickets, osteomalacia and osteoporosis*), nerve function, muscle contraction, blood clotting; retarded growth; hypocalcemia.	Cataracts; stones in gall bladder and kidney; calcification of tissues; inhibits the absorption of other essential metals; hypercalcemia.

Metal	Disease due to deficiency	Disease due to excessive accumulation
Cr	Impaired glucose and lipid metabolism.	Cr(VI) causes cancer and ulceration.
Mn	Skeletal abnormalities; gonadal failure; inhibited growth; impaired glucose metabolism.	Ataxia and damage to CNS.
Fe	Anemia.	Hemochromatosis (bronze diabetes); hemosiderosis; lesions in gastrointestinal tract; liver damage.
Co	Pernicious anemia	Coronary failure; polycythemia (increased RBC); thyroid dysfunction.
Ni	—	Dermatitis (sweating leads to complexation of Ni from Ni-plated jewelry with the skin protein keratin); gastrointestinal discomfort.
Cu	Anemia; kinky-hair syndrome; poor bone and connective tissues; pigmentation problem.	Wilson's disease; stomach irritation and nausea; reduced growth; liver damage.
Zn	Dwarfism; gonodal failure; delay in wound healing; affects lactation in woman.	*Metal-fume fever* due to inhaled Zn-fumes (pulmonary distress); may cause Cu-deficiency and anemia; impaired bone development.
Se	Liver necrosis; cancer; white muscle disease.	Cancer; alkali disease; hair and hoof loss; blind staggers.
Cd	Not known.	Nephritis; *itai-itai byo* (wrong bone metabolism)
Pb	Not known.	Impaired kidney function, multiple sclerosis; anemia; neurological problem; encephalitis.
Hg	Not known.	Encephalitis; impaired kidney function.