Name of the Teacher: Sutapa Chakrabarty

Subject: Chemistry

Class for which the note is prepared: Semester-6

Paper: C13T (Inorganic Chemistry)

Topic: Bioinorganic Chemistry

Part 3(last part)

Comments- Study the whole chapter thoroughly. Specially "**binding of O2 in haemoglobin, myoglobin, hamocyanine, hemerythrin, active site structure and catalytic cycle for hydration of CO2 by carbonic anhyrase,active site of cytochrome a,b,c , steps of photo synthesis, oxidized and reduced form of rubridoxin, ferredoxin, cycle of nitrogen fixation and chelation therapy"** must be read. Also complete the given assignment.

[N.B. - Acknowledgement of indebtedness to Mr.Sibshankar Das, my respected Teacher regarding collection of study materials in Inorganic Chemistry**]**

Carboxypeptidase-A (Hydrolytic enzyme)

Carboxypeptidase-A and thermolysin are two very important hydrolytic enzymes which catalyses the peptide hydrolysis reaction,

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

peptide hydrolysis is a difficult reaction in aqu solution. At neutral pH the uncatalysed reacti i.e, the hydrolysis of amides and peptides is a slow process with rate constant as low as 10^{-11} sec^{-1}, peptide hydrolysis in presence of catalyst can be attempt ~~K cat~~ k_{cat} values of 10^9 s.

At first in order to catalyse the reaction an enzyme must accomplish several things.

(1) Primarily it must facilitate the neucleophilic attack on the peptide carbonyl group by a neucleophile. This function can be accomplishe by producing a highly reacting neucleophile o by activating the carbonyl for attack by polarisation.

(2) Secondly it must stabilize the tetrahedral interm diate or the transition state that is generat following neucleophilic attack at carbonyl carbon

(3) finally it must stabilize the amide -N atom to make it a suitable leaving group, so that the tetrahedral intermediate can collapse upon -C-N bond cleavage.

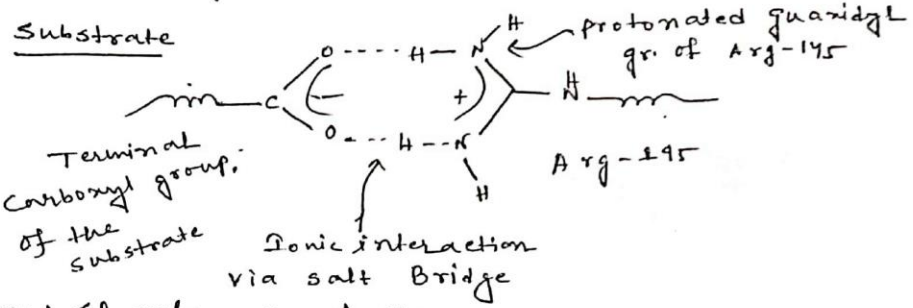
The peptidases are metalloenzymes containing Zn(II) in the active site. Depending upon the position of the peptide linkage to be attacked the peptidases are classified as—

Key features :-

① Hydrophobic pocket :-

This pocket is created by the polypeptide chain around the active site.

② Role of Arg-145 in ~~substr~~ substrate recognition.

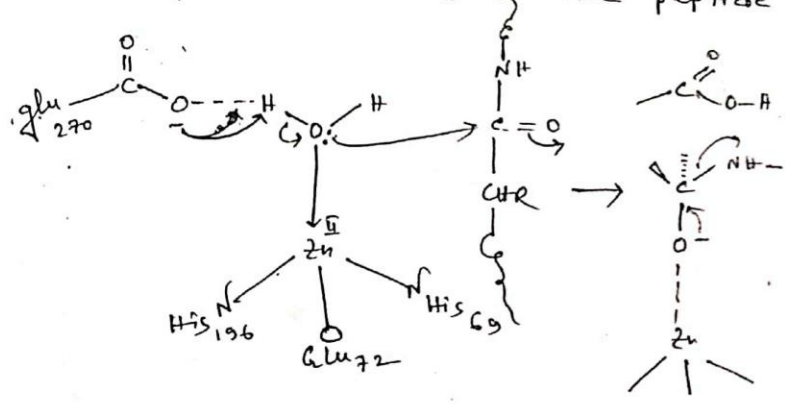


~~Terminal carbo. group of the~~

This keeps the substrate in a proper position and orientation required for the proper This is called substate recognation. This interaction helps to rupture the $-CN$ bond in the peptide linkage.

③ Role of Glu-270 to generate a potential nucleophile :

Carboxylate group of Glu-270 may interact with H_2O bound with $Zn(II)$ to generate the metal bound hydroxyde group which is a powerful nucleophile to attack the peptide linkage.



Generation of the enzyme bound $-OH$ group (a better nucleophile) by Glu-270 to attack the substrate in CpA activity}.

(iv) Role of $Zn(II)$ metal ion;

The main role of the metal ion is to activate the deprotonation of the co-ordinated H_2O to form a co-ordinated $-OH^{\ominus}$ ion. The H_2O is also hydrogen bonded to Glutamate 270 which also assists the formation of $OH^{\ominus}$ by transfering the proton to the carboxylate group of Glu-270 forming Glutamic acid. This $OH^{\ominus}$, a more potential nucleophile than H_2O affects a nucleophilic attack on the peptide $-CO$.

Ceruloplasmin

Ceruloplasmin is an intensely blue coloured Cu protein present in the blood plasma of the Vertibrates. 100 ml of human plasma contains 20–40 mg. of the protein. Beside blood it is also present in spinal and joint fluids and the secretion of eyes, ear and digestive system. Human ceruloplasmin (m. Wt. 130 KDa) consists of '4' peptide chains and the no. of Cu atoms per molecule is ~~1±1~~. 7 ± 1.

Ceruloplasmin is synthesised in ~~livers~~ and acts as a Cu transport protein. It provides Cu to cytochrome-c oxidase and other Cu containing oxidases. Albumin, which transports 'Cu' between intestine and liver in the vain also takes 'Cu' from ceruloplasmine.

Ceruloplasmin ~~acts~~ acts as a catalyst in the oxidation of polyphenoms, polyamines, adrinaline ~~cerat~~ ceranotine etc. It also has a role of sequestering excess 'Cu(II)' and store it to blood plasma, so the toxic effect of catalytic oxidative addition rexn. by free Cu(II) is prevented, and ceruloplasmin bound Cu(II) is made available to Cu containing enzymes as per their requirements.

Wilson's Disease:

Deficiency of ceruloplasmin i.e. the Cu-transport protein causes as desease called Wilson's disease. In this ~~genetic~~ disease Cu can't be stored in the bound form of ceruloplasmin in the body cell. So Cu gets deposited in liver, brain, eyes and kidney. this

leads to liver and kidney failure and various neurological disorders and formation of brown or green rings in the cornia of eyes.

Alzheimer's disease:

Alzheimer's disease is a neutrological disorder in which death of brain cells causes memory loss. The mental ability of the person gradually declines and reaches a stage where it becomes difficult for them to live normal life.

A no. of hypothesis have been advanced for the causes of A.D including genetic factors, neuro toxins (Al), acetyl chlorine defficiency and defficiency of Ca, Mg, Zn, Cu and Fe.

A neut natural food diet, avoidance of 'Al' exposure and suplimentary neutrition program may be helpful for the prevention of A.D.

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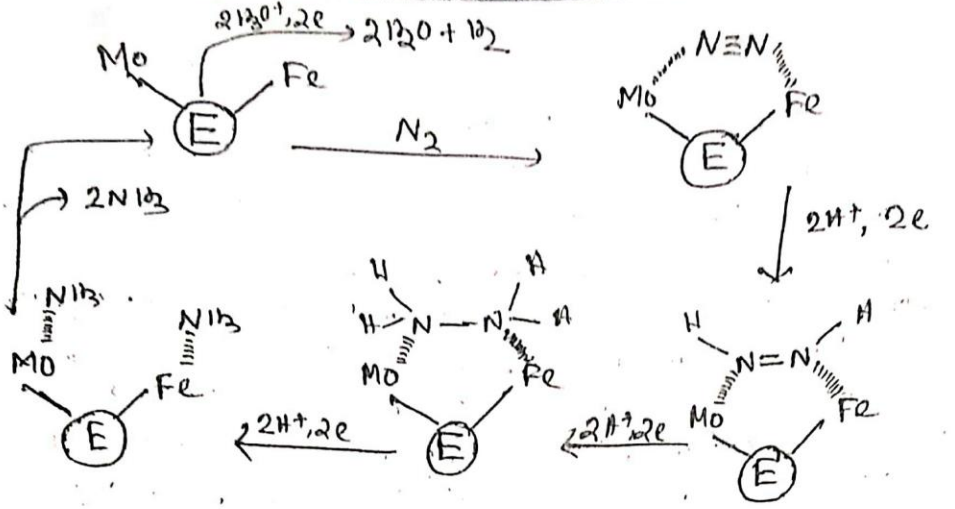
Nitrogen fixation :

Nitrogen fixation can be any reaction of N_2 (di-nitrogen) in which nitrogen gets co-valently bonded to any other elements. some plants. can synthesise nitrogenious bio-molecules by reducing atmospheric nitrogen in ambient condition. The state in which atmospheric nitrogen is reduced is known as Nitrogen fixation. The engyme necessary for catalytic reduction of di-nitrogen in vivo (inside any living body) is nitrogenage.

[vitro → outside anything body]

Active engyme in nitrogen fixation is nitrogenage. It is not a unique engyme but appears to differ some what from species to species. Nevertless the various engymes are very similar. Two protines are involved, the smaller has a molecular weight of 57,000 - 73,000 which contains Fe_4S_4 clusture. The larger protine is an $\alpha_2\beta_2$ tetramer with a molecular weight of 2,20,000 — 2,40,000 containing two Mo atoms, about 30 Fe atoms and around 30 labile sulphide ions.

Nitrogen reduction is strongly inhibited by CO, NO, N_2H_4 and O_2. H_2 competitively inhibits nitrogen reduction. In the absence of N_2 or other ~~a reducible~~ reducible substrates, the active engyme reduces H_3O^+ ions to evolve H_2 gas. Such H_2 evolution lowers the catalytic effeciencies of nitrogenage to about 75%. The overall stoichio-metric of N_2-reduction is shown below —



$$N_2 + 8H^+ + 8e \longrightarrow 2NH_3 + H_2$$

Fig: Nitrogenage catalysed nitrogen reduction

⊗ Metal ion toxicity:

Because of their non-biodegradeable nature, the intake of various metallic compounds causes local irritation, tissue damage, or systhemetic poisoning if the intake is sufficiently large. Metal ion toxicity may also disturb the electrolytic balance, damage specific organs (e.g; brain, kidney, Liver etc) and even the central nervous systems or interfare with the vital ~~engy~~ (enzymetic processes. There is considerable variations in the safe concentration levels of toxic metal ions. The toxicity caused by some metals are discussed below;

(i) Copper (Cu):

Although Cu in trace amounts is essential for life due to its role in the metalo enzymes and metalo proteins (e.g; super oxide dismutase, ~~&~~ hemocyanin, ~~etc~~ ceruloplasmin etc.)

But 'Cu' salts even in moderately low concentrations may cause vomiting (वमन) and considerable gastro-intestinal irritation. The

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with co-enzymes, and (iii) uncoupling of phosphorilation from metabolic oxidation.

(iii) Cadmium (Cd):

Cd is not an essential element for human. It is a toxic metal. Intake of Cd occurs mainly through the food chain by about 40mg per day. Exposure to metallic dust, or fume during industrial operations causes hypertension and cardiovascular problems which finally lead to damage of lungs. Zn appears to give some protection against the toxic effect of Cd. The reported hypertensive effect of Cd in man associated with a high cadmium/zinc ratio in the kidney. Cd depresses the growth and reduces the digestion of protein and fat. Once absorb, Cd is incorporated into protein by means of thiol groups. Enzyme bound Cd accumulates in kidney, liver and reproductive organs. The kidney is the main target of attack of Cd.

(iv) lead (Pb):

Lead is toxic and its hazardous effects due to industrial exposure are well documented. Tiredness, nervousness, dipression, lack of mind concentration, frequent cold and other infections may result from lead porsoning. Most of lead is accumulated from diet, air and water.

Lead is accumulated in bones and soft tissues particularly in brain

leading to dipressed functioning. It forms complexes with thiol groups of enzyme proteins, inhibits biosynthesis of heme, particularly in the conversion delta amino livulinic acid to pospho bilinogen. It also inhibits the formation of heme from Fe(II) and protoporphyrin - IX (P-IX). It depresses the formation of delta amino livulinic acid and decreases the conversion of pospho bilinogen to proto porphyrin-IX. It causes structural damage to mitochondria of Kidney cells. It is responsible for the loss of amino acids, glucose and phosphate in urine. It damages liver, Kidney and gastro-intestinal tract, muscle pains, weakness, joint pain, tremor, anaemia etc. It also causes abnormalities in fertility and pregnancy.

(v) Mercury (Hg):

The toxicity of Hg depends on it's chemical form which are given below;

(1) Hg :-

elemental Hg is fairly innert and non toxic. The vapour of Hg is quite toxic. When inhaled, enters into the brain through the blood stream leading to severe damage of nervous systems

(2) Hg^{2+} :-

It is fairly toxic in nature because of the higher affinity of the sulphur atoms. Thus it is attached with the sulphur atoms of proteins. It also forms bond with hemoglobin and cerum albumin, both of which contains sulphyAryl groups. (- SH)

(3) $R-Hg^{+}$:

The most toxic species are the organo mercurials; particularly CH_3-Hg^{+} which are soluble in fats, the lipid fraction of the membranes and brain tissues. The co-valent Hg—C bond is not easily

disrupted and the alkyl-mercury bond is retained in cells for long periods of time. The most dangerous aspect of $R-Hg^+$ is to move through the placental barrier and enter foetal tissues. Therefore CH_3-Hg^+ cation causes irreversible nerve and brain damages and inhibits transport of sugars across the membrance and causes mental retardation and inhibits cell division

Biological methylation :-

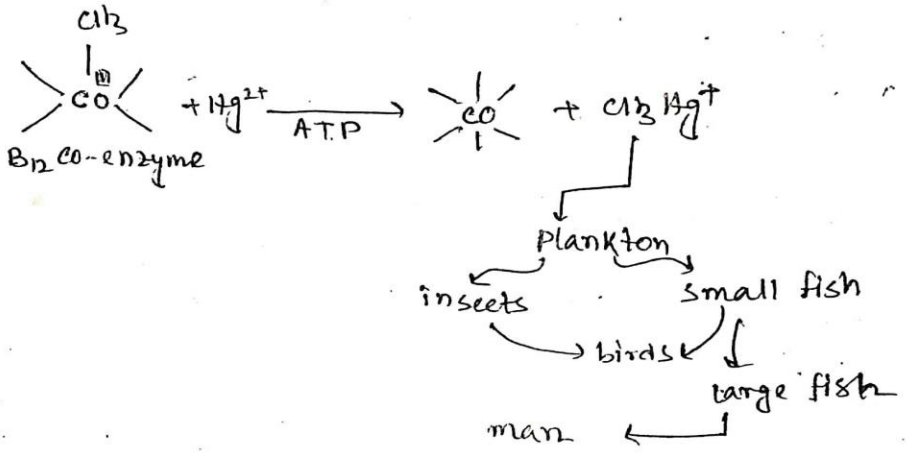

Fig : mechanism of propagation of Hg^{2+}

Hg or its salts can be converted into CH_3-Hg^+ cation, by anerobic CH_4 synthesising bacteria in water. This conversion is facilitated by Co(III) containing vit B_{12} co-enzyme. A methyl group bonded to the Co(III) on the methyl cobalamin co-enzyme is transferred enzymatically with Hg^{2+} to CH_3Hg^+. This CH_3-Hg^+ cation is then enters into the food chain through the plankton and is concentrated by the fish and then by man.

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- **Minamata incident in Japan:**

At Minamata bay of Japan in 1953 to 1960 more than 100 peoples lost their lives and many thousands were permenantly paralysed from eating Hg-contaminated fish. Genetic defects are observed in some babies (nearly 50) whose mothers had consumed the Hg-contaminated fish from this bay. The source of Hg was the effluent discharged into the bay from vinylchloride plant, Minamata Chemical company. This incident is the first known case where the natural bioaccumulation in fish of a toxic matter (CH_3-Hg^+ cation) killed the people and genetically damaged a large population this incident is known as Minamata incident

- **Chelation Therapy:**

(1) Detoxification of metal ions can be achieved by the use of suitable chelating agents, the process is known as chelation therapy.

Suitable chelating agents may be selected on the basis of SHAB principle and stability of metal-chelate complexes. According to SHAB principle the heavy metal (e.g; As, Pb, Hg etc) are soft acids. These are highly polarisable and have the tednancy to bind with ligand atoms in the order; $S \gg N > O$. On the other hand, alkali and alkaline earth metal ions are hard acids, they prefer to bind with ligand atoms in the order $O \gg N > S$. Border line metal ions like Co^{2+}, Cu^{2+}, Zn^{2+} etc. have intermediate preferences

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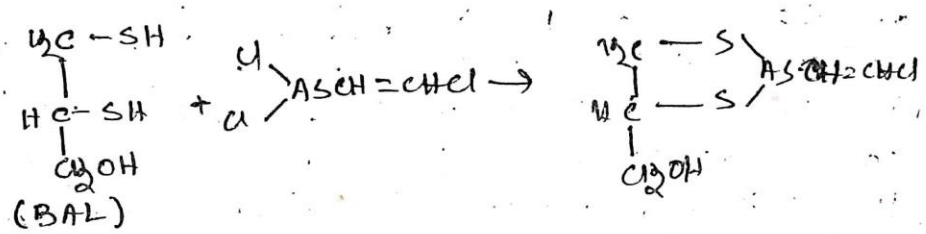
(i) Chelating agents as drugs :

Number of chelating agents are used as antidotes for metal poisoning (ভারী ধাতুর). If the doses are used carefully, the antidots circulate in the blood stream without causing much decrease of the body's essential metals. Such chelating agents have appropriate number of ionisable functional groups (e.g; $-SH$, $-SO_3H$, $-OH$, $-COOH$ etc) in their structures, so that the resulting metal complexes have an overall charge. The drugs should be hydrophilic in nature, so that these could be easily excreted through urine. e.g; the As bearing compound Lewisite ($Cl-CH=CH-AsCl_2$) was used as a poisonous gas during the world war II. Its antidote is 2,3-dimercepto propanol which is popularly known as british anti lewisite (BAL). It binds to As strongly and removes it from the system in the form of the following complex



At present BAL is widely used an administered (ইনজেকশন) intramascularly for the treatment of metal poisoning caused due to Hg, Sb, Bi, Cu and Au which also bind with the thiol group(-SH) of proteins

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(II) Metal chelates as drugs:

Free ethylene diamine tetra acetic acid (H_4EDTA) is toxic and causes reduction of Ca-level in blood but its disodium mono calcium salt ($Na_2CaEDTA$), could be injected intravenously.

It is widely used for the treatment of Pb-poisoning where by, PbEDTA complex ($Na_2PbEDTA$), together with unreacted $Na_2CaEDTA$ are excreted through urine.

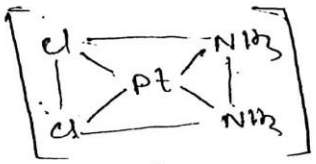
$$Pb\text{-}Enz + Na_2CaEDTA \rightleftharpoons Ca\text{-}Enz + Na_2PbEDTA$$

This drug ($Na_2CaEDTA$) is also used for the treatment of poisoning due to Zn and radioactive Sr. Fe(II)EDTA complex is used as an anti-anaemic drug. CaH_2EDTA is employed for the removal of stone from urinary tract.

(III) Gold drugs:

complexes of Au have also been used in chelation therapy. Thus, Na-Auro thio sulphate, $Na_3[Au(S_2O_3)_2] \cdot 2H_2O$ is used for the treatment of tuberculosis. K-Aurocyanide, $K[Au(CN)_2]$ is suitable for the treatment of tuberculosis and syphylis. Recently successful model studies on gold therapy have also been reported using phosphin complexes of the type, R_3PAuCl ($R = Et, Ph$) This Au-drugs are effective in case of inflammation in animal.

(IV) Metal complexes as anticancer drug:



cis platin or cis DDP

DDP is the best known example of the application of co-ordination compounds in medicine. Rosenburg and his collaborators started working with this a long 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 cancers and active against cancers of lungs, ovary, bladder, head, neck and cervics. cisplatin is administered as injection every few weaks — solutions are usually given in physiological saline. It is highly toxic agent. Chemoprotector like 'Na dimethyl dithio carbamate' is used to reduce the toxicity.

The mode of action of cis-platin based on its capacity to bind to DNA and blocked replication. Since the Cl^- ion concentration in extracellular fluids is large, the chloride ligands in cis-platin are significantly substituted by water molecules outside the cell. The chloride ion concentration being much less inside the cell, so the chloride ligands in cis-platin are replaced by H_2O forming products like $[Pt(NH_3)_2(H_2O)_2]^{2+}$ and $[Pt(NH_3)_2(OH)_2]^0$. The Pt(II) now binds to two N-atoms (N-7) of two adjacent guanin bases mostly in the same strained, to form a chelated interstrained cross link and upsets the normal replication of DNA. i.e. it prevents the replication of the DNA and inhibits the growth of cancer cells.

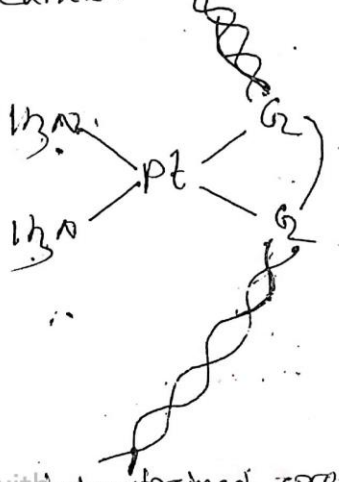


Fig. interstrained crosslink by cis-platin in DNA of cancer cell.

: Assignment :

1. What is co-operative interaction?
2. Show the active sites of Hb and Mb.
3. State how 'CO' affects biological system.
4. Draw catalytic cycle for the hydration of CO_2 by Carbonic anhydrase-B.
5. What is the function of Na^+/K^+ ATPase?
6. What is chelation therapy? State some uses of chelation therapy.
7. Draw the active site structure of Rubridoxin and Ferredoxins.