BIOINORGANIC CHEMISTRY

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Bioinorganic chemistry

Bioinorganic chemistry deals with the role of metals and non metals in biological systems. The inorganic elements, other than carbon, especially the metals are also vital to the functioning of bio- systems.

The two major components of bioinorganic chemistry are:

i) the study of naturally occurring inorganic elements in bio-systems and

ii) ii) introduction of these elements as probes or drugs into biological systems and studying inorganic models that mimic the behavior of various metallo-proteins.

The inorganic elements, especially the metals play an important role in biological systems. Based on the relative concentrations in the biological systems, the metals are divided into:

Bulk metals - Na, K, Mg & Ca

Trace metals - Zn, Fe, Co, Ni, Cu, Mo, V etc., which are present in low concentration and are used for biocatalysis.

H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac															

Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu
Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lr

Metals — Essential elements for humans (daily requirement: 25 mg)

Non metals — Presumably essential elements

- Symptoms of deficiency: Mg (muscle cramps), Fe (animea), Mn (infertility)
- Toxic effects in case of high doses (therapeutic width)
- Occurrence of non essential elements (e.g. Rb: 1.1 g / 70 kg) and of contaminations (e.g. Hg)

Metal content of a human body (70 kg)

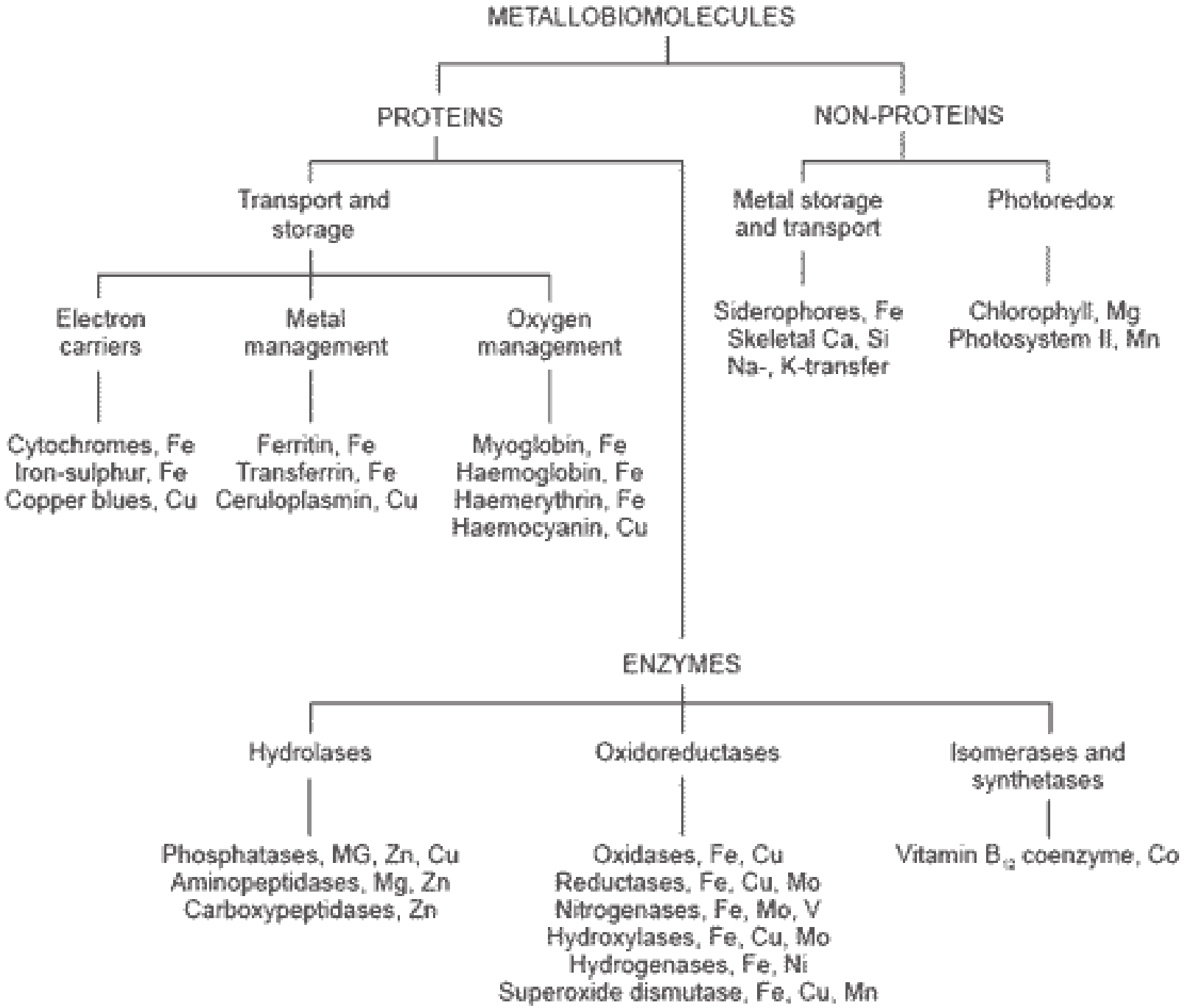
Ca	1000 g	Sn	20 mg
K	140 g	V	20 mg
Na	100 g	Cr	14 mg
Mg	25 g	Mn	12 mg
Fe	4.2 g	Mo	5 mg
Zn	2.3 g	Co	3 mg
Cu	72 mg	Ni	1 mg

- Non metals: O (45500 g), C (12600 g), H (7000 g), N (2100 g), P (700 g)

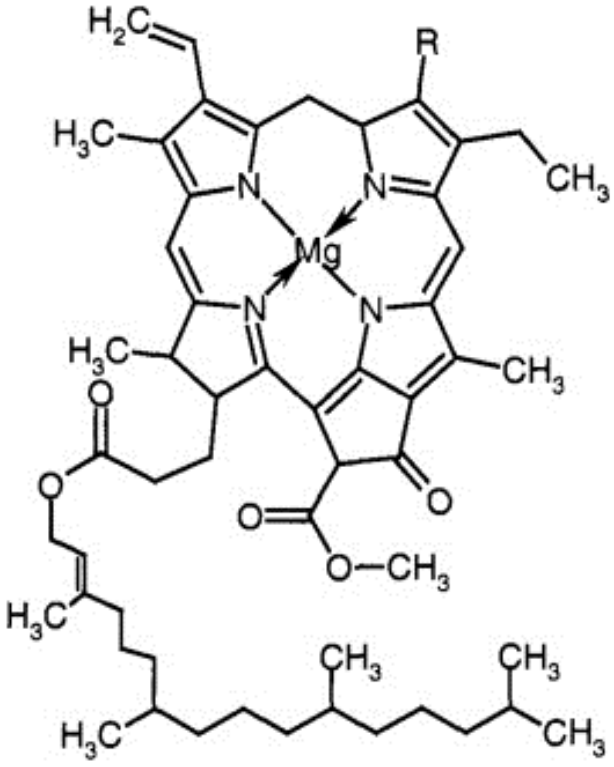
Earth's Crust	
Element	%
O	47
Si	28
Al	7.9
Fe	4.5
Ca	3.5
Na	2.5
K	2.5
Mg	2.2

Human Body	
Element	%
O	63
C	25.5
H	9.5
N	1.4
Ca	0.31
P	0.22
K	0.08
S	0.06

- Na^{+},K^{+}: Electrolytes
- Mg^{2+}: Chlorophyll, energy production (ATP $\rightarrow$ ADP), skeleton
- Ca^{2+}: muscle functions, Hydroxylapatite $Ca_5(PO_4)_3(OH)$, $CaCO_3$
- $V^{IV/V}$, $Mo^{IV/VI}$, $W^{IV/VI}$, $Mn^{II/III/IV}$, $Fe^{II/III}$, $Ni^{I/II/III}$, $Cu^{I/II}$: electron transfer
- Fe and Cu: transport and storage of oxygen
- Fe^{II}, Fe^{III}: Magnetite (Fe_3O_4)
- Co: Cobalamine, e.g. Vitamin-B_{12}
- Zn^{2+}: Enzymes, zincfinger (gen. transcription), stabilization of proteins
- Si^{IV}: bones; SiO_2/silicagel
- P^{V}: Hydroxylapatite, ATP, cell membrane, DNA
- Se^{-II}: Selenocysteine
- F^{-}: Fluorapatit ($Ca_5(PO_4)_3F$) teeth; Cl^{-}: besides HCO_3^{-} most important free anion, I^{-}: hormones of the thyroid, radiation therapy

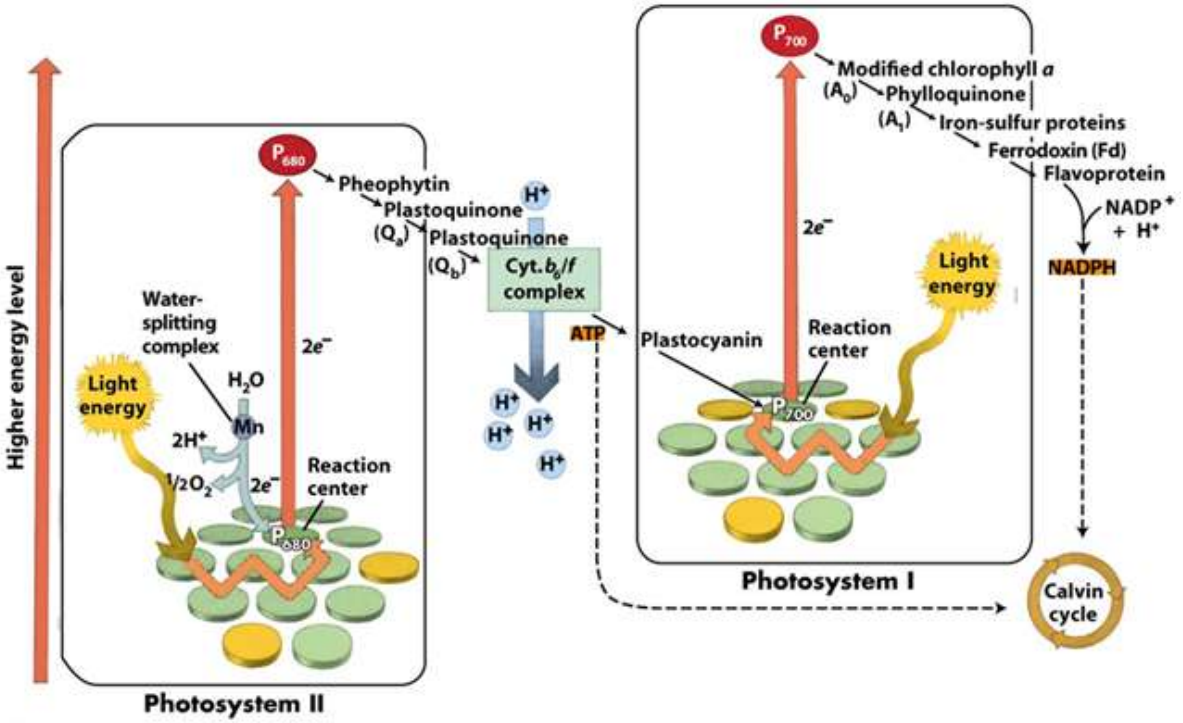

METALLOBIOMOLECULES
PROTEINS
NON-PROTEINS
Transport and storage
Metal storage and transport
Photoredox
Electron carriers
Metal management
Oxygen management
Siderophores, Fe
Skeletal Ca, Si
Na-, K-transfer
Chlorophyll, Mg
Photosystem II, Mn
Cytochromes, Fe
Iron-sulphur, Fe
Copper blues, Cu
Ferritin, Fe
Transferrin, Fe
Ceruloplasmin, Cu
Myoglobin, Fe
Haemoglobin, Fe
Haemerythrin, Fe
Haemocyanin, Cu
ENZYMES
Hydrolases
Oxidoreductases
Isomerases and synthetases
Phosphatases, MG, Zn, Cu
Aminopeptidases, Mg, Zn
Carboxypeptidases, Zn
Oxidases, Fe, Cu
Reductases, Fe, Cu, Mo
Nitrogenases, Fe, Mo, V
Hydroxylases, Fe, Cu, Mo
Hydrogenases, Fe, Ni
Superoxide dismutase, Fe, Cu, Mn
Vitamin B_{12} coenzyme, Co

Structure of Chlorophyll

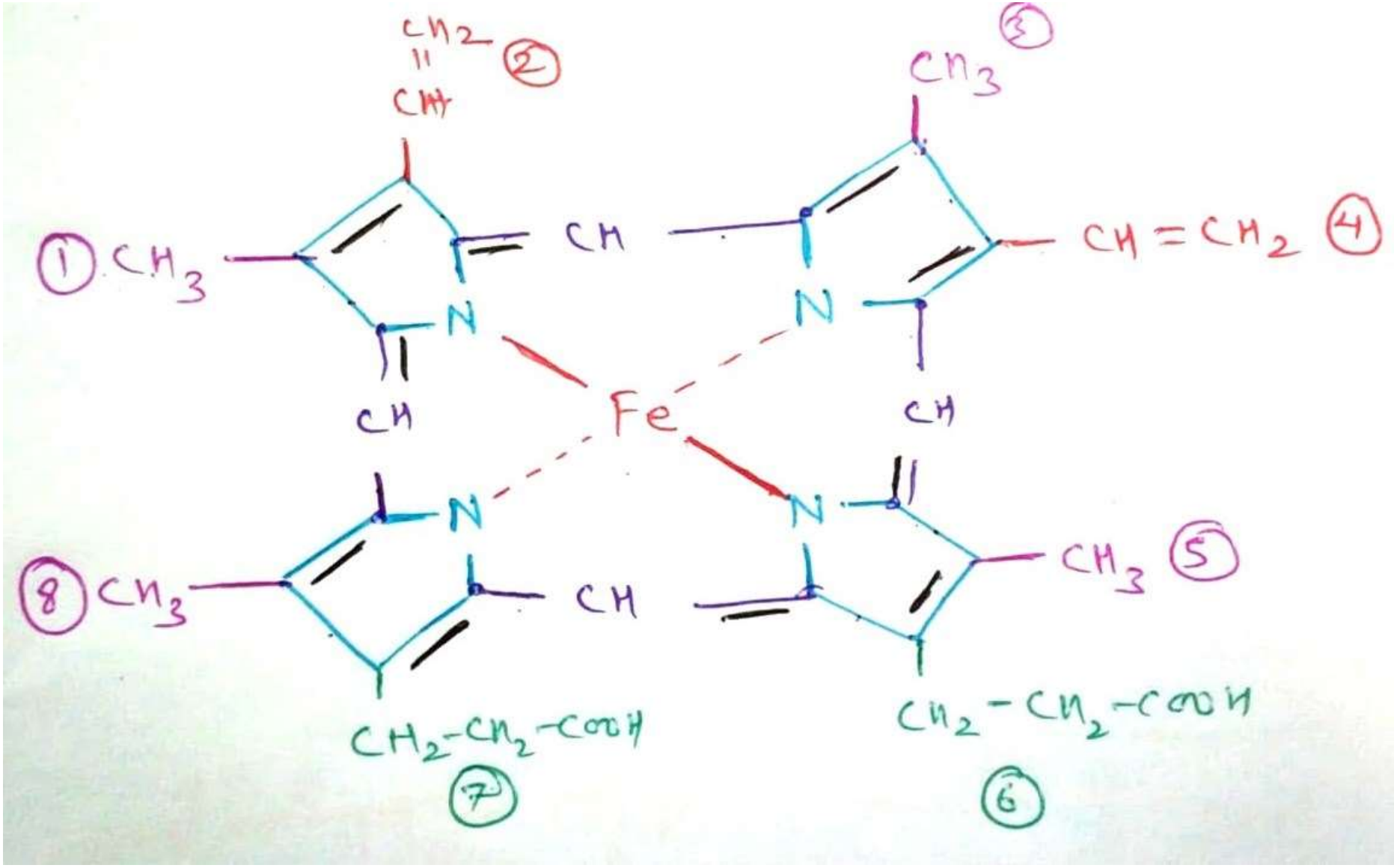


Chlorophyll a: R=CH3

Chlorophyll b: R=CHO



Structure of hemoglobin

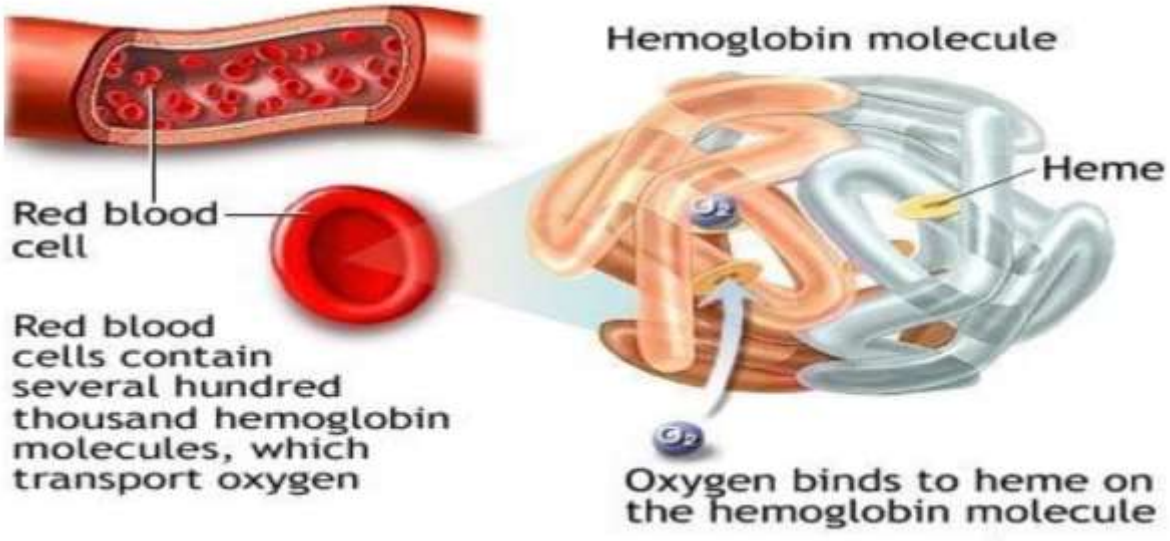


What Is Hemoglobin?

(Hemoglobin = Heme + Globin)

- **Hemoglobin(Hb)** is a **major Hemoprotein** of Human body
- **Hemoglobin Chemically is:**
  - **Conjugated Protein**
- **In Hemoglobin**
  - **Heme** is a **Prosthetic group**
  - **Globin** is a **Protein part**
- Hemoglobin(Hb) is **Red color pigment**
- **Location Of Hemoglobin-**

Inside Red blood cells/Erythrocytes of blood.

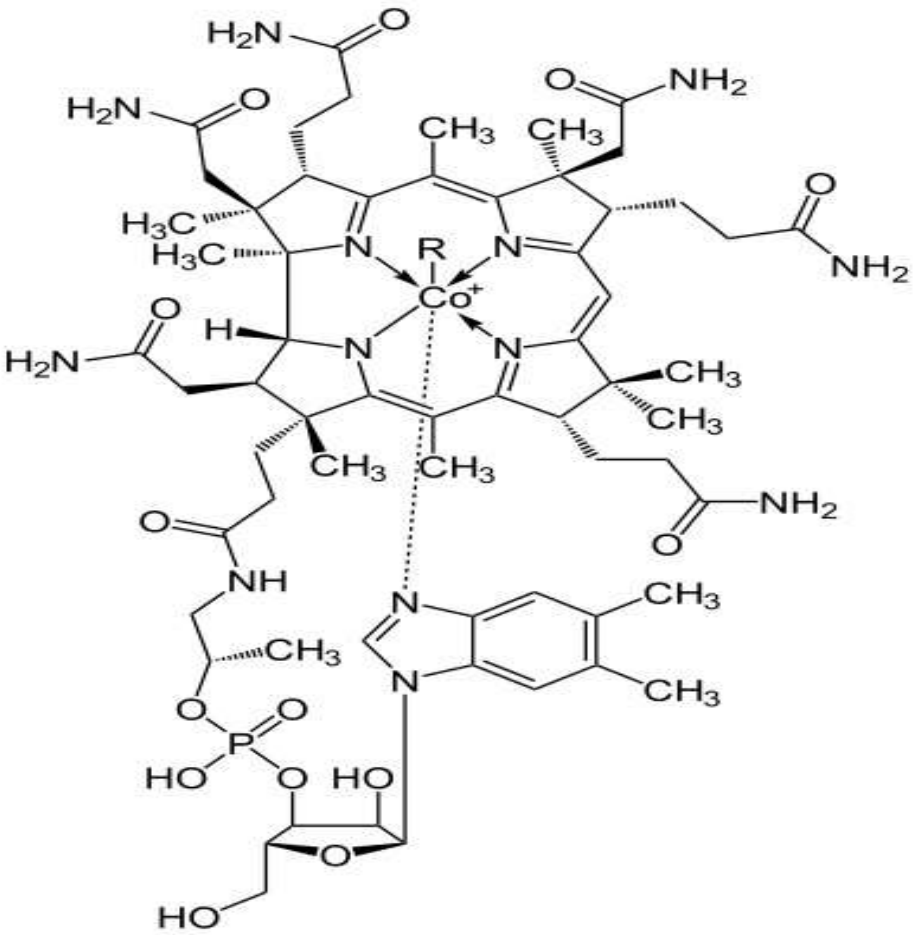


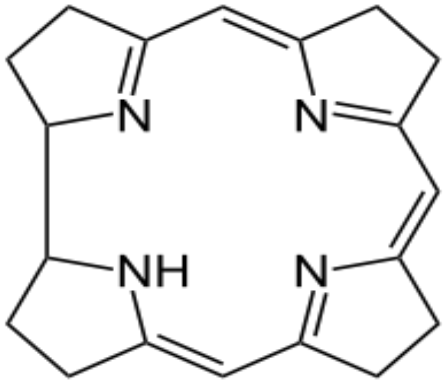
Corrin ring system – Cobalamin or Vitamin B12

- Cobalamin, or Vitamin B12, is the largest and the most complex out of all the types of Vitamins. The discovery of Cobalamin was made as scientists were seeking to find a cure for pernicious anemia, an anemic disease caused by an absence of intrinsic factor in the stomach.

- Cobalamin was studied, purified, and collected into small red crystals, and its crystallize structure was determined during an X ray analysis experiment conducted by Scientist Hodkin. A molecule structure of Cobalamin is simple, yet contains a lot of different varieties and complexes as shown in Figure 1.

- The examination of the vitamin's molecular structure helps scientists to have a better understanding of how the body utilizes Vitamin B12 into building red blood cells and preventing pernicious anemia syndromes.





- The metalloenzyme structure of Cobalamin presents a corrin ring with Cobalt, positioned right in the center of the structure by four coordinated bonds of nitrogen from four pyrrole groups.

- They are also connected to each other by a 3 C-CH methylene link on the other sides, by a C-H on one side and by two pyrroles directly coming together.

- The fifth ligand connected to Cobalt is a nitrogen coming from the 5,6-dimethhylbenzimidazole. It presents itself as an axial running straight down from the cobalt right under the corrin ring.

- This benzimidazole is also connected to a five carbon sugar, which eventually attaches itself to a phosphate group, and then straps back to the rest of the structure. Since the axial is stretched all the way down, the bonding between the Cobalt and the 5,6-dimethylbenzimidazole is weak and can sometimes be replaced by related molecules such as a 5-hydrozyl-benzimidazole, an adenine, or any other similar group.
- In the sixth position above the Corrin ring, the active site of Cobalt can directly connect to several different types of ligands.
- It can connect to CN to form a Cyanocobalamin, to a Methyl group to form a methylcobalamin, to a 5’-deoxy adenosy group to form an adenosylcobalamin, and OH, Hydroxycobalamin.
- Cobalt is always ready to oxidize from 1+ change into 2+ and 3+ in order to match up with these R groups that are connected to it. For example, Hydroxocobalamin contains cobalt that has a 3+ charge while Methyladenosyl contains cobalt that has a 1+ Charge.

- The point group configuration of Cobalamin is C4v. In order to determine this symmetry, one must see that the structure is able to rotate itself four times and will eventually arrive back to its original position.
- With Cobalt being the center metal of the molecule, Cobalamin carried a distorted octahedral configuration. The axial that connects Cobalt to the 5,6 dimethyl benzimidazole is stretched all the way down to the bottom. Its distance is several times longer than the distance from the Cobalt and the attached R group above it. This sometimes can also be referred to as a tetragonal structure. The whole shape overall is similar to an octahedral, but the two axial groups are different and separated into uneven distances.
- Cobalamin enzymes can catalyze a few different types of reactions. One of them is the reaction of Intramolecular rearrangements. During this rearrangement coenzyme is exchanged to the two groups attached to adjacent carbon atoms. Another reaction involves transferring the methyl group in certain methylation reactions, such as the conversion of homocysteine to methionine, biosysnthesis of choline and thymine etc. These interactions can bring beneficial values to the biological bodies.

- Cyanocobalamin, one type of cobalamin, works to generate the forming of red blood cells and heal many different damages in the nervous system.
- Cobalamin also serves as a vital role in the metabolism of fatty acids essential for the maintainence of myelin. Studies have shown that people with Vitamin B12 deficiency will reveal irregular destruction of the myeline shealth, which leads to parlysis and death.
- Some of the other symptoms of the lack of cobalamin are poor growth, megaloblastic bone marrow, Gi tract changes, Leucoopenia and hyper-segmented nutrophills, degenerative changes in spinal cord and nervous system and excretion of methyl malonic acid and homocystin in urine.
- Throughout the years, Vitamin B12 has shown to be essential for the functioning of the nervous system and the production of red blood cell. A study conducted by researchers at the National Institutes of Health, Trinity College Dublin, suggested that a deficiency in Vitamin B12 might increase the risk of neural tubes defect in children (Miller).
- Therefore, by studying the structure and function of Cobalamin, scientists can experiment and form Vitamin B12 in their laboratories and serve the community as a whole.

Nitrogen fixation

- Nitrogen fixation is an important step in nitrogen cycle, providing available nitrogen for plant nutrition. This occurs in the presence of nitrogenase, an enzyme which promotes the fixation of atmospheric dinitrogen. Nitrogen fixation occurs readily in various bacteria, blue green algae and in symbiotic bacteria-legume association under mild conditions. Nitrogen fixation is an important channel for providing nitrates to the plants.

Invitro-nitrogen fixation

- Dinitrogen was capable of forming stable complexes with transition metals which led to fixation through such complexes. Certain phosphine complexes of molybdenum containing dinitrogen yield ammonia in acetic media.

- $[MoCl_3(thf)_3] + 3e^- + 2N_2 + \text{excess dppe} \longrightarrow [Mo(N_2)_2(dppe)_2] + 3Cl^-$

- $[Mo(N_2)_2(dppe)_2] + 6H^+ \longrightarrow 2NH_3 + N_2 + Mo^{IV}$ products

Where thf = tetrahydrofuran, dppe = 1,2-bis(diphenylphosphino)ethane.

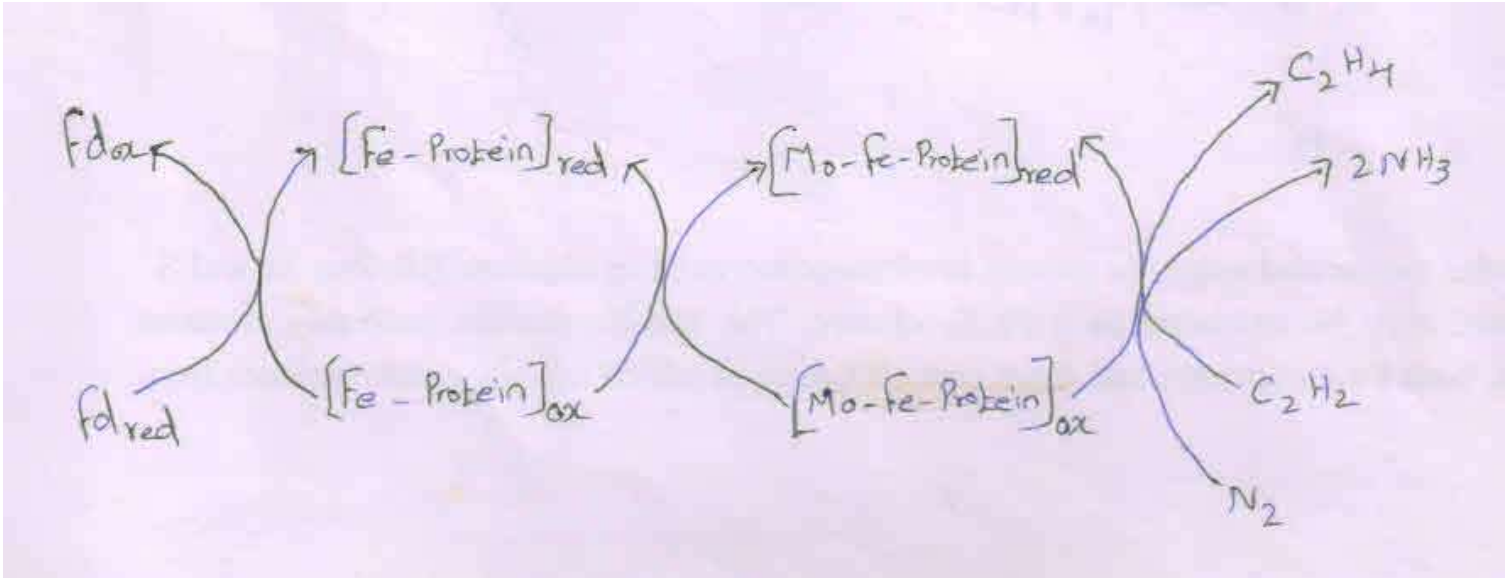
Both reaction takes place at room temperature and at atmospheric pressure. The reducing agent is a Grignard reagent.

Invivo-nitrogen fixation

- There are several bacteria and blue green algae that can fix molecular nitrogen In vivo. The most important nitrogen fixing species are Rhizobium living in root nodules of various species of legumes. The active enzyme in nitrogen fixation is nitrogenase.

Nitrogenase

- The enzyme system responsible for fixing N_2 is known as nitrogenase. It has been isolated from Rhizobium lupine. It is made up of two proteins (i) iron protein and (ii) molybdenum iron protein.
- Both protein appear to contain sulphide ions equal to number of Fe atoms. The iron is present mainly as Fe_4S_4 cubane clusters, which provide a system for electron storage. The major elements of the nitrogenase reaction are as follows.

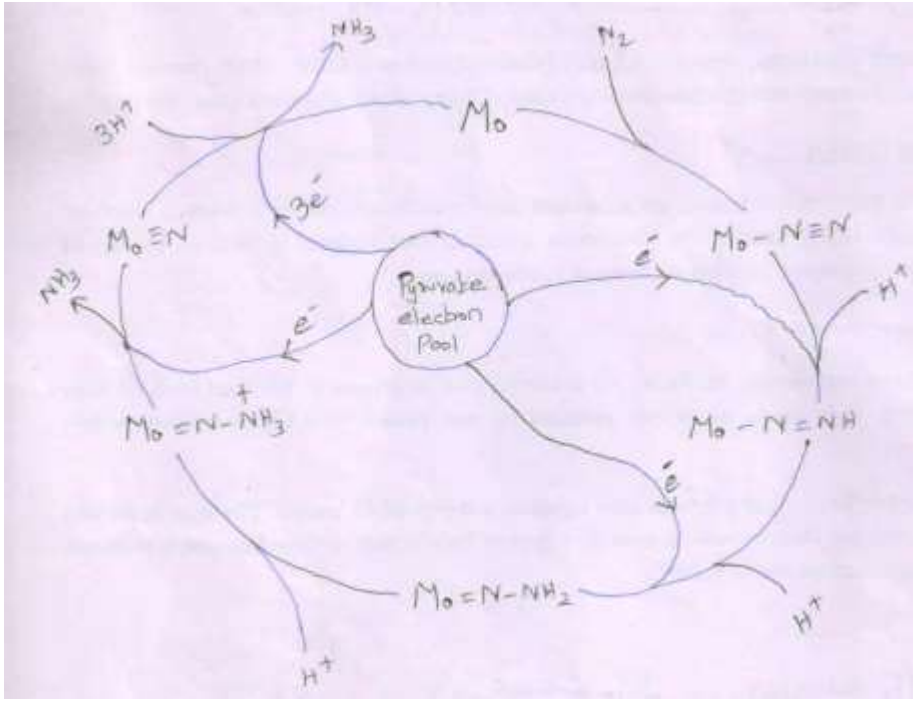


Electrons flow from a reducing agent into the Fe protein, then into Mo-Fe protein and finally on to the substrate.

$$N_2 + 6H^+ + 6e^- \longrightarrow 2NH_3$$

The two proteins together will catalyse the reduction of N_2 to NH_3 in the presence of Mg-ATP. The Fe protein acts as a specific electron carrier to the Mo-Fe protein, with which it forms a complex in the presence of Mg-ATP.

Pyruvate is the source of electrons which are transferred through ferredoxin to nitrogenase. Two Mo(III) atoms cycling through Mo(VI) would provide the six electrons necessary for reduction of dinitrogen. Since the enzyme is rich in ferredoxin type clusters, there should be a ready flow of electrons, and the molybdenum may stay in one or two oxidation states that most readily bind dinitrogen and its intermediate reductants. The overall catalytic cycle may be represented as below



- Many of the metal atoms and sulphide groups in nitrogenase exist in clusters. All four Fe and S^{2-} in the Fe protein may be extruded as a Fe_4S_4 cluster. The Mo-Fe protein probably contains several clusters, both Fe_4S_4 clusters and more complex ones in which molybdenum replaces iron.

Reactivity of Nitrogenase

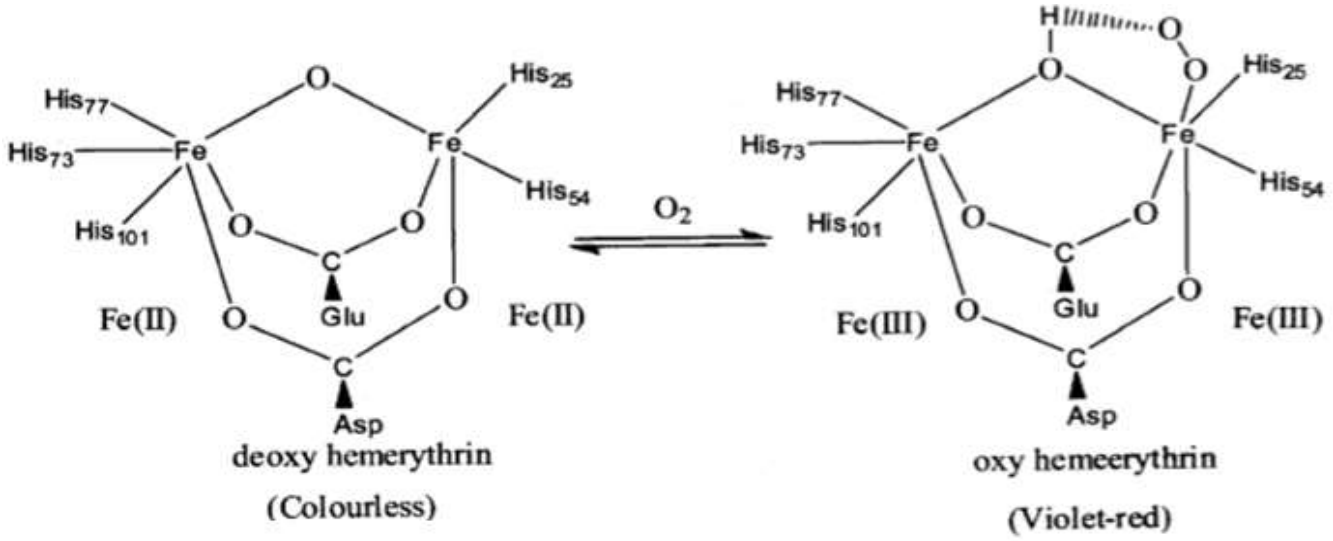
- Nitrogenase will catalyse the reduction of a range of other substrates. Many of these are triply bonded molecules. Acetylene is reduced to ethylene but not further reduced to ethane. If the reduction is carried out in D_2O the product is always cis-1,2-deuteroethylene. Acetylene is the most active substrate for the enzyme as it alone can complete 100% effectively with H_3O^+ as no dihydrogen is produced when acetylene is being reduced. The reduction of acetylene is commonly used as an assay for nitrogenase. Azide undergoes a two electron-reduction to N_2 and NH_3.

Redox properties of Nitrogenase

- Nitrogenase contains a number of redox active metal clusters to receive and transfer of electrons. Therefore electron transfer in this enzyme, particularly in the Mo-Fe proteins is a complex process. During nitrogenase activity, Mg-ATP binds to the Fe protein resulting in Mg-ATP-Fe protein which then binds to the Mo-Fe protein with transfer of electrons to the Mo-Fe protein and hydrolysis of the Mg-ATP.

Hemerythrin

- Hemerythrin is a non-heme iron protein used by two phyla of marine invertebrates for oxygen transfer and/or storage.
- The two iron atoms in hemerythrin are bound the imidazole rings of five histidine residues and the carboxylates of an aspartic acid and a glutamic acid. In addition, the complex contains an oxygen atom bridging between the two iron atoms.
- In deoxyhemerythrin, the bridge is a hydroxyl group, while in met- and oxyhemerythrin, the bridge is a mu-oxo atom. In deoxy- and metaquohemerythrin, one of the iron atoms is bound to six liganding atoms while the other is penta-coordinate. This extra site is where small molecules such as dioxygen or azide bind to the protein.



- In deoxyhemerythrin, the two iron atoms are in the ferrous oxidation state with a bridging hydroxyl group.
- As dioxygen is bound to the active site, the hydrogen atom from the hydroxyl bridge moves over onto the bound ligand, stabilizing the peroxo nature of the bound oxygen molecule.
- In met- derivatives of the protein, with and without small molecules bound to the complex, the iron atoms are both in the ferric oxidation state.
- Most hemerythrin do not bind dioxygen cooperatively. They show tight oxygen binding, but the subunits in the oligomeric forms generally act as individual binding sites.

Non-heme iron proteins

Fe-S proteins

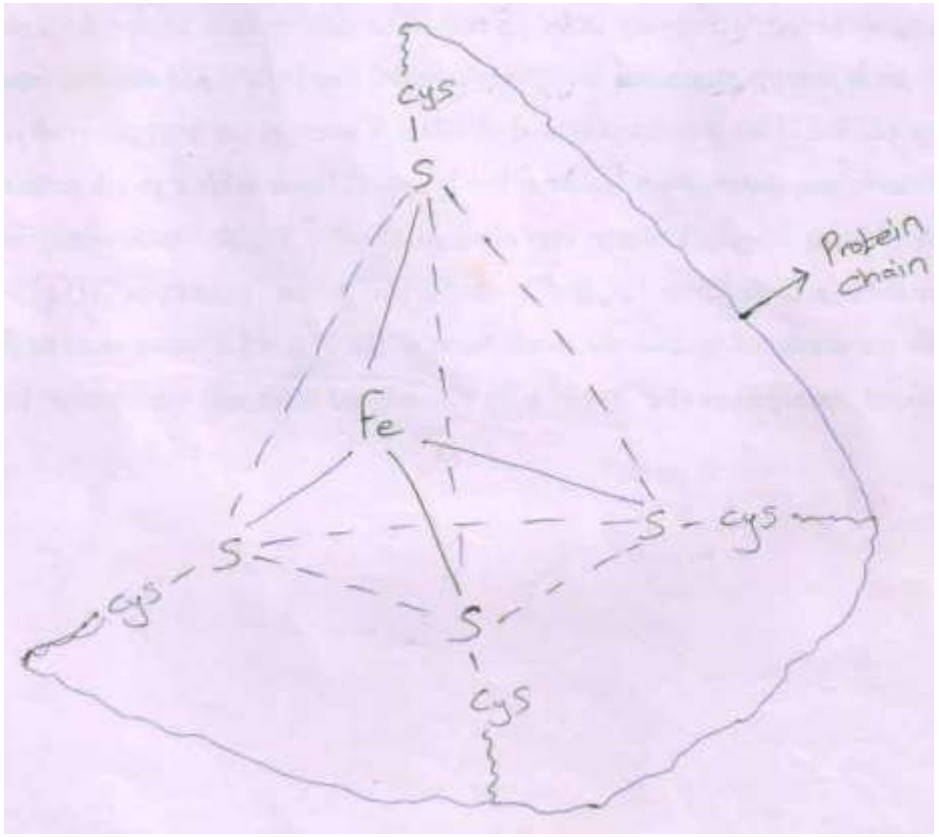
- Iron-sulphur proteins are non-heme proteins which are found in all living organism. They are involved in a wide range of electron transfer process. They are also involved in important redox process such as nitrogen fixation and electron transfer in mitochondria.
- In iron-sulphur proteins, both the cysteinyl sulphur and inorganic sulphur are present as S^{2-}. The inorganic sulphur are labile as they can be removed as H_2S on acidification.
- The iron-sulphur protein are represented as n Fe – m S, where n denotes the number of cations per protein molecule, S denotes the labile sulphur and m denotes the number of labile sulphur sites per protein molecule.

Rubredoxin (Rd)

It is the simplest bacterial iron-sulphur protein with mol.wt 6000. The protein chain is folded to create the tetrahedral cavity by four cysteinyl moieties with the Fe atom at the center.

It is a one electron transfer agent involving the couple Fe^{2+}/Fe^{3+} in which both Fe^{2+} and Fe^{3+} remain in high spin state in tetrahedral symmetry. The tetrahedral symmetry is distorted in both the oxidized and reduced forms.

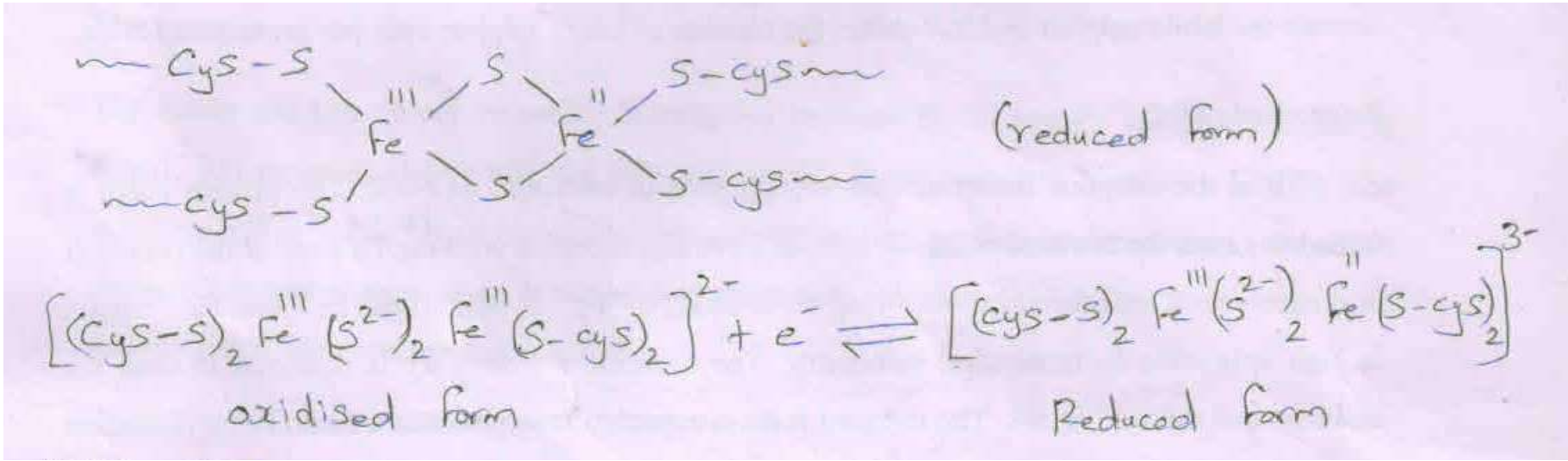
The reduced form is expected to experience a John-Teller distortion but the oxidized form does not experience any John-Teller distortion. This distortion is important for rapid electron transport through an outer sphere process.



Ferredoxins (Fd)

(i) 2Fe – 2S

It is also known as Fe_2S_2 protein. It is a binuclear moiety with two bridging inorganic sulphurs. Though there are two Fe centers but it acts as a one electron transfer agent i.e., in the oxidized form both the ions are in +3 state while in the reduced form, each iron centre remains in high spin state in a distorted tetrahedral symmetry. It acts as a one electron transfer protein. In the reduced form the metal centers are non-equivalent (Fe^{2+} and Fe^{3+}) while in the oxidized form both the centers are equivalent (both are Fe^{3+}). It act as a one electron transport protein.

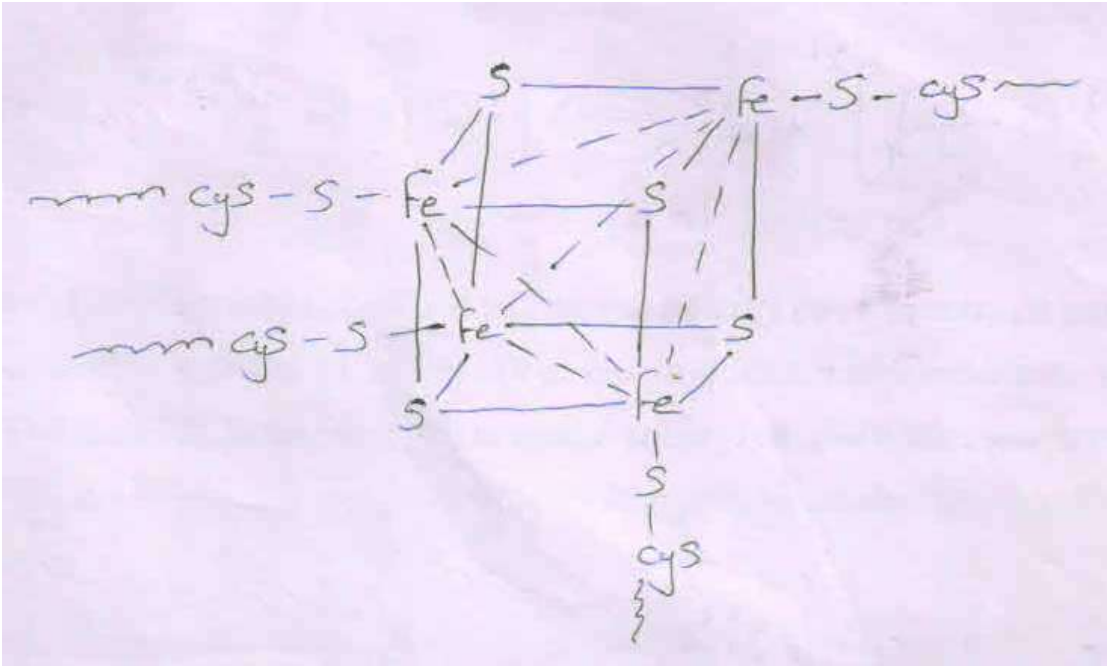


(ii) 4Fe – 4S (4Fe – Ferredoxin)

The [4Fe-4S] proteins are cubic with the iron occupying alternate corners of the cube with triply bridging sulphides occupying other corners. The iron is also ligated by cysteinyl residues. 4Fe-4S clusters usually undergo one electron transfer.

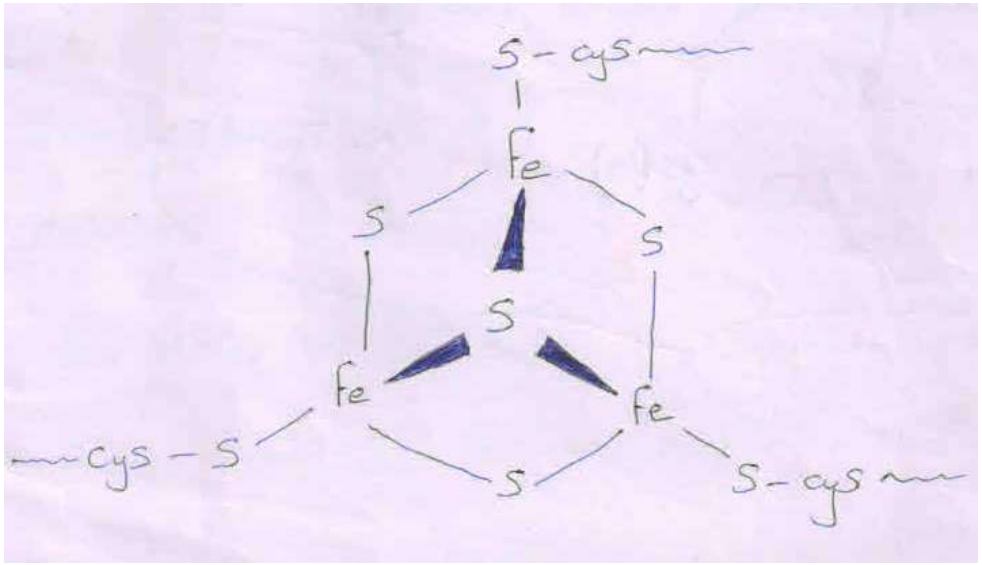
Cyclic voltammetry studies indicate that it can exist in three forms i.e., $(Fe^{3+})_3$ (Fe^{2+}), $(Fe^{3+})_2$ $(Fe^{2+})_2$ and (Fe^{3+}) $(Fe^{2+})_3$. The first and last forms are separated by two electrons.

Most of the 4Fe, 4S clusters exist as $(Fe^{3+})_2$ $(Fe^{2+})_2$ in the oxidized form and as (Fe^{3+}) $(Fe^{2+})_3$ in the reduced form.



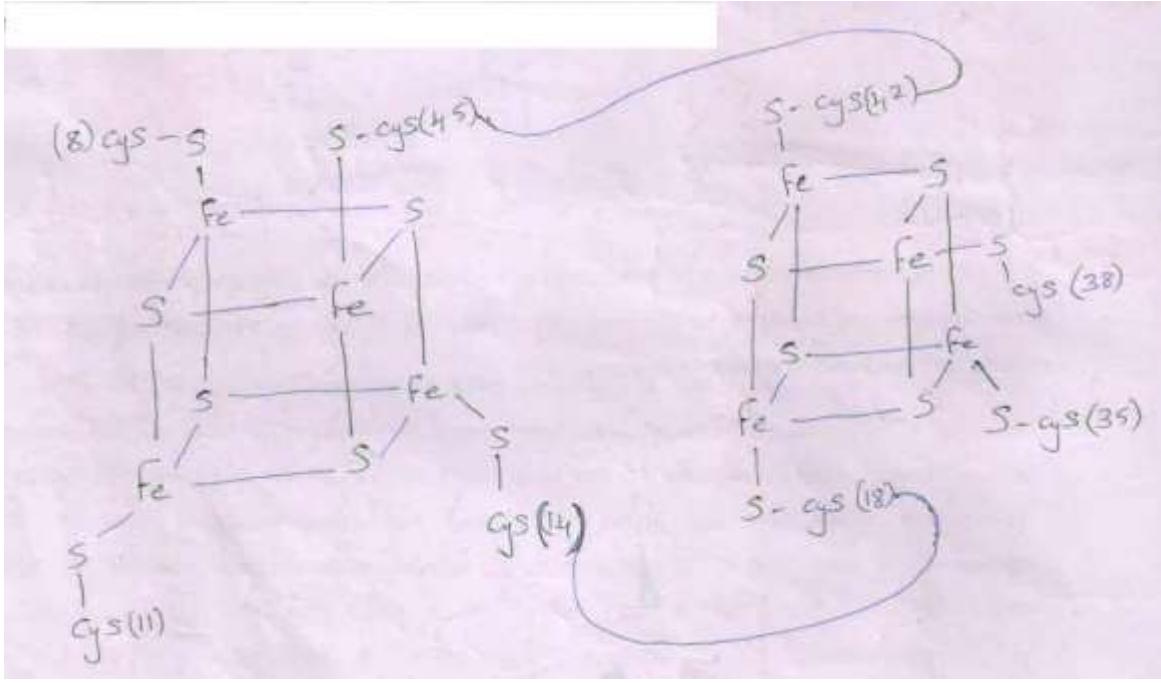
(iii) 3Fe – 4S

Fe_3S_4 cluster protein can be considered as Fe_3S_4 cubane type cluster where one Fe center is missing from one corner of the distorted cube. Hence 3Fe-4S ferridoxin is described as "Void-cubane" or "Fe-depleted cubane" protein



(iv) 8Fe – 8S

Fe_8S_8 cluster consists of two Fe_4S_4 cluster units separated by about 12 ^oA. Each Fe_4S_4 unit can act as one electron transfer center just like 4Fe-4S ferredoxin. 8Fe-8S ferridoxin can function as a two electron carrier. The two Fe_4S_4 cubes are linked through protein chains.



- **Blue copper proteins**

The functions of copper proteins include electron transport, copper storage and many oxidase activities. These proteins are present in different organisms from bacteria to humans. Several copper proteins are easily identified by their blue colour are called as ‘blue copper proteins’. Copper species present in proteins are of three types as follows

- **Type I copper**

Blue proteins with single type I copper are usually associated with electron transfer. These blue species give intense absorption band near 600 nm and have EPR spectrum with small hyperfine splitting constant and high redox potentials.

- **Type II copper**

These are less intensely coloured or colourless at normal concentrations. Bovine erythrocyte superoxide dismutase (BSOD) is an example of Cu protein containing type II center with λmax 680 nm.

- **Type III copper**

This is present in all multicopper oxidases and consists of two copper (II) ions which are diamagnetic. It can act as two electron donor/acceptor center and is essential for reduction of dioxygen. It gives strong absorption at 330 nm.

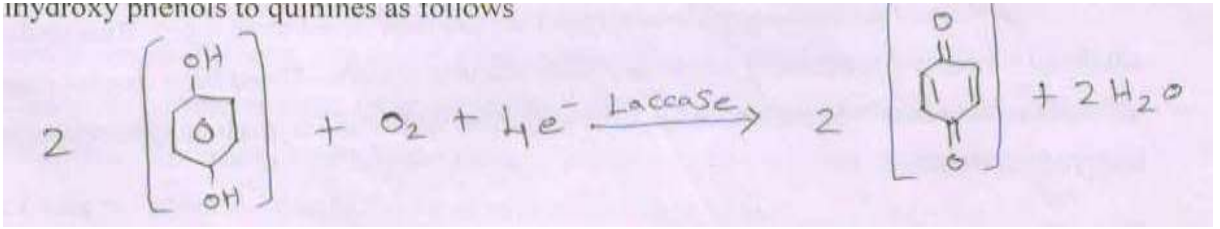
Blue copper proteins can be divided into two classes based on their functions.

- Oxidases
- Electron carriers

I. Blue – Oxidases with types I,II and III Copper:

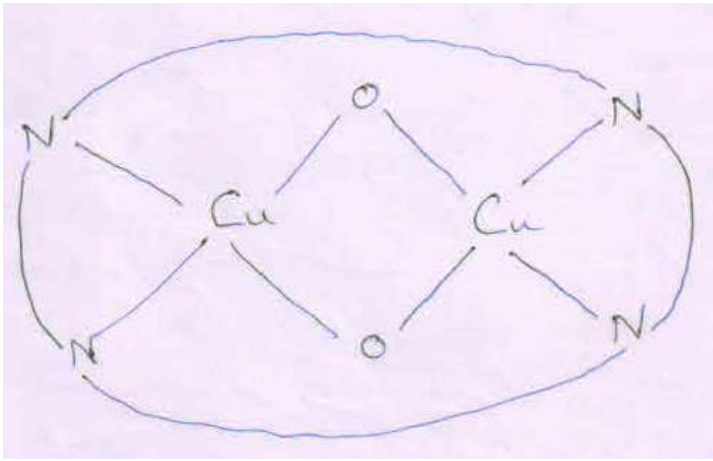
Laccase

It is a water soluble enzyme and easily purified. It contain type I and type II copper and two moles of antiferromagnetically linked type III copper. It's mol.wt is 1,20,000 amu. It shows three absorption bands at 532, 615, 730 nm. It catalyses the oxidation of o- and p-dihydroxy phenols to quinines as follows



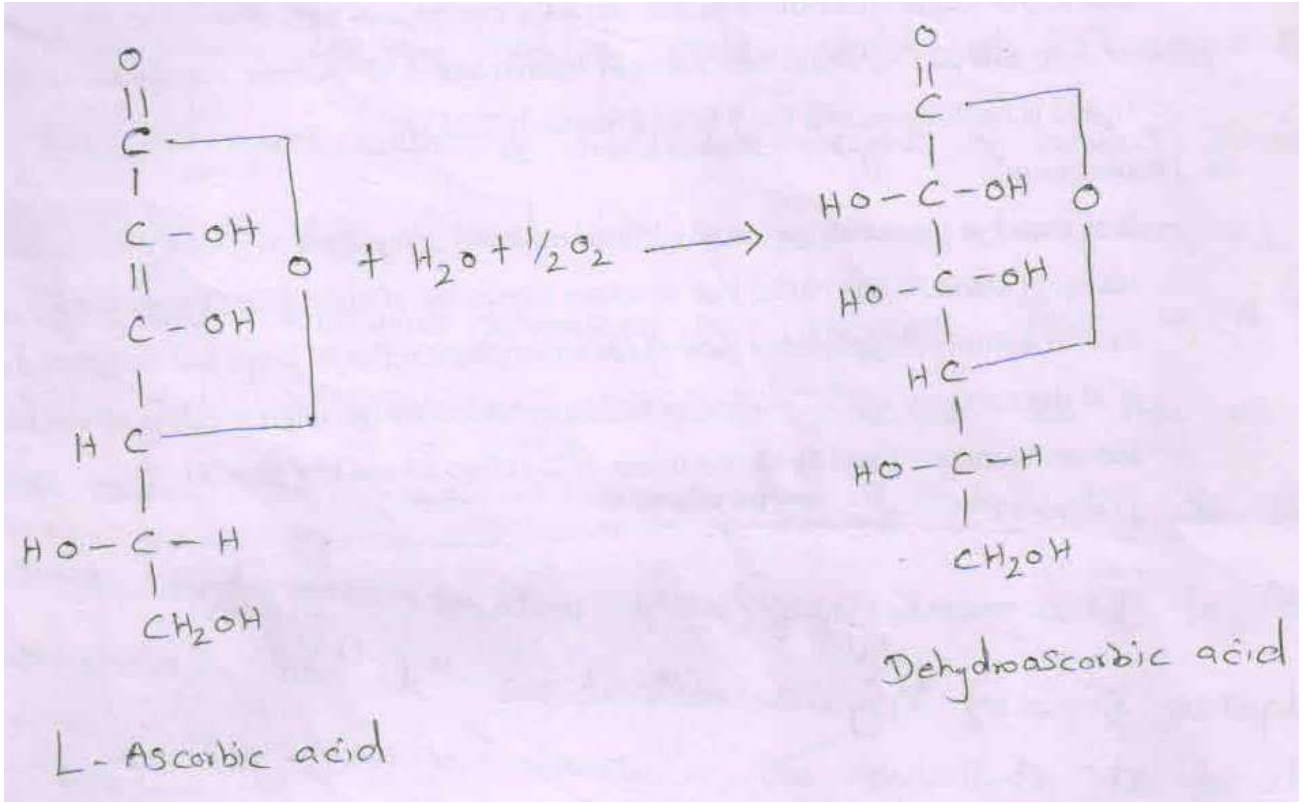
Ceruloplasmin

This protein is synthesized in the liver and contains7 or 8 Cu atoms per mole and two type I, one type II and four type IIIsites. The physiological role of ceruloplasmin is the oxidation of iron(II), while it may out as copper transport protein i.e., the Cu present in this enzyme is incorporated into cytochrome oxidase and other enzymes. Ceruloplasmin has been called enzymatic copper protein.



- **Ascorbic acid oxidase**

It is located within the soluble parts of the cytoplasm and in cell-wall material. It is deep blue green in colour and contains copper. The Cu bound to the protein is non-dissociable i.e., it doesn't exchange with Cu^{64}. The enzyme oxidises L-Ascorbic acid which is catalysed in the presence of oxygen as shown below



- **II. Blue-Electron transfer proteins with a single type I copper:**

The primary function of these proteins is electron transfer. The most studied proteins are azurins and plastocyanin.

- **Azurin**

Azurins contain one copper ion (type I) per mole of protein. Its mol.wt is 16,000. X-ray and EXAFS data on oxidised azurin show the presence of very short Cu-S bond distance of 2.10 ± 0.02 ^{o}A, while two nitrogen ligands are 1.97 ^{o}A from the metal. The fourth ligand is methionine and Cu-S bond distance is 2.25 ^{o}A.

- **Plastocyanin**

It is found in plant chloroplasts and is an essential component of photosynthetic electron transport chain. Cu is co-ordinated by sulphur atoms of cysteine-84 and methionine-92 and by the nitrogens of histidine-37 and histidine-87.