Monika Singh
Paper-IV,
Unit-I

UNIT 1

METAL IONS IN BIOLOGICAL SYSTEM : ESSENTIAL AND TRACE METALS

Bio-inorganic chemistry constitutes the discipline at the interface of the more classical areas of inorganic chemistry and biology. This has two major components: the study of naturally occurring inograníc elements in biology and the introduction of metals into biological systems.

The Chemistry of life involves many elements which are essential and indispensible in biological system. The elements are classified on the basis of their action in biological system and the molecules possessing one or more metallic elements are called metallobiomolecules. These metallobiomolecules are natural products and are usually complex

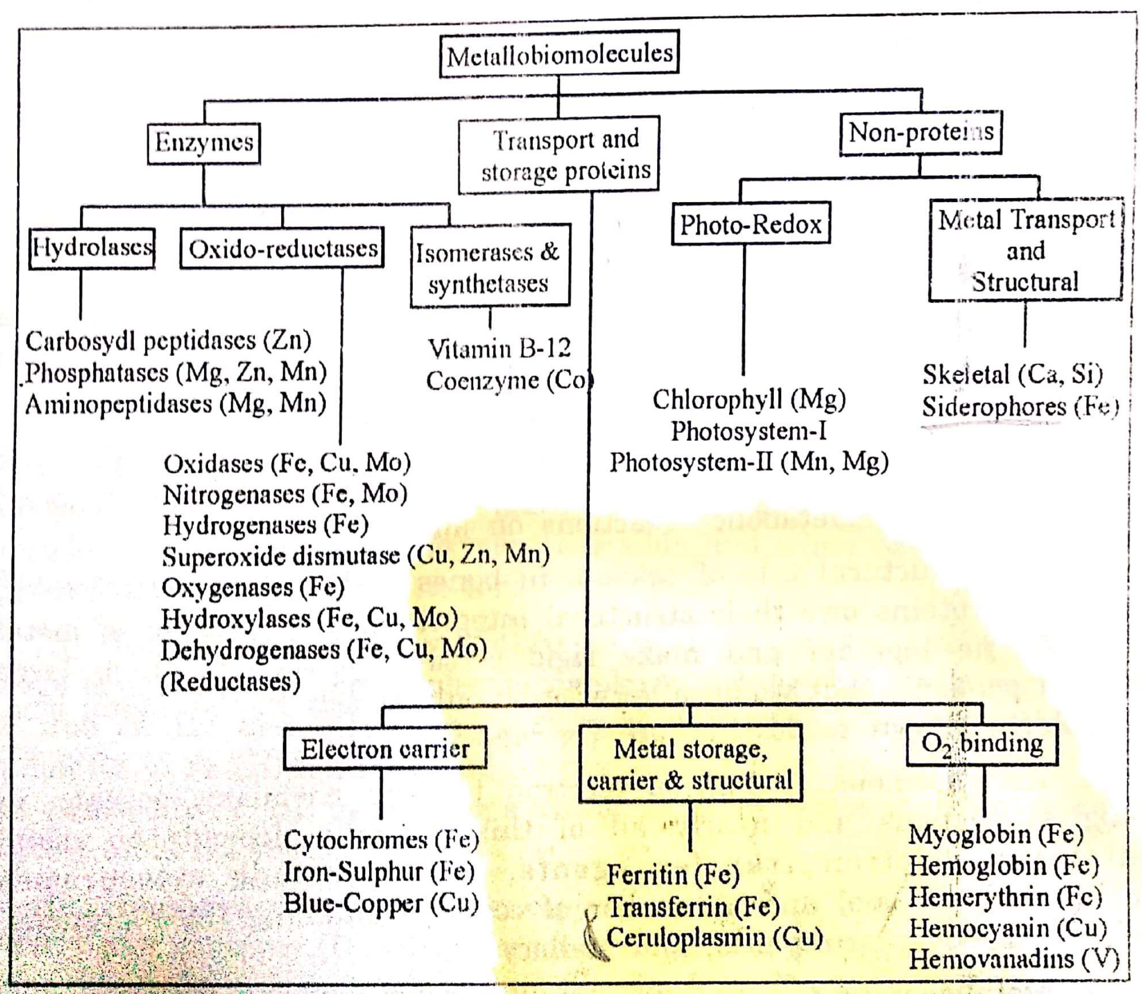

Fig. 1.1. Classification of Metallobiomolecules.

co-ordination compounds. The active sites (metal sites) contain various biochemical processes such as electron transfer binding of exogenous molecules and catalysis (Fig. 1.1)

Essential and Trace Elements :

There are thirty elements that have been recognized as essential and indispensible (absolutely necessary) to life. The elements that are required in relatively large quantity are called macronutrients and those which are required in small quantities are called micronutrients.

The essential elements can be further divided into two group :

(i) **Bulk elements :** essential elements found in higher concentration.

(ii) **Trace elements :** essential elements present in low concentration.

Table 1.1. Classification of essential elements

Bulk Structural elements	Mineral elements	Trace elements	Ultra trace elements	
			Metals	Non-metals
Hydrogen, **H** Carbon, **C** Nitrogen, **N** Oxygen, **O** Phosphorus, **P** Sulphur, **S**	Sodium, **Na** Potassium, K Magnesium, Mg Calcium, Ca Chlorine, Cl	Iron, Fe Copper, **Cu** Zinc, **Zn** Vanadium, V Nickel, **Ni** Cadmium, Cd Lithium, **Li** Lead **Pb** Tin, **Sn**	Manganese, **Mn** Molybdenum **Mo** Chromium, **Cr** Silicon, **Si** Arsenic, **As** Selenium, **Se**	Fluorine, **F** Iodine, **I** Boron, **B**

The metallic elements play a variety of roles in biological system. Important roles are as follows.

1. Regulatory action is exercised by Na^+, K^+, Mg^{+2} and Ca^{+2}. The flux of these ions through cell membranes and other boundary layers send signals that turn metabolic reactions on and off.

2. The structural role of calcium in bones and teeth is well known, but many proteins owe their structural integrity to the presence of metal ions that tie together and make rigid certain portions of these large molecule portions, that would otherwise be only loosely linked. Metal ions, particularly known to do this, are Ca^{+2} and Zn^{+2}.

3. An enormous amount of electron transfer chemistry goes on in biological systems and nearly all of this critically depends on metal containing electron transfer agents. These include cytochromes (Fe), Ferredoxin (Fe) and a number of copper containing "blue proteins" such as azurin, plastocyanin, and stellacyanin.

4. **Metalloenzymes** or metallocoenzymes are involved in a great deal of enzymatic activity, which depends on the presence of metal ions at the active site of the enzyme or in a key coenzyme. Of the latter, the best known is vitamin B_{12}, which contains CO. Important metalloenzymes

include carboxypeptidase (Zn), alcohol dehydrogenase (Zn), superoxide dismutase (Cu, Zn), urease (Ni) and cytochrome P-450 (Fe).

5. All aerobic forms of life depend on oxygen carriers, molecules that carry oxygen from the point of intake (such as the lungs) to tissues where O_2 is used in oxidative processes that generate energy carriers and all of them contain metal ions that peroxide the actual binding sites for the O_2 molecules. These types are:

Hemoglobins (Fe), found in all mammals.

Hemerythrins (Fe), found in various marine invertebrates.

Hemocyanins (Cu), found in arthropods and molluscs.

Functions of elements on the basis of their action in biological system:

The roles of different elements in the biological processes are :

A. Metals :

Commonly exist as metallobio-molecules and occur as such.

Metal storage and Transport

(i) Ceruloplasmin : It is blue copper binding plasma protein which exerts a catalytic activity (serum ferroxidase) in plasma to convert Fe (II) into Fe (III).

(ii) Transferrin : Iron released from the mucosal cell centers the portal blood mostly in Fe (II) state. Fe (II) is oxidised to Fe (III) and then picked up by iron binding protein, transferrin.

This protein is a glycoprotein of MW–77000 possessing two similar but not identical sites that bind two atoms of Fe (III) per molecule of protein to form a red ferric protein complex.

B. Oxygen binding metallo-biomolecules :

(i) Hemerythrin : It is a non-heme oxygen binding iron protein employed in great variety of marine worms and phyla of marine invertebrates. Iron (II) binds oxygen reversibly and when oxidised to Iron (III) (methemerythrin) shows no tendency to bind oxygen.

(ii) Hemoglobin and myoglobin : Function of oxygen transport and storage is provided by Iron-porphyrin complexes, hemoglobin and myoglobin. The iron in Hb and Mb is in (+ 2) oxidation state. Oxygen molecule coordinates to Fe (II) from one end of heme and globin protects the Fe (II) to be oxidised to Fe (III) by creating a hydrophobic environment so that water molecule could not reach the site.

(iii) Hemocyanin : It possesses copper and bind one molecule of oxygen for every pair of copper (I) ions. When oxygen binds to hemocyanin its colour is blue otherwise unbound hemocyanin is colourless. They are found only in molluscs and arthropods (crustacea and arachnids).

Electron Carriers : Fe–S

(i) Iron sulphur protein : Many non-heme iron sulphur proteins take part in electron transfer made up of peptide chains having cysteine (amino acids) moiety where cysteine sulphur is bounded to iron or cluster of iron sulphur atoms. Fe – S clusters are known as ferredoxins and these are the electron acceptors in photosynthesis.

(ii) Blue copper protein : This is an electron carrier in photosynthesis and is primarily present in algae, green leaves and other plants. The different forms of blue copper proteins are azurin, plastocyanin, stellacyanin and umecyanin.

(iii) Cytochromes : These are heme proteins which behave as electron carrier and take part in photosynthesis. It contains a porphyrin ring chelated to iron atom. Cytochromes are found in all aerobic forms of life. The oxidation state of iron can be either (II) or (III) and occurs as redox intermediates in electron transfer reactions. They are present in chloroplast for photosynthesis and in mitochondria for reverse process of respiration.

Non-protein metal transport :

Skeletal — (i) Calcium occurs in larger amount than any other mineral element. About 99% of the body Ca is in the skeleton, where it is present as deposits of Ca_3PO_4 in a soft fibrous matrix.

(ii) Phosphorus occurs in every cell of the body but most of it (about 80% of the total) is combined with Ca in bones and teeth, 10% is in combination with proteins, lipids and carbohydrates and in blood and muscles. The great importance of the phosphate ester in energy transfer is also known.

(iii) Silicon appears to be an integral component of acid mucopolysaccharides and have structural role in connective tissue, cartilage, skin and bone. Silicon is an essential element for growth and skeletal development.

(iv) Sodium is the major component of the cations and the extracellular fluids which occurs as chloride and bicarbonate. It helps in regulating acidbase equilibrium. It also helps in the maintenance of osmotic pressure of the body fluid.

(v) Lithium is in the form of Li_2CO_3 employed in the treatment of manic depressive illness. Li^+ is present in intracellular and extracellular fluids.

(vi) Potassium is present as cation in intracellular fluid as well as in extracellular fluid. It functions as sodium in extracellular fluid by influencing acidbase equilibrium osmotic pressure and water retention. It is necessary for metabolic functions including protein biosynthesis by ribosomes.

(vii) Zinc ions are essential for normal growth, reproduction and play a major role in tissue repair and wound healing.

(viii) Manganese essential for normal bone structure, reproduction and the functioning of central nervous system.

Thus life needs a continuous material exchange which principally, includes all chemical elements. The occurance of these elements in organisms depends on external and endogenous conditions. Elements can be bioavailable to variable extents but can also be enriched (bioaccumulated) by organisms employing energy utilising processes involving a local reduction of entropy.

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UNIT 2

ROLE OF METAL IONS IN BIOLOGICAL PROCESSES

SODIUM/POTASSIUM PUMP :

Introduction :

The cations Na^+, K^+ as such can not pass through the lipid layer of the double walled protein cell membrane. The encapsulation of metal ions facilitates their transport, the enclosed cations present in an organic lipid soluble surface called **Cavitands** can pass through the cell membrane. In the animal cells Na^+ ion concentration is 5–15 mM and K^+ ion concentration is 100-140 mM. In the extracellular space the situation being reverse (Na^+ is 100-140 mM and K^+ is 5-15 mM), this is essentially needed for the survival of the cell and many other physiological functions as well.

(i) Na^+, K^+ ions and other gradients are required for the transmission of nerve impulse.

(ii) They help in transport of amino acids, sugars, nucleotides and other substances.

(iii) They also regulate the cell volume and cell shape.

(iv) High cellular K^+ ion concentration is required for the optimal glycolysis and protein synthesis.

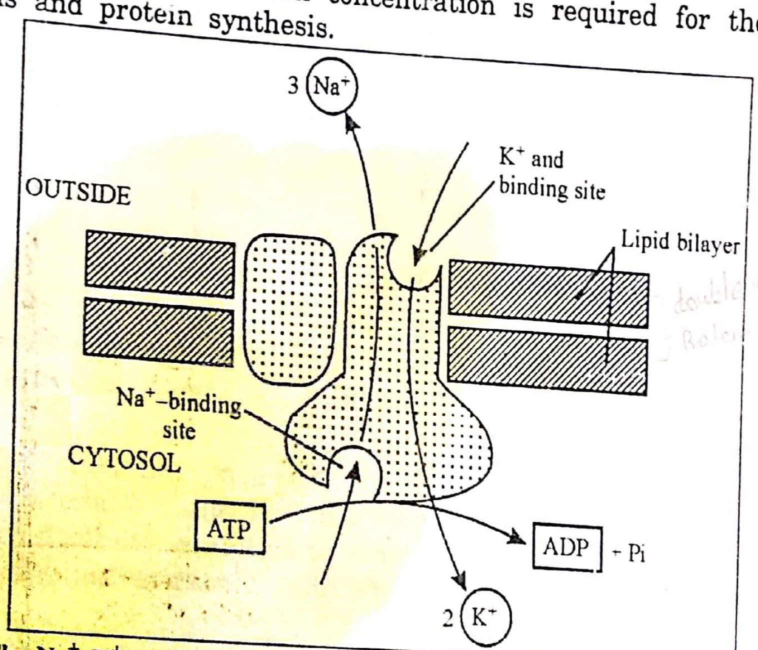

Fig. 2.1. The Na^+–K^+ ATPase pump for transport of three Na^+ out of a cell for every two K^+ into a cell against their gradients.

Thus upto 20-40% of the total metabolic energy of the cell is consumed in the maintenance of these ion ingredients, this energy consumption reaches upto 70% in the neural tissues. The energy to maintain high K^+ ion concentration and low Na^+ ion concentration in the cytosol is provided by a protein in the cell membrane, called Na^+, K^+ ATPase or Na^+/K^+ pump. The energy derived from ATPase causes the transport of Na^+, K^+ ions from lower concentration to higher concentration. This is known as active transport or pump.

Working : The Na^+, K^+ ATPase consists of two α, β subunits possessing cytoplasmic and extra-cellular domains, respectively. Each ATPase enzyme molecule binds sodium ion and hydrolyses ATP to ADP on the cytoplasmic side of the membrane (Fig. 2.1), to pump out three Na^+ ions out of the cell and two K^+ ions are transported into the cell. Since there is net movement of one positive charge outward per cycle, the Na^+/K^+ pump is considered **electrogenic** in nature. The net process can be represented as

$$\text{ATP} + H_2O + 3Na^+ \text{(inside)} + 2K^+ \text{(outside)} \longrightarrow \text{ADP} + \text{Pi} + H_2O$$
$$+ 3Na^+ \text{(outside)} + 2K^+ \text{(inside)}$$

The enzyme ATPase exists in two–forms–E_1 and E_2. The E_1 form has affinity for Na^+ and ATP, it readily gets phosphorylated in presence of Mg^{2+} to form E_1–P carrying three occluded Na^+ ions. E_1–P changes to E_2–P , having low affinity for Na^+ but high affinity for K^+. $E_2 - P$, therefore, releases $3\,Na^+$ ions and binds $2K^+$ ions on the outside of the cell giving rise to $E_2 - 2K^+$ (Fig. 2.2) which releases $2K^+$ ions in the interior of the cell.

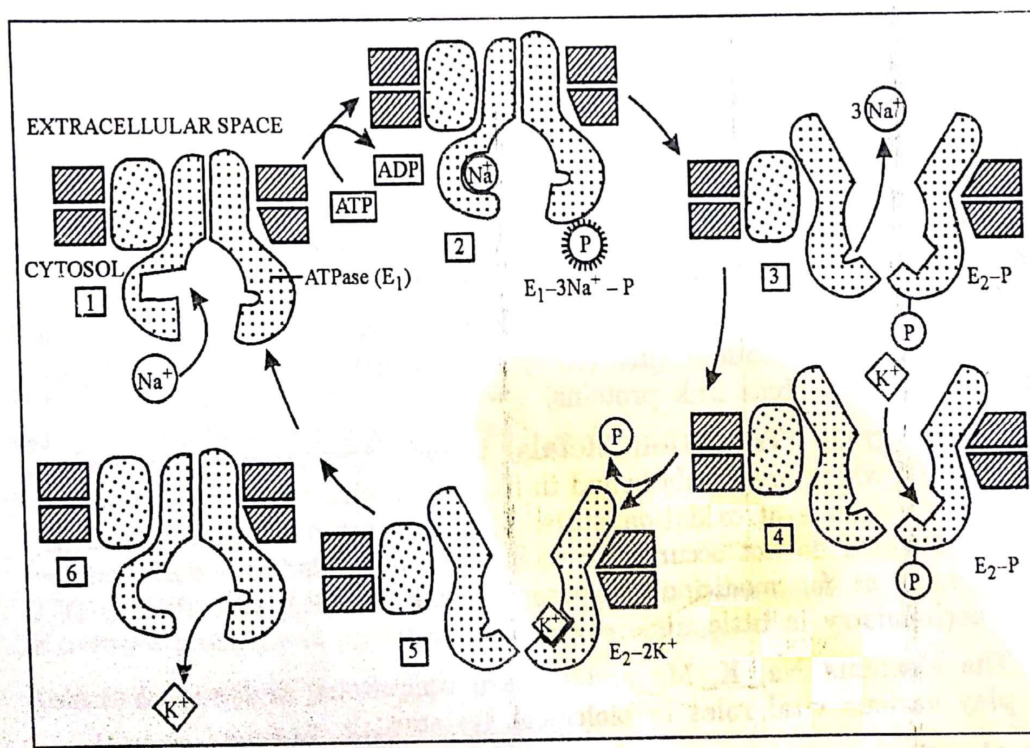

Fig. 2.2. A model for the pumping cycle of Na^+–K^+ ATPase involving reversible Na^+ dependent phosphorylation and K^+ dependent dephosphorylation of ATPase.

It has been shown that Na^+, K^+ ATPase, in its $E_2 - P$ state is used by cardiac glycosides for binding, forming a stable $E_2 - P -$ (cardiac glycoside) complex. This inhibits the function of Na^+, K^+ ATPase resulting in inhibition of sodium pump. This situation leads to accumulation of Na^+ ions in these cells, encouraging endosmosis thus leading to swelling/ bursting of cells and consequent narrowing of blood vessels creating hypertention.

ROLE OF METAL IONS IN BIOLOGICAL PROCESSES :

Living organisms store and transport the metal ions in appropriate concentration to carry out various biological processes. The important metal ions of biological system can be divided into four major groups. (according to periodic table), as shown in table (2.1) below.

Table 2.1. The Division of Metals in Biology

	Group IA	Group II A	Group II B	Transition Metals
Examples	Na^+, K^+	Mg^{2+}, Ca^{2+}	Zn^{2+}, Ni^{2+}	Mn, Fe, Co, Cu, Mo
Exchange rate	Fast	Medium	Do not Exchange	Do not exchange
Function	Osmotic Control Electrolytes Ion Currents	Triggers and Conformational control (Structures)	Acid Catalysts	Redox Catalysts

First group (IA) comprises of the metals which occur largely as free ions *e.g.*, Na^+ and K^+. They provide an osmotic balance and an electrolyte current.

Second group (IIA) of metal ions Mg^{2+} and Ca^{2+} are regulators of binding, since they can be pulsed in and out of biological chambers. They can control the protein triggers of biological activity.

Third group (IIB) of metals are usually represented by the element Zinc (Zn^{2+}), now include nickel (Ni^{2+}) also. These ions assist acid/base catalysis and can cross link proteins.

Fourth group (**Transition Metals**) include transition elements Fe, Cu, Mn, Mo, which are firmly bound to fixed sites and act in vast majority of cases by change of oxidation state. The last group of metals (not in the table) which do not occur in vivo, but can be introduced deliberately as poisonous or for medicinal purposes, *e.g.*, V, Cr, Hg, Au, Pt, Li, Bi, their biochemistry is little understood.

The elements Na, K, Mg and Ca are ubiquitous in living systems and play various vital roles in biological systems.

Role of sodium and Potassium :

In living organisms sodium and potassium occur as the salts of inorganic acids (chlorides, phosphates and carbonates), salts of proteins and organic acids. Sodium is the main extracellular cation, whereas potassium is the main intracellular cation. Important physiological functions which these ions perform in the body of plants and amimals are :

1. Maintainance of normal hydration of the body through osmotic regulations controlled by sodium/potassium pump.

2. Maintainance of normal acid base equilibrium by forming buffers, which play important role in the regulation of pH under different physiological conditions.

3. In the gaseous transport of CO_2.

4. Maintainance of normal neuromuscular irritability and excitability.

5. Maintainance of proper blood viscosity by keeping the globulins in physical solution and regulating the degree of hydration of plasma proteins.

6. Na^+, K^+ ions are responsible for secreting digestive juices like pancreatic, bile and gastric HCl etc.

7. Potassium is responsible for cell growth, repair and storage of proteins and glycogen.

8. Sodium and potassium salts get excreted by kidneys in the urine. Sodium salts are also excreted by skin in the form of prespiration.

Role of Calcium :

Calcium is the major cation in the structural materials, such as teeth, bones, shells and a number of other less well known calcium-rich deposits. These calciferous biological materials, like bones, though consist largely of calcium carbonate and phosphate is continually being deposited and reabsorbed and as well act as buffer for body. Calcium and phosphate ions are controlled by hormonal action. The form of calcium phosphate that occurs in bones and teeth has the same composition as the mineral apatite ($C_{10}(PO_4)_6X_2$, where X = F, Cl or OH).

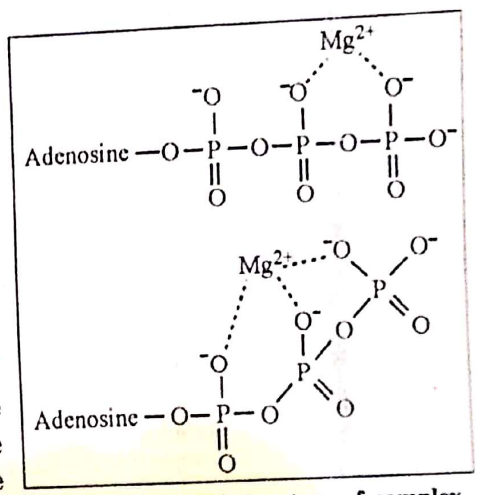

Fig. 2.3. Proposed structure of complex formed between Mg^{2+} and ATP.

Among the most vital functions of Ca^{2+} is its involvement in enzymatic systems, including its being a regulator of muscle contraction, a transmitter of nervous pulses, and an agent of blood coagulation.

Role of Magnesium :

Magnesium has high charge/radius ratio and consequent strong hydration as $[Mg(H_2O)_6]^{2+}$, therefore it plays important role in biological system. One of its major roles is as a counterion to negatively charged $ROPO_3H^-$ groups in nucleotides and polynucleotides. Magnesium helps to stabilize the three diamensional structure of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) and thus crucial to the proper functioning of genetic machinery of the cell.

The conversion of sugars to phospho-sugars is achieved in the presence of adenosine triphosphate (ATP) and catalysed by kinase enzyme. All kinase enzymes require the presence of divalent metal ions, Mg^{2+} being the most common complex with triphosphate group, facilitating the cleavage of a phosphorus-oxygen bond, (Fig. 2.3) Magnesium also has a unique role in green plants as the central atom of chlorophyll (Fig. 2.4).

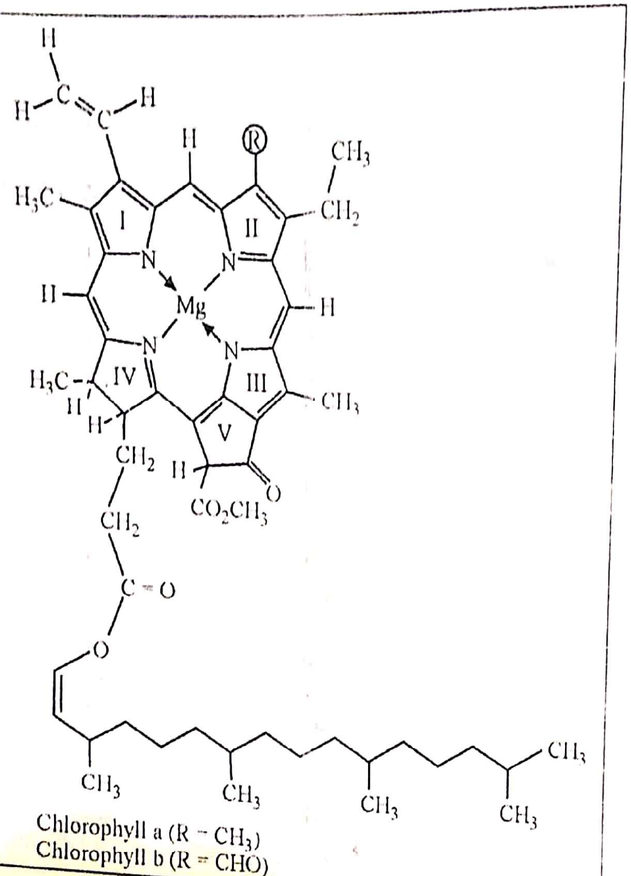

Fig. 2.4. The structure of chlorophyll a and b.

It is required for phosphoresence to occur during the process of photosynthesis.

Role of Zinc :

Zinc metal ion is relatively more abundant in biological materials. In the human body it is found in metalloprotein, which also binds copper, chromium, mercury, and other materials.

Zinc is a common element in nucleic acid polymerases and transcription factors, where its role is structural rather than catalytic. Zinc enhances the stereoselectivity of polymerization of nucleotides under prebiotic reaction conditions. It plays a structural role forming the peptide into multiple domains or zinc by coordination with cystein and histadine.

Carbonic anhydrase and carboxypeptidase (Fig. 2.5) are first recognized zinc enzymes, Later on 80 other zinc enzymes have been reported, which are responsible for staggered physiological functions in the human body, as maintaining the structure of proteins, that activate and deactivate genes.

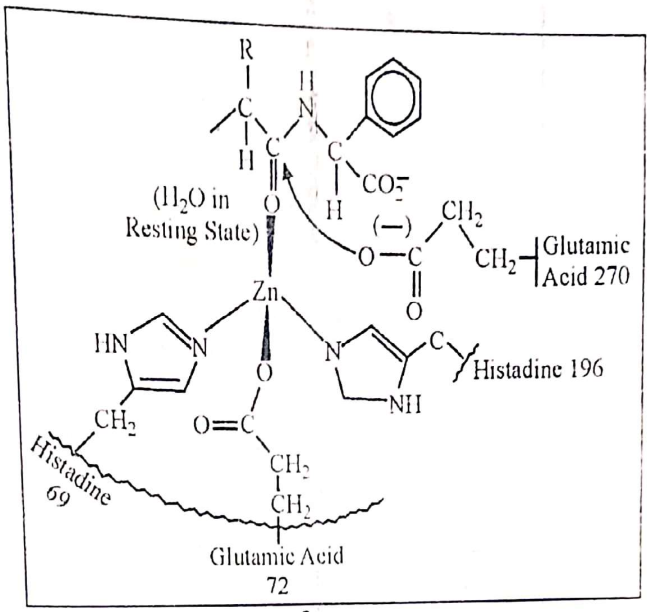

Fig. 2.5. The active Zn^{2+} site of carboxypeptidase A.

Role of Nickel :

Only recently, the biological importance of nickel has been recognized. All the nickel proteins known today are from plants or bacteria. Nickel is a lightly bound component of urease and is essential for the activity of the enzyme that catalyses the hydrolysis of urea to ammonia and carbonic acid. Methanogenic and several other types of bacteria are known to have nickel containing hydrogenases which catalyse the reaction $2H_2 \longrightarrow 2H^+ + 2e$, the reduction of sulphate ion, the production of methane and perhaps other reductive processes. Specific environments for nickel are also indicated for nucleic acts (or nucleic acid binding proteins), since nickel activates the gene for hydrogenase.

Role of Manganese :

Manganese plays a critical role in oxygen evolution catalysed by the proteins of the photosynthetic reaction center. Manganese is the energy source for a series of redox reactions which are dealt separately in photosystem (I) and (II).

In the photosystem (II) a strong oxidising agent is formed (P 680, where P stands for pigment, 680 is the wavelength of light absorbed,) and is responsible for the production of molecular oxygen. This oxidising agent is reduced and recycled for further use by interaction with a manganese complex. During the sequence of events leading to reduction, manganese displays three of its oxidation states. (Fig. 2.6)

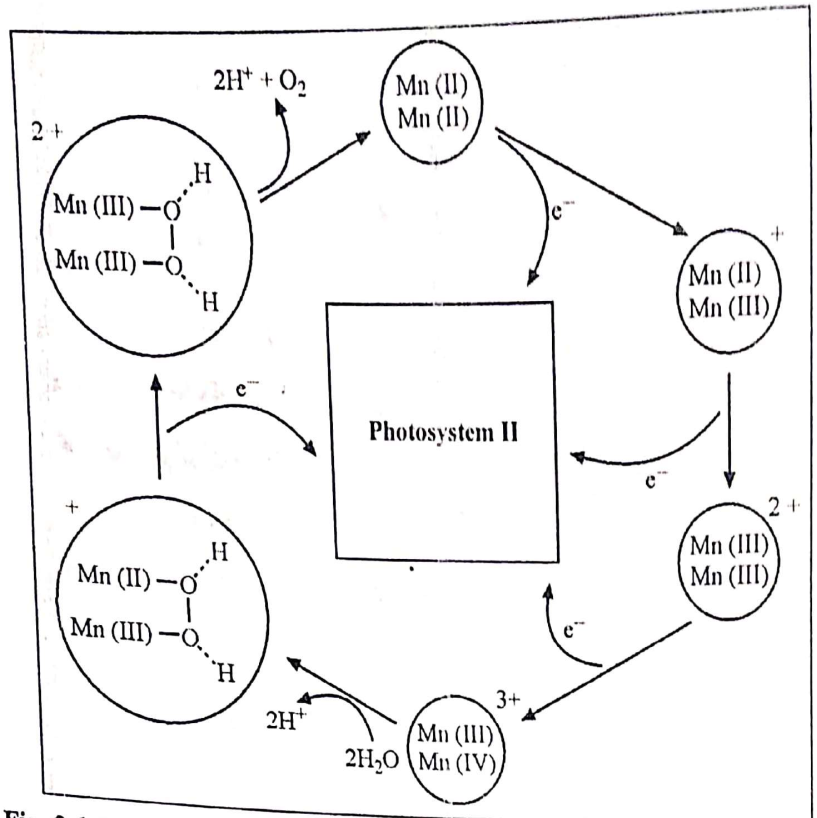

Fig. 2.6. Proposed changes in the oxidation state of manganese in photosystem (II)

Moreover, the superoxide dismutase of bacteria and mitochondria, as well as pyruvate carboxylase in mammals are also manganese proteins, responsible for various biological processes.

Role of Iron :

Iron is the most wide-spread transition metal found in the living system. It is most commonly found in the form of oxides and hydroxides. Iron hydroxides are relatively insoluble in water. To enable the uptake of iron by plants it is sometimes necessary to mark the iron in an organic complex. In the body of mammals iron is stored as iron (III) hydroxide particles surrounded by proteinaceous coat, known as **Ferritin.** Iron is transported through the blood stream via transferrins. In micro- organism solubilization and transportation is achieved by low molecular weight proteins called **sidophores.**

Table 2.2. Functions of iron containing proteins.

Protein	Nature of Iron Heme (H)/Non-heme (N)	Valence State of Iron	Function
Hemoglobin	H	Fe^{2+}	Oxygen transport in plasma
Myoglobin	H	Fe^{2+}	Oxygen storage in muscles
Transferrin	N	Fe^{2+}	Iron transport via plasma
Ferritin	N	Fe^{3+}	Iron storage in muscles
Hemosiderin	N	Fe^{3+}	Iron storage in cells.
Catalase	H	Fe^{2+}	Metabolism of H_2O_2
Cytochrome C	H	Fe^{2+}/Fe^{3+}	Terminal oxidation
Peroxidase	H		Metabolism of H_2O_2
Cytochrome oxidases	H	Fe^{2+}/Fe^{3+}	Terminal oxidation
Flavoprotein Dehydrogenases Oxidases and Oxygenases	N	Fe^{2+}	Oxidation reactions incorporation of molecular oxygen

The two main functions of iron containing materials are, (i) transport of oxygen and (ii) mediation in electron transfer chains. Table (2.2) shows the principal forms of iron containing proteins and their respective functions in the human body. Details of their structure and chemistry of functioning of different iron forms in living organisms shall be discussed in proceeding chapters.

Role of Cobalt :

Cobalt can display three oxidation states +3, +2, +1, the latter is extremely unstable but nevertheless is important in biology. Cobalt ion finds its place in (+3) Oxidation state at the core centre of corrin ring of vitamin B_{12} (Fig. 2.7). Vitamin B_{12} can undergo one electron or two electron reduction leading to Co (II) and Co (I), respectively. These reductions may be acheived by nicotinamide adenine dinucleotide (NADH) and flavinadenine dinucleotide (FAD)

$$[B_{12}\,(\text{Co III})] \xrightarrow{\text{NADH (Ie)}} [B_{12}\,(\text{Co II})]$$

or

$$\xrightarrow{\text{FAD (2e)}} [B_{12}\,(\text{Co I})]$$

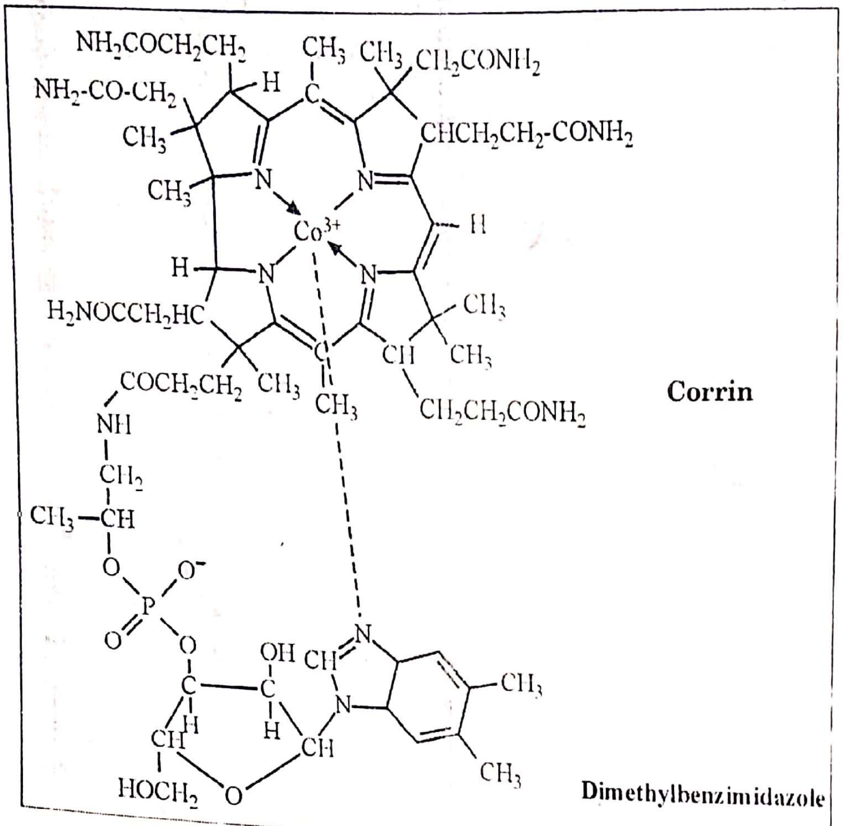

Fig. 2.7. The structure of vitamin B_{12}.

Role of Copper :

Copper is vital in plants and animals. Copper and iron proteins participate in many of the same biological reactions, such as :

(1) reversible binding of oxygen e.g., hemocyanin (Cu protein, A non-heme protein of lower animals such as snails and crabs), hemerythrin (Fe, protein)

(2) activation of dioxygen e.g., dopermine hydroxylase (Cu), tyrosinases (Cu) and catechol dioxygenases (Fe)

(3) electron transfer, e.g., plastocyanin (Cu), ferredoxin (Fe) and Cytochrome-c (Fe).

(4) dismutation of superoxide by Cu or Fe as the redox active metal.

The two metal ions together also function in proteins such as cytochrome oxidase which catalyses the transfer of four electrons to dioxygen to form water during respiration. Use of stored iron is reduced by copper deficiency, which suggests that iron metabolism may depend on copper proteins.

Role of Molybdenum :

Molybdenum displays four oxidation states (+ 6), (+ 4), (+ 3), and (+ 2), the first two being very important in biology. During metabolism of purines, adenine and guanine are broken to uric acid via xanthene. The oxidation of xanthene to uric acid is accomplished in the presence of the enzyme xanthine oxidase. This enzyme is a complex ensemble involving two molybdenum atoms, four iron sulphur clusters and two flavin adenine dinucleotide (FAD) moieties, Molybdenum converts from + 6 to + 4 oxidation state during the cascade of reactions involved in the production of uric acid, (Fig. 2.8)

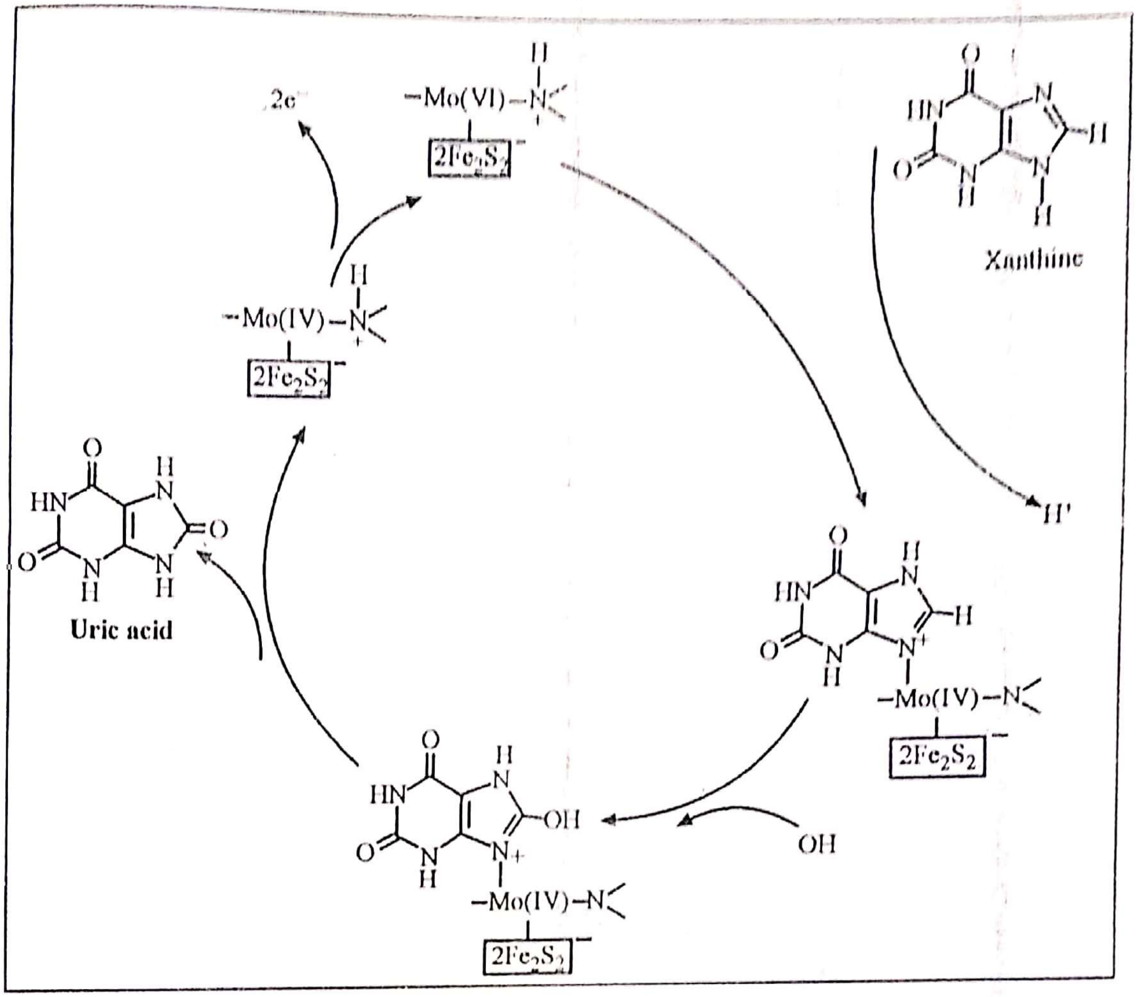

Fig. 2.8. Xanthine oxidation.

Nitrogen fixing bacteria use the enzyme nitrogenase for the fixation of atmospheric nitrogen. The enzyme nitrogenase is comprised of two protein chains, the smaller protein component contains an Fe_4S_4 cluster, while the larger protein (MoFe – protein) itself is tetrameric and has an array of two Mo atoms, large number of Fe and sulphur ions. Both the

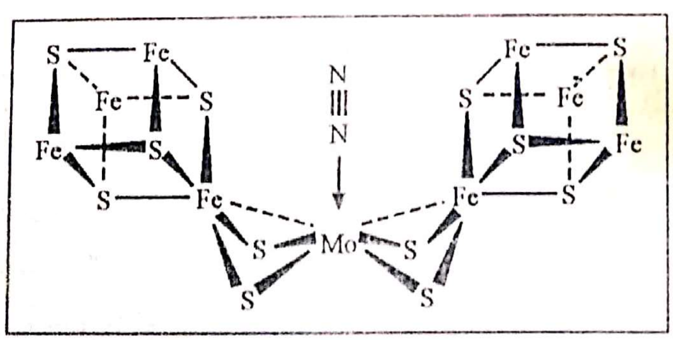

Fig. 2.9. Proposed arrangement of Mo, S and Fe in active site of nitrogenase.

proteins together are required for the nitrogen fixing activity. Molybdenum is vitally important, (Fig. 2.9) It has been shown that bacteria grown in presence of tungsten (VI) oxide rather than molybdenum (VI) oxide can incorporate tungsten, but show no nitrogen fixing activity. It is possible therefore, that nitrogen actually coordinates with molybdenum during the fixation process.

Role of Vanadium and Chromium :

Both vanadium and chromium are present in only small amounts in most organisms. Their biological role is largely unknown. However, vanadium is required for normal health and could act in vivo either as metal cation or as a phosphate analogue, depending on the oxidation state V (IV) or V (V), respectively. Vanadium in a sea squirt (a primitive vertebrate) is concentrated in blood cells apparantly as a major cellular transition metal, but whether it participates in the transport of dioxygen is not known. In proteins vanadium is a cofactor in an algal bromoperoxidase and in certain prokaryotic nitrogenases.

Chromium imbalance affects sugar metabolism and has been associated with the glucose tolerance factor in animals as Cr (III). But little is known about the structure of the factor or any other specific chromium complexes from plants, animals and bacteria. Role of metal ions in biological processes can be summarized as :

Metals	Biological Functions
Sodium	Charge carrier, osmotic balance
Potassium	Charge carrier, osmotic balance
Calcium	Structure; trigger; charge carrier
Magnesium	Structure hydrolase; isomerase
Zinc	Structure; hydrolase
Nickel	Hydrogenase; hydrolase
Manganese	Photosynthesis; oxidase; oxotransfer
Iron	Oxidase; dioxygen transport and storage; electron transfer; nitrogen fixation.
Cobalt	Oxidase, alkyl group transfer
Copper	Oxidase; dioxygen transport; electron transfer.
Molybdenum	Nitrogen fixation; oxidase; oxo-transfer.
Vanadium	Nitrogen fixation; oxidase
Chromium	Unknown, possible involvement in glucose tolerance.