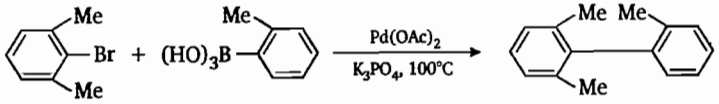
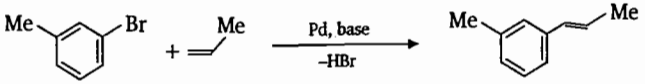
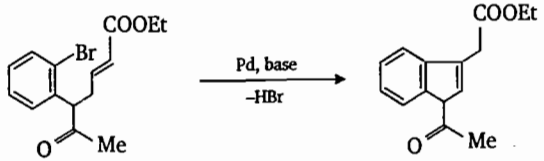
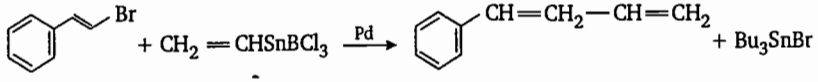
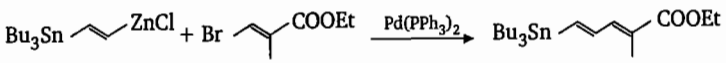
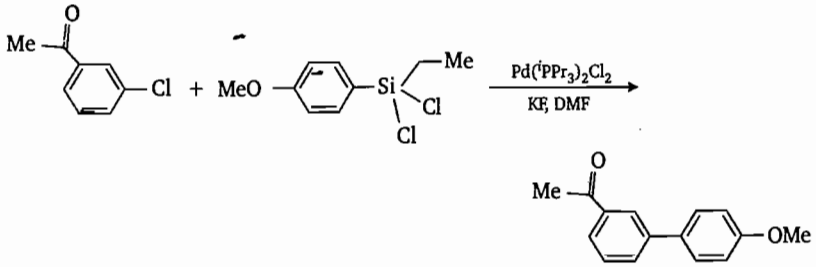
(c) 

(d) 

(e) 

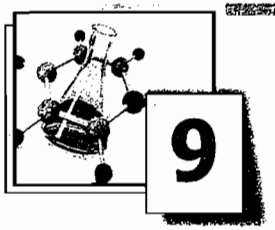
(f) 

(g) 

(h) 

2. What are the basic difference between the Stille and Suzuki coupling reactions ?
3. Write the short note on the following :
   (a) Suzuki coupling reaction
   (b) Stille reaction
   (c) Negishi coupling
   (d) Heck reaction
   (e) Kumada coupling
   (f) Hiyama coupling
   (g) Sonogashira coupling

□□□



9

Bioinorganic Chemistry

Bioinorganic chemistry is a relatively new and still growing interdisciplinary field of chemistry which largely focuses on the roles of metal ions in living systems.

Metalloporphyrins

The metalloporphyrins are the complexes in which a metal ion is coordinated to four nitrogen atoms inside the cavity of the porphyrin ring in a square planar geometry. The axial sites are available for other ligands. Some examples of metalloporphyrins are hemoglobin, myoglobin, cytochromes and chlorophylls.

The porphyrin rings are the dervatives of a macrocyclic ligand called porphine. The porphine molecule consists of unsubstituted tetra-pyrole connected by methylidyne (CH) bridges. These methylidyne carbon positions are labeled the $\alpha, \beta, \gamma, \delta$ and 5, 10, 15, 20 positions in porphine and porphyrin rings respectively. The 5, 10, 15, 20-tetraphenyl derivatives (tpd) are readily available because of their ease of synthesis and purification. In porphyrin rings various groups are attached to the perimeter of porphine molecule. The porphyrin ring can accept two hydrogen ions to form the dication (*i.e.*, + 2 diacid) or donate two protons to form dianion. In metalloporphyrin complexes the inner hydrogen atoms are replaced as protons by dipositive metal ions. Therefore the metal free porphyrin ligand has –2 charges. Since this macrocyclic ligand has a planar conjugated system of π-bonds around its perimeter, it is much more rigid macrocyclic ligand than the crown ethers. Therefore, the ligand is more selective for certain metal atoms than the crown ethers. It has a stronger preferences for the $d^8 Ni^{2+}$ ion. The other metal ions may add above or below the square plane. The structures of porphine molecule, metalloporphyrin and Fe-protoporphyrin IX or heme group are shown in Fig. 9.1.

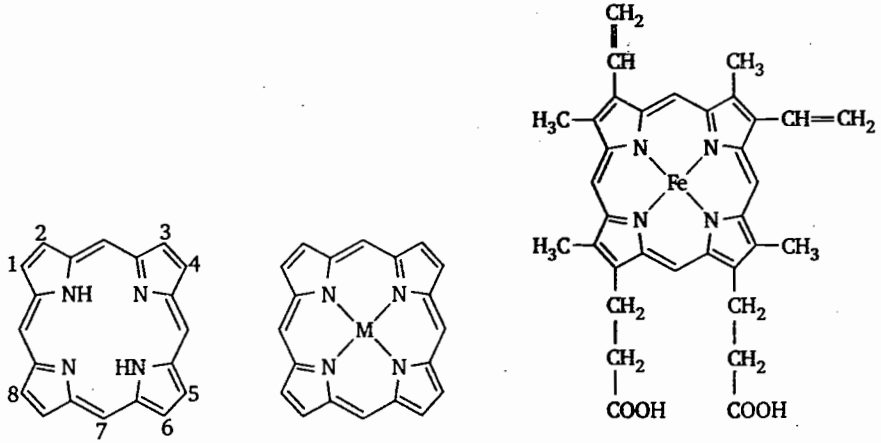


Fig. 9.1 Structure of (a) Porphine (b) Metalloporphyrin (c) Fe-protoporphyrin IX

The porphyrin rings are rigid because of the delocalization of the π-electrons around the perimeter. The size of the cavity in the centre of porphyrin ring is ideal for accommodation of metal ions of the first transition series. If the metal ion is too small such as Ni^{2+}, the ring becomes ruffled to allow closer approach of nitrogen atoms to the metal ion. On the other hand, if the metal ion is too large, it can not fit into the cavity and occupies position above the ring which also becomes domed.

Role of Iron in Living Systems

Iron is the most important transition metal involved in living systems, being vital for both plants and animals. In the living systems, iron has three well characterized systems :

(1) Proteins that contain one or more porphyrin rings such as hemoglobin, myoglobin and cytochrome P_{450}.

(2) Proteins that contain non-heme iron such as iron-sulphur compounds (ruberdoxin, ferredoxins, nitrogenase).

(3) The non-heme diiron oxo-bridged compounds such as carboxylates (hemerythrin, ribonucleotide reductase and methane monooxygenase).

Some important naturally occuring iron proteins and their functions in living systems are listed in Table. 9.1.

Table 9.1 : Iron Proteins and Their Functions

Protein	Molar mass (approximate)	No. of Fe atoms per molecule	Oxidation state	Source	Nature of iron, heme (H) or non-heme (NH)	Functions
Hemoglobin	64500	4	Fe^{II}	animals	H	Oxygen transport
Myoglobin	17500	1	Fe^{II}	animals	H	Oxygen storage
Cytochromes	12500	4	Fe^{II}	plants, animals, bacteria	H	electron transfer
Ferredoxin	6000-12000	2-8	Fe^{II},Fe^{III}	Bacteria, plants, animals	NH	Electron transfer
Ruberodoxin	6000	1	Fe^{III}	Bacteria	NH	Electron transfer
Ferritin	45000	20% Fe	Fe^{II}	animals	NH	Storage of iron
Transferritin	76000	2	Fe^{III}	animals	NH	Scavenging of iron
Hemerythrin	108000	2	Fe^{II}	marine invertib-rates	NH	Oxygen transport
FeMo Protein	220000	24-36	—	nitroge-nase enzyms	NH	Nitrogen fixation in bacteria
Catalase	280000	—	Fe^{III}	Living organism	H	decomposit-ion of H_2O_2
Peroxidase	44000	—	Fe^{III}	Living organism	H	decomposi-tion of H_2O_2

Hemoglobin and Myoglobin

Hemoglobin contains two parts : heme groups and globin proteins. A porphyrin ring containing an Fe atom is called a heme group. Cellular respiration is the process of using oxygen to break down glucose to produce CO_2, water and energy for use by the cell. It has molar mass of about 64500. Hemoglobin is found in red blood cells that are called erythrocytes and is resposible for their

characteristic colour. Without hemoglobin the blood is either colourless or a different colour. Hemoglobin picks up the weak ligand dioxygen from the lungs or gills and carries dioxygen in arterial blood to the muscles, where the oxygen is transferred to another heme containing protein, myoglobin which stores it untill oxygen is required to decompose glucose to produce energy, CO_2 and water. Hemoglobin then uses certain amino acid groups to bind CO_2 and carry it in venous blood back to the lungs.

Each hemoglobin molecule is made up of four subunits, each of which consists of a globin protein in the form of folded helix or spiral. The globin proteins are of two types : two are α and two are β. An α globin protein consists of 141 and an β globin protein consists of 146 amino acids. Each protein consists of one polar and one non-polar group. In hemoglobin which has no dioxygen attached (and is therefore called as deoxyhemoglobin or reduced hemoglobin), the protein is attached to Fe(II) protoporphyrin IX through imidazole nitrogen of histidine residue in such a way that the polar groups of each protein are on the outside of the structure leaving a hydrophobic interior. Therefore, the heme group is held in a water resistant protein pocket.

Perutz has suggested a mechanism for the cooperativity of the four heme groups in hemoglobin. In deoxyhemoglobin, iron is coordinated to four nitrogen atoms of the planar protoporphyrin IX and the fifth coordination site is occupied by nitrogen atom on imidazole of a proximal histidine of globin protein. The sixth vacant site *trans* to the imidazole nitrogen is vacant and reserved for dioxygen. In deoxyhemoglobin iron is present as high spin Fe(II) with one electron occupying the $d_{x^2-y^2}$ orbital that points directly toward the nitrogen atoms of protoporphyrin IX. The presence of this electron increases the size of Fe(II) in these directions by repelling the lone pair of electrons on nitrogen atoms. As a consequence, Fe(II) becomes too large to fit easily within the hole provide by the planar protoporphyrin IX ring. The Fe(II) ion is, therefore, lies about 40 pm out of the plane in the direction of the histidine group, and the heme group is slightly bent into a domed shape (Fig.9.2) The iron atom in deoxyhemoglobin has square based pyramidal coordination.

The steric interactions between the histidine residue, the associated globin chain and heme group inhibit the free movement of the iron atom into the porphyrin ring.

Although O_2 is not a strong ligand, the coordination of the dioxygen molecule *trans* to the histidine group as a sixth ligand alters the strength of the ligand field and causes the pairing of electrons on iron without affecting the oxidation state of iron. Therefore, Fe(II) becomes low spin and diamagnetic. In low spin Fe(II), the six *d*-electrons occupy the d_{xy}, d_{yz} and d_{zx} orbitals. The $d_{x^2-y^2}$ orbitals is now empty and the previous effects of an electron present in this orbital in repelling the porphyrin nitrogen atoms is dimnished. Therefore, the size of low spin Fe(II) becomes about 17 pm smaller than high spin Fe(II). Thus, the Fe(II) slips into the hole of an approximately planar porphyrin ring. As the iron slips into the hole, the imidazole side chain of histidine F_8 also moves toward Fe atom, and the complex has an octahedral geometry. Recent X-ray studies show that dioxygen is bound in a bent fashion with an Fe—O—O angle of approximately 130°. There is strong evidence for hydrogen bonding between an imidazole N—H of a distal histidine and the bound dioxygen.

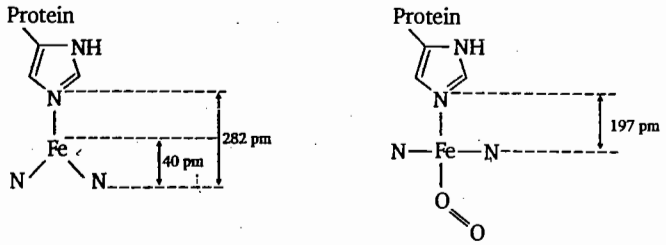


Fig.9.2 The changes in heme of hemoglobin upon oxygenation

There are four subunit in Hb —

The four subunits of hemoglobin are linked with each other through salt bridges between the four polypeptide chains. These salt bridges are formed mainly due to electrostatic interaction between the $-NH_3^+$ and $-COO^-$ groups present on all the four polypeptide chains of hemoglobin. The protein structures in hemoglobin consists of a peptide backbone with various side chains. These side chains consist of a variety of non-polar (hydrocarbons), cationic (such as $—NH_3^+$) and anionic (such as $-COO^-$) groups. These salt bridges between the polypeptide chains in hemoglobin are now believed to introduce strain in the molecule. Therefore the deoxy form of hemoglobin is called tense state (or T state).

The movement of iron atom and imidazole side chain of histidine-F_8 toward the porphyrin plane results in breaking of some of the salt bridges. The breaking of these salt bridges reduces the strain in hemoglobin molecule. Therefore, the oxyform of hemoglobin is called relaxed state(*i.e.*, R state). The T form of deoxyhemoglobin discourages the addition of first dioxygen molecule.

The bonding of one dioxygen molecule to a subunit of hemoglobin reduces the steric hindrance in the other subunits (due to breaking of salt bridges) and therefore encourages the bonding of dioxygen molecules to the iron atom of the second subunit which in turn encourages the third as well as fourth subunits. The binding of dioxygen molecule is most difficult in first subunit and easiest in the last subunit due to conformational change in the protein chain (or polypeptide chain). Initial addition of a dioxygen molecule to high spin Fe(II) triggers the oxygenation of deoxyhemoglobin. This is called cooperative effect.

Imp "The phenominon where the addition of dioxygen to one heme subunit encourages addion of the dioxygen molecules to other heme subunits is known as cooperative effect."

The successive equilibrium constants for binding of dioxygen molecules to each of the four iron atoms follow the order:

$$K_1 < K_2 < K_3 < K_4$$

$$Hb + O_2 \overset{K_1}{\rightleftharpoons} HbO_2$$

$$HbO_2 + O_2 \overset{K_2}{\rightleftharpoons} Hb(O_2)_2$$

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

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

The fourth equilibrium constant (K_4) is found to be much larger than the first (K_1). This indicates that last O_2 molecule is bound much more readily and tightly than the first. In the absence of conformational changes, K_4 would be much smaller than K_1. As a result, as soon as one or two dioxygen molecules are bound to iron atoms, all the four iron atoms are readily oxygenated. Conversely, as one O_2 molecule is removed from oxyhemoglobin the reverse conformational changes occur and successively decrease its affinity for oxygen. Therefore, initial removal of O_2 molecule from deoxyhemoglobin triggers the removal of remaining O_2 molecules. This phenominon is also called as cooperative effect.

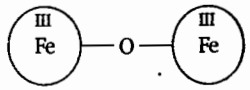


Fig. 9.3 : μ-oxo dimer (hematin)

The naked heme, the iron-porphyrin complex without accompanying the polypeptide chains is oxidized to Fe(III) by dioxygen molecule in aqueous solution and is converted immediately into a stable μ-oxo dimer (Fig. 9.3) known as hematin. In hematin iron is high spin Fe(III). The hematin is unable to transport oxygen. The polypeptide chain can be removed by treatment with HCl/acetone. The polypeptide chain in hemoglobin and myoglobin prevents oxidation of Fe(II) because:

(1) The hydrocarbon environment round the iron has a low dielectric constant and is hydrophobic and therefore act as a non-polar and provides non-aqueous environment.

(2) It provides steric hindrance and does not allow the formation of hematin.

The mechanism of the formation of hematin is as follows: The first step involves the binding of the O_2 molecule to Fe(II) of the heme group, PFe(II)

$$PFe^{II} + O_2 \rightleftharpoons PFe^{II}-O-O$$

Second step involves the coordination of bound oxygen to second heme group forming μ-peroxo complex.

$$PFe^{II}-O-O + PFe^{II} \longrightarrow PFe^{III}-O-O-Fe^{III}P$$

μ-peroxo complex

Third step involves the cleavage of the peroxo complex into two ferryl complexes in which iron is present in + 4 formal oxidation state.

$$PFe^{III}-O-O-Fe^{III}P \longrightarrow 2PFe^{IV}=O$$

ferryl complex

In the last step, the ferryl complex combines with an another heme group resulting in the formation of hematin.

$$PFe^{IV}=O + PFe^{II} \longrightarrow PFe^{III}-O-Fe^{III}P$$

μ-oxo-dimer

Myoglobin (Mb)

Myoglobin (or deoxy-myoglobin) is a protein which has only one heme group per molecule and serves as an oxygen storage molecule in the muscles. It has molar mass of about 17000 and binds

dioxygen molecule more strongly than hemoglobin. The myoglobin molecule is similar to a single subunit of hemoglobin. Myoglobin is a five coordinate high spin Fe(II) complex with four of the coordination positions occupied by N-atoms of the porphyrin ring. The fifth position is occupied by an N atom of an imidazole group of a histidine residue (a globin protein). The protein consists of 153 amino acids. This protein restricts access to the Fe(II) by a second heme and reduces the formation of a hematin like Fe(III) dimer. The result is that the Fe(II) porphyrin complex survives long enough to bind and release dioxygen molecule. Such five coordinate heme complexes of Fe(II) are always high spin $t_{2g}^4 e_g^2$ with one electron occupying the $d_{x^2-y^2}$ orbital that points directly toward the four porphyrin nitrogen atoms. The presence of this electron increases the size of Fe(II) in these directions by repelling the lone pair of electrons of the nitrogen atoms.

The size of Fe(II) is 92 pm in the square pyramidal arrangement which is considered to be pseudo-octahedral environment with the sixth ligand removed. The size of Fe(II) is so large that it can not fit into the hole of the planar porphyrin ring and therefore it lies about 40 pm away from the plane of the ring (Fig. 9.2). Therefore, high spin Fe(II) porphyrin complexes (in Hb and Mb) involves puckering and twisting of porphyrin ring.

When a dioxygen molecule binds to Fe(II) at sixth coordination site *trans* to imidazole group of histidine residue, the complex converts to low spin Fe(II) octahedral complex and the electronic configuration changes to t_{2g}^6 (*i.e.*, the six *d*-electrons occupy the d_{xy}, d_{yz} and d_{zx} orbitals leading to $d_{x^2-y^2}$ and d_{z^2} orbitals empty). The previous effect of two electrons occupying the $d_{x^2-y^2}$ and d_{z^2} orbitals in repelling the N atoms on X, Y and Z axes dimnishes. Therefore the low spin Fe(II) ion is smaller (75 pm) and slips into the hole in the planar pophyrin ring. As the Fe(II) ion moves, it pulls the imidazole group of histidine residue. Therefore, all the nitrogen atoms (including that of proximal histidine) approach more closer to the Fe(II) ion.

Hb → High affinity → High P for O_2

Mb → " → Low P

The Physisology of Hemoglobin and Myoglobin

Hemoglobin has relatively high affinity for dioxygen at high partial pressure of dioxygen whereas myoglobin has relatively high affinity for dioxygen at lower partial pressure of dioxygen. In vertibrates dioxygen enters the blood in the lungs or gills where the partial pressure of dioxygen is relatively high and hemoglobin is virtually saturated with dioxygen in lungs. When hemoglobin carries dioxygen to muscle tissues, it experiences the lower partial pressure of dioxygen and its affinity for dioxygen has fallen off rapidly and in this situation affinity of myoglobin for dioxygen is relatively high. Therefore, in muscle tissues dioxygen is thermodynamically favourable transferred from hemoglobin to myoglobin. The reactions occuring in lungs and muscles tissues are as follows:

lungs में O_2 ज्यादा होती है तो O_2 का partial pressure भी ज्यादा होता है

$$Hb + 4O_2 \longrightarrow Hb(O_2)_4$$ (In lungs) → High Pressure of oxygen

$$Hb(O_2)_4 + 4Mb \longrightarrow 4Mb(O_2) + Hb$$ (In muscle tissues) → Lower Partial Pressure of oxygen

The oxygenation equilibrium for myoglobin is represented as :

$$Mb + O_2 \rightleftharpoons Mb(O_2)$$

$$K_{Mb} = \frac{[Mb(O_2)]}{[Mb][O_2]}$$

If f is the fraction of myoglobin bearing oxygen and p_{O_2} is the equilibrium partial pressure of dioxygen, then

$$K = \frac{f}{(1-f)p_{O_2}} \Rightarrow$$

or

$$f = \frac{K p_{O_2}}{1 + K p_{O_2}}$$

The equilibrium constant K is called the binding constant of myoglobin for O_2.

This is the equation for the hyperbolic curve for myoglobin (Fig. 9.4).

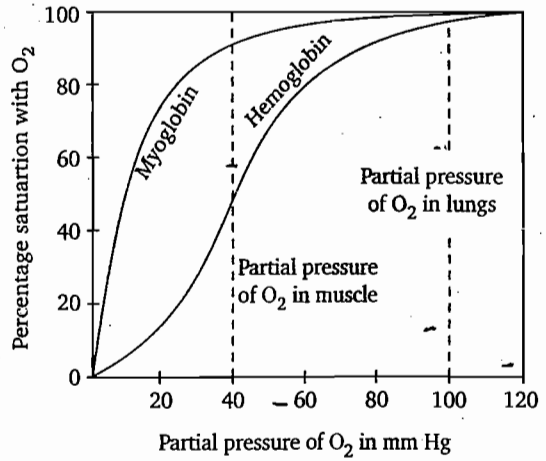


Fig. 9.4 Oxygen dissociation curves for hemoglobin and myoglobin, showing how hemoglobin is able to absorb O_2 efficiently in the lungs yet transfer it to myoglobin in muscle tissue

The hemoglobin curve does not follow such an equation. Hemoglobin has more complex behaviour as it has four heme subunits. It follows an emperically modified form with p_{O_2} replaced by $p^n_{O_2}$.

$$K = \frac{[Mb(O_2)_4]}{[Hb][O_2]^n}$$

$$K = \frac{f}{(1-f)p^n_{O_2}}$$

or

$$f = \frac{Kp^n_{O_2}}{1 + Kp^n_{O_2}}$$

Where the exponent n is called the Hill constant.

For hemoglobin $n = 2.8$ in the pH range of physiological importance in muscle tissues.

The exact value of n depends on pH of the biological system. When n exceeds the unity, the attachment of dioxygen to one heme group of hemoglobin increases the binding constant for next dioxygen which in turn increases the binding constant for the next one and so on. The exponent n =2.8 indicates that attachment of dioxygen to one heme subunit progressively increases its tendency to bind with the subsequent heme subunits of hemoglobin. Therefore, the exponent 2.8 reveals the cooperative effect of four heme subunits that produces the curves shown in Fig. 9.4.

If the four heme subunits of hemoglobin act independently, they will give curve identical to that of myoglobin. The cooperative effect favours the attachment of more dioxygen molecules to the heme subunits of hemoglobin. Conversely, if only one dioxygen molecule is attached to a heme subunit of hemoglobin, it dissociates more readily than from a more oxygenated hemoglobin. This indicates that at low partial pressure of dioxygen hemoglobin is less oxygenated and tends to release dioxygen where as at high partial pressure of dioxygen, hemoglobin is oxygenated almost to the same extent as if $n = 1$. This results in a sigmoidal curve for oxygenation of hemoglobin. (Fig. 9.4). The curve shows that mylglobin binds dioxygen more strongly than the first dioxygen of hemoglobin. However, the fourth binding constant of hemoglobin is larger than that for myoglobin by a factor of about 50.

Bohr's Effect

The cooperative effect is pH dependent. The affinity of hemoglobin for dioxygen decreases with decrease in pH. This is called Bohr effect. The CO_2 released in muscle tissues is the end product of break down of glucose. CO_2 being acidic, decreases the pH in muscle tissues and lovers the pH. The greater the muscular activity the more will be the release of CO_2. Therefore, in the muscles tissues, there is low p_{O_2}, low pH and high p_{CO_2}. In the muscle tissue CO_2 is produced and is transported to the lungs. Most of the CO_2 is transported in the form of soluble HCO_3^- ions. CO_2 enters the erythrocytes (red blood cells) where the enzyme carbonic anhydrase converts it to H_2CO_3 which dissociates into the HCO_3^- and H^+ ions.

$$CO_2 + H_2O \overset{\text{Carbonic anhydrase}}{\rightleftharpoons} H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

The formation of HCO_3^- ions is fascilitated by the portein chains of deoxyhemoglobin which acts as a buffer by picking up the accompanying protons. The HCO_3^- ions travel in the solution in the serum of the venous blood back to the lungs. The release of proton from hemoglobin on oxygenation produces H_2CO_3 from HCO_3^- ion.

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

The carbonic anhydrase converts H_2CO_3 in to CO_2 which is exhaled out.

$$H_2CO_3 \rightleftharpoons CO_2 + H_2O$$

Carbonic anhydrase

The oxygenated form of hemoglobin is more acidic than deoxygenated hemoglobin resulting in dissociation to produce protons from the molecule when dioxygen binds to it.

$$HbH_x^+ + 4O_2 \rightleftharpoons Hb(O_2)_4 + xH^+$$

Where x is about 1. It means oxyhemoglobin releases one H^+ for every dioxygen molecule binds. The protons are released from histidine residue of the protein chain.

About 10-15 % of CO_2 is transported in erythrocytes as carbaminohemoglobin. In this form the CO_2 is bound to the $-NH_2$ group of the protein.

$$CO_2 + HbNH_2 \rightleftharpoons HbNH.COO^- + H^+$$

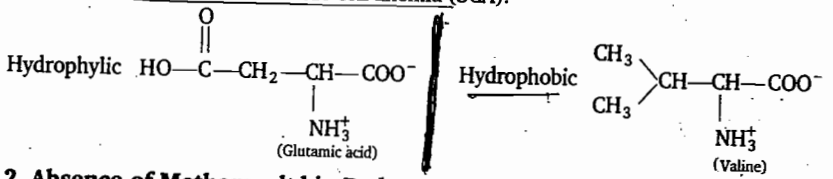
The reaction is rapid reversible and probably not catalyzed by an enzyme.

Poisoning Effect of CO and other ligands

Since O_2 is neither a strong nor a soft ligand, it can not coordinate to the fairly soft Fe^{2+} ion if soft-base and strong ligand such as CO and CN^- are present. The dioxygen molecule serves as a strong π-acceptor ligand in its interaction with Fe^{2+} centre. The ligands such as CO, CN^-, NO and PF_3 are stronger π - acceptors than O_2 and can bind to Fe^{2+} centre more strongly than O_2. CO binds comptitively at the same site on hemoglobin as does O_2 and shows similar cooperative and Bohr effects. CO binding is more than 200 times stronger than O_2 binding. The amount of O_2 that can be carried by the remaining Fe^{2+} centres of the hemoglobin molecule is reduced if CO is bound to one of the heme subunits. Moreover, the O_2 that is associated with hemoglobin moleculles containing some CO is bound more strongly making the transfer of dioxygen from these hemoglobin molecules to the muscles tissues more difficult. Therefore, it prevents the transport of dioxygen and causes death eventually due to Asphyxia.

Genetic Defects

1. Sickle cell Anemia : This is because of the mutant form of hemoglobin, where hydrophilic glutamic acid in one β-chain of globin porteins is replaced by hydrophobic valine which reduces the solubility of hemoglobin and prevents oxygen transport. During $R \rightarrow T$ conversion hemoglobin polymerizes and becomes distorted. Because of deformed shape of hemoglobin, red blood cells become sickle shaped and cause sickle cell anemia (SCA).



2. Absence of Methemoglobin Reductase : Methemoglobin is a metalloprotein in which the iron in the heme group is in the Fe^{3+} state, not in the Fe^{2+} of normal hemoglobin. The size of Fe^{3+} ion is so small that it can fit into porphyrin ring of hemoglobin without binding oxygen and therefore, it prevents transfer of dioxygen. In human blood a trace amount (about 3 %) of methemoglobin is normally produced spontaneously.

However, the Fe^{3+} ion has an increased affinity for dioxygen binding. The binding of dioxygen to methemoglobin results in an increased affinity of dioxygen to other three heme subunits that still contain Fe^{2+} ions within the same hemoglobin molecule. This leads to an overall reduced ability of the red blood cells to release oxygen to muscle tissues.

The NADH-dependent enzyme methemoglobin reductase (diaphorase) converts the methemoglobin back to hemoglobin. A higher level of methemoglobin causes a disease called as methemoglobinemia which is a disorder. NO_2^- / NO_3^- makes oxidation of Fe faster than its reduction and causes intoxication.

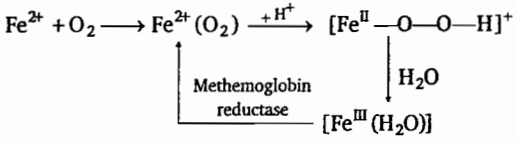


Fig. 9.5

During the formation of oxyhemoglobin from hemoglobin and dioxygen, one electron is partially transferred from Fe^{2+} of heme to the bound oxygen forming a ferric superoxide complex anion Fig. 9.5.

Electronic Spectra of Porphyrins and Metalloporphyrin

UV-visible spectroscopy is one of the most important technique for the characterization of macrocyclic ligands, porphyrins. The UV-visible absorption spectrum (Fig. 9.6) of the highly conjugated porphyrin ligand exhibit a strong absorption band at about 400 nm (the 'soret' band or B-band) and several weaker bands (Q-bands) at higher wavelengths (from 450 to 750 nm). Both the soret and Q bands in porphyrin systems arise from transition of electrons from porphyrin π-HOMO to the π*-LUMO. It is the nature of metal centre and the substituents on the ring that affect the energies of these transitions and intensities of bands. Protonation of two of the inner nitrogen atoms or the insertion/change of metal atoms into the porphyrin usually strongly change the absorption spectrum. The metal ions (d^0, d^2, d^3 or d^{10}) in which the $d\pi$ (d_{yz}, d_{zx}) orbitals are relatively low in energy and do not form metal to ligand π - bonds have little effect on the porphyrin π to π* energy gap in porphyrin absorption spectrum.

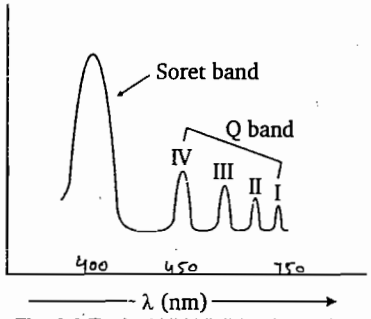


Fig. 9.6 Typical UV-Visible absorption spectrum of porphyrins

On the other hand the metal ions (d^n, $n = 4-9$) having filled $d\pi$ orbitals form metal to ligand π-bonds. This results in an increase in porphyrin π to π* energy gap causing the electronic absorptions to undergo hypsochromic (blue) shifts.

→ The porphyrin ligand contains two π-HOMO of a_{1u} and a_{2u} symmetries and two π* LUMO of e_g symmetry. An excited singlet state is formed with $a_{2u}^1\ e_g^1$ configuration by transfer of an electron from

a_{2u} orbital to an e_g orbital. These two excited states mix to form two new singlet states that are nearly 1 : 1 mixtures of the unmixed states.

An electronic transition to the higher energy mixed state, the S_2 state is strongly allowed whereas an electronic transition to the lower energy mixed state, the S_1 state is weakly allowed. The absorption band due to a transition to S_2 state ($S_0 \rightarrow S_2$) is the soret band and the band due to transition to the S_1 state ($S_0 \rightarrow S_1$) is the Q band.

Unlike most of the transition metal complexes, the colour of metallaporphyrins (either in oxidized or reduced form) is due to absorptions within the porphyrin ligand involving electronic transition from π -HOMO to π* LUMO of porphyrin ring.

When metal inserted in porphyrin ring. Soret band become broad and Q-band disappears (Fig. 9.7). The soret and Q bands are characteristic of porphyrins.

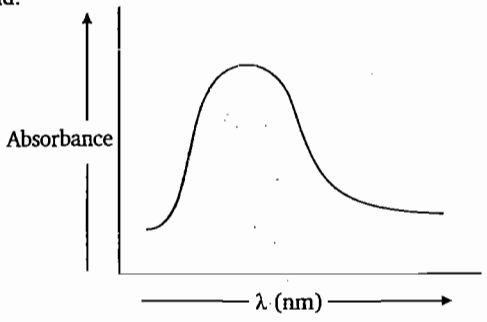


Fig. 9.7

Colour of hemoglobin (deoxy or oxy), myoglobin (deoxy or oxy), catalase (reduced or oxidized) and cytochromes is due to intraligand π to π* transition.

Hemerythrin

Hemerythrin is a non-heme iron containg protein found in marine invertibrates. Like hemoglobin and myoglobin, it contains Fe^{2+} ions and binds dioxygen reversibly. In the blood, hemerythrin (molecular mass = 108000) consists of eight subunits each with 113 amino acid residues and a two Fe(II) ions active site. In the muscle tissues it contains fewer subunits. A major difference between hemoglobin and hemerythrin is in the binding of dioxygen. In hemoglobin one dioxygen binds per Fe(II) ion whereas in hemerythrin one dioxygen binds to two Fe(II) ions. Unlike hemoglobin, hemerythrin exhibits no cooperativity between the subunits during oxygen binding. Each subunit in hemerythrin consists of the two iron active site connected by three bridging groups, two of which are carboxyl anions from the glutamate and asparatate and the other is either H_2O, OH^- or O^{2-} but probably OH^-. The remaining ligands which complete an octahedron about one Fe(II) and a five coordination about the other Fe(II) ion are three imidazole nitrogen atoms of histidine residues on one Fe(II) ion and two on the other [Fig.9.8(a)]. The two Fe(II) ions in deoxyhemerythrin are strongly antiferromagnetically coupled through the Fe—O—Fe bridge.

Dioxygen binds at the coordinatively unsaturated (vacant site) of the deoxyhemerythrin to give oxyhemerythrin. When dioxygen binds to the vacant site, two electrons are transferred from two Fe(II) centre (one electron from each Fe(II) of a subunit) to the dioxygen resulting in Fe(III) and peroxide (O_2^{2-}). The proton from the hydroxo bridge shifts to the bound peroxide resulting in HO_2^- group [Fig. 9.8 (b)]. The μ-oxobridging group is associated with bound HO_2^- group by hydrogen bonding.

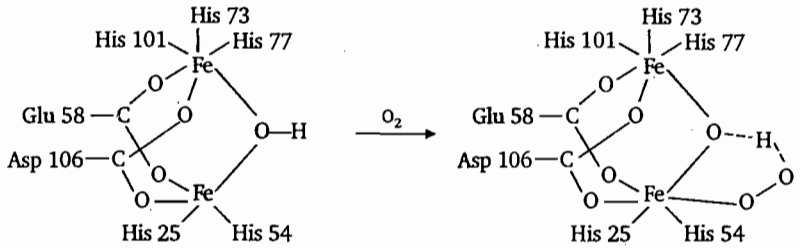


Fig. 9.8 Structure of (a) deoxyhemerythrin (b) oxyhemerythrin.

Mössbauer spectroscopy shows that the two Fe(III) ions in oxyhemerythrin are in different environment. It is due to the reason that the peroxide ion is coordinated to one Fe(III) ion not to the other. Raman spectroscopy has revealed the presence of a μ (O—O) stretching frequency at 845 cm^{-1}, a characteristic of a bound peroxide ion. Oxyhemerythrin contains antiferromagnetically coupled Fe(III) ions. Due to antiferromagnetically oxyhemerythrin is diamagnetic and EPR inactive. In oxyhemerythrin both Fe(III) ions are low spin ($t_{2g}^5 eg^0$).

When hemerythrin is oxidized to methemerythrin (the oxidized form of hemerythrin) which contains an oxo rather than hydroxo bridge, it does not bind dioxygen. The hemocyanin is the third system in nature after hemoglobin and hemerythrin for oxygen carrying from the point of intake to the muscle tissues where O_2 is required.

Hemocyanin

The name hemocyanin suggests the presence of heme group as well as the cyanide ion. But it has neither heme group nor the cyanide ion. The name simply means the blue blood. Hemocyanin is a copper containing protein which serves as an oxygen carrier in some invertibrates such as Mollusca (*e.g., whelks, snails, squid*) and Arthopoda (*e.g. crabs, lobsters, shrimps*). The deoxy form of hemocyannin contains Cu(I) ion and is colourless. The dioxygen binding results in the blue Cu(II) form. The blue clolur of oxyhemocyanin is due to $O^{2-} \rightarrow Cu^{2+}$ LMCT). Like hemoglobin, hemocyanin molecule have several subunits and binds dioxygen cooperatively. The active sites contain two Cu(I) ions (~360 pm apart) for binding of one dioxygen molecule. Each Cu(I) ion is bound by three histidine residues (Fig.9.9).

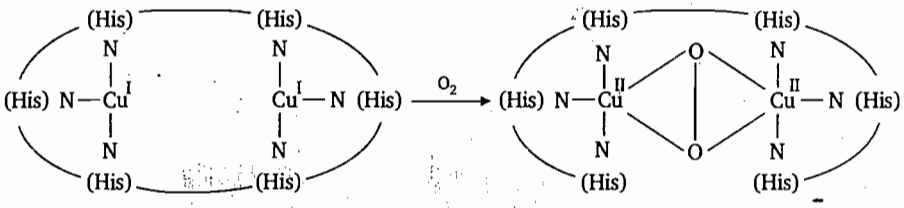


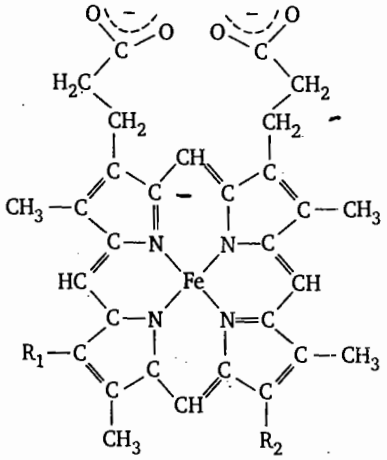
Fig. 9.9 : Structure of : (a) deoxyhemocyanin (b) oxyhemocyanin

The dioxygen molecule oxidizes each Cu(I) ion to Cu(II) ion and itself is reduced to the peroxide ion (O_2^{2-}). The two Cu(II) ions are bridged by the peroxide ion [Fig. 9.6(b)]. The resonance Raman spectroscopy reveals the formulation of $\overset{II}{Cu}-O-O-\overset{II}{Cu}$ linkage : ν(O—O) is about 750 cm^{-1} compared with about 800 cm^{-1} for free O_2^{2-} ion. The Cu(II) ions are coupled antiferromagnetically with the $\mu-[O_2]^{2-}$ ion being involved in a supexchange mechanism.

Cytochromes

Cytochromes are found in both plants and animals and serves as electron carriers. They contain heme like prosthetic groups. A prosthetic group is a compound required by an enzyme to facilitate a particular reaction, such compounds are also called coenzymes. The peptide portion of an enzyme that requires a prosthetic group is called an apoenzyme while complete enzyme with prosthetic group is called the holoenzyme. There are three main types of cytoctromes: Cytochrome a (Cyt-a), cytochrome b (Cyt-b) and cytochrome-c (Cyt-c). As in hemoglobin, the Fe(II) ion of heme group is attached to a N-atom of imidazole ring of histidine residue on one side of the porphyrin plane. The sixth coordination site of Fe(II) is occupied by a tightly bound S atom from a methionine residue of a protein. For this reason, the cytochromes are inert not only to oxygen but also to the poisons such as CO which affect the oxygen carriers.

The reduction potentials for cytochromes increase in the order : cyt-b (0.26 V) cyt-c (0.26 V) cyt-a (0.4 V) therefore the order of electron flow is : $b \rightarrow c \rightarrow a \rightarrow O_2$. The differences in reduction potentials for Fe(II) → Fe(III) oxidation results from changes in the porphyrin substituents (Fig. 9.10), changes in the protein and in some cases changes in axial ligands.



Heme A :

$R_1 = CH=CH_2$

$R_1 = C_{18}H_{30}OH$

(Found in cytochrome a)

Heme B :

(Protoporphyrin IX) :

$R_1 = R_2 = CH=CH_2$

(Found in Hb, Mb, cytochrome b, peroxydase)

Heme C :

$R_1 = R_2 = CH(CH_3)S$—Protein

(Found in cytochrome c)

Fig. 9.10 Structure of heme groups

The electron transfer reactions allow energy from glucose oxidation to be released gradually and to be stored in the form of adenosine triphosphate (ATP) which is used when required by the cell. Cytochrome-c oxidase, the terminal member of the cytoctrome chain which binds the inner memberane of the mitochondrion contains cytochrome-a, cytochrome-a_3 and two Cu(II) ions (Cu_A and Cu_B). Electron transfer involves the cytochrome-a and Cu_A, electron being transferred from cytochrome c to Cu_A and then to cytochrome-a. Cytochrome-a_3 and Cu_B provide site for dioxygen binding and conversion of O_2 to H_2O. Cytochrome a_3 and Cu_B are five and three coordinated respectively and therefore bind dioxygen. The overall reaction catalyzed the cytochrome c oxidaze enzyme is :

$$4\text{cyt } c^{2+} + O_2 + 8H^+_{inside} \longrightarrow 4\text{cyt } c^{3+} + 2H_2O + 4H^+_{outside}$$

This indicates that cytochrome chain involves transport not only of electrons but also of protons across the mitochondrial membrane. The oxidized form of cytochrome a_3 is susceptible to bind CN^- that renders cyanids toxic.

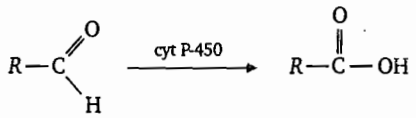
Cytochrome P-450

Cytochrome P-450 are a group of cytochromes found in plants, animals and bacteria. It is named as pigment that absorbs at 450 nm with their CO complexes. This is due to π-π^* transition (blue to red) and this band is called SORET bond. Cytochrome P-450 facilitates the cleavage of O_2 and functions as monooxygnase catalyzing the insertion of oxygen atoms into substrates. Oxygenases are enzymes that insert oxygen into the substrates. Monooxygenase inserts one oxygen atom and a dioxygenase inserts two oxygen atoms. Of the many possible substrates, the most important are molecules in which C—H bond is converted to C—OH groups. Some examples are:

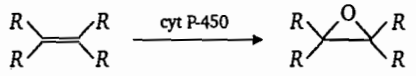
Conversion of an hydrocarbon *R*H to *R*OH.

$$R-H + O_2 + 2H^+ + 2e^- \xrightarrow{\text{cyt P-450}} R-OH + H_2O$$

Conversion of an aldehyde to the carboxylic acid.



Conversion of an alkene to epoxide.



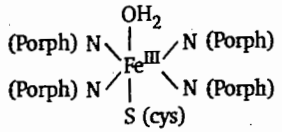
Cytochrome P-450 enzymes are found in kidney where they oxidize insoluble hydrocarbons to water soluble *R*—OH compounds which then be excreted in the urine.

One oxygen atom is inserted into an organic substrate and one atom is reduced to H_2O.

The active site in a cytochrome P-450 is heme similar to hemoglobin and myoglobin except that:

(i) Fe is present in Fe(III) state and it is low spin octahedral.

(ii) One S-atom of cysteine is coordinated to Fe(III) instead of histidine in the proximal position.
(iii) Sixth coordination site is occupied by H_2O.
The cytochrome P-450 enzymes have low spin octahedral Fe(III) active site.



Cytochrome P-450 enzymes have molar mass of about 50,000. The catalytic cycle for the action of cytochrome P-450 enzyme is shown in Fig. 9.11.

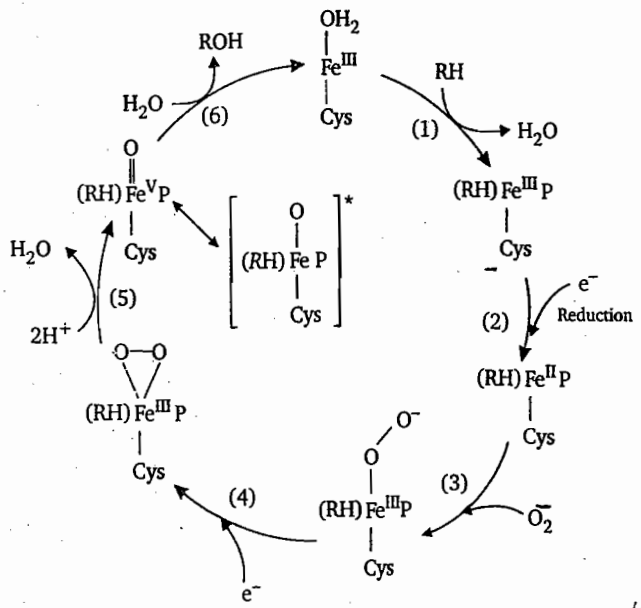


Fig. 9.11 Catalytic cycle for cytochrome P-450

In the first step, the organic substrate enters a hydrophobic pocket of the protein, near the Fe(III) centre expelling the water molecule from the iron axial coordination site to give a Fe(III) complex. In the second step, the Fe(III) complex is reduced by another enzymatic system to give high spin Fe(II) complex.

In the step 3, the dioxygen molecule binds to Fe(II) centre like hemoglobin and myoglobin followed by one electron transfer from Fe(II) to dioxygen to form an Fe(III)-superoxo complex. In the step 4, an another electron is added to give Fe(III)-peroxo complex. In step 5, the protonation of Fe(III)-peroxo complex leads to removing one oxide ion as water leaving an oxyferryl complex

Fe(V)=O or oxygen double bonded to Fe(IV) with one electron having been oxidized from the π -HOMO of the pophyrin ring leaving it as a radical cation. In step 6, the organic substrate (R—H) is oxidized to R—OH with concomitant binding of an H_2O ligand to the active site of the metalloenzyme which once again contains low spin Fe(III) centre.

Catalases and Peroxidases

Peroxidase is a heme protein catalyzing oxidation of variety of substrates such as ascorbate, ferrocyanide, and cytochrome c by hydrogen peroxide. Catalase enzyme is found in nearly all living organisms. Catalases catalyze the disproportionation of hydrogen peroxide and organic peroxides. They also catalyze the oxidation of substrates by hydrogen peroxide. Cataleses has one of the highest turnover numbers of all enzymes, one catalase molecule can convert millions of molecules of H_2O_2 to water and oxygen each second.

Peroxidases and catalases have similarities in both structure and reaction mechanisms. Both have high spin Fe(III) heme active site, with imidazole nitrogen of histidine residue occupying the fifth coordination site. The sixth coordination site is occupied by a water ligand in the resting enzyme.

The reaction is believed to occur in two steps:

$$[Fe^{III} - P]^+ + H_2O_2 \longrightarrow [O = Fe^{IV} - P^+]^+$$

$$[O = Fe^{IV} - P^+]^+ + H_2O_2 \longrightarrow [Fe^{III} - P]^+ + O_2$$

Where Fe^{III} — P is the heme group of the enzyme. $O = Fe^{IV}$ — P^+ is the mesomeric form of $O = Fe^{V}$ — P which indicates that Fe(III) is not completely oxidized to Fe(V) but it receives one electron from the porphyrin ring. It means that Fe(III) is oxidized to Fe(IV) and porphyrin ring (P) is oxidized by one electron to porphyrin$^+$ and the porphyrin ring containing one unpaired electron becomes a radical cation. The reactivity of the iron centre can be enhanced by the presence of Tyr-357 (tyrosinate at position 357) at the fifth (axial) position which assists the oxidation of the Fe(III) to Fe(IV). The optimum pH for human catalase is approximately 7. Hydrogen peroxide is harmful byproduct of several metabolic processes. To prevent damage to cells and tissues, it must be converted into H_2O and O_2. The colour of oxidized and reduced forms of catalse is due to π to π^* transition in the porphyrin ring.

Iron-Sulfur Proteins

These are non-heme iron proteins and are responsible for electron transfer in plants and bacteria. These have relatively low molar mass (6000-12000) and contain one, two, four or eight Fe atoms. The iron atoms are coordinated to four sulfur atoms in an approximately tetrahedral manner. Since iron atoms are coordinated to S atoms tetrahedrally, the iron atoms have high spin configuration. They have low range of reduction potentials (–0.05 V to –0.5 V), therefore, they act as reducing agents in biochemical processes. Different types of these are involved in photosynthesis, nitrogen fixation and metabolic oxidation of sugars prior to involvement of the cytochromes.

Ruberodoxin

Ruberodoxin is found in anaerobic bacteria where it participates in biological redox reactions. It is the simplest NHIP (non-heme iron protein) and contains only one Fe atom. The Fe atom is coordinated to four S atoms in distorted tetrahedral manner belonging to the amino acid cysteine in the protein chain. The iron is in the + 3 oxidation state. The Fe—S distance is 224 to 233 pm and S—Fe—S bond angle is 104 to 114°. It has no labile sulfur (*i.e.*, inorganic sulfur S^{2-} which can be liberated as H_2S by treatment with mineral acids, inorganic sulfur atoms are not part of the protein instead they form bridges between Fe atoms). The structure of ruberodoxin is shown in Fig. 9.12.

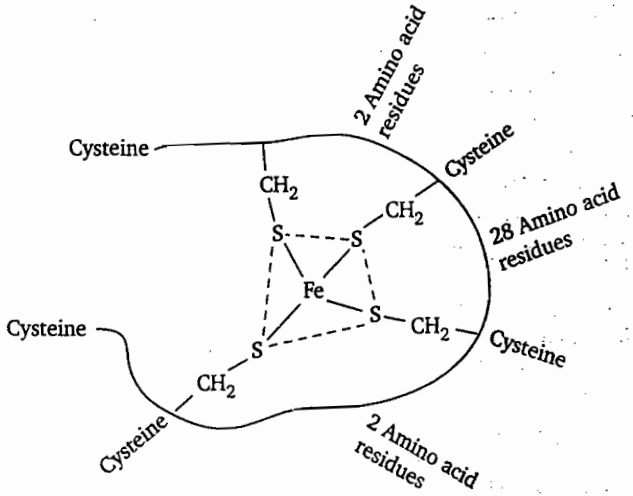


Fig. 9.12 The environment of the iron in the ruberodoxin molecule.

When Fe(III) is reduced to Fe(II), there is slight increases in the Fe—S distances but both Fe(III) and Fe(II) are high spin in tetrahedral geometry. Ruberodoxin is a one electron transfer agent.

Ferredoxins

NHIP with more than one iron atom are classified into three major catagories: [2Fe—2S], [3Fe—4S] and [4Fe—4S].

[2Fe—2S] or Fe_2S_2 Ferredoxins

They are called as plant ferredoxins and act as one electron transfer agents. These are very acidic proteins. The active sites consists of two di—μ-sulfido bridged high spin tetrahedral Fe(III) ions. The Fe(III) with $S = \frac{5}{2}$ are antiferromagnetically coupled *via* bridging atoms and therefore are

diamagnetic and ESR inactive. Each iron atom is also coordinated with two non-labile sulfur atoms of terminal cystine groups (Fig. 9.13)

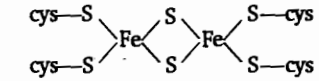
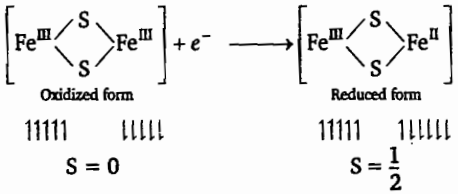


Fig. 9.13 Structure of [2Fe—2S] Ferredoxin

The ESR spectroscopy shows that the Fe^{n+} ions in reduced form are not equivalent but they exist as Fe(III) and Fe(II) rather than both having a fractional oxidation stat of + 2.5. In the reduced form the Fe(III) (d^5) and Fe(II) (d^6) electrons couple together resulting in one unpaired electron. Therefore it is EPR active.



If two cysteine units are replaced by two histidine residues, then the ferredoxin is called RIESKE protein (Fig. 9.14).

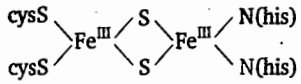


Fig. 9.14

[3Fe—4S] or Fe_3S_4 Ferredoxin

In recent years the existence of [3Fe—4S]–ferredoxins has been established by Mössbauer spectroscopy. It contains cubane [4Fe—4S] structure with one corner removed (Fig. 9.15).

The Fe_3S_4 cluster has 3Fe, four labile sulfurs and three non-labile sulfurs. In oxidized form it contains three high spin Fe(III) ions and in the reduced form it contains one Fe(II) and two Fe(III) ions. This suggests that it is a one electron transfer agent. In the reduced form Fe(II) and Fe(III) ions are also high spin. The two Fe(III) ions in oxidized form are coupled antiferromagnetically leaving the third Fe(III) ion paramagnetic ($S = \frac{5}{2}$) and therefore it ESR active. In the reduced form, the Fe(III) ions coupled antiferromagnetically leaving Fe(II) ion as paramagnetic (S = 2) and therefore reduced form is also ESR active.

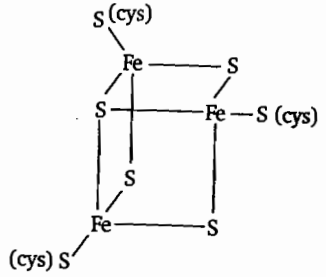


Fig. 9.15 Structure of [3Fe-4S] ferredoxin

[4Fe—4S] or Fe_4S_4 Ferredoxins

These are the most common and most stable ferredoxins. These are found in bacteria and involve in anaerobic metabolism. The Fe_4S_4 cluster consists of a cubane like cluster in which four Fe and four labile (or inorganic) S atoms are present on alternate corners of a cube. Therefore, it is called [4 Fe—4S] ferrodoxin. In addition, each Fe atom is bounded to S atom of crysteine ligand (Fig. 9.16). Each Fe atom has approximately tetrahedral coordination sphere.

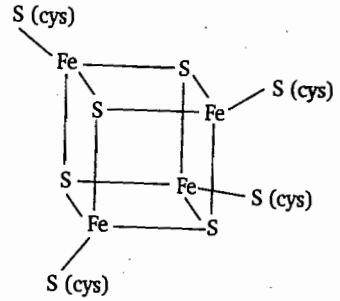


Fig. 9.16 Structure of Fe_4S_4 Ferredoxins

[4Fe—4S] cluster acts as one electron transfer agent. It contains two Fe(III) and two Fe(II) ions in the oxidized form and one Fe(III) and three Fe(II) ions in the reduced form. In both forms Fe(II) and Fe(III) ions are high spin.

$$2Fe(III) \cdot 2Fe(II) + e \rightleftharpoons Fe(III) \cdot 3Fe(II)$$

In oxidized form the two Fe(III) ions as well as the two Fe(II) ions are coupled antiferromagnetically resulting in S = 0 and therefore, the oxidized form is diamagnetic and EPR inactive.

Fe(III)	Fe(III)	Fe(II)	Fe(II)
d^5(HS)	d^5(HS)	d^6(HS)	d^6(HS)
↑↑↑↑↑	↓↓↓↓↓	↓↓ ↑↑↑↑	↓↓ ↓↓↓↓

S = 0

In the reduced form the Fe(III) and Fe(II) ions also couple antiferromagnetically resulting in $S = \frac{1}{2}$. Therefore, the reduced form is paramagnetic and EPR active.

Fe(III)	Fe(II)	Fe(II)	Fe(II)
d^5(HS)	d^6(HS)	d^6(HS)	d^6(HS)
↑↑↑↑↑	↓↓ ↓↓↓↓	↓↓ ↑↑↑↑	↓↓ ↓↓↓↓

$S = \frac{1}{2}$ S = 0

The Mössbauer spectroscopy has shown that all the four iron atom are equivalent and regarded as electronically delocalized systems with oxidation states of + 2.5 (in oxidized form) and + 2.25 in the reduced form.

The synthetic analogues, $[Fe_4S_4(SR)_4]^{x-}$ of ferrodoxins $[Fe_4S_4]^{n+}$ can be prepared by reacting $FeCl_3$, NaHS and an appropriate thiol. The magnetic properties and EPR, electronic and Mössbauer spectra of the synthetic $[Fe_4S_4(SR)_4]^{3-}$ anion are similar to those of reduced ferrodoxins.

$$[Fe_4S_4(SR)_4]^{3-} \underset{+e}{\overset{-e}{\rightleftharpoons}} [Fe_4S_4(SR)_4]^{2-}$$

Reduced form	Oxidized form
Oxidation state	Oxidation state
of Fe = +2.25	of Fe = + 2.5

The oxidized form $[Fe_4S_4(SR)_4]^{2-}$ can be further oxidized at high potentials of about + 0.35V. (therefore, these ferredoxins are called high potential iron sulfer proteins, HiPIP) to $[Fe_4S_4(SR)_4]^{-}$ which is paramagnetic with $S = \frac{1}{2}$ (due to antiferromagnetic coupling of Fe ions).

Nitrogen Fixation

Nitrogen in chemically bound forms such as the amino acids, proteins and porphyrins, is one of the most important elements of life. On earth nitrogen is found as the elemental N_2 and all forms of life are unable to utilize it as such. Nitrogen fixation is the process that converts atmospheric nitrogen into NH_3. There are two main processes for nitrogen fixation.

1. Biological process (By bacteria)
2. Industrial process (Haber-Bosch process)

In both the processes N_2 is converted into NH_3 breaking the N≡N triple bond which has the highest bond dissociation energy (945 kJ mol^{-1}) of any homonuclear diatomic molecule. The barrier to form NH_3 is not thermodynamic (ΔH_f for NH_3 = –50 kJ mol^{-1}) but kinetic. The two intermediates before breaking of N≡N triple bond completely, HN═ NH (diazene) and H_2N — NH_2 (hydrazine) are disfavoured thermodynamically because they have higher energies than either of the reactants or the products. (Fig. 9.17).

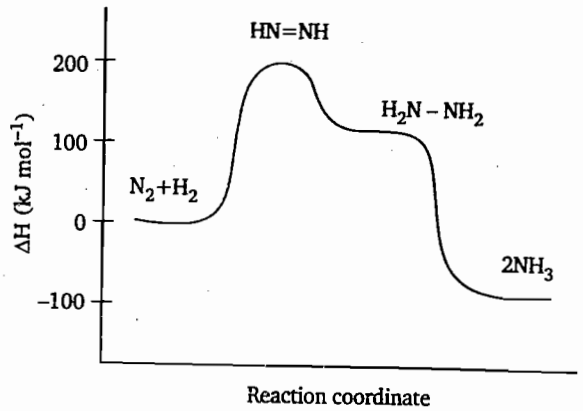


Fig. 9.17 Energies of reactants intermediate and the products along the reaction pathway

The Haber-Bosch process involves the high temperature (400 – 500°C) and pressure (200 atm) and Fe/Mo catalyst. Therefore, this process is very expensive.

$$N_2 + 3H_2 \xrightarrow[200\ atm,\ Fe/Mo]{400-500^\circ C} 2NH_3, \qquad \Delta H_f = -50\ kJ\ mol^{-1}$$

There is blue green algae, a wide variety of bacteria that can fix N_2 in vivo (life natural process) at ambient temperature and pressure. These bacteria are either free living or form symbiotic association with plants or other organisms. The important bacteria which fix the nitrogen are : *Clostridium pasteurianum*, *Azotobacter vinerlandii* and *Rhizobuim*, the best known of which is *Rhizobium* which is found in the root nodules of leguminous plants such as clover, beans, peas and soya.

The ammonia so formed is used in amino acids and protein synthesis by plants. There are three types of nitrogenase enzymes:

1. Vanandium nitrogenase
2. Iron nitrogenase
3. Molybdenum nitrogenase

Nitrogenases are composed of two metalloproteins: an Fe protein and the MFe (M = Mo, V and Fe) protein as cofactor. The vanadium nitrogenase (containing Fe protein, a 4Fe — 4S ferrodoxin and FeV cofactor) has less activity than the molybdenum nitrogenase.

The presence of molydenum is necessary component of most nitrogenses. These molybdenum nitrogenases consists of two different proteins: the Fe and MoFe proteins. The Fe protein has a molar mass of about 60,000. The Fe protein (called the P-cluster) has two Fe_4S_4 units connected by two cysteine ligand bridges. These two units are also linked by a S...S bond (Fig. 9.18). In one Fe_4S_4 unit there is a serine ligand attached to one of the iron atoms in addition to a cysteine ligand.

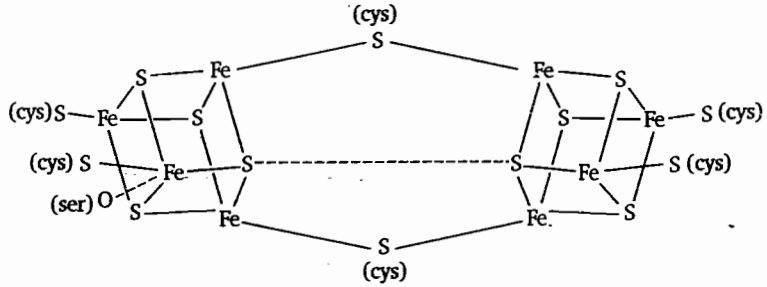


Fig. 9.18 Structure of P-cluster in nitrogenase

The molar mass of MoFe protein is 220,000 to 2,40,000. The most proposed structure of MoFe protein is shown in Fig. 9.19.

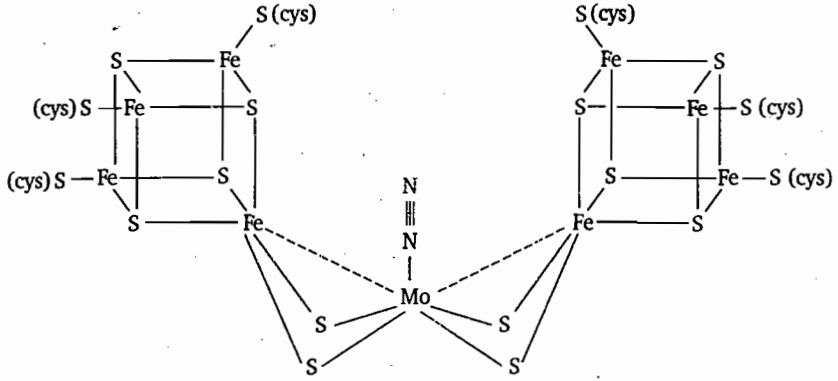


Fig. 9.19 Structure of the active site of the Mo-Fe protein nitrogenase

In MoFe protein, the Mo atom is linked to two Fe_4S_4 units by two sulphide groups and Mo-Fe bonds. It is believed that the reduction of molecule nitrogen occurs at the Mo site of the enzyme. It is not clear whether the initial coordination of N_2 occurs at an iron atom or a Mo atom, although most opinions favours the Mo atom.

The enzymes nitrogenases in various bacteria catalyzes the activation and reduction of dinitrogen to ammonia according to equation.

$$N_2 + 16MgATP + 8H^+ + 8e^- \longrightarrow 2NH_3 + 16MgADP + 16P_i + H_2$$

where P_i is inorganic phosphorus.

The electrons required in the reduction of N_2 are transferred to nitrogen by the reduced form of ferredoxins and flavodoxins. The source of these electrons is the oxidation of pyruvate. The electrons are first transferred to the smaller protein (Fe protein or P-cluster). The reduced Fe protein transfers its reducing electron to the MoFe protein and then to the dinitrogen bound to Mo. A series of such of electron transfer steps is:

$$Mo-N\equiv N \xrightarrow[2H^+]{2e^-} Mo-N=NH_2 \xrightarrow[2H^+]{2e^-} Mo-NH-NH_3 \xrightarrow[2H^+]{2e^-} Mo + 2NH_3$$

The energy for this process is provided by the hydrolysis of ATP to ADP and P_i.

Iron Storage and Transport : Ferritin and Transferrin

In humans and in many other higher animals iron is stored in ferritin and hemosiderin. Ferritin is composed of a protein coat (called as apoferritin) and an iron core. Twenty four proteins or peptide chains with about 175 amino acids are combined to form a protein coat (or apoferritin) which is a hollow sphere of about 100 Å in diameter. The iron core of ferritin contains about 45000 Fe(III) ions and some hydroxo as well as oxo and phosphate ligands. The iron core is similar to ferrihydrite, $(FeO\cdot OH)_8$ $(Fe\,O\cdot H_2PO_4)$. The iron core is fit into the hollow shell of apoferritin to form ferritin. The phosphate groups in the iron core functions as terminators and linking group to the protein shell. Ferritin is found in liver, spleen and bone marrow. Ferritin is a water soluble crystalline substance. Ferritin contains eight hydrophilic and six hydrophobic channels. It is thought that Fe(III) ions enter via the hydrophilic channels and leave via hydrophobic channels. The mechanism by which Fe(III) ions enter and leave ferritin is not clear. The iron core can be formed only from aqueous Fe(II) ions so that oxidation of Fe(II) give Fe(III) ions as shown below:

$$2Fe^{2+} + O_2 + 4H_2O \longrightarrow 2FeO(OH) + H_2O_2 + 4H^+$$

$$4Fe^{2+} + O_2 + 6H_2O \longrightarrow 4FeO(OH) + 8H^+$$

The oxidation of Fe(II) ions is catalyzed by Fe ferroxidase enzyme. Oxidation of Fe(II) and hydrolysis produces one electron and an average of 2.5 protons.

Mössbauer spectroscopy and EXAFS show that Fe(III) in ferritin is high spin and surrounded by six oxygen atoms at a distance of 195 pm and six Fe(III) ions at a distance about 300 pm followed by OH^- ions octahedrally. The individual protein and apoprotein have molar masses 18500 and 445000 respectively. The formation of full iron core of 4500 Fe(III) ions produces a total of 4500 electrons and 11250 protons. Hemosiderin contains larger proportions of hydrous metal oxide and is water insoluble and is usually located within an intercellular membrane (lysosomes).

Transferrin

Transferrin is a protein that binds Fe(III) very strongly [not Fe(II)] and transport it from the stomach into bloodstream. Transferrin proteins transport iron to the ferritin.

Humans and other animals absorb iron as Fe(II) from food in their digestive systems. As Fe(II) passes from the stomach (which is acidic) into the blood (pH = 7.4), it is oxidized to Fe(III) in a process catalyzed by the copper metalloenzyme ceruloplasmin. The Fe(III) is then binds with transferrin protein and transpoted to bone marrow where it is released from transferrin protein after the reduction of Fe(III) to Fe(II) because Fe(II) binds less effectively to the transferrin and stored as ferritin. Fe(II) ion is then used to synthesise the other iron compounds such as hemoglobin myoglobin, and the cytochromes. When a red blood cell become aged after an average of 16 weeks, the hemoglobin is decomposed and the iron is recovered by transferrin after oxidation to Fe(III).

Transferrim have molar mass of about 80000 and contain two similar but not identical sites and bind only two Fe(III) ions. The Fe(III) ion is in a distorted octahedral environment of one N, three O and a chelating CO_3^{2-} or HCO_3^-. The binding constant is sufficiently high (about 10^{26}), therefore, transferrin act as extremeny efficient scavenger of iron.

Siderophores

Siderophores are iron containing complexes which are found in most microorganisms and transport Fe(III), Siderophores are also known as siderochromes because some of them are intensely coloured. They have relatively low molar masses (500–1000). They are classified into several categories such as the ferrichromes, ferrioxamines and entrabactins. They are the chelating ligands and form chelates with Fe(III) ions. The complexes are high spin octahedral. These complexes are very stable but are labile which allow the iron to be transported and transferred within the bacteria.

Photosynthesis

Photosynthesis is a redox process by which green plants convert water and atmospheric CO_2 into carbohydrates such as glucose and release dioxygen by absorbing sun light. The overall reaction is:

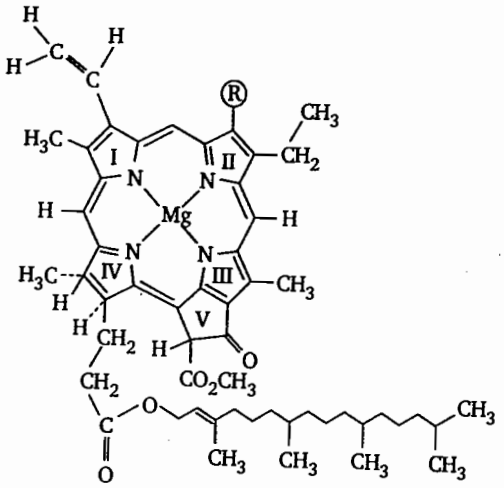
$$6CO_2 + 6H_2O \xrightarrow{h\nu} C_6H_{12}O_6 + 6O_2$$

This reaction involves the reduction of CO_2 and oxidation of H_2O to O_2. It is a two step reaction:

(i) $2H_2O \longrightarrow 4H^+ + O_2$

(ii) $6CO_2 + 12H^+ \longrightarrow C_6H_{12}O_6 + 3O_2$

Photosynthesis occurs in the chloroplasts of the cells of the green plants. Photosynthesis is essentially a process by which sun light energy is converted into chemical energy in the form of adenosine triphosphate (ATP) and reduced nicotinamide adenine dinucleotide phosphate(NADPH). This energy permits the fixation of CO_2 into carbohydrates with the liberation of dioxygen, O_2. Photosyntheis is initiated by the capture of light energy (usually referred to as light harvesting) in the photoreceptors of chlorophylls (chloros-green, phylon-leaf) found in chloroplasts in the green leaf. Many of the subsequent steps proceed in dark. The overall process is endothermic and involves more than one type of chlorophyll, ferredoxin, cytochromes, manganese complexes and a copper containing plastocyanin.



Chlorophyll-*a* (R = CH_3)
Chlorophyll-*b* (R = CHO)

Fig. 9.20 Structure of chlorophyll-a

Chlorophylls are complexes of Mg^{2+} with porphyrin ring in which one of the pyrrole rings has been reduced. A porphine ring with one double bond reduced is called a chlorin. Therefore, chlorophylls are the complexes of Mg^{2+} with substituted chlorin. A fused cyclopentanone ring is also present (Fig. 9.20). In green plants there are two chlorophylls (*a* and *b*) differing in one of the side groups. Chlorophyll a (Chl-*a*) is shown in Fig. 9.17. Chlorophyll-*b* is the same except that for the $-CH_3$ (in circle) which is —CHO in chlorophyll-*b*.

Chlorophylls in the chloroplast of plants leaf absorbs light in the red region of the visible spectrum (near 680–700 nm). The exact wave number depends upon the nature of the substituents. Chlorophylls are the green pigments. Chlorophyll-*a* has the absorption spectrum shown in Fig. 9.21.

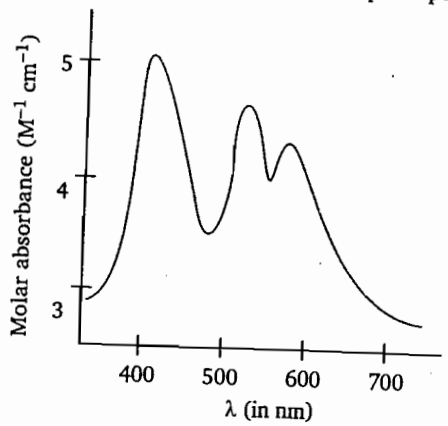


Fig. 9.21 The absorption spectrum of chlorophyll-a in the visible region

The two bands are observed in the absorption spectrum of chlorophyll-*a* : the strong absorption band in the red (called 'Q band') and a compratively weak band in blue to near UV (the Soret band). Both these bands arise from excitation of electrons from the porphytin ring πHOMO to π^* LUMO. The non-absorbing radiations between these two bands accounts for the characteristic green colour of the plants leaf.

The initial absorption of light radiations is from the singlet ground state to a singlet excited state of the chlorophyll Q band (^1Q). The chlorophylls in solution undergo rapid intersystem crossing into the lower energy spin triplet state (^3Q). This state causes the photochemical electron transfer reactions of molecules in solution. The light absorbed by chlorophylls is transferred to the reaction centre.

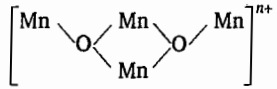
There are two photochemical reaction centres: Photosystem-I (PS-I) and Photosystem-II (PS-II). The initiating photosystem-II is called P_{680} as it absorbs at 680 nm and the subsequent photosystem-I is called P_{700} as it absorbs at 700 nm. The chlorophyll a site of PS-II absorbs quanta of higher energy than that of PS-I therefore, the two chlorophyll sites are distinguished as Chl-a_2 and a_1 or alternatively P_{680} and P_{700}.

In addition to the chlorophylls at PS-I and PS-II reaction centres, there are several other pigments associated with the light harvesting complex.

The photosystem I involves chlorophyll-*a* along with other pigments and does not produce dioxygen. This photosystem is involved in the anaerbic bacteria. These bacteria oxidize H_2S to S or oxidize organic molecules instead of the usual reaction where H_2O is oxidized to O_2.

Photosystem-II

In dark PS-II is in its ground, unexcited state in which it has no tendency to release an electron. When it absorbs sun light via antenna chlorophylls, it becomes excited such that it has a strong tendency to transfer its excited electron. It is, therefore, a strong reducing agent and it reduces the pheophytin (a chlorophyll like pigment laking the Mg^{2+} ion) of the PS-II electron transfer chain. The loss of an electron causes PS-II to become positively charged and it then attracts electron from manganese protein (an Mn_4 cluster).



The manganese protein contains three Mn(II) and one Mn(III). The oxidized manganese protein in turn attracts electron from water and catalyses the oxidation of H_2O to O_2 .

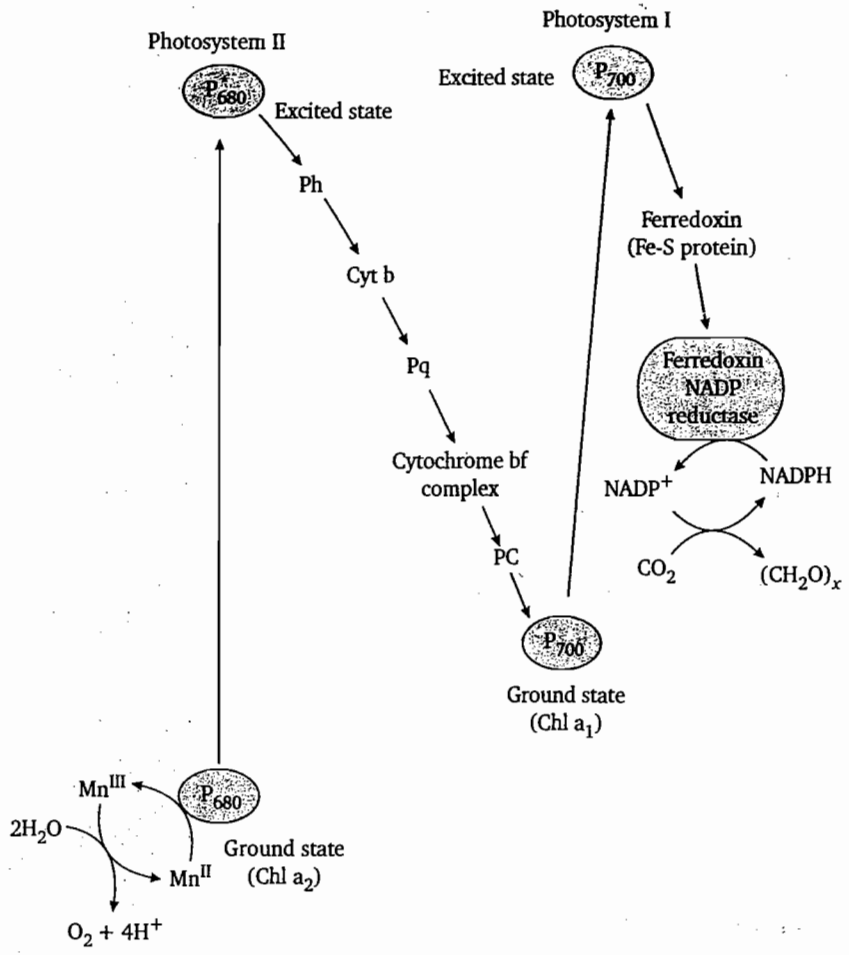


Fig. 9.22

The electron from pheophytin is then transferred through various redox systems like plastoquinone, cytochromes, iron-sulphur protein and plastocyanin (plastocyanin is a copper protein in which the copper ion alternates between the Cu^{2+} (oxidized) and Cu^+ (reduced) forms). At this point it leaves PS-II and moves on to PS-I. (Fig. 9.22)

Photosystem-I

Photosystem-I involves chlorophyll-*a* and *b*. When PS-I absorbs light energy from chlorophylls, the antema pigments (other pigments present in leaves to harvest some light and transfer its energy

to the reaction centre), the electron received from PS-II is, therefore, further excited and becomes a reducing agent. It passes on its electron to short chain of electron carriers, which reduces ferredoxin and the reduced form of ferredoxin reduces $NADP^+$ by the following reaction catalysed by the FAD enzyme called ferredoxin-NADP reductase (FAD or flavin adenine dinucleotide).

$$2(\text{Ferredoxin})_{\text{Red}} + NADP^+ + 2H^+ \longrightarrow 2(\text{Ferredoxin})_{\text{Ox}} + NADPH + H^+$$

In brief, an electron of P_{700} has been excited and transported to ferredoxin which inturn, reduces $NADP^+$. This left P_{700} an electron short. It is now P^+_{700}, an oxidizing agent. It accepts an electron from plastocyanin (PC)

$$P^+_{700} + PC_{Cu^+} \longrightarrow P_{700} + PC_{Cu^{2+}}$$

To go back further, we started with light absorption followed by exciting an electron on P_{680}, the reaction centre on PS-II, this leaves P^+_{680} which must have its electron restored so that it can revert to the ground state, ready for absorption of another light radiation to start a new cycle of reactions. The electron comes from water.

The net reaction of PS-II and PS-I is :

$$2H_2O + 2NADP^+ + 8\ \text{Photon} \longrightarrow 2NADPH^+ + 2H^+ + O_2$$

There are also cyclic dark reactions that produce ATP. NADPH and ATP involve in the conversion of CO_2 into glucose by the following net reaction.

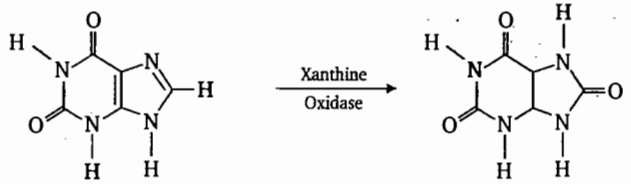
$$6CO_2 + 18ATP + 12NADPH + 12H^+ + 12H_2O \longrightarrow C_6H_{12}O_6 + 18ADP + 12NADP^+ + 18P_i$$

Role of Mg^{2+} Ion

Mg^{2+} in preferred in photosynthesis by chlorophyll because it binds strongly with chlorophyll and keep the macrocycle fairly rigid so that energy is not so readily dissipated by thermal vibrations.

Xanthine Oxidase

The enzyme xanthine oxidase contains two identical subunits, each containing one molybdenum, two Fe_2S_2 and FAD (flavin adenine dinucleotide) sites. It has molar mass of about 275000. Xanthine oxidase catalyzes the oxidation of xanthine to uric acid.



Xanthine Uric acid

An excess of uric acid accumulation leads to gout, which can be treated with inhibitors of xanthine oxidase. In xanthine oxidase, the Mo(VI) site carries out the two electron oxidation of xanthine to uric acid and it self reduces to Mo(IV). The Mo(VI) site is regenerated by transferring electrons, one at a time, to the Fe_2S_2 and FAD sites so that Mo(VI) becomes ready for oxidation of next equivalent of xanthine. The electron flow may be represented as :

$$\text{Xanthine} \longrightarrow \text{Mo(VI)} \longrightarrow 2Fe_2S_2 \longrightarrow \text{FAD} \longrightarrow O_2$$

Fe_2S_2 sites in xanthine oxidase play the same electron-transfer role as the Fe_2S_2 ferredoxins play in photosynthesis.

Alcohol Dehydrogenase, Aldehyde Oxidase, Aldehyde Dehydrogenase

When ethanol is consumed, it is oxidized to extremely poisonous acetaldehyde. The oxidation of ethanol to acetaldehyde occurs in the liver by alcohol dehydrogenase.

$$CH_3CH_2OH + NAD^+ \xrightarrow{\text{Alcohol dehydrogenase}} CH_3CHO + NADH + H^+$$

Alcohol dehydrogenases facilitate the oxidation of alcohols to aldehydes or ketones with the reduction of nicotinamide adenine dinucleotide (NAD^+) to NADH. Alcohol dehydrogenase is a dimer of two subunits. It has two Zn atoms per subunit.

An another minor route of oxidation of ethanol to acetaldehyde involves cytochrons P-450 (which uses molecular oxygen) and NADPH.

$$CH_3CH_2OH + NADPH + H^+ + O_2 \longrightarrow CH_3CHO + NADP^+ + 2H_2O$$

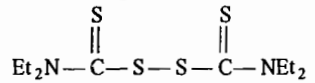
The oxidation of acetaldehyde to harmless acetic acid can be catalyzed by aldehyde oxidaze enzyme.

$$CH_3CHO \xrightarrow{\text{Aldehyde oxidase}} CH_3COOH$$

The enzyme aldehyde oxidase is closely related to xanthine oxidase. It also contains two identical subunits, each containing one Mo, two Fe_2S_2 and FAD sites. It has molar mass about 3,00,000. It oxidizes acetaldehyde to acetic acid via electron flow as shown below:

$$\text{Acetaldehyde} \longrightarrow \text{Mo(VI)} \longrightarrow 2Fe_2S_2 \longrightarrow \text{FAD} \longrightarrow O_2$$

The drug antabuse is used for treating alcoholism which is a sulfur containing ligand, disufiram.



Another route of oxidation of acetaldehyde to acetic acid involves aldehydede hydrogenase and NAD^+.

$$CH_3CHO + NAD^+ + H_2O \longrightarrow CH_3COOH + NADH + H^+$$

Vitamin B_{12} and Coenzyme B_{12} or Cyanocobalamin

Vitamin B_{12} and coenzyme B_{12} are nature's only organometallic compounds. Vitamin B_{12} was first isolated from liver extracts and it was observed that the deficiency of vitamin B_{12} or B_{12} coenzyme causes pernicious anemia in humans. In vitamin B_{12} the Co(III) ion is coordinated to four N-atoms of a corrin ring. The corin ring is modified porphyrin ring which has one less = CH- bridge between the two pyrrole rings than porphyrin ring. Therefore, corrin ring is less symmetric and less unsaturated than porphyrin ring. The fifth and sixth positions are occupied by an imidazole nitrogen and a cyanide ion. However in vivo, the cyanide ion is not present and the sixth position is occupied by loosely bound water molecule. (Fig. 9.23).

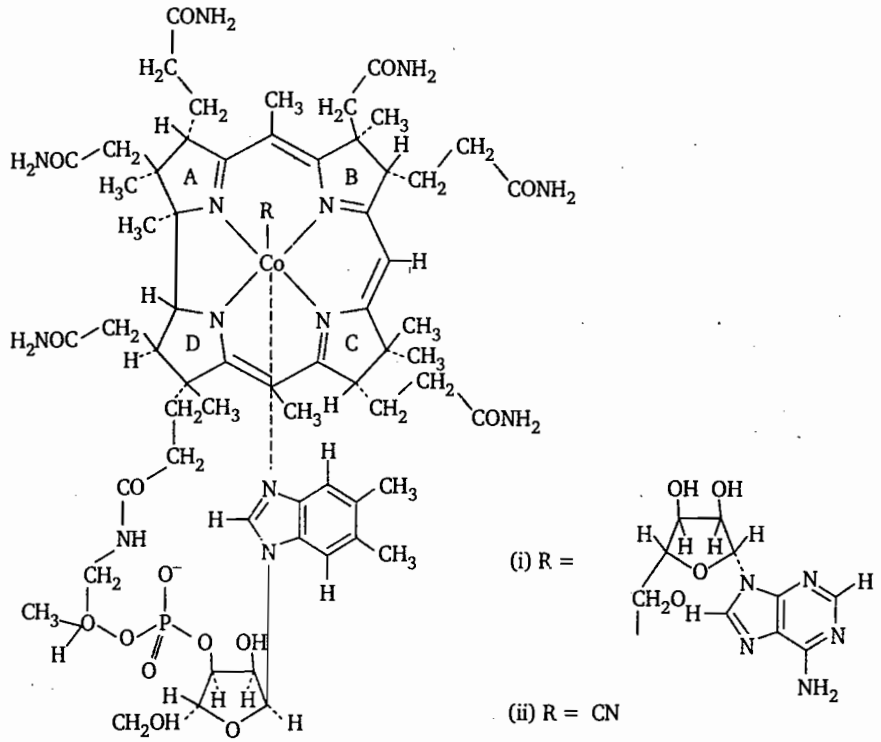
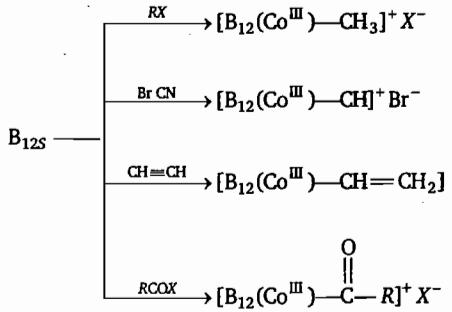


Fig. 9.23 Structure of (i) Coenzyme B_{12}, 5'-deoxyadenosylcobalamin, (ii) vitamin B_{12}, cyanocobalamin

Incorporation of Co(III) into the corrin ring modifies the reduction potentials of cobalt and therefore, can be reduced by one electron to give vitamin B_{12r} [Co(II) complex] or by two electrons to give vitamin B_{12s} [Co(I) complex]. These reductions can be carried out in vivo by reduced ferredoxin.. The later is highly nucleophilic so readily undergoes alkylation.

Some reactions of vitamin B_{12s} are given below:

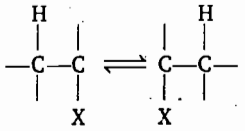


In Vitamin B_{12S}, the R sight is empty and the 5-coordinate cobalt(I) atom is extremely reactive. In vitamin B_{12} and its other derivative cobalt is present in + 3 oxidation state. Co(III) in these compounds is low spin octahedral field ($d^6 \rightarrow t_{2g}^6 eg^0$) and therefore they are diamagnetic and EPR inactive.

Vitamin B_{12} and vitamin B_{12r} are red and brown and these colours arise due to $\pi — \pi^*$ transitions in corrin rings. The later is EPR active due to presence of an unpaired electron in d_{z^2} orbital. Co(II) is also low spin vitamin B_{12r}. Co(I) in vitamin B_{12s} is also EPR active due to presence of two unpaired electrons and its blue-green colour is due to $\pi — \pi^*$ transitions.

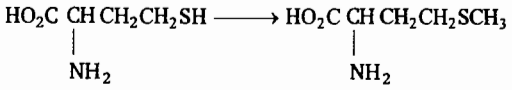
Unfortunately, certain bacteria can methylate not only sulfur in organic compounds but also some heavy metals such as Hg, Pb, Sn, Pd and Pt in aqueous solution to yield highly toxic species such as $Hg(CH_3)$ and $Pb(CH_3)_4$.

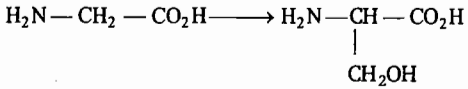
The reaction of adinosine triphosphate (ATP) with vitamin B_{12s} yields direct cobalt-carbon bond between adenosyl and cobalt. The resulting molecule is known as B_{12} coenzyme. It was the first organometallic compound discovered in living systems. In coenzyme B_{12}, Co(III) is coordinated to carbon atom of an adenosyl ligand in place of CN^- ligand. This coenzyme catalyses 1, 2 rearrangements of the general type:



Vitamin B_{12} with CN^- removed is called cobalamin, therefore, vitamin B_{12} is called cyanocobalamin.

Coenzyme B_{12} readily accepts a methyl group or hydroxy methyl group (bound to Co) that can be transferred to add a carbon to a substrate.





Coenzyme B_{12} also reduces —CH(OH) group to $—CH_2$ group as the ribonucleic acid (RNA) is reduced to deoxyribonucleic acid (DNA).

The precise mechanism of these reactions involve the cleavage of Co—C bond. It may be noted that cobalt porphyrin analogues of vitamin B_{12} can not be reduced to the Co(I) state under conditions where vitamin B_{12S} is obtained. Therefore, this inability of the porphyrin ligand to stabilize the Co(I) is the reason why the corrin ring has been selected in place of porphyrin ring in the evolution of the B_{12} cobalt complexes.

Enzymes

Enzymes are larger protein molecules that catalyze large number of biochemical reactions. They increase the rate of biochemical reactions about 10^6 times compared to the uncatalyzed rate. They lower the activation energy for the formation of one product rather than the other and therefore are highly specific. The enzymes are composed of proteins.

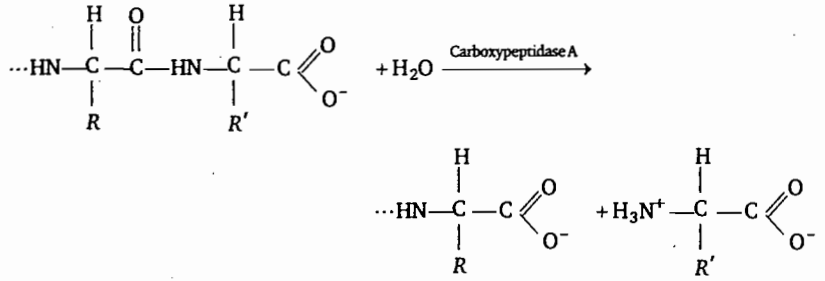
Metalloenzymes

A metalloenzyme is an enzymatic protein in which a metal as metal ion is embedded in the cavity of the enzyme and forms strong bonds with the donor atoms of the protein. The donor atoms of proteins may be either soft bases such as S or hard bases such as O and N. In the similar way the metals may be either soft metals such as Cu^+, Hg^+ and Cd^{2+} or hard such as Fe^{3+}, Zn^{2+}. The protein part is called as an apoenzyme and a metal ion or complex metal ion is called a prosthetic group. For example, heme is prosthetic group in hemoglobin. A reversible bound group that combines with an enzyme for a particular reaction and then is released to combine with another is called as coenzyme. Both the prosthetic group and coenzyme are sometimes called cofactors.

Zinc Metalloenzymes

Carboxypeptidase-A

Carboxypeptidase A is a pancreatic metalloenzyme which catalyses the hydrolysis of peptide bonds in protein during the process of digestion.



The enzyme consists of a single protein chain of 307 amino acids and one Zn^{2+} ion. Molar mass of this enzyme is about 34,800.

The metal ion is coordinated to two N-atoms of two histidine residues (His - 69 and His-196), to an oxygen atom of a glutamate residue (Glu-72) that acts as bidentate ligand and to a water molecule (Fig 9.24).

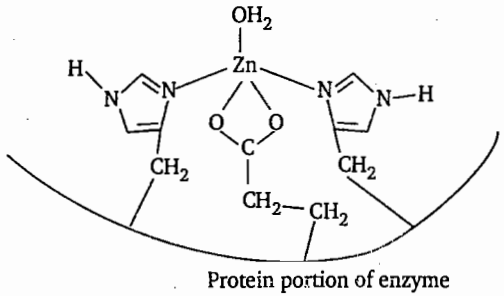


Fig. 9.24 Structure of carboxypeptidase

The cavity has a hydrophobic pocket close to Zn^{2+} ion that can accommodate organic group of the peptide undergoing hydrolysis and therefore accounts for the higher efficiency with which hydrophobic C-terminal peptides are cleaved. The hydrophobic C-terminals peptides containg branched side chain or aromatic side chain such as $C_6H_5 - CH_2 -$ or $-CH_2C_6H_4OH$. The carboxyl group of the substrate hydrogen bonds to an arginine (Arg-145) whereas the Zn^{2+} ion bonds to the oxygen of the peptide carbonyl group as shown in Fig. 9.25. The Arg-127 bonds to oxygen of carbonyl group of peptide of substrate and the phenoiic group of Tyr-248 residue hydrogen bonds to —NH group of peptide of the substrate. The oxygen of the water molecule coordinated to Zn^{2+} ion bonds to the carbon of the carbonyl group of the peptide and free glutamate residue of the enzyme hydrogen bonds to hydrogen of the water ligand and H^+ ion transfers from water to glutamate residue (Glu-270) leaving —OH group coordinated to Zn^{2+} ion.

In the next step C=O of carboxylic group of Glu-270 hydrogen bonds to H of — OH group coordinated to Zn^{2+} and H^+ form —COOH group of Glu-270 is transferred to —NH group of the

substrate with breaking of C—N peptide bond of the substrate (Fig. 9.26). The glutamate residue abstract a proton from —OH group coordinated to Zn^{2+} ion to form glutamic acid (Fig. 9.27). The hydrogen bonding to the carboxyl group by the Arg-145 and amide linkage by the Tyr-248 residues not only holds the substrate to the enzyme but also helps to break the C—N bond. In the final product of Zn^{2+}, the carbonyl of substrate binds in the bidentate fashion. Therefore, five coordination is maintained by switching the Glu- 72 metal ligand from bidentae to monodentate because the metal moves toward Arg-127.

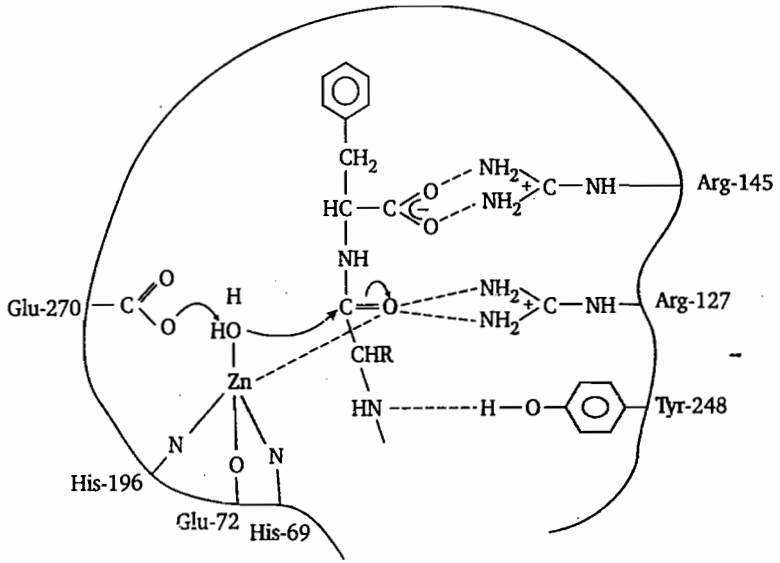


Fig. 9.25

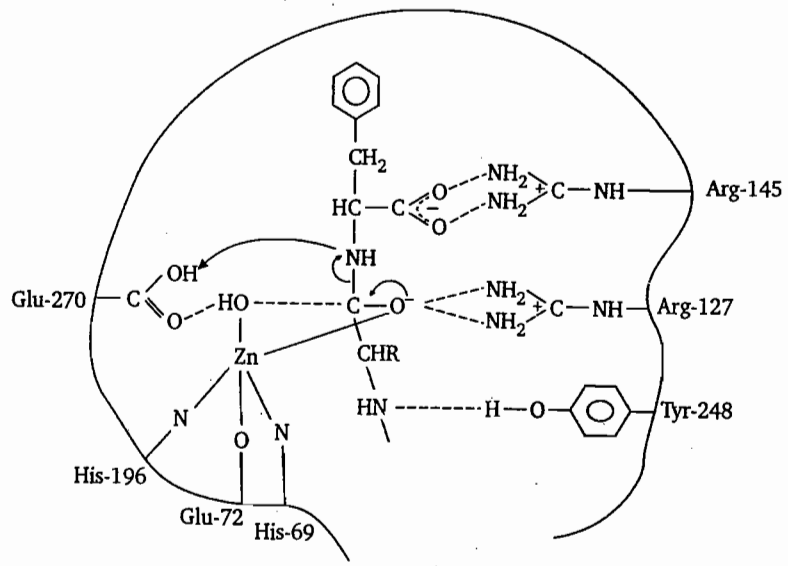


Fig. 9.26

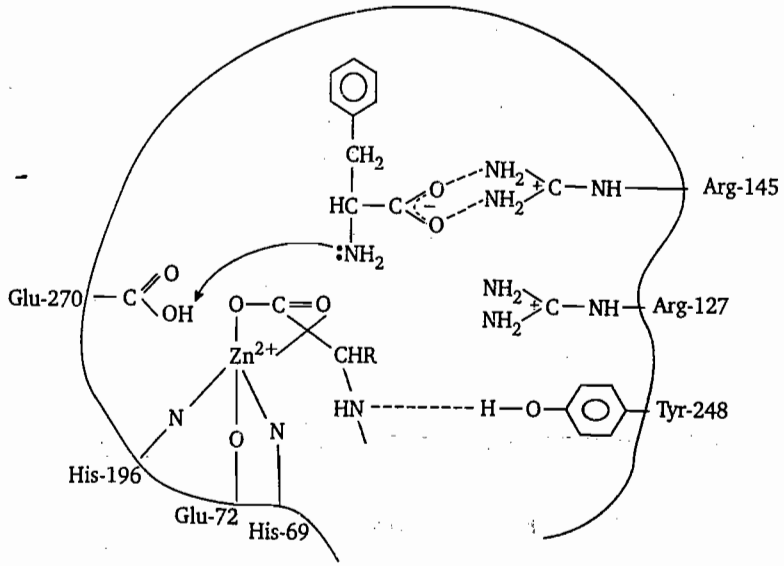


Fig. 9.27

Carbonic Anhydrase

The uncatalysed following equilibrium is relatively very slow.

$$CO_2 + H_2O \rightleftharpoons HCO_3^- + H^+$$

In the erythrocytes (red blood cells) the forward (hydration) reaction occurs during the uptake of CO_2 by blood in tissue whereas the backward (dehydration) reaction occurs when CO_2 is released in the lungs. The carbonic anhydrase enzyme increases the rate of this equilibrium by about one million times.

The molar mass of this enzyme is about 30,000 and it contains a single protein unit of 260 amino acids and the active site contains a Zn^{2+} ion coordinated tetrahedrally to three histidine imidazole nitrogen atoms (His-94, His-96 and His-119), and water molecule or hydroxide ion (Fig. 9.28). It contains other amino acids that may functions through hydrogen bonding, proton transfer etc.

The rates of forward and backward reactions in the CO_2 hydration equilibrium increase as the pH is raised. The Zn^{2+} ion is more acidic in carbonic anhydrase than in carboxy peptidase. The presence of a neutral or less basic histidine residue instead of the glutamate residue contribute to the greater acidity of Zn^{2+} ion. Also, the three histidine residues are pulled back, therefore, Zn^{2+} ion becomes more electronegative and more acidic toward the fourth position. Thus the coordinated water becomes more polarized and losses H^+ ion to give $Zn - OH^-$. The nucleophilic OH^- then attacks on the carbon atom of CO_2 captured in the hydrophobic pocket near the Zn^{2+} ion, and a transient five coordinate Zn^{2+} ion is formed in which a carbonato oxygen from HCO_3^- coordinates to the Zn^{2+} ion. After rearrangement, the HCO_3^- ligand is replaced by H_2O. The protonation of H_2O ligand coordinated to Zn^{2+} ion then regenerate $Zn - OH^-$ which then attacks another CO_2 with the continuation of the catalylic cycle (Fig. 9.29).

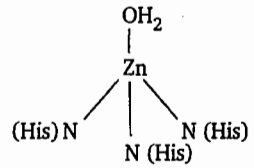


Fig. 9.28 Structure of carbonic anhydrase

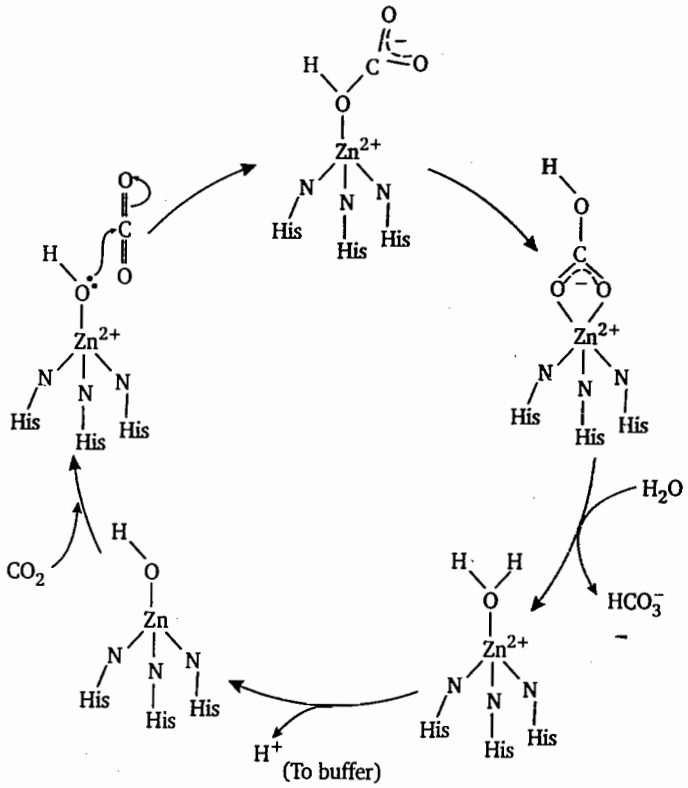


Fig. 9.29 Catalytic cycle for carbonic anhydrase

Superoxide Dismutases (SOD)

In biochemistry, dismutation means the disproportionation reaction. It has been well established that the initial step in the electron transfer reduction of O_2 produces the superoxide ion, O_2^-. The superoxide ion is toxic to cellular systems. The superoxide dismutase enzyme catalyses the dismutation (disproportionation) of superoxide ion into oxygen and hydrogen peroxide.

$$2O_2^- + 2H^+ \longrightarrow H_2O_2 + O_2$$

There are three types of superoxide dismutase:

(1) Copper-zinc superoxide dismutase, CuZnSOD

(2) Manganese superoxide dismutase, MnSOD

(3) Iron superoxide dismutase, FeSOD

The former is called as bovine superoxide dismutase and found in mitochondria of eukaryotic cells and the later two are found in bacteria (prokaryotes). A crystal structure determination of CuZnSOD has shown that the Cu^{2+} and Zn^{2+} ions are coordinated to the imidazole of a histidine residue as shown in Fig. 9.30. The Cu^{2+} ion is a distorted square pyramidal site bound to four imidazole histidine nitrogen atoms and a water molecule, the Zn^{2+} ion is tetrahedrally coordinated to three nitrogens of imidazole of histidine residues and an oxygen of aspartate residue.

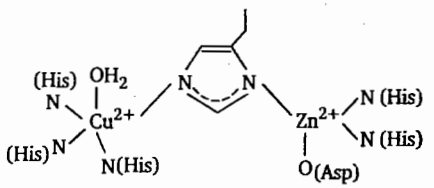


Fig. 9.30

The copper-zinc dismutase has molar mass of about 16000. It has been shown that the Cu^{2+} ion is the functional one whereas the Zn^{2+} ion is a supportive that holds the bridging imidazole histidine residue in place and provides structural stability.

The Cu^{2+} ion is more essential that can not be replaced by other metal while retaining activity. On the other hand, the Zn^{2+} ion can be replaced by other divalent metals such as Co or Cd with retention of most of the activity.

Ceruloplasmin

Ceruloplasmin is an intensely blue copper protein. Its molar mass is about 1,35,000 with six or seven Cu atoms per molecule. This protein is found in plasma of most animals. It apparantly plays an important role in the process of oxidizing Fe(II) to Fe(III) in the transfer of iron from ferritin to transferrin. It also participates in copper transport and storage. It is the deficiency of this protein which is responsible for Wilson's disease.

Blue Copper Proteins :

These proteins contain active copper centre and the copper centres can be divided into three main types:

Type 1 : This type of centre is characterized by an intense blue colour because of strong absorption in the electronic spectrum at about $\lambda_{max} = 600\,nm$ arising from cysteine (S) $\rightarrow Cu^{II}$ charge transfer. This type of centre is EPR active because of the presence of one unpaired electron on Cu^{2+} and a narrow hyperfine spliting is observed.

Type 2 : Normal monomeric Cu(II) site is tetragonally coordinated. It exhibits normal EPR spectrum. It also exhibits electronic spectroscopic characteristics typical of Cu(II) ion.

Type 3 : A pair of Cu(I) ions is about 360 pm apart and attached to protein through N-atoms of imidazole of histidine residues. The Cu_2-unit can function as two electron transfer centre and involves in the reduction and transport of O_2 according to the following reversible reaction:

$$\underset{\text{Colourless}}{2Cu^{I}} \overset{O_2}{\rightleftharpoons} \underset{\text{Bright blue}}{Cu^{II}-O-O-Cu^{II}}$$

Cu(I) and Cu(II) ions are EPR inactive because Cu(I) with its d^{10} electronic configuration is diamagnetic and Cu(II) ions interact antiferromagnetically and become diamagnetic.

Blue copper proteins conatin a minimum one Type 1 copper centre. Some important blue copper proteins are : Plastocyanin, stellacyanin, azurin, ceruloplasmin, laccase and ascorbic oxidase.

Type 1 Copper centres are found in **plastocyanin** and **azurin.** Plastocyanin is found in chloroplasts of green plants and blue-green algae. Plastocyanin has molar mass about 10500. It contain one copper atom per molecule. Plastocyanin is involved in electron transfer in photosynthesis (between PS I and PS II). The protein chain in a plastocyanin contains 97-104 amino acid residues. The copper centre in plastocyanin is coordinated to two N atoms of imidazoles of histidine residues, one S of methionine and one S of thiol of cysteine residues in distorted tetrahedral arrangement (or flattened tetrahedron) (Fig.9.31). The arrangement about copper centre involves three short bonds in an trigonal planar arrangement with fourth longer bond to S of methionine.

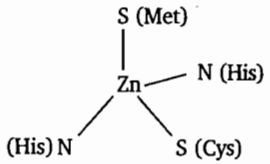


Fig. 9.31 Structure of plastocyanin

The blue copper protein **azurin** is found in bacteria and has molar mass about 16000. It also contains one Cu atom per molecule. The copper centre in azurin is trigonal bipyramid. The two N atoms of imidazole of histidine residues and one S atom of cysteine residue in trigonal planar arrangement, one S of methionine and one O of glycine residues above and below the plane. There are three short bonds in the trigonal plane and two large bonds along the axis of the trigonal bipyramid Cu—S (Met) and Cu—O (gly). (Fig. 9.32).

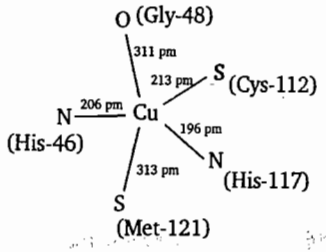


Fig. 9.32 Structure of blue copper protein (azurin)

The bond lengths shorten by 5-10 pm on going from Cu (I) to Cu(II). The coordination spheres can suite for both Cu(I) and Cu(II) and therefore, facilitate fast electron transfer. Since three donor atoms in trigonal plane are more closely bound to than the remaining donor atoms indicating the binding of Cu(I) is more favourable than that of Cu (II). This is supported by the high reduction potentials (+ .37 V for plastocyanin and + 0.31 V for azurin at about pH 7 for $Cu^{2+} \longrightarrow Cu^{+}$).

Multicopper Blue Proteins

Multicopper blue copper proteins include ascorbic oxidase and laccase (oxidases are enzymes that use O_2 as an electron acceptor). These are the metalloenzymes which are found in veriety of plants. They catalyze the four electron reduction of O_2 to H_2O and at the same time, they catalyze the oxidation of organic substrates such as phenols, amines and ascorbate by O_2 (a one electron oxidation).

$$O_2 + 4H^+ + 4e^- \rightleftharpoons 2H_2O$$

$$4RH + O_2 \longrightarrow 4R^{\bullet} + 2H_2O$$

$R^{\bullet}$ undergoes polymerization.

They contain all the three types of copper centres. Ascorbate oxidase contains a type 1 centre responsible for its blue colour along with triangular array of three other copper centres. Type 1 centre is indirectly connected to the Cu_3 unit by the protein chain. The coordination sphere of Type 1 centre is similar to that of oxidized form of plastocyanin with the metal bound by N atoms of imidazole of two histidine, S of one cysteine (Cu-S = 213 pm) and S of one methionine (Cu—S = 290 pm) residues. The trimer of Cu_3 unit lies within eight Histidine residues and can be subdivided into Type 2 and Type 3 Cu centres. Type 2 centre is coordinated to N atoms of imidazole of two histidine residues and an either H_2O or OH^- ligand. Type 3 centre contains two Cu atoms bridged by an O^{2-} ligand. The two Cu atoms are coupled antiferromagnetically. Reduction of O_2 occurs at Cu_3 unit with the remote Type 1 centre acting as the main electron acceptor, removing from the organic substrates, detail of mechanism is not clear.

Laccase also has Type 1 copper centre along with a trimer of copper site containing Type 2 and Type 3 copper centres. The structure of Cu_3 unit is similar to that in ascorbate oxidase but Type 1 copper centre is three coordinated to S of one cycteine and two histidine residues in trigonal planar array and lacks the axial ligand present in the Type 1 copper centre in ascorbate oxidase.

Non-Blue Copper Protein

Galactase oxidase is a non-blue copper protein and it is found in fungi where it catalyzes the oxidation of $-CH_2OH$ group in galactose to —CHO group by reducing O_2 to H_2O_2. It has molar mass about 68000. It contains one Cu atom coordinated to N atoms of imidazole of two histidine residues, O atoms of two tyrosine residues and O atom of an acetate ion in square pyramidal arrangement. The Cu (III) / Cu (I) couple involve in the 2-electron reduction of O_2.

Cytochrome-c oxidase containing Cu and Fe is also a non-blue copper enzyme. Cytochrome-c oxidase is the terminal member of the respiratory chain in plants, animals, aerobic yeasts and some bacteria. It catalyses the reduction of O_2 to H_2O.

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

It is a large, complex, multisubunit enzyme containing two copper ions and two heme iron units. There are two kinds of copper sites, Cu_A and Cu_B and two kinds of heme iron units, heme-*a* and heme a_3. Electron transfer ocurs from cytochrome-c to Cu_A and then to heme *a*.

Heme a_3 (or cytochrome a_3) and Cu_B provide the active site for O_2 binding and its reduction to H_2O. Cytochrome-c oxidase is found associated with the inner mitochondrial membrane. Heme *a* and Cu_B sites are involved in pumping H^+ ions (four H^+ ions per O_2 molecule) across the inner mitochondrial membrane. Cytochrome-c oxidase contains two Cu(I) ions and two Fe(II) heme centres. Oxidized from contains two Cu(II) ions and two Fe(III) heme centres.

Cytochrome-*a* in both oxidation states (Fe^{II} and Fe^{III}) is low spin octahedral with two axial Histidine residues. In its oxidized form, it is EPR active. Addition of CN^- ion to the oxidized form or CO to reduced form does not perturb this centre. This indicates that CN^- ion does not bind to heme which is consestent with a six coordinate heme. Cu_A is dicopper site bridged by cystine residue. It also acts as an electron transfer site with a Cu_2S_2 core. Histidine residue bound to Cu_A provide an electron transfer path between Cu_A and cytochrome *a*.

The cytochrome a_3 can bind ligands such as CN^- to the oxidized form Fe(III) and CO to the reduced form (Fe^{II}) which indicates that it is either five coordinate or it has readily displaceable ligand. It might be expected that two Cu(II) centres and two Fe(III) heme centres are EPR active. But It has been observed that low spin Fe(III) and Cu_A centres are EPR active and Fe(III) in cytoctrome a_3 and Cu_B centre are coupled antiferromagnetically and therefore are EPR inactive.

Dioxygen reacts with Fe(II) heme centre to form mononuclear dioxygen complex [Reaction–(i)]. These dioxygen complexes react rapidly with another Fe(II) heme centre to form binuclear peroxo bridged complex [Reaction (ii)]. These peroxo bridged complex are stable at low temperature but at higher temperature the peroxo linkage (O—O) breaks and two equivalents of Fe(IV) oxo complex are formed. [Reaction (iii)]. The subsequent reactions of peroxo-bridged complexes with Fe(IV) oxo complexes give the μ-oxo dimer [Reactons(iv)].

$$3Fe^{II}(P) + 3O_2 \longrightarrow 3Fe(P)(O_2) \quad \ldots(i)$$

$$3Fe(P)(O_2) + 3Fe^{II}(P) \longrightarrow 3(P)Fe^{III}—O—O—Fe^{III}(P) \quad \ldots(ii)$$

$$(P)Fe^{III}—O—O—Fe^{III}(P) \longrightarrow 2Fe^{IV}(P)(O) \quad \ldots(iii)$$

$$2Fe^{IV}(P)(O) + 2(P)Fe^{III}—O—O—Fe^{III}(P) \longrightarrow 2(P)Fe^{III}—O—Fe^{III}(P) + 2Fe(P)(O_2) \quad \ldots(iv)$$

Net Reaction $$4Fe^{II}(P) + O_2 \longrightarrow 2(P)Fe^{III}—O—Fe^{III}(P)$$

This reaction sequence indicates that, it is four electron reduction of one O_2 molecule and the final products are the binucuar, O^{2-} bridged two Fe(III) heme complexes.

Copper(I) complexes also react with dioxygen to form peroxo bridged binuclear complexes. But these complexes do not undergo readily cleavage of peroxy linkage (O — O bond) because Cu(III) ion is not easily obtained as the Fe(IV) ion in an iron porphyrin complex. The stable bridged peroxo complex of Cu(II) is also difficult to obtain because the peroxo complexes either is protonated to give H_2O_2 or is itself reduced by excess of Cu(I).

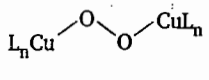
$$2Cu^I + O_2 \longrightarrow Cu^{II}—O—O—Cu^{II}$$

$$Cu^{II}—O—O—Cu^{II} + 2H^+ \longrightarrow 2Cu^{II} + H_2O_2$$

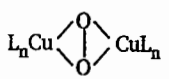
$$2Cu^I + H_2O_2 + 2H^+ \longrightarrow 2Cu^{II} + 2H_2O$$

Net Reaction $$4Cu^I + O_2 + 4H^+ \longrightarrow 4Cu^{II} + 2H_2O$$

The bridged peroxo complex of Cu(II) may have either of the following structures.



μ - 1, 2



μ^2-η^2, η^2

Essential and Trace Elements

There are many elements which are essential for biological reactions. Some of them are required in relatively large quantities and therefore, called macronutrients. These elements are Na, K, Mg, Ca, P, S, Cl along with four most abundant elements C, H, N, O in biological systems. There are also some elements which are required in small amounts, called as trace elements or micronutrients. These elements are : all the first row transition metals (except Sc and Ti) Mo and W (from second and third row transition metals respectively) and non-metals (B, Si, Se, F and I). Ni, Cd, Pd and As are ultratrace elements and are essential at very low concentrating (< 1 ppm). The elements are toxic at concentration above ultratrace level.

The Biological Roles of some metal ions

About 30% of enzymes have a metal ion at their active sites. The metalloenzymes facilitates the variety of biological reactions shown in Table 9.2.

Micronutrients 3d essential (except Sc, Ti)

4d only Mo

5d only W

Non-Metal → B, Si, Se, F and I

Ultratrace element

↓

Ni, Cd, Pd, As

Table 9.2

Metal	Related Compounds	Action
Fe	Hemoglobin	Oxygen transport
	Myoglobin	Oxygen storage
	Hemerythrin	oxygen storage
	Oxygenases	Insertion of oxygen atoms into substrate
	Hydrogenases	Oxidation of H_2
	Cytochrome P-450	Insertion of oxygen atom into substrate
	Catalase	Catalyze oxidation of substrates by H_2O_2
	peroxidase	Catalyze oxidation of substrates by H_2O_2
	Cytochromes	Electron transfer
	Ferredoxin	Electron transfer
	Ferritin	Iron storage
	Transferrin	Iron transport
	Siderophores	Iron transport
Co	Coenzyme B_{12}	Methylation of organic compound
Cu	Amine oxidase	Oxidation of amine to aldehyde
	Ceruloplasmin	Transport of Fe from ferritin to transferrin, Cu storage and transport
	Hemocyanin	Oxygen carrier
Zn	Carboxypeptidase	Hydrolysis of peptide bonds
Mg	Chlorophyll	Photosynthesis
	Phosphotransferase	Phosphate hydrolysis
Mn	Arginase	Electron transfer
Mg, Mn	Aminopeptidase	Catalyzes the cleavage of amino acid
Fe, Mo	Nitrogenase	Nitrogen fixation
Fe, Mo, Cu	Oxidase	Redox reaction involving O_2 as electron acceptor
	Reductase	Catalyzes reduction reactions
	Hydroxylases	Oxidative degradation of organic compounds
Cu, Zn, Mo	Superoxide dismutase	Dismutation of O_2^- into O_2 and H_2O_2
Mg, Cu, Zn	Phosphatases	Removed of phosphate group from substrate
Ni	Urease	Hydrolysis of urea into CO_2 and NH_3.

Na^+ and K^+ ions are involved in Na-K pump, C, H, N and O along with P involve in amino acids, protein, DNA, NADP, ADP and ATP. Cr is essential for glucose metabolism in humans. Iodine is essential for thyroid gland, calcium serves as structural material in teeth, bones, shells and a number

of less well known calcium rich deposits. Calcium in teeth and bones have the composition $Ca_{10}(PO_4)_6X_2$ where X = F, Cl or OH or a mixture of these. Fluorine is also essential for teeth and its deficiency causes dental caries.

Metal complexes in Medicine

Cisplatin: Anticancer Drug

In 1969, B. Rosenberg and Co-workers discovered the antitumor activity of simple square planar Pt(II) complex, *cis*-diamminedichloroplatinum(II) or cisplatiin, $[Pt(NH_3)_2Cl_2]$. This compound is used as chemotherapeutic agent to inhibit otherwise rapid division of tumor cells (*i.e.*, proliferation). The exact action of this complex is not known. Since the *trans*-isomer is inactive, therefore chelation or atleast coordination to donor atoms at *cis*-positions (*i.e.*, in close proximity) is an essential part of the activity. The proton nmr studies has suggested that platinum binds to N-7 atom of a pair of adjacent guanine bases of a fast growing tumor with the chloride ligands first being replaced by water molecules and then by a DNA base.

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

$$\downarrow \text{DNA}$$

$$cis\text{-}[Pt(NH_3)_2Cl]\text{—DNA}$$

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

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

Cisplatin interacts with two adjacent guanine bases on DNA usually within the same strand (intrastand linking) or occasionally between strands (interstrand linking). Recent X-ray studied on a 12-base pair fragment of double stranded DNA has suggested that the binding of Pt distorts the local DNA structure and therefore, inhibits the cell division inherent in the proleferation of cancer cells. Cisplatin is most effective against testicular cancer. Cisplatin has negative side effects in kidney and neuro-toxicity.

In order to avoid these serious side effects alternative platinum compounds have been developed. The most important of these is 'Carboplatin (Fig. 9.33) in which the *cis*-chloride ligands are replaced by the O-chelate, cyclobutanedicarboxylate.

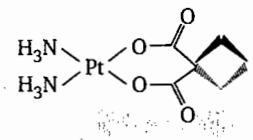


Fig. 9.33 Structure of carboplatin

Wilson's disease

Wilson's disease is caused by the overload of copper in the body. It is a genetic disease. Patients suffering from Wilson's disease have low levels of the copper storage protein ceruloplasmin and therefore, copper can not be tolerated even at normal levels. The Wilson's disease is responsible for liver disease, neurological damage and brown or green rings in the cornea of the eyes. Many chelating ligands can be used to remove the excess of copper but one of the best is **D-penicillamine.** This chelating ligand forms a complex with copper ions (Cu^{I} and Cu^{II}) Fig. 9.34 that has intense purple colour and the molecular formula of the compound is $[Cu^{I}_{8}\ Cu^{II}_{6}$ (penicillamine)$_{12}$ Cl). The sulfhydryl group of D-penicillamine effects removal of copper as Cu(I) complex.

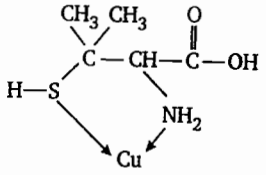


Fig. 9.34 Structure of complex of Cu with D-penicillamine chelating ligand

Chelation therapy using EDTA or 2, 3-dimercaptopropan-1-ol (BAL, British Anti-Lewisite) also causes the symptoms to disappear.

Anti-Arthritis

Complexes of Au(I) have been used most successfully for the treatment of arthritic disorders in humans. These complexes used to treat arthritis were painfully administered as intramuscular injections. These complexes include $Na_3[Au(S_2O_3)_2]$, called Sanochrysin, sodium salt of thiomalate, called Myochrysin (Fig. 9.35)

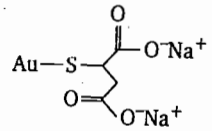


Fig. 9.35 Structure of Myochrysin

More recently, the compound auranofin (Fig. 9.36) has been developed. It has the advantage that it can be administered orally and is effective.

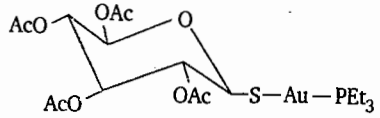


Fig. 9.36 Structure of Auranofin

Hypercalcemia

Hypercalcemia is a disease which causes the rapid loss of calcium from the bones of cancer patients. Gallium nitrate, $Ga(NO_3)_3$ has been found to be most effective for treatment of hypercalcemia.

Magnetic Resonance Imaging (MRI)

Nuclear magnetic resonance (NMR) spectroscopy can be used to image specific tissues of biological systems because of the differences in the relaxation times of water proton resonances usually brought about by metal ions which are paramagnetic. The useful metal ions for magnetic resonance imaging in humans are Gd(III), Fe(III) and Mn(II) ions. The paramagnetic character of these ions alters the relaxation of rate of nearby water protons and, therefore, the normal and diseased tissue can be distinguished. An advantage of paramagnetic MRI over radioisotopic imaging agents is that there is no possibility of radiation damage. Removal of excess of a metal from the body is called chelate therapy.

Siderosis Disease

An excessive intake of iron causes various problems known as siderosis. Chelation therapy is also used to treat the excess of iron. The patients who suffer from deposits of iron in liver, kidney and heart, lead to failure of these organs. The excess of iron can be removed by using chelating ligand sachas desferrioxamine-B, a polypeptide having a very high affinity for Fe (III) but not for other. The concepts of soft and hard metals and ligands can be used for the process of designing therapeutic chelating agents.

USE OF CHELATING AGENTS IN METAL POISONING : THE CHELATE THERAPY

LEAD

Lead is a very poisonous metal. It is a commulative poison, since it keeps on accumulating in the tissues of human body and plants.

Harmful Effects of Lead

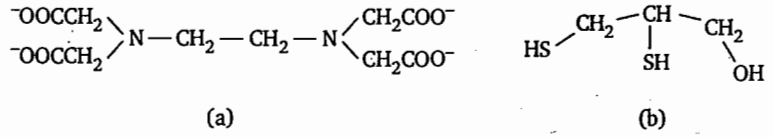
Lead has a strong affinity of complexing with oxo-groups of enzymes and inhibits all steps in the process of heme synthesis, porphyrin metabolism, protein synthesis by modifying *transfer*-RNA, acid phosphate, ATPase, carbonic anhydrase etc. Pb(II) also inhibits SH enzymes [less strongly than Cd(II) and Hg(II)] by interaction with cysteine residues in proteins.

Symptoms of Lead Poisoning

Symptoms of lead poisoning are : anaemia, loss of appetite, headaches, nervous disorders, brain damage, liver damage, kidney damage, cholic and skin diseases.

Treatment of Lead Poisoning

Lead poisoning can be treated by complexing and sequetering the lead by chelating ligands such as ethylenediamine tetraacetate $CaNa_2(EDTA)$ [Fig. 9.37(a)] or British anti-Lewisite, BAL [Fig. 9.37(b)] or penicillamine.



(a) (b)

Fig. 9.37 Structure of (a) EDTA (b) BAL

CADMIUM

Cadmium is extremely toxic. It accumulates in the kidneys and liver of human. It has a strong affinity for the —SH group of cysteine residues in protein and therefore inhibits SH enzymes. It also inhibits the action of zinc enzymes by displacing zinc.

Symptoms of Cadmium Toxicity

Symptoms of cadmium toxicity are : disfunctions of kidneys, vomiting, irritation, hypertension, anaemia and a disease called Hai Itai in which the whole body feels serious pains and the bones begins to facture very easily.

MERCURY

Mercury having an appreciable vapour pressure is also extremely toxic. Monomethyl mercury, CH_3Hg and dimethyl mercury, $(CH_3)_2Hg$ are more dangerous than metallic mercury itself and inorganic mercury compounds such as $HgCl_2$. These organomercury compounds are more readily absorbed in the gastrointestinal tract than Hg^{2+} salts because they have greater ability to penetrate biomembranes. They concentrate in blood and have immediate and permanent effect on brain and central nervous system because they bind to the —SH groups of cysteine residues in proteins.

Symptoms of Mercury Toxicity

The symptoms of mercury toxicity are : central nervous disorders, headaches, irritability, fatigue, inability to make decisions, sleeplessness, diarrhoea, etc.

Treatment of Mercury Toxicity

More rapid elimination of cadmium, mercury and lead requires the administration of chelating ligands such as 2, 3-dimercaptopropanol (*i.e.*, British anti-Lewisite, BAL), $HSCH_2CH(SH)CH_2OH$ and N-acetyl penicillamine.

A very intersting natural detoxification has been discovered in bacteria resistant to mercury. Bacteria have developed resistance to heavy metals and detoxifying process is initiated and controlled by metalloregulatory proteins that are able selectively to identify metal ions. The most studied metalloregulatory proteins is mer R. It is a small DNA binding protein that controls transcription of the mer genes.

DEFICIENCY SYMPTOMS OF SOME TRACE METALS

ZINC

Zinc is very important for proper functioning of the immune system. The body of an adult human contains about 2g of zinc. There is some zinc in every one of the cells in the body but most of it is in the skin, hair, nails and eyes and in the prostate gland for males. The most easily absorbed form of zinc is zinc gluconate, zinc citrate and zinc monomethionate. Zinc sulphate is likely to upset the stomach. The infection fighting white blood cells contains a lot of zinc present in the body. The best food for zinc are organ meats such liver or kidneys, leafy grains, root vegetables such as carrots and potatoes. Zinc is important for:

(i) **Fighting off cold or flu :** Zinc suppliments assist to reduce the cold symptoms such as runny nose, coughing and sore throat.
(ii) Zinc make skin, nails and hair healthy.
(iii) Zinc helps in healing wounds.
(iv) Zinc reduces the diabetic problems.
(v) Zinc increases production of testoterone and other male harmones. Therefore, it reduces the male infertility.
(vi) Zinc helps to preserve eye sight and improves memory.
(vii) Zinc may help teenagers with pimples.

Symptoms of Zinc Deficiency

(i) Reduced growth of children.
(ii) Reduced mental retardation.
(iii) Slow wound healing.
(iv) Frequent infections.
(v) Skin irritation.
(vi) Hair loss.
(vii) Loss of sense of taste.

Zinc Works Best with

(i) Vitamin A
(ii) Vitamin B_6
(iii) Insulin
(iv) Vitamin D
(v) Vitamin E
(vi) Glucose
(vii) Mg
(viii) Mn

Impacts of Excess of Zinc

Excess of zinc in the human body causes :

(i) Dysfunction fo the central nervous system
(ii) Anaemia
(iii) Diarrhoea
(iv) Dizziness
(v) Sore stomach
(vi) Nausea
(vii) Vomiting
(viii) Alcohol intolerance
(ix) Electrolyte imbalance
(x) Increase LDL cholesterol and lower HDL cholesterol

COPPER

Copper is required in the formation of hemoglobin, red blood cells and bones. It helps in the formation of elastin and collagen making it necessary for wound healing. Copper works closely with iron for these functions. Copper is a vital component of a number of enzymes. Copper is essential for connective tissue formation, iron metabolism. It also acts as an antioxidant.

Copper Sources

Main sources of copper are : Oysters (cooked), sunflower seeds, almonds, etc.

Symptoms of Copper Deficiency

Copper deficiency cause : Deficiency of iron which can lead to anaemia, infections, osteoporoses, thining of bones, thyroid gland dysfunction, heart disease, nervous system problems and increased blood fat level.

Copper works best with folic acid, vitamin B_6, vitamin B_{12}, amino acids, iron, zinc and Mn.

Impact of Excess of Copper

Excess of copper causes : Fever, high blood pressure, diarrhoea, dizziness, depression, fatigue, irritability, joint and muscle pain, nausea, premature ageing, vomiting, wrinkling of skin, headache etc.

COBALT

Cobalt is an essential trace mineral that is a constituent of vitamin B_{12}. Cobalt is a necessary cofactor for making the thyroid hormone thyroxine. The most of the body's cobalt is stored in liver.

Main sources of cobalt are liver, clams, milk, nuts, fish, red meat, oysters. Cobalt works best with vitamin B_{12}.

Deficiency of Cobalt

A deficiency of cobalt may lead to a deficiency of vitamin B_{12} and lead to pernicious anaemia. The symptoms of perniceous anaemia are :

(i) bleeding gums.
(ii) nausia, appetite loss and weight loss.
(iii) weakness and tingling in the arms and legs.
(iv) headache, confusion and poor memory.
(v) sore tongue.

Impact of Excess of Cobalt

The symptoms of excess of cobalt in the human body are :

(i) nausea
(ii) vomiting
(iii) diarrhoea
(iv) skin rashes

IRON

Iron is the most important transition element involved in the living systems. The adult humans body contains about 4 g of iron. It plays a crucial roles in the transport and storage of dioxygen and electron transport. The important sources of iron are : green vegetable, squash, pumkin seeds, liver, oysters beens, pulses, nuts (cashew, peanut, almond, pine, hazel nut), beef, lamb, Pork, wheat products, corn meal, strawberries, watermelon etc.

Deficiency of iron and oxygen causes anemia.

The symptoms of deficiency of iron an anemia are : difficulty maintaining body temperature, feeling tired and weak, decreased immune function which increases susceptibility to infection, fatigue, decreased memory,

Excess of iron causes stomach pain as the stomach lining becomes ulcerated, damage of internal organs particularly the brain and the liver.

Iron poisoning can be treated by chelate therapy using chelating agent such as deferoxamine.

Main Deficiency symptoms of some trace elements are given in Table. 9.3.

Table. 9.3 Deficiency Symptoms of Trace Elements

Element	Function	Main Deficiency Symptom
Chromium	Glucose metabolism	Impaired glucose metabolism
Cobalt	Vitamin B_{12}	Anemia
Copper	Oxidative enzymes	Anemia, skeletal defects
Manganese	Mucopolysaccharide metabolism	Growth retardation
Molybdenum	Purine metabolism, aldehyde oxidation	Joint pain
Zinc	Nucleic acid metabolism	Poor wound healing

Sodium Potassium Pump : Na^+, K^+-ATPase

Lipid soluble substance diffuse through the membrane lipid bilayer without the need for a carrier mechanism. This process is called passive diffusion. The movement of water soluble solutes and ions requires specific transmembrane proteins to fascilitate transfer across the lipid bilayer. This process is called faciliated diffusion. Many of the faciliated diffusion processes move a solute against a concentration gradient. This requires an input of energy which is usually provided by hydrolysis of ATP. This faciliated diffusion that requires energy input is called active membrane transport.

An interesting example of active transport is the Na^+ / K^+ pump present in animal cells. This pumps Na^+ and K^+ out of and into the cell respectively using energy released by hydrolysis of ATP. The animal cells have intracellular fluid with low concentration of Na^+ (0.012 *M*) and high concentration of K^+ (0.14 *M*) as compared to extracellular fluid (blood and lymph) which contains high concentration of Na^+ (0.15 *M*) and low concentration of K^+ (0.004 *M*). (Fig. 9.38). The concentration of Na^+ and K^+ on the two sides of the cell membrane are interdependent suggesting that the same carrier protein transports Na^+ and K^+ ions. The carrier is an ATP-ase and the energy released by hydrolysis of one ATP to ADP + Pi is enough to pumps three Na^+ ions out of the cell and two K^+ and one H^+ ions into the cell.

The concentration gradients (the difference in the intracellular and extracellular concentration) across the cell membrane produces an electrical potential difference across the cell membrane which is essential for functioning of nerve and muscle cells. To maintain this difference, Na^+ ions move into the cell *via* channels in the cell membrane but it is continuously pumped out again by means of the Na^+ / K^+ pump.

The Na^+ / K^+ pump is the key to functions such as cardiac and nenal activity as well as all general transport processes into and out of the cell. The pump thus forms the basis for our ability to absorb a considerable number of nutrients, excrete waste products from the kidneys and regulate the water balance in the cells. If this little pump stopped pumping Na^+ ions out of the cell the later would rapidly swell up because of the infilteration of water and finally burst.

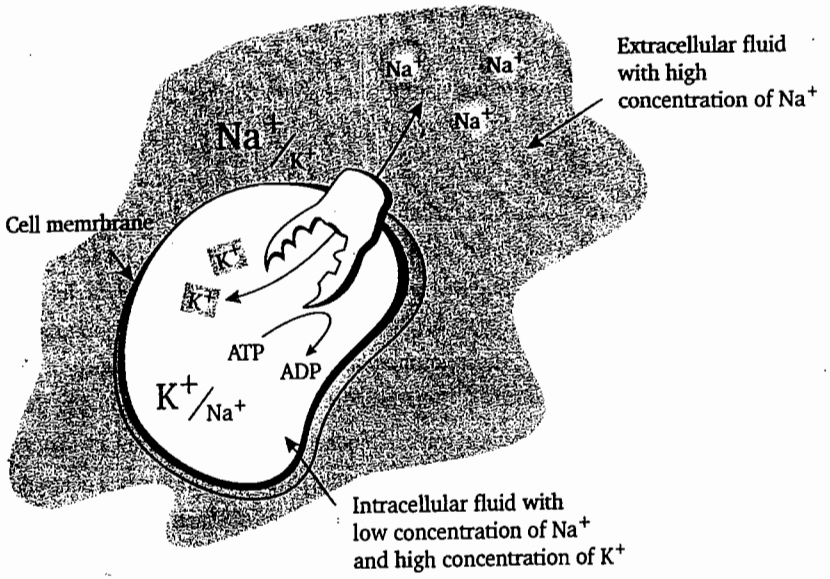


Fig. 9.38

The K^+ is required in the cell for glucose metabolism, protein synthesis and activation of many enzymes. Glucose and amino acids enter the cell in association with Na^+ which is favoured by the high concentration gradient. The Na^+ ions entering the cell in this way must then be pumped out again.

Skou did not use term Na^+ / K^+ pump regarding his discovery. He has stated that ATP-ase enzyme is responsible for the active transport of Na^+ and K^+ ions across the cell membrane and he described it as Na^+, K^+-ATPase. Therefore, Na^+ / K^+ pump is also called as Na^+, K^+-ATPase.

Mechanism

The mechanism for ion transport involves the natural polyether which are called as ionophores (ion-bearers). Some natural ionophores for Na^+ and K^+ ions have oxygen donor atoms from carboxylates and cyclic peptides. Some of these are antibiotics such as nonactin and valinomycin. Nonactin is a member of a family of naturally occurring cyclic ionophores known as the macrotetrolide antibiotics.

Valinomycin is a natural, lipid soluble molecule that binds K^+ and facilitates their transfer across cell membrane. Valinomycin and nonactin have selectivity for K^+ over Na^+ ion.

The Na^+ / K^+ pump is also called the Na^+, K^+-ATPase because ATP is hydrolysed to give ADP + Pi and released energy which is used to Na^+ and K^+ ions pump out and into the cell respectively. Na^+, K^+-ATPase is composed of two dissimilar subunits α and β. They are closely associated as dimeric pair (α, β) to form a tetramer $(\alpha\beta)_2$. Na^+, K^+-ATPase contains two high affinity binding sites for ATP, one on each α-subunit. These sites face the cytoplasmic surface of the cell membrane. The Na^+ binding sites also face the cytoplasmic surface whereas the binding sites for K^+ ions face the external surface of the cell membrane. Na^+, K^+-ATPase undergoes change in its shape (or conformation) due to the covalent attachment of phosphoryl group to the carboxyl group of a specific aspartic and residue of α- subunit. The phosphorylation occurs in the presence of Na^+ and Mg^{2+}-ATP. The catalytic cycle for Na^+ and K^+ ion transport is illustrated in Fig. 9.39. The form (a) of the enzyme opens up toward the inner side of cell and binds three Na^+ ions but not K^+ ions. Once the Na^+ ions are bound to the enzyme, an ATP molecule is bound to the enzyme and one of its phosphate groups (P) is transferred, *i.e.*, there is hydrolysis of ATP to produce ADP and phosphorylated protein and to relase energy. The enzyme changes its shape, opens up toward the outer side of the cell and releases Na^+ ions. There are now binding sites for two K^+ ions but not for Na^+ ions on the outer side of the cell. When K^+ ions bind, the phosphate group (P) is now hydrolyzed from the protein giving Pi. When K^+ ions have been released inside the cell, the enzyme returns to the first stage-ready to bind new Na^+ ions, so the cycle can begin all over again.

The net result is that hydrolysis of ATP pumps Na^+ out and K^+ in. The ratio is $3Na^+$ ions out and two K^+ ions in. The overall equation is :

$$3Na^+(in) + 2K^+(out) + ATP + H_2O \longrightarrow 3Na^+(out) + 2K^+(in) + ADP + Pi$$

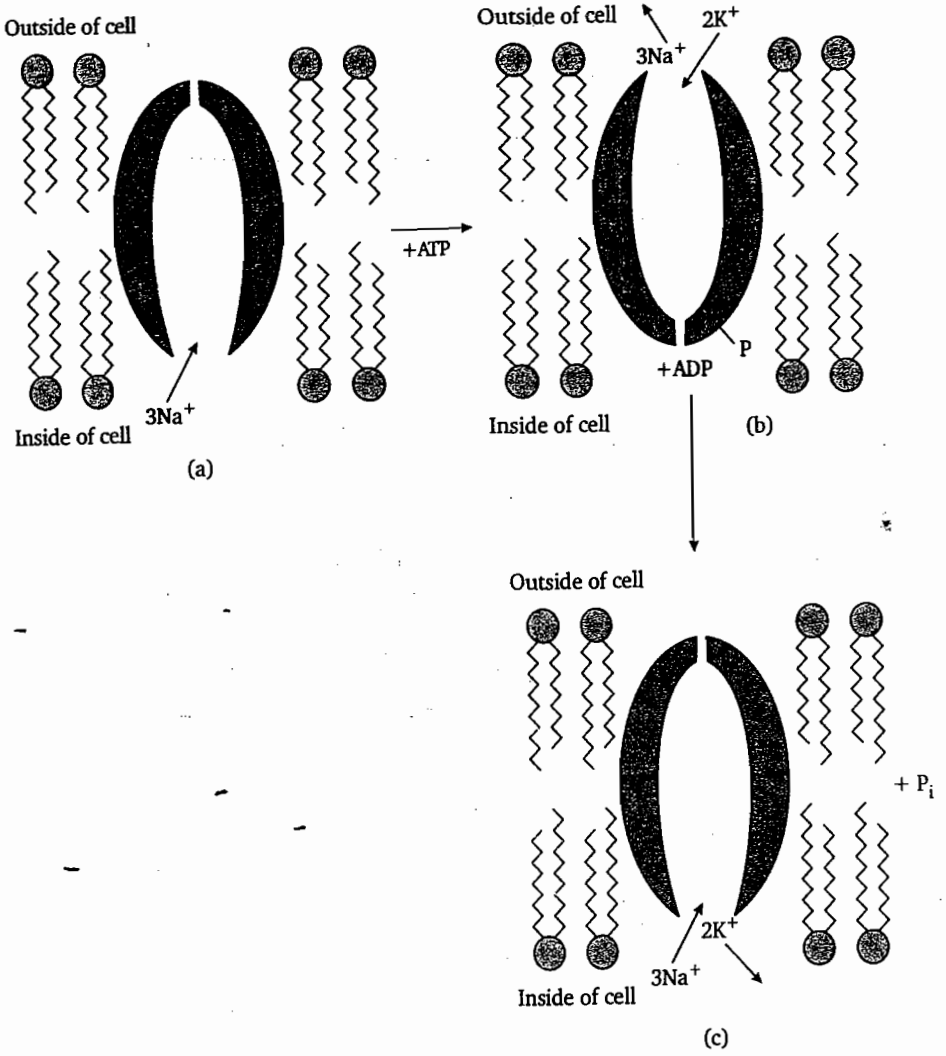


Fig. 9.39

NITROGEN FIXATION

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* Nitrogen fixation is a key reaction of the biological nitrogen cycle. Fixed nitrogen, in which 'N' is in molecules other than N_2, is frequently the limiting factor in plant growth.
* Since natural systems cannot provide enough fixed nitrogen for agriculture or animal husbandary, industrial processes have been developed to fix nitrogen chemically.
* The major process in use, referred to as ammonia synthesis is Haber Bosch Process, in which N_2 and H_2 are reacted at temperatures between 300 - 500°C and Pressures of more than 300 atm using catalyst based on metallic iron.
* Hundred of massive chemical plants are located throughout the world, some producing more than 1,000 tons of NH_3 / day. In contrast, in the biological Process, N_2 is reduced locally as needed at room temperature and ~ 0.8 atm by the enzyme system called "Nitrogenase".

$N_2 + 3H_2 \longrightarrow 2NH_3$ (Haber - Bosch Process)

* This biological Process amounts to about 1.2×10^8 tons of nitrogen fixed per year. it is evident that in the N_2-fixation Process, the bacterial efficacy is beyond the comparision with the chemist's efficiency. this biological fixation is very important in maintaining the natures nitrogen cycle.

Definition:

"Nitrogen fixation can be any reaction with dinitrogen (N_2) in which nitrogen gets covalently bonded to any other element. Certain Plants (leguminosa, clover, beans, peas, etc.) can synthesize nitrogenous biomolecules by reducing atmospheric nitrogen in their roots nodules under ambient condition."

The scope of biological Nitrogen fixation:

* Biological nitrogen fixation occurs naturaly only in certain Prokaryotic organisms. Although the majority of bacterial species are not nitrogen fixers, and it has been confirmed in atleast some members of many important phylogentic groups.

* The biological catalyst for reduction of nitrogen to ammonia is enzyme nitrogenase, which occurs in four types of microorganisms:

(i) free living bacteria : azotobacter (aerobic), clostridium Pasteurianium (anaerobic)

(ii) Symbiotic bacteria : rhizobium (leguminous plant, aerobic), Bradyrhizobium

(iii) photosynthetic blue green algae : anabina, nostoc (aerobic)

(iv) photosynthetic microorganism : chromatium (anaerobic) citrobactor freundii

Thermodynamic and kinetic Aspects of Nitrogen fixation:

* The N_2 molecule has the following parameters

- $N \equiv N$ bond distance = 110 Pm
- $\bar{\nu}$ ($N \equiv N$) = 2331 cm^{-1}
- $N \equiv N$ bond energy = 950 $kJ\ mol^{-1}$
- First ionisation energy of N_2 = 1503 $kJ\ mol^{-1}$
- electron affinity of N_2 : Thermodynamically unfavourable
- The HOMO of N_2 is Sigma (σ) bonding in character and makes the ionisation energy high whereas LUMO is π antibonding in character. Consequently, it is highly reluctant to accept an electron and only few strong reducing agents can transfer electrons to N_2 molecule leading to scission of $N \equiv N$ bond under drastic condition.

The chemical inertness of N_2 arises due to:

(i) Very high bond energy

(ii) high energy gap between HOMO and LUMO makes the molecule nonpolarisable and resistant towards electron transfer redox Process

(iii) Antibonding character of LUMO restricts it to accept any electron.

(iv) σ bonding character of HOMO makes the ionisation Process unfavourable

(v) low polarisability of N_2 disfavours it to accept the polar transition state ~~other than~~ required in nucleophilic and electrophilic displacement reactions

* The chemical inertness is mainly due to kinetic barrier not due to the thermodynamic barrier.

$N_2 + 3H_2 \longrightarrow 2NH_3$ ($\Delta G^\circ = -16.7\ kJ\ mol^{-1}$, $\Delta H^\circ = -46.1\ kJ\ mol^{-1}$)

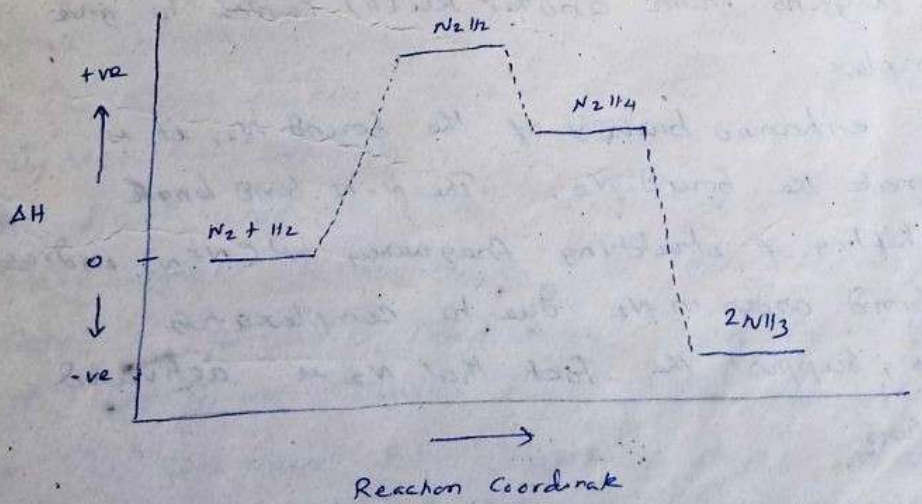
$N_2 + 6H^+ + 6e \longrightarrow 2NH_3$ ($E_0' = -0.34\ V$, at biological

* The E_0' value indicates the biological reductants can reduce N_2 to NH_4^+. The kinetic inertness of this reduction of N_2 to NH_3 can be understood by considering the steps involved in the 6e reduction of N_2. A single step involving 6e, $6H^+$ for direct reduction of N_2 to NH_3 is highly improbable. This process is likely to pass through the formation of N_2H_2 and N_2H_4 intermediates

$$N_2 + 2H^+ + 2e^- \longrightarrow N_2H_2 \quad (E_0' = -1.0 \text{ to } -1.5\ V)$$

$$N_2 + 5H^+ + 4e^- \longrightarrow N_2H_5^+ \quad (E_0' = -0.70\ V)$$

* The E_0' values are highly negative and the normal biological reductants cannot carry out the reduction of N_2 to these intermediates
* In terms of enthalpy of formation, the formation of N_2H_2 ($+210\ kJ\ mol^{-1}$) and N_2H_4 ($+105\ kJ\ mol^{-1}$) is thermodynamically unfavourable, though formation of NH_3 ($-80\ kJ\ mol^{-1}$) is thermodynamically favoured
* formation of these unstable intermediates put the barrier to measure the ~~inertness~~ inertness of N_2 towards its reduction
* To overcome the barrier, the intermediate may be stabilized through complexation. in other way formation of the unfavourable intermediates may be coupled with the thermodynamically favoured processes like ATP hydrolysis or the evolution of H_2 to make the overall process thermodynamically favourable



Dinitrogen Complexes and Activation of Dinitrogen through Complexation

* N_2 can act both as a monodentate ligand and as a bridging ligand and several N_2 complexes have been prepared. The segment $M-N\equiv N$ is linear in both mononuclear and binuclear complexes.
* As for carbonyls, the N_2 carbonyls are stable with d^4-d^9 metals than with the d^2, d^3 and d^{10} metals. It indicates that to stabilize the N_2 complexes, the π-donor properties of the metal is very important.
* For free N_2, $N\equiv N$ stretching frequency arises at 2331 cm^{-1}, while in complexes with terminal N_2, the frequency shift to 2200 - 1850 cm^{-1}. This shift is mainly due to the π-acceptor properties of the complexes N_2. it is supported from experiment establishing the fact that N_2 is a very poor σ-donor.
* The π-acceptor properties of N_2 is comparable with that of nitrile, but it is not as good as that of CO.
* The π-acceptor properties of N_2 largely depends on the chemical state of metal centre.
* The π-acceptance of N_2 indicates that in the complex $M-N\equiv N$, the N_2 moiety gains an additional electron density. This makes the bound N_2, a better base than the free N_2.

ex

$$\left[K(H_3)_5\ Ru\ (N_2)\right]^{2+} + \left[(H_2O)\,Ru(NH_3)_5\right]^{2+} \rightleftharpoons$$

$$\left[(NH_3)_5\ Ru-N_2-Ru\ (NH_3)_5\right]^{4+} + H_2O$$

Here the bound N_2 in Allen senoff's complex will displace a water ligand from another Ru(II) centre to give the binuclear complex.

* Because of the enhanced basicity of the bound N_2, it is possible to protonate the bound N_2. The N-N bond length elongation and shifting of stretching frequency $\nu(N\equiv N)$ indicate the lowering of bond order in N_2 due to complexation.

All these evidences, support the fact that N_2 is activated through complexation.

Nitrogenase in biological Nitrogen fixation:

* Nitrogenase contains both Mo and Fe metals, but in some cases Mo is substituted by V. and it is called as vanadium nitrogenase.

occurence of Nitrogenase:

* Nitrogenase mainly occur in Prokaryotic cells of the organisms such as bacteria and blue green algae etc.
* Both aerobic and anaerobic species may contain nitrogenase.

Composition of Nitrogenase:

* The Mo containing nitrogenase consists of two different components.

Component I : larger ~~molecular~~ Protein component (m.w ~ 230 k Da) Containing both Mo and Fe, called as Fe Mo cofactor, designated as [Fe Mo-Co]

Component II : Smaller component (m.w ~ 60 k Da) which is basically a Fe-Protein.

* Components I & II are also designated as Xy1 and Xy2 respectively where x and y are the first letters of the first and second name of the bacterial source.

* Av1 = Fe Mo protein of Azotobacter vinelandii
  Cp2 = Fe Protein of Clostridium Pasteurianum.

* Component I is a dimer of α, β units. each unit of this dimer contains one [Fe Mo-Co] and two P-clusters. if it is considered as $\alpha_2\beta_2$ (tertramer), it consists of four subunits.

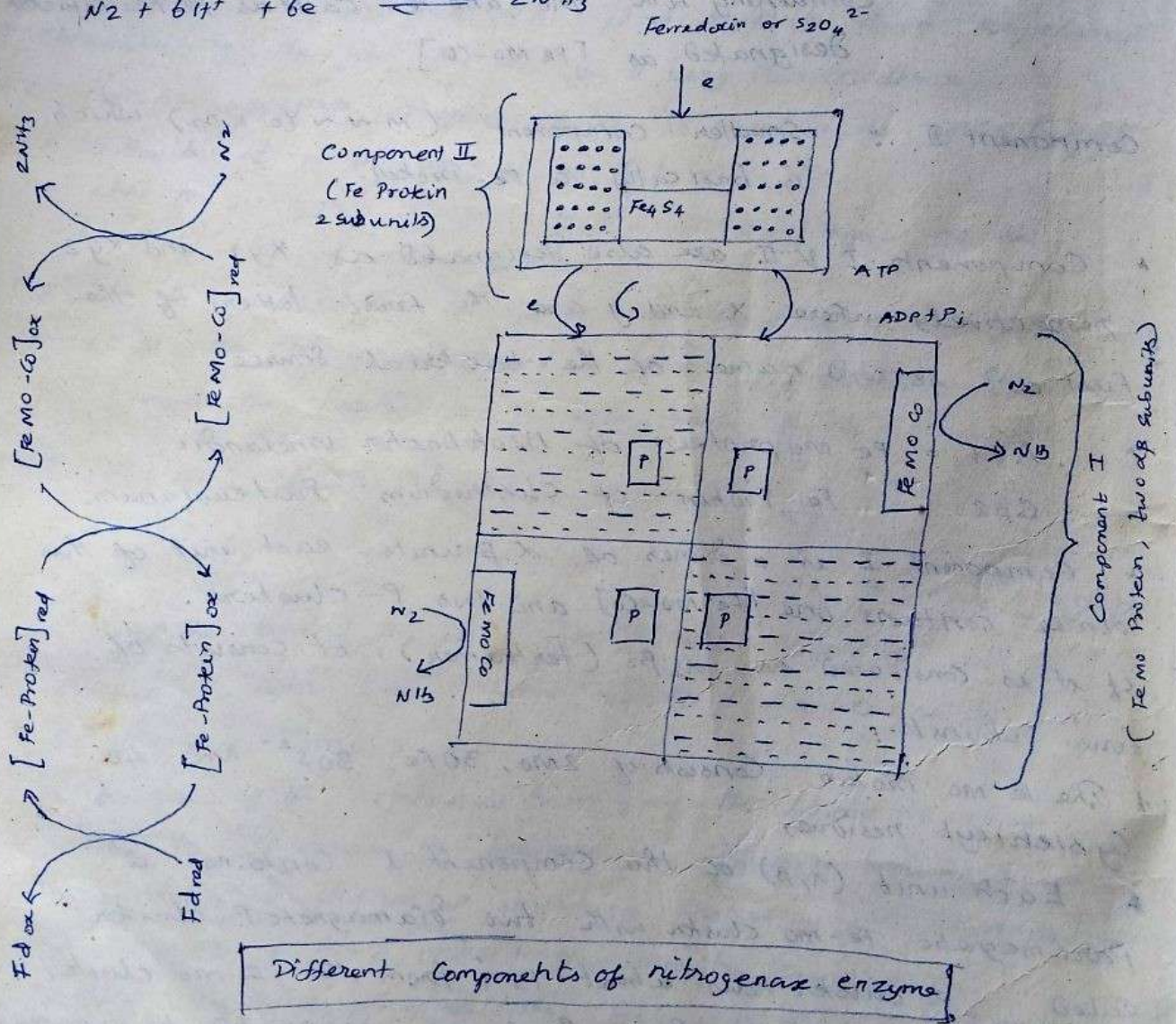
* The Fe Mo Protein Consists of 2Mo, 30Fe, $30S^{2-}$ and 40 Cysteinyl residues

* Each unit (α, β) of this Component I Contains a paramagnetic Fe-Mo cluster with two diamagnetic P-cluster Called P-cluster Pair about 15Å from the Fe-Mo cluster. the two Fe-Mo cluster Per molecule are separated by ~ 60-70 Å

* The component II Consists of two identical ~~two~~ sub units. One 4Fe-4S cluster bridges these two identical subunits at one end. The 4Fe-4S cluster experience a one electron redox process.

* The Fe-Protein is to bind two ATP units in order to deliver one electron to the Fe-mo Protein.
* To drive the nitrogenase reaction, both a reducing agent and an ATP binding agent are required.
* The redox Potential of 4Fe-4S cluster depend on the ATP or ADP level
  eg. CP2 $E_0' = -0.29V$ in the absence of Mg ATP while it is $-0.4V$ in the Presence of Mg ATP)
* The conformational change in Fe-Protein due to the hydrolysis of ATP is responsible for the change of redox Potential from $-0.29V$ to $-0.40V$. it thermodynamically allows the reduction of N_2.

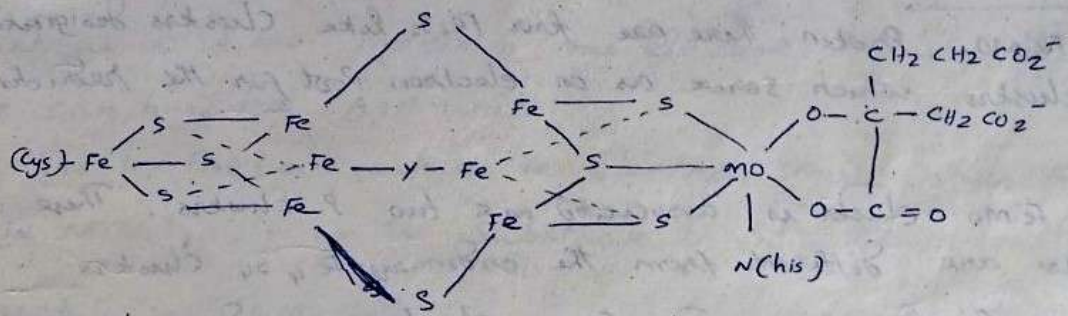
$$N_2 + 6H^+ + 6e \rightleftharpoons 2NH_3$$



Different Components of nitrogenase enzyme

Structural Aspects of Fe Mo-Co:

* From the X-ray studies, it is suggested that the [Fe Mo-Co] cluster consists of two voided cubane M_4S_4 units, each missing one bridging S^{2-} ion rather than a metal to leave an open Fe_3 face, facing each other.
* The two Fe_3 faces are eclipsed with respect to each other. It indicates that the six central Fe centres are probably arranged as a trigonal Prism. Thus the Fe_7MoS_8 cluster consists of the units Fe_4S_3 and Fe_3MoS_3 bridged by two S^{2-} ions and an unknown ligand (Y).
* The Mo centre is six ~~cent~~ coordinate (distorted octahedral) two O donors from a homocitrate group [$-C(OH)(COOH)-$], an α-His-442 N site, three cluster S^{2-} sites bridging three Fe sites.



Structural representation of $MoFe_7S_8$ cluster of Nitrogenase

* In the oxidised form Mo exists in +4 oxidation state. each unit consists of one [Fe Mo-Co] and two P-clusters. these two FeMo cofactors are separated by about 60-70 Å, but the P-clusters are only about 15Å away from the [Fe Mo-Co] cluster in each $\alpha\beta$ unit. The two [Fe Mo-Co] units act independently as the active sites.
* Mo is six coordinate (no vaccant coordination site is available) and thus the Mo-centre is not likely to accomodate the substrate N_2.
* The central bridge (Y) of the cluster is the site of N_2 binding and N_2 bridges the Fe-centres of the thiocubane fragments Probably this is the site of N_2 reduction. This proposition is quite reasonable as for different types of nitrogenases (Fe Mo-N_2 ase, Fe V-N_2 ase). Fe is always essential but Mo is not always essential

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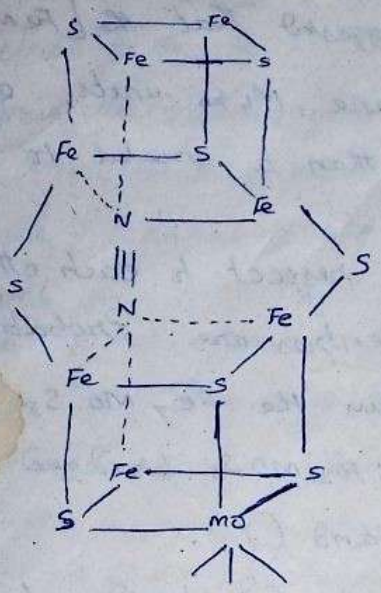
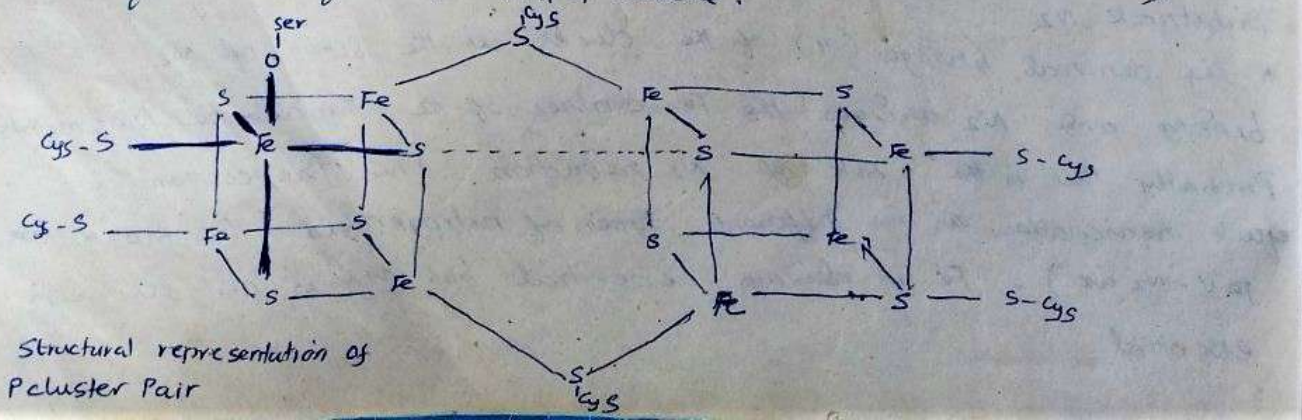


Speculated structure of MoFe$_7$S$_8$ binding N_2 (at the site of Y) as a central bridge between the thiocubane fragments

P-clusters:

* In FeMo-Protein, there are four Fe_4S_4 like clusters designated as P-clusters which serve as an electron Post for the reduction of N_2.
* Each FeMo cluster is associated with two P clusters. These P-clusters are differed from the ordinary Fe_4S_4 clusters found in ferredoxins. The four clusters ~~are~~ do not behave identically.
* The structure of P-cluster Pair involves a Fe_8S_8 unit in which two Fe_4S_4 cubane units are bridged by two Cys-S sites. there is a disulphide linkage bridging the two cubanes. This disulphide linkage is believed to be Potentially redox active in nitrogenase activity
* one Fe_4S_4 cubane contains cysteine ligands, but other Fe_4S_4 contains a serine ligand bound to one non bridged Fe centre. Presence of this Five-coordinate Fe ($3S^{2-}$, 1 Cys-S, 1 serine O) is quite unique in Fe_4S_4 cubane.



Structural representation of Pcluster Pair

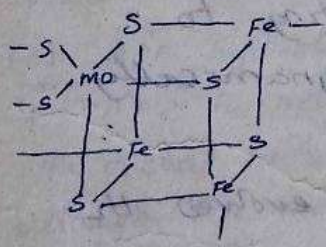
* The two [Fe Mo-co] units separated by about 60-70 Å act independently but each [Fe Mo-co] unit acts in cooperation with P-clusters which are only 15 Å away from [Fe Mo-co]. This 15 Å distance is not too far to allow the electron transfer from the P-cluster to [Fe Mo-co].

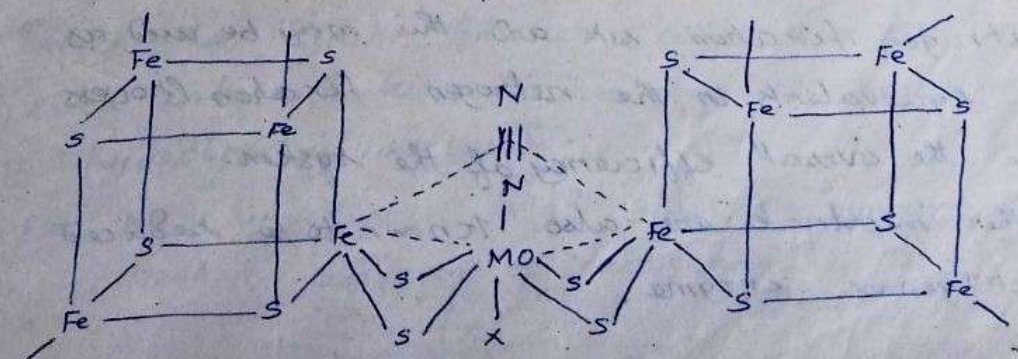
Fe-Protein:

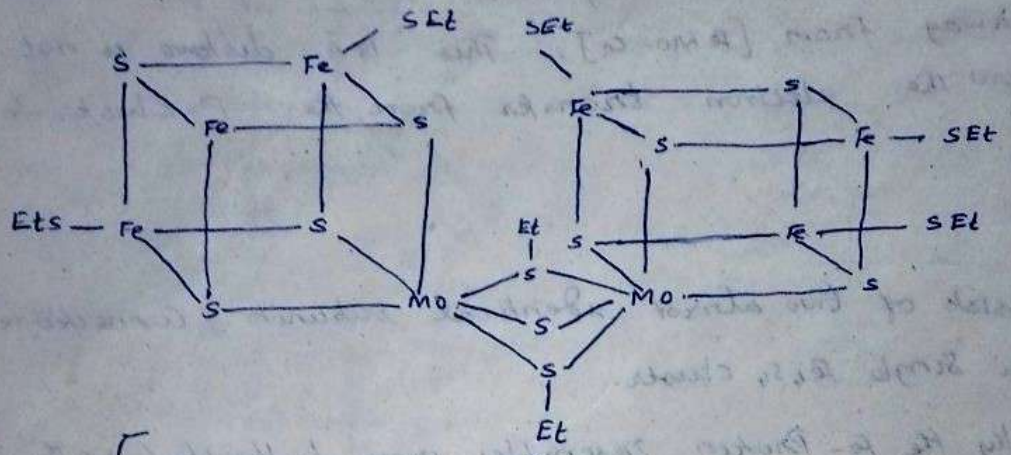
* This consists of two almost identical subunits connected at one end by a single Fe_4S_4 cluster.
* structurally the Fe-Protein resembles iron butterfly with the Fe_4S_4 cluster at its head.
* The Fe_4S_4 unit undergoes a one electron change where the oxidised form is diamagnetic and the reduced form is Paramagnetic.
* Fe-Protein acts as an ATP binding sit to deliver the electrons to Fe Mo Protein in one electron transfer steps. it can bind two ATP units.
* The Fe-Protein ach as the electron source while the [Fe Mo-co] acts as the substrate binding sit.

Models for [Fe Mo-co]

* Different model clusters have been suggested to explain the activities of [Fe Mo-co]. several 'double-cubane' structures have been suggested even before the Rees x-ray structure.







$[MO_2 Fe_6 S_9 (SEt)_3]^{3-}$

structural representation of different model Compounds for [Fe Mo-co]

Different types of Substrates Active towards Nitrogenase:

* N_2-ase enzyme Catalyse the reduction of N_2 to NH_4^+ with the Concomitant evolution of H_2. the overall reaction is:

$$N_2 + 10H^+ + e^- \xrightarrow{N_2\text{-ase}} 2NH_4^+ + H_2$$

* if N_2 is omitted, then all the electrons will be consumed in the evolution of H_2 even at 50 atm Pressure of N_2, H_2 is evolved along with the formation of NH_4^+.
* Probably, the evolution of H_2, a thermodynamically favoured Process, is Coupled with the reduction of N_2 to make the overall Process leading to unstable intermediate formation thermodynamically favourable.
* Here it is important to mention that the evolved H_2 may be Captured by the hydrogenase enzyme Present at the nitrogen fixation site and this may be used as reducing equivalents in the nitrogen fixation Process to increase the overall efficiency of the system.
* Many other substrates are also known to be reduced by this nitrogenase enzyme.

Different nitrogenase - substrate reactions

$2e^-$ reduction:

- $2H^+ + 2e \longrightarrow H_2$
- $C_2H_2 + 2H^+ + 2e \longrightarrow C_2H_4$
- $N_2O + 2H^+ + 2e \longrightarrow N_2 + H_2O$

$4e^-$ reduction:

- $HCN + 4H^+ + 4e \longrightarrow CH_3NH_2$
- $RNC + 4H^+ + 4e \longrightarrow RNHCH_3$

$6e^-$ reduction:

- $N_2 + 6H^+ + 6e \longrightarrow 2NH_3$
- $RNC + 6H^+ + 6e \longrightarrow RNH_2 + CH_4$
- $RCN + 6H^+ + 6e \longrightarrow RCH_3 + NH_3$

Multi electron reductions:

- $RNC \longrightarrow RNH_2 + (C_2H_6, C_2H_4, CH_4 \text{ etc})$
- $NCNH_2 + 8H^+ + 8e \longrightarrow CH_4 + 2NH_3$

* CO is a triple bonded compound, it is not reduced by this enzyme. Rather CO acts as a potent inhibitor to all the nitrogenase substrate reactions.
* All the nitrogenase substrate reactions envolve the transfer of 2e or multiples (ie 4e, 6e, 8e ....).

Functions of different units in the activity of Nitrogenase:

(a) ATP hydrolysis:

* ATP hydrolysis is essential for nitrogenase activity. The overall reduction of N_2 to NH_3 is thermodynamically allowed but the process passes through the formation of several unstable intermediates.
* Infact, the kinetic barrier arises due to the formation of such intermediates. To overcome this kinetic barrier ATP hydrolysis is required.
* For ATP hydrolysis Mg^{2+} plays an important Role. The Fe-Protein component of nitrogenase is the ATP binding site

$$ATP \xrightarrow{Mg^{2+},\ H_2O} ADP + P_i$$

$$N_2 + 16\,Mg\,ATP + 8H^+ + 16\,H_2O + 8e \xrightarrow{N_2\text{-ase}} 2NH_3 + 16\,Mg\,ADP + 16\,P_i + H_2$$

In the oxidation of glucose, ATP is generated through oxidative phosphorylation, but in the reduction of N_2, ATP is hydrolysed through the reductive dephosphorylation.

(b) Electron source:

* Different organisms rely on different sources. Some rely on Pyruvate oxidation, some depend on photosynthetic redox reactions, while the symbiotic Rhizobia and free living Azotobacter use the electrons from the ordinary electron transport chains.

(c) Leghemoglobin:

* This is a monomeric Fe-heme Protein used in binding O_2 which inhibits the nitrogenase activity. Probably O_2 binds itself to the active site of nitrogenase. Leg hemoglobin binds O_2 and Protects nitrogenase. Besides this, it makes a reservoir of O_2 to be used in bacterial respiratory system to generate ATP required in nitrogenase activity.

(d) Ferredoxins:

To transport the electrons from the source to Fe-Protein of nitrogenase. Ferredoxins act as the electron Carriers.

(e) Fe-Protein of N_2ase:

It acts as the ATP binding site to deliver the electrons to [Fe MO-Co] in one electron transfer steps. it carries electrons from ferredoxin to [Fe MO-Co].

* In Fe-Protein, one Fe_4S_4 unit works. the redox potential of Fe-Protein largely depends on the ATP level.

(f) P-clusters and [Fe MO-Co] of N_2ase:

* [Fe MO-Co] acts as the substrate binding site. The P-cluster Pair is believed to act as a reservoir of

low Potential electrons to be used by the [Fe Mo-Co] unit.

The electron flow can be expressed as

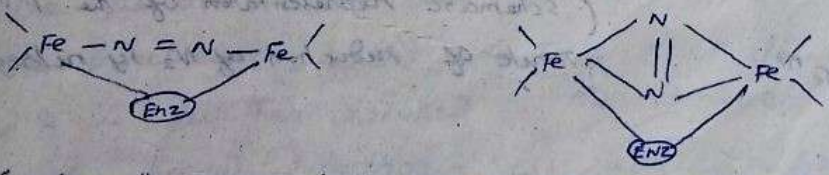
Photosynthesis (or) oxidative electron Transport → Fd → Fe-Protein → P-cluster → [Fe Mo-Co] → N_2

Reaction Pathway for Reduction of N_2 to NH_3

* The popular scheme to explain the Pathway for reduction of N_2 is given in the scheme. Considering the >Fe-Y-Fe< Segment Present in the active site, N_2 replaces the bridging ligand Y.
* Previously, the Mo-(En2)-Fe segment was Considered as the active site of N_2 reduction. But Ree's Crystal structure does not support the existence of the Mo-(En2)-Fe segment with a vaccant Coordination site on Mo in Fe Mo-N_2-ase.
* The structure of [Fe Mo-Co] indicates that the Mo centre is already octahedrally coordinated and there is no vaccant site around Mo to accomodate the N_2.
* In some other types of Nitrogenase (Fe V-N_2 ase) Mo is absent. it suggests that Mo is not essential for N_2 binding.

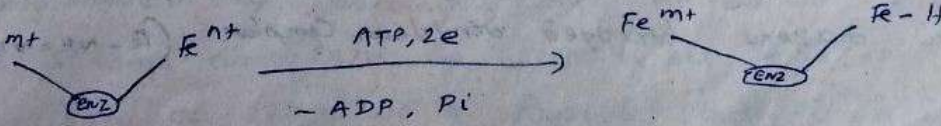
All these observations strongly suggest that the >Fe-Y-Fe< segment is the active site of N_2 binding and N_2 reduction.

* N_2 can symmetrically bridge the Fe-centres in two Pathways. (i) in the Fe-N=N-Fe segment where N_2 binds the Fe centres through an end-on bonding fashion.



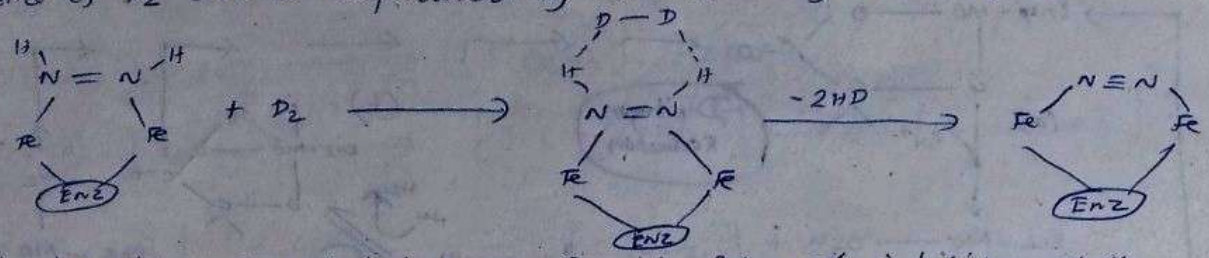
(Schematic representations of Possibilities of N_2 binding at the active site)

* The suggested scheme involves the formation of the active site through an ATP hydrolysis. This ATP reduction leads to Fe^{n+} to $Fe^{(n-2)+}$



(Generation of the active site in N_2-ase through an ATP hydrolysis)

(ii) inhibition of N_2 reduction by H_2 and formation of HD in the presence of D_2 can be explained by the following scheme



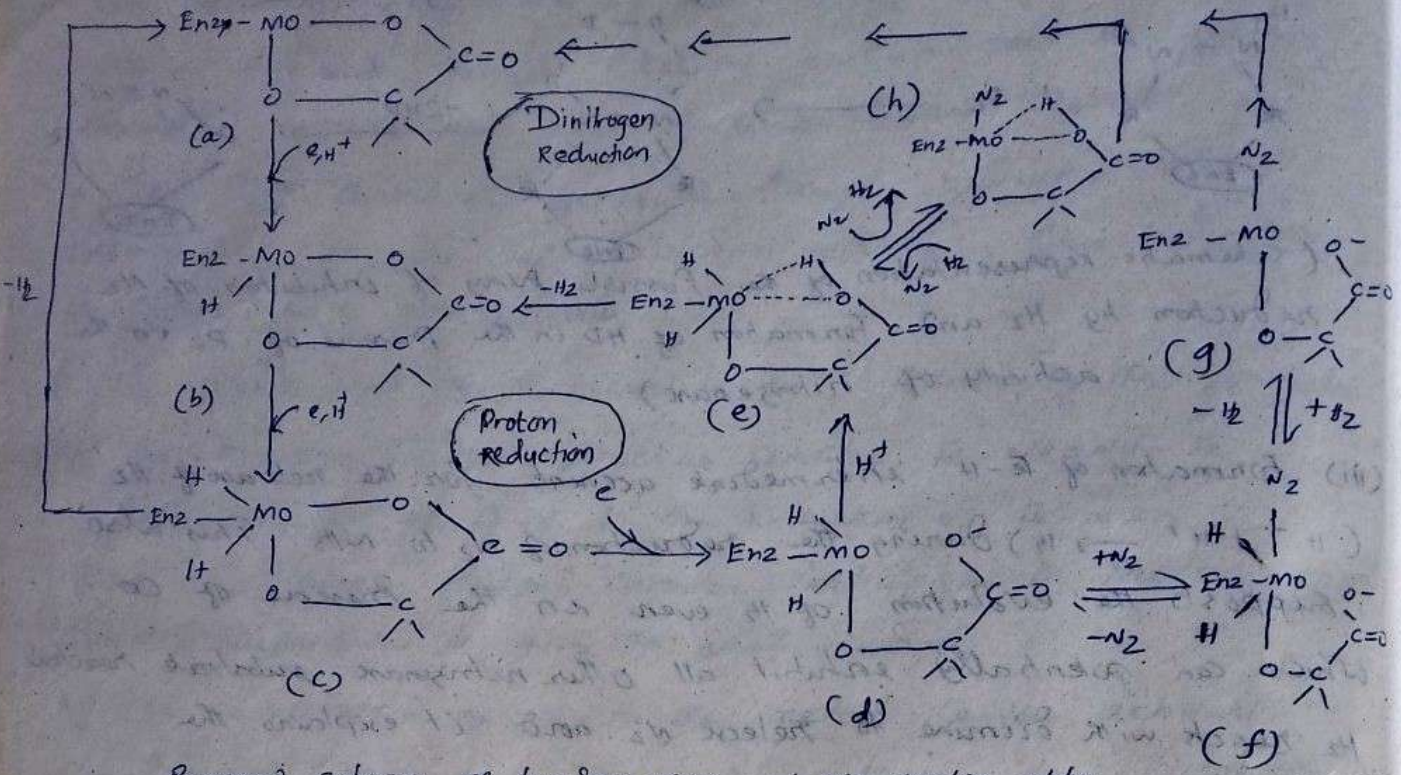
(schemahc representation of the Possible Way of inhibition of N_2 reduction by H_2 and formation of HD in the Presence of D_2 in the activity of nitrogenase)

(iii) Formation of Fe-H intermediate accounts for the release of H_2 ($H^- + H^+ \longrightarrow H_2$) during the reduction of N_2 to NH_3. This also supports the evolution of H_2 even in the Presence of CO which can potentially inhibit all other nitrogenase substrate reactions. H_2 reacts with diimine to release N_2 and it explains the inhibition by H_2

$$HN = NH + H_2 \longrightarrow N_2 + 2H_2$$

Possibility of N_2 binding at the Mo-centre:

* Though the Crystal structure does not support the existence of any vacant site around the Mo-centre to accomodate the substrate, dinitrogen, this Possibility has not been Completely ruled out.
* some workers have suggested that the Carboxylate group of homocitrate is dislodged from the Mo-centre after a 3electron reduction of the enzyme. this 3electron reduction followed by opening of the Mo-bound Carboxylate group of homocitrate allows the dinitrogen to bound with the Mo-centre.
* Here the dinitrogen does not bind with the resting enzyme.
* Thus the dinitrogen bound with the Partially reduced (3e) enzyme is further reduced to the Product.
* Here the bound dinitrogen is reduced after the release of H_2. it explains that H_2 evolution cannot be eliminated during N_2 reduction.
* The Complete reaction sequence leading to reduction of N_2 has been explained by Thorneley - Lowe which is a Complicated one. the scheme considers the step-wise reduction through the intermediacy of large no. of Possible states of the enzyme.



Proposed scheme of binding of N_2 at Mo-centre after reduction of the enzyme and the reaction sequence by Thorneley-Lowe mechanism

(i) H_2 evolution during N_2 reduction (route: c → a, e → h, f → g and e → b)

(ii) inhibition of N_2 reduction by H_2 (route h ⇌ e, d ⇌ f ⇌ g)

(iv) formation of HD in the Presence of D_2 (route A: g ⇌ f ⇌ d ⇌ e → b; D_2 can enter in to the cycle for the conversion g → f then HD may be eliminated at e → b keeping Mo-D. then c → a can also release HD.

route B: h ⇌ e → b; D_2 can enter for the conversion of h → e and then HD may be eliminated at the step e → b keeping Mo-D segment)

Abiological Nitrogen fixation through Complexation:

* After the discovery of $[Ru(NH_3)_5(N_2)]^{2+}$ several dinitrogen complexes have been Prepared, but the point of catalytic activity for the reduction of N_2, no Promising Complex is yet available.

* However, it has been established that through complexation N_2 can be activated due to the increased basicity and lowering of bond order in the bound N_2.

(i) Ti(II) alkoxides form dinitrogen complexes which may be subsequently reduced to NH_3 or N_2H_4

$$[Ti(OR)_4] + 2e \longrightarrow [Ti(OR)_2] + 2RO^-$$

$$[Ti(OR)_2] + N_2 \longrightarrow [Ti(N_2)(OR)_2] \xrightarrow{+4e} [Ti(N_2)(OR)_2]^{4-}$$

$$[Ti(N_2)(OR)_2]^{4-} + 4H^+ \longrightarrow [Ti(OR)_2] + N_2H_4$$

$$[Ti(N_2)(OR)_2]^{4-} + 2e \longrightarrow [Ti(N_2)(OR)_2]^{6-} \xrightarrow{6H^+} 2NH_3 + Ti(OR)_2$$

Here it requires fairly Powerful reducing agents and regenerating the starting material to maintain the catalytic cycle is not economic.

(ii) some Mo or Complexes after reduction to Mo(0) with a strong reducing agent (Na-Hg or Mg) can carry out the reduction of N_2 to NH_4^+ [(6e transfer, Mo(0) → Mo(VI)] in acid media at room temperature

$$[MoCl_3(thf)_3] \xrightarrow[\text{excess dpe, } N_2]{\text{Na-Hg or Mg}} [Mo(N_2)_2(dpe)_2] + 3Cl^- + 3thf$$

$$trans\text{-}[Mo(N_2)_2(dpe)_2] + 8H^+ \xrightarrow[\text{excess dpe, } N_2]{\text{Na-Hg or Mg}} 2NH_4^+ + N_2 + Mo(VI)\text{ Products}$$

thf = tetrahydrofuran dpe = 1,2-bis(diphenylphosphino)-ethane,

(iii) Some other Complexes of Mo(0) and W(0) such as $[M(N_2)_2(PPh_2Me)_4]$ can also used.

$$[M(N_2)_2(PPh_2Me)_4] \xrightarrow[CH_3OH]{H_2SO_4} N_2 + 2NH_4^+ + Mo(VI)\text{-Products}$$

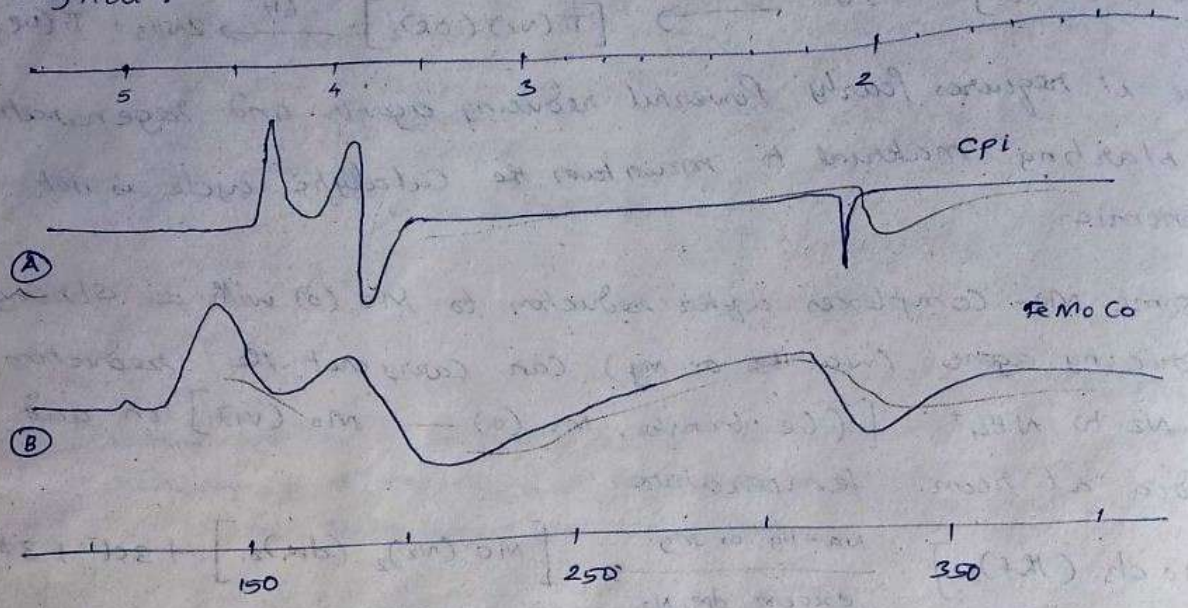
Regeneration of the initial complex from the Product is costly.

Spectroscopic studies of Nitrogenase and other evidences

* The two types of centers present in the nitrogenase FeMo Protein and FeMoCo display unique spectroscopic properties, but only FeMoCo continues to display most of those properties when it is extracted from the protein.

Fe MOCO

* The Presence of FeMo cofactor with in the FeMo Protein of nitrogenase is revealed through spectroscopic and redox studies.
* In the resting stat of [FeMo], as isolated in the Presence of dithionat, the FeMo center has a distinct $S = 3/2$ EPR Signal.



EPR spectra : (A) the $S = 3/2$ M center in Clostridium pasteurianum nitrogenase FeMo Protein
(B) the FeMoCo extracted into NMF from the Protein

* When the enzyme is turning over the EPR signal essentially disappears, leaving an EPR silent state in which the FeMoCo site is super reduced to what is Presumed to be its Catalytically active form
* In addition, a third state in which the $S = 3/2$ EPR signal disappears is Produced upon oxidation under non-turnover Conditions. Thus the FeMoCo center within the Protein shows three states of oxidation and these appear to have been reproduced in the FeMoCo extracted from the protein.

FeMoCo (oxidised) $\xrightarrow{e}$ FeMoCo (reduced) $\xrightarrow{e}$ FeMoCo (super reduced)

* The detailed characterization of FeMoCo site has involved parallel studies of the site within the Protein and in its extracted form

* The extracted FeMoco involved the production and use of mutant organisms that make an inactive FeMo protein that contains all subunits and P cluster.
* A mutant of Azotobacter vinelandii was first used to assay for isolated FeMo co.
* FeMoco can be extracted into many organic solvents provided proper combinations of cations and anions are present in the solvent. The role of the cation is to balance the charge of the negatively charged cofactor and the role of anion is to displace the cofactor from anion exchange columns. The ability to dissolve cofactor in such solvents should facilitate attempts at further characterization.
* The stoichiometry of the cofactor is $MoFe_{6-8}S_{7-10}$, with the variability likely due to sample inhomogeneity. The extracted cofactor resembles the FeMo Co center unit spectroscopically and structurally shown in Table.

	Fe Mo Protein (M center)	Fe Mo Co (in NMF)
EPR		
g values	4.27	4.8
	3.79	3.3
	2.01	2.0
EXAFS		
Mo-S	2.36 (4)	2.37 (3.1)
Mo-Fe	2.69 (3)	2.70 (2.6)
Mo-O (or) N	2.18 (1)	2.10 (3.1)
Fe-S		2.25 (3.4), 2.20 (3.0)
Fe-Fe		2.66 (2.3), 2.64 (2.2) 3.68 (0.8)
Fe-Mo		2.76 (0.4), 2.70 (0.8)
Fe-O (OR N		1.81 (1.2)

* Strong evidence to support FeMoCo as the site of substrate binding and reduction comes from the study of nif V mutants.

* FeMoCo can be extracted from nif V Protein and used to reactivate the FeMoCo-deficient mutants, such as nif B or UW-45. Nif V mutant have altered substrate specificity in vitro. Dihydrogen evolution by isolated nif V nitrogenase is inhibited by CO. Remarkably the reconstructed FeMo Protein has CO sensitive H_2 evolution which is characteristic of nif V.

* nif V phenotype is a property of FeMoCo site and not of the Protein. this result clearly indicates the FeMoCo site as an important part of the substrate reaction of the nitrogenase enzyme complex.

* Recently site-directed mutagenesis studies have shown that Cysteine residues are involved in binding FeMoCo to the subunits of [FeMo]. these studies implicate FeMoCo in the substrate reducing site.

P-clusters:

* The P-clusters are by no means ordinary Fe_4S_4 clusters, and may not be Fe_4S_4 clusters at all. P-clusters are manifest in electronic absorption and MCD and Mossbauer spectra of [FeMo]. these spectra are clearly not conventional ie they are no like those found in ferredoxins and have not yet been seen in model compounds

* In their oxidised forms the P-clusters are high spin probably $S = 7/2$ according to EPR studies.

* Mossbauer spectra reveal inequivalent Fe Populations indicating the Fe_4S_4 clusters are highly distorted or asymmetric.

* The four P-clusters do not appear to behave identically under many circumstances, and it is clear that they form at least two subsets

* Although spectroscopic studies of the P-cluster do not unequivocally reveal their structural nature, extrusion of these clusters from the Protein leads to the clear identification of three or four Fe_4S_4 clusters

* from the experimental results the P-clusters are thought to be involved in electron storage and transfer, and provide a reservoir of low potential electrons to be used by FeMoCo center. in substrate reduction

Electron nuclear double resonance — Electron spin echo envelop modulation

EPR, ENDOR and ESEEM studies:

* The FeMoCo (M center) has been identified spectroscopically within the Fe Mo Protein it has a distinctive EPR signal with effective g values of 4.3, 3.7, and 2.01 and originates from an $S = 3/2$ state of the M center.

* The signal arises from transition with in the $\pm 1/2$ ground state kramers doublet of the $S = 3/2$ system ($D = 5.1\ cm^{-1}$, $E/D = 0.04$).

* The isolated cofactor (FeMoCo) gives a similar EPR signal, with larger rhombicity ($E/D = 0.12$).

* The M-center EPR signal has proved useful in characterising the nature of the Sik, especially when more sophisticated magnetic resonance techniques such as ENDOR or ESEEM.

* Extensive ENDOR investigations have been reported using protein samples enriched with stable magnetic isotopes ^1H, ^{32}S, ^{57}Fe, ^{95}Mo, ^{97}Mo etc.

* Individual hyperfine tensors of five coupled ^{57}Fe nuclei are discernible and were evaluated by simulation of the polycrystalline ENDOR spectrum

* The data from ^{33}S and ^{95}Mo were analysed in less detail. ^{33}S gave a complex ENDOR spectrum, evidently with large hyperfine couplings.

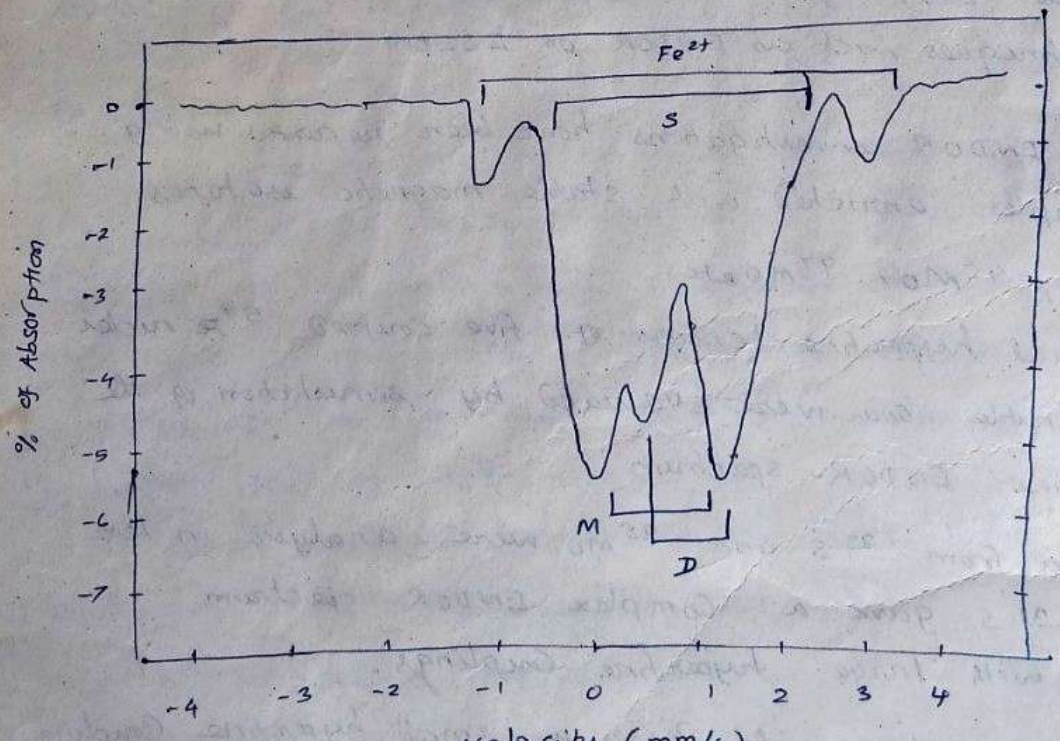
* ^{95}Mo was shown to Posses a small hyperfine coupling indicating the molybdenum Possesses very little spin density.

* No nitrogen splittings were reported in any of the ENDOR studies. But ^{14}N modulations are observed in the ESEEM of the M Center.

* The experiments suggest that the M-center arises from ~~a~~ a nitrogen atom that is associated with M-center, and probably from an amino acid side chain (most likely a histidine) ligated to the cluster.

Mossbauer Studies:

* Extensive Mossbauer investigation of nitrogenase and FeMoCo have been reported.
* unlike EPR which is used to investigate only the EPR active $S = 3/2$ oxidation state, all three available M-center oxidation states are accessible to Mossbauer spectroscopy.
* The fully reduced site was found to be diamagnetic with $S = 0$ where as the oxidised site was found to have $S \geq 1$.
* The zero-field spectrum of reduced C. Pasteurianum nitrogenase is shown in figure. the spectrum is comprised of four quadrupole doublets, one of which was concluded to originate from the M site.



Mossbauer spectrum of C. Pasteurianum nitrogenase Fe Mo Protein indicating the various components and their assignments.

M → (doublet) Cofactor signal

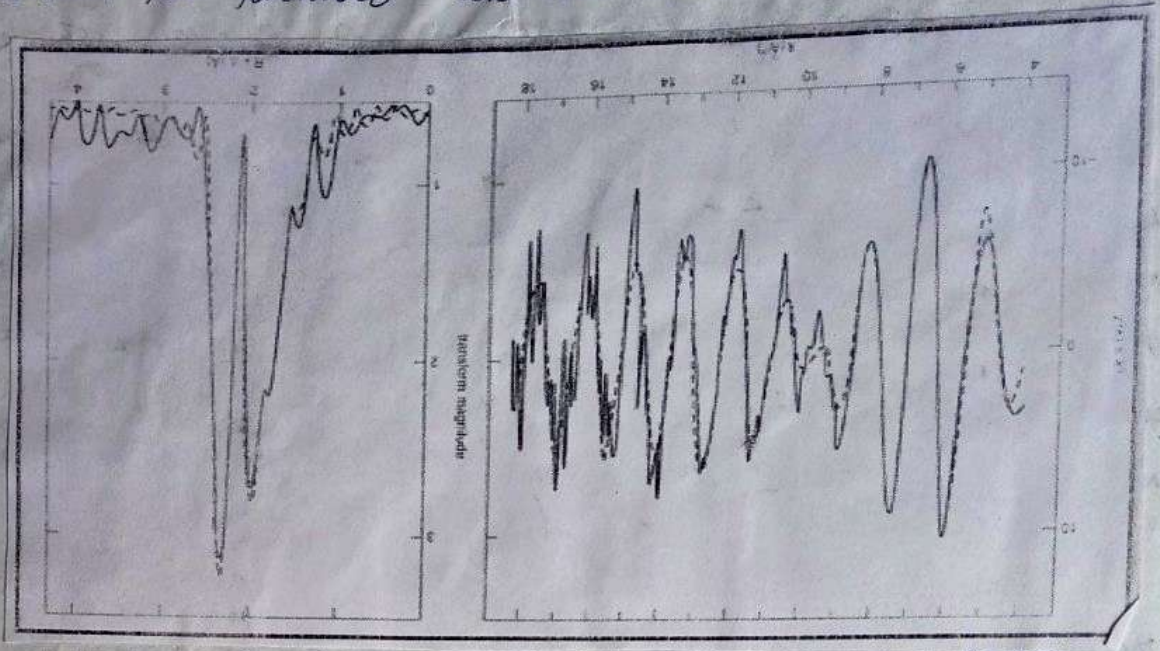
D, S & Fe^{2+} → P-clusters.

* Mossbauer spectra taken in the presence of applied magnetic fields were used to deduce the presence of four types of ^{57}Fe hyperfine coupling, these were called sites A1, A2, and A3 which have negative hyperfine couplings and B sites which have positive hyperfine couplings.
* A sites were quantitated as a single Fe each, the B sites were estimated to contain three irons. These conclusions were largely confirmed and extended by ENDOR investigations, although the B sites were ~~estimated to con~~ resolved as two inequivalent rather than three equivalent sites.
* ENDOR is rather more sensitive to the nature of the hyperfine couplings than mossbauer spectra.

X-ray absorption studies:

* one of the early triumphs of biological x-ray absorption spectroscopy was the deduction that the nitrogenase M center is an ~~Fe~~ Mo-Fe-S cluster.
* Early work indicated the presence of two major contributions to the Mo k-edge EXAFS, which were attributed to Mo-S ligands and more distant Mo-Fe contribution. (fig. 1)
* The best available analyses indicates that Mo is coordinated by three or four sulfur atoms at 2.4Å, one to three oxygens or nitrogens at 2.2Å with approximately three nearby iron atoms at 2.7Å.
* EXAFS evidence for the oxygen/nitrogen contribution is weakest. However, comparision of Mo k-edge and Mo L-edge XANES spectra with model compounds indicates strong similarities with $MoFe_3S_4$ thiocubane model compounds possessing MoS_3O_3 coordination and provide some support for the presence of O/N ligands
* The iron EXAFS of FeMoco has been independently examined by two groups. both groups agree that the iron is coordinated largely to sulfur at about 2.2Å, with more distant Fe-Fe interactions at about 2.6Å. They differ the presence of short (1.8Å) Fe-O interactions.

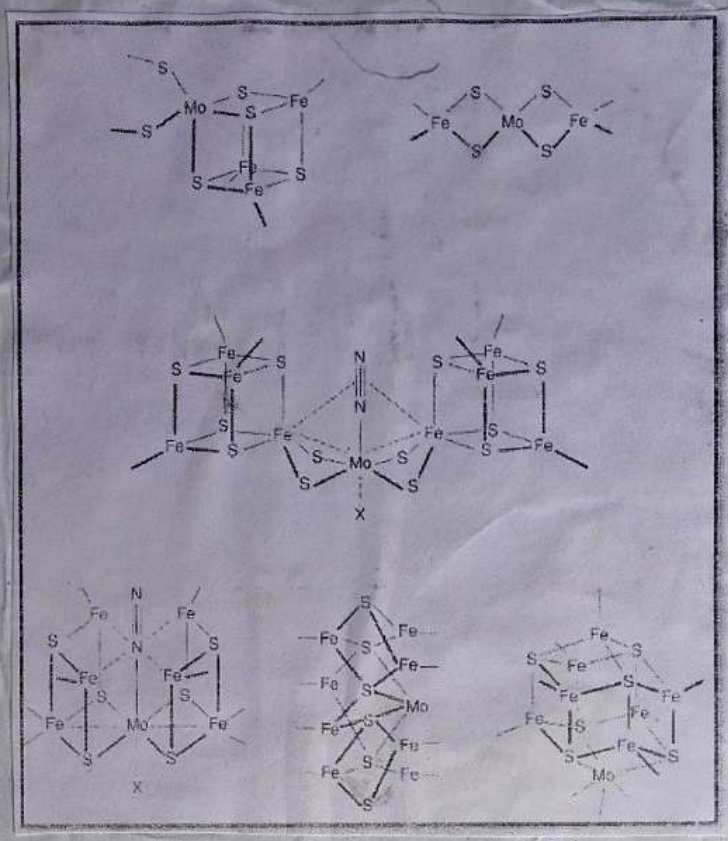
* Based on the Mo K-edge EXAFS results and model studies, several proposals for the structure of the M centre have been put forward and are illustrated in figure. 2



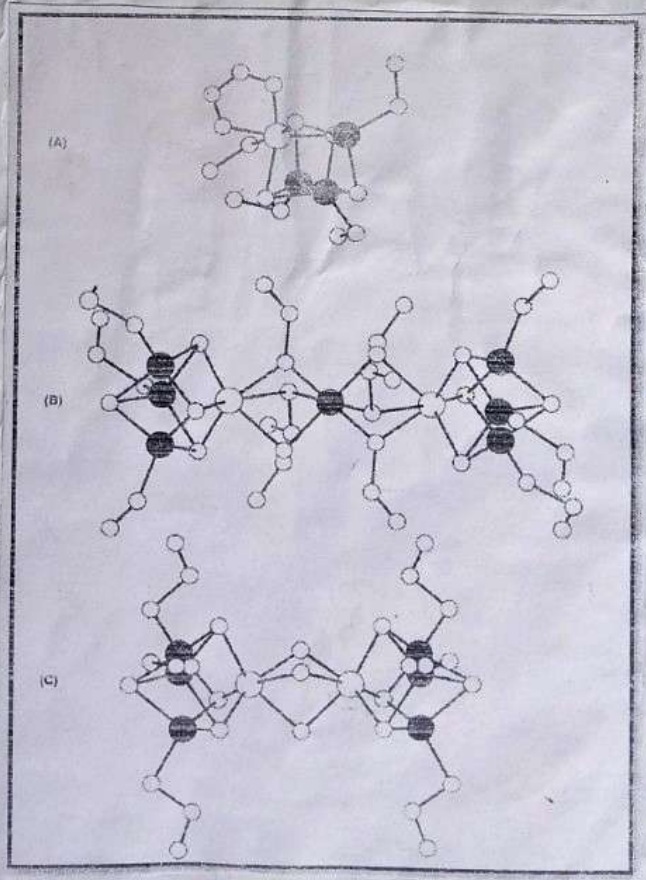
[Fig. 1. Mo K-edge EXAFS spectrum (left) and EXAFS fourier Transform (right) of Klebsiella Pneumoniae nitrogenase MoFe Protein. solid line is the experimental and dashed are calculated lines]

* The Mo Fe Proteins from clostridium Pasteurianum and from Azotobacter vinelandii have been Crystallised. For the former Protein, crystals of space group P2, are obtained with two molecules Per unit cell of dimensions 70 x 151 x 122 Å.
* There is good evidence for molecular two fold axis, which relates equivalent sites in the two $\alpha\beta$ dimers that make up the Protein molecule.
* Preliminary refinement reveals that the two FeMoCo units per Protein are about 70 Å apart and the four P cluster are grouped in two pairs.
* Single Crystal EXAFS studies have Provided important structural information on the molybdenum site. For different Crystal orientations, the amplitude of the Mo-Fe EXAFS Changes by a factor of 2·5, but the Mo-S EXAFS changes only slightly.
* Analysis of the anisotropy of the Mo-Fe EXAFS using the available Crystallographic information is Consistent with either a tetrahedral $MoFe_3$ geometry such as found in thiocubanes or a square Pyramidal $MoFe_4$ arrangement of metals. (Fig. 3)

* This interpretation tends to rule out some of the structural proposals given in (figure 4)



[Fig. 2 Proposed models for Fe Mo Co]



[Fig. 3 Structure of thiocubanes that display Mo-S and Mo-Fe distances similar to Fe Mo Co
(A) $(Fe_3 Mo S_4)_2 (SR)_9^{3-}$ (B) $(Mo Fe_3 S_4)_2 Fe (SR)_{12}^{3-/4-}$
(C) $Mo Fe_3 S_4 (SEt)_3 (Cat) CN^{3-}$]

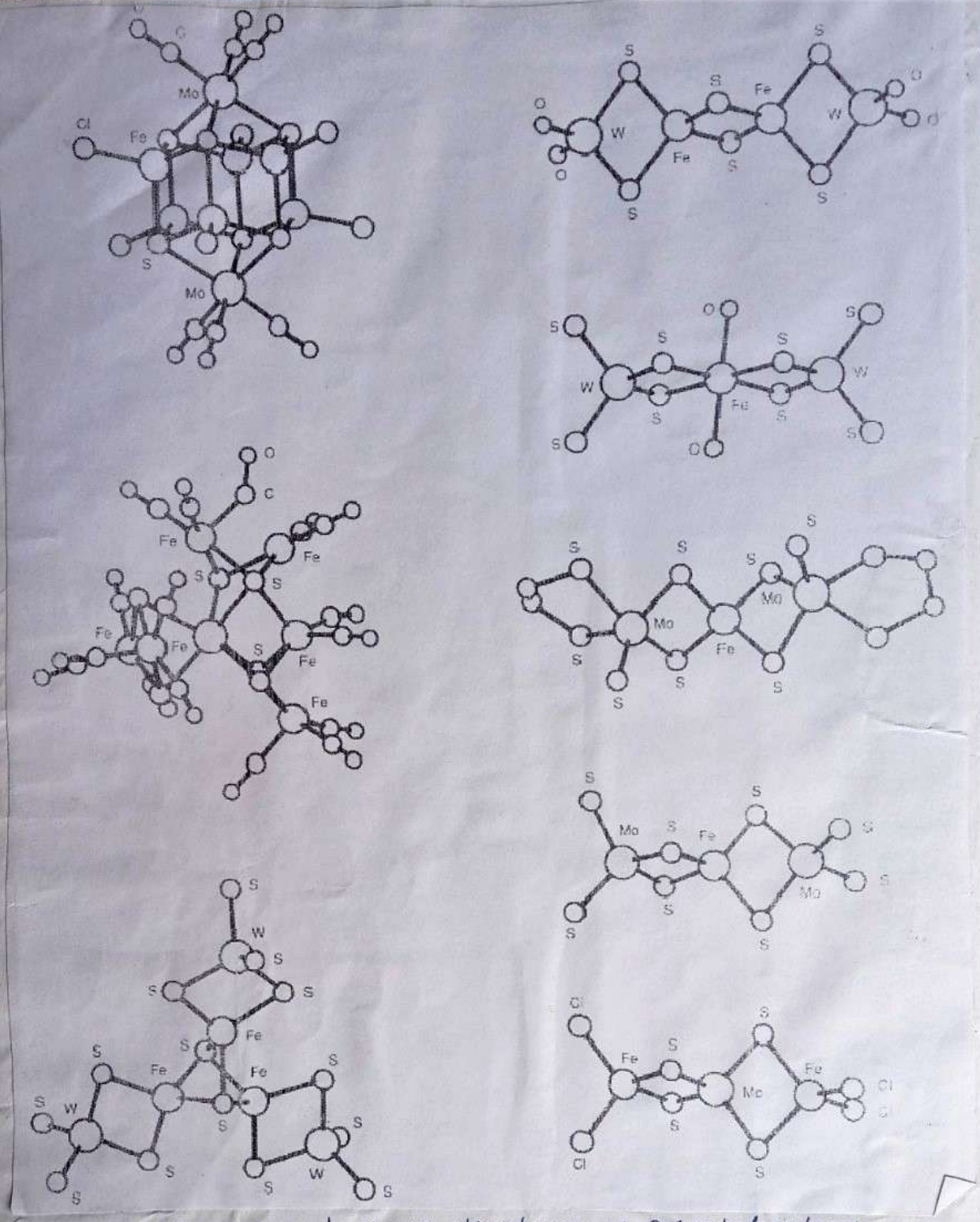


Fig. 4 FeMoS and FeWS structures of potential interet with respect to nitrogenase.

+ The observed orientation dependence of the iron amplitudes is too small for clusters containing a linear or planar arrangement of iron and molybdenum, and too large for arrangements that envolve regular disposition of iron about molybdenum

+ The lack of anisotropy of sulfur EXAFS argues against an $MoS_3(O/N)_3$ model that has molybdenum coordinated by sulfur atoms that bridge only to Fe atoms disposed to one side of the molybdenum

* Significant anisotropy for the Mo-S EXAFS would be expected for such an arrangement of sulfur atoms. However, the cubane model of figure III which provides the best model of both geometric and electronic structure, remains viable if one of the non bridging ligands to molybdenum is a sulfur atom with a bond length similar to that of the bridging sulfides.

The Alternative Nitrogenases:

Vanadium nitrogenase:

* Bortels reported in 1935 that vanadium stimulated nitrogen fixation. In the 1970s, attempts were made to isolate a vanadium nitrogenase.
* In 1971, two groups reported isolating a vanadium containing nitrogenase from A. vinelandii. The interesting notion was that vanadium might substitute for Mo in nitrogenase, not that there was a separate system.
* The isolated enzyme was reported to be similar to the Mo enzyme, but had a lower activity and an altered substrate specificity.
* The vandium was suggested to play a stabilizing role for [FeMo], allowing the small amount of active Mo-Containing protein to be effectively isolated.
* In 1986, two groups isolated the alternative nitrogenase Component Proteins from different species of Azotobacter, that one component Contained Vanadium and that neither Component contained molybdenum
* the two components of V-nitrogenase system is extremely similar to the Fe Protein of nitrogenax. This similarity is evident in the isolated Proteins from A. vinelandii. Both Fe Proteins have an α_2 Subunit structure and Contain a single Fe_4S_4 cluster that is EPR active in its reduced state.
* The FeV Proteins from Azotobacter Vinelandii and Azotobacter chroococcum each have an $\alpha_2\beta_2\delta_2$ Subunit structure. Metal Composition and Spectroscopic Comparision between the FeMo and FeV Proteins are shown in Table

Property	AvI[47]	AvI*[47]	AcI*[50]
Molecular weight	240,000	200,000	210,000
Molybdenum[b]	2	< 0.05	< 0.06
Vanadium[b]	—	0.7	2
Iron[b]	30-32	9.3	23
Activity[c]			
H^+	2200	1400	1350
C_2H_2	2000	220	608
N_2	520	330	350
EPR 'g' values	4.3	5.31	5.6
	3.7	4.34	4.35
	2.01	2.04	3.77
		1.93	1.93

AvI is the FeMo Protein of Azotobacter vinelandii.
AvI* is the FeV Protein of A. vinelandii
AcI* is the FeV Protein of A. chrococcum
b = Atoms Per molecule
c = nmol Product / min / mg of Protein

* There is the major difference involving in the Presence of V instead of Mo in the FeV Protein and in the Probable Presence of the small δ subunits, the two nitrogenase systems are Otherwise quite similar. In each, two highly oxygen sensitive Protein Carries out an ATP dependent N_2 reduction.
* The Fe Proteins have the same subunit structure and cluster Content, and spectroscopically Very similar.
* The FeV site still may be an $S = 3/2$ Center. The V-S and V-Fe distances as measured by EXAFS are similar to those in thiocubane VFe_3S_4 clusters and to Mo-S and Mo-Fe distances like those in turn similar to $MoFe_3S_4$ thiocubanes

* XANES indicates VS_3O_3 type coordination in [FeV] nitrogenase similar to MoS_3O_3 coordination by FeMo co.

* A major difference between V and Mo enzymes lies in substrate specificity and product formation. From the table the FeV nitrogenase has a much lower reactivity toward acetylene than does the Mo system.

* The FeMo system exclusively produces ethylene from acetylene, the FeV system yields significant amounts of four electron reduction product ethane. The detection of ethane in the acetylene assay may prove a powerful technique for detecting the presence of V nitrogenase in natural systems.

2. The All Iron nitrogenase :

* Another alternative nitrogenase came from genetic studies. A mutant of A. vinelandii was constructed with deletions in both nif HDK and nif H* D* K* ie. the structural genes for the Mo and V nitrogenases, the mutant strain nevertheless was able to fix nitrogen poorly.

* This mutant strain nitrogenase activity was clearly inhibited when either Mo or V was present in the culture medium. Preliminary studies indicate that the nitrogenase proteins produced by this organism are closely related to those previously isolated. A 4Fe-4S Fe protein nif H and a protein due to nif D was produced. This is the Fe Fe (all Iron protein) protein and its cofactor Fe Fe co.

* This nitrogenase seems to be the poorest of the set in reducing N_2 and makes ethane from ethylene.

* The concomitant absence of V and Mo suggests that nitrogen fixation need not directly involve the nonIron heterometal in the cofactor cluster. this result may explain the lack of direct implication of Mo in the nitrogen fixation mechanism.

Model systems for nitrogenase:

* Three types of model systems for nitrogenase may be considered.
* First, there are transition metal sulfide clusters that resemble the FeMoCo or FeVCo centers of the active Proteins
* A second approach uses the reactions of N_2 and related substrates or intermediates with metal centers in order to gain insights into the way in which transition metal systems bind N_2 and activate it toward reduction.
* Finally, there are other inorganic systems that display some of the structural and possibly some of the reactivity characteristics of the nitrogenase active sites without binding or reducing N_2 or precisely mimicking the active center.

(a). Transition metal sulfide models for nitrogenase sites:

* There has been great activity in synthetic Fe-S cluster chemistry, there is to date no example of a spectroscopic model for the P-cluster sites in nitrogenase.
* If the P-clusters are asymmetrically bound high spin Fe_4S_4 clusters, then the recent work on high-spin versions of Fe_4S_4 clusters and site-selectively derivatized Fe_4S_4 centers may hint that appropriate model systems are forthcoming.

(b). Fe-Mo-S cluster models for FeMoCo

* Despite the importance of P-clusters, the modeling of the FeMoCo center has received the most attention; the significant structural parameters that any model must duplicate are the Mo-S and Mo-Fe distances determined by EXAFS. The $S = 3/2$ EPR signal provides a stringent feature that model systems should aspire to mimic.
* Many Fe/Mo/S clusters have been prepared in the quest to duplicate the FeMoCo center, but none of these are reactive because of their lack of homocitrate.
* Despite the absence of homocitrate, some interesting model systems have been investigated. Heterothiocubane models were first synthesized using self assembly approaches.

$$MoS_4^{2-} + Fe^{3+} + SR \longrightarrow (MoFe_3S_4)_2(SR)_9^{3-} \text{ and } (MoFe_3S_4)_2Fe(SR)_{12}^{3-}$$

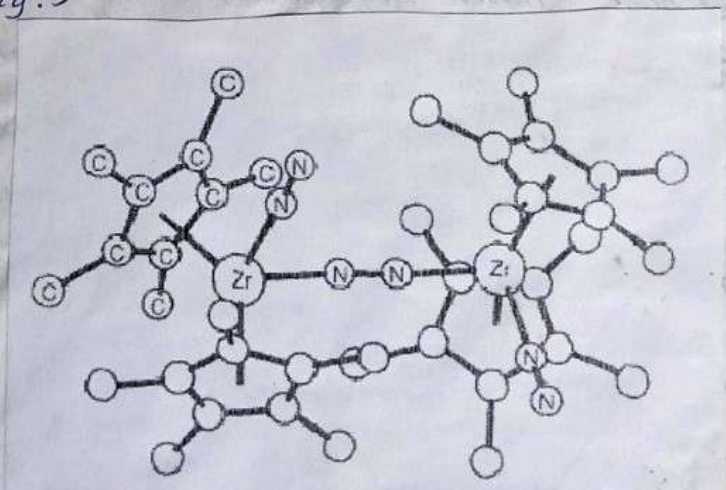
* This reaction uses tetrathiomolybdate MoS_4^{2-} as the source of Mo and leads to thiocubane (Fig 3A,B).

* The Fe_7MoS_8 was possible to complex the central Fe^{3+} iron atom with substituted ligands and isolate a single thiocubane unit (Fig. 3C). The single unit has $S = 3/2$ and Mo-S and Mo-Fe distances that match precisely those found by EXAFS for nitrogenase.

* other interesting Fe Mo S clusters with structurally distinct properties are shown in (Fig. 4). These include the linear $(MoS_4)_2Fe^{3-}$ ion, the linear $(WS_4)Fe(HCON(CH_3)_2]_2^{2-}$ ion, the linear ~~Cl~~ $Cl_2Fe_2S_2MS_2Fe_2Cl_2^{2-}$ (M = Mo, W), the linear $(MoS_4)_2Fe_2S_2^{4-}$ ion, the trigonal $(WS_4)_3Fe_3S_2^{4-}$, the capped thioprismane $Fe_6S_6X_6[M(CO)_3]_2^{3-}$ (X = Cl, Br, I; M = Mo, W) and the organometallic clusters $MoFe_6S_6(CO)_{16}^{2-}$, $MoFe_3S_6(CO)_6(PEt_3)_3$ and $MoFe_3S_6(CO)_6^{2-}$.

N_2 and Related Complexes:

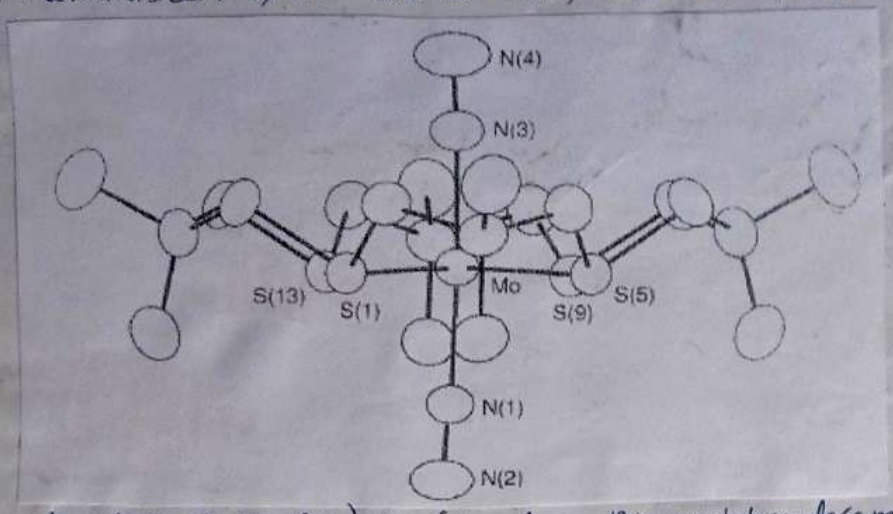
* The triple bond of N_2 has one σ and two π components. each nitrogen atom has a lone pair oriented along the N-N direction. The two lone pairs allow N_2 to bind in an end-on fashion in either a terminal or bridging mode. Both modes of binding are illustrated in the binuclear Zirconium complex shown in fig. 5



[Fig. 5 (x-ray crystal structure of $(Cp')_2Zr(N_2)(\mu_2\text{-}N_2)Zr(N_2)(Cp')$)]

* Here the N-N bond is not significantly lengthened and to be ~~a~~ insignificantly weakened in the complex. Here the complex not having long N-N distances, forms hydrazine quantitatively up on protonation

* binding of bound $N_2H_2^{2-}$ is consistent with the presence of similar bound species in nitrogenase.
* Further Protonation of these intermediates or treatment of the original complex with strong acid leads to the formation of NH_3 from the bound nitrogen.
* only few of the known N_2 complexes contain S-donor ligands. The Mo(0) complex $Mo(N_2)_2(S(CH_2C(CH_3)_2CH_2S)_3)$ has four thioether S-donor atoms bound to Mo(0). This complex shows reactivity reminiscent of the related phosphine complex.



[Fig.7 [The structure of $Mo(N_2)_2L$ (L = tetrathia cyclohexadecane)]]

* Another remarkable complex has been isolated in which two lone pairs of trans diimide bind to two Fe, concomitantly with H-binding of the two diimide hydrogen atom coordinated to sulfur.

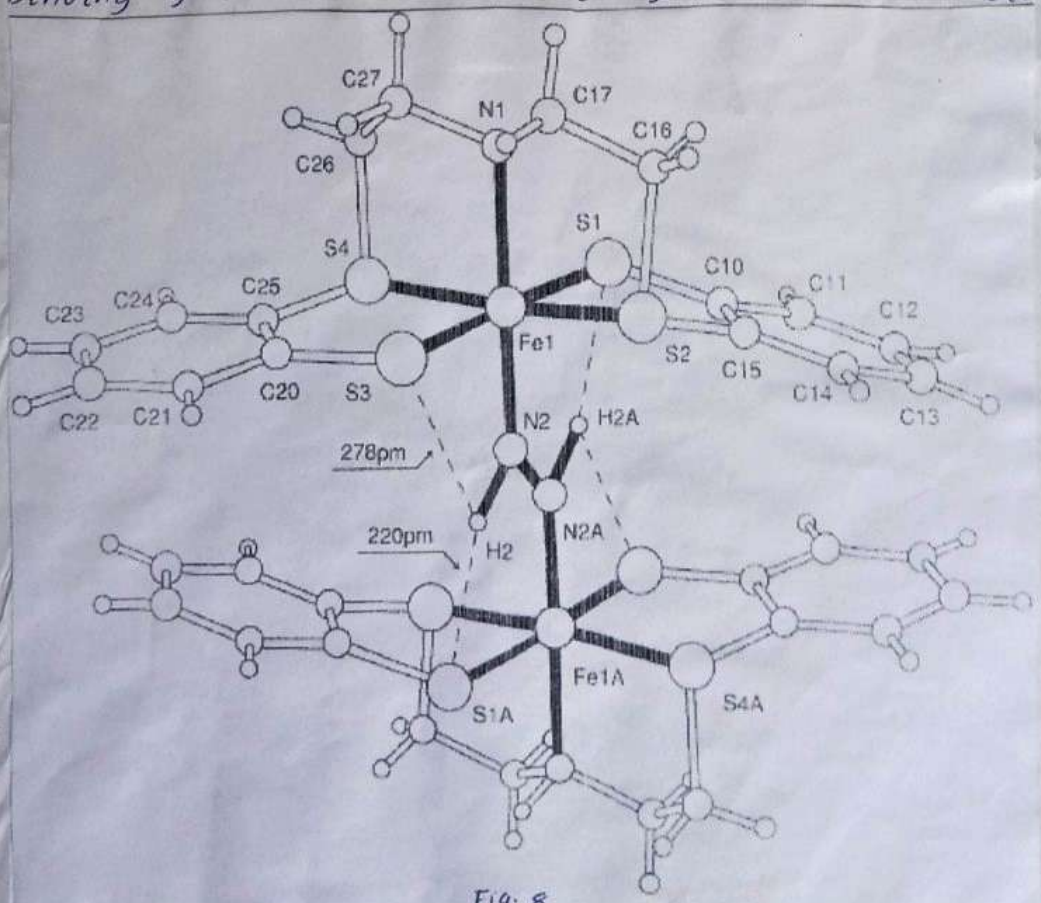


Fig. 8

[The structure of $FeL(N_2H_2)FeL$ ($L = S C_6H_4 S CH_2 CH_2 S CH CH_2 S C_6H_4 S$)]

* The ability of Fe-S system to stabilize the reactive trans N_2H_2 grouping adds support to the notion that similar metal-sulfide sites of nitrogenase may stabilize related intermediates along the $N_2 \rightarrow 2NH_3$ reaction path.

3
Current Status and Mechanism of Action of Platinum-Based Anticancer Drugs

Shanta Dhar and Stephen J. Lippard

3.1
Introduction

3.1.1
Platinum Chemotherapy and Cancer

Chemotherapy, surgery, and radiation therapy are the main pillars of cancer treatment. The term ''chemotherapy'' refers to the use of any chemical agent to stop cancer cell proliferation. Chemotherapy has the ability to kill cancer cells at sites remote from the original cancer. Thus chemotherapy is referred to as systemic treatment. More than half of all people diagnosed with cancer receive chemotherapy. ''Platinum chemotherapy'' is the term used for cancer treatment where one of the chemotherapeutic drugs is a platinum derivative. The spectacular and first such platinum-based drug is cisplatin, *cis*-diamminedichloridoplatinum(II). Subsequently, the cisplatin relatives carboplatin and oxaliplatin were introduced to minimize side effects (Table 3.1). Platinum compounds have been the treatment of choice for ovarian, testicular, head and neck, and small cell lung cancer for the past 20 years.

3.1.2
Palette of Current Platinum Chemotherapeutic Drugs

During the last 30 years, over 700 FDA-approved drugs have entered into clinical practice. The success of cisplatin [1] has been the main impetus for the expansion of the family of platinum compounds. Carboplatin [2] and oxaliplatin [3] (Table 3.1) are registered worldwide and have been a major success in clinical practice. Nedaplatin [4] is used in Japan to treat head and neck, testicular, lung, ovarian, cervical, and non-small cell lung cancers. Heptaplatin [3, 4] is used in gastric cancer in South Korea. Lobaplatin [5] is approved in China for the treatment of chronic myelogenous leukemia, metastatic breast, and small cell lung

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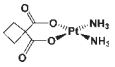
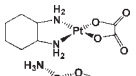
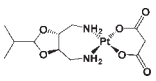
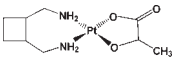
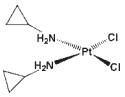
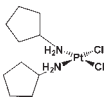
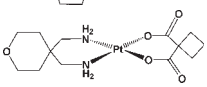
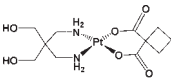
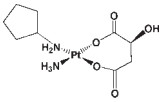
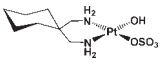
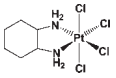
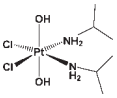
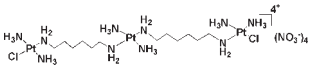
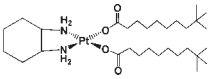
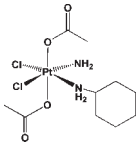
Table 3.1 List of platinum compounds.

Compound	Structure	Use	Current state
Cisplatin		Head and neck, testicular, lung, ovarian, cervical, and non-small cell lung cancers	FDA approved
Carboplatin		Head and neck, testicular, lung, ovarian, cervical, and non-small cell lung cancers	FDA approved
Oxaliplatin		Colon cancer	FDA approved
Nedaplatin		Head and neck, testicular, lung, ovarian, cervical, and non-small cell lung cancers	Phase II
Heptaplatin		Gastric, head and neck cancer, small cell lung cancer	Approved in South Korea
Lobaplatin		Chronic myelogenous leukemia (CML), metastatic breast, and small cell lung cancer, esophageal, ovarian cancers	Approved in China Phase II in USA
JM-11		Malignant disease	Abandoned
PAD		Leukemia	Failed in Phase I
Enloplatin		Refractory advanced ovarian carcinoma	Failed in Phase I
Zeniplatin		Ovarian cancer	Failed in Phase I
Cycloplatam		Ovarian and lung cancer	Failed in Phase I
Spiroplatin (TNO-6)		Ovarian cancer	Failed in Phase-II

Ormaplatin (Tetraplatin)		Melanoma, sarcoma, leukemia and breast cancer, refractory diseases	Failed in Phase I
Iproplatin (JM-9)		Small cell carcinoma of the lung, ovarian, metastatic breast, and head and neck cancer	Phase II and Phase III
Triplatin tetranitrate (BBR-3464)		Ovarian, small cell lung and gastric cancer	Failed in Phase II
Aroplatin (l-NDDP)		Colorectal and kidney cancer, malignant pleural mesothelioma	Phase II
Satraplatin (JM-216)		Prostate cancer	Failed in Phase III
Picoplatin		Non-small cell lung cancer	Phase III

cancer. These second-generation platinum drugs were developed to reduce the side effects generally shown by cisplatin, to enhance the therapeutic index, and for application against cisplatin-resistant tumors.

The clinical development of novel platinum compounds has been somewhat disappointing in view of the promise shown in preclinical studies. The vast majority of platinum compounds synthesized for cancer therapy have been abandoned because of low efficacy, high toxicity, and/or low water solubility. Included in this list (see Table 3.1) are JM-11, PAD, enloplatin [6], zeniplatin [7–10], cycloplatam [11], spiroplatin [12, 13], ormaplatin (tetraplatin) [14], iproplatin [15], the polynuclear platinum compound BBR-3464 [7], aroplatin [8], and other platinum conjugates. Although it is difficult to predict the clinical performance of a new platinum compound based solely on its geometry, structural features nonetheless provide important clues about its likely performance. Several platinum compounds are currently under clinical evaluation, including orally administered satraplatin [9] that showed promise against hormone refractory prostate cancer, the sterically hindered picoplatin [16] for small cell lung cancer, a liposomal cisplatin formulation, lipoplatin [10], as a first-line therapy in patients with non-small cell lung carcinoma (NSCLC), and a liposomal oxaliplatin, lipoxal [17]. Adverse side effects and low anticancer activity in Phase I and II clinical studies are the main reasons for the abandonment of platinum drugs. Of the two cisplatin liposomal formulations tested in the clinic, SPI-77 [12] failed in Phase II trials and was abandoned despite successful preclinical performance, whereas lipoplatin has progressed successfully through Phase III clinical trials in NSCLC with a response rate and stable disease

>70%. This result indicates that a formulation strategy, encapsulation of a platinum compound into tumor-targeted nanoparticles, could provide an attractive pathway for the development of clinically useful platinum compounds.

3.1.3 Early History of Cisplatin and Approved Platinum Drugs for the Clinic

The serendipitous discovery [13] of the anticancer properties of cisplatin and its clinical introduction in the 1970s represent a major landmark in the history of successful anticancer drugs. After the discovery of the biological activity of cisplatin, only two additional platinum compounds, carboplatin and oxaliplatin, have been approved by the FDA. Nedaplatin, lobaplatin, and heptaplatin are approved only in Japan, China, and South Korea, respectively. Cisplatin, carboplatin, oxaliplatin, and most other platinum compounds induce damage to tumors by apoptosis [14]. All these platinum drugs have characteristic nephrotoxicity and ototoxicity.

The present chapter focuses on nontraditional, strategically designed platinum(IV) complexes for targeted cancer therapy based on our knowledge of the mechanism of action of cisplatin.

3.2 Mechanism of Action of Cisplatin

The mechanism of cisplatin action is a multi-step process that includes (i) cisplatin accumulation, (ii) activation, and (iii) cellular processing.

3.2.1 Cisplatin Accumulation

The mechanism by which cisplatin enters cells is still under debate [18]. Originally, it was believed that cisplatin enters cells mainly by passive diffusion, being a neutral molecule. More recently, it was discovered that cisplatin might find its way into cells via active transport mediated by the plasma-membrane copper transporter Ctr1p present in yeast and mammals (Figure 3.1) [19]. Details about this active transport remain to be elucidated. Recent studies with Ctr1p−/− mouse embryonic fibroblasts exposed to 2 μM cisplatin or carboplatin revealed only 35% of platinum accumulation compared to that taken up by Ctr1p wild type cells, which supports such an active transport mechanism. Most likely there are multiple pathways by which the drug is internalized.

3.2.2 Cisplatin Activation

Cisplatin is administered to patients by intravenous injection into the bloodstream. The drop in Cl^- concentration as the drug enters the cytoplasm sets up a

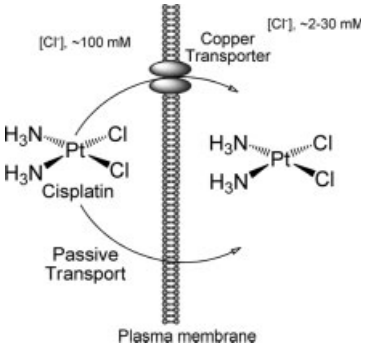


Figure 3.1 Cellular uptake of cisplatin by passive diffusion and via the copper influx transporter Ctr1.

complex pathway (Figure 3.2) for cisplatin activation. Several species form when water molecules enter the platinum coordination sphere, processes that essentially trap the activated form of cisplatin in the cell. The cationic, aquated forms of cisplatin can react with nuclear DNA, which contributes in a major way to the antitumor activity of cisplatin.

3.2.2.1 **Binding to DNA Targets**

There are significant consequences for the cell when cisplatin binds to nuclear DNA and forms covalent crosslinks with the nucleobases [20]. The 1,2-intrastrand d(GpG) crosslink is the major adduct, most likely responsible in large part for the ability of cisplatin to destroy cancer cells (Figure 3.3). Binding of cisplatin to DNA causes a significant distortion of the helical structure, which in turn results in inhibition of DNA replication and transcription. The platinated DNA adducts are recognized by different cellular proteins, including enzymes in DNA repair machinery, transcription factors, histones, and HMG-domain proteins [16, 21].

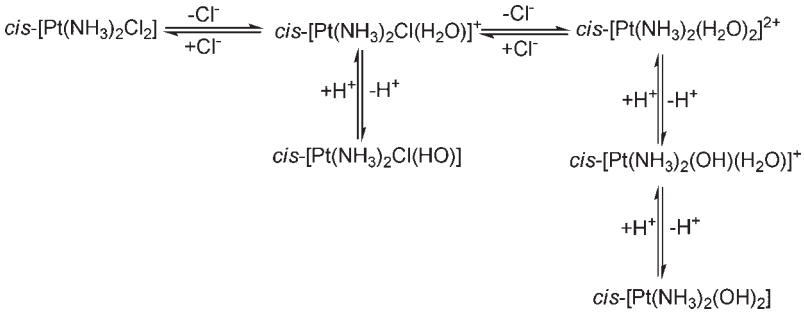


Figure 3.2 Intracellular activation of cisplatin.

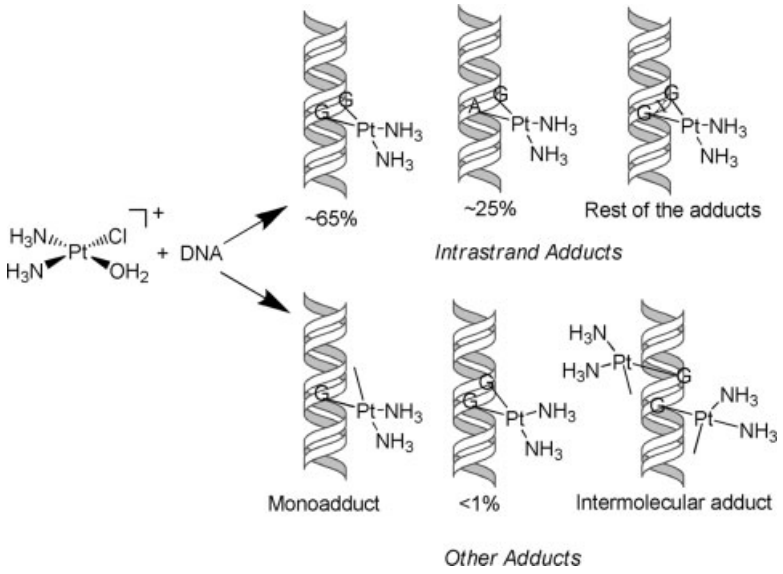


Figure 3.3 Different crosslinks formed by cisplatin.

3.2.2.2 **Binding to Non-DNA Targets**

Cysteine thiol groups in glutathione and metallothionein defend the cell against cisplatin [22]. Because of the high affinity of thiolate anions for Pt(II), after entering the cell a platinum complex may preferentially bind to sulfur atoms rather than to the bases of DNA [23]. Patients treated with cisplatin for the first time avoid this protective mechanism, but continuous exposure to the drug can build up resistance owing to increased levels of glutathione and metallothioneins [24]. The action of glutathione on cisplatin is catalyzed by glutathione *S*-transferases (GSTs), and the resulting complexes are exported from cells by the ATP-dependent glutathione *S*-conjugate export (GS-X) pump (Figure 3.4) [25].

3.2.3
Cellular Processing of Platinum-DNA Adducts

The therapeutic effect of cisplatin is due to the formation of adducts with nuclear DNA that inhibit DNA replication and/or transcription. The main mechanism for removing the intrastrand crosslinks is nucleotide excision repair (NER), but the efficacy of this process varies depending upon the nature of the adducts. NER in human cells depends on many factors, which include the XPA and RPA proteins [26], incision by structure-specific endonucleases, and repair DNA synthesis mediated by DNA polymerase (Figure 3.5). It is important to study the differential repair pathways of cisplatin–DNA intrastrand crosslinks to understand the intracellular processing of cisplatin and to design new platinum drug candidates. One potentially important factor is specific binding of high mobility group box

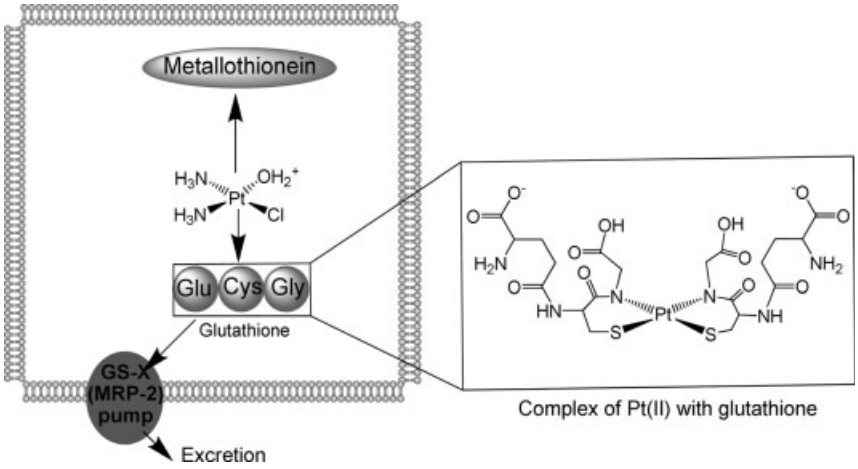


Figure 3.4 Cisplatin binding to glutathione and metallothionein.

(HMGB) proteins to 1,2-intrastrand cisplatin–DNA crosslinks, which shield these lesions from NER [27]. Signal-transduction pathways that control growth, differentiation, and stress responses, involving proteins such as ataxia telangiectasia and RAD3-related (ATR) [28], p53 [29], p73 [30], JUN amino-terminal kinase, and p38 mitogen activated protein kinase (MAPK14) [31], have also been implicated.

3.2.3.1 Cytotoxicity Associated with High Mobility Group (HMG) Proteins

HMG (high mobility group) domain proteins are non-histone chromosomal proteins that bind to specific structures in DNA or in chromatin with little or no sequence specificity [32]. There are two families of HMGB-domain proteins. The first contains two or more HMG domains, and includes the proteins HMGB1 and HMGB2, nucleolar RNA polymerase I transcription factor UBF, and mitochondrial transcription factor mtTF. In the second family, the proteins contain a single HMG domain and include tissue-specific transcription factors. Both families of proteins recognize the major 1,2-intrastrand crosslinks formed by cisplatin [27, 33, 34]. HMG-domain protein mediation of the cytotoxicity of cisplatin is the result of the recognition of DNA–cisplatin adducts by tissue-specific HMG proteins. Several HMG proteins, such as hSRY [35], are expressed in testis tissues and their presence might contribute to the efficacy of cisplatin in the treatment of testicular cancer. Binding of the HMG-domain proteins to cisplatin–DNA 1,2-intrastrand d-(GpG) crosslinks within nuclear DNA impairs the processing of DNA in tumor cells. The distortion caused by this adduct is well recognized by DNA-binding proteins containing HMG domains. The HMG protein forms a 1 : 1 complex with cisplatin–DNA adducts and acts as a protective shield against repair by NER. HMGB1 contains two tandem HMG domains, A and B, and a C-terminal acidic tail. The HMGB-1-induced inhibition of cisplatin–DNA adduct repair is accomplished through the acidic domain. A new member of HMGB family,

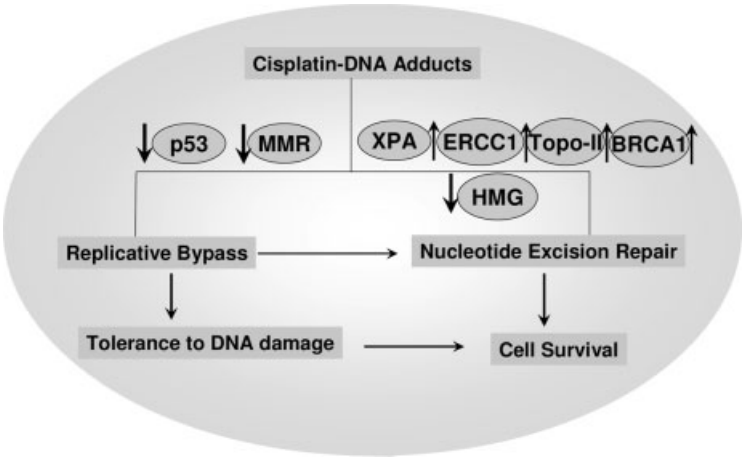


Figure 3.5 Proteins affect cytotoxicity of cisplatin.

HMGB4, was identified recently [36], which is preferentially expressed in the adult mouse testis and sperm cells. Sequence analysis reveals that a disulfide bond, which forms in HMGB1 between Cys23 and Cys45, cannot be formed in HMGB4 because of the absence of cysteine at position 23. The acidic C-terminal tail, which reduces the affinity of HMGB1 for DNA, is also absent in HMGB4. The fact that HMGB4 lacks the disulfide bond and an acidic tail would significantly improve its ability to shield cisplatin intrastrand d(GpG) crosslinks from NER and may contribute to the hypersensitivity of testicular cancer cells to treatment with cisplatin.

3.2.3.2 **Cytotoxicity Associated with Non-HMG Proteins**

Cisplatin adducts are recognized by mismatch repair proteins as well as ERCC-1 [37], an essential protein in NER (Figure 3.5). Human mismatch-repair protein, hMSH2 [38], also binds with modest specificity to DNA containing cisplatin adducts and displays selectivity for DNA adducts of therapeutically active platinum complexes. Similarly, the NER-related XPA gene [39], which contributes to enhanced repair, is overexpressed in cisplatin-resistant tumors. The sensitivity of testicular cancer to cisplatin has been related to a low expression of XPA and ERCC1/XPF [40]. Transcription-coupled nucleotide excision repair (TC-NER) is an important factor in the activity of cisplatin [41]. Both ERCC1 and XPA are involved in TC-NER. A gene that plays a key role in breast and ovarian cancer is BRCA1 [42]. Before the DNA repair machinery works on cisplatin–DNA crosslinks, these adducts are recognized by several specific proteins [16]. A futile attempt of MMR to repair cisplatin–DNA adducts leads to an apoptotic signal. The MMR complex consists of several proteins, including hMSH2, hMSH6, hMLH1, hMutL$_\alpha$, and hMutS$_\alpha$, with hMSH2 and hMutS$_\alpha$ involved directly in the recognition of cisplatin-d(GpG) intrastrand crosslinks.

The design of new platinum anticancer drug candidates can, in principle, benefit from this information by incorporating components that interfere with these processes or by using genes that provide improved platinum therapy. Of particular interest would be compounds that overcome cisplatin resistance.

3.3
Limitations of Current Platinum-Based Compounds: New Strategies

Despite its side effects, cisplatin-derived cancer therapy has been used successfully for three decades. Platinum-chemotherapy gives characteristic relief and modest improvement in survival. The unique pharmacological properties coupled with the side effects of cisplatin have led to the design of many analogs to broaden the spectrum of activity, reduce side effects, and overcome resistance. Although the cis configuration was initially identified as required for activity, trans-platinum complexes have shown significant antitumor activity in preclinical models. In addition to mononuclear platinum compounds, multinuclear platinum complexes are characterized by a different mode of interaction with DNA. One of the major limitations to the clinical efficacy of platinum compounds is drug resistance, and a most important feature of non conventional platinum compounds is their ability to overcome cellular resistance. The multifactorial nature of clinical resistance requires optimization of platinum-based therapy to include drug delivery approaches. The following discussion focuses on our recent studies to improve platinum therapy by introducing delivery systems that include single-walled carbon nanotubes (SWNTs), polymeric nanoparticles (NPs), and oligonucleotide-functionalized gold nanoparticles (DNA-Au NPs). We introduce a novel platinum(IV) compound, mitaplatin, which uses one of the unique pathways of cancer cell metabolism as a target for its selectivity towards cancer cells. We discuss the anticancer properties of these platinum constructs for their potential use in platinum-based chemotherapy.

3.4
Novel Concepts in the Development of Platinum Antitumor Drugs

The amount of platinum accumulated by cancer cells is an important factor that determines the efficacy of the drugs. Reduced cellular uptake or increased efflux is one reason for drug resistance [43]. A major goal is to develop platinum complexes that can overcome resistance by targeting them to cancer cells. Active and passive targeting of platinum compounds are attractive areas in the advancement of platinum-based drug development. For passive targeting, the vehicle for the drug exhibits prolonged circulation in blood. Active targeting results in higher therapeutic concentrations of the drug at the site of action. Normally, active targeting is achieved by using delivery systems that accumulate in cancer cells by a receptor-mediated mechanism. In passive targeting, the phenomenon known as the

selenocysteine residues. Cysteine proteases have been implicated in the pathophysiology of several diseases, including inflammatory airway diseases, bone and joint disorders, parasitic diseases and cancer [42] and the cathepsins B, K, and S [42–46] have been the subject of recent attention, along with tyrosine phosphatases [47], which are implicated in several disease states.

A particular focus of interest has been the thioredoxin system [48], which plays a key role in regulating the overall intracellular redox balance. Thioredoxin reductase (TrxR) has been implicated in several chronic diseases such as certain cancers, rheumatoid arthritis, and Sjögren's syndrome [49] and many emerging cancer therapies use TrxR as a target for drug development [50, 51]. Gold(I) complexes are the most effective and selective inhibitors of purified mammalian TrxR found to date [49, 51, 52], with auranofin being particularly potent [52]; the inhibition has been attributed to Au(I) binding to the -Cys-Sec- redox active center (Sec = selenocysteine) [51, 52]. Antiarthritic gold(I) drugs are known to interact with other selenoenzymes such as glutathione peroxidase [53], and notably the activity of glutathione reductase (closely related to TrxR but lacking the Sec residue) is inhibited at 1000-fold higher concentrations [49]. Recent studies have shown that different classes of cytotoxic gold compounds [both Au(I) and Au(III)] are potent inhibitors of TrxR. A unifying mechanism has been proposed that involves inhibition of mitochondrial TrxR by these gold compounds that ultimately leads to cell death [48]. Moreover, it has been shown that certain Au(I) phosphine and N-heterocyclic carbene (NHC) complexes are *selectively* toxic to cancer cells and not to normal cells and the mechanism may depend on the ability to selectively target mitochondrial TrxR in cancer cells [54, 55].

The potential application of gold-drugs against major tropical diseases has received recent attention [56] and is an area of growing importance due to the variety of thiol and selenol proteins that have been validated as drug targets. Similarly, targeting selenium metabolism with gold-based drugs offers a new avenue for antimicrobial development against selenium-dependent pathogens [57, 58].

These new developments in gold-based therapeutic agents are the focus of this chapter. Another area of emerging interest, not included here, is the potential application of gold nanoparticles for cellular imaging, diagnostic, or therapeutic purposes. This topic has been the subject of several recent reviews [59–61].

7.2 Biological Chemistry of Gold

There are several excellent reviews where the biological chemistry of gold has been discussed in detail [19, 24, 25] and so only a brief overview is given here. While various different oxidation states are known for gold, studies on gold-based therapeutic agents have been restricted to compounds in the two common oxidation states of $+1$ and $+3$.

7.2.1 Gold(I) Oxidation State

Gold(I) ($5d^{10}$), being a large ion with a low charge, is a ''soft'' Lewis acid and hence forms it most stable complexes with ''soft'' ligands such as CN, S-donors (RS^{H},

R_2S, and $S_2O_3^{2H}$), P-donors (PR_3), and Se ligands. In the absence of stabilization by "soft" ligands, disproportionation into metallic gold and gold(III) readily occurs in aqueous solution:

$$3Au(I) \rightarrow 2Au(0) + Au(III)$$

Gold(I) has a much higher affinity for thiolate S (cysteine) compared to thioether S (methionine) and a low affinity for N and O ligands. Hence antiarthritic gold(I) drugs contain thiolate and phosphine ligands, the biological chemistry is dominated by ligand exchange reactions with "soft" cysteine and selenocysteine binding sites on proteins, and DNA is not a target for gold(I) antitumor compounds. The highest affinity is for thiols with the lowest pK_a values. Consequently, in blood, most of the circulating Au from antiarthritic drugs is bound to the cysteine-34 of serum albumin ($pK_a \sim 5$) and transcription factors (Jun, Fos, NF-κB), which have cysteine residues flanked by basic lysine and arginine residues, are likely targets [62]. Gold(I) has a particularly high affinity for selenocysteine residues (e.g., in glutathione peroxidase and thioredoxin reductase) because Se is more polarizable (hence "softer") than S and the pK_a of selenocysteine (~ 5.2 [63]) is much lower than that of cysteine (8.5 [64]).

Linear, two-coordination is most common for Au(I) but higher (three and four) coordination numbers are known. Relativistic effects increase the 6s–6p energy gap of gold, which enhances the stability of the two-coordinate geometry compared to the lighter elements Ag(I) and Cu(I) [25]. Notably, tetrahedral four-coordination can be imposed by the use of bidentate phosphine ligands, and bis-chelated Au(I) diphosphine complexes such as $[Au(dppe)_2]^+$ have a high thermodynamic stability [65]. The formation of chelate rings can contribute to the driving force of unusual reactions [66]. For example, in the presence of thiols (SR) and blood plasma, bridged digold complexes RSAu(dppe)AuSR [linear two coordinate Au(I)] convert into the tetrahedral complex $[Au(dppe)_2]^+$ via the reaction [67]:

$$2[(AuSR)_2(dppe)] + 2RS^- \rightleftarrows [Au(dppe)_2]^+ + 3[Au(SR)_2]^-$$

For linear two-coordinate Au(I) compounds thiolate ligand exchange reactions are facile [64], occurring via an associative mechanism and a three-coordinate transition state. Hence, following administration of gold antiarthritic drugs, Au is readily transported by serum albumin and a thiol shuttle mechanism [68] is probably responsible for the transport of Et_3PAu^+ across cell membranes to key thiol/selenol protein target sites. In contrast, bis-chelated Au(I) diphosphine complexes such as $[Au(dppe)_2]^+$ are stable in the presence of thiols and in blood plasma [27], because ligand exchange reactions must occur by a ring-opening mechanism.

Small mononuclear Au(I) complexes show a pronounced tendency to self-associate and short sub-van der Waals Au· · ·Au distances of about 3.05 Å indicate the presence of an attractive (aurophilic) interaction [69] with a bond energy comparable to that of standard hydrogen bonds. Recent theoretical/computational studies indicate that "aurophilicity" results primarily from dispersion forces reinforced

by relativistic effects [70, 71]. Compounds displaying these short Au· · ·Au distances are often luminescent [72] and this native luminescence has been exploited recently to determine the intracellular distribution of a dinuclear Au(I)-NHC complex using fluorescence microscopy [73].

7.2.2
Gold(III) Oxidation State

Gold(III) is a d^8 metal ion, isoelectronic with Pt(II), and its complexes are generally four-coordinate and square planar. Ligand substitution reactions are likely to occur via five-coordinate intermediates and are faster for Au(III) than Pt(II) [74], but slower than for Au(I) [19]. However, while various ligand types form stable complexes with this oxidation state (Section 7.4.2), the biological chemistry is dominated by the strong oxidizing properties. Thus, *in vivo* most Au(III) compounds will be reduced to Au(I) or Au(0), driven by naturally occurring reductants such as thiols (cysteine), thioethers (methionine), and protein disulfides [24, 25, 75]. On the other hand, while the biological environment is strongly reducing, Au(I) compounds can be converted into Au(III) by strong oxidants, such as hypochlorite, which is produced in inflammatory situations during the oxidative burst. The immunological toxic side effects of antiarthritic gold(I) drugs are attributed to oxidation to Au(III) and subsequent interaction with proteins [75] (Section 7.3.1).

7.3
Gold Antiarthritic Drugs

7.3.1
Structural Chemistry and Biotransformation Reactions

The chemistry and pharmacology of gold(I) antiarthritic drugs were comprehensively reviewed in 1999 by Shaw [24, 25] and more recently by Messori and Marcon [76]. A few pertinent points are highlighted here.

Whereas the orally active complex auranofin is a crystalline monomeric complex [10], the injectable Au(I) thiolate complexes, such as aurothiomalate, are polymers with thiolate S bridging linear Au(I) ions. The crystal structure of aurothiomalate (Myocrisin) was determined only relatively recently [8] and shows linear S-Au-S units arranged into double-helical chains, in good agreement with the chain and cyclic structures indicated in early EXAFS and WAXS studies [77, 78].

After administration, these linear gold(I) complexes rapidly undergo ligand exchange reactions so that the administered drugs are unlikely to be the pharmacologically active species. For auranofin the phosphine ligand confers membrane solubility and affects the pharmacological profile, including uptake into cells. While release of the phosphine does not occur readily in most model

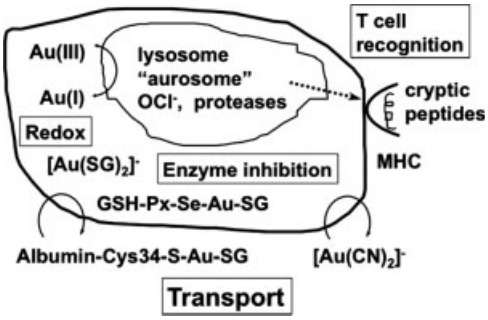


Figure 7.3 Biotransformations of gold antiarthritic drugs, from the article by Sadler and Guo [81]. Gold accumulates in the lysosomes of cells, forming gold rich deposits known as aurosomes. Oxidation of Au(I) to Au(III) can occur, due to the production of hypochlorite by the lysosomal enzyme myeloperoxidase during the oxidative burst in inflamed sites. The formation of Au(III) in lysosomes could lead to the modification of ''self proteins,'' which are degraded and transported to the cell surface. The presentation of these ''cryptic'' peptides at the cell surface, bound to the major histocompatibility complex (MHC) protein, could lead to T cell recognition and triggering of the immune response, accounting for the toxic effects of chrysotherapy [24, 87]. Enzyme inhibition includes the Se enzyme glutathione peroxidase (GSH-Px).
Reproduced from Reference [81] with permission.

reactions, studies with ^{195}Au, ^{35}S, and ^{32}P-radiolabeled auranofin in dogs have shown that the ^{35}S and ^{32}P are excreted more rapidly than ^{195}Au [79], and Et_3PO has been identified in the urine of auranofin-treated patients [16]. On binding to albumin the acetylthioglucose ligand is substituted first and the phosphine ligand is liberated slowly (with formation of Et_3PO) driven by the liberated acetylthioglucose ligand and thiol ligands such as glutathione (GSH) [80]. Once the phosphine is released, the products of auranofin metabolism could be similar to those of Au(I) thiolates. Understanding the mechanism of action of gold antiarthritic drugs is made difficult by the complicated biotransformation reactions that ensue (Figure 7.3 [81]). Albumin can transfer Au(I) into cells (via a thiol shuttle mechanism [68]) and the metabolite $[Au(SG)_2]^-$ can be excreted from cells and the Au(I) transferred back to albumin [82]. $[Au(CN)_2]^-$ is the major metabolite identified in the urine of patients treated with either injectable Au(I)-thiolate drugs or auranofin [83] and may play a key role in the pharmacology. The neutrophil enzyme myeloperoxidase converts aurothiomalate into $[Au(CN)_2]^-$ through the oxidation of thiocyanate [84]. $[Au(CN)_2]^-$ readily enters cells and can inhibit the oxidative burst of white blood cells, and thus may alleviate secondary effects of the chronic inflammation in the joints of RA patients. Under the oxidative conditions

that exist in inflamed joints, oxidation of Au(I) to Au(III) can occur and some of the immunological side effects (gold-induced dermatitis) observed in chrysotherapy are attributable to the production of Au(III) metabolites [85–87]. Hypochlorite (produced by the enzyme myeloperoxidase during the oxidative burst in inflamed sites) has been shown to oxidize Au(I) in aurothiomalate, auranofin, and $[Au(CN)_2]^-$ to Au(III) [24, 25]. The operation of a redox cycle [with Au(III) species reduced back to Au(I) by biologically occurring reductants] has also been proposed [24, 25, 87].

7.3.2 Mode of Action

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterized by the migration of activated phagocytes and leukocytes into synovial tissue, which causes progressive destruction of cartilage bone and joint swelling. Evidence suggests that gold drugs have multiple modes of action in this complex disease [24], and an overriding theme is the interaction with protein cysteine (or selenocysteine) residues. More recent studies on the mechanism of action of DMARDs, including gold drugs, have focused on their effects on macrophage signal transduction and the induction of proinflammatory cytokines (see References [88, 89] for reviews). Cytokines are low molecular weight peptides, proteins, or glycoproteins participating in intracellular signaling and are important mediators in many inflammatory diseases. Of particular importance in RA are tumor necrosis factor (TNF-α) and interleukin-1 (IL-1). Gold drugs have been shown to play a role in each of the different phases of the immune reaction. At the initiation stage gold is taken up by macrophages and inhibits antigen processing. Peptide antigens containing cysteine and methionine residues are especially important [87, 89]. Gold accumulates in the lysosomes of synovial cells and macrophages, forming gold laden deposits known as aurosomes. EXAFS measurements have shown that the gold in aurosomes is in the form S–Au(I)–S [22]. At the effector level, gold drugs inhibit degradative enzymes such as collagenase. Many of the degradative enzymes in the lysosome are cysteine dependent and of particular interest are the cathepsins, which are implicated in inflammation and joint destruction. They play a role in antigen processing and presentation and have been implicated in autoimmune disorders [43]. Recent studies have focused on understanding the mechanism of inhibition of cathepsin B by auranofin and in tuning the potency by alteration of the phosphine ligand [43–45]. Cathepsins K and S have been shown to play central roles in the inflammatory and erosive components of RA, and a recent study [46] shows efficient inhibition of both these cathepsins by auranofin and aurothiomalate; a crystal structure of a cathepsin K/aurothiomalate complex shows linear S–Au–S coordination with Au bound to the active site cysteine residue and a thiomalate ligand still coordinated [46].

At the transcription level gold(I) drugs downregulate a range of proinflammatory genes by inhibiting transcriptional activities of the NF-κB and AP-1 (Jun/Fos) [62] transcription factors. AP-1 controls the expression of genes for

collagenase and the cytokine IL-2, and NF-κB controls transcription of other inflammatory mediators, including TNF-α, IL-1, and IL-6. It was suggested that these transcription factors would be attractive targets for gold(I) drugs because they have conserved lysine-cysteine-arginine sequences in which the thiol pK_a of the cysteine residues is lowered by the positive charge of the flanking basic amino acid residues [62]. NF-κB activation is a complex process that can be triggered by many agents. The potential targets for gold drugs (via crucial cysteine/selenocysteine residues) include NF-κB itself [90], IκB kinase (thus preventing dissociation of NF-κB from the inhibitory protein IκB) [91, 92], and TrxR [93]. Gold drugs have been shown also to activate transcription factor Nrf2/small Maf, which leads to the upregulation of antioxidative stress genes, whose products contribute to the scavenging of reactive oxygen species and exhibit anti-inflammatory effects [94].

Gold drugs also act at the T-cell level [89], and have been shown to inhibit osteoclast bone resorption [95], recently attributed to the inhibition of the cathepsins [43, 46, 96]. RA patients have elevated levels of copper that can be correlated to the severity of the disease. Gold drugs could interfere with copper homeostasis by binding to Cu(I) responsive transcription factors and other Cu(I) transport proteins [97].

7.4
Gold Complexes as Anticancer Agents

7.4.1
Gold(I) Compounds

Analysis of the literature to date indicates that gold(I) antitumor compounds can be broadly divided into two distinct classes based on coordination chemistry, lipophilic-cationic properties, and propensity to undergo ligand exchange reactions with biological thiols and selenols [31, 39]. The two classes are (i) neutral, linear, two-coordinate complexes, such as auranofin and (ii) lipophilic cationic complexes such as $[Au(dppe)_2]^+$ and dinuclear Au(I) NHC complexes. For both classes tumor cell mitochondria are likely targets [98], with apoptosis induced by alteration of the thiol redox balance [31, 39, 48].

7.4.1.1 Auranofin and Related Compounds

Auranofin, in common with a large variety of other linear, two-coordinate Au(I) phosphine complexes, has been shown to inhibit the growth of cultured tumor cells *in vitro* [26, 34, 66, 99–104]. The cytotoxic activity of auranofin against HeLa cancer cells was first reported in 1979 by Lorber and coworkers [105], and subsequent studies showed that auranofin increased the survival times of mice with P388 leukemia [106]. Mirabelli and coworkers carried out an extensive study of the antitumor activity of auranofin against 15 tumor models in mice [26] and found that it was active only in ip (intraperitoneal) P388 leukemia, and required ip administration for activity. A comprehensive structure–activity study [99] of the *in*

11
Essential Metal Related Metabolic Disorders

Yasmin Mawani and Chris Orvig

11.1
Introduction: What is Essentiality?

The physiological importance of metals in humans, especially in blood, is well known. At low concentrations, essential metals play an important role in metabolism, enzymatic processes, and as functional components of proteins. At high concentrations, these metals can lead to serious health problems and even death [1]. The ability of our bodies to maintain a constant internal state with varying external conditions is essential for survival. This is called homeostasis, a state in which the nutrient flow within an organism is at controlled equilibrium. The importance of this equilibrium can be seen in Figure 11.1, where extreme deficiency or overload of the essential metal, if untreated, can lead to death.

For an element to be considered essential, it must have a specific role, where deficiency of that element results in adverse affects that are reversed upon resupply. Thus, it is important to distinguish nutritional effects from pharmacological effects, identifying an essential biochemical function for these metals [2]. In this chapter we overview some of the metabolic disorders that can lead to, or derive from, deficiency or overload of metal ions, and the effects that the perturbation of homeostasis of these metal ions can have on our body.

11.2
Iron Metabolic Diseases: Acquired and Genetic

11.2.1
Iron Homeostasis

Iron is an essential metal, necessary for cytochromes, hemoglobin, myoglobin, and for the function of many non-heme enzymes as well. Iron can be found in its ferric (Fe^{3+}) and ferrous (Fe^{2+}) states and thus is involved in many redox reactions. Excessive amounts can be toxic, with free iron leading, like copper, to Fenton chemistry, toxicity to the liver, and death. Too little iron can lead to cognitive

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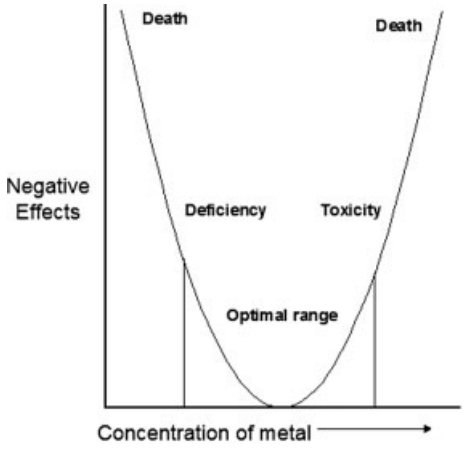

Figure 11.1 Dose–effect curve demonstrating the biological effect of the concentration of an essential metal.

decline, weakness, and death. Thus homeostasis of iron is important, especially since there is no active physiological pathway for excretion [3].

In a normal healthy adult, 1–2 mg is obtained from the diet, and 1–2 mg leaves the body each day (Table 11.1). Iron is absorbed in the duodenum of the small intestine, circulating in the plasma bound to transferrin. Premenopausal women have lower iron stores as a result of blood loss through menstruation [3].

Non-heme iron binds to mucosal membrane sites, is internalized, and then is either retained by the mucosal cell or is transported to the basolateral membrane where it is bound to transferrin (Tf) in the plasma pool. Acidity in the stomach, along with ferrireductase, reduces iron from its ferric to its ferrous state, increasing iron's solubility, making it more bioavailable. Divalent metal transporter DMT1 is a non-specific metal transporter that transfers iron across the apical membrane and into the cell through a proton-coupled process. Heme iron, on the other hand, does not require stomach acid to be solubilized. It is taken up by the enterocyte where it

Table 11.1 Distribution of iron in the body.

Dietary iron	1–2 mg day^{-1}
Muscle (myoglobin)	300
Bone marrow	300
Plasma transferrin (transport)	3
Circulating erythrocytes (hemoglobin)	1800
Liver parenchyma	1000
Reticuloendothelial macrophages	600
Menstruation, other blood loss	1–2

is either stored as ferritin or transferred across the basolateral membrane into the plasma. This receptor/transporter has not been identified [3, 4].

Diseases resulting from defects in iron metabolism are amongst the most common diseases in humans; herein we briefly discuss diseases of primary iron overload, secondary iron overload, and iron deficiency [3]. Primary iron overload disorders, also known as primary hemochromatosis, are caused by genetic defects leading to iron accumulation in tissues. Secondary or acquired iron overload, on the other hand, is iron accumulation caused by non-genetic disorders.

11.2.2 Diseases of Primary Iron Overload: Hemochromatosis

There are many causes of genetic iron overload disorders known as hemochromatosis. The most common is type 1 hereditary hemochromatosis (HH), which is caused by an inborn error of iron metabolism, leading to an increase in intestinal absorption of iron. Iron overload disorders lead to accumulation of the metal in the body, causing irreversible tissue and organ damage and fibrosis [5]. There are four types of hereditary hemochromatosis (HH) described below. Table 11.2 gives an overview of the four forms of HH.

11.2.2.1 Type 1 Hereditary Hemochromatosis

Type 1 hereditary hemochromatosis is the most common autosomal recessive disorder amongst Caucasians, presenting in 1 in 200–400 individuals. This hereditary disorder is caused by a mutation of the HFE (hemochromatosis) gene located on chromosome 6, resulting in an increase of iron absorption from the intestine, leading to liver cirrhosis, diabetes mellitus, and bronze skin pigmentation. It is caused by two mutations in the gene: a substitution of a tyrosine for a cysteine at position 282 (C282Y) and histidine for an aspartic acid at position 63 (H63D) [5].

Pathogenesis of HFE-related hemochromatosis is difficult to describe as the function of the HFE gene has not been clearly established. The HFE protein is found in the intestinal crypt cell of the duodenum where it complexes to transferrin receptor 1 (TfR1), which is the receptor by which cells acquire holotransferrin. Under normal conditions, the HFE and TfR1 help to regulate uptake of iron by crypt cells. In type 1 HH, the mutated HFE protein is believed to impair the TfR1 uptake of iron, causing a deficiency of iron in duodenal crypt cells. As a result of the low levels of iron in the crypt cells, an overexpression of DMT1 occurs, increasing iron absorption. Most patients suffering from type 1 HH absorb two to three times the amount iron, compared to that of a healthy individual, from dietary sources [3, 5].

11.2.2.2 Type 2 Hereditary Hemochromatosis: Juvenile Hemochromatosis

Juvenile hemochromatosis is a rare, autosomal recessive disorder caused by a mutation of the HJV gene (type 2A juvenile hemochromatosis gene) or of the HAMP (hepcidin antimicrobial peptide) gene [5]. It manifests as hypogonadotropic

Table 11.2 Genetic diseases of iron overload: genetic defects, pathology, and common symptoms.

Disease	Genetic defect	Pathology	Symptoms
Type 1 HH[a]	Mutation of the HFE (hemochromatosis) gene located on chromosome [5]	HFE gene is believed to facilitate uptake of transferrin iron into crypt cells, causing iron overload	Liver cirrhosis, diabetes mellitus, and bronze skin pigmentation (same as type 2 HH)
Type 2 HH[a]	Autosomal recessive disease caused by mutation of the HJV gene on chromosome 1 or in the HAMP gene on chromosome [5]	Mutations of the HAMP gene that encodes for hepcidin results in iron overloading. Function of HJV not fully understood	Hypogonadrotropic hypogonadism, cardiac disease, liver cirrhosis, diabetes, and skin pigmentation (same as type 1 HH)
Type 3 HH[a]	Autosomal recessive disorder caused by mutation of the TfR2 gene on chromosome 7 [4]	TfR2 gene is implicated in the uptake of iron by hepatocytes through a receptor-mediated endocytosis	Symptoms are the same as seen in type 1 HH
Type 4 HH:[a] African Iron Overload	Mutation in the SLC40A1 gene on chromosome 2q32, which encodes for the protein ferroportin [5]	Mutation of the SLC40A1 gene which encodes for ferroportin 1	Cirrhosis, cardiomyopathy, impaired immune function
Neonatal hemochromatosis	Unknown [3]	Unknown	Iron accumulation in the liver and fetal organs, leads to death
Aceruloplasminemia	Autosomal recessive disorder caused by a mutation in the CP gene [6]	It is believed that CP plays are role in ferric iron uptake by transferrin	Progressive neurodegeneration of the retina and basal ganglia
Friedrich's ataxia	Abnormal expansion of a GAA repeat in the *FRDA* gene on chromosome 9, encodes for the protein frataxin [7]	Unknown	Progressive gait and limb ataxia, lack of tendon reflexes, dysarthria and weakness of the limbs
Hallervorden-Spatz syndrome	Thought to be autosomal recessive [8]	Unknown. Causes iron deposition in the brain	Cognitive decline and extrapyramidal dysfunction

[a]HH denotes hereditary hemochromatosis.

hypogonadism, cardiac disease, liver cirrhosis, diabetes, and skin pigmentation. Iron overload occurs at an early age, leading to severe organ impairment before the age of 30, manifesting with increased severity to that of type 1 HH. Cardiac failure generally leads to death in individuals affected with juvenile hemochromatosis. Juvenile hemochromatosis, caused by mutations of the HAMP gene that encodes for hepcidin, results in more severe iron overloading. The function of HJV protein is unknown; however, patients with either type of juvenile HH present with low urinary hepcidin levels. It is thus believed that both the HJV and HAMP genes have the same pathophysiological effect [3, 5].

11.2.2.3 **Type 3 Hereditary Hemochromatosis**

Type 3 HH is an autosomal recessive disorder caused by mutations in the transferrin receptor 2 (TfR2) gene. While the role of TfR2 is not fully elucidated, there is evidence that it is highly expressed in the liver, and thus involved in iron uptake by hepatocytes through a receptor-mediated mechanism. Symptoms are the same as seen in type 1 HH [5].

11.2.2.4 **Type 4 Hereditary Hemochromatosis: African Iron Overload**

African iron overload is a hemochromatosis that occurs predominantly in those of African descent, affecting up to 10% of some rural populations is sub-Saharan Africa. Formerly known as ''bantu siderosis,'' it is a predisposition to iron overload that manifests because of excessive intake of dietary iron [3]. Unlike primary HH, it is not caused by a mutation in the HFE gene, but rather by mutations in the SLC40A1 gene on chromosome 2q32, which encodes for the protein ferroportin 1 [5]. Ferroportin is an export protein for iron, and mutations lead to an autosomal dominant hereditary condition characterized by high serum ferritin concentration, normal transferrin saturation, and iron accumulation [9]. It manifests itself in Africans who drink beer that is made in nongalvanized steel drums, because of high levels of iron in the beer [3].

11.2.2.5 **Neonatal Hemochromatosis**

Neonatal hemochromatosis (NH) is a rare condition that occurs during pregnancy, in which iron accumulates in the liver and extrahepatic sites of the fetus, causing extensive liver damage. It has similar pathology to HFE-associated hemochromatosis (type 1 HH). Without vigorous therapy it is fatal to the fetus, leading to the death within hours to days of birth [10]. The pathophysiology is unknown, but there is no genetic linkage to the HLA complexes. Though often unsuccessful, liver transplantation is the only primary treatment [3].

11.2.3
Diseases of Iron Overload: Accumulation of Iron in the Brain

Pathological brain iron accumulation is seen in common disorders, including Parkinson's disease, Alzheimer's disease, and Huntington disease. In disorders of

systematic iron overload such as hemochromatosis, there is no accumulation of brain iron. This suggests that there is a fundamental difference that exists between brain and systematic iron metabolism and transport [8]. Three iron-loading disorders of iron metabolism that result in accumulation in the brain are described below. A summary of these diseases can be found in Table 11.2.

11.2.3.1 **Aceruloplasminemia**

Aceruloplasminemia is an autosomal recessive iron metabolism disorder characterized by progressive neurodegeneration of the retina and basal ganglia. It is associated with inherited mutations in the ceruloplasmin gene leading to iron overload [6].

Ceruloplasmin is a blue copper oxidase that is synthesized in hepatocytes and secreted as a holoprotein binding six copper atoms. Copper does not affect the rate of synthesis or secretion of apoceruloplasmin, but failure to incorporate copper results in an unstable protein lacking oxidase activity. Though ceruloplasmin is a copper protein, the role of ceruloplasmin in copper uptake has not been elucidated; however, there is some evidence that demonstrates ceruloplasmin ferroxidase activity, suggesting a role for ceruloplasmin in ferric iron uptake by transferrin. This is consistent with evidence from animal studies that anemia that develops in copper-deficient animals is unresponsive to iron, but not to ceruloplasmin administration [6, 11]. The presence of neurological symptoms in aceruloplasminemia is unique among the known inherited and acquired disorders or iron metabolism [6].

11.2.3.2 **Hallervorden–Spatz Syndrome (HSS)**

This is an iron metabolic disorder that results in excessive iron storage in the brain [12]. Iron accumulation in the brain in an individual suffering from Hallervorden–Spatz syndrome (HSS) is so excessive that post-mortem the basal ganglia are rust colored. The pathophysiology of HSS is unknown; however, it is known that it is an autosomal recessive disorder manifesting as massive iron deposition in the globus pallidus and substantia nigra. It results in cognitive decline and extrapyramidal dysfunction [8].

11.2.3.3 **Friedreich's Ataxia**

Friedreich's ataxia (FRDA) is an autosomal recessive neurodegenerative disease that affects 1 in 50 000, and is caused by a mutation in the FRDA gene. It is believed that Friedrich ataxia is the result of accumulation of iron in mitochondria leading to excess production of free radicals, which results in cellular damage and death [7, 13]. The disease is characterized by progressive gait and limb ataxia, with lack of tendon reflexes in the legs, dysarthria, and weakness of the limbs. The gene associated with the disease has been mapped to chromosome 2q13 and encodes for the protein frataxin. The function of the protein is unknown, but a deficiency in the activity of iron-sulfur (Fe-S) cluster-containing subunits of mitochondrial

respirator complexes and increased iron content in the heart of patients suffering from FRDA have been reported [13].

11.2.4
Acquired Iron Overload Disorders

Acquired iron overload disorders are common because there is no physiological pathway for excretion of excessive iron. Secondary iron overload adversely affects the function of the heart, the liver, and other organs. As with other acquired iron overload disorders, it is generally treated by chelation therapy (Section 11.2.5) [14].

The main causes of iron overload in chronic hepatic diseases are alcohol-induced hepatocyte damage, chronic liver failure, and chronic iron transfusion therapy [14, 15]. Alcohol intake can lead to chronic liver failure, which induces increased iron absorption, chronic hemolysis, ineffective erythropoiesis, and increased ability of transferrin to deliver iron to the liver. Hepatocyte damage leads to an increase in iron and ferritin release to the extracellular fluid and plasma, as well as an increase in cytokine-mediated hepatocellular iron uptake [15].

11.2.5
Treatment of Iron Overload Disorders: Chelation Therapy

As there is no mechanism for iron excretion, iron loss is almost exclusively by blood loss. As a result, when absorption exceeds excretion, iron overload is inevitable; thus the use of chelators to remove excess iron is necessary to prevent oxidative stress and eventual organ failure [16]. These chelators must bind strongly to non-transferrin bound iron, as this iron is available for Fenton chemistry, while having limited access to both the brain and fetus, and must prevent the iron from participating in redox chemistry [17].

The three most commonly used iron (III) chelators (Figure 11.2) are: desferrioxamine B, deferiprone, and most recently deferasirox. Desferrioxamine B is a

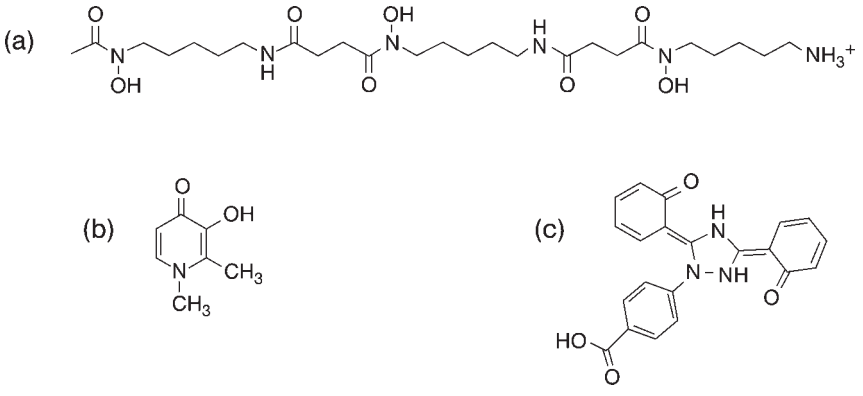

Figure 11.2 (a) Desferrioxamine B; (b) deferiprone; and (c) deferasirox.

siderophore, one of many strong chelators produced by microorganisms, containing either catecholate, hydroxamate, or hydroxy acid functionalities to bind to metals. Desferrioxamine B, which binds strongly to iron by its hydroxamates in a 1:1 ratio forming a charged octahedral complex (because of the NH_3^+ group), has poor oral availability, and a short retention time in the body, meaning it has to be administered intravenously. Deferiprone is an oral bidentate hydroxypyridonate ligand, binding to iron in a 3:1 ligand to metal ratio, forming a neutral complex with Fe(III) [16, 18]. Lastly, deferasirox belongs to a new class of oral tridentate chelators, containing an N-substituted bis-hydroxyphenyltriazole, forming a strong Fe(III) complex, binding in a 2:1 ligand to metal ratio [18, 19].

11.2.6 Iron Deficiency

Iron deficiency anemia (IDA) is caused by low iron levels and low hemoglobin, or abnormal levels of two out of the following three iron status tests: erythrocyte protoporphyrin, transferrin saturation, or serum ferritin [20]. Iron deficiency anemia overwhelmingly occurs in toddlers and women of a reproductive age [21]. According to the WHO, 35–75% of child-bearing women in developing countries and 18% in industrialized countries are anemic, while 43% of women in developing nations and 12% in industrialized nations suffer, or have suffered, from anemia [22]. In comparison IDA occurs in only 1–2% of men and 2% of women over the age of 50 years [21].

Transfer of iron from mother to fetus results in an increase in maternal iron absorption during pregnancy, which is regulated by the placenta. Significant decreases in serum ferritin is observed between 12 and 25 weeks of pregnancy. If maternal iron levels decrease, transferrin receptors to the fetus increase so as to increase the uptake of iron by the placenta. A lack of synthesis of placental ferritin often prevents excessive iron transport to the fetus. Evidence is accumulating that the capacity of this system may be inadequate to maintain iron transfer to the fetus when the mother is deficient, leading to detrimental effects to the cognitive function of the fetus [22].

Iron deficiency may be caused by prolonged low dietary intake, increased iron requirement due to pregnancy, loss of blood through gastrointestinal (GI) bleeding or menstruation, or gastrointestinal malabsorption of iron. In adults over the age of 50, GI blood loss is an important cause of iron deficiency. Amongst patients over the age of 50 suffering from IDA, 11% of these cases was a result of GI cancer [21].

Iron deficiency can have many negative effects on an individual's health, including changes in immune function, cognitive development, temperature regulation, energy metabolism, and work performance [20]. A decrease in cognitive function due to iron deficiency is not well understood; however, it is proposed that a decrease in iron-dependent dopamine D2 receptors in the cortex is observed, altering dopamine neurotransmission, causing a decrease in cognitive function [23].

11.3
Copper Metabolic Diseases

11.3.1
Copper Homeostasis

Copper is an essential metal that is required for cellular respiration, iron oxidation, pigment formation, neurotransmitter biosynthesis, antioxidant defense, peptide amidation, central nervous system development, and connective tissue formation [24]. Physiologically, copper exists in two redox states, cuprous (Cu^{+}) and cupric (Cu^{2+}), with many known enzymes requiring it. Copper is found complexed to proteins in its ionic form; free Cu ions, like free Fe ions, catalyze the formation of free radicals, resulting in Fenton chemistry [25].

Our ability to tightly regulate copper is cardinal to keep these processes in check. Imbalances in copper homeostasis can lead to neurodegeneration, growth retardation, and mortality [24, 26]. Table 11.3 describes the location and function of some important copper-dependent proteins [24–28].

Copper homeostasis is maintained by a balance between intestinal absorption and excretion. Copper is absorbed from the gut and transported to the liver, the main storage area for copper, where it is subsequently redistributed to all tissues and organs. Copper is then returned to the liver to be excreted by the bile, the principal route for copper elimination (Figure 11.3) [29]. While copper is also excreted in sweat and urine, this excretion is not significant enough to contribute to homeostasis.[26]

Ceruloplasmin accounts for approximately 90% of the copper content found in plasma, but is not believed to be involved as a specific copper transport vehicle. Those suffering from aceruloplasminemia (see Section 11.2.3.1), an autosomal recessive disorder, lack a functional form of the protein, but do not exhibit any signs of copper deficiency. In contrast, only 5% of the total copper in serum is

Table 11.3 Important copper-dependent proteins involved in copper transport and homeostasis.

Protein/enzyme	Location	Function
Cu/Zn superoxide dismutase	Cytosol	Antioxidant defense (superoxide dismutation)
Cytochrome *c* oxidase	Mitochondria	Mitochondrial respiration
Ceruloplasmin	Plasma	Iron and copper transport (ferrioxidase)
Lysyl oxidase	Elastin and collagen	Connective tissue formation
Dopamine-β-hydroxylase	Storage vesicle	Catecholamine production
Tyrosinase	Storage vesicle	Melanin formation
Peptidylglycine α-amidating mono-oxygenase	Storage vesicle	Peptide amidation (activation of peptides)
Metallothionein	Liver and kidneys	Storage and chaperon

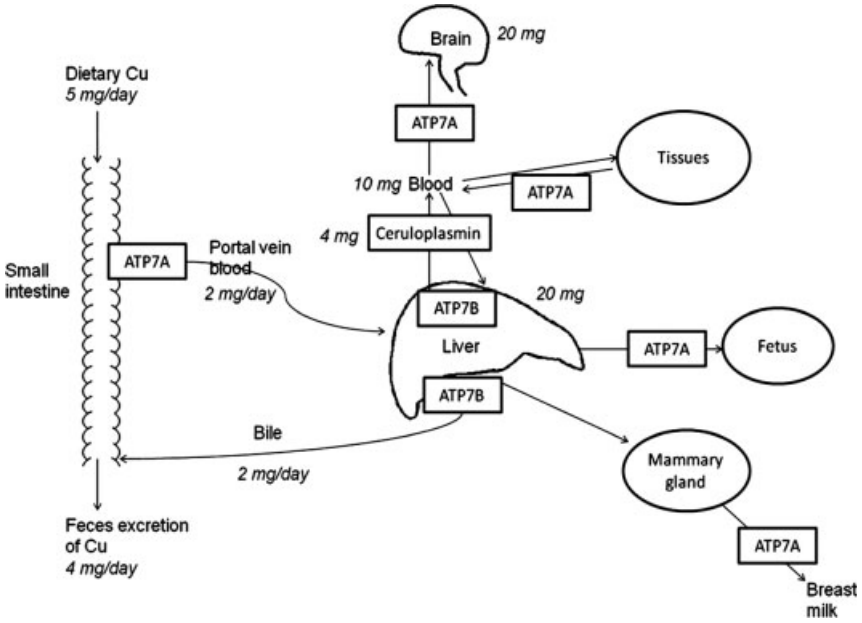

Figure 11.3 Transport and distribution of copper, and functions of ATP7A and APT7B.

bound to albumin, but this protein is considered to be very important for copper uptake [30]. Cellular copper uptake is mediated through energy-dependent transporters localized in the plasma membrane. Copper can enter cells by the high affinity carrier Ctr1, a membrane transport protein found in most tissues [31].

The mechanism of copper transport and homeostasis in the brain has not been completely elucidated; however, it has been well established that copper plays an important role in brain development. Copper transporting P-type ATPases possess six metal-binding sites (MBS) in the N-terminal part of the molecule to pump copper ions through physiological barriers, including the blood–brain barrier. Identification of ATP7A, a copper transporting ATPase that has a loss-of-function in Menkes disease (Section 11.3.2), has helped to gain insight into copper transport mechanisms in the brain [30, 31].

Once copper is transported into the cytosol by the Ctr1 transporter protein, the chaperone Atx1 shuttles copper to the ATPases (ATP7A/ATP7B), the copper chaperone for superoxide dismutase, CCS, delivers copper to superoxide dismutase (SOD) and Cox17, Sco1, and Sco3 are the chaperones involved in transporting copper to mitochondria and cytochrome oxidase (Cox). Metallothionein chelates most of the excess copper in the cell once Ctr1 transports it into the cell. Metallothionein plays an important role in scavenging free copper, along with other heavy metals, but it also may play a role in copper storage. ATP7A works to pump out excess copper in nonhepatic cells, while in hepatic cells this role is carried out by the P-type ATPase ATP7B, the protein that undergoes a loss-of-function in Wilson's disease (Section 11.3.3). The role of the ATPases ATP7A and

Table 11.4 Comparison of the hereditary disorders of copper metabolism.

	Wilson's disease	**Menkes disease**
Genetics	Autosomal recessive ATP7B	X-linked ATP7A
Onset	Late childhood: liver 20s–30s: neuropsychiatric problems	Early infancy
Pathogenesis	Copper overload caused by defected biliary copper excretion	Copper deficiency caused by defected copper transport across the brain, placenta, and GI tract
Presentation	Cirrhosis, liver disease, neuropsychiatric symptoms	Hypopigmentation, abnormal hair growth, failure to thrive, seizures, mental retardation

ATP7B can been seen in Figure 11.3. These two copper transporting proteins are the only ones that are presently known to be associated with specific copper metabolic diseases [24, 30]. Table 11.4 gives a comparison of the pathogenesis of Wilson's disease and Menkes disease [24].

11.3.2
Copper Deficiency: Menkes Disease

Menkes disease (MD) is an X-linked disorder that is diagnosed by stunted growth, hypopigmentation, brittle hair, arterial tortuosity (twisting of the arteries), and neurodegeneration. These characteristic disorders are caused by a mutation in the encoding of the copper transporting gene ATP7A, resulting in impaired activity of the cuproenzymes [24]. The CNS central nervous system pathology is less affected in patients suffering from MD, but that possess some ATP7A activity. This suggests that there is a hierarchic order of copper distribution, and that under copper deficiency copper will distribute preferentially in the brain.

Menkes is a rare, but serious, disease the affects 1 in 250 000 [32]. The defective gene, ATP7A, belongs to the large family of cation transporter P-type ATPases, and is found in muscle, kidney, lung, and brain. The functional role of ATP7A, originally described by Llanos and Mercer, can be seen in Figure 11.3 [33]. Only a trace amount is found in the liver, and is thus responsible for copper transport in non-hepatic cells. All P-type ATPases have similar amino acid sequences, where there is a conserved phosphorylation motif that contains an aspartic acid, an ATP-binding site, and a CPC (cysteine-proline-cysteine) domain that acts to bind copper when transferred from the metal binding site (MBS) [30]. The N-terminus contains six MBSs, each with the sequence GMTXCXXC, where X denotes a non-conserved amino acid, and copper binds to the CXXC motif [32].

When ATP7A is inactivated, copper becomes trapped in the endothelial cells of the blood–brain barrier (BBB) and the brain becomes severely deficient, leading to the profound neurological symptoms manifested in Menkes disease [30].

Deficiency in copper results in impaired function in some of the cuproenzymes and copper proteins described in Table 11.2. For example, a lack of function of lysyl oxidase results in connective tissue and skeletal defects [34]. Cytochrome *c* oxidase impairment results in deficient energy production (ATP production), causing altered nerve conduction, seizures and myopathy. Loss of function of tyrosinase results in hypopigmentation of the skin. Altered function of Cu/Zn SOD leads to oxidative stress to cells, which results in degeneration of the central nervous system and mitochondrial defects [26].

Treatment of MD with copper is not effective because copper transport into the brain is dependent on the function of ATP7A; however, administered copxper-histidine is taken up by the brain more efficiently, though the mechanism is unknown [24]. Copper-histidine therapy also results in normalization of serum copper, ceruloplasmin, dopamine, and norepinephrine levels in patients who have undergone this treatment course. Connective tissues disorders, however, still persist for these patients, indicating that copper-histidine does not bind effectively to lysyl oxidase [32].

11.3.3
Copper Overload: Wilson's Disease

Wilson's disease (WD) is an autosomal recessive disorder that causes cirrhosis, liver disease, progressive neurological disorders, or psychiatric illness, affecting 1 in 30 000 individuals [35]. There is an impairment of biliary copper excretion, leading to hepatocyte copper accumulation and copper-mediated liver damage. Leakage of copper can occur in the plasma, and eventually overload is seen in all tissues. ATP7B, the affected copper-transporter gene, is a P-type ATPase [24]. WD and MD P-type ATPases are functionally homologous, sharing 67% protein identity [33]. Their pathophysiologies are quite different, WD ATPase being found mainly in the liver and kidney, whereas ATP7A is in muscle, kidney, lung, and the brain [32]. Like Menkes, Wilson's disease ATPase possesses six Cys-X-X-Cys (CXXC) metal binding sites in the N terminus. There are some amino acid differences, but this does not affect the copper binding [32].

Copper can act as a prooxidant as it physiologically exists in two different valence states. Thus an excess of copper in the liver leads to organ damage caused by oxidative stress. Free copper ions participate in Fenton or Haber–Weiss chemistry, generating reactive oxygen species, which have been shown to form in HepG2 cells. Apoptosis commonly causes liver damage in Wilson's disease [35]. Although ATP7B is expressed in some regions of the brain, in WD, copper overload seen in extrahepatic tissues is due to accumulation in the plasma following liver injury. A complete reversal of non-hepatic tissue accumulation is seen after liver transplantation [24].

11.3.4
Treatment of Wilson's Disease: Chelation Therapy

Wilson's disease was once an untreatable disorder, inevitably leading to death; now, if caught at an early enough stage, the disease is treatable with copper chelators

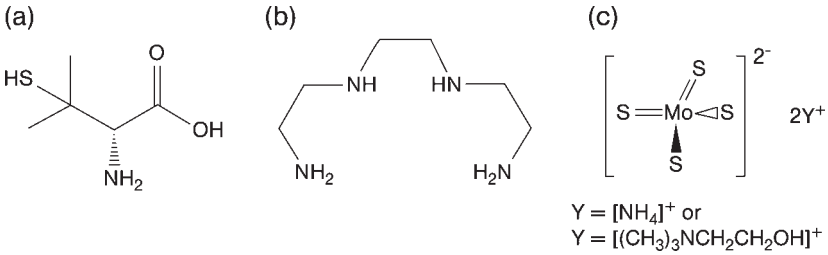

Figure 11.4 (a) D-Penicillamine; (b) trientine; and (c) tetrathimolybdate.

and zinc salt therapy [36, 37]. The three most common chelating agents are D-penicillamine, trientine, and tetrathiomolybdate. Penicillamine was the first oral agent for the treatment of WD. It is believed that penicillamine binds in a bidentate fashion, and can either bind in a 1:1 or a 1:2 ligand to metal ratio, forming a Cu(I, II) mixed-valence chelate that is unusually strong, binding the first copper through the amino nitrogen and thiol sulfur, and the second copper through a deprotonated carboxyl group, both acting as bidentate donors (Figure 11.4) [36, 38, 39].

Some patients have a hypersensitivity to penicillamine, a metabolite of the antibiotic penicillin. In these cases trientine is prescribed. Trientine is a tetradentate ligand, coordinating to copper in a 1:1 ratio by its four amines [36]. Tetrathiomolybdate is believed to form a polymetallic clusters with copper, binding up the three coppers per ligand with Mo(IV) in a tetrahedral arrangement and an overall 2– charge on the complex [40]. Tetrathiomolybdate is especially successful in treating patients with neurological manifestations from Wilson's disease [36].

More recently, zinc salts have been used in the treatment of copper overload. Zinc (Section 11.4) stimulates metallothionein production, helping to sequester copper. Since copper has a higher affinity for metallothionein than zinc, excess copper binds to it, helping to excrete excess copper [41]. Treatment is lifelong with either oral chelating agents or zinc salts. If unresponsive, liver transplantation is necessary for patients suffering from WD [35].

11.4
Zinc Metabolic Diseases

11.4.1
Zinc Homeostasis

Zinc is a co-factor in over 200 biologically important enzymes (e.g., alcohol dehydrogenase, carbonic anhydrase, carboxypeptidase), particularly enzymes involved in protein synthesis [41]. Between 3% and 10% of all proteins in mammals bind to zinc. The uniqueness of zinc is that unlike other abundant transition metals such as iron and copper it lacks redox activity [42]. It is one of the most abundant trace elements in the body, where it is present in all tissues and fluids. The average amount of zinc in healthy adults is 1.4–2.3 g [41]. Muscle and bone