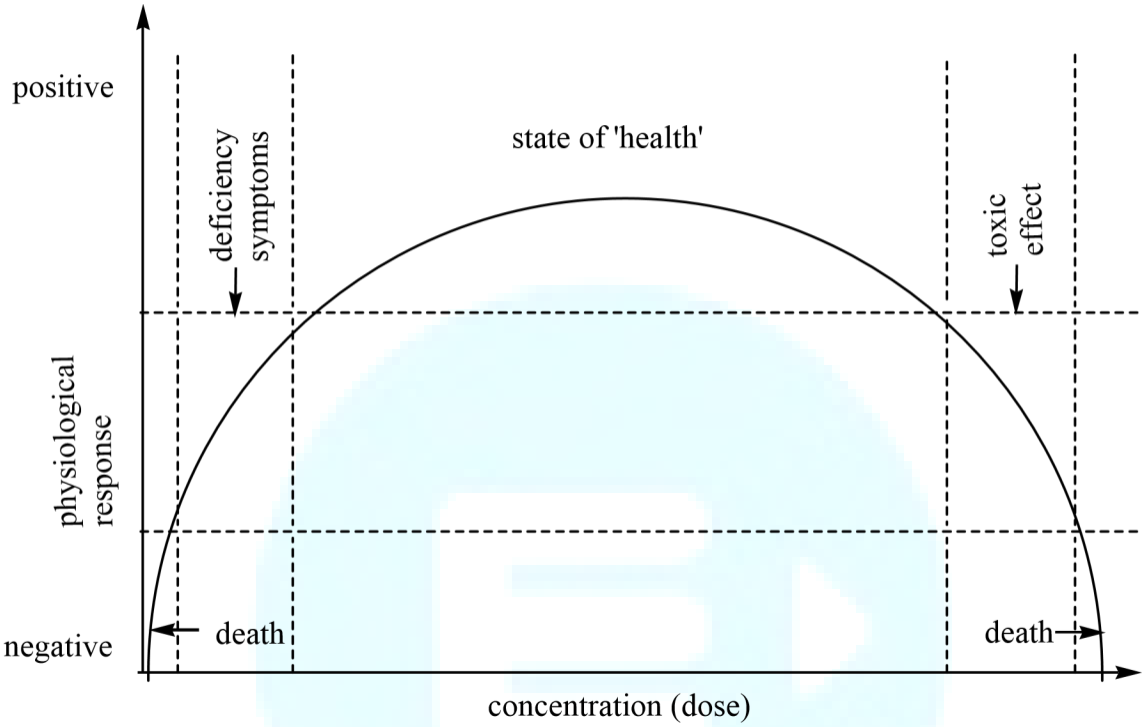
METAL IONS IN THE BIOLOGICAL SYSTEM

Metal ions their excess and deficiency

Concentration of metal ions in human being's system is controlled within very fine limits. This control is generally exercised by certain biological complexing agents.
The deficiency or excess of metal ions causes disorder, which leads to various diseases.



Calcium

Calcium is a critical element in all animals and man. A healthy human adult has about 1.05 kg Ca, of which 99% exists as phosphates resembling the mineral hydroxyapatite, $CaB_{10}(PO_B B_4)_B B_6 (OH)_{B\ 2}B$, in bones and teeth. The small remainder is in cellular $_B$ fluids, existing in partly ionized, or protein bound forms. The primary dietary source of Ca is milk (65-76%), with smaller amounts derived from meat, fish and eggs (5-10%), and still less from non-dairy foods such as nuts, fruits, beans etc. Dietary deficiency of Ca is not a common problem in nations with high dairy product and protein intake, particularly since normal individuals can regulate intestinal absorption and renal conservation mechanism with great precision. Hence, human health problems related to geochemical distribution of Ca, its entry into the human food chain and its bioavailability are relatively uncommon. Exceptions include very poor diets (such as those low in milk and animal proteins or unusual physiologic, other illness, such as intestinal malabsorption). In case of excess of Ca, it comes in to the blood as Ca is rejected by cell and its salts are not are not soluble. So excess of Calcium leads to the formation of stones, hardening of arteries, and cataracts in the eye.

Magnesium

Magnesium, an abundant element in the earth's crust, is vital to both plant and animal life. Chlorophyll pigment in plants is a Mg-porphyrin complex. All enzymatic reaction in

animals and men that are catalyzed by ATP require Mg as a cofactor. Oxidative phosphorylation, DNA transcription, RNA function, protein synthesis and critical cell membrane functions are all dependent upon optimal Mg concentrations. An average man has about 35g Mg, out of this 99% is either intracellular or in bone, of the 60% in bone, two-third is tightly incorporated into the mineral lattice, but one-third is in an apparently exchangeable bone surface pool. Dietary sources high in Mg include nuts, sea foods, legumes and vegetables, meat is intermediate in Mg content.

Potassium

An adult human has approximately 140 g K of which >90% is both intracellular and exchangeable (K is the predominant cation in intracellular water) since muscle contains most of the body's intracellular water, it also contains most of the K. Since K is found in most animal and vegetable foods, dietary deficiency is exceedingly rare except under unusual conditions (such as diets very high in refined sugars, alcoholic individuals deriving most of their calories from low-K alcoholic beverages in the states of starvation etc.).

Sodium

Sodium is the predominant extracellular cation in animals and man. An adult human has about 105 g Na, about 24% is located in bone and about 65% in extracellular water. Sodium ion equilibrium is maintained primarily by the kidney, the key organ in water and electrolyte balance. Sodium chloride (salt) is the predominant dietary source. Although excessive dietary Cl appear to have no significant ill effect on health, there is much evidence that excessive Na intake results in elevated blood pressure (hypertension) and that reduces Na intake or increased K intake helps to reduce high blood pressure.

Cobalt

Cobalt is an essential element for humans, but its pathway through the food chain to human being remain elusive. Only a little over 1 mg Co is present in an adult human. It is useful to man, insofar as is known, only in the form of vitamin B_{12} (cobalamin). Vitamin B_{12} is synthesized only by bacteria. Vitamin enters the human food chain as animal organs or muscle. In man dietary Co deficiency is only likely among strict vegetarians or when the intrinsic factor from the stomach that facilitates B_{12} absorption is absent or severely decreased as in pernicious anaemia.

Zinc

An adult has about 1.5-3.0 g Zn with the largest amounts being in liver and bone. There is evidence that Zn concentrations in blood and several tissues vary considerably in response to many stimuli. Zinc appears to be critical in many functions. Human Zn deficiency in an inherited form in infants is termed acrodermatitis enteropathica and is characterized by behavioral disturbances, diarrhoea, hair loss and severe peri-orificial skin rash, all of which respond with remarkable promptness to Zn administration. Similar syndromes have now been reported many times with penicillamine treatment of other disorders, presumably due to chelation of Zn, as well as during total parenteral nutrition when Zn was not added to the nutritional solutions for even as short a time as two weeks. A more chronic dietary deficiency of Zn (combined with other deficiencies) include dwarfism, hypogonadism and sexual immaturity, the latter devised with Zn therapy. There is much evidence for marginal dietary deficiencies in humans. The effects include decreased acuity of taste (hypogeusia), importance, delayed wound healing, hypogonadism and oligospermia, poor development

and possible foetal wasting and teratogenesis. Dietary source range in Zn concentrations from1400 µg g^{-1}P to 2 µg $g^{P\ -1}$P or ^{P} less in fresh fruit and vegetables. Bioavailability of Zn is especially high from animal tissues and is low from milk and from grains. The latter effect is apparently due to binding to phytic acid and fibre.

Molybdenum

The essentiality of Mo in animals and human beings is assumed from it's presence in the metalloemzymes xanthine oxidase and aldehyde oxidase. Mo is also part of the enzyme sulphite oxidase, an inherited deficiency of which cause severe neurologic disorders and early death in humans. However, no naturally occurring Mo deficiency has ever been documented in animals or man, even though several animal deficiencies have been produced experimentally, particularly by using the Mo antagonist. Molybdenum is present in very small quantities. Molybdenum appears to be readily absorbed from the GI tracts and excreted primarily through the kidneys (though human studies are lacking). In tissues with higher concentration, such as bone, liver and kidney the Mo content can be varied with dietary intake. There is evidence that dietary Mo affects Cu metabolism in animals and man, higher Mo intakes causing mobilization and excretion of Cu. These effects can be elicited in man even with naturally occurring dietary sources of Mo. Since Mo concentrations in grains and vegetables varies enormously (differences up to 500 times) and varies with soil content the possibility of Mo-induced Cu deficiency in man is conceivable, though not reported.

Chromium

The designation of Cr as an element essential to animals and man is quite recent. Insofar as is known, the major biological function of Cr is an integral part of an organic complex originally isolated from yeast termed "glucose tolerance factor" (GTF). This complex apparently includes one Cr (III) ion and two nicotinic acid molecules and may coordinate with three amino-acid molecules, probably glycine, cysteine and glutamic acid. Experimental data indicate that GTF functions in conjunction with insulin, and may in fact aid in binding insulin to sites of action. Other activities apparently include a lowering of serum cholesterol and triglycerides. An adult human has about 6 mg Cr. Trivalent Cr is absorbed in the upper gastrointestinal tract, but only in very small amounts (hexavalent Cr is better absorbed, but only trivalent Cr is biologically active as an essential element). Trivalent Cr as GTF is apparently absorbed much better. Thus conversion to GTF in the gastrointestinal tract may be important, and may vary with age of the individual. Chromium in excess amounts can be quite toxic, dependent upon the chemical species of Cr(III) is much less toxic than the hexavalent form. Chromium is a known carcinogen and toxic metal present abundantly in tannery effluents in India. As an estimate, about 80-90% of the tanneries use chromium as a tanning agent. Of this quantity, the hides take up only 50-70%, while the rest is discharged as effluent. This rest amounts to nearly 75, 000 tonnes per day. Today, the tannery industry mushrooming in North India has converted the Holy Ganges River into a dumping ground. Several analyses reveal high concentrations of chromium even in supposedly "treated" effluents. The residues can be traced even in crops cultivated with water taken from the river. The Department of Environment has identified the tannery industry as the 'biggest pollutant' across the country. Electroplating can also release chromic acid spray and air-borne Cr trioxide, both of which can result in direct damage to skin and lungs. Chromium dust has long been incriminated as potential cause of lung cancer and Cr has been shown to be mutagenic in micro-organism, causing infidelity (mis-reading)

during synthesis of DNA copies. Thus the story of Cr once again illustrates the principle of essentiality in small amounts and potential toxicity in large amounts.

Copper

Normal adult human has about 100-110 mg Cu, highest concentrations are found liver, kidney, heart and brain. The prototype functional deficiency of Cu in humans is an X-linked inherited disorder called Menke's syndrome (Kinky or steely hair syndrome). Cu insufficiency was first suspected by analogy with abnormal wool in Cu-deficient sheep. The defect appears to be decreased gastrointestinal absorption and /or cellular utilization of Cu. The essentiality of Cu is the consequence of its role in metalloenzymes involving several critical biochemical pathways. Several of these enzymes are noted here. Superoxide dismutase, which metabolize the potentially damaging superoxide anion. Lysyl oxidase is a monoamine oxidase required for cross-linking collagen and elastin, the structural macromolecules of connective tissue. Dopamine β-hydroxylase, amine oxidase and tyrosinase are all Cu containing enzymes that interconvert the major neurotransmitters dopamine, noradrenaline and adrenaline, probable accounting for the high concentration of Cu in the brain. The latter enzyme, tyrosinase, is also a key step in pigment production. Cytochrome C oxidase is the key and terminal enzyme of the respiratory chain, accounting for more than 90% of the energy of muscular contraction. Ferroxidase (better known as ceruloplasmin before its role in mobilizing and oxidizing Fe from storage sites was recognized) is believed to account for 95% of serum Cu, and appears to be a multi-functional protein serving as a major transport system for Cu as well. Copper is widely distributed in the food chain, a notable exception being cow's milk. Copper concentration ranges from 20-60 µg g^{-1} in animal tissues to less than 2 µg g^{-1} in leafy green vegetables and fruits to less than 0.2 µg g^{-1} in cow's milk, which is much less than in human milk. Infants, especially if premature and not breast-fed, are therefore most susceptible to dietary deficiencies; excessive loss of Cu from gastro-intestinal tract due to diarrhoea is the most common precipitating factor. In Wilson's disease, copper concentration increases up to one hundred times greater than normal. Copper is accumulated in a number of tissues but in particular is found in the liver, brain and kidney which leads to liver and kidney failure and various neurological abnormalities. Death results if the condition is not recognized and treated.

Iron

The average human adult has about 4-5 g Fe. Of this amount, about 60-70% is present in haemoglobin in red blood cell, 3-5% is in muscle myoglobin, 15% is bound to the Fe storage cellular protein, ferritin, 0.2% occurs as a component of critical respiratory enzymes and 0.004% is bound to the serum transport protein transferin. Iron deficiency causes anemia because red cells of blood containing less hemoglobin than in normal condition. Acute iron poisoning leads to vomiting, pallor, shock, circulatory collapse and coma. Chronic conditions are also known in which iron is deposited in tissues and organs of the body. This condition is known as siderosis.

Use of chelating agents in medicine; or chelation therapy

Treatment of Metal poisoning detoxification by chelating agents:

Mostly the treatment of metal poisoning is done by the use of chelating agents. It is hoped that the chelating agent will form soluble, stable and non-toxic complexes which are readily excreted.

Criteria for a potential chelating drug.

1. It must bind the metal strongly to complete for it with biological ligands and excreting as soluble chelate.
2. It should be selective for the metal ion. If it is non-selective then there will he harmful side effects from the removal of other metals, particularly calcium and zinc from the body.
3. Chelate must be of low toxicity and not metabolized i.e. it should remain unchanged in biological system.
4. It should be capable of penetrating in to metal storage sites.
5. Chelate should be less toxic than the 'free' metal ions.

A suitable multidentate ligand that can satisfy all the coordination positions of the metal ion would be ideally suited in the elimination of the metal ion. So that the chelated metal ion cannot bind to any binding sites of enzymes and proteins e.g. EDTA is the most familiar example of chelating agents used in chelation therapy.

(i) EDTA (Ethylenediamine tetraacetic acid)

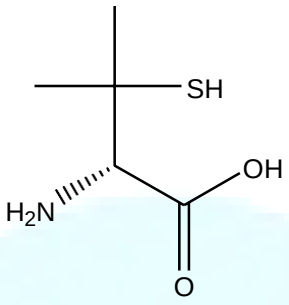
EDTA was synthesized in Germany in 1930 by Munz as a substitute for the expensive imported chemical citric acid, used as a calcium-sequestering agent in the textile industry. EDTA was patented for this use in 1935. Due to the greater demand of chelating agents for the removal of toxic elements and increasing risk of nuclear fission products entering the human body, Pfeiffer, Schwarzenbach and others introduced EDTA to medical research in 1945. The first administration to human beings was in the form of Ni-EDTA complex for the treatment of breast cancer, but it remained unchanged and excreted in the urine. Later in 1952 it was effectively used against lead poisoning. Sodium salt of EDTA depletes blood calcium levels and produces hypocalcaemic tetany. This danger is minimal if calcium disodium EDTA is used as the chelating agent and it has now become the material of choice in cases of lead poisoning. Calcium-EDTA has the lowest stability constant in comparison to other metal ions in the body. Hence these metals readily exchange in vivo to form soluble EDTA complexes that are excreted in the urine. Except in massive doses it is almost nontoxic and treatment with $[CaNa_2B\ EDTA]_B$ results in a rapid depletion of lead. Calcium EDTA used for the treatment of lead poisoning, acute iron poisoning and for the removal of radioactive strontium. Other than chelation therapy, in which it is generally administered by intravenous infusion, EDTA has also been used in creams and ointments, and in hair dyes. A novel application of an EDTA chelate is the use of the dicobalt chelate as an antidote in cyanide poisoning; the CN^{P-} ion forms a strong complex with the cobalt in the chelate to form a relatively non-^{P} toxic and readily excretable species.

(ii) British anti-Lewisite (BAL) or 2, 3-dimercapto-1-propanol : [CHB_2SH-$CHSH$-CH_B $B_2OH]_B$

It was used by British army during world war to treat patients poisoned by the gas Lewisite $ClCH{=}CHAsCl_2B$. It binds to the enzymes containing SH group. But BAL binds $_B$strongly to arsenic and removes it. It is also used for the treatment of poisoning caused by Hg, As, Gold etc.

(iii) D-Penicillamine :

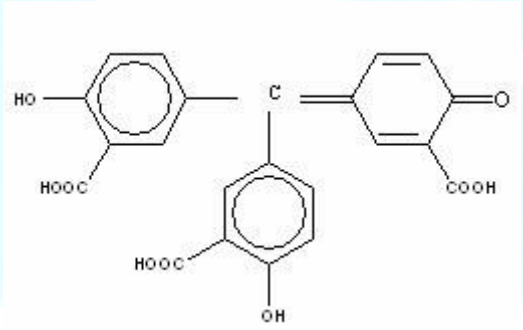
It is used for urinary excretion of copper in Wilson's disease.



D-penicillamine

(iv) Aurine tricarboxylic acid :

Effective in the treatment of Beryllium poisoning.

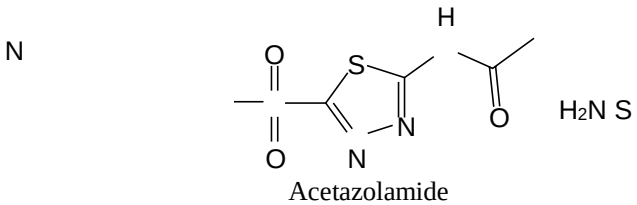


Drugs appear to involve interaction with metal ions:

Many drugs are suspected of acting via chelation e.g.

(v) Diuretics:

These are the drugs that promote the formation of urine e.g. Diacarb or acetazolamide.

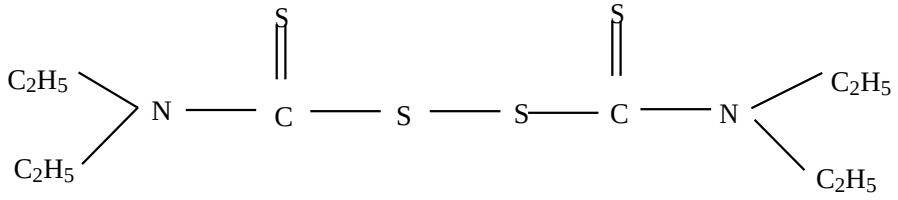


Acetazolamide

Coordinates with zinc of zinc containing enzyme carbonic anhydrase and stops the enzymatic activity of catalyzing the reaction. $CO_2 + H_2O \leftrightarrow H^+ + HCO_3^-$ in which water combines with CO_2 to form bicarbonate ion. When this reaction stopped, there will not be conversion of carbondioxide and water to bicarbonate ion, resulting in the formation of more urine.

(vi) Disulfiram:

Tetraethyl thiuram disulphide



Used in the treatment of chronic alcoholism. It inhibits Molybdenum containing metalloenzyme aldehydeoxidase. So the metabolism of ethanol stops with the formation of acetaldehyde producing unpleasant symptoms and discouraging further indulgence. Drug inhibits aldehydeoxidase presumably via the soft-soft Mo-S interaction.

(vii) Tetracycline and its analogues:

It has been shown that there is a correlation between the possession of antibacterial properties and the ability to form stable chelates. Tetracycline and its analogues have a number of sites to form metal chelates of fairly high stability.

Certain metal ions stabilize the DNA double helix. Unwinding of helix normally arises from the repulsion between negatively charged phosphate groups. Binding of cations to the phosphate neutralizes the charge and stabilizes the double helix. Mg^{2+} most effective in stabilizing the structure. It is now generally accepted that the action of the tetracyclines is directed towards the ribosomes of the bacterial cells and hence results in inhibition of protein synthesis. It seems that the ultimate target of tetracydines is the Mg^{2+} which is necessary for the stabilization and function of the ribosomes.

(viii) Platinum:

Chemotherapy is one of the main weapons in the fight against cancer. A crucial event happened in 1962 when B.Rosenberg, a physicist, who was investigating the effect of electric field (using platinum electrodes) on the division of cultured bacterial cell found that the field generated between platinum electrodes seemed to prevent the division of bacterial cell without simultaneous inhibition of bacterial growth which eventually led to the formation of long, filamentous cells. Further experiments showed that it was not the electric field but cis-diamminedichloroplatinum (II) $\{cis\ [Pt\ (NH_3)_2\ Cl_2]\}$, later known as cisplatin that were responsible for this effect. These species were formed by tiny amounts of platinum from the 'inert' electrodes reacting with the chloride and ammonia that were present in the electrolytic medium. In subsequent studies the antitumour activity of cisplatin has been studied in tumors induced in animals and the promising results led to

the first clinical trials in 1972. In 1978 it was officially approved as an anticancer drug in US. Since 1983, cisplatin has been the drug with the highest turnover in the United States; annual revenues are in excess of US$ 100 million, and about 30000 patients per year have regularly been treated successfully. For a long time, this compound has topped the list of the most successful patent application granted to American Universities (Michigan State University). Although effective against the broad spectrum of tumors, the compound is almost universally sued in the treatment of testicular and ovarian cancer, as well as some other types of cancer. The cure rate is approaching 100 percent, especially for early-recognized testicular cancer. The most common side effects of a cisplatin therapy include kidney and gastrointestinal problems, including nausea, which may be attributed to the inhibition of enzymes through coordination of the heavy metal platinum to sulfhydryl groups in proteins. Accordingly, a treatment with sulfur compounds such as sodium diethyldithiocarbamate or thiourea and subsequent diuretics may counteract these symptoms.

Now it is well established fact that the mechanism of cisplatin is based on PtDNA interaction. The first clue came from the filamentous growth observed by Rosenberg. This growth is to be caused by inhibiting DNA replication while the RNA synthesis and protein synthesis are relatively unaffected. Studies in vitro as well as in vivo indicate that Pt binds with NB_7 of two intrastand adjacent guanine bases of DNA. This $_B$ binding changes the active conformation of DNA leading to inhibiting of DNA replication or cell division. The binding of cisplatin to DNA seriously interferes with the ability of guanine bases to undergo Watson-Crick base pairing.

cis-Pt $(NHB_3)_B B_2 Cl_B B_2 + H_B B_2O$ ---------- cis- $[Pt (NH_B B_3)_B B_{2B} (OH B_2)_B Cl]^+P^P + Cl^-P^P$

Cisplatin is administered by intravenous injection as an aqueous saline solution. Approximately half the platinum binds to serum proteins and is excreted. The rest is distributed among various tissues. In serum, the drug remains largely as cis-Pt $(NH_3B)_B{}_2B_B ClB_2$, owing to the relatively high chloride concentration (0.1M). As a neutral molecule, $_B$ cisplatin diffuses passively across cell membranes into the cytoplasm, where it encounters a substantially lower chloride ion concentration (3mM). Hydrolysis produces cationic complexes such as cis- $[Pt (NH_3B)_B B_2 (OH_B B_2)_B Cl]^+P^P$ that diffuses to DNA, itself a polyanion, they bind to form cytotoxic lesions. The hydrolysis reactions of cisplatin are an important aspect of its biological activity.

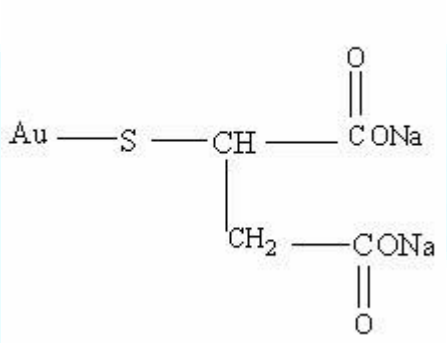
Since cis- as well as transplatin alter the double helical structure of DNA and its replication, the answer of the question that why only cis is active, is very interesting. The trans isomer is resorbed more rapidly than cis-platin; however the concentration of the DNA coordinated trans complex begins to decrease after six hours whereas the cis-isomer is then still accumulated in the cell nucleus. Only very little trans compound is still coordinated to DNA after 24 hours. These results indicate that the changes in the DNA structure caused by the trans isomer are sensed differently by the endogenous repair mechanism from those caused by coordination of cisplatin. Recently it has been found that as the cisplatin binds with DNA, a structure specific recognition protein (SSRP) recognizes the bending of DNA containing cis platin and binds with DNA. This protein binds specifically to DNA containing cisplatin and not with trans. Though the mechanism is not clear but this binding of SSRP may be the reason activity of cisplatin and inactivity of trans.

(ix) Lithium:

Lithium is used in the treatment of the manic phase of manic depressive patients. One in two thousand people in the U.K. receive such lithium treatment. Manic depressive psychoses involve alternating phases of depression and over excitement. Very little is known about the mechanism of lithium action. From a general point of view it is possible that lithium could be interfering with aspects of Na^{+}P P, K^{+}P P, Mg $^{2+}$P P or $Ca^{P_{2+P}}$ metabolism. The existenes of diagonal relationship suggests that competition between $Li^{P_{+}}$ Pand Mg^{2+}P P for $Mg^{P_{2+P}}$ sites must be considering as a priority.

(xi) Gold:

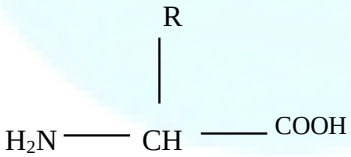
Gold compound were first used in 1927 for the treatment of rheumatoid arthritis and are still used. The major role of gold compounds in chemotherapy involves the treatment of rheumatoid arthritis with gold (I) compounds such as disodium gold (I) thiomalate (Myocrisin)



The actual role of Au (I) in controlling rheumatoid arthritis is uncertain.

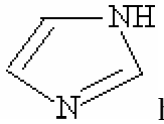
Some examples of biological complexing agents :

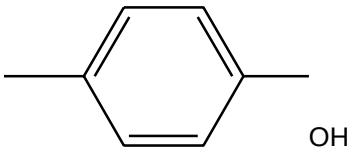
Amino Acids:



R= H Glycine, R= CHB3 Alanine, R= (CHBB3)BB2 CH- Valine, R= (CHB 3B)BB2 CH CHB 2B-B Leucine, R=

CHB3 S CHBB2 CHBB2-B methionine, R= HOCHB2-B Serine, R= -CHB2COOH-Aspartic acid, B

R= - CHB2CHBB2COOH Glutamic acid and. R = B  histidine R=



tyrosine

The binding of metals in metalloproteins is a difficult question to resolve with certainty. There are many alternative donor sites, the side chains, peptide and terminal – NHB_2 and – COOH groups. It is reasonable to suppose however that certain groups will $_B$ have particularly enhanced basic properties and tend to dominate the competition among the various potential binding sites for the metal. Two common amino acids have such outstanding binding properties. These are histidine, through its imidazole ring and cysteine through the thiol group.

The porphyrin and corrin ring systems are of great biological importance.

Four pyrrote units are linked by four –CH= bridges as shown but in Corrin ring one –CH=group is less. Porphyrins are derived from porphin, varying according to the nature of substituents. These rings are intensely colored and highly conjugated. Both the rings act as tetradentate ligands with four pyrrote like nitrogens surrounding a central site of metals. Great biological importance of these rings in the biosystem can be illustrated as:

i) Iron complex of the substituted porphin is Heme.

ii) When magnesium lies at the center of substituted porphin ring; the resulting complex is called chlorophyll.

iii) If Cobalt is the central metal atom of substituted corrin ring system, we have vitamin B_{12}.

STORAGE AND TRANSFER OF OXYGEN

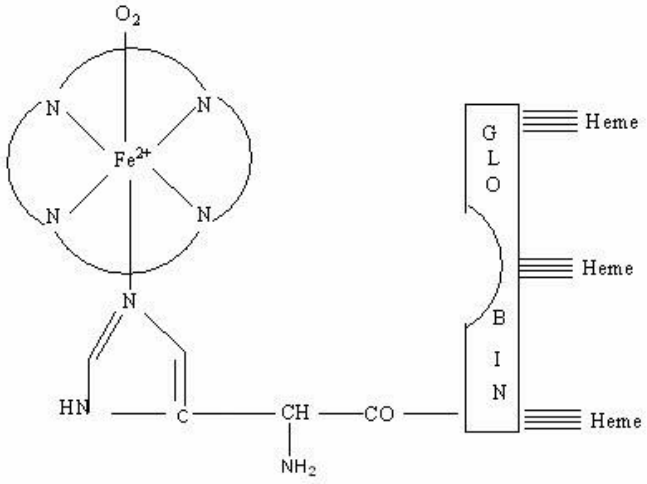
Transport and storage of molecular oxygen is an essential physiological function. It is carried out by a number of well known iron and copper containing species which occur in the blood. These are listed in Table.

S. No.	Examples	Metal	Mole ratio (OB_2/metal) $_B$	Function	Ligands
1.	Hemoglobin (Hb)	Fe (II)	1/1	Carrier	Porphyrin
2.	Myoglobin (Mb)	Fe (II)	1/1	Storage	Porphyrin
3.	Hemerythrin	Fe (II)	1/2	Storage	Protein
4.	Hemocyanin	Cu (I)	1/2	Carrier	Protein

Both heme and non-heme iron proteins are involved in oxygen transport and storage. Heme OB_2 carriers are responsible for red colour of human blood, absorb one $_B$ mole of oxygen per mole of iron (II), while the blue pigment of crab blood, hemocyanin, absorbs one mole of oxygen for every two moles of metal ions. Certain marine worms (e.g.Golfingia elongata) have a violet colour, which is due to the presence of non-heme iron protein hemerythrein. Besides these natural carriers, there are several synthetic oxygen carrying complexes, which provide model system for the natural carriers.
The OB_2 binding heme protein hemoglobin was:

- the first protein crystallized (1849)
- the first protein with a recognized physiological purpose (OB_2 transport, 1864; CO_B B_2 $_B$ transport, 1904)
- one of the first proteins whose molecular weight and primary sequences were established (ca 1930)
- one of the first proteins whose tertiary and quaternary structures were determined by X-ray crystallography (1960)

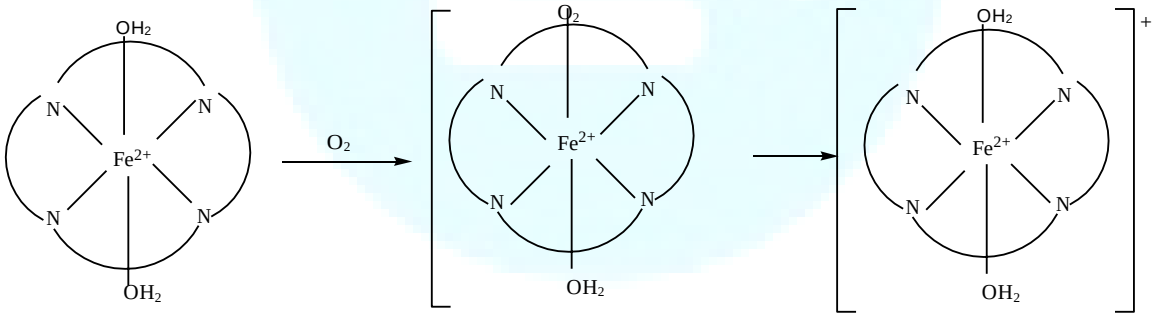
Hb has the molecular weight 64,500 daltons. Each Hb molecule has four heme groups bound to the globin on its surface. In each heme unit of Hb the four square-planar coordination positions of Fe (II) are being occupied by the four nitrogens of the protoporphyrin ring the fifth coordination position of Fe (II) is occupied by the imidazole nitrogen of histidine residue of the protein chain i.e. globin chain, and the sixth coordination position of Fe (II) is occupied by O_2 on oxygenation.



Hb consists of a tetrahedral arrangement of four heme groups each surrounded by its polypeptide (globin) chain. Normal adults have two α and two β type subunits and Hb can accordingly be represented as $\alpha B_2 \beta_B B_2$. The $_B$ α and β forms are distinguished by their different amino-acid sequences α-141, β-146.

Myoglobin (Mb) (MW 17, 800) is a monomer having only one molecule of heme. The peptide chains in Hb and Mb have extensive helical structure. There are 7 helical segments in the α chains and 8 in the β and myoglobin forms. These are linked by short non-helical segments.

In free heme molecule there are two coordination positions above and below the plane of the porphyrin molecule. These two positions are occupied by water molecules.



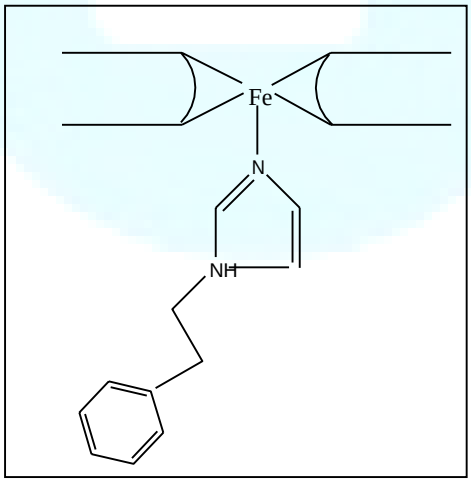
Heme Oxy-heme Hematin

The iron (II) ion in heme is very sensitive to oxygen and it undergoes combination with it readily to form a labile Fe (II)-O_2B complex (Oxy-heme) which changes into the Fe B (III) protopophyrin called hematin or hemin. Hence free heme is not favored for oxygen transport. Hence an important function of the globin in hemoglobin, apart from the action as a carrier of heme, has been found to stabilize the heme-OB_2 complex so that oxidation B of iron of heme does not take place and it can act as an effective carrier of oxygen. As a protein chain is folded around the heme group, the position of the protein chain reduces the access of water to Fe^{2+}P P and at the same time provides a hydrophobic surrounding. Thus the steric and chemical control permits the access of oxygen molecule but does not permit the simultaneous presence of oxygen and one or more molecules of water which appears to be necessary for oxidation of Fe^{2+}P P to $Fe^{P_{3+}}$

$$\text{Heme } (Fe^{P_{IIP}}) \xrightarrow[\text{Water}]{OB_{2\ B}} \text{Hematin } (Fe^{P_{IIIP}})$$

The iron in Mb and Hb in +2 oxidation state. The oxidized forms containing iron (III) called met Mb and met Hb, will not bind oxygen.

The stabilization of heme by the presence of hydrophobic surfaces has been well illustrated by embedding heme in a matrix of (bed of) polystyrene containing [1-(2phenylethyl)-imidazole]. The imidazole molecule approximated the function of a histidine group in Mb and Hb. This “synthetic hemoglobin” was found to bind oxygen reversibly in presence of water.



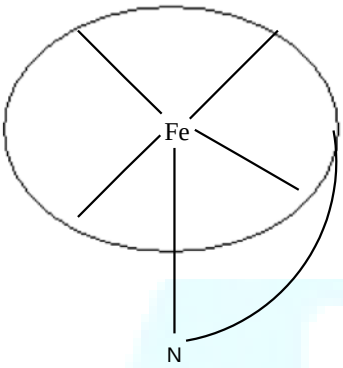
More recent work indicates that the protein chain also keep the heme group sufficiently apart so that formation of bridged Oxo dimers which degenerate to Fe^{III}P P – O - $Fe^{P_{III}}$ is prevented. P

The globin (protein) part is thought to be serving three principal functions in heme oxygenation :

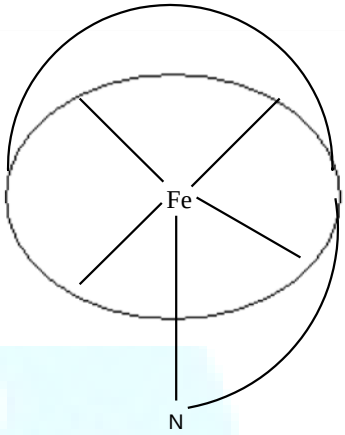
1. It furnishes a ligand to the iron i.e. the imidazole of the proximal histidine.

2. It provides the heme with " a medium of low dielectric strength", hydrocarbon environment of heme pocket has a low dielectric constant and so acts as a non polar "Solvent" that can not support extensive charge transfer from iron and reduction of oxygen to superoxide.
3. By restricting the motion of heme it prevents the formation of μ-OXO dimeric oxidation product.

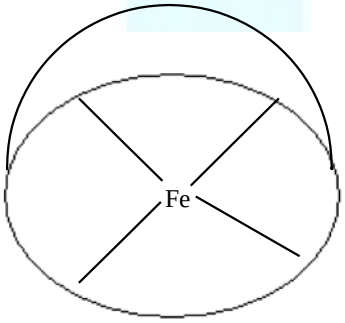
Attempts were made to mimic the natural O_2B – carrying process without a $_B$ polypeptide. The challenge to synthesis modified porphyrins in which steric hindrance has been created has produced much elegant work.



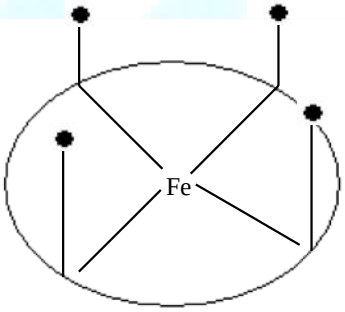
(i) Tailbase



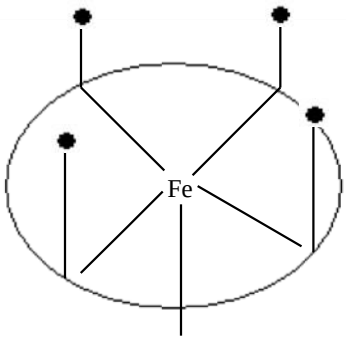
(ii) Strapped/tailbase



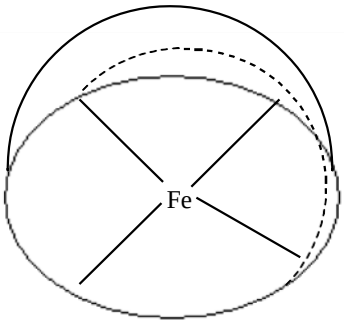
(iii) Strapped



(iv) Picket fence



(v) Picket fence/tailbase

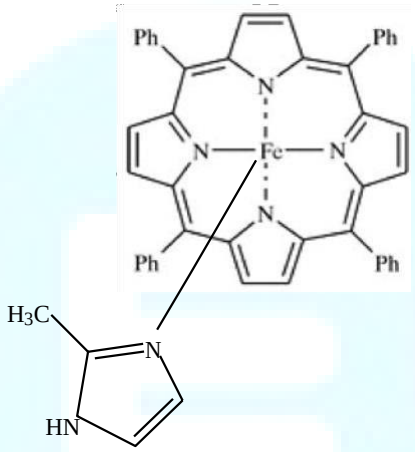


(vi) Capped

Initial attempt (1973) with Fe (II) – porphyrins having an imidazole covalently attached (tail base complexes) as in (i) gave 1:1 O_2B adducts only at low temperatures (-B 45°P °C). The strapped complex (II) and (III) with a hydrocarbon chain linked over the face of the porphyrin do not give reversible OB_2 addition, almost certainly because of lack of B rigidity of the chain, which can be pushed out of the way. The " picket fence" structure (IV) and (V) and 'capped' porphyrin (VI), provides satisfactory steric protection and stabilizes reversible O_2B addition at room temperature. A wide range of studies on the " B picket fence" complexes have been carried out by Collman and colleagues.

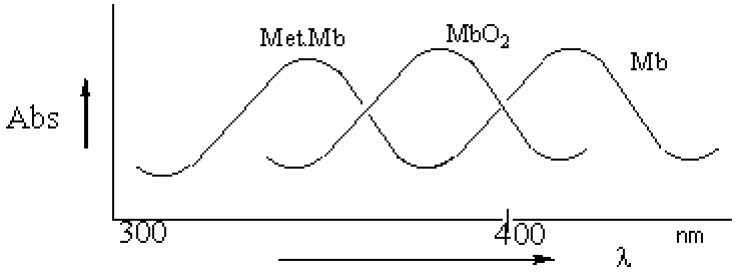
Importance of steric hindrance about the heme demonstrated from the study of " picket fence" compounds in which bulky rings or t-butyl groups protect the binding site allowingreversible OB_2 addition without the presence of the globin chain e.g. B

Fe (TPP) bound to an imidazole and attached to a silica gel support (to avoid dimer formation) exhibits reversible OB_2 up take. B



[Fe (TPP) (2-Me Im)]

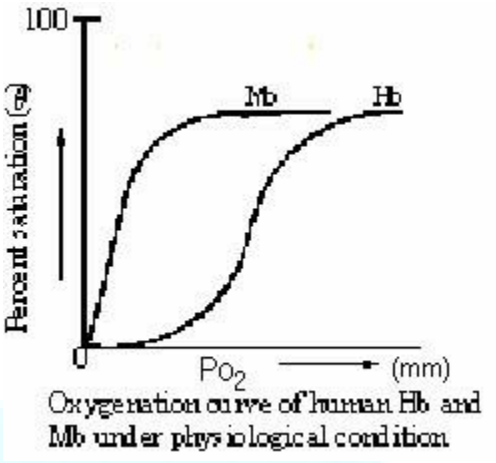
Oxygen binding to Hb and MbU



UV-visible spectra which are providing a ready means of identifying OB_2-B binding give no information as to the manner of the binding. By performing X-ray crystallographic analysis of one of the " picket fence" compounds (e.g. Fe (TPP)) by Collman et al., it has been found that the OB_2 molecule coordinates in the bent B arrangement.. Later on the structure of HbO_2B and $MbO_B B_2$ have been studied (but not so B accurately as picket fence) and it was found that Fe-O-O bond is bent.

OB_2 . Mb = $112_B{}^o$ P P; OB_2. Hb = $156_B{}^o$P P ; " picket fence" = 131^o P

Oxygenation behavior of Hb and Mb



Oxygenation curve of human Hb and Mb under physiological condition

Typical curves of oxygenation vs. oxygen pressure ($P_{O2}B$)$_B$ under physiological condition for Hb and Mb. Curves for oxygenation of Mb is hyperbolic while that of Hb is sigmoidal in nature. It is the cooperativity of the four-heme groups that produces two types of the curves shown in figure. Figure shows that Hb is about as good an OB_2 binder $_B$as Mb at high OB_2 pressure. It is much poorer at the lower pressures prevailing in muscle $_B$ and hence passes its oxygen on to Mb as required. Moreover, the need for O_2B will be $_B$greatest in tissues where O_2B is consumed followed by production of CO_B B_2. The CO_B B_2 lowers $_B$ the pH, thus causing the Hb to release even more oxygen to Mb. The pH-sensitivity (called the Bohr effect) as well as the progressive increase of OB_2 binding constants in Hb $_B$ are due to the interaction between the subunits; Mb behaves more simply because it consists of only one unit. It is clear that each of the two is essential in the complete oxygen transport process.

The oxygenation curve of Mb reflects a simple equilibrium

$$Mb + O_2 \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} MbO_2$$

$$KB_I = \frac{[MbO_2]}{[Mb]\,[OB_2]_B} \quad \text{----------(1)}\ _B$$

$$\text{Saturation } [\theta] = \frac{[MbOB_2]_B}{[Mb]_{total}B_B} \quad \text{----------} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(2)$$

$[Mb]_{total B} = [Mb] + [MbO_B B_2]_B$

$[Mb]_{B_{total}} = [Mb] + K_B B_I [Mb] [O_B B_{2B}]$ from equation (1) substituting the value of $[MbOB_2]_B$

$[Mb]_{B_{total}} = [Mb] [1+ K_{BI} B [O_B B_2]]_B$.

Substituting the value of $[Mb]_{B_{total}}$ in equation (2) $_B$

$$[\theta] = \frac{[Mb]\, KB_I\, [O]_B\, B_{2\,B}}{[Mb]\, [1+ KB_I\, [O]_B\, B_{2B}]}$$

value of $[MbOB_2]_B$ substituted from equation (1)

$$\theta = \frac{KB_I\, [O_B B_2]_B}{1+ KB_I\, [O_B B_2]_B} \quad \ldots\ldots\ldots\ldots (3)$$

$$1- \theta = \frac{1}{1+ KB_I\, [O_B B_2]_B} \quad \ldots\ldots\ldots\ldots (4)$$

Dividing equation (3) by equation (4)

$$\frac{\theta}{1- \theta} = KB_I\, [O_B B_2]_B$$

This equation is for Mb which is containing only one Heme unit.

Oxygenation curve of Hb may be approximated as

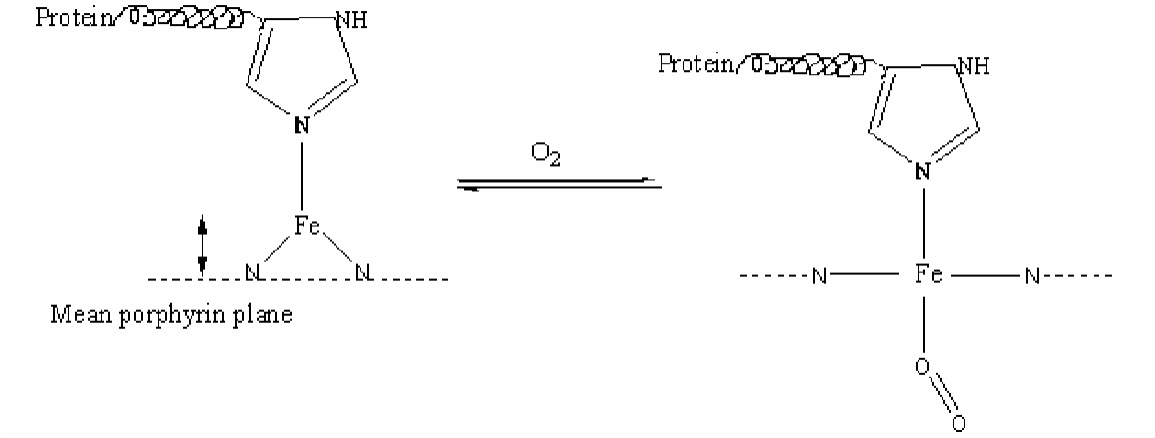
$$\frac{\theta}{1- \theta} = KB_I\, [O_B B_2]_B {}^{n} P^{P} \text{ with } n \sim 3$$

This equation is known as Hill equation and the exponent, n is called the Hill constant. In contrast to the hyperbolic curve obtained from the data for the monomeric heme (myoglobin), the data obtained by Hb shows “Sigmoidal” behavior, indicative of interaction between the subunits. The data obtained from Hb between 10 and 90% oxygenation can be fitted to the Hill equation to give values of n ~ 3 for normal Hb.

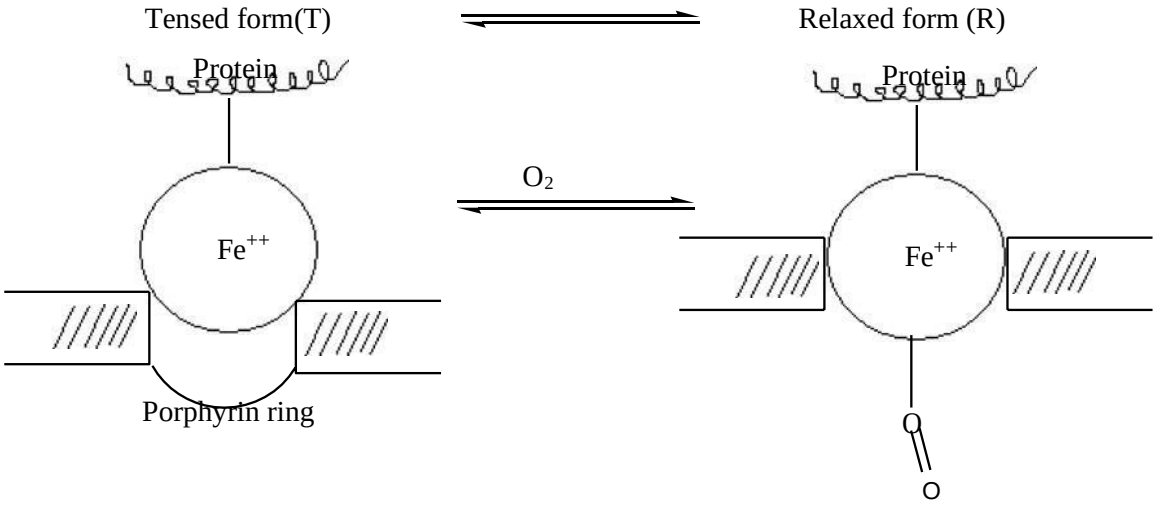
The form of oxygenation curve and that the fact if fits n>1 indicate that there is cooperative interaction between the subunits. The addition of oxygen to a subunit affects the oxygen affinities of other subunits, this is an example of Allosteric effect (Entatic effect) literally means “a stretched state or state of being under tension”. n<4 indicates that the cooperative interaction between heme units is rather moderate.

Mechanism of oxygenation in Hb and MbU

Hb may be viewed as tetrameric Mb. It has four heme groups bound to four protein chains. The differences between Hb and Mb in their behavior towards oxygen is related to the structure and movements of the four chains. It is found that, upon oxygenation of Hb, two of the heme groups move about 1A°P P towards each other while two other separate by about 7A°P P. These movements seem responsible for the cooperative effect observed. Detailed study of the mechanism has been accomplished by Perutz. According to Perutz, changes in the coordination of the iron play a crucial role. Deoxy Hb contains iron (II) in a high spin state, with two electrons occupying eg. orbitals, the bonding radius of the iron is so large (0.78A°P P) that it can not fit into the plane of four N atoms of the heme porphyrin. It therefore lies around 0.75A P oP out of the plane. The iron is thus pentacoordinate with square pyramidal coordination provided by four porphyrin nitrogen atoms in the basal position and an imidazole nitrogen atom from histidine in the apical position.



When an oxygen molecule is bound in the position opposite to this histidine, the iron atom goes in to a low spin state, eg. orbitals are then empty and the radius of the iron decreases (by 0.17 A P oP) so much that it now fits in to the plane of the porphyrin system. Thus the iron atom moves down when deoxy Hb becomes oxygenated. Since it remains attached to the side chain of histidine this shift is transmitted to various parts of the subunits, causing particularly important movements of the entire helical section.



Two forms tensed (T) and Relaxed (R) 0f Hb are present as an equilibrium mixture. With no OB_2 present, the T state is more stable than the R state and the Hb is thus found to be almost exclusively in the T form. The OB_2 affinity of Hb in the T state is much less than the OB_2 affinity of Hb in the R state. Thus the initial OB_2 affinity of Hb is significantly lower than that observed for the individual subunits. Addition of O_2B to deoxy Hb changes the equilibrium between T and R state. As the Hb picks up oxygen, the equilibrium shifts towards the R state. Thus the more OB_2 molecules bound to Hb, the higher the probability that Hb will be in the R state. As the oxygen affinity of the R state is approximately same as that of an isolated subunit, the OB_2 affinity of almost completely oxygenated Hb should be approximately equal to that of the isolated chain. It is this structural change, which occurs in the binding of the OB_2 to the heme that results in the decrease in the stability of T conformation relative to the R form and causes the observed cooperativity.

Synthetic oxygen carriers

Although the most common mode of reaction of molecular oxygen with transition metal complexes is oxidation i.e. extraction of electrons from the metal, it has been recognized in recent years that in appropriate circumstances the O_2B molecule, which we shall call dioxygen, can function as neutral ligand. The reaction of dioxygen with a complex so as to incorporate the dioxygen ligand without undergoing any reduction on oxygen is called oxygenation. This is contrast to oxidation reaction in which OB_2 looses its identity during the reaction. Some transition metal complexes act as reversible carriers of molecular OB_2 i.e. they take up and release OB_2 reversibly as follows:

$$\text{Carrier} + OB_2 \rightleftharpoons \text{carrier } (OB_2)$$

Molecular OB_2 can be reduced by one electron process without O-O bond being broken

$$O_2B \underset{-e}{\overset{+e}{\rightleftharpoons}} OB_2^{-} \underset{-e}{\overset{+e}{\rightleftharpoons}} OB_2^{2-}$$

and each of these species can act as a ligand towards transition metals.

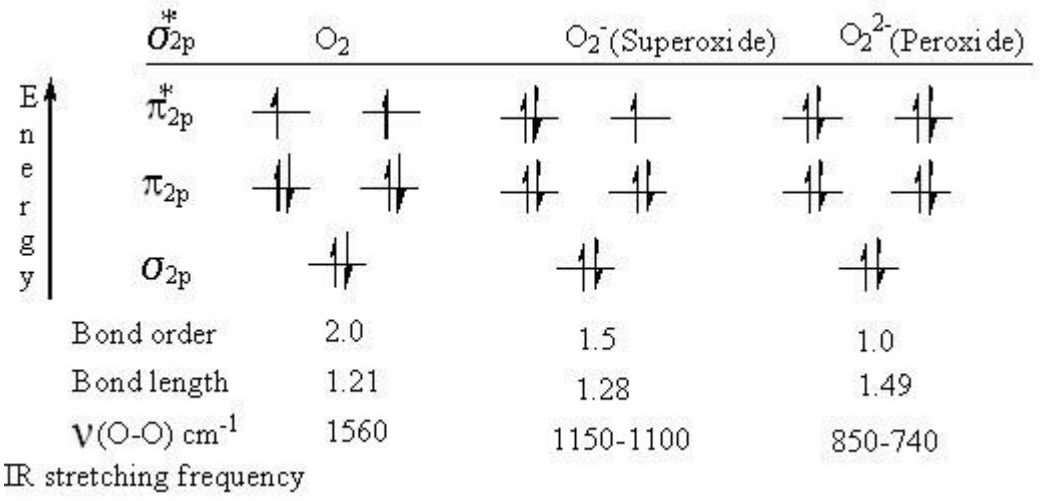
To understand all about synthetic oxygen carriers one should consider the following:

i) Electronic structure of OB_2 molecule

ii) Mode of linkage and possible structures of some model systems containing Co and Ir.

Electronic structure of O_2B molecule B

$$O_2 = \sigma 1s^2\ \sigma^* 1s^2\ \sigma 2s^2\ \sigma^* 2s^2\ \sigma 2p_x^2\ \pi 2p_z^2\ \pi 2p_y^2\ \pi^* 2p_y^1\ \pi^* 2p_z^1$$



All of these are useful guides to the assessment of extent of electron transfer from metal to dioxygen.

Cobalt Complexes

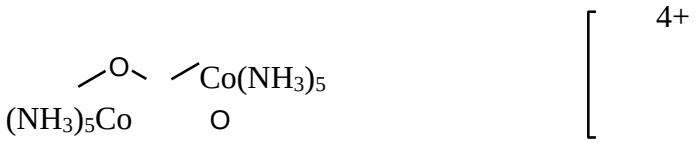
The reversible absorption of atmospheric oxygen by inorganic solids (ammoniacobalt salts) was first noted by Fremy in 1852. Fremy reported that the exposure of ammonical solutions of Co (II) salts to the atmosphere resulted in the formation of brown salts which he called oxo cobaltiates. They were later characterized by Werner in 1898 as

$[(NHB_3)_B B_5\ Co\ (O_B B_2)_B\ Co\ (NHB_3)_B B_5]_B^{4+} P^P$

$$2\ Co\ (NHB_3)_B B_6^{2+}{}_{BP}{}^P + OB_2 \underset{B}{\rightleftharpoons} [(NHB_{3B})B_{5B}\ Co\ –O\text{-}O\text{-}\ Co\ (NH\ \ B_3)_B B_5]_B^{4+} P +$$

$2\ NH^P B_{3\ B}$

(Yellow) (Brown)



O-O 1.47 A$^{P_{oP}}$

μ-peroxo-bis (pentaammine Cobalt) (4+) known to be as a μ-peroxo Co (III) complex

$$2Co^{2+}P\ P\ +\ OB_2\ B \rightleftharpoons [Co^{3+}P\ P\ (OB_{22\text{-}PB\ P})\ Co^{3+}P\ P]$$

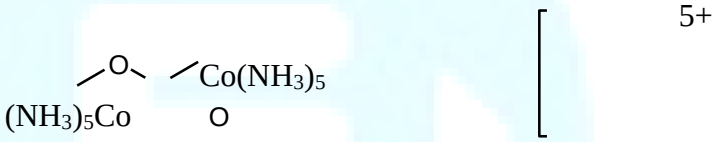
This complex reacts with oxidizing agents such as cerric ion to yield μ-superoxo cobalt (III) complex

Ce (IV)

$$[\ (NHB_3)_B B_5\ Co\ –O\text{-}O\text{-}\ Co\ (NH_{B3}B\ )_B B_5]_B{}^{4+}P\ P \longrightarrow [\ (NHB_{3B})B_5\ Co\ –O\text{-}O\text{-}\ Co\ (NH_B$$

$$B_3)_{B\ 5}B\ ]_B\ ^{P_{5+P}}$$

(Brown) (green)

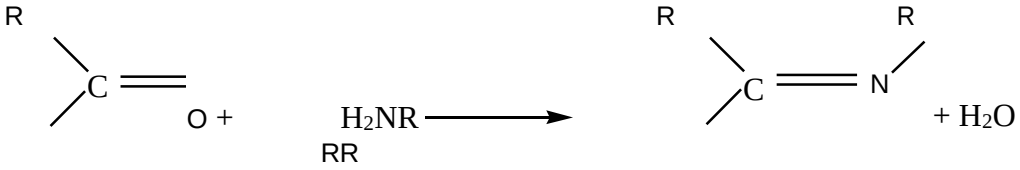


O-O 1.31 A^{o}P ^{P}

μ-superoxo-bis (pentaammine Cobalt) (5+)

Cobalt complexes with schiff base lignadsU

Schiff base are formed by shciff base condensation reactic



Schiff base used in these studies are generally tetradentate or pentadenate ligands and at least two of the ligating atoms are nitrogen atoms. Schiff base compounds are commonly referred by their abbreviations. The abbreviations are a combination of the ketone and amine precursors e.g. bis (acetyactone) ethylene diamine becomes acacen and bis(salicylaldehyde) ethylene diamine becomes salen. These are very effective oxygen

carriers. For a short period during world war II US Navy used these in the production of pure oxygen aboard a destroyer tender for use in welding and cutting.

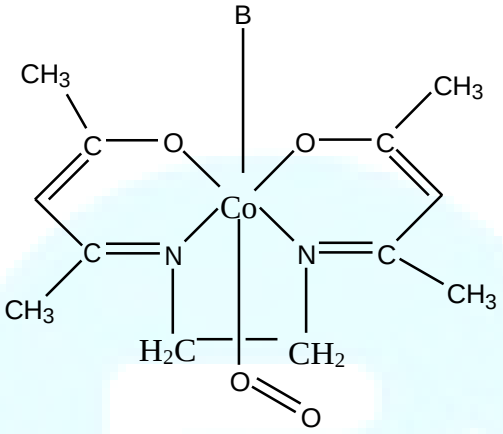
Acacen: Solid Co (acacen)B(O_2B)$_B$ is isolated where B is a base coordinated with cobalt. Crystal structure determination studies show:

Co-O-O angle 117^{o}P ^{P}

O-O bond length is 1.27 – 130 A$^{P\ oP}$ ν(O-O) IR

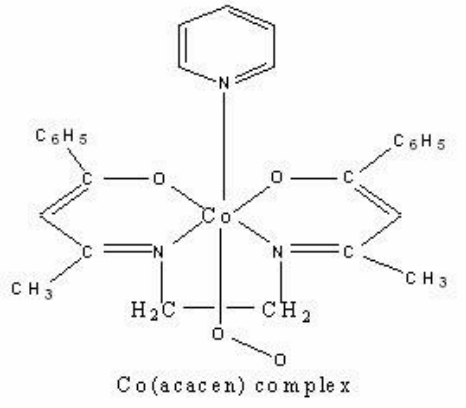
stretching frequency is 1120-40 cm^{-1}P ^{P}.

This was shown to involve Co (III) –O$B_2^-{}_{PB}$ ^{P} with the following structure.



Co(acacen) complex

The initial complex has one unpaired electron and so also do the oxygen adduct. But esr data indicate that in the latter the electron is heavily localized in oxygen atom. The adduct can be formulated as octahedral low spin Co (III) complexes containing a coordinated superoxide ($O_2^{B\ -P}{}_{PB}$) ion. The Co-O-O chain is bent.



Co(acacen) complex

Bis (benzoylacetone) ethylenediamine complex.

By crystal structure studies of Co (bzacen) (pyridine) OB_2. $_B$

Co-O-O angle is found to be 126$^{P\ oP}$ and O-O distance 1.26 A^{o}P ^{P}.

ν(O-O) stretching frequency is 1128 cm$^{P\ -1P}$

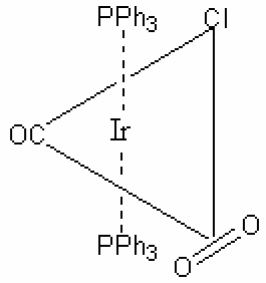
esr spectra is consistent with the presence of Co (III)- (O$B_2^-{}_{PB}$ ^{P}) as in the previous case.

After careful study of synthetic oxygen carriers, we can explain the binding of oxygen with Hb and Mb.

Let us take a picket fence complex. It binds OB_2, The Fe-O-O angle is 136B °P P and OO bond length is 1.25 A°P P. This model system provides the best guidance available about the structure of Mb and Hb oxygen complexes. That the complex is diamagnetic (lowspin), might be regarded as evidence of low-spin d^6P P Fe (II) complex of singlet OB_2 B which would mean that Fe and OB_2 reduction is less important than in Cobalt complexes. B However, we must be cautious with this interpretation because other evidence closer to the OB_2-PB values of 1145 cm^{-1}P P than the OB_2 value of 1560 cm-1P P. The low spin character could arise as the result of spin pairing of Fe (III) and OB-BP in which case the complex would be similar to the cobalt model compounds. That such a possibility is very real is underlined by the observation that a d^{P3P} Cr (III) porphyrin complex of oxygen has been synthesized and found to be having only two unpaired electrons. Since d^3P P Cr (III) must be high spin, the only explanation for the spin observed is spin pairing with an electron from OB_2-PB.

Vaska's Iridium complex

Complex chlorocarbonyl bis (triphenylphosphine)iridium. $Ir(PPHB_3)_B$ B_2COCl B behaves as an oxygen carrier. Oxygenated form is diamagnetic and has a trigonal bipyramidal structure containing Π- bonded oxygen.



From X-ray crystallographic studies it was found that it is 1:1 adduct of dioxygen in which O-O bond remains intact but is longer than in free OB_2 and two M-O distances B are equal. Several theories have been proposed to explain the diamagnetic nature of O_2B B adducts. Valence forms involving superoxide or cyclic peroxide complexes e.g.

Ir(I) + O_2 → Ir(II) O_2^-

Ir(I) + O_2 → Ir(III) O_2^{2-}

For the Ir (II) O_2B -PBP case it would be necessary to postulate that the spins on the d^7P P metal and superoxide cancelled each other in order to explain diamagnetic behavior.
Probably the most generally accepted view of bonding in these oxygen adducts is similar to that originally proposed by Dewar, Chatt and Duncanson for Pt (II)-ethylene complexes.

OB_2 acts as $_B$ Π-acids and electron rich metals in their low oxidation states are lewis bases. The bonding thus involves weak σ donation from a ligand Π-MO to an empty metal d-orbital and a stronger more significant "backbond" from a metal d-orbital to an empty Π MO on the ligand.

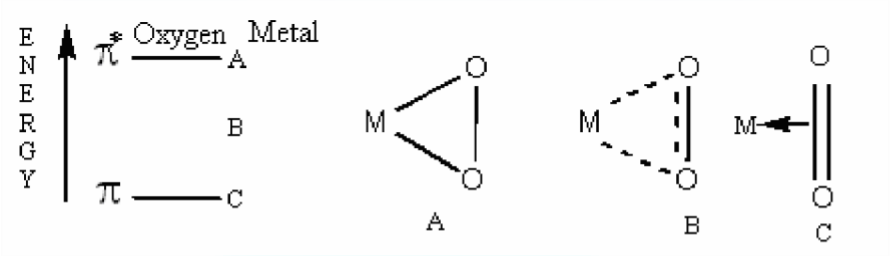
The relative bonding between metal and oxygen depends upon the relative energies of the metal orbitals and the dioxygen Π and $\Pi^{*}P^{P}$ orbitals. Three different situations can be imagined in the bonding of dioxygen to the metal.

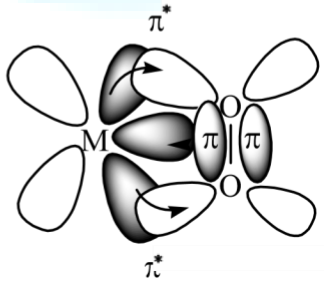
Case A: when the metal orbitals lie as high or higher than the $\Pi^{*}P^{P}$ orbitals of dioxygen. In this case the bonding orbitals formed from metal orbitals and dioxygen $\Pi^{*}P^{P}$ orbitals will be mostly dioxygen in character. Therefore, there will be little dioxygen to metal bonding. The Π bonding orbitals formed conceptually from electron transfer from the metal orbitals to dioxygen Π^{P*P} orbitals. If enough electron density is transferred to dioxygen $\Pi^{*}P^{P}$ orbitals, the O-O bond will become a single bond and hence longer.

Case C : when the metal orbitals lies as high as the Π orbitals of dioxygen.

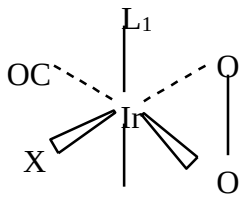
This case should produce largely σ bonding from dioxygen to metal.

Case B: This case is the intermediate one in which the metal orbitals lie in between Π and $\Pi^{*}P^{P}$ orbitals of dioxygen.





In this case both, σ bonding from dioxygen to metal and backbonding from metal d orbitals to $\Pi^{*}P^{P}$ orbitals of dioxygen are involved.

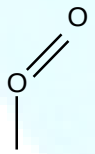


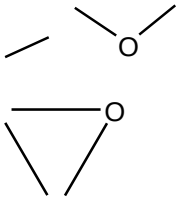
Structure:

LB1 B	X	O-O bond length (AoP P)
PPhB3 B	Cl	1.30
PPhB3 B	Br	1.36
PPhB3 B	I	1.51
PPhB2Et B	Cl	1.46

In this compound the O-O bond lengthens from 1.30 AoP P for X = Cl to 1.51 AoP P for X=I. The less electronegative more polarizable iodide ligand appears to have raised the metal valence orbitals to the level in case A, while the choride complex sams to be an example of B or C. Likewise for the complex with X = Cl, when triphenyl phosphine is substituted by ethyl diphenyl phosphine, a more weakly back bonding the O-O bond length increases from 1.30 to 1.46 AoP P reflecting a change in the metal valence orbital energies again from Case A to Case B or C.

General classification according to the bondingU

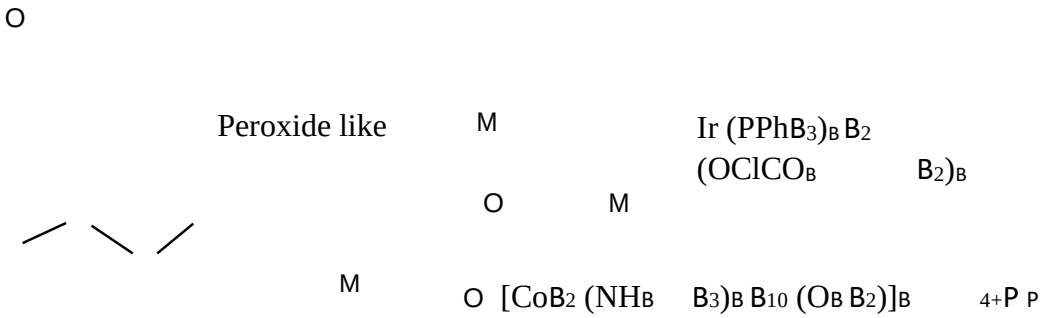




Superoxide like M Co (bzacen) (py) (OB2)B , Co (Acacen) (OB2)B

O M

M [CoB2 (NHBB3)B B10 (OB B2)]B 5+P



REDOX REACTION

Iron-Sulfur proteins

General name of Iron-sulfur proteins is Ferridoxins. These are non-heme ironsulfur proteins that are involved in electron transfer. They are widely dispersed in nature e.g. they are found in bacteria, algae, fungi, higher plants and mammals. They contain distinct iron-sulfur clusters composed of iron atoms, sulfhydryl group from cysteine residues and "inorganic" or "labile" sulfur atoms or sulfide ions. The latter are readily removed by washing with acid.

$$(RS)B_4\ Fe_B\ B_4S_B\ B_4 + 8H_B{}^{+}P^{P} \longrightarrow (RS)B_4\ Fe_B\ B_4{}^{+8}{}_{P\ B}{}^{P} + 4H_2B\ S_B$$

The cysteine moieties are incorporated within the protein chain and are not labile.

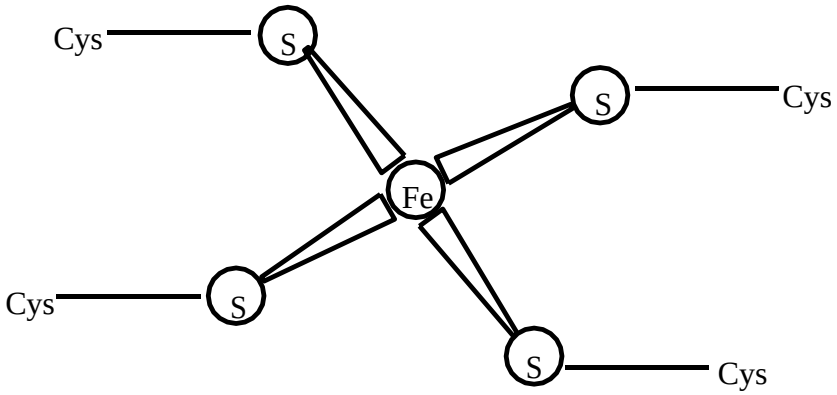
Classification:

S.No.	Fe-S U	Conventional name	No. of electrons involved in redox reaction
1.	1-0	Rubredoxin	1
2.	2-2	2-Iron ferredoxin	1 3. 3-4
		3-Iron ferredoxin	1
4.	4-4	4-Iron ferredoxin	1

Rubredoxin:

(Source: Clostridium pasteurianum)

Fe-S clusters are of several types. The simplest is bacterial rubredoxin $(Cys\text{-}S)_4B_B$ Fe (often abbreviated $FeB_1S_B B_0$) and contains only non-labile sulfur. It is a bacterial protein $_B$ of uncertain function. The single iron atom is at the center of an approximated tetrahedron of four cysteine ligands.



The rubredoxin is a one-electron transfer agent, with both Fe^{2+} and Fe^{3+} having high spin configurations.

$$[Fe(RS)_4]^{2-} \rightleftharpoons [Fe(RS)_4]^{-}$$

Fe (II) Fe (III)

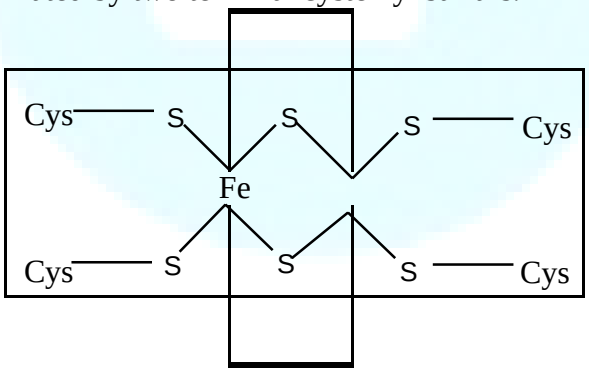
2-Iron ferridoxin

Chloroplast Ferridoxin

(Source: plants e.g. spinach)

The cluster in this ferridoxin molecule associated with photosynthesis in higher plants. It is having bridged structure.

These proteins are also one-electron transfer agents. The oxidized protein bears two high spin Fe^{3+} ions in the form of a S^{2-} bridged dimmer (the bridging sulfurs are labile); each iron is also coordinated by two terminal cysteinyl sulfurs.



$$[Fe_2S_2(RS)_4]^{4-} \rightleftharpoons [Fe_2S_2(RS)_4]^{3-} \rightleftharpoons [Fe_2S_2(RS)_4]^{2-}$$

2[Fe (II)] Fe (II) + Fe (III) 2[Fe (III)]

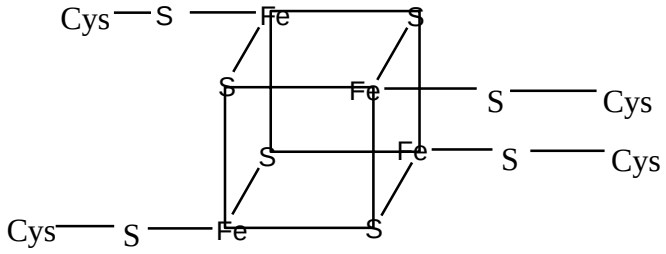
Fd $B_{super-red}$ Fd B_{red} Fd B_{ox}

The oxidized proteins bearing two high spin Fe^{3+} ions, are diamagnetic at very low temperatures, while the reduced form is paramagnetic corresponding to 1 electron. On reduction, one electron is accepted, formally giving one Fe (III) group (S=5/2) and one Fe (II) group (S=2), which interact giving a value for the dimmer of S=1/2. In other words, one electron has been added to the diamagnetic system.

4-Iron ferridoxin

HIPIP (Source: Chromatium purple sulfur bacteria)

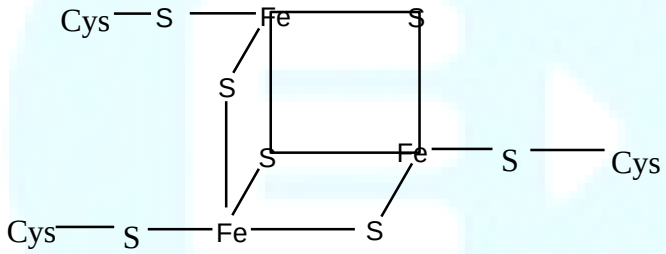
It contains one $FeB_4S_B B_4$ $(Cys)_B B_4$ unit on which iron and sulfur alternatively occupying $_B$ the edges of an approx.cube. The four Fe atoms are bonded with sulfur atoms of 4 Cys units. It is a bacterial iron sulfur protein.



3-Iron ferridoxin

(Source: Bacteria D.gigas)

The [3Fe-4S] ferridoxins have been isolated and studied from a number of species. The most intensively studied is D.gigas ferredoxin II, for which the structure has been determined. Structure is same (approx. cubane) as in the case of 4-Iron ferredoxins, except one iron is missing.



In oxidized form all the three Fe atoms are as $Fe^{P_{3+P}}$ and in reduced state it is containing 2 $Fe^{3+}P^P$ and 1 $Fe^{2+}P^P$.

$$[(RS)B_3\ Fe_B\ B_3S_B\ _4B\ ]_{B\ 2-}P\ _P \rightleftharpoons [\ B\ (RS)_BB_3\ Fe_B\ B_3S_B\ _4B\ ]_{B\ 3-}P\ _P$$

Bio-inorganic chemistry of NB_2-$_B$ fixation

Molecular nitrogen or dinitrogen $(N_2B)_B$ is an inert diatomic molecule. This molecule owes its lack of reactivity to the large energy difference between its filled and vacant molecular orbitals. The filled molecular orbitals are low in energy (≤ -15.6 ev) and its vacant orbitals are high (≥ -7 ev) in energy. As a result it is very difficult to add electrons to dinitrogen molecule or to remove them from it in the ground state. The nitrogen present in the atmosphere cannot be used by the higher organisms. It has to be "fixed", that is, incorporated in to a chemical compound. Nitrogen, in other words, has to be converted into ammonia or amino acids, so as to be of use to plants and animals.

Dinitrogen may be fixed industrially by a number of processes, of which the Haber's process is most important, but it required high temperature, high pressure and a catalyst.

$$NB_2 + 3H_B \quad B_2 \quad _B \rightleftharpoons 2NHB_3$$ (only 20% conversion) $_B$

$Fe/AlB_2O_B B_3/500\ _B{}^oP\ {}^PC/300bar$

At present what man can do with great difficulty, nature does apparently quite readily under mild aqueous conditions and at ambient conditions.

Biological nitrogen fixation is the process whereby some bacteria and blue green algae convert atmospheric nitrogen into ammonia.

Nitrogenase reaction:

$$NB_2 + 8H_B{}^+P\ {}^P + 8e^-P\ {}^P + 16\ Mg\text{-}ATP \longrightarrow 2NHB_3 + H_BB_2 + 16\ Mg\text{-}ADP + 16P_B$$
$$B_i \quad _B$$

In vitro nitrogen fixation

In 1965, Allen and Senoff obtained salts containing $[Ru\ (NHB_3)_B B_5\ N_B B_2]_B{}^{2+}P\ {}^P$ cation by the action of hydrazine hydrate on various compounds of tri and tetra positive ruthenium e.g. ruthenium trichloride.

$$RuClB_3\ 3H_BB_2O \xrightarrow{B} [Ru\ (NH_3B\ _B)\ B_5\ N_BB_2]_B\ ClB_{2\ B}$$

This discovery that molecular nitrogen was capable of forming stable complexes with transition metals led to extensive investigation of the possibility of fixation of nitrogen via such complexes. Of the various systems investigated, that employing titanium (II) was the first to be successful. Titanium (II) alkoxides form dinitrogen complexes which may then be reduces with subsequent release of ammonia or hydrazine.

$$Ti\ (ORB_4)_B + 2e^-P\ {}^P \quad Ti \longrightarrow (OR)B_2 + 2RO_BP^{-P}$$

$$Ti\ (OR)B_2 + N_B \quad B_2 \longrightarrow {}_B[Ti\ (OR)_2B\ N_B\ B_2\ ]_B$$

$$[Ti\ (OR)B_2\ N_BB_2\ ] + 4e_B{}^-P\ {}^P \quad [Ti\ (OR)B_2\ N_B{}_2B\ ]_B{}^{-4}P\ {}^P$$

$$[Ti\ (OR)_2\ N_2]^-_4 \xrightarrow{4H^+} N_2H_4 + Ti\ (OR)_2$$

$$2e\text{-} \qquad [Ti\ (OR)_2\ N_2]\text{-}6$$

$$[Ti\ (OR)B_2\ N_BB_2\ ]_B{}^{-6}P\ {}^P + 6HP^+\ {}^P \rightarrow 2NH_3B + Ti\ (OR)_B \qquad B_{2\ B}$$

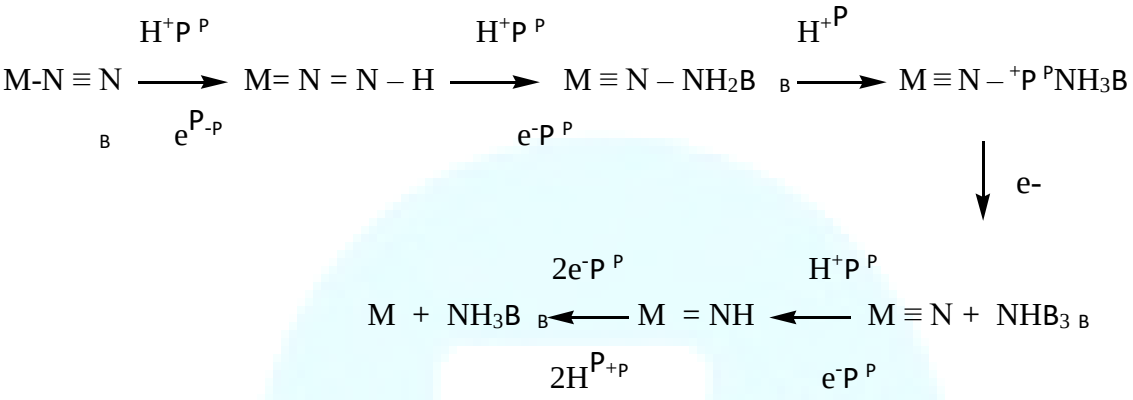
Such a process is not commercially competitive with the Haber process for the synthesis of ammonia but promises to be useful in the synthesis of other nitrogen compounds such as hydrazine and other organic nitrogen compounds.

All methods for converting dinitrogen complexes into ammonia required very powerful reducing agents, the dinotrogen in the complex was almost as unreactive as atmospheric nitrogen. An important development was the discovery that certain phosphine complexes of molybdenum and tungsten containing dinitrogen readily yield ammonia in acidic media.

Reaction occurs when compounds of the type $[M(NB_2)_B B_2(PR_B B_3)_B B_4]_B$ (M = Mo or W ; R = alkyl or aryl) are treated at room temperature with $HB_2SO_B B_4$ in $_B$methanol solution.

$$[M(NB_2)_B B_2(PR_B B_3)_B B_4]_B + H_2B_B SOB_4 \xrightarrow{MeOH} 2NHB_3 + N_B{}_2B + \text{other products}_B$$

At molybdenum and tungsten centers of this type, the bound NB_2 can be reduced by $_B$ protons at the terminal nitrogen, with electrons supplied by the metal to give the cycle of reduction.



Studies of the reaction intermediates support the proposed model. The partly reduced nitrogen species shown in cycle have been isolated when bound to a metal e.g. a recently obtained example of $=N-{}^{+}P\ {}^{P}NHB_3$ ligand is the X-ray structure of $(WCl(N\ NH_B\ \ B_3)(_B P(CHB_3)_B B_3)_B B_4)_B ClB_2$. If the system is quenched early in its reduction cycle, the intermediate $M_B{=}N-NH_2B$ produces hydrazine $N_B B_2H_B B_2$. It seems reasonable to propose this type of $_B$reduction cycle for nitrogenase. The sequence might closely resemble that of biological nitrogenase but here the cycle stops after one turn, giving two NHB_3 molecules per metal $_B$complex. This is due to be source of electrons being the metal, which is then completely oxidized after the conversion to NHB_3. To restart, more electrons must be supplied from $_B$the electron transfer system as in biological nitrogenase.

This reaction is important for two reasons:

1. It provides a model for vivo nitrogenase systems and to employ molybdenum.
2. It provides in sight into the development of useful catalyst for the industrial fixation of nitrogen.

In vivo nitrogen fixation:

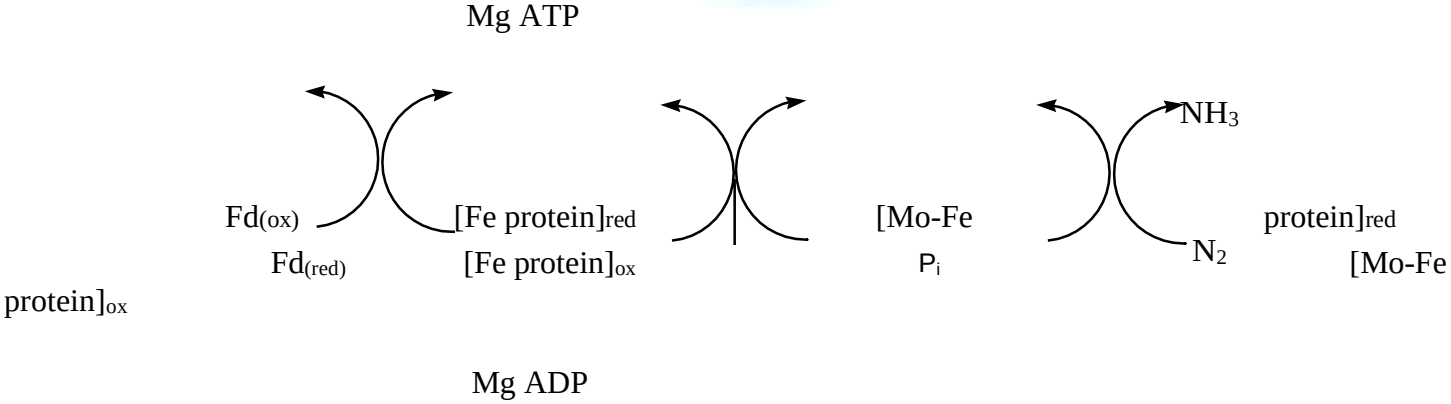
The enzyme system responsible for fixing nitrogen is known as nitrogenase. Nitrogenase plays the vital role of fixing gaseous nitrogen and making nitrogen compounds available for plants. It is distributed in a group of symbiotic bacteria and also in non-symbiotic or

asymbiotic bacteria. Symbiotic bacteria are those, which are fixing dinitrogen in association with plants e.g. the bacterium Rhizobium which is associated with the nodules on the roots of leguminous plants. Asymbiotic bacteria are certain free living bacteria which can fix atmospheric nitrogen e.g. Azotobacter. The enzymes isolated from the sources mentioned above are among the most complicated of all enzymes.

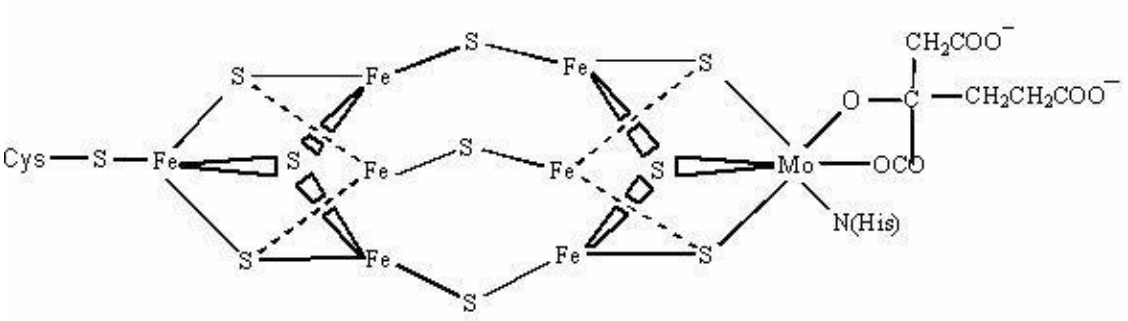
Long and intensive studies have revealed that nitrogenases are composed of two proteins; one is called the Mo-Fe protein and the other Fe protein. They are not active individually. The Mo-Fe protein contains molybdenum as well as iron-sulfur groups, and the Fe protein is an iron-sulfur protein. The smaller has a molecular weight of 57,000 – 73,000 and contains $FeB_4S_B B_4$ cluster. The larger protein has a molecular weight of 2,20,000 $_B$ – 2,40,000. Recently, X-ray studies have clarified the presence of two associated proteins in the enzyme nitrogenase viz. Fe-Mo and Fe proteins. Fe-Mo protein is having protein Pcluster and Mo-Fe cofactor. Fe-Mo cofactor structure model is recently deduced from single-crystal X-ray analysis for Fe-Mo proteins of Azotobacter vinelandii and Clostridium pasteurianum, which contains the cuboidal $FeB_4S_B B_3$ and $Fe_{B\ 3}B\ MoS_B B_3$ units bridge $_B$ by three sulfides. EXAFS analysis confirmed the presence of this core in both the isolated Fe-Mo protein and cofactor. N_2B molecule binding to the active site is still uncertain. At a $_B$ glance, a trigonal prismatic cavity surrounded by six coordinatively unsaturated Fe atoms seems to be susceptible to NB_2 insertion, giving the $\mu_B B_6$-$_B N_2B$ ligand, but the cavity size is $_B$ considered to be too small to accommodate NB_2. Alternatively, extended Huckel type $_B$ calculations suggested the coordination of NB_2 rather to the edge or the face of the $Fe_{B\ 6}B\ _B$ trigonal prism as a bridge between two cuboidal units. On the other hand, coordination of $CN^{P\text{-}P}$ to the isolated FeMo cofactor has been reported to take place at the Mo atom from the EXFAS criteria. Albeit this ambiguity of the binding and reduction mechanism of N_2B $_B$ in the biological system, it is apparent that the transition metals play an important role in promoting this transformation under mild conditions. Studies of the syntheses and reactions of NB_2 complexes are therefore of particular interest. $_B$

Electrons flow from a reducing agent $(FdB_{red})_B$ in to Fe-protein then in to Mo-Fe protein and finally on to the substrate.

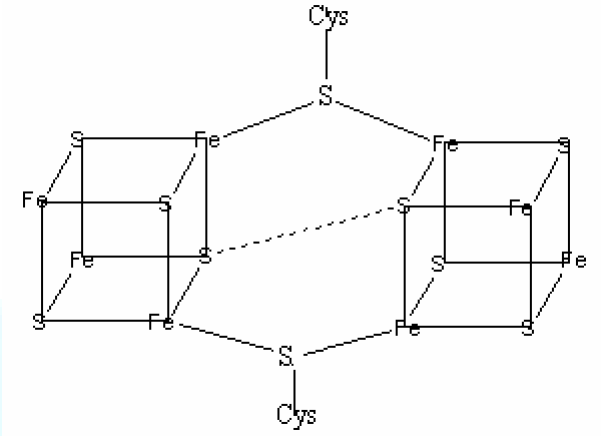
The major elements of the nitrogenase reaction are:



The structure of Fe-Mo cofactor

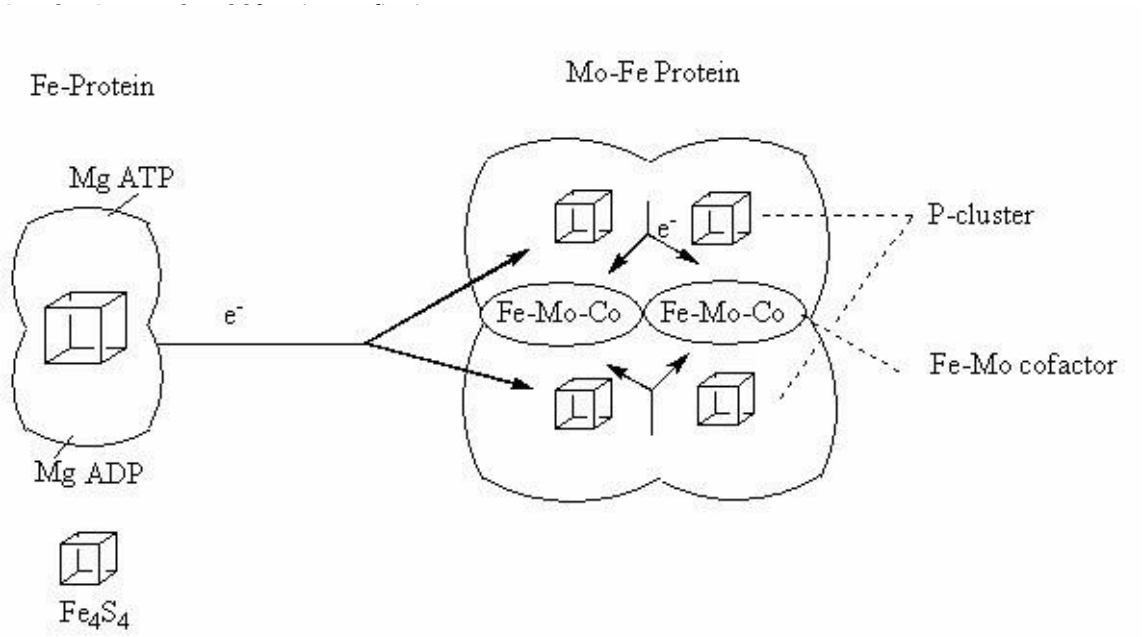


Structure of P-cluster



MgATP, which bind to the Fe-protein, is hydrolyzed as the substrate is reduced. The precise time at which MgATP binds and dissociates, and the exact role it plays in the reduction process have not been fully elucidated. The electrons that are utilized to reduce the substrates come through the enzyme, ultimately from such electron donors as ferredoxin. In vitro, nitrogenase requires the delivery of a considerable amount of energy by the act of ATP hydrolysis. The ATP requirement is highly specific and no other nucleotide works. For every NB_2 reduced by nitrogenase, one $H_B{}_2B$ is produced and as yet $_B$unexplained waste of electrons by the system. Indeed, some organisms incorporate a hydrogenase to recycle some of this HB_2. The stoichiometry of the biological reaction is $_B$thus

$$N_2B + 8H_B{}^+P^P + 8e^-P^P + 16\ \text{Mg-ATP} \longrightarrow 2NHB_3 + H_BB_2 + 16\ \text{Mg-ADP} + 16\ P_BB_i$$



Alkali and Alkaline earth metals

Sodium, Potassium, Megnesium and Calcium are four of the most important constituents of living systems (sodium being the principal extracellular and potassium the major intracellular monovalent cations). Alkaline and Alkaline earth metal cations also participate in the stabilization of cell membrane, enzyme, polynucleotide (DNA, RNA) conformations via electrostatic interactions and Osmotic effects. Nucleic acids are polyanions and as such, require, counter ions to neutralize partially or completely the negative charged phosphate groups, so that electrostatic repulsions do not overwhelm other stabilizing effects. This charge neutralization requirement is generally accomplished by cation such as Na^{+}, K^{+} and Mg^{2+}. The binding of alkali and alkaline earth metal cations to ligands is generally weak and thus often requires elaborate molecular constructions.

Calcium

The presence and central role of calcium in mammalian bones and other mineralized tissues were recognized soon after its discovery as element by Davy in1808. Calcium is used in various processes. No metal other than calcium is used in such large extent. Today it is widely recognized that Ca^{2+} ions are central to a complex intracellular messenger system that is mediating a wide range of biological process such as bone formation, muscle contraction, blood clotting, Secretion and as a co-factor for stabilization of various protein and ion transport.

Several extracellular enzymes have one or more Ca^{2+} ions as integral parts of their structure. In few of them the Ca^{2+} ion is bound at or near the active cleft, and appears

necessary for maintaining the catalytic activity (phospholipase A2, α-amylase, nucleases).

Magnesium

Magnesium is a biologically essential element with the average human adult requiring almost 0.5 g/day. Because it appears in chlorophyll, leafy green vegetables are an excellent source of Mg^{2+}. The typical adult contains about 25 g Mg^{2+}, with about 65% in the bones and 35% distributed widely and serving as a polynucliec acid stabilizer and enzyme activator. Virtually all enzymes with phosphate cofactors including ATP require Mg^{2+} for their function. Mg^{2+} also helps maintain the conformation of nucleic acids such as RNA and the stability of the ribosome. Until recently a Mg^{2+} deficiency was thought rare in humans. However, recent animal studies suggest that low- Mg^{2+} diets may be widespread and liked to diabetes and high blood pressure. Deficiency of Mg causes convulsions and excess causes anaesthetic feeling, treated using chelate agents.

Sodium & Potassium

Sodium is a vital element. The human diet must contain a sensible amount of sodium. The sodium cation is the main extracellular (outside cells) cation in animals and is important for nerve function in animals. Potassium salts are essential for both animals and plants. The potassium cation (K^+) is the major cation in intracellular (inside cells) fluids (sodium is the main extracellular cation). It is essential for nerve and heart function. A normal diet containing reasonable amounts of vegetables contains all the potassium necessary.

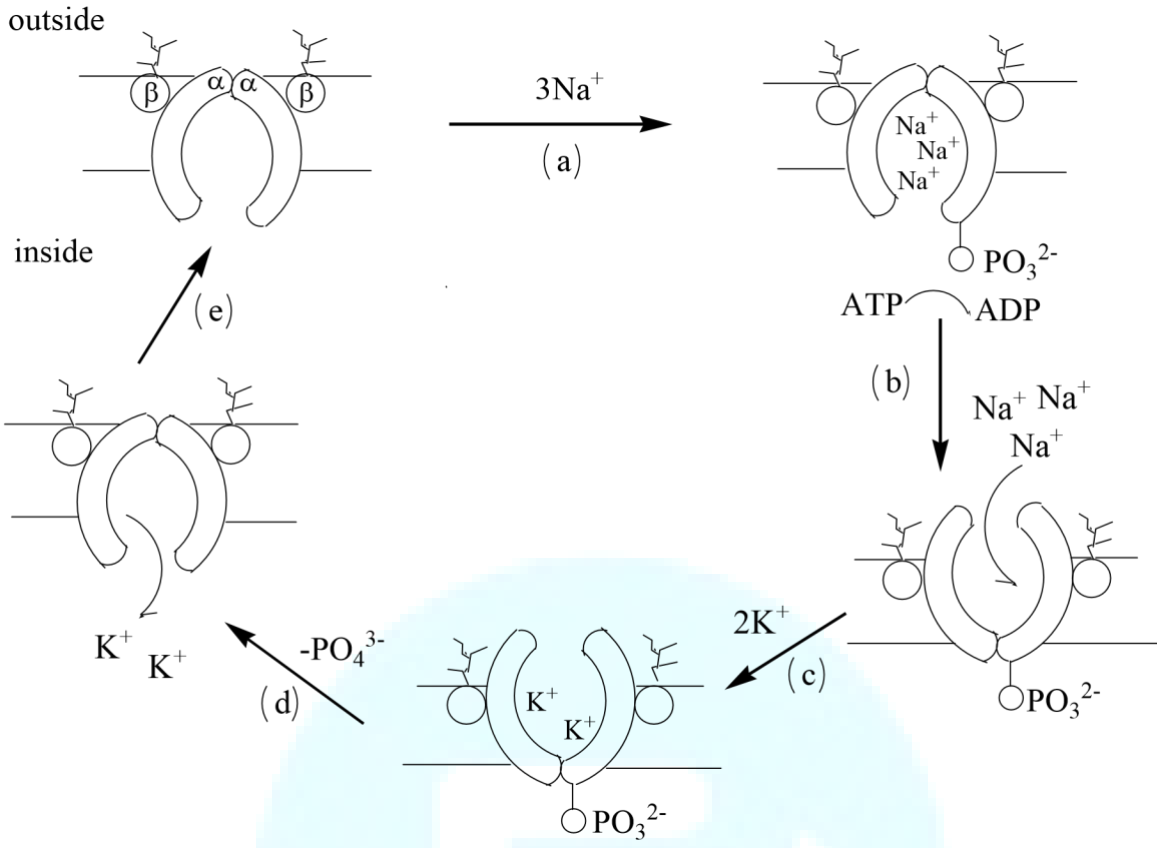
Sodium-Potassium Pump

In order to maintain the cell potential, cells must keep a low concentration of sodium ions and high levels of potassium ions within the cell (intracellular). Outside cells (extracellular), there are high concentrations of sodium and low concentrations of potassium, so diffusion occurs through ion channels in the plasma membrane. In order to keep the appropriate concentrations, the sodium-potassium pump pumps sodium out and potassium in through active transport.

The ionic transport conducted by sodium pump creates both an electrical and chemical gradient across the plasma membrane. Enzyme, Na^{+}-K^+ ATPase is the major component of the Na^+-K^+ pump, which is essential in creating membrane potential. This intrinsic membrane protein consists of two components; a 100KD catalytic subunit and a
45KD associated glycoprotein, organized in to a $\alpha_2\beta\beta_2$ tetramer.

Mechanism

$$3Na^+_{in} + 2K^+_{out} + ATP + H_2O \leftrightarrow 3Na^+_{out} + 2K^+_{in} + ADP + P_i$$



First of all the NaP^{+P}-KP^{+P} pump bound with ATP binds 3 intracellular NaP^{+P} ions (step a). This starts phosphorylation of an Asp residue leading to a conformational change, which weakens NaP^{+} ^{P}binding and moves NaP^{+} ^{P}out of the cell (step b). A conformational change in the pump exposes the NaP^{+} ^{P}ions to the outside, where they are released. ATP is hydrolyzed during this process with the release of ADP. Now in the changed conformational state pump binds 2 extracellular KP^{+P} ions (step c). Potassium binding leads to dephosphroylation and return to original conformation (step d). In this conformation ATP binds and the pump reorients to release KP^{+P} ions inside the cell (step e). The pump is ready to go again.

ENTRI

Coenzymes

- Some enzymes depend on their structure as protein for activity, while others also require one or more non-protein components for their activity ≡ cofactors.

- Cofactors may be metal ion or an organic molecule ≡ coenzyme. Some enzymes require both.

- The E-Cofactor complex is ≡ holoenzyme, and when the cofactor is removed, the remaining protein which is catalytically inactive ≡ apoenzyme.

- Although such cofactors may take p[art in the intermediate steps of the reaction catalyzed by the enzyme, they are not consumed during the process but are found in their original form at the end of catalysis. They may be regarded as the essential part of the catalytic mechanism.

- Coenzymes were originally discovered as vitamins and growth factors in nutritional and medical studies. Most coenzymes are modified form of vitamins.

- Vitamins are trace organic substances that are vital to the function of all cells and required in the diet of certain species. Vitamins were originally discovered in nutritional studies, in which they were purified from food stuffs and shown to cure various disorders in animals maintained on deficient diet.

- Vitamins were discovered by their absence rather by their presence.

Mode of Action

Majority of cofactors act in one of the following ways:

a) As interenzymic carriers:

b) As intraenzymic carriers

c) By changing the shape of the enzyme molecule

d) By subunit aggregation

e) As stablizers

f) As tempelates

g) As primers

h) As intermediates

Cofactors acting as carriers

a. Redox carriers

A very important group of cofactors consists of substances which are reduced by onesubstrate and oxidized by another.

$$AH_2 + C \xrightarrow{E_1} A + CH_2E_2$$

C = carrier

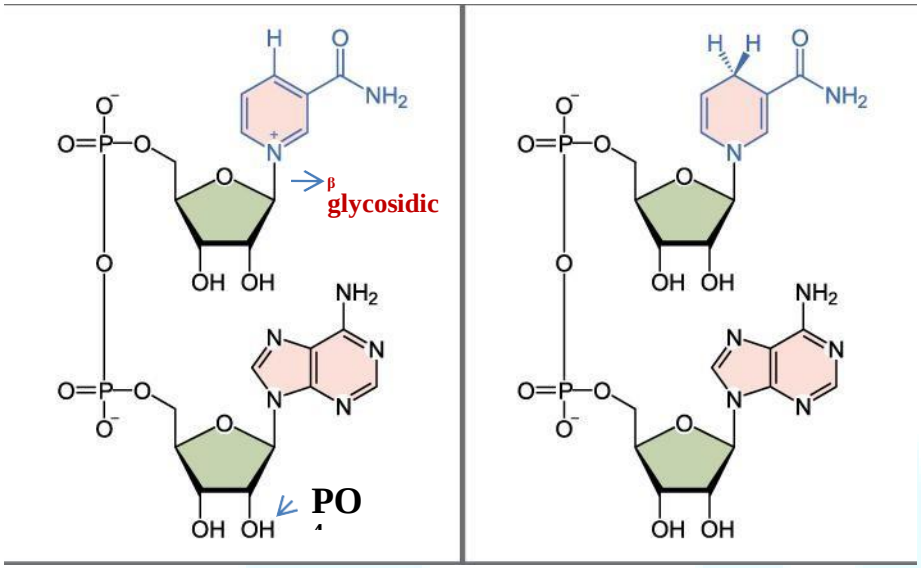
E_1 and E_2 are enzymes

H atoms represent reducing equivalents

i. NAD and NADP (DPN and TPN):

-Two coenzymes are closely related and their existence has been known since many years.The existence of thermostable coenzyme involved in fermentation [Coenzyme now identified as NAD] was shown by Harden and Young (1904) but it was not then isolated.

-It was isolated and purified by Von Euler et al and Warburg and Christian in 1936independently.



NAD^+ NADH

- Although NAD^+ is written as cation with one + charge at the nicotinamide gp., but in fact at pH 7.5, the two phosphates remain ionized giving one - charge on the molecule. While in $NADP^+$ the other PO_4 contributes to two more negative charges and the whole molecule bears three negative charges.

- NADP+ can be converted NAD+ by merely removal of the phospho group be alkaline phoaphatase.

Mode of Action

- These coenzymes can be reversibly reduced by either by chemical reducing agents such as dithionite (somewhat slowly) or much more rapidly by dehydrogenases for which they are specific.

- In the reduction two reducing equivalents per molecule are required.

- It is nicotinamide ring which is involved in reduction, when the coenzymes are reduced, theuv absorption undergoes a change:

  -NAD and NADP show only a band at 260 nm due to purine and pyrimifdine ring.

  -the reduced forms, NADH and NADPH, show an additional band at 340 nm. Thisband is due to quinoid bond structure of the reduced nicotinamide ring and is shown by no of simple derivatives of nicotinamide in the reduced state.

  -Nucleotides which do not contain nicotinamide do not give this band.

  -The transition between $NAD(P)^+$ = NAD(P)H is associated with the addition of one hydrogen atom and the removal of positive charge. Thus the oxidized is written as$NAD(P)^+$ and the reduced form as NAD(P)H.

  -Although both forms are negatively charged and the transition isNAD^- = $NADH^{--}$

  $NADP^{--}$ = $NADPH^{---}$

  because of the –ve charges on the phosphate gps. However it is customary to ignorethese charges and to write only the charges on the icotinamide part.

  -When it is desired to write coenzyme without specifying oxidized or reduced form,NAD(P) is written.

MECHANISM

- In the reduction of $NAD(P)^+$, one hydrogen atom and one electron are transferred to it, andthese together form a hydride ion (H^-), the reaction together is referred to as hydride ion transfer. But it is by no means follows that these components are transferred as a single particle and one cannot deduce the overall mechanism from the overall reaction.

- NAD+ is reduced by a large no. of substrates in the presence of their specificdehydrogenases while NADP+ is reduced by fewer enzymes. Usually dehydrogenases specific for one coenzyme or the other, but some can use either by the two (though not necessarily equally well).

- Without enzymes the reduced forms of the cofactor are not oxidized at significant rates by O_2 , or by dyes such as methylene

blue or by cytochromes; but they are oxidized by certain substances like phenazines, porphyrindienes and porphyrexides.

- Generally the dehydrogenases catalyze reversible reactions.
- The oxidation of NADPH by NAD^+ is catalyzed by $NADP^+$ transhydrogenase. Many of the reactions are rather specialized.
- A main function of these coenzymes is in the respiratory process . In mitochondria, the major reoxidation route for NADH is by way of ubiquinone and the respiratory chain.
- Another imp. Function is in anaerobic fermentation, where the reoxidation is due to other dehydrogenases acting in reverse.
- The stability of the oxidized and reduced forms vary with pH in opposite directions:
    - The reduced form is extremely unstable in acid but relatively stable in alkaline solns while
    - The oxidized form is fairly stable in acid but rather less stable in alkali
    - In neutral medium, the reduced form is less stable than the oxidized form.

ANALOGUES

A no. of modifications of NAD and NADP have been prepared and studied with respect toenzymatic properties:

- Changes in molecule, apart from the nicotinamide residue, like nicotinamidemethiodide and nicotimade mononucleotide, are completely inactive with dehydrogenases.
- If adenine is replaced by nicotinamide in NAD, it becomes inactive.
- If NH2 of adenine is replaced by –OH gp. Giving nicotinamide hypoxanthine dinucleotide, the activity is reduced to the extent which varies with dehydrogenases.
- Modification of sugar residue also effects the biological activity.

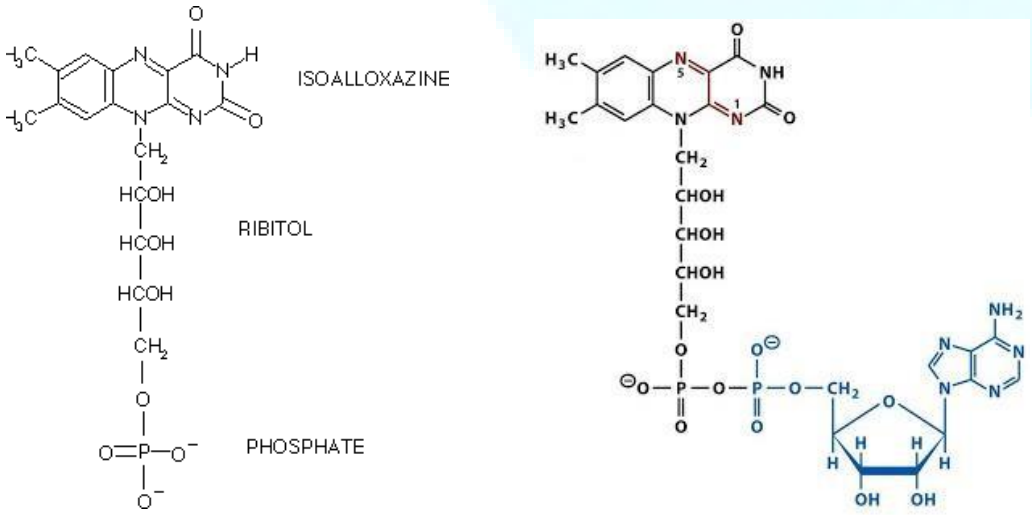
- NAD containing deoxyribose in the adenylate residue show only low activity with dehydrogenases.
- Thus the adenylate half of the molecule is no less important than the NMN half for the coenzyme activity,
- In NADP, the position of third PO_4 gp is also important. In acid solution, this gp migrates spontaneously from 2' to 3' position, giving an analogue which is inactive with those dehydrogenases which are specific for NADPbut is active to some extent as NADP itself with most of those which are able to use both coenzymes.
- When the nicotinamide gp is attached by an α-link, the dinucleotide is inactive, as is alsoNAD with ring fully reduced.
- By replacing both bases with $NAD(P)^+$ nucleosides, a series of inactive analogues are formed.
- It has been seen that CO gp. Attached to the ring or some other unsaturated structure, isnecessary for any coenzyme activity or even reduction by dithionite.
- Replacement of $CONH_2$ by $CSNH_2$ produces a dramatic increase of act. With some E but considerable fall in activity with others.
- Substitution or modification of NH_2 gp usually decreases but not abolish the activity, although replacement of $-NH_2$ gp by $-CH(CH_3)_2$ produces an enormous increase with one E and complete abolition of activity with another.
- The specificity shown by the different enzymes differ greatly, and analogues have been used to differentiate enzymes from different spp. And even isoenzymes in the same spp.

Other actions:

- NAD and NADP act as cofactors where it is not obvious that oxidation processes areinvolved eg with lyases
- The isomerases
- The transferases which however dose not involve a reduction
- Some other nucleotides have the property of bringing about assembly of inactive subunits of enzymes into complete active enzymes molecules or acting as cofactor of type (D). NAD has shown this type of action too, apart from its ability to act as redox carrier eg.
    - Four inactive subunits of G6PDH of erythrocytes are aggregated in the form oftetramer active enzyme in the presence of NAD.
    - The same enzyme from *Neurospora*, NADP has been found to pevent thetetramer from dissociating into subunits.

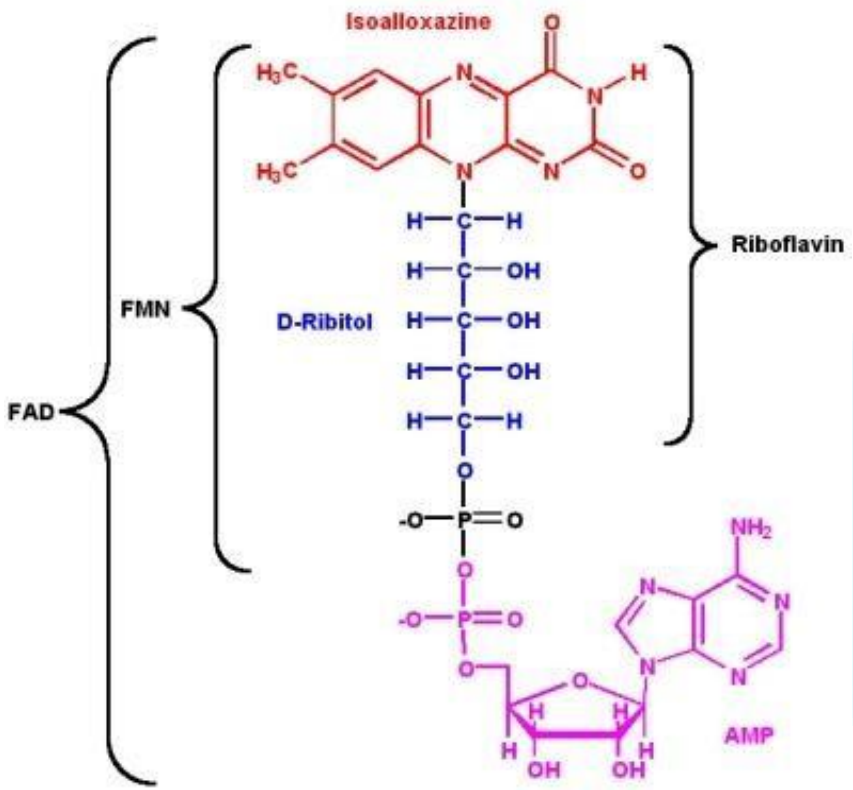
FLAVINS and FLAVOPROTEINS

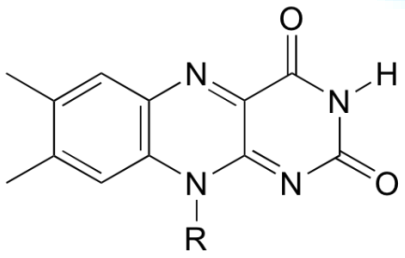
- The first flavoprotein enzyme, NADPH dehydrogenase [NADPH + acceptor = $NADP^+$+ $acceptor_{red}$ 1.6.99.1] was discovered by Warburg and Christian (1932) which they extracted from yeast and resolved into a yellow flavin gp. [Riboflavin-PO_4 or flavin



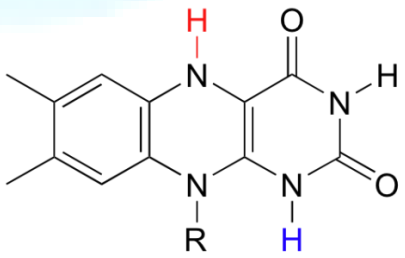
mononucleotide] and colloid which was latter shown to be a protein.

-The constitution of riboflavin was established by synthesis in 1935 by Kuhn et al and Karrer etal. Now no. flavin enzymes are known, a few of these have FMN as prosthetic gp. but the majority contain FAD.





FAD or FMN (oxidized flavin)



$FADH_2$ or $FMNH_2$ (reduced flavin)

- The oxidized forms are yellow fluorescent substances. These forms are readily reduced to the leuco forms by chemical reducers, and on reduction lose their yellow color and fluorescence, owing to the disappearance of the absorption band at 450 nm.

- The reduction involves addition of 2H atoms across the quinonoid structure in the isoalloxazine nucleus.

- Flavins bind to a no. of different specific proteins (apoenzymes) to give flavoprotein enzymes and generally one molecule of flavin is bound by each enzyme subunit. In majority of cases they are firmly and specifically bound to definite prosthetic gps although sometimes (eg in D-amino acid oxidase) the flavin may dissociate from the protein to someextent.

- The catalysis of redox reactions by flavoproteins is due to the alternate oxidation and reduction of their flavin gps.

- FAD differs from NAD, in that it completes its redox cycle while it is attached to one and the same enzyme protein, while NAD is reduced by one and oxidized by the other enzyme. FAD is therefore, an intraenzymic redox carrier.

- The properties of flavin are profoundly influenced by combination of the enzyme protein. In most cases the form of the absorption spectrum is not greatly changed on combination, although there may be slight modifications.
- The fluorescence properties of flavoproteins may be very different from those of the free flavins.Many flavoproteins do not floresce at all when oxidized and a very few show only a weak fluorescence (1-2% of that of the free flavins).

- The only one that has been found to approach the free flavins is dihydrolipoamide dehydrogenase (NAD^+). A small no. of flavoproteins have been found, unlike free flavins, to showsome fluorescence in the reduced form only (upto 1% of FMN).

- The fluorecence has not been correlated wany of the property of the flavoproteins.

- The protein part of the flavoprotein enzyme determines both the mechanism and the specificity of the reaction catalyzed. Apoproteins have several important functions:

    - Provide substrate binding site which is responsible for the activation of the substrate

      and the high substrate specificity of the enzyme.

    - In many cases it carries metal ions (Fe, Mo, Cu) as part of its structure, and these may play a part in catalytic mechanisms, enabling the E to react with substances that do notreaction with free flavins (eg nitrate reductase plays through Mo).

    - The protein also has a stabilizing effect on certain half-reduced forms of the flavin, including semiquinones which play an imporatnt part in some of the reaction catalyzed.

    - Finally the protein part establishes a pattern of specificity for the

flavin gp. towards the acceptors and therefore, determines functional class, to which the flavoprotein belongs In the free state, reduced flavins show very little specificity and are readily oxidized by many acceptors.

- Combination with proteins, selectively prevents the reaction with particular acceptors; moreover, it appears that each protein imposes a different acceptor-specificity pattern on the flavin. Some of these effects are quite remarkable and is difficult to explain.

- Catalysis by flavoproteins depends on oxidation and reduction of their flavin gps, it is not always the fully oxidized and fully reduced forms that are solely involved. Half reduced forms of various kinds play an essential part.

- Three main types of mechanisms have been distinguished:

i. depends on the oscillation between the fully oxidized and fully reduced forms of theflavins i.e.

F ==== FH_2 represented by glucose oxidase

here F is directly reduced to FH2 without formation of any intermediate (free radicalsemiquinone) and the acceptor oxidizes FH_2 directly to F.

Although the semiquinone form is not catalytically important, it is formed in large amount by reduction with dithionite, instead of glucose. It is also produced by NADH.

- In a variant of this mechanism, represented by cyt b5 reductase, F appears to be reduced to FH2 in one step by the substrate, but the oxidation of acceptor goes in two steps-

ii. Exemplified by dihydrolipoamide dehydrogenase (NAD^+): The oscillation is between the oxidized and the half reduced form, F→FH, and the FH_2 form is not involved in thecalatytic cycle.

iii. Represented by cytochrome c reductase, F form is not involved in catalysis and the cyle involves the half and fully reduced forms, FH→FH_2. FH2 is oxidized to FH formby oxygen, ferricyanide, cyt c menadione but FH form is not oxidized further by these acceptors.

Thus the acceptor specificities of the fully reduced and half reduced forms of the flavin aredifferent.

- In certain cases there is evidence that, in addition to flavins and metals, there are other

goups in the apoenzyme that undergo oxidation and reduction during the catalysis.

- Particularly in the cases of dihydrolipoamide dehydrogenase (NAD^+) and glutathione reductase (NADPH), an S-S group in the protein is oxidized and reduced at the same time as the flavin and mechanisms have been proposed involving the formation of complexes of half reduced flavin and thiol group.

Cytochromes

Cytochromes are redox-active proteins containing a heme, with a central Fe atom at its core, as a cofactor. They are involved in electron transport chain and redox catalysis. They are classified according to the type of heme and its mode of binding. Four varieties are recognized by the International Union of Biochemistry and Molecular Biology (IUBMB), cytochromes a, cytochromes b, cytochromes c and cytochrome d.

Cytochrome function is linked to the reversible redox change from ferrous (Fe(II)) to the ferric (Fe(III)) oxidation state of the iron found in the heme core.[2] In addition to the classification by the IUBMB into four cytochrome classes, several additional classifications such as cytochrome o[3] and cytochrome P450 can be found in biochemical literature.

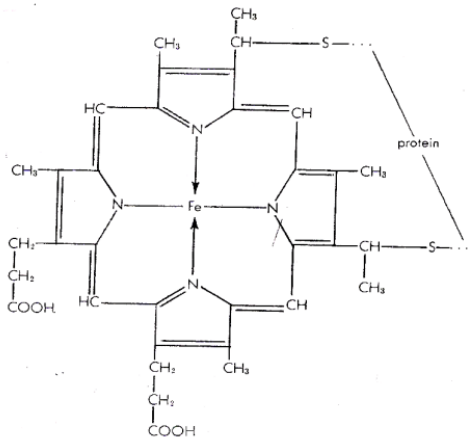
The heme group is a highly conjugated ring system (which allows its electrons to be very mobile) surrounding an iron ion. The iron in cytochromes usually exists in a ferrous (Fe^{2+}) and a ferric (Fe^{3+}) state with a ferroxo (Fe^{4+}) state found in catalytic intermediates. Cytochromes are, thus, capable of performing electron transfer reactions and catalysis by reduction or oxidation of their heme iron. The cellular location of cytochromes depends on their function. They can be found as globular proteins and membrane proteins.

In the process of oxidative phosphorylation, a globular cytochrome cc protein is involved in the electron transfer from the membrane-bound complex III to complex IV. Complex III itself is composed of several subunits, one of which is a b-type cytochrome while another one is a c-type cytochrome. Both domains are involved in electron transfer within the complex. Complex IV contains a cytochrome a/a3-domain that transfers electrons and catalyzes the reaction of oxygen to water. Photosystem II, the first protein complex in the light-dependent reactions of oxygenic photosynthesis, contains a cytochrome b subunit. Cyclooxygenase 2, an enzyme involved in inflammation, is a cytochrome b protein.

In the early 1960s, a linear evolution of cytochromes was suggested by Emanuel Margoliash[7] that led to the molecular clock hypothesis. The apparently constant evolution rate of cytochromes can be a helpful tool in trying to determine when various organisms may have diverged from a common ancestor.

In the respiratory chain the pathway of electron from NADH to oxygen involves many electron carriers, which remain tightly bound to proteins of respiratory chain. These proteins are now known to be organized into three enzyme complexes, each characterized by the electron carriers with which each interacts. Since many of the electron carriers in the respiratory chain absorb light and change colour, and since each of these carriers has a characteristic absorption spectrum, their behavior even in a crude mixture can be traced spectroscopically. These electron carriers were discovered in 1925 as compounds capable of undergoing rapid oxidation and reduction. The compounds called cytochromes were probably the first components of the electron transfer system to be associated with oxidation reduction reactions. As the name cytochrome (cell-color) indicates, each of these compounds is colored. Furthermore, the oxidized and reduced forms absorb light differently, giving different absorption bands when viewed with a spectroscope. In 1886 MacMunn observed that certain strong absorption bands of a cell suspension appeared as the oxygen of the solution was used up, and disappeared when oxygen was admitted to the system. This suggested that the compound was alternately oxidized and reduced. The term "cytochrome" was later coined for the substance involved, even though the precise structure was unknown. By examining cells and tissues with a spectroscope, three types of cytochromes (cytochrome a, b and c) were identified (actually five cytochromes a, b, c, c1 and a3 are involved is respiratory chain), although such a grouping is not functionally important. Cytochromes are the most 197 important electron carriers of the respiratory chain, which are related with each other by the presence of a bound heme group,

whose iron atom changes from ferric state (Fe^{3+}) to ferrous (Fe^{3+}) state when it accepts an electron. The heme group of cytochromes consists of a porphyrin ring holding an iron atom with its four nitrogen's. Similar porphyrin rings are found in hemoglobin of blood in animals and in chlorophyll of green plants. The three dimensional structure of cytochrome c, the most extensively studied of the five cytochromes is:



The Prosthetic Group of Cytochrome-c

The cytochromes excepting cytochrome oxidase are anaerobic dehydrogenases. They are involved as carriers of electrons from flavoproteins to cytochrome oxidase in the respiratory chain.

Type

Several kinds of cytochrome exist and can be distinguished by spectroscopy, exact structure of the heme group, inhibitor sensitivity, and reduction potential.

Cytochrome a - heme A
Cytochrome b - heme B
Cytochrome c - heme C (covalently bound heme b)
Cytochrome d - heme D (Heme B with γ-spirolactone)

There is no "cytochrome e," but cytochrome f, found in the cytochrome b_6f complex of plants is a c-type cytochrome. In mitochondria and chloroplasts, these cytochromes are often combined in electron transport and related metabolic pathways:

Cytochromes	Combination
a and a_3	Cytochrome c oxidase ("Complex IV") with electrons delivered to complex by soluble cytochrome c (hence

	the name)
b and c_1	Coenzyme Q - cytochrome c reductase ("Complex III")
b_6 and *f*	Plastoquinol—plastocyanin reductase

A distinct family of cytochromes is the cytochrome P450 family, so named for the characteristic Soret peak formed by absorbance of light at wavelengths near 450 nm when the heme iron is reduced (with sodium dithionite) and complexed to carbon monoxide. These enzymes are primarily involved in steroidogenesis and detoxification.

Cytochrome C:

1. It has a molecular wt. of 13000.

2. The iron porphyrin group of cytochrome c is attached to protein more firmly than in the hemoglobin.

3. It is quite stable to heat and acids.

4. The reduced form of cytochrome c is not autooxidizable.

5. The peptide chain of human heart cytochrome c contains 104 amino acids. Acetylglycine is the N-terminal amino acid and glutamic acid is the c-terminal amino acid. Two crystalline residues are located at positions 14 and 17. The linkage of iron in heme occurs through the imidazole nitrogen of histidine residue at position 17 in the peptide chain.

Owing to the differences of structure of the different cytochromes, they differ in reactivity, particularly in their ability to accept and to donate electrons. One of these compounds, thought to be cytochrome-b, is capable of accepting an electron from reduced coenzyme Q. The oxidized from of cytochrome-b has a ferric ion at the center of the porphyrin system; the reduced form a ferrous ion. The reduction of cytochrome-b involves only the transfer of a single electron. Two of these molecules are therefore necessary to complete the reoxidation of the coenzyme Q. Note that the hydrogens of the reduced coenzyme Q are not transferred to cytochromes; they are released as hydrogen ions to the medium. An electron is next passed from reduced cytochrome-b to one cytochrome after another with alternate oxidation and reduction of the iron atom. Finally a cytochrome called cytochrome-a; or more commonly cytochrome oxidase, is reached. This cytochrome is distinguished by its ability to undergo direct oxidation by molecular oxygen, a property rarely found in biological systems. As a class, enzymes catalyzing reactions involving oxygen are termed oxidases. It has been estimated that as much as 95 percent of the oxygen utilized by cells reacts in this single process, the oxidation of the reduced form of cytochrome oxidase to the oxidised form. The reaction is not completely understood. In addition to be iron-porphyrin portion prosthetic group, the enzyme cytochrome a3, conations a copper ion. There are indications that the cupric ion receives an electron from the ferrous-porphyrin portion of the enzyme, being reduced to cuprous ion, and that it is the enzyme-bound cuprous ion which is oxidized in the final step by molecular oxygen.

Whatever the details of the reaction mechanism, cytochrome oxidase is an exceedingly important enzyme. Inactivation of this enzyme , as occurs by its combination with carbon monoxide or cyanide ion in rather low concentrations, leads to the rapid death of the cells of most organisms.

Their utilization of oxygen is prevented, in turn prohibiting the formation of ATP in sufficient quantities to meet the energy demands. Cytochromes are also found in the endoplasmic reticulum (cytochromes P-450 and b5) plant cells, bacteria and yeast. Cytrochromes P450 is considered the most versatile biocatalyst. It has been shown by the use of 18O2 that one atom of oxygen enters R-OH and one atom enters water. This dual fate of the oxygen is responsible for naming of monooxygenases as "mixed-function oxidases". The reaction catalyzed by cytochrome P450 is represented below:

$$\underset{\text{Reduced cytochromie P450}}{RH + O_2} \rightarrow \underset{\text{Oxidised Cytochrome P-450}}{R\text{ - }OH + H_2O}$$

They are present in large amount in liver. They are present mainly in the membranes of the smooth endoplasmic reticulum in liver and most other tissues. In the adrenal, they are found in mitochondria as well as in the endoplasmic reticulum; the various hydroxylases present in that organ play an important role in cholesterol and steroid biosynthesis. The mitochondrial cytochrome P450 system differs from the microsomal system in that it uses an NADPH-linked flavoprotein, adrenodoxin reducates, and a non-heme iron-sulphur protein, adrenodoxin.

Chemical structure of Chlorophyll

Chlorophyll is a chlorin pigment, related to the porphyrin containing iron compound known as heme. At the centre of the ring is a magnesium ion. The sidechains vary somewhat between the different forms of chlorophyll found in different organisms - chlorophyll *a* is always present, but chlorophylls *b* and *c* also occur in various groups.

Chlorophyll a contains a magnesium ion encased in a large ring structure knownas a chlorin. The chlorin ring is a heterocyclic compound derived from pyrrole. Four nitrogen atoms from the chlorin surround and bind the magnesium atom. The magnesium centre uniquely defines the structure as a chlorophyll molecule.

Forms of chlorophyll

Chlorophyll consists of two forms, a and b.a:

$C_{55}H_{72}O_5N_4Mg$

b: $C_{55}H_{70}O_6N_4Mg$

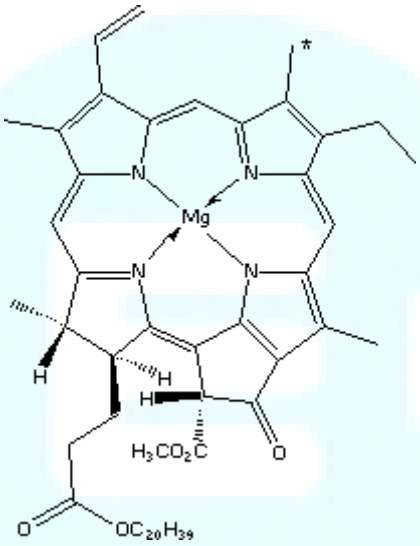
In both cases the magnesium atom is central in the molecule.

This green pigment is what gives green plants their colour. It is involved in photosynthesis by absorbing energy from visible light.

Chlorophyll is a green compound found in leaves and green stems of plants. Initially, it was assumed that chlorophyll was a single compound but in 1864 Stokes showed by spectroscopy that chlorophyll was a mixture. If dried leaves are powdered and digested with ethanol, after concentration of the solvent, 'crystalline' chlorophyll is obtained, but if ether or aqueous acetone is used instead of ethanol, the product is 'amorphous' chlorophyll.

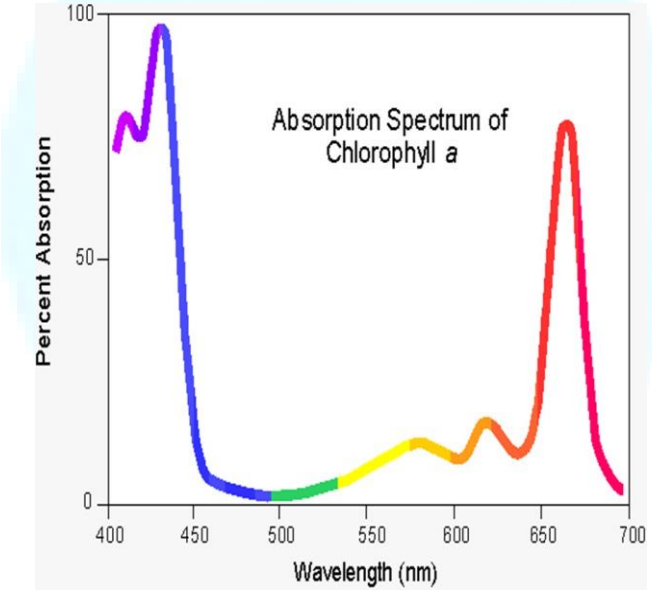
In 1912, Willstatter *et al.* showed that chlorophyll was a mixture of two compounds, chlorophyll-*a* and chlorophyll-*b*:

Apart from this fucoxanthin and phycoerythrin are important component of chlorohyll.



The two components were separated by shaking a light petroleum solution of chlorophyll with aqueous methanol: chlorophyll-*a* remains in the light petroleumbut chlorophyll-*b* is transferred into the aqueous methanol. Cholorophyll-*a* is a bluish-black solid and cholorophyll-*b* is a dark green solid, both giving a green solution in organic solutions. In natural chlorophyll there is a ratio of 3 to 1 (of *a*to *b*) of the two components.

The intense green colour of chlorophyll is due to its strong absorbencies in the red and blue regions of the spectrum, shown the below figure. Because of these absorbencies the light it reflects and transmits appears green.



The uv /visible adsorption spectrum for chlorophyll

Function of chlorophyll

Due to the green colour of chlorophyll, it has many uses as dyes and pigments. It is used in colouring soaps, oils, waxes and confectionary.

Chlorophyll's most important use, however, is in nature, in photosynthesis. It is capable of channelling the energy of sunlight into chemical energy through the process of photosynthesis. In this process the energy absorbed by chlorophyll transforms carbon dioxide and water into carbohydrates and oxygen:

$CO_2 + H_2O \rightarrow (CH_2O) + O_2$

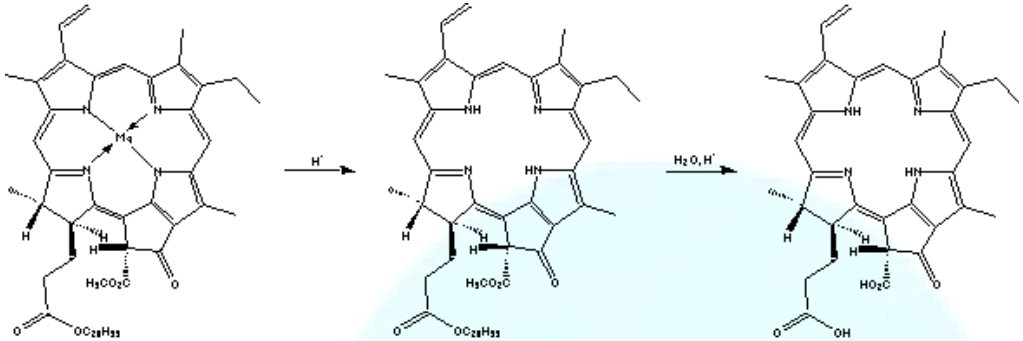
The chemical energy stored by photosynthesis in carbohydrates drivesbiochemical reactions in nearly all living organisms.

In the photosynthetic reaction electrons are transferred from water to carbon dioxide, that is carbon dioxide is reduced by water. Chlorophyll assists this transfer as when chlorophyll absorbs light energy, an electron in chlorophyll is excited from a lower energy state to a higher energy state. In this higher energy state, this electron is more readily transferred to another molecule. This starts a chain of electron-transfer steps, which ends with an electron being transferred tocarbon dioxide. Meanwhile, the chlorophyll which gave up an electron can accept an electron from another molecule. This is the end of a process which starts withthe removal of an electron from water. Thus, chlorophyll is at the centre of the photosynthetic oxidation-reduction reaction between carbon dioxide and water.

Simple reactions of chlorophyll

Treatment of cholorophyll-a with acid removes the magnesium ion replacing it with two hydrogen atoms giving an olive-brown solid, phaeophytin-a. Hydrolysis of this (reverse of esterification) splits off phytol and gives phaeophorbide-a. Similar compounds are obtained if chlorophyll-b is used.

Overall reaction scheme for the hydrolysis of chlorophyll.



Chlorophyll can also be reacted with a base which yields a series of phyllins, magnesium porphyrin compounds. Treatment of phyllins with acid gives porphyrins.