

BIOINORGANIC CHEMISTRY

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INTRODUCTION

Bio-inorganic Chemistry

Bioinorganic Chemistry is devoted to all aspects of “inorganic elements” (such as transition metals) as being vital for the growth and metabolism of living systems. Bioinorganic Chemistry is a multidisciplinary field which draws on expertise in biochemistry, chemistry, crystallography, genetics, medicine, microbiology together with the effective application of advanced physical methods.

Chairman,
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Organic chemistry at one time was thought to be the chemistry involved in living systems because compounds of carbon were found to be playing the key role in all biological processes. However slowly it was realized that metal ions play a vital role in a vast number of widely differing biological processes. Some of these processes are quite specific in their metal ion requirements in that only certain metal ions, in specified oxidation states can fulfill the necessary catalytic or structural requirements. A large number of metal ions are involved in biological processes. This can be verified by just going through the protein data banks e.g. in Brookhaven Protein Data Bank 52% proteins contain metals (excluding weakly bound metals such as sodium). This count includes proteins and enzymes with heme and corrin groups and mutated forms of certain metalloproteins and structures of the same enzyme with different substrates and inhibitors. Metal ions play essential roles in about one third of enzymes. These metal ions can modify electron flow in a substrate or enzyme, thus effectively controlling an enzyme-catalyzed reaction. They can serve to bind and orient substrate with respect to functional groups in the active site, and they can provide a site for redox activity if the

metal has several valence states. Without the appropriate metal ion, a biochemical reaction catalyzed by a particular metalloenzyme would proceed very slowly, if at all. So bio-inorganic chemistry comes in to the play. Bioinorganic chemistry encompasses a variety of disciplines, ranging from inorganic chemistry and biochemistry to spectroscopy, molecular biology, and medicine etc. The field is undergoing a phase of explosive growth, partly because of exposure and insights obtained by large number of x-ray structures of several metalloenzymes.

Bioinorganic chemistry is beginning to emerge as a major discipline, as can be judged by several published textbooks.

Elements in body

Consider the content of the elements in the body of an average healthy person (weighing 70 kg). It has been established that out of 70 kg man's weight

Oxygen accounts for (more than half the total weight)	45.5 kg
Carbon accounts for	12.6 kg
Hydrogen accounts for	7.0 kg
Nitrogen accounts for	2.10 kg
Phosphorus accounts for	700 g

Total weights of metals in gms

Calcium	1050.0
Potassium	140.0
Sodium	105.0
Magnesium	35.0
Iron	4.2
Zinc	2.3

The content of rest of metals does not exceed one gram particularly Cu –0.11 g and Mn – 0.02 g

So in human body only 2% metals but life depends much more upon these elements far more than this figure suggests.

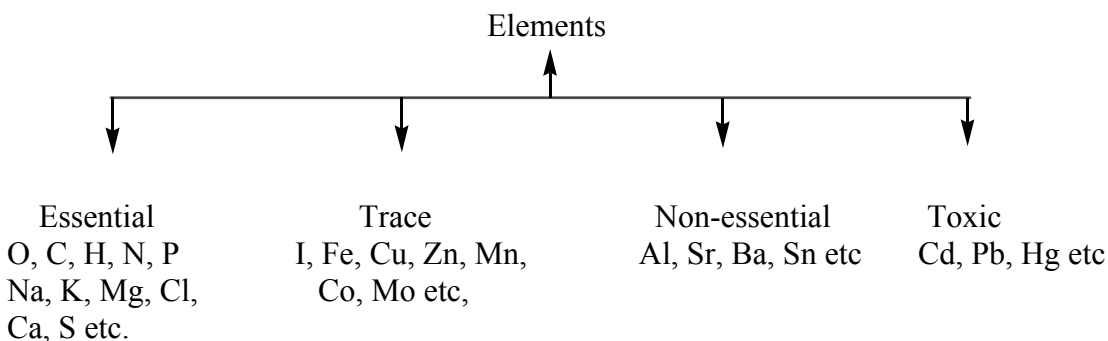
Geochemical effects on the distribution of metals

All the most essential elements, except Mo are fairly abundant in the earth's crust. Al (8.2%), Si (28.2%), Ti (0.57%) and Zr (0.02%) are although abundant but these are not essential elements. Because all these form insoluble oxides at biological pH values and do not form stable complexes with complexing agents of biological significance.

No common element is toxic at levels normally encountered though almost anything can be harmful at too high levels. All the well known toxic elements, which are currently of much concern in environmental pollution problems, are extremely rare in abundance in the earth crust.

As ($\sim 2 \times 10^{-4}\%$), Pb ($\sim 1.3 \times 10^{-3}\%$), Cd ($\sim 2 \times 10^{-5}\%$) and Hg ($\sim 5 \times 10^{-5}\%$).

Classification of elements according to their action in the biological system



Essential elements are absolutely essential or necessary for life processes.

Trace elements are also necessary for life processes.

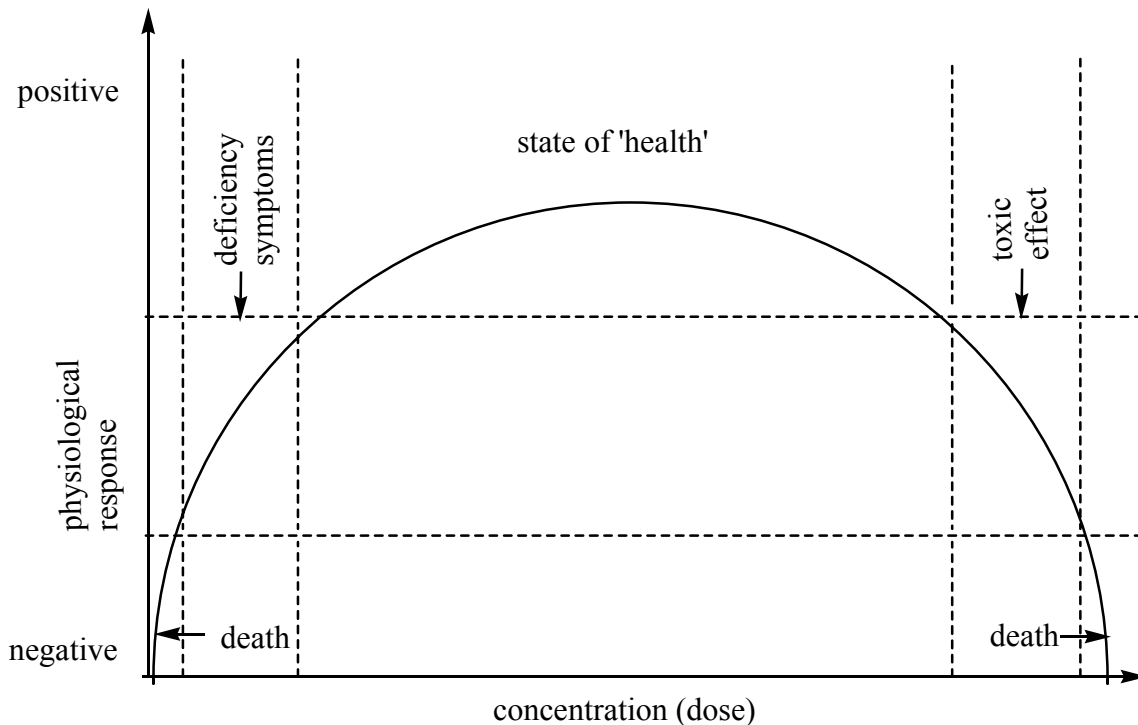
Non-essential elements are not essential. If they are absent other elements may serve the same function.

Toxic elements disturb the natural functions of the biological system.

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, $\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$, in bones and teeth. The small remainder is in cellular 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 bio-availability 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 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 B₁₂ (cobalamin). Vitamin B₁₂ 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 B₁₂ 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 from 1400 µg g⁻¹ to 2 µg g⁻¹ or 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 its presence in the metalloenzymes 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 $\mu\text{g g}^{-1}$ in animal tissues to less than 2 $\mu\text{g g}^{-1}$ in leafy green vegetables and fruits to less than 0.2 $\mu\text{g g}^{-1}$ 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 transferrin. 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.

Toxicity:

Mechanism of the toxicity of metals is very complicated. Generally toxicity of metals may result from one of the following:

- i) Blocking the essential biological functional groups of biomolecules such as enzymes.
Amino acid residues like serine is -OH functional group, cysteine is -SH group and histidine -N group often constitute the active sites of enzymes. A toxic metal ion may bind with these functional groups and block the activity of the enzyme.
- ii) Displacing the essential metal ions from biomolecules.
A biomolecule with a foreign metal ion loses its activity.
- iii) Modifying the active conformation of biomolecules.
Biomolecules are having specific active conformations and if this active conformation is lost due to the coordination of a metal ion, the activity of the biomolecule is lost.

Toxic Metals**Mercury:**

Sources of Mercury pollution are industrial waste, mining (as mercury is trace component of many minerals), pesticides, coal & lignite (containing about 100 ppb of Hg). It is a well known toxic metal came to lime light after the incidence of “Minamata disease” in 1953-60 in Japan. One hundred eleven cases of mercury poisoning were reported who had eaten mercury contaminated fish from Minamata Bay. Among them 45 people died. The sea fish were found to be containing 27-102ppm of Hg in the form of methyl mercury. The mercury source was the effluent (Hg containing catalyst wastes) from a vinyl chloride plant (Minamata Chemical Company) releasing into the bay. This was followed by more tragic report of mercury poisoning from Iraq in 1972, where 450 villagers died after eating wheat which had been dusted with mercury containing pesticides. These two tragic events boosted the awareness of mercury as pollutant; and ultimately resulting in its being studied more extensively than any other trace elements. Mercury can be toxic by ingestion or inhalation, but the toxicity depends upon the chemical form. Soluble inorganic mercury salts are highly toxic. In excess HgCl_2 causes corrosion of the intestinal tract, kidney failure and untimely death.

Reason for Toxicity: Toxicity of mercury is based on the strong affinity (very high formation constant between 10^{16} and 10^{22}) for the deprotonated forms of thiol ligands such as Cysteine; therefore, thiols, RSH , with sulfhydryl group, -SH are also called mercaptans (Mercurium captans). So Hg (II) binds strongly with the thiol group of proteins and enzymes and this binding changes the confirmation of protein about the active site. Mercury is a soft acid and -S of -SH group is a soft base so strong interaction between mercury and -SH group can be explained on the basis of stronger soft-soft binding.

Cadmium:

The source of Cd pollution in urban areas are metallurgical plants, Cd plating and battery fabricators. Acute Cd poisoning leads to nausea, salivation, vomiting, diarrhea and abdominal pain. Cd deposition tends to be cumulative in the kidney with lower

concentrations in the liver. Another characteristics of Cd poisoning is brittleness of bones. Cd occurs in nature in association with Zn minerals. A severe outbreak of chronic Cd poisoning occurred along the Jintsu river of North-West Japan and was known as Itai-Itai or ouch-ouch disease. Over 20 year period around 100 people died. In 1961 Cd was found to be the cause. The region was due to a old disused zinc mine and river water which was containing Cd used for irrigation of rice and people died after eating the rice.

Reason for toxicity: Cd is similar to zinc. Therefore Cd (II) can displace Zn (II) in many zinc enzymes. Like Hg (II), Cd (II) also binds strongly with the –SH groups of Cysteine residues of enzymes e.g., Carbonic anhydrase, dipeptidase, carboxy peptidase etc. Cd (II) like other toxic metal ions effects the active confirmation of biomolecules due to the strong binding.

Lead :

Sources of pollution : Battery industry is the largest single user of lead. But leaded petrol accounts for more than 20% of total lead consumed per year and 90% of lead released to atmosphere is from gasoline exhaust. The triethyl lead cation, $(C_2H_5)_3Pb^+$, is formed from tetraethyl lead by the dissociation of a carbanion. Toxicity; of this organometallic cation results from the permeability of membranes, including the very discrimination blood-brain barrier causing several disorders of central and peripheral nervous system (cramps, paralysis, loss of coordination). One of the characteristic symptoms of lead poisoning is anemia.

Reason for toxicity: Like Hg (II) and Cd (II) lead inhibits SH-enzyme but less strongly. Major biochemical effect of Pb is its interference with heme synthesis by inhibiting several of key enzymes involved in the overall process of heme synthesis.

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 be 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 $[\text{CaNa}_2\text{EDTA}]$ 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^- ion forms a strong complex with the cobalt in the chelate to form a relatively non-toxic and readily excretable species.

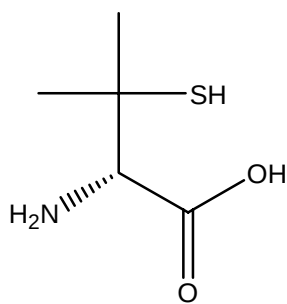
(ii) British anti-Lewisite (BAL) or

2, 3-dimercapto-1-propanol : $[\text{CH}_2\text{SH}-\text{CHSH}-\text{CH}_2\text{OH}]$

It was used by British army during world war to treat patients poisoned by the gas Lewisite $\text{ClCH}=\text{CHAsCl}_2$. It binds to the enzymes containing SH group. But BAL binds strongly to arsenic and removes it. It is also used for the treatment of poisoning caused by Hg, As, Gold etc.

(iii) D-Penicillamine :

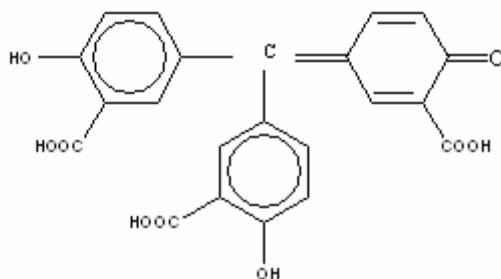
It is used for urinary excretion of copper in Wilson's disease.



D-penicillamine

(iv) Aurine tricarboxylic acid :

Effective in the treatment of Beryllium poisoning.

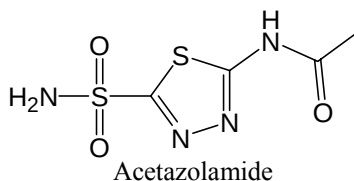


Drugs appear to involve interaction with metal ions:

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

(v) Diuretics:

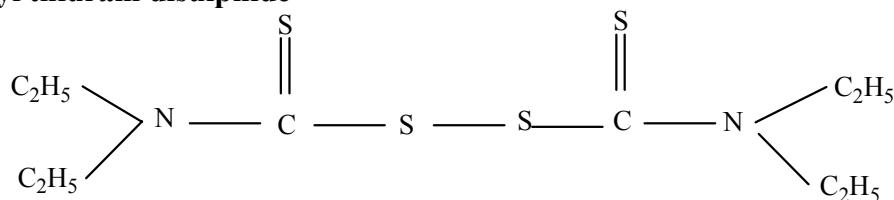
These are the drugs that promote the formation of urine e.g. Diacarb or acetazolamide.



Coordinates with zinc of zinc containing enzyme carbonic anhydrase and stops the enzymatic activity of catalyzing the reaction. $\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}^+ + \text{HCO}_3^-$ 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:

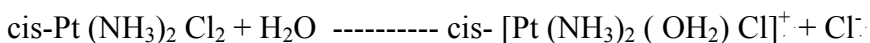
It has been shown that there is a correlation between the possession of antibacterial properties and the ability to form stable chelates. Tetracycline and its analogues have a number of sites to form metal chelates of fairly high stability.

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+} is 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 tetracyclines 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 used 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 Pt-DNA 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 N_7 of two intrastand adjacent guanine bases of DNA. This 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.



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 (NH₃)₂ Cl₂, owing to the relatively high chloride concentration (0.1M). As a neutral molecule, 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 (NH₃)₂ (OH₂) Cl]⁺ 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.

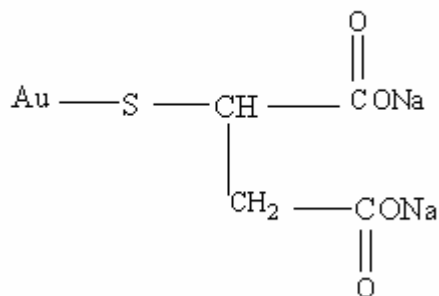
Since cis- as well as transplatin alter the double helical structure of DNA and its replication, the answer of the question that why only cis is active, is very interesting. The trans isomer is resorbed more rapidly than cis-platin; however the concentration of the DNA coordinated trans complex begins 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⁺, K⁺, Mg²⁺ or Ca²⁺ metabolism. The existence of diagonal relationship suggests that competition between Li⁺ and Mg²⁺ for Mg²⁺ sites must be considering as a priority.

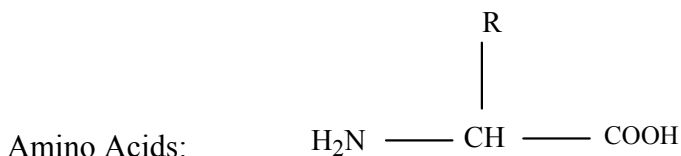
(xi) Gold:

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)

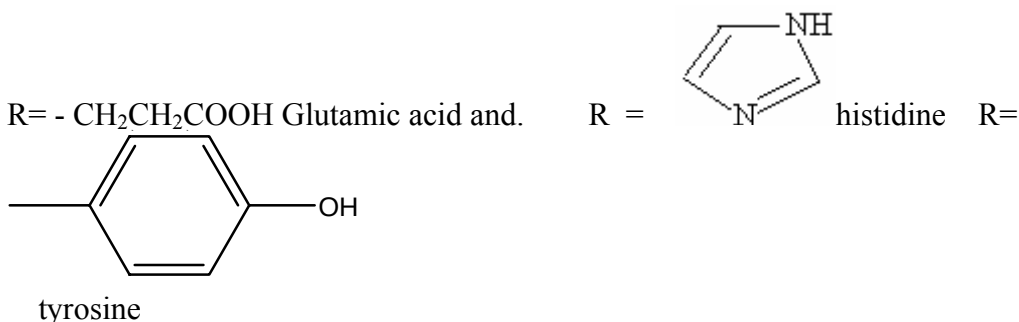


The actual role of Au (I) in controlling rheumatoid arthritis is uncertain.

Some examples of biological complexing agents :



R= H Glycine, R= CH₃ Alanine, R= (CH₃)₂ CH- Valine, R= (CH₃)₂ CH CH₂-Leucine, R= CH₃ S CH₂ CH₂-methionine, R= HOCH₂-Serine, R= -CH₂COOH-Aspartic acid,

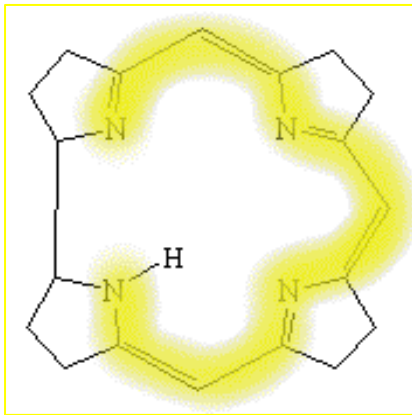
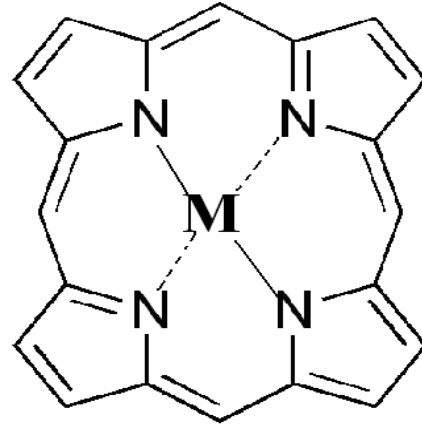
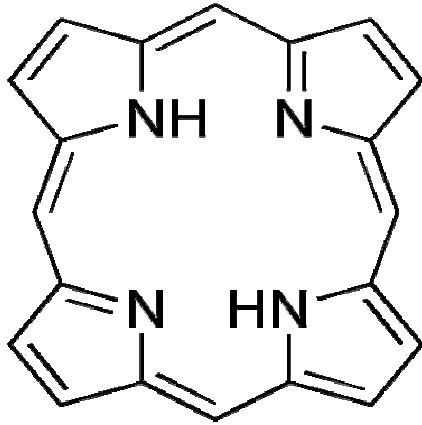


The binding of metals in metalloproteins is a difficult question to resolve with certainty. There are many alternative donor sites, the side chains, peptide and terminal –NH₂ and –COOH groups. It is reasonable to suppose however that certain groups will have particularly enhanced basic properties and 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.

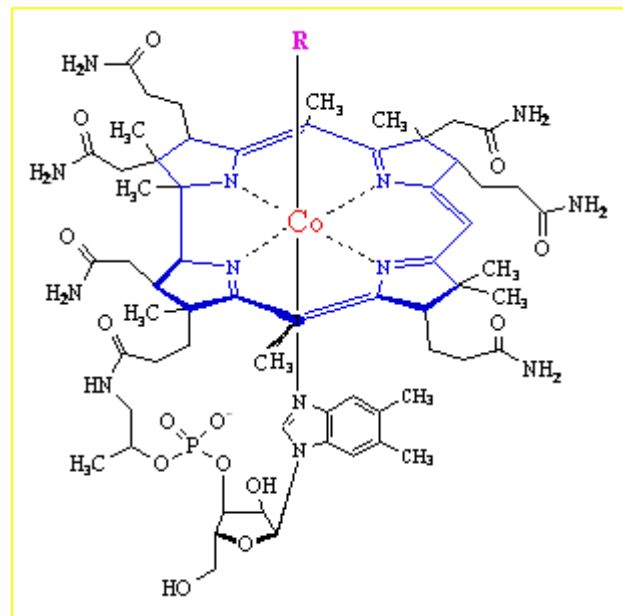
Four pyrrole units are linked by four –CH= bridges as shown but in Corrin ring one –CH=group is less. Porphyrins are derived from porphin, varying according to the nature of substituents. These rings are intensely colored and highly conjugated. Both the rings act as tetradentate ligands with four pyrrole 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 B₁₂.

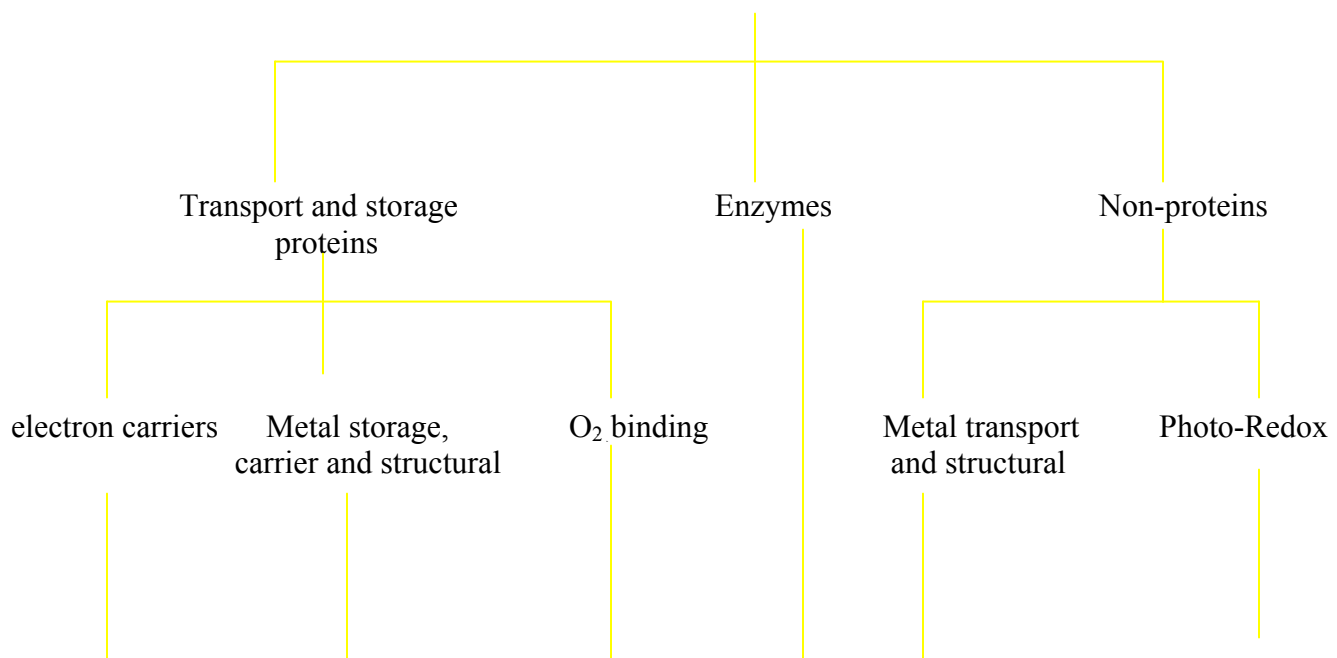


Corrin ring

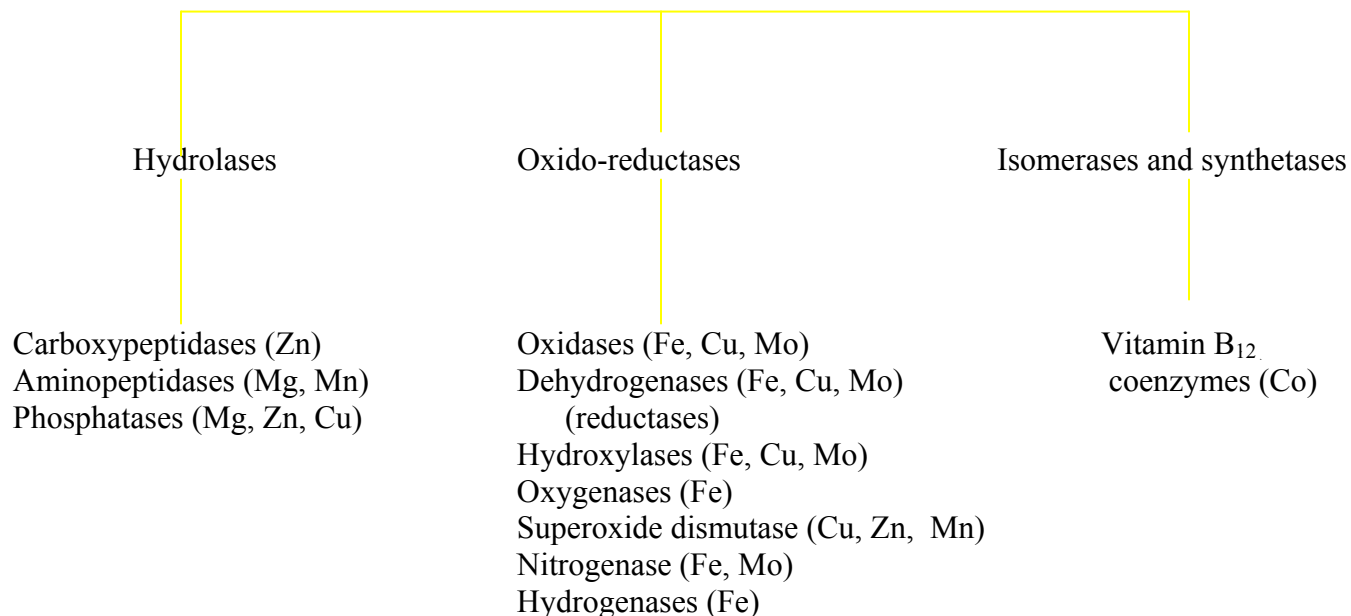
Vitamin B₁₂



Metallobiomolecules



Cytochromes (Fe) Ferritin (Fe) Myoglobin (Fe) Siderophores(Fe) Chlorophyll (Mg)
 Iron-sulfur (Fe) Transferrin (Fe) Hemoglobin (Fe) Skeletal (Ca, Si) Photosystem II (Mn, Mg)
 proteins



STORAGE AND TRANSFER OF OXYGEN

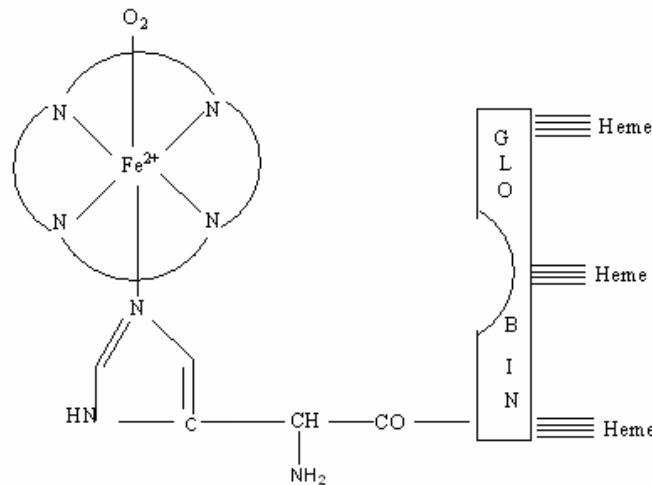
Transport and storage of molecular oxygen is an essential physiological function. It is carried out by a number of well known iron and copper containing species which occur in the blood. These are listed in Table.

S. No.	Examples	Metal	Mole ratio (O ₂ /metal)	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 O₂ carriers are responsible for red colour of human blood, absorb one 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 hemerythrin. Besides these natural carriers, there are several synthetic oxygen carrying complexes, which provide model system for the natural carriers. The O₂ binding heme protein hemoglobin was:

- the first protein crystallized (1849)
- the first protein with a recognized physiological purpose (O₂ transport, 1864; CO₂ 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)

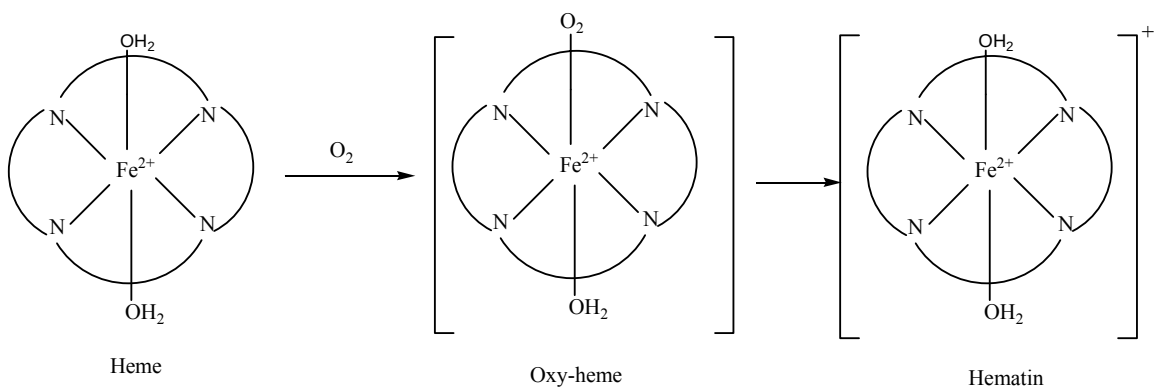
Hb has the molecular weight 64,500 daltons. Each Hb molecule has four heme groups bound to the globin on its surface. In each heme unit of Hb the four square-planar coordination positions of Fe (II) are being occupied by the four nitrogens of the protoporphyrin ring the fifth coordination position of 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 O₂ on oxygenation.



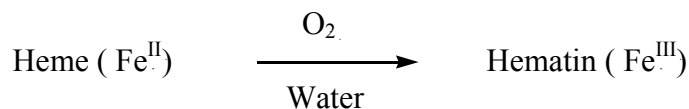
Hb consists of a tetrahedral arrangement of four heme groups each surrounded by its polypeptide (globin) chain. Normal adults have two α and two β type subunits and Hb can accordingly be represented as $\alpha_2\beta_2$. The α and β forms are distinguished by their different amino-acid sequences α -141, 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.

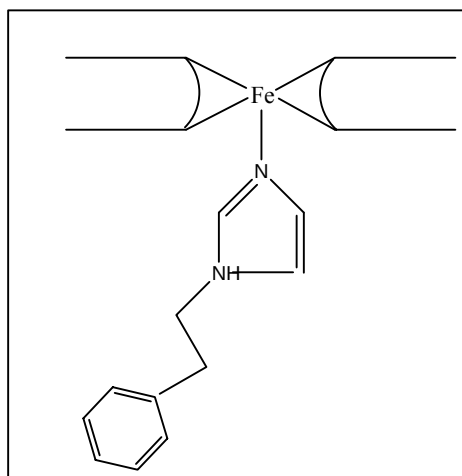


The iron (II) ion in heme is very sensitive to oxygen and it undergoes combination with it readily to form a labile Fe (II)- O_2 complex (Oxy-heme) which changes into the Fe (III) protoporphyrin called hematin or hemin. Hence free heme is not favored for oxygen transport. Hence an important function of the globin in hemoglobin, apart from the action as a carrier of heme, has been found to stabilize the heme- O_2 complex so that oxidation 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+} 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+} to Fe^{3+} .



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-(2-phenylethyl)-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.

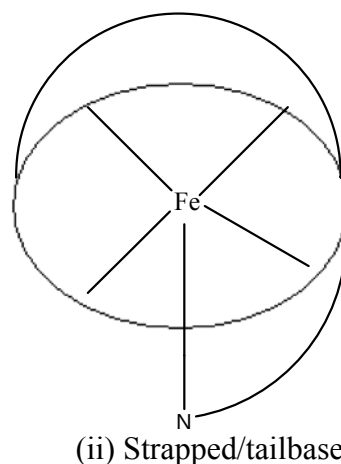
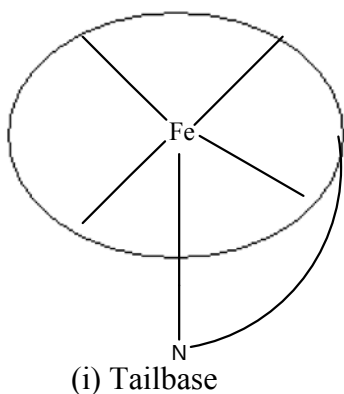


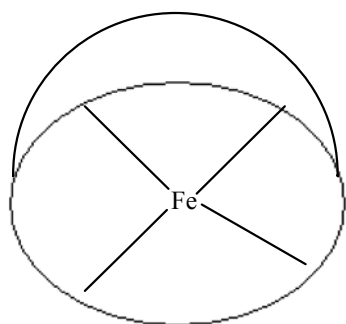
More recent work indicates that the protein chain also keep the heme group sufficiently apart so that formation of bridged Oxo dimers which degenerate to $\text{Fe}^{\text{III}}-\text{O}-\text{Fe}^{\text{III}}$ is prevented.

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

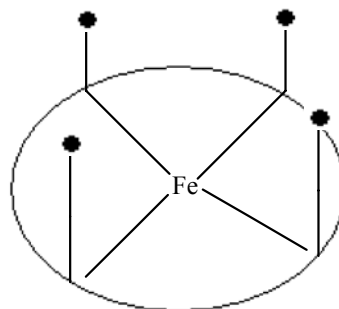
1. It furnishes a ligand to the iron i.e. the imidazole of the proximal histidine.
2. It provides the heme with “ a medium of low dielectric strength”, hydrocarbon environment of heme pocket has a low dielectric constant and so acts as a non polar “Solvent” that can not support extensive charge transfer 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_2 – carrying process without a polypeptide. The challenge to synthesis modified porphyrins in which steric hindrance has been created has produced much elegant work.

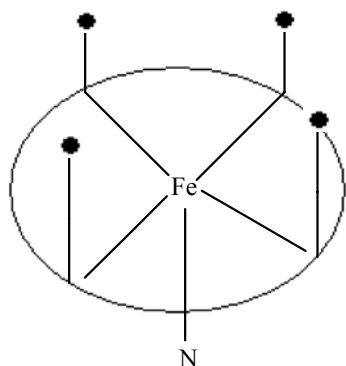




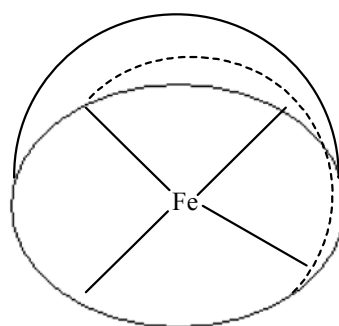
(iii) Strapped



(iv) Picket fence



(v) Picket fence/tailbase

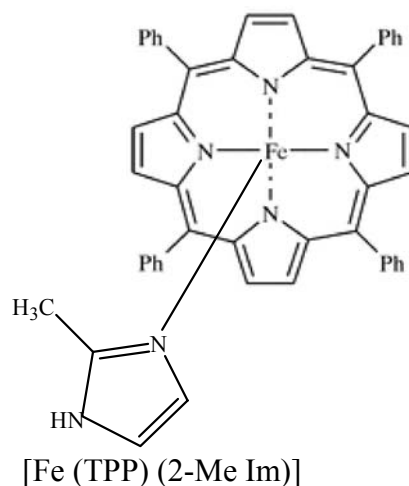


(vi) Capped

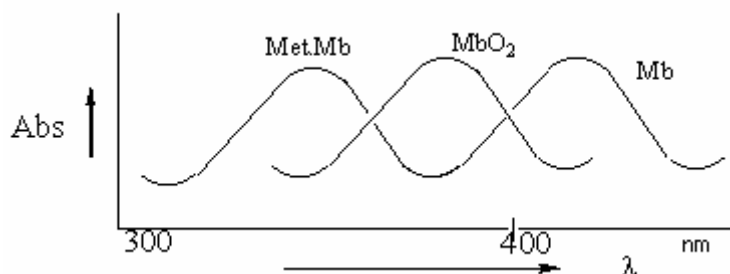
Initial attempt (1973) with Fe (II) – porphyrins having an imidazole covalently attached (tail base complexes) as in (i) gave 1:1 O₂ adducts only at low temperatures (-45°C). The strapped complex (II) and (III) with a hydrocarbon chain linked over the face of the porphyrin do not give reversible O₂ addition, almost certainly because of lack of 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 O₂ addition at room temperature. A wide range of studies on the “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 allowing reversible O₂ addition without the presence of the globin chain e.g.

Fe (TPP) bound to an imidazole and attached to a silica gel support (to avoid dimer formation) exhibits reversible O₂ up take.



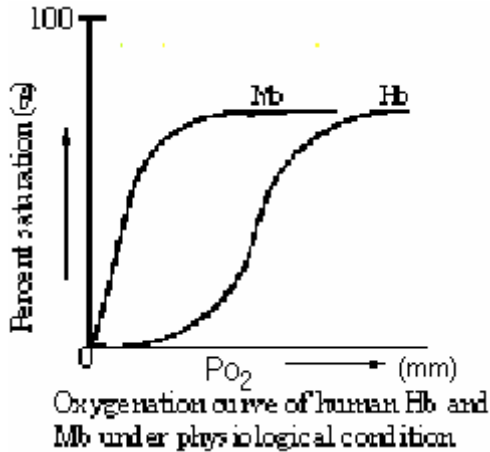
Oxygen binding to Hb and Mb



UV-visible spectra which are providing a ready means of identifying O₂-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 O₂ molecule coordinates in the bent arrangement.. Later on the structure of HbO₂ and MbO₂ have been studied (but not so accurately as picket fence) and it was found that Fe-O-O bond is bent.

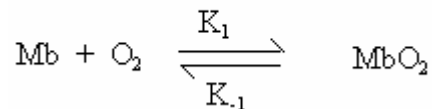
$$\text{O}_2 \cdot \text{Mb} = 112^\circ ; \text{O}_2 \cdot \text{Hb} = 156^\circ ; \text{“picket fence”} = 131^\circ$$

Oxygenation behavior of Hb and Mb



Typical curves of oxygenation vs. oxygen pressure (P_{O_2}) 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 O_2 binder as Mb at high O_2 pressure. It is much poorer at the lower pressures prevailing in muscle and hence passes its oxygen on to Mb as required. Moreover, the need for O_2 will be greatest in tissues where O_2 is consumed followed by production of CO_2 . The CO_2 lowers 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 O_2 binding constants in Hb 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



$$K_I = \frac{[MbO_2]}{[Mb][O_2]} \quad \text{----- (1)}$$

$$\text{Saturation } [\theta] = \frac{[MbO_2]}{[Mb]_{\text{total}}} \quad \text{..... (2)}$$

$$[Mb]_{\text{total}} = [Mb] + [MbO_2]$$

$$[Mb]_{\text{total}} = [Mb] + K_I [Mb][O_2] \text{ from equation (1) substituting the value of } [MbO_2]$$

$$[Mb]_{\text{total}} = [Mb] [1 + K_I [O_2]].$$

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

$$[\theta] = \frac{[\text{Mb}] K_I [\text{O}_2]}{[\text{Mb}] [1 + K_I [\text{O}_2]]}$$

value of $[\text{MbO}_2]$ substituted from equation (1)

$$\theta = \frac{K_I [\text{O}_2]}{1 + K_I [\text{O}_2]} \quad \dots\dots (3)$$

$$1 - \theta = \frac{1}{1 + K_I [\text{O}_2]} \quad \dots\dots (4)$$

Dividing equation (3) by equation (4)

$$\frac{\theta}{1 - \theta} = K_I [\text{O}_2]$$

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

Oxygenation curve of Hb may be approximated as

$$\frac{\theta}{1 - \theta} = K_I [\text{O}_2]^n \text{ with } n \sim 3$$

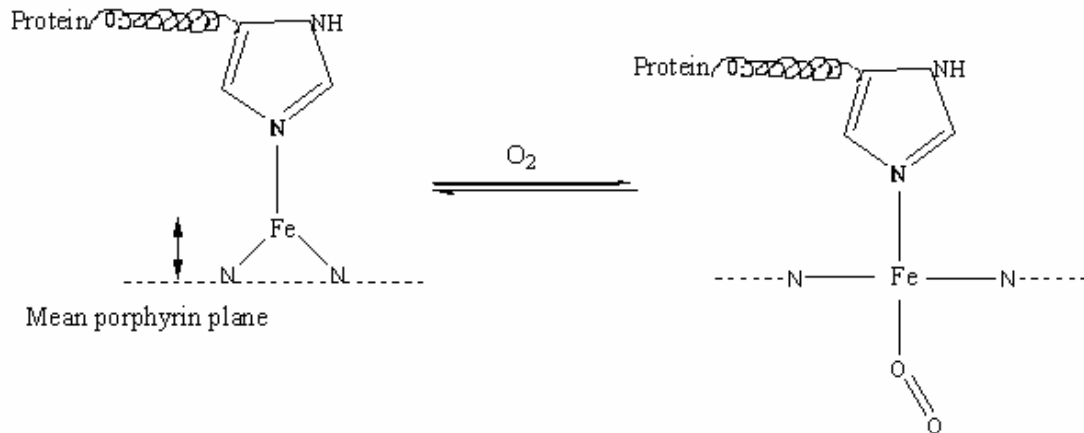
This equation is known as Hill equation and the exponent, n is called the Hill constant. In contrast to the hyperbolic curve obtained from the data for the monomeric heme (myoglobin), the data obtained by Hb shows “Sigmoidal” behavior, indicative of interaction between the subunits. The data obtained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

The form of oxygenation curve and that the fact it 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.

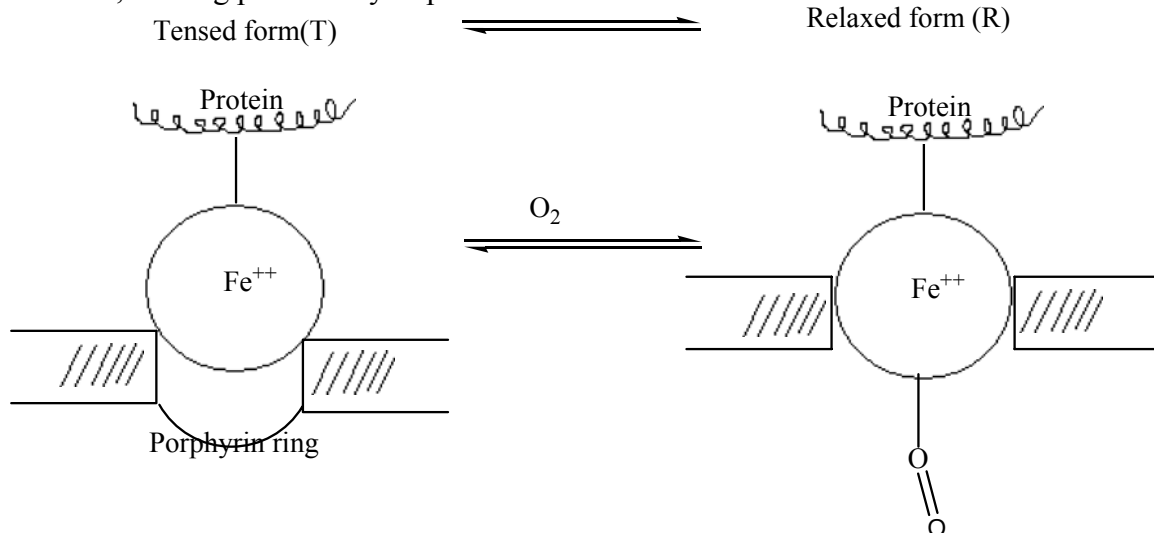
Mechanism of oxygenation in Hb and Mb

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 $1\text{\AA}$ towards each other while

two other separate by about $7\text{\AA}$. 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 ($0.78\text{\AA}$) that it can not fit into the plane of four N atoms of the heme porphyrin. It therefore lies around $0.75\text{\AA}$ 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 $0.17\text{\AA}$) 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.

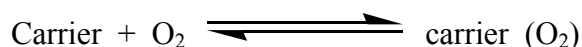


Two forms tensed (T) and Relaxed (R) of Hb are present as an equilibrium mixture. With no O_2 present, the T state is more stable than the R state and the Hb is thus found to be almost exclusively in the T form. The O_2 affinity of Hb in the T state is much

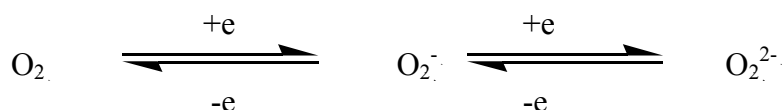
less than the O₂ affinity of Hb in the R state. Thus the initial O₂ affinity of Hb is significantly lower than that observed for the individual subunits. Addition of O₂ to deoxy Hb changes the equilibrium between T and R state. As the Hb picks up oxygen, the equilibrium shifts towards the R state. Thus the more O₂ 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 O₂ 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 O₂ 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 O₂ 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 O₂ loses its identity during the reaction. Some transition metal complexes act as reversible carriers of molecular O₂ i.e. they take up and release O₂ reversibly as follows:



Molecular O₂ can be reduced by one electron process without O-O bond being broken

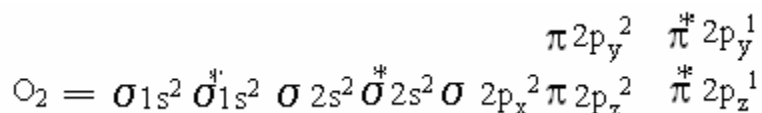


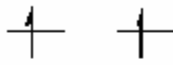
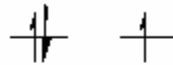
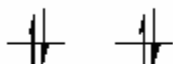
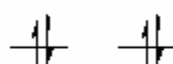
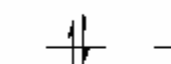
and each of these species can act as a ligand towards transition metals.

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

- i) Electronic structure of O₂ molecule
- ii) Mode of linkage and possible structures of some model systems containing Co and Ir.

Electronic structure of O₂ molecule

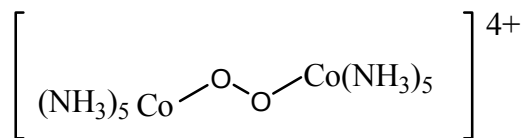
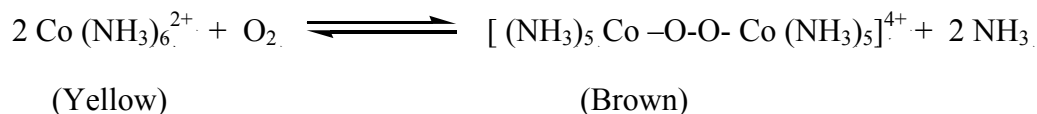


	σ_{2p}^*	O_2	O_2^- (Superoxide)		O_2^{2-} (Peroxide)	
Energy ↑	π_{2p}^*					
	π_{2p}					
	σ_{2p}					
Bond order		2.0		1.5		1.0
Bond length		1.21		1.28		1.49
$\nu(O-O) \text{ cm}^{-1}$		1560		1150-1100		850-740
IR stretching frequency						

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

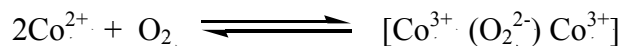
Cobalt Complexes

The reversible absorption of atmospheric oxygen by inorganic solids (ammonia-cobalt salts) was first noted by Fremy in 1852. Fremy reported that the exposure of ammoniacal solutions of Co (II) salts to the atmosphere resulted in the formation of brown salts which he called oxo cobaltates. They were later characterized by Werner in 1898 as $[(NH_3)_5Co(O_2)Co(NH_3)_5]^{4+}$.

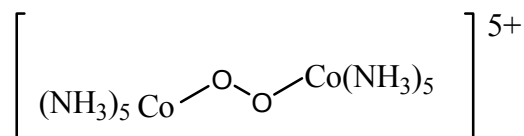
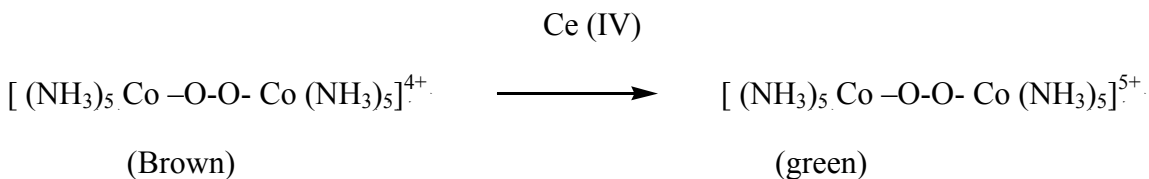


O-O 1.47 Å

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



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

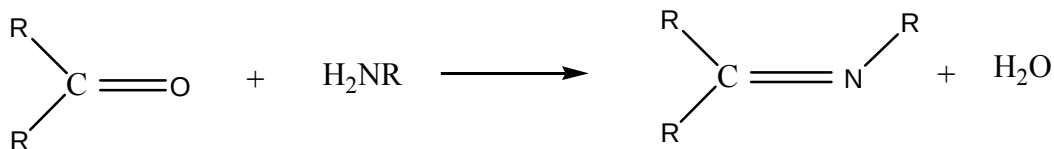


O-O 1.31 Å

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

Cobalt complexes with schiff base ligands

Schiff base are formed by schiff base condensation reaction



Schiff base used in these studies are generally tetradentate or pentadentate 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 (acetylactone) 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.

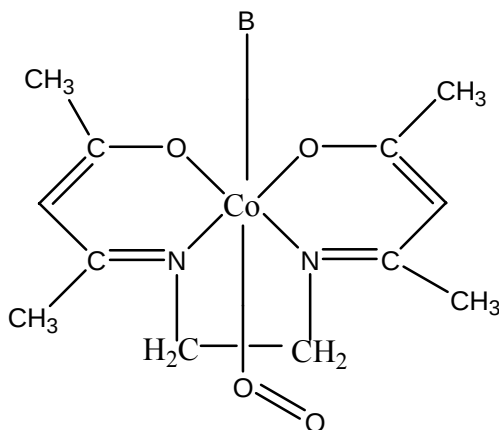
Acacen: Solid Co (acacen)B(O₂) is isolated where B is a base coordinated with cobalt. Crystal structure determination studies show:

Co-O-O angle 117°

O-O bond length is 1.27 – 1.30 Å

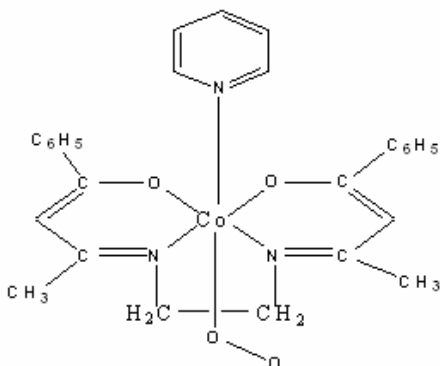
$\nu(\text{O}-\text{O})$ IR stretching frequency is 1120-40 cm⁻¹.

This was shown to involve Co (III) –O₂⁻ 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^-) ion. The Co-O-O chain is bent.



Co(acacen) complex

Bis (benzoylacetone) ethylenediamine complex.

By crystal structure studies of Co (bzacen) (pyridine) O_2 .

Co-O-O angle is found to be 126° and O-O distance $1.26 \text{ \AA}$.

$\nu(\text{O-O})$ stretching frequency is 1128 cm^{-1} .

esr spectra is consistent with the presence of Co (III)- (O_2^-) as in the previous case.

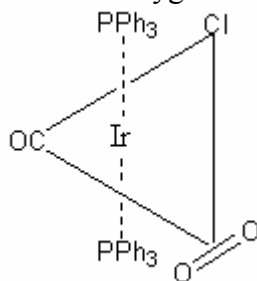
After careful study of synthetic oxygen carriers, we can explain the binding of oxygen with Hb and Mb.

Let us take a picket fence complex. It binds O_2 , The Fe-O-O angle is 136° and O-O bond length is $1.25 \text{ \AA}$. 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^6 Fe (II) complex of singlet O_2 which would mean that Fe and O_2 reduction is less important than in Cobalt complexes. However, we must be cautious with this interpretation because other evidence closer to the O_2^- values of 1145 cm^{-1} than the O_2 value of 1560 cm^{-1} . The low spin character could arise as the result of spin pairing of Fe (III) and O_2^- 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^3 Cr (III) porphyrin complex of oxygen has been synthesized and found to be having only two unpaired electrons. Since d^3 Cr (III) must be high spin, the only explanation for the spin observed is spin pairing with an electron from O_2^- .

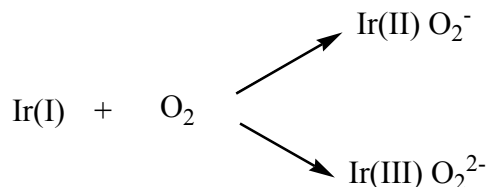
Vaska's Iridium complex

Complex chlorocarbonyl bis (triphenylphosphine)iridium. $Ir(PPH_3)_2COCl$ 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 O_2 and two M-O distances are equal.

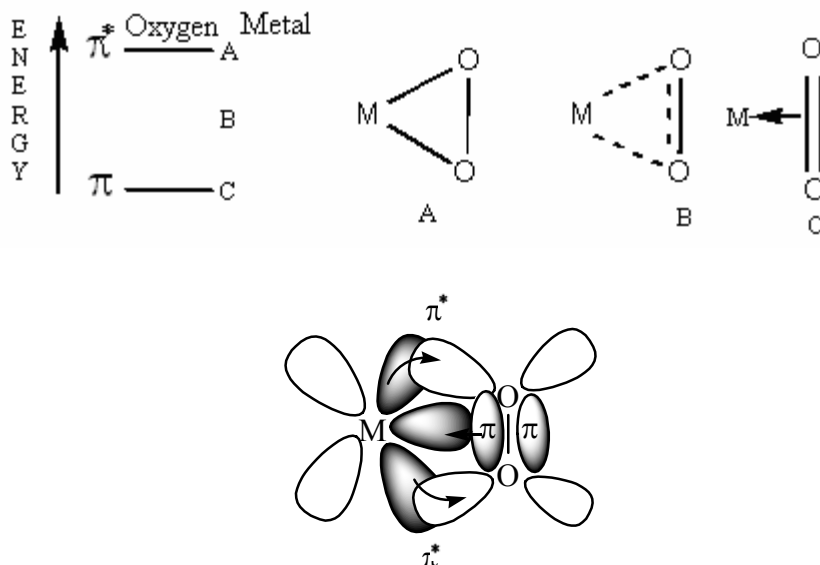
Several theories have been proposed to explain the diamagnetic nature of O_2 adducts. Valence forms involving superoxide or cyclic peroxide complexes e.g.



For the $Ir(II) O_2^-$ case it would be necessary to postulate that the spins on the d^7 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. O_2 acts as 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 Π^* 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 Π^* orbitals of dioxygen.

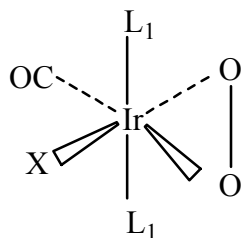
In this case the bonding orbitals formed from metal orbitals and dioxygen Π^* orbitals will be mostly dioxygen in character. Therefore, there will be little dioxygen to metal bonding. The Π bonding orbitals formed conceptually from electron transfer from the metal orbitals to dioxygen Π^* orbitals. If enough electron density is transferred to dioxygen Π^* 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 Π^* orbitals of dioxygen.

In this case both, σ bonding from dioxygen to metal and backbonding from metal d orbitals to Π^* orbitals of dioxygen are involved.



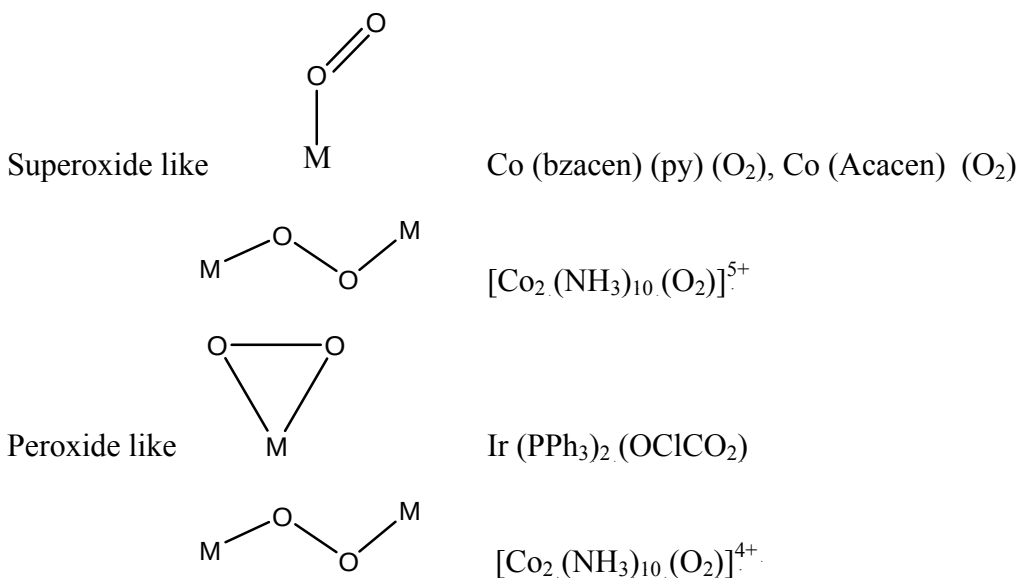
Structure:

L_1	X	O-O bond length ($\text{\AA}$)
PPh_3	Cl	1.30
PPh_3	Br	1.36
PPh_3	I	1.51
PPh_2Et	Cl	1.46

In this compound the O-O bond lengthens from $1.30 \text{ \AA}$ for $X = \text{Cl}$ to $1.51 \text{ \AA}$ for $X = \text{I}$. The less electronegative more polarizable iodide ligand appears to have raised the

metal valence orbitals to the level in case A, while the chloride complex seems 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 Å reflecting a change in the metal valence orbital energies again from Case A to Case B or C.

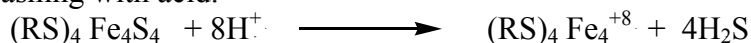
General classification according to the bonding



REDOX REACTION

Iron-Sulfur proteins

General name of Iron-sulfur proteins is Ferridoxins. These are non-heme iron-sulfur 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.



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

Classification:

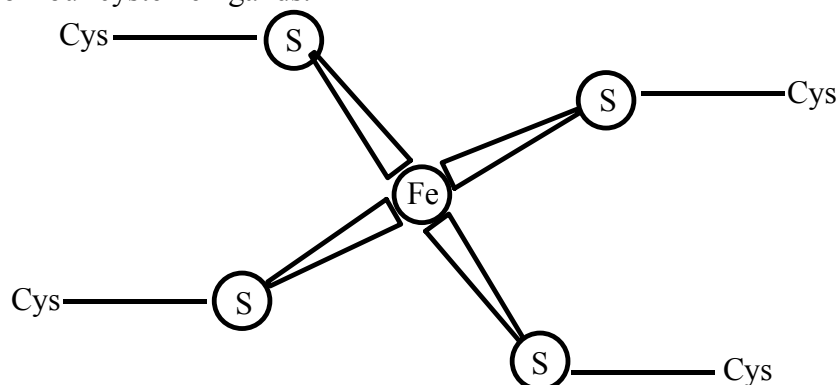
S.No.	Fe-S	Conventional name	No. of electrons involved in redox reaction
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1.	1-0	Rubredoxin	1
2.	2-2	2-Iron ferredoxin	1
3.	3-4	3-Iron ferredoxin	1
4.	4-4	4-Iron ferredoxin	1

Rubredoxin:

(Source: *Clostridium pasteurianum*)

Fe-S clusters are of several types. The simplest is bacterial rubredoxin (Cys-S)₄ Fe (often abbreviated Fe₁S₀) and contains only non-labile sulfur. It is a bacterial protein 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 Fe²⁺ and Fe³⁺ having high spin configurations.



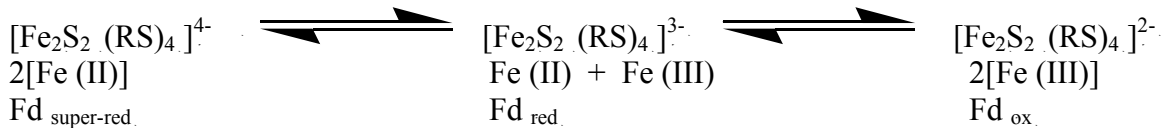
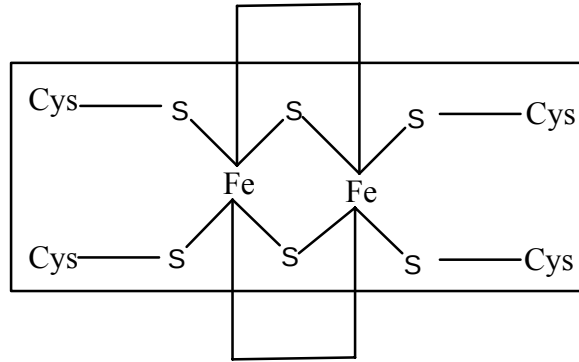
2-Iron ferridoxin

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 Fe³⁺ ions in the form of a S₂²⁻ bridged dimer (the bridging sulfurs are labile); each iron is also coordinated by two terminal cysteinyl sulfurs.

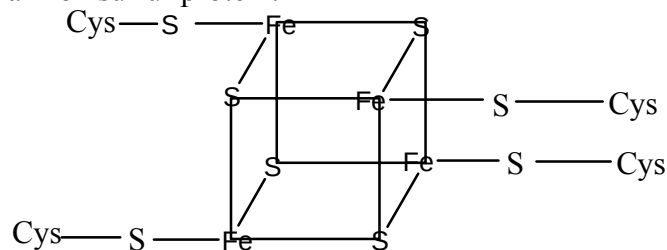


The oxidized proteins bearing two high spin Fe^{3+} ions, are diamagnetic at very low temperatures, while the reduced form is paramagnetic corresponding to 1 electron. On reduction, one electron is accepted, formally giving one Fe (III) group ($S=5/2$) and one Fe (II) group ($S=2$), which interact giving 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)

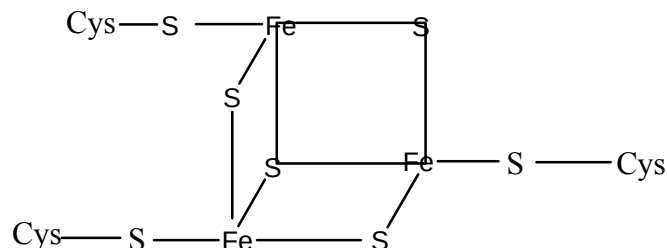
It contains one $\text{Fe}_4\text{S}_4(\text{Cys})_4$ unit on which iron and sulfur alternatively occupying the edges of an approx.cube. The four Fe atoms are bonded with sulfur atoms of 4 Cys units. It is a bacterial iron sulfur protein.



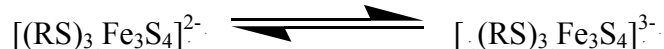
3-Iron ferridoxin

(Source: Bacteria D.gigas)

The $[3\text{Fe}-4\text{S}]$ 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^{3+} and in reduced state it is containing 2 Fe^{3+} and 1 Fe^{2+} :



Bio-inorganic chemistry of N_2 -fixation

Molecular nitrogen or dinitrogen (N_2) 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.

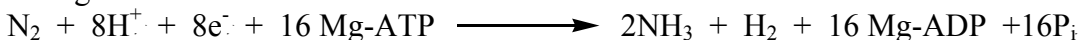
Dinitrogen may be fixed industrially by a number of processes, of which the Haber’s process is most important, but it required high temperature, high pressure and a catalyst.



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

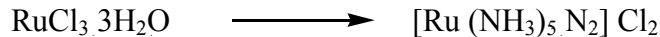
Biological nitrogen fixation is the process whereby some bacteria and blue green algae convert atmospheric nitrogen into ammonia.

Nitrogenase reaction:

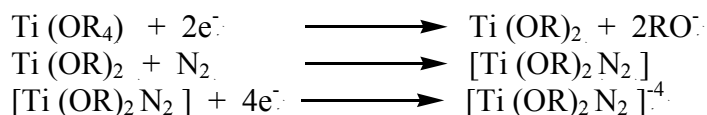


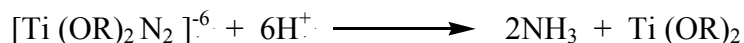
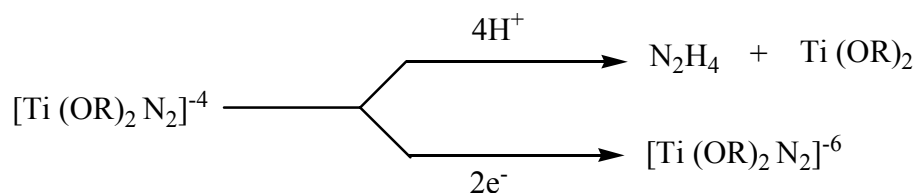
In vitro nitrogen fixation

In 1965, Allen and Senoff obtained salts containing $[\text{Ru}(\text{NH}_3)_5 \text{N}_2]^{2+}$ cation by the action of hydrazine hydrate on various compounds of tri and tetra positive ruthenium e.g. ruthenium trichloride.



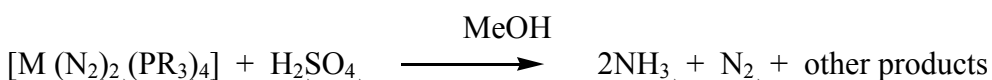
This discovery that molecular nitrogen was capable of forming stable complexes with transition metals led to extensive investigation of the possibility of fixation of nitrogen via such complexes. Of the various systems investigated, that employing titanium (II) was the first to be successful. Titanium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



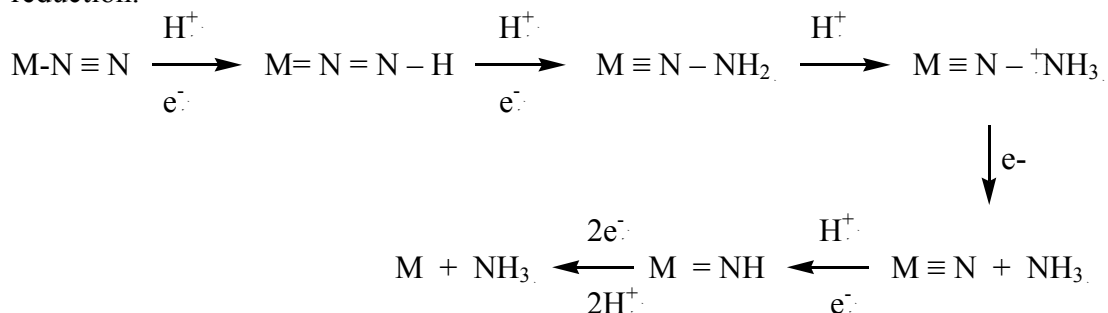


Such a process is not commercially competitive with the Haber process for the synthesis of ammonia but promises to be useful in the synthesis of other nitrogen compounds such as hydrazine and other organic nitrogen compounds.

All methods for converting dinitrogen complexes into ammonia required very powerful reducing agents, the dinitrogen 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 $[\text{M}(\text{N}_2)_2(\text{PR}_3)_4]$ ($\text{M} = \text{Mo}$ or W ; $\text{R} = \text{alkyl}$ or aryl) are treated at room temperature with H_2SO_4 in methanol solution.



At molybdenum and tungsten centers of this type, the bound N_2 can be reduced by 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 $=\text{N}-\text{NH}_3^+$ ligand is the X-ray structure of $(\text{WCl}(\text{N}(\text{NH}_3))(\text{P}(\text{CH}_3)_3)_4\text{Cl}_2$. If the system is quenched early in its reduction cycle, the intermediate $\text{M}=\text{N}-\text{NH}_2$ produces hydrazine N_2H_2 . It seems reasonable to propose this type of reduction cycle for nitrogenase. The sequence might closely resemble that of biological nitrogenase but here the cycle stops after one turn, giving two NH_3 molecules per metal complex. This is due to the source of electrons being the metal, which is then completely oxidized after the conversion to NH_3 . To restart, more electrons must be supplied from 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 insight into the development of useful catalyst for the industrial fixation of nitrogen.

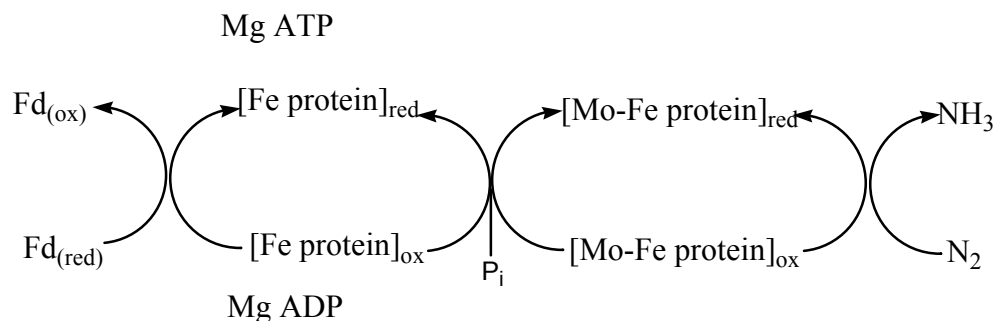
In vivo nitrogen fixation:

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.

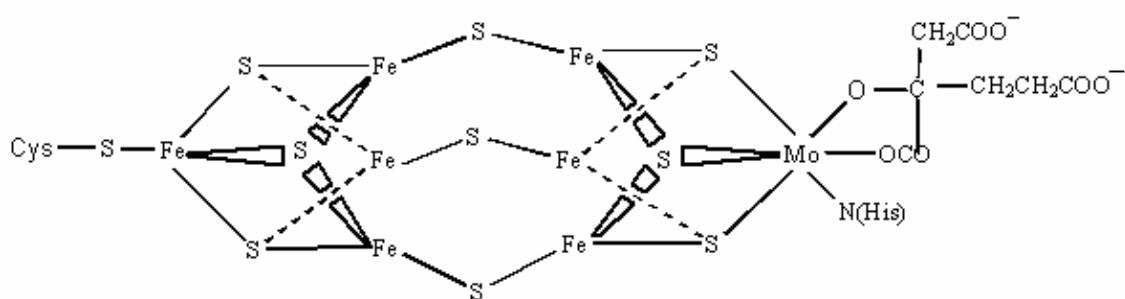
Long and intensive studies have revealed that nitrogenases are composed of two proteins; one is called the Mo-Fe protein and the other Fe protein. They are not active individually. The Mo-Fe protein contains molybdenum as well as iron-sulfur groups, and the Fe protein is an iron-sulfur protein. The smaller has a molecular weight of 57,000 – 73,000 and contains Fe_4S_4 cluster. The larger protein has a molecular weight of 2,20,000 – 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 P-cluster 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 Fe_4S_3 and Fe_3MoS_3 units bridge by three sulfides. EXAFS analysis confirmed the presence of this core in both the isolated Fe-Mo protein and cofactor. N_2 molecule binding to the active site is still uncertain. At a glance, a trigonal prismatic cavity surrounded by six coordinatively unsaturated Fe atoms seems to be susceptible to N_2 insertion, giving the $\mu_6\text{-N}_2$ ligand, but the cavity size is considered to be too small to accommodate N_2 . Alternatively, extended Huckel type calculations suggested the coordination of N_2 rather to the edge or the face of the Fe_6 trigonal prism as a bridge between two cuboidal units. On the other hand, coordination of CN^- 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_2 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 N_2 complexes are therefore of particular interest.

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

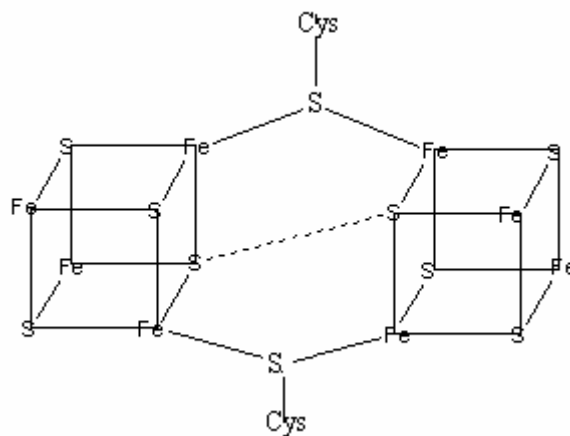
The major elements of the nitrogenase reaction are:



The structure of Fe-Mo cofactor

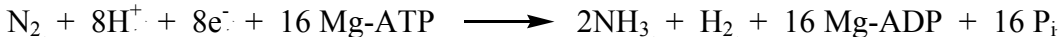


Structure of P-cluster

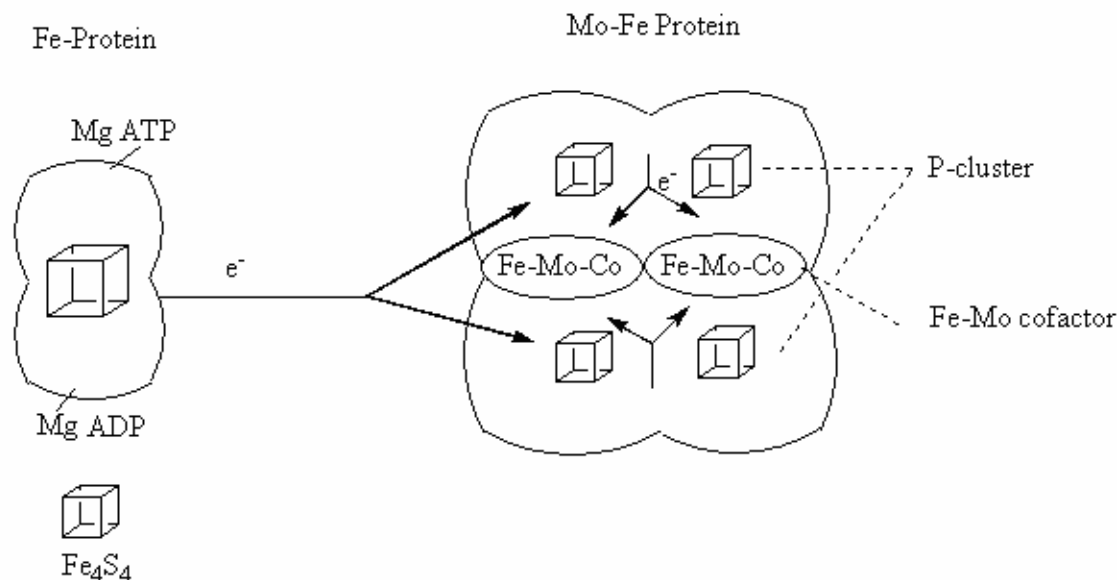


MgATP, which binds to the Fe-protein, is hydrolyzed as the substrate is reduced. The precise time at which MgATP binds and dissociates, and the exact role it plays in the reduction process have not been fully elucidated. The electrons that are utilized to reduce the substrates come through the enzyme, 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 N_2 reduced by nitrogenase, one H_2 is produced and as yet unexplained waste of electrons by the system. Indeed, some organisms incorporate a

hydrogenase to recycle some of this H_2 . The stoichiometry of the biological reaction is thus



Complete Structural Model for Nitrogen fixation



Alkali and Alkaline earth metals

Sodium, Potassium, Magnesium and Calcium are four of the most important constituents of living systems (sodium being the principal extracellular and potassium the major intracellular monovalent cations). Alkali 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 in 1808. 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 polynucleic 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 linked to diabetes and high blood pressure. Deficiency of Mg causes convulsions and excess causes anaesthetic feeling, treated using chelate agents.

Sodium & Potassium

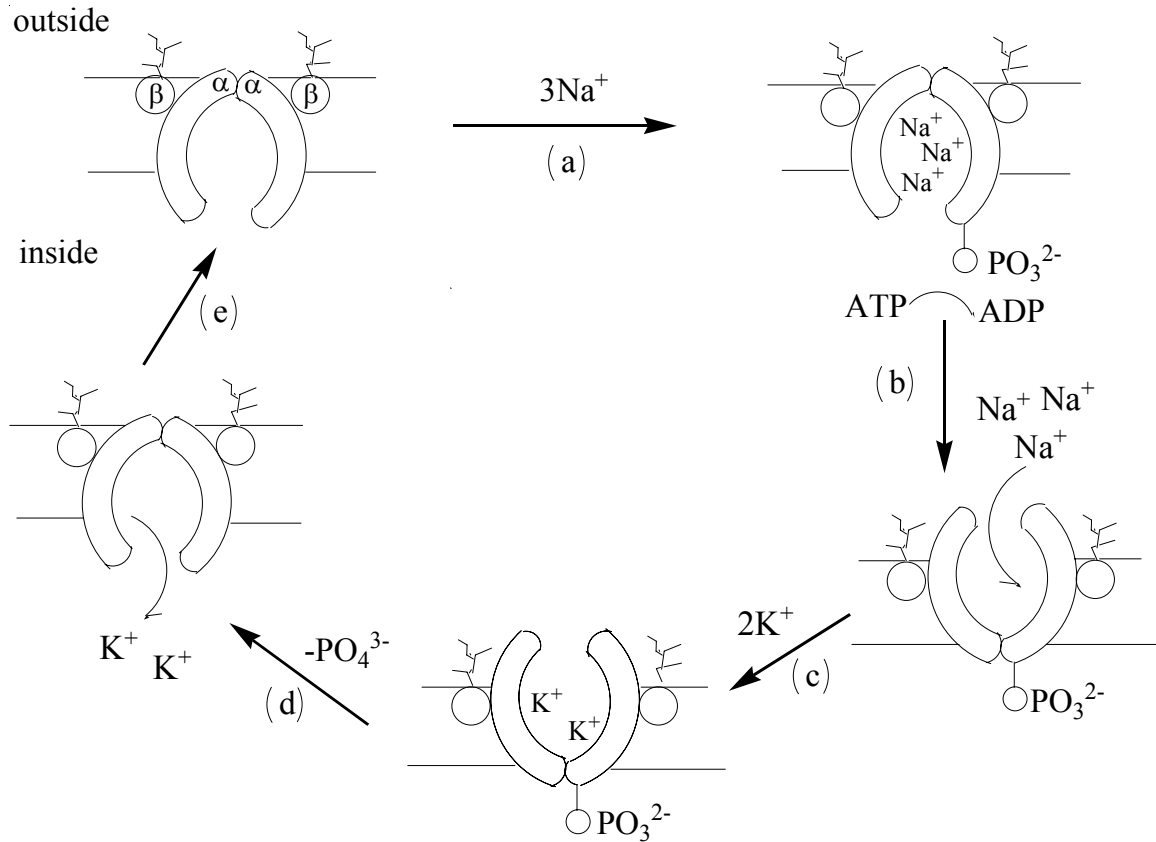
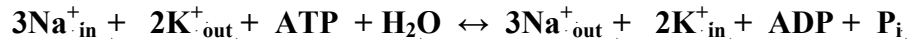
Sodium is a vital element. The human diet must contain a sensible amount of sodium. The sodium cation is the main extracellular (outside cells) cation in animals and is important for nerve function in animals. Potassium salts are essential for both animals and plants. The potassium cation (K^{+}) is 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, $\text{Na}^{+}\text{-K}^{+}\text{ATPase}$ is the major component of the $\text{Na}^{+}\text{-K}^{+}$ 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 $\alpha_2\beta_2$ tetramer.

Mechanism



First of all the Na⁺P-K⁺P pump bound with ATP binds 3 intracellular Na⁺P ions (step a). This starts phosphorylation of an Asp residue leading to a conformational change, which weakens Na⁺P binding and moves Na⁺P out of the cell (step b). A conformational change in the pump exposes the Na⁺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 K⁺P ions (step c). Potassium binding leads to dephosphorylation and return to original conformation (step d). In this conformation ATP binds and the pump reorients to release K⁺P ions inside the cell (step e). The pump is ready to go again.