PG Third Semester
Bioinorganic Chemistry
Unit IV

Contents:

Role of metal ions in biology and their toxic effects; Iron management in biological systems - siderophores, ferritin and transferrin; Dioxygen storage and transport - structure of myoglobin and haemoglobin, cooperativity of O_2 binding in haemoglobin, Bohr effect and Hill coefficients; Electron transfer proteins (structure and function) - Fe-S proteins, cytochromes and plastocyanin; Structure of nitrogenase and its role in di-nitrogen fixation; Structure and function of vitamin B_{12} and mechanism of 1,2-shift reaction; Inorganic therapeutics - chelate therapy, metal based drugs.

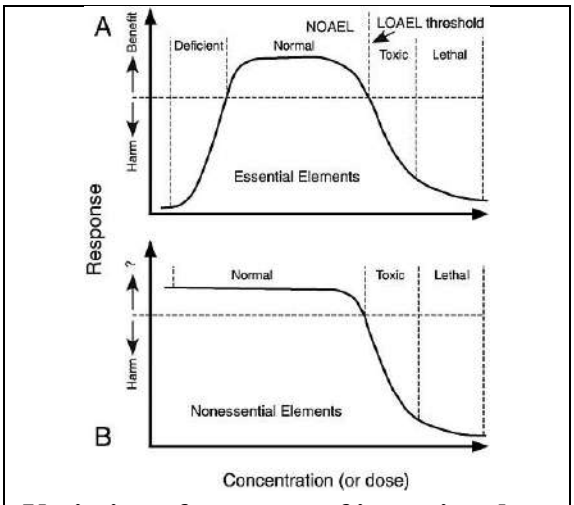
Role of metal ions in biology and their toxic effects
Lecture 1 & 2

Essential elements in biology

@ Essential metal ions are necessarily required in biological processes.
@ About 40 essential elements are present in biology.
@ Lighter elements (upto Z = 35) are biologically important (Exception: Mo, Sn, W, I)
@ Classification is based on percentage or availability with respect to Human Body Weight.
@ Three categories viz. bulk, trace and ultra trace.
@ Amount of required metal ions does not measure the importance of the metal

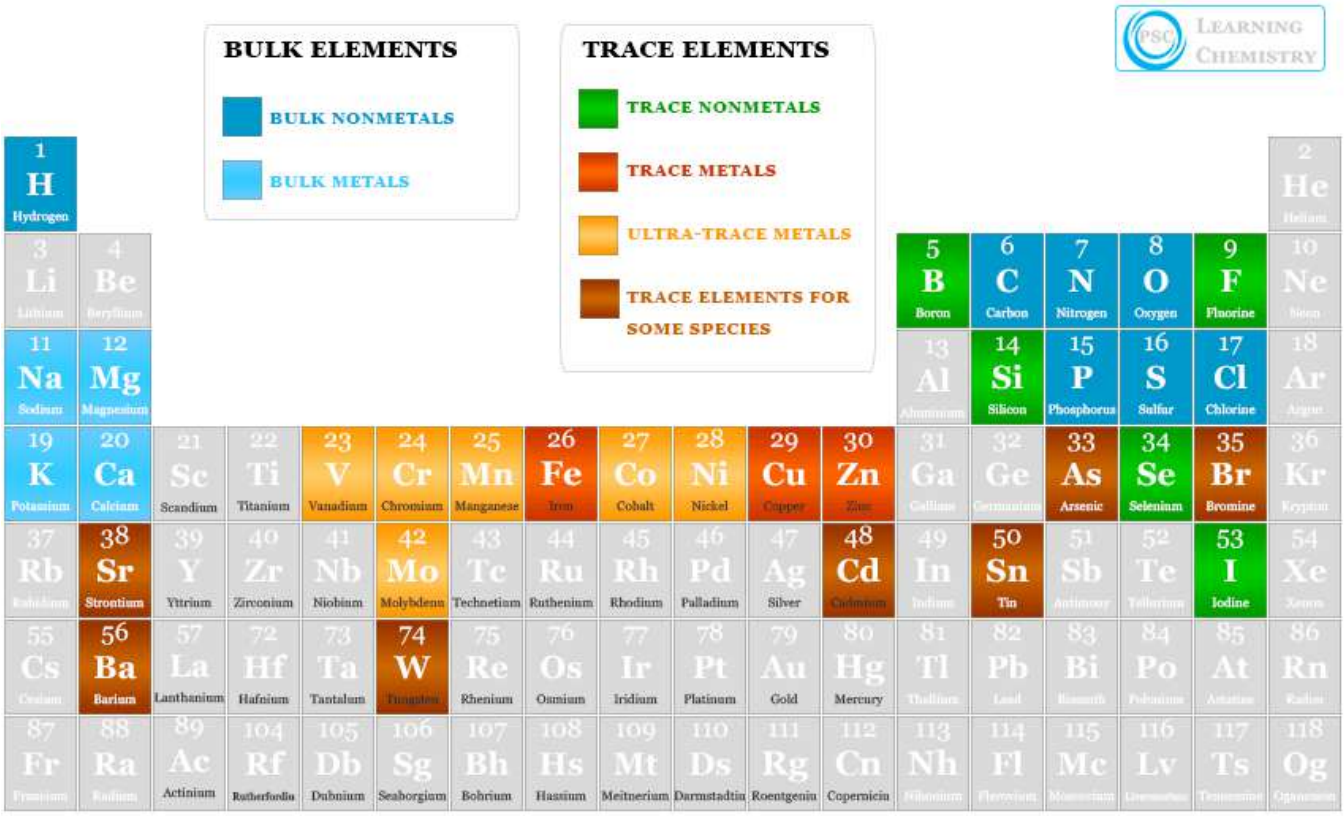
Bulk elements	**Trace elements**	**Ultra trace elements**
1-2% of HBW	< 0.01% of HBW, requirement 10^{-4}-10^{-1} g/mol	at ppm level, 0.0002% of HBW
H, C, N and O (constituent) Na, K, Ca, Mg, P, S, Cl	Fe (4-5 g), Cu, Zn Mn, Mo Co, F (2.6 g), I	Li, Si, V, Cr, Ni, Se, Br, Sn, W

@ Biometals are classified as essential and beneficial metals
@ Deficiency of essential metals lead to malfunctioning of biological processes (no survival)
@ In absence of beneficial metals, life process gets hampered (not death)
@ Role of metal ion can be structural (maintain structure) and functional (active site)
@ Good correlation between bioavailability and geochemical distribution of metal ions exists.
@ Pb, Cd, Hg are extremely toxic at trace amounts
@ Essential elements can be toxic at higher concentration, lead to deficiency disease at lower concentration

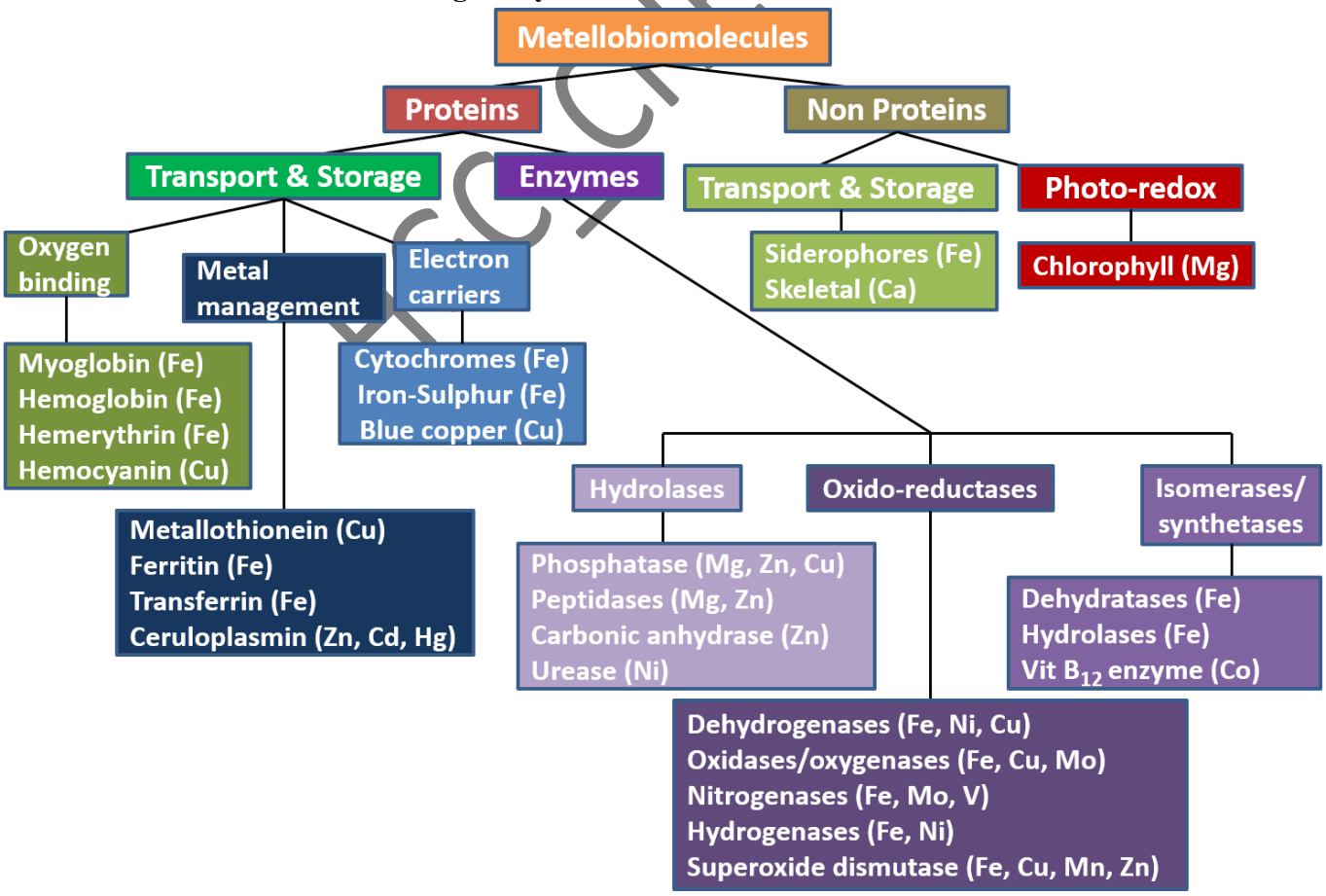


Variation of response of incoming dose

An overview of essential and trace elements in biological systems



An overview of metal ions in biological systems



Essential non-metals

Elements	Biological function	Deficiency sign
F	Structure of teeth and bones, used as CaF_2 by some mollusks	Growth depression, dental caries
B	Control of membrane function, nucleic acid biosynthesis, lignin biosynthesis (weak evidences)	Growth of angiosperms, impaired nitrogen fixation
Si	Structural role in connective tissues and ontogenetic cell	Growth depression, bone, and matrix deformities
P	Important constituents of DNA, RNA, bones, teeth, phospholipid, ATP, ADP and metabolic intermediates.	-
S	Essential in proteins (tertiary structure S-S links), involved in vitamins and fat metabolism.	-
Cl	Present in electrolyte and digestive juices.	Impaired growth in infants
I	Essential in many organisms, constituent of thyroid hormones-T_3 and T_4, important in metabolism and growth regulation	Goiter, reduced thyroid function
Se	Constituents of glutathione peroxidase, thioredoxin reductase enzymes, protection against oxidation of erythrocytes.	Muscle and pancreases degeneration, hemolysis

Essential metals

Elements	Biological function	Deficiency sign
Mn	Activates superoxide dismutase, carbohydrate metabolism, O_2-evolution reaction in photosynthesis	Growth depression, bone malformation
Mo	Used in enzymes with nitrogen fixation and nitrate reduction, Xanthine-oxidase	Growth depression
Co	Activates several enzymes (e.g., Vit-B_{12})	Pernicious anemia, growth retardation
Cr	Involved in glucose metabolism and diabetes, potentiates the effect of insulin.	Insulin resistance
V	Control of Sodium-pump, inhibition of ATP's, p-transferase	Reduced growth, impaired reproduction
Ni	Constituent of several enzymes like hydrogenases, plant ureases, CO dehydrogenases	Impaired liver function, reduced nitrogen utilization and iron metabolism.
Al	Activate succinic dehydrogenase and δ-aminolevulinate dehydrase (Heme synthesis)	-

Biochemical roles of Na

@ Sodium is a major cation of extracellular fluid (blood plasma and interstitial fluids).

@ Actual concentration differs for different type of the cell, ($[Na^+]_{out}/[Na^+]_{in} = 15$)

Functions:

a) Important in nerve-functioning and transmission of signals

b) Regulates uptake of nutrients and flow of water across the cell membrane.

c) Involved in the transport of sugars and amino acids into the cells.

d) Maintains of osmotic pressure of the body fluid and regulates blood pressure
e) Helps in muscle contraction
Deficiency: Initially nausea, vomiting, loss of energy and confusion. Serious deficiency results hyponatremia causing seizures, coma even death
Treatment: Intravenous fluid of sodium solution
Excess: Elevated blood pressure (hypertension)
Biochemical role of K
@ Potassium is a major cation of intracellular fluid.
@ Actual concentration differs for different type of the cell, ($[K^+]_{out}/[K^+]_{in}=25$)
Functions:
a) Participates in glucose metabolism to produce ATP, protein biosynthesis and activation of enzymes such as pyruvate kinase.
b) Essential in transmission of nerve impulse and cardiac function
c) Balance body fluids and regulates blood pressure.
d) Helps in muscle contraction.
Deficiency: Fatigue, irregular heartbeat, muscle weakness, increased urination, constipation
Treatment: at mild condition oral potassium pills and at severe condition potassium via intravenous mode

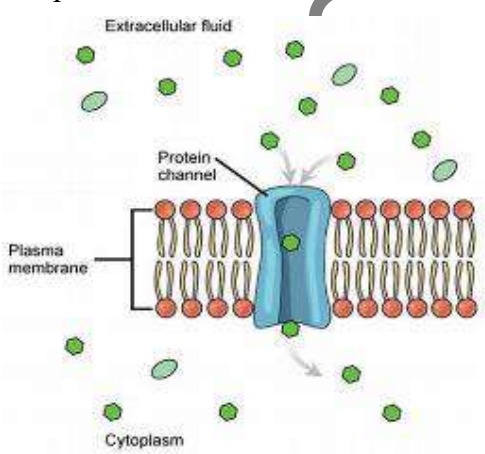
Transport of ions

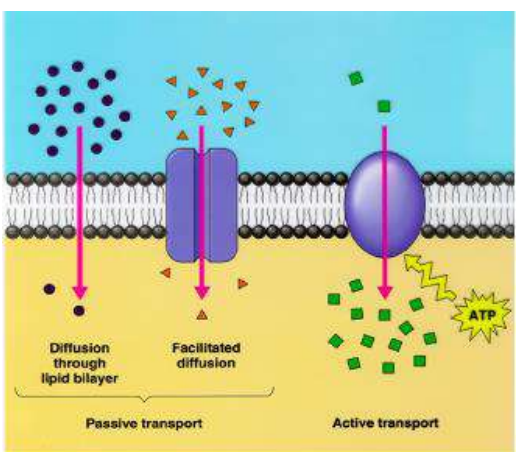
@ Active ion transport involves the movement of ions across a cell membrane against their concentration gradient, requires energy to drive the transport.

@ This process is typically facilitated by membrane proteins such as ion pumps.

@ Transport of ions across cell membranes is a critical process for various cellular functions, including signal transduction, muscle contraction, & regulation of enzyme activities.

@ Ions are transported across cell membranes through channels and pumps.





Channels vs Pumps

@ Channels & pumps are both proteins that help transport ions across cell membranes.

@ Channels allow ions to passively diffuse down their concentration gradient (high to low) and pumps actively transport ions against their concentration gradient (low to high) by consuming energy.

@ Channels are like gates that open & close to allow ions to flow through and pumps are like revolving doors that move ions across the membrane.

@ The main difference between channels & pumps is that channels require only a single gate, while pumps require at least two gates that should never be open at once.

Membrane Pumps (ATPases)

@ Membrane pumps play a crucial role in maintaining the appropriate ion concentrations inside and outside cells, contributing to the overall function and homeostasis of cells.

@ There are different types of membrane pumps.

@ Primary active transporters and secondary active transporters

@ Primary active transport is a mechanism of active transport that directly uses energy derived from the hydrolysis of ATP to transport ions or molecules across a cell membrane against their concentration gradient

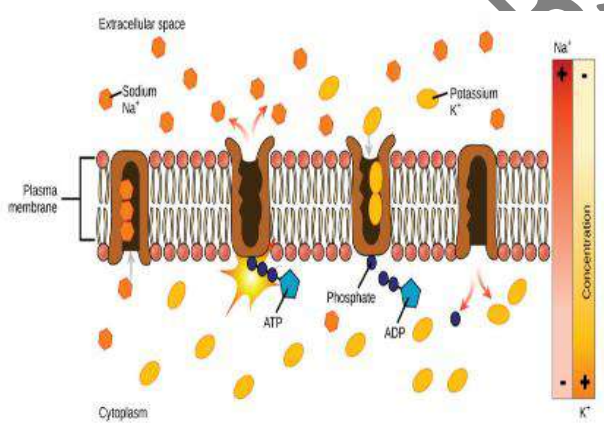
@ This process is typically mediated by specific proteins known as ATPases or pumps.

@ Three types of ATPase or pumps are involved in active transport of ions

a) Sodium-Potassium Pump (Na^+/ K^+ ATPases)

b) Sarco/Endoplasmic Reticulum Calcium ATPase (SERCA)

c) Proton Pump (H^+/K^+ ATPase)



Different types of ATP driven transporters

@ Considering the stoichiometry of the transport process, these are classified as

@ **Uniport**: movement of only one type of substance in certain direction

@ **Symport**: movement of two different types of substances in the same direction (Na^+-glucose transporter - indirectly powered by ATP hydrolysis)

@ **Antiport**: movement of two different types of substances in the opposite directions

@ These transporters are directly or indirectly powered by the ATP hydrolysis

@ Transporters directly powered by ATP hydrolysis: Ca^{2+} pump (uniport), Na^+-K^+ pump (antiport)

@ (Na^+-K^+ pump - directly powered by ATP hydrolysis,

@ Transporter indirectly powered by ATP hydrolysis: Na^+-Ca^{2+} exchanger (antiport) , Na^+-glucose transporter (symport)

@ Uphill flow - directly powered by ATP hydrolysis

@ Downhill flow - indirectly powered by ATP hydrolysis (cotransporter or secondary transporter)

Sodium potassium pump

@ Ion pump maintains the active transport of ions across the cell membrane.

@ The concentration gradient of Na^+ and K^+ ions across the cell-membrane is achieved by an energy repairing pump known as Na^+-K^+ pump (antiport).

@ The pump transports three Na^+ out of the cell in exchange for two K^+.

@ The pump is driven by an integral enzyme, Na^+/K^+ ATPase (P-type)

@ The energy for required for pumping these ions is obtained from hydrolysis of intracellular ATP catalyzed by Mg^{2+}-ions.

$$3Na^+_{intra} + 2K^+_{extra} + Mg^{2+}\text{-}ATP^{4-} + H_2O \rightarrow 3Na^+_{extra} + 2K^+_{intra} + Mg^{2+}\text{-}ADP^{3-} + H_3PO_4^{2-} + H^+$$

@ Different Na^+/K^+ ratio (and the correct concentrations of Na^+ and K^+) inside and outside the cell develops an electrical potential across the membrane (essential for functioning of nerve & muscle cells).

Na^+/K^+ ATPase

@ The Na^+/K^+ ATPase exists in two forms, depending on its orientation to the interior or exterior of the cell and its affinity for either Na^+ or K^+ ions.

@ The enzyme Na^+/K^+ ATPase (280 kD) is a tetrameric ($\alpha_2\beta_2$) protein.

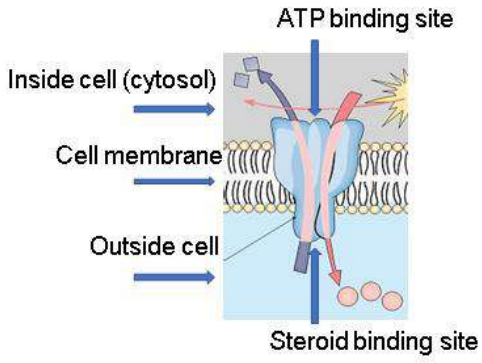
@ The larger unit (two α units, 100 kD) contains the ATP binding site (acts as revolving door, pass through plasma membrane).

@ The α-chains contain the selective metal binding sites and phosphorylation sites (one end).

@ Other end of α-chains has the steroid inhibitor binding site.

@ The α-chains traverse the plasma membrane

@ The smaller unit (two β units, 40 kD) primarily contains carbohydrate.

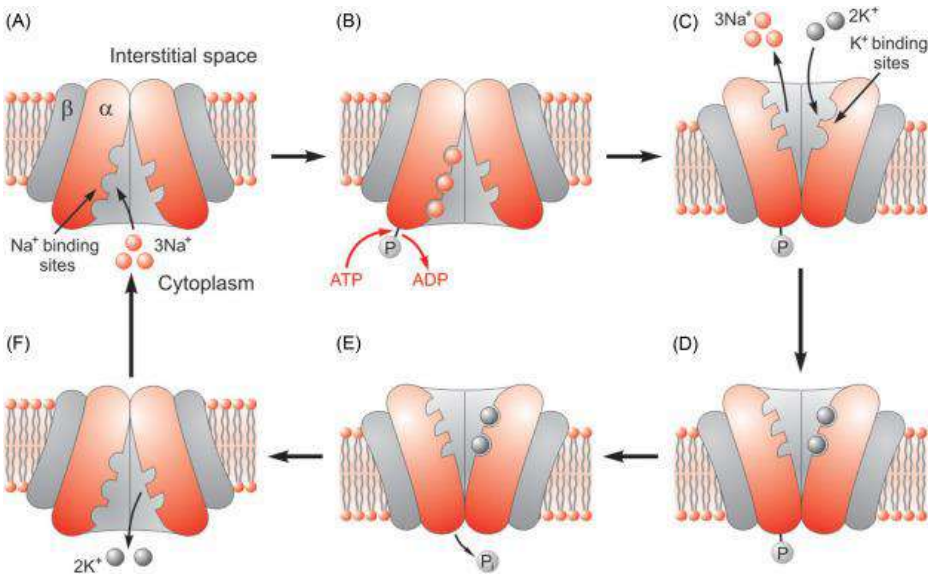


Mechanism of sodium potassium pump

@ Na^+/K^+ ions are pumped against their concentration gradients by the enzyme Na^+/K^+ ATPase coupled with hydrolysis of ATP catalyzed by Mg^{2+}-ions.

@ In the function of Na^+/K^+ pump, one cycle involves the transport of $3Na^+$ ions from inside the cell to the outside and $2K^+$ ions from outside the cell to inside the cell

@ Binding of $3Na^+$ ions with the protein (α_2 unit) changes the local polarities to facilitate the binding of ATP, α_2 unit is phosphorylated and ADP is released after hydrolysis



@ The phosphorylation changes the conformation (eversion) of protein (E1)

@ In this conformation, the Na^+-binding sites become open and three Na^+ is released to the extracellular fluid

@ The open channel binds two K^+ from outside causing dephosphorylation from the protein chain.

@ Conformational changes (eversion) then again occur (E2), opening the K^+-binding site to cytosol finally leading to release of two K^+

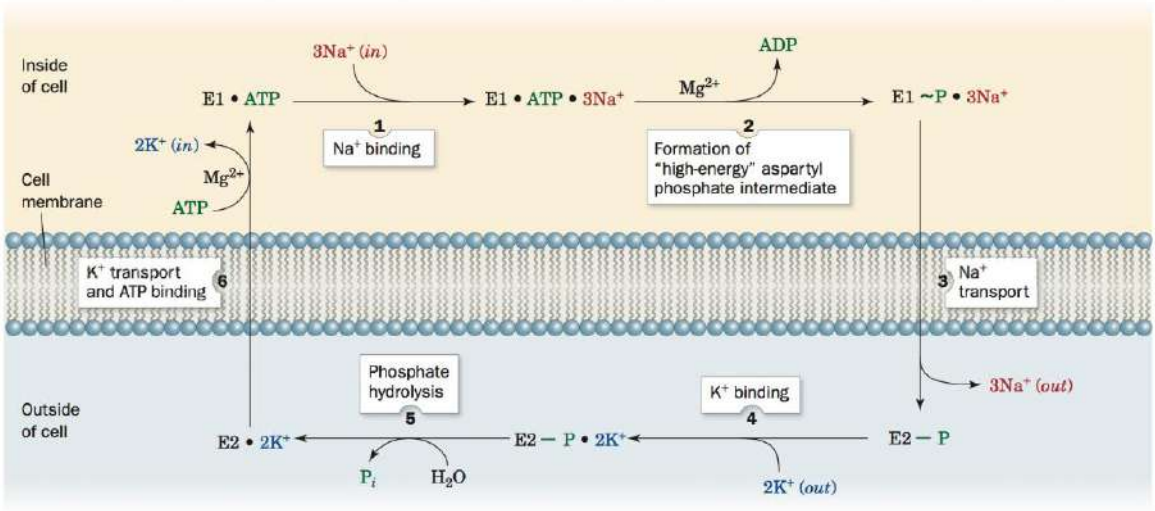
@ This leads to the original conformation of enzyme to initiate a new cycle again.

@ The overall process of the uphill transport of Na^+ and K^+ ion is

$$3Na^+_{intra} + 2K^+_{extra} + Mg^{2+}\text{-}ATP^{4-} + H_2O \rightarrow 3Na^+_{extra} + 2K^+_{intra} + Mg^{2+}\text{-}ADP^{3-} + H_3PO_4^{2-} + H^+$$

@ E1 projects the ion binding sites towards the cytosol, E2 projects the same outside the cell

@ Na^+ binding triggers phosphorylation (E1) and K^+ binding triggers dephosphorylation (E2)



Role of Mg^{2+} ion:

@ Mg^{2+} plays two crucial roles viz. catalyzes the ATP hydrolysis and structure forming effect to change the protein conformation.

Importance of Na^+-K^+ pump

@ Extrusion of Na^+ from the cytosol of the cell by this pump is important for animal cells to control water content osmotically

@ If $[Na^+]$ inside the cell were not depleted, the animal cells lacking in cell walls would swell and burst because the cell membrane is permeable to water

@ The concentration gradient $[Na^+]_{out}/[Na^+]_{in} \approx 15$ maintained by this pump powers

(a) the transport (symport) of glucose and amino acids in some cells

(b) The transport of Ca^{2+} through the Ca^{2+}-Na^+ exchanger (antiport)

@ The electrochemical gradient developed by this pump is responsible for the generation and propagation of nerve impulses

Biochemical role of Ca

@ Ca^{2+} ions (extra cellular fluid) is a major component of bones and shell (teeth)

@ Bones - hydroxy apatite, $[Ca_5(PO_4)_3.OH]$ and Teeth - fluorapatite, $3[Ca_3(PO_4)_2]CaF_2$.

@ It activates proteins and enzymes, participates in muscle contraction, blood clotting, glycolysis (metabolic degradation of glucose), gluconeogenesis (metabolic degradation of glucose) and messenger system for hormonal action

Deficiency: Osteoporosis, hypercalcemia or tetanin (spontaneous motor-neurons transmission), disturbed cardiac function.

@ Excessive Ca^{2+} ions into a cell may damage it or cause apoptosis by necrosis.

@ Excess of Ca^{2+} ions also lead to stone formation, hardening of arteries and cataract in eyes.

@ Ca^{2+} concentration in plasma is controlled by calcitriol, parathyroid and calcitonin hormones

@ Calcitriol promotes the absorption of Ca from gastrointestinal tract

@ Parathyroid hormones elevate the Ca level in plasma by decalcification and reabsorption

@ Calcitonin arrests gastrointestinal absorption of calcium from food and reduces its loss.

Muscle Contraction

@ Ca(II) in the cytoplasm of muscle fibers (sarcoplasm) plays a regulatory role in muscle contraction

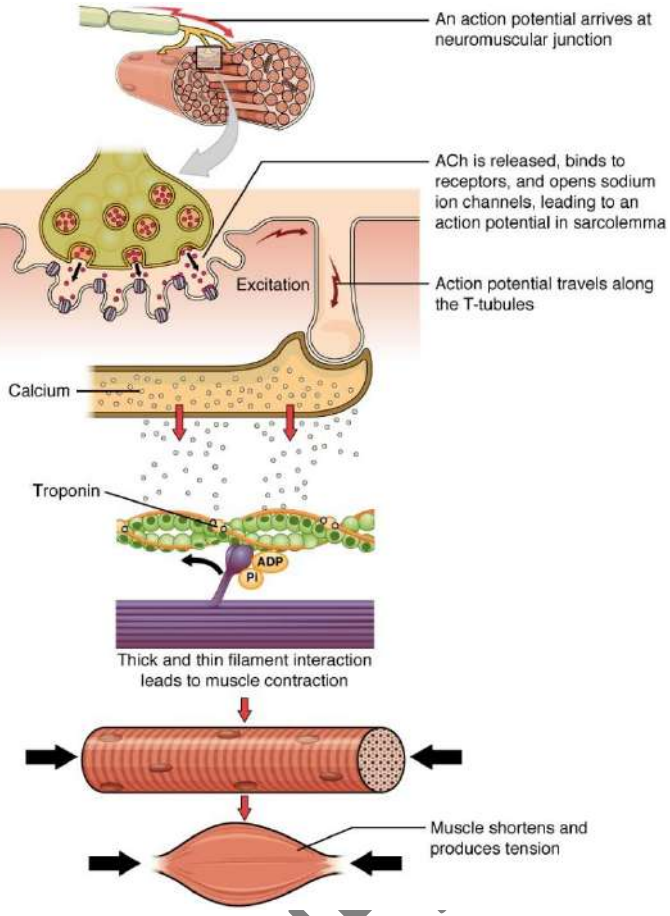
@ Ca binds to troponin-C and calmodulin calcium modulated proteins, necessary for promoting muscle contraction.

@ Release of Ca^{2+} ions from sarcoplasmic reticulum control the muscle contraction by allosteric mechanism

@ Muscle contraction force arises from the joint interaction of actin, myosin and ATP

@ Interaction between actin and myosin produces actomyosin.

@ Actomyosin carries out hydrolysis of ATP and energy released from hydrolysis is used for muscle contraction



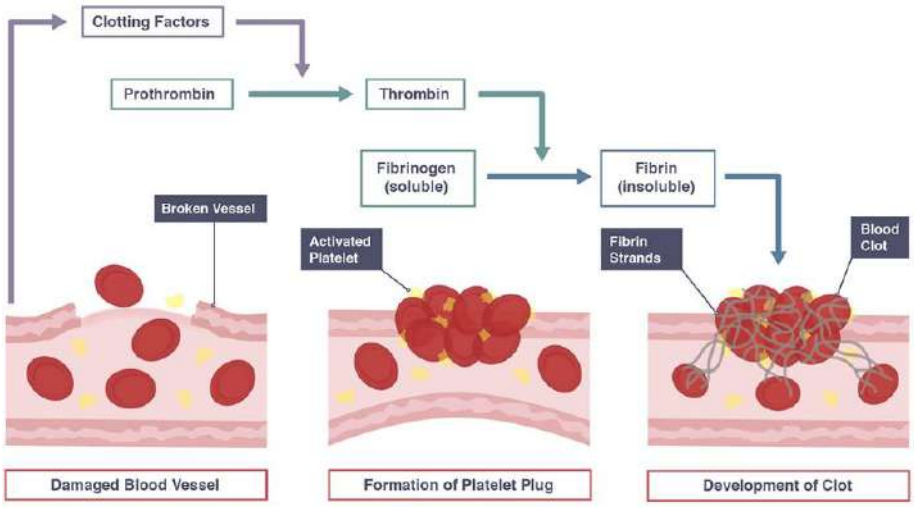
Blood clotting

@ Blood clotting process involves several proteins with the participation of Ca^{2+} ions

@ Formation of thrombin from prothrombin is of remarkable importance.

@ Blood contains prothrombin, a soluble protein which is converted to the enzyme thrombin by the action of prothrombin activation in presence of Ca-ion.

@ Thrombin, again aided by Ca-ion, clots the blood by converting soluble fibrinogen into insoluble fibrin.



Biochemical roles of Mg

@ Mg is essential to all organisms; ~25-30 g is present in human body.

@ ~ 60% is present in skeleton and rest is primarily present in cell.

@ The most important role is its involvement the photosynthetic activity chlorophyll.

@ Mg(II) acts as a cofactor of several enzymes that catalyzes the hydrolysis of phosphates.

@ Mg(II) is required in biological processes such as oxidative phosphorylation, DNA-transcription, RNA function, protein synthesis etc.

@ It plays important role in stabilizing DNA and RNA structure through neutralization of negative charge present on phosphate backbone.

@ Nerve impulse transmissions, muscle contraction and metabolism of carbohydrates are also associated to the interaction of Mg with nucleic acids.

Deficiency: Apatite, nausea, vomiting, weakness, fatigue, irregular heartbeat

Biochemical roles of Fe

@ Fe is the most abundant metal in biological system (4-5 g in human body)

@ All plants, animals, and bacteria use Fe, except for lactobacillus.

@ Its two oxidation sates viz. Fe (II) and Fe (III) are interconvertible (0.77 to -0.50 V). By varying the ligands, the redox potential can be monitored

@ An adult human requires ~ 10 mg of Fe-per day, for mensuration (~ 18mg) and pregnant or lactating women (~ 40mg) the amount is higher.

@ High spin Fe(II)/Fe(III) complexes with bioligands are labile while that with low spin (porphyrin) are inert.

@ The distribution of Fe in specific Fe-complexes depends strongly on their function.

@ About 70% of total Fe is present in Hb (oxygen-transport) and Mb (muscle oxygen-storage)

@ It is also incorporated in other metalloproteins (catalase, peroxidase, Fe-S protein, cytochromes)

@ Fe is also present in ferritin and transferrin

@ 70% of daily requirement is required in biosynthesis of blood (not absorbed from diet)

@ Destruction of RBC gives about 20 mg Fe and rest comes from Fe-storage site

Protein	**MW (kDa)**	**Function**	**Coordination sphere**
Hemoglobin, Fe (II)	64.5	Plasma O_2 transport	Heme (4-Fe)
Myoglobin, Fe (II)	17	Muscle O_2 transport	Heme (1-Fe)
Transferrin, Fe (III)	76	Plasma Fe transport	Non-heme (2-Fe)
Ferritin, Fe (III)	444	Cell Fe-storage	Non-heme (4500-Fe)
Hemosiderin, Fe (III)		Cell Fe-storage	Non-heme (5000-Fe)
Catalase, Fe (II)	280	H_2O_2 metabolism	Heme (1-Fe)
Cytochrome c, Fe (II/III)	12.5	Electron transport	Heme (1-Fe)

@ Fe is crucial to the survival of living organisms and plays role in

a) Ribonucleotide reduction (DNA synthesis),

b) Energy production (respiration)

c) Energy conversion (photosynthesis)

d) Nitrogen reduction

e) Oxygen transport (respiration, muscle contraction)

f) Oxygenation (steroid synthesis, solubilization and detoxification of arenes)

Fe-protein/enzyme	Function
Hemoglobin, myoglobin	Oxygen transport and storage
Cytochrome, Fe-S proteins	Respiration, electron transfer
Ferritin, hemosiderin	Fe storage
Transferrin	Fe transport
Metalloenzymes (oxidases, hydrogenases, reductases, nitrogenase, catalase, peroxidase)	oxygenation, H_2 production and consumption, nitrogen fixation, H_2O_2 metabolism

Deficiency: Anemia, β-thalassemia, heart palpitations, irregular heartbeat

@ Fe-deficiency is immediately reflected in terms of appearance of anemia

@ Anemic condition may also arise from Vit B_{12} deficiency (pernicious anemia), erratic Cu metabolism, Pb poisoning and even some time for genetic disorder (SCA)

Treatment: $FeSO_4$ pills coated with fructose or lactose, ferrous fumarate, ferrous gluconate etc. are clinically recommended. Sometime ascorbic acid is added with $FeSO_4$ to aid adsorption.

Toxicity: Hemochromatosis (bronze diabetes), hemosiderosis, lesions in gastrointestinal tract, liver damage.

Sickle cell anemia (SCA): Arises from the replacement of glutamic acid residue at 6-position in the β-chain with valine (hydrophobic side chain) in Hb

Biochemical roles of Cu

@ Cu is the third most abundant (200-300 mg in human body) metal in biology.

@ Essential to all organisms and constituents of redox enzyme and hemocyanin.

@ Also present in ceruloplasmin, cytochrome-c oxidase, catalase, superoxide dismutase (SOD).

@ Dietary requirement of Cu is nearly 2-3 mg per day

@ Absorbed in the intestines and carried to liver. Also found in heart, brain and even in kidney

Sources: organ meat, shellfish, fish, nuts and seeds as well as whole grains

Deficiency: demineralization of bones, anemia, decolorization of skin and hair, fragility of arteries, weight loss, muscle soreness, progressive brain disease in infants etc.

Treatment: Cu supplemented food, Cu(II)-(L-histidine) in Menkes' diseases

Excess: Wilson's disease (excess Cu in liver in brain due to its high intestinal absorption)

Treatment: Tetrathiomolybdate is used in treatment of Wilson disease.

Cu protein/enzymes	Metabolic functions
Ceruloplasmin	Oxidase activity and Cu transport, oxidation of Fe(II) and Fe-metabolism.
Cytochrome-c oxidase	Terminal oxidase enzyme in mitochondrial respiratory chain, involved in electron-transport.
Superoxide dismutase (SOD)	Intracellular and extracellular enzymes involved in defense against reactive oxygen species, destruction of superoxide radical
Tyrosinase	Enzymes catalyzing mechanism and other pigment production.

Blue copper protein and hemocyanin	Electron transfer and O_2 transport (molluscs/Arthropoda) respectively
Human serum albumin	Cu(II) transport

Biochemical roles of Zn

@ Zn is the second most abundant (2-3 g in human body) metal in biology

@ Dietary requirement of Zn is about 10-15 mg per day.

@ Zn is stored in kidneys and liver in metallothionine. The prostate gland is very rich in Zn.

@ Essential constituent of enzymes (>70) such as carbonic anhydrase, carboxypeptidase, alcohol dehydrogenase, alkaline phosphatase, superoxide dismutase etc.

@ Biochemical function of Zn is based on its Lewis acid character.

@ Zn stabilizes coiled ribosomes and plays a significant role in sexual maturation (male) and reproduction (female-growth factor)

Sources: Coriander, prawn, garlic, mushroom, pea, nuts, fruit

Deficiency: Retarded growth, inhibition of sexual maturation, anemia, loss of appetite, test sensitivity, acne and rashes, poor neurological function etc.

Treatment: Zn-supplemented food and $ZnSO_4$ capsule is clinically recommended

Zn protein/enzyme	**Functions**
Carbonic anhydrase (known first, 1939)	Hydration of CO_2 and dehydration of H_2CO_3 (conversion of CO_2 to H_2CO_3 and vice versa)
Carboxypeptidase A (known second, 1955)	Hydrolysis of C-terminal peptide linkages during digestion of protein
Zn-finger protein	Recognize DNA base sequences during replication and transcription of DNA
Alcohol dehydrogenase	Catalyses the hydride transfer from alcohol to NAD^+
DNA polymerase	Polymerization of DNA with the formation of phosphate ester
Superoxide dismutase	Controls and stabilizes the enzyme SOD

Biochemical roles of Mn

@ Its deficiency induces retarded growth, skeletal abnormalities, transient dermatitis, hypocholesterolemia, ataxia in infants, reproductive failure

@ In glycoprotein synthesis, the Mn-dependent enzymes like glycosyl transferase, glactosyl transferase play important role. the impaired glycoprotein synthesis leads to skeletal abnormalities and ataxia.

@ In glucose metabolism, Mn^{2+} actively participates in smooth functioning of pyruvate kinase.

Source: Whole grains, mussels, nuts, soybeans, leafy vegetables, black peeper, legumes, brown rice etc.

Treatment: Mn enriched food and sometimes $MnSO_4$ is clinically recommended.

Biochemical roles of Cr

@ Cr is part of GTF (glucose tolerance factor) which includes one Cr^{3+} and provides aid in insulin binding to the site of action

@ It helps in lowering the cholesterol and triglyceride levels

@ Excess of Cr can be carcinogenic, causes skin and lung cancer

Biochemical roles of Co

@ It is the metal center in Vit B_{12} (Cobalamin)

@ It promotes RBC formation and activates some enzymes

@ Excess of Co can result in vomiting and nausea, heart problems, thyroid damage

@ Co deficiency may cause anemia

Some metal dependent Human systems

Human systems	**Metal disbalance**	**Diseases**
Nerve	Na, K, Mg, Ca	Epilepsy, personality change
Muscular	Na, K, Fe	Myotonia
Cardiovascular, Heart, Blood	Mg, Ca, Na	Hypertension
Blood Vessels	Na, K, Fe, Cu	Heart failure
Digestive, Liver	Zn, Fe Cu	Liver cirrhosis Wilson disease
Urinary	K, Mg, Ca	Renal insufficiency
Bone and skeleton	Ca, Mg	Osteoporosis

Chemical Toxicology

@ Chemical toxicology is the study of toxic chemicals and their modes of action.

@ Toxicity is the degree to which a chemical can damage an organism.

@ Toxic chemicals disturb the biochemical processes.

@ Metals listed as environmental hazards (Al, Co, Pb, Hg, Mo, Ag, Sn, Zn etc.) can be essential in trace amount.

@ Defining the essential and toxic limit of an element is confusing.

@ Schwartz coined the term "concentration window" to draw arbitrary lines of demarcation.

Essential at trace levels for sustaining life, deficient at lower level than essential limit and toxic at higher level than essential limit.

@ Toxicity can occur by the pathway of administration (applied on skin, ingested, inhaled, injected), the time of exposure (short/long term), the number of exposures (a single/multiple doses over time), the physical form of the toxin (solid, liquid, gas), the genetic makeup of an individual.

@ Toxic metals can sometime imitate the action of an essential element and thereby interfering with the metabolic process.

@ Metals in one oxidation state may be essential (Cr(III)) while in other it can be toxic (Cr(VI- carcinogenic)

@ Toxic chemicals can be classified according to their function and effect exerted on the body (mutagens, carcinogens etc.)

@ Toxic chemicals can attack at the active site of enzymes/metalloenzymes inhibiting their function/action

@ For example, Hg(II)/As(III) ions can attack at S-atoms present in the active sites of enzymes and Cd(II) can substitute Zn(II) in metalloenzyme, resulting in toxicity.

General aspect of mechanism of toxicity

@ Replacement of certain active moiety (phosphate by arsenate)

@ Deposition of excess metals in vital organs (severe irritations)

@ Cell damage by radiation from the radioactive elements (malignancy or mutation)

@ Interference through competitive inhibition (Se may replace S in amino acids)

@ Interference with the proteins and enzymatic process (heavy metals affinity for -SH group).

Toxicity of Iron

@ Fe is essential to all organisms and is not excreted

@ Excessive intake for long duration may lead to Fe-deposition and Fe-toxicity.

@ The excess of Fe is deposited primarily in liver, heart and kidney.

@ Acute Fe toxicity results from an accidental intake of Fe(II) tablets causing erosion of the gastrointestinal tract.

@ Fe overload leading to chronic Fe poisoning arises in some genetically disordered diseases.

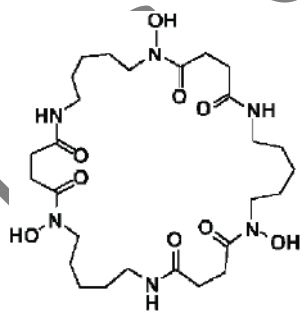
@ Chronic Fe poisoning may also arise from regular excess intake of iron from cooking vessels. For example, African siderosis (hemosiderosis) found in the members of Bantu tribe in South Africa, who consume beer brewed in iron pots.

@ In hemosiderosis, Fe is deposited in different parts of the body among the patients receiving repeated blood transfusions.

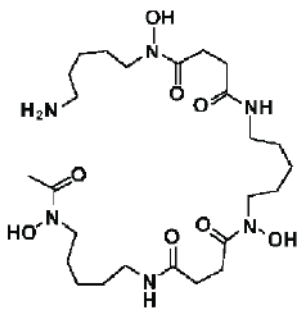
@ In hemochromatosis (a genetic disorder), deposition of Fe occurs in organs like liver, spleen, pancreas and skin. It may result in liver cirrhosis, pancreatic fibrosis and bronze pigmentation on the skin (bronze diabetes).

@ Fe poisoning is a leading cause of death in children (prenatal/paediatric).

@ The chelating antidote used for detoxification of Fe is the siderophore desferrioxamine, having a very high thermodynamic affinity specially for Fe(III).



desferrioxamine E



desferrioxamine B

Toxicity of Aluminium

@ Al is toxic to most plants and slightly toxic to mammals.

@ Commercial deodorant, baking powder also contain Al. Moreover, Al foil and Al cookware that we use also chief source of Al deposition in body.

@ Al(III) is a hard cation and has tendency to bind strongly to N- and O-donor ligands in biomolecule and deactivate them.

@ Alzheimer's disease arises from increased AI(III) concentration in brain tissues. Al(III) crosses the blood brain barrier and is progressively deposited in large pyramidal neurons of the hippocampus, cortex and other regions vulnerable in Alzheimer's disease.

@ It is called the soft in head mineral because it is associated with memory loss and dementias

@ Once absorbed, Al accumulates in bone (majority), brain, liver and kidney leading to osteoporosis

@ Al(III) can inhibit δ-aminolaevulinic acid dehydratase (ALAD) involved in biosynthesis of heme. ALAD binds eight Zn(II) ions for its enzymatic activity. It probably competes with Zn(II), inhibits the enzyme resulting in anaemia.

@ Al(III) can also inhibit different Mg-dependent enzymes like kinases and ATPase.

@ Al-toxicity are also associated with renal function, and breast and prostate cancer

@ Tea plants accumulate AI(III) and stores it in leaves. On addition of milk, insoluble $AIPO_4$ is formed and reducing its bioavailability. But in lemon tea, formation of soluble AI(III)-citrate complex facilitates the absorption of AI(III) in gastrointestinal tract.

@ Presence of $AI(OH)_3$ and $Si(OH)_4$ in AI(III)-based antacids results the formation of hydroxyaluminosilicates (stable in intestine), thereby reducing bioavailability of AI(III).

@ 1,2-dimethyl-3-hydroxypyrid-4-one (L1) and desferrioxamine are recommended for Al detoxification.

Toxicity of Copper

@ Cu is essential for all forms of life.

@ Cu is primarily absorbed in brain and organs like liver, kidney and intestine.

@ Problem arises when it is in excess. To be toxic, Cu intake must be in gram amounts or continual intake of ~ 250 mg/day.

@ Excess Cu leads to irritation of gastro-intestinal tract.

@ Wilson's disease arises due to genetic disorder in Cu-metabolism. Cu-metabolism is prohibited due to interference in synthesis of ceruloplasmin or any impairment of Cu- binding to this protein.

@ In Wilson's disease, large amount of copper is present in blood stream, damaging the erythrocyte membrane. Cu is finally deposited in liver and brain developing the hepatic and neurologic disorders respectively.

@ Symptom of Wilson's disease are hepatic cirrhosis (liver damage), neurological damage, brown/green rings in the cornea of the eyes, lack of coordination (ataxia), progressive mental deterioration.

@ Some other features Wilson's disease are: low levels of Cu in plasma and increased excretion in urine, high intestinal absorption of Cu, renal damage due to deposition of Cu leading to an increased excretion of amino acids, proteins, hemoglobin through urine.

@ To reduce the Cu-overload, the chelating drugs like $Na_2Ca(EDTA)$, 2,3-dimercaptopropan-1-ol (BAL), D-penicillamine are clinically recommended.

@ Zn-salts are also recommended for the treatment of Wilson's disease.

@ Trien(triethylenetetramine) can also be used to allow the excretion of copper through urine.

@ Tetrathiomolybdate prevents the absorption of Cu by forming insoluble Copper thiomolybdate in the gut and can also be used in treatment of Wilson's disease.

Calcium toxicity

@ Ca-salts are not soluble and precipitated resulting in formation of stones in kidney, gall bladder and cataract in eyes.

@ Stone formation may also lead to hardening of arteries.

Radionuclide toxicity

@ Radionuclides (even trace amount) show toxicity because of their ionizing radiation which can damage the living tissues. Nuclear radiation can interact with biomolecules too.

@ Radionuclide like ^{239}Pu emits α-particles which induces malignancy in bone, liver, lung and lymph nodes.

@ ^{90}Sr is known to produce bone cancer.

@ ^{137}Cs can follow the biochemical pathway of potassium and distributed throughout the soft tissue and it irradiates to cause cancer.

@ Organs affected: ^{42}K (muscle), ^{60}Co (liver), ^{35}S (skin), ^{85}Kr (ovaries), ^{131}I (thyroid), ^{90}Sr (bone), ^{222}Rn (lungs), ^{226}Ra (bones), ^{137}Cs (whole body).

Manganese toxicity

@ Arises due to inhalation of Mn-ores through dust.

@ May led to hepatolenticular degeneration resembling Parkinson's disease.

Nickel toxicity

@ Can produce bronchial cancer

@ It causes dermatitis and interferes with the activities of the enzymes like isocitrate dehydrogenase, cytochrome c oxidase etc.

Vanadium toxicity

@ Inhibits the synthesis of amino acids, phospholipids and cholesterol.

@ Inhibits the activities of enzymes like tyrosinase, nitrate reductase.

@ Vanadate which is similar to phosphate can inhibit Na^+-K^+ ATP-ase.

Cobalt toxicity

@ Heart failure (excessive consumption of beer)

@ Affects the Hb content and sometime can produce polycythaemia.

Zinc toxicity

@ Zn dust ingestion causes respiratory problems known as zinc fume fever.

@ Chronic Zn poisoning can also cause anorexia, paralysis, diarrhoea, dyspepsia etc.

Chromium toxicity

@ Cr(VI) can transport in cell as CrO_4^{2-}.

@ On reduction by –SH group (glutathione) produces Cr(V) and Cr(IV) intermediates which interact with DNA to induce carcinogenicity.

Molybdenum toxicity

@ Impaired growth, diarrhoea, skin disease, loss of hair.

@ Diminishes intestinal absorption of copper.

Metals as carcinogen

@ Ni, Cr and Cd are the three most effective carcinogenic metals.

Toxicity of Arsenic (III)

@ Excessive withdrawal of ground water is the main cause of As-contamination in water.

@ As - content in drinking water ranges from 0.05-3.5 mg/L & permissible limit is 0.05 mg/L

@ Arsenic compounds are mostly found in insecticides, fungicides, and herbicides.

@ It has been used as a therapeutic agent and as a poison (perhaps Napoleon was poisoned)

@ Arsenic exposure is usually suicidal, homicidal, or occupational

@ As(III) is the most toxic and is a carcinogen (lung and skin cancer)

@ Three major biochemical actions of As(III) are coagulation of protein, complexation with coenzymes and uncoupling of phosphorylation.

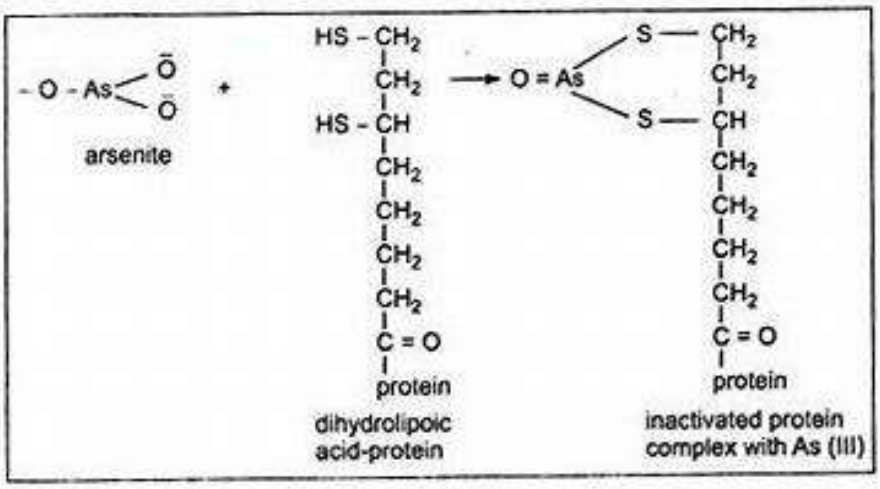
@ The toxicity due to As-compounds arises from three possible routes

@ Mechanism: Inhibition of –SH (sulfhydryl) in cellular enzymes and replacement of phosphate molecules in "high energy" compounds

Blocking of -SH group in enzymes

@ As(III) being soft binds with -SH group containing enzymes, thereby inhibiting the enzyme action. The enzymes which generate cellular energy in citric acid cycle are adversely affected. The inhibitory action is based on activation of pyruvate dehydrogenase by complexation with As(III) thereby preventing the generation of ATP.

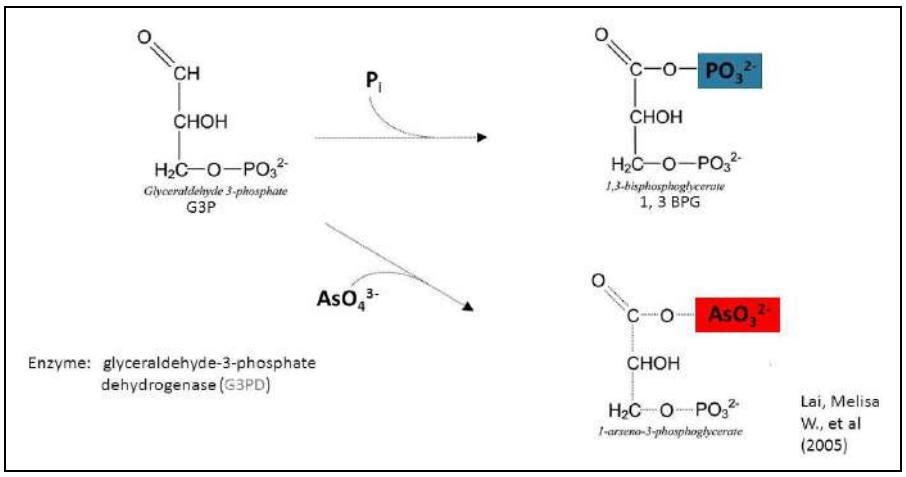
@ As(III) can also inhibit the enzyme which is involved in DNA repair mechanism i.e., poly(ADP-ribose)polymerase. It is also responsible for inducing heavy atom effect by binding methyl transferase

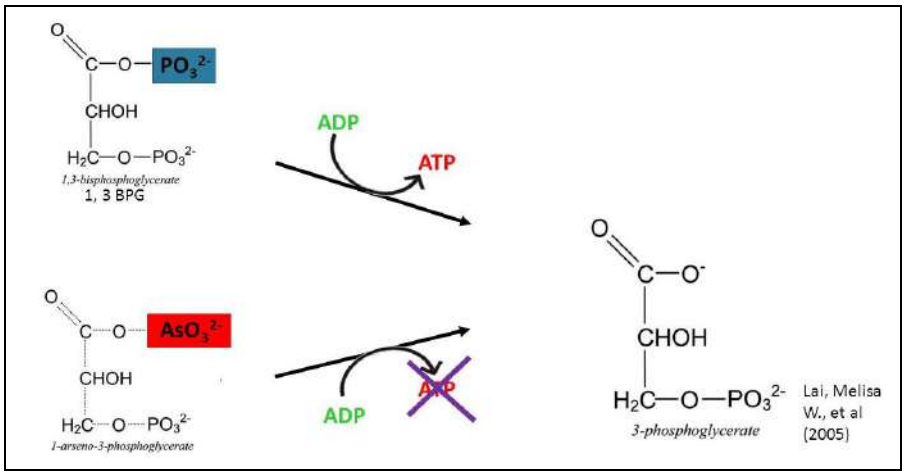


Competitive inhibition of different enzymes

@ As(III) interferes with biochemical processes involving P such as enzymatic synthesis of 1,3-diphosphglycerate from glyceraldehyde-3-phosphate through oxidative phosphorylation thereby producing 1-arseno-3-phosphoglycerate which hydrolyses without generating ATP (AsO_3^{3-} vs PO_4^{3-})

@ As-compounds can inhibit phosphoenolpyruvate mutase required for the biosynthesis of C-P bonds in living bodies





Denaturation of proteins

@ Excess of As(III) can denature (coagulate) proteins by attacking the -SH groups required in maintaining the its secondary and tertiary structures.

Clinical symptoms of Arsenic poisoning

@ Initial stage: gastroenteritis, dermatitis, keratosis.

@ Second stage: depigmentation and hyperkeratosis, peripheral neuropathies, melanosis.

@ Last stage: Gangrene of feet (Blackfoot disease), ulceration in the limbs and skin cancer.

@ Urine sample provide the most reliable diagnostic testing

@ Antidote should be capable of binding As(III).

@ Should have -SH group

@ BAL or dimercaprol was first used

@ DMSA is currently in use

Toxicity of Lead

@ Pb is the most abundant heavy metal, occurs as Pb minerals.

@ Major source of air borne Pb is the combustion of leaded petrol or gasoline along with paints, batteries etc.

@ Pb intake is mostly from diet (200-300 µg/day), air and water contribute ~ 10-15 µg/day.

@ 200 µg/day of Pb is excreted while almost 25 µg/day is stored in bones.

@ Almost 70-90% of Pb is accumulated in bones followed by liver and kidney.

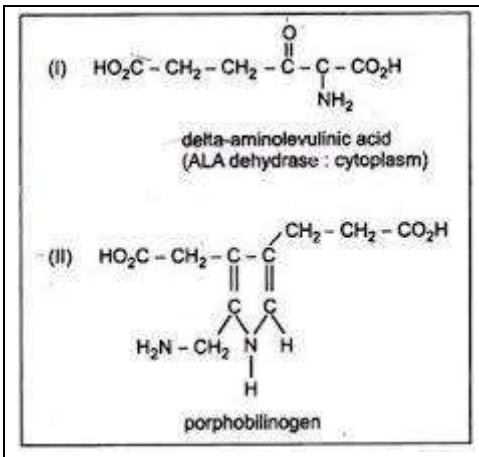
@ Pb(II) readily replaces Ca(II) in bones, either firmly fixed or reversibly fixed.

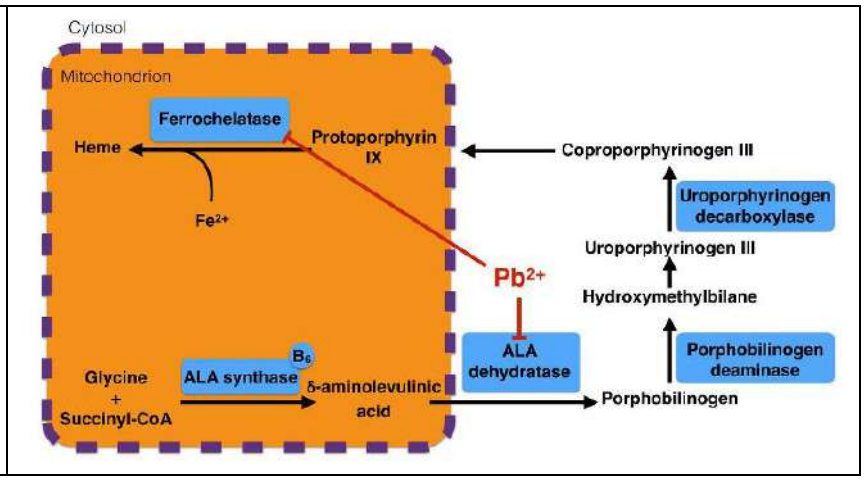
@ On reversible binding, Pb may get released in blood stream from bone tissue.

@ At the initial stage, Pb is stored in bones and when the body requires essential elements like Ca/P, blood starts leaching out these elements from bone and thereby exerting toxic action of Pb.

@ Major biochemical effect of Pb is its interference with heme (porphyrin) synthesis. It interacts with the enzyme δ-aminolevulinate dehydrase to inhibit the formation of porphobilinogen which acts as the building block unit for the biosynthesis of porphyrin skeleton. Pb(II) probably competes with Zn(II) center required for the activity.

@ Pb poisoning (Pb-content in blood > 0.8 ppm) in severe cases leads to anemia, damages nervous system by irreversible brain damage.





@ Pb can damage the mitochondria of kidney allowing the loss of glucose, amino acids and phosphate through urine. It can also damage the liver and gastrointestinal track.

@ Pb poisoning also cause enzyme inhibition, cellular dysfunction, chronic nephritis, neurological problems, and even exerts reproductive and teratogenic effects.

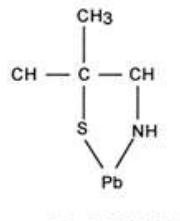
@ Pb poisoning is common in children (developing brain) due to their propensity of chewing objects containing the Pb based paints having sweet taste

System	Symptoms
General	Anaemia
Digestive	Constipation, loss of appetite, pain in abdomen
Muscular	Loss of coordination and strength, tiring
Nervous	Peripheral motor paralysis, insomnia, dizziness
Vascular	Diminished hemoglobin, arteriosclerosis, hypertensin
Other organs	Lead line in gums, lower sperm count, miscarriages, loss of vision, join pain

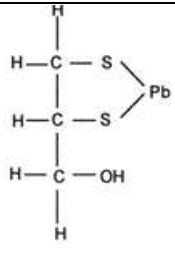
@ Pb poisoning can be cured by treatment with chelating agents that binds Pb effectively.

@ Ca-chelating agent like $CaNa_2EDTA$ in solution is fed to the patient with Pb poisoning, Pb displaces Ca from the chelate and excreted via urine.

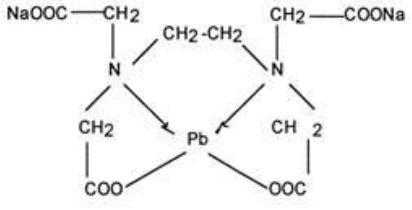
@ Typical chelating agents used are EDTA, BAL, D-penicillamine and Succimer.



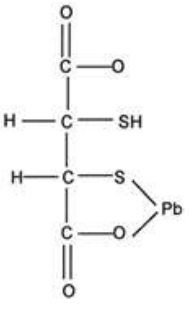
Pb- PENICILLAMINE



Pb - BAL



Pb - $CaNa_2EDTA$



Pb - SUCCIMER

Toxicity of Mercury

@ Hg is the most toxic heavy metal with natural abundance in soil is ~ 0.1 ppm

@ Minamata Disease in Japan (1953), the effluent from a vinyl chloride plant was the main source of Hg.

@ Hg-poisoning from wheat in Iraq (1972) and in US (1996).

@ Hg compound is used as pesticides and fungicides (results its distribution in environment). It is also widely used as electrodes and in different electrical apparatus

@ Mercury contamination of tuna - currently a problem

@ The inorganic Hg-compounds are very often absorbed on sediments and may be biomethylated subsequently.

@ Hg toxicity or poisoning is a disease caused by exposure to Hg or its compounds. The toxicity of Hg depends on its chemical form.

@ Elemental Hg is inert and non-toxic. If swallowed, it is excreted without serious damage.

@ Hg vapor when inhaled (due to its low vapor pressure), enters the brain through the blood stream, leading to severe damage of the central nervous system.

@ Hg_2^{2+} ion forms insoluble salts chloride ions. Our stomach contains a fairly high concentration of chloride and hence Hg_2^{2+} ion is not toxic.

@ Hg^{2+} ion is toxic. Because of its high affinity for S-atoms, it is easily attached to the S-containing amino acids of proteins. It also forms bonds with hemoglobin and serum albumin, both of which contain sulphydryl groups. Hg^{2+} ion does not travel across biological membranes and hence does not get access into biological cells.

@ Organomercurials (CH_3Hg^+ at 0.5 ppm) is the most toxic of all. RHg^+ is soluble in fat, lipid fraction of membrane and the brain tissue and therefore retained in cell for prolonged period. The most dangerous aspect is its ability to move through the placental barrier and enter foetal tissues (teratogenic effect).

@ CH_3Hg^+ may inhibit the normal functioning of the brain (neurological disorder).

@ Attachment of Hg to cell membrane is likely to inhibit active transport of sugar across the membrane and allow the passage of K^+ to the membrane. In brain cell this would result energy deficiency and disorder in the transmission of nerve impulses.

@ Babies born to mother subjected to CH_3Hg^+ poisoning suffer from irreversible damage to central nervous system such as mental retardation etc.

@ CH_3Hg^+ poisoning also leads to segregation of chromosome (chromosome breakage and inhibition of cell division)

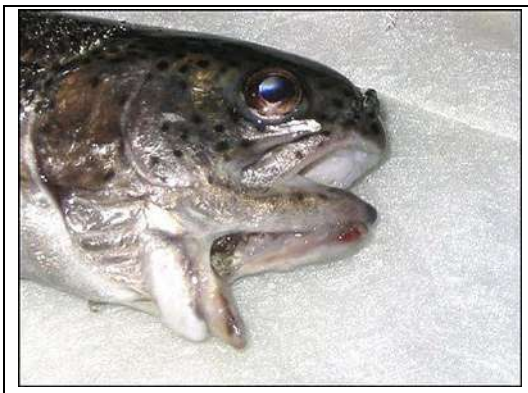
Species	**Biochemical impact**
Hg	Non-toxic, vapour is highly toxic when inhaled
Hg_2^{2+}	Insoluble as chloride, low toxicity
Hg^{2+}	Toxic, not easily transported across biological membrane
CH_3Hg^+	Highly toxic, causes irreversible nerve and brain damage, easily transported to biological membrane and stored in fat tissue.
$(CH_3)_2Hg$	Low toxicity, can be toxic on its conversion to CH_3Hg^+ in acidic medium

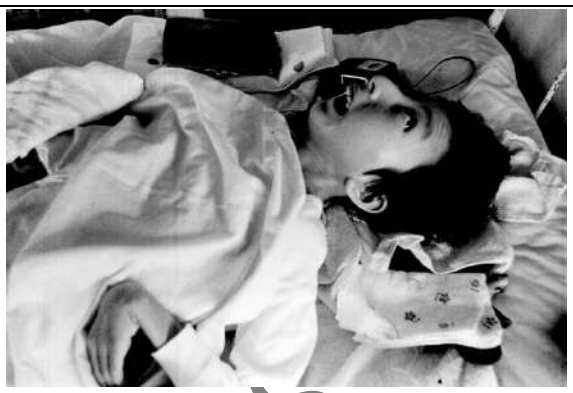
Tragedy of Minamata

@ Minamata disease is a neurological disease caused by severe Hg-poisoning. Signs and symptoms include numbness in the hands and feet, general muscle weakness, loss of peripheral vision, and damage to hearing

and speech. In extreme cases insanity, paralysis, coma, and death follow within weeks of the onset of symptoms.

@ Minamata disease (Minamata city, Japan in 1956) was caused by the release of CH_3Hg^+ in the industrial wastewater from the chemical factory (Chisso Corporation). This highly toxic chemical bioaccumulated and biomagnified (CH_3Hg^+) in fish and shellfish in Minamata Bay, which when eaten by the local population, resulted in mercury poisoning.





@ All forms of Hg are toxic to the fetus, but methylmercury most readily passes through the placenta and maternal exposure can lead to spontaneous abortion or other issues.

@ Clinically see: visual disturbances, ataxia, hearing loss, mental deterioration, muscles tremors, paralysis and even death.

@ For detoxification of Hg(II) or CH_3Hg^+, D-penicillamine (DPA-$C_5H_{11}NO_2S$), N-acetyl-D-penicillamine derivative (NAPA-$C_7H_{13}NO_3S$) and unithiol (2,3-dimercapto-1-propansulfonic acid) are recommended.

@ In detoxification of CH_3Hg^+, NAPA is a better antidote than DPA because of the presence of the lipophilic acetyl group in NAPA.

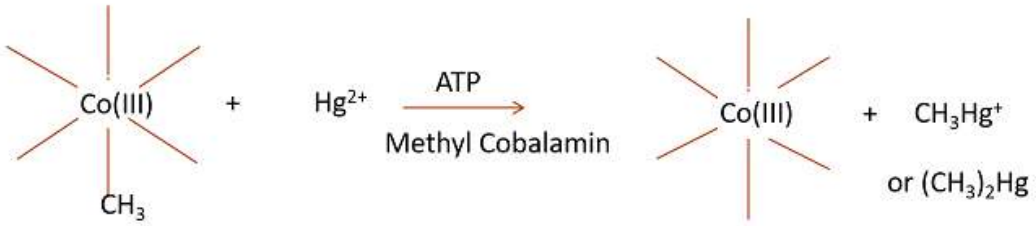
@ Natural Chelators: A detoxification mechanism has been traced in some Hg-resistant bacteria. Chlorella (from algae) is a natural immune stimulant and has a high affinity for heavy metals (it contains sulfur bound amino acids and acts as a natural chelator)

@ However, the reduction of use of Hg(II) products like Hg-electrodes, Hg-based pesticides, Hg-based electrical appliances are desired for environmental remedial.

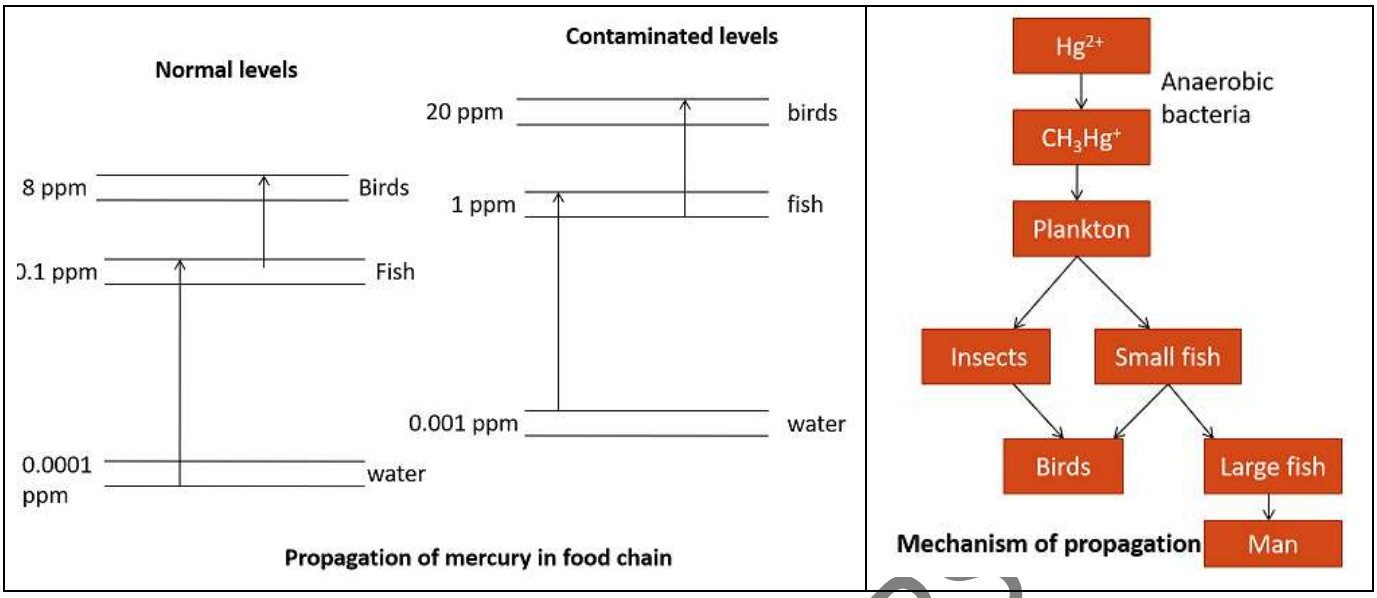
Biological methylation: Amplification in food chain

@ Hg or its salts can be converted into methyl mercury by anaerobic methane synthesizing bacteria in water (Biological methylation process). This conversion is facilitated by Co(III)-containing vitamin B_{12} coenzyme. A CH_3-group bonded to Co(III) on the coenzyme is transferred enzymatically by methyl cobalamin to Hg^{2+}, yielding CH_3Hg^+ or $(CH_3)_2Hg$.

@ Acidic medium promotes the conversion of $(CH_3)_2Hg$ to CH_3Hg^+ which is soluble in water and it enters the food chain through plankton and further concentrated by fish by a factor 1000 or more as passes through food chain.



Propagation of Hg in food chain



Toxicity of Cadmium

@ Cd occurs in nature in association with Zn minerals.

@ Source: Pigments (CdS, CdSe), Ni-Cd battery, nuclear reactors (used to slow down the neutron flux), semiconductors, electroplating industries, welding electrodes, etc.

@ Majority ingested Cd is trapped on the kidneys and mostly got eliminated.

@ A small fraction is bound effectively by metallothionine (-SH sites) in kidney, leading to its disfunction and the remaining is stored in body and gradually accumulate with age.

@ Ca^{2+} deficient diet enhances Cd^{2+} accumulation, older person and pregnant women are most at the risk.

@ Ingestion of excessive Cd^{2+} replaces Zn^{2+} ion at the key enzymatic sites causing metabolic disorder.

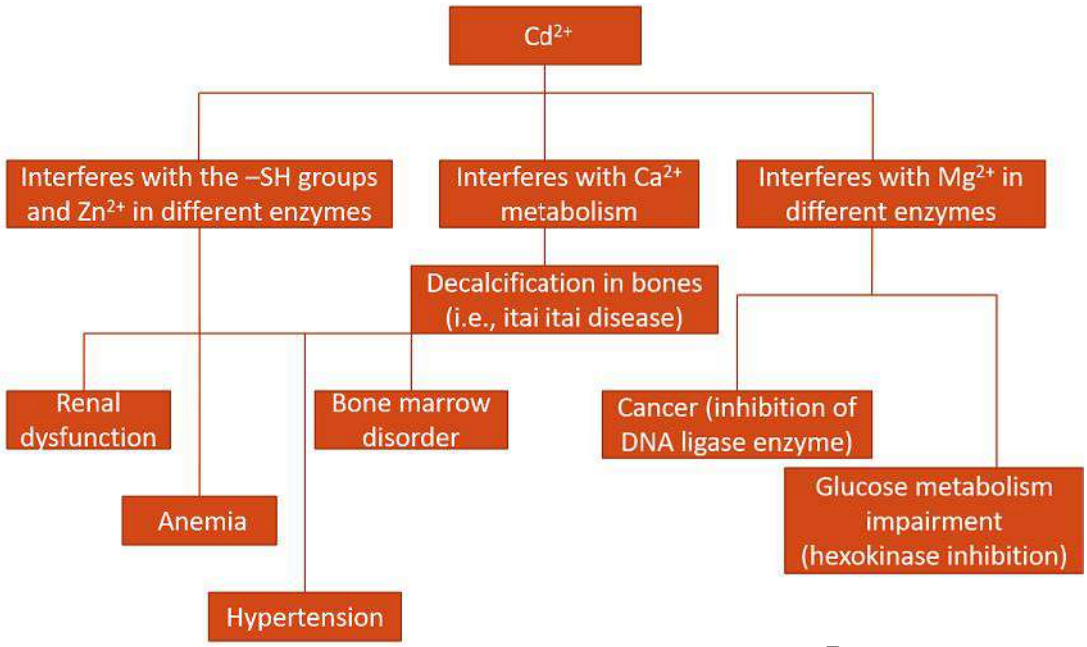
@ Cd^{2+} leads to decalcification (through competitive inhibition) in bones and the bones become fragile.

@ At high level, Cd^{2+} causes kidney problems, anemia and bone marrow disorder.

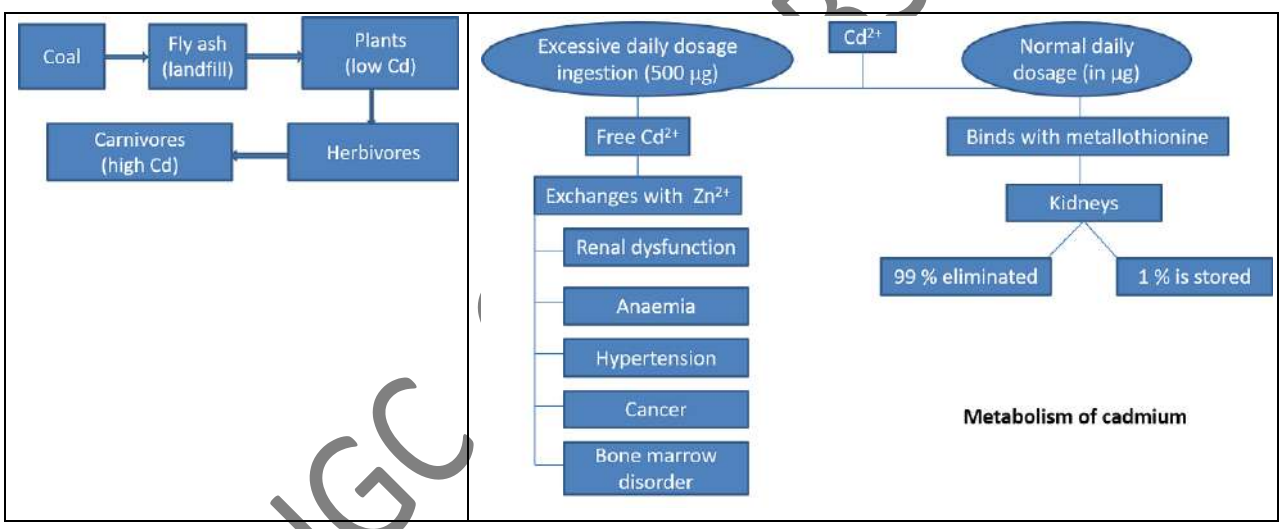
@ An outbreak of Cd poisoning occurred in Japan in the form of itai itai or "Ouch ouch" disease where the victims suffered from fragile bones. It is also accompanied by renal dysfunction.

@ Respiratory and pulmonary damages occur on breathing Cd vapor or particulates.

In summary,



Bio amplification of Cd



Exercises

1. The metal that is non-prevalent in biology

(a) Pt (b) Mn (c) Co (d) Ni

2. The metal ions with highest mobility in biological media are

(a) Zn(II) & Ni(II) (b) Fe(II) & Cu(II) (c) Na(I) and K(I) (d) Mg(II) and Ca(II)

3. Toxic properties of Hg and its compounds are due to their

(a) High affinity for reaction with thiols (b) Interference with oxygen transport

(c) Binding to histidines (d) Inhibition of Vit B_{12} synthesis

4. Which metal are very toxic?

(a) Hg, Cd, As, Fe, Cr(VI) (b) Hg, As, Pb, Cr(VI), Cd

(c) Cd, Hg, Pb, Zn, Co (d) As, Pb, Pt, Au, Mg

5. How is Hg released into the environment? (more than one option)

(a) Coal burning and fungicides (b) batteries and paint

(c) Tube light and fungicides (d) coal burning

6. Which metal is used for nitrogen fixation

(a) W, Cu (b) Ni, Ti (c) V, Mo (d) only Mo

7. Which metal deficiency causes anemia

(a) Fe (b) Co (c) Cu (d) All

8. Which of the following complex is used for the treatment of breast cancer?

(a) Ca-EDTA (b) Ni-EDTA (c) cis-platin (d) Carboplatin

9. Which of the following element causes the Alzheimer's disease

(a) Pb (b) Cr (c) Pd (d) Al

10. Which of the following metal disbalance causes the Wilson disease?

(a) Cu (b) Zn (c) Fe (d) Na

Iron management in biological systems

Lecture 3-5

In-vivo storage and transport of iron

@ Two main problems

(i) Insolubility of Fe (III): At physiological O_2 concentrations, Fe (II) is readily oxidized to Fe (III), which is highly insoluble in aqueous solution at normal pH.

(ii) Toxicity of free Fe species through the generation of free radicals causing severe cell damage.

@ Nature has developed sophisticated chemical system to execute and acquisition to its subsequent transport, storage, and utilization in tissue.

@ Fe storage system must be able to respond to 'supply and demand' (store the excess, and must mobilize & release when needed), as the amount of Fe in the diet is variable.

@ Ferritin stores and transferrin transports Fe in mammals.

@ Siderophore stores and transports Fe in microorganisms.

Storage of Iron

@ Three properties of Fe accounts for its extensive use in biological processes

a) facile redox reactions of iron ions;

b) an extensive range of redox potentials available by ligand substitution

c) abundance and availability under conditions apparently existing when terrestrial life began

@ The combination of the reactivity of Fe (II) ion and the relatively large amounts of Fe used by cells have necessitated its storage

@ The transition in the atmosphere (about 2.5 billion years ago) resulted in drop in bioavailability of Fe thereby increasing the need for its storage.

@ Comparison of the solubility of Fe^{3+} at physiological conditions ($\sim 10^{-18}$ M) to the Fe content of cells ($\sim 10^{-5}$ to 10^{-8} M) emphasizes the difficulty of acquiring sufficient Fe

Ferritin

@ Ferritin is a storage protein for Fe in non-toxic form.

@ It is present in liver, spleen, and bone marrow, and in plants and bacteria.

@ Ferritin is known to release Fe to the developing fetus

@ It consists of Fe-mineral core (hydrated Fe (III) oxide) surrounded by a protein coat/sheath (apoferritin) with varying amount of phosphate

@ The diameter of the core is ~ 80Å and ~ 4500 Fe atoms can be reversibly stored inside the protein coat.

@ The lipophilic sheath makes the Fe (III)-complex soluble in biological fluid.

@ Apoferritin allows controlled access to the core though eight hydrophilic channels (enter) and six hydrophobic channels (leave)

@ Fe (III) ion is of high spin nature and is subjected to strong antiferromagnetic coupling

@ The role of the stored iron in ferritin varies (intracellular use for biosynthesis of Fe-proteins or mineralization, long term storage and detoxification of excess Fe)

@ Iron regulates the synthesis of ferritin; large amounts of ferritin is associated with Fe excess and its small amounts is associated with Fe deficiency.

@ Ferritin is also known to be a precursor of several forms of Fe in living organisms (hemosiderin in lysosomes of animals – Fe complex with protein is insoluble).

@ Magnetite (Fe_3O_4) is another form of biological Fe derived from the Fe in ferritin. It plays a role in the behavior of magnetic bacteria, bees, and homing pigeons

Structure of ferritin

@ The structure of ferritin can be considered to consist of three units: the protein coat, iron-protein interface, and Fe-core

Protein coat/sheath (Apoferritin)

@ The protein sheath consists of subunits of 24 polypeptide chains (about 175 amino acid) folded into ellipsoids (lozenge like shape; MW=20000 D).

@ Each subunit is approximately cylindrical & linked together to form a hollow sphere with two-fold, three-fold and four-fold axes.

@ The hollow sphere is about 100 Å, the organic surface is about 10 Å thick.

@ Two ends of the apoferritin subunit are designated as N (the polar N-terminal end of the protein) and E (the non-polar helical segment)

@ At 8 places, three subunits meet with their N-ends to form a polar channel of three-fold symmetry (C_3) through which Fe-can be transferred in or out.

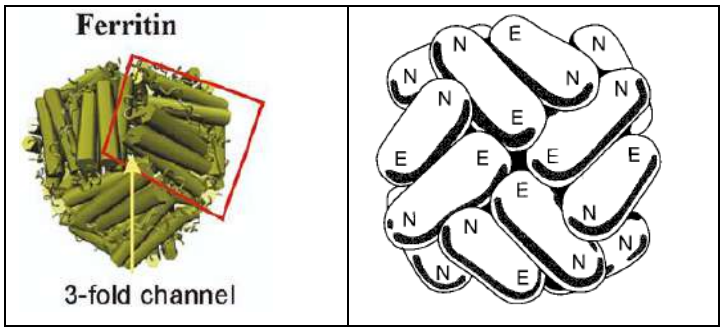
@ These three-fold polar channels are lined up with hydrophilic aspartate or glutamate residues

@ There are 6 non-polar channels of C_4 symmetry produced by the meeting of four subunits with their E-ends.

@ These 4-fold non-polar channels are lined up with hydrophobic amino acid residues.

@ The protein coat is stable with or without Fe, and hence the center of the hollow sphere may be filled with solvent and/or $Fe_2O_3.H_2O$.

@ Amino acids required to form the shape of the protein coat & the ligands for Fe-core is not established (perhaps tyrosine acts as an Fe (III)-ligand)



Iron-Protein interface

@ At the Fe-protein interface, smaller clusters of Fe(III) centers (bridged to each other by oxo/hydroxo bridges) are attached to the protein.

@ The coordination of Fe to the protein occurs via carboxyl groups from glutamic (Glu) & aspartic (Asp) acids (EXAFS - Extended X-ray Absorption Fine Structure & Mossbauer spectra)

Iron core

@ The composition of the microcrystalline core is $[(FeOOH)_8(FeO).H_2PO_4]$

@ The core is primarily consists of a sheet structure of Fe(III)-oxide, similar to the mineral ferrihydrite ($5Fe_2O_3.9H_2O$)

@ All the Fe-centers are octahedrally surrounded by oxygen

@ The core provides a close packed matrix of O^{2-} ions with Fe(III) ions randomly distributed in the octahedral holes (EXAFS-study)

@ The HS Fe(III) centers undergo strong antiferromagnetic coupling.

@ Hydroxide and phosphate groups present in the core helps in counterbalancing the charge and binding at the protein surface

@ Fe(III) atom in ferritin is surrounded by six O-atoms at a distance of 1.95 Å and six Fe- atoms at distances of 3.0 to 3.3 Å (Mossbauer spectroscopy and EXAFS study)

@ Ferritin cores vary in their degree of structural, magnetic ordering and/or the level of hydration.

@ Structural differences in the iron core have been associated with variations in the anions present (phosphate or sulfate), and with the electrochemical properties of iron.

@ Formation of ferritin core is a representative example of biomineralization process. This biomineral is insoluble but remains soluble in biological fluids as a complex having protein sheath.

Mechanism of Fe-core formation

@ Fe(II) and dioxygen are required for core formation especially in the early stages.

@ The core is formed from aqueous Fe(II) & its oxidation to Fe(III) follows its incorporation.

@ Fe-gets into the core through channels in the protein and then transferred into the cavity to form first diiron-oxo dimers.

@ Aggregates and clusters are formed via a progression of oligomers related to iron hydrolysates.

@ Oxidation to Fe(III) and hydrolysis produce one e^- and 2.5 H^+ for incorporation of each Fe-atom into the iron core (for 4500 Fe-atoms, 4500 e^- & 11250 H^+)

@ The protons are released & electrons are transferred to dioxygen (the relative rates of proton release, oxo-bridge formation, & electron transfer are not known in detail).

@ If all the protons were retained, the pH would drop to 0.4

@ When large numbers of Fe(II) atoms are added, the protein coat appears to stabilize the encapsulated Fe(II).

@ An Fe(III)-tyrosinate (Fe(III)-ligand) complex is proposed to be a transient precursor to polynuclear cluster formation (UV-Vis and resonance Raman spectroscopy)

Iron storage mechanism (proposed)

@ The mechanism for the reversible incorporation of Fe in ferritin involves its transport in and out as Fe(II).

@ There are 8 hydrophilic and 6 hydrophobic pores of apoferritin.

@ The 24 subunits arranged in such a way that at 8 places, 3 subunits meet with their N-ends to form a polar channel (hydrophilic pores) through which Fe can be transferred in or out.

@ There are also 6 non-polar channels (hydrophobic pores) produced by the meeting of four subunits with their E-ends.

@ Iron is taken up in the labile ferrous form prior to oxidation.

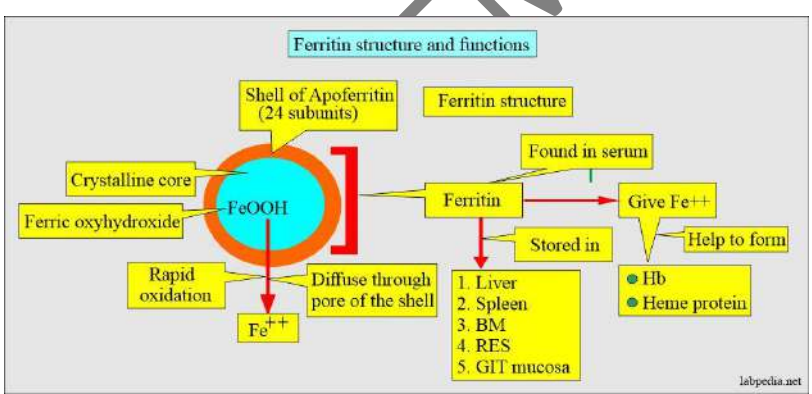
@ Release of iron requires reduction of the core (Fe(III) to Fe(II)) with a biological reductant (NADH – nicotinamide adenine dinucleotide + hydrogen)

@ The mobile Fe(II) is oxidized to Fe(III) at specific di-iron binding sites known as 'ferroxidase centers', present in each of the subunits.

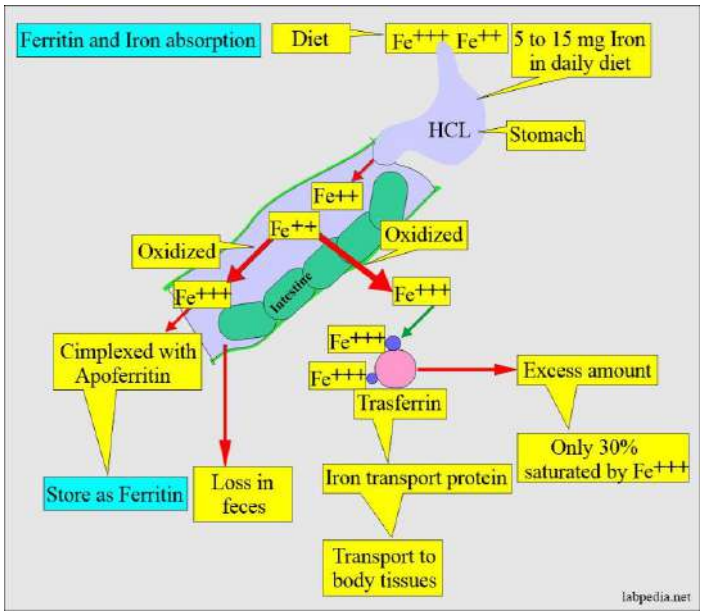
@ Oxidation to Fe(III) involves the coordination of O_2 and inner sphere electron transfer:

$$2Fe(II) + O_2 + H^+ \rightarrow 2Fe(III) + H_2O_2$$

Summary of Ferritin structure and functions



Summary of ferritin and Iron absorption



Iron transport -Transferrin (Tf)

@ Transport of Fe-from ferritin or hemosiderin occurs via the serum-transport protein transferrin.

@ Serum transferrin is a monomeric glycoprotein with high MW ≈ 80000 D

@ Serum transferrin (blood plasma - most studied), conalbumin or ovatransferrin (egg white) and lactoferrin (mother's milk) are examples of Fe-transport protein.

@ Serum transferrin mainly functions to transport iron from the Fe-core and breakdown cells of RBC to the place of biosynthesis of RBC. Excess iron is also transported back to the ferritin.

@ Tf is also present in tears, serving to cleanse eyes after irritation.

@ Ovatransferrin acts as antibacterial agent, lactoferrin is a potent antibacterial Tf (protects from infectious disease) and serum Tf is a potential Fe-transporter.

@ Transferrin is bilobal, with each lobe reversibly (independently) binding Fe(III) ion (binding constant $>10^{20}$ M^{-1})

@ This complexation of the metal cation occurs via prior complexation of a synergistic HCO_3^- or CO_3^{2-} ion.

@ LMCT from phenolate to Fe accounts for salmon pink color of transferrin

@ Binding affinity for Fe(III) decreases progressively with decreasing pH

@ Tf binds only two Fe-atoms (efficient) and delivers iron by interacting with the tissues where required

Apo-transferrin

@ Transferrin not bound to iron (free Tf)

@ It has two iron binding sites per molecule and are similar but not identical.

@ For each site, the binding constant (K_A) under physiological condition is ~10^{26}.

@ It binds Fe(III) so strongly that no other protein can snatch the iron effectively from it.

@ Although the two iron-binding sites of transferrin are different, their coordination environments are quite similar.

Structure of Transferrin

@ Fe site in human lactoferrin has been determined by XRD (E. Baker *et al.*)

@ Transferrin is an ellipsoidal protein with two subdomains or lobes (almost similar), each of which binds iron.

@ Polypeptide chain contains 679 amino acids.

@ Serum transferrin contains about 6% carbohydrates linked to the protein, and affect the recognition & conformation of the native protein.

@ Fe-atom in Tf is coordinated to four amino acid residues viz. two tyrosines (one phenolate oxygen each), one histidine (N-atom) & one aspartate (O-atom of carboxylate group).

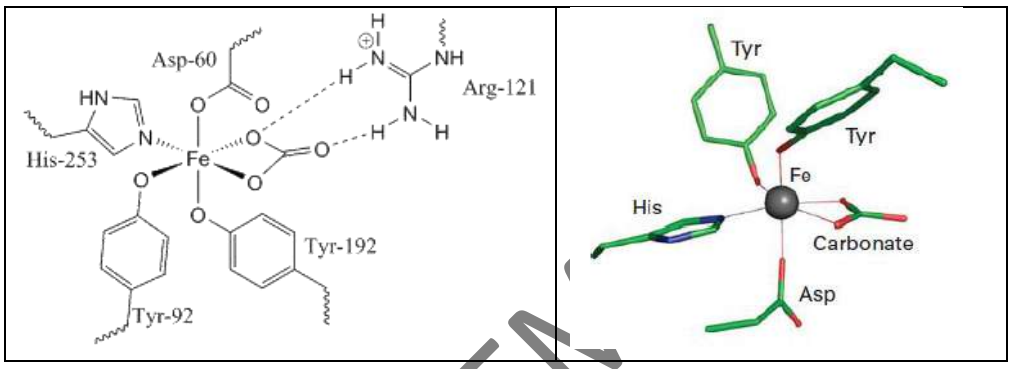
@ A bidentate CO_3^{2-} ion occupies the remaining two sites of the distorted octahedral environment.

@ It sits in a pocket between the Fe-atom & two positively charged protein chains viz. an arginine side chain and a helix N-terminal.

@ Sometime, a carboxylate ligand can also bind instead of CO_3^{2-} ion.

@ Fe is buried deep in cleft between two protein domains, with two polypeptide strands behind it.

@ Flexing of these strands alters the conformation providing driving force for Fe-release



Binding and transport/release of Fe

@ Fe(II) in stomach is oxidized to Fe(III), catalyzed by ceruloplasmin (Cu-protein)

@ On passing from stomach to blood (pH=7.4), oxidation of Fe(II) occurs

@ Fe(III) binds to apo transferrin effectively (no other bioligand can compete)

@ Fe(III) complexation involves binding of CO_3^{2-} and release of proton.

$$\text{apo-Tf} + \text{Fe(III)} + CO_3^{2-} \rightarrow \text{Fe(III)-Tf-}CO_3^{2-} + H^+$$

@ The protein chain provides four binding sites with two cis-sites vacant for the synergistic CO_3^{2-} ion (bidentate coordination, may also be linked to Fe(III) release)

@ The release of the Fe from Tf occurs at low pH of the endosome (inside the cell), and Apo-Tf is returned to the outside of the cell for delivering of another pair of Fe-atoms (millions of Fe-atoms per cell per minute)

@ The reduction potential of Tf (-0.05V) is too negative to be reduced by common biological reducing agents

Mechanism of Transport/release

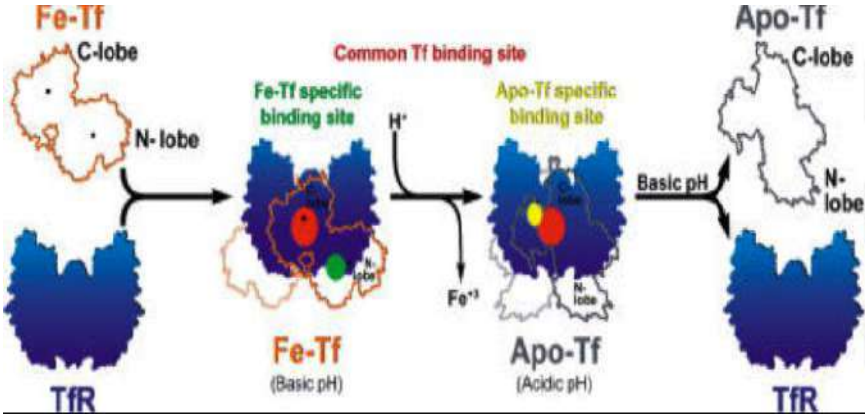
@ A transferrin receptor (glycoprotein) influences the stability of Fe-transferrin complex on the cell binding site

@ The complex binds to receptor and gets transported into the cell in a vesicle by receptor-mediated 'endocytosis'.

@ The pH of the vesicle is reduced (~ 5.5) by H^+ pumps (structural variation in protein), causing transferrin to release its Fe ions thereby forming a complex with cell receptors

@ The vesicle then splits and the Tf-receptor complex is returned to the plasma membrane by exocytosis, and Fe(III) is released to the cytoplasm.

@ The path of Fe from the endosome to Fe-proteins has not been established; and the form of transported intracellular iron is not known.



Synergistic action of CO_3^{2-}/HCO_3^-

@ Without CO_3^{2-} within the coordination sphere of Fe(III), transferrin fails to retain Fe(III)

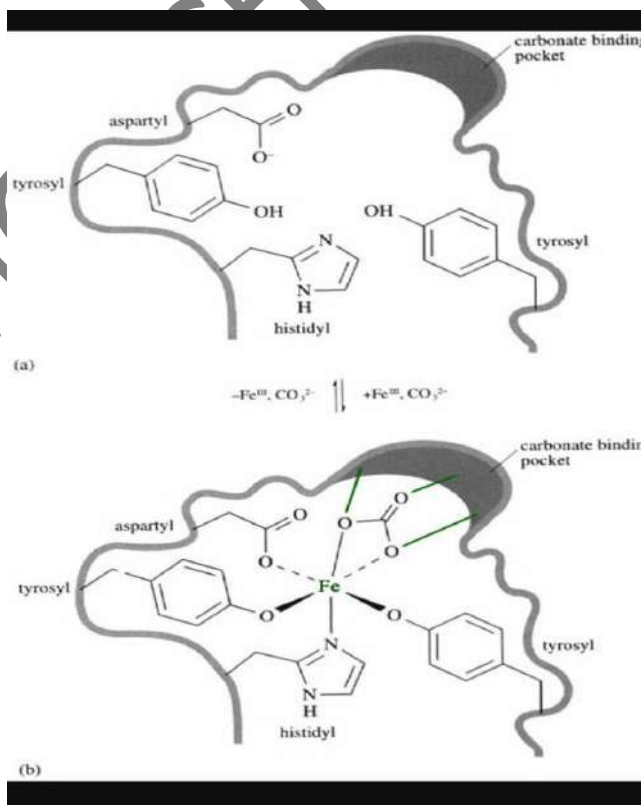
@ CO_3^{2-} establishes the stabilizing interaction from H-bonding (folding protein chain) and coordination of Fe(III) in the pocket

@ The anion bridges between Fe(III) and the cationic sites of encircling protein (Fe-O hard-hard interaction).

@ This coordinated synergistic anion minimizes the electrostatic repulsion between the metal center and the cationic sites of the protein chain.

@ Removal of CO_3^{2-}/HCO_3^- from Tf destabilizes Fe-Tf interaction, a key step in Fe-release

@ This explains the synergistic function of the anion.



Fe-storage and transport in lower organisms

@ Almost all microorganisms including plants have Fe as an essential element

@ Iron transfer compounds in microorganisms (bacteria and fungi)

@ Small polydentate ligand with high affinity for Fe(III)

@ They have peptide backbones and are strong chelating agents.

@ They sequester Fe to give a soluble complex that re-enters the organism at a specific receptor. Once inside the cell, the Fe is released.

@ In reducing environment, Fe was substantially available as Fe(II) compounds (relatively soluble at ~ neutral pH) and this was one of the factors for bioavailability of Fe(II).

@ In oxidizing environment, microorganisms were forced to deal with insoluble Fe(III)-hydroxide

@ On Fe deficiency, a high-affinity iron binding site called siderophores comes into play

@ Siderophores capture Fe and transfer it through the cell wall.

@ Siderophore-mediated iron-uptake usually occurs in aerobic conditions.

@ In *E. coli,* Fe-uptake can occur under anaerobic conditions

Siderophores

@ Siderophores are small polydentate ligand with low MW (500-1000 D)

@ They have peptide backbones and are strong chelating agents.

@ It solubilizes and transports iron as Fe(III) and have high affinity for Fe.

@ They sequester Fe to give a soluble complex that re-enters the organism at a specific receptor. Once inside the cell, the Fe is released.

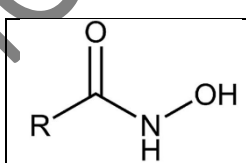
@ They are grouped into two categories viz. one having hydroxamate ligands (ferrichromes, ferrioxamines) & other having catecholate (o-dihydroxybenzene) ligands (enterobactin).

@ Hydroxamates occur mainly in fungi and yeast, while the catecholates mainly occur in bacteria,

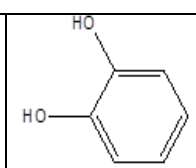
@ These molecules are polydentate ligands with many potential ligating atoms (HSAB principle -hard O-donor sites) to form chelate.

@ Readily form extremely stable octahedral complexes with high spin Fe(III).

@ Although stable, the complexes are labile (HS, d^5, no CFSE) enough to allow transport and transfer of iron within the bacteria



hydroxamate



catecholate

@ At the site of iron release, Fe(III) is reduced to Fe(II) which does not bind so strongly with the siderophores.

@ Iron containing siderophores look red brown and this is why, siderophores are also named as siderochromes.

@ The colour originates from LMCT (ligand to Fe(III)) band

@ Enterobactin-mediated iron uptake in *E. coli* is one of the best-characterized of the siderophore-mediated iron-uptake processes

@ The ferric-enterobactin complex interacts with a specific receptor in the outer cell membrane, and the complex is taken into the cell by active transport.

Hydroxamate and catecholate ligands

@ Ferrichromes (a cyclic hexapeptide consisting of three glycine and three N- hydroxyl-1-ornithines) and ferrioxamines are trihydroxamic acids which form neutral tris chelates from three bidentate hydroxamate monoanions.

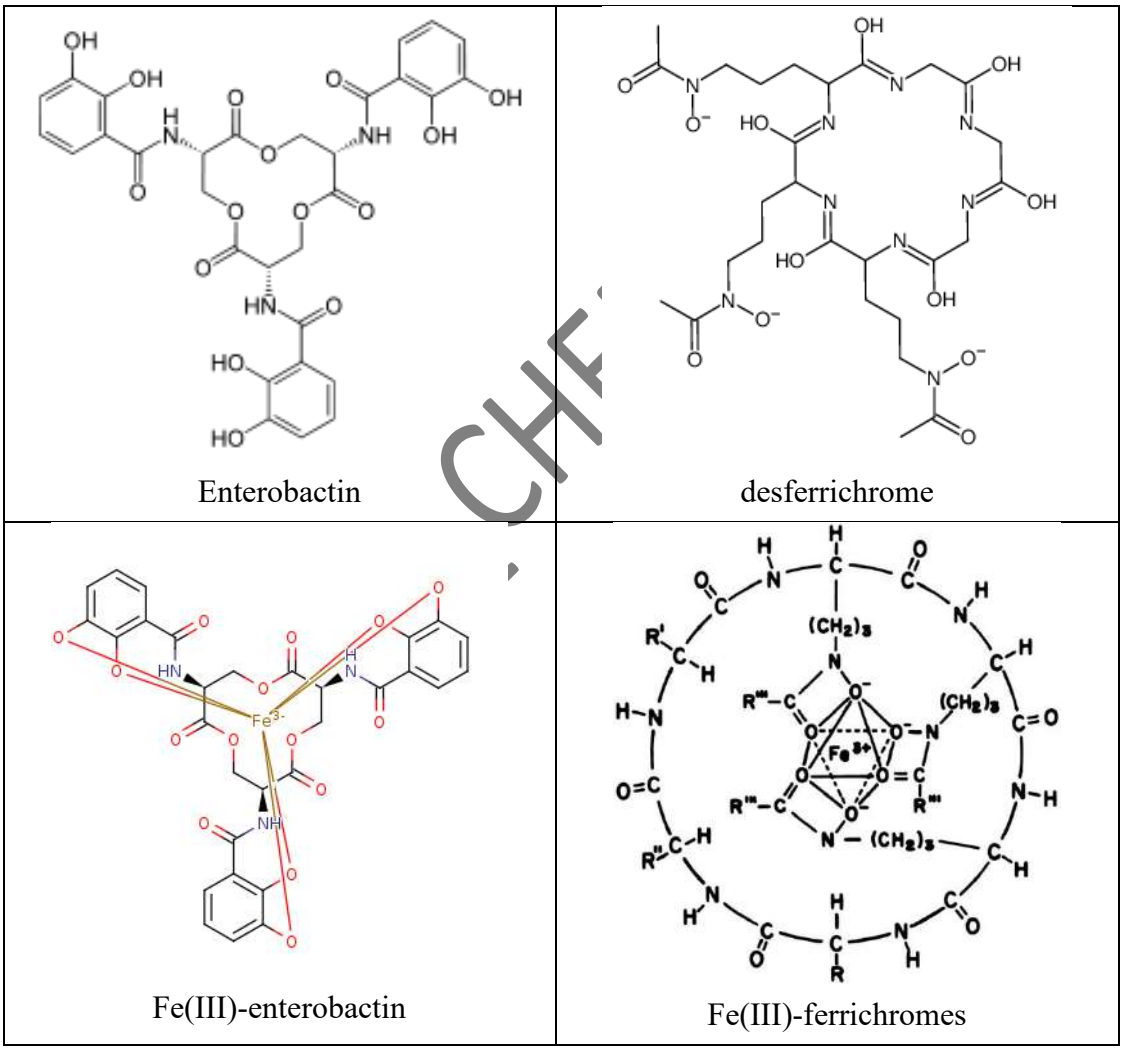
@ In enterobactin, each catechol group behaves as a dianion for a total charge of -6 for the ligand (association constant for Fe(III) is approximately 10^{50}).

@ In addition to form globular complexes, siderophore molecules consists of a symmetric hydrophilic portion that plays a key role in transport of iron in cell membrane.

@ Tris chelated octahedral complexes can provide optical isomer and thereby can be chiral.

@ Since the complexes are labile, ligand or metal exchange can be incurred in these complexes. For example, compared of Fe(III), Cr(III) (d^3 with significant CFSE) analogues are significantly inert. Similarly, V(IV) although a bit smaller, can replace Fe(III) in enterobactin.

@ Other synthetic analogues and treatment of Fe toxicity by desferrioxamine B.



Enterobactin | desferrichrome

Fe(III)-enterobactin | Fe(III)-ferrichromes

Fe uptake mechanism of siderophore

@ Three different types of mechanism have been proposed for siderophores activity

@ First, the siderophore (ferrichrome) transports the metal across the cell membrane to the cell interior where it is released by a non-destructive process and hence ligands are available for reuse.

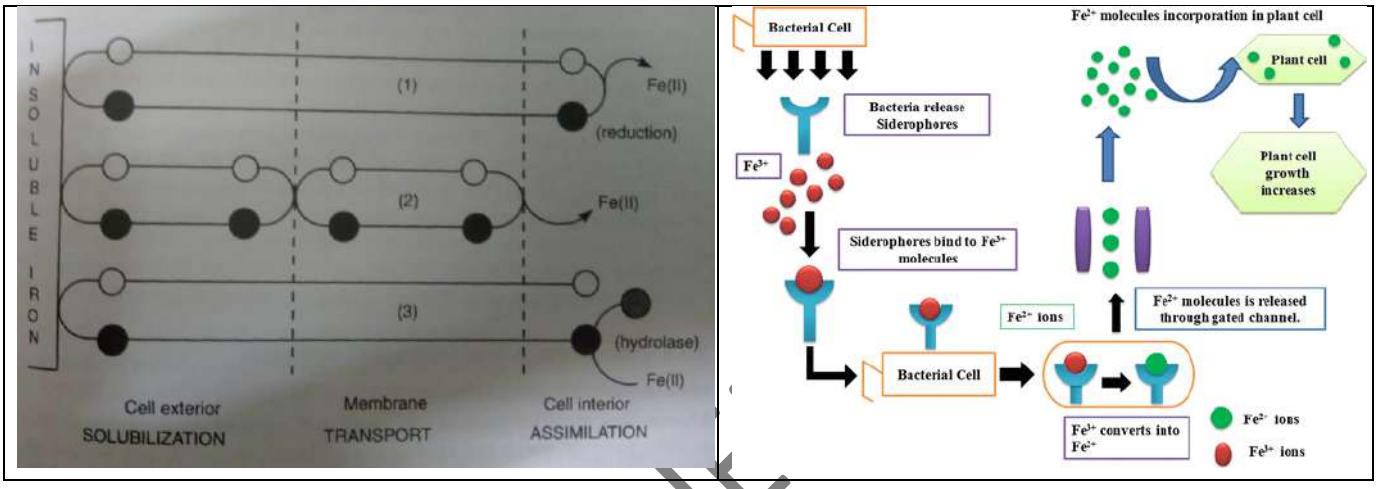
@ Second, the siderophore (ferrioxamine) delivers the metal to the outer cell membrane surface where it is transferred to a secondary transport device which carries it to the cell interior where it is released.

@ Third, the metal is transported across the cell membrane by the siderophores (enterobactin) to the cell interior where the complex is broken up, by a hydrolase, so destroying the ligand and giving an example of built in obsolescence

@ Enterobactin-mediated Fe uptake in *E. coli* is one of the best characterized of iron uptake processes in microorganisms.

@ Ferric enterobactin accumulated in *E. coli*, must pass through the outer membrane, the periplasm and cytoplasm membrane, and is probably subjected to reduction of the metal in a low pH compartment or to ligand destruction.

@ After complexation, the Fe(III)-enterobactin complex interacts with a specific receptor in the outer cell membrane and then taken into the cell by active transport.



Dioxygen storage and transport

Lecture 5-7

Dioxygen in Living Organism

@ Dioxygen is produced in biosphere through photolysis of water during photosynthesis in cyanobacteria, green algae and plants.

@ Most organisms require dioxygen to survive.

@ Mostly consumed in the terminal (or primary) step of oxidative phosphorylation.

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

@ Some small animals and plants with large surface-to-volume ratio, an adequate supply of dioxygen can be obtained from diffusion across cell membranes.

@ For other organisms (scorpions to whales), diffusion does not supply sufficient dioxygen for respiration.

@ Dioxygen carrier protein is required for higher organisms.

@ The carrier must bind and release dioxygen at a rapid rate.

General features of dioxygen carrier proteins: Three components

@ First, the active site (dioxygen binding site), a complex either of Cu or Fe (hemoglobin, hemerythrin, hemocyanin etc.)

@ Second, the dioxygen carrier protein (facilitates sequestration of dioxygen), (lungs, gills etc.)

@ Third, the delivery system (blood plasma, heart etc.)

Metalloprotein	Active site (deoxy)	Color change (deoxy to oxy)	MW (D)	Subunits

Hemoglobin (vertebrate)	Heme Fe II	Purple to red	64,000 (Human)	4
Hemoglobin (invertebrate) Erythrocruorin Chlorocruorin	Heme Fe II Chloroheme Fe II	Purple to red Purple to green	Upto 3.3 x 10^6 Upto 3.1 x 10^6	192 192
Hemocyanin Mollusc Arthropod	Cu I….Cu I	Colorless to blue	~ 9 x 10^6 ~ 9 x 10^5	160 (mollusc) 12 (arthropod)
Hemerythrin	Fe II….Fe II	Colorless to burgundy	108,000	8

Where hemoglobin is found?

@ Hb is the iron containing dioxygen transport metalloprotein in the RBC of all vertebrates (possible exception, the Antarctic fish *Cyclostomata*).

@ Hb is found in the tissues of some invertebrates (including some insect larvae), molluscs, almost all annelid worms (erythrocruorins (Er) in arthropods and chlorocruorins (Ch) in some annelid).

@ Hb is found in some plants (e.g., leghemoglobin in the nitrogen-fixing nodules of legumes).

@ Some organisms (the clam *Scapharca equivalvis*) also feature a dimeric hemoglobin.

			
Molluscs - Hemocyanin	Sipunculan - Hemerythrin	Marine worm - Chlorocurin	Annelids - Erythocruorin

Myoglobin and its structure

@ Mb (MW ≈ 17000 D) is found in cytosol within the cell. Present in muscles and bone marrow

@ It is a single heme group surrounded by protein chain.

@ Heme unit is a Fe (II) porphyrin ring (tetrapyrrole ring) with high spin Fe (II) (d^6).

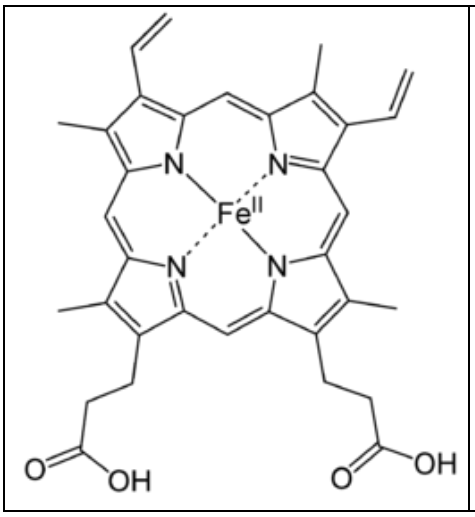
@ 153 amino acid residues in protein chain folded about single heme group.

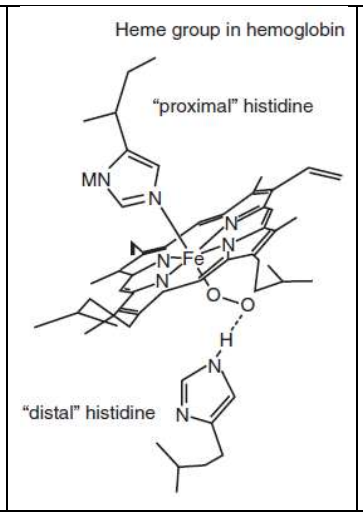
@ Fifth co-ordination site of Fe(II) is occupied by N-atom from imidazole side chain of histidine segment.

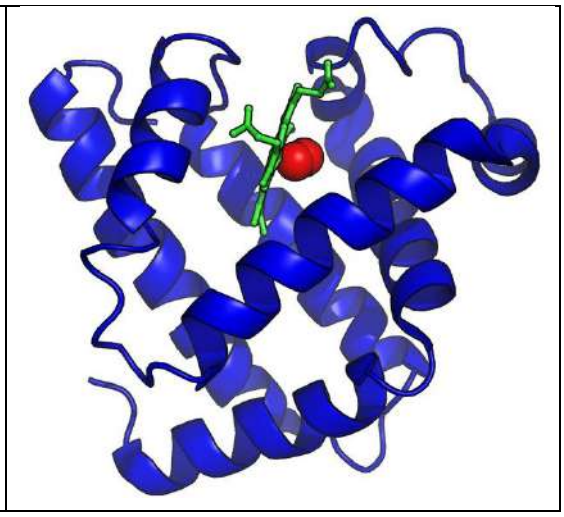
@ Eight side chains (four methyl, two vinyl and two propionic acid)

@ High spin Fe(II) ($t_{2g}^4 e_g^2$) has ionic radius ≈ 92 pm and is large enough to get fit in the hole of porphyrin ring, deoxy form is pseudo-octahedral (out of the plane).

@ Fe(II) is low spin ($t_{2g}^6 e_g^0$) in oxy form and has ionic radius ≈ 75 pm. Fe(II) drops into the hole in the porphyrin ring (F-O-O angle ~ 115°).







Hemoglobin

@ Discovered by Hunefeld in 1840.

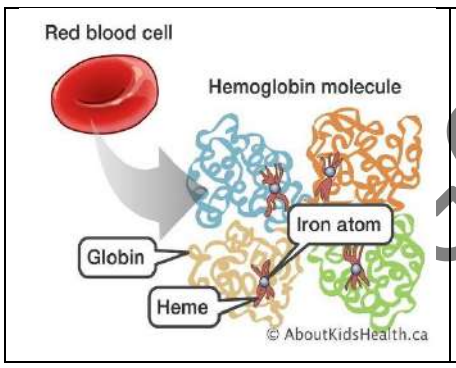
@ Max Perutz (1959) determined the molecular structure of hemoglobin (XRD).

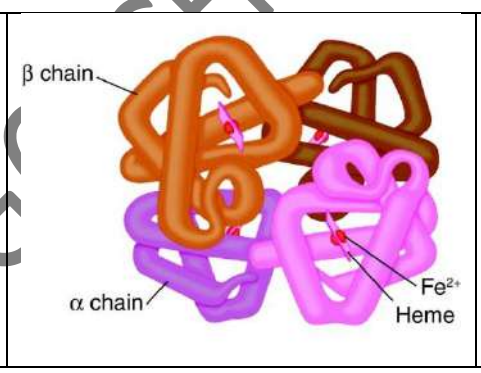
@ Resulted in his sharing of Noble prize with John Kendrew (1962), for their studies of the structures of globular proteins.

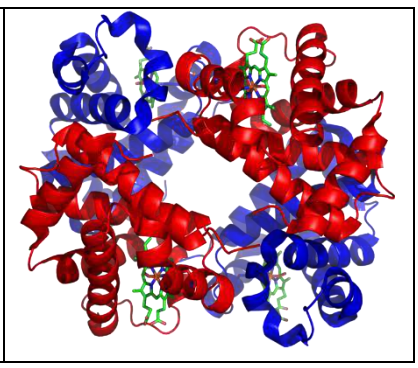
@ Hb is considered as an approximate tetramer Mb, MW around 68000 D

@ Hb has a quaternary structure, characteristic of many multi-subunit globular proteins.

@ Most of the amino acids in hemoglobin form alpha helices, and are connected by short non-helical segments (loops)







Structure of Hemoglobin

@ Hb structure is **heme** and **globin**.

@ Hb is an approximate tetramer Mb (four heme groups).

@ Heme group is an Fe(II) porphyrin complex (tetra pyrrole unit).

@ Fe(II) ion coordinates to the four nitrogen atoms of four pyrrole rings.

@ The fifth co-ordination site of Fe(II) is occupied by an imidazole N-atom of from a histidine segment (proximal histidine) below the porphyrin ring.

@ Pyrrole units are connected at the α-carbon by methylidene bridges

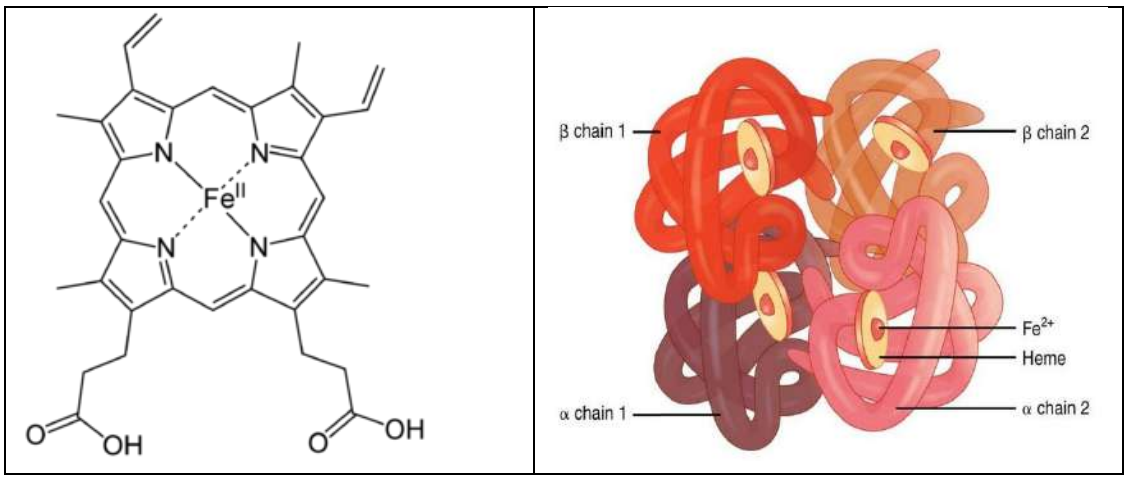
@ Eight side chains (4-methyl, 2-vinyl, 2-propionic acid) are present at the tetrapyrrole moiety.

@ Globin is made up of four polypeptide chains.

@ Two alpha (α) and two beta (β) chains with 141 (α) and 146 (β) amino acids respectively ($\alpha_2\beta_2$).

@ Fe(II) is high spin ($t_{2g}^4e_g^2$) and resides out of the porphyrin plane.

@ In deoxy form, weakly bonded H_2O is proposed to fill the sixth co-ordination site forming a distorted octahedron.



Hemoglobin binding to dioxygen-Conformation Change

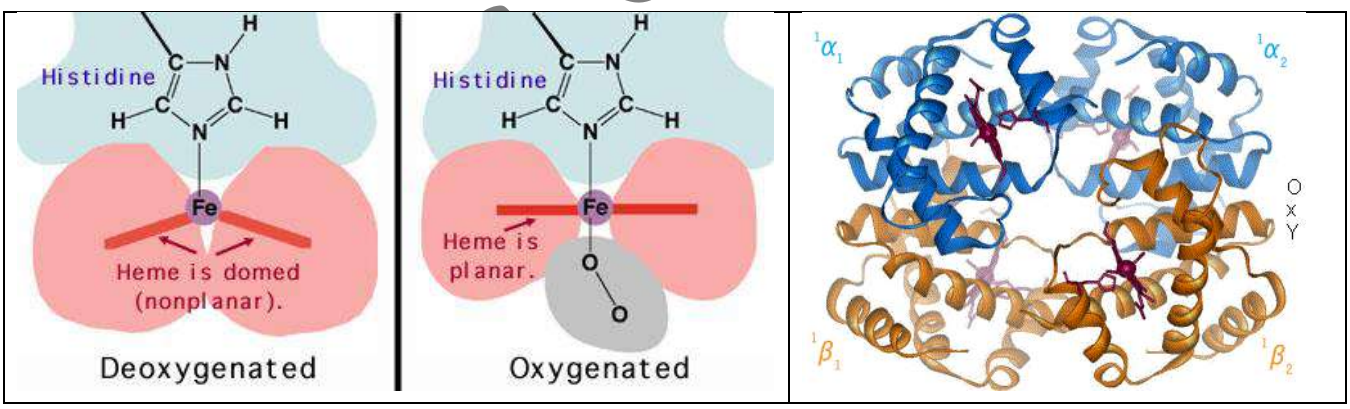
@ Sixth position of Hb is reversibly coordinated by O_2 via coordinate covalent bond, resulting in an octahedral arrangement.

@ Conformations of the deoxy and oxy-Hb are called the T (tensed) and R (relaxed) state respectively.

@ Dioxygen binds in an "end-on bent" geometry where one oxygen atom binds to Fe and the other protrudes at an angle (Fe-O-O angle about 153°).

@ One αβ half of the molecule rotates around 15° relative to other half, change in the quaternary structure.

@ Upon oxygenation, two of the heme groups move about 100 pm towards each other while the two others separated by about 700 pm.



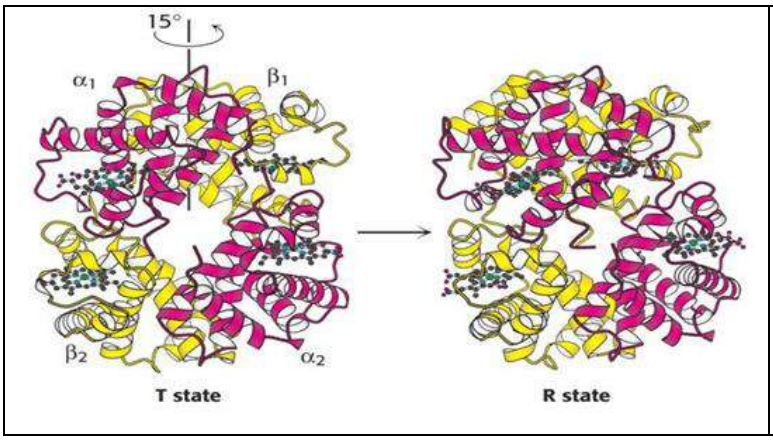
@ Fe(II) is about 0.6 Å out of the heme plane in the deoxy state and upon oxygenation, it is pulled back into the heme plane.

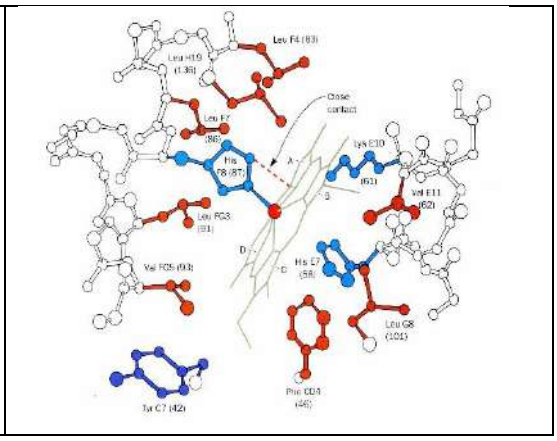
@ This motion is particularly important for Hb because it pulls on the proximal histidine ligand and helix F moves.

@ Binding of dioxygen on one heme is more difficult, but its binding causes a shift in the α_1 and β_2 contacts and moves the distal His E7 and Val E11 away from the oxygen's path to the Fe on the other subunits.

@ This process increases the affinity of heme toward oxygen.

@ The T state with reduced oxygen affinity will be changed to the R state without binding oxygen because the other subunits switch upon oxygen binding.





Physiology of Hemoglobin and Myoglobin

@ Hb carries O_2 from lungs to tissues and gets transferred to Mb (stored) for metabolic processes.

@ To be thermodynamically favourable, Mb must have greater affinity for O_2 than Hb in the tissues/cell.

@ Equilibrium constant for Mb-O_2 complexation is simple. If total amount of Mb ([Mb]+ [Mb-O_2] is held constant and O_2 concentration is varied, hyperbolic curve is obtained (in cell Mb is largely oxygenated).

@ Equilibrium constant for $(Hb\text{-}O_2)_4$ complexation is complex and the exponent 2.8 arises from cooperative binding. This results in a sigmoid curve for oxygenation of Hb.

@ Binding of O_2 by Hb is also pH dependent and at higher pH it is favorable (Bohr effect).

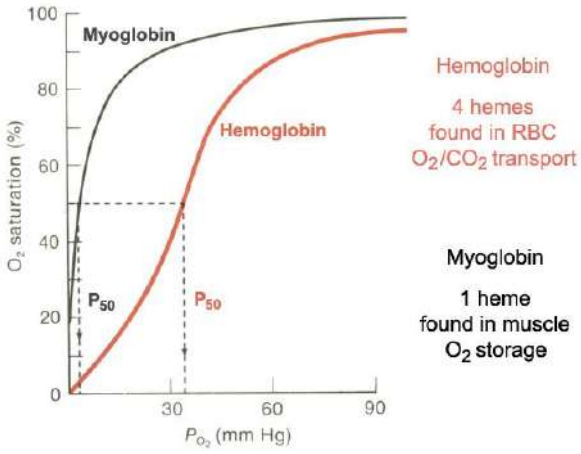
Lungs: $Hb + 4O_2 \longrightarrow Hb(O_2)_4$

Tissues: $Hb(O_2)_4 + 4Mb \longrightarrow 4Mb(O_2) + Hb$

Equilibrium constants for $Mb(O_2)$ & $Hb(O_2)_4$ complexation are

$$K_{Mb} = [Mb(O_2)] / [Mb][O_2]$$

$$K_{Hb} = [Hb(O_2)_4] / [Hb][O_2]^n \qquad n < 4 \text{ , } n \sim 2.8.$$



Cooperativity of Hemoglobin

@ The binding of four O_2 molecules to hemoglobin is interdependent.

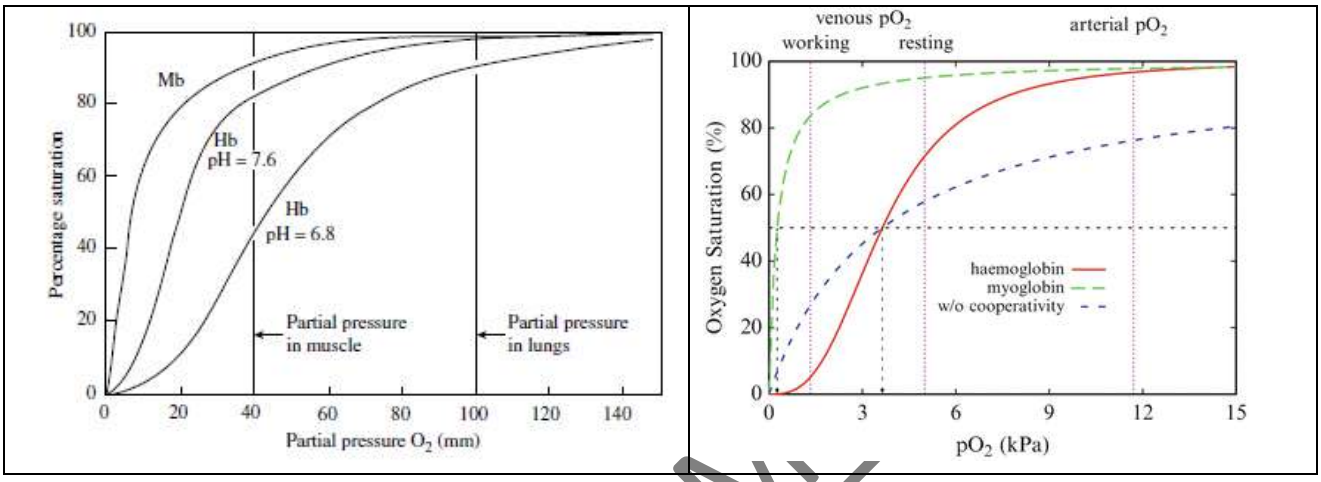
@ Cooperativity: Presence of several bound O_2 molecules favors further oxygenation and conversely, if only one O_2 molecule is present, it dissociates more readily than a highly oxygenated species.

@ At low O_2 concentrations Hb is less oxygenated (tends to release) and at high O_2 concentrations Hb is oxygenated to larger extent.

@ The Hb-O_2 binding constant depends on oxygen partial pressure.

@ Cooperativity allows the oxy-Hb to carry the maximum amount of O_2 to the tissues and then allows the deoxy-Hb to release the maximum amount of O_2 into the tissues.

@ Results in sigmoidal behavior for oxygenation of Hb. Mb upon oxygenation yields a hyperbolic curve.



Trigger action - mechanism of co-operativity

@ Hb can exist in two different forms: T-state (deoxy) & R-state (oxy)

@ T-state has a much lower O_2 affinity than the R-state (almost equal to isolated α and β chains).

@ Increasing the partial pressure of O_2 causes the conversion of T-state to R-state.

@ O_2 binding at the four heme sites in Hb is not independent and simultaneous.

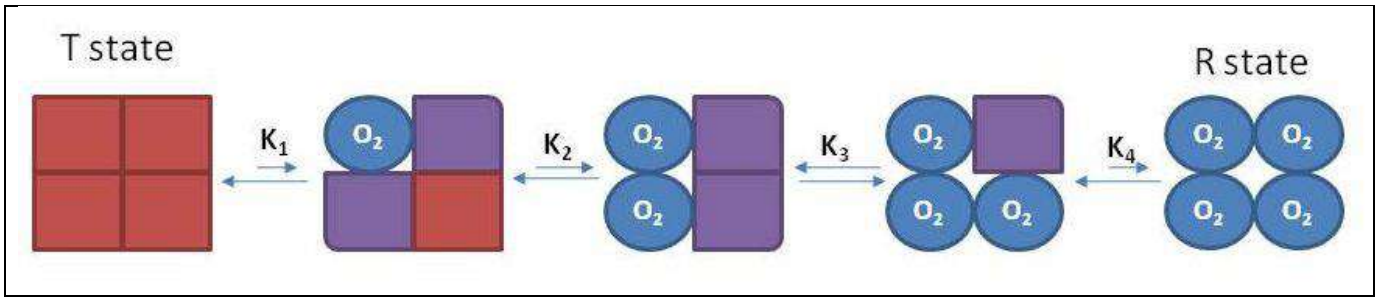
@ Binding of 1st O_2 molecule is difficult but 2nd, 3rd & 4th gets progressively easier and easier.

@ As the body circulates, the O_2 level drops and Hb releases its bound O_2 into the tissues.

@ The key or trigger in the Perutz mechanism is the high spin Fe(II) atom in deoxy-heme.

@ The Fe(II) is forced to sit above the porphyrin plane with Fe-N distance of about 206 pm.

@ The heme group is domed upward towards the proximal histidine.



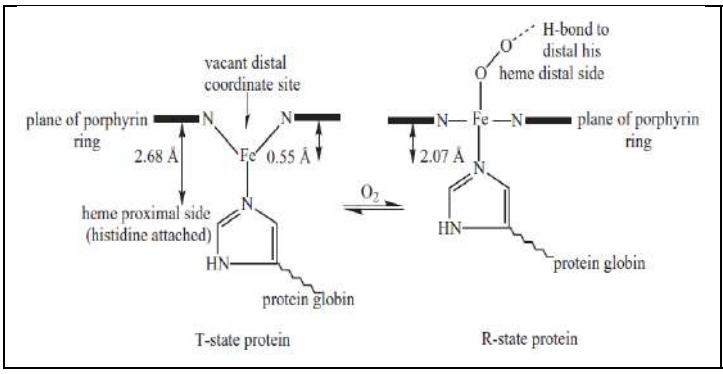
@ Upon oxygenation, the Fe(II) is low spin and smaller, move towards the porphyrin plane with Fe-N distance of 198 pm (attributed to steric interactions between histidine, globin chain and heme unit).

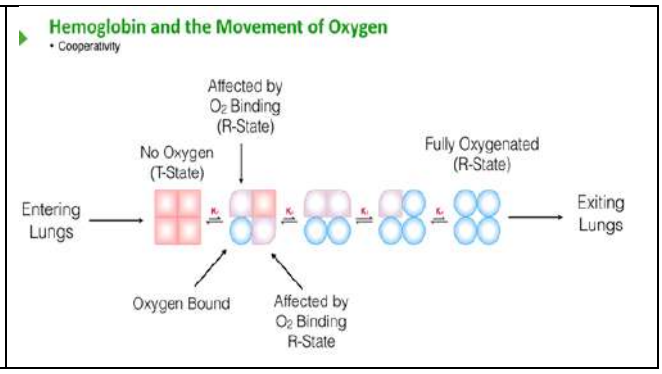
@ These results considerable strain on the oxy-heme and associated tertiary structure of the globin within the T-state, discouraging the addition of first O_2 and pushes the last O_2 off in the tissue.

@ Addition of second O_2 molecule takes place with similar results.

@ Addition of third O_2molecule results in interconversion to R state and Fe(II) moves into porphyrin hole.

@ This allows the fourth heme to bind the last O_2 molecule without any constrains.





Thermodynamic factors

@ Hb must bind and release dioxygen at a rapid rate. For the process (M-oxygen carrier),

$$M + O_2 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} MO_2 \qquad \text{.....(1)}$$

$$K_c = [MO_2]/[M][O_2] \text{ …….. (2)}$$

@ The solvent dependent quantity $[O_2]$ in equation (2) can be replaced by solvent independent quantity $P(O_2)$, the partial pressure of dioxygen. The new equilibrium for the process (1) is given by,

$$K_p = [MO_2]/[M]P(O_2) \text{ …….. (3)}$$

@ The affinity can thus be conveniently expressed as the partial pressure of dioxygen required for half-saturation of the species M, $P_{1/2}(O_2)$. Under such conditions, $[M] = [MO_2]$ and we have

$$P_{1/2}(O_2) = 1/K_p \text{ ……. (4)}$$

where $P_{1/2}(O_2)$ is given in torr or mm Hg. The dioxygen affinity is composed of enthalpic ΔH and entropic ΔS components, with

$$\Delta G° = - RT \ln K = \Delta H° - T \Delta S° \qquad \text{……..(5)}$$

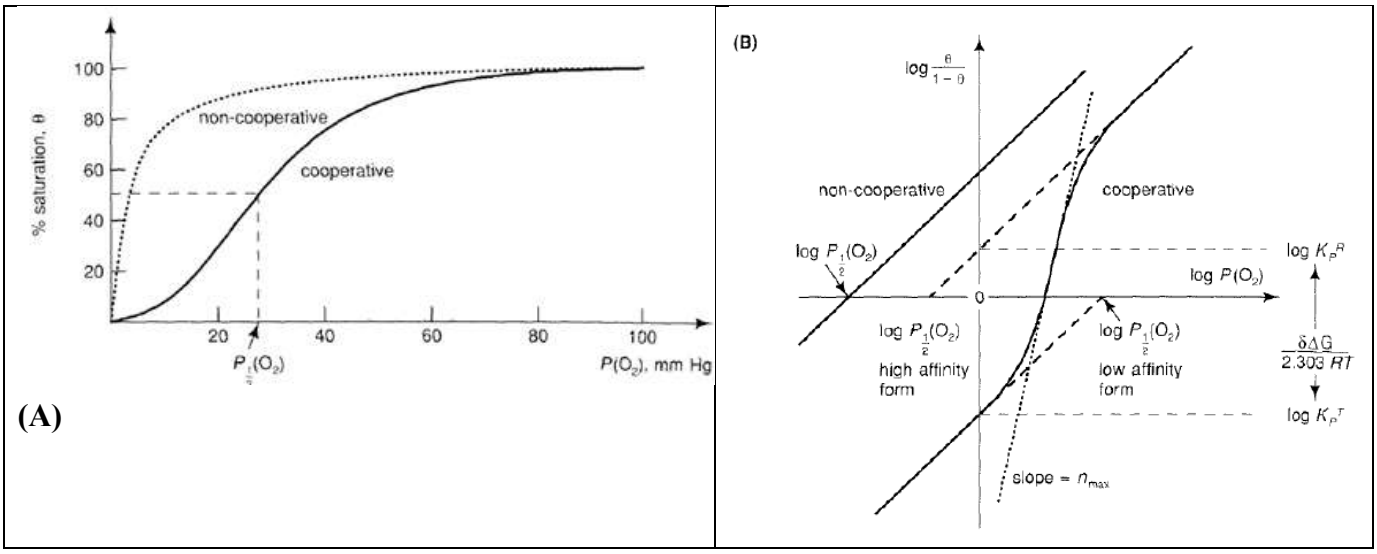
@ Within a family of oxygen carriers, the values of $\Delta H°$ and $\Delta S°$ are usually similar. Large deviations are therefore indicative of a change in nature of the oxygen binding process.

Non-Cooperative Dioxygen Binding

@ If the oxygen-binding sites M are mutually independent and non-interacting, the plot of concentration of species MO_2 as a function of the partial pressure of O_2 is analogous to Langmuir isotherm. The plot of the fractional saturation of dioxygen binding sites (θ) versus $P(O_2)$ is hyperbolic curve labeled "non-cooperative" (Fig. A), where;

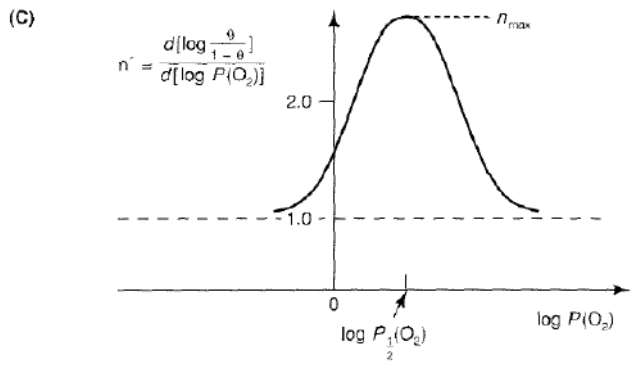
$$\theta = [MO_2]/([M]+ [MO_2]) = K_pP(O_2)/((1 + K_pP(O_2)) \text{ ……. (6)}$$

@ Alternatively, plot of log (θ/(1 - θ)) vs log $P(O_2)$, called as Hill plot, gives a straight line with a slope of unity and an intercept of -log $P_{1/2}(O_2)$ (Fig B). ($n_H = 1$, for Mb, non-cooperative)



@ A differential form is shown as the dotted line (Fig. C).

@ Such binding, where the dioxygen sites are independent of each other, is termed non-cooperative



Cooperative Dioxygen Binding

@ Dioxygen binding in Hb is interdependent. The binding or release of dioxygen at one site may affect the affinity and kinetics of ligand binding or release at a neighboring site (sigmoidal curve, Fig. A). Thus, the dioxygen binding is co-operative in nature.

Degree of co-operativity can be characterized in several ways:

@ By means of a Hill plot of $\log(\theta/(1-\theta))$ versus $\log P(O_2)$, the limiting slopes (which should be unity) at high O_2 pressure and low O_2 pressure may be extrapolated as shown in Fig. B to $\log(\theta/(1-\theta)) = 0$, where $\theta = 0.5$

Two limiting values for $P_{1/2}(O_2)$ are obtained:

1. $P_{1/2}(O_2)$ characterizing the regime of high partial pressure of dioxygen, where the O_2 affinity is high (positive cooperativity).
2. $P_{1/2}(O_2)$ value characterizes the regime of low partial pressure of dioxygen, where affinity is relatively low.

The difference in affinities can be converted into a difference between the free-energy change upon O_2 binding in the low-affinity state (K_p^T) and the high-affinity state (K_p^R).

$$\delta\Delta G^\circ = - RT \ln (K_p^T / K_p^R) \quad \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

@ A second way to characterize cooperativity involves fitting the oxygen-binding data at intermediate saturation ($0.2 < \theta < 0.8$), that is, about the inflection point in a Hill plot - to the Hill equation

$$\theta/(1-\theta) = K_p P^n(O_2)$$

$$\log(\theta/(1-\theta)) = -\log(P_{1/2}(O_2)) + n\log(P(O_2)) \quad \ldots\ldots\ldots\ldots\ldots\ldots(8)$$

@ The Hill coefficient (n) is an empirical coefficient and has value of unity for non-cooperative binding.

@ Any number greater than unity indicates positive cooperativity (For Hb, n_H (max slope)= 3.0-3.5, $n_H > n$). The fit is only approximate, since the Hill plot is only approximately linear about the inflection point (Fig B).

@ Intercepts with broken black line at the 0 value for log (θ/(1 - θ)) indicate $P_{1/2}(O_2)$ and so O_2 binding affinity (lower $P_{1/2}(O_2)$ = higher affinity

@ Hb high affinity O_2 binding log $P_{1/2}(O_2)$ = upper asymptote intercept

@ Hb low affinity O_2 binding log $P_{1/2}(O_2)$ = lower asymptote intercept

@ A more precise value of n may be obtained by plotting the slope in the Hill plot (n') as a function of log $P(O_2)$ (Fig. C).

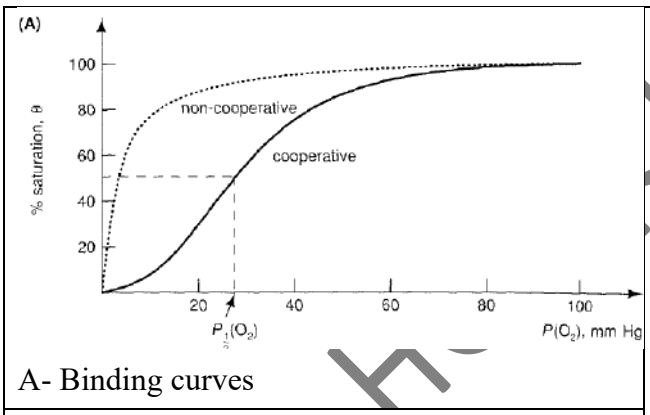
$$n' = d[\log(\theta/(1-\theta))]/d[\log(P_{1/2}(O_2)] \quad \ldots\ldots..(9)$$

Non-Cooperative and Cooperative Dioxygen Binding Plots

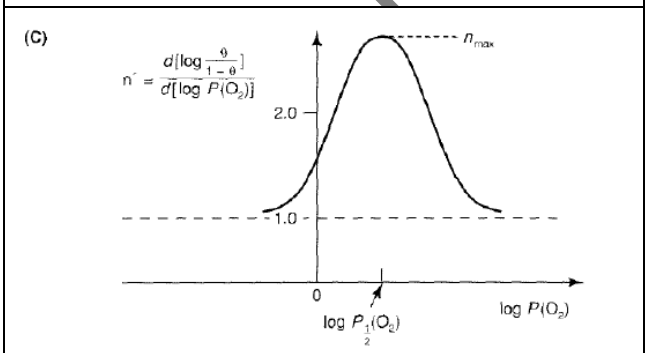
@ The maximum value of n' is taken as the Hill coefficient n.

@ The maximum in this first-derivative plot of the binding curve will occur at $P_{1/2}(O_2)$ only if the Hill plot is symmetric about its inflection point.

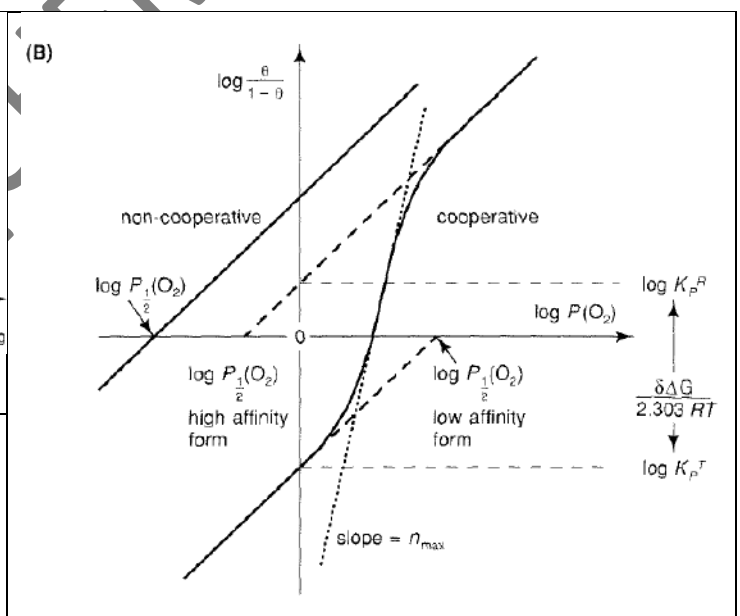
@ For tetrameric hemoglobin, a maximum Hill coefficient of around 3 and for hemocyanin (n) may be as high as 9.



A- Binding curves



C- first derivatives (slope) of the Hill plot



B- Hill plot of binding curves

Benefits of Cooperative Ligand Binding

@ With cooperative binding, the problem of inefficient and inflexible O_2 delivery disappears. If hemoglobin bound O_2 non-cooperatively, then the hyperbolic binding curve (c) in Figure D would represent the O_2 binding. Instead, the observed binding follows curve (d).

@ Since the partial pressure of dioxygen in the lungs and arterial blood of vertebrates is around 100 Torr, but in the tissues and venous blood it is around 40 Torr, then at these pressures a typical myoglobin ($P_{1/2}(O_2)$ = 1 Torr) remains effectively saturated.

@ On the other hand, about 25 % of the available dioxygen can be delivered, even in the absence of myoglobin. With venous blood remaining 75 percent oxygenated, hemoglobin has substantial capacity to deliver more O_2 at times of exertion or stress when $P(O_2)$ in the tissues falls below 40 Torr.

@ The net result is that whole blood, which contains about 15 g of hemoglobin per 100 ml, can carry the equivalent of 20 ml of O_2 (at 760 Torr) per 100 ml, whereas blood plasma (no hemoglobin) has a carrying capacity of only 0.3 ml of O_2 per 100 ml.

Allosteric effectors

@ The molecule/ligand whose co-ordination (presence) with a center influences the binding/release of other ligand (same/different) at that site is known as allosteric effector.

@ The cooperative interaction where binding of one molecule of a substance influences the binding of next molecule of the same kind is described as homotropic allosteric interaction.

@ A heterotropic allosteric interaction occurs when both the two interacting ligands are different.

@ Oxygen binding in vivo is significantly modulated by allosteric effectors (both homo and hetero) through the interaction with the protein.

@ For Hb, natural allosteric effectors are H^+, CO_2 and 2,3-diphosphoglycerate (2,3-DPG or BPG, present in RBC).

@ Increasing concentrations of H^+ and CO_2 progressively lower the affinity of deoxy-Hb towards O_2, thereby enhancing the release of coordinated O_2 (curve e).

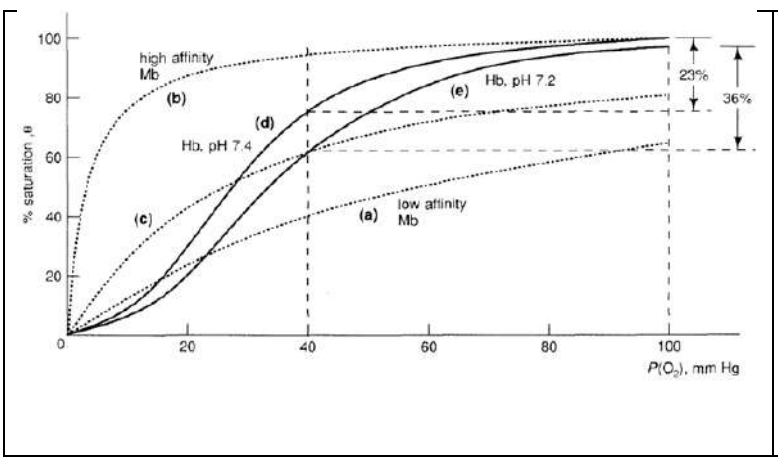
@ When O_2 consumption outpaces O_2 delivery, glucose is incompletely oxidized to lactic acid (instead of CO_2). The lactic acid produced lowers the pH, and O_2 release from oxy-Hb is stimulated (curve e) - Bohr effect (here the allosteric effector is H^+). Thus in an activity where O_2 is needed much, due to Bohr effect oxy-Hb releases O_2.

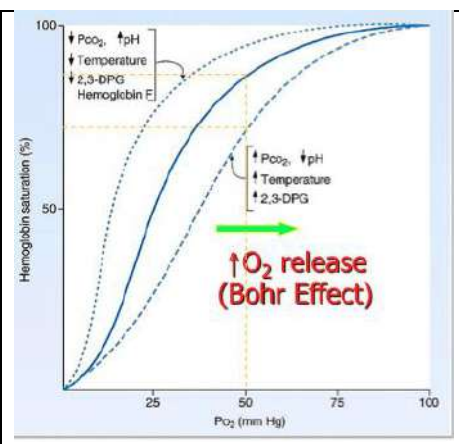
@ 2,3-DPG shows a heterotropic allosteric effect due to which O_2 affinity of Hb decreases with increase in its concentration and thereby stimulating the release of O_2.

@ 2,3-DPG is part of a subtle mechanism by which O_2 is transferred from mother to fetus across the placenta. The subunits comprising fetal Hb and adult Hb are slightly different.

@ In absence of allosteric effectors, the oxygen binding curves are identical.

@ However, 2,3-DPG binds less strongly to fetal Hb than to adult Hb. Thus, fetal Hb has a slightly higher affinity for O_2, thereby enabling O_2 to be extracted from mother's Hb.





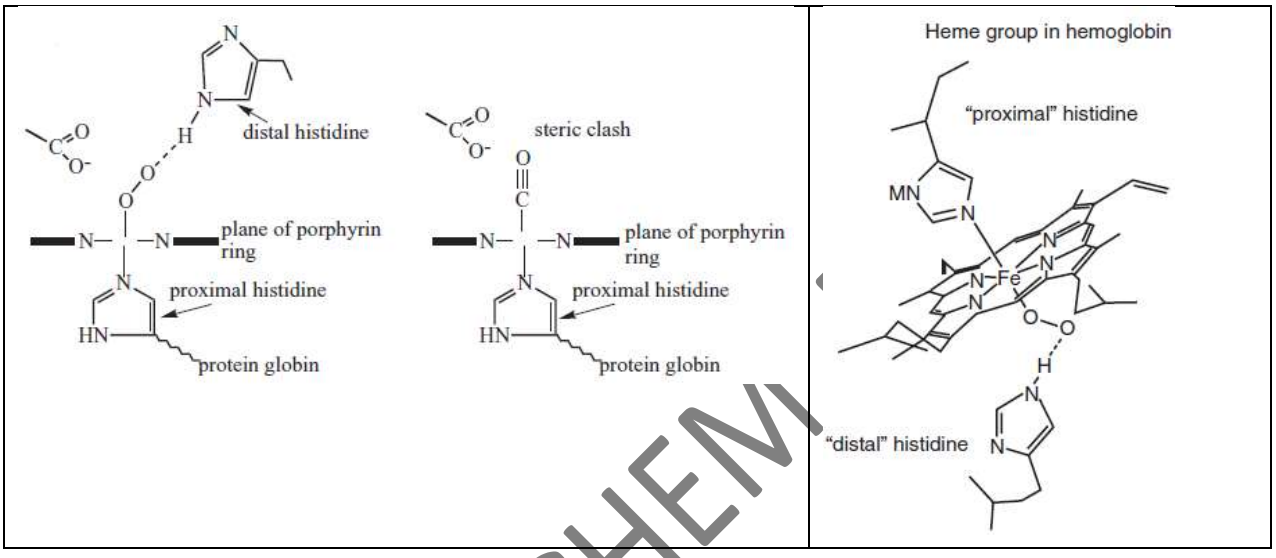
Role of distal and proximal histidine

@ Proximal histidine residue binds the fifth coordination site via its imidazole moiety and distal histidine resides in the region of sixth coordination site (does not coordinate to Fe(II))

@ The imidazole group from proximal histidine (F8) residue acts as a good σ-donor to facilitate the Fe(II) to act as better π-donor, making O_2 a better π-acid ligand to induce spin pairing at Fe.

@ CO is a powerful poison to Hb and Mb (binds CO strongly)

@ The globin protein drastically reduces the CO affinity. The presence of imidazole moiety from distal histidine (E7) residue in the region of sixth coordination site does not allow CO to form linear Fe-CO bond. The bent Fe-CO bond is very weak (for O_2, angular binding is normal)



Bohr effect- effect of pH

@ Hb binds one proton (H^+) for every two O_2 molecules released.

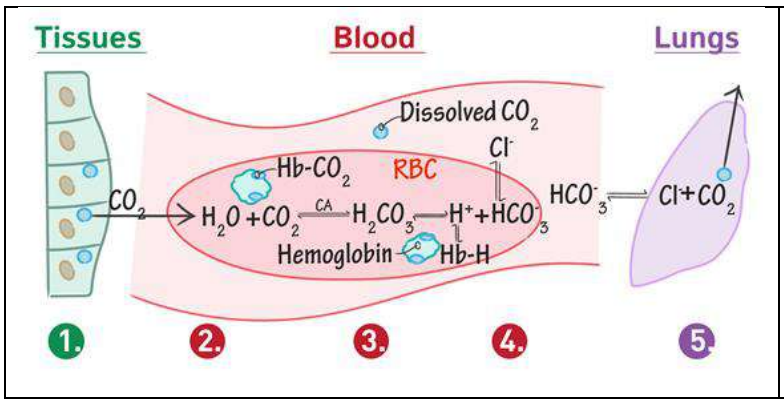
@ This favors the conversion of CO_2 (tissue metabolite) into HCO_3^- ion promoting its transport back to the lungs.

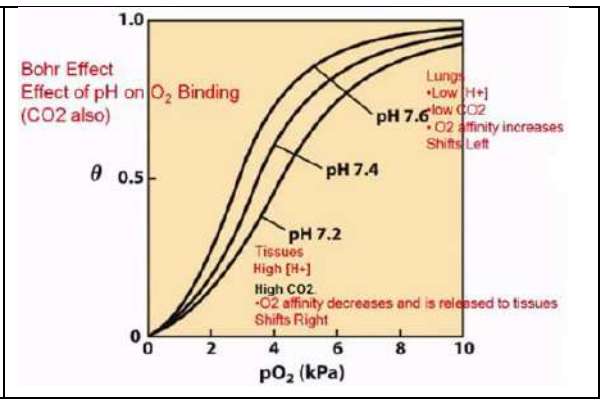
@ Similarly, the production of CO_2 from cell respiration and of lactic acid from anaerobic metabolism favors the release of O_2 to the tissues.

@ Beneficial at the tissue level where lower pH decreases O_2 affinity and promotes O_2 release.

@ As the pH increases the P_{50} value decreases, indicating an increase in O_2 binding and vice versa.

@ Root Effect: A very large Bohr effect, where O_2 affinity decreases sharply with pH.





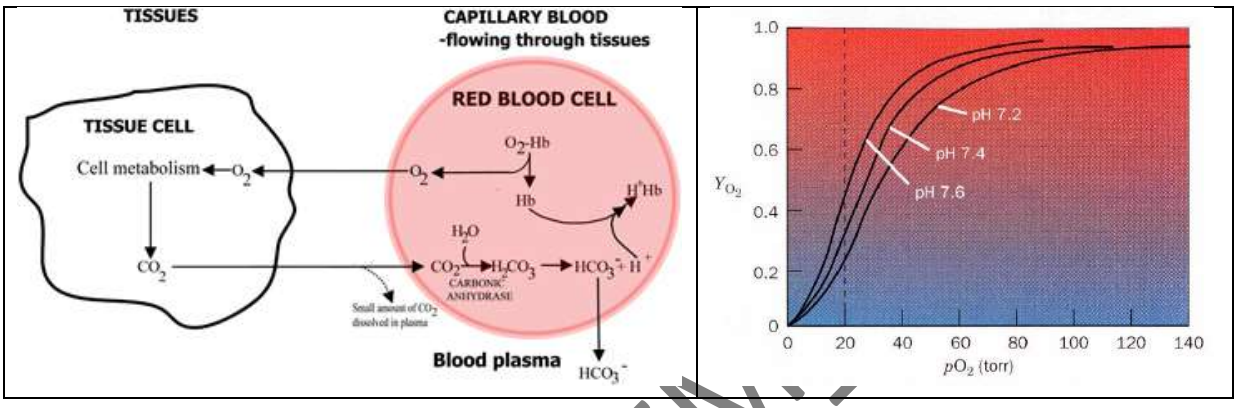
@ The concentration of H^+ ions can alter the affinity of Hb towards oxygen. This is because hemoglobin in the T-state has a higher affinity for H^+ ions than it does for oxygen.

@ As pH goes down ([H^+] goes up), Hb enters the T state and its affinity for oxygen goes down. More oxygen is needed to achieve maximum percentage saturation (Bohr effect).

@ It allows oxygen to dissociate at tissues with a lower pH: a good indicator of rate of cellular respiration.

@ Lower the pH, the more the dissociation curve shifts to right.

@ **Origin of Bohr effect:** When hemoglobin is in T state the N-terminal groups of α-subunits and C-terminal of β- subunits are protonated, allowing the ionic interaction with nearby carboxyl groups. This interaction helps to hold the hemoglobin in T state. Thus, oxygen affinity of hemoglobin decreases. At low pH oxygen binding affinity decreases.



Uptake of CO_2 by hemoglobin

@ In RBC, the enzyme carbonic anhydrase catalyzes the conversion of dissolved CO_2 to H_2CO_3, which rapidly dissociates to HCO_3^- and a free H^+.

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO_3^-$$

@ Histidine residues in Hb can accept H^+ and act as buffers.

@ Deoxygenated Hb is a better proton acceptor than the oxygenated form.

@ Stabilization of the produced H^+ will shift the reaction to the right, thus the enhanced affinity of deoxyhemoglobin for protons and thereby enhancing the synthesis of bicarbonate and increasing the capacity of deoxygenated blood for CO_2.

Oxygen-Hemoglobin dissociation curve

@ It is a plot of Hb saturation vs O_2 partial pressure and provides some insight of how blood carries and release O_2.

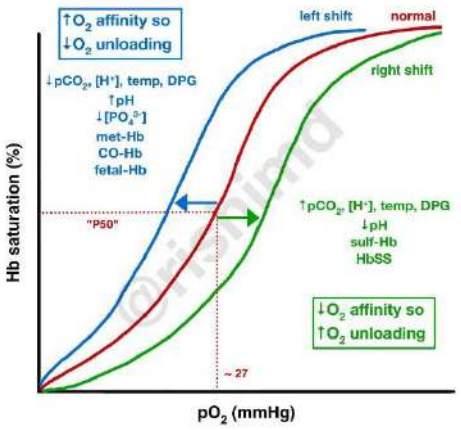
@ Strength with which O_2 binds to Hb is affected by several factors. These shift the curve.

@ Blood pH: Lowering of blood pH reduces the affinity of Hb for O_2, more O_2 is delivered.

@ CO_2 concentration: Higher the CO_2 concentration in tissue, less the is the affinity of Hb for O_2, the more O_2 is released.

@ Blood Temperature: Increased blood temperature reduces the affinity Hb for O_2. Higher temperatures mean oxygen has more kinetic energy, making it more likely to dissociate.

@ 2,3-DPG (2,3-diphosphoglycerate): It decreases the affinity of hemoglobin for oxygen. At high altitudes, 2,3-DPG level is elevated to decrease the O_2 affinity and thereby facilitating its release in tissues.



Rightward shift indicates decreased affinity, easier of release O_2.
Leftward shift indicates increased affinity of Hb to bind O_2.

Dioxygen Toxicity

@ Dioxygen toxicity takes place due to irreversible binding of O_2.

@ Free heme in aqueous solution is immediately converted to a new μ-oxodimer, hematin.

@ The first step is the binding of O_2 molecule, as in hemoglobin

@ The bound O_2 can co-ordinated to a second heme, forming a μ-peroxo complex.

@ Cleavage of the peroxo complex gives two molecules of ferryl complex with the Fe atom in +4 OS.

@ Finally, attack of the ferryl complex on another heme results in the formation of hematin.

@ Formation of μ-peroxo complex (dimerization) is less favorable at low temperatures (<-40°C) or by sterically preventing the bimolecular contact of an Fe^{III}-O_2^{I-} moiety with an Fe(II) moiety.

P-Fe(II) + O_2 ⟶ P-Fe(II)O_2

P-Fe(II)O_2 + P-Fe(II) ⟶ P-Fe(III)–O–O–Fe(III)-P
μ-peroxocomplex

P-Fe(III)–O–O–Fe(III)-P ⟶ 2P-Fe(IV)═O
ferryl complex

P-Fe(IV)═O + P-Fe(II) ⟶ P-Fe(III)–O–Fe(III)-P
hematin

Function of globin

@ Histidine, F8 binds to the proximal side and the oxygen binds to the distal side.

@ Heme without globin interacts with oxygen and Fe(II) is oxidized to Fe(III), no longer binds oxygen.

@ Globin introduces steric hindrance on one side of the heme plane thereby preventing dioxygen toxicity. The globin acts to (a) modulate oxygen binding affinity & (b) make reversible oxygen binding possible.

@ Globin weakens the interaction of CO with the heme and stabilizing the binding of dioxygen by distal histidine (E7) residues.

@ The protonation and deprotonation sites in globin chain are important in maintaining the biological pH and CO_2 transport.

@ Moreover, improper sequencing of amino acids results in several genetic disorder.

Anemia

Sickle-cell anemia – abnormal hemoglobin (due to defects in globin chain)

@ Sickle-cell anemia (Hb) is the most common form of abnormal hemoglobin

@ The hydrophilic glutamic acid at β-6 position is replaced by hydrophobic valine.

@ Causes a distortion of cell into sickle shape.

@ Hemoglobin polymerizes and precipitates – severe deformation in RBC

Cooly's anemia

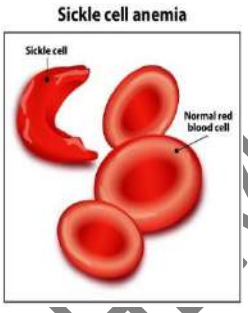
@ Genetic blood disease, results from insufficient production of β-chains

Thalassemia

@ α and β chains are wrongly produced.

@ α-thalassemia is caused by an erratic synthesis or total absence of α-globulin chains of Hb

@ β-thalassemia is caused from the defect in the β-globulin chains of Hb



Synthetic Dioxygen Carrier

@ Nature prevents the irreversible binding of dioxygen and protects Hb from hematin formation.

@ Steric hindrance provided by the globin part of the molecule prevents one oxoheme from attacking another heme. The embedded heme then binds dioxygen reversibly.

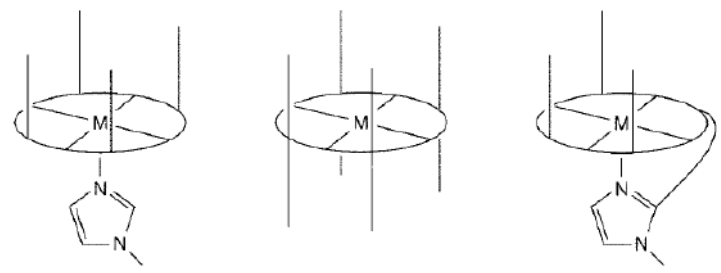
@ Some simple compounds to mimic the natural dioxygen carrier have been synthesized.

@ The model compounds contain the basic iron-porphyrin unit but attempt to simulate the globin protein in a simpler way.

@ Like natural systems, importance of steric hindrance and hydrophobic environment has been illustrated in different models and synthetic dioxygen carriers.

@ In model compounds, the steric hindrance has been introduced in different possible ways.

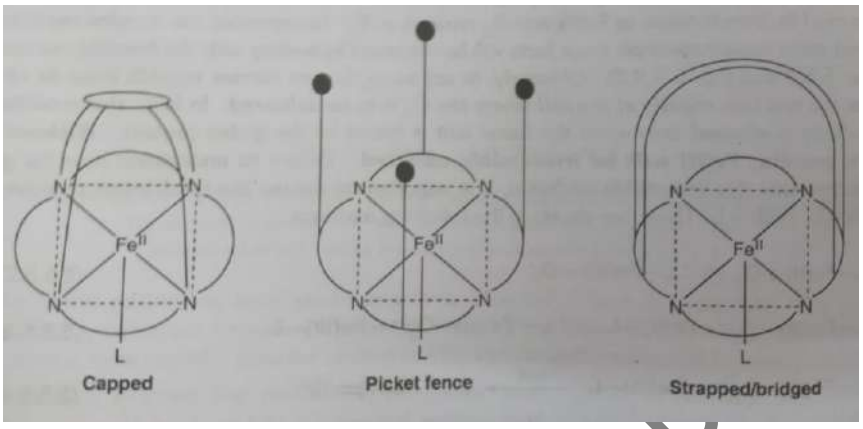
@ All these compounds can bind dioxygen reversibly and illustrate the role of bulky globin unit.

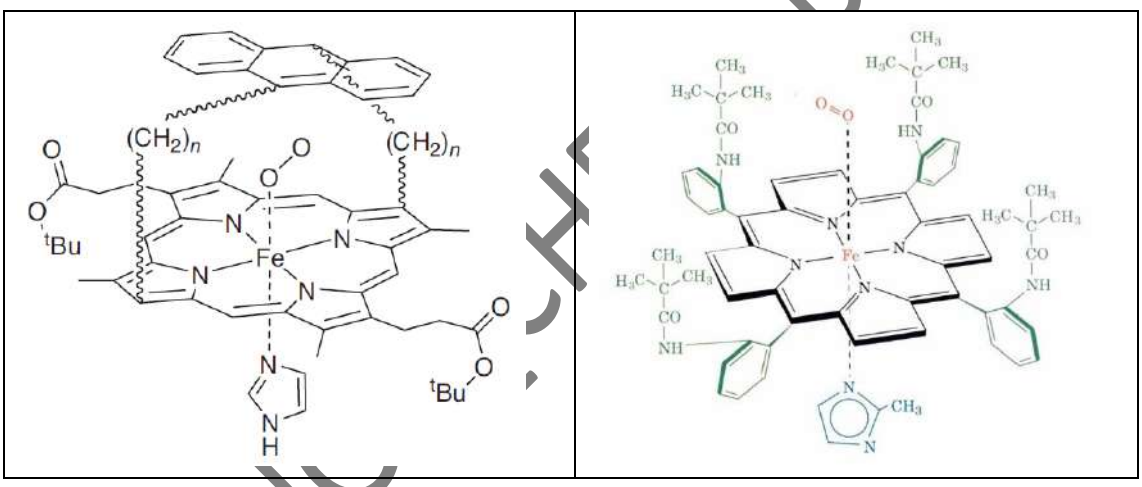


@ **Picket fence model:** Three of four large groups are projected on one side of the porphyrin plane and the other side is kept unhindered for imidazole. These bulky groups create a fence on one side of the plane to prevent the formation of a binuclear complex to be produced in the irreversible oxidation.

@ **Strapped/bridged model:** One chain extends one side of the porphyrin ring leaving room for dioxygen in the same side.

@ **Roofed/capped model:** The chains make a complete enclosure on one side of the ring providing sterically hindered binding sites even to the dioxygen molecules.



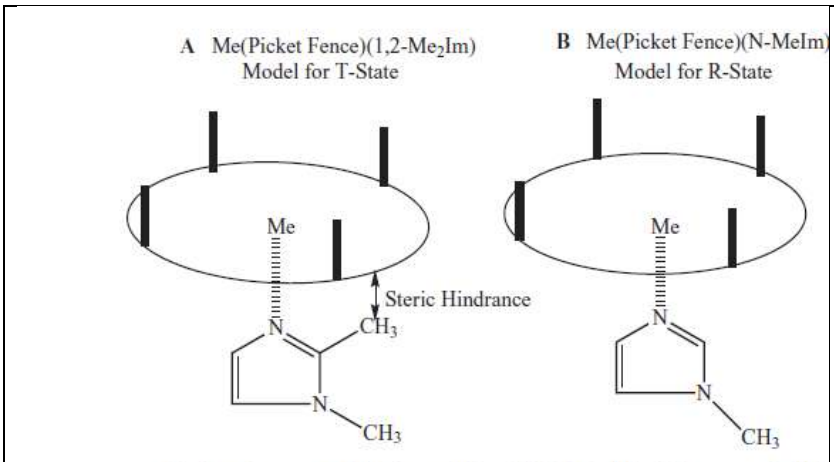


@ In summary, researchers found a number of methods for avoiding μ–oxo dimer formation and preserving a five - coordinate Fe(II) in iron containing model compounds, through:

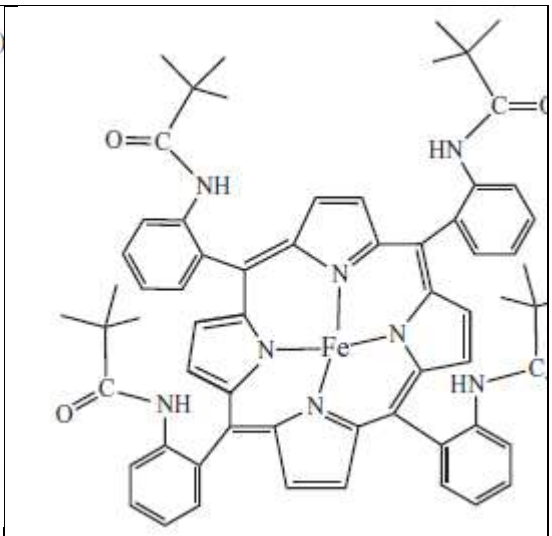
@ Modifying the imidazole to preserve the T – state

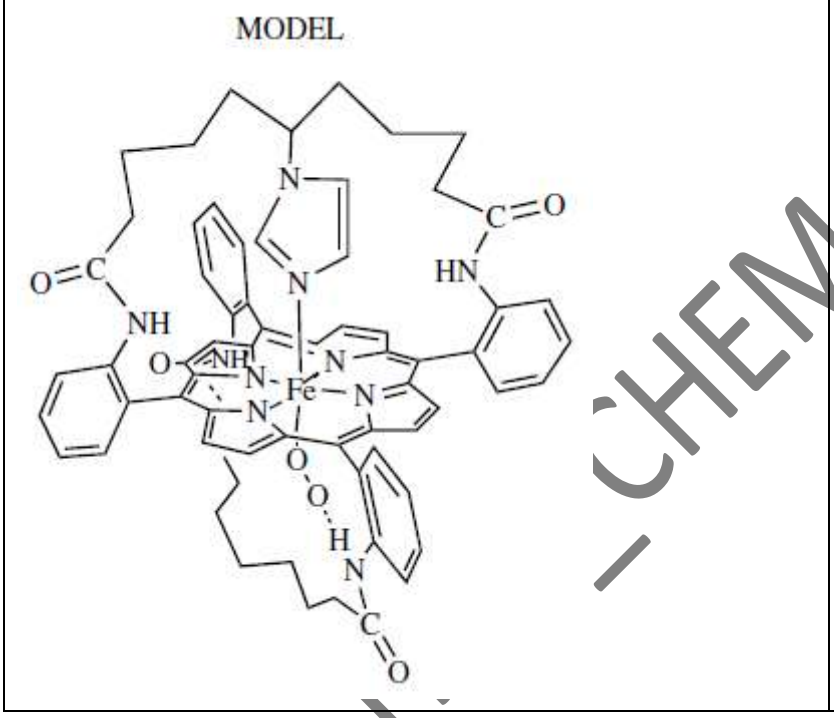
@ Modifying the porphyrin to sterically prevent addition of a large sixth ligand. The most well - known version of these is the "picket-fence" porphyrin illustrated.

@ Attaching the five - coordinate system to a rigid support, reducing its mobility and ability to add a sixth ligand.



Picket-fence porphyrin models. (A) Model for T-state and (B) R-state.





Haldane effect

@ The Haldane effect relates the number of protons released with a change in saturation of binding site (θ) at constant pH

@ In alveolar capillaries of lungs, the high concentration of O_2 unloads H^+ and CO_2 from hemoglobin through oxygenation. The release of proton stabilizes the deoxy form through the formation of salt bridge interaction. This property is the Haldane effect.

$$H^+ + HbO_2 \rightleftharpoons H^+Hb + O_2$$

@ The significance of this equation lies in realizing that oxygenation of Hb promotes dissociation of H^+ from Hb, which shifts the bicarbonate buffer equilibrium towards CO_2 formation; therefore, CO_2 is released from RBCs.

$$CO_2 + H_2O \longrightarrow H_2CO_3 \longrightarrow H^+ + HCO_3^-$$

@ In the oxygen-rich capillaries of the lung, this property causes the displacement of carbon dioxide to plasma as low-oxygen blood enters the alveolus and is vital for alveolar gas exchange.

Nature of Heme-Dioxygen bonding

@ Dioxygen can bind to metal in several ways, three are known in biology viz. superoxo (oxyHb), peroxide (oxyhemocyanin) and hydroperoxo (oxyhemerythrin).

@ The geometry is a function of the metal, its oxidation state and associated ligands.

@ In binding, oxygen temporarily and reversibly oxidizes Fe(II) to Fe(III) while oxygen temporarily turns into the superoxide, O_2^- ion

@ O_2 acts as a one electron acceptor leading to low spin Fe(III) with t2g5 configuration and O_2^- ion assuming octahedral geometry.

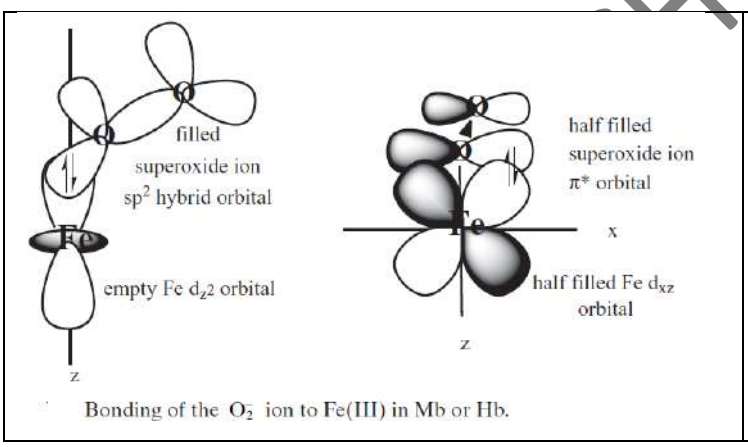
@ The unpaired t2g5 electron of Fe(III) undergoes antiferromagnetic coupling with the unpaired electron of antibonding π* orbital of O_2^- ion giving rise to diamagnetic properties.

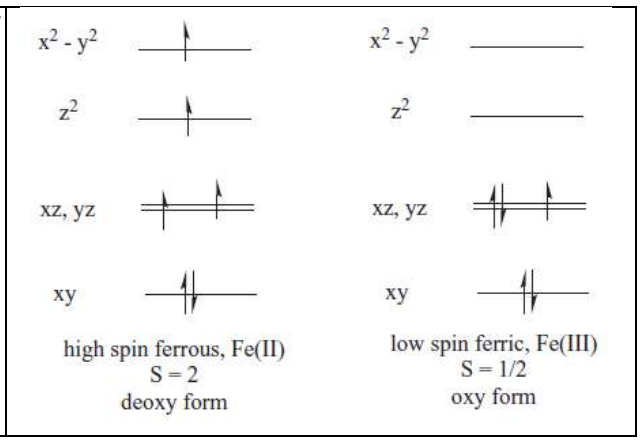
@ During oxygenation, O_2 makes a bent bond with metal center viz. one σ-type interaction between metal dz2 orbital and π* orbital of O_2 and another π-type interaction between dx2-y2 orbital and orthogonal π* orbital of O_2. The order of the O-O bond is about 1.5

@ In oxyhemoglobin, the stretching frequency for O-O bond appears at 1106 cm^{-1} with O-O separation is around 1.30 Å. This is significantly close to the O-O stretching of 1097 cm^{-1} in O_2^- ion

@ Although such O-O separations and vibrations are consistent with coordinated peroxide or superoxide moieties, the net amount of charge is transferred onto the dioxygen ligand from the metal.

@ The nearest formal oxidation state of iron in Hb-O_2 is the +3 state, with oxygen in the −1 state (as superoxide O_2^-).

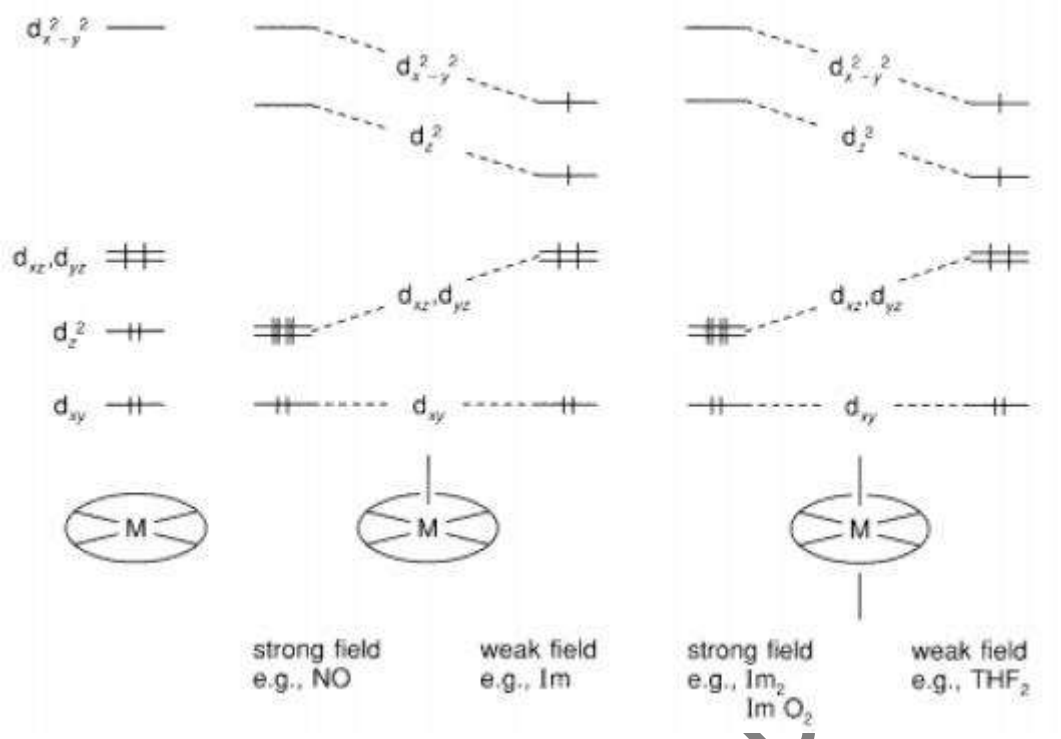




@ Iron porphyrins may be octahedral (two axial ligands), square pyramidal (one axial ligand), or square planar (no axial ligand). Metal d orbitals, now having partial porphyrin π* character, are split as shown

@ Radius of HS Fe(II)(S=2)/Fe(III)(S=5/2) metal is much greater the LS Fe(II)(S=0)/Fe(III)(S=1/2). This difference influences Fe-$N_{porphyrin}$ separations, porphyrin conformation, and the displacement of the iron center with respect to the porphyrin plane.

@ For iron(II)-porphyrins, a pair of imidazole or imidazole and CO ligands occupy the axial position giving diamagnetic complexes (S = 0) with approximate t_{2g6} symmetry.



Hemoglobin vs Myoglobin

Hemoglobin	**Myoglobin**
Four polypeptide chain (MW=64000 D)	Single polypeptide chain (MW=16.7 D)
Exhibits cooperative binding	Does not exhibit cooperative binding
Low affinity for oxygen, depends on oxygen concentration	High affinity for oxygen, does not depend on oxygen concentration
Found in blood stream	Found in muscle
Takes oxygen from lungs and transports to the rest of the body	Stores oxygen in the muscle cells and releases it when needed
Hb A (primarily), Hb A2 and Hb F are the types found in humans	A single type is found in the cells

Electron transfer Proteins - Structure and function

Lecture 8, 9 & 10

Electron transfer

@ Electron transfer reactions (ET) are central to many metabolic processes.

@ It depends on the approach of electron donor and electron acceptor

@ ET reactions are of two types

@ Inner sphere ET, coordination sphere of the reactants shares one ligand/forming a bridge

@ Outer sphere ET, coordination sphere of the reactants remains intact

@ In biology, outer sphere ET is common and can be intramolecular and intermolecular

@ Intramolecular ET occurs at fixed sites within single protein while intermolecular ET occurs between sites on different proteins

@ Intermolecular ET leads to ET chain, functions as a series of consecutive ET reactions between pairs of proteins

@ Electrons are generally transferred between metal sites that are arranged within a protein or complex of proteins via the intervening peptide units

@ The reactions of ET proteins are generally examined in terms of Marcus theory.

@ It correlates the rate constants of the reaction (k_{12}) with electron self-exchange rate constant of the reactants (k_{11} & k_{22}) and the equilibrium constant (K) for the ET reaction using the equation $(k_{12})^2 = k_{11}.k_{22}.K.f$; where $f \approx 1$.

@ The product $k_{11}.k_{22}$ reflects the intrinsic barrier to ET and K is a measure of the overall free energy $\Delta G°$.

@ The structure of the protein-the intrinsic separation, the nature of the intervening groups, and the orientation of the donor and acceptor site plays key role in determining the reaction rate constant.

Cytochromes

@ It is an ET protein with heme as prosthetic group

@ It is involved in photosynthesis (chloroplast) & aerobic/anaerobic respiration (mitochondria)

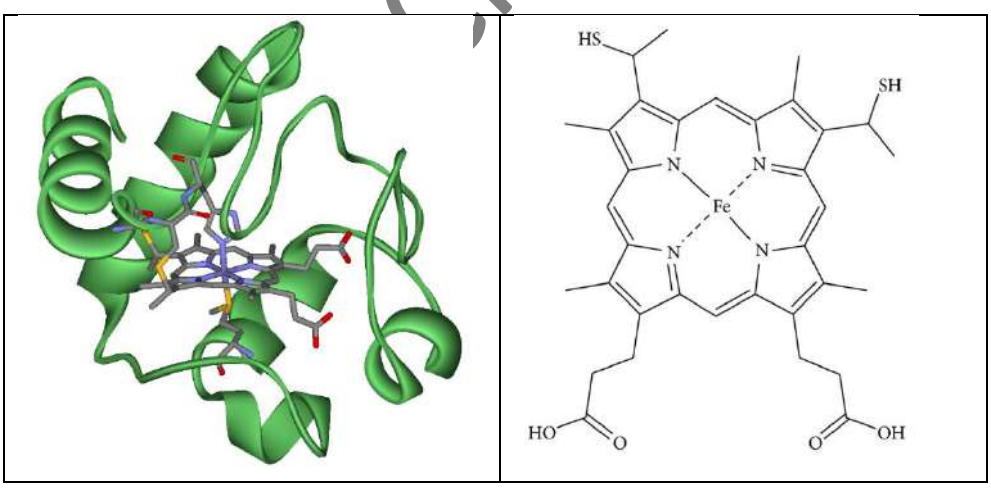
@ Porphyrin ring is a macrocyclic pyrrole system (Fe-protoporphyrin IX unit) with conjugated double bonds and various substituents that tune the redox properties.

@ Porphyrin donates two H^+ ions (di-anionic) and complexes with metal (II) ions forming porphyrin complex.

@ Size of the hole in the centre of porphyrin ring is ideal for accommodating 3d metal ions.

@ Porphyrin ring is rigid and its rigidity is attributed to the delocalization of π-electrons.

@ M-N bond distance is do not vary significantly (small metal ion - ruffled ring, large metal ion - domed ring).



Structural features

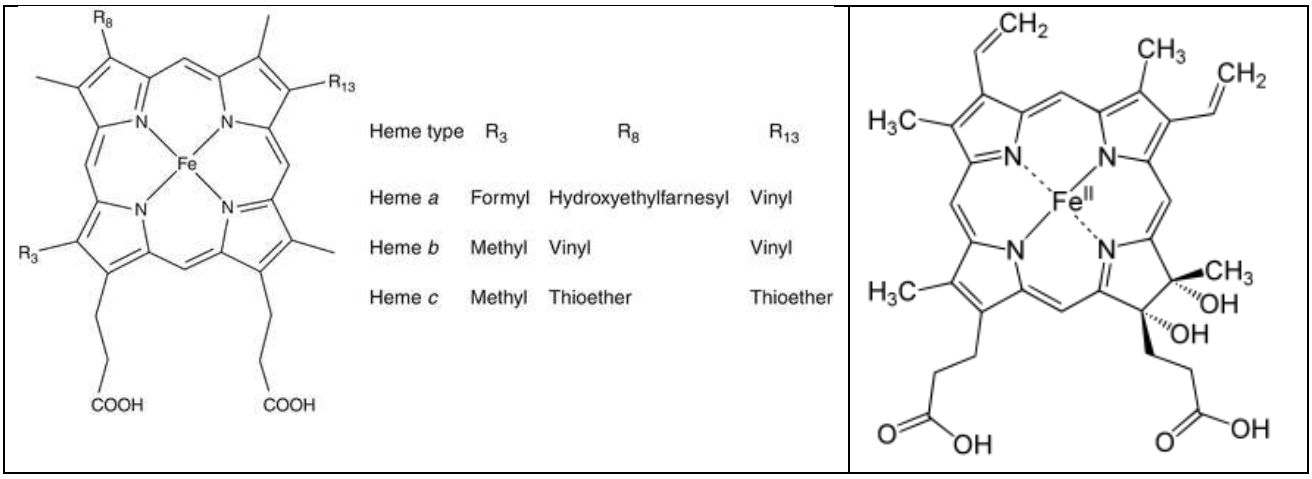
@ It is a porphyrin ring chelated to an Fe atom (square planar coordination for Fe).

@ One electron carrier by shuttling between Fe(II) and Fe(III) at their active site.

@ The fifth and sixth coordination sites (axial) may be occupied ligating sites from the protein chain (provides hydrophobic environment) to give octahedral geometry.

@ One of the axial ligands are often histidine-N but sometimes methionine-S or other protein chains. In some cases, sixth coordination site remain vacant for coordination by O_2.

@ Cytochromes present in different organisms differ only in their amino acid sequence.



Different groups of cytochromes

@ Cytochromes a: Heme has a formyl group.

@ Cytochromes b: protein is not covalently bonded to Heme.

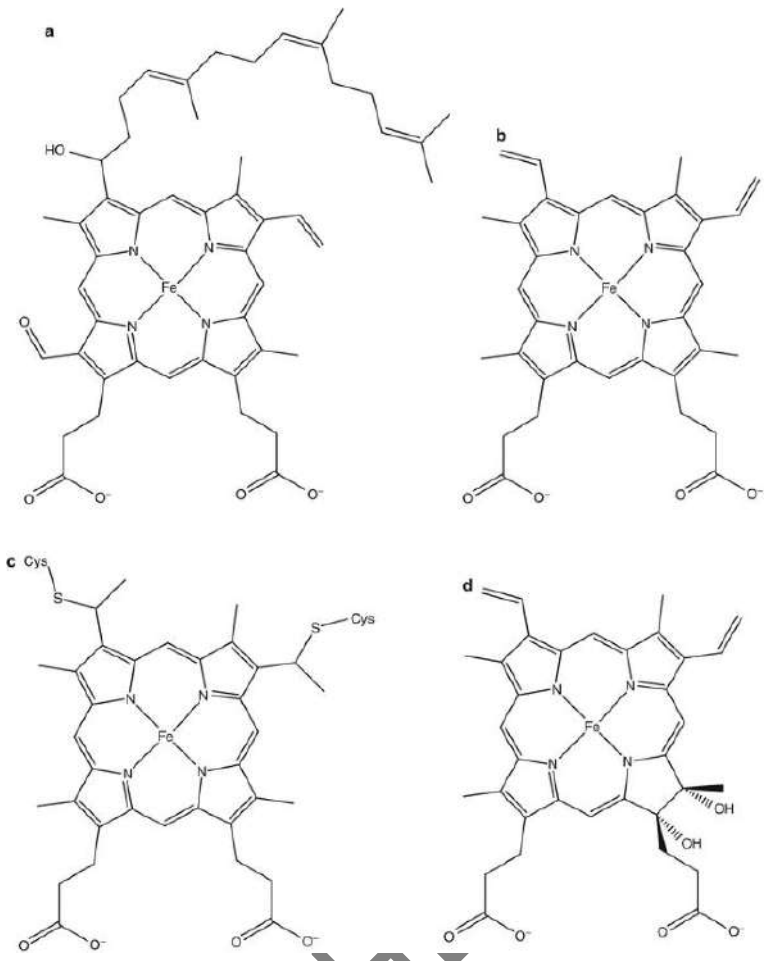
@ Cytochromes c: covalent links between protein and Heme.

@ Cytochromes d: the di-porphyrin group is present.

@ Cytochrome b and c generally have two strong field axial ligands and are low spin. They are hexa-coordinated, have no position for further coordination and therefore only interact by an electron transfer mechanism.

@ Cytochrome c, isolated from mitochondrial membranes where its role is to transfer electrons from cytochromes c_1 to cytochrome c oxidase, most widely studied cytochromes.

@ Some cytochrome a-types are five co-ordinate and account for unusual toxicity of CN^- binding at the Fe (III) site (prevents its reduction). CN^- can bind to Hb at the dioxygen sites & inhibition of cytochrome a activity is more serious than inhibition of oxygen binding.



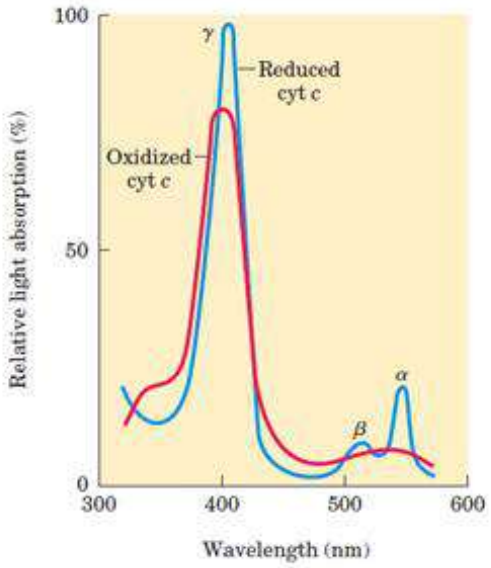
a-Cytochrome a, $C_{18}H_{30}OH$, b-Cytochrome b, c-Cytochrome c and d-Cytochrome d

Cytochromes- Electron Carriers

@ Cytochromes show strong absorption in visible light, can be attributed to their iron containing heme prosthetic groups.

@ The cytochromes a (600 nm), b (560 nm) and c (550 nm) can be distinguished by differences in their light-absorption spectra.

@ Each cytochrome in its reduced (Fe^{2+}) state has three absorption bands in the visible range.



Absorption spectra of cytochrome c in its oxidized (red) and reduced (blue) forms

Electron transport

@ The mitochondrion transfers the carbohydrate derived electrons to dioxygen and uses chemical potential of the reaction to dive the phosphorylation of ADP to ATP.

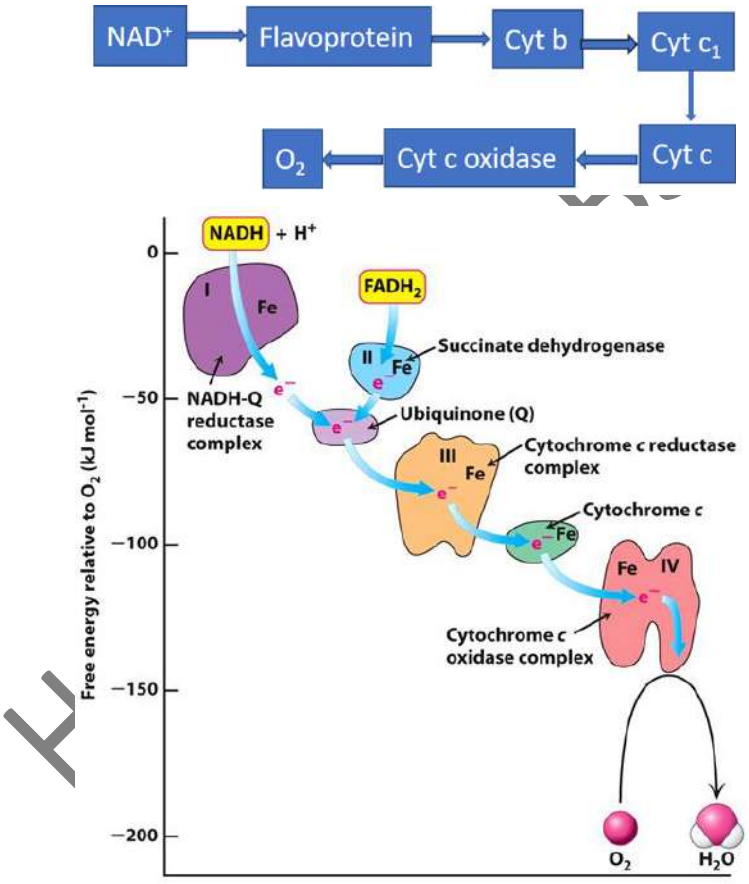
@ The electron flow occurs through a series/chain of several cytochromes.

@ No conformational changes occurs during electron transfer/redox process.

@ Three types of electron transfers occur in oxidative phosphorylation:

1. Direct transfer of electrons, as in the reduction of Fe^{3+} to Fe^{2+}
2. Transfer as a hydrogen atom ($H^+ + e^-$) and
3. Transfer as a hydride ion ($:H^-$), which bears two electrons.

@ Electron transfer from the reductase and to the oxidase occurs over a large distance (edge to edge distance of 16 Å) and distance between metal centers separated by a distance over 10 Å (relatively rapid).



Part of the mitochondrial electron transfer chain involving cytochrome c

Cytochrome c

@ Cytochrome c is present in both plants and animals, maintains the function of mitochondrial respiratory chain

@ Source: Mitochondrial inner membrane, endoplasmic reticulum, in chloroplast of plants & photosynthetic microorganisms

@ The different cytochrome c from different sources primarily differ in the amino acid sequences

@ It bridges between cytochrome c reductase and cytochrome c oxidase in the respiratory chain

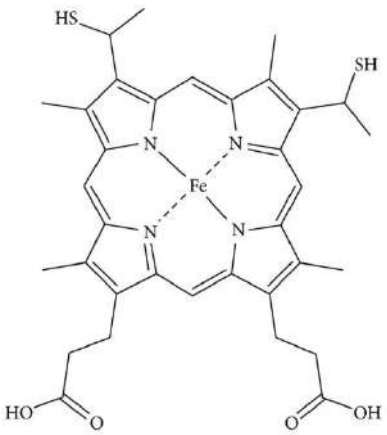
@ It is water soluble with low molecular weight, MW = 12,400 D

@ The active site of cytochrome c is the Heme group which is bonded to polypeptides chains of 104 amino acids residues.

@ Fe remains octahedrally coordinated in both the oxidized and reduced forms.

@ One axial site is occupied by the imidazole N-atom of histidine and the other axial position is occupied by the thioether S-atom of methionine

@ Heme group is surrounded by hydrophobic polypeptide chain, 3D & roughly spherical



Cytochrome c oxidase

@ Cytochrome c oxidase (inner mitochondrial membrane) is the terminal member of the electron transfer chain in mitochondrial respiration.

@ It has four active metal ions, two Cu and two heme Fe.

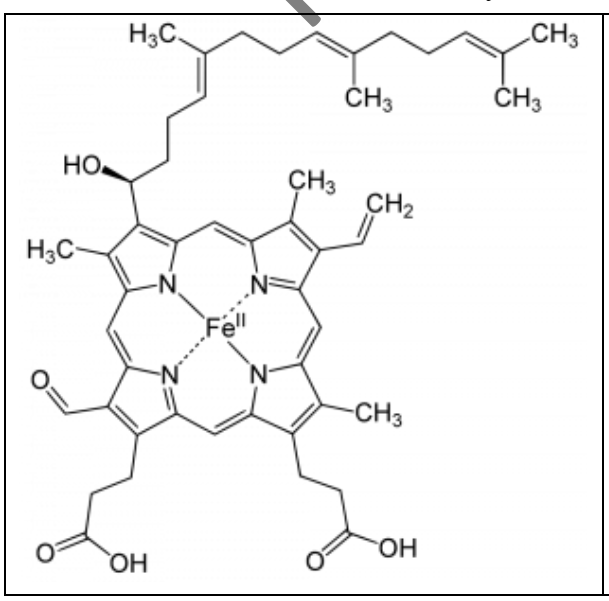
@ It catalytically reduces dioxygen to water, $O_2 + 4H^+ \rightarrow 2H_2O$ in four steps

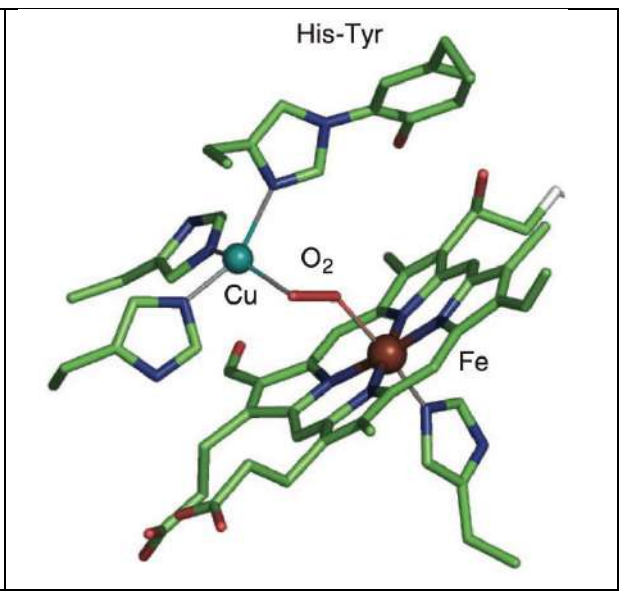
@ **Step 1**: One copper atom (Cu_A) and one heme unit (cyt a) are involved in electron transfer process while the second copper atom (Cu_B) and the second heme carry dioxygen through the four reduction steps to water.

@ **Step 2**: They neutralize four protons and pump four protons across the membrane in which the enzyme sits.

@ **Step 3**: It is proposed that, the dioxygen must coordinate to the sixth coordination site in cyt a and forms a hetero-di-metallic complex with Cu_B, $Cu_B(I)Fe_{a3}(II)$

@ **Step 4**: Electron transfer through cytochrome c oxidase occurs from cytochrome c to the Cu_A center, to heme a, to the heme a_3-Cu_B center, and finally to O_2





@ For every four electrons passing through this complex, the enzyme consumes four "substrate" H^+ from the matrix (N side) in converting O_2 to $2H_2O$.

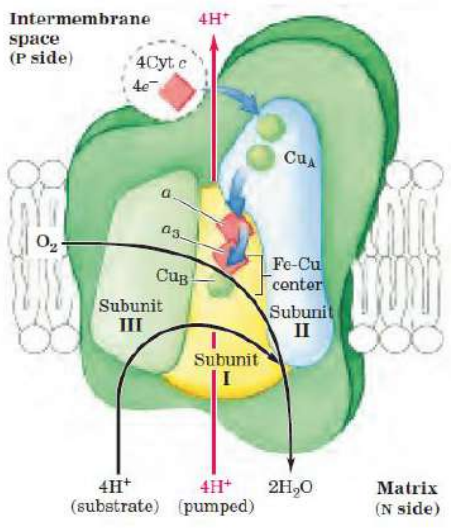
@ It also uses the energy of this redox reaction to pump one H^+ outward into the inter-membrane space (P side) for each electron that passes through,

@ This adds to the electrochemical potential produced by redox-driven H^+-transport

$$4\text{Cyt c(reduced)} + 8H^+_N + O_2 \rightarrow 4\text{Cyt c(oxidized)} + 8H^+_P + 2H_2O$$

@ This four-electron reduction of O_2 involves redox centers that carry only one electron at a time, and it must occur without the release of incompletely reduced intermediates such as H_2O_2 or $OH^{\bullet}$ very reactive species that would damage cellular components.

@ The intermediates remain tightly bound to the complex until completely converted to water.



Electron flow path in cytochrome c oxidase

Mechanism O_2 reduction by Cytochrome c oxidase

@ The reaction of cytochrome c oxidase involves

1. Reduction of the four metal centers by four equivalents of reduced cytochrome c,
2. Binding of dioxygen to the partially or fully reduced enzyme,
3. Transfer of four electrons to dioxygen, coupled with
4. Protonation of O_2 by four equivalents of protons to produce two equivalents of water, without giving any substantial amount of superoxide or hydrogen peroxide.

@ At low temperatures, the reaction can be slowed down and individual steps in the O_2 reduction can be visualized. For the purpose, fully reduced enzyme with CO bound is used. Binding of CO to the Fe(II) heme center in reduced cytochrome c oxidase inhibits the enzyme and makes it unreactive to O_2.

@ O_2 reacts very rapidly with the fully reduced enzyme to produce a species that appears to be the dioxygen adduct of cytochrome a_3 *(5.48)*. Such a species is presumed to be like other mononuclear oxy-heme derivatives.

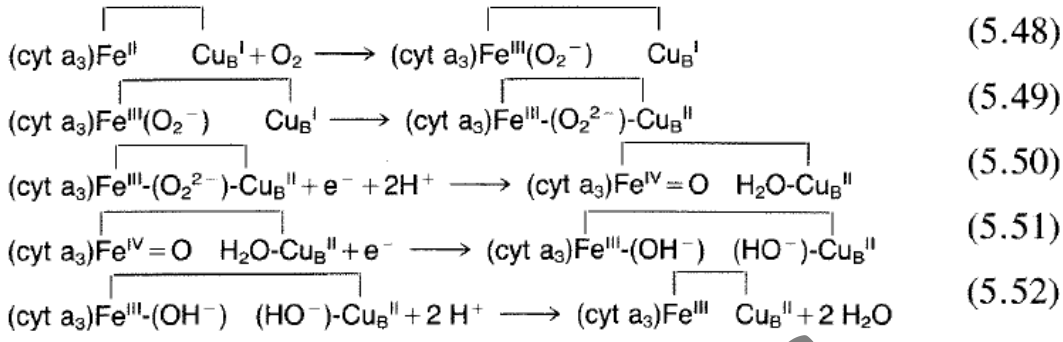
@ The dioxygen ligand in this species is then rapidly reduced to peroxide by the nearby Cu_B, forming what is believed to be a binuclear μ-peroxo species (*5.49*).

@ The μ-peroxo Fe(III)-(O_2^{2-})-Cu(II) species is then reduced by a third electron, resulting in cleavage of the O-O bond (*5.50*)

@ One of the oxygen atoms remains with Fe in the form of a ferryl complex, i.e., an Fe(IV) oxo, and the other is protonated and bound to copper in the form of a Cu(II) aquo complex.

@ Reduction by another electron leads to hydroxo complexes of both the Fe(III) heme and the Cu(II) centers (*5.51*)

@ Protonation then causes dissociation of two water molecules from the oxidized cytochrome *a_3-Cu_B center* (*5.52*)



Cytochrome P-450

@ These are heme enzymes which acts as mono-oxygenases and used dioxygen to catalyze aromatic/aliphatic hydroxylation or oxygenate a wide variety of substrates

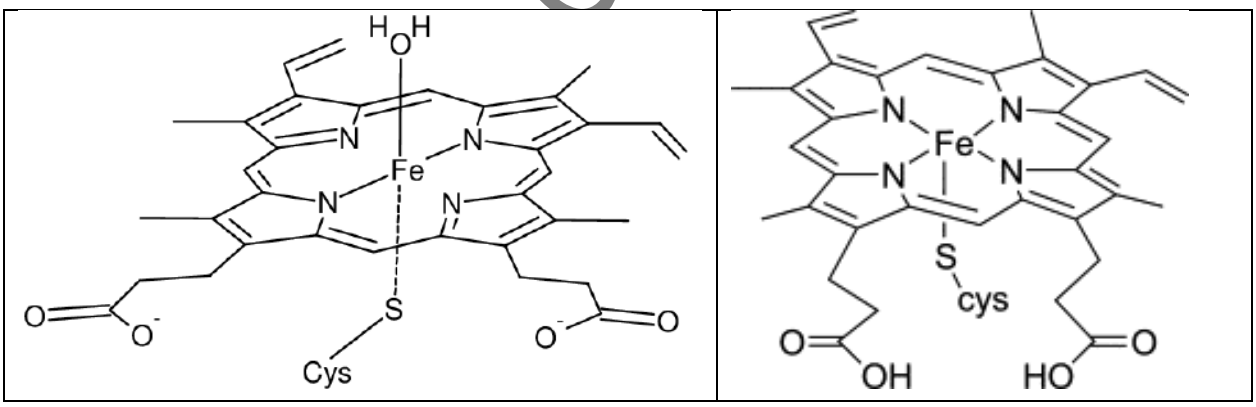
@ Source: plants, animals, and bacteria

@ The name of the enzymes derives from the fact that their CO adducts have absorption bands at 450 nm (Soret band, high energy π→π* transition attributed to axial thiolate ligand)

@ Fifth coordination site is occupied by a thiolate side chain of cysteine residues

@ No covalent attachment between the porphyrin ring and protein

@ In resting state, the Fe(III) is low spin with probably water molecule as the second axial ligand.



Mechanism of activity

@ The resting enzyme is shown at the top of the cycle.

@ Substrate binds to the enzyme at a position close to the iron center, but it is not directly coordinated to the metal ion.

@ The enzyme-substrate complex is then reduced to the ferrous form.

@ Dioxygen then binds to form an oxy complex (not shown).

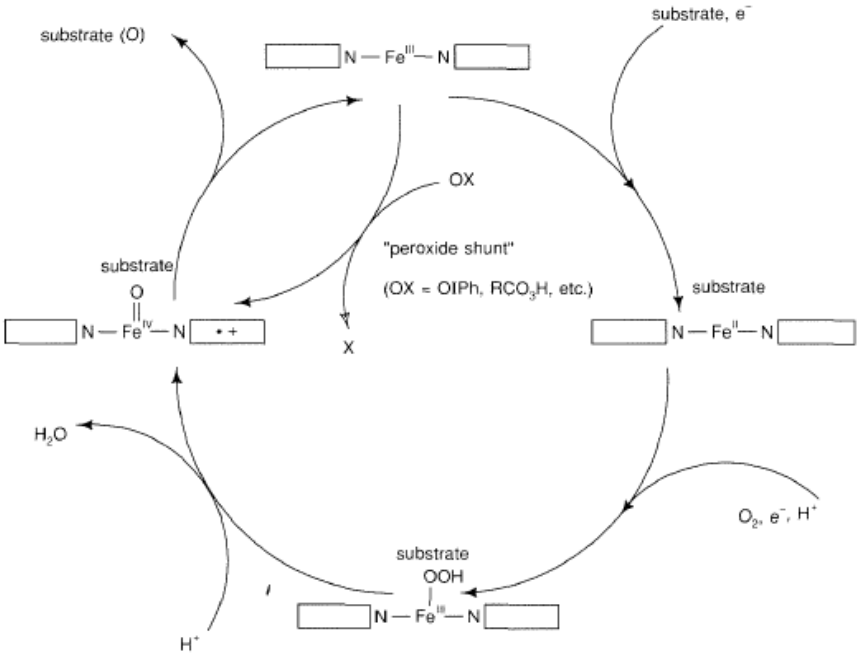
@ The oxy complex is then reduced by another electron and protonated, giving a ferric-hydroperoxy complex shown at the bottom of the cycle.

@ The ligand bound here to the Fe(III) center is HO_2^-, i.e., deprotonated hydrogen peroxide.

@ The ferric hydroperoxy form of the enzyme-substrate complex then undergoes heterolytic O-O bond cleavage, giving a high-valent Fe(IV) oxo center, with the porphyrin ligand oxidized by one equivalent.

@ This species then transfers a neutral oxygen atom to the bound substrate, which is then released, giving the oxygenated product and regenerating the resting form of the enzyme.

@ The "peroxide shunt" refers to the mechanism proposed for the cytochrome P450-catalyzed oxygenation of substrates by single-oxygen-atom donors.



@ Three species are potential candidates for "active oxygen," the oxygen-containing species that attacks the substrate, in cytochrome P-450. They are:

(1) a ferric peroxo (la) or hydroperoxo complex (1b), formed from one electron reduction of the oxy complex (Reaction 5.65);

(2) an iron(IV) oxo complex (2) formed by homolytic O-O bond cleavage of a ferric hydroperoxo complex (Reaction 5.66); and

(3) a complex at the oxidation level of an iron(V) oxo complex (3) formed by heterolytic O-O bond cleavage of a ferric hydroperoxo complex (Reaction 5.67).

$$Fe^{II}P + O_2 \longrightarrow FePO_2 \xrightarrow{e^-} \underset{\textbf{1a}}{[Fe^{III}P(O_2^{2-})]^-} \xrightarrow{H^+} \underset{\textbf{1b}}{Fe^{III}P(O_2H^-)} \qquad (5.65)$$

$$\underset{\textbf{1b}}{Fe^{III}P(O_2H^-)} \longrightarrow \underset{\textbf{2}}{Fe^{IV}P(O)} + HO^{\cdot} \qquad (5.66)$$

$$\underset{\textbf{1b}}{Fe^{III}P(O_2H^-)} \longrightarrow \underset{\textbf{3}}{[Fe^{V}(P^{2-})(O)^+ \longleftrightarrow Fe^{IV}(P^-)(O)^+]} + HO^- \qquad (5.67)$$

(P^{2-} = porphyrin ligand; P^- = one-electron oxidized porphyrin ligand)

Iron-sulfur proteins: Electron Carriers

@ Fe-S proteins (Helmut Beinert) are non heme electron transfer proteins, found in all living organisms

@ Fe-S proteins are relatively low MW high-spin Fe(II) or Fe(III) coordinated tetrahedrally by four S-donors.

@ Fe is uniquely bound either by S-atom from cysteinyl residue (sulphydryl group) or by inorganic (labile) S-atoms/S^{2-} ions present in the protein.

@ Fe-S clusters are represented by square brackets with the number of Fe and inorganic S (labile) atoms [nFe-mS]. E.g., [2Fe–2S], [4Fe–4S], [3Fe–4S] etc.

@ Rieske iron-sulfur proteins (John S. Rieske) are a variation on this theme, in which one Fe atom is coordinated to two histidyl residues rather than two cysteinyl residues.

@ Fe-S proteins participate in one-electron transfer, one of the Fe-atom is oxidized or reduced.

@ Reduction potential (-0.65 V to +0.45 V) of Fe-S proteins are sensitive to the conformation of the protein chain

@ Functions – involved in redox process such as nitrogen fixation, oxidation of NADH to $[NAD]^{+}$ in mitochondria, and also in catalytic sites in hydrogenases

Rubredoxins [1Fe-0S]

@ Rubredoxins (MW ≈ 6000 D), Rd the simplest iron-sulphur protein, is found in bacteria.

@ Inorganic sulphur is absent, and Fe atom is bound to four cysteinyl residues in a distorted tetrahedral manner.

@ The metal site lies in a pocket of the folded protein chain, the Fe-S bond length in Fe(III) form are within 2.24-2.33 Å and increased by 0.05 Å in reduced Fe(II) form.

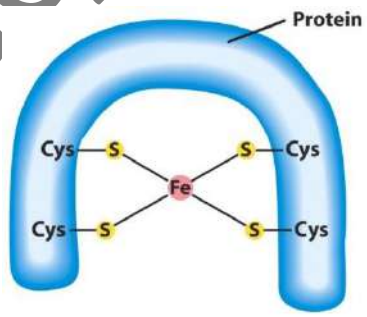
@ Rd is considered to be in entatic state and can act as one electron donor-acceptors.

@ It is a high spin Fe(III)/Fe(II) couple, reduced form experiences J. T. distortion which is important for rapid electron transport.

@ For Rd *Clostridium pasteurianum* E°= -0.57 V, negative E° indicates the stabilization of oxidized form resulting from S^{2-} ligand

$$Fe^{3+} + e^{-} \rightleftharpoons Fe^{2+} \qquad E^{o} = -0.15 \text{ to } 0.40 \text{ V}$$

S=5/2 S=2



S-Fe-S angle ≈ 104° to 114°

Ferredoxin [2Fe-2S]

@ Ferredoxin [2Fe-2S] is isolated from mammalian, plant & bacterial sources.

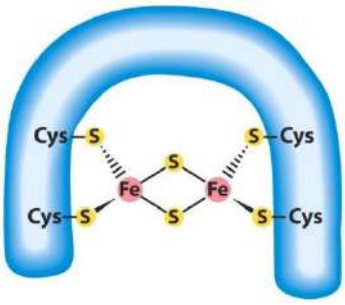
@ It contains two Fe centers, bridged by two S^{2-} ligands with the tetrahedral coordination sphere of each metal completed by two cysteinyl S-residues

@ The Fe-Fe distance in the oxidized form is 2.69 Å

@ The cluster transfers & accepts one electron, and on reduction gives a mixed valance Fe(II)-Fe(III) cluster. It is EPR active with HS d^5-HS d^6 pair.

@ In the reduced form, the metal centres are non-equivalent (quenching is not complete).

@ Diamagnetism in the oxidized form arises from the antiferromagnetic coupling of Fe(III) (two HS d^5) centres through the bridging sulphur sites.



$[2Fe-2S]^{2+} + e^- \rightarrow [2Fe-2S]^+ \qquad E^{\circ} = 0 \text{ to } -0.4 \text{ V}$

2 Fe(III) {Fe(III):Fe(II)}

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

[2Fe-2S] Rieske's protein

@ Rieske proteins belong to the 2Fe-2S class with at least one (two is common) imidazole N of histidine residues linked to Fe.

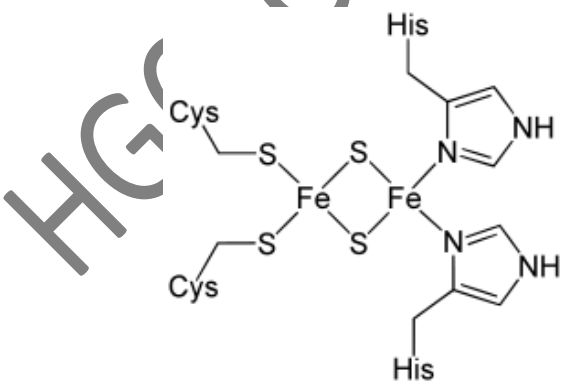
@ It is isolated from bovine mitochondria and photosynthetic b6*f* complex

@ Presence on non-sulphur remarkably influence the reduction potential (+0.35 to -0.15V)

@ It is non-symmetrical with one Fe linked to two cysteinyl residues and other Fe linked to two histidine residues.

@ Number of labile Sulphur remains unchanged (same in both Fd and RP)

@ The potential depends strongly on pH, indicates possible role in coupling proton and electron transfer processes.



$[Fe^{III}S_2Fe^{III}]^{2+} + e^- \rightleftharpoons [Fe^{III}S_2Fe^{II}]^+ \qquad E^{o} = -0.15 \text{ to } 0.40 \text{ V}$

S=0 S=1/2

Ferredoxin [4Fe-4S]

@ [4Fe-4S] protein is found in certain bacteria involved in anaerobic metabolism.

@ It is a distorted cube with alternate corners occupied by Fe and inorganic sulphur (S^{2-}).

@ Each Fe is coordinated to three sulphide and one cysteine thiolate.

@ The cubane like [4Fe-4S] cluster is considered as the combination of two Fe_2S_2 cluster

@ [4Fe–4S] ferredoxin containing four Fe(II) centers is never accessed in biology, Fe(II) and Fe(III) centers delocalized over the cluster core.

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

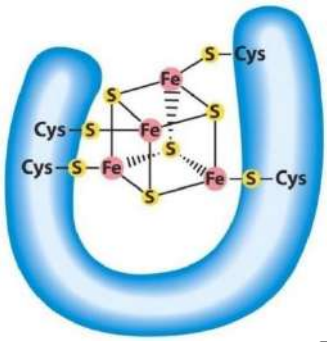
@ Reduced form is paramagnetic and indicate the presence of antiferromagnetic coupling

@ Peptococcus aerogenes contains two [4Fe-4S] clusters separated by 12Å with E° value of -0.4V

$$[4Fe-4S]^{2+} + e^- \rightarrow [4Fe-4S]^{+} \qquad E^{\circ} = -0.2 \text{ to } -0.7 \text{ V}$$

{2Fe(III):2Fe(II)} {Fe(III):3Fe(II)}

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



High potential iron sulphur protein, HiPiP

@ Some [4Fe-4S] clusters are also known as HiPiP or clostridial ferredoxin

@ HiPiP found in photosynthetic *Chromatium vinosum* has a single [4Fe-4S] cluster with a redo potential of +0.35 V

@ Unlike [4Fe-4S] ferredoxin, the reduced HiPiP cluster is significantly distorted

@ Oxidized HiPiP is paramagnetic while the reduced protein is diamagnetic (EPR silent).

@ 2Fe-2S ferredoxin is a one electron transfer agent so 4Fe-4S should be two electron transfer agent (CV-studies supports).

@ However, structural effect (protein environment) play crucial role in making HiPiP to acts as one electron transfer protein.

@ In biological systems, there are three available cluster oxidation levels but in a given system only one pair is employed.

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

3Fe(III)1Fe(II) 2Fe(III)2Fe(II) 1Fe(III)3Fe(II)

HiPiP-ox HiPiP-red

Fd-ox Fd-red

@ No single ferredoxin is known to undergo reversible electron transfer between all three oxidation levels

Ferredoxin [3Fe-4S]

@ Found in bacteria *Desulfovibrio gigas* and *Azobacter vinlandii,* in inactive form of pig heart aconitase, an enzyme that converts citrate to isocitrate.

@ The structure is quite similar to that of [4Fe-4S] ferredoxins with one Fe removed to leave a voided cuboidal cluster.

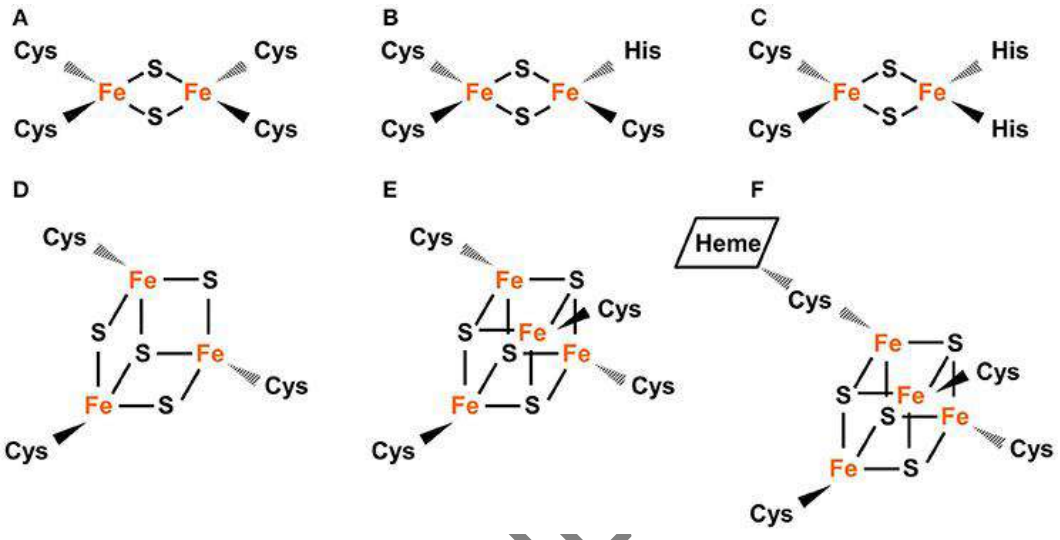
@ [3Fe-4S] clusters possess the ability to undergo interconversion reaction to [4Fe-4S] clusters with minimum structural change (dehydration-rehydration reaction).

@ The cuboidal [3Fe-4S] form of aconitase is inactive, reactivated by addition of Fe(II) under anaerobic conditions followed by interconversion to the active cuboidal [3Fe-4S] centres.

$$[3Fe{-}4S]^{+} + e^{-} \rightarrow [3Fe{-}4S] \qquad E^{\circ} = +0.1 \text{ to } -0.4 \text{ V}$$

3Fe(III)	{2Fe(III):Fe(II)}
$S = \frac{1}{2}$	$S = 2$

Structures of Fe-S proteins



Blue copper protein

@ Blue copper protein are redox system in bioinorganic chemistry, ideal for electron exchange

@ Blue Cu proteins are classified according to their spectroscopic properties viz. Type I, II & III

@ Type I or blue, has an intense adsorption in the visible region (597 nm) arising from S(cysteine)→Cu (II) (d_{x2-y2}) charge transfer (LMCT). Also, an EPR spectrum with unusually narrow hyperfine splitting due to an asymmetric environment at the metal.

@ Type II or normal, has limited absorption and EPR spectrum typical of small Cu(II) complexes

@ Type III, has a strong absorption near UV region (330 nm) and no EPR signal, believed to be consists of a pair of antiferromagnetically coupled Cu(II) ions

@ Blue copper protein: stellacyanin (no methionine ligand), plastocyanin (higher plants, green algae) and azurin (denitrifying bacteria)

@ Reduction potentials for Cu(II)/Cu(I) couple is within the range 0.15-0.8 V, so more oxidizing than cytochromes

Plastocyanin

@ Plastocyanin is a Cu protein (Type I) with molecular weight around 10,000 D

@ It transports electrons between PS I and II in photosynthetic chain

@ ET proceeds vis transition state structures which are intermediate between those of reactant and product.

@ Interconversion of geometries from Cu(I) (tetrahedral) to Cu(II) tetragonal is energetically demanding.

@ Plastocyanin is pseudo tetrahedral, oxidation state shuttling between Cu(I),d^{10}/Cu(II), d^{9}

@ Electron exchange involve no change in spin state

@ No or little movement of the ligands and small Frank Condon activation barrier.

@ Blue color (597 nm) is due to the Cu-S (Cys) bond ($S_{p\pi} \rightarrow Cu_{dx^2-y^2}$, LMCT charge transfer).

@ EPR spectrum (Cu(II) - one unpaired electron) shows narrow hyperfine splitting

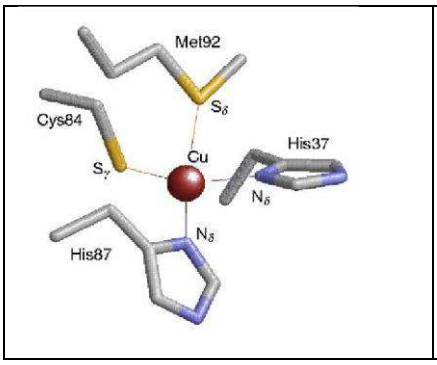
Structure

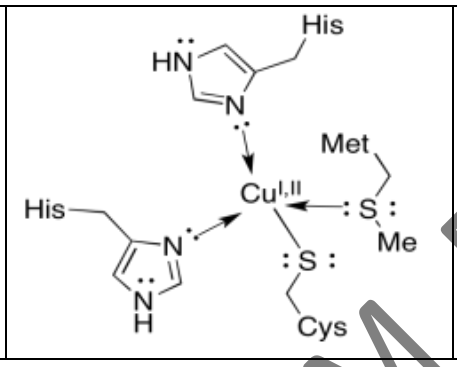
@ Cu(I) is in tetrahedral environment and Cu(II) is octahedrally coordinated with JT distortion to square planar coordination

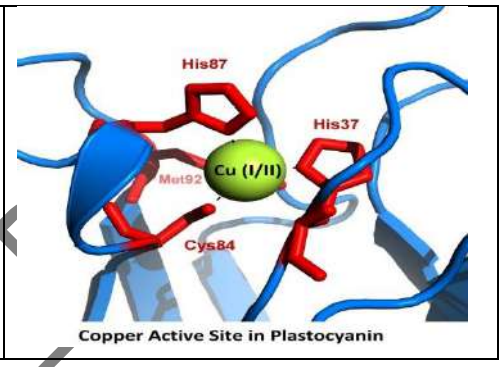
@ The protein chain in a plastocyanin comprises between 97 and 104 amino acid residues

@ Exhibits a distorted tetrahedral geometry (distorted trigonal pyramidal) with an unusually long Cu-S methionine (2.90 A°) distance in both the oxidation states.

@ The Cu centre lies within a pocket in the chain, composed of two N-atoms from two His residues, a S-atom from Cys residue & another S-atom from an axial Met residue forms the apex.







Plastocyanin	Cu(I)	Cu(II)
Cu-N-His37	2.12	2.04
Cu-S-Cys84	2.11	2.13
Cu-N-His87	2.25	2.10
Cu-S-Met92	2.90	2.90

@ The geometry of the copper binding site is described as a 'distorted trigonal pyramidal'.

@ Distortion occurs due difference in Cu-S bond lengths, Cu-S(Cys) contact is shorter than Cu-S(Met).

@ The elongated Cu-S bond destabilizes the Cu II form and increases the redox potential of the protein.

@ In the reduced form, His-87 will become protonated (pKa = 4.4). Protonation restricts its role as ligand & the geometry becomes trigonal planar.

@ While the molecular surface of the protein near the copper binding site varies slightly, all plastocyanin have a hydrophobic surface surrounding the exposed Histidine of the copper binding site

Function of Plastocyanin

@ Plastocyanin has adequate structure for electron transfer and involved in electron transfer between PSI (acceptor - P700) & PSII (donor - Cytochrome b_6f).

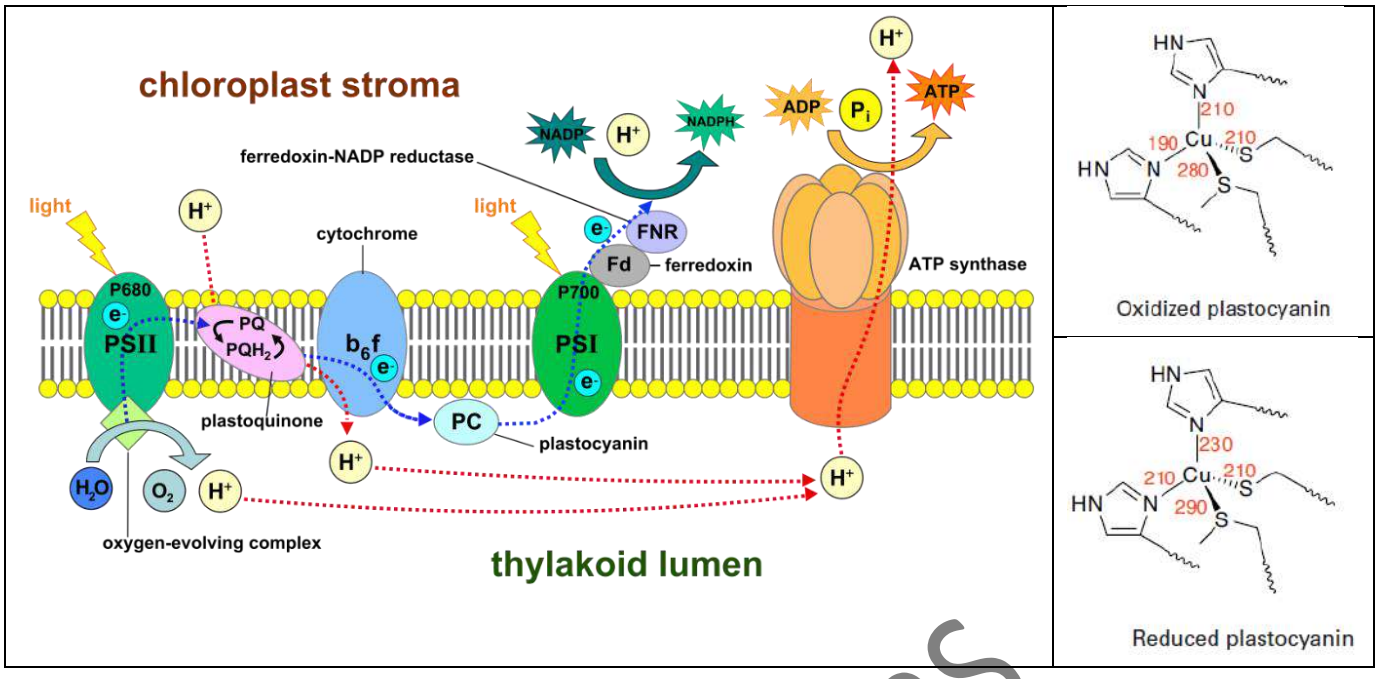
@ Due to water solubility, it can move through the inner space of the thylakoids.

@ Its Cu center acts as the catalyst in the redox reaction.

@ Plastocyanin ($Cu^{2+}PC$) is reduced by cytochrome: $Cu^{2+}PC + e^- \rightarrow Cu^{+}PC$

@ After dissociation, $Cu^{+}Pc$ diffuses through the lumen space until recognition/binding occurs with P700 & finally gets oxidized: $Cu^{+}PC \rightarrow Cu^{2+}PC + e^-$

@ Plastocyanin maintains the redox potential (370 mV) and the isoelectric pH is about 4.



@ The crystal structures of oxidized, reduced and apo forms reveal that the ligands remain in essentially the same position in all cases

@ As a result, the blue Cu center is well suited to undergo fast and efficient electron transfer because the reorganization energy is small.

@ Cu in a coordination environment that does not change upon electron transfer.

Oxidative Phosphorylation (Electron transport)

Components in respiratory chain

1. Cytochromes
2. Fe-S proteins
3. Dehydrogenase systems

Proton coupled electron transfer reactions (PCET): Reduced molecules

@ NADH (nicotinamide adenine dinucleotide): Produced in glycolysis and fatty acid oxidation

@ $FADH_2$ (flavin adenine dinucleotide): Produced in Kreb's cycle or citric acid cycle

@ Quinone system: Found in most living organisms. Reduced form is hydroquinone (H_2Q) and oxidized form is Q

@ Photosynthetic systems requires plastoquinone and respiratory systems requires ubiquinone.

@ Ubiquinone is also described as coenzyme (CoQ)

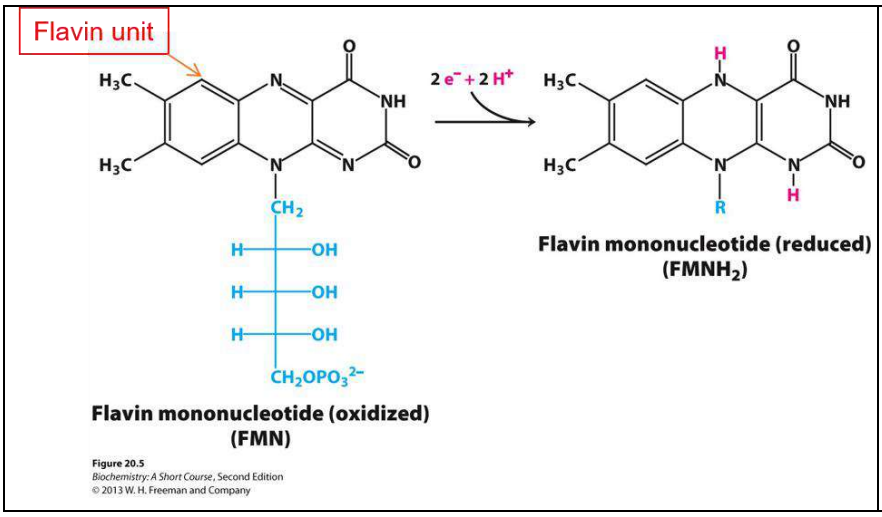
@ CoQ is the only electron carrier which is not covalently bonded to protein chain in the respiratory system. Thus, it functions as mobile carrier of electron.

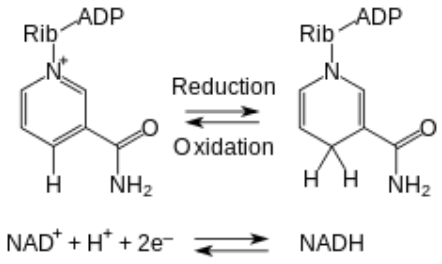
Flavodoxins Electron Carriers

@ NADH and NADPH are water-soluble electron carriers that associate reversibly with dehydrogenases.

@ NADH carries electrons from catabolic reactions (CAC) to their point of entry into the respiratory chain.

@ Flavoproteins contain a tightly bound flavin nucleotide either FMN or FAD. The oxidized flavin nucleotide can accept either one electron (yielding the semiquinone form) or two (yielding $FADH_2$ or $FMNH_2$).





Prosthetic group of ETC protein (complex I)

1- or 2-electron acceptor

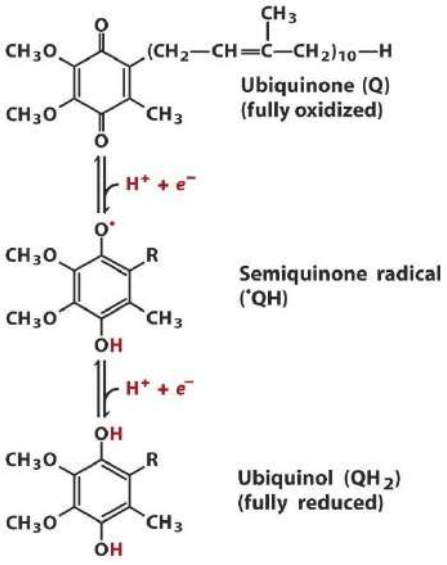
ETC carriers: Coenzyme Q

@ Ubiquinone (CoQ, or simply Q) is a lipid-soluble benzoquinone with a long isoprenoid side chain

@ Ubiquinone can accept one electron to become the semiquinone radical (QH) or two electrons to form ubiquinol (QH_2) and, like flavoprotein carriers, it can act at the junction between a two-electron donor and a one-electron acceptor.

@ Because ubiquinone is both small and hydrophobic, it is freely diffusible within the lipid bilayer of the inner mitochondrial membrane and can shuttle reducing equivalents between other, less mobile electron carriers in the membrane.

@ And because it carries both electrons and protons, it plays a central role in coupling electron flow to proton movement (proton coupled transfer ($H^+ + e$).



Mobile electron carrier within the bilayer

1- or 2-electron acceptor

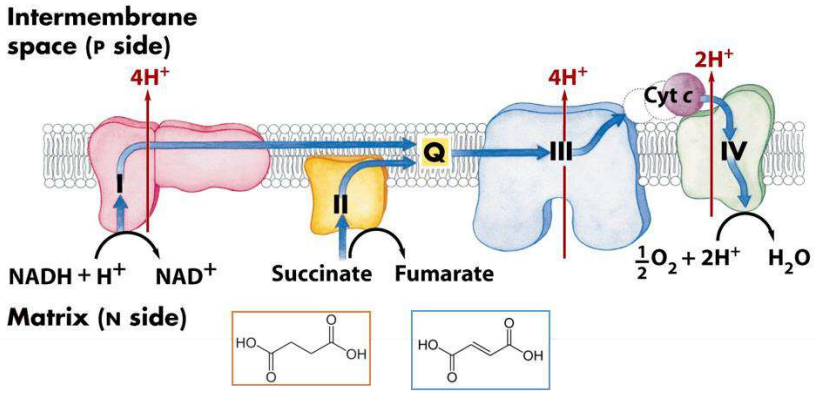
Electron transport in Oxidative phosphorylation

@ The mitochondrial respiratory chain consists of a series of sequentially acting electron carriers, most of which are integral proteins with prosthetic groups capable of accepting and donating either one or two electrons.

@ Three types of electron transfers occur in oxidative phosphorylation:

1. Direct transfer of electrons, as in the reduction of Fe^{3+} to Fe^{2+};
2. Transfer as a hydrogen atom ($H^+ + e^-$); and
3. Transfer as a hydride ion ($:H^-$), which bears two electrons.

@ Electron transport chain



Complex I: NADH to Ubiquinone

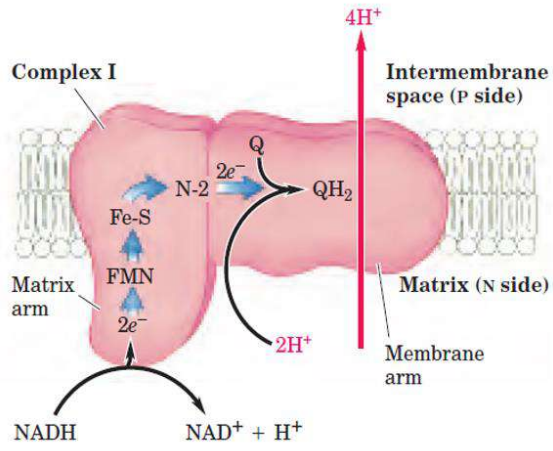
@ Complex I (NADH-ubiquinone oxido-reductase or NADH dehydrogenase), a large enzyme composed of 42 different polypeptide chains, including an FMN-containing flavoprotein and at least six Fe-S centers.

@ Complex I catalyzes two simultaneous and obligately coupled processes:

1. the exergonic transfer to ubiquinone of a hydride ion from NADH and a proton from the matrix, expressed by $NADH + H^+ + Q \rightarrow NAD^+ + QH_2$
2. the endergonic transfer of four H^+ from the matrix to the inter-membrane space

@ Complex I is therefore a proton pump driven by the energy of electron transfer matrix, which becomes negatively charged with the departure of H^+ to the intermembrane space, which becomes positively charged.

@ Overall reaction $NADH + 5H^+_N + Q \rightarrow NAD^+ + QH_2 + 4H^+_P$



$$NADH + H^+ + Q \longrightarrow NAD^+ + QH_2 \quad (19\text{–}1)$$

$$NADH + 5H^+_N + Q \longrightarrow NAD^+ + QH_2 + 4H^+_P \quad (19\text{–}2)$$

Complex II: Succinate to Ubiquinone

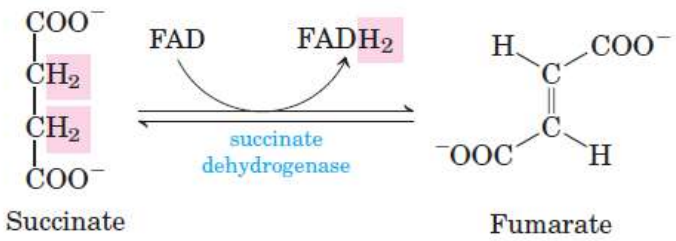
@ Succinate dehydrogenase, the only membrane-bound enzyme in citric acid cycle.

@ It has four different protein subunits, shown in figure given in next page.

@ Subunits A and B extend into the matrix (or the cytosol); they contain three 2Fe-2S centers, bound FAD, and a binding site for the substrate, succinate.

@ Subunits C and D are integral membrane proteins. They contain a heme b, and a binding site for ubiquinone (final electron acceptor) in the reaction catalyzed by Complex II.

@ The heme b of Complex II is apparently not in the direct path of electron transfer; it may serve instead to reduce the frequency with which electrons "leak" out of the system, moving from succinate to molecular oxygen to produce the reactive oxygen species (ROS) hydrogen peroxide (H_2O_2) and the superoxide radical ($O_2^{\bullet-}$).



$\Delta G'^{\circ} = 0$ kJ/mol

Structure of Complex II (succinate dehydrogenase of *E. coli)*

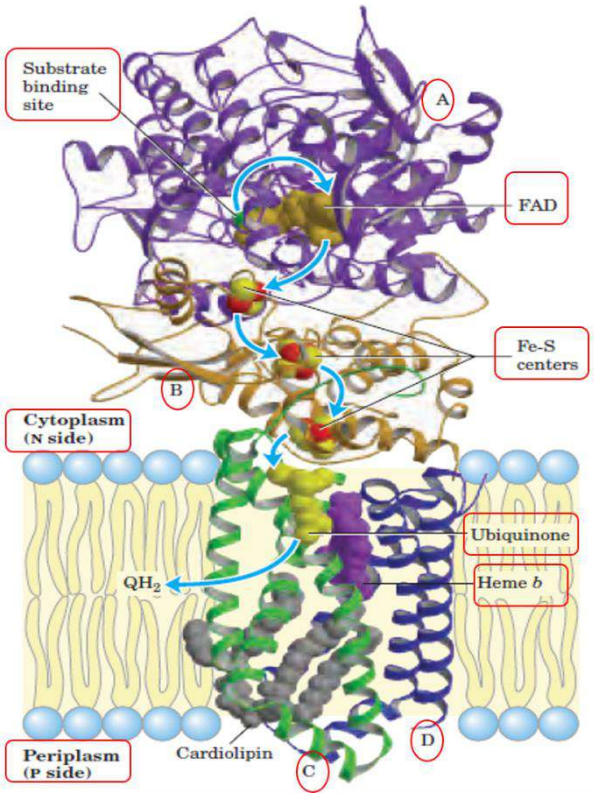
@ The enzyme has two transmembrane subunits, C (green) and D (blue); the cytoplasmic extensions contain subunits B (orange) and A (purple).

@ Subunit B has three sets of Fe-S centers (yellow and red);

@ Ubiquinone (yellow) is bound to subunit C; and heme *b (purple) is sandwiched between subunits C and D.*

@ Electrons move (blue arrows) from succinate to FAD, then through the three Fe-S centers to ubiquinone.

@ The heme *b is not on the main path of electron transfer* but protects against the formation of reactive oxygen species (ROS) by electrons that go astray.



Complex III: Ubiquinone to Cytochrome c

@ *The next respiratory* complex, Complex III, also called cytochrome *bc_1 complex or ubiquinone: cytochrome c oxido-reductase,*

@ Couples the transfer of electrons from ubiquinol (QH_2) to cytochrome *c with the vectorial* transport of protons from the matrix to the inter-membrane space.

@ The determination of the complete structure of this huge complex and of Complex IV by x-ray crystallography, achieved between 1995 and 1998, were landmarks in the study of mitochondrial electron transfer, providing the structural framework to integrate the many biochemical observations on the functions of the respiratory complexes.

@ The net equation for the redox reactions of this Q cycle is

$$QH_2 + 2\text{Cyc c1(oxidized)} + 2H^+_N \rightarrow Q + 2\text{Cyt c1 (reduced)} + 4H^+_P$$

Cytochrome *bc_1 complex (Complex III)*

@ It is a dimer of identical monomers.

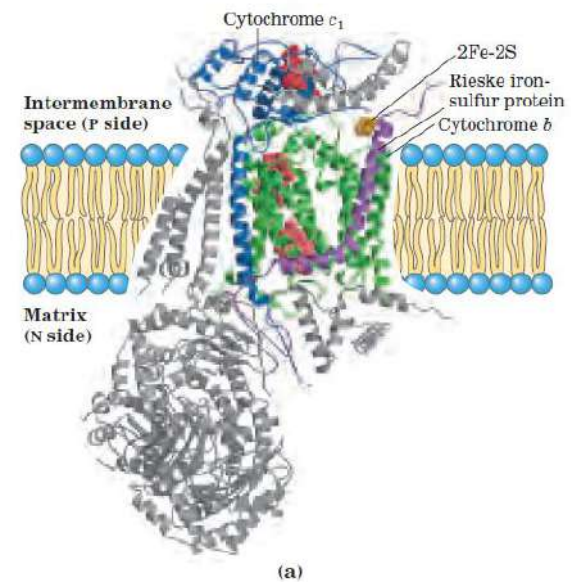
Structure of a monomer

@ Cytochrome *b (green) with its two hemes (b_H and b_L, light red);*

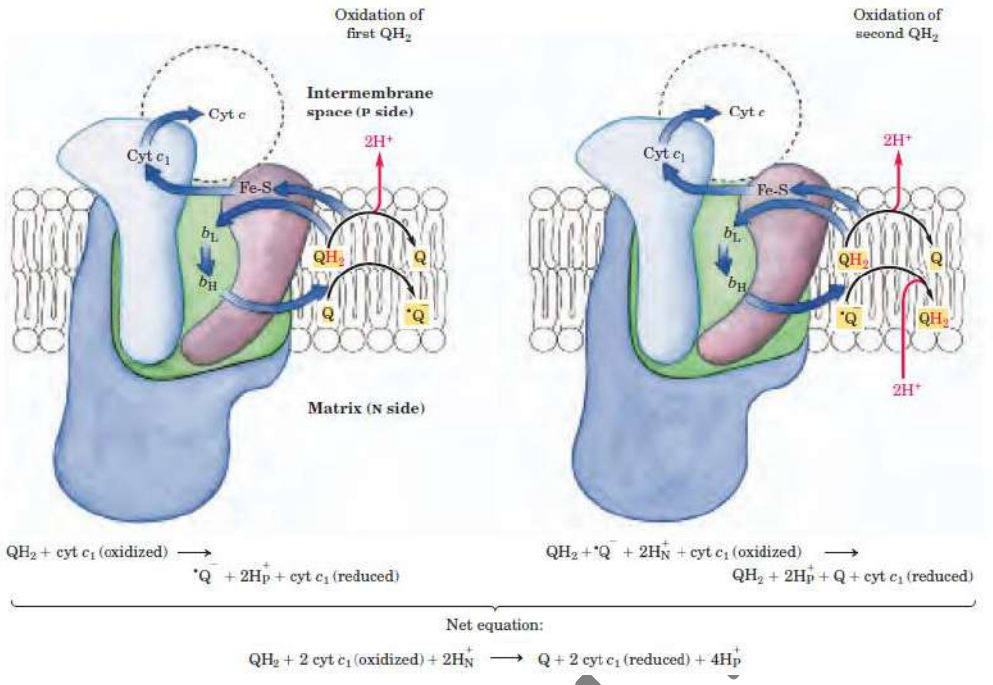
@ The Rieske iron-sulfur protein (purple) with its 2Fe-2S centers (yellow);

@ and cytochrome *c_1 (blue) with its heme (red).*

@ Cytochrome *c_1 and the Rieske iron-sulfur protein* project from the P surface and can interact with cytochrome *c* in the intermembrane space. The complex has two distinct binding sites for ubiquinone, Q_N and Q_P, which correspond to the sites of inhibition by two drugs that block oxidative phosphorylation.



Q cycle in Complex III



Q cycle: The path of electrons is shown by blue arrows. On the P side of the membrane, two molecules of QH_2 are oxidized to Q near the P side, releasing two protons per Q (four protons in all) into the intermembrane space. Each QH_2 donates one electron (via the Rieske Fe-S center) to cytochrome *c_1, and one electron (via cytochrome b) to a molecule of Q near the* N side, reducing it in two steps to QH_2. This reduction also uses two protons per Q, which are taken up from the matrix.

Cytochrome *c* Oxidase or Complex IV

@ In the final step of the respiratory chain, Complex IV or cytochrome c oxidase carries electrons from cytochrome c to O_2, reducing it to H_2O.

@ Complex IV is a large enzyme of the inner mitochondrial membrane.

@ Electron transfer through Complex IV is from cytochrome c to the Cu_A center, to heme a, to the heme a_3-Cu_B center, and finally to O_2.

@ For every four electrons passing through this complex, the enzyme consumes four "substrate" H^+ from the matrix (N side) in converting O_2 to $2H_2O$.

@ It also uses the energy of this redox reaction to pump one proton outward into the inter-membrane space (P side) for each electron that passes through,

@ This adds to the electrochemical potential produced by redox-driven proton transport through Complexes I and III.

@ The $4e^-$ reduction of O_2 involves redox centers that carry only one electron at a time, and it must occur without the release of incompletely reduced intermediates such as H_2O_2 or $OH^{\bullet}$ very reactive species that would damage cellular components.

@ The intermediates remain tightly bound to the complex until completely converted to water.

$$4\text{Cyt c(reduced)} + 8H^+_N + O_2 \rightarrow 4\text{Cyt c(oxidized)} + 4H^+_P + 2H_2O$$

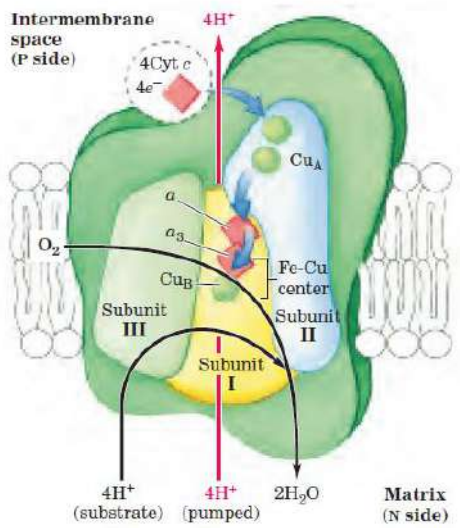
@ Most of the O_2 consumed by aerobic organisms is used to produce energy in a process referred to as oxidative phosphorylation

@ It is a series of reactions in which electron transport is coupled to the synthesis of ATP and in which the driving force for the reaction is provided by the four-electron oxidizing power of O_2.

@ The next to the last step in the electron transport chain produces reduced cytochrome c, a water-soluble electron-transfer protein.

@ It is the terminal member of the respiratory chain which then transfers electrons to cytochrome c oxidase, where they are ultimately transferred to O_2.

@ This enzyme is always found associated with a membrane (membrane bound), and the diversity of the four redox metal sites, i.e., two copper ions and two heme iron units, each of which is found in a different type of environment within the protein.



Electron flow path in Complex IV

Electron Transport Chain: The Proteins components of the Mitochondrial Electron Transfer Chain

Enzyme complex/protein	Mass (kDa)	Number of subunits	Prosthetic group (s)
I NADH dehydrogenase	850	43	FMN, Fe-S
II Succinate dehydrogenase	140	4	FAD, Fe-S
III Ubiquinone cytochrome c oxidoreductase	13	11	Heme, Fe-S
Cytochrome c		1	Heme
IV Cytochrome oxidase	160	13	Heme, Cu_A, Cu_B

Nitrogen Fixation

Lecture 11 & 12

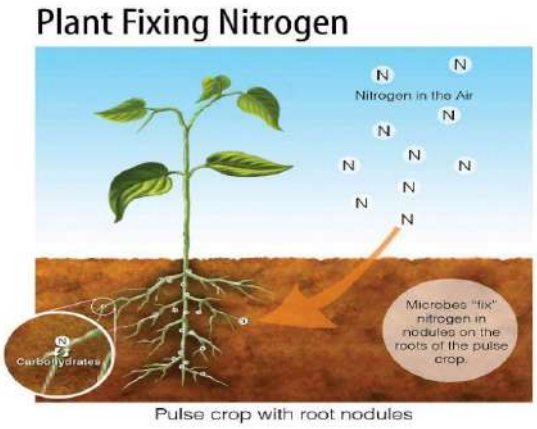
Nitrogen Fixation

@ A key reaction of biological system, converts atmospheric dinitrogen (N_2) to other N-containing species (nitrate/nitrite/other essential compounds)

@ Fixed nitrogen is essential for the synthesis of amino acids and nucleic acid.

@ Natural systems cannot provide adequate amount of fixed nitrogen for agriculture and animal husbandry.

@ Industrial processes have been developed to fix nitrogen chemically.



Pulse crop with root nodules

Haber-Bosch Process

@ Dinitrogen is almost chemically inert (bond energy 945 $kjmol^{-1}$) and activation energy is prohibitively high (kinetically unfavourable).

@ Breaking and reducing N≡N triple bond is a challenging task.

@ Requires about 300-500 $^{\circ}C$ and 300 atmospheric pressures with Haber's catalyst, usually metallic Fe or its oxide (thermodynamically favourable).

$$N_2 + 3H_2 \rightarrow 2NH_3 \qquad \Delta H = -50\ kjmol^{-1}, \qquad \Delta G = -16.6\ kjmol^{-1}$$

@ At pH=7, E° value is easily accessible to biological reductant such as low potential ferredoxins

$$N_2 + 8e^- + 6H^+ \rightarrow 2NH_4^+; \qquad E^{\circ} = -280\ mV$$

Biological nitrogen fixation

@ Nitrogen fixation with the help of microorganisms (nitrogen fixing bacteria).

@ Two types - symbiotic and non-symbiotic

@ Non-symbiotic - Fixation by free living organisms (aerobic, anaerobic and blue green algae)

Free living aerobic: *Azotobacter vinelandii*

Free living anaerobic: *Clostridium pasteurianum*

Facultative aerobes: *Klebsiella pneumoniae*

Free living photosynthetic: *Rhodobacter capsulatus* - purple,

Blue green algae: *Anabaena cylindrica* (cyanobacterium)

@ Symbiotic - Fixation by microorganisms in soil living symbiotically inside the plants.

Nodule formation in leguminous plants: *Rhizobium* and *Bradyrhizobium*

Nodule formation in non-leguminous plants: *Frankia*

Non-nodulation: *Anabaena azollae*

Symbiotic Nitrogen Fixation

@ Rhizobium (gram negative, aerobic) is present in nodules on the roots of legumes (peas, beans, clover, alfalfa and soya)

@ Red colour inside the nodules is due to leghaemoglobin (a plant O_2-binding protein)

@ The reaction takes place at 0.8 atmospheric pressure and ambient temperature.

@ Intensive efforts have been made to determine the bacterial mechanism.

@ The nitrogen fixing bacteria contains an enzyme called nitrogenase.

@ Nitrogenase catalyses the reduction of N_2 to NH_3 in a reaction coupled to the hydrolysis of 16 ATP molecules and production of H_2.

$$N_2 + 16MgATP^{2-} + 8e^- + 8H^+ \rightarrow 2NH_3 + 16MgADP^- + 16H_2PO_4^- + H_2$$

@ Fe-Mo nitrogenase is mostly studied - reduction of N_2 probably occurs at Mo-site

@ Fe-V nitrogenase - V plays (other metal can also) the role of Mo

@ Fe-Fe nitrogenase - similar metals can also affect fixation

Biochemistry of nitrogen fixation

Basic requirements

@ Nitrogenase and hydrogenase enzyme

@ Protective mechanism against Oxygen (leghaemoglobin binds oxygen tightly and protects nitrogenase that cannot operate in presence of oxygen.)

@ Ferredoxin

@ Hydrogen releasing systems or electron donor (pyruvic acid or glucose/sucrose

@ Constant supply of ATP

@ Coenzymes and cofactors TPP, CoA, inorganic phosphate and Mg^{2+}

Nitrogenase enzyme

@ Active in anaerobic condition

@ Consists of two protein subunits

Non-heme Fe-protein: P clusters (dinitrogen reductase, smaller)

Fe-Mo protein: Di-nitrogenase (larger)

@ Fe-protein reacts with ATP and reduces second subunit which ultimately reduces N_2 to NH_3,

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

@ Larger protein is an $\alpha_2\beta_2$ tetramer with molecular weight 220,000-240,000 D. Contains 2-Mo atoms, almost 30-Fe atoms and about 30-labile (inorganic) sulphur atoms.

@ P-cluster has the molecular weight of 57,000-73,000 D with an Fe_4S_4 cluster (dimer of two identical subunits bridged by an Fe_4S_4 cluster). It acts as a redox centre.

Fe-Mo nitrogenase

@ The protein free cofactor is soluble and contains Mo and Fe (1 Mo, 7-8 Fe and 4-6 S^{2-} ions)

@ Recombination of the cofactor with inactive nitrogenase restores the activity.

@ Reaction: $N_2 + 16MgATP^{2-} + 8e^- + 8H^+ \rightarrow 2NH_3 + 16MgADP^- + 16\ PO_4^{3-} + H_2$

@ Electrons required for the reaction are transferred to nitrogenase by reduced form of ferredoxins and flavodoxins.

@ The source of these electrons is the oxidation of pyruvate (2-oxo-propionate).

@ The e^-s first transferred to the Fe-protein, the reduced form of which forms a complex with Mg-ATP and Fe-Mo-protein.

@ The reducing e^- is transferred to Fe-Mo-protein and then to N_2 in a series of steps accompanied by H^+ transfer from H_2O to N_2 produces NH_3, Mg-ADP and *i*-PO_4^{3-}.

@ Regeneration of proteins.

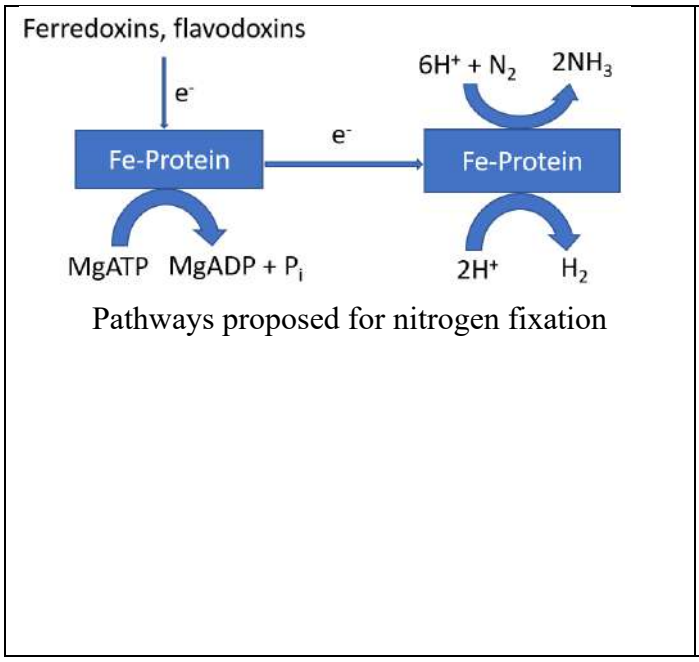
@ The energy released from hydrolysis of ATP drives the reaction.

$$ATP \rightarrow ADP + PO_4^{3-} \quad \Delta G = -30.5\ kjmol^{-1}$$

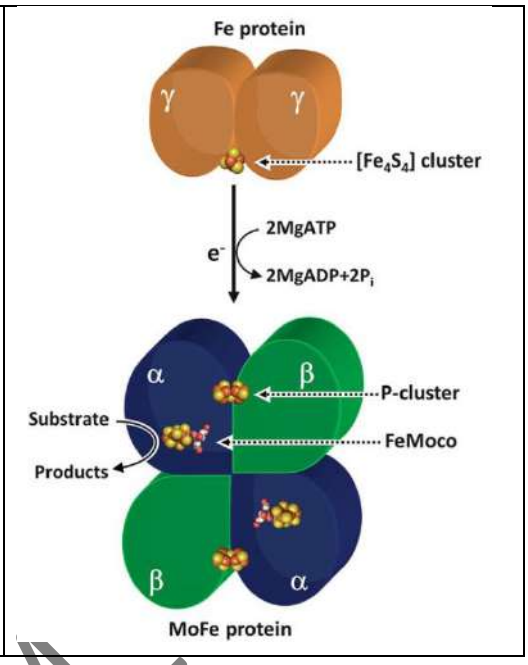
@ Fe-Mo protein contains the site of N_2 binding (Fe-Mo cofactor) where the N_2 is reduced.

@ P cluster is believed to assist the reduction of N_2 by transfer of electrons.

$$N_2 + 16MgATP^{2-} + 8e^- + 8H^+ \rightarrow 2NH_3 + 16MgADP^- + 16\ PO_4^{3-} + H_2$$



Pathways proposed for nitrogen fixation



Fe-Mo-protein

@ Two Fe-Mo cofactors are located at about 70 Å apart.

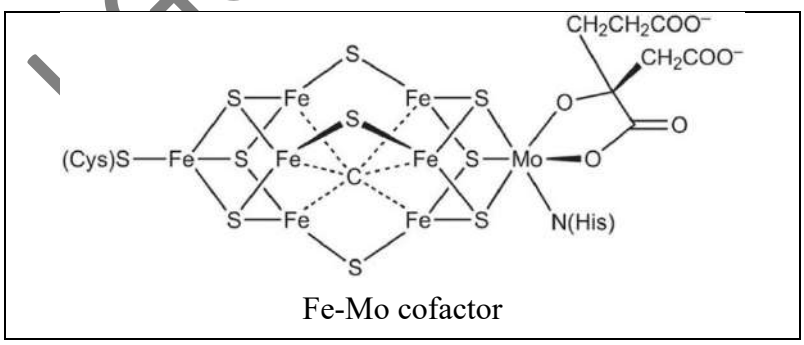
@ It is an $\alpha_2\beta_2$ tetramer with molecular weight of 220000-240000 D (2Mo, 30Fe & 30 labile S^{2-})

@ Consists of cuboidal $[Fe_4S_4]$ and $[Fe_3MoS_3]$ fragments linked by two sulphide bridges

@ A third bridge might be derived from O or N donor.

@ The cluster is bound to the protein via a cysteine at Fe and a histidine at the Mo.

@ The coordination sphere of the six coordinated Mo (probably in IV OS) is completed by the homocitrate anion.



Fe-Mo cofactor

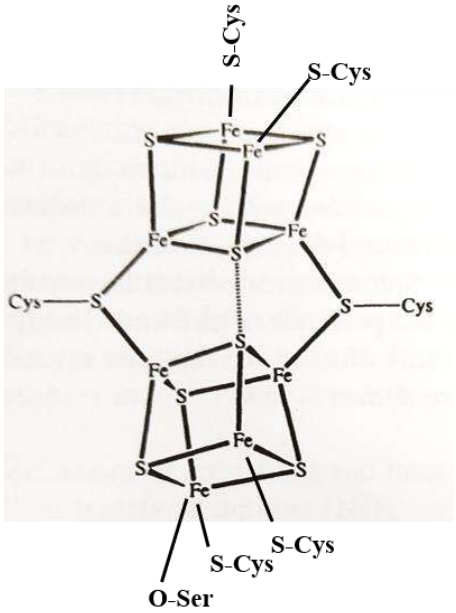
P-cluster

@ It is smaller protein with molecular weight 57000-73000 D

@ Contains a pair of $[Fe_4S_4]$ units linked by two bridging cysteine thiolate group

@ A disulphide bridge formed between S atoms of each $[Fe_4S_4]$ cubane cluster.

@ Unusual, EPR studies suggests its oxidized form to be high spin with S=7/2 and Mossbauer spectra reveals inequivalent Fe-population indicating $[Fe_4S_4]$ cluster to be distorted or asymmetric.



Mechanism of nitrogen fixation

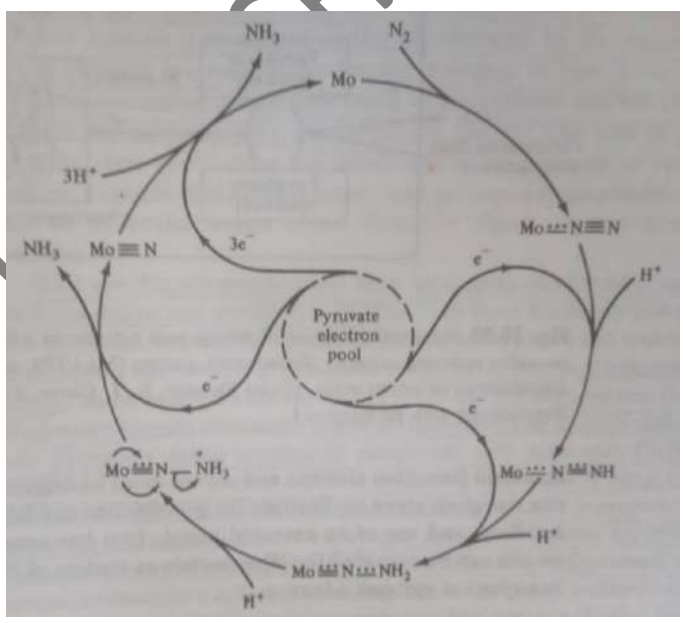
@ The active site for dinitrogen binding involves Mo atom.

@ The coordination sphere consists of several S-atoms at distances of about 2.35 Å

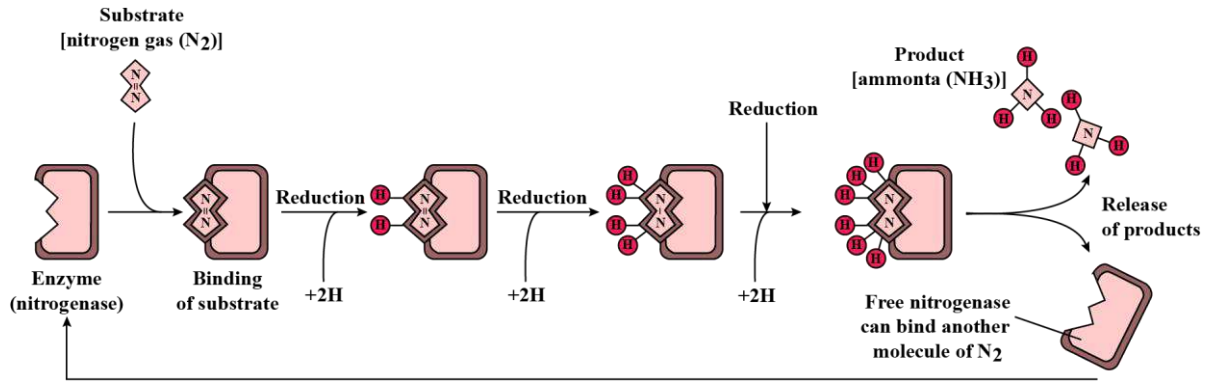
@ The source of reductive capacity is pyruvate, and e⁻s are transferred via ferredoxin to nitrogenase

@ Two Mo(III) atoms cycling through Mo(VI) would provide six electrons required for the reduction.

@ Alternatively, there should be ready flow of electrons, and the Mo may stay in the one or two oxidation states that most readily bind dinitrogen and its intermediate reductants.



Schematic diagram of nitrogenase activity in bacterial cell



Steps of conversion of atmospheric nitrogen to ammonia by nitrogenase enzyme complex found found in nitrogen - fixing bacteria

Vitamin B_{12}

Lecture 13

Vitamins

@ Organic nutrients required (small quantity) for several biochemical functions.

@ Cannot be synthesized in body.

@ Must be acquired from diet.

@ Two classes

Water soluble: B-complex, Vit C (ascorbic acid)

Fat soluble: Vit A (retinol), Vit D (calciferol), Vit E (tocopherol)

@ Vitamin C and Vitamin E function as antioxidant.

@ Both excess and deficiency intake of Vitamin can be potentially harmful.

Vitamin B_{12}: Cyanocobalamin

@ First naturally occurring organometallic compound (the only vitamin with metal)

@ Produced by micro-organisms (bacteria) and fungi, and not by higher plants

@ Dietary intake for adult is 2-3µg/day.

@ Pregnancy and lactation 6 µg/day

@ Body stores vitamin B_{12} of about 3-4 mg, primarily in liver (sufficient enough for 3 years if its dietary intake is ceased).

@ Vitamin B_{12} is relatively stable and little is lost during cooking.

@ Cyanocobalamin: anti-pernicious anemia factor

Sources of Vitamin B_{12}

@ Foods of animal origin (red meat, liver, fish, eggs, seafood, dairy products)

Structure of Vitamin B_{12}

@ D. C. Hodgkin, Noble Prize 1964

@ Six coordinated, Planar Co(III) corrin ring (tetrapyrrole),

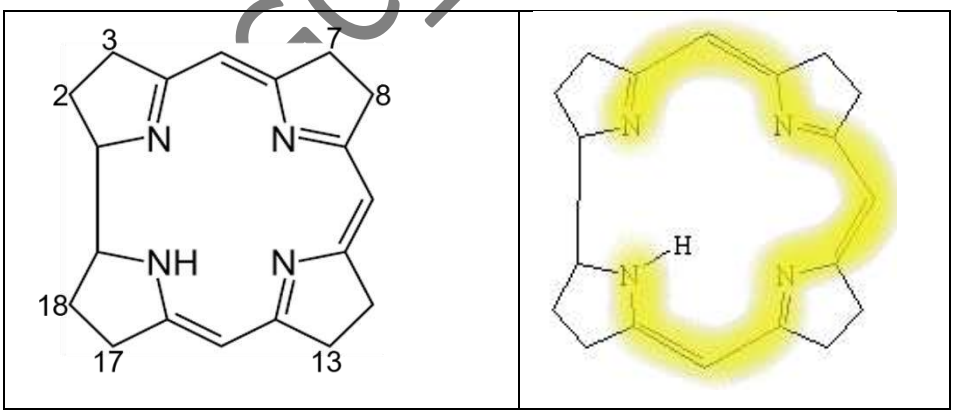
@ Corrin is similar to porphyrin with one methylidyne linkage (-CH=) between two of the pyrrole type ring is missing, contracting the ring.

@ π-delocalization disrupt, making corrin ring flexible and adopt different conformations required for biochemical function

@ At 2, 7 & 18 positions - three acetamide, $-CH_2CONH_2$

@ At 3, 8 & 13 positions - three propionamide, $-(CH_2)_2CONH_2$

@ At 1, 2, 5, 7, 15 & 17 positions – one Me and at 12 – two Me

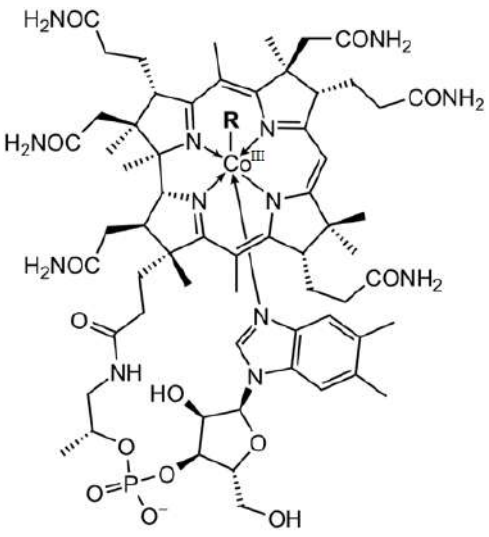


@ At 17 position, one N-substituted propionamide (isopropyl connected to unusual ribonucleotide with 5,6-dimethylbenzimidazole group)

@ Fifth coordination site is occupied by N-atom from imidazole ring of 5,6-dimethyl benzimidazole group (phosphate, ribose sugar and organic base)

@ Sixth coordination site is occupied by CN^- ion (in biological system, H_2O molecule is loosely bound to Co-center)

@ Inclusion of CN is an artifact & does not exist in nature



R = 5'-deoxyadenosyl, CH_3, OH, CN

Structure of Vitamin B_{12} coenzyme

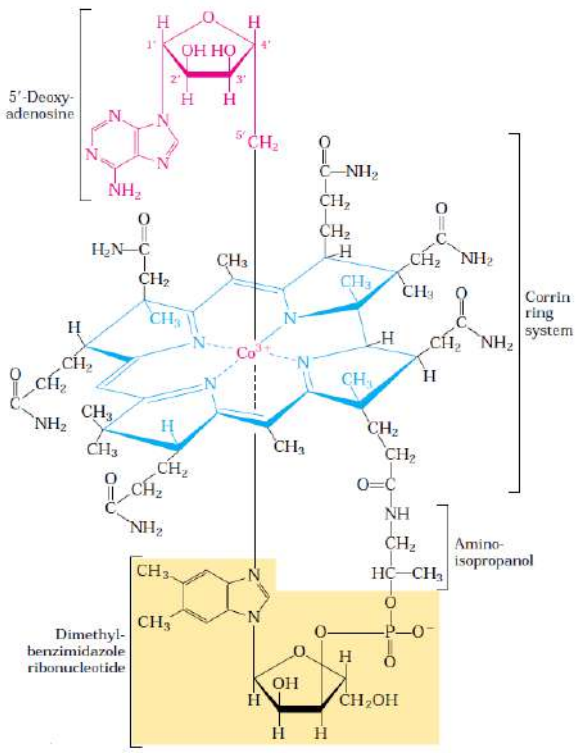
@ D. C. Hodgkin, Noble prize 1964

@ Structure is similar to Vitamin B_{12}

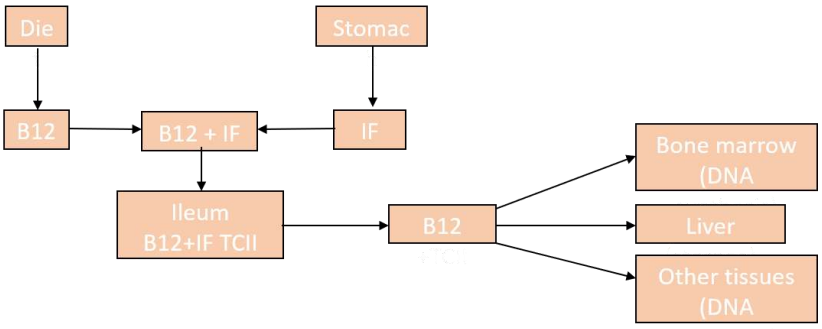
@ Sixth coordination site is occupied by 5'-deoxyadenosyl ligands.

@ Acts as prosthetic group in different enzyme

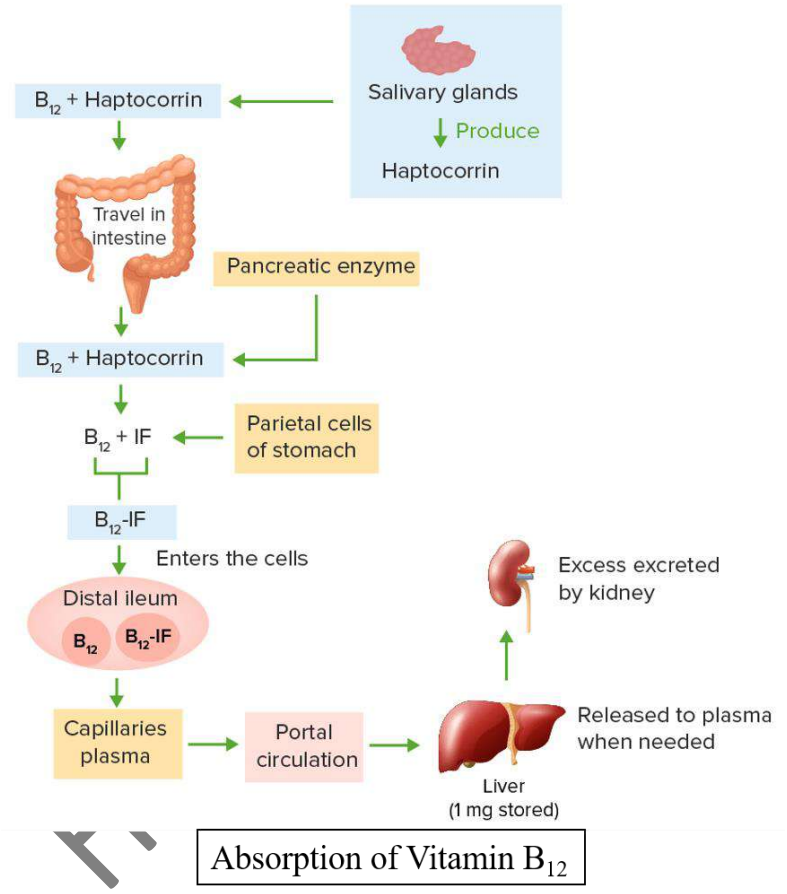
@ It is a diamagnetic orange red crystal



Transport and metabolism of Vitamin B_{12}



Transportation path of Vit B_{12}



Absorption of Vitamin B_{12}

Biochemical function of Vitamin B_{12}

@ Vit B_{12} plays a significant role in one carbon transfer reactions, important in

1) DNA and Fatty acid synthesis and energy production

2) Biosynthesis of amino acids such as serine, methionine, glycine etc.

@ Enzymes that require Vit B_{12} as cofactor (essential in addition to enzyme)

1) Methylmalonyl-coA mutase: Isomerization of methyl malonyl CoA from odd number of carbon containing fatty acids which is an essential step for catabolism of fatty acids.

2) Methionine synthase: Synthesis of Methionine from Homocysteine

@ Vit B_{12} may be reduced by one electron (Vit B_{12r}) or two electrons (Vit B_{12s}) to form Co(II) and Co(I) respectively. In biological condition, the two-electron reduction is accomplished by NADH (nicotinamide adenine dinucleotide + H) and flavin adenine dinucleotide (FAD).

@ Vit B_{12s} is strongly nucleophilic and readily undergoes alkylation via oxidative addition

$$[B_{12}(Co^{I})] + CH_3I \rightarrow [B_{12}(Co^{III})\text{-}CH_3]^{+}I^{-}$$

Synthesis of Methionine from Homocysteine

@ This step is important in intracellular synthesis of folate coenzyme.

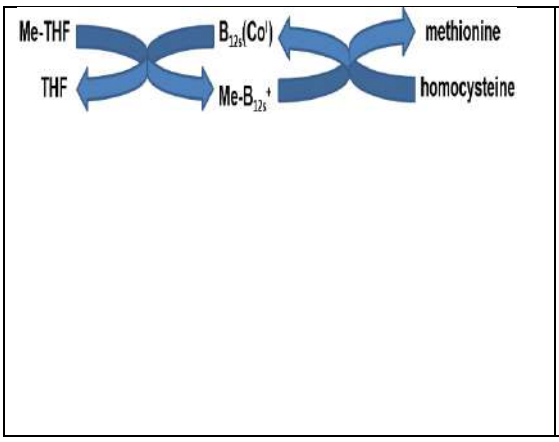
@ Both Vit B_{12} and folic acid are involved

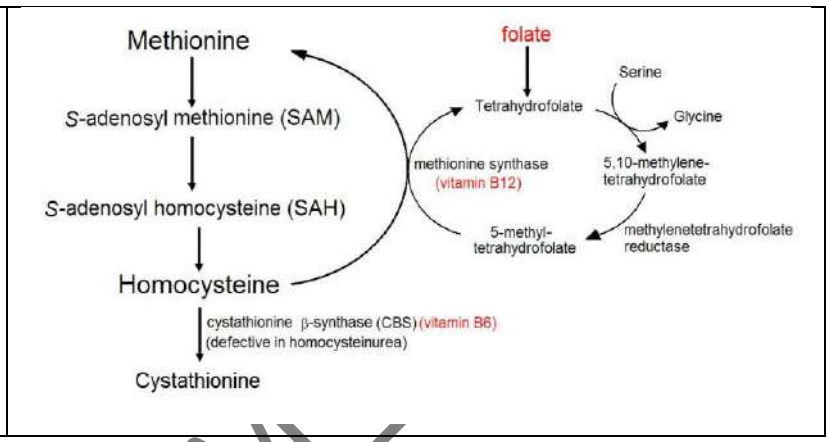
@ Vit B_{12} acts as a co-enzyme (methylcobalamin, MeCo) for methyltransferase

@ Vit B_{12} accepts Me group from CH_3THF to give methylcobalamin

@ MeCo participates in biomethylation in biosynthesis of methionine (terminal step)

@ Me transfer from methylcobalamin involves cleavage of Co-C bond.





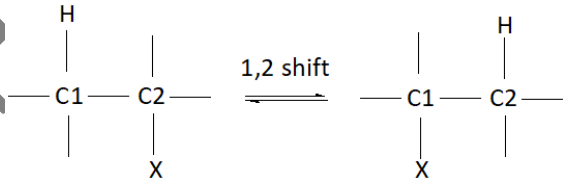
1,2-shift reaction

@ An enzymic reaction of B_{12} having 5/-deoxycobalamin as the prosthetic group.

@ Reaction of B_{12s} with ATP results in the formation of Co-C bond between adenosyl and Co, forming B_{12} coenzyme.

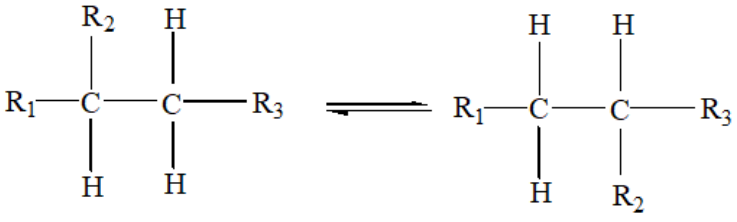
@ It is very effective in inducing 1,2-shift, very important in metabolism (C-C, C-N and C-O bond cleavage).

@ The shift is followed by internal condensation to give the final product.



X = group with C/N

Representative of 1,2-shift reactions



	R_1	R_2	R_3
Diol dehydratase	CH_3	OH	OH
Ethanolamine deaminase	H	NH_2	

Glutamate mutase	H	$CH(NH_2)COOH$	COOH
Methylmalonyl CoA mutase	H	CO-CoA	COOH
Glycerol dehydratase	CH_2OH	OH	OH

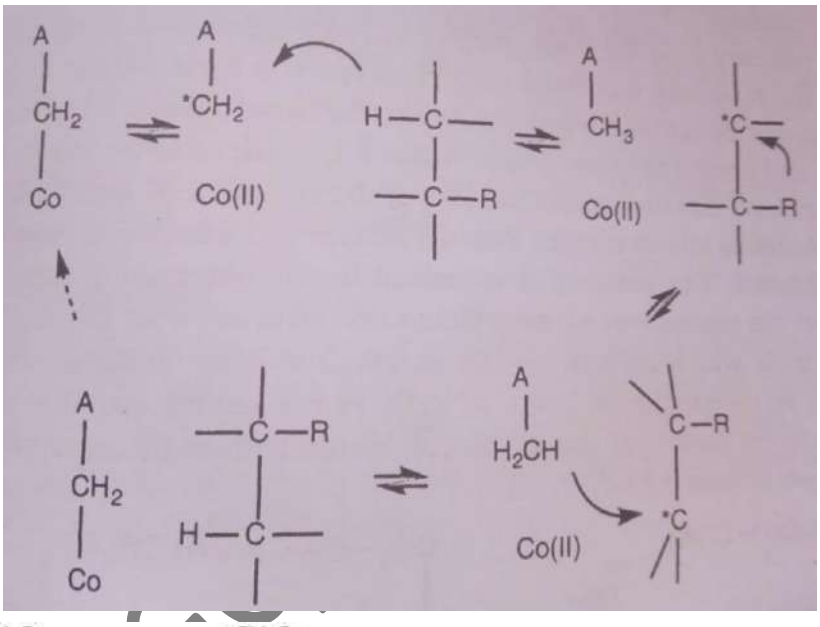
Mechanism of 1,2-shift/isomerase reaction

@ Proposed to occur via a radical pathway.

@ Homolytic cleavage of the Co-deoxyadenosine bond yields B12r and deoxyadenosyl radical that abstracts H-atom from the substrate to give 5/-deoxyadenosine and substrate radical

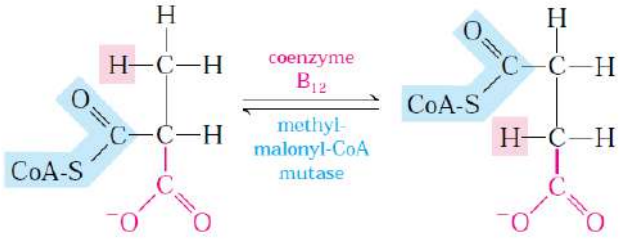
@ Rearrangement of the substrate radical takes place with enzymic intervention and then abstraction of H-atom from 5/-deoxyadenosine to shift it to its new position in the product.

@ The deoxyadenosyl radical formed reacts with B12r to generate the coenzyme.



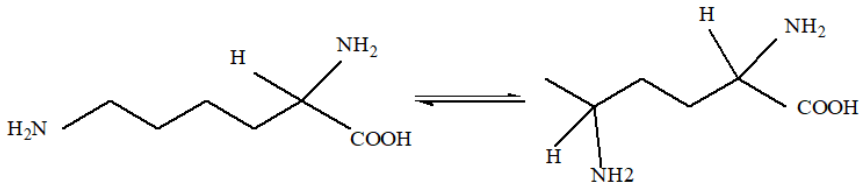
Some Examples

@ Vit B_{12} is important in conversion/isomerization of methylmalonyl CoA to succinyl CoA in Krebs cycle. In this reaction B_{12} acts as co-enzyme for methylmalonyl Co A mutase. This is important substrate in Heme synthesis

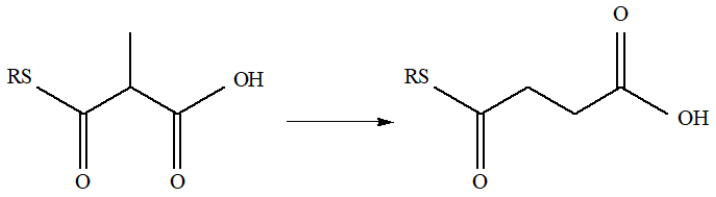


L-Methylmalonyl-CoA Succinyl-CoA

@ Isomerization of an amino group from a primary to secondary carbon



@ Insertion of CH_2 group



@ Free radical reaction, homolytic cleavage of Co-C bond giving Co(II) atom and 5'-deoxyadenosyl radical (B12 r)

@ 5'-deoxyadenosyl radical abstracts a H-atom from Me group

@ Migration of –C(O)SR group followed by return of H-atom from 5'-deoxyadenosyl to the substrate

@ Regeneration of 5'-deoxyadenosyl radical and formation of Co-C bond giving back the coenzyme.

Factors in favour of Vit B_{12} for its usefulness in biochemical functions

1. Existence of three oxidation states Co^{I}, Co^{II} and Co^{III} which are stable in aqueous phase
2. d^6/d^8 (16e-/18e-) systems are ideal for oxidative addition and reductive elimination.
3. Flexibility of Corrin ring allows changes in conformation which is crucial in reducing Co(III) to Co(I)

Vitamin B_{12} Deficiency

@ Liver can store up to six years' worth of vitamin B_{12}, hence deficiencies are rare.

@ Upto 50% of vegetarians are Vit B_{12} deficient

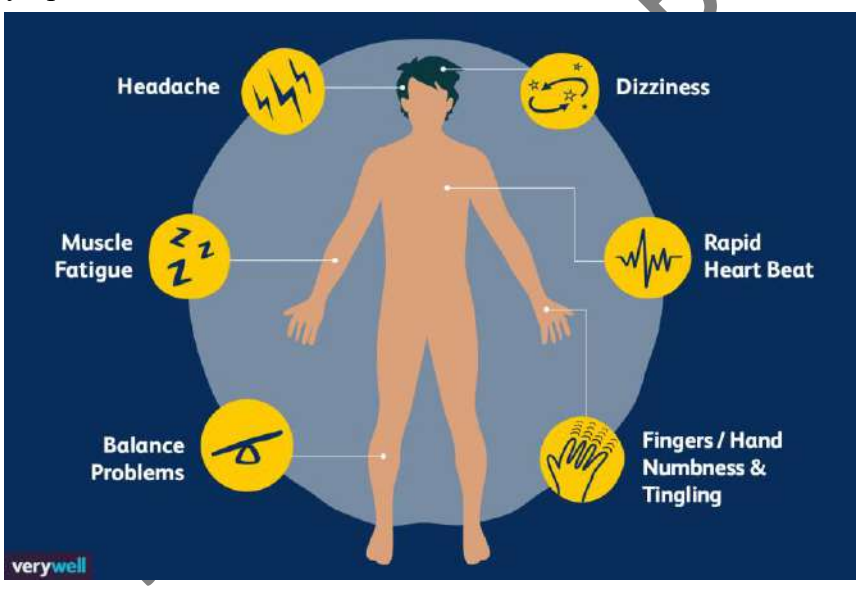
@ About 40% have low normal levels of Vit B_{12}

@ Vit B_{12} deficiency develops as a result of a lack of intrinsic factor in the stomach leading to malabsorption of the vitamin.

@ **Pernicious anaemia**: Pernicious anemia is failure of the absorption of Vit B_{12} (through gut wall) rather than dietary deficiency. A disease characterized By Gastric parietal atrophy leading to decreased secretion of intrinsic factor and other gastric juices. This results an increase in excretion of methylmalonic acid as the body fails to convert it to succinic acid.

@ Neurological complications (Neuro psychiatrique symptoms).

@ Gastrointestinal symptoms



Treatment of Vitamin B_{12} Deficiency

@ Simple blood test

@ Folic acid and Vit B_{12} both must be given to halt CNS Symptoms

@ To administer Vit B_{12} injection and take Vit B_{12} supplements

Inorganic Therapeutics: Metal based drugs

Lecture 14 & 15

History of Inorganic Medicinal Chemistry

@ Medicinal inorganic chemistry is not a new discipline

@ Cu was used to sterilize water in Egypt as far back as 3000 BC

@ Chinese and Arabian were using Au in several medicines over 3,500 years ago.

@ Hg_2Cl_2 was used as diuretic during the Renaissance period in Europe

@ Paul Ehrlich (founder of chemotherapy) developed the arsenical-Salvarsan, as a drug in treatment of Syphilis in early twentieth century.

@ Medicinal inorganic chemistry was kickstarted by the discovery of bacteriostatic and anticancer properties of Ru-polypyridyl complexes (F. Dwyer in 1950's) and strengthened by discovery of anticancer properties of Pt-ammine complexes (B. Rosenberg in 1969).

Chemistry of Inorganic elements in medicine

@ Coordination compounds have been extensively used in the treatment, management and diagnosis of disease.

@ Therapeutic chelating agents have been used to remove excess metals from body (metal detoxification).

@ The ideal ligand (antidote) should be specific for toxic metal and non-toxic itself and its complexes.

@ The ligand should be selective and form highly stable complexes with toxic metal.

@ The complexes should be water soluble, readily excretable and should not be metabolized.

@ HSAB principle is important for selectivity in metal detoxification.

@ The most important aspect is the identification of donor group.

@ Medicinal Inorganic Chemistry: Mineral supplements, Therapeutic agents, Diagnostic agents & Chelation therapy

Chelate Therapy

@ Therapeutic chelating agents have been in use to remove excess metals ions from the body such as Wilson disease (Cu-excess), Fe-overload, toxic metals (Pb(II), Cd(II), Hg(II))

@ Development of chelate therapy is based on

1. Determination of the site of action of the poison
2. Determination of donor group arrangement responsible for binding the metal (HSAB)
3. Synthesis of chelating agents having the same donor group arrangements
4. Chelate compounds can reverse the enzyme inhibition caused by the metal
5. Critical evaluation of the compound to assess its ability to perform efficiently in vivo.

@ The ideal ligand should be specific for the toxic metal &, non-toxic itself & as its complex.

@ Chelating agent should form highly stable complex with the metal ion to be removed that are water soluble, & so readily excretable & it should not be metabolized

Chelating drugs with –SH groups

2,3-dimercapto-1-propanol (BAL)

@ First used in the treatment of poisonous gas Lewisite ($ClCH{=}CHAsCl_2$) during World War II

@ BAL can be used against As poisoning by complexation, excreted through urine.

@ In acute Cu-poisoning and in Wilson's disease BAL used as antidote.

@ Although Hg is soft, BAL cannot be used because their resulting complex is highly toxic.

@ In case of As, the complex is unstable in aqueous solution with respect to aerial oxidation and local anaesthetic is required for intramuscular administration of BAL.

@ Sometimes hypertension, vomiting and sweating are also observed on BAL administration

@ The use of BAL is therefore questionable

2,3-dimercapto-1-propan-sulfonic acid or Unithiol (DMPS)

@ DMPS is water soluble and has several clinical advantages over BAL.

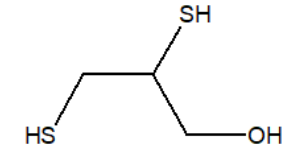
@ Due to its strong complexing power, high LD50 value (less toxicity) and water solubility, unithiol is used in the detoxification of metals like As, Hg, Tl etc.

Disodium meso-2,3-dimercaptosuccinate (DMSA)

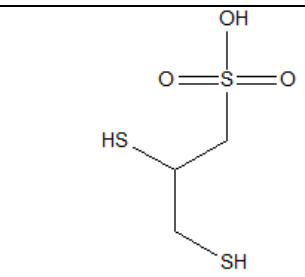
@ DMPS is also water soluble and can be given in drinking water.

@ It is of low toxicity with LD50 value 30 times more than BAL.

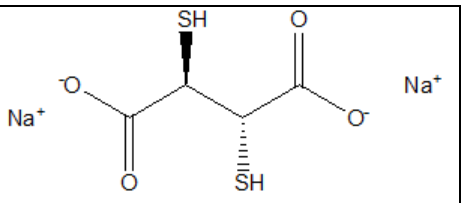
@ It can also detoxify As, Hg and many other soft metals under suitable conditions



2,3-dimercapto-1-propanol



2,3-dimercapto-1-propansulphonic acid



disodium meso-2,3-dimercaptosuccinate

D-penicillamine (DPA - 3,3′-dimethyl cysteine) and N-acetyl- D-penicillamine (NAPA)

@ D-penicillamine is therapeutically active but corresponding L isomer is toxic

@ LD_{50} value is much higher than BAL and can be orally administered.

@ DPA uses its S, N, O binding site to bind Hg(II), CH_3Hg(II), Cu(II), Au (I), Pb(II) etc.

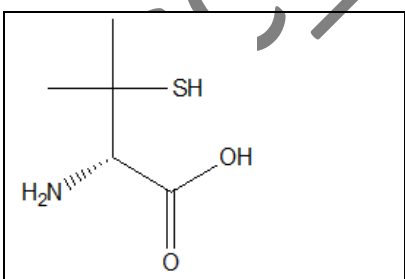
@ Possibility of depletion of essential elements like Zn and Cu are also there with DPA.

@ In Wilson's disease, use of DPA is clinically recommended (intense purple coloured multinuclear complex linked through S and N atoms).

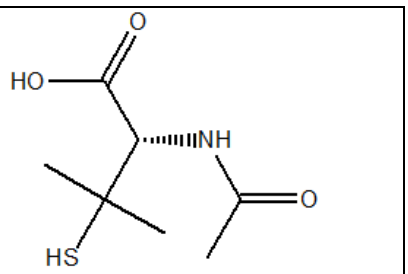
@ DPA has also been used in the treatment of rheumatism and arthritis.

@ In NAPA, presence of acetyl group makes it more lipophilic and less toxic than DPA.

@ Due to its lipophilicity, NAPA is used more effectively to detoxify CH_3Hg^+ from erythrocyte cells.



D-penicillamine



N-acetyl-D-penicillamine

Polyaminocarboxylic acid as chelating agent

@ Polyaminocarboxylic acids (EDTA, CDTA, DTPA, puchel) are administered intravenously or intramuscularly as solution of their Ca-salts.

@ Use of H_4EDTA is prohibited as they can form complexes with both toxic and essential metal ions (deplete serum calcium).

@ Na_4EDTA salt is toxic and therefore mixed complexes Na_2CaEDTA is used to prevent rapid Ca depletion.

@ Similarly, to avoid depletion of Zn(II), Zn_2EDTA, Na_3ZnDTPA are used.

@ In Pb(II) poisoning, Na_2CaEDTA is administered intravenously, Pb replaces Ca in the chelating agent and excreted via urine.

@ EDTA complexes can also be used for removing Hg(II), Fe and other metals.

@ $Na_2CaEDTA$ can also detoxify Co and Cd poisoning.

@ DTPA and its derivative Puchel can be used for Pu detoxification.

Desferrioxamine (DFO) as chelating agent

@ Naturally occurring siderophores (desferrioxamine) with hydroxamic acid groups.

@ Highly specific to reduce Fe toxicity

@ Desferrioxamine B (desferal) injection is clinically well established to remove Fe load from the patients suffering from genetic disorders (Cooley's anaemia, thalassemia, hemosiderosis, hemochromatosis etc.)

@ Desferrioxamine is poorly absorbed and may lead to allergic and skin reactions, neurological and renal effects.

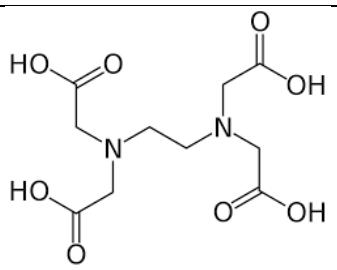
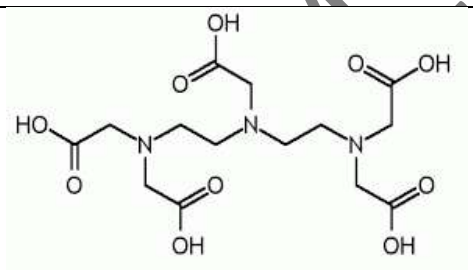
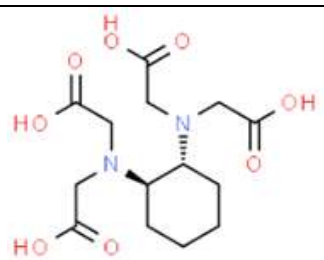
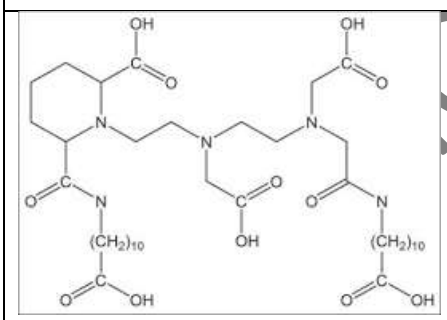
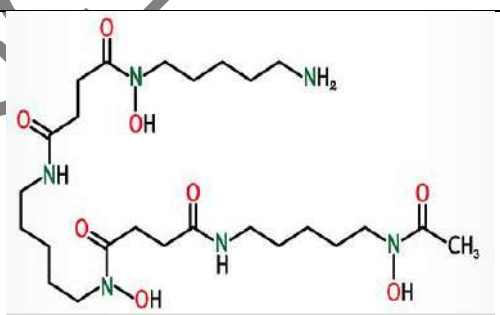
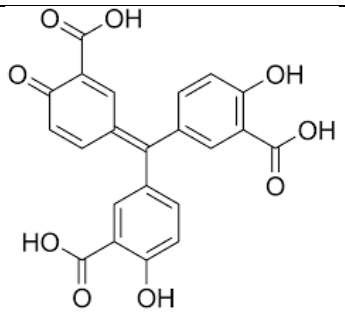
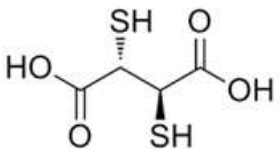
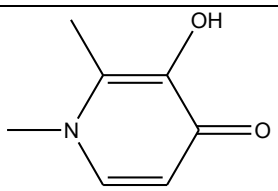
@ 1,2-dimethyl-3-hydroxypyridine-4-one (L1, promising, rapidly absorbed and commercially cheaper) is under clinical trial.

@ Can be useful for treating Fe and Al overload. However, is associated with some side effects like gastric, joint pain and even Zn depletion

Aurintricarboxylic acid as chelating agent

@ Be poisoning is common amongst workers in the light alloy industries.

@ Aurintricarboxylic (aluminon) is recommended to treat excess Be.

 EDTA	 DTPA	 CDTA
 Puchel	 Desferrioxamine B	 Aluminon
 Succimer (DMSA)	 1,2-dimethyl-3-hydroxypyridine-4-one	

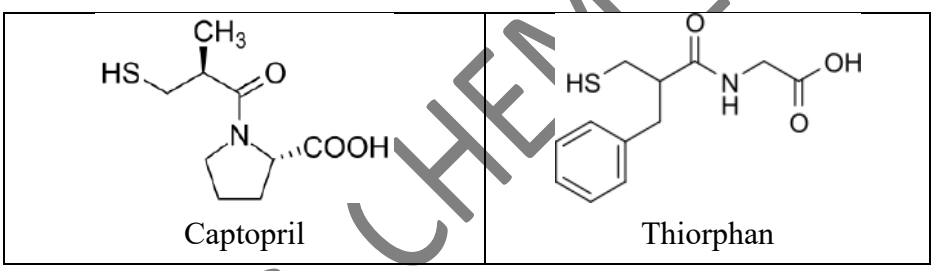
Chelating antidotes for metal detoxification

Metal	Chelating agent	Metal	Chelating agent
Al	DFO, L1	Cu	DPA, DMSA, DMPS, trien
Sb	DMSA, DMPS	Au	DMSA, DMPS
As	DMSA, DMPS, BAL, DPA	Fe	DFO, L1
Be	Aluminon	Pb	$Na_2CaEDTA$, DPA, BAL, DMSA
Bi	DMSA, DMPS	Mn	$Na_2CaEDTA$, $Na_2Ca(DTPA)$
Cd	$Na_3Ca(DTPA)$	Hg	DPA, NAPA, DMSA, DMPS
Ni	$Na_2CaEDTA$, DTCA	Pt	DTCA, DMSA

@ Ligands may also be used to target metalloenzyme and inhibit their undesirable activity which appear at the root of many physiological disorders.

@ Zn-enzyme (Angiotensin converting enzyme) may catalyse the cleavage of decapeptide to octapeptide, raises blood pressure (hypertension). This may be controlled by EDTA or captopril, controls hypertension by binding with Zn.

@ Thiorphan binds to Zn and deactivate the enzyme enkephalinases, and thereby reduces and controls the pain thereby acting as analgesic.



Captopril Thiorphan

Limitations of chelate therapy

@ Chelating ligands more often or not produce undesirable symptoms like diarrhoea, skin rashes, vomiting tendency, irritations etc.

@ It increases the concentration of toxic metal ions in kidneys, impairs kidney function

@ Prolong chelation therapy may induce loss of essential trace metal ions

@ In pregnancy, chelating agents may harm the embryo (congenital malformation)

@ Sometimes chelating drug may induce undesirable translocation of metal complexes to enhance toxicity.

Metals in medical treatment

@ Metals have been used in treatments since ancient times.

@ The Ebers Papyrus from 1500 BC is the first written account of the use of metals for treatment and describes the use of Cu to reduce inflammation and the use of Fe to treat anemia.

@ The application of metals to medicine is a rapidly developing field and novel therapeutic and diagnostic metal complexes are now having an impact on medical practice.

@ Examples of metal-based drugs

Metal	Product name	Active compound	Medical usage
Li	Camcolit	Li_2CO_3	manic depression

Mg	Magnesia	MgO	laxative
Fe	Fenelmin	$Na_4Fe(II)(citrate)$	anemia
Co	Cobaltamin S	Vitamin B_{12}	supplement
Zn	Calamine	ZnO	skin ointment
Ba	Baridol	$BaSO_4$	X-ray contrast medium
Pt	Cisplatin	cis-$[Pt(NH_3)_2Cl_2]$	anticancer
Au	Auranofin	$Au(I)(PEt_3)$ (acetylthioglucose)	rheumatoid arthritis
Bi	De-Nol	$K_3[Bi(III)(citrate)_2]$	antiulcer

Metals/compounds with medicinal applications

Metal	**Applications**	**Metal**	**Applications**
Lithium	Treatment of bipolar disorders	Gallium	^{67}Ga - SPECT radio-imaging, $^{66/68}$Ga - PET radio-imaging
Magnesium	Laxative, antacid, dietary supplement	Arsenic	Treatment of leukemia
Aluminium	Adjuvant, antacid, treatment of hyperphosphatemia and peptic ulcer	Selenium	Dietary (trace mineral) supplement
Calcium	Antacid	Rubidium	^{82}Rb - PET radio-imaging
Chromium	Dietary (trace mineral) supplement	Strontium	Osteoporosis
Manganese	MRI contrast agent	Yttrium	^{90}Y - therapeutic radionuclide, ^{86}Y - PET radio
Iron	MRI contrast agent, dietary supplement	Zirconium	^{89}Zr - PET radio-imaging
Cobalt	Treatment and diagnosis of pernicious anemia, dietary supplement	Molybdenum	Treatment for Wilson's
Nickel	Dietary (trace mineral) supplement	Technetium	^{99m}Tc - SPECT radio-imaging
Copper	Treatment for Menke's disease, ^{64}Cu PET - radio-imaging, intrauterine contraception	Silver	Antimicrobial, Treatment of burns
Zinc	Treatment of eczema, dietary supplement	Barium	Radiographic contrast medium
Lanthanum	Treatment of hyperphosphatemia	Rhenium	^{188}Re - therapeutic radionuclide
Cerium	Treatment of burns	Gold	Antiarthritic
Samarium	^{153}Sm - therapeutic radionuclide	Bismuth	treatment of Antibacterial gastrointestinal
Gadolinium	MRI Contrast agent		

Metal and metal compounds in diagnosis

@ Co-ordination complexes or specific metals has been in use in imaging ($BaSO_4$ in imaging gastro intestinal tract.

@ Techniques like ultrasonography, computerized Tomography (CT) and magnetic resonance imaging (MRI) also involve different metal or metal compounds.

@ B-10 isotope may be used in detecting tumour (neutron capture therapy).

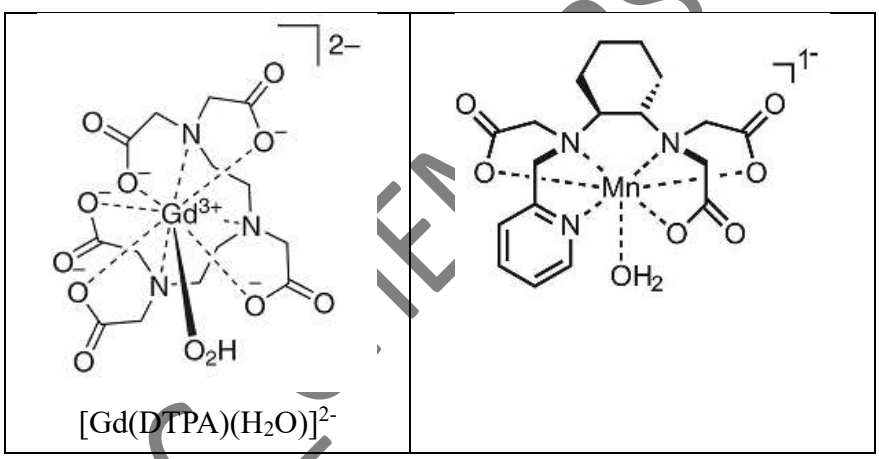
@ Metal compounds with radioactive nuclei (selective and should not damage the tissues) is extensively used for investigations like tumours, organs and other tissues.

@ Some common nuclei that are used in diagnosis are ^{57}Co, ^{57}Ga, ^{99}Tc (most suitable), ^{111}In, ^{113}In, ^{123}I, ^{169}Yb, ^{197}Hg, ^{201}Tl etc.

@ The species $[Tc(CNR)_6]^+$ where R = t-butyl, CH_2COOBu^+ is used in hard imaging phosphonate. Tc-methylene diphosphonate complex is used in imaging bone malformities

@ MRI is based on NMR spectroscopy (paramagnetic metal ion changes the water-proton relaxation time around the tissue – distribution of metal ion in normal and abnormal tissue is different – provide means for diagnosis).

@ Mn(II), Fe(III) and Gd(III) ions have been known to produce good proton relaxation enhancement in human. $[Gd(DTPA)(H_2O)]^{2-}$ has been successfully used in diagnosis of brain tumours. DTPA-diethylenetriamine pentaacetate ion**.**



$[Gd(DTPA)(H_2O)]^{2-}$

Co-ordination compounds as drug

@ Coordination compounds are extensively used as therapeutic agent (Au-arthritis, Cu-rheumatism and anti-inflammatories, Pt-anticancer agent).

@ Efficiency of many organic drugs are also enhanced through metal binding (ibuprofen is more effective as its Zn-complex, anticancer drug bleomycin is activated as Fe(II) complex).

Lithium compounds as medicine

@ Lithium carbonate is widely used in the treatment and prevention of manic depression (Li(II)).

@ In the manic stage, neuronal communication becomes high. Lithium ion possibly binds to inositol phosphates reducing their degradation to inositol and thereby suppress neuronal communication.

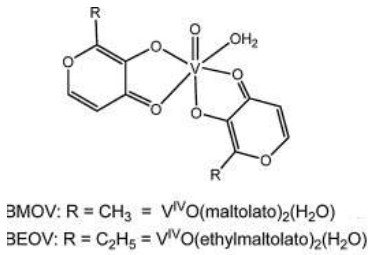
@ Lithium ion may also disturb neuro transmission by inhibiting the formation of the key signalling molecule adenosine monophosphate (AMP).

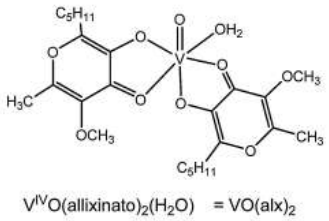
Vanadium compounds as medicine

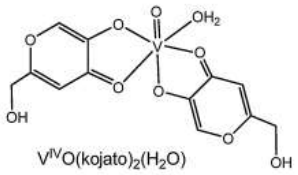
@ V(IV) compounds have been reported to exhibit insulin like properties (toxicity prevents their use).

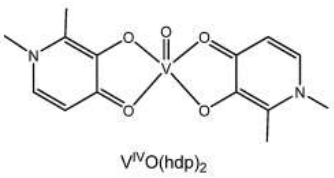
@ (Dipicolinato) oxovanadate (V), $[VO_2dipic]^-$ is less toxic (adsorbed in acidic environment of stomach and intestine) is used orally in animals.

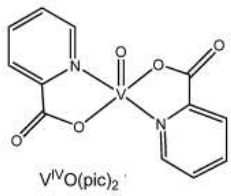
@ Some Vanadium compound also known to have anticancer activity.

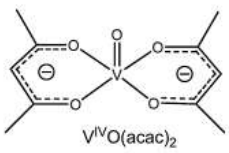


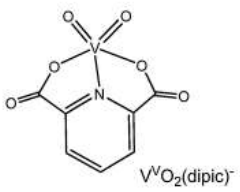


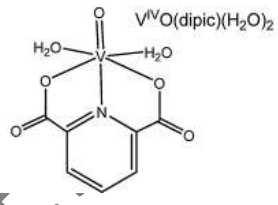












Gold compounds in medicine

@ Use of Gold in medicine (chrysotherapy) was initiated in 1929 by French physician Forestier.

@ He used Au(I)thiolates for the treatment of rheumatoid arthritis.

@ Up to 2% of the global population (120 million) are affected by rheumatoid arthritis (RA),

- An inflammatory condition.
- Progressive destruction of the articular cartilage lining the bone surfaces in joints.
- Tissue damage results from action of lysosomal enzymes including collagenase and other proteases.

@ Number of gold(I) thiolate drugs including sodium aurothiomalate (Myocrisin), aurothioglucose (Solganol), sodium aurothiopropanolsulfate (Allochrysine) and sodium aurothiosulfate (Sanochrysin).

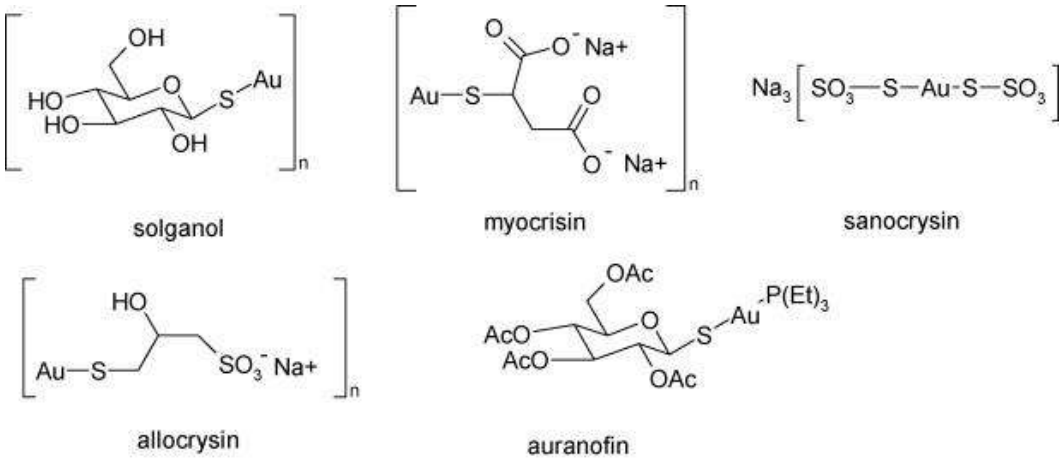
@ Myochrisin (sodium aurothiomalate), solganol (aurothioglucose) are now used as intramuscular injection curing arthritis.

@ Auranofin is administered orally, binds to –SH and S-S units of proteins (blood serum albumin) – prolonged treatment is necessary.

@ All of the clinically used gold(I) drugs have linear two coordinate geometry.

@ The intramuscular administered drugs are oligomeric/polymeric in nature consisting of bridging thiolates between gold(I) ions (e.g., myocrisin)

@ Auranofin, some Au-thiolate and Au-NHC drugs are also used as anticancer agents.



Platinum-based anticancer drug

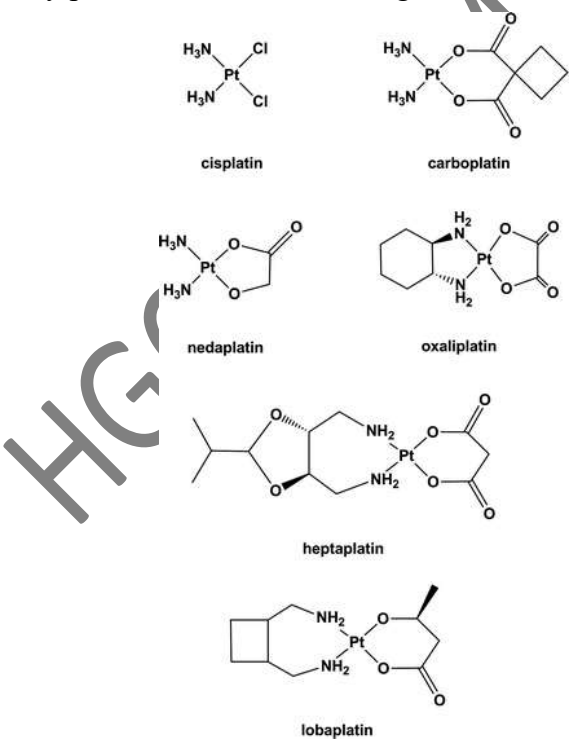
@ cis-diamminedichloroplatinum (II) (cis-DDP or cis platin) is the best-known example.

@ It is an effective antitumor agent causing regression of both slow- and fast-moving tumours and also inhibitor of DNA synthesis.

@ Effective against testicular cancer and active against ovarian, lung, bladder, neck and cervical cancers.

@ It is administered as intravenous injection, usually given physiological saline electrolyte.

@ But associated with kidney problem, nausea, vomiting as adverse side effect.

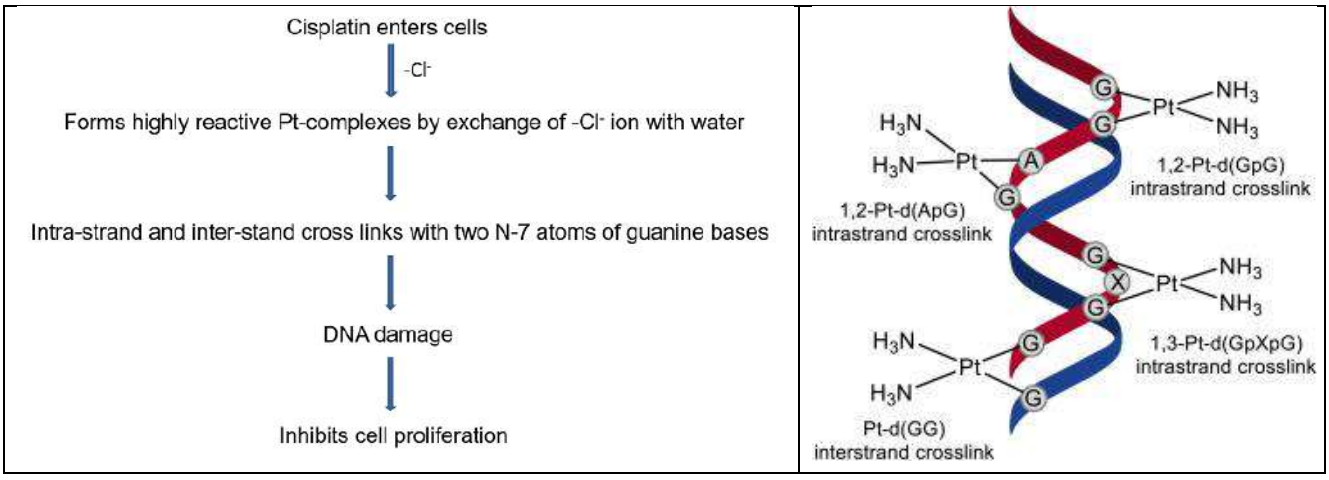


@ Mechanism

@ Extracellular fluids have high Cl^- concentration and therefore hydrolysis of cis platin is suppressed.

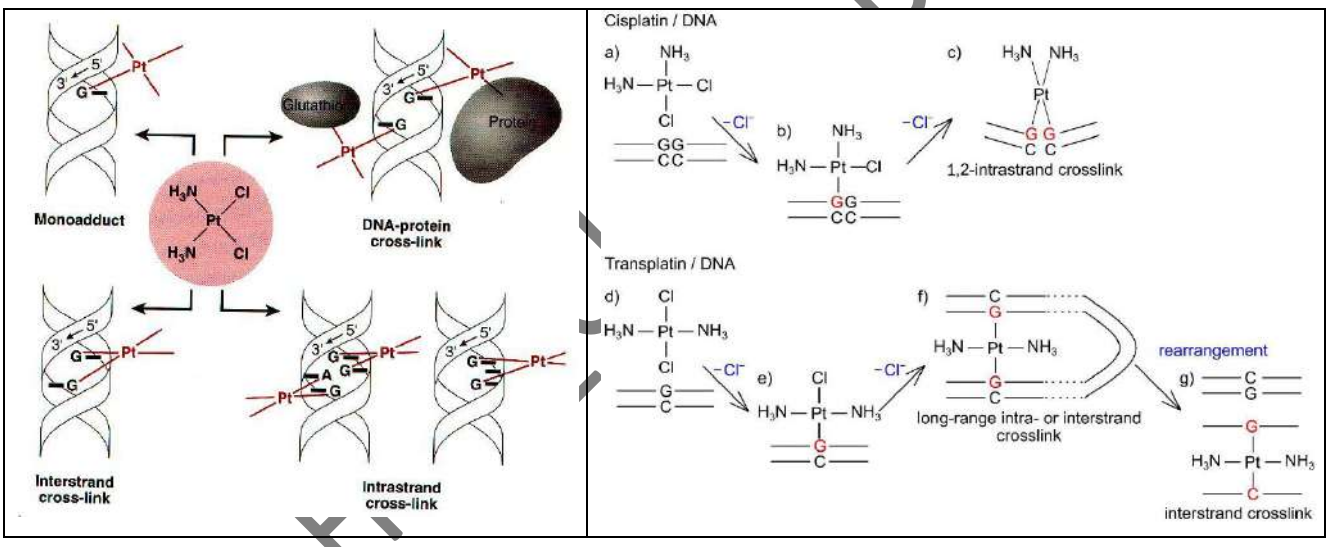
@ Inside the cell, the Cl^- concentration is low and hence cis platin hydrolyses to (exchange of Cl^- by water) diaqua complex $[Pt(NH_3)_2(OH_2)_2]^{2+}$, which reacts with the N-atoms (N-7) of the guanine (major) and adenine (lesser extent) bases, to produce either intra- (more important) or inter- stand cis-$Pt(NH_3)_2$ bridges viz. dinucleotide complex $[Pt(NH_3)_2\{d(pGpG)\}]$ with adjacent guanine bases.

@ The result is a kink in the DNA helix, with angles upto 34º (prohibits self-replication).



@ The interaction of cis-platin would interfere with the process of DNA replication and thereby synthesis of messenger RNA by prohibiting the base pairing (DNA perturbation).

@ The isomer, trans-platin has no such activity. It cannot form crosslink between adjacent guanine bases within the same strand (intra-strand bridge) due to steric reasons. It is labile and undergoes rapid, non-specific nucleophilic substitution before reaching the target.



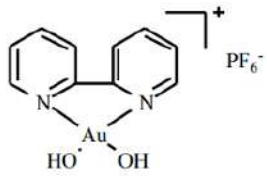
Non-platinum anticancer drug

@ The primary goal is to find Pt-resistance anticancer or antitumor drug.

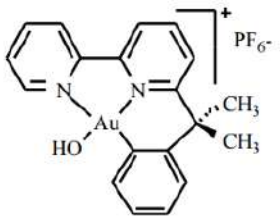
@ The secondary goal is that activity of non-platinum antitumor drug should be associated with severe toxicity like Pt-drugs.

@ The different toxicity may be attributed to their different co-ordination geometry, binding preference, ligand exchange rates and hence different mechanism of action and different biological properties.

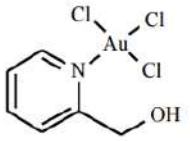
@ Radiometals aside, arsenic is the only other metal whose compounds are approved for the treatment of cancer. A crude solution of As_2O_3 and trace amounts of mercury, as a potential treatment for acute promyelocytic leukemia.



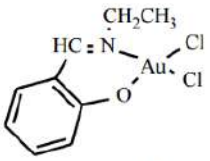
[Au(bipy)(OH)$_2$][PF$_6$]



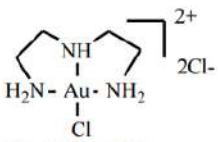
[Au(bipy-H)(OH)](PF$_6$)



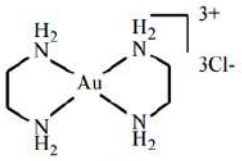
AuCl$_3$(Hpm)



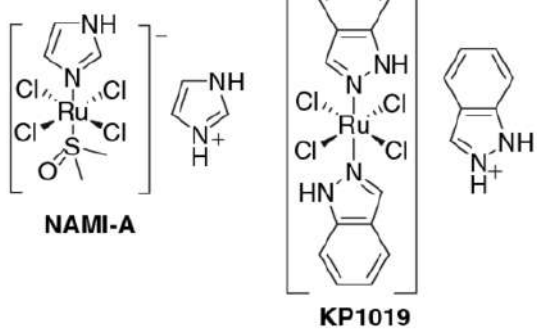
AuCl$_2$(esal)

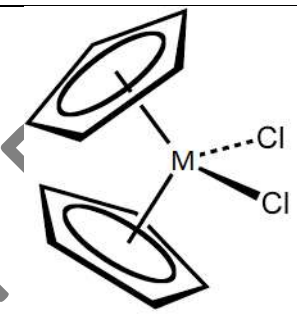


[AuCl(dien)]Cl$_2$



Au(en)$_2$]Cl$_3$





M = Ti (Cl may be Br), V, Mo, Nb

Ru-based drugs

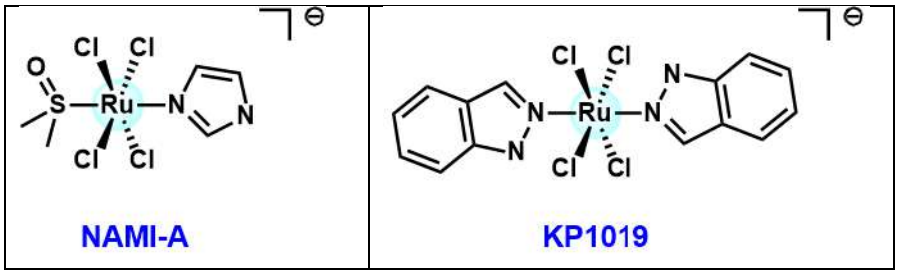
@ In recent years, ruthenium-based molecules have emerged as promising antitumor and antimetastatic agents.

@ Ruthenium compounds are usually less toxic and no cross resistant than platinum counterparts, therefore better tolerated in vivo.

@ In animal models, ruthenium compounds are effective in the treatment of cancer types which cannot be treated by platinum compounds, most probably due to a different mode of action.

@ NAMI-A and KP1019 are two potential ruthenium drugs in phase II clinical trials.

@ Former strongly inhibits metastasis without effects on the primary tumor & latter induces apoptosis in colorectal tumor in which cisplatin is inactive, via intrinsic mitochondria apoptosis pathway.



Anti-infective – Ag as medicine

@ Ag is commonly used in the treatment for burns and wounds, venereal diseases, abscesses, removal of granulation tissue and newborn conjunctivitis, the majority of which rely on the antibacterial action of Ag(I) ions.

@ A dilute preparation of $AgNO_3$ (0.5%) (1960s) was used in treatment for burns based on its anti-bacterial action against a range of pathogens such as S. aureus.

@ Silver sulfadiazine cream (Silvadene, Silvazine 1970s), containing 1% Ag(I) and a sulfonamide antibacterial drug, came into use as a broad-spectrum antibacterial agent for burns treatment.

@ $AgNO_3$ is widely used for cauterization in the treatment of epistaxis (nose bleed) in children and is the recommended first line medical treatment for cauterization of cutaneous pyogenic granulomas.

@ AgNP wound dressing for use on burns and ulcers.

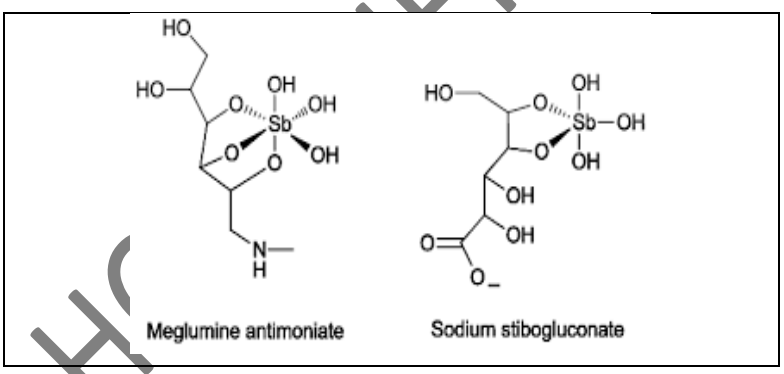
@ AgNP impregnated polyurethane ventricular catheter for neurosurgical use

Antimony Agents

@ Sb was first used to treat leishmaniasis in Brazil in the form of Tartar 65 Emetic (Potassium antimony tartrate)

@ The drug which contained Sb(III) increased survival rates despite its toxicity and later succeeded less toxic Sb(V) drugs with improved therapeutic and toxicity profiles such as stibamine, stibosan and neostibosan in 1920s

@ Sb(III) targets Zn-finger binding domains responsible for regulatory functions like DNA recognition, RNA packaging, protein folding and assembly, transcriptional activation, cell differentiation, growth and apoptosis.



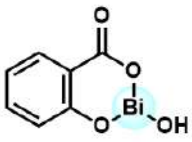
Bi-based drugs

@ Bismuth is a highly acidic metal ion and may allow it to block Ca^{2+} channels and to disrupt the cell walls of bacteria.

@ These are also active against the bacterium Helicobacter pylori which is associated with the mucus layer of ulcers and cancers

@ Bismuth drugs are used to treat occasional upset stomach, heartburn, and nausea. It is also used to treat diarrhea

@ Bismuth subsalicylate is a drug used to treat temporary discomforts of the stomach and gastrointestinal tract. Commonly known as pink bismuth, it is the active ingredient in popular medications such as Pepto-Bismol



Bismuth subsalicylate

Al-based drugs

@ In addition to Bi, Al-compounds play a role as adjunctive treatments for H. Pylori infection given its association with treatments for peptic ulcer disease.

@ Al containing drugs, as antacids, antisecretory medication and mucosal protecting agents, are therefore known to help manage acute and chronic gastroduodenal ulcerations and contribute to ulcer healing.

@ $Al(OH)_3$ containing antacids for example are known to adsorb heat shock proteins, cytotoxin VacA and urease, all of which are implicated in H. pylori associated ulceration.

As-based drugs

Salvarsan

@ Salvarsan, the first chemotherapeutic compound introduced in 1910 as the effective treatment for syphilis.

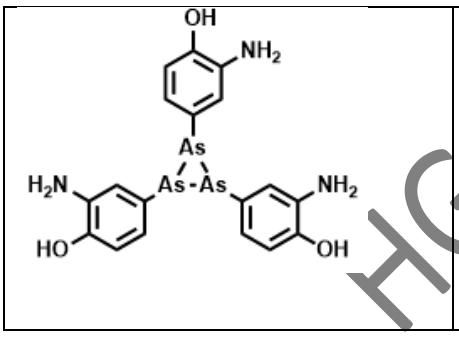
@ Risk of side effects, used until 1943 when penicillin became available.

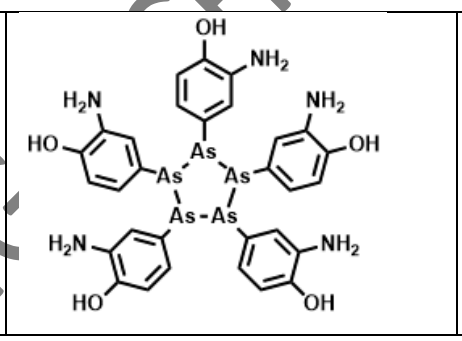
Melarsoprol

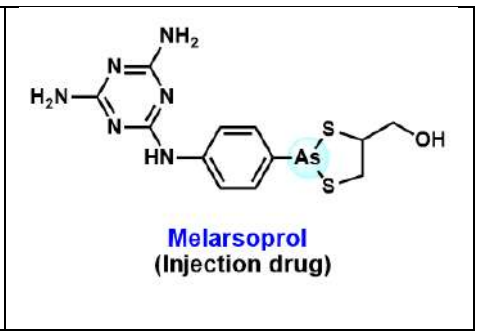
@ Melarsoprol is widely used in the treatment of late-stage human African trypanosomiasis (sleeping sickness, a parasitic disease).

@ 48,000 people died of it in 2008

@ Melarsoprol is toxic to humans, causing severe brain disease in some cases.







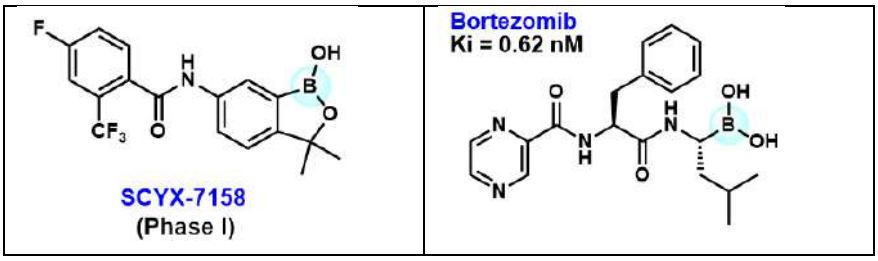
Melarsoprol
(Injection drug)

B-based drugs

@ Boric acid has antiseptic, antifungal and antiviral properties and for these reasons is applied as a water clarifier in swimming pool water treatment and eye antiseptics.

@ Benzoxaboroles were developed by Anacor as oral treatment for human African trypanosomiasis (sleeping sickness). SCYX-7158 was shown to be safe and exhibited excellent in vivo PK and in vivo efficacy

@ Bortezomib is the first-in-class proteasome inhibitor for the treatment of multiple myeloma (a plasma cell cancer) approved in the US in 2003.

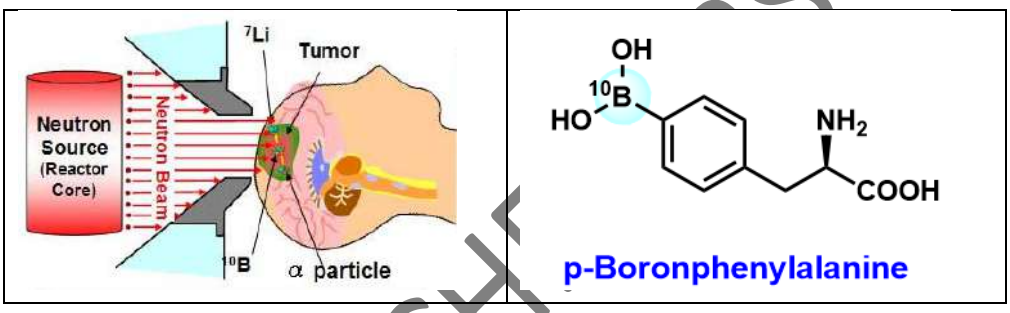


Boron neutron capture therapy (BNCT)

@ BNCT is a combination of treatment with boron and low energy neutrons. Radiotherapy using neutrons can get rid of glioblastoma cells.

@ The boron molecules give off radiation within the brain tumor cells when the external neutron radiation hits them.

1. ^{10}B compound which accumulates in the cancer cell is injected in a patient
2. The neutron beam is irradiated to the lesion
3. The cancer cells are selectively destroyed using a-particles which are generated by the ^{10}B

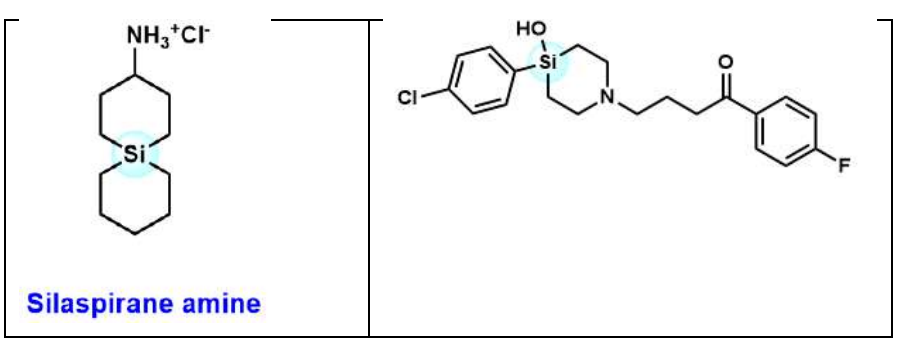


Si-based drugs

@ Silicones (silicon containing polymers) are used in the applications requiring high biocompatibility such as bandages, breast implants and contact lenses.

@ In the influenza A virus M2 proton channel inhibitor, hydrophobicity is known to play a critical role in improving the antiviral potency. The larger size and increased lipophilicity of silicon can provide a better hydrophobic contact between the inhibitor and the channel.

@ A clinically useful antipsychotic drug, haloperidol is associated with a problematic metabolic pathway. The sila-analogs show a higher potency and selectivity than haloperidol and avoid the formation of a toxic metabolite.



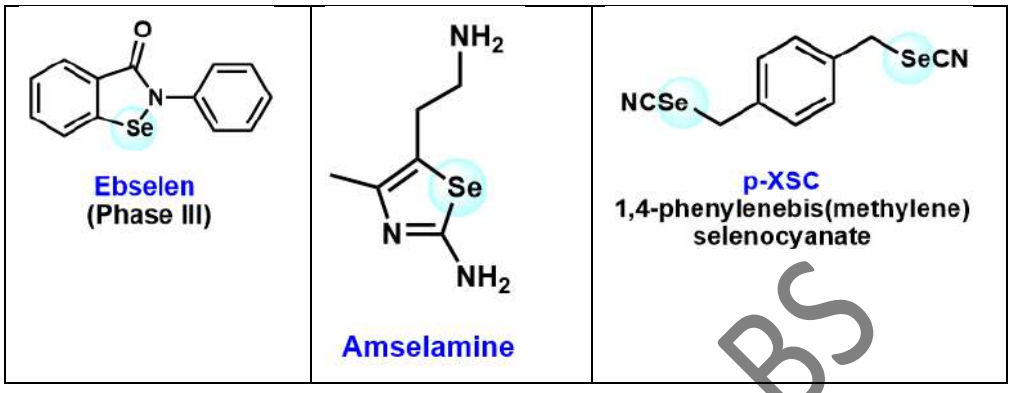
Se-based drugs

@ Se-deficiency is associated with several serious or chronic diseases like cancer, diabetes, AIDS & tuberculosis.

@ Ebselen is a mimic of the antioxidant enzymes glutathione peroxidase (GPx), which is a potent scavenger of hydrogen peroxide as well as hydroperoxides. It is being investigated as a possible treatment for stroke, tinnitus, and manic depression.

@ Amselamine, which is the seleno analog of amthamine, behave as a histamine H_2-agonist with a higher potency than histamine and amthamine. Moreover, amselamine exerts hardly any activity for histamine H_1 and H_3-receptors, which make it selective for the H_2-receptor.

@ p-XSC exerts chemo-preventive activity for carcinogenesis in colon, lung, liver, intestine and oral tissues.



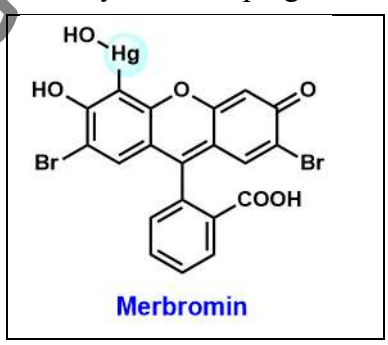
Hg-based drugs

@ In China, Hg use was thought to prolong life, heal fractures, and maintain generally good health, although it is now known that exposure to mercury leads to serious adverse health effects.

@ Today, the use of mercury in medicine has greatly declined in all respects, especially in developed countries.

@ Merbromin is a topical antiseptic drug discovered in 1918. This chemical soon became popular among parents and physicians for everyday antiseptic uses. It is readily available in most countries, but because of its mercury content, it is no longer sold in the US, Germany and France.

@ It is still an important antiseptic, particularly in developing nations, due to its "unbelievably low cost".

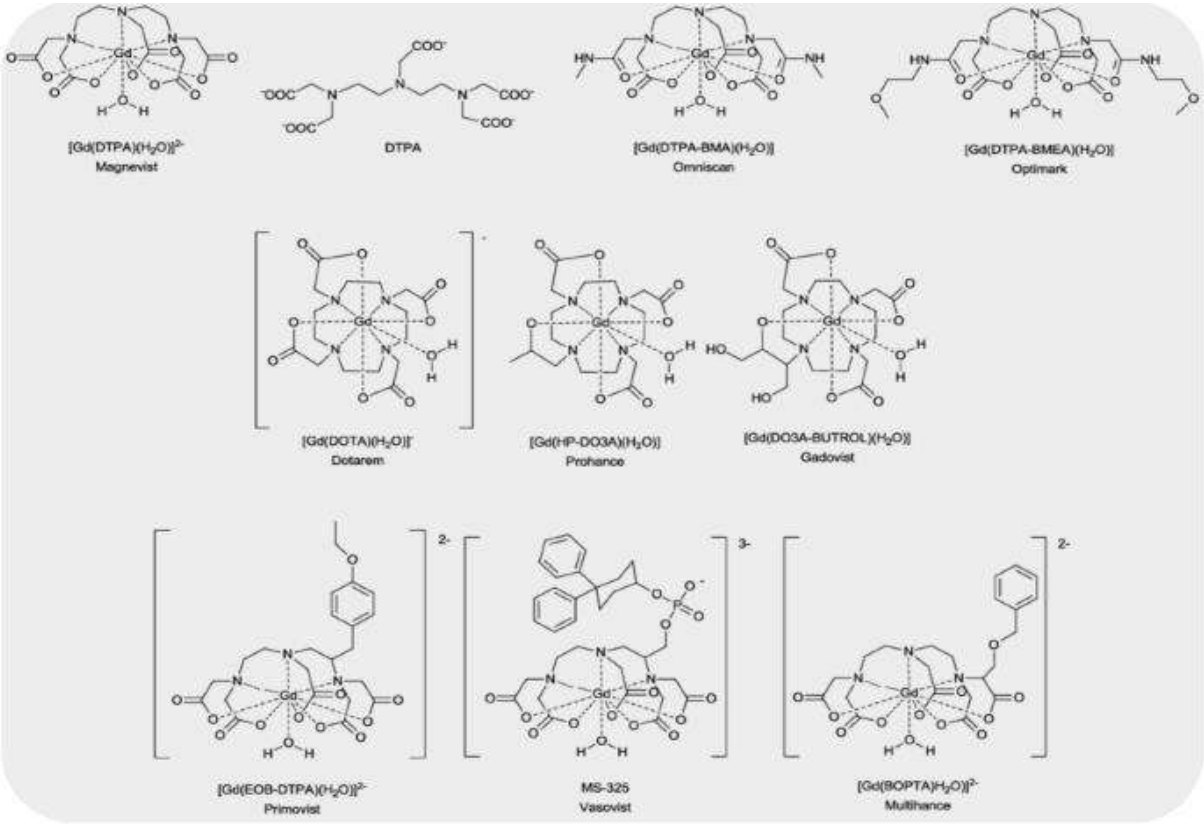


MRI Contrast Agents

@ MRI is an invaluable non-invasive diagnostic tool, which provides high resolution and 3-dimensional images of internal physiological structures.

@ MRI employs nuclear magnetic resonance (NMR) to image hydrogen protons in free water and organic molecules such as lipids and proteins inside the body.

@ Metal complexes can enhance MR images by shortening the T_1 and T_2 relaxation times of water molecules that encounter the complexes.



Radiotherapeutic and Radio diagnostic Agents

@ Radiopharmaceuticals provide a valuable source of ionizing radiation and have important clinical applications in the diagnosis and treatment of various diseases. Metallic nuclides or radiometals, also play an important role.

@ Radio diagnostic imaging is a non-invasive procedure where radiopharmaceuticals are introduced in to the human body at suitably low concentrations, with a view to locating a potential disease, assessing a pre-existing disease or monitoring the effects of treatment

@ Diagnostic radiometals emit suitably energized γ rays

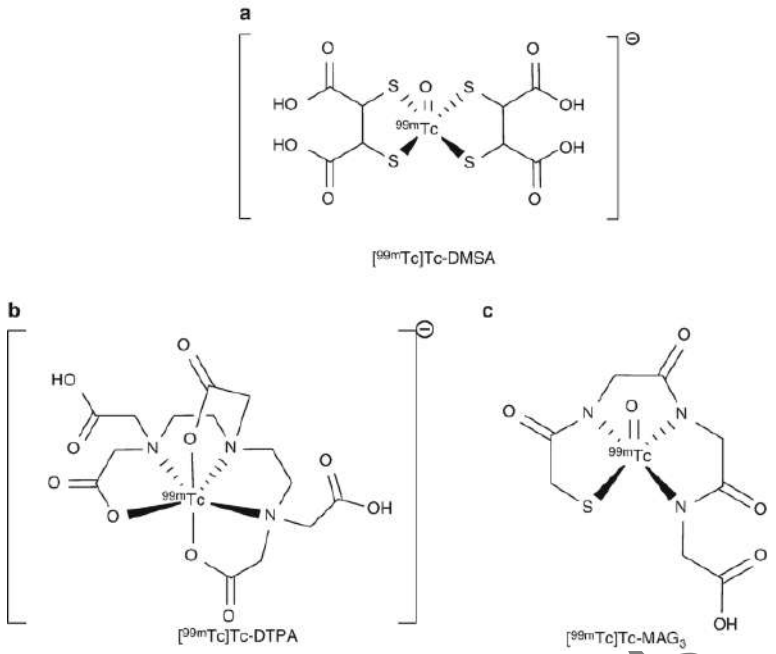
@ There are two primary diagnostic modalities in clinical use viz. positron emission tomography (PET) and single photon emission computed tomography (SPECT).

@ In PET a positron emitter, for example gallium-68, is administered

@ In contrast for SPECT a γ emitting radiopharmaceutical is administered and γ emissions from the radioisotope are collected by a γ camera, providing vital information regarding the source of the rays.

@ Nearly 80% of all clinically-used radiopharmaceuticals are technetium-based

Radiometal	**Half-Life (hours)**	**Decay Mode (% branching mode)**	**Applications**
Tc	6.01	Isomeric transition (99.99)	SPECT
In	67.39	Electron capture (100)	SPECT
Ga	78.28	Electron capture (100)	SPECT
	12.70	Electron capture	PET
Zr	78.41	Electron capture	PET



Consulted Books

1. Bioinorganic Chemistry by Valentine, Gray, Lippard and Bertini
2. Bioinorganic Chemistry by Das and Das
3. Inorganic Chemistry by Huheey, Keiter, Huheey and Medhi
4. Inorganic Chemistry by Atkins
5. Inorganic Chemistry by Missler and Tar
6. EPG-Pathshala
7. IGNOU-MSc Materials
8. Bioinorganic Chemistry by Fenton
9. Inorganic Chemistry – Shriver, Atkins, Langford, Overton