DIRECTORATE OF DISTANCE & CONTINUING EDUCATIONS

MANONMANIAM SUNDARANAR UNIVERSITY

TIRUNELVELI – 627012

OPEN AND DISTANCE LEARING(ODL) PROGRAMMES

(FOR THOSE WHO JOINED THE PROGRMMES FROM THE ACADEMIC YEAR 2023 – 2024)

M.Sc. CHEMISTRY

COURSE MATERIALS

ELECTIVE – IV – BIOINROGANIC CHEMISTRY

COURSE CODE: SCHE22

BIO-INORGANIC CHEMISTRY SYLLABUS

UNIT-I: Essential trace elements: Selective transport and storage of metal ions: Ferritin, Transferrin and siderophores; Sodium and potassium transport, Calcium signalling proteins. Metalloenzymes: Zinc enzymes–carboxypeptidase and carbonic anhydrase. Iron enzymes–catalase, peroxidase. Copper enzymes – superoxide dismutase, Plastocyanin, Ceruloplasmin, Tyrosinase. Coenzymes - Vitamin-B12 coenzymes.

UNIT-II: Transport Proteins: Oxygen carriers -Haemoglobin and myoglobin - Structure and oxygenation Bohr Effect. Binding of CO, NO, CN– to Myoglobin and Haemoglobin. Biological redox system: Cytochromes-Classification, cytochrome a, b and c. Cytochrome P-450. Non-heme oxygen carriers-Hemerythrin and hemocyanin. Iron-sulphur proteins- Rubredoxin and Ferredoxin-Structure and classification.

UNIT-III: Nitrogen fixation-Introduction, types of nitrogen fixing microorganisms. Nitrogenase enzyme - Metal clusters in nitrogenase- redox property - Dinitrogen complexes transition metal complexes of dinitrogen - nitrogen fixation via nitride formation and reduction of dinitrogen to ammonia. Photosynthesis: photosystem-I and photosystem-II-chlorophylls structure and function.

UNIT-IV: Metals in medicine: Metal Toxicity of Hg, Cd, Zn, Pb, As, Sb. Therapeutic Compounds: Vanadium-Based Diabetes Drugs; Platinum-Containing Anticancer Agents. Chelation therapy; Cancer treatment. Diagnostic Agents: Technetium Imaging Agents; Gadolinium MRI Imaging Agents. temperature and critical magnetic Field.

UNIT-V: Enzymes -Introduction and properties -nomenclature and classification. Enzyme kinetics, free energy of activation and the effects of catalysis. Michelis - Menton equation - Effect of pH, temperature on enzyme reactions. Factors contributing to the efficiency of enzyme.

Recommended Text

1. Williams,D.R. – Introdution to Bioinorganic chemistry.
2. F.M. Fiabre and D.R. Williams– The Principles of Bioinorganic Chemistry, RoyolSoceity of Chemistry, Monograph for Teachers-31
3. K.F. Purcell and Kotz., Inorganic chemistry, WB Saunders Co., USA.
4. G.N. Mugherjea and Arabinda Das, Elements of Bioinorganic Chemistry - 1993.
5. R. Gopalan, V. Ramalingam, *Concise Coordination Chemistry*, S. Chand, **2001**.

Reference Books

1. M.Satake and Y.Mido, Bioinorganic Chemistry- Discovery Publishing House, New Delhi (1996)

2. M.N. Hughes, 1982, The Inorganic Chemistry of Biological processes, II Edition, Wiley London.

3. R. W. Hay, Bio Inorganic Chemistry, Ellis Horwood, 1987.

4. R. M. Roat-Malone, Bio Inorganic Chemistry, John Wiley, 2002.

5. T. M. Loehr, Iron carriers and Iron proteins, VCH, 1989.

Website and e-learning source

1. https://www.pdfdrive.com/instant-notes-in-inorganic-chemistry-the-instant-notes-chemistry-series-d162097454.html
2. https://www.pdfdrive.com/shriver-and-atkins-inorganic-chemistry-5th-edition-d161563417.html

UNIT -1

Ferritin

- Ferritin is discussed in greater detail under Iron Storage.
- A protein having 24 subunits of 500kD**a**
- It binds 4000 iron molecules which accounts for a larger iron storage
- Synthesized by cells that store iron and is later used to synthesize hem.
- Ferritin in serum is derived from the breakdown of macrophages of the RES (liver, spleen and Bone marrow). Its measurement is used to assess iron stores in the body.
- Low Ferritin levels indicate depletion of iron stores and used as an early indication of iron Deficiency.
- Increased Ferritin levels are observed in liver diseases such as hepatitis, liver cirrhosis due to Alcohol consumption and hepatic carcinoma.
- Increased Ferritin levels are also observed in: leukemia, Hodgkin's lymphoma and chronic Inflammatory disease

Metallothionein

- Cytoplasmic metal- binding protein.
- Involved in ion storage & detoxification.
- Small, cysteine-rich proteins that bind Zn2+,Cd2+ Cu2+ & cys ligands.
- Found in cyanobacteria, fungi, plants, insects & vertebrates.
- Bind metal ions with high affinity.

Transferrins

Transferrins are group of non-heme iron-binding glycoproteins that can effectively control the level of free iron in biological fluids. Transferrin glycoproteins bind iron tightly but reversibly in the cleft of each of two homologous lobes and hence capable of transporting it to the sites of absorption, utilization and storage. They are widely distributed in the physiological fluids of most vertebrates as well as invertebrates including serum, egg white, mammalian milk, tears, and leukocytes. These transferrin families are customarily divided into following major subcatagories based on the amino acid sequence, function and source from which each is isolated.

Serum transferrin: Serum transferrin also known as serotransferrin, siderophilin and '13a metalbinding globulin is generally present in the serum of vertebrates and some other biological fluids. It plays a vital role in transport of iron within the circulatory system of the vertebrates between different biological tissues.

Lactotransferrin: Lactotransferrin also termed as lactoferrin is generated by mucosal epithelial cells of mammals and therefore it is found abundantly in mammalian milk. In addition, it is also present in specific granules of polymorphonuclear leukocytes and other secreted fluids such as tears, pancreatic juice etc. It plays pivotal role in body defense against infections owing to numerous outstanding properties like antioxidant, antiinflammatory and antimicrobial activities.

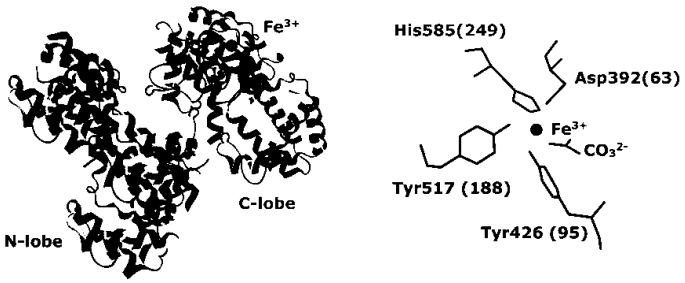
Ovotransferrin (conalbumin): Ovotransferrin or conalbumin which is commonly found in oviduct secretions, eggs of birds and reptiles possess antimicrobial property that is vital for bird's innate immunity . The structural protein of ovotransferrin is similar to that of serum transferrin since they are derived from the same gene.

Melanotransferrin (a p97 cell surface protein): Melanotransferrin is one of the first cell surface markers associated with melanomas. The exact biological function of melanotransferrins is unknown, however, it has been predicted that it may play major role in cell proliferation, migration, angiogenesis and tumorigenesis.

Role of transferrin

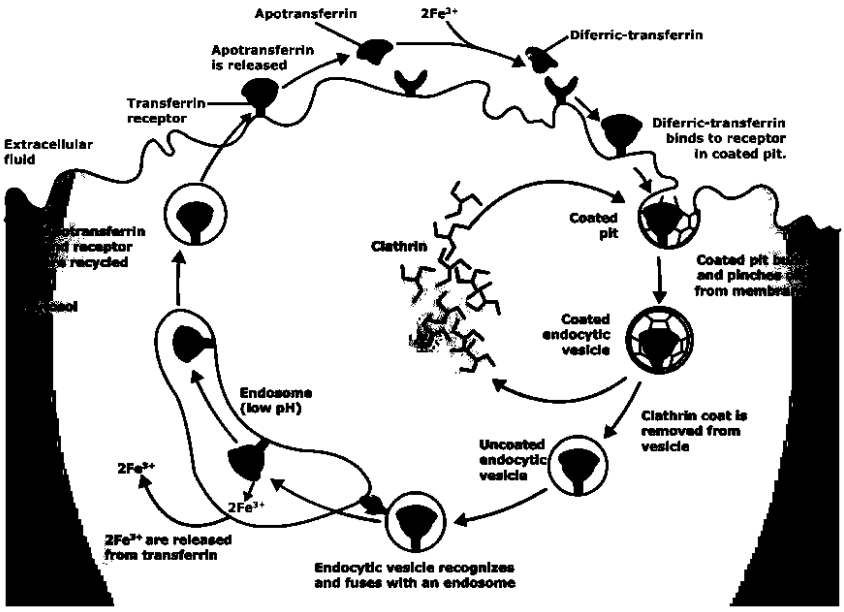
Transferrin is mainly produced by the liver cells which is then secreted into plasma. The main function of transferrin is to transport iron from absorption centers to the sites of utilization, storage and haemoglobin degradation. It also plays a key role in areas where erythropoiesis and active cell division occur.

Structure of Human Serum Transferrin



Human serum transferrin is a 79.6 kDa blood plasma glycoprotein made up of polypeptide chain containing 679 amino acids. It has a bilobal structure with two homologous lobes of almost equal size namely amino- (N lobe) and carboxy- (C lobe) terminal lobe joined by linking polypeptide. N lobe consists of amino acids 1 to 331 while C lobe is formed by amino acids 339 to 679. Each lobe is further divided into subdomain, N1(1-92, 247-331), N2(93-246), C1(339-425, 573-679) and C2(426-572) which are interconnected by two antiparallel β strands that acts as a hinge. Both N and C lobes are capable of binding one iron atom with the help of six ligands; Four ligands (an aspartic acid, two tyrosines, and a histidine) are provided by the protein and two ligands are provided by a synergistically bound carbonate anion.

Mode of action



Iron enters into cells by receptor-mediated endocytosis in a form of monoferric and diferric transferrin. First of all, the iron-loaded transferrin binds to receptors present on the outer surface of the plasma membrane. Subsequently, transferrin is internalized via invagination of clathrin-coated pits followed by development of endocytic vesicles. After that, the clathrin is removed with the help of uncoating enzymes and the vesicle combines with endosomes. The pH of endosomes is reduced to about 5.5 due to the activity of V-type ATPases in their membranes which weakens the association between iron and transferrin. This would eventually facilitates the removal of iron from transferrin. Transferrin along with its receptor is then transported through the endocytic cycle back to the cell surface which becomes ready for another round of iron uptake.

SIDEROPHORES

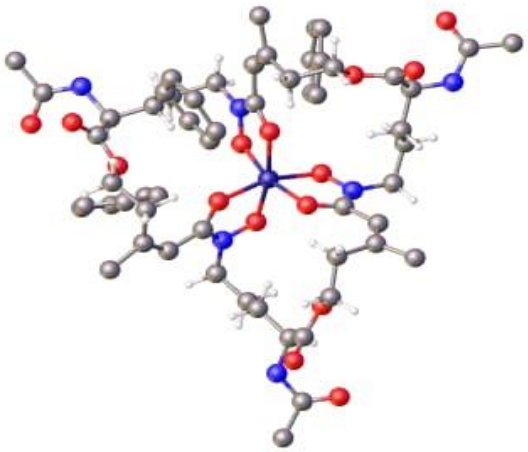
INTRODUCTION:

- Biological control of plants pathogens has been the subject of much research in recent years.
- It can potentially help us limit the use of chemical pesticides that are harmful to the environment.
- The use off plant growth-promoting rhizobacteria(PGPR), such as siderophores-producing bacteria, represents a potentially attractive alternative disease management approach, since they have the capacity to increase yield and protect crops simultaneously .
- Few organisms like *pseudomonas fluorescens, p.putida* are a special group of organisms which are widely used as bio control agents.

ROLE 0F SIDEROPHORES:

- High affinity system of Fe^{3+} acquisition, utilization and storage.
- Sometimes, required for virulence.
- Helps in growth, colonization and sexual sporulation.
- Elicit the plant defense through an antagonism mechanism between SA and JA signaling casades.

STRUCTURE:



TYPES:

1.HYDROXAMATE:

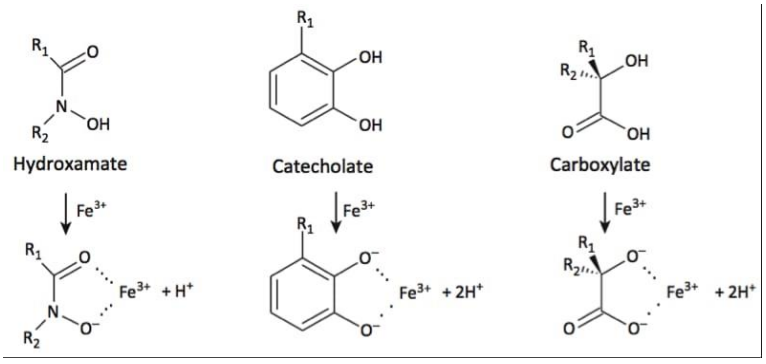
- Hydroxamate group-bearing siderophores are mainly synthesized by fungi and Gram-positive filament- forming bacteria (streptomycetes).
- In fungal systems the hydroxamic acid chelating group is commonly derived from acylated Nõ-acyl- No-hydroxy-L-ornithine.

2.CATECHOLATE:

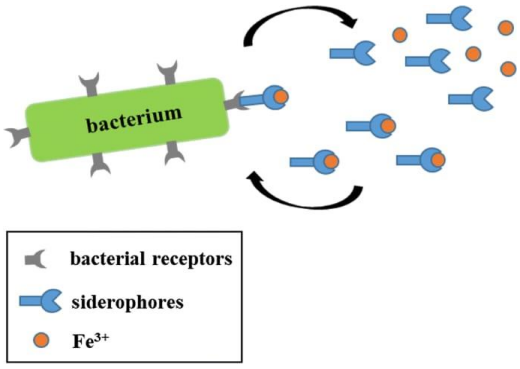
- Each catecholate group provides two oxygen atoms for chelation with iron so that a hexadentate octahedral complex is formed as in the case of the hydroxamate siderophores. Linear catecholate siderophore are also produced in certain species.
- Agrobactin and parabactin are produced by Agrobacterium tumefaciens and Paracoccus denitrificans respectively.

3.CARBOXYLATE:

- The best characterized carboxylate type siderophore with a novel structure is rhizobactin.
- Rhizobactin is produced by Rhizobium meliloti strain DM4 and is an amino poly (carboxylic acid) with ethylenediaminedicarboxyl and hydroxycarboxyl moieties as ironchelating groups.
- Staphyloferrin A, produced by Staphylococcus hyicus DSM20459, is another member of this class of complexon siderophores.



SIDEROSPORE STRUCTURE IN BACTERIA:



FUNCTIONS:

Siderophores function as biocontrols, biosensors, and bioremediation and chelation agents, in addition to their important role in weathering soil minerals and enhancing plant growth.

CONCLUSION :

- Siderophore system constitutes a key position in Iron-homeostasis in many plant pathogens.
- The role of siderophores in Iron homeostasis depend largely on the pathogen-host system.
- Siderophore system affects growth, oxidative stress resistance as well as asexual and sexual development.
- Common virulence determinant, at least in some plant pathogenic fungi and bacteria.
- Modulates plant defense through an antagonistic mechanism between SA & JA signaling cascade.

Selective transport

Selective transport is the process by which membranes in cells determine what molecules can go in and what molecules can go out of a cell.

This helps the cell control its functions, such as removing waste and maintaining its other functions needed to keep it alive. Selective transport is also known as selective permeability due to the cell using its membranes to allow molecules in and out of it and it being permeable for molecules to enter and exit. Also, the cell is selective regarding what molecules it lets in and out. If a cell was not selective about what molecules it lets in and out, and let everything in and out of it, it would not function well and would die.

Selective transport helps a cell live and function properly by making it selective when it comes to what molecules should come in and what molecules should go out.

Selective transport is a process that occurs in the cell membrane, regulating the concentration of specific molecules inside the cell1. This process is facilitated by specialized proteins in the cell membrane.

Here are some key points about selective transport:

Selective Permeability: The cell membrane has the ability to differentiate between different types of molecules, only allowing some molecules through while blocking others. This property stems from the intrinsic diffusion rates for different molecules across a membrane.

Regulation of Passage: The regulation of passage through the membrane is due to selective membrane permeability, a characteristic of biological membranes which allows them to separate substances of distinct chemical nature. In other words, they can be permeable to certain substances but not to others.

Transport Mechanisms: Transport across a membrane can be considered from an energy story perspective. For instance, at the beginning of the process, a generic substance X may be either on the inside or outside of the cell. At the end of the process, the substance will be on the opposite side from which it started.

Substance Types: The substances include ions such as Ca 2+, Na +, K +, and Cl –; nutrients including sugars, fatty acids, and amino acids; and waste products, particularly carbon dioxide (CO 2), which must leave the cell.

Selective transport is a crucial process that helps maintain the homeostasis of the cell by controlling the movement of substances in and out of the cell

SODIUM – POTASSIUM PUMP:

Introduction

The Na+ K+ pump is an electrogenic transmembrane ATPase first discovered in 1957 and situated in the outer plasma membrane of the cells; on the cytosolic side. The Na+ K+ ATPase pumps 3 Na+ out of the cell and 2K+ that into the cell, for every single ATP consumed. The plasma membrane is a lipid bilayer that arranged asymmetrically, containing cholesterol, phospholipids, glycolipids, sphingolipid, and proteins within the membrane. The Na+ K+-ATPase pump helps to maintain osmotic equilibrium and membrane potential in cells.

The sodium and potassium move against the concentration gradients. The Na+ K+-ATPase pump maintains the gradient of a higher concentration of sodium extracellularly and a higher level of potassium intracellularly. The sustained concentration gradient is crucial for physiological processes in many organs and has an ongoing role in stabilizing the resting membrane potential of the cell, regulating the cell volume, and cell signal transduction. It plays a crucial role on other physiological processes, such as maintenance of filtering waste products in the nephrons (kidneys), sperm motility, and production of the neuronal action potential. Furthermore, the physiologic consequences of inhibiting the Na+-K+ ATPase are useful and the target in many pharmacologic applications.

Na, K-ATPase is a crucial scaffolding protein that can interact with signaling proteins such as protein kinase C and phosphoinositide 3-kinase .

Cellular Level

Structurally, the Na+ K+ ATPase is composed of a catalytic alpha subunit and an auxiliary beta subunit. Some Na-K ATPases include a subunit that is tissue-specific and belongs to the FXYD protein family.The alpha subunit contains a transmembrane region which is composed of 10 helices, referred to as MA1-M10. Within these ten helices, ion binding sites, specifically three binding sites that bind to Na+ in the E1 state and two binding sites that bind to K+ in the E2

state.The structure of the Na-K ATPase is composed of three sites. Site one and two overlap within both the E1 and E2 states. However, site three is exclusively in the E1 state and is between the M5, M6, and M8 transmembrane helices, which bind to Na+ and catalyze H+ transport as well, dependent on the Na+, K+, and H+ concentrations.According to previous studies, the pump's E2 state selectivity for K+ may be due to ion binding pocket protonation.

Function

Sodium and potassium gradients function in various organ systems' physiologic processes.The kidneys have a high level of expression of the Na, K-ATPase, with the distal convoluted tubule expressing up to 50 million pumps per cell. This sodium gradient is necessary for the kidney to filter waste products in the blood, reabsorb amino acids, reabsorb glucose, regulate electrolyte levels in the blood, and to maintain pH.

Sperm cells also use the Na, K-ATPase, but they use a different isoform necessary for preserving fertility in males. Sperm needs the Na, K ATPase to regulate membrane potential and ions, which is necessary for sperm motility and the sperm's acrosome functioning during penetration into the egg.

The brain also requires NA, K ATPase activity. Neurons need the Na, K ATPase pump to reverse postsynaptic sodium flux to re-establish the potassium and sodium gradients which are necessary to fire action potentials. Astrocytes also need Na, K ATPase pump to maintain the sodium gradient as the sodium gradient maintains neurotransmitter reuptake. Na, K ATPases in the gray matter consumes a significant amount of energy, up to three-quarters of energy is absorbed by Na, K ATPases in the gray matter while merely a quarter of the total energy gets utilized for protein synthesis and molecular synthesis.

Pathophysiology

The Na+-K+ ATPase plays a prominent role in thyroid pathophysiology. In hyperparathyroidism, there is an increase in heat intolerance, increased sweating, and increased weight loss due to the increased synthesis of Na+-K+ ATPase induced by the excessive thyroid

hormone. This increased synthesis of Na+-K+ ATPase then increases basal metabolic rate, which then increases oxygen consumption, respiratory rate, body temperature, and calorigenesis.

Clinical Significance

As the Na+-K+ ATPase is essential for maintaining various cellular functions, its inhibition could result in diverse pathologic states. Studies show that patients with heart failure have a 40% lower concentration of total Na, K-ATPase. One significant clinical application is in cardiovascular pharmacology. For example, ouabain is a cardiac glycoside that inhibits the Na+-K+ ATPase by binding to the K+ site. Other cardiac glycosides such as digoxin and digitoxin directly inhibit the Na+-K+ ATPase.This inhibition causes a buildup of excessive K+ extracellularly, and accumulation of excessive Na+ intracellularly as the Na+-K+ ATPase can no longer pump K+ into the cell or pump Na+ out of the cell. This buildup of intracellular Na+ hinders the concentration gradient that usually drives the Na+/Ca 2+ channel exchanger, which generally pumps Na+ into the cell and Ca 2+ out of the cell because the concentration gradient is not favorable for Na+ to enter the cell as excessive Na+ has built up intracellularly. This indirect inhibition of Na+/Ca 2+ exchange, therefore, causes a buildup of Ca 2+ intracellularly because the exchanger cannot allow Ca 2+ to exit the cell since it cannot accept Na+ into the cell. This increased intracellular Ca 2+ then increases cardiac contractility. This positive inotropy stimulates the vagus nerve, causing a decrease in heart rate. This physiology is clinically significant in the treatment of heart failure as it increases the contractility of the heart. It is also clinically significant in the treatment of atrial fibrillation as it decreases the conduction of the atrioventricular node and causes depression of the sinoatrial node.Diuretic therapy has also been shown to reduce myocardial Na, K-ATPase when there is potassium loss. In contrast, angiotensin-converting enzyme inhibitors could stimulate the activity of the Na, K pump.

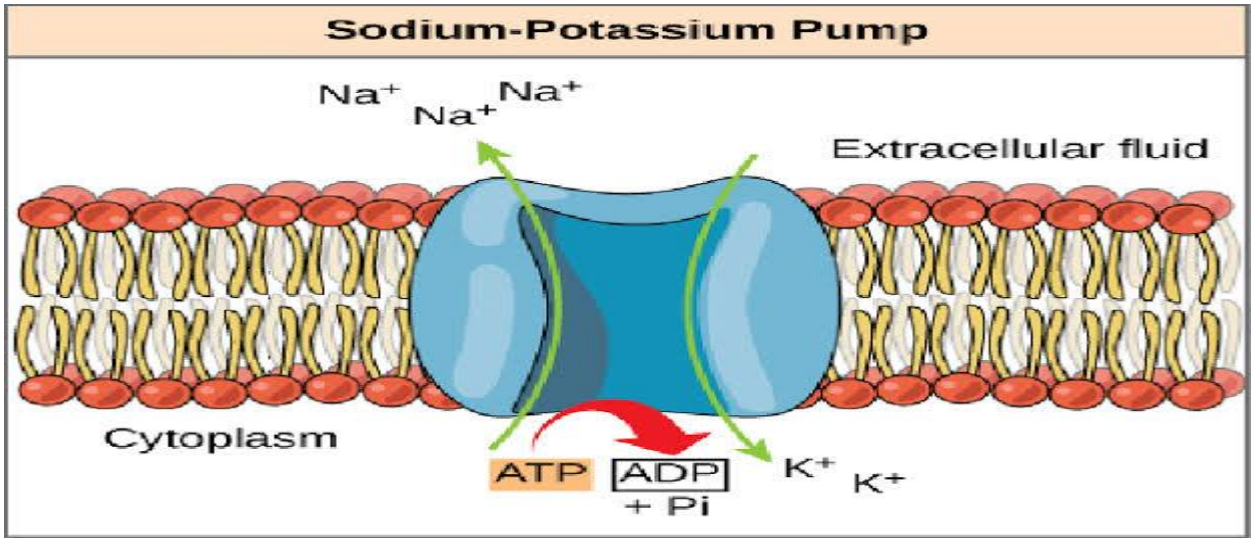
Another significant clinical application includes the effect of beta-adrenergic agonists in increasing the number of Na+/K+ ATPase channels; this is because beta-adrenergic agonists can enhance the gene expression of the Na+-K+-ATPase pump, which ultimately results in an increased quantity of the enzyme and therefore increased the activity of the enzyme. Because of this increased quantity of Na+/K+ ATPase, more potassium is pumped into the cell, causing a buildup of intracellular potassium. Therefore, extracellularly, this inward shift of potassium results in hypokalemia in the extracellular blood. Thus beta-adrenergic agonists can cause

increased Na+ transport out of the cell as well. Increased Na+ transport extracellularly across alveolar epithelial cells for example, which would then cause lung liquid to follow this flow of Na+, ultimately stimulating lung liquid clearance.

Insulin also causes clinically significant effects on the Na+/K+ ATPase. Insulin increases the number of Na+/K+ ATPase pumps in the membrane as well, this leads to an intracellular shift of potassium, causing hypokalemia in the extracellular space of the blood.

There are reports of abnormal expression levels, or activity of the Na+K+ pump in diabetes, hypertension, Alzheimer's disease, and in various tumors including glioblastoma, non-small cell lung carcinoma, breast cancer, melanoma, colorectal carcinoma, and bladder cancer.

Na+ K+-ATPase and its endogenous regulators, the endogenous cardiac steroids (ECS), play a role in the etiology of bipolar disorder and are a potential target for drug development for the treatment.



Metalloenzymes

- Enzymes are the catalysts for biological systems.
- Their basic structure is built of proteins
- Enzymes which are composed of a proteins structure are called **epoenzymes**
- They have a small prosthetic group which may be simple or a complexed metal ion.
- **Coenzymes** –a group that combines reversibly with an enzyme for a particular reaction and is released to combine with other enzymes
- The prosthetic groups and coenzymes are called **cofactors**

- More than 1500 metalloenzymes are identified so far
- Their names are derived by adding '**ase**' to the name of process catalyzed of the name of molecules on which the enzymes acts.
- The molecules on which the enzymes acts is called the **substrate**
- Metal ions (metal cofactors) that are directly bound to the protein or to enzyme-binding nonprotein components are found in metalloenzymes (prosthetic groups).
- Metalloenzymes make up around a third of all enzymes discovered so far. Other metalloproteins, in addition to enzymes, are involved in non-enzyme electron transfer reactions (cytochromes), and can serve as storage (for example, ferritin for iron) or transport proteins (e.g., transferrin for iron).
- Metal storage is reversible in the latter groups of proteins, and the metal is only a transient component.
- In a larger sense, ribozymes, i.e. RNA molecules with enzyme function, may contain structurally and/or functionally significant metal ions (usually divalent metal ions like Mg2+) and are thus referred to as metalloenzymes.
- Natural metalloenzymes are well-known proteins that include one or more transition metal ions such as Fe, Cu, Zn, Ni, and Co. These metalloenzymes are capable of catalyzing a wide range of biosynthesis and metabolic events.
- These metal ions serve mostly as Lewis acids or redox-active sites. Further more, several enzymes have been used to manufacture important chemical molecules in both laboratory and industrial-scale operations. In one prominent case, nitrile hydratase, which possesses a Co(III) ion in the reactive site, has been utilized to produce acrylamide in commercial quantities.
- Metal complexes comprising valuable metals such as Ru, Rh, or Pd, on the other hand, have been used as catalysts in the creation of a wide range of chemicals and drug precursors.
- Several research groups have looked into altering such metal complexes to improve not only their individual catalytic reactivities, but also their stereo- and regio- selectivities, as well as their substrate specificity.
- Examples of metalloenzymes:
    - superoxide dismutase (Zn and Cu)
    - carboxypeptidase A (Zn)
    - carbonic anhydrase (Zn)
    - cytochrome oxidase (Fe and Cu)

- xanthine oxidase (Co and Fe)

Metal	Type of enzyme	Role of metal	Redox active?
Mg	Kinases,Phosphatases,Phosphodiesterases	Binding of phosphates/polyphosphates	X
Zn	Metalloproteases,dehydrogenases	Lewis acid carbonyl activation	X
Fe	Oxygenases(P450 non-haem) [FeS] Clusters	Binding and activation of oxygen Electron transport,hydratases	✓
Cu	oxygenases	Activation of oxygen	✓
Mn	Hydrolases,hydratases	Lewis acid	✓
Co	Vitamin B12 coenzyme	Homolysis of co-carbon bond	✓
Mo	Nitrogenase	Component of Mo/Fe cluster	✓

Sr.no	Name of Metalloenzyme	Metal present
1.	Phosphohydrolases Phosphotransferases	Magnesium
2.	Cytochromes Peroxidase catalase Ferridoxine	Iron
3.	Tyrosinase Amine oxidase Cytochrome oxidase Ascorbate oxidase Galactose oxidase	Copper
4.	Arginase Oxaloacetone decarboxylase	Manganese
5.	Alcohol dehydrogenase Alakaline phosphatase Carbonic anhydrase Carboxy peptidase	Zinc

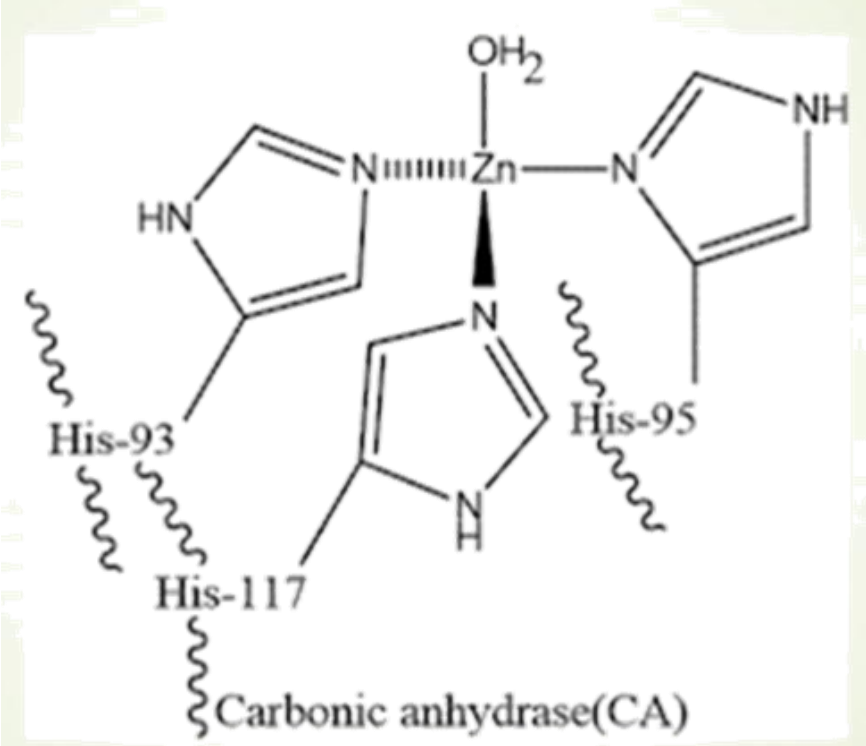
Introduction

- It is a zinc enzyme.
- Catalyse hydration of carbon dioxide and dehydration of carbonic acid.
- It has a molar mass of 30,000
- It occurs in animals as well as plants.
- It can hydrate 106 molecules of hydration of carbon dioxide
- The zinc iron lies in a deep pocket created by the coiled epoenzymes.
- Coordinated with three nitrogen atoms of three imidazole rings of histidine groups of epoenzyme.
- The fourth coordination is occupied with water molecule when the enzyme is at rest.
- The sterochemistry of zinc in the enzyme is tetrahedral.
- Structure of anhydrase

Zinc based enzyme

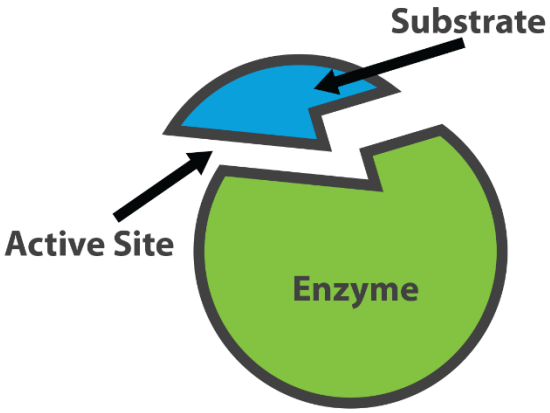
• Carbonic anhydrase is a zinc-based enzyme that the catalyst conversion of carbon dioxide to carbonic acid. It was found that the molar concentration of carbon dioxide in solution decrease from 220mol the rate constant is the first order.

Zinc Enzyme -Carboxypeptidase

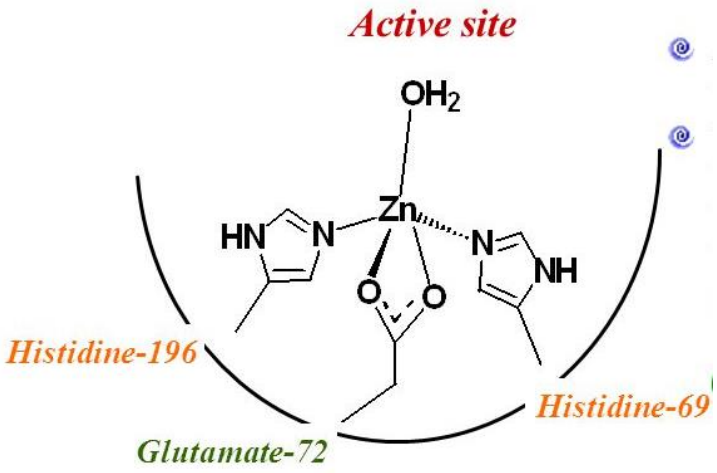


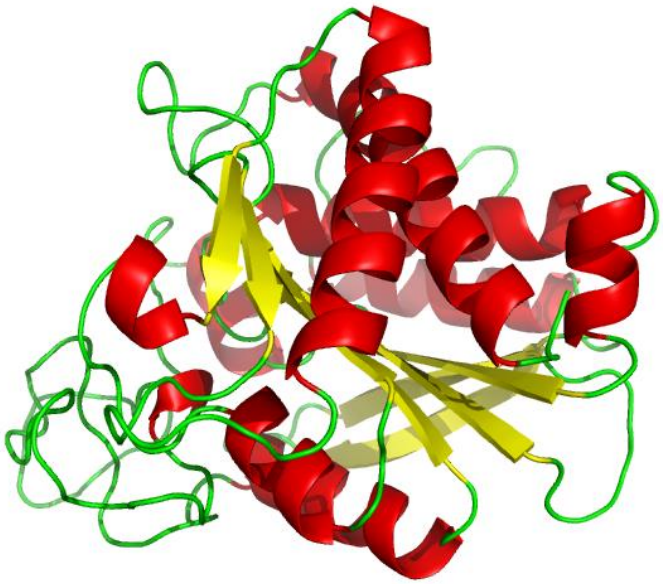
Enzyme:

Enzymes are proteins comprised of amino acid linked together in one or more polypeptide chain.



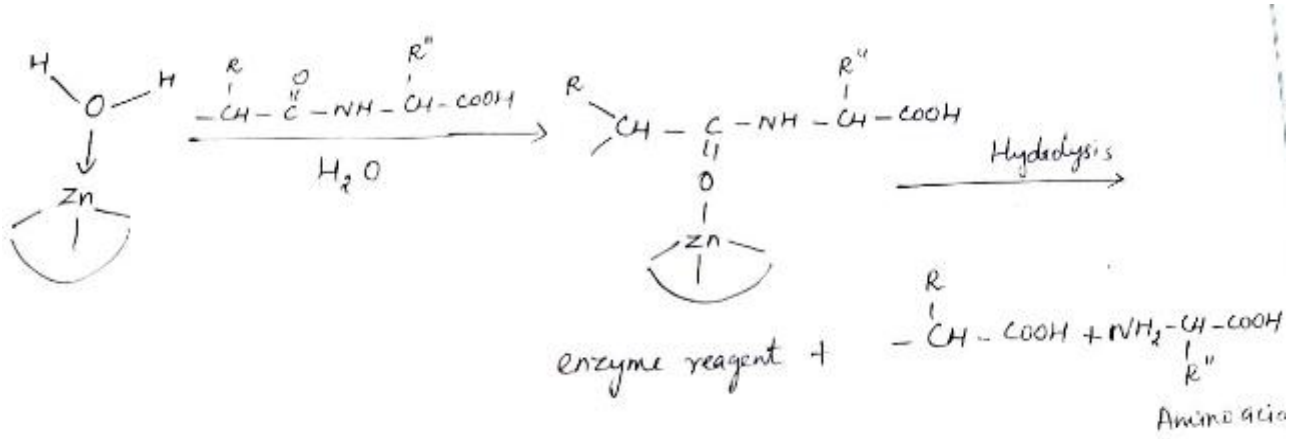
Carboxypeptidase (Zinc Enzyme):





- Zinc present in active site. It is also containing Zn enzyme.
- It has tetrahedral geometry, held in position through complex formation with Glutamic acid 72, Histidine 196, Histidine 69 and water molecule.
- The Zinc metal a strong electrophilic lewis acid catalyst stabilizes the negative intermediates that form throughout the hydrolytic reaction.
- It hydrolysis the terminal peptide bond of peptide chain from the side of its carboxy end.
- C-terminal amino acid is released but it should be a phenyl group.

Reaction:



The major classification of carboxypeptidase:

Carboxypeptidase A:

Carboxypeptidase A cleaves off aromatic or branched chain amino acids. For example, a drug that treats high blood pressure, Captopril, was designed based on a carboxypeptidase A inhibitor.

Carboxypeptidase B:

Carboxypeptidase B cleaves off basic amino acids. Thrombin-activatable fibrinolysis inhibitor (TAFI) is a 55-kDa carboxypeptidase B–like proenzyme synthesized in the liver that circulates in blood at a plasma concentration of 4 to 15 µg/mL (70 to 275 nmol/L).

APPLICATIONS:

- Carboxypeptidases (CPs) perform many diverse physiological functions by removing C-terminal amino acids from proteins and peptides. Some CPs function in the degradation of proteins in the digestive tract while other enzymes play biosynthetic roles in the formation of neuropeptides and peptide hormones.
- Recent biomedical research on collagenase, enkephalinase, and angiotensin-converting enzyme used carboxypeptidase A for inhibitor synthesis and kinetic testing.

CATALASE ENZYME

CATALASE:

Bacteria that conduct aerobic metabolism (biological) Reaction that require O_2) produce H_2O_2 as a toxic Product of their metabolism. Toxic H_2O_2 can cause Intracellular damage such as

damage to DNA, lipid, proteins. To remove H_2O_2 and other similar compounds, Cells produce Catalase enzyme to breakdown H_2O_2 into liquid water (H_2O) and oxygen (O_2),

$2H_2O_2 \rightarrow 2H_2O + O_2$

Bacteria can only make catalase if they have the gene for catalase in their DNA.

CLASSIFICATION OF CATALASE:

1. Monofunctional Catalase Ex:Pseudomonas Syringae
2. Catalase Peroxidases Ex:Bacillus pumilus
3. Mn-catalases Ex:Thermus thermophillus
4. Minor catalases Ex:Caldariomyces Fumago

PRINCIPLE:

- Catalase is an enzyme that converts hydrogen peroxide into water and oxygen.
- The bacteria that contain this enzyme usually aerobic or facultative anaerobic.

CATALASE TEST:

Catalase test is used to detect the presence of the enzyme catalase in bacteria PURPOSE**:**

- Identification for gram positive and gram-negative Organisms.
- It is primary test used in the differentiation of Staphylococcus and streptococcus

PROCEDURE:

SLIDE CATALASE TEST:

- Transfer a large amount of growth to a microscope Slide. Aseptically place one or two drops of 3% Hydrogen peroxide directly onto the bacteria and immediately observe for the formation of bubbles.
- The formation of bubbles is observed against a dark
- Background indicates a positive result.
- **POSITIVE**: Rapid bubbles formation
- **NEGATIVE**: No bubble formation

APPLICATION:

Food Industry

- Detection of ethanol from food samples

- Detection of calcium in milk

Therapeutics

- Prevention of H1N1 Induced Pneumonia.
- Enzyme complex as blood substitute

Environment

- Treatment of textile and Bleaching industry

Effluent

- Degradation of phenolic compound.

PEROXIDASE:

- Peroxidase are the group of enzymes that catalyzes oxidation – reduction reactions.
- It is also called oxidoreductase.
- Peroxidase is an enzyme found in a wide variety of organisms, from plants to humans to bacteria.
- Its function is to breakdown the hydrogen peroxidase (H_2O_2), which is one of the toxins produced as a byproduct of using oxygen for respiration.
- Peroxidase as electron acceptor for catalysing different oxidative reaction.

Source:

Fungi – Aspergillus niger

Bacteria – bacillus subtills

Medium:

glucose +yeast extract +ammonium nitrate + magnesium sulphate + dipotassium phosphate.

Inoculum:

Aspergillus niger

Stock:

- Stock culture is prepared by inoculating Aspergillus niger on a potato dextrose agar slants at 4℃ for 24 hours.
- Fermentation medium + inoculum incubate at 22 ℃ in a rotatory shaker at 160rpm for 7 days followed by filtrations discard the residue filtrate.
- Centrifuge the filtrate at 500rpm for 15 min at 4℃.
- Ammonium sulphate added as supernatant.
- Collect the precipitate, washed, dried and collect the product.

Difference between peroxide and catalase

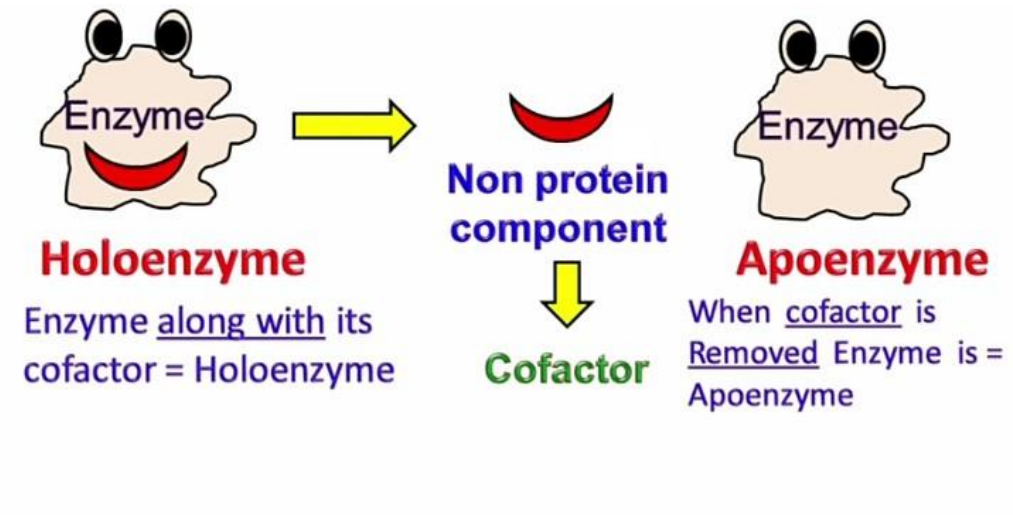
- Peroxidase detoxifies hydrogen peroxide; Catalase detoxifies hydroxyl radicals.
- Catalase produces oxygen, peroxidase does not.

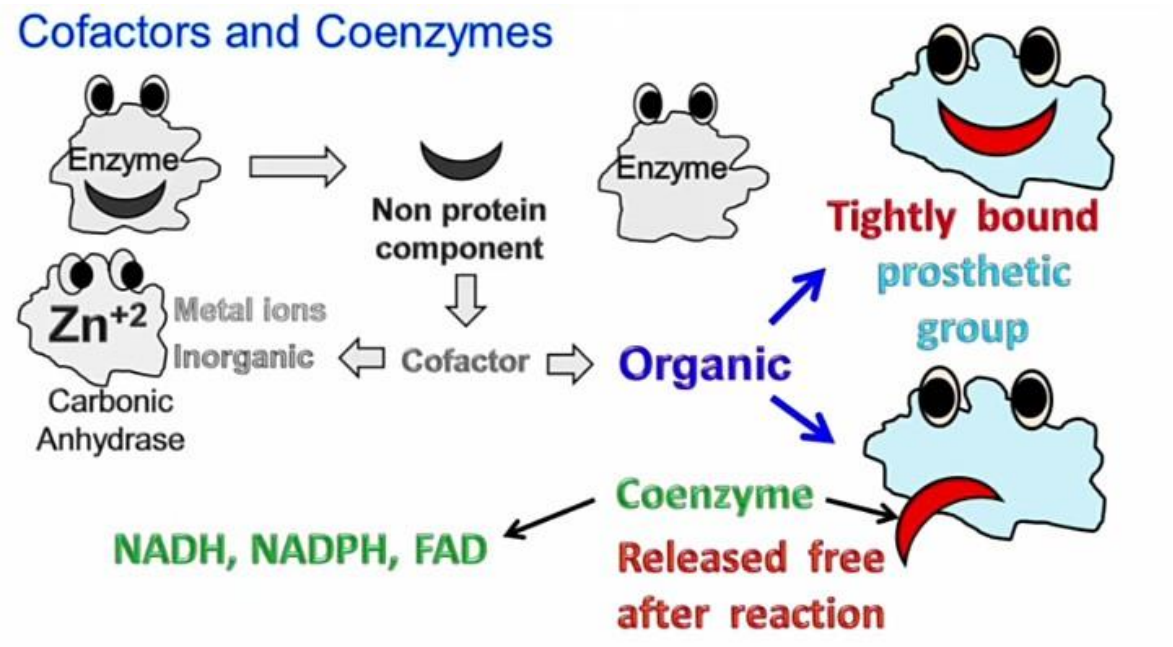
Coenzyme – Vitamin B 12 Coenzymes

Coenzyme

- ✓ An enzyme is a protein that functions as a catalyst to mediate and speed a chemical reaction.
- ✓ Coenzyme is a substance that enhances the action of an enzyme.
- ✓ The catalytic activity of enzymes mostly depends on the presence of non-proteins compounds called coenzymes.
- ✓ Coenzymes cannot be isolated from apoenzymes without denaturation of the enzyme's proteins.
- ✓ Coenzymes are small molecules.
- ✓ They cannot by themselves catalyse a reaction but they can help enzymes to do so.
- ✓ In technical terms, coenzymes are organic molecules that bind with the protein (apoenzyme) to form the active enzyme (haloenzymes).
- ✓ A number of the water vitamins such as vitamins B1, B2 and B6 serve as coenzymes.

- ✓ The coenzyme forms of vitamin B_{12} are methylcobalamin (Figure 2) and deoxyadenosylcobalamin.
- ✓ These assist in the conversion of homocysteine to the amino acid methionine, the oxidation of amino acids and odd-chain fatty acids, and the removal of a methyl group from methyl folate, which regenerates tetrahydrofolate.





Copper Enzymes

Copper enzymes are a class of enzymes that utilize copper ions (Cu^{2+}) as cofactors to carry out catalytic reactions. Copper is a transition metal that can exist in different oxidation states (Cu^{+} and Cu^{2+}) and plays a crucial role in the catalytic activity of these enzymes.

There are two main types of copper enzymes based on their copper-binding sites and functions:

1. Mononuclear Copper Enzymes: These enzymes contain a single copper ion within their active site. The copper ion can undergo redox reactions, where it alternates between the +1 and +2 oxidation states, facilitating electron transfer reactions. Examples of mononuclear copper enzymes include copper-containing oxidases and oxygenases. Copper oxidases, such as ascorbate oxidase and ceruloplasmin, catalyze the oxidation of substrates by reducing molecular oxygen to water. Copper oxygenases, like dopamine β-monooxygenase, incorporate oxygen atoms into organic substrates.

2. Binuclear Copper Enzymes: These enzymes have two copper ions that are closely associated within their active site. The copper ions can interact with molecular oxygen and other substrates to perform various reactions, including oxidation and reduction processes. Binuclear copper enzymes are involved in important biological functions such as electron transfer, oxygen activation, and radical chemistry. Examples of binuclear copper enzymes include tyrosinase, which catalyzes the hydroxylation of monophenols to o-diphenols and the oxidation of o-diphenols to o-quinones.

Copper enzymes are essential for various metabolic processes in living organisms, including respiration, antioxidant defense, and the biosynthesis of important biomolecules like hormones and pigments. The unique redox properties of copper ions make them versatile catalysts in enzymatic reactions, contributing to the diversity and complexity of biological processes.

There are several types of copper enzymes, each with specific functions and structures. Some notable examples include:

Cytochrome c oxidase: This enzyme, found in mitochondria, is involved in the electron transport chain during cellular respiration. It uses copper ions to facilitate the transfer of electrons and the reduction of molecular oxygen to water, generating ATP (adenosine triphosphate) for cellular energy.

Superoxide dismutase (SOD): SOD is an antioxidant enzyme that helps protect cells from oxidative stress by converting superoxide radicals into oxygen and hydrogen peroxide. Some forms of SOD, such as copper/zinc SOD (Cu/Zn SOD), contain copper ions at their active sites.

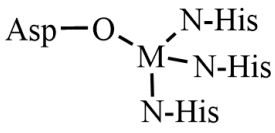
Tyrosinase: Tyrosinase is an enzyme involved in melanin production, which gives color to hair, skin, and eyes. Copper ions in tyrosinase catalyze the oxidation of tyrosine to dopaquinone, a key step in melanin biosynthesis.

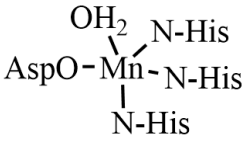
Lysyl oxidase: This enzyme plays a crucial role in the cross-linking of collagen and elastin fibers in connective tissues. Copper ions in lysyl oxidase facilitate the formation of covalent bonds between lysine and hydroxylysine residues, contributing to the structural integrity of tissues.

Dopamine β-monooxygenase: This enzyme is involved in the synthesis of neurotransmitters like norepinephrine from dopamine. Copper ions assist in the hydroxylation of dopamine, a critical step in neurotransmitter biosynthesis.

Superoxide dimutases

Superoxide dimutases exist either as mono nuclear or dinuclear metalloenzymes. The X-ray crystal structures of mononuclear compounds are similar and show the metal to be four-coordinated, to three histidine residues and one tyrosine residue.





M = Fe, Mn

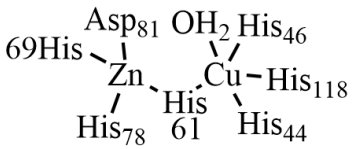
They have distorted trigonal bipyramidal geometry with one apical site left empty. In iron enzyme, the apical position is empty; and for manganous enzyme, it is occupied with water (e.g. Thermus thermophilus)

The mechanism of action of mononuclear SOD is not clear, but for the iron system the following sequence could occur.

$O_2^- + LFe(III) \rightarrow LFe(II) + O_2$

$O_2^- + LFe(III) \rightarrow LFe(III) + H_2O_2$

The structure of Cu-Zn binuclear BESOD shows that the coordination at the Cu(II) is distorted square pyramidal with three histidines, a water molecule and a bridging imidazolate residue from His-61 which is shared with zinc. The zinc is tetrahedral, the coordination being completed by an additional two histidines and an aspartate residue. The Cu(II) – Zn separation is 5.4 Å



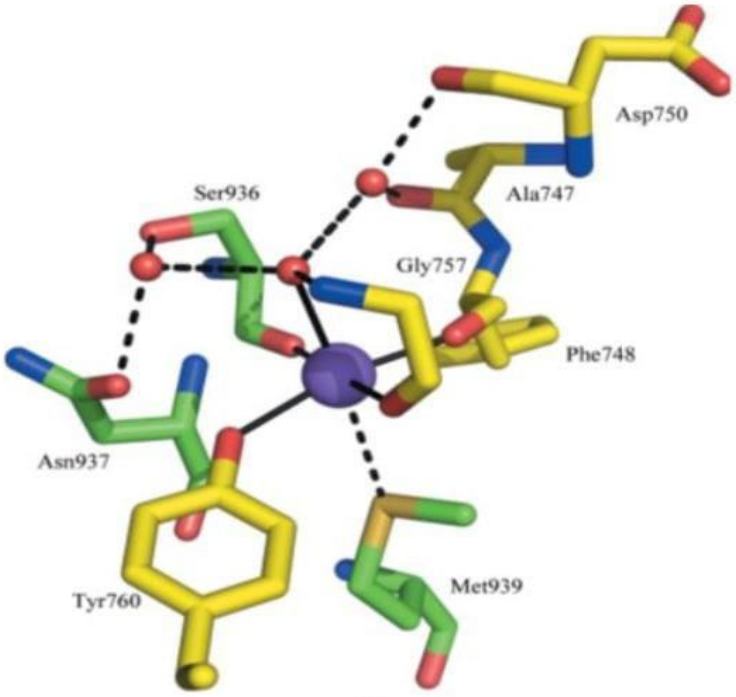
A proposed mechanism for the enzymatic activity of BESOD requires that the superoxide replaces the water molecule on the copper (II). The bounded superoxide reduces the Cu(II) to Cu(I)

Copper Enzyme

Copper is one of the translation elements frequently found at the active site of proteins. The copper conta111mg enzymes and proteins constitute an important class of biologically active compounds.The biological functions of copper enzymes include electron transfer,dioxygen transfort,oxygenation.

CERULOPLASMIN

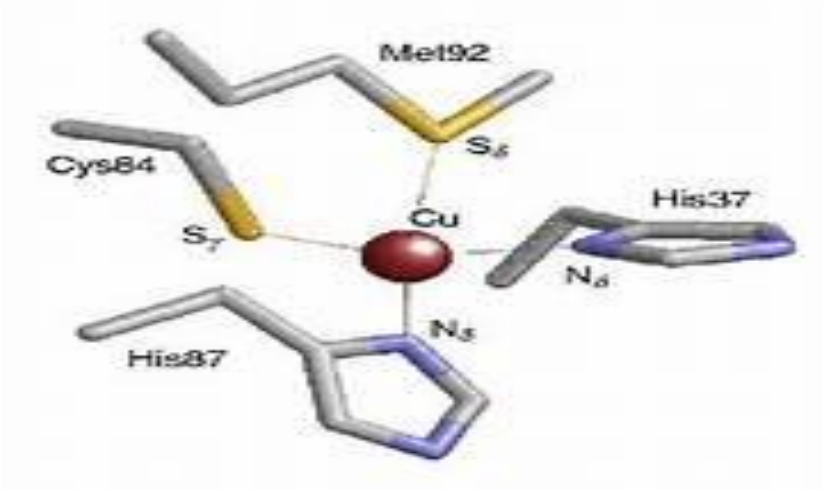
- Ceruloplasmin is a protein made in your liver.It stores and carries the mineral copper around your body.Ceruloplasmin carries 65% to 90% of the copper found in blood.copper is vital many processes in your body.These include building strong bones and melanin.But having too much copper in your body can be toxic.
- Your liver normally takes copper from your blood stream and puts it into ceruloplasmin proteins.The ceruloplasmin is then released into blood plasma.Ceruloplasmin carries copper around your body to the tissues that need it.
- Copper is mainly distributed in liver ,kidneys and intestines.Copper is bound by albumin and histinide and is transported in liver and is transfered to ceruloplasmin.This ceruloplasmin is the major transporter of copper in the human body.



Plastocyanin

1. Plastocyanin is a copper-containing protein involved in electron-transfer. The protein is monomer, with a molecular weight around 10,000 Daltons, and 99 amino acids in most vascular plants
2. It is a member of the plastocyanin family of copper-binding proteins. It carries electrons at one point in the electron transport chain in its reduced form, it gives electrons directly to the systems that reduce nitrate and sulfate and via NADPH to the system that reduces carbon dioxide.
3. Plastocyanin molecules are water soluble and can move through the inner space of the thylakoids.

Structure of Plastocyanin



Plastocyanin was the first of the blue copper proteins to be characterized by X-ray crystallography. The tertiary structure is a beta-barrel — common in proteins which bind to other proteins. The geometry of the copper binding site is described as a 'distorted trigonal pyramidal.

The trigonal plane of the pyramidal base is composed of two nitrogen atoms (N1 & N2) from separate histidine residues and a sulfur atom (S1) from a cysteine residue.

A second sulfur atom (S2) from an axial methionine residue forms the apex. The distortion occurs in the bond lengths between the copper atom and sulfur ligands.

The Cu-S1 contact is much shorter (207 picometers) than Cu-S2 (282 picometers). The elongated Cu-S2 bonding destabilizes the Cu II form and increases the redox potential or the protein.

Metals induces oxidative stress in aquatic organisms – by producing ROS – by impairing antioxidant defences

Metal-binding proteins → ferritin, ceruloplasmin, metallothioneins play major role in oxidative defence

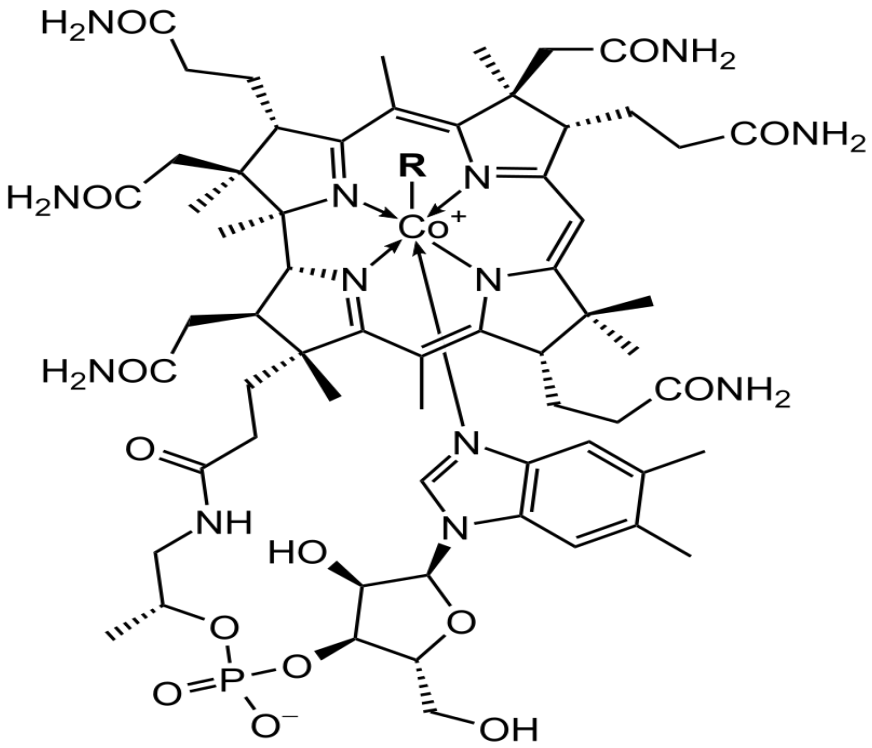
Cp acts as an antioxidant in plasma through its ferroxidase activity θ Ceruloplasmin expression - highly modulated by copper exposure

Pre-exposure to copper -- ↑ Cp activity in fish serum

Vitamin B12

- **Vitamin B_{12}**, a complex water-soluble organic compound that is essential to a number of microorganisms and animals, including humans.
- Vitamin B_{12} aids in the development of red blood cells in higher animals.
- The vitamin, which is unique in that it contains a metallic ion, cobalt, has a complex chemical structure
- Vitamin B_{12} is a coordination complex of cobalt, which occupies the center of a corrin ligand and is further bound to a benzimidazole ligand and adenosyl group.

- The most common cause of vitamin B_{12} deficiency in developed countries is impaired absorption due to a loss of gastric intrinsic factor (IF) which must be bound to a food-source of B_{12} in order for absorption to occur.
- A second major cause is an age-related decline in stomach acid production (achlorhydria), because acid exposure frees protein-bound Vitamin.
- For the same reason, people on long-term antacid therapy, using proton-pump inhibitors, H_2 blockers or other antacids are at increased risk.
- Vitamin B_{12} was discovered as a result of pernicious anemia, an autoimmune disorder in which the blood has a lower than normal number of red blood cells, due to a deficiency of vitamin B_{12}.
- The ability to absorb the vitamin declines with age, especially in people over 60.



R = 5'-deoxyadenosyl, CH_3, OH, CN

- cyanocobalamin, the adenosyl ligand in vitamin B_{12} is replaced by cyanide.
- hydroxocobalamin, the adenosyl ligand in vitamin B_{12} is replaced by hydroxide.
- methyl cobalamin, the adenosyl ligand in vitamin B_{12} is replaced by methyl.

Synthesis of Vitamin B12

- Vitamin B_{12} is synthesized by microorganisms that occur in the rumen (the first stomach chamber) of cows and sheep.
- From the rumen it is transferred to the muscle and other tissues, which other animals and humans eat. Good dietary sources of vitamin B_{12} are eggs, meat, and dairy products.
- Because vitamin B_{12} is found in animal but not vegetable foods, strict vegetarians (vegans) who do not eat dairy products, meats, fish, eggs, or vitamin B_{12}-fortified foods may develop a deficiency if they do not receive supplements of the vitamin.

Symptoms of Vitamin B12

- Weakness, tiredness**,** Nerve problems
- Heart palpitations and shortness of breath
- Pale skin, smooth tongue

- Constipation, diarrhea, loss of appetite
- Vision loss

Deficiency of Vitamin B12

- Pernicious anaemia
- Gastritis
- Surgery & Alcohol use disorder
- Transcobalamin II Deficiency

UNIT-2

Oxygen carriers

Oxygen carriers are special types of metalloproteins which have a transition metal from 3d series bound to a protein. Only those transition metals can form dioxygen complexes with O_2 which have vacant site/s for binding and exist in higher oxidation states also.

$nML + O_2 \rightleftharpoons MLn\ O_2$

In the above ML acts as a dioxygen carrier. In order for transition metal complexes to form dioxygen complexes there must be a vacant site (or sites) for binding and an accessible higher oxidation state (or states).

There are three known classes of dioxygen transport proteins. These are:

1. Hemocyanins
2. Hemerythrins
3. The Hemoglobin- Myoglobin family

HEMOGLOBIN

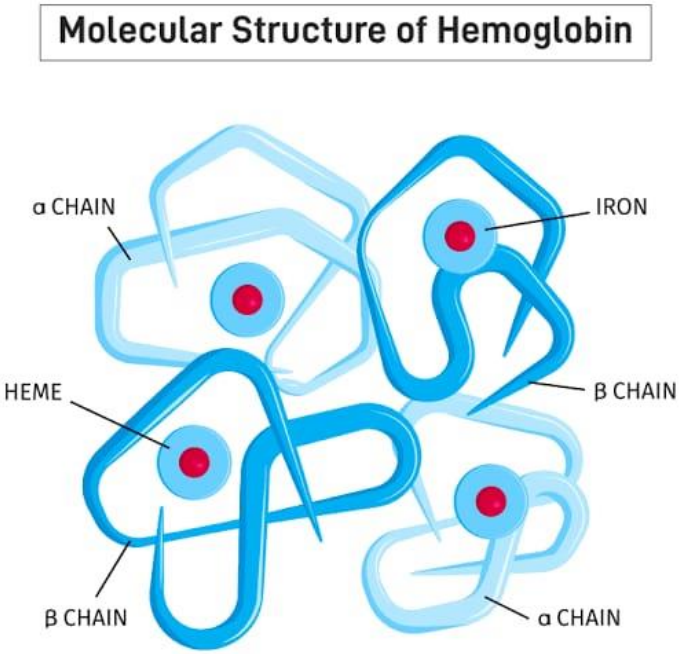
- Hemoglobin is a globular, iron containing metalloprotein with a quaternary structure
- It is found in red blood cells (RBC) of mammals and other animals.
- It transports dioxygen from the lungs to the tissues, where it is used to oxidize glucose.
- This process serves as a source of energy required for cellular metabolic processes.
- Hemoglobin also plays a role in transport of carbon dioxide and hydrogen ions.

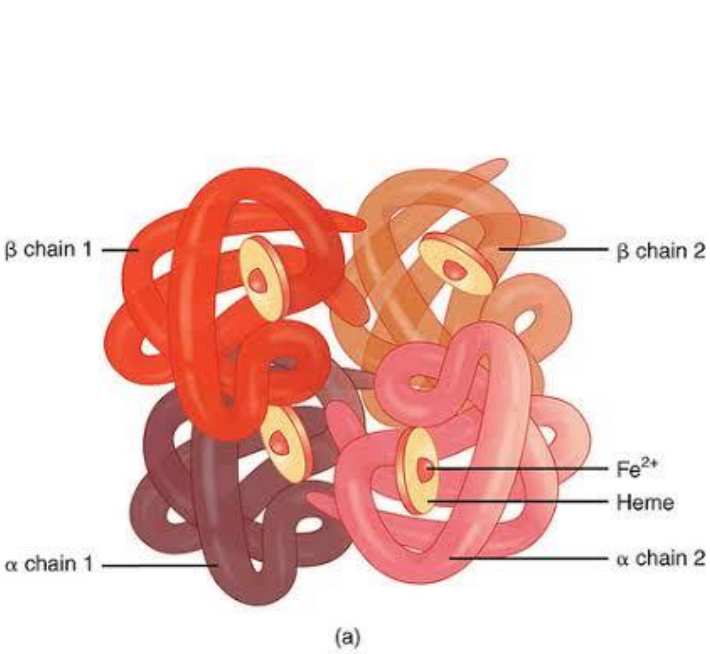
- Synthesis occurs in the developing RBCs in the bone marrow.
- It is the first protein to have been crystallized (1849).
- The first protein with a recognized physiological purpose (O_2 transport, 1864; CO_2 transport, 1904).
- One of the first proteins to have its molecular weight and primary sequences
- established (1930).
- One of the first proteins to have its tertiary and quaternary structures determined by Xray crystallography (1960)
- Hemoglobin levels are measured in **grams per deciliter (g/dL)** of blood.
- Males typically have higher levels than females.
- denoted as Hb or Hgb
- A healthy human has 12 to 20 grams of hemoglobin in every 100 mL of blood.
- Hemoglobin can bind and transport up to four oxygen molecules.

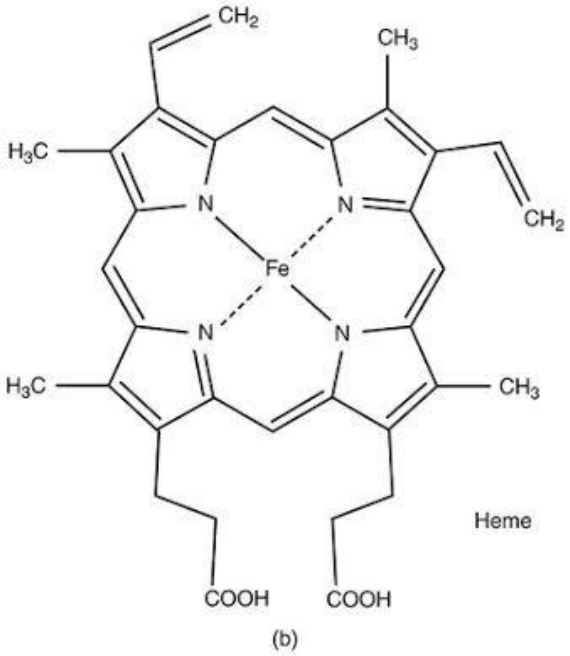
In addition to oxygen, haemoglobin also transports other gases. It carries some of the body's respiratory carbon dioxide (about 20–25% of the total) as carbaminohaemoglobin, where CO_2 binds to the heme protein[1]. Haemoglobin also carries the important regulatory molecule nitric oxide bound to a thiol group in the globin protein, releasing it at the same time as oxygen.

Abnormal levels of haemoglobin can be a sign of several health problems. If your haemoglobin levels are low, your cells might not get enough oxygen, which can result in you frequently feeling tired, weak, or dizzy. High levels of haemoglobin can also be a sign of a serious health condition.

STRUCTURE


Molecular Structure of Hemoglobin
α CHAIN
IRON
HEME
β CHAIN
β CHAIN
α CHAIN


β chain 1
β chain 2
Fe2+
Heme
α chain 1
α chain 2
(a)


CH2
CH3
H3C
N
N
CH2
Fe
N
N
H3C
CH3
Heme
COOH
COOH
(b)

- The structure of hemoglobin was solved by groups of Andrew Kendrew and Max Perutz. It is a tetramer with four subunits
- Hemoglobin is a complex protein that has a quaternary structure. It is composed of four subunits, each containing a polypeptide chain (globin protein) attached to a prosthetic heme group. Each heme group contains an iron atom at its center, surrounded by a complex of four nitrogen atoms.
- The four subunits of hemoglobin are made up of two alpha chains and two beta chains. The interactions between these subunits in the hemoglobin molecule is called cooperativity. This structure allows hemoglobin to bind and transport up to four oxygen molecules.
- The sequence of amino acids in the polypeptide chains of hemoglobin varies in different species. The heme part of hemoglobin is synthesized in the mitochondria and cytoplasm of immature red blood cells, while the globin protein is synthesized in the cytoplasm by ribosomes

STRUCTURE AND MECHANISM OF MYOGLOBIN

BACKGROUNDL;

1. Myoglobin, the first protein whose structure was determined by X-ray crystallography, is a small protein with relatively simple oxygen-binding behavior.
2. Its X-ray structure was determined by John Kendrew in 1959.

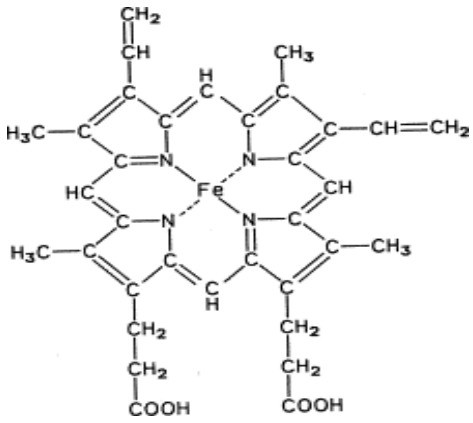
MYOGLOBIN;

- Myoglobin (symbol Mb or MB) is an iron- and oxygen-binding protein .
- Found - cardiac and skeletal muscle tissue of vertebrates in all mammals.
- High concentrations of myoglobin in muscle cells allow organisms to hold their breath for a longer period of time.
- Diving mammals – whales, seals have muscles with particularly high abundance of myoglobin.

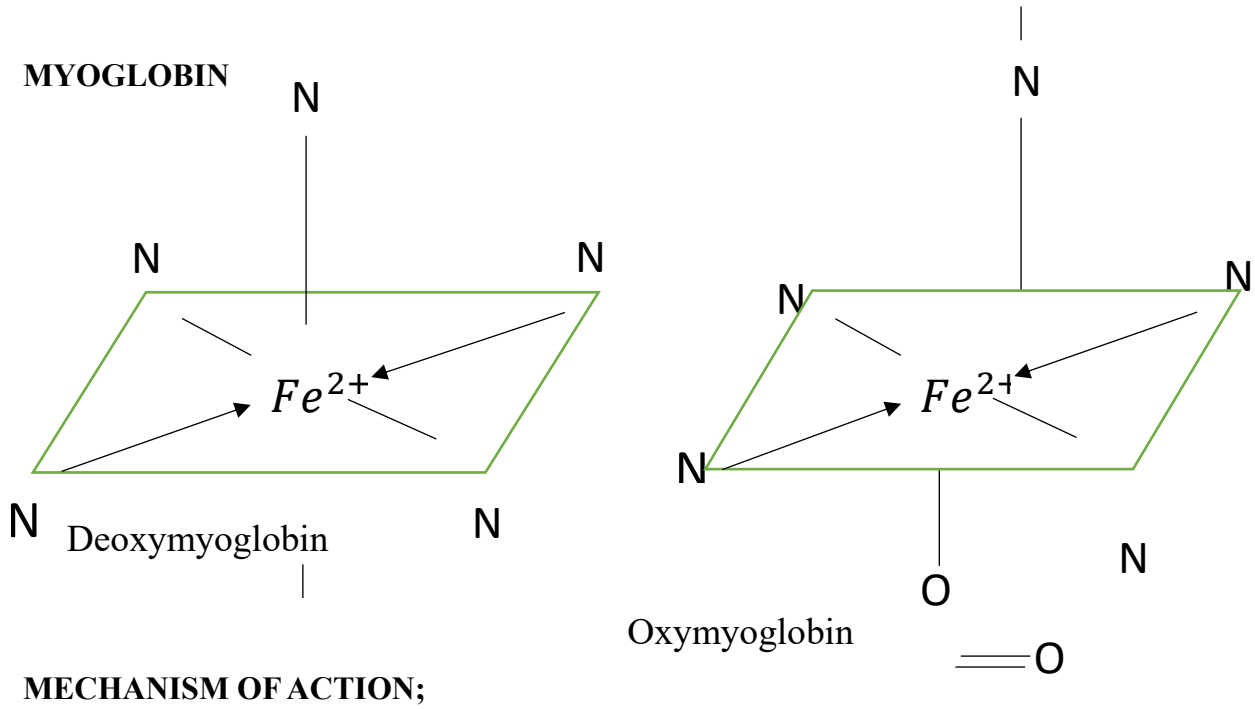
STRUCTURE OF MYOGLOBIN;

- Myoglobin is a heme protein containing one heme group and one globin polypeptide chain.
- It is present in skeletal muscle. The globin polypeptide chain consists of 153 amino acids.

- It is a globular protein with 45x35x25A⁰ dimensions. The heme group of myoglobin is similar to that of hemoglobin.
- The porphyrin nitrogen atoms bind iron (Fe^{+2}) through four of its six coordination positions.
- The proximal histidine is bound to the iron atom through its fifth coordination position.
- The sixth position of the iron atom is vacant in deoxymyoglobin or occupied by O^2 in oxymyoglobin or occupied by H_2O in ferrimyoglobin (Fe^{3+}).
- Myoglobin resembles one submit of hemoglobin.



MYOGLOBIN



MECHANISM OF ACTION;

Myoglobin function as a reservoir for oxygen. It has greater affinity for O_2 than hemoglobin. Therefore, in the tissue's myoglobin takes up oxygen from hemoglobin.

Hb $(O_2)_4$ + 4Mb $\rightleftharpoons$ 4MbO_2 + Hb

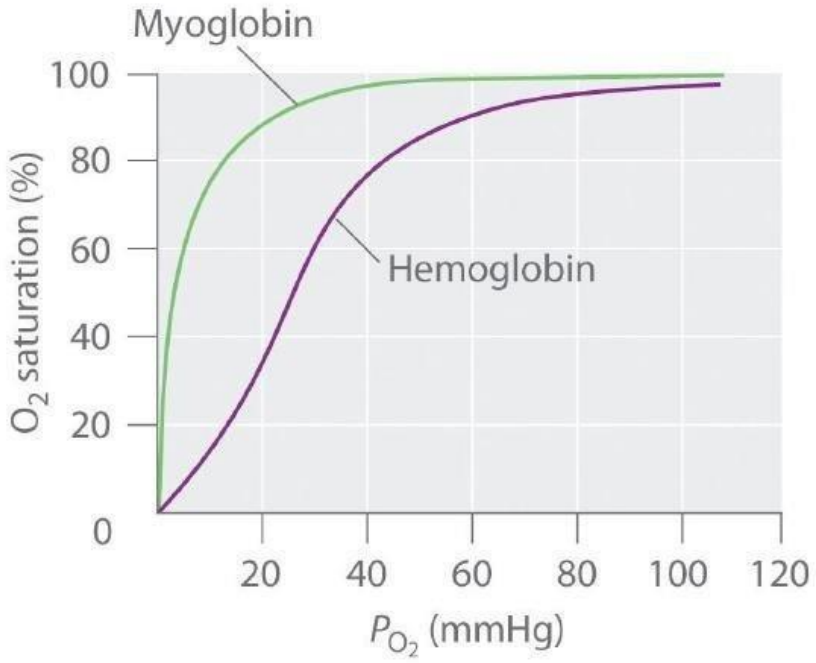
Hemoglobin myoglobin

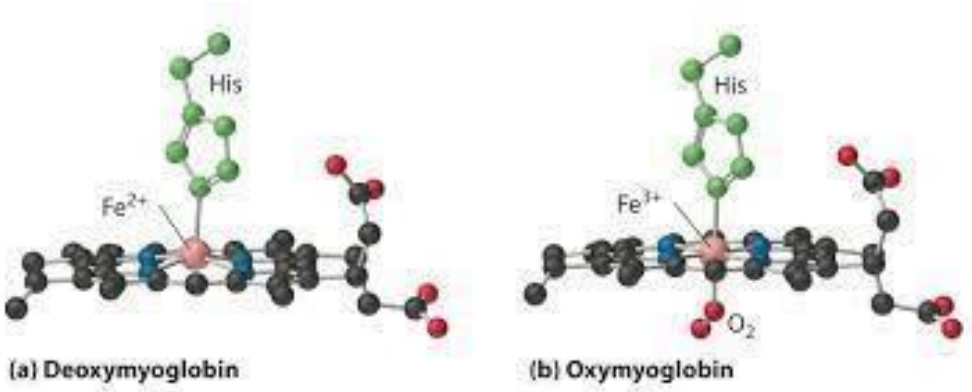
- Oxygen dissociation curve of myoglobin is "hyperbolic". The equilibrium constants for the formation of oxymyoglobin (KMb) and oxymyoglobin (KHb) may be given as follows;

KMb = [MbO_2] / [Mb] [O_2] KHb = [Hb$(O_2)_4$] / [Hb] [O_2]$^{2.8}$

Mechanism of oxygen binding in myoglobin

- The O_2 binding process by myoglobin is accompanied by a substantial structural change at the iron center:
- Firstly, the radius of the iron atom shrinks considerably so much so that it fits into the plane of the porphyrin rings.
- Secondly, a spin-pairing phenomenon occurs: The five-coordinate ferrous deoxy form(Fe2+) with a high spin is converted into the six coordinate oxy form containing a diamagnetic and low spin Fe^{3+}.





- The oxygen binding process in myoglobin occurs reversibly which may be reflected by the simple equilibrium reaction:

$$Mb + O_2 \; MbO_2 \; Keq = ([Mb]\,[O_2]) / [Mb\text{-}O_2]$$

O_2 dissociation from Mb commonly described by its fractional saturation, YO_2, and $[O_2]$ in partial pressure, pO_2.

$$YO_2 = [Mb\text{-}O_2] / [Mb] + [Mb\text{-}O_2]$$

- Substitution from the equilibrium expression gives: **$YO_2 = ([pO_2] / [p1/2 + (pO_2)]$**
- Thus, as described by this equation, the O_2 binding curve for myoglobin follows a hyperbolic pathway. When the degree of saturation of myoglobin with oxygen is plotted against oxygen pressure, a steady rise is observed until complete saturation is approached and the curve levels off.

BOHR'S EFFECT

- Bohr's effect tells us about the dissociation of oxygen from oxyhaemoglobin from the RBC and the oxygen is transferred to the tissue.
- In this first, the oxygen is released from the lungs at high pressure and it is diffuse into the blood vessels.

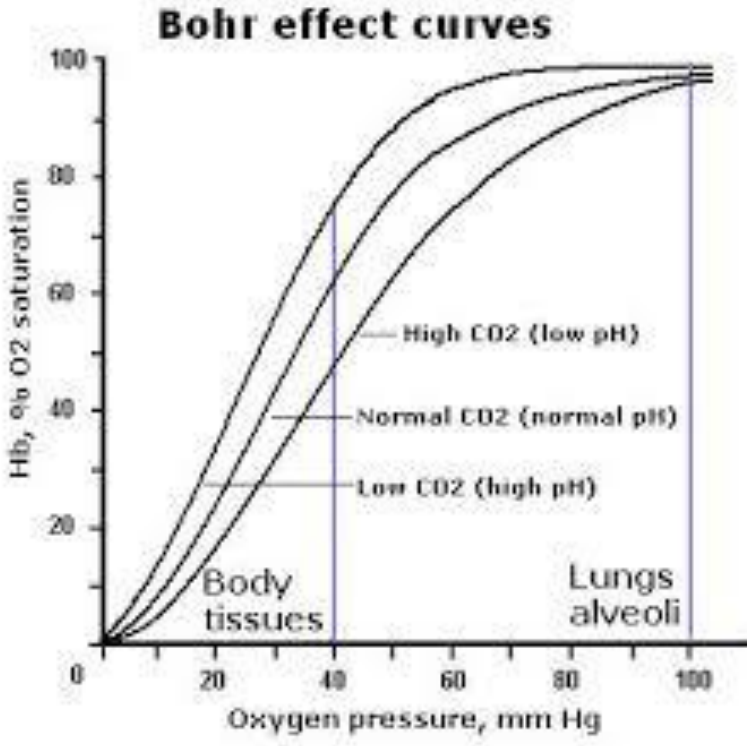
- Further, it is combines with Hb of RBC to form oxyhemoglobin
  - In this, tissue is in working or metabolic so its produce large amount of CO2 and it is diffuse into the plasma of blood vessels and its combines with water to form carbonic acid which is weak acid and its readily dissociates into H+ and HCO_3^-.

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

  - In the tissues, the glucose undergoes glycolysis to form 2,3- diphosphoglycerate, it is responsible for effect of dissociation of oxygen from oxyhemoglobin.
  - The CO_2 produced from the tissues and it is bind to hemoglobin and produce carbamoyl hemoglobin and the oxygen is transferred to tissues.
  - The binding of oxygen to hemoglobin decreases with low PH and the hemoglobin is exposed to increased partial pressure of CO_2.
  - Bohr effect causes a shift in the oxygen dissociation curve to right.
  - The binding of oxygen to hemoglobin increases with high PH and the haemoglobin is exposed to decreased partial pressure of CO_2.
  - Thus, Bohr effect causes a shift in the oxygen dissociation curve to left.

FACTORS:

- The factors which are responsible for dissociation of O_2 from oxyhaemoglobin as we know the tissue is working continuously so producing;
- CO_2- partial pressure of CO_2.
- Temperature or heat is increasing.
- 2,3- diphosphoglycerate – a product from glycolysis.
- PH is low.

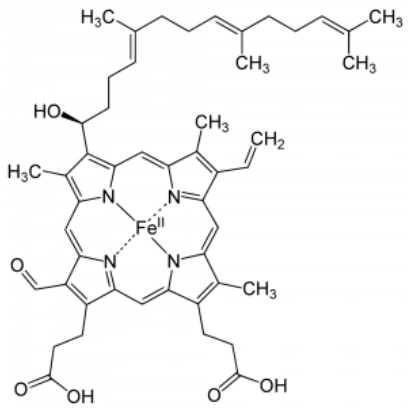


CYTOCHROMES

Cytochromes are redox-active proteins containing a heme, with a central iron (Fe) atom at its core, as a cofactor. Cytochromes are proteins containing one or more heme cofactors.

The cytochromes are the most thoroughly characterized class and were among the first to be identified in cellular extracts due to their unique optical properties and prominent intense absorption in the region of 410-430 nm known as the Soret band.

Structure:



Functions:

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

Types of Cytochromes:

There are three most commonly encountered types of heme:

1. heme a, possesses a long phytyl "tail" and is found in cytochrome c oxidase

2. heme b is found in b-type cytochromes and globin's.

3. heme c is covalently bound to c-type cytochromes via two thioether linkages.

Cytochrome a - Cytochromes are redox-active proteins containing a heme, with a central iron (Fe) atom at its core, as a cofactor. They are involved in the electron transport chain and redox catalysis.

Cytochrome b - In plant chloroplasts and cyanobacteria, there is an homologous protein, cytochrome b6. These complexes are involved in electron transport, the pumping of protons to create a proton-motive force (PMF). This proton gradient is used for the generation of ATP.

Cytochrome c - Cytochromes *c* cytochromes, or heme-containing proteins, that have heme C covalently attached to the peptide backbone via one or two thioether bonds.

Cytochrome P450:

The cytochrome P450 is a superfamily of mono oxygenases Heme containing enzymes OR hemoproteins. It is a large and diverse group of enzymes that catalyze the oxidation of organic substances.

They absorb light at a wavelength of 450 nm. The substrates of CYP enzymes include

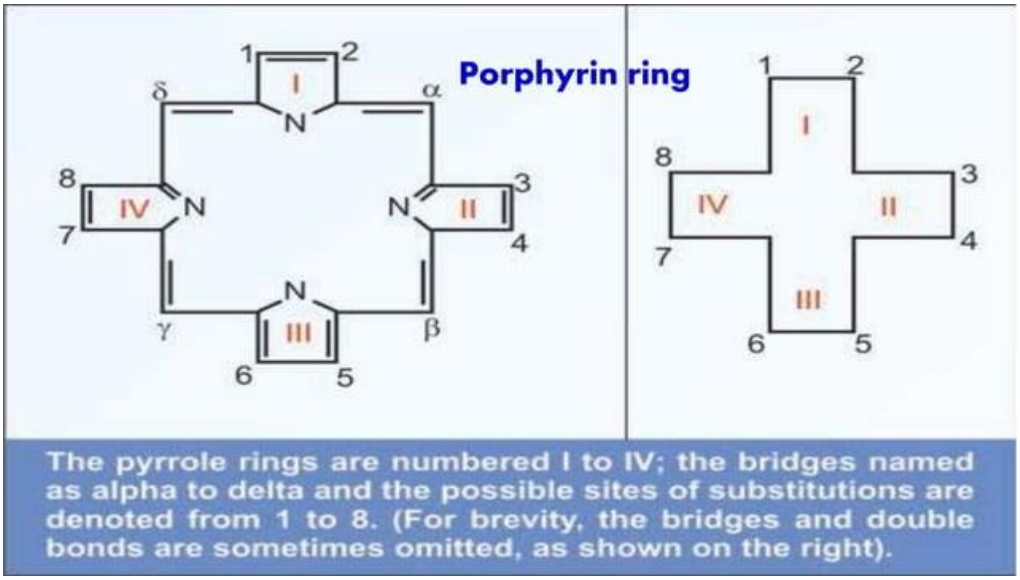
1. CYPs are the major enzymes involved in drug lipids and steroidal hormones

2. Xenobiotics such as drugs and toxic chemicals

Metabolism and bioactivation, accounting for about 75% of the total number of different metabolic reactions

TYPE OF HEME

- Heme is propyhyrin compound
- Porphyrins are cyclic compound formed by fusion of 4 pyrrole ring linked by methynyl [=CH-] bridges
- Since of atom and iron is present , heme is ferroprotoporphyrine
- Pyrrole ring are named as I, II,III,IV and the bridges as alpha beta gamma and delta



Types of heme

- ❖ Heme a
- ❖ Heme b
- ❖ Heme c
- ❖ Heme d

Heme a

- Heme a is made up of propyhyrin iron and atom
- Heme a differ from heme b because formyl group present in the heme a at a ring position 8 is replaced methyl group in Heme b
- Hydroxy ethyl for nesyl group heme a in replace by vinyl group in Heme b
- Heme a work in the electron transport chain as per of cytochrome c

Heme b

- Is the most abundant heme
- Is present in hemoglobin and myoglobin of human blood

Heme c

- Heme c is differs from Heme b
- Thioether linkage in heme c with the a aproprotein
- Due to the present of Thioether linkage heme c has difficulty in dissociating from holoprotein as in cytochrome c & help cytochrome c in its function
- Heme c plays a crucial role in apoptosis leading to cell destruction

Heme d

- Heme d is another form of heme B
- Instead, the hydroxylated propionic acid side chain forms a gamma – spirolactone group
- Heme d reduces oxygen of bacteria present in water with a low oxygen tension

CYTOCHROME P450

Cytochrome

The cytochrome are the most thoroughly characterized class extracts due to their unique optical properties and prominent intense absorption in the region of 410- 430nm known as the soret band.

By definition, cytochromes are proteins containing one or more heme cofactors. These proteins generally classified on the basis of heme type.

Classification of Cytochrome

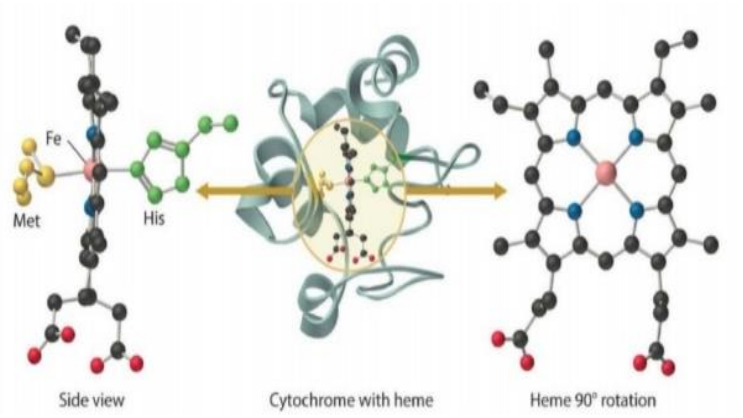
There are three most commonly encountered types of heme:

1. Heme a possesses a long phytyl “tail” and is

found in cytochrome c oxidase.

2. heme b is found in b- type cytochromes and globin’s.

3. heme c is covalently bound to c-type cytochromes via two thioether linkages.



Cytochrome P450

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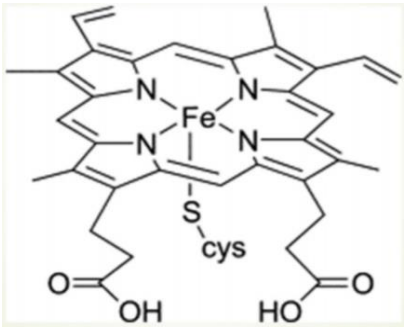
STRUCTURE OF CYTOCHROMES P450

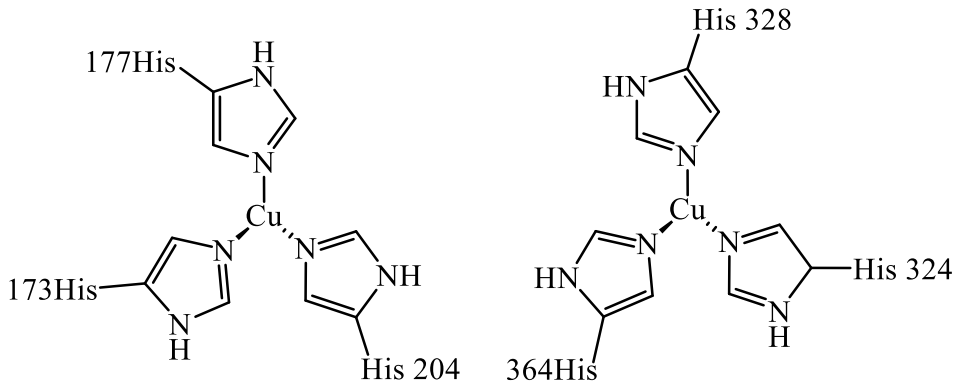


Haemocyanins

- Hemocyanins (or haemocyanins) are oxygen carrying proteins/oxygen carriers in invertebrates such as molluscs (eg. octopus, snails, squids) and arthropods (eg. scorpions, crabs, lobsters etc.).
- It is extracellular protein and is present in hemolymph.

Active site structure of deoxyhemocyanin

- Hemocyanin is a copper containing metalloprotein.
- Each monomer contains two cuprous ions [Cu(I)] that reversibly bind one dioxygen.
- An empty cavity is present between the two cuprous ions to accommodate the dioxygen.
- The Cu(I)- Cu(I) bond distance is 460 pm.
- The coordination number of each Cu(I) is three and is satisfied by three histidines residues from the protein.
- This results in a distorted trigonal pyramidal geometry.
- Two phenylalanine residues which are in close proximity to the histidines residues provide a hydrophobic environment at the active site.



Active site structure of oxyhemocyanin

The binding of dioxygen to the Cu(I) at the active site leads to the following changes:

- Cu(I) is oxidized to Cu(II).
- O_2 is reduced to O_2^{2-}.

• Colour of protein changes from colorless to blue.

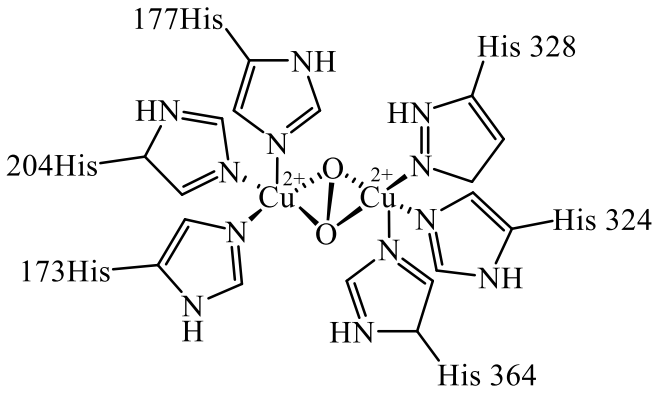
• Coordination number of copper changes to five from three.

• Geometry of copper changes to square pyramidal from trigonal pyramidal.

• The equatorial plane has two histidyl imidazole nitrogens, the bound oxygens and the third histidyl nitrogen is axially coordinated to copper.

• The Cu-Cu distance decreases to 360pm.

• In hemocyanin oxidative addition of dioxygen occurs.

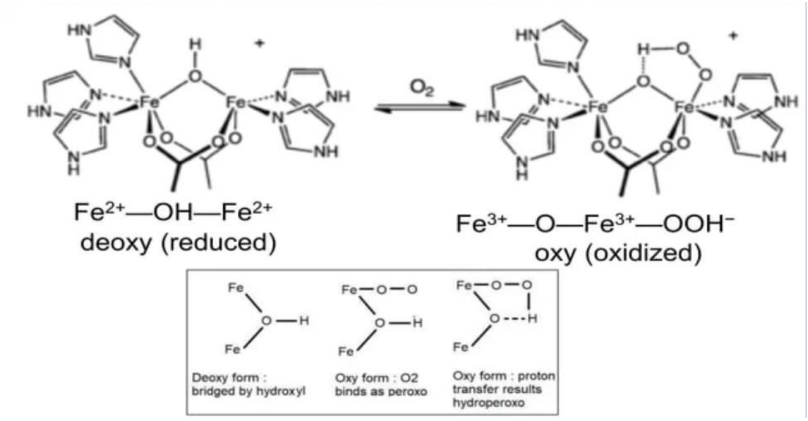


HEAMERYTHRINS (Hr):-

- In invertebrate animals' oxygen carrying proteins are often non-heme pigments, hemerythrin and hemocyanin in place of the familiar hemoglobin.
- Both hemoglobin and hemerythrin have iron as the oxygen carrying metal.
- Hemerythrin (as well as hemocyanin), it does not contain a heme group.
- Hemerythrin combines with molecular oxygen in a ratio 2Fe: O2 rather than Fe: O2 found in hemoglobin.
- In spite of these differences both hemerythrin and hemoglobin function effectively as oxygen carriers.
- Hemerythrin is found in four different invertebrate phyla: sipunculids, poly-chalets, priapulids and bachiopods.

]

O_2 Binding:



STRUCTURE: -

Active site structure of Deoxy hemerythrin

- Each monomeric unit contains an active site which has two high spin ferrous ions [Fe (II)].
- The ferrous ions are bridged together by a hydroxyl group and two carboxyl groups from an aspartate residue and a glutamate residue of the protein chain.
- One of the ferrous is hexacoordinated with an octahedral geometry and the other is penta coordinated with a distorted trigonal bipyramidal geometry.
- The remaining coordination sites of hexacoordinated ferrous and penta coordinated ferrous are satisfied by three and two imidazole nitrogens respectively from histidine residues of the protein chain.
- The hydroxyl group serves as a bridging ligand but also functions as a proton donor to the O_2 substrate.

Active site structure of Oxyhaemerythrine

- One monomeric unit of hemerythrin binds one dioxygen.
- The dioxygen adds only to the coordinatively unsaturated ferrous.
- The dioxygen adds to hemerythrin in an oxidative manner resulting in the formation of two Fe (III) centers and peroxide (O_2).

- The oxidative addition is followed by the shifting of proton from the bridged OH to the bound peroxide resulting in the formation of hydroperoxo (HO^{2-}) group.
- This proton-transfer result in the formation of a single oxygenatom (u-oxo) bridge in oxyhemerythrin.
- The hydroperoxo group is hydrogen bonded
- with the p-oxo group.

Functions:

- Hemerythrin is a nonheme, dioxygen binding pigment. ▸. Hemerythrin contains iron (II) which binds oxygen reversibly.
- There is an octameric form with molecular weight of about 108,000 that transports dioxygen in the blood.
- There are lower molecular weight monomers, dimers, trimers or tetramers.
- Homerythrin consists of eight subunits very much similar in quaternary structure to myohemerythrin.
- A major difference between the hemoglobins and hemerythrins is in the binding of dioxygen.
- The Oxyhaemerythrin & Deoxyhemerythrin may be oxidised to Fe (II) space.
- Oxyhaemerythrin is dymagnetic to so the spin coupling or the electron on the to Fe (II).

IRON – SULFUR PROTEINS

Electron transfer system can be classified into three types they are iron-sulfur protein, cytochrome and blue-copper.

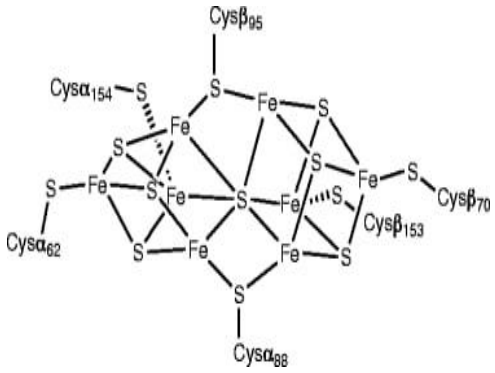
Iron-sulfur proteins can be again classified as two types they are Rubridoxin, Ferredoxin.

Electron transport chain:

- A protein cluster that transfers electron through a membrane within mitochondria to form proteins.
- That derives the production of ATP.
- The cell that uses ATP as energy source for metabolic process and cell functions.

Iron-sulphur proteins:

- A group of proteins only the iron-sulphur (Fe-S) complex as prosthetic group.
- These proteins participate in all major pathways: photosynthesis, respiration, hydroxylation and bacterial hydrogen and nitrogen fixation.
- Iron-sulphur proteins are proteins characterized by the presence of Fe-S clusters containing sulfide linked di, tri and tetra iron centers in variable oxidation states.



Fe-S protein:

- Electron transfer and redox regulation
- Enzyme activation
- DNA damage and repair
- Environmental sensing
- Post-transcriptional gene regulation
- Substrate binding and activation
- Protein stability
- Iron and sulphur storage

Ferredoxin:

- Ferredoxin is an Fe-S containing protein.
- It occurs in bacteria and plants.
- It contains inorganic sulphur.
- Its redox potential is comparatively low.

Rubredoxin:

- Rubredoxin is a Fe containing protein.
- It occurs in bacteria and archaea.
- It contains no inorganic sulphur.

- It‘s redox potential is comparatively high.

RUBREDOXIN

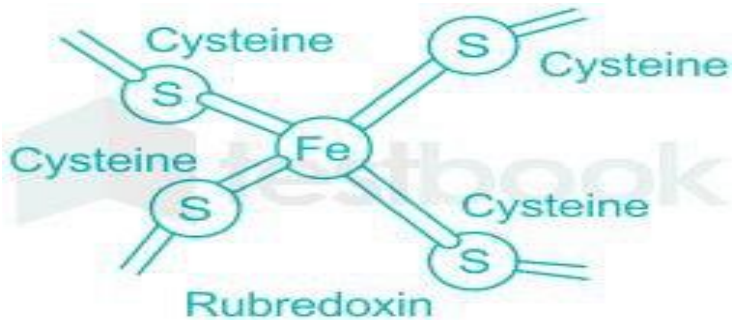
INTRODUCTION

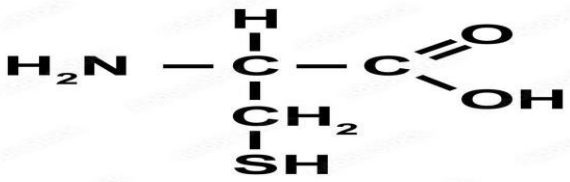
- Rubredoxin is an Iron Sulphur protein.
- Non – heme iron protein.
- Electron transfer agent in plants and bacteria.
- Low reduction potential but oxidize well.
- Involved in Photosynthesis, nitrogen fixation and metabolic oxidation of sugar.
- There are two iron Sulphur protein, they are
- Rubredoxin

1. **Ferredoxin**

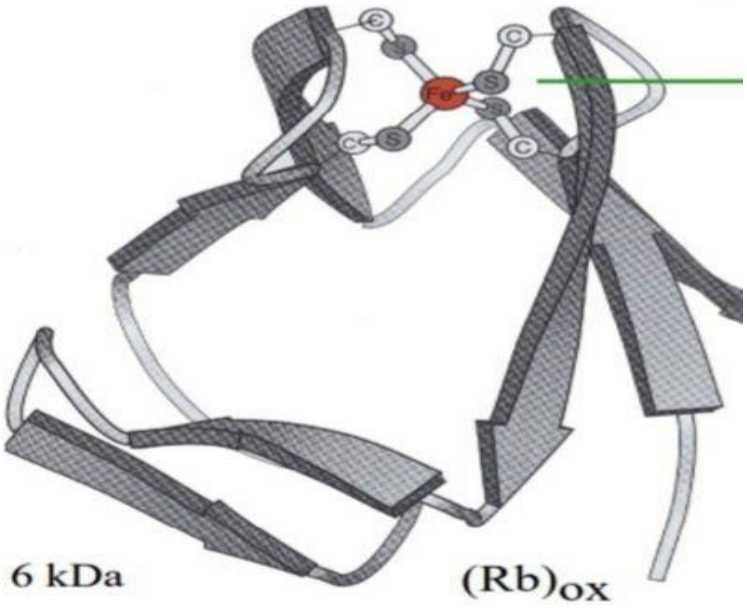
RUBREDOXIN

- Rubredoxin protein belongs to bacterial origin and was first isolated from Clostridium pasteurianum.
- Later found in number of anaerobic bacteria.
- It is also called as 1Fe – 0S cluster.
- It act as a one electron transfer agent.





Cysteine
(Cyc/C)



C. pasteuriabun Rubredoxin
bacteria

- These comprise of one iron atom which will in +3 oxidation state.
- One iron atom is teterahedrally coordinated to four Sulphur atom from four cysteine amino acid.
- The distance between Fe^{3+} - S is 2.28 A.
- The bond angle between S - Fe^{3+} − S is 104 to 1140.
- Sulphur atom is not labile (not S^{2-}), this is the reason for 0S cluster naming.
- The Fe(III) is reduced to Fe(II), where the Fe^{2+} - S distance is increased by 2-3 %.
- Both forms are high spin proved by EPR and Mossbauer spectroscopy with Teterahedral geometry.
- Fe (III) is in reddish in colour due to the Ligand Metal Charge Transfer (LMCT). After it reduced to Fe (II) it is colourless.
- Redox potential range is $-50\ to\ +50$ mV at pH 7.

FUNCTION

- Rubredoxin plays an important role in the reduction of superoxide.
- It acts as an electron carrier in many biochemical process

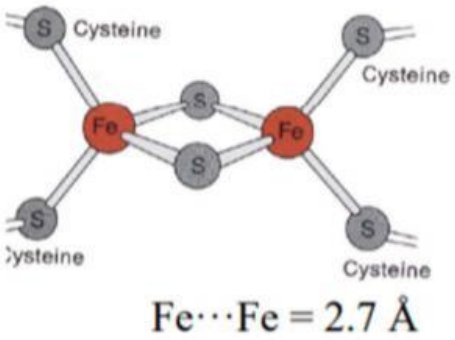
Ferredoxins

Introduction

Ferredoxins (Fed) are small, mostly acidic, soluble proteins found ubiquitously in biological organisms. They possess a highly negative redox potential and use their iron-sulphur cluster to act as electron distributors in various metabolic pathways. Ferredoxins are nonheme-iron-containing proteins and are mainly found in anaerobic bacteria and in chloroplasts (11). The first isolation was from Clostridium pasteurianum and the actual name was introduced about 1962 (63).

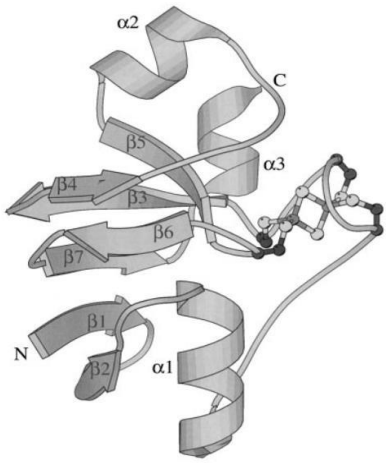
A well-studied example is ferredoxin:thioredoxin reductase (FTR), which can be found in organisms ranging from cyanobacteria to plants. Recently, a team of researchers has examined a ferredoxin:disulfide reductase (FDR) from the methane-producing Archaea Methanosarcina acetivorans.

STRUCTURE

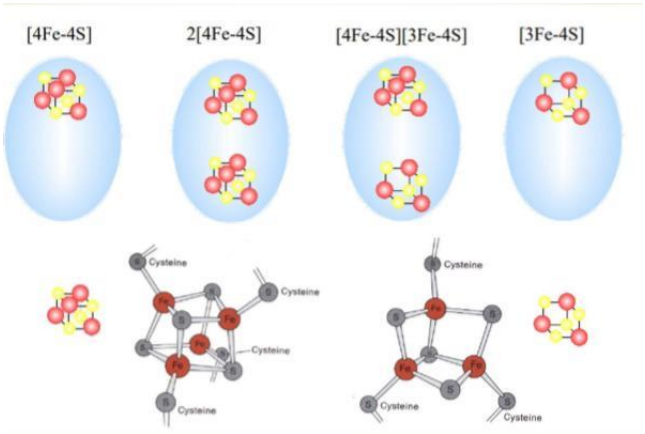


Classification of ferredoxin

The Fed proteins can be classified according to the nature of their iron-sulfur center



([2Fe-2S], [3Fe-4S] and [4Fe-4S]) and the organisms in which they were isolated for the first time. Hence, the ferredoxins with a [2Fe-2S] cluster can be divided into plant-type or bacterial-type Feds.

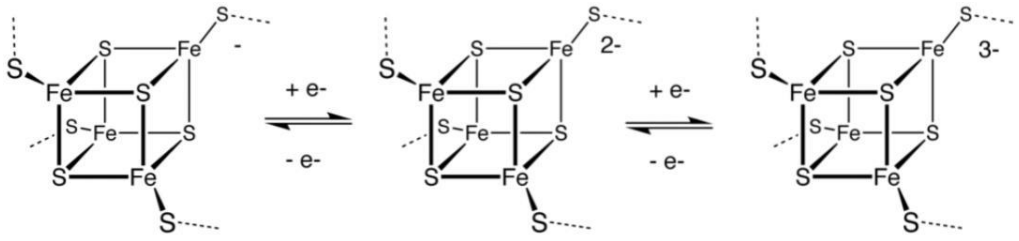


2Fe-2S Ferredoxin

Members of the 2Fe–2S ferredoxin superfamily have a general core structure consisting of beta (2)-alpha-beta (2), which includes putidaredoxin, terpredoxin, and adrenodoxin. They are proteins of around one hundred amino acids with four conserved cysteine residues to which the 2Fe–2S cluster is ligated. This conserved region is also found as a domain in various metabolic enzymes and in multidomain proteins, such as aldehyde oxidoreductase (N-terminal), xanthine oxidase (N-terminal), phthalate dioxygenase reductase (C-terminal).

4Fe-4S Ferredoxin

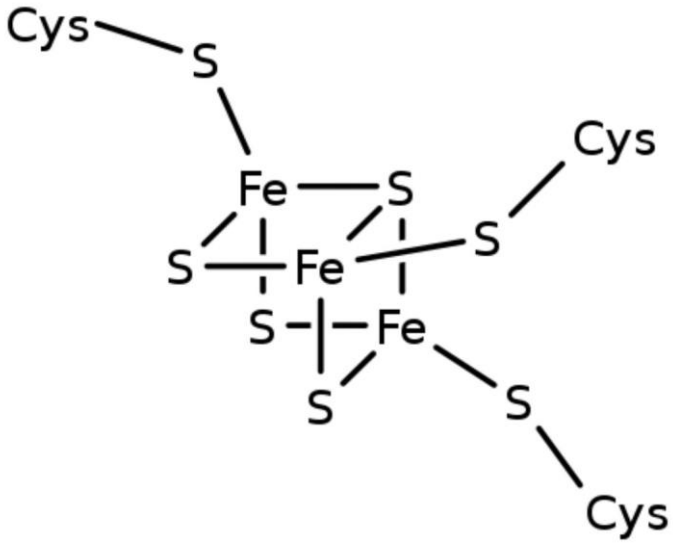
The [Fe4S4] ferredoxins may be further subdivided into low-potential (bacterial-type) and high-potential (HiPIP) ferredoxins. Low- and high-potential ferredoxins are related by the following redox scheme.



3Fe-4S Ferredoxin

During the evolution of bacterial-type ferredoxins, intra sequence gene duplication, transposition and fusion events occurred, resulting in the appearance of proteins with multiple

iron–sulphur centers. In some bacterial ferredoxins, one of the duplicated domains has lost one or more of the four conserved Cys residues. These domains have either lost their iron–sulphur binding property or bind to a [Fe3S4] cluster instead of a [Fe4S4] cluster [31] and dicluster-type.



Function of Ferredoxin

Ferredoxin serves as an electron transfer agent in biological redox processes in this way. Ferredoxin is a protein that facilitates electron transfer events like photosynthesis. Ferredoxin, which facilitates electron transport and comprises an iron-sulphur cluster, is present in chloroplasts.

It is involved in the photosynthesis process where its iron atoms accept or discharge electrons when they are being oxidized or reduced.

In non-cyclic photophosphorylation, ferredoxin is the last electron acceptor thus reducing the enzyme NADP+ reductase. It accepts electrons produced from sunlight-excited chlorophyll and transfers them to the enzyme ferredoxin: NADP+ oxidoreductase EC 1.18.

UNIT 3

NITROGEN FIXATION

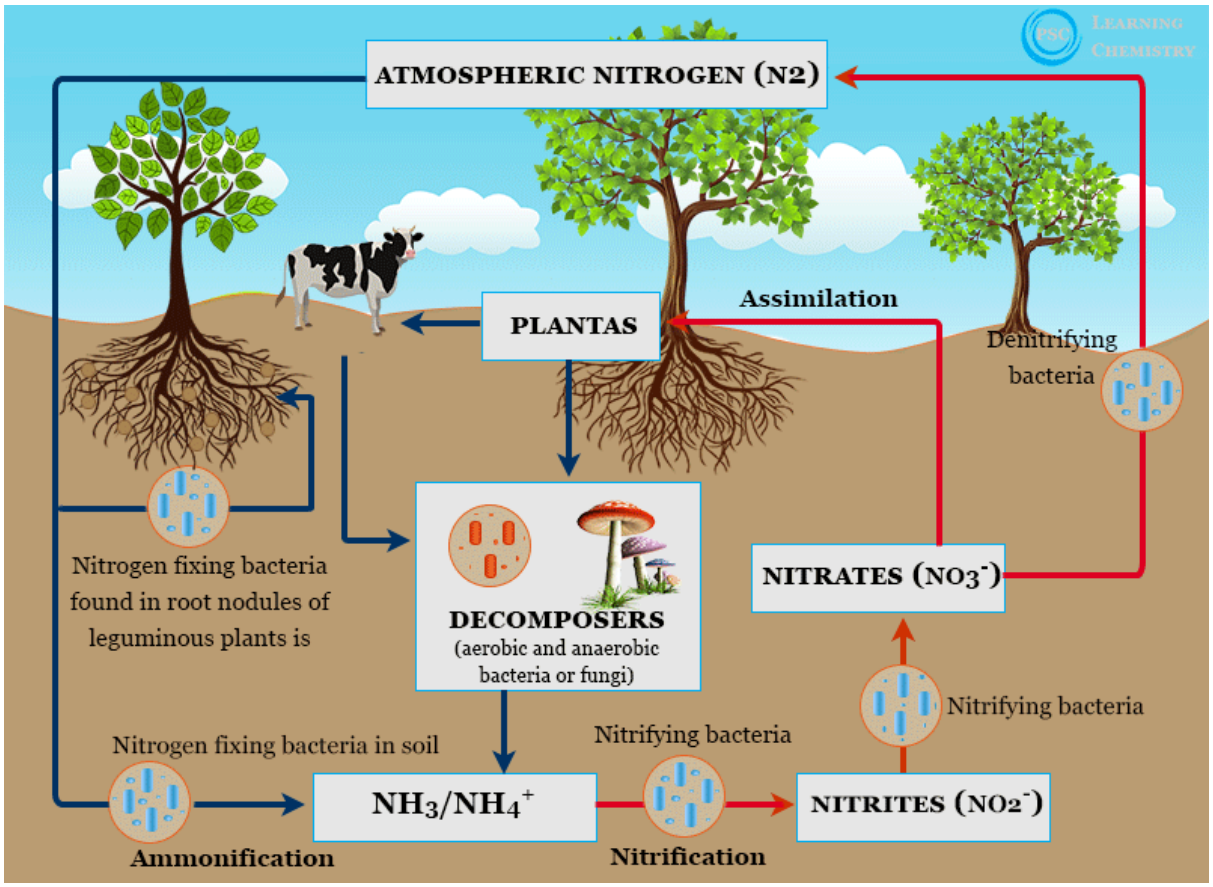
Nitrogen is an essential constituent of all biomolecules both in plants and in animals.

Most of the plants obtain nitrogen from soil in the form of nitrate or ammonium ion, but it is limited.

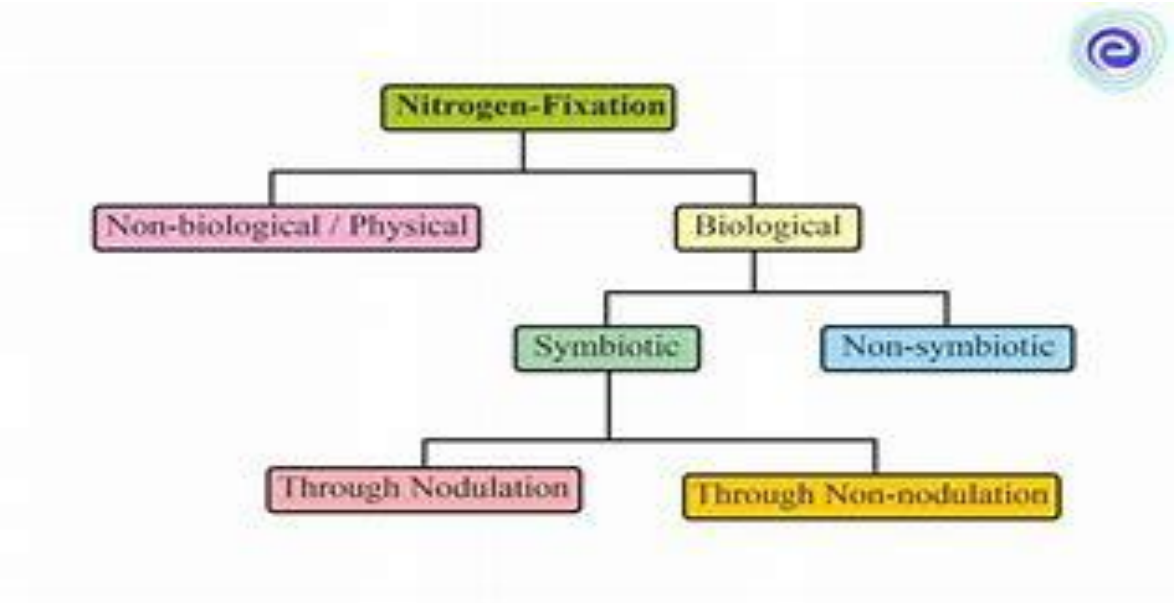
Atmosphere consists 78% of molecular nitrogen but plants unable to convert this molecular nitrogen into a useful form because the lack of nitrogenase.

Only prokaryote species possess this enzyme.

Nitrogen fixation:



The conversion of free nitrogen into nitrogenous salts to make it available for absorption of plants.



Types of nitrogen fixation

1. Non biological fixation
2. Biological fixation

Non biological fixation is classified into atmospheric/physical and Industrial/chemical nitrogen fixation.

Biological fixation in classified into symbiotic and non-symbiotic fixation.

NON-BIOLOGICAL NITROGEN FIXATION

Lightning converts NOx (Nitrogen oxide) to N_2 and O_2. Similarly, non-biological nitrogen fixation NOx reacts with H20 to form Nitrous acid and Nitric acid, steeps into land and make soil fertile.

Lightning + N_2 + O_2 → 2NO

The nitrous oxide formed converted with oxygen to form nitric oxide.

$2NO + O_2 \rightarrow 2NO_2$

Nitric oxide readily dissolves in water to produce nitric and nitrous acids.

$2NO_2 + H_2O \rightarrow HNO_3 + HNO_2$

These acids readily release the hydrogen, forming nitrate and nitrite ions.

The nitrate can be readily utilized by plants and microorganisms.

$HNO^3 \rightarrow H^+ + NO^{3-}$(Nitrate ions)

$HNO^2 \rightarrow H^+ + NO^{2-}$(Nitrite ions)

BIOLOGICAL NITROGEN FIXATION

The biological nitrogen fixation is carried out by some bacteria, cyanobacteria and symbiotic bacteria. Biological nitrogen fixation occurs in the presence of the enzyme nitrogenase which is found inside the nitrogen fixing prokaryote.

NON-SYMBIOTIC NITROGEN FIXATION

Fixation carried by microorganism. ex: aerobic, anaerobic and blue green algae, etc.

The blue green algae fixed atmospheric N_2. This alga contains nitrogen fixing enzymes called as nitrogenase. Nitrogenase enzyme has two proteins. This enzyme converts N_2 into NH_3 and reduces H_2.

$N_2 + 8H^+ + 8e^- \rightarrow 2NH_3 + H_2$

SYMBIOTIC NITROGEN FIXATION

Fixation of the free nitrogen in the soil by microorganism. Ex: Nodule formation, Rhizobium

Rhizobium→ Conversion of nitrogen into nitrate

Processes responsible for nitrogen fixation in Biosphere

Typically, all the nitrogen fixation happening in the biosphere can be attributed to three processes

• Atmospheric fixation

• Industrial fixation

• Biological fixation

Atmospheric Nitrogen Fixation

• Non-biological nitrogen fixation can happen by electric discharge

(lightning) in the troposphere, and by cosmic radiation in the stratosphere.

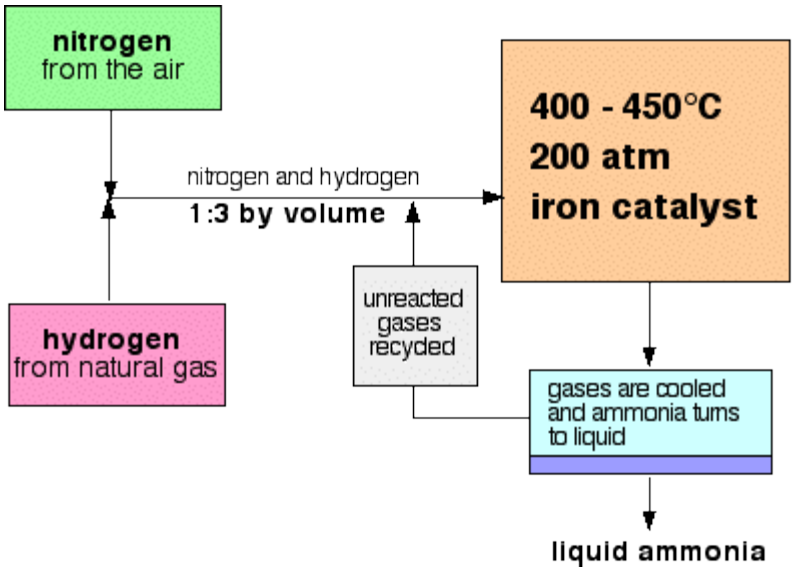
• Equation involved: $N_2 \rightarrow 2N$; $N + O_2 \rightarrow NO + O \rightarrow NOx$

• It accounts for only 10 % of N_2 fixation.

• The high energy of lightening breaks N_2 molecules apart & aids the nitrogen atoms to combine with O_2 forming nitrogen oxides.

• They dissolve in rain water forming nitrates and are carried to the ground with the rain and then plants use nitrates to grow.

Industrial Nitrogen Fixation



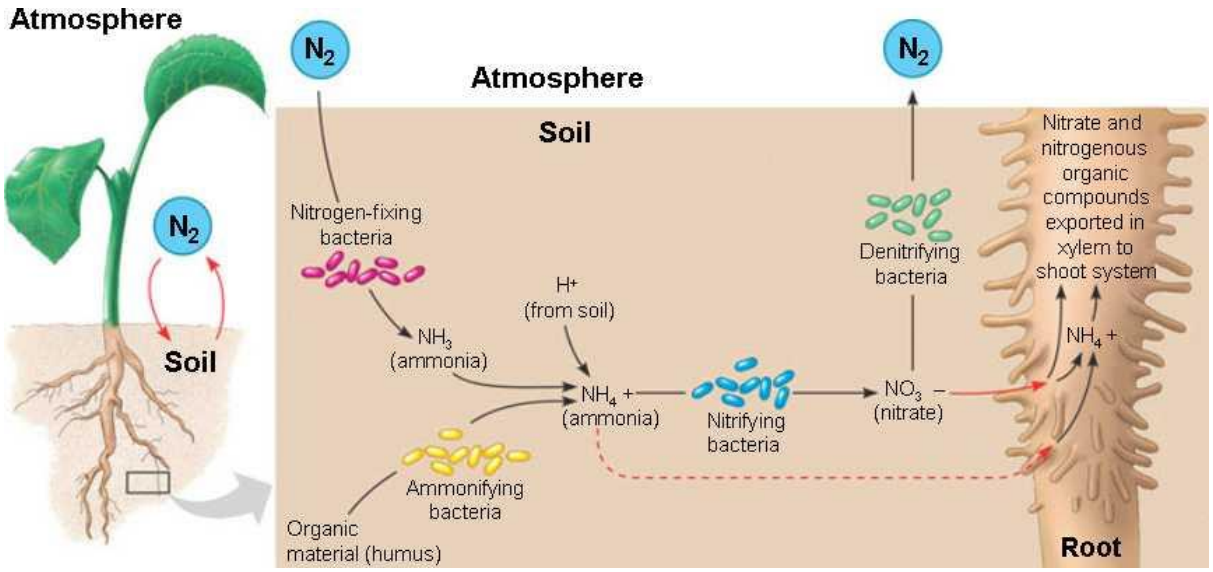
• The Haber-Bosch process: This process directly synthesizes ammonia from nitrogen and hydrogen. In 1909, the German chemist named Fritz Haber ascertained that atmospheric nitrogen could be combined with hydrogen under extremely high temperature and pressure condition which is catalyzed by an iron catalyst to yield an extremely high proportion of ammonia, which is the starting point for the production of a wide range of nitrogen compounds. This process was made commercially feasible by Carl Bosch and now called as the Haber-Bosch method or the synthetic ammonia process. The Haber-Bosch process is now one of the largest and most-basic processes of the chemical industry throughout the world.

$N_2 + 3H_2$ $2NH_3$ (only 20% conversion)

• NH_3 so produced can be used directly as fertilizer, but most of it is further processed to urea and ammonium nitrate (NH_4NO_3).

• Besides this, combustion of fossil fuels (natural gas, coal, crude oil) and products produced from crude oil (petrol, diesel, gasoline) contributes to nitrogen fixation.

Biological Nitrogen Fixation (BNF)



Biological nitrogen fixation (Figure 4) plays an essential part in providing nitrogen for other forms of life on earth, since it contributes about 60% of the total N_2 fixed in the biogeochemical nitrogen cycle. BNF is therefore called a key for sustenance of agriculture and reduction of soil fertility decline. In 1901, Beijerinck discovered the BNF which is carried out by a specific group of prokaryotes. These organisms utilize an enzyme called nitrogenase to catalyze the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3). Plants can conveniently absorb NH3 to synthesize the aforementioned nitrogenous biomolecules. This biological process though less efficient, occurs at room temperature and pressure. Therefore, the natural process of BNF deals an economic means for the reduction of environmental problems and improvement of the internal resources.

In this process microorganisms transform atmospheric nitrogen to ammonia, assailable by associated plants. These microorganisms need 16 moles of adenosine triphosphate (ATP) for the reduction of each mole of nitrogen and they obtain this energy by oxidation of organic molecules. Free-living microorganisms which are non-photosynthetic in nature, receive these molecules from other organisms, while photosynthetic microorganisms, such as cyanobacteria, use sugars synthesized in the process of photosynthesis

$N_2 + 8H+ + 8e- + 16$ Mg-ATP→ $2NH_3 + H_2 + 16$ Mg-ADP + 16 Pi

Associative and symbiotic nitrogen-fixing microorganisms obtain these compounds from their host plants' rhizospheres.

• Asymbiotic Bacteria or free-living bacteria- fixes 30 % of N_2

• Symbiotic Bacteria- fixes 70 % of N_2 & live in a symbiotic relationship with plants of the legume family (e.g., alfalfa, soybeans)

Mechanism of biological nitrogen fixation

Introduction

The mechanism of biological nitrogen fixation is a process in which nitrogen is converted into usable form with the help of living organisms. This can be done by free living bacteria and symbiotic bacteria or symbiotic microorganisms. Nitrogen fixation occurs when molecular nitrogen is converted to ammonia.

Examples of free-living bacteria are Azotobacter, Rhodospirillum, etc. An example of sympathetic bacteria is rhizobium (symbiotic with leguminous plants) etc. Certain cyanobacteria, such as Nostoc and Anabaena, help in nitrogen fixation in this category.

Nostoc and Anabaena can fix nitrogen in a free state and a symbiotic state, whereas rhizobium helps in nitrogen fixation only in a symbiotic condition.

Rhizobium leguminosarum is a symbiotic species of rhizobium. In a symbiotic condition, it helps in nitrogen fixation. Rhizobium is an aerobic bacterium. It turns anaerobic when it is in a nitrogen-fixing state. Rhizobium has an enzyme called nitrogenase, which helps in nitrogen fixation.

Nitrogenase is the molybdenum iron-containing protein (MoFeP). This one is active only in an anaerobic condition due to the presence of the enzyme, which helps in nitrogen fixation.

Mechanism of biological nitrogen fixation in legumes

The mechanism of biological nitrogen fixation in legumes takes place in the following steps:

1.Nodule formation

In nodule formation, the infection of rhizobium bacteria to the plant occurs through the root hair. These nitrogenases are present in the soil, making an infection thread. In the formation of a nodule, the following steps take place:

i) Formation of infection thread

Infection thread is a chain of these bacteria, and this chain contacts through the root hair and curls the root hair.

ii) Curling of root hair

- After the infection, root hair curls and infection thread reach up to cortex cells in the root and the bacteria changes into two types. Some of them change into bacteroids.
- These bacteroides initiate cell division in cells of the cortex in the root, and when cortical cells divide, a mass of cells is formed and is called the nodule.
- Bacteroides initiate cell division, and nodule formation takes place.
- The first step is the formation of an infection thread that is rhizobium. Infection thread enters the root hair, and it shows curling. This infection thread then reaches up to the cortex.
- Some of those bacteria change into bacteroides and initiate cell division of the cortical cell, forming nodules.
- Some cells of the cortex get modified into nitrogen-fixing cells. These are specialised cells having thick walls where nitrogen fixation can take place.
- These special cells are nitrogen fixation cells (tightly packed cells) which create an anaerobic condition for the nitrogenase enzyme to work. Nitrogen-fixing cells maintain an anaerobic condition.

2. Nitrogen fixation

- The conversion of molecular nitrogen into ammonia is known as nitrogen fixation.
- In the process of nitrogen fixation, the nitrogenase enzyme is essential.
- This nitrogenase enzyme has certain vital properties. It is a molybdenum iron-containing protein (MoFeP) made up of two subunits – iron-containing protein and iron and molybdenum-containing protein. These two subunits make the nitrogenase enzyme.
- We know that the nitrogenase enzyme can work only in an anaerobic condition, so there is a pigment present in a leguminous plant known as Leg Hb (leguminous haemoglobin).
- It acts as an oxygen scavenger. It removes all the oxygen and creates an anaerobic condition for nitrogenase to work.

- That is why rhizobium can work with leguminous plants, and this is a symbiotic association.
- Nitrogenase can work only in an anaerobic condition, and the roots of leguminous plants have leg haemoglobin which removes the oxygen and creates an anaerobic condition.
- Hydrogen donors are required for nitrogen fixation. Hydrogen donors can be many such as pyruvic acid, glucose, sucrose, etc.
- ATP as the source of energy is also required. One more carbon compound is needed to bind the ammonia molecules which are produced.

3. Nitrification

- In nitrification, ammonia is converted into nitrites and nitrates.
- In this process, ammonia is first converted into an ammonium ion, and then this ammonium ion gets oxidised in the presence of some bacteria.
- These bacteria are found in soil. For example, nitrosomonas and nitrococcus.
- With the help of these bacteria, ammonium ion is oxidized into nitrite, and a water molecule is released.
- Now, nitrites are oxidised further with the help of some bacteria like nitrobacter and converted into nitrates again, and water molecules are produced. These nitrates are used by the plants.

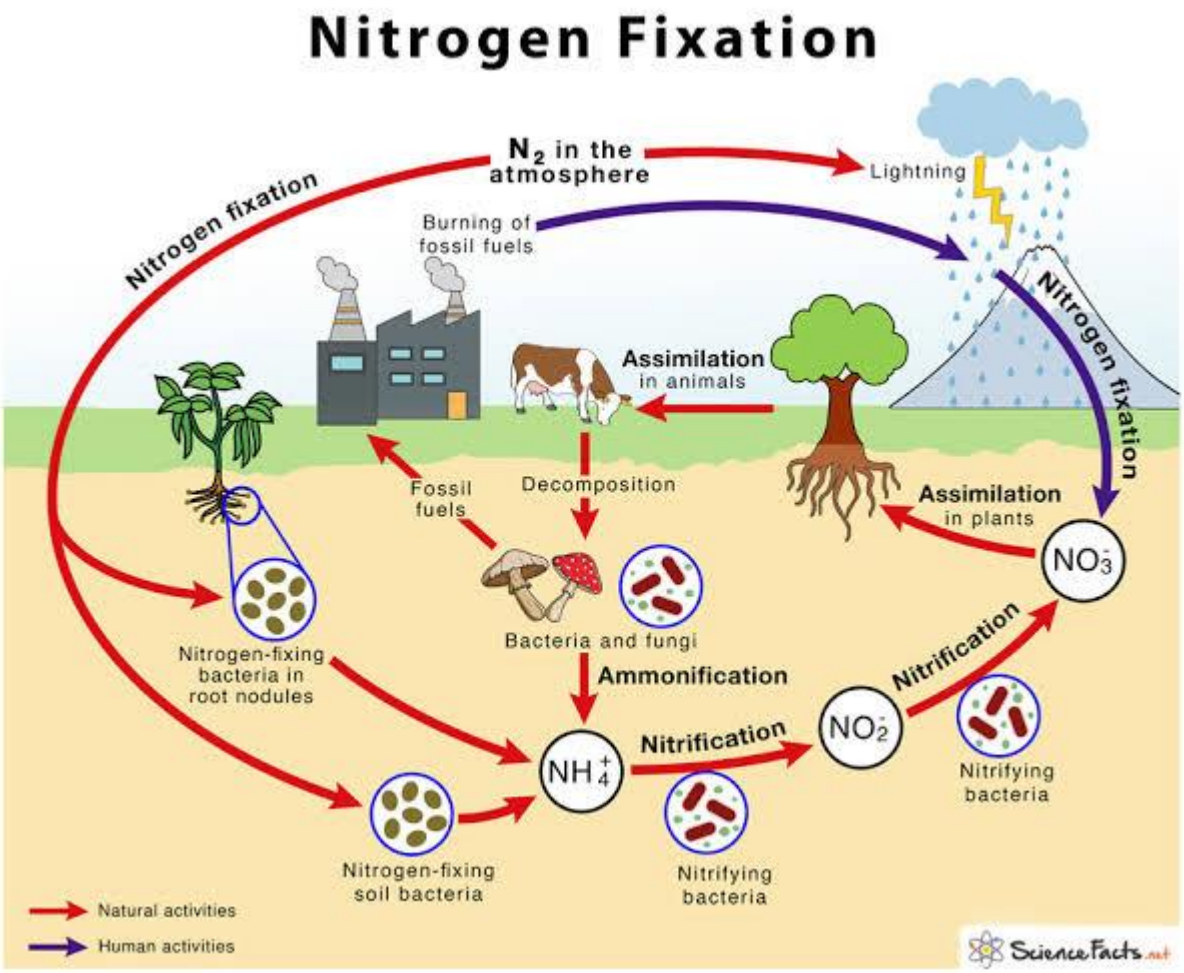
4. Ammonification

- Ammonification means the conversion of organic matter or any nitrogenous compounds into ammonia.
- Plants and animals decompose after their death, and this organic matter, with the help of bacteria, is converted into ammonia. This process is called ammonification.
- Similarly, animals excrete nitrogenous waste. These could be urea or uric acid. With industrial nitrogen fixation, we can also get urea as a fertiliser, and this urea is also converted into ammonia.
- Any organic matter on the nitrogen-containing compound is converted into ammonia, and this process is known as ammonification.

Conclusion

In the mechanism of biological nitrogen fixation in legumes, two nitrogen molecules are combined with two hydrogen molecules in the presence of 8 electrons, 8 protons, and 16 ATPs. Then, we get 2 ammonia molecules, 16 ADP (adenosine diphosphate) molecules, 16 inorganic phosphate, and two hydrogen molecules.

So, two nitrogen, 8 electrons, 8 protons, and 16 ATP would give us 2 ammonia molecules and ADP, and inorganic phosphate would be released. This is called the mechanism of biological nitrogen fixation. This reaction takes place only in an anaerobic conditions .



CHEMICAL NITROGEN FIXATION

- Nitrogen fixation is a chemical process that converts atmospheric nitrogen into ammonia, which is absorbed by organisms.
- Nitrogen fixation is essentially converting atmospheric nitrogen into a form that plants can more readily utilize.
- Reduction of N_2 into NH_3 by the chemical process is called chemical **nitrogen fixation.**
- In the use of Haber's process.
- Haber's process in use of high pressure and high temperature.
- The method is used in the agricultural and industry.

HABER-BOSCH PROCESS

Haber -Bosch Process, is the main industrial procedure for the production of ammonia.

The German chemists Fritz Haber and Carl Bosch developed it in the first decade of the 20th century.

The process converts atmospheric nitrogen (N_2) to ammonia (NH_3) by the reaction with hydrogen (H_2) using an iron metal catalyst under high temperature and pressures.

$$N_2(g)+3H_2(g) \rightleftharpoons 2NH_3\,(g)$$

Raw Material

NITROGEN from air

HYDROGEN from natural gas

$$N_2(g)+3H_2\,(g) \rightleftharpoons 2NH_3(g) \quad \Delta H = -92.44\ KJ/mol$$

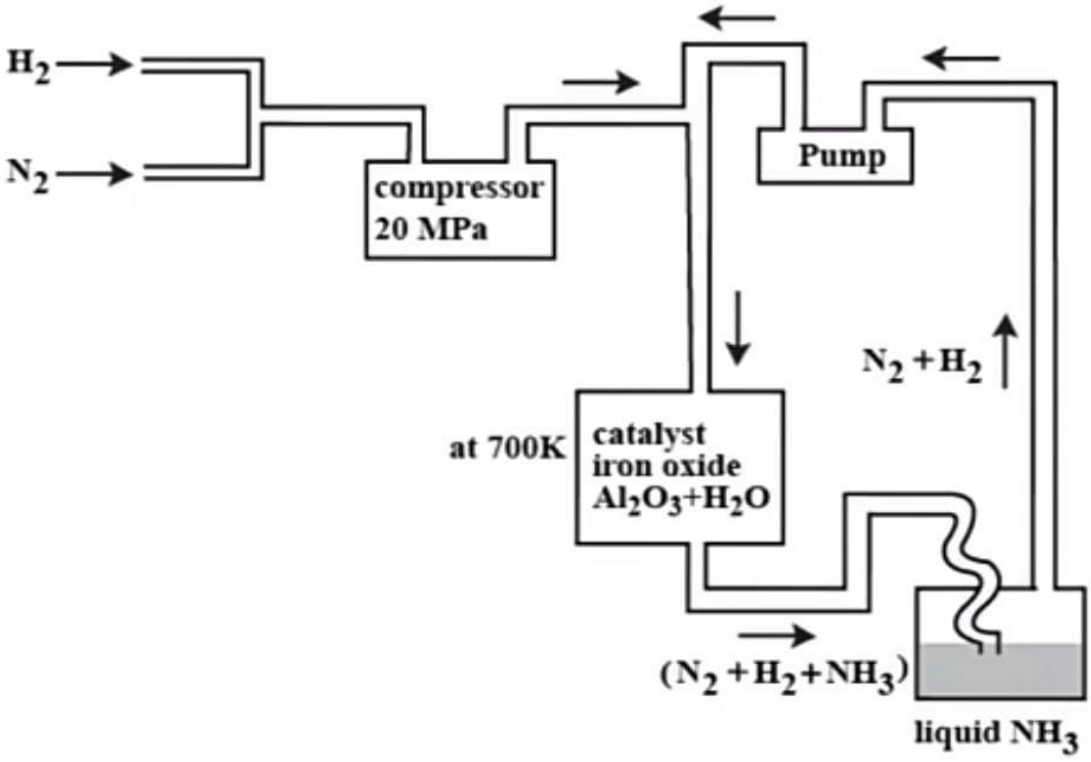
CONDITION

Temperature : 700k

Pressure : 200 atm

Catalyst : Iron oxide

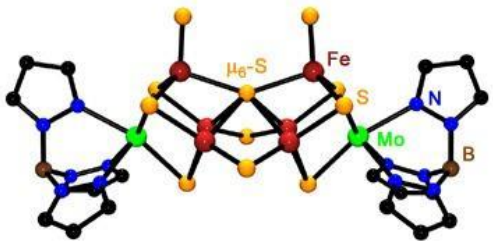
Promoter : $K_2O + Al_2O_3$



OPTIMIZING REACTION RATE AND YIELD

- Forward reaction is exothermic.
- Ammonia yield is favoured at low temperatures.
- But reactions are slower at lower temperatures.
- Fe catalyst increases reaction rate.

Metal Cluster in Nitrogenase

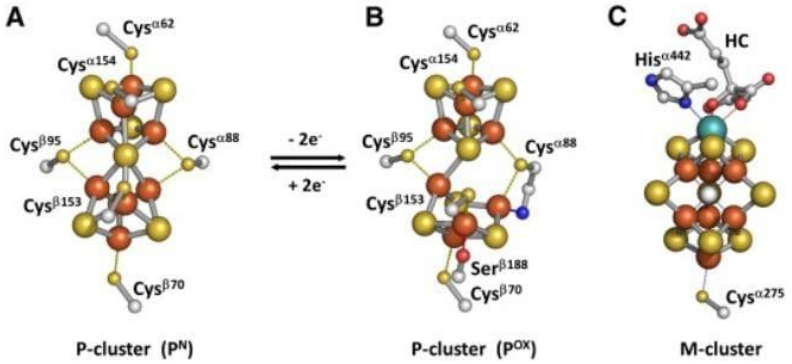


- The nitrogenase enzyme catalyses the key reductive step of dinitrogen to ammonia in the global biological nitrogen. The Mo-dependent nitrogenase contains two component proteins that are designated the Fe protein and the MoFe protein. The Fe protein acts as an

agent for electron transfer by delivering the necessary reducing equivalents to the MoFe protein, the location of substrate activation and reduction. The enzyme relies on three high nuclearity metal-sulfur complexes to carry out this complicated process: an Fe4S4 cubane unit in the Fe protein; and a P-cluster and iron-molybdenum cofactor (FeMo-cofactor) in the MoFe protein. The macromolecular structure determination of the MoFe protein has revealed a [MoFe7S9] cluster composed of two sulfur-voided [M4S3] cuboidal fragments linked by three μ2-sulfur bridges. Higher-resolution (2.0-Å) protein crystal structures have subsequently revealed the P-cluster to be a symmetric [Fe8S7] structure comprising, in the native state (PN), two [Fe4S3] cuboidal halves, vertex-fused through a μ6-sulfide and further interconnected by two μ2-cysteinate bridges. All these cluster structures are remarkable from a synthetic perspective, and we are exploring new chemistry to better understand the properties of these cluster units.

-
- Pursuant to these goals, we have demonstrated that the reduction of phosphine-ligated [MFe3S4] cubanes (M = Fe, Mo, V) yield edge-bridged double-cubane [M2Fe6S8] clusters that approach the core composition and overall extended geometry of symmetrized versions of the FeMo-cofactor. In an effort to realize clusters with cofactor-exact 8:9 metal/sulfide stoichiometries, these double-cubane products can be treated with sulfide reagents to incorporate sulfur into the core. The rearranged cluster core that is generated contains the desired [M8S9] composition. Despite the constitutional similarity, the resultant clusters do not adopt cofactor-like cores but instead bear a striking near-congruence to the native form of the nitrogenase PN-cluster (if bridging μ2-sulfides are substituted for the μ2-cysteinates in the biological system). We are currently investigating how the edge-bridged double cubane is transformed into the PN-cluster arrangement, and aim to use this information to incorporate a heteroatom into the assembly to form a structural analogue to the FeMo-cofactor.
-
- The M-cluster serves as the active site for substrate reduction. Buried within the α-subunit of NifDK, it is located 14 Å away from the P-cluster. Structurally, the M-cluster can be viewed as [Fe4S3] and [MoFe3S3] partial cubanes bridged by three μ2-sulfides in between. It also contains a homocitrate moiety at the Mo end and a μ6-interstitial carbide in the center. The M-cluster is coordinated to NifDK by only two ligands: Cysα275, which ligates the apical Fe atom; and Hisα442, which ligates the opposite Mo atom. In addition, Lysα426 provides an anchor for the homocitrate moiety. The “simple” coordination pattern of M-

cluster facilitates the extraction of this cluster as an intact entity into organic solvents, such as N-methylformamide (NMF).



➢

➢ Crystal structures of the P^N (A) and P^{OX} (B) states of the P-cluster and the M-cluster (C). The clusters are shown as ball-and-stick models, with the atoms colored as described in the legend of the above figure.

Heavy metals and toxicity

Introduction

Heavy metals are metallic elements with high atomic weights and densities. While many heavy metals play essential roles in biological processes at trace levels, their accumulation in the environment can lead to toxicity, posing risks to human health and ecosystems. This essay provides a brief overview of common heavy metals and their potential toxicity.

Common Heavy Metals:

1. **Lead (Pb):**

- Sources: Lead is found in various industrial processes, lead-based paints, and contaminated soil and water.
- Toxicity: Lead exposure can cause neurological damage, especially in children, leading to developmental issues and learning disabilities.

2. **Mercury (Hg):**

- Sources: Mercury is released into the environment through industrial processes, coal combustion, and certain natural sources.
- Toxicity: Methylmercury, a bioaccumulative form of mercury, can lead to neurological damage, particularly affecting the nervous system.

3. **Cadmium (Cd):**

- Sources: Cadmium is released from industrial activities, phosphate fertilizers, and cigarette smoke.
- Toxicity: Chronic exposure to cadmium can lead to kidney damage, respiratory issues, and cardiovascular problems.

4. **Arsenic (As):**

- Sources: Arsenic is naturally occurring and can be found in groundwater, as well as in some pesticides and industrial processes.
- Toxicity: Long-term exposure to arsenic can lead to skin issues, cardiovascular diseases, and an increased risk of certain cancers.

5. **Copper (Cu):**

- Sources: Copper is present in various industrial processes, plumbing, and fungicides.
- Toxicity: While copper is essential for certain biological functions, excessive exposure can lead to gastrointestinal issues and liver damage.

Potential Health and Environmental Impacts:

Human Health:

- Heavy metal toxicity can lead to a range of health issues, including neurological disorders, kidney damage, respiratory problems, and an increased risk of certain cancers.
- Vulnerable populations such as children, pregnant women, and the elderly may be more susceptible to the adverse effects of heavy metal exposure.

Environmental Impact:

- Heavy metals can accumulate in soil and water, affecting plant and animal life.
- Bioaccumulation in the food chain can lead to elevated concentrations in species higher up the food web, posing risks to ecosystems.

PHOTOSYNTHESIS

Photosynthesis is a process by which phototrophs convert light energy into chemical energy, which is later used to fuel cellular activities. The chemical energy is stored in the form of sugars, which are created from water and carbon dioxide.

In other words, the process of photosynthesis is defined that solar energy is harvested and converted to chemical energy in the form of glucose using water and carbon dioxide. Oxygen is released as a byproduct.

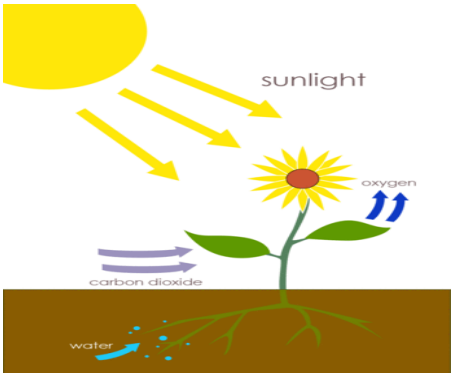


Fig 1: Process of photosynthesis

Photosynthesis Equation

Photosynthesis reaction involves two reactants, carbon dioxide and water. These two reactants yield two products, namely, oxygen and glucose. Hence, the photosynthesis reaction is considered to be an endothermic reaction. Following is the photosynthesis formula:

$$6CO_2 + 6H_2O \longrightarrow C_6H_{12}O_6 + 6O_2$$

Unlike plants, certain bacteria that perform photosynthesis do not produce oxygen as the by-product of photosynthesis. Such bacteria are called anoxygenic photosynthetic bacteria. The bacteria that do produce oxygen as a by-product of photosynthesis are called oxygenic photosynthetic bacteria.

Process Of Photosynthesis

At the cellular level, the photosynthesis process takes place in cell organelles called chloroplasts. These organelles contain a green-coloured pigment called chlorophyll, which is responsible for the characteristic green colouration of the leaves.

As already stated, photosynthesis occurs in the leaves and the specialized cell organelles responsible for this process is called the chloroplast. Structurally, a leaf comprises a petiole,

epidermis and a lamina. The lamina is used for absorption of sunlight and carbon dioxide during photosynthesis.

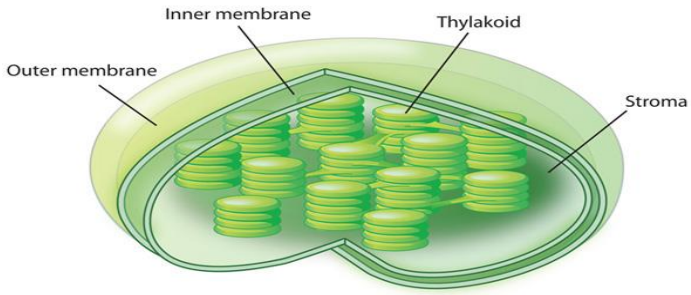


Fig:2 Structure of chloroplast

Chloroplast is an organelle that contains the photosynthetic pigment chlorophyll that captures sunlight and converts it into useful energy, thereby, releasing oxygen from water.

Photosystem

The light-absorbing pigments of thylakoid or bacterial membranes arranged in functional arrays are called photosystems. All the pigments of the photosystem can absorb photons that are being used in photosynthetic reactions but only a few molecules of the pigment are directly involved in the transduction of photon's light energy to electrochemical energy.

Constituents of Photosystem

Photosystem contains mainly two types of pigment that are, chlorophyll and accessory pigments. The main function of chlorophyll is to absorb light energy in the form of photons to perform photosynthesis whereas accessory pigments as the name suggest act as secondary light-absorbing pigments which enhances the overall performance of chlorophyll. An example of accessory pigment is carotenoids.

The carotenoid pigments absorb light at a wavelength not absorbed by the chlorophyll, they may be yellow, red, or purple. The most important is beta carotene, which is a red-orange isoprenoid, and yellow carotene lutein.

The main constituents of the photosystem are photochemical reaction centers and antenna molecules, also known as the light-harvesting complex. Photochemical reaction system constitutes chlorophyll and is the site of photosynthetic reaction, specialized chlorophyll molecule converts light energy into electrochemical energy Light-harvesting complex constitutes

of the accessory pigments which absorbs a photon and transfers it to the reaction system. This is known as exciton transfer. Together these systems form a complete functioning photosystem.

Types of Photosystems

The photosynthetic apparatus of modern cyanobacteria, algae, and vascular plants are highly evolved and complex as compared to their primitive counterparts found in bacteria. These evolved organisms constitute 2 photosystems namely Photosystem I and photosystem II, both of these works in a coordinated fashion to carry out photosynthesis. The coordinated pathway of PSI and PSII is known as Z-scheme.

1. Photosystem I
2. Photosystem II

Photosystem I is one of two photosystems in the photosynthetic light reactions of algae, plants, and cyanobacteria. Photosystem I is an integral membrane protein complex that uses light energy to catalyse the transfer of electrons across the thylakoid membrane from plastocyanin to ferredoxin.

Photosystem II is the first protein complex in the light-dependent reactions of oxygenic photosynthesis. It is located in the thylakoid membrane of plants, algae, and cyanobacteria.

Comparison between Photosystem 1 and Photosystem 2:

Photosystem 1	**Photosystem 2**
Function	
Utilizes light energy to transform NADP+ into NADPH2	These are protein complexes that absorb lig energy and function in the separation of water molecule
Location in the cell	
Located on the external surface of the grana thylakoid membrane	Found on the internal surface of the grana thylakoid membrane
Reaction center	

P700	P680
Wavelength of light absorbed	
Roughly 700 nm	Approximately 680 nm
Core complex composition	
Contains fewer proteins (approximately l subunits)	It's a multi subunit complex (around 25-3 subunits)
Type of reaction center	
Iron-sulphur type reaction center	Quinone type reaction center
Rich content of pigment	
Higher concentration of chlorophyll a compared chlorophyll b	More chlorophyll b than chlorophyll a
Involvement in water photolysis	
No	Yes
Participates in which type of photophosphorylation	
Both cyclic and non-cyclic photophosphorylatio	Only non-cyclic photophosphorylation
Pigments involved	
Consists of chlorophyll A-695, chlorophyll A-670, chlorophyll A-680, chlorophyll A-700, chlorophyll B, and carotenoids.	Includes chlorophyll A-670, chlorophyll A-660, chlorophyll A-695, chlorophyll A-680, chlorophy A-700, chlorophyll B, phycobilins, ar xanthophylls.
Key Function	
In cyclic photophosphorylation, it synthesiz ATP, and in non-cyclic photophosphorylation, it synthesizes NADPH.	Involved in water hydrolysis and ATP synthesis.

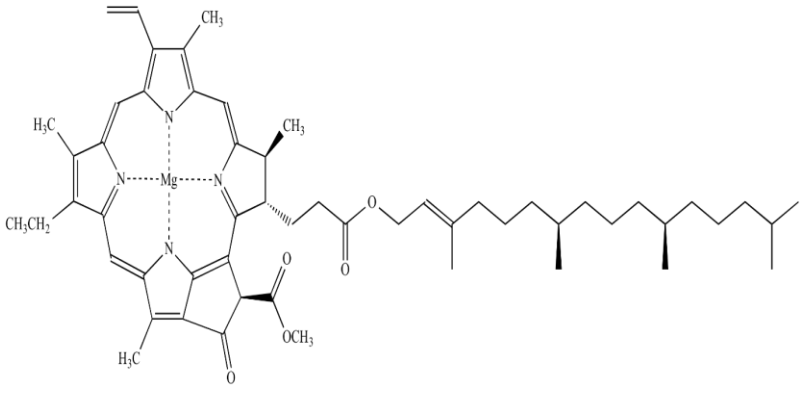
CHLOROPHYLL

DEFINITION:

Chlorophyll is a pigment present in all green plants and a few other organisms. It is required for photosynthesis, which is the process by which light energy is converted into chemical energy. The chlorophyll pigment is responsible for the green colouration in plants.

STRUCTURE:

- The chlorophyll molecule consists of a central metal core surrounded by a nitrogen-containing structure, resulting in a porphyrin ring.
- All the chlorophyll molecules are characterized by the presence of four pyrrole-like rings (thus termed tetrapyrroles) along with an additional fifth ring.
- Besides, a number of side chains are attached to the ring structure, of which an important one is, the side chain of a long hydrocarbon, called phytol ring.
- The molecular formula of chlorophyll is $C_{55}H_{72}MgN_4O_5$.
- Chlorophylls are classified as chlorins , which are close relatives of porphyrins like hemoglobin.
- There are different types of chlorophylls that might differ in their chemical structures as they occur in different living organisms.
- Among all different types of chlorophylls, the structure of chlorophyll a is the most studied.
- Structurally, chlorophylls are different from other photosynthetic pigments in that they have networks of alternating single and double bonds which make them quite effective towards photoreception.
- The electrons in the molecules are not localized but remain distributed throughout the bonds, which enable the pigment to absorb light more readily.



FUNCTIONS:

- Production of Carbohydrates**:** Chlorophylls are present in the chloroplasts of plant cells where photosynthesis takes place. Proteins are arranged in the thylakoid membranes of the chloroplast, which work together with chlorophyll by absorbing sunlight. They produce energy in the form of ATP which then goes through the Calvin cycle, and fixes the CO2 to produce sugars.
- Production of Oxygen**:** Oxygen is produced as a by-product of photosynthesis. This oxygen is then used up by plants for cellular respiration, and is also released into the environment supporting the living beings.

APPLICATIONS:

- Different products produced as derivatives of chlorophylls are used for coloring purposes in industries.
- The pigment is also used in various pharmaceutical and cosmetic products as a wound healing or coloring agent.
- Chlorophyll also acts as a biomordant to enhance the dyeing process of textile products.
- Chlorophyll and chlorophyll derivatives can also be used as coating or protective agents due to their ability to form laminar films with either hydrophilic or hydrophobic properties.
- Chlorophyll is also used as chelating agents due to its complex molecular structure.
- Due to the unique structure of chlorophyll molecules, these act as oxidation-reduction catalysis in both chemical and photosensitized reactions.
- Some amount of chlorophyll is also added to sun protection creams due to its ability to provide protection against polychromatic radiation.
- A derived form of chlorophyll, chlorophyllin, is taken as a health supplement by immune-compromised individuals.

Health Benefits:

- Chlorophyllin is known to reduce inflammation of the skin and prevent bacterial growth on skin wounds.
- Chlorophyll also increased the production of blood cells (both RBCs and WBCs) while also improving the quality of these cells.
- Based on different studies, chlorophyll indicates anti-carcinogenic properties which help in the reduction of risks of cancer.
- Chlorophyll has the ability to absorb toxins which helps in the detoxification of the digestive system.
- The use of chlorophyll is also associated with weight loss; however, the research on this topic is quite limited.
- Chlorophyll acts as an internal deodorant that helps with odors from bad breath, sweat, urine, and food odors.
- Chlorophyll helps in digestion by acting as a regulator of intestinal fermentation. It reduces the production of gas in the intestine and even has an antibacterial effect.

Unit 4

Toxicity of Arsenic and Lead

Nitrogenase Enzyme

The nitrogenase complex, a highly conserved protein complex, is responsible for biological nitrogen fixation. Molybdenum nitrogenase, Vanadium nitrogenase, and iron only nitrogenase are the three forms of nitrogenase found in diverse nitrogen fixing bacteria. Molybdenum nitrogenase has been explored and characterised in more depth. All nitrogenase are made up of two proteins: Fe protein denitrogenate reductase and Mo-Fe protein dinitrogen's reductase. During catalysis, electron move from a pair of ATP molecules within component II to the Fe-S cluster, where N_2 is reduced to NH_3.

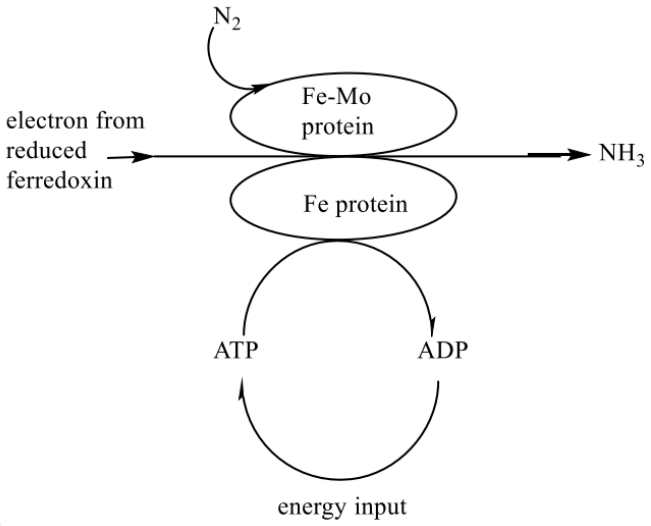


Fig. Nitrogenase Complex

- The smaller protein has a molecular weight of 60000 and is often termed as iron protein.
- It contains a Fe_4S_4 cluster and called reductase.
- The other protein has a molecular weight of 240,000 and is called the Mo-Fe protein.
- This is an α_2-β_2 tetramer and contain two molybdenum atoms, about 30 iron atom and around 30 inorganic/ labile sulphur.
- The iron sulphur cluster seems to act as redox centers. A soluble protein free cofactor containing molybdenum and iron has been isolated.
- Neither of the protein is separately active, but on mixing them the activity is restored.

Arsenic

- Occurrence: drinking water, food (Seafood and fish)
- Uses: wood preservatives, insecticides, and herbicides.
- Other Sources:

Industrial processes

Semiconductor manufacturing

Fossil fuels

Smelting (copper, zinc, lead)

Glass manufacturing

Antiparasitic drugs

Folk remedies

- Mechanism of Toxicity:

Accumulate in mitochondria and inhibiting succinic dehydrogenase activity and oxidative phosphorylation, a process that results in disruption of all energy dependent cellular functions.

- Trivalent forms:

-bind to sulfhydryl groups leading to inhibition of enzymatic systems.

-inhibit the Krebs cycle and oxidative phosporylation. These lead to inhibition of ATP production.

- Pentavalent forms:

-can replace the stable phosphate ester bond in ATP and produce an arsenic ester stable bond which is not a high energy bond.

-Endothelial damage, loss of capillary integrity, capillary leakage, volume loss, shock.

- Toxicokinetics:

-t1/2 of inorganic arsenic in the blood is 10 hrs and of organic arsenic is around 30 hours.

-2-4 weeks after the exposure ceases, most of the remaining arsenic in the body is found in keratin-rich tissues (nails, hair, skin).

-Renally excreted (30-50% of inorganic arsenic is excreted in about 3 days). Both forms are excreted depend on the acuteness of the exposure and dose.

- Signs and Symptoms of Acute Toxicity:

-GI distress, watery or bloody diarrhea

-Pulmonary edema, haemorrhagic bronchitis, and respiratory distress may be seen with acute oral poisoning

-Hypotension, tachycardia

-Complaint of a metallic taste in the mouth and garlic odor on the breath.

- Treatment of Acute Poisoning:

-consumption of large volumes of water, gastric lavage, or cathartics initiated within a few hours of exposure after oral ingestion of As.

-Activated charcoal does not bind well inorganic arsenic.

-Whole bowel irrigation with polyethylene glycol.

-Skin decontamination in dermal exposure.

-Chelation therapy should be instituted promptly (minutes to hours).

–BAL (British anti-Lewisite)- IM

–Succimer (DMSA)- PO

–DMPS – PO, IV

–D-Penicillamine- less effective

Lead:

- Occurrence and Uses:

  -Batteries and in sheathing electric cables, protective shielding from X rays and radiation from nuclear reactors, pigments in paint, "antiknock" agent in gasoline, until it was banned as an environmental pollutant in the United States in the 1970s.

- Other Sources:

  -Soil and dust

  -Paint chips

  -Contaminated water

- Mechanism of Toxicity:

  -Principal targets for Pb intoxication are the bone marrow and blood-forming pathways, GI tract, CNS, and neuromuscular system.

  -Pb increases intracellular levels of Ca in brain capillaries, neurons, hepatocytes, and arteries that trigger smooth muscle contraction, thereby inducing hypertension.

- Toxicokinetics and Toxicoynamics:

  Absorption:

  Skin:

  1.Inorganic lead is not absorbed

  2.Organic lead is well absorbed.

  Excretion:

  Kidney:

  1.Effects of Pb on blood formation and heme biosynthesis, Effects of Pb on heme synthesis also impact skeletal, renal, and neurological functions.

  2.In bone, Pb alters circulating levels of 1,25dihydroxyvitamin D, affecting Ca homeostasis and osteocyte function.

- Signs and Symptoms of Acute Toxicity:

  -Rare: result in cramping, colicky abdominal pain, and constipation, vomiting; bloody, black stools; and a metallic taste, Arthralgias and myalgias, neurotoxicity.

- Treatment of Acute Poisoning:

-In patients with kidney impairment, BAL is recommended since excretion is primarily in bile rather than urine

-EDTA also mobilizes Pb from bone to soft tissue and may aggravate acute toxicity if not given in conjunction with BAL.

Cadmium

Cadmium is known for its toxicity in animals and man as it is not used in these species. Its only role in biology is as a zinc replacement at the catalytic site of a particular class of carbonic anhydrases in some marine diatoms. The toxicity of cadmium continues to be a significant public health concern as cadmium enters the food chain and it is taken up by tobacco smokers. The biochemical basis for its toxicity has been the objective of research for over 50 years. Cadmium damages the kidneys, the lungs upon inhalation, and interferes with bone metabolism. Evidence is accumulating that it affects the cardiovascular system. Cadmium is classified as a human carcinogen. It generates oxidative stress. This chapter discusses the chemistry and biochemistry of cadmium (II) ions, the only important state of cadmium in biology. This background is needed to interpret the countless effects of cadmium in laboratory experiments with cultured cells or with animals with regard to their significance for human health. Evaluation of the risks of cadmium exposure and the risk factors that affect cadmium's biological effects in tissues is an on-going process. It appears that the more we learn about the biochemistry of cadmium and the more sensitive assays we develop for determining exposure, the lower we need to set the upper limits for exposure to protect those at risk. But proper control of cadmium's presence and interactions with living species and the environment still needs to be based on improved knowledge about the mechanisms of cadmium toxicity; the gaps in our knowledge in this area are discussed herein.

Zinc

Zinc toxicity is a medical condition involving an overdose on, or toxic overexposure to, zinc. Such toxicity levels have been seen to occur at ingestion of greater than 50 mg of zinc. Excessive absorption of zinc can suppress copper and iron absorption. The free zinc ion in solution is highly toxic to bacteria, plants, invertebrates, and even vertebrate fish.Zinc is an essential trace metal with very low toxicity in humans

Sign and symptoms

Following an oral intake of extremely high doses of zinc (where 300 mg Zn/d – 20 times the US RDA – is a "low intake" overdose[]), nausea, vomiting, pain, cramps, and diarrhea may occur. There is evidence of induced copper deficiency, alterations of blood lipoprotein levels, increased levels of LDL, and decreased levels of HDL at long-term intakes of 100 mg Zn/d. The USDA RDA is 15 mg Zn/d. There is also a condition called the "zinc shakes", "zinc chills", or metal fume fever that can be induced by the inhalation of freshly formed zinc oxide formed during the welding of galvanized materials

Treatment

Treatment of zinc toxicity consists of eliminating exposure to zinc. However, no antidotes are available

Cross-reaction toxicity

Supplemental zinc can prevent iron absorption, leading to iron deficiency. Zinc and iron should be taken at different times of the day

Antimony

Antimony toxicity occurs either due to occupational exposure or during therapy. Occupational exposure may cause respiratory irritation, pneumoconiosis, antimony spots on the skin and gastrointestinal symptoms. In addition antimony trioxide is possibly carcinogenic to humans. Improvements in working conditions have remarkably decreased the incidence of antimony toxicity in the workplace. As a therapeutic, antimony has been mostly used for the treatment of leishmaniasis and schistosomiasis. The major toxic side-effects of antimonials as a result of therapy are cardiotoxicity (~9% of patients) and pancreatitis, which is seen commonly in HIV and visceral leishmaniasis co-infections. Quality control of each batch of drugs produced and regular monitoring for toxicity is required when antimonials are used therapeutically.

TOXICITY OF MERCURY

MERCURY

Mercury is a compound found in rocks in the earth’s crust. It has a shiny silver appearance which gives the nick name “liquid silver”. Mercury is the element on the periodic table with the symbol Hg and the atomic number 80. Mercury is unique because it’s classified as a metal and comes in

both liquid and solid form depending on the temperature. Mercury has several uses because it's a conductor. This means that the compound allows electricity and heat to flow through it.

MERCURY POISONING

Mercury is toxic and harmful to the human body. Mercury poisoning occur when you exposed yourself to too much mercury and your body react negatively to the compound.

There are three different types of mercury that are harmful to the human body including:

Elemental mercury (liquid mercury, quicksilver): You'll find elemental mercury in glass thermometers, electrical switches, fluorescent lightbulbs and dental fillings.

Inorganic mercury: You'll find inorganic mercury in batteries, certain types of disinfectants and in chemistry labs.

Organic mercury: You'll find organic mercury in coal fumes, fish that ate methylmercury (a form of organic mercury) and older antiseptics (germ killers like red mercurochrome).

Effect of mercury poisoning:

Your body will negatively react if you eat, touch or inhale mercury. Once inside your body, mercury travels to your heart, central nervous system and kidneys. Your body knows that mercury is not supposed to be there, so you'll experience symptoms, caused by your immune system trying to get the compound out in the same way it would attack bacteria or germs.

Symptoms and Causes

Symptoms of mercury poisoning are different for each type of mercury and range in severity from person to person.

Elemental mercury poisoning symptoms

Elemental mercury is usually harmless if you touch or swallow it because its slippery texture won't absorb into your skin or intestines. Elemental mercury is extremely dangerous if you breathe it in and it gets into your lungs. Often, elemental mercury becomes airborne if someone is trying to clean up a mercury spill with a vacuum.

Symptoms of elemental mercury poisoning occur immediately after inhaling the chemical and include:

Coughing.

Trouble breathing.

Metallic taste in your mouth.

Nausea or vomiting.

Bleeding or swollen gums.

Inorganic mercury poisoning symptoms

Inorganic mercury is poisonous when swallowed. When the chemical enters your body, it travels through your bloodstream and attacks your brain and kidneys.

Symptoms of inorganic mercury poisoning include:

Burning sensation in your stomach and/or throat.

Nausea or vomiting.

Diarrhea.

Blood in vomit or stool.

Urine color changes.

Organic mercury poisoning symptoms

Organic mercury causes symptoms if you inhale it (breathe it in) or touch it. Symptoms don't occur immediately and usually arise after long periods of contact (could be years or decades) with the compound. Though not always common, being exposed to a large amount of organic mercury at one time can cause symptoms.

Symptoms of organic mercury poisoning from long-term exposure include:

Feeling numb or dull pain in certain parts of your body.

Tremors (uncontrollable shaking).

Unsteady walk.

Double vision or blurry vision; blindness.

Memory loss.

Seizures.

People who are pregnant and exposed to large amounts of methylmercury (a type of organic mercury) can cause brain damage to developing fetuses. Most healthcare providers recommend people who are pregnant eat a limited number of fish or remove fish from their diet, especially swordfish, during their pregnancy.

Long-term organic mercury exposure is deadly. If you frequently come into contact with organic mercury, wear proper personal protective equipment, like a mask and gloves, to reduce your risk of health problems associated with the compound.

Prevention

You can prevent mercury poisoning by:

Limiting the number of fish (that contain mercury) you eat.

Avoid fish (containing mercury) if you're pregnant or breastfeeding (chestfeeding).

Wearing personal protective equipment when handling chemicals and compounds.

Avoid areas in your environment where mercury is present.

Replacing old amalgam fillings in your teeth with a safer alternative

Chelation Therapy

- Chelation therapy is a treatment that uses medicine to remove metals in the body. It is used to treat metal poisoning.
- Some alternative health care providers also use it to treat heart disease, and Alzheimer's disease. But there's very little evidence it works for those conditions. In fact, chelation therapy can cause serious side effects -- including death -- especially if it's used in the wrong way.
- Chelation therapy uses special drugs that bind to metals in your blood.
- Once the drug has attached to the metal, your body removes them both through your pee.

Chelators

Chelators are organic compounds with 2 or more electronegative groups that form stable bonds with cationic metal atoms. These stable complexes lack the toxicity of the free metals and often

are excreted readily. Chelators, which function as chemical antagonists, are used as antidotes in the treatment of heavy metal poisoning.

What means chelate in medicine?

It is a method of removing certain heavy metals from the bloodstream, used especially in treating lead or mercury poisoning. Chelators used clinically include dimercaprol (BAL), succimer, unithiol, penicillamine, edetate (EDTA), and deferoxamine.

1. Dimercaprol

Dimercaprol is used in acute arsenic and mercury poisoning and, in combination with EDTA, for lead poisoning.

2. Succimer

Succimer (DMSA) is a water-soluble bidentate congener of dimercaprol.used for the oral treatment of lead toxicity in children and adults. It is as effective as parenteral EDTA in reducing blood lead concentration. Succimer is also effective in arsenic and mercury poisoning, if given within a few hours of exposure.

3. Penicillamine

Penicillamine is a derivative of penicillin, is another bidentate chelator. The major uses of penicillamine are in the treatment of copper poisoning. It is sometimes used as adjunctive therapy in gold, arsenic, and lead intoxication and in rheumatoid arthritis. The agent is water-soluble, well absorbed from the gastrointestinal tract, and excreted unchanged.

4. Unithiol

water-soluble derivative of dimercaprol, unithiol can be administered orally or intravenously. Intravenous unithiol is used in the initial treatment of severe acute poisoning by inorganic mercury or arsenic.

5. EDTA

EDTA is an efficient polydentate chelator of many divalent cations, including calcium, and trivalent cations. The primary use of EDTA is in the treatment of lead poisoning. Because the agent is highly polar, it is given parenterally. To prevent dangerous hypocalcemia, EDTA is usually given as the calcium disodium salt.

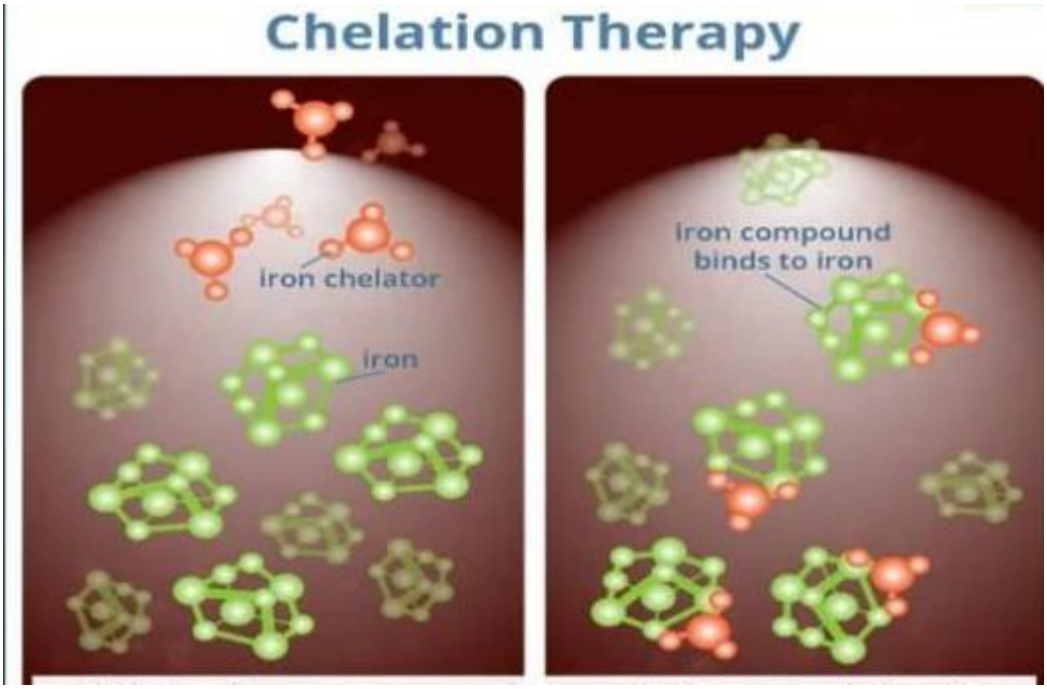
6. Deferoxamine

Deferoxamine is a polydentate bacterial product that has an extremely high and selective affinity for iron. Fortunately, the drug competes poorly for heme iron in hemoglobin and cytochromes. Deferoxamine is used parenterally in the treatment of acute iron intoxication and in the treatment of iron overload caused by blood transfusions in patients with diseases such as thalassemia.

BIO-INORGANIC CHEMISTRY

Definition:

Chelation therapy is a medical procedure that employs chelating agents. Chemical compounds with a high affinity for certain metals to bind and remove heavy metals from the body. While traditionally used for heavy metal poisoning the therapy has garnered attention. For its potential application in cancer treatment.



Chelating Agents:

Chelating agents, such as ethylenediaminetetraacetic acid (EDTA) and dimercapto succinic acid (DMSA) function by forming stable complexes with heavy metals. These complexes enhanced the elimination of metals through various excretory pathways.

It is introduced a iron chemical compound into the body that can find to the unwanted heavy metal (iron) the compound renders the unwanted metal chemically inert by binding to it and thus allows the metals to harm safety pass through the body without causing any further. Chelates are used in cancer as cytotoxic agent, as radioactive agent in imaging studies and in radioimmunotherapy.

Various chelates based on ruthenium, copper, zinc, organocobalt, gold, platinum, palladium, cobalt, nickel and iron are reported as cytotoxic agent. Monoclonal antibodies labeled with radioactive metals such as yttrium-90, indium-111 and iodine-131 are used in radioimmunotherapy. This review is an attempt to compile the use of chelates as cytotoxic drugs and in radioimmunotherapy.

PROPOSED MECHANISM IN CANCER:

Antioxidant Properties:

Chelation therapy is theorized to exhibit antioxidant properties particularly in mitigating metal induced oxidative stress associated with cancer development this oxidative stress in known to contribute to the initiation and progression of tumors.

Angiogenesis inhibition:

Some studies suggest that chelation agents may interfere with angiogenesis a pivotal process in tumor growth where new blood vessels form to supply nutrients and oxygen to the growing tumor.

INHIBITORS OF ANGIOGENESIS

Naturally occurring angiogenic inhibitors	Thrombospondin, Interferon, Metalloproteinase inhbitors
Tumor derived inhibitors	Angiostatin, Endostatin
Pharmacological inhibitors	Thalidomide, Carboxyamidotriazole (CAI)
Synthetic inhibitors	Synthetic Protease Inhibitors, Anti-adhesive Peptides [3]

Immune Modulation:

Chelation therapy might influence the immune system. This modulation could potentially enhance the body's ability to recognize and eliminate cancer cells thereby contributing to an anti-tumor effect.

Clinical Evidence:

The clinical evidence supporting the efficacy of chelation therapy in cancer treatment is limited and predominantly consists of preclinical studies and observational data. Few well-designed clinical trials have been conducted and their outcomes have been varied rendering the overall effectiveness uncertain.

The Trial to Assess chelation Therapy (TACT) didn't provide enough evidence to support routine use of this treatment for heart disease. But it did find that chelation therapy offered moderate protection against future cardiovascular events, such as stroke and heart attack, in those with diabetes.

COMMONLY USED CHELATORS CLINICALLY

CHELATORS:

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Chelation is useful in applications such as providing nutritional supplements, in chelation therapy to remove toxic metals from the body, as contrast agent in MRI scanning, in manufacturing using homogeneous catalysts, in chemical water treatment to assist in the removal of metals, and in fertilizers.

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VANADIUM BASED DIABETIC DRUG

DIABETES MELLITUS:

A group of disease that results in too much sugar in the blood [high glucose level].

CLASSIFICATION:

- **Type 1 diabetes:**

  A chronic condition in which pancreas produce little or no insulin

- **Type 2 diabetes:**

  A chronic condition where your produce still produces insulin but it doesn't make enough of it or it doesn't use in efficiently.

NEED FOR THE ADVANCED TREATMENT:

- In 21st century, patients suffering from diabetes mellitus will increase more than in 20th century.
- Diabetes mellitus is threatening due to several secondary complications like atherosclerosis, migroangiopathy, renal dysfunction and failure, cardiac abnormalities, diabetic retinopathy and ocular disorder.
- Using insulin injection can be painful both mentally and physically.
- The synthetic therapeutics substances used over a long term of time have side effects.
- Thus, the creation and development of new class of pharmaceuticals for diabetes mellitus would be extremely diserable.

Vanadium:

- Symbol: V
- Atomic no: 23
- Appearance: Hard, silvery grey, malleable transition metal
- 20th most abundant element in Earth's crust

ARISE OF VANADIUM COMPLEXES:

- After many trials, scientist noticed vanadium must be very useful in treatment of diabetes mellitus.
- In 1921, insulin was discovered by Banting and Best.
- Before its discovery in 1899, oral administration of sodium vanadate [$NaVO_3$] was reported to improve human diabetes mellitus.

- Researches where made to find why vanadium exhibits insulin-mimetic or blood glucose lowering effect in invitro and in vivo experiments.
- Then Vanadyl sulfate [$VOSO_4$] and its complexes with several types of ligands have been proposed as useful for treating diabetes mellitus.

IMPORTANTCE:

Mechanism of action:

- Vanadium compounds exhibits insulin mimetic properties helping to improve insulin sensitivity.
- They may activate key enzymes involved in glucose metabolism, such as tyrosine kinase and phosphatase.

Antidiabetic effects:

- Vanadium compounds have shown promising results in lowering blood glucose levels in both animal and human studies

Insulin sensitization:

- Vanadium has been suggested to enhance insulin signaling pathways, potentially overcoming insulin resistance observed in type -2 diabetes.
- This insulin sensitizing effect may contribute to better glucose utilization by cells.

Antioxidant properties:

- Vanadium compounds possess antioxidant properties, which could protect pancreatic beta cells from oxidative stress.
- Oxidative stress is is implicated in the progression of diabetes and vanadium's antioxidant effect may also mitigate this.

Clinical studies:

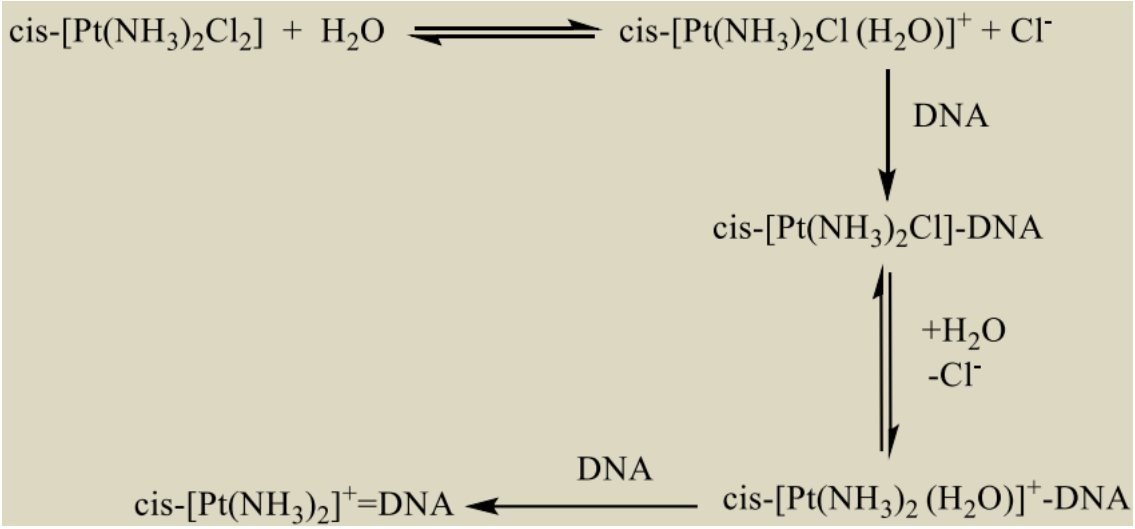
- Various vanadium compounds such as vanadyl sulfate and bis(maltolato)oxovanadium (IV), have been investigated in clinical trials.
- Clinical studies have explored their safety, efficacy and potential side effects in diabetic patients.

Other medical use:

- Vanadium also lowers LDL [Low Density Lipoprotein] that causes heart disease and stroke.

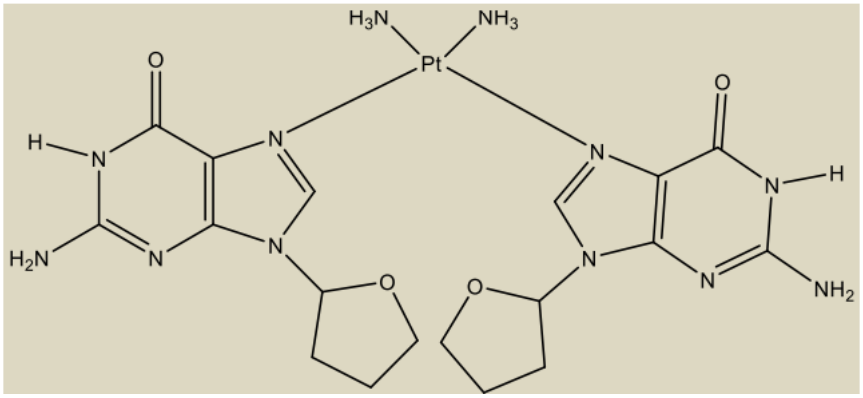
Platinum containing Anti-cancer drug

Cisplatin: Anticancer Drugs In 1969, B. Rosenberg and co-workers discovered the antitumor activity of simple square planar Pt (II)complex, cis- diamminedichloroplatinum (II) or cisplatin, $[Pt(NH_3)_2Cl_2]$. This compound is used as chemotherapeutic agent to inhibit otherwise rapid division of tumour cells (i.e. proliferation). Chemotherapy is the use of anticancer drug designed to inhibit growth of rapid dividing cancer cell in the body. The exact action of this complex is not known. Since the trans-isomer is inactive, therefore chelation or atleast coordination to Donor atoms at cis position is an essential part of activity. The proton NMR studies has suggested that Platinum binds to N-7 atom of a pair of adjacent quinine bases of a fast-growing tumour with the chloride ligands first being replaced by water molecules and then by a DNA base.

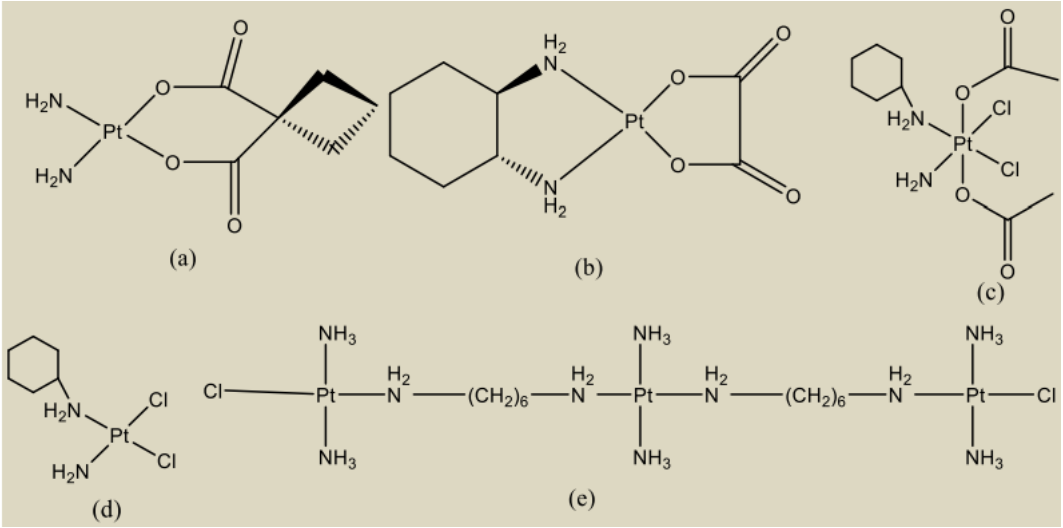


Cis-platin interact with N atoms of two adjacent guanine bases N-7 on DNA usually within the same strand (intra-stand linkage) or occasionally between strands (interstrand linking). The N7 position of guanine base is much base is much more basic than that of adenine and provides, therefore, a stronger site for the attack by platinum. Recent X-ray studied on a 12 - base pair fragment of double stranded DNA has suggested that the binding of Pt distorts the local DNA structure and therefore, inhibits the cells division inherent in the proliferation of cancer cells. Cisplatin has side effect in kidney and neuro-toxicity. Alternative Platinum compounds have been

developed to avoid these serious side effects. The most important of these is carboplatin, which replaces the Cis Chloride right ligands with O-chelate cyclobutanedicarboxylate.



Interaction of cis-platin with two guanine bases on a DNA strand



(a) Carboplatin (b)Oxaliplatin (c) Sataraplatin (d)cis-dichloroamine(cyclohexylamine) Pt(II) (e) trinuclear Pt(II) anticancer drug

Platinum containing anti-cancer agent

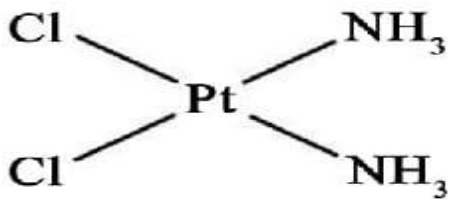
Cisplatin is an anti-cancer ("antineoplastic" or "cytotoxic") chemotherapy drug. This medication is classified as an alkylating agent.

Therapeutic agent

Treatment of advanced bladder cancer, metastatic ovarian cancer, and Metastatic testicular cancer. neuroblastoma sticular, ovarian, bladder, head and neck, Esophageal ,small and non-

small cell lung, breast, cervical, stomach and prostate Cancers. Also to treat Hodgkin's and non-Hodgkin's lymphomas,

Structure of cisplatin

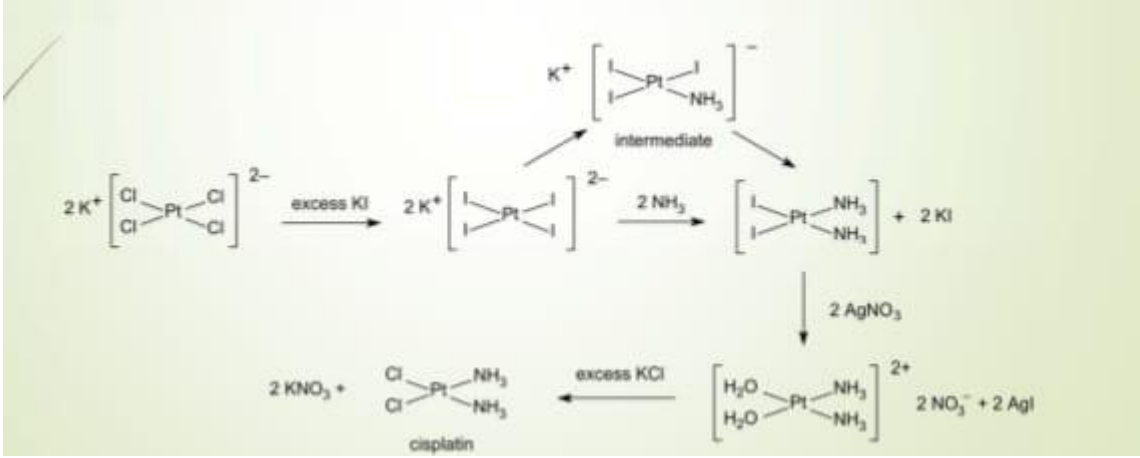


Cisplatin

- Cisplatinum, also called cis- diamine dichloroplatinum (II), is a metallic (platinum) coordination compound with a square planar geometry.
- Cisplatin for injection, USP, a platinum- based drug for intravenous use, is a White to light yellow lyophilized powder. The molecular formula is Cl2H6N2Pt And a molecular weight is 300.05.
- Cisplatin is a heavy metal complex containing a central atom of platinum Surrounded by two chloride atoms and two ammonia molecules in the cis Position.
- It is soluble in water or saline at 1 mg/mL and in dimethylformamide at 24 Mg/mL. It has a melting point of 207°C. It is soluble in water or saline at 1Mg/mL and in dimethylformamide at 24 mg/mL. It has a melting point of 207°C.

Methods of synthesis

Potassium tetra chloroplatinate is reacted with excess of potassium iodide. The Tetraiodide so formed is reacted with ammonia to form K2[PtI2(NH3)2], a yellow compound. The insoluble silver iodide is precipitated as the yellow compound is treated with silverNitrateinwater. Addition of the potassium chloride gives the final product.



Chelation therapy

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3**.Proposed mechanism in cancer**

•Antioxidant Properties: Chelation therapy is theorized to exhibit antioxidant properties, particularly in Mitigating metal-induced oxidative stress associated with cancer development. This oxidative stress is Known to contribute to the initiation and progression of tumors.

•Immune Modulation: Chelation therapy might influence the immune system. This modulation could Potentially enhance the body's ability to recognize and eliminate cancer cells, thereby contributing to an anti-tumor effect.

4.clinical Evidence:

The clinical evidence supporting the efficacy of chelation therapy in cancer treatment is limited and predominantly consists of preclinical studies and observational data. Few well-designed

clinical Trials have been conducted, and their outcomes have been varied, rendering the overall effectiveness uncertain.

DIAGNOSTIC IMAGING

Diagnostic imaging describes various techniques of viewing the inside of the body to help figure out the causes of an illness or injury and confirm a diagnosis. It has used and treated to patient's body which responds to treatment for a fracture or illness.

It allows physicians to view the inside of your body to help them find any indications of a health condition. It has been used to scan injury site by various instruments and technical methods which can produce pictures of the activities and structures inside your body.

Many imaging tests are non-invasive, easy and painless. Some techniques will require you to remain still inside the machine for a long time, however, which can get a little uncomfortable. Some tests involve a small amount of radiation exposure.

TYPES OF TECHNIQUES:

MAGNETIC RESONANCE IMAGING (MRI):

MRI is a non-invasive imaging technology that produces three dimensional detailed anatomical images. It is often used for disease detection, diagnosis, and treatment monitoring. It is based on sophisticated technology that excites and detects the change in the direction of the rotational axis of protons found in the water that makes up living tissues.

MRIs employ powerful magnets which produce a strong magnetic field that forces protons in the body to align with that field. When a radiofrequency current is then pulsed through the patient, the protons are stimulated, and spin out of equilibrium, straining against the pull of the magnetic field. When the radiofrequency field is turned off, the MRI sensors are able to detect the energy released as the protons realign with the magnetic field. The time it takes for the protons to realign with the magnetic field, as well as the amount of energy released, changes depending on the environment and the chemical nature of the molecules.

It is often used in the detection of tumours, strokes and bleeds. It also can be used to visualise the functionality of suspected masses and tumours.

Computerized tomography (CT) or computerized axial tomography (CAT):

This technique combines data from several X-rays to produce a detailed image of structures inside the body. A CT scanner emits a series of narrow beams through the human body as it moves through an arc. This is different from an X-ray machine, which sends just one radiation beam. The CT scan produces a more detailed final picture than an X-ray image. The CT scanner's X-ray detector can see hundreds of different levels of density. It can see tissues within a solid organ.

Ultrasound Imaging:

Ultrasound Imaging is a non-invasive diagnostic technique used to provide image inside the body. Ultrasound probes, called transducers, which produce sound waves that have frequencies above the threshold of human hearing (above 20KHz), but most transducers in current use operate at much higher frequencies (in the megahertz (MHz) range). Most diagnostic ultrasound probes are placed on the skin. However, to optimize image quality, probes may be placed inside the body via the gastrointestinal tract, vagina, or blood vessels.

Ultrasound produces images of internal organs or other structures. It has also given information such as the movement and velocity of tissue or blood, softness or hardness of tissue.

X-Ray Imaging:

X-rays are a form of electromagnetic radiation, which having higher energy and can pass through most objects, including the body. X-ray imaging is used to generate images of tissues and structures inside the body.

It has been used to Detects bone fractures, certain tumours and other abnormal masses, pneumonia, some types of injuries, calcifications, foreign objects, or dental problems.

Scintigraphy Radionucleotide:

Scintigraphy has also known as a gamma scan, is a diagnostic test in nuclear medicine, where radioisotopes in which the radioactive elements (having high nuclear energy) are attached to drugs that travel to a specific organ or tissue (radiopharmaceuticals) are taken internally and the emitted gamma radiation is captured by gamma cameras, which are external detectors that form two-dimensional images in a process similar to the capture of x-ray images.

It has to be done to diagnose obstruction of the bile ducts by a gallstone (cholelithiasis), a tumour. It can also diagnose gallbladder diseases.

Diagnostic agents

- A field of medicine used in determining physiology, managing disease, and locating abnormalities in the body.
- Diagnostic is a process of distinguishing symptoms, and the chemicals which are used to distinguish symptoms are known as diagnostic agents.
- Basically, diagnostic agents include chemical compounds of inorganic or organic nature, most of these being modified in their structural moeity, so as to become specific for their test reactions. These modifications make them biochemicals, depending upon their constitution and functional groups.
- Inorganic chemicals are not directly functioning as diagnostic agents.
- Broadly, we can divide the various compounds used as diagnostic agents into four major types as:
- Inorganic and organic compounds used directly.
- Dyes and stains specifically for use in end point or initial rate colorimetry.
- Radioactive tracers.
- Culture-media chemical-basic constituent being Agar

Classification

(A) Inorganic and Organic Compounds Used

i. Ferric ammonium citrate: it is used as bacteriological ingredient.

ii. Sodium chloride: it is used as tissue culture grade.

iii. Bees wax: It is used for histology.

iv. Digitonin: it is used for cholesterol determination

v. p-Aminoacetophenone, C8H9ON

It is a chemical reagent used in a simple method for the chemical determination of urinary thiamine based upon the PrebludaMcCollum reaction

vi. Bilirubin, $C_{33}H_{36}O_6N_4$

Standard in the calorimetric determination of bilirubin in blood, i.e. the estimation of serum

vii. Dichloroquinone Chlorimide, $C_6H_2ONCl_3$

This reagent is used in the diagnostic study of the urinary excretion of vitamin B6 by a colorimetric method.

viii. Dichlorophenol-indophenol, $C_{12}H_6O_2NCl_2Na$

Used in the diagnosis of vitamin C-sub-nutrition by urine analysis, with a note on the antiscorbutic value of human milk.

(B) Dyes and Stains

i. Acid fuchsin: A widely used plasma stain for connective tissue and stain for bacteriology.

iii. Acridine Orange: A biological stain, fluorescent dye for cytochemical staining.

iv. Basic fuchsin: Used for staining bacilli, especially influenzae and tubercle, in tissues

v. Giemsa Stain: Stain used for blood and malarial parasites.

vi. Methyl Green, A biological stain used as general tissues stain for differentiation of bacteria.

vii. Methylene Blue: A stain for elastic fibres and connective tissue and for tubercle and leprae bacilli in mammalian tissue.

viii. Neutral Red: A general histological and bacteriological stain.

ix. Orange-G: A collagen stain for connective tissue.

x. Orcein: A histological staining reagent.

Classification based on test functionalities

They are classified according to their test functionalities as

i) Gall bladder function, cholecystography and cholangiography:

eg. Iocetamic acid, Iodipamide, Tyropanoate sodium.

ii) Gastric function: eg. Pentagastrin, Congored.

iii) Liver function: eg. Indocyanine green.

iv) Ophthalmic diagnostic aid: eg. Fluorescein sodium.

v) Pancreatic function: eg. Bentiromide.

vi) Intestinal function: eg. Barium sulfate, Xylose.

vii) Kidney function: eg. Aminohippurate sodium, Indigotin disulfonate, Phenolsulphophthalein.

viii) Lymphatic system: eg. Isosulfan blue.

ix) Bronchial airway hyperacidity: eg. Methacholine.

x) Drug – hyper sensitivity: eg. Benzyl penicilloyl polylysine.

xi) Drugs used in X- ray contrast medium: eg. Diatriazoic acid, Iocetamic acid, Iothalamic acid, Propyliodone.

xii) Miscellaneous: eg. Erythrosin sodium, Evans blue

MRI CONTRAST AGENTS

- MRI contrast agents are a group of contrast media which are used body structures in magnetic resonance imaging (MRI).
- These are widely used to increase the contrast difference between normal and abnormal tissues.
- The most commonly used compounds for contrast enhancement are gadolinium-based
- MRI contrast agents have become an indispensable part of contemporary magnetic resonance imaging.
- MRI was initially hoped to provide a means of making definite diagnosis without administering contrast media,it has been found that the addition of contrast agents in many cases improves sensitivity and/or specificity.
- MRI contrast agents alter the relaxation times of atoms within body tissues where they are present after oral or intravenous administration.
- Most clinically used MRI contrast agent work through shortening the T1 relaxation time of protons located nearby.
- T1 shortens with an increase in rate of stimulated emission from high energy states(Spin anti-aligned with the main field) to low energy states(Soin aligned)
- To enhance the inherent contrast between tissues MRI contrast mus alter rate of relaxation of the proton within tissue.
- Relaxation must vary for different tissues in order to produce differential enhancement of signal.

- MRI contrast agents must exert a large magnetic field density (a property imparted by their unpaired electron) to interact with the magnetic moment of proton in the tissue and shorten their relaxation time.
- The electron magnetic moment also causes the local change in magnetic field promoting more rapid proton dephasing and shortens the relaxation time.
- Agents with unpaired electron pain spins used a MRI contrast agents.

CLASSIFICATION :

This may be classified into two groups

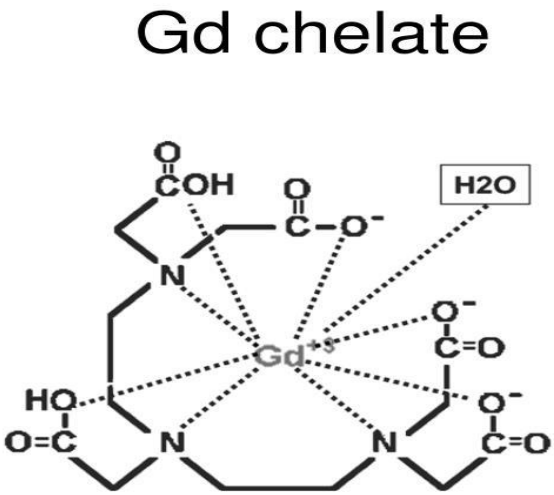
Paramagnetic:

- The paramagnetic contrast agents are usually made from dysprosium,the lanthanide metal gadalonium or the transition metal manganese
- Possess water soluble properties
- The most commonly selected metal atom used in MRI contrast agent is the lanthanide ion gadalonium as it possesses a high magnetic moment and it is most stable ion with unpaired electron
- Contrasting agent containing gadalonium shorten the T1 (or longitudinal) and T2(transverse) relaxation time of neighbouring water proton
- T1 shortening occurs at lower gadalonium concentration whereas T2 shortening occurs at higher gadalonium concentration which is of limited clinical use due to the increased risk of toxicity

SUPER PARAMAGNETIC:

- The super paramagnetic contrast agent are usually made of iron oxide particles
- Contrast agent containing transition metal ions such as high spin manganese and super magnetic iron oxide such as iron oxide affect the T2 relaxation strongly.

- When the applied during imaging, they reduce the intensity of T2 signals in the tissue which absorb the contrast agent.



CHELATE:

- Chelates surround an ion and make a cage around it
- A chelate of gadolinium occupies all available space around the ion except water molecule
- Water molecules exchange in and out of that one spot.
- When in that spot, the spins have an extremely short T1.
- This accelerates the overall relaxation rate, shortening T1.
- Gadolinium chelates (e.g.. gadopentate dimeglumine
- (Magnevist), are intravenous contrast agents used to
- enhance vascular structures during diagnostic magnetic resonance imaging.

SIDE EFFECTS OF MRI CONTRAST :

- The most common, so called normal, side effects of
- MRI contrast agent are mild and temporary in nature.
- They include warmth ,pain or burning at the injection
- site, low blood pressure, minor skin rash, mild
- headaches, changes in blood clotting, light headedness and nausea.
- Typically these side effects do not require any treatments

ALLERGIC REACTION :

The most common allergic reaction symptoms to MRI contrast agents include

- -swelling of the face.
- -rashes.
- -itching.
- -sweating,
- -watery or itchy eyes, and
- -shortness of breath.

TECHNETIUM IMGAGE CONSTRAT AGENT

Technetium-99m (99mTc) is a radionuclide nuclear agent that is FDA approved for diagnostic imaging of the brain, bone, lungs, kidneys, thyroid, heart, gall bladder, liver, spleen, bone marrow, salivary and lachrymal glands, blood pool, and sentinel nodes.

1. Technetium pharmaceuticals are metal-electron donor complexes
2. Tc is an electron deficient metal and therefore interacts with electronegative
3. Produced using the molybdenum-99/technetium-99 system where molybdenum is obtained as a fission product of uranium

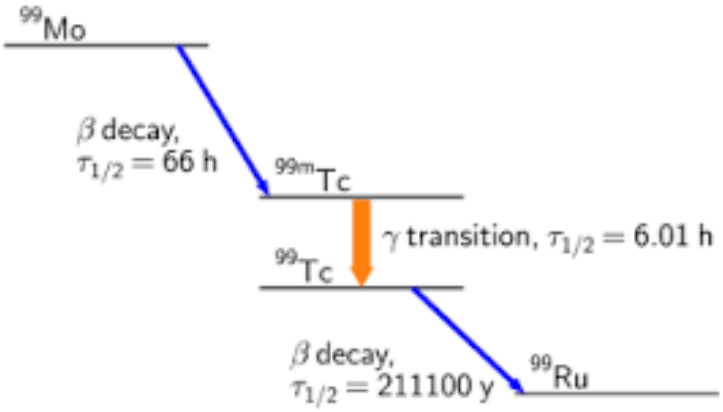
Technetium-99m (Tc-99m) is a metastable nuclear isomer of technetium-99 used in many radiology diagnostic procedures. Its primary use in medical imaging is to detect the presence of biological interactions and abnormalities through the formation of target compounds within the body. Through the emission of gamma rays, Tc-99m radioisotopes within the target compounds can be detected via Single Photon Emission Computed Tomography (SPECT)

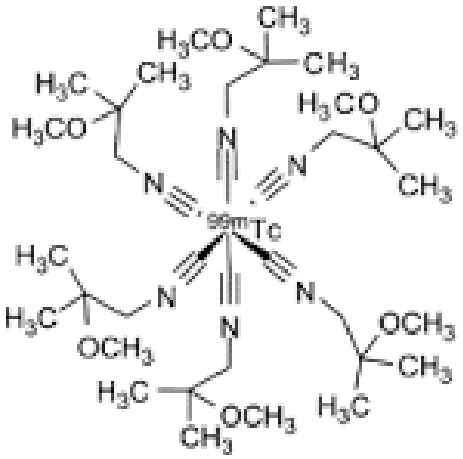
Examples:

•Tc-99m HMPAO labelled WBC

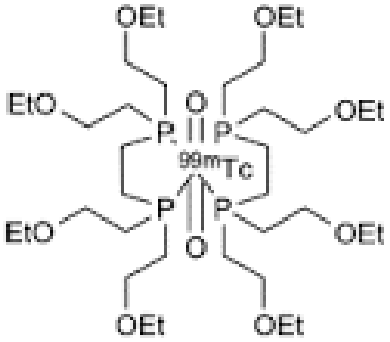
•Tc-99m Pertechnetate

•Tc-99m Methyl Diphosphonate (MDP)





^{99m}Tc-Sestamibi



^{99m}Tc-Tetrofosmin

Applications

• Tc-99m results in lower toxicity than other radiopharmaceuticals.

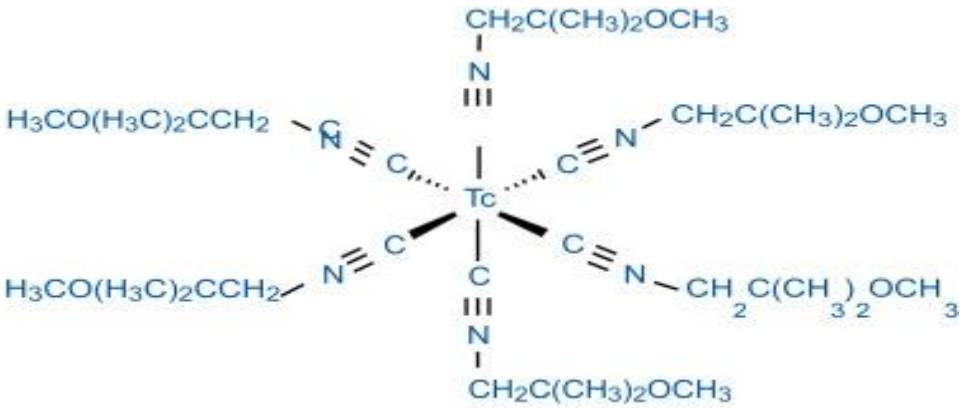
• Tc-99m is more cost effective compared to other radionuclides (Cr, I, Hg).

• Tc-99m results in reduced background allowing further tests to be carried out in a few days.

Limitations

• Radiopharmaceuticals cannot differentiate between cysts and tumors.

• Resolution of images may not be as clear as CT or MRI.



Tc-hexakis-2-methoxyisobutylisonitrile (Tcsestamibi).

UNIT-5

Introduction of Enzymes and its properties:

Enzyme, a substance that acts as a catalyst in living organisms, regulating the rate at which chemical reactions proceed without itself being altered in the process.

The biological processes that occur within all living organisms are chemical reactions, and most are regulated by enzymes. Without enzymes, many of these reactions would not take place at a perceptible rate. Enzymes catalyze all aspects of cell metabolism. This includes the digestion of food, in which large nutrient molecules (such as proteins, carbohydrates, and fats) are broken down into smaller molecules; the conservation and transformation of chemical energy; and the construction of cellular macromolecules from smaller precursors. Many inherited human diseases, such as albinism and phenylketonuria, result from a deficiency of a particular enzyme.

Enzymes also have valuable industrial and medical applications. The fermenting of wine, leavening of bread, curdling of cheese, which have been practiced from earliest times. Since then, enzymes have assumed an increasing importance in industrial processes that involve

organic chemical reactions. The uses of enzymes in medicine include killing disease-causing microorganisms, promoting wound healing, and diagnosing certain diseases.

Properties:

- Enzymes are proteins that start and speed up biological reactions.
- The acidity of the media affects enzyme activity (pH specific). At a certain pH, each catalyst is most active.
- For example, pepsin has a pH of 2 whereas trypsin has a pH of 8.5. The pH of most internal enzymes is close to neutral.
- Enzymes have the ability to speed up a reaction in either way. All enzymes have active sites, which are engaged in biological activities.
- Enzymes are extremely unstable molecules that can only be dissolved in dilute glycerol, NaCl, and dilute alcohol.
- Enzymes are active when the temperature is just perfect. Enzymes are proteins in nature, although not all proteins are enzymes.
- Enzymes lower the amount of energy needed to activate a substance molecule, allowing the biochemical reaction to take place at body temperature, which is 37°C.

Catalytic Properties:

Enzymes are biological catalysts with catalytic characteristics. The higher quantities of chemicals are catalyzed by a small number of enzymes. It signifies that enzymes have a great capacity for converting large amounts of substrate to product. Enzymes accelerate reactions while remaining unaffected by the reactions they catalyse.

Enzyme specificity:

Enzymes are extremely specific in nature, meaning that only one enzyme can catalyze a single process. Enzyme sucrase, for example, can exclusively catalyse sucrose hydrolysis.

CHARACTERISTICS OF ENZYMES:

- Enzymes being proteins exhibit all properties of proteins.
- They have their specific isoelectric points at which they are least soluble.
- They can be denatured by changes in pH and temperature.

- Higher reaction rate: The reaction rates of enzyme catalyzed reaction are veryHigh and are 106 to 1012 times higher than uncatalyzed reactions.

Midler reaction conditions:

- Enzymatic reactions occur at relatively low temperatures, below 100oC, atmosphericPressure and nearly neutral pH.

Capacity for regulation:

- The catalytic activity and the number of enzymes synthesized are highly regulated.
- The mechanisms of these regulatory processes include allosteric control, covalent modification **Greater reaction specificity:**
- Reactions catalyzed by enzymes are highly specific and three types of specificities are observed.

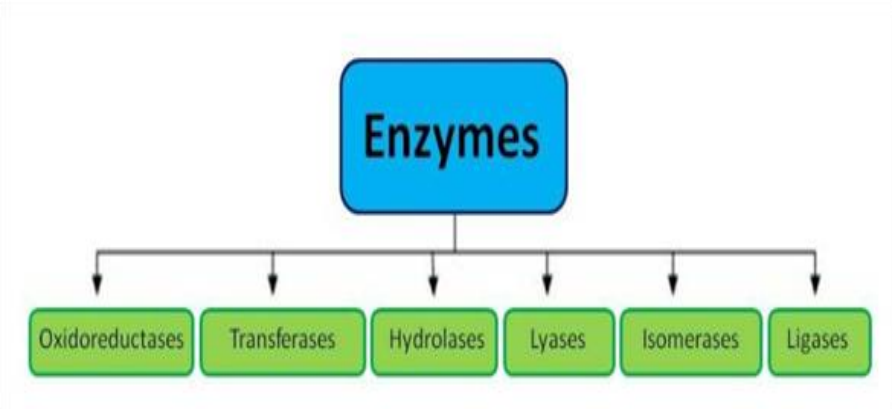
Absolute specificity:

- When enzymes catalyse only one particular reaction, they are said to exhibit absolute Specificity. **E.g.** Urease acts only on urea.

Group specificity:

- Enzymes acting on a group of substances that possess a particular type of linkageCommon to that group of substances is called group specificity. **E.g.** Enzyme actingUpon starch, dextrin and glycogen which have the same type of glycosidic linkage.

CLASSIFICATION AND NOMENCLATURE OF ENZYME



Oxidoreductases

These catalyze oxidation and reduction reactions. Example for pyruvate dehydrogenase, catalysing the oxidation of pyruvate to acetyl coenzyme A.

Transferases

These catalyze transferring of the chemical group from one to another compound. An example is a transaminase, which transfers an amino group from one molecule to another.

Hydrolases

They catalyze the hydrolysis of a bond. For example, the enzyme pepsin hydrolyzes peptide bonds in proteins.

Lyases

These catalyze the breakage of bonds without catalysis, e.g. aldolase (an enzyme in glycolysis) catalyzes the splitting of fructose-1, 6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone phosphate.

Isomerases

They catalyze the formation of an isomer of a compound. Example: phosphoglucomutase catalyzes the conversion of glucose-1-phosphate to glucose-6-phosphate (phosphate group is transferred from one to another position in the same compound) in glycogenolysis (glycogen is converted to glucose for energy to be released quickly).

Ligases

Ligases catalyze the association of two molecules. For example, DNA ligase catalyzes the joining of two fragments of DNA by forming a phosphodiester bond.

Types	Biochemical Property

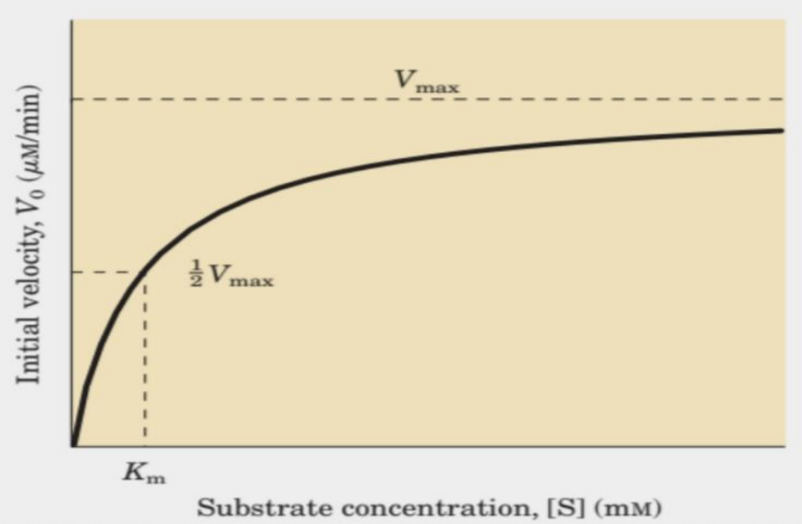
Oxidoreductases	The enzyme Oxidoreductase catalyzes the oxidation reaction where the electro tend to travel from one form of a molecule to the other.
Transferases	The Transferases enzymes help in the transportation of the functional grou among acceptors and donor molecules.
Hydrolases	Hydrolases are hydrolytic enzymes, which catalyze the hydrolysis reaction b adding water to cleave the bond and hydrolyze it.
Lyases	Adds water, carbon dioxide or ammonia across double bonds or eliminate the to create double bonds.
Isomerases	The Isomerases enzymes catalyze the structural shifts present in a molecule, th causing the change in the shape of the molecule.
Ligases	The Ligases enzymes are known to charge the catalysis of a ligation process.

ENZYME KINETICS

- Enzyme kinetics is the study of the chemical reactions that are catalyzed by enzymes.
- In enzyme kinetics, the reaction rate is measured and how get changes in response to changes in experimental parameters such as substrate concentrations, enzyme concentration etc.
- This is the oldest approach to understanding enzyme mechanism and remains the most important.
- The initial rate (or initial velocity),designated V_o, when [S] is much greater than the concentration of enzyme [E] can be measured by Michaelis-Menton kinetics. It is one of the simplest and best-known models of enzyme kinetics.

EFFECT OF SUBSTRATE CONCENTRATION ON ENZYME KINETICS

- The concentration of substrate [S] is a key factor affecting the rate of a reaction catalyzed by an enzyme.



- Fig. Effect of substrate concentration on the initial velocity of an enzyme catalyzed reaction.

EFFECT OF CATALYST

Catalyst:

The substance that alters or changes the rate of reactions without itself being consumed is known as a catalyst.

It is chemically unchanged at the end of the reaction.

The mass of the catalyst is exactly the same as the initial mass when the reaction is finished.

Ex: Sulfuric acid used in some esterification reactions.

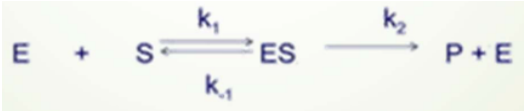
Effect of catalyst:

- A positive catalyst increases the rate of reaction.
- A negative catalyst decreases the rate of the reaction.
- The catalyst does not influence the amount of product.
- In the presence of a catalyst, an alternative pathway of reaction with lower activation energy is made available.
- More collisions of reactants are successful because less energy is required for success.
- After the reaction over, the catalyst can be used again and again.

Enzyme kinetics

- Enzyme Kinetics– Quantitative measurement of the rates of enzyme catalysed reactions and the systematic study of factors that affect these rates

- Enzyme kinetics began in 1902 when Adrina Brown reported an investigation of the rate of hydrolysis of sucrose as catalysed by the yeast enzyme invertase.
- Brown demonstrated– when sucrose concentration is much higher than that of the enzyme, reaction rate becomes independent of sucrose concentration
- Brown proposal– overall reaction is composed of two elementary reactions in which the substrate forms a complex with the enzyme that subsequently decomposes to products and enzymes



Here E, S, ES and P symbolize the enzyme, substrate, enzyme-substrate complex and products

k1 k2 E + S ES P + E k-1

According to this model,

- When the substrate concentration becomes high enough to entirely convert the enzyme to the ES form, the second step of the reaction becomes rate limiting step.
- The overall reaction rate becomes insensitive to further increase in substrate concentration.
- The general expression of the velocity (rate) of this reaction is

$$v = \frac{d[P]}{dt} = k_2[ES]$$

The overall rate of production of [ES]– Difference between the rates of elementary reactions leading to its appearance and those resulting in its disappearance.

$$E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E$$

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES]$$

At this point, an assumption is required to achieve an analytical solution.

- ✓ The rapid equilibrium assumption -Michaelis- Menten Approach
- ✓ The steady-state assumption - Briggs and Haldane Approach.

Michaelis-Menten Approach

The rapid equilibrium assumption: Assumes a rapid equilibrium between the enzyme and substrate to form an [ES] complex.

$$E+S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P+E$$

$$k_1[E][S]=k_{-1}[ES]$$

$$K_m=\frac{k_{-1}}{k_1}=\frac{[E][S]}{[ES]}$$

The equilibrium constant Km can be expressed by the following equation in a dilute system. Since the enzyme is not consumed, the conservation equation on the enzyme yields. Then rearrange the equilibrium constant equation. Substituting [E] in the above equation with enzyme mass conservation equation

$$[E]=[E_0]-[ES]$$

$$K_m=\frac{k_{-1}}{k_1}=\frac{[E][S]}{[ES]} \qquad [ES]=\frac{[E][S]}{K_m}$$

$$[ES]=\frac{([E_0]-[ES])[S]}{K_m}$$

$$[ES] = \frac{([E_0]-[ES])[S]}{K_m}$$

$$[ES]K_m = [E_0][S]-[ES][S]$$

$$[ES]K_m + [ES][S] = [E_0][S]$$

$$[ES](K_m + [S]) = [E_0][S]$$

$$[ES] = \frac{[E_0][S]}{K_m + [S]}$$

Then the rate of production formation v can be expressed in terms of [S]

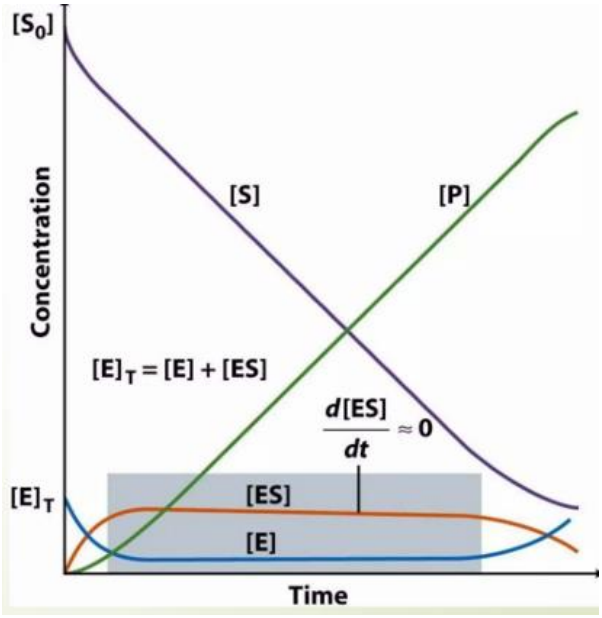
$$v = \frac{d[P]}{dt} = k_2[ES] = \frac{k_2[E_0][S]}{K_m + [S]} = \frac{V_{max}[S]}{K_m + [S]}$$

- Where $V_{max} = k_2[E_0]$

steady state assumption

Progress curve for the components of a simple Michaelis Menten reaction

- Except the transition phase of the reaction (before shaded block) [ES] remains constant until the substrate is nearly exhausted.
- Hence synthesis of ES must equals to its consumption over the course of reaction i.e. ES maintain steady state



$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

steady state assumption,

d[ES]/dt = 0

d[ES]/dt = k1[E][S] –k-1[ES] – k2[ES] = 0

(steady state assumption)

solve for [ES] (do some algebra)

[ES] = [E][S] k1/(k-1 + k2)

Define KM (Michaelis Constant)

KM = (k-1 + k2)/k1 => [ES] = [E][S]/KM SSA and Rate Equation

- Substitute $[E] = [E_0] - [ES]$ in K_M = [E][S]/[ES]

$$K_m = \frac{([E_0] - [ES])[S]}{[ES]}$$

$$K_m[ES] = ([E_0] - [ES])[S];\ [ES]K_m = = [E_0][S] - [ES][S]$$

$$[ES]K_m + [ES][S] = = [E_0][S]$$

$$[ES](K_m + [S]) = = [E_0][S]$$

$$[ES] = = \frac{[E_0][S]}{K_m + [S]}$$

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$$v = \frac{d[P]}{dt} = k_2[ES] = \frac{k_2[E_0][S]}{K_m + [S]} = \frac{V_{max}[S]}{K_m + [S]}$$

- Where $V_{max} = k_2[E_0]$
- Michaelis Menten Equation

$$v = \frac{V_{max}[S]}{K_m + [S]}$$

Michaelis-Menten equation, the rate equation for a one-substrate enzyme catalysed reaction. It is a statement of the quantitative relationship between the initial velocity V0, the maximum velocity Vmax, and the initial substrate concentration [S], all related through the Michaelis constant Km. Numerical relationship emerges from the Michaelis-Mentenequation in the special case whenV0isexactlyone-halfofVmax On dividing by Vmax we obtained

- Solving for Km,

$$\frac{V_{\max}}{2} = \frac{V_{\max}[\mathrm{S}]}{K_{\mathrm{m}} + [\mathrm{S}]}$$

$$\frac{1}{2} = \frac{[\mathrm{S}]}{K_{\mathrm{m}} + [\mathrm{S}]}$$

Km + [S] = 2[S] Km = [S] when

$$v_0 = \frac{1}{2} V_{max}$$

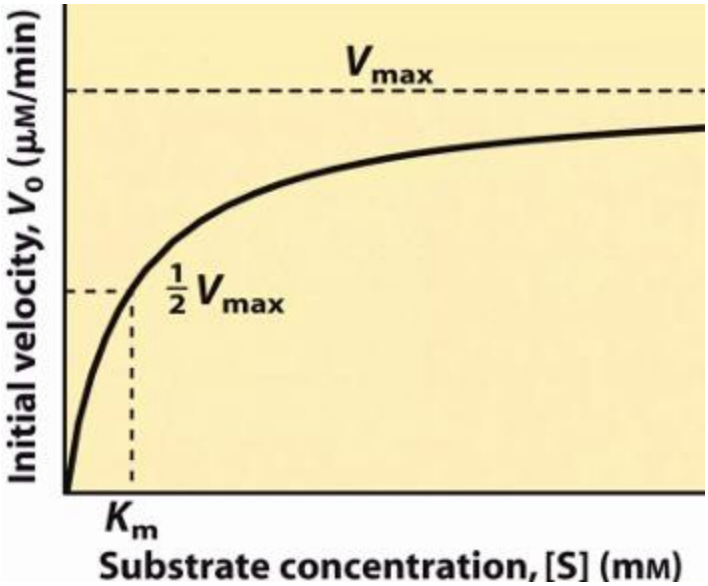
KM

- ✓ KM is the substrate concentration required to reach half-maximal velocity (Vmax/2).

- ✓ KM is a measure of a substrate's affinity for the enzyme. A small KM means the substrate binds tightly to the enzyme and saturates

Vmax

- ✓ Considering the total enzyme concentration the maximal rate, that the enzyme can attain is Vmax,.
- ✓ Vmax is equal to the product of the catalytic rate constant (K cat) and the concentration of the enzyme.
- ✓ The Michaelis-Menten equation can then be rewritten as V= K cat [Enzyme] [S] / (Km + [S]).
- ✓ K cat is equal to K2, and it measures the number of substrate molecules "turned over" by enzyme per second. The higher the K cat is, the more substrates get turned over in one second



Factors

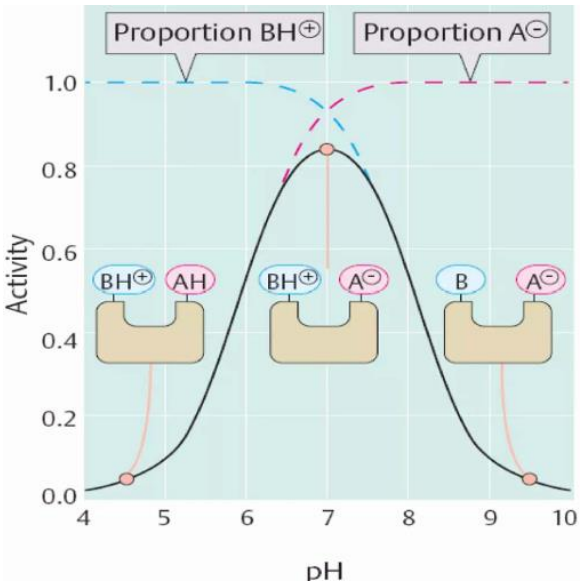
The catalytic properties of enzymes, and consequently their activity, are influenced by numerous factors. These factors include,

- ✓ Physical quantities (temperature, pressure),

- ✓ The chemical properties of the solution (pH value, ionic strength),
- ✓ The concentrations of the relevant substrates, cofactors, and inhibitors

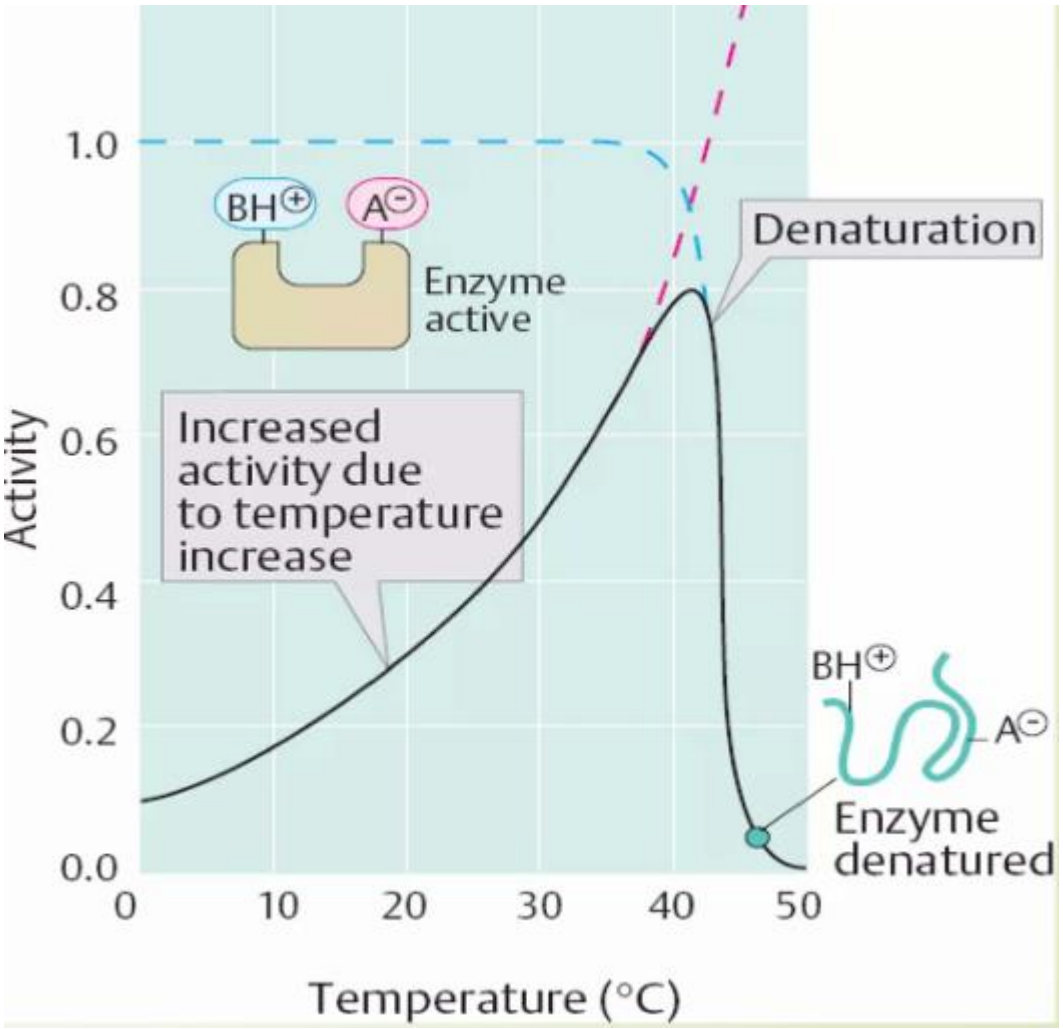
pH Dependency of Enzyme Activity

- ✓ Effect of enzymes is strongly dependent on the pH.
- ✓ Activity is plotted against pH, a bell-shaped curve is usually obtained.
- ✓ Bell shape of the activity–pH profile results from the fact that amino acid residues with ionizable groups in the side chain are essential for catalysis
- ✓ A basic group B (pKa = 8), which has to be protonated in order to become active.
- ✓ A second acidic amino acid AH (p Ka = 6), which is only active in a dissociated state.
- ✓ At the optimum pH of 7, around 90% of both groups are present in the active form.
- ✓ At higher and lower values, one or the other of the groups increasingly passes into the inactive state.



The temperature dependency of enzymatic activity

- ✓ The temperature dependency of enzymatic asymmetric. activity is usually
- ✓ With increasing temperature, the increased thermal movement of the molecules initially leads to a rate acceleration.
- ✓ At a certain temperature, the enzyme then becomes unstable, and its activity is lost within a narrow temperature difference as a result of denaturation.



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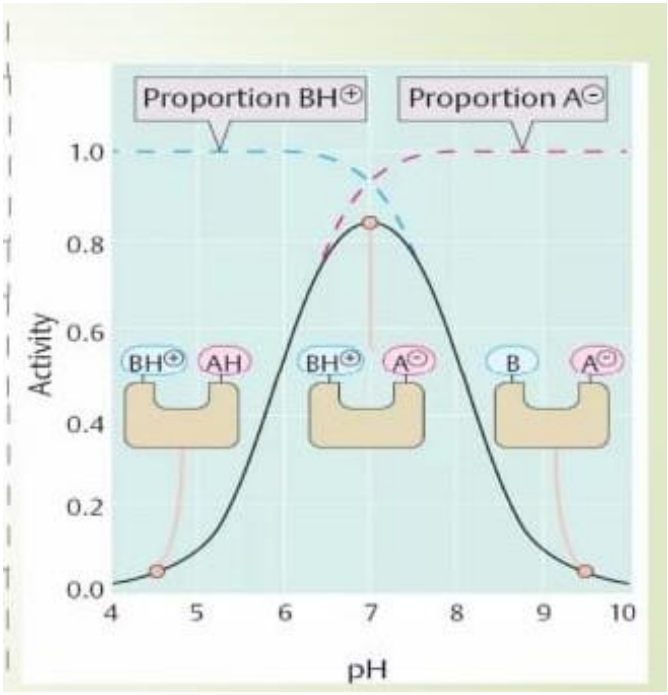

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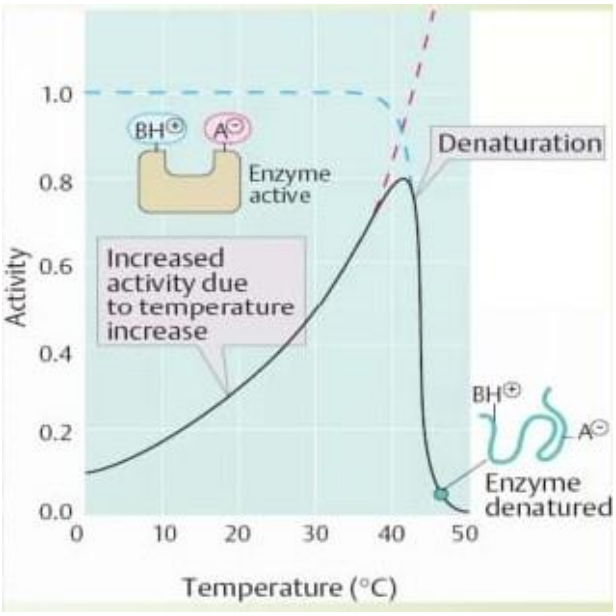

Factors contributing to the Efficiency of Enzyme

Enzyme

An enzyme is a biological catalyst and is almost always a protein. It speeds up the rate of a specific chemical reaction in the cell. The enzyme is not destroyed during the reaction and is used over and over. A cell contains thousands of different types of enzyme molecules, each specific to a particular chemical reaction.

Factors contributing to the Efficiency of Enzyme

Enzymes are protein molecules acting as biological catalysts to accelerate chemical reactions within the cell. The enzyme is a series of amino acids that link together to form a polypeptide chain; they are chemical catalysts similar to other stimulants/catalysts. They take part in the response but are unaffected. Scientists studying the fermentation process discovered the existence of enzymes in the mid-nineteenth century. In other words, enzymes increase the rate of chemical reactions within cells without consuming them: the hydrogen ion concentration (pH) and temperature impact enzymes. In comparison to other catalysts, enzymes are particular, with each enzyme specialised for a single reactant chemical.

Factors influencing enzyme action-

1. **Enzyme Concentration-**

When the enzyme's concentration increases, the reaction rate proportionately increases. This unique property of enzymes helps determine the serum's activities in diagnosing diseases.

2. **Substrate Concentration**-

The enzymatic reaction rate proportionally increases as the substrate concentration increases when there is a specific enzyme amount. But it increases to a limiting reach. After this, there are no reaction changes irrespective of the substrate concentration changes. Because there is so much substrate at this time, almost all of the enzyme active sites are coupled to it. In other words, the substrate has saturated the enzyme molecules. The left-over substrate molecules cannot react until the substrate-bound to the enzymes reacts and is released (or been released without the reaction). To put it simply, Substrates interact with enzymes and then transform
Enzyme denaturation kills life when the temperature becomes too high. Low temperatures also alter the structures of enzymes. Cold-sensitive enzymes lose activity as a result of the shift. As a result, enzymes are harmed by both cold and heat extremes. The ideal temperature for enzymes is between 37 and 40 degrees Celsius.

into products. When the concentration of substrate is increased, the enzymes' velocity increases.

3. **Effect of temperature on enzyme action-**

Enzymes work best when they're kept at a temperature that's comfortable for them. However, high temperatures can damage enzymes because they are protein molecules. When it is boiled, the curdling of milk is one example of such destruction, known as protein denaturation. In addition, the pace of chemical reactions tends to rise with temperature; therefore, increasing the temperature has two impacts on an enzyme: first, the reaction velocity increases slightly; and second, the enzyme becomes increasingly denatured. As a result, increasing the temperature only boosts the metabolic rate within a certain range.

4. **Effect of pH on enzyme action-**

Acids are liquids with a pH less than 7, whereas bases or alkaline are liquids with pH more than 7. At 25 degrees Celsius, pH seven drinks are neutral and have the same acidity as pure water. The pH indicators can be used to determine the pH of any solution. Proteins with acidic carboxylic groups (-COOH) and basic amino groups are known as enzymes (NH2). As a result, changing the pH value impacts the enzymes.

There is an ideal pH concentration for the enzyme to work, just as there is an optimum temperature. The activity of enzymes is reduced as the pH falls or rises. Some enzymes have strong catalytic activity in acidic environments, while others have good activity in alkaline environments. Every enzyme has a pH range where its activity is at its peak.

5. **Effect of activators-** For optimal activity, several enzymes require inorganic metallic cations such as Mn2+, Mg2+, Zn2+, Co2+, Ca2+, Cu2+, K+, Na+, and others. Anions are occasionally needed for enzyme function, such as a chloride ion (CI–) for am