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## KANPUR UNIVERSITY'S

# QUESTION BANK

Based On NEP 2020

## M.Sc. III SEM

# BIOINORGANIC, BIOORGANIC AND BIOPHYSICAL CHEMISTRY

- Brief and Intensive Notes
- Long & Short Answers

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## M. Sc. (III) Semester

## CHEMISTRY

## Bioinorganic, Bioorganic, and Biophysical Chemistry

## B020901T


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# SYLLABUS

## A. BIO-INORGANIC CHEMISTRY

**1. Metal Ions in Biological Systems:** Essential and trace metals.

**2. Na+/K+ Pump:** Role of metals ions in biological processes,

**3. Bioenergetic and ATP Cycle:** DNA polymerization, glucose storage, metal complexes in energy transmission, chlorophylls, photosystem I and photosystem II in water cleavage, model systems.

**4. Transport and storage of Dioxygen:** Heme protein and oxygen uptake, structure and function of hemoglobin, myoglobin, and hemerythrin, model synthetic complexes of iron and copper

## B. BIO-ORGANIC CHEMISTRY

**1. Enzymes:** Introduction and historical perspective, chemical and biological catalysis, remarkable properties of enzymes like catalytic power, specificity and regulation, nomenclature and classification, extraction and purification. Fischer's lock and key and Koshland's induced fit hypothesis, concept, and active site identification using inhibitors, affinity labeling, and enzyme modification by site-directed mutagenesis.

**2. Mechanism of enzyme action:** Transport state theory, orientation, steric effect, acid-base catalysis, covalent catalysis, strain or distortion, examples of some typical enzyme mechanisms for chymotrypsin, ribonuclease.

**3. Co-Enzyme Chemistry:** Cofactors as derived from Vitamins, coenzymes, prosthetic groups, apoenzymes, structure, and biological functions of coenzyme A,

thiamine pyrophosphate, pyridoxalphosphate, NAD+, NADP+, FMN, FAD, lipoic acid, vitamin B12, mechanism of reactions catalyzed by the above cofactors.

## C. BIOPHYSICAL CHEMISTRY

**1. Biological cell and its constituents:** Biological cell, structure and functions of proteins, enzymes, DNA and RNA in living systems, Helix coil transition.

**2. Bioenergetics:** Standard free energy change in biochemical reactions, exergonic, endergonic, hydrolysis of ATP, synthesis of ATP from ADP.

**3. Thermodynamics of biopolymer solutions:** Thermodynamics of biopolymer solutions, osmotic pressure, membrane equilibrium, muscular contraction, and energy generation in the mechanochemical system ns in Biological Systems: Essential and trace metals.

# Bioinorganic Chemistry

## NOTES

### Metal Ions in Biological Systems

Metal ions are integral to many biological processes and are involved in various biochemical functions, including catalysis, structural stabilization, and electron transfer. These metal ions typically exist in either a free form or as part of metal-containing biomolecules (metalloproteins, metalloenzymes, etc.).

### 1. Classification of Metal Ions in Biological Systems

- **Essential Metal Ions** are metal ions required to function properly in biological systems. They are crucial for maintaining life.
    - **Major Essential Metals**: Iron (Fe), Copper (Cu), Zinc (Zn), Manganese (Mn), Magnesium (Mg), Calcium (Ca).
    - **Role**: These metals often serve as cofactors in enzyme activity, structural components of proteins, or regulating various biochemical pathways.
- **Toxic Metal Ions**: Some metal ions are toxic in excess, such as mercury (Hg), lead (Pb), and cadmium (Cd). They disrupt cellular functions by binding to proteins, displacing essential metals, or producing oxidative stress.

### 2. Roles of Metal Ions in Biological Systems

- **Enzyme Catalysis**: Many enzymes require metal ions for their activity. For example, zinc in carbonic anhydrase or magnesium in ATPases.
- **Electron Transport**: Metal ions are involved in the electron transfer chain in cellular respiration. Iron and copper, for instance, are found in cytochrome complexes that transfer electrons.
- **Structural Role**: Some metal ions are key to maintaining the structure of biomolecules, such as zinc in zinc fingers that stabilize protein structures.
- **Signaling**: Calcium ions ($Ca^{2+}$) act as intracellular messengers in muscle contraction, neurotransmitter release, and cell division.

- **Oxygen Transport and Storage**: Iron (in hemoglobin) and copper (in hemocyanin) play roles in oxygen transport and storage in the blood.

**3. Types of Metal Ions**

- **Monovalent Ions**: Sodium ($Na^+$) and Potassium ($K^+$) are primarily involved in maintaining osmotic balance and nerve function.
- **Divalent Ions**: Calcium ($Ca^{2+}$), Magnesium ($Mg^{2+}$), Zinc ($Zn^{2+}$), Copper ($Cu^{2+}$) – involved in enzymatic activity, structural roles, and signaling.
- **Trivalent Ions**: Iron ($Fe^{3+}$), Aluminum ($Al^{3+}$) – iron plays a role in electron transport and oxygen binding, and aluminum is often considered toxic.

**4. Metal Ions in Disease**

- **Deficiency**: Deficiencies in metal ions, such as iron (leading to anemia) or zinc (causing immune dysfunction), can result in various diseases.
- **Excess**: Metal ion overload, such as in Wilson's disease (excess copper) or hemochromatosis (excess iron), can lead to toxicity and organ damage.
- **Free Radical Formation**: Metal ions, particularly iron and copper, can catalyze the formation of reactive oxygen species (ROS), leading to oxidative stress and cell damage.

**Essential and Trace Metals**

**1. Essential Metals**

Essential metals are required for various biochemical functions. They can be classified as **macrominerals** (needed in large amounts) and **microminerals** (needed in trace amounts).

- **Macrominerals**: These metals are required in larger quantities and play fundamental roles in cellular function.
    - **Calcium ($Ca^{2+}$)**: Necessary for bone and teeth formation, muscle contraction, neurotransmitter release, and blood clotting.
    - **Magnesium ($Mg^{2+}$)**: Involved in over 300 enzyme reactions, including protein synthesis, muscle function, and nerve transmission. It also stabilizes the structures of DNA and RNA.
    - **Potassium ($K^+$)**: Essential for maintaining cell membrane potential, nerve impulse transmission, and fluid balance.

  - **Sodium ($Na^+$)**: Key in maintaining fluid balance, osmotic pressure, and nerve function.

- **Microminerals (Trace Metals)**: These are required in smaller amounts but are just as essential.

  - **Iron (Fe)**: Vital for oxygen transport in the blood (hemoglobin) and muscle (myoglobin), as well as electron transport in mitochondria.

  - **Copper (Cu)**: Plays a role in redox reactions, iron metabolism, and the formation of hemoglobin. It is a component of enzymes like cytochrome c oxidase and superoxide dismutase.

  - **Zinc (Zn)**: Involved in hundreds of enzymes related to DNA synthesis, protein synthesis, immune function, and cell division.

  - **Manganese (Mn)**: Involved in enzyme activation and cofactor in oxidative stress protection and bone formation processes.

  - **Cobalt (Co)**: A component of vitamin B12, crucial for red blood cell production and DNA synthesis.

**2. Trace Metals in Detail**

- **Iron (Fe)**: Essential for oxygen transport in hemoglobin and myoglobin. Iron deficiency leads to anemia. Iron is also a cofactor in various enzymes involved in cellular metabolism.

- **Copper (Cu)**: Essential for the function of enzymes involved in energy production, iron metabolism, and neurotransmitter synthesis. Excessive copper can lead to toxicity (e.g., Wilson's disease).

- **Zinc (Zn)**: Required for the activity of over 300 enzymes. Zinc is involved in immune function, wound healing, protein synthesis, and DNA synthesis. Deficiency can lead to stunted growth, skin lesions, and impaired immune response.

- **Manganese (Mn)**: Important for bone development, wound healing, and blood sugar regulation. It also functions as an antioxidant by activating the enzyme superoxide dismutase.

- **Iodine (I)**: Critical for thyroid function, where it is a component of thyroid hormones (T3 and T4), which regulate metabolism. Iodine deficiency leads to goiter and developmental problems.

- **Selenium (Se)**: Important for antioxidant protection, thyroid function, and immune defense. Selenium is a cofactor for enzymes like glutathione peroxidase.

**3. Toxicity of Essential Metals**

- While essential metals are vital for health, excessive intake can be harmful.
    - **Iron overload** (hemochromatosis) can cause liver damage, diabetes, and heart problems.
    - **Copper toxicity** (Wilson's disease) can lead to neurological damage and liver cirrhosis.
    - **Zinc toxicity** can lead to gastrointestinal problems and impair copper absorption.
    - **Manganese toxicity** (manganism) is associated with neurological issues resembling Parkinson's disease.

**4. Deficiency of Essential Metals**

- **Iron deficiency** leads to anemia, fatigue, and impaired immune function.
- **Zinc deficiency** can cause growth retardation, hair loss, and delayed wound healing.
- **Iodine deficiency** can lead to goiter, hypothyroidism, and developmental delay.
- **Magnesium deficiency** can cause muscle cramps, fatigue, and irregular heartbeat.

**5. Trace Metals with Potential Toxicity**

Despite being essential in small quantities, some trace metals can be toxic at higher concentrations. These include:

- **Lead (Pb)**: Accumulation in the body can cause neurological damage, developmental delays, and organ damage.
- **Mercury (Hg)**: Exposure leads to neurological and kidney damage and disrupts protein function.
- **Cadmium (Cd)**: Associated with kidney damage, bone loss, and cancer risk.

## Short answer type question:

**Q. Discuss the biological role of $Mg^{2+}$ and $Ca^{2+}$ in living cells. (CSJMU 2020)**

**Ans. Magnesium**

- Magnesium is a macronutrient that the body needs in substantial quantities. About 25 gms of Mg is found in the human body. Seventy percent of the body's mg is found in the bones and teeth.
- Mg2+ is a phosphatase enzyme activator and an important cation in intracellular fluid. Magnesium, calcium, and phosphorous combine to produce a complex salt of bones.
- It is an important part of chlorophyll.
- It also participates in the ATP hydrolysis process (a universal energy source). Magnesium, like calcium, helps with muscular contraction, blood coagulation, lung function, and blood pressure management. It is involved in a variety of life-sustaining processes.

**Calcium**

Calcium ions (Ca2+) play a vital role in many biological processes, including:

- **Cell signalling**
- Ca2+ is a second messenger that translates signals inside and outside the cell into intracellular effects. It controls many cellular processes, including muscle contraction, gene transcription, and cell proliferation.
- **Blood clotting**
- Ca2+ is a key part of the coagulation cascade, which maintains homeostasis. Ca2+ activates platelets and coagulation factors, such as coagulation Factor XIII (FXIII).
- **Calcium homeostasis**
- The concentration of Ca2+ inside cells must be carefully regulated to balance cell function and cell death. Cells use a variety of mechanisms to generate and interpret Ca2+ signals and to maintain calcium homeostasis.

**Q. What is the role of $Zn^{2+}$ in the human body? (CSJMU 2013)**

Ans. Zn is the second most abundant dress material. The average Zinc content in the body is approximately 2g. It is distributed in different body parts, like bones, teeth, skin, kidneys, and muscle acceptors. It is essential for normal human body growth, wood healing, and tissue repairing. It regulates insulin's function and maintains the normal concentration of vitamin A.

**Essential properties of zinc ion**

- Zinc supplements to reduce cold symptoms such as age, runny nose, and coughing. Zinc makes skin, nails, and hair healthy. Zinc helps in healing wounds. Zinc reduces diabetic problems. Zinc increases the production of testosterone and other male hormones.

Therefore, it reduces male infertility. Zinc helps to preserve eye sight and improves memory. Zinc may help teenagers with pimples.

**Q. What is the role of cobalt in vitamin $B_{12}$? (CSJMU 2015)**

**Ans.** One of the most important trace metals is cobalt. Itis one the most ancient biocatalysts. Only around 1.5 mg of cobalt is found in the human body, most of which is in the form of cobalamin, or vitamin B12. Cobalt is complexed in B12 by a special macrocycle called corrin. A benzimidazole that is covalently linked to the corrin ring is known as cobalamin. Cobalamins are cofactors in enzymes that catalyze alkyl transfer reactions and many radical-based rearrangements. Cobalamin-containing enzymes show strong UV–visible absorption bands; EPR spectra are observed for Co (II). Animals or plants cannot synthesize vitamin B12. In reality, only a few microorganisms are capable of producing it. Humans get 100% of their vitamin B12 from animal sources, particularly meat. Because vitamin B12 is only required in trace amounts, vitamin B12 deficiency is uncommon. However, vegans who eat no animal products have been observed to have vitamin B12 deficiency.

**Q. What is the role of iron in biological systems? (CSJMU 2016, 2015, 2014, 2012)**

**Ans.** Iron is a vital trace element for the human body. The average human body has 2.4 grams of iron. It has been found in the active centers of proteins involved in O2 transport (like hemoglobin and myoglobin) and electron transport (like cytochromes) and in the active sites of metalloenzymes like nitrogenase, reductase, and hydrogenase. Essential (or function) iron and storage iron are the two types of iron found in the human body. Essential iron is involved in the normal metabolism of cells.

**Q. What is the role of manganese in oxygen evolution? (CSJMU 2013,2017)**

**Ans.** Manganese plays a role in oxygen evolution in several ways, including:

**1. Photosynthesis**

Manganese is involved in the water-splitting metalloenzyme that produces molecular oxygen. It is also required for hydrolysis in the oxygen-evolving complex of photosystem II.

**2. Evolution of life**

Manganese helped cells survive the rise of oxygen.

**3. Oxygen evolution reaction (OER)**

Manganese-based oxides (MnO) are promising materials for OER. They have several properties that make them suitable for OER, including a large number of unsaturated edge sites on the surface and improved active area.

**4. Electrodeposited manganese oxide films**

These films are stable oxygen evolution catalysts that can catalyze the OER in acidic solutions. Their performance can be improved by "activating" them with potential cycling protocols.

# Long answer type question:

**Q. Discuss the role of metal ions in biological systems. (CSJMU 2018,17,15)**

Ans. Metal ions play a vital role in biological systems, participating in many biochemical processes and contributing to the proper functioning of cells, tissues, and organs:

- **Cofactors for proteins**: Metal ions are essential cofactors for many proteins and are used in enzyme design and protein-protein interaction design.
- **Biochemical reactions**: Metal ions catalyze many biochemical reactions, including those in bacteria.
- **Intra- and intercellular communication**: Metal ions communicate between cells and tissues.
- **Electrical charges and osmotic pressure**: Metal ions help maintain electrical charges and osmotic pressure.
- **Photosynthesis and electron transfer**: Metal ions participate in photosynthesis and electron transfer processes.
- **DNA transcription**: Metal ions regulate DNA transcription.
- **Nerve cells, muscle cells, brain, and heart**: Metal ions contribute to the properly functioning of these cells and organs.
- **Oxygen transport**: Metal ions are involved in the transport of oxygen.
- Some examples of metal ions and their roles in biological systems include:

**1. Calcium**: Essential for bone and teeth formation and signal transduction.

**2. Sodium and potassium**: Involved in opening and closing ion channels, which is crucial for transmitting nerve impulses.

**3. Copper and iron** play a significant role in the brain.

Metal ions can cause health problems if they are deficient or in excess.

**Q. Write the classification of essential elements. (CSJMU 2015)**

**Ans. Essential and Trace elements:**

Only about seven elements are known to be needed for the efficient functioning of the human body out of more than 100 identified elements. Essential elements include Na, K, Mg, Ca, P, Fe, Mn, S, Zn, Cu, Co, Cr, Mo, Cl, F, I, and Se. They are said to be crucial since the organism would not be able to thrive without them. Essential components are divided into two categories based on their absolute quantities in the body:

A. Macronutrients B. Micronutrients

The rest of the elements, which are only required in small amounts by the body, are called micronutrients. They are also known as trace elements because they are only needed in trace levels in the body.

**Metal ions in biological Systems**

1. Sodium: Sodium is the principal electrolyte found in high concentrations in extracellular fluid (140 mmol/L) Na+ is the principal. It regulates the body's osmotic pressure. In the body, sodium is found chiefly in chloride and bicarbonate, as NaCl and $NaHCO_3$, respectively. Adults require between 1 and 3.5 grams of salt each day. It enhances glucose and amino acid absorption. In conjunction with chloride and bicarbonate, it maintains an acid-base balance. It is involved in the control of membrane potential. The most frequent source of sodium in cooking is table salt (NaCl). Bread, cheese, carrots, cauliflower, egg nuts, spinach, and other foods are among the other sources. A lack of sodium causes headaches and abdominal muscle cramps. On the other hand, high blood pressure is caused by a high intake of table salt.

2. Potassium: K+ is the principal cation of the intracellular fluid. It increases the activity of cardiac muscles. Along with Na+, it maintains the osmotic pressure of the body. It also maintains an acid-base balance. It increases the activity of the enzymes like pyruvate kinase. It also plays a prominent role in blood coagulation and the synthesis of ribosomes. The sound sources of potassium are chicken, beef liver, banana, orange juice, pineapple, etc., and deficiency of potassium leads to depression and also affects the nervous system.

3. Magnesium is a macronutrient that the body needs in substantial quantities. About 25 gms of Mg is found in the human body. Seventy percent of the body's mg is found in the bones and teeth. $Mg^{2+}$ is a phosphatase enzyme activator and an important cation in intracellular fluid. Magnesium, calcium, and phosphorous combine to produce a complex salt of bones. It is an important part of chlorophyll. It also participates in the ATP hydrolysis process (a universal energy source).

Magnesium, like calcium, helps with muscular contraction, blood coagulation, lung function, and blood pressure management. It is involved in a variety of life-sustaining processes. Role of magnesium in enzyme action, energy production, Nerve conduction, muscle protein formation, nucleic acid stabilization, and DNA synthesis. Nuts, soybeans, and seafood are good sources of magnesium. Mg deficiency results in neuromuscular dysfunction.

4. Calcium is the most abundant mineral in the human body. It is the principal constituent of teeth and bones. About 90% of the body's calcium is in the skeleton, maintained as a deposit of calcium phosphate. Calcium and phosphorus are the principal minerals of bone and teeth, where they exist as the double salt of calcium and phosphate, CaCO3.nCa3(PO4)2 (n ranging from 2 to 3). These minerals lend hardness and strength to these tissues. A little calcium is scattered in soft tissue like muscles and organs. Calcium helps in blood coagulation. It plays a prominent role in muscle contraction. Ca is a cofactor of various enzymes like protein kinase, lipase, adenylate, and cyclase. It also helps with nerve action. The chief sources of calcium are milk, eggs, nuts, beans, cabbage, cauliflower, etc. Deficiency of calcium leads to the disease rickets in children (weakness of bones) and osteoporosis in adults. However, excess calcium adversely affects the body, giving rise to the formation of stones.

5. Phosphorus: Phosphorous, in the form of phosphate, is present in the body. The total body phosphate content is approximately 700 g. More than 85 percent is found in bones, with only about 15 percent in soft tissues and 1 percent in extracellular fluid. 90% of daily dietary phosphate absorption is found in bone and teeth in conjunction with calcium. It is also a component of DNA and RNA, which serve as the foundation for life and growth. Furthermore, it is required for the phosphorylation-mediated regulation of enzyme activity. Vitamin D boosts intestinal phosphate absorption by increasing the expression of the Na-P co-transporter in the small intestine.

# Notes

**Na+/K+ Pump (Sodium-Potassium Pump):** The **Na+/K+ pump** (also known as **sodium-potassium ATPase**) is a vital membrane-bound enzyme found in the plasma membranes of most animal cells. It plays a crucial role in maintaining cellular homeostasis by actively transporting sodium ($Na^+$) and potassium ($K^+$) ions across the cell membrane against their concentration gradients.

**1. Structure and Mechanism:**

- **Protein Composition**: The Na+/K+ pump consists of two main subunits:

- **Alpha subunit (α)**: Responsible for binding ATP and transporting the ions. It has ten transmembrane domains and is the catalytic subunit.
- **Beta subunit (β)**: Helps in the correct positioning and functioning of the alpha subunit, though it is not directly involved in ion transport.
- **Gamma subunit (optional)**: Some pump variations include a gamma subunit, which can modulate activity, though it is not essential for function.

- **Mechanism**: The Na+/K+ pump operates through an **active transport** mechanism using energy from ATP hydrolysis. It follows these steps:

1. **Binding of $Na^+$**: Three $Na^+$ ions bind to the intracellular side of the pump.

2. **ATP Hydrolysis**: ATP is hydrolyzed into ADP and inorganic phosphate (Pi), which induces a conformational change in the pump.

3. **$Na^+$ Transport**: This conformational change leads to the pump releasing the three $Na^+$ ions to the outside of the cell against their concentration gradient.

4. **Binding of $K^+$**: Two $K^+$ ions from the extracellular fluid bind to the pump.

5. **Dephosphorylation**: The phosphate group is released, causing the pump to revert to its original shape.

6. **$K^+$ Transport**: This causes the two $K^+$ ions to be transported into the cell against their concentration gradient.

**2. Function and Importance:**

- **Maintaining Ion Gradients**: The Na+/K+ pump helps to maintain high concentrations of $K^+$ inside the cell and high concentrations of $Na^+$ outside the cell. This is critical for various cellular functions.
  - **$Na^+$ Gradient**: Helps generate the resting membrane potential, enabling nerve impulse transmission.
  - **$K^+$ Gradient**: Essential for maintaining cell volume, osmotic balance, and proper cellular function.
- **Electrochemical Gradient**: The pump contributes to the **electrochemical gradient**, which is the combined gradient of the concentration and electrical potential differences across the

membrane. This is crucial for **nerve signaling**, **muscle contraction**, and **nutrient transport**.

**3. Energy Use:**

- **ATP Hydrolysis**: The pump uses energy from the hydrolysis of ATP to move ions against their concentration gradients. Each cycle of the Na+/K+ pump uses one ATP molecule to transport three $Na^+$ ions out of the cell and two $K^+$ ions into the cell.
- The continuous activity of the pump consumes a significant portion of the cell's ATP, typically around **30% to 70%** of the total cellular ATP consumption, depending on the cell type and activity level.

**4. Role in Cell Homeostasis:**

- **Cell Volume Regulation**: By pumping out $Na^+$ ions and taking in $K^+$ ions, the Na+/K+ pump helps to prevent excessive water influx into the cell, thus maintaining osmotic balance and preventing cell swelling.
- **Action Potential**: In neurons and muscle cells, the Na+/K+ pump helps reset the membrane potential after an action potential has been propagated, ensuring the cell is ready for the next signal.

**5. Clinical Relevance:**

- **Cardiac Function**: The Na+/K+ pump plays a key role in heart muscle function. Inhibition of the pump (e.g., by **digoxin**, a drug used to treat heart failure) can increase intracellular calcium concentration, enhancing the force of contraction.
- **Neurological Disorders**: Disruption of Na+/K+ pump activity can result in **neurological disorders**. For example, mutations in the pump's alpha subunit are associated with **familial hemiplegic migraine**, and other pump defects can cause **muscular dystrophy** or **periodic paralysis**.
- **Cellular Edema**: If the pump is dysfunctional, it can lead to the accumulation of $Na^+$ inside the cell, causing water influx and cellular swelling, which can result in cell death or tissue damage (e.g., in stroke or ischemia).

**6. Physiological Impact:**

- **Nerve Signaling**: The Na+/K+ pump is critical for maintaining the resting membrane potential of neurons. Without it, the cell would lose the ability to generate action potentials essential for nerve signal transmission.
- **Muscle Contraction**: The Na+/K+ pump helps reset the membrane potential in muscle cells after each contraction, which is necessary for proper muscle function.

**7. Evolutionary Perspective:**

- The Na+/K+ pump is highly conserved across species, indicating its critical importance in cellular function. The fundamental ion exchange and ATP hydrolysis mechanism has been maintained from **single-celled organisms** to complex **multicellular organisms** like humans.

The **Na+/K+ pump** is a fundamental enzyme responsible for maintaining sodium and potassium concentration gradients across the cell membrane, essential for numerous physiological processes like **nerve conduction**, **muscle contraction**, and **cellular homeostasis**. It functions through active transport mechanisms powered by ATP hydrolysis, and its proper functioning is crucial for the overall health and activity of cells in various tissues. Metal ions play crucial roles in various biological processes, acting as cofactors or components in enzymes, proteins, and other biomolecules. These metal ions are vital for adequately functioning many biochemical pathways and cellular processes. Below is a detailed overview of the significant metal ions involved in biological processes:

**1. Common Metal Ions in Biological Systems**

- **Iron ($Fe^{2+}/Fe^{3+}$)**, **Magnesium ($Mg^{2+}$)**, **Calcium ($Ca^{2+}$)**, **Zinc ($Zn^{2+}$)**, **Copper ($Cu^{2+}/Cu^{+}$)**, **Manganese ($Mn^{2+}$)**, **Nickel ($Ni^{2+}$)**, **Cobalt ($Co^{2+}$)**

**2. Iron (Fe)**

- **Role in Oxygen Transport:**
    - Hemoglobin and myoglobin contain iron in their heme groups, which bind to oxygen. Iron allows for the reversible binding and release of oxygen in the bloodstream and muscle tissue.
- **Role in Enzymatic Reactions:**
    - Iron is a cofactor in several enzymes involved in electron transfer, such as cytochrome P450 and ribonucleotide reductase.
- **Iron Deficiency and Disease:**

- Iron deficiency leads to anemia, characterized by reduced oxygen-carrying capacity in the blood.

- **Oxidation States:**
  - Iron can exist in two oxidation states ($Fe^{2+}$ and $Fe^{3+}$), allowing it to participate in redox reactions.

**3. Magnesium ($Mg^{2+}$)**

- **Role in Enzyme Function:**
  - Magnesium is a cofactor for more than 300 enzymes, particularly in ATP-dependent processes. It stabilizes the phosphate groups in ATP and helps enzymes like DNA polymerase function properly.

- **Structural Role:**
  - Magnesium stabilizes the structure of ribosomes and nucleic acids (e.g., DNA and RNA).

- **Bone and Muscle Function:**
  - Magnesium is critical for maintaining bone health and proper muscle function. It plays a key role in muscle contraction and relaxation by interacting with calcium.

**4. Calcium ($Ca^{2+}$)**

- **Role in Signal Transduction:**
  - Calcium ions are critical in intracellular signaling pathways. They act as secondary messengers in signal transduction, influencing processes like muscle contraction, neurotransmitter release, and gene expression.

- **Muscle Contraction:**
  - In muscle cells, releasing calcium from the sarcoplasmic reticulum is essential for contraction. Calcium binds to troponin, which triggers the contraction mechanism in skeletal and cardiac muscle fibers.

- **Bone Formation:**
  - Calcium is a major component of bones and teeth, contributing to their structure and strength.

- **Blood Clotting:**
    - Calcium is required to activate clotting factors in the coagulation cascade, leading to blood clot formation.

**5. Zinc ($Zn^{2+}$)**

- **Cofactor for Enzymes:**
    - Zinc is involved in more than 300 enzymes, playing a role in protein synthesis, nucleic acid metabolism, and cell division. It is critical for enzymes like DNA polymerase and carbonic anhydrase.
- **Role in Immune Function:**
    - Zinc is essential for normal immune function, including developing and activating T-lymphocytes. Zinc deficiency can lead to impaired immune responses.
- **Antioxidant Properties:**
    - Zinc has antioxidant properties and protects cells from oxidative damage. It is also a component of the enzyme superoxide dismutase (SOD).
- **Gene Regulation:**
    - Zinc fingers, a structural protein motif, help regulate gene expression by binding to DNA.

**6. Copper (Cu)**

- **Redox Reactions:**
    - Copper functions as a redox-active metal ion, cycling between $Cu^{2+}$ and $Cu^{+}$ states. It is a component of enzymes such as cytochrome c oxidase (involved in cellular respiration) and superoxide dismutase (SOD).
- **Iron Metabolism:**
    - Copper is involved in iron metabolism by aiding in the conversion of $Fe^{2+}$ to $Fe^{3+}$, which is required to function iron-containing enzymes and proteins properly.
- **Collagen Synthesis:**

- Copper is essential for the activity of lysyl oxidase, an enzyme involved in the formation of collagen and elastin fibers, contributing to the structural integrity of connective tissues.

**7. Manganese ($Mn^{2+}$)**

- **Cofactor in Enzymes:**
  - Manganese is a cofactor for several enzymes, including manganese superoxide dismutase (MnSOD), which protects cells from oxidative stress by dismutating superoxide radicals.
- **Bone Development:**
  - Manganese is involved in bone formation and is required for synthesizing glycosaminoglycans, which are important for cartilage and connective tissues.
- **Metabolism of Amino Acids:**
  - Manganese plays a role in the metabolism of amino acids, including the breakdown of carbohydrates and the synthesis of fatty acids.

**8. Nickel ($Ni^{2+}$)**

- **Enzyme Activity:**
  - Nickel is an essential cofactor for several enzymes, particularly those involved in nitrogen metabolism, such as urease (which catalyzes the hydrolysis of urea).
- **Role in Cellular Respiration:**
  - Nickel is important for enzymes involved in electron transfer in some organisms, particularly bacteria.

**9. Cobalt ($Co^{2+}$)**

- **Vitamin $B_{12}$ Coenzyme:**
  - Cobalt is a critical component of vitamin $B_{12}$ (cobalamin), which is required for DNA synthesis, red blood cell formation, and proper neurological function.
- **Methylation Reactions:**
  - Cobalt in vitamin $B_{12}$ is involved in methylation reactions, crucial for synthesizing methionine and other important compounds.

**10. Other Trace Metals**

- **Chromium (Cr):**
    - Chromium plays a role in insulin function by enhancing binding to its receptor, thus aiding in glucose metabolism.
- **Selenium (Se):**
    - Selenium is a component of selenoproteins, such as glutathione peroxidase, which protect cells from oxidative damage.

**11. Metal Ion Imbalance and Diseases**

- **Deficiency:**
    - Deficiency in essential metal ions can lead to various health conditions, such as anemia (iron deficiency), osteoporosis (calcium or magnesium deficiency), and immune dysfunction (zinc deficiency).
- **Toxicity:**
    - Excessing specific metal ions can lead to toxicity, such as copper toxicity causing Wilson's disease or iron overload leading to hemochromatosis.

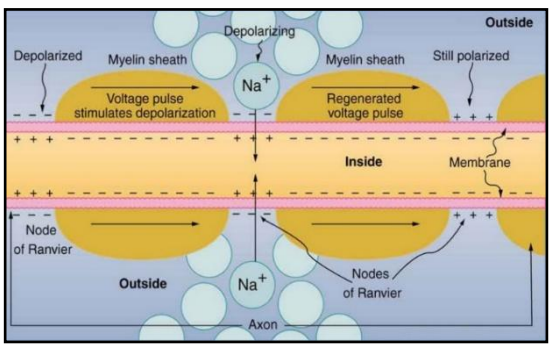
**12. Role in Redox Reactions**

- Many metal ions, like iron, copper, and manganese, are involved in redox reactions that drive essential biochemical processes such as respiration, photosynthesis, and detoxification. Their ability to switch between different oxidation states facilitates electron transfer, which is crucial for cellular metabolism.

## Short answer type question:

**Q.1- Write a short note on nerve conductance. (CSJMU2018,2015,2014)**

**Ans.** Nerves are like cables that carry electrical impulses or signals between your brain and the rest

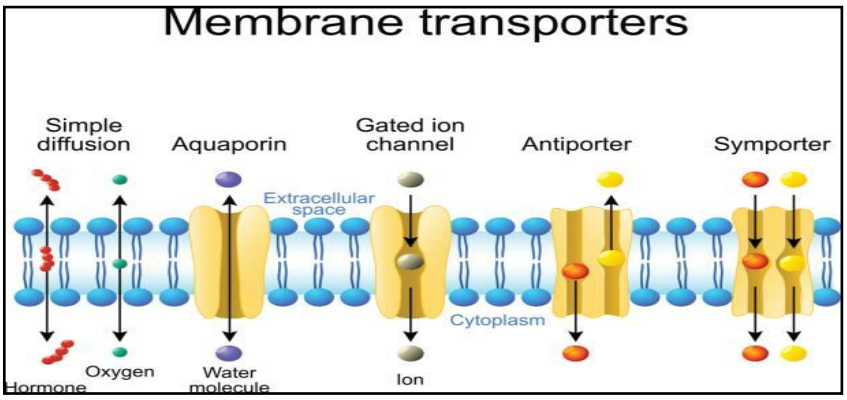


of your body. These impulses help you feel sensations and move your muscles. A nerve conduction study tests the transmission of these signals, especially the speed at which they travel and their "strength." The study involves wires (electrodes) taped to your skin in specific places along a nerve pathway. A provider stimulates the nerve with a mild electrical shock. As the electrical current travels down the nerve, the electrodes record the current and how fast it travels. If the provider stimulates a motor nerve, they measure the response of the muscle it controls. If they stimulate a sensory nerve, they record the response somewhere along the nerve**.** In healthy nerves, electrical signals can travel up to 120 miles per hour. If your nerve is damaged, the current will be slower and weaker. The provider can determine the specific site of the nerve injury or issue by stimulating the nerve at various places.

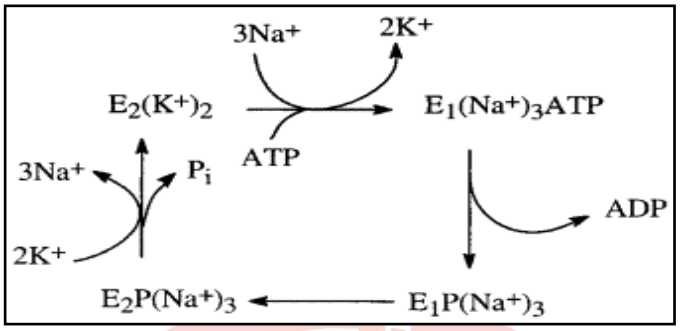
## Long answer type question:

**Q. How are Na+ and K+ ions transported through the cell membrane? Write the importance of the $Na^+$ / $K^+$ pump. (CSJMU2020, 2019,2017,2015,2014,2013)**

**Ans.** Jens Skou discovered the Na+, K+ -ATPase, a transmembrane protein 1957. With the hydrolysis of intracellular ATP, this enzyme is also known as the Na+, -K+ pump because it pumps three Na+ out and two K+ into the cell. In animal cells, the Na+-K+ pump keeps the cell's Na+ concentration low while maintaining a high K+ concentration in the extracellular medium. The transmembrane electric potential is created by ion transport. E1 and E2 are the two conformations of the Na+-K+-ATPase. E1 has a high-affinity Na+ binding site, while E2 has a high-affinity K+ binding site. The E1 conformation enzyme binds three Na* from the cell's interior. The phosphate group is transferred to the aspartic acid residue of the transport protein after E1, 3Na+ binds ATP, which is hydrolyzed. This aspartyl phosphate causes a conformation change from E to E within the transept protein. The Na+- K+ -ATPase E1 conformation has a low affinity for Na+ and a high affinity for K+. As a result, the transporter discharges 3Na+ into the environment and binds 2K+ from the surrounding medium.



The phosphate group is hydrolyzed, and the enzyme returns to its E1 shape. Because the E1 conformation of the enzyme has a high affinity for Na but a low affinity for K+, the transporter releases 2K+ to the cell's interior. A trans membrane potential of -50 to -70 mV is generated by the migration of 3Na+ ions from the cell and 2K+ ions inside the cell. As a result, this ion transport is called electrogenic, or the creation of electric potential.




Here are detailed notes on each of the listed topics:

**1. DNA Polymerization**

DNA polymerization refers to the process by which DNA molecules are synthesized. It occurs during DNA replication, where a cell copies its genetic material to pass on to its daughter cells. The steps involved are:

- **Initiation**: DNA polymerization begins at specific locations on the DNA and is called the "origins of replication." The enzyme **helicase** unwinds the double helix, while **single-strand binding proteins (SSBs)** prevent the DNA from re-annealing. The **primase** enzyme synthesizes a short RNA primer complementary to the DNA strand.
- **Elongation**: **DNA polymerase** adds nucleotides to the 3' end of the primer, building a new DNA strand in the 5' to 3' direction. The template strand is read in the 3' to 5' direction. The sliding clamp (often PCNA) holds it in place as the DNA polymerase moves.
- **Leading and Lagging Strands**: DNA polymerase can synthesize continuously on the leading strand. DNA polymerase works in the opposite direction on the lagging strand, creating **Okazaki fragments,** which are later joined together by **DNA ligase**.
- **Termination**: Replication ends when the replication machinery reaches the end of the DNA or a termination sequence. The RNA primers are replaced with DNA, and the final phosphodiester bond is formed between adjacent nucleotides.

**2. Glucose Storage**

Glucose is stored in organisms primarily as **glycogen** in animals and **starch** in plants. Both of these polysaccharides are made of glucose molecules linked by **glycosidic bonds**.

- **Glycogen**: In humans, glucose is stored in the liver and muscles as glycogen. It is a highly branched molecule, allowing for rapid glucose mobilization when energy is needed. Glycogen breakdown (glycogenolysis) occurs through the action of enzymes like **glycogen phosphorylase**.
- **Starch**: In plants, glucose is stored as starch in the form of amylose (unbranched) and amylopectin (branched). Starch breakdown occurs through enzymatic activity (like **amylase**) to release glucose when the plant needs energy.
- **Glycogenesis**: This is the process of glucose storage in the form of glycogen. In the liver, insulin promotes glycogenesis by activating enzymes like **glycogen synthase**, which adds glucose units to the growing glycogen chain.

**3. Metal Complexes in the Transmission of Energy**

Metal complexes play crucial roles in energy transmission in biological systems, particularly in **electron transfer** and **photosynthesis**:

- **Iron-sulfur clusters** are important in electron transport chains, such as in the **mitochondrial respiratory chain** and **photosynthesis**. Iron in these clusters can cycle between oxidation states, facilitating electron transfer.
- **Copper-containing proteins**: In photosynthesis, copper is involved in electron transfer in cytochrome c oxidase, part of the electron transport chain.
- **Chlorophylls**: In energy transfer, chlorophylls are metal-containing molecules that absorb light and convert it into chemical energy through **photosynthesis**. Magnesium is the central metal in the porphyrin ring structure of chlorophyll.

**4. Chlorophylls**

Chlorophylls are a group of green pigments essential for photosynthesis in plants, algae, and some bacteria. The key features of chlorophyll are:

- **Structure**: Chlorophylls have a porphyrin ring structure with a central **magnesium ion ($Mg^{2+}$)**. This allows them to absorb light, primarily in the blue and red regions of the electromagnetic spectrum.

- **Types**: The most common types are **chlorophyll-a** and **chlorophyll-b**. Chlorophyll-a is the primary pigment involved in the light reactions of photosynthesis, while chlorophyll-b assists by broadening the absorption spectrum.
- **Role in Photosynthesis**: Chlorophyll absorbs light energy and uses it to excite electrons, initiating the electron transport chain in the **light-dependent reactions** of photosynthesis, which produces ATP and NADPH for the **Calvin cycle**.

**5. Photosystem I and Photosystem II in Water Cleavage**

Photosystem I (PSI) and Photosystem II (PSII) are integral parts of the light-dependent reactions of photosynthesis. They are both involved in the capture and conversion of light energy:

- **Photosystem II (PSII)**: PSII absorbs light energy, excites electrons, and drives water splitting (photolysis). This reaction releases **oxygen** and provides **electrons** to replace those lost by chlorophyll. The electrons are passed through a series of carriers, including the **plastoquinone** pool, to the **cytochrome b6f complex**, creating a proton gradient across the thylakoid membrane.
- **Photosystem I (PSI)**: PSI absorbs light at a slightly longer wavelength (around 700 nm) and uses the energy to excite electrons, which are passed to the **NADP+** to form **NADPH**. PSI works with PSII to produce both ATP (via the proton gradient) and NADPH, which are used in the **Calvin cycle** to fix carbon into glucose.
- **Water Splitting**: In PSII, splitting water molecules ($H_2O \rightarrow 2H^+ + \frac{1}{2}O_2 + 2e^-$) releases oxygen as a byproduct and provides the electrons that drive the electron transport chain.

**6. Model Systems**

Model systems are experimental organisms or techniques used to study biological processes due to their simplicity, ease of manipulation, and relevance to more complex systems. Examples include:

- **E. coli**: A bacterium used extensively in molecular biology for studying gene expression, DNA replication, and protein function. It is easy to manipulate genetically and proliferates.
- **Saccharomyces cerevisiae (yeast)**: A eukaryotic organism that studies cell division, gene regulation, and metabolism.
- **Drosophila melanogaster (fruit fly)**: A model organism in genetics and developmental biology. Its short lifespan and well-mapped genome make it ideal for studying inheritance patterns and developmental processes.

- **Arabidopsis thaliana (plant)**: A small, fast-growing plant used as a model for studying plant genetics, development, and responses to environmental stresses.
- **C. elegans (roundworm)**: A model for studying developmental biology, aging, and neurobiology due to its transparency and well-mapped nervous system.
- **Mouse (Mus musculus)**: Used in biomedical research, particularly in studying diseases, genetics, and drug development due to its similarity to human physiology and genetic manipulability.

Each model system provides valuable insights into fundamental biological processes conserved across species.

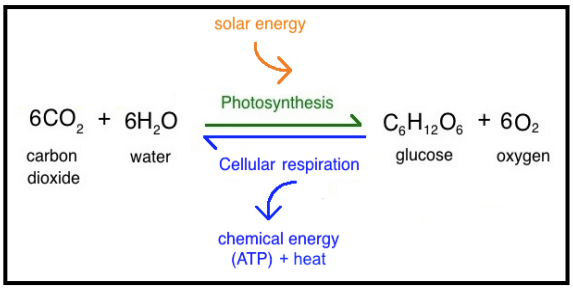
## Short answer type question:

**Q.1- Write a short note on the model system. (CSJMU2019,2017,2015,2014)**

**Ans.** The Role of Model Systems Because of the size and complexity of most biochemical molecules and processes, it is often advantageous to find smaller and simpler models upon which controlled experiments can be more easily performed and with which hypotheses can be tested. Bioinorganic chemistry has been an especially fruitful area for the use of model systems, mainly where transition metals are involved; of course it is not always possible to find or develop suitable models, and it can be dangerously misleading should overly simplistic models be used naively. Even in the best circumstances, a model can only give a partial view of how the actual system works. If these limitations are recognized, the model system approach can provide valuable guidance to the eventual study of the actual systems.

**Q.2- What is the function of the photosystem? (CSJMU2019)**

Ans. Photosynthesis is a crucial process not just in terms of quality but also in terms of quantity. Photosynthesis transforms between 200 and 500 billion tonnes of carbon every year. As a result, photosynthesis is a quantitatively important activity as well. At the expense of solar energy, the light reaction of photosynthesis produces energy-rich NADPH and ATP. These products are utilized in the carbon-assimilation process, which reduces CO2 to produce carbohydrates and occurs in light



or darkness. Green plants, algae, and photosynthetic bacteria use photosynthesis to capture solar energy and use it to fuel the synthesis of carbohydrates from carbon dioxide and water.

**The function of the photosystem:**

1. Light absorption

Photosystems absorb light at different wavelengths. The primary pigment chlorophyll a absorbs light, while accessory pigments chlorophyll b and carotenoids broaden the light that can be absorbed.

2. Energy and electron transfer

Photosystems transfer light energy to electrons, which are then transferred to the electron transport chain. Plants use the energy from these electrons to produce sugar.

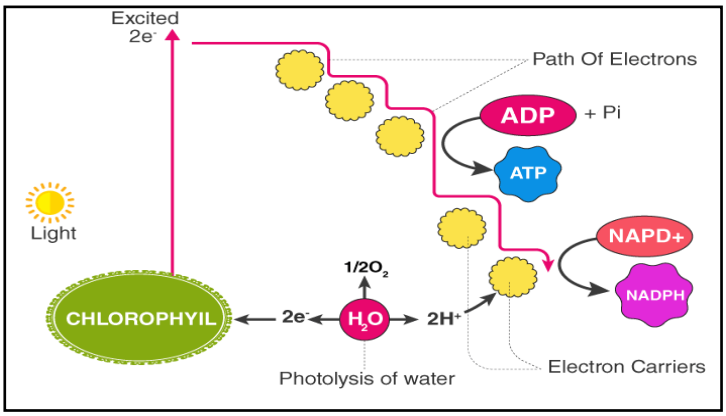
**Q.3- What is non – cyclic photophosphorylation? (CSJMU2012)**

**Ans**. Non-cyclic photophosphorylation is the "standard" form of light-dependent reactions. It is important for the functioning of various organs and tissues in the human body. Disruptions in these processes can lead to metabolic disorders.

Non-cyclic photophosphorylation is a light-dependent reaction that occurs during photosynthesis. It is a two-stage process that uses light energy to produce ATP molecules:

**1. Photolysis**: A water molecule is broken down into 2H+ + 1/2 O2 + 2e-. The electrons are kept in photosystem II, while the 2H+ and 1/2O2 are left out.

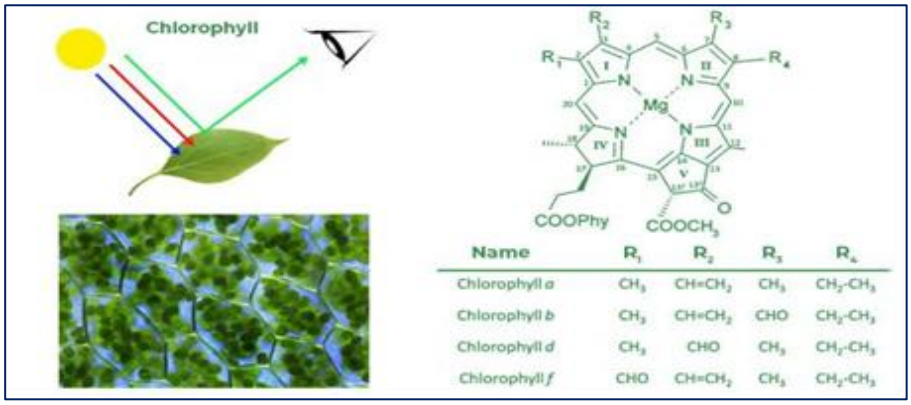
**2. Light excitation**: Light excites electrons in chlorophyll pigments, which sets off a chain reaction. The electrons are transferred from photosystem II to photosystem I to an enzyme called Ferredoxin-NADP+ reductase.




**3. Electron transfer**: The electrons catalyze a reaction that reduces NADP to NADPH2.

**Q.4- Give the structure of chlorophyll. (CSJMU 2014)**

Ans. Chlorophyll is a member of the most important class of pigments involved in photosynthesis,




| Name | $R_1$ | $R_2$ | $R_3$ | $R_4$ |
|---|---|---|---|---|
| Chlorophyll *a* | $CH_3$ | $CH{=}CH_2$ | $CH_3$ | $CH_2{\cdot}CH_3$ |
| Chlorophyll *b* | $CH_3$ | $CH{=}CH_2$ | CHO | $CH_2{\cdot}CH_3$ |
| Chlorophyll *d* | $CH_3$ | CHO | $CH_3$ | $CH_2{\cdot}CH_3$ |
| Chlorophyll *f* | CHO | $CH{=}CH_2$ | $CH_3$ | $CH_2{\cdot}CH_3$ |

the process by which light energy is converted to chemical energy by synthesizing organic compounds. Chlorophyll is found in virtually all photosynthetic organisms, including green plants, cyanobacteria, and algae. Chlorophyll is a green pigment in plants that has several functions, including:

**1. Photosynthesis**

Chlorophyll absorbs light energy from the sun and converts it into chemical energy that plants use to create glucose and oxygen. Plants use glucose for growth.

**2. Colour**

Chlorophyll absorbs red and blue light from sunlight, so leaves appear green.

**3. Photoreceptor**

Chlorophyll is a photoreceptor, which means it is a protein that detects and responds to light.

**4. Other applications**

Chlorophyll has other uses, including:

**a. Biosensors**: Chlorophyll's fluorescence activity can be used to develop optical biosensors.

**b. Organic lasers**: Chlorophyll's mild lasing action can be used to make organic lasers.

**c. Solar cells**: Chlorophyll has a natural solar cell-like function that traps insolation.

**d. Herbicide concentration indicator**: An intact chlorophyll system can indicate herbicide concentration.

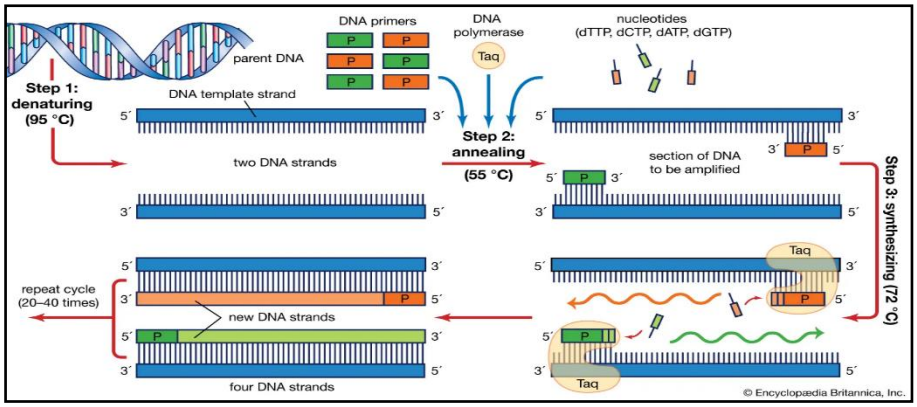
## Long answer type question:

**Q.1- Write a note on DNA polymerization. (CSJMU 2019)**

Ans. DNA replication is the most crucial biological activity in all living species, which involves copying a double-stranded DNA molecule into two identical duplicates. DNA replication is the most important biological process in all living organisms by which a double-stranded DNA molecule is copied to produce two identical replicas. DNA Polymerase is the enzyme responsible for DNA replication. This enzyme catalyzes the synthesis of polynucleotide chains by adding nucleotides produced from deoxy nucleoside triphosphates one after the other. It usually acts in pairs to split a single DNA molecule into two identical DNA strands. A DNA polymerase requires the following components for replication:

1. The triphosphate versions of four nucleotides are ATP, GTP, TTP, and CTP. Single-stranded DNA is used as a template. 2. A primer, an existing strand of nucleic acids with a free 3' end primer. 3. An existing strand of nucleic acids with a free 3' end.

DNA usually comprises two polynucleotide chains coiled around each other as a double helix. DNA polymerization is the synthesis of polynucleotide chains by the addition of successive nucleotides. The enzymes that catalyze the synthesis of polynucleotide chains of DNA are known as DNA polymerases. DNA polymerases require three components for DNA synthesis: template, primer, and four nucleotides (d ATP, d GDP, dTTP, and d CTP). The template is a single-stranded (ss) DNA that will direct addition complementary to the 3’ end template. The primer provides a free 3’ OH group that DNA polymerase extends by adding nucleotides. A nucleotide comprises three components: a nitrogenous base, a pentose sugar, and three phosphate groups. DNA polymerase catalyzes the synthesis of DNA by the addition of nucleotides to the 3’OH group of primer. The newly synthesized DNA has a base complementary to the template DNA.




**DNA Polymerization**

During DNA polymerization, the primer's 3' OH group assaults the incoming nucleotide's -P. The pyrophosphate is released when the incoming nucleotide is joined to the primer's 3' -OH group. The hydrolysis of pyrophosphate into two inorganic phosphates by an enzyme known as pyrophosphatase provides the driving power for this process.

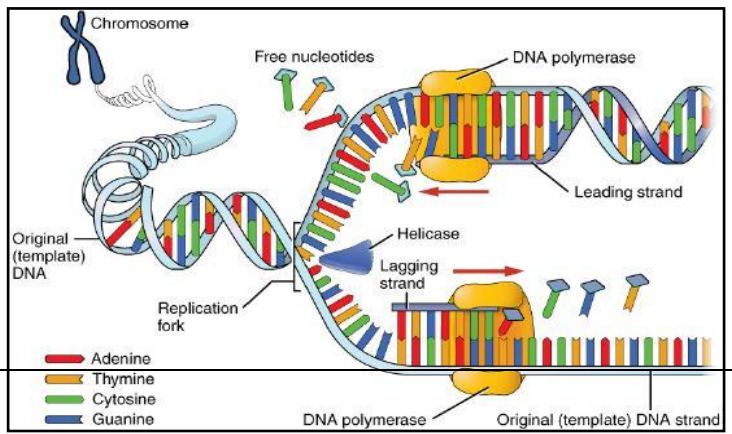
**Detailed description of the DNA polymerization process:**

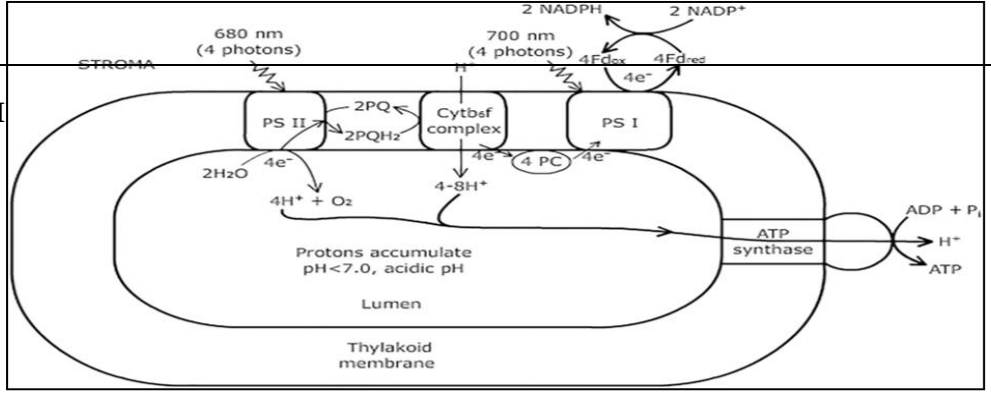
The steps are as follows

(1) **Initiation**: DNA replication begins when an enzyme called helicase loosens the two strands of DNA molecule by breaking hydrogen bonds between each nucleotide. The origin of replication is when a short section of DNA helix opens up, and the resulting structure is known as the Replication Fork. SSB proteins (Single-stranded DNA-binding proteins) then bind to the unwound single strands of DNA, preventing them from breaking and reannealing.

**(2) Elongation:** However, while the helicase splits the strands, RNA primase attaches to each strand briefly and creates an RNA primer, which serves as a starting point for DNA synthesis. DNA polymerase III begins creating a new complementary strand by adding the reciprocal sequences of the DNA to a single unwinding polynucleotide strand after the primer is in place. Because DNA polymerase can only add nucleotides in a 5' (prime) to 3' (prime) direction, this process occurs oppositely. This means that bases are added toward the origin of replication on the leading strand, but DNA is copied in the opposite direction of fork movement on the lagging strand. As a result, the lagging strand is produced in short, Okazaki-like pieces. The RNase enzyme removes the primer RNA fragments from both strands and DNA Polymerase fills in the gaps with the required nucleotides. Through the activity of DNA Ligase, a single nick on the leading strand and numerous nicks on the lagging strand are created, subsequently filled to produce two continuous double strands of DNA.

**(3) Termination:** Termination is the final phase of DNA replication, which occurs when the DNA polymerase enzyme reaches the end of the strands, where no further replication is feasible. The RNA primer is removed from the last segment of the lagging strand, which is not duplicated. Telomeres are regions of the genome that contain a repetitive, non-coding sequence of nucleotides.






A portion of the telomere is lost after each replication cycle, resulting in shorter strands after each cycle. Finally, enzymes such as nucleases "proofread" the new double helix structures, removing any reduced nucleotides that occurred during DNA replication. As a result, the removed bases leave a few gaps, which DNA Polymerase I eventually repairs.

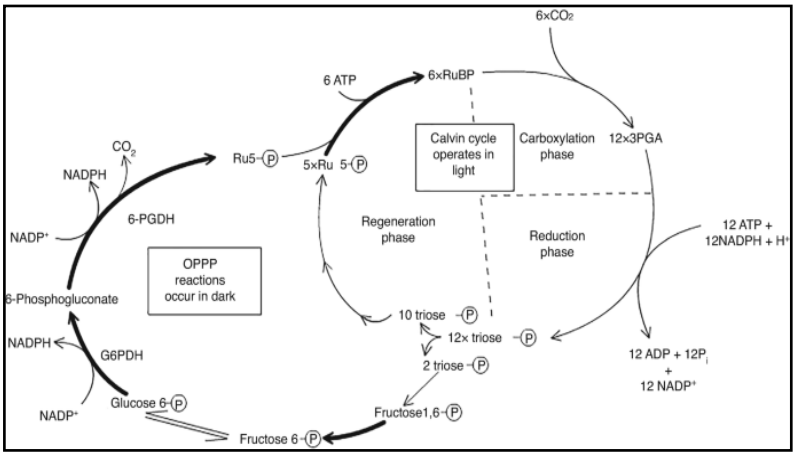
**Q.2- Discuss bio-energetics and the ATP cycle. (CSJMU 2013)**

**Ans. Generation of ATP via cyclic electron flow:**

When ferredoxin has reduced virtually all of the NADP+, it gives an electron to the cytochrome bf complex. The resultant proton gradient created by the H+ pump, cytochrome bf complex, subsequently drives ATP production. ATP is generated during photophosphorylation without the formation of NADPH, and no O2 is produced since PSII is not engaged. In summary, when electron transport through PSI AND PSII operates in a noncyclic mode, the products are NADPH and ATP. The sole result of cyclic electron transport, on the other hand, is ATP.

**The Dark reaction of photosynthesis, the Calvin Cycle:**

The dark reaction, the carbon-fixation process, converts CO2 into carbohydrates using the ATP and NADPH generated by photosynthesis' light reaction. Sucrose and starch are the end products. The metabolite 3-phosphoglycerate is converted to glucose by a sequence of processes similar to glucogenesis in the liver, except that NADPH is employed as the reductant instead of NADH.

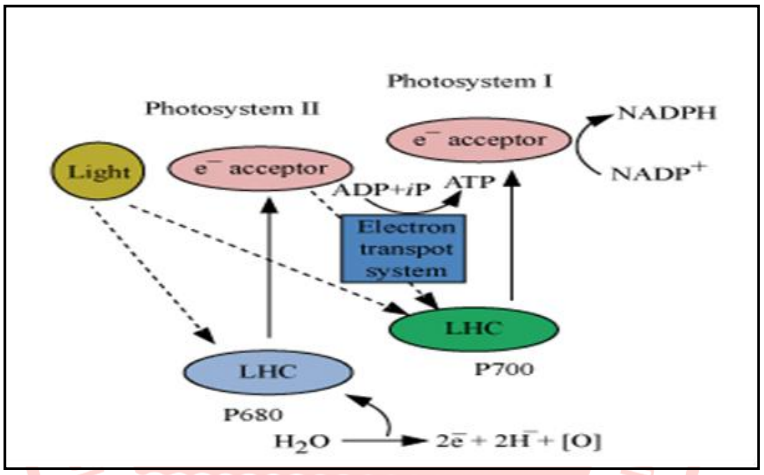


**Q. What is the significance role of photosystem – I and photosystem – II? (CSJMU 2013)**

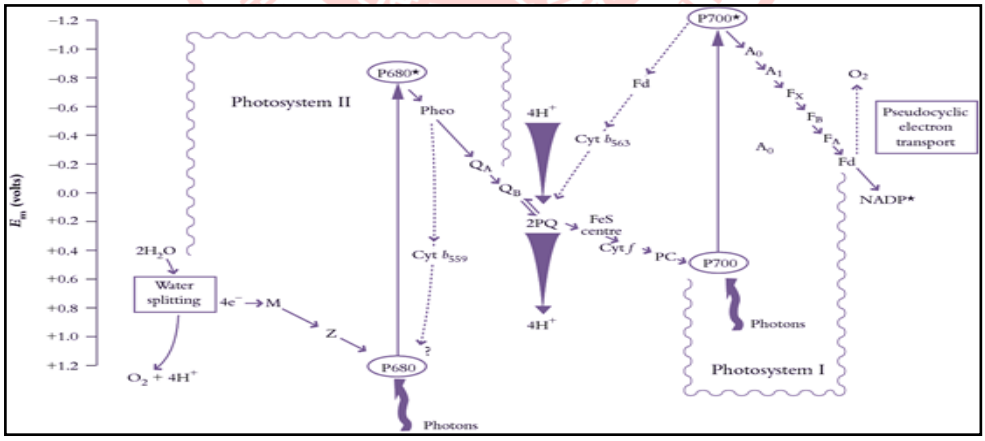
Ans. Photosystem I (PSI) and photosystem II (PSII) are the two types of photosystems used by green plants and algae.

Photosystem 1: These are located on the outer surface of the thylakoid membrane, which synthesizes ATP and NADPH.

Photosystem 2: These are located on the inner surface of the thylakoid membrane, which Synthesizes ATP.




PSI's reaction center chlorophyll has an absorption maximum of 700 nm and is therefore known as P700 (P for pigment), while PSII's reaction center chlorophyll has an absorption maximum of 680



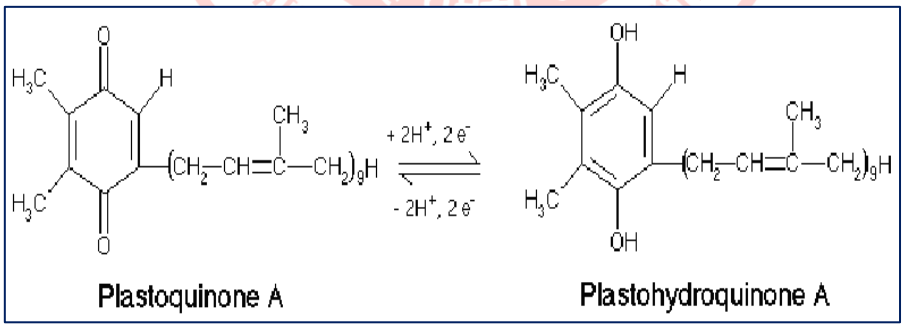

nm and is thus known as P680. Other electron carriers, particularly the cytochrome bf complex, connect the two photosystems. The different components from the so-called Z scheme are organized according to their redox potential because the overall form of the redox diagram provides a look of z. Plastoquinone, plastocyanin, ferredoxin, and other highly mobile electron carriers are used in these events.

**Mechanism of light-dependent reduction**:

In photosynthesis, electron transport pathways from H2O to NADP+ are defined. The absorption of light by PSII and PSII allows for this endergonic process. When a PSII chlorophyll in a PSII reaction center is in its ground, unexcited state, it does not incline to give up an electron. When a photon's energy reaches it through the antenna chlorophyll, it is excited and has a high inclination to pass on its excited electron. In reality, it is a reducing agent. The high-energy electron is transferred to plastoquinone (Q), a mobile quinone in the thylakoid membrane with a structure similar to ubiquinone in the mitochondrial electron transport chain. P680 is the P680+ cation as a result of this. Plastoquinone is converted to plastoquinone (QH2) by absorbing two electrons and two H+ ions. It is worth noting the following:

- The light-driven splitting of H2O is catalyzed by a Mn-containing protein complex, producing O2. This strong reductant transfers its electron to NADP+ to create NADPH.
- Four electrons abstracted from water do not pass directly to P680+, which can accept only one electron at a time. Instead, a remarkable molecular device, the water-splitting complex, passes four electrons simultaneously to $P680^{+}$.
- Reduced plastoquinone formed by PSII now passes electrons into the cytochrome bf complex. Pc is a copper-containing protein that accepts electrons by the copper cycling between Cu2+ and Cu+ states.

An Mn-containing protein complex catalyzes the light-driven splitting of H2O, resulting in the production of O2. This strong reductant donates an electron to NADP+, resulting in NADPH.




**1. Heme Protein and Oxygen Uptake**

- **Heme Proteins**: Heme proteins are a group of proteins that contain a heme group, which is a metal-containing compound crucial for the protein's function. The heme group contains an iron ion ($Fe^{2+}$ or $Fe^{3+}$) that binds to oxygen ($O_2$) or other ligands. These proteins play key roles in oxygen transport and storage.

- **Structure of Heme**: The heme group comprises a porphyrin ring structure surrounding a central iron ion. This iron atom is capable of binding to an oxygen molecule. The iron is coordinated with the nitrogen atoms of the porphyrin ring and has one additional coordination site for oxygen or other ligands.
- **Oxygen Uptake**: In heme proteins like hemoglobin and myoglobin, the iron in the heme group binds reversibly with oxygen, allowing for oxygen uptake, transport, and release. Oxygen binding is influenced by the protein's structure, which can alter the affinity for oxygen based on environmental factors such as pH and oxygen concentration.

**2. Structure and Function of Hemoglobin**

- **Structure**:
  - Hemoglobin (Hb) is a tetrameric protein composed of four subunits, typically two α-chains and two β-chains in adult human hemoglobin (HbA).
  - Each subunit contains a heme group so that each hemoglobin molecule can bind up to four oxygen molecules.
  - Hemoglobin exhibits cooperative binding: the binding of oxygen to one subunit increases the affinity of the remaining subunits for oxygen. This is known as positive cooperativity.
  - The quaternary structure of hemoglobin changes between two states: the T (tense) state, which has a low affinity for oxygen, and the R (relaxed) state, which has a high affinity for oxygen.
- **Function**:
  - Hemoglobin's primary function is to transport oxygen from the lungs to tissues and organs and to return carbon dioxide ($CO_2$) from tissues to the lungs.
  - The oxygen-binding capacity of hemoglobin is influenced by pH (Bohr effect), $CO_2$ concentration, and temperature, enabling efficient oxygen delivery in metabolically active tissues.
  - In tissues with lower pH (due to increased $CO_2$), hemoglobin's affinity for oxygen decreases, promoting oxygen release.

**3. Structure and Function of Myoglobin**

- **Structure**:
    - Myoglobin is a monomeric protein found primarily in muscle tissue, with a structure similar to a single hemoglobin subunit.
    - It consists of a single polypeptide chain that binds one oxygen molecule via a single heme group.
    - Myoglobin has a higher affinity for oxygen than hemoglobin, allowing it to store oxygen in muscle cells efficiently.
- **Function**:
    - Myoglobin's primary function is to store oxygen in muscles and facilitate oxygen diffusion to muscle cells during periods of high metabolic activity.
    - Myoglobin does not exhibit cooperative binding like hemoglobin. It binds oxygen tightly, even at low concentrations, making it an efficient reservoir.
    - It also helps transport oxygen within muscle fibers from the capillaries to mitochondria during exercise or muscle activity.

**4. Structure and Function of Hemerythrin**

- **Structure**:
    - Hemerythrin is a non-heme iron-containing protein in certain invertebrates, such as annelids (earthworms) and marine arthropods.
    - Unlike hemoglobin, hemerythrin does not have a heme group. Instead, it contains a binuclear iron center coordinated by histidine and other amino acids.
    - The iron atoms are bridged by oxygen, and the protein exists in two states: deoxygenated (iron is in a ferrous state) and oxygenated (iron is in a ferric state).
- **Function**:
    - Hemerythrin is an oxygen carrier in some invertebrates, similar to hemoglobin in vertebrates. It binds oxygen reversibly at its iron center.
    - It is found in the blood of certain invertebrates, where it facilitates the transport and release of oxygen, like hemoglobin, but without a porphyrin ring.

- The oxygen binding and release in hemerythrin are not cooperative like hemoglobin, and its function is highly adapted to the specific environments of the organisms that use it.

**5. Model Synthetic Complexes of Iron and Copper**

- **Iron Complexes**:
  - **Iron(II) Complexes**: Iron in the +2 oxidation state ($Fe^{2+}$) can form complexes with various ligands, such as porphyrins, organic molecules, and anions. These complexes are often used as models for studying oxygen binding and electron transfer in biological systems.
  - **Synthetic Hemoglobin Models**: Synthetic iron(II)-containing complexes, such as iron porphyrin derivatives, are designed to mimic the oxygen-binding properties of hemoglobin and myoglobin. These complexes can undergo reversible oxygen binding similar to heme proteins, and they are valuable tools in bioinorganic chemistry.
  - **Iron-Sulfur Complexes**: These complexes, which contain iron atoms coordinated with sulfur ligands, play a role in electron transfer and are involved in redox reactions in biological systems (e.g., in mitochondrial electron transport).
- **Copper Complexes**:
  - Copper involves various biological processes, including electron transport and oxygen binding.
  - **Copper(II) Complexes**: In the +2 oxidation state ($Cu^{2+}$), copper can form complexes with nitrogen and sulfur-containing ligands. In biological systems, copper is present in enzymes like cytochrome c oxidase, which participates in cellular respiration.
  - **Copper as a Model for Oxygen Binding**: Synthetic copper complexes are designed to model the behavior of copper in biological systems, particularly in enzymes involved in oxygen activation. These models provide insight into how copper interacts with oxygen and facilitates electron transfer.
  - **Copper-Tetraazamacrocycle Complexes**: These complexes, which contain copper coordinated to nitrogen-containing ligands, have been studied as models for copper-containing enzymes like hemocyanins, which transport oxygen in some arthropods and mollusks.

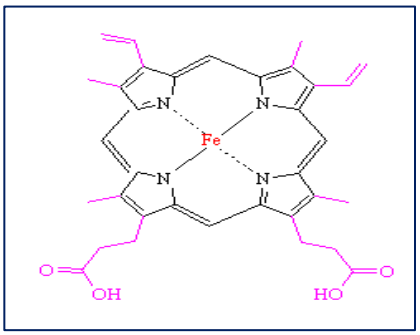
**Summary:**

- **Heme proteins** (hemoglobin, myoglobin, hemerythrin) are central to oxygen transport and storage in animals, utilizing a metal center (iron) to bind oxygen reversibly.
- **Hemoglobin** has a cooperative structure that allows efficient oxygen uptake and release in response to environmental conditions.
- **Myoglobin** is an oxygen reservoir in muscle tissues, binding oxygen tightly and enabling rapid diffusion to muscle cells.
- **Hemerythrin** functions as an oxygen carrier in certain invertebrates without a heme group, using a binuclear iron center.
- **Synthetic iron and copper complexes** are used as models for studying the oxygen-binding properties of heme proteins and copper-containing enzymes, providing insights into the biological mechanisms of oxygen transport and redox reactions.

## Short answer type questions:

**Q.1- Write notes on hemoglobin. (CSJMU 2020,2015,2012)**

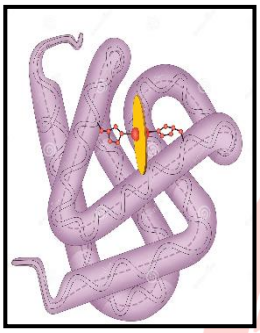
Ans. **Haemoglobin:** Haemoglobin (molecular weight 645000) can be considered an animal oxygen carrier. In most animals, the pigment provides the color of blood. The color of blood is red if hemoglobin is present with oxygen. In the absence of oxygen in hemoglobin, the color of the blood is blue. It is due to the transfer of electrons between the ring and the iron atom's π and π* orbital. In the human body, nearly 4 g of iron is present. Out of thin, approximately 0.8 g is used to produce red color in RBC by hemoglobin. The remaining iron is stored in the form of ferritin.

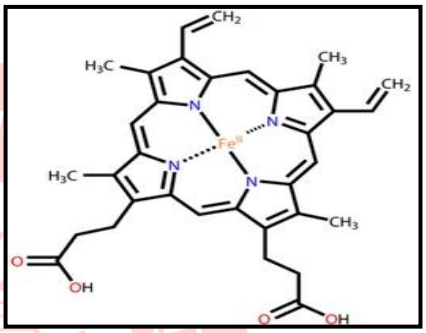



**Q.2- Write notes on myoglobin. (CSJMU 2020,2015,2012)**

**Ans. Myoglobin:**

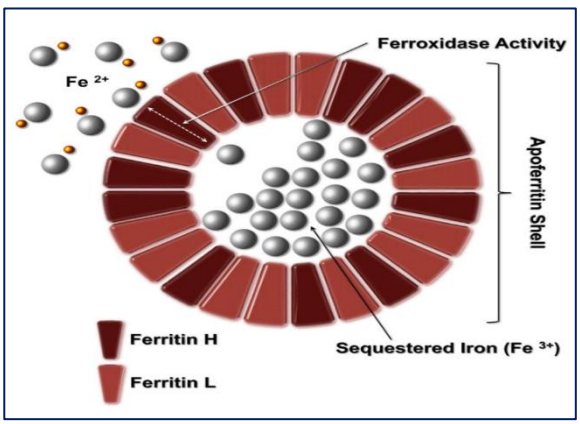
Myoglobin (Mb) is the oxygen-binding protein found principally in muscle tissues of vertebrates. It comprises a single polypeptide chain of 153 amino acids called globin, comprising seven a-helical and six non-helical segments. Attached to the chain by coordination to the imidazole ring of a histidine residue is the dioxygen-binding prosthetic group, iron (II) protoporphyrin IX. The structure of sperm-whale myoglobin has been determined in all three (deoxy, oxy, and met) forms. Deoxy Mb has a pentacoordinate iron(I1) center in which the metal atom lies 92 pm out of the plane of the four pyrrole-ring donor nitrogen atoms(scheme). It is displaced toward the bonded imidazole group, called the heme's proximal side. The iron atom moves toward the Fe N planes upon binding of dioxygen. In oxy Mb, the dioxygen molecule is bonded end-on to iron, forming a bent structure with a Fe-0-0 bond angle of 115.






**Q.3- Write notes on the ferritin. (CSJMU 2020,18)**

Ans. Inside and outside of cells, ferritin is an iron-binding protein. Iron is stored primarily in ferritin, an iron storage protein. For the production of hemoglobin, myoglobin, and cytochrome, ferritin release is required. This is important because unbound free iron is highly toxic and causes cellular damage by promoting the production of free radicals. In mammalian tissues such as the liver, spleen, and bone marrow, ferritin is a significant iron storage. Apoferritin, as shown in the picture, creates a roughly spherical container within which ferric iron is kept as the ferrihydrite mineral ferric iron (Apoferritin refers to the iron-free form of the protein; the iron-containing form is termed holoferritin or simply ferritin).



There are 24 subunits in the apoferritin shell. The two sorts of subunits are H and L. The ratio of these subunits varies depending on the tissue type, and it can alter a lot under inflammatory and infectious situations. H-subunit-rich tissue ferritins (found primarily in the heart and kidney) to L-subunit-rich tissue ferritins (found predominantly in the liver and spleen). Each apoprotein molecule has a diameter of around 450,000 d. The L monomer comprises 174 amino acids and has a molecular weight 174. The H monomer has a molecular mass of 21,000 d and is made up of 182 amino acids.

**Q.4- Write notes on the transferrin. (CSJMU 2019)**

Ans. Several structurally important and related Fe-proteins, all of which are glycoproteins, make up transferrins. Their molecular weights (molar masses) are approximately 80 k Da. New hemoglobin is produced in the bone marrow, while old red cells are destroyed in the spleen and liver. Transferrins [as a class of protein, serum protein, lactoferrin (milk), and ovatransferrin (egg)] are iron-binding proteins that transport iron in higher animals via the bloodstream to the site of synthesis of other iron-containing compounds such as hemoglobin, cytochrome, and others, where it is inserted into the porphyrin ring via enzyme.

**Function of Transferrin:**

a. Transferrin binds Iron in the +3 oxidation state.
b. Transferrin binds Fe3+ (from the stomach), enters the blood, and transportFe3+ to bone marrow. In bone marrow, Fe3+ is reduced to Fe2+ and is delivered to ferritin. Before and after binding in transferrin, Fe is in a +2 oxidation state; when binds to transferrin, Fe is in a +3 oxidation state.
c. Transferrin delivers ions when reduced to Fe+2 in the bone marrow. Fe+2 is further oxidized by ceruloplasmin (ferroxidase) to Fe+3 and picked up by ferritin (ferritin is found in bone marrow, spleen, and livers.
d. Transferrin is the best scavengers of iron in the animals.

**Q. Give the name and main functions of heme proteins. (CSJMU 2019,2016,2014)**

**Ans**. One study found that hem group I hemoglobin and myoglobin can bind O2 molecules and their subsequent release without the iron atom becoming permanently oxidized to the Fe (III) state. The following points are of significance:

**The primary function of heme protein-**

- 1. Oxygen transport: Hemoglobin and myoglobin are heme proteins that bind oxygen in the lungs and tissues. Hemoglobin is responsible for the round shape of red blood cells.
- 2. Electron transfer: Heme proteins can act as electron carriers.

- 3. Catalysis: Heme proteins are involved in catalysis.
- 4. Signalling: Heme proteins control the activities of signal transducers and transcriptional regulators.
- 5. Gas sensing: Heme proteins can sense diatomic gases like carbon monoxide and nitric oxide.
- 6. Protein stability: Heme proteins regulate protein stability.
- 7. Circadian rhythm: Heme proteins are involved in circadian rhythm.
- 8. Cell growth and differentiation: Heme proteins are required to function and differentiate many types of cells properly.

**Q.5- Discuss the biological function of hemoglobin. (CSJMU 2018)**

**Ans. The biological function of hemoglobin:**

The following types function of hemoglobin in the human body are:

- Hemoglobin is an oxygen carrier.
- Hemoglobin is a carbon dioxide carrier.

3. Hemoglobin gives a red color to blood.

- Hemoglobin maintains the shape of the red blood cells. Hemoglobin acts as a buffer. Hemoglobin interacts with other ligands. Hemoglobin degradation accumulates physiologically active catabolites. Hemoglobin binds $O_2$ to its heme iron in the lungs and delivers $O_2$ to myoglobin, which stores the $O_2$ until it is required for metabolic oxidation.
- A second task of Hb is to bring the $CO_2$ by-product of oxidation back to the lungs to get rid of it:
    - $R\text{-}NH2 + CO2 \rightarrow R\text{-}NH\text{-}COO^- + H^+$
- CO2, as well as buffering, results from the reversible formation of HCO3.
    - $CO2 + H2O \rightarrow HCO^-_3 + H^+$

**Q.6- Name the three Heme proteins and mention their primary function. (CSJMU 2017,2016)**

**Ans. 1. Hemoglobin (Hb)**: The primary oxygen carrier in humans, hemoglobin uses a heme iron active site. Hemoglobin is a protein in red blood cells that carries oxygen. The hemoglobin test measures how much hemoglobin is in your blood. Hemoglobin is the most important component of red blood cells. It is composed of a protein called heme, which binds oxygen.

**2. Hemocyanin (Hcy)**: In the blood of mollusks and some arthropods, hemocyanin uses a di copper active site. Hemocyanin is so foreign to humans that it's a significant cause of shellfish allergies. Hemocyanin is labile, meaning oxygen can bind and unbind quickly to and from the copper center. This allows oxygen to dissociate to oxygenate tissues quickly.

**3. Hemerythrin (Hr)**: Another iron-containing oxygen carrier. Hemerythrin is a respiratory protein that binds and transports oxygen in the blood cells of some marine worms. In bacteria, it acts as an oxygen and redox sensor, helping the bacteria adapt to changes in oxygen concentration and redox status.

**Q.7 Write the similarities between hemoglobin and myoglobin. (CSJMU 2014)**

Ans. Hemoglobin and myoglobin are both globular proteins that bind oxygen and contain heme, a molecular constituent that gives them a red-brown color:

**1. Structure**: Both are part of the globin family of heme-containing proteins and have a similar structure to one of hemoglobin's β subunits.

**2. Function**: Both bind oxygen to the heme prosthetic group's ferrous iron (Fe2+) atom.

**3. Colour**: Both give color to blood and muscles, respectively.

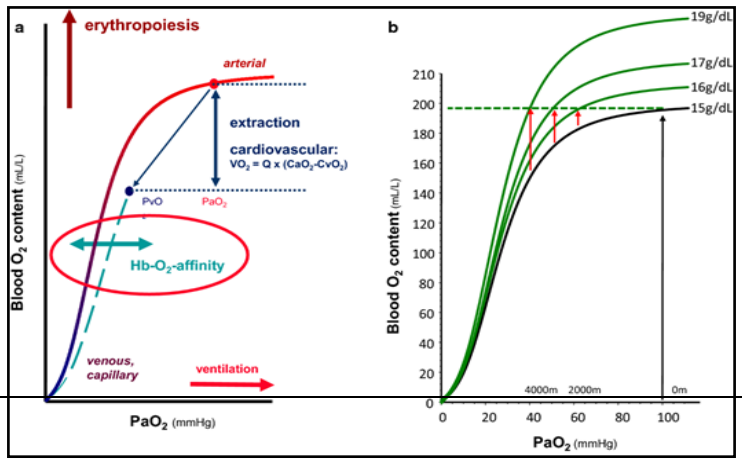
**4. Chemical similarity**: Both have a close chemical similarity.

## Long answer type question:

**Q.1- Explain the kinetics of hemoglobin oxygenation. (CSJMU 2016,2013)**

Ans. **Kinetics of hemoglobin:**

The Bohr effect is a phenomenon first described in 1904 by the Danish physiologist Christian Bohr. The Bohr effect generally describes the effect of pH on the blood-O2-binding affinity. The affinity of Hemoglobin (Hb) towards the O2 is pH dependent. The affinity of Hb decreases with decreases in pH. The affinity of myoglobin (Mb) towards O2 is pH-independent. The Oxygen (O2) competitively and reversibly binds to hemoglobin, with specific changes within the environment altering the affinity in which this relationship occurs. The sigmoidal shape of the oxygen dissociation curve illustrates



hemoglobin's propensity for positive cooperativity, as hemoglobin undergoes conformational changes to increase its affinity for oxygen as molecules progressively bind to each of its four available binding sites. The Bohr effect describes hemoglobin's lower affinity for oxygen secondary to increases in the partial pressure of carbon dioxide and/or decreased blood pH. This lower affinity, in turn, enhances the unloading of oxygen into tissues to meet the oxygen demand of the tissue.

The phenomenon where the binding of one O2 molecule to a sub-unit encourages the binding of O2 molecules to another sub-unit is called the cooperative effect. The cooperative effect describes the ability of the four identical hemoglobin sub-units to change their conformation. The cause of this change is the acceptance or release of an O2 molecule by one of the sub-units, which increases the ability of the other hemoglobin domains to accept or release oxygen. Of great importance to the physiological functions of Hb and Mb is the beautifully sophisticated bioinorganic system devised by nature in which dioxygen binds to Hb in the lungs and is transferred to Mb in tissues or to fatal Hb in the uterus of pregnant mammals. At the heart of this system is the motion of iron toward the plane of the porphyrin ring upon conversion of deoxy to oxy Hb, which serves as a trigger for cooperative binding of dioxygen by the multi-subunit hemoglobin protein. The protein is presumed to have two different quaternary structures: R, for relaxed, and T, for tense. The former has a high affinity for $O_2$, similar to that of isolated subunits, whereas the latter, tense state, has a diminished $O_2$ affinity. These two conformational states are in equilibrium with one another. When all four sub-units are ligated in the T state, inter-sub-unit interactions are believed to constrain the proximal histidine to resist movement into the porphyrin-ring plane and diminish the $O_2$ binding constant.

**Q. Write the properties of three oxygen – binding metallobiomolecules. (CSJMU 2015)**

**Ans:** Metallobiomolecules contain metal ions and play a significant role in regulating biological processes. All of these oxygen-binding metallobiomolecules use transition-metal complexes to transport oxygen.

Three metallobiomolecules that bind oxygen are hemoglobin (Hb), hemocyanin (Hcy), and hemerythrin (Hr).

**1. Hemoglobin (Hb)**: The primary oxygen carrier in humans, hemoglobin uses a heme iron active site. Hemoglobin is a protein in red blood cells that carries oxygen. The hemoglobin test measures how much hemoglobin is in your blood. Hemoglobin is the most important component of red blood cells. It is composed of a protein called heme, which binds oxygen.

**2. Hemocyanin (Hcy)**: In the blood of mollusks and some arthropods, hemocyanin uses a di copper active site. Hemocyanin is so foreign to humans that it's a significant cause of shellfish

allergies. Hemocyanin is labile, meaning oxygen can bind and unbind quickly to and from the copper center. This allows oxygen to dissociate to oxygenate tissues quickly.

**3. Hemerythrin (Hr)**: Another iron-containing oxygen carrier. Hemerythrin is a respiratory protein that binds and transports oxygen in the blood cells of some marine worms. In bacteria, it acts as an oxygen and redox sensor, helping the bacteria adapt to changes in oxygen concentration and redox status. Hemerythrin has a non-heme diiron center, which consists of two iron atoms bridged by an oxygen atom. The iron atoms are bound to five histidine residues, one glutamic acid residue and one aspartic acid residue.

**Q. What are the functions of hemocyanin and hemoerythrin? (CSJMU 2013)**

**Ans. Hemocyanin:** Hemocyanin is a protein found in the hemolymph of mollusks and arthropods that has multiple functions, including:

**1. Oxygen transport**

Hemocyanin is a metalloprotein that carries oxygen in the blood of invertebrates. It has two copper atoms that reversibly bind to a single oxygen molecule.

**2. Immune response**

Hemocyanin is a precursor to antimicrobial peptides and helps agglutinate microbes and viruses.

**3. Other functions**

Hemocyanin also helps with osmotic pressure control, metabolism, signal transduction, and maintaining the microbial balance of hemolymph.

Hemocyanin is a large, copper-containing molecule with at least six subunits. It is produced in the digestive gland by RI cells.

**Hemerythrin:** Hemerythrin is a protein that binds and transports oxygen reversibly and has multiple functions:

- **Oxygen transport**

Hemerythrin is a primary oxygen-transporting protein in marine invertebrates. It is a better oxygen carrier than hemoglobin in low-oxygen environments, such as at the bottom of the ocean.

- **Oxygen sensing**

Hemerythrin is also proposed to be an oxygen sensor in bacteria. Bacteria use hemerythrin to sense changes in oxygen concentration and redox status and adapt to these changes to maintain physiological processes.

- **Colour change**

Hemerythrin is colourless when deoxygenated but turns violet-pink when oxygenated.

Hemerythrin is found in marine phyla, arthropods, mollusks, vertebrates, and invertebrates. It contains iron atoms that are firmly bound to sulfur**.**

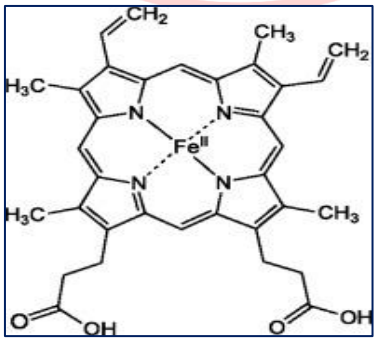
## Short answer type questions:

**Q. Write a short note on the chemical nature of cytochrome. (CSJMU 2020,2018,2015)**

**Ans**. Cytochromes are iron-containing hemeproteins central to them, which are home groups primarily responsible for generating ATP via electron transport. They are either monomeric proteins (e.g., cytochrome c) or subunits of larger enzymatic complexes that catalyze redox reactions.

**Cytochrome P-450:**

Cytochrome P-450 is a family of cytochromes found in plants, animals, and microorganisms. It is called a pigment because its CO compounds absorb light at 450 nm. This is owing to the π-π* (blue to red) transition, and this bond is known as the SORET bond. Cytochrome P-450 aids in O2 cleavage and acts as a monooxygenase, facilitating the insertion of oxygen atoms into the substrate. Oxygenases are enzymes that add oxygen to a food source. A monooxygenase introduces one oxygen atom into the substrate, while a dioxygenase inserts two oxygen atoms into the substrate. The C-H bond is transformed to COH groups in the most significant compounds.




**Q. Write a short note on auxochromes. (CSJMU 2019)**

**Ans. Important points of auxochromes:**

1. Auxochromes, also known as color helpers, function as additional chromophores. By themselves, auxochromes are colorless. However, when attached to a chromophore, auxochromes can modify the wavelength of light absorbed, changing the object's color.

2. Auxochromes change the wavelength and intensity of light that a chromophore absorbs. This is because auxochromes provide additional electrons to the system that can absorb light with more energy.

3. Auxochromes are covalently attached to the chromophore skeleton of a dye molecule. Unlike chromophores, they can undergo n-electron transitions, which cannot undergo π–π* transitions.

**Q. What is the function of iron – sulfur proteins? (CSJMU 2019,2016,2012)**

**Ans.** Ron–sulfur (Fe/S) proteins are involved in numerous key biological functions such as respiration, metabolic processes, protein translation, DNA synthesis, and DNA repair. The simplest types of Fe/S clusters include [2Fe–2S], [3Fe–4S], and [4Fe–4S] forms that sometimes are present in multiple copies.

**The function of iron-sulfur proteins:**

**1. Electron transfer**: Fe/S proteins are responsible for rapid electron transfer, essential for photosynthesis and cellular respiration.

**2. Catalysis**: Fe/S proteins are involved in redox and non-redox catalysis. For example, hydrogenases and nitrogenases are exclusively Fe/S dependent.

**3. Gene regulation**: Some Fe/S proteins regulate gene expression.

**4. DNA synthesis and repair**: Fe/S proteins are involved in DNA synthesis and repair.

**5. Protein translation**: Fe/S proteins are involved in protein translation.

**6. Metabolic processes**: Fe/S proteins are involved in metabolic processes.

**7. Sulfur donor**: Fe/S proteins act as sulfur donors in biotin and lipoic acid biosynthesis.

**8. Radical generation**: Fe/S proteins are responsible for generating radicals.

## Long answer type questions:

**Q. Describe the function of Ferri chromes and ferrioxamines. (CSJMU 2016)**

**Ans**. **Ferri chrome:** Ferri chrome is a siderophore, a metal chelating agent with a low molecular mass produced by microorganisms and plants growing under low iron conditions.

Ferri chrome has multiple functions, including:

- **Iron chelation**: Ferri chrome is a siderophore, a metal chelating agent that helps make iron available to plant and microbial cells. It does this by extracting ferric iron (Fe3+) from insoluble minerals in the environment.
- **Suppressing ammonium's metabolic action**: Ferri chrome can help fission yeast grow in high ammonium media.
- **Facilitating growth in low glucose conditions**: Ferri chrome can help wild-type and sib1-deficient cells grow in low glucose conditions.
- **Tumor suppression**: Ferri chrome produced by the bacterium Lacticaseibacillus case may have a more significant tumor-suppressive effect than other drugs used to treat colon cancer.
- **Iron transport**: Ferri chrome may transport iron between organelles.

**Ferrioxamine:** Ferrioxamine E from Streptomyces antibiotic is a siderophore that facilitates the supply of iron (III), an essential trace element, to bacteria involved in food poisoning, including Salmonella, Enterobacter saka-zakii, and Yersinia enterocolitica. It promotes rapid growth by reducing the lag phase in culture media and reactivates dormant bacteria.

Ferrioxamine has multiple functions, including:

- **Treating iron poisoning**

Ferrioxamine is a water-soluble complex formed when deferoxamine (DFO) chelates iron. DFO is an iron chelator used to treat acute iron poisoning, chronic iron overload, thalassemia, and sickle cell anemia. Ferrioxamine is excreted through the urine and can give it a rusty-red appearance.

- **Bacterial siderophore**

Ferrioxamine is a bacterial siderophore produced by various bacteria, including S. glaucescent, M. luteus, and P. Mendocino. Ferrioxamines are the sole iron source for Salmonella and have been used to detect Salmonella in food and industrial applications.

- **Growth promoting agent**

Ferrioxamines promote growth in some fungi and bacteria that cannot produce the siderophore.

**Q. What is oxidation phosphorylation? Explain about a synthetic model of electron transfer biology. (CSJMU 2015)**

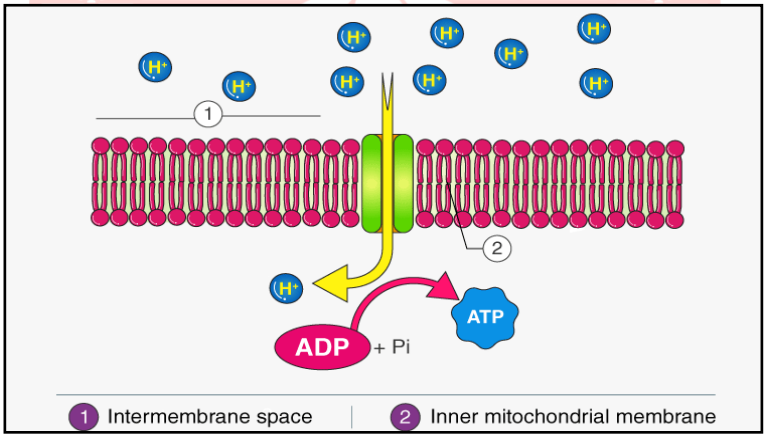
**Ans. Oxidative Phosphorylation:**

*"Oxidative phosphorylation is the process of ATP formation when electron carriers transfer electrons from NADH or FADH2 to oxygen."*

Oxidative phosphorylation is the final step in cellular respiration. It occurs in the mitochondria. It is linked to a process known as the electron transport chain. The electron transport system is located in the inner mitochondrial membrane. The electrons are transferred from one member of the transport chain to another through a series of redox reactions.

**Electron Transport Chain**

Most biochemical catabolic processes, like the citric acid cycle, glycolysis, beta-oxidation, etc., produce the coenzyme NADH. It consists of electrons having high transfer potential. These reactions release a considerable amount of energy on oxidation. These reactions are also known to be uncontrollable reactions since the energy within the cells is not released at once. The electrons are separated from the NADH and then passed to oxygen, where a series of enzymes release a




small amount of energy. All these series of enzymes have complexes known as electron transport chains. This chain can be seen in the inner layer or membrane of mitochondria. This electron chain transport system also oxidizes the salts of succinic acid. In the case of eukaryotes, the enzymes make use of energy that has been released in the electron transport system from the oxidation of NADH to pump protons across the inner membrane of the mitochondria. This results in the generation of the electrochemical gradient across the membrane. This can be considered one of the best examples of understanding the concept of oxidative phosphorylation.

# Bioorganic Chemistry

## NOTES

### 1. Introduction and Historical Perspective, Chemical, and Biological Catalysis

**Historical Perspective:**

- **Catalysis** refers to the acceleration of a chemical reaction by a substance (catalyst) that is not consumed in the process. Early work on catalysis dates back to the 19th century when scientists like **Jöns Jacob Berzelius** coined the term "catalysis" and recognized the role of catalysts in speeding up reactions without being altered.
- **Enzymes** were recognized as biological catalysts in the early 20th century. Their discovery grew from understanding how certain substances in living organisms could accelerate biochemical reactions.

**Chemical vs. Biological Catalysis:**

- **Chemical catalysts** involve non-living, synthetic catalysts like metals or acids. It often works in inorganic reactions and can function in various environments, including extreme conditions like high temperature or pressure.
- **Biological Catalysis** involves enzymes, particularly proteins, that catalyze biochemical reactions in living organisms. Enzymes are typically more specific and operate under mild conditions (ambient temperature, neutral pH, and atmospheric pressure).

### 2. Remarkable Properties of Enzymes

**Catalytic Power:**

- Enzymes can accelerate reactions by factors of millions, achieving reaction rates far more significant than those seen in non-catalyzed reactions. This is due to their ability to lower the activation energy, often through precise molecular interactions in the active site.

**Specificity:**

- Enzymes exhibit remarkable specificity for their substrates, meaning they catalyze only one or a few related reactions. This specificity is due to the highly structured active site that fits only specific substrate molecules (e.g., the **lock-and-key and induced-fit models**).

**Regulation:**

- Enzyme activity is often tightly regulated. This regulation can occur through mechanisms such as **feedback inhibition**, where the product of an enzymatic pathway inhibits an enzyme earlier in the pathway, or through **allosteric regulation**, where molecules bind to a site other than the active site to modulate the enzyme's activity.

**3. Nomenclature and Classification**

- Enzymes are classified into six main classes based on the type of reaction they catalyze:
    1. **Oxidoreductases**: Catalyze oxidation-reduction reactions.
    2. **Transferases**: Transfer functional groups from one molecule to another.
    3. **Hydrolases**: Catalyze hydrolysis (breaking bonds with water).
    4. **Lyases**: Add or remove groups to form double bonds.
    5. **Isomerases**: Convert molecules into their isomers.
    6. **Ligases**: Join two molecules using energy from ATP.

**Fischer's Lock-and-Key Hypothesis:**

- Proposed by **Emil Fischer**, it suggests that the enzyme's active site is rigid and fits the substrate like a key fits a lock. The specific complementary shapes of the enzyme and substrate facilitate catalysis.

**Koshland's Induced Fit Hypothesis:**

- **Daniel Koshland** expanded on Fischer's idea, proposing that the enzyme active site undergoes conformational changes upon substrate binding, which helps to stabilize the transition state and increase catalysis.

**Active Site Identification:**

- **Inhibitors**, **affinity labeling**, and **enzyme modification** identify the active site and study enzyme-substrate interactions.
    - **Inhibitors**: Molecules that block or decrease enzyme activity (competitive, non-competitive, uncompetitive).
    - **Affinity Labeling**: Covalent attachment of a label to the enzyme active site.
    - **Site-Directed Mutagenesis**: Altering specific amino acids in the enzyme to study their role in catalysis.

## 4. Mechanism of Enzyme Action

**Transport State Theory:**

- This theory suggests that enzymes stabilize the transition state between the substrate and product, lowering the activation energy required for the reaction.

**Orientation and Steric Effects:**

- Enzymes bring substrates into optimal orientation to facilitate the reaction, ensuring that the correct bonds are formed and broken.
- **Steric effects** involve the positioning of atoms or molecules in a way that reduces the system's free energy, allowing for efficient catalysis.

**Acid-Base Catalysis:**

- Enzymes can donate or accept protons ($H^+$) to stabilize reaction intermediates, facilitating the breaking or forming of bonds.

**Covalent Catalysis:**

- In covalent catalysis, the enzyme forms a transient bond with the substrate, stabilizing a reaction intermediate and facilitating the conversion to the product.

**Strain or Distortion:**

- Enzymes induce strain or distortion in the substrate, destabilizing bonds and lowering the activation energy for the reaction.

**Examples of Enzyme Mechanisms:**

- **Chymotrypsin** is a protease enzyme that breaks peptide bonds via acid-base and covalent catalysis.
- **Ribonuclease**: An enzyme that cleaves RNA using acid-base catalysis and stabilizes the transition state through hydrogen bonds.

## 5. Co-Enzyme Chemistry

**Cofactors and Coenzymes:**

- **Cofactors** are non-protein molecules and enzymes required to function, often derived from vitamins. They can be inorganic (e.g., metal ions) or organic molecules known as **coenzymes**.

**Apoenzymes and Prosthetic Groups:**

- **Apoenzyme**: The protein part of the enzyme, which requires a cofactor to become active.
- **Prosthetic Group**: A tightly bound, non-polypeptide unit required for the enzyme's activity.

**Common Coenzymes and Their Functions:**

- **Coenzyme A (CoA)**: Involved in the transfer of acyl groups, crucial for fatty acid metabolism.
- **Thiamine Pyrophosphate (TPP)**: Derived from vitamin B1, involved in decarboxylation reactions.
- **Pyridoxal Phosphate (PLP)**: Derived from vitamin B6, it plays a role in amino acid metabolism.
- **$NAD^+$ and $NADP^+$** are electron carriers, with $NAD^+$ involved in catabolic reactions and $NADP^+$ in anabolic reactions.
- **FMN and FAD**: Flavin mononucleotide and flavin adenine dinucleotide are involved in electron transfer in oxidation-reduction reactions.
- **Lipoic Acid**: Acts as an electron carrier in reactions involving pyruvate decarboxylation.
- **Vitamin B12**: Involved in methyl group transfers, particularly in DNA synthesis and fatty acid metabolism.

**Mechanisms of Cofactor-Catalyzed Reactions:**

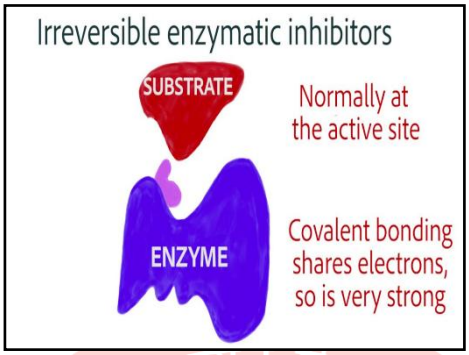
- Each of these coenzymes participates in a specific reaction mechanism, often involving electron or group transfer to and from the enzyme active site. For example, **$NAD^+$** accepts electrons in redox reactions, while **Coenzyme A** carries acyl groups in metabolic cycles like the citric acid cycle.

## Short answer type questions:

**Q. What is irreversible inhibition? (CSJMU 2019,2016,2013)**

**Ans. Irreversible Inhibition:** These substances bind tightly to the activity of the enzyme molecule, alternating them so that they permanently lose their catalytic properties. These inhibitors bind with or destroy the functional groups of the enzyme molecules necessary for their catalytic activities. For example, the compound diisopropylfluorophosphate (DFP) is an irreversible inhibitor, inhibiting the

enzyme acetylcholinesterase, which is important in transmitting nerve impulses. Diisopropylfluorophosphate is very reactive and combines with the hydroxyl group of an essential serine residue at the enzyme's active site to form a catalytically inactive derivative. Once this derivative is formed, the enzyme inhibitor is iodoacetamide, which can react with sulfhydryl (-SH) groups of essential cysteine residues or with the imidazole group of essential histidine residues.




**Q. Write a short note on synthetic enzymes. (CSJMU 2017)**

**Ans.** Synthetic enzymes, also known as artificial enzymes or synzymes, are organic molecules or ions that mimic or recreate the functions of natural enzymes. They can be designed to:

1. Mimic the catalytic ability and specificity of a natural enzyme 2. Catalyze chemical reactions that do not occur naturally

Here are some examples of synthetic enzymes:

**A. Syn-F4**: The first synthetic enzyme that could sustain life in living organisms

**B. Neu5Ac aldolase**: A pyruvate-dependent enzyme that can synthesize Neu5Ac from ManNAc.

**C. Sialic acid aldolase**: Can be used in combination with CSS and SiaT to synthesize sialyllactose tri saccharides

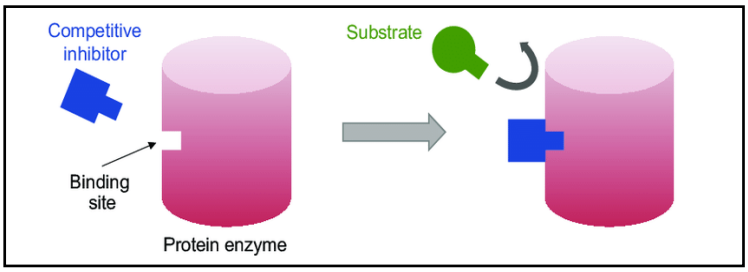
## Long answer type questions:

**Q. What is reversible inhibition? (CSJMU 2018)**

**Ans. Reversible Inhibition**

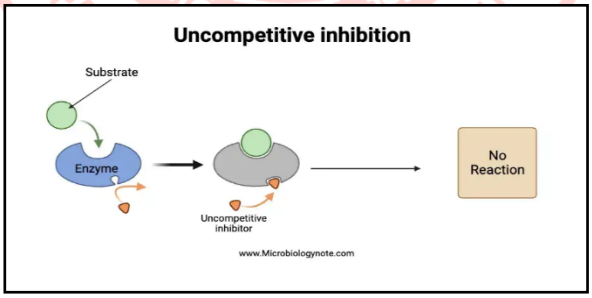
These substances bind less tightly to enzymes, and their inhibiting effect can be reversed. There are three main types:

(i) Competitive inhibitors (ii) Uncompetitive inhibitors (iii) Non-competitive inhibitors



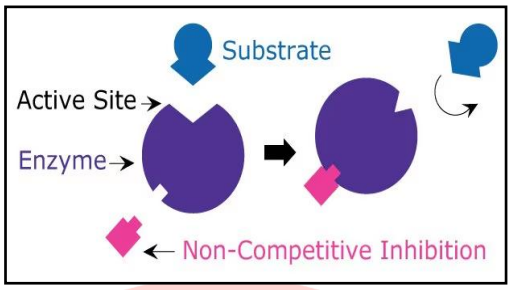

**Competitive inhibition:** These types of inhibitors compete with the substrate for binding to the enzyme's active site, but once bound, the enzyme cannot transform them. Their effect can be reversed or relieved simply by increasing the substrate concentration. These inhibitors generally resemble the standard substrate in a three-dimensional structure. As a result, the ability of the substrate to bind to the enzyme is reduced. Because of resemblance, the competitive inhibitor tricks the enzyme into binding to the active site. However, the enzyme molecules cannot attack the inhibitor molecules since their active site is occupied. A typical example of competitive inhibition is the inhibition of succinate dehydrogenase by the malonate anion. Succinate dehydrogenase is involved in the citric acid cycle, which catalyzes the removal of two hydrogen atoms from succinate one from each methylene group.

**Uncompetitive inhibition:** These enzyme inhibitors are not very specific and bind at a site on the enzyme other than the active site. This binding alters the enzyme molecule's conformation so that the active site's reversible inactivation occurs. These inhibitors bind reversibly to the free enzyme and the enzyme-substrate complex to form inactive complexes.




The uncompetitive inhibitors are the compounds that reversibly combine with only the enzyme-substrate complex but not with the free enzyme, and a high concentration of the substrate does not overcome this type of inhibition. Increasing substrate concentration increases the degree of inhibition instead of releasing the inhibition. An uncompetitive inhibitor binds with an already formed enzyme-substrate complex and has equal effects on Km and Vmax. This type of inhibition is rare in a one-substrate reaction but causes product inhibition in reactions with multiple substrates and products.

**Non-competitive inhibition:** These inhibitors are the reversible type, which does not bind to the active sites of an enzyme. These have not competed with the substrate for the active site and do not prevent S from binding to the enzyme. Non-competitive inhibitors bind to the enzyme and cause a conformational change in it. The enzyme thus becomes inactive. The inhibitors reduce Vmax for the reaction but do not change Km.




**Q. Discuss the concept of the lock-key fit theory of enzyme action. (CSJMU 2018)**

**Ans. Fisher's lock & key hypothesis:**

Enzymes are particular; therefore, the reasonable question is what is their mechanism of action. According to Arrhenius, enzymes catalyze the reaction by forming an unstable intermediate. The Lock and Key model proposed by Fischer is the simplest model to explain enzymatic action. This model assumes that the enzyme has a rigid three-dimensional body, the surface of which has active sites that have slots for fitting definite substrates just as a key fit in a particular lock.

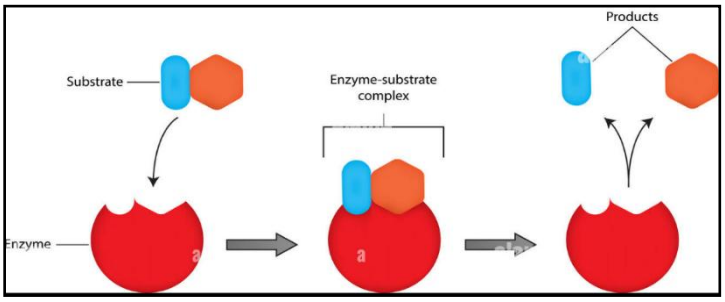
An enzyme molecule is huge (consisting of 100 to 200 amino acid residues), but active sites, which combine with substrate and have a definite shape in which substrate can be fixed, are comparatively small (with few amino acid residues). Amino acids of active sites are located at different places in the chain, whereas other amino acids, which are not part of active-sites, are located in a definite sequence. This is because this sequence allows the whole enzyme molecule to fold exactly as required.

$$E + S \rightarrow ES \rightarrow E + \text{Product}$$

A hypothetical example of the mechanism of enzyme-action is given in above. This is referred to as a "Lock and Key Mechanism."

An enzyme-substrate complex is formed when the enzyme reacts chemically with the substrate (Michaelis-Mention hypothesis).

The enzyme-substrate complex then breaks down to give the products of a reaction. The enzyme is released and can be used over and over again.




**Lock and Key Mechanism**

The Lock and Key model explains the action of many enzymes. However, there is evidence that this model is too restrictive for other enzymes. Enzyme molecules are in a dynamic state, not a static one. There are constant motions within them, so the active site has some flexibility.

**Q. Concept and identification of active site by the use of inhibitors. Explain. (CSJMU 2015,2013)**

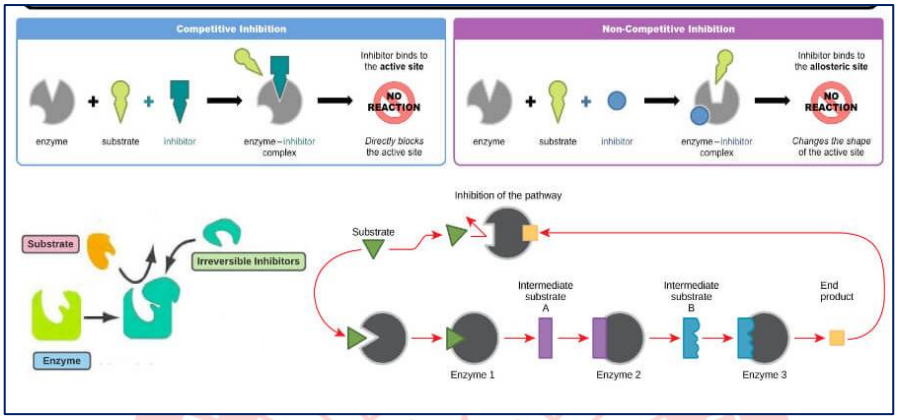
**Ans. Concept and identification of active (enzyme inhibition-reversible and irreversible):**

The substrate is attached to a specific cavity or site in an enzyme. The cleft has an active core, where the amino acids are clustered together to allow them to mix with a substrate. A long distance in the polypeptide chain may separate the reactive amino acids. The chain, on the other hand, folds in such a way that the reactive amino acids are brought together in the active site. When the substrate molecule connects to the active site, it is thought that the Parts are held together so that chemical bonds are distorted, i.e., the bonds are weakened. The substrate or chemical bonds' reactivity is increased due to this distortion, which speeds up the reaction rate. The strain model of catalysis describes how the reaction products are liberated because they are less tightly bound. Inhibitors are chemicals that slow down the process rate catalyzed by an enzyme. The following is a diagram of inhibition.

*Enzyme inhibitors are molecular agents that interfere with catalysis to show or eliminate enzymatic activity.*

These compounds can combine with certain enzymes but do not serve as substrates:

Instead, these after or even block enzymatic catalysis. Poison acts upon living bodies by inhibiting enzymes. For instance, Carbon monoxide poisoning is combined with the hemoglobin, thus making it useless fort performing its usual role as a carrier of oxygen. Cyanide poisoning due to its combination with natural substances, particularly with a metallic center of Cytochrome. The poisonous effect of arsenate is due to its blocking of enzyme sites in place of phosphates. A wide range of naturally occurring and synthetic compounds can bind reversibly or irreversibly to specific enzymes.




**Q. How are the active sites of enzymes identified? Explain with suitable examples. (CSJMU 2014)**

**Ans. The active site of an enzyme-catalytic power of enzymes:** Enzymes display their remarkable feat of catalysis by presenting their three-dimensional environment (made out of L-amino acids) to the substrates. The active sites of an enzyme, *i.e.*, the functional portion of an enzyme, occupy only a very small portion of the enzyme molecule. The active side site of an enzyme may be shown overall by either of the representations. When a substrate binds to an enzyme, the enzyme changes the substrate's structure, causing it to move toward the transition state. An enzyme's active site usually has a form that closely resembles the transition state rather than the substrate. For this region, transition state analogs are potent inhibitors.

1. In the tertiary structure of protein enzyme (folding of enzyme), the active sites (amino acid residues) are relatively nearer, whereas in the primary structure, the active sites are far from each other. The letters A, B, and C show these amino acid residues.

2. Catalysis takes place in the active site of every enzyme molecule. The binding site is located within the active site, and it is here that the amino acid residues (R-groups) bind the substrate in the correct location for reaction. In an intramolecular reaction, this is analogous to the appropriate location of the reactive group. Hydrogen bonding, electrostatic interactions, and other factors play a

role in this binding. These favorable interactions with amino acid residues in the active site stabilize the transition state, lowering the process's active activation energy and increasing the reaction rate.

3. There is also a pocket (P) in the active site, which, *e.g.*, in the case of carboxypeptidase, can accept the side chain of terminal amino acid when this enzyme splits the terminal amino acid from a peptide chain.

4. Cofactors are non-protein molecules that many enzymes require for the reaction. These cofactors are metal ions or small organic molecules called coenzymes (NAD+, NADP+). Most coenzymes are bound by ionic bonds or other non-covalent bonding interactions; others are bound covalently and are termed prosthetic groups. In carboxypeptidase A, zinc ions are essential for enzymatic activity. Zinc ions are located in a groove near the enzyme's surface and held in position via coordinate to two histidine side chains, a glutamate side chain, and a water molecule.

5. After the substrate is properly bound in the enzyme's active site, the R group of other amino acids brings about the catalytic activity.

**Q. What are enzymes, and can you explain their important properties? (CSJMU 2014,2011)**

**Ans.** *"Enzymes can be defined as biological polymers that catalyze biochemical reactions."*

Perhaps the oldest known biochemical and bio-organic phenomenon is the fermentation of juices into alcoholic beverages. Also, this was an Ist chemical transformation catalyzed by enzymes within living Yeast cells. It was discovered in the 18th century that fermentation converts sugar into carbon dioxide and alcohol. The 19th century witnessed the identification of fermentation as a physiological act of yeast cells and the introduction of forces to review Pasteur's view that life and fermentation are inseparable. By now, the extraction of enzymes from bio-cells was known, and this Discovery, like most scientific discoveries, was accidental. In 1897, E. Buchner required a quantity of purified protein for therapeutic purposes. He ground yeast and sand, filtered the broken cells, and added a large amount of sugar to the filtrate as a preservative. Enzymes are biochemical catalysts (biocatalysts) needed in almost all the biochemical reactions in a living system. Enzymes are proteins in nature, which can be defined as biocatalysts synthesized by living cells. Kuhne used the word enzyme, which comprises two words, Greek- en: in and zyme: yeast. Berzelius coined the term catalysis, which means to dissolve (Greek: to dissolve) 1836, and it could catalyze the fermentation reaction.

**Remarkable properties of enzymes as catalysts:**

1. Enzymes are highly effective catalysts, enhancing the reaction rate by 105-1017. 2. Then, the substrate is tightly bound in the active site to form an ES complex. 3. Like other catalysts, enzymes

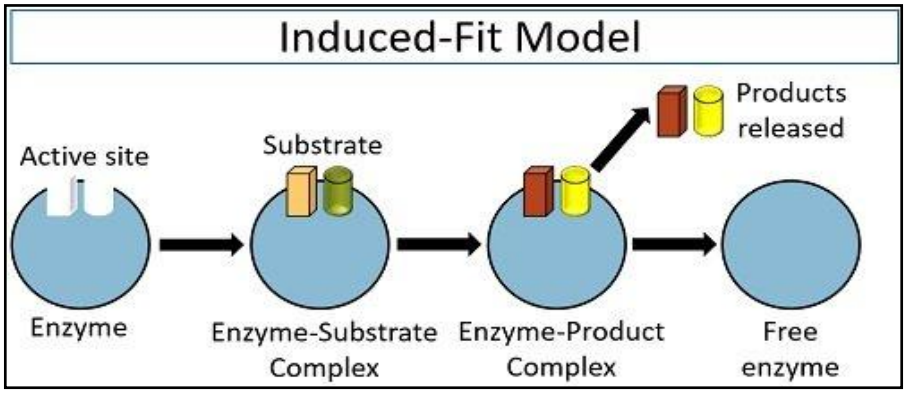
lower the activation energy for a reaction and enhance the reaction rate. The enzyme does not affect the reaction's equilibrium. 4. The energy used for enzymatic rate enhancement is largely derived from weak interactions like hydrogen bonding ionic and hydrophobic interactions. 5. The active sites provide these interactions, stabilizing the transition state. 6. The general acid bas4e catalysis, covalent catalysis, and metal ions catalysis help to provide a lower energy path. 7. The binding energy helps lower the substrate's entropy and can bring about a conformational change in the enzyme to induce fit. 8. Binding energy also justifies the enzymes' behavior as a catalyst.

**Q. Discuss the concept of Koshland's induced fit hypothesis. (CSJMU 2012)**

**Ans.** Koshland gave this model, the Fischer model (1966). In the Fischer model, i.e., the Lock and Key model, the active-site is presumed to be reshaped to fit the substrate. In the induce -fit theory, the substrate induces a conformational change in the enzyme. This aligns amino acid residue or the other groups on the enzyme in the correct spatial orientation for substance-binding catalysis. At the same time, the other amino acid residues may get buried in the interior of the enzyme.

In Fig, in the absence of substrate, the catalytic and the substrate-binding groups are removed by several bond distances. When the substrate approaches, a conformational change occurs in the enzyme protein, aligning the groups correctly for binding and catalysis. At the same time, a change in the spatial orientation of the other regions also occurs.

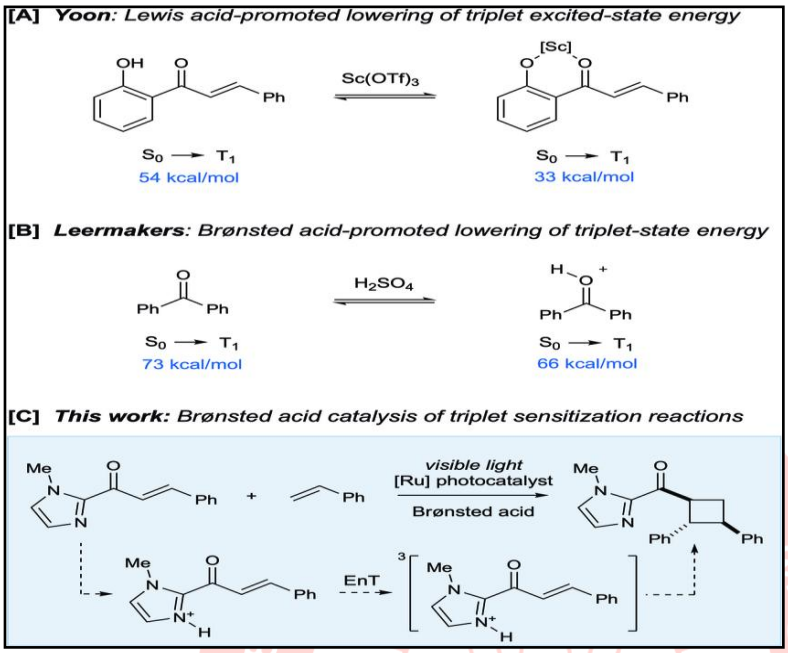
The main evidence favoring the induced fit model is a demonstration of conformational changes during substrate binding catalysis with creatine kinase, phosphoglucomutase, and several other enzymes. Until now, the exact sequence of events in a substrate-induced conformational change has not been established. There may be several possibilities, as shown in fig. Even if one knows the complete primary structure of an enzyme, it is not easy to decide exactly which residues constitute.




## Short answer type questions:

**Q. Write a short note on acid – base catalysis. (CSJMU 2018,2017)**

**Ans.** In acid catalysis, a Bronsted acid donates a proton to a substrate, allowing it to attain a high-energy transition state and facilitating its conversion to a product. In the case of a base-catalyzed reaction, a Bronsted base extracts a proton from the substrate, leading to the formation of a transition state intermediate.




**Q. Explain the role of ribonuclease. (CSJMU 2017,2016,2014,2012)**

**Ans.** Ribonuclease (RNase) is an enzyme that cleaves specific RNA sequences. It involves various cellular processes, such as DNA replication and rRNA processing. The exact function of RNase in cell cycle control is still unknown. RNase MRP is a specific type of RNase found in different organisms, including yeast.

**1. RNA maturation**: RNases are involved in the maturation of tRNAs for protein translation and rRNA precursors.

**2. DNA replication**: RNases break down RNA primers that promote DNA replication.

**3. Host defence**: Some RNases, like hRNase2/EDN and hRNase3/ECP, help the body defend against pathogens.

**3. Membrane receptor biology**: hRNase5/ANG plays a role in membrane receptor biology by binding to EGFR and activating it.

## Long answer type questions:

**Q. What is the enzyme mechanism for chymotrypsin? (CSJMU 2013)**

**Ans.** Chymotrypsin is an enzyme that belongs to the general class of serine proteases. Chymotrypsin is a proteolytic enzyme that acts in the digestive systems of many organisms and is produced and secreted by the pancreas. It facilitates the cleavage of peptide bonds by a hydrolysis reaction, a process that, albeit thermodynamically favorable, occurs extremely slowly in the absence of a catalyst.

The active site of Chymotrypsin, marked by serine 195, lies in a cleft on the enzyme's surface. The enzyme action occurs via the concerted action of the three amino acid residues in the catalytic triad. Serine 195, Histidine 57, and Aspartate 102 are these three amino acids. Placement of these amino acids in a linear array forms what is known as the catalytic triad. The linear arrangement of these three amino acid residues allows a charge relay to take place from Aspartate to Serine, activating the Serine195 residue.

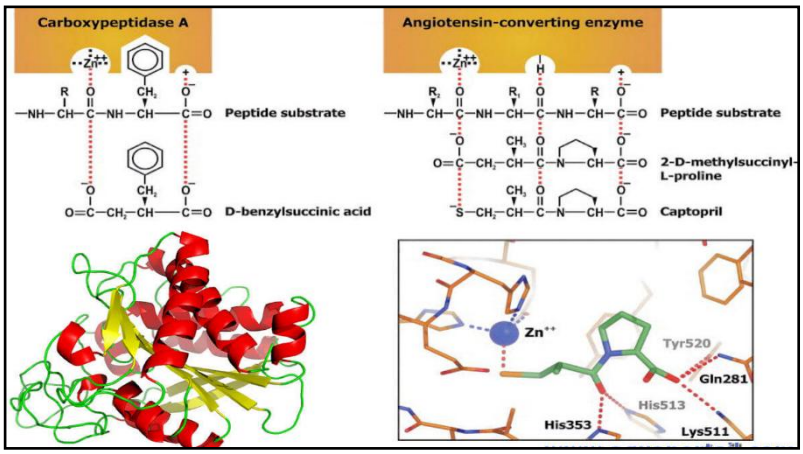
Step 1: When substrate (polypeptide) binds, the side of the chain of the residue next to the peptide bond cleaves and nestles in a hydrophobic pocket on the enzyme, positioning the peptide bond for attack. In the substrate binding pocket of the enzyme, Histidine 57 extracts one proton from serine to form an alkoxide ion. This serine ion reacts with the substrate.

Step 2: The carboxylate R-group of Asp102 forms a hydrogen bond with N-δ hydrogen of His 57, increasing the pKa of its ε nitrogen and thus making it able to deprotonate serine 195. This deprotonation of Ser195 by His57 turns it into a strong nucleophile that can now attack the substrate. Oxygen develops a partially negative charge in the oxyanion hole.

**Q. Write a short note on the followings: (CSJMU 2011)**

**1. Carboxypeptidase A and its mechanism.**

**Ans.** Carboxypeptidase A is a digestive enzyme secreted by the pancreas into the small intestine. It functions to digest proteins and peptides in the small intestine into smaller peptides by hydrolyzing the peptide bonds. It preferentially hydrolyses the peptide bond at the carboxy end of amino acid residues with aromatic or branched hydrocarbon side chains. This enzyme is also referred to as CPA. Besides digestion, Carboxypeptidase A is also involved in post-translational modification of proteins, blood clotting, and reproduction. Coordination of the enzyme with zinc ion is crucial for its activity, and hence, this enzyme is also classified as a metallopeptidase. Loss of zinc leads to loss of enzyme activity. However, other divalent metal ions, such as cobalt and nickel, can replace zinc.




**Structure and Mechanism of Carboxypeptidase A**

Carboxypeptidase A is a single polypeptide chain enzyme of 307 amino acids. It is a compact globular protein consisting of alpha helices and beta-pleated sheets. When the enzyme is not bound to its substrate, the zinc ion within the active site of the enzyme is coordinated to five amino acid residues viz., with two imidazole Nδ1 nitrogens of His 69 and His 196, the two carboxylate oxygens of glutamate-72, and a water molecule to form a distorted tetrahedral. Altogether, five amino acid residues are involved in substrate binding: Arg-71, Arg127, Asn-144, Arg-145, Tyr-248, and Glu-270. Once the substrate is bound to the enzyme, the coordination number of the zinc ion can vary from five to six. The zinc ion polarizes the carbonyl bond, which makes the carbonyl carbon more susceptible to nucleophilic attack.

$$Zn + O\ \delta^{-} \text{---} C\ \delta^{+}$$

The mechanism of Carboxypeptidase A mediated cleavage of the peptide bond at the C-terminal end of aromatic amino acid residues in a protein chain is diagrammed in figure 7 below using glycyl-tyrosine as an example of a substrate: The substate fits into the nonpolar pocket in the enzyme active site.

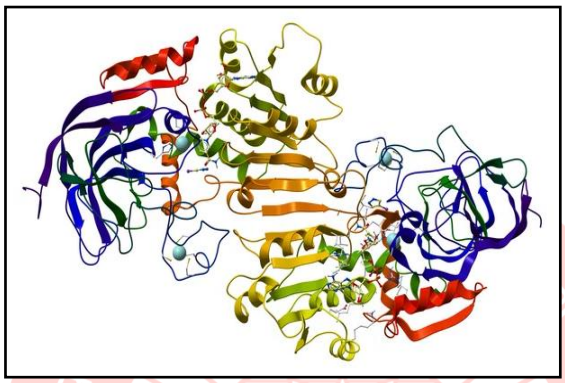
**2. Isoenzymes and its mechanism.**

**Ans.** Enzyme exists in multiple forms to perform different biological functions. Isozymes are different forms of the same enzyme with minor differences in the amino acid sequence (similar structure) but perform different functions of the same chemical reaction. R L Hunter and Clement Markert first described isozymes in 1957 as ‘different variants of the same enzyme having identical functions and present in the same individual,’ However, with the discovery of new Isozymes, the same metabolic reaction replaced the same function with different functions. Isozymes are encoded by different genes and expressed in a distinct organelle or at a distinct stage of development. The purpose of isozymes is to allow fine adjustment of metabolism to meet the needs of different development

stages and help the different tissues and organs function properly depending on their physiological makeup and in what kind of environment they function. Regarding kinetics, isoenzymes can fine-tune their enzymatic rate constants $K_m$ and Kcat. This adaptation allows for the proper use of the enzyme based on its environment.

**Method of characterization and separation of the Isozymes**

They differ from each other structurally, electrophoretically, and immunologically. Therefore, the methods that characterize the structure, electrophoretic movement, and immunological recognition

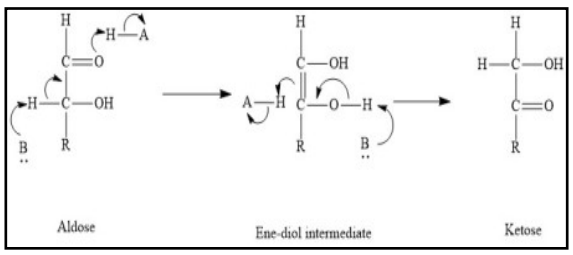


of isozyme can be used to separate the isozyme. The methods that distinguish the structure include circular dichroism (differentiate secondary structure), NMR (differentiate tertiary structure in solution), Fluorescence (differentiate tertiary structure in solution), and IR (differentiate secondary structure), spectroscopy. Therefore, they are utilized to characterize the isozymes structurally. Electrophoresis separates molecules based on charge, shape, and size of the molecules. Isozymes differ in the amino acid composition and can be easily separated by electrophoresis. Isozymes have differences in sequence; therefore, if the sequential antibody is synthesized against the isozyme, the antibody can be used as an affinity ligand to purify the isozyme.

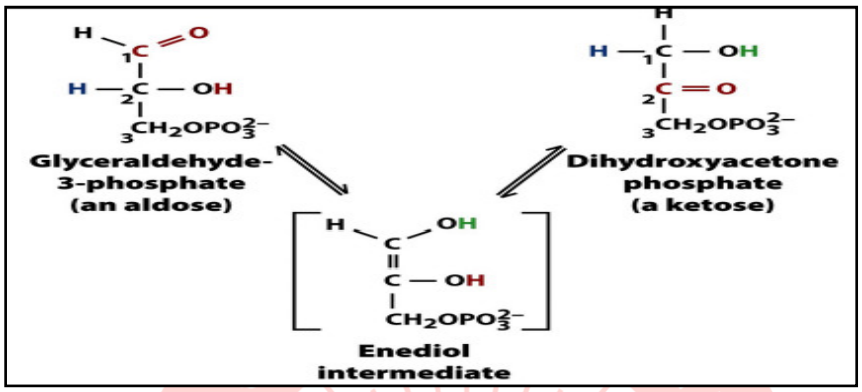
**Q. Discuss the role of enzymes on enolic intermediates in isomerization reactions. (CSJMU 2012)**

**Ans. Enolic intermediates in isomerization reactions:**

Isomerization reactions are those in which an H-atom is moved intramolecularly to alter the location of a double bond. In these reactions, a proton is moved from one C-atom to another. The process proceeds through an intermediate called enediol.

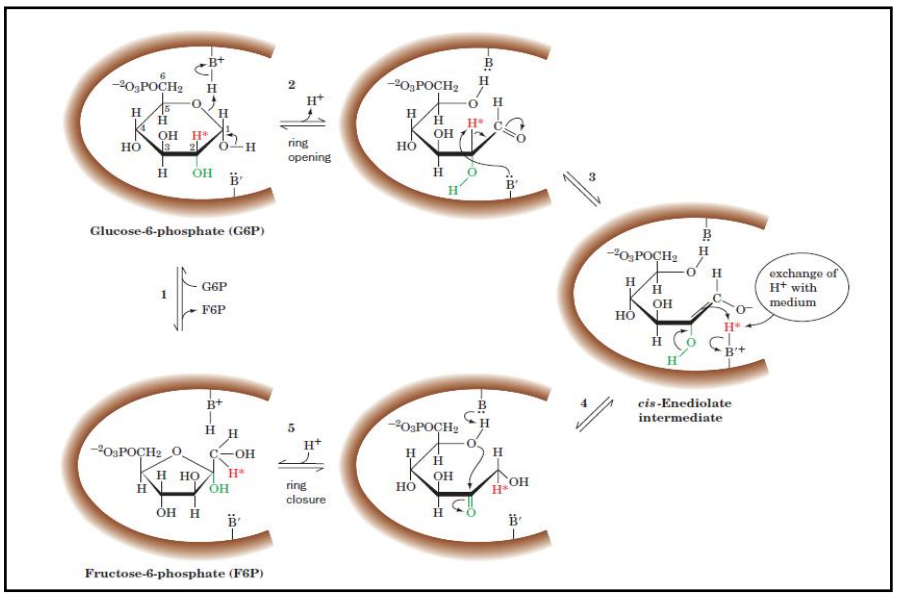


The two ionizable groups on the enzyme are X1 and X2. In the first step, X1 provides a proton, and X2 donates an electron to produce a C=C bond and the intermediate enediol. The second phase involves the donation of a proton by X1 and the abstraction of a proton by X2, which allows the C=O bond to form. The C—H bond is created due to the displacement of an electron pair from the C=C bond. The enzyme triose phosphate isomerase catalyzes the conversion of dihydroxy acetone phosphate to glyceraldehyde-3-phosphate, which illustrates an isomerization reaction. The enediol intermediate is formed as the reaction progresses.




The catalytic mechanism of the enzyme triose phosphate isomerase involves the abstraction of a proton from the substrate by glutamate residue and the donation of a proton by lysine residue, resulting in the creation of the intermediate enediol. Glyceraldehyde-3-phosphate is produced in the second stage by proton donation by glutamate and proton abstraction by lysine.

The transformation of glucose-6-phosphate into fructose-6-phosphate, catalyzed by the enzyme phosphoglucose isomerase, is another illustration of an isomerization reaction. In order to create a cis-enediol intermediate, a base from the imidazole component of the His-Glu dyad abstracts a proton from the C-2 atom. In a whole proton transfer, a proton is transferred onto the C-1 atom.



**Q. Describe the enzyme-catalyzed bio-organic carboxylation and decarboxylation reaction. (CSJMU 2011)**

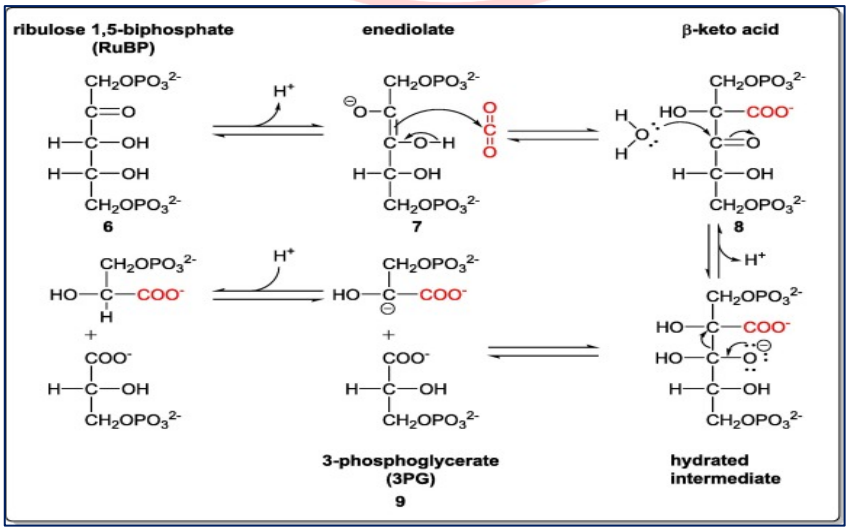
**Ans. Enzyme-catalyzed carboxylation and Decarboxylation:**

The most important C—C bond-building and bond-breaking reactions in biological processes result in the gain or loss of one carbon in the form of CO2. Carboxylation refers to adding a CO2 unit to a substrate molecule, whereas decarboxylation refers to removing carbon in the form of CO2. RuBisCO and biotin-dependent carboxylases catalyze the bulk of carboxylation reactions in metabolic pathways. The enzyme ribulose-1, 5-bisphosphate carboxylase oxygenase (RuBisCO) catalyzes the synthesis of 3-phosphoglycerate from ribulose-1, 5-bisphosphate (RuBP).

The most prevalent protein on the planet, this enzyme contains up to 50% of leaf proteins. The 500–560 KD protein known as RuBisCO from higher plants comprises eight large (L) subunits encoded by chloroplast DNA and eight tiny (S) subunits encoded by a series of nuclear genes. At the top and bottom of the protein, eight small subunits are grouped as two caps (tetramer), while eight big subunits are present in the space between the two caps. The L-subunit contains the enzyme's catalytic site.

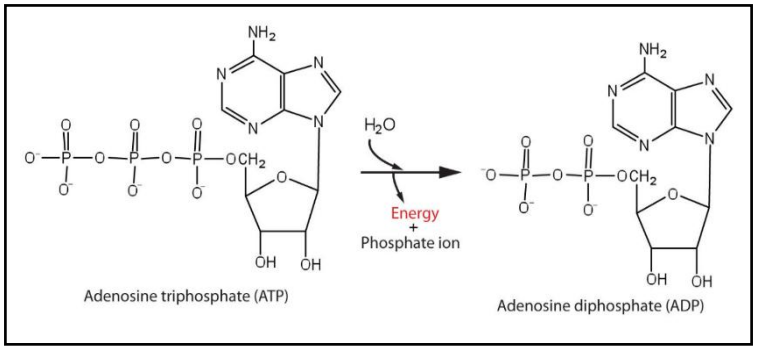
The removal of a proton from RuBP's C-3, which produces an enediolate, initiates the carboxylation process of RuBisCO. The intermediate enediolate then conducts a nucleophilic attack on CO2 to produce a β-keto acid. This β-keto acid then reacts with water to produce an adduct, which splits to produce a molecule of 3-phosphoglycerate and an intermediate carbanion. In order to create a second molecule of 3-phosphoglycerate, the carbanion is protonated. The finding that the homolog of the -keto acid intermediate, 2-carboxyarabinitol-1-phosphate (CA1P), binds closely to the active site of the enzyme from spinach supports this enzyme's mechanism.

(A) Production of an enediolate intermediate that attacks CO2 nucleophilically to produce a keto acid



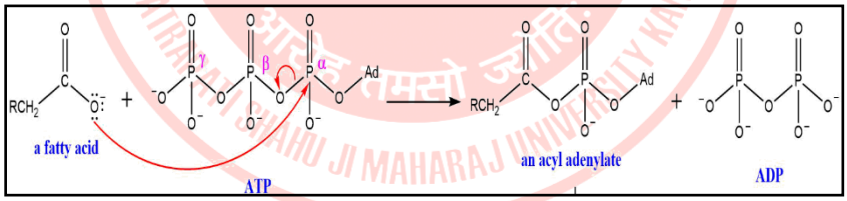
**Q. Write a short note about nucleophilic displacement on phosphorus atoms. (CSJMU 2013)**

**Ans. Nucleophilic displacement on phosphorus atom:**




**1. Important in living things is the chemical ATP:**

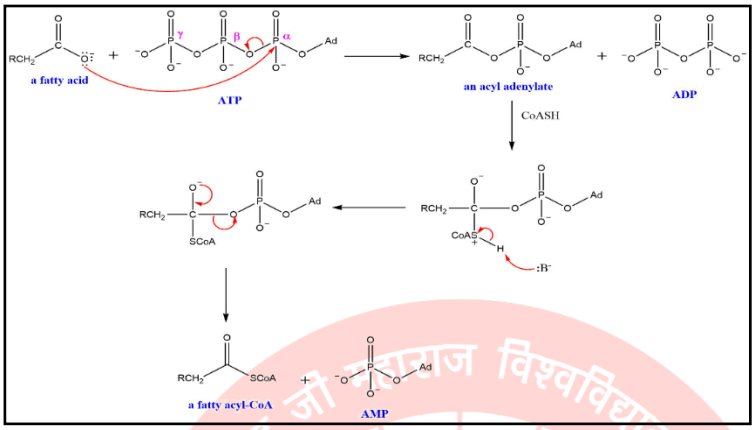
As a "high-energy" phosphate, adenosine triphosphate (ATP) is frequently mentioned. In biological terms, energy is released when ATP phosphorylates an acceptor (nucleophile) and transfers a phosphate group to that acceptor. Breaking phosphate bonds is crucial for energy transmission in a biological system. The type of nucleophile used in the phosphorylation affects how much energy is released. With water serving as the reference (Fig), ATP is hydrolyzed to produce adenosine diphosphate (ADP), which releases energy at a rate of around 7 kcal/mol (30 kJ/mol).



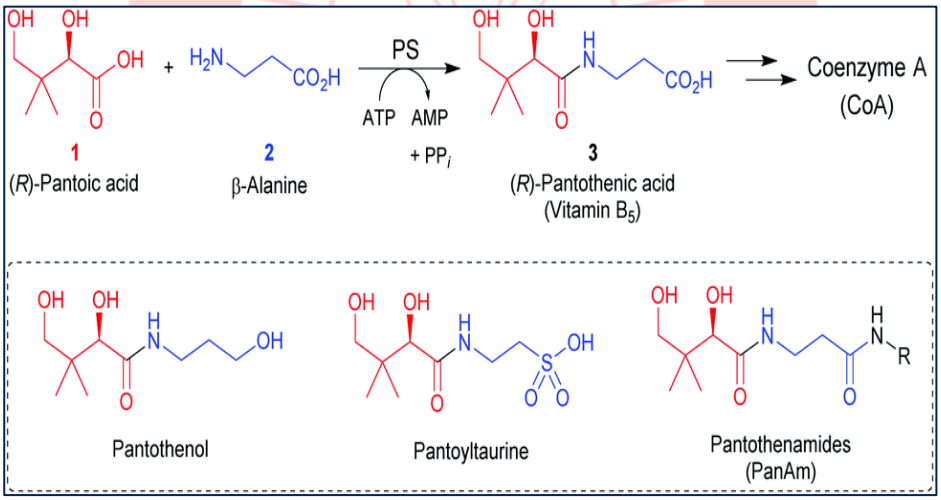

**Multiple displacement reactions of phosphorus**

In biological systems, ATP reacts with a variety of nucleophiles. These are oxygen from alcohol or carboxylate, nitrogen from creatine, oxygen from the side chain of arginine, or oxygen from the histidine side chain. Depending on the enzyme initiating the reaction, each of the three phosphates of ATPα-, β-, orγ-is susceptible to nucleophilic displacement and gives a unique product.

When a pyrophosphate is hydrolyzed, it yields two equivalents of phosphate. So, when a pyrophosphate is produced due to nucleophilic displacement on phosphorus, its subsequent hydrolysis moves the reaction to the right, guaranteeing its irreversibility. When such irreversibility is necessary, biochemical processes occur by nucleophilic displacement on α or β phosphorus of ATP.




**Q. Explain in short co-enzyme and Apoenzyme. (2020,19)**



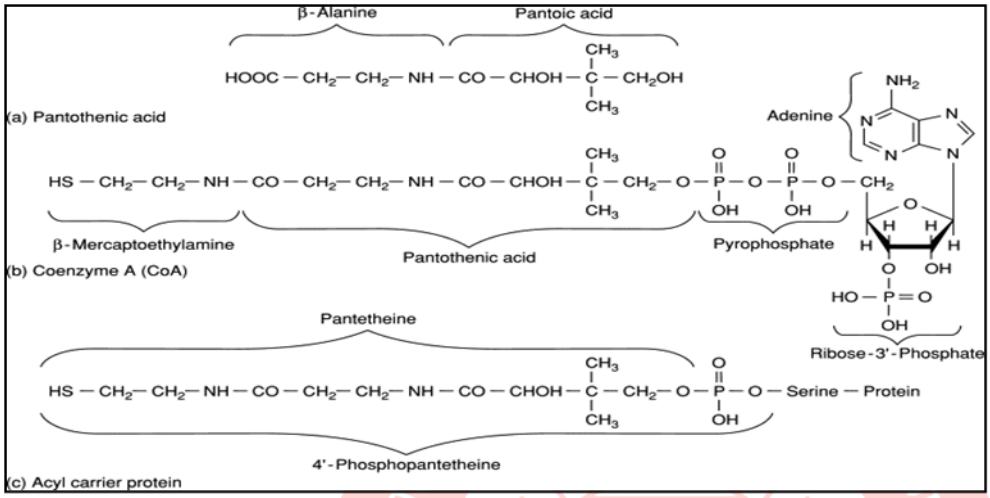

**Ans.** Coenzyme A is an acyl-activating enzyme derived from vitamin pantothenic acid. Pantothenic acid is a pentomic acid, and β alanine joined in an amide linkage. Coenzyme A can be divided into two components, adenosine 3, 5 diphosphate, and pantetheine, formed by the combination of pantothenic acid and β mercaptoethylamine.

**Structure**

The SH group of thioethanolamine amine moiety is an active group acting as a carrier, and coenzyme A is abbreviated as CoA or CoASH. The rest of the molecule provides an enzyme binding site. In acylated derivatives, such as acetyl-coenzyme A, the acyl group is linked to the thiol

group to form an energy-rich thioester. CoA reacts with many compounds to form important derivatives such as:

• Acetyl CoA • Succinyl CoA • HMG CoA • Fatty acyl CoA, Acetyl CoA, and succinyl CoA are important intermediates at cross roads of many metabolic pathways. Acyl CoA is formed as an intermediate in fatty acid biosynthesis and oxidation.




**Apoenzyme**

**Ans.** An enzyme that requires a cofactor but does not have one bound. An apoenzyme is an inactive enzyme, and activation of the enzyme occurs upon binding to an organic or inorganic cofactor. Coenzyme - A coenzyme is a substance that works with an enzyme to initiate or aid the function of the enzyme. It may be considered a helper molecule for a biochemical reaction. Coenzymes are small, nonproteinaceous molecules providing a functioning enzyme transfer site. They are intermediate carriers of an atom or group of atoms, allowing a reaction to occur. Coenzymes are not considered part of an enzyme's structure. They are sometimes referred to as cosubstrates.

**Q. Discuss the structure of FAD and its biological functions. (CSJMU 2019,2018,2015,2012)**

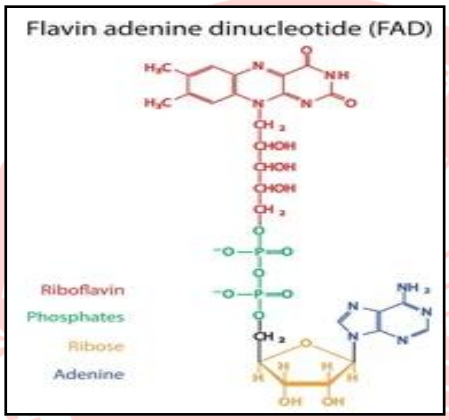
**Ans. Flavin Adenine Dinucleotide:**

1. Flavin adenine dinucleotide is a redox-active coenzyme in various proteins that participate in metabolic enzymatic processes.
2. The reaction begins with the conversion of riboflavin to flavin mononucleotide, which is mediated by riboflavin kinase.
3. The dinucleotide nomenclature is, therefore, deceptive, yet the flavin mononucleotide group is physically and chemically extremely similar to a nucleotide.

4. Flavin Adenine Dinucleotide is light, acid, and basic sensitive.

**Uses of FAD:**

1. Flavin adenine dinucleotide is an electron acceptor during the oxidation of succinate in the citric acid cycle.
2. Reduced anionic flavin adenine dinucleotide is the critical cofactor in DNA photolyase (PL) to repair cyclobutane pyrimidine dimers (CPD) in UV-damaged DNA.
3. Dihydrolipoamide dehydrogenase uses FAD to transfer reducing equivalents to NAD.
4. It also serves in nucleotide biosynthesis, beta-oxidation of fatty acids, amino acid catabolism, and synthesizing cofactors such as CoA, CoQ, and heme groups.




**Q. What cofactors are derived from vitamins, co-enzymes, prosthetic groups, and apoenzymes? Give the mechanism and biological function of any two cofactors. (2016,13)**

**Ans.** Cofactors are inorganic or small organic molecules that bind enzymes to enable or enhance their activity. Common inorganic cofactors are metals, including but not limited to magnesium, manganese, zinc, molybdenum, cobalt, and copper. Inorganic cofactors generally bind allosterically to enzymes and, in doing so, change the structure and chemical potential of the active site.

Inorganic cofactors can expose amino acids capable of donating or absorbing electrons in the active site, reducing the energy required to convert the enzyme's substrate to its products, thereby increasing the enzyme's catalytic activity. Cofactor binding can also change the active site's structure to increase the substrate's affinity for the active site, which increases the rate at which substrates bind, thus increasing enzymatic activity.

Organic cofactors, called coenzymes, are organic molecules that donate or accept an inorganic molecule or chemical group during enzyme catalysis. The chemical groups that can be donated or

accepted range from phosphate and methyl groups to lipids and sugars. All vitamins (e.g., nicotinamide adenine dinucleotide (vitamin B3) and ascorbic acid (vitamin C)) function as cofactors. Energetic molecules (e.g., ATP, ADP), proteins containing iron-sulfur clusters (e.g., metalloproteins), and even the nucleotide sugars of DNA can function as coenzymes.

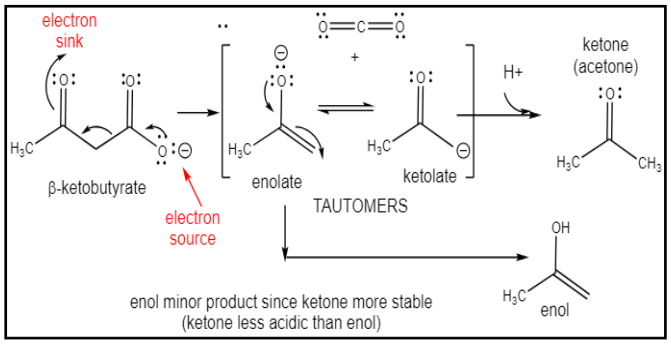
**Cofactor Function**

1. Enzymes use cofactors to change the structure of the enzyme's active site or to provide chemical groups not found within the enzyme that facilitate or regulate enzymatic activity.

2. These chemical groups can be used in multiple ways, including increasing substrate affinity, stabilizing intermediate products within the active site, contributing chemical groups to an enzyme product, or accepting a chemical group from the substrate. Common donor/acceptor groups include electrons, phosphate, oxygen, methyl groups, sugars, and fatty acids.

We present plausible mechanisms for prototypical reactions using some of the cofactors. Each shows the flow of electrons from a source to a sink. The source is often a pair of electrons on an anion, formed by a general base's prior removal of a proton from the atom. A sink could be a carbonyl O, which receives a pair of electrons from one of the C=O bonds of the carbonyl. As a bond is made to the carbonyl, one of the double bonds must break with the electrons going (temporarily if the reaction is a nucleophilic substitution reaction) to the carbonyl O, an excellent sink since it is so electronegative. An even better sink is a positive N of an iminium ion; examples are shown below. Just the "business parts" of the cofactors are shown below.

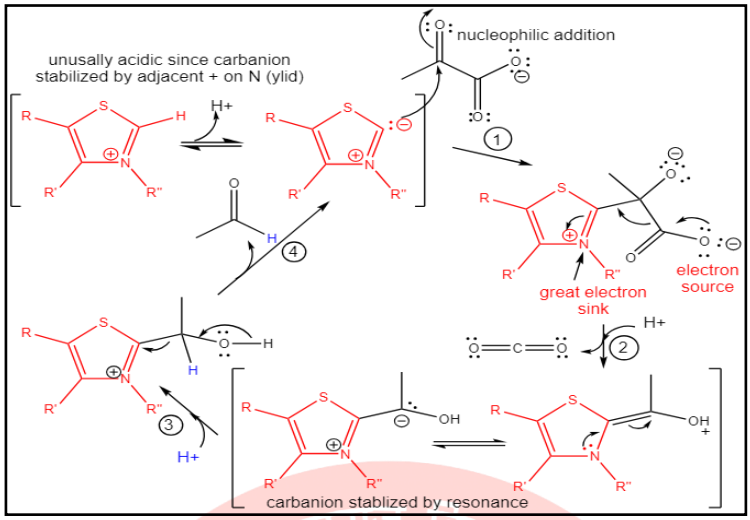
To appreciate the mechanism cofactors use and show a clear example of an electron source/sink, let us look at a reaction that does not require a cofactor, the spontaneous decarboxylation of a β-keto acid.




**Spontaneous decarboxylation of a β-keto acid**

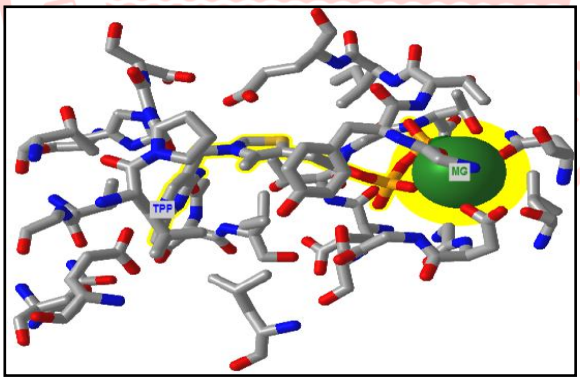
**Q. What is the function of thiamine pyrophosphate? (CSJMU 2013)**

**Ans.** Thiamine pyrophosphate (TPP) facilitates the decarboxylation of α-keto acids. TPP is a




derivative of thiamine, vitamin B1, whose deficiency causes beriberi.

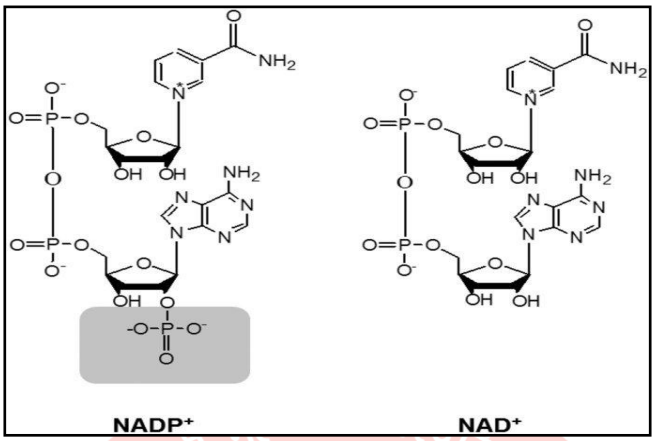
TPP is covalently attached to the enzyme, such as in pyruvate dehydrogenase and alpha-ketoglutarate dehydrogenase, which catalyze the decarboxylation of α-keto acids. The structure and "business" end of TPP and catalytic activity are shown. Interactive iCn3D model of the thiamine diphosphate-dependent enzyme pyruvate decarboxylase from the yeast Saccharomyces cerevisiae (1pvd).




**Q. What are the functions of NAD? (CSJMU 2012)**

**Ans.** These coenzymes are derived from vitamin niacin [vitamin B3]. Niacin is synonymous with nicotinic acid. NAD+ and NADP+ are the active form of niacin. They are called nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate, respectively. Collectively, they are called pyridine nucleotides. NAD+ is a dinucleotide comprising two nucleotides joined by a phosphodiester bond. One nucleotide comprises nicotinamide [N-base] with ribose and phosphate. The second is AMP. In NADP+, the second carbon-hydroxyl group of ribose of AMP is phosphorylated to form NADP -Nicotinamide, nicotinic acid, and tryptophan are all the precursors of

synthesis of nicotinamide mononucleotide (NMN), which is then converted to NAD+ and NADP+. In the oxidized form, the N-atom of nicotinamide is positively charged and written as NAD+ and NADP+. In the reduced form, this charge is neutralized. So, they are in the form of NADH and NADPH.




Nicotinamide adenine dinucleotide (NAD+) is a coenzyme that plays a vital role in many cellular processes, including:

**Energy metabolism**: NAD+ is a key factor in enzymatic reactions that produce energy, such as glycolysis, oxidative phosphorylation, and the TCA cycle.

**DNA repair**: NAD+ is a substrate for poly(ADP-ribose) polymerases (PARPs), which are involved in DNA damage and repair.

**Immune response**: NAD+ is involved in the immune response through proteins like CD38.

**Aging**

NAD+ levels decline with age, and this can lead to metabolic and aging-related disorders.

**Adaptation to environmental changes**

NAD+ helps cells adapt to environmental changes like nutrient perturbation, inflammation, and infection.

**Q. What is the biological function of co-enzyme-A. Which form is effective, and how is it produced naturally in the organism? (CSJMU 2011,12)**

**Ans.** Coenzyme A is an acyl-activating enzyme derived from vitamin pantothenic acid. Pantothenic acid is a pentomic acid, and β alanine joined in an amide linkage. Coenzyme A can be divided into two components, adenosine 3, 5 diphosphate, and pantetheine, formed by the combination of pantothenic acid and β mercaptoethylamine.

Based on the chemical nature, coenzymes can be classified as (i) vitamins or vitamin-derived coenzymes and (ii) nonvitamins or metabolite coenzymes. Vitamins help the body produce coenzymes and water-soluble vitamins like B-complex and vitamin C play major roles in the production of coenzymes.

**The function of co-enzyme-A:**

Coenzyme A (CoA) is a small, low-molecular-weight thiol that plays a key role in cell metabolism:

**1. Carrier of acyl groups**

CoA carries activated acyl groups within cells, forming reversible thioester bonds with carbon chains.

**2. Metabolic cofactor**

CoA is a metabolic cofactor that participates in many catabolic and anabolic reactions, including the metabolism of carbohydrates, lipids, proteins, ethanol, bile acids, and xenobiotics.

**3. Precursor to steroids and other compounds**

CoA is a precursor to steroids and other naturally occurring compounds, such as terpenes and acerogenins, which are present in plants.

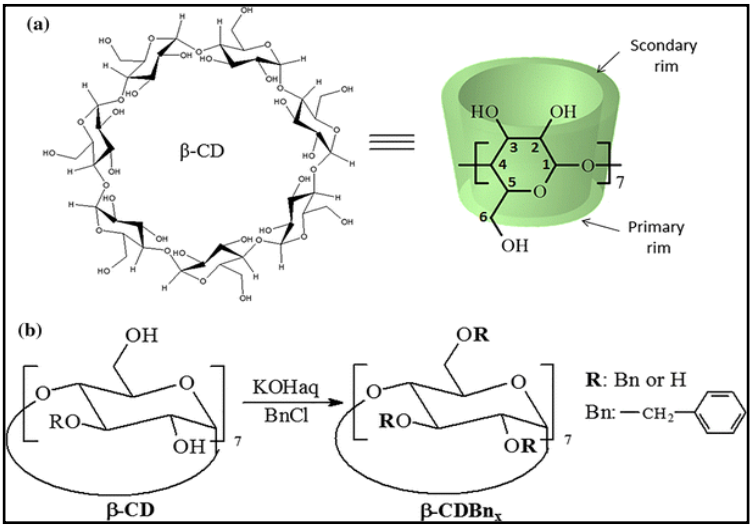
**4. Prolongs drug half-life**

CoA is used in certain drugs to prolong the half-life of the medication, extending the effect it has on the body.

**Q. Discuss any cyclodextrin-based enzyme model. (CSJMU 2016)**

**Ans.** Cyclodextrins (CDs) are a structurally related group of natural products formed during bacterial digestion of cellulose. CDs are cyclic oligosaccharides that contain (α1,4)-linked α-D-glucopyranose units and contain a sort of lipophilic central cavity and a hydrophilic surface on the outer side (Fig). Cyclodextrins have a huge range of applications in varied areas of drug delivery and the pharmaceutical industry because of their ability to be complex and have other versatile characteristics. The most commonly known pharmaceutical application of cyclodextrins is their ability to enhance drug molecules' stability, solubility, bioavailability, and safety.

**Structural Details:**




As the name indicates, these are cyclic oligosaccharides primarily consisting of (α1,4)-linked α-D-glucopyranose units, containing a relatively lipophilic central cavity and a hydrophobic outer area. The glucopyranose having the chair conformation is why cyclodextrins have a truncated cone rather than a perfect cylindrical shape. The hydroxyl functional groups are orientated to the cone's exterior side along with the sugar residues' primary hydroxyl moieties at the cone's narrow edge and the secondary hydroxyl moieties at the wider edge. The central cavity is lined by the glucose residues' skeletal carbons and ethereal oxygens, which gives cyclodextrins a lipophilic character. The polarity of the cavity formed has been estimated to be close to that of an aqueous ethanolic solution. The naturally occurring α-, β- and γ-cyclodextrins mainly contain six, seven, and eight glucopyranose units, respectively. The naturally occurring cyclodextrins, particularly β-cyclodextrin, are of limited aqueous solubility. This indicates that complexes resulting from the interaction of lipophiles with these cyclodextrins can be of limited solubility, which results in the precipitation of solid cyclodextrin complexes from water and other aqueous systems. Interestingly, the aqueous solubility of the naturally occurring cyclodextrins is much lower than that of analogous acyclic saccharides. There is a strong intermolecular hydrogen bonding, which is responsible for this property in the crystal state.

**Q. What are cryptate? (CSJMU 2015)**

**Ans.** Cryptates are more complex versions of crown ethers. They are three-dimensional analogs of crown ethers and are often called cryptands. Cryptands enables the encapsulation of ions, effectively hiding or 'encrypting' them – hence the term cryptand.

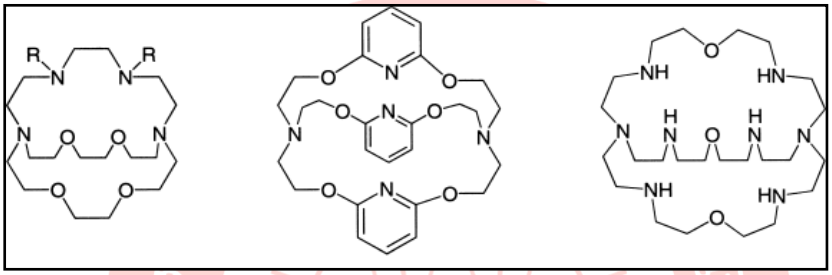
Properties

The three-dimensional interior cavity of a cryptand provides a binding site - or nook - for "guest" ions. The complex between the cationic guest and the cryptand is called a cryptate. Cryptands form complexes with many "hard cations," including $NH_4^+$, lanthanides, alkali, and alkaline earth metals. In contrast to typical crown ethers, cryptands bind the guest ions using nitrogen and oxygen donors.

Their three-dimensional encapsulation mode confers some size-selectivity, enabling discrimination among alkali metal cations (e.g., $Na^+$ vs. $K^+$).

**Uses**

Cryptands, although more expensive and difficult to prepare, offer better selectivity and binding strength than other complex ants for alkali metals, such as crown ethers. They can extract otherwise insoluble salts into organic solvents. Cryptands increase the reactivity of anions in salts since they effectively break up ion-pairs. They can also be used as phase transfer catalysts by transferring ions from one phase to another. Cryptands enabled the synthesis of the alkalis and electrizes. They have also crystallized Zintl ions, such as $Sn_9^{2-}$.



**Q. Citing any one example, write the role of the model system in bio-organic chemistry. (CSJMU 2011)**

**Ans.** Because of the size and complexity of most biochemical molecules and processes, it is often advantageous to find smaller and simpler models upon which controlled experiments can be more easily performed and with which hypotheses can be tested. Bioinorganic chemistry has been an especially fruitful area for using model systems, particularly where transition metals are involved.

## Short answer type questions:

**Q.1 Write the role of any one enzyme in the brewing industry. (CSJMU 2014,12)**

**Ans.** Several recent advances have been made concerning the use of enzymes in brewing. There is increasing interest in the possible use of blended a-amylase and protease preparations as replacements for malt in brewing. This is attributable to the expense and limited supplies of malt and the possibility that better quality control might be achieved. The industry has also used amyloid glucosidases recently to make "light" beer. Amylo glucosidases hydrolyze the α-1,6- glucosidic bonds of the amylopectin fraction, permitting the complete fermentation of starch. The use of β-glucanases may solve the high viscosity/slow filtration rate problems caused by mannans from cell

walls. The most exciting advance, however, is using acetolactate decarboxylase, now cloned into brewer yeast, to shorten the fermentation time by avoiding diacetyl formation.

**Q. What do you mean by anticancer drugs? How are they used for curing cancer? (CSJMU 2011)**

**Ans.** Anticancer drugs, also known as chemotherapy drugs, are used to treat cancer by killing, shrinking, or slowing the growth of cancer cells. The goal of anticancer drugs is to Eliminate cancer cells, shrink tumors, prevent cancer from spreading, and Relieve cancer symptoms.

There are many different types of anticancer drugs, including:

**1. Alkylating agents**: Damage cell DNA to prevent cancer cells from dividing

**2. Antimetabolites**: Prevent cancer cells from making the genetic material they need to create new cells

**3. Anthracyclines**: Anti-tumour antibiotics that bind with DNA so it cannot make copies of itself

**4. Mitotic disrupters**: Disrupt the formation of mitotic spindles, which are molecular railroads that help split DNA during cell division

The type of anticancer drug used depends on many factors, including the type and location of the cancer, its severity, and whether surgery or radiation therapy can be used. Most anticancer drugs are administered intravenously.

## Long answer type question:

**Q. Write down one method for the immobilization of enzymes. (CSJMU 2012)**

**Ans.** Enzymes linked to a solid and inert solid are called immobilized enzymes. Many enzymes catalyzed reactions of industrial use are carried out on such solid matrices. Besides enabling easy recovery of the enzymes after the completion of the concerned reactions, it also enhances the useful properties of the enzymes. This module describes the various support systems used to immobilize enzymes and the chemical methods used. Immobilization of enzymes offers many advantages that afford important applications in the industry, which are also described in this module.

**1. Procedures for immobilizing enzymes:**

**A. Covalent coupling to a polymeric matrix:**

In this method of enzyme coupling, functional groups on the amino acid residues on the enzyme are used to react to the matrix. The most reactive functional groups on the enzyme are the ones in its active site. These residues will preferentially react with the matrix during the reaction. In order to protect the active site, the reaction is carried out in the presence of a competitive inhibitor molecule. Reactive functional groups that usually participate in such a coupling reaction include the amino groups: the N-terminal alpha amine, the ε-amino group of lysine, or the guanidine group of arginine. The carboxylic functional group is also reactive, such as the alpha, beta, and gamma carboxylic acid groupings of the C-terminal amino acid residue, aspartic acid, and glutamic acid, respectively.

(i) Diazonium coupling: Tyrosine residues on the enzyme react with diazonium salts on a resin to generate a spacer arm between the matrix and the enzyme, which reduces the steric interaction between the enzyme and matrix. This method achieves a higher enzyme-carrier conjugation ratio.

(ii) Metal-linked Enzymes: Transition metal salts can be used to make solid support for enzyme systems. These metals can transform materials such as cellulose, nylon, borosilicate glass, soda glass, filter paper, and yeast cells. A solution of the metal salt is used to steep the solid matrix. This is followed by filtration and washing, after which the enzyme is added. The metal salts commonly used are $TiCl_4$, $TiCl_3$, $SnCl_4$, $ZnCl_4$, $VCl_3$, $FeCl_2$ and $FeCl_3$.

**Q. Describe the clinical uses of enzymes and the scope of enzyme therapy. (CSJMU 2019)**

**Ans. Clinical uses of enzymes:**

Some important clinical significance reactions are such that they estimate the levels of important enzymes. Levels of certain enzymes in key organs like the kidney and liver serve as indicators of good physiological health. The following part of the module discusses what these enzymes are, how they are estimated, and what their normal levels are in our bodies.

**1. Estimation of Alkaline and Acid Phosphatase –**

Phosphatases in the cell act on the organic phosphate esters, releasing phosphate. They are classified into two categories based on their pH optima: alkaline phosphatase (ALP) and acid phosphatase (ACP). ALP helps transport phosphate groups across cell membranes. A commonly used substrate for this enzyme in our blood is Para nitrophenyl phosphate (PNPP). Hydrolysis of this substrate yields para-nitrophenol, which is alkaline pH ionized and yellow-colored. Para nitrophenol is estimated calorimetrically at 405 nm, and its concentration is correlated with ALP in the blood sample. The enzyme activity is usually expressed in international units (IU/ml), i.e., micromoles of p-nitrophenol released per milliliter per minute.

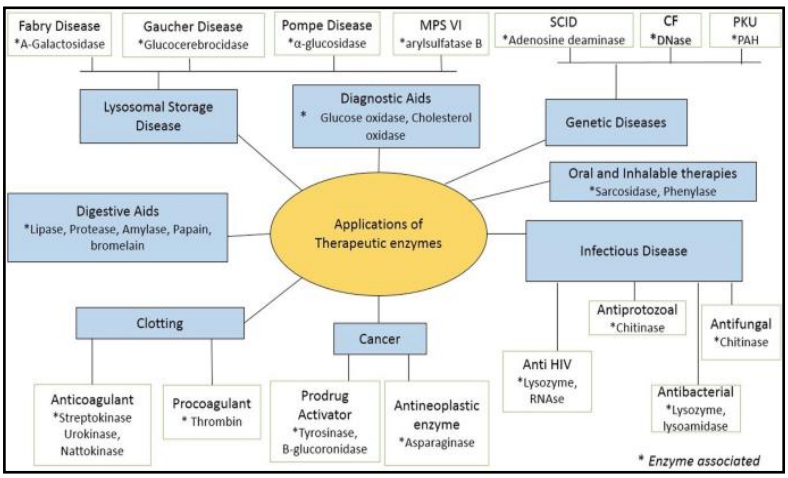
**Enzyme Therapy:**

**Potential of Enzymes in Therapeutics:** The administration of enzymes for therapeutic purposes is being excessively approached for treating diseases. The trials and successes have been in cancer, dissolving blood clots, and genetic deficiency diseases. The first enzyme drug was human tissue plasminogen, which dissolves clots and treats heart attacks.

**Treatment of genetic deficiency diseases:** A number of genetic diseases known whose underlying molecular basis is an enzyme deficiency. Any person afflicted with a deficiency of any enzyme in cholesterol metabolism is advised to have a diet low in cholesterol to minimize the harmful effects of the defect. The best treatment for such a case to fulfill what is missing in the person is to insert a gene that codes for the deficient enzyme in the person's chromosomes. The most important aspect of enzyme therapy is to deliver the enzyme at the site where it is required. For example, a deficiency of lysosomal enzymes can be treated if the enzyme can be delivered to the lysosomes where its function is required. So, targeted delivery of the enzyme is required for the success of enzyme therapy. One of the finest examples of understanding enzyme therapy is in treating severe combined immunodeficiency (SCID), in which the immune system of the patient's body is severely dysfunctional. ADA (Adenosine deaminase) deficiency is responsible for SCID. ADA breaks a toxic substance, deoxyadenosine, and does not let it accumulate in the body. A person deficient in ADA cannot break this substance, and this toxin accumulates in the body, weakening the immune system.

Enzyme replacement therapy using glucocerebrosidase has shown success in treating Gaucher's disease, a lysosomal storage disease (LSD).

**Treatment of Cancer:** Asparaginase treats acute lymphocytic leukemia (cancer of white blood cells). Asparaginase catalyzes the hydrolysis of asparagine to aspartic acid and ammonia. Normal cells can synthesize the non-essential amino acid asparagine, but cancer cells cannot synthesize it and hence die upon asparaginase treatment. These enzyme treatments are given with chemotherapy.



**Q. Describe any method for extracting and purifying enzymes. (CSJMU 2019)**

**Ans. Purification of enzymes:**

There are various methods for isolating enzymes from tissues. Acetone powders of tissues or cells are made by blending the tissue (1 vol) with acetone (5-10 vol) at 0 C in one of the techniques. To remove room moisture and lipids, the smooth slurry is often filtered and rinsed with acetone. It is then dried after being rinsed with ether. A powdery residue, which could be a mixture of enzymes, is left behind. The powdered substance is separated, and the different fractions are evaluated for catalytic activity in vitro. The fraction with the required activity is chosen for fractional crystallization, resulting in the pure state of the desired enzyme. Chemical or physical fractionation processes are used in the purifying process.

The goal is to keep most of the targeted enzyme while freeing it from other proteins, nucleic acids, and other contaminants. Heat guide the cell-free extract to 500C for 5 minutes to remove denatured protein. This can be accomplished by precipitating ammonium sulfate and using ion exchange chromatographic methods, gel filtering, etc.

**Separation and Extraction of Enzymes:**

**Extraction**

Material availability is critical in the extraction and purification of enzymes. A single enzyme's concentration can change between tissues. It is crucial to choose a tissue with a high enzyme content. As a result, yeast, bacteria, and fungi provide certain advantages as source materials. The benefit is that these cells can be cultivated in a favorable environment. However, there is one disadvantage: obtaining large quantities of microbial cells other than yeast is difficult.

After selecting the starting material, some techniques can be used to extract and isolate the enzyme. The following are some specific examples of various methods:

**1. Sedimentation:** Many mitochondrial and other particle cell bodies remain constant when liver tissue is homogenized using the Potter-Elvehjem apparatus rather than usual blending devices. They easily sediment out of the solution, taking a slew of enzymes. Only in the early stages of separation is physical separation by e-sedimentation useful.

**2. Extraction:** Previously, enzymes were divided into two categories: soluble or lysozyme and bound or desmon enzyme. Desmon zymes are likely enzymes for which suitable techniques have yet to be developed; hence, this is a bad classification.

Because of its fat-free nature, acetone-powder (from which enzymes can be extracted using buffer) is often the easiest to extract enzymes. The initial stage, in any case, is a fine-grinding. Autolysis, lysozyme digestion, grinding, freezing and thawing, sonic disintegration, shaking with solvents, shaking with fine-glass beads, and ultimately, explosion by quick pressure release are some methods for eliminating enzymes from microorganisms.

**Q. Discuss the application of enzymes in the food and drink industry. (CSJMU 2018,2016)**

Ans. Enzymes are used in the beverage industry to improve the quality and yield of drinks and to create new ingredients:

**Drink industry:**

**1. Fruit and vegetable juices**: Enzymes clarify juices, remove turbidity, and increase the extraction yield. Pectinases are often used to break down the pectin in plant cell walls, improving the juice's quality and reducing waste.

**2. Beer**: Enzymes form sugars during fermentation, control viscosity, and are chill-proof.

**3. Wine**: Enzymes clarify, extract color, and stabilize the protein.

**4. Coffee and tea**: Enzymes are used in fermentation to make tea soluble in cold water.

**Enzymes in the Food Industry:**

Enzymes can also be added to foods during processing to change their characteristics, and some of these changes will be discussed. Microbial enzymes left after the destruction of the microorganisms continue to affect the quality of processed and reformulated food. For example, starch-based sauces can undergo undesirable changes in consistency because of heat-stable microbial α-amylases that survive a heat treatment sufficient to destroy the microorganisms. Because of their high specificity, enzymes are also the ideal catalysts for the biosynthesis of highly complex chemicals.

**1. Role of Endogenous Enzymes in Food Quality:**

**Color:** The quality of many fresh vegetables and fruits is judged based on their "greenness." On ripening, the green color of many of our fruits decreases and is replaced with red, orange, yellow, and black colors. In green beans and English green peas, maturity leads to a decrease in chlorophyll levels.

**2. Enzymatic Removal of Undesirable Compounds:** Raw food materials often contain toxic or anti-nutrient compounds that are sometimes removed by proper heat treatment, extraction, or

enzymatic reactions. More than 12,000 plants in the world may have potential as food sources. Many are not used because of undesirable properties, some of which could be overcome by the proper use of enzymes.

**4. Enzymes in Milk and Dairy:** Products Bovine milk contains many enzymes, and other enzymes are added during processing. The use of chymosin (rennet) is of most importance economically in the production of several kinds of cheese. β-Galactosidase is potentially of great importance in the commercial hydrolysis of lactose in milk and dairy products so that these products can be consumed by individuals who are deficient in. βgalactosidase. β-Galactosidase is also used to convert whey lactose to glucose and galactose, sweeteners with greater commercial demand.

**5. Enzymes in Brewing:** Several recent advances have been made concerning using enzymes in brewing. There is increasing interest in the possible use of blended a-amylase and protease preparations as replacements for malt in brewing. This is attributable to the expense and limited supplies of malt and the possibility that better quality control might be achieved. The industry has also used amyloglucosidases recently to make "light" beer. Amyloglucosidases hydrolyze the α-1,6-glucosidic bonds of the amylopectin fraction, permitting the complete fermentation of starch.

**6. Enzymes for Control of Microorganisms:** Enzymes can potentially destroy microorganisms by several means. The means range from hydrolysis of cell-wall compounds, such as β-glucans, chitin, and peptidoglycans, to production of H202 and O2, which oxidize the essential - SH group of key sulfhydryl enzymes or polyunsaturated fatty acids in cell walls. These are interesting possibilities that are worthy of being tested at the commercial level.

**7. Flavour and Aroma Changes in Foods:** Enzymes cause food flavors and aromas, particularly during storage. Improperly blanched foods like green beans, English green peas, corn, broccoli, and cauliflower develop noticeable off flavors and off aromas during frozen storage.

**8. Production of high-fructose corn syrup and sweeteners:**

This involves a relatively heat-stable α-amylase, glucoamylase, and glucose isomerase:

$$Starch \xrightarrow{\alpha\text{-amylase}} dextrins$$

$$glucose \xrightarrow{glucose\ isomerase} fructose$$

Starch is heated to 105°C, Bacillus licheniformis a-amylase is added, and the endo-splitting enzyme produces dextrin's of DP 10-12. The soluble digest is passed through giant columns (6-10 ft in diameter and 20 ft high) of immobilized glucoamylase, where glucose is produced. The glucose-containing stream is then run through giant columns of immobilized glucose isomerase, where approximately equimolar concentrations of glucose and fructose are produced.

## 1. Procedures for immobilizing enzymes:

### A. Covalent coupling to a polymeric matrix:

In this method of enzyme coupling, functional groups on the amino acid residues on the enzyme are used to react to the matrix. The most reactive functional groups on the enzyme are the ones in its active site. These residues will preferentially react with the matrix during the reaction. In order to protect the active site, the reaction is carried out in the presence of a competitive inhibitor molecule. Reactive functional groups that usually participate in such a coupling reaction include the amino groups: the N-terminal alpha amine, the ε-amino group of lysine, or the guanidine group of arginine. The carboxylic functional group is also reactive, such as the alpha, beta, and gamma carboxylic acid groupings of the C-terminal amino acid residue, aspartic acid, and glutamic acid, respectively.

(i) Diazonium coupling: Tyrosine residues on the enzyme react with diazonium salts on a resin to generate a spacer arm between the matrix and the enzyme, which reduces the steric interaction between the enzyme and matrix. This method achieves a higher enzyme-carrier conjugation ratio.

# Biophysical chemistry

## NOTES

### 1. Biological Cell

The biological cell is the fundamental unit of life. All living organisms, from single-celled bacteria to multicellular plants and animals, are composed of cells. Cells are responsible for carrying out the essential functions of life, including metabolism, energy production, reproduction, and response to environmental stimuli.

**Types of Cells:**

- **Prokaryotic cells**: Simple cells without a nucleus. They lack membrane-bound organelles and include organisms like bacteria and archaea.
- **Eukaryotic cells**: Complex cells with a defined nucleus and membrane-bound organelles (e.g., mitochondria, chloroplasts in plants, and the Golgi apparatus).

**Key Organelles in Eukaryotic Cells:**

- **Nucleus**: Contains the cell's genetic material (DNA) and controls cell activities.
- **Mitochondria**: Known as the cell's powerhouses, they generate energy in the form of ATP through cellular respiration.
- **Endoplasmic Reticulum (ER)**: A network of membranes involved in protein and lipid synthesis. The rough ER has ribosomes on its surface, while the smooth ER is involved in lipid metabolism.
- **Golgi Apparatus**: Modifies, sorts, and packages proteins and lipids for transport or secretion.
- **Lysosomes**: Contain enzymes that break down waste materials and cellular debris.
- **Cytoskeleton**: Provides structural support and helps in cell movement and division.

**Functions of a Cell:**

- **Metabolism**: The chemical reactions that occur within a cell to maintain life. These include catabolic reactions (breaking down molecules for energy) and anabolic reactions (building molecules like proteins and DNA).

- **Reproduction**: Cell division occurs through processes like mitosis (for somatic cells) and meiosis (for gametes).
- **Response to stimuli**: Cells respond to environmental changes through signaling pathways.
- **Growth and Development**: Cells grow in size and undergo differentiation to form specialized structures and functions.

**2. Structure and Functions of Proteins**

Proteins are complex molecules made up of amino acids linked by peptide bonds. They play a vital role in the structure, function, and regulation of the body's cells, tissues, and organs.

**Structure of Proteins:** Proteins are structured in four levels:

- **Primary Structure**: The sequence of amino acids in a polypeptide chain.
- **Secondary Structure**: The folding of the polypeptide chain into structures like alpha-helices or beta-pleated sheets, stabilized by hydrogen bonds.
- **Tertiary Structure**: The three-dimensional folding of the protein, determined by interactions such as hydrogen bonds, ionic bonds, and hydrophobic interactions.
- **Quaternary Structure**: The association of multiple polypeptide chains into a functional protein complex, such as hemoglobin.

**Functions of Proteins:**

- **Catalysis**: Enzymes are proteins that accelerate biochemical reactions.
- **Transport**: Transport proteins like hemoglobin carry oxygen in the blood.
- **Structural**: Proteins like collagen provide structural support to cells and tissues.
- **Defense**: Antibodies are proteins that help defend against infections.
- **Signaling**: Hormones such as insulin are proteins involved in cellular communication.
- **Movement**: Motor proteins like actin and myosin are responsible for muscle contraction and cell movement.

**3. Enzymes**

Enzymes are proteins that act as biological catalysts, speeding up chemical reactions in living organisms. They are highly specific to the substrates they bind to and are crucial for digestion, metabolism, and DNA replication.

**Structure and Mechanism of Action:**

- **Active Site**: The part of the enzyme where the substrate binds and undergoes a chemical reaction.
- **Substrate Specificity**: Enzymes are specific to the shape and size of their substrates, fitting them like a lock and key.
- **Induced Fit Model**: The enzyme changes shape slightly when the substrate binds, facilitating the reaction.
- **Cofactors and Coenzymes**: Some enzymes require non-protein molecules to assist in catalysis. Cofactors are usually metal ions, while coenzymes are organic molecules (e.g., vitamins).

**Factors Affecting Enzyme Activity:**

- **Temperature and pH**: Enzymes have an optimal temperature and pH range for activity. Extreme conditions can denature the enzyme.
- **Concentration of Substrate and Enzyme**: Enzyme activity increases with substrate concentration, up to a point where the enzyme becomes saturated.
- **Inhibitors**: Molecules that reduce enzyme activity. Competitive inhibitors compete with the substrate for the active site, while non-competitive inhibitors bind elsewhere on the enzyme.

**4. DNA and RNA in Living Systems**

DNA (Deoxyribonucleic acid) and RNA (Ribonucleic acid) are nucleic acids that carry genetic information and play critical roles in protein synthesis.

**DNA:**

- **Structure**: DNA is a double-stranded helix of nucleotides, each consisting of a sugar (deoxyribose), a phosphate group, and a nitrogenous base. The nitrogenous bases are adenine (A), thymine (T), cytosine (C), and guanine (G). Pairs with T, and C pairs with G.

- **Function**: DNA stores genetic information that dictates the synthesis of proteins. It is inherited from parents to offspring and undergoes replication before cell division.
- **Replication**: DNA replication is the process by which DNA makes a copy of itself before cell division. This involves enzymes like DNA polymerase.
- **Transcription**: The process of copying a segment of DNA into RNA.

**RNA:**

- **Structure**: RNA is a single-stranded molecule composed of nucleotides (adenine, uracil, cytosine, and guanine) with the sugar ribose.
- **Types of RNA**:
  - **mRNA (messenger RNA)**: Carries genetic information from DNA to the ribosomes for protein synthesis.
  - **tRNA (transfer RNA)**: Brings amino acids to the ribosome during translation.
  - **rRNA (ribosomal RNA)**: Part of the structure of ribosomes, which are the sites of protein synthesis.
- **Transcription and Translation**: mRNA is transcribed from DNA and then translated into a protein at the ribosome with the help of tRNA.

**5. Helix-Coil Transition**

The helix-coil transition refers to the reversible change in the secondary structure of polypeptides (and nucleic acids) between a helical (ordered) structure and a random coil (disordered) state. This transition is important in various biological processes and is influenced by temperature, pH, and ionic strength factors.

**Protein Helix-Coil Transition:**

- The helix-coil transition can occur between alpha-helices (secondary structure) and random coils (unstructured regions) in proteins. The stability of these structures depends on environmental conditions.
- **Temperature and pH**: An increase in temperature or change in pH can disrupt the hydrogen bonds that stabilize alpha-helices, leading to a transition to the coil form.

- **Molecular Crowding**: High concentrations of other molecules can also influence this transition, affecting protein function and folding.

**DNA Helix-Coil Transition:**

- In DNA, the helix-coil transition refers to the denaturation (unwinding) of the double-stranded helical structure of DNA into two separate single strands (denaturation). This process can be induced by heat, which disrupts the hydrogen bonds between complementary base pairs, and it is central to processes like PCR (polymerase chain reaction) and DNA replication.
- **Renaturation**: After denaturation, DNA strands can reanneal (renature) when the temperature is lowered, allowing the complementary strands to re-bind.

The helix-coil transition plays a role in understanding how proteins fold and how genetic material behaves under different conditions, which is important for areas like drug design, disease mechanisms, and molecular biology techniques.

## Short answer type questions:

**Q. Give a comparison between DNA and RNA. (CSJMU 2020)**

**Ans.** Life on Earth is very diverse, from single-celled protozoans to complex multicellular plants and animals. Nevertheless, at the molecular level, all life is fundamentally made up of the same building blocks – DNA and RNA. One of the primary differences between DNA and RNA is that DNA is double-stranded while RNA is single-stranded.

**Q. Name the different RNA of metal ions in biological systems. (CSJMU 2018,2012)**

| S. No. | DNA | RNA |
|---|---|---|
| 1 | Sugar moiety is Deoxy ribose | Sugar moiety is Ribose |
| 2 | The bases present are Adenine, Thymine, Guanine and Cytosine. Uracil is not present. | The bases present are Adenine, Uracil, Guanine and Cytosine. Thymine is rarely present. |
| 3 | Double stranded molecules | Single stranded molecules |
| 4 | Obeys Chargaff's rule | Does not obey Chargaff's rule |
| 5 | Bases are not modified | Bases are modified |
| 6 | It is stable and not hydrolysed easily by alkalis | It is unstable and hydrolysed easily by alkalis |
| 7 | DNA content is constant in all the cells except during cell division | Varies from cell to cell |
| 8 | The life time of DNA is comparatively high. | RNA is short lived. |
| 9 | No natural DNA is catalytic | RNA can be catalytic |
| 10 | Present in the nucleus, mitochondria and chloroplast | Present in the nucleus, mitochondria, nucleolus, ribosomes and cytosol. |

**Ans.** Ribonucleic acid (RNA) is a molecule present in most living organisms and viruses. It comprises nucleotides, ribose sugars attached to nitrogenous bases, and phosphate groups. The nitrogenous bases include adenine, guanine, uracil, and cytosine. RNA mostly exists in the single-stranded form, but some special RNA viruses are double-stranded. The RNA molecule can have a variety of lengths and structures.

Three main types of RNA are involved in protein synthesis. They are messenger RNA (mRNA), transfer RNA (tRNA), and ribosomal RNA (rRNA).

**mRNA**

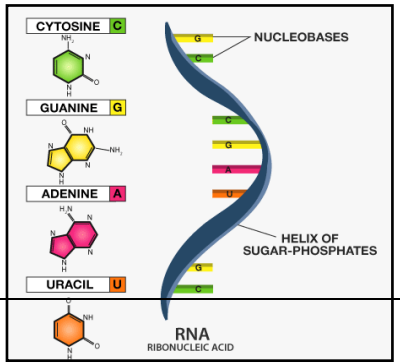
mRNA is transcribed from DNA and contains the genetic blueprint to make proteins. Prokaryotic mRNA does not need to be processed and can proceed to synthesize proteins immediately. A freshly transcribed RNA transcript in eukaryotes is considered a pre-mRNA and needs to undergo maturation to form mRNA. A pre-mRNA contains non-coding and coding regions known as introns and exons. During pre-mRNA processing, the introns are spliced, and the exons are joined together. A 5’ cap known as 7-methylguanosine is added to the 5’ end of the RNA transcript, and the 3’ end is polyadenylated. Polyadenylation refers to the process of adding a poly(A) tail, a sequence of adenine nucleotides, to the transcript. The 5’ cap protects the mRNA from degradation, and the 3’ poly(A) tail contributes to the stability of mRNA and aids it in transport.

**tRNA**

tRNAs are RNA molecules that translate mRNA into proteins. They have a cloverleaf structure that consists of a 3’ acceptor site, 5’ terminal phosphate, D arm, T arm, and anticodon arm. The primary function of a tRNA is to carry amino acids on its 3’ acceptor site to a ribosome complex with the help of aminoacyl-tRNA synthetase. Aminoacyl-tRNA synthetases load the appropriate amino acid onto a free tRNA to synthesize proteins. Once an amino acid is bound to tRNA, the tRNA is considered an aminoacyl-tRNA. The type of amino acid on a tRNA depends on the mRNA codon, a sequence of three nucleotides that codes for an amino acid. The anticodon arm of the tRNA is the site of the anticodon, which is complementary to an mRNA codon and dictates which amino acid to carry. tRNAs also regulate apoptosis by acting as a cytochrome c scavenger.

**rRNA**

rRNA forms ribosomes, which are essential in protein synthesis. A ribosome contains a large and



small ribosomal subunit. A small 30S and large 50S ribosomal subunit in prokaryotes make up a 70S ribosome. In eukaryotes, the 40S and 60S subunits form an 80S ribosome. The ribosomes contain an exit (E), peptidyl (P), and acceptor (A) site to bind aminoacyl-tRNAs and link amino acids together to create polypeptides.

**Q. Describe what is α-Helix. (CSJMU 2018, 2016, 2014, 2012)**

**Ans. α-helix:** α-helix exists in hair protein keratin. Various conformations can be assumed for a protein by rotating around single and rigid peptide bonds. The simplest arrangement of a polypeptide chain is α-helix. The polypeptide coils around an imaginary axis with side groups protruding from the helix. The single turn of the helix is 5.4 Angstrom, which is the repeating unit of the α-helix.

**Q. Discuss the structure and function of DNA. (CSJMU 2017,2015,2013,2012)**

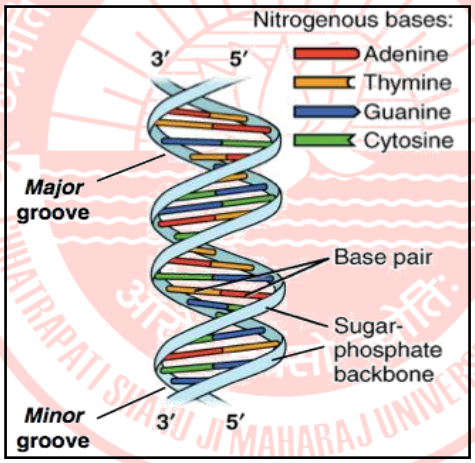
**Ans. Structure of DNA (Deoxyribonucleic acid):**

1. Watson and Crick hypothesized DNA's structure.
2. It comprises two anti-parallel strands that wrap around one another to create a right-handed DNA helix.
3. Phosphorus, nucleotides, and sugar make up the building blocks of DNA.
4. In DNA, there are roughly ten nucleotides per turn.
5. Major and minor grooves can be found on it.
6. On one side of the helix compared to the other, the strand backbones are closer together.
7. Where a large distance separates the backbones, the major groove appears, and when they are close together, the minor groove.
8. On opposite sides of the molecule, the grooves encircle it.
9. Purines and pyrimidines are nitrogen-based compounds.
10. Adenine (A) and guanine (G) are purines, while cytosine (C) and thymine (T) are pyrimidines.
11. There are three hydrogen bonds between cytosine and guanine and two hydrogen bonds between adenine and thymine.

12. Chargaff's rules state that DNA from any species of any organism should have a 1:1 stoichiometric ratio of purine and pyrimidine bases and, more specifically, that the amount of guanine should be equal to cytosine and the amount of adenine should be equal to thymine.
13. This pattern is found in both strands of the DNA.

**Functions of DNA:**

1. DNA serves as the hereditary material for the cell.
2. It is the storage medium for genetic information.
3. DNA replication is the process by which the information in the DNA is passed along from parents to children.
4. When DNA is replicated, a duplicate copy of its DNA is made.
5. **These DNA copies are correctly distributed to each daughter cell during cell division**.




## NOTES

**1. Standard Free Energy Change in Biochemical Reactions**

- **Definition**: The standard free energy change (ΔG°) is the change in the free energy of a biochemical reaction when the reactants are converted to products under standard conditions (298 K, 1 M concentration for all reactants and products, 1 atm pressure, and pH 7).
- **Importance**: ΔG° helps determine the direction of a chemical reaction. If ΔG° is negative, the reaction is spontaneous (exergonic). If positive, the reaction is non-spontaneous (endergonic).

- **Interpretation**: A negative ΔG° indicates a reaction that releases free energy, making it exergonic (spontaneous), while a positive ΔG° indicates a reaction that requires an input of energy, making it endergonic (non-spontaneous).

**2. Exergonic Reactions**

- **Definition**: Exergonic reactions are reactions that release energy, meaning that the free energy of the products is lower than the free energy of the reactants (ΔG < 0).
- **Characteristics**:
    - Spontaneous reactions that occur naturally under standard conditions.
    - They are often coupled with endergonic reactions to drive processes that require energy.
    - Examples include the breakdown of glucose in cellular respiration, such as converting glucose to pyruvate.
- **Applications**: Exergonic reactions power various cellular processes, like muscle contraction, active transport, and biosynthesis.

**3. Endergonic Reactions**

- **Definition**: Endergonic reactions require an input of energy, meaning the free energy of the products is higher than the free energy of the reactants (ΔG > 0).
- **Characteristics**:
    - Non-spontaneous reactions do not occur unless energy is supplied from an external source.
    - Often coupled with exergonic reactions to make the overall process energetically favorable.
    - Examples include the synthesis of glucose during photosynthesis or the formation of complex macromolecules like proteins from amino acids.
- **Applications**: Endergonic reactions are essential for building and storing energy within cells, such as synthesizing ATP, DNA, and other biomolecules.

**4. Hydrolysis of ATP**

- **Definition**: Hydrolysis of ATP (adenosine triphosphate) refers to the process in which ATP reacts with water to break one of its high-energy phosphate bonds, producing ADP (adenosine diphosphate), inorganic phosphate (Pi) and releasing energy.

This highly exergonic reaction has a standard free energy change of about -30.5 kJ/mol.

- **Importance**:
    - The hydrolysis of ATP is a key process in cellular metabolism, providing the energy required for many cellular processes, such as muscle contraction, active transport, and protein synthesis.
    - The released energy drives endergonic reactions, making ATP hydrolysis a central player in cellular energy transfer.
- **Mechanism**: The reaction involves breaking the high-energy bond between the second and third phosphate groups of ATP. This bond is a phosphoanhydride bond, which stores a significant amount of energy.

**5. Synthesis of ATP from ADP**

- **Definition**: ATP synthesis from ADP (adenosine diphosphate) and inorganic phosphate (Pi) is an endergonic process that requires energy input. It is the process by which cells regenerate ATP to power cellular activities.
- **Process**:
    - **Cellular Respiration**: ATP is synthesized in the mitochondria through oxidative phosphorylation, where energy from nutrients (such as glucose) is transferred to ADP and Pi, forming ATP.
    - **Photosynthesis**: In plants, ATP is synthesized in the chloroplasts during light-dependent reactions, where solar energy is used to convert ADP and Pi to ATP.
- **Mechanism**:
    - **Chemiosmosis**: In cellular respiration and photosynthesis, a proton gradient is established across a membrane (mitochondrial inner or thylakoid membrane). Protons flow back through ATP synthase, which uses this proton motive force to synthesize ATP.

- **Substrate-level Phosphorylation**: In some metabolic pathways, such as glycolysis and the Krebs cycle, ATP is synthesized by directly transferring a phosphate group from a high-energy substrate to ADP.

- **Importance**: ATP synthesis is essential for providing the energy required for cellular functions. Without ATP, cells could not perform processes such as protein synthesis, DNA replication, and active transport.

These concepts are central to understanding cellular energy metabolism, as ATP is the primary energy currency in all living cells, facilitating both the release and storage of energy required for various biochemical reactions.

**Q. Discuss the following: (CSJMU 2020,2018,2014)**

**1. Hydrolysis of ATP**

**Ans.** The living objects require a continuous supply of free energy mainly for the following purposes:

(a) Synthesize macromolecules from simpler and smaller precursors,

(b) Transport molecules and ions across membranes against gradients,

(c) Perform mechanical work, as in the muscle contraction, and

(d) Ensure fidelity of information transfer.

The free energy in these processes is derived from the environment. The phototrophs obtain this energy by trapping light energy from the sun. On the other hand, the chemotrophs obtain it by the oxidation of foodstuffs. This free energy (derived from light or the oxidation of foodstuffs) is partly transformed into a special form before it is used for biosynthesis, transport, motion, and fidelity. This special carrier of free energy is adenosine triphosphate (ATP). ATP plays a central role in the transference of free energy from the exergonic (= energy-yielding) to the endergonic (= energy-requiring) processes in the cells. During the breakdown of energy-rich foodstuffs or fuel molecules, some free energy is harnessed to make ATP from adenosine diphosphate (ADP) and inorganic phosphate (Pi), a process that requires an input of free energy. ATP then donates much of its chemical energy to energy-requiring processes by undergoing a breakdown to ADP and Pi

**2. Synthesis of ATP from ADP**

**Ans. Free energy of hydrolysis of ATP**

When ATP is hydrolyzed, it loses its terminal γ phosphate group to form ADP and orthophosphate or inorganic phosphate (Pi).

$$ATP + H2O \rightarrow ADP + Pi$$

This reaction's standard free energy change, ΔG°′, is –7.3 kcal/mol (Figure 5). Standard free energy changes have also been determined for the hydrolysis of other phosphorylated compounds or organophosphates. Some phosphates yield more, and some yield less free energy than ATP upon hydrolysis under standard conditions. This intermediate position enables ATP to function efficiently as a carrier of phosphoryl groups. Thus, concerning the ΔG°′ value of hydrolysis of ATP, two classes of oregano phosphates are recognized: high-energy phosphates exemplified by enol phosphates (e.g., phosphoenolpyruvate), phosphoguanidines (e.g., creatine phosphate and arginine phosphate), etc., which have ΔG°′ values larger than that of ATP and low energy phosphates, exemplified by ester phosphates found in the intermediates of glycolysis, which have ΔG°′ values smaller than that of ATP. However, the designations 'high' and 'low' are unclear. Indicate that there are three classes of phosphates. Such phosphates as phosphoenolpyruvate, creatine phosphate, etc., whose ΔG°′ values are higher than that of ATP, should better be designated as 'super' high energy phosphates, the ATP then be designated as 'high' energy phosphate and the ester phosphates as 'low' energy phosphates.

**Q. Explain the concept of standard free energy change in biological reactions. (CSJMU 2013)**

**Ans. Standard free energy change in biochemical reactions:** In biochemical reactions, the standard free energy change is usually expressed as G, which is the free energy change of a reaction in an aqueous solution at pH = 7, roughly corresponding to the conditions inside a cell. Most of the biological reactions (such as the synthesis of macromolecules) are thermodynamically unfavorable (G > 0) under cellular conditions. In order for such reactions to take place, an additional source of energy is required. For example, consider the reaction:

$$A \rightleftharpoons B \qquad \Delta G = -10 kJ/mol$$

The conversion from A to B is energetically unfavorable, so the reaction proceeds in reverse rather than forward. However, the reaction could be driven in the forward direction by coupling the A to B-conversion with an energetically favorable reaction, such as

$$C \rightleftharpoons D, \Delta G = +20 kJ/mol$$

When these two reactions are mixed, the coupled reaction can be written as follows: -

$$A+C \rightleftharpoons B+D, \Delta G = -10 kJ/mol$$

The G of the combined reaction is the sum of free energy changes of its components, so the coupled reaction is energetically favorable and will proceed as described. Thus, the energetically unfavorable conversion of A to B is driven by coupling to a second reaction and a large decrease in

free energy. Enzymes are responsible for the coordinated execution of such coupled reactions. The cell uses this fundamental mechanism to drive many energetically unfavorable reactions that must take place in biological systems. Adenosine-5-triphosphate (ATP) is central to storing free energy inside the cell.

## NOTES

### 1. Thermodynamics of Biopolymer Solutions

Biopolymer solutions, such as proteins, polysaccharides, or nucleic acids in a solvent (typically water), are influenced by various thermodynamic principles that govern their behavior. Studying these solutions is crucial for understanding biological processes, from protein folding to cell signaling.

**Key Concepts:**

- **Free Energy and Phase Behavior**: The thermodynamic free energy (G) dictates the spontaneity of biopolymer solutions. For polymer solutions, the Helmholtz or Gibbs free energies must be considered to describe equilibrium states. The phase behavior (solvent-solute interactions, solvent quality) determines whether a biopolymer will remain in solution or phase-separate.
- **Flory-Huggins Theory**: A popular model used to describe polymer-solvent mixtures, predicting the behavior of biopolymers in solutions based on their interaction parameters. It considers the entropy of mixing and enthalpy due to the interaction between polymer and solvent molecules.
- **Enthalpy and Entropy Contributions**: The enthalpy (ΔH) reflects the heat exchange in interactions between solvent molecules and the biopolymer. The entropy (ΔS) governs the disorder introduced into the system. The balance between enthalpy and entropy determines whether a biopolymer dissolves or aggregates.
- **Critical Micelle Concentration (CMC)**: For amphiphilic biopolymers (e.g., surfactant proteins), CMC represents the concentration at which they begin to aggregate into micelles, a thermodynamically favored structure to minimize the exposure of hydrophobic regions to the solvent.
- **Solubility and Hydration**: Biopolymers often interact strongly with water, contributing to their solubility. Water structure around biopolymers (hydration shells) significantly influences the solution thermodynamics. The entropic cost of ordering water molecules around hydrophobic regions impacts protein folding and aggregation.

**2. Osmotic Pressure**

Osmotic pressure is the pressure required to stop the osmotic flow of solvent into a solution, driven by concentration differences of solutes (like biopolymers) across a semipermeable membrane.

**Key Concepts:**

- **Van't Hoff Equation**: For ideal solutions, osmotic pressure (π) is given by the formula:

$$\pi = nRT/V$$

Where:

  - n is the number of moles of solute
  - R is the universal gas constant.
  - T is the temperature.
  - V is the volume of the solution.

In the case of biopolymer solutions, it may be replaced with the molar concentration of biopolymer.

- **Semi-permeable Membranes**: These membranes allow solvent molecules to pass but block solutes. Osmosis refers to the movement of solvent molecules through the membrane, driven by differences in solute concentration.
- **Colloid Osmotic Pressure**: Biopolymers (like proteins in the blood) are often colloidal particles, and their osmotic pressure plays a crucial role in maintaining fluid balance in cells and tissues.
- **Osmotic Pressure and Biopolymer Size**: Larger biopolymers exert greater osmotic pressure due to their larger molecular size, which can affect properties like viscosity and the overall structure of the solution.
- **Applications**: Osmotic pressure is key in cellular functions such as maintaining cell shape, nutrient transport, and waste removal. It is also essential in processes like dialysis.

**3. Membrane Equilibrium**

Membrane equilibrium refers to the balance achieved when the fluxes of substances (e.g., ions, water, small molecules) across a membrane are equal in both directions, maintaining homeostasis within a cell or organism.

**Key Concepts:**

- **Diffusion and Permeability**: Membrane permeability governs how substances diffuse across the lipid bilayer. The permeability depends on the solubility of molecules in the lipid phase and their size.
- **Nernst Equation**: Describes the equilibrium potential (voltage) across a membrane for a particular ion based on its concentration gradient inside and outside the cell:

$E=RT/zF \times \ln[C]out/[C]in$

Where:

  - E is the equilibrium potential
  - R is the gas constant
  - T is temperature
  - F is the Faraday constant
  - [C]out and [C]in are ion concentrations outside and inside the membrane

- **Active and Passive Transport**: Membrane equilibrium is dynamic and often involves active transport (requiring energy) or passive transport (driven by concentration gradients). Active transport proteins, such as pumps (e.g., Na+/K+ ATPase), maintain ion gradients essential for cell function.
- **Gibbs-Donnan Equilibrium**: Describes the distribution of ions across a membrane that is selectively permeable to some ions but not others. It leads to unequal ion distribution, generating osmotic pressure and affecting cell volume.
- **Electrochemical Gradients**: These gradients result from both the concentration of ions and the charge across the membrane. They are essential for processes like nerve signal transmission and muscle contraction.

**4. Muscular Contraction**

Muscle contraction is a complex biochemical and mechanical process that involves the interaction between actin and myosin filaments in muscle fibers powered by ATP hydrolysis.

**Key Concepts:**

- **Sliding Filament Theory**: Muscle contraction occurs when myosin filaments slide over actin filaments, shortening the muscle fiber. This interaction is driven by ATP hydrolysis and the conformational changes in the myosin heads.

- **Calcium Ions**: The release of calcium ions from the sarcoplasmic reticulum initiates muscle contraction. Calcium binds to troponin, a protein on the actin filament, causing a conformational change that exposes myosin-binding sites on actin.
- **Cross-Bridge Cycle**: The process involves binding myosin heads to actin, pulling the actin filaments towards the center of the sarcomere (the functional muscle unit). ATP is required for both the detachment of myosin from actin and its re-cocking to its high-energy state.
- **ATP and Energy Transduction**: ATP provides the energy needed for the actin-myosin interaction and pumps calcium ions back into the sarcoplasmic reticulum to end contraction.
- **Force Generation**: The force generated during contraction is proportional to the number of cross-bridges formed between actin and myosin. The strength of contraction can be modulated by the frequency of stimulation (tetanus) and the length of the muscle fiber.

**5. Energy Generation in the Mechanochemical System**

Mechanochemical systems convert chemical energy (from ATP) into mechanical work, such as in muscle contraction or molecular motors (e.g., kinesin, dynein). In these systems, energy is transduced through chemical reactions (e.g., ATP hydrolysis) that result in conformational changes, allowing the movement of molecules or cellular components.

**Key Concepts:**

- **ATP Hydrolysis**: ATP hydrolysis (ATP → ADP + Pi) is the primary energy source for mechanochemical systems. The breakdown of ATP releases energy to power conformational changes in proteins or enzymes.
- **Molecular Motors**: Proteins like kinesin and dynein are examples of molecular motors that move along microtubules, converting chemical energy from ATP hydrolysis into mechanical work, such as transporting cellular cargo.
- **Coupling of Chemical and Mechanical Energy**: In muscle contraction, ATP hydrolysis is coupled with the movement of myosin along actin filaments. The energy released from ATP hydrolysis is stored in the myosin head as mechanical work, which is used to generate force and movement.
- **Efficiency and Power Output**: The efficiency of mechanochemical systems, like muscles or molecular motors, can vary depending on factors like the ATP turnover rate, the mechanical properties of the system, and the environmental conditions. In muscles, the ATP usage rate can determine the contraction speed and the force generated.

- **Energy Recycling**: In muscles, ATP is regenerated through cellular respiration (aerobic or anaerobic) and phosphocreatine stores. Similarly, molecular motors may use secondary energy sources like ion gradients or the release of stored mechanical energy.

These processes are central to many biological functions, ranging from cellular movement to organisms' physical actions.

**Q. Explain the structure and functions of protein. (CSJMU 2018,2020,2014,2018,2019)**

**Ans.** Protein's function is highly governed by its stable structure. This structure has four levels: Primary, secondary, tertiary, and quaternary. Multiple weak interactions stabilize protein structure. The primary structure is the sequence of amino acids joined to each other by peptide bonds. The secondary structure is the local folding of a part of the polypeptide. Next level, the tertiary structure is a mixture of α-helix and β-sheets. At the same time, the quaternary structure is the subunit composition of a protein. This module will give us deeper insights into the protein structure and its various levels.

(1) Primary Structure: describes a sequence of amino acids and all the covalent bonds (peptide bonds, disulfide bonds) linking the various amino acids in the polypeptide chain.

(2) Secondary Structure: structural patterns made from the arrangements of amino acid residues.

(3) Tertiary Structure: Three-dimensional folding of the protein.

(4) Quaternary Structure: This considers the spatial arrangement of subunits.

**Primary Structure of Protein**

In 1953, Frederick Sanger elucidated the first amino acid sequence of a protein, bovine insulin. Bovine insulin is 51 amino acids long and is composed of two polypeptide chains, A (21 amino acids) and B (30 amino acids), joined to each other by intra- and inter-chain disulfide bonds (Figure 2). Sequencing is carried out by first deciphering the N-terminal amino acid of the polypeptide chain. This is done by labeling the amino group of amino acid at the N-terminus by 1-fluoro-2, 4-dinitro benzene (FDNB), damsel chloride, or damsel chloride. N-terminal determination of bovine insulin gave two amino acid residues—Phe and Gly, suggesting that it is a heterodimer.

**Secondary Structure of Protein**

Structural patterns from the amino acid residue arrangements are termed a protein's secondary structure. α-helix and β-sheets are two prominent secondary structures that occur in proteins.

**1. α-helix:** α-helix exists in hair protein keratin. Various conformations can be assumed for a protein by rotating around single and rigid peptide bonds. The simplest arrangement of a polypeptide chain is α-helix. The polypeptide coils around an imaginary axis with side groups protruding from the helix. The single turn of the helix is 5.4 Angstrom, which is the repeating unit of the α-helix.

**2. β-sheet**: The β-sheet is a zig-zag extended polypeptide conformation. The intra-molecular hydrogen bonding is formed between adjacent segments of a polypeptide (Figure 6). The adjacent segments can be in parallel or antiparallel orientation. Apart from β-sheets, β-turns are important components of the protein structure that connect the two adjacent segments of the antiparallel β sheet by hydrogen bonding between the first and fourth amino acids. Gly and Pro appear in the βturns.

**Tertiary Structure**

The three-dimensional arrangement of all atoms in a protein is termed the tertiary structure. When the polypeptides fold spherically, they are called globular proteins, while fibrous proteins have extended conformation.

**Quaternary Structure**

A protein's arrangement of subunits (same or different) forms its quaternary structure.

α-keratin and collagen are examples of fibrous proteins. They have been assigned structural functions. Hydrophobic amino acids are present in the interior and on the surface of fibrous proteins, conferring them the property of insolubility in water. For example, α-keratin is rich in Ala, Leu, Ile, Val, Met, and Phe. α-keratins are found in hair and nails and comprise the outer skin layer. The helix of α-keratin has right-handedness and a coiled-coil structure. Two parallel strands wrap around each other to form the coiled-coil with left-handedness. Hydrophobic residues of the two α-helices interact to form this coiled-coil structure.

**Q. Explain the denaturation of protein. (CSJMU 2017,2014)**

**Ans.** Denaturation is the loss of a protein's secondary, tertiary, and quaternary structures by a chemical or physical agent that leaves the primary structure intact. For example, heat cleaves hydrogen bonds, so boiling a protein solution destroys the a-helical and β-pleated sheet structure. In globular proteins, heat causes the unfolding of the polypeptide chains; because of subsequent intermolecular protein-protein interactions, precipitation or coagulation then takes place. That is what happens when we boil an egg. Denaturation changes secondary, tertiary, and quaternary structures. It does not affect primary structures (the sequence of amino acids that make up the

chain). If these changes occur to a small extent, denaturation can be reversed. For example, removing a denatured protein from a urea solution and returning it to water often reassumes its secondary and tertiary structures. This process is called reversible denaturation. In living cells, some denaturation caused by heat can be reversed by chaperones. These proteins help a partially heat-denatured protein regain its native secondary, tertiary, and quaternary structures. Some denaturation, however, is irreversible. We cannot unboil a hard-boiled egg.

Heavy metal ions (Pb2+, Hg2+, and Cd2+) also denature protein by attacking the -SH groups. They form salt bridges in –S- Hg2+ -S-. This feature is taken in advance in the antidote for heavy metal poisoning: raw egg whites and milk. The metal ions denatured the egg and milk proteins, forming insoluble precipitates in the stomach. These must be pumped out or removed by inducing vomiting.

Other chemical agents, such as alcohol, denature proteins, coagulating them. This process is used to sterilize the skin before injections. At a concentration of 70%, ethanol penetrates bacteria and kills them by coagulating their proteins, whereas 95% alcohol denatures only surface proteins.

**Q. What is meant by protein folding? (CSJMU 2017,2014)**

**Ans.** Protein folding is a process by which a polypeptide chain folds to become a biologically active protein in its native 3D structure. Protein structure is crucial to its function. Various molecular interactions hold together folded proteins. During translation, each protein is synthesized as a linear chain of amino acids or a random coil that does not have a stable 3D structure. The amino acids in the chain eventually interact with each other to form a well-defined, folded protein. The amino acid sequence of a protein determines its 3D structure. Folding of proteins into their correct native structure is key to their function. Failure to fold properly produces inactive or toxic proteins that malfunction and cause several diseases.

**Four stages of protein folding**

- Primary structure refers to the linear sequence of amino-acid residues in the polypeptide chain.
- Secondary structure is generated by the formation of hydrogen bonds between atoms in the polypeptide backbone, which folds the chains into either alpha helices or beta-sheets.
- A tertiary structure is formed by folding the secondary structure sheets or helices into one another. The tertiary structure of a protein is the geometric shape of the protein. It usually has a polypeptide chain as a backbone, with one or more secondary structures. The

interactions and bonding of the amino acid side chains in the protein determine the tertiary structure.

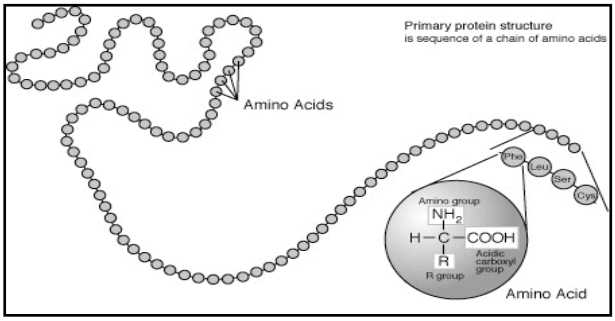
- Quaternary structure results from folded amino-acid chains in tertiary structures interacting further to give rise to a functional protein such as hemoglobin or DNA polymerase.

**Q. Discuss briefly the structure of the polypeptide chain. (CSJMU 2013)**

**Ans.** The definition of a polypeptide chain is a string of amino acids connected by peptide bonds. The word *poly* means many, and the word *peptide* refers to proteins. So, a polypeptide chain is a chain of the building blocks of proteins or amino acids. Polypeptide chains are important because they make up proteins. Proteins are macromolecules that serve many important functions inside the cell. Proteins make up structural components of the cell, such as the cytoskeleton.

They also make up enzymes, which speed up chemical reactions inside the cell. Proteins regulate cell signaling and behavior, such as transcription, translation, and cell division. The structure of a polypeptide chain is a linear sequence of amino acids. Each amino acid is connected to the next with a peptide bond. There are twenty different types of amino acids in cells.

Each amino acid has a central carbon attached to an amino group, a carboxyl group, and a variable, the *R* group. The *R* group is important because it gives different properties to the amino acids. Some amino acids have large R groups, creating steric hindrance in the polypeptide chain, while others may have acidic or basic R groups.




**The structure of a polypeptide**

**Q. Write are Biopolymers? Explain the forces that interact in these compounds. (CSJMU 2016)**

**Ans.** Biopolymers are polymers that are produced by living organisms. They are generally polymers of starch. These are composed of monomeric units. There are three main classes of biopolymers, classified according to the monomers used, and the structure of the biopolymer formed: polynucleotides, polypeptides, and polysaccharides.

**The noncovalent force:**

- In contrast to a covalent bond, a non-covalent contact involves more diffused electromagnetic interactions between molecules or inside a molecule and does not share electrons.
- Large molecules like proteins and nucleic acids have a three-dimensional structure that non-covalent interactions must maintain.
- Additionally, they participate in various biological processes in which big molecules briefly yet specifically bond.
- These interactions significantly impact the synthesis of numerous organic molecules as well as medication design, crystallinity, and material design, notably for self-assembly.
- Four primary noncovalent bond types are frequently cited.
- Hydrophobic interactions, hydrogen bonds, van der Waals interactions, and electrostatic interactions are some of them.
- Primary and secondary bonds (covalent bonds) hold polymers together (van der Waals and hydrogen bonds).
- To complete an octet (a group of eight electrons) around atoms, covalent bonds require the sharing of valence electrons (the s and p shells).

**Q. What are the various forces involved in biopolymer interactions? (CSJMU 2013, 2014)**

**Ans.** Various kinds of weak interactions are important in establishing secondary and tertiary structures. These weak bonds are all non-covalent; the main types are as follows:

Ionic or electrostatic bonds result from the attractive force between ionized groups having opposite charges. Hydrogen bonds result when a H+ (proton) is shared between neighboring electronegative atoms. Thus, the forces that stabilize biopolymer structures are as follows:

(a) Hydrogen bonding: Weak force of attraction between partially positive hydrogen and a partially negative atom such as oxygen, fluorine, or nitrogen on the same or another molecule.

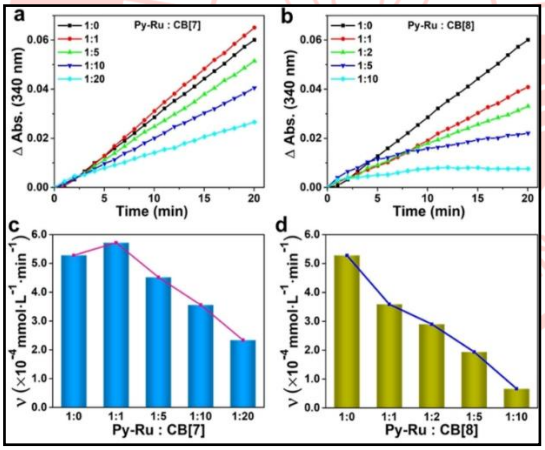
(b) Ionic bonding: Cross-linking can occur due to bonding between anionic and cationic side chains.

(c) Covalent bonding: The most common form of inter-chain bonding is the disulfide bond between the sulfur atoms of two cysteine residues. The insulin consists of two polypeptide chains linked together by this type of bridge.

(d) Hydrophobic bonding: Several amino acid residues have hydrophobic (water-hating) side chains. Proteins in aqueous solutions fold so that most hydrophobic chains cluster inside the folds. The polar side chains, which are hydrophilic (water-loving), lie on the protein's outside or surface.

**Q. Explain the hydrogenation ion titration curves. (CSJMU 2013)**

**Ans.** The hydrogenation of NaF/9NaH + 5MgB2 and NaF/2NaH + 1.5MgB2 reactive hydride composites (RHC) was studied by volumetric titration (kinetics and PCI curves), in situ synchrotron radiation powder X-ray diffraction (SR-PXD), high-pressure differential scanning calorimetry (HP-DSC), Fourier transform infrared spectroscopy (FT-IR), and scanning electron microscope (SEM). A hydrogen uptake between 4.1 and 4.8 wt % was observed when the H2 pressure was between 25 and 50 bar, and the temperature was kept constant at 325 °C. PCI curves indicate a hydrogenation equilibrium pressure of 2 and 8 bar at 325 °C for NaF/9NaH + 5MgB2 and NaF/2NaH + 1.5MgB2, respectively. Synchrotron radiation powder X-ray diffraction revealed the formation of solid solutions of NaF–NaH after milling and a change in the reaction pathway compared to a reported nonadopted 2NaH + MgB2 reactive hydride composite. Formation of the stable side-product NaMgH2F was a drawback for hydrogen storage capacity and reversibility. FT-IR indicates no hydrogen-to-fluorine substitution in the NaBH4 product.




**Q. Explain how bioenergetics obey the laws of thermodynamics. (CSJMU 2012)**

**Ans.** Bioenergetics includes the terms bio, which means “within the living organism,” and energetics, which means “production of energy.” Bioenergetics is a biochemistry and cell biology field concerned with energy flow through living systems. Bioenergetics studies energy transfer and utilization within living organisms or biological systems. A human requires a constant energy supply to power all biological processes, including physical activity.

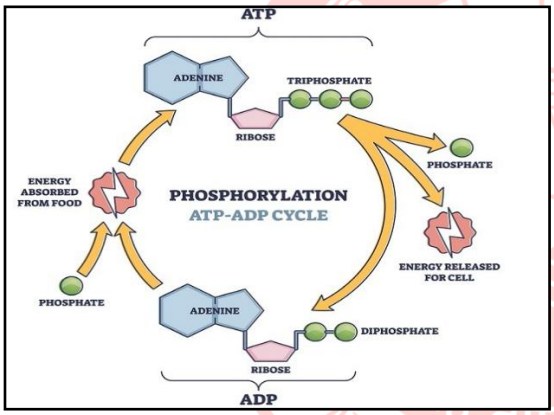
Laws of Bioenergetics: Thermodynamic laws

The laws of thermodynamics help us to understand why energy flows in certain directions and ways.

**1st Law of Thermodynamics**

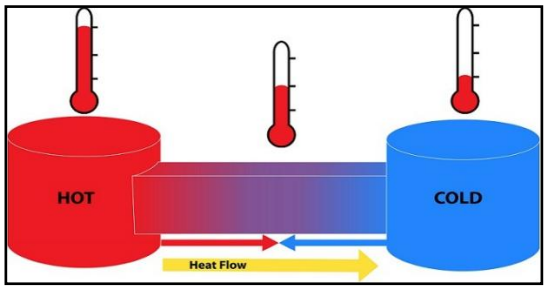
In concern to the changes in energy, according to this law, energy cannot be created nor destroyed, but instead, it is shown to get transferred from one form of energy to another form that may be from potential energy to kinetic energy to heat energy, etc. Here, the total energy of the system and surroundings always remains constant unless there is any physical change or chemical reaction.

**Example** – In humans, energy is transferred through ATP and stored in the two links of outer phosphate groups. The energy is released by the hydrolysis of ATP into ADP and inorganic phosphate and hydrogen ions. This released energy is used for muscle contraction during the workout. However, ATP stored in muscle cells is limited, so our body activates three metabolic pathways for the continuous supply of ATP to the body during exercise. These are the Phosphagen, glycolytic, and oxidative systems, where all three synthesize ATP.

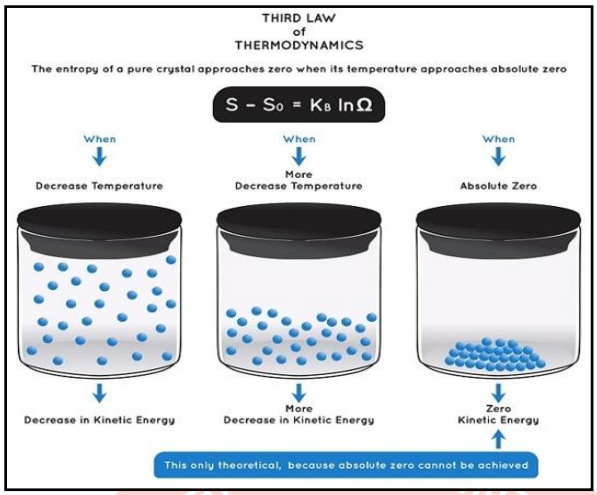



**2nd Law of Thermodynamics**: Here, the concept of entropy comes into play. This can be understood by the word disorder. The second law states that the entropy of a system and its surroundings must always increase. Within a system, there is also a tendency towards higher entropy. Here, the correlation between both forms of energy can be stated by this law.

**Example – 1.** The solid-state of water is more ordered and has greater entropy, and the liquid state is disordered and has less entropy. 2. Heat energy from a hot coffee cup flows to the solid surface.



**3rd law of Thermodynamics** states that at an absolute zero, the perfect crystalline solid molecule has zero entropy, as this is the most ordered state a substance can be in. Entropy is measured in joules per kelvin and is not a measure of energy but the distribution of energy within a system.




## Conclusion

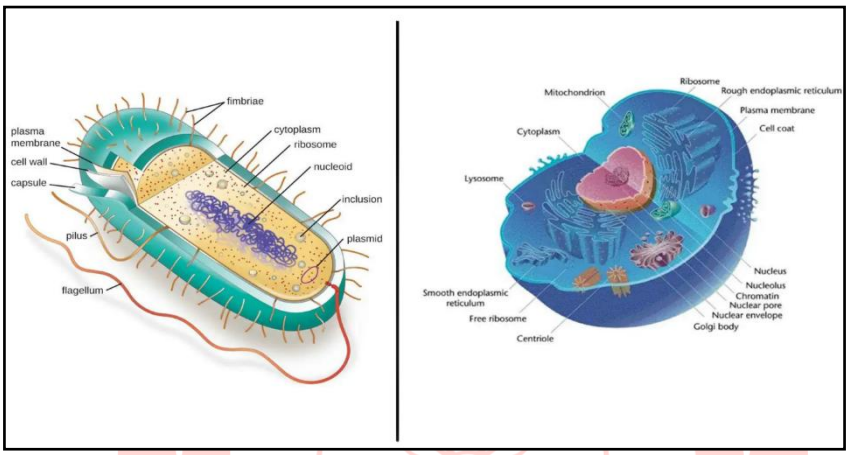
Bioenergetics studies biochemistry and deals with how organisms manage their energy source, adenosine triphosphate (ATP), by storing, producing, or consuming. The change in free energy, which is the energy to do work, is always expressed in terms of enthalpy, temperature, and entropy change.

Enthalpy is the energy within the system, and entropy is the energy distribution. If the change in free energy is negative, then the process is said to be spontaneous, and when it is positive, the process is non-spontaneous. One must know the thermodynamic laws to understand the energy flow within a system. There are mainly three thermodynamic laws. These laws are important for understanding the metabolism through the bioenergetics of humans.

**Q. Describe the various parts and their function organizing a biological cell. (CSJMU 2014)**

**Ans.** A cell is the basic unit of an organism as diverse as a bacterium, fish, or man. A cell consists of various biomolecules in an aqueous solution called cytoplasm enclosed or bound by a lipid layer and frequently, in addition by a tough wall (cell wall), which protects the cell from the environment.

Based on the features within the cell, there are two types: prokaryotes and eukaryotes. Prokaryotes are simpler in structure, enclosing their genetic material only in the cell envelope. Bacteria fall into this group and are usually single-celled organisms. The second group, called eukaryotes, has additional compartments within their cells that are enveloped by a single or double lipid membrane. These compartments are called organelles. It is thought that during early evolution, prokaryotes engulfed other prokaryotes, leading to larger eukaryotes with membrane-bound genetic material that formed nuclei and other organelles, notably mitochondria and chloroplasts, which have a double membrane-enveloped structure. This theory is known as the Endosymbiosis theory.

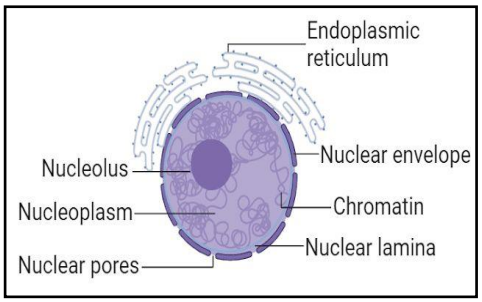



**Eukaryotes and their organelles:**

Eukaryotes consist of single-cell eukaryotes, such as yeast and amoeba, and multicellular organisms, such as plants and animals. The cells of eukaryotes contain organelles, which include mitochondria, lysosomes, Golgi, chloroplasts, peroxisomes, endoplasmic reticulum, etc., which are best illustrated in the following diagram. The functions of these organelles in the cell's life have also been tabulated.

List of eukaryotic cell organelles and their function:

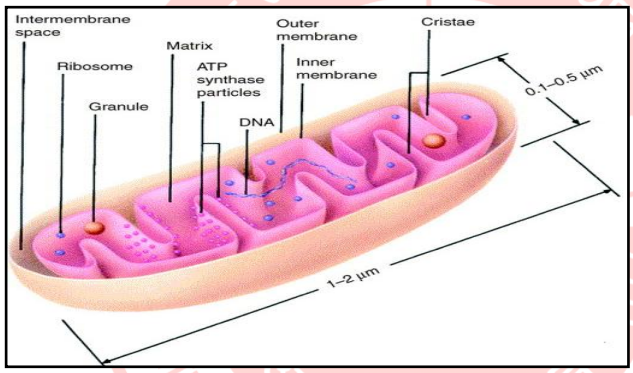
**1. Nucleus:**




**Functions**

Contains genetic material that is replicated to propagate the cell and transcribed into RNA to express genes and, eventually, proteins.

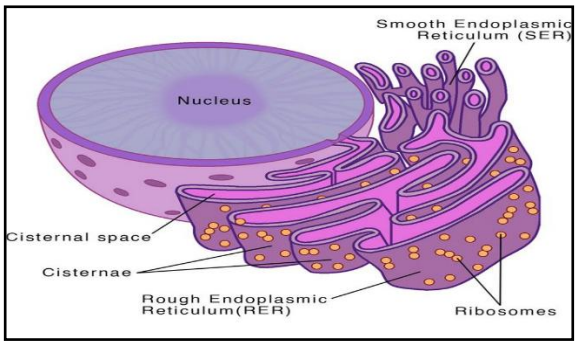
**2. Mitochondria**




**Functions**

Powerhouses generate ATP (energy currency) and break down sugars and lipids for the same.

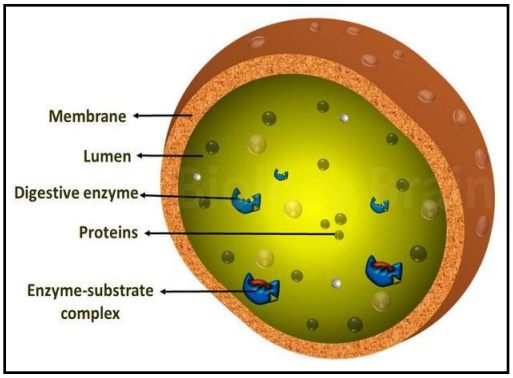
**3. Endoplasmic reticulum**




**Functions**

Synthesis of lipids and proteins for export.

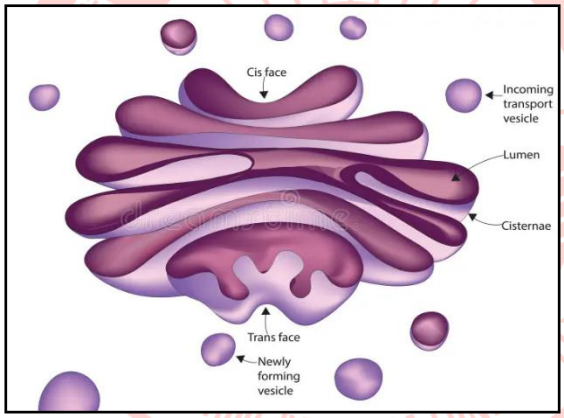
### 4. Lysosomes




### Functions

Breakdown of various molecules by hydrolytic enzymes.

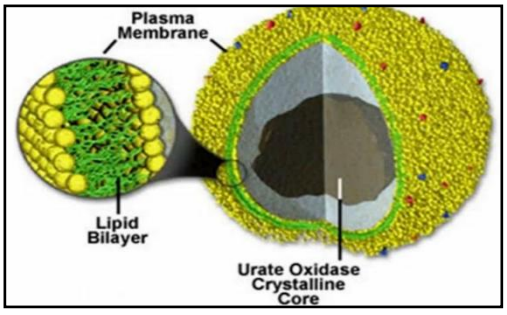
### 5. Golgi apparatus




### Functions

Glycosylation of proteins and sorting for export.

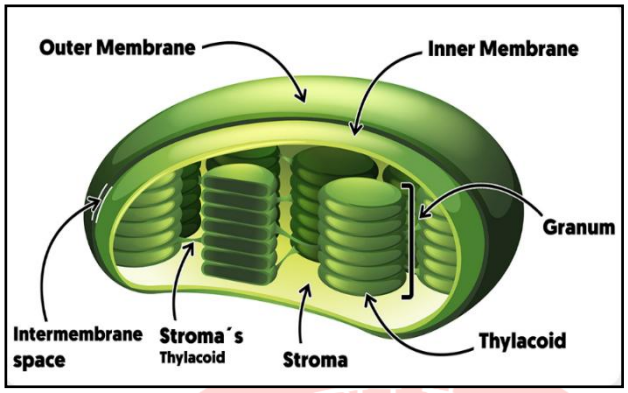
### 6. Peroxisomes



**Functions**

Perform oxidative reactions using oxygen and hydrogen peroxide.

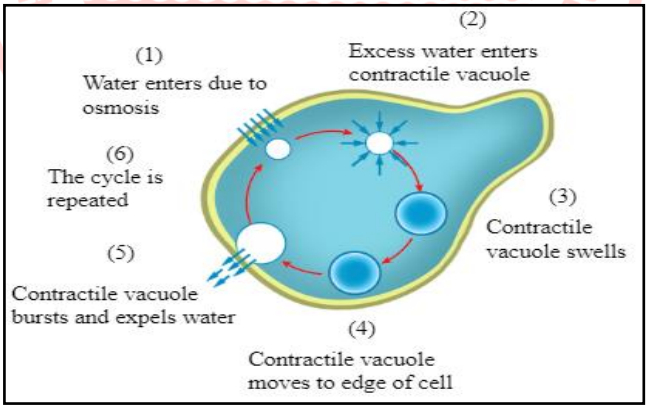
**7. Chloroplast**




**Functions**

Generate sugar and carbohydrates (photosynthesis) by splitting water using the energy of sunlight.

**8. Vacuoles (in plant cells, fungal cells, and some animal cells)**

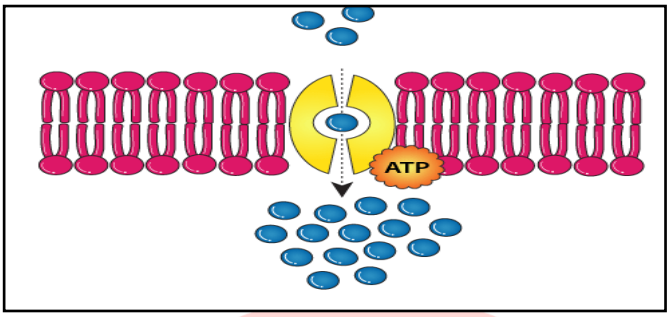



**Functions**

Used for storing water salts and removing molecules, this may be toxic to the cell.

**Q. Discuss the function of cell membrane ion transport through the cell membrane. (CSJMU 2013,2017)**

**Ans.** Active transport is a kind of cellular transport in which substances like amino acids, glucose, and ions are transported across cell membranes to a region with a high concentration of such substances. As a result, active transport employs chemical energy like ATP to move substances against their concentration gradient. This type of transport is commonly found in the small intestine wall and root hair cells.




Active transport is performed by a special type of protein molecules of the cell membrane called the transport proteins or pumps. They consume energy in the form of ATP molecules.

**Primary Active Transport**

Photon energy and redox energy are two energy sources for primary active transport. The mitochondrial electron transport chain, which uses the reduction energy of NADH to transport protons across the inner membrane of mitochondria against their concentration gradient, is an example of primary active transport using redox energy. The proteins involved in photosynthesis are an example of primary active transport using photons or light energy.

Primary active transport is demonstrated by glucose uptake in the human intestine.

**Secondary Active Transport**

Secondary active transport allows one solute to move downward (along its electrochemical potential gradient) to generate enough entropic energy to drive the transport of the other solute upward (from a low-concentration region to a high-concentration region). This is also known as coupled transport. There are two types of coupled transport – antiport and symport. Antiport transport involves the movement of two ion or other solute species in opposite directions across a membrane, whereas symport transport involves the movement of two species in the same direction.

**Q. How does the membrane potential develop? Explain. (CSJMU 2012)**

**Ans.** The influx and expulsion of ions within the cell cause an action potential. The action potential, a nerve impulse, is the electrical potential difference across the plasma membrane. Specifically,

potassium and sodium ions are involved. The sodium-potassium pump and channels transport ions in and out of the cell.

**Electrical Cell Membranes**

Electrical currents flow across cell membranes. They produce a charge that extends the entire length of the cell membrane by using ions from both the extracellular and intracellular sides of the cell. It is said that the cell membrane has a resting potential when not much is happening.

**Ion Channels**

Since ions are hydrophilic, they cannot pass through a membrane's lipids. Specially structured proteins that form channels or tunnels are necessary to move in and out of the membrane. These channels transporting ions are called ion channels.

**Chemical Synapses**

Small chemicals called neurotransmitters can potentially activate ion channels in postsynaptic cells. Neurotransmitters are released into the synaptic cleft in response to action potentials reaching the synaptic knobs. The action potential initiation causes the presynaptic membrane to open voltage-sensitive calcium channels.

**Electrical Synapses**

neurotransmitter's "middleman," electrical synapses connect the postsynaptic and presynaptic cells.

**Phases or Steps**

Depolarisation, overshoot or peak phase, repolarisation, and refractory period are the phases of an action potential. The membrane potential has two more phases connected to the action potential. First, there is hypolarisation, which comes before depolarisation, and then there is hyperpolarisation, which comes after repolarisation.

- **Hypo polarisation:** The initial rise in membrane potential to the threshold potential is known as hypo polarisation.
- **Depolarisation:** A significant influx of sodium ions is produced when the threshold potential activates voltage-gated sodium channels. This period is known as depolarization.
- **Overshoot or Peak Phase:** As the cell depolarizes, the inside of the cell becomes increasingly electropositive, approaching the electrochemical equilibrium potential of sodium, which is +61 mV. This phase of intense electro-positivity is the peak phase or overshoot phase.

- **Repolarisation:** Following the action potential firing, the potassium ion channels that remove this cation from the cell open, and the sodium ion channels close. The voltage-gated potassium channels are opened when the cell potential overshoots, which results in a significant potassium outflow and a reduction in the electro-positivity of cells. This is the repolarisation phase, and its main objective is the restoration of the resting potential of the membrane.
- **Hyperpolarisation:** This phase possesses more electronegativity in the membrane potential than in the usual resting membrane potential.
- **Refractory Period:** The duration after a nerve impulse that a neuron must travel before it can fire again is referred to as the refractory period. Every action potential is preceded by a refractory period, subdivided into an absolute and a relative refractory period. Another action potential cannot be evoked during the absolute refractory period, and the relative refractory period requires a stronger stimulus than usual. Modifications in potassium and sodium channel molecule states drive the two refractory phases.

**Q. Name the different methods used to determine the molecular weight of biopolymer. (CSJMU 2019,2016,2015)**

**Ans. Molecular Weight Determination of Biopolymers:**

It was Avogadro who developed the concept of a mole. According to him, a mole consists of many substances containing the same number of atoms in 12 gm of C-12. This has a value of 6.0229×1023. The weight of a mole in grams is numerically the same as that of a single molecule in atomic mass units (AMU). The weight of one gram atom of an element is called gram-atomic weight, and the weight of one mole of molecules is called gram molecular weight. They are simplified as atomic and molecular weight. One amu is equal to 1.66×10-24 gm. The molecular weight is the sum of all the atoms in the molecule.

**(a) Number Average Molecular Mass ($\overline{Mn}$)**

It is obtained by dividing the sum of masses of all the molecules of different monomer units of different masses by the total number of molecules. We can understand it by considering a polymer comprising three monomeric mass units: M1, M2, and M3. If N1 molecules of monomer of mass M1, N2 molecules of mass M2 and N3 molecules of mass M3 constitute the polymer then,

the total mass of N1, molecules = $N_1 M_1$ (i)

the total mass of N2 molecules = $N_2 M_2$ (ii)

the total mass of N3 molecules = $N_3 M_3$ (iii)

Adding (i), (ii), and (iii), we will get the total mass.

Total molecular mass = $N_1 M_1 + N_2 M_2 + N3 M_3$ (iv)

and total molecules = $N_1 + N_2 + N_3$ (v)

Number Average Molecular mass = $N_1 M_1 + N_2 M_2 + N3 M3 \div (N1+N2+N3)$

$$\overline{(Mn)} = \frac{\sum Ni\ Mi}{\sqrt{Ni}}$$

**where $\sum Ni = N1 + N2 + N3 \ldots \ldots$**

**where $\sum Ni\ Mi = N1M1 + N2M2 + N3M3 \ldots \ldots$**

$\overline{(Mn)}$ is generally determined by osmotic pressure measurement, depression in freezing point, and elevation in boiling point.

**(b)Weight average molecular weight ($\overline{Mn}$)**

It is obtained by multiplying the sum of the total molecular masses of different monomeric units by their respective molecular masses, adding all the molecular masses, and then dividing by the total mass of all the molecules.

If a polymer consists of N1 molecules of a monomeric unit of molecular mass M1, N2 molecules of another monomeric unit of molecular mass M2, and N3 molecules of the third monomeric unit of molecular mass of M3, then

**Weight average molecular weight ($\overline{Mw}$) = $\frac{N1M1 \times M1 + N2M2 \times M2 + N3M3 \times M3}{N1M1 + N2M2 + N3M3}$**

$$\overline{(Mw)} = \frac{\sum Ni\ Mi^2}{\sum Ni\ Mi}$$

where,

$\sum NiMi^2 = N1M12 + N2M22 + N3M32$

$\sum Ni\ Mi = N1\ M1 + N2M2 + N3M3 \ldots \ldots$

($\overline{Mw}$) is generally determined by ultracentrifugation or sedimentation, e.g., the number of average molecular mass and the weight average molecular mass of a polymer sample containing 30 molecules of molecular mass 10,000, 30% of molecular mass 20,000 and the remaining 40% of molecular mass 30,000, will be

$$Mn = \frac{30 \times 10000 + 30 \times 20000 + 40 \times 30000}{30 + 30 + 40}$$

$$= 21000$$

$$M w = \frac{30 \times 10000 \times 10000 + 30 \times 20000 \times 20000 + 40 \times 30000 \times 30000}{30 \times 10000 + 30 \times 20000 + 40 \times 30000}$$

$$= 24286$$

$\overline{\quad}$ $\overline{\quad}$

The ratio of M w and M w is called the poly dispersion index

$$(PDI)\ PDI = \frac{\overline{M\ w}}{\overline{Mn}}$$

For natural polymers, PDI = 1, whereas for synthetic fibers, PDI >1.

**Q. Describe the powder method for the determination of the structure of crystalline biopolymer. (CSJMU 2018,2015,2014,2017)**

**Ans.** One-dimensional (1D) (spherically averaged) powder diffraction diagrams are commonly used to determine the degree of cellulose crystallinity in biomass samples. Here, molecular modeling shows how disorder in cellulose fibrils can lead to considerable uncertainty in conclusions drawn concerning crystallinity based on 1D powder diffraction data alone. For example, cellulose microfibrils containing crystalline and nanocrystalline segments can lead to powder diffraction diagrams lacking identifiable peaks, while microfibrils without crystalline segments can lead to such peaks. This leads to false positives, assigning disordered cellulose as crystalline, and false negatives, categorizing fibrils with crystalline segments as amorphous. The reliable determination of the crystallinity fraction in any given biomass sample will require a more sophisticated approach combining detailed experiments and simulation.

**Q. Discuss briefly the sedimentation equilibrium method and sedimentation velocity method for the determination of molecular weight of macromolecules. (CSJMU 2016,2012,2018,2017,2013,2020)**

**Ans. Sedimentation Equilibria:**

On ultracentrifuging the polymer solution, several boundaries are observed, revealing the presence of different components. This method is useful only when there is sufficient molecular weight difference. This fact is used in the Sedimentation equilibrium method for molecular weight determination. In this method, at the equilibrium stage, the rate at which the solute is driven outwards by the centrifugal force is equal to the rate at which it diffuses inwards due to the concentration gradient.

$$\text{The sedimentation rate} = Cw2 \times M\,(1 - vp)\,(I/f) \qquad \text{(i)}$$

$$\text{The diffusion rate} = -\frac{KRT\,dc}{f\,dx} \qquad \text{(ii)}$$

$$dc/C = -(1 - vp)\,w^2/dx\,RT \ldots\ldots \text{(iii)}$$

Integrating (ii) $$M\,w = \frac{CRT \ln (C_2\ C_1)}{(1 - vp)\,w^2\,(x22\text{-}x12) \ldots\ldots \text{(iv)}}$$

This method requires the time for equilibrium, which was found too long for substances with molecular weights greater than 500. Shortly after the centrifuge is brought to speed, concentrations at the top meniscus and bottom of the cell are determined, and the equilibrium values are given.

**Sedimentation Velocity Method**

The macromolecules, which are large and have heavy masses, settle out of dispersion under gravitational force. The force F causing sedimentation of spherical particles is given by

$$\mathbf{F = 4 / 3\ \pi r3(p - p\theta)\ g = 6\pi\eta r\ dx / dt}$$

The particle's radius can be determined by its path in a definite time. It was observed that a particle of radius $10^{-7}$ mm with a density of 2.5 gm per cm3 will take about 100 years to settle down. Wiegner, Kelly, and Stamm have designed equipment to measure the sedimentation velocity of colloidal particles. Svedberg and others developed analytical ultracentrifuges to determine the velocity of sedimentation. A particle of mass m at a distance x from the center of rotation will experience a centrifugal force, fc. given by

$$fc = m \times w$$

## Short answer type questions:

**Q. What is the role of X-ray diffraction? (CSJMU 2019,2018,2013)**

**Ans. Role of X-ray diffraction:**

1. X-rays are extremely useful for the determination of molecular structure. Two fundamentally different methods using X-rays are currently used: diffraction and absorption.

2. X-ray diffraction is used for the structural determination of macromolecules, including metal coordination geometries. Even small molecules can often be determined with uncertainties in the bond distances of 0.01-0.001 Å.

3. X-ray absorption spectroscopy determines the oxidation state of the metal ions of interest. Details about metal-coordination geometries can also be extracted using XAS.

4. EPR spectroscopy is ideally used to study metalloproteins and to isolate and purify iron-containing proteins, such as ferredoxins.

5. NMR methods are often quite useful for kinetic studies.

**Q. Describe the role of X-ray diffraction in macromolecules. (CSJMU 2020,2015,2012)**

**Ans.** X-ray diffraction X-ray crystallography offers the most powerful structural probe of macromolecular structure, including metal coordination geometries. Small molecule structures can often be determined with uncertainties in the bond distances of 0.01-0.001Å. The order of precision of macromolecular structures is usually less than that for small inorganic complexes. Because of high water content, protein crystals do not diffract X-rays like small crystals. They differ at around 50%, which is usually highly disordered. Resolution, defined by Bragg's law, where θ is half the scattering angle between the incident and diffracted X-ray beams.

$$d = \lambda / (2 \sin\theta) = 1.5418Å / (2 \sin\theta) \text{ (for Cu radiation)}$$

In order to assign the electron density to specific amino acid residues, it is important to know the amino acid sequence of metalloproteins. This requirement, in addition to the need for heavy atom derivatives to determine the phase angles for the thousands of measured reflections from which the maps are generated (isomorphous replacement technique), makes the method very laborious.

For the study of polycrystalline fiber of DNA, the X-ray diffraction technique is used, and its results also had great significance in the discovery of the double helical structure for DNA.

**Q. What is the role of X-ray diffraction? (2019,2018,2013)**

**Ans. Role of X-ray diffraction:**

1. X-rays are beneficial for the determination of molecular structure. Two fundamentally different methods using X-rays are currently used: diffraction and absorption.

2. X-ray diffraction is used for the structural determination of macromolecules, including metal coordination geometries. Even small molecules can often be determined with uncertainties in the bond distances of 0.01-0.001 Å.

3. X-ray absorption spectroscopy determines the oxidation state of the metal ions of interest. Details about metal-coordination geometries can also be extracted using XAS.

4. EPR spectroscopy is ideally used to study metalloproteins and to isolate and purify iron-containing proteins, such as ferredoxins.

5. NMR methods are often quite helpful for kinetic studies.

## Long answer type questions:

**Q. How will you distinguish between prokaryotic and eukaryotic cells? (CSJMU 2019)**

**Ans. Prokaryotic Cell:**

The term “prokaryote” is derived from the Greek words “*pro*”, (meaning before) and “*karyon*” (meaning kernel). It translates to “*before nuclei”.* Prokaryotes are one of the most ancient living organisms, with fossil records dating back almost 3.5 billion years. These prokaryotes thrived in the earth’s ancient environment, some using chemical energy and others using the sun’s energy. These extremophiles thrived for millions of years, evolving and adapting. Scientists speculate that these organisms gave rise to the eukaryotes. Prokaryotic cells are comparatively smaller and much simpler than eukaryotic cells. The other defining characteristic of prokaryotic cells is that they do not possess membrane-bound cell organelles, such as a nucleus. Reproduction happens through the process of binary fission.

| | Prokaryotes | Eukaryotes |
|---|---|---|
| **Type of Cell** | Always unicellular | Unicellular and multi-cellular |
| **Cell size** | Ranges in size from 0.2 µm – 2.0 µm in diameter | Size ranges from 10 µm – 100 µm in diameter |
| **Cell wall** | Usually present; chemically complex in nature | When present, chemically simple in nature |
| **Nucleus** | Absent. Instead, they have a nucleoid region in the cell | Present |
| **Ribosomes** | Present. Smaller in size and spherical in shape | Present. Comparatively larger in size and linear in shape |
| **DNA arrangement** | Circular | Linear |
| **Mitochondria** | Absent | Present |

**Eukaryotic Cell:**

The term "Eukaryotes" is derived from the Greek words "*eu*" (meaning: good) and "*karyon*" (meaning: kernel), therefore translating to "*good or true nuclei*." Eukaryotes are more complex and much larger than prokaryotes. They include almost all the significant kingdoms except kingdom Monera. Structurally, eukaryotes have a cell wall supporting and protecting the plasma membrane. The plasma membrane surrounds the cell, controlling the entry and exit of certain substances. The nucleus contains DNA, which is responsible for storing all genetic information. The nuclear membrane surrounds the nucleus. Within the nucleus exists the nucleolus, which plays a crucial role in synthesizing proteins. Though these two classes of cells are quite different, they possess some common characteristics. For instance, both possess cell membranes and ribosomes, but the similarities end there. The complete list of differences between prokaryotic and eukaryotic cells is summarized as follows:

**Q. What is meant by ORD? Why does the cotton effect arise? Discuss the Various factors that affect ORD. (CSJMU 2012,2017)**

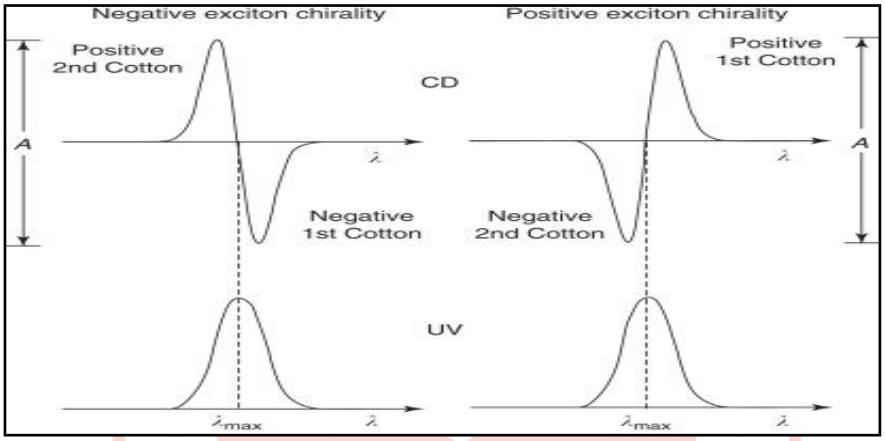
**Ans.** It is observed by the dependence of optical rotation on wavelength. The optical Rotatory Dispersion method measures the ability of the optically active compound to rotate plane-polarized light as a function of the wavelength. ORD based on the index of refraction.

1. If the refractive indices of the sample for the left- and right-handed polarized light are different when the components are recombined, the plane-polarized radiation will be rotated through an angle α. 2. nl and nr are the indices of the refraction for left-handed and right-handed polarized light.

3. α is in radians per unit length [α = nl-nr/λ]. ORD curve plots molar rotation [α] or M vs. λ. 4. Clockwise rotation is plotted positively; counter-clockwise rotation is plotted negatively 5. ORD is based solely on the index of refraction ¬ plain curve is the ORD for a chiral compound that lacks a chromophore ¬ Chiral compounds containing a chromophore can give anomalous, or Cotton effect, curves**.**

**Factors affect the ORD:**

• Nature of substance. Length of the column. Concentration. of the solution Temperature of the sol. Nature of the solvent. The wavelength of the light used.




**Cotton Effect:**

The characteristics change in ORD/or CD near an absorb band. The combination of both (circular birefringence and circular dichroism) effect in the region in which optically active absorption bands are observed gives rise to a phenomenon called the cotton effect.

**Cotton effect curves:** It has the following points:

1. These curves will show the high peak ns, which depends on the absorbing groups.

2. These curves will be obtained for the compounds with asymmetric carbon chromophores that absorb near the UV region.

3. A chromophore with a +ve cotton effect causes a right rotation at low frequency.

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