GLYCOGENOLYSIS

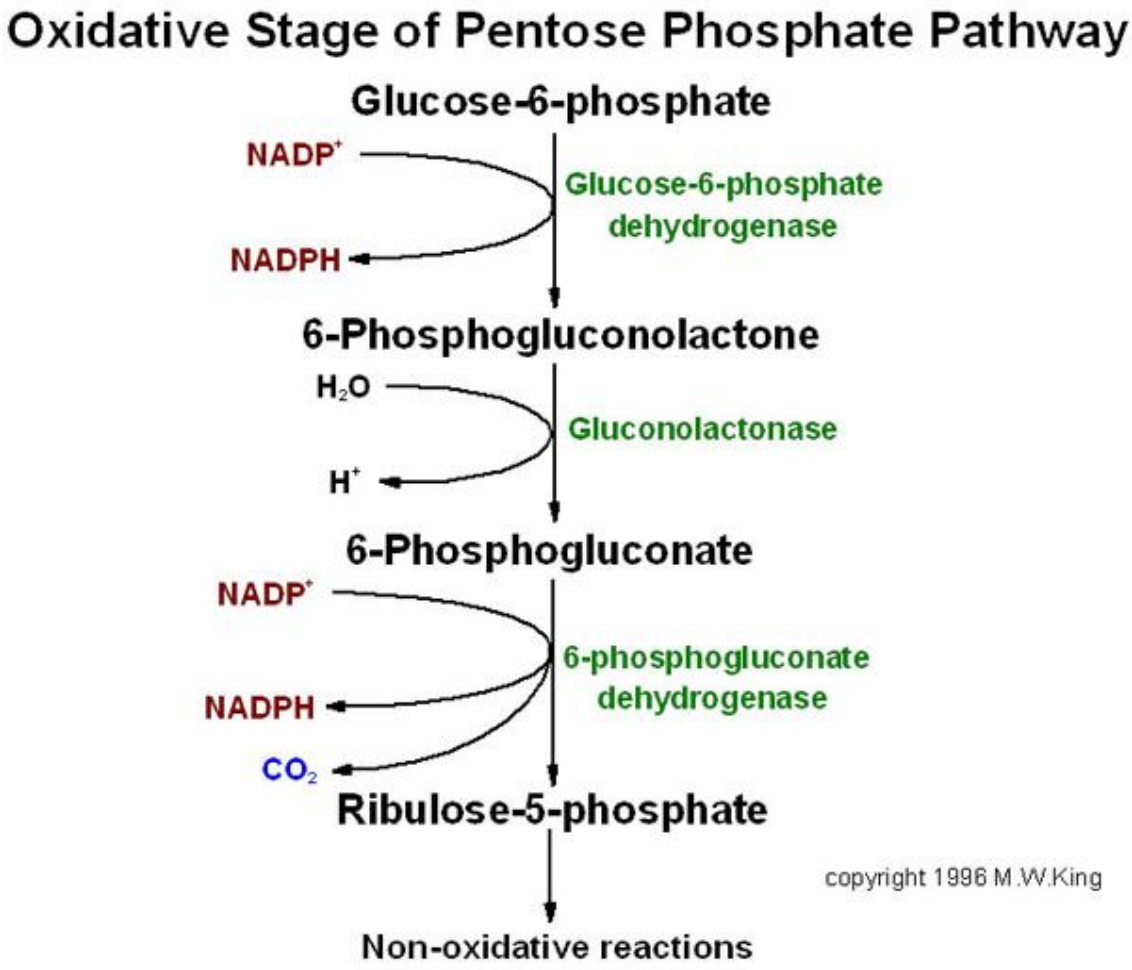
This is the breakdown of glycogen to glucose. This process is initiated by the phophorylase enzyme which brings about phosphorylytic cleavage of the α1-4 linkage to yield G-1-P. The enzyme can exists as active or inactive forms (phosphorylated or dephophorylated).

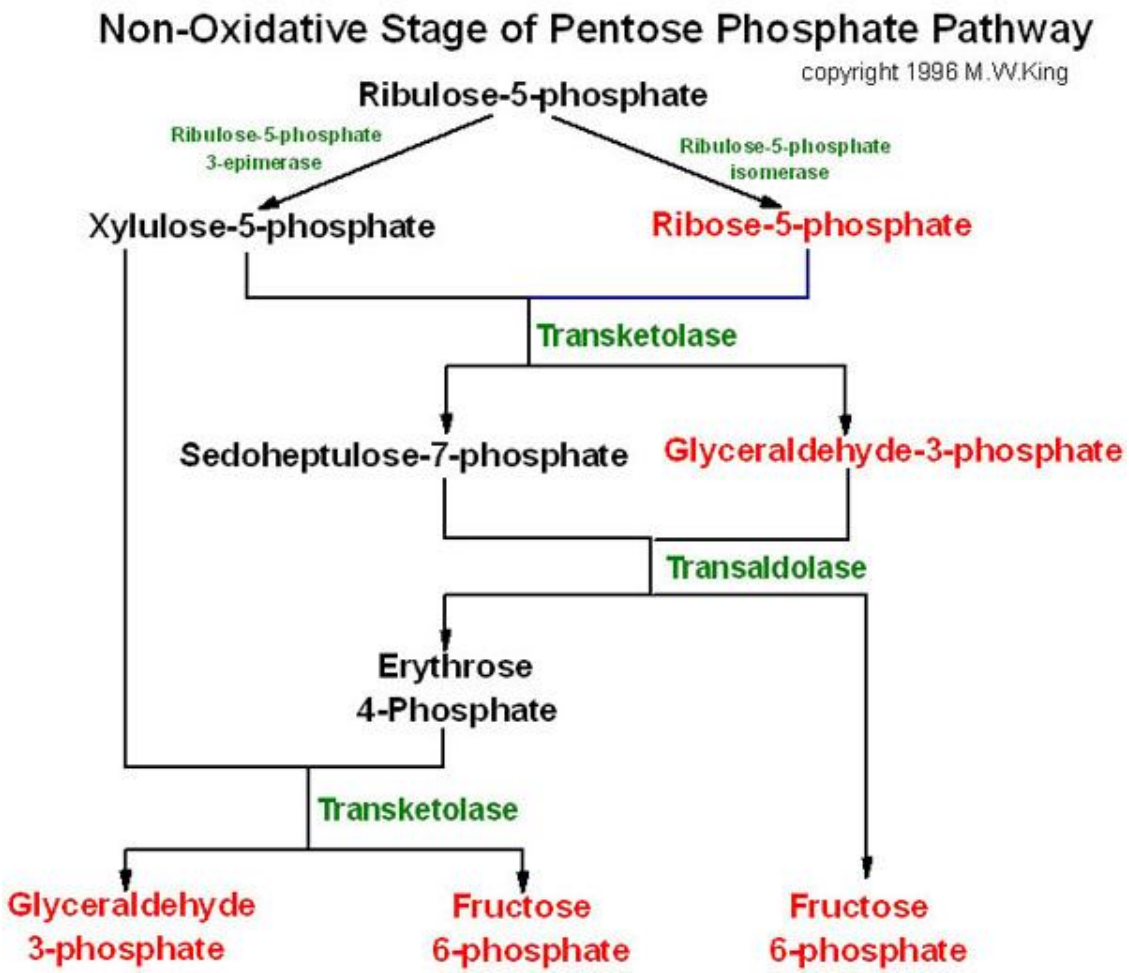
- The phosphorylase step is the first step and rate limiting and key enzyme in glycogenolysis with proper activation and in the presence of inorganic phosphate (Pi) the enzyme breaks down the glucosyl α1-4 linkages and removes by phosphorylytic cleavage the1-4 glucosyl residues from outermost chains of the glycogen molecule until approximately4 glucose residues remain on either side of a α1-6 branch (limit dextran).
- The phosphorylase liberates glucose as G-1-P.
- When 4 glucose residues are left from the branch point the another enzyme α1,4-1,6 glucan transferase transfers a trisaccharide unit from one side to the other thus exposing α1-6 branch point.
- The hydrolytic splitting of the α1-6 glucosidic linkage requires the action of a specific debranching enzyme (amylo 1-6 glucosidase).
- As the α1-6 linkage is hydrolytically split one molecule of free glucose is produced rather than one molecule of G-1-P as the case of phosphorylase cleavage.

PENTOSE PHOSPHATE PATHWAY/HEXOSE MONPHOSPHATE SHUNT/PENTOSE CYCLE/PHOSPHOGLUCONATE PATHWAY.

This is an alternate pathway of glucose metabolism. Energy is however not produced from this pathway but rather NADPH which is required for various reductive synthesis in the body ; pentoses (5-carbon sugars) required for nucleic acid synthesis.

NADPH is used as an electron donor in such pathways as:extramitochondrial de novo fatty acid synthesis, cholesterol and steroid synthesis, in conversion of oxidized glutathione G-S-S-G to reduced glutathione GSH (which serves to remove H_2O_2 from RBCs,maintenance of lens proteins), in synthesis of sphingolipids, microsomal denaturation of fatty acids, cytoplasmic synthesis of L-glutamate by the L-glutamate dehydrogenase, conversion of phenylalanine to tyrosine, as coenzyme for methemoglobin reductase for its conversion to Hemoglobin(Hb), in the uronic acid pathway, for production of superoxide ion by NADPH reductase useful for leucocytes phagocytosis etc

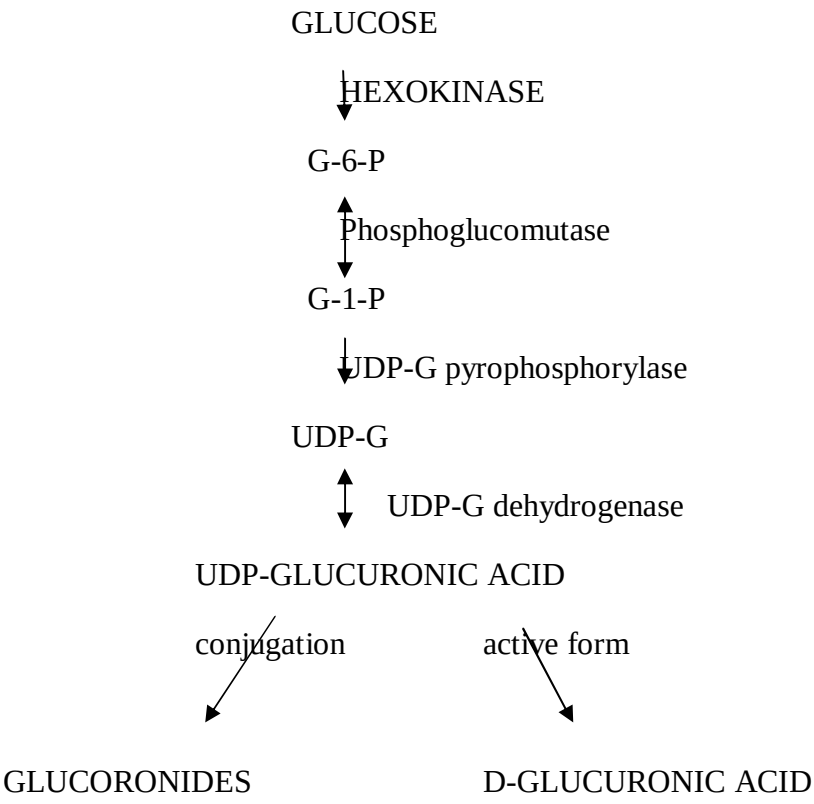




URONIC ACID PATHWAY.

Another alternate pathway of glucose oxidation also devoid of energy production but useful for the production of glucuronic acid which is mainly utilized for detoxification of foreign chemicals (xenobiotics). It is also important in synthesis of mucoploysaccharides.

Inherited defieciency of na enzyme in this pathway leads to a condition called inherited pentosuria.

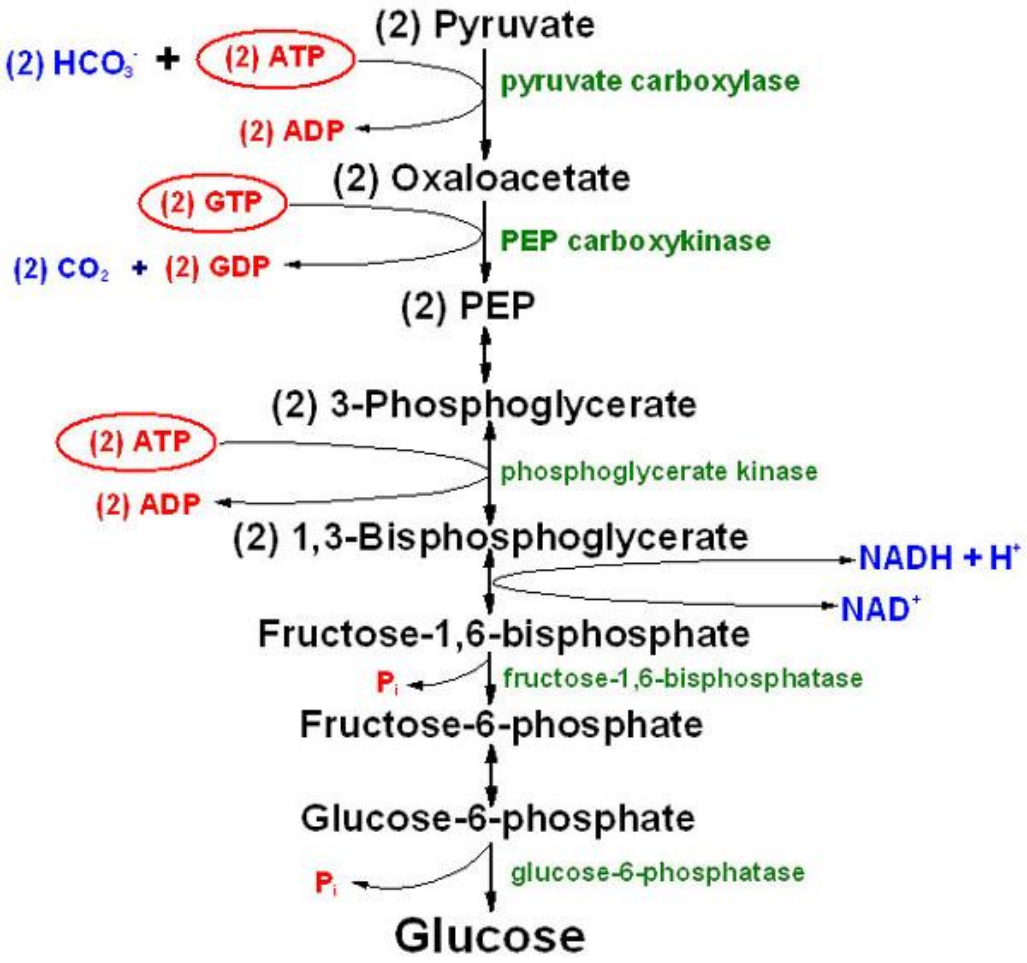


GLUCONEOGENESIS

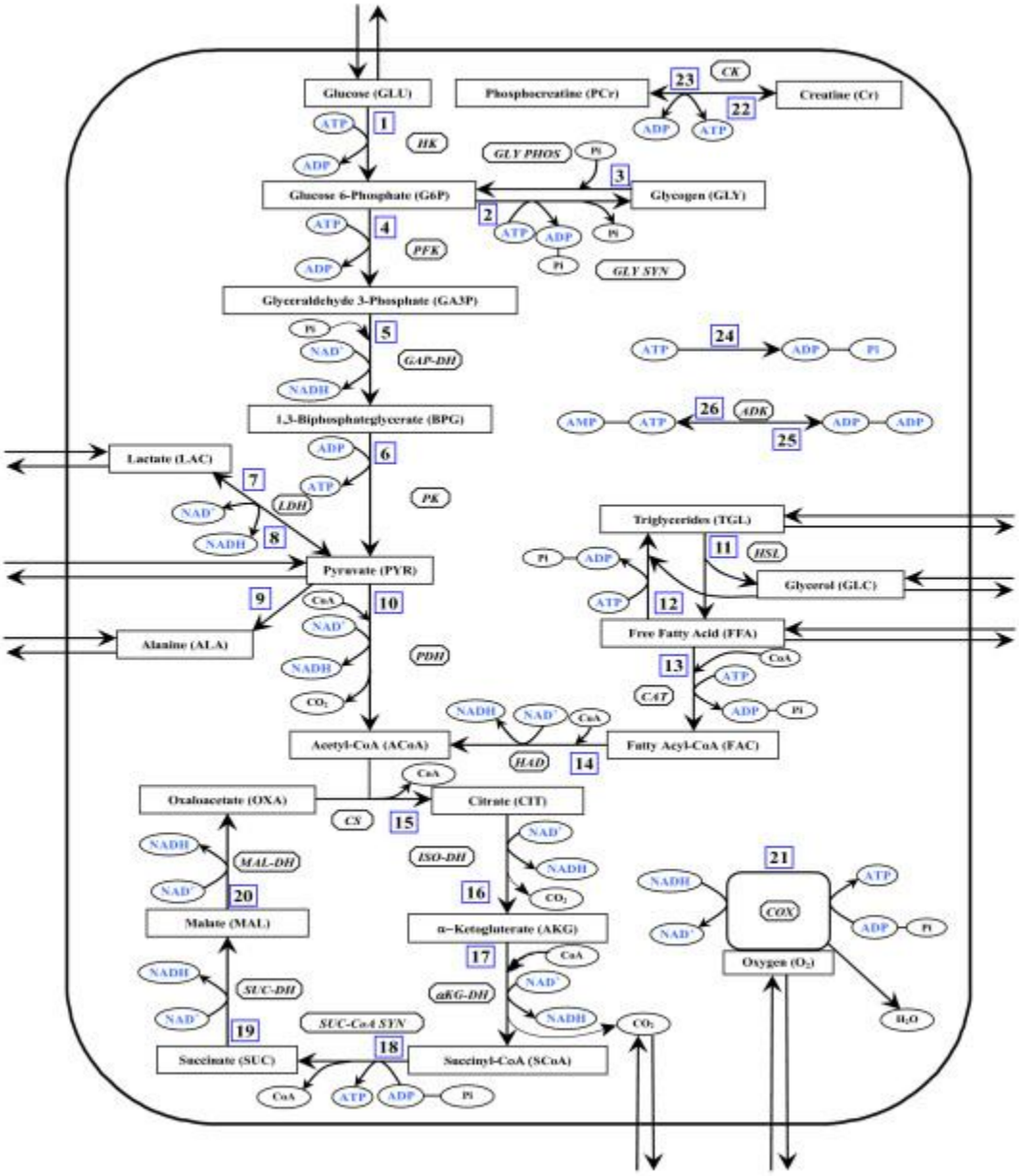
This is the formation of glucose from non-carbohydrate sources. It is required to meet the body's need for glucose when carbohydrates are not available or insufficient. And is also required for the maintaining levels of intermediates of the CAC and to clear products of metabolism of other tissues from blood e.g. lactic acid produced by muscles, glycerol by adipose tissue etc.

The principal site of gluconeogenesis is liver, kidney as they poetesses the full complement of enzymes necessary for the pathway.

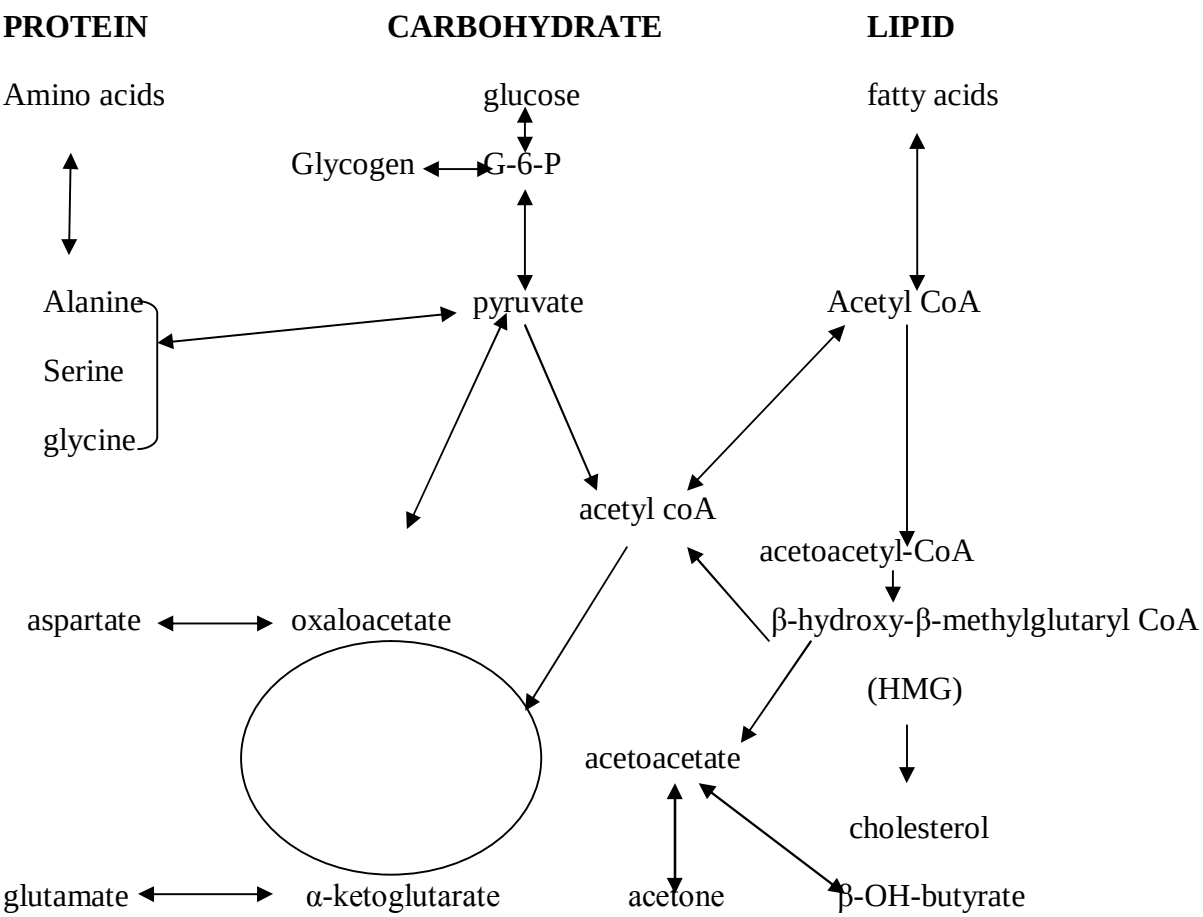
Gluconeogenesis substrates gluconeogenic amino acids forming pyruvate, oxaloacetate and α-ketoglutarate (aspartate, glycine, alanine, serine, cysteine, threonine, glutamate, glutamine, proline, arginine, histidine, lysine); lactates and pyruvate, glycerol and propionic acid.


(2) Pyruvate
(2) HCO3- + (2) ATP
pyruvate carboxylase
(2) ADP
(2) Oxaloacetate
(2) GTP
PEP carboxykinase
(2) CO2 + (2) GDP
(2) PEP
(2) 3-Phosphoglycerate
(2) ATP
phosphoglycerate kinase
(2) ADP
(2) 1,3-Bisphosphoglycerate
NADH + H+
NAD+
Fructose-1,6-bisphosphate
Pi
fructose-1,6-bisphosphatase
Fructose-6-phosphate
Glucose-6-phosphate
Pi
glucose-6-phosphatase
Glucose

INTERRELATIONSHIP BETWEEN CARBOHYDRATE, LIPID AND PROTEIN METABOLISM



Intergration of carbohydrate, lipid and protein metabolism.


PROTEIN
CARBOHYDRATE
LIPID
Amino acids
glucose
fatty acids
Glycogen
G-6-P
Alanine
Serine
glycine
pyruvate
Acetyl CoA
acetyl coA
acetoacetyl-CoA
aspartate
oxaloacetate
β-hydroxy-β-methylglutaryl CoA
(HMG)
acetoacetate
cholesterol
glutamate
α-ketoglutarate
acetone
β-OH-butyrate

DISEASES/DISORDERS OF CARBOHYDRATE METABOLISM

- Diabetes mellitus;
- Hyperinsulinism
- Hypoglycemia of pigs (undeveloped gluconeogenic mechanisms)
- Glycogen storage diseases (GSD).

ENZYMES

OCCURRENCE

Enzymes are produced by all living organisms including humans and present only in small amounts.

MEDICAL AND BIOLOGICAL IMPORTANCE

1. Enzymes are the chemical work horses of the body. Enzymes are biological catalysts that speed up the pace of chemical reactions.
2. A chemical reaction without an enzyme is like a drive over a mountain. The enzyme bores a tunnel through it so that passage is far quicker and takes much less energy.
3. Enzymes make life on earth possible, all biology from conception to the dissolution that follows death depends on enzymes.
4. Enzymes regulates rate of physiological process. So, defects in enzyme function cause diseases.
5. When cells are injured enzymes leak into plasma. Measurement of activity of such enzymes in plasma is an integral part of modern day medical diagnosis.
6. Enzymes are used as drugs.
7. Immobilized enzymes, which are enzymes attached to solid supports are used in clinical chemistry laboratories and in industry. For example glucose in blood or urine is detected by using immobilized glucose oxidase. In pharmaceutical industry, glucose isomerase is used to produce fructose from glucose.
8. Enzymes are used as biosensors.
9. AIDS detection involves use of enzyme dependent ELISA technique.
10. Enzymes are used as cleansing agents in detergent industry.

CHEMICAL NATURE OF ENZYMES (PROPERTIES)

1. All the enzymes are proteins except ribozymes and number of enzymes are obtained in crystalline form.
2. In 1878, Kuhne, introduced term ‘Enzyme’ to indicate biological catalyst.
3. Enzymes cut big molecules apart and join small molecules to form big molecules.
4. Most of the chemical reactions in the body are enzyme catalysed.
5. The substance upon which an enzyme acts is called as substrate. By the action of

enzyme it is converted to product. An enzyme-catalysed reaction consist of substrate, enzyme and product as shown below.

Substrate → Product

6. The enzymes are big particles. Their molecular (size) weight ranges from few thousands to millions.

7. Enzymes have enormous power of catalysis. They increase rate of reaction to 105 to 1010 folds. For example, carbonic anhydrase can hydrate to 106 molecules of CO2 per second. In the absence of enzyme hydration of CO2 is 10–1 per second.

8. Enzymes are far more efficient compared to non-enzyme (man made) catalysts.

9. Enzymes are not consumed in the overall reaction.

10. Enzymes accelerate the rate of reaction but does not alter the equilibrium constant (Keq).

To know how enzymes work, physical chemistry of catalysts must be explored because enzymes are catalysts.

Catalyst

A catalyst does not change the chemical reaction but it accelerates the reaction. They are not consumed in overall reaction. But they undergo chemical or physical change during reaction and returns to original state at the end of reaction. Transition state theory was proposed to explain action of catalyst.

For a chemical reaction A → B to occur, energy is required. When enough energy is supplied. A undergoes to transition state which is an unstable state. So, it gets converted to product B which is more stable. The amount of energy needed to convert a substance from ground state to transition state is called *activation energy*. In presence of catalyst, A undergoes to transition state very fast and requires less energy . Hence, a catalyst accelerate the rate of reaction by decreasing the energy of activation. Likewise enzymes also speed up reaction by lowering energy of activation. Further, the activation energy is very much less for a reaction in presence of enzyme than non-enzyme catalyst . Therefore enzymes are more efficient than non-enzyme catalyst.

ENZYME SPECIFICITY

Enzymes are highly specific compared to other catalyst. An enzyme catalyzes only specific reaction. Some general types of enzyme specificity are:

1. Substrate Specificity

Enzymes are specific towards their substrates. For example, glucokinase catalyzes the transfer of phosphate from ATP to glucose. Galactokinase catalyzes transfer of phosphate from ATP to galactose. Though both enzymes catalyzes transfer of phosphate from ATP they act only on specific substrate. Similarly, transminase which catalyze transfer of amino group are specific to substrate. Aspartate transminase catalyzes the transfer of amino group from aspartate and alanine transminase catalyzes transfer of amino group from alanine only. So, they are specific towards substrate.

2. Reaction Specificity

A given enzyme catalyze only one specific reaction. For example, lipases only hydrolyze lipids, urease hydrolyzes urea. They do not catalyze any other type of reaction. Likewise amino acid oxidase catalyze oxidation of amino acid and decarboxylase catalyze only decarboxylation of amino acids.

3. Group Specificity

Some lytic (hydrolases) enzymes act on specific groups. Proteases are specific for peptide groups, glycosidases are specific to glycosidic bonds.

Proteins Amino acids

4. Absolute Group Specificity

Certain lytic enzymes exhibit high order group specificity. For example, chymotrypsin is protein splitting enzyme *i.e.,* it hydrolyzes peptide bonds. But it preferentially hydrolyzes peptide bonds in which carboxyl group is contributed by aromatic amino acids phenylalanine, tyrosine and tryptophan. Likewise, trypsin another peptide bond hydrolyzing enzyme preferentially hydrolyzes peptide bonds in which carboxyl group is contributed by basic amino acids.

Amino peptidase Chymotrypsin Trypsin Carboxypeptidase

H N Ala 2 Gly Val Tyr Glu Ala Ile Arg Ala Gln Asp COOH

Similarly, carboxy peptidase removes one amino acid each time from carboxy terminus and amino peptidase removes one amino acid each time from N-terminus. Thrombin of blood clotting process is highly specific for Arg-Gly-bonds.

5. Optical Specificity

Several enzymes exhibit optical specificity of substrate on which they act. It means enzymes are able to recognise optical isomers of the substrate. For example, enzymes of amino acid metabolism act only on L-isomers (L-amino acid) but not D-isomers (D-amino acids). Likewise enzymes of carbohydrate metabolism act only on D-sugars but not on L-sugars.

Enzyme Classification and Nomenclature

International Union of Biochemistry classified all enzymes into six major classes based on the type of reaction they catalyze and reaction mechanism.

Nomenclature

The name of an enzyme has two parts. The first part indicates name of its substrate and second part ending in ‘ase’ indicates the type of reaction it catalyzes. Further, each enzyme has code (EC) number. It is a four-digit number. The first digit indicates major class, second digit indicates sub class, third digit denotes sub sub class and final digit indicates specific enzyme.

The six major classes of enzymes with some example are:

1. Oxidoreductases

They catalyze oxidation and reduction reactions.

2. Tranferases

They catalyze transfer of groups

3. Hydrolases

They catalyze hydrolysis of peptide, ester, glycosyl etc. bonds.

4. Lyases

They catalyze removal of groups from substrates by mechanisms other than hydrolysis forming double bonds.

5. Isomerases

They catalyze interconversion of optical, functional and geometrical isomers.

6. Ligases

They catalyze linking together of two compounds. The linking is coupled to the breaking of phosphate from ATP.

MECHANISM OF ENZYME ACTION

The mechanism of enzyme action deals with molecular events associated with conversion of a substrate to product in an enzymatic reaction.

Medical Importance

1. Some drugs are designed based on mechanism of enzyme action. For example, X-ray crystallographic studies on mechanism of carboxy peptidase action lead to design of specific inhibitor to angiotensin converting enzyme like captopril which is used in treatment of hypertension.
2. Enzymes with specific properties can be designed based on mechanistic studies. They may be introduced into humans to correct specific abnormalities associated with disorders.

The larger size of an enzyme molecule relative to smaller size of its substrate always puzzled biochemists. Ultimately it led to the concept that small portion of enzyme is required for enzyme action. This part of the enzyme is known as active site.

CHARACTERISTICS OF AN ENZYME ACTIVE SITE

1. It consists of two parts.

(*a*) **Catalytic site.** It is the portion (part) of the enzyme that is responsible for catalysis. It determines reaction specificity. Occasionally, catalytic site and active site are used synonomously.

(*b*) **Binding site.** It is the part of the enzyme that binds with substrate. It determines substrate specificity.

2. The active sites of enzyme are clefts within the enzyme molecule. For example, the active site of ribonuclease lies within cleft .
3. Active site consists of few amino acid residues only.
4. Active site is three dimensional.
5. The active site is contributed by amino acid residues that are far apart in the enzyme molecule. During catalysis, they are brought together.
6. The amino acids at the active site are arranged in a very precise manner so that only specific substrate can bind at the active site.
7. Usually serine, histidine, cysteine, aspartate or glutamate residues make up active site.

Enzymes are named according to the active site amino acid. For example, trypsin is a serine protease and papain is cysteine protease.

MODELS OF ACTIVE SITE

Some active site models are proposed to explain enzyme specificity.

A. Lock and Key Model

1. According to this model the active site is a rigid portion of the enzyme molecule and its shape is complementary to the substrate like lock and key.
2. The complimentary shape of substrate and active site favours tightly bound enzyme. Substrate complex formation followed by catalysis.
3. This model was unable to explain the possibility of rigid active site combining with the product to form substrate in reversible reaction.

B. Induced Fit Model

1. According to this model, the active site is flexible unlike rigid type of the lock and key model.
2. In the enzyme molecule the amino acid residues that make up active site are not oriented properly in the absence of substrate.
3. When substrate combines with enzyme, it induces conformational change in the enzyme molecule in such way that amino acids that make active site are shifted into correct orientation to favour tightly bound enzyme-substrate complex formation followed by catalysis.
4. The enzyme molecule is unstable in the induced conformation and returns to its native conformation in the absence of substrate.

FACTORS AFFECTING ENZYME ACTION

Rates of enzyme catalyzed reactions are affected by:

1. Enzyme concentration
2. Temperature
3. Hydrogen ion concentration or pH
4. Substrate concentration
5. Inhibitors and cofactors

MEDICAL AND BIOLOGICAL IMPORTANCE

1. For normal health, all enzymatic reactions must occur in the body and they must proceed at appropriate rates. Alterations in the rates of enzymatic reactions may disturb tissue homeostasis.
2. Any alteration in intracellular pH disturbs rates of enzyme reactions.
3. Organs for transplantation, blood and serum are preserved at low temperature as soon as they are removed from body because enzymatic reactions proceed at much lower rate at low temperature. Under such conditions, O2 demand of cells decreases, so cells of the

organs or fluids survive with available O2 for some time.
4. Rates of enzymatic reactions are altered in fever and hypothermia because temperature influences rate of enzyme reaction.
5. An understanding of factors affecting enzyme action is required for development of drugs. Many drugs act by decreasing rate of key metabolic reaction by blocking that particular enzyme. For example, AZT used in treatment of AIDS is an inhibitor of HIV virus enzyme. Lovastatin is used in treatment of atherosclerosis is an inhibitor of HMGCoA reductase, a cholesterol producing enzyme, captopril used in the treatment of hypertension is an inhibitor of angiotensin converting enzyme an enzyme of blood pressure regulation.
6. Some poisons work by abolishing (affecting) essential enzymatic reactions.
1. Enzyme concentration
The rate of enzyme catalyzed reaction is directly proportional to the concentration of enzyme.
2. Temperature
Like any chemical reaction, enzyme activity increases with increase in temperature initially. After a critical temperature, the enzyme activity decreases with increase in the temperature. When the effect of temperature on enzyme activity is plotted, cone-shaped curve is obtained The figure indicates that there is an optimal temperature at which enzyme is optimally active. It is called as *optimum temperature*. For most of the enzymes, the optimum temperature is the temperature of the cell or body in which they occur. For example, human trypsin the optimum temperature in 37 °C which is the normal body temperature. The first half of the curve approaching the optimum temperature indicates that enzyme activity increased with increase in the temperature due to the increased kinetic energy of reacting molecules. The other half which corresponds to decreased catalytic activity with increased temperature is due to denaturation of enzyme.
Enzymes of plants and micro-organisms growing in hot climates or hot springs may exhibit optimal temperature close to the boiling point of water. Examples are enzymes of thermophilic bacteria, snake venom phospholipase and urease (55 °C).
3. Effect of pH or hydrogen ion concentration
Most of the enzymes are not maximally active throughout pH scale (1-14). Several enzymes has optimum activity between pH of 5 to 9. When enzyme activity measured at several pH values is plotted a bell shaped curve is obtained.
Since enzymes are proteins pH changes affects:
1. Charged state of catalytic site.
2. Conformation of enzyme molecules.
In addition low or high pH causes denaturation of enzymes. It accounts for the less activity of enzymes at acidic or alkaline pH . For most of the enzymes, optimum pH is the pH of body or cell in which they occur. However, for some enzymes optimum pH may not be in the neutral range.
In the case of oligomeric enzymes, optimum pH is required for the association of

protomers. When the pH is altered, the protomers dissociate with loss of biological activity.

4. Effect of substrate concentration

If the concentration of the substrate (S) is increased while other conditions are kept constant, the initial velocity *v*0 (velocity measured when little substrate is reacted) increases proportionately in the beginning. As the substrate concentration continues to increase, the increase in *v*0 slows down and reaches maximum Vmax and no further . The plot of (S) versus *v*0 is rectangular hyperbola. It is called as *Michaelis plot*. To explain the reason for characteristic shape of the curve, Michaelis proposed that in an enzyme catalyzed reaction, the enzyme (E) combines with substrate (S) to form and enzyme-substrate (ES) complex which decomposes to form product (P) and free enzyme.

Based on this, reasons for the three phases of the curve can be interpreted.

1. In the first phase, substrate concentration is low and most of the enzyme molecules are free so they combine with the substrate molecules. Therefore, velocity is proportional to substrate concentration. At this state, enzymatic reaction shows first-order kinetics.
2. In the second phase, half of the enzyme molecules are bound to substrate, so the velocity is not proportional to substrate concentration. At this stage, enzymatic reaction shows mixed-order kinetics.
3. In the third phase, all the enzyme molecules are bound to substrate, so velocity remain unchanged because free enzyme is not available though the substrate is in excess. At this stage enzymatic reaction shows zero-order kinetics.

The Michaelis plot is used to determine Michaelis constant a characteristics of enzyme and type of enzyme inhibition.

Michaelis Constant or Km

The substrate concentration that produces half the maximal velocity (Vmax/2) is known as Michaelis constant. Apart from graph Km also can be determined from Michaelis-Menten equation. It is a simple equation and describes the dependence of initial velocity (*v*0) on the concentration of enzyme and substrate. It is the theoretical expression for rectangular hyperbola.

Significance of Km

1. It is an enzyme kinetic constant.
2. It indicates the substrate concentration required for the enzyme to work efficiently.
3. Low Km indicates high affinity of enzyme towards substrate. High Km indicates low affinity of enzyme towards substrate. Hence, Km and affinity are inversely related. (Km α 1/affinity)

Example: Hexokinase and glucokinase both phosphorylates glucose. However, hexokinase can phosphorelate glucose 2000 times more efficiently than glucokinase because Km of hexokinase is low (1 × 10–5 M) whereas Km of glucokinase is high (2.0 × 10–2 M).

4. Km is required when enzymes are used as drugs.
5. Use of enzymes in immunodiagnostics (ELISA) require Km of the enzyme.

Line Weaver–Burk Plot

1. Michaelis plot gives only approximate Km and Vmax values because proper Vmax is difficult to obtain at very high substrate concentration.
2. By using Line Weaver-Burk Plot accurate Km and Vmax are obtained.
3. Line Weaver-Burk Plot is obtained by taking reciprocals of both sides of Michaelis-Menten equation

The equation represents $Y = ax + b$ straight line equation with slope of Km/Vmax. Further straight line is obtained by plotting 1/S against 1/V. Since 1/S and 1/V are reciprocals of S and V, respectively. This plot is known as "double reciprocal plot".

4. The straight line intersects y-axis, which corresponds to Vmax value. A line extended from point of intersection to x-axis of second quadrant provides Km value (Fig. 4.6 *c*).
5. In addition to Km and Vmax values, type of inhibition is determined using this plot.
6. Inhibition constant (Ki) of inhibitor is also determined using this plot.

Significance of Ki

1. Ki indicates affinity of inhibitor towards enzyme. Like Km, Ki is inversely related to affinity.
2. Use of inhibitors as drugs requires knowledge of Ki. Since Ki and affinity are inversely related inhibitors of low Ki are highly potent drugs.

Direct Linear Plot

1. Determination of Km and Vmax values of enzymes, which are inhibited by substrate at high concentration is not feasible with Line Weaver-Bunk plot.
2. In such cases, direct linear plot is used for Km and Vmax determination. They are read directly from plot without involving any calculation.
3. In this plot, each S and V are marked on X and Y axes, respectively. Then a straight line passing through two points and extending into first quadrant is drawn. When lines for all S and V values are drawn they intersect at common point which provides Km and Vmax.

INHIBITORS

Substances that decrease the catalytic activity of enzymes are called as inhibitors. They may be protein or non-protein inhibitors. The decrease in enzyme activity is called as inhibition. More than two types of enzyme inhibition exist based on the mode of action of inhibitors.

Competitive Inhibition

Competitive inhibition occurs at active site. Competitive inhibitor is structurally similar to that of substrate. Hence, it competes with substrate to bind at active site. Inhibition occurs when it binds at the active site of enzyme molecule. It is reversible. If the substrate concentration is increased then the competitive inhibition is relieved. Further, the rate of formation of product from (ES) complex is same as that of in the absence of inhibitor. So, velocity (Vmax) is not altered in competitive inhibition but Km increases (affinity of enzyme towards substrate

decreases) because of competition of substrate and inhibitor to bind at active site. The interaction of enzyme (E) substrate (S) and competitive inhibitor (I) is represented as equations below:
In addition at high substrate concentration, the number of enzyme molecules available for the inhibitor are far less. So, the inhibition is masked. (becomes reversible.) Michaelis plot also indicates Km alternation and unaffected Vmax in the presence of competitive inhibitor
A classical example for reversible competitive inhibition is succinate dehydrogenase enzyme. Malonate competitively inhibits the enzyme because it is structurally similar to the substrate succinate.

Competitive Inhibitors as Chemotherapeutic Agents

When used in clinical situations, the competitive inhibitors are called as *antagonists* or *anti metabolites* of the substrate with which they compete. The use of anti-metabolites in the treatment of diseases is called as *chemotherapy*. Therefore, competitive inhibitors are useful chemotherapeutic agents. They are used

1. Antibiotics
2. Anti-cancer drugs
3. In the treatment of metabolic diseases like gout, atherosclerosis and hypertension.

1. Sulfonamide antibiotics are used in the treatment of bacterial infections. Bacteria synthesize folic acid from p-aminobenzoic acid (PABA). Since these sulfonamide drugs contain sulfonilamide a structural analog of PABA , when used as chemotherapeutic agent, it blocks the synthesis of folic acid in bacteria. The lack of folic acid leads to death of bacteria . Sulfonamide act as competitive inhibitor for the enzyme involved in the formation of folic acid using PABA as substrate.
Precursor Folic acid Bacterial growth arrest PABA blocked
2. Competitive inhibitors used in the treatment of cancer are aminopterin and amethopterin (methotrexate). They are structural analog of folic acid. They are competitive inhibitors for the enzyme dihydrofolate reductase. They are used in the treatment of leukaemia, a type of cancer.
When used these drugs block formation of nucleic acids. For cell proliferation, nucleic acid are needed. So, lack of nucleic acids lead to arrest of tumour growth and advancement of cancer is prevented.
3. Allopurinol is a drug used in the treatment of gout. Gout is due to excessive production of uric acid. Xanthine oxidase is an enzyme involved in the formation of uric acid from hypoxanthine. Allopurinol is a structural analog of hypoxanthine and hence it is an antimetabolite of hypoxanthine. When it is used it blocks formation of uric acid by inhibiting the enzyme xanthine oxidase .
4. Lovastatin is a competitive inhibitor of enzyme HMG-CoA reductase, when used it blocks production of cholesterol. In atherosclerosis, cholesterol is more. Lovastatin reduces cholesterol formation thus arrest the advancement of atherosclerosis.

5. Competitive inhibitors used in the treatment of hypertension are captopril, lisinopril and enalapril. They competitively inhibit angiotensin converting enzyme, which is involved in regulation of blood pressure. When used they lower blood pressure by reducing activity of angiotensin converting enzyme.

Non-Competitive Inhibition

In this type of enzyme inhibition no competition occurs between substrate and inhibitor to bind at active site of enzyme. Inhibitor is not structurally related to substrate. In addition inhibitor binds to some other site of enzyme which is far off from active site.

In non-competitive inhibition, the inhibitor can react with free enzyme as well as enzyme substrate complex, because its binding site is away from active site. In addition, the formation of product from enzyme substrate-inhibitor complex is not same as that of in absence of inhibitor. So, the Vmax is decreased and Km (affinity) remains same because no competition of substrate and inhibitor in non-competitive inhibition. Michaelis plot also indicates same in the presence of non-competitive inhibitor.

Examples for Non-competitive Inhibition

Reversible non-competitive inhibitors are rare. Most of the known non-competitive inhibitors are irreversible. They are referred as enzyme poisons.

1. Iodoacetate blocks the formation of 1,3-bisphosphoglycerate from glyceraldehyde-3-phosphate by inhibiting enzyme glyceraldehyde-3-phosphate dehydrogenase
2. Fluoride blocks the action of enolase, which converts 2-phosphoglycerate to phosphoenol pyruvate.
3. Heavy metals like Hg^{2+}, Ag^{+}, Pb^{2+} and Arsenite are also enzyme poisons. They interact with –SH group of enzyme and activate it.

Hg^{2+} inhibits –SH containing pyruvate dehydrogenase. Similarly, arsenite inhibits –SH containing α-ketoglutarate dehydrogenase.

4. Some non-competitive inhibitors are used as pesticides. DDT, melathion and parathion are inhibitors of enzyme choline esterase that catalyzes hydrolysis of acetylcholine.
5. Di-isopropyl fluro phosphate (DFP) is a non-competitive inhibitor used as nerve gas in World War II. It is an active site directed irreversible non-competitive inhibitor. It forms covalent linkage with –OH groups of serine residue of choline esterase. When used DFP causes constriction of larynx, pain in eyes and mental confusion.
6. CN^{-} inhibits activity of cytochrome oxidase an enzyme of respiratory chain. Bitter almonds contain some cyanide.
7. Ethylene diaminotetra acetic acid (EDTA) inhibits metalloenzymes by forming complex with metal ion.
8. Tubers, bananas and beans contain inhibitors to trypsin, chymotrypsin and elestase.

FEEDBACK INHIBITION

Inhibition of activity of enzyme of a biosynthetic pathway by the end product of that pathway is called as feedback inhibition.

For example, formation of a substance D from A is catalyzed by three enzymes E1, E2 and E3.

A □E□1→ B □E□2→ C □E□3→ D

When enough D is formed it inhibits the activity of E1. By inhibiting E1, D regulates its own synthesis.

Examples:

1. Inhibition of aspartate trans carbamoylase by CTP.
2. Inhibition of HMG-CoA reductase by cholesterol.
3. Inhibition of ALA-synthase by heme.
4. Inhibition of anthranilate synthetase by tryptophan.

CO-FACTORS

1. Cofactors are non-protein molecules required for activity of some enzymes. They may be involved in catalysis or in structure maintenance.
2. There are two types of cofactors:

1. Organic cofactors, and
2. Inorganic cofactors.

The organic cofactors are further subdivided into. 1. Prosthetic groups 2. Co-enzymes.

1. Prosthetic Groups

These organic molecules are covalently attached to the enzyme and they undergo change during catalysis but return to native state at the end of the reaction.

2. Co-enzymes

These organic molecules are loosely (non-covalent) attached to enzyme molecules. They undergo change during reaction. Since they undergo change along with substrate they are referred as co-substrates.

Apo-enzyme + Co-enzyme = Holo enzyme
(Protein) (Non-protein) (Active).

Examples of Organic Co-factors

The major function of water soluble vitamins is to serve as co-factors, some of them as such serve as co-factor otherwise their derivatives serve as co-factors. They are divided on the basis of their function, in enzymatic reaction.

1. Co-enzymes of oxidation reduction reactions.

(*a*) Co-enzymes derived from niacin. They are NAD+, NADH + H+ and NADP+, NADPH + H+. These co-enzymes are loosely bound to apo-enzymes.

(*b*) Co-enzymes derived from riboflavin. They are FMN, FMNH2 and FAD, FADH2. They are covalently linked to apo-enzymes. So, they are prosthetic groups.
2. Coenzymes of group transfer reactions.
(*a*) Co-enzyme of pantothenic acid. It is co-enzyme of A(CoA, CoASH). It is involved in CoA transfer reaction.
(*b*) Co-enzyme of thiamin. It is thiamnin pyro(di)phosphate (TPP, TDP). It is prosthetic group of several enzymes.
Pyruvate Pyruvate dehydrogenase Acetyl-CoA
TPP CoASH
(*c*) Co-enzyme of pyridoxine. It is pyridoxal phosphate (P-PO4). It is prosthetic group of enzymes involved in amino group transfer. Other reactions where it serve as coenzyme are decarboxylation, transulfuration etc.
(*d*) Co-enzymes of folic acid. It is tetrahydrofolate (FH4). It participates in one carbon transfer reaction.
(*e*) Biotin is the only water-soluble vitamin that function as coenzyme as such. It is the prosthetic group of carboxylases.
(*f*) Co-enzyme of vitamin B12 or cyanocobalamin. It is methylcobamide. It is involved in methyl transfer reactions.
3. Many nucleotides also function as co-enzymes. They are adenosine triphosphate (ATP), cytidine diphosphate (CDP), uridine diphosphate (UDP), phosphoadenosine phosphosulfate (PAPS) and S-adenosyl methionine (SAM).

INORGANIC CO-FACTORS

Many enzymes require metal ions. They are required for maintenance of protein (enzyme) conformation and catalysis. Metal ions participate in enzymatic reactions in three ways.

1. Metallo Enzymes

Metal is tightly bound to enzyme molecule and it is an integral part of enzyme molecule. Metals are attached to enzyme through coordinate bonds. They participate in catalysis.

Examples:

(*a*) **Iron (Fe2+):** It is required for cytochrome oxidase, catalase, xanthine oxidase, succinate dehydrogenase.
(*b*) **Copper (Cu2+):** It is required for cytochrome oxidase, superoxidedismutase, lysyloxidase and ceruloplasmin.
(*c*) **Zinc (Zn2+):** It is required for carbonic anhydrase, carboxy peptidase, alkaline phosphatase, alcohol dehydrogenase etc.

2. Metal-dependent Enzymes

Metal is loosely associated with enzyme molecule or it may be required for enzyme substrate complex formation. In the absence of metal, enzyme may not interact with substrate molecule or with co-enzyme molecule.

Examples:

(*a*) **Magnesium (Mg2+):** It is needed by enzymes using ATP. Formation of Mg: ATP complex is essential. They include hexokinase, galactokinase, pyruvate kinase etc.

(*b*) **Calcium (Ca2+):** It is required for the activity of calpain, a calcium-dependent protease. Others are Na+/K+-ATPase and Ca2+ ATPase.

3. Metal-activated Enzymes

In presence of metals, some enzymes get activated *i.e.,* their activity increases many folds.

Examples:

(*a*) **Chloride (Cl–):** It activates amylase and angiotensin converting enzyme.

(*b*) **Calcium (Ca2+):** It activates trypsin.