KARPAGAM ACADEMY OF HIGHER EDUCATION
(Deemed to be University Established Under Section 3 of UGC Act 1956)
Coimbatore – 641 021.

Semester II

18BCU202 ENZYMES 4H-4C

SCOPE

This course provides a clear understanding of enzymes and its structure, classification, catalysis, kinetics, mechanism of action of enzymes, regulation of enzyme activity, coenzymes, immobilized enzymes and application of enzymes in medicine and industry.

OBJECTIVES

To have a clear understanding on enzyme structure, classification, kinetics of enzymes, enzyme catalysis, coenzymes, immobilized enzymes and application of enzymes in medicine and industry.

Unit 1

Introduction to enzymes and enzyme catalysis

Introduction - Nature of enzymes - protein and non-protein (ribozyme). Cofactor and prosthetic group, apoenzyme, holoenzyme. IUBMB classification of enzymes. Features of enzyme catalysis- Factors affecting the rate of chemical reactions, collision theory, activation energy and transition state theory, catalysis, reaction rates and thermodynamics of reaction. Catalytic power and specificity of enzymes (concept of active site), Fischer's lock and key hypothesis, Koshland's induced fit hypothesis.

Unit 2

Enzyme kinetics

Relationship between initial velocity and substrate concentration, steady state kinetics, equilibrium constant - monosubstrate reactions. Michaelis-Menten equation, Lineweaver-Burk plot, Eadie-Hofstee and Hanes plot. Km and Vmax, Kcat and turnover number. Effect of pH, temperature and metal ions on the activity of enzyme. Bisubstrate reactions - Types of bi bi reactions (sequential – ordered and random, ping pong reactions). Differentiating bi substrate mechanisms (diagnostic plots, isotope exchange).

Unit 3

Mechanism of action of enzymes and Enzyme inhibition

Mechanism of action of enzymes - General features - proximity and orientation, strain and distortion, acid base and covalent catalysis (chymotrypsin, lysozyme). Metal activated

enzymes and metalloenzymes, transition state analogues. Enzyme Inhibition - Reversible inhibition (competitive, uncompetitive, non-competitive, mixed and substrate). Mechanism based inhibitors - antibiotics as inhibitors.

Unit 4

Regulation of enzyme activity

Control of activities of single enzymes (end product inhibition) and metabolic pathways, feedback inhibition (aspartate transcarbomoylase), reversible covalent modification phosphorylation (glycogen phosphorylase). Proteolytic cleavage- zymogen. Multienzyme complex as regulatory enzymes. Occurrence and isolation, phylogenetic distribution and properties (pyruvate dehydrogenase, fatty acyl synthase) Isoenzymes - properties and physiological significance (lactate dehydrogenase).

Unit 5

Coenzymes in enzyme catalysed reactions and Applications of enzymes. Structure and Functions of TPP, FAD, NAD, pyridoxal phosphate, biotin, coenzyme A, tetrahydrofolate, lipoic acid. Applications of enzymes - Application of enzymes in diagnostics (SGPT, SGOT, creatine kinase, alkaline and acid phosphatases), enzyme immunoassay (HRPO), enzyme therapy (Streptokinase). Immobilized enzymes – Preparation techniques and its applications.

REFERENCES

Nelson, D.L. and Cox, M.M., (2013). Lehninger: Principles of Biochemistry 6th ed., W.H. Freeman and Company (New York), ISBN:13: 978-1-4641-0962-1 / ISBN:10:1-4292-3414-8.

Donald, V. and Judith G.V., (2011). Biochemistry 4th ed., John Wiley & Sons Asia Pvt. Ltd. (New Jersey), ISBN:978-1180-25024.

Nicholas C.P., and Lewis S., (1999). Fundamentals of Enzymology 3rd ed., Oxford University Press Inc. (New York), ISBN:0 19 850229 X.

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LECTURE PLAN
DEPARTMENT OF BIOCHEMISTRY

STAFF NAME: Ms.P.LOGANAYAKI
SUBJECT NAME: ENZYMES

SUB.CODE: 18BCU202

SEMESTER: II **CLASS: I B.Sc (BC)**

Sl. No	Duration of Period	Topics to be Covered	Support material
UNIT I Introduction to enzymes and enzyme catalysis			
1	1	Introduction - Nature of enzymes - protein and non-protein (ribozyme)	T1:190-192
2	1	Cofactor and prosthetic group, apoenzyme, holoenzyme	T2:463-464
3	1	IUBMB classification of enzymes	T1192-193 T4:6-12
4	1	Features of enzyme catalysis- Factors affecting the rate of chemical reactions	T4:91-94
5	1	activation energy and transition state theory, catalysis, reaction rates and thermodynamics of reaction.	T2:474-477 T1:195-202
6	1	Catalytic power and specificity of enzymes (concept of active site)	T2:460-463 T1:196-202
7	1	Fischer's lock and key hypothesis	T3:93
8	1	Koshland's induced fit hypothesis.	T3:97-98
		Total no of hours planned for UNIT I = 8	
Unit II: Enzyme kinetics			
1	1	Relationship between initial velocity and substrate concentration, steady state kinetics, equilibrium constant - monosubstrate reactions	T1:202-203
2	1	Michaelis-Menten equation	T1:203-205
3	1	Lineweaver-Burk plot, Eadie-Hofstee and Hanes plot	T1:206-207
4	1	Km and Vmax, Kcat and turnover number	T4:113-116 T2:480
5	1	Effect of pH, temperature and metal ions on the activity of enzyme	T1:207 T3:87-88
6	1	Bisubstrate reactions - Types of bi bi reactions, ping	T2:487-490

		pong reactions	
7	1	Differentiating bi substrate mechanisms (diagnostic plots).	T2:490
8	1	Isotope exchange	T2:491
		Total no of hours planned for UNIT II = 8	
Unit III: Mechanism of action of enzymes and Enzyme inhibition			
1	1	Mechanism of action of enzymes - General features - proximity and orientation, strain and distortion,	T5:154-159
2	1	acid base catalysis - chymotrypsin, covalent catalysis - lysozyme	T2:496-501 T5:156-159
3	1	Metal activated enzymes and metalloenzymes, transition state analogues	T2:501-504 T1:220 T2:505-507
4	1	Enzyme Inhibition - Reversible inhibition and competitive inhibition	T1:209-210
5	1	Uncompetitive and non-competitive	T2:483-485
6	1	Mixed and substrate inhibition	T1:210-211
7	1	Mechanism based inhibitors	T2:485-486
8	1	Antibiotics as inhibitors	T1:211-212
		Total no of hours planned for UNIT III = 8	
Unit- IV Regulation of enzyme activity			
1	1	Control of activities of single enzymes and metabolic pathways	T5:218-242
2	1	End product inhibition,	T5:218-242
3	1	feedback inhibition aspartate transcarbomoylase	T2:465-470
4	1	Reversible covalent modification phosphorylation (glycogen phosphorylase).	T5:256-263 T1:228
5	1	Proteolytic cleavage- zymogen.	T1:231-232
6	1	Multienzyme complex as regulatory enzymes	T5:281-282
7	1	Occurrence and isolation, phylogenetic distribution and properties (pyruvate dehydrogenas	T5:282-288
8	1	Isoenzymes - properties and physiological significance (lactate dehydrogenase).	T5:12 T5:200-201
		Total no of hours planned for UNIT IV = 8	
UNIT V Coenzymes			
1	1	Structure and Functions of TPP and FAD, NAD Structure and Functions of pyridoxal phosphate	T4:209-215 T4:206-209
2	1	Structure and Functions of biotin and coenzyme	T4:217-218
3	1	Structure and Functions of tetrahydrofolate and lipoic acid as enzymes.	T4:218-219
4	1	Application of enzymes in diagnostics SGPT, SGOT,	T4:340-350

		creatine kinase	
5	1	Application of enzymes in diagnostics alkaline and acid phosphatase	T4:340-350
6	1	enzyme immunoassay (HRPO) enzyme therapy (Streptokinase)	T5:422-424 T5:424-426
7	1	Immobilized enzymes – Preparation techniques	T4:356-358
8	1	Application of immobilized enzymes	T4:359-365
		Total no of hours planned for UNIT V = 8	
Total planned Hours	**40**		

Support Materials

T1: Nelson, D.L. and Cox, M.M., (2013). Lehninger: Principles of Biochemistry 6th ed., W.H. Freeman and Company (New York), ISBN:13: 978-1-4641-0962-1 / ISBN:10:1 4292-3414-8.

T2: Donald, V. and Judith G.V., (2011). Biochemistry 4th ed., John Wiley & Sons Asia Pvt. Ltd. (New Jersey), ISBN:978-1180-25024.

T3: U. Sathyanarayana (1999). Biochemistry, Books and Allied Pvt Ltd, Calcutta.

T4: Trevor Palmer (2004). Enzymes – Biochemistry, Biotechnology, Clinical Chemistry; 1st edition, East West Press Private Ltd., New Delhi.

T5: Nicholas C.P., and Lewis S., (1999). Fundamentals of Enzymology 3rd ed., Oxford University Press Inc. (New York), ISBN:0 19 850229 X.

UNIT-I

SYLLABUS

Introduction - Nature of enzymes - protein and non-protein (ribozyme). Cofactor and prosthetic group, apoenzyme, holoenzyme. IUBMB classification of enzymes. Features of enzyme catalysis- Factors affecting the rate of chemical reactions, collision theory, activation energy and transition state theory, catalysis, reaction rates and thermodynamics of reaction. Catalytic power and specificity of enzymes (concept of active site), Fischer's lock and key hypothesis, Koshland's induced fit hypothesis.

INTRODUCTION TO ENZYMES

There are two fundamental conditions for life. (i) The living entity must be able to self-replicate and (ii) The organism must be able to catalyze chemical reactions efficiently and selectively. Living systems make use of energy from the environment. Many of us, for example, consume substantial amounts of sucrose—common table sugar—as a kind of fuel, whether in the form of sweetened foods and drinks or as sugars itself. The conversion of sucrose to CO_2 and H_2O in the presence of oxygen is a highly exergonic process, releasing free energy that we can use to think, move, taste, and see. However, a bag of sugar can remain on the shelf for years without any obvious conversion to CO_2 and H_2O. Sucrose is consumed by a human (or almost any other organism), it releases its chemical energy in seconds. The difference is catalysis. Without catalysis, chemical reactions such as sucrose oxidation could not occur on a useful time scale, and thus could not sustain life. The reaction catalysts of biological systems is the enzymes, the most remarkable and highly specialized proteins. Enzymes have extraordinary catalytic power, often far greater than that of synthetic or inorganic catalysts. They have a high degree of specificity for their substrates, they accelerate chemical reactions tremendously, and they function in aqueous solutions under very mild conditions of temperature and pH. Enzymes are central to every biochemical process. Acting in organized sequences, they catalyze the hundreds of stepwise reactions that degrade nutrient molecules, conserve and transform chemical energy, and make biological macromolecules from simple precursors. Through the action of regulatory enzymes, metabolic pathways are highly coordinated to yield a harmonious interplay among the many activities necessary to sustain life.

The study of enzymes has immense practical importance. In some diseases, especially inheritable genetic disorders, there may be a deficiency or even a total absence of one or more enzymes. For other disease conditions, excessive activity of an enzyme may be the cause. Measurements of the activities of enzymes in blood plasma, erythrocytes, or tissue samples are important in diagnosing certain illnesses. Many drugs exert their biological effects through interactions with enzymes and enzymes are important practical tools, not only in medicine but in the chemical industry, food processing, and agriculture.

HISTORY OF ENZYMES

Biological catalysis was first recognized and described in the late 1700s, in studies on the digestion of meat by secretions of the stomach, and research continued in the 1800s with examinations of the conversion of starch to sugar by saliva and various plant extracts. In the 1850s, Louis Pasteur concluded that fermentation of sugar into alcohol by yeast is catalyzed by "ferments." He postulated that these ferments were inseparable from the structure of living yeast cells; this view, called vitalism, prevailed for decades. Then in 1897 Eduard Buchner discovered that yeast extracts could ferment sugar to alcohol, proving that fermentation was promoted by molecules that continued to function when removed from cells. Frederick W. Kühne called these molecules **enzymes.** As vitalistic notions of life were disproved, the isolation of new enzymes and the investigation of their properties advanced the science of biochemistry.

The isolation and crystallization of urease by James Sumner in 1926 provided a breakthrough in early enzyme studies. Sumner found that urease crystals consisted entirely of protein, and he postulated that all enzymes are proteins. In the absence of other examples, this idea remained controversial for some time. Only in the 1930s was Sumner's conclusion widely accepted, after John Northrop and Moses Kunitz crystallized pepsin, trypsin, and other digestive enzymes and found them also to be proteins. Haldane made the remarkable suggestion that weak bonding interactions between an enzyme and its substrate might be used to catalyze a reaction. This insight lies at the heart of our current understanding of enzymatic catalysis. Since the latter part of the twentieth century, research on enzymes has been intensive. It has led to the purification of thousands of enzymes, elucidation of the structure and chemical mechanism of many of them, and a general understanding of how enzymes work.

Most Enzymes Are Proteins

Almost all enzymes are proteins with the exception of a small group of catalytic RNA molecules. Their catalytic activity depends on the integrity of their native protein conformation. If an enzyme is denatured or dissociated into its subunits, catalytic activity is usually lost. If an enzyme is broken down into its component amino acids, its catalytic activity is always destroyed. Thus the primary, secondary, tertiary, and quaternary structures of protein enzymes are essential to their catalytic activity. Enzymes, like other proteins, have molecular weights ranging from about 12,000 to more than 1 million. Some enzymes require no chemical groups for activity other than their amino acid residues. Others require an additional chemical component called a **cofactor**—either one or more inorganic ions, such as Fe^{2+}, Mg^{2+}, Mn^{2+}, or Zn^{2+} or a complex organic or metalloorganic molecule called a **coenzyme.** and one or more metal ions for activity. A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme protein is called a **prosthetic group.** A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions is called a **holoenzyme.** The protein part of such an enzyme is called the **apoenzyme** or **apoprotein.** Coenzymes act as transient carriers of specific functional groups. Most are derived from vitamins, organic nutrients required in small amounts in the diet. Finally, some enzyme proteins are modified covalently by phosphorylation, glycosylation, and other processes. Many of these alterations are involved in the regulation of enzyme activity.

Cofactors for enzymes

S.No	Inorganic ion (cofactor)	Specific for enzyme
1	Cu^{2+}	Cytochrome oxides
2	Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
3	K^{+}	Pyruvate kinase
4	Mg^{2+}	Hexokinase, glucose-6-phosphatase, pyruvate kinase
5	Mn^{2+}	Arginase, ribonucleotide reductase
6	Mo	Dinitrogenase

7	Ni^{2+}	Urease
8	Se	Glutathione peroxidase
9	Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Some coenzymes that serve as transient carriers of specific atoms or functional groups

S.No	Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
1	Biotin	CO_2	Biotin
2	Coenzyme A	Acyl groups	Pantothenic acid and other compounds
3	5' –Deoxyadenosyl cobalamine (Coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
4	Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
5	Lipoate	Electrons and acyl groups	Not required in diet
6	Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin)
7	Pyridoxal phosphate	Amino groups	Pyridoxine (Vitamin B_6)
8	Tetrahydrofolate	One carbon group	Folate
9	Thiamine pyrophosphate	Aldehydes	Thiamine (Vitamin B_1)

Non protein enzymes (Ribozymes)

Ribozymes (ribonucleic acid enzymes) are RNA molecules that are capable of catalyzing specific biochemical reactions, similar to the action of protein enzymes. The 1982 discovery of ribozymes demonstrated that RNA can be both genetic material (like DNA) and a biological catalyst (like protein enzymes), The RNA catalysts called ribozymes are found in the nucleus, mitochondria, and chloroplasts of **eukaryotic** organisms. Some viruses, including several bacterial viruses, also have ribozymes. The most common activities of natural or in vitro-evolved ribozymes are the cleavage or ligation of RNA and DNA and peptide bond formation. Within the ribosome, ribozymes function as part of the large subunit ribosomal RNA to link amino acids during protein synthesis. They also participate in a variety of RNA processing reactions, including RNA splicing, viral replication, and transfer RNA biosynthesis.

The ribozymes discovered to date can be grouped into different chemical types, but in all cases the RNA is associated with metal ions, such as magnesium (Mg^{2+}) or potassium (K^{+}), that play important roles during the catalysis. Almost all ribozymes are involved in processing RNA. They act either as molecular scissors to cleave precursor RNA chains (the chains that form the basis of a new RNA chain) or as "molecular staplers" that **ligate** two RNA molecules together. Although most ribozyme targets are RNA, there is now very strong evidence that the linkage of amino acids into proteins, which occurs at the ribosome during **translation**, is also catalyzed by RNA. Thus, the ribosomal RNA is itself also a ribozyme.

In some ribozyme-catalyzed reactions, the RNA cleavage and ligation processes are linked. In this case, an RNA chain is cleaved in two places and the middle piece (called the intron) is discarded, while the two flanking RNA pieces (called exons) are ligated together. This reaction is called splicing. Besides ribozyme-mediated splicing, which involves RNA alone, there are some splicing reactions that involve RNA-protein complexes. These complexes are called small nucleus ribonucleoprotein particles, abbreviated as snRNPs. This class of splicing is a very common feature of messenger RNA (mRNA) processing in "higher" eukaryotes such as humans. It is not yet known if snRNP-mediated splicing is catalyzed by the RNA components. Note also that some RNA splicing reactions are catalyzed by enzymes made of only protein.

Catalytic Power and Specificity of Enzymes

Enzymes are extraordinary catalysts. The rate enhancements they bring about are in the range of 5 to 17 orders of magnitude .

Some rate enhancements produced by enzymes

S.No	Name of the enzyme	Rate enhancement
1	Cyclophyllin	10^5
2	Carbonic anhydrase	10^7
3	Triose phosphate isomerase	10^9
4	Carboxypeptidase A	10^{11}
5	Phosphoglucomutase	10^{12}
6	Succinyl CoA transferase	10^{13}
7	Urease	10^{14}
8	Orotidine monophosphate dehydrogenase	10^{17}

Classification of enzymes

There are six major classes of enzymes

1.OXIDO REDUCTASES – Catalyses oxidation reduction reactions.

2.TRANSFERASES – Catalyse the transfer of some group or radical R from one molecule A to another molecule B.

3.HYDROLASES – Catalyses hydrolysis of ester, ether, peptide, glycosyl, acid anhydride by the addition of water.

4.LYASES - Catalyses removal of groups from substrate by mechanism

other than hydrolysis leaving double bonds.

5.ISOMERASES – Catalyses inter conversion of optical, geometric or positional isomers.

6. LIGASES - Catalyses the linking together of two compounds couple to the breaking of a pyrophosphate bond in ATP or a similar compound.

1. OXOREDUCTASES:

A(red)+B(oxi)=A(oxi)+B(red)

Subgroups:

1. oxidass –Use oxygen as hydrogen acceptor. Eg: tryosinase,cytochrome oxidase,uricase.
2. Anaerobic dehydrogenases - Use some other substance as hydrogen acceptor. Eg : Malate dehydrogenases, succinate dehydrogenases, Lactate dehydrogenases.
3. Hydroperoxidases – use H_2O_2 as substrate. Eg peroxidase, , catalase.
4. Aerobic dehydrogenases – Use either O_2 or another substance as hydrogen acceptor. Eg D and L amino acid oxidase,Xanthine oxidase, Aldehyde oxidase.
5. oxyggenases- act on single hydrogen donors with incorporation of O_2. eg :tryptophan oxygenase.
6. hydroxylases- act on paired donors with incorporation of O_2 into one donor. Eg steroid hydroxylases, phenylalanine-4-hydroxylases.

2. TRANSFERASES (TRANSFERRING ENZYMES)

It catalyse the transfer of some group or radical R from one molecules A to another molecules B

The subgroups includes,

1. transphosphorylases- eg Hexokinase, Phospho glucomutase, Phosphoglycerate kinase.

Hexokinase

ATP + D- Hexose ↔ ADP + D – hexose – 6- phosphate

2. Transglycosidases eg., Phosphorylase
3. Transaminases eg SGOT and SGPT.
4. Transacylase eg choline acetyl transferase , aeto acetate transacetylase, amino acid transacetylase.

Choline acetyl transferase

Acetyl CoA + choline ↔ CoA + Acetyl choline

5. transmethylase

3. HYDROLASES

Catalyses hydrolysis of ester, ether, peptide, glycosyl acid anhydride by the addition of water. The group includes the extra cellular digestive enzymes and many intacellular enzymes.

The group includes the extra cellular digestive enzymes and many intra cellular enzymes.

i) Enzymes acting on glycosyl compounds. Eg., β – galactosidase

B-D-galactoside + H_2O = An alcohol + D- galactose

ii) Enzymes acting on peptide bonds – eg., pepsin, rennin, chymotrypsin.

iii) Esterases - eg., lipases, phosphatases, sulphatases

iv) Amydases - eg., urease, arginase, glutaminase

v) Hydrolytic deaminases - eg., guanine deaminase

Monomeric: eg., ribonuclease, trypsin carboxypeptidase

Oligomeric: eg., acetyl coA carboxylase, lactate dehydrogenase

4. LYASES

Catalyse the removal of groups from substrates by mechanisms other than hydrolysis leaving double bonds.

X –C-C-X = X -Y + C=C.

i) Aldehyde lyases eg., aldolase

Fructose-1-phosphate ↔ Dihydroxy acetone phosphate + glyceraldehydes

ii) Carbon oxygen lyases - eg., Fumarase

L – Malate ↔ Fumarate + H_2O

5. ISOMERASES:

Enzymes catalyzing interconversion of optical, geometric or positional isomers.

i) Racemases and epimerases eg., alanine racemase

L –Alanine ↔ D - Alanine

ii) Cis-trans isomerase eg., retinene isomerase

All trans retinene ↔ II cis retinene

iii) Enzymes catalyzing interconversion of aldoses and ketoses eg., triose phosphate isomerase

D-Glyceraldehyde-3-phosphate ↔ Dihydroxy acetone phosphate

6. LIGASES:

Enzymes catalyzing the linking together of two compounds couple to the breaking of a pyrophosphate bond in ATP or a similar compound.

i) Enzymes catalyzing formation of C –S bonds eg., succinate thiokinase

GTP + Succinate + CoA ↔ GDP + Pi + Succinyl CoA

ii) Enzymes catalyzing the formation of C-N bonds eg., glutamine synthetase

ATP + L-Glutamate + NH_4^+ ↔ADP + ortho phosphate + L-Glutamine

iii) Enzymes catalyzing formation of C-C bonds eg., Acetyl CoA carboxylase

ATP + Acetyl CoA + CO2 ↔ ADP+ Pi + malonyl CoA

Enzymes can catalyze reactions through a variety of mechanisms. Some of these include:

- Bond strain: enzymes can destabilize bonds within the substrate.
- Proximity and orientation: conformational changes in the enzyme upon substrate binding can bring reactive groups closer together or orient them so they can react.
- Proton donors and acceptors: the presence of acidic or basic groups can affect bond polarization and reaction speed.
- Electrostatic catalysis: electrostatic attractions between the enzyme and the substrate can stabilize the activated complex.
- Covalent catalysis: covalent bonding to side chains or cofactors can lower the energy of the transition state.

As such, enzymes show that evolutionary biology has produced highly effective catalysts.

There are several factors that affect the speed of a reaction:

- Nature of the reactants
- Particle size of the reactants
- Concentration of the reactants
- Pressure of gaseous reactants
- Temperature

- Catalysts

Nature of the reactants

In order for a reaction to occur, there must be a collision between the reactants at the reactive site of the molecule. The larger and more complex the reactant molecules, the less chance there is of a collision at the reactive site.Sometimes, in very complex molecules, the reactive site is totally blocked off by other parts of the molecule, so no reaction occurs. There may be a lot of collisions, but only the ones that occur at the reactive site have any chance of leading to chemical reaction.

Particle size of the reactants

Reaction depends on collisions. The more surface area on which collisions can occur, the faster the reaction. You can hold a burning match to a large chunk of coal and nothing will happen. But if you take that same piece of coal, grind it up very, very fine, throw it up into the air, and strike a match, you'll get an explosion because of the increased surface area of the coal.

Concentration of the reactants

Increasing the number of collisions speeds up the reaction rate. The more reactant molecules there are colliding, the faster the reaction will be. For example, a wood splint burns okay in air (20 percent oxygen), but it burns *much* faster in pure oxygen.

In most simple cases, increasing the concentration of the reactants increases the speed of the reaction. However, if the reaction is complex and has a complex *mechanism* (series of steps in the reaction), this may not be the case. Determining the concentration effect on the rate of reaction can give you clues as to which reactant is involved in the rate-determining step of the mechanism.

Pressure of gaseous reactants

The pressure of gaseous reactants has basically the same effect as concentration. The higher the reactant pressure, the faster the reaction rate. This is due to the increased number of collisions. But if there's a complex mechanism involved, changing the pressure may not have the expected result.

Temperature

Increasing the temperature causes molecules to move faster, so there's an increased chance of them colliding with each other and reacting. But increasing the temperature also increases the average kinetic energy of the molecules.

Catalysts

Catalysts are substances that increase the reaction rate without themselves being changed at the end of the reaction. They increase the reaction rate by lowering the activation energy for the reaction. In the preceding figure, if you shift to the left that dotted line representing the minimum amount of kinetic energy needed to provide the activation energy, then many more molecules will have the minimum energy needed, and the reaction will be faster.

Catalysts lower the activation energy of a reaction in one of two ways:

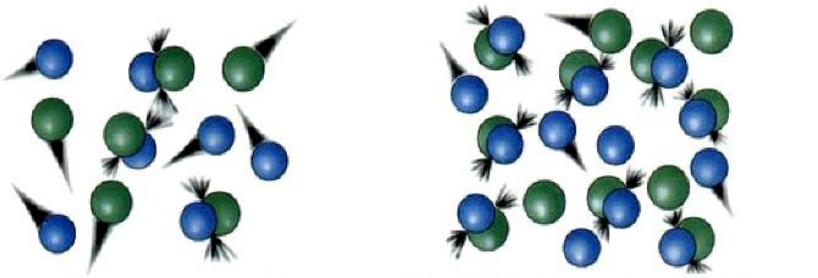
- Providing a surface and orientation
- Providing an alternative mechanism (series of steps for the reaction to go through) with a lower activation energy

Collision Theory

Collision Theory provides a qualitative explanation of chemical reactions and the rates at which they occur. A basic principal of collision theory is that, in order to react, molecules must collide. This fundamental rule guides any analysis of an ordinary reaction mechanism.

Consider the elementary bimolecular reaction: $A+B \rightarrow products$

If the two molecules A and B are to react, they must come into contact with sufficient force so that chemical bonds break. We call such an encounter a collision. If both A and B are gases, the frequency of collisions between A and B will be proportional to the concentration of each gas. If we double the concentration of A, the frequency of A-B collisions will double, and doubling the concentration of B will have the same effect. Therefore, according to collision theory, the rate at which molecules collide will have an impact on the overall reaction rate.



Low concentration = Few collisions High concentration = More collisions

Activation Energy and Temperature

When two billiard balls collide, they simply bounce off of one other. This is also the most likely outcome when two molecules, A and B, come into contact: they bounce off one another, completely unchanged and unaffected. In order for a collision to be successful by resulting in a chemical reaction, A and B must collide with sufficient energy to break chemical bonds. This is because in any chemical reaction, chemical bonds in the reactants are broken, and new bonds in the products are formed.

Therefore, in order to effectively initiate a reaction, the reactants must be moving fast enough (with enough kinetic energy) so that they collide with sufficient force for bonds to break. This minimum energy with which molecules must be moving in order for a collision to result in a chemical reaction is known as the activation energy. As we know from the kinetic theory of gases, the kinetic energy of a gas is directly proportional to temperature. As temperature increases, molecules gain energy and move faster and faster. Therefore, the greater the temperature, the higher the probability that molecules will be moving with the necessary activation energy for a reaction to occur upon collision.

Molecular Orientation and Effective Collisions

Even if two molecules collide with sufficient activation energy, there is no guarantee that the collision will be successful. In fact, the collision theory says that not every collision is successful, even if molecules are moving with enough energy.

The reason for this is because molecules also need to collide with the right orientation, so that the proper atoms line up with one another, and bonds can break and re-form in the necessary fashion. For example, in the gas-phase reaction of dinitrogen oxide with nitric oxide, the oxygen end of N2O must hit the nitrogen end of NO; if either molecule is not lined up correctly, no reaction

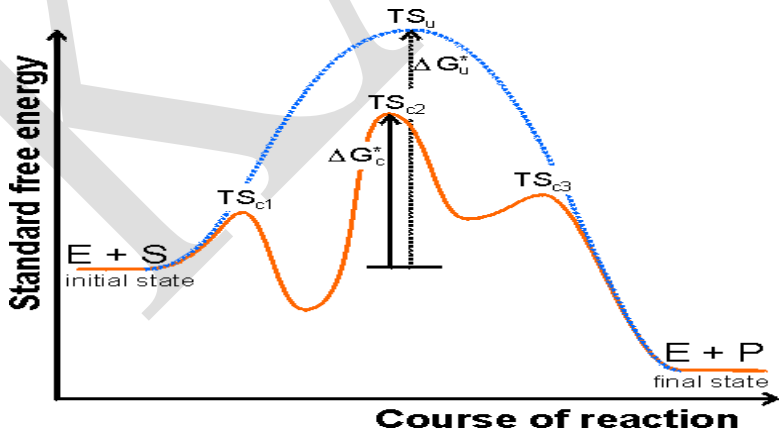
will occur upon their collision, regardless of how much energy they have. However, because molecules in the liquid and gas phase are in constant, random motion, there is always the probability that two molecules will collide in just the right way for them to react.

According to the collision theory, the following criteria must be met in order for a chemical reaction to occur:

- Molecules must collide with sufficient energy, known as the activation energy, so that chemical bonds can break.
- Molecules must collide with the proper orientation.
- A collision that meets these two criteria, and that results in a chemical reaction, is known as a successful collision or an effective collision.

Catalysis

In order for a reaction to occur, reactant molecules must contain sufficient energy to cross a potential energy barrier, the activation energy. All molecules possess varying amounts of energy depending, for example, on their recent collision history but, generally, only a few have sufficient energy for reaction. The lower the potential energy barrier to reaction, the more reactants have sufficient energy and, hence, the faster the reaction will occur. All catalysts, including enzymes, function by forming a transition state, with the reactants, of lower free energy than would be found in the uncatalysed reaction.



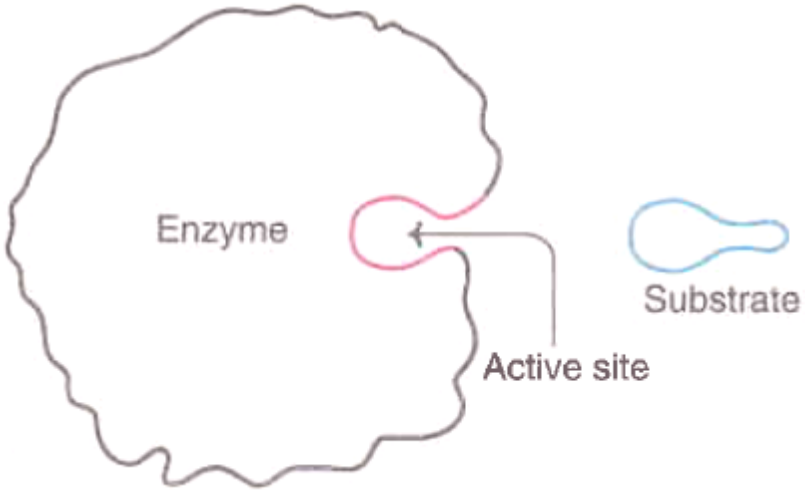
an enzyme catalysed reaction involving the formation of enzyme-substrate (ES) and enzyme-product (EP) complexes, i.e.

$$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$$

Active site:

Enzymes are big in size compared to substrates which are relatively smaller. Evidently, a small portion of the huge enzyme molecule is directly involved in the substrate binding and catalysis.

The active site (or active centre) of an enzyme represents as the small region at which the suhstrate(s) binds and participates in the analysis.



Salient features of active site

1 . The existence of active site is due to the tertiary structure of protein resulting in three dimensional native conformation.

2. The active site is made up of amino acids (known as catalytic residues)which are far from each other in the linear sequence of amino acids (primary structure of protein). For instance, the enzyme lysozyme has 129 amino acids. The active site is formed by the contribution of amino acid residues numbered 35, 52, 62, 63 and 101.

3. Active sites are regarded as clefts or crevices or pockets occupying a small region in

a big enzyme molecule.

4. The active site is not rigid in structure and shape. lt is rather flexible to promote the specific substrate binding.

5. Cenerally, the active site possesses a substrate binding site and a catalytic site. The latter is for the catalysis of the specific reaction.

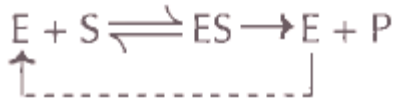
6. The coenzymes or cofactors on which some enzymes depend are present as a part of the catalytic site.

7. The substrate(s) binds at the active site by weak non covalent bonds.

8. Enzymes are specific in their function due to the existence of active sites.

9. The commonly found amino acids at the active sites are serine, aspartate, histidine, cysteine, lysine, arginine, glutamate, tyrosine etc. Among these amino acids, serine is the most frequently found.

10. The substrate (S) binds the enzyme (E) at the active site to form enzyme-substrate complex (ES). The product(P) is released after the catalysis and the enzyme is available for reuse.

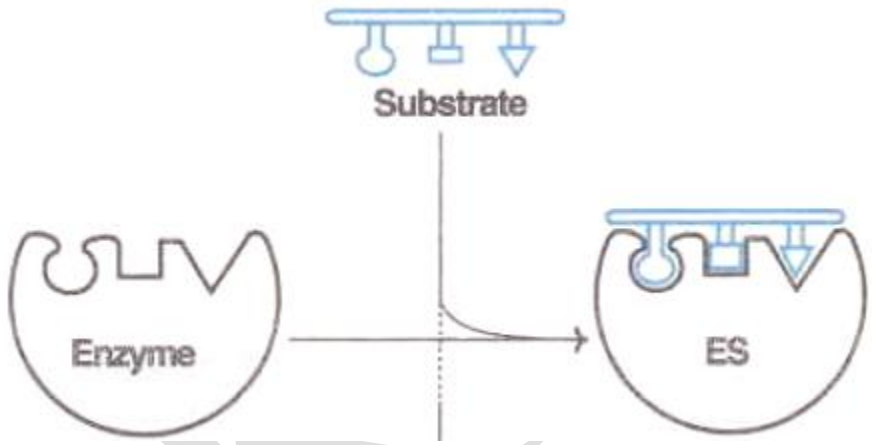


11.Those amino acid residues in the active site which do not have a binding or catalytic function may nevertheless contribute to the specificity of the enzyme. Their side chains must be of suitable size, shape and character not to interfere with the binding of the substrate, but they might interfere with the binding of other, chemically similar substances.

12. The active site often includes both polar and non polar amino acid residues, creating an arrangement of hydrophilic and hydrophobic micro environments not found elsewhere on an enzyme molecule. Thus the function of an enzyme may depend not only on the special arrangement of binding and catalytic sites but also on the environment in which these sites occur.

Lock and Key model (or) Fischer's template model:

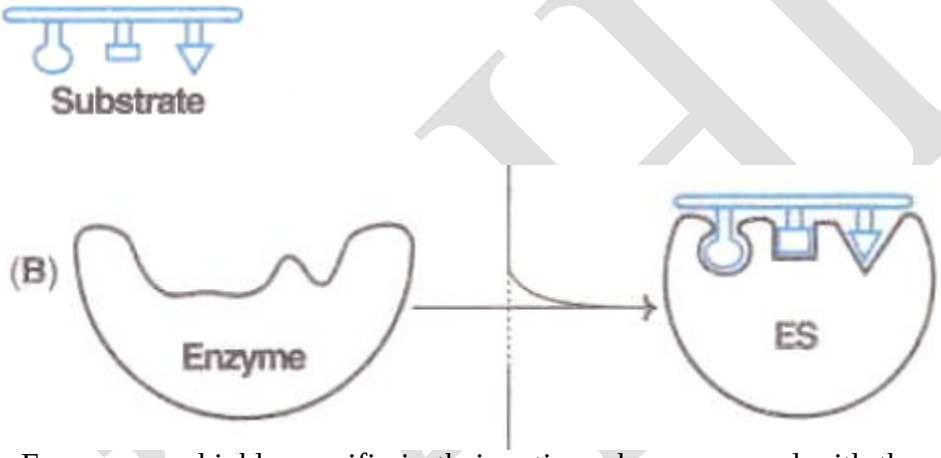
This is the first model proposed to explain an enzyme catalyzed reaction. The theory was proposed by a German Biochemist Emil Fischer. According to this model, the structure or conformation of the enzyme is rigid. The substrate fits to the binding site (active site) just as a key fits into the proper lock or a hand into the proper glove. Thus the active site of an enzyme is a rigid and pre-shaped template where only a specific substrate can bind.



This model does not give any scope for the flexible nature of enzymes, hence the model totally fails to explain many facts of enzymatic reactions the most important being the effect of allosteric modulators. Eventhough it has some limitations, this model is still useful for understanding certain properties of enzymes.

Induced fit theory or Koshland's model:

This model was proposed by Koshland in 1958. It is the more acceptable and realistic model for enzyme-substrate complex formation. As per this model, the active site is not rigid and pre-shaped. The essential features of substrate binding site are present at the nascent active site. The interaction of the substrate with the enzyme induces a fit or a conformational change in the enzyme, resulting in the formation of a strong substrate binding site. Further, due to induced fit, the appropriate amino acids of the enzymes are repositioned to form the active site and bring about the catalysis. Induced fit theory has sufficient experimental evidence from the x-ray diffraction studies. Koshland's model also explains the action allosteric modulators and competitive inhibition on enzymes



Specificity of enzymes:

Enzymes are highly specific in their action when compared with the chemical catalysts. The occurrence of thousands of enzymes in the biological system might be due to the specific nature of enzymes.

Specificity is a characteristic property of the active site.

Reaction specificity:

i) An enzyme catalyses only a very few reactions (frequently only one). Eg.,arginase, catalase and urease only attack arginine, H_2O_2 and urea respectively.

ii) Most enzymes can catalyse the same type of reaction (phosphate transfer, oxidation, reduction) with several structurally related substrates.

iii) Reactions with alternate substrates takes place if they are present in high concentration and also dependent on their affinity to enzyme. Eg Histidase acts on histidine. This enzyme acts on tryptophan when it is present in high concentration and it is also dependent on due affinity of that enzyme.

iv) An amino acid can undergo transamination, oxidative deamination, decarboxylation, racemization etc., The enzymes however are different for each of these reactions. A substrate can undergo many reactions but in a reaction specificity one enzyme can catalyse only one of the various reactions.. eg., In the presence of acetyl coA the enzyme citrate synthetase acts on or catalyse oxalo acetate to citrate. But when only oxalo acetate is present then another specific enzyme that is malate dehydrogenase will catalyse it and forms different products that is malate.

Optical specificity:

Stereo isomers are the compounds which have the same molecular formula but differ in their structural configuration.

Enzymes show absolute optical specificity for atleast a portion of substrate molecule eg., maltase catalyse the hydrolysis of α glycosides but not β glycosides, while Embden-Meyerhof and direct oxidative pathway catalyse the interconversion of D-phospho sugar but not L-phospho sugar.

There are certain exceptions

Eg., D-amino acid oxidase in kidney acts on L-amino acid.

The glycosidases which catalyse the hydrolysis of glycosidic bonds between sugars and alcohols are highly specific for the sugar portion but relatively non-specific for the alcohol portion or aglycone.

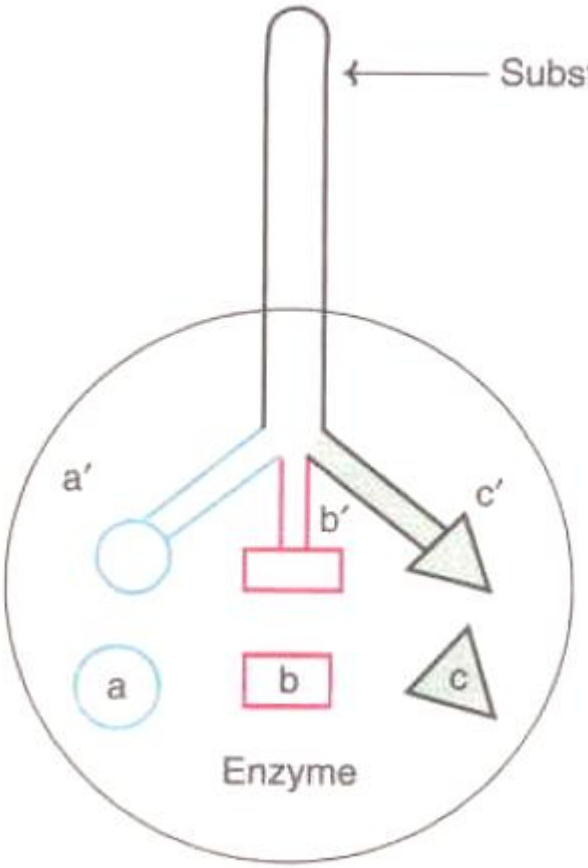
When enzyme acts on a substrate and produces one kind of isomer which will be the product. Eg., succinate dehydrogenase while acts on succinic acid will produce only fumaric acid and not malic acid which is an isomer of it.

The enzymes acts only on one isomer and therefore exhibit stereo specificity. Eg., L-amino acid oxidase and D-amino acid oxidase act on L and D amino acids respectively. For example

hexokinase acts on D-Hexoses and glucokinase acts on D-glucose. Amylase acts on α glycosidic linkages. Cellulase acts on β glycosidic bonds.

Stereo specificity is explained by considering three distinct regions of substrate molecule specifically binding with three complementary regions on the surface of the enzyme. The class of enzymes belonging to isomerases do not exhibit stereo specificity since they are specialized in the inter conversion of isomers.

Stereo specificity-three point attachment of substrate to the enzyme.



Bond specificity:

A particular enzyme acts on particular chemical bond. Eg., glycosidases on glycosides. Alcohol dehydrogenases on alcohol. Pepsin and trypsin on peptide bond. Esterases on ester linkages.

Group specificity:

A particular enzyme act on particular group. Eg., chymotrypsin hydrolyses peptide bond in which the carboxyl groups is contributed by the aromatic amino acids such as tyrosine, tryptophan etc. Carboxypeptidases split off carboxyl group. Amino peptidases split off amino group from the polypeptide chain.Oxido reductases function in biosynthetic processes (eg., fatty acid synthesis) tend to use NADPH as reductant but those which function in degradative processes tend to use NAD^+ as oxidant. In liver about 90% of the NADP specific enzyme occurs extramitochondrially. But NAD^+ specific enzyme of mitochondria is activated by ADP.

Substrate specificity:

The substrate specificity varies from enzyme to enzyme. It may be either absolute, relative or broad.

Absolute substrate specificity:

Certain enzymes act only on one substrate eg., glucokinase acts on glucose to give glucose-6-phosphate. Urease cleaves urea to ammonia and CO_2.

Relative substrate specificity:

Some enzymes act on structurally related substances. This in turn may be dependent on the specific group or a bond present. Eg., the action of trypsin and chymotrypsin. Trypsin hydrolyses peptide linkage involving arginine or lysine. Chymotrypsin cleaves peptide bonds attached to aromatic amino acids (Phenyl alanine, tyrosine, tryptophan).

Broad specificity:

Some enzymes act on closely related substrates which is commonly known as broad substrate specificity. Eg., Hexokinase acts on glucose, fructose, mannose and glucosamine and not on galactose. It is possible that some structural similarity among the first four compounds makes them a common substrate for the enzyme hexokinase.

UNIT I

Short Answers (2 marks)

1. Write a note on ribozymes.
2. Define cofactor.
3. What is meant by prosthetic group? Add a note on it.
4. Define apoenzyme.
5. Define holoenzyme.
6. Write a note on collision theory.
7. Define activation energy.
8. Define active site.
9. How are enzymes classified?
10. What is meant by specificity of enzymes?

Long Answers (8 marks)

1. Explain in detail the classification of enzymes.
2. What are the factors affecting the rate of chemical reactions? Add a note on it.
3. Discuss on the specificity of enzymes.
4. Define active site. Write its salient features.
5. Write about Fisher's Lock and Key hypothesis.
6. Explain Koshland's induced fit theory.
7. Define the following: i. cofactor ii. Apoenzyme iii. Holoenzyme

KARPAGAM UNIVERSITY
DEPARTMENT OF BIOCHEMISTRY
I B.Sc., BIOCHEMISTRY
MULTIPLE CHOICE QUESTIONS
ENZYMES-18BCU202

S.NO	QUESTION	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER
1	The inactive protein component of an enzyme is termed as	Holoenzyme	**apoenzyme**	prosthetic group	cofactor	**apoenzyme**
2	An active enzyme including the cofactor is termed as	**Holoenzyme**	apoenzyme	prosthetic group	cofactor	**Holoenzyme**
3	When a cofactor bound tightly to the enzyme it is called as	Holoenzyme	apoenzyme	**prosthetic group**	cofactor	**prosthetic group**
4	The term enzyme was first proposed by _____	**Kuhne**	Pasteur	Liebig	Sumner	**Kuhne**
5	The term enzyme was proposed by Kiihne in the year	**1878**	1864	1964	1823	**1878**
6	Who crystallized the enzyme urease first from Jack-bean extracts?	Pasteur	**Sumner**	Kiihne	Liebig	**Sumner**
7	First crystallization of urease enzyme was done in the year	1924	1933	**1926**	1864	**1926**
8	Removal of a group from substrate other than hydrolysis is	Ligases	**Lyases**	Isomerases	Hydroxylases	**Lyases**
9	The enzymes catalyse the transfer of H atom, O atom or electrons from one substrate to another is called as	**Oxido reductases**	lyases	ligases	isomerases	**Oxido reductases**
10	Transfer of H atom to O2 is catalysed by	**Oxidases**	aerobic dehydrogenases	oxigenases	hydroperoxidases	**Oxidases**
11	Transfer of H to an acceptor other than O2 is catalysed by the enzyme	Oxidases	**dehydrogenases**	oxigenases	hydroperoxidases	**dehydrogenases**
12	The conversion of isocitrate to 2 oxo glutarate is catalysed by	**Iso citrate dehydrogenase**	iso citrate lyase	oxo glutarate dehydrogenase	2 oxo acid oxidase	**Iso citrate dehydrogenase**

13	Transcarboxylase catalyses the transfer of a carboxyl group from methyl melonyl coA to	lactate	**pyruvate**	acetyl CoA	malate	**pyruvate**
14	Fe^{2+} or Fe^{3+} is required as a cofactor for all the enzyme except	cytochrome oxidase	catalase	peroxidase	**hexokinase**	**hexokinase**
15	Cu^{2+} is a cofactor for the enzyme	**cytochrome oxidase**	catalase	peroxidase	hexokinase	**cytochrome oxidase**
16	Alcohol dehydrogenase utilizes _____ as a cofactor	Mg^{2+}	**Zn^{2+}**	Fe^{2+}	Mn^{2+}	**Zn^{2+}**
17	Carbonic anhydrase uses _____ as a cofactor	Mg^{2+}	**Zn^{2+}**	Fe^{2+}	Mn^{2+}	**Zn^{2+}**
18	Mg^{2+} is a cofactor for all the following enzymes except	hexokinase	**peroxidase**	glucose-6-phosphatase	pyruvate kinase	**peroxidase**
19	Arginase enzyme requires _____ as a cofactor	**Mn^{2+}**	Mg^{2+}	Zn^{2+}	Fe^{2+}	**Mn^{2+}**
20	Ribonucleotide reductase requires _____ as a cofactor	**Mn^{2+}**	Mg^{2+}	Zn^{2+}	Fe^{2+}	**Mn^{2+}**
21	K^{+} is a cofactor for the enzyme	**pyruvate kinase**	hexokinase	peroxidase	catalase	**pyruvate kinase**
22	Ni^{2+} is a cofactor required by the enzyme	catalase	peroxidase	**urease**	arginase	**urease**
23	Mo is a cofactor for the enzyme	**dinitrogenase**	urease	arginase	pyruvate kinase	**dinitrogenase**
24	Se is a cofactor required by the enzyme	**glutathione peroxidase**	urease	arginas	pyruvate kinase	**glutathione peroxidase**
25	Thiamine pyrophosphate is involved as coenzyme in the transfer of	electrons	**aldehydes**	acyl group	CO_2	**Aldehydes**
26	Flavin adenine dinucleotide is derived from the vitamin	B_1	B_6	**B_2**	B_1	**B_{22}**
27	Coenzyme A is required for the transfer of	aldehyde	**acyl group**	CO_2	Electrons	**acyl group**
28	Pyridoxal phosphate is involved in transfer of	acyl group	CO_2	**amino group**	electron	**amino group**
29	Pyridoxal phosphate is derived from the vitamin	B_1	B_2	**B_6**	B_{12}	**B_6**
30	The coenzyme involved in one carbon group transfer is	biotin	**tetrahydro folate**	lipoic acid	coenzyme A	**tetrahydro folate**
31	The only coenzyme not required in diet is	Biotin	tetrahydrofolate	coenzyme A	**lipoic acid**	**lipoic acid**

32	CO_2 group transfer is carried out by the coenzyme	coenzyme A	**Biotin**	Lipoic acid	Tetra hydrofolate	**Biotin**
33	Transition state theory was developed by	Fischer	Koshland	**Eyring**	Pasteur	**Eyring**
34	Acids stabilize the transition state by	**donating a proton**	accepting a proton	increasing the pH	decreasing the pH	**donating a proton**
35	Bases stabilize the transition state by	donating a proton	**accepting a proton**	increasing the pH	decreasing the pH	**accepting a proton**
36	In metal ion catalysis the transition state is stabilized by _____ force with a metal ion	hydrogen bonding	vanderwaals forces	disulphide bond	**electrostatic interactions**	**electrostatic interactions**
37	Which one of the following enzyme exhibit group specificity	**Hexokinase**	Glucokinase	catalase	peroxidase	**Hexokinase**
38	L amino acid oxidase is an example for	group specificity	absolute specificity	**stereo chemical specificity**	product specificity	**stereo chemical specificity**
39	The Lock and Key hypothesis was proposed by	**Emil Fischer**	Koshland	Pasteur	Sumner	**Emil Fischer**
40	The Induced fit hypothesis was proposed by	Emil Fischer	**Koshland**	Pasteur	Sumner	**Koshland**
41	The Fischer’s Lock and Key hypothesis was proposed in the year	**1890**	1864	1872	1958	**1890**
42	The Koshland’s induced fit hypothesis was proposed in the year	**1890**	1864	1872	**1958**	**1958**

UNIT-II

SYLLABUS

Enzyme kinetics

Relationship between initial velocity and substrate concentration, steady state kinetics, equilibrium constant - monosubstrate reactions. Michaelis-Menten equation, Lineweaver-Burk plot, Eadie-Hofstee and Hanes plot. Km and Vmax, Kcat and turnover number. Effect of pH, temperature and metal ions on the activity of enzyme. Bisubstrate reactions - Types of bi bi reactions (sequential – ordered and random, ping pong reactions). Differentiating bi substrate mechanisms (diagnostic plots, isotope exchange).

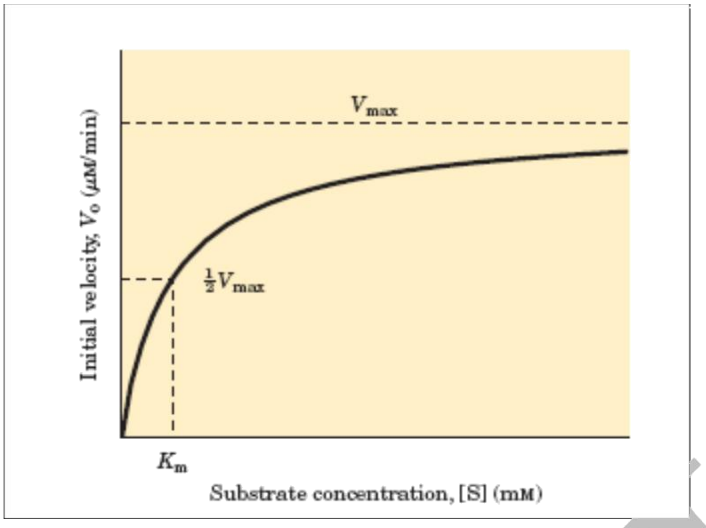
Relationship between initial velocity and substrate concentration

Enzyme kinetics

Enzyme kinetics is the central approach to study the mechanism of an enzyme catalysed reaction to determine the rate of the reaction and how it changes in response to changes in experimental parameters.

It is the oldest and most important approach to understand enzyme mechanisms. A key factor affecting the rate of a reaction catalysed by an enzyme is the concentration of the substrate [S]. Studying the effect of substrate concentration is complicated because [S] changes during the course of a reaction as substrate is converted to product. One simplified approach is measuring the initial velocity (V0), when [S] is much greater than the [E]. At relatively low concentration of substrate, V0 increases almost linearly with an increase in [S]. At higher substrate concentration V0 increases by smaller and smaller amount in response to increase in [S]. Finally a point is reached beyond which increases in V0 is vanishingly small as [S] increases. This plateau like V0 region is close to maximum velocity (Vmax).

Figure 1: Effect of substrate concentration on the initial velocity of an enzyme-catalyzed reaction



$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \qquad (6\text{–}7)$$

$$ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P \qquad (6\text{–}8)$$

substrate, so that further increases in [S] have no effect on rate. This condition exists when [S] is sufficiently high that essentially all the free enzyme has been converted to the ES form. After the ES complex breaks down to yield the product P, the enzyme is free to catalyze reaction of another molecule of substrate. The saturation effect is a distinguishing characteristic of enzymatic catalysts and is responsible for the plateau observed in Figure 6–11. The pattern seen in Figure 6–11 is sometimes referred to as saturation kinetics. When the enzyme is first mixed with a large excess of substrate, there is an initial period, the **pre–steady state,** during which the concentration of ES builds up. This period is usually too short to be easily observed, lasting just microseconds. The reaction quickly achieves a **steady state** in which [ES] (and the concentrations of any other intermediates) remains approximately constant over time. The concept of a steady state was introduced by G. E. Briggs and Haldane in 1925. The

measured *V*0 generally reflects the steady state, even though *V*0 is limited to the early part of the reaction, and analysis of these initial rates is referred to as **steady-state kinetics.**

The Relationship between Substrate Concentration and Reaction Rate

The curve expressing the relationship between [S] and Vo has the same general shape for most enzymes (it approaches a rectangular hyperbola), which can be expressed algebraically by the Michaelis-Menten equation. Michaelis and Menten derived this equation starting from their basic hypothesis that the rate limiting step in enzymatic reactions is the breakdown of the ES complex to product and free enzyme. The equation is

$$V_0 = \frac{V_{max}[S]}{K_m + [S]} \qquad (6\text{–}9)$$

The important terms are [S], *V*0, *V*max, and a constant called the Michaelis constant, *K*m. All these terms are readily measured experimentally. Here we develop the basic logic and the algebraic steps in a modern derivation of the Michaelis-Menten equation, which includes the steady-state assumption introduced by Briggs and Haldane. The derivation starts with the two basic steps of the formation and breakdown of ES (Eqns 6–7 and 6–8). Early in the reaction, the concentration of the product, [P], is negligible, and we make the simplifying assumption that the reverse reaction, P →S (described by k_{-2}) can be ignored. This assumption is not critical but it simplifies our task. The overall reaction then reduces to

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \qquad (6\text{–}10)$$

*V*0 is determined by the breakdown of ES to form product, which is determined by [ES]:

$V0 = k_2[ES]$ (6-11)

Because [ES] in Equation 6–11 is not easily measured experimentally, we must begin by finding an alternative expression for this term. First, we introduce the term [Et], representing the total enzyme concentration (the sum of free and substrate-bound enzyme). Free or unbound enzyme can then be represented by [Et] - [ES]. Also, because [S] is ordinarily far greater than [Et], the amount of substrate bound by the enzyme at any given time is negligible compared with the total [S]. With these conditions in mind, the following steps lead us to an expression for Vo in terms of easily measurable parameters.

Step 1The rates of formation and breakdown of ES are determined by the steps governed by the rate constants k_1 (formation) and $k_{-1} + k_2$ (breakdown), according to the expressions

$$\text{Rate of ES formation} = k_1([E_t] - [ES])[S] \qquad (6\text{–}12)$$

$$\text{Rate of ES breakdown} = k_{-1}[ES] + k_2[ES] \qquad (6\text{–}13)$$

Step 2 We now make an important assumption: that the initial rate of reaction reflects a steady state in which [ES] is constant—that is, the rate of formation of ES is equal to the rate of its breakdown. This is called the **steady-state assumption.** The expressions in Equations 6–12 and 6–13 can be equated for the steady state, giving

$$k_1([E_t] - [ES])[S] = k_{-1}[ES] + k_2[ES] \qquad (6\text{–}14)$$

Step 3 In a series of algebraic steps, we now solve Equation 6–14 for [ES]. First, the left side is multiplied out and the right side simplified to give

$$k_1[E_t][S] - k_1[ES][S] = (k_{-1} + k_2)[ES] \qquad (6\text{–}15)$$

Adding the term k_1[ES][S] to both sides of the equation and simplifying gives

$$k_1[E_t][S] = (k_1[S] + k_{-1} + k_2)[ES] \qquad (6\text{–}16)$$

We then solve this equation for [ES]:

$$[ES] = \frac{k_1[E_t][S]}{k_1[S] + k_{-1} + k_2} \qquad (6\text{–}17)$$

This can now be simplified further, combining the rate constants into one expression:

$$[ES] = \frac{[E_t][S]}{[S] + (k_2 + k_{-1})/k_1} \qquad (6\text{–}18)$$

The term (*k*2 _ *k*_1)/*k*1 is defined as the **Michaelis constant, *K*m.** Substituting this into Equation 6–18 simplifies the expression to

$$[ES] = \frac{[E_t][S]}{K_m + [S]} \qquad (6–19)$$

Step 4 We can now express *V*0 in terms of [ES]. Substituting the right side of Equation 6–19 for [ES] in Equation 6–11 gives

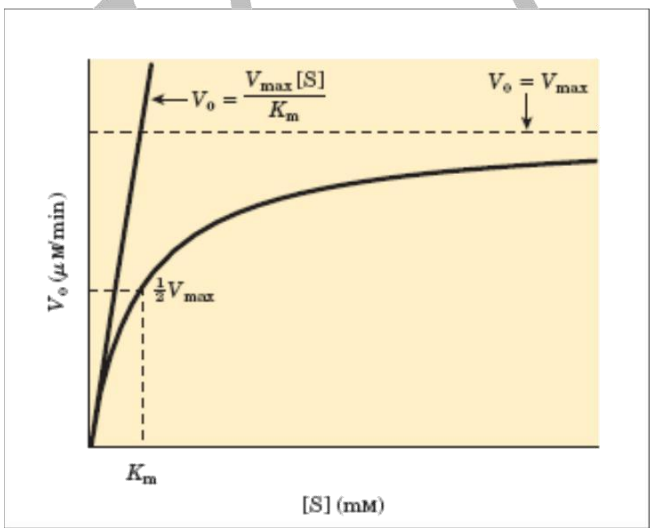
$$V_0 = \frac{k_2[E_t][S]}{K_m + [S]} \qquad (6–20)$$

This equation can be further simplified. Because the maximum velocity occurs when the enzyme is saturated (that is, with [ES] -[Et]) *V*max can be defined as k_2[Et]. Substituting this in Equation 6–20 gives Equation 6–9:

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

This is the **Michaelis-Menten equation,** the **rate equation** for a one-substrate enzyme-catalyzed reaction. It is a statement of the quantitative relationship between the initial velocity V_0, the maximum velocity *V*max, and the initial substrate concentration [S], all related through the Michaelis constant *K*m.

Figure 6-12: Dependence of initial velocity on substrate concentration.



Definition of km:

Km the Michaelis constant is given by the formula

Km =

An important numerical relationship emerges from the Michaelis-Menten equation in the special case when V_0 is exactly one-half *V*max (Fig. 6–12). Then

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]} \qquad (6\text{–}21)$$

On dividing by *V*max, we obtain

$$\frac{1}{2} = \frac{[S]}{K_m + [S]} \qquad (6\text{–}22)$$

Solving for *K*m, we get *K*m + [S] = 2[S], or

$$K_m = [S], \text{ when } V_0 = \frac{1}{2}V_{max} \qquad (6\text{–}23)$$

This is a very useful, practical definition of *K*m: *K*m is equivalent to the substrate concentration at which V_0 is one-half *V*max.

Km or Michaelis-Menton constant is defined as the substrate concentration (expressed in terms moles/litre) to produce half maximum velocity in an enzyme catalysed reaction.

It indicates that half of the enzyme molecules (that is 50%) are bound with the substrate molecules when the substrate concentration equals the km value.

Km value is a constant and a characteristic features of a given enzyme.

It is a representative for measuring the strength of [ES] complex.

A low km value indicates a strong affinity between enzyme and substrate whereas a high km value reflects a weak affinity between them. For majority of enzymes the km values are in the range of 10^{-5} to 10^{-2} moles.

Transformations of the Michaelis-Menten Equation: The Double-Reciprocal Plot (Line Weaver Burk plot)

The Michaelis-Menten equation

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

can be algebraically transformed into equations that are more useful in plotting experimental data. One common transformation is derived simply by taking the reciprocal of both sides of the Michaelis-Menten equation:

$$\frac{1}{V_0} = \frac{K_m + [S]}{V_{max}[S]}$$

Separating the components of the numerator on the right side of the equation gives

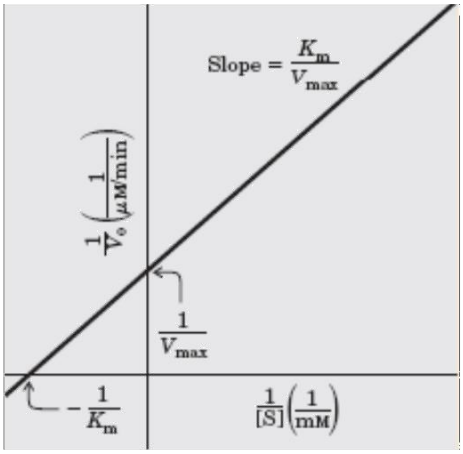
$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$

which simplifies to

$$\frac{1}{V_0} = \frac{K_m}{V_{max}[S]} + \frac{1}{V_{max}}$$

This form of the Michaelis-Menten equation is called the **Lineweaver-Burk equation.** For enzymes obeying the Michaelis-Menten relationship, a plot of $1/V_0$ versus 1/[S] (the ―double reciprocal‖ of the V_0 versus [S] plot we have been using to this point) yields a straight line. This line has a slope of *K*m/*V*max, an intercept of 1/*V*max on the $1/V_0$ axis, and an intercept of -1/*K*m on the 1/[S] axis. The double-reciprocal presentation, also called a Lineweaver-Burk plot, has the great advantage of allowing a more accurate determination of *V*max, which can only be *approximated* from a simple plot of V_0 versus [S].

The double-reciprocal plot of enzyme reaction rates is very useful in distinguishing between certain types of enzymatic reaction mechanisms and in analyzing enzyme inhibition.



Eadie-Hofstee plot:

It takes the Lineweaver-Burk equation as its starting point. Both sides of equation are multiplied by the factor V_0V_{max}

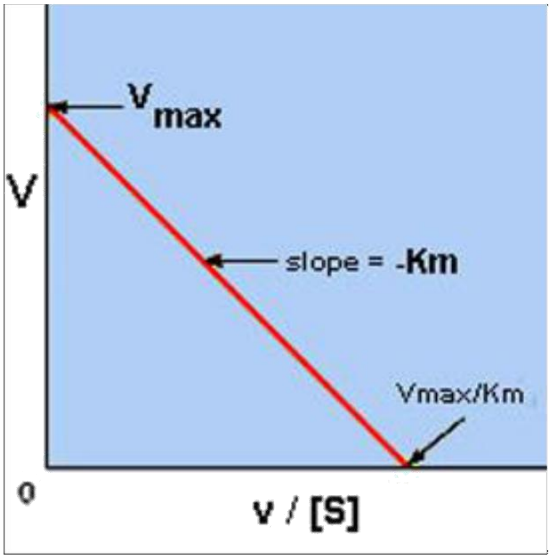
$\frac{1}{}$ Vo Vmax = $\frac{1}{}$ Vo Vmax + $\frac{1}{}$ VoVmax

Therefore,

Vo = -km[ ] + Vmax

This is also an equation of the straight line graph from which Vmax and km can be determined.

Figure: Eadie Hofstee plot



Hanes plot

Hanes–Woolf plot is a graphical representation of enzyme kinetics in which the ratio of the initial substrate concentration [S] to the reaction velocity *v* is plotted against [S]. It is based on the rearrangement of the Michaelis–Menten equation shown below:

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

where K_m is the Michaelis–Menten constant and $V_{\max}$ is the maximum reaction velocity.

The equation can be derived from the Michaelis–Menten equation as follows:

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

invert and multiply by [S]:

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

Rearrange:

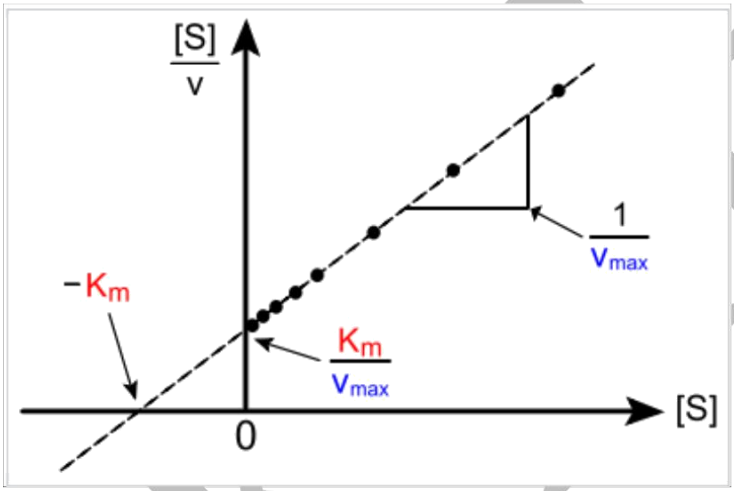
$$\frac{[S]}{v} = \frac{1}{V_{\max}}[S] + \frac{K_m}{V_{\max}}$$

As is clear from the equation, perfect data will yield a straight line of slope $1/V_{max}$, a *y*-intercept of K_m/V_{max} and an *x*-intercept of $-K_m$.

Like other techniques that linearize the Michaelis–Menten equation, the Hanes–Woolf plot was used historically for rapid determination of the important kinetic arameters K_m, V_{max} and V_{max}/K_m, but it has been superseded by nonlinear regression methods that are significantly more accurate and no longer computationally inaccessible. It remains useful, however, as a means to present data graphically.

One drawback of the Hanes–Woolf approach is that

neither ordinate nor abscissa represent independent variables: both are dependent on substrate concentration. As a result, the typical measure of goodness of fit, the correlation coefficient *R*, is not applicable.



Turnover number

Turnover number (k_{cat}) is defined as the maximum number of molecules of substrate that an enzyme can convert to product per catalytic site per unit of time and can be calculated as follows:

$K_{cat} = V_{max}/[E]_t$

For example, carbonic anhydrase has a turnover number of 400,000 to 600,000 s^{-1}, which means that each carbonic anhydrase molecule can produce upto 600,000 molecules of product per second.

Factors affecting enzyme activity

The contact between the enzyme and substrate is the most essential pre-requisite for enzyme activity. The important factors that influence the velocity of the enzyme reaction are as follows:

1. **Effect of temperature**

Velocity of an enzyme reaction increases with increase in temperature up to a maximum and then declines. A bell-shaped curve is usually observed.

Temperature coefficient or Q_{10} is defined as increase in enzyme velocity when the temperature is increased by 10ºC. For a majority of enzymes, Q_{10} is 2 between 0ºC and 40ºC. Increase in temperature results in higher activation energy of the molecules and more molecular (enzyme and substrate) collision and interaction for the reaction to proceed faster.

The optimum temperature for most of the enzymes is between 40ºC and 45ºC. However, a few enzymes (e.g. venom phosphokinases, muscle adenylate kinase) are active even at 100ºC. Some plant enzymes like urease have optimum activity around 60ºC. This may be due to very stable structure and conformation of these enzymes.

In general, when the enzymes are exposed to a temperature above 50ºC, denaturation leading to derangement in the native (tertiary) structure of the protein and active site are seen. Majority of the enzymes become inactive at higher temperature (above 70ºC).

It is worth noting here that the enzymes have been assigned optimal temperatures based on the laboratory work. These temperatures, however, may have less relevance and biological significance in the living system.

2. **Effect of pH**

Increase in the hydrogen ion concentration (pH) considerably influences the enzyme activity and a bell-shaped curve is normally obtained. Each enzyme has an optimum pH at which the velocity is maximum. Below and above this pH, the enzyme activity is much lower and at extreme pH, the enzyme becomes totally inactive.

Most of the enzymes of higher organisms show optimum activity around neutral pH (6-8). There are however, many exceptions like pepsin (1-2), acid phosphatase (4-5) and alkaline phosphatase (10-11). Enzymes from fungi and plants are most active in acidic pH (4-6).

Hydrogen ions influence the enzyme activity by altering the ionic charges on the amino acids (particularly at the active site), substrate, ES complex etc.

3. Effect of activators

Some of the enzymes require certain inorganic metallic cations like Mg^{2+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Na^{2+}, K^{+} etc. for their optimum activity. Rarely, anions are also needed for enzyme activity e.g. chloride ion (Cl^{-}) for amylase. Metals function as activators of enzyme velocity through various mechanisms-combining with the substrate, formation of ES-metal complex, direct participation in the reaction and bringing a conformational change in the enzyme.

Two categories of enzymes requiring metals for their activity are distinguished

(a) Metal-activated enzymes: The metal is not tightly held by the enzyme and can be exchanged easily with other ions e.g. ATPase (Mg^{2+} and Ca^{2+}); Enolase (Mg^{2+}).

(b) Metalloenzymes: These enzymes hold the metals rather tightly which are not readily exchanged. E.g. alcohol dehydrogenase, carbonic anhydrase, alkaline phosphatase, carboxypeptidase and aldolase contain zinc. Phenol oxidase (copper); pyruvate oxidase (manganese); xanthine oxidase (molybdenum); cytochrome oxidase (iron and copper).

REACTIONS INVOLVING 2 SUBSTRATES (BISUBSTRATE REACTIONS)

Approximately 60% of enzyme-catalyzed reactions have 2 substrates & 2 products

bisubstrate reaction:

$S_1 + S_2 <==> P_1 + P_2$

There are 2 different types of bisubstrate kinetic mechanisms:

- sequential (single displacement) reactions, which can be of either of 2 subtypes: ordered sequential and random sequential
- "ping-pong" (double displacement) reactions

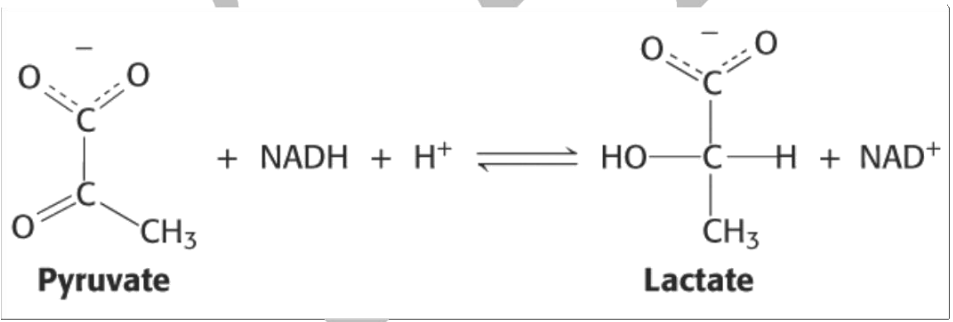
Sequential kinetic mechanisms (single displacement reactions):

- All substrates must bind to enzyme before any product is released.
- A ternary complex (3 components: E, S_1 and S_2, all bound in same E•S_1 •S_2 complex) must form before any chemistry can occur.
- 2 sub-types, depending on whether the substrates can bind randomly or must bind in a required order:

- ordered sequential: substrate binding has to occur in a certain order to form the ternary complex
- random sequential: the substrates can bind randomly -- doesn't matter which one binds first on the way to forming the ternary complex

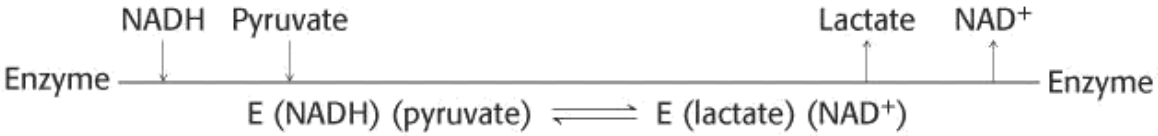
Ordered sequential kinetic mechanisms (single displacement reactions)

- Second substrate's binding site isn't there or isn't available until first substrate binds.
- e.g., many enzymes that use the coenzyme (cosubstrate) NAD+/NADH, such as lactate dehydrogenase, which catalyzes a redox reaction (so it's an oxidoreductase)
- the coenzyme (cosubstrate) has to bind first, and the other substrate then can bind
- product release is also *ordered*: the other product is released first, and other form of the coenzyme is released last.
- EXAMPLE: the **lactate dehydrogenase** reaction
- a very important enzyme in glucose metabolism -- reversible reaction in which **pyruvate** (an *-keto* acid) is **reducedto lactate** (the corresponding *-hydroxy* acid); the **2-electron donor** is the coenzyme **NADH**; the products are the reduced product (lactate) and the **oxidized coenzyme, NAD$^+$**
- an ***ordered bi bi*** reaction



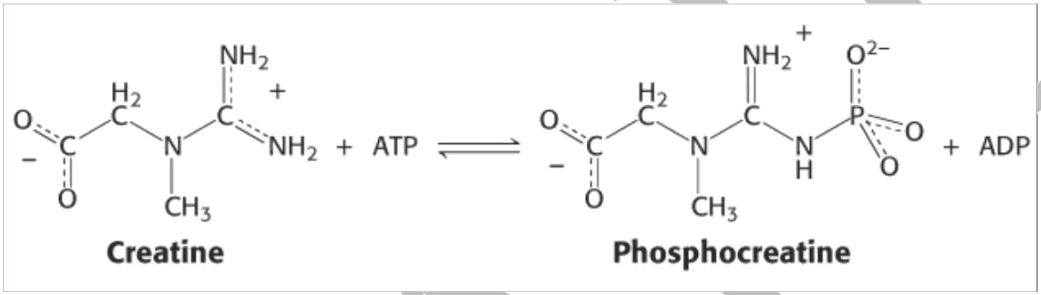
Kinetic mechanism of the LDH reaction (modified Cleland notation):

Is a ternary complex formed?



Random sequential kinetic mechanisms

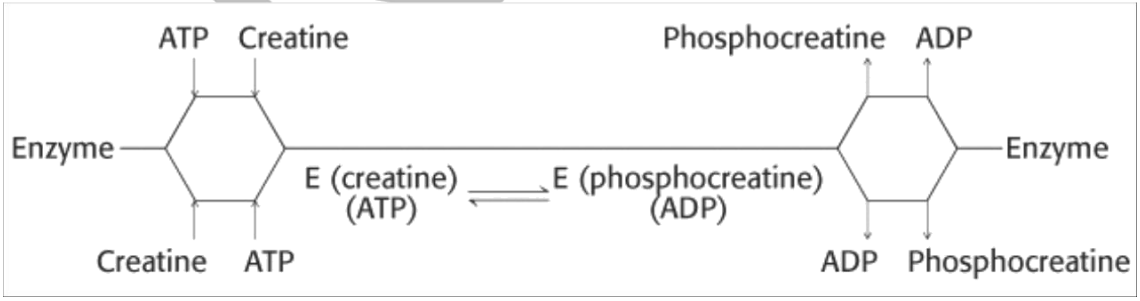
- Both substrates' binding sites are available on the free enzyme.
- EXAMPLE: the **creatine kinase** reaction (phosphoryl group transfer from ATP to creatine, or from phosphocreatine to ADP)
- a ***random bi bi*** reaction



Kinetic mechanism of the creatine kinase reaction (modified Cleland notation):

Is a ternary complex formed?

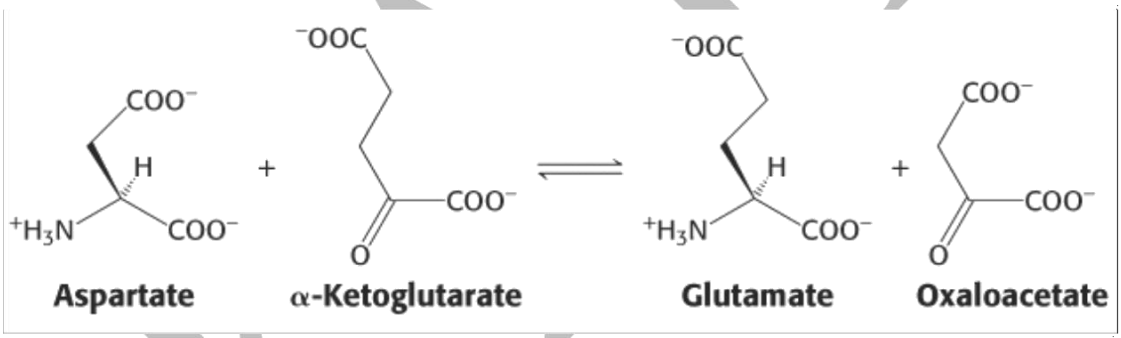
Either creatine can bind first and then ATP, or vice versa; likewise, the order of product release is *random.*



Ping pong kinetic mechanisms (double displacement reactions)

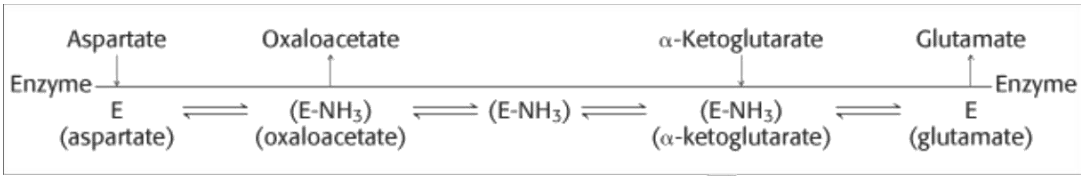
- NO ternary complex is formed.

- One or more products are released before all substrates have been added.
- Substrates don't react directly with each other in active site of enzyme.
- 1st substrate binds and reacts with enzyme, converting enzyme to another stable enzyme form (E'), a CHEMICALLY modified form of the enzyme
- 1st product (the "remains" of first substrate) is released
- 2nd substrate binds to E' and reacts with E', forming 2nd product and regenerating original stable enzyme form (E)
- 2nd product is released
- There are thus 2 HALF REACTIONS in the kinetic mechanism.
- Example: the aspartate aminotransferase reaction (one of a number of metabolically very important aminotransferase reactions (enzymes sometimes called transaminases), that all use the coenzyme pyridoxal phosphate (PLP)



- a ping pong bi bi reaction
- The amino group donor substrate (an -AMINO acid) transfers its amino group to PLP and the resulting product, an -KETO acid, dissociates from the "modified enzyme"
- The second substrate, a different -keto acid, which is to receive the amino group, binds to the modified enzyme, and the coenzyme transfers the amino group to the recipient - keto acid, generating the second product of the reaction, a different -amino acid.
- kinetic mechanism of aspartate aminotransferase
- Is a ternary complex formed?

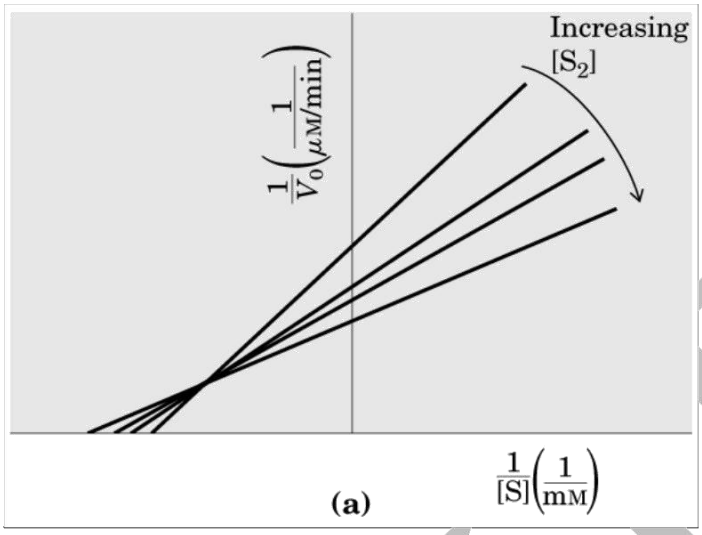
- Identify the 2 half reactions, connected by the modified enzyme form, E-NH_3 (the amino group is actually "attached" to the pyridoxal cofactor on the enzyme.)



Double reciprocal plots for bisubstrate reactions (S_1 + S_2 --> product(s))

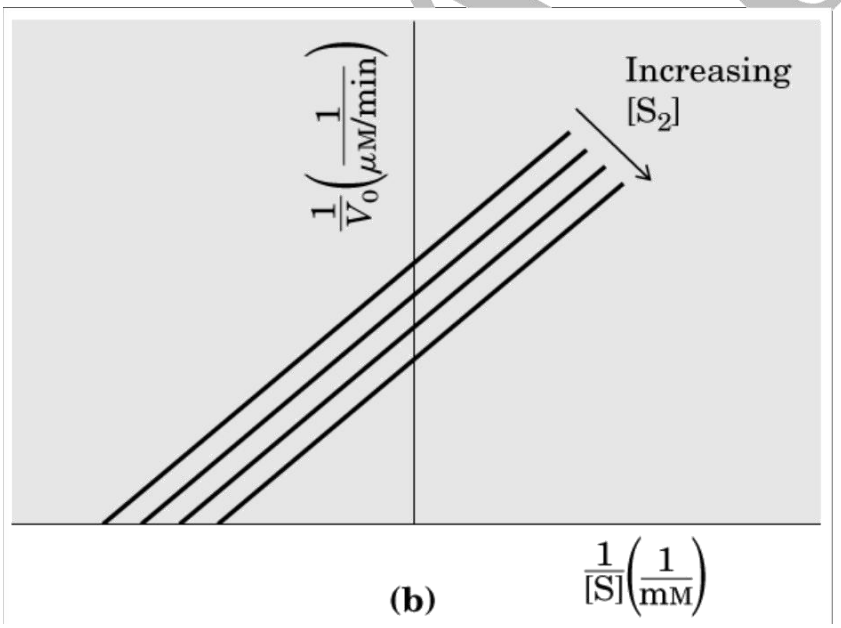
- Concentration of substrate 1 is varied while the concentration of S_2 is held constant and velocities are measured.
- This is repeated for several concentrations of S_2.
- 1/velocity as a function of $1/S_1$ is plotted as a straight line (Lineweaver-Burk/double reciprocal plot) for each concentration of S_2, generating several separate lines.
- Pattern of those lines, specifically whether or not they intersect permits identification of the kinetic mechanism as sequential or ping-pong.
- Intersecting lines ($1/v_o$ vs. $1/S_1$, with a different line for each concentration of S_2) are diagnostic of a sequential kinetic mechanism.

Fig. Intersecting lines indicate that a ternary complex is formed in the reaction.



- *Parallel* lines ($1/v_o$ vs. $1/S_1$, with a different line for each concentration of S_2) are diagnostic of a ping-pong kinetic mechanism.

Fig. Parallel lines indicate a ping-pong (double displacement) pathway. (No ternary complex is formed in the reaction.)



- True V_{max} for bisubstrate reactions is observed only at SATURATING concentrations of BOTH S_1 and S_2, and the true Km value for one substrate is that required to give 1/2 V_{max} when the other substrate is saturating.

Isotope exchange

The mechanism of the reaction catalyzed by adenosine triphosphate: creatine phosphotransferase has been studied by measuring the initial velocities of the exchange with isotopically labeled substrates. The rates of the creatine-phosphocreatine, ATP-ADP, and ADP-ATP exchanges at equilibrium are approximately equal and dependent on the concentrations of Mg^{2+} and ADP^{3-}. The ADP-ATP exchange rate increases hyperbolically to a maximum value as the concentration of the creatine-phosphocreatine pair is raised while the creatine-phosphocreatine exchange rate increases initially and then decreases with increasing concentrations of the MgATP-MgADP pair. The decrease in the exchange rate was shown to be due to the inhibitory effect of NaCl which is introduced when the reactants are formed from $MgCl_2$ and the sodium salts of the nucleotides. The formation of a dead end enzyme-MgADP-creatine complex has been confirmed, but the experimental data were not in accord with the formation of a dead end enzyme-MgATP-phosphocreatine complex. These data confirm the results from initial velocity and product inhibition studies which indicate that the mechanism of the reaction is rapid equilibrium, random.

Short Answers (2 marks)

1. Write Michaelis menton equation.
2. Write a note on Km.
3. What is meant by Vmax? Add a note on it.
4. Derive Eadie and Hofstee plot.
5. Derive Hanes plot.
6. Define turnover number.
7. What is meant by bi substrate reaction?.
8. Write the types of bi substrate reaction
9. What is the effect of Ph on enzyme catalysed reaction.
10. What is the effect of temperature on enzyme catalysed reaction.
11. What is the effect of metal ions on enzyme catalysed reaction?
12. Write a note on isotope exchange.
13. Differentiate the two types of bi substrate reactions.
14. What is meant by single displacement reaction?
15. Write a note on double displacement reaction.

Long Answers (8 marks)

16. Derive Michaelis Menton equation for single substrate reaction.
17. Write the transformation of MM equation.
18. What are the factors affecting enzyme catalysed reaction? Explain.
19. Explain the types of bisubstrate mechanism.
20. How will you differentiate bi substrate mechanism? Explain.
21. Add a note on the following: i. Km ii. Vmax iii. Kcat

Karpagam academy of higher education
Department of biochemistry
Enzymes (18BCU202) MCQ
UNIT II

S.NO	QUESTION	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER
1	The Vo against substrate concentration plot	parabola	**hyperbola**	sigmoidal	linear	**hyperbola**
2	The substrate concentration of the	the molecular	**km value**	isoelectric Ph	dissociation	**km value**
3	The Michaelis-Menton hypothesis	postulates the	enables us to	states that rate	**states that**	**states that the**
4	Km and reaction rate are	**directionally**	inversely	independent with	same	**directionally**
5	When the substrate concentration equals Km in an enzyme-catalysed reaction	A few of the enzyme molecules are present as ES	Majority of the enzyme molecules are	**Half of the enzyme molecules are**	All of the enzyme molecules are	**Half of the enzyme molecules are present as ES**
6	When the velocity of an enzymatic reaction equals Vmax, Substrate concentration is	Half of Km	Equal of Km	Twice the Km	**Far above the Km**	**Far above the Km**
7	When the velocity of an enzymatic reaction is half of Vmax	substrate concentration is half	**substrate concentration is**	substrate concentration is	substrate concentration is	**substrate concentration is**
8	In lineweaver-Burk plot, the y-intercept represents	Vmax	**1/Vmax**	Km	1/Km	**1/Vmax**
9	In lineweaver-Burk plot, the x-intercept represents	Vmax	Km	reciprocal of Vmax	**reciprocal of Km**	**reciprocal of Km**

10	If the substrate concentration is much below the Km of the enzyme, the velocity of the reaction is	**directly proportional to substrate**	not affected by enzyme concentration	nearly equal to Vmax	inversely proportional to substrate	**directly proportional to substrate**
11	The slope of Lineweaver Burk plot for Michaelis Menton equation is	**Vmax/Km**	Km/Vmax	1/Km	Km **.** Vmax	**Vmax/Km**
11	Which of the following statements about the active site of an enzyme is correct?	The active site of an enzyme binds the substrate of the reaction it catalyses more tightly than it	**The active site of an enzyme binds the substrate of the reaction it**	The active site of an enzyme binds the product of the reaction it catalyses more	The active site of an enzyme is complementary to the substrate	**The active site of an enzyme binds the substrate of the reaction it**
12	Sucrose phosphorylase reaction occurs via	sequential	compulsory	random ordered	**ping pong**	**ping pong**
13	Maltose phosphorylase reaction occurs via	**sequential**	non sequential	allosteric	ping pong	**sequential**
14	Sequential mechanism is a	double displacement	**single**	both a and b	neither a nor b	**single**
15	The enzyme lactate dehydrogenase follows	**ordered sequential kinetic mechanism**	random sequential kinetic	non sequential kinetic	ping pong kinetic	**ordered sequential kinetic**
16	Non sequential mechanism is a	**double displacement**	single displacement	both a and b	neither a nor b	**double displacement**
17	The Michaelis Menton equation states that V_0 is equal to	Vmax x [S] / Km	**Vmax x [S] / Km + [S]**	Vmax / Km + [S]	Vmax x 2[S] / Km + [S]	**Vmax x [S] / Km + [S]**
18	The slope of Hanes plot is	**1/Vmax**	Vmax	Vmax/2	-1/Km	**1/Vmax**
19	The x intercept in Eadie and Hofstee plot is	Vmax	**Vmax/Km**	–Km	-1/Km	**Vmax/Km**
20	The y intercept in Eadie and Hofstee plot is	**Vmax**	Vmax/Km	-Km	-1/Km	**Vmax**
21	The slope of Eadie and Hofstee plot is	Vmax	Vmax/Km	**–Km**	-1/Km	**–Km**
22	The y intercept in Hanes plot is	Vmax	**Km/Vmax**	–Km	-1/Km	**Km/Vmax**

23	The transformation of MM equation is otherwise called as	Hanes plot	**Line Weaver Burk plot**	Eadie and Hofstee plot	Cornish Bowden plot	**Line Weaver Burk plot**
24	Two substrate two product reaction is otherwise called as	Bi reaction	**bi bi reaction**	secondary reaction	quaternary reaction	**bi bi reaction**
25	Ternary complex is formed in all except	sequential mechanism	random ordered mechanism	compulsory ordered	ping pong mechanism	
26	Both the substrates bind together with the enzyme before the first product is released out in	sequential mechanism	non sequential mechanism	ping pong mechanism	both a and b	
27	Ping pong mechanism is	sequential mechanism	**non sequential mechanism**	random ordered mechanism	compulsory ordered	**non sequential mechanism**
28	The enzyme activity will be maximum at its	acidic pH	basic pH	neutral pH	**optimum pH**	**optimum pH**
29	The enzyme activity will be maximum at its	**optimum**	40°C	-20°C	60°C	**optimum**
30						
31						
32						
33						
34						

UNIT-III

SYLLABUS

Mechanism of action of enzymes and Enzyme inhibition

Mechanism of action of enzymes - General features - proximity and orientation, strain and distortion, acid base and covalent catalysis (chymotrypsin, lysozyme). Metal activated enzymes and metalloenzymes, transition state analogues. Enzyme Inhibition - Reversible inhibition (competitive, uncompetitive, non-competitive, mixed and substrate). Mechanism based inhibitors - antibiotics as inhibitors.

Proximity and orientation

Proximity:

Reaction between bound molecules doesn't require an improbable collision of 2 molecules -- they're already in "contact" (increases the local concentration of reactants)

Orientation:

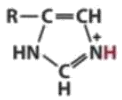
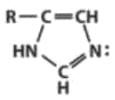
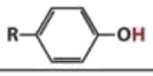
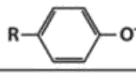
Reactants are not only near each other on enzyme, they're oriented in optimal position to react, so the Improbability of colliding in correct orientation is taken care of

Acid-base catalysis

Very often-used mechanism in enzyme reactions, e.g., hydro lysis of ester/ peptide bonds, phosphate group reactions, addition to carbonyl groups, etc

The mechanism of acid- and base-catalyzed reactions is explained in terms of the Brønsted–Lowry concept of acids and bases as one in which there is an initial transfer of protons from an acidic catalyst to the reactant or from the reactant to a basic catalyst. In terms of the Lewis theory of acids and bases, the reaction entails sharing of an electron pair donated by a base catalyst or accepted by an acid catalyst.

Amino acids in general acid-base catalysis

Amino acid residues	General acid form (proton donor)	General base form (proton acceptor)
Glu, Asp	R—COOH	R—COO⁻
Lys, Arg	R—⁺NH(H)—H	R—NH₂
Cys	R—SH	R—S⁻
His		
Ser	R—OH	R—O⁻
Tyr		

- Many organic reactions are promoted by proton donors (general acids) or proton acceptors (general bases).
- The active sites of some enzymes contain amino acid functional groups, that can participate in the catalytic process as proton donors or proton acceptors.

Acid catalysis is employed in a large number of industrial reactions, among them the conversion of petroleum hydrocarbons to gasoline and related products. Such reactions include decomposition of high-molecular-weight hydrocarbons (cracking) using alumina–silica catalysts (Brønsted–Lowry acids), polymerization of unsaturated hydrocarbons using sulfuric acid or hydrogen fluoride (Brønsted–Lowry acids), and isomerization of aliphatic hydrocarbons using aluminum chloride (a Lewis acid).

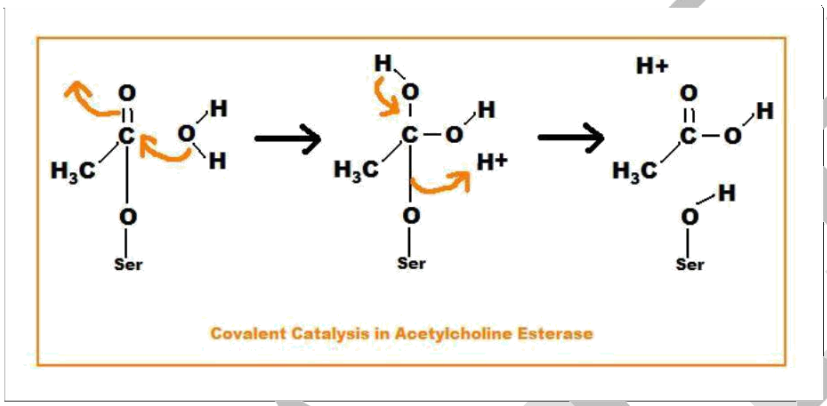
Covalent Catalysis

Covalent Catalysis is one of the four strategies that an enzyme will employ to catalyze a specific reaction. Covalent catalysis occurs when the substrate(s) in an enzymatic reaction become temporarily covalently attached to the enzyme during the catalytic reaction. In this reaction the enzyme contains a reactive group, usually a nucleophilic residue which reacts with the substrate through a nucleophilic attack. This is usually carried out by pyridine, which is a better nucleophile than water that has a pKa of 5.5. The charge loss in the reaction during transitional state will then cause hydrolysis to accelerate. The residue becomes covalently attached to the substrate throughout the catalytic reaction adding an additional intermediate which helps stabilize later transition states by lowering the activation energy. The covalent bond is then broken to regenerate enzymes.

Examples of Enzymes that Participate in Covalent Catalysis

Examples of enzymes that participate in covalent catalysis include the proteolytic enzyme Chymotrypsin and trypsin in which the nucleophlie is the hydroxyl group on the serine. Chymotrypsin is a degradative protease of the digestive system. It catalyzes the cleavage of peptide bonds that are adjacent to large aromatic or nonpolar residues. It cleaves the peptide bond on the carboxyl terminus side of the protein. The chymotrypsin has three main catalytic

residues termed as the catalytic triad. These are His 57, Asp 102 and Ser 195. Upon deprotonation the serine residue becomes a powerful nucleophile due to its alkoxide that will attack the relatively unreactive carbon of the carbonyl in the protein.



Metal activated enzymes

Enzyme-activated MR contrast agents are compounds that cause a detectable change in image intensity when in the presence of the active form of a certain enzyme. This makes them useful for in vivo assays of enzyme activity. They are distinguished from current, clinical MR contrast agents that give only anatomical information,such as aqueous gadolinium compounds, by their ability to make molecular processes visible. Enzyme-activated contrast agents are powerful tools for molecular imaging. To date, β-galactosidase-activated contrast agents have attracted the most attention in the literature, although there no theoretical reason that other enzymes could not be used to activate contrast agents. Also, mechanisms other than enzyme activation, such as Ca2+-dependent activation, can theoretically be used.

In general, enzyme-activated agents contain a paramagnetic metal ion which can affect the T1 or T2 relaxation times for nearby water molecules. However, the metal ions are unable to interact with the water until an enzyme-catalyzed reaction takes place. Steric hindrance or coordination with other ions prevents water from accessing the paramagnetic center prior to the enzymatic reaction.

Metalloenzyme

An enzyme that contains a metal ion, usually held by co-ordinate-covalent bonds on the amino acid side chains, or bound to a prosthetic group (e.g., heme). The metal ions function like coenzymes, imparting activity to the enzymes.

Examples

Alcohol dehydrogenase (zinc), ascorbic acid oxidase (copper), cytochrome (iron), cytochrome oxidase (copper), glutamate mutase (cobalt), glutathione peroxidase (selenium), urease (nickel), and xanthine oxidase (molybdenum).

Transition state analogues

Transition states are the balance point of catalysis. Bonds are partially made and/or broken at the transition state, and the energy of the extended system provides near-equal probability that the system forms products or reverts to reactants. Enzymatic catalytic sites provide dynamic electronic environments that increase the probability that the transition state will be formed. Alignment of reactants in the Michaelis complex and motion of the catalytic site architecture are necessary to achieve the transition state. Transition state lifetimes are a fraction of a picosecond, preventing chemical equilibrium in extended covalent systems. Thus, dynamic descriptions of enzymatic transition states are required. Stable analogues similar to the transition state capture dynamic excursions that generate the transition state and convert them into thermodynamic binding energy. These analogues bind with extraordinary affinity relative to reactants.

Enzyme inhibition

Enzyme inhibitor is defined as a substance which binds with the enzyme and brings about a decrease in catalytic activity of that enzyme. This inhibitor may be organic or inorganic in nature. There are three broad categories of enzyme inhibition.

(i) Reversible inhibition

(ii) Irreversible inhibition

(iii) Allosteric inhibition

Reversible inhibition

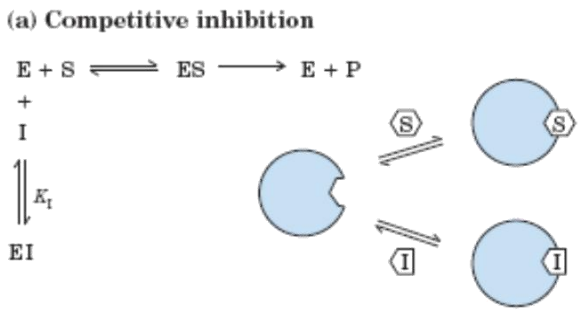
The inhibitor binds non covalently with enzyme and the enzyme inhibition can be reversed if the inhibitor is removed.

The reversible inhibition is further subdivided into:

(i) Competitive inhibition

(ii) Non competitive inhibition

(iii) Uncompetitive inhibition



The inhibitor I which closely resembles the real substrate (S) is regarded as a substrate analogue. The inhibitor competes with substrate and binds at the active site of the enzyme but does not undergo any catalysis. As long as the competitive inhibitor holds the active site, the enzyme is not available for the substrate to bind. During the reaction, ES and EI complexes are formed. The relative concentration of the substrate and inhibitor and their respective affinity with the enzyme determines the degree of competitive inhibition. The inhibition could be overcome by a high substrate concentration. In competitive inhibition, the km value increases whereas Vmax remains unchanged.

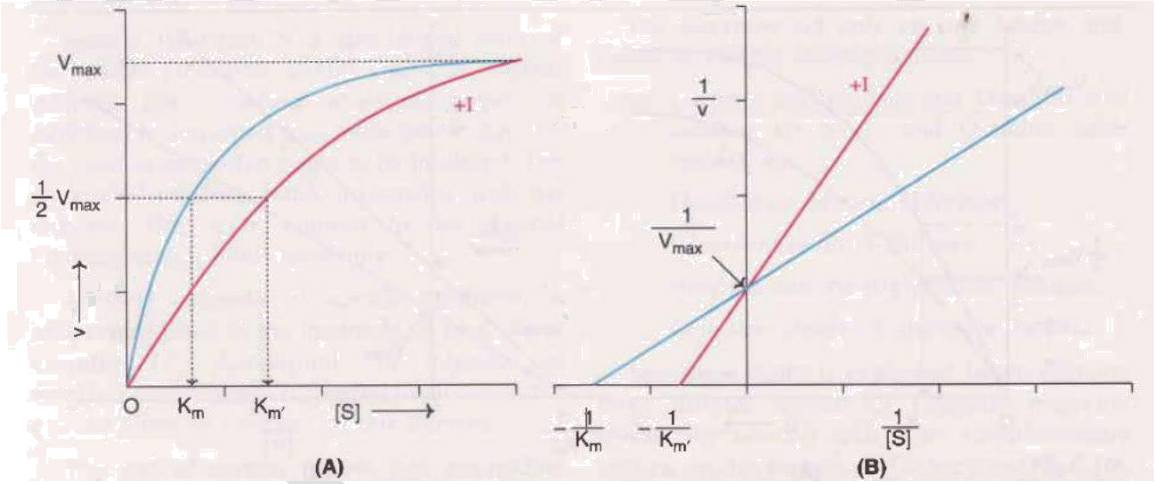
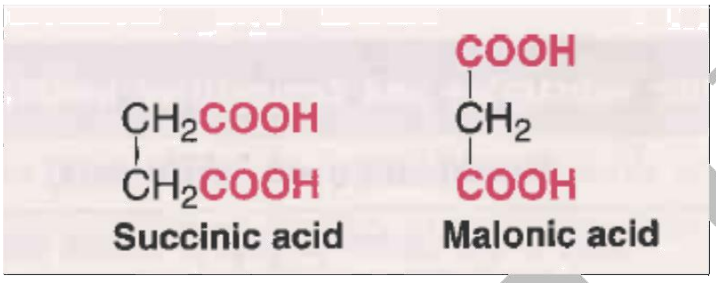


Figure: Effect of competitive inhibitor (I) on enzyme velocity (A) Velocity (v) versus substrate (S) plot. (B) Lineweaver-Burk ptot (Red lines with inhibitor; campetitive inhibitor increases Km, unalters Vmax..

The enzyme succinate dehydrogenase (SDH) is a classical example of competitive inhibition with succinic acid as its substrate. The compounds, namely, malonic acid, glutaric acid

and oxalic acid, have structural similarity with succinic acid and compete with the substrate for binding at the active site of SDH.



Methanol is toxic to the body when it is converted to formaldehyde by the enzyme alcohol dehydrogenase (ADH). Ethanol can compete with methanol for ADH. Thus, ethanol can be used in the treatment of methanol poisoning.

Some more examples of the enzymes with substrates and competitive inhibitors (of clinical and pharmacological significance) are as follows:

Table: Selected examples of enzymes with their respective substrates and competitive inhibitors

Enzyme	Substrate	Inhibitor(s)	Significance of inhibitor(s)
Xanthine oxidase	Hypoxanthine xanthine	Allopurinol	Used in the control of gout to reduce excess production of uric acid from hypoxanthine.
Monoamine oxidase	Catecholamines (epinephrine, norepinephrine)	Ephedrine amphetamine	Useful for elevating catecholamine levels
Dihydrofolate reductase	Dihydrofolic acid	Aminopterin, Amethopterin	Employed in the treatment of leukemia and other cancers.

		methotrexate,	
Acetylcholine esterase	**acetylcholine**	**Succinyl choline**	**Used in surgery for muscle relaxation, in anaesthetized patients**
Dihydropteroate synthase	**Para aminobenzoic acid (PABA)**	**Sulfonilamide**	**Prevents bacterial synthesis of folic acid**
Vitamin K epoxide reductase	**Vitamin K**	**Dicumarol**	**Acts as an anticoagulant**
HMG CoA reductase	**HMG CoA**	**Lovastatin, compactin**	**Inhibit cholesterol biosynthesis**

Antimetabolites:

These are the chemical compounds that block the metabolic reactions by their inhibitory action on enzymes. Antimetabolites are usually structural analogues of substrates and thus are competitive inhibitors. They are in use for cancer therapy, gout etc. The term antivitamins is used for the antimetabolites which block the biochemical actions of vitamins causing deficiencies, e.g., sulphonilamide, dicumarol.

Non-competitiveinhibition :

The inhibitor binds at a site other than the active site on the enzyme surface. This binding impairs the enzyme function. The inhibitor has no structural resemblance with the substrate. However, there usually exists a strong affinity for the inhibitor to bind at the second site. In fact, the inhibitor does not interfere with the enzyme-substrate binding. But the catalysis is prevented possibly due to a distortion in the enzyme conformation.

The inhibitor generally binds with the enzyme as well as the ES complex. The overall

relation in non-competitive inhibition is represented below

For non-competitive inhibition, the Km value is unchanged while Vmax is lowered

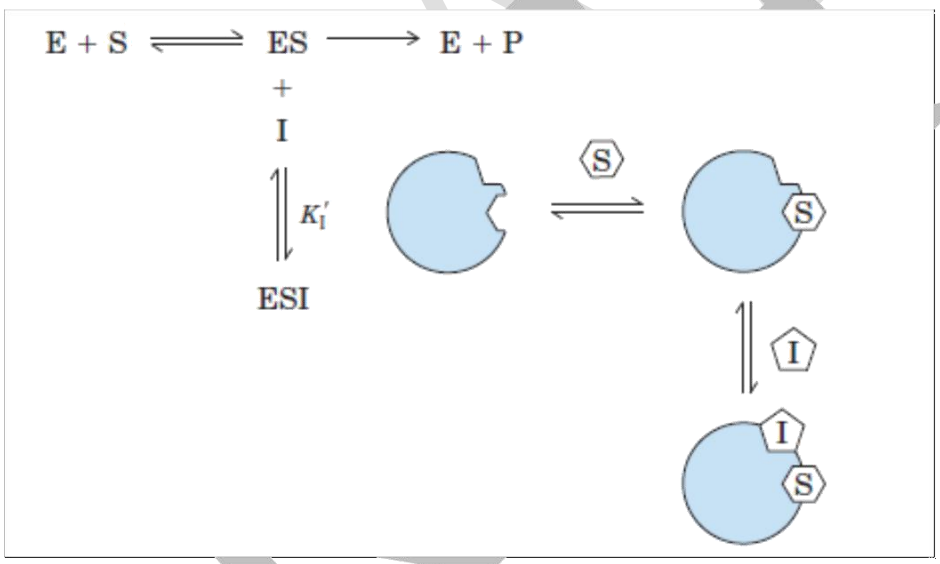
Figure: Effect of non-competitive inhibitor (I) on enzyme velocity (A) Velocity (v) versus substrate (S) (B) Lineweaver-Burk plot (Red lines with inhibitor, non-competitive inhibitor does not change Km but decreases Vmax.)

Heavy metal ions (Ag^{+}, Pb^{2+}, Hg^{2+} etc.) can non-competitively inhibit the enzymes by binding with cysteinyl sulfhydryl groups. The general reaction for Hg^{2+} is shown below.

Heavy metals also lead to the formation of covalent bonds with carboxyl groups and histidine, often resulting in irreversible inhibition.

Uncompetitive inhibition

Uncompetitive and non-competitive inhibition, though often defined in terms of one substrate enzymes, are in practice observed only with enzymes having two or more substrates. An **uncompetitive inhibitor** binds at a site distinct from the substrate active site and, unlike a competitive inhibitor, binds only to the ES complex.



An uncompetitive inhibitor lowers the measured *V*max. Apparent *K*m also decreases.

Allosteric inhibition and covalent modifications

The activities of regulatory enzymes are modulated in a variety of ways. **Allosteric enzymes** function through reversible, noncovalent binding of regulatory compounds called **allosteric modulators** or **allosteric effectors,** which are generally small metabolites or cofactors. Other enzymes are regulated by reversible **covalent modification.** Both classes of regulatory enzymes tend to be multisubunit proteins, and in some cases the regulatory site(s) and the active site are on separate subunits.

Allosteric inhibition

Allosteric proteins are those having ―other shapes‖ or conformations induced by the binding of modulators. The modulators for allosteric enzymes may be inhibitory or stimulatory. Often the modulator is the substrate itself; regulatory enzymes for which substrate and modulator are identical are called homotropic. The effect is similar to that of O_2 binding to hemoglobin: binding of the ligand—or substrate, in the case of enzymes—causes conformational changes that affect the subsequent activity of other sites on the protein. When the modulator is a molecule other than the substrate, the enzyme is said to be heterotropic. Although the latter bind at a second site on the enzyme, they do not necessarily mediate conformational changes between active and inactive forms, and the kinetic effects are distinct. The properties of allosteric enzymes are significantly different from those of simple non regulatory enzymes. Some of the differences are structural. In addition to active sites, allosteric enzymes generally have

one or more regulatory, or allosteric, sites for binding the modulator.

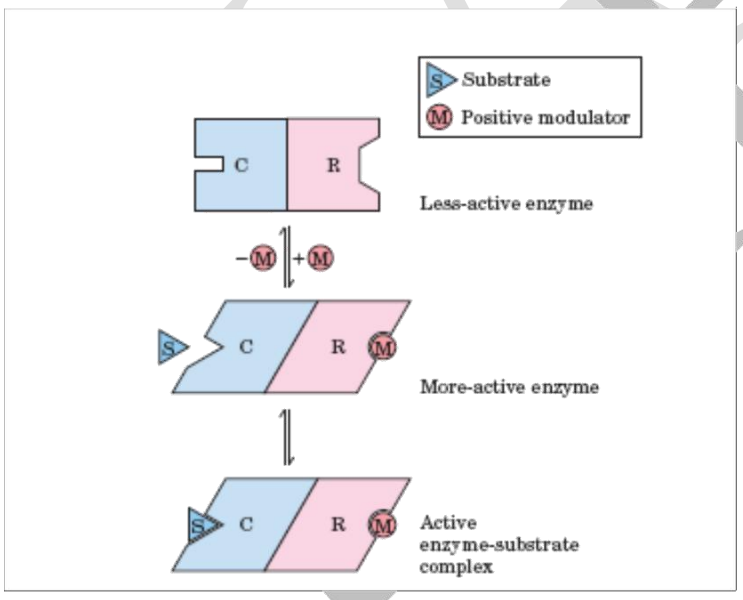


Figure: Subunit interactions in an allosteric enzyme, and interactions with inhibitors and activators. In many allosteric enzymes the substrate binding site and the modulator binding site(s) are on different subunits, the catalytic (C) and regulatory (R) subunits, respectively.

Binding of the positive (stimulatory) modulator (M) to its specific site on the regulatory subunit is communicated to the catalytic subunit through a conformational change. This change renders the catalytic subunit active and capable of binding the substrate (S) with higher affinity. On

dissociation of the modulator from the regulatory subunit, the enzyme reverts to its inactive or less active form.

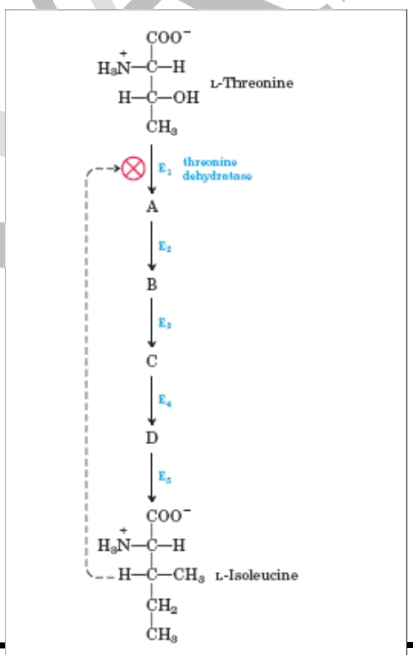
Just as an enzyme's active site is specific for its substrate, each regulatory site is specific for its modulator. Enzymes with several modulators generally have different specific binding sites for each. In homotropic enzymes, the active site and regulatory site are the same. Allosteric enzymes are generally larger and more complex than non allosteric enzymes. Most have two or more subunits.

Aspartate transcarbamoylase, which catalyzes an early reaction in the biosynthesis of pyrimidine

nucleotides, has 12 polypeptide chains organized into catalytic and regulatory subunits.

Example of pathway Regulation by allosteric enzymes

In some multienzyme systems, the regulatory enzyme is specifically inhibited by the end product of the pathway whenever the concentration of the end product exceeds the cell's requirements. When the regulatory enzyme reaction is slowed, all subsequent enzymes operate at reduced rates as their substrates are depleted. The rate of production of the pathway's end product is thereby brought into balance with the cell's needs. This type of regulation is called **feedback inhibition.** Buildup of the end product ultimately slows the entire pathway. One of the first known examples of allosteric feedback inhibition was the bacterial enzyme system that catalyzes the conversion of L-threonine to L-isoleucine in five steps.



Feedback inhibition. The conversion of L-threonine to L-isoleucine is catalyzed by a sequence of five enzymes (E1 to E5). Threonine dehydratase (E1) is specifically inhibited

allosterically by L-isoleucine, the end product of the sequence, but not by any of the four intermediates (A to D). Feedback inhibition is indicated by the dashed feedback line and the ⊗ symbol at the threonine dehydratase reaction.

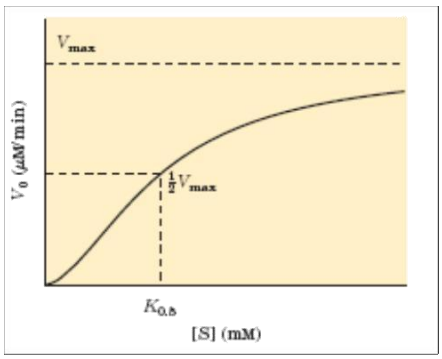
In this system, the first enzyme, threonine dehydratase, is inhibited by isoleucine, the product of the last reaction of the series. This is an example of heterotropic allosteric inhibition. Isoleucine is quite specific as an inhibitor. No other intermediate in this sequence inhibits threonine dehydratase, nor is any other enzyme in the sequence inhibited by isoleucine. Isoleucine binds not to the active site but to another specific site on the enzyme molecule, the regulatory site. This binding is non covalent and readily reversible; if the isoleucine concentration decreases, the rate of threonine dehydration increases. Thus threonine dehydratase activity responds rapidly and reversibly to fluctuations in the cellular concentration of isoleucine.

The Kinetic Properties of Allosteric Enzymes

Allosteric enzymes show relationships between *V*0 and [S] that differ from Michaelis-Menten kinetics. They do exhibit saturation with the substrate when [S] is sufficiently high, but for some allosteric enzymes, plots of *V*0 versus [S] produce a sigmoid saturation curve, rather than the hyperbolic curve typical of non regulatory enzymes. On the sigmoid saturation curve we can find a value of [S] at which *V*0 is half-maximal, but we cannot refer to it with the designation *K*m, because the enzyme does not follow the hyperbolic Michaelis- Menten relationship. Instead, the symbol [S]0.5 or *K*0.5 is often used to represent the substrate concentration giving half-maximal velocity of the reaction catalyzed by an allosteric enzyme. Sigmoid kinetic behavior generally reflects cooperative interactions between protein subunits. In other words, changes in the structure of one subunit are translated into structural changes in adjacent subunits, an effect mediated by non covalent interactions at the interface between subunits. The principles are particularly well illustrated by a nonenzyme: O_2 binding to hemoglobin.

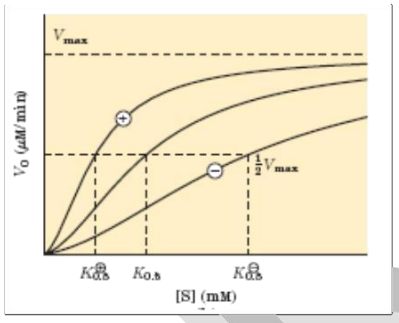
Homotropic allosteric enzymes generally are multisubunit proteins and, the same binding site on each subunit functions as both the active site and the regulatory site. Most commonly, the substrate acts as a positive modulator (an activator), because the subunits act cooperatively: the binding of one molecule of substrate to one binding site alters the enzyme's conformation and enhances the binding of subsequent substrate molecules. This accounts for the sigmoid rather than hyperbolic change in *V*0 with increasing [S]. One characteristic of sigmoid kinetics is that small changes in the concentration of a modulator can be associated with large changes in activity.

Figure: 3a



As is evident in Figure 3a, a relatively small increase in [S] in the steep part of the curve causes a comparatively large increase in *V*0. For heterotropic allosteric enzymes, those whose modulators are metabolites other than the normal substrate, it is difficult to generalize about the shape of the substrate-saturation curve. An activator may cause the curve to become more nearly hyperbolic, with a decrease in *K*0.5 but no change in *V*max, resulting in an increased reaction velocity at a fixed substrate concentration (*V*0 is higher for any value of [S]; Fig.3b, upper curve).

Figure: 3b



Other heterotropic allosteric enzymes respond to an activator by an increase in *V*max with little change in *K*0.5 (Fig. 3c).

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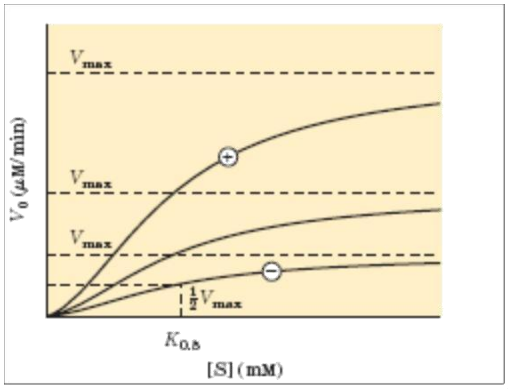
Figure: 3c

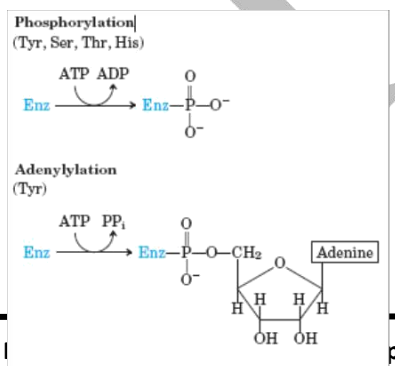


A negative modulator (an inhibitor) may produce a *more* sigmoid substrate-saturation curve, with an increase in *K*0.5 (Fig. 3b, lower curve). Heterotropic allosteric enzymes therefore show different kinds of responses in their substrate-activity curves, because some have inhibitory modulators, some have activating modulators, and some have both.

Covalent Modification

In another important class of regulatory enzymes, activity is modulated by covalent modification of the enzyme molecule. Modifying groups include phosphoryl, adenylyl, uridylyl, methyl, and adenosine diphosphate ribosyl groups (Fig. 6–30). These groups are generally linked to and removed from the regulatory enzyme by separate enzymes.

Figure: Covalent modifications



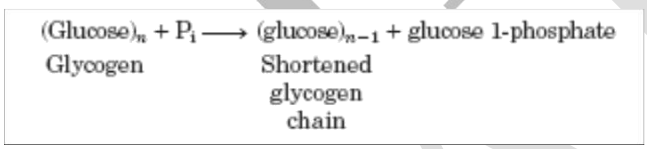
An example of an enzyme regulated by methylation is the methyl-accepting chemotaxis protein of bacteria. This protein is part of a system that permits a bacterium to swim toward an attractant (such as a sugar) in solution and away from repellent chemicals. The methylating agent is *S*-

adenosyl methionine (adoMet). ADP-ribosylation is an especially interesting reaction, observed in only a few proteins; the ADP-ribose is derived from nicotinamide adenine dinucleotide (NAD). This type of modification occurs for the bacterial enzyme dinitrogenase reductase, resulting in regulation of the important process of biological nitrogen fixation. Diphtheria toxin and cholera toxin are enzymes that catalyze the ADP-ribosylation (and inactivation) of key cellular enzymes or proteins. Diphtheria toxin acts on and inhibits elongation factor 2, a protein involved in protein biosynthesis. Cholera toxin acts on a G protein that is part of a signaling pathway, leading to several physiological responses including a massive loss of body fluids and, sometimes, death. Phosphorylation is the most common type of regulatory modification; one-third to one-half of all proteins in a eukaryotic cell are phosphorylated. Some proteins have only one phosphorylated residue, others have several, and a few have dozens of sites for

phosphorylation. This mode of covalent modification is central to a large number of regulatory pathways.

Phosphoryl Groups Affect the Structure and Catalytic Activity of Proteins by covalent modifications

The attachment of phosphoryl groups to specific amino acid residues of a protein is catalyzed by **protein kinases;** removal of phosphoryl groups is catalyzed by **protein phosphatases.** The addition of a phosphoryl group to a Ser, Thr, or Tyr residue introduces a bulky, charged group into a region that was only moderately polar. The oxygen atoms of a phosphoryl group can hydrogen-bond with one or several groups in a protein, commonly the amide groups of the peptide backbone at the start of an αhelix or the charged guanidinium group of an Arg residue. The two negative charges on a phosphorylated side chain can also repel neighboring negatively charged (Asp or Glu) residues. When the modified side chain is located in a region of the protein critical to its three dimensional structure, phosphorylation can have dramatic effects on protein conformation and thus on substrate binding and catalysis. An important example of regulation by phosphorylation is seen in glycogen phosphorylase (*M*r 94,500) of muscle and liver , which catalyzes the reaction



The glucose 1-phosphate so formed can be used for ATP synthesis in muscle or converted to free glucose in the liver. Glycogen phosphorylase occurs in two forms: the more active phosphorylase *a* and the less active phosphorylase *b*. Phosphorylase *a* has two subunits, each with a specific Ser residue that is phosphorylated at its hydroxyl group. These serine phosphate residues are required for maximal activity of the enzyme.

Figure: Regulation of glycogen phosphorylase activity by covalent modification

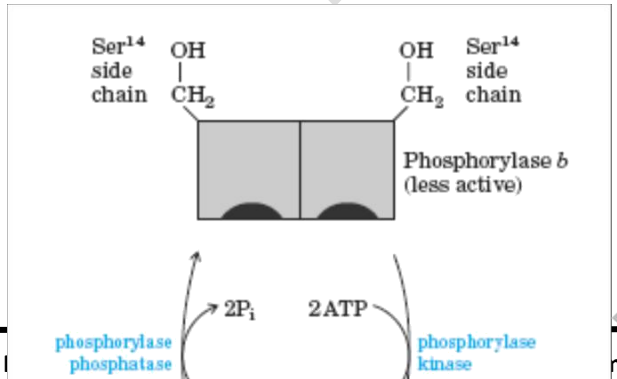


Figure: In the more active form of the enzyme, phosphorylase *a*, specific Ser residues, one on each subunit, are phosphorylated. Phosphorylase *a* is converted to the less active phosphorylase *b* by enzymatic loss of these phosphoryl groups, promoted by phosphorylase phosphatase. Phosphorylase *b* can be reconverted (reactivated) to phosphorylase *a* by the action of phosphorylase kinase.

The phosphoryl groups can be hydrolytically removed by a separate enzyme called phosphorylase phosphatase:

Phosphorylase a + $2H_2O$ → Phosphorylase b + 2Pi

In this reaction, phosphorylase *a* is converted to phosphorylase *b* by the cleavage of two serine phosphate covalent bonds, one on each subunit of glycogen phosphorylase. Phosphorylase *b* can in turn be reactivated—covalently transformed back into active phosphorylase *a*—by another enzyme, phosphorylase kinase, which catalyzes the transfer of phosphoryl groups from ATP to the hydroxyl groups of the two specific Ser residues in phosphorylase *b*:

2ATP + Phosphorylase b → 2ADP + Phosphorylase a

(less active) (more active)

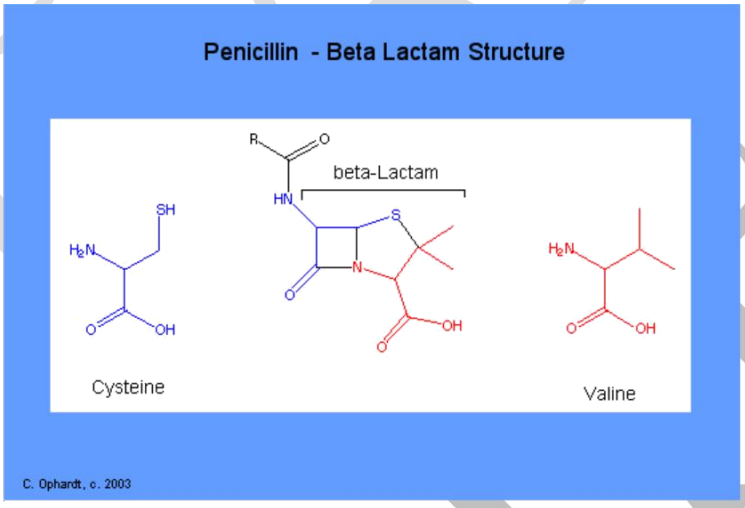
The breakdown of glycogen in skeletal muscles and the liver is regulated by variations in the ratio of the two forms of glycogen phosphorylase. The *a* and *b* forms differ in their secondary, tertiary, and quaternary structures; the active site undergoes changes in structure and, consequently, changes in catalytic activity as the two forms are inter converted. The regulation of glycogen phosphorylase by phosphorylation illustrates the effects on both structure and catalytic activity of adding a phosphoryl group. In the unphosphorylated state, each subunit of this protein is folded so as to bring the 20 residues at its amino terminus, including a number of basic residues, into a region containing several acidic amino acids; this produces an electrostatic interaction that stabilizes the conformation. Phosphorylation of Ser14 interferes with this interaction, forcing the amino-terminal domain out of the acidic environment and into a confirmation that allows interaction between the P –Ser and several Arg side chains. In this conformation, the enzyme is much more active. Phosphorylation of an enzyme can affect catalysis in another way: by altering substrate-binding affinity. For example, when isocitrate dehydrogenase (an enzyme of the citric acid cycle) is phosphorylated, electrostatic repulsion by the phosphoryl group inhibits the binding of c itrate (a tricarboxylic acid) at the active site.

Mechanism-Based Enzyme Inhibitors

Natural product scaffolds are highly evolved to bind a biological protein target with exquisite potency. Unique subsets of natural products possess latent reactivity that is realized only within the confines of its biological target and often results in the formation of a covalent enzyme-inhibitor complex. These types of molecules are often referred to as ―Mechanism-Based Inhibitors‖ and can offer many therapeutic advantages (lower dosing and longer target dwell times). Our lab will elucidate the molecular mechanism of enzyme inhibition of naturally occurring mechanism-based enzyme inhibitors and exploit this knowledge to design and synthesize more effective synthetic inhibitors. We are interested in developing Tabtoxinine-β-Lactam (TβL) as a potent mechanism-based inhibitor of the enzyme Glutamine Synthetase to treat multidrug resistant Mycobacterium tuberculosis infections.

Antibiotics:

Antibiotics are specific chemical substances derived from or produced by living organisms that are capable of inhibiting the life processes Antibiotics are specific chemical substances derived from or produced by living organisms that are capable of inhibiting the life processes of other organisms. The first antibiotics were isolated from microorganisms but some are now obtained from higher plants and animals. Over 3,000 antibiotics have been identified but only a few dozen are used in medicine. Antibiotics are the most widely prescribed class of drugs comprising 12% of the prescriptions in the United States.



Penicillins as well as cephalosporins are called beta-lactam antibiotics and are characterized by three fundamental structural requirements: the fused beta-lactam structure (shown in the blue and red rings, a free carboxyl acid group (shown in red bottom right), and one or more substituted amino acid side chains

Short Answers (2 marks)

1. Write a note on proximity and orientation.
2. Define metal activated enzymes.
3. Define metallo enzymes.
4. Define enzyme inhibition.
5. Write a note on competitive inhibition.
6. Add a note on non competitive inhibition.
7. Write a note on uncompetitive inhibition.
8. Define acid base catalysis.
9. Define covalent catalysis.

Long Answers (8 marks)

1. Explain the mechanism of acid base catalysis with an example.
2. Explain the mechanism of covalent catalysis with an example.
3. Describe the mechanism of action of chymotrypsin.
4. Elaborate the mechanism of action of lysozyme.
5. Differentiate metal activated enzymes and metalloenzymes.
6. Write a note on transition state analogues.
7. Explain the mechanism of competitive inhibition with example.
8. Explain the mechanism of non competitive and un competitive inhibition with examples.
9. Comment on antibiotics as inhibitors.

CLASS: I B.Sc **COURSE NAME: ENZYMES**

BATCH: 2018-2021 **COURSE CODE: 18BCU202**

Karpagam academy of higher education
Department of biochemistry
Enzymes (18BCU202) MCQ
UNIT III

S.NO	QUESTION	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER
1	Name of inhibition in which Vmax unaltered	**competitive**	uncompetitive	non-competitive	irreversible	**competitive**
2	Antimetabolites act by	non-competitive inhibition	**competitive inhibition**	binding with substrate	enzyme denaturation	**competitive inhibition**
3	The inhibition of succinate dehydrogenase by melonate is an example of	**competitive inhibition**	irreversible inhibition	un competitive inhibition	mixed inhibition	**competitive inhibition**
4	Competitive inhibitors	decrease in Km	decrease in	**increase in Km**	increase in Vmax	**increase in Km**
5	Competitive inhibition can be relieved by raising the	enzyme concentration	**substrate concentration**	inhibitor concentration	coenzyme concentration	**substrate concentration**
6	Cholinesterase is competitively inhibited by	Aminopterin	Acetylcholine	**Neostigmine**	Allopurinol	**Neostigmine**
7	Physostigmine is a competitive inhibitor of	Xanthine oxidase	**Cholinesterase**	Carbonic anhydrase	Monoamine oxidase	**Cholinesterase**
8	Dihydrofolate reductase is competitively inhibited by	Amethopterin	neostigmine	Acetazolamide	**allopurinol**	**allopurinol**
9	Non-competitive inhibitors	decrease the Km	**decrease the**	increase the Km	increase the Vmax	**decrease the Vmax**
10	A competitive inhibitor of an enzyme is usually	a highly reactive compound	a metal ion such as Hg2+ or Pb2+	**structurally similar to the substrate**	water insoluble	**structurally similar to the substrate**
11	A classical noncompetitive inhibitor has	no effect on substrate binding	no effect on substrate binding and vice versa	significant effect on substrate binding	**significant effect on substrate binding and vice versa**	**significant effect on substrate binding and vice versa**
12	A noncompetitive inhibitor of an enzyme-catalysed reaction	increases Km and increases Vmax	increases Km and reduces	reduces Km and increases Vmax	**unchanged Km and reduces Vmax**	**unchanged Km and reduces Vmax**

13	A classical uncompetitive inhibitor is a compound that binds	**reversibly to the enzyme substrate complex yielding an inactive ESI complex**	irreversibly to the enzyme substrate complex	reversibly to the enzyme substrate complex yielding an active ESI complex	irreversibly to the enzyme substrate complex yielding an active ESI complex	**reversibly to the enzyme substrate complex yielding an inactive ESI complex**
14	Which of the following common drugs is not a specific enzyme inhibitor?	iodine	methotrexate	sulfonilamide	**penicillin**	**penicillin**
15	In a Lineweaver-Burk plot, competitive inhibitor shows which of the following effect?	it moves the entire curve	it moves the entire curve to left	**it changes the x-intercept**	it has no effect on the slope	**it changes the x-intercept**
16	The types of inhibition pattern based on Michaelis-Menton equation are	competitive inhibition	non-competitive	**uncompetitive inhibition**	feedback inhibition	**uncompetitive inhibition**
17	Biological molecules (proteins) which catalyze a biochemical reaction and remain unchanged after completion of reaction are called	Cofactor	Coenzymes	Activator	**Enzymes**	**Enzymes**
18	Which statement about enzyme is incorrect	Some of them consist solely of protein with no non protein part	They catalyze a chemical reaction without	**All enzymes are fibrous Proteins**	They without their cofactor are called apoenzyme	**All enzymes are fibrous Proteins**
19	It is the measure of the stability of the ES complex	It is the measure of the stability of the affinity of an enzyme for	its substrate	A high Km indicates weak substrate binding	**all of these**	**all of these**
20	Competitive inhibition is	**always reversible**	always	sometimes reversible	none of these	**always reversible**
21	Which one of the following statements regarding enzyme inhibition is correct?	**competitive inhibition is seen a substrate competes with an enzyme for binding to an inhibitor protein**	non competitve inhibition of an enzyme can be overcome by adding large	competitive inhibition is when the substrate and the inhibitor compete for the active site on the	competitive inhibition is seen when the substrate and the inhibitor compete for the	**competitive inhibition is seen a substrate competes with an enzyme for binding to an inhibitor protein**
22	Which of the following is an example for irreversible inhibitor Iodoacetamide, a cys residue modifierat the active sites	Di-isopropylphospho fluoridate (DIPF) with the enzyme	acetylcholineeste rase, involved in nerve impulse transmission	Penicillin with glycopeptides transpeptidase enzyme involved in bacterial cell wall	**all of these**	**all of these**

23	The inhibition caused by the final end product of a reaction is called	Non competitive inhibition	competitive inhibition	**Allosteric inhibition**	All of these	**Allosteric inhibition**
24	Which of the following is/ are irreversible?	competitive inhibition	**Non competitive**	Allosteric inhibition	None of these	**Non competitive inhibition**
25	Which of the following are allosteric enzymes	RUBISCO	PEP Carboxylase	Phosphofructokinase	**All of these**	**All of these**
26	The competitive inhibitor malonic acid resembles	malic acid	fumaric acid	**succinic acid**	oxaloacetate acid	**succinic acid**
27	Competitive inhibition overcome by adding substrate show that	**enzymes are pH dependent**	enzymes are made up of	enzyme are biocatalysts	enzymes are specific in nature	**enzymes are pH dependent**
28	Inhibition of enzyme cytochrome oxidase by carbon monoxide is an example for	Feed back inhibition	competitive inhibition	**Non competitive inhibition**	None of the above	**Non competitive inhibition**
29	Which of the following amino acids would be prone to a reaction with an irreversible inhibitor?	**Serine**	Tyrosine	Alanine	Tryptophan	**Serine**
30	Which of the following terms best describes a drug that binds to an active site and inhibits the enzyme, and where inhibition decreases when substrate concentration is increased?	**reversible inhibitor**	rreversible	competitive inhibition	**Non competitive inhibition**	**reversible inhibitor**
31	Which of the following terms best describes a drug which inhibits the enzyme, but binds to a binding site other than the active site?	**allosteric inhibitor**	rreversible	competitive inhibition	reversible inhibitor	**allosteric inhibitor**
32	Which of the following is not true of transition-state analogues?	**They react irreversibly with the enzyme.**	They react reversibly with	They react irreversibly with the	reversible inhibitor	**They react irreversibly with the**
33	Which of the following is not true of suicide substrates?	**They self-destruct as a result of an enzyme-catalysed reaction**	It is the measure of the stability of the ES complex	It is the measure of the stability of the affinity of an enzyme	A high Km indicates weak substrate binding	**They self-destruct as a result of an enzyme-catalysed reaction**
34	Which enzyme inhibitors represent the most common used clinical agents?	allosteric inhibitor	rreversible	competitive inhibition	**reversible inhibitor**	**reversible inhibitor**

35	Which of the following agents act as irreversible inhibitors	**Penicilllins**	Kanamycine	Serine	Alanine	**Penicilllins**
36	Which of the following drugs can be classed as a suicide substrate?	**Clavulanic acid**	Gallic acid	Hcl	Sulphuric acid	**Clavulanic acid**
37	Ribosomal resistance occurs with	Sulphonamides	Penicillin	Fluoroquinolones	**Macrolides**	**Macrolides**
38	Aminoglycoside	Have a b lactam ring	**Can produce neuromuscular blockade**	Normally reach high CSF concentrations	Have good oral absorption but high first pass	**Can produce neuromuscular blockade**
39	All of the following are true regarding metronidazole EXCEPT	It is used to treat giardia	It causes a metallic taste in	**It inhibits alcohol dehydrogenase**	It is used to treat gardnerella	**It inhibits alcohol dehydrogenase**
40	Vancomycin	Binds to the 30S unit on the ribosome and inhibits protein synthesis	60% of vancomycin is excreted by glomerular filtration	Parenteral vancomycin is commonly used for treatment of infections caused by	**Adverse reactions to vancomycin are encountered in about 10% of patients**	**Adverse reactions to vancomycin are encountered in about 10% of patients**
41	Pharmacokinetics of doxycycline	20% bound by serum proteins	60-70% absorption after oral	**Absorption is impaired by divalent cations,**	Widely distributed especially into the CSF	**Absorption is impaired by divalent cations, Al^{3+}, and**
42	Resistance to Penicillin and other b lactams is due to	Modification of target PBPs	Presence of an efflux pump	Inactivation of antibiotics by b	**All of the above**	**All of the above**
43	Which of the following is the general mechanism of action for erythromycin	Inhibition of a metabolic enzyme	Inhibition of cell wall synthesis	**Disruption of protein synthesis**	Inhibition of nucleic acid transcription and replication	**Disruption of protein synthesis**
44	Which of the following antibiotics is a macrolide	Chloramphenicol	Doxycycline	**Erythromycin**	Streptomycin	**Erythromycin**
45	Which of the following antibiotics is a tetracycline?	Chloramphenicol	**Doxycycline**	Erythromycin	Streptomycin	**Doxycycline**
46	**w**hich of the following antibiotics is responsible for Gray Baby Syndrome?	**Chloramphenicol**	Doxycycline	Erythromycin	Streptomycin	**Chloramphenicol**
47	What crucial feature of a penicillin is involved in its mechanism of action?	Modification of target PBPs	Presence of an efflux pump	Inactivation of antibiotics by b	**β-lactam ring**	**β-lactam ring**

48	Regarding the “azole” group of antifungals	Fluconazole has low water solubility	Ketoconazole may be given IV/PO	Clotrimazole is the treatment of choice for systemic candidiasis – given orally	**They work by reduction of ergosterol synthesis by inhibition of fungal cytochrome P_{450} enzymes**	**They work by reduction of ergosterol synthesis by inhibition of fungal cytochrome P_{450} enzymes**
49	The cephalosporin with the highest activity against gram positive cocci is	Cefaclor	**Cephalothin**	Cefuroxime	Cefepime	**Cephalothin**
50	Regarding the penicillins	**Penicillin ix excreted into breast milk to levels 3-15% of those present in the serum**	Absorption of amoxyl is impaired by food	Benzathine penicillin is given PO	Penicillins are 90% excreted by glomerular filtration	**Penicillin ix excreted into breast milk to levels 3-15% of those present in the serum**
51	Rifampicin	Inhibits hepatic microsomal enzymes	Inhibits DNA synthesis	**Is bactericidal for mycobacteria**	Is predominantly excreted unchanged in the urine	**Is bactericidal for mycobacteria**
52	Regarding resistance to antibiotics	Penicillinases cannot inactivate cephalosporins	Macrolides can be inactivated by transferases	**Mutation of aminoglycoside binding site is its main mechanism of resistance**	Tetracycline resistance is a marker for multidrug resistance	**Mutation of aminoglycoside binding site is its main mechanism of resistance**
53	Concerning toxicity of antibiotics	Enamel dysplasia is common with aminoglycosides	A disulfiram like reaction can occur with macrolides	**Haemolytic anaemias can occur with sulphonamide** use	Nephritis is the most common adverse reaction with isoniazid	**Haemolytic anaemias can occur with sulphonamide** use
54	An example of competitive inhibition of an enzyme is the inhibition of	**succinic dehydrogenase by malonic acid**	cytochrome oxidase by	carbonic anhydrase by carbon dioxide	hexokinase by glucose-6-phosphate	**succinic dehydrogenase by**
55	Blocking of enzyme action by blocking its active sites is	allosteric inhibition	feedback inhibition	**competitive inhibition**	non-competitive inhibition	**competitive inhibition**
56	Many catalysts are	noble gases	**transition**	non-metals	inert gases	**transition metals**
57	Optimal temperature for enzymes is	room temperature	**35-37 °C**	40-45 °C	above 45 °C	**35-37 °C**
58	uses of enzymes does not include	production of insulin	production of cancer drugs	**drug to combat plague**	drug to fight dwarfism	**drug to combat plague**

59	Value needed for enzyme action is	Low Km	Low Ki	High Km	**High Ki**	**High Ki**
60	Enzymes are polymers of	Hexose sugar	**Amino acids**	Fatty acids	Inorganic	**Amino acids**

UNIT-IV

SYLLABUS

Regulation of enzyme activity

Control of activities of single enzymes (end product inhibition) and metabolic pathways, feedback inhibition (aspartate transcarbomoylase), reversible covalent modification phosphorylation (glycogen phosphorylase). Proteolytic cleavage- zymogen.Multienzyme complex as regulatory enzymes. Occurrence and isolation, phylogenetic distribution and properties (pyruvate dehydrogenase, fatty acyl synthase) Isoenzymes - properties and physiological significance (lactate dehydrogenase).

Introduction

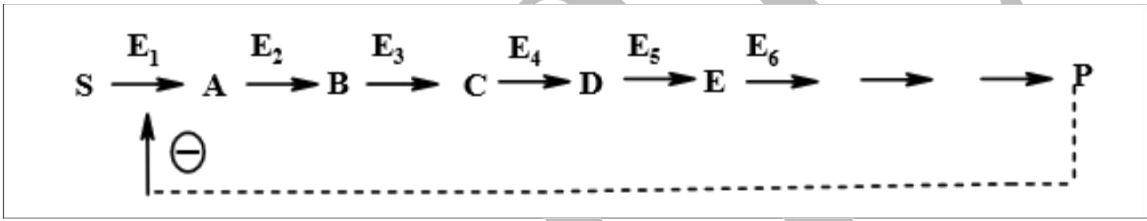
Metabolic pathways are made up of many chemical reactions and these reactions are catalysed by enzymes. Often, the product of the last reaction in the pathway inhibits the enzyme that catalyses the first reaction of the pathway. This is called end-product inhibition and it involves non-competitive inhibitors. The product of the last reaction of the metabolic pathway will bind to a site other than the active site of the enzyme that catalyses the first reaction. This site is called the allosteric site. When it binds to the allosteric site it acts as non-competitive inhibitor and changes the conformation of the active site. Therefore, it makes the binding of the substrate to the enzyme unlikely. Once the inhibitor is released from the allosteric site, the active site returns to its original conformation and the substrate is able to bind again. There is a clear advantage in using end-product inhibition for controlling metabolic pathways. When there is an excess of end-product, the whole metabolic pathway is shut down as the end product inhibits the first enzyme of the pathway. Therefore less of the end product gets produced and by inhibiting the first enzyme it also prevents the formation of intermediates. When the levels of the end product decrease, the enzymes start to work again and the metabolic pathway is switched on.

Feedback Inhibition

The term feedback inhibition or **end-product** inhibitionmeans that the activity of the enzyme is inhibited by thefinal product of the biosynthetic pathway. When in a metabolic pathway a substrate, S, is transformed into a product, P, through a series of enzymatic reactions and if Paccumulates in amounts that are not immediately needed by the cell then this product specificallyinhibits the action of the first enzyme, E1, of the pathway. Thus, further transformation of S in that direction is stopped. This is called feedback inhibitionor end-product inhibition. Two noteworthy points of this inhibition are following:

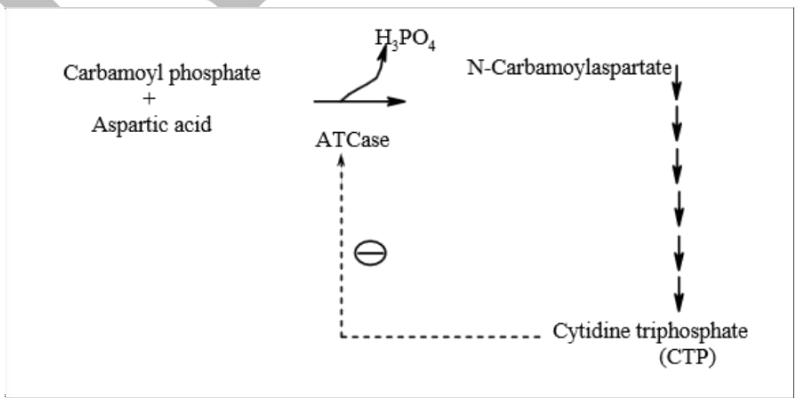
i. None of the intermediate products inhibits the enzyme E1.

ii The other enzymes in the pathway except E1 are not inhibited by P.



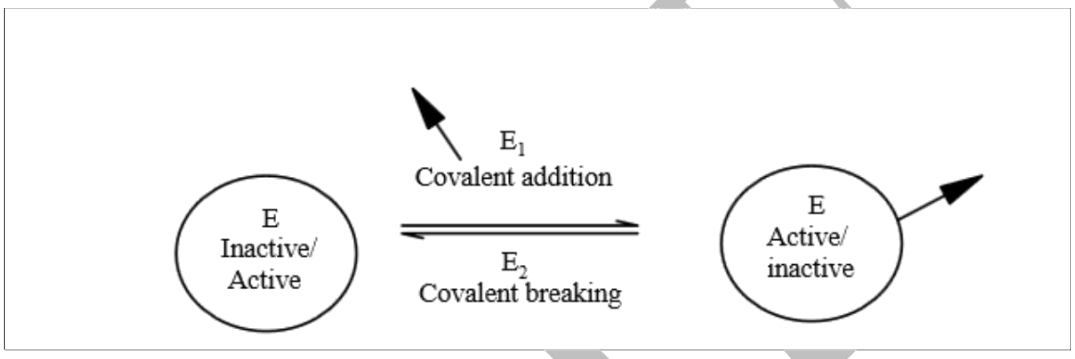
So, only regulatory enzymes are subjected tofeedback inhibition. These enzymes are allosteric in nature. The following are the well established examples showing feedback inhibition:

1. Inhibition of enzyme aspartate transcarbamoylase (ATCase) by nucleotide cytidine Triphosphate (CTP). ATCase catalyzes the synthesis of nucleoside triphosphate, CT P, from aspartic acid and carbamoyl phosphate through a sequence of reactions. The end product of the pathway, CTP, is responsible for inhibition of first enzyme when needed, as shown below:

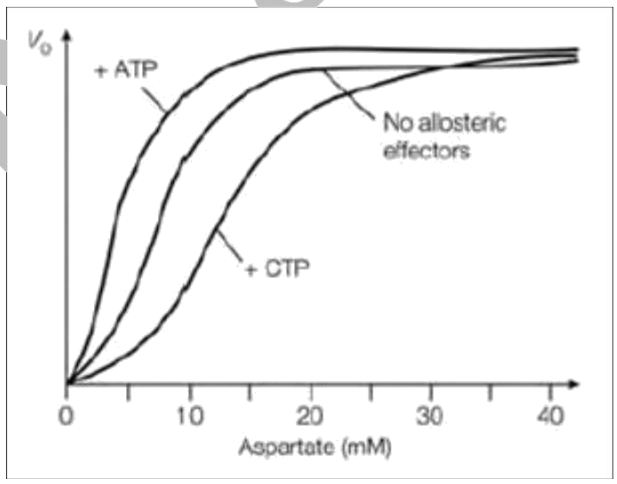


Reversible covalent modification

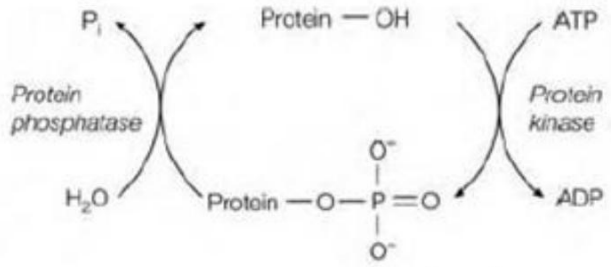
This is also one of the major ways of controlling the enzyme activity to exercise a regulatory control over metabolism. In this the enzyme protein gets activated or inhibited by undergoing through a covalent modification. These modifications are reversible and require two enzymes. Depending on the metabolic milieu of the cell one enzyme incorporates a covalently linked group and the other enzyme removes it from the enzyme protein whose activity is being controlled



Reversible covalent modification is the making and breaking of a covalent bond among a nonprotein group and an enzyme molecule. While a range of nonprotein groups may be reversibly attached to enzymes that affect their activity, the mainly general modification is the removal and addition of a phosphate group phosphorylation and dephosphorylation, respectively. The Phosphorylation is catalyzed through protein kinases, frequently using ATP as the



Plot of initial velocity (V0) against substrate concentration for the allosteric enzyme aspartate transcarbamoylase.



The reversible phosphorylation and dephosphorylation of an enzyme.phosphate donor and dephosphorylation is catalyzed through protein phosphatases in above figure. The removal and addition of the phosphate group causes modification in the tertiary structure of the enzyme which alter its catalytic activity. One class of the protein kinases transfers the phosphate specifically on to the hydroxyl group of Thr or Ser residues on the goal enzyme serine or threonine protein kinases, typified through 3′,5′-cyclic adenosine monophosphate (cAMP)-dependent protein kinase, although a second class transfers the phosphate on to the hydroxyl group of Tyr residues tyrosine kinases. The Protein phosphatases catalyze the hydrolysis of phosphate groups from proteins to regenerate not modify hydroxyl group of the amino acid and release Pi which is shown in figure.

A phosphorylated enzyme may be either less or more active than its dephosphorylated form.Therefore phosphorylation or dephosphorylation may be used as a rapid reversible that is switched to turn a metabolic pathway on or off according to the requirement of the cell. For instance glycogen phosphorylase an enzyme included in glycogen breakdown is active in its phosphorylated form and glycogen synthase, included in glycogen synthesis is much active in its unphosphorylated form.

Other kinds of reversible covalent modification which are used to regulate the activity of certain enzymes involves adenylylation the transfer of adenylate from ATP and ADP-ribosylation the transfer of an ADP (adenosine diphosphate)-ribosyl moiety from NAD.

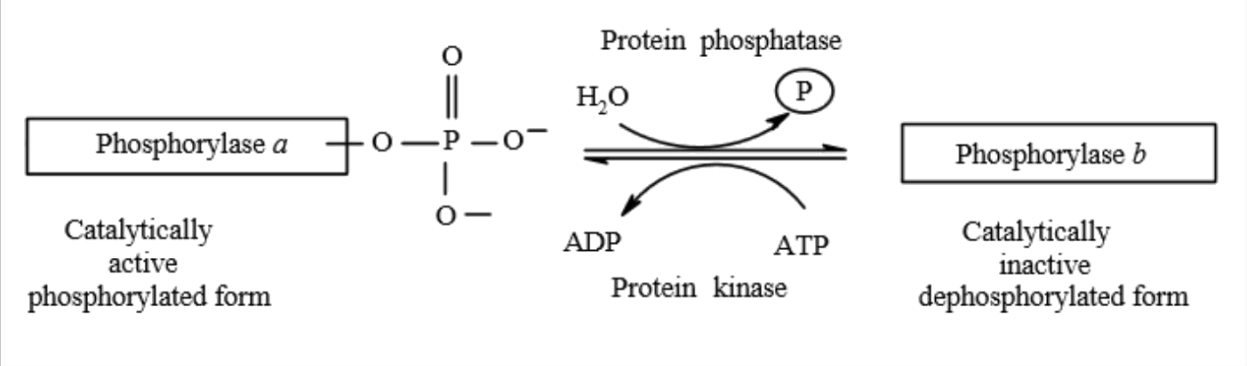
Glycogen phosphorylase:

Activated by phosphorylation of enzyme protein. The enzyme liberates glucose-1-phosphate fromglycogen in muscle. A glucose residue atthe non-reducing end of the chain is

removed by breaking the glycosidic bond involving a phosphoric acid molecule. Thus, a molecule of glucose -1-phosphate is released that acts as a source of energy and glycogen chain becomes shorter by one glucose unit at each step as shown below:

$$(\text{Glycogen})_n + H_3PO_4 \xrightarrow{\text{pholsphorylase a}} (\text{Glycogen})_{n-1} + \text{Glu cos e} - 1 - \text{phosphate}$$

The enzyme phosphorylase in active form is phosphorylated and called phosphorylase. Under the conditions where the breakdown of glycogen is not needed this active enzyme is converted into inactive form called phosphorylase. These two forms of enzyme are interconvertible with the help oftwo enzymes; a protein phosphataseand a protein kinase as shown below:



Zymogen

Zymogen, also called Proenzyme, any of a group of proteins that display no catalytic activity but are transformed within an organism into enzymes, especially those that catalyze reactions involving the breakdown of proteins. Trypsinogen and chymotrypsinogen, zymogens secreted by the pancreas, are activated in the intestinal tract to trypsin and chymotrypsin. Activation is effected by the cleavage of one or more peptide bonds of the zymogen molecule and may be catalyzed by a separate enzyme—*e.g.*,enterokinase converts trypsinogen to trypsin—or by the active form itself—trypsin also converts trypsinogen to more trypsin. Zymogenic cells synthesize and store zymogens in inactive form.

Multi-enzyme Complexes

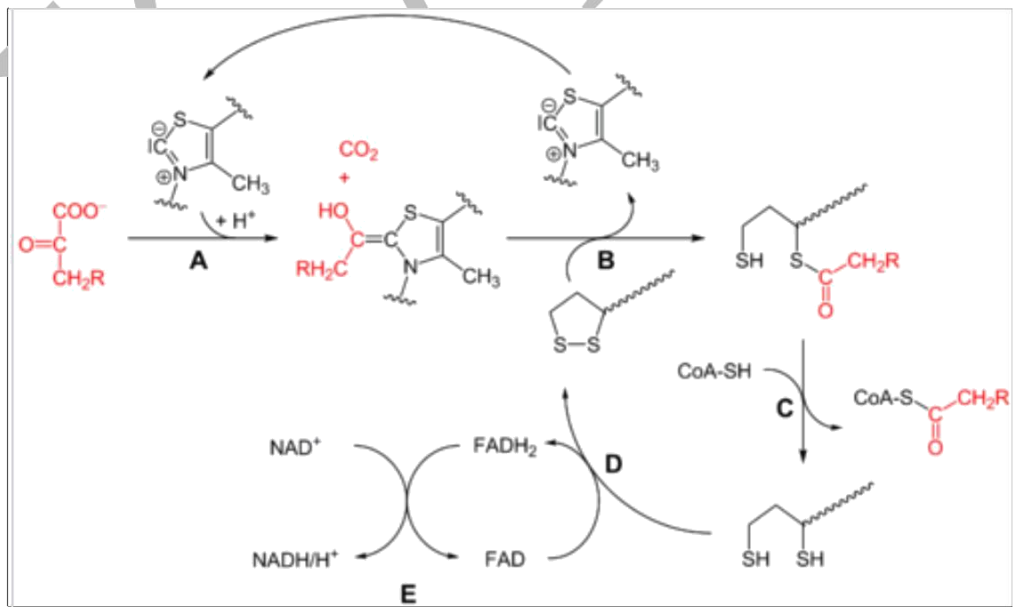
Multienzyme systemA complex of enzymes within a cell that form a reaction sequence of a biochemical pathway so that the product of the first enzyme reaction is transferred directly to the next enzyme and immediately undergoes a second reaction, and so on. The rate of an enzyme reaction often depends on the concentration of the enzyme and the substrate, both being required in relatively high amounts.

Multienzyme systems, such as those involved in RNA and protein synthesis, help maintain a high rate of cellular metabolism since the intermediate products are transferred directly to the next enzyme and are therefore not required in large concentrations.

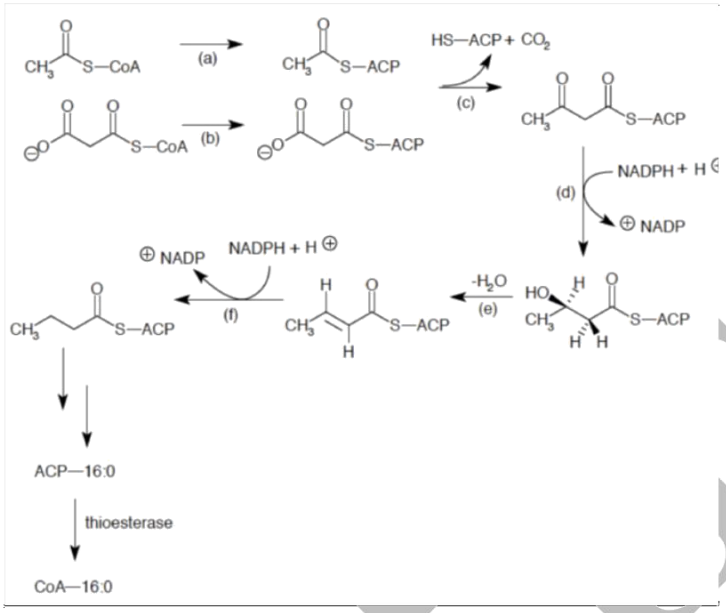
Many enzymes in living cells catalyse chains of reaction in a sequential order either in a biosynthetic or a catabolic pathway. In the preceding paragraphs hypothetical examples of such pathways have been cited.

The series of enzymes catalyzing such chains of reactions are said to form a multi-enzyme system. In its simplest form, the enzymes of such a system remain free in the cytosol as independent entities each interacting with its own substrate which is also present in -the cytosol. The product formed from each reaction is liberated and is acted upon by another enzyme of the sequence. Some of the multi-enzyme systems may operate in a different way, when the enzymes are closely associated with each other to form a multi-enzyme complex.

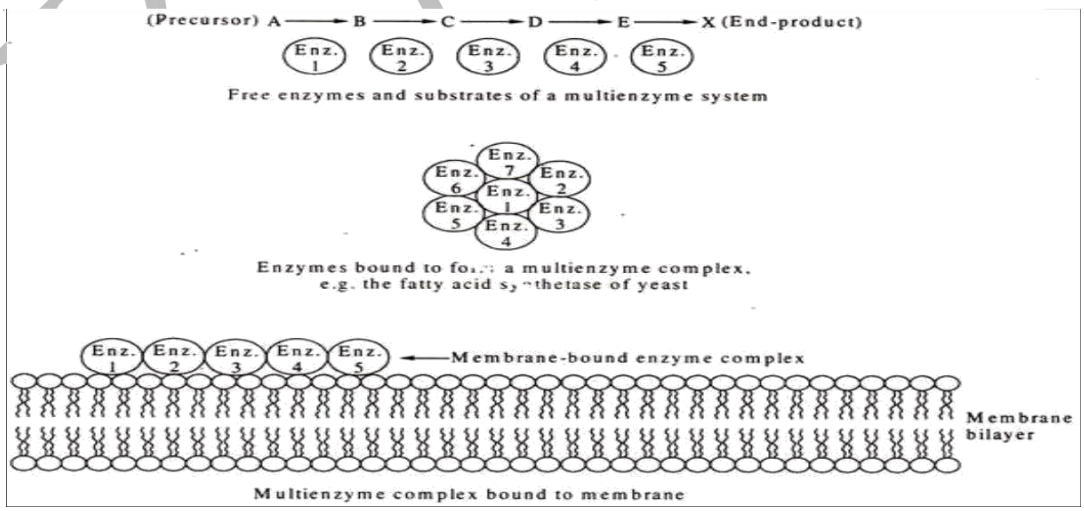
Pyruvate dehydrogenase



Fatty acyl synthase - Mechanism



The fatty acid synthetase of yeast provides an example of a multi-enzyme complex. It consists of seven different enzymes which form a tightly bound cluster. Each enzyme of the complex catalyzes a different reaction, ultimately producing a long- chain fatty acid.



They are **physically distinct forms of the same enzymeactivity**. Multiple molecular forms of an enzyme aredescribed as iso-enzymes or isozymes. If 50 paise coinsridges on the rims andnumber of dots below the year. In the market, all these coins have the same face value;but to an experienced numismatist, these variations willexplain from which mint it was produced. In the sameway, different molecular forms of the same enzymesynthesized from various tissues are called iso-enzymes.Hence, study of iso-enzymes is very useful to understandDiseases of different organs. If the subunits are all the same,the protein is a homomultimer represented by a singlegene. If the subunits are different, protein is said to be aheteromultimer, produced by different genes.

Iso-enzymes may be formed in Different Ways

1. They may be products of different genes (more than one locus) inwhich case they are known as **true iso-enzymes**. The genes maybe located on different chromosomes, e.g. salivary and pancreaticamylase.

2. In certain cases, all the different forms are present in the sameindividual, e.g. **Lactate dehydrogenase** (LDH) alleles (alternateforms). Such alleleiciso-enzymes are called **allozymes**. In thiscase, only one form will be present in one individual; but all thedifferent forms will be in total population.

For example, morethan 400 distinct forms of **glucose-6-phosphate dehydrogenase**(GPD) have been identified; all of them are produced by the samelocus on the X-chromosome. When iso-enzymes due to variationat a single locus occur with appreciable frequency (more than 1%in population), it is said to be **polymorphism.**

4. Molecular heterogeneity of enzymes may also be produced afterthe protein is synthesized (post-translational modification). Theseare called **iso-forms**, e.g. sialic acid content in alkaline phosphatase(ALP) iso-enzymes. Different types of iso-forms may be seen inthe same individual.

Identification of Iso-Enzymes

1. In Agar gel or polyacrylamide gel **electrophoresis**, the isoenzymeshave different mobility. LDH, CK and ALP iso-enzymescan be separated by electrophoresis.

2. **Heat stability**: One of the iso-enzymes may be easily denatured byheat, e.g. bone iso-enzyme of ALP (BALP).

3. **Inhibitors**: One of the iso-enzymes may be sensitive to oneinhibitor, e.g. tartrate labile ACP.

4. Km value or **substrate specificity** may be different for iso-enzymes,e.g. glucokinase has high Km and hexokinase has low Km for glucose.

5. **Co-factor** requirements may be different for iso-enzymes.Mitochondrial isocitrate dehydrogenase is NAD+ dependent andthe cytoplasmic iso-enzyme is NADP+ dependent.

Short Answers (2 marks)

1. What are the various methods of regulation of enzyme activity?
2. How is the activity of single enzyme regulated?
3. Write a note on end product inhibition.
4. Write a note on feedback inhibition.
5. Add a note on reversible covalent modification.
6. Define zymogen.
7. Define multi enzyme complex.
8. Add a note on multi enzyme complex with example.
9. Define isoenzymes.
10. Give a brief note on isoenzymes with example.

Long Answers (8 marks)

1. How are enzymes regulated by end product inhibition? Explain with example.
2. Explain feedback inhibition with example.
3. How is glycogen phosphorylase regulated?
4. Explain about proteolytic cleavage.
5. Explain multi enzyme complex with one example.
6. Describe fatty acyl synthase as multienzyme complex.
7. Define isoenzyme. Write the properties and physiological significance of lactate dehydrogenase.

Karpagam academy of higher education
Department of biochemistry
Enzymes (18BCU202) MCQ
UNIT IV

S.NO	QUESTION	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER
1	The negative allosteric effector of ATCase is	ATP	**CTP**	UTP	Mg2+	**CTP**
2	ATCase is inhibited by	**end product**	substrate	external inhibitor	alanine	**end product**
3	The positive allosteric effector of aspartate transcarbamoylase is	CTP	**ATP**	UTP	GTP	**ATP**
4	An allosteric enzyme is	present at the end of a pathway	generally catalyses a reversible reaction	**generally catalyses a functionally irreversible reaction**	possesses only substrate site	**generally catalyses a functionally irreversible reaction**
5	An allosteric enzyme is generally inhibited by	initial substrate of the pathway	substrate analogues	product of the reaction catalysed by allosteric enzyme	**product of the pathway**	**product of the pathway**
6	Initial velocity versus substrate concentration plot of allosteric enzymes is	straight	hyperbolic	hyperparabolic	**sigmoidal**	**sigmoidal**
7	Binding of an allosteric inhibitor to an allosteric enzyme is	covalent	**reversible**	co-operative	all of the above	**reversible**
8	Kinetics of an allosteric enzyme are explained by	MM equation	LB plot	**Hill plot**	Eadie and Hofstee plot	**Hill plot**
9	Allosteric enzymes have all the following properties except	**they are made up of a single polypeptide chain**	enzyme substrate binding is co-operative	substrate concentration versus velocity plot is sigmoidal	they are subject to positive as well as negative regulation	**they are made up of a single polypeptide chain**
10	The M4 isoform of LDH is present in all except	liver	**heart**	skeletal muscle	RBC’s	**heart**
11	H4 activity is stable and M4 activity is affected at the temperature	160°C for 30 minutes	50°C for 15 minutes	**60°C for 30 minutes**	70°C for 30 minutes	**60°C for 30 minutes**
12	The substrate used for H4 activity is	oxalate	**hydroxy butyrate**	isocitrate	urea	**hydroxy butyrate**
13	The isoform of LDH, H4 is strongly inhibited by	**oxalate**	hydroxy butyrate	isocitrate	urea	**oxalate**

14	The isoform of LDH, M4 is inactivated by	oxalate	hydroxy butyrate	isocitrate	**urea**	**urea**
15	Feedback inhibition of pyrimidine nucleotide synthesis can occur by which of the following ?	Increased activity of Carbamoyl phosphate synthetase	Increased activity of Aspartate transcarbamoylase	**CTP allosteric effects**	UMP competitive inhibition	**CTP allosteric effects**
16	Which is the rate limiting step of pyrimidine synthesis that exhibits allosteric inhibition by cytidine triphosphate	**Aspartate transcarbamoylase**	Hypoxanthine Guanine phosphoribosyl	Thymidylate synthase	Xanthine oxidase e) PRPP synthetase	**Aspartate transcarbamoylase**
17	Which of the following contributes nitrogen atoms to both purine and pyrimidine rings ?	**Aspartate**	Carbamoyl phosphate	Carbon dioxide	Glutamate	**Aspartate**
18	Which of the following is an analogue of hypoxanthine ?	Ara C	**Allopurinol**	Ribose phosphate	PRPP	**Allopurinol**
19	Which statement best describes Xanthine ?	It is a direct precursor of Guanine	It covalently binds to Allopurinol	**It is oxidized to form Uric acid**	It is oxidized to form Hypoxanthine	**It is oxidized to form Uric acid**
20	The predominant isozyme of LDH in cardiac muscle is:	**LD-1**	LD-2	LD-3	LD-5	**LD-1**
21	An enzyme that catalyzes the conversion of an aldose sugar to a ketoses sugar is classified as	transferases	ligases	coxidoreductases	**isomerizes**	**isomerizes**
22	In noncompetitive enzyme action	apparent Km is increased	apparent Km is decreased	Vmax is increased	**concentration of active enzyme molecule is**	**concentration of active enzyme molecule is reduced**
23	In competitive enzyme action	**apparent Km is increased**	apparent Km is decreased	Vmax is increased	Vmax is increased	**apparent Km is increased**
24	How many isoenzyme forms are there for LDH enzyme.	2	4	**5**	8	**5**
25	The protein portion conjugated enzyme is called	**Apo enzyme**	co enzyme	holo enzyme	cofactor	**Apo enzyme**
26	which of the following could be a component of a conjugated enzyme.	Apo enzyme	co enzyme	**more than one correct response**	co factor	**more than one correct response**
27	Enzyme cofactors that bind covalently at the active site of an enzyme are referred as	cosubstrates	**prosthetic groups**	holo enzyme	cofactor	**prosthetic groups**

28	An allosteric activator	increases the binding affinity	decreases the binding affinity	stabilizes the R state of the protein	**both a and b**	**both a and b**
29	an apoenzyme	includes non-protein compounds such as metalions	cconsists of complex organic structure	**Is the protein portion of the enzyme without the cofactors**	stabilizes the R state of the protein	**Is the protein portion of the enzyme without the cofactors**
30	NAD^+ ,FAD and FMN are all factors for	transferases	ligases	**oxidoreductases**	isomerizes	**oxidoreductases**
31	A competitive inhibitor of an enzyme works by	**fitting into the enzyme's active site**	fitting into the allosteric site of the	increases the binding affinity	decreases the binding affinity	**fitting into the enzyme's active site**
32	If an enzyme is described by the Michaels menten equation , a competitive inhibitor will.	decrease the Km and decrease Vmax	decrease the Km , but not the Vmax	always just change the Vmax	**increase the Km but not change the Vmax**	**increase the Km but not change the Vmax**
33	the most likely effect of a non competitive inhibitor on an Michaels menten enzyme is to	Increase the Vmax	**Decrease the Vmax**	always just change the Vmax	increase the Km but not change the Vmax	**Decrease the Vmax**
34	An organic substance bound to an enzyme and essential for its activity is called	**Apo enzyme**	co enzyme	holo enzyme	iso enzyme	**Apo enzyme**
35	Glycogen phosphorylase which mobilizes glycogen for energy , requires which of the following as a cofactor	**pyridoxal phosphate**	tetra hydrofolate	adenosyl cobalamine	co enzyme	**pyridoxal phosphate**
36	the enzymes present in lysosomes are	transferases	isomerases	**hydrolases**	lyases	**hydrolases**
37	all the following gastrointestinal enzymes are secreted as zymogens except.	**ribonucleases**	pepsin	cymotrypsin	trypsin	**ribonucleases**
38	LDH belongs to which main class of enzyme	transferases	ligases	**oxidoreductases**	isomerizes	**oxidoreductases**
39	The enzyme LDH5 is typically increased in patients	**acute viral hepatitis**	bacute myocardial infarction	acute pancreatitis	viral hepatitis	**acute viral hepatitis**
40	Enzyme-catalyzed modifications are	reversible	irreversible	**both A and B**	none of above	**both A and B**
41	Covalent modification is means of changing	**chemical properties**	physical properties	bonding on amino	pH of amino acids	**chemical properties**
42	Which of the following statements about the control of enzyme activity by phosphorylation is correct?	Enzyme control by phosphorylation is irreversible.	Phosphorylation of enzymes is carried out by phosphoprotein	Phosphorylation of enzymes only occurs at specific tyrosine residues.	**phosphorylation of an enzyme results in a conformational**	**phosphorylation of an enzyme results in a conformational change**

43	The enzymes involved in feedback inhibition are called	**Allosteric enzymes**	Holo enzymes	Apo enzymes	Coenzymes	**Allosteric enzymes**
44	Which of the following statements best describes an allosteric binding site	It is a binding site containing amino acids with aliphatic side chains.	It is a binding site that can accept a wide variety of differently shaped molecules.	**It is a binding site, which is separate from the active site, and affects the activity of an enzyme when it is occupied**	t is a description of an active site which has undergone an induced fit	**It is a binding site, which is separate from the active site, and affects the activity of an enzyme when it is occupied by a ligand.**
45	The first step in the zymogen activation of chymotrypsinogen is	Binding of trypsinogen activator	**Cleavage by trypsin**	Folding into the native structure	Self-digestion by chymotrypsin	**Cleavage by trypsin**
46	The aspartate transcarbamylase (ATCase) reaction is controlled by CTP acting as a	allosteric substrate	feedback product	**heterotrophic inhibitor**	homotropic inhibito	**heterotrophic inhibitor**
47	An enzyme which requires a biological change in order to become active is called	Transferase	**zymogen**	hydrogenase	trypsin	**zymogen**
48	On top of active site, allosteric enzymes contain	inhibitors	substrate	**allosteric site**	polypeptide chains	**allosteric site**
49	irreversible modifications require synthesis of	enzymes	carbohydrates	vitamins	**proteins**	**proteins**
50	In non-competitive inhibition extent of inhibition depends only on	concentration of enzyme	concentration of substrate	**concentration of inhibitor**	**both A and B**	**concentration of inhibitor**
51	in competitive inhibition, two things that binds to enzyme active site are	substrate	inhibitor	catalyst	**both A and B**	**both A and B**
52	which statement is false about covalent modification	it is reversible	it is slower than allosteric regulation	**it is irreversible**	phosphorylation is a common covalent modification	**it is irreversible**
53	allosteric enzymes are	similar to simple enzyme	smaller than simple enzyme	**larger and more complex than simple**	all of the above	**larger and more complex than simple**
54	de novo synthesis of fatty acids is catalyzed by a multi enzyme complex which contains	one –SH groups	two –SH groups	three –SH groups	**four –SH groups**	**four –SH groups**
55	formation of acetyl CoA from pyruvate for de novo synthesis of fatty acids requires	pyruvate dehydrogenase	citrate synthase	ATP citrate lyase	**all of the above**	**all of the above**

56	the enzyme regulating extramitochondrial fatty acid synthesis is	thioesterase	**acetyl CoA carboxylase**	acyl transferase	multi enzyme complex	**acetyl CoA carboxylase**
57	acetyl CoA required for de novo synthesis of fatty acids is obtained from	breakdown of existing	ketone bodies	acetate	**pyruvate**	**pyruvate**
58	**Which of the following amino acids would be prone to a reaction with an irreversible inhibitor?**	RUBISCO	PEP Carboxylase	Phosphofructokinase	**All of these**	**All of these**
59	β oxidation of fatty acids is inhibited by	NADPH	**Malonyl CoA**	Acetyl CoA	Pyruvate dehydrogenase	**Malonyl CoA**
60	Which of the following is a typical example ‘feedback inhibition’?	Cyanide and cytochrome reaction	Sulpha drugs and folic acid synthesizer bacteria	**allosteric inhibition of hexokinase by glucose6 phosphate**	Reaction between succinic dehydrogenase and succinic acid	**allosteric inhibition of hexokinase by glucose6 phosphate**

UNIT-V

SYLLABUS

Coenzymes in enzyme catalysed reactions and Applications of enzymes. Structure and Functions of TPP, FAD, NAD, pyridoxal phosphate, biotin, coenzyme A, tetrahydrofolate, lipoic acid. Applications of enzymes - Application of enzymes in diagnostics (SGPT, SGOT, creatine kinase, alkaline and acid phosphatases), enzyme immunoassay (HRPO), enzyme therapy (Streptokinase). Immobilized enzymes – Preparation techniques and its applications.

Coenzymes:

Definition: Coenzymes are organic compounds required by many enzymes for catalytic activity. They are often vitamins or derivatives of vitamins. Sometimes they can act as catalysts in the absence of enzymes but not so effectively as in conjunction with an enzyme. Coenzymes which are prosthetic groups form an integral part of the active site of an enzyme and undergo no net charge as a result of acting as a catalyst.

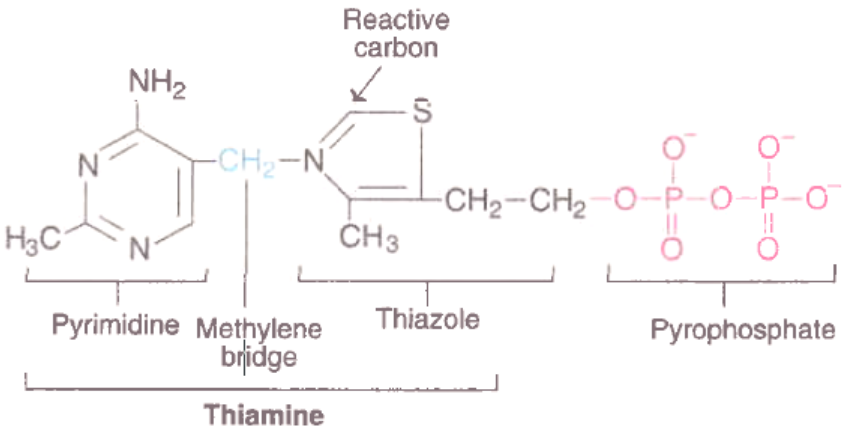
The functional enzyme is referred to as holoenzyme which is made up of a protein part (apoenzyme) and a non protein part (coenzyme). The term prosthetic group is used when a non protein moiety is tightly bound to the enzyme which is not easily separable by dialysis.

Coenzymes are second substrates:

Coenzymes are often regarded as the second substrates or co-substrates. Since they have affinity with the enzyme comparable with that of the substrates. Coenzymes undergo alterations during the enzymatic reactions which are later regenerated. This is in contrast to the substrate which is converted to the product.

Thiamine pyrophosphate:

Structure:



Properties:

It is derived from vitamin B_1 thiamine. The thiazole ring can lose a proton to produce a negatively charged carbon atom.

This is a potent nucleophile and can participate in covalent catalysis, particularly with α keto (oxo) acid decarboxylase, α keto acid oxidase, transketolase and phosphoketolase enzymes.

For example,

1. Pyruvate decarboxylase found in yeast and some other microorganisms utilizes TPP to catalyse the production of acetaldehyde from pyruvate.

The actual decarboxylation step is facilitated by electrophilic catalysis as the thiazole ring withdraws electrons. The reaction will proceed in the absence of enzyme but the acetaldehyde formed tends to react with the TPP – C – OH complex to produce acetoin as

CH_3

the final product. It is likely that the enzyme stabilizes the TPP-acetaldehyde complex and prevents this condensation from occurring.

Another example is the multi enzyme complex known as pyruvate dehydrogenase also catalyses the decarboxylation of pyruvate. But it utilizes a second coenzyme lipoic acid to introduce an oxidation step and a third coenzyme coenzyme A to react with the acetyl lipoamide complex

giving acetyl coA as a final product. The TPP – C – OH complex is

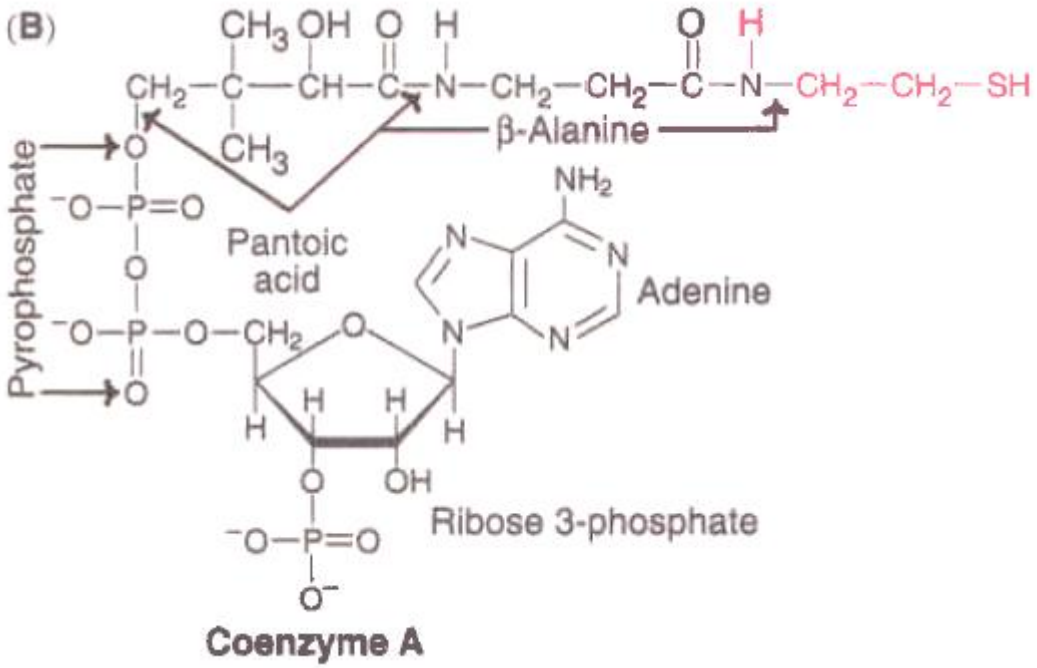
CH_3

Formed.

1. The enzyme pyruvate dehydrogenase catalyses (oxidative decarboxylation) the irreversible conversion of pyruvate to acetyl CoA. This reaction is dependent on TPP, besides the other coenzymes.
2. cr-Ketoglutarate dehydrogenase ls an enzyme of the citric acid cycle. This enzvme is comparable with pyruvate dehydrogenase and requiresTPP.
3. Transketolase is dependent on TPP. This is an enzyme of the hexose monophosphate shunt (HMP shunt).
4. The branched chain a-keto acid dehydrogenase (decarboxylase) catalyses the oxidative decarboxylation of branched chain amino acids (valine, leucine and isoleucine) to the respective keto acids. This enzyme also requires TPP.
5. TPP plays an important role in the transmission of nerve impulse. lt is believed that TPP is required for acetylcholine synthesis and the ion translocat on of neural tissue.

Coenzyme A:

Structure:



Coenzyme A

It was first originally discovered as a coenzyme for acetylation in liver and microorganisms by Lipmann in 1947.

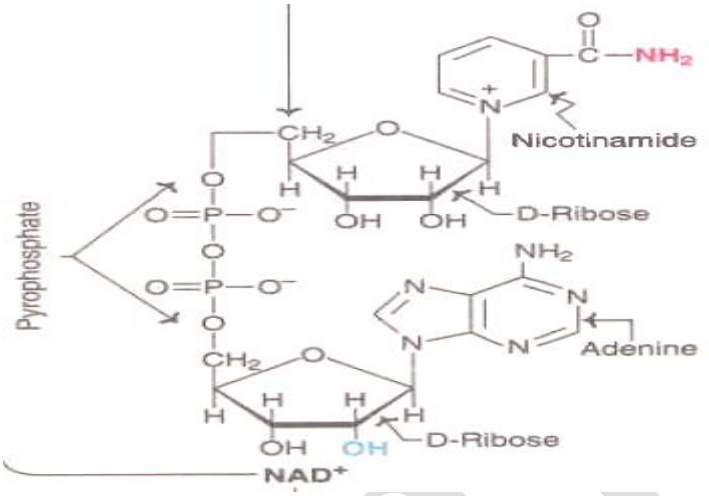
Properties:

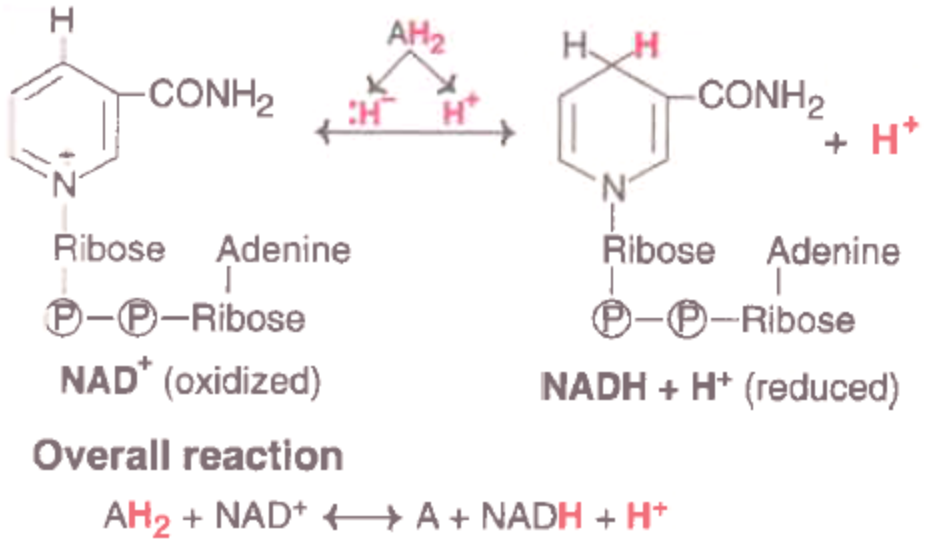
It is a colourless substance having an absorption band in the uv at 257 nm due to the adenine residue. It is derived from the vitamin panthothenic acid. It is mainly involved in acyl group transfer reactions. Mainly it act as coenzyme for thiokinase enzymes.

- The acyl groups can be added to the thiol group of coenzyme A either by the transfer of the acyl group from another molecule or by a synthetase reaction.
- All the enzymes of the sub group 6.2.1 bring about the formation of coenzyme A thiolesters from free acids making use of the energy of ATP or in two cases GTP.
- Of the 63 acyl transferases of group 2.3.1. 56 transfer acyl groups to or from coenzyme A. In 32 of these cases it is an acetyl group that is transferred. The acyl transfer may be from combination with another S atom (6 cases), from N (19 cases), from O (22 cases), from C (7 cases) or from phosphate (2 cases). In these systems coenzyme A is acting as an acyl carrier in the transfer of acyl groups from one molecule to another.
- It is also involved in reactions of another type, in which the acyl group transferred to the coenzyme A is removed from the substrate by a lyase-type reaction leaving a double bond. Of the 31 lyases of groups 4.1.3.16 involve coenzyme A as the acyl-acceptor.
- In the reverse direction the acyl group (usually acetyl) is added to a double bond in the substrate with the synthesis of a more complex molecule. The synthase reactions of this type produce a number of important metabolites including citrate, malate and hydroxyl methyl glutaryl coA.
- A number of enzymes using coenzyme A can also use simple analogues of the coenzyme for example N- acetyl cysteamine; others, however are specific for coenzyme A itself.

Nicotinamide nucleotides:

It is derived from the vitamin niacin.





Overall reaction

$$AH_2 + NAD^+ \longleftrightarrow A + NADH + H^+$$

The reduction of NAD^+ to NADH requires two reducing equivalents per molecule: one electron (e^-) and one hydrogen atom ($H=H^+ + e^-$), which together may be regarded as a hydride ion (H^-) add to the pyridine ring of nicotinamide. $NADP^+$ is also identical to NAD^+ except that the 2' position of D-ribose unit attached to adenine is phosphorylated. Both are acting as coenzymes. Enzymes utilizing NAD^+ usually have a catabolic function. The NADH produced being an energy source for the cell. Anabolic enzyme utilize NADPH as coenzyme.

Both NAD^+ and $NADP^+$ act as coenzyme in a changed form at the endof the reaction. Horse liver alcohol dehydrogenase involve NAD^+ as coenzyme in the catalytic oxidation of primary or secondary alcohols. NAD^+ is the first substrate to bound and NADH is the last product to leave. The dissociation of NADH from the enzyme being the rate limiting step of the

overall reaction. This enzyme is a dimer, each subunit containing one binding site for NAD^+ and two sites for Zn^{2+}.

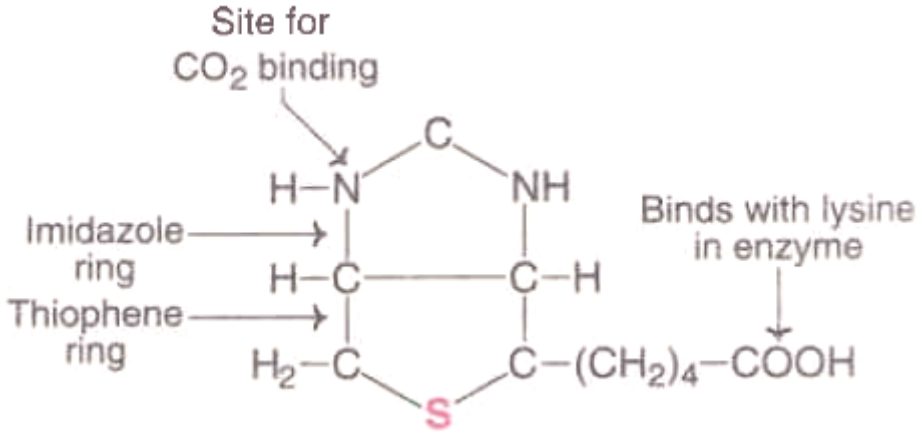
Another example is dog fish muscle lactate dehydrogenase which catalyses the reaction:

It is a tetrameric enzyme, each subunit having a binding site for NAD^+ ; no metal ions are bound. The coenzyme first binds to the enzyme and bringing about a conformational change in the enzyme which enables the substrate to bind. The substrate binding in turn causes a peptide loop of the enzyme to close over the active site. The reaction is completed by the transfer of a hydride ion to NAD^+ , in the same orientation as with alcohol dehydrogenase.

Biotin:

Biotin is always found firmly bound to a side chain amino group of one of the lysine residuesof a protein. Protein bound biotin can link with CO_2 to form a biotin carboxyl carrier protein (BCCP), which is important in carboxylation reactions. Example is that catalysed by acetyl coA carboxylase.

Structure:



Properties:

Acetyl coA carboxylase from *E-coli* dissociate into three distinct subtypes:

(1) BCCP (2) Biotin carboxylase(3) Carboxyl transferase

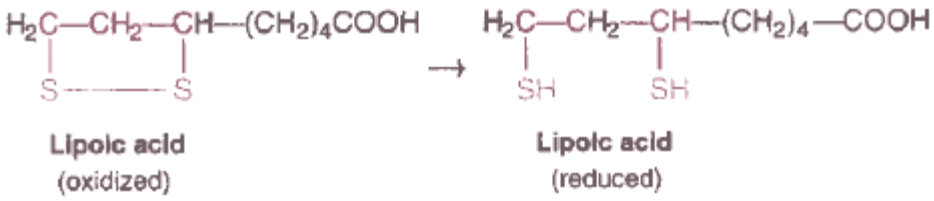
The BCCP appears to act as a flexible arm transporting the CO_2^- from the active site of biotin carboxylase to that of carboxyl transferase where it is presented to the acetyl coA.

Lipoic acid:

It is an important and widely distributed hydrogen carrier.

Structure:

Lipoic acid exist in both oxidized and reduced form.



The essential change is the reduction of a disulphide group to two thiol groups. Here one of the carbon atom is asymmetric. So two optical isomers exist. The natural isomer of the oxidized form is dextro rotator but gives rise on reduction to the laevo rotator reduced form. Only the natural isomers are active in the pyruvate oxidase system. A considerable part of the lipoate in cells appears to exists bound to protein, attached by a peptide bond to a lysine group and can be removed by an enzyme from yeast or bacteria.

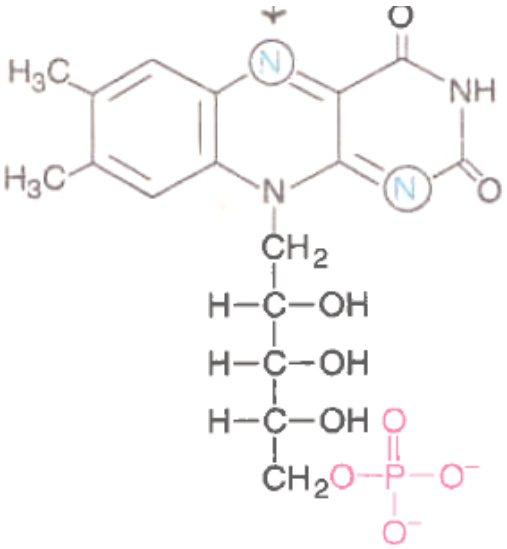
Properties:

In the oxidized form the substance is yellowish with an absorption band at about 335 nm. Is due to the five membered ring this contains. A powerful reducing agents such as zinc in GCl open the ring by the reduction of the disulphide bond. It is reduced by pyruvate and by 2-oxoglutarate in the presence of their respective dehydrogenases and thiamine diphosphate. In these cases the reduction is accompanied by the transfer of an acyl group from the substrate through thiamine to the lipoate, giving acetyl hydrolipoate and succinyl hydro lipoate respectively. The reduced lipoate after removal of any attached acyl group is oxidized by NAD^+ in the presence of dihydro lipoamide dehydrogenase. It may also be oxidized by mild chemical oxidizing agents such as iodine.

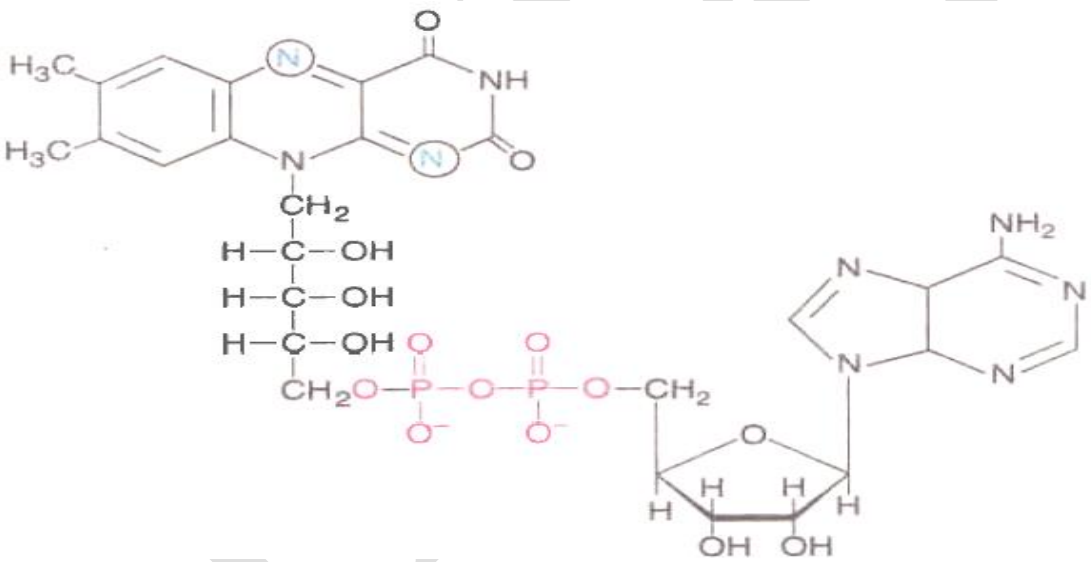
Flavin nucleotides (FMN and FAD):

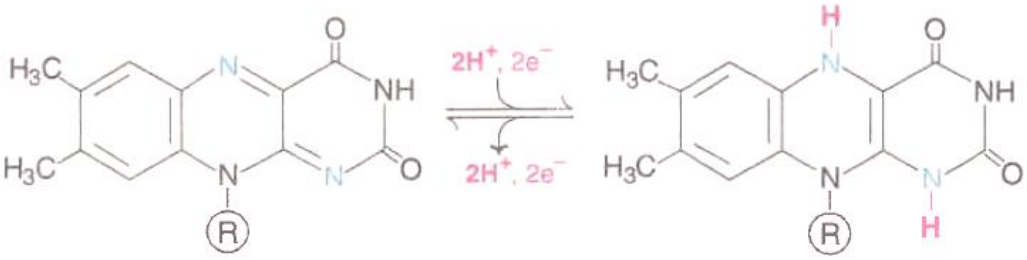
Flavin nucleotides are derived from riboflavin vitamin B_2.

They function in oxidation / reduction reactions. The reducing equivalents being carried by the fused three ringed system of flavin as shown below:



Flavin mononucleotide (FMN)





Oxidized flavin (FMN or FAD)

Reduced flavin ($FMNH_2$ or $FADH_2$)

TABLE 7.2 Selected examples of FAD and FMN dependent enzymes along with their respective reactions

Enzyme	*Reaction*
FAD dependent	
I. ***Carbohydrate metabolism***	
(a) Pyruvate dehydrogenase complex*	Pyruvate ⟶ Acetyl CoA
(b) α-Ketoglutarate dehydrogenase complex*	α-Ketoglutarate ⟶ Succinyl CoA
(c) Succinate dehydrogenase	Succinate ⟶ Fumarate
II. ***Lipid metabolism***	
(d) Acyl CoA dehydrogenase	Acyl CoA ⟶ α, β-Unsaturated acyl CoA
III. ***Protein metabolism***	
(e) Glycine oxidase	Glycine ⟶ Glyoxylate + NH_3
(f) D-Amino acid oxidase	D-Amino acid ⟶ α-Keto acid + NH_3
IV. ***Purine metabolism***	
(g) Xanthine oxidase	Xanthine ⟶ Uric acid
FMN dependent	
L-Amino acid oxidase	L-Amino acid ⟶ α-Keto acid + NH_3

* *Dihydrolipoyl dehydrogenase component requires FAD*

FMN and FAD are prosthetic groups. They cannot be separated from the protein without denaturing it. The protein flavin nucleotide complex being termed a flavoprotein. Because the flavin nucleotide doesnot have an independent existence, reactions catalysed by flavoproteins usually involves the transfer of reducing equivalents from a donor via the flavin to some specific external acceptor. For example, glucose oxidase, which catalyses the reaction which utilizes FAD as prosthetic group and O_2 as hydrogen acceptor.

With some flavoproteins the reduction of the flavin has been shown to be a two step process involving an unstable free radical semiquinone as intermediate:

Many flavoproteins are also metalloproteins and one of the roles of the metal ion could be to stabilize this semiquinone.

Not all the reactions involving flavin nucleotides as coenzymes proceed via an identical mechanism: the reaction catalysed by NADH dehydrogenase has been shown to involve semiquinone formation, but that catalysed by glucose oxidase does not. Similarly, the reoxidation of E – $FADH_2$ can proceed by a variety of mechanisms:where the molecular oxygen is the acceptor, the products may be H_2O_2 (with oxidases), H_2O and hydroxylated products (with hydroxylases), or the superoxide anion ($O2^{\cdot -}$) and flavin semiquinone.

Flavin dependent disulphide oxidoreductase enzymes, of which glutathione reductase is an example, use FAD to shuttle reducing equivalents from NADPH (or sometimes NADH) to a cysteine residue, which generally involves the cleavage of a disulphide bridge. Usually two electrons are transferred simultaneously to FAD, so it never passes through the semiquinone form. Finally, the substrate is reduced by the cysteine.

Pyridoxal Phosphate (PAL):

Pyridoxal phosphate is a coenzyme associated with — transaminases which catalyse transfer of amino groups from amino acids to keto acids. In this transfer process, PAL acts as the acceptor of the amino group and is converted to pyridoxamine phosphate (PAM).

PAM can react with a keto acid to produce an amino acid. PAL and PAM remain bound to the protein part of the transaminase enzyme during these transfer of amino group. The reactions catalysed by transaminases can be represented in a simple way as shown in Fig. 8.36. Pyridoxal phosphate has a simple molecule containing the B-vitamin, pyridoxine.

The structures of PAL and PAM are shown in Fig. 8.36:

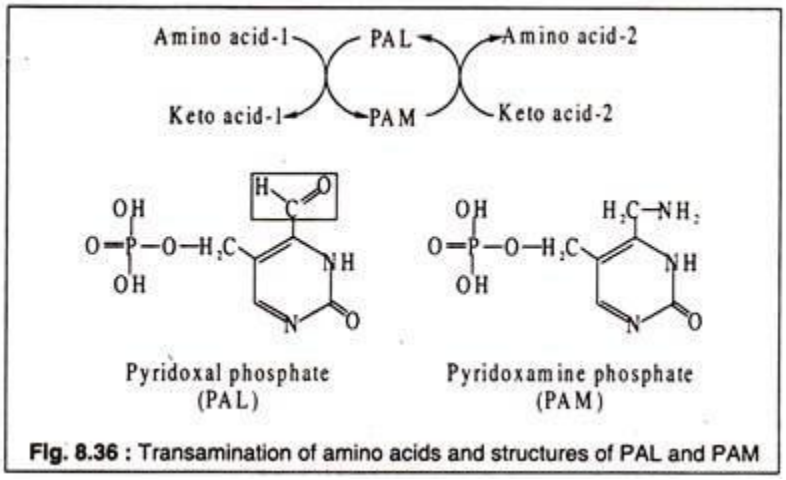


Fig. 8.36 : Transamination of amino acids and structures of PAL and PAM

The aldehyde group of PAL is the reactive group of the coenzyme which binds to the amino acid forming a Schiff s base.

The details of transaminase reaction

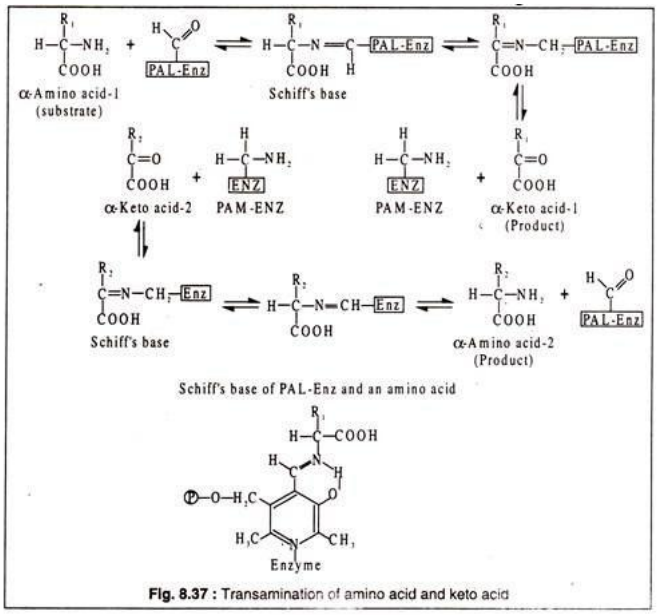
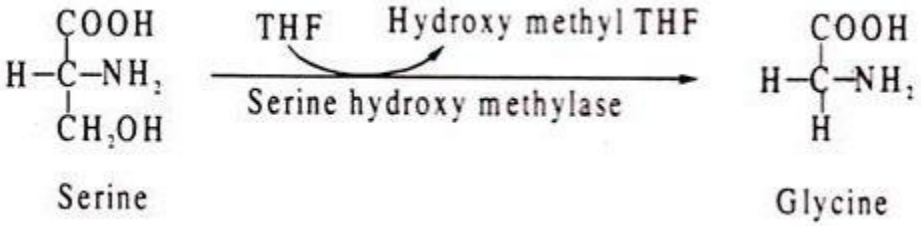


Fig. 8.37 : Transamination of amino acid and keto acid

Tetrahydrofolic acid

Tetrahydrofolic acid (THF)

Tetrahydrofolic acid (THF) acts as coenzyme for enzymes involved in transfer of one-carbon fragments, like formyl, methyl and methenyl groups. An example of a reaction involving THF is conversion of homocysteine to methionine. The methyl group of methionine is added from methyl-THF. Another THF mediated reaction is conversion of serine to glycine where the hydroxy-methyl group of serine is removed by THF.



Diagnostic applications of enzymes

Introduction:

Assays of some of the enzymes present in blood plasma or serum are carried out routinely in most clinical chemistry laboratories and these play an important role in diagnosis. If the cells of a particular tissue are affected by disease many of them no longer have intact membranes. Then their contents will leak out into the blood stream at an increased rate and the enzymes associated with these cells will be found in plasma in elevated amounts. Plasma enzyme assay can help to identify the location of damaged cells.

Enzymes are biocatalysts and are made up of amino acid or they are proteins. All enzymes are protein but all proteins are not enzymes. Enzymes can be differentiated between one another and they can be used as diagnostic tool to detect some diseases in human. Enzyme concentration varies in diseased condition therefore variation in enzyme concentration can be used as tool in detecting disease or disorders related to the particular part of the human body. Enzyme concentration can be detected at plasma level, serum level or cellular level. Enzyme concentration depends on factors like enzyme formation, release into circulation and also enzyme clearance and cellular leakage of enzymes. Variation in enzyme concentration from the normal indicates disease or disorder in human.

Lactate Dehydrogenase and Disease:

1. Lactate dehydrogenase converts puyruate to lactate in a reversible manner. This enzyme exists in 5 forms such as LDH-1, LDH-2, LDH-3, LDH-4 and LDH-5. In other words lactate dehydrogenase is an isoenzyme and its normal concentration in body is 60-250 IU/L.
2. Lactate dehydrogenase is an important biological marker.
3. Disease of liver, heart, muscle and some form of cancer can be detected using Lactate dehydrogenase.
4. Lactate dehydrogenase concentration increases within 12hrs after myocardial infarction, therefore this can be used as indicator of myocardial infarction.
5. Lactate dehydrogenase concentration also increases during leukemia, renal cell necrosis, hepatic necrosis, carcinomas, muscular dystrophy and many more conditions.

Creatinine Phosphokinase and Disease:

1. Creatinine phosphokinase enzyme converts creatinine into phosphocreatinine using energy rich molecule such as ATP. This also exists in three different forms such as CPK-1, CPK-2, CPK-3. Normal concentration of creatinine phosphokinase is 4-60 IU/L.

2. CPK-1 concentration increases in brain injury may be occurred due to trauma, stoke or bleeding in the brain.
3. CPK-1 concentration also increases during lung injury in human.
4. CPK-2 level increases after myocardial stroke or heart attack, and also during viral infection of the heart muscle cells.
5. CPK-3 concentration increases in skeletal muscle injuries, muscular dystrophy and also after strenuous exercise.

Alkaline Phosphatase and Disease:

1. Alkaline phosphatase catalyses the reaction of phosphoric acid from monophosphate esters. It is also isoenzymes and occurs in six different forms.
2. Alkaline phosphatase level increases during liver damage due to hepatocarcinoma, liver cirrhosis, and much more.
3. Alkaline phosphatase level increases during ostioblastic activity in children.
4. Alkaline phosphatase level increases during hyperparathyroidism.
5. Alkaline phosphatase level increases during rickets, ostiomalacia.
6. Alkaline phosphatase level decreases during anemia.
7. Alkaline phosphatase level decreases during scurvy.
8. Alkaline phosphatase level decreases during defective calcification

Acid Phosphatase and Disease:

1. Acid phosphatase enzyme catalyses formation of phosphoric acid from monophosphate ester. Occur in two forms such as prostatic ACP and non-prostatic ACP.
2. Acid phosphatase enzyme used as biological marker of prostate cancer.
3. Acid phosphatase enzyme level are increased during conditions such as breast cancer, hyperparathyroidism, leukemia.

Serum Glutamate Oxaloacetate Transferase and Disease:

1. Serum glutamate oxaloacetate trasferase enzyme act as marker of myocardial infraction and also other forms of heart diseases.
2. Serum glutamate oxaloacetate trasferase enzyme level also increases during kidney damage, liver damage, skeletal muscle damage.

Conclusion: Enzymes not only help in biological reactions of the body but also can be used as markers in detecting disease or any other abnormal conditions in the body of a human.

ENZYME IMMUNO ASSAY

INTRODUCTION

Enzyme immunoassay (EIA) or enzyme linked immunosorbant assay (ELISA) is a powerful technique used for detecting and quantifying antigens and antibodies in clinical samples. It is widely used in clinical laboratories for diagnoses, prognoses and for monitoring immune responses.

The general principle of this technique is based on the binding of conjugated enzyme molecule with specific antibodies to detect and quantify the presence of either antigens or antibodies in the test sample. This is followed by adding appropriate colourless substrate which catalyses the interaction complex to produce a visible coloured product. There are many chromogenic substrates used in ELISA technique but the most common are alkaline phosphatase (AP) and horseradish peroxidase (HRP). The end product can be determined by using spectrophotometer and the intensity of colour is directly proportional to the presence of either antigens or antibodies in the test samples.

TYPES

There are several types of ELISA which include: indirect, sandwich, competitive, chemiluminescence and elispot assay. Indirect ELISA can be used to detect the antibodies that are present in patient's serum. With this method, patient's serum containing antibodies is added to the microtitre wells which are coated with the antigen and the intensity of the end colour product is directly proportional to the amount of antibodies that are present in the patient's sample.

The second type of enzyme linked immunosorbant assay is called sandwich ELISA, in which the target antigen that is to be detected must be located between two antibodies. The antigen of interest that is present in the clinical sample is added to a microtitre wells which is coated with the antibody and the end product is determined by spectrophotometer to measure the amount of antigen in the clinical sample.

The development of biotechnology has become widespread and one example of its progress is the modification of ELISA by using chemiluminescence substrate rather than chromogenic substrate to increase the sensitivity of the reaction. With this method, chemilumescence (CL) substrate and some enhancing agents, which have the ability to bind an antibody or antigen in the reaction, are used to create light emissions which can be measured by specific spectrometry. This approach has been used to diagnose several types of tumour disease, and quantify numerous compounds at low concentration.

Another example of ELISA modification is enzyme linked immunospot (ELISPOT) assay. It is a quantitative technique often used to detect cytokines that are secreted from single cell in response to the antigen. During the incubation period, the cytokines of interest is released and captured by immobilised anti-cytokines on the microtitre plate. After washing them and adding

labelled anti-cytokines antibody and substrate, the end colour product displays as a "footprint" around each of the secreting cells, which can be estimated. This technique has great potential for monitoring immune responses. In addition, sensitive ELISPOT assay has the ability to detect low level of secreted IFN- α compared with other IFN-α assays.

APPLICATION

There are many applications of enzyme immunoassays in clinical laboratories and the largest application is related to the detections of viruses. For example, the screening and diagnosis of hepatitis virus is routinely used in the lab. These include the detection of HBsAg and HBeAg by direct ELISA, anti-HBc antibody and anti-HBe antibody by competitive ELISA and anti-HBs antibody by indirect ELISA (Tsitsilonis et al. 2004).What is more, ELISA can detect HCV antibodies in serum samples as screening tests and the positive samples are conformed by molecular HCV RNA.

The beneficial effect of using an enzyme immunoassay in clinical hematology is that it has the ability to identify alloantibodies in patients who frequently receive platelet transfusions for therapeutic purposes. It has been demonstrated that ELISA is more sensitive to the detection of alloantibodies than other immunoassays, such as lymphocytotoxic test (LCT) and the platelet immunofluorescence test (PIFT), and it is performed in a short period of time. Furthermore, enzyme immunoassays are able to detect anti-platelet antibodies, platelet associated IgG (PAIgG) , HLA and circulating antibodies in patients with Idiopathic thrombocytopenic purpura (ITP).

There are several pieces of evidence which suggest that a ubiquitous herpes virus, in particular Epstein-Barr virus (EBV), is implicated in many types of lymphoma such as Hodgkin's disease and Burkitt's lymphoma, so the early detection of EBV antigen specific T cells is essential and therefore ELISPOT assay can be used to analyse T cell responses.

Another of its feature is related to acquired von Willebrand syndrome, which is caused by autoantibodies against von Willebrand factor (vWF). This plays an essential role in hemostasis and is characterised by prolonged bleeding due to the defect in vWF activity. Detection of anti-vWF antibody can be performed by ELISA and mention that competitive ELISA is a useful technique in the detection of anti-vWFA antibodies in a clinical samples.

Despite the benefits of ELISA in the detection of HLA class I specific antibodies, it is less sensitive than flow cytometry, so before platelet transplantation, flow cytometry must be used. Although chemiluminescence immunoassay has great sensitivity in detecting HBsAg in blood donor samples, it has less sensitivity than the polymerase chain reaction (PCR) so to increase the safety of blood from HIV-1 , HCV, and HBV, the nucleic acid amplification technique (NAT) has been applied for the screening of blood donor samples.

CONCLUSION

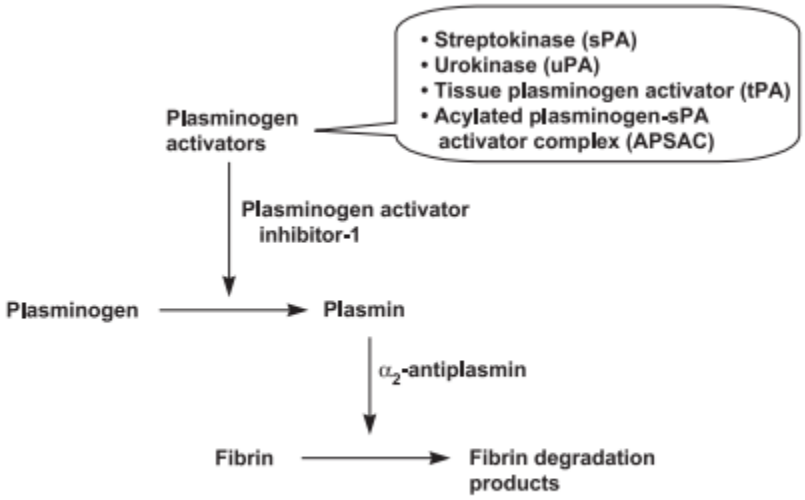
Enzyme Immunoassay is one of the most significant techniques to have been used in clinical laboratories to analyse the antigen-antibody reaction in a short period of time. It is sensitive to screening a large number of clinical samples and therefore has been applied in many clinical laboratories for diagnoses, prognoses and for monitoring immune responses.

Enzyme therapy - Streptokinase

In 1944 it was found that some cultures of streptococci can dissolve the clots of coagulated blood. Active factor here is lytkinaa, later received the name of streptokinase. It does not act directly on fibrin, and makes inert preactivator contained in blood, active substance, which then activates the plasminogen, turning it in plasmin. The first drugs streptokinase poorly tolerated; to introduce them intravenous was possible only subject to a number of precautions. In recent years, thanks to special treatment methods were obtained drugs significantly less side effects. Although in the human body there is no permanent, hereditary deterministic antistreptokinase or inhibitors of streptokinase, each person meets a certain amount of antibodies to streptokinase. Blood contains two such antistreptokinase. One of them is an inhibitor, formed as a result of immune reactions in previous streptococcal infection. As everyone has probably been such infections, this factor can be found almost always, but the title of his different people has very different. Second nonspecific antistreptokinase. Thus, for successful treatment streptokinase is necessary to overcome the effect of antistreptokinase. Therapeutic effect depends on the speed of recovery of initial concentrations of inhibitors streptokinase. As streptokinase is quickly destroyed in the body, and plazmin irreversibly inactivated by relatively large numbers of antiplasmin, effective enough thrombolytic therapy is only possible when continuous addition of streptokinase. If, for example, the patient has a high resistance to streptokinase, when normal dose can develop a heightened state of coagulation with advanced thrombosis; on the contrary, with a small number of inhibitors in the body the same dose may be excessive and may lead to increased fibrinolysis and hemorrhagic diateza.
Individual doses can be calculated, using the test of resistance to streptokinase, or on the basis of thrombo elastographic samples with plasma or whole blood. In the test for resistance to streptokinase determine the least amount of this enzyme, lyse clot formed from nitrate blood of the patient under the action of thrombin. Based on the found values and taking into account the total volume of blood in the body, calculate the number of streptokinase, capable of neutralizing any existing antibody (titrated initial dose"-"titrated initial dose", TID). This dose slowly injected for about 10 minutes, and then at intervals of 1 hour add 2/3 of this amount as a maintenance dose. In recent years the method of determining TID refused. Currently, after a large initial doses (500 000-750 000 E) hourly impose on 100 000 E streptokinase. This method is mobilized all the resources of plasminogen; the danger of hemorrhage minimize, working simultaneously on different clotting factors. With fibrinolytic therapy particular problem secondary thrombosis, since after the dissolution of a blood clot damaged in this area endothelium is present and the emergence of new blood clot especially high here. So after fibrinolytic therapy in each case you should type anticoagulants. This method of treatment makes it possible to dissolve an existing clot; in this case, there is a causal therapy.
When treated with streptokinase decisive criterion for success is not the restoration of patency of the vessel, and restores his lost functions. But back to normal in this sense is possible only in case if fibrinolytic therapy results even before the development of necrosis of tissues for the resumption of blood flow closer to normal. Earlier initiation of therapy is a necessary condition

for optimal results. As shown by clinical experience of recent years, treatment streptokinase apply in the following cases: when fresh (not existed for more than 5 days) for venous or arterial blood clots, heart lung and other diseases thrombotic nature, myocardial infarction and some heart attacks the brain. The success of therapy is possible under certain conditions. Treatment of acute occlusion should be taken within the first 5 days (according to others, 3 days); need some minimal level of collateral circulation, ensuring the delivery of a drug to a blood clot; there should be no specific contraindications.

The General consensus among clinicians, contraindications are: hemorrhagic diathesis; local factors - disintegrating cancer, fresh surgical wounds the first few days postpartum period; from cardiovascular diseases, arteriosclerosis, severe diabetic arteriopathy, subacute bacterial endocarditis. Fibrinolytic therapy was effective in 7 out of 12 cases of acute ischemia of extremities: in these patients after successful use of anticoagulants has been no recurrence. Winkelmann describes his experience of treatment of thromboses of various localization. Out of 23 patients 12 were completely cured and had no recurrence. In 36 cases of acute occlusion of arteries limbs was that when death results are significantly better than in fresh thrombosis. Very good results were obtained when emboli external iliac artery, popliteal arteries and arteries of the upper limb. Thrombolytic therapy is indicated if the obstructions that will not lead to severe ischemia, or by any reason cannot be removed in a surgery way. In acute closures of large vessels (e.g., internal iliac artery) shows the operation. Patel reports on the application of streptokinase for acute blockage of blood clots or emboli. He watched good results in 18 out of 31 cases. Partial success was achieved in 8 cases, 5 failed. If the blockage has been more than 3 days, success is possible only as an exception. There are significant individual differences in the response of blood clots following side effects: fever, and leukocytosis in the first day, allergic reactions, which managed to remove the eye, nausea and bruising at the site of injection. A significant disadvantage of this method of treatment is associated with the antigenicity of streptokinase, leading to sensitization (in most cases 12-14 days). The titer of antibodies to streptokinase often reaches several million units. Therefore repeat its use with an interval of less than one year is possible only in exceptional cases, and only with special precautions. Treatment streptokinase can be very effective if it is started early enough. Fever and blood circulation disorders, often observed before, using modern highly purified preparations are rare, however, to avoid them completely unable.



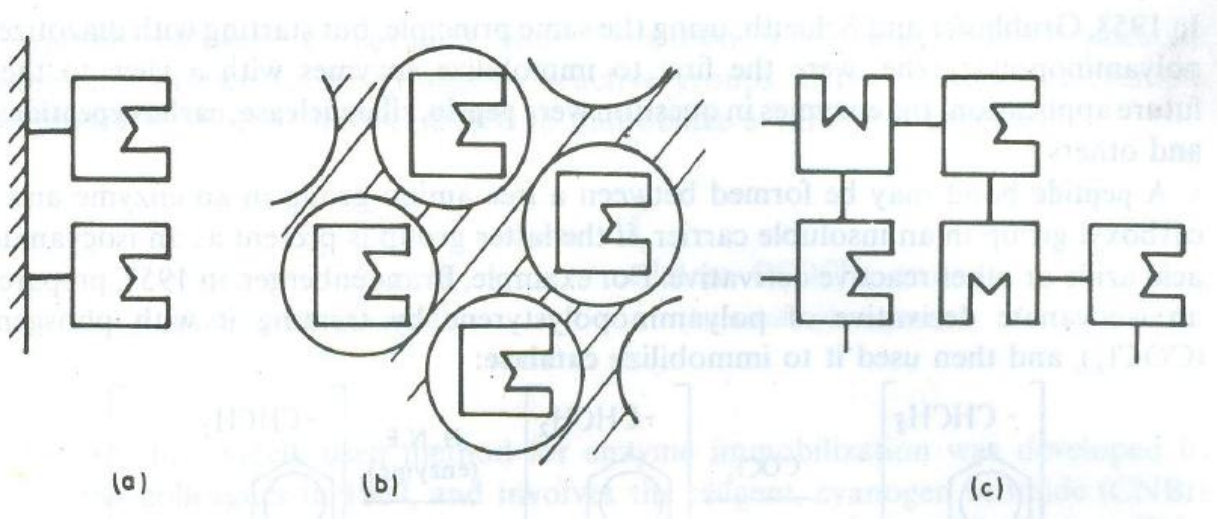
Schematic representation of fibrinolysis.

Immobilized enzymes

Introduction

An immobilized enzyme is one which has been attached to or enclosed by an insoluble support medium (carrier) or one where the enzyme molecules have been cross linked to each other, without loss of catalytic activity.

Figure: Enzyme immobilization



(a) Attachment to an insoluble support medium

(b) Entrapping by an insoluble support medium

(c) Cross linking of enzyme molecules

Physical adsorption

On to an inert carrier is a very simple procedure for immobilizing an enzyme. For it requires just the mixing of enzyme solution with the carrier. Eg., invertase could be adsorbed on to activated charcoal without any change in enzymatic activity.

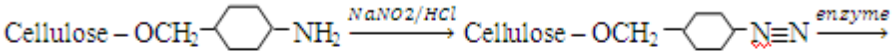
Ionic binding

It provides a slightly more specific way of attaching an enzyme to a carrier therefore, many ion exchange resins eg., DEAE, sephadex and CM-cellulose have been used as support media.

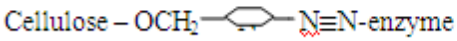
Covalent binding

Covalent binding can provide even more permanent linkages between enzyme and carrier. Many procedures depends on the coupling of phenolic imidazole or free amino groups on an enzyme to a diazonium derivative of a carrier.

For example for linking albumin to diazotized ρ-amino benzyl cellulose to form an immobilized antigen has been widely used to prepare immobilized enzyme.



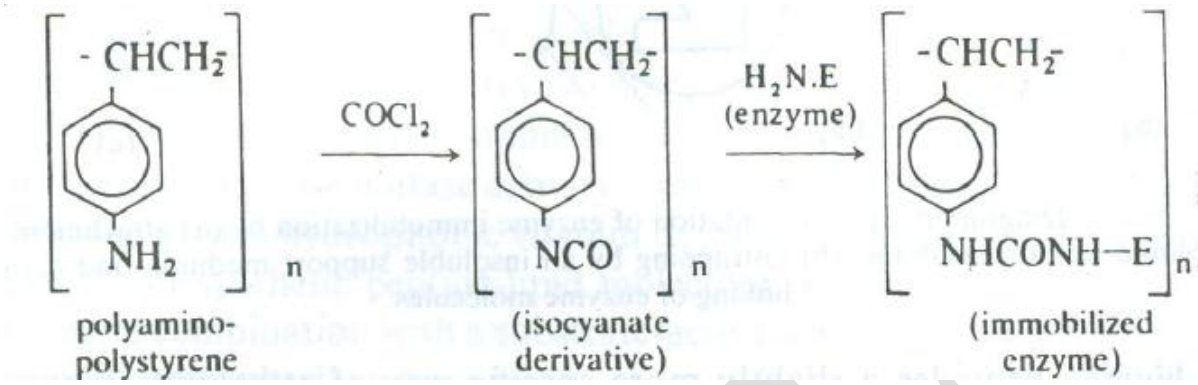
P-amino benzyl cellulose **diazonium derivative**



Immobilized enzyme

A peptide bond may be formed between a free amino group in an enzyme and a carboxyl group in an insoluble carrier.

Brandenberger, in 1957 prepared an isocyanate derivative of polyamino polystyrene by treating it with phosgene and then used it to immobilize catalase.



Many enzymes have been immobilized by reacting with the azide derivative of a carrier, whose formation may involve treatment with hydrazine (NH_2NH_2). For example,

cellulose-$OCH_2CO_2H \xrightarrow{CH20H\ and\ HCl}$ cellulose-$OCH_2CO_2CH_3 \xrightarrow{NH2NH2}$

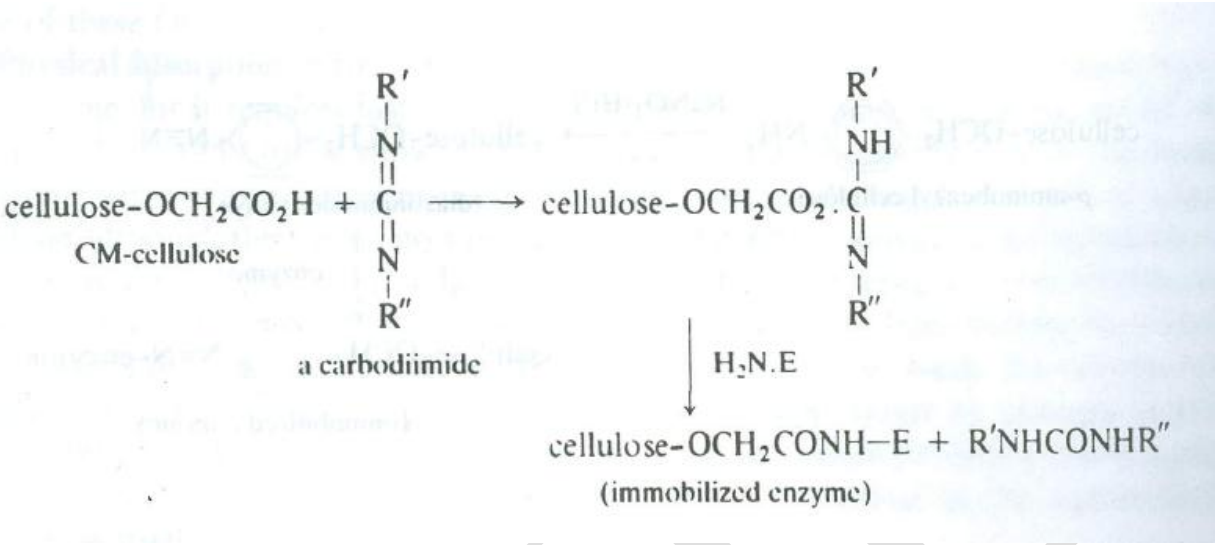
CM-Cellulose **Methyl ester**

cellulose-$OCH_2CONHNH_2 \xrightarrow{NaNO2/HCl}$ cellulose-$OCH_2CON_3 \xrightarrow{H2N-E}$ cellulose-OCH_2CONH-E

Hydrazide derivative **azide derivative**

Immobilized enzyme

Peptide bond between enzyme and carrier may also be formed by the use of condensing reagents such as carbodiimides. For example,



Alkylation

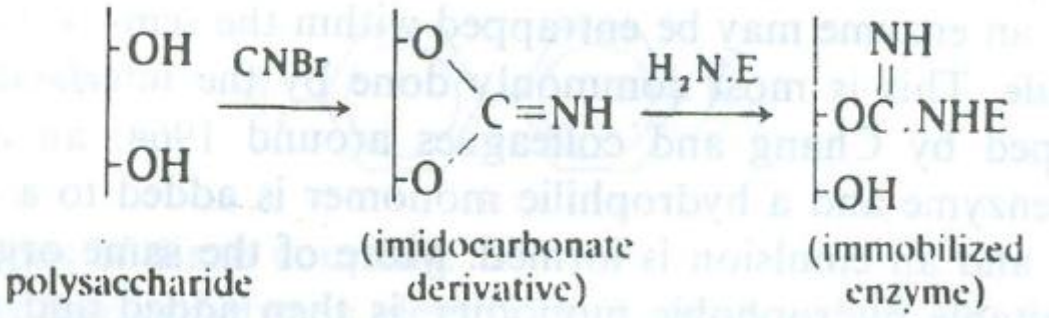
It is an another form of covalent bond formation. Alkylation of phenolic, sulphydryl or free amino groups by reactive groups in the carrier.

For example, Bromoacetyl cellulose used to immobilize enzymes.

$$\text{Cellulose-OCOCH}_2\text{Br} \xrightarrow{enzyme} \text{cellulose-OCOCH}_2\text{-Enz}$$

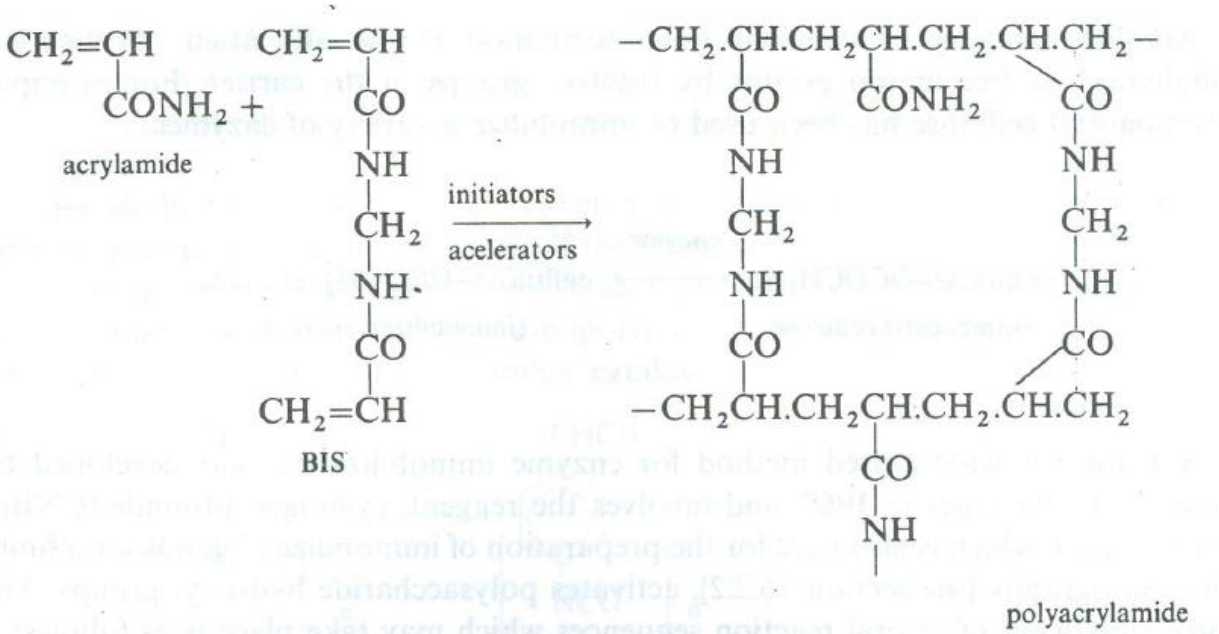
Immobilized enzyme

Cyanogen bromide involved immobilization



Entrapment

It is commonly achieved within the lattice of a polymerized gel. The most widely used is polyacrylamide, which may be synthesized from an aqueous solution of acrylamide and N-N-Methylene bis acrylamide in the presence of initiators and accelerators.



Microcapsule

An enzyme may be entrapped within the semi permeable membrane of a microcapsule. For example, nylon microcapsule may be formed if the hydrophyllic monomer is 1, 6-hexamethylene diamine, the hydrophobic monomer sebacoyl chloride and the organic solvent a cyclo hexane-chloroform mixture. Such microcapsule are typically 10-100 µm in diameter.

Disadvantages

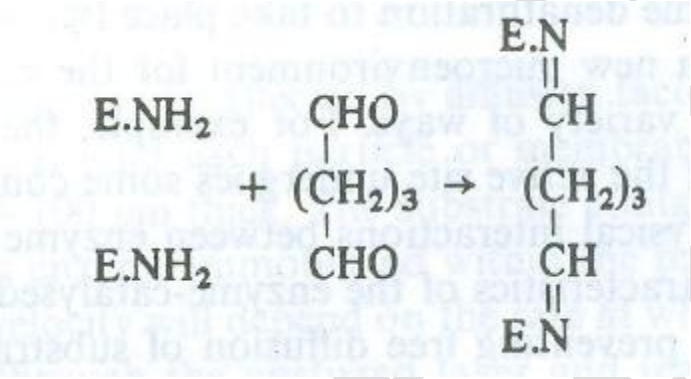
- In some instances free radical generated during the polymerization procedure may cause some loss of enzymic activity.
- Since entrapped enzyme does not escape because of their size, a very large substrate will not be able to diffuse in to reach the enzyme.
- Hence this procedure is not suitable for proteolytic enzymes or others whose substrate is macromolecule.

Liposomes

Amphipathic lipids such as phosphatidyl choline and cholesterol are dissolved in chloroform and spread as a film over the walls of a rotating flask; an aqueous solution of enzyme is added and rapidly dispersed and liposomes are formed as lipid membranes enclose the water droplets.

Cross linking

Cross linking molecules of enzyme is most commonly brought about by the action of glutaraldehyde whose two aldehyde groups form schiff's base linkages with amino groups.



Applications

- Sometimes changes in property during immobilization is advantageous.
- If an enzyme catalysed reaction cannot be linked directly to another because of incompatible pH activity ranges, it may be possible to immobilize the enzymes in such a way that their pH activity ranges now overlap thus allowing them to be used in a single rather than a two step process.
- Component enzymes of a coupled system may be immobilized together. This increases the efficiency of the coupled process.
- Use of liposomes to study in vitro, the effect of a lipid environment on the activity of enzymes which are associated with membrane in vivo.
- They can be easily separated from reaction mixture.
- Further the enzyme will still be active and largely uncontaminated and so can be used again.
- So used in continuous operated processes.
- Immobilized enzymes may also be used as components of analytical systems, either in dry reagent or automated techniques. For example, tubular and packed bed reactors have been incorporated into continuous flow analysers.

POSSIBLE QUESTIONS

Short Answers (2 marks)

1. Define coenzyme.
2. Write the structure of TPP.
3. Write the structure of biotin.
4. Write the structure of tetra hydrofolate.
5. Write the structure of lipoic acid.
6. Write the structure of pyridoxal phosphate.
7. List out the applications of SGOT in diagnostics.
8. List out the applications of SGPT in diagnostics.
9. List out the applications of AST in diagnostics.
10. List out the applications of ALT in diagnostics.
11. Write the principle of enzyme immunoassay.
12. Define enzyme immobilization.

Long Answers (6 marks)

1. Describe the structure and functions of FAD.
2. Describe the structure and functions of NAD.
3. Describe the structure and functions of coenzyme A.
4. Describe the structure and functions of TPP and biotin.
5. Describe the structure and functions of pyridoxal phosphate and lipoic acid.
6. Explain the application of enzymes in diagnostics.
7. Describe the principle, technique and applications of enzyme immune assay.
8. Write a note on enzyme therapy with streptokinase.
9. Explain the preparation techniques of immobilized enzymes.
10. Define immobilization of enzymes. Write its applications.

Karpagam academy of higher education
Department of biochemistry
Enzymes (18BCU202) MCQ
UNIT I

S.NO	QUESTION	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER
1	Biotin is a coenzyme for	pyruvate dehydrogenase	**pyruvate carboxylase**	PEP carboxykinase	glutamate pyruvate transaminase	**pyruvate carboxylase**
2	Biotin is bound to	**Lysine residues of carboxylases**	Lysine residues of decarboxylases	Serine residues of carboxylases	Serine residues of decarboxylases	**Lysine residues of carboxylases**
3	Coenzymes combines with	Proenzymes	**Apoenzymes**	Holoenzymes	Antienzymes	**Apoenzymes**
4	The following coenzyme takes part in hydrogen transfer reactions:	Tetrahydrofolate	Coenzyme A	**FAD**	Biotin	**FAD**
5	The following coenzyme does not takes part in hydrogen transfer reactions:	FAD	NAD	c) NADP	**Cobamides**	**Cobamides**
6	The following coenzyme takes part in oxidation	pyridoxal phosphate	**lipoic acid**	thiamine di phosphate	coenzyme A	**lipoic acid**
7	Coenzyme A contains a vitamin which is	thiamin	ascorbic acid	**pantothenic acid**	niacinamide	**pantothenic acid**
8	A coenzyme required in carboxylation reaction is	lipoic acid	coenzyme A	**biotin**	all of the above	**biotin**
9	Which of the following is not a component of coenzyme A?	adenylic acid	pantothenic acid	β-mercapto ethylamine	**deoxy adenylic acid**	**deoxy adenylic acid**
10	Dehydrogenases utilize as coenzymes, all of the following except	FAD	NAD+	NADP+	**FH4**	**FH4**
11	Lipoic acid is involved in the action of following enzyme	**pyruvate dehydrogenase**	acetyl coA carboxylase	pyruvate kinase	pyruvate carboxylase	**pyruvate dehydrogenase**
12	The coenzyme involved in one carbon transfer is	biotin	lipoic acid	coenzyme A	**folate coenzyme**	**folate coenzyme**
13	The tightly bound non-protein part of enzyme is	apo enzyme	**coenzyme (or) prosthetic group**	metallo enzyme	holo enzyme	**coenzyme (or) prosthetic group**
14	---------- coenzyme involved in acyl group transfer TPP	Biotin	TPP	**Coenzyme A**	PALP	**Coenzyme A**

15	Antimetabolites act by	non-competitive	**competitive**	binding with substrate	enzyme denaturation	**competitive**
16	In microorganisms, the production of acetaldehyde from pyruvate by the enzyme pyruvate decarboxylase requires	biotin	**TPP**	FAD	NAD	**TPP**
17	---------- enzyme is extracted by disruption of yeast cell wall in the	amylase	cellulase	**invertase**	papain	**invertase**
18	Immobilised urease is used in	urea formation	**hemodialysis**	pharmacy industry	leather industry	**hemodialysis**
19	In microorganisms, the production of acetaldehyde from pyruvate by the enzyme pyruvate decarboxylase requires	biotin	**TPP**	FAD	NAD	**TPP**
20	---------- enzyme is extracted by disruption of yeast cell wall in the	amylase	cellulase	**invertase**	papain	**invertase**
21	Immobilised urease is used in	urea formation	**hemodialysis**	pharmacy industry	leather industry	**hemodialysis**
22	The most widely used enzyme immobilization technique involves	**covalent attachment to insoluble support**	encapsulation	ionic interacton with matrix	adsorpsion on to a matrix	**covalent attachment to**
23	---------- is a cross linking agent in enzyme immobilization.	**glutaraldehyde**	DEAE cellulose	butanol	tributylin	**glutaraldehyde**
24	A very simple procedure for immobilizing enzyme is	**physical adsorpsion**	ionic binding	covalent binding	microcapsule	**physical adsorpsion**
25	The first immobilized enzyme prepared is	amylase	**invertase**	lipase	trypsin	**invertase**
26	An isocyanate derivative of polyaminopolystyrene is used to immobilise	amylase	invertase	lipase	**catalase**	**catalase**
27	The condensing reagent used to form a peptide bond between an enzyme and a carrier is	hydrazide	**carbodiimides**	polystyrene	isocyanate	**carbodiimides**
28	In affinity chromatography the preparation of immobilized ligands makes use of a reagent	FDNB	**CNBR**	sanger’s reagent	millon’s reagent	**CNBR**
29	The following is the encapsulation technique used to immobilize enzymes	disulphide bridge formation	ionic bonding	covalent bonding	**liposomes**	**liposomes**
30	Immobilisation by cross linking procedure is used first to immobilize the enzyme	catalase	invertase	**carboxy peptidase A**	peroxidase	**carboxy peptidase A**

31	The reagent used to attach enzymes to amino groups of carriers such a Aminoalkylated porous glass is	**glutaraldehyde**	DEAE cellulose	butanol	tributylin	**glutaraldehyde**
32	The coenzyme involved in LDH activity is	**NAD+**	FAD	Biotin	Lipoic acid	**NAD+**
33	A markedly raised plasma activity of ALT indicates a disease condition known as	myocardial infarction	**viral hepatitis**	inflammatory diseases	asthma	**viral hepatitis**
34	The Regan isozyme of ALP is found in patients with	**cancer**	rheumatoid arthritis	liver necrosis	asthma	**cancer**
35	The intestinal and placental forms of ALP are strongly inhibited by the The amino acid	L-Alanine	D-Alanine	**L-Phenyl alanine**	D-Valin	**L-Phenyl alanine**
36	The bone isozymes of ALP are strongly inactivated by	oxalate	hydroxy butyrate	isocitrate	**urea**	**urea**
37	Increased plasma activities of AST, ALT, LDH and GGT indicates	Muscle damage	**liver damage**	diabetes	myocardial infarction	**liver damage**
38	Increased activities of ALP, 5’ NT and GGT is an indication of	Diabetis	liver damage	myocardial infarction	**cholestasis**	**cholestasis**
39	AST and ALT level were increased in	Myocardial infarction	**muscular dystrophy**	liver damage	cholestasis	**muscular**