SEMESTER IV

17BTU404B **ENZYMOLOGY** **3H - 3C**

Total hours/week: L:3 T:0 P:0 **Marks: Internal: 40 External: 60 Total: 100**

Scope: This course includes the isolation, purification and characterization of enzymes and their applications.

Objective: This paper will enable the students to learn in detail about the fundamentals of enzymes and their functions.

UNIT-I

Isolation, crystallization and purification of enzymes: homogeneity of enzyme preparation, methods of enzyme analysis. Enzyme classification (rationale, overview and specific examples) Zymogens and their activation (Proteases and Prothrombin). Enzyme substrate complex: concept of E-S complex, binding sites, active site, specificity, Kinetics of enzyme activity, Michaelis-Menten equation and its derivation, Different plots for the determination of Km and V_{max} and their physiological significance, factors affecting initial rate, E, S, temp. & pH. Collision and transition state theories, Significance of activation energy and free energy.

UNIT-II

Enzyme-Substrate reactions: Two substrate reactions (Random, ordered and ping-pong mechanism) Enzyme inhibition types of inhibition, determination of Ki, suicide inhibitor. Mechanism of enzyme action: General mechanistic principle, factors associated with catalytic efficiency: proximity, orientation, distortion of strain, acid-base, nucleophilic and covalent catalysis. Techniques for studying mechanisms of action, chemical modification of active site groups, specific examples-: chymotrypsin, Iysozyme, GPDH, aldolase, RNase, Carboxypeptidase and alcohol dehydrogenase. Enzyme regulation: Product inhibition, feed back control, covalent modification.

UNIT-III

Allosteric enzymes: Allosteric enzymes with special reference to aspartate transcarbomylase and phosphofructokinase. Qualitative description of concerted and sequential models. Negative

cooperativity and half site reactivity. Enzyme - Enzyme interaction, Protein ligand binding, measurements analysis of binding isotherm, cooperativity, Hill and scatchard plots, kinetics of allosteric enzymes. Isoenzymes– multiple forms of enzymes with special reference to lactate dehydrogenase. Multienzyme complexes. Ribozymes. Multifunctional enzyme-eg Fatty Acid synthase.

UNIT-IV

Properties of Enzymes: Thermal stability and catalytic efficiency of enzyme, site directed mutagenesis and enzyme engineering– selected examples, Delivery system for protein pharmaceuticals, structure function relationship in enzymes, structural motifs and enzyme evolution. Methods for protein sequencing. Methods for analysis of secondary and tertiary structures of enzymes. Protein folding *in vitro* & *in vivo*.

UNIT-V

Enzyme Technology: Methods for large scale production of enzymes. Immobilized enzyme and their comparison with soluble enzymes, Methods for immobilization of enzymes. Immobilized enzyme reactors. Application of Immobilized and soluble enzyme in health and industry. Application to fundamental studies of biochemistry. Enzyme electrodes.

References

1. Robert Murray, K., David Bender, A., Kathleen Botham, M., Peter Kennelly, J., Victor Rodwell, W., Anthony Weil, P. (2009). *Harper's illustrated Biochemistry* (28th ed.). McGrawHill.
2. Lubert Stryer, (2006). *Biochemistry* (6th ed.). WH Freeman.
3. Donald Voet, & Judith Voet, (1995). *Biochemistry* (2nd ed.). John Wiley andSons.
4. Mary K., & Shawn O.Farrell, (2005). *Biochemistry* (5th ed.). Cenage Learning.
5. Nicholas Price, & Lewis Stevens (1999) *Fundamentals of Enzymology*. Oxford University Press.

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Deemed to be University Established Under Section 3 of UGC Act 1956)

Coimbatore – 641 021.

LECTURE PLAN
DEPARTMENT OF BIOTECHNOLOGY

STAFF NAME: Dr. R S. SARANYA

SUBJECT NAME: ENZYMOLOGY SUB.CODE:17BTU404B

SEMESTER: II CLASS: II B.Sc. (BT)

S.No	Lecture Duration Period	Topics to be Covered	Support Material/Page Nos
		UNIT-I	
1	1	Homogeneity of enzyme preparation, methods of enzyme analysis. Enzyme classification (rationale, overview and specific examples	T2: 521 ; T1: 86
2	1	Zymogens and their activation (Proteases and Prothrombin)	T1: 102-103
3	1	Enzyme substrate complex: concept of E-S complex, binding sites, active site, specificity	T1: 98-100
4	1	Kinetics of enzyme activity, Michaelis-Menten equation and its derivation	T3: 188-191
5	1	Different plots for the determination of Km and V_{max} and their physiological significance, factors affecting initial rate, E, S, temp. & pH	T3:191-193
6	1	Collision and transition state theories, Significance of activation energy and free energy.	T3: 185
	Total No of Hours Planned for Unit I =06		
		UNIT-II	
1	1	Two substrate reactions (Random, ordered and ping-pong mechanism)	T1:652
2	1	Enzyme inhibition types of inhibition, determination of Ki, suicide inhibitor. Mechanism of enzyme action: General mechanistic principle, factors associated	T4: 487; T1:98-100

		with catalytic efficiency	
3	1	Proximity, orientation, distortion of strain, acid-base, nucleophilic and covalent catalysis	T4:520
4	1	Techniques for studying mechanisms of action, chemical modification of active site groups	T4:524
5	1	Chymotrypsin, Iysozyme, GPDH, aldolase, RNase, Carboxypeptidase and alcohol dehydrogenase	T4: 504,525,
6	1	Enzyme regulation: Product inhibition, feed back control, covalent modification	T3: 196-200
	Total No of Hours Planned for Unit II = 06		
UNIT-III			
1	1	Allosteric enzymes with special reference to aspartate transcarbomylase and phosphofructokinase. Qualitative description of concerted and sequential models	T3: 185,203 T4:1050
2	1	Negative cooperativity and half site reactivity. Enzyme - Enzyme interaction	T4:1185
3	1	Protein ligand binding, measurements analysis of binding isotherm, cooperativity	T4:1182
4	1	Hill and scatchard plots, kinetics of allosteric enzymes. Isoenzymes– multiple forms of enzymes with special reference to lactate dehydrogenase	T4:754
5	1	Multienzyme complexes-Multifunctional enzyme-eg Fatty Acid synthase.	T4:738
6	1	Unit test	
	Total No of Hours Planned for Unit III = 06		
UNIT-IV			
1	1	Thermal stability and catalytic efficiency of enzyme. site directed mutagenesis and enzyme engineering– selected examples	J1
2	1	Delivery system for protein pharmaceuticals	J2
3	1	structure function relationship in enzymes-structural motifs and enzyme evolution	
4	1	Methods for protein sequencing	J3

5	1	Methods for analysis of secondary and tertiary structures of enzymes. Protein folding *in vitro* & *in vivo*	J4
6	1	Unit test	
	Total No of Hours Planned for Unit IV = 06		
UNIT-V			
1	1	Methods for large scale production of enzymes. Immobilized enzyme and their comparison with soluble enzymes	T1: 106
2	1	Methods for immobilization of enzymes	T1: 106-109
3	1	Immobilized enzyme reactors. Application of Immobilized and soluble enzyme in health and industry	W1
4	1	Application to fundamental studies of biochemistry- Enzyme electrodes	W2
5	1	Unit test	
6	1	Unit test	
	Total No of Hours Planned for Unit V = 06		
Total Planned Hours	**40**		

TEXT BOOK

1. Dr. U. Satyanarayana. Biochemistry (2007).Books and Allied (P) Ltd.

2. Lubert Stryer, (2006). Biochemistry (6th ed.). WH Freeman.

3. H.P.Gajera, S.V.Patel, B. A. Golakiya. Fundamentals of Biochemistry (2005). International Book Distributing Co.

4. Donald Voet, & Judith Voet, (1995). Biochemistry (2nd ed.). John Wiley andSons.

5. A Textbook Robert Murray, K., David Bender, A., Kathleen Botham, M., Peter Kennelly, J., Victor Rodwell, W., Anthony Weil, P. (2009). Harper's illustrated Biochemistry (28th ed.). McGrawHill.

6. Mary K., & Shawn O. Farrell, (2005). Biochemistry (5th ed.). Cenage Learning.

7. Nicholas Price & Lewis Stevens (1999). Fundamentals of Enzymology. Oxford University Press.

JOURNALS:

1. http://www.rna.uzh.ch/dam/jcr:ffffffff-b34e-2810-ffff-ffff9f2b6d4b/03082016_TJC_Enzyme_engin.pdf
2. www.ncbi.nlm.nih.gov/pmc/articles/PMC2857543/pdf/nihms165289.pdf
3. https://nptel.ac.in/courses/102103017/pdf/lecture%2018.pdfS
4. www.ncbi.nlm.nih.gov/pmc/articles/PMC3984617/

WEBSITES:

1. www.slideshare.net/abhigiri02/immobilized-enzyme-reactors-batch-and-continuous-types
2. www.slideshare.net/taidomang/fundamentals-of-biochemistry-by-jain

UNIT-I

SYLLABUS

Isolation, crystallization and purification of enzymes: homogeneity of enzyme preparation, methods of enzyme analysis. Enzyme classification (rationale, overview and specific examples) Zymogens and their activation (Proteases and Prothrombin). Enzyme substrate complex: concept of E-S complex, binding sites, active site, specificity, Kinetics of enzyme activity, Michaelis-Menten equation and its derivation, Different plots for the determination of Km and Vmax and their physiological significance, factors affecting initial rate, E, S, temp. & pH. Collision and transition state theories, Significance of activation energy and free energy.

Enzymes are biological catalysts and are protein in nature with an exception of ribozyme, an RNA catalyzing the RNA splicing in eukaryotes. Almost, all the metabolic reactions, in the living systems, are catalyzed by the enzymes. Enzymes are catalytically active in isolated form too. In other words, living cells are not essential to get the enzyme activity. This property of enzymes has been exploited by the biochemists to study enzymes *in vitro*. While studying enzymes *in vitro*, it is believed that whatever we are getting *in vitro*, the same is *in vivo*. Although in almost all the cases, it is true but still there may be exceptions.

Enzyme solubilization techniques Since almost all the enzymes (with few exceptions) are heat labile and not much stable at room temperature, the entire process of enzyme isolation, purification is carried out at 0-4oC using a cold room. However, enzyme work can also be done without a cold room if 2 precautions of cold conditions are followed. All the isolation medium components should be in chilled condition (which is done by putting them in a refrigerator overnight). The component of the homogenization technique like pestle and mortar, bowl of the Waring blender should also be in chilled condition. While homogenizing in a pestle and mortar, it should be surrounded by the ice flakes. In case of Waring blender bowl, many people also

wrap a cloth wet with chilled water. Enzyme may be present in the cytoplasm or may be localized in an organelle (applicable only in eukaryotic cells, there are no distinct organelles in prokaryotes). Even in the organelle, the enzyme may be present in the matrix of the organelle or may be bound with the membrane. If the enzyme is localized in a particular organelle, it is preferable first to isolate the organelle in an intact form and afterwards conditions may be applied to rupture the organelle. In this process, the enzyme protein will not get contaminated with the proteins present in other organelles. If it is a soluble enzyme present in cytoplasm, then generally the complete cell is ruptured without taking care of getting organelles in intact form. If one has to isolate the organelle in an intact form, one will have to use an isotonic isolation medium and there should not be any detergent in the medium capable of rupturing the organelles. It is important to select a suitable cell rupturing technique and an optimal isolation medium.

Selection of the isolating medium

In case of animal tissues and microbes, many people just use distilled water as isolating (homogenizing) medium. However, generally a buffer of a suitable ionic concentration and pH is preferred in order to maintain the pH and ionic concentration in the medium. A certain ionic strength of the buffer is essential for maintaining the buffering capacity. However, much higher ionic strength is also avoided since some times, high ionic concentration may be inhibitory to the activity of the enzyme. All the enzymes are pH sensitive. Every enzyme is stable in a particular pH range only. Every enzyme shows enzyme activity in a particular pH range only. However, mostly for isolation, pH of the medium is little different than the pH at which enzyme activity is measured. The pH of the buffer is maintained according to the nature of the enzyme. Selection of the pH should be such that isolated enzyme should be in fully active form. In case of plants, presence of the buffer in the isolating medium is more important since there is accumulation of acids in plant cell vacuoles and these acids get released in the medium after rupturing. The released acids will influence (decrease) the pH of the medium and if buffer has good buffering capacity, it will counteract the change in pH by the acids. However, sometimes, for isolation of

the enzymes which act in acidic pH range like acid phosphatase, it is preferable to use water instead of buffer since release of the acids will not decrease the pH of water lesser than the optimum pH of the enzyme. In addition to buffer or water as the case may be, other components are also used as per need. The other commonly used components in the isolating medium are described below: If the enzyme under study requires free sulfhydryl group(s) in its structure for exhibiting activity or in case of plant tissues especially if that tissue accumulates phenolics, many workers prefer to use a reducing agent. The commonly used reducing agents are cysteine, 2- mercaptoethanol (β- mercaptoethanol), sodium metabisulfite, dithiothreitol, dithioerythritol, reduced glutathione etc. and all are used generally in the concentration range 10 to 50 mM. In some cases, sodium metabisulfite has been used even up to 100 mM. The reducing agent may get oxidized by air, therefore, it is always dissolved freshly (except 2-mercaptoethanol 3 which is commercially available as concentrate, 14.5 M, in the liquid form). After rupturing of the cell, phenolics (if present) get oxidized to quinones. The quinones bind with the proteins to make protein-quinone complex which makes the enzyme protein inactive. The reducing agent keeps the phenolics in the reduced form. Sometimes, only reducing agent is not sufficient to keep the phenolics in the reduced form. Under the conditions, it is preferred to use a non-ionic detergent like Triton-X-100 in the isolating medium (generally up to 1%). The Triton-X-100 dissociates protein quinone complex. Triton-X-100 also ruptures the cell organelles. If organelles have to be kept intact, then under the conditions, it is preferred to use phenolfixing agents like poly vinyl pyrrolidone (PVP)**,** which is used generally at a final concentration of 1% (w/v). PVP fixes the phenolics and therefore, phenolics are not oxidized to quinones. Sometimes, proteases get released from the organelles on rupturing the cells and chew the enzyme protein. To prevent this a protease inhibitor like phenyl methyl sulfonyl fluoride (PMSF) in 10 to 20 mM concentration is added in the isolating medium. A metal chelating agent like ethylene diamine tetra acetate (EDTA) or ethylene glycol bis (2- aminoethyl ether), N, N, N/, N/ tetra acetate (EGTA) (in the concentration range 10 to 50 mM) is added in the isolating medium to prevent the denaturation of the enzyme protein by heavy metals already present in the tissue/ cell. Sometimes**,** presence of metal ion**s** like Mg+2, Mn+2, Zn+2**,** etc. **is** essential in the isolating medium for getting the enzyme in the active form. If both metal chelating agent and metal ions are required in the isolating medium, amount of the metal ion is put more compared to the amount of the metal

chelating agent. If one wishes to rupture only the cell wall and cell membrane keeping the cell organelles intact, isotonic conditions have to be maintained. Generally, 0.25 M mannitol or 0.5 M sucrose is used in the isolating medium. These are considered comparatively to be the inert substances on which enzymes do not act. If one is studying invertase enzyme, then sucrose is not used**,** since it will be degraded by the enzyme. There may be a special requirement in the isolating medium for a particular enzyme like the presence of an effector molecule. Therefore, composition of the isolating medium has to be optimized before starting the actual work.

Techniques used for enzyme isolation

As mentioned above, generally enzymes are isolated in the cold condition (at 0 to 4oC). For the purpose, homogenizing medium as well as container should be in the chilled condition. It is preferable to homogenize the tissue in a cold room. The following are the commonly used techniques for enzyme homogenization:

Pestle and mortar

Pestle and mortar is a moderate technique for tissue homogenization. Mechanical breakdown occurs during the process. Sometimes, grinding is done in the presence of purified sand or glass beads for aberration. Pestle and mortar is considered to be a moderate grinding technique and rupturing of the cell organelles does not occur if isotonic grinding medium without detergent is used.

Blenders

Waring blender (commonly called as mixie) is comparatively harsh technique of grinding the tissue compared to pestle and mortar and is mostly used for homogenizing the harder tissues (generally the plant tissues). If the worker is interested in isolating intact cell organelles, then Waring blender is not a preferred technique. Waring blender is first operated at low speed for 4 few seconds and then at medium speed(s) for few seconds before bringing it at high speed. Time of grinding at various speeds is decided according to the nature of the tissue being ground. If homogenization has to be done for a little longer time, then it is generally done after few seconds interval after every minute of grinding at high speed to avoid heating during operation of the Waring blender.

Ultra- Sonicator

This technique of rupturing the cells is generally used for microbial/ bacterial cells. Ultrasonicator generates low as well as high wavelength ultrasonic waves. For the purpose, a suitable probe depending on the volume of the homogenizing medium is selected and connected with the ultra-sonicator. The container having cells and homogenizing (isolating) medium is put in chilled condition by covering the container with ice. There is much generation of heat during ultra-sonication, therefore, ultrasonic waves are thrown in the sample after few seconds interval**,** every 10 to 15 seconds ultrasonication.

Vir-Tis homogenizer

This is considered to be a mild technique and generally used for homogenization of soft tissues such as animal tissues. Here a motorized pestle with teeth like aberrations is used. With Vir-Tis homogenizer, generally no rupturing of cell organelles occurs during grinding provided isotonic medium with no detergent is used.

Potter Elvejm homogenizer

This is also a mild technique and is used for homogenization of soft animal tissues. Potter Elvejm Homogenizer is a simple equipment having a pestle like glass rod with teeth like aberrations on its tip. There are down aberrations in the tube too on which teeth of the rod are fitted during up and down process of the rod. Up and down process of the pestle is done manually by hand or by mechanical device.

Razor blade

It is comparatively very mild technique. It is generally used only for isolation of intact cell organelles for the purpose of studying the intracellular localization of the enzyme proteins. In the technique, razor blade is used for chopping the tissue in the presence of isolating medium. Although the technique is good for the isolation of intact organelles, but it is unable to rupture all the cells. Therefore, there is low recovery of the enzyme due to left out of unruptured or partially ruptured cells. These un-ruptured or partially ruptured cells are removed as cell debris after centrifugation.

Extrusion method This method relies on the principle that forcing a cell suspension at high pressure through a narrow orifice will provide a rapid pressure drop. This is a powerful mean of disrupting cells especially from bacteria.

Lytic enzymes

Cell wall and cell membrane lytic enzymes like cellulase, pectinase, xylanase**,** pectin methyl esterase, lysozyme etc. can be used for rupturing the cells. Enzymes being costly are not

commonly used for making cell free preparation for isolation of enzymes. In plant tissue culture, lytic enzymes are used to prepare protoplast.

Freeze-Thaw

With certain susceptible microbes and eukaryotic cells, repeated freezing and thawing results in extensive membrane lesions with release of periplasmic and intracellular proteins.

Acetone powder

Drying with acetone is a good method for rupturing the cell membrane. Using acetone, powder of the tissue may be prepared which may be stored in a Deep freezer for a long time. It forms a convenient starting material from which the enzyme may be extracted with the isolating medium, whenever required. However, one has to take much precautions of low temperature (generally –20oC), otherwise, acetone may denature the enzyme protein. Isolation of enzymes from sub-cellular organelles requires rupturing of the organelle. Generally for the purpose, organelle is isolated in intact form thus removing the contaminating proteins of the cytoplasm and other cell organelles. Afterwards, cell organelle is ruptured in the presence of a suitable detergent like tween, teepol, digitonin etc.

Enzyme classification

Since many enzymes have common names that do not refer to their function or what kind of reaction they catalyze, an enzyme classification system was established. There were six classes of enzymes that were created so that enzymes could easily be named. These classes are:Oxidoreductases,Transferases, Hydrolases, Lyases, Isomerases, and Ligases. This is the

international classification used for enzymes. Enzymes are normally used for catalyzing the transfer of functional groups, electrons, or atoms. Since this is the case, they are assigned names by the type of reaction they catalyze. The enzymes were numbered 1-6 and from here, they were divided into subdivisions. This allowed for the addition of a four-digit number that would precede EC(Enzyme Commission) and each enzyme could be identified. The reaction that an enzyme catalyzes must be known before it can be classified.

Oxidoreductases catalyze oxidation-reduction reactions where electrons are transferred. These electrons are usually in the form of hydride ions or hydrogen atoms. When a substrate is being oxidized it is the hydrogen donor. The most common name used is a dehydrogenase and sometimes reductase will be used. An oxidase is referred to when the oxygen atom is the acceptor. EC 1.1 includes oxidoreductases that act on the CH-OH group of donors (alcohol oxidoreductases)

EC 1.2 includes oxidoreductases that act on the aldehyde or oxo group of donors

EC 1.3 includes oxidoreductases that act on the CH-CH group of donors (CH-CH oxidoreductases)

EC 1.4 includes oxidoreductases that act on the CH-NH2 group of donors (Amino acid oxidoreductases, Monoamine oxidase)

EC 1.5 includes oxidoreductases that act on CH-NH group of donors

EC 1.6 includes oxidoreductases that act on NADH or NADPH

EC 1.7 includes oxidoreductases that act on other nitrogenous compounds as donors

EC 1.8 includes oxidoreductases that act on a sulfur group of donors

EC 1.9 includes oxidoreductases that act on a heme group of donors

EC 1.10 includes oxidoreductases that act on diphenols and related substances as donors

EC 1.11 includes oxidoreductases that act on peroxide as an acceptor (peroxidases)

EC 1.12 includes oxidoreductases that act on hydrogen as donors

EC 1.13 includes oxidoreductases that act on single donors with incorporation of molecular oxygen (oxygenases)

EC 1.14 includes oxidoreductases that act on paired donors with incorporation of molecular oxygen

EC 1.15 includes oxidoreductases that act on superoxide radicals as acceptors

EC 1.16 includes oxidoreductases that oxidize metal ions

EC 1.17 includes oxidoreductases that act on CH or CH2 groups

EC 1.18 includes oxidoreductases that act on iron-sulfur proteins as donors

EC 1.19 includes oxidoreductases that act on reduced flavodoxin as a donor

EC 1.20 includes oxidoreductases that act on phosphorus or arsenic in donors

EC 1.21 includes oxidoreductases that act on X-H and Y-H to form an X-Y bond

EC 1.97 includes other oxidoreductases

- **Transferases** catalyze group transfer reactions. The transfer occurs from one molecule that will be the donor to another molecule that will be the acceptor. Most of the time, the donor is a cofactor that is charged with the group about to be transferred. Example: Hexokinase used in glycolysis. EC 2.1 includes enzymes that transfer one-carbon groups (methyltransferase)
- EC 2.2 includes enzymes that transfer aldehyde or ketone groups
- EC 2.3 includes acyltransferases
- EC 2.4 includes glycosyltransferases
- EC 2.5 includes enzymes that transfer alkyl or aryl groups, other than methyl groups
- EC 2.6 includes enzymes that transfer nitrogenous groups (transaminase)
- EC 2.7 includes enzymes that transfer phosphorus-containing groups (phosphotransferase, including polymerase and kinase)
- EC 2.8 includes enzymes that transfer sulfur-containing groups (sulfurtransferase and sulfotransferase)
- EC 2.9 includes enzymes that transfer selenium-containing groups

- **Hydrolases** catalyze reactions that involve hydrolysis. This cases usually involves the transfer of functional groups to water. When the hydrolase acts on amide, glycosyl, peptide, ester, or other bonds, they not only catalyze the hydrolytic removal of a group from the substrate but also a transfer of the group to an acceptor compound. These enzymes could also be classified under transferases since hydrolysis can be viewed as a transfer of a functional group to water as an acceptor. However, as the acceptor's reaction with water was discovered very early, it's considered the main function of the enzyme which allows it to fall under this classification. For example: Chymotrypsin. EC 3.1: ester bonds (esterases: nucleases, phosphodiesterases, lipase, phosphatase)
- EC 3.222: sugars (DNA glycosylases, glycoside hydrolase)
- EC 3.3: ether bonds
- EC 3.4: peptide bonds (Proteases/peptidases)
- EC 3.5: carbon-nitrogen bonds, other than peptide bonds
- EC 3.6 acid anhydrides (acid anhydride hydrolases, including helicases and GTPase)
- EC 3.7 carbon-carbon bonds
- EC 3.8 halide bonds
- EC 3.9: phosphorus-nitrogen bonds
- EC 3.10: sulfur-nitrogen bonds
- EC 3.11: carbon-phosphorus bonds
- EC 3.12: sulfur-sulfur bonds
- EC 3.13: carbon-sulfur bonds

Lyases catalyze reactions where functional groups are added to break double bonds in molecules or the reverse where double bonds are formed by the removal of functional groups. For example: Fructose bisphosphate aldolase used in converting fructose 1,6-bisphospate to G3P and DHAP by cutting C-C bond. EC 4.1 includes lyases that cleave carbon-carbon bonds, such as

decarboxylases (EC 4.1.1), aldehyde lyases (EC 4.1.2), oxo acid lyases(EC 4.1.3) and others (EC 4.1.99)

EC 4.2 includes lyases that cleave carbon-oxygen bonds, such as dehydratases

EC 4.3 includes lyases that cleave carbon-nitrogen bonds

EC 4.4 includes lyases that cleave carbon-sulfur bonds

EC 4.5 includes lyases that cleave carbon-halide bonds

EC 4.6 includes lyases that cleave phosphorus-oxygen bonds, such as adenylate cyclase and guanylate cyclase

EC 4.99 includes other lyases, such as ferrochelatase

Isomerases catalyze reactions that transfer functional groups within a molecule so that isomeric forms are produced. These enzymes allow for structural or geometric changes within a compound. Sometime the interconversion is carried out by an intramolecular oxidoreduction. In this case, one molecule is both the hydrogen acceptor and donor, so there's no oxidized product. The lack of a oxidized product is the reason this enzyme falls under this classification. The subclasses are created under this category by the type of isomerism. For example: phosphoglucose isomerase for converting glucose 6-phosphate to fructose 6-phosphate. Moving chemical group inside same substrate. EC 5.1 includes enzymes that catalyze racemization (racemases) and epimerization (epimerases)

EC 5.2 includes enzymes that catalyze the isomerization of geometric isomers (cis-trans isomerases)

EC 5.3 includes intramolecular oxidoreductases

EC 5.4 includes intramolecular transferases (mutases)

EC 5.5 includes intramolecular lyases

EC 5.99 includes other isomerases (including topoisomerases)

Ligases are used in catalysis where two substrates are ligated and the formation of carbon-carbon, carbon-sulfide, carbon-nitrogen, and carbon-oxygen bonds due to condensation

reactions. These reactions are couple to the cleavage of ATP. EC 6.1 includes ligases used to form carbon-oxygen bonds

EC 6.2 includes ligases used to form carbon-sulfur bonds

EC 6.3 includes ligases used to form carbon-nitrogen bonds (including argininosuccinate synthetase)

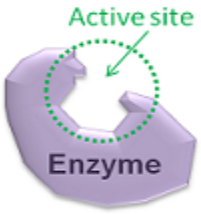
EC 6.4 includes ligases used to form carbon-carbon bonds

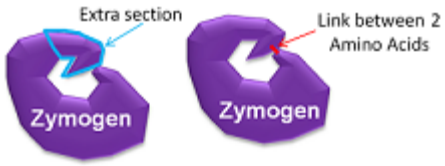
EC 6.5 includes ligases used to form phosphoric ester bonds

EC 6.6 includes ligases used to form nitrogen-metal bonds

ZYMOGEN

A zymogen is like a wrapped candy bar. In order to get to the good stuff, you need to tear away what's keeping you from it. **Zymogens**, or **proenzymes**, are enzymes that aren't functioning yet because their action is blocked by a 'wrapper'. The 'wrapper' can be a link between two **amino acids** (the building blocks of proteins), like a piece of string keeping a box closed. Or it can be an extra section of protein, like a jar lid.



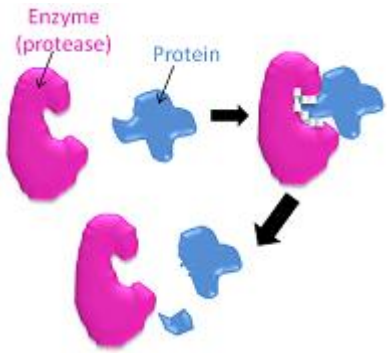


Enzymes have active sites. ***Zymogens are inactive enzymes.***

But what are enzymes? **Enzymes** are proteins that help chemical reactions happen faster via special places called **active sites**.

Imagine you want to make a fruit smoothie. You could hand-mush the fruit or put the fruit in a blender. The blender is like an enzyme; the active site is like the blades of the blender. The fruit becomes a fruit smoothie faster in the blender than if you squish it by hand.

Enzymes help make many things in the cell, but they can also unmake them. Enzymes that chop up proteins are called **proteases**.



Enzymes that degrade other proteins are proteases.

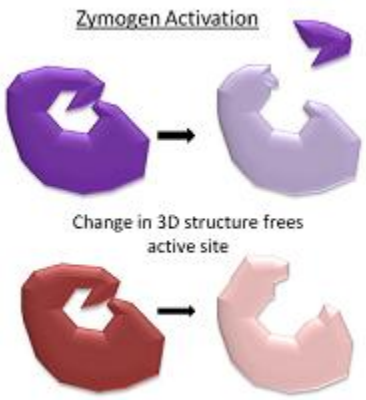
When cells make enzymes, especially proteases, they often make them as zymogen, an inactive form of the enzyme. This is so they don't go crazy and are only used when needed. Imagine your reaction if your blender suddenly hopped about on the counter, out of control, spewing half-chopped fruit everywhere. The counter would be a mess, and so would the cell.

Zymogens also ensure the enzyme folds properly (has the correct 3-D form), make the enzyme stable in unfavorable environments, and allow the enzyme to go to the proper place so that it doesn't function where it's not supposed to.

. Enzymes that begin with 'pro-' or end with '-gen' are often the zymogen form. For example: PROthrombin is the zymogen form of thrombin, an enzyme involved in blood clotting. PepsinoGEN is the zymogen form of pepsin, the enzyme found in your stomach that helps digest food.

Zymogen Activation

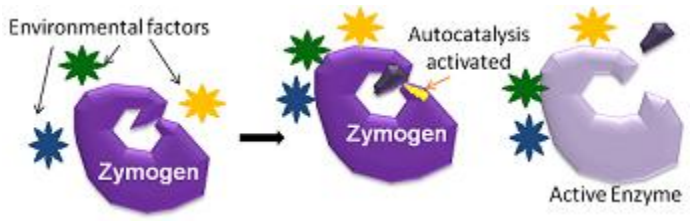
Zymogens are activated by snipping the bonds between two or more amino acids, rather like cutting a balloon string so that it floats away. When the bonds are cut, the enzyme changes its **conformation**, its 3-D structure, so that the active site is free or able to become active.



Activated zymogens change their 3-D structure.

Upon activation, sometimes pieces of the protein completely leave the enzyme, like taking the wrapper off a candy bar. Other times, the pieces of protein fold in and become part of the enzyme, like a catapult being pulled back.

Zymogens can be activated by proteases that cut the amino acid bonds. They can also be activated by the environment and become autocatalytic. **Autocatalysis** is self-activation, and happens when something in the environment allows the zymogen to cut its own chemical bonds.



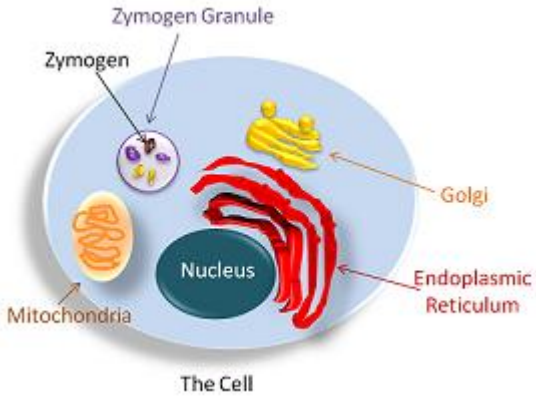
Environment can activate zymogen autocatalysis

Pepsinogen, for example, does not become pepsin until the pH is around 2-3. The extra hydrogens found in the lower pH makes the molecule cut its own bonds that are preventing it from functioning as a digestive enzyme.

What Are Zymogen Granules?

Inside the cells of your pancreas and salivary glands are proteases that can activate digestive enzymes. To make sure the proteases inside the cell can't change into the zymogen form before they are released into your digestive system, the cell uses special holding rooms called granules.

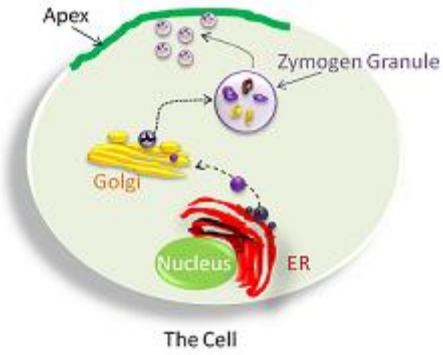
Zymogen granules are places in the cell that keep zymogens safe from the proteases inside the cell. They are like little rooms, or little bubbles, full of different types of zymogens.



Zymogen granule inside a cell

They are mostly found in acinar cells. **Acinar cells** are cells found in the pancreas and salivary glands that group together like the bumps on a raspberry.

Most zymogen granules begin formation where the zymogens are first formed - the endoplasmic reticulum (ER). The ER is like the cell's factory; it's where proteins are made. From there, they are sent to the Golgi. The Golgi is like the cell's post office; it's where finished products are packed and shipped to different parts of the cell. From the Golgi, fully formed zymogen granules begin to gather at the apex, or the top of the cell.

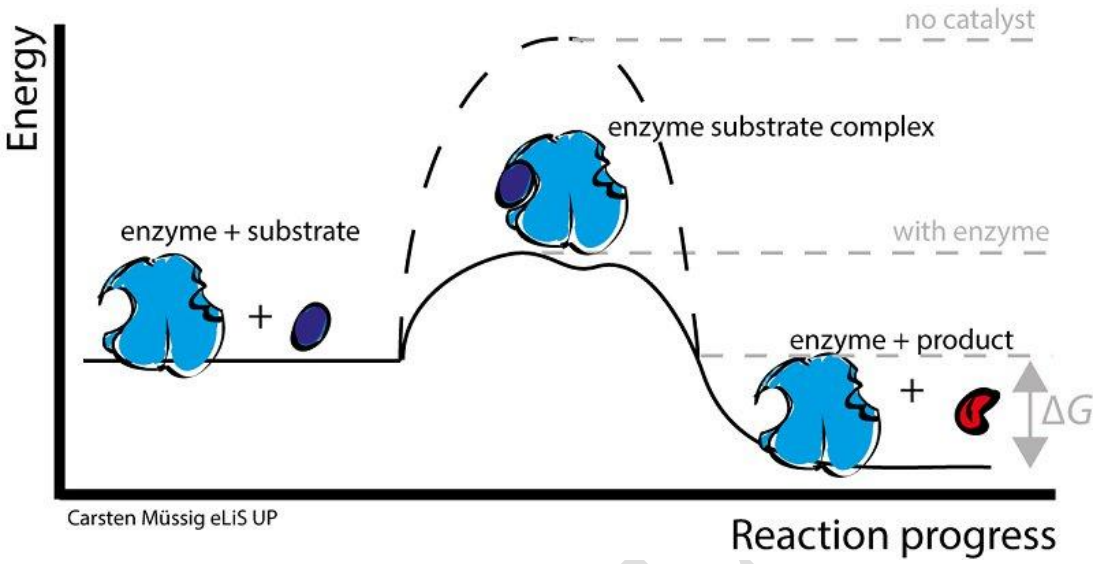


Zymogen granules move from the ER, to the Golgi, and then to the apex.

Enzyme Substrate Complex

The enzyme substrate complex is a temporary molecule formed when an enzyme comes into perfect contact with its substrate. Without its substrate an enzyme is a slightly different shape. The substrate causes a conformational change, or shape change, when the substrate enters the active site. The active site is the area of the enzyme capable of forming weak bonds with the substrate. This shape change can force two or more substrate molecules together, or split individual molecules into smaller parts. Most reactions that cells use to stay alive require the actions of enzymes to happen fast enough to be useful. These enzymes are directly coded for in the DNA of the organism.

The enzyme substrate complex is extremely important for a number of reasons. First, the enzyme substrate complex is only temporary. This means that once the substrate has changed, it can no longer bind to the enzyme. The products are released and the enzyme is ready for another substrate molecule. A single enzyme can operate repeatedly millions of times, meaning only a small amount of enzyme is needed in each cell.



Enzymes are complex molecules, like little machines meant for one purpose. Built out of a chain of amino acids, this long string experiences interactions between the different amino acids and twists and turns into complex structures. These structures can operate like hinges, wedges, and all sorts of other shapes intended to speed certain reactions. Different mutations give rise to slightly different forms of enzyme. In mutations that are beneficial to the organism, the enzyme substrate complex is changed in a way that effects the output of product or the function of the enzyme as a whole. This change in the organism is only beneficial if it somehow helps the organism reproduce more.

Enzymes are usually named after the substrate that they work on, and have the -ase suffix to designate they are enzymes. Each enzyme has a certain specificity for the substrate it works on, which determines which molecules they can bind to. Some molecules that are similar in structure to the substrate may get stuck in the active site, because they cannot undergo the reaction intended by the enzyme. In this warped enzyme substrate complex, the competitive inhibitor binds to the enzyme and inhibits its further action. Other inhibitors do not copy the substrate, but modify the enzyme in other ways so the enzyme substrate complex cannot be formed.

Examples of Enzyme Substrate Complex

Amylase and Amylose

Amylose is a complex sugar produced by plants. In our saliva is an enzyme, amylase, used to break amylose apart. Amylase uses one substrate molecule of amylose and a cofactor of one water molecule to produce an enzyme substrate complex. The complex severely reduces the amount of energy required to start the reaction, which increases the time in which it happens. A typical sugar molecule would take millions of years to break apart, were it not for the actions of enzymes such as amylase.

In fact, enzymes are so important in digesting the foods we eat that our body produces an enzyme for almost every type of food the body is evolutionarily prepared to consume. New foods are poorly processed, because the enzymes have not had time to adjust their efficiency. For instance, the modern diet of processed foods is leading to an obesity epidemic because the process foods are rich in easily accessible nutrients, but only to the pathways that are used to storing fat. As a result, much of the population experiences weight related illnesses. Many nutritionists are pushing for more natural, whole-food, plant based diets that tend to support the enzymes our bodies have naturally developed.

Allosteric Regulation in Enzymes

Although the enzyme substrate complex forming quickly is important for most reactions, in some cases it is important to "turn off" the enzyme to conserve energy or resources. Many enzymes are regulated in this way to provide just the right amount of energy and products. One of the most important places this happens is in the production of adenosine triphosphate (ATP), or the molecule that provides energy to cellular processes. Many of the enzymes in the pathway that creates ATP are inactivated by ATP. In this way, when too much ATP is produced, the enzyme shuts off. This is known as feedback inhibition, or the ability to self-regulate. In the same way, the enzymes can be reactivated by the presence of adenosine diphosphate ADP, an ATP that has used a phosphate group to provide energy to a process or reaction.

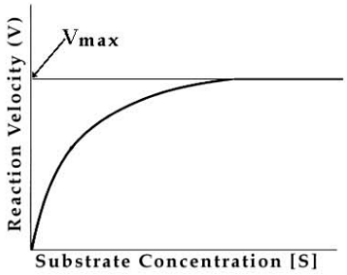
Many bodily processes are controlled in this manner, and the enzyme substrate complex in these cases can only be formed with the proper molecules present. Many of the cofactors that activate enzymes are vitamins, minerals, and other inorganic molecules present in the diet.

MICHAELIS- MENTEN EQUATION

maximum velocity is called optimum temperature. The enhancement of reaction velocity with the increase in temperature from low to optimum is due to: (a) With increase in temperature the initial energy the substrate becomes higher which in turn lowers the activation energy and lowers the energy barrier of the reaction. (b) With the rise in temperature the no of collision between enzyme and substrate increases. Decrease in enzyme activity with the rise in temperature beyond optimum temperature is due to denaturation of the enzyme mainly.

Michaelis- Menten equation

For an enzyme catalyzed reaction, the plot of intial velocity against substrate concentration gives a hyperbolic curve. The nature of the curve can be explained with the help of Michaelis- Menten equation.



It has been assumed that the overall reaction occurs in two steps. In the first step, enzyme (E) interacts with substrate (S) in a reversible manner to form complex (ES). In the next step, the complex dissociates to give enzyme and product (P) irreversibly. This sequence of event is

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

Now, the initial velocity (rate) of the reaction is

$$v = k_2[ES] \qquad \text{..... (i)}$$

[ES] can not be measure experimentally. But [ES] is directly related with total enzyme concentration (E_t) by the following equation

$$[E_t] = [E] + [ES] \qquad \text{..... (ii)}$$

$$\text{Rate of ES formation} = k_1[E][S] \qquad \text{..... (iii)}$$

$$= k_1([E_t]-[ES])[S] \quad \text{..... (iv)}$$

$$\text{Rate of ES breakdown} = (k_{-1}+k_2)[ES] \quad \text{..... (v)}$$

Applying the rule of steady state approximation for ES, we have,

Rate of ES formation = Rate of ES breakdown

$$k_1([E_t]-[ES])[S] = (k_{-1}+k_2)[ES]$$

$$[ES](k_{-1}+k_2+k_1[S]) = k_1[E_t][S]$$

$$[ES] = \frac{k_1[E_t][S]}{k_1[S]+(k_2+k_{-1})} \quad \text{..... (vi)}$$

Dividing the numerator and denominator by k_1

$$[ES] = \frac{[E_t][S]}{[S]+\left(\frac{k_2+k_{-1}}{k_1}\right)} \quad \text{..... (vii)}$$

Now defining, $\left(\frac{k_2+k_{-1}}{k_1}\right)$=$K_m$, known as Micheelis- Menten constant, we have,

$$[ES] = \frac{[E_t][S]}{[S]+K_m} \quad \text{..... (viii)}$$

So, the initial velocity (rate) of the reaction is

$$v = k_2[ES] \quad \text{..... (ix)}$$

$$v = \frac{k_2[E_t][S]}{[S]+K_m} \quad \text{..... (x)}$$

At high substrate concentration, all enzyme is saturated with substrate i.e. all enzyme in the ES form and the velocity of the reaction attains its maximum value.
Mathematically,

When $[E_t] = [ES]$, then $v = V_{max}$

Putting these relations in equation (ix) we have,

$$V_{max} = k_2[E_t] \quad \text{..... (xi)}$$

Combining eqn. (x) and (xi), we obtain

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

This eqn. is known as Michaelis- Menten equation

If the initial velocity, $v = \frac{1}{2}V_{max}$, applying in eqn. (xii) we have

$$\frac{1}{2}V_{max} = \frac{V_{max}[S]}{[S] + K_m}$$

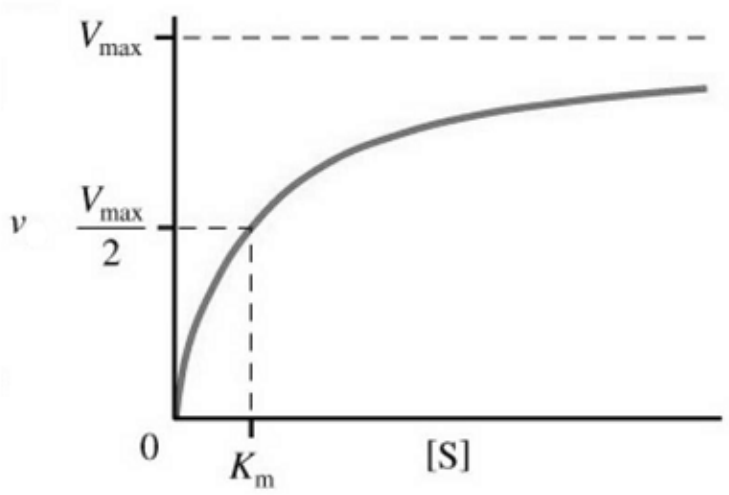
Simplifying, $K_m = [S]$

Hence, K_m is the substrate concentration when the velocity of the reaction becomes half of its maximum value.

Small Km value for a enzyme indicates that the reaction acquires maximum catalytic efficiency at low substrate concentration.

Small Km value indicates tight binding between enzyme and substrate whereas high value of Km indicates weak binding.

Unit of K_m: It has the same unit as that of the substrate concentration.



The Lineweaver-Burk plot: *Determination of Km and Vmax*

The direct plot of initial velocity (*v)* vs substrate concentration ([S]) using Micheelis- Menten equation does not give accurate measurement of Vmax and hence Km. In this curve, the initial velocity (*v*) approaches Vmax asymptotically at very high substrate concentration. But even at 10 time's greater substrate concentration than Km, the initial velocity (*v*) is only 91% of that Vmax. So the value of Vmax, obtained from the extrapolation of the asymptote will not be accurate. To overcome this problem, Hans Lineweaver and Dean Burk use the reciprocal of eqn. (xii).

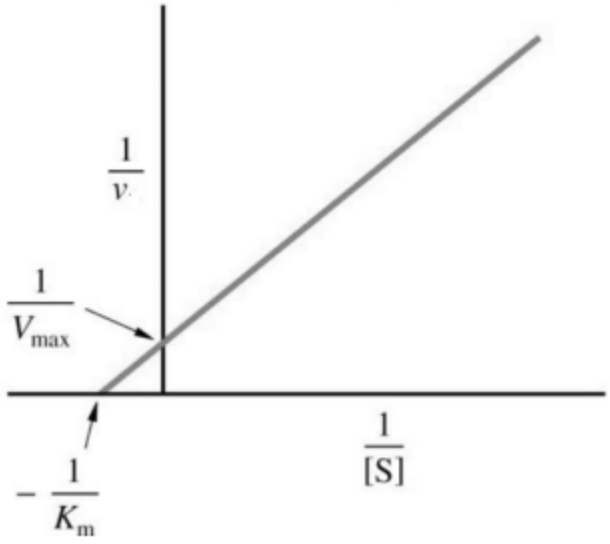
$$v = \frac{V_{max}[S]}{[S] + K_m}$$

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

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

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

The plot of 1/*v* (X- axis) against 1/[S] (Y-axis) generates a straight line with slope = Km/Vmax. This plot is known as Lineweaver- Burk plot or double reciprocal plot. Extrapolation of the straight line gives intercepts 1/Vmax in the Y-axis and -1/Km in the X-axis from which Vmax and Km can be determine easily.



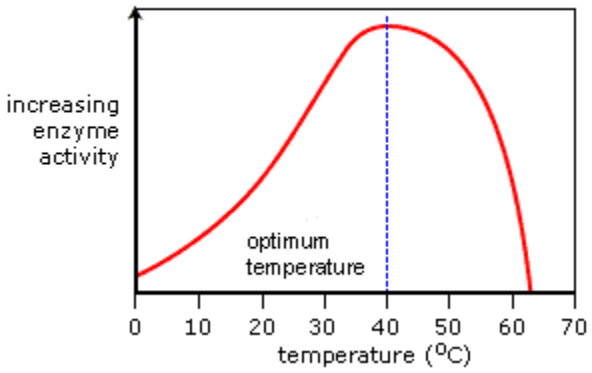
Factors affecting Enzyme Activity

The activity of an Enzyme is affected by its environmental conditions. Changing these alter the rate of reaction caused by the enzyme. In nature, organisms adjust the conditions of their enzymes to produce an Optimum rate of reaction, where necessary, or they may have enzymes which are adapted to function well in extreme conditions where they live.

Temperature

- Increasing temperature increases the Kinetic Energy that molecules possess. In a fluid, this means that there are more random collisions between molecules per unit time.
- Since enzymes catalyse reactions by randomly colliding with Substrate molecules, increasing temperature increases the rate of reaction, forming more product.
- However, increasing temperature also increases the Vibrational Energy that molecules have, specifically in this case enzyme molecules, which puts strain on the bonds that hold them together.
- As temperature increases, more bonds, especially the weaker Hydrogen and Ionic bonds, will break as a result of this strain. Breaking bonds within the enzyme will cause the Active Site to change shape.
- This change in shape means that the Active Site is less Complementary to the shape of the Substrate, so that it is less likely to catalyse the reaction. Eventually, the enzyme will become Denatured and will no longer function.
- As temperature increases, more enzymes' molecules' Active Sites' shapes will be less Complementary to the shape of their Substrate, and more enzymes will be Denatured. This will decrease the rate of reaction.

In summary, as temperature increases, initially the rate of reaction will increase, because of increased Kinetic Energy. However, the effect of bond breaking will become greater and greater, and the rate of reaction will begin to decrease.

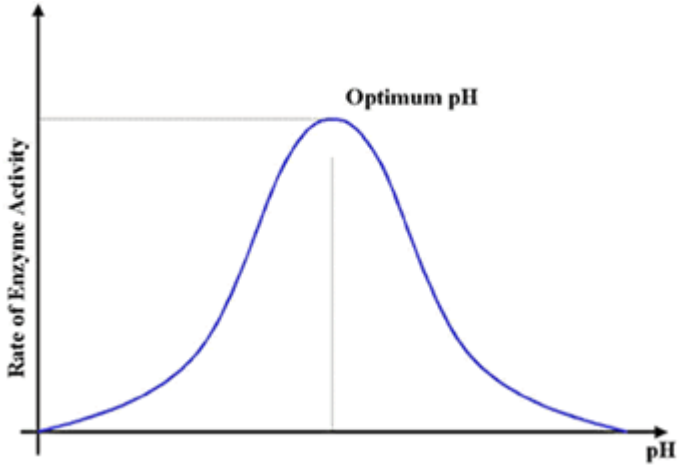


The temperature at which the maximum rate of reaction occurs is called the enzyme's Optimum Temperature. This is different for different enzymes. Most enzymes in the human body have an Optimum Temperature of around 37.0 °C.

pH - Acidity and Basicity

- pH measures the Acidity and Basicity of a solution. It is a measure of the Hydrogen Ion (H+) concentration, and therefore a good indicator of the Hydroxide Ion (OH-) concentration. It ranges from pH1 to pH14. Lower pH values mean higher H+ concentrations and lower OH- concentrations.
- Acid solutions have pH values below 7, and Basic solutions (alkalis are bases) have pH values above 7. Deionised water is pH7, which is termed 'neutral'.
- H+ and OH- Ions are charged and therefore interfere with Hydrogen and Ionic bonds that hold together an enzyme, since they will be attracted or repelled by the charges created by the bonds. This interference causes a change in shape of the enzyme, and importantly, its Active Site.
- Different enzymes have different Optimum pH values. This is the pH value at which the bonds within them are influenced by H+ and OH- Ions in such a way that the shape of their Active Site is the most Complementary to the shape of their Substrate. At the Optimum pH, the rate of reaction is at an optimum.

- Any change in pH above or below the Optimum will quickly cause a decrease in the rate of reaction, since more of the enzyme molecules will have Active Sites whose shapes are not (or at least are less) Complementary to the shape of their Substrate.



- Small changes in pH above or below the Optimum do not cause a permanent change to the enzyme, since the bonds can be reformed. However, extreme changes in pH can cause enzymes to Denature and permanently lose their function.
- Enzymes in different locations have different Optimum pH values since their environmental conditions may be different. For example, the enzyme Pepsin functions best at around pH2 and is found in the stomach, which contains Hydrochloric Acid (pH2).

Concentration

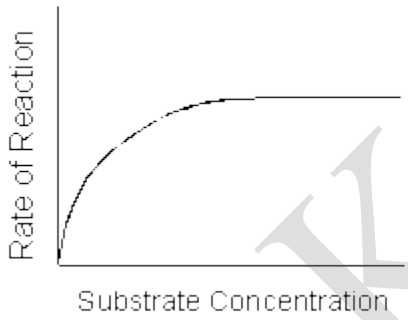
- Changing the Enzyme and Substrate concentrations affect the rate of reaction of an enzyme-catalysed reaction. Controlling these factors in a cell is one way that an organism regulates its enzyme activity and so its Metabolism.
- Changing the concentration of a substance only affects the rate of reaction if it is the limiting factor: that is, it the factor that is stopping a reaction from preceding at a higher rate.
- If it is the limiting factor, increasing concentration will increase the rate of reaction up to a point, after which any increase will not affect the rate of reaction. This is because it will

no longer be the limiting factor and another factor will be limiting the maximum rate of reaction.

- As a reaction proceeds, the rate of reaction will decrease, since the Substrate will get used up. The highest rate of reaction, known as the Initial Reaction Rate is the maximum reaction rate for an enzyme in an experimental situation.

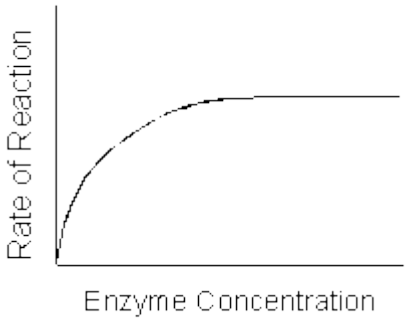
Substrate Concentration

- Increasing Substrate Concentration increases the rate of reaction. This is because more substrate molecules will be colliding with enzyme molecules, so more product will be formed.
- However, after a certain concentration, any increase will have no effect on the rate of reaction, since Substrate Concentration will no longer be the limiting factor. The enzymes will effectively become saturated, and will be working at their maximum possible rate.



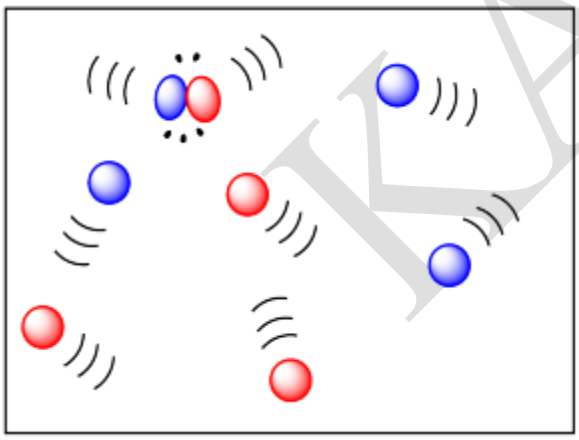
Enzyme Concentration

- Increasing Enzyme Concentration will increase the rate of reaction, as more enzymes will be colliding with substrate molecules.
- However, this too will only have an effect up to a certain concentration, where the Enzyme Concentration is no longer the limiting factor.



Collision Theory

If two molecules need to collide in order for a reaction to take place, then factors that influence the ease of collisions will be important. The more energy there is available to the molecules, the faster they will move around, and the more likely they are to bump into each other. Higher temperatures ought to lead to more collisions and a greater frequency of reactions between molecules. In the drawing below, the cold, sluggish molecules on the left are not likely to collide, but the energetic molecules on the right are due to collide at any time.

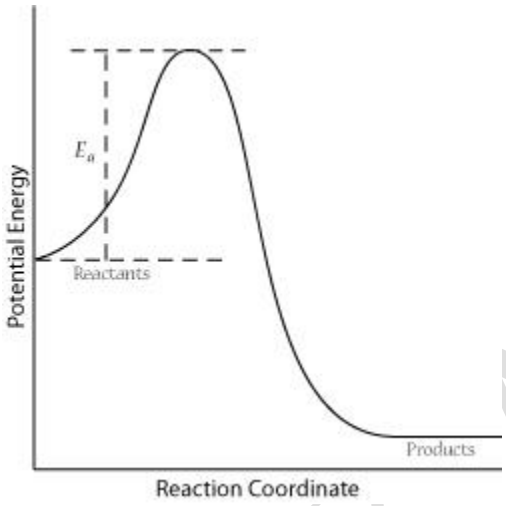


The rate at which molecules collide which is the frequency of collisions is called the collision frequency, Z, which has units of collisions per unit of time. Given a container of molecules A and B, the collision frequency between A and B is defined by:

The concepts of collision frequency can be applied in the laboratory: (1) The temperature of the environment affects the average speed of molecules. Thus, reactions are heated to increase the

reaction rate. (2) The initial concentration of reactants is directly proportional to the collision frequency; increasing the initial concentration will speed up the reaction.

For a successful collision to occur, the reactant molecules must collide with enough kinetic energy to break original bonds and form new bonds to become the product molecules. This energy is called the activation energy for the reaction; it is also often referred to as the energy barrier.



Transition-State Theory

Transition state theory (TST) provides a more accurate alternative to the previously used Arrhenius equation and the collision theory. The transition state theory attempts to provide a greater understanding of activation energy, Ea , and the thermodynamic properties involving the transition state. Collision theory of reaction rate, although intuitive, lacks an accurate method to predict the probability factor for the reaction. The theory assumes that reactants are hard spheres rather than molecules with specific structures. In 1935, Henry Eyring helped develop a new theory called the transition state theory to provide a more accurate alternative to the previously used Arrhenius equation and the collision theory. The Eyring equation involves the statistical frequency factory, v, which is fundamental to the theory.

According to TST, between the state where molecules are reactants and the state where molecules are products, there is a state known as the transition state. In the transition state, the reactants are combined in a species called the activated complex. The theory suggests that there are three major factors that determine whether a reaction will occur:

The concentration of the activated complex

The rate at which the activated complex breaks apart

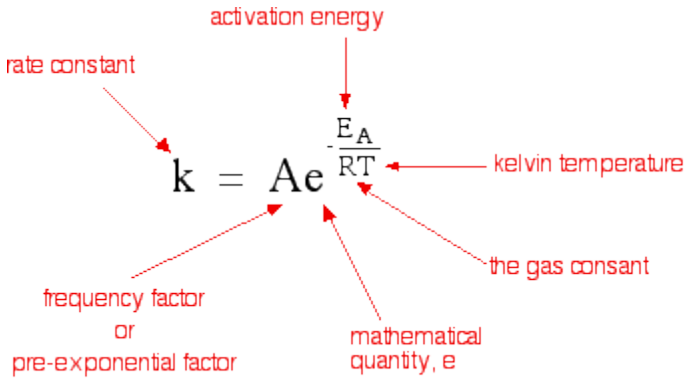
The way in which the activated complex breaks apart: whether it breaks apart to reform the reactants or whether it breaks apart to form a new complex, the products.

Collision theory proposes that not all reactants that combine undergo a reaction. However, assuming the stipulations of the collision theory are met and a successful collision occurs between the molecules, transition state theory allows one of two outcomes: a return to the reactants, or a rearranging of bonds to form the products.

Significance of activation energy and free energy

All molecules possess a certain minimum amount of energy. The energy can be in the form of kinetic energy or potential energy. When molecules collide, the kinetic energy of the molecules can be used to stretch, bend, and ultimately break bonds, leading to chemical reactions. If molecules move too slowly with little kinetic energy, or collide with improper orientation, they do not react and simply bounce off each other. However, if the molecules are moving fast enough with a proper collision orientation, such that the kinetic energy upon collision is greater than the minimum energy barrier, then a reaction occurs. The minimum energy requirement that must be met for a chemical reaction to occur is called the activation energy, Ea.

The activation energy is related to the rate constant for the reaction according to the Arrhenius' equation.



Activation energy, in chemistry, the minimum amount of energy that is required to activate atoms or molecules to a condition in which they can undergo chemical transformation or physical transport. In transition-state theory, the activation energy is the difference in energy content between atoms or molecules in an activated or transition-state configuration and the corresponding atoms and molecules in their initial configuration. The activation energy is usually represented by the symbol Ea in mathematical expressions for such quantities as the reaction rate constant, k = Aexp(−Ea/RT), and the diffusion coefficient, D = Doexp(−Ea/RT).

Activation energies are determined from experimental rate constants or diffusion coefficients that are measured at different temperatures.

UNIT-II

SYLLABUS

Enzyme-Substrate reactions: Two substrate reactions (Random, ordered and ping-pong mechanism) Enzyme inhibition types of inhibition, determination of Ki, suicide inhibitor. Mechanism of enzyme action: General mechanistic principle, factors associated with catalytic efficiency: proximity, orientation, distortion of strain, acid-base, nucleophilic and covalent catalysis. Techniques for studying mechanisms of action, chemical modification of active site groups, specific examples-: chymotrypsin, Iysozyme, GPDH, aldolase, RNase, Carboxypeptidase and alcohol dehydrogenase. Enzyme regulation: Product inhibition, feed back control, covalent modification.

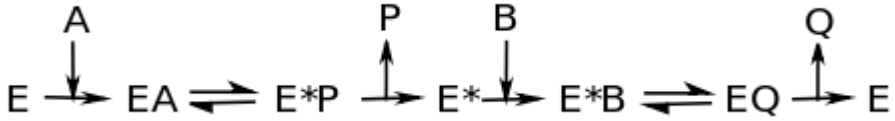
The "Ping-Pong" Mechanism

In order to understand the ping-pong mechanism, it is important to have an underlying knowledge of enzymes. Particularly, focus on enzyme and substrate chemistry. The simplest of enzymes will involve one substrate binding to the enzyme and producing a product plus the enzyme. However, the majority of enzymes are more complex and catalyze reactions involving multiple substrates. Binding of two substrates can occur through two mechanisms: sequential mechanism and non-sequential mechanism. In sequential mechanisms both substrates bind the enzyme and the reaction proceeds to form products which are then released from the enzyme. This mechanism can be further subdivided into random and ordered reactions. For random reactions the order in which the substrates bind does not matter. In ordered reactions one substrate must bind the enzyme before the second substrate is able to bind. Non-Sequential mechanism does not require both substrates to bind before releasing the first product. This page will focus on the non-sequential mechanism, which is also known as the "ping-pong" mechanism. It is called this because the enzyme bounces back and forth from an intermediate state to its standard state.The enzyme acts like a ping-pong ball, bouncing from one state to another.

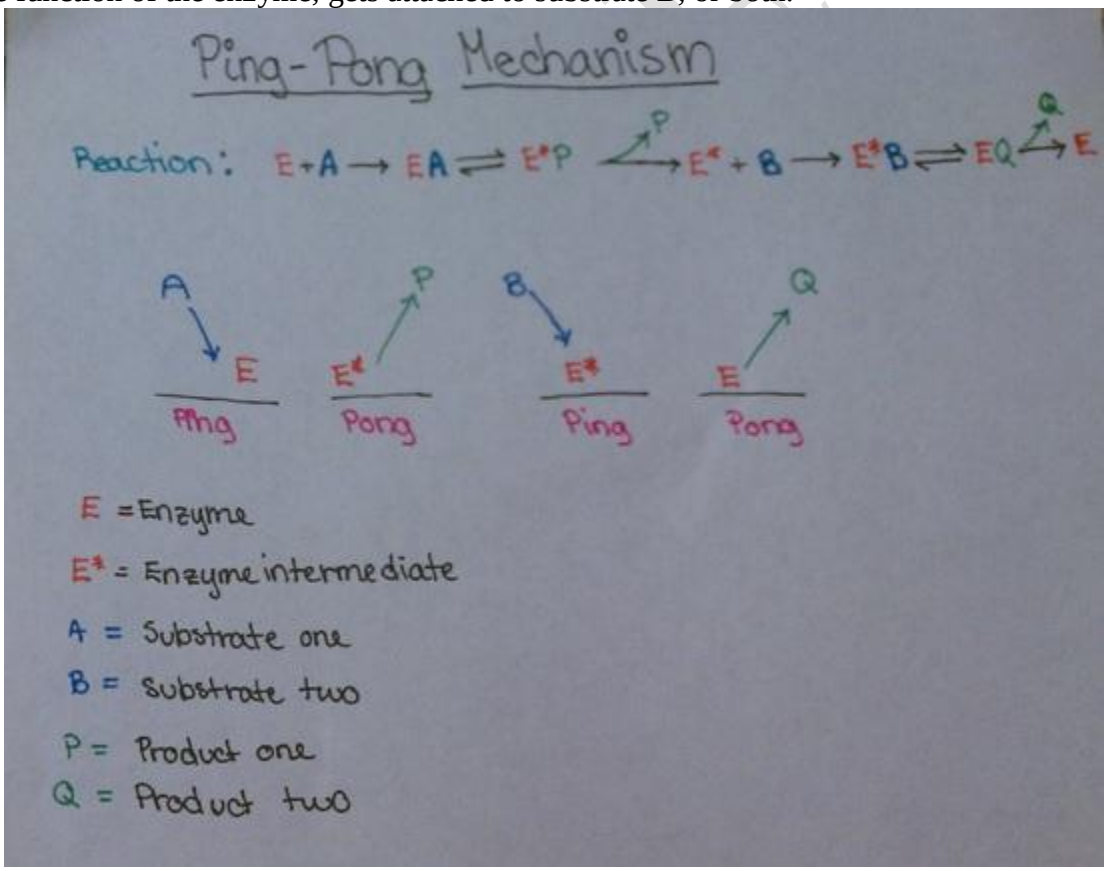
Introduction

Ping-pong mechanism, also called a double-displacement reaction, is characterized by the change of the enzyme into an intermediate form when the first substrate to product reaction

occurs. It is important to note the term intermediate indicating that this form is only temporary. At the end of the reaction the enzyme MUST be found in its original form. An enzyme is defined by the fact that it is involved in the reaction and is not consumed. Another key characteristic of the ping-pong mechanism is that one product is formed and released before the second substrate binds. The figure below explains the Ping Pong mechanism through an enzymatic reaction.



This image shows that as substrate A binds to the enzyme, enzyme-substrate complex EA forms. At this point, the intermediate state, E* forms. P is released from E* , then B binds to E*. B is converted to Q, which is released as the second product. E* becomes E, and the process can be repeated. Often times, E* contains a fragment of the original substrate A.This fragment can alter the function of the enzyme, gets attached to substrate B, or both.



CHYMOTRYPSIN

An example of the ping-pong mechanism would be the action of chymotrypsin. When reacted with p-nitrophenyl acetate (A), the reaction of chymotrypsin is seen to occur in two

steps. In the first step, the substrate reacts extremely fast with the enzyme, leading to the formation of a small amount of p-nitrophenolate (P). In the second step, the substrate-enzyme interaction results in the formation of acetate ion (Q). The action of chymotrypsin is a ping-pong reaction because the binding of the two substrates causes the enzyme to switch back and forth between two states.

PYRUVATE CARBOXYLASE

Another example of an enzyme that exhibits a ping-pong mechanism is pyruvate carboxylase. This enzyme catalyzes the addition of carbon dioxide to pyruvate in order to form oxaloacetate. (leads to gluconeogenesis) This biotin-containing enzyme works by binding CO_2 (A) to form carboxybiotin (EA). The biotin swings over towards pyruvate (E*P) and releases CO_2. (P, due to the fact that it had been moved from its original binding site) Pyruvate (B), in close proximity to CO_2, attacks the partial positive of Carbon in CO_2 (E*B). Oxaloacetate is formed within the enzyme (EQ) and gets released (Q). While this attack is occurring, biotin swings back to its initial position, (E* --> E) and is ready to bind another CO_2.

MECHANISM OF ACTION OF LYSOZYME

Lysozyme exerts its action as an anti-bacterial agent by hydrolyzing the peptidoglycan layer of the bacterial cell wall. The peptidoglycan layer is composed of a polymer of alternating NAM (Nacetyl muramic acid) and NAG (N-acetyl glucosamine) residues linked together by β-(1,4) glycosidic linkages. Lysozyme acts to hydrolyse these β-(1,4) glycosidic linkages.HEW lysozyme catalyses the reaction at a rate of 1010 fold greater than that of the uncatalysed reaction. The substrate binding cleft of the enzyme can accommodate a polysaccharide composed of as many as six sugar residues. If the six sugars are labeled A to F as shown in figure 3, it is the β-(1,4) glycosidic bond that links the sugar residue D with sugar residue E that is the exact site of attack by the enzyme. Specifically, it is the β-(1,4) glycosidic bond between the NAM residue at the fourth position and the NAG residue at the fifth position that is hydrolyzed by the enzyme.

The C6 and O6 atoms of the D residue is in very close physical proximity to Gln 57 , Trp108 of the enzyme as well as to the acetoamido group of residue C. This steric hindrance makes the binding of residue D to the enzyme very unstable. In order to accommodate the D

residue, the sugar ring distorts itself such that these contacts are minimized. The D residue changes its conformation from the chair to the half chair form (figure 4). In other words, this results in shifting of the atoms C(1), C(2), C(5) and O(5) of the D residue to a coplanar arrangement and movement of the ---C(6)H2OH group from its sterically unfavourable equatorial position to axial position where they no longer make such close contacts with enzyme. Instead, this group now makes hydrogen bond with the Gln57 residue of the enzyme.

GAPDH

GAPDH uses covalent catalysis and general base catalysis to decrease the very large and positive activation energy of the second step of this reaction. First, a cysteine residue in the active site of GAPDH attacks the carbonyl group of GAP, creating a hemithioacetal intermediate (covalent catalysis). Next, an adjacent, tightly bound molecule of NAD+ accepts a hydride ion from GAP, forming NADH while GAP is simultaneously oxidized to a thioester in a concerted series of steps. This thioester species is much higher in energy than the carboxylic acid species that would result in the absence of GAPDH (the carboxylic acid species is so low in energy that the energy barrier for the second step of the reaction (phosphorylation) would be too high, and the reaction, therefore, too slow and equilibrium too unfavorable for a living organism). Donation of the hydride ion by the hemithioacetal is facilitated by its deprotonation by a histidine residue in the enzyme's active site (general base catalysis). Deprotonation encourages the reformation of the carbonyl group in the thioester intermediate and ejection of the hydride ion. NADH leaves the active site and is replaced by another molecule of NAD+, the positive charge of which stabilizes the negatively charged carbonyl oxygen in the transition state of the next and ultimate step. Finally, a molecule of inorganic phosphate attacks the thioester and forms a tetrahedral intermediate, which then collapses to release 1,3-bisphosphoglycerate, and the thiol group of the enzyme's cysteine residue.

ALDOLASE

The generic fructose bisphosphate aldolase enzyme cleaves a 6-carbon fructose sugar into two 3-carbon products in a reverse aldol reaction. This reaction is typified by the formation of a Schiff base intermediate with a lysine residue (lysine 229) in the active site of the enzyme; the formation of a Schiff base is the key differentiator between Class I (produced by animals) and Class II (produced by fungi and bacteria) aldolases. After Schiff base formation, the fourth hydroxyl group on the fructose backbone is then deprotonated by an aspartate residue (aspartate

33), which results in an aldol cleavage. Schiff base hydrolysis yields two 3-carbon products. Depending on the reactant, F1P or FBP, the products are DHAP and glyceraldehyde or glyceraldehyde 3-phosphate, respectively.

The ΔG°' of this reaction is +23.9 kJ/mol. Though the reaction may seem too uphill to occur, it is of note that under physiological conditions, the ΔG of the reaction falls to close to or below zero. For example, the ΔG of this reaction under physiological conditions in erythrocytes is -0.23 kJ/mol.

Carboxypeptidase and Substrate Binding

In the human body, proteins are essential molecules in organisms and have a multitude of functions ranging from providing tensile strength to bones and tendons to providing storage and transportation of necessary substances such as O_2 and iron throughout the body. Hence, within the body's cells, proteins from foods must first be separated into their constituent amino acids. Then these amino acids are used to construct the proteins needed by our body.

To break down a protein into its constituent amino acids, the cell uses a hydrolysis reaction. The protein reacts with a water molecule to produce an amino acid and a new smaller protein. The enzyme carboxypeptidase A is secreted by the pancreas and is used to speed up this hydrolysis reaction., this enzyme consists of a single chain of 307 amino acids. It assumes a compact, globular shape containing regions of both a helices and b pleated sheets. This globular shape contains a region resembling a pocket, where a substrate can fit. This region is the active site of the enzyme.

Carboxypeptidase A is a good illustration of the induced-fit theory, because the active site changes appreciably when the substrate binds. Figures 2 and 3 show three-dimensional representations of the carboxylase protein with and without a bound substrate. Note how the active site changes shape when it is complexed with a substrate. As the protein substrate binds to carboxypeptidase, the active site closes in around it. Hydrolysis of the peptide bond is most likely to occur if the terminal residue has an aromatic or bulky hydrocarbon side chain. A zinc ion (Zn^{2+}) is tightly bound near the active site and assists in catalysis. Three hydrogen bonding

and electrostatic interactions are critical for the enzyme to recognize the terminal amino acid in the peptide chain. The intermediate is stabilized by interactions with Zn^{2+} and the carboxypeptidase molecule. The last step is a proton transfer and cleavage of the peptide bond. This entire process requires considerable mobility of the carboxypeptidase A protein itself.

ALCOHOL DEHYDROGENASE

Alcohol dehydrogenase (ADH) is located in the cytosol of stomach and liver cells and functions as the main enzyme for alcohol metabolism. ADH has a low Km and becomes saturated, reaching its Vmax, even at low concentrations of ethanol. Therefore, the enzyme appears to show zero-order kinetics because once the enzyme is saturated, the reaction rate is no longer dictated by the concentration of the ethanol.

ADH is an oxidoreductase enzyme that oxidizes alcohol to acetaldehyde while subsequently reducing an NAD+ cofactor to NADH. A Zn^{2+} atom is coordinated in the active site by Cys-174, Cys-46 and His-67 and functions to position the alcohol group of ethanol in the active site. Ser-48 and His-51 function similarly to a catalytic dyad, acting as a charge-relay network to help deprotonate the ethanol and activate it to be oxidized to the aldehyde. Before ethanol enters, a water molecule is initially positioned in the active site, but dissociates when the ethanol enters. At the end of the mechanism, water again enters the active site when the oxidized substrate—acetaldehyde—leaves.

Modes of Enzyme Action:

There are two view points by which enzymes are supposed to bring about chemical reaction.

i. Lock and Key Hypothesis:

It was put forward by Emil Fischer in 1894. According to this hypothesis, both enzyme and substrate molecules have specific geometrical shapes. 'In the region of active sites the surface configuration of the enzyme is such as to allow the particular substrate molecules to be held over it. The active sites also contain special groups having —NH2, —COOH, —SH for establishing contact with the substrate molecules.

The contact is such that the substrate molecules or reactants come together causing the chemical change. It is similar to the system or lock and key. Just as a lock can be opened by its specific

key, a substrate molecule can be acted upon by a particular enzyme. This also explains the specificity of enzyme action.

After coming in contact with the active site of the enzyme, the substrate molecules or reactants form a complex called enzyme-substrate complex. In the complexed state the molecules of the substrate undergo chemical change.

The products remain attached to the enzyme for some time so that an enzyme-product complex is also formed. However, the products are soon released (Fig. 9.34) and the freed enzyme is able to bind more substrate molecules.

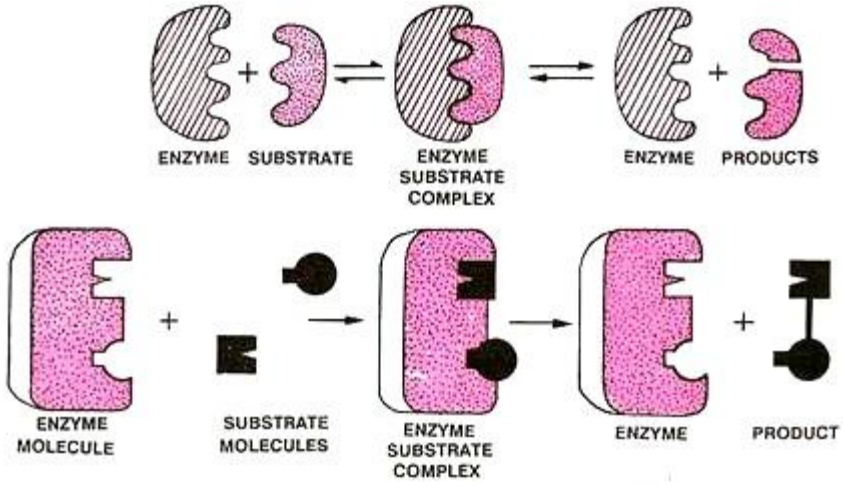


Fig. 9.33. Lock and key theory of enzyme action.
Upper Series – Breakdown Reaction Lower Series – Biosynthetic Reaction

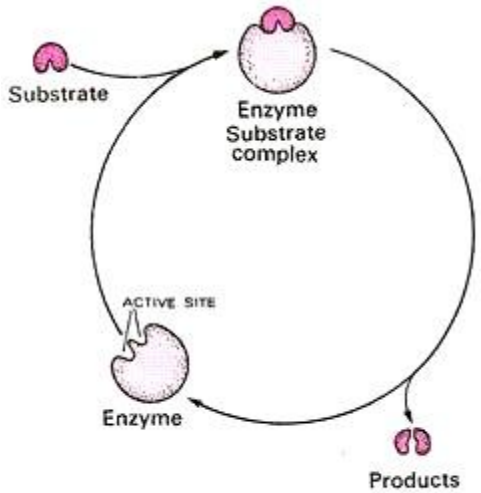


Fig. 9.34. The method by which the same enzyme molecule can be used again and again.

Enzyme + Substrate ⇋ Enzyme – Substrate Complex

Enzyme – Substrate Complex ⇋ Enzyme – Products Complex

Enzyme – Products Complex ⇋ Enzyme + Products

Thus we see that the chemical reactants do not cause any alteration in the composition or physiology of the enzyme. The same enzyme molecule can be used again and again (Fig. 9.35). Hence, enzymes are required in very small concentrations.

Evidences:

1. Blow and Steitz (1970) have found the formation of complex between the enzyme chymotrypsin and its substrate.

2. Keilen and Maun have observed that the absorption spectra of the same enzyme are different in the free state and in the presence of the substrate.

3. The theory explains how a small concentration of enzyme can act upon a large amount of the substrate.

4. Lock and key theory explains how the enzyme remains unaffected at the end of chemical reaction.

5. It is able to predict the increase in the rate of chemical reaction on the addition of more enzyme or substrate.

6. The theory explains how a substance having a structure similar to the substrate can work as competitive inhibitor.

ii. Induced-Fit Theory (Fig. 9.35):

It is modification of lock and key hypothesis which was proposed by Koshland in 1959. According to this theory the active site of the enzyme contains two groups, buttressing and catalytic. The buttressing group is meant for supporting the substrate. The catalytic group is able to weaken the bonds of reactants by electrophilic and nucleophilic forces.

The two groups are normally at a distance. As soon as the substrate comes in contact with the buttressing group, the active site of the enzyme undergoes conformational changes so as to bring the catalytic group opposite the substrate bonds to be broken.

Catalytic group helps in bringing about chemical reaction. The substrate is converted into product. The product is unable to hold on the buttressing site due to change in its structure and bonds. Buttressing group reverts to its original position. The product is released.

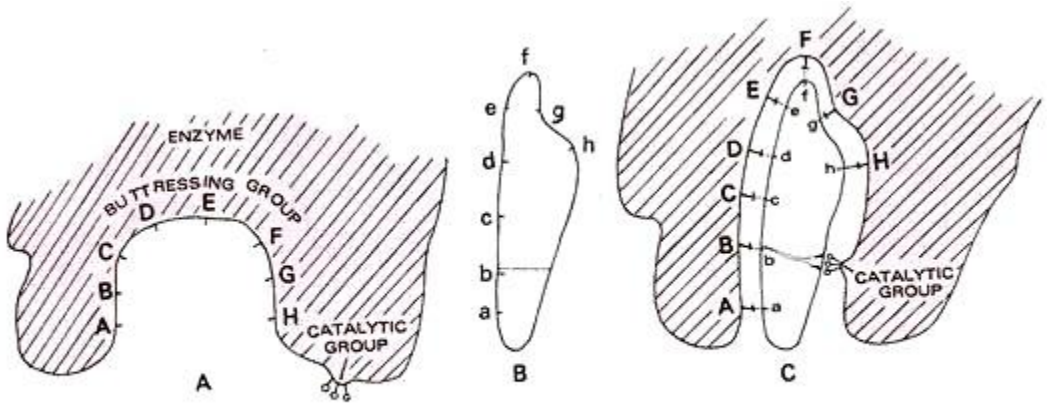


Fig. 9.35. Induced-fit theory of enzyme action. A, active site of enzyme. B, substrate molecule. C, enzyme-substrate complex with conformational changes so as to bring the catalytic group against the substrate bonds to be broken.

Note # 16. Inhibition of Enzyme Action:

Reduction or stoppage of enzyme activity due to presence of adverse conditions or chemicals is called enzyme inhibition. It is of several types. Inhibition can be classified into two (a) Reversible and irreversible (b) Competitive and non-competitive.

Reversible inhibition is that inhibition which can be overcome by withdrawal of the inhibitor because the effect of the latter is of temporary nature due to blocking of active site or binding to linkages required for maintenance of active site. Dilution and dialysis reduces or eliminates the effect of reversible inhibition. Irreversible inhibition is of permanent nature as the enzyme conformation is harmed.

Denaturation of enzyme is an example of irreversible inhibition. Heavy metals (e.g., Ag^{+}, Hg^{2+}, As^{+}) and iodoacetic acid cause irreversible inhibition by combining with —SH groups and destroying protein structure. Dilution and dialysis have little effect once irreversible inhibition has set in.

Competitive inhibition is caused by swamping of the active sites by a chemical which is similar in structure to the substrate but does not undergo chemical change. Competitive inhibition is usually reversible. Non-competitive inhibition is caused by alteration of conformation of the

enzyme by a chemical that binds to a site other than the active site. It may be reversible or irreversible.2

Four common types of enzymes inhibition are as follows:

i. Protein Denaturation:

Enzyme activity is dependent upon the maintenance of tertiary structure of the protein moiety. The latter is destroyed by several factors like heat, high energy radiations and salts of heavy metals.

ii. Competitive inhibition:

It is the inhibition of enzyme activity by the presence of a chemical that competes with the substrate for binding to the active site of the enzyme. The inhibitor chemical is also called substrate analogue or competitive inhibitor.

It resembles the substrate in structure and gets bound up to the active site of the enzyme without getting transformed by the latter (Fig. 9.37). As a result, the enzyme cannot participate in catalytic change of the substrate. This is similar to the jamming of a lock by a key similar to original one.

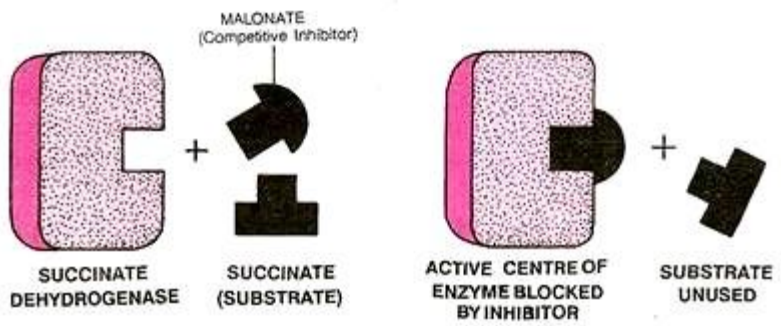
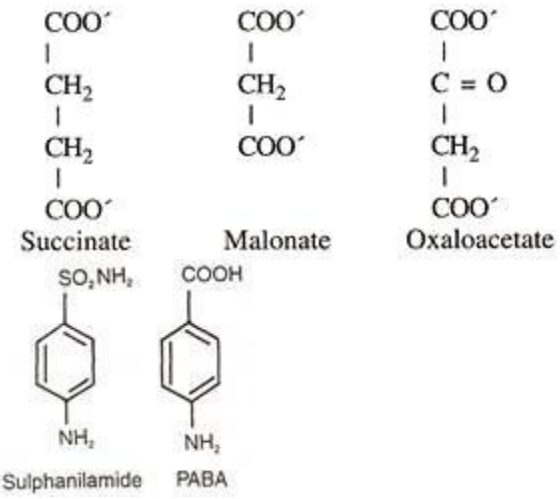


Fig. 9.37. Competitive inhibition of enzyme action.

Equilibrium constant for inhibitor binding is called K_i. A high $K_i$reduces enzyme activity while a low K_i allows enzyme activity to continue though at a reduced rate. Classical example of competitive inhibition is reduction of activity of succinate dehydrogenase by malonate, oxaloacetate and other anions which resemble succinate in their structure.



Competitive inhibition is usually reversible since the addition of more substrate tends to reduce the effect of the inhibitor.

The inhibition is important in that:

(i) It gives evidence for lock and key hypothesis of enzyme action,

(ii) Substrate analogues are not metabolized by enzymes,

(iii) Control of bacterial pathogens has been effected through competitive inhibition.

Sulpha drugs (e.g., sulphanilamide) inhibit the synthesis of folic acid in bacteria by competing with p-amino benzoic acid (PABA) for the active site of enzyme. Preformed folic acid is obtained by animal cells. Therefore, sulpha drugs do not harm them.

iii. Non-competitive Inhibition:

It is an irreversible inhibition of enzyme activity by the presence of a substance that has no structural similarity with the substrate. It is of two types, reversible and irreversible.

The irreversible non-competitive inhibitor destroys or combines irreversibility with a functional group of enzyme that is essential for its catalytic function. Cyanide inhibits the activity of cytochrome oxidase by combining with its metallic ions.

It has no structural similarity with the substrate of the enzyme, namely cytochrome c. Cytochrome oxidase is a respiratory enzyme. In its inhibition, the animal is unable to perform the respiration properly and gets killed. Di-isopropyl fluorophosphates (DFP, a nerve gas) prevents impulse transfer by combining irreversibly with amino acid serine of acetylcholine esterase.

It also poisons a number of other enzymes like trypsin, chymotrypsin, phosphoglucomutase, elastase, etc. lodoacetamide inhibits enzymes having sulphahydryl (—SH) or imidazole group.

iv. Allosteric Modulation or Feed Back Inhibition:

It is a type of reversible inhibition found in allosteric enzymes. The inhibitor is non-competitive and is usually a low molecular intermediate or product of a metabolic pathway having a chain of reactions involving a number of enzymes. It is, therefore, also called end product or feedback inhibition.

The inhibitor is also called modulator. Modulator is a substance that attaches with an allosteric enzyme at a site other than catalytic one but influences the latter, either inhibiting or activating the same. An example of feed back or allosteric inhibition is stoppage of activity of enzyme hexokinase (glucokinase) by glucose-6-phosphate, the product of reaction catalysed by it (Fig. 9.38).

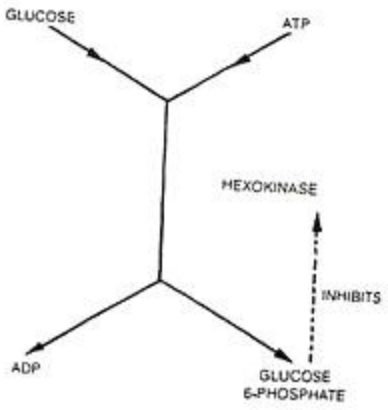


Fig. 9.38. Feedback or allosteric inhibition of hexokinase.

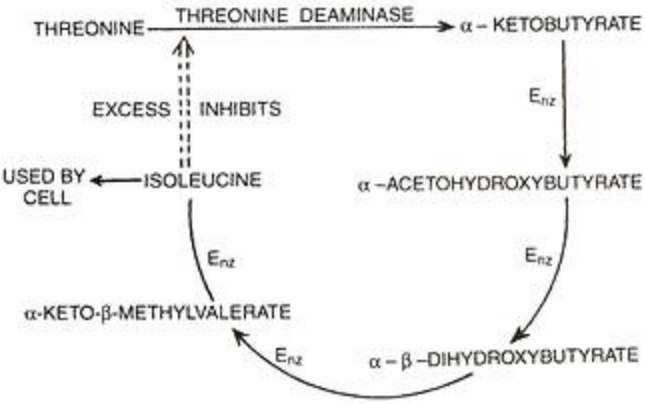


Fig. 9.39. Feedback or end product in case of isoleucine.

Another example is inhibition of threonine deaminase by isoleucine (Fig. 9.3). Amino acid isoleucine is formed in bacterium Escherichia coli in a 5-step reaction from threonine. Each step requires a separate enzyme. When isoleucine accumulates beyond a threshold value, its further production stops.

Isoleucine added to the medium of bacterium also stops its internal production showing that its excess prevents some step of the reaction. The latter was found out to be enzyme threonine deaminase which is involved in the first step of the reaction (threonine to a-ketobutyrate).

Importance:

(i) It has a regulatory role on enzyme activity,

(ii) Enzyme inhibitors have been used in the study of metabolic pathways,

(iii) Some inhibitors are used in controlling pathogenic activity, e.g., sulpha drugs,

(iv) Use of inhibitors have shown the mechanism of enzyme action.

Feedback Inhibition of Enzymes:

Feedback inhibition (also called end-product inhibition or allosteric modulation) is one in which the end- product of the reaction acts as inhibitor and inhibits the activity of regulatory enzyme, usually, enzyme of the first step of a biosynthetic pathways. In multi-enzyme system synthesis of a product is completed in a number of steps, each step being catalyzed by a specific enzyme.

In some of such 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 requirement. When the reaction catalyzed by the regulatory enzyme is slowed, all subsequent enzymes act at reduced rates due to the depletion of their substrates.

The rate of production of the pathway's end-product is thereby brought into balance as per the requirement of the cell. Feedback inhibition is beautifully illustrated by biosynthesis of L-isoleucine from L-threonine.

In this system, the first enzyme, threonine dehydratase, is inhibited by L-isoleucine. No other intermediate in the sequence inhibits threonine dehydratase, nor is any other enzyme of the system inhibited by L-isoleucine.

L-isoleucine binds not to the active site, but to regulatory site on the enzyme molecule; this is called allosteric modulation. However, when the concentration of the end-product drops sufficiently, the enzyme reactivates and the end-product is resynthesized.

Specificity of Enzyme:

One characte-ristic that distinguishes an enzyme from all other types of catalysts is its substrate specificity (Fig. 8.19). Enzyme specificity is a result of the uniqueness of the active site of each enzyme.

Enzyme specificity

Enzyme specificity is arbitrari-ly grouped as:

i. Absolute specificity:

Enzymes having absolute specificity will catalyse a particular substrate only and will have no catalytic effect on substrates that are closely related. E.g., Urease will catalyse the hydrolysis of urea but not of methyl urea, thiourea or biuret:

ii. Stereo-chemical specificity:

Most enzymes show a markedly high degree of specificity toward one stereo-isomeric form of the substrate, e.g:

(1) Lactic acid dehydro-genase catalyses the oxidation of the L-lactic acid found in muscle cells but not the D-lactic acid found in certain microorga-nisms.

(2) Fumerase adds water to fumaric acid but not to its cis-isomer-maleic acid.

iii. Group specificity:

Enzymes having group specificity are less selective in that they will act upon structurally similar molecules having the same functional groups, e.g., many of the peptidases.

(1) Pepsin will hydrolyse all peptides having adjacent aromatic amino acids.

(2) Carboxy-peptidase attacks peptides from the carboxyl end of the chain, cleaving the amino acids one at a time.

iv. Linkage specificity:

Enzymes hav-ing linkage specificity are the least specific of all, because they will attack a particular kind of chemical bond, irrespective of the struc-tural features in the vicinity of linkage, e.g., lipases catalyse the hydrolysis of ester link-ages in lipids.

ENZYME REGULATION: PRODUCT INHIBITION

Introduction

The cells of your body are capable of making many different enzymes, and at first you might think: *great, let's crank all of those enzymes up and metabolize as fast as possible!* As it turns

out, though, you really don't want to produce and activate all of those enzymes at the same time, or in the same cell.

Needs and conditions vary from cell to cell and change in individual cells over time. For instance, stomach cells need different enzymes than fat storage cells, skin cells, blood cells, or nerve cells. Also, a digestive cell works much harder to process and break down nutrients during the time that follows a meal as compared with many hours after a meal. As these cellular demands and conditions changes, so do the amounts and functionality of different enzymes.

Because enzymes guide and regulate the metabolism of a cell, they tend to be carefully controlled. In this article, we'll take a look at factors that can affect or control enzyme activity. These include pH and temperature (discussed in the active site article), as well as:

- **Regulatory molecules.** Enzyme activity may be turned "up" or "down" by activator and inhibitor molecules that bind specifically to the enzyme.
- **Cofactors.** Many enzymes are only active when bound to non-protein helper molecules known as cofactors.
- **Compartmentalization.** Storing enzymes in specific compartments can keep them from doing damage or provide the right conditions for activity.
- **Feedback inhibition.** Key metabolic enzymes are often inhibited by the end product of the pathway they control (feedback inhibition).

In the rest of this article, we'll examine these factors one at a time, seeing how each can affect enzyme activity.

Regulatory molecules

Enzymes can be regulated by other molecules that either increase or reduce their activity. Molecules that increase the activity of an enzyme are called **activators**, while molecules that decrease the activity of an enzyme are called **inhibitors**.

There are many kinds of molecules that block or promote enzyme function, and that affect enzyme function by different routes.

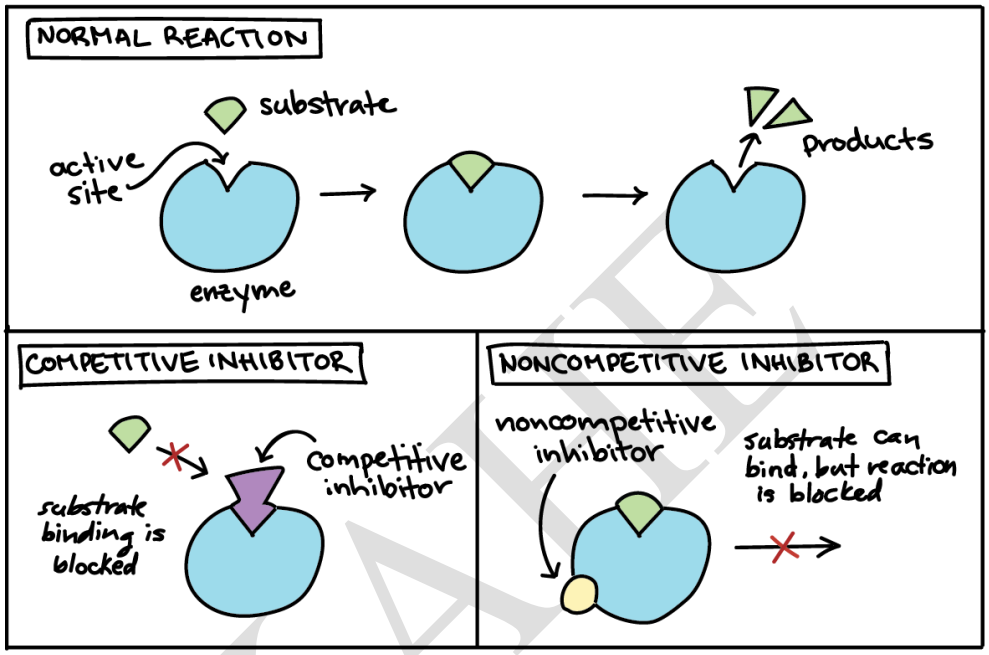
Competitive vs. noncompetitive

In many well-studied cases, an activator or inhibitor's binding is reversible, meaning that the molecule doesn't permanently attach to the enzyme. Some important types of drugs act as reversible inhibitors. For example, the drug tipranivir, which is used to treat HIV, is a reversible inhibitor.^11 It blocks activity of a viral enzyme that helps the virus make more copies of itself.

Reversible inhibitors are divided into groups based on their binding behavior. We won't discuss all of the types here, but we will look at two important groups: competitive and noncompetitive inhibitors.

- An inhibitor may bind to an enzyme and block binding of the substrate, for example, by attaching to the active site. This is called **competitive inhibition**, because the inhibitor "competes" with the substrate for the enzyme. That is, only the inhibitor or the substrate can be bound at a given moment.
- In **noncompetitive inhibition**, the inhibitor doesn't block the substrate from binding to the active site. Instead, it attaches at another site and blocks the enzyme from doing its job. This inhibition

is said to be "noncompetitive" because the inhibitor and substrate can both be bound at the same time.

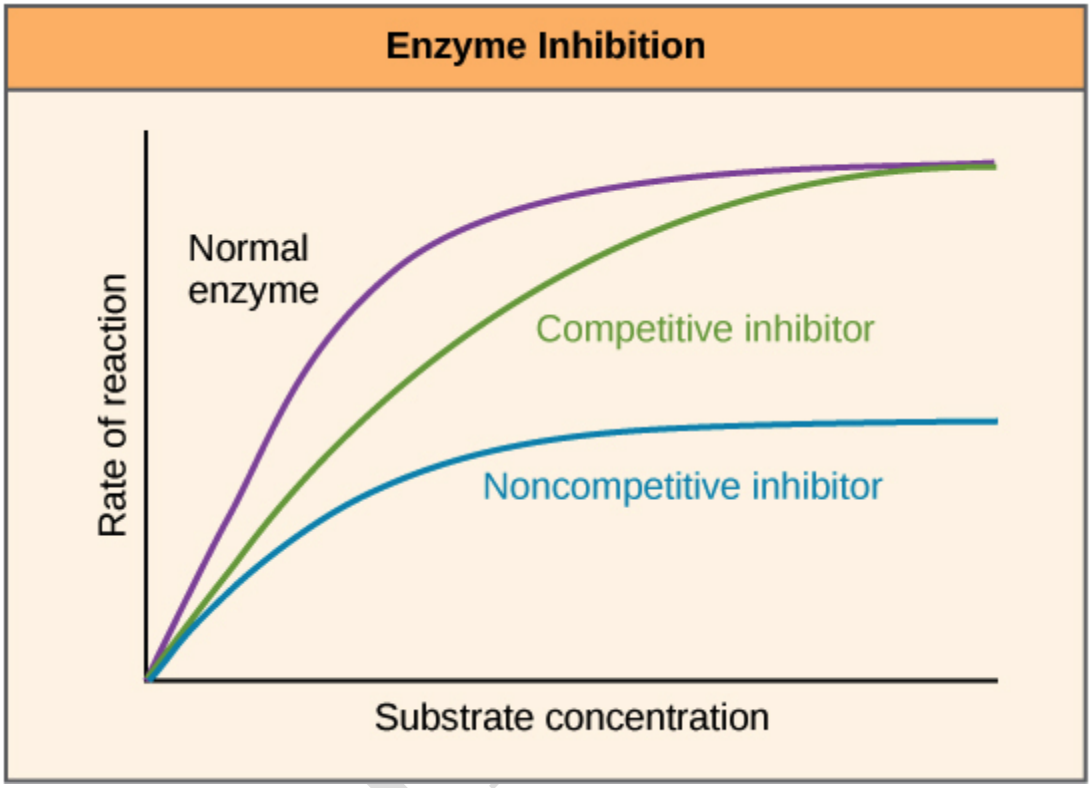


Competitive and non-competitive inhibitors can be told apart by how they affect an enzyme's activity at different substrate concentrations.

- If an inhibitor is competitive, it will decrease reaction rate when there's not much substrate, but can be "out-competed" by lots of substrate. That is, the enzyme can still reach its maximum reaction rate given enough substrate. In that case, almost all the active sites of almost all the enzyme molecules will be occupied by the substrate rather than the inhibitor.
- If an inhibitor is noncompetitive, the enzyme-catalyzed reaction will never reach its normal maximum rate even with a lot of substrate. This is because the enzyme molecules with the

noncompetitive inhibitor bound are "poisoned" and can't do their job, regardless of how much substrate is available.

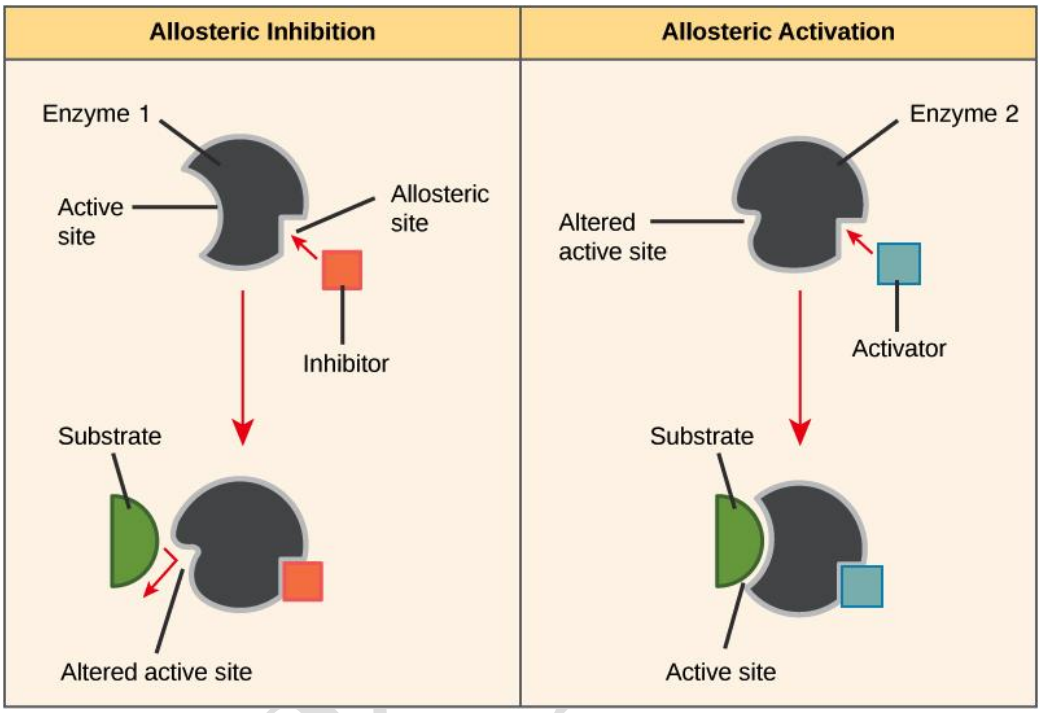
On a graph of reaction velocity (y-axis) at different substrate concentrations (x-axis), you can tell these two types of inhibitors apart by the shape of the curves:



This plot shows rate of reaction versus substrate concentration for an enzyme in the absence of inhibitor, and for enzyme in the presence of competitive and noncompetitive inhibitors. Both competitive and noncompetitive inhibitors slow the rate of reaction, but competitive inhibitors can be overcome by high concentrations of substrate, whereas noncompetitive inhibitors cannot.

Allosteric regulation

Allosteric regulation, broadly speaking, is just any form of regulation where the regulatory molecule (an activator or inhibitor) binds to an enzyme someplace other than the active site. The place where the regulator binds is called the **allosteric site**.



The left part of this diagram shows allosteric inhibition. The allosteric inhibitor binds to an enzyme at a site other than the active site. The shape of the active site is altered so that the enzyme can no longer bind to its substrate.

The right part of this diagram shows allosteric activation. The allosteric activator binds to an enzyme at a site other than the active site. The shape of the active site is changed, allowing substrate to bind at a higher affinity.

Pretty much all cases of noncompetitive inhibition (along with some unique cases of competitive inhibition) are forms of allosteric regulation.

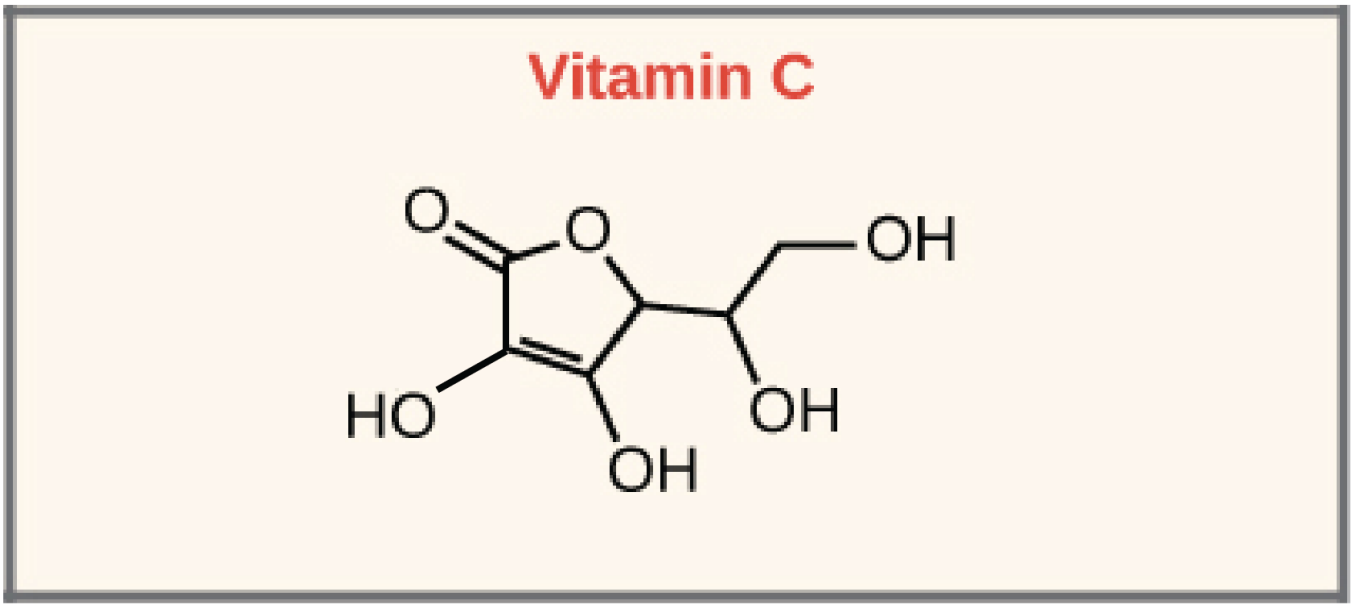
However, some enzymes that are allosterically regulated have a set of unique properties that set them apart. These enzymes, which include some of our key metabolic regulators, are often given the name of **allosteric enzymes**. Allosteric enzymes typically have multiple active sites located on different protein subunits. When an allosteric inhibitor binds to an enzyme, all active sites on the protein subunits are changed slightly so that they work less well.

There are also allosteric activators. Some allosteric activators bind to locations on an enzyme other than the active site, causing an increase in the function of the active site. Also, in a process called **cooperativity**, the substrate itself can serve as an allosteric activator: when it binds to one active site, the activity of the other active sites goes up. This is considered allosteric regulation because the substrate affects active sites far from its binding site.

Cofactors and coenzymes

Many enzymes don’t work optimally, or even at all, unless bound to other non-protein helper molecules called **cofactors**. These may be attached temporarily to the enzyme through ionic or hydrogen bonds, or permanently through stronger covalent bonds.Common cofactors include inorganic ions such as iron \text {(Fe}^{2+})(Fe2+) and magnesium (\text {Mg}^{2+})(Mg2+). For example, the enzyme that builds DNA molecules, DNA polymerase, requires magnesium ions to function.

Coenzymes are a subset of cofactors that are organic (carbon-based) molecules. The most common sources of coenzymes are dietary vitamins. Some vitamins are precursors to coenzymes and others act directly as coenzymes. For example, vitamin C is a coenzyme for several enzymes that take part in building the protein collagen, a key part of connective tissue.



Chemical structure of vitamin C, which acts as a coenzyme for several enzymes.

Enzyme compartmentalization

Enzymes are often compartmentalized (stored in a specific part of the cell where they do their job) -- for instance, in a particular organelle. Compartmentalization means that enzymes needed for specific processes can be kept in the places where they act, ensuring they can find their substrates readily, don't damage the cell, and have the right microenvironment to work well.

For instance, digestive enzymes of the lysosome work best at a pH around 5.05.0, which is found in the acidic interior of the lysosome (but not in the cytosol, which has a pH of about 7.27.2). Lysosomal enzymes have low activity at the pH of the cytosol, which may serve as "insurance" for the cell: even if a lysosome bursts and spills its enzymes, the enzymes will not begin digesting the cell, because they will no longer have the right pH to function.

Feedback inhibition of metabolic pathways

In the process of **feedback inhibition**, the end product of a metabolic pathway acts on the key enzyme regulating entry to that pathway, keeping more of the end product from being produced.

This may seem odd – why would a molecule want to turn off its own pathway? But it's actually a clever way for the cell to make just the right amount of the product. When there's little of the product, the enzyme will not be inhibited, and the pathway will go full steam ahead to replenish the supply. When there's lots of the product sitting around, it will block the enzyme, preventing the production of new product until the existing supply has been used up.

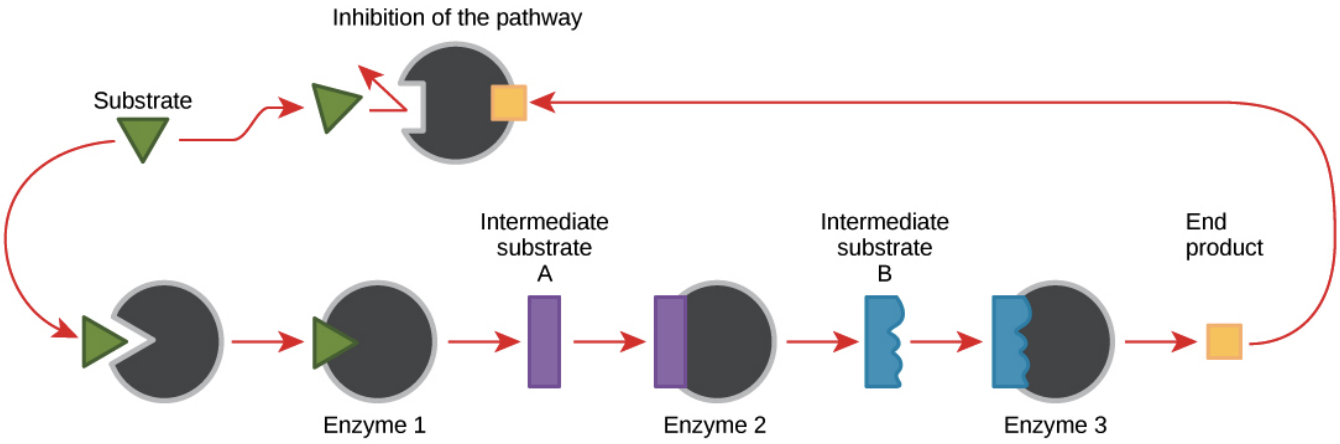


Diagram illustrating feedback inhibition. The end product of a multi-step metabolic pathway binds to an allosteric site on the enzyme that catalyzes the committed step of the pathway, reducing the enzyme's activity. This regulation helps slow the pathway down when levels of the end product are already high (when more is not needed).

Typically, feedback inhibition acts at the **first committed step** of the pathway, meaning the first step that's effectively irreversible. However, feedback inhibition can sometimes hit multiple points along a pathway as well, particularly if the pathway has lots of branch points. The pathway steps regulated by feedback inhibition are often catalyzed by allosteric enzymes.

For example, the energy carrier molecule ATP is an allosteric inhibitor of some of the enzymes involved in cellular respiration, a process that makes ATP to power cellular reactions. When there is lots of ATP, this feedback inhibition keeps more ATP from being made. This is useful because ATP is an unstable molecule. If too much ATP were made, much of it might go to waste, spontaneously breaking back down into its components (ADP and P_i*i*).

ADP, on the other hand, serves as a positive allosteric regulator (an allosteric **activator**) for some of the same enzymes that are inhibited by ATP. For instance, ADP may act by binding to an enzyme and changing its shape so that it becomes more active.

UNIT-III

SYLLABUS

Allosteric enzymes: Allosteric enzymes with special reference to aspartate transcarbomylase and phosphofructokinase. Qualitative description of concerted and sequential models. Negative cooperativity and half site reactivity. Enzyme - Enzyme interaction, Protein ligand binding, measurements analysis of binding isotherm, cooperativity, Hill and scatchard plots, kinetics of allosteric enzymes. Isoenzymes– multiple forms of enzymes with special reference to lactate dehydrogenase. Multienzyme complexes. Ribozymes. Multifunctional enzyme-eg Fatty Acid synthase.

Allosteric Inhibition:

Inhibition of threonine deaminase by isoleucine is an example of allosteric inhibition. Threonine deaminase deaminates threonine to α-ketrobutyrate. The final product of the reaction is isoleucine.

Whenever the accumulation of isoleucine occurs, conversion of threonine to α-ketobutyrate and consequently formation of other intermediaries in the biosynthesis of isoleucine is stopped. When isolcucine is used up, threonine deaminase is reactivated and reactions for the biosynthesis of isoleucine start again.

ii. Allosteric Activation:

Activation of glycogen synthetase by glucose-6-phosphate is an example of allosteric activation. Another example of allosteric regulation (of both inhibitory and activating type) is observed during Pasteur effect. Pasteur effect is the inhibition of glycolysis and fermentation by oxygen. The molecular basis of this effect is the allosteric inhibition of enzyme phosphofructokinase by ATP and citrate and its activation by AMP.

Like many others, this kind of regulation is also of adaptive significance. As the level of AMP increases due to increased use of ATP in the cell, glycolysis is increased by the activation of phosphofructokinase with the result of more formation of ATP. When ATP level exceeds normal

requirement of the cell, inhibition of glycolysis occurs through the same enzyme phosphofructokinase and ATP synthesis is stopped.

iii. Mechanism of Allosteric Regulation:

Regarding the mechanism of allosteric regulation, it is proposed that allosteric enzymes have two active centers; one for the substrate and the other for effector. These two sites lie either on same or on two different subunits. Binding of a effector molecule to one type of subunit changes the structure of the enzyme molecule in such a way that binding of the substrate to the other subunit is affected.

To explain the mechanism, an example of allosteric regulation of aspartate transcarbamylase may be cited. Asparate transcarbamylase contains two types of subunits. These two types of subuntis may be split apart by treatment with mercurials; with one type retaining the ability to bind with the substrate, whereas the other to recognize the inhibitor.

When these two species of subunits are together (active enzyme molecule), binding of the inhibitor to one type of subunit changes the structure of other subunits in such a way that the binding of the substrate is inhibited. When the subunits containing binding sites for the inhibitor are removed enzyme is not affected by the inhibitor.

Further, it gives a typical Michaelis-Menten curve with the substrate concentration. Similarly, the binding of activator may change the molecular structure in such a way that the binding of substrate is facilitated.

II. Isozyme Formation:

Another phenomenon that controls cellular metabolism is the formation of isozymes (isoenzymes). Isozymes are different physical forms of the same enzyme performing the same general function at different rates. They differ to some extent in their amino acid composition also, so that they may be separated by electrophoresis. Lactate dehydrogenase is a classic example of isozyme formation. It catalyses the oxidation of lactate to pyruvate with the help of NAD+.

Lactate dehydrogenase enzyme is a tetramer composed of two distinct types (H and M types) of subunits.

Depending upon the relative number of two types of subunits, lactate dehydrogenase forms 5 isozymes as follows:

LD1 = HHHH

LD2 = HHHM

LD3 = HHMM

LD4=HMMM

LD5 = MMMM

The molecular weight of the enzyme is 13,500 but when it is treated with urea or guanidine hydrochloride, it dissociates into subunits each having a molecular weight of about 35,000. The regulation of different isozymes is different. LD1 (HHHH) type of lactate dehydrogenase is found in the heart muscles.

This species is most active at low pyruvate concentration and is inhibited by high concentrations of pyruvate. LD5 (MMMM) type of enzyme is found in skeletal muscle cells and it remains active at high pyruvate concentrations.

Another example of isozyme formation is that of aspartokinase. This enzyme catalyzes the reaction between aspartic acid and ATP to form aspartyl phosphate. Amino acids lysine, methionine, and threonine are final products of the reaction.

The enzyme aspartokinase exists in three forms—aspartokinase I, aspartokinase II and aspartokinase III. Aspartokinase I is inhibited by threonine and III by lysine. Aspartokinase II is insensitive to any of these amino acids. Thus, when any one of these amino acids accumulates, the synthesis of the other is affected very little.

III. Multienzyme System:

Some enzymes exist not as individuals but as aggregates of several enzymes and coenzymes. This they do to channel the metabolities in a pathway efficiently. In an aggregate, each component is arranged in a way that the product of one enzyme becomes the substrate for the other and so on.

An example of enzyme aggregation is that of pyruvic acid dehydrogenase of E. coli. This complex consists of three enzymes- pyruvate decarboxylase, dihydrolipoic dehydrogenase and lipoyl reductase transacetylase. The coenzyme associated with the complex are thiamine pyrophosphate (TPP) and flavin adenine dinucleotide (FAD). A schematic diagram of pyruvate dehydrogenase complex is given in Fig. 27.13.

Pyruvate dehydrogenase complex system

The stepwise reactions catalyzed by this complex may be written as follows:

Pyruvate + thiamine pyrophosphate → α-hydroxythyl thiamine + pyrophosphate + CO_2

α -hydroxyethyl thiamine + lipoate → Thiamine pyrophosphate + acetyl dihydrolipoate

Acetyl dihydrolipoate + CoASH → Acetyl CoA + dihydrolipoate

Dihydrolipoate + NAD+/FAD+ → Lipoate + NADH/FADH2

IV. Regulation by Adenylate Energy Charge:

The importance of adenosine phosphates in metabolic processes has been well recognised for living systems. The adenylate energy charge is the measure of total pool of adenosine phosphates in the form of ATP, ADP and AMP. D.D. Atkinson (1969) defines adenylate energy charge as follows.

In most systems, an increase in adenylate energy charge in the physiological range results in stimulation of regulatory enzymes. Although it is a well-known phenomenon in animals and microorganisms, some instances have been recorded from plants also.

It has been shown that the adenylate energy charge affects the activity of pyrophosphomevalonate decarboxylase, which is the key enzyme in the biosynthesis of kaurene from mevalonate. An increase in enzyme activity is observed between adenylate energy charge of 0.8 and 1.0.

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

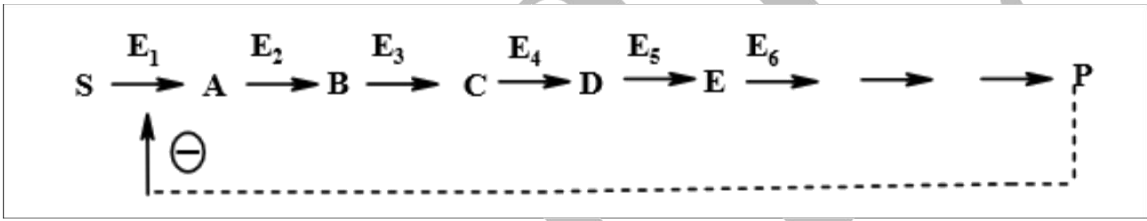
Metabolic pathways are made up of many chemical reactions and these reactions are catalysed by enzymes. Often, the product of the last reaction in the pathway inhibits the enzyme that catalyses the first reaction of the pathway. This is called end-product inhibition and it involves non-competitive inhibitors. 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:

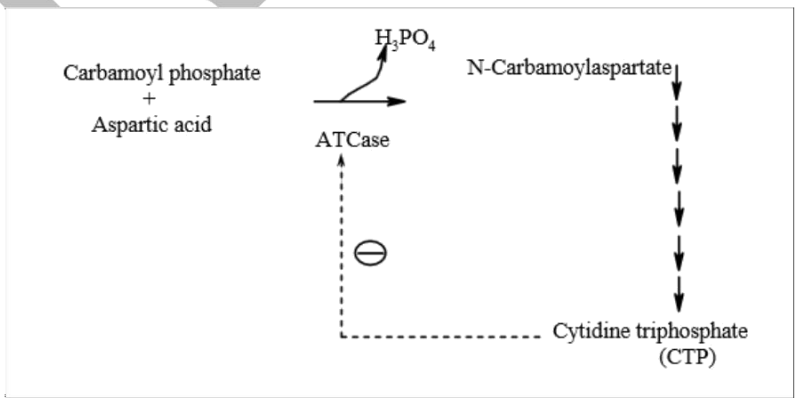
i. None of the intermediate products inhibits the enzyme E1.

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



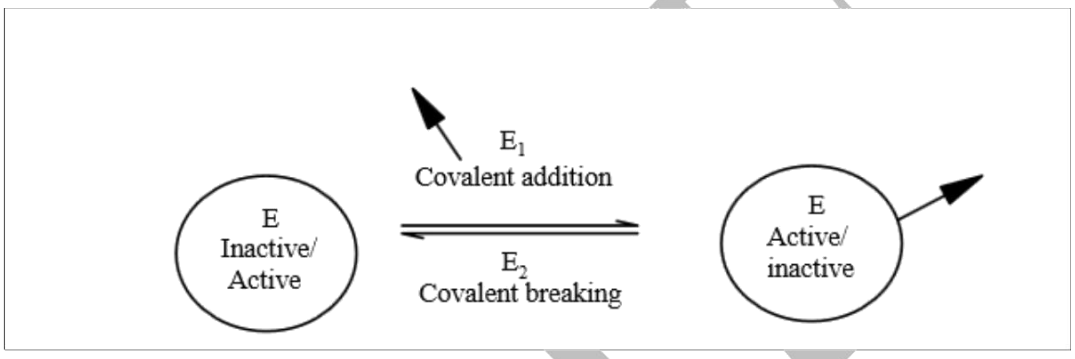
So, only regulatory enzymes are subjected tofeedback inhibition. These enzymes are allosteric in nature. The following are the well established examples showing feedback inhibition:

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:

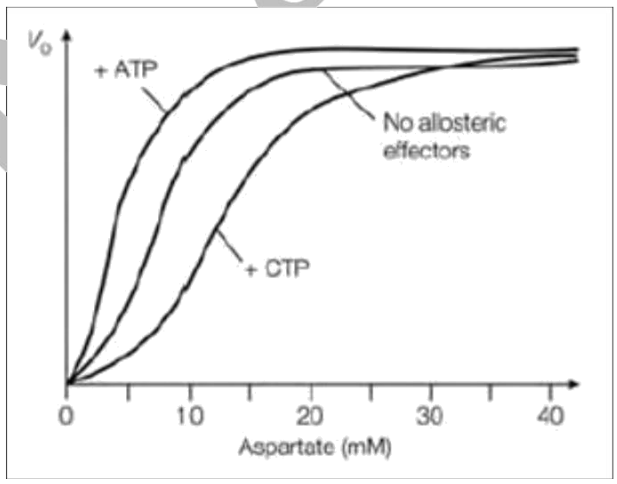


Reversible covalent modification

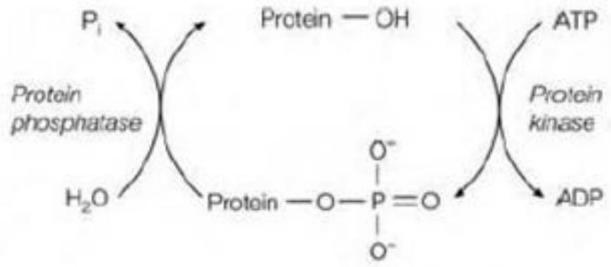
This is also one of the major ways of controlling the enzyme activity to exercise a regulatory control over metabolism. In this the enzyme protein gets activated or inhibited by undergoing through a covalent modification. These modifications are reversible and require two enzymes. Depending on the metabolic 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.

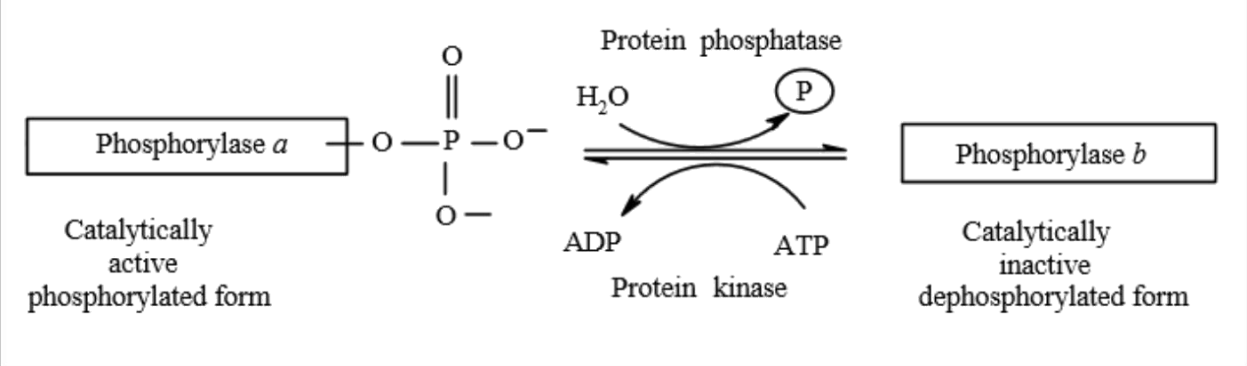
Glycogen phosphorylase:

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

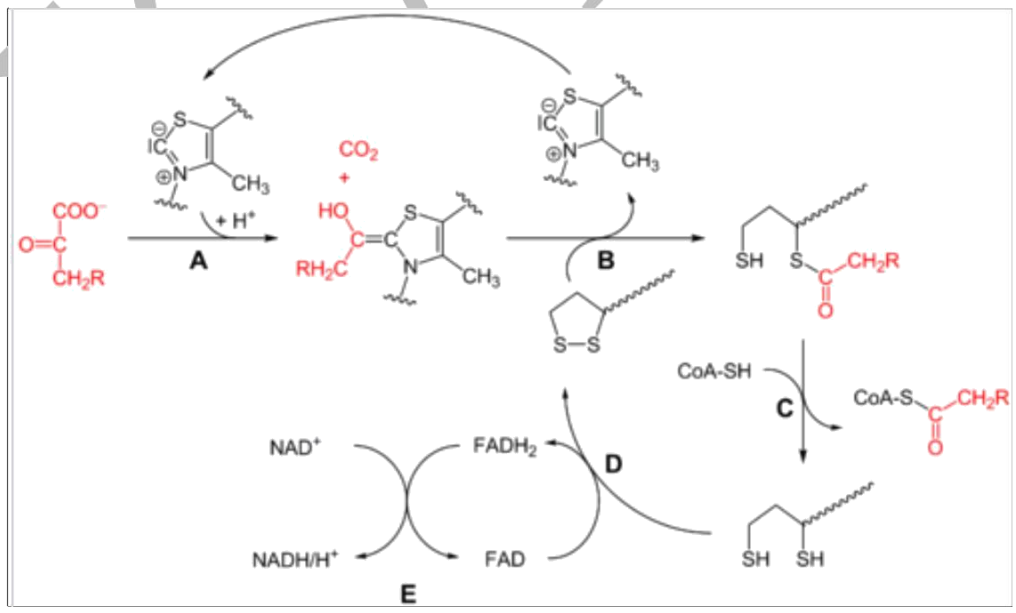
Multienzyme systemA complex of enzymes within a cell that form a reaction sequence of a biochemical pathway so that the product of the first enzyme reaction is transferred directly to the next enzyme and immediately undergoes a second reaction, and so on. The rate of an enzyme reaction often depends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

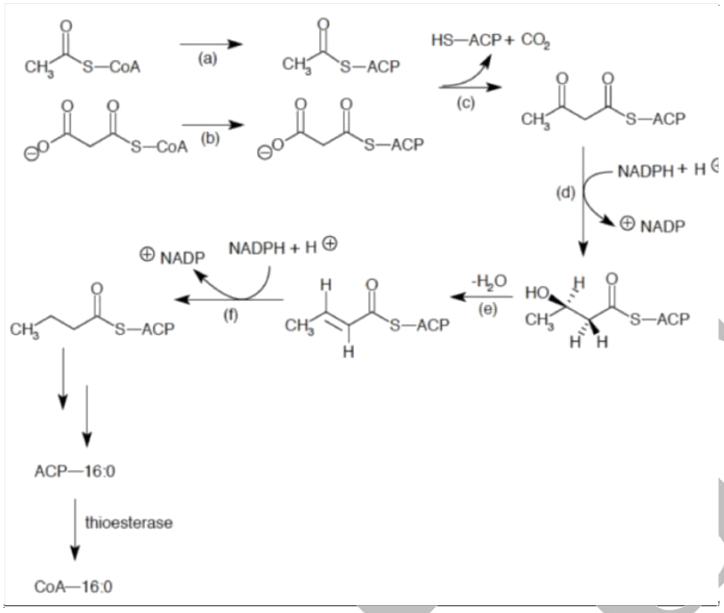
Many enzymes in living cells catalyse chains of reaction in a sequential order either in a biosynthetic or a catabolic pathway. In the preceding paragraphs hypothetical examples of such pathways have been cited.

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.

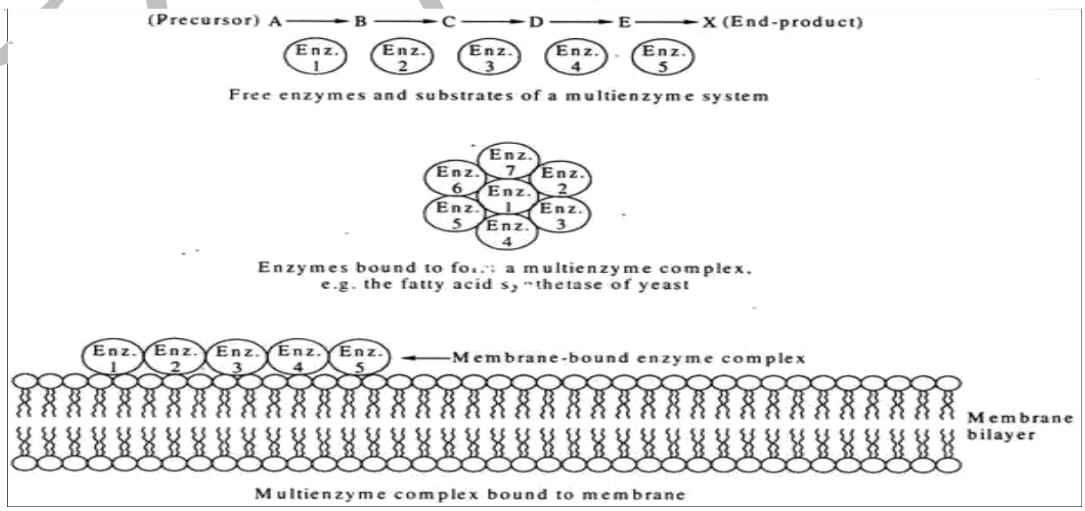
Pyruvate dehydrogenase



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.

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

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

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.

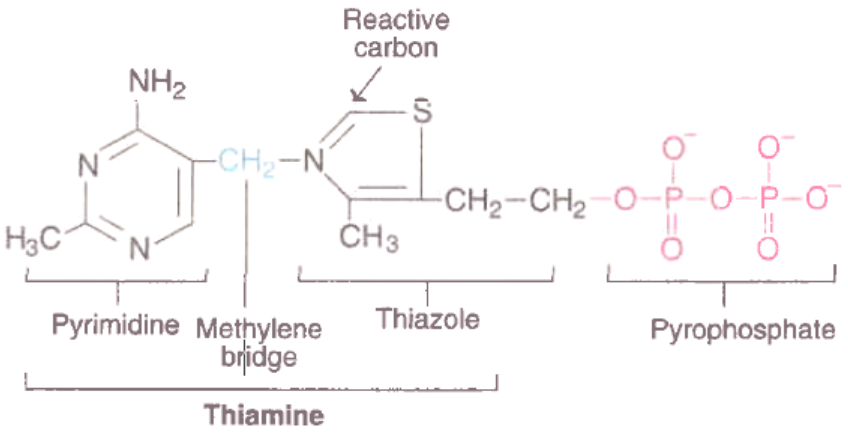
The functional enzyme is referred to as holoenzyme which is made up of a protein part (apoenzyme) and a non protein part (coenzyme). The term prosthetic group is used when a non protein moiety is tightly bound to the enzyme which is not easily separable by dialysis.

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

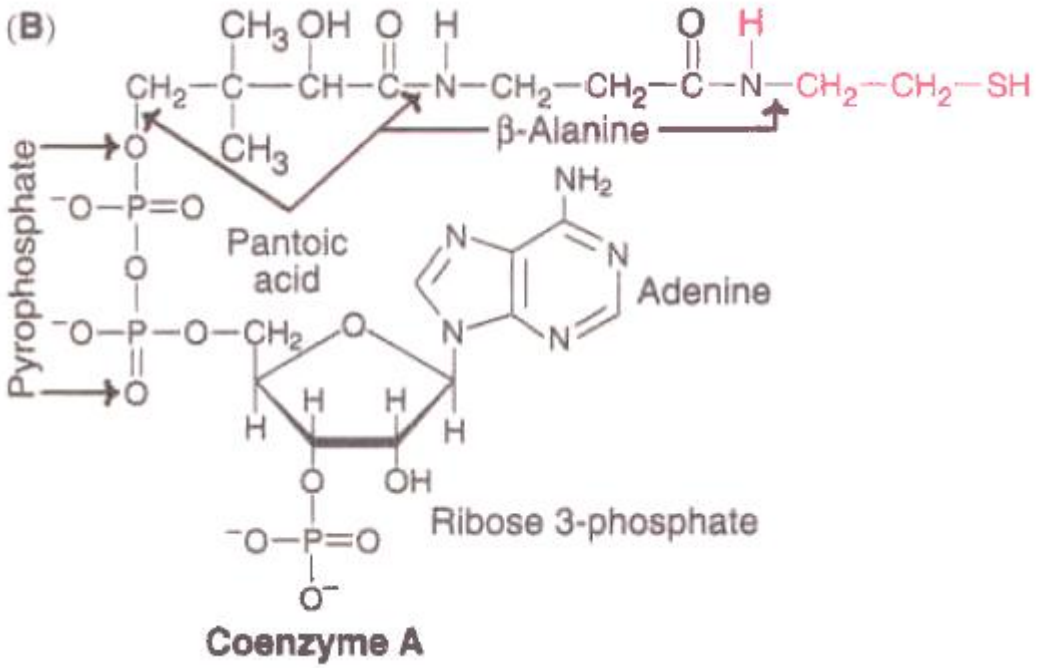
CH_3

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:



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

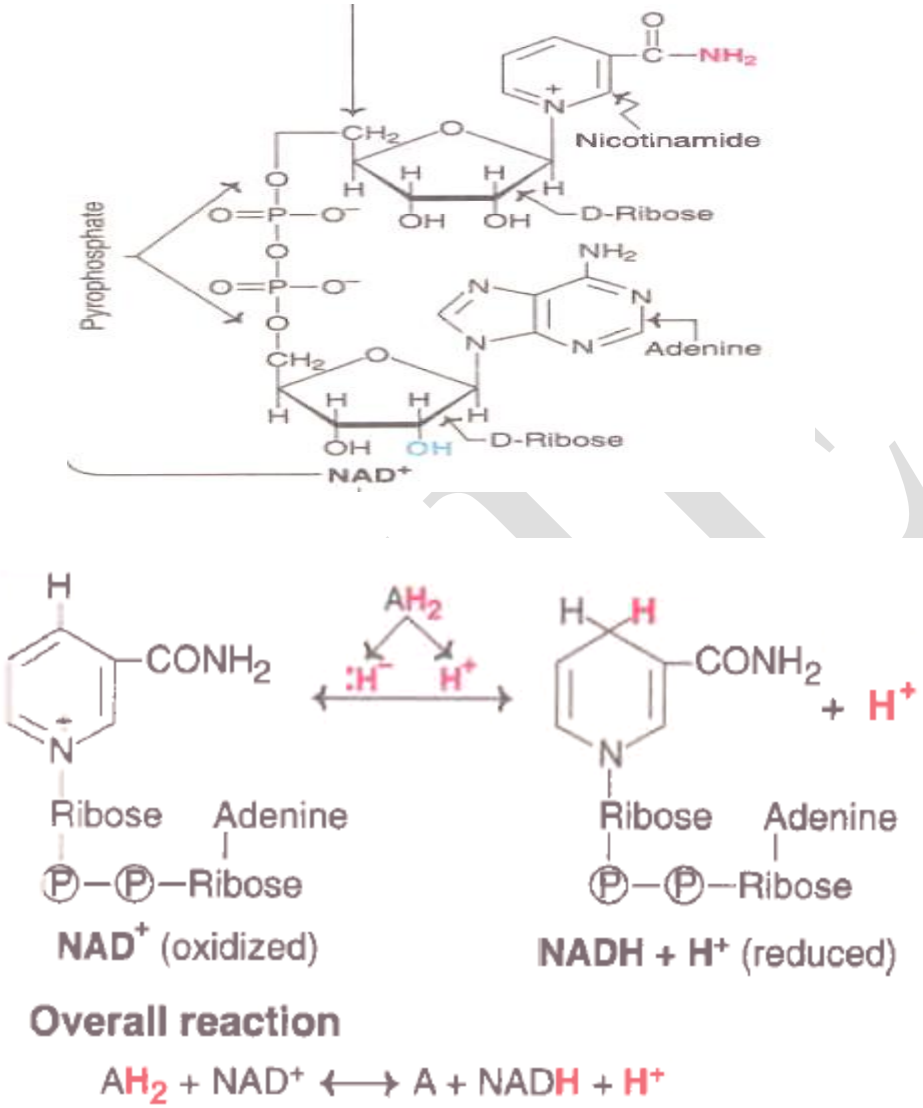
Properties:

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.



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+}.

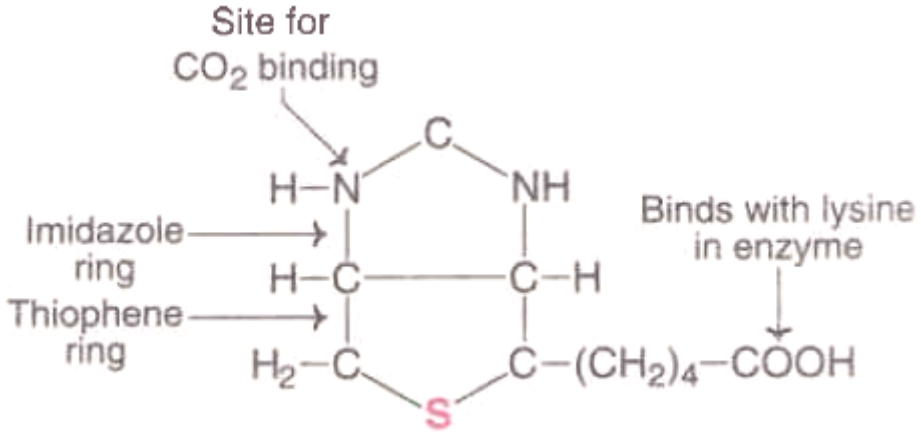
Another example is dog fish muscle lactate dehydrogenase which catalyses the reaction:

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

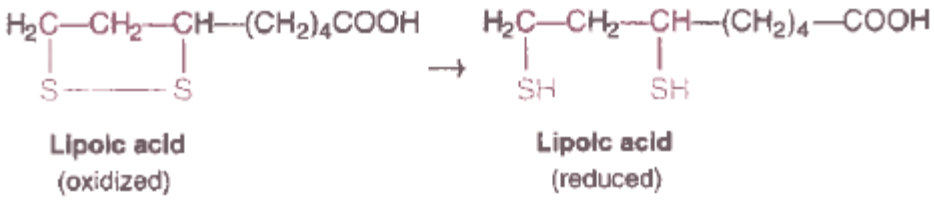
The BCCP appears to act as a flexible arm transporting the CO_2^- from the active site of biotin carboxylase to that of carboxyl transferase where it is presented to the acetyl coA.

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.

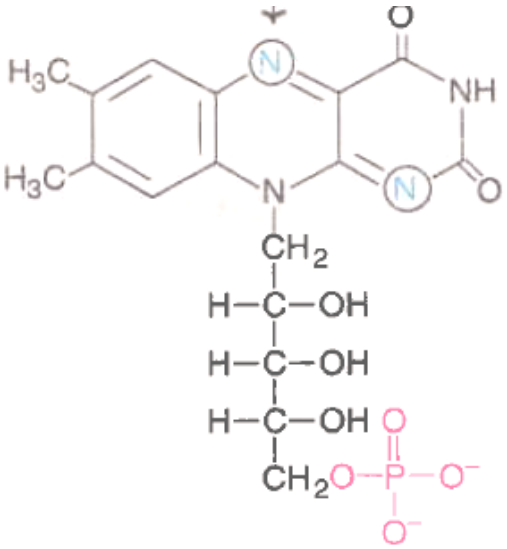
Properties:

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.

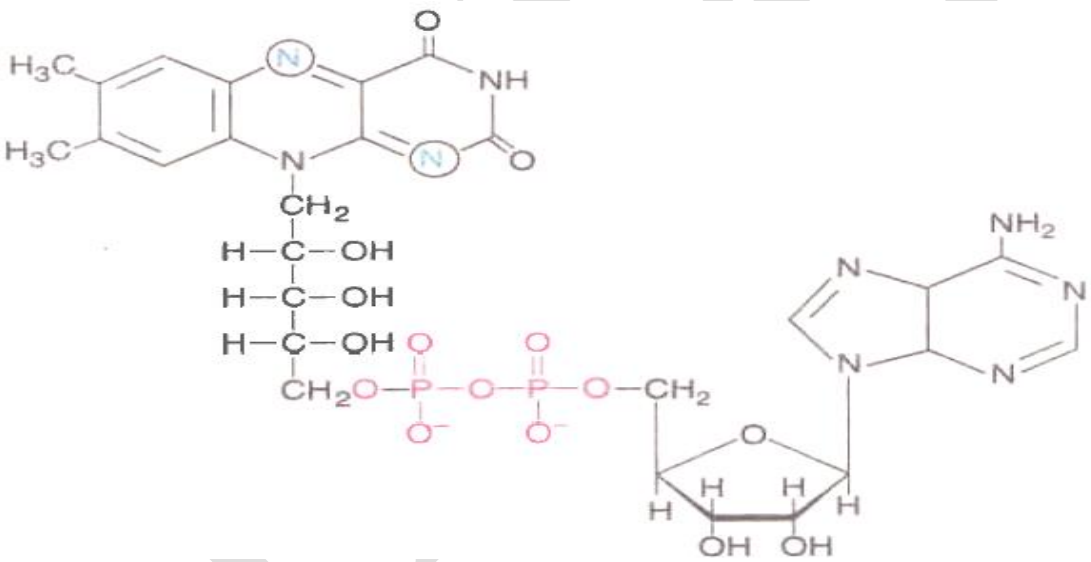
Flavin nucleotides (FMN and FAD):

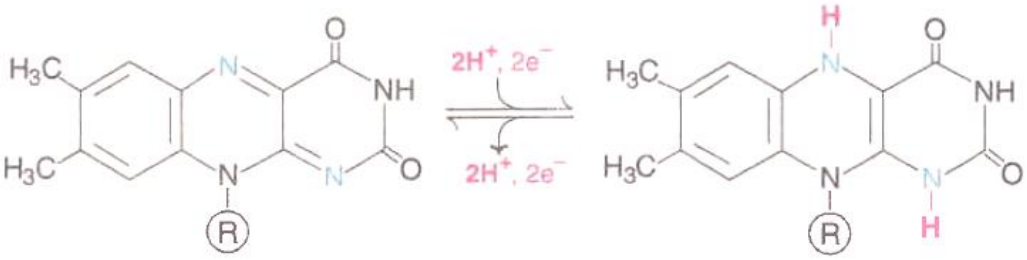
Flavin nucleotides are derived from riboflavin vitamin B_2.

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:

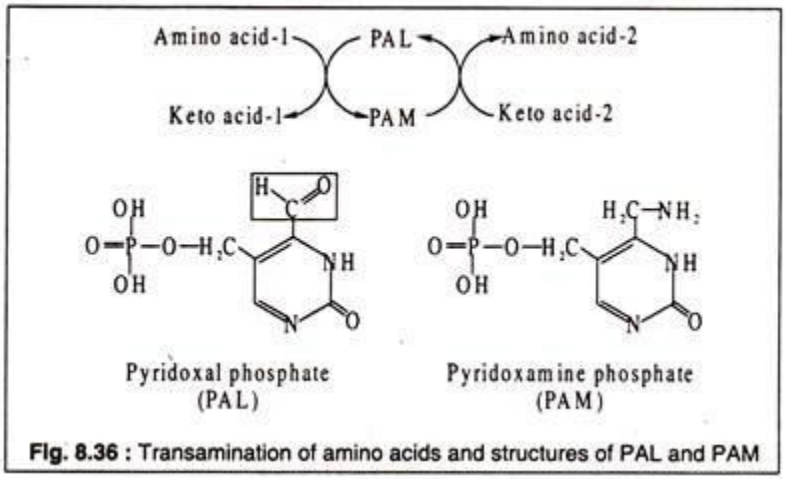


Fig. 8.36 : Transamination of amino acids and structures of PAL and PAM

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

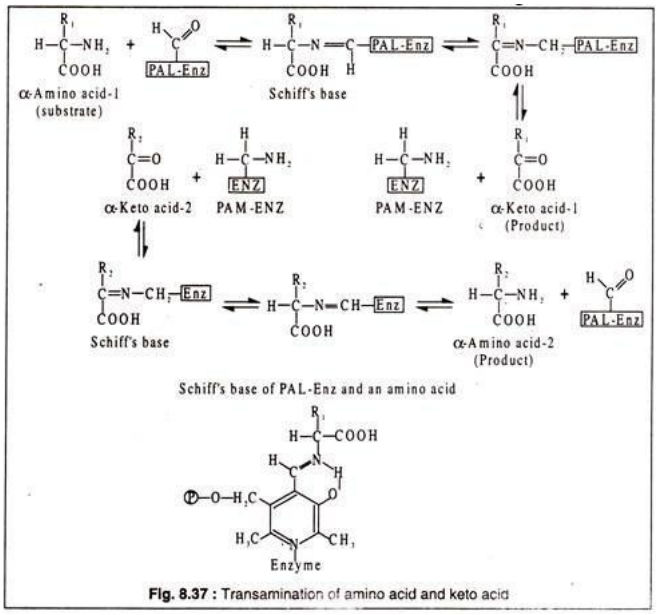
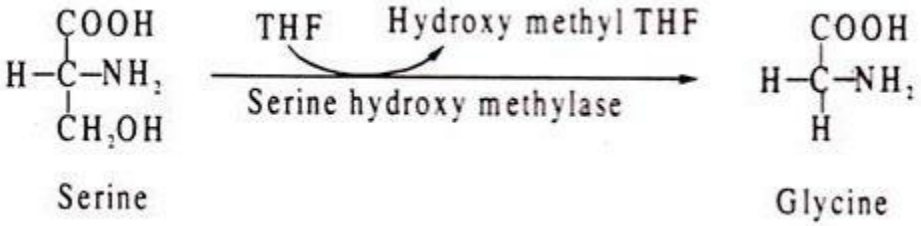


Fig. 8.37 : Transamination of amino acid and keto acid

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

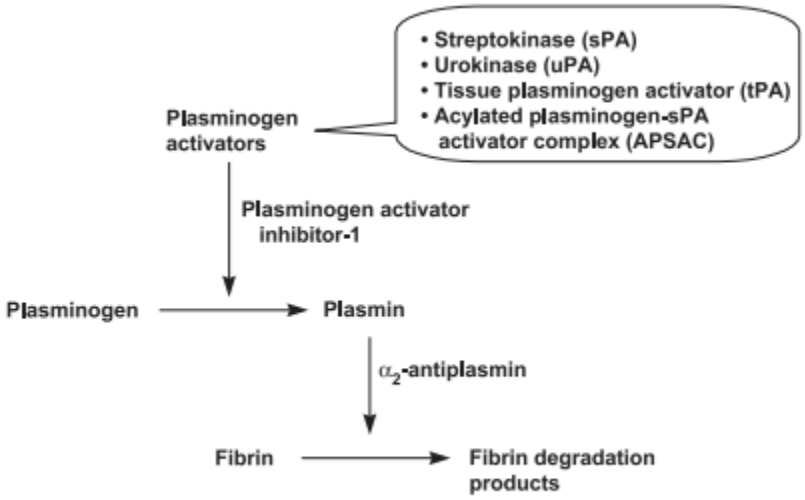
Enzyme Immunoassay is one of the most significant techniques to have been used in clinical laboratories to analyse the antigen-antibody reaction in a short period of time. It is sensitive to screening a large number of clinical samples and therefore has been applied in many clinical laboratories for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



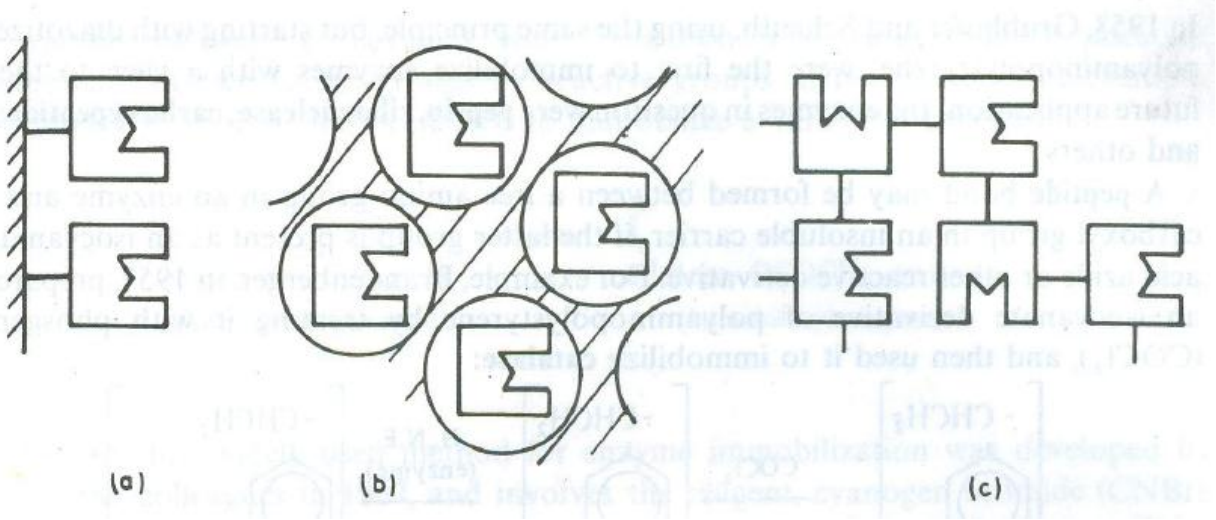
Schematic representation of fibrinolysis.

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.

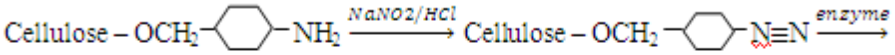
Ionic binding

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.

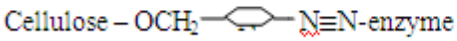
Covalent binding

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.



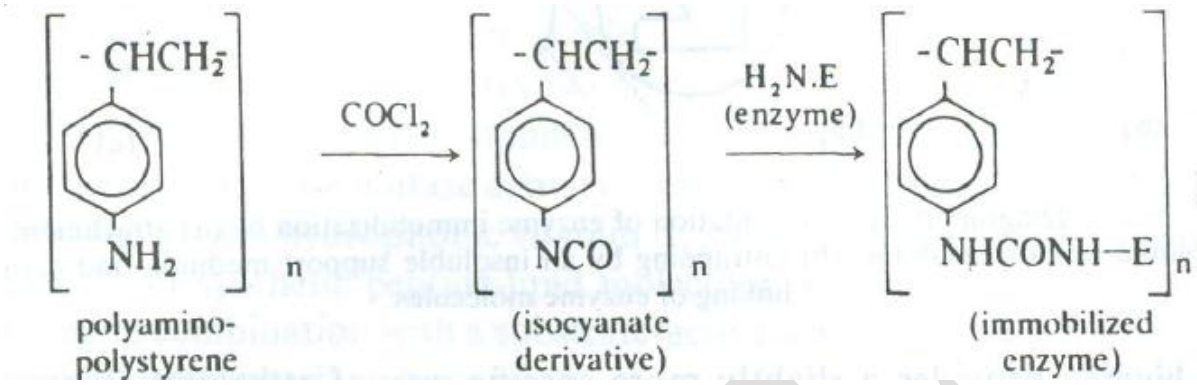
P-amino benzyl cellulose **diazonium derivative**



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-$OCH_2CO_2CH_3 \xrightarrow{NH2NH2}$

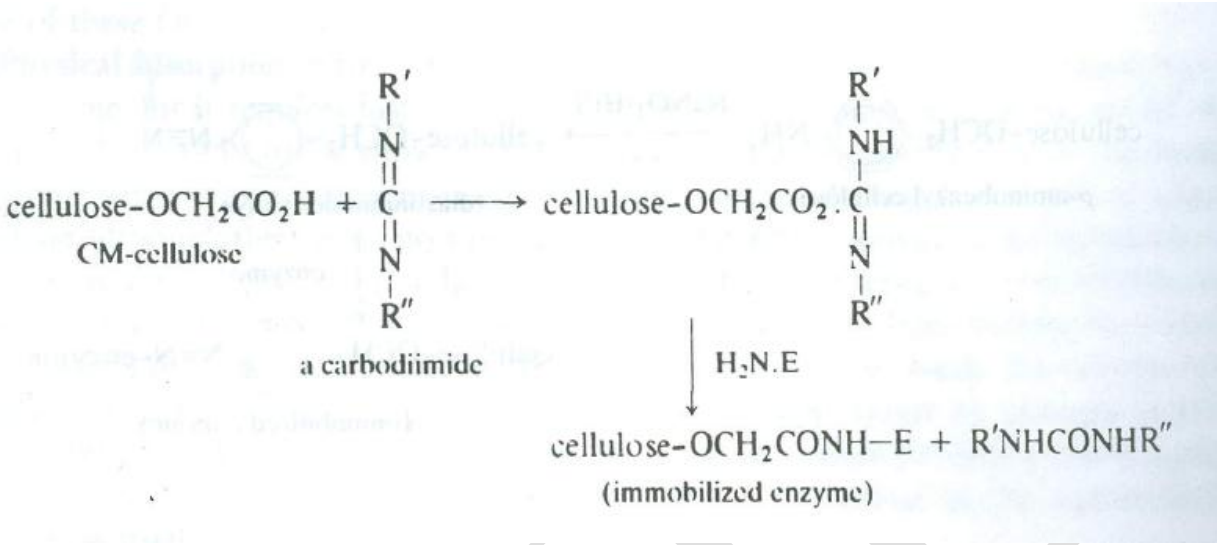
CM-Cellulose **Methyl ester**

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

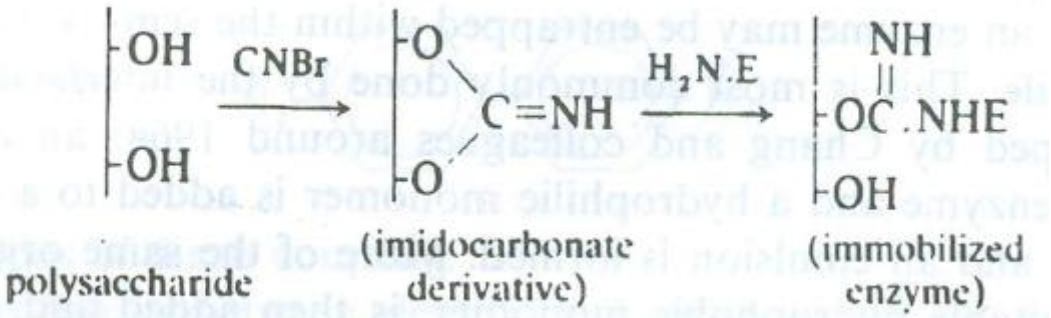
It is an another form of covalent bond formation. Alkylation of phenolic, sulphydryl or free amino groups by reactive groups in the carrier.

For example, Bromoacetyl cellulose used to immobilize enzymes.

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

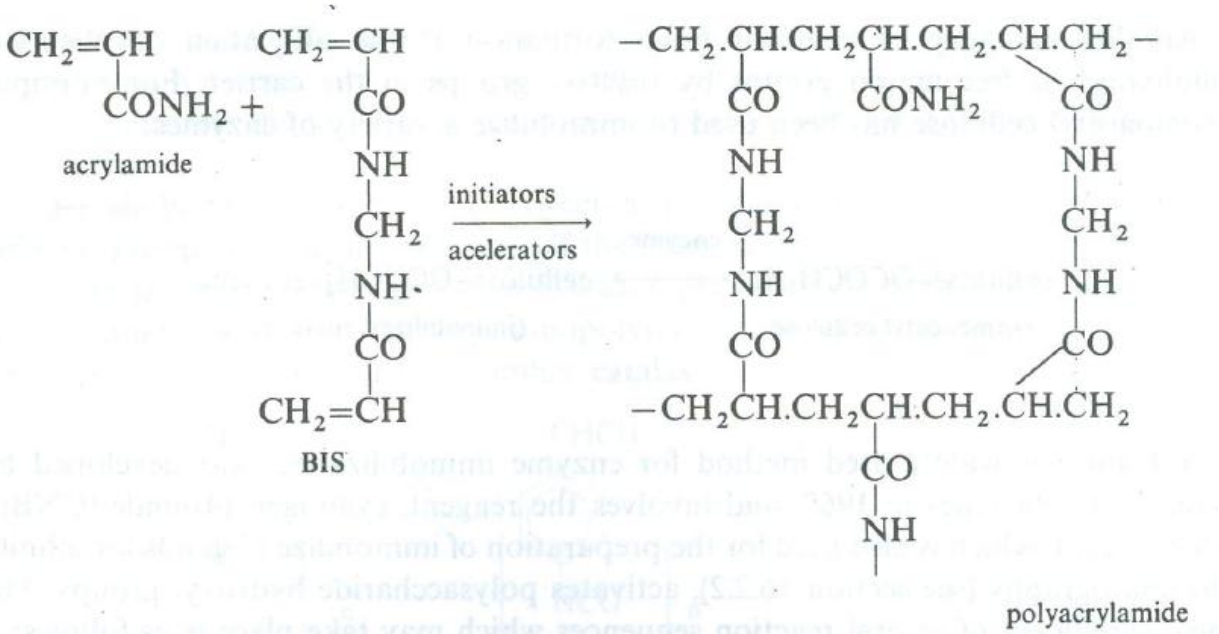
Immobilized enzyme

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

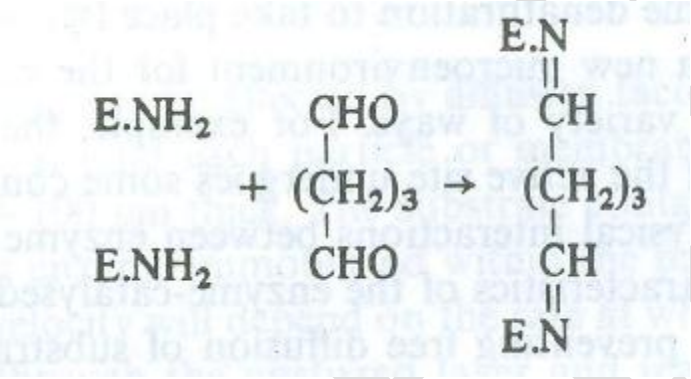
- In some instances free radical generated during the polymerization procedure may cause some loss of enzymic activity.
- Since entrapped enzyme does not escape because of their size, a very large substrate will not be able to diffuse in to reach the enzyme.
- Hence this procedure is not suitable for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

Reg. No……

[17BTU404B]

KARPAGAM ACADEMY OF HIGHER EDUCATION,
COIMBATORE
FIRST INTERNAL EXAMINATIONS, DECEMBER 2018
(For Candidates admitted from 2017 and onwards)
Fourth Semester
Department of Biotechnology

ENZYMOLOGY

Date: 18.12.2018 (AN) **Class : II B.Sc BT**
Time: 2 hours **Maximum: 50 marks**

PART –A
Answer All Questions **20 X 1 = 20 marks**

1. Which of the following is an enzyme?
   a) Lactose b) Lactase c) Lactol d) Glucose
2. Nature of an enzyme is……..
   a) Protein b) Lipid c) Carbohydrate d) Fat
3. An enzyme was crystallized first by……
   a) John Northrop b) Edward Buchner c) J.B. Sumner d) James Watson
4. Enzymes are classified into ….types
   a) Two b) three c) five d) six
5. Majority of the enzymes are inactive are
   a) At 25º C b) At 15º C c) between 30-40 º C d) above 70 º C
6. SI unit of enzyme activity is
   a) Bel b) mho c) Hertz d) Katal
7. Which of these enzymes is related to molecular biology?
   a) DNA ligases b) DNA topoisomeras c) Polymerase d) All of the above
8. Enzymes that differ in amino acid sequence but catalyze the same reaction are
   a) Co-factors b) Co-enzymes c) Apo enzymes d) Isoenzymes
9. The optimum pH for breaking human salivary amylase into starch is:
   a) 6.0 b) 6.2 c) 6.4 d) 6.7
10. The class of enzymes that catalyze synthetic reactions where two molecules are joined together and ATP is used is….
    a) Hydrolases b) Ligases c) Lyases d) Isomerases
11. Pepsin and ureases are examples of which class of enzymes
    a) Hydrolases b) Lyases c) Oxidoreductases d) Ligases
12. Enzymes are basically made up of
    a) Fat b) Proteins c) Nucleic acids d) Vitamins
13. The coenzyme is
    a) Often a metal b) Always a protein
    c) Often a vitamin d) Inorganic compound
14. An enzyme that joins the ends of two strands of nucleic acid is
    a) Polymerase b) Ligase c) Synthetase d) Helicase
15. Diastase takes part in digestion of _____.
    a) Protein b) Starch c) Amino acids d) Fat
16. Enzyme catalyzing rearrangement of atomic groupings without altering molecular weight or number of atoms is
    a) Ligase b) Isomerase c) Oxidoreductase d) Hydrolase
17. Enzymes are polymers of
    a) Hexose sugar b) Amino acids c) Fatty acids d) Inorganic phosphate
18. Enzymes bind with chemical reactant called
    a) Products b) Reactants c) Substrates d) Inhibitor
19. The molecule which acts directly on an enzyme to lower its catalytic rate is
    a) Repressor b) Inhibitor c) Modulator d) Regulator
20. Where does inhibitor binds on enzyme in mixed inhibition?
    a) At active site b) Allosteric site
    c) Does not bind on enzyme d) Binds on substrate

PART – B **3 X 2 = 6 marks**
Answer All the Questions

21. Define enzyme activity.
22. What is active site?
23. Comment on specificity

PART – C **3 X 8 = 24 marks**
Answer all questions choosing either a or b. All questions carry equal marks.

24. a. Write in detail on enzyme classification.
    (Or)
    b. Give the significance of activation energy and free energy.

25. a. Explain in detail about the Zymogens and their activation
    (Or)
    b. Explain in about Michaelis-Menten equation and its derivation
26. a. Give brief note on: Ping-pong mechanism.
    (Or)
    b. Explain the different types of enzyme inhibition.

Reg. No……

[17BTU404B]

KARPAGAM ACADEMY OF HIGHER EDUCATION, COIMBATORE
SECOND INTERNAL EXAMINATIONS, FEBRAUARY 2018
(For Candidates admitted from 2017 and onwards)
Fourth Semester
Department of Biotechnology
ENZYMOLOGY

Date: **Class : II B.Sc BT**
Time: 2 hours **Maximum : 50 marks**

PART –A **Answer All Questions** **20 X 1 = 20 marks**

1. The slope of Hanes plot is…….
   a) 1/Vmax b) Vmax c) Vmax/2 d) -1/Km
2. The enzyme activity will be maximum at its……..
   a) Optimum temperature b) Low temperature c) High temperature d) Freezing temperature
3. Competitive inhibitors……
   a) Decrease in Km b) Decrease in Vmax
   c) Increase in Km d) Increase in Vmax
4. Which of the following amino acids would be prone to a reaction with an irreversible inhibitor?
   a) Serine b) Tyrosine c) Alanine d) Tryptophan
5. Which of the following agents act as irreversible inhibitors?
   a) Penicilllins b) Kanamycine c) Serine d) Alanine
6. Hill constant means
   a) Measure of cooperativity b) measure of allosteric
   c) Measure of Zero cooperativity d) katal
7. Scatchard equations discovered by_____
   a) Archibald Hill b) filmer
   c) George Scatchard d) Koshland, Nemethy and Filmer
8. LDS stands for
   a) Lactate dehydrogenase b) Lactate hydrogenase
   c) Lactic acid d) Lactose dehydrogenase
9. Lactate dehydrogenase is composed of_____ subunits (tetramer)
   a) 4 b) 5 c) 6 d) 7
10. Catalytic RNA molecules are called
    a) Lysozymes b) Multizymes c) Ribozymes d) Peptin
11. Positive cooperativity
    a) h>3 b) h>1 c) h>2 d) h=1
12. Concerted models also know as
    a) KNF model b) symmetry model c) MWC model d) fit model
13. Phosphofructokinase genes in human are
    a) 1 b) 2 c) 3 d) 4
14. Variety of RNA processing reactions, including….
    a) RNA splicing b) viral replications
    c) RNA synthesis d) tRNA synthesis
15. Negative cooperativity
    a) o b) H>0 c) h<1 d) h>2
16. Multienzyme complexes are
    a) Pyruvate dehydrogenase b) Proteasome
    c) Fatty acid synthetase d) Glutamine
17. Two substrate two product reaction is otherwise called as….
    a) Bi reaction b) bi bi reaction c) Secondary reaction d) quaternary reaction
18. The x intercept in Eadie and Hofstee plot is…….
    a) Vmax b) Vmax/Km c) –Km d) -1/Km
19. Maltose phosphorylase reaction occurs via…..
    a) Sequential mechanism b) non- sequential mechanism
    c) Allosteric mechanism d) ping pong mechanism
20. The transformation of MM equation is otherwise called as….
    a) Hanes plot b) Line Weaver Burk plot
    c) Eadie and Hofstee plot d) Cornish Bowden plot

PART – B **Answer All the Questions** **3 X 2 = 6 marks**

21. Define covalent catalysis.
22. What is isoenzyme?
23. Comment on allosteric enzyme.

PART – C **3 X 8 = 24 marks**

Answer all questions choosing either a or b. All questions carry equal marks.

24. a. Write in detail on proximity and orientation mechanism associated with enzyme catalytic efficiency. **(Or)**
    b. Explain in detail about acid-base and covalent catalysis mechanism.
25. a. Write short notes on significance of transcarbomylase and phosphofructokinase enzymes. **(Or)**
    b. Give brief note on: (i) concerted ii) sequencial models.
26. a. Significance of multienzyme complexes.
    (Or)
    b. Explain the unique features of Hill and Scarchard plot.

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

S.NO	QUESTION	OPTION 1	OPTION 2	OPTION 3	OPTION 4	ANSWER
1	The Vo against substrate concentration plot for single substrate reaction for an enzyme	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 formation of ES	enables us to calculate the vI	states that rate of chemical	states that the reaction rate	states that the reaction rate is
4	Km and reaction rate are	directionally proportional	inversely proportional	independent with each other	same	directionally proportional
5	When the substrate concentration equals Km in an enzyme-catalysed reaction	A few of the enzyme molecules are present as ES complex	Majority of the enzyme molecules are present as ES complex	Half of the enzyme molecules are present as ES complex	All of the enzyme molecules are present as ES complex	Half of the enzyme molecules are present as ES complex
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 of Km	substrate concentration is equal to Km	substrate concentration is twice the Km	substrate concentration is not related to Km	substrate concentration is equal to Km
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 concentration	not affected by enzyme concentration	nearly equal to Vmax	inversely proportional to substrate concentration	directly proportional to substrate concentration
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 does the transition state intermediate.	The active site of an enzyme binds the substrate of the reaction it catalyses less tightly than it does the transition state intermediate.	The active site of an enzyme binds the product of the reaction it catalyses more tightly than it does the transition state intermediate	The active site of an enzyme is complementary to the substrate of the reaction it catalyses.	The active site of an enzyme binds the substrate of the reaction it catalyses less tightly than it does the transition state intermediate.
12	Sucrose phosphorylase reaction occurs via	sequential mechanism	compulsory ordered mechanism	random ordered mechanism	ping pong mechanism	ping pong mechanism
13	Maltose phosphorylase reaction occurs via	sequential mechanism	non sequential mechanism	allosteric mechanism	ping pong mechanism	sequential mechanism
14	Sequential mechanism is a	double displacement reaction	single displacement reaction	both a and b	neither a nor b	single displacement reaction

15	The enzyme lactate dehydrogenase follows	ordered sequential kinetic mechanism	random sequential kinetic mechanism	non sequential kinetic mechanism	ping pong kinetic mechanism	ordered sequential kinetic mechanism
16	Non sequential mechanism is a	double displacement reaction	single displacement reaction	both a and b	neither a nor b	double displacement reaction
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 mechanism	ping pong mechanism	sequential 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	random ordered mechanism	sequential mechanism
27	Ping pong mechanism is	sequential mechanism	non sequential mechanism	random ordered mechanism	compulsory ordered mechanism	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 temperature	40°C	-20°C	60°C	optimum temperature
30						
31						

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 Vmax	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 Vmax	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 Vmax	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 yielding an inactive ESI	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 inhibition	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 being utilized.	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	its product	its substrate
20	Competitive inhibition is	always reversible	always irreversible	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 amount of substrate.	competitive inhibition is when the substrate and the inhibitor compete for the active site on the enzyme	competitive inhibition is seen when the substrate and the inhibitor compete for the active site on the enzyme.	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 synthesis	enzyme are biocatalyst	Penicillin with glycopeptides transpeptidase enzyme involved in bacterial cell wall synthesis
23	The inhibition caused by the final end product of a reaction is called	Non competitive inhibition	competitive inhibition	Allosteric inhibition	irreversible inhibition	Allosteric inhibition
24	Which of the following is/ are irreversible?	competitive inhibition	Non competitive inhibition	Allosteric inhibition	reversible inhibitor	Non competitive inhibition
25	Which of the following are allosteric enzymes	RUBISCO	PEP Carboxylase	Phosphofructokinas e	Alanine	Phosphofructokinase
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 protein	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	allosteric inhibition	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 the enzyme.	They react irreversibly with the enzyme.	reversible inhibitor	They react irreversibly with the enzyme.
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 for its substrate	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 metabolism	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 the mouth	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 methicillin susceptible staphylococci	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 administration	Absorption is impaired by divalent cations, Al^{3+}, and antacids	Widely distributed especially into the CSF	Absorption is impaired by divalent cations, Al^{3+}, and antacids
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 lactamase	Inactivation of antibiotics	Presence of an efflux pump
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	which 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 lactamase	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	cytochrome oxidase by	carbonic anhydrase by carbon dioxide	hexokinase by glucose-6-phosphate	succinic dehydrogenase by malonic acid
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 metals	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 phosphate	Amino acids

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 Transferase	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 reduced	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	unstabilizes the R state of the protein	increases the binding affinity
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 enzyme	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	reversible and irreversible	non-standard	reversible and irreversible
41	Covalent modification is means of changing	chemical properties	physical properties	bonding on amino acids	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 phosphatases.	Phosphorylation of enzymes only occurs at specific tyrosine residues.	phosphorylation of an enzyme results in a conformational change	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 by a ligand.	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	substrate and inhibiotr	inhibitor and catalyst	substrate and inhibiotr
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 enzyme	simple enzyme	larger and more complex than simple enzyme
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	ATP citrate lyase, citrate synthase and pyruvate	ATP citrate lyase, citrate synthase and pyruvate
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	PFK,RUBISCO, PEP Carboxylase	PFK,RUBISCO, PEP Carboxylase
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

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	coenzyme D	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 inhibition	competitive inhibition	binding with substrate	enzyme denaturation	competitive inhibition
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 insoluble support
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 dystrophy