CHEMISTRY OF ENZYMES

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**Major Concepts:**

A. To study what are enzymes, their general properties and classification.

B. To learn the mechanisms of enzyme catalysed reactions and various factors affecting enzyme activity.

C. To learn various types of enzyme inhibition and how the enzyme activity is regulated.

**Specific Objectives:**

A. 1. Define enzyme.

2. What is meant by catalytic activity of enzymes?

3. Note that enzymes are protein in nature.

4. Learn what coenzymes are.

5. Study the role of metal ions in enzymes.

6. Study the nomenclature and classification of enzyme as approved by International Union of Biochemistry (IUB). Learn at least two examples from each class.

B. 1. Know what is enzyme catalysed reaction and how an enzyme functions by lowering the energy of activation.

2. Define specificity of enzyme and learn different types of specificity.

3. Study Lock-and-Key theory and induced fit theory of mechanism of action of enzymes.

4. Learn various factors that affect the activity of enzyme, such as, pH, temperature, substrate concentration, enzyme concentration, product concentration, presence of inhibitors or activators.

5. Know the Michaelis-Menten equation and significance of each term.

6. Know the importance and application of double reciprocal or Lineweaver-Burk plot and calculate enzyme velocity when S >> Km, S = Km and S << Km.

C. Learn what is enzyme inhibition and various types of inhibition.

1. Nonspecific inhibition: List the various agents responsible for it.

2. Competitive inhibition.

3. Noncompetitive reversible inhibition, and noncompetitive irreversible inhibition.

4. Make a tabular form to show the difference between competitive and noncompetitive inhibition.

5. Learn examples of competitive inhibition in biological system: Clinically used drugs.

6. Study the various mechanisms by which enzyme activity is regulated, study allosteric enzyme.

7. Learn about control of enzyme degradation: Ubiquitin-protease pathway.

**INTRODUCTION**

Enzymes are another important group of biomolecules synthesized by the living cells. They are ***catalysts of*** ***biological systems (hence are called as biocatalysts),*** ***colloidal, thermolabile and protein in nature.*** They are remarkable molecular devices that determine the pattern of chemical transformations. They also mediate the transformation of different forms of energy. The striking characteristics of enzymes are their catalytic power and specificity. Actions of most enzymes are under strict regulation in a variety of ways. ***Substances on which*** ***enzymes act to convert them into products are called*** **substrates.**

**Catalytic Activity of Enzymes:** Enzymes have immense catalytic power and accelerate reactions at least a million times, by ***reducing the energy of activation***. Before a chemical reaction can occur, the reacting molecules are required to gain a minimum amount of energy, this is called the **energy of activation**. It can be decreased by increasing the temperature of the reaction medium. But in human body which maintains a normal body temperature fairly constant, it is achieved by enzymes.

**Protein Nature of Enzymes:** In general with the exception of *ribozymes* which are few RNA molecules with enzymatic activity, ***all the enzymes are protein in nature*** ***with large mol. wt.*** Few enzymes are simple proteins while some are conjugated proteins. In such enzymes the ***non-protein part is called prosthetic group or coenzyme*** and the protein part is called as ***apoenzyme***. ***The complete*** ***structure of apoenzyme and prosthetic group is called*** ***as holoenzyme.***

***Holoenzyme = Apoenzyme + Coenzyme***

***(Protein part) (Prosthetic group)***

Certain enzymes with only one polypeptide chain in their structure are called as ***monomeric enzymes***, e.g. *ribonuclease*. Several enzymes possess more than one polypeptide chain and are called as ***oligomeric enzymes***, e.g. *lactate dehydrogenase, hexokinase*, etc. Each single polypeptide chain of oligomeric enzymes is called as ***subunit***. When many different enzyme catalysing reaction sites are located at different sites of the same macromolecule, it is called as ***multienzyme complex***. The complex becomes inactive when it is fractionated into smaller units each bearing individual enzyme activity, e.g. *fatty acid synthetase, carbamoyl phosphate synthetase II,* *pyruvate dehydrogenase, prostaglandin synthase*, etc.

**Coenzymes:**

Certain enzymes require a specific, ***thermostable, low mol. wt, non-protein*** organic substance called as ***coenzyme***. A coenzyme may bind covalently or noncovalentlyto the *apoenzyme*. ***The term prosthetic group denotes a covalently bonded enzyme.*** It is generallyobserved that reactions involving oxidoreductions, grouptransfers, isomerisation and covalent bond formationrequire coenzyme.Since the involvement of coenzyme in a given reactionon a substrate is so intimate that coenzyme is often calledas ***cosubstrate or second substrate*.**

Many coenzymes are derived as the physiologically active forms from the constituents of vitamin B-complex viz, **Pantothenic acid: *CoASH,* Vitamin B12: *Cobamide,*** **Folic Acid: *Tetrahydrofolate (F.H4)*, Niacin: *NAD+,*** ***NADP*, Riboflavin: *FMN, FAD,* Pyridoxine: *Pyridoxal*** ***phosphate,* Thiamine: *TPP*.**

**Classification of Coenzymes:**

Coenzymes can be **classified according to the group whose transfer they facilitate**. Based on this concept wemay classify coenzymes as follows:

***(a) For transfer of groups other than hydrogen***

• Sugar phosphates, CoASH

• Thiamine pyrophosphate (TPP)

• Pyridoxal phosphate

• Folate coenzymes

• Biotin

• Cobamide coenzyme

• Lipoic acid

***(b) For transfer of hydrogen***

• NAD+, NADP+

• FMN, FAD

• Lipoic acid

• Coenzyme Q.

In addition heme acts as coenzyme in cytochromes, peroxidases and PG synthase complex. Many coenzymes contain adenine, ribose and phosphate and are derivatives of adenosine monophosphate (AMP) such as NAD, FAD.

**Role of Metal Ions in Enzymes:**

The activity of many enzymes depends on the presence of certain metal ions such as K+, Mg++, Ca++, Zn++, Cu++.

• **Metal activated enzymes:** In certain enzymes the metals ***form a loose and easily dissociable complex.*** Such enzymes are called ***metal-activated enzymes***. The metal ions can be removed by dialysis or any other such method from the enzyme without causing any denaturation of apoenzyme.

• ***Metalloenzymes:*** The second category of metal enzymes is called as ***metalloenzymes***. In this case ***metal ion is bound tightly to the enzyme*** ***and is not dissociated*** even after several extensive steps of purification.

**Metals play variety of roles such as:**

**•** They help in either maintaining or producing (or both), active structural conformation of the enzyme,

**•** Formation of enzyme-substrate complex,

**•** Making structural changes in substrate molecule,

**•** Accept or donate electrons,

**•** Activating or functioning as nucleophiles, and

**•** Formation of ternary complexes with enzyme or substrate.

**Table 9.1: Examples of some metalloenzymes**

|  |  |
| --- | --- |
| Metal | Enzymes |
| Copper | Superoxide dismutase, cytochrome oxidase, tyrosinase, lysyl oxidase. |
| Calcium | Lipase, lecithinase |
| Iron | Catalase, xanthine oxidase, peroxidase cytochrome oxidase. |
| Manganese | Hexokinase, enolase, phosphoglucomutase, glycosyl transferase |
| Magnesium | Hexokinase, enolase, glucose-6-phosphatase, phosphofructokinase |
| Molybdenum | Xanthine oxidase |
| Zinc | Carbonic anhydrase, alcohol dehydrogenase, carboxy peptidase, alkaline phosphatase (ALP), lactate dehydrogenase (LDH). |

**NOMENCLATURE AND CLASSIFICATION OF ENZYMES:**

Enzymes are generally named after adding the suffix ***‘ase’*** to the name of the substrate, e.g. enzymes acting onnucleic acids are known as *nucleases,* enzymes hydrolysingdipeptides are called *dipeptidases*. Eventhough fewexceptions such as trypsin, pepsin, and chymotrypsin arestill in use. Further, **few enzymes exist in their inactiveforms** and are called as **proenzymes or zymogens,** e.g.*pepsin* has *pepsinogen* as its zymogen. The zymogens ***become active after undergoing some prior modification*** in its structure by certain agents. ***Many times the active form of enzyme acts on zymogen and catalyses its conversion into active form and this process is called as autocatalysis.***

In order to have a uniformity and unambiguity in identification of enzymes, ***International Union of Biochemistry*** ***(IUB)*** adopted a nomenclature system ***based*** ***on chemical reaction type and reaction mechanism.***

According to this system, enzymes are grouped in **six main classes.**

• Each enzyme is characterised by a code number (enzyme code No. or E C No) comprising four figures (digits) separated by points, **the first being that of** **the main class (one of the six).**

• ***The second figure*** indicates the type of group involved in the reaction.

• ***Third figure*** denotes the reaction more precisely indicating substrate on which the group acts.

• ***The fourth figure*** is the serial number of the enzyme. Briefly, the four digits characterise class, sub-class, sub-sub-class and serial number of a particular enzyme.

**Six classes are:**

1. ***Oxidoreductase:*** Enzymes involved in oxidations and reductions of their substrates, e.g. *alcohol dehydrogenase,* *lactate dehydrogenase, xanthine oxidase, glutathione reductase,* *glucose-6-phosphate dehydrogenase.*

2. ***Transferases:*** Enzymes that catalyse transfer of a particular group from one substrate to another, e.g. *aspartate and* *alanine transaminase (AST/ALT), hexokinase, phosphoglucomutase,* *hexose-1-phosphate uridyltransferase, ornithine* *carbamoyl transferase,* etc.

3. ***Hydrolases:*** Enzymes that bring about hydrolysis, e.g. *glucose-6-phosphatase, pepsin, trypsin, esterases, glycoside* *hydrolases,* etc.

4. ***Lyases:*** Enzymes that facilitate removal of small molecule from a large substrate, e.g. *fumarase, arginosuccinase,* *histidine decarboxylase*.

5. ***Isomerases:*** Enzymes involved in isomerisation of substrate, e.g. *UDP-glucose, epimerase, retinal isomerase,* *racemases, triosephosphate isomerase*.

6. ***Ligases:*** Enzymes involved in joining together two substrates, e.g. *alanyl-t. RNA synthetase, glutamine* *synthetase, DNA ligases.* Many times the word **‘OTHLIL’** is used to remember the six classes.

**SPECIFICITY OF ENZYMES:**

Another important property of enzymes is their specificity. The specificity is of ***three different types*** namely:

***1. Stereochemical specificity,***

***2. Reaction specificity,*** and

***3. Substrate specificity.***

**Stereospecificity:**

**1. Optical Specificity**

***There can be many optical isomers of a substrate. However, it is only one of the isomers which acts as a substrate for an enzyme action***, e.g. for the oxidationof *D*- and *L*-amino acids, there are two types ofenzyme which will act on *D*- and *L*-isomers of aminoacids. Secondly there can be a product of enzymeaction which can have isomers. However, it is onlyone kind of isomer which will be produced as aproduct, e.g. *Succinic dehydrogenase* while acting onsuccinic acid will give only fumaric acid and not malicacid which is its isomer.

**2. Reaction Specificity**

A substrate can undergo many reactions but in a reaction specificity ***one enzyme can catalyse only one*** ***of the various reactions***. For example, oxaloacetic acid can undergo several reactions but each reaction is catalysed by its own separate enzyme which catalyses only that reaction and none of the others.

**3. Substrate Specificity**

The extent of substrate specificity varies from enzyme to enzyme. There are two types of substrate specificity viz, absolute specificity and relative specificity

• **Absolute specificity** is comparatively rare such as *urease* which catalyses hydrolysis of urea.

• **Relative substrate specificity** is further divided as:

**• Group dependent** or

**• Bond dependent.**

Examples of group specificity are trypsin, chymotrypsin. *Trypsin* hydrolyses the residues of only *lysine* and *arginine*, while chymotrypsin hydrolyses residues of only aromatic amino acids.

**4. Bond Specificity**

Bond specificity is observed in case of proteolytic enzymes, *glycosidases* and *lipases* which act on peptide bonds, glycosidic bonds and ester bonds respectively.



Template or lock-and-key model



Models for enzyme-substrate interaction

**MECHANISM OF ENZYME ACTION:**

**Michaelis and Menten** have proposed a hypothesis for enzyme action, which is most acceptable. According to their hypothesis, ***the enzyme molecule (E) first combines*** ***with a substrate molecule (S) to form an enzymesubstrate*** ***(ES) complex which further dissociates to form*** ***product (P) and enzyme (E) back.*** Enzyme once dissociated from the complex is free to combine with another molecule of substrate and form product in a similar way. The ***ES complex is an intermediate or transient*** ***complex*** and the bonds involved are weak non-covalent bonds, such as H-bonds, Van der Waals forces, hydrophobic interactions. Sometimes two substrates can bind to an enzyme molecule and such reactions are called as **bisubstrate reactions**. The site to which a substrate can bind to the enzyme molecule is extremely specific and is called as ***active site or catalytic site***. Normally the molecular size and shape of the substrate molecule is extremely small compared to that of an enzyme molecule. The active site is made up of several amino acid residues that come together as a result of foldings of secondary and tertiary structures of the enzyme. So, the active site possesses a complex three dimensional form and shape, provides a predominantly non-polar cleft or crevice to accept and bind the substrate. Few groups of active site amino acids are bound to substrate while few groups bring about change in the substrate molecule.

**MODELS OF ENZYME-SUBSTRATE COMPLEX FORMATION:**

These interactions have been described basically of two types.

**1. Template or Lock-and-Key Model**

This model was originally proposed by **Fischer** which states that the active site already exists in proper conformation even in absence of substrate. Thus the ***active site by itself provides a rigid, pre-shaped*** ***template*** fitting with the size and shape of the substrate molecule. **Substrate fits into active site of** **an enzyme** as the key fits into the lock and hence it is called the **lock-and-key** model. This model proposes that substrate binds with rigid pre-existing template of the active site, provides additional groups for binding other ligands. But this cannot explain change in enzymatic activity in presence of allosteric modulators **.**

**2. Induced-Fit or Koshland Model**

Because of the restrictive nature of lock-and-key model, another model was proposed by **Koshland** in 1963 ***which is known as induced-fit model. The*** ***important feature of this model is the flexibility of*** ***the region of active site.*** According to this, active site does not possess a rigid, preformed structure on enzyme to fit the substrate. On the contrary, ***the*** ***substrate during its binding induces conformational changes in the active site to attain the final catalytic shape and form* (Fig. 9.2)*.*** This explains several mattersrelated to enzyme action such as:

• Enzymes become inactive on denaturation,

• Saturation kinetics,

• Competitive inhibition, and

• Allosteric modulation.



Induced-fit model

**KINETIC PROPERTIES OF ENZYMES:**

Kinetic analysis of enzymes was used for characterization of enzyme-catalysed reactions even before enzymes had been isolated in pure form. One of the first things that is measured in kinetic analysis is the variation in rate of reaction with substrate concentration. For this purpose a fixed low concentration of enzyme is used in a series of parallel experiments in which only the substrate concentration is varied. Under these conditions initial velocity increases until it reaches a substrate independent maximum velocity at substrate concentration **.** The saturation effect is believed to reflect the fact that all the enzyme binding sites are occupied with substrate. This interpretation of the substrate saturation curve led **Hensi, Michaelis and Menten** to develop a general treatment of kinetic analysis of enzyme catalysed reactions. As already mentioned:



The hyperbolic curve of reaction velocity (V) against substrate

concentration (S). Km is the substrate concentration at ½ Vmax

K1 K3

E + S E-S E + P …….. ...(1)

K2 K4

Where E is the free enzyme, S is the substrate, ES is enzyme-substrate complex, P is the product, K1 is the rate constant for the formation of ES, K2 is the rate constant for the dissociation of ES to E and S and K3 is the rate constant for the dissociation of ES complex into E and P.

***Rate of formation of ES***

Rate of formation = *K1* [Et] – [ES] [S] …………………………….... (2)

***Rate of dissociation of ES***

Rate of dissociation = *K2* [ES] + K3 [ES] ............................................ (3)

***Steady state*** is attained when rate of formation of ES is equal to rate of dissociation,

*K*1 [Et] – [ES] [S] = *K*2 [ES] + *K*3 [ES] ……………………………....(4)

***Separation of rate constants***

The left side of equation (4) is multiplied to give:

*K*1 [Et] [S] – *K*1 [ES] [S]

and right side is simplified to give

[*K*2 + *K*3] [ES].

We then have,

*K*1 [Et] [S] — K1 [ES] [S] = [*K*2 + *K*3] [ES].

On transposing and changing sign we get,

*K*1 [Et] [S] = *K*1 [ES] [S] + [*K*2 + *K*3] [ES].

On further simplifying and rearranging,

*K*1 [Et] [S]

[ES] = ………………………………………….. (5)

*K*1 [S] + *K*2 + *K*3

***Definition of initial velocity Vo in terms of [ES]:***

The initial velocity, according to Michaelis and Menten theory is determined by the rate of dissociation of [ES] in reaction (1) whose rate constant is *K3*. So we get,

Vo = *K*2 [ES]

Substituting value of ES from equation (S) we get

*K*2 [E] [S]

Vo = ………………………………………. (6)

[S] + [*K*3 + *K*2]/*K*1

Now let us simplify further by defining Km (the Michaelis-Menten Constant) as:

*K*3 + *K*2

*K*1

and by defining Vmax as *K2* [Et], i.e. the rate when all the available E is present as ES. On substituting these terms in equation (6).

**Vmax [S]**

**Vo** =

**[S] + Km.**

This is called the **Michaelis-Menten equation,** the rate equation for one substrate-enzyme catalysed reaction. It is a statement of the quantitative relationship between the initial velocity Vo, the maximum velocity Vmax and the initial substrate concentration, all related through the Michaelis-Menten constant Km.

• An important relationship is observed when the initial reaction rate is exactly one-half the *V*max. Then,

Vmax Vmax [S]

=

2 Km + [S]

Divide by *V*max:

[S]

1/2 =

Km + [S]

Solving for Km, we get

Km + [S] = 2[S]

Km = [S]

The Michaelis-Menten equation can be algebraically transformed into equivalent equations that are useful in the practical determination of Km and Vmax. Therefore, ***Km is equal to substrate concentration at which the*** ***velocity is half the maximum.***

The initial velocity Vo is directly proportional to the molar concentration [S] of the substrate when substrate concentration is very low as compared to Km. In this stage, a single substrate enzyme reaction is a first order reaction, its rate depending on conc. of single reactant.

• When

S << Km.

∴ Km + [S] ≅ Km

Vmax [S] Vmax [S]

∴ Vo= = K [S]

Km + [S] Km

Vmax

Where K is a new constant equalling because both Vmax and Km are constants for a particular enzyme. Km

• When S>> Km, the initial velocity attains its *V*max and becomes independent of [S]. The reaction now turns into a zero-order reaction.

**Lineweaver-Burk Double-Reciprocal Plot:**

It is difficult to estimate Vmax from the position of an asymplote, as in the rectangular hyperbola**,** linear transforms of the Michaelis-Menten equation are often used.

1 1 Km 1

= + =

V Vmax Vmax  [S]

(Y = *b* + mx) gives a straight line where m is the slope and (*b*) is *y* intercept of the regression of *y* on *x.* Figure 9.4 shows the straight line graph obtained by plotting 1/V against 1/[S]. Where *y* intercept = 1/*V*max, the *x* intercept = –1/Km and the slope = Km/*V*max.

Factors affecting enzyme activity:

Activity of enzymes is markedly affected by several factors such as temperature, pH, conc. of other substances, presence of activators or inhibitors, etc.

**1. Effect of Temperature**

Each enzyme is most active at a specific temperature which is called its ***optimum temperature*.** Temperature increases the total energy of the chemical system with the result the activation energy is increased. The exact ratio by which the velocity changes of 10 o C temperature rise is the **Q10 or temperature coefficient. Reactions velocity almost doubles with 10oC rise (Q10 = 2) in many enzymes*.*** Activity of enzyme progressively decreases when the temperature of reaction is below or above the optimum temperature. However, increase in temperature also causes denaturation of enzyme.

**Note:**

The shape of the curve is ***bell-shape.*** Most of the enzymes of human system have an optimum temperature within the range of 35–40oC. Thus, **the optimum temperature is** **that temperature at which the activity of the enzyme is** **maximum.**



Effect of temperature on enzymatic reaction

**2. Effect of pH**

The rate of enzymatic reaction also depends on pH of the medium. The enzymatic activity is maximum at a particular pH which is called its ***optimum pH.*** The optimum pH of most enzymes lies in the range of 4–9**.**

• Hydrogen ions in the medium may alter the ionisation of active site or substrates. Ionisation is a requirement for ES complex formation and pH may influence the separation of coenzyme from holoenzyme complex. At a very low or high pH the H-bonds may be inactivated in the protein structure, destroying its 3D structure. The optimum pH may vary from substrate to substrate for an enzyme acting on a number of substrates because of the ES complex formation and ionisation will vary from substrate to substrate.



Effect of pH on enzymatic ‘reaction

**3. Effect of Enzyme Concentration**

In the beginning velocity of the enzymatic reaction is directly proportional to the enzyme concentration. When the substrate conc. is in large excess exceeding that of Vmax, because enzyme is the limiting factor in the enzyme-substrate reaction and providing more enzyme molecules enables the conversion of progressively larger numbers of substrate molecule.

**4. Effect of Product Concentration:**

Products formed as a result of enzymatic reaction may accumulate and this excess of product may lower the conditions of high concentration of products a reverse reaction may be favoured forming back the substrate.



Effect of enzyme concentration on enzymatic reaction

**5. Effect of Substrate Concentration**

As already described a known quantity of enzyme, the reaction is directly proportional to the substrate concentration. However, this is true only up to a certain concentration after which the increasing concentration of substrate does not further increase the velocity of reaction.



Effect of substrate concentration on enzymatic reaction

**6. Effect of Activators and Coenzymes**

The activity of certain enzymes is greatly dependent of metal ion activators and coenzymes. The role of metal ions and coenzymes is already discussed.

**7. Effect of Modulators and Inhibitors**

Whenever the active site is not available for the binding of the substrate the enzyme activity may be reduced. The substances which stop or modify the enzymatic reaction are called ***inhibitors or*** ***modulators.*** Presence of these substances in reaction medium can adversely affect the rate of enzymatic reaction.

**8. Effect of Time**

The time required for completion of an enzyme reaction increases with decreases in temperature from its optimum. However under the optimum conditions of pH and temperature time required for enzymatic reaction is less.

**ENZYME INHIBITION:**

Enzymes are protein and they can be inactivated by the agents that denature them. The chemical substances which inactivate the enzymes are called as ***inhibitors*** and the process is called as ***enzyme inhibition.*** Inhibitors are sometimes referred to as ***negative modifier***. They may be small inorganic ions, or organic substances. Enzyme inhibition is classified under **three major groups:**

***• Competitive inhibition (Reversible).***

***• Non-competitive inhibition (Irreversible or reversible).***

***• Allosteric inhibition.***

**1. Competitive Inhibition**

***When the active site or catalytic site of an enzyme is occupied by a substance other than the substrate of that enzyme, its activity is inhibited.*** The type ofinhibition of this kind is known as competitiveinhibition. This is a type of ***reversible inhibition*.** Insuch inhibition both the ES and EI (Enzyme-Inhibitor)complexes are formed during the reaction. However,the actual amounts of ES and EI will depend on:

• Affinity between enzyme and substrate/inhibitor,

• Actual concentrations (amounts) of substrate and inhibitor present, and

• Time of preincubation of enzyme with the substrate or inhibitor.

So the affinity of the substrate for the enzyme is progressively decreased with the increase in conc. Of inhibitor lowering the rate of enzymatic reaction. Thus, ***the Km is high***, but ***Vmax is the same*** in competitive inhibition. However, when the concentrated substrate is increased, the effect of inhibitor can be reversed forcing it out from EI complex.



Competitive inhibition

**2. Non-competitive Inhibition:**

This is of two different types namely (i) ***reversible*** and (ii) ***irreversible***. This occurs when the substances not resembling the geometry of the substrate do not exhibit mutual competition. Most probably the ***sites of attachment of the substrate and inhibitor are different.*** The inhibitor binds reversibly with a site on enzyme other than the active site. So the inhibitor may combine with both free enzyme and ES complex. This probably brings about the changes in 3D structure of the enzyme inactivating it catalytically. In noncompetitive inhibition Vmax is lowered, but Km is kept constant. If the inhibitor can be removed from its site of binding without affecting the activity of the enzyme, it is called as Reversible-Non-competitive. However, if the inhibitor can be removed only at the loss of enzymatic activity, it is known as Irreversible Non-competitive Inhibition. However, the kinetic properties in case of both are the same. Combine with both free enzyme and ES complex.



Non-competitive inhibition

This probably brings about the changes in 3D structure of the enzyme inactivating it catalytically. In noncompetitive inhibition ***Vmax is lowered***, but ***Km is kept*** ***constant***. If the inhibitor can be removed from its site of binding without affecting the activity of the enzyme, it is called as ***Reversible-Non-competitive*** ***Inhibition***. However, if the inhibitor can be removed only at the loss of enzymatic activity, it is known as ***Irreversible Non-competitive Inhibition***. However, the kinetic properties in case of both are the same.



Lineweaver-Burk plot for normal and competitive and Sigmoid kinetics, allosteric inhibition

noncompetitive inhibition

**The above diagram** gives graphical presentation of Lineweaver- Burk double reciprocal plot in case of competitive and non-competitive inhibition.

**Examples of Non-competitive Irreversible Inhibitors**

• **Iodoacetate:** An irreversible inhibitor of enzymes like glyceraldehyde-3-p dehydrogenase and papain. It **combines with–SH group** at the active site of the enzyme inactivating the enzyme.

• **Heavy metal ions** like Ag, Hg also act as irreversible noncompetitive inhibitor.

• **Fluoride:** Inhibits the enzyme emolase by removing Mg++ and Mn++ and stops glycolysis.

• **BAL (British anti Lewesite):** Called **Dimercaprol**, used as antidote for heavy metal poisoning. The heavy metals act as enzyme poisons by reacting with –SH groups. BAL has several –SH groups with which the heavy metal ions react, thus removing their poisonous

effects.

• **Disulfiram (Antabuse):** Used in treatment of alcoholism, the drug irreversibly inhibits the enzyme ***aldehyde dehydrogenase*** preventing further oxidation of acetaldehyde which accumulates and produces sickening effect leading to aversion to alcohol.

• **Di-isopropyl fluorophosphate (DFP):** Inhibits enzymes with serine in their active site e.g. acetylcholine esterase.

**3. Allosteric Inhibition and Allosteric Enzymes**

There is a mixed kind of inhibition when the inhibitor binds to the enzyme at a site other than the active site but on a different region in the enzyme molecule called ***allosteric site***. ***Allosteric inhibition does not*** ***follow the Michaelis-Menten hyperbolic kinetics.*** ***Instead it gives a sigmoid kinetics****.* Allosteric inhibitors shift the substrate saturation curve to the right. However as opposite to inhibitors, the presence of activators shifts the curve to the left.

**Types:** Allosteric enzymes are of *K* and *M* series according to their kinetics.

• In ***K-enzymes***, e.g. *aspartate carbamoylase* and *phosphofructokinase*, the allosteric inhibitor lowers the substrate affinity to raise the Km of the enzyme; but the ***Vmax*** is unchanged.

• ***In M-enzymes***, e.g. ***acetyl-CoA carboxylase***, the allosteric inhibitor reduces the maximum velocity but no change in Km or substrate affinity. Allosteric activators produce a fall in K enzymes and a rise in ***Vmax*** in *M* enzymes.

• When the final product allosterically inhibits the enzyme, it is called as feedback allosteric inhibition.

**Allosteric Enzyme:**

**Aspartate transcarbamoylase is** **a model allosteric enzyme:**

Aspartate transcarbamoylase (ATCase) catalyses the first reaction unique to pyrimidine biosynthesis. ATCase is feedback inhibited by cytidine triphosphate (CTP). Following treatment with mercurials, ATCase loses its sensitivity to inhibition by CTP but retains its full activity for carbamoyl aspartate synthesis. This suggests that CTP is bound at a different (allosteric) site from either substrate. ATCase consists of multiple catalytic and regulatory protomers. Each catalytic protomer contains four aspartate (substrate) sites and each regulatory protomer atleast two CTP (regulatory sites).

Aspartic acid + carbamoyl PO4

Aspartate trans- carbamoylase

Carbamoyl aspartate

↓

↓ Series of reactions (six)

↓

Cytidine triphosphate (CTP) (End product)

**Another example of allosteric enzyme and inhibition:**

***Synthesis of isoleucine from threonine involves at least*** ***5 steps*** of enzymatic reactions. Isoleucine, the end product, inhibits the first enzyme ***threonine deaminase*** and stops its own synthesis.

Threonine

Threonine deaminase

α-ketobutyrate

↓ (4 steps more)

↓

↓

Isoleucine (End product)

• A metabolite may also cause feed-forward allosteric activation of an enzyme for a subsequent step of its metabolism, e.g. F 1,6-biphosphate allosterically activates *pyruvate kinase* catalysing subsequent step.

• An allosteric effector oppositely influences two allosteric enzymes catalysing reverse reactions. For example, AMP allosterically activates *phosphofructo-* *kinase* and allosterically inhibits *FDpase.* Following box gives some examples of allosteric modulation.

|  |  |  |
| --- | --- | --- |
| **Name of enzyme** | **Allosteric activator** | **Allosteric inhibitor** |
| • Glutamate | ADP | ATP, NADH |
| Dehydrogenase |  |  |
| • Hexokinase, ICD | ADP | G-6-P, ATP |
| • Protein kinases | c-AMP | — |
| • Pyruvate carboxylase | Acetyl-CoA | ADP |

• **In oligomeric enzymes**, the allosteric site and active site are located on different subunits. ***Changes in the enzyme-substrate interaction due to the allosteric effects of regulatory molecules other than the substrate are called heterotropic allosteric modulations***. Allos- teric activators and inhibitors exhibit respectively posi- tive and negative cooperativities with the substrates. Binding of substrate to one protomer enhances the binding of the same to another protomer or another substrate binding site on the same enzyme molecule. ***When the binding of a substrate enhances the interaction*** ***between the allosteric enzyme and more molecules*** ***of the same substrate it is homotropic allosteric effect.*E**

***Feedback regulation vs feedback inhibition:***

***Feedback regulation and feeback inhibition are not synonymous and they are different.*** In both mammalian and bacterial cells, end-products “feedback” and control their own synthesis. In many instances, this involves feedback inhibition of an early biosynthetic enzyme. It is necessary to distinguish between “feedback regulation” and feedback inhibition, a mechanism for regulation of many bacterial and mammalian enzymes.

***Example***

• Dietary cholesterol restricts the synthesis of cholesterol from acetate in mammalian tissues. This is feedback regulation.

This feedback regulation, however, does not appear to involve “feedback inhibition” of an early enzyme of cholesterol biosynthesis. An early enzyme *‘HMG-CoA reductase’* is affected, but the mechanism involves curtailment by cholesterol or a cholesterol metabolite of the expression of the gene that encodes *HMG-CoA reductase’*, i.e. enzyme repression. Choles- terol added directly to *‘HMG-CoA reductase’* has no effect on its catalytic activity.

**Uses of Enzymes:**

Enzymes are used as follows:

**(a) Enzymes estimation in serum and body fluids for** **diagnosis and prognosis**

**(b) Enzyme used as laboratory reagent**

**(c) Therapeutic uses of enzymes**

(***a) Enzyme estimation in serum and body fluids:*** Various enzyme estimations in serum and body fluids viz. CS fluid, peritoneal/pleural fluids have been **used for diagnosis and prognosis of diseases.** Serial estimations of Alanine transaminase (AL-T) in serum have been used

for prognosis of viral hepatitis (for details—refer to

chapter on “Enzymes and Isoenzymes of Clinical

Importance”).

***(b) Enzyme used as laboratory reagent:*** Some enzymes are used for estimation of biomolecules in serum.

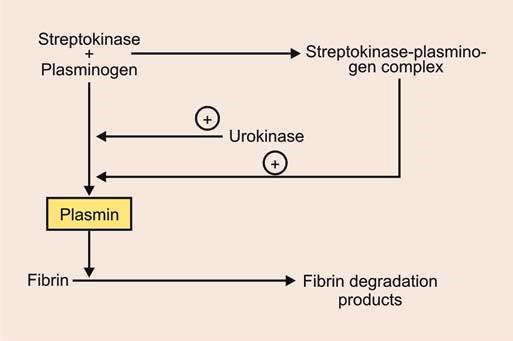
***Examples***

• ***“Glucose oxidase”*** enzyme is used for estimation of ***“true glucose”*** in blood and body fluids.

• Enzyme ***“uricase”*** is used for estimation of serum uric acid.

• Enzyme ***“urease”*** is used for estimation of urea in blood and body fluids.

***(c) Therapeutic uses of enzymes:*** Enzymes have been used for treatment purposes. Some of the enzymes used therapeutically are given in the box.



Showing mechanism of action of streptokinase and urokinase

**I. Questions (Essay type and short notes)**

1. What are enzymes? Classify them giving suitable example.

2. Describe the factors which influence the rate of enzyme action.

3. Describe various factors affecting enzyme action.

4. What are enzymes? Describe various processes of inhibition of enzymes activity.

5. Classify enzymes with suitable examples. Explain the mechanism of enzyme action.

6. Give the classification of enzymes.

7. Classify enzymes giving a few examples in each group. Add a note on enzyme inhibitors.

**Short Notes**

a. Competitive inhibition of enzymes.

b. Allosteric inhibition

c. Enzyme inhibition

d. Enzyme specificity

e. Enzyme inhibitors

f. Classification of enzymes

g. Enzymes—their classification and mechanism of action.

h. Allosteric enzymes

i. Therapeutic uses of enzymes

**Differentiate**

a. Competitive and non-competitive inhibition.

b. Coenzymes and isoenzymes

**therapeutic uses of enzymes:**

***Name of the enzyme Availability Mechanism of action Indications***

**A. Enzymes used systemically**

• ***Streptokinase and*** Pure stabilised Increases amounts of proteolytic • Acute myocardial infarction

• ***Urokinase*** • Streptokinase available enzyme ***“plasmin”*** by either • Acute thrombosis of arteries

750,000 to 15,00,000 IU vial • Increasing the circulating level • Deep vein thrombosis (DVT

• Urokinase—50,000 to 500,000 of its precursor ***“plasminogen”*** or • Pulmonary embolism

IU vial • Increasing the conversion of

plasminogen to plasmin.

Plasmin acts directly on “fibrin”

breaking it down to achieve

thrombolysis **(Fig. 9.14)**

• ***L-Asparaginase*** Available as ***“Leunase”.*** 10,000 Certain tumour cells require • Acute leukaemia

KU of L-Asparaginase per vial L-Asparagine for growth • Malignant lymphomas

L-Asparaginase hydrolyses

L-Asparagine and growth of

tumour cell suffer

• ***Digestive enzymes*** Available as tablets and syrup Replacement therapy in pancreatic • Cystic fibrosis

***Amylase, lipase*** insufficiency • Chronic pancreatitis

***and protease*** • Following pancreatectomy

• α***-chymotrypsin*** 5.775 mg sublingual tablets Mucolytic and proteolytic activity **Used as adjunct therapy**

• In management of inflam-

matory oedema due to injury

Postsurgical infections and

dental procedures

• ***Serratopeptidase*** 5 mg tablet Fibrinolytic activity, high bradykinin • Effective adjunct in inflam-

decomposing activity, and potent mation after traumatic injury

caseinolytic activity and after operation

• Subconjunctival bleeding

**B. Enzymes used locally**

• Hyaluronidase Available as ***“Hyalase”*** 1500 Brings about depolymerisation of • Promotes diffusion of fluids

IU per ml. ground substance and helps in given subcutaneously (SC)

absorption of fluids • Intra-articular injection

in joints to alleviate pain

in osteoarthritis