Unit-I Enzymes, enzyme kinetics

1.1 Importance and definition,

i. structure of enzyme , physico chemical nature, Apoenzyme and cofactors. Prosthetic group, coenzymes and metal cofactors.

ii Active site and its silent features

iii. Classification of enzymes

iv. General properties of enzyme

v. Types of enzymes: extracellular, intracellular, constitutive, inducible

vi. Mechanism of enzyme action –Lock and key hypothesis, induced fit model.

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B. Sc.Third year (Semester -V)
Subject : Microbiology

Course – Biocatalyst and Microbial Metabolism

Course Teacher: Dr. K.G.Maske

Importance and definition

History:

Louis Pasteur-living Yeast cells convert grape juice in to wine. Refered as ferment.

Buchner used cell lysate of yeast cells and produced alcohol from sugar.

Enzyme: en G.=in, Zyme =in yeast

•Term **Enzyme** coined by Fredrich Kuhne

•Eduard Buchner :Use of cell free extract =Zymase , Nobel Prize 1907

•James Sumner : Father of Modern Enzymology-Preparation of enzymes and proteins in pure form and viral proteins -Nobel prize 1946

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•Sumner -Isolated , purified and crystallize enzyme urease.

Definition: Group of simple or combined proteins acting as specific catalyst (Sumner)

Exception: Ribozyme

•Biological catalyst formed by living cells which catalyse a particular reaction or a group of closely related reactions.

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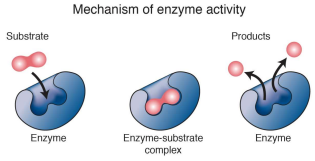

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Intracellular and extracellular enzymes

- **Intracellular**
  - enzymes are *synthesized and retained in the cell for the use of cell itself.*
  - They are found in the cytoplasm, nucleus, mitochondria and chloroplast.

  Example: Oxydoreductase catalyses biological oxidation, Enzymes involved in reduction in the mitochondria.
- **Extracellular**
  - enzymes are *synthesized in the cell but secreted from the cell to work externally.*

  Example : Digestive enzyme produced by the pancreas, are not used by the cells in the pancreas but are transported to the duodenum.

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INTRACELLULAR ENZYMES VERSUS EXTRACELLULAR ENZYMES

INTRACELLULAR ENZYMES	EXTRACELLULAR ENZYMES
The enzymes, which act inside the cell	The enzymes made by the cell but, work in the outside of the cell
AKA: Endoenzymes	AKA: Exoenzymes
Account for the majority of enzymes	Account for the minority of enzymes
Breakdown large polymers into smaller chains of monomers	Act on the end of the polymer to breakdown its monomers one at a time
Undergo intracellular digestion	Undergo extracellular digestion

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Types: Depending upon formation
1.Extracellular: digestive enzymes, Amylases.
Lipoprotein lipase.
Pectinase.
Pepsin.
Trypsin.

Intracellular: DNAand RNA polymerases
ATP synthatases,respiratory,
Depending of site of action on polymer molecule
Exoenzymes –Proteases,exonucleases
•Endoenzymes—endonucleases, synthetases
Types depending upon their production :
▪Induced enzymes -Galactocidases
▪Constitutive enzymes

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Types:
Simple enzymes-Contain simple proteins only,---a.a, degradation produce only amino acids

Conjugated enzymes-

Holoenzymes-These are conjugated proteins,

they have protein component called apoenzyme

(apo^{G} =away from),not active

Non protein- component prosthetic gr,cofactor,coenzyme/cofactor

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Constitutive enzymes:
The enzymes which are always present in the organism in constant amounts regardless of its metabolic state are called as constitutive enzymes.
For example, the enzymes involved in central pathway of catabolism such as glycolysis are constitutive enzymes.

Inducible or inductive enzymes:

- Sometimes, the enzymes appear in cells only when they are needed in the presence of their substrates or other agents .
- Such enzymes are called as inducible or inductive enzymes or induced enzymes and this process of their synthesis is called as enzyme induction.
- The substrate or any other agent capable of inducing the synthesis of an enzyme is called as inducer or inducing agent.
- In bacterium *Escherichia coli* (*E. coli*) an example of the inducible enzyme is β-galactosidase which catalyses the hydrolysis of lactose to yield D-Glucose and D-Galactose

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Cofactors: They bind to an enzyme also known as helper molecules.

- Some cofactors covalently bound the enzyme.
- It can be removed only by denaturation.
- Chemical compound , inorganic substance ex. Metal ions such as k+, Zn 2+
- Metal activators :Metaloenzymes Zn^{+2},Mn^{+2},Fe^{+2},Mg^{+2},Ca^{+2},K^{+},Na^{+}
- Two types of cofactors: Coenzyme and prosthetic groups. Increase the speed of reaction.

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Coenzyme: It carries chemical groups between enzymes.

- Also known as cosubstrate. Co+Apo
- Coenzyme loosely bound to enzymes.
- Can be easily removed. These are Organic substances. It is a type of cofactor. Gr,Hydrogen atom
- They act as carriers ex. Biotin, Vitamin, Coenzyme A
- Ex. NAD,NADP,TPP, Dehydrogenases

The prosthetic group

- The prosthetic group may be organic (such as a vitamin, sugar, RNA, phosphate or lipid)
- ex. Succinate dehydrogenase :FAD
- or inorganic (such as a metal ion).
- Prosthetic groups are bound tightly to proteins and may even be attached through a covalent bond ,ex. Haemoprotein peroxidase- Porphyrein

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- Conjugated Enzymes : Also known as coenzyme
- 1.Protein component –Apoprotein or apoenzyme
- 2.Non protein component –
- **Cofactors**
- Prosthetic gr- firmly
- Coenzyme –Organic molecule, dialyzable NAD,NADP,TPP
- Metal activators :MetaloenzymesZn^{+2},Mn^{+2},Fe^{+2},Mg^{+2},Ca^{+2},K^{+},Na^{+}
- DNA Polymerase, alcohol dehydrogenase-Zn^{+2} ATPase, phosphatase ,kinase Mg

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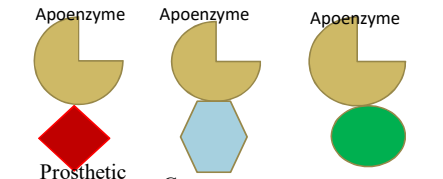

Metal ion or organic comp covalentlly bound

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General Characteristics of enzymes

- **1. Most enzymes are proteins**
- All enzymes are proteins with exception of few catalytic RNA molecules i.e. ribonucleases.
- Their catalytic activity depends upon integrity of their native protein conformation.
- Its catalytic activity lost, if a protein dissociated or denatured into its subunits .
- Thus primary, secondary, tertiary and quaternary structure of is essential for their activity
- Enzymes have molecular weight ranging from about 12,000 to more than 1 million.

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•Due to their large molecular size ,the enzyme molecules possess extremely low rate of diffusion and form colloidal system in water.
•Being colloidal in nature ,the enzymes are nondialyzable in nature . Coenzymes are dialyzable.

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- **2. Remain unaltered at the end of reaction**
- Enzymes accelerate rate of biochemical reaction .
- They do not alter at the end of reaction.
- They are recovered at the end of reaction as such without undergoing any qualitative and quantitative change.
- This enzyme may enter into another reaction with similar substrate.
- They can participate in many individual reaction over and over again .
- Hence they are present in the cell in very small concentrations to carry out reaction.

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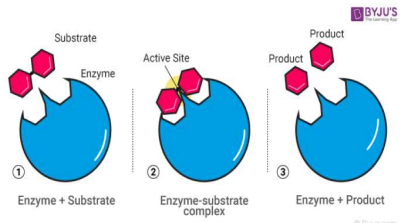
Enzyme Remain unaltered at the end of reaction



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- **3. Enzymes required in small quantities**
- Enzymes
- Ex.Carbonic unhydase –Act on 600000 substrate molecules per minute
- Catalase, single molecule of enzyme convert 50,00000 hydrogen peroxide molecule in to water and oxygen in one minute
- Turn over number-Number of mole of substrate converted to the product,in one minute by one mole of the particular enzyme.
- Turn over number also known as molecular activity
- Defined as the number of substrate molecules converted into product per unit time, when the enzyme is fully saturated with substrate.
-

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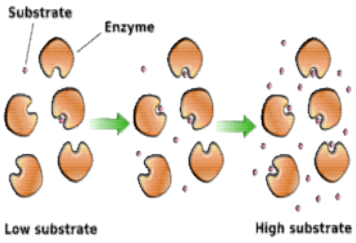
Enzyme is fully saturated with substrate.



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- For most of the enzymes turnover number falls between 1 to 10^4 per second.
- Specific activity is the number of enzyme units per milligram of protein.
- International enzyme commission recomonded a new unit for measurement of enzyme activity known as **Katal /Kat**
- Katal defined as the number of substrate molecules converted in to its product per second assuming the enzyme is fully saturated.

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Enzyme Turnover Number: Examples

	Enzyme	Turnover Number *(per second)*
1	Carbonic anhydrase	600000
2	Acetylcholinesterase	280000
3	Penicillinase	2000
4	Chymotrypsin	100
5	DNA Polymerase I	15
6	Lysozyme	0.5

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- **4. Accelerate rate of reaction without altering chemical equilibrium**
- Enzymes are capable of carrying about reversible reaction.
- Enzyme accelerate rate of reaction without altering chemical equilibrium of reaction
- The equilibrium between substrate and product will remain constant.
- Rate of forward and backward reaction is also increased.

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- **5. Reversibility of a reaction:**
- Enzymes are capable of bringing about reversion in a reaction
- Digestive enzymes catalyse the hydrolytic reactions which are reversible.
- This property is significant in metabolism.
- ex. Dehydrogenation of succinic acid by succinate dehydrogenase
- Succinic acid +A $\longleftrightarrow$ Fumaric acid +AH_2Reduced substrate

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- **6. Specificity of enzyme action**
- Enzymes are specific in their action Enzyme substrate interaction are selective in nature
- 1. they may act on only one type of substrate molecule
- 2.on a group of structurally related molecules .
- 3. On of the two optical isomers
- 4. Only one of the two geometrical isomers

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- **1. Absolute specificity:**
- Some enzyme act only on one type of specific substrate.
- Ex. 1.Urease- Substrate urea
- Urea Ammonia+Carbon dioxide
- 2.Carbonic unhydrase: bring about union of carbon dioxide and water to carbonic acid
- 3.Maltase
- 4.Gelatinase: protein
- 5.Caseinase: milk protein
- 6.Lecithinase

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2.Group specificity

- Some other enzymes capable of catalysing reaction of structurally related group of substrates
- This is broad specificity
- Such enzymes are economical for cell
- Ex. Carboxy peptidases is digestive enzyme act on protein chains in digestive tract
- It removes one amino acid at a time from carboxy terminus irrespective of type of amino acids

Esterases act upon esters of fatty acids with variety of alcohols.

- Lactate dehyrogenase ,Alcohol dehydogenases ,glycosidases

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3.Optical specificity

- Particular enzyme will act on only one of the two optical isomers
- ex arginase act on only L arginine not D arginine
- Although they exhibit optical specificity , **recemase** inter convert L-amino acid to D-amino acid

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4.Geomatric specificity

- Some of the enzymes exhibit specificity towards cis and trans forms
- Fumarase
- Does not react with malic acid which is cis isomer of fumaric acid (trans)

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Mechanism of enzyme action

- **Active site:**
- Specific regions or sites on the enzyme involved in the binding of the substrate molecule is called as active site or catalytic site or substrate site.
- Important general features of the enzyme are
- 1.The active site occupies relatively small portion of the enzyme molecule.
- 2. Active site is 3D entity it is neither a point nor a line or even a plane
- 3.It is made up of groups that comes from different parts of the linear amino acid sequence. For ex. Enzyme lysozyme made up of 129 amino acid residues, of these 35,52,59,62,63, and 107 are located at the active site.

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- 4. Substrates are bound to the enzyme molecule with forces of noncovalent nature-ionic and hydrogen bonds, van der waals forces.
- 5. The specificity of substrate binding depends on the precise arrangement of atoms in catalytic site
- 6. Active sites in the enzymes are grooves or cervices from which water is largely excluded during substrate binding. It contains amino acids such as aspartic acid , glutamic acid , glycine, serine etc. The side chain groups like –COOH,-NH_2,-CH_2OH
- etc. serve as catalytic groups in the active sites

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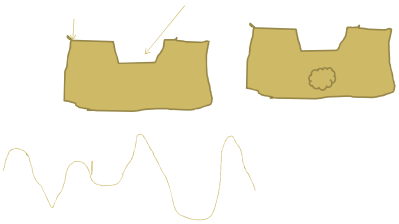
- Enzyme Active site Catalytic
- allosteric site: NonCatalytic:repressor



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- E+S ⟷ ES ⟷ E+P

Enzyme substrate Enzyme substrate complex Enzyme Product

Activation energy is the energy required to bring all molecules in a chemical reaction into the reactive state.

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Activation energy

- Activation energy is the energy required to bring all molecules in a chemical reaction into the reactive state.
- Every chemical reaction requires a certain amount of energy in order to proceed – this is the activation energy (E_A).
- Enzymes speed up the rate of a biochemical reaction by *lowering* the activation energy
- When an enzyme binds to a substrate it stresses and destabilises the bonds in the substrate
- This reduces the overall energy level of the substrate's transitionary state, meaning less energy is needed to convert it into a product and the reaction proceeds at a faster rate

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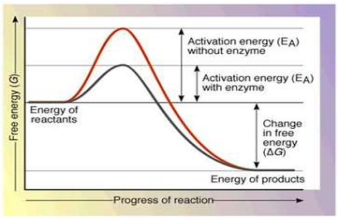

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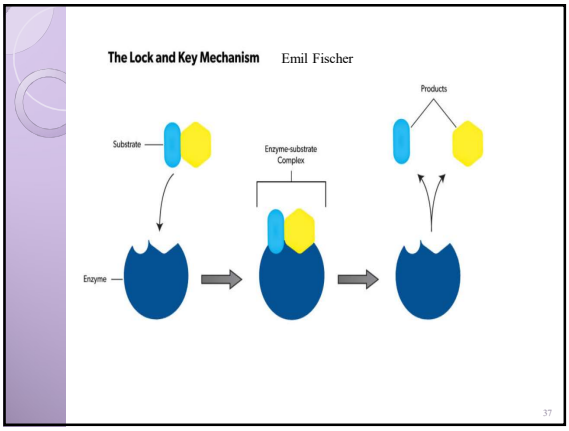
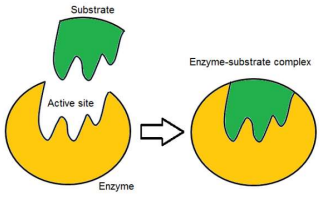


Fischer, Lock and key model

- Interaction of enzyme and substrate visualized in terms of Lock and Key model proposed by **Emil Fischer**
- According to this model union of the substrate take place at active site
- More or less in a manner in which lock and key fits.
- Active site is rigid and preshaped.
- Only substrate molecule having complementary shape can fit.
- Hence model also known as concept of intermolecular fit.

- Enzyme -substrate E-S is highly unstable – transition state. Union results in release of energy. It is the energy which elevates energy level of the substrate.
- Thus inducing to activated state. In this activated state certain bonds of the substrate molecule becomes susceptible to cleavage.
- Complex is immediately broken down in to end product of the reaction and enzyme is regenerated.

Koshlands induced fit model



Koshlands induced fit model:

Daniel Koshlands Modified Fischer's model

Explained enzyme properties precisely.

Active site is flexible

Contact of the substrate induce conformational changes in active site

Hypothesis confirmed by Lipscomb

Hydrophobic and charged groups in active sites involved in substrate binding.

Confirmed in phosphoglucomutase,carboxypeptidase

- Nomenclature and classification

1.substrate acts upon by the enzyme

Duclaux (1883)Named enzyme adding suffix -ase

- Ex.maltase,urease, lecithinase

2. Type of reaction catalysed

Hydrolases,oxidases,isomerase,dehyrogenase

3.Substrate acted upon and type of reaction catalysed

Ex succinate dehydrogenase,L –Glutamic acid dehydrogenase

4.Substance synthesized ex fumarase

- International union of Biochemistry IUB
- Enzyme Commission
- Given precise ,descriptive and informative system of classification.
- Major features are--

1.Reactions and enzymes catalyzing them divided in to six major classes, each with 4 to 13 subclasses

2. Each enzyme name has two parts .the first part is the name of the substrate and the second part which end in the suffix –ase indicate the type of the reaction catalysed.

3.Each enzyme has been allotted systemic code number called enzyme commission (E.C.) number.

Each enzyme is designated by four numbers.

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- First place number represents **major class** to which enzyme belongs.
- Two median numbers denoting the **subclass** and the subsub class of the enzyme within the major class
- Last place number or fourth digit number represent serial number of the enzyme within subsub class
- Ex. **E.C.2.7.1.1** represents the
- Class-2 (Transferase)
- Subclass -7(transfer phosphate group)
- Sub sub class 1(an alcohol group as phosphate acceptor)
- Final digit indicate the enzyme serial number hexokinase

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- **Classes of enzymes**

1.Oxidoreductases

Enzymes earlier called as dehydrogenases .RETS

A(red)+ B(ox) ⟷ A(ox)+ B(red)

Oxidoreduction of groups C=O,CH-CH ,CH-OH, CH-NH2andCH=NH groups

Dehydrogenase- removal of two hydrogen atom

Oxidases--red. of oxygen

Oxygenases-- incorporation of molecular oxygen in to substrate

Peroxidases– Use hydrogen peroxide as oxident.

Hydroxylases- introduce –OH group

oxidative deaminases—Catalyse oxidation of amino compounds with the release of ammonia

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2.Transferases-exchange of gr between two substrate

AB+CD ⟷ AC+BD

Aminotransferase, kinase, actyl transferase

- 3.Hydrolases –catalyse hydrolysis reactions
- $AB+H_2O$ ⟷ AOH+HB
- Hydrolases are hydrolytic enzymes, which catalyze the hydrolysis reaction by adding water to cleave the bond and hydrolyze it.
- Peptidase, glycocidase, esterase, phosphatases.

- 4.Lyases- Adds water, carbon dioxide or ammonia across double bonds or eliminate these to create double bonds.
- remove gr of the sub non hydrolytically
- Decarboxylase, aldolase dehydratase

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- 5.Isomerases.
- Enzyme catalyse isomerization of substrates.
- Ex. Recemases ,epimerases

6.Ligases:

Catalyse joining together of two molecules coupled with the breakdown of a pyrophosphate bond in ATP.

Ex. Sythatase-bring about formation of C-O,C-S,C-N bonds

Reaction require expenditure of energy with simultaneous breakdown of ATP.

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- Enzyme kinetics:
- Enzyme kinetics is **the study of the rates of enzyme-catalysed chemical reactions**.
- In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction are investigated.
- Kinetics study is important to understand rate of biochemical reactions in a cell.
- The Michaelis- Menten Model:

In enzymatically catalysed reactions rate of reaction is influence by physical conditions temp pH,

Concentration of enzyme and substrate are important variables.

Rate of reaction varies with the change in the substrate concentration.

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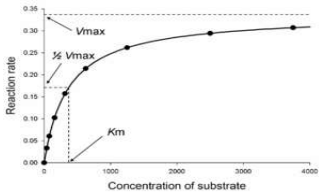

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- To determine the maximum rate of an enzyme mediated reaction,
- the **substrate** concentration (*[S]*) is increased until a constant rate of product formation is achieved. This is the *maximum velocity* (V_{max}) of the enzyme.
- In this state enzyme active sites are saturated with substrate.
- Note that at the *maximum velocity*, the other factors that affect the rate of reaction (ie. pH, temperature, etc) are at optimal values.
- Starch [S] –
- Lag
- A. 1,2,3,4 mg--------- Vmax increased, $\frac{Vmax}{2}$
- Amylase [E]

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In 1913, Leonor Michaelis and Maud Menten proposed a simple model to account for these kinetic characteristics.

$$E + S \underset{K2}{\overset{K1}{\rightleftarrows}} ES \underset{K4}{\overset{K3}{\rightleftarrows}} E + P$$

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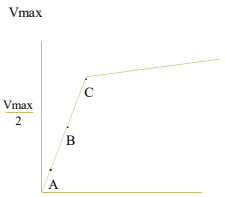

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$$\frac{d[ES]}{dt} = k_1([E] - [ES])[S]$$

The rate of breakdown of ES is given by

$$-\frac{d[ES]}{dt} = k_2[ES] + k_3[ES]$$

$$k_1([E] - [ES])[S] = k_2[ES] + K_3[ES]$$

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The Michaelis- Menten Equation

$\frac{d[ES]}{dt} = K_1([E]-[ES])[S]$ Rate of formation of ES

Rate of breakdown of ES is given by

$-\frac{d[ES]}{dt} = K_2[ES]+K_3[ES]$ Rate of breakdown of ES

$K_1([E]-[ES])[S] = K_2[ES]+K_3[ES]$

$\frac{([E]-[ES])[S]}{[ES]} = \frac{K_2 + K_3}{K_1}$

By combining constants we have

$\frac{K_2 + K_3}{K_1} = Km$

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ES= $\frac{K_2 + K_3}{K_1}$

Since initial velocity of reaction is V_0

$V = K_3[ES]$

At high substrate concentration velocity of reaction reaches maximum Vmax

$V_{max} = K_3[E]$

The value of ES can be substituted from previous equation

$V = K_3 \frac{[E][S]}{Km+[s]}$

$\frac{V}{Vmax} =$

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$\frac{v}{V_{max}} = k_3 \frac{[E][S]}{K_m + [S]}$ $k_3[E]$

$V = \frac{Vmax[S]}{Km+[S]}$

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Lineweaver Burk Plot

- Michaelis Menten equation is utilized to determine Km at various substrate concentrations. These values can be used in predicting rate limiting steps .
- Therefore Vmax and Km should be carefully determined.
- Reciprocal of the substrate concentration and velocity of the reaction is taken to draw plot to determine these values precisely . This plot is called as Lineweaver Burk Plot.
- One of the major application of this plot is to study enzyme inhibition.
- Michaelis Menten equation can be transformed by taking double reciprocal of both sides of the equation:

$V = \frac{Vmax[S]}{Km+[S]}$

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$1/v = 1/ \frac{Vmax[S]}{Km+[S]}$

$= \frac{Km+[S]}{Vmax[S]}$

Double reciprocal plot by taking 1/V values on Y-axis and 1/S on X-axis a straight line is obtained which is used to determine Km.

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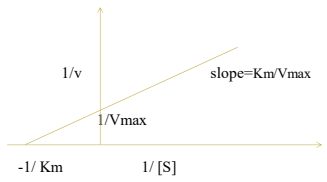

Lineweaver Burk Plot

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Factors affecting enzyme activity

Enzyme concentration

- To determine effect of enzyme concentration the substrate must be saturating.
- The rate of velocity of the reaction is independent of concentration of the substrate
- Product produce per unit of time is related directly to the enzyme concentration.
- Formation of the product is linear with the time.
- As time doubles, concentration of the product doubles.
- When concentration of enzyme doubled Product also doubled.
- When substrate concentration reduce or depleted , there is loss of zero order rate .
- There is no proportionality between enzyme concentration and a enzyme activity.

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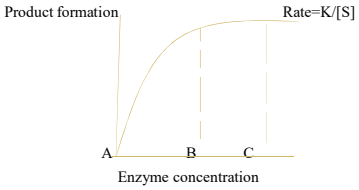

Fig: Effect of Enzyme concentration on rate of reaction

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Effect of substrate concentration

- MM Constant Km
- Small Km (10^{-6} to 10^{-7} M) indicate only small amount of substrate are necessary to saturate the enzyme.
- A high Km (10^{-2} to 10^{-3} M) indicates that large amount of substrate is required to obtain maximum velocity.

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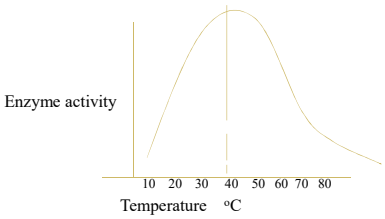
- Effect of temperature



- As temperature increase by 10 °C rate of reaction doubles it is called as Q_{10} Value
- For most of enzymes opt temp 25 °C to 40 °C .
- Ribonuclease ,lecithinase are stable at water boiling temperature

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Effect of P^H on Enzyme activity

- Enzymes are also proteins, which are also affected by changes in pH.
- The change of pH will lead to the ionization of amino acids atoms and molecules, change the shape and structure of proteins, thus damaging the function of proteins.
- Very high or very low pH will lead to the complete loss of the activity of most enzymes.
- The pH value at which the enzyme is most active is called the optimal pH value.

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- For example, pH can affect the ionization state of acidic or basic amino acids.
- There are carboxyl functional groups on the side chain of acidic amino acids.
- There are amine-containing functional groups in the side chain of basic amino acids.
- If the ionized state of amino acids in the protein is changed, the ionic bonds that maintain the three-dimensional shape of the protein will change.
- This may lead to changes in protein function or inactivation of enzymes

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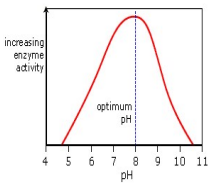

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- Assignment.