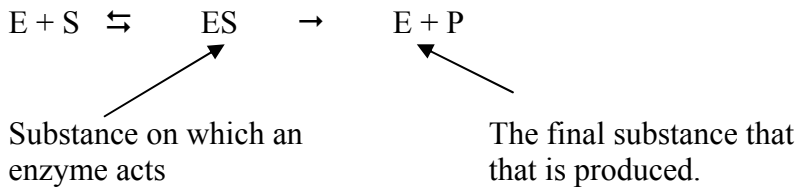
INTRODUCTION TO ENZYMOLOGY

Enzymes are protein specialized to catalyse biological reactions (Biocatalyse):

- Increase the speed of reactions
- Thumbers do not changes in the reaction.
- Very important to life as life depends on biological reactions (example digestion).
- Any change in a single enzyme can have very harmful effects.
- Very specific
- Enzymes (E) act on certain substances which are known as substrate(s). They form an Enzyme-Substance Complex (ES) which is broken or changed to give the Product (P).



Cofactor: Some enzymes depend for activity only their structure and proteins, while others require one or more non-protein component. This is known as the Cofactor. Cofactor may be metal coin or an organic molecule called Coenzyme. Some enzymes require both:

Apoenzyme (Catalytically inactive)	+	Cofactor	→	Haloenzyme active (enzyme)

Cofactors are generally stable to heat while the enzyme protein loses its activity on heating.

Metal as cofactor

e.g.	Alcohol dehydrogenase	-	Zn^{++}
	Kinases (phosphotransformer)	-	Mg^{++}
	Cytochromes	-	Fe^{++} or Fe^{+++}
	Cytochrome oxidase	-	Ge^{++}

Coenzymes

Papidoxal phosphate	-	Amino transferase
NAD^{+}	-	in H. transfer
NADP	-	" "
FAD	-	" "
FMN	-	" "
COQ	-	" "
COA	-	Amyl group transfer

- Most of the coenzymes contain as part of its structure and molecule of one or another of the vitamins. Coenzyme usually function as intermediate carriers of functional groups of specific atoms or of electrons.
- Where the coenzyme is tightly bond to the enzyme molecule, it is usually called a prosthetic group e.g. biocyte group of acetyl CoA carboxylases.

  In some cases the co-enzyme is only loosely bound to the enzyme protein.
- The enzyme combined with the substrate at a specific site known as the active site:

  E Active site

- Inhibitors some substances combined with the enzyme and reduce its activity.

  These are known as inhibitors.

- Activators. Others they combine with the enzyme or increase its activity, these are known as activators.
- Activation: Process by which the enzyme molecule becomes more active.

Brief History of Development of Enzymology

1833

Payen and Persoz – found that alcohol precipitate of malt extract contained a substance that was thermolalite and that converted starch into stager. They called it disease (now known as amylase) of its power of separating soluble dextrin from the insoluble starch grains.

1894

Duclaux proposed that to name an enzyme the name of the substance on which the enzyme acted. This is still used (a few enzymes contain "in" as the last 2 letters especially those of the digestive treat). (Now IUB system of nomenclature).

1860

Lonis Pasteur recognized that for mutation.

1878

W. Kuhine introduced the same 'enzyme"

1897

Edward Buchners succeeded in extracting from yeast cells the enzyme catalyzing alcohol for mutation.

1922-1928

Willstalter and his colleagues carried out studies to purify enzyme.

1926

J.B. Summer purified increase from extracts of Jack bear. He crystallized the increase.

1948

Crystallize proteolytic enzymes were isolated by Northrop and his colleagues.

Todate

Over 2000 enzymes are known and ~ 200 have been crystallized.

Recent world has known light on structure of enzymes, mechanism of enzyme reactions, control of enzyme synthesis. Complete detailed 3D structure of enzyme:

Classification of Proteins (briefly)

e.g. Enzymes are proteins:

- Exist as Zwitterious isoelectric pH. Above this pH – newly charged, below and newly charged
    - can move in an electric field (useful in separation methods).
- Can be denatured loss of structure and shape: loss of function. pH, heat, radiations, high salt concentration, detergents, 8M urea/β-mcorpts ethanol metal ions.
- Mol nt: ranging from 1000 m several thousand.
- Have buffering capacity (acid base property)
- Absorption spectra – One toTmp, Tye, Phe absorb light in the visible range (in the uV) at 280.

Enzyme reduce the energy of activation

Speed up the reaction.

Reaction A ⇆ B goes the rough a transition

- The rate of the formed reaction depends on kan temp and on the difference in free energy between A and the transition state.
- Enzyme ↑ reactions by ↓ ∇G^{+}, the activation barrier. The combination of E+S creates a new reaction pathway whose transition state energy is lower than reaction with E.
- Enzymes are highly specific
  - Absolute specificity
  - Reaction specificity
  - Sterio specificity
  - Group (class specificity)

  e.g. substitution (bacteria) can break all peptide bonds

  Types C^{106} - N IH

  Agr Lys

  Chymotrypsin

  Phe Thy Trp

  Peps Ale Tyr Top

- DNA polymerase : very specific
- However, enzymes vary in the degree of specificity

    - Group specificity – relatively non specific for a group of diameter compounds

- e.g. alkaline phosphate hydrolyses many different esters of phosphoric acid carboxypeptidase catalysis the hydrolysis of c-terminal peptide bond of peptides.
- Since most proteins contain Tyr residue measurement of light absorption at 280 nm in the spectrophotometer is an extremely rapid and convenient method for certain proteins in solution.
- Optically active (contains only C aa)
- Free N-terminal that can react with ninhydrin to form a colored derivative.
- Binver reaction is given by peptide and proteins but not by a.a. P + Cm^{++} alleli, Purple Cm^{++} - peptide complex.
- Reduction of disulphate (by β-mercapto ethanol).

Enzyme Specificity

1. Enzymes act on either one substance or a group of closely related substrates – i.e. they are specific.
2. Very important characteristic of all C.
3. Most important biological phenomenon
4. E → determine velocity change
   E → determine which substance undergo change

   E – help in organized to organize the metabolic pathway
   1. Organization by specificity
5. Degree of specificity varies with different E . Some E only only one one 5' and catalyse only one reaction.
   1. Others act on a small No. of closely related substrates, carrying out the same reaction in all cases (A common chemical structure can be recognized in all the substrates on which the E acts. For less specific E this group is a male molecule e.g. aldehydic or ester.

Investigation of Specificity: For satisfactory investigation:

1. Pure E (preferably recrustallized several times)
2. Free of other E which act on similar S
3. Pure S and free of other S on which E may act.
4. 2 opt active isomers – must be tested separately (some of them may inhibit the reaction).
5. 1st use most readly attached biological 5' then examine other S.
6. For each S work out Kim and Vmax (using time-weaker and bul plot)

7. For ionizing S – Kim and Vmax must be obtained for a wide range of pH. Obtain opt pH curve.
8. Study effect of competitive I to investigate the influence of structure.
9. Use low conc. of I to avoid reactions due to traces of other E.
10. Make small chemical modifications in E and study effect of this on the activity and reactivity.
11. Formulate the minimal structure necessary for combination and for reaction.

Types of Specificity

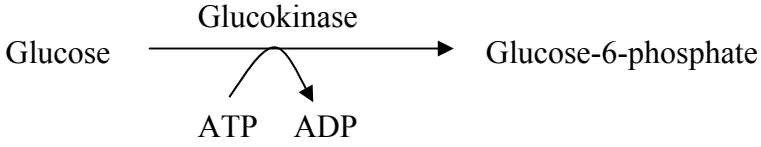
The specificity is due to substrate binding site which has on the enzyme surface. The specificity is also due to the specific arrangement of a.a. that participate in the bond making and bond breaking phase of catalysis:

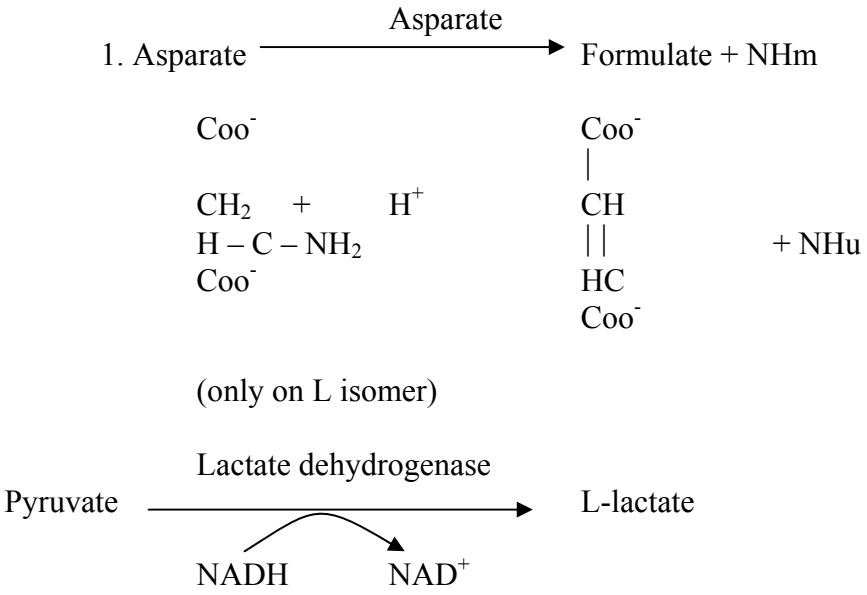
Models to explain substrate specificity

1. Lock and key model (Fischer)
2. Induced fit model

1. Absolute Specificity

The E com act only on 1 specific substrate:





2. Group Specificity

- Bound specificity
- E act an a group of related S
- The substrates have a common group on which the E acts.

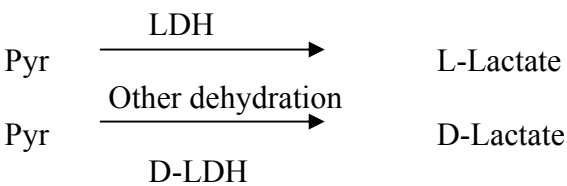
e.g. esteroses can act on esteri proteases ------ protein.

3. Stereo Specificity

- E can act only on one type of optical isomer or geometrical isomer – (usually absolute specificity).

  e.g. chymotrypsin acts on β-isomers of acetyle-L-tryptophenamide, Acetyle-L-tysosinamide, and N=catamyl-L-ltryptophin.

- Aim etc., but not E the D-isomer. In fact the D-isomer acts as an inhibitor.
- If the S is symmetric but the product is asymmetric, the E gives only 1 isomers of the product e.g.



$$\alpha\text{-ketoglu} + NH_3 \xrightarrow{GDH} \text{L-glutamate}$$

- The antipode may act as a inhibitor of the E.

<u>Cis-trans Isomerism</u>

- Often the enzyme acts on only 1 form of genometric isomers cis or trans

H H H

C C or C C

H

e.g. Furmarate hydrotase

CooH CooH

CH C H

C – H for hydrolase HO – C

CooH CooH

Furmarate Molate

<u>Highly specific</u>

- Dehydrogenase e.g. alcohol dH.

aldehyde DH

Acyl-CoA du

Highly specific

Only 1 pair of S is known (i.e. H-donor and H-acceptor)

- Glucose oxidate Highly specific – acts only on D-glucose

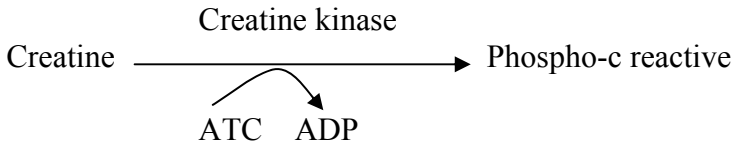
on D –maumase D-Altrose

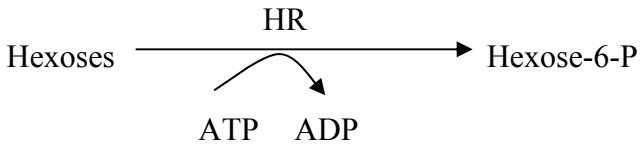
D Gal, only very slow rate.

- Flaro protein

- Amino acid oxidase

- Kinases

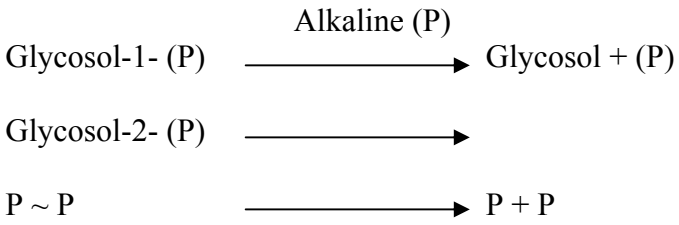




Estors e.g. Liposes
(acetylcholine ester)

Acetylcholine
Hydrolyse choline and non-choline esters.

<u>Phosphatase</u>



<u>Nucleases</u> Break nucleic acid

<u>Glycosidase</u>

<u>Peptidase</u>

Increase - First considered to have absolute specificity

- But now know that it may act on urea derivatives.

<u>Highly Specific</u>

May lyases e.g. Pyr decarboxylase

Furmate hydrolase

L – malate ⇆ Furmate

Emolase L- phosphor – D – glycerate

Emolase

P E P

Isomerase

Aldolase 1- epimerase

α - Sugar $\leftrightarrows$ β - sugars

ENZYME STRUCTURE

- Protein in nature
- Four levels of protein structure.
    - Primary
    - Secondary
    - Tertiary
    - Quartering
- Classification of proteins
    - Basis of information
    - Catalytic enzyme
    - Transport function

      Albumin
    - Protective (immunological)

      Antibodies
    - Coagulation

      Thrombin
    - Mechanical support

      Collagen, Kerantin
    - Contractile function

      Action and lyosin in muscle
    - Enzyme inhibitors
    - Storage

      Aproferritin → Ferritin

      Myoglobin

- On the basis of composition
  - Simple proteins
  - Conjugated proteins
    - Glycoproteins
    - Hemoproteins
    - Metaloproteins
    - Lipoproteins
    - Phosphoproteins
- On the basis of confirmation
  - Globular
  - Fibrous

- Action site
- Modulating site (alloitric site)
- Lock and key model – Emil Fischer
- Induced fit
- Stained induced fit.

- Isoenzymes
- Factors affecting E catalyzed reactions:
  - Substrate conc.

- Temperature - Q10 Temp, coefficient for every 10°C → rate doubled

  Optimal temp.

- pH (i) E denaturation

  (ii) Alterations in the charged state of the E and/or

  $E \pm SH^+ \rightarrow ESH$

  $E \pm H^+ \rightarrow$ EujH at low pH.

  At high pH $SH^+ \rightarrow S + H^+$

- Equilibrium - Conc. - Not changed by an enzyme
- Key is a dynamic state.

- Enzyme conc. - (α [E]

ENZYMES

- General nature - Biocatalysis
    - Protein – increase the speed of a reaction.
    - Act on specific called substrate to form an ES complex which is the changed to produce:
        - Do not change during the reaction
        - Do not change the equilibrium constant of a reaction.
        - Increase the rate at which the reaction approaches equilibrium
        - Sensitive to effect of pH, temp ionic composition → are denatured and lose activity
        - Apo E catalytically inactive — Cofactor or prosthetic → gp — HbE active

          Small organic or inorganice needed for activity mol. of E
          Prosthetic gp is tightly bound to the E
        - Active site
        - V. specific - Diff. enzymes differ in this
            - Absolute specificity
            - Group
            - Reaction
            - Optical specificity
        - All is added at the name of the E

Classification: International Union of Biochemistry:

- 6 major classes
- Each divided into several subclasses which are further subdivided.
- A No. is assigned to each class, subclass and sub-subclass.

- Some trivial names

Class I – Oxidoreductase

- Involved in oxidation and reduction
- e.g. alcohol NAD oxidoreductase catalysis conversion of alcohol to aldehyde bt removing 2 e and 2 H.

$$R - \underset{H}{C} - OH + NAD^{+} \leftrightarrows R - \underset{H}{\overset{O}{C}} - H + NADH + H^{+}$$

Dehydrogenase also act on the following functional groups as electron donors:

$- CH_2 - CH_2 - \quad -CH_2 \quad -CH_2 \quad -CH = NH$ + Nucliotide

NADH + NADPH

Oxidase transfer 2 electron from the donor to ozygen resulting in H_2H_2 formation:

β - D – glucose → O_2 → δ gluconolactone + H_2O_2

Ozygenase catalyse the incorporation of both atoms of O_2 into a single S. Hydrxtlases – incorporate on atom of mol. O_2 into the δ and the second O_2 appears as water.

Peroxidase use H_2O_2 as oxidant

NADH + H^+ + H_2O_2 as H^+ donor and accepts

$H_2O_2 + H_2O_2 \leftrightarrows O_2 + 2H_2O$

Class 2 Transferases

- Transfer functional grps between donors and acceptors

- Groups transferred include amino, acyl, phosphate one carbon and glycosynl grps.

  e.g. Transaminase : transfer = NH_2 gp from amino acids to ketoacids to for a new amino acid.

Kinases – are phosphosylating Es that catalyse the transfer of the phsophoryl gp from ATP to another nucleotide triphosphate, to alcohol or amino gp acceptor.

$$\text{ATP + Glucose} \xrightarrow[\text{Glucokinase}]{} \text{ADP} \rightarrow \text{glucose-6-phosphate}$$

Glycosyltransferase – required for glycogen syn. catalyse the transfer of a activated glycosyl residue to a glycogen primer.

$$\text{UDP – glucose} + \underset{\text{Primer (n)}}{\text{Glycogen}} \rightarrow \text{UDP} + \underset{\text{(n+1)}}{\text{Glycogen}}$$

Class 3 Hydrolases

- Clear bonds by addition of water.
- The general reactions involve the cleavage of C-O, C-N, O-P and C-S grps

$$\underset{\text{R – C – NH – R}_2}{\overset{\text{O}}{}} + \text{H}_2\text{O} \xrightarrow[\text{Peptidase}]{} \underset{\text{R – C – OH -+ NH}_2\text{ – R}}{\overset{\text{O}}{}}$$

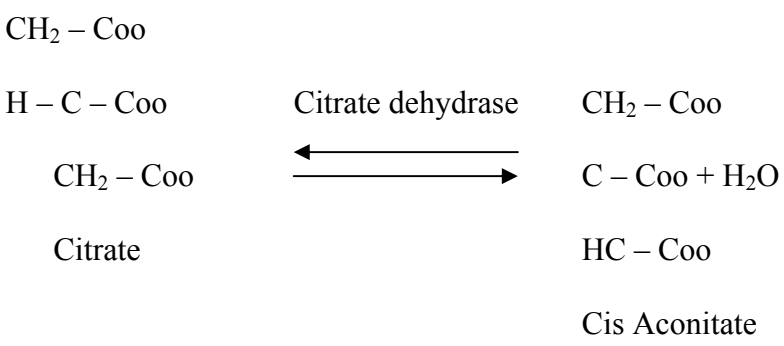
Class 4 Lyases

Lyases remove or add water, ammonia or CO_2 to produce bonds (in same cases)

Decarboxylases remove CO_2

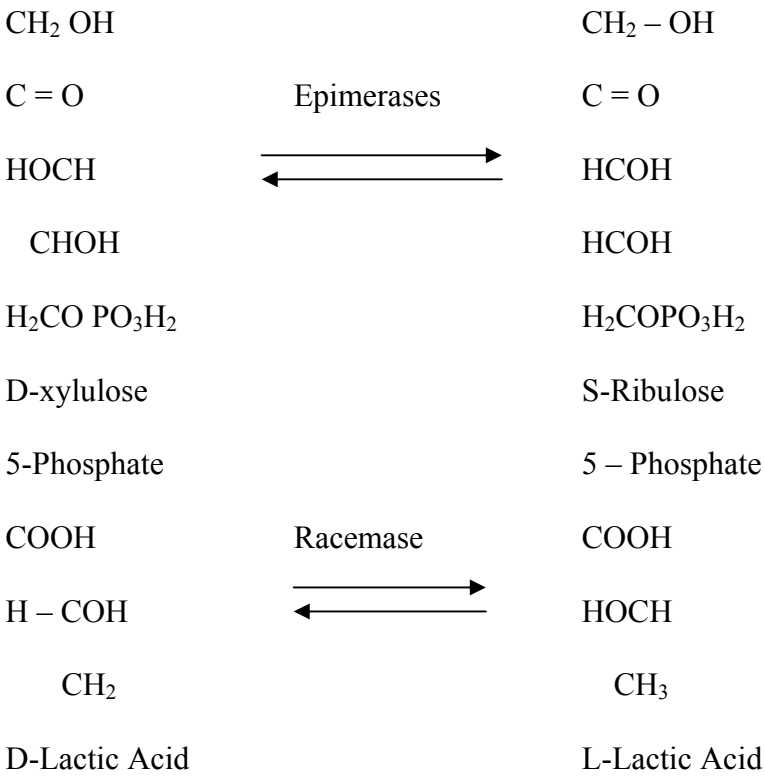
$$\text{R – C – C – O} \longrightarrow \text{R – C – H + CO}_2$$

Dehydrases remove H_2O



Class 5 – Isomerases

Catalyse isomerization include cis-trans, lato-emol and aldose-ketose intercoversions. Isomerases that catalyze the interconversion at asymmetric carbons are either epimerases or racemerases.



Mutases catalyse the intramolecular transfer of a gp such as the phosphoxyl gp e.g. phsophoglycerate mutase catalyzes the conversion of 2-phosphoglycerate to 3-phosphoglycerate.

PGM

COOH	COOH
H – C – O (P)	H – C – OH
CH_2OH	CH_2O – (P)
2-phosphoglycocerate	3-phosphoglycerate

Class 6 – Ligases

Ligate means to bind. These E are involved in synthesis reactions where two mol are joined at the expense of an ATP – high energy phosphate bond. Also known as synthetases

e.g. amino acid ERNA synthetases

Glutamine synthetase

Pyruvate carboxylase

		COOH		COOH
$ATP + CO_2$	+	C = O	(Bisline) ⟶	C = O + ADP + P
		CH_3		CH_2
				CooH
		Pyr		Oxaloacetate

ENZYMES

- Enzymes bind S at the active site to form on ES complex.
- E are highly selective for S.
- Active site is that region of a E that bind the S and the prosthetic group if any contributes the residues that participate directly in making a breaking if bonds. These residues are called the catalytic groups.
- The active site is a relatively small part of the total vol. of an E.
- The active site is 3D entity. It is not a point or online and is made of groups which are far away from each other in the polypeptide chain.
- S binds to the active site by weak forces.
- Active sites are clefts or crevices from those water is usually excluded.
- The specificity of binding depends on the precisely defined arrangement of atoms in an active site.
- Lock and key model

Active site has a shape complimentary to the active site.

- Induced fit model. The active site has a shape complementary to that of the S only after the S is bound.

- Enzyme decrease the activation energey of reaction catalyzed by the

$S \leftrightarrows P$

$\triangle G^{\#}$ = G transition state G Substrate

Transition state has higher energy

the S or P

$\triangle G^{\#}$ Gibbs free energy of activation

E ↑ the rate of a reaction by ↓ $\triangle G^{\#}$

Factors affecting the rate of E catalyzed reactions

- Substrate

$$E + S \underset{K_2}{\overset{K_1}{\leftrightarrows}} ES \xrightarrow{K_3} E + P$$

V is and to S when S is very small

Km (Michaelis constant) = $\frac{K_2 + K_3}{K_1}$

V = K_3 (ES) (none of the ES goes back to E + S). The initial stage of the reaction).

Rate of function of ES = K_1 [E][S]

Rate of breakdown of ES = (K_2 + K_1) [ES]

L = steady state . Rate of formation of ES remains constant while S and P change.

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

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

FACTORS AFFECTING RATE OF ENZYME CATALYZED REACTIONS

- Enzyme Concentration

  $r \alpha [E]$

- Substrate concentration

  Rate of catalysis K_1, varies with [S].

- Hypebolic way

  When [S] is small, V is almost proportional to [S]. At high [S] is nearly independent to [S]:

$E + S \underset{K_2}{\overset{K_1}{\leftrightarrows}} ES \xrightarrow{K_3} E + P$ $V = K_3 [ES]$	Proposed by Mchalis and Menten in 1913. • ES formed • None of the perverts back to ES a condition that hold in the initial stages of a reaction when P is ↓.

Rate of formation of ES = $K_1 [E][S]$

Rate of breakdown of ES = $(K_{+2} + K_{+3}) [ES]$

We are interested in the catalytic rates under steady state conditions. In a steady state, the concentration of intermediate stay the same while the concentration of starting maternal and products are changing:

Rate of formation [ES] = rate of breakdown of - [ES]

$K_1 [E][S] = (K_{+2} + K_{+3}) [ES]$ Rearrange

$$[ES] = \frac{[E][S]}{(K_{+2} + K_{+3}) [ES]}$$

$$Km = \frac{K_{+2} + K_3}{K_1} = \text{Michaelis constant}$$

$$[S]([E]-(ES)) = [ES]\,Km$$

Divide by [S]

$$[S]-[ES] = \frac{[ES]\,Km}{[S]}$$

Divide by [ES]

$$[ES] = \frac{[E][S]}{Km} \qquad \frac{Et}{[ES]} - 1 \qquad \frac{Km}{[S]}$$

$$\frac{Et}{[ES]} = \frac{Km}{[S\}} + 1 = \frac{Km + [S]}{}$$

The concentration of [S] is nearly equal to total S concentration, provided concentration of [E} is much lower that [S]. The concentration of uncombined [E] is equal to

$$ES = \frac{([E]-[ES])\,(SS)}{Km}$$

solving to ES

or $$ES = [Et]\,\frac{[S]/Km}{1+[S]/Km}$$

or $$ES = [Et]\,\frac{[S]}{[S]+Km}$$

or $$V = K_3\,[Et]\,\frac{[S]}{[S]+Km}$$

At a very high S concentration $\frac{[S]}{[S]+Km} = 1$ (saturation of (S/with [E])

$$Vmax = K_3\,[Et]$$

or $$V = \frac{Vmax\,[5]}{[S]+Km}$$ Michaelis Menlin equalation

At low [S] when [S] is much lower than Km

$$V = \frac{[5]\,Vmax}{Km}$$ i.e. rate x [S]

At high [S] whn [S] is much greater the Km

V = Vmax i.e. rate is maximal, independent of S soon.

when [S] = $V = \frac{Vmax}{2}$ Km is equal to the constant come at which the reaction rate is half the maximal value.

Vmax and Km can be determined by varying [S].

Reciprocal of Michaeles –Menlin e.g.

$$\frac{1}{V} \qquad \frac{1}{Vmax} \qquad \frac{Km}{Vmax} \qquad \frac{1}{[S]}$$

Significance of Km and Vmax

The Km form C depends on the particular [S] and on the environmental conditions e.g. temp and ionic strength.

- Km is the [S] concentration at ½ Vmax – at which half the active sites are filled.

  Km: is the dissociation constant (consider K_2 is much greater than K_3 i.e. dissociation of ES to E+P in which less than to [E] + [S] $K_2 >> K_3$

  Km is equal to the dissociation concentration of the ES complex if K_3 is much smaller than K_2.

- Km is the measure of the strength of ES. A high Km indicate weak binding, a low Km indicates strong binding.

- Vmax reveals the turnover No. of E if the concentration of active site [Et] is known

  $$Vmax = K_3 [Et]$$

- The turnover No. of a enzyme is the No. of substrate mol. converted to predict per unit time when the E is fully saturated with [S].

  e.g. 10^{-6}M sol. of carbonic anhydrase catalyses the formation of 0.6M H_2CO_3 / sec when it is fully saturated with [S]

  $$K_3 = 6 \times 10^5 \text{ sec}^{-1}$$

  K_3 is the turnover No.

  The turnover No. 600,000 sec^{-1} for carbonic anhydrase is the longest known.

Kinectus is the study of the rate of change of the initial state of reactant and products to the final state of reactant and products (the term velocity, is often used).

Velocity is expressed in terms of change in the concentration of S or P / min time.

No.	Class	Type of reaction catalyzed
1.	Oxidoreductase	Transfer of electron (Hydridian or H -----
2.	Transferase	Group transfer reaction
3.	Hydrolases	Hydrolyse in relation (transfer of functional group to water).
4.	Lyases	Addition of groups to double and or additional of double bonds by removal of groups.
5.	Isomerase	Transfer of groups with mol. to infield isomeric forms
6.	Ligases	Formation of C-C, C-S, C-O and C-N bonds by condensation reaction coupled to ATP cleavage.

	Oxidoreductase	$A^- + B \quad \leftrightarrows \quad A + B^-$
	Transferase	$A - B + C \quad \leftrightarrows \quad A + B - C$
	Hydrolases	$A - B + H_2O \quad \leftrightarrows \quad A - H + B - OH$
	Lyases	X Y A B $\leftrightarrows$ A – B X – Y
	Isomerase	X Y Y - X A B $\leftrightarrows$ A – B
	Ligases	$A + B \quad \leftrightarrows \quad A - B$

INTERNATIONAL CLASSIFICATION OF ENZYMES

- Suffix 'ase' to the name of the S or the word or phrase describing their activity

  e.g. increase – acts on urea

  protease

  lipase

  DNA polymerase

  (some enzymes e.g. trypsin, pepsin, chymotrypsin do not denote their substrates)

- Some time some enzyme has 2 or more names and or 2 different enzymes have the same names.
- To avoid such a problem, there is an evere increasing number of enzymes, a system for classification and naming of enzymes has been devised and adopted by International Union of Biochemistry (IUB). According to this:

  (i) all enzymes are placed in 6 major chains, each with subclasses and sub-subclasses, based on the type of reaction catalyzed (4-13 subclasses).

  (ii) The enzyme name has two parts. The first name the substrate or substrates. the second, ending in 'ase', indicates the type of reaction catalyzed.

  (iii) Additional information, if needed to clarify, is e.g. the co-enzyme category) e.g. L. molate + NAD^+ ⇆ pyruvate + Cis + NADH + H is designated 1.1.1.37 tomolate NAD^+ xodiase reductase (decarboxylating).

  (iv) Each E has a code No. (EC) that characterize the reaction type as to which class (first digit), subclass (second digit) and sub-subclass (third digit). the 4^{th} digit for the specific E.

e.g. EC 2.7.1.1. denotes

Calss 2	Transferase
Subclass 7	Transfer of phosphate
Sub-subclass	1. An alcohol or phosphate acceptr.
Final digit	Hezokinase, or ATP: O-Hexose-6- phosphotransferase

-