Unit-II Enzyme inhibition and Regulation

2.1 Enzyme inhibition

i. Reversible Inhibition
ii. Competitive Inhibition
iii. Non-Competitive Inhibition
iv. Uncompetitive Inhibition
v. Irreversible Inhibition
vi. Substrate and Product Inhibition,
vii. Allosteric Inhibition

2.2 Allosteric enzymes

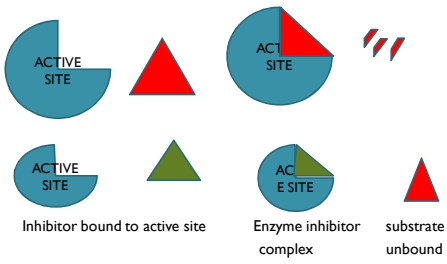
2.3 Isoenzymes

1

- **Enzyme inhibition** –Major control mechanism
  A number of substances may cause a reduction in the rate of an enzyme catalysed reaction.
- Some of these (e.g. urea) are non-specific protein denaturants. Others, which generally act in a fairly specific manner, are known as inhibitors. Ex cyanide, CO, Oxalic acid etc

2

- Enzyme substrate Enzyme substrate complex Product



Inhibitor bound to active site | Enzyme inhibitor complex | substrate unbound

3

Types of inhibition

a.Irreversible inhibition

b.Reversible inhibition

Irreversible inhibition

1.Inhibitor bound covalently with enzyme

2.Can not dissociate from enzyme

3.Prevent catalytic activity of enzyme

4.The inhibited enzyme is totally inactive. Suicidal inhibition

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- The inhibitor-enzyme bond is so strong that the inhibition cannot be reversed by the addition of excess substrate.
- The nerve gases, especially DIPF (diisoprofyl phosphofluridate irreversibly inhibit certain enzymes ex acetyl choline esterase an enzyme that has important role in the transmission of nerve impulse.
- by forming an enzyme-inhibitor complex with a specific OH group of serine situated at the active sites
- Heavy metal ions silver, mercury, lead
- .

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Reversible inhibition:

- A **reversible inhibitor** inactivates an enzyme through noncovalent, more easily reversed, interactions.

1. E+I+S --->EIS--->E+P+I

- E+S-> P
- E+I→ EI
- Reversible inhibitor can dissociate from the enzyme. Reversible inhibitors include
- competitive inhibitors and noncompetitive inhibitors. (There are additional types of reversible inhibitors.)

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- Irreversible and Reversible inhibition

Comparison:

Reversible	Irreversible
Binds via non covalent interactions	Binds via covalent interactions
Do not perform any chemical changes	Inhibitor binds to the substrate and prevent catalytic activity of enzymes.
Can be reversed, as there is no bonding between the inhibitor and substrate.	Irreversibility due to strong covalent bonding.

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Competitive inhibition:

- E+I→ EI
- E+S→ES
- A competitive inhibitor is any compound that bears a structural resemblance to a particular substrate.
- Inhibitor competes with that substrate for binding at the active site of an enzyme.
- The inhibitor is not acted on by the enzyme but does prevent the substrate from approaching the active site.

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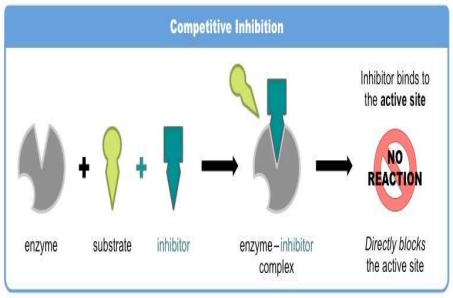

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- The degree to which a competitive inhibitor interferes with an enzyme's activity depends on the relative concentrations of the substrate and the inhibitor.
- If the inhibitor is present in relatively large quantities, it will initially block most of the active sites. Inhibitor diminishes rate of reaction by reducing proportion of enzyme molecule bound to the substrate.

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- But because the binding is reversible, some substrate molecules will eventually bind to the active site and be converted to product.
- Increasing the substrate concentration promotes displacement of the inhibitor from the active site.

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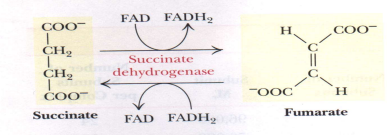
- Competitive inhibition can be completely reversed by adding substrate so that it reaches a much higher concentration than that of the inhibitor.
- Reaction exhibits normal Vmax. However Km will increase in presence of inhibitor.
- 

- Malonate is inhibitor of Succ. Dehydrogenase 

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•Ex.2
•PABA is analogue of sulfa drugs sulfanilamide

•Bacteria synthesize folic acid from PABA

•sulfa drugs act as enzyme inhibitor and block active site of enzymes catalyzing this reaction.
•MM kinetics can be applied

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Non competitive inhibition:
No competition between I and S
Non isosteric with S
In noncompetitive inhibition, an inhibitor molecule binds to the enzyme at a location other than the active site (an allosteric site).

Both combine at different sites simultaneously, no overlapping
Formation of ES,EI an
ESI,ES, broken down to P

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The substrate can still bind to the enzyme, but the inhibitor changes the shape of the enzyme so it is no longer in optimal position to catalyze the reaction.
Can not overcome by increasing Substrate conc.
Noncompetitive inhibition act by lowering turnover number.

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- Inhibitor lowers availability of active sites of E hence
- lowers Vmax. No km affected.

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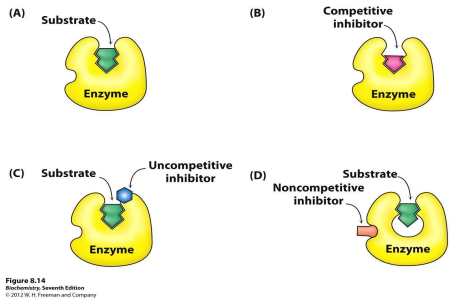

Figure 8.14
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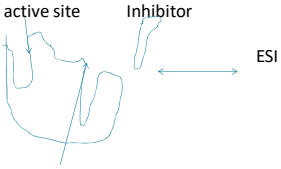
Enzyme S,I=ESI

active site Inhibitor



ESI

Allosteric site

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Kinetic behaviur

+S

E+I EI EIS EI+P

S+

ES

I+

ESI

EI+P

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Uncompetitive Inhibition

Some inhibitors combine reversibly with ES complex only hence are called as uncompetitive inhibitors.

Inhibitors not resemble substrate .

Inhibitors have no affinity to the substrate so no product synthesized.

+I

E+S ⟷ ES ⟷ ESI no product formation

Inhibition is found in multi substrate reactions.

Uncompetitive inhibition typically occurs in reactions with two or more substrates or products.While **uncompetitive inhibition** requires that an enzyme-substrate complex must be formed

characterized by a decrease in both substrate K_m and V_{max}

This mode of action is attractive for drug design as the inhibitors bind to the enzyme target only when the target is active and substrate present.

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- **Uncompetitive inhibition**, also known as **anti-competitive inhibition**, takes place when an enzyme inhibitor binds only to the complex formed between the enzyme and the substrate(the E-S complex
- Uncompetitive inhibition is distinguished from competitive inhibition by two observations:
- first uncompetitive inhibition cannot be reversed by increasing [S] and second, as shown, the Lineweaver–Burk plot yields parallel rather than intersecting lines

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Competitive inhibition	Non-competitive inhibition
1. In this type of inhibition, the chemical structure and shape of substrate and inhibitor are quite similar.	1. In this type of inhibition, the chemical structure and shape of substrate and inhibitor are different.
2. In this, inhibitors bind to the active site of enzyme.	2. In this, inhibitors bind to the allosteric site of enzyme.
3. Here, inhibitor does not change the shape of the active site of enzyme.	3. Here, inhibitor changes the shape of the active site of enzyme.
4. If substrate concentration is increased, then inhibition rate is decreased.	4. Here, no effect of substrate concentration is on the inhibition rate of enzyme.
5. Example is succinate dehydrogenase substrate is inhibited by malonate inhibitor.	5. Example is pyruvate kinase is inhibited by alanine inhibitor.

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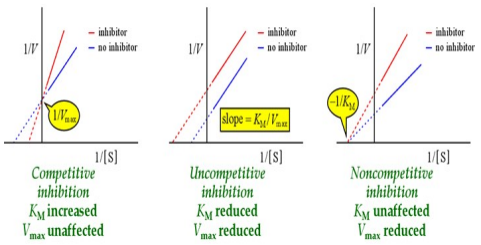
The Lineweaver-Burk plots for inhibition



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- Allosteric inhibition
- ALLOSTERIC INHIBITION
- Allos^{G}= other
- Stereos ^{G}=space or site
- Term ALLOSTERIC SITE has been introduced by Jacob and Monod which denotes a enzyme site different from the active site. Regulator, modulator
- Which noncompetitively binds molecules other than the substrates and may influence enzyme activity.
- A Modulator is a low molecular weight metabolite which when bound to the allosteric site of the enzyme, alters its kinetic characteristics.
- Modulator may be produced at the end of metabolic pathway in which each step is catalysed by an independent enzyme. Ex EMP pathway.A-→E
- A->B->C
-

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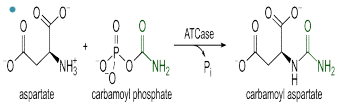
- End Product inhibition or feed back inhibition
- Aspartate transcarbamylase ATCase
-



N-Carbamyl-L-aspartate+Pi

The catalysis by ATCase serves as the rate limiting step in pyrimidine biosynthesis because it alters its catalytic velocity in response to cellular levels of both pyrimidines and purines. The end-product of the pyrimidine pathway, CTP , decreases catalytic velocity, whereas ATP the end-product of the parallel purine pathway, increases catalytic velocity.

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- ATCase is a highly regulated enzyme that catalyses the first committed step in pyrimidine biosynthesis, the condensation of L Aspartate and carbamyl phosphate to form N Carbamyl L aspartate

Aspartate Transcarbamoylase catalyzes the first step in the biosynthesis of pyrimidines (specifically called N-carbamoyl-aspartate) which ultimately yield pyrimidine nucleotides such as CTP (Cytidine Triphosphate).

- The cell must precisely regulate the amount of CTP in the cell because making it can be energetically expensive.
- Therefore, the rate of reaction catalyzed by ATCase is fast at low [CTP] but slows as [CTP] increases. However, CTP is quite different than the active site of ATCase,
- so at high levels it effectively inhibits the enzyme by binding to an allosteric/regulatory site rather than the active site.

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End Product inhibition or feed back inhibition

Product inhibition is a type of enzyme inhibition where the product of an enzyme reaction binds to the enzyme and inhibits its activity.

End-product inhibition (or feedback inhibition) is a form of negative feedback by which metabolic pathways can be controlled

- In end-product inhibition, the final product in a series of reactions inhibits an enzyme from an earlier step in the sequence
- The product binds to an allosteric site and temporarily inactivates the enzyme (via non-competitive inhibition)
- As the enzyme can no longer function, the reaction sequence is halted and the rate of product formation is decreased.

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- End-product inhibition functions to ensure levels of an essential product are always tightly regulated
- If product levels build up, the product inhibits the reaction pathway and hence decreases the rate of further product formation
- If product levels drop, the reaction pathway will proceed unhindered and the rate of product formation will increase.
- Isoleucine is an essential amino acid, meaning it is not synthesised by the body in humans (and hence must be ingested)
- In plants and bacteria, isoleucine may be synthesised from threonine in a five-step reaction pathway
- In the first step of this process, threonine is converted into an intermediate compound by an enzyme (threonine deaminase)

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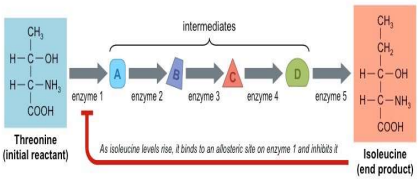
- Isoleucine can bind to an allosteric site on this enzyme and function as a non-competitive inhibitor.
- **<u>Threonine → Isoleucine Pathway</u>**



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- As excess production of isoleucine inhibits further synthesis, it functions as an example of end-product inhibition
- This feedback inhibition ensures that isoleucine production does not cannibalise available stocks of threonine.

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Allosteric enzymes

- You will remember that enzymes are classed as **biological catalysts**.That is, they help to **accelerate the rate** of a reaction, but **remain unchanged** during the entire process.
- Allosteric enzymes are enzymes which have an **additional site**, as well as the active site – it comes from the Greek 'allo', which means 'other'. These are called **allosteric sites**, and enzymes can have more than one.
- They are unique in that they have the ability to **respond to multiple different conditions** in their immediate environment.
- Also, when allosteric enzymes are shown on a graph as velocity against substrate concentration, they show a **sigmoid curve** rather than the usual hyper parabolic curve.
- The image below shows a generic allosteric enzyme.

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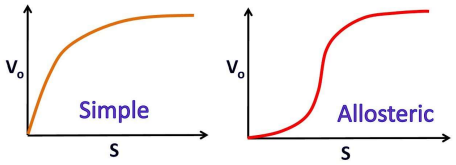
Quickly understand

Simple vs allosteric Enzymes



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Properties of allosteric enzymes

- Allosteric sites are **binding sites** on the enzyme – they are different from the active site and the substrate binding site.
- The molecule that binds to the allosteric site is called an **effector** (it can also be called a modulator), and it **regulates the activity** of the enzyme it binds to.
- The activity of the enzyme is **increased when a positive allosteric effector binds** to the allosteric site.
- This means that the activity of the enzyme is decreased when a **negative allosteric effector** binds to the allosteric site – they **inhibit** the enzyme.
- Allosteric enzymes are **larger and more complex** than non-allosteric enzymes and often have **many sub-units**.

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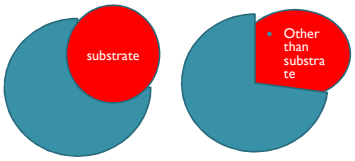
Homotrophic Heterotrophic



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- The **substrate** binding site is on the catalytic subunit – often referred to as the **C subunit**.
- The effector binding site is on the **regulatory subunit** – often referred to as the **R subunit**.
- When an effector molecule at one binding site causes a conformational change in that subunit, a conformational change is then caused in the other subunits in the protein
- this means that a huge portion of the binding energy of the effector is used to **change the conformation of the whole protein complex**.

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- This interaction between all of the subunits can be expressed using the **Hill coefficient** – this is also called a cooperativity coefficient.
- When **n=1**, there will be **no interaction** between the subunits in the enzyme.
- The **larger the Hill coefficient** (cooperativity coefficient), the **stronger the interactions** between all of the subunits in the enzyme.
- Allosteric enzymes can also **'switch'** between their active form and their inactive form.
- When an effector binds to an enzyme, it is called **cooperative binding**.

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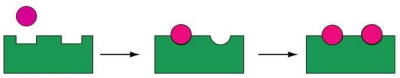
- **Homotropic Regulation**
- A **homotropic allosteric effector** is a substrate for the enzyme, as well as a regulatory molecule – the prefix 'homo' refers to them being the **same**.
- In homotropic enzymes the active site and regulatory site are the same.

  They are usually **activators** of the enzyme.
- The below image shows a homotropic allosteric effector.



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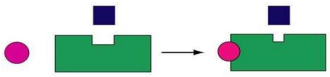
- A good example of a homotropic allosteric effector is **oxygen** (O_2) – it acts as an effector of haemoglobin in the human body.
- **Heterotropic Regulation**
- A **heterotropic allosteric effector** is a regulatory molecule which is **not also the substrate** for the enzyme.
- It can either **activate or inhibit** the enzyme it binds to. The below image shows a heterotropic allosteric effector.



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- A good example of a heterotropic allosteric effector is **carbon dioxide** (CO_2) – it also acts as an effector of haemoglobin but is not the enzyme's substrate.
- **Essential Activators**
- **Essential activators** are allosteric activators that, without which, the **enzyme activity** would be so low it would be **negligible**. For example, N-acetylglutamate is an essential activator for carbamoyl phosphate synthetase I. They are the exact opposite of enzyme inhibitors.
-

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- **The Concerted and Sequential Models for Allosteric Enzymes**
- The two principal models for the behavior of allosteric enzymes are the concerted model and the sequential model.

1. Concerted or symmetry model for allosteric Enz

In 1965, Jacques Monod, Jeffries Wyman, and Jean-Pierre Changeux proposed the **concerted model** for the behavior of allosteric proteins

In this model the protein has two conformations-

1. The active R (relaxed) conformation, which binds substrate tightly.
2. The inactive T (tight, also called taut) conformation, which binds substrate less tightly.

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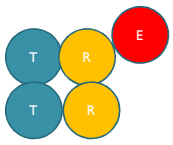

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- The distinguishing feature of this model is that the conformations of *all* subunits change simultaneously.
- Figure shows a hypothetical protein with two subunits.
- Both subunits change conformation from the inactive T conformation to the active R conformation at the same time; that is, a concerted. Symmetry is conserved in this model
- The equilibrium ratio of the T/R forms is called L and is assumed to be high-that is, more enzyme is present in the unbound T form than in the unbound R form.
- The binding of substrate to either form can be described by the dissociation constant of the enzyme and substrate, *K*, with the affinity for substrate higher in the R form than in the T form.

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Sequential model for allosteric ENZYMES

- The name Daniel Koshland is associated with the direct **sequential model** of allosteric behavior.
- The distinguishing feature of this model is that the binding of substrate induces the conformational change from the T form to the R form.
- The type of behaviour postulated by the induced-fit theory of substrate binding.
- A conformational change from T to R in one subunit makes the same conformational change easier in another subunit.
- This is the form in which cooperative binding is expressed in this model .

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Binding of inhibitor to one subunit causes a conformational change such that the T form is even less likely to bind substrate than before.

This conformational change is passed along to other subunits, making them also more likely to bind inhibitor and less likely to bind substrate.

This is an example of cooperative behavior that leads to more inhibition of the enzyme.

Likewise, binding an activator causes a conformational change that favours substrate binding, and this effect is passed from one subunit to another.

This phenomenon, called **negative cooperativity,** has been observed in a few enzymes.

- The concerted model makes no provision for negative cooperativity.

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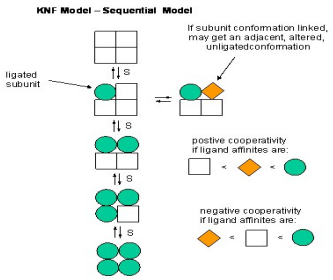

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Isoenzymes

Many enzymes occur in more than one molecular form in the same species, in the same tissues or even in the same cell.

Isoenzymes are a group of enzymes that catalyse the same reaction but have different molecular form

- Isozymes (also known as isoenzymes) are homologous enzymes that catalyze the same reaction but differ in structure.
- The differences in the isozymes allow them to regulate the same reaction at different places in the specie.
- In particular they differ in amino acid sequences.
- They display different kinetic parameters as well as regulatory properties.
- For example, isozymes have different K_M and V_{max} values, and can be distinguished from one another by biochemical properties such as electrophoretic mobility.

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Isoenzymes are wide spread in nature

LDH occur in five different forms in various organs of most vertebrates. It catalyze reversible oxidation reduction reaction.

Lactate+NAD $\longleftrightarrow$ Pyruvate+NADH+H

Two types

- Heart LDH --- HHHH
- Muscle LDH----MMMM

The five "classical" isozymes of Lactate dehydrogenase (LDH) arise from combinations of the two polypeptide chains.

LDH isozymes consist of two genetically distinct polypeptide chains, Subunit A (or M for muscle type) and B (or H for heart type),

Mol.Wt same 35000, but different amino acid sequence.

These polypeptdie chains form varying combinations of tetrameric structures .

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- Hybrid form –3

1.HHHH----H4

2.HHHM -----H3M

3.HHMM----H2M2

4.HMMM----HM3

5.MMMM-----M4

2.Aspartokinase –three forms—*E.coli*

3.Hexokinase

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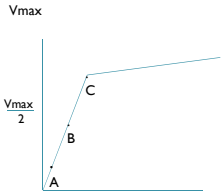

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$$\frac{d[ES]}{dt} = k_1([E] - [ES])[S]$$

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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$$\frac{[S]([E] - [ES])}{[ES]} = \frac{k_2 + k_3}{k_1}$$

By combining constants k_1, k_2 and k_3, we have

$$\frac{k_2 + k_3}{k_1} = K_m \text{ (constant)}$$

By replacing three constants with Km

$$[ES] = \frac{k_2 + k_3}{k_1}$$

By replac

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Since the initial velocity of reaction is v_o,

$$v = k_3[ES]$$

At high substrate concentration t
the velocity reaches V_{max}, we c

$$V_{max} = k_3[E]$$

The value of ES can be substituted fro

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

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

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MM equation

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

A.
S > Km
V depends upon 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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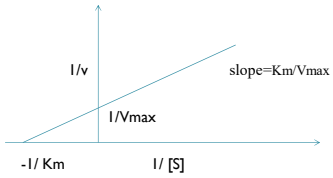

Lineweaver Burk Plot

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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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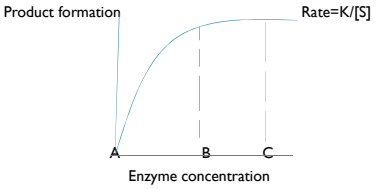

Fig: Effect of Enzyme concentration on rate of reaction

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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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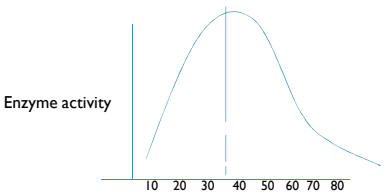
- Effect of temperature



- 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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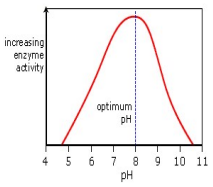

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