ENZYMES

Enzymes
2nd Week

Course Subjects

1) Kinetics of Enzyme Catalyzed Reactions

-Michaelis Menten Kinetics

-Basics and interpretation of Michealis Menten equation

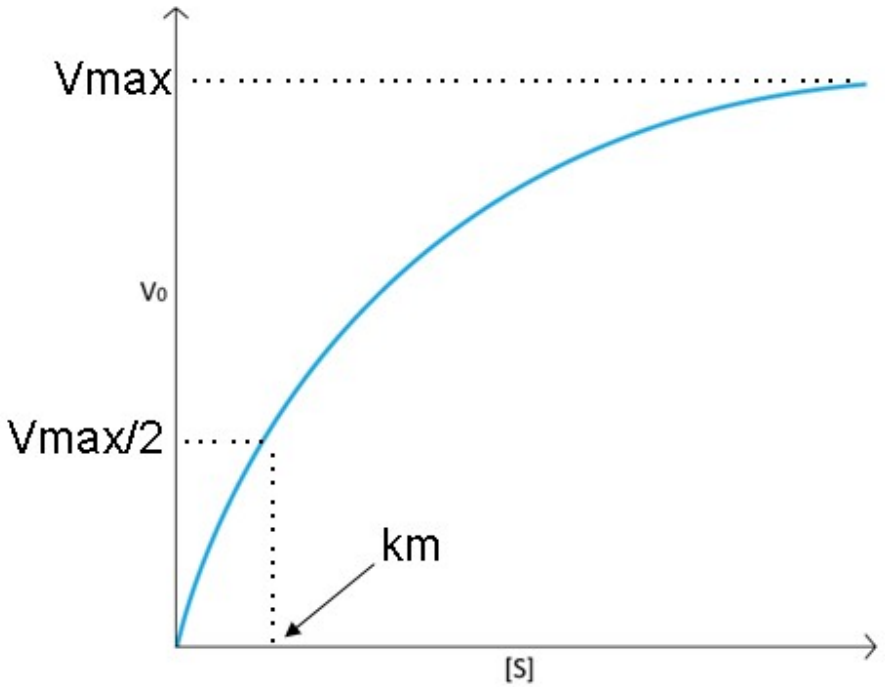
-Physiological meaning of the terms -km and kcat

2) Inhibitors of Enzymes

-Irreversible Inhibitors

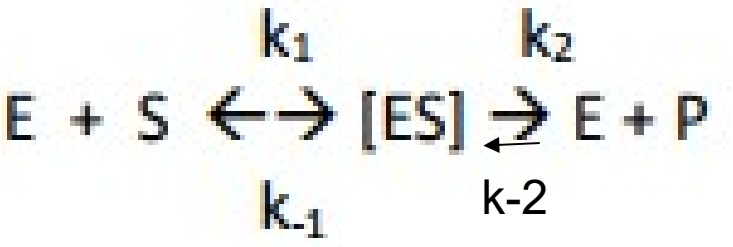
-Reversible Inhibitors

KINETICS OF ENZYME CATALYSED REACTIONS



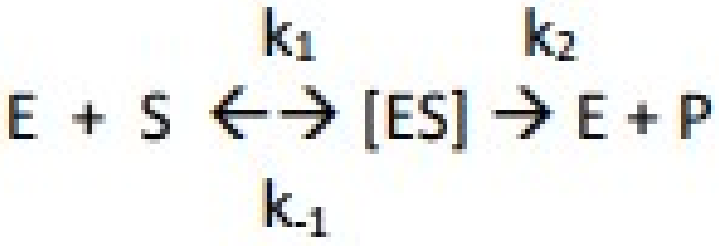
Relationship between reaction velocity and substrate concentration in an enzyme catalysed reaction.

Basics of Michaelis Menten Equation

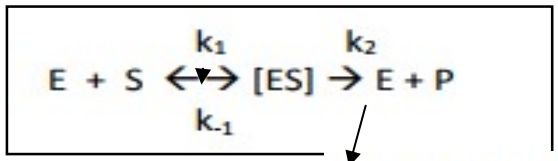


This equation is acquired when the reaction is in equilibrium.

To simplify this equation, assume that in the beginning of the reaction when t=0, very little product is formed and the reverse reaction to ES from P is neglected.



Derivation of Michaelis Menten Equation



1)Velocity equation of the reaction $V_0 = k_2[ES]$

2) Velocity for formation of [ES] $k_1[E][S]$ =

3) Velocity for dissociation of [ES] $k_{-1}[ES] + k_2[ES]$

4) In equilibrium, the formation of [ES] is equal to the dissociation of [ES]

$$k_1[E][S] = k_{-1}[ES] + k_2[ES]$$

5) $[E_T] = [E] + [ES]$

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

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

$$[ES] = \frac{k_1[E_T][S]}{k_{-1} + k_2 + k_1[S]}$$

7) $$[ES] = \frac{[E_T][S]}{\frac{k_{-1} + k_2}{k_1} + [S]}$$

$$[ES] = \frac{[E_T][S]}{K_M + [S]}$$

8) $$V_0 = \frac{k_2[E_T][S]}{K_M + [S]} = \frac{k_{cat}[E_T][S]}{K_M + [S]}$$

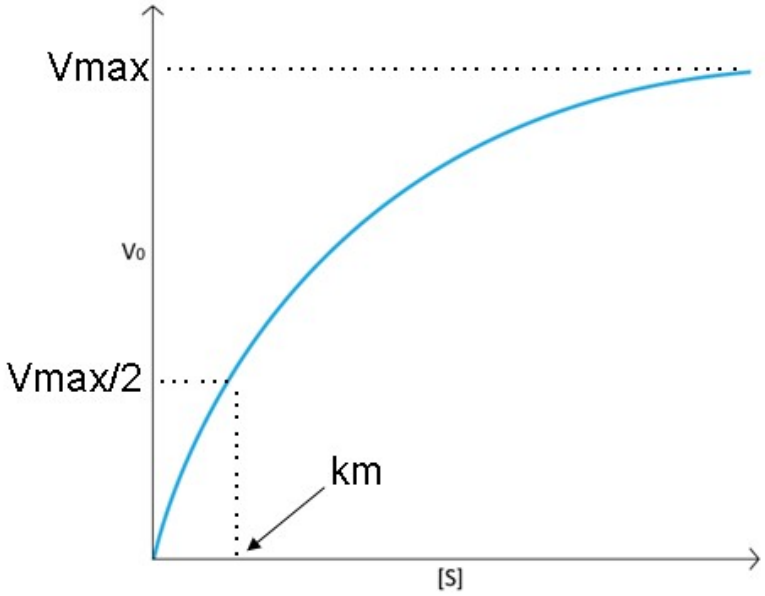
[ES]= k1 [E] [S]/k-1+k2

[ES]= [E] [S]/km

Michaelis Menten Equation:

$$V_0 = \frac{V_{max}[S]}{K_M + [S]}$$

Interpretation of Michaelis Menten Equation



$$V_0 = \frac{V_{max}[S]}{K_M + [S]}$$

1) When Km=[S] V0=Vmax/2

2) When Km>>>>> [S] V0=Vmax. [S] /Km

Reaction is first order and directionally proportional to substrate concentration.

3) When Km< < < < [S] V0=Vmax

When substrate concentration is very high, reaction is zero order.

Physiological meaning of Km

-Km is known as Michaelis Menten constant

-Km can be effected by temperature or pH.

Km has two main definitions:

1)

$$V_0 = \frac{V_{max}[S]}{K_M + [S]}$$

When V=Vmax/2→Km=[S]

2)

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

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

When k-1>>>>k2

km= k-1/k1

km defines the dissociation tendency of enzyme from its substrate.

kcat (Turnover number)

V0=k2[ES]

[ES] =[E]total

Vmax=k2. [E]total

k2, which is also known as kcat defines the turnover number of an enzyme.

Turnover number of an enzyme is the number of substrate that is converted to product by one active site of an enzyme in a unit of time.

Example: If the Vmax of an enzyme at [Et]=0.2 M is 400000 M/second what is the kcat value for this enzyme?

Derivation of Michaelis Mente Equation

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

$$[ES] = \frac{k_1[E_T][S]}{k_{-1} + k_2 + k_1[S]}$$

7) $$[ES] = \frac{[E_T][S]}{\frac{k_{-1} + k_2}{k_1} + [S]}$$

$$[ES] = \frac{[E_T][S]}{K_M + [S]}$$

$V_o = k_{cat}[ES]$

$V_o = \frac{k_{cat}}{K_M} [E]_{Total}[S]$

[ES]= k1 [E] [S]/k-1+k2

[ES]= [E] [S]/km

8) $$V_0 = \frac{k_2[E_T][S]}{K_M + [S]} = \frac{k_{cat}[E_T][S]}{K_M + [S]}$$

Michaelis Menten Equation:

$$V_0 = \frac{V_{max}[S]}{K_M + [S]}$$

Velocity of an enzyme catalyzed reaction depends on….

$V_0 = k_{cat}[ES]$

$[ES] = [E]\,[S]/km$

$V_0 = k_{cat}/km.\ [E]_{total}\,[S]$

!!!!! kcat/km defines the catalytical eficiency of an enzyme

1) Total enzyme concentration
2) Substrat concentration
3) kcat/Km constant

Physiological meaning of km-Example

Hexokinase→→ Low Km (0.1 mM) Enables to start glucolysis even if blood glucose is relatively low.

Glucokinase→→ High Km (10 mM) This enzyme is most active when blood glucose is high after a carbohydrate rich meal.

Lineweaver Burk Equation-Graph

$$V_0 = \frac{V_{max}[S]}{K_M + [S]}$$

Double reciprocal of Michaelis Menten equation gives the Lineweaver Burk plot.

$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$$

ENZYME INHIBITION

•Reduction or complete elimination of in vivo and/or in vitro activities of enzymes by various compounds is called inhibition.

•The compounds that cause this inhibitory activity are called inhibitors. Inhibitors can be small molecular weight compounds or ions.

• Inhibition of enzymatic activity is very important as it establishes a control mechanism in biological systems.

• Many drugs and toxic compounds perform their functions in this way.

• It also has benefits for the investigation of mechanisms of enzyme activity.

Inhibition of enzymes:

1) Irreversible Inhibition

2) Reversible Inhibition

- Competetive Inhibition
- Uncompetetive Inhibition
- Non competetive Inhibition

1) Irreversible Inhibition

The irreversible inhibitor binds to the enzyme either covalently or forms a complex that is difficult to dissociate.

Examples:

- Sit c oxidase inh. (Cyanide)
- Protease inh. (Mercuri benzoate)
- Choline esterase inh (Diisopropyl fluorophosphate)
- Sistein peptidase inh.Glycolysis inh. (Iodoacetate)

Irreversible inhibition-Examples (Drugs)

Various drugs exhibit their effects by irreveresible inhibition.

- Penicillin → → → Inhibition of glycopeptide transpeptidase

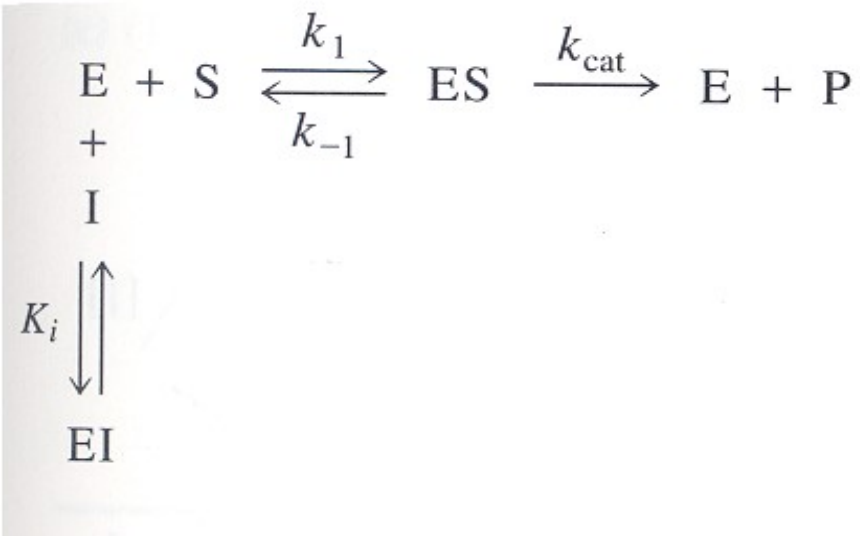
- Aspirin → → → COX inhibition

2) Reversible Inhibitions:

1. Competetive Inhibition
2. Uncompetetive Inhibition
3. Non-competetive Inhibition

1. Competetive Inhibition

- This kind of inhibition is made by substances which are structurally similar in structure to the substrate.
- The inhibitor binds to the active site to form the enzyme-inhibitor complex.
- Such inhibition is called competitive inhibition because these inhibitors compete with substrate molecules.



Competetive inhibition-examples

- The succinate dehydrogenase enzyme, which acts in the TCA cycle, is also competitively inhibited by malonate.

- In methanol poisoning, ethanol is used as a competetive inhibitor of methanol as a treatment.

- Methotrexate has a similar structure to folic acid and it inhibits nucleotide synthesis by inhibiting dihydrofolate reductase and used as a chemotherapeutical agent.

- The sulfonylamide group antibiotics act as competitive inhibitors because they are very similar to the p-amino benzoic acid structure and inhibit bacterial proliferation.

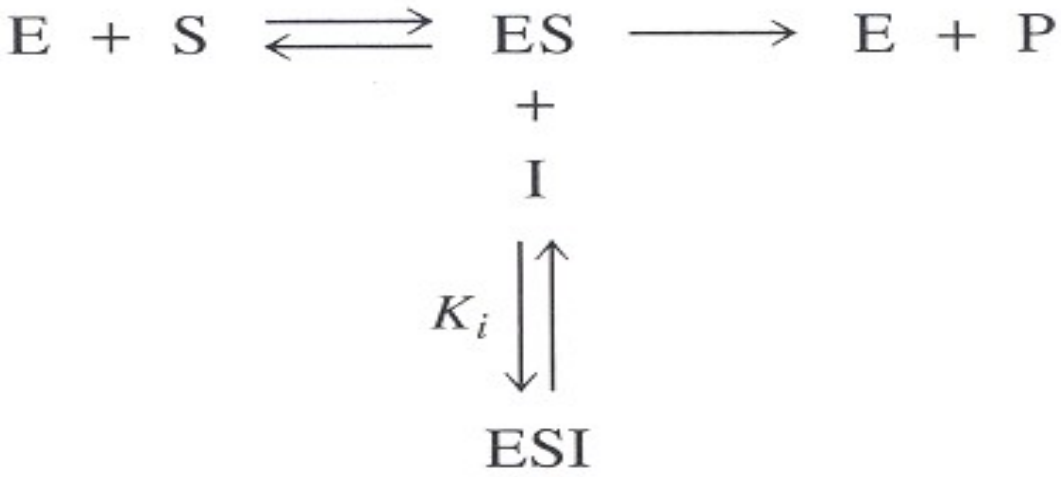
Effects of Competetive inhibition on reaction kinetics

In a competitive inhibition,

- **Km increases**.
- **Vmax** does **not change**.

2. Uncompetetive Inhibition

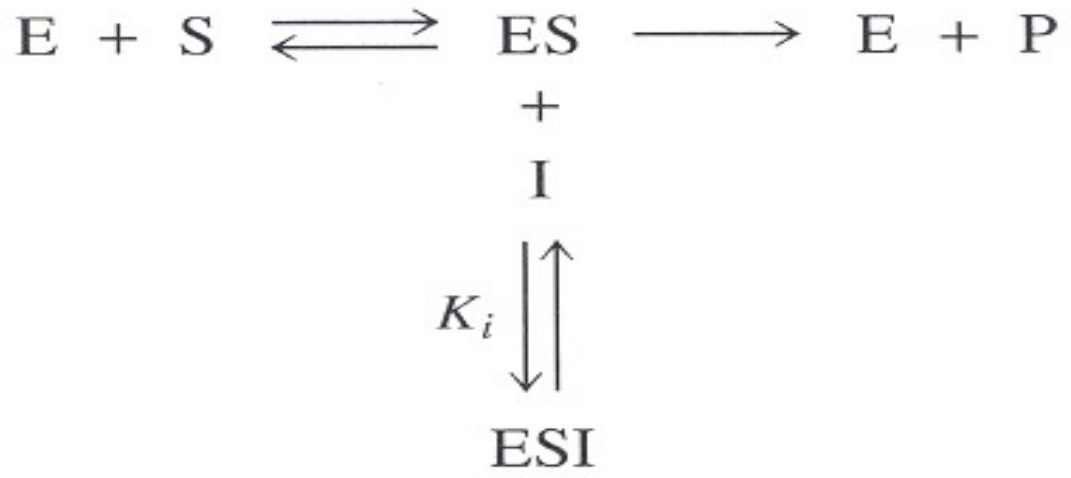
An uncompetetive inhibitor binds to a region other than the active site and only binds to the enzyme substrate complex.



Effects of Uncompetetive inhibition on reaction kinetics

- As the ES complex moves away from the reaction, the value of **Vmax decreases.**

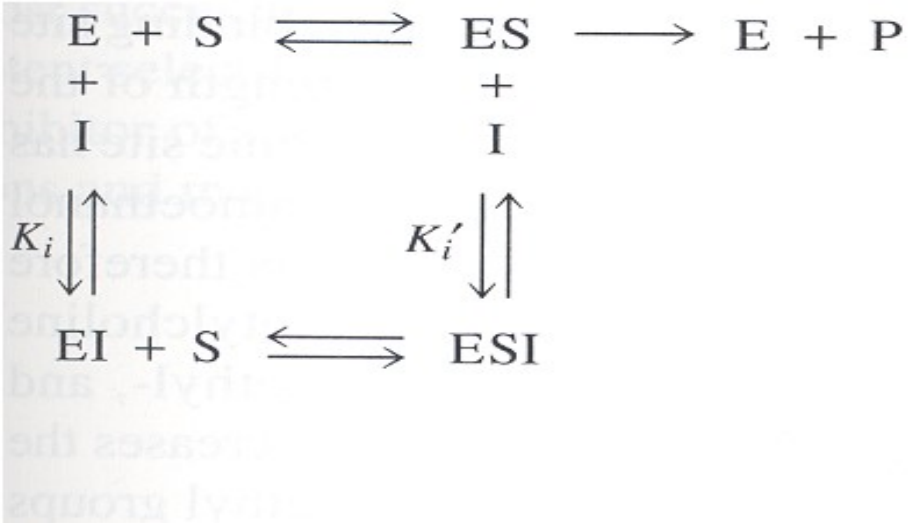
- Substrate binding to the enzyme acts as a stimulant for inhibitor binding, so the value of the **km decreases.**



3. Non-competetive Inhibition

•If an inhibitor binds to a region other than the active site of the enzyme and results in inhibition, this type of inhibition is called the noncompetitive inhibition. These inhibitors can bind to the free enzyme or ES complex.

► Such an inhibitor exhibits its effect by reducing the turnover number of an enzyme, i.e. its catalytic activity. Some of the noncompetitive inhibitions are reversible if they are part of the reversal !!!.



Non-competetive inhibition-examples

- The activity of enzymes that require catalytic metal ions can be inhibited noncompetitively by binding certain compounds to these metal ions. For example, CN- can inhibit iron-containing enzymes, while EDTA can inhibit enzymes that use Mg + 2 ions.

Effects of Non-Competetive inhibition on reaction kinetics

- In noncompetetive inhibition, the Lineweaver-Burk plot decreases in the y-axis, that is, **Vmax decreases**.
- In contrast to Vmax, **Km** is **not affected** by competing inhibition.
- This inhibition can not be prevented by increasing the substrate concentration.

Inhibitor can bind to the free enzyme or enzyme substrate complex and therefore the affinity of the enzyme to its substrate does not change.