ENZYMES - II

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Enzyme kinetics

Enzyme kinetics

- is the field of biochemistry concerned with the quantitative measurement of the rates of enzyme-catalyzed reactions and the study of factors that affect these rates.

A complete, balanced set of enzyme activities is of fundamental importance for maintaining homeostasis.

An understanding of enzyme kinetics thus is important to understanding how physiologic stresses such as anoxia, metabolic acidosis or alkalosis, toxins, and pharmacologic agents affect that balance. The involvement of enzymes in virtually all physiologic processes makes them the targets of choice for drugs that cure or ameliorate human disease.

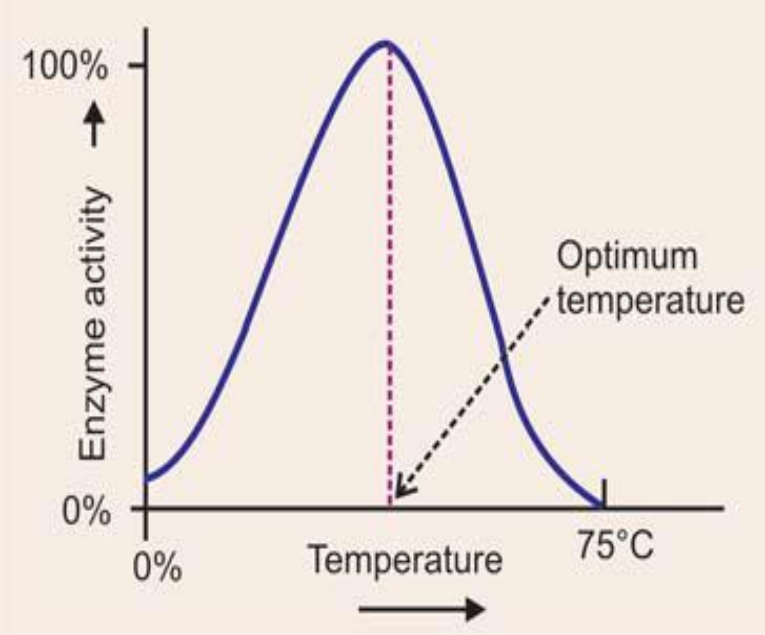
The factors that affect the rates of enzyme catalyzed reactions

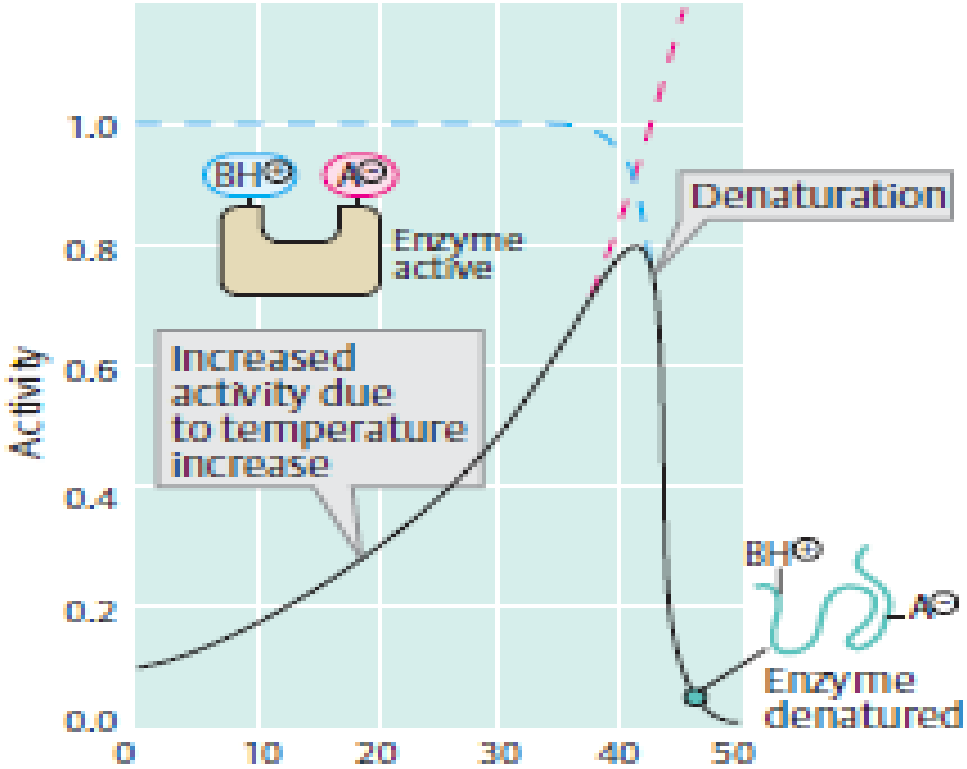
Multiple **factors** affect the rates of enzyme catalyzed reactions

These factors include:

- physical quantities (**temperature**)
- the chemical properties of the solution (**pH** value, **ionic strength**)
- the concentrations of the relevant
  - **substrates**,
  - **enzyme,**
  - **cofactors**,
  - **inhibitors**

Temperature

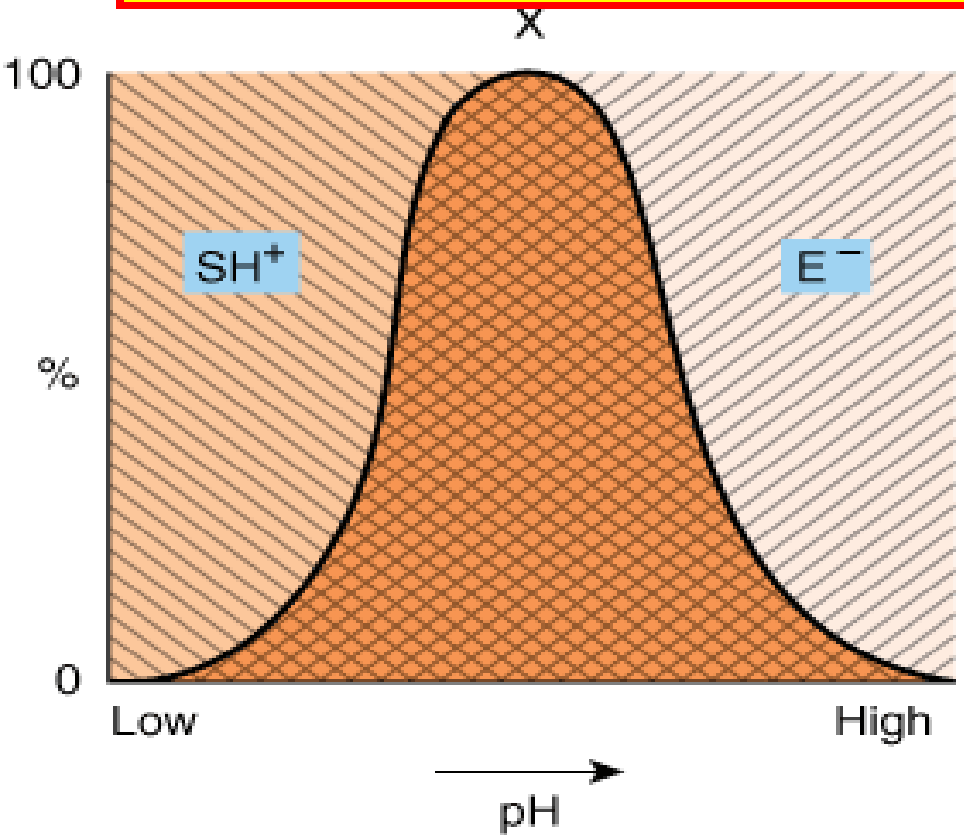


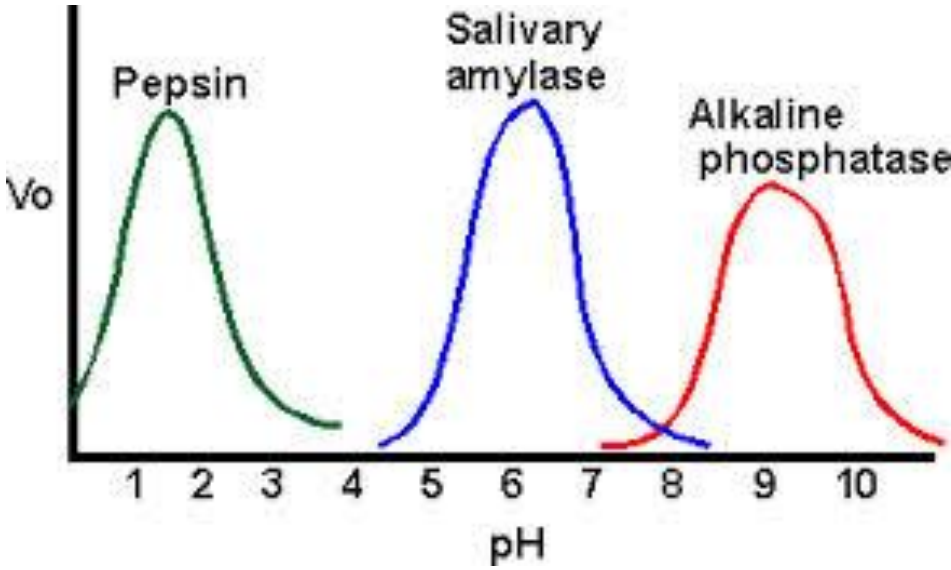


With increasing **temperature**, the increased thermal movement of the molecules initially leads to a rate acceleration.

At a certain temperature, the enzyme then becomes unstable,and its activity is lost as a result of **denaturation**.

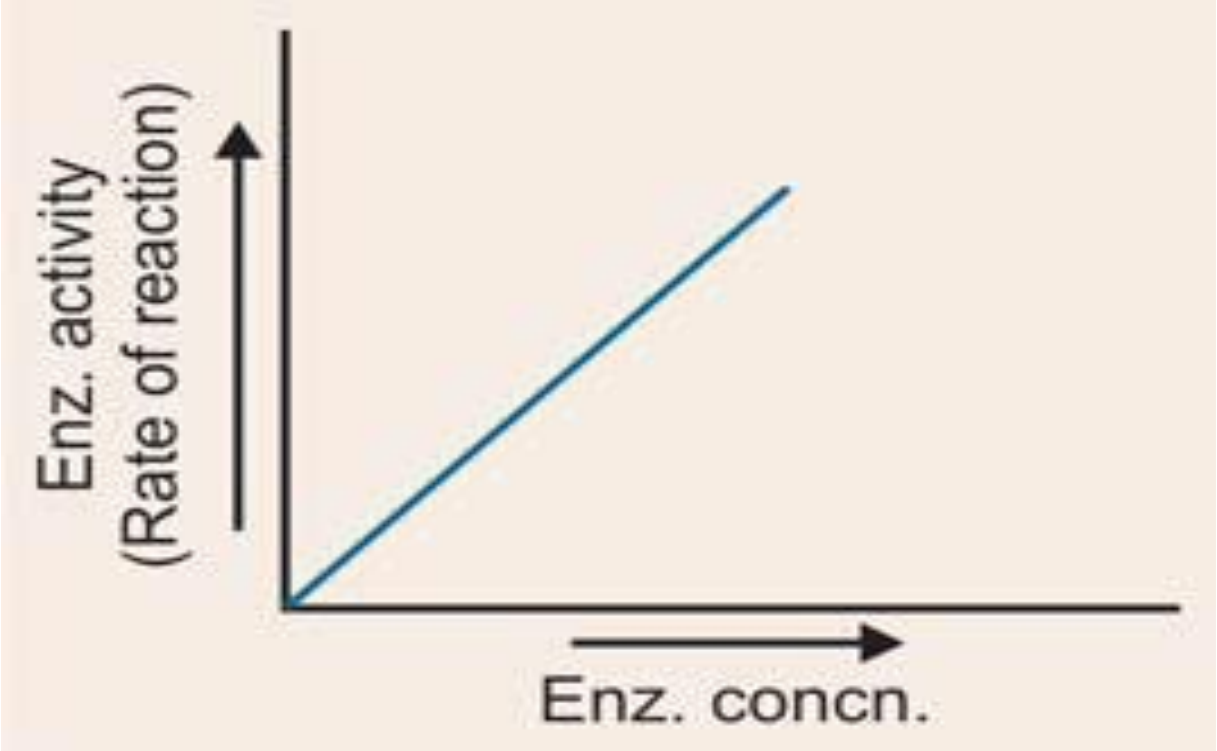
Hydrogen Ion Concentration (pH)





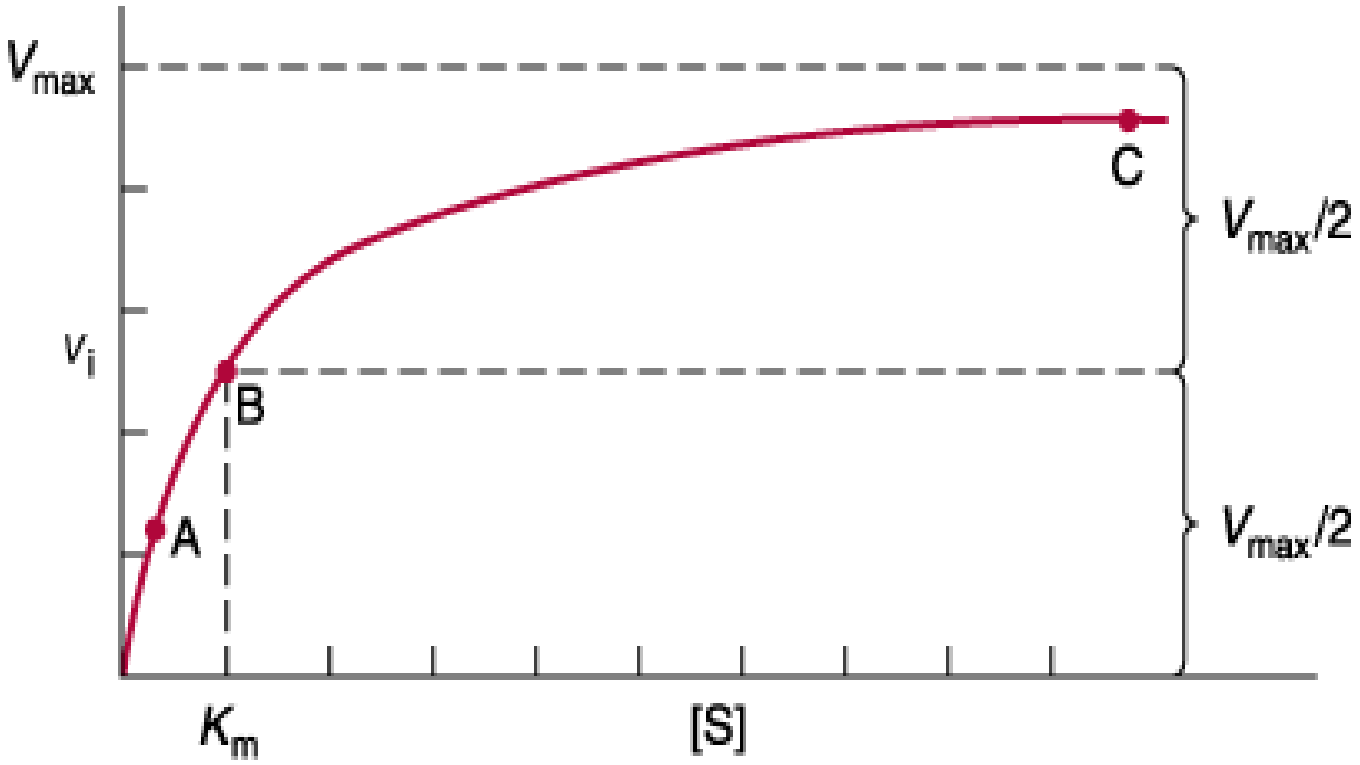
Changes in **pH** affect the **ionic charge** of amino acid side chains of enzymes, and can have a dramatic effect on enzymatic activity.

ENZYME CONCENTRATION AFFECTS THE REACTION RATE



Enzyme activity rate increases in proportion to the amount of enzyme

SUBSTRATE CONCENTRATION AFFECTS THE REACTION RATE



Enzyme Kinetics Equation

L. Michaelis and M. Menten have proposed a hypothesis for enzyme action, which is most acceptable (1913).

According to their hypothesis,

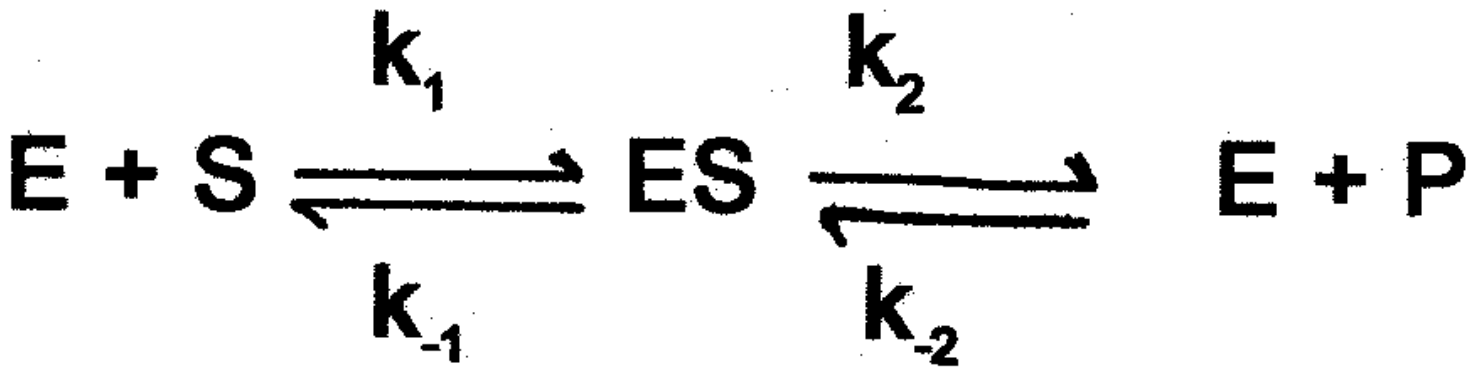
the enzyme molecule (E)

first combines with a substrate molecule (S) to form an enzyme/substrate (ES) complex

which further dissociates to form product (P) and enzyme (E) back.

$$\mathrm{E} + \mathrm{S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}$$

Enzyme Kinetics Equation



S = substrate P = product

E = enzyme

ES = enzyme-substrate complex

k_1, k_{-1}, k_2, k_{-2} are rate constants

The **Michaelis–Menten equation** illustrates in mathematical terms the relationship between initial reaction velocity V_i and substrate concentration [***S***].

It is named after German biochemist

Leonor Michaelis and

Canadian physician **Maud Menten**

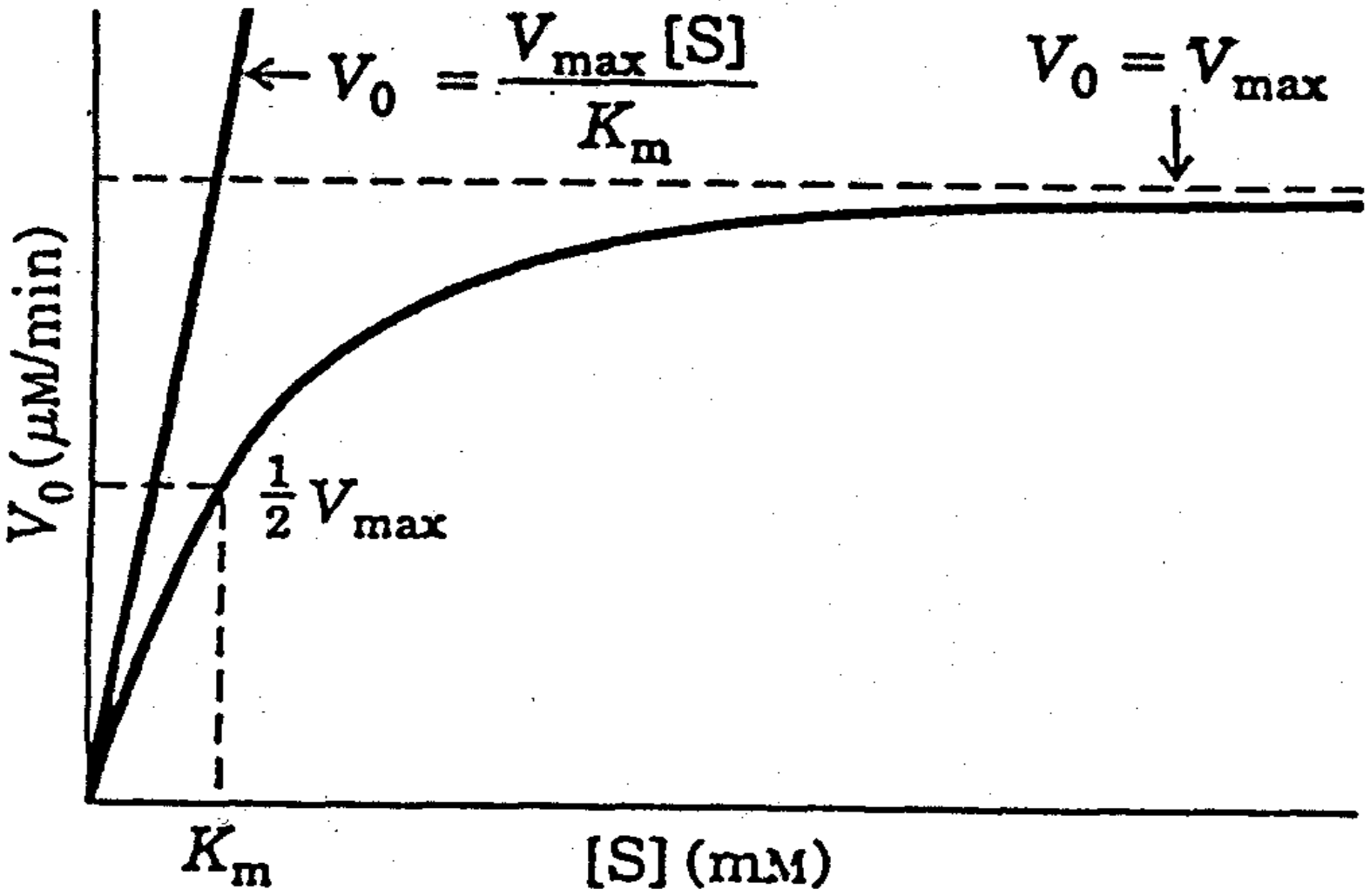
in 1913.

Michaelis-Menten Equation

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

This equation is called the **Michaelis–Menten equation**.
Here, V_{max} represents the **maximum rate** achieved by the system, at saturating substrate concentration.
The **Michaelis constant - K_M** is the substrate concentration at which the reaction rate is **half of V_{max}**

Michaelis-Menten Curve



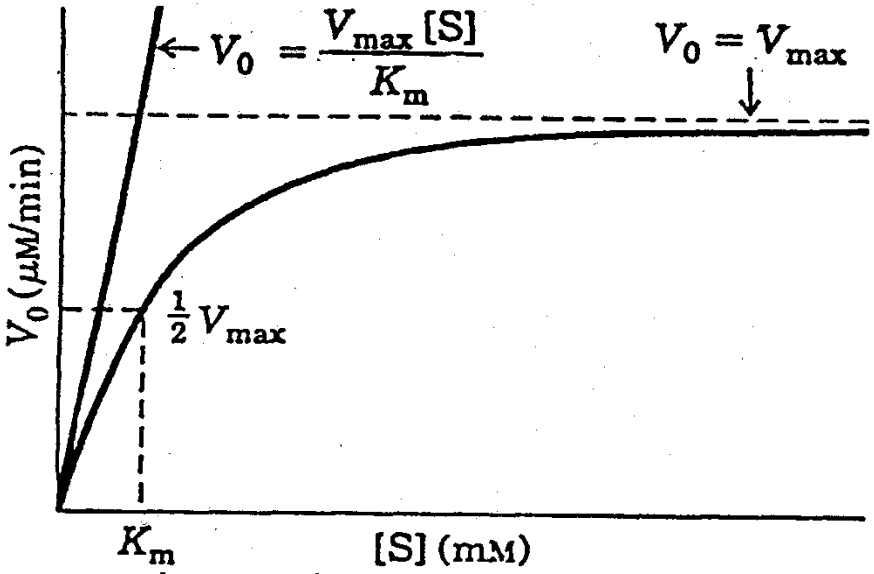
Initial velocity (V_i) and [S]

Studying the effects of [S] on the velocity of a reaction is complicated by the reversibility of enzyme reactions. To overcome this problem, the initial velocity (V_i) measurements are used.

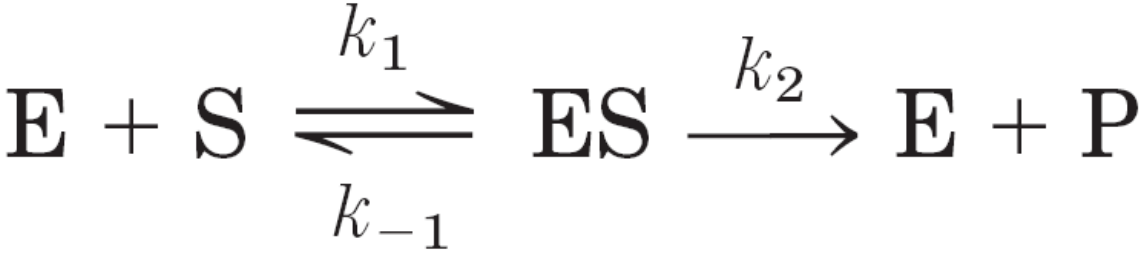
At the start of a reaction, [S] is in large excess of [P], thus the initial velocity of the reaction will be dependent on substrate concentration

Initial velocity (V_i) and [S] (cont)

When initial velocity is plotted against [S], a hyperbolic curve results, where V_{max} represents the maximum reaction velocity. At this point in the reaction, if [S] >> [E], all available enzyme is "saturated" with bound substrate, meaning only the ES complex is present.



The saturation effect is believed to reflect the fact that all the enzyme binding sites are occupied with substrate.

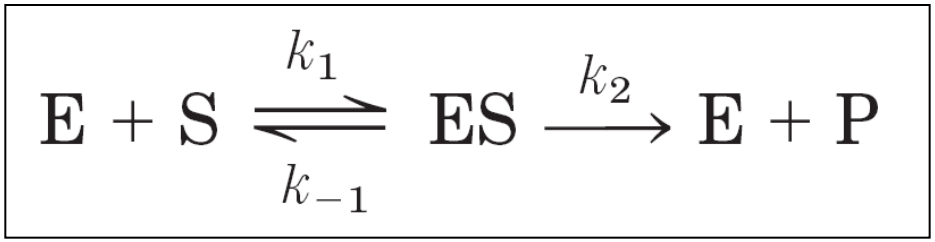


$$V_i = k_2[ES]$$

$[E_t]$ – the **overall level** of an enzyme involved in the reaction at the moment.

The concentration of the remaining **free** enzyme:

$[E_t]$ - $[ES]$ - the concentration of **free** enzyme .



(Formation) $[ES] = k_1([E_t] - [ES])[S]$

(Dissociation) $[ES] = k_{-1}[ES] + k_2[ES]$

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

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

open brackets - ()

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

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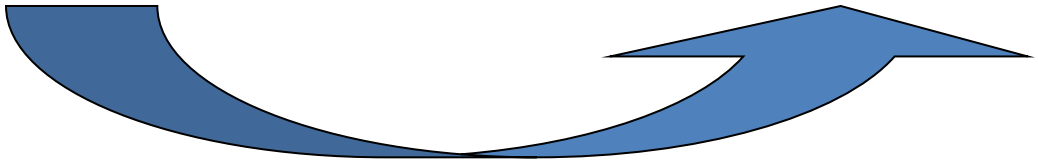


$$k_1[E_t][S] = k_1[ES][S] + k_{-1}[ES] + k_2[ES]$$

put out of brackets [**ES**]

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

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

solve the equation in [ES]:

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

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

divide the denominator by K_1

$$[\mathrm{ES}] = \frac{[\mathrm{E_t}][\mathrm{S}]}{[\mathrm{S}] + (k_2 + k_{-1})/k_1}$$

The **ratio** of the sum of the rate of decay constants to a constant rate of synthesis

$$\frac{(k_2 + k_{-1})}{k_1}$$

named **Michaelis constant** - K_m

$$[\mathrm{ES}] = \frac{[\mathrm{E_t}][\mathrm{S}]}{K_\mathrm{m} + [\mathrm{S}]}$$

$$V_i = k_2[ES]$$

$$V_0 = \frac{k_2[E_t][S]}{K_m + [S]}$$

$[ES] = [E_t]$

$V_{max} = k_2[E_t]$

Michaelis-Menten Equation

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

Meaning of K_m

An important relationship that can be derived from the Michaelis-Menten equation is the following:

if V_i **is equal to 1/2** V_{max}**, then** the relation

$$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]}$$

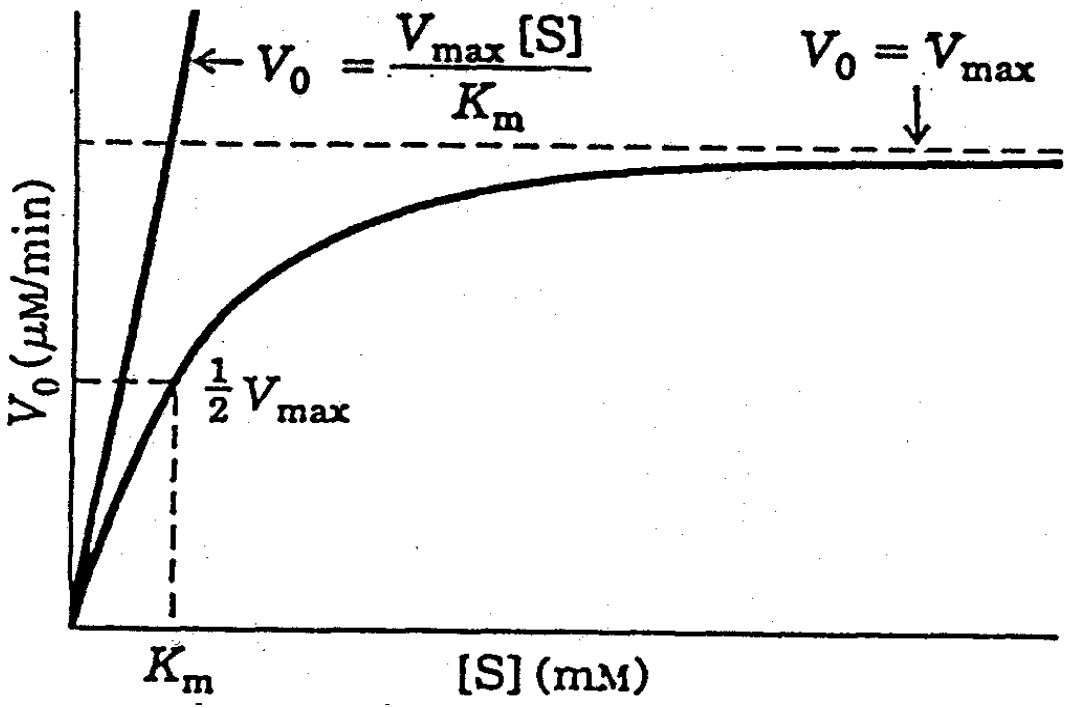
can be simplied to

$$K_m + [S] = 2[S], \text{ or}$$

$$K_m = [S].$$

This means that at ½ of the maximal velocity, the substrate concentration will be equal to the K_m.

The Michaelis constant K_m is the substrate concentration at which V_i is half the maximal velocity ($V_{max}/2$)



Uses of K_m

Experimentally, K_m is a useful parameter for characterizing the number and/or types of substrates that a particular enzyme will utilize.

It is also useful **for comparing** similar enzymes from different tissues or different organisms.

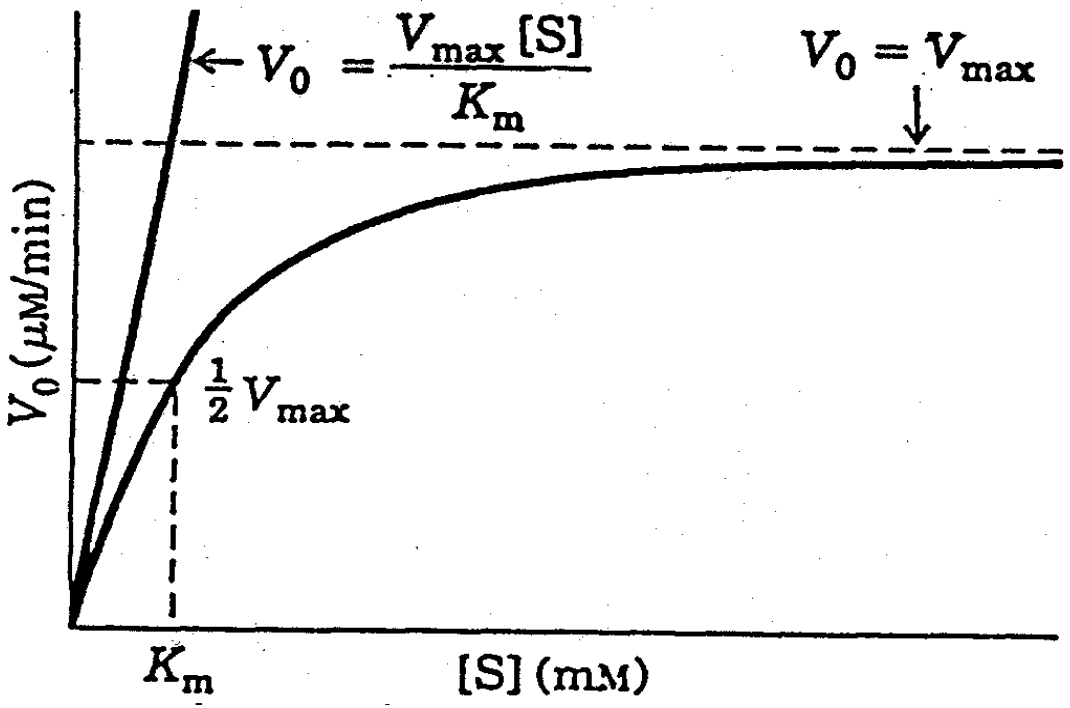
Clinically, K_m comparisons are useful for evaluating the effects mutations have on protein function for some **inherited genetic diseases**.

Meaning of V_{max}

The values of V_{max} will vary widely for different enzymes and can be used as an **indicator** of an enzymes catalytic efficiency.

It does not find much clinical use.

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



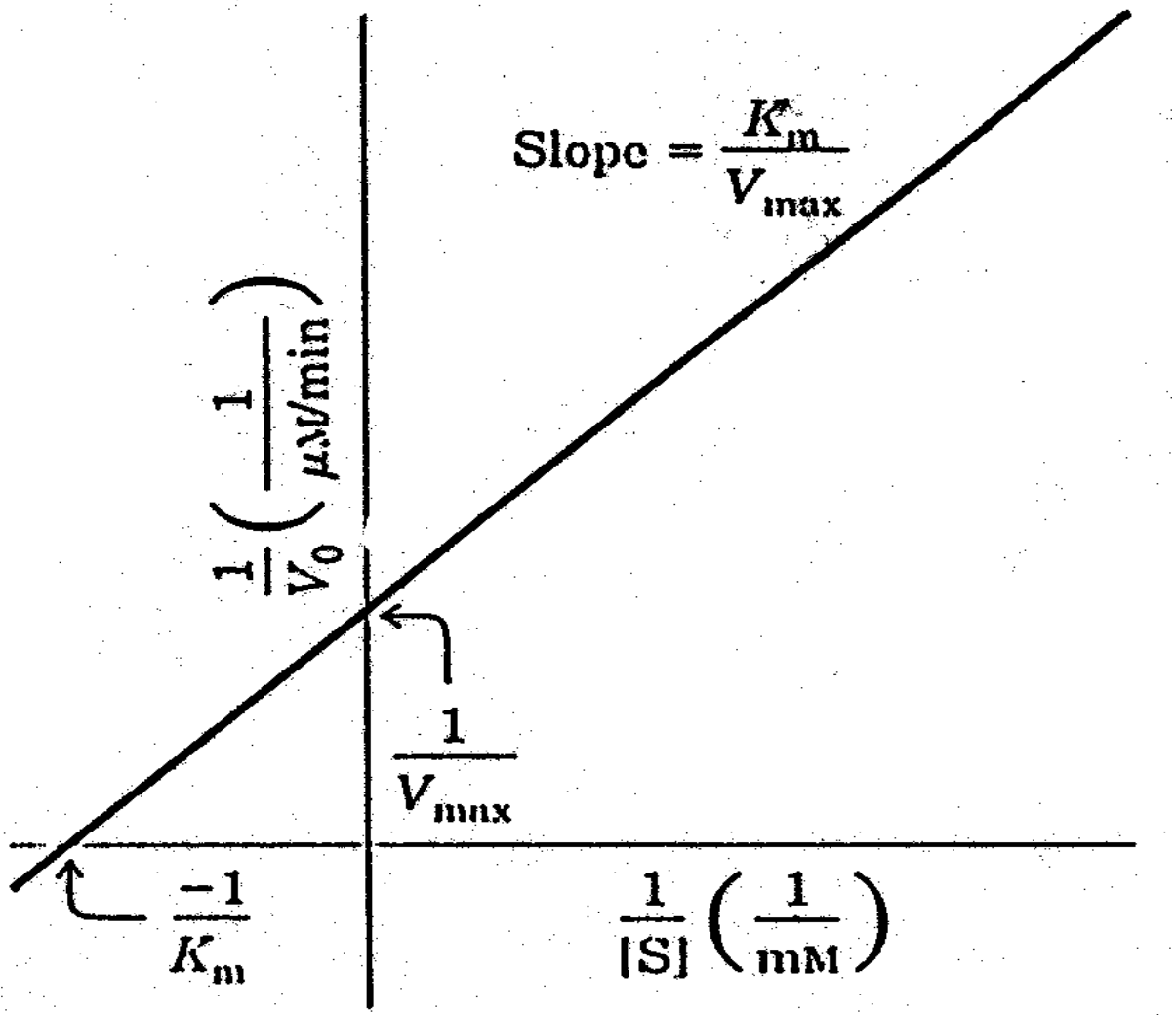
Lineweaver-Burk
(double reciprocal plot)

- If the reciprocal (1/X) of the Michaelis-Menten equation is done, after algebraic simplification the following equation results:

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

- This relation is written in the format of the equation for a **straight line**, (y = **m**x + b), where **y** = $1/v_o$, **m** (slope) = K_m/V_{max}, **x** = 1/[S]. When this relation is plotted, the result is a **straight line graph**

Lineweaver-Burk
(double reciprocal plot)



Uses of double reciprocal plot

The **x** intercept value is equal to $-1/K_m$.
The biggest advantage to using the double reciprocal plot is a more accurate determination of V_{max}, and hence K_m.

It is also useful in characterizing the effects of enzyme **inhibitors** and distinguishing between different enzyme mechanisms.

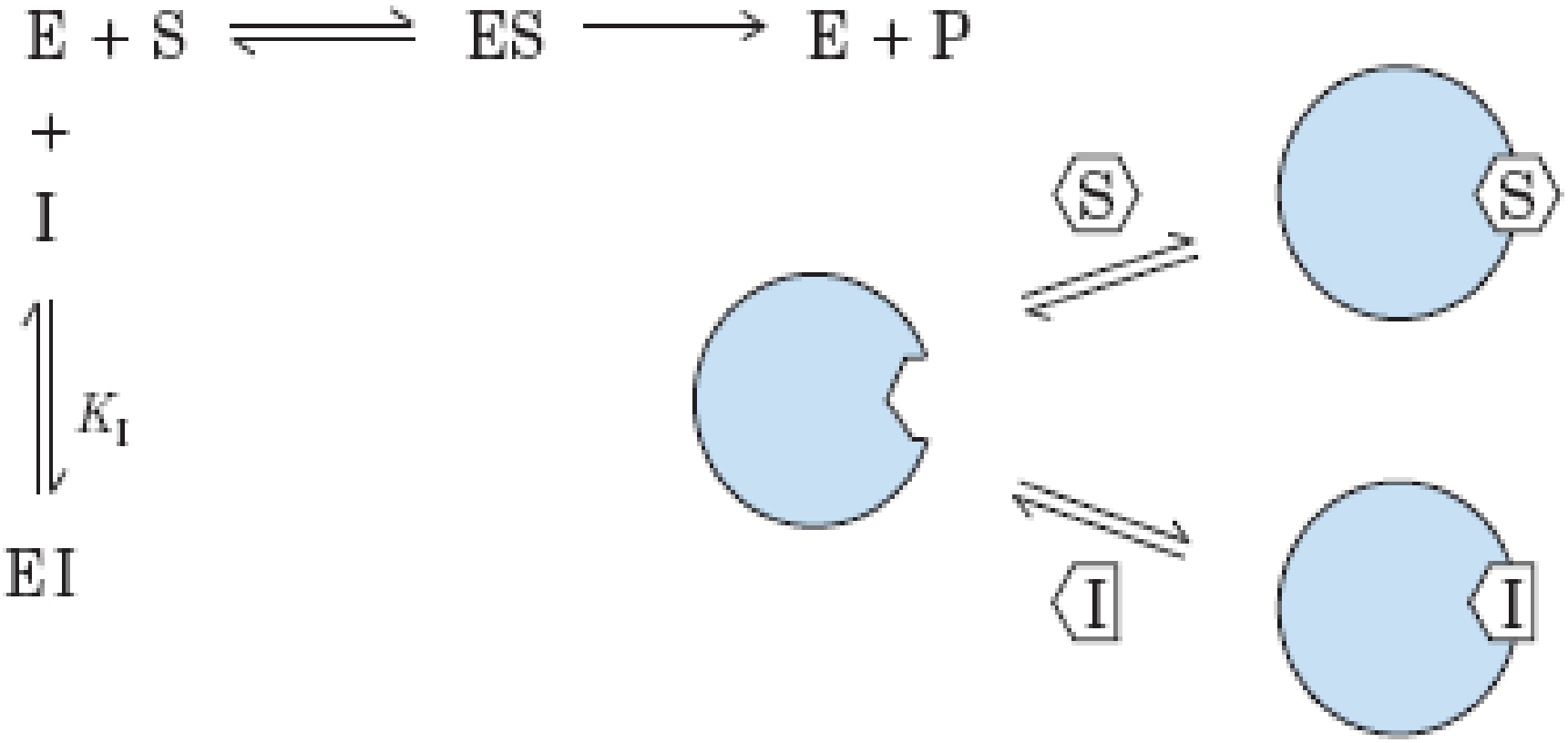
Enzyme inhibitor

Enzyme **inhibitor** types

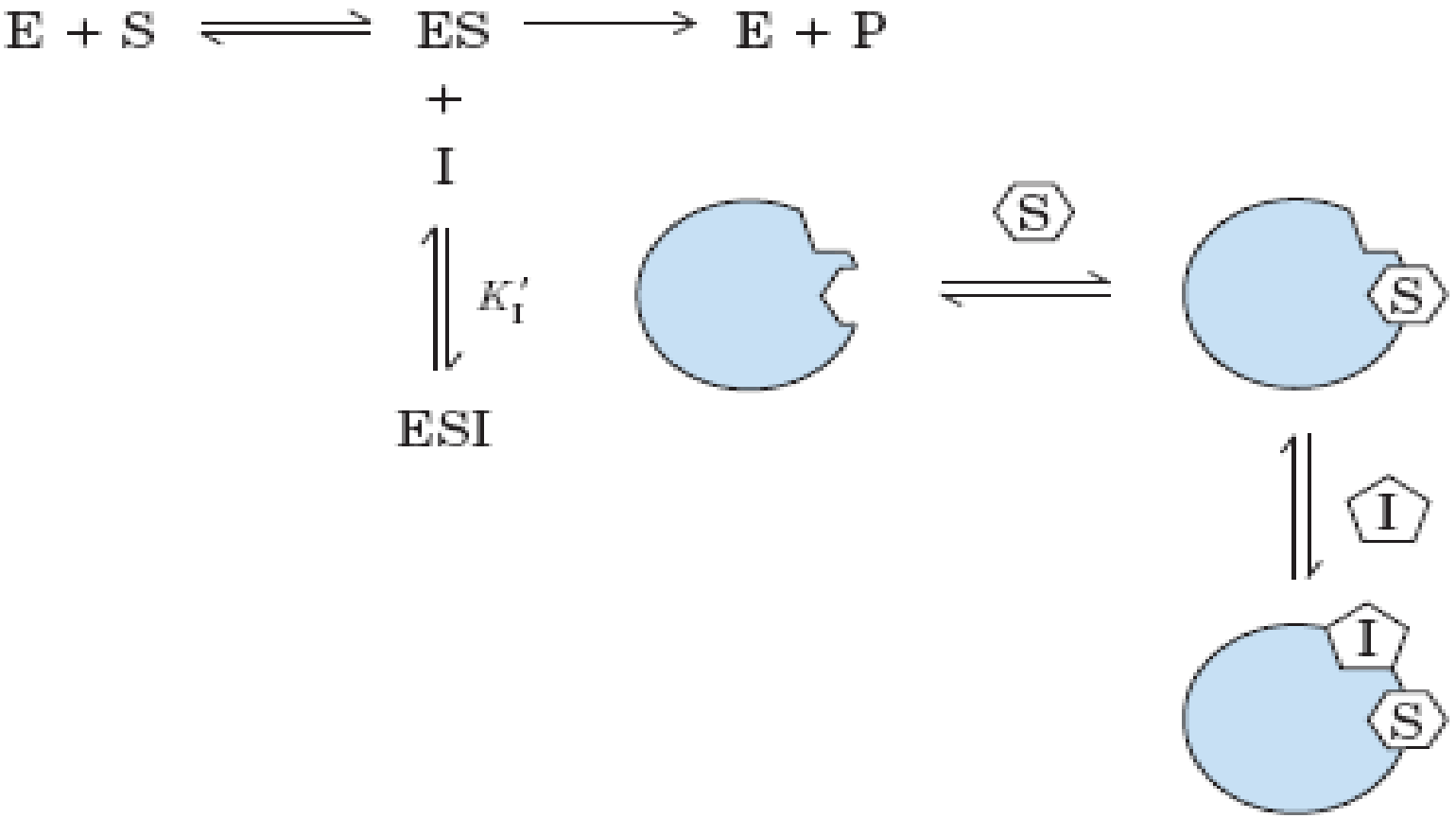
The modes and types of **inhibitors** have been classified by their kinetic activities and sites of actions. These include:

- **Irreversible Inhibitors**
- **Reversible**
  - **Competitive Inhibitors,**
  - **Non-Competitive Inhibitors,**

Competitive Inhibition



Non-Competitive Inhibition



Uses of K_i

- **K_i values** are used to characterize and compare the effectiveness of **inhibitors** relative to **K_m**. This parameter is especially useful and important in evaluating the potential therapeutic value of **inhibitors** (**drugs**) of a given enzyme reaction.

In general, the lower the K_i value, the tighter the binding, and hence the more effective an inhibitor is.

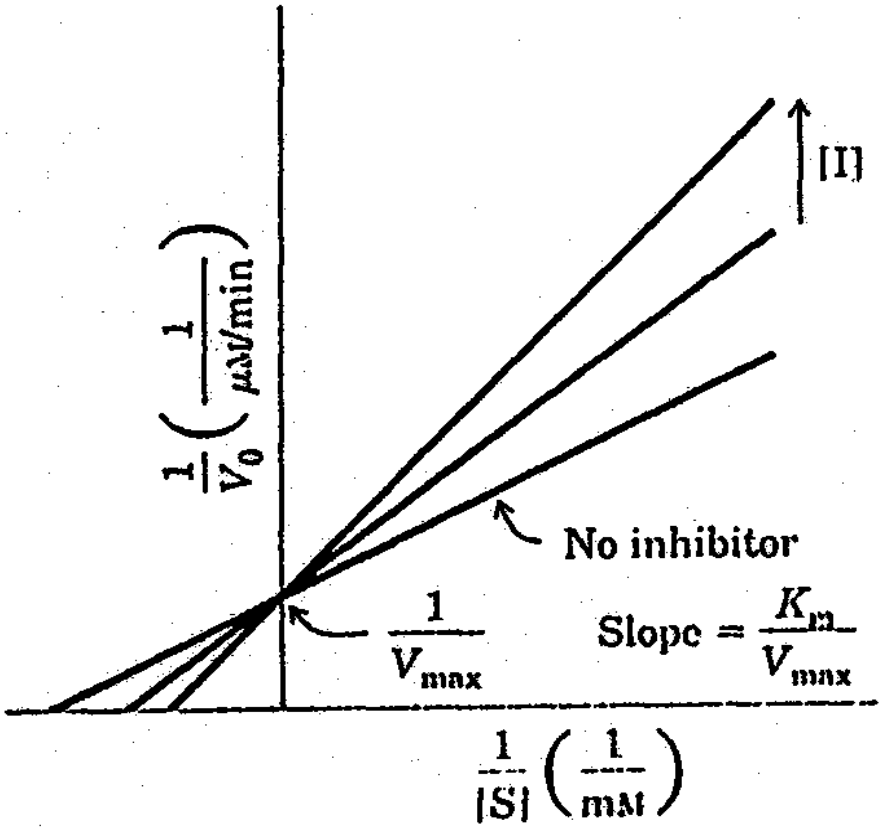
Reversible competitive inhibition

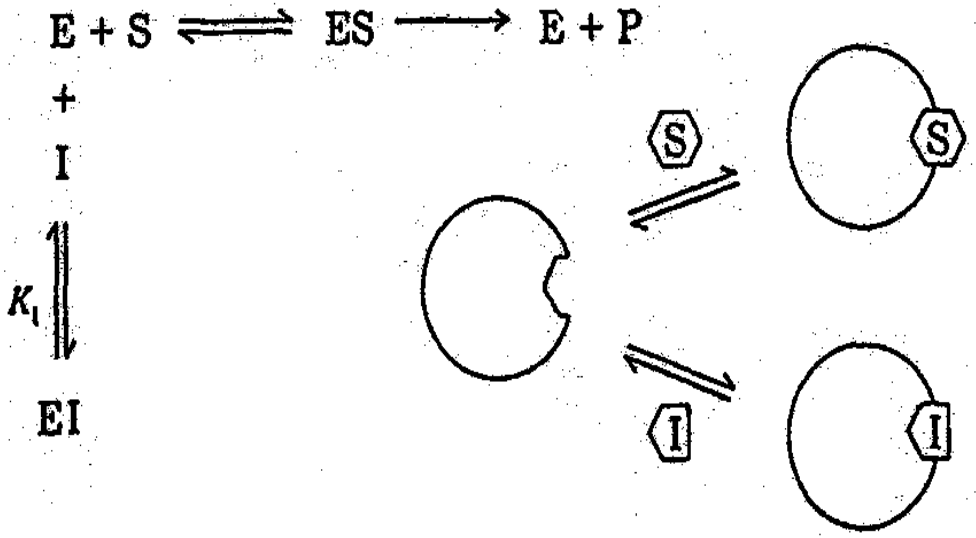
Competitive inhibitors compete with the substrate for binding at the active site.

In the double reciprocal plot for a **competitive inhibitor** acting at the substrate site for the following reasons, notice with increasing concentration of **inhibitor**, the V_{max} **does not change; however, the** K_m **of the substrate is increased**.

This also reflects the reversible nature of the inhibitor; there is always some concentration of substrate which can displace the **inhibitor**.

Competitive Inhibition





V_{max} - no change

K_m - INCREASES - indicates a direct interaction of the inhibitor in the active site

Non-Competitive Inhibition

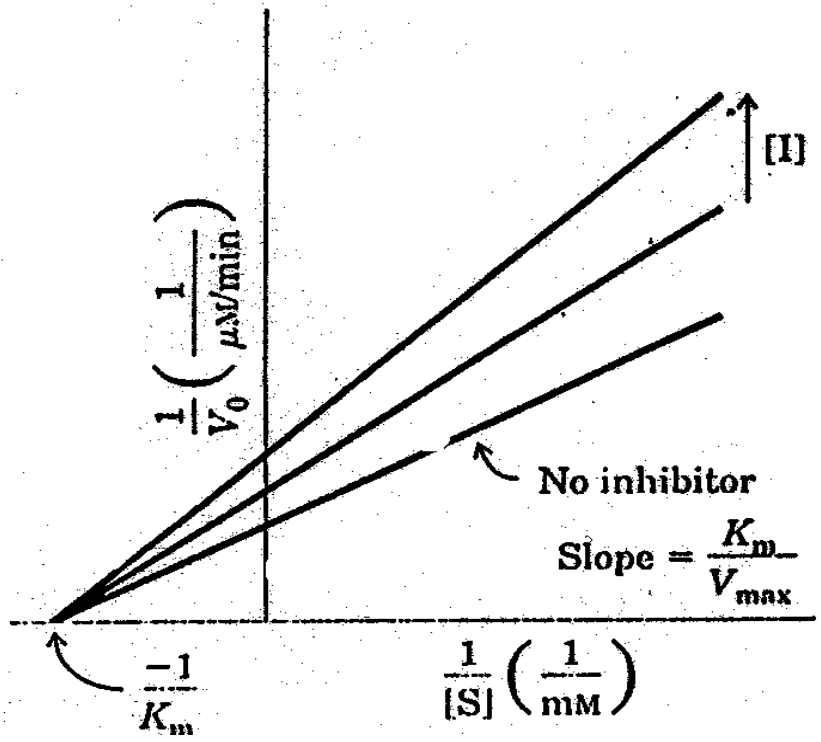
Non-competitive inhibitors combine with both the enzyme (E + I) and the enzyme-substrate (EI + S) complex. The **inhibitor** binds to a site other that the substrate site, and is thus independent of the presence or absence of **substrate**. This action results in a **conformational change** in the protein that affects a catalytic step and hence decreases or eliminates enzyme activity (formation of P).

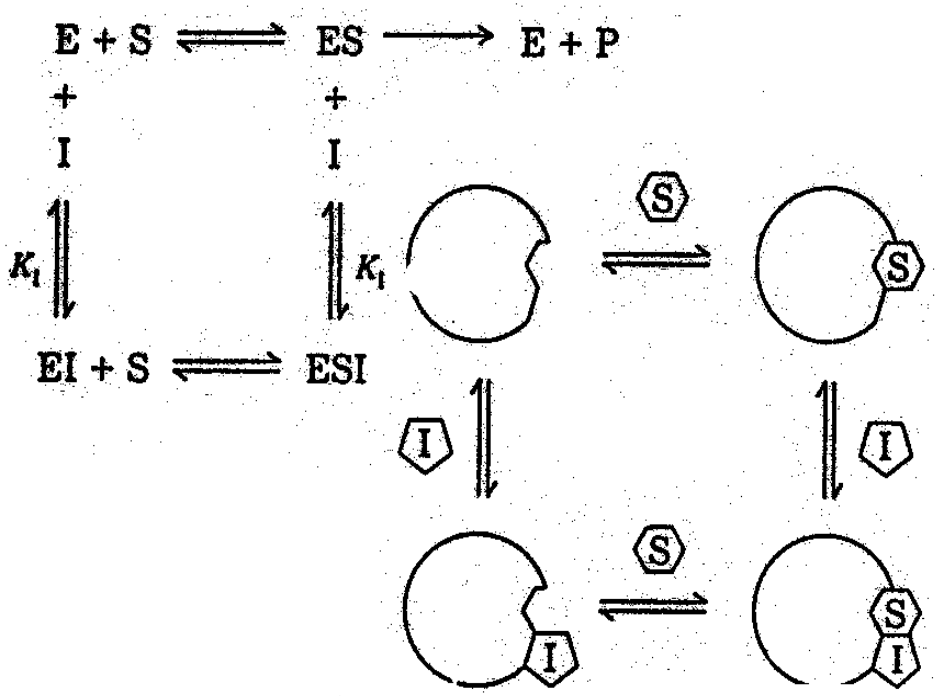
Non-Competitive Inhibition

- a non-competitive **inhibitor** does not affect the binding of the substrate (K_m), but it **does result in a decrease in** V_{max}.

This can be explained by the fact that since **inhibitor** bound to an enzyme inactivates it, the more [EI] formed will lower [ES] and thus lower the overall rate of the reaction V_{max}.

Non-Competitive Inhibition





V_{max} DECREASES - inhibitor affects rate of reaction by binding to site **other** than substrate **active-site**

K_m - No change

Irreversible Inhibitors

Irreversible inhibitors generally result in the destruction or modification of an essential amino acid required for enzyme activity. **Frequently, this is due to some type of covalent link between enzyme and inhibitor.**

These types of **inhibitors** range from fairly simple, to complex **inhibitors** that interact specifically and irreversibly with active site amino acids

termed **suicide inhibitors**.

Irreversible Inhibitors (cont.)

- **suicide inhibitors** are designed to mimic the natural substrate in recognition and binding to an enzyme active site. Upon binding and some catalytic modification, a **highly reactive inhibitor product** is formed that binds irreversibly and inactivates the enzyme. Use of **suicide inhibitors** have proven to be very clinically effective

The inhibitor of **polyamine biosynthesis**,
α-difluoromethylornithine (**DFMO**),
which is an analogue of the amino acid ornithine, and is used to treat **African trypanosomiasis** (***sleeping sickness***).

Ornithine decarboxylase can catalyse the decarboxylation of DFMO instead of ornithine. This decarboxylation reaction is followed by the elimination of a **fluorine** atom, which converts this catalytic intermediate into a conjugated imine. This reactive then reacts with either a **cysteine** or **lysine** residue in the active site **to irreversibly inactivate the enzyme**

Irreversible Inhibitor: **Penicillin**

β-Lactam antibiotics inhibit the formation of **peptidoglycan cross-links** in the **bacterial cell wall**; this is achieved through binding of the four-membered **β-lactam ring** of penicillin to the enzyme ***DD-transpeptidase.***

As a consequence, ***DD-transpeptidase*** cannot catalyze formation of these cross-links, and an imbalance between cell wall production and degradation develops, causing the **bacterial cell** to **rapidly die**.

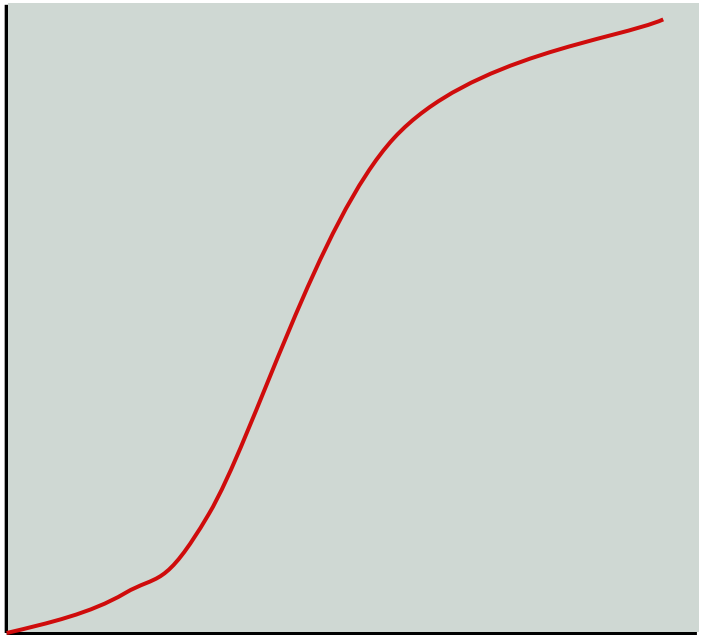
Enzyme Regulation

- Methods used by cell to regulate rate of reactions:
  - [**S**], [**P**] (substrate level regulation)
  - **pH**, **salt** (various ions)
  - **Inhibitors** (presence and concentration)
  - **Covalent** modification
  - ***Allosteric*** modification

Allosteric Enzymes

- **Do not exhibit Michaelis-Menten kinetics.**
- **Plot of V_o vs [S] is sigmoidal not hyperbolic.**
- **Generally are multisubunit enzymes.**
- **Exhibit cooperativity.**

  The binding of substrate to one active site changes the 3-D conformation, which alters the affinity other subunits have for the substrate.



One Model of **Allosteric Mechanism**

- The binding of substrate **switches conformation** of only the subunit to which it is bound.

- Conformational change in one subunit may ↑ or ↓ the **affinity of other subunits** have for the substrate.

- Allows for **+** or **-** cooperativity.

Allosteric Enzyme Regulation
Feedback Inhibition

- A → B → C → D → P
  - E1 E2 E3 E4

Inhibition

Threonine deaminase is regulated by **isoleucine** in the cell.
Isoleucine binds to enzyme at allosteric site and acts as **allosteric inhibitor**