

Topic 6

Enzymes

Nelson & Cox (2016)

Ch 6 pp. 189-213

Nelson & Cox (2017)

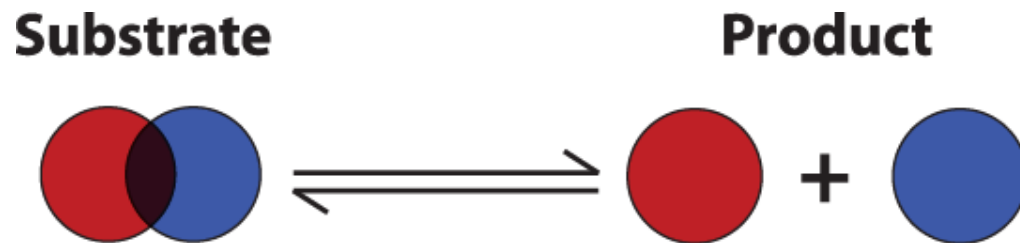
Ch 6 pp. 187-212

What you should know...

- Describe how enzymes show specificity
- Understand what is meant by the term 'activation energy', and how enzymes lower this energy state.
- Discuss the factors and mechanisms that govern enzyme activity
- Explain the role of coenzymes in the function of enzymes
- Understand how the rate of a reaction changes in response to changes in experimental conditions?
- Describe the kinetics of enzyme activity
- Explain the terms $V_{\max}$ and K_m
- Discuss the derivatives of the Michaelis-Menten graph (including Lineweaver-Burk plot)
- Describe the function and kinetics of different types of enzyme inhibitors.

Enzymes

- Is a molecule that **increases the rate** of biochemical reactions by **decreasing the activation energy** needed. It does this by interacting with **substrates** in the **active site** of the enzyme, which **promotes/stabilises** their **transition state**



- Although thermodynamically favourable, nearly all biological reactions in the body would naturally occur too slowly to support life without enzymes
- Because enzymes are specific for certain substrates, they determine which metabolic pathways are active within a cell

Enzymes

- Key characteristics of enzymes
 - Are **biological catalysts**
 - Most are **proteins** (exception: **ribosomes**, RNA based enzymes)
 - Most are **globular proteins**
 - Enzymes **DO NOT** alter the **equilibrium** of a reaction
 - Instead, enzymes **enhance** the **rate of a reaction** (millions of times faster)



Enzymes

- Key characteristics of enzymes cont....
 - Enzymes **are not consumed** in the chemical reaction
 - Enzymes are **very specific** for certain substrates
 - Enzymes promote the **conversion** of **substrate(s)** into **product(s)**.

Enzymes

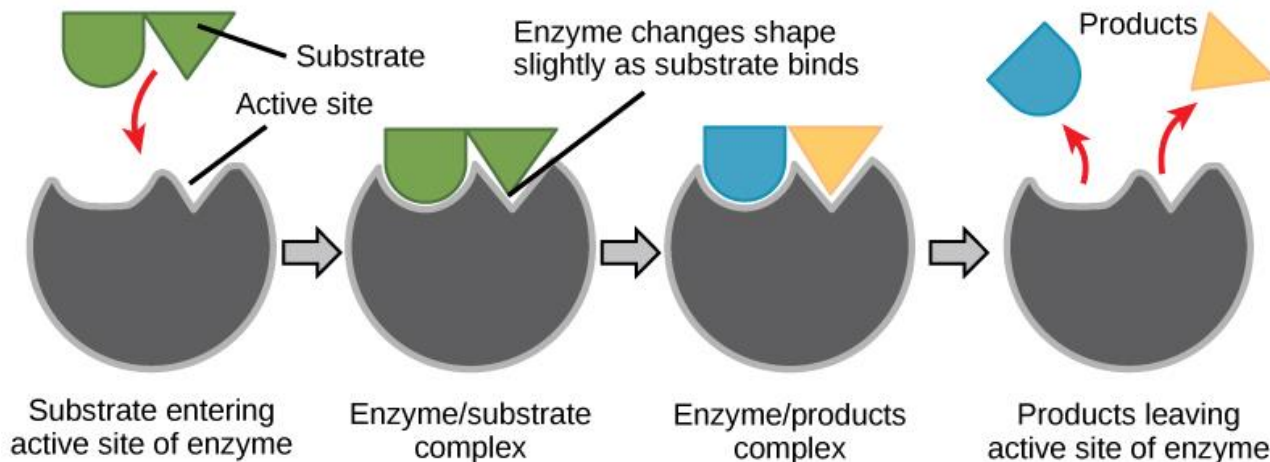
General Formula



Full Formula

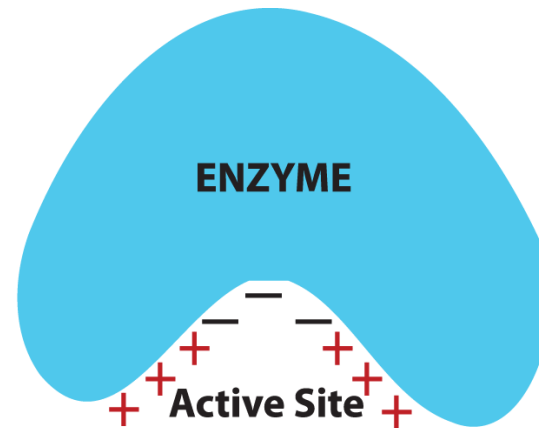


**S: Substrate, ES: Enzyme/substrate complex (transition state(1)),
EP: Enzyme/product complex (transition state (2)), P: Product**



Enzyme Specificity

Specificity of an enzyme comes from the **shape** and characteristics of the **R-groups** of the amino acid residues that form the active site of the enzyme.

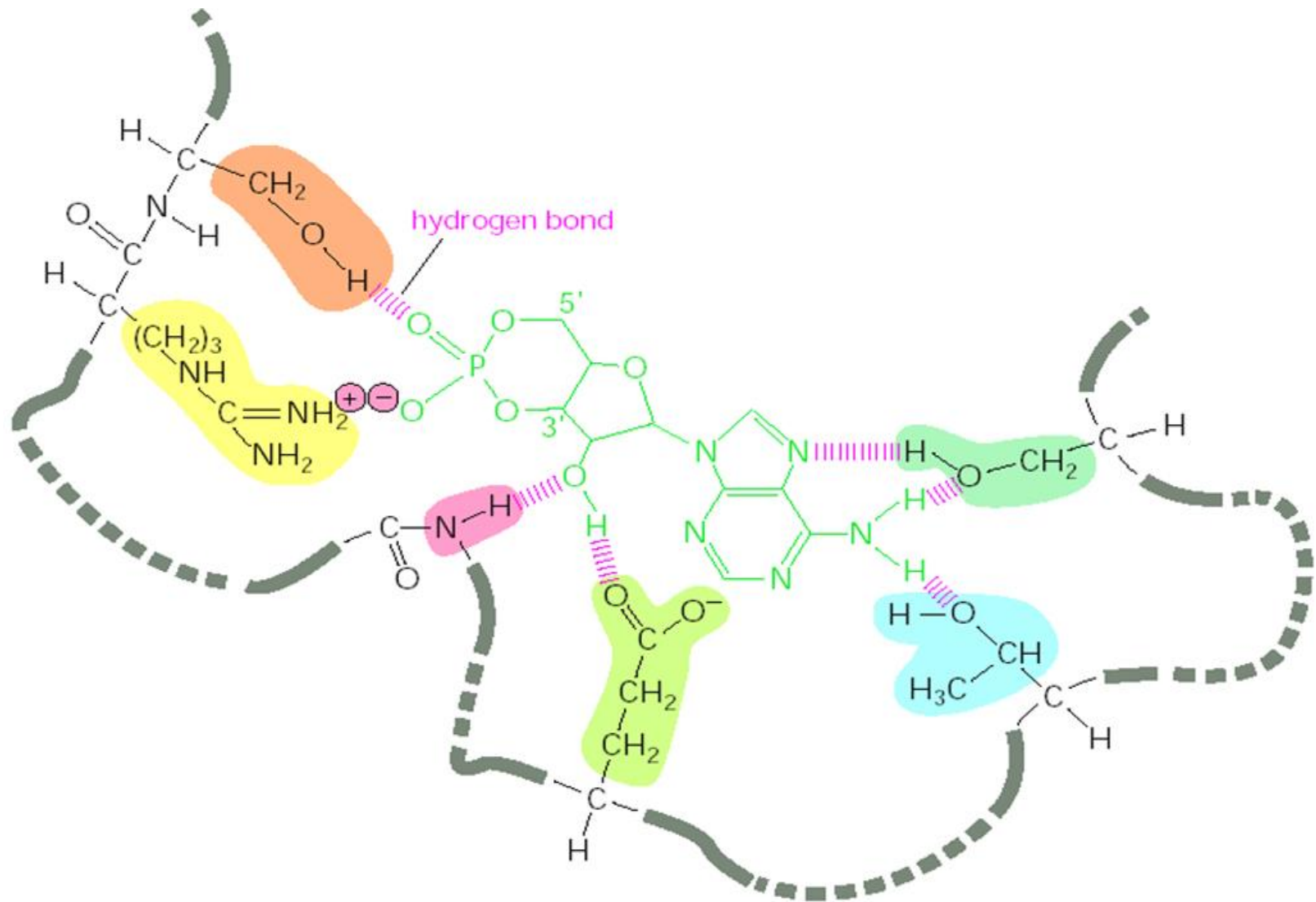


Compatible for:
Shape
R-groups



Not compatible for:
Shape
R-groups

Enzyme Specificity



Cofactors

- In addition to the **R-groups** present, and the **3-dimensional** structure, some enzymes require additional chemical components, termed **cofactors**.
- Cofactors
 - **Inorganic** (Ions: Mg^{2+} , Ca^{2+} etc.)
 - Ionic interactions – help orientate substrate and/or stabilise charged intermediates.
 - Oxidation/reduction reactions (electron transfer (redox reactions))
 - **Organic (co-enzymes:** many derived from vitamins)
 - More diverse functions
 - Transfer of functional groups
 - Substrate binding stabilisation
 - Oxidation/reduction reactions (electron transfer (redox reactions))
 - Acid/base reactions (H^+ ion transfer)

Cofactors

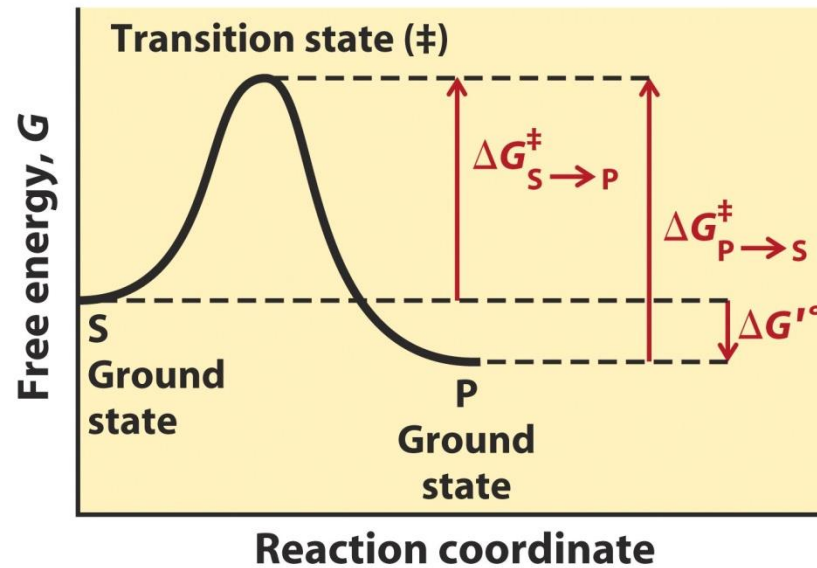
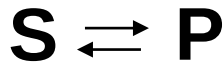
- Some enzymes require both a co-enzyme and a metal ion.
- **Prosthetic group**: a cofactor that is very tightly or even covalently bound to an enzyme
- **Apoenzymes** or **apoproteins**: refers to an enzyme that requires a cofactor, but does not currently have one bound.
- **Holoenzyme**: a complete, catalytically active enzyme with its bound cofactors.

How do Enzymes Work?

- Enzymes provide a **specific environment** in which chemical reactions are **more favorable**.
 - Alignment of substrate functional groups in the correct catalytic orientation
 - Increase the likelihood of interactions between substrates, co-substrates and co-factors (entropy reduction)
 - Removes substrate(s) from the solvent shell of hydrogen bonded water that surrounds and stabilises these biomolecules (bury substrate in catalytic active site, replacing water bonds)
 - Induces distortion in the substrate that is normally required for a reaction to occur (formation of the transition state)
 - Formation of covalent and non-covalent bonds to 'offset' the energy needed to break the original bonds
- In doing the above, reduces the initial input energy needed (activation energy) to convert a substrate into a product
- Many biochemical reactions are **unfavorable** or **unlikely** to occur on their own in the **cellular environment**. Enzymes help **lower the activation energy of a reaction**, making the biological reaction more favorable, and hence increasing the rate of the reaction.

What is 'Activation Energy' and how do Enzymes Lower it?

- A simple chemical reaction can be described as;



- Here, Gibbs free energy (G) of the ground state of P is lower than that of S .
- Thus, the reaction has a $-\Delta G'^{\circ}$, so the equilibrium for this reaction favours the formation of product (P).
- Catalysts **do not** effect the position or direction of equilibrium.

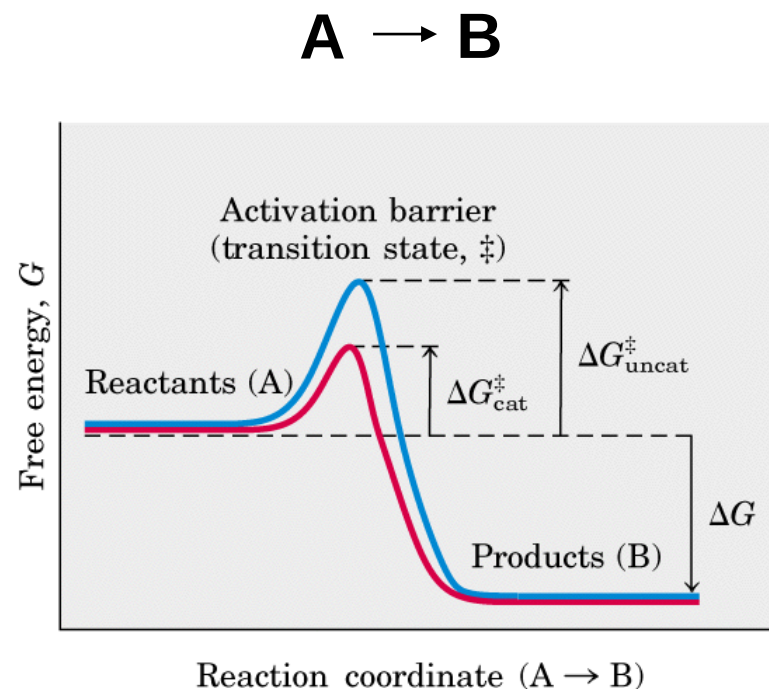
Activation Energy

- The energetically favorable conversion of substrate (A) to product (B) does not necessarily occur at a detectable rate.

e.g. glucose to CO_2 and H_2O .

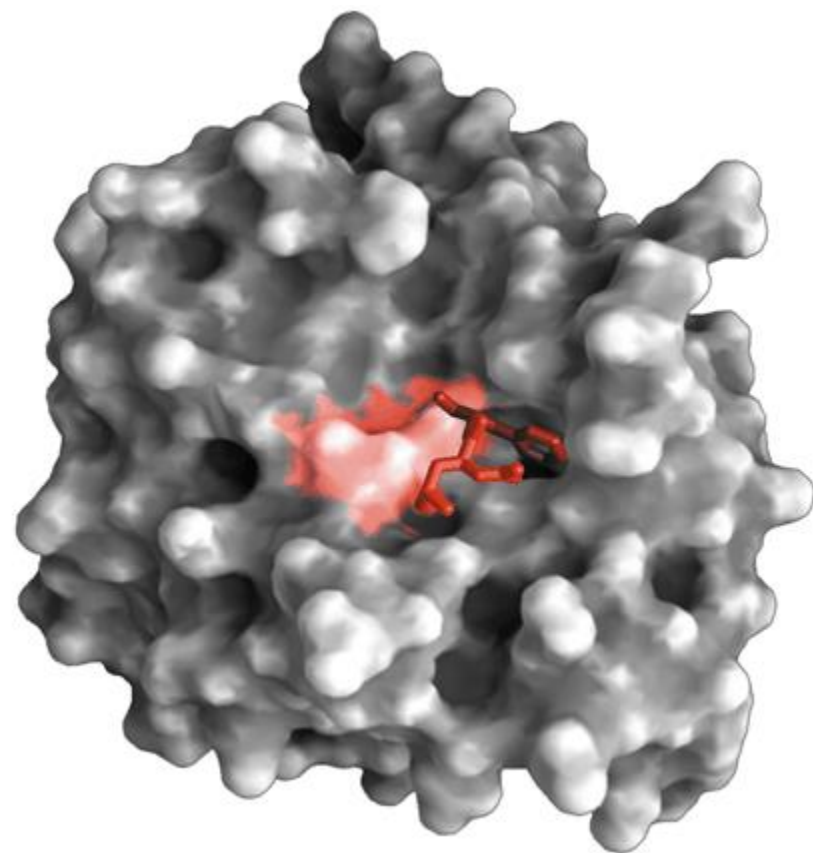
- The path from A to B involves an energy barrier, called the **activation barrier**.

- The energy expended to overcome the activation barrier, is termed **activation energy ($\Delta G^{\ddagger}$)**. This **activation energy** is used to produce transient unstable charges and/or bond rearrangement, present in the high energy **transition state**.



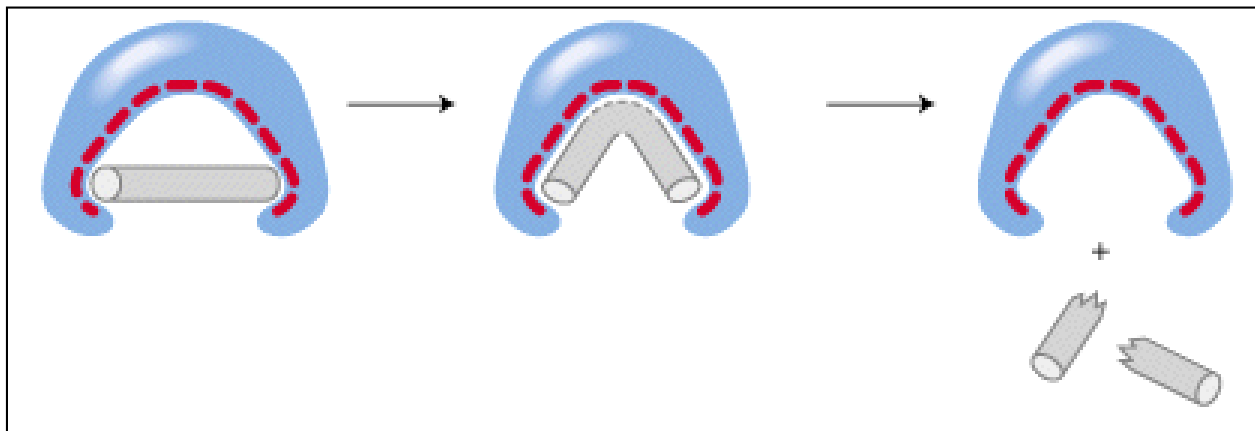
Enzyme Active Site

- A highly **specific pocket** located within the enzyme where the biological reaction occurs
- The **functional groups** of the amino acids within the active site bind specifically to the **substrate(s)** prior to **catalyzing** the chemical reaction.
- Promote the formation of the **transition state**, thus **lowering** the **activation energy** of the reaction.



The enzyme **chymotrypsin**.
Substrate is in red. Key amino acids in pink.

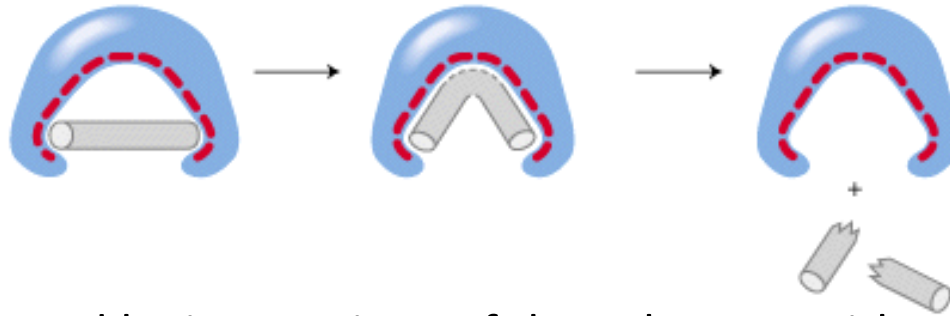
Transition State



- The **transition state** represents an unstable chemical species with high free energy, that can decay to either substrate or product.
- Interactions between the **substrate** and aa's within the **active site** destabilize the stick, promoting the formation of the **transition state** before cleavage.
- The **transition state** requires less input energy to form in a catalysed reaction than in an uncatalysed reaction.

Enzymes Lower the Activation Energy (ΔG^{++})

- How do enzymes lower the required activation energy of a reaction?
 - By removing **substrate(s)** from the **solvent shell** of hydrogen bonded water that surrounds and stabilises these biomolecules (bury substrate in catalytic active site, replacing water bonds).
 - By altering the **shape** (transition state) of the substrate.
 - By the **release of free energy**, due to the formation of **covalent** and **non-covalent** bonds, to 'offset' the energy needed to break the original bonds.



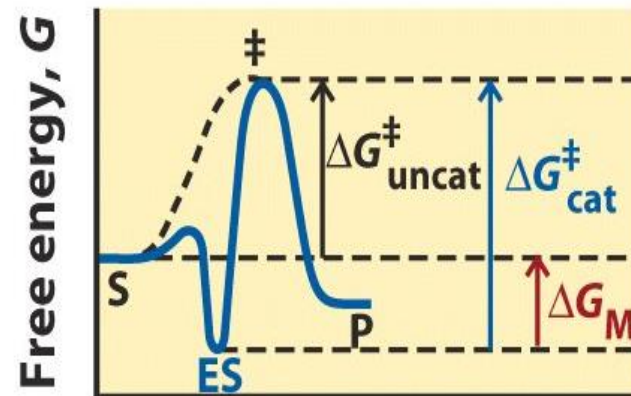
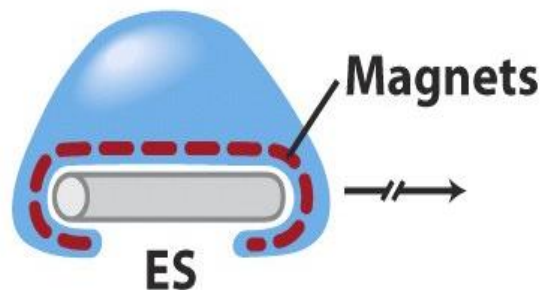
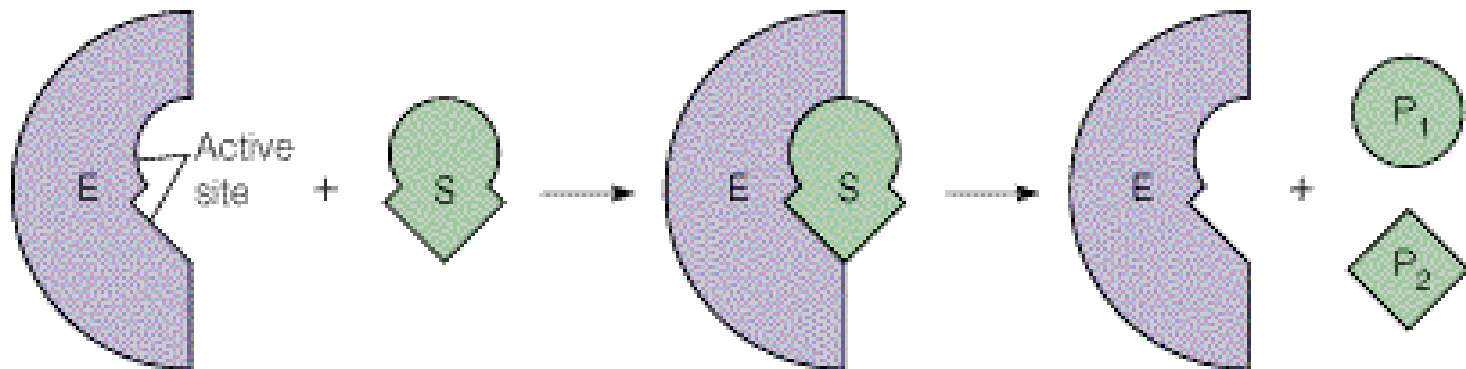
- Changes are induced by interactions of the substrate with reactive side groups of amino acids in the active site.
- In this manner, enzymes enhance the **rate** of chemical reactions in the order of **5-17 times** the magnitude of non-catalyzed reactions.

Enzyme-Substrate Interactions

- There are two models explaining enzyme-substrate interactions
 - Lock and key model
 - Induced fit model

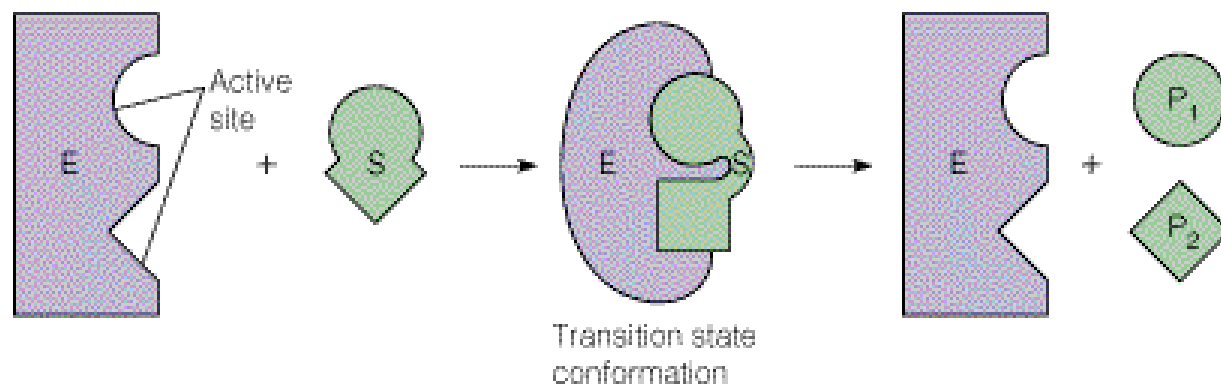
Lock and Key Model

- In this model, the active site of the enzyme fits the substrate like a lock does a key.



Induced Fit Model

- Substrate binds → change in conformation (induced by the many weak interactions that occur between the substrate and active site of the enzyme = induced fit)
- Induced fit
 - Active site continues to change in shape until the substrate is completely bound.
 - Optimal binding of the substrate occurs only when the transition state is formed
 - Can promote the formation of the substrate transition state.
 - May also bring specific functional groups within the active site into position to catalyse the reaction.
 - Enhances the catalytic abilities of an enzyme.



(b) Induced fit model

Remember...

- Enzymes are highly specific and can **discriminate** between very similar compounds (substrates).
- The shape of the **active site** and the arrangement of **functional groups** within this site, helps determine **enzyme specificity**. Functional groups are arranged so as to form **numerous weak interactions** with a specific substrate(s).
- Enzymes **increase the rate** of biochemical reactions by:
 - Bringing substrates together in the **correct orientation** to react
 - Increasing the likelihood of interactions between substrates
 - Reducing the initial input energy (**activation energy**) needed to convert a substrate into a product. Enzymes achieve this:
 - By **'offsetting'** the energy needed to break bonds within the substrate by the formation of temporary **covalent** and **non-covalent** bonds with the substrate.
 - By **promoting/stabilising** a substrates **transition state** through **numerous weak interactions** that occur within the **active site**.
 - By removing **substrate(s)** from their water **solvent shell**

Enzyme Activity

- Measured in **units**.
- **1.0 unit of enzyme activity** is defined as the amount of enzyme causing transformation of 1 μmol of substrate (S) to product (P) per minute at 25°C.

LEARN THIS!



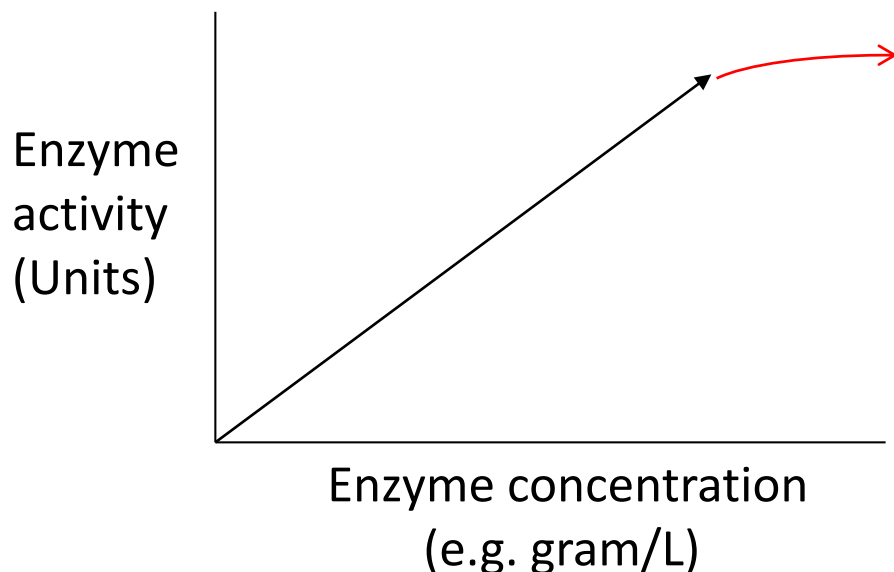
- The amount of **enzyme activity** in a solution equals the total number of units in the solution.

Factors that Influence Enzyme Activity

- The rate at which an enzyme catalyses a chemical reaction is dependent on several factors:
 1. Enzyme concentration
 2. pH
 3. Temperature
 4. Substrate concentration
 5. Inhibitors

1. Enzyme Concentration

- There exists a linear relationship between enzyme concentration and enzyme activity.
- In the clinical setting, the enzyme activity in serum is measured to give an indicator of the amount of enzyme present.

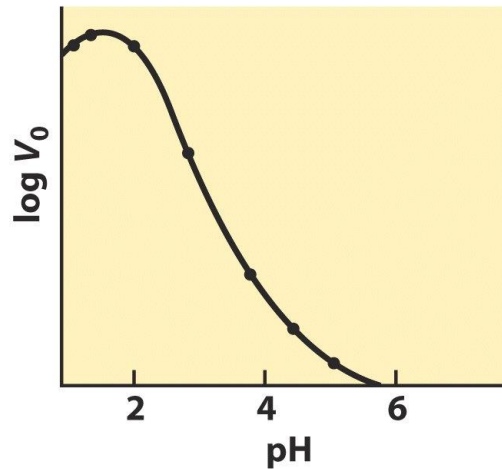


NOTE - This graph holds true only:

1. When there is unlimited substrate present
2. Until a concentration maximum is reached for the enzyme

2. The Effect of pH

- In general, the optimum pH for the activity of an enzyme is close to the pH of the environment in which that enzyme is found.
- *E.g.* pepsin is an enzyme released by the stomach (degrades proteins). The pH of the stomach is acidic (~ 2).



(a) Pepsin

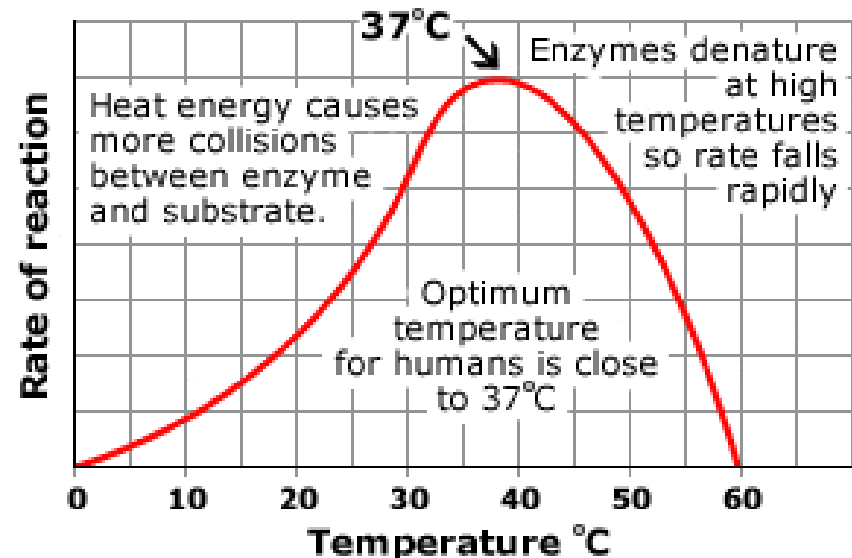
- Based on what you have learnt, why would changing the pH effect the activity of an enzyme (the same is true for the substrate)?

3. The Effect of Temperature

- The rate of most non-enzyme catalyzed chemical reactions generally increases with temperature.
 - $\uparrow$ Temp you $\uparrow$ free energy, therefore, overcome activation barrier.
- However, the 3D structure of enzymes (proteins) are disrupted by high temperatures (denaturation).
- Enzymes therefore lose activity beyond their optimal temperature.

Real life Examples:

- *Boiling water when camping*
- *Pasteurization of milk*



4. The Effect of Substrate Concentration

- The generalized reaction, in which substrate (S) is converted into product (P) by an enzyme (E), can be described by the equation:

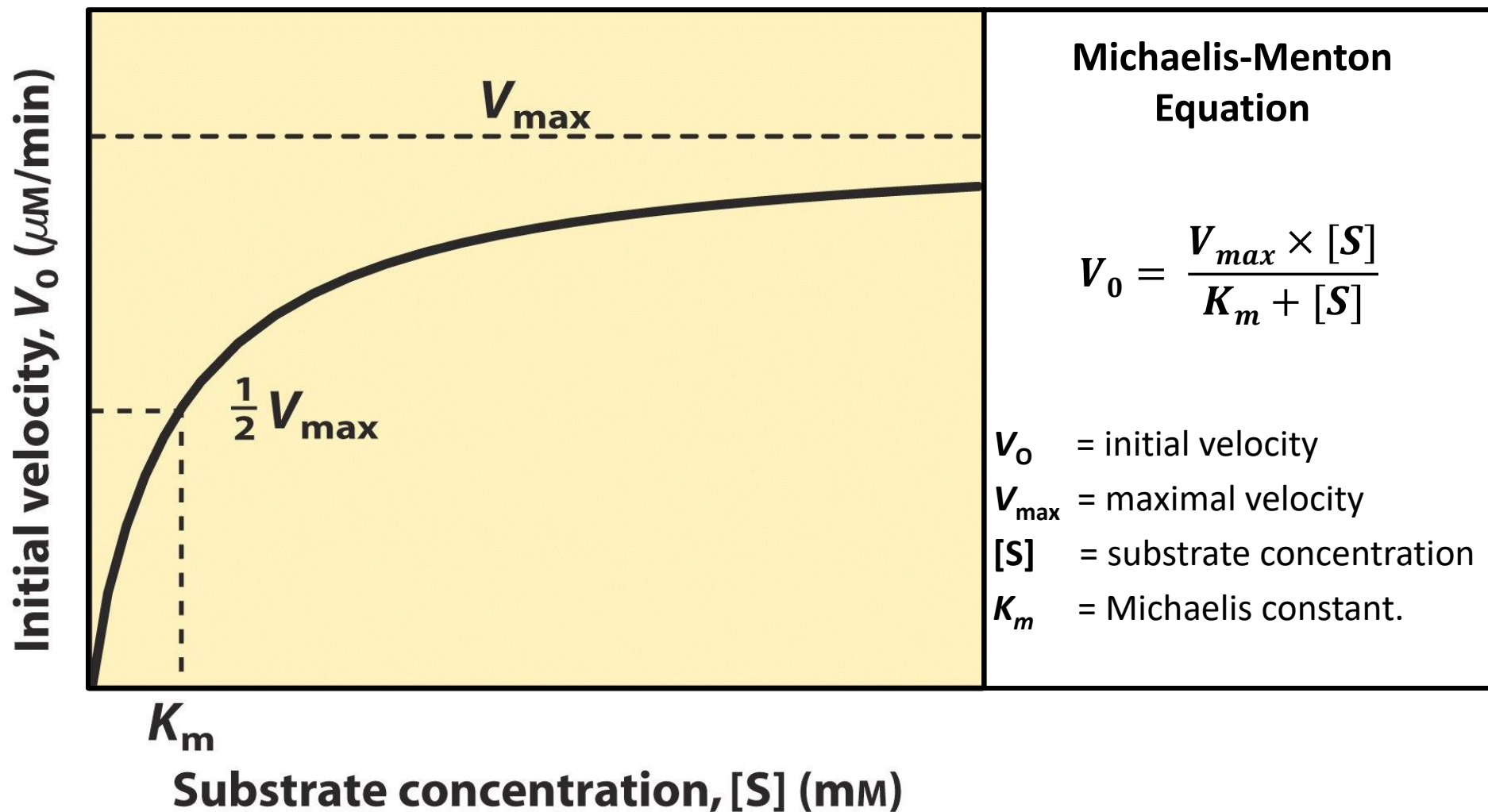


- The binding of an enzyme to its substrate is reversible. It is assumed here that the formation of product (P) is irreversible due to the small amount initially present.
- When the second step (K_2), in which the product is formed, is slower than the first (K_1), the second step limits the rate of the overall reaction.

Influence of [S] on Enzyme Activity

27

- It is difficult to study the effect of substrate concentration on the rate of enzyme catalyzed reactions.
 - [S] changes as the reaction progresses
 - I.e. [S] ↓ during the reaction as it is converted to product.
- A common method is to measure the **initial rate/velocity (V_o)** of an enzyme catalyzed reaction (allows us to assume [S] is constant).
- **In lab, you will measure enzyme activity in the 1st 2 minutes after adding enzyme.**



If an enzyme follows Michaelis-Menten kinetics, initial velocity (V_o) will show a hyperbolic response to increasing $[S]$.

Maximal Velocity ($V_{\max}$)

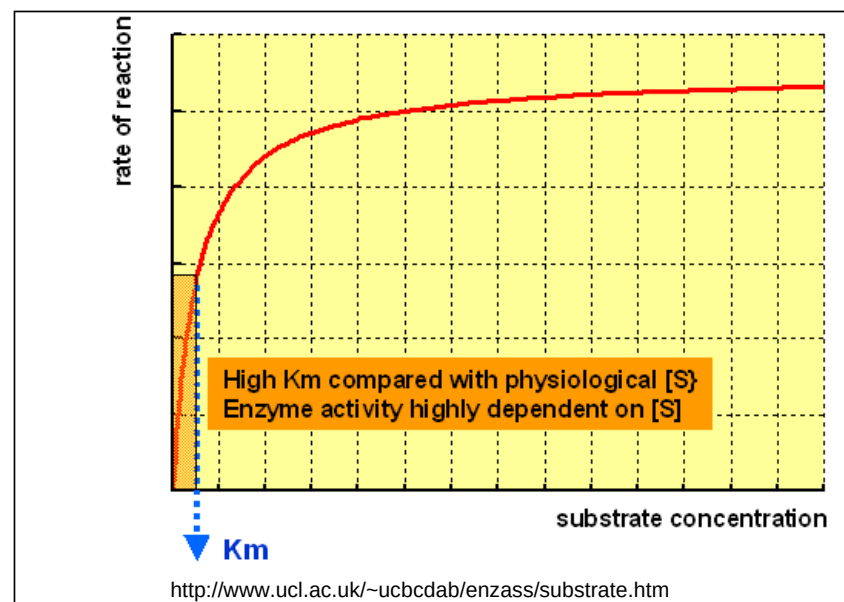
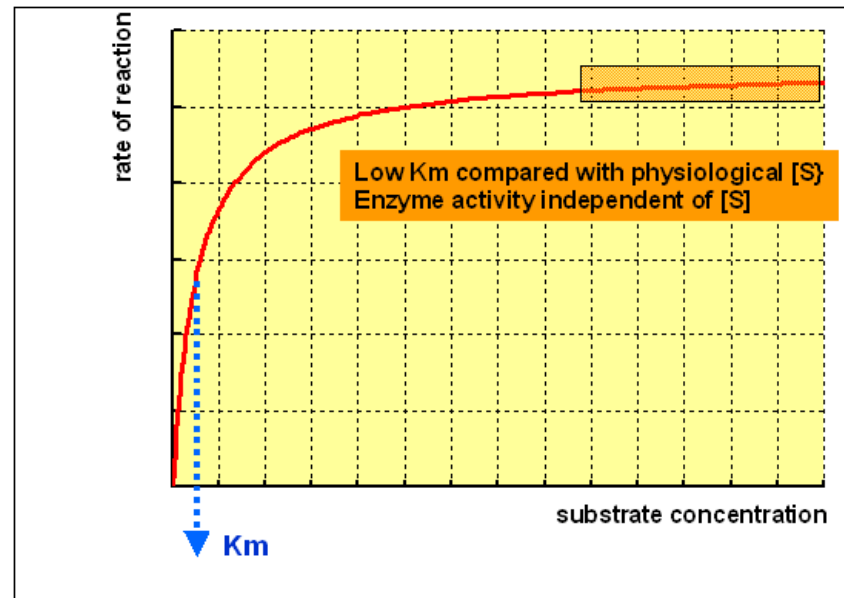
- The **maximal velocity** of a reaction ($V_{\max}$) is the point at which all the available substrate binding sites on the enzyme are occupied by substrate, and the enzyme is said to be saturated. At this point V_o approximately equals $V_{\max}$
- Any further increase in $[S]$ has no effect on V_o . Therefore; **at high $[S]$ (approaching $V_{\max}$), the rate of the reaction is independent of $[S]$, and is dependent on the enzymes kinetics ($K_m \ll [S]$)**
- It is difficult to determine $V_{\max}$ from the plot of V_o against $[S]$, as the graph only **approaches $V_{\max}$** . Therefore for we commonly use a **Lineweaver-Burk plot** (discussed later)

Influence of [S] on Enzyme Activity

- The enzyme can exist in one of two forms;
 - The free form (E), or as a complex with the substrate (ES)
- At low [S], most of the enzyme is in the free form (E)
 - At low [S], the rate of the reaction is dependent on [S] ($K_m \gg [S]$)
- As the [S] $\uparrow$, [ES] $\uparrow$; as the [ES] $\uparrow$, [E] $\downarrow$
 - At high [S] (approaching V_{max}), the rate of the reaction is independent of [S], and is dependent on the enzymes kinetics ($K_m \ll [S]$)
- The maximum rate of an enzyme-catalyzed reaction (V_{max}) occurs when all the enzyme exists as the ES complex

Michaelis-Menten Constant (K_m)

- If the K_m for an enzyme is below physiological $[S]$, the enzyme will normally be saturated and will act at maximal rate
 - At high $[S]$ (V_{max}), the rate of the reaction is independent of $[S]$ ($K_m \ll [S]$).
- If the K_m for an enzyme is above physiological $[S]$, enzyme activity will vary with substrate concentration.
 - At low $[S]$, the rate of the reaction is dependent on $[S]$ ($K_m \gg [S]$)



Michaelis-Menten Constant (K_m)

- An enzyme with a high K_m requires a higher $[S]$ to achieve a given reaction velocity than an enzyme with a low K_m .
- For the simplest reactions: the lower the K_m the higher the affinity for S

TABLE 6-6 K_m for Some Enzymes and Substrates

<i>Enzyme</i>	<i>Substrate</i>	K_m (mM)
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5

Michaelis-Menten Constant (K_m)



$$K_m = \frac{K_2 + K_{-1}}{K_1}$$

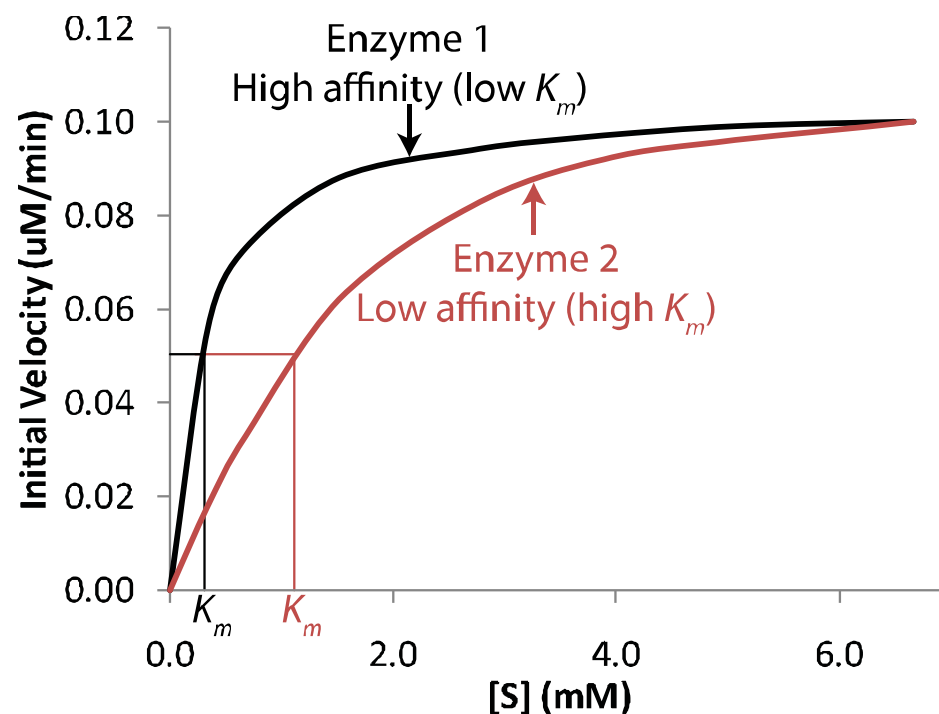
Breakdown of ES (reduced affinity)

Formation of ES (increased affinity)

- Increases in either K_2 and K_{-1} , or a reduction in K_1 , represents a reduction in ES formation and therefore a theoretical reduction in enzyme affinity (increased K_m)
- Increases in K_1 , or a reduction in K_2 or K_{-1} , represents an increase in ES formation and therefore a theoretical increase in enzyme affinity (decreased K_m)

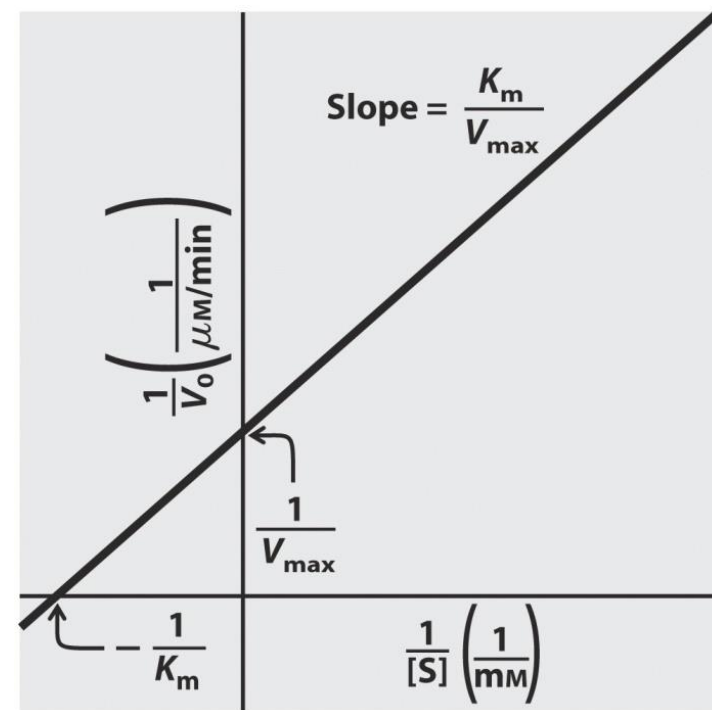
Enzyme Efficiency/Affinity

- Enzyme A has a lower K_m , therefore a greater affinity for the substrate than enzyme B
 - Low K_m = higher affinity for S
- This holds true ONLY for simple two-step reactions where K_2 is the rate limiting step



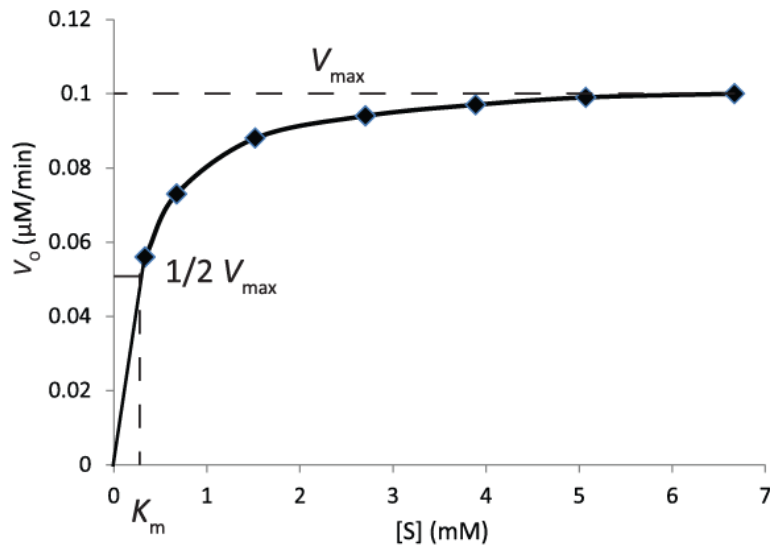
Lineweaver-Burk Plot

- Double reciprocal plot
- **Originally** allowed better graphical representation of the Michaelis-Menten equation
 - Straight line, therefore, can determine enzyme kinetics more easily (e.g. $V_{\max}$ and K_m)
 - Allows for visual interpretation of enzyme inhibitors
 - **However**, prone to enhancing error due to the taking of the reciprocal.



Need to be able to determine: $V_{\max}$ and K_m from either a Michaelis-Menton or Lineweaver-Burk plot

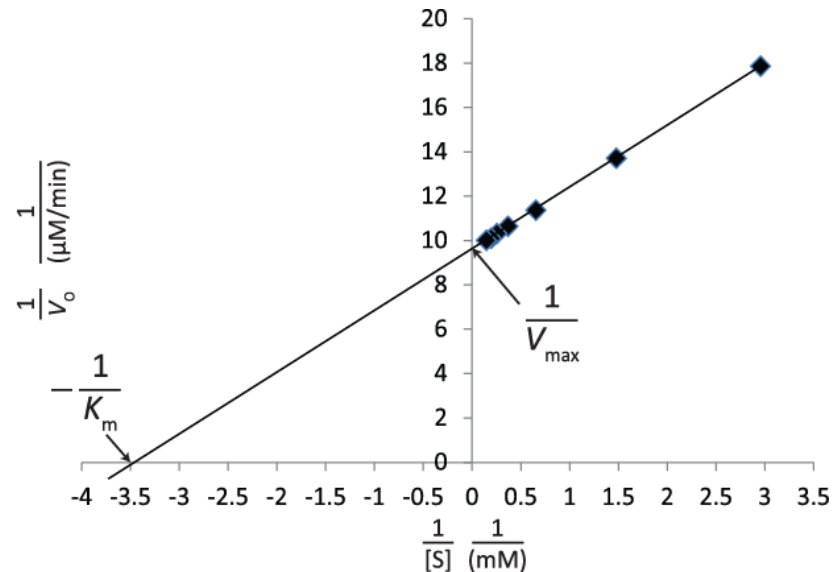
Determining $V_{\max}$ and K_m



$[S]$	V_o
0.338	0.056
0.676	0.073
1.521	0.088
2.705	0.094
3.888	0.097
5.071	0.099
6.671	0.100

$$V_{\max} = 0.100 \mu\text{M}/\text{min}$$

$$K_m = 0.250 \text{ mM}$$



$1/[S]$	$1/V_o$
2.959	17.857
1.479	13.699
0.657	11.364
0.370	10.638
0.257	10.309
0.197	10.101
0.150	10.000

$$1/V_{\max} = 9.500 \text{ } 1/(\mu\text{M}/\text{min})$$

$$1/K_m = -3.500 \text{ } 1/(\text{mM})$$

$$V_{\max} = 1/9.5 = 0.105 \mu\text{M}/\text{min}$$

$$K_m = -1/-3.5 = 0.290 \text{ mM}$$

5. Enzymes are subject to reversible and irreversible inhibition

- Enzyme inhibitors are molecules that slow or completely inhibit enzyme catalyzed reactions.
- Two broad types of enzyme inhibitors:
 - Reversible inhibitors.
 - Irreversible inhibitors.

Reversible Enzyme Inhibitors



E = enzyme

I = inhibitor

EI = enzyme-inhibitor complex,
non-covalent interactions!

- The enzyme is inactive when the inhibitor is bound.
- Therefore, product (P) cannot be formed
- This reaction is reversible, as EI dissociates to E + I.
- 3 types of reversible inhibitors;
 - Competitive
 - Uncompetitive
 - Mixed and non-competitive

(a) Competitive inhibition

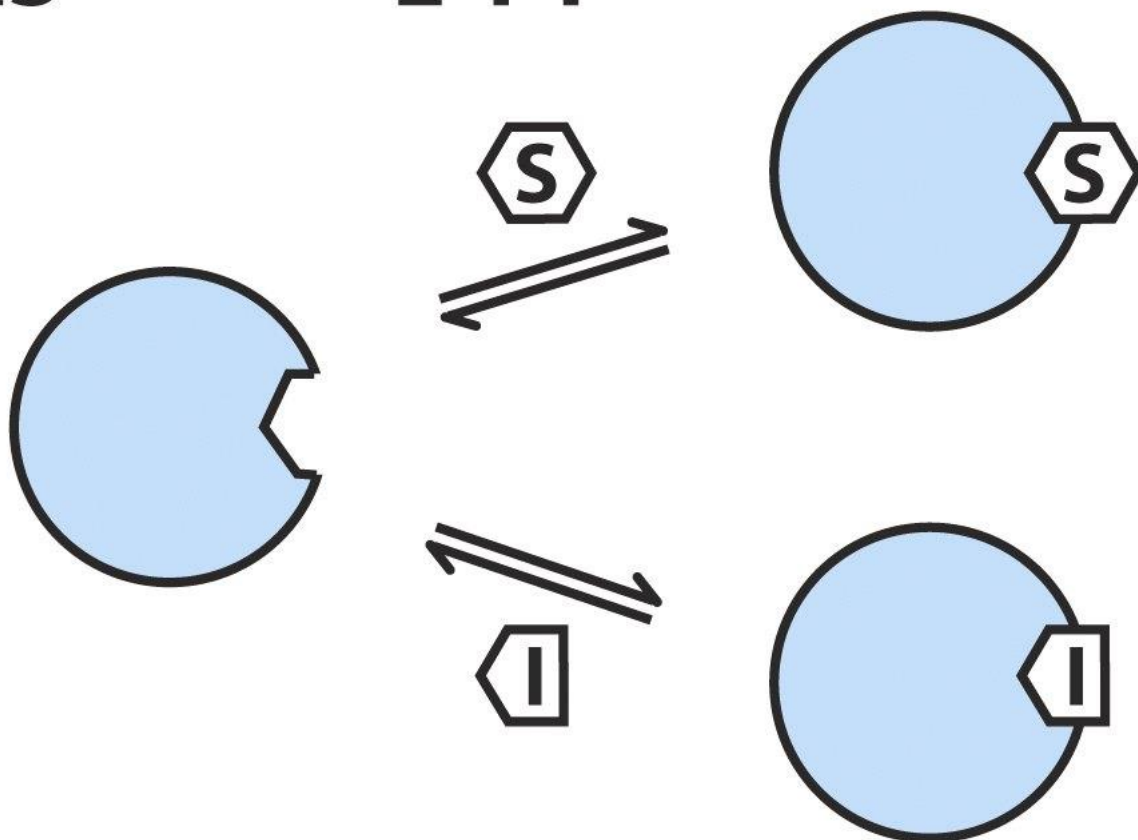


Fig 6-15a

Competitive Inhibitors

- Increasing $[S]$ will reduce the ability of the inhibitor to bind to the enzyme.
- $V_{\max}$ is therefore unchanged
- K_m however is increased

La Chatelier's principle (equilibrium)



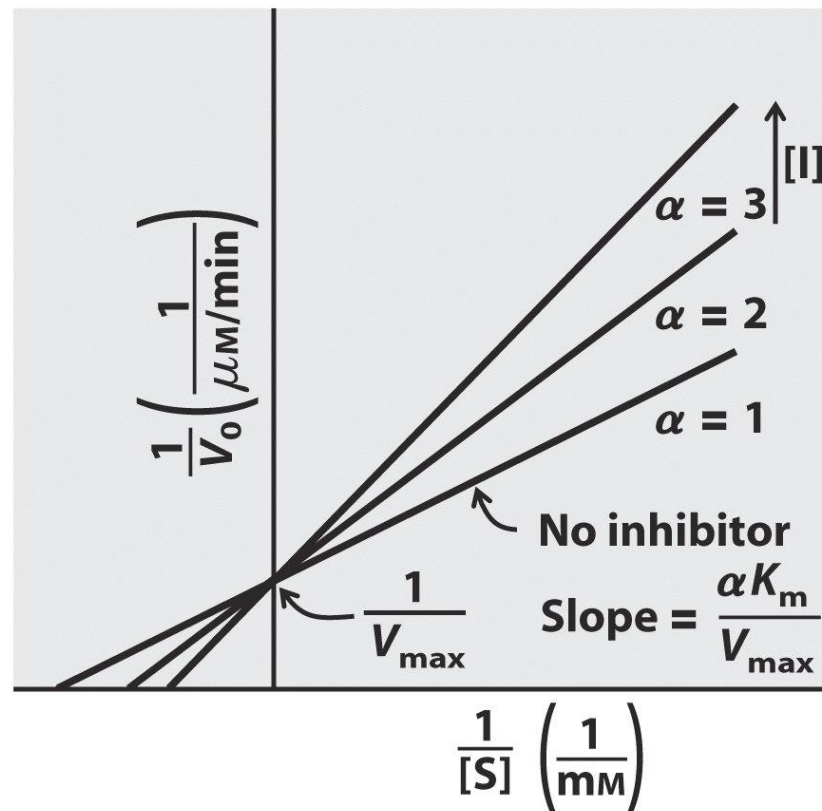
Remove E, equation moves to the left (K_m **increases**)

Remove ES, equation moves to the right (K_m **decreases**)

Free E removed. Equation pushed to the left (away from ES formation), K_m increased (apparent lower affinity)

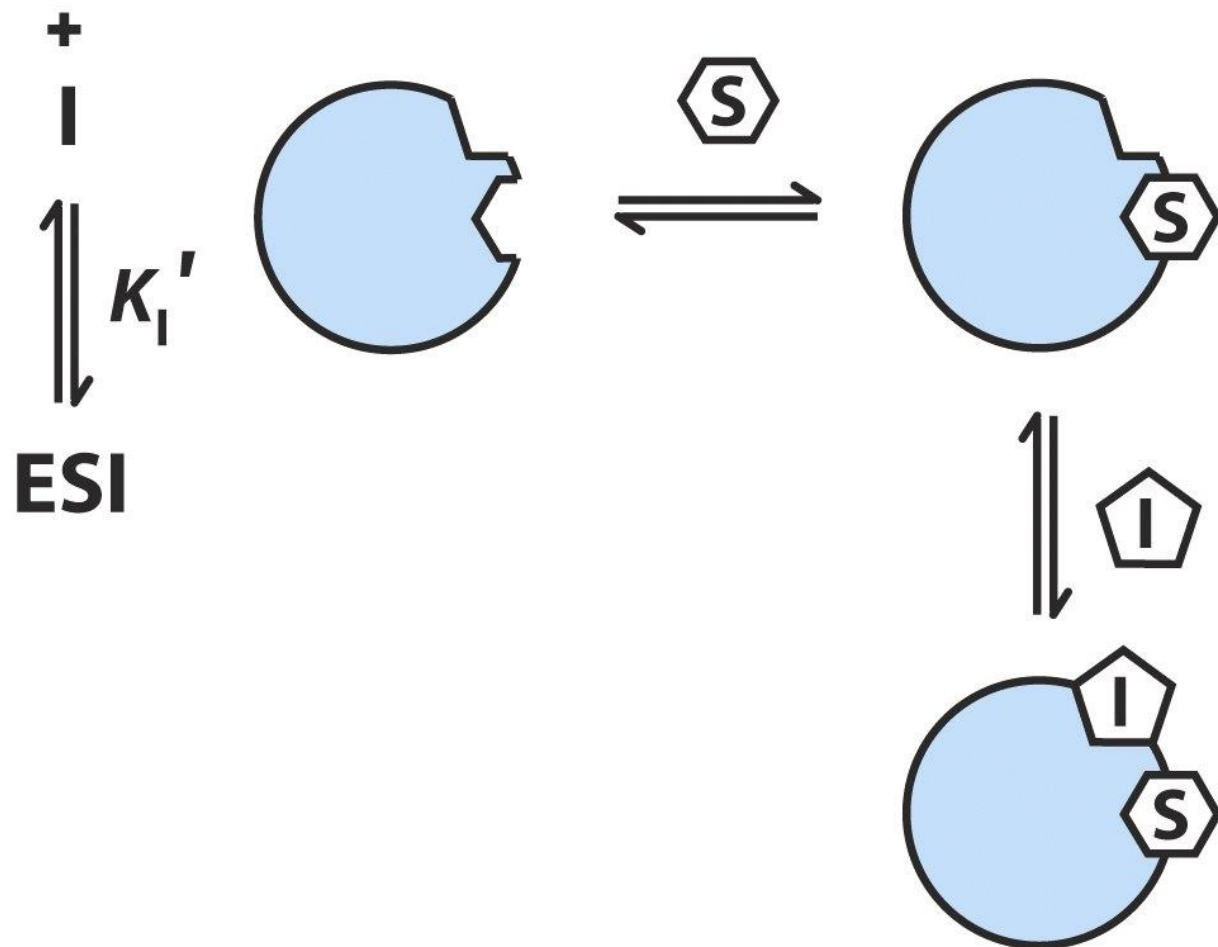


$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{\max}} \right) \frac{1}{[S]} + \frac{1}{V_{\max}}$$



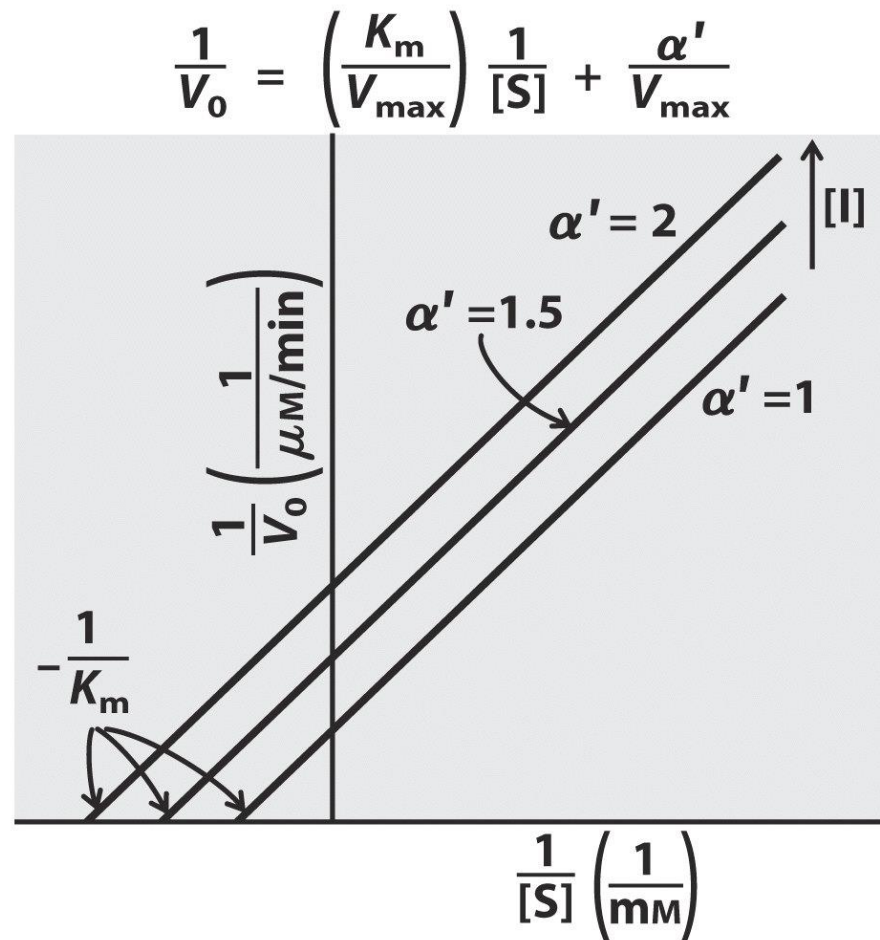
Formation of P unaffected,
 $V_{\max}$ unaffected

(b) Uncompetitive inhibition



Uncompetitive Inhibitors

- Increased $[S]$ does not affect the ability of the inhibitor to bind to the enzyme.
- $V_{\max}$ is decreased
- K_m is decreased



ES removed as ESI. Equation pushed to the right (towards ES formation), K_m decreased (apparent higher affinity)

Formation of P affected by ESI, $V_{\max}$ reduced

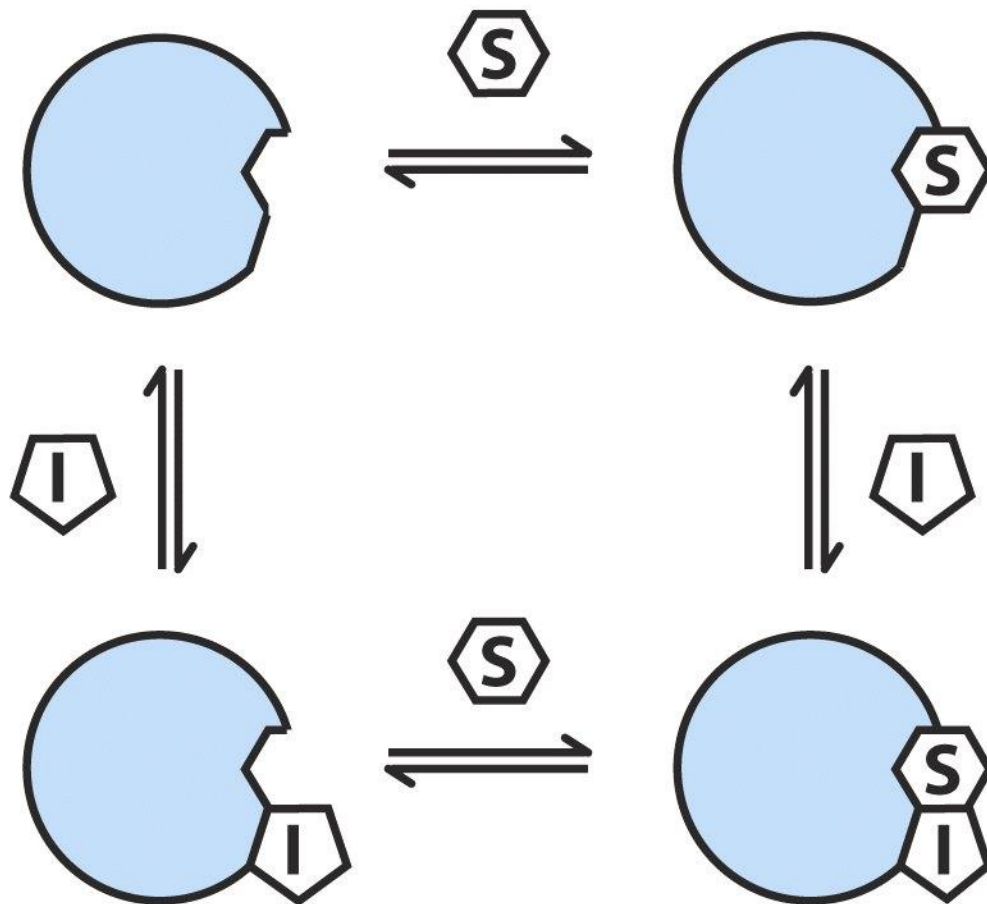


(c) Mixed inhibition



+
I

+
I

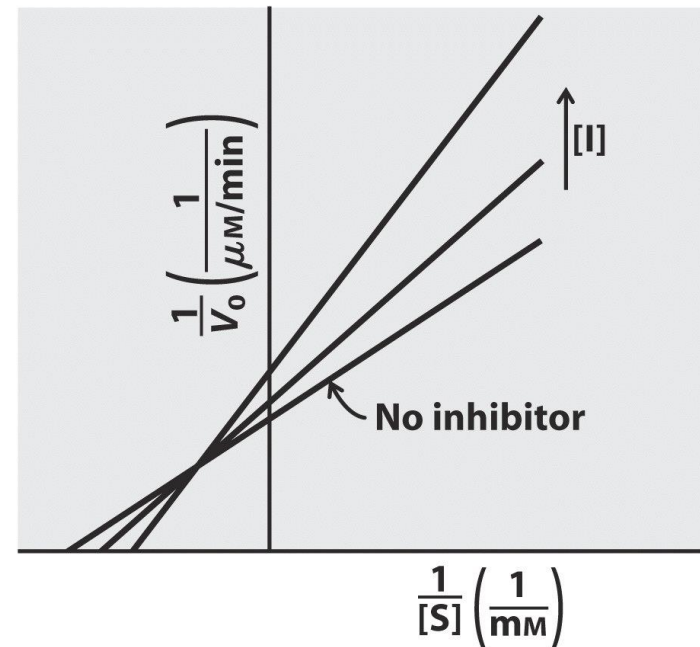


6-15c

Mixed Inhibitors

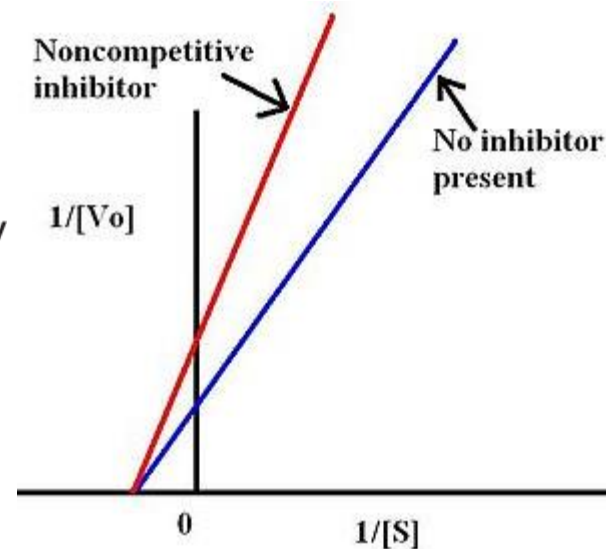
- $V_{\max}$ is decreased
- If EI is greater than ESI , K_m is increased (less affinity) – mixed inhibitor
- If $EI = ESI$, K_m will be unchanged – non-competitive inhibitor

$$\frac{1}{V_0} = \left(\frac{\alpha K_m}{V_{\max}} \right) \frac{1}{[S]} + \frac{\alpha'}{V_{\max}}$$



Both E and ES are affected. However, in this case E is more affected, so equation is pushed to the left (away from ES formation). K_m increases (apparent lower affinity)

Formation of P affected by EI and ESI, $V_{\max}$ reduced



Summary - Enzyme Inhibitors

Inhibitor	$V_{\max}$	K_m
Competitive	Unchanged	Increased
Uncompetitive	Decreased	Decreased
Mixed	Decreased	Increased
Non-competitive	Decreased	Unchanged

Quiz

The following data were obtained in a study of an enzyme known to follow Michaelis-Menten kinetics:

V_0 (mmol/min)	Substrate added (mmol/L)
217	0.8
325	2
433	4
488	6
647	1,000
647	2,000

The K_m for this enzyme is approximately:

- a) 1 mM
- b) 2 mM
- c) 4 mM
- d) 6 mM
- e) 1,000 mM
- f) 2,000 mM

In a plot of $1/V$ against $1/[S]$ for an enzyme-catalyzed reaction, the presence of a competitive inhibitor will alter the:

- a) curvature of the plot.
- b) intercept on the $1/[S]$ axis.
- c) intercept on the $1/V$ axis.
- d) pK of the plot.
- e) $V_{\max}$.

What's Next?

- ✓ • Topic 1 – Foundations in Biochemistry
- ✓ • Topic 2 – Amino Acids
- ✓ • Topic 3 – Proteins
- ✓ • Topic 4 – Lipids and Membranes

- ✓ • Topic 5 – Signal Transduction
- ✓ • Topic 6 – Enzymes
- Topic 7 – Nucleotides and Nucleic Acids
- Topic 7 – DNA Replication
- Topic 8 – Transcription
- Topic 9 – Translation

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