

Biomedical Science

Molecular Genetics



BMS 23110c | Fall 2026-2027

Enzymology

The Mechanism Of Enzyme Action II

جميع الأوراق والمحتويات التابعة للمنصة محمية بموجب حقوق الملكية الفكرية.
يُمنع منعاً باتاً نسخ، أو نشر، أو مشاركة أي محتوى أو مستندات تخص المنصة بأي شكل
دون إذن مسبق. ويُعد أي تجاوز أو مخالفة لهذا القرار خرقاً قانونياً يعرض مرتكبه للمساءلة
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Enzyme Kinetics

DEFINITION

Enzyme kinetics is the study of the **rates of enzyme-catalysed** chemical reactions. In enzyme kinetics, the reaction rate is measured and the effects of varying the conditions of the reaction are investigated.

STUDYING AN ENZYME'S KINETICS IN THIS WAY CAN REVEAL



Catalytic mechanism

The catalytic mechanism of this enzyme



Role in metabolism

Its role in metabolism



Control of activity

How its activity is controlled



Drugs & modifiers

How a drug or a modifier (inhibitor or activator) might affect the rate

WHY STUDY ENZYME KINETICS?

01

Allow studying **complex dynamic** of enzyme behavior

02

Systematic evaluation of enzyme function

WHERE IT BEGAN



1902

Enzyme kinetics began when **Adrian Brown** reported an investigation of the rate of hydrolysis of **sucrose** as catalyzed by the yeast enzyme **invertase**.



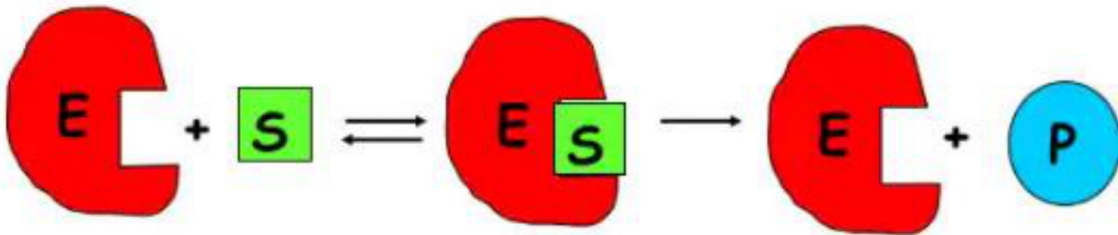
Finding

Brown demonstrated that when **sucrose concentration** is much higher than that of the enzyme, the reaction rate becomes **independent of sucrose concentration**.

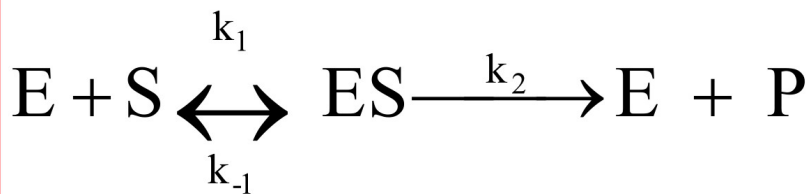


The Enzyme–Substrate Complex

An **enzyme (E)** is typically a protein molecule that promotes a reaction of another molecule, its **substrate (S)**. This binds to the **active site** of the enzyme to produce a transition **enzyme–substrate complex ES**, and to form a **product P**.



The overall reaction is composed of **two elementary reactions** in which the substrate forms a complex with the enzyme that subsequently decomposes to products and enzyme.



E Enzyme

S Substrate

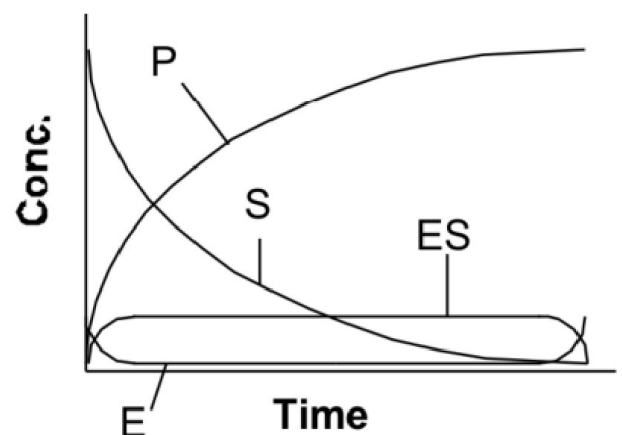
P Product

ES Enzyme–Substrate complex

k_1 Rate constant for the forward reaction

k_{-1} Rate constant for the breakdown of the ES to substrate

k_2 Rate constant for the formation of the products

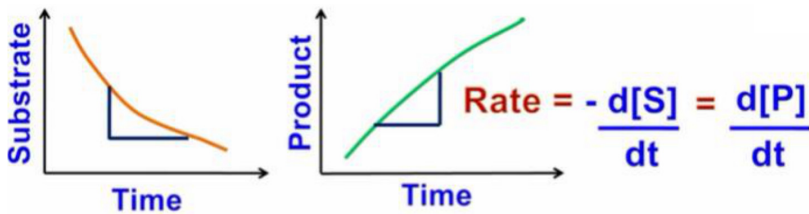




Michaelis–Menten Equation

WHAT IT DESCRIBES

The Michaelis–Menten equation describes the **velocity of enzymatic reactions (v)** by relating it to **[S]**, the concentration of a substrate S.



Changes in concentrations of S and P with respect of time

VELOCITY

Velocity of reaction is actually the **instantaneous rate** of reaction.

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

V_0

Initial velocity (moles/
time)

[s]

Substrate concentration
(molar)

$V_{\max}$

Maximum velocity

K_m

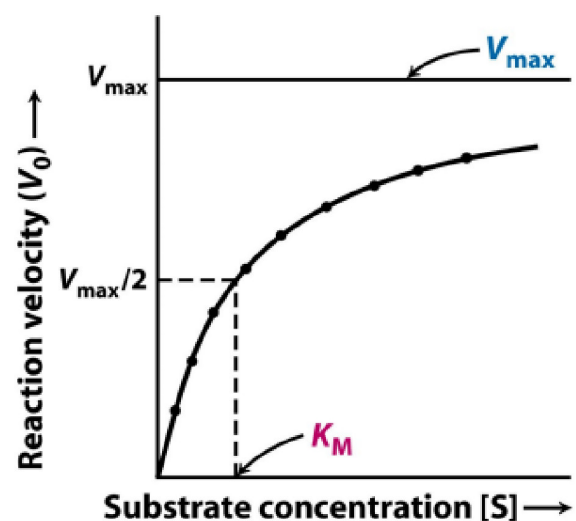
Substrate concentration
at half $V_{\max}$

$V_{\max}$

Represents the **maximum rate** achieved by the system, at maximum (saturating) substrate concentrations.

K_m – Michaelis constant

The substrate concentration at which the reaction rate is **half of $V_{\max}$** .

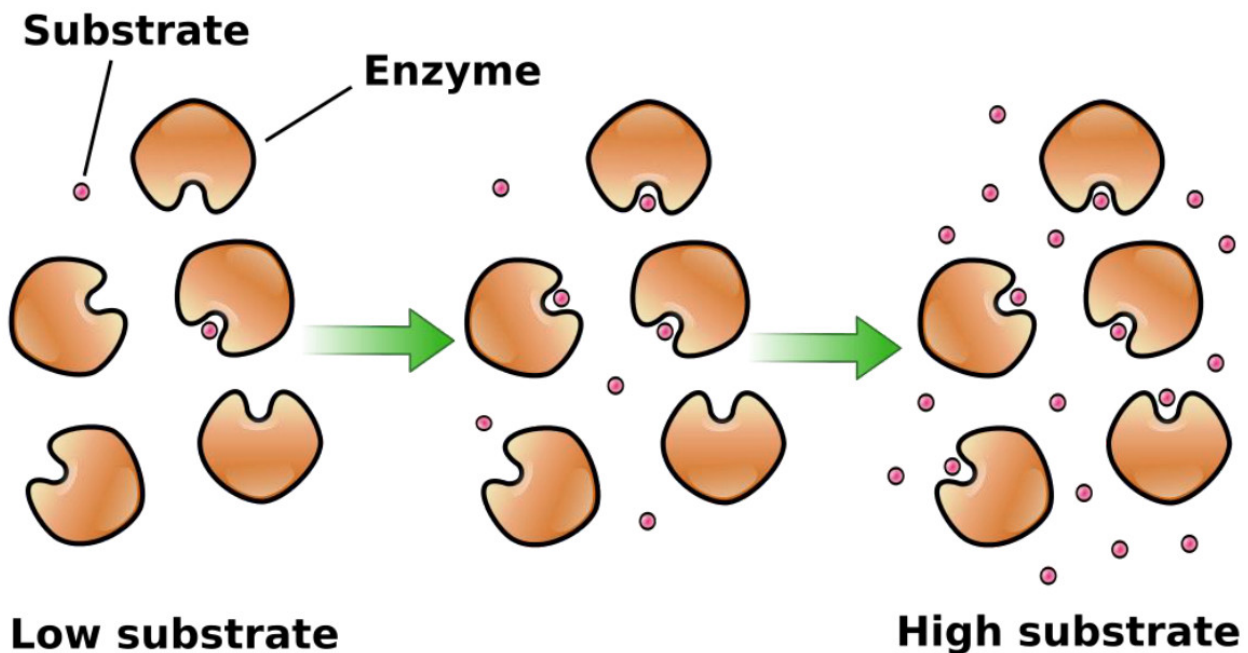




Substrate Concentration & Saturation

When there is **no S**, V_0 is also **0**. As S gets big, V_0 approaches $V_{\max}$ — **"Saturation"**

As larger amounts of substrate are added to a reaction, the available enzyme binding sites become filled to the limit of $V_{\max}$.



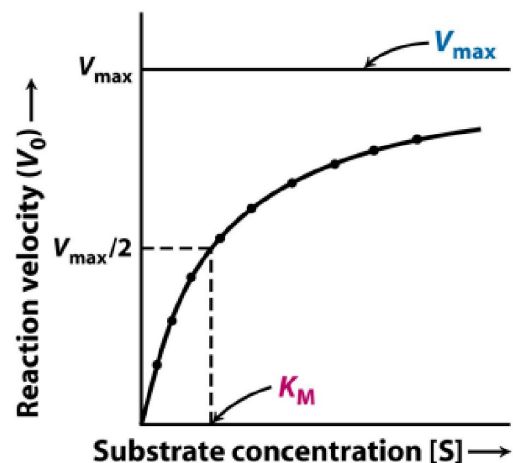
Beyond this limit the enzyme is **saturated with substrate** and the reaction rate **ceases to increase.**



Factors Affecting Enzyme Reaction Velocity

SUBSTRATE CONCENTRATION [S]

- 1 Increasing [S] increases the rate of the reaction **hyperbolically** in proportion with the increase in [S] until all active sites are saturated.
- 2 The reaction reaches its maximal velocity ($V_{\max}$) and further addition of substrate has no effect on the reaction rate, i.e. the reaction is **no longer dependent on [S].**





Effect of [S]: The K_m

MICHAELIS-MENTEN CONSTANT

The [S] at which the reaction rate is **half V_{max}** is a constant for the particular enzyme and substrate, and is known as the K_m .

It is a measure of the **affinity** of the enzyme for the particular substrate.



Characteristic

K_m is characteristic of an enzyme and its particular substrate and reflects the **affinity** of the enzyme for that substrate.



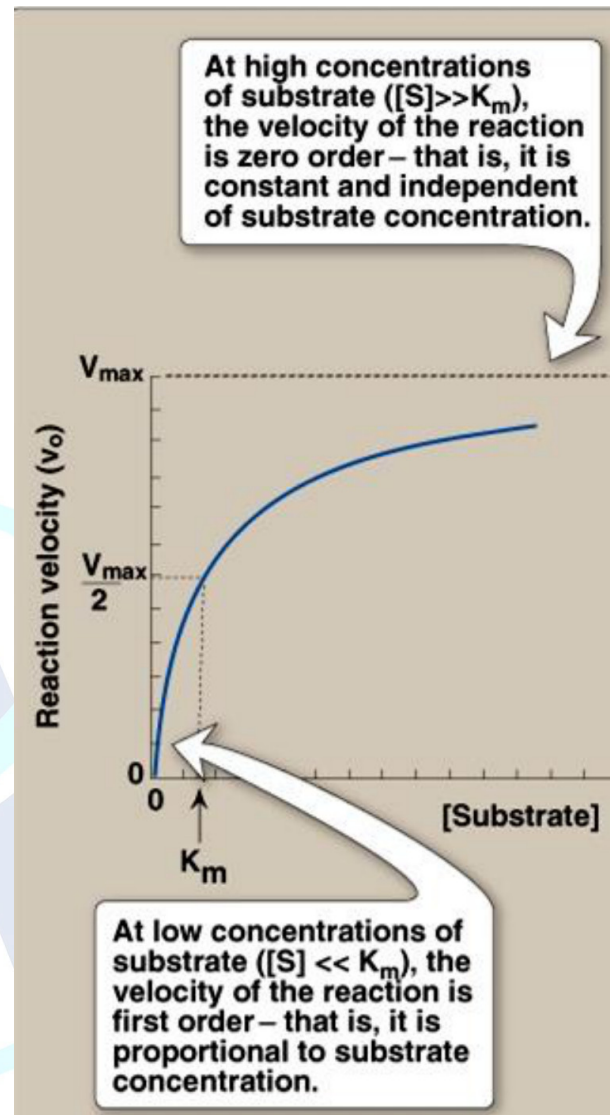
Numerically equal

K_m is numerically equal to the substrate concentration at which the reaction velocity is equal to **one half V_{max}** .



Constant

K_m **does not vary** with enzyme concentration.

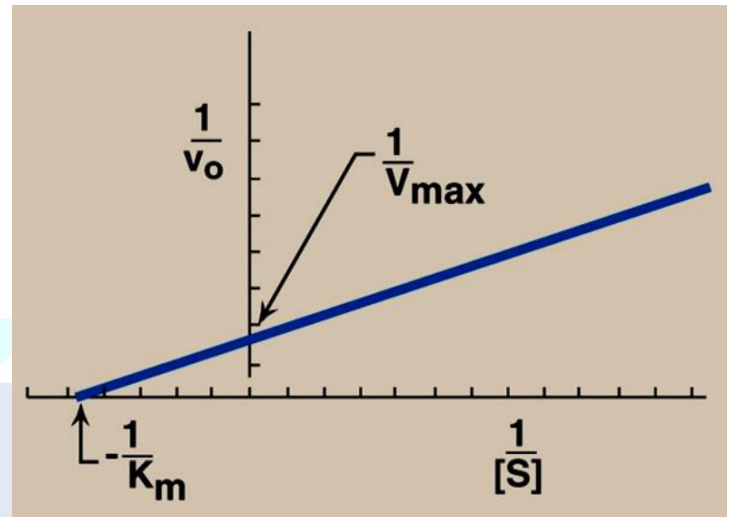
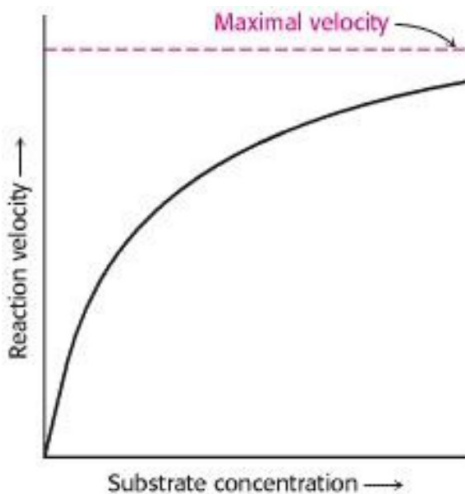


$$V_0 = \frac{V_{max}[S]}{\{K_m + [S]\}} \quad K_m = [S] \left\{ \left[\frac{V_{max}}{V_0} \right] - 1 \right\}$$



Lineweaver–Burk Plot

When it is difficult to determine $V_{\max}$ and hence the K_m of a reaction from the hyperbolic plot, you should plot the **reciprocal** of $[S]$ against the reciprocal of the velocity (**$1/v$ against $1/[S]$**).



This changes the hyperbolic curve into a **straight line**, which intercepts the x-axis at **$-1/K_m$** and the y-axis at **$1/V_{\max}$** . This plot is known as the **Lineweaver–Burk plot**.

$$-1/K_m$$

x-axis intercept

$$1/v_{\max}$$

y-axis intercept



Small vs Large K_m

Small $K_m \rightarrow$ high affinity

A numerically small (low) K_m reflects a **high affinity** of the enzyme for substrate, because a **low** concentration of substrate is needed to half-saturate the enzyme—that is, to reach a velocity that is one half $V_{\max}$.

VS

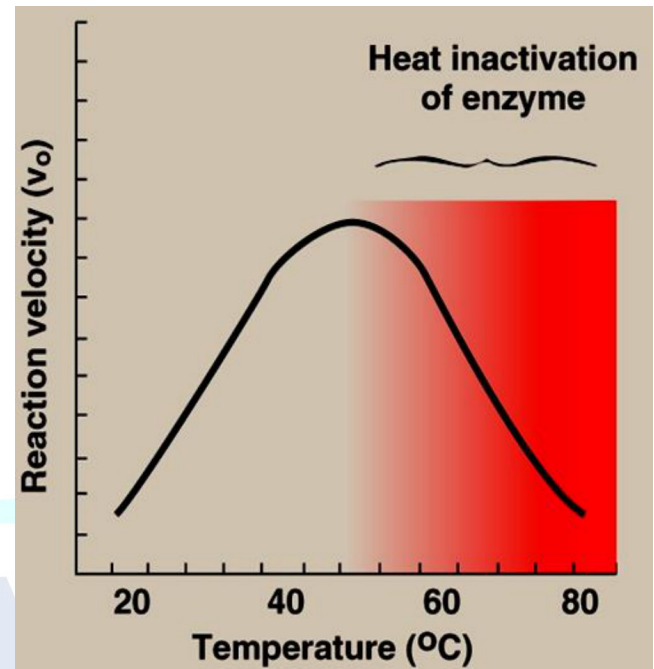
Large $K_m \rightarrow$ low affinity

A numerically large (high) K_m reflects a **low affinity** of enzyme for substrate because a **high** concentration of substrate is needed to half-saturate the enzyme.

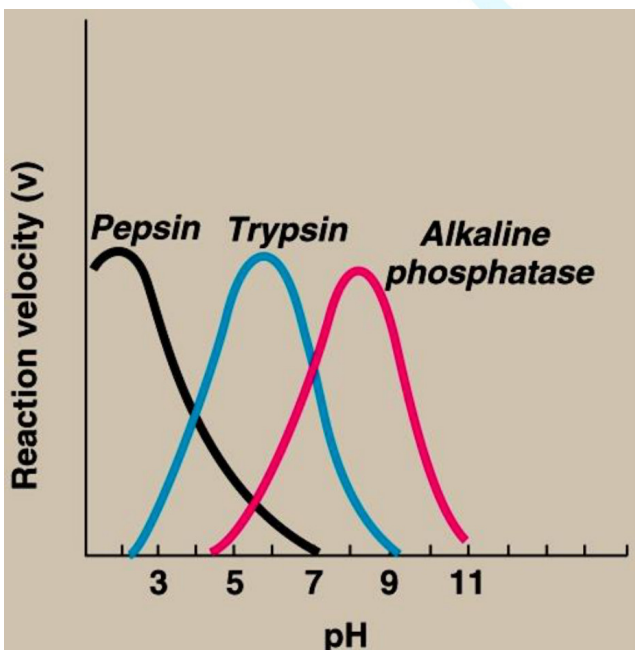


Effect of Temperature

- 1 Increasing **temperature** increases the rate of the reaction to a maximum known as the **optimum temperature**.
- 2 Above this temperature the enzyme protein is **denatured** and the reaction rate decreases.
- 3 If a person develops **fever**, all enzymes will have higher reaction velocities.



Effect of pH



A change in pH...

- Alters the **ionization state** of ionisable residues
- This occurs on both the **enzyme surface** and in the **active site**

The reaction rate changes to reach maximum at the enzyme **optimal pH**.

Further changes in pH may **denature** the enzyme, leading to decreased reaction rate.



Enzyme Inhibition

ENZYME INHIBITORS

Molecules & factors that interact with the enzyme, and **prevent it from working** (decrease velocity).

CLASSIFIED INTO

Nonspecific inhibitors

These affect **all enzymes** in the same way.

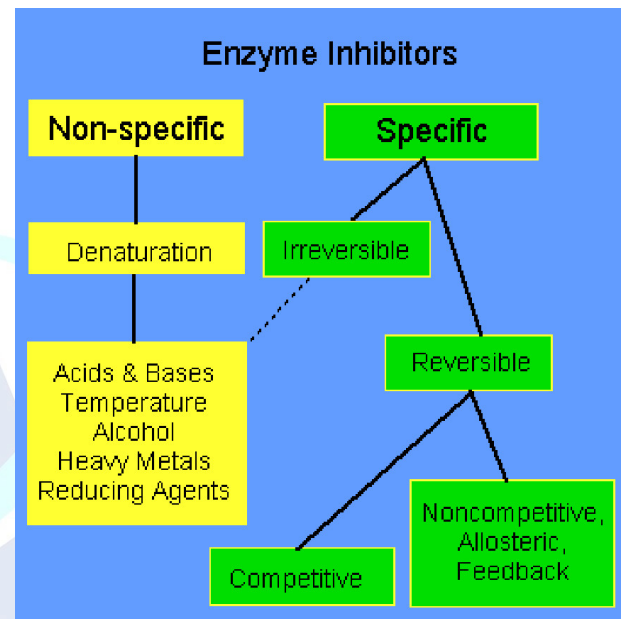
Specific inhibitors

These exert their effects on a **single enzyme**. They are classified into:

Reversible: the inhibitor can dissociate from the enzyme, and catalytic activity is regained:

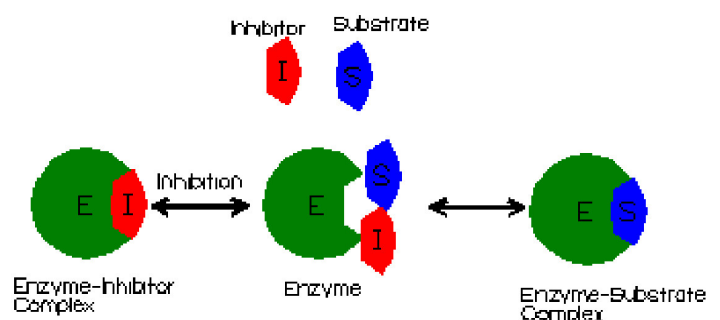
- **Competitive:** with respect of the substrate
- **Non-competitive:** do not resemble the enzyme's substrate, thus do not bind enzyme at the active site but at other site, inducing the enzyme to undergo conformational changes

Irreversible: when the enzyme is permanently inactivated



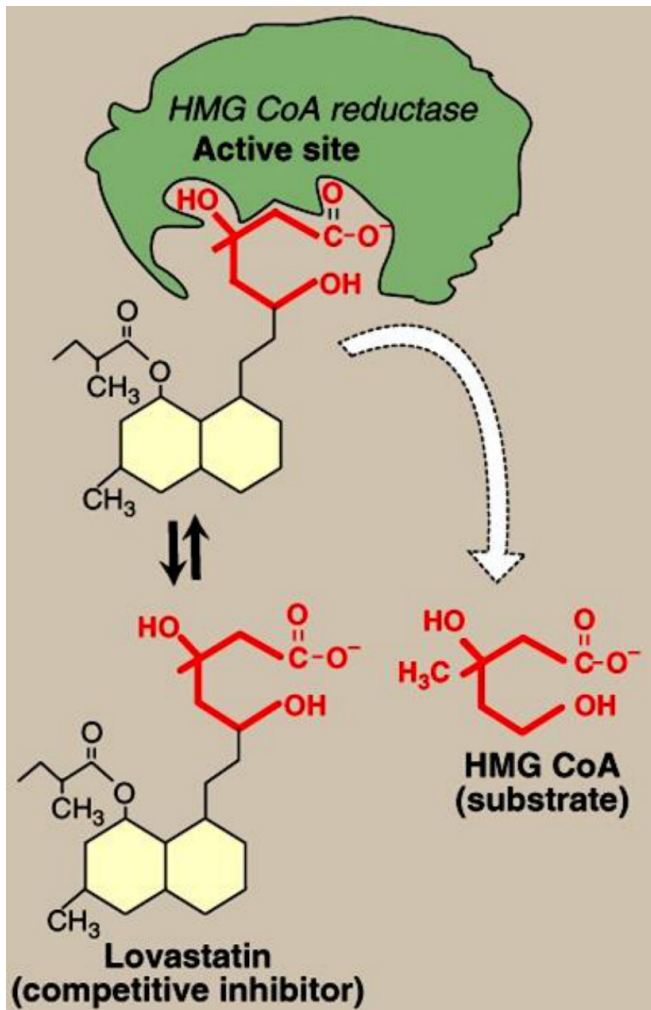
A Competitive Inhibitor is...

- ✓ Structurally **similar to the substrate**
- ✓ Competes with the substrate for binding to the enzyme **active site**
- ✓ At high [S] or low inhibitor concentration [I], the inhibitor will cause **little disruption** to normal enzyme function





Competitive Enzyme Inhibition



Increasing **[S]** will **displace the inhibitor** and allows the reaction to reach **V_{max}** .

$$K_m \uparrow$$

K_m increases

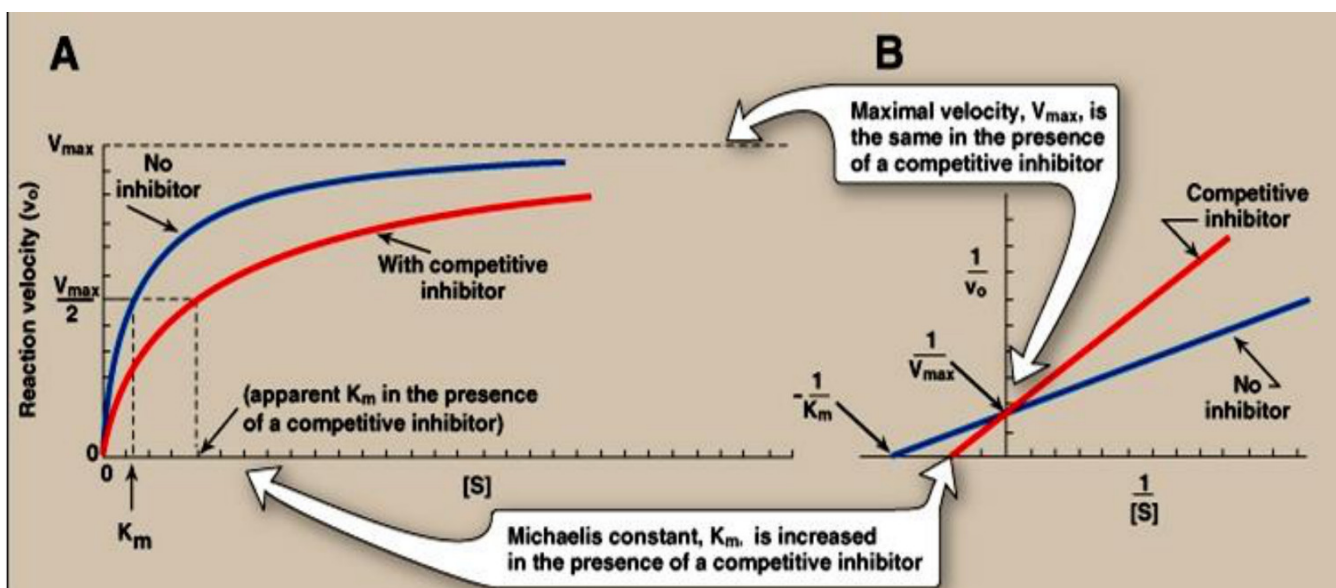
$$V_{max} =$$

V_{max} remains unchanged

EFFECT ON K_M

A competitive inhibitor **increases the apparent K_m** for a given substrate. This means that, in the presence of a competitive inhibitor, **more substrate** is needed to achieve one half V_{max} .

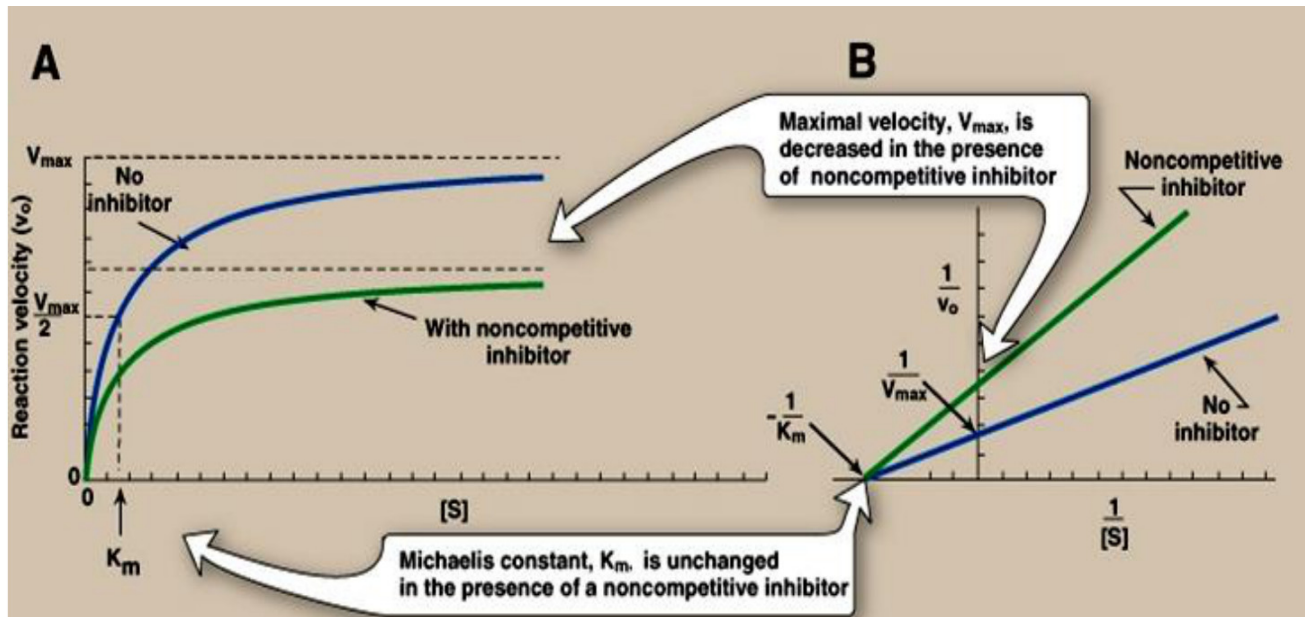
Lovastatin (competitive inhibitor) vs HMG CoA (substrate) at the HMG CoA reductase active site





Noncompetitive Enzyme Inhibition

The inhibitor **does not resemble the substrate** and may bind to a part on the enzyme other than the active site. Increasing $[S]$ will not displace the inhibitor, therefore $V_{\max}$ will decrease while the K_m will remain unchanged.



Different sites

Noncompetitive inhibition occurs when the inhibitor and substrate bind at **different sites** on the enzyme. The inhibitor can bind either **free enzyme or the ES complex**, preventing the reaction from occurring.

Effect on $V_{\max}$

Cannot be overcome by increasing the concentration of substrate. Therefore, noncompetitive inhibitors **decrease the apparent $V_{\max}$** .

K_m unchanged

They do not interfere with the binding of substrate to enzyme, so the enzyme shows the **same K_m** in the presence or absence of the inhibitor.

$V_{\max}$ ↓

$V_{\max}$ decreases

K_m =

K_m remains unchanged



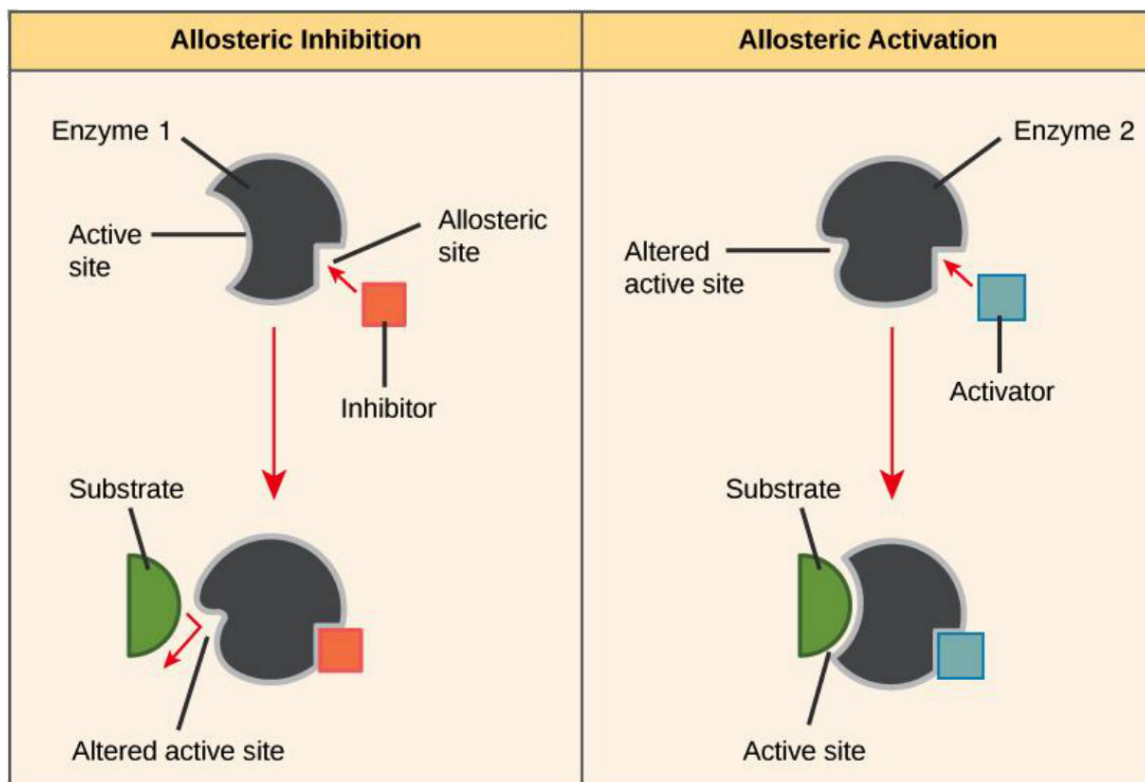
Regulation of Enzyme Activity

Allosteric sites

Some enzymes have binding sites **other than the active site** (**allosteric enzymes**).

Effector binding

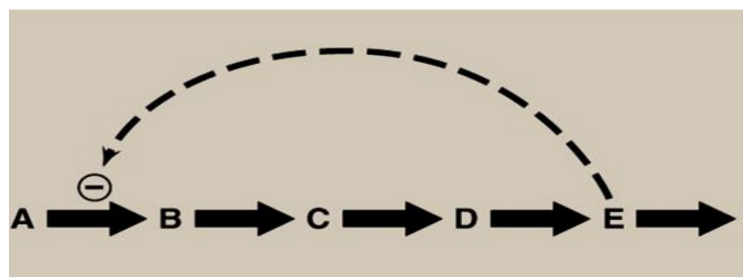
When an **effector** binds to that allosteric site, the resulting change in conformation of the enzyme may **increase (+)** or **decrease (-)** the affinity of the enzyme for its substrate and the maximal catalytic activity of the enzyme (V_{max}).



Feedback Inhibition

The **effector** molecule may be the **end product** of a metabolic pathway.

This may cause **negative feedback** (feedback inhibition) to stop the production of more of this end product.





Covalent Modification of Enzyme Activity

Enzyme activity can be modified by **covalent attachment of a phosphoryl group** to an amino acid residue, which can be:

Active

Stimulation of enzyme

Inactive

Inhibition of enzyme activity

Fast

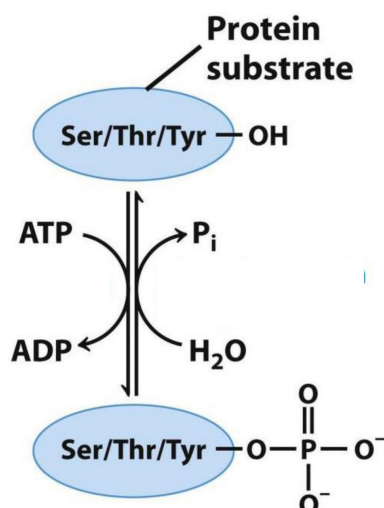
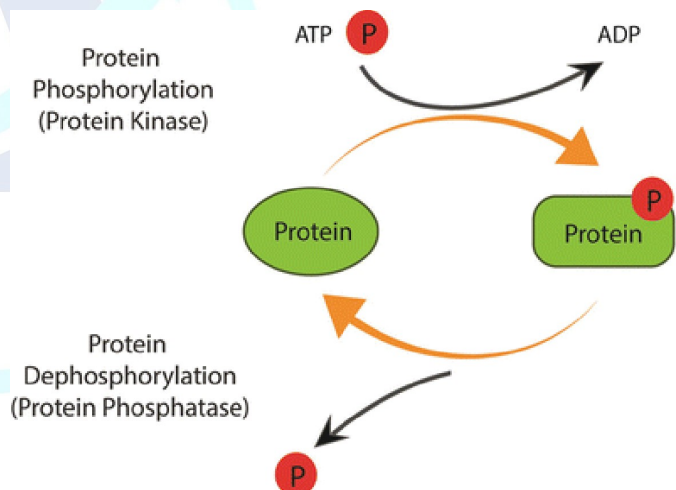
The response is usually very fast (acute)

Binding a molecule covalently to an enzyme will **alter its conformation**, and therefore its activity.



Phosphorylation Affects Enzyme Activity

- 1 Protein phosphorylation is catalyzed by **kinases**
- 2 Dephosphorylation is spontaneous, or catalyzed by **protein phosphatases**
- 3 Typically, **hydroxyl groups** of Ser, Thr, or Tyr are phosphorylated



MOST COMMON COVALENT MODIFICATION

Adding a **phosphate group** to the —OH group of:

Serine

Tyrosine

Threonine



Induction & Repression of Enzyme Synthesis

The previous mechanisms of regulating enzyme activity do **not** involve any change in the **amount of enzyme protein**.

Cells may regulate the amount of enzyme (and therefore the total activity, $V_{\max}$) by changing the **rate of synthesis** of the enzyme protein.

Gene

Occurs at the level of the gene

Induction

(stimulation)

Repression

(inhibition)

It is a **slow process** (hours – days).



Check Yourself

Q1

Molecule 'X' is an enzyme inhibitor that reversibly binds to an enzyme at a site that is distinct from its active site. Molecule 'X' must NOT be what type of inhibitor?

- ☐ 1 Non-specific inhibitor
- ☐ 2 Noncompetitive inhibitor
- ☐ 3 Competitive inhibitor
- ☒ 4 1 and 3

Q2

The K_m (Michaelis constant) of an enzyme-catalyzed reaction?

- ☐ 1 is a measure of the turnover rate of the enzyme
- ☐ 2 has a lower numerical value when the enzyme has high affinity for its substrate
- ☐ 3 is the substrate concentration when the reaction rate is half of the maximum rate
- ☒ 4 2 and 3



To Summarise...

KINETICS V

Enzyme kinetics

- Study of the rates of enzyme-catalysed reactions
- $E + S \rightleftharpoons ES \rightarrow E + P$ (k_1 , k_{-1} , k_2)
- Began in 1902 with Brown's invertase study

EQUATION V_0

Michaelis–Menten

- $V_0 = V_{\max} [S] / (K_m + [S])$
- $V_{\max}$: maximum rate at saturating $[S]$
- Increasing $[S]$ raises rate hyperbolically until saturation

AFFINITY K_m

Michaelis constant

- $[S]$ at which rate is half $V_{\max}$
- Small K_m = high affinity; large K_m = low affinity
- Does not vary with enzyme concentration

PLOT $1/v$

Lineweaver–Burk

- Plot $1/v$ against $1/[S]$
- Straight line: x-intercept $-1/K_m$
- y-intercept $1/V_{\max}$

FACTORS $T \cdot pH$

Temperature & pH

- Rate rises to an optimum temperature, then enzyme denatures
- pH alters ionization of residues (surface & active site)
- Rate peaks at the optimal pH

INHIBITION 2

Reversible inhibitors

- Competitive: $K_m \uparrow$, $V_{\max}$ unchanged
- Noncompetitive: $V_{\max} \downarrow$, K_m unchanged
- Irreversible: enzyme permanently inactivated

REGULATION OF ENZYME ACTIVITY

Allosteric sites ($\pm$)


Feedback inhibition



Phosphorylation — fast



Induction / repression — slow