CORE IDEA 1: THE CELL AND BIOMOLECULES OF LIFE
TOPIC 1.3: ENZYMES

Learning Outcomes:

(p) Explain the mode of action of enzymes in terms of an active site, enzyme-substrate complex, lowering of activation energy and enzyme specificity using lock-and-key and induced-fit hypotheses.

(q) Investigate and explain the effects of temperature, pH, enzyme concentration and substrate concentration of an enzyme-catalysed reaction by measuring rates of formation of products (e.g. measuring gas produced using catalase) or rate of disappearance of substrate (e.g. using amylase, starch and iodine).

(r) Describe the structure of competitive and non-competitive inhibitors with reference to the binding sites of the inhibitors.

(s) Explain the effects of competitive and non-competitive inhibitors (including allosteric inhibitors) on the rate of enzyme activity.

References

Campbell, Urry, Cain, Wasserman, Minorsky, Reece (2018) **Biology – A Global Approach (11th Edition)(Global Edition) Chapter 6: Energy and Life pg. 151 – 161** (Pearson Publication) ISBN-10 1-292-17043-3

Lecture Outline:

1 Introduction

2 Properties of Enzymes

3 Mode of Action of Enzymes

4 Enzyme Kinetics

5 Factors Affecting Enzyme Activity

6 Enzyme Inhibition

7 Allosteric Regulation

1 INTRODUCTION

Enzymes are biological catalysts which speed up the rate of metabolic reactions while remaining unchanged at the end of the reaction.

Metabolic reactions are spontaneous (can occur without enzymes) but they occur at a very slow rate. Thus, enzymes are needed to speed up these reactions. They are involved in almost all biochemical reactions in living organisms including respiration, photosynthesis, digestion and biosynthesis of macromolecules.

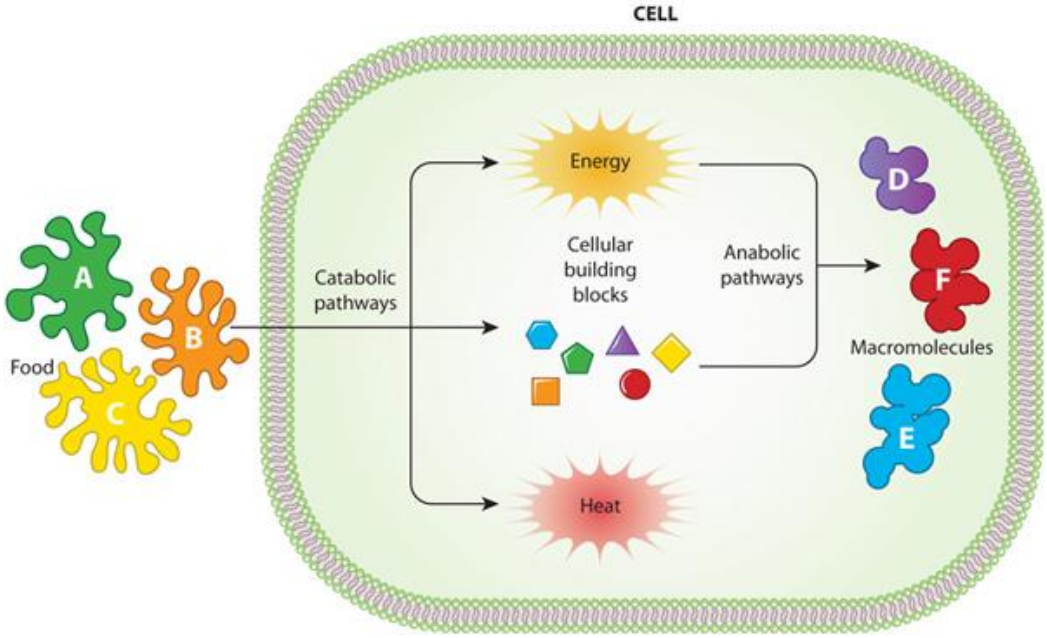
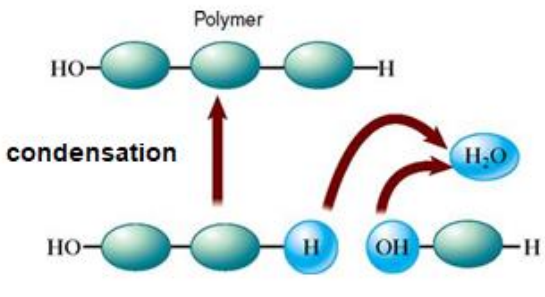


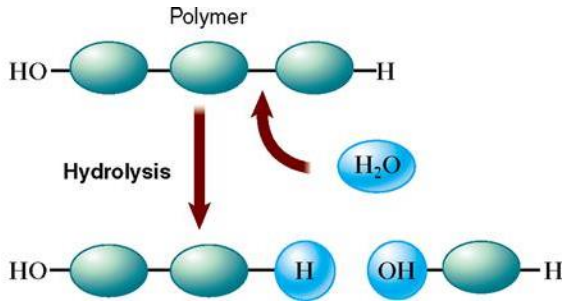
Fig. 1.1: Overview of metabolic reactions in a generalised animal cell

Metabolic reactions can be classified into catabolic and anabolic reactions:

Anabolic Reactions	Catabolic Reactions
Synthesis of complex molecules from simpler molecules	Breakdown of complex molecules into simpler molecules
Energy required	Energy released
E.g. Formation of glycogen from glucose, catalysed by glycogen synthase	E.g. Breakdown of sucrose to glucose and fructose, catalysed by sucrase



(a) Anabolic reaction via condensation



(b) Catabolic reaction via hydrolysis

Fig. 1.2: Anabolic and catabolic reactions

DID YOU KNOW?

Not all enzymes are protein in nature. Molecules like RNA can also have catalytic functions. RNA that functions as enzymes are known as ribozymes.

E.g. An RNA molecule found in ribosome, called peptidyl transferase, catalyses formation of peptide bond between amino acids during protein synthesis. *(Details will be covered in Core Idea 2)*

2 PROPERTIES OF ENZYMES

2.1 Enzymes are specific

Enzymes have a specific active site which recognises and binds to specific groups of atoms or type of bond present in the substrates.

Thus, most enzymes are specific to one particular type of substrate molecule e.g. amylase hydrolyses amylose but not cellulose.

2.2 Enzymes are effective in minute amounts

Enzymes can catalyse a reaction repeatedly without undergoing permanent chemical changes itself i.e. it **remains unchanged** at the end of the chemical reaction.

2.3 Some enzymes require the aid of cofactors to perform their functions

Cofactors are **non-protein** components that are required for the functioning of the enzyme.

Types of cofactors include:

1. **Inorganic ions**
   E.g. Zn^{2+} acts as a cofactor for carbonic anhydrase

2. **Coenzymes**
   Coenzymes are organic molecules (contains C and H) that are loosely bound to enzymes.

   E.g. Nicotinamide adenine dinucleotide (NAD^{+}) is a coenzyme for dehydrogenase involved in cellular respiration. *(Details will be covered in Core Idea 3)*

3. **Prosthetic groups**
   Prosthetic groups are organic molecules that remain tightly bound to enzymes.

   E.g. haem is an iron-containing porphyrin ring found in catalases and peroxidases, which catalyse the decomposition of hydrogen peroxide into water and oxygen.

3 MODE OF ACTION OF ENZYMES

Learning Outcome (p):
Explain the mode of actions of enzymes in terms of active site, enzyme-substrate complex, lowering of activation energy and enzyme specificity using lock-and-key and induced-fit hypothesis.

Enzymes are mostly globular proteins, each having a **specific three-dimensional conformation**.

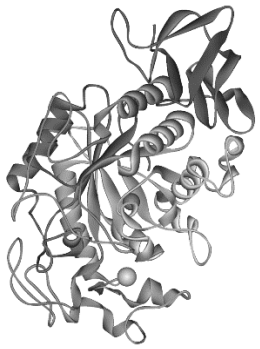


Fig. 3.1: 3D conformation of amylase

Amino acids that make up the enzyme have different functions (Ref. to Fig. 3.2):

1. **Contact residues**. Amino acids that recognise and **bind substrate(s)** to active site via multiple weak non-covalent interactions.

2. **Catalytic residues**. Amino acids that **catalyse the conversion of substrate to product.**

Contact and catalytic residues are found in the **active site** of the enzyme.

3. **Structural residues**. Amino acids are involved in maintaining the **overall three-dimensional conformation** of the enzyme.

4. **Non-essential residues** are generally found on the surface of the protein. They are amino acids which do not play specific functions.

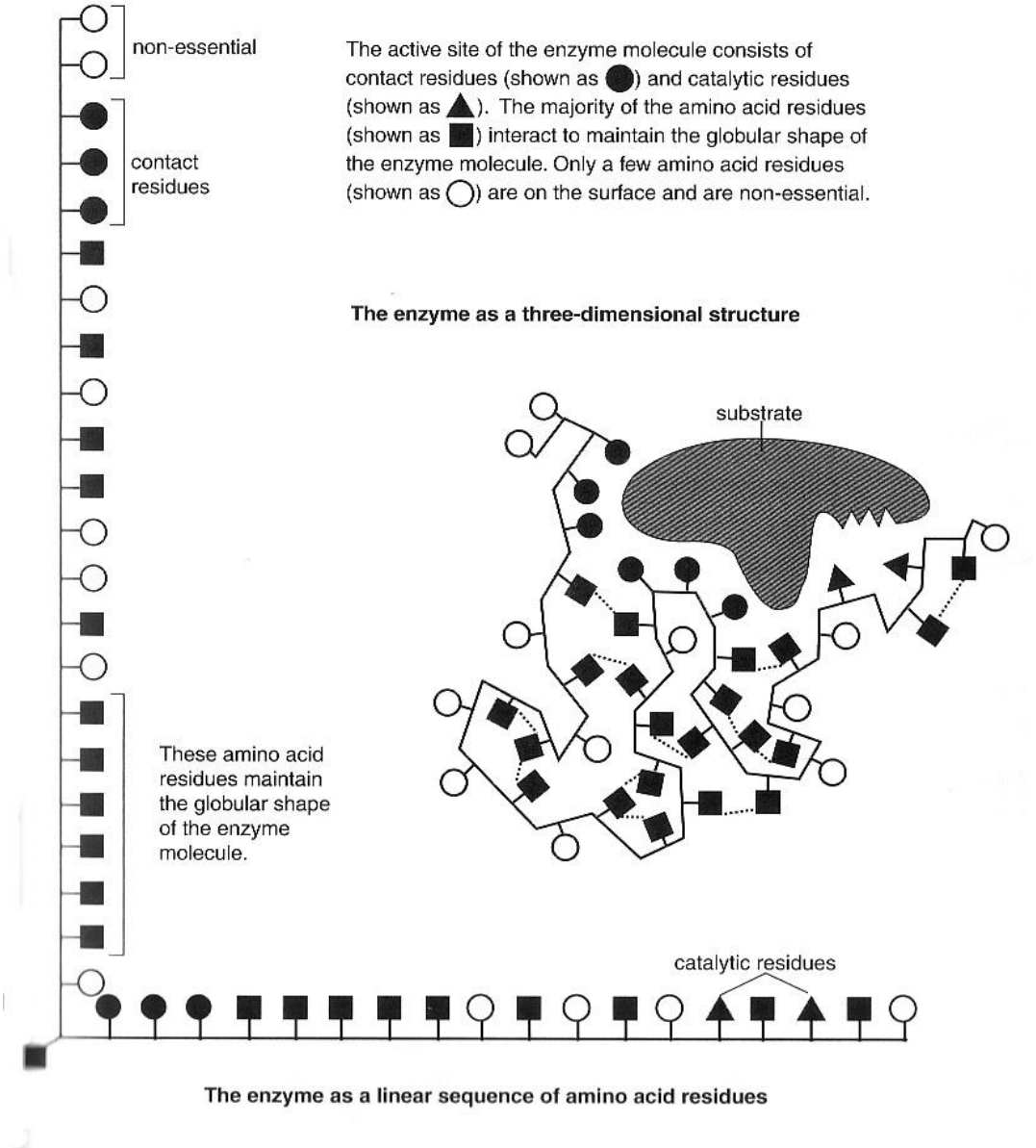


Fig. 3.2: Folding of a linear polypeptide chain to form the 3D conformation of an enzyme.

3.1 Active Site

Although the enzyme molecule is usually a relatively large molecule, only a small part of the enzyme, known as the **active site**, actually comes into **direct contact** with the substrate.

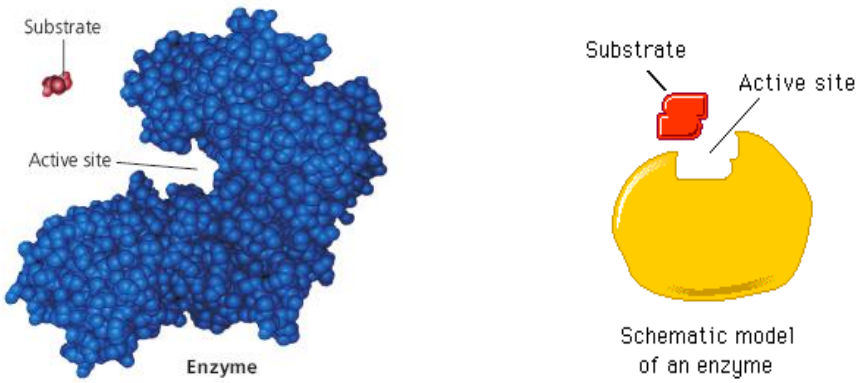


Fig. 3.3: Active site of enzyme that binds to the substrate

The active site is formed by **small number of amino acids from different parts of a single polypeptide chain** brought together through the precise folding of the polypeptide chain.

REMEMBER: Contact and catalytic residues are found in the **active site** of the enzyme.

It has a specific three-dimensional conformation that is **complementary** to its substrate in terms of **shape, size, charge and orientation**.

3.2 Enzyme-Substrate Complex

The substrate binds to active site of enzyme to form an **enzyme-substrate complex** (ES complex). The substrates are held in active site by contact residues via non-covalent bonds such as **hydrogen bonds, ionic bonds and hydrophobic interactions**.

enzyme + substrate(s) → enzyme-substrate complex → enzyme + product(s)

The R groups of the of the catalytic amino acid residues catalyse the conversion of substrate to product. Once the products are formed, they are no longer complementary to the active site and thus will leave the enzyme. The enzyme is then available to act on other substrates.

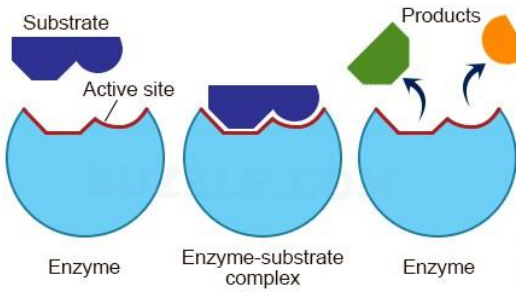


Fig. 3.4: Enzymatic reaction

3.3 Mode of Action

There are two hypotheses as to how an enzyme recognises and binds to its substrate:
1 Lock-and-key hypothesis
2. Induced fit hypothesis

(i) Lock-and-key hypothesis

The enzyme's active site is **perfectly complementary** to the substrate in terms of shape, size, charge and orientation. The substrate binds to enzyme's active site **precisely** to form the enzyme-substrate complex.

This mode of activation is more probable for enzymes that work on only one type of substrate.

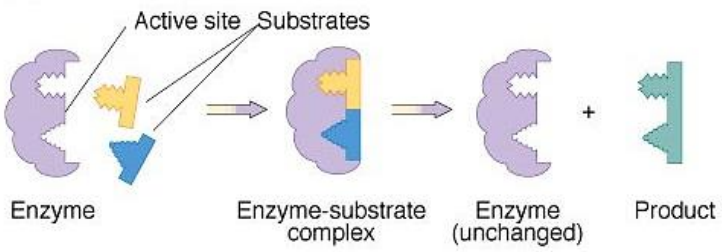


Fig. 3.5: Lock-and-key hypothesis

(ii) Induced fit Hypothesis

The enzyme's active site is **not perfectly complementary** to the substrate in terms of shape, size, charge and orientation.

Upon forming some bonds with the substrate, the enzyme undergoes a **conformational change**, which leads to a **precise fit** to form the enzyme-substrate complex.

This mode of action is more probable for enzymes that work on a group of closely-related substrates, e.g. lipases.

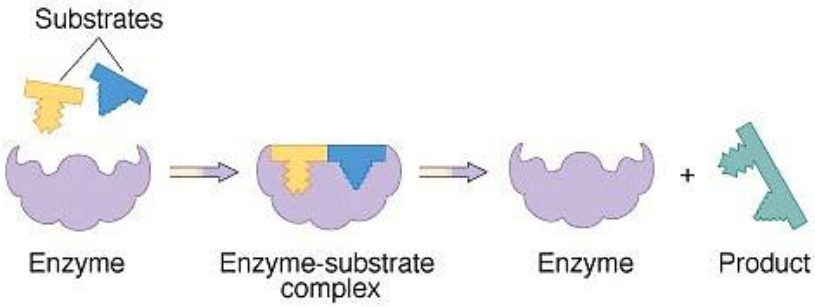


Fig. 3.6: Induced fit hypothesis

3.4 Lowering Activation Energy

Activation energy (E_A) is the initial investment of energy that reactant molecules must possess to overcome an energy barrier, for a reaction to begin.

For uncatalysed reactions, E_A is usually in the form of thermal energy (heat) that reactant molecules absorb from surroundings.

With increase in temperature, reactant molecules gain kinetic energy, thus colliding more frequently and more forcefully. When the molecules have absorbed sufficient energy for bonds to break, the reactants have reached an unstable condition known as the transition state and eventually react to form products.

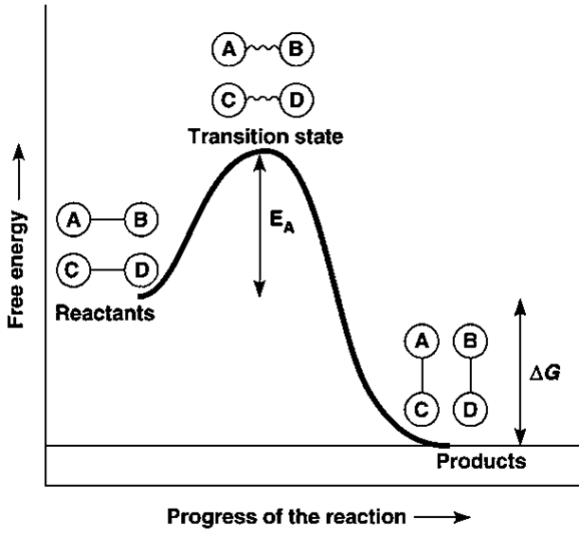


Fig 3.7: Acquisition of energy to voercome E_A of a uncatalysed reaction

Enzymes speed up biological reactions by providing an alternative pathway, which has **lower activation energy (E_A)** as compared to the uncatalysed reaction. The reactions can then occur at a faster rate without an increase in temperature.

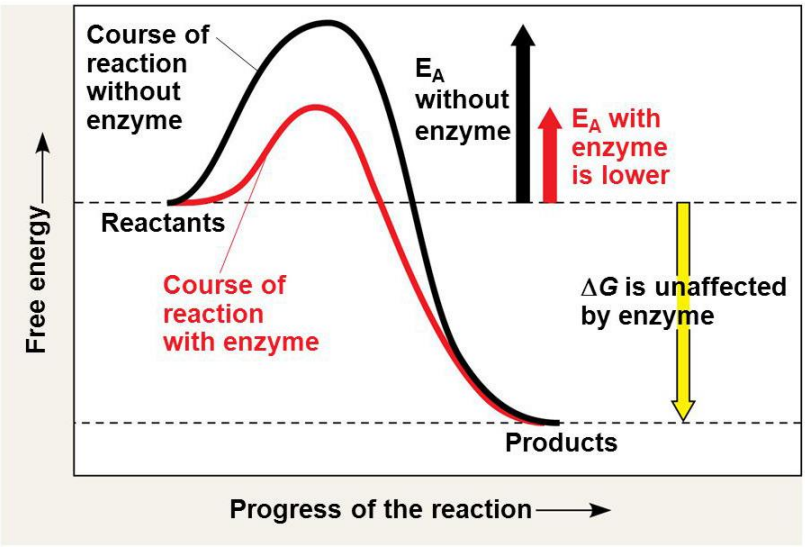


Fig 3.8: Enzymes lower the E_A of a catalysed reaction

Enzymes lower the E_A of a reaction by forming an enzyme-substrate complex that promotes formation of **transition state** by:

1. Bringing substrates into **close proximity** and **correct orientation** at the active site
   This occurs during formation of enzyme-substrate complex where reacting functional groups of substrates will be brought near to each other, allowing catalytic residues in the active site to catalyze the reaction.

   E.g. cellulose synthase catalyzing β-1,4-glycosidic bond in cellulose synthesis

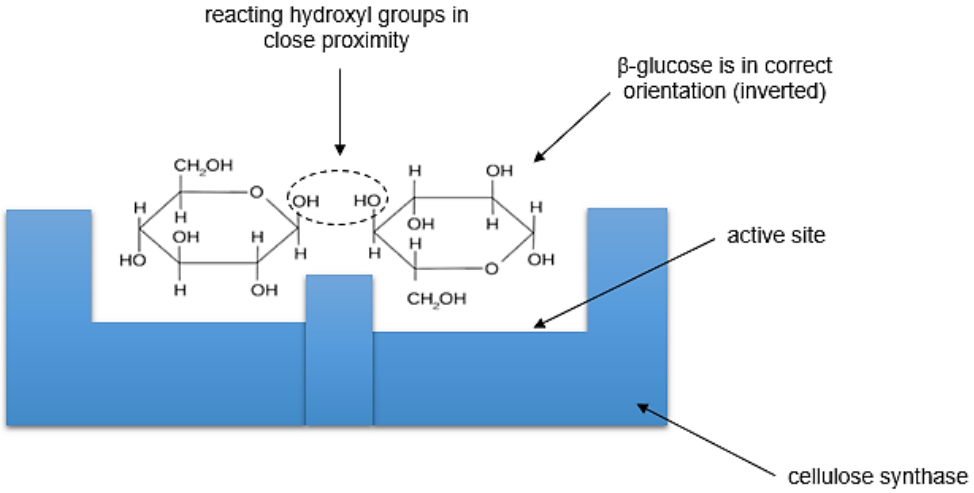


Fig 3.9: Mode of enzyme action

2. **Inducing bond stress** in the substrate
   By putting physical stress on bonds to increase the likelihood that the substrates will reach transition state.

3. Providing a **conducive microenvironment** for reaction
   R groups of catalytic residues at the enzyme's active site may **alter the charge** or **electron distribution** of bonds within substrate to increase its reactivity.

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Checkpoint 1

Identify the mechanisms by which enzymes lower the activation energy of a reaction.

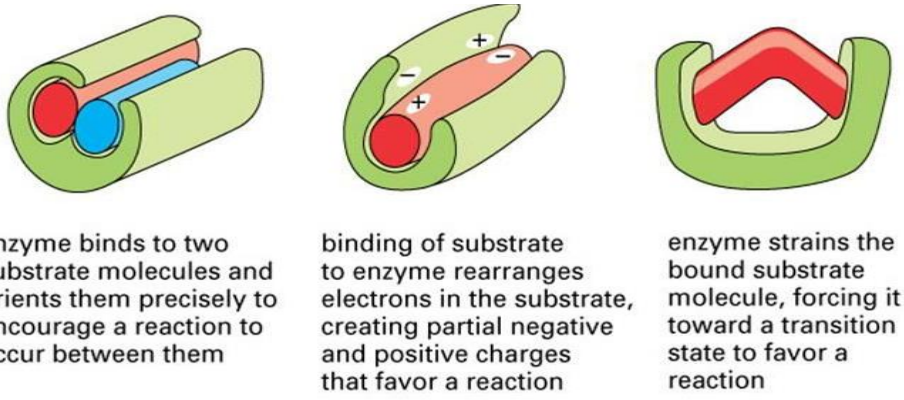


Fig. 3.10: Mechanisms to lower activation energy

4 ENZYME KINETICS

Learning Outcome (q):
Investigate and explain the effects of temperature, pH, enzyme concentration and substrate concentration of an enzyme-catalysed reaction by measuring rates of formation of products (e.g. measuring gas produced using catalase) or rate of disappearance of substrate (e.g. using amylase, starch and iodine).

Enzyme kinetics is the study of the **rate** of chemical reactions which are catalysed by enzymes. The study of enzyme kinetics reveals the catalytic mechanism of an enzyme, its role in metabolism, how its activity is controlled, and how a drug or poison might inhibit the enzyme.

4.1 Investigating Rate of Enzymatic Reactions

The reaction rate of enzymes can be measured by:
- the **amount of substrate depleted** per unit time
- the **amount of product formed** per unit time

(Note: Per unit time may be in h^{-1}, min^{-1} or s^{-1} etc.)

When investigating the effect of a particular factor (variable) on the rate of an enzyme reaction, only the variable studied is changed. All other variables should be kept **constant**.

The **initial rate of reaction** (rate of reaction soon after the reaction starts) is measured in the investigation, as the concentration of substrate would decrease with time, affecting the reaction rate. The initial rate of reaction is determined by calculating the gradient of a product formed vs time graph or amount of substrate depleted vs time graph.

(i) Rate of Product Formation

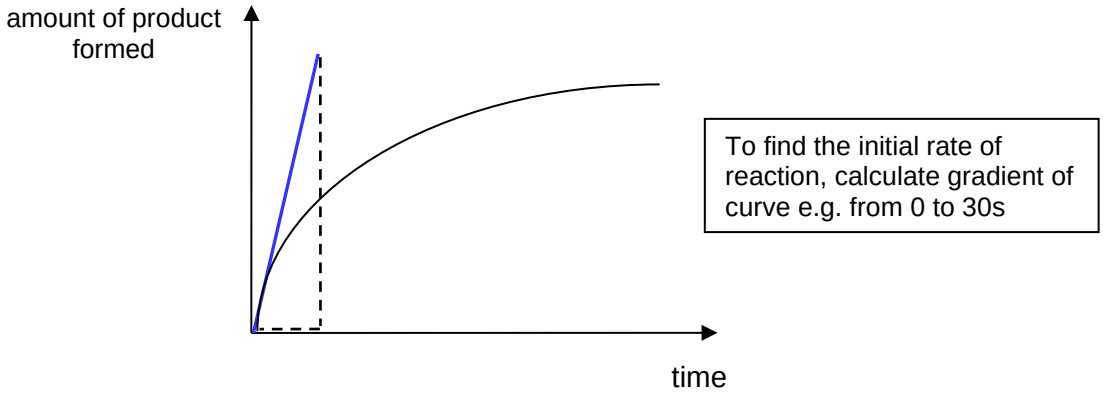


Fig. 4.1: Graph of amount of product formed vs time

(ii) Rate of Substrate Disappearance

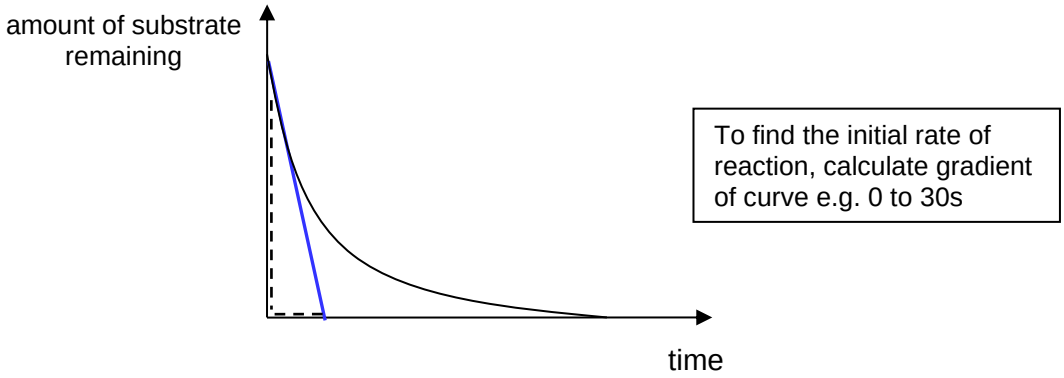


Fig. 4.2: Graph of amount of substrate remaining vs time

4.2 Enzyme Kinetics

V_{max} and Michaelis constant (K_M) values are commonly used in the study of enzyme kinetics.

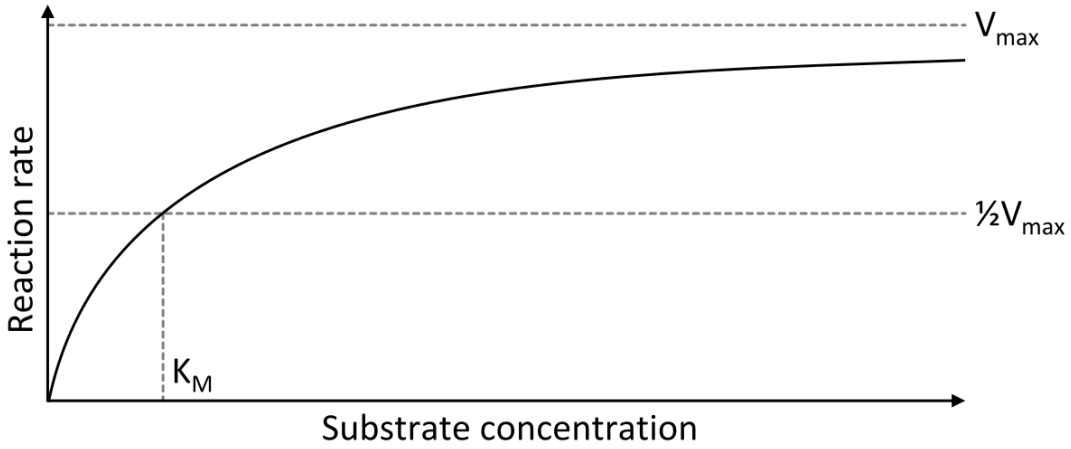


Fig. 4.3: Graph of initial rate of reaction against substrate concentration

(i) V_{max}

V_{max} is the maximum rate of reaction. At V_{max}:

- all enzyme active sites are saturated (bound to substrate)
- rate of ES complex fomation is at its maximum
- rate of product formation is at its maximum

(ii) Michaelis constant (K_M)

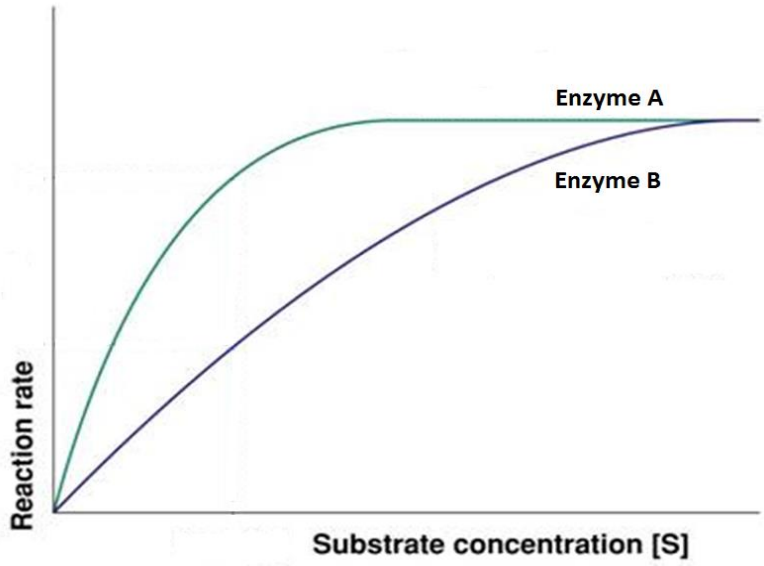
Michaelis constant (K_M) of an enzyme is the **substrate concentration** at which the rate of reaction catalyzed by the enzyme equals to half its maximum rate (i.e. **½ V_{max}**).

K_M is a measure of the affinity of enzyme for its substrate i.e. how readily enzyme reacts with its substrate:

- **Low K_M** indicates that enzyme has **high affinity** for substrate because ½ V_{max} is achieved at low substrate concentration.
- **High K_M** indicates that enzyme has **low affinity** for substrate because ½ V_{max} is achieved at high substrate concentration.

Checkpoint 2

Based on the graphs below, which enzyme has a higher affinity for its substrate?



5 FACTORS AFFECTING ENZYME ACTIVITY

As enzymes are protein in nature, they are affected by factors that affect the structure of proteins such as temperature, pH, substrate concentration and enzyme concentration.

5.1 Temperature

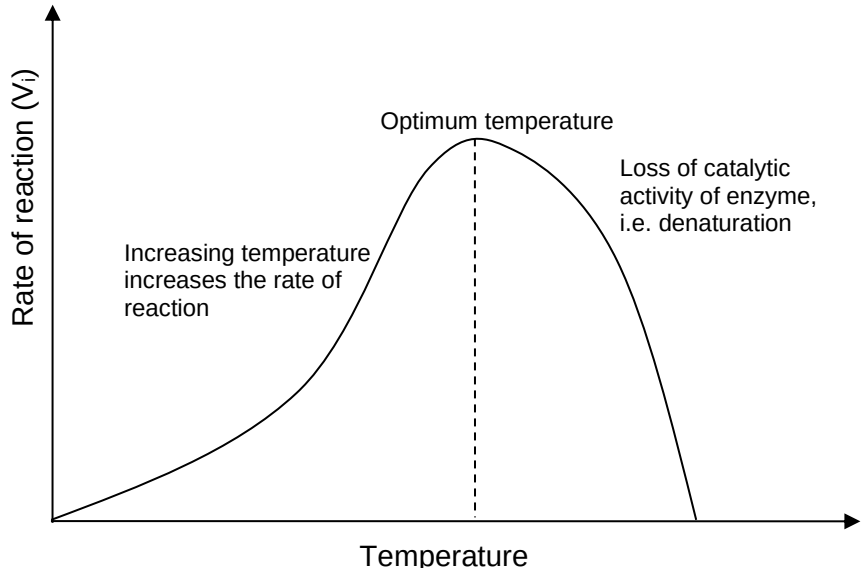


Fig. 5.1: Graph of initial rate of reaction against temperature

(i) At low temperatures

- Enzyme and substrate molecules possess low kinetic energy. Thus, rate of effective collisions is low and rate of formation of ES complexes is low. Enzymes are **inactive**.
- As temperature increases towards optimum, **kinetic energy** of the enzyme and substrate molecules increase. The rate of **effective collisions** between enzyme and substrate molecules increases thus rate of formation of **ES complexes** increases.
- As temperature increases towards optimum temperature, the **rate doubles for every 10°C rise** in temperature. The temperature coefficient, $\mathbf{Q_{10} = 2}$, where

$$\text{Temperature coefficient } (Q_{10}) = \frac{\text{Rate of enzyme-catalyzed reaction at } (x+10)^oC}{\text{Rate of enzyme-catalyzed reaction at } x^oC}$$

(ii) At optimum temperature

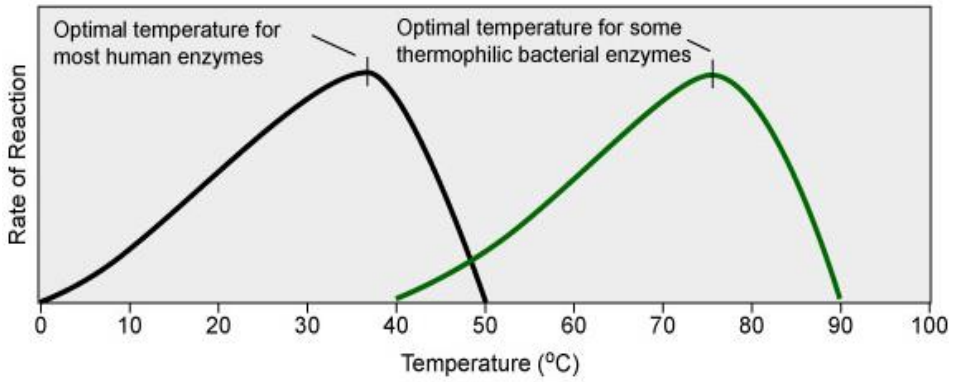
- There is **maximum rate of effective collisions** between enzyme and substrate molecules, thus maximum rate of ES complexes formation. Rate of reaction has reached its maximum (V_{max}).

(iii) At temperature above optimum temperature

- As temperature increases beyond optimum temperature, **kinetic energy** of the enzyme and substrate molecules increase.
- **Thermal agitation** of enzyme molecules **disrupts weak non-covalent bonds** like hydrophobic interactions and hydrogen bonds which stabilise the secondary and tertiary structures of enzyme.
- The enzyme loses its specific 3-dimensional conformation. The active site is no longer complementary to the substrate, hence ES complexes cannot be formed. This results in a drastic decrease in rate of reaction. The enzyme said to be **denatured**.

CHALLENGE YOURSELF!

The optimum temperature for an enzyme varies considerably. Many Arctic plants have enzymes that function optimally at around 10°C, whereas those in bacteria inhabiting hot springs function optimally at around 80°C. For many human enzymes, the optimum temperature is around 37-40°C, and denaturation occurs at around 50°C.



With reference to your knowledge of protein structure, what type of bonds do you expect to find in higher proportion in thermophilic bacterial enzymes? Why?

5.2 pH

- Enzymes function effectively **over a narrow pH range**.
- Each enzyme has an **optimum pH** at which it functions most efficiently (e.g. pepsin at pH 2; sucrase at pH 4.5).
- Unlike the effects of heat on enzymes, the effects of pH are usually reversible, within limits. Restoring the pH to the optimum level usually restores the rate of reaction.

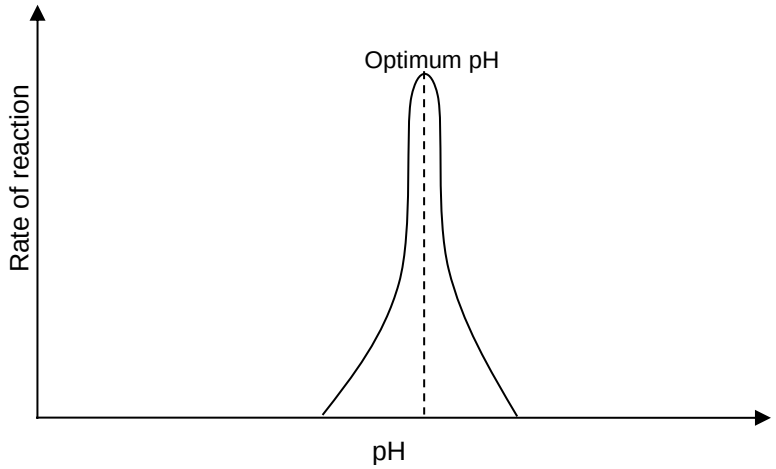


Fig. 5.2: Graph of initial rate of enzymatic reaction against pH

(i) At optimum pH

- All the ionic and hydrogen bonds between R groups of amino acids are intact. Hence enzyme active site is complementary to substrate molecule.
- There is **maximum rate of effective collisions** between enzyme and substrate thus **maximum rate of ES complexes formation**. Rate of reaction is at its maximum.

(ii) At pH higher or lower than optimum pH

- pH lower than optimum (more acidic due to increase in H^+ concentration) or pH higher than optimum (more basic due to increase in OH^- concentration) disrupts intramolecular **ionic bonds** and **hydrogen bonds** which stabilise the secondary and tertiary structures of enzyme.
- The enzyme **unfolds** and loses its specific 3-dimensional conformation. The active site is **no** the **longer complementary** to the substrate, hence the enzyme is unable to form ES complex and rate of reaction decreases. The enzyme said to be **denatured**.

5.3 Substrate Concentration

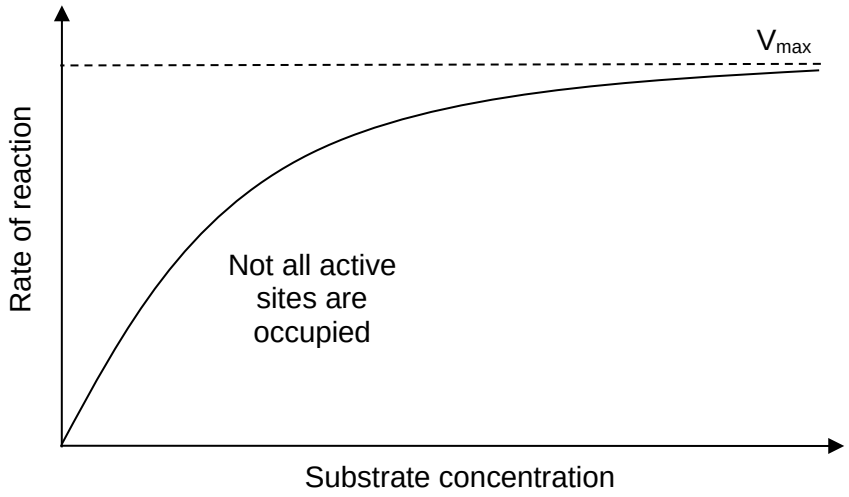


Fig. 5.3: Graph of initial rate of enzymatic reaction against substrate concentration

(i) At low substrate concentration

- As substrate concentration increases, rate of reaction **increases sharply.**
- This is due to an **increase in rate of effective collisions** between enzyme and substrate molecules thus **increasing the rate of ES complexes formation** and the rate of reaction increases.
- At this point, not all active sites of the enzyme molecules are occupied. **Substrate concentration** is the **limiting factor**.

> Limiting factor is defined as any factor that when increased in quantity, increases the rate of reaction.

(ii) At high substrate concentration

- As substrate concentration increases, the rate of reaction **remains constant / plateaus off.** The **maximum rate of reaction** (V_{max}) is reached.
- Enzymes are **saturated** i.e. all active sites of enzymes are occupied by substrate. At this point, substrate concentration is no longer limiting; other factors like enzyme concentration are now the **limiting factor**.

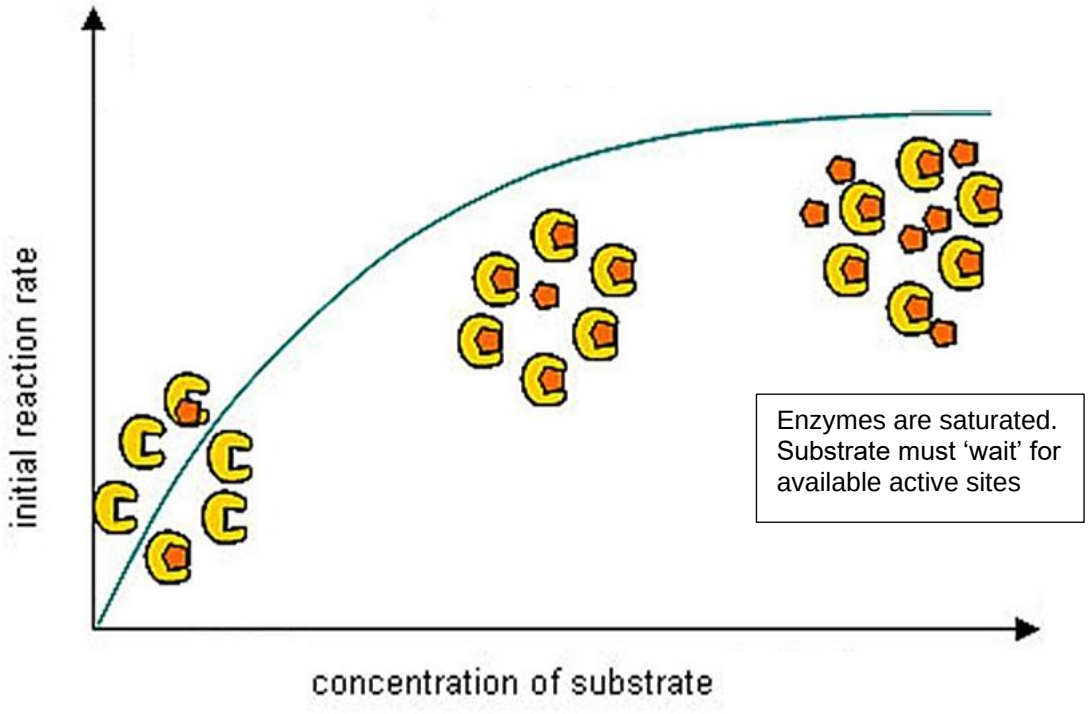


Fig. 5.4: Enzyme saturation at high substrate concentration

5.4 Enzyme Concentration

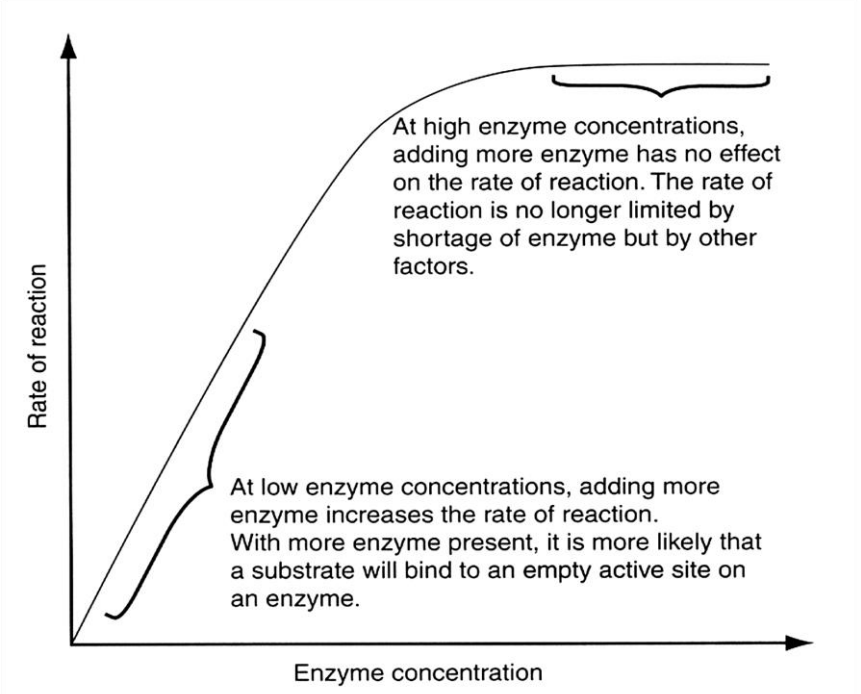


Fig. 5.5: Graph of initial rate of enzymatic reaction against enzyme concentration

(i) At low enzyme concentration

- As enzyme concentration increases, rate of reaction **increases sharply.**
- This is due to an **increase in rate of effective collisions** between enzyme and substrate molecules thus **increasing the rate of ES complexes formation** and the rate of reaction increases.
- At this point, **enzyme concentration** is the **limiting factor**.

(iii) At high enzyme concentration

- As enzyme concentration increases, the rate of reaction **remains constant / plateaus off.** The **maximum rate of reaction** (V_{max}) is reached.
- At this point, enzyme concentration is no longer limiting; other factors like substrate concentration are now the **limiting factor**.

6 ENZYME INHIBITION

Learning Outcome (r):
Describe the structure of competitive and non-competitive inhibitors with reference to the binding sites of the inhibitors.

Learning Outcome (s)
Explain the effects of competitive and non-competitive inhibitors (including allosteric inhibitors) on the rate of enzyme activity.

Enzyme activity can be reduced by addition of an **inhibitor**. This results in formation of an **enzyme-inhibitor (EI) complex**, preventing the enzyme from binding to its substrate.

Inhibition can be reversible or irreversible:

- The inhibition is **reversible** if the EI complex can dissociate.
  Weak bonds like hydrogen bonds are formed between the enzyme and the inhibitor. The binding of the inhibitor to the enzyme is loose.

  These weak bonds break upon collision with neighbouring molecules, causing inhibitors to leave the enzyme. The enzyme's catalytic activity is restored.

- The inhibition is **irreversible** if the EI complex cannot dissociate.
  Strong bonds like covalent bonds are formed between the enzyme and inhibitor. The binding of the inhibitor is **permanent**.

  These strong bonds do not break upon collision from neighbouring molecules. Binding of the inhibitor to the enzyme causes permanent change to the enzyme's conformation, such that the enzyme is unable to carry out its catalytic activity.

Inhibitors can be **competitive** or **non-competitive**. They have different effects on the rate of reaction and affect V_{max} and K_M of the enzyme differently.

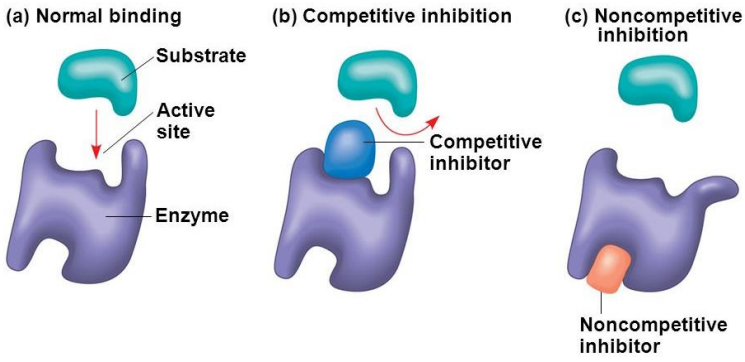


Fig. 6.1: Comparison between normal enzymatic action and different types of inhibition

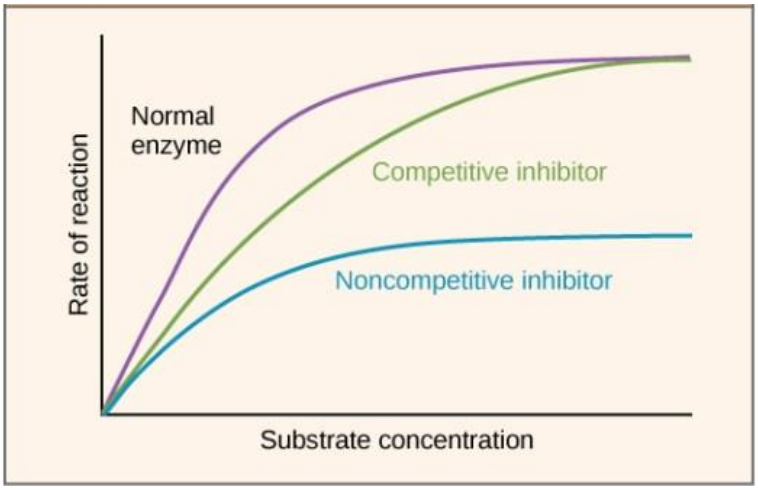


Fig. 6.2: Graphs of initial rate of various enzymatic reactions (uninhibited, competitive and non-competitive inhibition) against substrate concentration

6.1 Competitive inhibitors

Competitive inhibitors are **structurally similar** (in terms of shape, size, charge) to the substrate. Inhibitors compete with substrate for **binding to the active site** of the enzyme forming EI complexes.

Binding of competitive inhibitors occurs via weak bonds and hence the inhibition is reversible .

(i) At low substrate concentration

The formation of EI complex reduces the number of active sites available for the substrates to bind to form ES complex, resulting in a decrease in the rate of reaction.

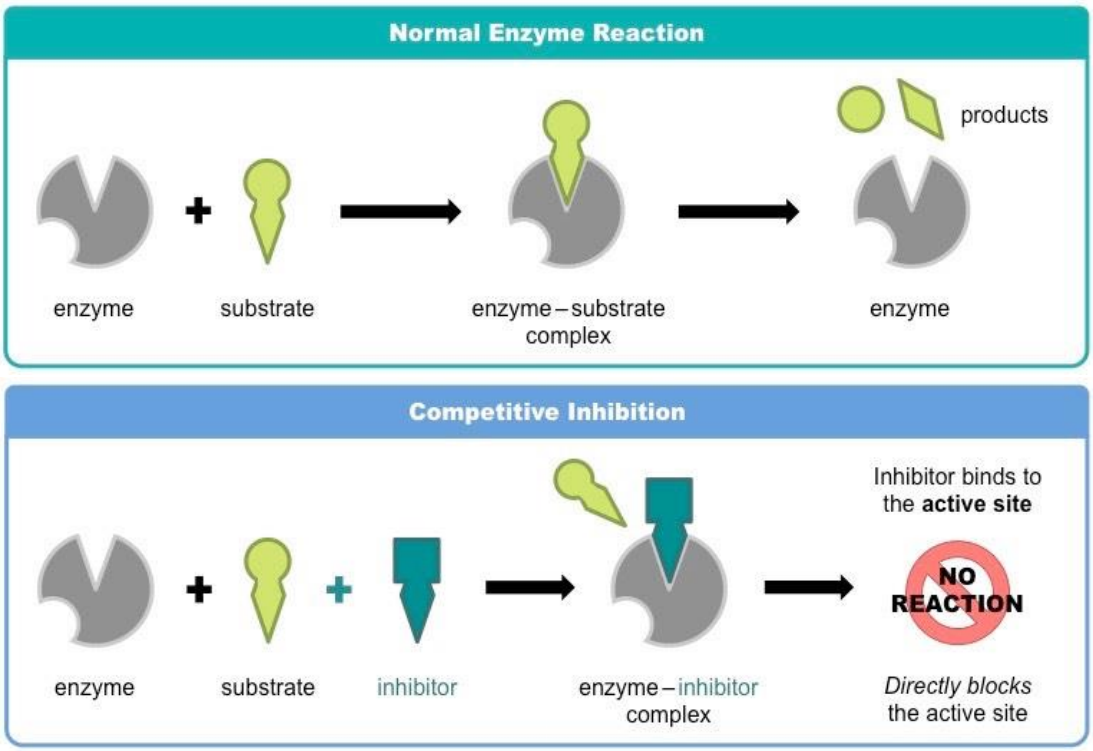


Fig. 6.3: Competitive inhibition

(ii) At high substrate concentration

Increasing substrate concentration increases the number of substrate molecules competing with inhibitor molecules for enzyme active site.

This increases the **probability** of the substrate binding to the active site to form ES complexes. At high substrate concentration, substrate outcompetes inhibitors and **V_{max} is reached**.

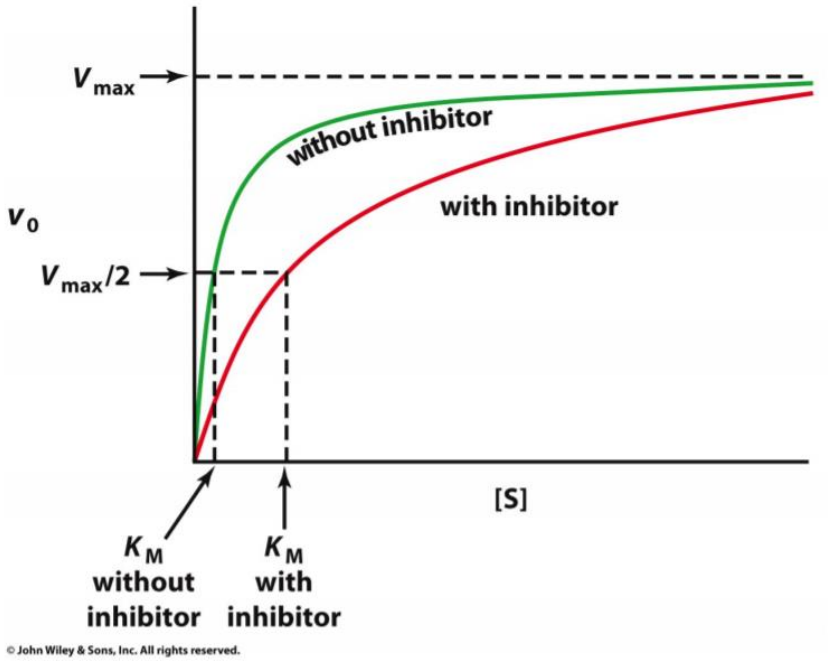


Fig. 6.4: Effect of competitive inhibitor

Thus, competitive inhibitors decrease rate of reaction at low substrate concentration but V_{max} is achievable at high substrate concentration. However, **K_M** of the reaction is higher because the affinity of the enzyme for its substrate is decreased in the presence of competitive inhibitors.

6.2 Non-competitive inhibitors

Non-competitive inhibitors are **not structurally similar** to the substrate. Inhibitors **binds at a site away from the active site** of the enzyme to forming EI complex.

Inhibitor binding results in a **conformational change** of the enzyme. This causes the active site to **no longer be complementary** to the substrate. Thus, the substrate is unable to bind to form ES complex and the rate of reaction decreases. Binding of non-competitive inhibitors occurs via strong covalent bonds and is irreversible.

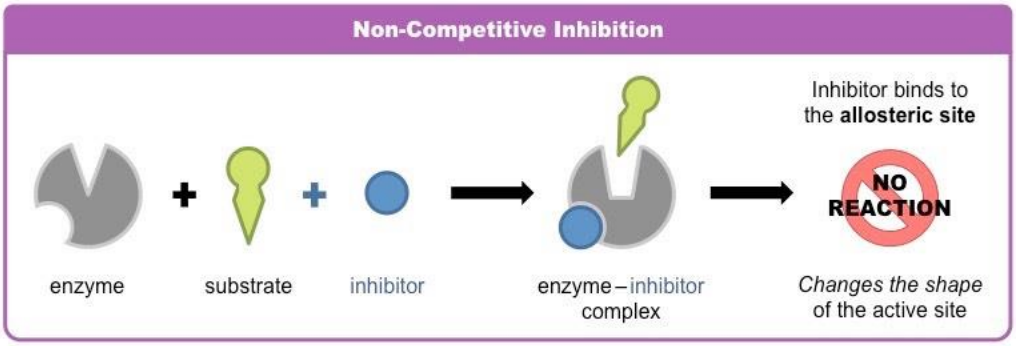


Fig. 6.5: Non-competitive inhibitor

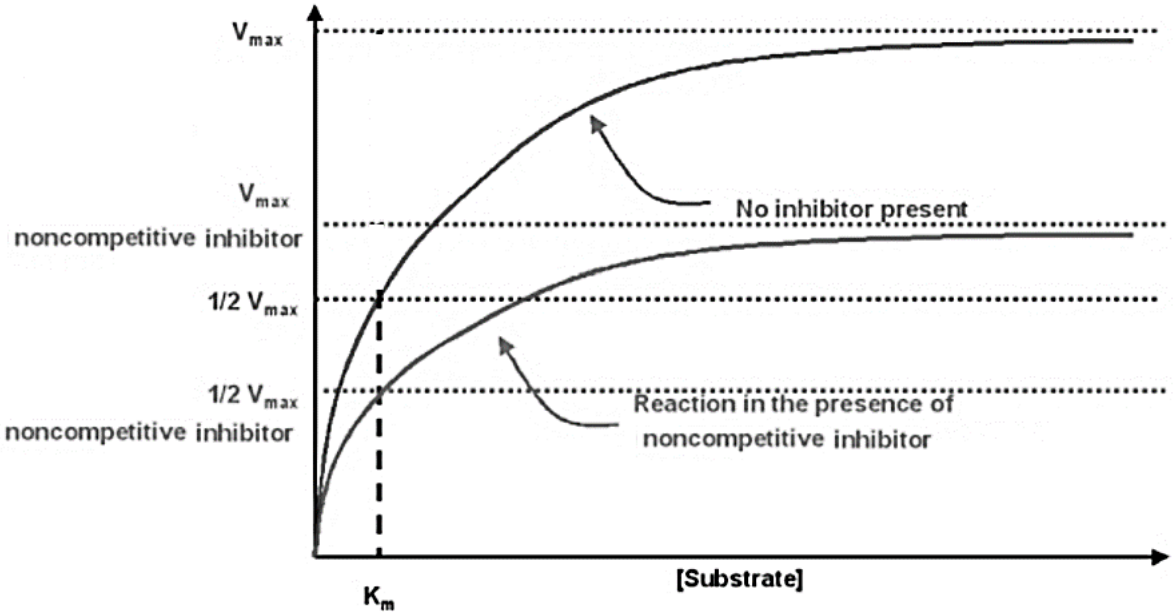


Fig. 6.6: Effect of non-competitive inhibitor

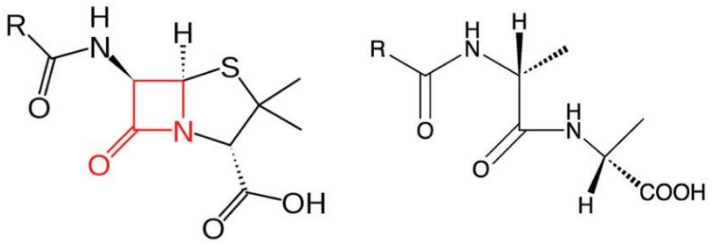
Increasing substrate concentration does not overcome the effect of a non-competitive inhibitor as the number of functional enzymes has been permanent reduced. $\mathbf{V_{max}}$ is thus **lowered** and not achievable even at high substrate concentrations.

$\mathbf{K_M}$ of enzyme remains **unchanged** because non-competitive inhibitors do not compete with the substrate in binding to the enzyme, thus affinity of the enzyme for substrate is not affected.

Challenge Yourself!

Bacterial transpeptidase catalyzes the final step in cell wall biosynthesis where peptidoglycan are cross-linked.

Penicillin is an inhibitor that binds irreversibly to transpeptidase. Fig 6.7 shows the structure of penicillin and a peptidoglycan monomer.



penicillin peptidoglycan monomer

Fig. 6.7

(a) Explain if penicillin is likely to be a compeitive or non-competitive inhibitor.

(b) Using the information provided, suggest if V_{max} of transpeptidase can be reached at high substrate concentration.

CHECKPOINT 3

Fill in the blanks in the table to compare competitive and non-competitive inhibition.

Feature	Competitive inhibition	Non-competitive inhibition
Structure of inhibitor		
Site of binding of inhibitor		
Effect of binding		
Effect on K_M		
Effect on rate of reaction		

7 ALLOSTERIC REGULATION

In a cell, there are molecules that bind to enzymes to regulate their activity. These regulatory molecules known as **allosteric effectors**. They bind to a specific site on the enzyme other than the active site known as the **allosteric site**. Binding of allosteric effectors is **reversible**.

- Allosteric effectors induce a **conformational change** in the enzyme and its active site to result in either:
  (i) **Allosteric activation**: Increase in rate of enzymatic reaction
  (ii) **Allosteric inhibition**: Decrease in rate of enzymatic reaction

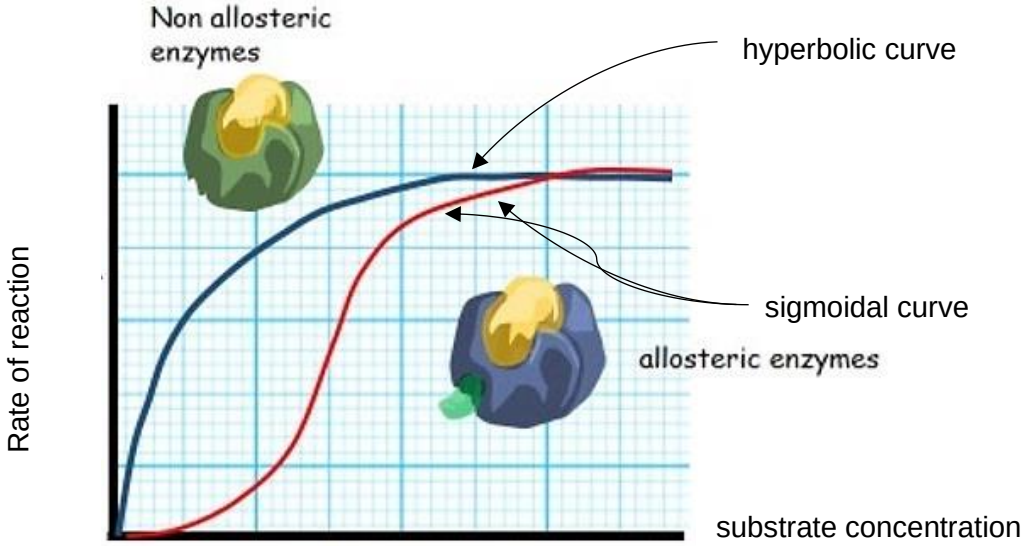


Fig 7.1: Effect of allosteric effectors on rate of reaction

The reaction curve of an allosterically regulated enzymes tend to be **sigmoidal** in shape (S-shape) compared to the reaction curve of non-allosterically regulated enzymes, which tend to be hyperbolic in shape.

7.1 Allosteric Activation

When an allosteric enzyme is bound to its allosteric activator, the activator **induces a conformational change** in the enzyme to increase the affiinity of the enzyme for its substrate.

Thus, the enzyme binds its substate more readily to form ES complex, increasing the rate of reaction.

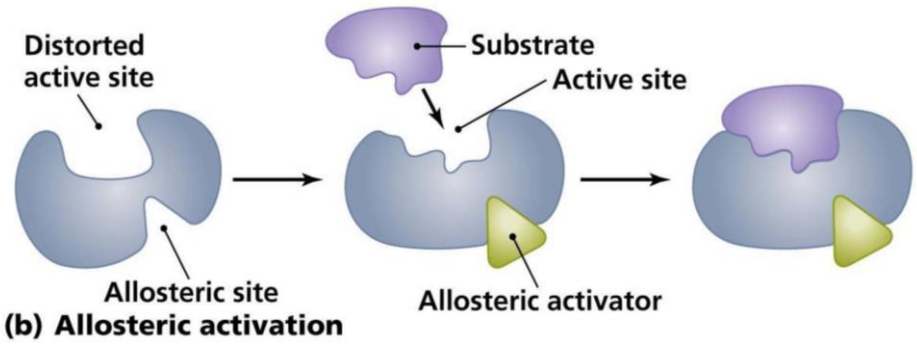


Fig 7.2: Effect of allosteric activator on enzymatic reaction

7.2 Allosteric Inhibition

When an allosteric enzyme is bound to its allosteric inhibitor, the inhibitor **induces a conformational change** in the enzyme such that the enzyme active site is **no longer complementary** the substrate molecule.

Thus the enzyme cannot bind to its substrate to form ES complex, decreasing the rate of reaction.

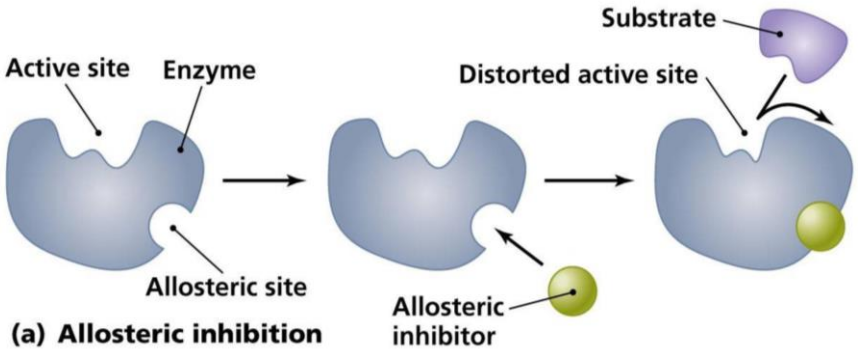


Fig 7.3: Effect of allosteric inhibitor on enzymatic reaction

In a cell, products are often produced through a series of reactions. This series of reactions is known as a **metabolic pathway** where each reaction is catalysed by different enzymes.

The end-product of some pathways, when reaching a threshold level, can act as an allosteric inhibitor of one of the enzymes in the pathway. This form of allosteric inhibition is known as **end-product inhibition** or feedback inhibition.

This reduces the rate of reaction of the downstream reactions, thereby slowing the synthesis of the end-product. It acts as a means to control metabolic reactions by **negative feedback**.

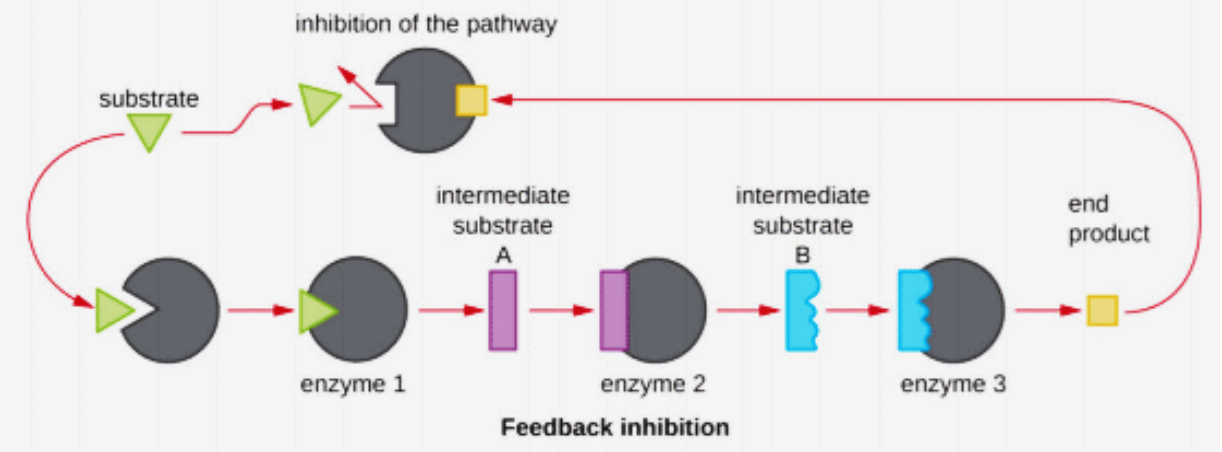


Fig. 7.4: End-product inhibition in a metabolic pathway

E.g. Isoleucine (an amino acid) is synthesised from threonine (another amino acid) via a series of enzyme-catalysed reactions. Isoleucine, the end-product of the metabolic pathway, acts as an allosteric inhibitor of threonine deaminase (the first enzyme of the pathway).

It binds to the allosteric site of the enzyme, causing a conformational change to the enzyme active site such that it is no longer complementary to threonine. Thus, the rate of formation of isoleucine decreases.

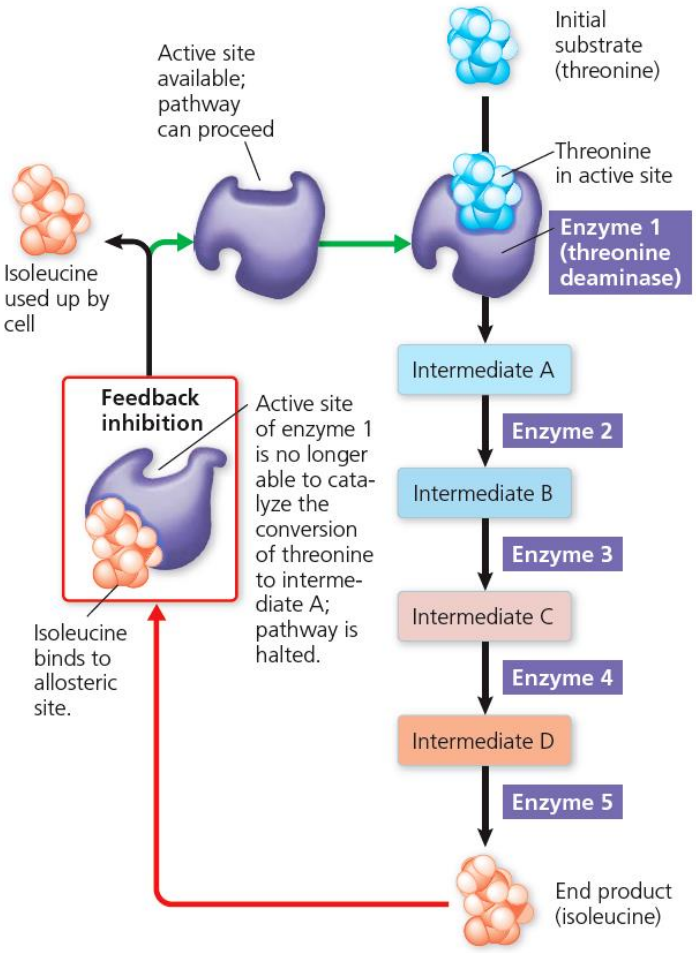


Fig. 7.5: Feedback / end-product inhibition allows metabolic pathway to be self-regulatory

Challenge Yourself!

Suggest what happens to the rate of isoleucine production as it is being used up within the cell.

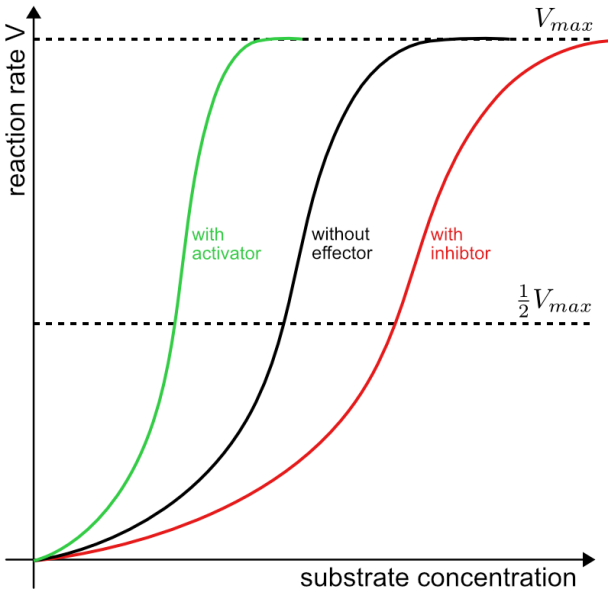
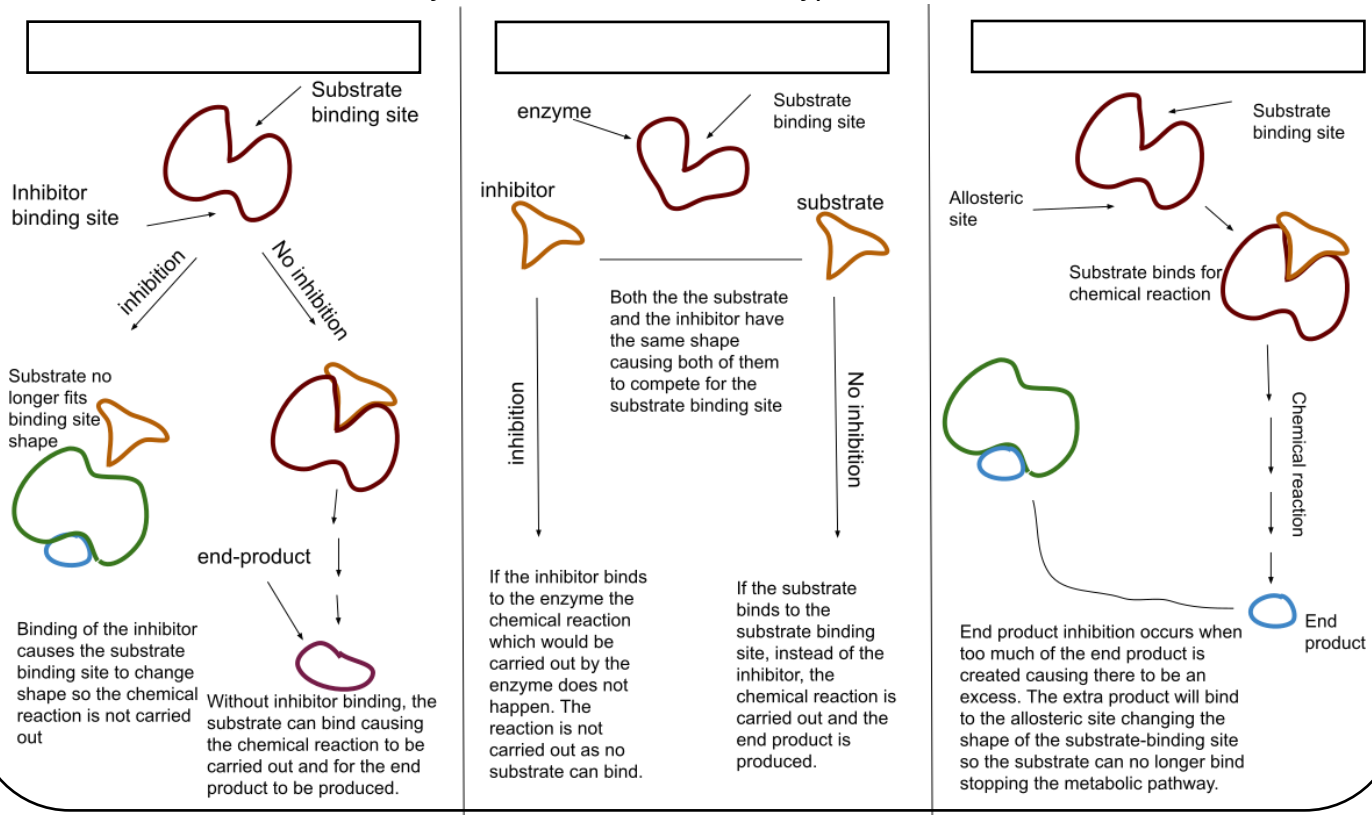


Fig. 7.6: Summary of effect of allosteric regulation

Checkpoint 4

Identify and describe the different types of inhibition.



Answers

Please take note that the phrasing of the answers in SLS module may deviate from the phrasing in this section. You may choose to follow either phrasing as the intended meaning remains the same.

Checkpoint 1

Identify the mechanisms by which enzymes lower the activation energy of a reaction.

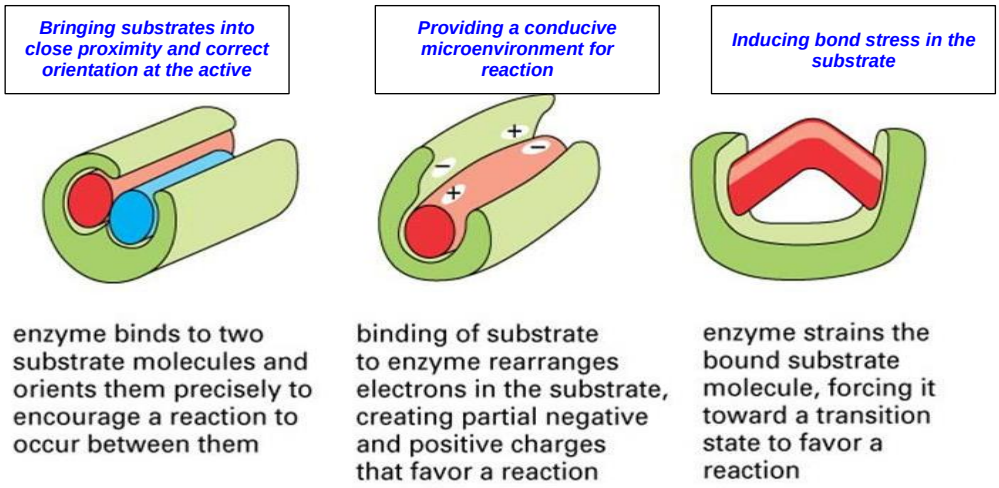
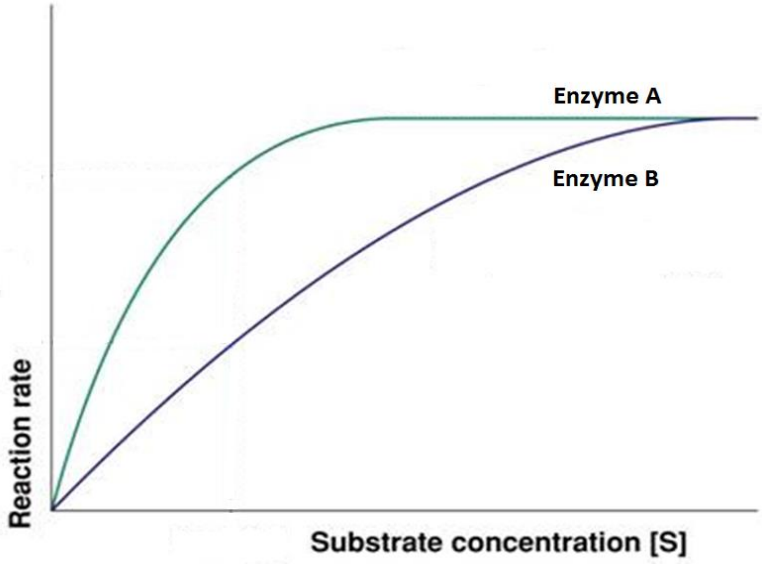


Fig. 3.10: Mechanisms to lower activation energy

Checkpoint 2

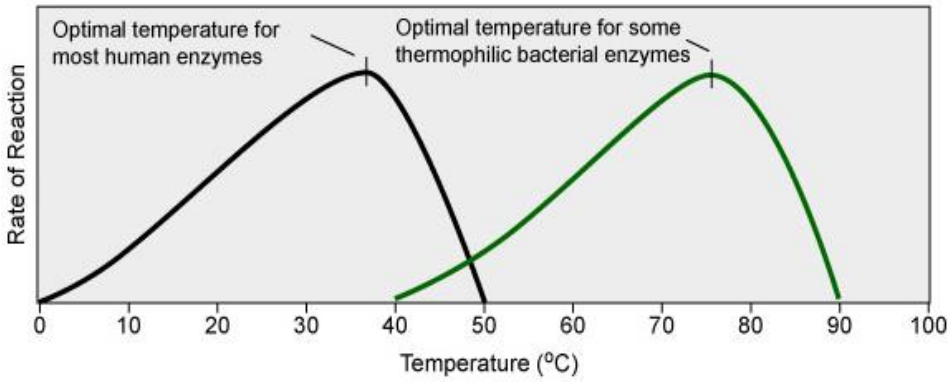
Based on the graphs below, which enzyme has a higher affinity for its substrate?



Answer: Enzyme A
Explanation: Low KM indicates that enzyme has high affinity for substrate because ½ Vmax is achieved at low substrate concentration.

CHALLENGE YOURSELF!

The optimum temperature for an enzyme varies considerably. Many Arctic plants have enzymes that function optimally at around 10°C, whereas those in bacteria inhabiting hot springs function optimally at around 80°C. For many human enzymes, the optimum temperature is around 37-40°C, and denaturation occurs at around 50°C.



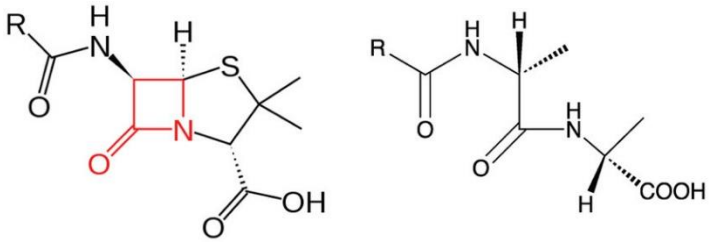
With reference to your knowledge of protein structure, what type of bonds do you expect to find in higher proportion in thermophilic bacterial enzymes? Why?

Answer: disulfide bond; disulfide bond is thermal stable and strong OR disulfide bond can withstand high temperature without being broken ;

Challenge Yourself!

Bacterial transpeptidase catalyzes the final step in cell wall biosynthesis where peptidoglycan are cross-linked.

Penicillin is an inhibitor that binds irreversibly to transpeptidase. Fig 6.7 shows the structure of penicillin and a peptidoglycan monomer.



penicillin peptidoglycan monomer

Fig. 6.7

(a) Explain if penicillin is likely to be a compeitive or non-competitive inhibitor.
Answer: competitive ; penicillin is structurally similar to peptidoglycan monomer, hence penicillin will bind to the active site.

(b) Using the information provided, suggest if V_{max} of transpeptidase can be reached at high substrate concentration.
Answer: No, V_{max} will not be reached at high substrate concentration.
Explanation: Penicillin is an inhibitor that <u>binds irreversibly</u> to transpeptidase.

CHECKPOINT 3

Fill in the blanks in the table to compare competitive and non-competitive inhibition.

Feature	Competitive inhibition	Non-competitive inhibition
Structure of inhibitor	***Structurally similar to substrate***	***Structurally dissimilar to substrate***
Site of binding of inhibitor	***Active site***	***Site away from active site*** ***® allosteric site***
Effect of binding	***Physically block substrate from binding to the active site, hence lower effective collisions and the rate of reaction***	***Causes the conformation change to the active site to prevent substrate from binding to the active site, hence lower effective collisions and the rate of reaction***
Effect on K_M	***Increase***	***No change***
Effect on rate of reaction	***Rate of reaction lowered at low substrate concentration*** ***V_{max} achievable at high substrate concentration***	***Rate of reaction lowered at low substrate concentration*** ***V_{max} is not achieved even high substrate concentration, V_{max} is lowered***

Challenge Yourself!

Suggest what happens to the rate of isoleucine production as it is being used up within the cell.

Answer:

1. ***Isoleucine bound to threonine deaminase will dissociate ;***
   Threonine deaminase no longer inhibited by isoleucine ;

2. ***Threonine deaminase free to catalyse the conversion of threonine in a series of reaction to form isoleucine ;***
   Isoleucine concentration will gradually increase which acts as an allosteric inhibitor to control the rate of its own production ;

Checkpoint 4

Identify and describe the different types of inhibition.