TOPIC C: ENZYMES

Learning Outcomes

Core Topic 1: Cellular Functions

Candidates should be able to:

(h) Explain the mode of action of enzymes in terms of an active site, enzyme/ substrate complex, lowering of activation energy and enzyme specificity.

(i) Follow the time course of an enzyme-catalysed reaction by measuring rates of formation of products (e.g. using catalase) or rate of disappearance of substrate (e.g. using amylase).

(j) Investigate and explain the effects of temperature, pH, enzyme concentration and substrate concentration on the rate of enzyme catalysed reactions, and explain these effects.

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

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References

1. Boyle, M. and Senior, K. (2002) Biology. Chapter 5: Enzymes and Metabolism. Harper Collins Publishers Limited.
2. Campbell, N. A. and Reece J.B. (2008) Biology. Chapter 8: An Introduction to Metabolism. Eighth Edition. Pearson Education, Inc.
3. Jones, M. and Jones, G. (2004) Advanced Biology. Chapter 2: Enzymes. Cambridge University Press.

1. Introduction

Enzymes **catalyse** biological reactions. Thus, they are also called **biological catalysts**. Most biological catalysts are **globular proteins**.

However, some enzymes are RNA molecules known as ribozymes. For example, **peptidyl transferase** activity found in ribosome is mediated by the ribosomal RNA in the large subunit.

They are important as many metabolic reactions, though spontaneous, 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.

Enzymes are usually classified according to the type of reaction they catalyse and they can be named according to their **substrates** (i.e. reactants that enzymes act on).

Classification of Enzymes

Class of Enzyme	Type of Reaction Catalysed	Examples
Oxidoreductase	Transfers electrons, oxygen atom or hydrogen atom between molecules, i.e. oxidation-reduction reactions	Dehydrogenases Oxidases
Transferase	Transfers a functional group from one molecule to another molecule e.g. phosphate group	Kinases Phosphorylases
Hydrolase	Carries out hydrolysis reactions	Sucrase Lipases Proteases
Lyase	Removes a functional group from one molecule without hydrolysis	Decarboxylases
Isomerase	Rearranges groups of atoms within a molecule	Isomerases
Ligase	Forms bonds between two molecules using energy derived from the breakdown of ATP	Synthetases Ligases

*Note: The names of enzymes usually ends with **'-ase'**.*

2. Characteristics of Enzymes

Enzymes are defined as **biological catalysts** which speed up the **rate of metabolic reactions** (both catabolic and anabolic) while remaining chemically **unchanged** at the end of the reaction.

Metabolic reactions can be classified into catabolic and anabolic reactions.

(i) Anabolic reactions refer to reactions where complex molecules are built from simpler molecules using energy (e.g. photosynthesis).

(ii) Catabolic reactions refer to reactions where complex molecules are broken down into simpler molecules and energy is usually released (e.g. respiration).

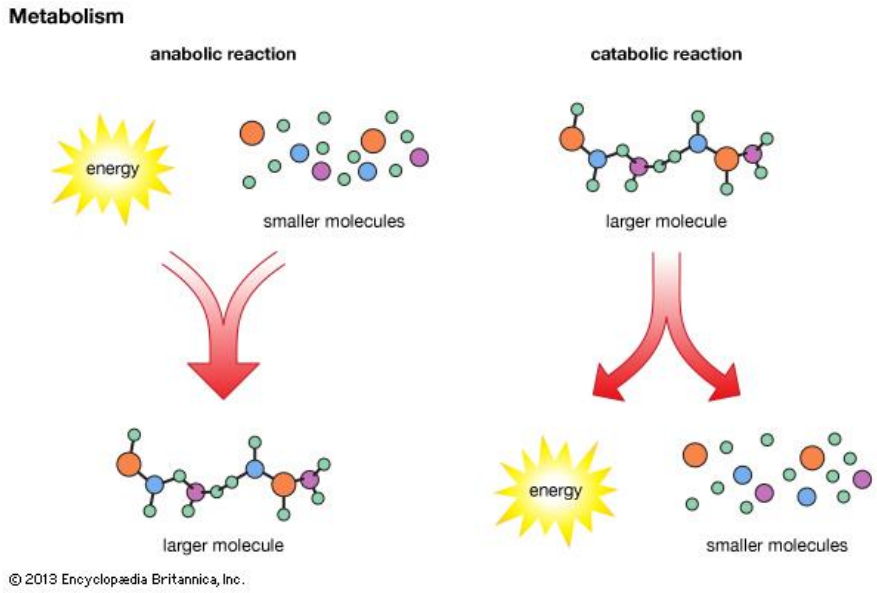


Diagram showing anabolic reaction and catabolic reaction

(a) Common Properties of Enzymes

(i) Enzymes are **highly specific**.

- Most enzymes are highly specific to one particular type of substrate molecule, e.g. amylase hydrolyses amylose but not cellulose.
- This is due to the specificity of enzyme's **active site** which recognises specific groups of atoms or particular type of bond / linkage present in the substrates.

(ii) Enzymes are effective in **small amounts** as they have **high turn-over rates**.

- The turn-over rates of enzymes is the amount of substrate that a unit of enzyme can convert into its products over a period of time when enzyme is saturated.
- E.g. one molecule of catalase can break down 6 x 10^5 molecules of hydrogen peroxide into oxygen and water per second.

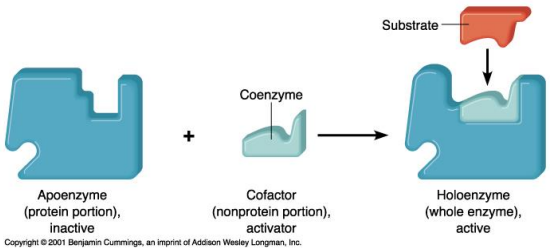
(iii) Enzymes **remain chemically unchanged** at the end of the chemical reaction.

- Enzymes can be used repeatedly without undergoing permanent chemical damage. Therefore, enzymes can be **reused**.
- A small amount of enzymes can effect a great change in the rate of the chemical reaction.

(iv) Enzymes are affected by factors such as **substrate concentration, enzyme concentration, temperature and pH**.

(v) 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.
- They may vary from simple inorganic ions to complex organic molecules.
- They may either remain chemically unchanged at the end of a reaction or be regenerated by a later process.
- The enzyme-cofactor complex is called a **holoenzyme**.
- The enzyme portion, without its cofactor, is called an **apoenzyme**.



Apoenzyme and Holoenzyme

- There are three types of cofactor: inorganic ions, coenzymes and prosthetic groups.
  - **Inorganic ions**
    - E.g. Zn^{2+} acts as a cofactor for carbonic anhydrase.
  - **Coenzymes** are organic molecules which bind loosely to enzyme.
    - E.g. Nicotinamide adenine dinucleotide (NAD^+), derived from vitamin niacin, is a coenzyme to dehydrogenase involved in cellular respiration.
  - **Prosthetic groups** are organic molecules which 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.

(vi) The activity of enzymes is **tightly regulated**.

- Enzyme activity may be enhanced or reduced.

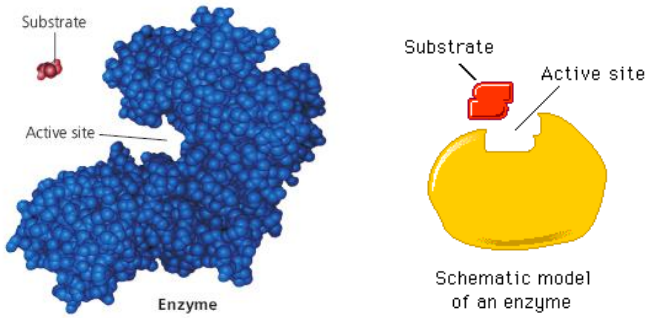
(vii) Some enzymes allow reactions to **reach equilibrium within a shorter period of time**.

- Some enzymes are able to catalyse both the forward and backward reactions of a reversible reaction.
- Enzymes do not affect the position of the equilibrium.

3. Mode of Action of Enzymes

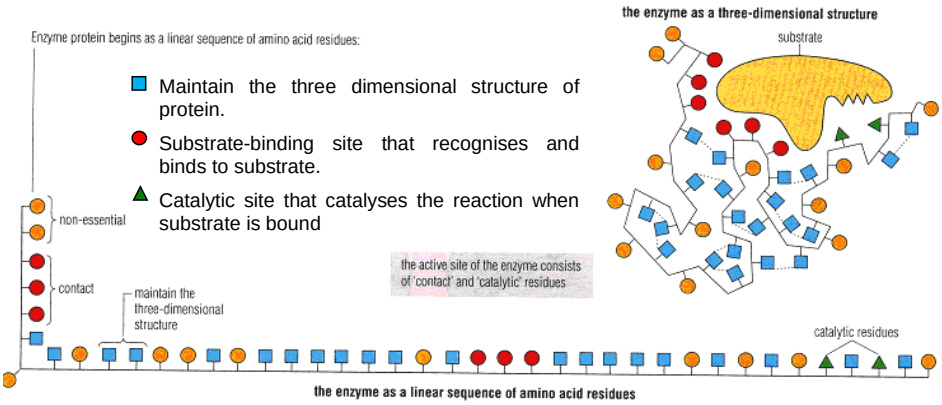
(a) Active Site

Enzymes have a **unique three dimensional conformation** with an **active site** (i.e. catalytic site and substrate-binding site) of the enzyme.



Active site of hexokinase

- The **active site** is formed by **3 to 12 amino acids from different parts of a single polypeptide chain** held together by hydrogen bonds, ionic bonds, disulfide bonds and/or hydrophobic interactions.
- Other amino acids are involved in maintaining the overall three dimensional structure of the enzyme.



Structure of an enzyme

- The active site comprises substrate binding site and catalytic site.
  - Substrate binding site
    - Amino acid residues that recognise and bind to substrate thus determining enzyme specificity.
  - Catalytic site
    - Amino acid residues that catalyse the reaction when substrate is bound.

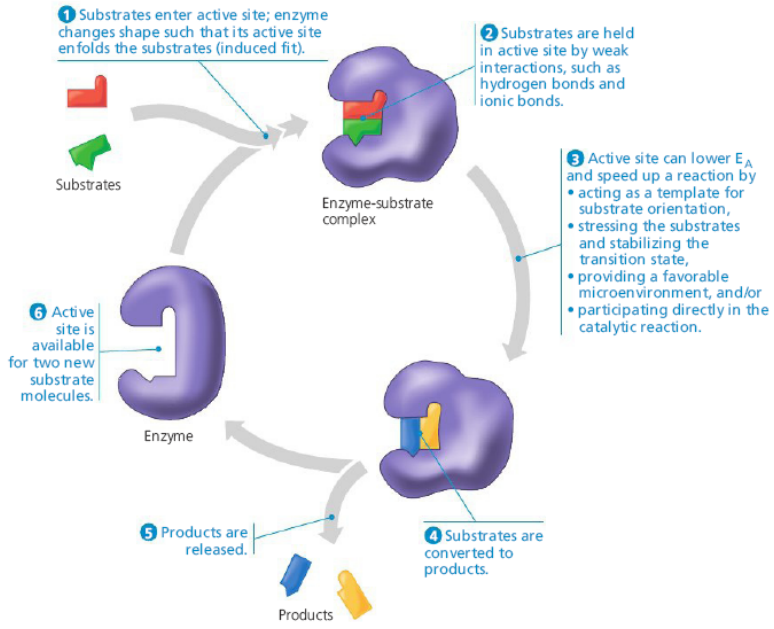
(b) Enzyme specificity

- The **enzyme specificity** is determined by the fit between the shape of the enzyme's active site and its substrate.
- The **active site** of an enzyme is **complementary to its substrate in terms of shape, size, charge and orientation**.
- Enzymes are specific to only one particular substrate or one group of similar substrates e.g. lipases hydrolyses only lipids.
- The enzyme specificity is a result of its three dimensional conformation, which is consequence of its amino acid sequence. *(refer to Biomolecules: Proteins)*

(c) Enzyme-Substrate Complex

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

- When the substrate binds to the active site of the enzyme, the **enzyme-substrate complex** (E-S complex) is formed.
- The substrates are held in active site by weak bonds such as **hydrogen bonds, ionic bonds and hydrophobic interactions**.
- The R groups of a few of the amino acids that make up the **active site** (catalytic site) catalyse the conversion of substrate to product
- Once the **products** are formed, they are no longer complementary to the active site and thus, they will leave the enzyme.
- The enzyme is then available to act on other substrates.

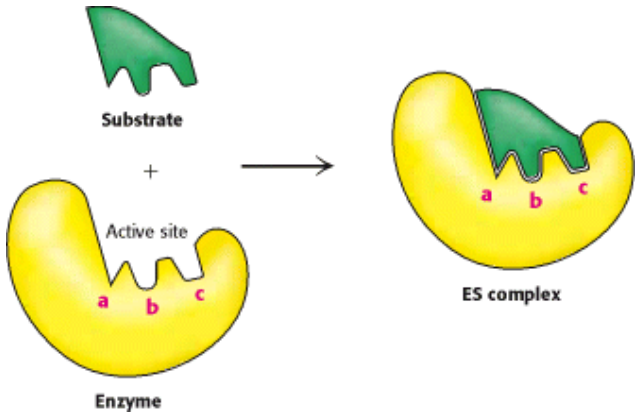


Mode of action of enzyme

There are 2 hypotheses as to how an enzyme recognises and binds to its substrate, namely the **lock-and-key hypothesis** and the **induced fit hypothesis**.

(i) **Lock-and-key hypothesis**

- The enzyme acts as a lock and the substrate acts as a key, which fits **precisely**.
- The active site of the enzyme is **perfectly complementary** to the substrate in terms of shape, size, charge and orientation.
- The substrate binds to enzyme's active site to form the enzyme-substrate complex.
- This mode of activation is more probable for enzymes that work on only one type of substrate.

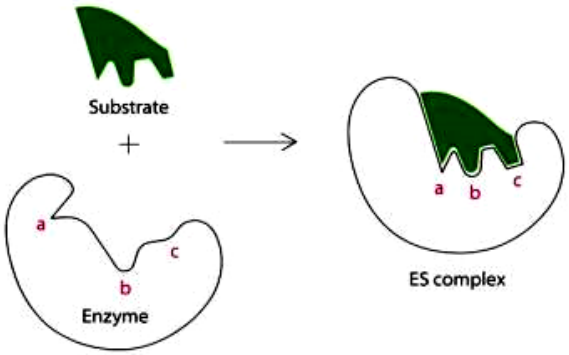


Lock-and-key hypothesis

(Source: http://neurobio.drexel.edu/GalloWeb/lock&key.gif)

(ii) **Induced fit Hypothesis**

- Enzymes may work in a more **flexible** manner.
- The active site is **not perfectly complementary** to the substrate in terms of shape, size and orientation.
- However, upon forming some bonds with the substrate, the enzyme **changes its shape**, 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.



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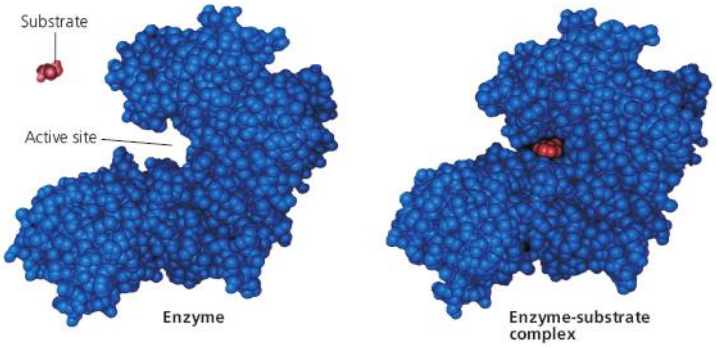


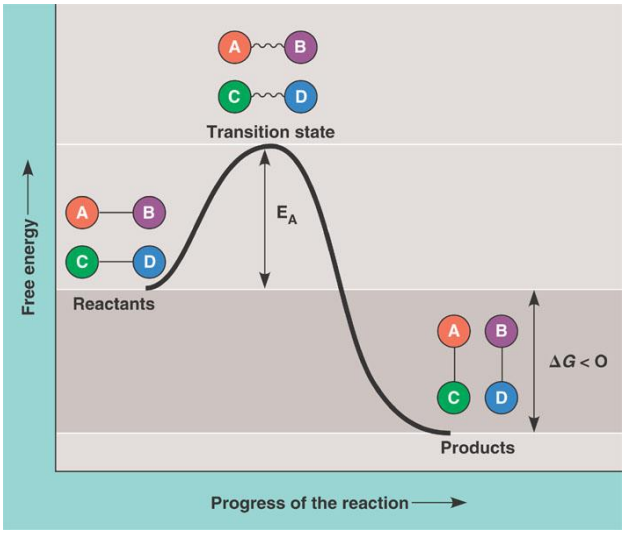
(a) In this computer graphic model, the active site of this enzyme (hexokinase, shown in blue) forms a groove on its surface. Its substrate is glucose (red).

(b) When the substrate enters the active site, it forms weak bonds with the enzyme, inducing a change in the shape of the protein. This change allows additional weak bonds to form, causing the active site to enfold the substrate and hold it in place.

Induced fit hypothesis

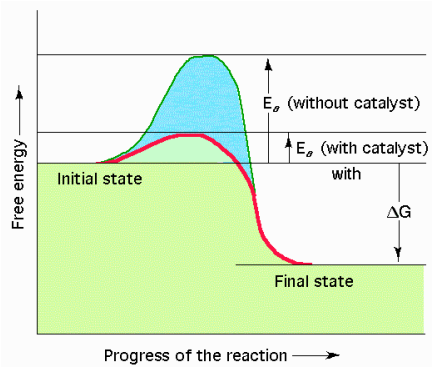
(d) Activation Energy

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



Energy Profile of a Exergonic reaction

- Activation energy is often supplied in the form of thermal energy (heat) that the reactant molecules absorb from the surroundings.
- With increase in temperature, the reactants **gain kinetic energy**, thus colliding more frequently and more forcefully.
- **Thermal agitation** of atoms in the molecules contorts the reactants, making the bonds more likely to break.
- When the molecules have absorbed enough energy for the bonds to break, the reactants are in an unstable condition known as the **transition state**.
- After bonds have broken, new bonds are formed releasing energy to the surroundings.



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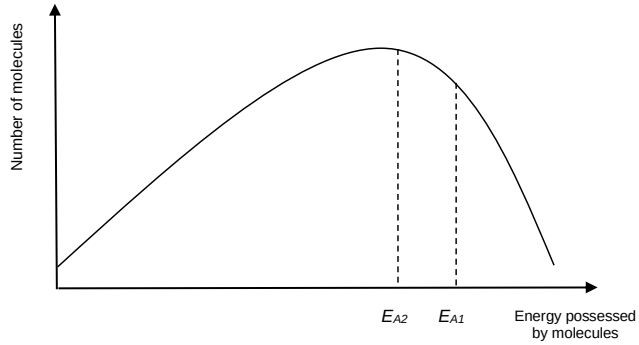


E_{A1} – energy possessed by molecules in an uncatalysed reaction
E_{A2} – energy possessed by molecules in a catalysed reaction

- Enzymes speed up biological reactions as they provide **an alternative pathway,** which has a **lower activation energy (E_A)** as compared to the uncatalysed reaction.
- Thus, more reactant molecules possess energy equal or more than the activation energy required for the catalysed reaction. As such, the reactions occur at a faster rate and a high temperature is not required.
- Enzymes lower the activation energy of a reaction by promoting formation of transition state via a number of mechanisms, such as:
  - allowing **close proximity** of reactants due to temporary binding of substrates on the enzyme;
  - ensuring **correct orientation** of reactants to facilitate the reaction taking place;
  - **destabilising the bonds** of reactants as enzymes contort reactant molecules to facilitate formation of transition state;
  - providing a **microenvironment conducive for reaction**

    e.g. creating a water-free zone due to the presence of hydrophobic amino acids at the active site**,** in which non-polar reactants may react more easily.

4. Enzyme Kinetics

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

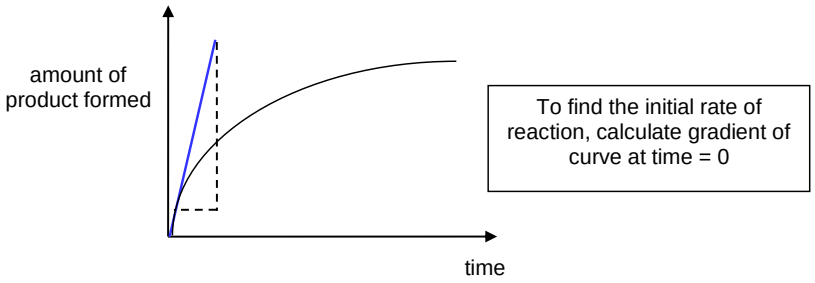
(a) Measurement of Enzyme Kinetics

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

- The method is chosen based on the ease at which the reactant depleted or product formed can be measured.
- When studying the effect of a factor on the rate of an enzymatic reaction, it is important that only one specific factor varies while all other factors are kept **constant** and at its **optimum** for the particular enzyme.
- It is also important to measure the **initial rate of reaction** as the concentration of substrate would decrease once the reaction starts.
- The initial rate of reaction is determined by the gradient of the linear slope in a graph of amount of product formed / amount of substrate depleted against time.

(i) Rate of Formation of Product

Graph of amount of product formed against time



Time course of an enzymatic reaction

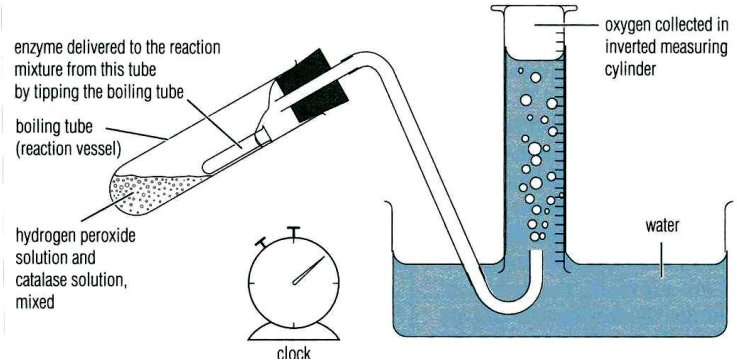
- The time course of an enzymatic reaction can be followed by measuring the **amount of product formed** over a period of time.
- The **initial rate of reaction** is determined by calculating the gradient of the linear slope at time = 0.
- An example is **catalase** catalyse the break down of hydrogen peroxide to form oxygen.

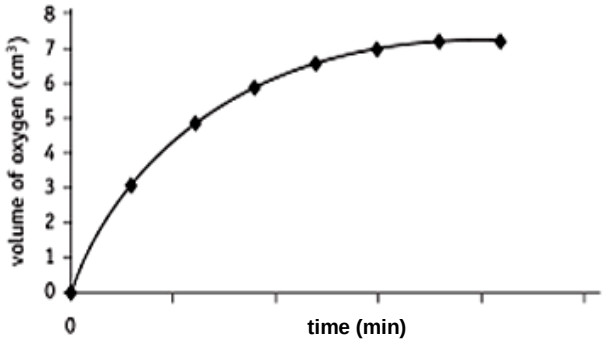
$$\text{Hydrogen peroxide} \xrightarrow{\text{catalase}} \text{oxygen + water}$$

- The **volume of oxygen formed** is measured at fixed time intervals.

Experiment to follow a time couse of an enzyme catalysed reaction by catalase

1. Using a **syringe**, add 5.0 cm^3 of 5% hydrogen peroxide solution into a boiling tube.
2. Using a **syringe**, add 1.0 cm^3 of 2% catalase into a small vial.
3. Place the small vial held by a string into the boiling tube containing hydrogen peroxide and seal with rubber bung.
4. Place boiling tube into water bath of optimum temperature for catalase (37^0C) and **equilibrate** for 5 minutes.
5. Connect a delivery tube from the boiling tube to an inverted measuring cylinder in a water bath to collect the oxygen evolved as shown in diagram.
6. Tip the boiling tube to deliver enzyme to hydrogen peroxide solution and start timing.
7. The reaction is followed by measuring the volume of oxygen evolved at 1 minute interval for 10 minutes.

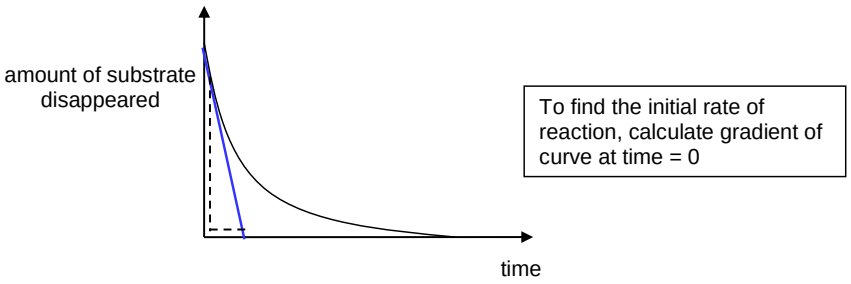




Measuring volume of oxygen formed from hydrogen perioxide catalysed by catalase

(ii) Rate of Disappearance of Substrate

Graph of amount of substrate disappeared against time



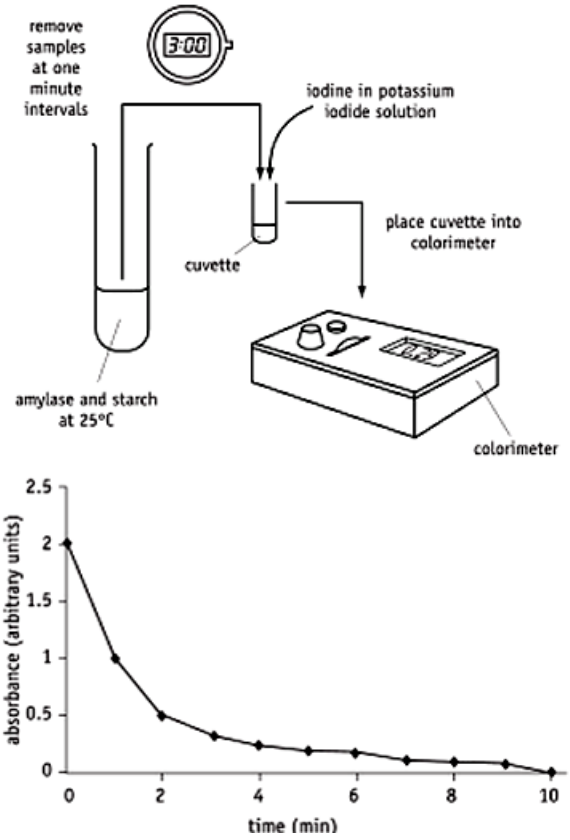
Time course of an enzymatic reaction

- The time course of an enzymatic reaction can be followed by measuring the **amount of substrate disappeared** over a period of time.
- The **initial rate of reaction** is determined by the calculating the gradient of the linear slope at time = 0.
- An example is **amylase** catalyse the hydrolysis of starch to form maltose.

  Starch $\xrightarrow{\text{amylase}}$ maltose
- The **absorbance** of reaction mixture is measured at fixed time intervals.

Experiment to follow a time couse of an enzyme catalysed reaction by amylase

1. Using a **measuring cylinder**, add 15.0 cm^3 of 1% starch solution into a boiling tube.
2. Using a **syringe**, add 5.0 cm^3 of 1% amylase and 10.0 cm^3 of buffer solution pH7 to another boiling tube.
3. Place both boiling tubes into water bath of optimum temperature for amylase (37^0C) and **equilibrate** for 5 minutes.
4. Pour the starch solution into the boiling tube containing amylase in a buffer solution and start timing.
5. The reaction is followed by withdrawing a 1.0 cm^3 of sample using a syringe at 1 minute intervals for 10 minutes.
6. Each sample is tested for the presence of starch using 0.1 cm^3 of iodine solution in a cuvette.
7. Starch turns iodine blue-black and in the absence of starch, iodine remains yellow.
8. The absorbance is measured using a colorimeter. A dark blue black colour with iodine gives a high value of absorbance.



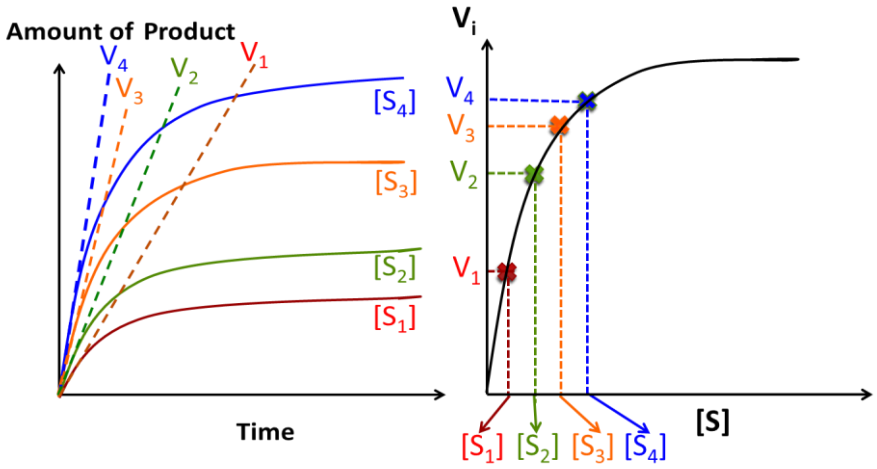
Measuring absorbance (starch disapperance) catalysed by amylase

(b) Measurement of Enzyme affinity

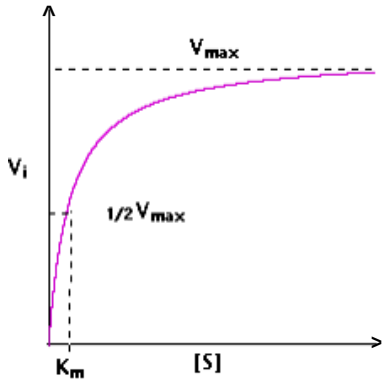
Different enzymes catalysing different reactions will show different Michaelis constant values.

The **Michaelis constant** or **K_m** of an enzyme is the:

- **substrate concentration** at which the **rate of reaction** catalysed by the enzyme equals to half its maximum rate (i.e. **½ V_{max}**).
- indication of the affinity of the enzyme for its substrate molecules i.e. how readily the enzyme reacts with its substrate.
  - low K_m means that there is a high affinity between the enzyme and substrate, i.e. the enzyme reacts readily with the substrate molecule.
  - high K_m means that there is a relatively low affinity between the enzyme and substrate.



Graph of initial rate of reaction against substrate concentration



V_i = initial velocity (mol/ time)

[S] = substrate concentration ($moldm^{-3}$)

V_{max} = maximum velocity

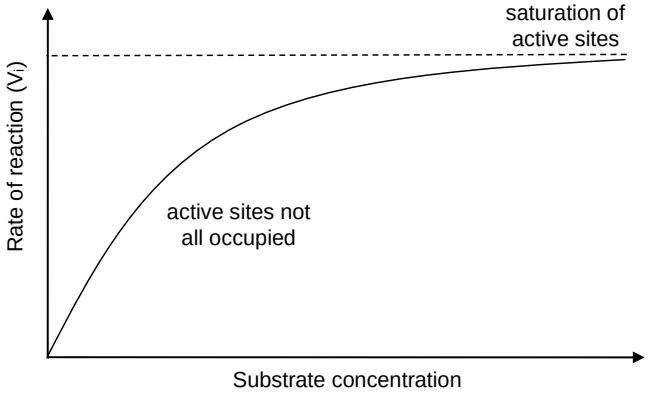
K_m = [S] when V_i = ½ V_{max}

5. Factors Affecting Enzyme Activity

Enzymes are affected by factors such as **substrate concentration, enzyme concentration, temperature and pH**.

(a) Substrate Concentration

Graph of rate of enzymatic reaction against substrate concentration



- Description:
  - At low substrate concentration, there is a **linear / proportional increase** in rate of reaction with increase in substrate concentration.
  - As substrate concentration continues to increase, the increase in rate of reaction **slows down**.
  - Any further increase in substrate concentration will not increase the rate of reaction. Rate of reaction **plateaus off** and **maximum rate of reaction** is reached.

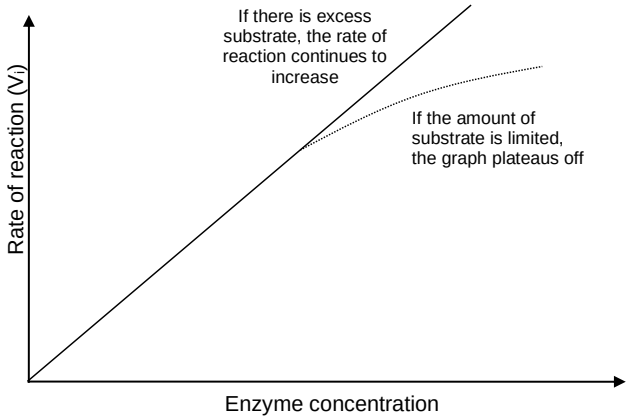
- Explanation:

  At low substrate concentration,
  - increase in substrate concentration **increases the number of effective collisions** between enzyme and substrate molecules. Not all the active sites of the enzyme molecules present will be occupied at any one time.
  - There is an **increase in the number of enzyme-substrate complex formed per unit time**. Hence, there is a proportional **increase in the rate of formation of products**.
  - **Substrate concentration** is limiting the rate of reaction.

  At high substrate concentration,
  - further increase in substrate concentration results in **all active sites of enzymes being occupied** by the substrate at any one time / all active sites of enzyme are **saturated** with substrate.
  - **Maximum number of enzyme-substrate complex formed per unit time**. Hence, the rate of formation of products has reached the maximum.
  - Other factors like **enzyme concentration** is now limiting the rate of reaction.

(b) Enzyme Concentration

Graph of rate of enzymatic reaction against enzyme concentration



- Description:
  - At low enzyme concentration, there is a **linear / proportional increase** in the rate of reaction with increase in enzyme concentration.
  - As enzyme concentration continues to increase, the increase in rate of reaction **slows down**.
  - Any further increase in enzyme concentration will not increase the rate of reaction. Rate of reaction **plateaus off** and **maximum rate of reaction** is reached.

- Explanation:

  At low enzyme concentration,
  - increase in enzyme concentration increases number of **active sites** thus, **increases the number of effective collisions** between enzyme and substrate molecules.
  - There is an **increase in the number of enzyme-substrate complex formed per unit time**. Hence, there is a proportional **increase in the rate of formation of products**.
  - **Enzyme concentration** is limiting the rate of reaction.

  At high enzyme concentration,
  - substrate molecules are not enough to occupy all the active sites of enzyme molecules available.
  - **No further increase in number of enzyme-substrate complex formed per unit time**. Hence, the rate of formation of products has reached the maximum.
  - Other factors like **substrate concentration** or temperature is now limiting the rate of reaction.

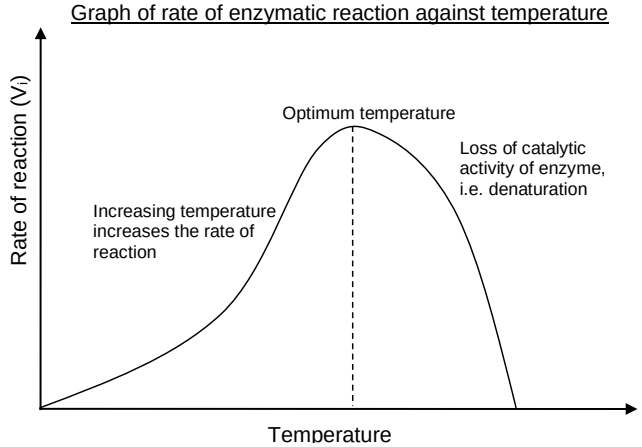
As enzymes are protein in nature, they are also affected by factors affecting the structure of proteins such as temperature and pH.

(c) Temperature

- Temperature affects enzyme kinetics is two ways:
  - Increase in temperature will increase the **kinetic energy** of the substrate and enzyme molecules.
  - Increase in temperature affects the **stability of the protein structure**.
- This results in an **asymmetrical** graph with an **optimum temperature** whereby the rate of enzyme reaction is at its maximum.
- Different enzymes have different optimum temperature.

  e.g. enzymes in humans function optimally between 37°C and 40°C; in plants at around 25°C; in thermophilic bacteria at around 80°C.

Graph of rate of enzymatic reaction against temperature



- Description:
  - At very low temperatures, the **rate of reaction is very slow**.
  - As the temperature increases towards optimum temperature, the **rate doubles for every 10°C rise** in temperature, i.e. temperature coefficient: **Q_{10}= 2**, where

$$Q_{10} = \frac{\text{rate of reaction at}(x+10^{o}C)}{\text{rate of reaction at } x^{o}C}$$

  - At optimum temperature, the **rate of enzyme reaction is at its maximum**.
  - At temperatures slightly above optimum temperature, the rate of enzyme reaction **decreases slowly**.
  - At very high temperatures, there is a **drastic fall** in rate of reaction and it **eventually falls to zero**.

- Explanation:

  At very low temperatures,

  - substrate and enzyme molecules have **low kinetic energy**.
  - Thus, they **move very slowly** and there are **very few effective collisions** between enzyme and substrate molecules. The rate of enzyme-substrate complex formation is thus very low.

  As the temperature increases to optimum temperature,

  - the **kinetic energy of molecules increases** and molecules **move faster**.
  - Thus, there are **more effective collisions** between enzyme and substrate molecules. There is an **increase in the number of enzyme-substrate complex formed per unit time**.
  - Also, substrate molecules at higher energy levels have a higher probability to **overcome the activation energy barrier** and form products.

  At optimum temperature,

  - **Maximum number of enzyme-substrate complex formed per unit time**. Hence, the rate of formation of products has reached the maximum.

  At temperatures slightly above optimum temperature,

  - **thermal agitation** of enzyme molecules **disrupts the weaker bonds** such as hydrophobic interactions, hydrogen bonds, and ionic bonds.
  - This **distorts the specific 3-dimensional conformation** of the enzyme.
  - Thus the **active site is distorted** and **no longer complementary to the substrate**.
  - As a result, substrate molecules cannot fit the active site of enzyme molecules and **E-S complex cannot be formed**. The enzymes are said to be **denatured**.

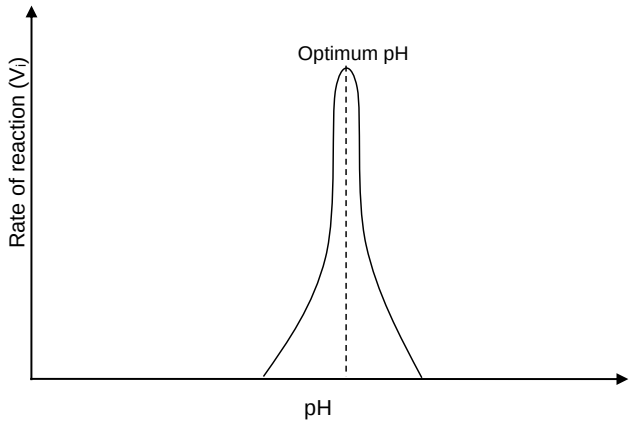
  At very high temperatures,

  - there are also an increasing percentage of enzymes that are denatured. When all enzymes are eventually denatured, the rate of reaction then falls to zero.

(d) 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; salivary amylase at pH 7; trypsin at pH 8.5.
- 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.

Graph of rate of enzymatic reaction against pH



- Description:
  - The graph is **bell-shaped**, i.e. **symmetrical** about the optimum pH.
  - The rate is high over a narrow range of pH and peaks at optimum pH.
  - At pH values **slightly** above or below optimum pH, rate of reaction falls **drastically**.

- Explanation:

  At optimum pH,
  - all the **ionic and hydrogen bonds** between R groups of amino acids are **intact**.
  - The active sites are complementary to the substrate molecule. As such, **maximum number of enzyme-substrate complex can be formed per unit time**.

  A **slight change** in pH from optimum pH
  - will change the **charge** found on the acidic and basic R-groups of amino acids at the active site.
  - This reduces the binding ability of substrate to the active site and hence the rate of formation of E-S complex will decrease.

  A **drastic change** in pH from optimum pH
  - will disrupt the **ionic bonds** between the acidic and basic R-groups of the amino acids and **hydrogen bonds** between polar R-groups.
  - This **distorts the specific 3-dimensional conformation** of the enzyme.
  - Thus the **active site is distorted** and **no longer complementary to the substrate**.
  - As a result, substrate molecules cannot fit the active site of enzyme molecules and **rate of E-S complex formation** will decrease drastically. The enzymes are said to be **denatured**.

6. Enzyme Inhibition

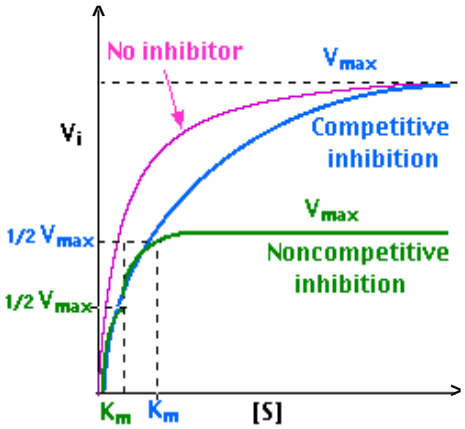
Enzyme activity can be reduced by **inhibitors**.

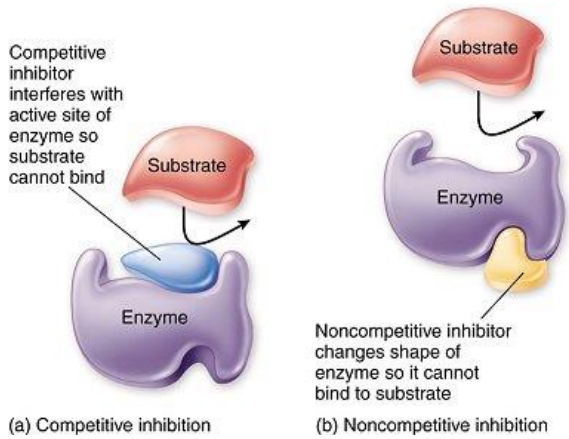
This can be achieved by the formation of **enzyme-inhibitor (E-I) complex**.

There are two main types of inhibition: **competitive** and **non-competitive** inhibition.

They have different effects on the rate of reaction. They affect the V_{max} and K_m of the enzyme differently.

Graph of initial rate of reaction against substrate concentration

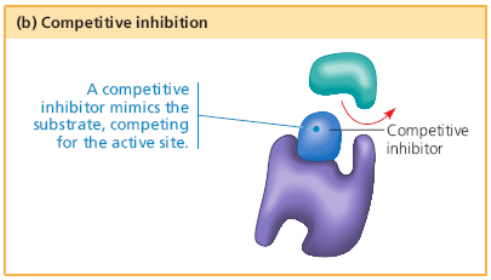




Competitive inhibition and Non-competitive inhibition

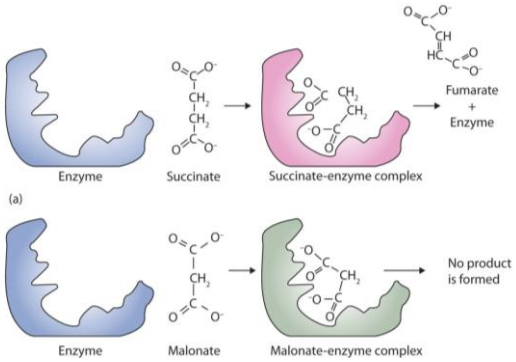
(a) Competitive Inhibition

- Competitive inhibitors
  - are **structurally similar** (in terms of shape, size, charge and orientation) to the substrate molecule.
  - bind to the active site of the enzyme and thus **competes with the substrate for the active site**.
  - **reduce the number of active sites available for the substrates** to bind and form enzyme-substrate (E-S) complex.



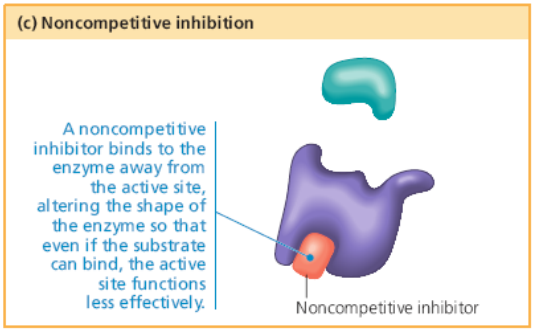
Competitive inhibition

- **K_m of the enzyme will increase** in the presence of competitive inhibitor.
- **V_{max} can be reached eventually at higher substrate concentration**.
- When substrate concentration is low,
  - it is more likely for the enzyme to collide with competitive inhibitor molecules and form **enzyme-inhibitor (E-I) complex**.
  - Thus, the number of active sites available for substrate molecules will be reduced and the **rate of E-S complex formation will decrease**.
- When substrate concentration is high,
  - it is more likely for the enzyme molecules to collide with substrate molecules and form **enzyme-substrate (E-S) complex**.
  - Thus, the competitive inhibitor has no effect on maximum rate of reaction.
- E.g. malonate (inhibitor) competes with succinate (substrate) for succinate dehydrogenase (a Krebs cycle reaction in respiration).



(b) Non-Competitive Inhibition

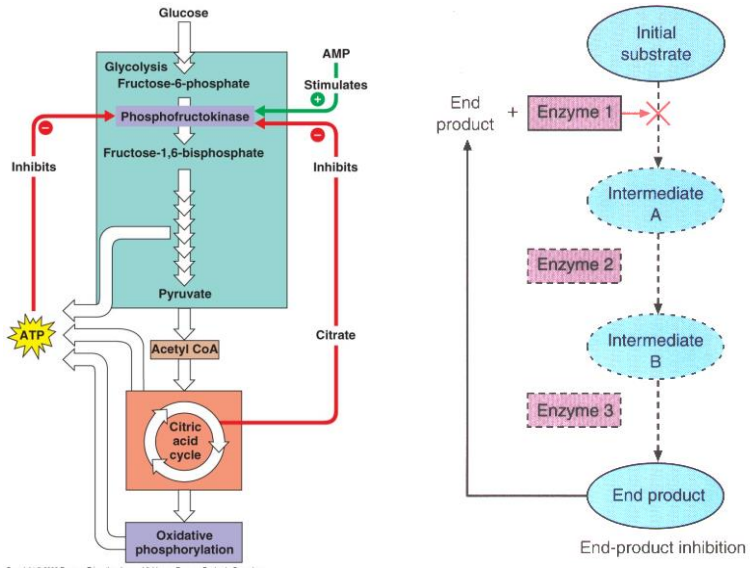
- Non-competitive inhibitors
  - are **not structurally similar** (in terms of shape, size, charge and orientation) to the substrate molecule.
  - **bind at a site away from the active site**
  - This interaction **alters the specific 3-dimensional conformation** of the enzyme molecule such that
    - **active site is distorted** and **no longer complementary to substrate**, **thus not able to bind to the substrate properly** or
    - substrate can still bind to active site but the enzyme is not able to catalyse the conversion of substrate to product



Non-competitive inhibition

- **K_m of the enzyme remains unchanged** in the presence of non-competitive inhibitor since non-competitive inhibitors do not compete with substrate molecules for active site.

  (i.e. the affinity of the substrate for the functional enzyme remains unaffected).
- **V_{max} is lowered** as non-competitive inhibitors reduce the number of functional enzymes. Thus V_{max} will not be restored even if substrate concentration is increased.
- E.g. cyanide is an irreversible non-competitive inhibitor of cytochrome oxidase, an enzyme involved in respiration.
- E.g. phosphofructokinase is inhibited by ATP and citrate, both of which are products of enzymatic reactions in cellular respiration. This form of inhibition is also known as **end product inhibition**. It acts as a means to control metabolic reactions by **negative feedback**.



End-product inhibition

(c) Reversible Inhibition

- If the E-I complex can dissociate, the inhibition is **reversible**.
- The association of enzyme with inhibitor is a loose one and can be easily removed. Removal of inhibitor restores the activity of the enzyme to normal.
- Inhibition is reversible only when the inhibitor and enzyme are held by **weak bonds** such as hydrogen bonds.
- These weak bonds eventually break as neighbouring molecules collide and the inhibitors leave the enzyme molecule.

(d) Irreversible Inhibition

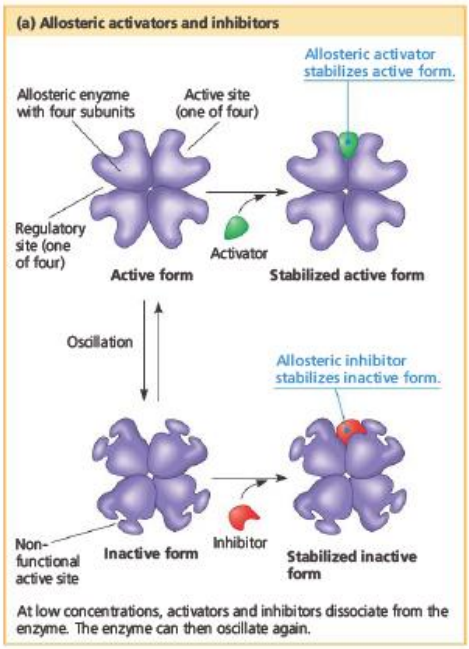
- If the E-I complex cannot dissociate, the inhibition is **irreversible**.
- Irreversible inhibitors **bind permanently** to the enzyme and cause **permanent damage to the enzyme**, such that the enzyme is unable to carry out catalytic activity.
- **Strong covalent bonds** are formed between the enzyme and inhibitor.
- E.g. Heavy metals, e.g. mercury, arsenic and silver, disrupt disulfide bonds which help to maintain the structure of the enzyme molecule.
  - ➢ They react with sulphydryl (-SH) groups in the enzyme and cause the protein to precipitate.
  - ➢ Once the disulfide bonds are broken, the enzyme's molecular structure becomes irreversibly altered with the permanent loss of its catalytic properties.

Additional Reading

Allosteric Regulation of Enzymes

In many cases, the molecules that naturally regulate enzyme activity in a cell behave something like reversible noncompetitive inhibitors: These regulatory molecules change an enzyme's shape and the functioning of its active site by binding to a site elsewhere on the molecule, via weak interactions.

Allosteric regulation is the term used to describe any case in which a protein's function at one site is affected by the binding of a regulatory molecule to a separate site. It may result in either inhibition or stimulation of an enzyme s activity.



Allosteric Activation and Inhibition

- Most enzymes known to be allosterically regulated are constructed from **two or more** subunits, each composed of a polypeptide chain with its own active site.
- The entire protein complex alternates between two different shapes, one catalytically active and the other inactive.
- In the simplest kind of allosteric regulation, an activator or inhibitor binds to an allosteric site, often located where subunits join.
- The binding of an **activator** to a regulatory site stabilizes the shape that has functional active site, whereas the binding of an **inhibitor** stabilizes the inactive form of the enzyme.
- The subunits of an allosteric enzyme fit together in such a way that a change in shape in one subunit is transmitted to all others. Through this interaction of subunits, a single activator or inhibitor molecule that binds to one regulatory site will affect the active sites of all subunits.
- Changes in concentrations of regulators can cause a sophisticated pattern of response in the activity of cellular enzymes.
- The products of ATP hydrolysis (ADP and Pi), for example, play a complex role in balancing the anabolic and catabolic pathways by their effects on key enzymes. ATP binds to several catabolic enzymes allosterically, lowering their affinity for substrate and thus inhibiting their activity.
- ADP, however, functions as an activator of the same enzymes. This is logical because catabolism functions in regenerating ATP. If ATP production lags behind its use, ADP accumulates and activates the enzymes that speed up catabolism, producing more ATP. If the supply of ATP exceeds demand, then catabolism slows down as ATP molecules accumulate and bind to the same enzymes, inhibiting them.
- An example of an enzyme that is allosterically regulated is phosphofructokinase 1 (PFK1) which catalyzes the conversion of **fructose 6-phosphate and ATP to fructose 1,6-bisphosphate and ADP in glycolysis.**

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