

CAIE Biology A-level

Topic 3: Enzymes

Notes

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Enzymes

Enzymes are **globular proteins** that increase the **rate of reaction** by lowering the **activation energy** of the reaction they catalyse. The **active site** is the area of the enzyme where the reaction with the **substrate** takes place. Each enzyme has a **specific** shape that must be **complementary** to the substrate, meaning that only one type of substrate fits into the active site of each enzyme (enzyme specificity). When the enzyme and substrate form a **complex**, the structure of the enzyme is altered so that the active site of the enzyme fits around the substrate. This is called the **induced fit model**.

Enzymes can be **intracellular** (function inside cells), for example DNA polymerase. They can also be **extracellular**, such as the enzymes used in digestion.

Lock and Key Theory:

Proposed by Fischer in 1894

- Active site and substrate have **complementary shapes prior to binding**
- The enzyme binds with substrate forming an enzyme-substrate complex
- Products are released from active site and enzyme can be reused
- Only one substrate can fit each active site

Induced Fit Theory:

Proposed by Koshland in 1958

- Enzyme has active site
- Enzyme is moulded around substrate as it enters to **become complementary** forming an enzyme-substrate complex
- Bonds form between oppositely charged groups on substrate and R groups to induce a better fit. This puts a strain on the substrate molecule so reactions occur more easily.

Factors affecting the rate of enzyme-controlled reactions:

- **Enzyme concentration** – the rate of reaction increases as enzyme concentration increases as there are more active sites for substrates to bind to, however increasing the enzyme concentration beyond a certain point has no effect on the rate of reaction as there are more active sites than substrates so substrate concentration becomes the limiting factor.
- **Substrate concentration** – as concentration of substrate increases, rate of reaction increases as more enzyme-substrate complexes are formed. However, beyond a certain point the rate of reaction no longer increases as enzyme concentration becomes the limiting factor.
- **Temperature** – rate of reaction increases up to the optimum temperature as kinetic energy increases. Rate of reaction decreases beyond the optimum temperature. At very high temperatures, bonds in the enzymes tertiary structure break, changing the shape of the active site so reactions cannot occur. This is called denaturation.



- **pH** –As the pH moves away from the enzymes optimum, rate of reaction decreases. The pH is a measure of the concentration of hydrogen ions. Each enzyme has an optimum pH: the wrong pH alters the charges on the amino acids which make up the active site, breaking the bonds in the enzyme's tertiary structure and leading to denaturation. Thus, when the enzyme is not in its optimum pH, the substrate can no longer become attached to the active site and the enzyme-substrate complex cannot form.
- **Concentration of competitive reversible inhibitors** – as concentration of competitive reversible inhibitors increases, rate of reaction decreases as the active sites are temporarily blocked by inhibitors so substrates cannot bind to them.
- **Concentration of non-competitive reversible inhibitors** – as concentration on non-competitive reversible inhibitors increases, rate of reaction decreases as the shape of the enzyme (not the active site) is altered by the inhibitors.

Investigating enzyme-catalysed reactions:

You can investigate enzyme activity over a period of time using the following methods

Catalase

- Catalyses the **breakdown of hydrogen peroxide**
- Products: oxygen and water
- Measure the rate of oxygen produced over a period of time
- You can plot a graph of **time vs volume of oxygen produced**

Amylase

- Catalyses the **breakdown of starch**
- Products: maltose
- Amylase is added to the starch samples
- At regular timed intervals take samples
- Use iodine/KI solution to test for the presence of starch (colour change to orange-brown when starch breakdown is complete)
- Measure the **absorbance** in a colorimeter (make sure colorimeter is calibrated and sample has been mixed properly with the iodine)
- The darker the colour the higher the starch concentration hence a higher absorbance
- Plot a graph of **time vs absorbance**

Inhibitors

Inhibitors are substances which stop the enzyme from binding to its substrate. They can therefore control the progress of a reaction.



Types of inhibition:

- **Competitive inhibition** – this is when an inhibitor molecule binds to the active site of the enzyme and stops the substrate from binding to it; it can be reversed by increasing the substrate concentration as the inhibitor is diluted.
- **Non-competitive inhibition**- an inhibitor doesn't bind to the active site but binds to a different part of the enzyme which changes the shape of the enzyme; it decreases the reaction rate as the substrate cannot bind to the enzyme.
- **Feedback inhibition** – this occurs when the end product binds to the enzyme at the start of the reaction/pathway and this stops the pathway until the concentration of the end product decreases.
- **Reversible inhibition** - they can be competitive or non-competitive. Once they are removed from the enzyme, inhibition stops and it can work again.

Michaelis-Menten Equation

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

V_0 = Initial velocity (moles/times)

$[S]$ = substrate concentration (molar)

V_{\max} = maximum velocity

K_m = substrate concentration at half V_{\max}

Michaelis-Menten equation can be used to calculate the **maximum rate of reaction (V_{\max})** by relating the **velocity of enzyme reactions (V)** to **concentration of a substrate $[S]$** . V_{\max} represents the maximum rate of reaction achieved by the system at maximum substrate concentration.

Immobilising enzymes in alginate

When enzymes are **in solution**, they can only be **used once** as it is very difficult and time consuming to separate them from the product. Therefore they are **immobilised by attaching them to an insoluble, inert material e.g. calcium alginate** which forms a gel capsule around them thus holding them in place during the reaction. **This process enables enzymes to be reused** as they can be easily separated from the products. Immobilised enzymes are used in industry because it **enables the reaction to flow continuously**. Moreover, the use of immobilised enzymes is **much cheaper** than using enzymes in solution as they can be reused.

