SL IB Biology

Your notes

Enzymes & Metabolism

Contents

Metabolism: Enzymes & Reactions

Your notes

Increasing Reaction Rates in Cells

Enzymes as catalysts

- Most chemical reactions either **do not occur spontaneously** or **occur very slowly**
- In laboratory or industrial settings, some chemical reactions require some sort of catalyst in order to form a sufficient concentration of **product molecules**
  - Other conditions that may speed up the reaction rate include:
    - High temperatures or pressures
    - Extremes of pH
    - High concentrations of the reactants
- Cells are very sensitive to extreme temperatures, pressures and pH-levels, so the chemical reactions occurring in them cannot be sped up by these means
- **Enzymes** are proteins that **act as biological catalysts** in cells and allow chemical reactions to occur at a suitable rate in the conditions found in living organisms
  - They are **reusable**, so only a small number is needed to catalyse reactions
  - They **remain unchanged** by the reactions that they catalyse
- Without the presence of enzymes, the rate of chemical reactions in organisms would be **too low** to support life
- To form product molecules, the reactants would need to collide at the **correct angle and speed** in order for a reaction to occur
  - The chances of this occurring under normal conditions would be so low, that this would be an insignificant event
- Enzymes ensure that molecules (called substrate **molecules**) are orientated correctly and close enough for a reaction to occur
- The cell has control over the enzymes being produced, which in turn gives the cell **control** over the **chemical reactions** occurring in the cytoplasm

Metabolism: Role of Enzymes

What is metabolism?

- **Metabolism** is a catch-all term used to describe **all the chemical reactions** that take place within cells and organisms
- Metabolism can be thought of as **the chemical reactions of life**
  - The molecules involved are **metabolites**
- Many reactions of metabolism take place in **multiple stages**
  - Each stage is **catalysed** by a separate **enzyme**
- A series of interlinked metabolic reactions is called a **metabolic pathway**
- Metabolic reactions can be classified broadly as **anabolic** or **catabolic**

Role of enzymes in metabolism

- Enzymes are **globular proteins**
- Critical to the enzyme's function is the **active site** where the substrate binds
- Enzymes are **specific** to the substrate
  - The shapes of the enzyme and substrate and their **chemical properties** are **complementary**, to allow the substrate to fit into the active site, like two jigsaw pieces fitting together
  - This is called **enzyme-substrate specificity**
- Due to this specificity, thousands of enzymes are needed throughout an organism, to carry out **individual chemical reactions**
- This means that **control over metabolism** can be exerted through these enzymes

Examiner Tip

Avoid the common mistake in an exam to say that the shapes of the enzyme active site and substrate molecule are the same, they are not. Complementary means that they fit together because of the specific differences in their shapes.

Your notes

Anabolism & Catabolism

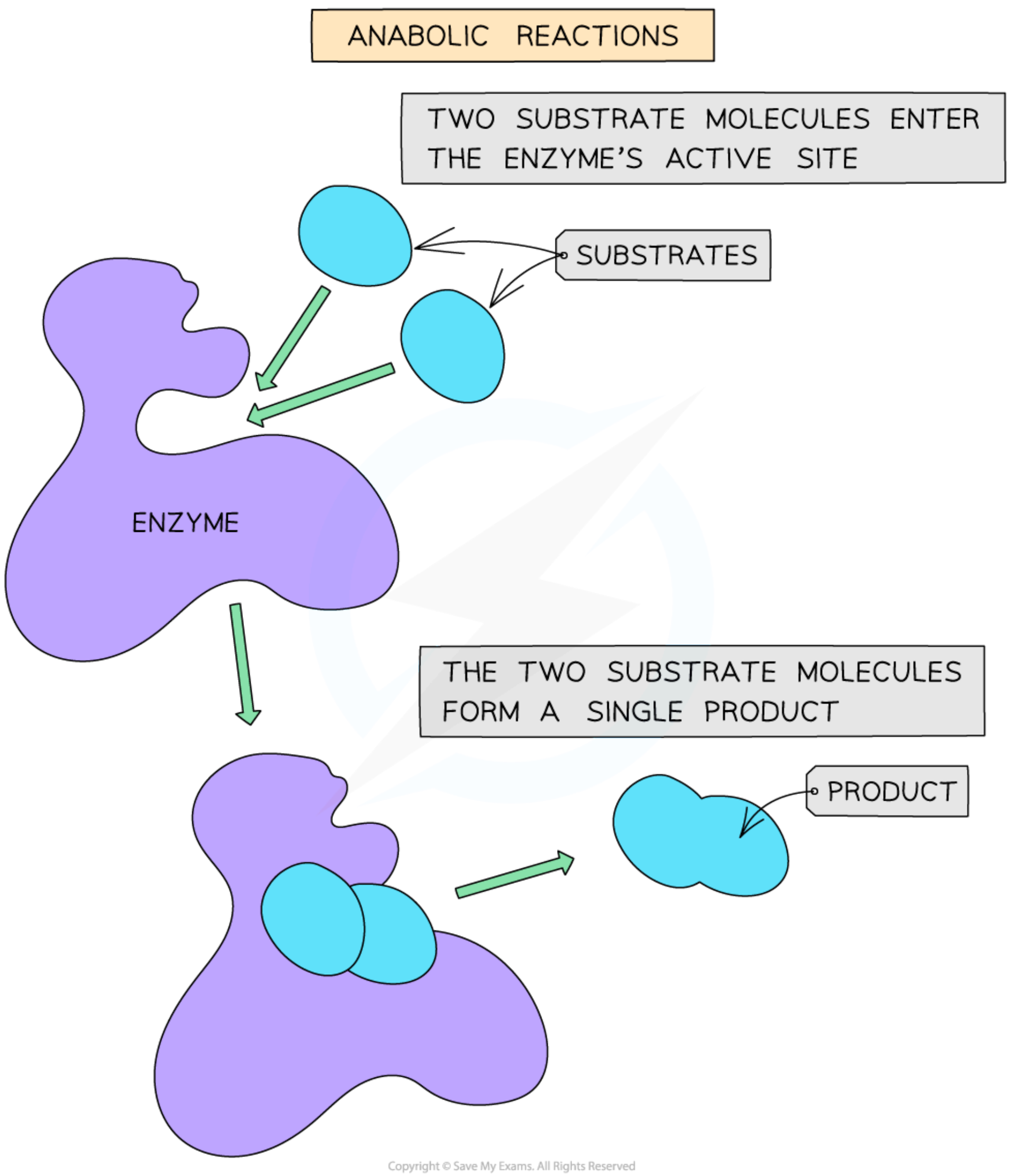
Your notes

Anabolic reactions

- Anabolic reactions are involved with the **building of large molecules from smaller ones**
- Examples include;
  - Photosynthesis, where CO_2 and water are built up into complex sugars
  - Protein synthesis, where amino acids are joined together in sequence
  - The formation of glycogen by linking glucose molecules together
- Anabolic reactions often include condensation reactions
- Anabolic reactions are **endergonic** (they require an input of energy to take place)
  - Energy-storing products are the end result

Enzyme-catalysed anabolic reactions diagram

Your notes



Anabolic reactions involve the linking of more than one substrate molecule to form a more complex product molecule

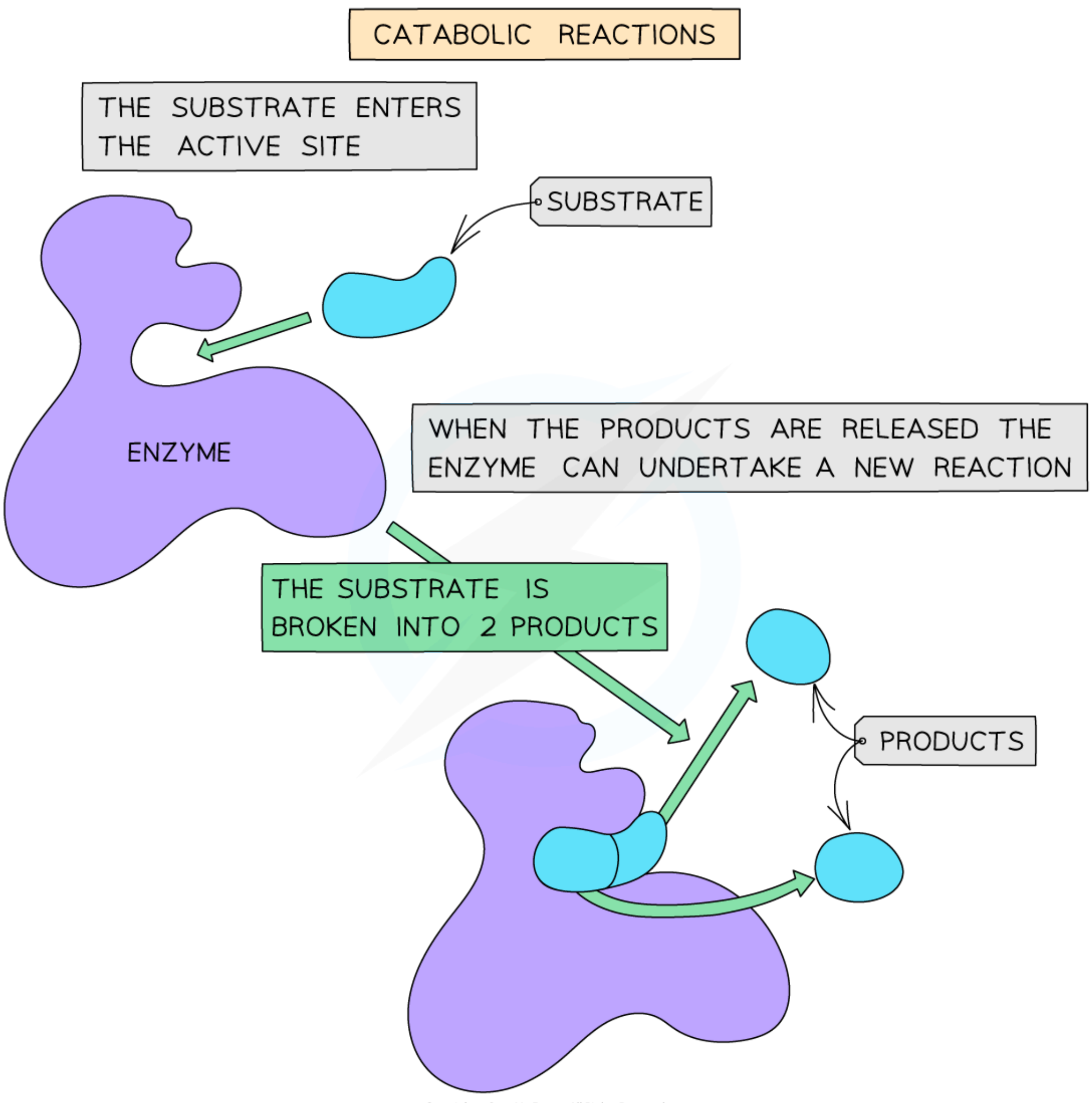
Catabolic reactions

- Catabolic reactions are involved with **breaking down large molecules** into smaller, simpler ones
- These reactions are often carried out to **release energy** for cellular processes and for the **excretion** of waste
- Examples include:
  - **Respiration**, where CO_2 and water are produced from the oxidation of sugars

Your notes

- **Deamination** of proteins to release urea
- **Breakdown of macromolecules** into monomers during digestion
- Catabolic reactions often include hydrolysis reactions
- Catabolic reactions are **exergonic** (free energy is released for cellular processes or as excess heat)

Enzyme-catalysed catabolic reactions diagram



Catabolic reactions happens when a single substrate molecule is drawn into the active site and broken apart into two or more product molecules

Comparison of anabolism and catabolism table

Anabolism	Catabolism
Requires an input of energy (endergonic)	Releases energy (exergonic)
Builds large molecules from small ones	Breaks down large molecules into smaller ones
Used to store energy in chemical form	Used to release chemical energy as heat and for other activities such as movement and active transport
Involves condensation reactions	Involves hydrolysis reactions
Used for growth, repair and energy storage	Performs several activities such as digestion, excretion and energy supply
Both are made up of enzyme-catalysed reactions	
Both are coupled to ATP, the principle energy carrier in cells	

Your notes

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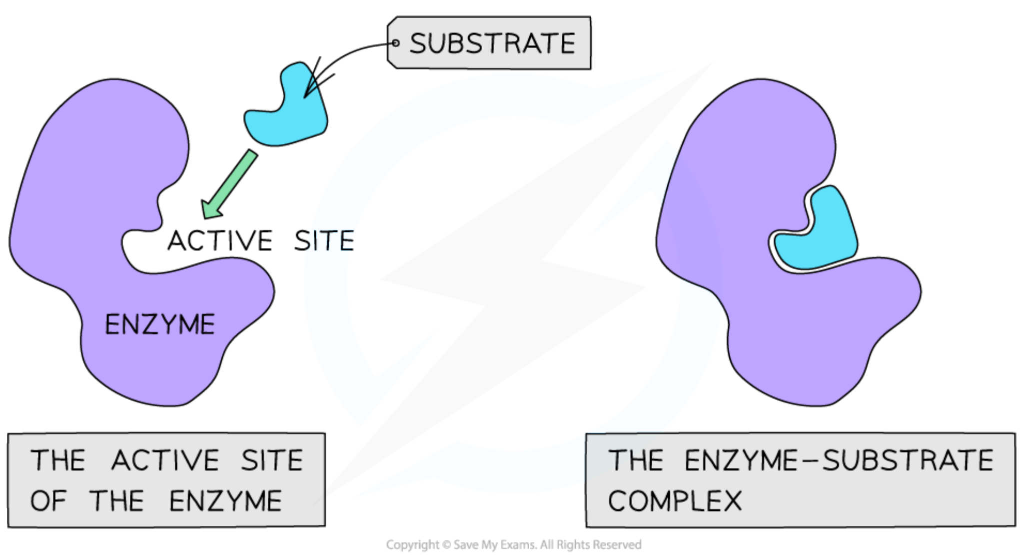
Enzyme Action

Structure of Enzymes

The structure of enzymes

- Enzyme catalysis involves molecular motion and the collision of substrates with the active site
- For an enzyme-catalysed reaction to take place, substrates **collide at random** with the enzyme's active site
- This must happen at the correct **orientation** and **speed** in order for a reaction to occur
  - **Unsuccessful collisions** can occur when the molecules are not correctly aligned with each other at the moment of collision
  - The molecules 'bounce' off each other and **no reaction** takes place
- Some enzymes have **two substrates** that must each collide with a separate active site **at the same time**
- Substrates bind to enzymes, forming a temporary **enzyme-substrate complex**
- The **active site** of an enzyme has a **specific shape** and **chemical properties** to bind with a specific substrate
- The reaction occurs within the enzyme-substrate complex which leads to changes in the **chemical structure of the substrate**
- **Products** are formed, which **detach** and **move away** from the active site, which can be re-used

Enzyme action diagram

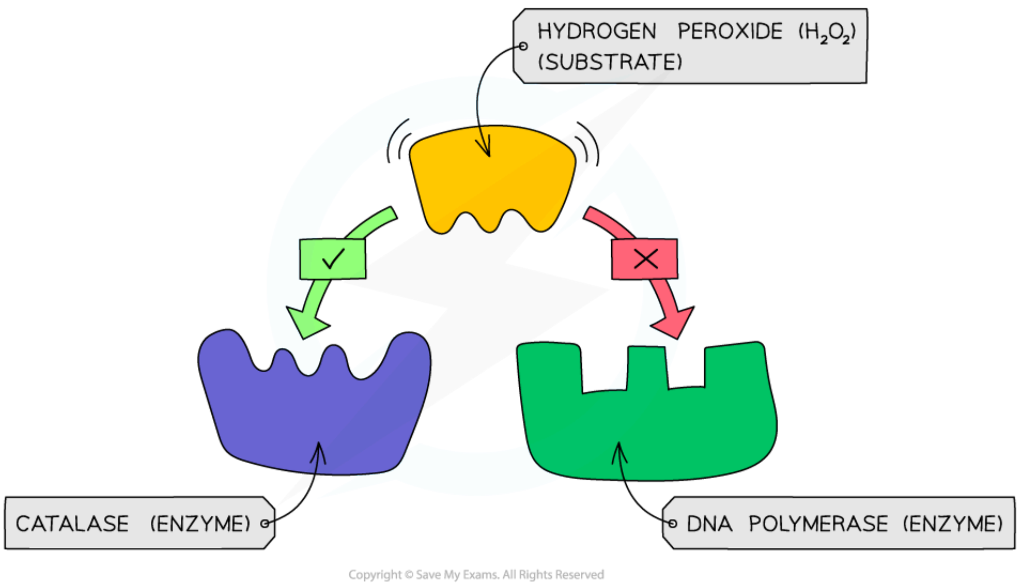


The active site of an enzyme has a specific shape to fit a specific substrate (when the substrate binds an enzyme-substrate complex is formed)

Your notes

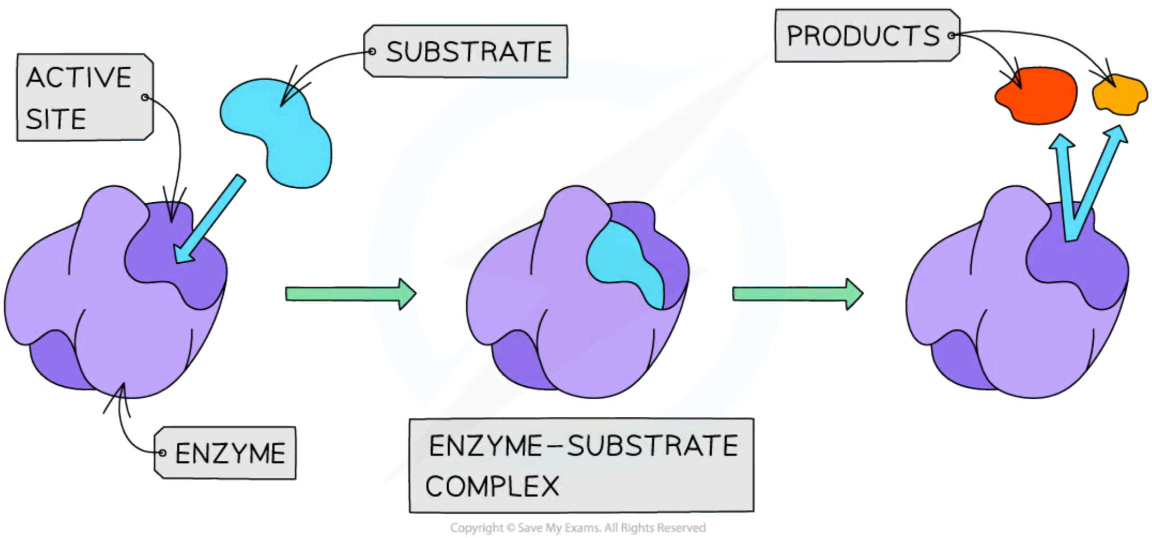
- The **specificity** of an enzyme is a result of the **complementary nature** between the shape of the active site on the enzyme and its substrate(s)
- The **shape** of the active site (and therefore the specificity of the enzyme) is determined by the **complex 3D shape** of the protein that makes up the enzyme
  - The active site is made of only a few amino acids but the **interaction of these amino acids** within the 3D shape of the enzyme ensures that **catalysis** can occur
  - This is achieved by:
    - Binding to the **substrate molecule**
    - **Holding it in position** for a chemical reaction to occur
    - **Lowering the energy needed** for the reaction to occur

Enzyme specificity diagram



An example of enzyme specificity – the enzyme catalase can bind to its substrate hydrogen peroxide as they are complementary in shape, whereas DNA polymerase is not

Formation of enzyme-substrate complex diagram



The temporary formation of an enzyme-substrate complex

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Examiner Tip

Don't forget that both enzymes and their substrates are highly specific to each other - this is known as enzyme-substrate specificity.

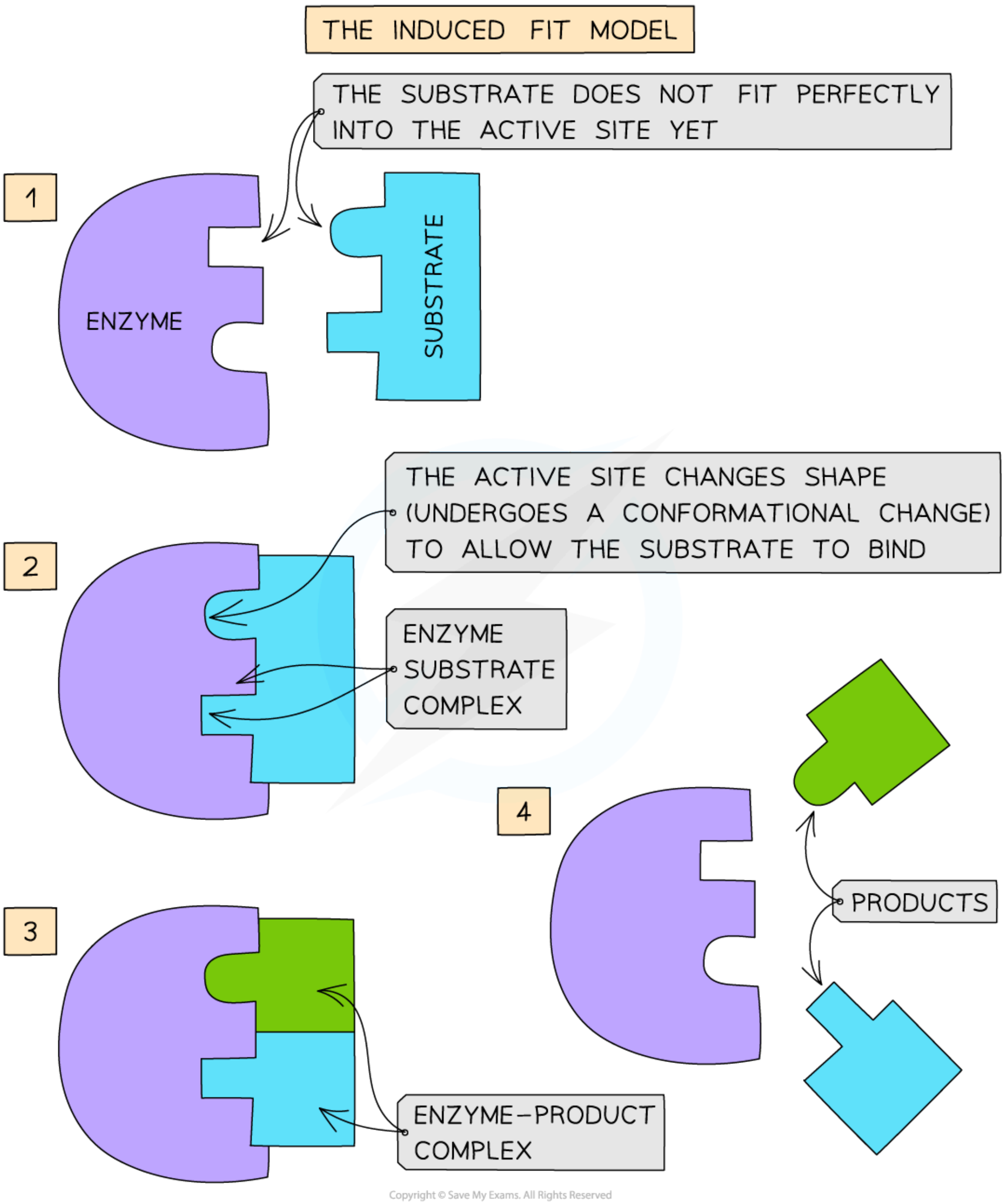
Induced-fit Binding

The induced-fit hypothesis

- The original model explaining interactions between enzymes and their substrate molecules was called the **lock-and-key model**
  - This model proposed that the enzyme active site is **precisely complementary** to the shape of the substrate molecule
  - The substrate molecule therefore fits into the active site like a key in a lock
- The **modified model** of enzyme activity is known as the '**induced-fit hypothesis**'
- Although it is very similar to the lock and key hypothesis, in this model the enzyme and substrate **interact** with each other:
  - The enzyme and its active site (and sometimes the substrate) can **change shape** slightly as the substrate molecule enters the enzyme
  - These changes in shape are known as **conformational changes**
  - This ensures an **ideal binding arrangement** between the enzyme and substrate is achieved
  - This **maximises the ability of the enzyme to catalyse the reaction**

Induced-fit model diagram

Your notes



The induced-fit hypothesis of enzyme action

Examiner Tip

Don't forget - our current understanding of enzyme-substrate interactions is based on the induced-fit hypothesis.

Your notes

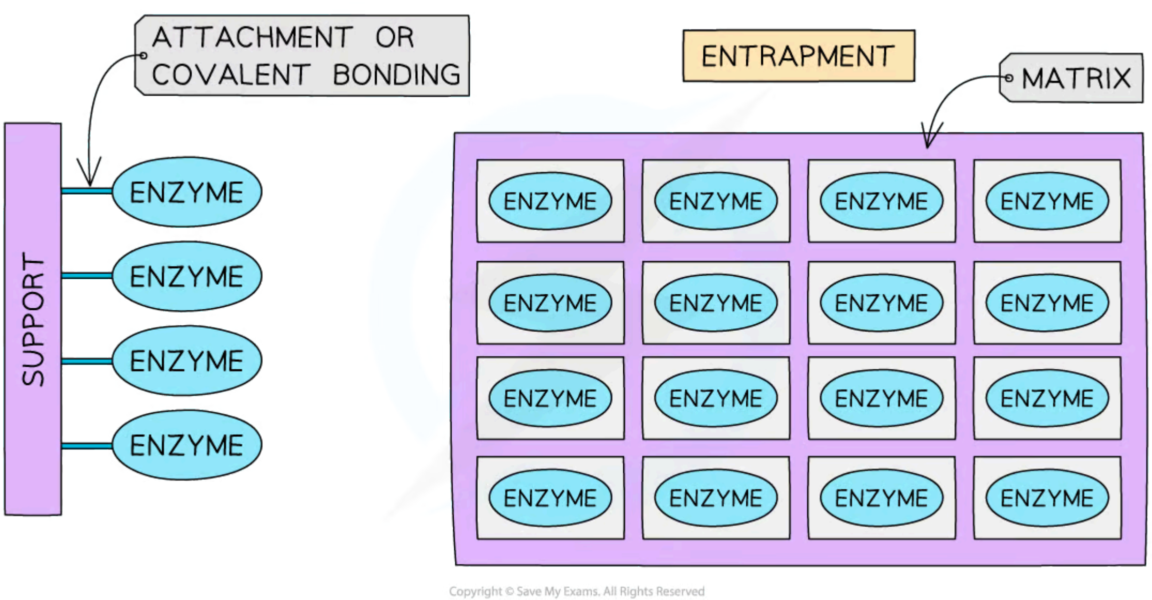
Your notes

Enzyme Catalysis

The role of molecular motion and substrate-active site collisions

- In order for the substrate molecule to collide with and ultimately bind to the enzyme active site, **movement is required**
  - This movement is the result of the **kinetic energy** that molecules have
  - The greater the kinetic energy of the molecules, the **faster the movement** and the higher the probability of enzyme and substrate colliding
  - This leads to **more enzyme-substrate complexes** forming and the production of more product molecules
- In some cases, **large substrate molecules** are immobilised, while in other cases it is possible to **immobilise enzymes** by embedding them in membranes
- These immobilised enzymes can be used in a **range of industries** such as food processing, environmental management, pharmaceuticals and manufacturing processes
- There are different methods by which enzymes can be immobilised including:
  - Attachment to an **inert substance** e.g. glass
  - Entrapment within a **matrix** e.g. alginate gel
  - Entrapment within a **partially permeable membrane**

Examples of immobilised enzymes diagram



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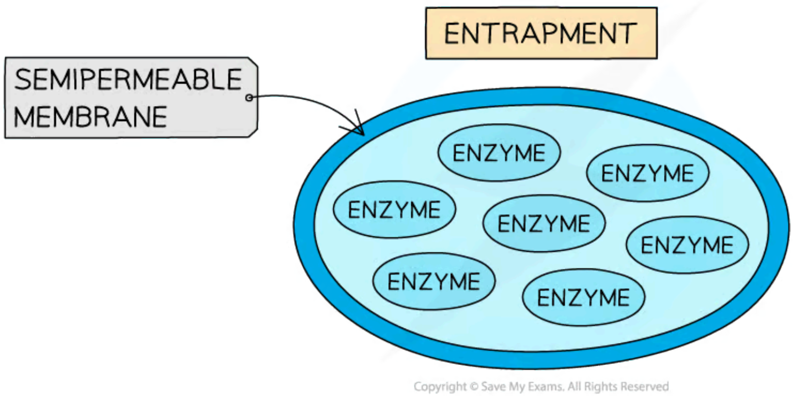


There are many different ways in which enzymes can be immobilised

Advantages of immobilised enzymes

- There is **no enzyme in the product** (the product is **uncontaminated**) and therefore there is no need to further process or filter the end product
- The immobilised enzyme can be **reused multiple times** which is both efficient and **cost-effective** (many enzymes are **expensive**)
  - Reusing the enzyme also **avoids the need to separate** the enzyme from the product in downstream processing
- Immobilised enzymes have a **greater tolerance of temperature and pH changes** (immobilisation often makes enzymes more **stable**)
- Substrates can be exposed to **higher enzyme concentrations** than when using enzymes in solution, increasing the rate of throughput
- Conditions can be **controlled carefully**, allowing immobilised enzymes to function **close to their optimum conditions** and be **more stable**

Denaturation: Enzymes

- Enzymes can be denatured when it is exposed to **high temperatures** or **extremes of pH**
- **Bonds** (e.g. hydrogen bonds) holding the enzyme molecule in its precise 3D shape start to **break**
  - Take note that the peptide bonds holding the amino acids together are not broken
- This causes the **3-dimensional shape** of the protein (i.e. the enzyme) to **change**
- This permanently **changes the shape** of the **active site**, preventing the **substrate** from **binding**
- **Denaturation** has occurred if the **substrate can no longer bind**
- The reaction that was previously catalysed **now no longer takes place**
- Denaturation often causes the enzyme to **become insoluble** and form a **precipitate**
- Very few human enzymes can function at temperatures **above 50°C**
  - This is because humans maintain a body temperature of about 37°C, therefore even temperatures exceeding 40°C will cause the denaturation of enzymes
  - High temperatures cause increased vibrations in the bonds **between the R-groups of amino acids** so they start to break, changing the conformation of the enzyme

Examiner Tip

Don't forget that enzymes are always proteins and so anything that could denature a protein, rendering it non-operational (extremes of heat, temperature, pH etc.) would also denature an enzyme. Avoid using the term 'destroyed' or saying that the enzyme is 'killed' when describing the disruption to enzyme structure.

Your notes

Your notes

Enzyme Activity: Skills

Effects of Temperature, pH & Substrate Concentration

NOS: Describing patterns and trends in graphs

- You are required to **describe the relationship** between variables shown in graphs
- **Generalised sketches** of these relationships are **examples of models** in Biology
- Enzyme experiments can be conducted to **investigate the effects** of the following factors on the **rate of enzyme activity**:
  - Temperature
  - pH
  - Substrate concentration
- Sketch graphs can be drawn and evaluated using the results from these experiments

Designing experiments to test the effect of temperature, pH and substrate concentration on the activity of enzymes

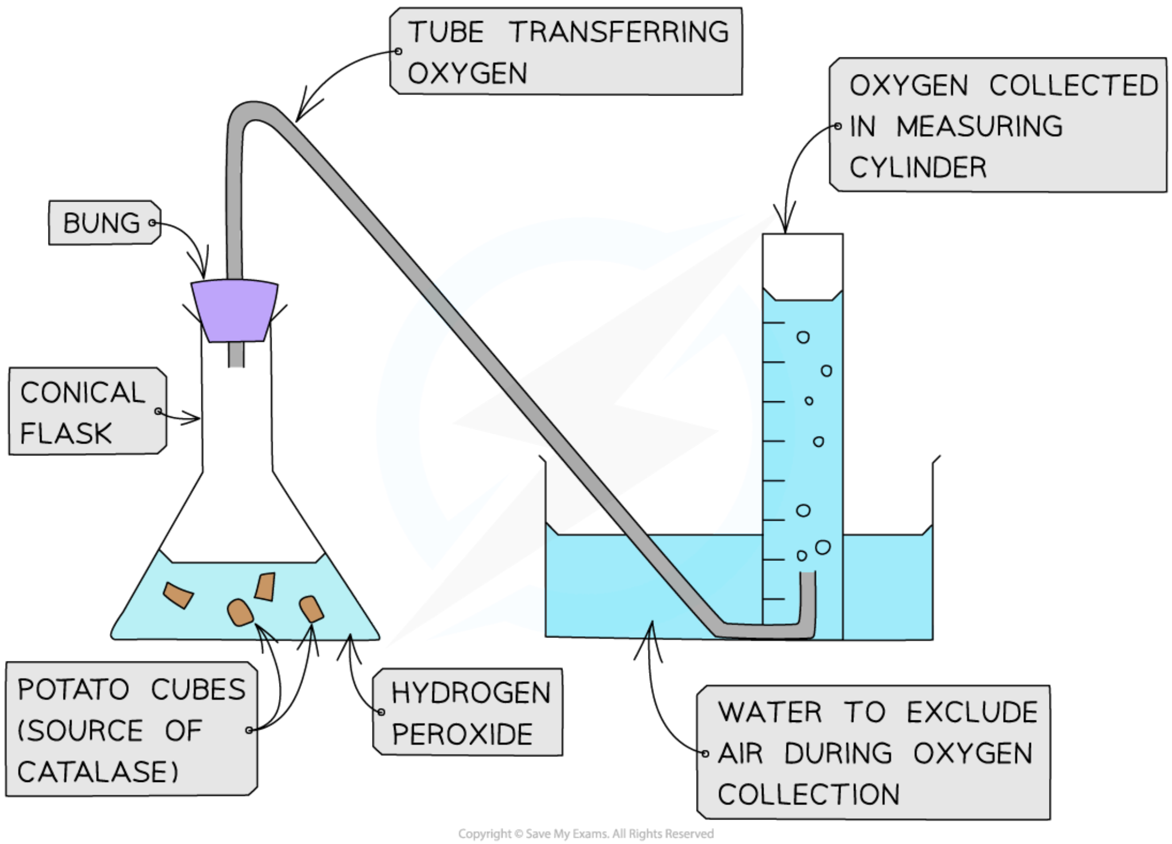
- Three different **independent variables** can be tested
  - Temperature
  - pH
  - Substrate concentration
- You should plan how the **dependent variable is going to be measured**
  - With appropriate units
- Also, what **intervals of the independent variable** are going to be chosen
- These factors dictate the **choice of apparatus** and other equipment required for the experiment
- The **control variables** need to be identified and monitored e.g. temperature when measuring the effect of pH

Investigating the effects of temperature or pH on catalase activity

- The **progress** of **enzyme-catalysed reactions** can be investigated by:
  - Measuring the **rate of formation of a product**
  - Measuring the **rate of disappearance of a substrate**
- In this investigation, the rate of **product formation** is used to measure the rate of an enzyme-controlled reaction:
  - **Hydrogen peroxide** is a common but **toxic** by-product of metabolism
  - This means it must be **broken down** quickly
  - **Catalase** is an enzyme found in the cells of most organisms that **breaks down hydrogen peroxide into water and oxygen**
  - Hydrogen peroxide and catalase are combined and the **volume of oxygen generated** is measured in a set time
  - The **rate of reaction** can then be calculated

Investigating catalase diagram

Your notes



Experimental set-up for investigating the rate of formation of a product using catalase

- If measuring the effect of temperature on enzyme activity, the conical flask containing potato pieces can be held in a water bath at the required temperature
  - The water level in the water bath must be higher than the level of H_2O_2 in the conical flask, to ensure even heating
  - The conical flask can also be swirled gently to mix the contents and maintain an even temperature

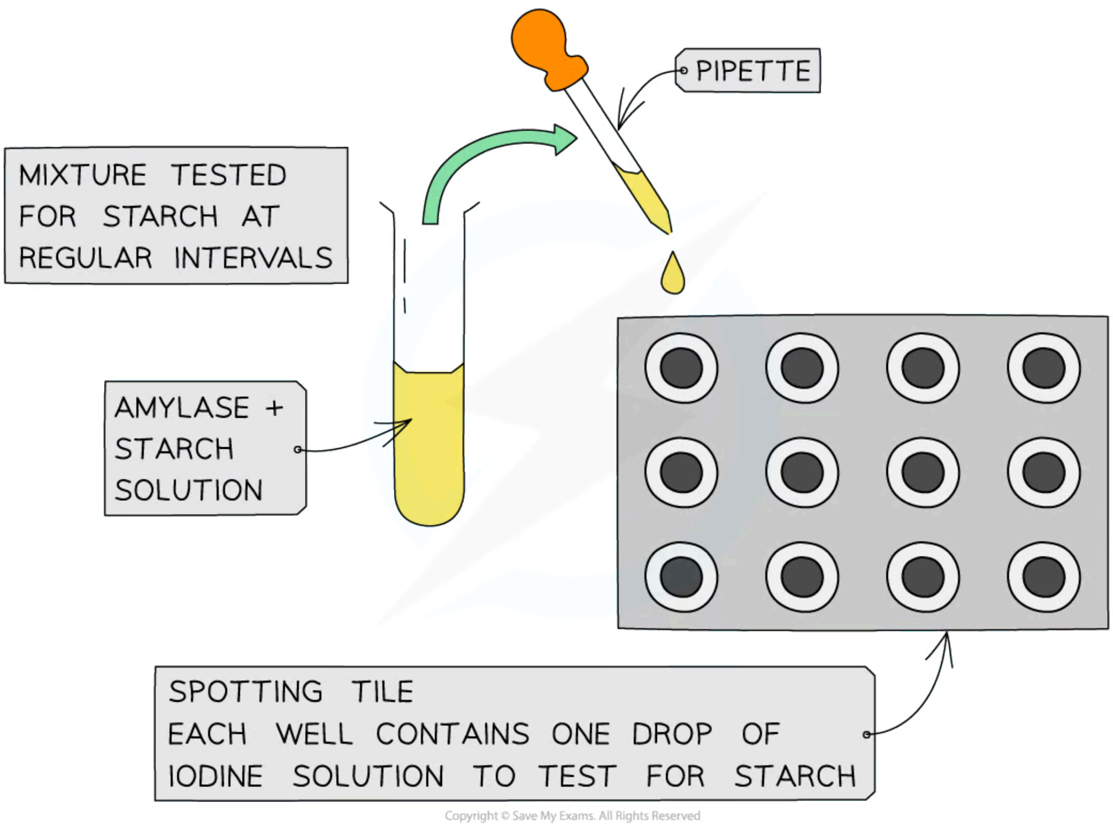
Investigating the effect of substrate concentration on amylase activity

- In this investigation, the rate of **substrate disappearance** is used to compare rates of reaction under different conditions
- **Amylase** is a digestive enzyme that **hydrolyses starch into maltose and glucose**
- Amylase functions best at pH 7 and 37^{o}C (all enzymes operate best under specific conditions)
- **Amylase and starch are combined** and this reaction mixture is then **tested for starch** at regular time intervals
- This can be done by taking samples from the reaction mixture at each time interval and adding each sample to some **iodine in potassium iodide solution**
  - Starch forms a **blue-black** colour with this solution
  - If no starch is present, the iodine solution remains yellow-brown

Your notes

- In this way, the time taken for starch to be broken down can be measured
- The investigation can be **repeated under different starch concentrations** and the **reaction rates can then be compared**
  - This experiment also can be adapted to measure the effects of altering pH, temperature or enzyme concentration

Investigating amylase diagram



Experimental set-up for investigating the rate of disappearance of a substrate using amylase

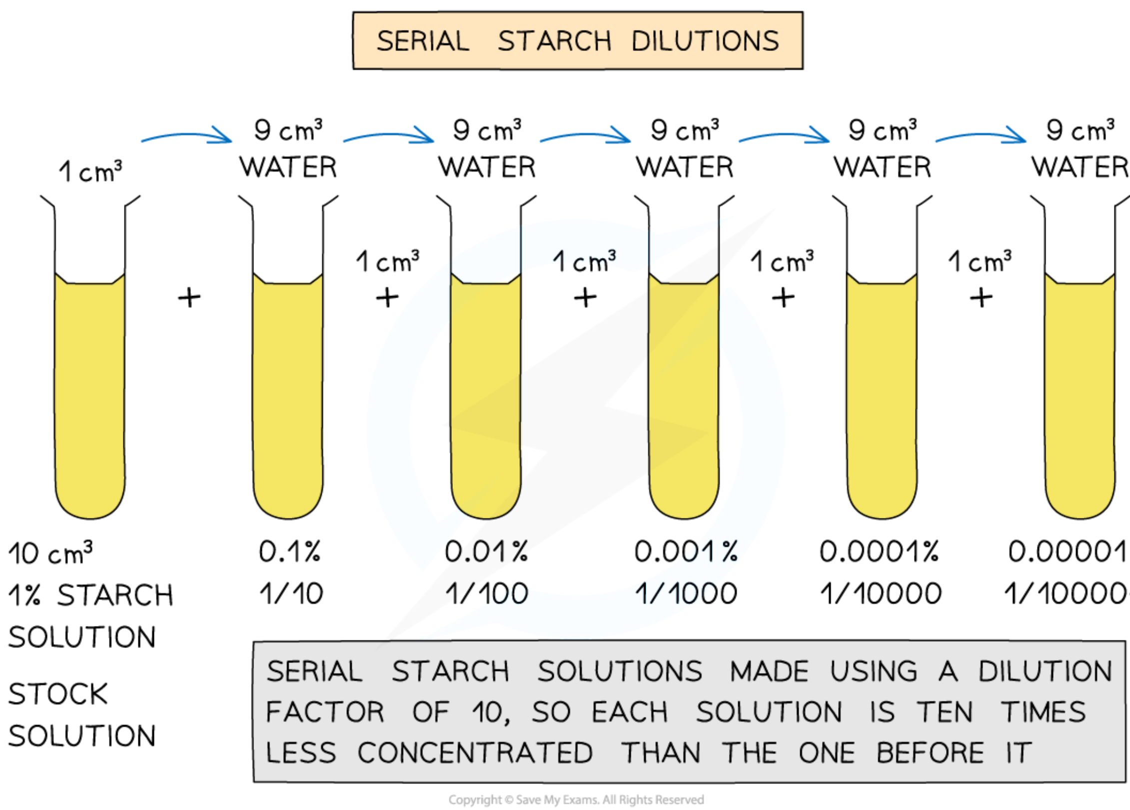
Investigating the effect of starch concentration on amylase using colorimetry

- A **colorimeter** is able to measure **light absorbance** (how much light is absorbed) or **light transmission** (how much light passes through) a substance
- Colorimetry can be used in any enzyme-catalysed reaction that involves a **colour change**
- As the colour breaks down the **transmission increases** or **light absorption decreases** and this can be used to **measure the rate of the reaction**
- For example, a colorimeter can be used to follow the progress of a **starch-amylase catalysed reaction** as the amylase breaks the starch down into maltose
- This can be carried out as follows:

Your notes

- **Colorimeter calibration**: this is an important step in a colorimetric investigation and in this case, a weak iodine solution can be used to calibrate the colorimeter as the endpoint (or 100% transmission)
- Preparation of a starch solution of **known concentration** (stock solution), from which a range of concentrations are made using **serial dilutions** (method outlined in diagram below)
- Following calibration and switching on the red filter (to maximise the percentage transmission or absorbance), the colorimeter is used to **measure the percentage absorbance or percentage transmission values**
- Sometimes a reagent or indicator is used to produce the colours detected by the colorimeter and sometimes the solutions themselves absorb light waves
- A **calibration graph** is then plotted of starch concentration (*x*-axis) vs percentage absorbance or percentage transmission (*y*-axis)

Serial starch dilutions diagram



Serial dilution of starch to make a range of concentrations

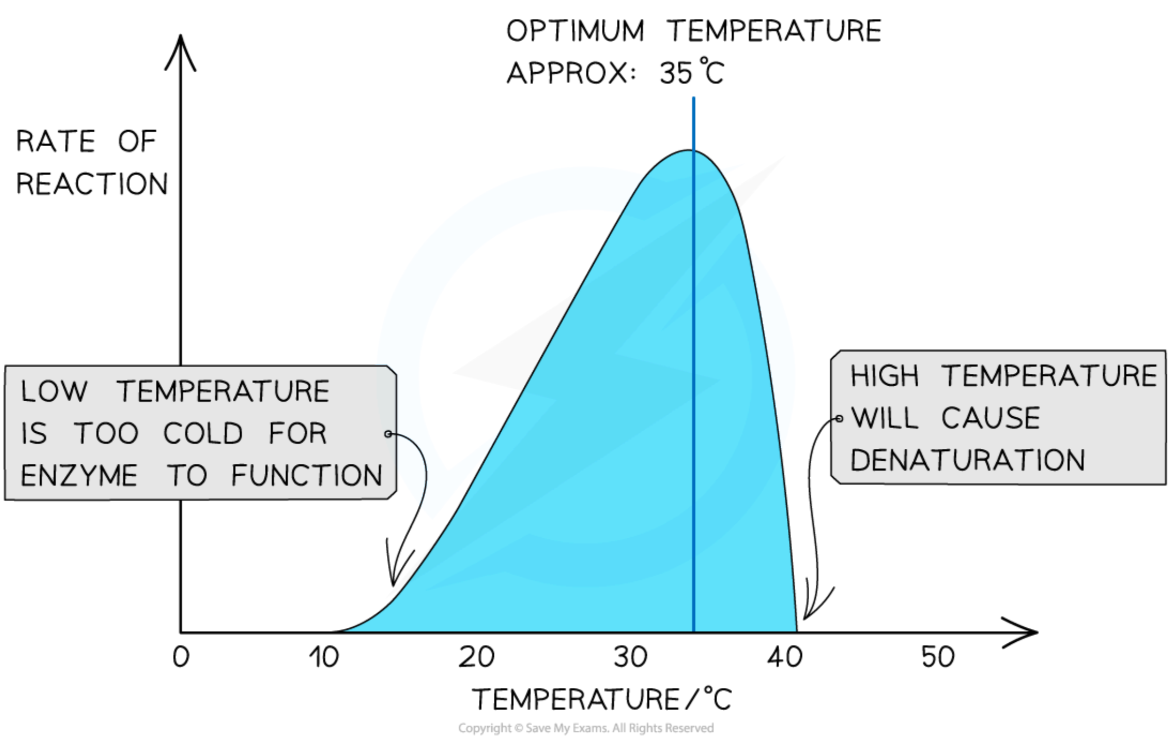
Interpreting graphs on the effects of temperature, pH and substrate concentration on the rate of enzyme activity

- Temperature, pH and substrate concentration affect the rate of activity of enzymes

Your notes

- Enzymes have a **specific optimum temperature** – the temperature at which they catalyse a reaction at the **maximum rate**
- **Lower temperatures** either **prevent** reactions from proceeding or **slow them down**:
  - Molecules move relatively **slowly** due to having **less kinetic energy**
  - **Lower frequency of successful collisions** between a substrate molecule and the active site of enzyme
  - Less frequent enzyme-substrate complex formation
  - Substrate and enzyme collide with **less energy**, making it less likely for bonds to be formed or broken (stopping the reaction from occurring)
- **Higher temperatures speed up reactions**:
  - Molecules move more **quickly** due to having **more kinetic energy**
  - **Higher frequency of successful collisions** between a substrate molecule and the active site of enzyme
  - More frequent enzyme-substrate complex formation
  - Substrate and enzyme collide with **more energy**, making it more likely for bonds to be formed or broken (allowing the reaction to occur)
- However, as temperatures continue to increase, the rate at which an enzyme catalyses a reaction **drops sharply**, as the enzyme begins to denature

The effect of temperature on enzyme action diagram



The effect of temperature on the rate of an enzyme-catalysed reaction

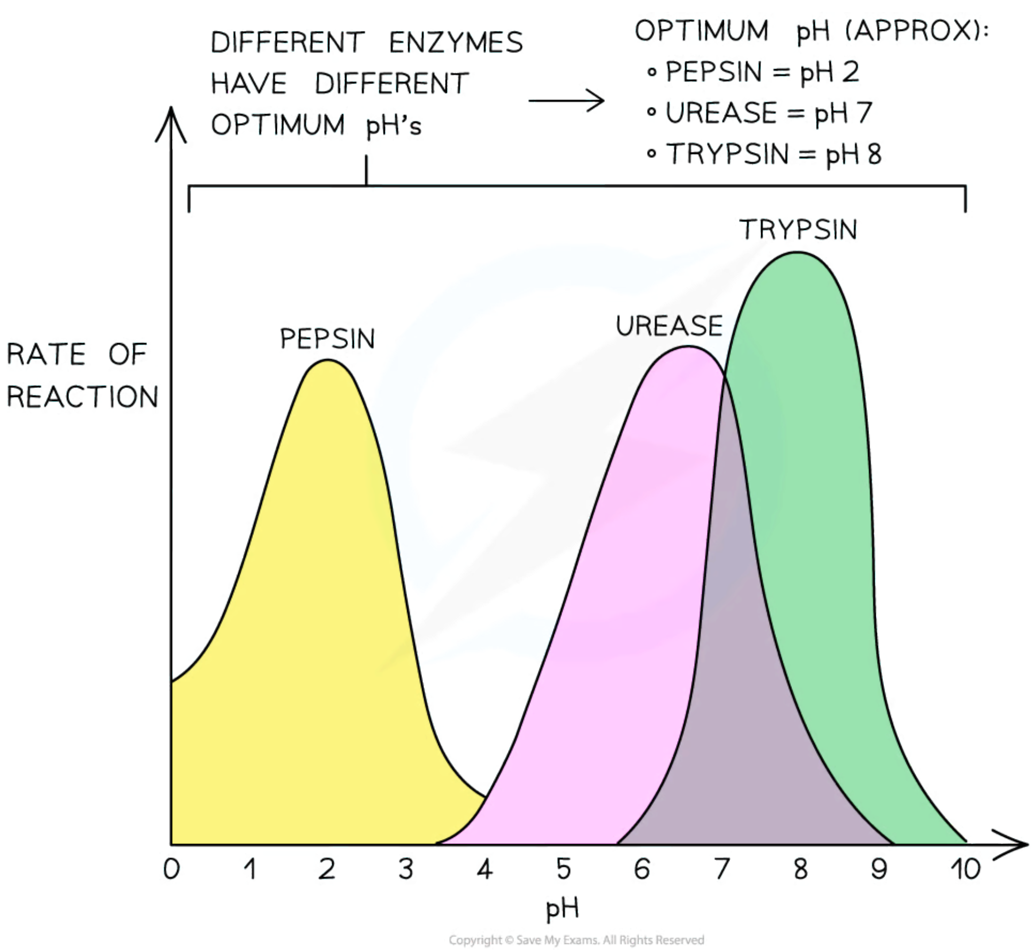
Changes in pH

Your notes

- pH is a result of the **hydrogen ion concentration** in a solution
- A **low pH** is **acid** and has a **high** hydrogen ion concentration
- A **high pH** is **alkaline** and has a **low** hydrogen ion concentration
- A **10× increase** in hydrogen ion concentration lowers the pH by **1 unit**
  - pH is therefore measured on a **logarithmic scale** of hydrogen ion concentration, **not a linear scale**

- Water has a pH of 7, regarded as **neutral**
- **Extremes of pH** can also alter hydrogen bonding within an enzyme's structure and cause irreversible **denaturation**
- Each enzyme has an **optimum pH**
- Not all enzymes have an optimum pH near to neutral. For example
  - The **stomach enzyme** pepsin is adapted to work best at **pH 2**
  - Certain bacterial enzymes work at **pH 9–10**, in line with the pH of the bacteria's main habitat

The effect of pH on enzyme action diagram



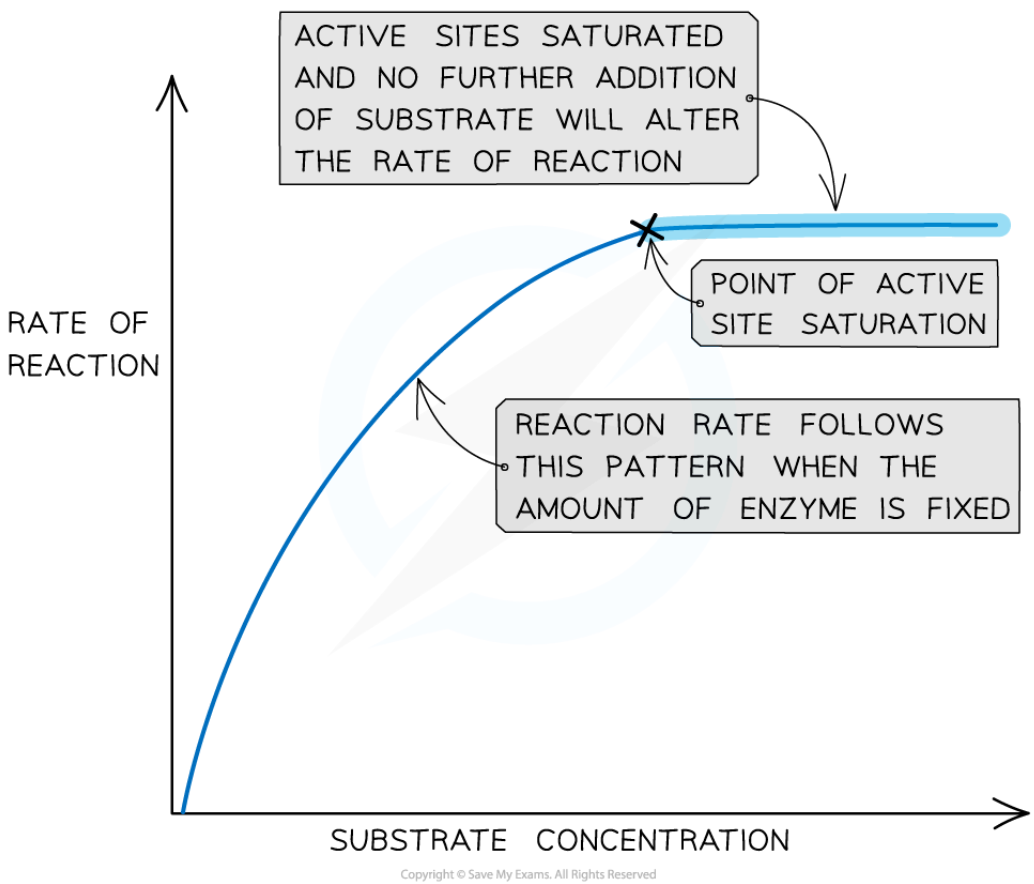
The effect of pH on three enzymes' rates of reaction

Your notes

Changes in substrate concentration

- The more substrate molecules are present in a solution, this **increases the frequency of collisions** with the enzyme's active site
- Active sites are **occupied** or 'blocked' by substrates whilst the reaction is taking place
- The more active sites are occupied, **the fewer are available to catalyse other substrate** molecules
- As substrate concentration rises, the slower the rise in the rate of the enzyme-catalysed reaction
- The active sites have become **saturated**
- At the **point of active site saturation**, increasing the substrate concentration will cause **no further increase** in the rate of reaction
- At the point of active site saturation, a method of increasing the rate of reaction would be to make more active sites available by **increasing the enzyme concentration**

The effect of substrate concentration on enzyme action diagram



The effect of substrate concentration on enzyme activity

Your notes

Examiner Tip

When answering questions about reaction rates for enzyme-catalysed reactions, make sure to explain how the temperature affects the speed at which the molecules (enzymes and substrates) are moving and how this, in turn, affects the number of **successful collisions**.You should memorise the sketch graphs of temperature, pH and substrate concentration and be able to sketch new curves for changed conditions.

Enzyme Reaction Rates: Skills

Determining Enzyme Reaction Rates

- Enzyme catalysed reactions can be affected by changes in **pH, temperature or substrate concentration**
- The **rate of reaction** can be determined by measuring the **rate of disappearance of a substrate** or the **rate of product accumulated** in a **given time period**
- This may be shown as a change in quantity (usually volume or mass) of substrate or product over a measured time period:

$$\text{RATE OF A REACTION} = \frac{\text{CHANGE IN AMOUNT OF REACTANTS OR PRODUCTS (mol dm}^{-3}\text{)}}{\text{TIME (s)}}$$

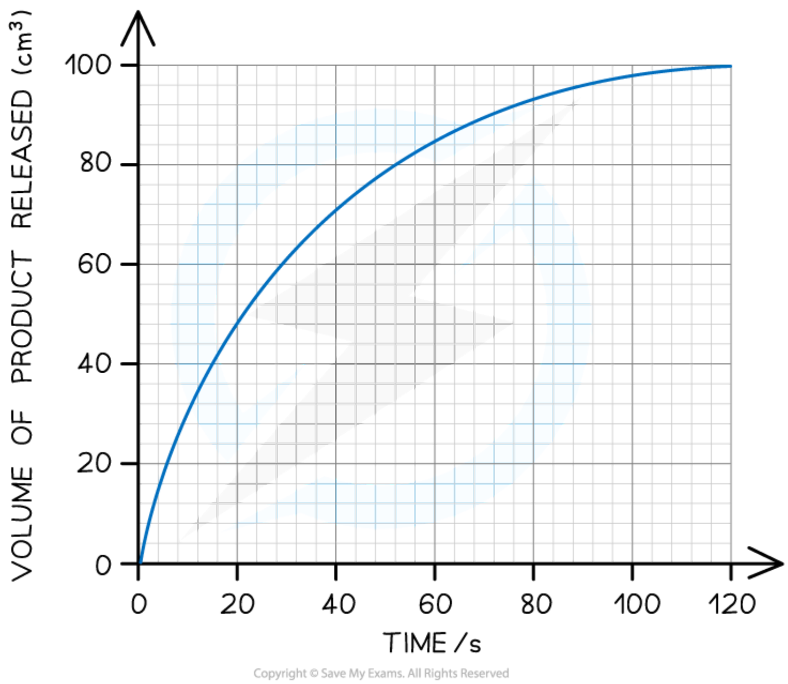
- **Or,** if we cannot collect quantitative data on the amount of substrate or product, we can calculate the rate of reaction **based on the time measured** using the following equation:

$$\text{RATE OF REACTION} = \frac{1}{\text{TIME TAKEN (s}^{-1}\text{)}}$$

  - **1 ÷ time taken (seconds)** and should include the units s^{-1}
- A **high** rate of reaction is when the reaction happens in **less time** i.e. it is faster
- A **low** rate of reaction is when the reaction happens in **more time** i.e. it is slower
- The rate of a reaction is likely to change throughout a reaction as the **substrate concentration will decrease** as the reaction proceeds
  - This leads to a graph that starts out as a **directly proportional** straight line (the value on the X increases at the same rate as the value on the Y) but then **plateaus as the reaction slows down**
- The **steeper the line the faster the rate of reaction**
- The rate of reaction can be calculated from a graph plotted where the reaction **time** is shown on the X-axis and the **quantity of product or substrate** is shown on the Y-axis

Volume of a product produced against time graph

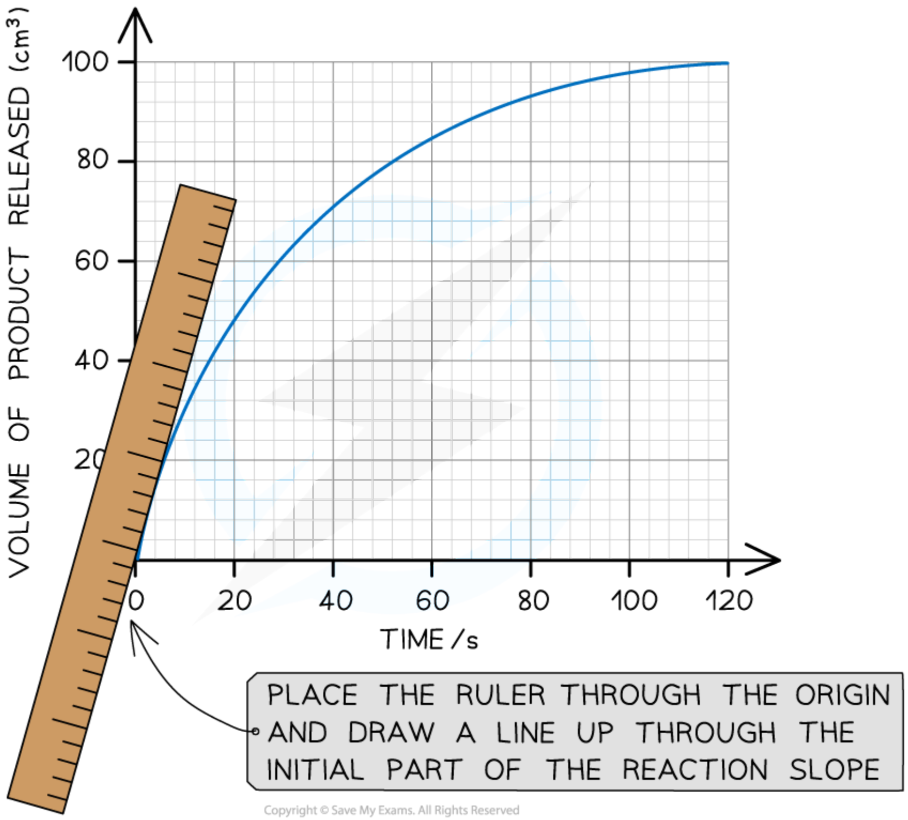
Your notes



Graph produced when plotting the volume of a product produced against time

- The gradient is calculated from a point on the graph and used as a measure of the rate of reaction at that point in time
- A **tangent** must be drawn to calculate the change in x and y so the rate of reaction can be calculated
  - E.g. if calculating the initial rate of reaction
    - Place a ruler on the point of **origin** and draw a line that corresponds to the curve during the early part of the reaction
    - **Extend the line** as far as is convenient to perform the calculations e.g. to 60 seconds

Drawing a tangent to calculate initial rate of reaction diagram



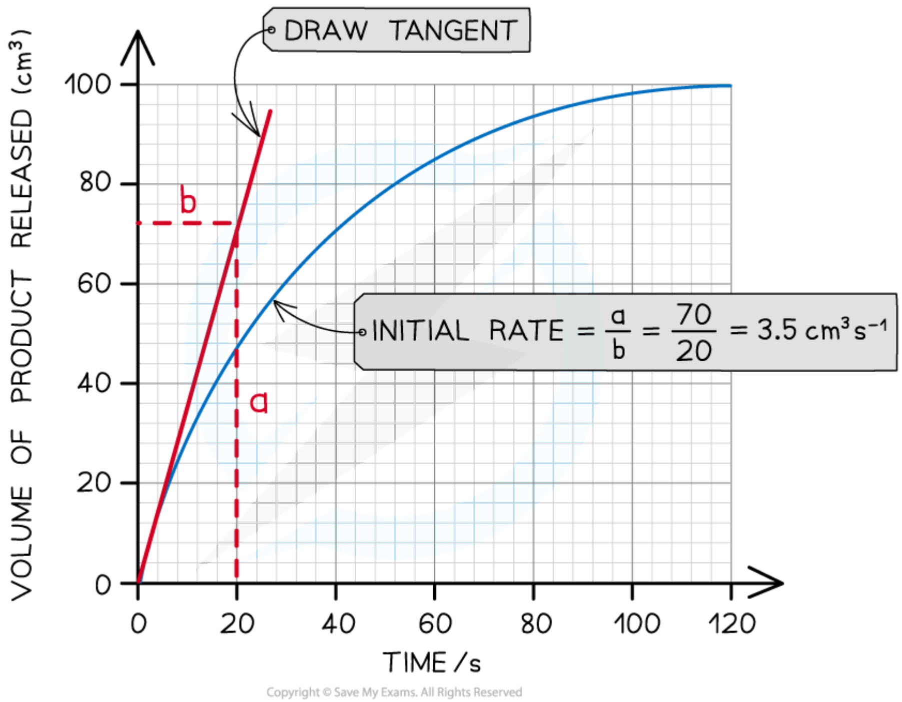
Drawing a tangent against the line through the origin to calculate the initial rate of reaction

Calculating the rate of reaction

- Once the tangent is drawn you can calculate the **gradient** of the line which is equal to the rate of the reaction
  - Initial rate = a ÷ b
  - Where
    - a = change in volume and
    - b = change in time
  - The units will be **cm^3 sec^{-1}** (this means volume per sec)

Your notes

Calculating the rate of reaction from the tangent diagram



Rate of reaction is calculated by finding the gradient of the tangent from the origin

Activation Energy: Skills

Your notes

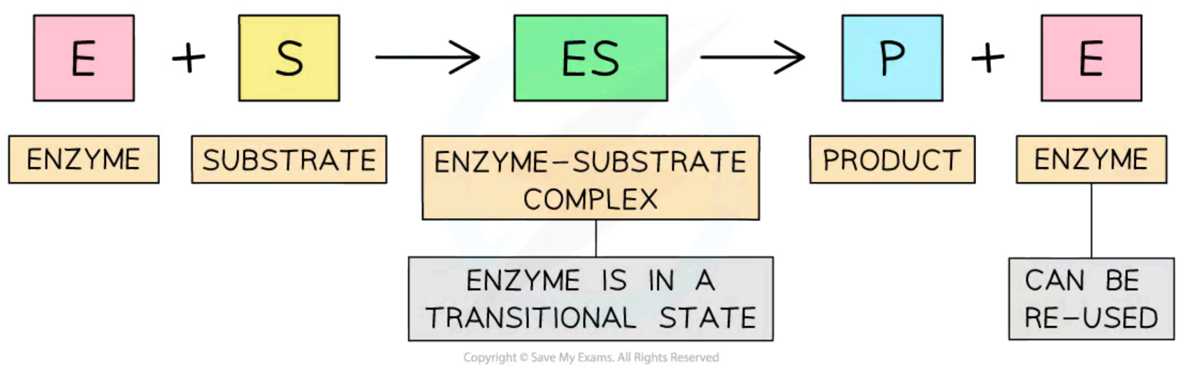
Activation Energy

- **Metabolic pathways** are controlled by **enzymes** in a biochemical cascade of reactions
  - Virtually every metabolic reaction within living organisms is catalysed by an enzyme
  - Enzymes are therefore essential for life to exist
- Enzymes are **biological catalysts**
  - 'Biological' because they function in **living systems**
  - 'Catalysts' because they **speed up** the rate of chemical reactions without being used up or undergoing permanent change

The Enzyme-Substrate Complex

- The starting point of a metabolic pathway is a **substrate** which is converted to an end product
- The enzyme works by binding to the substrate at a special site on the enzyme called the **active site**
  - The active site of an enzyme has a specific shape to fit a specific substrate
- Substrates **collide** with the enzyme's active site and this must happen at the **correct orientation** and speed in order for a reaction to occur
- An **enzyme-substrate complex** is formed, temporarily, when the substrate binds to the active site
  - The substrate is said to be in a **transitional state** at this moment
- The product is formed and enzyme is released to take part in another reaction
- The reaction can be shortened to a simple equation

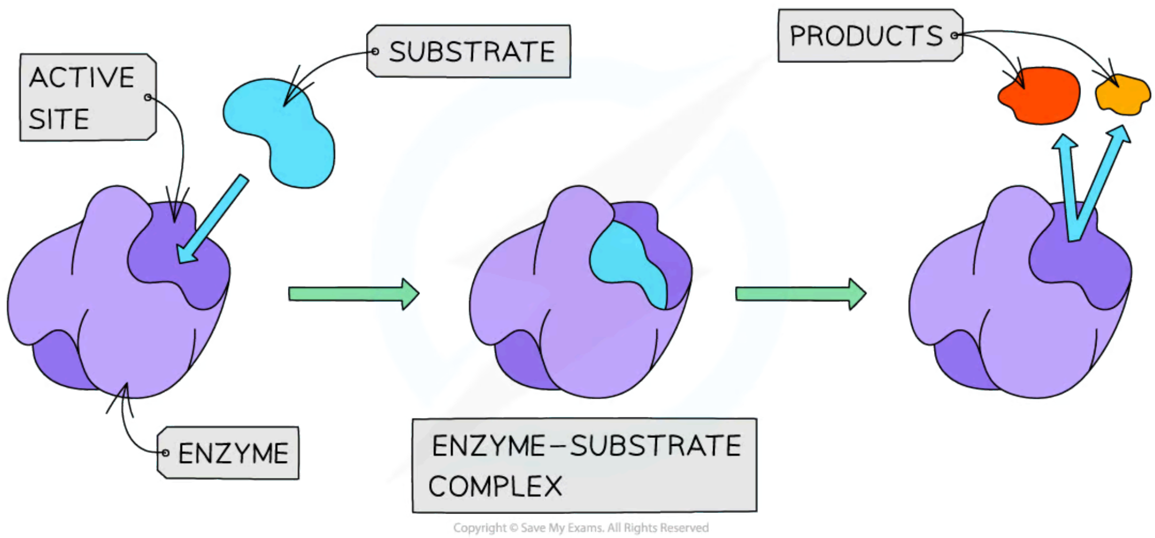
Process of enzyme-catalysed reactions diagram



The simple equation can show how an enzyme reaction proceeds

Formation of enzyme-substrate complex diagram

Your notes

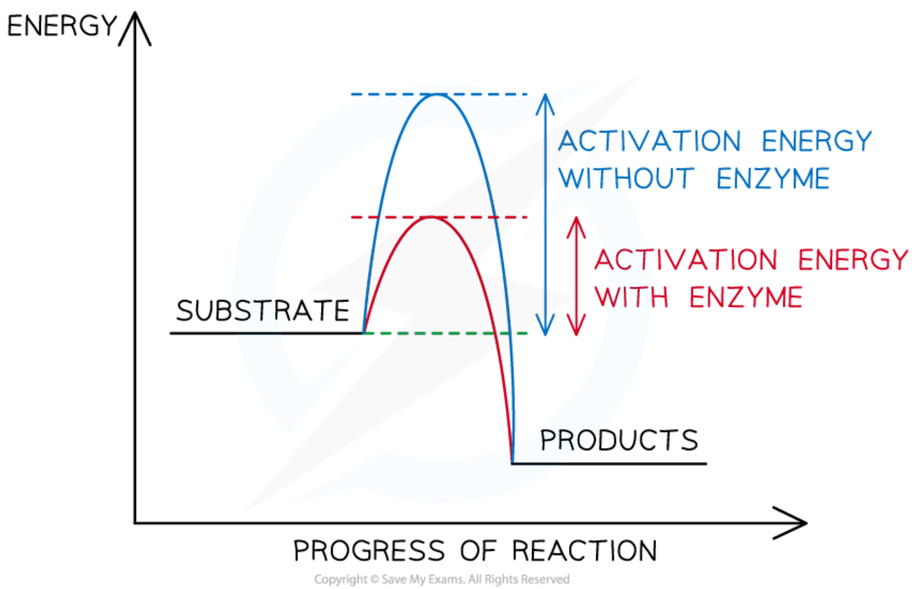


The formation of the enzyme-substrate complex where the substrate is said to be in a transitional state, before forming the product(s)

Enzymes and the lowering of activation energy

- All chemical reactions, including metabolic pathways, are associated with **energy changes**
- Energy may either be released or absorbed during a reaction
  - If energy is released to the surroundings it is an **exergonic** reaction
  - If energy is absorbed from the surroundings it is an **endergonic** reaction
- For a reaction to proceed there must be enough **activation energy**
- Activation energy is the amount of **energy** needed by the substrate to become **unstable** enough for a reaction to occur and for **new products** to be formed
- Enzymes **speed up** chemical reactions because they reduce the **stability of bonds** in the substrate
- Enzymes **lower the activation energy** needed to catalyse a reaction
  - The energy released is unchanged but the activation energy required is lowered
  - The rate of reaction is therefore quicker

The effect of enzyme action on activation energy diagram



The graph shows how an enzyme lowers the activation energy required for a reaction

Examiner Tip

Endergonic and **exergonic** reactions are defined by the net the intake or output of energy (respectively) this differs from endo**thermic** and exo**thermic** reactions which are defined by the intake or output of **thermal energy** only.