Key Terms

Primary Structure	Formed from the order of **amino acids**
Condensation	Reaction occurs **joining** 2 molecules together into a larger one with the **elimination of water**
Secondary Structure	Formed from the **folding** of the **primary structure** into 2 main forms: the **alpha helix** or **beta pleated sheet**
Tertiary Structure	Formed from the **folding** of the **secondary structure** into a **3D shape**
Hydrolysis	The **breaking down** of large molecules into smaller ones by the **addition** of a molecule of **water**

Metabolism Terms

Metabolism	A series of **enzyme-controlled** reactions in the body. There are 2 main types:
Anabolic Reactions	**Protein synthesis** where amino acids are **built up** into more **complex polypeptides**

Metabolism Terms (cont)

Catabolic Reactions	**Digestion of proteins** where **complex** polypeptides are **broken down** into simple **amino acids**

Key Terms

Enzyme-substrate complex	An **intermediate structure** formed during an **enzyme-catalysed reaction** in which the **substrate** and **enzyme** bind temporarily, such that the substrates are **close enough** to **react**
Activation energy	The **minimum energy** that must be put into a **chemical system** for a **reaction** to occur

Key Points about Enzymes

They are **proteins** that **speed up** chemical reactions

They **lower** the **activation energy** needed for the **reaction** to take place

They **don't** actually **take part** in the reaction

They are only needed in **small quantities**

They can be **re-used**

They convert **substrates** into **products**

Key Points about Enzymes (cont)

Therefore they can be described as **biological catalysts**

Inhibitors

Enzymes can be **inhibited** by other substances, which can **either combine** with the **active site directly** or **bind** to **another** part of the enzyme to **prevent** the **formation** of an **enzyme-substrate complex.**

2 forms of inhibition exist, **competitive** and **non-competitive** inhibition, which may be either **reversible** (where inhibitor binds **temporarily**) or **irreversible** (where the inhibitor binds **permanently**).

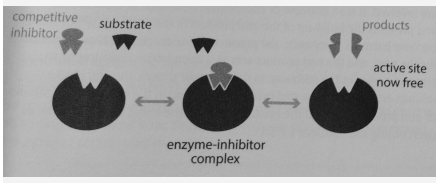
Competitive Inhibition

This is where a **molecule** has a **similar shape** to the **substrate** and so it also has a **complimentary shape** to the **active site.**

The **first molecule** to **collide successfully** with the **active site** will form a **complex.**

By **increasing** the **concentration of substrate**, the **inhibition** is overcome, so long as the inhibition is **reversible**, as it is more likely that a **substrate** molecule will **form** an **enzyme-substrate complex.**

Competitive Inhibition diagram



Competitive Inhibition Summary

The **substrate** and **competitive inhibitor** both 'compete' for the **active site**. It can be **overcome** by **increasing substrate concentration.**

Biosensors

Contain **immobilised enzymes** that can be used to **detect small concentrations** of **specific molecules** in a **mixture**, e.g. glucose in a sample of blood.

A **biosensor** consists of a **specific immobilised enzyme**, a **selectively permeable membrane**, and a **transducer** connected to a **display.**

The **selectively permeable membrane** allows the **metabolite** to **diffuse** through to the **immobilised enzyme**, whilst **preventing** the **passage** of other **molecules.**

The **metabolite binds** to the **active site** of the enzyme, and is **converted** into a **product**, which in turn **combines** with the **transducer** turning the **chemical energy** into an **electrical signal.**

The **higher** the **concentration** of **metabolite** present, the **greater** the **electrical signal.**

This **technique** is used to **accurately measure** the **blood glucose** of **diabetic parents** whose **blood glucose** should normally be kept between **3.89 and 5.83mmol dm-3.**

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Enzyme Structure

Complex folded **polypeptide chains** that are held together in a **complex 3D shape**

each **amino acid** in **primary structure** is joined to the next by a **condensation reaction** which forms a **peptide bond**

This structure is then **folded** into an **alpha helix** or **beta pleated sheet**, held together by **hydrogen bonds** called the **secondary structure**

The **secondary structure** is folded again to form a **3D shape** that is held together by **hydrogen, ionic and disulphide bonds**

This creates an **active site** where **substrates** can **bind**

The **bonds** that hold the **tertiary structure** in place are **susceptible** to changes in **temperature, pH** and the action of **reducing agents**

Enzymes act in an **aqueous environment** because they are **soluble** and **catalyse** many reactions including **hydrolysis**

How enzymes work

In a **catabolic** reaction, the **substrate** binds to the **active site**, forming the **enzyme-substrate complex.**

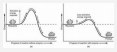
The reaction proceeds and **products are released**, the **active site** is now **free** to **catalyse** another reaction.

In **anabolic** reactions, several **substrates** bind and one or more **products** are **released.**

How enzymes work (cont)

As **biological** reactions, enzymes **lower** the **activation energy** needed to start a reaction by providing **energy** to **break bonds** in existing molecules so **new** ones can form in new molecules. By doing so, **chemical reactions** are **sped up.**

Enzyme work



Intracellular and Extracellular

Enzymes may act **intracellularly**, e.g. during **protein synthesis** where the **formation** of a **peptide bond** between **2 amino acids** is **catalysed.**

Or **extracellularly** e.g. when **pancreatic amylase** is **released** from **pancreatic cells** and **travels** to the **small intestine** via the **pancreatic duct** where it **catalyses** the **breakdown** of **starch** to **maltose.**

Factors affecting the rate of reaction

Rate of reaction = number of reactions that occur per second or unit time

Enzyme action is affected by 5 things:

1. Substrate concentration
2. Temperature
3. pH
4. Enzyme concentration
5. Presence of inhibitors

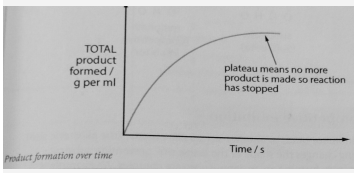
Product formation

Product formation is **different** from the **rate of reaction** as it shows the **total product** made.

Once a **plateau** is reached, **no more product** is formed and the **reaction** has **stopped.**

With a **rate of reaction graph**, the rate would **drop to zero** at this point.

Product formation diagram



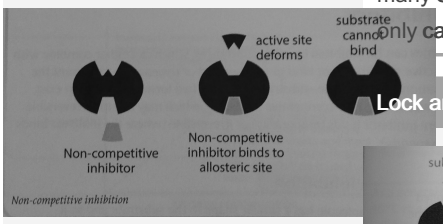
Non-competitive inhibition

An inhibitor **binds** to **another site** on the enzyme (the **allosteric site).**

This binding **changes** the **shape** of the **active site**, preventing **substrate** molecules from forming an **enzyme-substrate complex.**

An example is **cyanide** that **binds** to cytochrome oxidase **inhibiting respiration.**

Non-competitive inhibition diagram



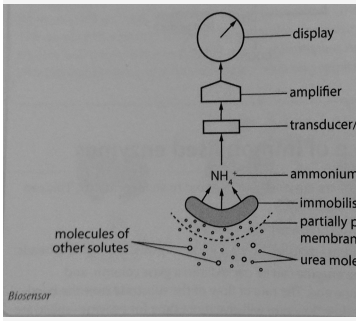
Non-competitive inhibition summary

The **inhibitor** binds to an **allosteric site** deforming the **shape** of the enzyme's **active site**. It **cannot** therefore be **overcome** by **increasing substrate concentration.**

Biosensor definition

A **device** that **combines** a **biomolecule** such as an **enzyme**, with a **transducer**, to **produce** an **electrical signal** which **measures** the **concentration** of a **chemical.**

Biosensor diagram

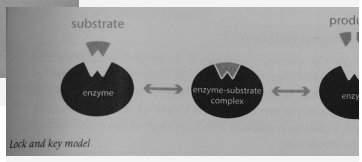


Lock and Key Model

The **substrate** has a **complimentary shape** to the enzyme's **active site**, like a key fitting into a lock.

This explains the **specificity** of many **enzymes** i.e. that many only **catalyse** one **substrate.**

Lock and key diagram



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Induced fit model

Many **observations** show that the enzymes **active site** was being **altered** by the **binding substrate** molecule.

The **induced fit theory** suggests that the **active site** is able to **change** slightly to **accommodate** the substrate.

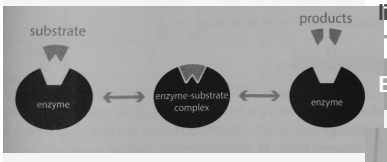
This change places **strain** on the **substrate** molecule, helping to **break bonds** and so **lowering** the **activation energy**.

This explains why in some cases **several molecules** with **similar shapes** are able to **bind** to the **active site**.

This is shown by the **enzyme lysosome**, which is an **anti-bacterial** enzyme found in **human tears** and **saliva**.

The **active site** consists of a **groove**, which closes over the **polysaccharides** found in the **bacterial cell walls**, and the enzyme molecule **changes shape**, which allows **hydrolysis** to occur.

Induced fit diagram



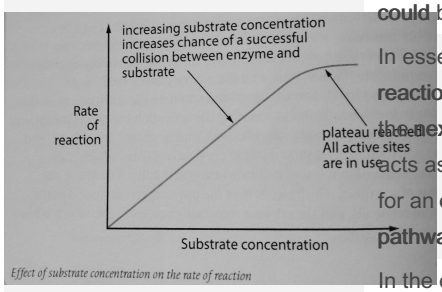
Substrate concentration

When the **substrate concentration increases** in an **enzyme-controlled reaction**, there is a **greater chance** of a successful **collision** between the **substrate** and the **enzyme** resulting in **more enzyme-substrate complexes** forming which **increases** the **rate of reaction**.

Substrate concentration (cont)

When all the **enzyme active sites** are **occupied**, a **plateau** is reached which represents the **maximum rate of reaction** for their **conditions**.

Substrate concentration diagram

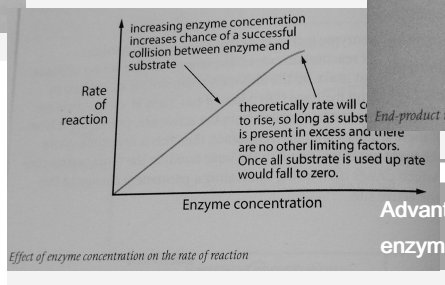


Enzyme concentration

When the **substrate concentration increases** in an **enzyme-controlled reaction**, there is a **greater chance** of a **successful collision** between the **substrate** and **enzyme** so more **enzyme-substrate complexes** are formed, thus **increasing** the **rate of reaction**.

As long as **substrate** is **present in excess** this will continue to **rise** so long as there are **no limiting factors**.

Enzyme concentration diagram



End product inhibition

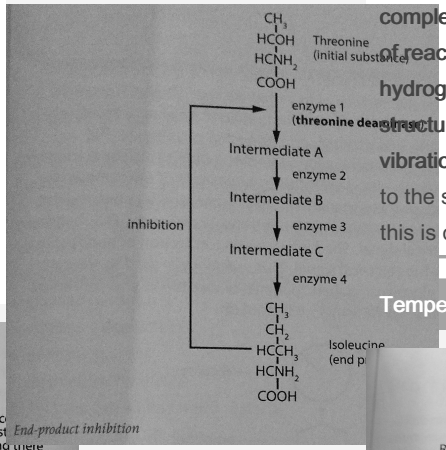
Often seen in **complex metabolic pathways** where **several enzymes** are involved.

It is an example of **competitive inhibition** at work in cells, and **prevents** the **build-up** of the **end product** in the pathway, which **could** become **harmful**.

In essence, the **product** of **one reaction** acts as the **substrate** for the **next**, and the **end product** acts as a **competitive inhibitor** for an **enzyme** earlier in the **pathway**.

In the example below, the **end product inhibits enzyme 1**: as the **end product** is **used up** in the **cell**, the concentration of **end product falls** and the **concentration** of the **initial substrate rises**, so **overcoming** the inhibitor's effect

End product inhibition diagram



Advantages of immobilised enzymes

1. **Enzymes** can be **easily recovered** and **reused**.

2. Product is **not contaminated** by the enzyme .

Advantages of immobilised enzymes (cont)

3. More **stable** at **higher temperature**.

4. **Catalyse** reactions at a **higher** range of **pH**.

The result is that **several enzymes** with **different temperature** and **pH** optima can be used at the **same time**.

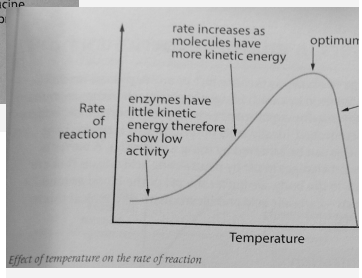
Enzymes can also be **easily added** or **removed** giving **greater control** over the **reaction**.

Temperature

When the **temperature** of an **enzyme** and **substrate increases** in an **enzyme-controlled reaction**, both the **enzyme** and **substrate** molecules **gain more kinetic energy** and so move **faster**, **increasing** the chance of a **successful collision** between them.

As more **enzyme-substrate complexes** are formed, the **rate of reaction decreases** rapidly as **hydrogen bonds** in the **tertiary structure break** due to **increased vibrations** resulting in a change to the shape of the **active site** - this is called **denaturing**.

Temperature diagram



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pH

When the **pH** of an enzyme **increases or decreases** either side of the **optimum**, the **rate of reaction decreases.**

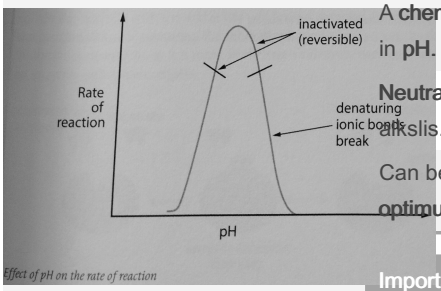
The **charges** on the **amino acid side chains** (R groups) that make up the **enzyme's active site** are influenced by **free hydrogen** (H+) and **hydroxyl** (OH-) ions.

If **too many** H+ or OH- ions are **present**, the **substrate** can be **repelled** from the **active site**, preventing it from **binding.**

If these **changes** are **relatively minor**, then it is **reversible.**

More **excessive changes** in **pH** will result in the **ionic bonds** in the **tertiary structure** breaking which causes **denaturing** by creating a **permanent change** to the **shape** of the **active site.**

pH diagram



Effect of pH on the rate of reaction

pH Optimum

Use of buffers in enzyme experiments

The rate of an **enzyme-controlled reaction**, is greatly influenced by **small changes** in **pH.**

It is therefore **essential**, when carrying out any **enzyme experiment** (where pH is not the independent variable), that **pH is controlled**, ideally at its **optimum**, so it is **not limiting** the **rate of reaction.**

This can be **achieved** by using a **pH buffer.**

A **buffer** is a solution that can **resist changes** in pH by **neutralising acid**/alkalis that are **added** to the **solution.**

In the body, we **buffer** the **pH of the blood** around **7.4** by using **2 chemicals - carbonic acid** and **bicarbonate.**

Buffer Summary

A **chemical** that **resists changes** in **pH.**

Neutralises excess acids or alkslis.

Can be used to **maintain** the **optimum pH** for a given reaction.

Importance of immobilised enzymes

Immobilised enzymes are enzymes that are **fixed** to an **inert matrix.** This can be achieved in **2 main ways:**

1. **Entrapment** - held inside a **gel** e.g. silica gel.

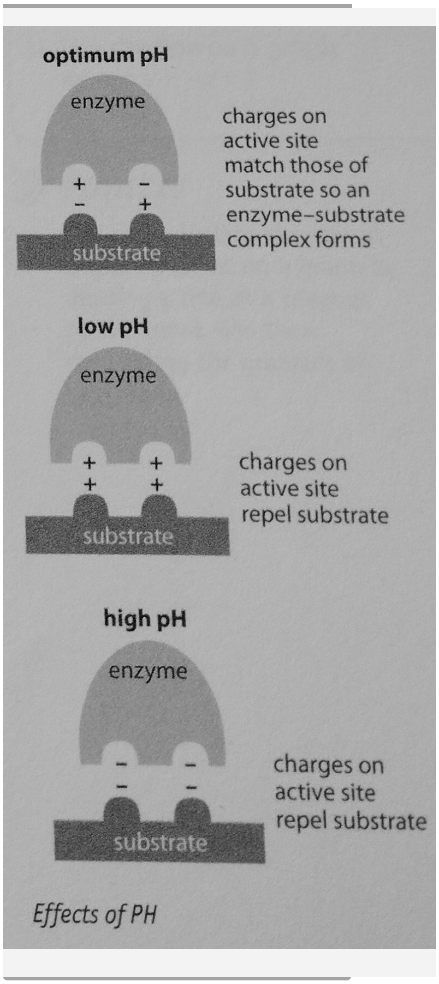
2. **Micro-encapsulation** - trapped inside a **micro-capsule** e.g. alginate beads.

Importance of immobilised enzymes (cont)

Beads containing the **enzyme** can be packed into a **glass column**, and **substrate** added at **one end.**

The **rate of flow** of the **substrate** over the **beads** can be **controlled**: a **slow flow rate** will give **more time** for the **enzyme-substrate complexes** to **form**, and therefore **yield more product.**

Because the **enzymes** are **contained** within their own **'micro-environment'**, the enzymes are **less susceptible** to changes in **pH, temperature** and the action of **chemicals** such as **organic solvents.**



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