

ENZYMES

Proteins that function as biological catalysts are called enzymes.

Enzymes speed up specific metabolic reactions.

Low contamination, low temperature and fast metabolism are only possible with enzymes.

Metabolism is fast, with the product made to a high degree of purity.

General Properties

- Catalysts
- Protein
- Specific
- Reversible — can catalyse the reaction in both directions
- Denatured by high temperature and change in pH
- Rate of action affected by temperature and pH

Protein Nature of Enzymes

- Composed of C, H, O and N. Sulphur (S) may also be present.
- One or more polypeptide chains - large number of linked amino acids.
- Formed on the ribosomes (70s, 80s) – translation of mRNA during protein synthesis.
- Denatured by high temperature and unfavourable pH.

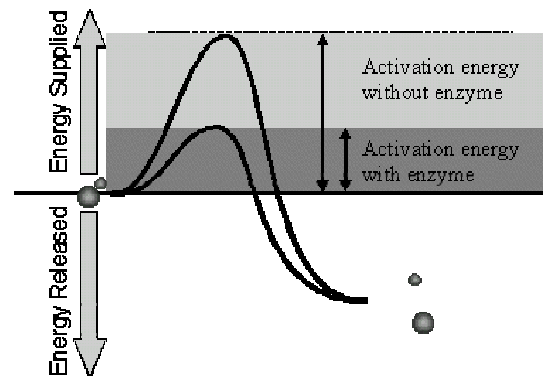
Folded Shape of Enzymes

- The polypeptide chains are folded into a particular three-dimensional shape (**H-bonds**).
- The correct folded shape is essential for enzyme action '*tertiary structure*'.
- The shape gives the enzyme special areas known as active sites.
- The compatible substrate molecules bind to the **complementary** active site.
- Different enzymes have a differently shaped active site.
- REMEMBER: '**enzyme-substrate complex**'; '**active site**';
- Higher temperatures = **more kinetic energy, so more collisions**
- '**Induced fit**' means enzyme and substrate **both** get distorted; bonds bent, so reaction faster.

Role of Enzymes in Living Things:

Enzymes catalyse **all** metabolic reactions.

- They **lower the activation energy** – the energy input needed to bring about the reaction.
- Regulate the thousands of different metabolic reactions in a cell and in the organism.
- The activity of a cell is determined by which enzymes are active in the cell at that time.
- Cell activity is altered by removing specific enzymes and/or synthesising new enzymes.



Active Site Theory

“Lock and Key Hypothesis and Induced Fit”

- The enzyme's active site has a **shape complementary to the substrate**.
- The substrate locks into the **active site** of the enzyme.
- The active site alters its shape holding the substrate more tightly and straining it.
- An **enzyme-substrate complex** is formed.
- The substrate undergoes a chemical change – a new substance (product) is formed.
- The product is released from the active site.
- The free unaltered active site is ready to receive fresh substrate.

Denatured Enzyme: an enzyme that cannot operate because the shape of its active site is altered thus the substrate cannot combine with it – change in shape resulting in loss of biological function.

Denaturation

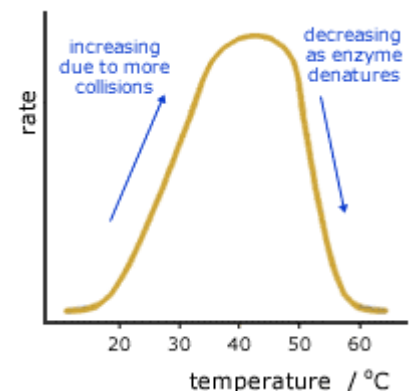
- Heat is a form of energy. The addition of heat can cause a change in the **tertiary structure** of a protein (*mainly by breaking H-bonds*).
- The new shape results in a change in the chemical properties of the protein.
- The protein is denatured if the shape change causes it to lose its normal biological activity.
- Heat-induced denaturation is not usually reversible.
- PH-induced denaturation is usually reversible

Factors Affecting Enzyme Action

- Enzyme action occurs when the enzyme and substrate collide ‘**enzyme-substrate complex**’
- During the collision the substrate slots into the active site of the enzyme.
- Collisions happen because of the rapid random movement of molecules in liquids.

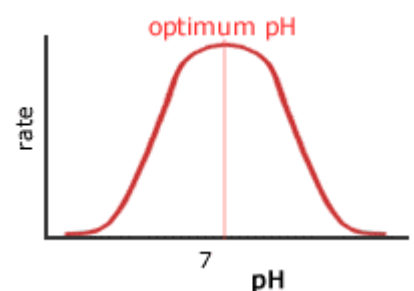
(i) Temperature

- at 0°C enzyme action is low because the movement of molecules is low
- the collision frequency between enzyme and substrate is therefore low
- increasing the temperature speeds up the movement of molecules (> kinetic energy)
- more ‘enzyme-substrate complexes’ are formed
- therefore enzyme action increases
- maximum enzyme action at 40°C - maximum collision frequency between active enzymes and substrates
- enzyme action decreases above 40°C because the enzymes are denaturing more quickly
- when all the enzymes are denatured enzyme action stops (>60°C)



(ii) pH

- enzyme action is greatest within a narrow range of pH, because:
- all the enzymes are active
- changing pH changes H-bonds, thus shape of the active site
- therefore substrate can no longer bind to the active site
- and so enzyme action decreases
- a major pH change denatures the enzyme so enzyme action stops



Optimum Enzyme Activity

Enzymes function best within a narrow range of temperature and pH. Human intracellular enzymes work best at 37°C and pH 7.

Industrial Uses of Enzymes

Bioprocessing is the use of biological materials (organisms, cells, organelles, enzymes) to carry out manufacturing or treatment procedures of commercial or scientific interest.

Examples include:

- **Glucose isomerase**: production of fructose from glucose (sweeter).
- **Sucrase**: production of glucose and fructose from sucrose (much sweeter).
- **Glucose oxidase**: testing of blood/urine for glucose (diabetics)
- **Pectinase**: production of **clear** juice from apples/grapes etc for cider/wine-making

Immobilised enzymes are not free in solution

- they can be held in a bead of soft permeable gel
- or coat the internal surface of a porous solid.

Advantages of Immobilised Enzymes

- Easier purification of the product as the separation of the enzyme beads is not a problem.
- Easy to recover and recycle the enzymes – more economical process.
- The enzymes remain functional for much longer as it is a gentler process
- Therefore cheaper
- Best to use **thermostable** enzymes as these
 - last longer
 - and work at higher temperature, thus faster

Bioprocessing Procedure

- Bioprocessing with immobilised enzymes is carried out in a bioreactor.
- The gel beads, with the immobilised enzymes, are held in suspension in the nutrient medium.
- The bioreactor is sterile – micro-organisms would
 - contaminate the product and
 - reduce yield.
- Temperature, pH, substrate and product concentration and waste level are checked constantly (*computer*).
- The product can be produced by continuous flow (better) or by batch processing.

Practicals

To Determine the **Effect of pH** on the Rate of Enzyme Action.

- Substrate: starch. Enzyme: amylase.
- Use the same volume of the same substrate and enzyme solutions.
- Temperature at 37°C: heated water bath and thermometer.
- Different pH values: use buffer solutions – pH 3, pH 5, pH 7, pH 9, pH 11.
- Experiment: starch + buffer + amylase.
- Control: starch + buffer + water.
- Each minute test a small sample of experiment and control for starch using iodine.
- Control Results: no starch break down as blue-black is the constant result.
- Experiment Results: record the time at which each first produced a yellow-brown result.
- Yellow-brown means starch is not present, therefore starch breakdown occurred.
- Calculate rate of enzyme activity: Rate = 1/time
- Repeat many times to verify the results.
- Graph the results – pH on x-axis.

To Determine the **Effect of Temperature** on the Rate of Enzyme Action

- Use the same volume of the same substrate and enzyme solutions.
- Constant pH: – use a buffer solution.
- Different Temperatures: 0°C – use ice bath, 20°C – room temperature, use a heated water bath and thermometer for temperatures greater than room temperature (30°C, 40°C, 50°C....)
- Control: substrate + buffer + water (? + **boiled** enzyme).
- Each minute test a small sample of experiment and control
- Control Results: no change
- Experiment Results: record the time at which change visible
- Calculate rate of enzyme activity: Rate = 1 /time
- Repeat >3 times to verify the results.
- Graph the results – temperature on x-axis.

Prepare an Enzyme Immobilisation and Examine its Application

Preparation of Immobilised Enzyme

- Prepare a solution of calcium chloride.
- Mix sodium alginate and amylase solutions.
- Draw the mixture into a syringe.
- Gently squeeze drops of the mixture into the calcium chloride solution.
- Allow the beads of immobilised amylase to harden in the calcium chloride solution.
- Collect the beads in a strainer and wash with distilled water.

Examination of the Application of Immobilised Enzyme

- Control Jar: amylase solution mixed with a starch solution.
- Experiment Jar: immobilised enzyme beads in the same volume of the starch solution.
- Temperature: 20°C – room temperature.
- Swirl both jars equally.
- Every minute test a sample from each for starch using iodine.
- Record the time to achieve a yellow-brown colour – starch breakdown completed.
- Now test for reducing sugar using Benedict's Reagent – brick-red colour forms.

Note that it is much easier to remove the immobilised enzyme than the free enzyme from the product solution and the immobilised enzyme beads can be easily reused.

Regulation of Enzymes

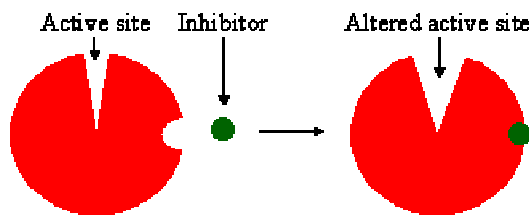
Competitive Inhibition

Inhibitor is a similar-shaped molecule competes with the substrate for active sites.



Non-competitive Inhibition

Another form of inhibition involves an inhibitor that binds to an *allosteric site* of an enzyme. An allosteric site is a different location than the active site.



The binding of an inhibitor to the allosteric site alters the shape of the enzyme, resulting in a distorted active site that does not function properly.

The binding of a non-competitive inhibitor is usually temporary. Poisons are inhibitors that bind irreversibly. For example, penicillin inhibits an enzyme needed by bacteria to build the cell wall.

Feedback Inhibition

Negative feedback inhibition is like a thermostat. When it is cold, the thermostat turns on a heater which produces heat. Heat causes the thermostat to turn off the heater. Heat has a negative effect on the thermostat; it feeds back to an earlier stage in the control sequence as shown below:



Many enzymatic pathways are regulated by feedback inhibition. As an enzyme's product accumulates, it turns off the enzyme just as heat causes a thermostat to turn off the production of heat. The end product of the pathway binds to an allosteric site on the first enzyme in the pathway and shuts down the entire sequence.

