UNIT 3 - ENZYMES

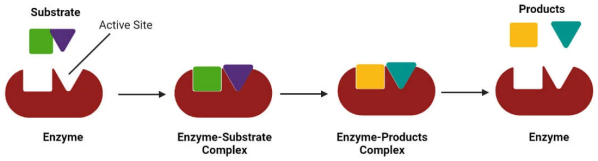
Enzymes - enzymes are biological catalysts that work to speed up metabolic reactions by lowering the activation energy required for that reaction to take place.

- Enzymes are globular proteins that have a precise 3-dimensional shape
- Almost all metabolic reactions are catalysed by enzymes
- Many enzyme names end with "ase" e.g. amylase, catalase, and DNA polymerase

Enzymes can be intracellular (e.g. the hydrolytic enzymes found in lysosomes) or be secreted by cells to become extracellular enzymes (e.g. lysozyme in tears).

- All enzymes, due to their precise shapes, have an active site that is unique to the enzyme's substrate e.g. if an enzyme's substrate is protein, then the shape of the active site will be specific to that protein's shape.

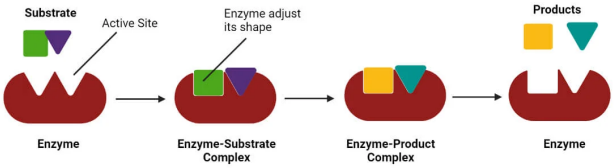
The Lock and Key Hypothesis



This hypothesis was proposed by Emil Fischer in 1894. The Lock and Key hypothesis states that an enzyme's active site can only bind to its specific substrate, and that the substrate fits perfectly into the active site to form an enzyme substrate complex (ESC).

In 1959 this hypothesis was modified when scientists discovered that enzymes were more flexible than the lock-and-key stated.

The Induced Fit Model of Enzyme Action



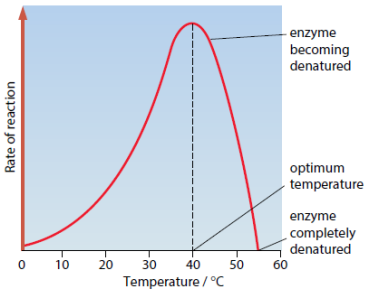
The induced fit model suggests that the enzyme's active site changed shape slightly to bind to the substrate more perfectly and create a more precise ESC. It has been suggested that the induced fit model is also an explanation for the efficiency and affinity the enzyme has for its substrate.

Active site - an area on an enzyme molecule where the substrate can bind.

Enzymes work by lowering the activation energy.
They achieve this by influencing the stability of bonds in the reactants (substrates). There are 4 main factors that affect enzyme activity and the rate at which a product is formed:

1) Temperature
2) pH level
3) Concentration of the enzyme & Concentration of the substrate

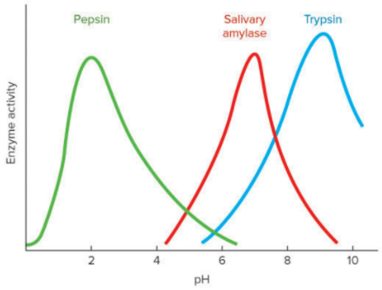
1) Temperature



- From 0°C to 37°C, the higher the temperature, the higher the enzyme activity (as temperature increases so does enzyme activity)
- At 37°C, enzyme activity peaks, indicating that this is the **optimum temperature** for the enzyme. This is when the most number of ESCs are being formed.
- After 37°C, the high temperatures begin to break the hydrogen and ionic bonds holding the enzyme's active site in place, and the enzyme begins to denature.
- At about 39.8°C the enzyme is fully denatured and no more ESCs can form.

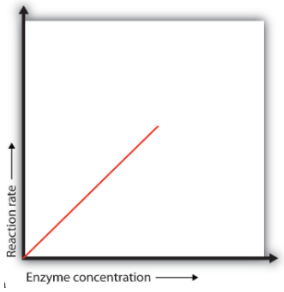
Most mammalian enzymes possess an optimum temperature of 37°C, as this is the normal body temperature maintained in most mammals. Though temperature can stay constant across species, pH can vary depending on the location of the enzyme within the organism.

2) pH levels



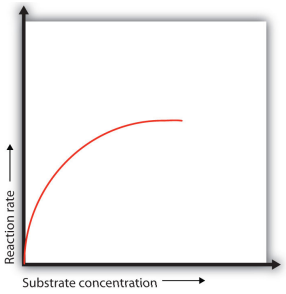
- Depending on the location of the enzyme in the body, optimum pH varies, e.g. salivary amylase is found in the mouth, a pH neutral part of the body, so its optimum pH is around 7. Pepsin on the other hand is found in the highly acidic environment of the stomach, so has an optimum pH of about 2.
- When the conditions get too alkaline (excess of OH^- ions) or too acidic (excess H^+ ions), it will affect the bonds in the enzyme that contain a charge; ionic and hydrogen bonds.
- Again, different enzymes denature at different pHs depending on their location in the body.

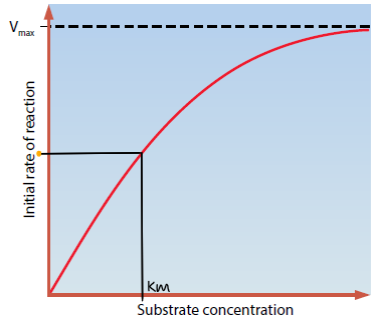
3) Concentration of the Enzyme and Concentration of the Substrate



The greater the enzyme concentration, the faster the rate of reaction. This is because as the number of enzymes increases, so does the maximum number of enzyme-substrate complexes that can be formed, and so the rate of reaction steadily increases.

However, it is different for substrate concentration. For a bit, as the substrate concentration increases, so does the rate of reaction but eventually the line plateaus. This is because there isn't enough of the enzyme and all the active sites available have been filled. The point where the line plateaus is called $\underline{V_{max}}$.





- Vmax can only be increased from this point by increasing the enzyme concentration.

Vmax - the theoretical maximum rate of an enzyme-controlled reaction, obtained when all the enzymes' active sites are occupied.

Michael's-Menten constant (Km) - the substrate concentration at which an enzyme works at ½ the maximum efficiency rate (½ Vmax) – it is used as a measure for the efficiency of the enzyme.

- The LOWER the value of Km, the MORE efficient the enzyme. This is because it tells us that a lower concentration of substrate is required to reach the same value of ½ Vmax, indicating that the enzyme has a high affinity for its substrate.

Enzyme Inhibitors

- An enzyme inhibitor is a molecule that binds to an enzyme instead of a substrate and blocks or hinders the active site.
- Inhibitors can be <u>competitive</u> or <u>non-competitive</u>.

Competitive inhibitors:

- They have a shape similar to the substrate and so compete with the substrate for the active site of the enzyme.
- The effects of competitive inhibitors are usually reversible by increasing the substrate concentration to outnumber the amount of inhibitors.

Non-competitive inhibitors:

- Can bind to the active site. When this happens, effects are irreversible and the inhibitor binds permanently. Examples include cyanide and gaseous nerve poisons.
- They can also bind to areas other than the active site. This changes the shape of the active site. It can be reversible (e.g. ATP*), or irreversible (e.g. silver or iodine poisons).

*ATP is an example of ed-product inhibition which is where the product of a metabolic reaction goes on to inhibit the very same process. ATP does this when the body is in a stage where it does not require as much energy (e.g. during sleep), and so ATP inhibits its own production.

- This process is reversible and the effects are reversed when the body is in need of more energy (e.g. once we wake up)
- ATP is a non-competitive inhibitor because it binds to a place on the enzyme other than the active site

Immobilised enzymes

Immobilised enzymes - enzymes that have been fixed to a surface or trapped inside beads of agar gel.

- A disadvantage of simply adding an enzyme to a substrate solution is that it may be difficult to separate the enzyme from the product once the reaction is complete. This could mean that we cannot reuse the enzyme, which, for commercial use is expensive.
- Immobilised enzymes are therefore crucial in industriall processes because...:
  - They allow the enzyme to be separated from the solution easily and so can be reused, saving money since enzymes are expensive
  - Immobilised enzymes are protected by whatever substance they're trapped in, and so are more resistant to temperature and pH changes. They may therefore last longer
  - They allow for continuous processing rather than a batch system, allowing for smaller industrial setups, reducing costs
  - The production of an immobilised enzyme is greatly increased as it may be mmore fully used at higher substrate concentrations for longer peroiods of time compared to free enzymes