Enzymes
2.1.4

26 January, 2017

2.1.4 Enzymes

Metabolism in living organisms relies upon enzyme-controlled reactions. Knowledge of how enzymes function and the factors that affect enzyme action has improved our understanding of biological processes and increased our use of enzymes in industry.

	Learning outcomes	Additional guidance
	Learners should be able to demonstrate and apply their knowledge and understanding of:	
(a)	the role of enzymes in catalysing reactions that affect metabolism at a cellular and whole organism level	To include the idea that enzymes affect both structure and function.
(b)	the role of enzymes in catalysing both intracellular and extracellular reactions	To include catalase as an example of an enzyme that catalyses intracellular reactions and amylase and trypsin as examples of enzymes that catalyse extracellular reactions.
(c)	the mechanism of enzyme action	To include the tertiary structure, specificity, active site, lock and key hypothesis, induced-fit hypothesis, enzyme-substrate complex, enzyme-product complex, product formation and lowering of activation energy.

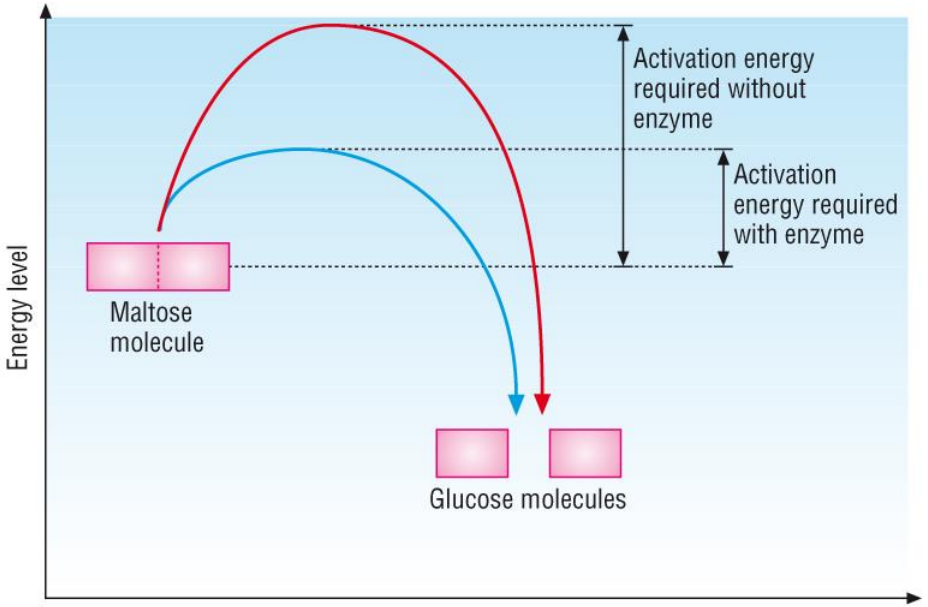
2.1.4 Enzymes

Metabolism in living organisms relies upon enzyme-controlled reactions. Knowledge of how enzymes function and the factors that affect enzyme action has improved our understanding of biological processes and increased our use of enzymes in industry.

	Learning outcomes	Additional guidance
	Learners should be able to demonstrate and apply their knowledge and understanding of:	
(d)	**(i)** the effects of pH, temperature, enzyme concentration and substrate concentration on enzyme activity	To include reference to the temperature coefficient (Q_{10}).
(e)	the need for coenzymes, cofactors and prosthetic groups in some enzyme-controlled reactions	To include Cl^- as a cofactor for amylase, Zn^{2+} as a prosthetic group for carbonic anhydrase and vitamins as a source of coenzymes. **PAG4**
(f)	the effects of inhibitors on the rate of enzyme-controlled reactions.	To include competitive and non-competitive and reversible and non-reversible inhibitors with reference to the action of metabolic poisons and some medicinal drugs, and the role of product inhibition **AND** inactive precursors in metabolic pathways (covered at A level only).

An enzyme is a BIOLOGICAL CATALYST.

Enzymes serve to LOWER THE ACTIVATION ENERGY of a reaction.



Adding the enzyme maltose reduces the amount of activation energy required for the reaction to take place

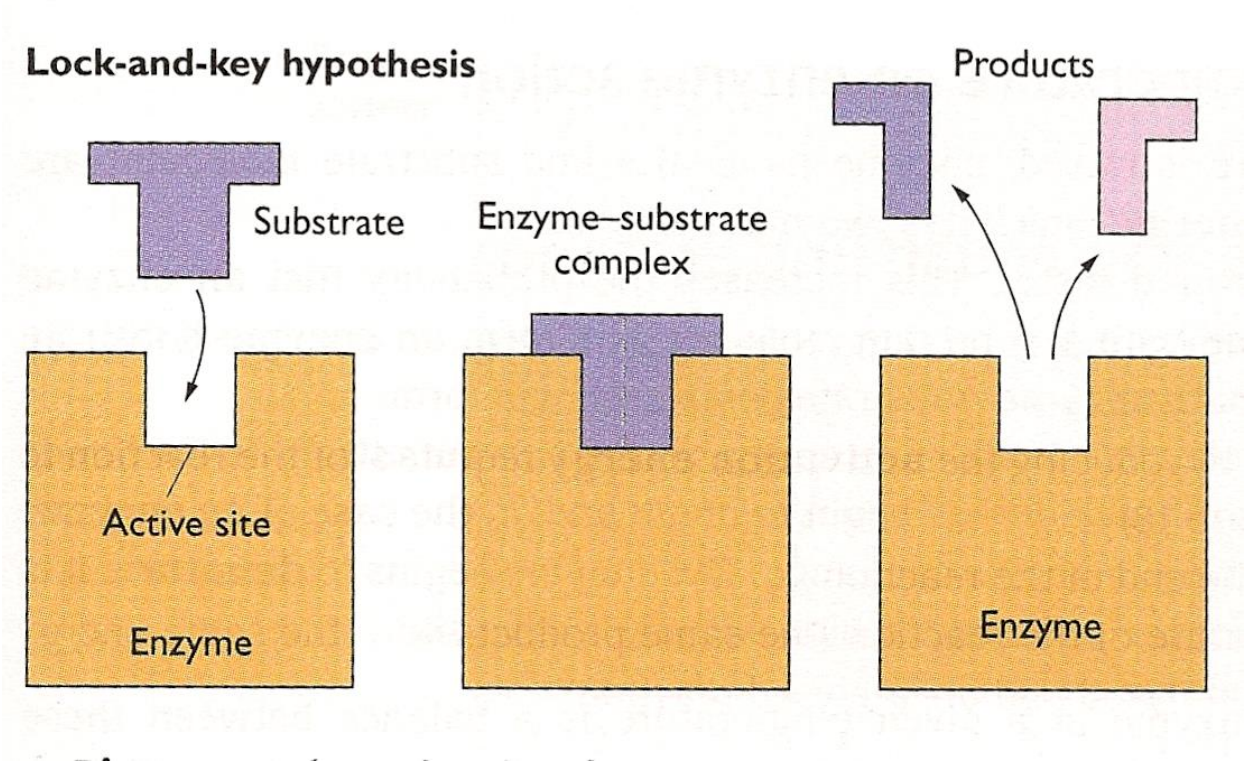
Enzymes contain an ACTIVE SITE which is able to bind with its SUBSTRATE..

Each active site is unique and therefore SPECIFIC to only ONE substrate.

There are two models to explain how enzymes work

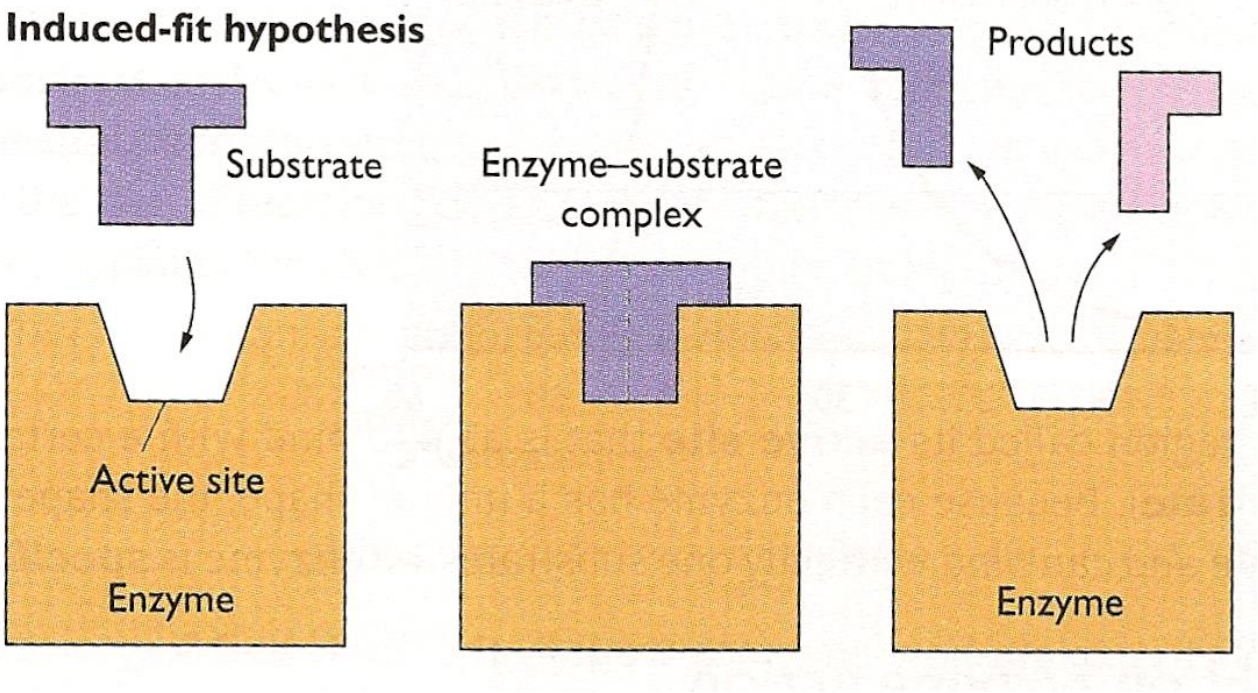
Lock and Key model:

- Active site of enzyme and substrate have COMPLEMENTARY SHAPES.
- The substrate 'fits' into the active site and binds with it to form an enzyme-substrate complex.
- After the reaction, the products leave the active site.



INDUCED FIT model:

- Initially, the shape of the active site is not quite complementary to the substrate.
- As the active site begin to bind the substrate, it changes shape and 'moulds' itself around the substrate molecule.
- After the reaction, the products leave the active site and the active site reverts back to its original shape



Induced fit is the preferred model as it explains the effect of non-competitive inhibitors.

Intracellular Enzymes

Enzymes that act *WITHIN* cells

1. Catalase breaks down hydrogen peroxide, a toxic product of many metabolic pathways, into hydrogen and oxygen

Extracellular Enzymes

Enzymes that are *RELEASED* from cells, e.g. in digestion

1. Starch is broken down into Maltose by the action of AMYLASE in the saliva and in pancreatic secretions.
2. Maltose is then broken down into Glucose by the action of MALTASE present in the small intestine.

3. TRYPSIN is a protease, produced in the pancreas.

Activity of enzymes is affected by:

Temperature.

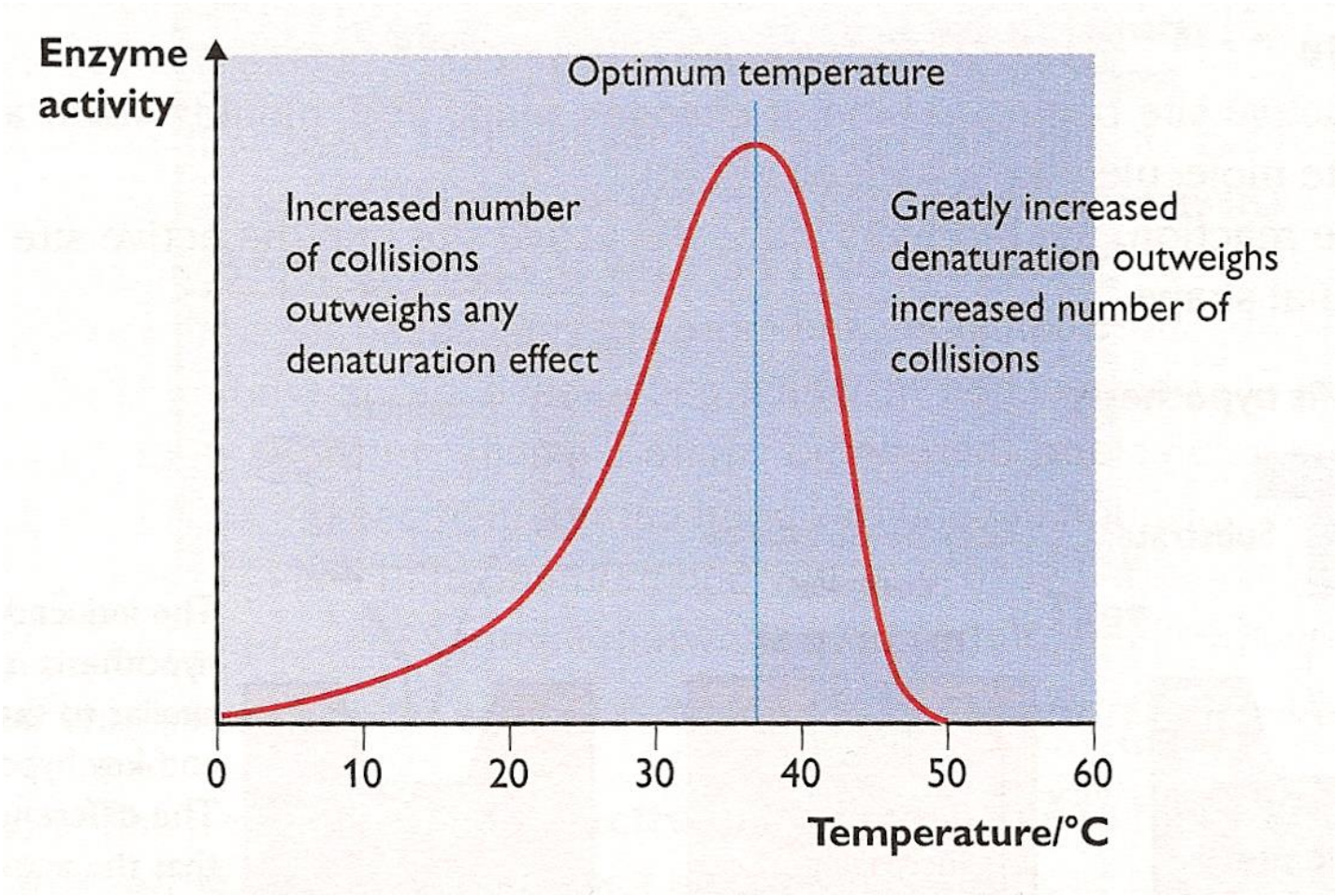
When temperature is raised, the kinetic energy of both the enzyme and the substrate is increased, increasing the likelihood that they will collide and form an enzyme-substrate complex.

Increased Temperature = Increased Enzyme-Substrate Complexes.

TO A POINT.

As temperature increases, particles within the enzyme vibrate more energetically putting strain on the bonds that hold the atoms in place. These bonds begin to break and the shape of active site changes. The enzyme begins to DENATURE, making it more difficult for E-S complexes to form.

(Think of frying an egg – the higher the temperature the more the egg proteins denature. If a cooked egg was an enzyme, it couldn't bind its substrate!)



Enzyme activity at any given temperature is a balance between increased kinetic energy and increased denaturation.

Q_{10} – Temperature Coefficient.

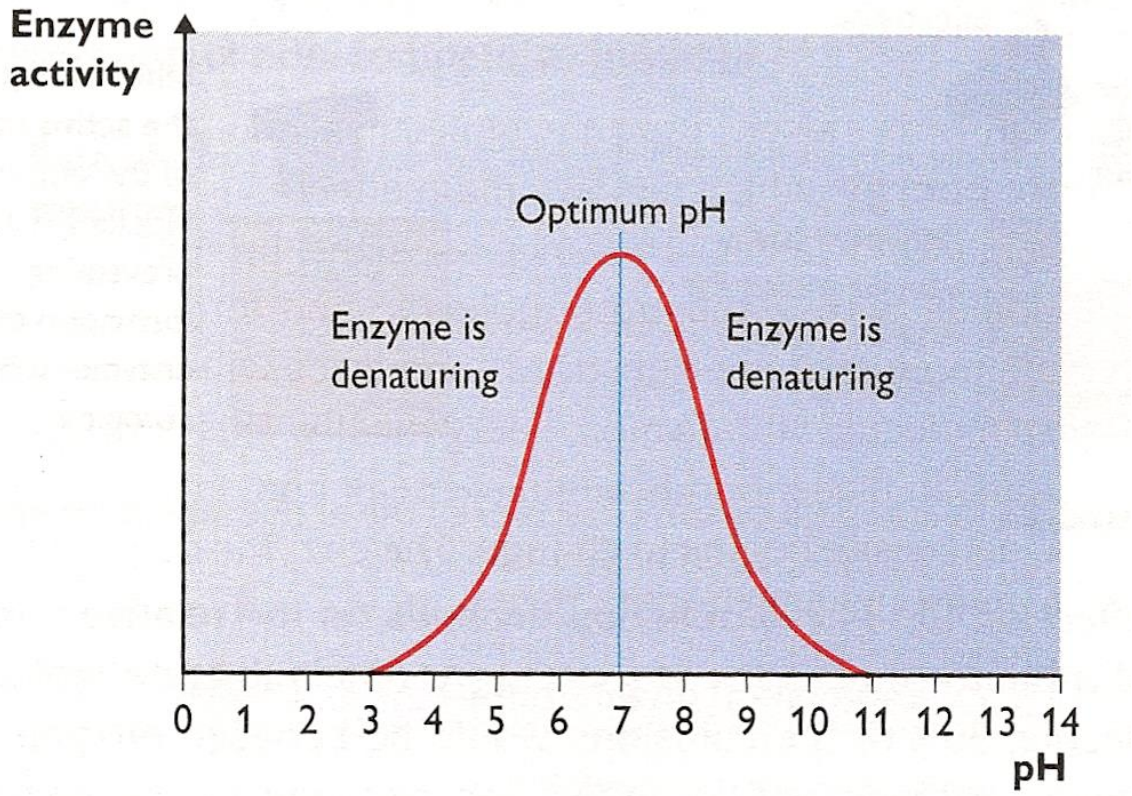
Is a measure of how much the rate of reaction increases with a 10°C increase in temperature.

Q_{10} = Rate at higher temp / Rate at lower temp.

For enzyme controlled reactions, this value is usually 2, indicating that the rate has doubled.

pH:

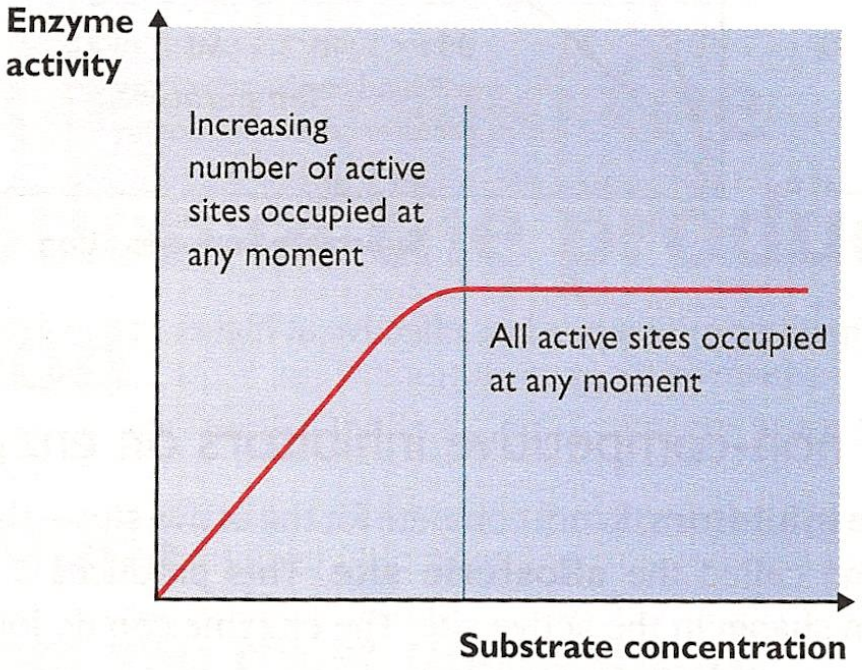
Changes in pH can affect enzymes by either breaking ionic bonds that hold the protein's tertiary structure in place, leading to DENATURATION, or, altering the charge of some of the amino acids that form the active site, making it more difficult for the substrate to bind (pH <7 is acidic, meaning an excess of H^+ ions, pH >7 is alkali, meaning an excess of OH^- ions)



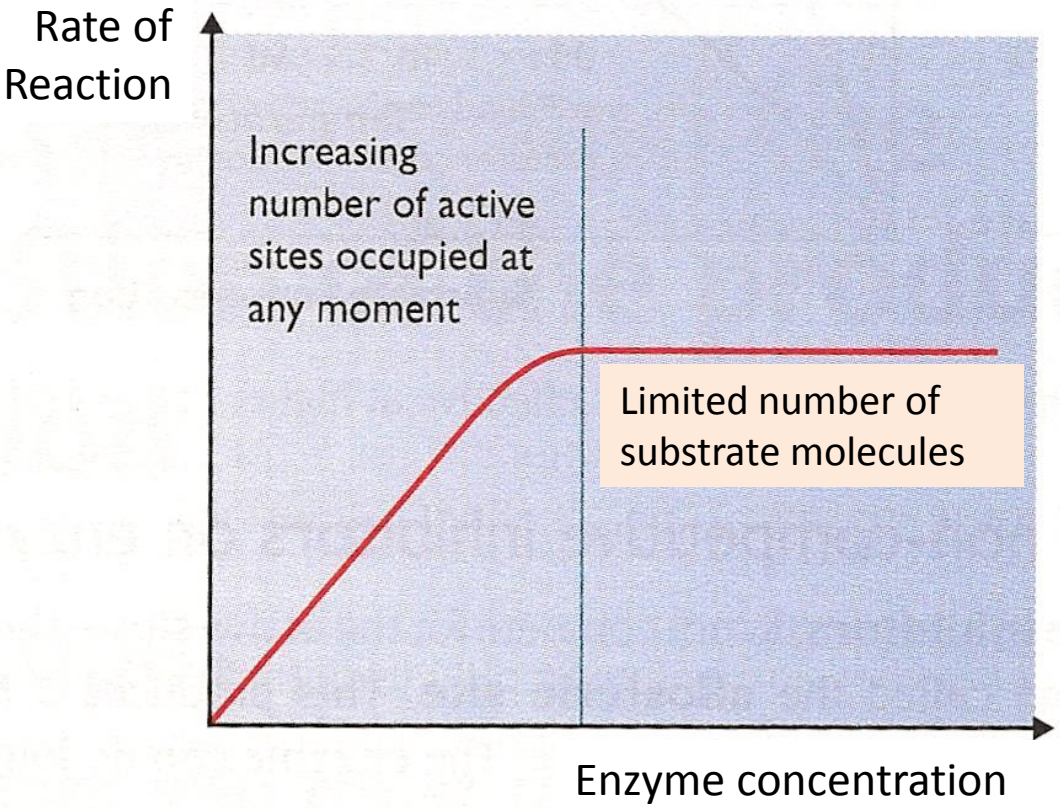
Substrate concentration:

Small number of substrate molecules = relatively few collisions and a lower rate of reaction.
Increasing the number of substrate molecules means more collisions and an increased rate of reaction.

At a certain concentration of substrate, all available active sites on the enzymes will be continually occupied by substrate molecules.
Increasing the substrate concentration beyond this point will not increase the rate of reaction (maximum rate is referred to as V_{max}).



The same is also true for enzyme concentration. Increased enzyme concentration will result in an increased rate of reaction as more enzymes are able to form E-S complexes with the substrates. However, the number of substrate molecules is limited and at a certain point, the substrate concentration becomes the limiting factor, shown by a plateau in the rate of reaction.

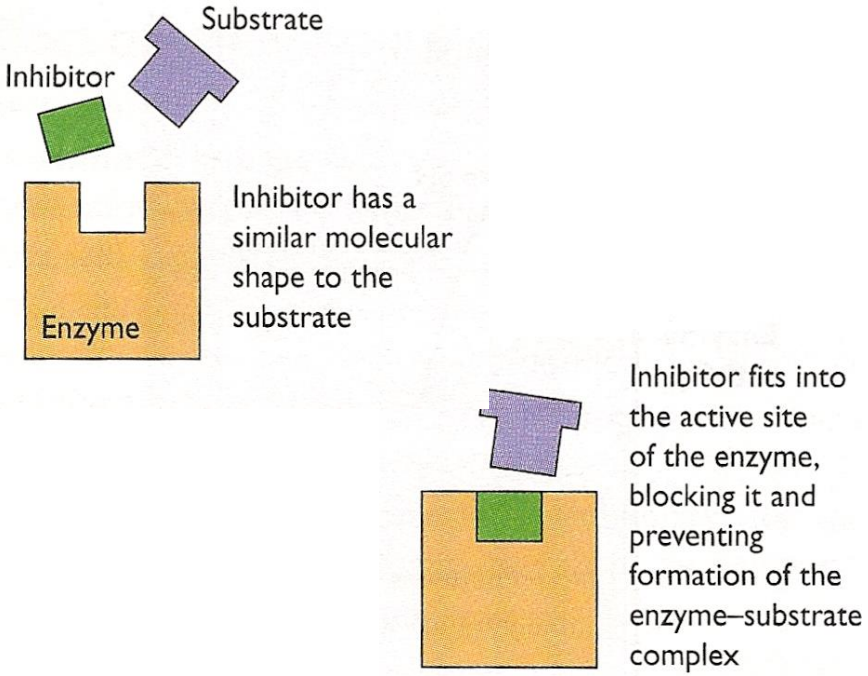


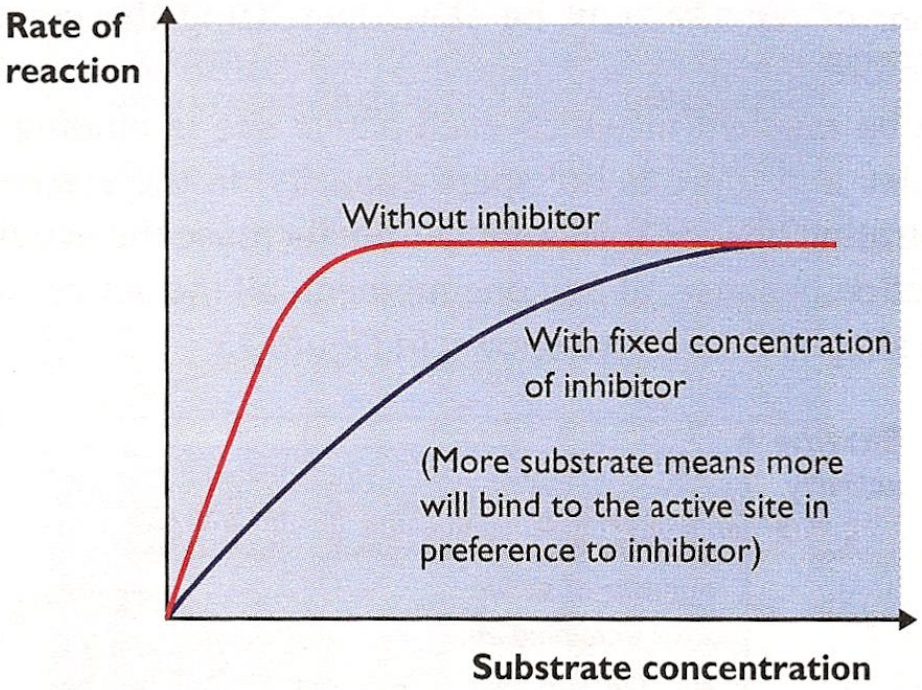
Inhibitors.

There are two types of Inhibitor – COMPETITIVE and NON-COMPETITIVE.

Competitive inhibitors are molecules that have a complementary shape to all or part of the active site.
These inhibitors temporarily bind in the active site of the enzyme, preventing a substrate molecule from doing so.

Because the bindings are temporary, increasing substrate concentration will ‘dilute’ the effect of the inhibitor, meaning that the same rate of reaction can be achieved. iust over a greater time.

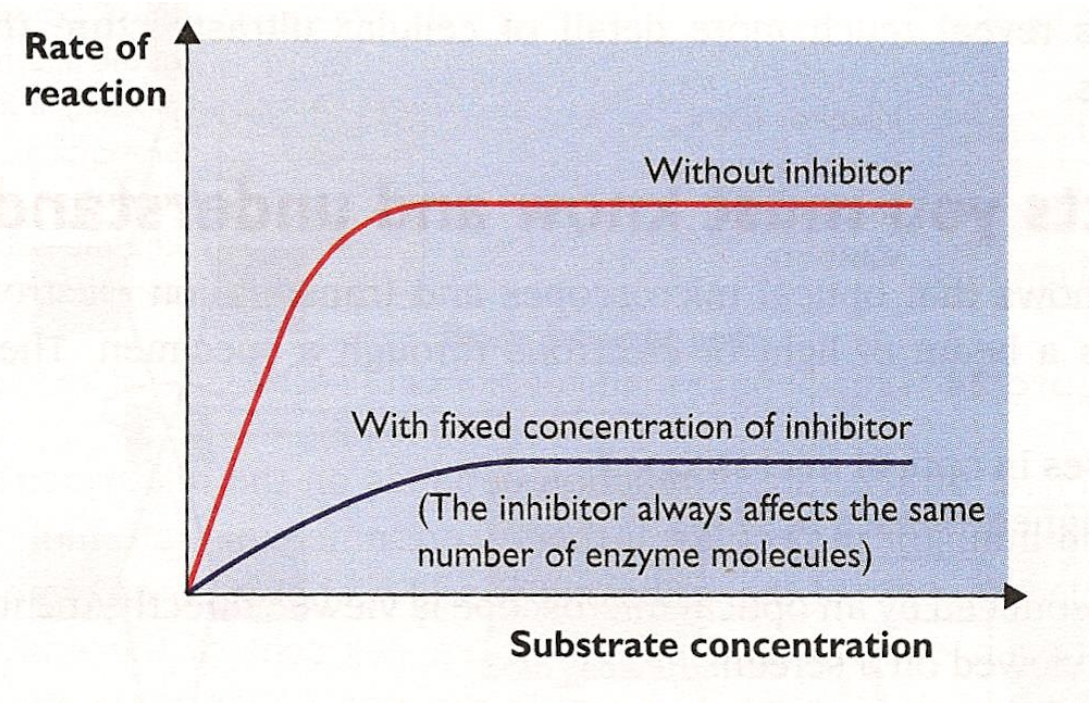




NON-COMPETITIVE Inhibitors do not compete for the active site – they bind to another part of the enzyme called the ALLOSTERIC SITE.
This produces an CONFORMAITONAL change (change in shape) in the active site meaning that the substrate can no longer bind.

The effect of a non-competitive inhibitor is not affected by the concentration of the substrate because a non-competitive inhibitor does not bind to the same part of the enzyme.

The binding of a NCI is usually permanent – if a NCI binds to 80% of enzymes in a solution, then the reaction rate will drop to 20% of its maximum.



END-PRODUCT INHIBITION When the product of a reaction acts as an inhibitor to the enzyme that produces it.
This is an example of negative feedback. It is a non-competitive, reversible inhibition.

For example:

Respiration breaks down glucose to produce ATP.
The first step involved the addition of two phosphate groups to the glucose. This addition is catalysed by the enzyme Phosphofructokinase (PFK). This enzyme is non-competitively inhibited by ATP. ATP therefore inhibits its own production:

- When the levels of ATP are high, more ATP binds to the allosteric site on PFK, preventing the addition of the second phosphate group to glucose. Glucose is not broken down and ATP is not produced at the same rate.

- As ATP is used up, less binds to PFK and the enzyme is able to catalyse the addition of a second phosphate group to glucose. Respiration resumes, leading to the production of more ATP.

Cofactors, coenzymes, prosthetic groups

Some enzymes need a non-protein 'helper' component in order to carry out their function.

Cofactors:

Inorganic ions.
May transfer atoms or groups from one reaction to another, or form part of the enzyme's active site.
E.g. Amylase contains a chloride ion that is necessary for the formation of a correctly shaped active site.

Coenzymes:

Organic molecules.
Usually derived from vitamins.
E.g. Vitamin B3 is used to synthesise NAD, a coenzyme used a lot in respiration. Vitamin B5 is used to make coenzyme A, also used in respiration

Prosthetic Groups:

Similar to cofactors – inorganic ions.
Required by some enzymes to carry out their functions.
Prosthetic groups are tightly bound and form a permanent feature of the protein.
E.g. Zinc ions form an important part of the structure of carbonic anhydrase, involved in the metabolism of CO_2.

Precursor Activation

Some enzymes are produced in an inactive form – known **as inactive precursor enzymes**.
This is particularly the case for enzymes that might cause damage to the cell or those whose action needs to be controlled and only activated under certain conditions.

Precursor enzymes often need to undergo a change in shape, particularly to the active site, to be activated:

- This can be achieved by the addition of a cofactor.
- Before this, the precursor is called an **apoenzyme**.
- When the cofactor is added and the enzyme is activated, it is called a **holoenzyme**.

Precursor Activation

Sometimes the change in shape is brought about by the action of another enzyme, such as a protease, which cleaves certain bonds in the molecule.

In some cases a change in conditions, such as pH or temperature, results in a change in tertiary structure and activates a precursor enzyme. These types of precursor enzymes are called **zymogens** or **proenzymes**.

E.g. When inactive pepsinogen is released into the stomach to digest proteins, the acid pH brings about the transformation into the active enzyme pepsin. This adaptation provides protection against the digestive action of pepsin.