3 Enzymes

Enzymes are essential for life to exist. Their mode of action and the factors that affect their activity are explored in this topic. Prior knowledge for this topic is an understanding that an enzyme is a biological catalyst that increases the rate of a reaction and remains unchanged when the reaction is complete.

There are many opportunities in this topic for students to gain experience of carrying out practical investigations and analysing and interpreting their results.

3.1 Mode of action of enzymes

There are many different enzymes, each one specific to a particular reaction. This specificity is the key to understanding the efficient functioning of cells and living organisms.

By the end of this section you should be able to:

a) explain that enzymes are globular proteins that catalyse metabolic reactions
b) state that enzymes function inside cells (intracellular enzymes) and outside cells (extracellular enzymes)
c) explain the mode of action of enzymes in terms of an active site, enzyme/substrate complex, lowering of activation energy and enzyme specificity (the lock and key hypothesis and the induced fit hypothesis)
d) investigate the progress of an enzyme-catalysed reaction by measuring rates of formation of products or rates of disappearance of substrate

Introducing enzymes and their role in metabolism

Enzymes are globular proteins that catalyse the many thousands of metabolic reactions taking place within cells and organisms. **Metabolism** is the name we give to these chemical reactions of life. The molecules involved are collectively called **metabolites**. Many of these metabolites are made in organisms. Other metabolites have been imported from the environment, such as from food substances taken in, water and the gases carbon dioxide and oxygen.

Metabolism actually consists of chains (linear sequences) and cycles of enzyme-catalysed reactions, such as we see in respiration (page 242), photosynthesis (page 266), protein synthesis (page 120) and very many other pathways. These reactions may be classified as one of just two types, according to whether they involve the build-up or breakdown of organic molecules.

- In **anabolic reactions**, larger molecules are built up from smaller molecules. Examples of anabolism are the synthesis of proteins from amino acids and the synthesis of polysaccharides from simple sugars.
- In **catabolic reactions**, larger molecules are broken down. Examples of catabolism are the digestion of complex foods and the breakdown of sugar in respiration.

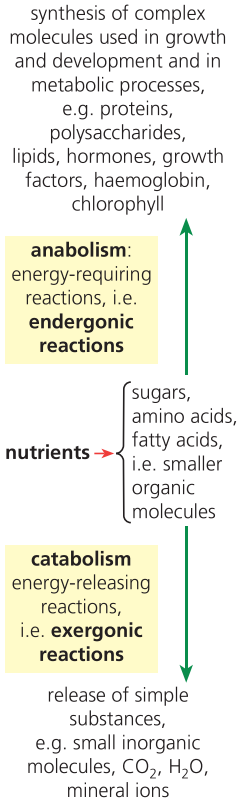


Figure 3.1 Metabolism: an overview

Enzymes as globular proteins

In Topic 2 we saw that the tertiary structure of **globular proteins** was typically spherical. In these molecules their linear chain of amino acids (primary structure) is precisely folded and held in a globular three-dimensional shape containing α-helices and β-sheets. Also, the R-groups of the amino acids present on the exterior of the molecule are hydrophilic groups, making the protein water soluble. Remember, this structure contrasted with that of the fibrous proteins, such as collagen.

Enzymes as biological catalysts

A catalyst is a molecule that speeds up a chemical reaction but remains unchanged at the end of the reaction. Most chemical reactions do not occur spontaneously. In a laboratory or in an industrial process, chemical reactions may be made to occur by applying high temperatures, high pressures, extremes of pH and by keeping a high concentration of the reacting molecules. If these drastic conditions were not applied, very little of the chemical product would be formed. In contrast, in cells and organisms, many of the chemical reactions of metabolism occur at exactly the same moment, at extremely low concentrations, at normal temperatures and under the very mild, almost neutral, aqueous conditions we find in cells.

How is this brought about?

For a reaction between two molecules to occur there must be a successful collision between them. The molecules must collide with each other in the right way and at the right speed. If the angle of collision is not correct, the molecules bounce apart. Alternatively, if the speed of the collision speed is wrong or the impact is too gentle, for example, then there will be insufficient energy for the rearrangement of electrons. Only if the molecules are lined up and collide with the correct energies does a reaction occur.

The 'right' conditions happen so rarely that the reaction doesn't happen to a significant extent normally. If we introduce extreme conditions, such as those listed above, we can cause the reaction to happen. On the other hand, if we introduce an enzyme for this particular reaction then the reaction occurs at great speed. Enzymes are amazing molecules in this respect.

Where do enzymes operate?

Some enzymes are exported from cells, such as the digestive enzymes. Enzymes, like these, that are parcelled up, secreted and work externally are called **extracellular enzymes**. However, very many enzymes remain within the cells and work there. These are **intracellular enzymes**. They are found inside organelles, in the membranes of organelles, in the fluid medium around the organelles (the cytosol) and in the cell surface membrane.

Enzymes control metabolism

There is a huge array of enzymes that facilitate the chemical reactions of the metabolism. Since these reactions can only take place in the presence of specific enzymes, we know that if an enzyme is not present then the reaction it catalyses cannot occur.

Many enzymes are always present in cells and organisms but some enzymes are produced only under particular conditions or at certain stages. By making some enzymes and not others, **cells can control what chemical reactions happen in the cytoplasm**. Sometimes it is the presence of the substrate molecule that triggers the synthesis of the enzyme. In Topic 4 we see how protein synthesis (and therefore enzyme production) is directly controlled by the cell nucleus.

How enzymes work

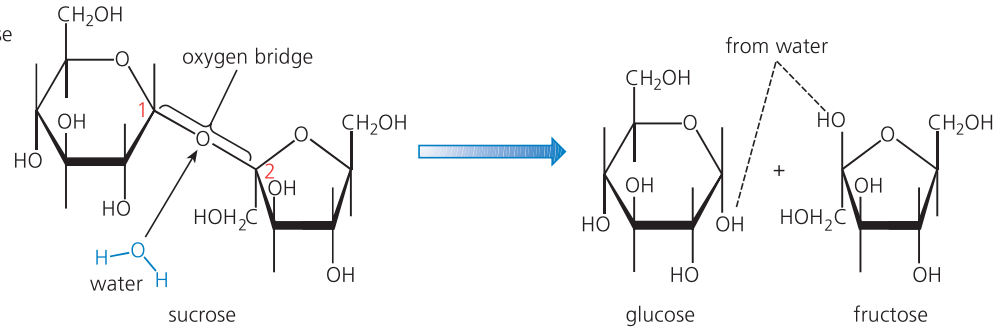
So, enzymes are biological catalysts made of protein. They speed up the rate of a chemical reaction. The general properties of catalysts are:

- they are **effective in small amounts**
- they remain **unchanged at the end of the reaction**.

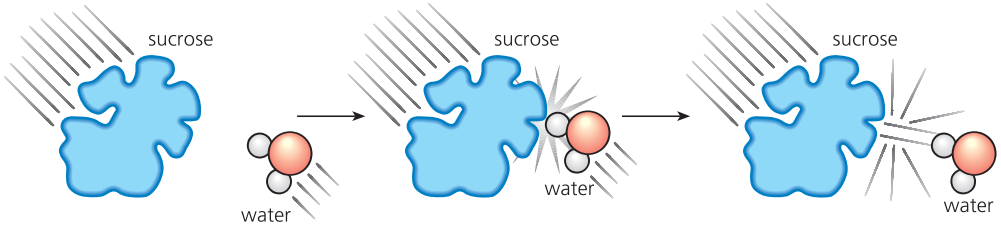
The presence of enzymes enables reactions to occur at incredible speeds, in an orderly manner, yielding products that the organism requires, when they are needed. Sometimes reactions happen even though the reacting molecules are present in very low concentrations.

How is this brought about?

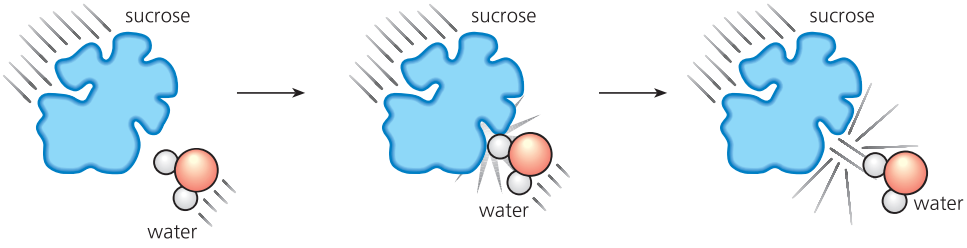
1 The reaction: hydrolysis of sucrose to form glucose and fructose



2 Random collision possibilities: when sucrose and water collide at the wrong angle

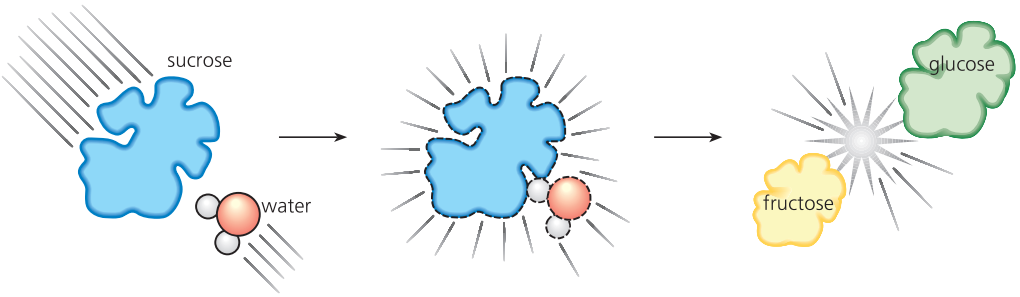


when sucrose and water collide at the wrong speed



These events are what happens at most random collisions.

For the reaction to occur, sucrose and water must collide in just the right way – glucose and fructose are formed



Under normal conditions this happens so very infrequently it is an insignificant event.

3 In the presence of one molecule of the enzyme sucrase (invertase), approximately 3.0×10^4 molecules of sucrose are hydrolysed each minute!

Figure 3.2 Can a reaction occur without an enzyme?

Enzymes lower the activation energy

As molecules react they become unstable, high energy intermediates, but they are in this state only momentarily. We say they are in a transition state because the products are formed immediately. The products have a lower energy level than the substrate molecules. Energy is needed to raise molecules to a transition state and the minimum amount of energy needed to do this is called the **activation energy**. It is an energy barrier that has to be overcome before the reaction can happen. Enzymes work by lowering the amount of energy required to activate the reacting molecules.

A model of what is going on is the boulder (the substrate) perched on a slope, prevented from rolling down by a small hump (the activation energy) in front of it. The boulder can be pushed over the hump. Alternatively, the hump can be dug away (the activation energy can be lowered), allowing the boulder to roll and shatter at a lower level (into the products).

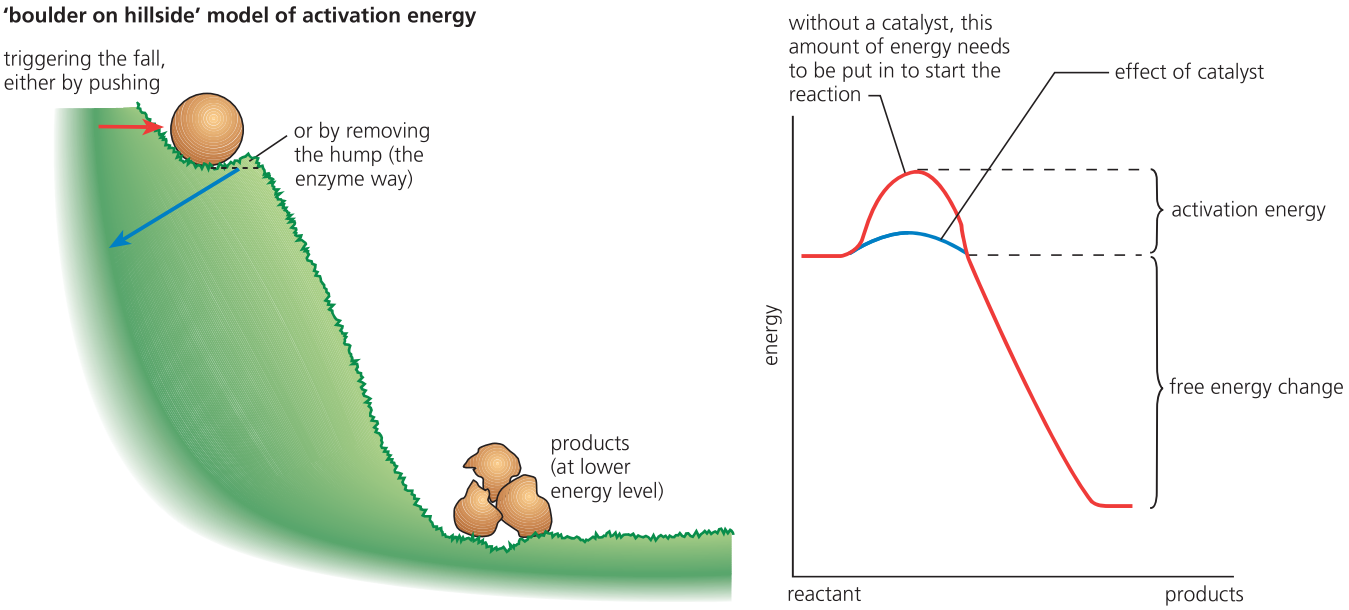


Figure 3.3 Activation energy

The enzyme has an active site

In a reaction catalysed by an enzyme, the starting substance is called the **substrate**. It is converted to the **product**. The way an enzyme works is for the substrate molecule to become attached (we say 'bind to') the enzyme at a specially formed pocket in the enzyme – very briefly. This binding point is called the **active site**. The active site takes up a relatively small part of the total volume of the enzyme.

So, an enzyme (**E**) works by binding to its substrate (**S**) molecule at a specially formed pocket in the enzyme. This concept is referred to the **'lock and key'** hypothesis of enzyme action. As the enzyme and substrate form a complex (**E–S**), the substrate is raised in energy to a transition state and then breaks down into products (**Pr**) plus unchanged enzyme.

$$E + S \rightarrow E\text{–}S \rightarrow Pr - E$$

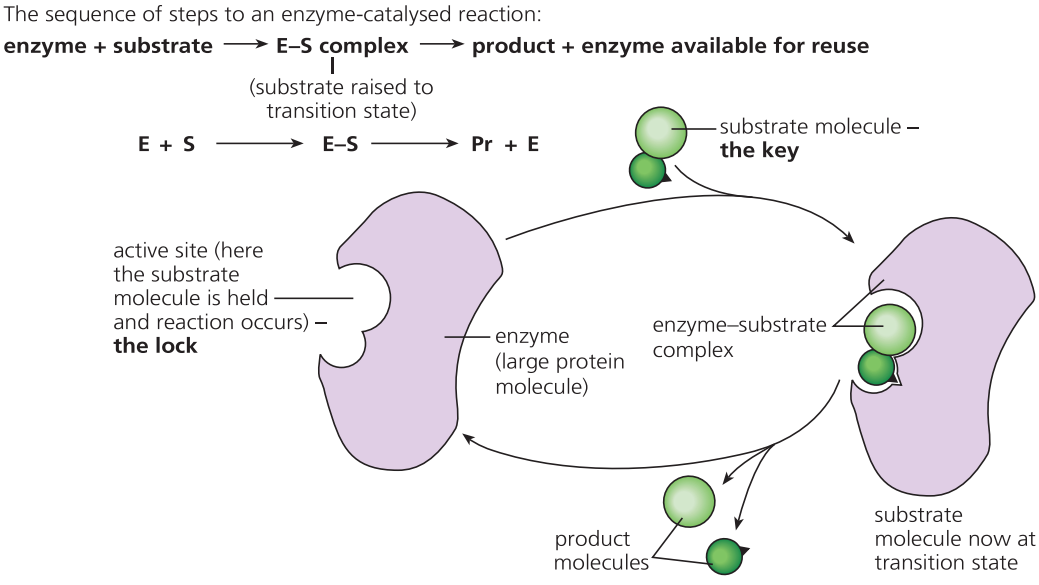


Figure 3.4 The lock and key hypothesis of enzyme action

Enzymes are typically large **globular protein** molecules. Most substrate molecules are quite small molecules by comparison. Even when the substrate molecules are very large, such as certain macromolecules like the polysaccharides, only one bond in the substrate is in contact with the active site of the enzyme.

Enzymes are highly specific

Enzymes are highly specific in their action. They catalyse only one type of reaction or only a very small group of very similar reactions.

This means that an enzyme 'recognises' a very small group of substrate molecules or even only a single type of molecule. This is because the active site where the substrate molecule binds has a **precise shape** and **distinctive chemical properties** (meaning the presence of particular chemical groups and bonds). Only particular substrate molecules can fit to a particular active site. All other substrate molecules are unable to fit and so cannot bind.

Question

1 Explain why the shape of globular proteins that are enzymes is important in enzyme action?

Catalysis by 'induced fit'

We have seen that enzymes are highly specific in their action. This makes them different from most inorganic catalysts. Enzymes are specific because of the way they bind with their substrate at a pocket or crevice in the protein. The 'lock and key' hypothesis, however, does not fully account for the combined events of 'binding' and simultaneous chemical change observed in most enzyme-catalysed reactions.

At the active site, the arrangement of certain amino acid molecules in the enzyme exactly matches certain groupings on the substrate molecule, enabling the enzyme–substrate complex to form. As the complex is formed, an essential, critical **change of shape** is caused in the enzyme molecule. It is this change of shape that is important in momentarily raising the substrate molecule to the transitional state. It is then able to react.

With a transitional state achieved, other amino acid molecules of the active site bring about the breaking of particular bonds in the substrate molecule, at the point where it is temporarily held by the enzyme. It is because different enzymes have different arrangements of amino acids in their active sites that each enzyme catalyses either a single chemical reaction or a group of closely related reactions.

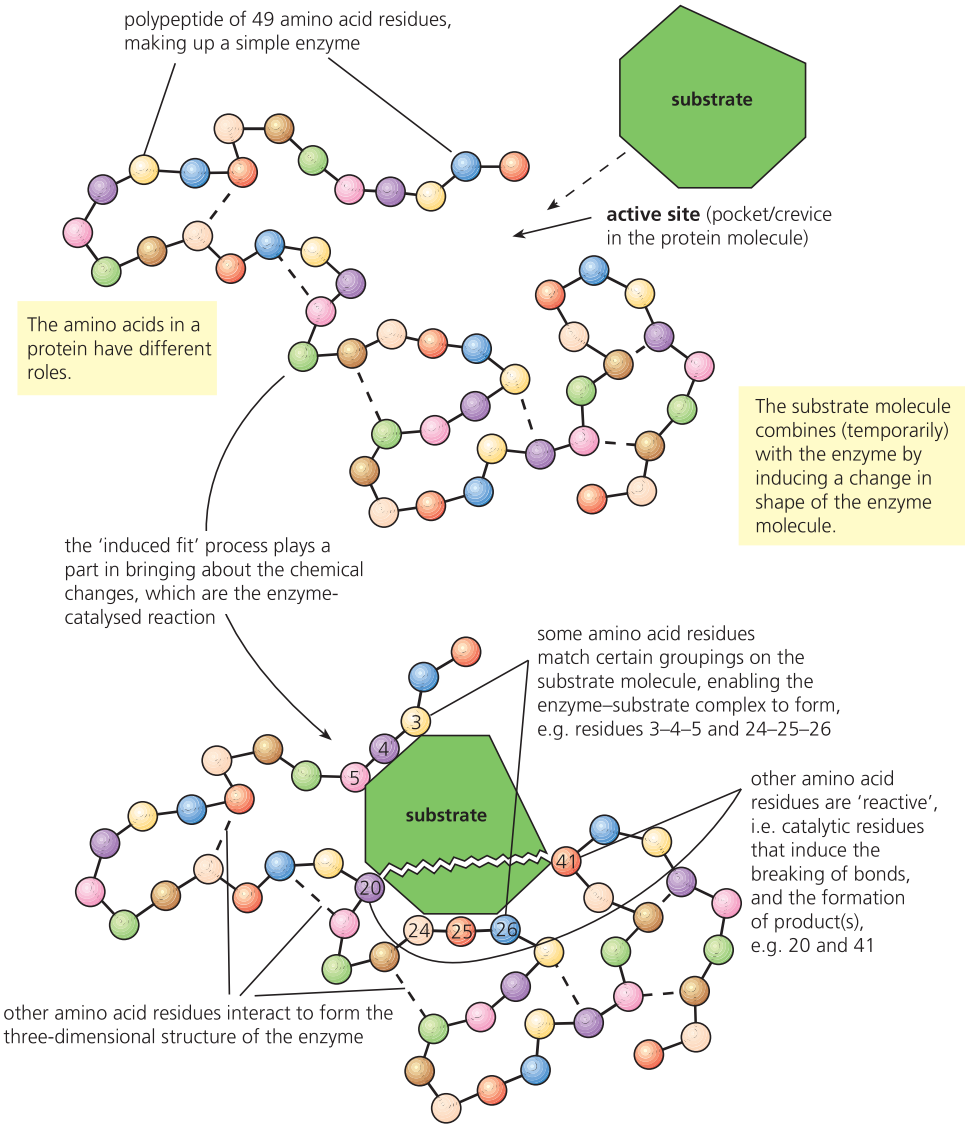


Figure 3.5 The induced fit hypothesis of enzyme action

Specificity:

- Some amino acid residues allow a particular substrate molecule to 'fit'
- Some amino acid residues bring about particular chemical changes.

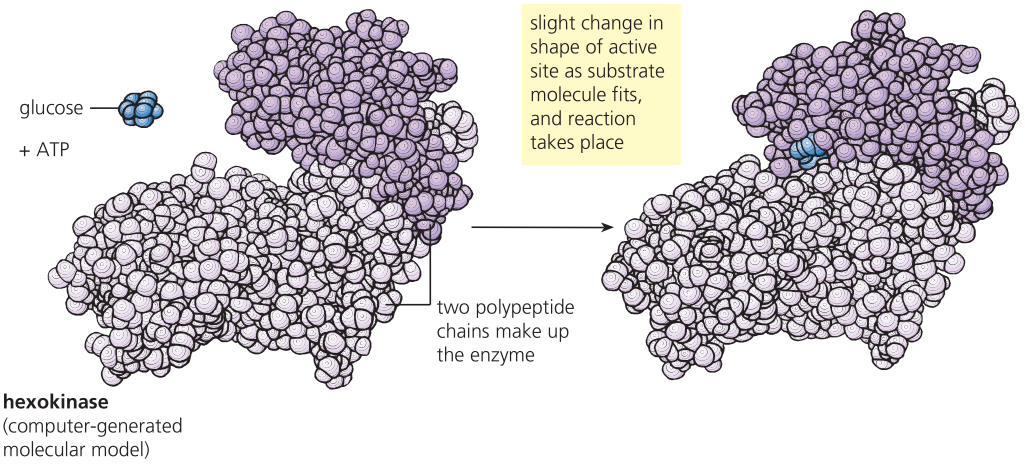


Figure 3.6 Computer-generated image of the induced fit hypothesis in action

Naming enzymes

Many enzymes have a name based on the name of their substrate, with the ending *-ase* added. For example, **lactase** hydrolyses lactose and **amylase** hydrolyses amylose.

Other enzymes have been given names that tell us little or nothing about what they do, such as many of the enzymes of digestion, for example, **pepsin**, **trypsin** and **rennin**.

Today, systematic naming of enzymes is based on an agreed classification of enzymes and on the name of the substrate catalysed. These types of names are long and detailed. They are outside the scope of this book. They are used in the communications of enzymologists but not in everyday usage. However, you are already familiar with certain enzymes. For example, the enzymes that catalyse the formation of two products from a larger substrate molecule by a hydrolysis reaction are classified as 'hydrolases'. Can you name a hydrolase?

Question

2 **a** Define the term 'catalyst'.
b List two differences between inorganic catalysts and enzymes.

Studying enzyme-catalysed reactions

Enzyme-catalysed reactions are fast reactions. We know this from the very large numbers of substrate molecules converted to products by a mole of enzyme in one minute. The mole is a unit for the amount of a substance. It is explained in Appendix 1 on the CD.

The enzyme catalase catalyses the breakdown of hydrogen peroxide. This enzyme is a good example to use in studying the rate of enzyme-catalysed reactions.

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

Catalase occurs widely in the cells of living things. It functions as a protective mechanism for the delicate biochemical machinery of cells. This is because hydrogen peroxide is a common by-product of some of the reactions of metabolism. Hydrogen peroxide is a very toxic substance – a very powerful oxidising agent, in fact (Appendix 1). Catalase inactivates hydrogen peroxide as soon as it forms, before damage can occur.

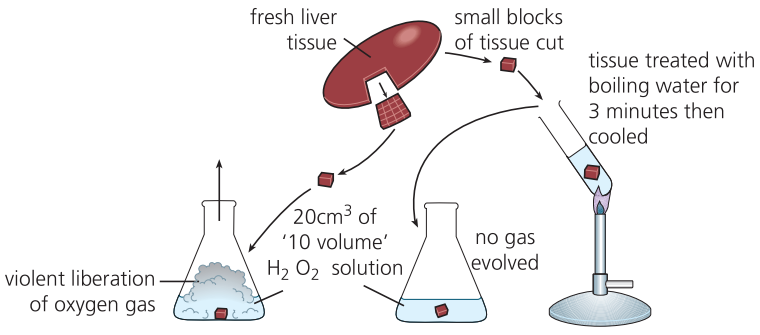


Figure 3.7 Liver tissue in dilute hydrogen peroxide solution

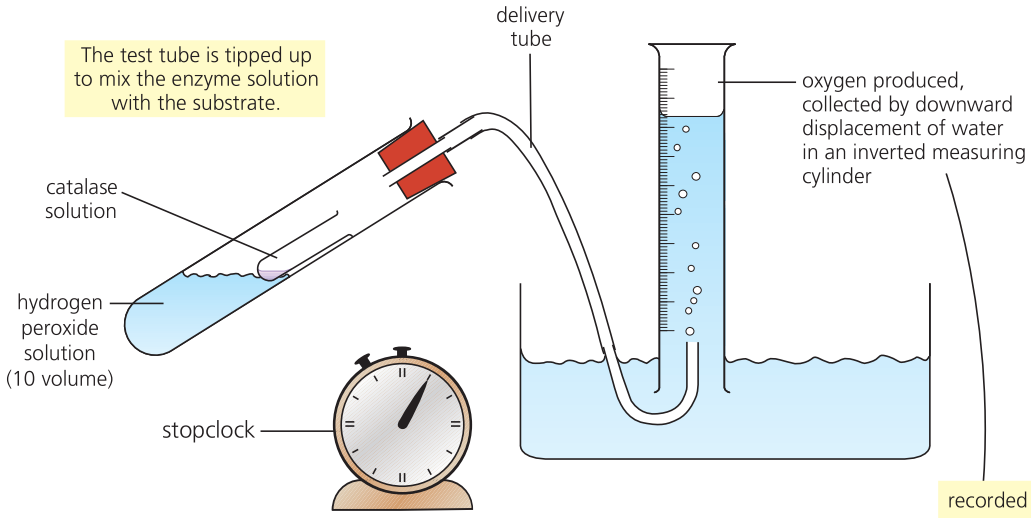
You can demonstrate the presence of catalase in fresh liver tissue by dropping a small piece into dilute hydrogen peroxide solution (Figure 3.7). Compare the result obtained with that from a similar piece of liver that has been boiled in water (high temperature denatures and destroys enzymes, including catalase). If you do not wish to use animal tissues, then you can use potato or soaked and crushed dried peas instead – the results will be equally dramatic!

Measuring the rate of reaction

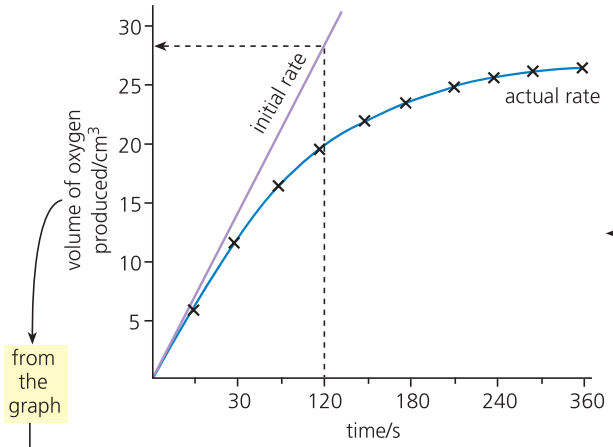
In practical terms, the rate of an enzyme-catalysed reaction is taken as the amount of substrate that has disappeared from a reaction mixture in a given time. Alternatively, the amount of product that has accumulated in a period of time can be measured. For example, in Figure 2.38, the enzyme amylase is used to digest starch to sugar, and here it is the rate that the substrate starch disappears from a reaction mixture that is measured.

Working with catalase, however, it is easiest to measure the rate at which the product (oxygen) accumulates. In the experiment illustrated in Figure 3.8, the volume of oxygen that has accumulated at half minute intervals is recorded on the graph.

In both of these examples we find that, over a period of time, the initial rate of reaction is not maintained but, rather, falls off quite sharply. This is typical of enzyme actions studied outside their location in the cell. Can you think of reasons why?



The rate of an enzyme reaction is greatest at the start, the **initial rate**.



results plotted

Time/s	Gas volume collected/cm^3
30	6
60	12
90	16
120	19
150	22
180	23
210	24
240	25
270	25.5
300	26

from the graph

If the initial rate of O_2 production continued for 120 s, then 28 cm^3 of O_2 would be produced.
Therefore the initial rate = 28/120 cm^{3}s^{-1}
= 0.23 cm^{3}s^{-1}

Figure 3.8 Measuring the rate of reaction using catalase

The fall-off can be due to a number of reasons. Most commonly it is because the concentration of the substrate in the reaction mixture has fallen. Consequently, it is the initial rate of reaction that is measured. This is the slope of the tangent to the curve in the initial stage of reaction. How this is calculated is shown in Figure 3.8.

Question

3 In an investigation of oxygen production from hydrogen peroxide solution by the enzyme catalase the following results were obtained.

Time of readings (s)	0	20	40	60	80	100	120
Volume of O_2 produced (cm^3)	0	43	66	78	82	91	92

a Plot a graph to show oxygen production against time.

b From your graph, find the initial rate of the reaction.

3.2 Factors that affect enzyme action

Investigating the effects of factors on enzyme activity gives opportunities for planning and carrying out experiments under controlled conditions.

By the end of this section you should be able to:

a) investigate and explain the effects of the following factors on the rate of enzyme-catalysed reactions:
- temperature
- pH (using buffer solutions)
- enzyme concentration
- substrate concentration
- inhibitor concentration

b) explain that the maximum rate of reaction (V_{max}) is used to derive the Michaelis-Menten constant (K_m) which is used to compare the affinity of different enzymes for their substrates

c) explain the effects of reversible inhibitors, both competitive and non-competitive, on the rate of enzyme activity

d) investigate and explain the effect of immobilising an enzyme in alginate on its activity as compared with its activity when free in solution

Investigating factors that affect the rate of reaction of enzymes

Enzymes are sensitive to environmental conditions – very sensitive, in fact. Many factors within cells affect enzymes and therefore alter the rate of the reaction being catalysed. Investigations of these factors, including temperature, pH and the effect of substrate concentration in particular, have helped our understanding of how enzymes work.

Temperature

Examine the investigation of the effect of temperature on the hydrolysis of starch by the enzyme amylase shown in Figure 3.9. When starch is hydrolysed by the enzyme amylase, the product is maltose, a disaccharide. Starch gives a blue–black colour when mixed with iodine solution (iodine in potassium iodide solution) but maltose gives a red colour. The first step in this experiment is to bring samples of the enzyme and the substrate (the starch solution) to the temperature of the water bath before being mixed – a step called 'pre-incubation'.

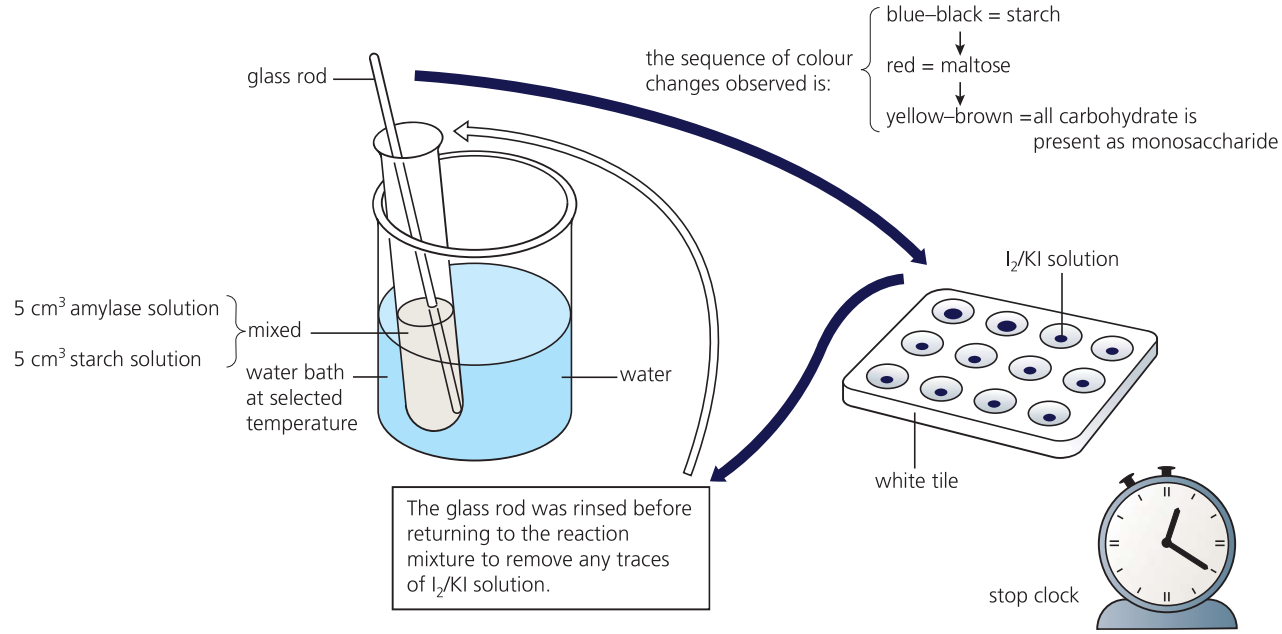


Figure 3.9 The effect of temperature on hydrolysis of starch by amylase

The experiment is **repeated at a range of temperatures,** such as at 10, 20, 30, 40, 50 and 60°C.

A **control tube** of 5 cm^3 of starch solution + 5 cm^3 of distilled water (in place of the enzyme) should be included and tested for the presence/absence of starch, at each temperature used.

Other variables – such as the concentrations of the enzyme and substrate solutions – were kept constant.

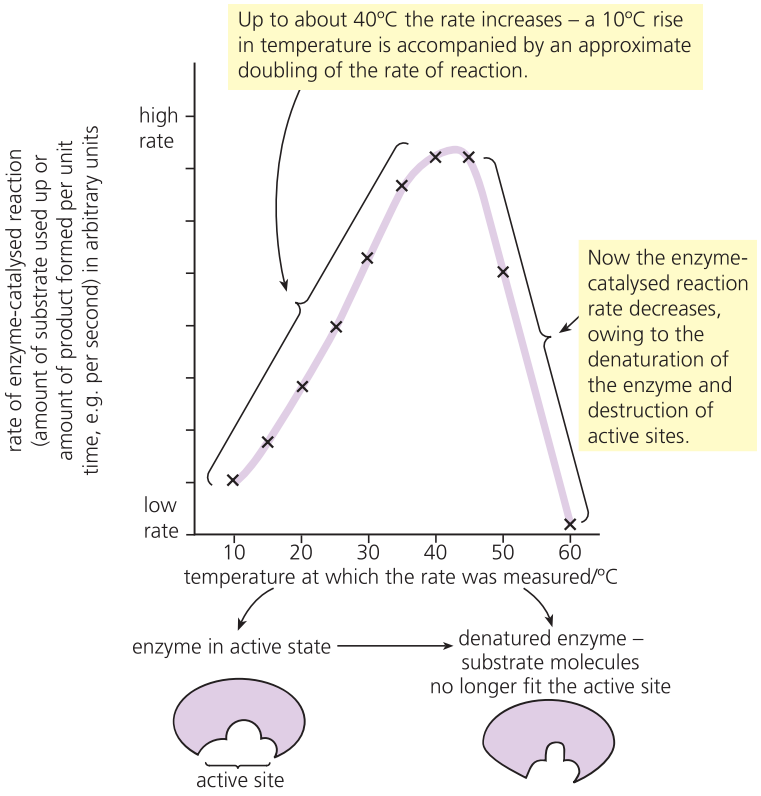


Figure 3.10 The effect of temperature on the rate of an enzyme-catalysed reaction

The progress of the hydrolysis reaction is then followed by taking samples of a drop of the mixture on the end of the glass rod, at half minute intervals. These are tested with iodine solution on a white tile. Initially, a strong blue–black colour is seen confirming the presence of starch. Later, as maltose accumulates, a red colour forms. The end point of the reaction is when all the starch colour has disappeared from the test spot.

Using fresh reaction mixture each time, the investigation is repeated at a series of different temperatures, say at 10, 20, 30, 40, 50 and 60°C. The time taken for complete hydrolysis at each temperature is recorded and the rate of hydrolysis in unit time is plotted on a graph. A characteristic curve is the result – although the 'optimum' temperature varies from reaction to reaction and with different enzymes (Figure 3.10).

How is the graph interpreted? Look at Figure 3.10.

Question

4 In studies of the effect of temperature on enzyme-catalysed reactions, suggest why the enzyme and substrate solutions are pre-incubated to a particular temperature before they are mixed.

As the temperature is increased, molecules have more energy and reactions between them happen more quickly. The enzyme molecules are moving more rapidly and are more likely to collide and react. In chemical reactions, for every 10°C rise in temperature the rate of the reaction approximately doubles. This property is known as the **temperature coefficient (Q_{10})** of a chemical reaction.

However, in enzyme-catalysed reactions the effect of temperature is more complex because proteins are **denatured by heat**. The rate of denaturation increases at higher temperatures, too. So as the temperature rises above a certain point the amount of active enzyme progressively decreases and the rate is slowed. As a result of these two effects of heat on enzyme-catalysed reactions, there is an apparent optimum temperature for an enzyme.

Not all enzymes have the same optimum temperature. For example, the bacteria in hot thermal springs have enzymes with optimum temperatures between 80 and 100°C or higher, whereas seaweeds of northern seas and the plants of the tundra have optimum temperatures closer to 0°C. Humans have enzymes with optimum temperatures at or about normal body temperature. This feature of enzymes is often exploited in the commercial and industrial uses of enzymes today.

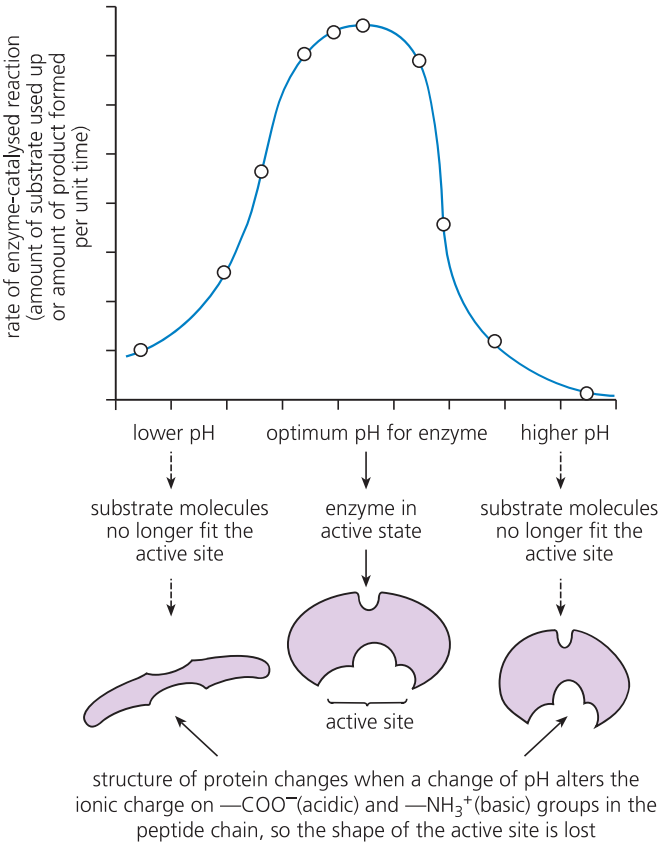


Figure 3.11 The effect of pH on enzyme shape and activity

pH

Change in pH can have a dramatic effect on the rate of an enzyme-catalysed reaction. Each enzyme has a range of pH in which it functions efficiently – often at or close to neutrality. The pH affects the rate of reaction because the structure of a protein (and therefore the shape of the active site) is maintained by various bonds within the three-dimensional structure of the protein (Figure 2.22). A change in pH from the optimum value alters the bonding patterns. As a result, the shape of the enzyme molecule is progressively changed. The active site may quickly become inactive. However, the effects of pH on the active site are normally reversible (unlike temperature changes). That is, provided the change in surrounding acidity or alkalinity is not too extreme. As the pH is brought back to the optimum for that enzyme, the active site may reappear (Figure 3.11).

Some of the digestive enzymes of the gut have different optimum pH values from the majority of other enzymes. For example, those adapted to work in the stomach, where there is a high concentration of acid during digestion, have an optimum pH which is close to pH 2.0 (Figure 3.12).

Question

5 **a** Explain what a buffer solution is.
b Why are they often used in enzyme experiments?

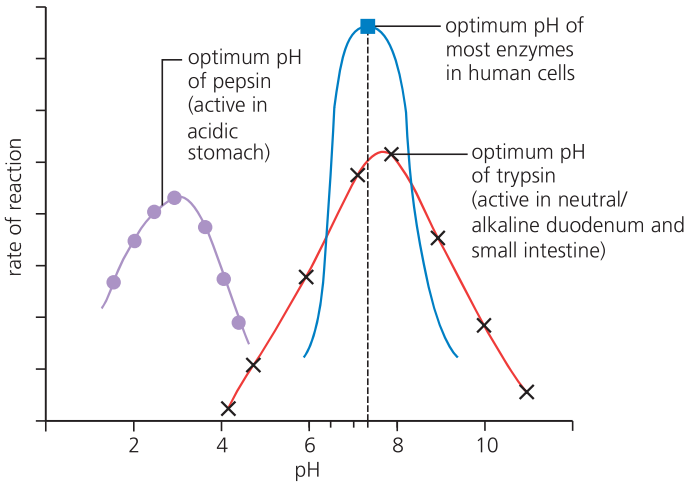


Figure 3.12 The optimum pH of different human enzymes

Substrate concentration

The effect of different concentrations of substrate on the rate of an enzyme-catalysed reaction can be shown using the enzyme catalase.

Look again at the investigation of the initial rate of reaction using catalyse (Figure 3.8). We saw that, when working with catalase, it is easy to measure the rate the product (oxygen) accumulated (recorded at half minute intervals).

To investigate the effect of substrate concentration on the rate of this enzyme-catalysed reaction, the experiment shown in Figure 3.8 is repeated **at different concentrations of substrate**. The initial rate of reaction plotted in each case. Other variables such as temperature and enzyme concentration are kept constant.

When the initial rates of reaction are plotted against the substrate concentration, the curve shows two phases. At lower concentrations the rate increases in direct proportion to the substrate concentration but at higher substrate concentrations, the rate of reaction becomes constant and shows no increase (Figure 3.13).

We can see that the enzyme catalase does work by forming a short-lived enzyme–substrate complex. At low concentrations of substrate, all molecules can find an active site without delay. Effectively, there is excess enzyme present. The rate of reaction is set by how much substrate is present – as more substrate is made available the rate of reaction increases.

At higher substrate concentrations there are more substrate molecules than enzyme molecules. Now, in effect, substrate molecules have to 'queue up' for access to an active site. Adding more substrate increases the number of molecules awaiting contact with an enzyme molecule. There is now no increase in the rate of reaction. This relationship between amount of substrate and the rate of reaction is shown in Figure 3.13.

Question

6 When there is an excess of substrate present in an enzyme-catalysed reaction, explain the effects on the rate of reaction of increasing the concentration of:
- **a** the substrate
- **b** the enzyme.

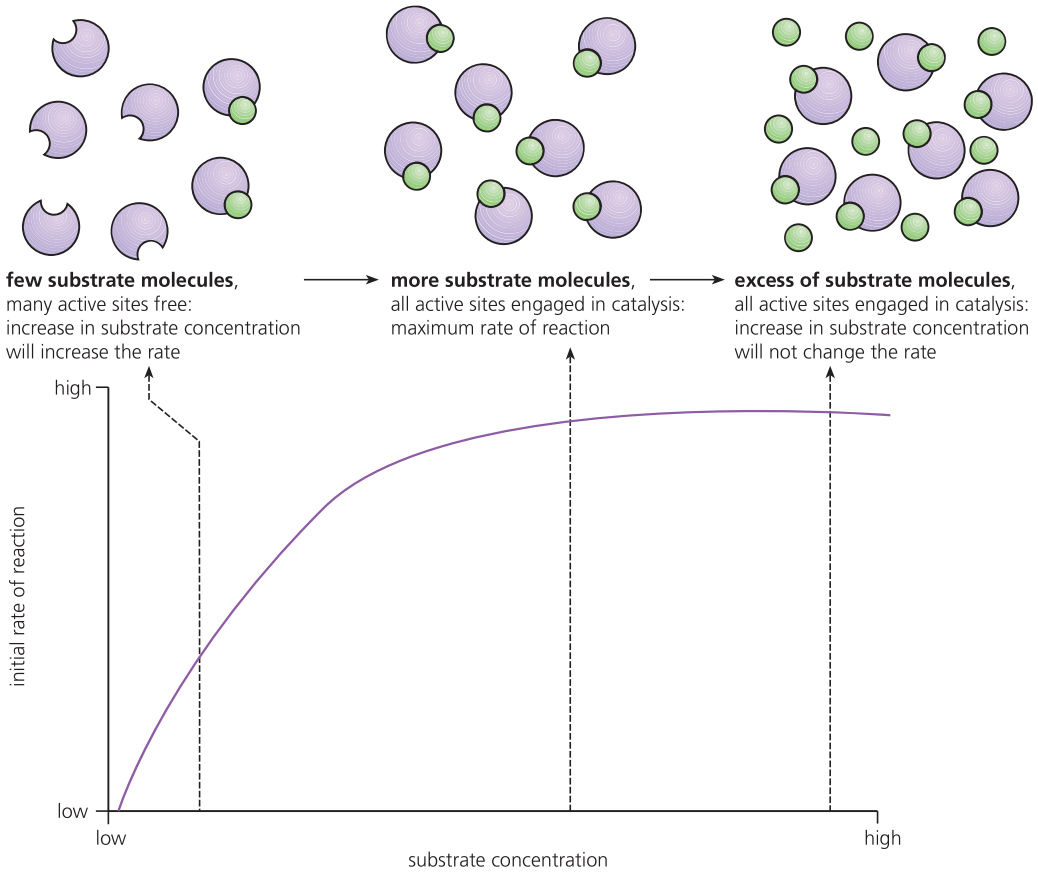


Figure 3.13 The effect of substrate concentration on the rate of an enzyme-catalysed reaction

Enzyme concentration

If there are plenty of substrate molecules in a reaction mixture, then the more enzyme that is added the faster the rate of reaction will be. This is the situation in a cell where an enzyme reaction occurs with a small amount of enzyme present. Any increase in enzyme production will lead to an increased rate of reaction, simply because more active sites are made available.

Introducing the Michaelis-Menton constant (K_m) and its significance

On page 62 we saw how the initial rate of an enzyme-catalysed reaction was measured and why (Figure 3.8).

Remind yourself 'how' and 'why' now.

When the initial rate of reaction of an enzyme is measured over a range of substrate concentrations (with a fixed amount of enzyme) and the results plotted on a graph, a typical example of the resulting curve is shown in Figure 3.14. You can see that, with increasing substrate concentration, the velocity increases – rapidly at lower substrate concentrations. However, the rate increase progressively slows, and above a certain substrate concentration, the curve has flattened out. No further increase in rate occurs. This tells us the enzyme is working at maximum velocity at this point. On the graph, this point of maximum velocity is shown as V_{max}.

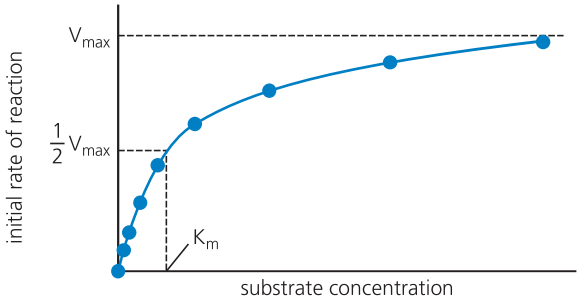


Figure 3.14 Graph of initial rate of an enzyme-catalysed reaction against substrate concentration

In 1913 the biochemists Michaelis and Menten studied this aspect of enzyme reactions and introduced a constant, now known as the Michaelis-Menten constant. The **Michaelis-Menten constant (K_m)** is defined as the substrate concentration that sustains half maximum velocity ($\frac{1}{2}V_{max}$). It measures the degree of attraction or affinity of an enzyme for the substrate – the smaller the K_m the higher the affinity. K_m can be experimentally determined at a specified pH and temperature, and is expressed in units of molarity.

Actual values of K_m have been measured for a great many enzymes. These values fall between 10^{-3} and 10^{-5} mol dm^3 of substrate. This is a very wide range of concentrations. It means that some enzymes are able to work at maximum velocity at very low concentrations of substrate, whilst others only function effectively at much higher concentrations.

Take the case of two enzymes that catalyse the transformation of the same substrate molecule but in different reaction sequences. If the 'pool' or reserves of that substrate are low and its supply restricted, then the enzyme with the lowest K_m will claim more – and one particular metabolic pathway will benefit at the expense of the other. Knowing the K_m of enzymes is an important part of understanding metabolism, both quantitatively and qualitatively.

Inhibitors of enzymes

Certain substances present in cells (and some which enter from the environment) may react with an enzyme, altering the rate of reaction. These substances are known as **inhibitors**, since their effect is generally to lower the rate of reaction. Studies of the effects of inhibitors have helped our understanding of:

- the chemistry of the active site of enzymes
- the natural regulation of metabolism and which pathways operate
- the ways certain commercial pesticides and many drugs work (by inhibiting specific enzymes and preventing particular reactions).

For example, molecules that sufficiently resemble the substrate in shape may compete to occupy the active site. They are known as **competitive inhibitors**. For example, the enzyme that catalyses the reaction between carbon dioxide and the CO_2-acceptor molecule in photosynthesis, known as ribulose bisphosphate carboxylase (rubisco), is competitively inhibited by oxygen in the chloroplasts.

Because these inhibitors are not acted on by the enzyme and turned into 'products' as normal substrate molecular are, they tend to remain attached. However, if the concentration of the substrate molecule is raised, the inhibitor molecules are progressively displaced from the active sites (Figure 3.15).

Alternatively, an inhibitor may be unlike the substrate molecule, yet still combine with the enzyme. In these cases, the attachment occurs at some **other part** of the enzyme. This may be quite close to the active site. Here the inhibitor either partly blocks access to the active site by substrate molecules or it causes the active site to change shape so that it is then unable to accept the substrate.

These are called **non-competitive inhibitors**, since they do not compete for the active site. Adding excess substrate does not overcome their inhibiting effects (Figure 3.15). An example is the effect of the amino acid alanine on the enzyme pyruvate kinase in the final step of glycolysis (Topic 12).

When the initial rates of reaction of an enzyme are plotted against substrate concentration, the effects of competitive and non-competitive inhibitors are seen to be different.

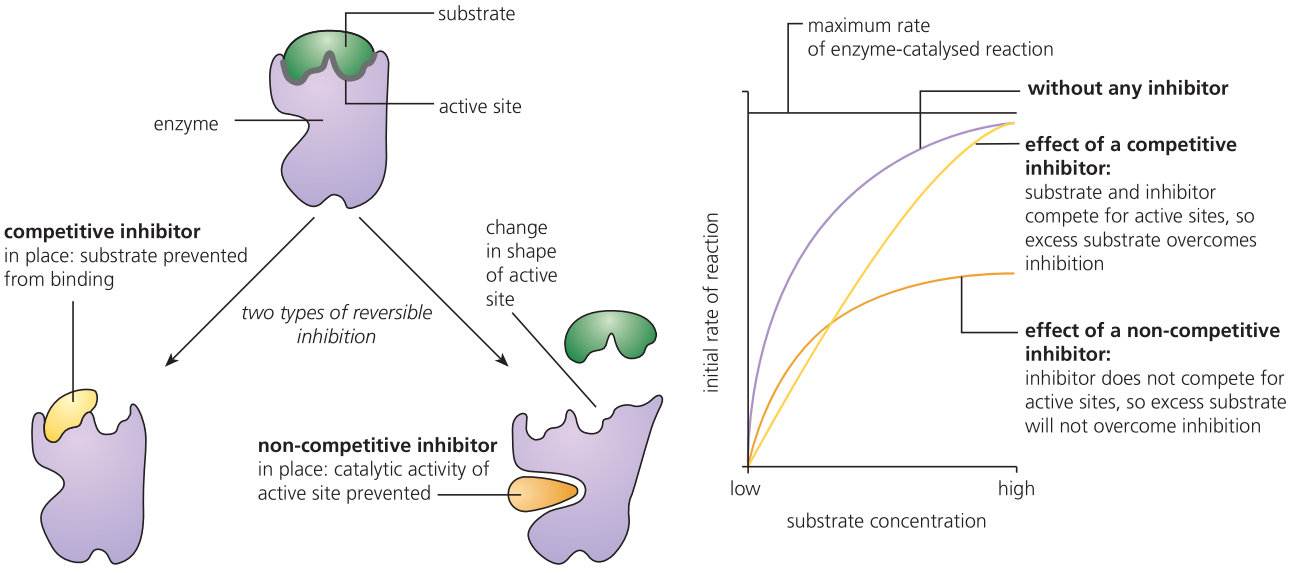


Figure 3.15 The principles of competitive and non-competitive inhibition

Table 3.1 A comparison of competitive and non-competitive inhibition of enzymes

Competitive inhibition	Non-competitive inhibition
Inhibitor chemically resembles the substrate molecule and occupies (blocks) the active site	Inhibitor chemically unlike the substrate molecule but reacts with the bulk of the enzyme, reducing access to the active site
With a low concentration of inhibitor, increasing concentration of substrate eventually overcomes inhibition as substrate molecules displace inhibitor	With a low concentration of inhibitor, increasing concentration of substrate cannot prevent binding of inhibitor – some inhibition remains at high substrate concentration
Example: O_2 competes with CO_2 for active site of rubisco	Example: alanine non-competitively inhibits pyruvate kinase

Certain **irreversible inhibitors** bind tightly and permanently to an enzyme and destroy its catalytic properties entirely. These drastic effects occur at low concentrations of inhibitor and we may describe these substances as **poisons**. Examples include:

- cyanide ions which block cytochrome oxidase in terminal oxidation in cell aerobic respiration
- the nerve gas sarin blocks a neurotransmitter (acetyl cholinesterase) in synapse transmission

Use of enzymes as industrial and laboratory catalysts

Enzymes as biological catalysts are important components of many industrial processes. Their use is widespread because they are:

- highly specific, catalysing changes in one particular compound or one type of bond
- efficient, in that a tiny quantity of enzyme catalyses the production of a large quantity of product
- effective at normal temperatures and pressures, and so a limited input of energy (as heat and high pressure) may be required.

The enzymes selected by industry are frequently produced from microorganisms – typically from species of fungi or bacteria. Table 3.2 lists some examples.

Table 3.2 Enzymes with industrial applications obtained from microorganisms

	Enzyme	Source	Application
Bacterial	Protease	*Bacillus*	'Biological' detergents
	Glucose isomerase	*Bacillus*	Fructose syrup manufacture
Fungal	Lactase	*Kluyveromyces*	Breakdown of lactose to glucose and galactose
	Amylase	*Aspergillus*	Removal of starch in woven cloth production

Using enzymes *in vitro*

Enzymes may be used as cell-free preparations added to a reaction mixture, or they may be immobilised enzymes, with the reactants passed over them.

Enzyme immobilisation involves the attachment of enzymes to insoluble materials, which then provide support for the enzyme. For example, the enzyme may be entrapped between inert fibres, or it may be covalently bonded to a matrix. In both cases the enzyme molecules are prevented from being leached away. The immediate advantages of using an immobilised enzyme are:

- it permits re-use of the enzyme preparation
- the product is obtained enzyme free
- the enzyme may be much more stable and long lasting, due to protection by the inert matrix.

Clearly, there are advantages in using industrial enzymes in the immobilised condition, where this is possible. We will return to this issue shortly. First, we can investigate the process of immobilisation of an enzyme and compare its activity when alternatively used free in a solution.

Immobilised enzyme – a laboratory demonstration

The steps to this demonstration are:

1 A solution of sodium alginate (a polysaccharide obtained from the walls of brown algae, capable of holding 200 times its own mass in water) is prepared by dispersing 2 g of the alginate in 100 cm^3 of distilled water at a temperature of 40 °C.

2 An alginate-enzyme solution is prepared by stirring 2 cm^3 of invertase concentrate into 40 cm^3 of the cooled alginate solution. (Remember, invertase catalyses the hydrolysis of sucrose [a disaccharide] to two monosaccharide molecules [glucose and fructose] from which it is formed in a condensation reaction.)

3 Immobilised enzyme pellets are produced by filling a syringe with the alginate-invertase solution and arranging for it to drip into a beaker of calcium chloride solution (100 cm^3 of $CaCl_2$, 0.1 mol dm^3). The insoluble pellets formed may be separated with a nylon/plastic sieve. They should be washed with distilled water at this stage, and allowed to harden.

4 These hardened pellets may be tested by placing them in a tube with a narrow nozzle at the base (the barrel of a large, plastic syringe is suitable). A quantity of sucrose solution (30 cm^3 of 5% sucrose w/v) may be slowly poured down the column of pellets and the effluent collected. Note the time taken for the solution to pass through.

5 Test the effluent solution for the presence of glucose, using Clinistix™ (see below).

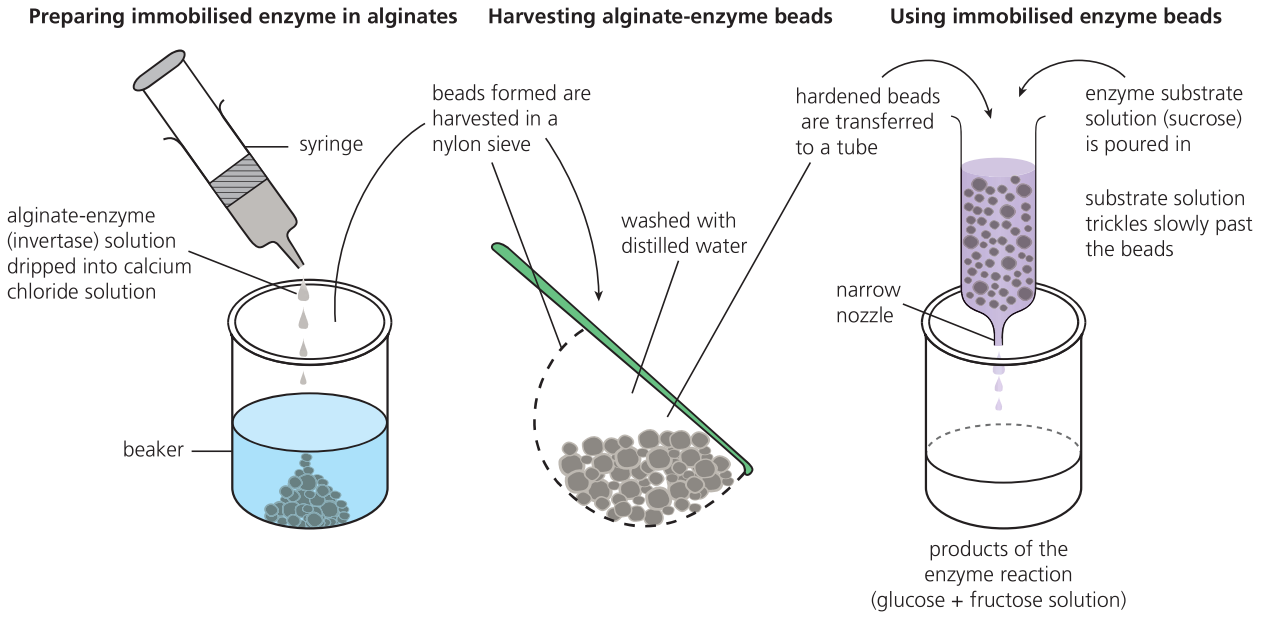


Figure 3.16 Preparing an immobilised enzyme

Investigating the efficiency of immobilised enzyme compared to its use free in solution

A comparison of the efficiency of immobilised invertase compared with free enzyme can be made by stirring into a second sample of the sucrose solution (again, 30 cm^3 of 5% sucrose w/v), 2 cm^3 of the invertase concentrate solution. The free enzyme should be allowed to catalyse hydrolysis of the sucrose for the same time period as it took for the earlier sucrose solution sample to pass through the alginate bead column. Once again, the solution should be tested for the presence of glucose, using Clinistix™ (see Figure 3.17).

Estimating the glucose concentration using Clinistix™

Clinistix™ exploit immobilised enzymes in dipsticks for the quantitative measurement of glucose. (An important medical application of the Clinistix™ has been the measurement of glucose in urine samples in patients with diabetes.)

The Clinistix™ strip contains two enzymes, glucose oxidase and peroxidase, together with a colourless hydrogen donor compound called chromogen. When the strip is dipped into a sample, if glucose is present it is oxidised to glucuronic acid and hydrogen peroxide. The second enzyme catalyses the reduction of hydrogen peroxide and the oxidation of chromogen. The product is water and the oxidised dye, which is coloured. The more glucose present in the sample the more coloured dye is formed. The colour of the test strip is then compared to the printed scale to indicate the amount of glucose present (Figure 3.17).

Note that this is another example of the industrial exploitation of biological catalysts.

How effective was the immobilised enzyme, compared to its activity, free in solution?

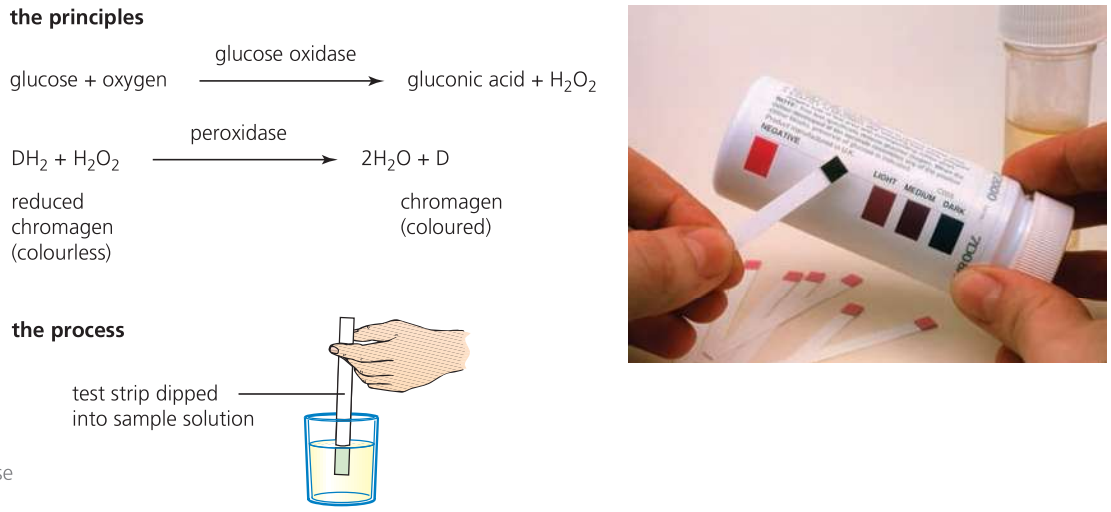


Figure 3.17 Measuring glucose in urine using a Clinistix™

What are the advantages and disadvantages of immobilisation?

The most obvious advantage is the recovery of the enzyme and its availability for re-use. Other issues are listed in Table 3.3.

Table 3.3 Advantages and disadvantages of immobilisation

Advantages	Disadvantages
The enzyme is held in a form that can be manipulated easily. It is absent from the product, so no purification steps are required.	An immobilisation mechanism that does not alter the shape or the catalytic ability of the enzyme must be selected.
The enzyme is available for multiple re-use, since it functions as an effective catalyst in pellet form.	The creation of stable, hardened pellets is an added expense that is inevitably reflected in the cost of the industrial product.
An immobilised enzyme is stable at the temperatures and pH at which it is held and used.	If the enzyme becomes detached it will appear in the product as a contaminant, possibly unnoticed.

Summary

- **Metabolism**, all the chemical reactions of life, consists of **anabolic reactions**, the build up of complex molecules from smaller ones, e.g. protein synthesis, and **catabolic reactions**, the breakdown of complex molecules, e.g. oxidation of sugar in respiration.
- All reactions of metabolism are made possible by **enzymes**. Enzymes are **biological catalysts** and most are made of globular protein. An enzyme is **highly specific** to the type(s) of substrate molecule and type of reaction that they catalyse.
- Enzymes work by forming a **temporary complex** with a **substrate** molecule at a special part of the enzyme surface, called the **active site** (the lock and key hypothesis). Enzymes work by lowering the **activation energy** needed for a reaction to occur.
- A slight **change in shape** of the substrate molecule when it binds to the active site helps raise the molecule to a **transition state** (the induced fit hypothesis), from which the products may form. The enzyme is released for reuse.
- The **rate of an enzyme-catalysed reaction** is found by measuring the disappearance of the substrate or the accumulation of the product in a given period of time. The **initial rate of reaction** is taken since the reaction rate falls with time under experimental conditions.
- The **factors that affect the rate of an enzyme-catalysed reaction** include **pH** and **temperature** – through their effects on protein structure. When molecules of substances recognised as **inhibitors** are in contact with enzyme molecules, the rate of reaction may be lowered in characteristic ways.
- **The Michaelis-Menten constant (K_m)** is the substrate concentration that sustains half maximum velocity ($\frac{1}{2}$ V_{max}) of an enzyme-catalysed reaction. It is a measure of the degree of affinity of an enzyme for its substrate. K_m can be measured experimentally, and is expressed in units of molarity. Values of K_m have been measured for a great many enzymes, and it has been shown that some enzymes are able to work at maximum velocity at very low concentrations of substrate.
- Industries use enzymes as biological catalysts, often immobilised, with the reactants passed over them. In these cases, the advantages are the recovery of the enzyme and its availability for re-use. Being immobilised may affect the enzyme's efficiency compared to use of the same enzyme when free in the substrate solution.

Examination style questions

1 The enzyme sucrase catalyses the breakdown of the glycosidic bond in sucrose.

A student investigated the effect of increasing the concentration of sucrose on the rate of activity of sucrase. Ten test-tubes were set up with each containing 5 cm^3 of different concentrations of a sucrose solution. The test-tubes were placed in a water bath at 40°C for ten minutes. A flask containing a sucrase solution was also put into the water bath. After ten minutes, 1 cm^3 of the sucrase solution was added to each test-tube. The reaction mixtures were kept at 40°C for a further ten minutes.

After ten minutes, the temperature of the water bath was raised to boiling point. Benedict's solution was added to each test-tube. The time taken for a colour change was recorded and used to calculate rates of enzyme activity.

The results are shown in Fig. 1.1.

a) i) Name the type of reaction catalysed by sucrase. [1]

ii) Explain why the temperature of the water was raised to boiling point. [2]

b) Describe **and** explain the results shown in Fig. 1.1. [5]

[Total: 8]

(Cambridge International AS and A Level Biology 9700, Paper 21 Q4 June 2011)

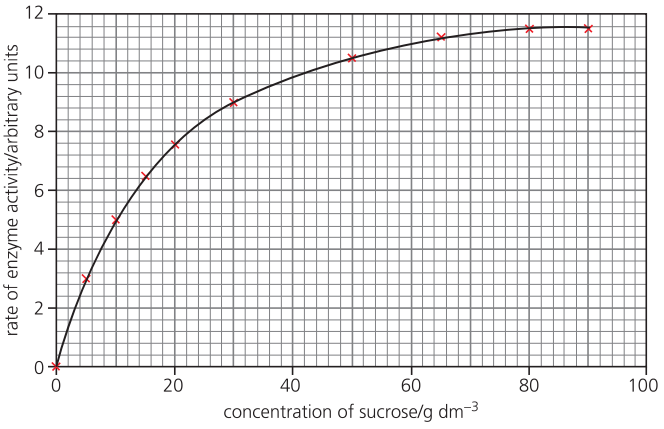


Fig. 1.1

2 **a)** Describe how enzymes take part in chemical reactions. [4]

Starch phosphorylase is an enzyme found in plant cells. In potato tuber cells, the enzyme takes part in the breakdown of starch when the tuber begins to grow.

starch phosphorylase

starch + phosphate ions → glucose-1-phosphate

A student investigated the effect of pH on this reaction using two buffer solutions.

The student prepared four test tubes, **A** to **D**, as shown in the table and described below.

The student made an extract of potato tissue that contained the enzyme. Some of this extract was boiled.

A solution of potassium dihydrogen phosphate was added to some tubes as a source of phosphate ions.
The test tubes were left for ten minutes in a water bath at 30 °C and then samples were tested with iodine solution.

b) i) State what the student would conclude from a positive result with iodine solution. [1]

ii) Explain why the student boiled some of the extract in this investigation. [2]

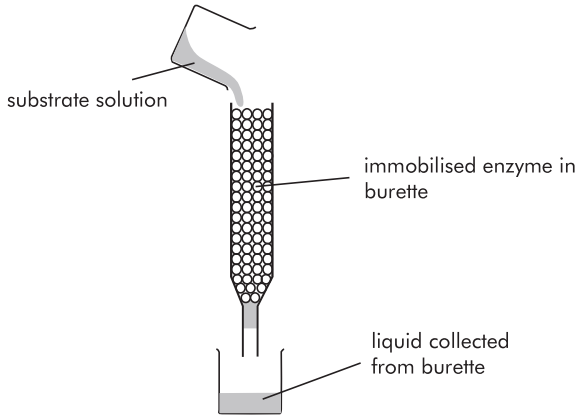
c) Explain the results shown in the table. [4]

[Total: 11]

(Cambridge International AS and A Level Biology 9700, Paper 02 Q2 June 2007)

Test tube	Contents					Results with iodine solution after 10 minutes
	Volume of starch solution / cm^3	Volume of glucose-1-phosphate solution / cm^3	Volume of potassium dihydrogen phosphate solution / cm^3	pH of buffer solution	Enzyme extract	
A	2		0.5	6.5	unboiled	negative
B	2		0.5	2.0	unboiled	positive
C	2		0.5	6.5	boiled	positive
D		2		6.5	boiled	negative

3 The diagram shows an apparatus used in an investigation using immobilised enzymes. It is **not** expected that you will have done this investigation.
A solution of a substrate was poured into a burette containing an enzyme immobilised onto alginate beads. The liquid passing through the burette was collected into a beaker and the concentration of substrate and the concentration of the product measured. The table shows the results obtained by five students.



	Enzyme concentration				Enzyme concentration			
	0.2 gdm^{-3}		0.4 gdm^{-3}		0.2 gdm^{-3}		0.4 gdm^{-3}	
	Substrate concentration / gdm^{-3}				Product concentration / gdm^{-3}			
	Repeat 1	Repeat 2	Repeat 1	Repeat 2	Repeat 1	Repeat 2	Repeat 1	Repeat 2
student A	24	26	14	13	32	33	60	64
student B	25	22	12	12	34	39	60	63
student C	22	23	10	13	35	32	59	61
student D	18	24	11	12	34	33	62	68
student E	25	28	13	18	30	32	65	64

a) Identify two variables and explain how each might be controlled. [2]

b) On a copy of the table above, indicate by placing a circle around the value, **two** results that are anomalous. [2]

c) A student drew the following conclusion from this investigation:
Doubling the enzyme concentration doubled the rate of reaction of the enzyme.

i) State **one** way in which the evidence in the table above supports the conclusion. [1]

ii) State **two** ways in which the reliability of the results might be improved. [2]

[Total: 7]

(Cambridge International AS and A Level Biology 9700, Paper 05 Q3 June 2007)