Enzyme structure and mode of action

Enzymes are globular proteins which catalyse metabolic reactions. A catalyst alters the rate of a chemical reaction without itself undergoing permanent change. It can therefore be used repeatedly and so is effective in tiny amounts. Enzymes do not make a reaction happen; they simply speed up ones which already occur, sometimes by a factor of many millions.

**Enzyme structure**

As globular proteins, enzymes have a specific three-dimensional shape which is determined by their sequence of amino acids (Topic 2.7). Despite their large overall size, enzyme molecules only have a small region that is functional. This is known as the **active site**. Only a few amino acids of the enzyme molecule make up this active site. The active site forms a hollow depression within the much larger enzyme molecule. The substrate molecule is held within the active site by bonds that temporarily form between the R groups of the amino acids of the active site and groups on the substrate molecule. This structure is known as the **enzyme–substrate complex** (Figure 1).

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**Figure 1 Enzyme–substrate complex showing the six out of 50 enzyme amino acids that form the active site**

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**Enzymes and activation energy**

Consider a typical chemical reaction:

\[
\text{sucrose} + \text{water} \rightarrow \text{glucose} + \text{fructose}
\]

(substrates) (products)

For such a reaction to occur naturally, the energy of the products (glucose + fructose) must be less than that of the substrates (sucrose + water). Such reactions, however, need an initial boost of energy to get them kick-started. This is known as the **activation energy**. It can be likened to a small stone lying on a hillside. If initial energy is applied to the stone, e.g. by giving it a push, then it will begin to move. It will move downhill, rather than uphill, because this lowers its potential energy (it is a fundamental law of thermodynamics that materials will naturally tend towards a state of low energy). Once set in motion, however, the stone gathers its own momentum and reaches the bottom with no
Enzymes further input of energy. This comparison shows how an initial input of energy (activation energy) can cause a reaction to continue on its own. In other words, there is an energy hill, or barrier, which must be overcome before the reaction can proceed. What enzymes do is to lower this activation energy level so that the reaction can happen more easily (Figure 2). For example, they allow many reactions to take place at a lower temperature than normal. As a result, some metabolic processes occur rapidly at the human body temperature of 37°C, which is relatively cool in terms of chemical reactions. These would take place too slowly to sustain life as we know it were they to take place without enzymes.

How enzymes work

In one sense, enzymes work in the same way as a key operates a lock: each key has a very specific shape which, on the whole, fits and operates only one lock. In the same way, a substrate will only fit the active site of one particular enzyme. Enzymes are therefore specific in the reactions that they catalyse. The shape of the substrate (key) exactly fits the active site of the enzyme (lock). This is known as the lock and key hypothesis and explains, in a simple way, what happens (Figure 4). In practice, for most enzymes, the process is slightly different. Rather than being a rigid lock, the enzyme actually changes its form slightly to fit the shape of the substrate. In other words, it is flexible and moulds itself around the substrate, just as a glove moulds itself to the shape of someone’s hand. The enzyme has a certain basic shape, just as a glove has, but this becomes slightly different as it alters in the presence of the substrate. As it alters its shape, the enzyme puts a strain on the substrate molecule and thereby lowers its activation energy. This whole process is called the induced fit hypothesis of enzyme action.

Figure 2 How enzymes lower activation energy

SUMMARY TEST 3.1

Enzymes act as biological (1). They are (2) proteins that have a specific shape within which there is a functional portion known as the (3). Enzymes lower the (4) of a reaction, allowing it to proceed at a lower temperature than it would normally. In an enzyme-controlled reaction, the general term for the substance on which the enzyme acts is (5) and the substances formed at the end of the reaction are known as the (6). The enzyme molecule and the substance it acts on fit together very precisely, giving rise to the name (7) hypothesis of enzyme action. In practice, the enzyme changes shape slightly and so moulds itself to the shape of the substance it acts on. This is called the (8) hypothesis of enzyme action.
Before considering how pH and temperature affect enzymes, it is worth bearing in mind that, for an enzyme to work, it must:

- come into physical contact with its substrate
- have an active site which fits the substrate.

Almost all factors that influence the rate at which an enzyme works do so by affecting one or both of the above two circumstances. In order to investigate how enzymes are affected by various factors we need to be able to measure the reactions they catalyse.

### Measuring enzyme-catalysed reactions

To measure the progress of an enzyme-catalysed reaction we usually measure its time-course, i.e. how long it takes for a particular event to run its course. The two ‘events’ most frequently measured are:

- the formation of products of the reaction, e.g. the volume of oxygen produced when catalase acts on hydrogen peroxide (Figure 1)
- the disappearance of the substrate, e.g. the reduction in concentration of starch when it is acted upon by amylase (Figure 2).

Although the graphs in Figures 2 and 3 differ, the explanation for their shapes is the same:

- At first there is a lot of substrate (hydrogen peroxide / starch) but no product (water and oxygen / maltose).
- It is very easy for substrate molecules to come into contact with the empty active sites on the enzyme molecules.
- All enzyme active sites are filled and the substrate is rapidly broken down into its products.
- The amount of substrate decreases as it is broken down, resulting in an increase in the amount of product.
- As the reaction proceeds, there is less and less substrate and more and more product.
- The product produced per unit time decreases as there are fewer substrate molecules and so some active sites may not be filled at any one moment.
- The rate of reaction continues to slow as the substrate concentration decreases.
- The graphs flatten out because all the substrate has been used up and so no new product can be produced.

### Effect of temperature on enzymes

A rise in temperature increases the kinetic energy of molecules, which therefore move around more rapidly and collide with one another more often. In an enzyme-catalysed reaction, this means that the enzyme and substrate molecules come together more often in a given time, so that the rate of reaction is increased. Shown on a graph, this gives a rising curve. However, the temperature rise also increases the energy of the atoms that make up the enzyme molecule. Its atoms begin to vibrate and cause bonds to break, with weaker bonds such as hydrogen bonds, breaking first. Gradually, the shape of the active sites is changed. At first, the substrate fits less easily into the active site slowing the rate...
Enzymes of reaction. For many human enzymes this may begin at temperatures of around 45°C. At some point, usually around 60°C, the tertiary structure of the enzyme and shape of the active site is so changed that it stops working altogether. It is said to be **denatured**. Shown on a graph, the rate of this reaction follows a falling curve. The actual effect of temperature on the rate of an enzyme reaction is a combination of these two factors, increased kinetic energy of molecules and denaturation of the enzyme (Figure 4). The optimum working temperature differs from enzyme to enzyme. Some work best at around 10°C, while others continue to work well at 80°C. Each enzyme in the human body has a different optimum working temperature. Our body temperatures have, however, evolved to be 37°C because:

- Although higher body temperatures would increase the metabolic rate slightly, the advantages are offset by the additional energy (food) that would be needed to maintain the higher temperature.
- Proteins, other than enzymes, may be denatured at higher temperatures.
- At higher temperatures, any further rise in temperature, e.g. during illness, might denature the enzymes.

Denaturing enzymes at high temperatures is used to prevent the spoilage (breakdown) by various enzymes found in food materials. This is the basis for heating food before canning or bottling it and for blanching vegetables before freezing.

**Effect of pH on enzymes**

The pH of a solution is a measure of its hydrogen ion concentration. Each enzyme has an optimum pH, i.e. a pH at which it works fastest (Figure 5). This is because the exact arrangement of the active site of an enzyme is partly fixed by hydrogen and ionic bonds between —NH₂ and —COOH groups of the polypeptides that make up the enzyme. Changes in pH can affect this bonding, causing changes of shape in the active site. As a result, the substrate can no longer become attached to the active site and the enzyme–substrate complex cannot be formed. In a similar way to a rise in temperature, this reduces the effectiveness of an enzyme and eventually causes it to stop working altogether, i.e. it becomes denatured. This is why foods can be preserved in vinegar: the low pH denatures the enzymes that would otherwise cause the food to break down. Solutions, known as **buffer solutions**, can be used to prevent fluctuations in pH.

### SUMMARY TEST 3.2

We can measure the progress of an enzyme-catalysed reaction by measuring its (1). This is usually done by measuring either the (2) of the substrate or the formation of the (3). For example, in the case of the enzyme amylase, we could either measure the rate at which (4) is produced or the rate at which (5) is used up. If the temperature is increased, the rate of enzyme action will (6) up to a point at which its molecular structure is disrupted and the shape of its (7) is altered so that the substrate no longer fits it. At this point the enzyme is said to be (8). Many human enzymes have an optimum working temperature of (9). Enzymes also have an optimum pH at which they operate. Some, like pepsin, work fastest at a pH of (10) while others, such as (11), function fastest in neutral conditions.
Effect of enzyme and substrate concentration on enzyme action

In addition to external factors such as temperature and pH, the substrate and enzyme concentrations affect the rate of enzyme-catalysed reactions. An enzyme reaction is always most rapid at first because the enzyme and substrate molecules can freely collide with one another. As the reaction proceeds substrate molecules decrease in concentration and there are fewer successful collisions between enzyme and substrate molecules. The rate of reaction therefore slows.

Effect of enzyme concentration on the rate of reaction

Once an active site on an enzyme has acted on its substrate, it is free to repeat the procedure on another substrate molecule. This means that enzymes are not used up in the reaction and therefore work efficiently at very low concentrations. In some cases, a single enzyme molecule can act on millions of substrate molecules in one minute.

As long as there is an excess of substrate, an increase in the amount of enzyme leads to a proportionate increase in the rate of reaction. A graph of the rate of reaction against enzyme concentration will initially show a proportionate increase (straight line). This is because there is more substrate than the enzyme’s active sites can cope with. If we therefore increase the enzyme concentration, more substrate will be acted upon and the rate of reaction will increase. If, however, the substrate is limiting, i.e. there is not sufficient to supply all the enzyme’s active sites at one time, then any increase in enzyme concentration will have no effect on the rate of reaction. The rate of reaction will therefore stabilise at a constant level, i.e. the graph will level off. This is because the available substrate is already being used as rapidly as it can be by the existing enzyme molecules. These events are summarised in Figure 1.

EXTENSION
Non-protein biological catalysts

It used to be thought that all biological catalysts were enzymes and were therefore made of protein. We now know that some reactions in cells are catalysed by RNA molecules, also known as ribozymes. Some ribozymes work on other RNA molecules, such as those that cut out unwanted sections from messenger RNA. This could answer the ‘chicken or egg’ question – which came first, the enzyme (protein) needed to make nucleic acids, or the nucleic acids, needed to make enzymes? The answer could be RNA which, in this sense at least, is both nucleic acid and ‘enzyme’.

Figure 1 Effect of enzyme concentration on the rate of enzyme action

46
Effects of substrate concentration on the rate of enzyme action

If the concentration of enzyme is fixed at a constant level and substrate concentration is increased, the rate of reaction increases in proportion to the increase in substrate concentration. If a higher concentration of substrate is used the active sites become fully occupied. They are said to be fully saturated at the point where they are all working as fast as they can. The rate of reaction is at its maximum ($V_{\text{max}}$). After that, the addition of more substrate will have no effect on the rate of reaction. In other words, when the substrate is in excess the rate of reaction levels off. A summary of the effect of substrate concentration on the rate of enzyme action is given in Figure 2.

There are too few substrate molecules to occupy all the available active sites in this example. The rate of reaction is therefore only half the maximum possible for the number of enzyme molecules available.

With twice as many substrate molecules available in this example, all the active sites are occupied at one time. The rate of reaction has doubled to its maximum because all the active sites are filled.

A higher concentration of substrate has no effect as all active sites are already occupied at one time. There is no increase in the rate of reaction.

**Figure 2** Effect of substrate concentration on the rate of enzyme action

**Michaelis–Menten constant**

The Michaelis–Menten constant is the substrate concentration needed for an enzyme reaction to proceed at half of its maximum rate (Figure 3). The constant is the same for any one enzyme but varies for different enzymes. It gives a measure of how easily an enzyme reacts with its substrate. In other words, it measures the affinity of an enzyme for its substrate. A high Michaelis–Menten constant shows that an enzyme has a low affinity for its substrate, whereas a low constant shows that it has a high affinity for its substrate. The Michaelis–Menten constant can therefore be used to compare the efficiency of different enzymes for their substrates.

**Summary Test 3.3**

Enzymes work fastest at the start of a process and this is called the (1). As the enzyme concentration increases, the rate of reaction (2), provided there is excess substrate. The graph may later ‘tail off’ if the concentration of substrate is limited because not all the (3) of the enzyme molecules are filled. If the substrate concentration of an enzyme-controlled reaction is halved then the rate of reaction will be (4), but when the substrate is in excess the rate of reaction will (5).

**Figure 3**
Enzyme inhibition

Enzyme inhibitors are substances that directly or indirectly interfere with the functioning of the active site of an enzyme and so reduce its activity. Most inhibitors only make temporary attachments to the active site. These are called reversible inhibitors and are of two types:

- **Competitive** (active site directed) – inhibitor binds to the active site of the enzyme
- **Non-competitive** (non-active site directed) – inhibitor binds to the enzyme at a position other than the active site.

### Competitive (active site directed) inhibitors

Competitive inhibitors have a molecular shape that is complementary to that of the substrate, which allows them to occupy the active site of an enzyme. They therefore compete with the substrate for the available active sites (Figure 1). It is the difference between the concentration of the inhibitor and the concentration of the substrate that determines the effect this has on enzyme activity: if the substrate concentration is increased, the effect of the inhibitor is reduced. The inhibitor is not permanently bound to the active site and so, when it leaves, another molecule can take its place. This could be a substrate or inhibitor molecule, depending on how much of each type is present. Sooner or later, all the substrate molecules will find an active site, but the greater the concentration of inhibitor, the longer this will take. Examples of competitive inhibitors include malonate, which inhibits succinic dehydrogenase in the Krebs cycle (stage of aerobic respiration).

### Non-competitive (non-active site directed) inhibitors

Non-competitive inhibitors attach themselves to the enzyme at a binding site which is not the active site. This is known as the allosteric site (allosteric = ‘at another place’). Upon attaching to the enzyme, the inhibitor alters the shape of the enzyme’s active site in such a way that substrate molecules can no longer occupy it, and so the enzyme cannot function (Figure 3). As the substrate and the inhibitor are not competing for the same site, an increase in substrate concentration does not decrease the effect of the inhibitor (Figure 2).
Enzymes

Substrate molecule occupying the active site of the enzyme

1. Inhibitor absent – The substrate attaches to the active site of the enzyme in the normal way. Reaction takes place as normal.

2. Inhibitor present – The inhibitor prevents the normal enzyme–substrate complex being formed. The reaction rate is reduced.

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**Figure 3 Non-competitive inhibition**

**Immobilising enzymes**

One method of immobilising enzymes is to trap them in small alginate beads. This is carried out in a laboratory as follows.

- The enzyme to be immobilised is mixed with a solution of sodium alginate.
- Tiny droplets of this mixture are added to a solution of calcium chloride, one at a time using a syringe.
- A reaction takes place between the sodium alginate–enzyme mixture and the calcium, causing calcium ions to replace sodium ions.
- As a result of this reaction, jelly-like beads are formed in which the enzyme is trapped.

To catalyse reactions, the beads are packed into a long column in a vessel and the substrate is poured in at the top. As the substrate enters the beads, it is converted to the product by the enzymes immobilised in the beads. Although the process cannot continue indefinitely, as impurities accumulate, it can proceed for a considerable time without renewing the enzyme.

Enzymes are used in a wide range of industrial processes, including the production of foods, agrochemicals and drugs. In many cases the enzyme and substrate are mixed together to form a product. This is then extracted and the rest of the mixture, including the enzyme, is discarded. Enzymes are costly to produce so this is both wasteful and expensive. As enzymes are not used up in reactions, keeping them for future use clearly has a cost benefit. One way of doing this is to immobilise the enzymes so that they can be retained rather than discarded.

An example of the commercial use of immobilised enzymes is in the manufacture of lactose-free milk for people with lactose intolerance. In this case the immobilised enzyme is β-galactosidase. Milk contains the sugar lactose which is the cause of the discomfort experienced by lactose intolerant individuals. To produce lactose-free milk, the milk is passed over the immobilised β-galactosidase which catalyses the hydrolysis of lactose to glucose and galactose. Figure 4 illustrates the process.

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**Advantages of using immobilised enzymes or cells**

There are a number of advantages to immobilising enzymes or cells.

- With enzyme immobilisation, the enzyme can be used repeatedly as it is not lost in the process making it more economic, especially where the enzyme is expensive.
- Enzymes are vulnerable to changes in temperature and pH. The beads in which they are trapped can buffer them against these changes.
- The immobilised enzymes and cells, being held in place, cannot contaminate the substance being made leading to a purer product.
- With whole cell immobilisation, a number of enzymes can act together at the same time in a single process.

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**Figure 4 Using immobilised enzymes to produce lactose-free milk**

Substrate in (e.g. milk containing lactose)

Tap to control flow of substrate into the column

Alginate beads containing immobilised enzyme e.g. β-galactosidase

Long column containing the beads

Tap to control flow of substrate out of the column

Product out (e.g. milk with glucose and galactose, but no lactose)
3 Examination Questions

1 The enzyme, catechol oxidase, causes a brown colour to develop when slices of many fruits, such as apples, are exposed to air.

The enzyme catalyses the following reaction:

\[
\text{catechol} + \text{oxygen} \xrightarrow{\text{catechol oxidase}} \text{quinone} + \text{water}
\]

Quinone is then immediately further oxidized in air to a brown-coloured substance. Catechol and quinone are colourless.

A student investigated the rate of this reaction under different conditions.

a State how the student could follow the progress of this reaction.

(1 mark)

In the first investigation, the student measured the initial rate of the reaction in varying concentrations of catechol. The results are shown in Figure 1.

b Explain the results shown in Figure 1.

(5 marks)

c In a second investigation, the student repeated the experiment, but this time added a competitive inhibitor, para-hydroxybenzoic acid (PHBA), to each reaction mixture.

i Sketch on Figure 1 the results that would be obtained for this second investigation.

(2 marks)

d Lemon juice contains citric acid. Adding even a small amount of diluted lemon juice to apple slices slows the appearance of the brown colour.

Suggest an explanation for this observation.

(2 marks)

(Total 12 marks)

2 Sucrase is the enzyme that catalyses the hydrolysis of sucrose. A student investigated the effect of substrate concentration on the activity of this enzyme.

Six test-tubes were set up each containing 10 cm³ of different concentrations of sucrose solutions. The test-tubes were left in a water bath at 30 °C for ten minutes.

After ten minutes, 5 cm³ of a sucrase solution at 30 °C was added to each test-tube and the reaction mixtures were stirred.

After a further five minutes, the temperature of the water bath was raised to above 85 °C and the same volume of Benedict’s solution added to each test-tube in turn. The student recorded the time when a green colour first became visible in each test-tube.

The concentrations used and the student’s results are shown in Table 2.

<table>
<thead>
<tr>
<th>concentration of sucrose/g dm⁻³</th>
<th>time taken for green colour to appear/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>278</td>
</tr>
<tr>
<td>10</td>
<td>145</td>
</tr>
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<td>15</td>
<td>95</td>
</tr>
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<td>50</td>
<td>47</td>
</tr>
<tr>
<td>100</td>
<td>45</td>
</tr>
</tbody>
</table>

a Explain why the temperature of the water bath was raised to above 85 °C.

(2 marks)

b Copy the axes and sketch a graph to show the effect of substrate concentration on the rate of hydrolysis of sucrose by sucrase.

(2 marks)

c With reference to the student’s results, describe and explain the effect of increasing substrate concentration on the rate of hydrolysis of sucrose by sucrase.

(5 marks)

(Total 9 marks)
3 Practice Questions

Enzymes can function at a wide-range of temperatures. Shrimps that live in Arctic waters have enzymes that function best around 4 °C and are denatured at around 15 °C. By contrast, bacteria that live in hot springs have enzymes that function best at 95 °C and continue to operate effectively above 100 °C. These bacteria are called thermophilic (heat-loving) bacteria.

Enzyme X is produced by thermophilic bacteria and hydrolyses many proteins, including haemoglobin and egg albumin.

Enzyme Y is found in the stomach of young mammals, where it acts on a single soluble protein found in milk, causing it to coagulate (clot).

3 a i From the descriptions, comment on the differences in the specificity of the two enzymes.
   ii Enzymes X and Y are each used for different commercial purposes. Suggest what this might be in each case.
   iii Suggest a possible purpose of enzyme Y in the mammalian stomach.
   iv Use the information about the two enzymes to suggest a possible difference in the type of bonding found in the tertiary structure of each. Explain your reasoning.

b An experiment was carried out with enzyme X in which the time taken for it to fully hydolyse 5 g of its protein substrate was measured at different temperatures. The following data were obtained:

<table>
<thead>
<tr>
<th>Temperature/°C</th>
<th>Time for hydrolysis of protein/min</th>
<th>Rate of reaction 1 time</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>5.8</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>35</td>
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</tr>
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<td>65</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>7.1</td>
<td></td>
</tr>
</tbody>
</table>

i Calculate the values for 1/time for each of the temperatures.
ii Plot a graph that shows the effect of temperature on the rate of reaction of enzyme X.
iii Measure the optimum temperature for the action of enzyme X.
iv Suggest how you might determine this optimum temperature more precisely.

4 a Explain why enzymes function less well at lower temperatures.
   b Explain how high temperatures may prevent enzymes from functioning at all.
   c Enzymes produced by microorganisms are responsible for spoiling food. Using this fact and your knowledge of enzymes suggest a reason in each case why the following procedures are carried out:
   i Food is heated to a high temperature before being canned.
   ii Some foods, such as onions, are preserved in vinegar.

5 The figure below represents an enzyme and its substrate. Of the other four molecules shown, one is a competitive inhibitor and one is a non-competitive inhibitor of the enzyme.

   a What is the number of the molecule that is a competitive inhibitor?
   b What is the number of the molecule that is a non-competitive inhibitor?