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Second edition

CJ Clegg

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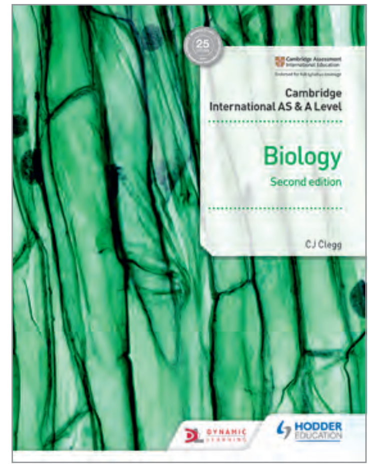

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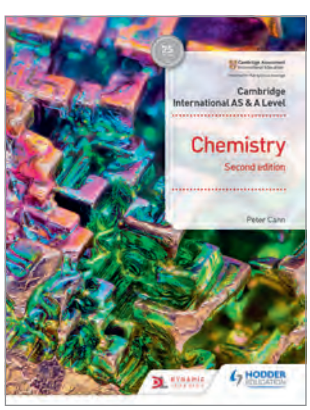

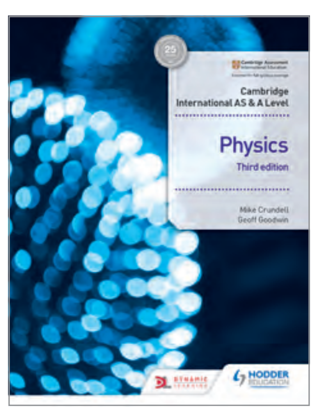

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3 Enzymes

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.

Learning Outcomes

By the end of this topic, you should be able to:

3.1.1 state that enzymes are globular proteins that catalyse reactions inside cells (intracellular enzymes) or are secreted to catalyse reactions outside cells (extracellular enzymes)

3.1.2 explain the mode of action of enzymes in terms of an active site, enzyme-substrate complex, lowering of activation energy and enzyme specificity, including the lock-and-key hypothesis and the induced-fit hypothesis

3.1.3 investigate the progress of enzyme-catalysed reactions by measuring rates of formation of products using catalase and rates of disappearance of substrate using amylase

3.1 Mode of action of enzymes

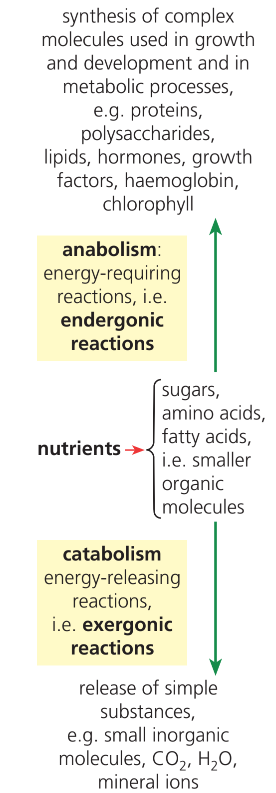
There are many different enzymes, each one specific to a particular reaction. This specificity is the key to understanding the efficient functioning of cells and living organisms.

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, photosynthesis, protein synthesis and 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).



▲ **Figure 3.1 Metabolism: an overview**

Enzymes as globular proteins

In Topic 2 we saw that the tertiary structure of **globular proteins** is 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 contrasts 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 (Figure 3.2). If the angle of collision is not correct, the molecules bounce apart. Alternatively, if the speed of the collision 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 does not 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, 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 Chapter 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.

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

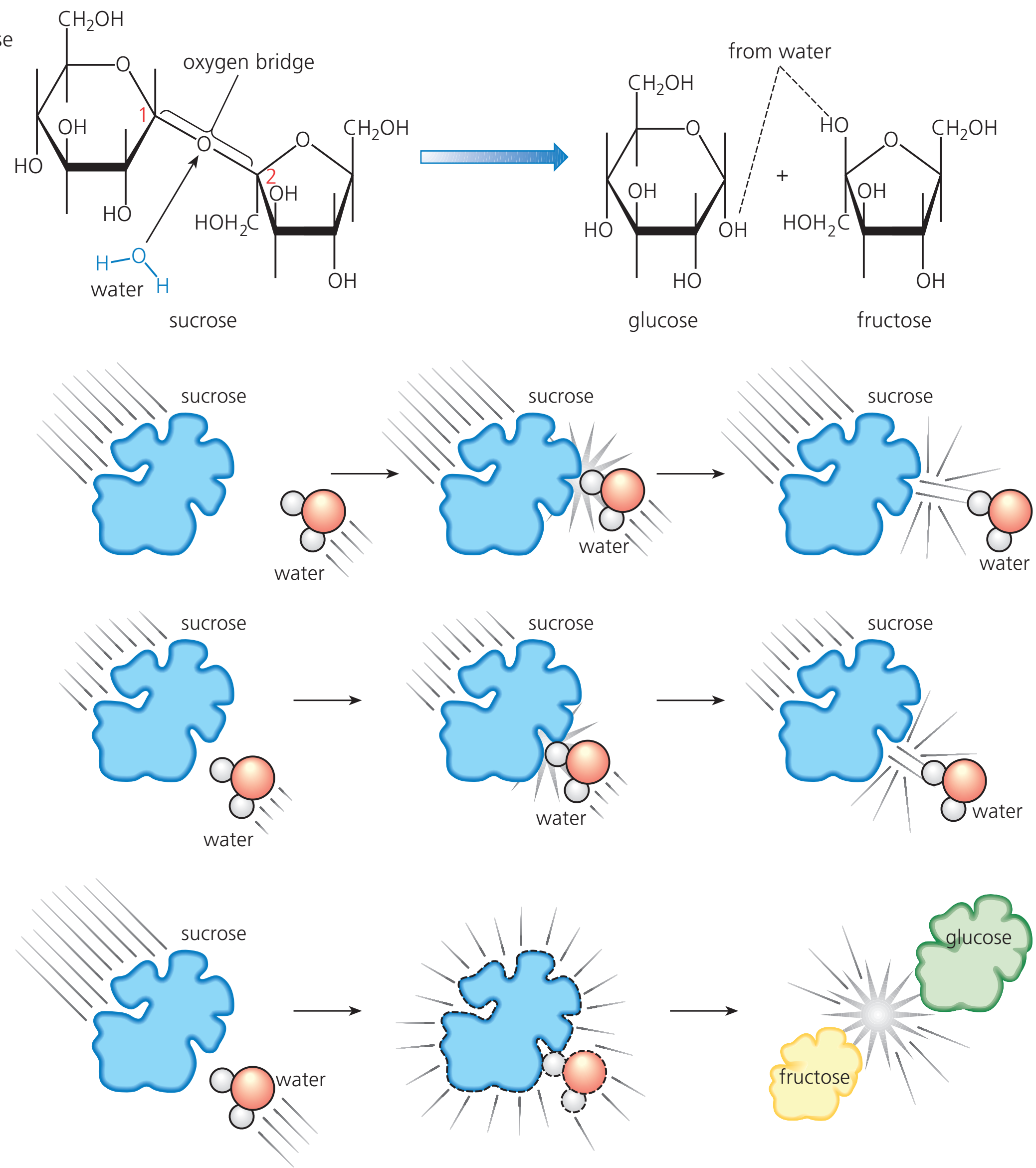
2 Random collision possibilities: when sucrose and water collide at the wrong angle

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 infrequently that it is an insignificant event



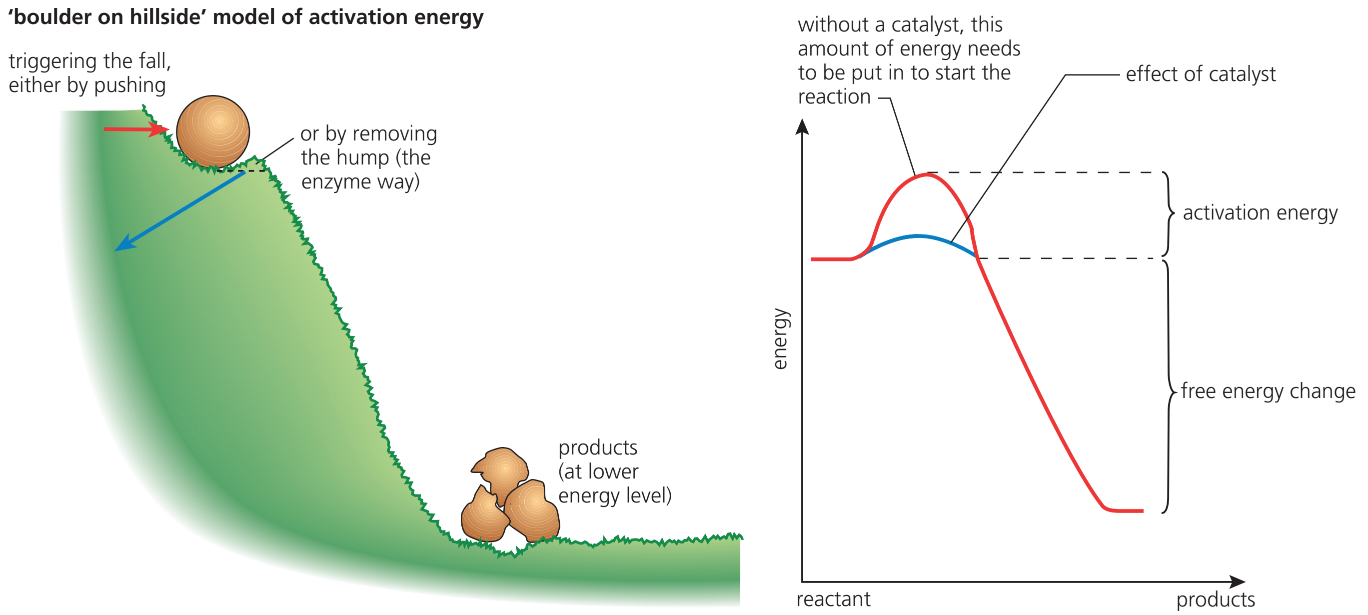
3 In the presence of one molecule of the enzyme sucrase (invertase), approximately 3.0×10^4 molecules of sucrose are hydrolysed each minute!

▲ **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** (Figure 3.3**)**. 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).



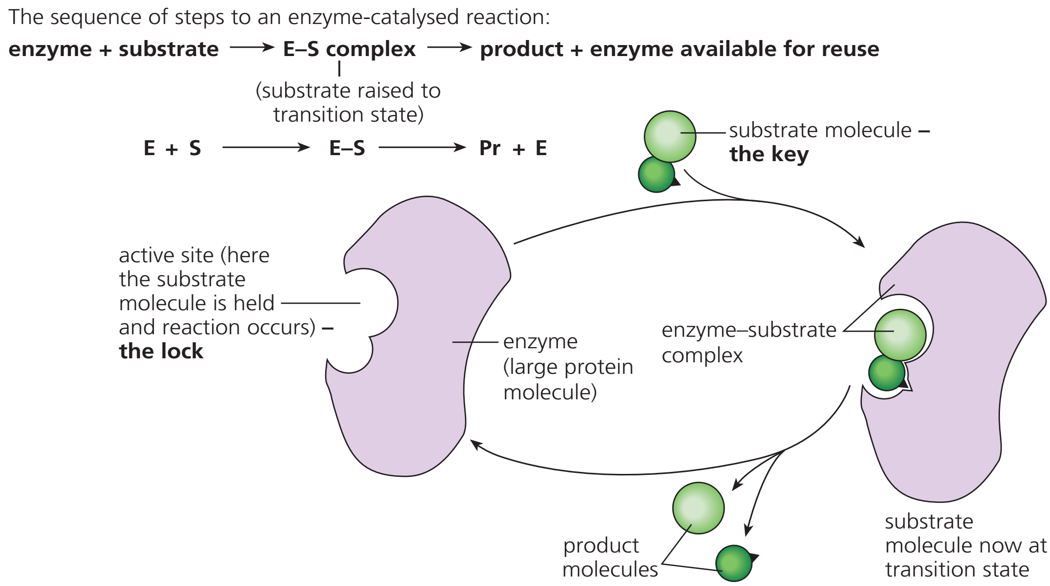
▲ Figure 3.3 Activation energy

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 to (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 (Figure 3.4).

$$E + S \rightarrow E - S \rightarrow Pr + E$$



▲ **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

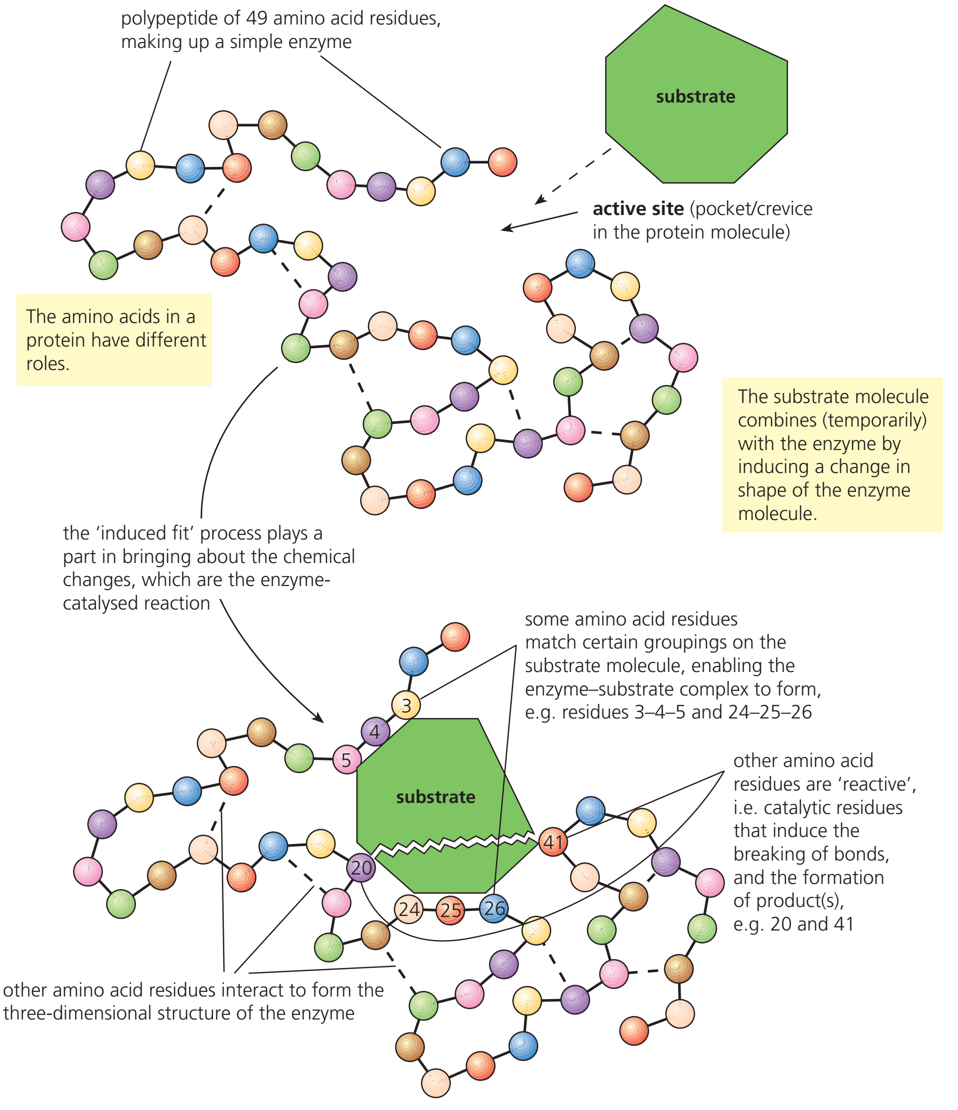
1 Explain why the shape of globular proteins that are enzymes is important in enzyme action.

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 (Figures 3.5 and 3.6).

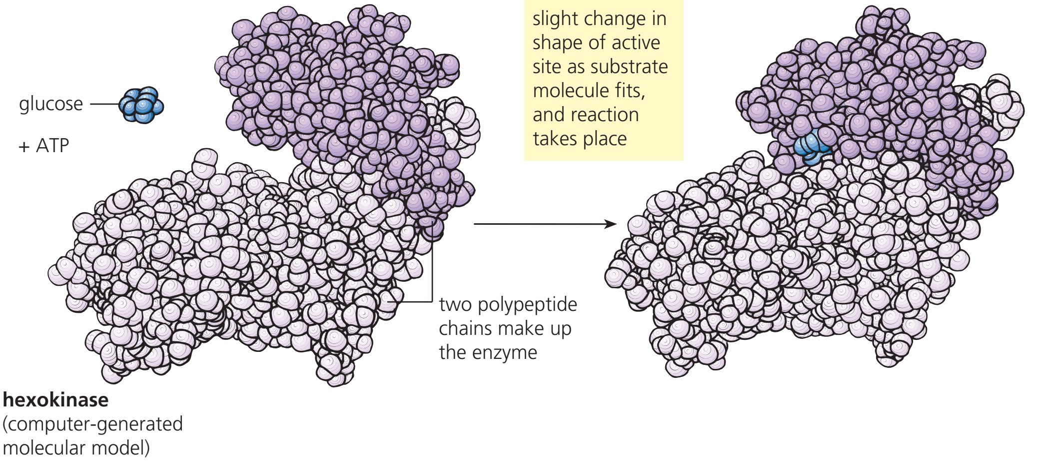
With a transitional state achieved, other amino acid molecules of the active site bring about the breaking of particular bonds in the substrate molecule, at the point where it is temporarily held by the enzyme. It is because different enzymes have different arrangements of amino acids in their active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



Specificity:

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

▲ **Figure 3.5 The induced fit hypothesis of enzyme action**



▲ **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 name are long and detailed and 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.

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 it catalyses.
- 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.

Examination practice 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 10 minutes. A flask containing a sucrase solution was also put into the water bath.

After 10 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 10 minutes.

After 10 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.

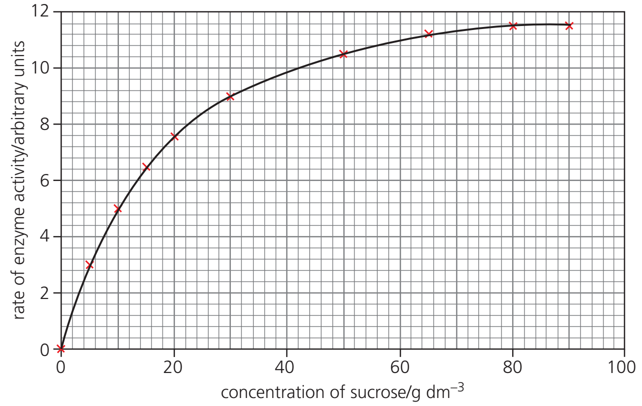
The results are shown in the graph on the next page.

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 the graph. [5]

[Total: 8]



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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.

$$\text{starch + phosphate ions} \xrightarrow{\text{starch phosphorylase}} \text{glucose-1-phosphate}$$

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 10 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]**

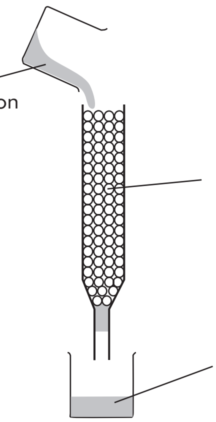
c Explain the results shown in the table below. **[4]**

[Total: 11]

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

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

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**			
	$0.2\,g\,dm^{-3}$		**$0.4\,g\,dm^{-3}$**		**$0.2\,g\,dm^{-3}$**		**$0.4\,g\,dm^{-3}$**	
	Substrate concentration/$g\,dm^{-3}$				**Product concentration/$g\,dm^{-3}$**			
	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

b Identify **two** variables and explain how each might be controlled. **[2]**

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

d 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]

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