Enzymes

Unit 3

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3.3 Factors affecting the functions of enzymes (page 93)	• Explain factors that affect enzyme activity. • Investigate the destruction of an enzyme by heat. • Show how temperature, pH, substrate concentration and enzyme concentration affect enzyme activity. • Explain allosteric regulation and feedback control mechanism of enzyme activity. • Appreciate the role of enzymes in controlling our metabolic activities.

KEY WORDS

active site *the part of an enzyme molecule that binds with its substrate so that the enzyme can catalyse the chemical reaction*

substrate *a substance upon which an enzyme acts in a biochemical reaction*

enzyme–substrate complex *the intermediate formed, temporarily, when an enzyme binds to its substrate*

3.1 Nature of enzymes

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

- Define enzymes and explain the properties of enzymes.
- Explain how enzymes are named and then classify them according to their structure.
- Conduct an experiment to show the specificity of an enzyme.
- Appreciate the importance of enzymes in industries and local products.

What are enzyme molecules like?

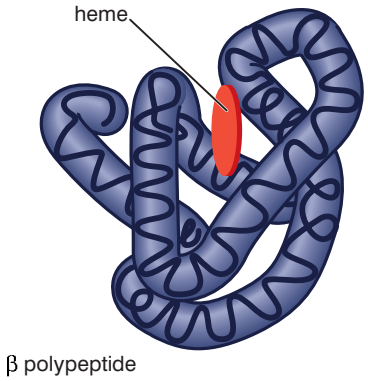


Figure 3.1 *The human lipase enzyme*

First, all enzymes are globular proteins. We learned in unit 2 that globular proteins all have a unique tertiary structure, which gives them a unique shape. Figure 3.1 shows a model of the tertiary structure of the human lipase enzyme that hydrolyses lipids into fatty acids and glycerol.

You should be able to identify regions where there is:

- an α-helix
- a β-pleated sheet
- no folding into a secondary structure.

Second, within that very complex structure is a region called the **active site**. This is the part of the enzyme molecule that binds with its **substrate** so that the enzyme can catalyse the chemical reaction. The active site of an enzyme is shaped to allow:

- binding with a particular substrate and that substrate only, and
- binding in such a way that the reaction can take place requiring less energy than if the enzyme was not present.

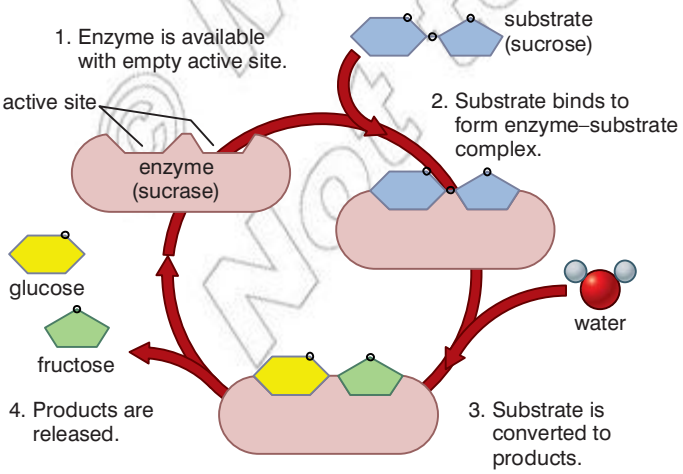


Figure 3.2 *The hydrolysis of sucrose by sucrase*

We can use the example of the enzyme sucrase catalysing the hydrolysis of sucrose into glucose and fructose to illustrate this. The substrate for the enzyme is the molecule of sucrose. This binds with the active site to form an **enzyme–substrate complex**.

A molecule of water then reacts with the sucrose to hydrolyse the molecule into glucose and fructose. These are then released from the active site, which can then accept another molecule of sucrose. It is important to note that the enzyme is unaltered by the reaction.

This is shown in figure 3.2.

We are now in a position where we can define more precisely what we mean when we are talking about an enzyme:

An enzyme is a globular protein with a uniquely shaped active site; it acts as a biological catalyst for a specific reaction, but remains unaltered by the reaction.

KEY WORD

catalyst *a substance that speeds up a chemical reaction and remains unchanged at the end of the reaction*

What are the properties of enzymes?

- They are all proteins.
- They are biological catalysts: they speed up a reaction without being used up, so they can be used over and over again.
- They are specific: they catalyse one reaction only.
- A small amount of enzyme can bring about a change in a large amount of its substrate.
- Enzymes are affected by pH and temperature. They can be destroyed by excessive heat. They are also affected by the concentration of their substrate and the presence of certain substances that act as inhibitors.

What are catalysts?

A **catalyst** is a substance that speeds up a reaction; the reaction itself is unaltered. There is no overall change to:

- the nature of the products
- the energy change that takes place during the reaction
- the catalyst itself

Enzymes allow biochemical reactions inside cells to take place quickly, at a temperature that will not damage the structure of the cell.

DID YOU KNOW?

Not all biological catalysts are proteins. Recently, it has been shown that some RNA molecules can catalyse some biological reactions.

Why are enzymes specific?

This is also a function of the active site. Because of the conformation of the active site (the way in which it is shaped), only a certain substrate or combination of substrates can bind with it.

Because only one substrate (or substrate combination) can bind, there is only one possible reaction that can be catalysed. This is illustrated in figure 3.3.

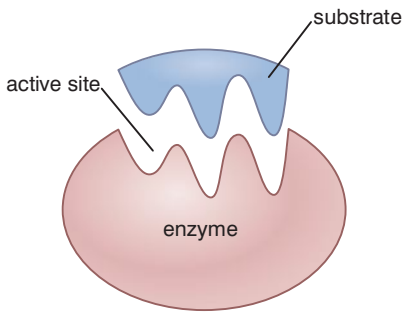


Figure 3.3 *An enzyme can only bind with one substrate because of the shape of its active site.*

How are enzymes affected by pH and by temperature?

Temperature affects enzyme action in two ways:

- a higher temperature gives the enzyme molecules (and their substrate molecules) more kinetic energy; they move around faster and form more enzyme–substrate complexes
- a higher temperature affects the chemical bonds holding the tertiary structure of the enzyme in place (particularly those in the active site); as more and more of these bonds break, the shape of the active site changes and it can no longer bind with its substrate

Activity 3.1

Make a poster which lists the four criteria for naming enzymes. Include a chart or key showing how you would classify the main types of enzymes (you will need to look at p83 before you can complete this activity).

pH affects the enzyme molecule in a similar way to high temperatures. A pH that is too low (too acid) or too high (too alkaline) will cause charges on the active site to alter and cause the active site to lose its conformation. The substrate cannot bind and so the reaction is no longer catalysed.

How do we name and classify enzymes?

Common or working enzyme nomenclature (naming of enzymes)

Table 3.1 gives some examples of enzymes and the reactions they catalyse.

Table 3.1 *Examples of enzymes and the reactions they catalyse*

Name of enzyme	Reaction catalysed
Lipase	Hydrolysis of lipids
ATPase	Hydrolysis of ATP
Succinate dehydrogenase	Removal of hydrogen ions from succinate (during respiration)
DNA polymerase	Joining of nucleotides to form DNA
Pepsin	Digestion of proteins in the stomachs of mammals

Different enzymes are named in different ways.

- Most commonly enzymes are named by adding 'ase' to part of the name of the substrate. For example, *lipase* (lipid hydrolysing enzyme), *sucrase* (sucrose hydrolysing enzyme).
- Sometimes the enzymes are named on the basis of the reaction that they catalyse. For example, *polymerase* (aids in polymerisation – joining similar units together), *dehydrogenase* (removal of hydrogen atoms or ions).
- Some enzymes have been named based on the source from which they were first identified. For example, *papayin* from papaya. Others are named according to where they act. For example, *intestinal* protease acts on proteins in the intestine.
- The names of some enzymes end with 'in', indicating that they are basically proteins. For example, *pepsin*, *trypsin*, etc. These enzymes usually have alternative names that tell you rather more about them. For example, the alternative name for pepsin is gastric protease. This tells you that it acts on proteins and it does so in the stomach.

Because of the varied ways in which enzymes had been named, biologists at the **Enzyme Commission** decided to produce a systematic way of naming enzymes, based on the ways in which the enzymes act. To appreciate this, we must first look at how enzymes are classified.

KEY WORD

Enzyme Commission *body set up to produce a systematic way of naming enzymes*

Enzyme classification and the systematic nomenclature of enzymes

Enzymes are generally classified on the basis of the type of reactions that they catalyse. Six groups of enzymes can be recognised on this basis. Table 3.2 lists these groups along with examples.

***Table 3.2** Classification of enzymes*

Class	Reaction catalysed	Examples
1. Oxidoreductases	Transfer of hydrogen and oxygen atoms or electrons from one substrate to another	Dehydrogenases Oxidases
2. Transferases	Transfer of a specific group (a phosphate or methyl, etc.) from one substrate to another	Transaminase Kinases
3. Hydrolases	Hydrolysis of a substrate	Esterases Digestive enzymes
4. Isomerases	Change of the molecular form of the substrate	Phosphohexoisomerase Fumerase
5. Lyases	Nonhydrolytic removal of a group or addition of a group to a substrate	Decarboxylases Aldolases
6. Ligases (Synthetases)	Joining of two molecules by the formation of new bonds	Citric acid synthetase

Each class of enzymes contains several different, but related, subclasses. Each subclass is further divided into sub-subclasses. Within the sub-subclasses, each enzyme has a number.

So, in the systematic naming of enzymes, an enzyme will have a 'name' such as EC 3.4.11.1. Each part of the description tells us something about the enzyme:

- EC stands for Enzyme Commission
- the first number shows to which of the six main classes the enzyme belongs
- the second figure indicates a subclass
- the third figure gives a sub-subclass
- the fourth figure is the serial number of the enzyme in its sub-subclass.

Enzyme EC 3.4.11.1 is:

- a hydrolase – all the enzymes in class 3 hydrolyse some kind of bond
- a peptidase – all the enzymes in subclass 4 of class 3 are peptidases and hydrolyse peptide bonds
- an amino-peptidase – all the enzymes in sub-subclass 11 of sub-class 4 are amino-peptidases; they hydrolyse peptide bonds at the amino end of a polypeptide chain
- leucyl-amino-peptidase – this particular amino-peptidase is number 1 of this sub-subclass

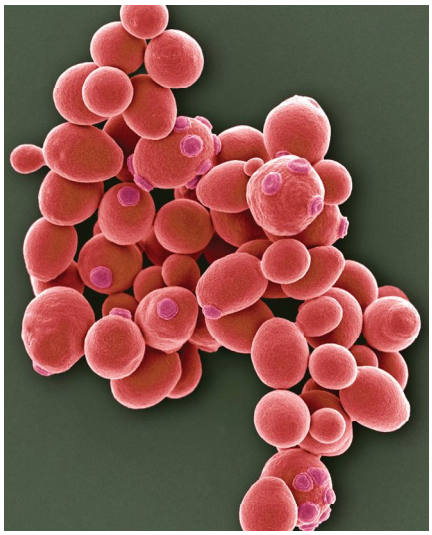


Figure 3.4 *Yeast cells*

Activity 3.2: Library search

If you have access to the internet, you could visit site: www.chem.qmul.ac.uk/iubmb/enzyme/ and find out more about the naming of enzymes. What is enzyme 1.1.1.1?

What do enzymes do for you?

We have been using enzymes for thousands of years – although the people who used them then didn't know quite what they were using! Unknowingly, they used enzymes (in yeast) to make bread and beer. These are almost certainly the first uses of 'enzyme technology'.

Yeast is a unicellular fungus that ferments carbohydrates to produce carbon dioxide and alcohol. The enzymes in yeast control the reactions of fermentation. Figure 3.4 shows some yeast cells.

DID YOU KNOW?

How long people have been brewing beer?

The oldest proven records of brewing are about 6000 years old in the ancient country of Sumeria, in the Middle East. A document 4000 years old is a Sumerian 'Hymn to Ninkasi', who was the *goddess* of brewing! The 'hymn' is also a recipe for making beer.

Of course, these people did not know they were using enzymes. They did not know at first that they were using yeast! But as time progressed people found that it was the yeast that fermented carbohydrates into alcohol. Now we know that several enzymes are involved in the brewing process and can control it much more efficiently.

Figure 3.5 *An ancient Egyptian tomb model showing a woman brewing beer*

Figure 3.6 *Injera*

We still use yeast to brew alcoholic drinks, such as tella, and to bake breads, such as injera. Both these Ethiopian products are often made at home, as well as professionally.

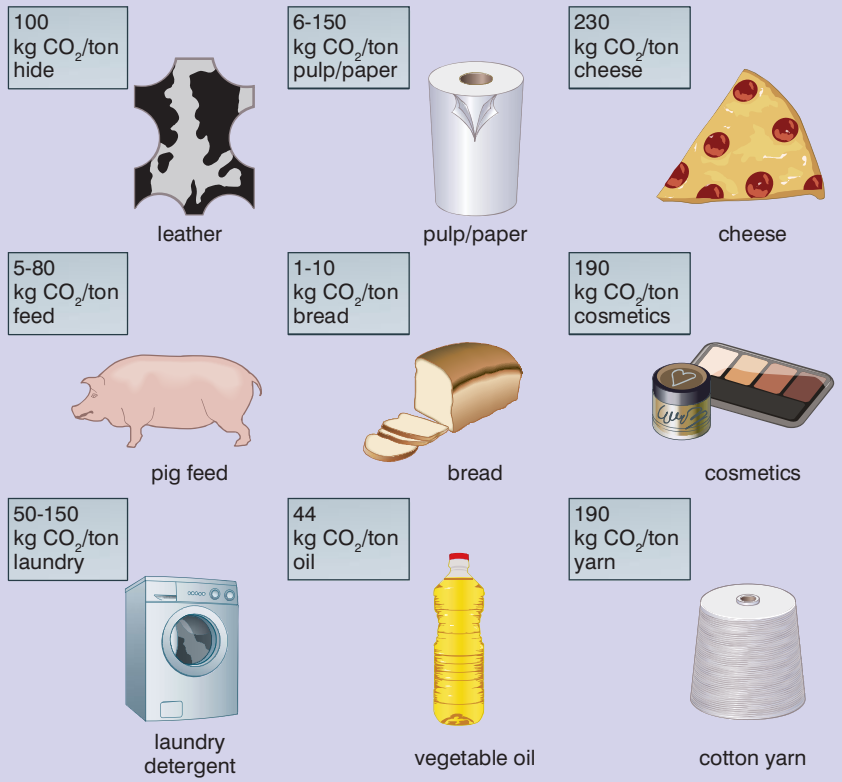
When dough is baked to produce the bread, the tiny amount of alcohol formed is lost and the carbon dioxide expands to make the dough 'rise' to form a loaf of bread. When beer is brewed, it is the carbon dioxide that is lost and the alcohol remains!

However, enzyme technology is now very big business. Enzymes are used in many industries. They are used to produce washing powders – the enzymes in the washing powders digest the stains in the clothes. 'Stone-washed' denim jeans are now given their stone-washed look by the action of enzymes. Table 3.3 shows just a few of the areas where enzyme technology is used.

Table 3.3 *Some industrial uses of enzyme technology*

Sector	Application area	Benefits
Dairy	• Biochymosin to produce cheese • Lactase to produce lactose-free milk	• Supplies of natural rennet from calves livers are limited • Lactose-intolerant people suffer fewer cramps
Detergents	• Use of proteases, lipases and amylases in biological washing powders • Use of proteases and amylases in dishwasher detergents	• Many biological stains are removed efficiently at low temperatures (saving energy) • Remove food particles at lower temperatures and require fewer bleaching products to be added
Textiles	• Proteases to remove hair and lipases to degrease animal hides • Use of cellulase to 'bio-polish' cotton fabrics • Use of cellulase to 'bio-stone' denim	• Process is carried out much quicker than by traditional methods • Produces a smoother and glossier finish • The enzyme gives the 'stone-washed' effect much more easily
Food processing	• Pectinase to process fruit juice • Inverase to produce liquid-centre chocolates	• Clarifies fruit juice • Sucrose paste in the chocolate is made liquid by injection of the enzyme
Pulp and paper	• Amylases used in starch conversion • Use of xylanase enzymes in pre-bleaching the pulp • Use of esterases in control of 'stickies' (glues introduced during paper recycling)	• Reduces the quantity of starch in the paper and improves quality • Produces a whiter paper • Stickies would otherwise clog the machinery and reduce the quality of the paper
Medicine	• Glucose oxidase in clinistix strips, tests for glucose • Liver enzymes • Pulmozyme to treat cystic fibrosis	• Allows easy diagnosis of diabetes by testing urine • Testing for high levels of these in the blood confirms liver damage • Reduces viscosity (stickiness) of mucus
Pharma-ceutical	• Streptokinase to dissolve clots of heart-attack patients • Production of abacavir sulphate is controlled by enzymes	• Restores blood supply to area of heart muscle • Abacavir sulphate is an important anti-AIDS drug

One of the appeals of using enzymes in industry is that they allow the reactions involved in the processes to be carried out at much lower temperatures. This means less energy (and therefore less money) is spent on heating the reactants. Because less heating is required, less carbon dioxide is produced and this can benefit the environment as carbon dioxide is a greenhouse gas and its accumulation in the atmosphere can lead to global warming.



DID YOU KNOW?

Using enzymes in industry can help the environment. Because enzymes allow some manufacturing processes to be carried out at lower temperatures, less carbon dioxide is produced in raising the temperature of the reactants. Figure 3.7 shows the mass of carbon dioxide emissions saved in some processes that now use isolated enzymes rather than the traditional method.

Figure 3.7 *Carbon dioxide emission reductions in some industrial processes that now use enzymes*

Activity 3.3: Discussion

The importance of enzymes in local manufacturing

As you have seen from the material presented in this book, some enzymes have been used for many hundreds of years in manufacturing processes and the number being used is increasing all the time. In this activity, you will discuss the importance of enzymes in these local processes. You might bear in mind:

- the importance of enzymes as catalysts in the processes
- whether or not there are other options to using enzymes in the processes that might be more cost-effective

The activity will follow the following procedure:

Your teacher will describe some of the uses of enzymes in the manufacture of products in your locality.

Your teacher will then ask you for your opinions as to how crucial you think the role of enzymes is in these processes. You may then make your point of view but, during this stage, it is important that:

- you do not interrupt anyone else; they also have the right to put their point of view
- you only put your point of view when your teacher allows you to – the discussion cannot degenerate into a row!

At the end of the discussion, your teacher will summarise the views of the class.

You will write a summary of the main views held by different people in the group.

Review questions

Choose the correct answer from A to D.

1. All enzymes are:
   - A globular proteins that digest large molecules
   - B globular proteins that catalyse reactions
   - C fibrous proteins that catalyse reactions
   - D fibrous proteins that digest large molecules
2. Enzymes are specific because:
   - A they are globular proteins
   - B they are affected by temperature and pH
   - C their tertiary structure gives them a uniquely shaped active site
   - D they may have cofactors
3. The shape of an enzyme's active site and its substrate are:
   - A complementary
   - B the same
   - C similar
   - D related
4. Catalysts:
   - A speed up a reaction and are used up in the process
   - B slow down a reaction and are used up in the process
   - C slow down a reaction and are not used up in the process
   - D speed up a reaction and are not used up in the process
5. The name of an enzyme often ends in:
   - A -ase
   - B -ese
   - C -ise
   - D -ose
6. Some enzymes' names are derived from their substrate. An example of this is:
   - A pepsin
   - B papayin
   - C nuclease
   - D amylase
7. How many classes of enzymes are there in the Enzyme Commission classification?
   - A 3
   - B 4
   - C 5
   - D 6
8. Which of the following is NOT a reason why enzymes are often used in industrial processes?
   - A they allow reactions to be carried out at lower temperatures
   - B they reduce heating costs
   - C more energy is used and so more carbon dioxide is produced during the process
   - D less energy is used and so less carbon dioxide is produced during the processes
9. An environmental benefit of using enzymes in industrial processes is that it can:
   - A reduce use of paper in packaging the product
   - B reduce carbon dioxide emissions
   - C increase purity of the product
   - D reduce the costs involved
10. One advantage of the Enzyme Commission systematic naming of enzymes is:
    - A all enzymes are 'named' in the same way
    - B biologists from all countries can understand the 'name' equally
    - C no local knowledge is necessary to understand the name
    - D all of the above

3.2 Functions of enzymes

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

- Explain how enzymes lower activation energy.
- Explain the mechanism of enzyme action.
- Discuss the action of apo- and coenzymes.
- Give examples of vitamins and minerals in food that act as cofactors.

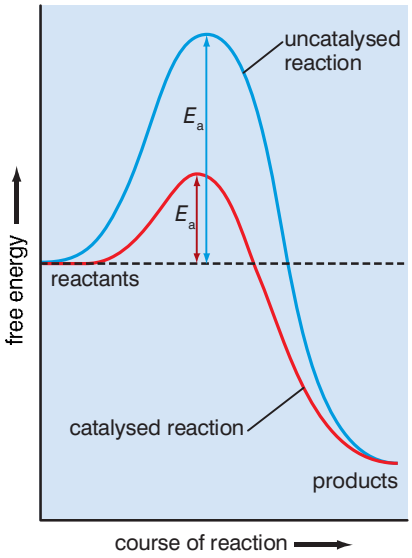


Figure 3.8 *Activation energy for an uncatalysed reaction and the same reaction with a catalyst*

How do enzymes act as catalysts?

Catalysts speed up chemical reactions. In order for molecules to react, they must have sufficient energy. This energy to start off the reaction is called **activation energy** (or E_a). Imagine a reaction in which substance A reacts with substance B to form substance AB. We can write an equation for this as: A + B → AB

However, this does not tell the whole story. The equation gives only the reactants (starting materials) and the products. It does not show how the energy changes as the reaction takes place.

The reactant must 'climb an activation energy hill' before anything happens. Under normal conditions, very few molecules of A and B have sufficient kinetic energy to 'climb the activation energy hill', so the reaction proceeds slowly. A catalyst lowers the activation energy required for the reaction. More reactant molecules can meet this lower energy requirement and so the reaction proceeds more quickly. Because the enzyme molecule is unaltered by the reaction, it can be used over and over, and so a small amount of enzyme can affect a large amount of substrate. This is shown in figure 3.8.

How do enzymes lower activation energy?

There are two models of enzyme action; the **lock-and-key model**, first proposed in 1894 by a German biochemist named Fischer and the **induced-fit model**, proposed in 1958 by Koshland. Both of these models suggest that the enzyme catalyses the reaction by lowering the activation energy. However, they differ in the way that they explain how this happens. In particular, they differ in explaining how the substrate binds to the active site of the enzyme.

The lock-and-key model

This model proposes that the shapes of the substrate molecules are *complementary* to that of the active site, rather like the shape of a key is complementary to that of the lock it fits. A useful way of thinking of complementary shapes is to think of an egg sitting in an egg cup. The egg can sit inside the egg cup because the shapes are complementary. One egg cannot sit inside another egg because the shapes are the same.

KEY WORDS

activation energy *the energy required to start off a chemical reaction*

lock-and-key model *proposes that the shapes of the substrate molecules are complementary to that of the active site*

induced-fit model *the active site and substrate do not complement each other but the binding of substrate molecules produces a change in shape in the active site, allowing the substrate to fit the active site*

The complementary substrate molecule binds with the active site of the enzyme to form the enzyme–substrate complex. The complex causes the reactants to enter a transition state in which the activation energy of the reaction is lowered. The reaction takes place and the products formed are released. The lock-and-key model of enzyme action suggests that the enzyme lowers the activation energy by providing an alternative pathway for the reaction.

For example:

Non-catalysed pathway:
Reactant A + Reactant B ➞ Product AB

Enzyme-catalysed pathway:
Reactant A + Reactant B + enzyme ➞ Intermediate ➞ Product AB + enzyme

This model sees the enzyme–substrate complex as the intermediate, which is part of a pathway that requires less energy than the normal pathway. However, a weakness of this model is that it does not explain how the intermediate reduces activation energy.

Figure 3.9 *Complementary shapes*

The induced-fit model

This model suggests that the active site and the substrate aren't naturally complementary in shape, but the binding of substrate molecules produces a conformational change (change in shape) in the active site. This allows the substrate and active site to bind fully. The conformational change also puts the substrate molecules under tension, so they enter a 'transition state' and are able to react because of the lowered activation energy. In the transition state, bonds in the reactants are put under strain and break more easily and rejoin with other bonds to form the products. The products formed leave the active site. This is shown in figure 3.11.

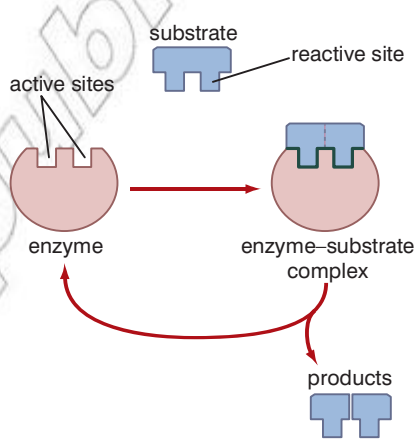


Figure 3.10 *The lock-and-key model of enzyme action*

Most biologists now prefer the induced-fit model over the lock-and-key model as it explains other properties of enzymes, such as enzyme inhibition, in a more complete manner than the lock-and-key model.

The rate of a chemical reaction is the rate at which reactants are converted into products. In the case of an enzyme-controlled reaction, this is determined by how many molecules of substrate bind with enzyme molecules to form enzyme–substrate complexes. The number of molecules of reactants that form enzyme–substrate complexes with each molecule of an enzyme, per second, is the **turnover rate** of the enzyme.

KEY WORD

turnover rate *the number of molecules of reactants that form enzyme–substrate complexes with each molecule of an enzyme, per second*

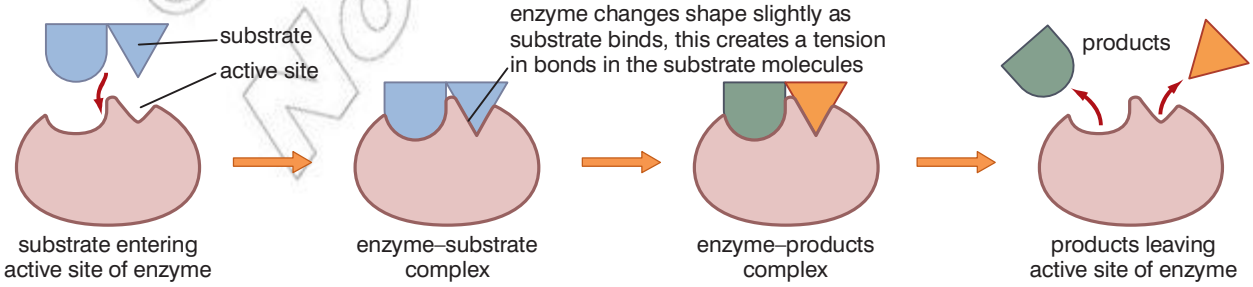


Figure 3.11 *The induced-fit model of enzyme action*

DID YOU KNOW?

Just how much faster enzyme-catalysed reactions proceed

Table 3.4 shows how much faster reactions proceed with the enzymes than without the enzymes.

Table 3.4 The rate enhancement of some enzymes

Enzyme	Rate enhancement
OMP decarboxylase	1.4×10^{17}
Staphylococcal nuclease	5.6×10^{14}
Adenoisine deaminase	2.1×10^{12}
AMP nucleosidase	6.0×10^{12}
Cytidine deaminase	1.2×10^{12}
Phosphotriesterase	2.8×10^{11}
Carboxypeptidase A	1.9×10^{17}
Ketosteroid isomerase	3.9×10^{17}
Triosephosphate isomerase	1.0×10^{9}
Chorismate mutase	1.9×10^{6}
Carbonic anhydrase	7.7×10^{6}
Cyclophilin, human	4.6×10^{5}

Why do some enzymes need cofactors?

Sometimes an active enzyme isn't just a single molecule, but is made from two molecules, neither of which has enzymic activity without the other. The two parts are the apoenzyme and the cofactor. We can define these in the following way:

Apoenzyme – a protein that combines with a cofactor, to form an active enzyme. The protein is inactive on its own.

Cofactor – a small non-protein particle essential for the activity of some enzymes. The cofactor combines with the apoenzyme to produce an active enzyme.

Where an active enzyme molecule comprises an apoenzyme and a cofactor, the whole is sometimes referred to as the **holoenzyme**. Figure 3.12 shows this.

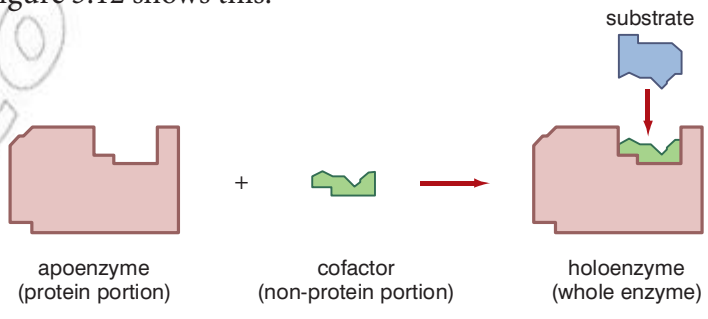


Figure 3.12 The apoenzyme and the cofactor make the holoenzyme.

Cofactors include:

- coenzymes
- mineral ions

Coenzymes are organic molecules and many are derived from vitamins. They bind with the enzyme to give catalytic activity.

Table 3.5 shows some common co enzymes, the vitamins they are derived from, the enzyme with which they bind, and their functions.

***Table 3.5** Common coenzymes and their functions*

Coenzyme	Vitamin	Enzyme	Function
Nicotinamide adenine dinucleotide (NAD)	Niacin	Oxidoreductase in respiration	Oxidation or hydrogen transfer in respiration
Flavin adenine dinucleotide (FAD)	Riboflavin	Oxidoreductase in respiration	Oxidation or hydrogen transfer in respiration

Some enzymes can only function in the presence of certain mineral ions. These bind loosely with the enzyme to give it its catalytic activity. Table 3.6 shows some examples of enzymes that require mineral ions as cofactors.

***Table 3.6** Enzymes that require mineral ions as cofactors*

Enzyme	Mineral ion	Function
Carbonic anhydrase	Zinc ions (Zn^{++})	Causes CO_2 to react with water to form hydrogen carbonate
Alcohol dehydrogenase	Zinc ions (Zn^{++})	Oxidises alcohol
Cytochrome oxidase	Copper ions (Cu^{++} or Cu^{+})	Transfers electrons to oxygen during respiration

Activity 3.4: Field visit

Field visit to study the use of enzymes by local manufacturers

You may be able to visit a nearby manufacturing plant that uses enzymes in some of its processes. If this is possible you should:

- make careful notes when you are there about:
- the processes themselves
- the role of enzymes in these processes
- write a report on your return that describes how important the use of enzymes is in this particular manufacturing plant

Review questions

Choose the correct answer from A to D.

1. Enzymes speed up biological reactions by:
   - A reducing the kinetic energy of the reacting molecules
   - B reducing the activation energy of the reaction
   - C increasing activation energy of the reaction
   - D increasing the kinetic energy of the reacting molecules
2. Which of the following statements about a lock and key model of enzyme action are not true?
   - A the substrate and the active site bind because they have shapes that fit together like a key fits in a lock
   - B the substrate and the active site have complementary 3-D shapes
   - C nothing can interfere with the way the substrate and the active site bind together
   - D high temperatures stop enzymes working as they denature the protein and change the shape of the active site

Activity 3.5

Design a 3-dimensional model to show how an enzyme works. You can plan to use a variety of resources from a carved fruit to modelling clay, from papier mache to paper and card. You may have the opportunity to actually make your model and display it to the rest of the class.

3. The induced-fit model of enzyme action suggests that, when enzyme and substrate bind, there is a conformational change in:
   A the substrate
   B the active site
   C both substrate and active site
   D neither substrate nor active site
4. An apoenzyme is:
   A a protein with enzymic activity
   B a non-protein with enzymic activity
   C a non-protein with no enzymic activity
   D a protein with no enzymic activity
5. Which of the following does not act as a cofactor to an enzyme?
   A niacin
   B copper ions
   C pepsin
   D riboflavin
6. A coenzyme is:
   A an organic molecule that binds tightly with the apoenzyme
   B an organic molecule that binds loosely with the apoenzyme
   C an inorganic molecule that binds loosely with the apoenzyme
   D an inorganic molecule that binds tightly with the apoenzyme
7. Mineral ions needed for enzyme activity:
   A bind tightly with the apoenzyme
   B bind loosely with the apoenzyme
   C bind loosely with the coenzyme
   D bind tightly with the coenzyme
8. Many coenzymes are derived from:
   A vitamins
   B hormones
   C lipids
   D proteins
9. The turnover rate of an enzyme is:
   A the number of enzyme molecules used per second
   B the number of product molecules formed per second
   C the number of reactant molecules used per second
   D all of the above
10. According to the induced-fit model of enzyme action, reacting molecules enter a transition state in which:
   A reacting molecules assume a complementary shape
   B apoenzyme and cofactor assume a complementary shape
   C bonds in reacting molecules are put under tension
   D bonds in the apoenzyme and coenzyme are put under tension

3.3 Factors affecting the functions of enzymes

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

- Explain factors that affect enzyme activity.
- Investigate the destruction of an enzyme by heat.
- Show how temperature, pH, substrate concentration and enzyme concentration affect enzyme activity.
- Explain allosteric regulation and the feedback control mechanism of enzyme activity.
- Appreciate the role of enzymes in controlling our metabolic activities.

The turnover rate and, therefore, the activity of the enzyme are influenced by a number of external factors, including:

- temperature
- pH
- substrate concentration
- the presence of inhibitors

How hot must it be?

When the temperature is raised, particles are given more kinetic energy. This has two main effects:

- 'Free' particles move around more quickly. This increases the probability that a substrate particle will collide with an enzyme molecule.
- Particles within a molecule vibrate more energetically. This puts strain on the bonds that hold the atoms in place. Bonds begin to break and, in the case of an enzyme, the shape of the molecule, and the active site in particular, begin to change. The enzyme begins to lose its tertiary structure (remember it is a protein) and **denature**. Figure 3.13 shows this.

KEY WORD

denature *the alteration of the tertiary structure of a protein; in living cells this is reversible*

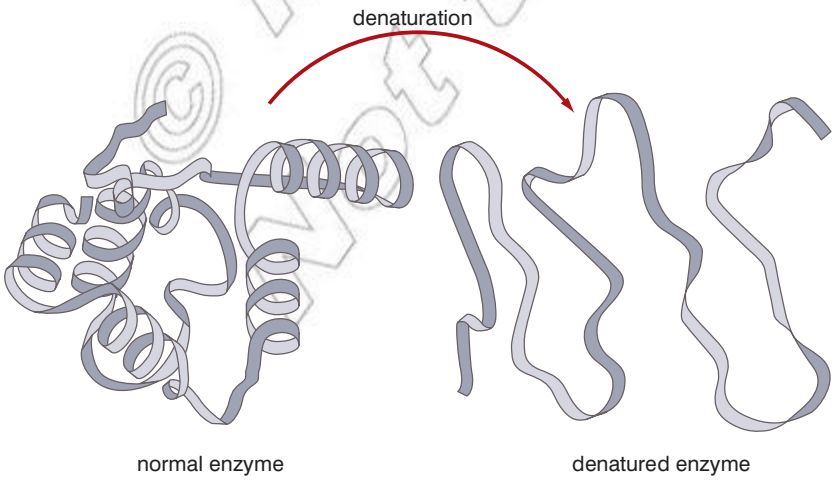


Figure 3.13 *How an enzyme denatures*

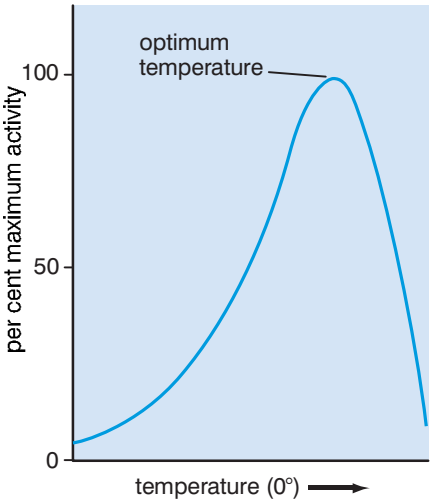


***Figure 3.14** The effect of temperature on enzyme activity*

The activity of an enzyme at a given temperature is a balance between these two effects. If the raised temperature results in little denaturation but a greatly increased number of collisions, the activity of the enzyme will increase. If the higher temperature causes significant denaturation then, despite the extra collisions, the activity of the enzyme will probably decrease. The temperature at which the two effects just balance each other is the **optimum temperature** for that enzyme. Any further increase in temperature will cause increased denaturation that will outweigh the effects of extra collisions. A decrease in temperature means that fewer collisions will occur. Figure 3.14 shows this.

Note that the graph is not symmetrical. Above the optimum temperature, the enzyme denatures very quickly to the point at which the shape of the active site has changed so much that an enzyme–substrate complex cannot form. At this point the reaction rate is zero.

DID YOU KNOW?

Optimum temperatures

Enzymes do not all have the same optimum temperature; they are adapted to work most efficiently within the organism in which they are found. For example, the optimum temperature for enzymes:

- in human beings is around 37 °C (normal body temperature)
- in plants growing in the Arctic may be less than 5 °C
- in bacteria that live in hot springs (thermophilic bacteria) may be over 90 °C.

How acidic must it be?

The **pH scale** is a measure of the hydrogen ion concentration of a solution or other liquid system. The pH scale ranges from 0 to 14. Solutions with a pH of less than 7 are acidic, those with a pH of more than 7 are alkaline and a solution with a pH of exactly 7 is neutral.

The majority of enzymes in most mammals function most efficiently within the pH range 6.0–8.0, although the optimum pH of pepsin (an enzyme found in the stomach) is between pH 1.0 and pH 3.0. Significant changes in pH can affect an enzyme molecule by:

- breaking ionic bonds that hold the tertiary structure in place; this leads to denaturation of the enzyme molecule
- altering the charge on some of the amino acids that form the active site; this makes it more difficult for substrate molecules to bind

These effects occur if the pH becomes either more acidic or more alkaline. Figure 3.15 shows this effect.

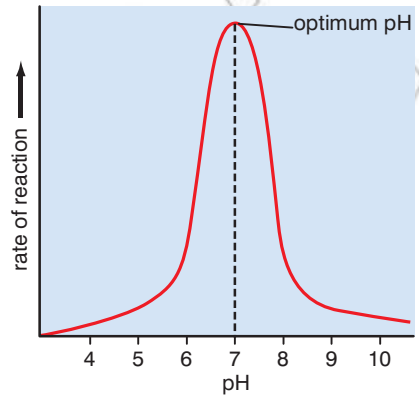


***Figure 3.15** The effect of pH on enzyme activity*

DID YOU KNOW?

About pH

The pH scale of acidity/alkalinity is an inverse logarithmic scale! Each pH unit represents a tenfold change in hydrogen ion (H^+) concentration. pH 0 represents the highest H^+ concentration and is the most acid. A pH 1.0 solution has one-tenth (0.1) of this H^+ concentration; a pH 4 solution has one ten-thousandth (0.0001). pH 14 represents the lowest H^+ concentration and is the most alkaline. pH 7 is neutral.

DID YOU KNOW?

About the pH in your gut

The pH of the intestinal tract of humans changes from one region to the next. The pH in the mouth varies from being slightly alkaline (pH 7.5) to quite acidic (pH 5.0) depending on whether or not we have eaten and also what we have eaten. The pH in the stomach can be as low as pH 1.5, whereas the pH of the small intestine is slightly alkaline at pH 7.5. Digestive enzymes from the different regions have optimum pHs that reflect the region in which they are secreted. Figure 3.16 shows the optimum pHs of salivary amylase (mouth), pepsin (stomach) and trypsin (small intestine).

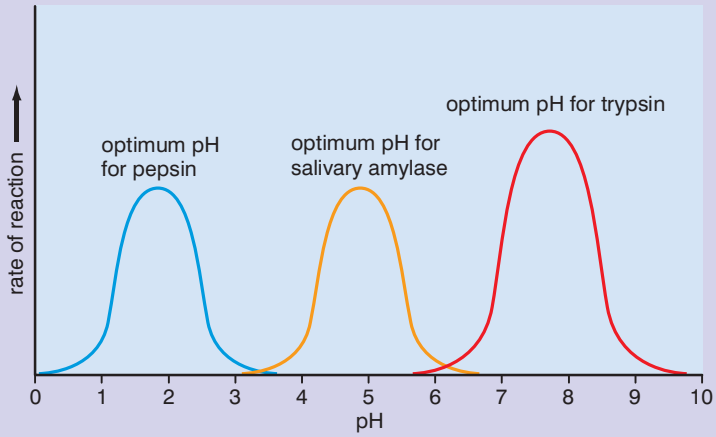


***Figure 3.16** The optimum pHs of some human digestive enzymes*

Does the concentration of the substrate matter?

The activity of an enzyme depends on the number of substrate molecules per second that bind to form enzyme–substrate complexes. So the number of substrate molecules present must have an effect. A small number of substrate molecules means few collisions and so only a few enzyme–substrate complexes form. Increasing the concentration of the substrate means more collisions and more enzyme–substrate complexes. So, the overall rate of reaction is increased. Eventually, because of the high substrate concentration, each enzyme molecule could be working at maximum turnover – that is, each active site is binding with substrate molecules all the time and there is no 'spare capacity' in the system. Increasing the substrate concentration beyond this point will have no effect on the activity of the enzyme because all the active sites are occupied all the time. Figure 3.17 shows this effect.

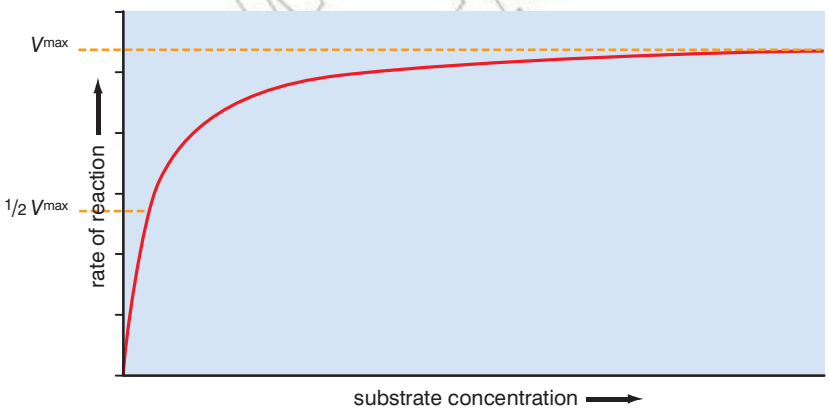


***Figure 3.17** The effect of substrate concentration on enzyme activity. V^{max} is the maximum rate of enzyme action.*

KEY WORDS

optimum temperature *temperature at which an enzyme works most efficiently*

pH scale *measure of the hydrogen ion concentration of a solution*

KEY IDEA

Think about what will happen to the concentration of substrate molecules as an enzyme-controlled reaction takes place. As the reaction proceeds, more and more of the substrate molecules react, so there will be fewer remaining. The concentration of the substrate will decrease. With fewer substrate molecules left, the number of collisions per second between enzyme and substrate will also decrease, and the rate of reaction will slow down. This is because the turnover rate of each enzyme molecule decreases with time.

How much enzyme should there be?

Assuming a constant large supply of substrate molecules, each enzyme molecule will work at maximum turnover. Therefore, the reaction rate will be directly proportional to the number of enzyme molecules – the concentration of the enzyme. Increasing the concentration will increase the reaction rate.

However, increasing the concentration of the enzyme will not increase the activity of the enzyme. Each enzyme molecule will be working at maximum turnover, so the activity of the enzyme is likely to remain constant.

Activity 3.6: How can we measure the rate of an enzyme-controlled reaction?

We can do this in one of two ways. We can:

- measure the rate at which the substrate is used up, or
- measure the rate at which the product is formed.

Usually, it is more convenient to do the latter – measure the rate at which product is formed.

The enzyme catalase is commonly used in these sorts of investigations. This is because it is found in almost all cells and there are many readily available sources that contain significant amounts of catalase. These include:

- yeast
- liver
- potato

Catalase catalyses the decomposition of hydrogen peroxide to water and oxygen. The equation for the reaction is:

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

Because oxygen is a gas, the volume of oxygen collected in a certain time is a measure of how fast the reaction is proceeding. There are several ways of carrying out the investigation. One of these is shown in figure 3.18. This investigation uses potato, but it could just as easily be carried out with yeast or pieces of liver.

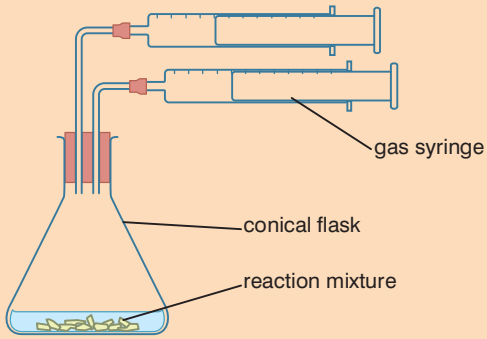


Figure 3.18 *Apparatus set-up*

You will also need:

- a potato
- access to a balance
- 10 volume hydrogen peroxide solution (safety note: this is an oxidising agent, take care)
- a scalpel and a tile on which to cut the potato
- a stopwatch

The experiment is carried out as follows:

1. Peel a potato and chop into small pieces (less than 1 cm square).
2. Weigh out 10 g of the potato and place it in the conical flask.
3. Attach the gas syringe (you will need to support it with a clamp and stand).
4. Make sure that the gas syringe:
   - is horizontal
   - reads zero

5. Draw 20 cm^3 hydrogen peroxide into the second syringe and attach it to the conical flask.
6. Check again that all seals are tight, the gas syringe reads zero and is horizontal.
7. Add all the hydrogen peroxide solution to the potato quickly and start the stopwatch.
8. Record the volume of gas collected in the gas syringe every minute for ten minutes.

Now that you have a set of results, you can plot them as a graph. You may well end up with a graph that looks like the one below:

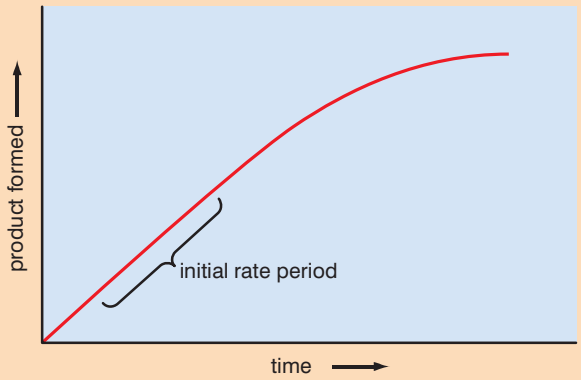


Figure 3.19 *A graph of the results.*

Notice that the line is starting to level off (yours may have completely levelled off). This is because as the reaction proceeds, substrate is used up, fewer enzyme substrate complexes form and the reaction rate slows down. Not as much oxygen is formed per minute as a result.

This procedure is a very basic one and there are a number of reasons why the results obtained might not be reliable. These include:

- We did not control the temperature; it might have increased or decreased during the investigation, speeding up the reaction or slowing it down.
- We did not control the pH of the reaction mixture; it too might have increased or decreased during the investigation, speeding up the reaction or slowing it down.
- We only carried out the investigation once; we may have obtained an anomalous (freak) result.

However, we did control:

- The concentration of the substrate (we used 10 volume hydrogen peroxide).
- The concentration of the enzyme (we used a specific mass of potato).

We can improve our investigation fairly easily, as shown in table 3.7.

Table 3.7 *Improving the investigation*

Factor controlled	How controlled	Note
Temperature	Use of water bath at the required temperature	Stand the potato pieces in the conical flask and hydrogen peroxide in the water bath separately for 10 minutes. This is called equilibration.
pH	Use of buffer solutions at required pH	Buffer solutions resist changes in pH and maintain a more or less constant pH. Add the buffer solution to the potato pieces at the start.
Repeats	Carry out the experiment three or five times	Carrying out the experiment more than once allows us to spot anomalous results and eliminate them. This is easier if you have an odd number of results.

We can use this basic experiment, with the improvements, to investigate how the different factors affect the rate of enzyme action. Before we do, however, we must be quite clear about what we are trying to find out.

Reminder from Unit 1

- The factor that you change is the independent variable (IV).
- The factor that you record as the results is the dependent variable (DV).

Activity 3.7

Plan an investigation into the effect of temperature on the rate of an enzyme controlled reaction such as the breakdown of starch by the enzyme amylase in saliva or the breakdown of hydrogen peroxide by the enzyme catalase in potato or liver. Explain how you would vary the temperature of the reacting mixture and suggest what results you would expect to see.

KEY IDEA

The 'rate of enzyme action', like any rate, means 'how much per unit of time'. We cannot just say 12 cm^3 oxygen. We must convert this to volume per minute, or volume per second. Then we have a rate.

It is also best if we can compare the rates of enzyme action when they are working to maximum or near maximum capacity for the conditions. So, before we proceed to the main investigations, we should:

- *carry out our improved basic experiment three times*
- *plot the graphs of our results*
- *determine the point on each where the graph starts to level*
- *take an average of these times*

This is the time we will use for our main investigations.

You are now in a position to use this procedure to design your own investigations into:

- *the effect of temperature on enzyme activity*
- *the effect of pH on enzyme activity*
- *the effect of substrate concentration on enzyme activity*

When you are investigating one factor, then all the others need to be controlled – kept constant – so that they cannot influence the results. If you were investigating the effect of temperature then pH, substrate concentration and enzyme concentration would need to be controlled, as would the duration of the experiment.

For each of your investigations, you should think about each of the following:

- How will I change the independent variable?
- How will I measure the dependent variable?
- What other factors need to be controlled?
- How will I control them?
- How many different values of the IV shall I use? Usually five is the minimum requirement.
- What values shall I use? These need to be reasonably spaced, for example, temperatures of 20 °C, 30 °C, 40 °C, 50 °C, 60 °C are better than temperatures of 20 °C, 22 °C, 30 °C, 52 °C, 60 °C. Can you see why?
- How many times shall I repeat each condition? Usually three times is the minimum requirement.
- How will I record my results? You should have a table prepared before you commence the investigation.

If, for some reason, you were unable to carry out the investigation, here are some results you could analyse.

Substrate concentration

Concentration of hydrogen peroxide/volume	Reaction rate/cm^3 s^{-1}			
	Trial 1	Trial 2	Trial 3	Mean
0	0	0	0	
5	0.7	0.7	0.4	
10	1.6	1.9	1.6	
15	2.5	3.1	2.8	
20	2.9	3.0	3.7	

You can copy the table, calculate the mean and plot a graph of the mean reaction rate against the concentration of hydrogen peroxide.

Temperature

These results come from a class who varied the procedure slightly. They timed how long it took to produce 30 cm^3 oxygen at different temperatures. So before you can plot your graph of reaction rate, you must first:

- calculate the mean result for each temperature, and
- convert this to a volume per second (or per minute).

Temperature/°C	Time taken to collect 30 cm^3 oxygen in seconds			
	Trial 1	Trial 2	Trial 3	Mean
10	54	47	43	
20	12	14	16	
30	5	5	5	
35	9	5	4	
40	9	6	9	
45	14	11	11	
50	73	71	57	
55	119	109	132	

Once you have calculated the mean rates for each temperature, plot a graph of reaction rate against temperature.

How do other substances affect enzyme activity?

Inhibitors are substances that bind to enzymes and prevent them from forming enzyme–substrate complexes and, as a result, stop, or slow down, the reaction. There are two main types of inhibitors:

- irreversible inhibitors, and
- reversible inhibitors.

Irreversible inhibitors bind strongly to enzymes, usually by a covalent bond, permanently altering the structure of the enzyme

Activity 3.8

Plan an investigation into the effect of pH on the rate of an enzyme controlled reaction such as the breakdown of starch by the enzyme amylase in saliva or the breakdown of hydrogen peroxide by the enzyme catalase in potato or liver. Explain how you would vary the pH of the reacting mixture and suggest what results you would expect to see.

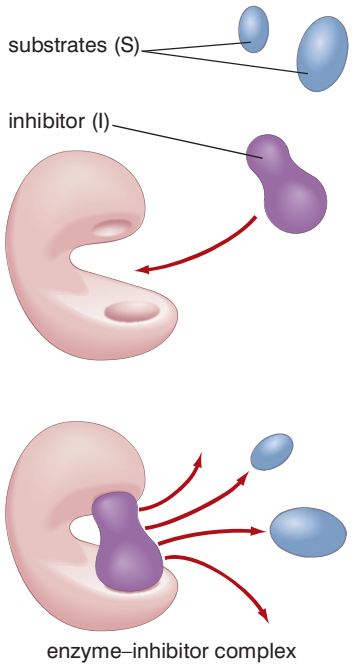


Figure 3.20 *Competitive inhibition*

molecule and inactivating it. The painkiller aspirin is an example of an irreversible inhibitor. It binds with the enzyme cyclo-oxidase-2, which is an important enzyme in producing prostaglandins which give the sensation of pain.

Reversible inhibitors bind to enzymes only weakly and the bond that holds them breaks easily releasing the inhibitor. This allows the enzyme to become active again. There are two main kinds of reversible inhibitors:

- **competitive inhibitors**, and
- **non-competitive inhibitors**.

Competitive inhibitors

Competitive inhibitors have molecules with shapes that are complementary to all, or part, of the active site of an enzyme. They are often similar in shape to the substrate molecules. They can bind with the active site and prevent substrate molecules from binding. The binding is only temporary and the competitive inhibitor is quickly released. A competitive inhibitor blocks the active site so substrate molecules cannot bind.

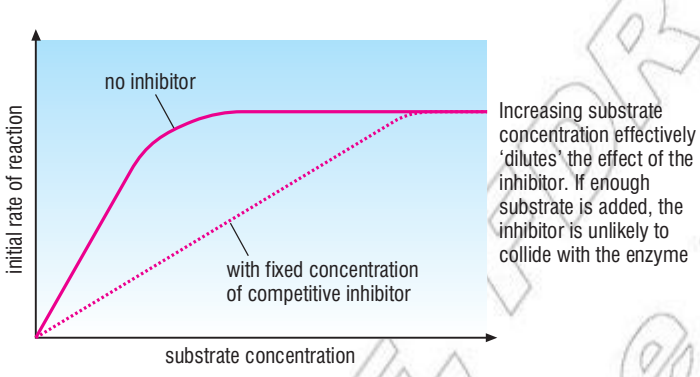


Figure 3.21 *Effect of substrate concentration on inhibition by a competitive inhibitor*

The overall effect on the rate of reaction depends on the relative concentrations of substrate and inhibitor molecules. Each molecule of competitive inhibitor can inhibit (temporarily) one enzyme molecule – but only if it can collide with the enzyme molecule and bind with the active site. To do this, it must compete with the substrate molecules for the active site – hence the name, competitive inhibitor. If there were 99 substrate molecules for every inhibitor molecule, then 99% of the collisions would be between enzyme and substrate and the reaction would proceed at 99% of the maximum rate. If the ratio were 90 substrate molecules to ten inhibitor molecules, there would be 10% inhibition and the reaction rate would fall to 90% of maximum.

The painkiller ibuprofen acts as a competitive inhibitor of the enzyme cyclo-oxidase-2, competing with the precursors of prostaglandins, which are the substrate of cyclo-oxidase-2. The metabolic poison cyanide acts as a competitive inhibitor of the enzyme cytochrome oxidase, an important enzyme in the release of energy in respiration.

KEY WORDS

competitive inhibitor *a molecule that inhibits enzyme activity by competing with the substrate for the active site*

non-competitive inhibitor *a molecule that alters the conformation of the active site by binding with the allosteric site of the enzyme; it prevents the substrate from binding and inhibits enzyme activity*

Non-competitive inhibitors

Non-competitive inhibitors do not compete for the active site. Instead, they bind to another part of the enzyme called the allosteric site. This produces a conformational change in the part of the enzyme molecule that includes the active site. Because of this, the active site is a different shape and can no longer bind with the substrate to catalyse the reaction.

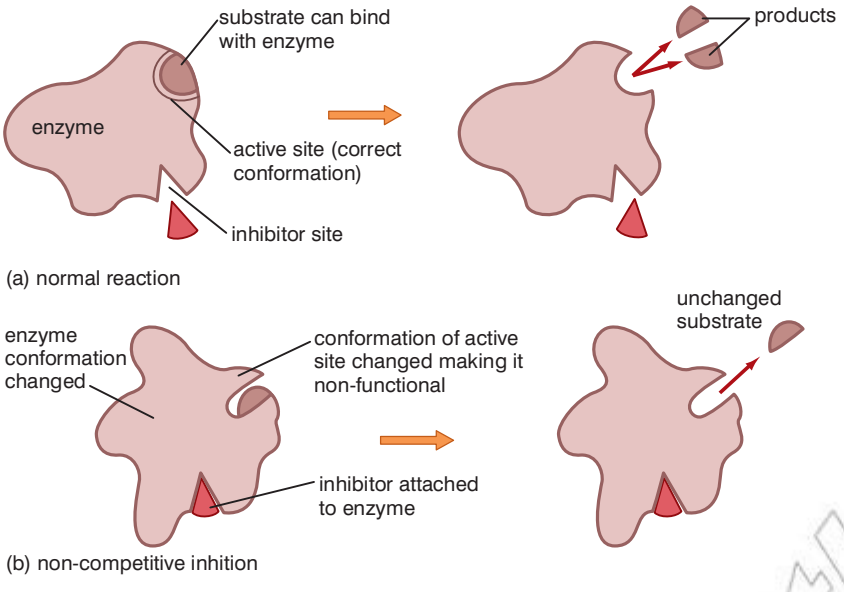


Figure 3.22 *Non-competitive inhibition*

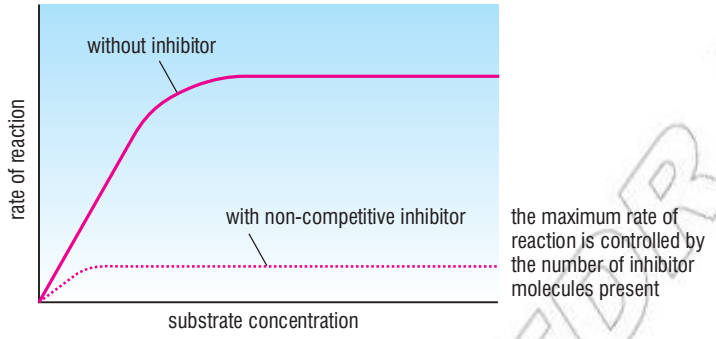


Figure 3.23 *The effect of substrate concentration on a non-competitive inhibitor*

The effectiveness of a non-competitive inhibitor is in no way affected by the concentration of the substrate. Suppose there are enough inhibitor molecules to bind with the allosteric sites of 80% of the enzyme molecules. 80% of the enzyme molecules will be inhibited irrespective of the number of substrate molecules (as the two are not competing for the same site) and the reaction rate will drop to 20% of maximum.

Non-competitive inhibitors are particularly important in regulating metabolic pathways in cells.

How do inhibitors control enzyme activity in living cells?

Many substances are produced in cells as a result of a metabolic pathway (a series of reactions), which can be represented as:

$$\text{Substance A} \xrightarrow{E1} \text{Substance B} \xrightarrow{E2} \text{Substance C} \xrightarrow{E3} \text{Substance D}$$

E1, E2 and E3 are enzymes catalysing the reactions.

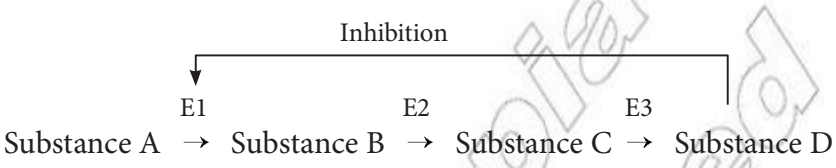
All the reactions in this sequence are enzyme controlled. Therefore, inhibition of any of these enzymes will interrupt the process. However, the main function of this pathway is to produce substance D for use by the cell. If the requirement for substance D in the cell decreases, then the concentration of D will increase. This is at

KEY WORDS

end-product inhibition *when an end product inhibits the enzyme controlling the first stage of a reaction sequence*

activator *a substance that removes an inhibitor*

least inefficient (producing something that is not being used) and may be potentially harmful because high concentrations could be toxic. Such reaction sequences are often controlled by **end-product inhibition**. The end product (D) inhibits the enzyme controlling the first stage of the reaction sequence, as shown in the diagram.



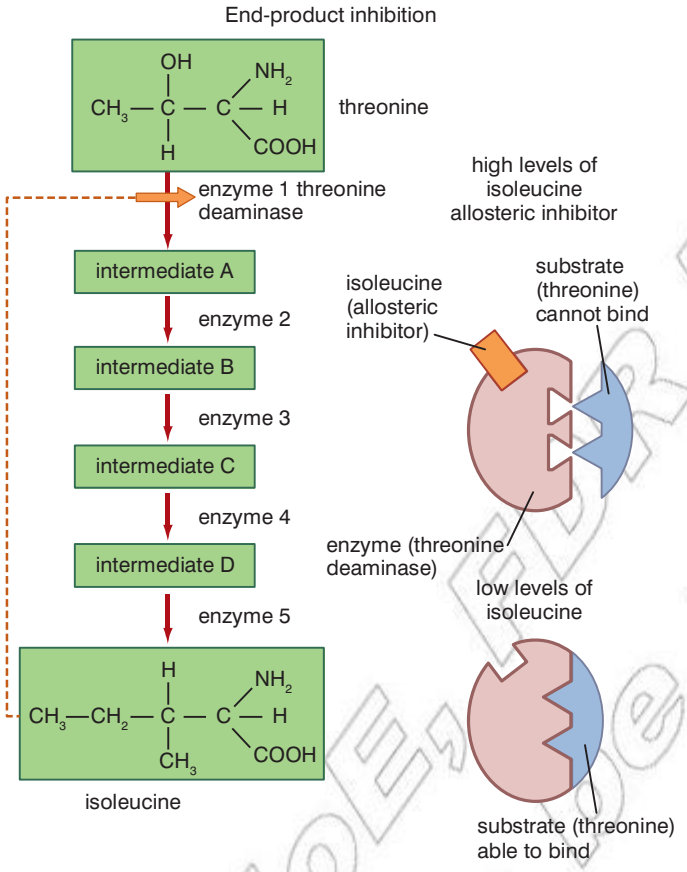


Figure 3.24 *A metabolic pathway controlled by end-product inhibition*

Substance D normally acts as a non-competitive inhibitor, which prevents the enzyme E1 from catalysing the reaction that converts A to B. As a result, the entire reaction sequence is halted. When the inhibition is removed, the metabolic pathway can start again. Sometimes, a decrease in the concentration of D is sufficient to remove the inhibition. In other cases, the inhibitor must be removed by a substance known as an **activator**. Enzymes are often kept inactive in a cell by an inhibitor and are activated by a specific activator only when they are required.

Figure 3.24 shows an example of a metabolic pathway controlled by end-product inhibition. In this pathway, the amino acid threonine is converted to another amino acid, isoleucine. Isoleucine acts as a non-competitive (allosteric) inhibitor. High concentrations of isoleucine inhibit more and more of the enzyme catalysing the first reaction in the pathway, until the pathway is completely inhibited.

Review questions

Choose the correct answer from A to D.

1. When an enzyme is subjected to excess heat:
   - A bonds in the active site are strained
   - B some of the bonds in the active site break
   - C the active site undergoes a conformational change
   - D all of the above

2. Extreme pHs can inactivate enzymes because they:
   A alter the charge on the amino acids in the allosteric site
   B alter the charge on the amino acids in the active site
   C alter the charge on amino acids away from the active site and allosteric site
   D all of the above
3. The optimum temperature of an enzyme is the temperature at which:
   A there is no denaturation
   B the maximum number of enzyme–substrate complexes are formed
   C there is the maximum number of collisions between enzyme and substrate
   D the particles have the most kinetic energy
4. A non-competitive enzyme inhibitor…
   A does not compete for the active site
   B binds with the allosteric site
   C binds with the active site of the enzyme
   D is not affected by the substrate concentration
5. If the ratio of non-competitive inhibitor molecules to substrate molecules is 3:7, the enzyme controlling the reaction will be:
   A 70% inhibited
   B 30% activated
   C 30% inhibited
   D three-sevenths inhibited
6. When investigating the effect of temperature on enzyme activity, we should control:
   A pH
   B substrate concentration
   C enzyme concentration
   D all of these
7. End-product inhibition of a metabolic pathway occurs when:
   A the last product of the pathway inhibits the enzyme controlling the first reaction
   B the last product of the pathway inhibits the enzyme controlling the last reaction
   C the first product of the pathway inhibits the enzyme controlling the last reaction
   D the last product of the pathway inhibits the enzyme controlling the first reaction

Activity 3.9

Plan an investigation into the effect of changing the substrate concentration on the rate of an enzyme controlled reaction such as the breakdown of starch by the enzyme amylase in saliva or the breakdown of hydrogen peroxide by the enzyme catalase in potato or liver. Explain how you would vary the concentration of the substate and suggest what results you would expect to see.

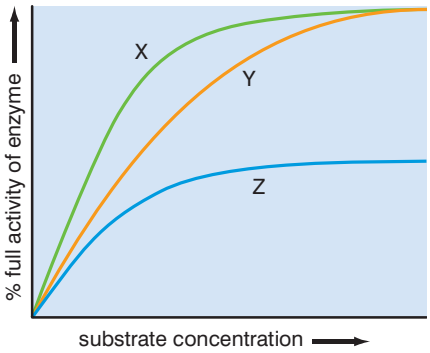


Figure 3.25

8. Figure 3.25 shows the activity of an enzyme at different substrate concentrations under three conditions:
   - no inhibitor present
   - a competitive inhibitor present
   - a non-competitive inhibitor present

   Which of the following represents the correct interpretation of the graph:

   A X is with the competitive inhibitor, Y with the non-competitive inhibitor and Z with no inhibitor

   B X is with the non-competitive inhibitor, Y with the competitive inhibitor and Z with no inhibitor

   C X is with no inhibitor, Y with the non-competitive inhibitor and Z with the competitive inhibitor

   D X is with no inhibitor, Y with the competitive inhibitor and Z with the non-competitive inhibitor

9. As temperature increases up to the optimum, the rate of an enzyme-controlled reaction increases because:

   A the particles have more kinetic energy

   B there are more collisions between enzyme and substrate

   C there are more enzyme–substrate complexes formed

   D all of these

10. If substrate concentration is kept permanently high and the enzyme concentration is gradually increased, the rate of activity of the enzyme will:

    A increase

    B increase and then decrease

    C increase and then level off

    D stay the same

Summary

In this unit you have learnt that:

Catalysts

- A catalyst speeds up a chemical reaction with no effect on:
  - the products formed
  - the energy change
  - the nature of the catalyst itself
- A catalyst speeds up a reaction by lowering the activation energy required for reactants to enter the transition state.
- Nearly all biological catalysts are enzymes. They are globular proteins with a specific tertiary shape, part of which forms an active site.

- A substrate molecule binds with the active site to form an enzyme–substrate complex. This then forms the products. The products are released from the enzyme molecule, which is unaltered.

Models of enzyme action

- The lock-and-key model of enzyme action suggests a rigid structure for the enzyme molecule, with the shape of the substrate and active site being complementary to each other. This model explains enzyme specificity but not how the transition state is achieved.
- The induced-fit model of enzyme action suggests that binding of the substrate induces a conformational change in enzyme structure, which puts the substrate molecule under tension, causing it to enter the transition state.
- The number of substrate molecules that bind to the active site of an enzyme molecule per second is the turnover rate.

Factors affecting enzyme activity

- Temperature – below the optimum temperature, the low level of kinetic energy limits the number of enzyme–substrate complexes formed; above the optimum temperature, denaturation of the enzyme prevents binding of the substrate.
- pH – above and below the optimum pH, changes occur in the tertiary structure of the enzyme molecule and in the charges on the amino acids making up the active site; both prevent binding of the substrate.
- Substrate concentration – if the concentration of enzyme remains constant, increasing the substrate concentration increases the number of enzyme–substrate complexes formed until, at any one time, all the active sites are occupied; the rate of reaction increases to its maximum.
- Enzyme concentration – if the substrate concentration is high and constant, increasing the enzyme concentration increases the rate of reaction.
- Inhibitors:
  - competitive inhibitors have molecules that are often similar in shape to the substrate molecules and that compete for the active site; the extent of the inhibition depends on the ratio of substrate molecules to inhibitor molecules
  - non-competitive (allosteric) inhibitors bind to a region away from the active site, producing a conformational change in the enzyme that prevents the substrate from binding; the extent of the inhibition is independent of the substrate concentration
  - allosteric inhibition can control metabolic pathways; the final product of a series of reactions inhibits the enzyme controlling the first reaction in the series; this is also known as end-product inhibition.

Activity 3.10

You know that a high temperature can denature an enzyme but it can be difficult to imagine how this happens. However you can demonstrate the effect very easily. Take an egg and separate the yolk from the white. Then divide the white between two test tubes. Egg white is pure protein and the coiled protein molecules are similar to the protein molecules which form enzymes.

Keep one tube at room temperature. Place the other in a beaker of boiling water and leave it for several minutes. Observe what happens and explain what you see in terms of changes to the coiled protein molecules in the raw egg white.

End of unit questions

1. a) What is a cofactor?

   b) The table shows the various groups that can combine to form a holoenzyme. Copy and complete the table by placing a tick (✓) or a cross (✗) in each box.

Type of group	Organic	Protein	Binds tightly
Apoenzyme			
Coenzyme			
Ion			

2. When conducting investigations into the activity of enzymes, a number of factors need to be controlled. Copy and complete the table to describe the reasons for controlling these factors.

Factor controlled	How controlled	Reason for controlling factor
Temperature		
pH		Changes in pH can alter charge on amino acids in the active site.
Substrate concentration	Equal strength solutions	

3. Figure 3.26 shows the activity of two enzymes at different temperatures.

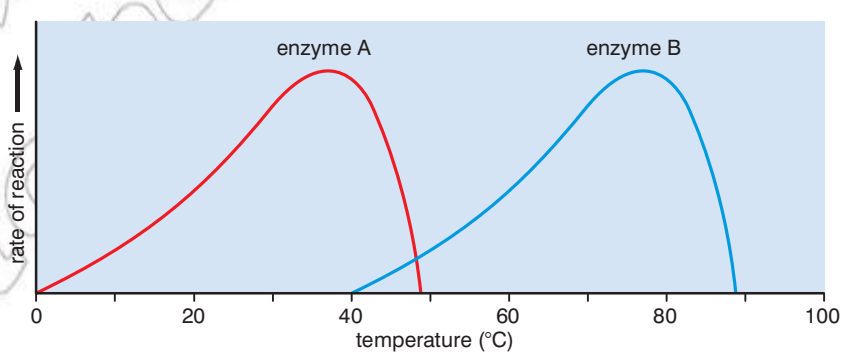


Figure 3.26

   a) What is the optimum temperature of each enzyme? Give reasons for your answers.

   b) Which enzyme may have come from a thermophilic bacterium? Give the reasons for your answer.

   c) Describe and explain the shape of the curve from 20 °C to 35 °C for enzyme A. Explain your answer.

4. Figure 3.27 shows the rate of reaction of an enzyme at 25 °C at different substrate concentrations.

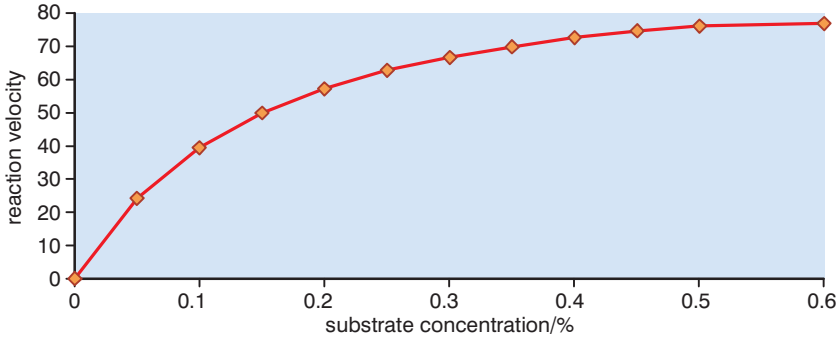


Figure 3.27

   a) Describe and explain the shape of the graph in terms of kinetic theory and enzyme–substrate complex formation:

      (i) from substrate concentration 0.05% to 0.4%

      (ii) from substrate concentration 0.4% to 0.6%

   b) Copy the graph and sketch, on your copy, the curve you would expect if the experiment had been carried out at 35 °C rather than 25 °C.

5. Figure 3.28 shows an energy level diagram of a reaction proceeding without an enzyme and the same reaction with an enzyme.

   a) Describe *two* ways in which the energetics of the reactions are similar.

   b) Describe and explain the differences between the regions marked X and Y on the diagram.

   c) Explain why enzymes speed up biological reactions.

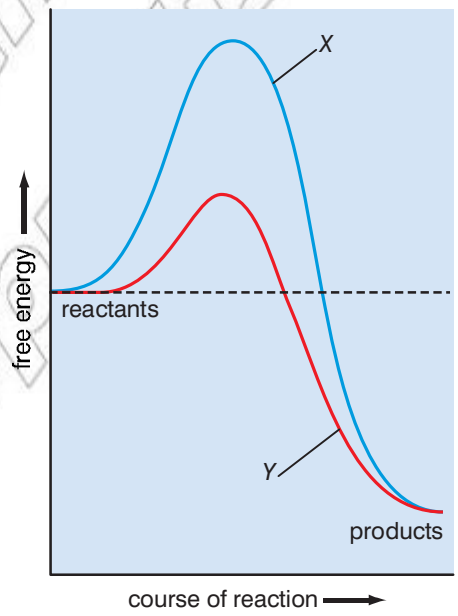


Figure 3.28

6. Enzymes are increasingly being used in industrial processes.

   a) Give three examples of industrial processes that use enzymes.

   b) Give two reasons why enzymes are being increasingly used in industrial processes.

   c) Explain one way in which the increased use of enzymes may benefit the environment.

7. Students investigated the effect of temperature on the rate of activity of the enzyme catalase. They timed how long it took for potato tissue to produce 50 cm^3 oxygen at different temperatures. Figure 3.29 shows the graph that one student drew after averaging all the students' results.

   a) (i) According to this graph, what is the optimum temperature of catalase?

      (ii) Explain why this might not be an accurate estimate of the optimum temperature.

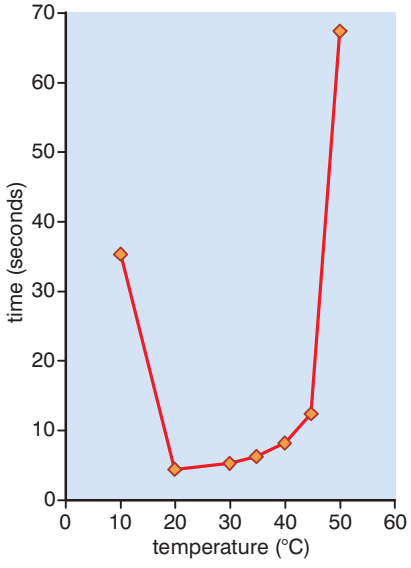


Figure 3.29

b) In a control experiment (no enzyme present but all other factors the same as in the other experiments) carried out at 20 °C, 0.5 cm^3 of oxygen was collected. Assuming no experimental error, explain why this small amount of oxygen was produced.

c) (i) Explain the difference in the volumes of oxygen collected at 10 °C and at 20 °C.

(ii) Explain the difference in the volumes of oxygen collected at 35 °C and at 50 °C.

8. a) Explain what is meant by non-competitive (allosteric) inhibition.

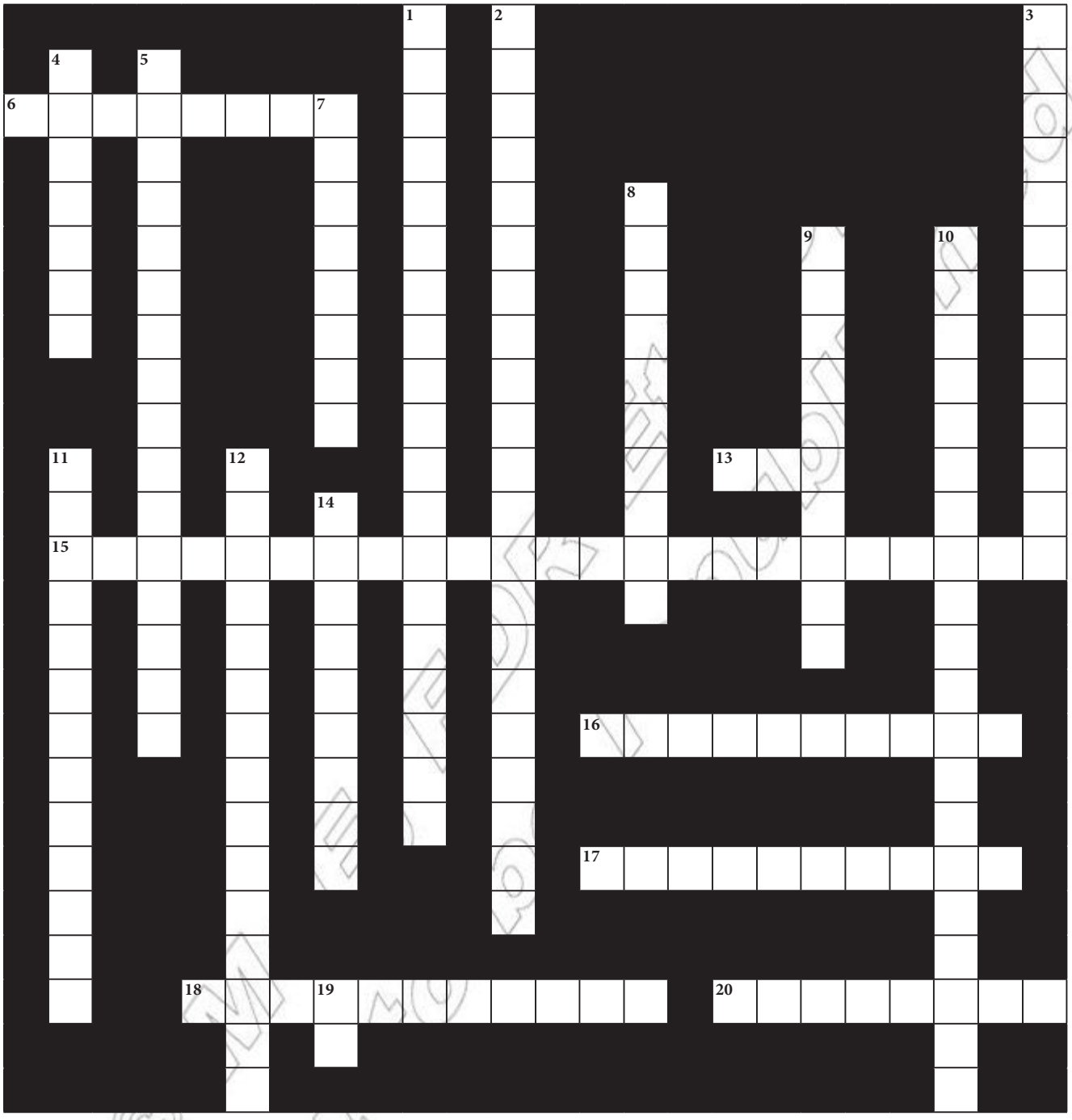
b) A metabolic pathway consists of a series of reactions controlled by enzymes, as shown below.

$$\text{Substance A} \xrightarrow{E1} \text{Substance B} \xrightarrow{E2} \text{Substance C} \xrightarrow{E3} \text{Substance D}$$

(i) Use this example to explain what is meant by end-product inhibition.

(ii) Explain how end-product inhibition can control enzyme activity in living cells.

Copy the crossword puzzle below into your exercise book (or your teacher may give you a photocopy) and solve the numbered clues to complete it.



Across

6. Means that enzymes only catalyse one reaction (8)
13. The names of most enzymes ends in these three letters (3)
15. This slows a reaction by binding to the allosteric site of an enzyme (3-11, 9)
16. A model of enzyme action in which the active site changes shape as the substrate binds (7, 3)
17. Literally means 'water-splitting' (10)

18. If this is too high, enzymes are denatured (11)
20. A substance (sometimes a vitamin) necessary for the functioning of an enzyme (8)

Down

1. Enzymes are sometimes described as these (10, 9)
2. This is formed when an enzyme and its substrate bind (6-8, 7)
3. This cleaning substance for clothes often contains enzymes (7, 6)
4. The temperature at which an enzyme is most active is called this (7)
5. The energy needed before a reaction will proceed (10, 6)
7. A substance that speeds up a chemical reaction, but remains unchanged itself (8)
8. A model of enzyme action in which enzyme and substrate fit together like an egg and egg cup (4, 3, 3)
9. The part of an enzyme that binds with its substrate (6, 4)
10. This slows a reaction by competing with the substrate for the active site of an enzyme (11, 9)
11. The amount of substrate per 100 cm^3 is its … (13)
12. All enzymes are this type of molecule (8, 7)
14. The main part of an enzyme that consists of two molecules (9)
19. This can influence how active enzymes are (2)