CHAPTER

18 Uses of proteins

Proteins provide the molecular machinery within biological systems. They have also been used to perform functional roles outside the body. For example, the enzyme systems within yeast have been used for thousands of years to assist in the production of bread and alcoholic beverages. Similarly, in more recent years, enzymes have been increasingly used to catalyse reactions involved in industrial processes. In Chapter 17, you learnt about the interactions that govern the folding of proteins into their functional three-dimensional shapes. In this chapter, you will learn about how the shape of proteins is related to their function, focusing particularly on the role of enzymes both within biological systems and in industrial applications.

Science as a human endeavour

- The Protein Data Bank (PDB) houses an international repository of structural data of proteins. The information is accessed and contributed to by scientists worldwide. The function of a protein is closely linked to its structure.

Science understanding

- secondary structures of proteins (α-helix and β-pleated sheets) result from hydrogen bonding between amide and carbonyl functional groups; hydrogen bonding between amide and carbonyl functional groups within a peptide chain leads to α-helix structures while hydrogen bonding between adjacent polypeptide chains leads to β-pleated sheets
- the tertiary structure of a protein (the overall three dimensional shape) is a result of folding due to interactions between the side chains of the α-amino acid in the polypeptide, including disulfide bridges, hydrogen bonding, dipole–dipole interactions, dispersion forces and ionic interactions
- enzymes are protein molecules which are biological catalysts and can be used on an industrial scale to produce chemicals that would otherwise require high pressure or temperature conditions to achieve an economically viable rate, including fermentation to produce ethanol versus hydration of ethene

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18.1 Investigating proteins

Proteins are required for the structure, function and regulation of all the processes that take place within cells. Therefore, investigating the structure and function of proteins is critically important to scientists. A greater knowledge of proteins provides insight into biological processes and offers potential targets for drug discovery. For this reason, scientists share information about protein structure and function in online databases.

ROLE OF PROTEINS IN BIOLOGICAL SYSTEMS

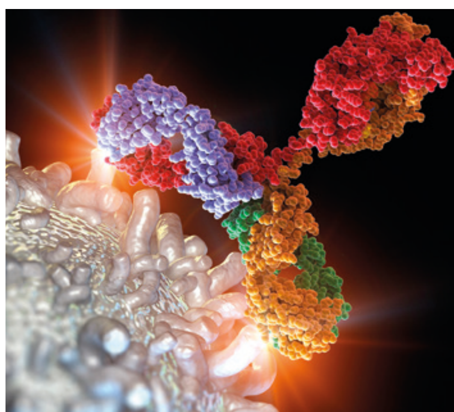

FIGURE 18.1.1 The Y-shape of this protein, an antibody (multi-coloured), enables it to interact with and attack a foreign substance (white) that has entered the body.

In order to survive, your body produces thousands of different proteins, each one with its own unique function. For example, proteins can act as enzymes to catalyse biochemical reactions, as hormones, as structural components in cells, and as part of the immune system, and some proteins assist with the transport of substances across cell membranes. Proteins are critical to almost every chemical process within our bodies.

The function of a protein is closely linked to its structure. As you learnt in Chapter 17, the specific sequence of amino acids in a protein results in a unique three-dimensional shape that enables the protein to function correctly.

The function of a protein depends on its three-dimensional shape.

For example, antibodies are large Y-shaped proteins that your immune system uses to recognise foreign materials such as bacteria and viruses (Figure 18.1.1). The shape of the antibody molecules allows them to recognise and bind to these pathogens. All antibodies have a very similar structure and shape, with the exception of the region at the tip of the Y-shape. Your body contains millions of different antibodies, each with a slightly different structure that allows each one to recognise a slightly different pathogen.

CHEMISTRY IN ACTION

Investigating protein function—knockout mice

One way in which scientists can investigate protein function is through using genetically engineered knockout mice. Scientists are able to inactivate or 'knock out' genes in the mice, preventing their cells from synthesising specific proteins.

For example, leptin is a protein hormone that regulates appetite in mammals by signalling to the brain when the animal is full or satiated. The function of this protein was discovered by examining a strain of mice with a mutation that prevents them from producing leptin. These mice gain weight rapidly throughout their lives and often reach a weight three times that of unaffected mice (Figure 18.1.2).

The leptin knockout mice have been used to investigate the causes of obesity and many of the processes involved in obesity-related diseases such as type 2 diabetes.

FIGURE 18.1.2 A leptin knockout mouse next to a normal mouse. The knockout mice grow up to three times larger than normal mice despite eating a low-fat diet. Examining the differences between the two mice enables scientists to determine the function of the leptin protein.

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PROTEIN DATA BANK

The **Protein Data Bank** (PDB) is a database that contains the amino acid sequence and three-dimensional shapes of large biological macromolecules such as proteins and nucleic acids. It provides research scientists with a means of rapidly sharing their findings with one another. More than 100 000 structures have been posted on the PDB since 1973 and are freely available to all. The PDB can be accessed online.

Most of the structural data is obtained from X-ray crystallography experiments, as discussed in section 17.4. More recently, nuclear magnetic resonance (NMR) spectroscopy has also been used to determine the three-dimensional structure of proteins. Once uploaded to the PDB, the protein structure can be visualised in a number of different ways (Figure 18.1.3).

The PDB also includes a large amount of additional information such as the known functions of proteins, their location within the cell and any molecules that they are known to interact with. Understanding the structure of a large molecule helps in understanding its function. Such knowledge can be used to gain a better understanding of the role of a protein in human health and disease.

For example, cyclooxygenases are enzymes that catalyse one of the steps involved in the production of prostaglandins. Prostaglandins are involved in inflammation and pain signalling. The PDB entry for cyclooxygenase shows the three-dimensional shape of the enzyme and lists the molecules that are known to interact with it and affect its function. These include the common pain killer salicylic acid (aspirin) and many other anti-inflammatory drugs such as ibuprofen (Figure 18.1.4)

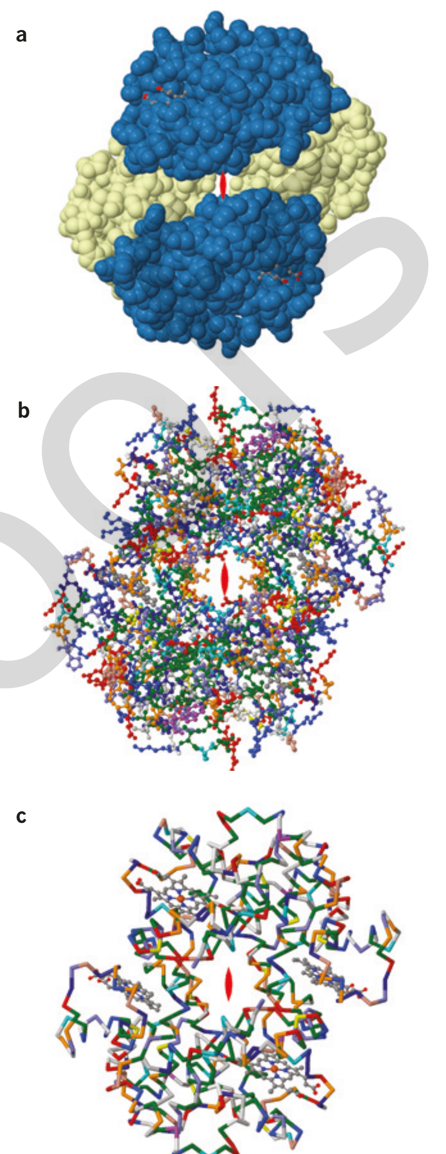

FIGURE 18.1.3 The Protein Data Bank enables scientists to view protein structures in a number of different ways. (a) A space-filling model showing four subunits of the protein haemoglobin. (b) A ball and stick model showing all amino acids in different colours. (c) The backbone of the protein haemoglobin, with amino acids in different colours. Hydrogen bonds are shown as dashed lines. The position of the haem group in the protein is visible, with the orange Fe^{2+} ion in the centre.

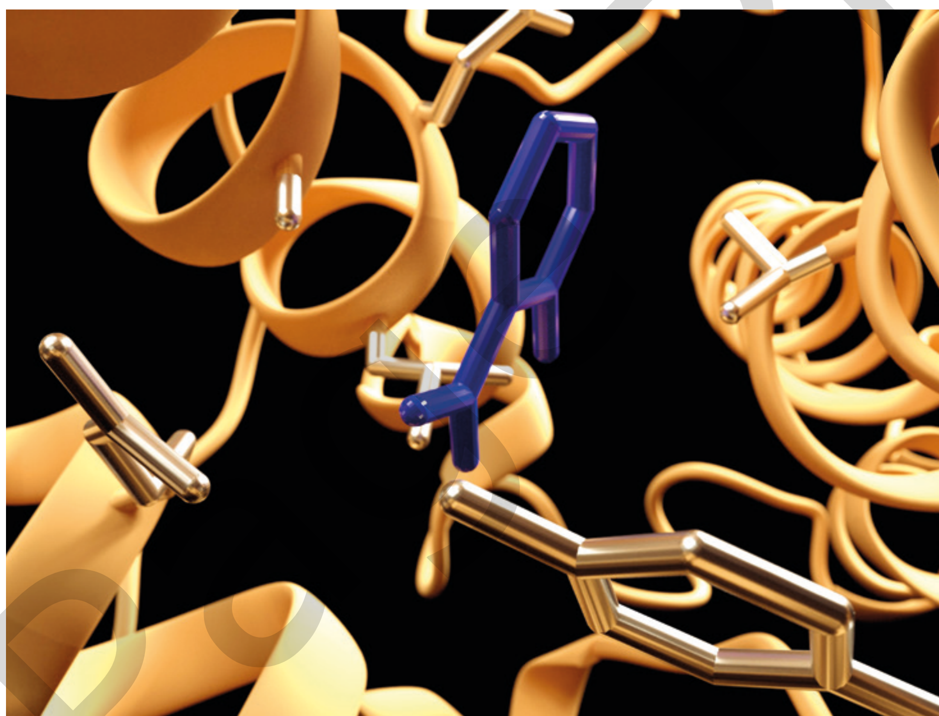

FIGURE 18.1.4 Aspirin is converted to salicylic acid and ethanoic (acetic) acid in the body. The binding of salicylic acid (blue) deactivates the cyclooxygenase enzyme (yellow) and prevents it from producing prostaglandins that are involved in pain signalling and inflammation.

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CHEMFILE

Drug discovery

Historically, drugs were discovered either by chance or by analysing the compounds present in traditional remedies. For example, in the 1970s, one of the compounds present in the herb sweet wormwood (*Artemisia annua*) was found to have anti-malarial properties. This herb had been used for over 2000 years in China as a traditional treatment against the malaria-causing parasite *Plasmodium*, carried by mosquitoes (Figure 18.1.5). The isolated compound, known by the name artemisinin, is now widely used as an anti-malarial drug throughout the world. The 2015 Nobel Prize in Physiology or Medicine was awarded for this discovery. However, the problem with this approach to drug discovery is that it can take a long time and there are often issues with isolating the active compound in sufficient quantities.

FIGURE 18.1.5 Coloured scanning electron micrograph of a female mosquito, carrier of the parasite *Plasmodium*, which causes malaria.

With greater access to protein structural data, it is now common for scientists to use a more targeted approach to discover potential drugs. Using this approach, scientists begin by identifying the proteins that are involved in particular disease conditions. They can then screen chemical libraries, containing large numbers of small molecules, to identify compounds that bind to the particular protein of interest. These compounds are then tested in cells and animal models to test their biological action. Alternatively, the large number of molecules in chemical libraries can be tested directly in diseased cells in order to identify potential drugs. The development of databases such as the Protein Data Bank assist in this drug discovery process, because they hold information about protein structure and the molecules that are known to interact with them, as well as having sophisticated search functions.

Many drugs have been developed in this way in recent years. One such example is a new drug (DDD107498) that also shows promise as an anti-malarial treatment. The development of this drug is extremely timely because there are signs of malaria parasites developing resistance to artemisinin. DDD107498 was discovered by testing a chemical library containing 4371 compounds against the malaria parasite. One compound was found to destroy the parasite at each stage in its life cycle. It was then modified slightly to improve its suitability as a single-use drug, and is currently undergoing further testing.

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18.1 Review

SUMMARY

- Proteins perform many functional roles within living organisms. The function of every protein is closely linked to its three-dimensional shape.
- The Protein Data Bank (PDB) is a database of three-dimensional structures of large biological molecules such as proteins.

KEY QUESTIONS

1. When a scientist determines the primary structure of a new protein, the Protein Data Bank can enable the function of the protein to be predicted. Explain this process.
2. Why is it important that the Protein Data Bank is freely available?
3. Oxytocin is a hormone that causes contractions during child birth. It is a polypeptide containing nine amino acids in the sequence:
   Cys–Tyr–Ile–Gln–Asn–Cys–Pro–Leu–Gly
   The image of oxytocin in Figure 18.1.6 is a 'ball and stick' computer model. The colour code of the atoms is as follows: carbon (grey), oxygen (red), nitrogen (blue), hydrogen (white) and sulfur (yellow). Use this model and the labels to describe the chemical structure of this polypeptide. In your answer, name the type of chemical bond labelled A and explain the significance of the groups labelled B–D.

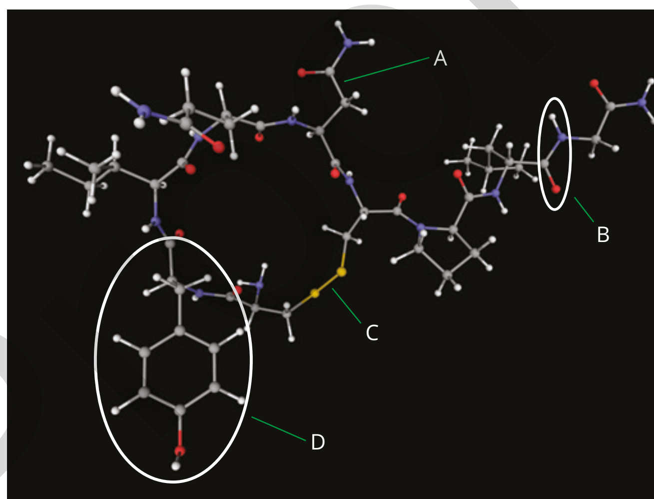

FIGURE 18.1.6 A ball and stick model of the hormone oxytocin

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18.2 Enzymes

Thousands of chemical reactions are involved in sustaining life. These reactions occur in a highly organised, sequential fashion. The biological catalysts that accelerate the rate of chemical reactions in cells are a particular type of protein called **enzymes**.

In this section, you will learn about several models that biochemists use to describe enzyme catalysis. Enzymes are highly efficient catalysts that can increase reaction rates by as much as a factor of 10^{10}. This is the equivalent of taking 1 second to complete a task that normally takes 300 years.

ROLE OF ENZYMES

As you learnt in Chapter 1, for a reaction to occur, the reactants need to collide with each other with sufficient energy and the correct orientation. Catalysts are able to increase the rate of reaction by providing an alternative reaction pathway with a lower activation energy, thereby increasing the proportion of reactant particles with sufficient energy to react (Figure 18.2.1). Inorganic catalysts and biological catalysts (enzymes) act in a similar way and share the following characteristics. Both inorganic catalysts and enzymes:

- are only needed in relatively small amounts
- are not used up or changed at the end of the reaction
- do not alter a reaction's equilibrium position.

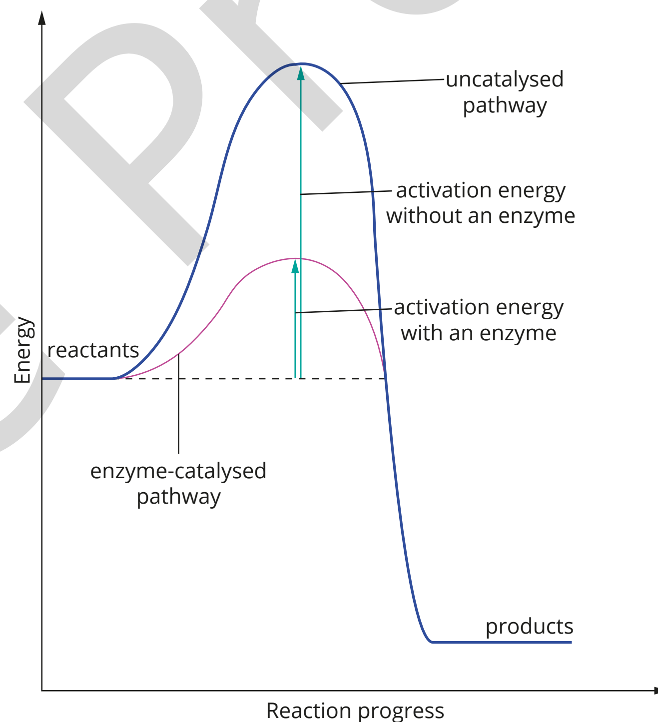

FIGURE 18.2.1 Both inorganic catalysts and enzymes lower the activation energy of a reaction by providing an alternative pathway by which the reaction can occur.

However, there is a significant difference between the behaviour of enzymes and the behaviour of inorganic catalysts. An inorganic catalyst, such as metallic platinum, can catalyse many different reactions often involving a variety of reactants. Enzymes can only catalyse one specific reaction or a reaction that involves a particular chemical bond or functional group. This characteristic is often referred to as the 'specificity of enzymes'. For example, despite being very similar, the two sugars lactose and sucrose are broken down by two different enzymes in our bodies. The models of enzyme action developed by scientists must account for this specificity. Enzymes are also more sensitive than inorganic catalysts to changes in reaction conditions.

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Living organisms are very complex systems. With so many reactions required to sustain each living organism, thousands of different enzymes are needed. It is important that chemists understand the role of enzymes and their action when they develop new medications. It is also important to investigate the potential of these complex catalysts for use in greener industrial processes.

The catalytic action of enzymes is specific for a single reaction or type of reaction.

LOCK-AND-KEY MODEL OF ENZYME ACTION

The catalytic activity of an enzyme is highly specific and depends on its overall three-dimensional structure. Because enzymes are proteins, their overall three-dimensional structure is dictated by their tertiary structure. The specific part of the enzyme molecule with which a reactant can interact is known as its **active site**. The active site is usually a uniquely shaped flexible hollow or cavity within the protein where the reaction occurs. The reactant molecule that binds with the active site is referred to as the **substrate**.

One early model for the catalytic action of an enzyme is the **'lock-and-key' model**. This model provides an explanation for the critical importance of the three-dimensional shape of the enzyme. In the lock-and-key model, the substrate molecule fits into the enzyme like a key in a lock, forming an **enzyme–substrate complex**, allowing the enzyme to break the bonds in the substrate. Figure 18.2.2 shows the steps involved in an enzyme-catalysed reaction according to the lock-and-key model. The enzyme is specific for a particular substrate, so binding of a different molecule will not result in a reaction.

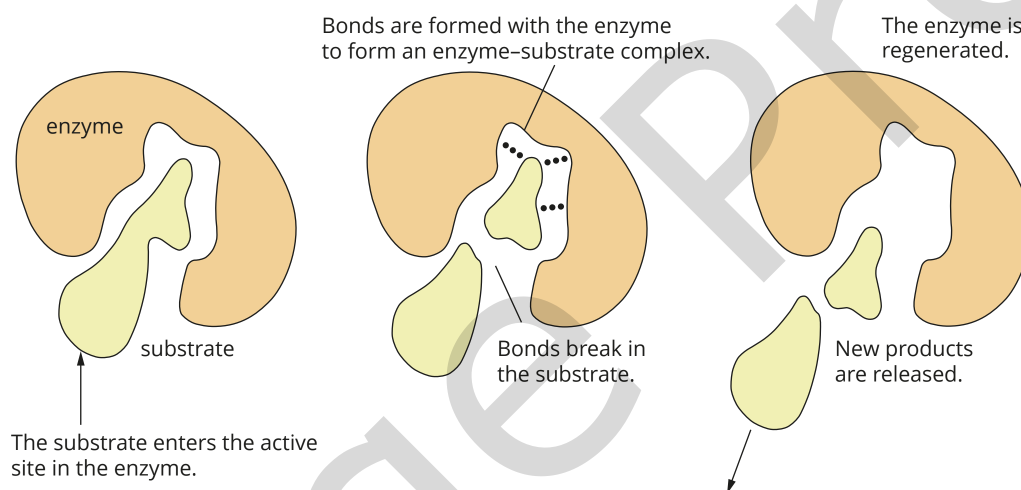

FIGURE 18.2.2 Steps in the action of an enzyme, according to the lock-and-key model

The shape of the substrate molecule must match the shape of the active site. Only reactant molecules that have a suitable 'key' shape can enter and form the necessary intermolecular interactions with the active site ('lock') of the enzyme.

Example—hydrolysis of proteins

The digestion of proteins into smaller polypeptide molecules by enzymes called proteases can be understood using the lock and key model. As you learnt in Chapter 17, amino acids are joined together by peptide bonds to form a protein in a condensation reaction. This reaction can be reversed and the peptide bonds broken by the addition of water in a hydrolysis reaction. However, due to the strength of the peptide bonds, this hydrolysis reaction does not occur very rapidly without the presence of an enzyme.

Trypsin is an enzyme that breaks a protein chain next to a lysine or arginine amino acid (on the C-terminal side). It binds to the protein chain at that point and weakens that particular peptide bond, reducing the amount of energy required for it to react with a water molecule (Figure 18.2.3).

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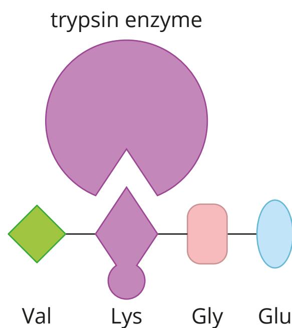
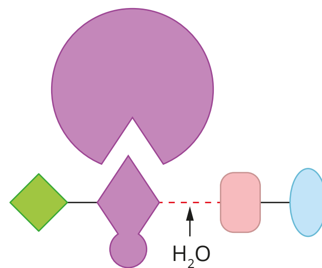
The enzyme trypsin binds to the protein chain on the C-terminal side of a lysine residue, forming an enzyme–substrate complex.



The peptide bond is weakened, enabling it to react with a water molecule (hydrolysis).



Two shorter polypeptides are formed and the enzyme is regenerated and able to catalyse another reaction.

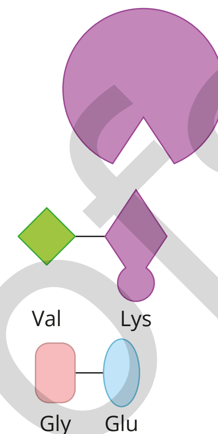


FIGURE 18.2.3 Trypsin is an enzyme that catalyses the hydrolysis of a protein chain next to an arginine or lysine amino acid. Its specific shape enables it to bind to the protein chain at that point and weaken a peptide bond, meaning that less energy is required for it to be broken by reaction with a water molecule.

The types of intermolecular bonds formed between an enzyme and a substrate are the same as those that determine the tertiary structure of proteins. The intermolecular forces are determined by the side chains of the amino acids in the peptide sequence and can include hydrogen bonds, ionic interactions, dipole–dipole attractions and dispersion forces. In the example above, both lysine and arginine contain amine groups (NH_2) on their respective side chains. These amine groups form hydrogen bonds with the active site of the trypsin enzyme, forming the enzyme–substrate complex.

INDUCED FIT MODEL OF ENZYME ACTION

Since the lock-and-key model for enzyme action was proposed, chemists have realised that enzymes have flexible structures. The shape of an enzyme's active site can be modified markedly by the binding of a substrate. This discovery led to a modification of the lock-and-key model and the development of the **'induced fit' model**. The induced fit model for enzymes can be applied to a larger number of chemical reactions.

As Figure 18.2.4 shows, the flexible active site can mould itself to achieve a better fit for substrate molecules. After the reaction, the products are released from the active site and the active site returns to its initial shape.

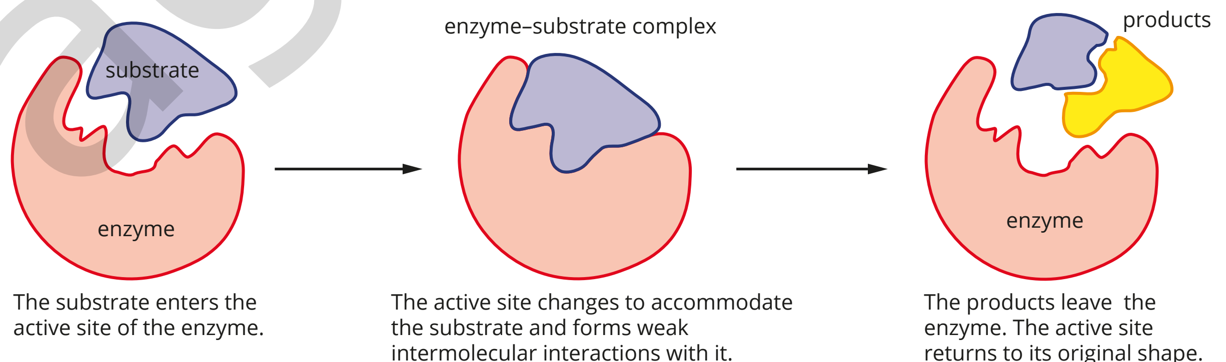


FIGURE 18.2.4 Steps in the action of an enzyme, according to the induced fit model

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CHEMISTRY IN ACTION

From lock-and-key to induced fit

The development of the lock-and-key and induced fit models of enzymes is a good example of how scientific ideas change over time as new evidence becomes available.

Emil Herman Fischer (Figure 18.2.5) was a remarkable chemist. He was awarded the Nobel Prize in Chemistry in 1902 for his work on identifying the 16 isomers of the aldo-hexoses, the family of sugars that glucose belongs to. His work on biomolecules and the precise stereochemistry of their different forms led him to propose the lock-and-key model of enzyme–substrate interactions.

This model formed the main explanation for the interactions in enzyme active sites and receptor sites until the American chemist Daniel Koshland (Figure 18.2.6) proposed the induced fit model of enzyme interactions.

Koshland described his model as more like a hand in glove, where the enzyme is flexible, not rigid, and can change shape slightly on binding of the substrate. Figure 18.2.7 shows a simplified version of Fischer's lock-and-key model and Koshland's induced fit model. Koshland was able to build on the hypothesis put forward by Fischer to develop what is now considered to be a more accurate representation of enzyme interactions.

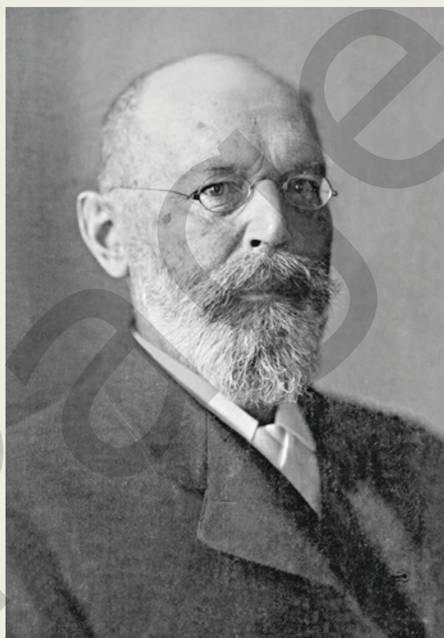
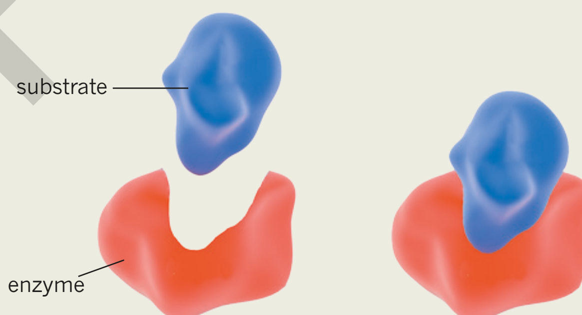


FIGURE 18.2.5 Emil Hermann Fischer (1852–1919) proposed the lock-and-key model of enzyme–substrate interactions.

FIGURE 18.2.6 Daniel Koshland (1920–2007) further developed Fischer's lock-and-key model by proposing the revised induced fit model for enzyme interactions.

a Lock-and-key model



b Induced fit model

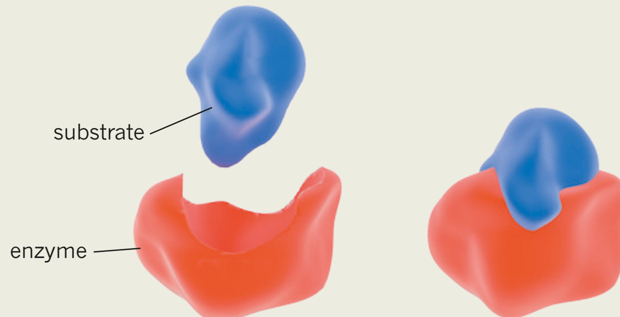


FIGURE 18.2.7 The two models of enzyme interaction proposed by (a) Fischer and (b) Koshland. The development of these models is an example of how scientific ideas change over time, being refined and adapted as new insights and discoveries are made.

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EXTENSION

Coenzymes

Many enzymes cannot function without the presence of a coenzyme. A coenzyme is a non-protein organic compound that interacts with an enzyme and changes its functionality. Coenzymes are small compared to protein molecules. Similarly, some enzymes require inorganic atoms or molecules such as metal ions to function correctly. These are called cofactors rather than coenzymes.

Many coenzymes are derived from vitamins. Vitamins are essential micronutrients that are required in small amounts as part of a balanced diet. Figure 18.2.8 shows the coenzyme folic acid bound into dihydrofolate reductase, an enzyme found in *Escherichia coli* bacteria in the human gut.

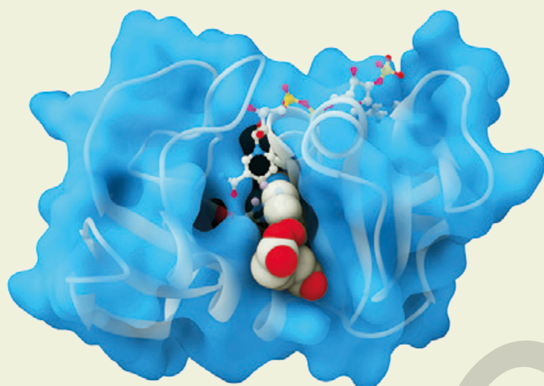

FIGURE 18.2.8 Folic acid acts as a coenzyme, binding to the dihydrofolate reductase enzyme.

Coenzymes interact with the enzyme during catalysis and their role is often to act as carriers of electrons or specific groups of atoms. They change the surface shape of the enzyme and hence the binding properties of the active site, allowing the enzyme to better interact with the substrate.

Before binding to the coenzyme, the enzyme is inactive. You can see this in Figure 18.2.9. Once the coenzyme is bound, the newly formed enzyme–coenzyme complex can interact fully with the substrate and the catalytic process can occur.

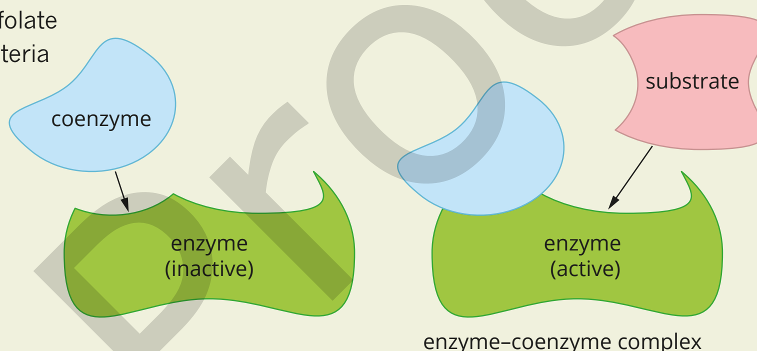

FIGURE 18.2.9 Coenzymes work by binding to particular enzymes. The newly formed complex can bind to the substrate, allowing the reaction to be catalysed.

Unlike an enzyme, a coenzyme may be changed as a result of the reaction as it accepts or donates an electron or group of atoms. If it accepts a particular group of atoms in one biochemical reaction it helps catalyse, there will be other reactions in which it is restored to its original form by the loss of the group.

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18.2 Review

SUMMARY

- Enzymes are proteins that catalyse biochemical reactions by providing an alternative reaction pathway with a lower activation energy.
- Enzymes are not changed as a result of the process of catalysis.
- Enzymes are highly specific; enzymes may only catalyse one specific reaction or a reaction with a particular chemical bond or functional group.
- There are thousands of enzymes in the human body to catalyse different biochemical reactions.
- Enzyme molecules have uniquely shaped active sites that interact with specific reactant molecules (substrates), weakening or breaking the bonds in the reactant molecules.
- The earliest model to account for enzyme action was the lock-and-key model.
- A newer model for enzyme action is the induced fit model. This model accounts for the flexibility of many enzymes' active sites.

KEY QUESTIONS

1 The enzyme glucokinase catalyses the first step in the oxidation of sugar in human liver cells, in a process called glycolysis. Which one of these statements about this process is correct?

A Many other enzymes can also catalyse this reaction.
B Glucokinase increases the activation energy of this reaction.
C Glucokinase is able to catalyse many other reactions.
D Glucokinase is a protein.

2 **a** The steps in the action of an enzyme involve, in particular, an active site and a substrate. Use a diagram to describe in detail the action of an enzyme (according to the lock-and-key model of enzyme action).

b The forces of attraction that enable a substrate to bind to an active site can vary. Identify four such forces.

3 Identify whether each of the statements about the induced fit model of enzyme catalysis is true or false.

a This model explains why enzymes do not catalyse a wide range of reactions.
b The enzyme molecule has an active site.
c The active site does not change shape as the substrate enters.
d An enzyme–substrate complex forms.

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18.3 Enzymes—dependence on pH and temperature

As you learnt in the previous section, enzymes share many characteristics with inorganic catalysts. By lowering a reaction's activation energy barrier, both enzymes and inorganic catalysts increase reaction rate without altering the equilibrium position or being changed themselves by the process. One significant difference between the two types of catalysts is the sensitivity of enzymes to reaction conditions such as temperature and pH (Figure 18.3.1).

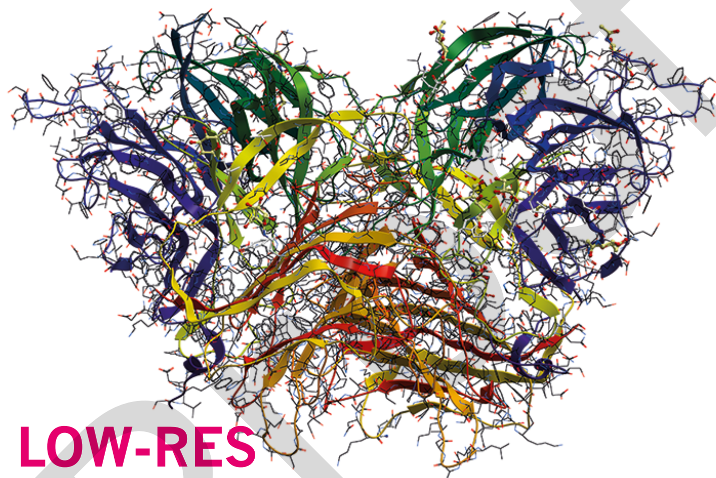

FIGURE 18.3.1 A ribbon model of the enzyme invertase (also called sucrase), which catalyses the hydrolysis of sucrose. It operates under mild conditions but is very sensitive to changes in temperature and pH.

DEPENDENCE ON pH

Enzymes operate effectively only within a narrow pH range, as the graph in Figure 18.3.2 shows. Salivary amylase catalyses the breakdown of starch to a disaccharide, maltose, in the relatively neutral environment of the mouth. Pepsin catalyses the breakdown of proteins to amino acids in the acidic conditions of the stomach. Activity of these enzymes drops off drastically outside their normal conditions.

Pepsin is active only at pH values below 3 and amylase is active between pH 5 and 9. The pH at which the **enzyme activity** is greatest is known as the enzyme's **optimum pH**. The optimum pH of pepsin is 1.5, while the optimum pH of salivary amylase is 7.2.

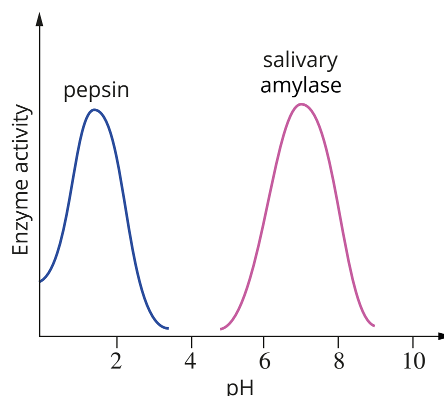

FIGURE 18.3.2 Enzymes are only effective in a narrow pH range. Pepsin is a protein-digesting enzyme secreted into the stomach. Salivary amylase is the enzyme in human saliva. These enzymes are most effective at very different pH values.

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Acid–base properties of enzymes

You will recall that α-amino acids (those found in living systems) can form zwitterions, with the general structure shown in Figure 18.3.3. A zwitterion has both a positive and negative charge within the molecule.

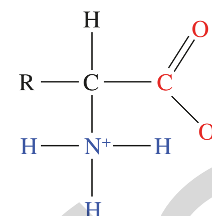
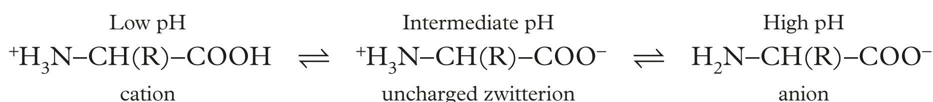


FIGURE 18.3.3 The general structure of a zwitterion of an amino acid. Different amino acids have different side chains, represented by the R group.

Amino acids have different charges depending on the pH of the surrounding environment.

- At high pH, the $-NH_3^+$ group can act as an acid, donating a proton to become an $-NH_2$ group.
- At low pH, the $-COO^-$ group can act as a base, accepting a proton to become a –COOH group.

As the following equation shows, the charge on the predominant form of the amino acid present in a solution depends on the pH of the solution.



Just as the ionisation of the amino and carboxyl groups in amino acids depends on pH, some of the side chains of the amino acids may also be affected by changing the pH.

Intermolecular attractions between the side chains of a polypeptide maintain a protein's overall three-dimensional structure. Some bonds that determine the tertiary structure of an enzyme may be disrupted as changes in pH alter the ionisation of some side chains.

For example, the ionic interactions shown in Figure 18.3.4 might occur at pH 7 between side chains of the amino acid units along the protein chain, but not at pH 3 or 10. The change from neutral pH to an acidic pH of 3 would mean the carboxylic acid on the side chain is not likely to ionise and will therefore not carry the negative charge required for the ionic interaction to occur. The opposite situation would be true with a change from neutral pH to the basic pH 10. At the higher pH, the amino group in the other side chain would not act as a base, remaining uncharged and unable to participate in the ionic interaction.

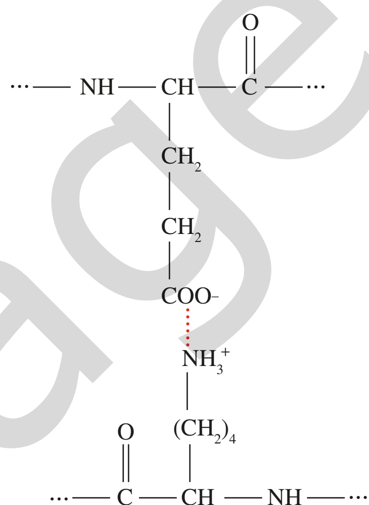


FIGURE 18.3.4 An ionic interaction linking two parts of a polypeptide chain. Ionic interactions between side chains of amino acids in proteins are dependent on pH. One R group contains $-NH_3^+$ and another R group contains $-COO^-$.

In this way, changes in pH can have a large impact on the stability of enzyme structure. As the tertiary structure of the enzyme is disrupted, the enzyme's active site changes shape and enzyme activity decreases. Extremely high or low pH values generally cause complete loss of activity for most enzymes. Drastic changes to pH can result in a permanent change to the shape of an enzyme through a process called **denaturation** (see below).

Changes in pH alter the charge of side chains containing amino and carboxyl functional groups.

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DEPENDENCE ON TEMPERATURE

Enzyme activity is also affected by temperature. The graph in Figure 18.3.5 shows the effect of temperature on the rate of a reaction involved in carbohydrate metabolism. You can see from the steep sides of the curve that the rate of reaction drops off quickly either side of a narrow temperature range (30–40°C).

The temperature at which the enzyme activity is greatest is known as the enzyme's **optimum temperature**. Enzymes that operate inside human cells have an optimum temperature of about 37°C.

At temperatures above and below the optimum temperature, enzyme function is impaired. This is one of the reasons why conditions such as hypothermia and fever (when you have an abnormally low or high temperature) are life-threatening.

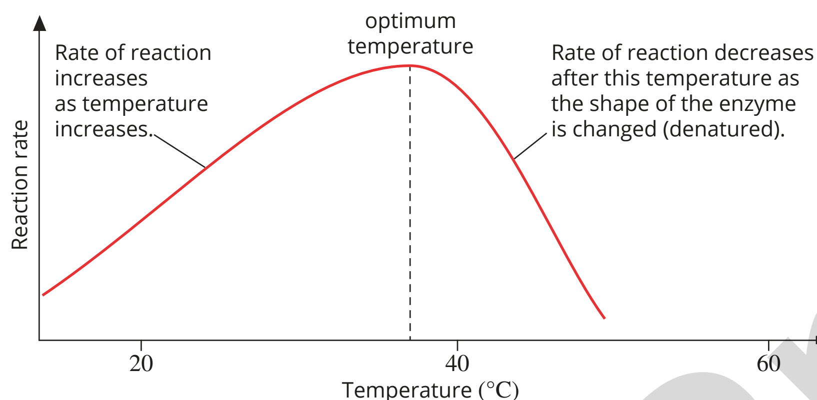

FIGURE 18.3.5 Effect of temperature on rate of reaction for an enzyme-catalysed reaction. Enzymes are only effective in a relatively narrow range of temperatures. Reaction rate is highest at the optimum temperature.

An increase above or decrease below the optimum temperature has a different effect on an enzyme.

- As the temperature increases above the optimum temperature, the increased kinetic energy of the molecules disrupts the structure of the enzyme. The increased movement throughout the enzyme breaks some of the intermolecular forces responsible for the tertiary structure. This change in three-dimensional shape of the enzyme means the active site can no longer effectively catalyse the reaction so the reaction rate decreases rapidly.
- As the temperature decreases below the optimum temperature, the enzyme and substrate molecules have lower kinetic energies, resulting in less frequent and less energetic collisions between the molecules. Additionally, under the induced fit model discussed in section 18.2, enzymes need a certain amount of flexibility so that the shape of the active site can change upon substrate binding. When the temperature is too low, the enzyme is not flexible enough for this change of shape to take place, and therefore it cannot function properly.

Denaturation

Once the temperature becomes too high, the increased kinetic energy of the polypeptide chains of the enzyme breaks some of the bonds between side chains of the amino acid units, and new bonds are formed. A change in the enzyme's tertiary structure causes a change in the shape of the active site and the enzyme loses its catalytic activity. It is said to be denatured. This change to the protein structure is often irreversible (Figure 18.3.7).

CHEMFILE

Hyperthermophiles

A hyperthermophile is a type of bacteria that thrives in extremely hot environments, at temperatures of 60°C and higher. Many hyperthermophiles can also withstand other environmental extremes, such as high acidity or radiation. Hyperthermophiles were first discovered in 1965, in the hot springs in Yellowstone National Park (Figure 18.3.6). Since then, more than 70 different species of bacteria capable of withstanding these high temperatures have been discovered.

FIGURE 18.3.6 An aerial photograph of the large geothermal spring in the Yellowstone National Park, Wyoming, USA. The water's temperature can reach more than 70°C. The bright colours in the pool are due to minerals that are deposited from the evaporation of water. The colour differences within the pool are due to different species of hyperthermophilic bacteria.

In order for hyperthermophiles to survive under these extreme conditions, their proteins must be able to maintain their three-dimensional shape at high temperatures rather than being denatured. Indeed, many of the proteins in these organisms have higher levels of hydrogen bonding and ionic interactions stabilising their three-dimensional shape than similar proteins in normal bacterial cells.

These hyperthermostable proteins are of interest commercially, because they may be able to catalyse industrial processes at higher temperatures, increasing the rate of reaction.

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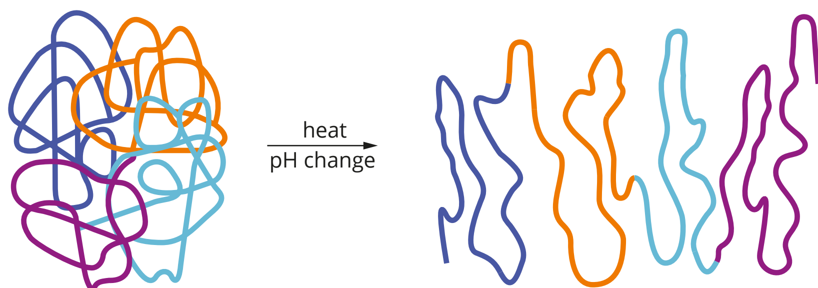

FIGURE 18.3.7 The specific three-dimensional shape of enzymes is lost when denaturation occurs.

Enzymes can also be denatured by a change in pH. As discussed earlier, when the pH is above or below the enzyme's optimum pH, the enzyme's overall three-dimensional shape can also be disrupted. Substantial changes in pH can change an enzyme's structure permanently.

Comparing denaturation with hydrolysis

Even though increased temperature and variations in pH can permanently change the tertiary structure of an enzyme, the primary structure of the protein, the covalently bonded sequence of amino acids, remains intact.

You will recall from Chapter 17 that the breakdown of proteins occurs through a process called hydrolysis. The hydrolysis of a protein molecule involves breaking the covalent peptide bonds. This is shown in Figure 18.3.8.

Polypeptide found in food

H_2O enzyme-catalysed hydrolysis in the stomach

Dipeptides

H_2O enzyme-catalysed hydrolysis in the intestine

Amino acids

absorbed into the bloodstream from the small intestine

FIGURE 18.3.8 Digestion of protein in food. The blue dashed lines indicate where peptide links are broken during hydrolysis of the polypeptide.

Page Proofs

The products of hydrolysis are shorter polypeptide chains or individual amino acids. Note that water is a reactant in this process. One water molecule is consumed for each peptide bond broken.

In the laboratory, extreme conditions are required to hydrolyse a protein. Typically, the protein sample is heated in 6 mol L^{-1} hydrochloric acid for 24–72 hours.

CHEMFILE

Denaturing egg white

When egg white is heated, the clear liquid turns into an opaque solid. This is an everyday example of the denaturing of a protein. Egg white contains a protein called albumin. The denatured protein has very different chemical and physical properties from the original protein.

The protein in egg white can be denatured in a variety of ways. Some are irreversible, such as the cooking of an egg; others are reversible.

If you add salts such as ammonium sulfate to egg white, the water that surrounds the proteins is drawn away and forms ion–dipole bonds with the ions of the salt. Without the protective layer of water stabilising its tertiary structure, the protein denatures and precipitates out of solution. If you add enough water to hydrate the ions and the protein strands, the protein refolds and dissolves back into solution. In this instance, the denaturation of the protein is reversible.

When denaturation occurs because the hydrogen bonds in the protein molecules are disrupted, the process is irreversible. This can happen when the protein is heated, such as when you boil or fry an egg (Figure 18.3.9), or when protein is mixed with strong acids such as nitric acid. When an egg white is heated, the hydrophobic amino acid side chains that were hidden within the folded albumin molecules are exposed and aggregate together by forming dispersion forces, changing the texture and colour of the egg white.

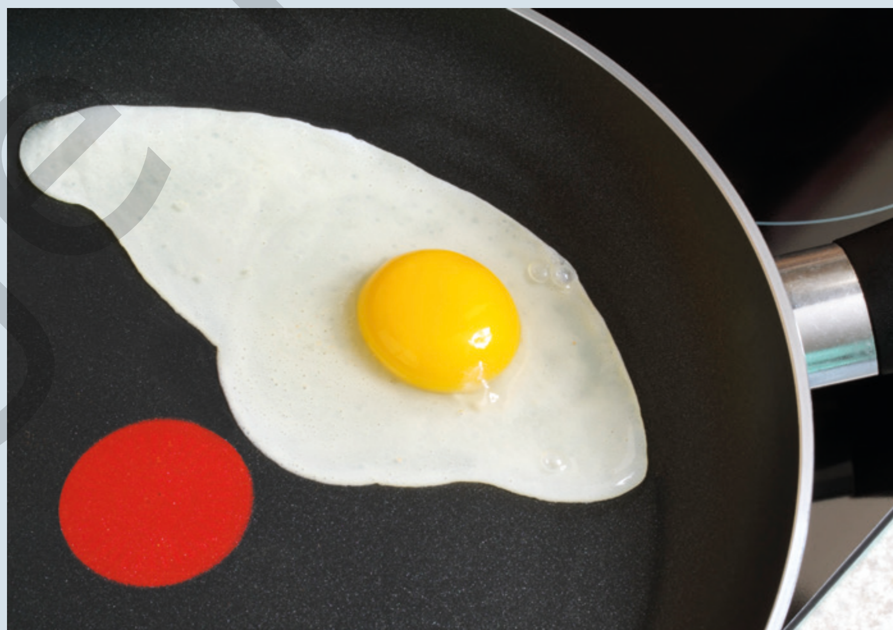

FIGURE 18.3.9 When an egg white is heated, the hydrogen bonds maintaining the tertiary structure are disrupted and the protein albumin irreversibly unfolds (denatures).

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18.3 Review

SUMMARY

- Enzymes operate over an extremely mild and narrow set of conditions when compared with inorganic catalysts.
- Enzymes are very sensitive to changes in pH and temperature.
- An enzyme is said to be denatured if its tertiary structure is disrupted; for example, by high temperatures or pH changes.
- When an enzyme is denatured, the shape of its active site is changed and its catalytic activity is lost.
- During the hydrolysis of a protein, the primary structure is broken down as peptide links are broken. This occurs during digestion of food.
- The temperature at which the enzyme activity is greatest is the enzyme's optimum temperature.
- High temperature denatures an enzyme because the increased kinetic energy of the polypeptide chain disrupts the enzyme's tertiary and quaternary structure.
- At lower temperatures, fewer, less energetic collisions occur per unit time between the enzyme and substrate so the rate of reactions is slower.
- The pH at which the enzyme activity is greatest is the enzyme's optimum pH.
- The charges on some side chains in the polypeptide chain of an enzyme depend on the pH of the solution.
- Changes to the charges on the side chains that occur as pH changes can result in a new tertiary structure for an enzyme.
- The optimum pH and temperature of an enzyme usually match the conditions in which the enzyme operates.

KEY QUESTIONS

1 The structure of proteins can be disrupted by denaturation or hydrolysis. Describe each process and the bonds that are disrupted for each.

2 Enzyme activity is influenced by changes in pH. Changing pH can alter the charge on some functional groups because they act as weak acids or bases. The side chains of a polypeptide chain are important in maintaining the enzyme's tertiary structure. Complete the following table for each of the side chains listed.

Structure of side chain	Is the side chain acidic or basic or neither?	Is the side chain positively charged, negatively charged or neutral?	
		In a solution of pH 2	In a solution of pH 11
$-CH_2COOH$			
$-CH_2CH(CH_3)_2$			
$-CH_2OH$			
$-(CH_2)_4NH_2$			

3 The enzyme carbonic anhydrase catalyses the decomposition of carbonic acid molecules to carbon dioxide and water in the lungs. When heated above 60°C, the enzyme becomes denatured.

a What is meant by 'denatured'?

b Describe what usually occurs to the structure of an enzyme when the enzyme is denatured.

c Does the primary structure of the carbonic anhydrase enzyme change during the process?

d Why is the function of the enzyme closely related to its tertiary structure?

4 One of the key differences between enzymes and inorganic catalysts is how they are affected by their environment.

a How can a change in pH effect enzyme activity?

b What effect does decreasing the temperature have on enzyme activity?

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18.4 Enzymes in industry

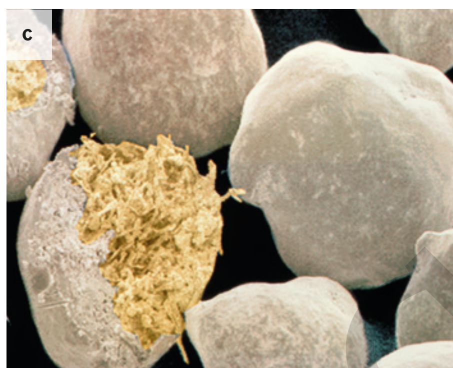

FIGURE 18.4.1 Enzymes are used in a number of different commercial applications. (a) Malted barley is mixed with water and heated during the brewing of beer. Enzymes in the malt break down the starch into simple sugars. (b) The enzymes in yeast consume sugars and produce carbon dioxide, causing bread to rise. (c) A scanning electron micrograph of a biological washing powder. The capsules contain enzymes that break down fats, oils or proteins that may have stained the clothing.

Enzymes have been used for thousands of years to produce food such as cheese, wine and bread. More recently, they have been increasingly used in industrial processes and in a range of commercial applications (Figure 18.4.1). For example, washing powders often contain enzymes such as proteases, lipases and amylases, which break down proteins, fats and starches respectively. These enzymes are able to break down many of the molecules found in common stains and have the advantage of being biodegradable.

Enzymes are also being used increasingly in industrial processes to catalyse reactions. For example, several companies are using the enzyme lipase to catalyse the production of biodiesel as an alternative to the traditional base-catalysed process. This process is discussed in greater detail in Chapter 16.

ADVANTAGES AND DISADVANTAGES

There are advantages and disadvantages to using enzymes as catalysts in industrial processes. These are summarised in Table 18.4.1.

TABLE 18.4.1 Advantages and disadvantages of using enzymes as industrial catalysts

Advantages	Disadvantages
• Enzymes are specific so that they only catalyse one particular reaction or type of reaction. • In general, enzymes are effective at biological temperatures and pH levels. This saves energy and cost as high temperatures and pressures are not required. • Enzymes are not consumed in the reaction, so they can be used for a long period of time. • Enzymes are biodegradable and therefore cause less environmental pollution.	• Enzymes are very sensitive to changes in temperature and pH. Reaction conditions must therefore be tightly controlled. • Certain chemicals can also change the structure of enzymes and cause them to lose their function. • Enzymes can be expensive to produce. • Enzyme-catalysed reactions generally take place in aqueous solutions. It can be difficult to separate the products from the reaction mixture.

The key advantage of using enzymes as industrial catalysts is that, in general, they function optimally under biological conditions. This removes the need for high pressures and temperatures to be used to maintain a fast rate of reaction, thereby reducing the energy costs and safety considerations involved in the process. This addresses some of the ideas of green chemistry discussed in Chapter 12. Due to their unique shape, enzymes are also extremely specific, catalysing only one particular reaction or type of reaction. This can increase the efficiency of the industrial process in that side-reactions do not take place.

The major disadvantage of using enzymes as industrial catalysts is that they are very sensitive to changes to pH and temperature. As discussed in section 18.3, changes in temperature and pH affect the interactions between amino acids on the surface of the enzyme. This can lead to a change in the enzyme's three-dimensional shape and a loss of enzyme function. The presence of other molecules in the reaction mixture may also adversely affect the shape of the active site and hence the function of the enzyme.

PRODUCTION OF ETHANOL

The production of ethanol is a large global industry. In 2015, over 100 billion litres of ethanol were produced worldwide. Ethanol has many uses, including industrial solvents, antiseptics, precursors for other chemical reactions, and in alcoholic beverages. However, its major use is as a fuel source, particularly in Brazil and the United States. It can be produced by two different processes: by fermentation, which is an enzymatic process using corn, sugar cane or other grains as a starting material; or by the hydrolysis of ethene, which uses crude oil as a starting material.

Page Proofs

Fermentation

For thousands of years, humans have used enzymes to convert starches from grains and sugars to ethanol in the process known as **fermentation**. In this process, amylase enzymes are used to catalyse the breakdown of the polysaccharide starch to glucose. Then the fermentation process uses other enzymes from yeast organisms to convert small sugar molecules, such as glucose and fructose, into ethanol and carbon dioxide, according to the following equation:

$$C_6H_{12}O_6(aq) \rightarrow 2CH_3CH_2OH(aq) + 2CO_2(g)$$

In wine-making, yeasts are found naturally present on the surface of grapes and in wine cellars and do not need to be added to the fermentation mixture. However, in beer brewing, yeast is added to the barley mixture to catalyse the fermentation process. Margaret River, in the south of Western Australia, is a region known for its winemaking (Figure 18.4.3). Many boutique breweries have also opened recently in the region.

FIGURE 18.4.3 Palmer Wines is in the Margaret River Region, Western Australia.

The fermentation process is also used commercially to produce purified forms of ethanol for other purposes, such as as a fuel. While starches and sugars are often used as the raw materials for fermentation, another polysaccharide called cellulose, which is found in biomass such as woody plants, can also serve as a raw material for fermentation. Aside from producing a valuable chemical, the use of cellulose to produce ethanol reduces the need to dispose of waste materials in landfill or by burning.

The grains and other matter used for industrial fermentation are prepared by grinding and crushing, adding water and then heating to 85–105°C in the presence of cellulase and amylase enzymes. This process breaks down the polysaccharides present in the raw materials into simple sugars called monosaccharides that can undergo fermentation.

After being cooled, the processed raw materials undergo fermentation in fermentation tanks. The process stops when the ethanol content is 15–18%, at which point the yeast cells and their enzymes can no longer function. The fermented mixture then undergoes repeated distillations to purify the ethanol. When cooled, the resulting liquid contains about 96% ethanol and 4% water. This process is depicted in Figure 18.4.4.

CHEMFILE

Producing enzymes for industry

Industrial applications generally require large quantities of enzymes that are only produced naturally in tiny quantities. Scientists use microorganisms such as yeast and bacterial cells to produce the quantities of enzymes required (Figure 18.4.2). The microorganisms are genetically modified so that the enzyme of interest is expressed (synthesised) at much higher concentrations than usual. After sufficient enzyme has been produced, the cells are removed from the reaction mixture and the enzyme is purified.

FIGURE 18.4.2 A microbe fermentation unit for the production of drugs, hormones and enzymes for medical and industrial use. It is used to ferment microbes that have been genetically engineered to produce a drug or enzyme.

Enzymes that are to be used for industrial applications require very little processing and purification. However, if they are to be used for medical or therapeutic applications, a far more rigorous purification process is needed. Enzymes such as lipase, used to catalyse the production of biodiesel, is manufactured by industrial means. Similarly, the protein insulin, used to treat diabetes, is also produced for medicinal use by a microbial system.

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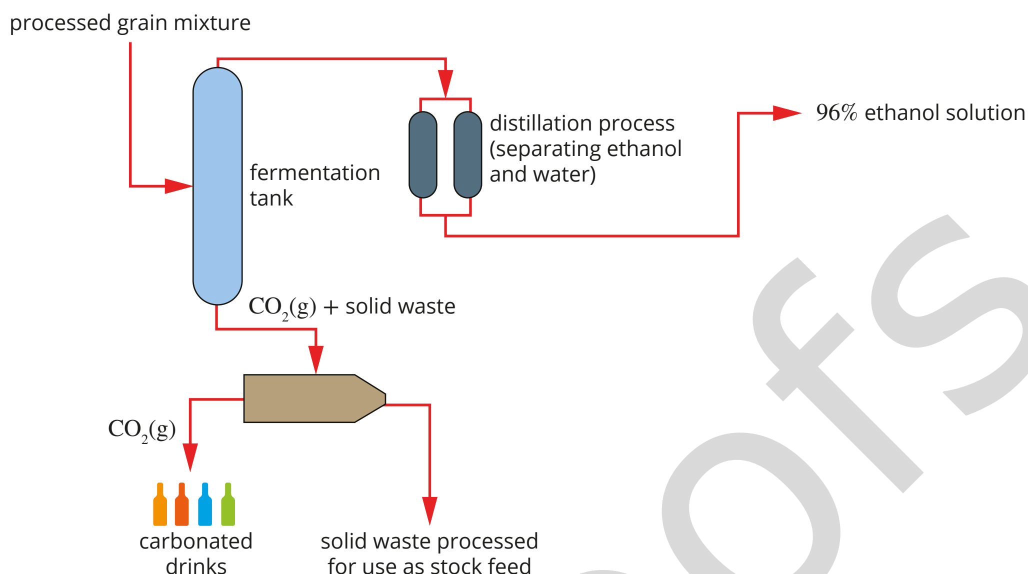


FIGURE 18.4.4 The industrial process for manufacturing ethanol. The products of the fermentation process are separated by distillation.

Once the fermentation and distillation processes are complete, the 96% ethanol mixture is then dehydrated, leaving ethanol that is 99.7% pure. If the ethanol is not being used for alcoholic beverages, the final ethanol is then poisoned by adding up to 5–10% of another chemical such as methanol, to make it unsuitable for consumption as drinking alcohol.

CHEMISTRY IN ACTION

Brewing beer

Beer is one of the world's oldest alcoholic drinks, dating back to over 5000 years ago. Indeed, during the building of the Great Pyramids in Giza, Egypt, there are reports that workers were given a daily ration of 4–5 litres of beer to help them perform their work.

There are four major ingredients in beer: barley, hops, water and yeast, although other additives are often used.

The first step in the process of brewing beer involves extracting the sugars from the barley. To do this, the barley is heated and dried a number of times (a process called malting), before being steeped in hot water for about an hour (a process called mashing). Hops, the small fruit of a vine plant, are then added to provide bitterness to the mixture, balancing the sugar content. Other spices are often added at this stage. After the mixture is filtered, yeast is then added and the fermentation process begins (Figure 18.4.5). Enzymes in the yeast convert the sugar to ethanol and carbon dioxide. This process may take place over a period of up to several weeks, depending on the type of beer being made. Some beer is artificially carbonated to produce its fizz, whereas other beer is left to age for a period of time, allowing the carbon dioxide from the fermentation process to build up.

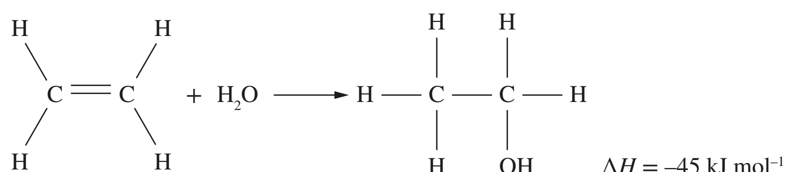
FIGURE 18.4.5 Copper kettles at Becks Brewery in Bremen, Germany

Page Proofs

Industrial production of ethanol by hydration of ethene

While the fermentation process is the most common way of producing ethanol industrially, it is not the only method. Around 7% of the total ethanol produced is produced by the hydration reaction between ethene and water.

Ethene is obtained from the catalytic cracking of larger hydrocarbon molecules in crude oil. The ethene is then reacted with steam to produce ethanol according to the following addition reaction:



The conditions for this reaction must be carefully selected to ensure a compromise is reached between the reaction rate and yield. The forward reaction in this process is exothermic and there are more reactant gas particles than product gaseous particles. A moderate temperature of 270–300°C is used together with a catalyst of phosphoric acid coated on a porous solid.

A relatively high pressure of 6000–7000 kPa is used, as the cost associated with maintaining the reactants at higher pressures is outweighed by the increases to reaction rate and yield.

Each pass of the reaction mixture through the reactor only yields a conversion of about 5% of the ethene to ethanol. The reaction mixture is cooled to liquefy the ethanol, but this also liquefies unreacted water, meaning that a solution of ethanol and water is collected. Unreacted ethene is heated and cycled back into the reactor as depicted below in Figure 18.4.6.

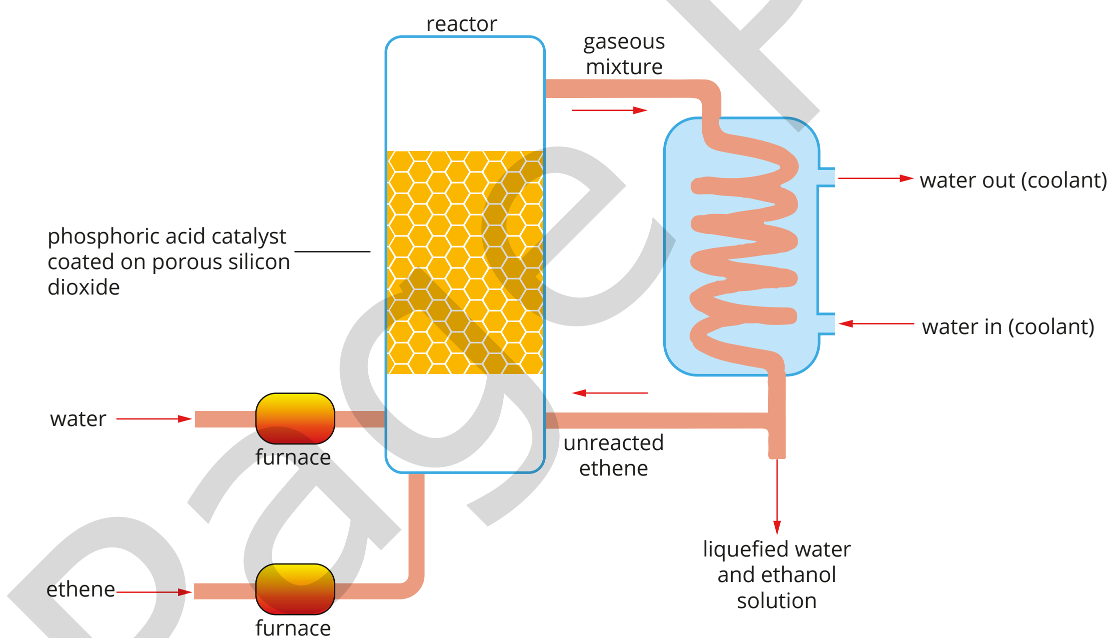


FIGURE 18.4.6 Steps in the industrial process for the hydration of ethene to ethanol

Comparison of industrial methods for ethanol production

The two methods for production of ethanol, by fermentation of glucose and hydration of ethene, have advantages and disadvantages. Table 18.4.2 summarises the major differences between the processes.

The use of enzymes as catalysts in fermentation, as opposed to inorganic phosphoric acid, means that fermentation is run at much lower temperatures and pressures. The reduced temperatures and pressures for fermentation result in a significant saving in costs, compared to the hydration of ethene.

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TABLE 18.4.2 Comparison of fermentation process and hydrolysis of ethene

Factor	Fermentation process	Hydrolysis of ethene
Temperature	Low temperatures	Moderate temperatures
Pressure	Normal pressures	High pressures
Catalysts	Amylase and cellulase to produce simple sugars and yeast enzymes for fermentation	Phosphoric acid
Purification required	Many distillations required	Limited distillations required
Raw materials used	Monosaccharides from grains and other plant material	Ethene from crude oil
Renewable	Yes—plant matter is renewable	No—crude oil is not renewable
Cost	Low	High

The advantage of producing ethanol from the hydration of ethene is that the reaction has no by-products, so the products are only ethanol and water. In the fermentation process, many different organic molecules are produced or present in small quantities due to the many different enzymes in yeast and the different compounds present in the starting mixture. Also, in some locations there are limited crops to source raw materials for fermentation, whereas crude oil may be readily available.

Fermentation is usually the preferred method for production due to the renewable nature of the process and lower cost of the ethanol produced.

Page Proofs

18.4 Review

SUMMARY

- The general advantages and disadvantages of using enzymes versus inorganic catalysts in the chemical industry are:
  - enzymes are effective at low temperatures, whereas inorganic catalysts usually do not function well at low temperatures. This reduces the cost of maintaining high temperatures
  - enzymes catalyse specific reactions, whereas inorganic catalysts often catalyse multiple reactions, generating unwanted by-products and reducing yields
  - enzymes have to be cultivated and collected, which can be a lengthy and expensive process.
- The main differences between the fermentation process and the hydration of ethene for producing ethanol are:
  - fermentation uses enzymes as the catalyst, whereas hydration of ethene uses phosphoric acid
  - fermentation uses lower temperatures and pressures, but requires more distillation cycles
  - the raw materials used for fermentation are renewable, usually a local grain, whereas the hydration of ethene uses crude oil, which is a non-renewable source of chemicals.

KEY QUESTIONS

1 What is the percentage yield if 100 g of ethene is converted into 150 g of ethanol using the hydrolysis of ethene reaction?

2 A moderate temperature of around 300°C is used in a reactor when producing ethanol using the hydration process. What is the reason for using a moderate temperature?

3 There is a significant cost involved in maintaining the high pressures used in the hydration process. Use your knowledge of chemical equilibrium to explain why high pressures are employed.

Chapter review

18

KEY TERMS

active site	enzyme–substrate complex	optimum pH
denaturation	fermentation	optimum temperature
enzyme	induced fit model	Protein Data Bank
enzyme activity	lock-and-key model	substrate

Investigating proteins

1 Indicate whether the following statements are true or false. If false, explain why.

a Proteins with similar tertiary structures are likely to have a similar function.

b The Protein Data Bank enables protein structures to be visualised in different ways.

c A mutation that causes one amino acid in a protein to be exchanged will always result in a loss of protein function.

2 Protein structures need to be determined experimentally. They cannot be predicted from the primary structure. X-ray crystallography analyses often reveal that amino acids that are far apart in the primary structure of a protein are very close to each other in the protein's three-dimensional shape. Explain the process by which this occurs.

Enzymes

3 **a** Explain why the action of enzymes justifies the statement 'Enzymes make life possible'.

b Why is the action of an enzyme often described as operating like a lock and key?

4 Indicate whether the following statements summarising the properties of enzymes are true or false.

a Enzymes are made of proteins.

b Enzymes do not change the position of equilibrium.

c Enzymes are consumed by the reaction.

d Enzymes increase the activation energy of a reaction.

e Enzymes increase the rate of reaction.

f Enzymes are sensitive to conditions such as pH changes or temperature increase which denatures the enzyme.

g Enzymes are highly specific for the biochemical reactions they catalyse because of the shapes of their active site.

5 There is a mutation that causes one of the amino acids in the active site of an enzyme to be substituted for another. Although the mutation does not dramatically alter the three-dimensional shape of the protein, it causes a reduction in enzyme activity. Account for this observation.

6 On the diagram in Figure 18.5.1:

a identify the model of enzyme action shown

b label the parts of the diagram as indicated to show the first two steps of enzyme action

c draw and label a diagram to show the next step in this process.

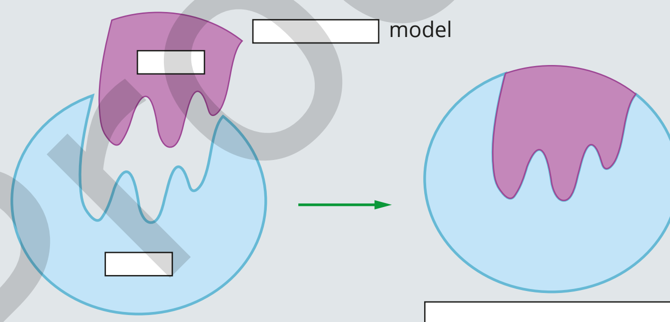


FIGURE 18.5.1

Enzymes—dependence on pH and temperature

7 Medicinal proteins such as insulin are administered by injection, rather than being ingested. Suggest why insulin cannot be given orally as tablets or capsules.

8 Jellied pineapple dessert cannot be made by using gelatine and fresh pineapple because an enzyme in the pineapple causes molecules in the gelatine to break down instead of setting. Suggest how jellied pineapple might be prepared.

9 Which one of these formulas gives the correct structure of tyrosine ($NH_2CH(C_6H_4OH)COOH$) in the highly acidic conditions present in the human stomach?

A $^{+}NH_3CH(C_6H_4OH)COOH$

B $NH_2CH(C_6H_4OH)COO^{-}$

C $^{+}NH_3CH(C_6H_4OH)COO^{-}$

D $NH_2CH(C_6H_4OH)COOH$

10 Draw the structure of the zwitterion form of the amino acid valine. You should refer to the table of amino acids (Table 17.1.1, page 460).

Page proofs

11 The graph in Figure 18.5.2 shows the effect of temperature on the enzyme activity for a metabolic reaction. For each of the parts of the graph labelled A, B and C, explain the variation in enzyme activity with temperature.

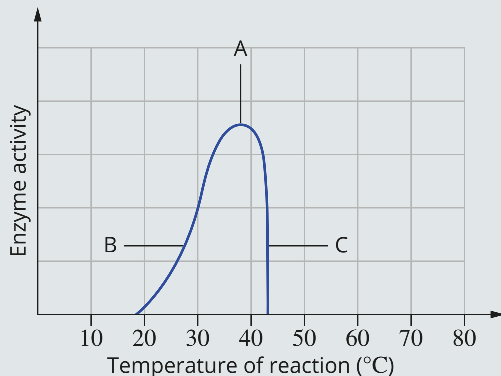


FIGURE 18.5.2

Enzymes in industry

12 Give the chemical equation that describes the fermentation of glucose.

13 Describe three differences between the two processes for the formation of ethanol, the fermentation of glucose and the hydration of ethene.

14 The equation for the reaction for the hydration of ethene is:

$CH_2{=}CH_2(g) + H_2O(g) \rightleftharpoons CH_3CH_2OH(g)\ \Delta H = -45\,kJ\,mol^{-1}$

Use your knowledge of Le Châtelier's principle, equilibrium and collision theory to predict how the listed changes will affect both the equilibrium yield and reaction rate.

a Increasing pressure

b Decreasing temperature

15 Give two disadvantages and two advantage of using enzymes as catalysts for industrial processes, rather than inorganic catalysts.

16 In order to purify the ethanol produced by either fermentation of glucose or the hydration of ethene, the process of distillation is used. Explain how distillation can be used to purify ethanol from a mixture of ethanol and water.

Connecting the main ideas

17 Biological washing powders contain enzymes such as lipases (to break down oil) and proteases (to break down proteins). Evaluate the advantages and disadvantages of using a biological washing powder over a traditional washing powder.

18 Unless distillation is used, beer and wine both have a maximum ethanol content of roughly ~15%. This is because high concentrations of ethanol can cause the enzymes in yeast to denature. Explain this process of denaturation and describe the types of interactions that ethanol is likely to form with the protein.

Page Proofs

UNIT 4 • ORGANIC CHEMISTRY AND CHEMICAL SYNTHESIS

REVIEW QUESTIONS

Section 1: Multiple choice

1 The systematic name for $CH_3CH_2CH_2CH(CH_3)_2$ is:
- **A** 1,1-dimethylbutane.
- **B** 2-methylpentane.
- **C** 2-methylpentene.
- **D** propyldimethylmethane.

2 Oxidation of a secondary alcohol produces:
- **A** an aldehyde.
- **B** a ketone.
- **C** a carboxylic acid.
- **D** an ester.

3 Which of the following compounds would be expected to have the highest boiling point?
- **A** $CH_3CH_2CH_2OH$
- **B** $CH_3CH_2CH_2CH_3$
- **C** $CH_3CH_2CH_2Cl$
- **D** $CH_3CH_2CH_3$

4 Which of the following statements are true of the homologous series of primary alcohols?
- **I** The members differ by one CH_2 unit.
- **II** They are all strong bases.
- **III** They can be oxidised to form carboxylic acids.
- **A** I and II
- **B** II and III
- **C** I and III
- **D** I, II and III

5 Chloroform (trichloromethane, $CHCl_3$) is synthesised commercially by the chlorination of methane. Reaction occurs according to the equation:

$CH_4 + 3Cl_2 \rightarrow CHCl_3 + 3HCl$

The final mixture contains hydrogen chloride and chloroform along with chloromethane, dichloromethane and tetrachloromethane. Distillation allows separation of the chloroform, and at one plant yields of 75% are obtained. What mass of methane (in g) would be needed to generate 1.0 kg of chloroform, assuming 75% yield?
- **A** 101
- **B** 134
- **C** 179
- **D** 197

6 The ester methyl ethanoate could be made by reacting together which of the following?
- **A** CH_3CH_2OH and CH_3COOH
- **B** CH_3CH_2OH and $HCOOH$
- **C** CH_3OH and CH_3CH_2COOH
- **D** CH_3OH and CH_3COOH

7 The volume of carbon dioxide (in L) collected at STP when 6.32 g of ethane undergoes complete combustion is:
- **A** 4.77
- **B** 9.25
- **C** 9.55
- **D** 18.5

8 Which of the following molecules is most likely to undergo an addition polymerisation reaction?
- **A** FCH_2CH_2COOH
- **B** $HOCH_2CONH_2$
- **C** $C_6H_5CHCHOH$
- **D** $CH_3COCH_2CH_3$

9 Listed below are the names of groups found in some organic compounds.
- **I** alcohol
- **II** amide
- **III** aldehyde
- **IV** ketone

Which of these groups are always found at the end of a hydrocarbon chain?
- **A** I and II only
- **B** III only
- **C** I, II and III only
- **D** I, III and IV only

10 Consider the following reaction pathway.

ethene $\xrightarrow{\text{I}}$ chloroethane $\xrightarrow{\text{II}}$ ethanol $\xrightarrow{\text{III}}$ ethanoic acid

The reactions that occur in steps I, II and III of the pathway are:
- **A** substitution, addition, hydrolysis
- **B** chlorination, substitution, addition
- **C** addition, substitution, oxidation
- **D** addition, reduction, hydrolysis.

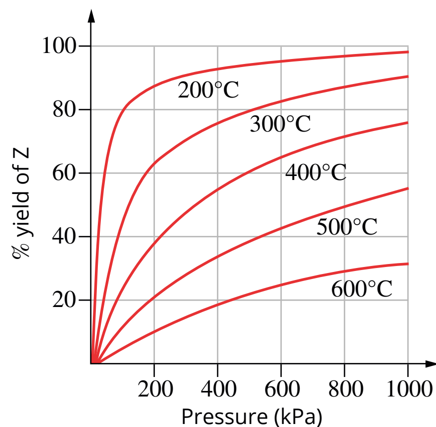
11 A triglyceride molecule with a molar mass of 878 $g\,mol^{-1}$, when fully hydrolysed, yields a single type of fatty acid molecule. The molar mass of this fatty acid ($g\,mol^{-1}$) is closest to:
- **A** 262
- **B** 280
- **C** 292
- **D** 310

Page Proofs

12 The industrial production of chemical Z proceeds in the presence of a catalyst according to the equation:

$$X(g) + 2Y(g) \rightarrow zZ(g)$$

The graph below shows the variation in the equilibrium yield of Z with pressure at a range of temperatures



It can be deduced that the production of Z is an:

A endothermic reaction and the value of *z* could be 2.

B endothermic reaction and the value of *z* could be 4.

C exothermic reaction and the value of *z* could be 2.

D exothermic reaction and the value of *z* could be 4.

Section 2: Short answer

1 Enzymes are biological catalysts, some of which can be used for industrial scale synthesis reactions. One example is the synthesis of ethanol from glucose, using enzymes derived from yeast. Ethanol can also be produced by the catalysed hydration of ethene. Ethanol can be used as a fuel in modified vehicles, or mixed with petrol for use in existing vehicles.

a Write a balanced equation, using molecular formulas for organic compounds, for the enzyme-catalysed reaction of glucose to produce ethanol.

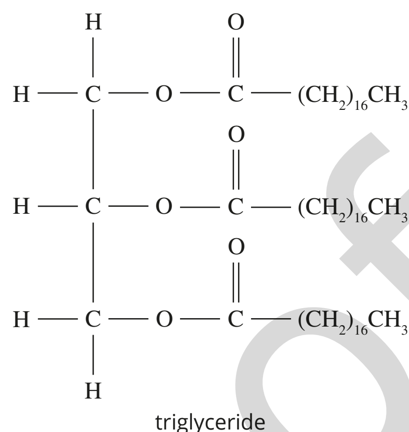
b i Write a balanced equation, using semistructural formulas for organic compounds, for the catalysed hydrolysis of ethene. Include the name of a suitable catalyst.

ii Which one or more of the following terms could be used to describe the type of reaction occurring in part b(i): substitution, addition, acid–base, redox, condensation?

iii The synthesis reaction in part **b(i)** takes place in industry at pressures of around 60 times atmospheric pressure, and a temperature near 300°C. In terms of the rate and extent of the reaction, account for the use of such high pressure.

c Suggest two reasons why the enzyme catalysed synthesis reaction in part a may be the preferred method for large scale, long term synthesis of ethanol for use as a fuel.

2 A triglyceride molecule found in a fat is shown in the following diagram.

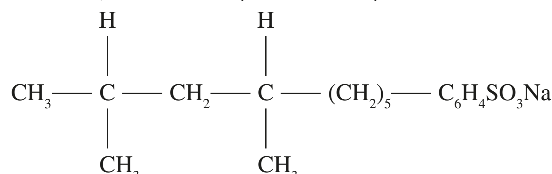


triglyceride

a i Draw the structures of the glycerol molecule and the anionic form of the long chain fatty acid produced by the base catalysed hydrolysis of this triglyceride.

ii The saponification reaction in part a(i) produces soap. Name the functional group in the anionic particle drawn in part a(i).

b The semistructural formula of a synthetic detergent which can be used in place of soap is shown below.



i Is the detergent shown cationic, anionic or polar?

ii Name the type of bonding which occurs between the detergent ions and water molecules.

iii Name the type of bonding which occurs between the detergent ions and oil molecules from a greasy plate.

c Synthetic detergents have replaced natural detergents such as soap for many cleaning purposes.

i State one advantage of detergents over soap.

ii State one disadvantage of detergents over soap.

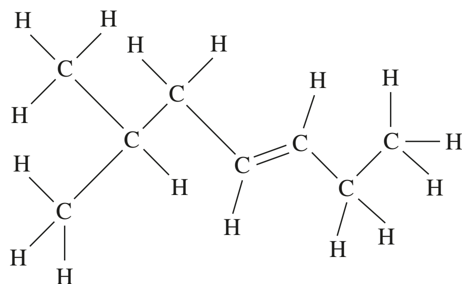
d Biodiesel is an increasingly important alternative fuel which is synthesised from triglycerides and methanol using either a hydroxide catalyst or a lipase catalyst.

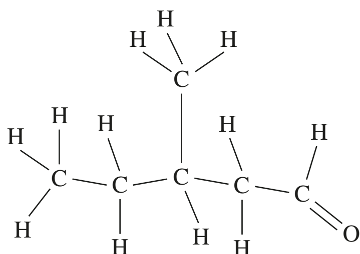
i Draw the structure of a biodiesel molecule containing a total of 18 carbon atoms

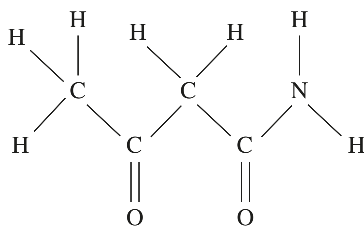
ii Name the types of bonding which exist between biodiesel molecules.

Page Proofs

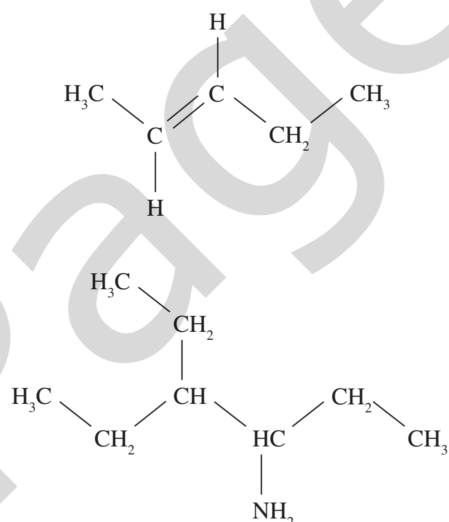
3 a For each of the following structures, write a condensed (semistructural) formula.

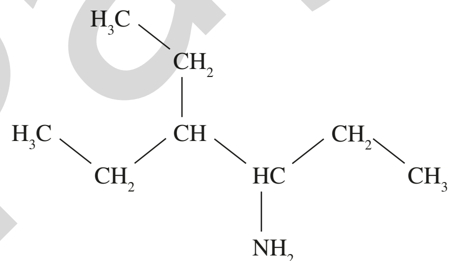


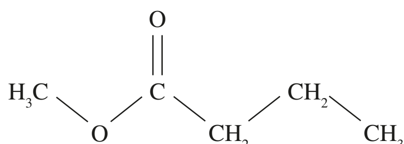




b Give the correct IUPAC names for each of the following structures.







c State appropriate reagents and conditions to effect the conversion of:

i butan-2-ol to butanone

ii cyclohexene to cyclohexane.

d Write balanced chemical equations (excluding states) using semi-structural formulas for the:

i condensation reaction of ethanol and propanoic acid

ii acid–base reaction between ethanamine and propanoic acid.

4 An experiment was conducted to synthesise ethanal. When 3.00 g of ethanol was treated with 25.0 mL of a 1.00 mol L^{-1} solution of acidified potassium dichromate under appropriate conditions 2.42 g of ethanal was obtained. The reaction is represented by the equation:

$$3CH_3CH_2OH(l) + Cr_2O_7^{2-}(aq) + 8H^+(aq) \rightarrow 3CH_3CHO(l) + 2Cr^{3+}(aq) + 7H_2O(l)$$

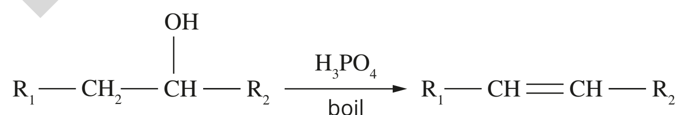
a i State the oxidation number of carbon in ethanal.

ii Write a balanced half-equation for the oxidation process occurring in the reaction.

b i Calculate the percentage yield for the synthesis experiment.

ii Suggest two reasons why the percentage yield is less than 100%.

5 Alcohols can be dehydrated by heating in acid to produce alkenes, as shown in the following generalised reaction.



When hexan-3-ol was heated with acid, the resultant mixture was found to contain four distinct isomeric products.

a i Draw a structural formula for hexan-3-ol.

ii What type of alcohol (primary, secondary or tertiary) is hexan-3-ol?

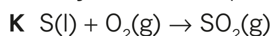
iii Hexan-3-ol has a very low solubility in water compared with ethanol, which is completely miscible in water. Account for this difference in solubilities.

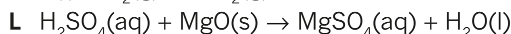
b Two of the products of the dehydration of hexan-3-ol are structural isomers, differing only in the position of the functional group. Draw and name these two isomers.

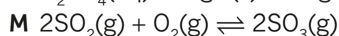
c Each of the isomers drawn in part b can exhibit *cis–trans* isomerism. For one of the compounds drawn in part b, draw and label the *cis* and *trans* isomers.

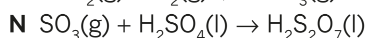
Page Proofs

6 A number of reactions involving sulfur containing compounds are shown in the equations K–O. Some of these reactions occur during the production of sulfuric acid by the contact process.


K $S(l) + O_2(g) \rightarrow SO_2(g)$


L $H_2SO_4(aq) + MgO(s) \rightarrow MgSO_4(aq) + H_2O(l)$


M $2SO_2(g) + O_2(g) \rightleftharpoons 2SO_3(g)$


N $SO_3(g) + H_2SO_4(l) \rightarrow H_2S_2O_7(l)$

O $H_2SO_4(l) + 3H_2S(aq) \rightarrow 4S(s) + 4H_2O(l)$

a Select the letter K–O corresponding to a reaction:
- **i** showing sulfuric acid acting as an oxidising agent
- **ii** in which the oxidation number of sulfur is unchanged
- **iii** in which the substance called oleum appears.

b The reaction represented by the letter M is an important step in the manufacture of sulfuric acid by the contact process.
- **i** To improve the yield of SO_3 in this reaction should the temperature be raised or lowered? Explain your choice.
- **ii** State one disadvantage of using the temperature chosen in part **b(i)**.
- **iii** The yield of SO_3 in this reaction would be improved by the use of high pressure. Explain why the reaction is carried out at near atmospheric pressure during industrial production of sulfuric acid.

c The reaction represented by the letter M is conducted in the presence of a catalyst. Does the presence of this catalyst increase, decrease or not change each of the following characteristics of the reaction?
- **i** ΔH value
- **ii** Equilibrium yield of SO_3
- **iii** Rate of reverse reaction

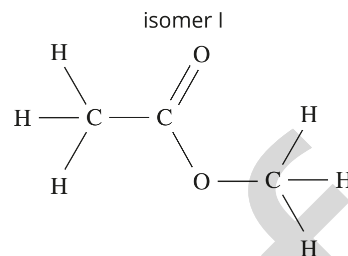
Section 3: Extended answer

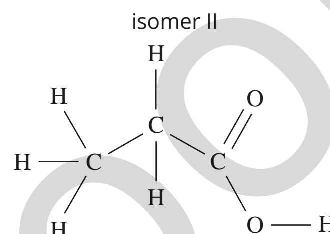
1 A 6.500 g sample of an organic compound ($C_xH_yO_z$) was burnt in excess oxygen. 4.74 g of water, and 11.60 g of carbon dioxide were produced.

In a separate experiment, a 5.01 g sample of the compound was vaporised. The vapour occupied 2.22 L at 200°C and 1.20×10^2 kPa.

a Show that the molecular formula of the compound is $C_3H_6O_2$

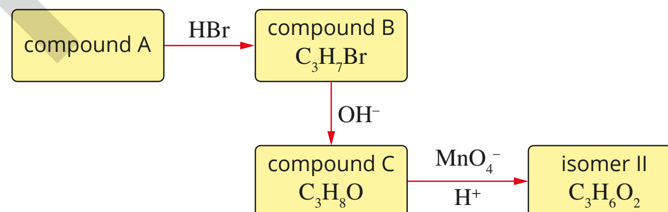
b Two possible molecular structures for the compound are shown below.





- **i** Give the systematic name of isomer I.
- **ii** Describe a simple laboratory test, including the results of the test, which could distinguish between samples of the isomers I and II above.
- **iii** Draw a structural formula for, and give the name of, another isomer of $C_3H_6O_2$.

c Isomer II may be produced using the reaction sequence shown below.



- **i** Draw the structural formula for compound B.
- **ii** Compound C, C_3H_8O, has a molar mass of 60 g mol^{-1} and a boiling point of 97°C.
  Propanone (C_3H_6O) has a molar mass of 58 g mol^{-1} and a boiling point of 56°C.
  Account for the difference in the boiling point of these two compounds of similar molar mass.
- **iii** Write the expected observations which could be made when substance C reacts with an acidified solution of potassium permanganate to produce isomer II. Include the appearance of reactants and products in your answer.

Page Proofs

2 Enkephalins are short polypeptides involved in the nervous system's detection of pain and harm. The structure of met-enkephalin, so-called because it contains a methionine residue, is shown below. Met-enkephalin may undergo acid catalysed hydrolysis to release the four different amino acids.

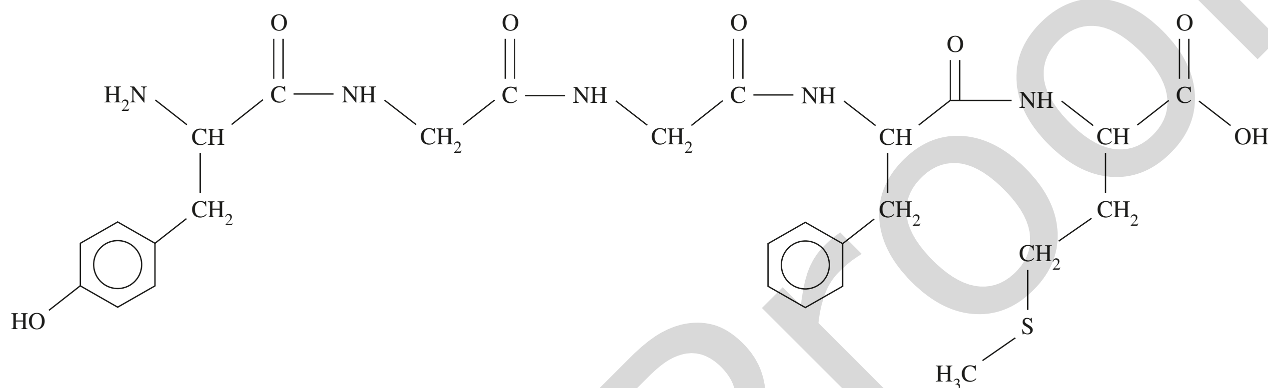
a **i** Refer to a table of amino acids in your Chemistry Data Booklet and use it to name the amino acids (other than methionine) present in met-enkephalin.

ii On the structure shown below, circle and label two peptide linkages.

iii On the structure shown below, circle and label the terminal carboxyl and amino groups.

iv Draw the structure of the methionine amino acid as it would exist in the acidic hydrolysis solution.

v At a particular pH, an amino acid has both a positive and negative charge and is known as a zwitterion. Draw the zwitterion structure for one of the amino acids in met-enkephalin other than methionine.



b Enkephalins are short polypeptides. Much longer polypeptides form proteins, whose structure may be described in terms of primary, secondary and tertiary structures.
Give explanations for the following facts relating to protein structure and function.

i Formation of the primary structure of proteins produces water as a by-product but formation of secondary, tertiary and quaternary structures do not.

ii Despite having unique and very different amino acid sequences, almost all proteins are able to form α-helical and β-pleated regions.

iii All proteins are denatured by extreme pH changes, but some enzymes can lose their activity as a result of even relatively small pH changes without being fully denatured.

iv The tertiary structures of some proteins are more easily disrupted by an increase in temperature than are those of others.

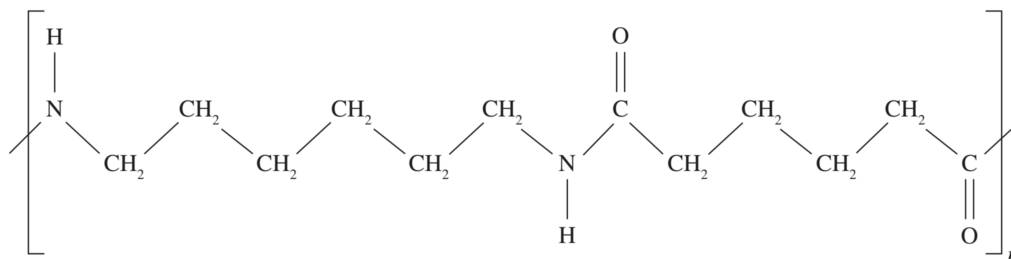
c Proteins are formed by condensation polymerisation of amino acids. Another similar condensation polymer is nylon. The structure of one nylon molecule is shown below.
This nylon is composed of two alternating monomers. One of these monomers shows a strongly basic character.

i Draw the structural formula of this basic monomer.

ii Write an equation to show the reaction of this monomer with excess hydrochloric acid (states are not required).

iii State one structural similarity between proteins and nylon.

iv State one structural difference between proteins and nylon.



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