23 Option B Biochemistry

23.1 Introduction to biochemistry

Revised

Essential idea: Metabolic reactions involve a complex interplay between many different components in highly controlled environments.

Introduction to biochemistry

Revised

- The diverse functions of biological molecules depend on their structures and shapes.
- Metabolic reactions take place in highly controlled aqueous environments.
- Reactions of breakdown are called catabolism and reactions of synthesis are called anabolism.
- Biopolymers form by condensation reactions and are broken down by hydrolysis reactions.
- Photosynthesis is the synthesis of energy-rich molecules from carbon dioxide and water using light energy.
- Respiration is a complex set of metabolic processes providing energy for cells.

Metabolism

Revised

Biochemistry is the study of chemical processes in living cells at the molecular level.

Cells are the basic unit of structure and function in all living organisms. Complex organisms contain vast numbers of cells, which together carry out all the life processes. At any one time within the microscopic volume of a living cell, thousands of chemical reactions occur involving a range of biochemically important molecules that have a series of diverse roles. Some metabolic reactions take place in the cytoplasm of the cell, the synthesis of proteins on ribosomes, for example, and some in membrane-bound organelles, such as the chloroplast, the site of all the reactions involved in photosynthesis in green leaves. Many metabolic pathways involve redox reactions, which involve the transfer of electrons.

Living organisms convert energy from one form into another. The enzyme-controlled reactions that place inside living cells are collectively known as **metabolism**. Reactions in which organic molecules are broken down to release energy are catabolic reactions. Reactions that use energy to synthesize larger molecules are known as anabolic reactions. Metabolism involves **anabolism** and **catabolism**.

The biochemical reactions involved in metabolism are organized into *metabolic pathways*, each step of which is catalysed by a specific enzyme in an aqueous environment (within a narrow range of conditions) (Figure 23.1).

Key definitions

Metabolism – the total of all the enzyme-catalysed reactions in a cell or organism. It involves the breakdown of molecules with the release of energy and the synthesis of molecules that are required by the cell.

Catabolism – the breakdown of complex molecules into simpler molecules. Such processes release energy.

Anabolism – the synthesis of more complex molecules from simpler precursor molecules. Such processes require energy.

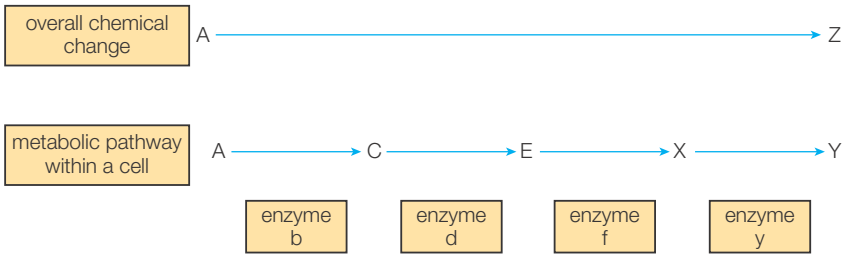


Figure 23.1 A metabolic pathway achieves a chemical conversion in a sequence of enzyme-catalysed steps

Metabolic pathways may be linear or cyclic (Figure 23.2). Compounds taking part in metabolism are known as *metabolites*.

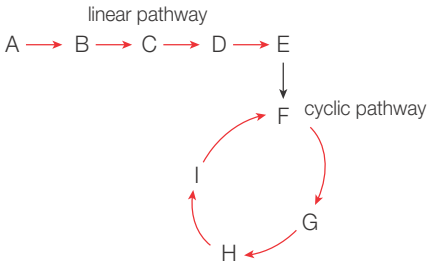


Figure 23.2 Linear and cyclic metabolic pathways

Expert tip

The shape of a molecule determines the way it behaves inside cells and metabolic pathways. The chemical nature of a molecule is dependent upon the shape as well as the distribution of the electron density in its covalent bonds. The shape of a molecule and its polarity influence the physical and chemical properties of the elements involved.

Respiration and *photosynthesis* are two key metabolic processes central to life on the Earth.

- Respiration is a process by which cells generate energy through the breakdown of glucose. As such it is a catabolic process. It is common to the metabolism of all cell types, whether bacterial, fungal, plant or animal.
- Photosynthesis is an anabolic process in which carbon dioxide from the atmosphere is taken in by plants and combined with water to form energy-rich molecules such as glucose.

Photosynthesis

Revised

Photosynthesis (Figure 23.3) is an anabolic process used by plants and some bacteria to synthesize glucose from carbon dioxide and water using light energy absorbed by chlorophyll. During photosynthesis water molecules are 'split' and oxygen is released as a by-product. It is a complex series of enzyme-controlled reactions occurring inside chloroplasts.

Photosynthesis can be summarized by the following overall chemical equation:

$$6CO_2(g) + 6H_2O(l) \rightarrow C_6H_{12}O_6(aq) + 6O_2(g)$$

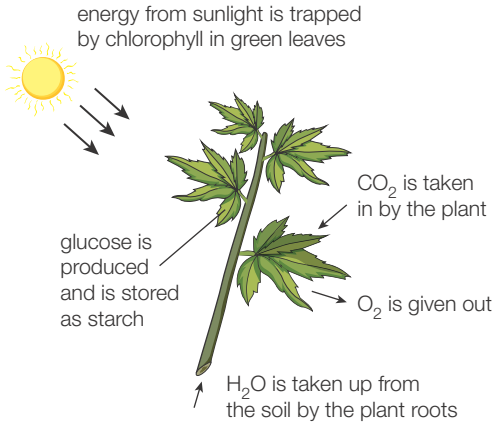


Figure 23.3 An overview of photosynthesis, showing the importance of sunlight as the source of energy

Expert tip

Photosynthesis is responsible for the origin and maintenance of oxygen in the atmosphere. It essentially 'fixes' carbon (in the form of carbon dioxide) into biomass.

Photosynthesis and respiration are largely responsible for the global balance of carbon dioxide and oxygen in the atmosphere, though levels of carbon dioxide are increasing, largely due to emissions of carbon dioxide from the combustion of fossil fuels.

Respiration

Revised

Aerobic respiration

Respiration is a catabolic process operating in all living organisms. Aerobic respiration involves the breakdown of glucose in the presence of molecular oxygen to form carbon dioxide and water. The overall equation is the reverse of the process of photosynthesis, but it is a complex series of enzyme-controlled steps involving a large number of intermediates (in linear and cyclic metabolic cycles) (Figure 23.4).

$$C_6H_{12}O_6(aq) + 6O_2(g) \rightarrow 6CO_2(g) + 6H_2O(l)$$

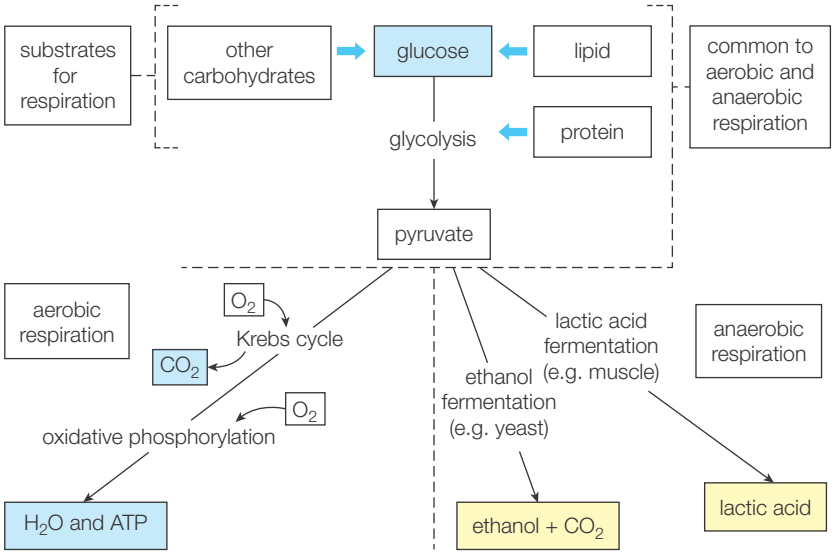


Figure 23.4 The metabolic processes involved in cellular respiration

The overall products of aerobic respiration are carbon dioxide and water, with the generation of a large number of molecules of ATP (**a**denosine **tri**phosphate), the short-term energy store in cells.

Anaerobic respiration

Respiration can also occur in the absence of oxygen. In humans, lactic acid (2-hydroypropanoic acid) is formed, but in plants and yeast ethanol is formed (Figure 23.4), which can be used as biofuel (Option C Energy). Anaerobic respiration releases less energy to cells than aerobic respiration as fewer molecules of ATP are formed. Anaerobic respiration in yeast is known as fermentation:

$$C_6H_{12}O_6(aq) \rightarrow 2CO_2(g) + 2C_2H_5OH(l)$$

The two processes of photosynthesis and respiration help to maintain the balance between carbon dioxide and oxygen in the atmosphere.

In addition to carbohydrates, lipids and proteins can be used as energy sources for ATP synthesis. This happens during starvation. After hydrolysis, these large molecules form fatty acids plus glycerol and amino acids. These three types of molecule can also be catabolized to release energy and so produce ATP.

Worked example

The blood plasma of a typical human adult contains 20.00 g of glucose. Calculate the mass of oxygen needed to completely oxidize it to water and carbon dioxide.

$$\text{amount of glucose} = \frac{20.00\,\text{g}}{180.16\,\text{g mol}^{-1}} = 0.111\,\text{mol}$$

During aerobic respiration:

1 mole of glucose requires 6 moles of oxygen to react with it, so the amount of oxygen required is

$$6 \times 0.111\,\text{mol} = 0.666\,\text{mol}$$

hence the mass of oxygen required is

$$0.666\,\text{mol} \times 32.00\,\text{g mol}^{-1} = 21.3\,\text{g}$$

NATURE OF SCIENCE

One of the most important events that happened during 1900s in the history of biochemistry is the experiment done by Buchner. He prepared a cell-free extract of yeast which he called the zymase. It fermented glucose and produced carbon dioxide and ethanol. He introduced the concept of an 'enzyme' and falsified the earlier idea that only cytoplasm could carry out metabolic processes.

Expert tip

The removal of metabolic waste is known as excretion. Excretory products in humans are carbon dioxide and urea.

Condensation and hydrolysis reactions

Revised

Biological macromolecules and polymers (biopolymers), such as proteins, lipids and DNA, are formed by enzyme-controlled condensation reactions (Figure 23.5). These involve the reaction between the functional groups of two smaller molecules to form one large molecule with the release of a water molecule. Condensation reactions are an addition reaction followed by an elimination reaction.

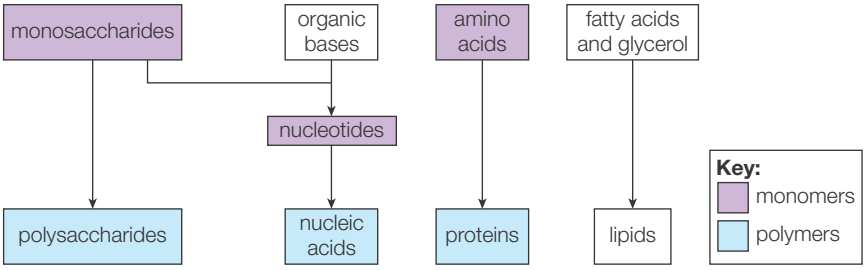


Figure 23.5 The synthesis of macromolecules from smaller monomers

For condensation polymerization (Figure 23.6) to occur each of the reacting molecules must possess at least two reactive functional groups. Examples include the condensation of amino acids to form proteins, the condensation of nucleotides to form DNA, the condensation of propane-1,2,3-triol (glycerol) and fatty acids to form lipids and the condensation of glucose to form starch and cellulose.

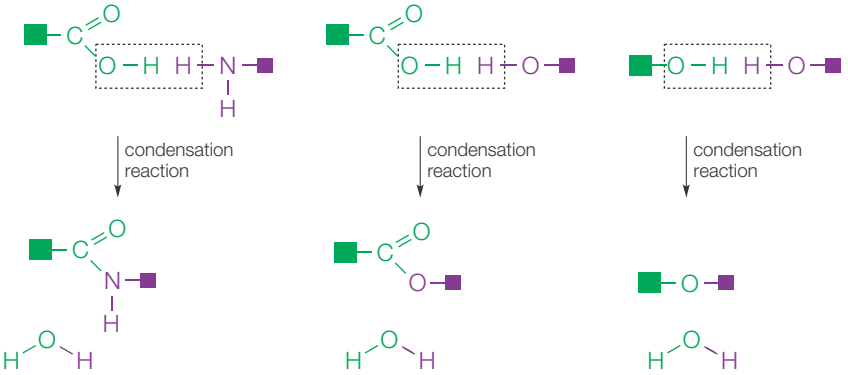


Figure 23.6 The condensation reactions involved in the formation of proteins, lipids and polysaccharides

For example, the two amino acids alanine, $H_2N–CHCH_3–COOH$, and cysteine, $H_2N–CH(CH_2SH)–COOH$, can condense together to form two different possible dipeptides provided each dipeptide contains one of each of two amino acid residues.

Each end of the dipeptides contains a reactive group so can undergo further condensation reactions (in the presence of enzymes) with additional amino acids to produce a polypeptide chain (protein).

Hydrolysis is the reverse of condensation (Figures 23.7 and 23.8). A molecule is hydrolysed when a water molecule reacts with a large molecule to break a specific bond (ester or amide) and form two smaller molecules. The hydrolysis of proteins produces amino acids and the hydrolysis of starch and cellulose (polysaccharide) produces glucose. Sucrose (a disaccharide) can be hydrolysed to form glucose and fructose. The hydrolysis reactions can also occur in the presence of acid without the presence of enzymes.

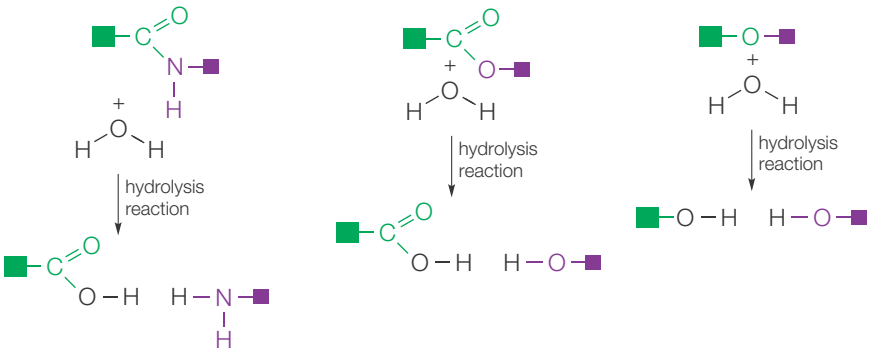


Figure 23.7 Hydrolysis reactions involve the breaking of a covalent bond by the addition of the fragments of water (H– and –OH) across the bond

Figure 23.8 summarizes the interrelationship between condensation and hydrolysis, and the key role of water, in the formation of biopolymers.

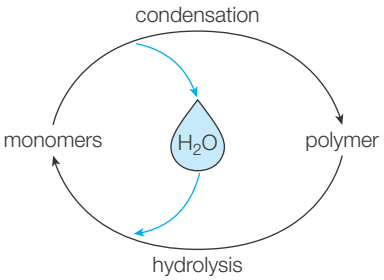


Figure 23.8 Summary of the general interrelationship between condensation reactions and hydrolysis

QUICK CHECK QUESTIONS

1 Distinguish between catabolic and anabolic reactions in metabolism. Classify photosynthesis and respiration as one or other of these types of reaction, giving your reasons.

2 Name the product(s) of anaerobic respiration in the following two different biological situations:

a in yeast

b in human muscle.

3 Write balanced equations to show the catabolism of glycerol, $C_3H_8O_3$, and oleic acid, $C_{18}H_{34}O_2$, a fatty acid, to carbon dioxide and water.

4 Hydrogen peroxide is broken down to water and oxygen and under the control of the enzyme, catalase.

Write a balanced equation for the reaction. State and explain what type of metabolic reaction this is an example of.

5 Maltotriose is formed when three molecules of glucose react together to form water. Write a balanced equation to describe this reaction and state what type of reaction it is. Suggest how the trisaccharide can be hydrolysed to form glucose.

Expert tip

Water behaves as a solvent and hydrates all biological molecules, as a reactant (during hydrolysis), and as a product (during condensation reactions). These all rely on its polar nature and its ability to form intermolecular hydrogen bonds.

23.2 Proteins and enzymes

Revised

Essential idea: Proteins are the most diverse of the biopolymers responsible for metabolism and structural integrity of living organisms.

Proteins and enzymes

Revised

- Proteins are polymers of 2-amino acids, joined by amide links (also known as peptide bonds).
- Amino acids are amphoteric and can exist as zwitterions, cations and anions.
- Protein structures are diverse and are described at the primary, secondary, tertiary and quaternary levels.
- A protein's three-dimensional shape determines its role in structural components or in metabolic processes.
- Most enzymes are proteins that act as catalysts by binding specifically to a substrate at the active site.
- As enzyme activity depends on the conformation, it is sensitive to changes in temperature and pH and the presence of heavy metal ions.
- Chromatography separation is based on different physical and chemical principles.

Amino acids

Revised

Amino acids contain an amine and a carboxyl group bonded to a central common atom. Figure 23.9 shows the generalized molecular formula of amino acids.

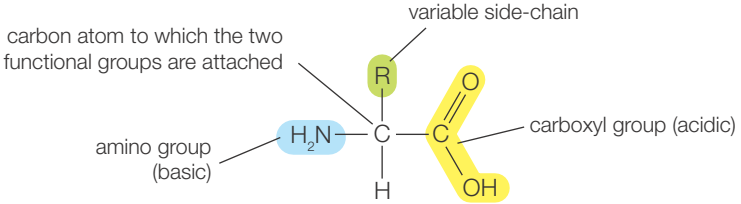


Figure 23.9 Generalized structure of amino acids

There are 20 amino acids. Each a different variable side-chain (a full table is available in the IB Chemistry *data booklet*). Amino acids can be classified based on their polarity and their acid–base properties (Table 23.1).

With the exception of glycine (2-aminoethanoic acid), all of the 2-amino acids contain a chiral carbon atom and therefore exhibit optical isomerism.

Table 23.1 Some examples of the different types of variable side-chains, R, in amino acids

Type of amino acid	R group contains	Example	Structure
Non-polar/hydrophobic	Hydrocarbon chain	Alanine, Ala	$H_2N-CH(CH_3)-COOH$
Polar, uncharged	Hydroxyl (–OH), sulfhydryl (–SH) or amide ($-CONH_2$) groups	Serine, Ser	$H_2N-CH(CH_2-OH)-COOH$
Basic (positively charged at pH 6.0–8.0)	Amino group ($-NH_2$)	Lysine, Lys	$H_2N-CH(CH_2-CH_2-CH_2-CH_2-NH_2)-COOH$
Acidic (negatively charged at pH 6.0–8.0)	Carboxyl group (–COOH)	Aspartic acid, Asp	$H_2N-CH(CH_2-COOH)-COOH$

Amino acids are white crystalline solids under standard conditions. They exist in the solid state and in neutral aqueous solution as zwitterions (Figure 23.10). This is due to an internal acid–base reaction. A hydrogen ion is released from the carboxyl group and protonates the amine group.

The presence of zwitterions leads to ionic bonding in crystalline amino acids involving electrostatic forces of attraction between oppositely charged ends of the zwitterions. This results in high melting (or decomposition) points and good solubility in water.

Expert tip

They are referred to as 2-amino acids as the amine group is attached to the first carbon after that of the carboxyl functional group in the chain.

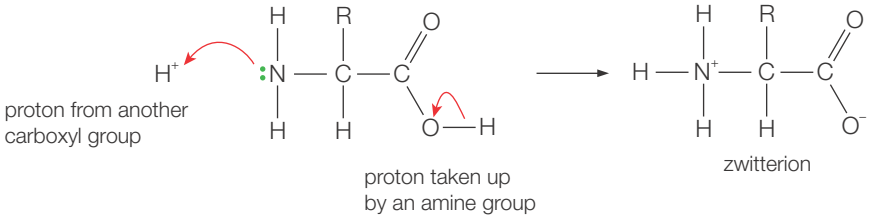


Figure 23.10 Zwitterion formation in amino acids

During protein synthesis in cells, amino acids condense (in the presence of enzymes) through the formation of the amide link (–CONH–), or peptide bond, to form a polypeptide chain (Figure 23.11), which then folds to form a biologically active protein.

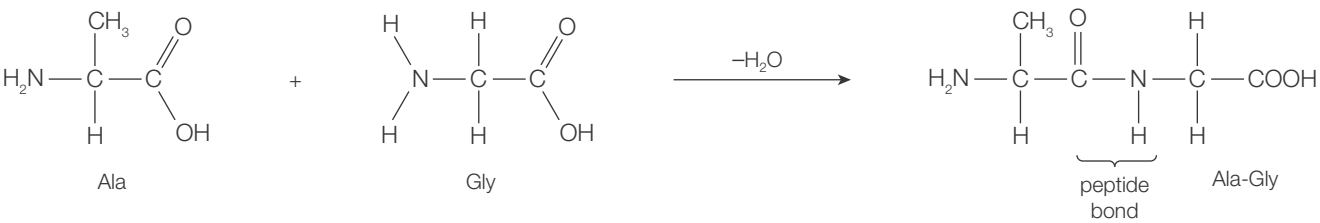


Figure 23.11 Peptide formation between two amino acids

Peptide bonds experience restricted rotation (Figure 23.12) due to resonance (π delocalization), giving rise to two possible conformations: the *trans* conformation is generally more stable.

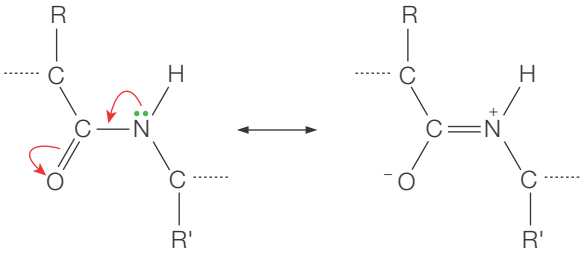


Figure 23.12 Resonance structures of the peptide bond (in the *trans* conformation)

Common mistake

Figure 23.11 shows the formation of a dipeptide, Ala-Gly, formed by condensation of the two amino acids, alanine and glycine. If this reaction is carried out in a test tube then remember that this is just one of two possible options. Gly-Ala is equally likely to be formed. When writing, or drawing, an equation for the reaction, do not forget the molecule of water eliminated in the condensation reaction.

Each particular protein has a specific number of amino acid residues bonded in a specific sequence between an amine group and a carboxyl group. This is known as the primary structure (see Figure 23.13). A small change in a primary structure can result in a large change in biological function.

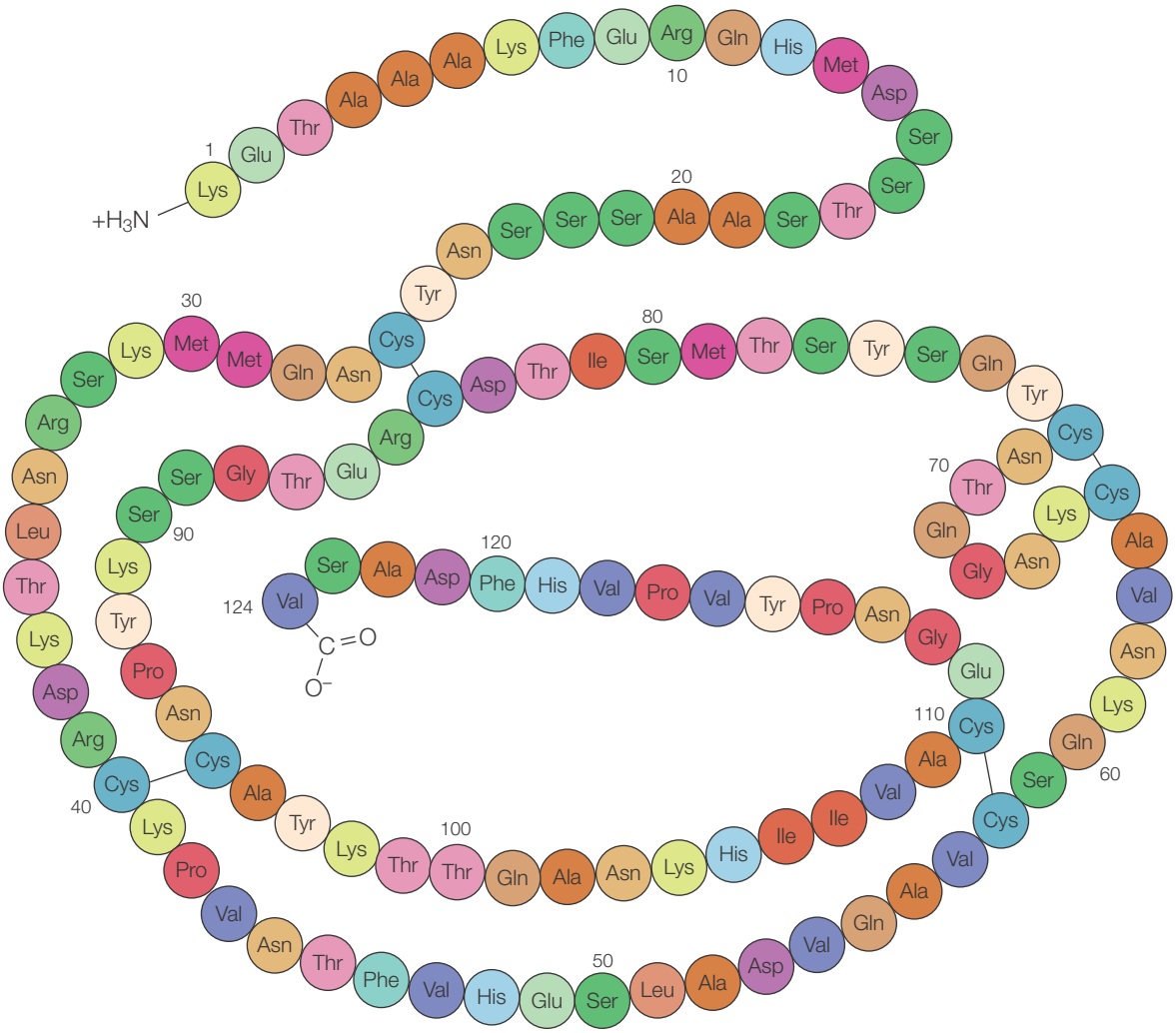


Figure 23.13 The primary sequence of the enzyme ribonuclease A (bovine pancreatic ribonuclease). Note the four disulfide bridges between cysteine residues

Ribonuclease A is a relatively short polypeptide of 124 amino acid residues and it illustrates the key features of the primary chain structure of polypeptides. The sequence is written with the $-NH_2$ group which has not been converted into a peptide link at the left-hand end. The unchanged –COOH group is written at the right-hand end. The end of the peptide chain with the $-NH_2$ group is known as the amino(N)-terminal end, and the end with the –COOH group

is the carboxyl(C)-terminal end. This reflects the fact that in biological cells the synthesis of a protein in cells is directional, with the protein chain being synthesized from the N-terminal end to the C-terminal end.

In order to sequence an entire protein, the polypeptide chain is broken down into smaller fragments using either chemicals (concentrated acid or alkali), or proteases (Figure 23.14). The resulting smaller fragments are then sequenced. The complete sequence is assembled by analysing overlapping fragments generated by cleaving the polypeptide chain with different reagents.

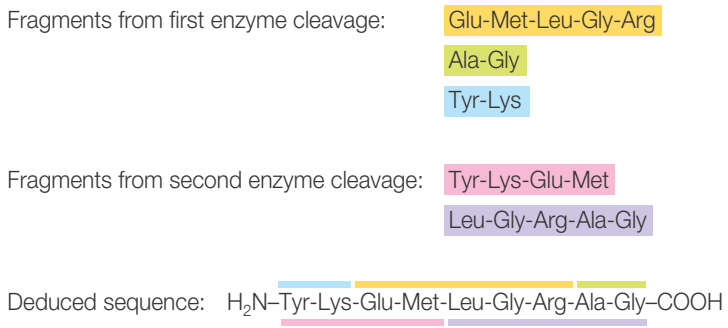


Figure 23.14 Establishing the primary structure of a protein by partial hydrolysis

Amino acids are amphoteric or, more specifically, amphiprotic, because, depending on pH, they can act as either an acid (via proton loss at the carboxyl group), or as a base (via protonation at the amino group). Amino acids have two pK_a values, one for the carboxyl functional group and one for the amine functional group. Amino acids with basic or acidic side-chains will have a third pK_a value.

The isoelectric point (pI) of an amino acid is the pH at which the concentrations of the zwitterionic form reaches its maximum value (Figure 23.15). During electrophoresis, amino acids are separated based on their pI values.

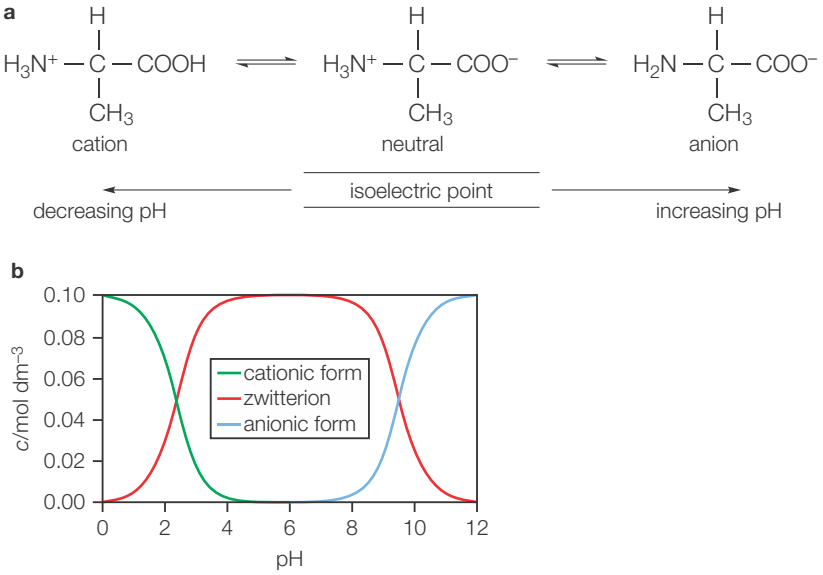


Figure 23.15 The isoelectric point (pI) of alanine: **a** the ionic forms at different pH and **b** the acid–base equilibria in a 0.1 mol dm^{-3} solution (pI = 6.0)

Structure of proteins

The secondary structure of a protein refers to three-dimensional conformations of localized regions of the protein, in particular, an α-helix (Figure 23.16) or a β-pleated sheet (Figure 23.17). The tertiary structure of a protein is the arrangement of the α-helix or β-pleated sheet into a configuration characteristic of the protein.

Expert tip

The term amino acid residue has to be used since a peptide chain is not made up of amino acids. When the amino acids chemically combine together, a water molecule is lost. The peptide chain is made up from what is left after the water is eliminated. Relatively short chains of amino acid residues are called peptides.

Expert tip

When working with enzymes it is important to buffer the pH of the solution. This prevents denaturation and hence loss of activity.

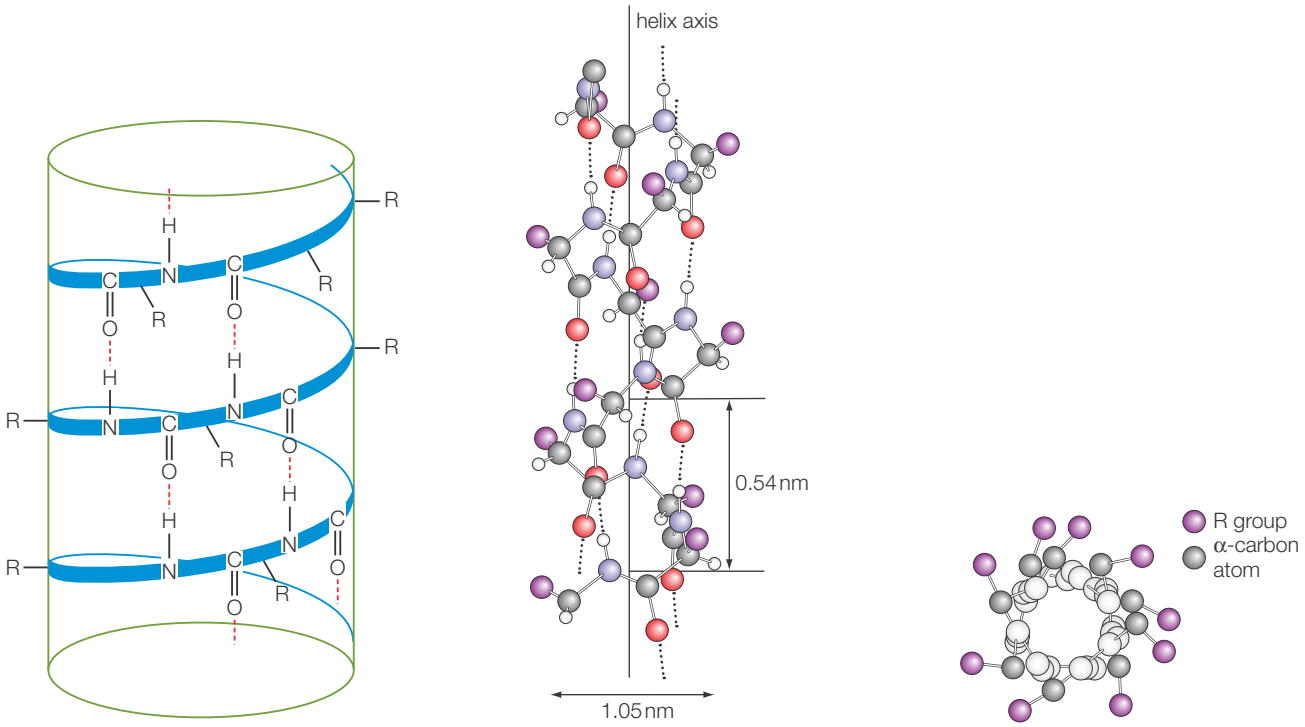


Figure 23.16 An α-helix

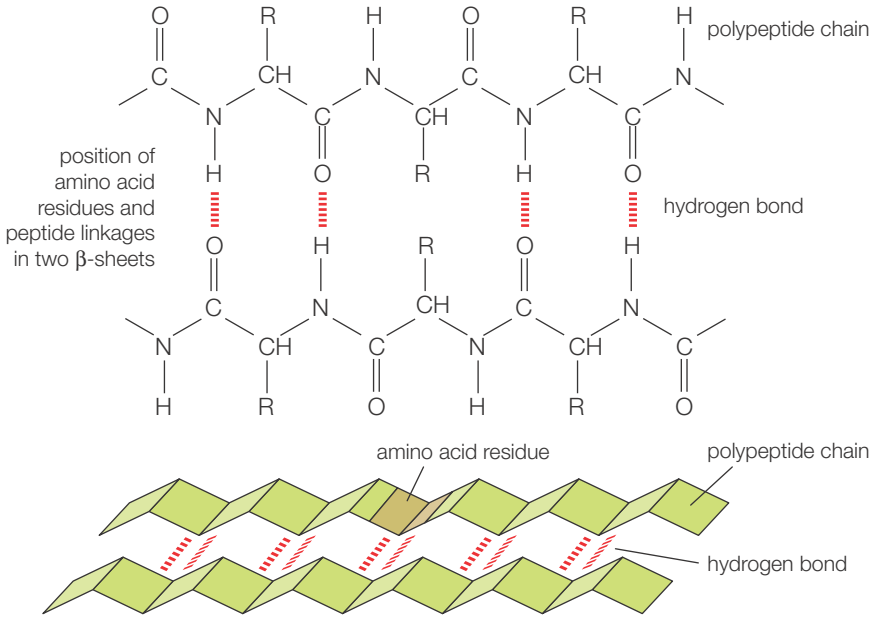


Figure 23.17 A β-sheet

Expert tip

In a parallel β-pleated sheet the hydrogen bonds run in the same direction; in an antiparallel β-pleated sheet the hydrogen bonds run in the same direction.

A variety of bonds and intermolecular forces are involved in maintaining tertiary structure (Figure 23.18). The quaternary structure of a protein is the grouping of two or more tertiary structures into a unit (the functional protein). Thus, myoglobin is an oxygen-carrying protein that consists of a single chain and has a conformation defined up to the tertiary level, whereas hemoglobin, consisting of four sub-units, has quaternary structure too.

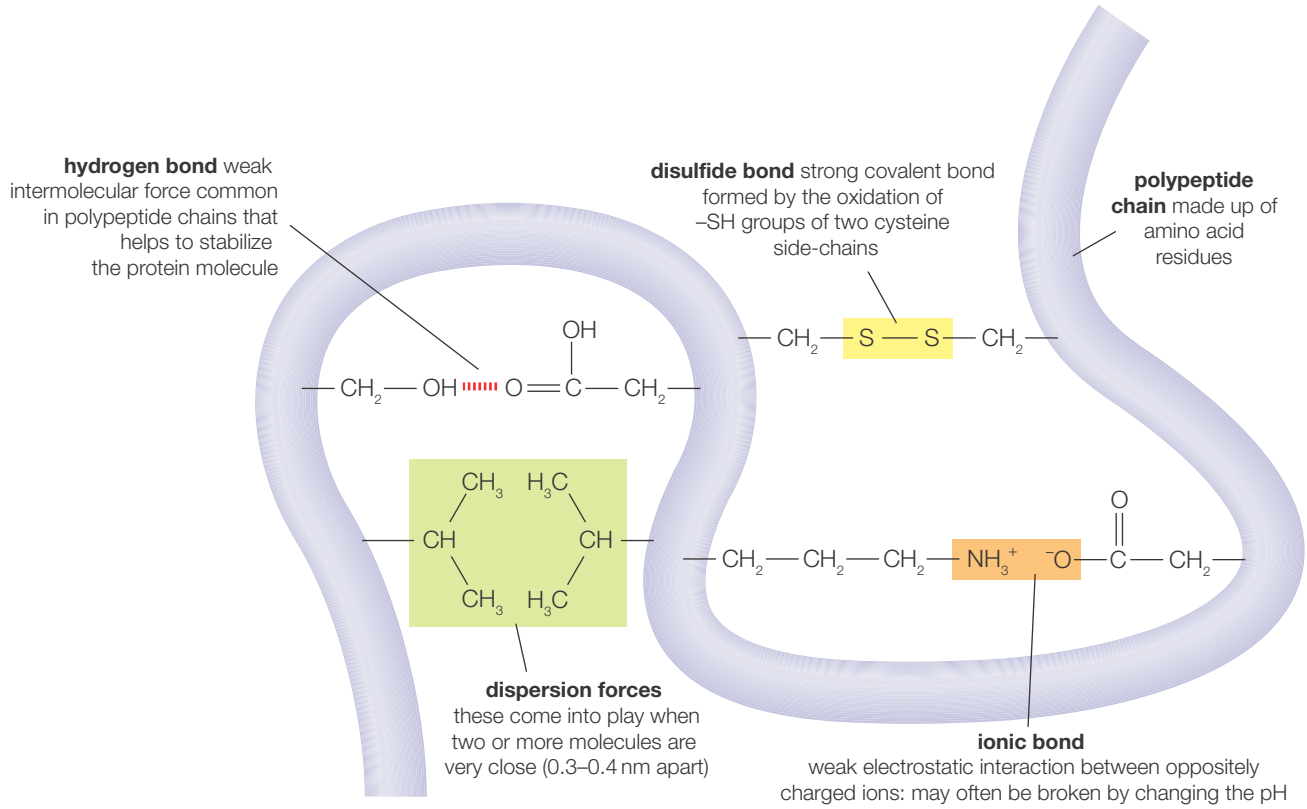


Figure 23.18 Interactions maintaining protein tertiary structure

Expert tip

The position of covalent disulfide bonds between cysteine residues is also included in the primary structure.

Cysteine residues are capable of being covalently bonded to another (under enzyme control) via the formation of disulfide bridges (Figure 23.19). Their formation contributes to the stability of the secondary and tertiary structures of a protein.

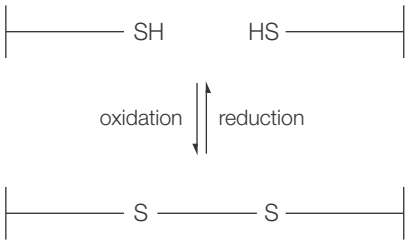


Figure 23.19 The formation of a disulfide bridge

Table 23.2 summarizes the key features of the different levels of protein structure.

Table 23.2 A summary of the bonds and forces involved in protein structure

Level of protein structure	Nature of interactions
Primary	Sequence of amino acids linked by covalent bonds (peptide/amide links)
Secondary	Folding of chain involving hydrogen bonding between the peptide link regions of the chain (α-helix and β-pleated sheet)
Tertiary	Folding of the polypeptide chain involving interactions between the R-groups of the amino acid residues: London dispersion forces, hydrogen bonds, ionic interactions and disulfide bridges
Quaternary	Folding and assembly of multiple sub-units of a protein, involving the same interactions as tertiary structure

QUICK CHECK QUESTIONS

6 Alanine is a white crystalline solid that readily dissolves in water. State the special features about the structure of the molecule that account for:

a its crystalline nature

b its solubility in water.

7 The following diagram represents the two ends of the primary structure of a myoglobin molecule:

X-Val-Leu-Ser-Glu-Gly---------Gly-Tyr-Gln-Gly-COOH

where Val, Leu, Ser, etc. are the shorthand forms referring to the different amino acids in the chain.

a Which functional group is represented by **X**?

b Name the covalent bond formed between **each** pair of amino acids in the chain.

c Draw a diagram of this link region showing clearly the atoms present and the bonds present.

d Describe the bonding responsible for the secondary structure of myoglobin and other proteins.

e Explain what is meant by the *secondary*, *tertiary* and *quaternary* structure of a protein.

8 Polypeptides and proteins are formed by the condensation reactions of amino acids.

a Give the general, condensed structural formula of a 2-amino acid.

b Give the condensed structural formulae of the two dipeptides formed by the test tube reaction of alanine and glycine.

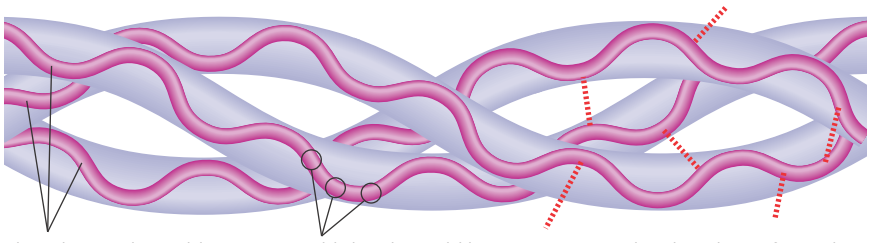
State the other inorganic substance formed during this reaction.

c How many different tripeptides can be formed from glycine, serine and alanine if each tripeptide molecule contains one residue from each of these three amino acids?

Fibrous and globular proteins

Biological molecules, especially proteins, are very diverse in nature and their functions depend upon their chemical structures and overall molecular shape (conformation).

Fibrous proteins, for example, collagen (Figure 23.20), consist of linear polypeptide chains that are bundled and associated together. Globular proteins, for example, hemoglobin (Figure 23.21) are polypeptide chains that are coiled into compact shapes. Structural proteins, such as keratin, are fibrous proteins that provide structural rigidity in nails and hair.



three long polypeptide molecules, coiled together to form a triple helix

every third amino acid is glycine (the smallest amino acid) and the other two amino acids are mostly proline and hydroxyproline

covalent bonds are formed between the polypeptide chains – together with many hydrogen bonds

Figure 23.20 The structure of collagen, a fibrous protein

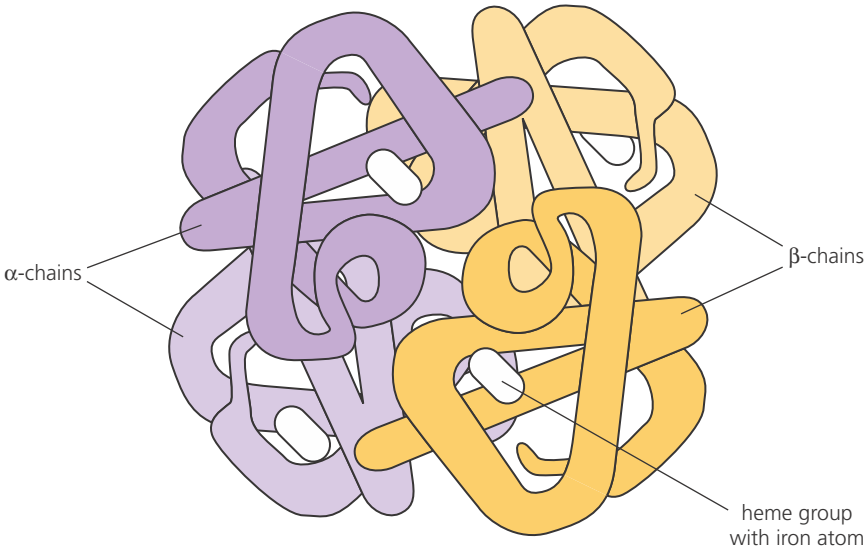


Figure 23.21 Structure of hemoglobin

Transport proteins, such as hemoglobin, are used to transport molecules or ions from one location to another. Hemoglobin consists of a protein unit covalently bonded to a non-protein prosthetic group which contains an iron(II) ion that reversibly bonds to an oxygen molecule. It is an oxygen transport protein.

Expert tip

Some enzymes require of the presence of cofactors, small non-protein units, to function. Cofactors may be inorganic ions or complex organic molecules called coenzymes. A cofactor that is covalently bonded to the enzyme is called a prosthetic group.

Table 23.3 The roles of different proteins

Protein	Function	Where found in body
Collagen	Structural protein, giving strength and elasticity	Skin, tendon, bone
Keratin	Structural	Hair, nails
Myosin	Muscle contraction	Muscle tissue
Actin	Muscle contraction	Muscle tissue
Chymotrypsin	Digestive (proteolytic) enzyme, breaks down proteins in food	Small intestine
Pepsin	Digestive (proteolytic) enzyme, breaks down proteins in food	Stomach
Insulin	Peptide hormone, enables use of glucose for energy through respiration	Blood, pancreas
Immunoglobulin	Antibodies	Blood, lymph
Hemoglobin	Oxygen transport from lungs to other tissues	Blood

Enzymes

Enzymes are biological catalysts and greatly accelerate biochemical reactions without being altered by the reaction. This effect is achieved by providing an alternative reaction pathway that has a lower activation energy than the uncatalysed reaction (Figure 23.22). Enzymes achieve their catalysis via the stabilization of the transition state (activated complex) formed by the reactants (substrate) during the reaction.

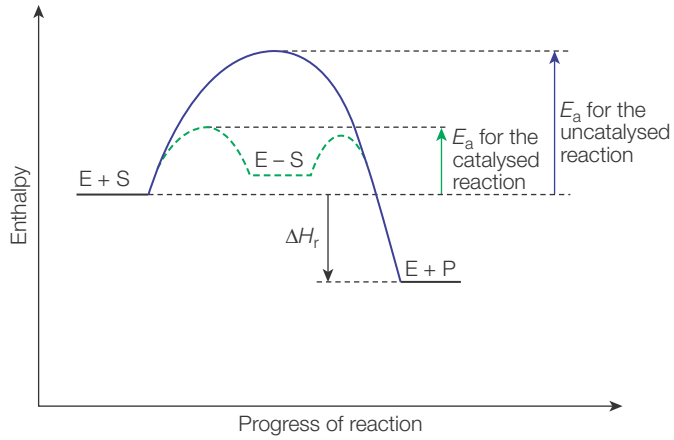


Figure 23.22 The enthalpy profile of an enzyme-catalysed reaction (ΔH_r, E_a, S, E and P represent enthalpy change of reaction, activation, substrate, enzyme and product, respectively)

Table 23.4 Differences between enzymes and inorganic catalysts

Enzymes	Inorganic catalysts
Enzymes are complex globular proteins	Inorganic catalysts are generally ions or simple molecules
Enzymes are synthesized by living cells	Inorganic catalysts are not produced by living cells
Enzymes are usually highly specific in action	Inorganic catalysts are usually less specific in action
Enzymes are sensitive to changes in pH and temperature	Inorganic catalysts are usually less sensitive to changes in pH and temperature
Enzymes only function in aqueous solution	Some inorganic catalysts function in aqueous solution

Enzymes are globular proteins and are able to catalyse in aqueous solution under mild conditions of temperature and pH (unless the enzymes have evolved to function at extremes of temperature or pH). Although very specific compared with inorganic catalysts (see Table 23.4), enzymes vary considerably in their degree of specificity. Some are absolutely specific for a particular substrate, and will not bind and attack even the enantiomer (optical isomer), whereas others will react with a whole class of molecules but at widely differing rates. The function of an enzyme depends on its three-dimensional shape (conformation), in particular the precise shape of the active site. This region of the enzyme's surface recognizes and binds (via non-covalent interactions) the specific substrate of the enzyme.

The chemical basis of enzyme catalysis involves the stabilizing of the transition state of the reaction by helping to orient the substrate(s) in the active site. This brings together functional groups on the enzyme and the substrate so that enzyme functional groups can participate in the chemical catalysis events, which may involve covalent bond formation or acid–base catalysis.

The process of enzyme–substrate recognition and binding is often referred to as the 'lock and key' model (Figure 23.23). The active site of an enzyme is the binding site for the substrate and a catalytic site which completes the enzyme-catalysed reaction.

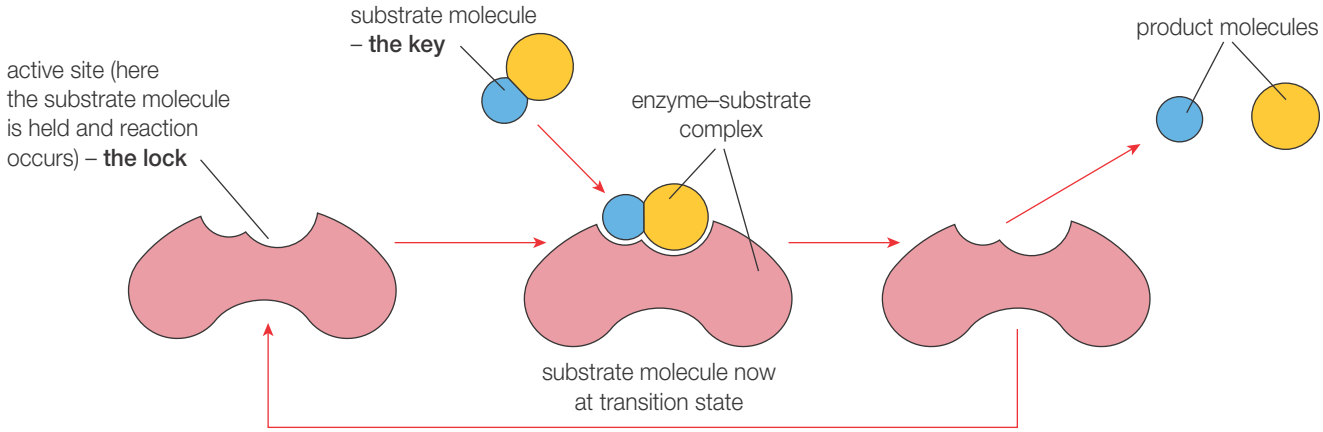


Figure 23.23 The lock and key model of enzyme action

Expert tip

The catalytic rate of an enzyme can be lowered by inhibitor molecules. Many inhibitors exist, including normal body metabolites, foreign drugs and toxins.

Enzyme-catalysed biochemical reactions are sensitive to conditions of temperature and pH. They are usually active within a narrow range and show characteristic temperature and pH optima (Figures 23.24 and 23.25, respectively).

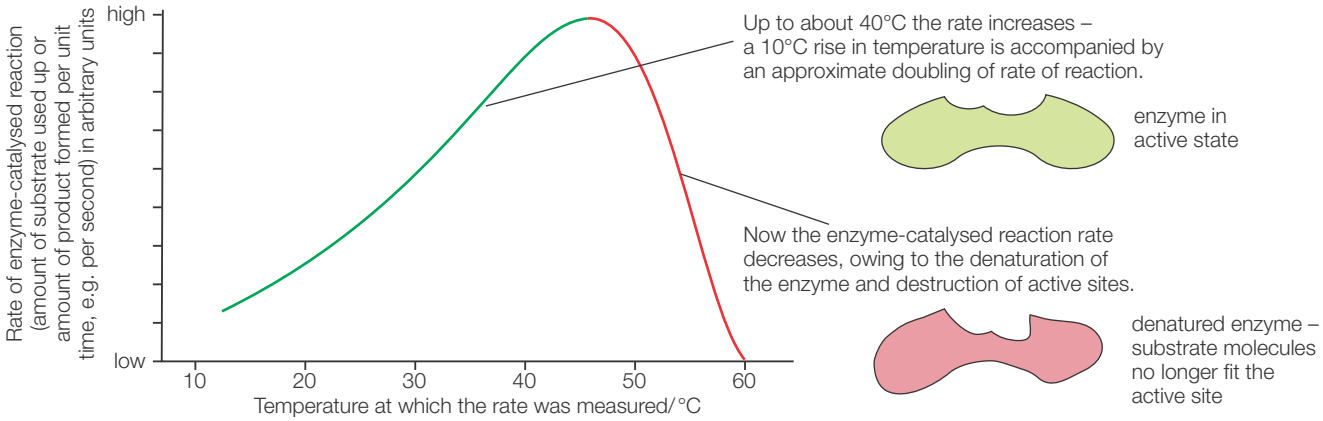


Figure 23.24 The effect of temperature on enzyme shape and activity

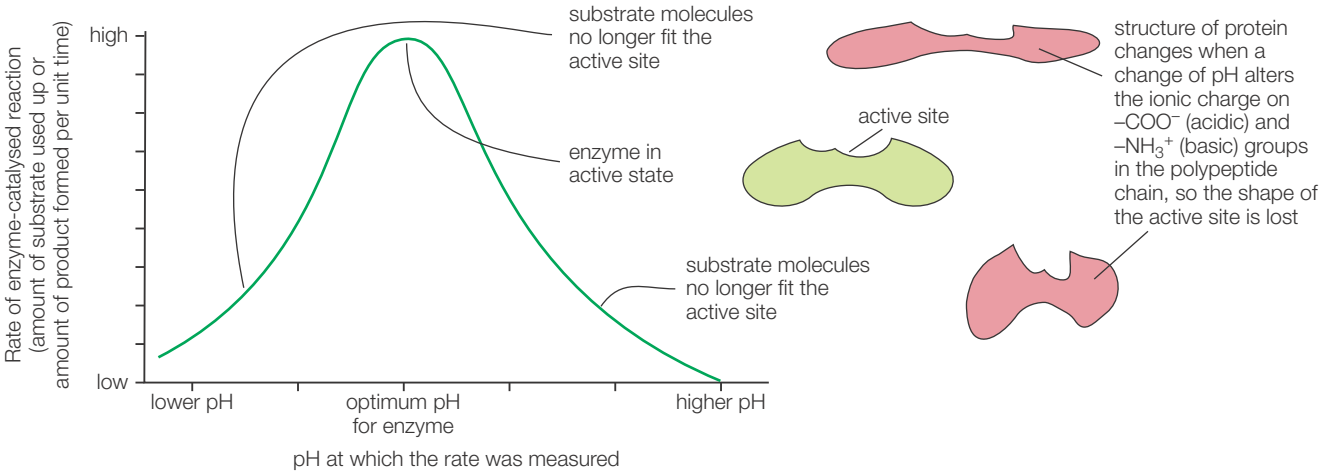


Figure 23.25 The effect of pH on enzyme shape and activity

Temperature affects the rate of enzyme-catalysed reaction by increasing the average kinetic energy of the substrate molecules. This increases the proportion of molecules with sufficient kinetic energy to overcome the activation barrier and hence increases the rate of the reaction.

In addition, the kinetic energy of the component molecules of the enzyme is increased, which leads to an increased rate of denaturation of the enzyme protein due to the disruption of the non-covalent interactions holding the structure together.

Large deviations in pH lead to denaturation of the enzyme due to changes in the ionization of amino acid residues and the disruption of non-covalent interactions, especially hydrogen bonds.

Inhibitors which bind irreversibly to an enzyme often form a covalent bond to an amino acid residue at or near the active site, and permanently inactivate the enzyme. Susceptible amino acid residues include serine and cysteine residues

which have reactive –OH and –SH groups, respectively. Heavy metals and their ions react with the side-chain of cysteine residues (Figure 23.26).

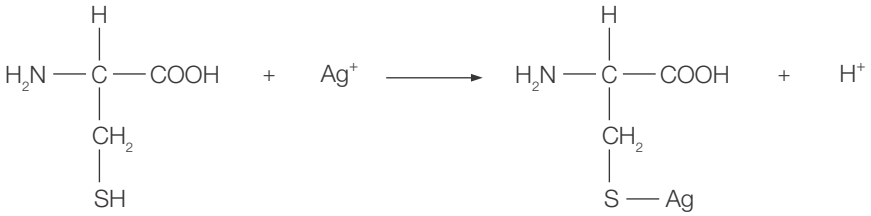


Figure 23.26 The action of silver ions on the –SH functional group of cysteine

Analysis of proteins

The primary structure of a small protein can be determined by paper chromatography or, more efficiently, by gel electrophoresis. Chromatography and electrophoresis both separate and identify substances in complex mixtures. The protein is hydrolysed to amino acids by boiling with concentrated hydrochloric acid in a sealed tube for six hours. The three-dimensional tertiary structure of the protein can be confirmed by X-ray analysis of the crystalline protein.

Paper chromatography

In paper chromatography, the mobile phase (a solvent mixture) moves the amino acids over the stationary phase (hydrated cellulose). Separation occurs by the transfer of amino acids to the stationary phase by portioning between the two liquids. The amino acids are identified by their retention values (R_f) (Figure 23.27) after they have been made visible by reaction with the locating agent ninhydrin.

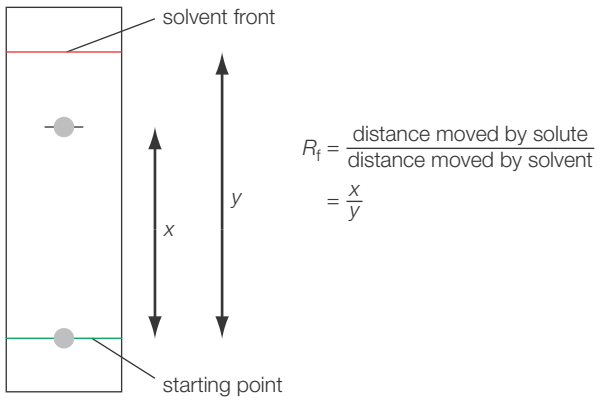


Figure 23.27 Calculating the retention factor, R_f, from a paper chromatogram

Common mistake

Remember when measuring distances to calculate R_f values to start from the pencil line (origin) not the bottom of the paper.

Two-dimensional chromatography consists of two successive acts of chromatographic separation, done with different solvents, in directions at 90° to each other. It operates on the principle that amino acids left unseparated by one solvent will be resolved by the second.

Electrophoresis

In gel electrophoresis (Figure 23.28) the mixture of amino acids is supported on a semi-solid gel. The amino acids molecules are charged and move in an applied electric field, where they separate according to their charge and the shape and size of their molecules.

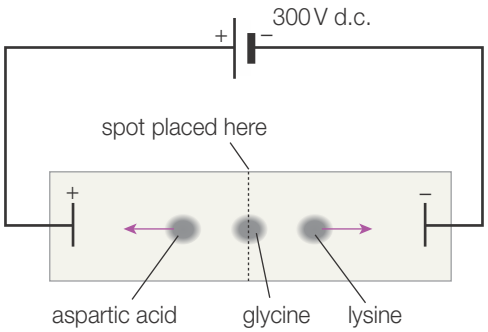


Figure 23.28 Electrophoresis of a mixture of glycine, lysine and aspartic acid

Expert tip

A buffer solution is used as the electrolyte in electrophoresis. For amino acids (and protein fragments), the net charge on the molecule depends on the pH of the medium.

Remember that an amino acid or protein will be positively charged if the pH of the running buffer is below its isoelectric point, and negatively charged if the pH is above its isoelectric point.

QUICK CHECK QUESTIONS

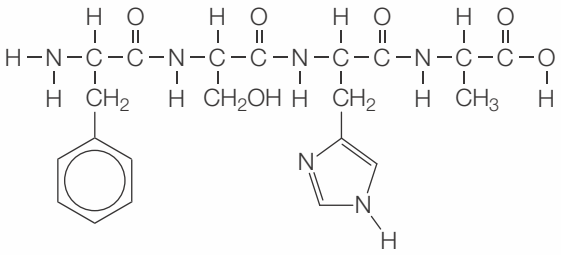
9 Electrophoresis can be used to identify the amino acids present in any given protein. To carry out this analysis the protein must first be hydrolysed.

a State the reagent and conditions needed to hydrolyse the protein, and identify the bond that is broken during hydrolysis.

b Explain how the amino acids could be identified using electrophoresis.

10 The isoelectric point (pI value) of the amino acid serine (2-amino-hydroxypropanoic acid) is 5.7. Draw the main structural formula of serine, in the solid state and in an aqueous solution at pH values of 1, 14 and 5.7.

11 Name and give the structures of the amino acids that would be formed by the hydrolysis of the polypeptide below. Use the information on their structures given in Section 33 of the IB Chemistry *data booklet* to help identify them.



12 a Sketch the energy profile of an uncatalysed exothermic reaction, showing:

i the activation energy (E_a)

ii the enthalpy change of reaction (ΔH_r).

b Sketch a similar energy profile for the reaction in part **a** when the reaction is enzyme catalysed.

23.3 Lipids

Revised

Essential idea: Lipids are a broad group of biomolecules that are largely non-polar and therefore insoluble in water.

Lipids

Revised

- Fats are more reduced than carbohydrates and so yield more energy when oxidized.
- Triglycerides are produced by condensation of glycerol with three fatty acids and contain ester links. Fatty acids can be saturated, monounsaturated or polyunsaturated.
- Phospholipids are derivatives of triglycerides.
- Hydrolysis of triglycerides and phospholipids can occur using enzymes or in alkaline or acidic conditions.

- Steroids have a characteristic fused ring structure, known as a steroidal backbone.
- Lipids act as structural components of cell membranes, in energy storage, thermal and electrical insulation, as transporters of lipid-soluble vitamins and as hormones.

Fats and oils

Revised

Fats and oils and steroids are collectively termed lipids. Lipids are a structurally diverse group of biological compounds that are grouped together due to their poor solubility in water and excellent solubility in organic solvents. Lipids can be extracted from cells, using non-polar solvents, such as ethers and hydrocarbons.

Lipids act as structural components of cell membranes (as phospholipids), in energy storage (as adipose tissue), thermal and electrical insulation (around nerves) and as transporters of lipid-soluble vitamins and as hormones (vitamin D).

Lipids are a concentrated chemical energy source of carbon and hydrogen. They are more reduced than carbohydrates (owing to the presence of fewer oxygen atoms). Lipids release more energy per gram during respiration (oxidation) than do carbohydrates. Animal fats and vegetable oils contain a mixture of lipids.

Fats and oils are triglyceryl esters (Figure 23.29), esters of glycerol (propane-1,2,3-triol) with fatty acids, aliphatic acids with 16–22 carbon atoms. They are formed by enzyme-controlled reactions between three fatty acid molecules and glycerol (propane-1,2,3-triol) (Figure 23.30). The resulting triglyceride is said to contain three fatty acid residues.

Expert tip

Fats are semi-solid at room temperature; oils are liquids.

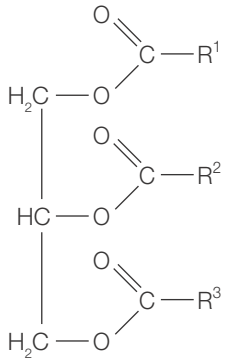


Figure 23.29 The generalized formula of a triglyceride, where R^1, R^2 and R^3 represent fatty acid chains, which may be the same or different

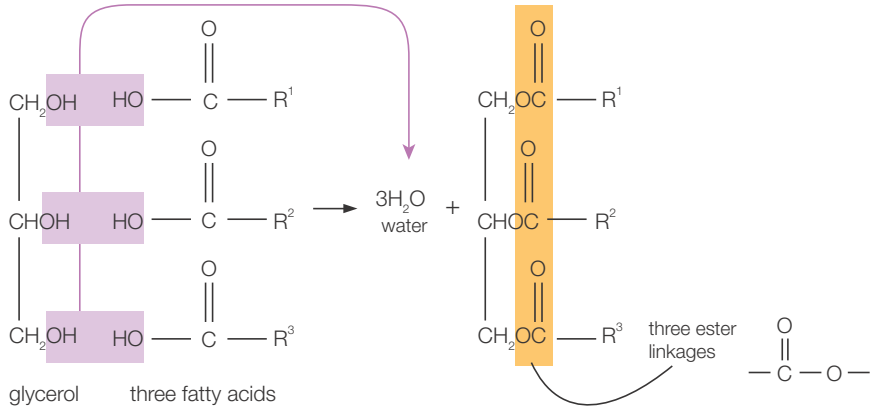


Figure 23.30 Formation of a triglyceride from fatty acids and a glycerol molecule by condensation reactions

The chemical difference between fats and oils is that oils contain unsaturated fatty acid chains and fats contain saturated fatty acid chains (Figure 23.31). Many oil molecules contain one or several carbon–carbon double bonds and are described as polyunsaturated.

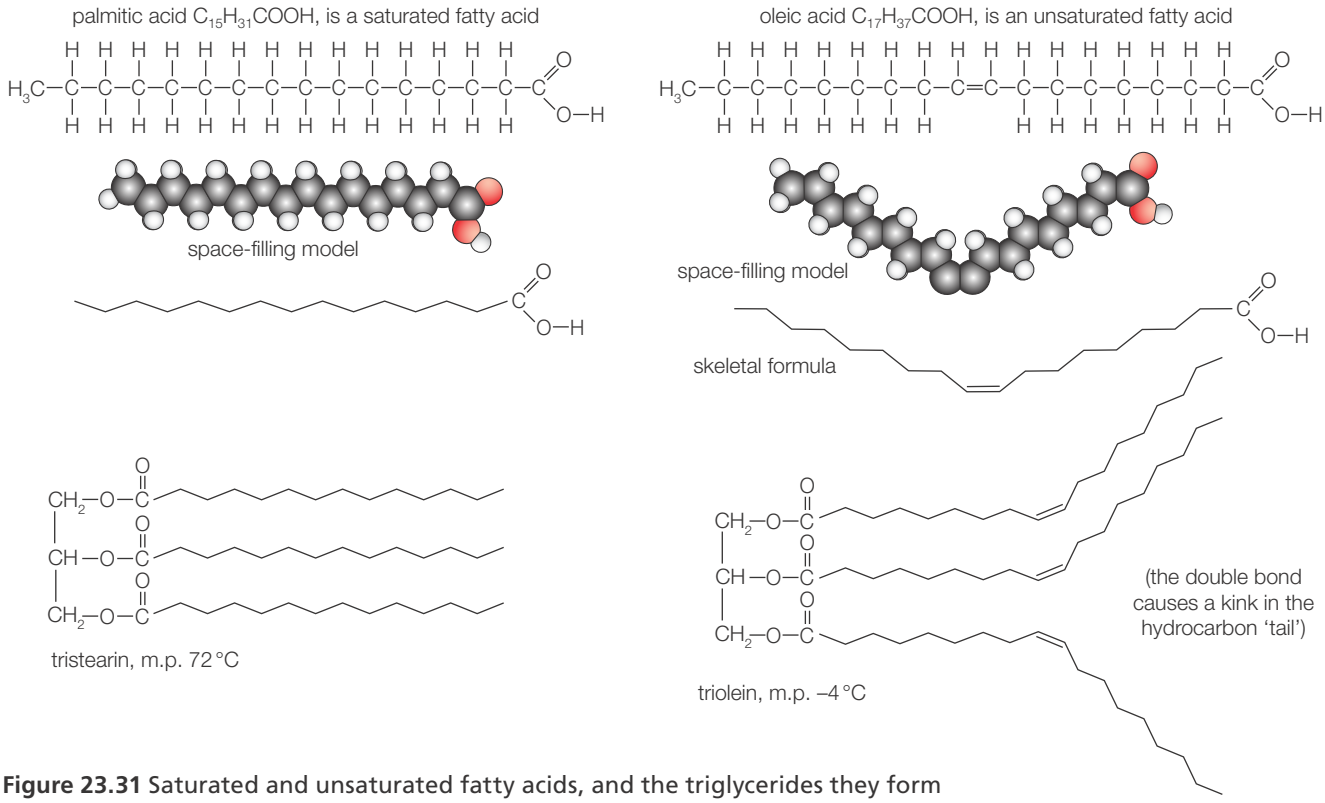


Figure 23.31 Saturated and unsaturated fatty acids, and the triglycerides they form

The melting points and other properties of lipids depend on the identity of the fatty acid groups. Fats containing unsaturated fatty acids melt at lower temperatures than those with saturated fatty acids (Table 23.5).

Table 23.5 The melting points of selected saturated and unsaturated fatty acids

Name of fatty acid	Number of carbon atoms	Number of C=C double bonds	Melting point/°C
Saturated fatty acids			
Lauric acid $CH_3(CH_2)_{10}COOH$	12	0	44.2
Myristic acid $CH_3(CH_2)_{12}COOH$	14	0	54.1
Palmitic acid $CH_3(CH_2)_{14}COOH$	16	0	62.7
Stearic acid $CH_3(CH_2)_{16}COOH$	18	0	69.6
Arachidic acid $CH_3(CH_2)_{18}COOH$	20	0	75.5
Unsaturated fatty acids			
Palmitoleic acid $CH_3(CH_2)_5CH=CH(CH_2)_7COOH$	16	1	–0.1
Oleic acid $CH_3(CH_2)_7CH=CH(CH_2)_7COOH$	18	1	10.5
Linoleic acid $CH_3(CH_2)_4CH=CHCH_2CH=CH(CH_2)_7COOH$	18	2	–5.0
Linolenic acid $CH_3CH_2CH=CHCH_2CH=CHCH_2CH=CH(CH_2)_7COOH$	18	3	–11.9
Arachidonic acid $CH_3(CH_2)_4(CH=CHCH_2)_4(CH_2)_2COOH$	20	4	–49.0

This trend is a steric effect and occurs because the introduction of a carbon–carbon double bond prevents the triglyceride molecules from approaching each other closely and hence interacting via London dispersion forces. Long-chain saturated fatty acids have a regular tetrahedral arrangement of carbon atoms and so can pack closely together. The dispersion forces between chains are strong because of their extended surface area.

In unsaturated fatty acids the bond angle in the chains changes around the double bond and the structure becomes rigid at that point. This introduces a kink in the chain (Figure 23.31) and they are unable to pack so closely together.

Expert tip

Where two lipids have similar degrees of unsaturation, the one with the higher molecular mass will tend to have the higher melting point.

Phospholipids

Phospholipids are formed by the condensation of two fatty acid molecules and a phosphate group (or a derivative of a phosphate group). The simplest type of phosphoglyceride is a phosphoric acid monoester, called a phosphatidic acid. Phosphoglycerides that contain choline are called lecithins.

Phospholipid molecules are amphipathic, having hydrophobic (non-polar) hydrocarbon chains and a hydrophilic (polar) phosphate group (Figure 23.32). Phospholipid bilayers form as molecules line up with the phospholipids hydrophilic 'heads' on the outside and hydrophobic 'tails' on the inside of the double layer of the cell membrane.

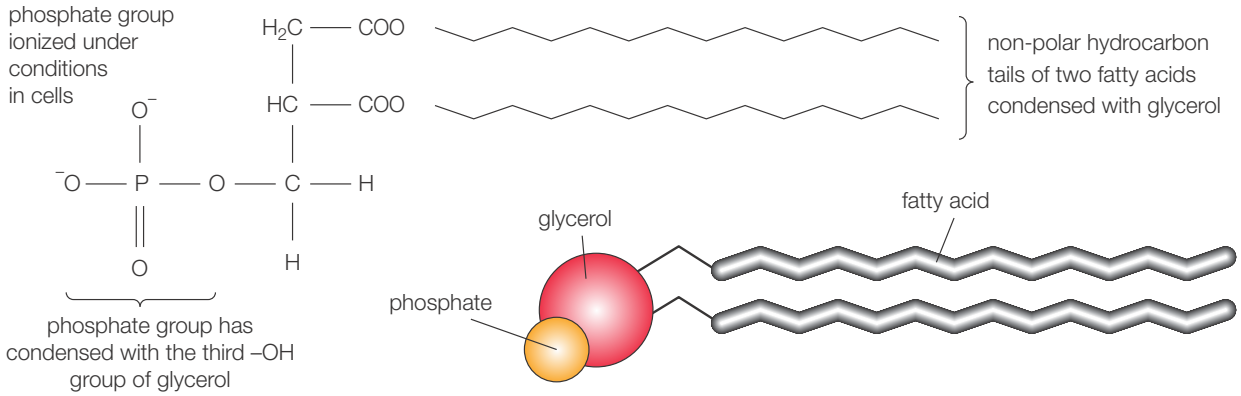


Figure 23.32 Generalized structure of a simple phospholipid

Reactions of lipids

Hydrolysis of lipids occurs slowly to produce fatty acids and other products with a rancid smell. This is termed hydrolytic rancidity.

The alkaline hydrolysis of fats and oils by sodium hydroxide produces soaps: the sodium salt of fatty acids. The process is called saponification (Figure 23.33).

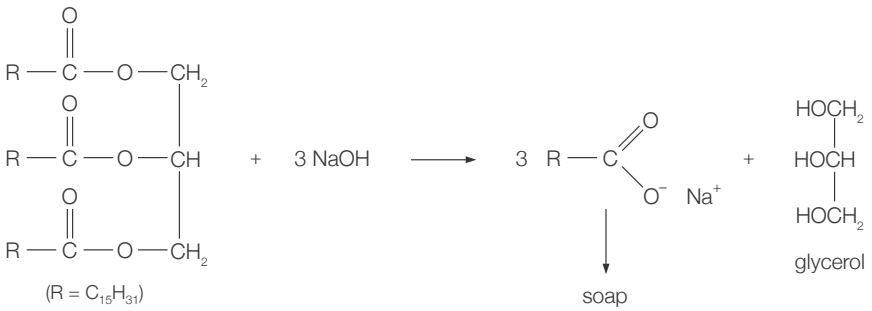


Figure 23.33 Production of soap

The saponification value of a lipid is a measure of the amount of fatty acids that is formed when one gram of lipid is completely hydrolysed by a strong base. This technique can be used to find the fatty acid composition of food.

Unsaturated oils can be hydrogenated, for example, in the production of margarine. Oils contain *cis*-unsaturated fatty acids which are 'healthier' than saturated fats as they increase levels of HDL cholesterol. However, during the hydrogenation process partial hydrogenation may occur, leading to the production of *trans*-unsaturated fats (Figure 23.34). These increase the formation of LDL cholesterol which raises the risk of heart disease.

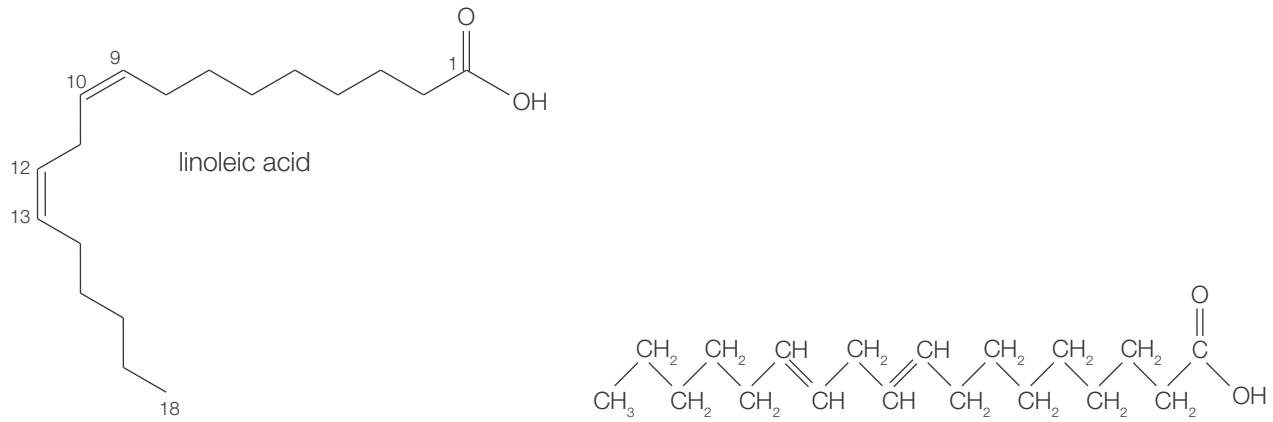


Figure 23.34 **a** The structures of the *cis,cis* form of linoleic acid and **b** the straightening of the *trans,trans* form of linoleic acid

Fats and oils can undergo addition reactions with iodine (in the presence of a non-polar solvent). The iodine value is a measure of the degree of unsaturation.

The iodine number is the mass of iodine in grams that reacts with 100 g of an unsaturated lipid. A moles calculation can be used to deduce the number of carbon–carbon double bonds since each mole reacts with 1 mole of iodine molecules (Figure 23.35).

Transesterification of triglycerides can be achieved via either acid or base catalysis to produce biodiesel (Option C Energy).

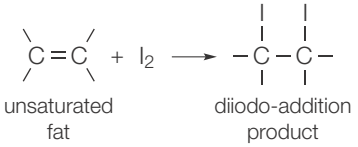


Figure 23.35 Addition reaction between carbon–carbon double bond (in fatty acid) and iodine molecule

Worked example

Oleic acid, $[CH_3(CH_2)_7CH{=}CH(CH_2)_7COOH$, is present in many animal and vegetable fats and oils. Calculate the iodine number.

It is monounsaturated and hence each carbon–carbon double bond reacts with one molecule of iodine.

molar mass of oleic acid = $(18 \times 12.01) + (34 \times 1.01) + (16.00 \times 2)$

$= 282.52\ g\ mol^{-1}$

molar mass of iodine = (126.90×2)

$= 253.80\ g\ mol^{-1}$

282.52 g of lipid reacts with 253.80 g of iodine; by ratios, 100 g of fat reacts with $\frac{(253.80 \times 100)}{282.52} = 90$ g of iodine, and hence the iodine number is 90.

Expert tip

Similar addition reactions occur with the more reactive iodine monochloride, ICl, which was referred to in Topic 20 Organic chemistry.

Rancidity

Lipids become rancid as a consequence of hydrolytic or oxidative processes.

- *Hydrolytic rancidity* occurs where ester links are broken by water, producing glycerol and unpleasant smelling/tasting fatty acids. This condition is favoured by high water content, acidic or alkaline conditions, high temperature and the presence of lipase enzymes.
- *Oxidative rancidity* occurs where fatty acid chains are broken down when oxygen reacts with the C=C bonds of unsaturated lipids. Unpleasant smelling and tasting ketones, alcohols and aldehydes are produced. This condition is favoured by a high proportion of unsaturated fatty acids, high temperature, a high oxygen availability, high light intensity and the presence of metals such as copper and nickel.

Expert tip

You will need to know the conditions that favour rancidification.

QUICK CHECK QUESTIONS

13 State **three** functions of lipids (fats and oils) in the human body.

14 Glycerol can react with three molecules of stearic acid, $C_{17}H_{35}COOH$, to form a triglyceride.

a Deduce the number of carbon atoms in one molecule of the triglyceride formed.

b An oil sample containing 0.0100 mol of oil was found to react with 7.61 g of iodine, I_2, in a test to determine the degree of unsaturation of the oil.

Determine the number of double bonds present in each molecule of the oil.

15 Fats and oils are made from a molecule of propane-1,2,3-triol bonded to three molecules of fatty (alkanoic) acids.

a Give the structural formula of propane-1,2,3-triol.

b Give the name and formula of the functional group present in all alkanoic acids and draw the structural formula of the saturated fatty acid containing eight carbon atoms per molecule.

c Explain the difference between saturated and unsaturated fats in terms of their molecular structures.

d Explain briefly how the degree of unsaturation of lipids can be determined experimentally.

16 A sample of vegetable oil (2.50 g) reacted completely with 19.00 cm^3 of a 0.50 mol dm^{-3} solution of iodine.

a Calculate the iodine number of the oil.

b Estimate the average number of carbon–carbon double bonds per molecule of this oil if its average molecular mass is 865 g mol^{-1}.

c Explain why is the figure in **b** necessarily an average figure for the number of double bonds per molecule.

17 **a** Predict and explain which fatty acid in each group has the highest melting point:

i butanoic acid, palmitic acid and stearic acid

ii linoleic acid, oleic acid and linolenic acid.

b Chocolate is a food made from cocoa, sugars, unsaturated vegetable fats, milk whey and emulsifiers. Chocolate bars sold in hot climates are made with a different blend of vegetable fats from those sold in colder climates.

i Explain why fats with different structures, and hence physical properties, are used for making chocolate in different climates.

ii Suggest how the fat molecules used in a hot climate differ chemically from those used in a cold climate.

18 The average figures for the percentage fatty acid composition of some common fats and oils are presented in the table below.

Source of fat/oil	Saturated fatty acids/%	Monounsaturated fatty acid (oleic acid)/%	Unsaturated fatty acids/%	
			Linoleic acid	Linolenic acid
Beef fat	59	38	3	–
Corn oil	25	26	47	2
Olive oil	15	78	7	–
Soya bean oil	14	28	50	8
Coconut oil	90	8	2	–

a Deduce which of these fats/oils has the highest iodine number. Give a reason for your answer.

b The 'P/S index' is sometimes used to compare the relative health benefits of different lipids in the diet. It is the ratio of polyunsaturated fat to saturated fat present in the fat or oil. Calculate the P/S index of soya bean oil, olive oil and beef fat.

c Which of the fats or oils listed above is most likely to turn rancid when exposed to the air? Explain your choice.

Steroids

Cholesterol (Figure 23.36) is present in animal cell membranes (where it controls fluidity) and has the characteristic fused four ring structure possessed by all steroids (Figure 23.37). This is a tetracyclic system, involving three six-membered rings and one five-membered ring that form a rigid system.

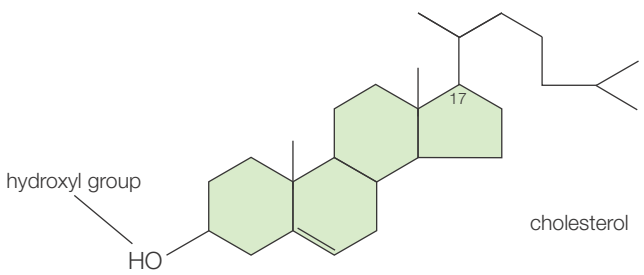


Figure 23.36 The skeletal structure of cholesterol. This is the most abundant steroid in humans

The presence of cholesterol in mammalian cell membranes has the effect of disturbing the close-packing of the phospholipids, influencing the flexibility and fluidity of the membrane, and controlling its permeability to some solutes. Cholesterol molecules have a hydroxyl (–OH) group and a hydrocarbon chain either side of the carbon ring structures. Each cholesterol's hydroxyl group aligns with the phosphate heads of the phospholipids, and the hydrocarbon chain with the fatty acid chain on the nearest adjacent phospholipid (Figure 23.38). This helps maintain a stable structure on the outer surface of the membrane, making it less permeable to very small water-soluble molecules that could otherwise easily pass through.

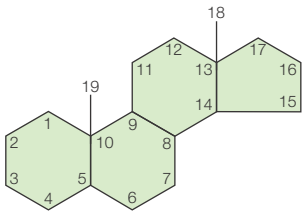


Figure 23.37 The steroid 'nucleus' of four fused rings

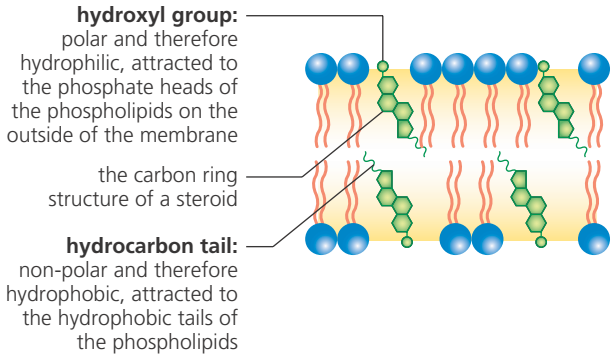


Figure 23.38 The interaction of cholesterol with the phospholipids of animal cell membranes

Cholesterol is transported around the body in the blood plasma by lipoproteins. Low-density lipoproteins (Figure 23.39) (LDL) transport cholesterol through the arteries, where it can line the walls of the arteries as plaque, leading to heart diseases. Smaller lipoproteins, known as high-density lipoproteins (HDL), can remove the plaque (mainly cholesterol) from the arteries and transport it back to the liver to be metabolized.

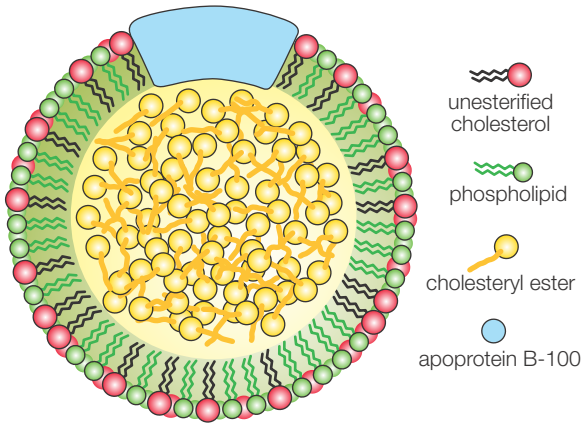


Figure 23.39 Structure of LDL

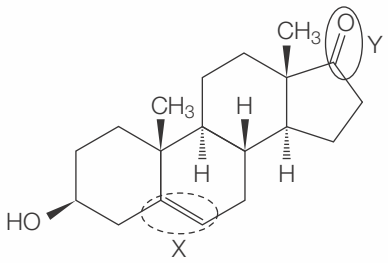
Human sex hormones are steroids that regulate tissue growth and reproductive processes.

Expert tip

You need to be able to describe the use and abuse of steroids.

■ QUICK CHECK QUESTION

19 The prohibited list of the World Anti-Doping Agency (WADA), which manages drug testing for the Olympics and other sports, includes the steroid hormone dehydroepiandrosterone (DHEA). The structure of DHEA is given below:



a State the name of the functional groups circled in the DHEA molecule shown above.

b Identify the characteristic region of this structure that means that this molecule is classified as a steroid.

c The production of banned synthetic steroids has ethical implications. Suggest a reason why steroid research might be supported.

23.4 Carbohydrates

Revised

Essential idea: Carbohydrates are oxygen-rich biomolecules, which play a central role in metabolic reactions of energy transfer.

Carbohydrates

Revised

- Carbohydrates have the general formula $C_x(H_2O)_y$.
- Haworth projections represent the cyclic structures of monosaccharides.
- Monosaccharides contain either an aldehyde group (aldose) or a ketone group (ketose) and several –OH groups.
- Straight chain forms of sugars cyclize in solution to form ring structures containing an ether linkage.
- Glycosidic bonds form between monosaccharides forming disaccharides and polysaccharides.
- Carbohydrates are used as energy sources and energy reserves.

■ Monosaccharides

Carbohydrates are important in nutrition and have the general formula $C_x(H_2O)_y$. The simplest carbohydrates are monosaccharide sugars: trioses (C3 sugars), for example, ribose, $C_5H_{10}O_5$, pentoses (C5 sugars), and hexoses (C6 sugars), for example, glucose and fructose, $C_6H_{12}O_6$ (see Table 23.6). Monosaccharides are used to release energy during respiration or act as precursors for other biomolecules.

Table 23.6 Examples of different monosaccharides

Value of *n* in $C_nH_{2n}O_n$	Example	Formula	Type of sugar
3	Glyceraldehyde (2,3-dihydroxypropanal)	$C_3H_6O_3$	Triose
4	Erythrose	$C_4H_8O_4$	Tetrose
5	Ribose	$C_5H_{10}O_5$	Pentose
6	Glucose	$C_6H_{12}O_6$	Hexose

Expert tip

Deoxysugars contain less oxygen; there is one less hydroxyl group attached to the chain. For example, 2-deoxyribose ($C_5H_{10}O_4$), which is present in DNA, contains one less oxygen atom than ribose ($C_5H_{10}O_5$), the sugar present in RNA.

Monosaccharides are classified as aldoses (containing a terminal aldehyde group), for example, glucose (Figure 23.40), or as ketoses (containing a ketone group) (Figure 23.41). Monosaccharides contain chiral carbon atoms and two or more hydroxyl groups.

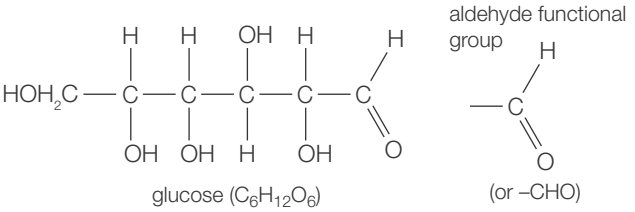


Figure 23.40 The structure of D-glucose, an aldose

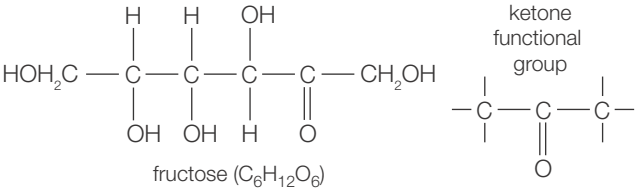


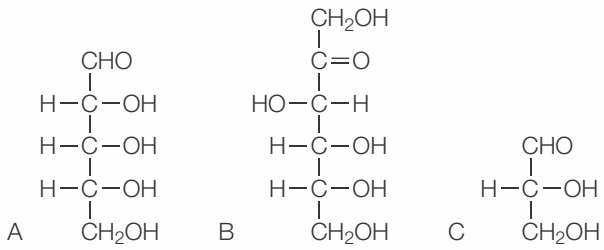
Figure 23.41 The structure of D-fructose, a ketose

Expert tip

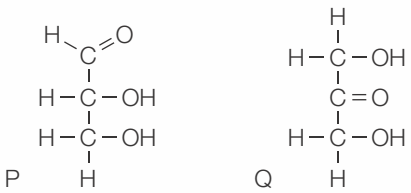
Monosaccharides are known as simple sugars because they cannot be hydrolysed. You'll note that in the monosaccharides the values of *x* and *y* in the general molecular formula $C_x(H_2O)_y$ are the same.

QUICK CHECK QUESTIONS

20 Which of the following is (or are) a ketone, an aldehyde, a triose, a pentose and a hexose?



21 The structures of two molecules, **P** and **Q**, having the same formula, $C_3H_6O_3$, are shown below.



a Explain why both these molecules are classed as carbohydrates.

b Distinguish between these molecules on the basis of the carbonyl functional group present in each molecule.

22 Glucose, $C_6H_{12}O_6$, is a monosaccharide that our bodies can use as a source of energy.

a Give the equation for the cellular respiration of glucose.

b Calculate the energy, in kJ, produced from 15.0 g of glucose given that its enthalpy of combustion, is –2803 kJ mol^{-1}.

Disaccharides

Monosaccharides condense (in the presence of enzymes) by the formation of glycosidic bonds (ether linkages) to form dimers known as disaccharides, for example, sucrose (Figure 23.42), lactose and maltose (Figure 23.43) ($C_{12}H_{22}O_{11}$). Disaccharides condense (in the presence of enzymes) to form polysaccharides, for example, amylose and amylopectin, (starch) glycogen and cellulose.

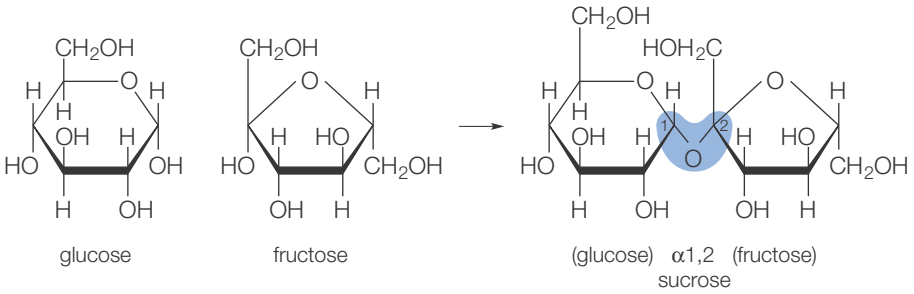


Figure 23.42 The formation of sucrose from glucose and fructose

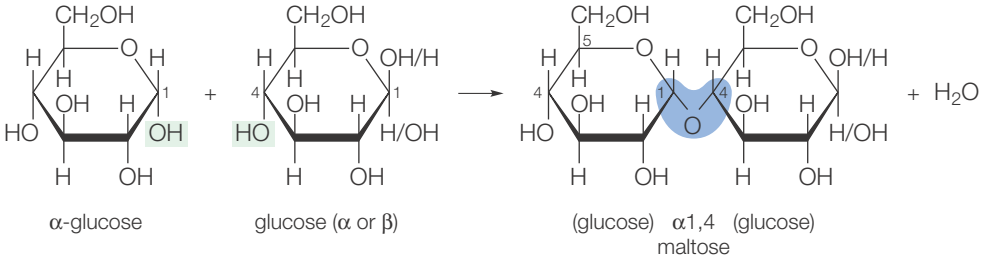


Figure 23.43 The formation of maltose from glucose

Monosaccharides and disaccharides are very soluble because their hydroxyl groups form hydrogen bonds with water molecules. Polysaccharides, such as starch and glycogen, have limited solubility and therefore make better food storage materials.

Polysaccharides

Starch is the food storage material of plants. It is a polysaccharide, which occurs in two forms: amylose and amylopectin. Both are condensation polymers of glucose and poorly soluble in water.

Amylose is an unbranched polymer of glucose molecules linked by α-1,4-glycosidic bonds. Amylopectin is a branched polymer of glucose, but has many branches arising from α-1,4-glycosidic bonds. Starch contains variable amounts of amylose and amylopectin (Figure 23.44).

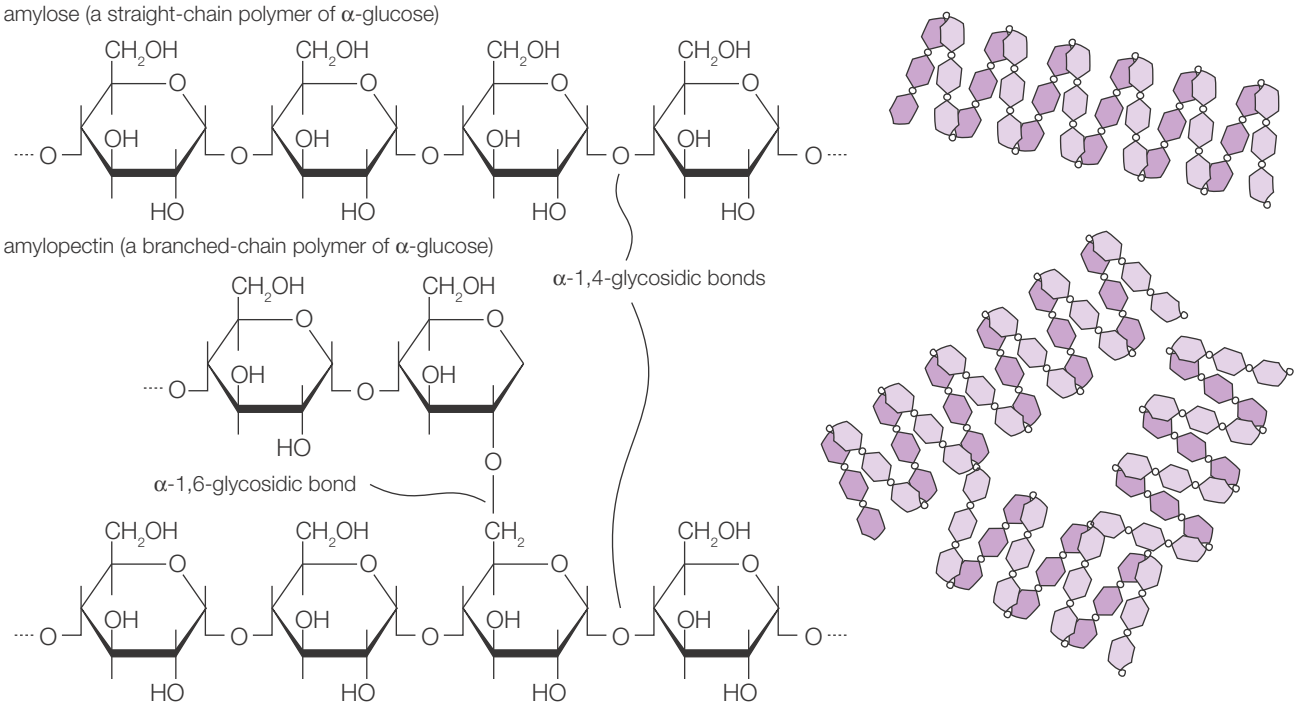


Figure 23.44 Structures of amylose and amylopectin

Glycogen, the food storage material in an animal, is a polysaccharide which is similar in structure to amylopectin (a component of starch), but more branches occur. Glycogen is stored in the liver and muscles.

Cellulose (Figure 23.45) is the structural component of plant cell walls. It is a polysaccharide consisting of glucose units joined by 1, β-4-glycosidic linkages. Few animals have the cellulose enzyme that can hydrolyse these linkages, so allowing them to digest cellulose.

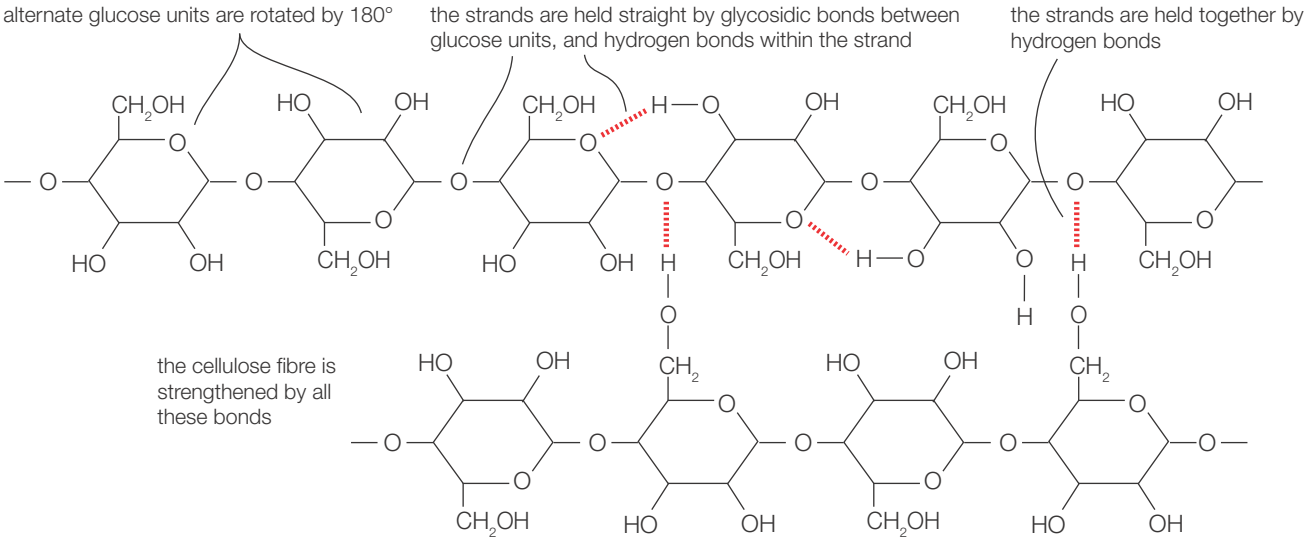


Figure 23.45 Structure of cellulose

Cellulose molecules are linear because of the orientation of the glucose residues. The molecules are held together by hydrogen bonds between hydroxyl groups and are assembled into cellulose fibres which give tensile strength to plant cell walls.

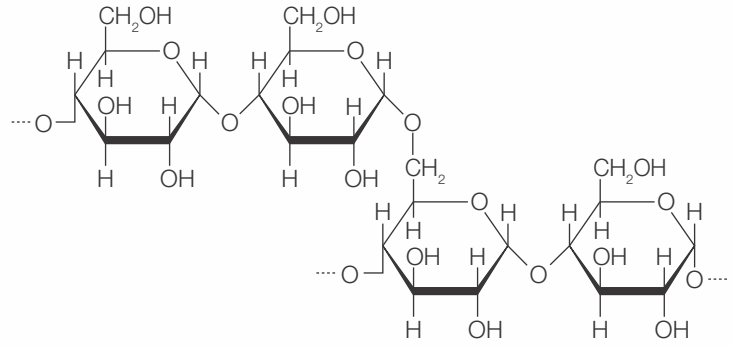
Polysaccharides can be hydrolysed by heating with dilute hydrochloric acid. Polysaccharides can also be hydrolysed by specific enzymes, which convert polysaccharides to monosaccharides when required by the cell. Hence the chemical energy stored in these molecules is available to the organism. Table 23.7 presents the major features and roles of the different polysaccharides.

Table 23.7 A summary of the major features of polysaccharides

Polysaccharide		Monomer	Type of glycosidic link	Shape of macromolecule	Function
Starch	Amylose	α-Glucose	α-1,4	Unbranched chains wound into a helix	Carbohydrate storage in plants
	Amylopectin	α-Glucose	α-1,4 and also α-1,6 at branches	Tightly packed branched chains	Carbohydrate storage in plants
Glycogen		α-Glucose	α-1,4 and also α-1,6 at branches	Very branched, compact molecules	Carbohydrate storage in animals
Cellulose		β-Glucose	β-1,4	Linear	Structural component in plant cell walls

QUICK CHECK QUESTIONS

23 The diagram below shows a part of the structure of a carbohydrate storage polymer which can be broken down by enzyme action.



a What types of glycosidic linkages are present in this structure?

b Name this type of breakdown reaction.

c Draw the structure of the monomer unit produced.

d Suggest which carbohydrate polymer this drawing represents.

e Suggest why the polymer functions as an energy store but the monomer does not.

24 Describe the difference in structure between starch and cellulose. How does this difference affect human nutrition?

The structure of glucose

Glucose is one of 16 stereoisomers, 8 pairs of enantiomers, which include fructose and galactose. The glucose molecule exists in solution as an equilibrium between a straight chain form (with a free aldehyde group) and the more favourable six-membered pyranose ring, which exists in α and β forms and can be represented by Haworth projections (Figure 23.46).

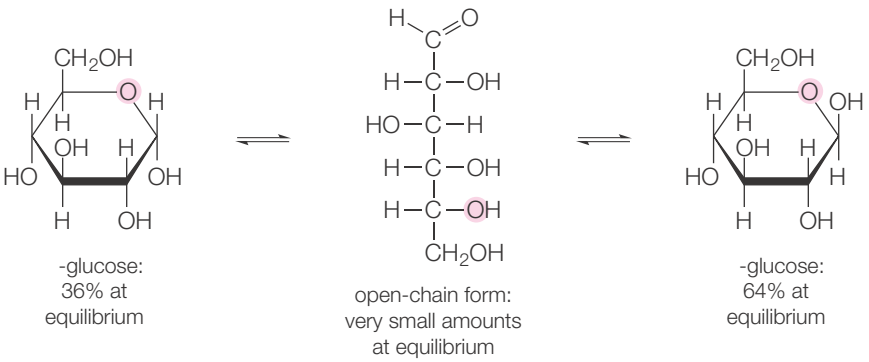


Figure 23.46 The equilibrium composition of an aqueous solution of glucose

Expert tip

A pyranose is a six-membered cyclic (ring) form of a monosaccharide. They can exist in chair and boat conformations (see Topic 20 Organic chemistry). The rings can open and re-close, allowing rotation to occur about the carbon with the carbonyl group allowing the formation of alpha and beta isomers, known as anomers.

NATURE OF SCIENCE

The stereochemistry of the cyclic (ring) forms of sugars is often represented by their Haworth projections. These are standardized ways of showing the position of hydroxyl groups in space. Haworth projections show the stereochemistry of sugars. The plane of the ring is attached is tilted to the plane of the paper. The attached groups are above or below the plane of the ring. The ring oxygen is always placed in the back right-hand corner of the ring, with the anomeric carbon (C-1) on the right-hand side and the primary alcohol group drawn up from the back left-hand corner (C-5).

Tests for carbohydrates

Aldoses, such as glucose, are reducing sugars in solution because they contain a terminal carbonyl (aldehyde) group and are easily oxidized under relatively mild conditions. Benedict's solution and Fehling's solution (both of which contain copper(II) ions) are used to test for reducing sugars with an aldehyde, –CHO, group. A red-brown precipitate of copper(I) oxide is formed. The presence of starch (even at low concentrations) can be detected using iodine: an intense blue-black complex is formed.

23.5 Vitamins

Revised

Essential idea: Vitamins are organic micronutrients with diverse functions that must be obtained from the diet.

Vitamins

Revised

- **Vitamins** are organic micronutrients which (mostly) cannot be synthesized by the body but must be obtained from suitable food sources.
- The solubility (water or fat) of a vitamin can be predicted from its structure.
- Most vitamins are sensitive to heat.
- Vitamin deficiencies in the diet cause particular diseases and affect millions of people worldwide.

Key definition

Vitamins – organic micronutrients that cannot (except for vitamin D) be synthesized in the body and must be obtained from appropriate food sources.

Vitamins and micronutrients

Revised

Micronutrients are substances required in tiny amounts by the body unlike protein, carbohydrates and lipids, which are termed macronutrients. They mainly function as co-factors for enzyme activity and include not only vitamins but trace minerals such as iron (Fe), iodine (I_2) and zinc (Zn).

NATURE OF SCIENCE

The term vitamin was derived from '*vitamine*', a combination of *vital* and *amine*, because it was originally suggested that the organic micronutrient food factors which prevented beri beri and perhaps other similar dietary deficiency diseases, might be amines. This proved incorrect for the micronutrient class, and the word was shortened.

Vitamins can be classified as either fat soluble or water soluble

Non-polar groups such as hydrocarbon rings (cycloalkenyl groups, for instance) and long alkyl chains decrease water solubility but increase fat solubility (Table 23.8). Fat-soluble vitamins include vitamins A, D, E and K. They can accumulate in the fatty tissues of the body; sometimes an excess of a fat-soluble vitamin can be as detrimental to good health as a deficiency.

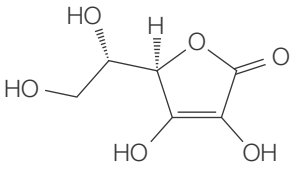
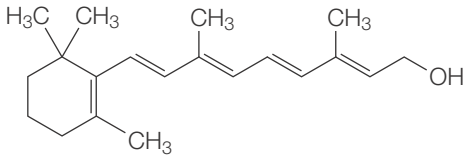
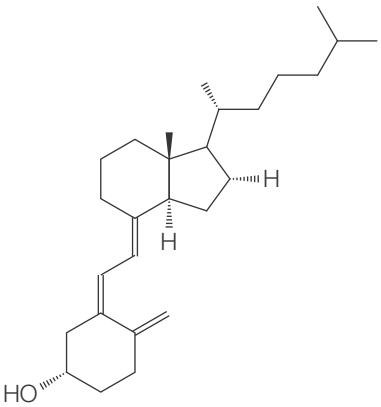
The presence of functional groups capable of taking part in hydrogen-bonding promotes water solubility; these include hydroxyl, carboxyl, amine and amide groups. Vitamin B group members (of which there are eight) and vitamin C are water-soluble molecules. As a result of their solubility, these vitamins are excreted readily in the urine, they do not accumulate in the body and so require regular daily intake from the diet.

Expert tip

You need to be able to identify the key functional groups present in each vitamin molecule and explain how the structures affect water and/or fat solubility.

- Vitamins containing many hydroxyl (–OH) groups and/or several very electronegative atoms (such as nitrogen or oxygen) are generally water-soluble.
- Those that consist almost entirely of carbon and hydrogen are fat-soluble.

Table 23.8 The solubility and function of certain vitamins in relation to functional groups present

	Vitamin C	Vitamin A	Vitamin D
Skeletal structural formula			
Functional groups	alkenyl hydroxyl ester	alkenyl cycloalkenyl alkanyl hydroxyl	alkenyl cycloalkenyl alkanyl cycloalkanyl hydroxyl
Solubility	Contains four hydroxyl groups, interaction with water through hydrogen bonding; highly soluble in water	Predominantly hydrophobic hydrocarbon chains; hydroxyl group has minimal effect; fat soluble, virtually insoluble in water	
Function	Acts as a co-factor in some enzyme reactions; important in tissue regeneration and wound healing; can act as an antioxidant	Involved in the visual cycle of the eye; particularly important for night vision (low light intensity)	Stimulates the absorption of calcium from the gut; important in whole body calcium homeostasis and the health of bones and teeth

Sensitivity to heat

Some vitamins, particularly those that are water soluble, vitamin C and thiamin (vitamin B1), are highly sensitive to heat; they decompose or are chemically altered at temperatures involved in food processing and cooking, causing them to lose their biological effect. Note also that water-soluble vitamins may leach into cooking water and be lost.

The hydrocarbon backbones of the fat-soluble vitamins A and D are relatively stable to heat and do not decompose significantly when food is steamed or boiled. Over-cooked or fried food can lose more than 50 per cent of its fat-soluble vitamin content and virtually all its vitamin C.

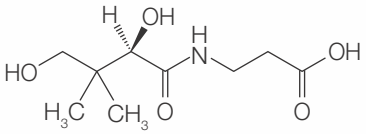
Vitamins A and C, containing carbon–carbon double bonds and –OH groups, are more sensitive to light and air than vitamin D as they are susceptible to free radical and redox reactions.

QUICK CHECK QUESTIONS

25 a Draw out these vitamin structures, **X** and **Y**, and identify the following functional groups in each by circling the appropriate grouping.

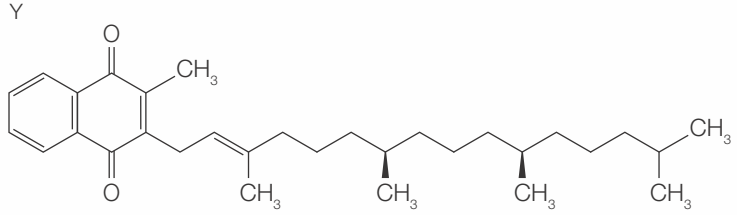
i Identify **A** an ester group, **B** an amide group and **C** a hydroxyl group.

X



ii Identify **A** a ketone group, **B** a phenyl group and **C** an alkenyl group.

Y



b State whether vitamin molecules X and Y are fat-soluble or water-soluble.

26 State two reasons why the vitamin C content of vegetables and fruits decreases when they are left in the open and then boiled in water.

Vitamin deficiencies

An inadequate intake of vitamins is termed *vitamin deficiency*. Severe vitamin deficiencies may lead to diseases or conditions that, if left untreated, can cause significant harm or, in the most severe cases, death, e.g. beri beri caused by vitamin B1 deficiency. The World Health Organization (WHO) has identified vitamin A as the most important vitamin deficiency in global health terms.

The causes of vitamin deficiency vary from country to country. Typical reasons include:

- harsh agricultural conditions
- famine
- poverty
- lack of prolonged periods sunlight for part of year (vitamin D only)
- poor access to or supply of vitamin supplements.

Scurvy, which involves a deficiency of vitamin C, is one of the accompanying diseases of malnutrition and thus is still widespread in areas of the world depending on external food aid. Though rare, there are also documented cases of scurvy due to poor dietary choices by people living in industrialized nations.

Programmes involving the biofortification of diet using crops rich in pro-vitamin A have shown the complexity of the dietary situation where people are under-nourished. Vitamin A is fat soluble, so if the level of body fat is low then the vitamin is not absorbed. An adequate fat intake is essential for the β-carotene from golden rice – a variety of rice rich in provitamin A – to be absorbed.

There are several ways to combat these causes.

- **Addition of vitamins to food-stuffs:** Fortification of foods with vitamin A has proved a successful strategy for combating this deficiency. Programmes also exist to encourage farmers to grow varieties of foods richer in β-carotene (provitamin A; which can be converted into vitamin A in the body) – this is called *biofortification*. For instance, the introduction of orange-fleshed sweet potato into Uganda to replace the indigenous white-fleshed variety has met with some success in reducing vitamin A deficiency. The provision of golden rice should also help with reducing vitamin A deficiency.

  Many foods, such as breakfast cereals, are fortified with niacin and thiamin (vitamin B), and deficiency is rare in developed countries.

  Mandatory vitamin D fortification is increasing; fortification is usually applied to milk and margarine, as the vitamin is fat soluble. Fortification of dairy products with vitamin D means that deficiency is now rare in some industrialized countries; though not all European countries do this and vitamin D tablets may need to be prescribed to some individuals.
- **Genetic modification of crops:** A more controversial form of biofortification involves genetic modification (GM) of foodstuffs to make them richer in a

Expert tip

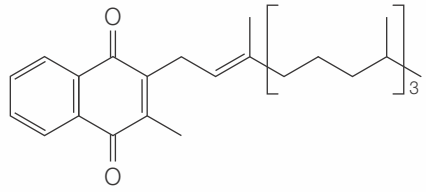
Vitamin deficiencies: note that the guidance in the syllabus explicitly says that you will not be expected to know names of particular deficiency diseases. However, the discussion of such deficiencies does raise certain key issues relating to health and the interplay of general and specific dietary well-being and overall lifestyle.

particular vitamin. Genetic modification has been used to produce golden rice, fortified with vitamin A. It is hoped that the use of golden rice will make a significant difference to vitamin A deficiency in countries such as India, Bangladesh and Vietnam.

- **Education:** Debate is widespread on the extent to which a balanced diet can provide sufficient quantities of vitamins, or whether supplementation is necessary for optimum health. The existence of conflicting information and advice, and the pressure of advertising, can make it difficult for individuals to make an informed choice. Apart from vitamin D, there is little need for supplements for people with a good mixed diet – but there are problems of low vitamin A intake in many developing countries, as well as iron and iodine. Results from intervention trials with antioxidant supplements (especially β-carotene and vitamin E) in developed countries are mixed, with many studies showing increased all-cause mortality among those taking antioxidant supplements.
- **Medical programmes:** Scientists have a duty to share their findings with the public in ways that help people to understand the issues and make valid judgements.
- **Spend more time outdoors:** Vitamin D deficiency it is still a problem in some developing countries where intake of dairy products may be low or where religious or social customs or climatic conditions prevent an adequate exposure to sunlight. More widespread use of sunscreen lotions during the summer months seems to have resulted in greater concern over an increase in vitamin D deficiency even in affluent populations. Current recommendations for vitamin D intake are greater than can be achieved readily from diet, especially in winter above about 40°N or S.

QUICK CHECK QUESTION

27 Vitamin K_1 is required for the synthesis of certain proteins necessary for blood clotting. Its structure is shown below.



a Define the term vitamin.

b State and explain whether vitamin K is classified as a water-soluble or fat-soluble vitamin.

c It is present in green leafy vegetables. Some meal replacement bars and shakes are *fortified* with vitamin K. Explain this term.

23.6 Biochemistry and the environment

Revised

Essential idea: Our increasing knowledge of biochemistry has led to several environmental problems, while also helping to solve others.

Biochemistry and the environment

Revised

- Xenobiotics refer to chemicals that are found in an organism that are not normally present there.
- Biodegradable/compostable plastics can be consumed or broken down by bacteria or other living organisms.

- Host–guest chemistry involves the creation of synthetic host molecules that mimic some of the actions performed by enzymes in cells, by selectively binding to specific guest species, such as toxic materials in the environment.
- Enzymes have been developed to help in the breakdown of oil spills and other industrial wastes.
- Enzymes in biological detergents can improve energy efficiency by enabling effective cleaning at lower temperatures.
- Biomagnification is the increase in concentration of a substance in a food chain.
- Green chemistry, also called sustainable chemistry, is an approach to chemical research and engineering that seeks to minimize the production and release to the environment of hazardous substances.

Host–guest chemistry

Revised

Host–guest chemistry involves the design, synthesis and investigation of simpler organic compounds that imitate the working features of naturally occurring compounds: molecular recognition, transport, regulation and catalysis.

A molecular recognition process can be described as a specific interaction between two molecules through multiple non-covalent contacts (via intermolecular forces). Molecular recognition forms basis for many processes in biology: receptor–substrate binding; enzyme catalysis (e.g. lock and key principle); assembly of multi-protein complexes, e.g. ribosomes; and transport across cell membranes.

The host is an organic molecule containing convergent binding sites. These are synthetic counterparts to receptor sites in enzymes, genes and antibodies. The guest is a molecule or ion containing divergent binding sites. The binding sites are the counterparts to substrates, inhibitors, cofactors and antigens.

The host and guest components of a complex are held together in solution (Figure 23.47) by an enthalpy contribution and an entropic contribution to their combination.

- The enthalpy contribution: consists of electrostatic forces of interaction such as ion-pairing, hydrogen bonding, metal ion-to-ligand attraction, ring-stacking, dipole–dipole interaction, and London (dispersion) forces.
- The entropic contribution: results from the process of desolvation – an increase in entropy due to the freeing up of solvent molecules as the host and guest associate.

The host–guest complex is also known as a *supramolecule*.

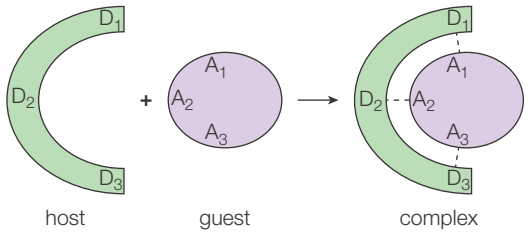


Figure 23.47 Host–guest complex formation

Expert tip

A 'supramolecule' literally means a structure 'beyond the molecular'; the key being that the association is brought about by non-covalent interactions, which distinguishes these structures from complex ions.

A well-studied class of host molecules is that of the crown ethers whose guests are group 1 cations (Figure 23.48). They selectively bind group 1 cations whose size matches that of the cavity. The cavity is polar; but the outside is non-polar.

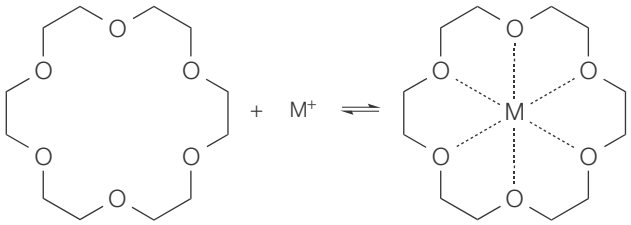
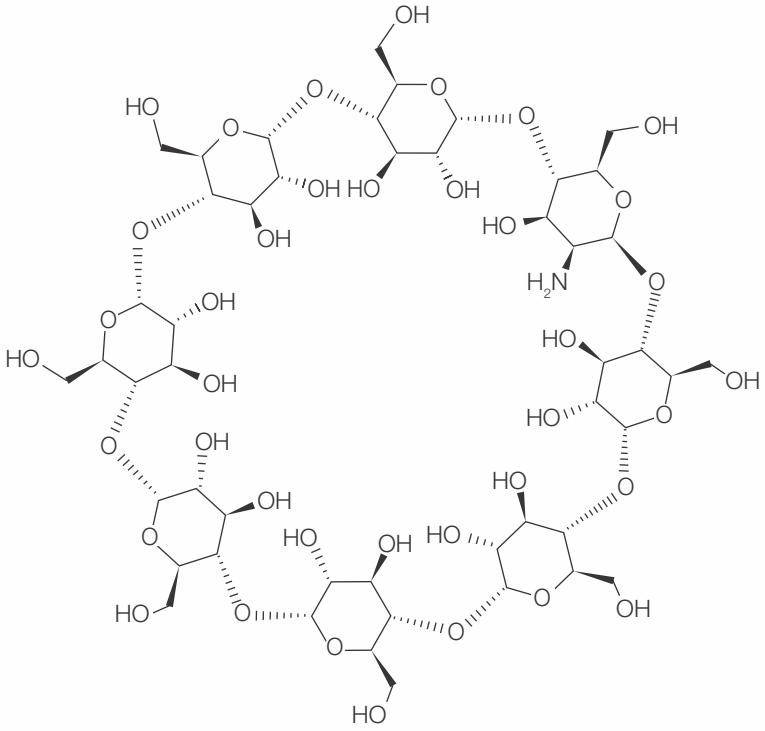


Figure 23.48 The coordination of a group 1 metal ion by a crown ether (here 18-crown-6 ether)

Host–guest complexes have been used to deliver poorly soluble drug molecules more effectively in patients by increasing the solubility and bioavailability of the drug. Cyclodextrins are chemically stable, water-soluble oligosaccharide hosts derived enzymatically from starch. Because their interiors are relatively lipophilic and their exteriors hydrophilic, cyclodextrins can complex hydrophobic guests to form inclusion complexes in aqueous solution.

Worked example

Gamma cyclodextrin consists of eight glucopyranose units joined together in a ring. Deduce an equation for the complete hydrolysis of gamma cyclodextrin. Cyclodextrins can be used to carry drugs, such as ibuprofen.



Cyclic polymers do not have terminal groups, so the formula of gamma cyclodextrin is $(C_6H_{10}O_5)_8$. Because each glucose residue needs one oxygen and two hydrogen atoms to produce glucose, the number of water molecules in the equation will also be eight:

$$(C_6H_{10}O_5)_8 + 8H_2O \rightarrow 8C_6H_{12}O_6$$

Host–guest systems have been utilized to remove hazardous substances from the environment. They can be made in different sizes and different shapes to trap a variety of chemical guests. One application is the ability of BOBCalix6 to trap a caesium ion (Figure 23.49).

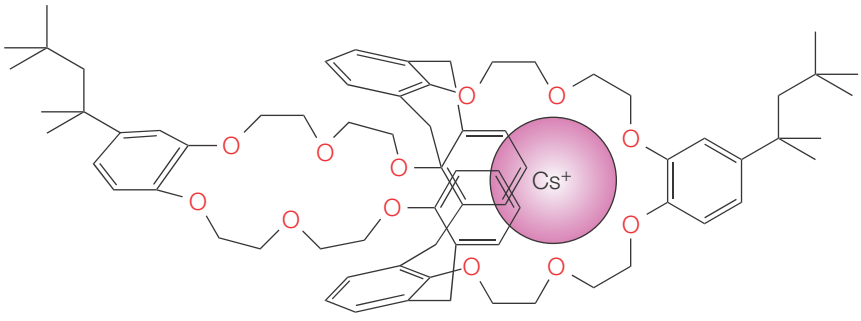


Figure 23.49 Host molecule (BOBCalix6) shown with a positively charged caesium ion held inside one of its cavities

Caesium-137 is radioactive and there is a need to remove it from nuclear waste in an efficient manner. Host–guest chemistry has also been used to remove carcinogenic aromatic amines, and their N-nitroso derivatives from water. These waste materials are used in many industrial processes and found in a variety of products such as pesticides, drugs and cosmetics.

Expert tip

You will need to be able to give a named example of host–guest chemistry – the calixarenes are such an example: used to remove radioactive caesium-137 from waste water by forming ion–dipole interactions.

QUICK CHECK QUESTIONS

28 Explain why 18-crown-6 is unable to bind calcium ions, but has a high affinity for oxonium ions (H_3O^+). Draw a diagram of the complex between 18-crown-6 and an oxonium ion.

29 Small molecules, such as nitrogen, methane and argon, can be trapped within the spaces of the ice lattice when it forms under high pressure. This is an example of a structure known as a clathrate; it is not a true compound, but an example of supramolecular chemistry.

a Suggest **one** way that the ice clathrate could be made to release its gas molecules.

b Suggest why helium is unable to form a clathrate.

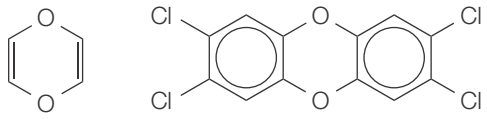
c Identify the interaction between the gas particle and the water molecules.

Xenobiotics

Revised

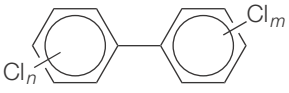
Xenobiotics are chemicals found in organisms which are not normally present or are present in higher than usual concentrations. They are chemical compounds, often drugs (including antibiotics which enter the water supply and those given to livestock), pesticides or carcinogens that are foreign to the living organism.

Some xenobiotics may be natural compounds, but most are pollutants. Three well-studied classes of xenobiotics are dioxins, dioxin-like substances, such as polychlorinated dibenzodioxins (PCCDs, Figure 23.50), and polychlorinated biphenyls (PCBs, Figure 23.51).



1,4-dioxin 2,3,7,8-tetrachlorodibenzodioxin

Figure 23.50 The structure of 1,4-dioxin and 2,3,4,8-tetrachlorobenzodioxin (an example of a PCCD)



polychlorinated biphenyl

Figure 23.51 The generalized formula of PCBs

Dioxins are produced as by-products in the manufacture of some chlorinated organic compounds and the incineration of plastics. They are highly carcinogenic, especially the chlorinated dioxins, and they can disrupt the endocrine system (hormone action) and lead to cellular and genetic damage.

PCBs contain one to ten chlorine atoms attached to a biphenyl molecule (Figure 23.51). They are highly stable, with high electrical resistance and were used as coolants, plasticizers, lubricants and insulating liquids.

A major issue with xenobiotics is **biomagnification,** which involves an increase in concentration as a substance passes up the food chain via digestion and assimilation. DDT (Figure 23.52) (dichlorodiphenyltrichloroethane) is an insecticide (now banned in many countries) against the malaria mosquito which accumulates in birds of prey and makes their eggs non-viable (Figure 23.53). Heavy metals, such as mercury compounds, can also be involved in biomagnification in marine environments.

Key definition

Biomagnification – the increase in the concentration of the pollutant as it moves up through the food chain.

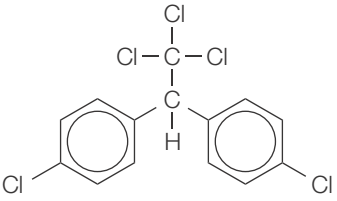


Figure 23.52 The structure of DDT

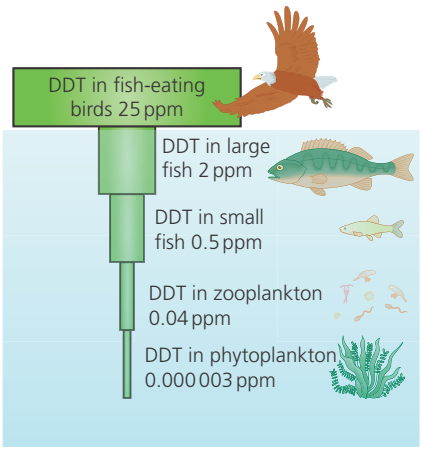


Figure 23.53 The biomagnification of DDT concentration as it passes up the food chain

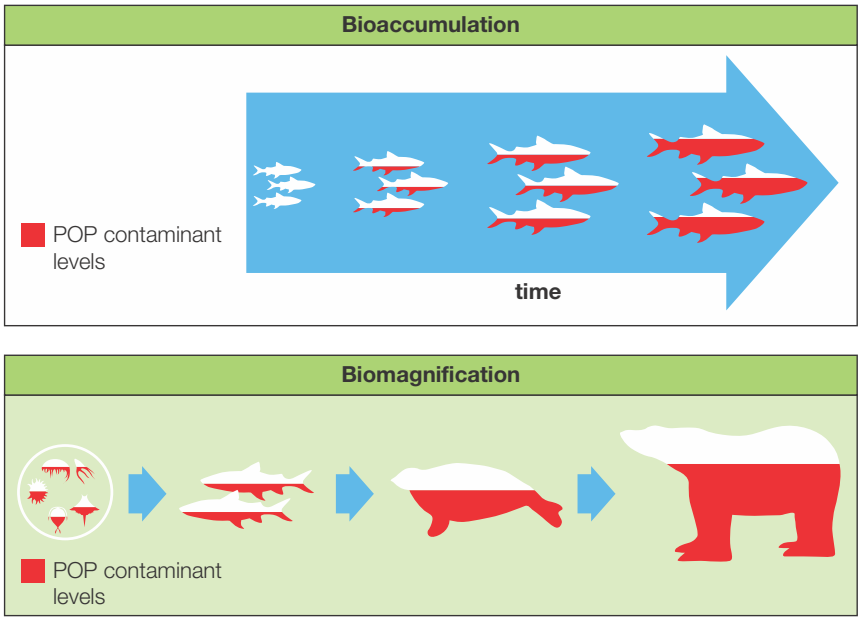


Figure 23.54 The processes of **bioaccumulation** and biomagnification of POPs (**P**ersistent **O**rganic **P**ollutants)

Key definition

Bioaccumulation – the increase in the concentration of a pollutant in an organism as it absorbs or it ingests it from its environment (Figure 23.54).

Worked example

A 500.0 cm^3 water sample is analysed for DDT using chromatography and mass spectrometry. The water sample undergoes chromatography and is then rinsed with 20.0 cm^3 of water. The DDT-containing fraction is removed with 20.0 cm^3 of methanol, evaporated and then transferred to a 2.00 cm^3 volumetric flask using methanol. A 20 μdm^3 aliquot is injected for analysis and the concentration of DDT in this extract is found to be 27.1 $ng\,cm^{-3}$.

Determine the concentration of DDT in $ng\,dm^{-3}$ in the water sample, assuming 100% transfer efficiency.

mass isolated in DDT extract = $(27.1\,ng\,cm^{-3})(2.00\,cm^3) = 54.2\,ng$

(note that 2.00 cm^3 = final extract volume)

initial water volume = 500.0 cm^3 = 0.500 dm^3

$$\text{initial water concentration} = \frac{\text{mass of DDT isolated}}{\text{water volume}}$$

$$= \frac{54.2\,ng}{0.500\,dm^3}$$

$$= 108\,ng\,dm^{-3}$$

Expert tip

Persistent Organic Pollutants (POPs) are a group of chemicals that are very toxic and can cause cancer and other adverse health effects. POPs are persistent in the environment and travel vast distances via air and water. POPs are organic chemical compounds which bioaccumulate in animals and humans.

Green chemistry

Revised

Green chemistry promotes the design and application of chemical products and chemical processes that are compatible with human health and preserve the environment. The areas in which green chemistry can operate to improve environmental quality include choices of solvents and reagents for chemical reactions, development of alternative processes and improvements in existing chemical processes or practices.

Some of the major principles of green chemistry are:

- It is better to avoid producing waste than to clean or treat it after it has been created.
- When synthesizing new substances, the method used should generate as little waste as possible. The substances generated should have little or no toxicity to human health or towards the environment.
- Catalysts that allow the use of common and safe chemical reagents should be used whenever possible.
- The raw materials for chemical processes should be renewable feedstock when it is technologically and economically feasible.
- Auxiliary substances, such solvents, should be eliminated or made as harmless as possible.
- Chemical processes should be designed to be as energy efficient as possible, avoiding high temperatures and pressures.

Expert tip

You are not expected to learn this list, or the 12 principles of green chemistry, but you will be expected to be able to discuss the broad issues and apply them in a discussion of the 'greenness' of a particular product.

A further key component of green chemistry is the assessment of *atom economy* (see Topic 1 Stoichiometric relationships and worked example).

Worked example

An ultrasound imaging agent can be made by the reaction of butane and fluorine as shown in the following equation.

$$C_4H_{10} + 10F_2 \rightarrow C_4F_{10} + 10HF$$

Calculate the atom economy for the formation of decafluorobutane, C_4F_{10}, to three significant figures.

$$\text{atom economy (\%)} = \frac{\text{molecular mass of atoms in useful products}}{\text{molecular mass of atoms in reactants}} \times 100$$

$$= \frac{(238 \times 100)}{438} = 54.3\%$$

Biodegradability

Revised

Synthetic plastics are organic and usually based on hydrocarbons. Thus they are generally not biodegradable and cause pollution in the sea and in landfills. Biodegradable plastics are plastics capable of being decomposed by bacteria and fungi, ultimately to carbon dioxide and water. They are based on naturally occurring polymers usually with ester functional groups, such as PLA (polylactic acid) or glycosidic (ether) linkages, such as starch.

Bacterial and fungal enzymes can be used to degrade biological pollutants, such as the hydrocarbons released in crude oil spills, a process known as bioremediation. Proteases, lipases and other enzymes are used in biological washing powders. Biological detergents allow for use at lower temperatures than non-biological ones and so save energy. Immobilized enzymes (enzymes attached to a solid support) have been used in the clean-up of industrial waste water.

QUICK CHECK QUESTIONS

30 Corn starch is made up of two carbohydrate polymers, amylose and amylopectin. It can be used to make replacements for plastics derived from petroleum, especially for packaging. Discuss **one** potential advantage and **one** disadvantage of this use of starch.

31 The organic compound 1-phenylethanone ($C_6H_5COCH_3$) is being investigated as a potentially useful enzyme inhibitor. There are two possible ways of synthesizing 1-phenylethanone from 1-phenylethanol under consideration.

Method 1:

$$3C_6H_5CH(OH)CH_3 + 2CrO_3 + 3H_2SO_4 \rightarrow C_6H_5COCH_3 + Cr_2(SO_4)_3 + 6H_2O$$

Method 2 (using a catalyst):

$$C_6H_5CH(OH)CH_3 + \tfrac{1}{2}O_2 \rightarrow C_6H_5COCH_3 + H_2O$$

a Calculate the atom economy of both these methods.

b Identify **two** other considerations which would need to be borne in mind, besides the atom efficiencies, when deciding which method is 'greener' and therefore the preferred option.

23.7 Proteins and enzymes

Revised

Essential idea: Analyses of protein activity and concentration are key areas of biochemical research.

Proteins and enzymes

Revised

- Inhibitors play an important role in regulating the activities of enzymes.
- Amino acids and proteins can act as buffers in solution.
- Protein assays commonly use UV–Vis spectroscopy and a calibration curve based on known standards.

Enzyme activity

At the active site of the enzyme, the amino acid side-chains of the folded protein are precisely positioned so they favour the formation of the high-energy transition states that the substrate(s) must pass through to be converted to product.

NATURE OF SCIENCE

The 'lock and key model' explains enzyme specificity, but it does not explain X-ray diffraction data indicating that the active site of some enzymes change shape when the substrate binds. This finding led to the development of the induced-fit model of enzyme activity (Figure 23.55).

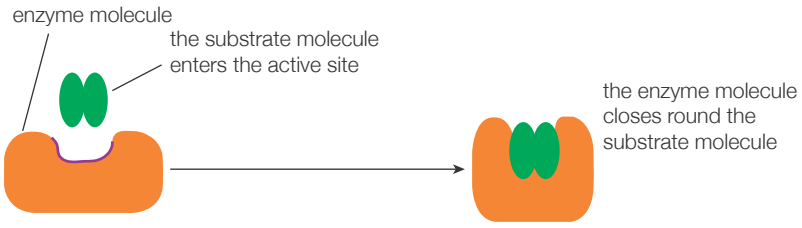


Figure 23.55 The induced fit model of enzyme action

Enzyme kinetics

When reaction rate is plotted against substrate concentration, a hyperbolic curve with an asymptotic plateau is reached. This is known as saturation kinetics (Figure 23.56) and occurs when the rate at which substrate enters active sites is equal to the rate at which products leave active sites.

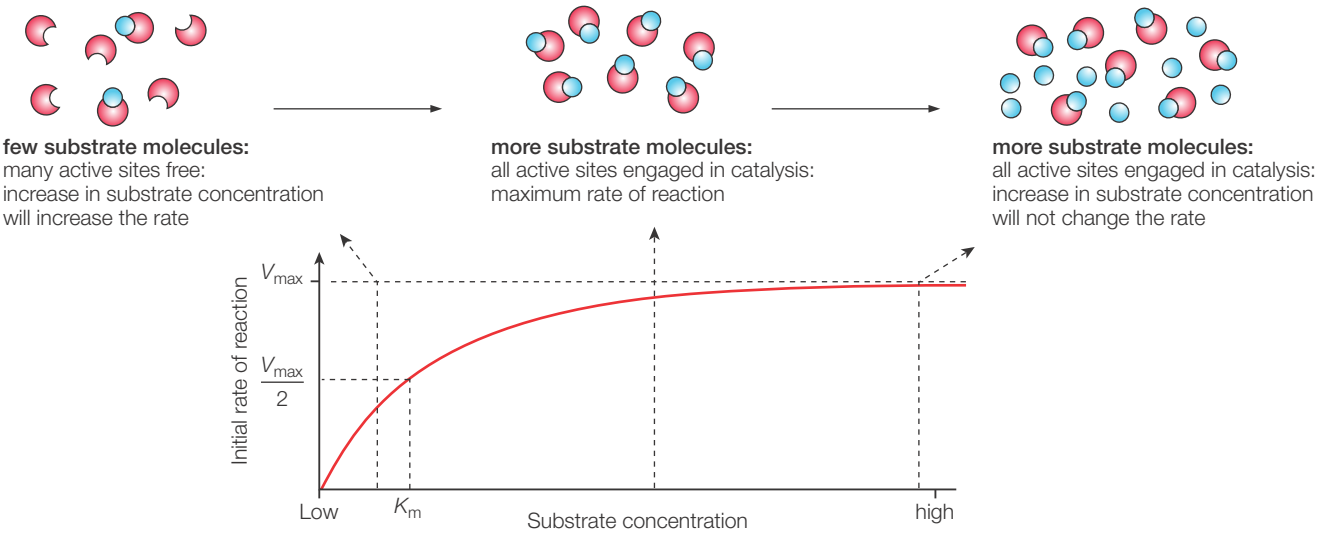


Figure 23.56 Interpreting the change in initial reaction rate with substrate concentration of an enzyme-catalysed reaction

The kinetics of enzyme-controlled reactions (known as Michaelis–Menten kinetics) identifies two important parameters: V_{max} and K_m (the Michaelis constant), the concentration of substrate that results in half the maximum rate.

A low value of the Michaelis constant, K_m, indicates that the enzyme works efficiently even if the concentration of the substrate is low, while a high value of the Michaelis constant indicates that the enzyme requires a high concentration of the substrate before being relatively active.

An enzyme assay measures the conversion of substrate to product, under conditions when the enzyme is optimally active. High substrate concentrations are used so that the initial rate is proportional to the enzyme concentration. Either the rate of appearance of product or the rate of disappearance of substrate is measured, often by following changes in absorbance using a spectrophotometer.

Expert tip

The maximum reaction rate for given a concentration of enzyme at a specified temperature and other conditions, e.g. pH, is referred to as V_{max}.

Inhibition

There are two common types of reversible inhibition of enzymes, **competitive** and **non-competitive inhibition** (Figure 23.57). They can be distinguished by their effects on enzymes.

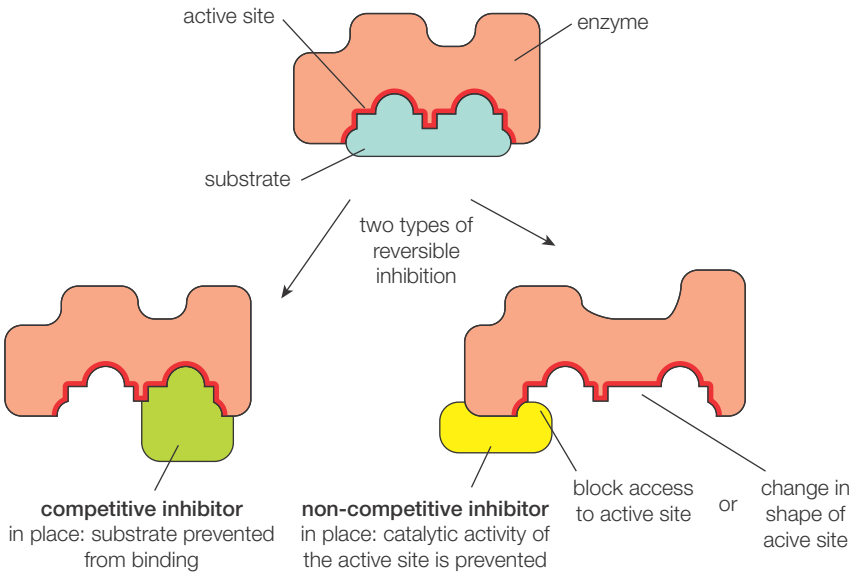


Figure 23.57 The principles of competitive and non-competitive inhibition

In competitive inhibition the inhibitor has a similar shape to the substrate and competes with the substrate to bind with the active site. In non-competitive inhibition the inhibitor does not bind to the active site but another binding site. This binding alters the shape (conformation) of the enzyme and prevents the catalysed reaction occurring.

Key definitions

Competitive inhibition – involves a molecule binding to the active site of an enzyme and thus preventing substrate from binding. The inhibitor has a similar structure to the substrate. Competitive inhibition does *not* affect the value of V_{max} but increases K_m.

Non-competitive inhibition – involves a molecule binding to the enzyme at a site distinct from the active site. This alters the shape of the enzyme, affecting the active site so that it no longer binds the substrate. Non-competitive inhibition *decreases* the value of V_{max} but has no effect on the value of K_m.

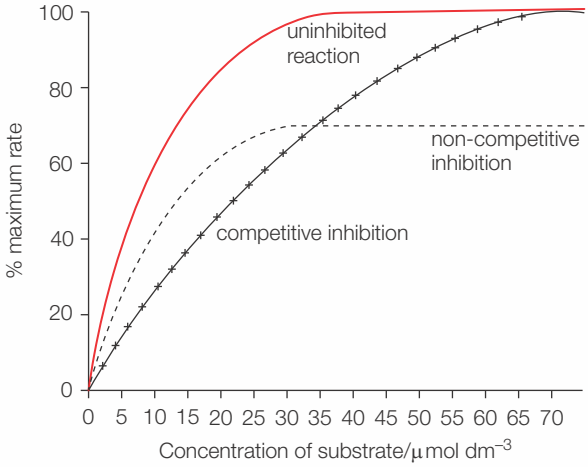
Expert tip

The rates of enzyme reactions are often so fast they are limited by diffusion. Rates can be increased if the enzymes are part of a multi-enzyme complex or if confined within a membrane.

QUICK CHECK QUESTIONS

32 Outline the general principle that describes how enzymes work as catalysts. Describe the effect, if any, that the enzyme has on the free energy difference between the initial and the final states of the reaction. What is the major source of free energy required for the catalytic power of enzymes?

33 The graphs show how the rate of an enzyme-catalysed reaction varies with substrate concentration for the uninhibited enzyme, for competitive inhibition and for non-competitive inhibition.



a Explain in both energy and structural terms how enzymes are able to catalyse specific biological reactions.

b Describe the difference in the way that competitive and non-competitive inhibitors interact with the enzyme.

The activities of most enzymes within cells are strictly regulated. One of the most common forms of inhibition is feedback inhibition (Figure 23.58), in which an enzyme early in a metabolic pathway is inhibited by its binding to one of the metabolic pathway's end products.

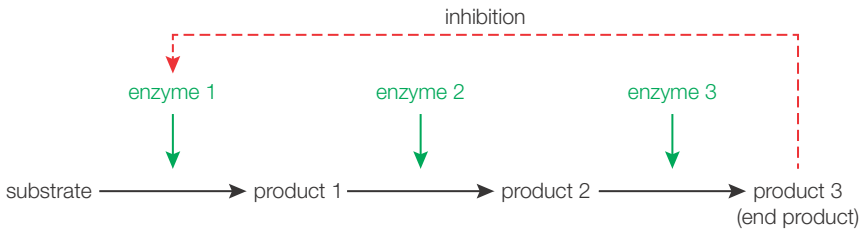


Figure 23.58 Feedback control of a metabolic pathway. The rate of reaction of the whole sequence is controlled by the concentration of the end product

Uses of enzymes

Proteases are widely used in biological washing powders to destroy organic material in stains and to condition fabrics. The enzymes are selected for their activity at high temperatures. Most of the enzymes used in industry are extracellular and secreted by bacteria into the growth medium. The main methods of immobilizing enzymes are cross-linking, entrapment and adsorption (Figure 23.59).

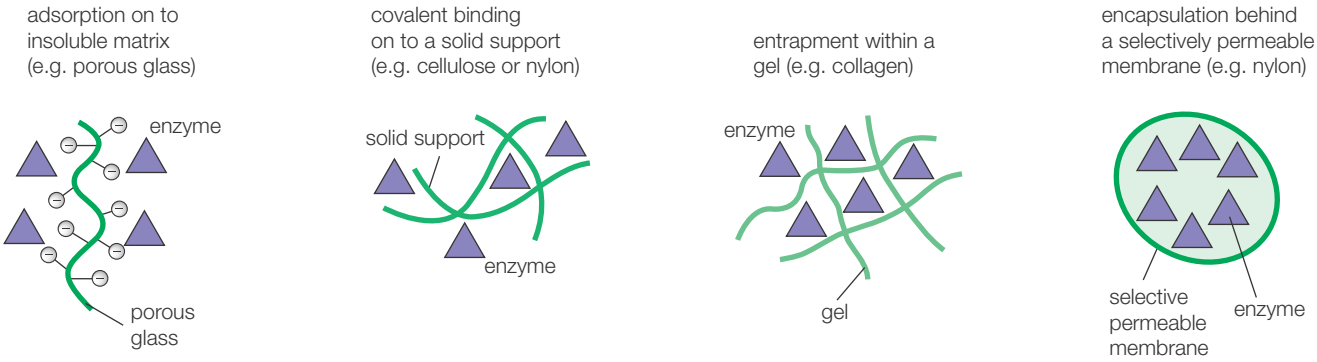


Figure 23.59 Methods of immobilizing enzymes

Isolated enzymes are used commercially and their effectiveness is increased if the enzyme is immobilized. Immobilized enzymes can be readily removed from a reaction mixture to avoid feedback inhibition by products. Immobilization of an enzyme can also improve its thermal stability. Immobilized enzymes are important in biosensor and diagnostic tests.

Buffers

Biological fluids including the cytoplasm and fluids, such as blood, are buffered. The major components in most biological fluids are the dihydrogen phosphate ion ($H_2PO_4^-$, pK_a 6.82) and the hydrogencarbonate ion (HCO_3^-, pK_a 6.35).

However, many biological molecules, including amino acids, proteins, nucleic acids and lipids have multiple acid–base groups that are effective at buffering in the physiological pH range (pH 6–8).

The carboxyl and amino groups bonded to the central carbon-2 atom of an amino acid act as acid–base groups, donating or accepting a proton as the pH is changed (Figure 23.60). At low pH, both groups are fully protonated, but as the pH is increased first the carboxylic acid group and then the amino group lose a hydrogen ion (H^+). Those amino acids with an ionizable side-chain with an additional side-chain have an additional acid–base group with a distinctive pK_a.

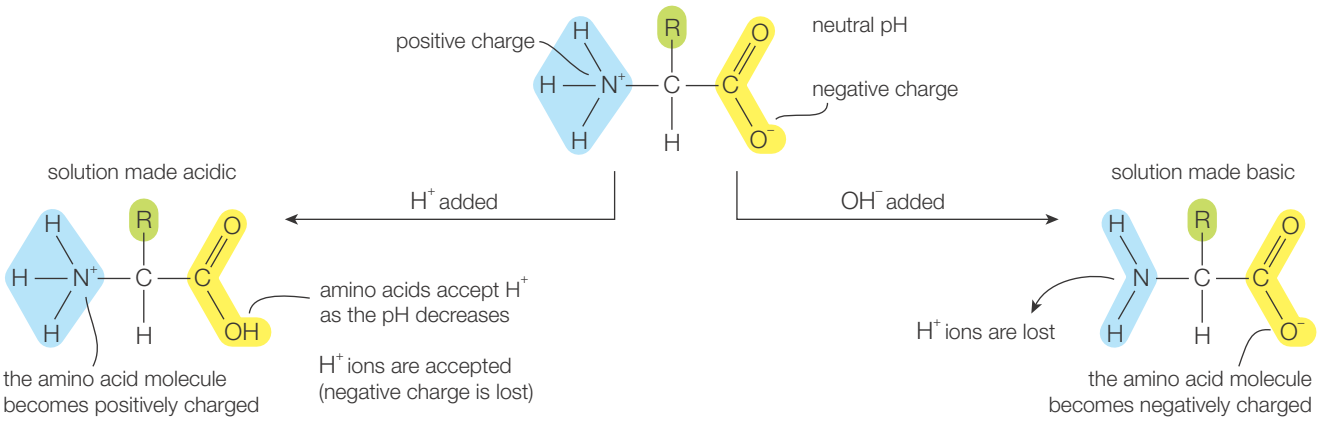


Figure 23.60 The buffering action of amino acids in aqueous solution

An acid–base conjugate pair can act as a buffer, resisting changes in pH. It is important to realize that an amino acid does not act as a buffer around its isoelectric point because there is only one species present. The titration curve for alanine (isoelectric point = 6.0) is shown in Figure 23.61, with the buffering regions being the almost horizontal regions of the curve (around pK_{a1} and pK_{a2}). The buffering capacity of the acid–base pair is in the range of the $pK_a \pm 1$.

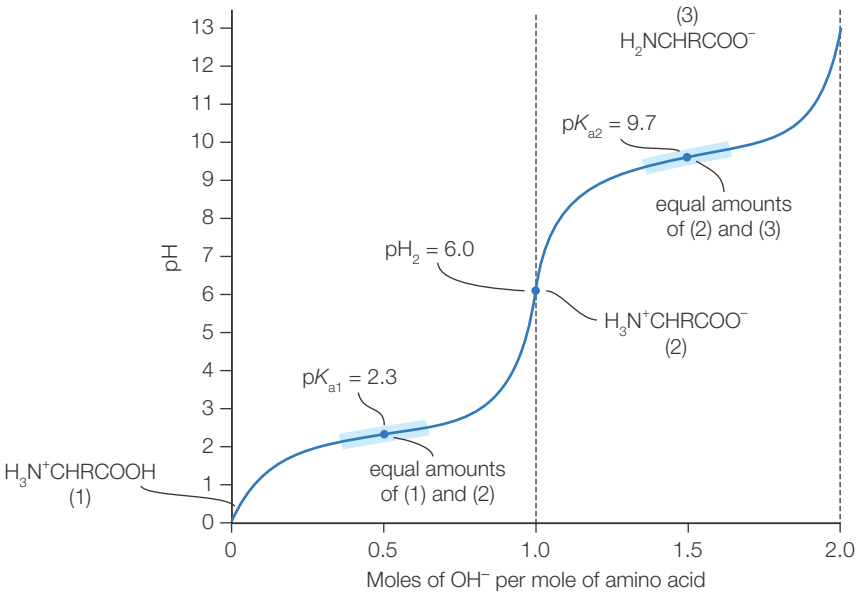


Figure 23.61 Titration curve for alanine

Solutions with an acidic pH (pH < pI) contain a mixture of the zwitterion and the cation, while alkaline solutions (pH > pI) contain zwitterions and anions. The exact ratio of these forms depends on the solution pH and the pK_a value of the conjugate acid present in the solution. Since $pH = -\log_{10}[H^+]$, the pK_a expression can be modified into the *Henderson–Hasselbalch* equation:

$$pH = pK_a + \log \frac{[\text{conjugate base}]}{[\text{conjugate acid}]}$$

For amino acid buffers this can be adapted as follows:

acidic pH (pH < pI)

$$pH = pK_{a1} + \log \frac{[\text{zwitterion}]}{[\text{cationic form}]}$$

alkaline solutions (pH > pI)

$$pH = pK_{a2} + \log \frac{[\text{anionic form}]}{[\text{zwitterion}]}$$

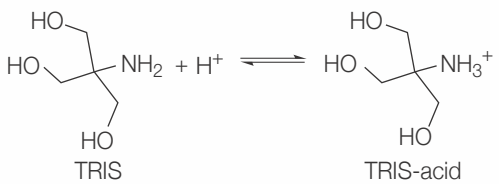
■ QUICK CHECK QUESTIONS

34 The following buffer systems are used in working with biochemical systems:

a Calculate the pH of a solution containing 0.200 mol dm^{-3} ethanoic acid ($K_a = 1.74 \times 10^{-5}$ mol dm^3) and 0.250 mol dm^3 sodium ethanoate.

b Phosphate buffers are routinely used in biochemical experiments, particularly for buffering around neutral pH. Calculate the pH of a buffer solution containing 0.055 mol dm^3 $H_2PO_4^-$ ($pK_a = 7.21$) and 0.045 mol dm^{-3} HPO_4^{2-}.

c TRIS is a buffer system used frequently in biochemistry. A buffer solution is prepared by adding hydrochloric acid to TRIS to form a mixture of TRIS and its protonated form (TRIS–acid). The equilibrium that exists in the buffer solution is:



Calculate the pH of a buffer solution containing 0.650 mol dm^{-3} TRIS–acid ($pK_a = 8.30$) and 0.750 mol dm^{-3} TRIS.

35 Calculate the pH of an aqueous solution that contains 0.8 mol dm^{-3} zwitterionic and 0.2 mol dm^{-3} anionic forms of serine. (For serine, $pK_{a1} = 2.2$ and $pK_{a2} = 9.1$.)

Expert tip

When calculating the pH of a buffer solution, you can check whether or not your answer is reasonable. If the solution contains a higher concentration of acid than base, the pH of the solution will be lower than the pK_a of the acid; if there is a higher concentration of base than acid, the pH will be higher than the pK_a.

Protein assay by ultraviolet–visible spectroscopy

The concentration of a protein in solution can be determined by UV–Vis spectroscopy (Figure 23.62). Proteins are colourless and only weakly absorb UV radiation so the protein is complexed with a dye or redox reagent to form a highly coloured complex.

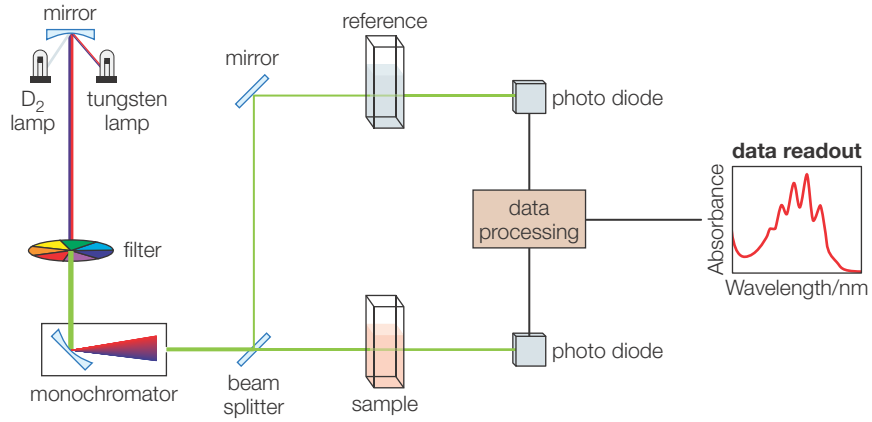


Figure 23.62 A schematic diagram of a UV–Vis spectrophotometer. The combination of the filter and monochromator ensures that only light of a particular wavelength is shone through the sample

One common approach known as the Bradford assay is to add a dye called Coomassie Brilliant Blue to bind to the protein in solution. The intensely coloured complex with the dye absorbs light with maximum absorption at a wavelength of 595 nm.

The principle of quantitative spectroscopy depends on the Beer–Lambert law (Figure 23.63). This states that for dilute solutions at a fixed wavelength absorbance is directly proportional to concentration:

$$\log_{10}\left(\frac{I}{I_0}\right) = \varepsilon l c$$

where I_0 is the intensity of the incident radiation, I is the intensity of the transmitted radiation, ε the molar absorption coefficient ($cm^{-1}\,mol^{-1}\,dm^3$), l is the path length (in the cuvette) of the absorbing solution (usually 1 cm) and c is the concentration ($mol\,dm^{-3}$) of the solution.

Ultraviolet spectrometers measure $\log_{10}\left(\frac{I}{I_0}\right)$ directly as absorbance if the path length is kept the same by using the same cuvette sample tube and all the readings are taken at the wavelength of maximum absorption, λ_{max}.

The Beer–Lambert Law describes the behaviour of solutions of proteins complexed with Coomassie Brilliant Blue covering the range of protein concentrations from 0 to approximately 1500 $\mu g\,cm^{-3}$.

To determine the concentration of the solution of the protein with an unknown concentration, it is necessary to obtain a calibration curve (line) by using a range of known concentrations of a specific purified soluble protein (such as serine albumin from a cow) and measuring the associated absorbance values.

A line of best fit (Figure 23.64) is constructed and once the absorbance of the unknown sample has been measured its concentration can be determined by interpolation of the graph.

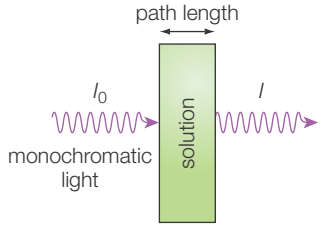


Figure 23.63 The Beer–Lambert law relates the absorbance of light by a solution to the path length and concentration

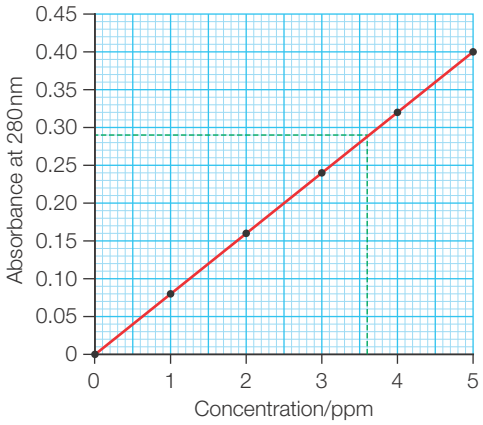


Figure 23.64 A calibration curve for the UV assay of proteins using absorbance at 280 nm (note that 1.00 mg dm^{-3} is the same concentration as 1 ppm)

Expert tip

You should note that absorbance has no units since it is a logarithmic scale.

QUICK CHECK QUESTION

36 Using the Beer–Lambert law, calculate the concentration of each of the following protein solutions. All absorbance values were measured at 280 nm in a cuvette of path length 1.0 cm.

a The molar absorptivity at 280 nm for a particular protein solution is 500 cm^{-1} mol^{-1} dm^3 and the absorbance = 0.31.

b The molar absorptivity at 280 nm for a particular protein solution is 63.5 cm^{-1} mol^{-1} dm^3 and the absorbance = 0.23.

23.8 Nucleic acids

Revised

Essential idea: DNA is the genetic material that expresses itself by controlling the synthesis of proteins by the cell.

Nucleic acids

Revised

- Nucleotides are the condensation products of a pentose sugar, phosphoric acid and a nitrogenous base – adenine (A), guanine (G), cytosine (C) and thymine (T) or uracil (U).
- Polynucleotides form by condensation reactions.
- DNA is a double helix of two polynucleotide strands held together by hydrogen bonds.
- RNA is usually a single polynucleotide chain that contains uracil in place of thymine, and a sugar ribose in place of deoxyribose.
- The sequence of bases in DNA determines the primary structure of proteins synthesized by the cell using a triplet code, known as the genetic code, which is universal.
- Genetically modified organisms have genetic material that has been altered by genetic engineering techniques, involving transferring DNA between species.

Nucleic acids

Revised

There are two forms of nucleic acid: DNA (deoxyribonucleic acid), in which the sugar is deoxyribose, and RNA (ribonucleic acid), where the sugar is ribose. Both these sugars are pentose sugars containing five carbon atoms.

DNA and RNA are condensation polymers consisting of: two chains of sugar–phosphate backbone (the sugar is 2-deoxyribose in DNA and ribose in RNA), nitrogen-containing bases attached to each sugar; the bases are adenine (A), guanine (G), cytosine (C) and thymine (T) (in DNA only) and uracil (in RNA only) (Figure 23.65).

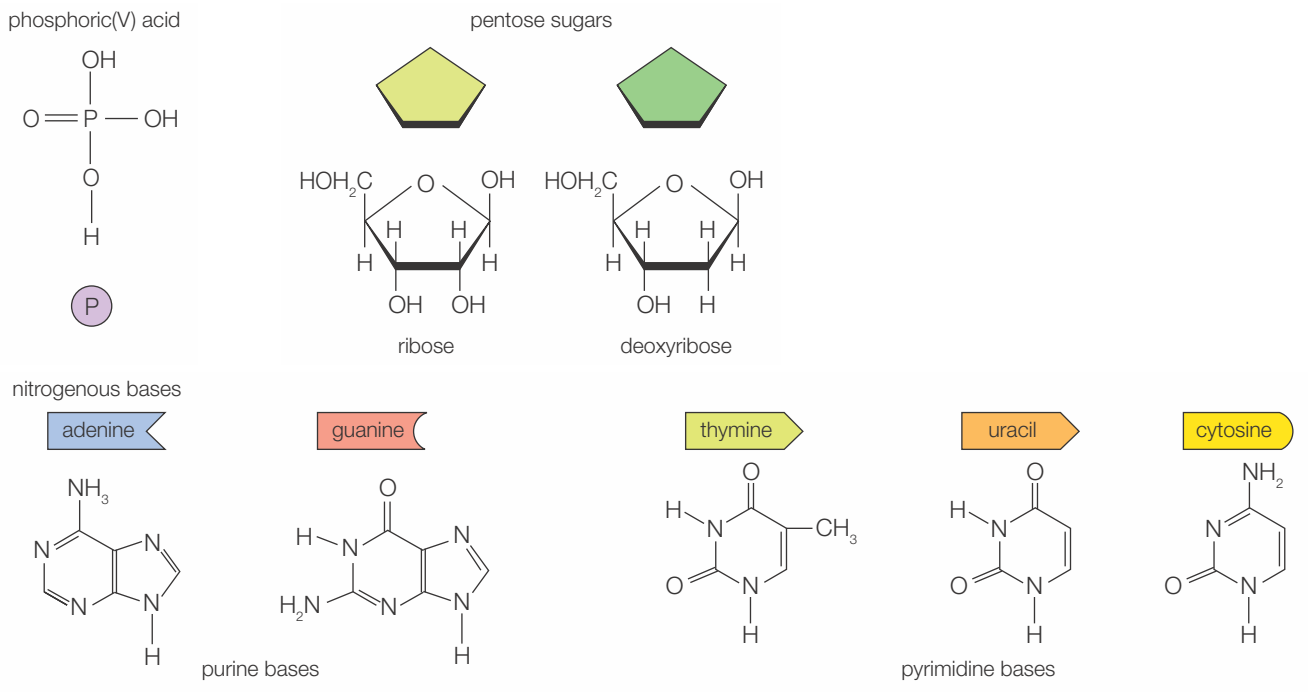


Figure 23.65 Components of DNA and RNA nucleotides

Nucleotides are the monomers from which the nucleic acids are synthesized by enzyme-controlled condensation reactions (Figure 23.66). The nucleotides themselves are made from a pentose sugar, a nitrogen-containing base and a phosphate group (which is ionized and negatively charged under physiological conditions).

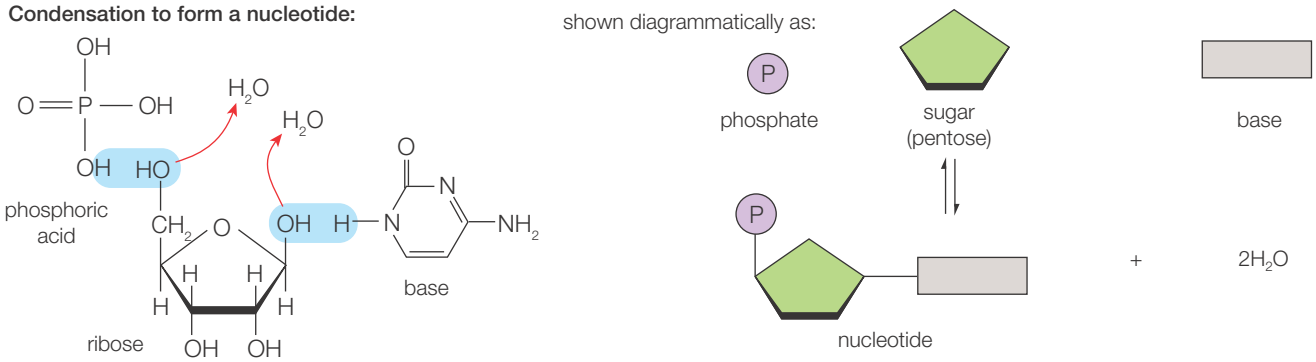


Figure 23.66 Condensation to a base, phosphate and pentose sugar to form a nucleotide

Expert tip

The nitrogenous bases present in RNA and DNA nucleotides are heterocyclic ring structures classified as purines or pyrimidines. The *pyrimidines* (thymine, uracil and cytosine) contain just a single ring (Figure 23.65), whereas the *purines* (guanine and adenine) contain two fused rings (Figure 23.65).

The two sugar–phosphate chains run in opposite directions in double-stranded DNA (they are anti-parallel) with the two chains twisted round each other to form a right-handed double helix (Figure 23.67). The sugar–phosphate backbone is on the outside and the bases are in the centre.

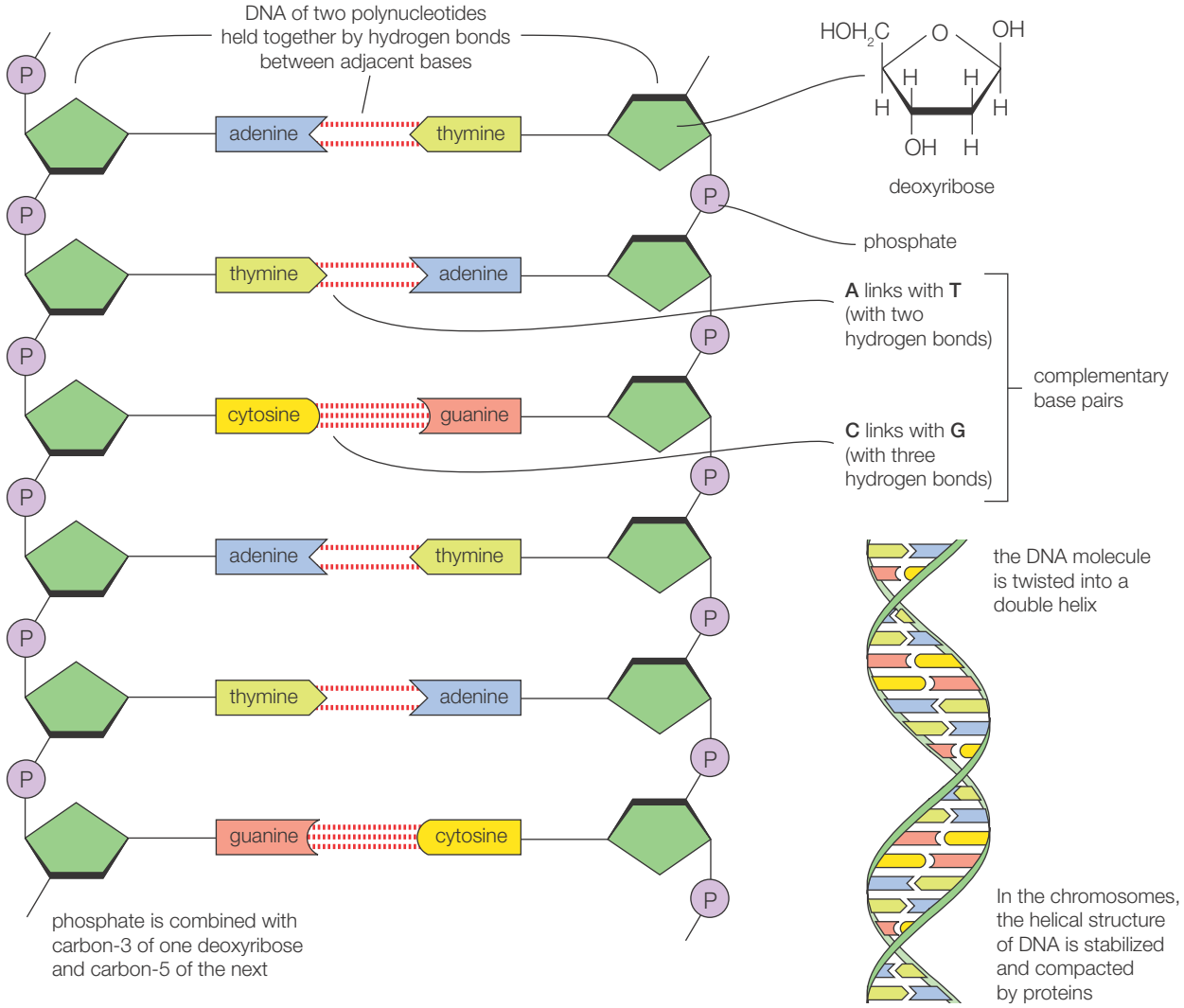


Figure 23.67 The double helix formed from two DNA strands

Pairs of bases (complementary base pairs) are stacked at right angles to the long axis of the helix. A always pairs with T, while C always pairs with G. The pairing is mediated through hydrogen bonding which stabilizes the double helix structure; note that the AT pairing involves two hydrogen bonds, whereas the GC pairing involves three such bonds. London (dispersion) forces due to the presence of the flat-ringed bases stacked above and below each other also contribute to maintaining the secondary structure of DNA.

NATURE OF SCIENCE

During the early 1950s a number of scientists were studying crystals of proteins and other large molecules by irradiating them with X-rays and examining the photographic evidence produced by the X-rays after they had been diffracted by the layers of molecules in the crystal. Rosalind Franklin and Raymond Gosling, working in London in association with Maurice Wilkins, found that one crystalline form of DNA produced a cross-like pattern characteristic of a helical structure. However, Franklin focused on another DNA form which she felt would yield more information but did not give a diffraction pattern characteristic of a helix. James Watson and Francis Crick realized the importance of the X-ray results from Franklin's group, which they coupled with crucial chromatographic data on base pairing (from Erwin Chargaff) and accurate model building to propose a double helical structure for DNA.

Worked example

If a given sample of double-stranded DNA is analysed for its base composition and shown to contain 40% cytosine, what is the expected percentage of adenine?

By Chargaff's ratios, 40% cytosine means the sequence must have 40% guanine as C always pairs with C. That leaves 20% of the bases as being A and T. Again, by Chargaff's pairing ratios, the %A must equal %T (A always pairs with T). Therefore, A and T split the remaining 20%, and there must be 10% adenine and 10% thymine in the DNA.

RNA (Figure 23.68) has an almost identical structure to DNA, except that the sugar is ribose, thymine is replaced by uracil, and it is single stranded and less chemically stable than DNA.

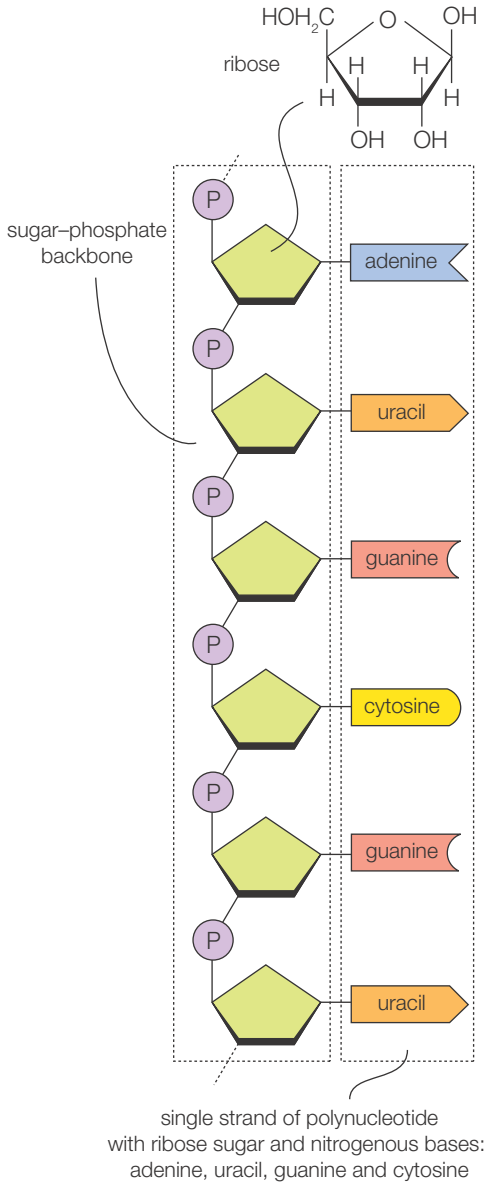


Figure 23.68 Structure of RNA

Replication

DNA produces new copies of itself (under enzyme control) during cell division. This is called replication (Figure 23.69). When DNA replicates itself identical copies of the base sequence are produced.

After cell division the new DNA molecules consist of one parent DNA strand and one 'new' strand. Complementary base pairing is the molecular basis for DNA replication.

The process of replication is semi-conservative; after cell division the new DNA molecules consist of one parent strand and one daughter strand (Figure 23.70).

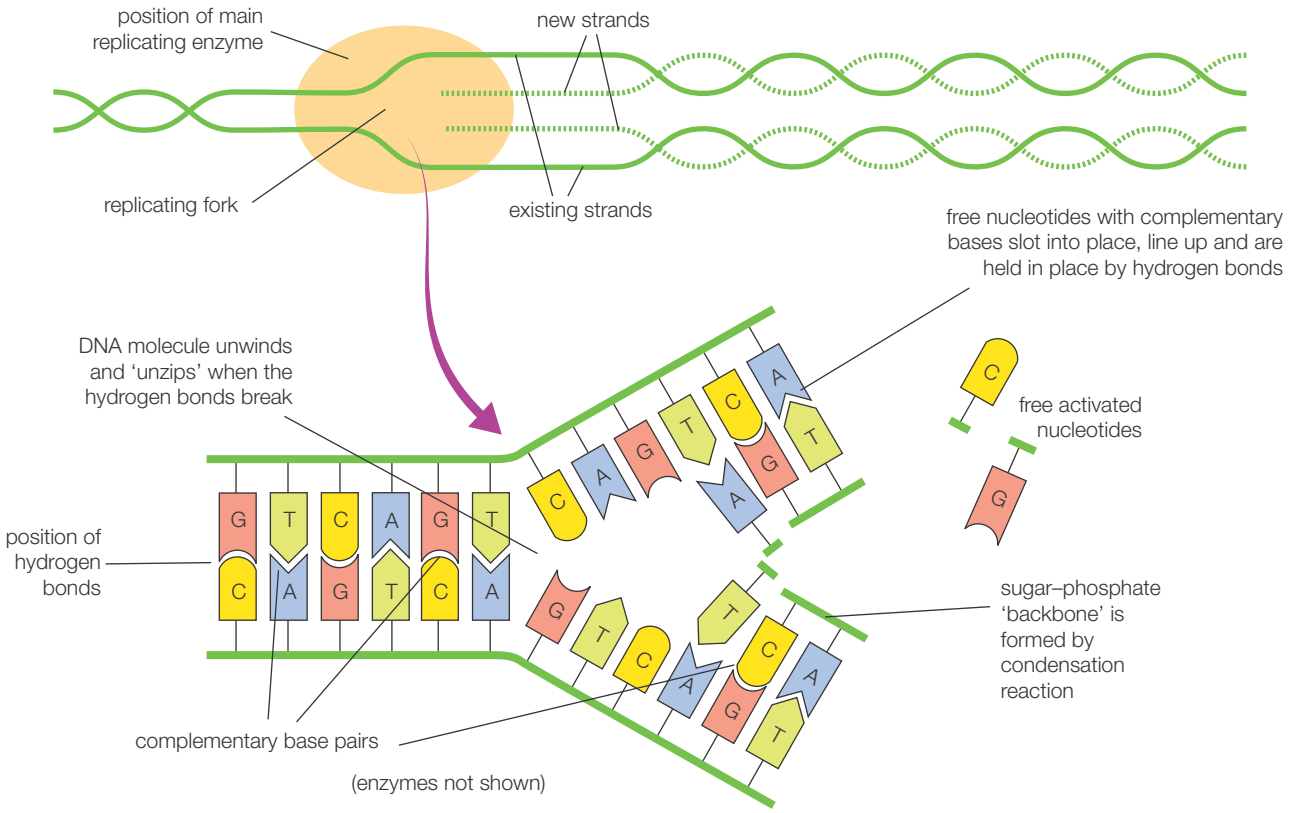


Figure 23.69 Simplified summary of the replication of DNA

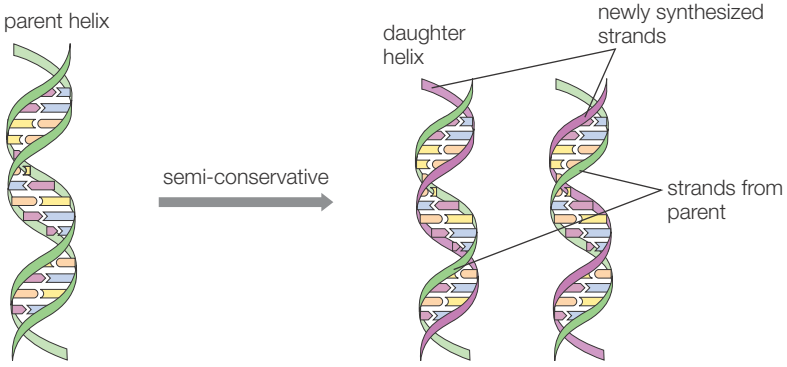


Figure 23.70 The outcome of semi-conservative replication

■ QUICK CHECK QUESTIONS

37 DNA (deoxyribonucleic acid) molecules and their fragments have been analysed in a variety of ways, one of which is by polyacrylamide gel electrophoresis. State the name of the component of DNA responsible for the migration of its fragments to the positive electrode in gel electrophoresis.

38 a What role do hydrogen bonds play in the accurate replication of DNA?

b DNA is replicated semi-conservatively. What is meant by this term?

c i What type of interaction takes place between the bases in the two DNA strands?

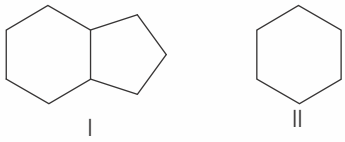
ii Condensation reactions are involved in the formation of DNA. What is the name given to the links which form the backbone of a DNA strand?

➜

39 a State two consistent differences between the structure of DNA and that of RNA.

b i Name two nitrogenous bases present in DNA, one a purine and the other a pyrimidine.

ii Suggest the most appropriate name for each of the following outline structures: purine or pyrimidine.



40 DNA is the molecule that carries genetic information in nearly all cells. Two months before Watson and Crick published their paper describing the double helical nature of DNA in 1953, Linus Pauling published a suggested structure for DNA based on a triple helix. Pauling's model soon proved to be incorrect; it had the phosphate groups facing into the core of the helix and the nitrogenous bases facing out.

a Suggest why Pauling's model would not have been a stable structure for DNA.

b DNA has the unusual property of being able to replicate. State the type and position of the bonds that break at the start of the replication process.

Transcription and translation

DNA stores the genetic information for the production of proteins. Each gene stores the information for a single polypeptide chain (protein). The flow of information in all living cells is DNA → RNA → protein. This is known as the central dogma (Figure 23.71). The conversion of the genetic code in DNA into RNA and proteins is termed gene expression.

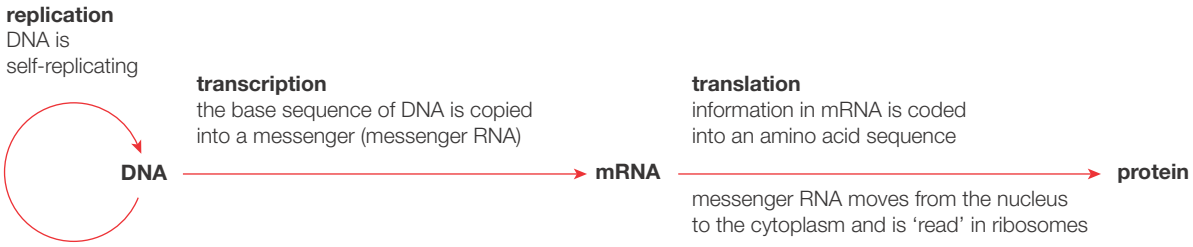


Figure 23.71 The central dogma

Using the genetic code to synthesize proteins involves several types of RNA: messenger RNA (mRNA), synthesized in the nucleus, transfer RNA (tRNA) to interact with amino acids and ribosomal RNA (rRNA) to catalyse the formation of a polypeptide chain from amino acids.

The enzyme-controlled processes involved in synthesizing proteins based on the genetic code in DNA of a gene are known as *transcription* and *translation* (Figure 23.72). Transcription involves the copying of the gene from the DNA into an mRNA molecule within the nucleus. The RNA molecule is then translated into the polypeptide chain in a complex process involving the ribosomes (composed of proteins and rRNA molecules) of a cell.

The mRNA molecules contain a triplet code in which three successive bases (a triplet) in the RNA sequence code for one amino acid in the polypeptide chain. The correspondence between the amino acids and the mRNA codons is specified by the genetic code. The possible combinations of the four different nucleotides in mRNA give 64 different codons ($4^3 = 64$) in the genetic code. Most amino acids are specified by more than one codon (Figure 23.73); for this reason the genetic code is described as being redundant.

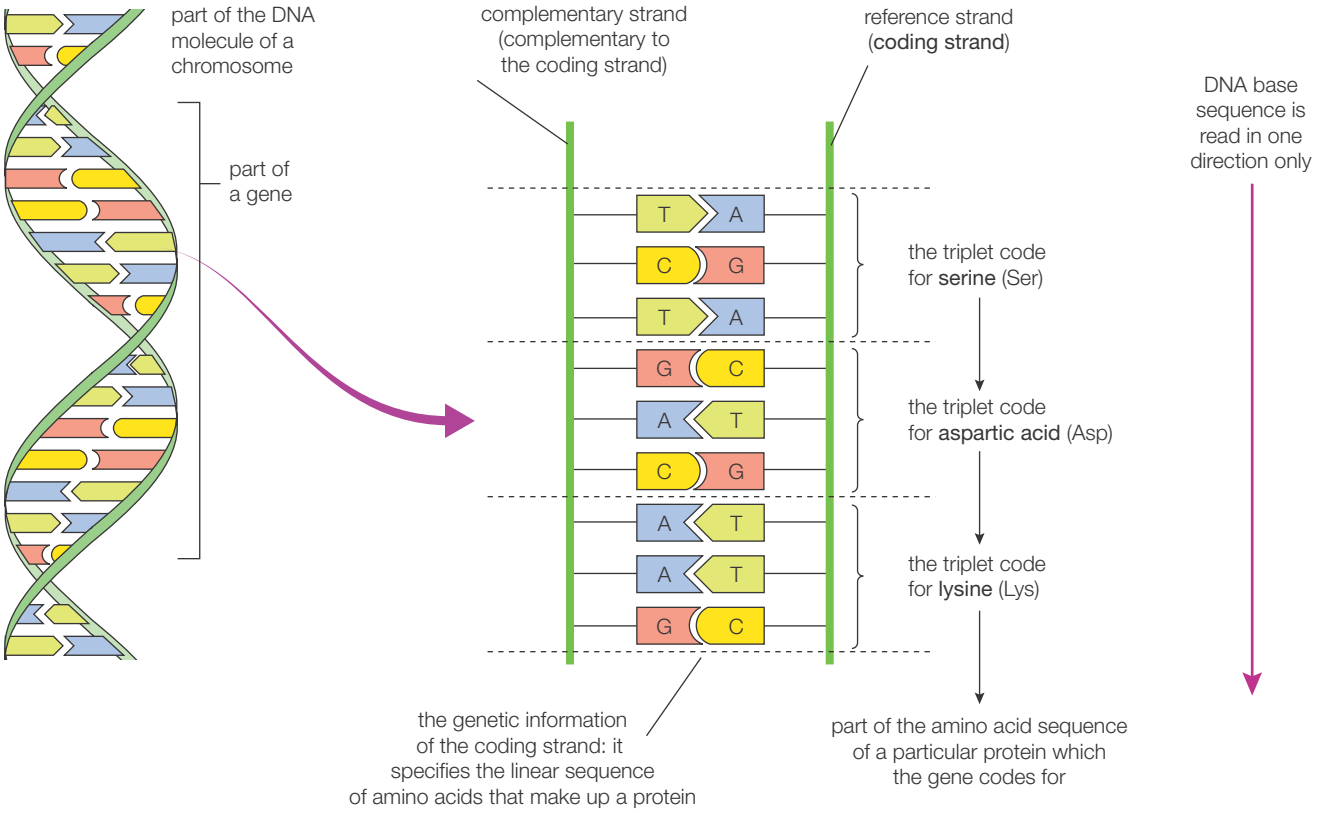
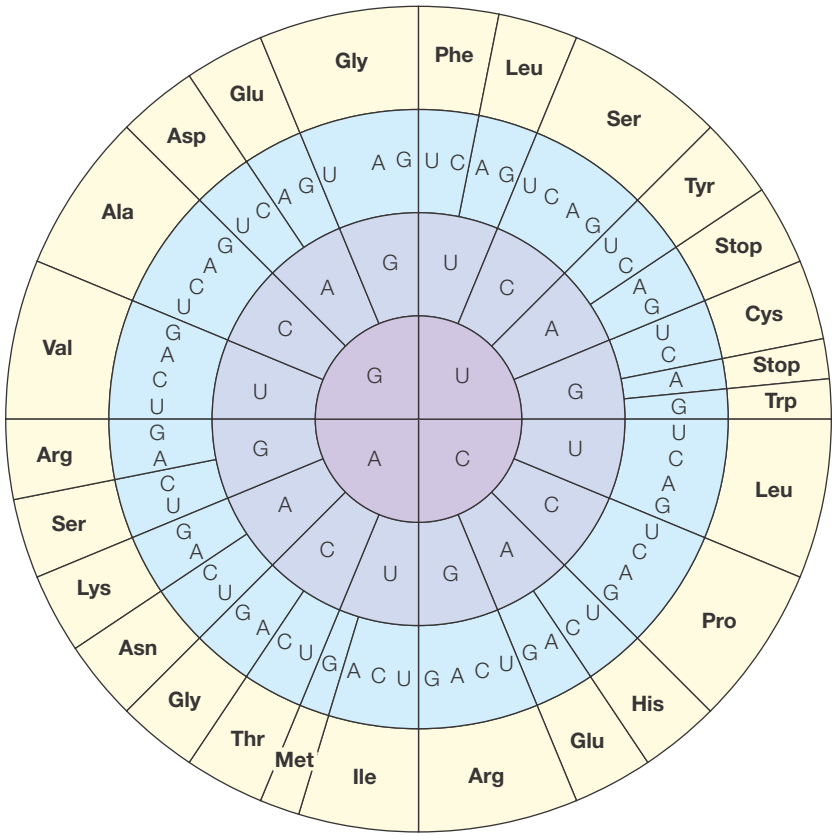


Figure 23.72 Part of a gene and how its DNA codes for amino acids

The genetic code in circular form
The codons are those of the DNA complementary strand and of messenger RNA (where uracil, U, replaces thymine, T).

Read the code from the centre of the circle outwards along a radius. For example, serine is coded by UCU, UCC, UCA or UCG, or by AGU or AGC.



In addition, some codons stand for **stop**, signalling the end of a polypeptide/protein chain.

Figure 23.73 The genetic code

Specific tRNA molecules bring specific amino acids from the cytoplasm to the ribosomes and bind to specific codons (via complementary base pairing) in the mRNA molecules. Protein synthesis (Figure 23.74) then takes place in three stages within the ribosome: initiation, elongation (amino acid polymerization) and termination (release and folding of the polypeptide to form a protein).

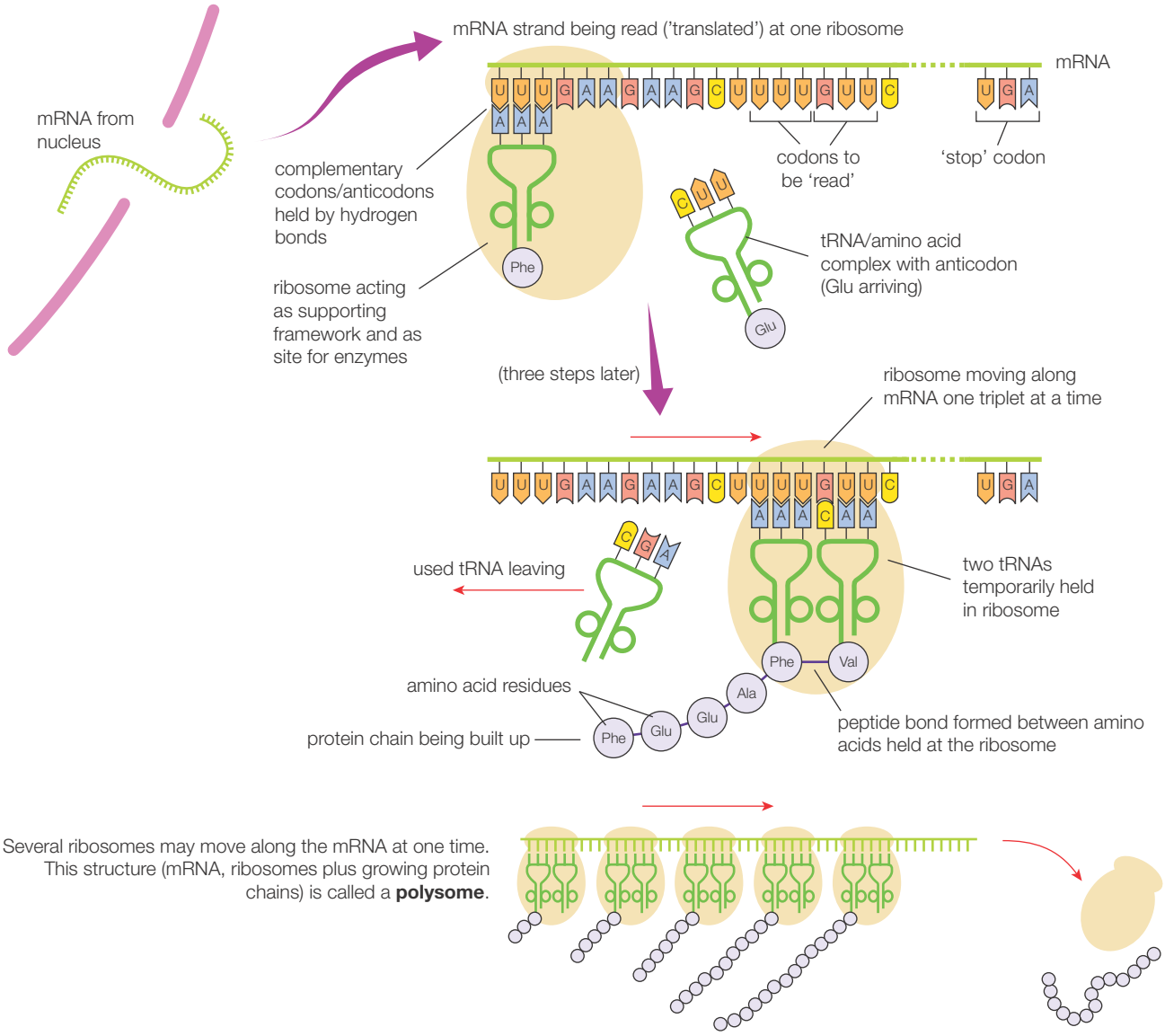


Figure 23.74 Translation: protein synthesis

The genetic material of cells with nuclei is contained in a set of paired chromosomes, each formed from a long DNA molecule with many genes. *Chromosomes* consist of DNA bound to specialized, highly basic proteins which help fold the DNA into a compact form. These basic proteins are known as histones and they have a high content of amino acid residues with basic positively charged R-groups. The first level of association between the DNA strand and histones is the formation of *nucleosomes*. A nucleosome consists of a length of DNA of about 150 base pairs, wrapped around a core of eight histones. These nucleosomes are spaced along the DNA, and thus form a 'string of beads' referred to as *chromatin*. Nucleosomes help to *supercoil* the DNA while still ensuring appropriate access to it. Figure 23.75 shows the relationship between the DNA strand, the nucleosomes, and a chromosome.

Expert tip

Details of transcription and translation are not required for the examination. For the examination limit your revision of the expression of DNA to the concept of a 4-unit base code determining a 20-unit amino acid sequence.

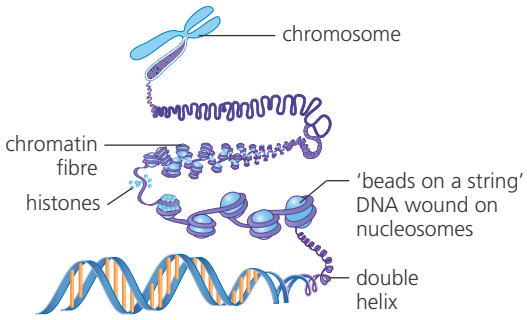


Figure 23.75 The packaging of DNA into a chromosome

The human genome contains approximately 3×10^9 nucleotide base pairs divided among 23 pairs of chromosomes (in humans). Only a small percentage of the DNA codes for proteins and RNA. The remainder is a mixture of 'junk' and sections that regulate the decoding, or expression, of the genes themselves. The functional regions of DNA are known as *genes*.

Individual humans differ from each other by an average of 1 nucleotide pair in every 1000; this variation is the basis for our genetic individuality and is the basis for identifying individuals by DNA 'fingerprinting'.

Expert tip

Certain viruses (retroviruses such as HIV) have been discovered which function initially in the opposite direction to the 'central dogma'. The genetic information of these viruses is coded in viral RNA which is then copied into DNA using a reverse transcriptase enzyme. Such enzymes have proved immensely useful in research into the isolation and identification of genes for specific protein chains.

Expert tip

You will note that the majority of the terms used in connection with this section are words that are associated with language and coding; terms such as transcription and translation. The overall term for the reading of the information contained in the genetic information is gene expression – the information contained within the genes is expressed in the proteins generated, and ultimately in the organisms that make up the living world.

■ QUICK CHECK QUESTIONS

41 Human blood is recovered from a knife found at a crime scene. Outline briefly the stages in preparing a DNA profile from the blood sample.

42 The base composition of a virus was found to be 11% A, 32% G, 18% U and 39% C.

a i Deduce whether this a DNA or RNA virus. Explain how you reached your conclusion.

ii Deduce whether this genetic material is single-stranded or double-stranded. Explain your suggestion.

b A fragment of DNA strand that is used as a template for mRNA synthesis has the following sequence: 5′-ACTGTCGCACAGT-3′.

Write the sequence of the mRNA molecule (starting from the 5′-end) synthesized from this DNA. Can this mRNA adopt a 3-dimensional structure by itself?

Recombinant DNA technology

Revised

The universal nature of the genetic code makes it possible for DNA from one organism to be expressed by directing protein synthesis when it is transferred into the DNA of a different species. This is the basis of *genetic engineering* which gives rise to **genetically modified organisms (GMOs)**.

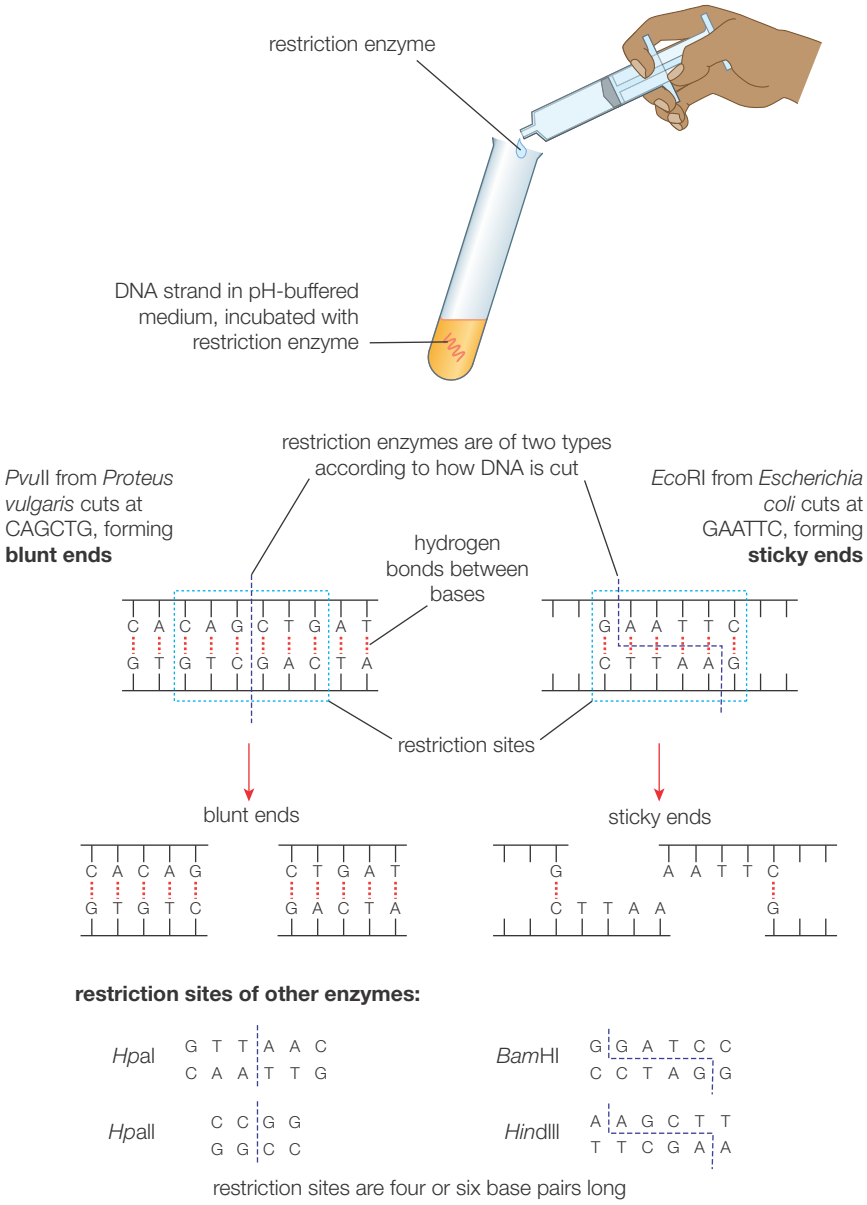
The DNA sequences used in the construction of **recombinant DNA (rDNA)** molecules can originate from any species. For example, plant DNA may be joined to bacterial DNA, or human DNA may be joined with fungal DNA. In addition, DNA sequences that do not occur anywhere in nature may be created by the chemical synthesis of DNA and incorporated into recombinant molecules. Using recombinant DNA technology and synthetic DNA, literally any DNA sequence may be created and introduced into any of a very wide range of living organisms.

Recombinant DNA technology allows biochemists to pick out a specific gene from a cell's genome and determine the molecular structure of the gene. An important technique is the ability to cut a long DNA molecule into a specific and reproducible set of fragments using restriction enzymes (Figure 23.76), each of which cuts the DNA double helix only at a particular nucleotide sequence.

Key definitions

Genetically modified organisms (GMOs) – organisms that have genetic material that has been changed in some way by genetic engineering. This can often be the result of the insertion of DNA from a different species.

Recombinant DNA (rDNA) – these molecules are DNA molecules formed by laboratory methods of genetic recombination which bring together genetic material from multiple sources, creating sequences that would not otherwise be found in the genome.



Restriction enzymes are named after the microorganisms they are found in. Roman numbers are added to distinguish different enzymes from the same microorganism.

Figure 23.76 The role of restriction endonucleases (restriction enzymes)

DNA fragments can be separated from one another on the basis of size by gel electrophoresis. Nucleic acid hybridization (Figure 23.77) can detect any specific RNA or DNA sequence in a mixture of nucleic acid fragments. This technique relies on the fact that a single strand of DNA or RNA will form a double helix only with another nucleic acid strand of the complementary nucleotide sequence.

Genetic engineers isolate, cut out and transfer genes between organisms. In order to transfer DNA in a living cell the DNA is introduced by a vector, usually a plasmid (circular DNA from bacteria). Once the foreign DNA is inside the host cell it may be incorporated by the host cell. Genes in the foreign DNA are expressed using the host cell's ribosomes. The foreign DNA is replicated (copied) each time the cell divides.

Molecular cloning and PCR are two techniques for the rapid replication of modified DNA. The fundamental difference between the two methods is that molecular cloning involves replication of the DNA within a living cell (often a bacterial cell), while PCR replicates DNA in the test tube, free of living cells. Recombinant DNA is widely used in biotechnology, medicine and research. The most common application of recombinant DNA is in basic research, but many additional practical applications of recombinant DNA are found in industry, food production, human and veterinary medicine, agriculture and bioengineering. Some specific examples are listed below.

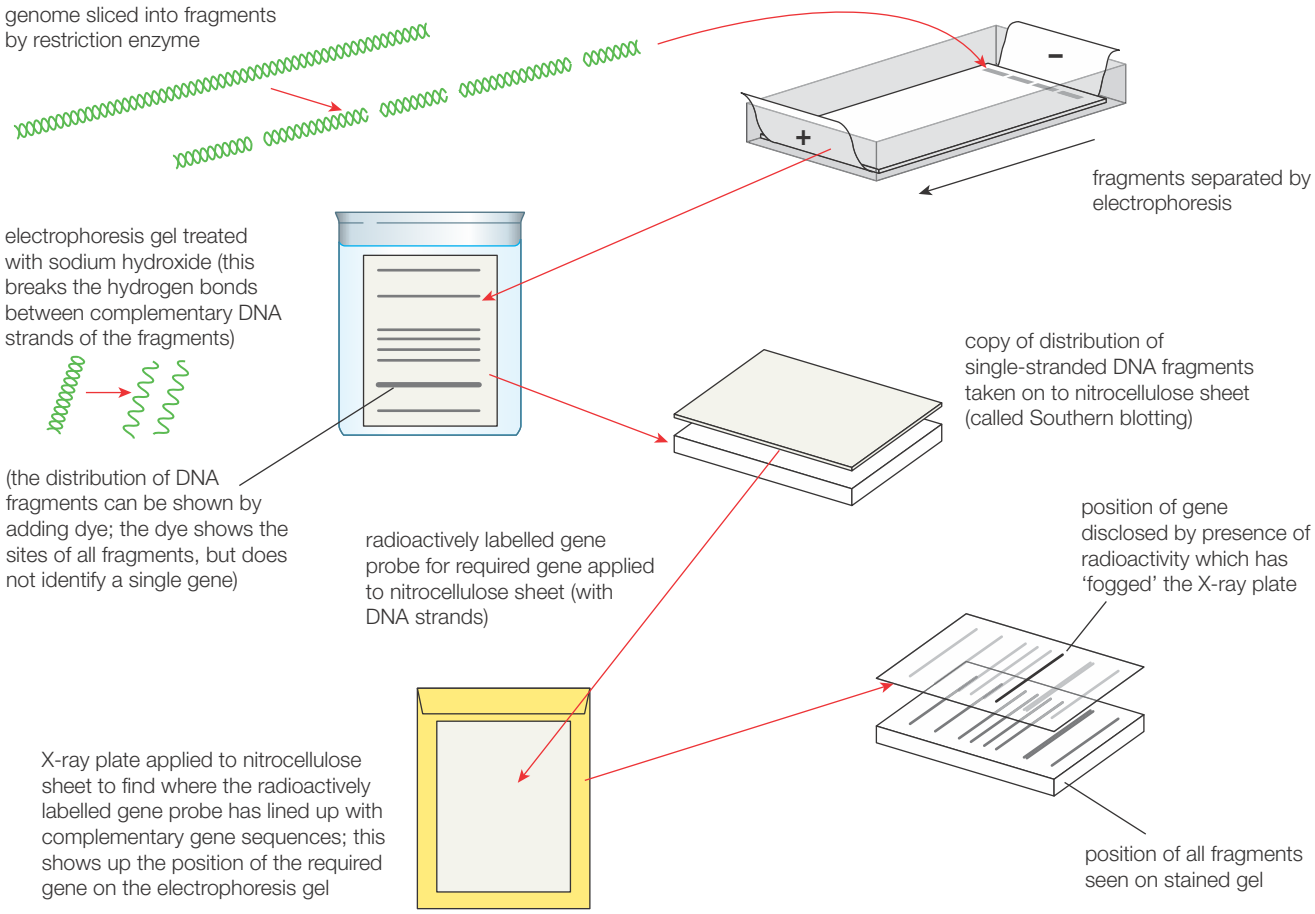


Figure 23.77 Gene probe technology

- **Recombinant human insulin:** This form of insulin has almost completely replaced insulin obtained from animal sources for the treatment of insulin-dependent diabetes. Recombinant insulin is synthesized by inserting the human insulin gene into *E. coli*, or yeast (*Saccharomyces cerevisiae*), which then produces insulin for human use. This example is of importance because of its impact on patients and the fact that it was essentially the first successful application of this ground-breaking technique. A similar approach has been taken in the following cases.
- **Recombinant human growth hormone (HGH, somatotropin):** Recombinant HGH is now administered to patients whose pituitary glands generate insufficient quantities to support normal growth and development. The use of the recombinant protein eliminated problems associated with HGH from cadavers (dead corpses), which had been the previous source.
- **Recombinant hepatitis B vaccine:** Recombinant hepatitis B vaccine contains a form of the hepatitis B virus surface antigen that is produced in yeast cells. The development of this recombinant vaccine was an important and necessary development because hepatitis B virus, unlike other common viruses such as the polio virus, cannot be grown *in vitro*.

These examples indicate the importance of recombinant DNA techniques using rapidly growing organisms such as bacteria or yeast. However, a number of GMOs have been developed for commercial use involving animals and plants that produce pharmaceuticals or other compounds. Within the field known as *pharming*, intensive research has been conducted to develop transgenic animals that produce biotherapeutics. The first human biological drug produced from such an animal, a goat, is the drug anti-thrombin (ATryn), which is an anticoagulant that reduces the probability of blood clots during surgery or childbirth. The anticoagulant is extracted from the goat's milk.

Genetically modified crops

Biotechnology is the manipulation of organisms and cells for the benefit of people in agriculture, medicine and food production. Biotechnology is used to modify plants used in food production (Figure 23.78). Techniques of inter-species DNA transfer are now advanced, and a wide range of genetically modified foods, known as GM *foods*, are marketed in many countries. The genetic modification may:

- add a gene to yield a new product
- inactivate a gene to remove undesired an undesired property
- modify a gene for higher yields of its protein.

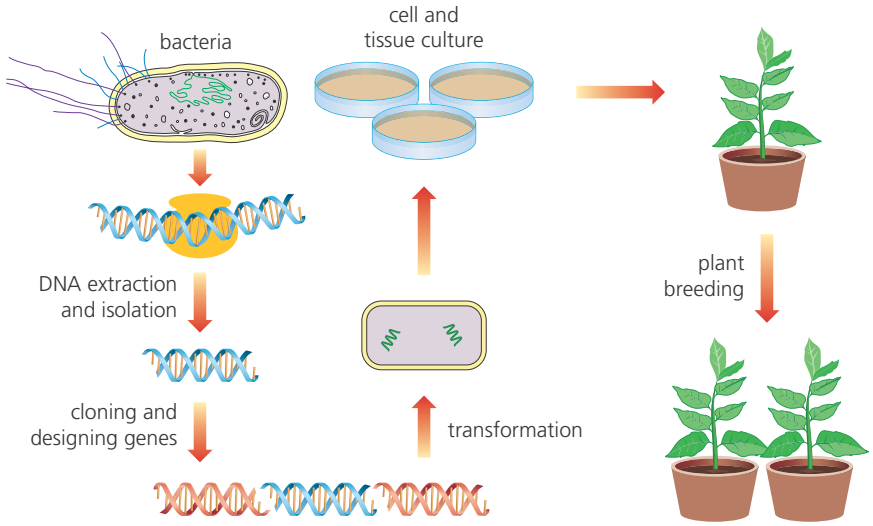


Figure 23.78 The process of genetic modification through isolation of a gene and insertion into the genetic sequence of a host organism (a plant)

Examples include corn which contains a bacterial gene that produces a natural pesticide, herbicide-resistant crops, rice which produces higher concentrations of vitamin A ('golden rice'), and tomatoes that remain fresh for longer.

While genetically modified foods such as these promise certain benefits, their development also raises many issues and is the subject of ongoing debate. Some aspects of both sides of this debate are summarized here.

Benefits of GM foods: Some GM foods and GMOs have the following qualities:

- longer shelf-life
- improved flavour, texture and nutritional value
- increased resistance to diseases and pests, reducing the use of pesticides
- produce a supply of substances such as vitamins and vaccines
- increased crop yields
- tolerance of a wider range of growing conditions, such as drought resistance.

Concerns over GM food: Many people, however, express the following concerns:

- lack of information about long-term effects
- changes to the natural ecosystem through cross-pollination
- possible links to increased allergies
- risk of altering natural composition of food
- concerns of breeding species that are resistant to control
- in some cases lack of information through food labelling.

QUICK CHECK QUESTION

43 Many modern food items contain genetically modified components.

a Explain what is meant by the term *genetically modified food*.

b Describe **two** advantages and **one** concern about the use of genetically modified food.

23.9 Biological pigments

Revised

Essential idea: Biological pigments include a variety of chemical structures with diverse functions which absorb specific wavelengths of light.

Biological pigments

Revised

- Biological pigments are coloured compounds produced by metabolism.
- The colour of pigments is due to highly conjugated systems with delocalized electrons, which have intense absorption bands in the visible region.
- Porphyrin compounds, such as hemoglobin, myoglobin, chlorophyll and many cytochromes are chelates of metals with large nitrogen-containing macrocyclic ligands.
- Hemoglobin and myoglobin contain heme groups with the porphyrin group bound to an iron(II) ion.
- Cytochromes contain heme groups in which the iron ion interconverts between iron(II) and iron(III) during redox reactions.
- Anthocyanins are aromatic, water-soluble pigments widely distributed in plants. Their specific colour depends on metal ions and pH.
- Carotenoids are lipid-soluble pigments, and are involved in harvesting light in photosynthesis. They are susceptible to oxidation, catalysed by light.

Biological pigments

Revised

Biological pigments are coloured compounds which are produced by metabolism (enzyme-controlled reactions inside cells). Melanin is a biological pigment responsible for the colour of skin, hair and eyes. The colour of pigments results from the absorption of certain wavelengths of visible light. All pigment molecules have intense absorption bands in the visible region of the spectrum. The colour seen is the light that is not absorbed but instead is reflected (Figure 23.79).

White light can be described as a mixture of red, green and blue light. These are known as the primary colours and when mixed together (in equal intensities) produce white light. All the colours can be generated from the three primary colours.

The colour that we see is white light minus the colour being absorbed. The colour we see is called the complementary colour to the colour being absorbed. A colour wheel (Figure 23.80) illustrates the approximate complementary relationship between the wavelengths of light absorbed and the wavelengths transmitted or reflected.

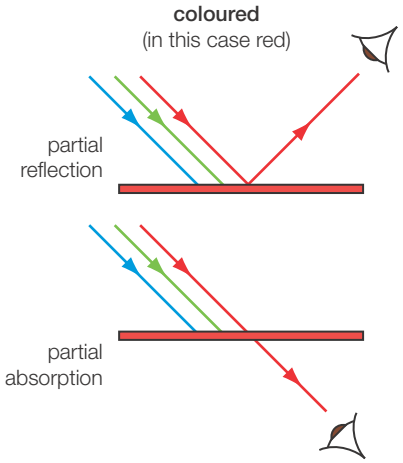


Figure 23.79 Perception of a red coloured pigment

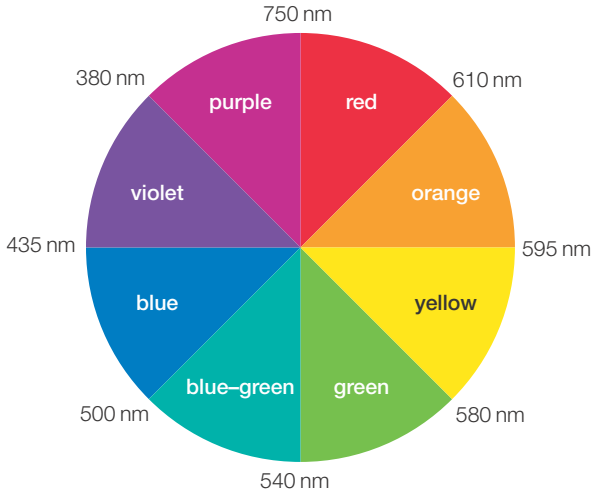


Figure 23.80 A simple colour wheel

Examples of naturally occurring coloured organic compounds include the anthocyanins, carotenoids (e.g. β-carotene) and the porphyrins (e.g. chlorophyll, hemoglobin, cytochromes and myoglobin).

NATURE OF SCIENCE

A study of the interaction of light (or other electromagnetic radiation) with matter is an important tool for chemists. Much of our knowledge of chemical substances comes from their specific absorption or emission of light. In recent years spectrophotometric methods have become the most frequently used and important methods of quantitative analysis. They are applicable to many industrial and clinical problems involving the quantitative determination of compounds that are coloured or that react to form a coloured product.

Chromophores

Groupings within organic molecules that cause colour are chromophores. They are typically delocalized electron systems, often benzene rings or with functional groups with lone pairs, such as >C=O, –N=N– and $–NO_2$. A common chromophore is an arrangement of alternating single and double carbon–carbon bonds (Figure 23.81). The more extensive the conjugation, the lower the energy (longer the wavelength) of the light (photons) absorbed.

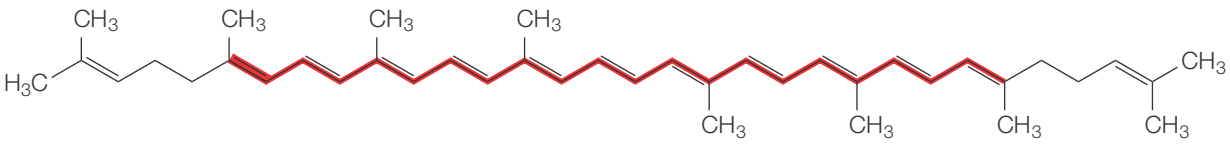


Figure 23.81 Lycopene, the red pigment in tomatoes, has 11 conjugated double bonds (highlighted). Note that not all the >C=C< bonds in lycopene are part of the conjugated system

The absorption of light (of specific energy) causes electrons in π-bonds or lone pairs (*n*) being excited and undergoing a transition to a molecular orbital of higher energy, usually an anti-bonding orbital (Figure 23.82).

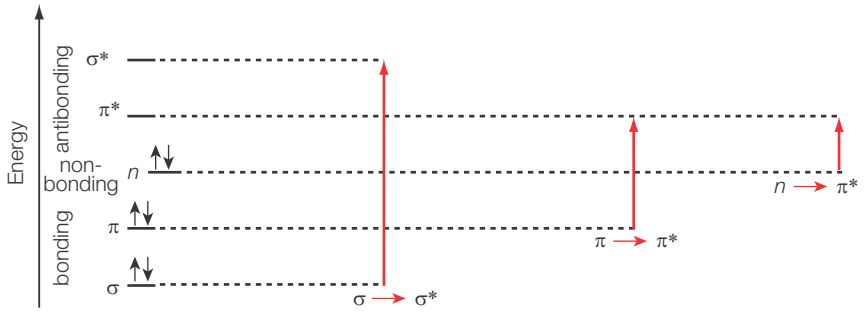


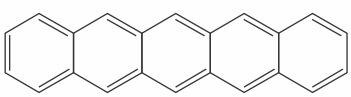
Figure 23.82 Possible electron transitions: only π to π* transitions and *n* to π* transitions that occur in the UV–Vis region are observed

QUICK CHECK QUESTIONS

44 Lycopene (Figure 23.81) is a carotenoid and is responsible for the red colour in tomatoes. When bromine is slowly added to some tomato juice, the colour of the juice gradually changes from red to yellow.

Explain this colour change in terms of changes in bonding in lycopene.

45 Pentacene (structure below) is purple while benzene is colourless. Both molecules are conjugated. Explain these observations.



Expert tip

Conjugation occurs when three or more p orbitals overlap. This can be achieved via π–σ–π bonds or can be achieved by an atom (such as nitrogen or oxygen) with a lone pair that is sp^2 or sp^3 hybridized.

Anthocyanins

Anthocyanins are aromatic, water-soluble polyphenol pigments found in fruits, vegetables and flowers. Their structures are based on the hydrocarbon flavan structure (Figure 23.83) which has a $C_6–C_3–C_6$ skeleton with two benzene rings (conjugated systems) isolated by an oxygen-containing pyran ring.

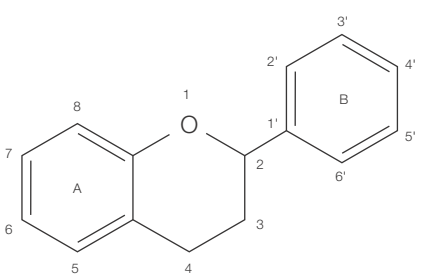


Figure 23.83 The central flavan structure of the anthocyanins

The colour of anthocyanin molecules is affected by the presence of cations, pH and temperature. They can act as acid–base indicators.

Anthocyanin molecules can act as ligands (Lewis bases) via their oxygens and can coordinate to cations, such as aluminium, iron(II) and iron(III) to form intensely coloured complexes.

A complex equilibrium exists in aqueous solution (Figure 23.84) with four different structural forms with different extents of conjugation:

A	⇌	AH^+	⇌	B	⇌	C
quinoidal base	⇌	flavylium cation	⇌	carbinol	⇌	chalcone
purple/red	⇌	red	⇌	colourless	⇌	yellow

Low values of pH favour the red flavylium form; high values of pH favour the yellow chalcone form. At intermediate pH values the purple quinoidal base and colourless carbinol form are at high concentrations in the equilibrium mixture. At low temperatures the red flavylium form predominates; at high temperatures the yellow chalcone form predominates.

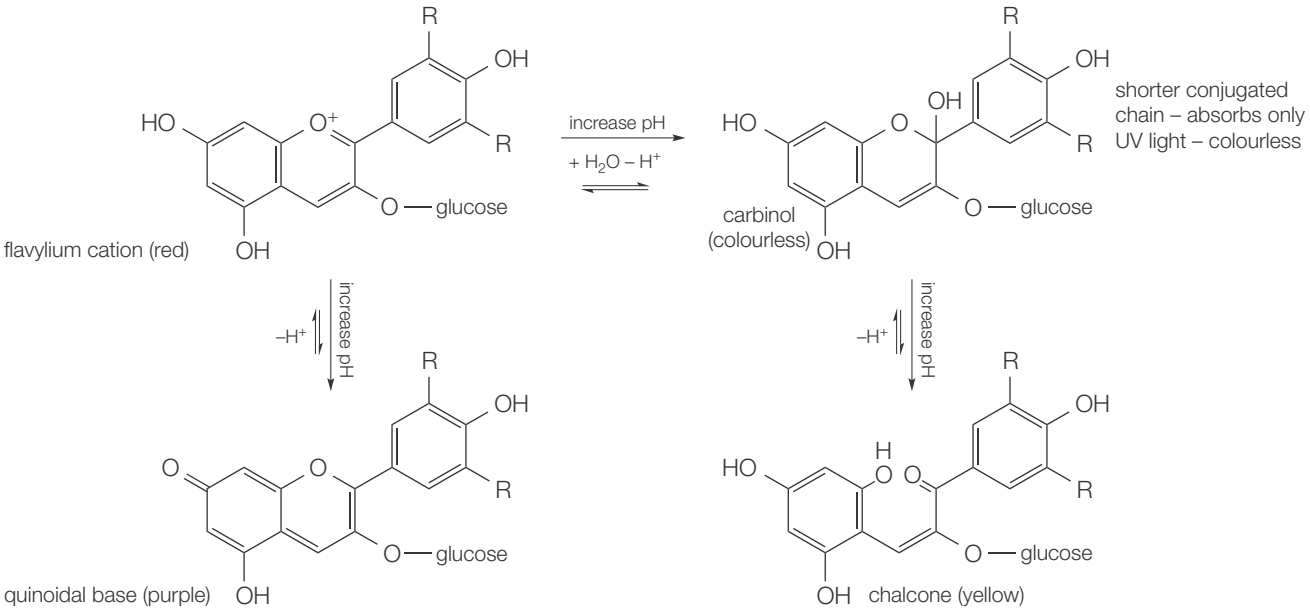
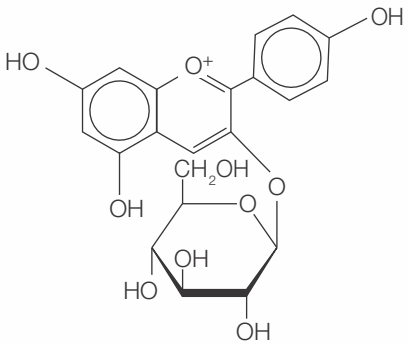


Figure 23.84 A complex equilibrium, which is very sensitive to pH, exists in an anthocyanin solution

■ QUICK CHECK QUESTIONS

46 The colour of many flowers and fruits is due to the presence of anthocyanin pigments. The red colour of ripe strawberries is largely due to the anthocyanin pigment having the structure below.



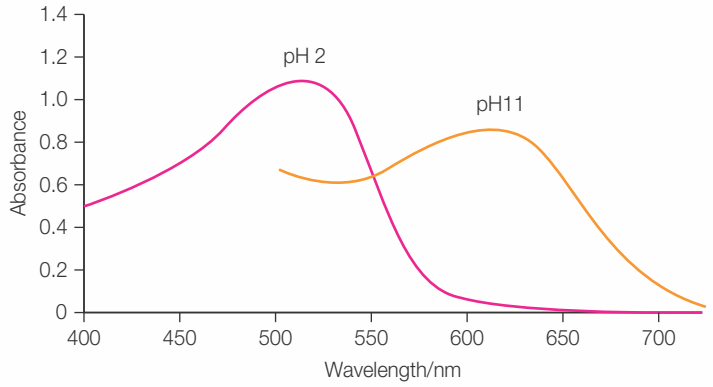
a Explain why this molecule absorbs visible light.

b By referring to its chemical structure, outline whether this pigment is found in aqueous solution in the cells or in the lipid-based membranes.

➜

47 Anthocyanins can act as pH indicators. An extract from blackberry juice produced the following absorption spectra at different pH values when analysed using a UV–Vis spectrophotometer:

Using information from section 17 of the IB Chemistry *data booklet*, deduce the colour of the extract at each of the pH values shown.



Carotenoids

Carotenoids are lipid-soluble plant pigments involved in the absorption of blue light during photosynthesis. The conjugated system in carotenoids is due to the presence of a long hydrocarbon chain consisting of an alternating series of single and double carbon–carbon bonds.

Many carotenoids, such as beta-carotene (Figure 23.85), are derived from a polyene chain containing 40 carbon atoms, which may have terminal cyclic groups and oxygen-containing functional groups.

β-Carotene (a precursor to vitamin A) is found in carrots and has a characteristic orange colour. It has a conjugated π-system involving 11 conjugated carbon–carbon double bonds. It appears orange when viewed in white light since its molecules absorb strongly in the violet-blue (400–510 nm region) of the electromagnetic spectrum.

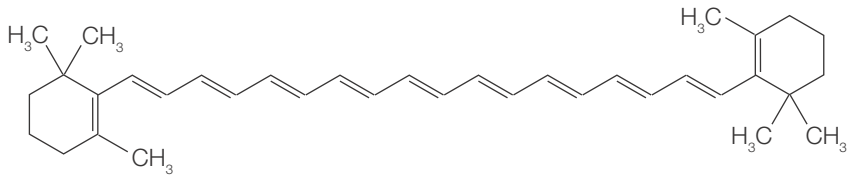


Figure 23.85 The structure of β-carotene

Owing to their polyunsaturated nature, carotenoids are susceptible to oxidation. This is a complex free radical reaction catalysed by light, transition metal cations and hydroperoxides (ROOH). At high temperatures carotenoids isomerize from the all-*trans* form into a mixture of *cis* isomers.

Expert tip

The carotenoids are divided into two groups: the carotenes, which are hydrocarbons, and the xanthophylls, which contain oxygen.

Porphyrins

The porphyrins are a series of related nitrogen-containing macrocyclic (large single ring with multiple donor sites) conjugated ligands that are able to strongly coordinate a specific metal cation. Examples of porphyrins and their cations are chlorophyll (Mg^{2+}), hemoglobin (Fe^{2+}), myoglobin (Fe^{2+}) and the cytochromes (Fe^{2+} and Fe^{3+}, depending on their oxidation state).

Hemoglobin

Hemoglobin is a protein found in mammals that transports molecular oxygen in the red blood cells. Oxygen binds cooperatively to hemoglobin, resulting in a sigmoidal (S-shaped) oxygen dissociation curve. It is a tetrameric protein – four protein molecules associated together. Each of the four globin proteins contains a heme group with an iron(II) ion at the centre (Figure 23.86).

Expert tip

This effect is due to small changes that occur in the quaternary structure as oxygen binds. A conformational shift caused by the binding of oxygen at one heme group makes the other heme groups have a higher affinity for oxygen.

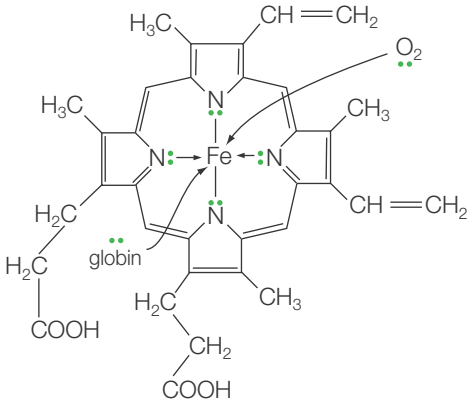


Figure 23.86 The structure of the heme group in hemoglobin

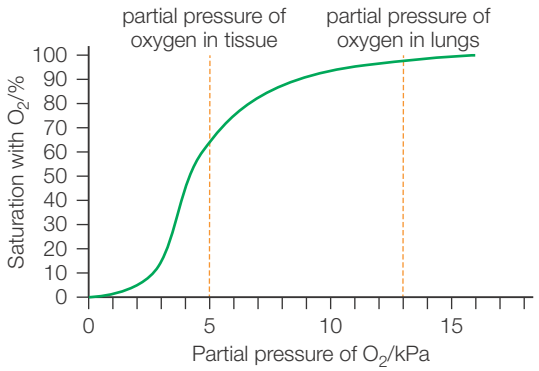


Figure 23.87 The oxygen binding or dissociation curve for hemoglobin. The partial pressure of oxygen is the pressure of the oxygen in a mixture of gases

The oxygen saturation of hemoglobin (Figure 23.87) is influenced by the following factors.

Common mistake

Do not confuse oxygen pressure with blood pressure. Oxygen pressure, oxygen partial pressure and oxygen tension are the same concept and are simply a measure of the concentration of oxygen in the blood.

pH

Hemoglobin can be protonated (at an amino acid side-chain) at a low value of pH (high concentration of protons), resulting in the dissociation (release) of oxygen as the protein undergoes a conformational change:

$$HbO_2 + H^+ \rightleftharpoons HbH^+ + O_2$$

Carbon monoxide

Carbon monoxide is a competitive inhibitor of oxygen at the iron(II) ion in the heme group. Hemoglobin has a higher affinity for carbon monoxide than oxygen. A strong coordinate bond is formed between the iron(II) ion and the lone pair of electrons on the carbon atom of carbon monoxide.

Carbon dioxide

Carbon dioxide reacts reversibly with water to form carbonic acid (H_2CO_3), which dissociates to form hydrogen carbonate, HCO_3^-(aq) and hydrogen ions, H^+(aq). This lowers the pH so that more H^+ binds to hemoglobin and causes a release of oxygen. Hemoglobin also binds carbon dioxide, but not at a different site from oxygen. This carbon dioxide reacts with the $-NH_2$ group on the terminal amino acid of each polypeptide chain that makes up hemoglobin (Figure 23.88) which releases H^+ and also changes the shape of the protein; both of these reduce the affinity of hemoglobin for oxygen.

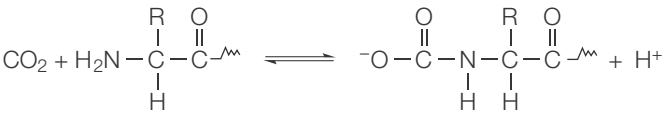


Figure 23.88 The reaction of carbon dioxide with the terminal amino acid of a polypeptide chain

Common mistake

A frequent source of confusion is in distinguishing 'carboxyhemoglobin' from 'carbaminohemoglobin'. The first is hemoglobin combined with CO and the second is hemoglobin carrying CO_2.

Temperature

The dissociation of oxyhemoglobin ($HbO_2 \rightarrow Hb + O_2$) is an endothermic process. Hence, the position of equilibrium will shift to the right as the temperature is increased and less oxygen binds to hemoglobin.

Expert tip

When muscles are metabolically active they release heat and the hemoglobin provides with the increased oxygen required for respiration.

Fetal hemoglobin and myoglobin

Fetal hemoglobin is a different form of hemoglobin only present in the blood of the developing fetus. It has a higher affinity for oxygen than maternal hemoglobin (which replaces it after birth) (Figure 23.89). This adaptation allows the efficient transfer of oxygen from the mother's blood to the fetal blood in the placenta.

Myoglobin also has an oxygen dissociation curve to the left of that of hemoglobin, which means it has a greater affinity for oxygen and can accept oxygen from hemoglobin for storage (in the striated muscles).

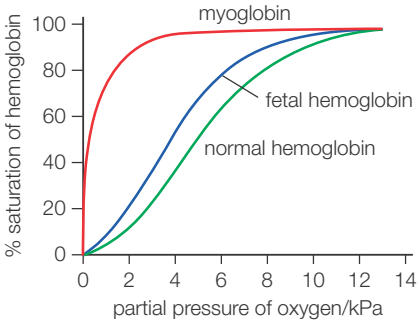


Figure 23.89 Dissociation curves for fetal hemoglobin and myoglobin

QUICK CHECK QUESTION

48 Hemoglobin contains a heme group with an iron(II) ion.

a Outline how the oxygen saturation of hemoglobin is affected by changes in the blood plasma

b Explain why fetal hemoglobin has a greater affinity for oxygen than adult hemoglobin.

Expert tip

The dissociation curve for myoglobin is not sigmoidal in shape as there can be no cooperative binding within its one heme structure.

Cytochromes

Cytochromes are a varied group of electron transport proteins that contain a heme group which has an iron ion that alternates between iron(II) and iron(III). It is able to accept electrons from one substance and donate them to another (with a less negative electrode potential), functioning as part of the electron transport chain which is involved in the last step of the aerobic respiration of glucose. Cytochrome c passes its electrons to the terminal acceptor oxygen with the formation of water:

$$4Fe^{2+}_{(\text{cytochrome c})} + 4H^+ + O_2 \rightarrow 4Fe^{3+}_{(\text{cytochrome c})} + 2H_2O$$

Expert tip

Cyanide ions are a very effective poison because they block this reaction and prevent respiration.

Chlorophyll

Chlorophylls are the main photosynthetic pigments in leaves and are involved in absorbing the light needed in the process of photosynthesis. Chlorophyll molecules ionize and donate an electron to the electron transport chain.

Chlorophyll (Figure 23.90) occurs in plants in two closely related forms: chlorophyll a (blue green) and chlorophyll b (yellow green).

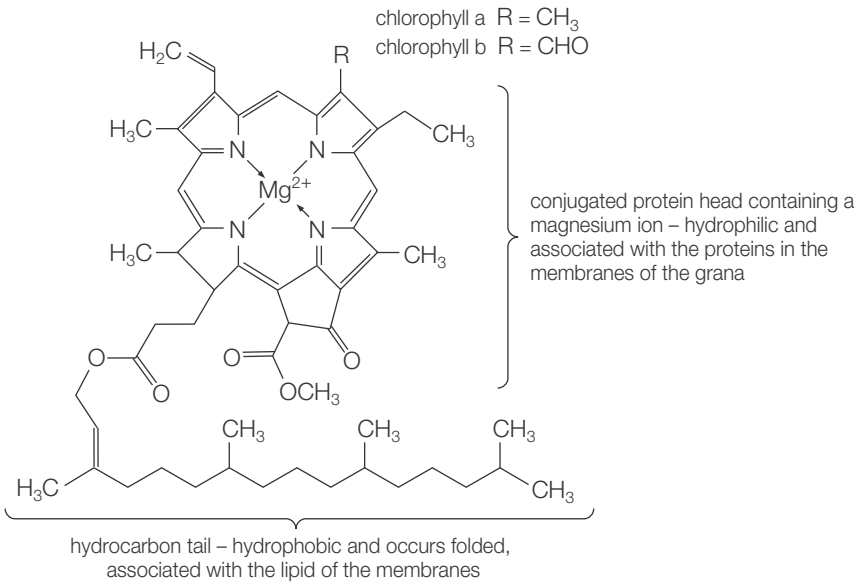


Figure 23.90 The structure of chlorophyll

Chlorophyll absorbs light strongly in the blue part of the spectrum and to a lesser extent in the red (see Figure 23.91) and hence leaves appear green (when viewed in white light). The chromophore is the conjugated porphyrin ring system.

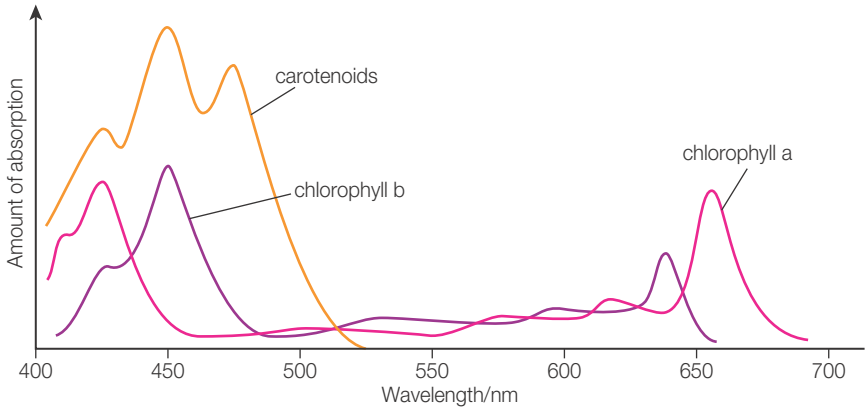


Figure 23.91 Ultraviolet spectrum of chlorophyll a, chlorophyll b and carotenoids

The stability of chlorophyll is affected by pH: in highly acidic conditions (pH < 3) the central magnesium ion, Mg^{2+}, is replaced by a proton (H^+). Chlorophyll will undergo decomposition at high temperatures and in the presence of high intensity ultraviolet radiation.

Chromatography

Chromatography is based upon the differential retention of compounds in a mobile phase as they pass through or across a stationary phase. It can be used to separate, identify, identify and quantify the component pigments (or dyes) in a mixture.

In paper chromatography, the stationary phase is a liquid adsorbed onto the surface of the paper. The paper has many pores that can adsorb and strongly hold hydrogen bond water molecules to form the stationary phase. The water can be displaced by other liquids to give different stationary phases.

Pigments that are more soluble in the solvent than they are in the water molecules of the stationary phase move rapidly up the paper, while those that are more soluble in the water are not carried as far up the paper (Figure 23.92).

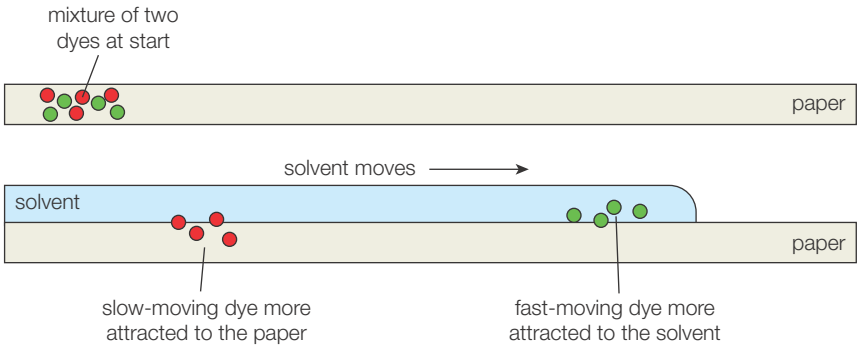


Figure 23.92 The principles of paper chromatography

Thin layer chromatography (TLC) uses a stationary phase of silica or alumina particles bonded to a thin layer of glass or plastic. TLC (Figure 23.93) separates a mixture of pigments based on how strongly they are adsorbed on the stationary phase and dissolved in the mobile phase (a liquid or mixture of liquids). This equilibrium is known as partitioning. The greater the affinity of the pigment for the stationary phase, the more slowly it moves along the surface of the TLC plate.

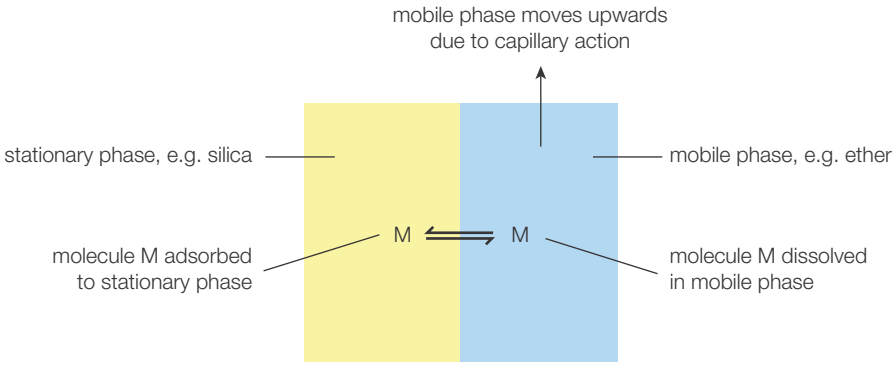


Figure 23.93 Principles of TLC

Expert tip

TLC is faster, more sensitive and has better resolution than paper chromatography.

23.10 Stereochemistry in biomolecules

Revised

Essential idea: Most biochemical processes are stereospecific and involve only molecules with certain configuration of chiral carbon atoms.

Stereochemistry in biomolecules

Revised

- With one exception, amino acids are chiral, and only the L-configuration is found in proteins.
- Naturally occurring unsaturated fat is mostly in the *cis* form, but food processing can convert it into the *trans* form.
- D and L stereoisomers of sugars refer to the configuration of the chiral carbon atom furthest from the aldehyde or ketone group, and D forms occur most frequently in nature.
- Ring forms of sugars have isomers, known as α and β, depending on whether the position of the hydroxyl group at carbon 1 (glucose) or carbon 2 (fructose) lies below the plane of the ring (α) or above the plane of the ring (β).
- Vision chemistry involves the light-activated interconversion of *cis*- and *trans*-isomers of retinal.

Chirality

Revised

Enantiomers are a pair of molecules related as non-superimposable images ('mirror images'). Molecules with sp^3 hybridized carbon atoms bonded to four different atoms/ groups show chirality due to their tetrahedral shape. Figure 23.94 shows the enantiomers ('optical isomers') of the amino acid, alanine. All amino acids can exist as a pair of enantiomers except glycine (2-amino ethanoic acid).

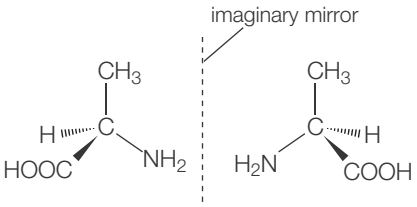


Figure 23.94 Enantiomers of alanine

Fischer projections

Fischer projections (Figure 23.95) attempt to show three-dimensional structure using a two-dimensional framework of vertical and horizontal bonds. The main carbon chain is drawn as a vertical line, and bonds to all substituents (atoms or groups of atoms) are drawn as horizontal lines. All vertical lines represent bonds behind the plane of the page, and horizontal lines bonds coming out of the plane towards the viewer.

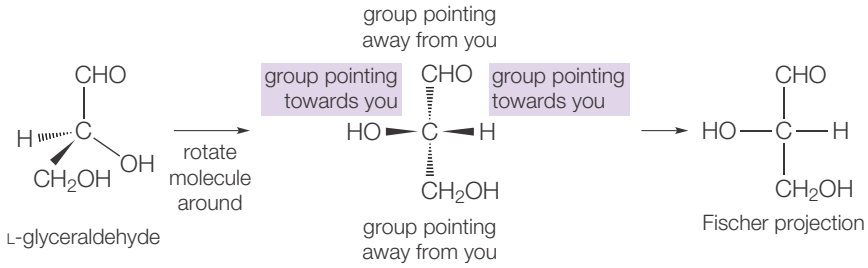


Figure 23.95 Deriving a Fischer projection for glyceraldehyde, starting from a tetrahedral model and moving to a line drawing

When shown in Fischer projection, if the –OH group on the highest numbered chiral carbon is on the right, the molecule is assigned the label D; if the –OH group is on the left, it is given the label L. These notations are known as absolute configurations and often used to describe amino acids and monosaccharides.

Expert tip

The amino acids that make up the majority of proteins exist in the L configuration; most naturally monosaccharides exist in the D configuration.

Stereoisomerism in monosaccharides

Fischer projections are a method of representing the structure of any straight-chain forms of a sugar by projection on to a plane. In a Fischer projection, the sugar molecule is shown with the carbon numbered 1 at the top – according to the normal naming rules the aldehyde/ketone group will be given the lowest possible number (Figure 23.96).

In sugars, such as ribose or glucose (see Figure 23.97), the tetrahedral centres are simply stacked on top of each other, with the aldehyde group at the top.

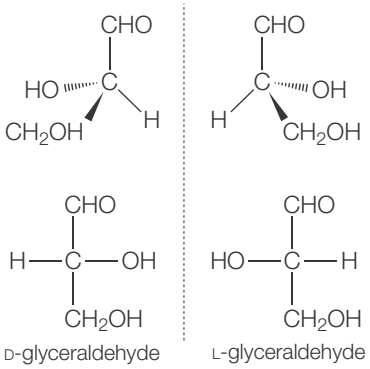


Figure 23.96 The structures of D- and L-glyceraldehyde (a C_3 monosaccharide), showing the Fischer projections

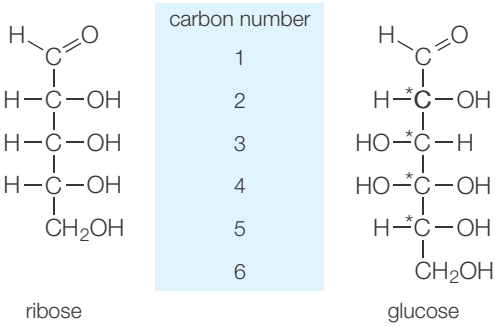


Figure 23.97 Open-chain structures of ribose and glucose. These structures are drawn as Fischer projections, which enables stereoisomers to be compared easily

Cyclic forms of monosaccharides

The conversion of sugars in the straight-chain form to the ring form creates α- and β-isomers (Figure 23.96) known as anomers. These differ by the relative position of the hydroxyl substituent attached to the carbon atom derived from the aldehyde or ketone carbon in the open chain form of the sugar.

When a sugar cyclizes from the straight-chain (linear) form, an extra chiral carbon is formed (Figure 23.98). The lone pair of electrons on the oxygen atom on carbon 5 can attack the carbonyl group (>C=O) from either above the plane of the group or below. Two cyclic molecules can therefore be formed: α- and β-forms. If the –OH on the new chiral carbon (anomeric carbon) is above the ring when the structure is drawn with the ring oxygen at the rear then it has β configuration (the –OH is on the same side of the ring as carbon-6), and if it is beneath then it is α.

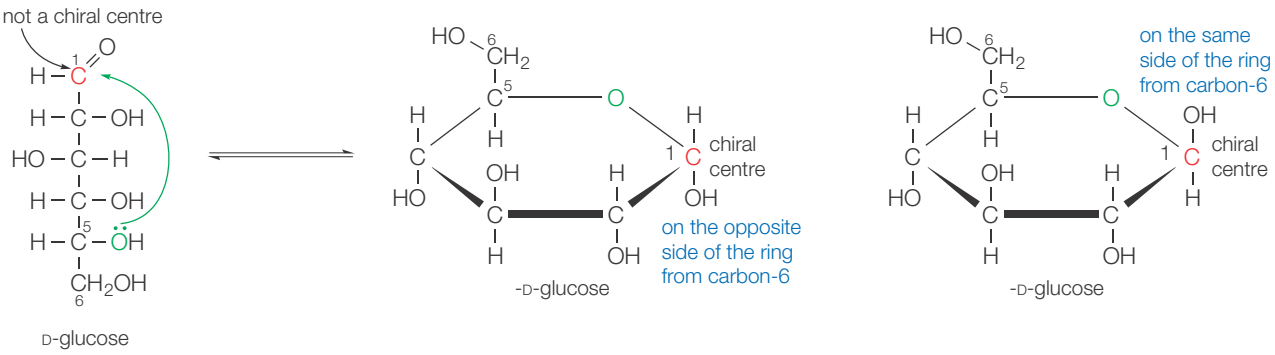
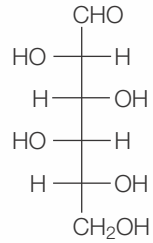


Figure 23.98 The structures of glucose in aqueous solution; the formation of the cyclic (ring) structures

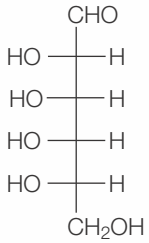
QUICK CHECK QUESTIONS

49 Examine the structures of monosaccharides **A**, **B** and **C** and determine whether each of the sugars shown below is the D-form or the L-form:

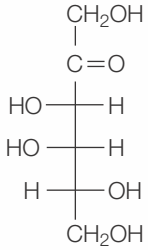
A



B

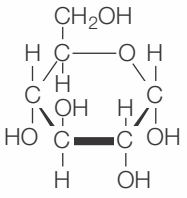


C

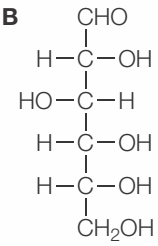


50 Glucose is an example of an aldohexose sugar which dissolves in water. In aqueous solution, glucose exists in three different structures which are in equilibrium with each other. The three structures are shown below:

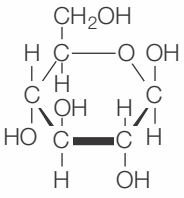
A



B



C



a What chemical term is used to describe the different forms shown above?

b Explain why glucose is very soluble in water.

c Which of the two cyclic forms of glucose can be described as α-glucose?

Vision chemistry

The visual cycle (Figure 23.99) is a process by which light (photons) are converted into an electrochemical signal in the cells of the retina lining the eye. Rhodopsin consists of the protein opsin and a covalent bonded co-factor retinal, which is synthesized from vitamin A. Retinal acts as the chromophore.

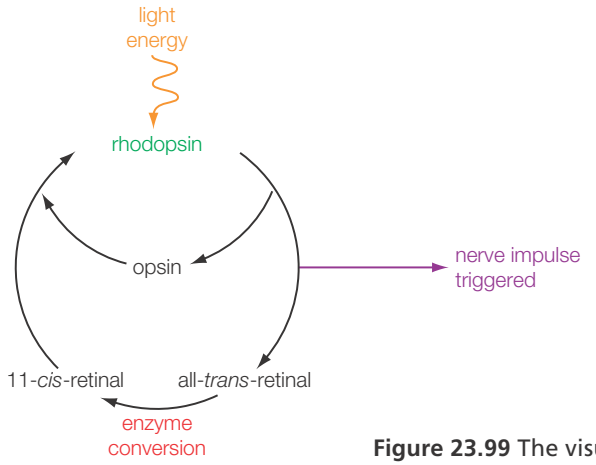
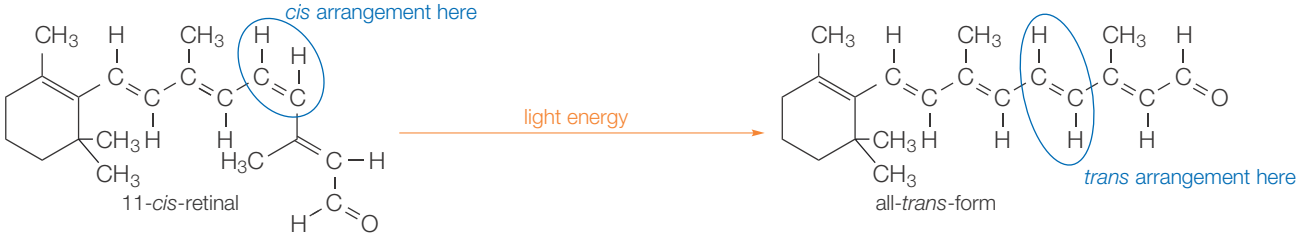


Figure 23.99 The visual cycle

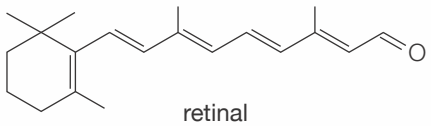
The absorption of light converts the *cis* form of retinal (11-*cis*-retinal) to the *trans* form (all *trans*-retinal) (Figure 23.100). This causes the all-*trans*-isomer to dissociate from the opsin, which ultimately causes a nerve impulse to the brain. Rhodopsin is regenerated from opsin and 11-*cis*-retinal after the all-*trans* form has isomerized back to the 11-*cis* form in a series of steps catalysed by enzymes.

Figure 23.100 The light-induced transformation of 11-*cis*-retinal to all-*trans*-retinal



■ QUICK CHECK QUESTIONS

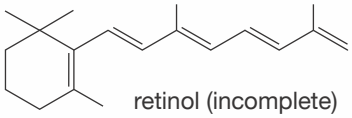
51 a The rod cells in the retina at the back of the eye contain a primary alcohol called retinol which is responsible for their sensitivity to visible light. Retinol is oxidized by an enzyme-catalysed reaction to the aldehyde retinal.



retinal

i Deduce the molecular formula of retinal from its skeletal formula shown above.

ii Suggest the structure of the alcohol retinol by completing the skeletal formula below.



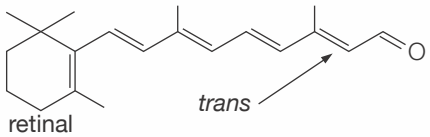
retinol (incomplete)

iii Identify a functional group which is present in both the retinol and retinal molecules.

iv Outline the reagents and conditions could be used to convert an alcohol to an aldehyde in a laboratory.

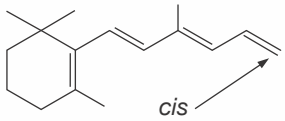
v Deduce how many moles of hydrogen molecules you would expect to react with one mole of retinol (in the presence of a heated nickel catalyst).

b When light enters the rod cells of the eye, an enzyme-catalysed reaction occurs. This changes the arrangement around the double bond at position 11 from *trans* to *cis*, as indicated in the following structure.



retinal

i Suggest the structure of *cis*-retinal by completing the skeletal formula below.



cis-retinal (incomplete)

ii The *cis*-retinal bonds covalently to the protein opsin to form rhodopsin. Part of the mechanism of this reaction is shown below. State the name of the functional group on opsin which is reacting with the aldehyde group on the *cis*-retinal molecule.

iii State the type of reaction mechanism which starts in step 1 and is completed in step 2.

iv Deduce a structure for compound **X** that would be drawn in the box below.