Introduction to biochemistry

METABOLISM

The chemical reactions that occur in living organisms are collectively known as metabolism. Essentially these enzyme-catalysed reactions allow organisms to grow and reproduce, maintain their structures and respond to their environment. Reactions in which organic matter is broken down to produce energy involve the process of *catabolism* whereas reactions which use energy to synthesize larger molecules such as proteins and nucleic acids are known as *anabolism*.

The chemical reactions involved in metabolism are organized into metabolic pathways. Each step is catalysed by an enzyme and normally occurs in a controlled aqueous environment. Biological molecules are diverse in nature and their functions depend upon their precise structure and shape.

Typical examples of biological molecules include:

Proteins – made from linking amino acids by peptide bonds.

Lipids – these include fats and steroids.

Carbohydrates – they have the general formula $C_x(H_2O)_y$ and include monosaccharides (e.g. glucose and fructose) and polysaccharides (e.g. starch and cellulose).

Nucleotides – consist of a phosphate group, a ribose sugar group and a nitrogenous base. Polymers of nucleotides include DNA and RNA.

Co-enzymes – compounds such as adenosine triphosphate (ATP) that are metabolic intermediates whose function is to carry chemical groups (or energy) between different reactions.

PHOTOSYNTHESIS AND RESPIRATION

Photosynthesis is the process used by plants and other organisms to synthesize energy-rich molecules such as carbohydrates from carbon dioxide and water using light energy. During photosynthesis oxygen is released. This is the origin of the oxygen in the atmosphere and photosynthesis is responsible for maintaining atmospheric oxygen levels. Essentially photosynthesis is the process of converting carbon into biomass. The process is complicated and needs the presence of chlorophyll but can be simplified by the overall equation:

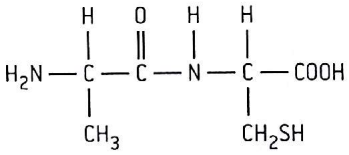
$$6CO_2(g) + 6H_2O(l) \xrightarrow{\text{light}} C_6H_{12}O_6(aq) + 6O_2(g) \quad \Delta H \text{ positive}$$

Respiration is also a complex set of reactions in which energy-rich molecules such as carbohydrates are broken down in the presence of oxygen to provide energy for cells. Ultimately the products of respiration are carbon dioxide and water, which are released into the atmosphere. The overall equation is the reverse of photosynthesis.

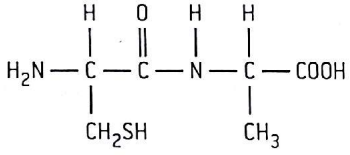
$$C_6H_{12}O_6(aq) + 6O_2(g) \rightarrow 6CO_2(g) + 6H_2O(l) \quad \Delta H \text{ negative}$$

CONDENSATION AND HYDROLYSIS REACTIONS

Biological polymers (biopolymers) are formed by condensation reactions. These involve the reaction between two smaller molecules to form one larger molecule with the evolution of a small molecule such as water. For condensation polymerization to occur each reacting molecule must possess at least two reactive functional groups. Classic examples include the condensation of amino acids to form proteins and the condensation of sugars to form starch. 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 if each dipeptide contains one of each of the two acid residues.



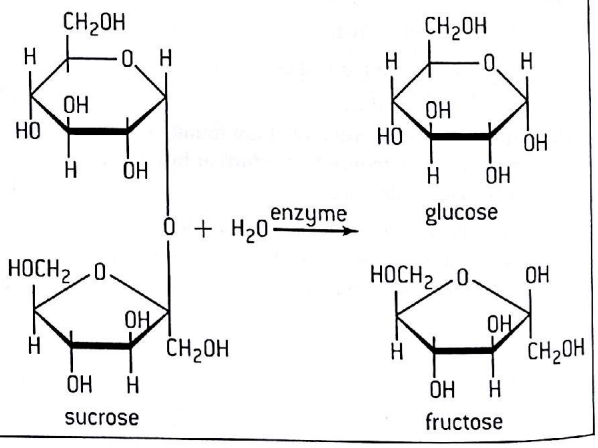
H_2N-Ala-Cys-COOH



H_2N-Cys-Ala-COOH

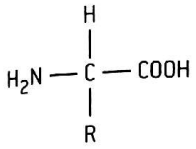
Each end of the dipeptides contains a reactive group so can undergo further condensation reactions with more amino acids to produce polypeptides.

Hydrolysis is the reverse of condensation. A molecule is hydrolysed when a water molecule (often in the presence of acid or alkali) reacts with a larger molecule to break a bond and form two smaller molecules. The hydrolysis of proteins produces amino acids and the hydrolysis of starch (polysaccharide) produces sugars (monosaccharides). For example, sucrose, a disaccharide, can be hydrolysed to form glucose and fructose.



Structure of proteins

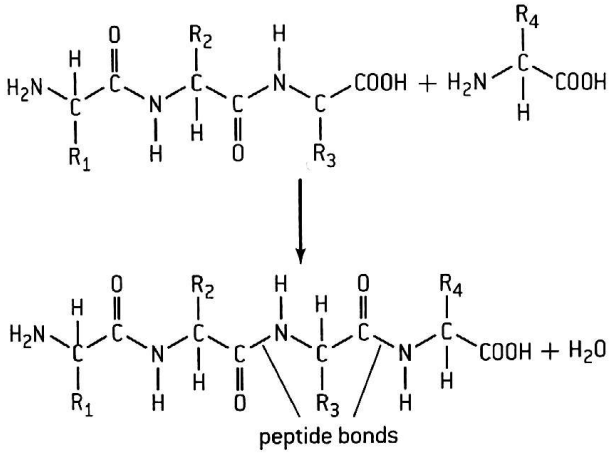
AMINO ACIDS AND THE STRUCTURE OF PROTEINS



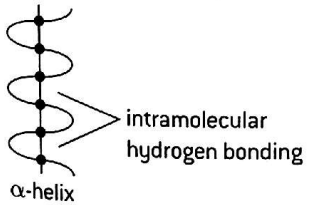
2-amino acid

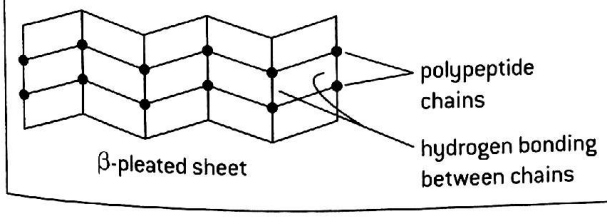
Amino acids contain both an amine functional group and a carboxylic acid functional group. When both are attached to the same carbon atom they are known as 2-amino acids (or α-amino acids). They are solids at room temperature and have quite high melting points. This is because they can exist as zwitterions in which the hydrogen atom from the carboxylic acid group protonates the amine group to form a carboxylate anion and a substituted ammonium cation within the same compound (see page 126).

Proteins are large macromolecules made up of chains of 2-amino acids. There are about twenty 2-amino acids that occur naturally. A full list of the 2-amino acids together with their common names, abbreviations and structural formulas is given in Section 33 of the IB data booklet. The amino acids bond to each other through condensation reactions resulting in the formation of a polypeptide, in which the amino acid residues are bonded to each other by a carboxamide link (peptide bond).



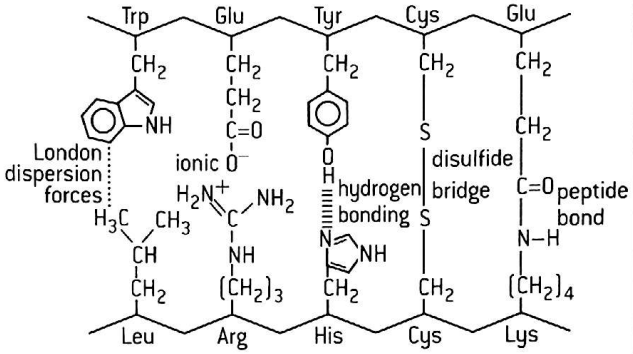
Each protein contains a fixed number of amino acid residues connected to each other in strict sequence. This sequence, e.g. gly-his-ala-ala-leu- ... is known as the primary structure of proteins. The secondary structure describes the way in which the chain of amino acids folds itself due to intramolecular hydrogen bonding. The folding can either be α-helix in which the protein twists in a spiralling manner rather like a coiled spring, or β-pleated to give a sheet-like structure.



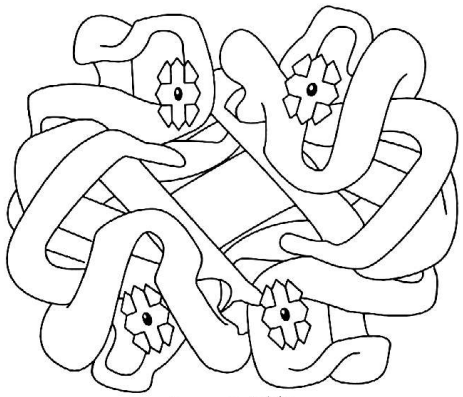


The tertiary structure describes the overall folding of the chains by interactions between distant amino acids to give the protein its three-dimensional shape. These interactions may be due to hydrogen bonds, London dispersion forces between non-polar side groups, and ionic attractions between polar groups. In addition two cysteine residues can form **disulfide bridges** when their sulfur atoms undergo oxidation.

Examples of interactions between side groups on polypeptide chains:



Separate polypeptide chains can interact together to give a more complex structure – this is known as the quaternary structure. Haemoglobin has a quaternary structure that includes four protein chains (two α-chains and two β-chains) grouped together around four haem groups.



haemoglobin

Fibrous and globular proteins

Haemoglobin is an example of a globular protein. Globular proteins have complex tertiary and sometimes quaternary structures (e.g. haemoglobin) folded into spherical (globular) shapes. They are usually soluble to some extent in water as the hydrophobic side chains tend to be in the centre of the structure. Fibrous proteins, such as collagen, have little or no tertiary structure and form long parallel polypeptide chains. Fibrous proteins have cross-linking at intervals to form long fibres or sheets and have mainly structural roles such as keratin in hair and collagen, which is found in skin and the walls of blood vessels and acts as connective tissue.

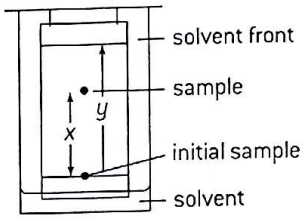
Analysis of proteins

The primary structure of proteins can be determined either by paper chromatography or by electrophoresis. In both cases the protein must first be hydrolysed by hydrochloric acid and heat to successively release the amino acids. The three-dimensional structure of the complete protein can be confirmed by X-ray crystallography.

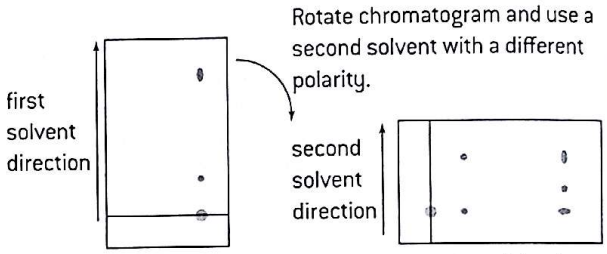
PAPER CHROMATOGRAPHY

A small spot of the unknown amino acid sample is placed near the bottom of a piece of chromatographic paper. Separate spots of known amino acids can be placed alongside. The paper is placed in a solvent (eluent), which then rises up the paper due to capillary action. As it meets the sample spots the different amino acids partition themselves between the eluent and the paper to different extents, and so move up the paper at different rates. When the eluent has nearly reached the top, the paper is removed from the tank, dried, and then sprayed with an organic dye (ninhydrin) to develop the chromatogram by colouring the acids. The positions of all the spots can then be compared.

If samples of known amino acids are not available the R_f value (retention factor) can be measured and compared with known values as each amino acid has a different R_f value. It is possible that two acids will have the same R_f value using the same solvent, but different values using a different solvent. If this is the case the chromatogram can be turned through 90° and run again using a second solvent.



$$R_f \text{ value} = \frac{\text{distance travelled by sample}}{\text{distance travelled by solvent}} = \frac{x}{y}$$



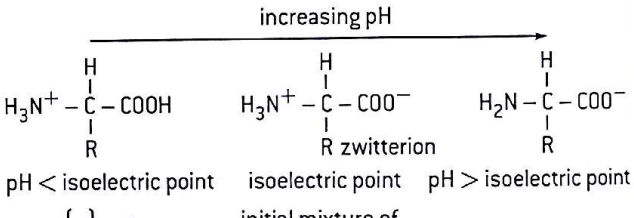
Three-component mixture split into two by first solvent.

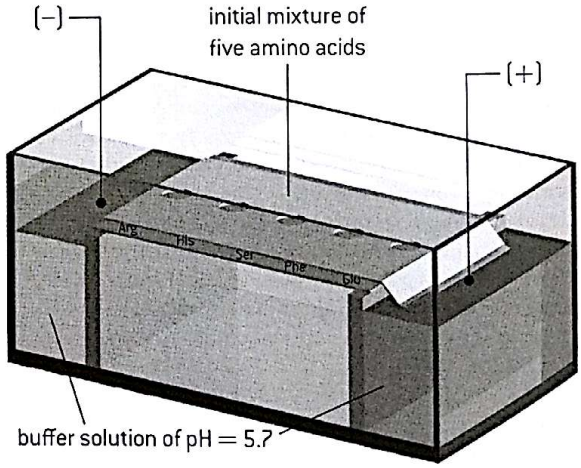
Separation of the three components is achieved.

ELECTROPHORESIS

The structure of amino acids alters at different pH values. At low pH (acid medium) the amine group will be protonated. At high pH (alkaline medium) the carboxylic acid group will lose a proton. This explains why amino acids can function as buffers. If H^+ ions are added they are removed as $-NH_4^+$ and if OH^- ions are added the $-COOH$ loses a proton to remove the OH^- ions as water. For each amino acid there is a unique pH value (known as the **isoelectric point**) where the acid will exist as the zwitterion.

The medium on which electrophoresis is carried out is usually a polyacrylamide gel. So the process is known as PAGE (polyacrylamide gel electrophoresis). The sample is placed in the centre of the gel and a potential difference applied across it. Depending on the pH of the buffer the different amino acids will move at different rates towards the positive and negative electrodes. At its isoelectric point a particular amino acid will not move as its charges are balanced. When separation is complete the acids can be sprayed with ninhydrin and identified by comparing the distance they have travelled with standard samples, or from a comparison of their isoelectric points.





2-amino acid	pH of isoelectric point
glutamic acid (Glu)	3.2
phenylalanine (Phe)	5.5
serine (Ser)	5.7
histidine (His)	7.6
arginine (Arg)	10.8

Separation of a mixture of five amino acids by electrophoresis
Serine does not move as its isoelectric point is the same pH as the buffer. Histidine and arginine contain $-NH_3^+$ at pH 5.7, so move towards the negative electrode. Glutamic acid and phenylalanine contain $-COO^-$ at pH 5.7, so move towards the positive electrode.

Enzymes

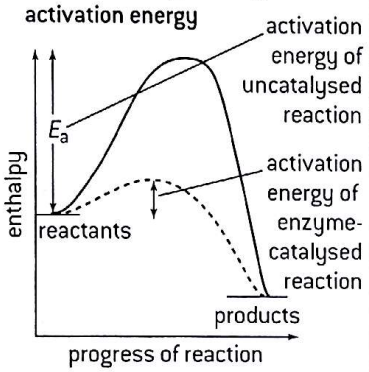
USES OF PROTEINS

Proteins have many different functions in the body. They can act as biological catalysts for specific reactions (enzymes). They can give structure (e.g hair and nails consist almost entirely of polypeptides coiled into α-helices), and provide a source of energy. Some hormones are proteins, e.g. FSH (follicle stimulating hormone), responsible for triggering the monthly cycle in females.

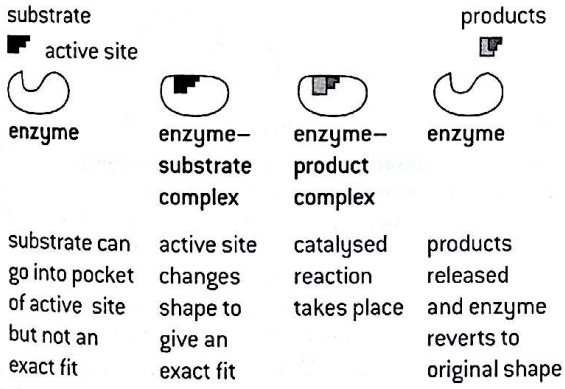
CATALYTIC ACTIVITY OF ENZYMES AND ACTIVE SITE

Enzymes are protein molecules that catalyse biological reactions. Each enzyme is highly specific for a particular reaction, and extremely efficient, often being able to increase the rate of reaction by a factor greater than 10^8. Enzymes work by providing an alternative pathway for the reaction with a lower activation energy, so that more of the reactant particles (substrate) will possess the necessary minimum activation energy.

Effect of adding an enzyme on activation energy



The specificity of enzymes depends on their tertiary and quaternary structure. The part of an enzyme that reacts with the substrate is known as the active site. This is a groove or pocket in the enzyme where the substrate will bind. The site is not necessarily rigid but can alter its shape to allow for a better fit – known as the induced fit theory.



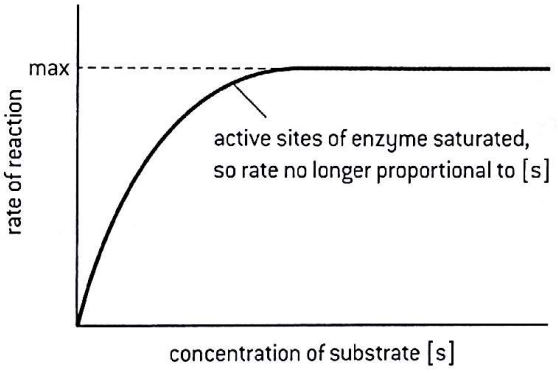
Induced fit theory of enzyme catalysis

The induced fit theory replaces the old 'lock and key' theory which assumes that enzymes have a fixed shape into which the substrate fits.

ENZYME KINETICS

At low substrate concentrations the rate of the enzyme-catalysed reaction is proportional to the concentration of the substrate. However at higher concentrations the rate reaches a maximum. This can be explained in terms of enzyme saturation. At low substrate concentrations there are enough active sites present for the substrate to bind and react. Once all the sites are used up the enzyme can no longer work any faster.

Effect of concentration of substrate on rate of enzyme-catalysed reaction

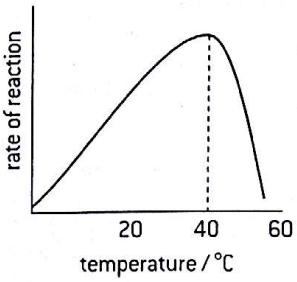


EFFECT OF TEMPERATURE, PH AND HEAVY METAL IONS ON ENZYME ACTIVITY

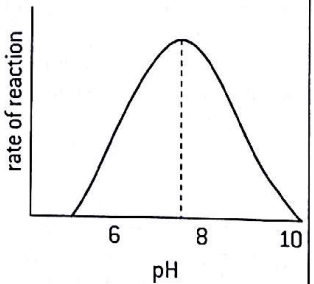
The action of an enzyme depends on its specific shape. Increasing the temperature will initially increase the rate of enzyme-catalysed reactions, as more of the reactants will possess the minimum activation energy. The optimum temperature for most enzymes is about 40°C. Above this temperature enzymes rapidly become denatured as the weak bonds holding the tertiary structure together break.

At different pH values the charges on the amino acid residues change affecting the bonds between them, and so altering the tertiary structure and making the enzyme ineffective. Heavy metals can poison enzymes by reacting with –SH groups replacing the hydrogen atom with a heavy metal atom or ion so that the tertiary structure is altered.

Effect of temperature on enzyme activity



Effect of pH on enzyme activity

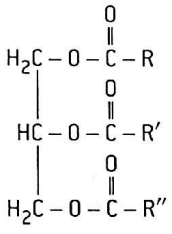


Lipids (1)

Lipids are organic molecules with long hydrocarbon chains that are soluble in non-polar solvents. They are mainly used for energy storage, insulating and protecting vital organs, forming cell membranes and, in some cases, acting as hormones. Three important types of lipids are triglycerides (fats and oils), phospholipids (lecithin) and steroids (cholesterol).

FATS AND OILS

Fats and oils are triesters (triglycerides) formed from the condensation reaction of propane-1,2,3-triol (glycerol) with long chain carboxylic acids (fatty acids).



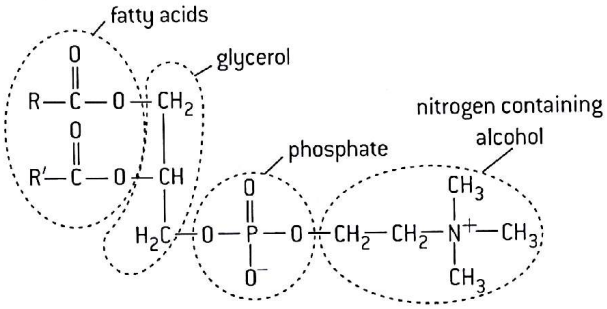
R, R′, and R″ are long chain hydrocarbons formed from carboxylic acids which may be the same or different.

General formula of a fat or oil.

Fats are solid triglycerides; examples include butter, lard and tallow. Oils are liquid at room temperature and include castor oil, olive oil and linseed oil. The essential chemical difference between them is that fats contain saturated carboxylic acid groups (i.e. they do not contain C=C double bonds). Oils contain at least one C=C double bond and are said to be unsaturated. Most oils contain several C=C double bonds and are known as polyunsaturated.

PHOSPHOLIPIDS

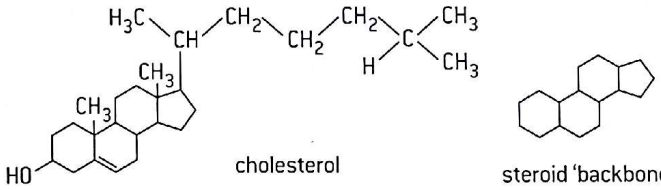
Phospholipids form an integral part of all cell membranes. They are essentially made of four components. A backbone such as propane-1,2,3-triol (glycerol), linked by esterification to two fatty acids and a phosphate group which is itself condensed to a nitrogen-containing alcohol. There are many different phospholipids. They can be exemplified by phosphatidyl choline – the major component of lecithin, present in egg yolk.



The structure of phosphatidyl choline showing the origins of the four distinct components.

CHOLESTEROL

Cholesterol has the characteristic four-ring structure possessed by all steroids.

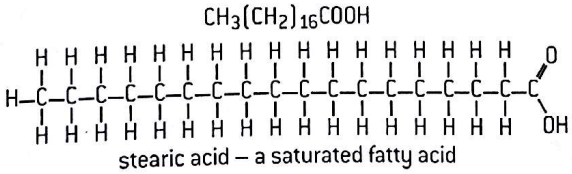


It is transported around the body by lipoproteins. **Low density lipoproteins** (LDL) are in the order of 18–25 nm and transport cholesterol to the arteries where it can line the walls of the arteries leading to cardiovascular diseases. The major source of these low density lipoproteins are saturated fats, in particular those derived from lauric (C_{12}), myristic (C_{14}) and palmitic (C_{16}) acids. Smaller lipoproteins, in the order of 8–11 nm, known as **high density lipoproteins** (HDL) can remove the cholesterol from the arteries and transport it back to the liver.

FATTY ACIDS

Stearic acid (m.pt 69.6 °C) and linoleic acid (m.pt −5.0 °C) both contain the same number of carbon atoms and have similar molar masses. However, linoleic acid contains two double bonds. Generally the more unsaturated the fatty acid the lower its melting point.

The regular tetrahedral arrangement of saturated fatty acids means that they can pack together closely, so the London dispersion forces holding molecules together are stronger as the surface area between them is greater. As the bond angle at the C=C double bonds changes from 109.5° to 120° in unsaturated acids it produces a 'kink' in the chain. They are unable to pack so closely and the London dispersion forces between the molecules become weaker, which results in lower melting points. This packing arrangement is similar in fats and explains why unsaturated fats (oils) have lower melting points.



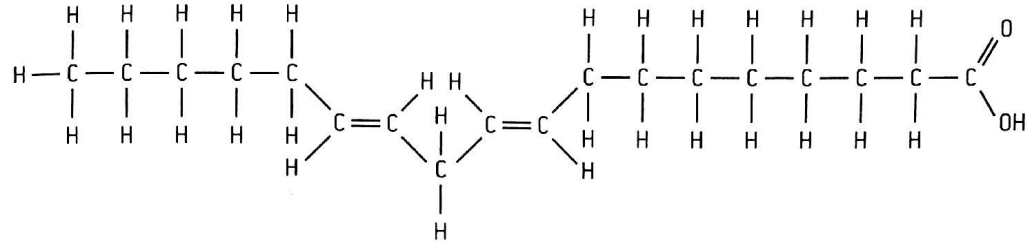
stearic acid – a saturated fatty acid

Name		Number of C atoms per molecule	Number of C=C 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
unsaturated fatty acids				
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

Lipids (2)

HYDROGENATION

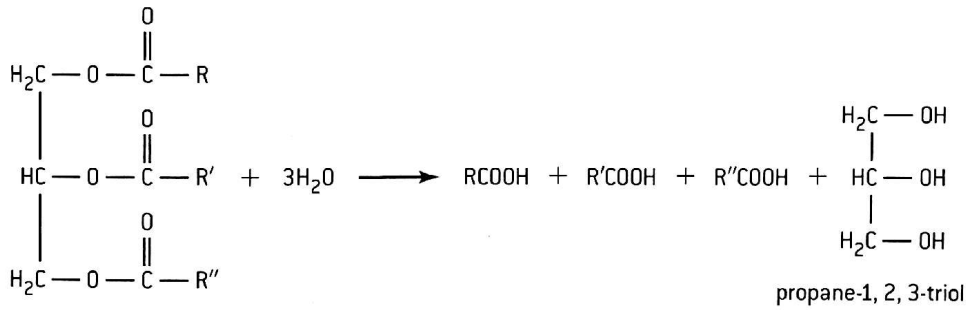
Oils naturally contain only cis-unsaturated fatty acids. These are generally healthier than saturated fats as they increase HDL cholesterol. Unsaturated fats can be hydrogenated to saturated fats with a higher melting point by adding hydrogen under pressure in the presence of a heated nickel catalyst. However during the hydrogenation process, partial hydrogenation can occur and the trans-isomers may be formed. Trans-unsaturated fatty acids are present in fried foods such as French fries and some margarines. Unlike natural mono- and poly-unsaturated oils, trans-unsaturated fats increase the formation of LDL cholesterol ('bad' cholesterol) and thus increase the risk of heart disease.



the structure of the trans, trans- form of linoleic acid

HYDROLYSIS AND RANCIDITY OF FATS

Fats and oils are hydrolysed in the body by enzymes, known as lipases, to glycerol and fatty acids. These in turn are broken down by a series of redox reactions to produce ultimately carbon dioxide, water and energy. Because they are essentially long-chain hydrocarbons with only two oxygen atoms each on the three carboxyl atoms fats are in a less oxidized form than carbohydrates so weight for weight produce more energy.



hydrolysis of a fat

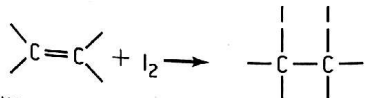
propane-1, 2, 3-triol (glycerol)

Lipids (fats and oils) in food become rancid when our senses perceive them to have 'gone off' due to a disagreeable smell, texture or appearance. This may be caused either by hydrolysis of the triesters (hydrolytic rancidity) as shown above to produce disagreeable smelling fatty acids or by oxidation of the fatty acid chains.

Oxidative rancidity is typically due to the addition of oxygen across the C=C double bonds in unsaturated fatty acids. Oily fishes, such as mackerel, contain a high proportion of unsaturated fatty acids and are prone to oxidative rancidity. The process proceeds by a free radical mechanism catalysed by light in the presence of enzymes.

IODINE NUMBER

Unsaturated fats can undergo addition reactions. The addition of iodine to unsaturated fats can be used to determine the number of C=C double bonds, since one mole of iodine will react quantitatively with one mole of C=C double bonds. Iodine is coloured. As the iodine is added to the unsaturated fat the purple colour of the iodine will disappear as the addition reaction takes place. Once the colour remains the amount of iodine needed to react with all the C=C double bonds can be determined. Often fats are described by their iodine number, which is the number of grams of iodine that add to 100 g of the fat.



unsaturated fat

diiodo-addition product

THE ROLES OF LIPIDS IN THE BODY

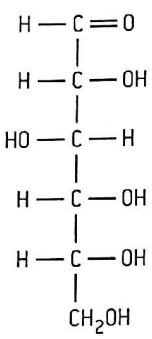
- Energy storage. Because they contain proportionally less oxygen than carbohydrates they release more energy when oxidized.
- Insulation and protection of organs. Fats are stored in adipose tissue, which provides both insulation and protection to parts of the body.
- Steroid hormones. Examples include female and male sex hormones such as progesterone and testosterone and the contraceptive pill. Sometimes steroids are abused. Anabolic steroids have similar structures to testosterone and are taken to build up muscle.
- Cell membranes. Lipids provide the structural component of cell membranes.

More controversially, lipids are thought to affect health, particularly heart disease. Although the evidence is disputed by many, some think that saturated fatty acids, particularly lauric (C_{12}), myristic (C_{14}) and palmitic (C_{16}) acids increase LDL, as do trans-unsaturated fats, causing heart problems. Conversely omega-3-polyunsaturated fatty acids such as natural unsaturated fats (e.g. olive oil) are thought to lower the level of LDL and consequently are thought to be good for you.

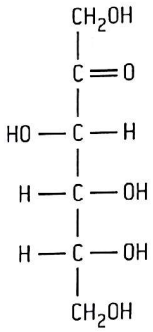
Carbohydrates

MONOSACCHARIDES

All monosaccharides have the empirical formula CH_2O. In addition they contain a carbonyl group (C=O) and at least two –OH groups. If the carbonyl group is an aldehyde (RCHO) they are known as an aldose, if the carbonyl group is a ketone (RCOR') they are known as a ketose. Monosaccharides have between three and six carbon atoms.



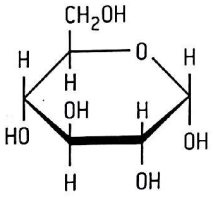
Straight chain glucose (an aldose)



Straight chain fructose (a ketose)

Monosaccharides with the general formula $C_5H_{10}O_5$ are known as pentoses (e.g. ribose) and monosaccharides with the general formula $C_6H_{12}O_6$ are known as hexoses (e.g. glucose).

Many structural isomers of monosaccharides are possible. In addition several carbon atoms are chiral (asymmetric) and give rise to optical isomerism. As well as this, open chain structures and ring structures are possible. The form of glucose that is found in nature is known as D-glucose. Note that the ring structures are cyclic ethers as they contain an oxygen atom bonded on either side by a carbon atom within the ring.



D-glucose

Six-membered ring monosaccharides are known as pyranoses. Hexoses can also have a furanose structure where they have a five-membered ring containing an oxygen atom.

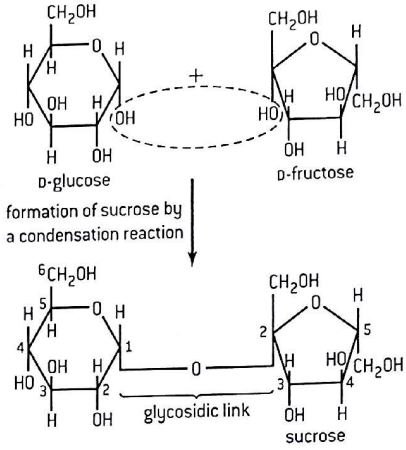
MAJOR FUNCTIONS OF POLYSACCHARIDES IN THE BODY

Carbohydrates are used by humans:

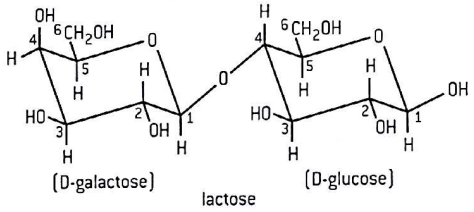
- **to provide energy:** foods such as bread, biscuits, cakes, potatoes and cereals are all high in carbohydrates.
- **to store energy:** starch is stored in the livers of animals in the form of glycogen – also known as animal starch. Glycogen has almost the same chemical structure as amylopectin.
- **as precursors** for other important biological molecules, e.g. they are components of nucleic acids and thus play an important role in the biosynthesis of proteins.
- **as dietary fibre:** dietary fibre is mainly plant material that is not hydrolysed by enzymes secreted by the human digestive tract but may be digested by microflora in the gut. Examples include cellulose, hemicellulose, lignin and pectin. It may be helpful in preventing conditions such as diverticulosis, irritable bowel syndrome, obesity, Crohn's disease, haemerrhoids and diabetes mellitus.

POLYSACCHARIDES

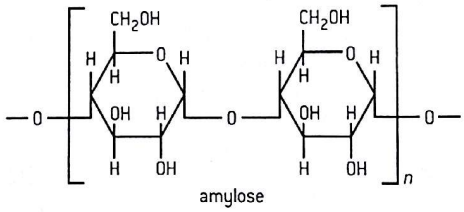
Monosaccharides can undergo condensation reactions to form disaccharides and eventually polysaccharides. For example, sucrose, a disaccharide formed from the condensation of D-glucose in the pyranose form and D-fructose in the furanose form.



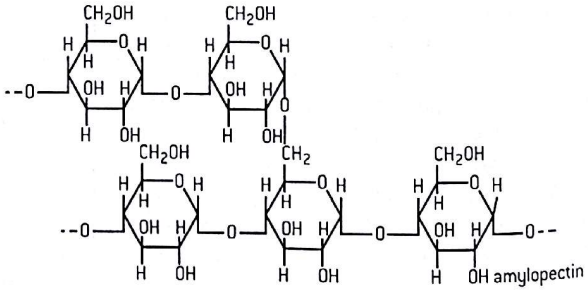
The link between the two sugars is known as a glycosidic link. In the case of sucrose the link is between the C-1 atom of glucose and the C-2 atom of fructose. The link is known as a 1,2 glycosidic bond. Maltose, another disaccharide is formed from two glucose molecules condensing to form an 1,4 glycosidic bond. Lactose is a disaccharide in which the D-galactose is linked at the C-1 atom to the C-4 atom of D-glucose to form a 1,4 glycosidic bond.



One of the most important polysaccharides is starch. Starch exists in two forms: amylose, which is water soluble, and amylopectin, which is insoluble in water. Amylose is a straight chain polymer of D-glucose units with 1,4 glycosidic bonds:



Amylopectin also consists of D-glucose units but it has a branched structure with both 1,4 and 1,6 glycosidic bonds:



Most plants use starch as a store of carbohydrates and thus energy. Cellulose, a polymer of D-glucose contains 1,4 linkages. Cellulose, together with lignin, provides the structure to the cell walls of green plants. Most animals, including all mammals, do not have the enzyme cellulase so are unable to digest cellulose or other dietary fibre polysaccharides.

Vitamins

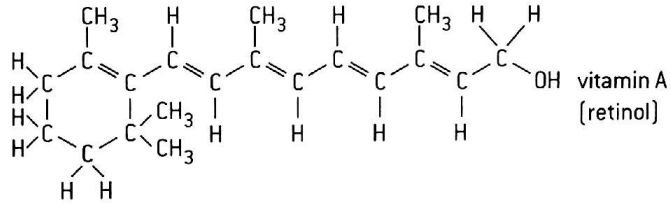
VITAMINS

Vitamins are micro-nutrients. Micro-nutrients are substances required in very small amounts (mg or μg). They mainly function as a co-factor of enzymes and include not only vitamins but also trace minerals such as Fe, Cu, F, Zn, I, Se, Mn, Mo, Cr, Co and B.

Vitamins can be classified as fat soluble or water soluble. The structure of fat soluble vitamins is characterized by long, non-polar hydrocarbon chains or rings. These include vitamins A, D, E, F and K. They can accumulate in the fatty tissues of the body. In some cases an excess of fat soluble vitamins can be as serious as a deficiency. The molecules of water soluble vitamins, such as vitamin C and the eight B-group vitamins, contain hydrogen attached directly to electronegative oxygen or nitrogen atoms that can hydrogen bond with water molecules. They do not accumulate in the body so a regular intake is required. Vitamins containing C=C double bonds and -OH groups are readily oxidized and keeping food refrigerated slows down this process.

VITAMIN A (RETINOL)

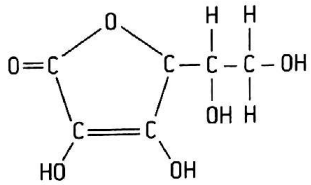
Although it does contain one -OH group, vitamin A is fat soluble due to the long non-polar hydrocarbon chain. Unlike most other vitamins it is not broken down readily by cooking. Vitamin A is an aid to night vision.



vitamin A (retinol)

VITAMIN C (ASCORBIC ACID)

Due to the large number of polar -OH groups vitamin C is soluble in water so is not retained for long by the body. The most famous disease associated with a lack of vitamin C is scorbutus ('scurvy'). The symptoms are swollen legs, rotten gums and bloody lesions. It was a common disease in sailors, who spent long periods without fresh food, until the cause was recognized.

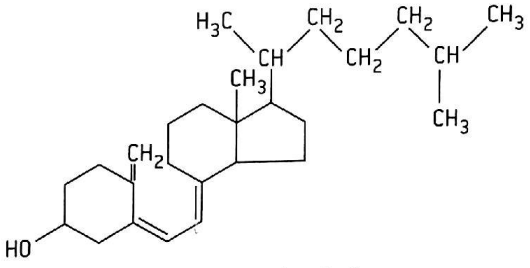


vitamin C (ascorbic acid)

VITAMIN D (CALCIFEROL)

Vitamin D is essentially a large hydrocarbon with one –OH group and is fat soluble.

A deficiency of vitamin D leads to bone softening and malformation – a condition known as rickets.



vitamin D

MALNUTRITION

Malnutrition occurs when either too much food is consumed, which leads to obesity, or the diet is lacking in one or more essential nutrients. Specific micro-nutrient deficiencies include:

- Fe – anaemia
- I – goitre
- vitamin A (retinol) – xerophthalmia, night blindness
- vitamin B_3 (niacin) – pellagra
- vitamin B_1 (thiamin) – beriberi
- vitamin C (ascorbic acid) – scurvy
- vitamin D (calciferol) – rickets.

Solutions to combat malnutrition include:

- eating fresh food rich in vitamins and minerals
- adding nutrients that are missing in commonly consumed foods
- genetic modification of food
- providing nutritional supplements.

Biochemistry and the environment

GREEN CHEMISTRY

Our increasing knowledge and use of biochemistry has led to solutions to some issues but has also caused environmental problems in other areas. Scientists have a responsibility to be aware of the impact of their research on the environment and should actively find ways to counter any negative impact their work may have on the environment. Examples of negative impact include the use of enzymes in biological detergents and the overuse of antibiotics in animal feed. Green chemistry, which is sometimes also known as sustainable chemistry, encourages the reduction and prevention of pollution at source. It does this by trying to minimize the use and formation of substances harmful to the environment. One way in which this can be achieved is to make use of atom economy (see page 6).

BIODEGRADABILITY

Although most plastics are organic in origin they are petroleum-based so cannot easily be broken down by natural organisms and cause big pollution problems. Biodegradable plastics are plastics capable of being broken down by bacteria or other organisms, ultimately to carbon dioxide and water. They are based on natural renewable polymers containing ester or glycosidic links, such as starch, that can be hydrolysed. In theory, starch-based bioplastics produced as biomass could be almost carbon neutral but there are problems such as using land that could otherwise be used for growing food and the release of the greenhouse gas methane if the plastics are decomposed anaerobically in landfill sites.

Enzymes can also be used to biodegrade pollutants. Enzymes are used to aid the breakdown and dispersal of oil spills. This reduces the effect of dispersal agents, such as 2-butoxyethanol, but does not replace them completely. The oil still needs to be broken into smaller droplets before the microbes containing the enzymes can be effective. The use of enzymes in biological detergents is also well known. This has the advantage to the environment of lowering the temperature at which clothes need to be washed so making the process more efficient, i.e. saving on energy and fossil fuels.

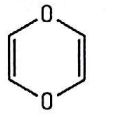
HOST–GUEST CHEMISTRY

Host–guest complexes are made of two or more molecules or ions bonded together through non-covalent bonding, which is critical in maintaining the 3-D structure of the molecule. Non-covalent interactions include hydrogen bonds, ionic bonds and van der Waals' forces. These forces, which are weaker than covalent bonding, allow large molecules to bind specifically but transiently to one another to form supramolecules. They work by mimicking some of the actions performed by enzymes by selectively binding to 'guest' species. For example, they have been used to deliver drugs more effectively in humans by increasing the solubility and availability of the drug and reducing drug resistance. They are also used to remove toxic materials (xenobiotics) from the environment. For example, radioactive 137-caesium from nuclear waste and carcinogenic amines from polluted water.

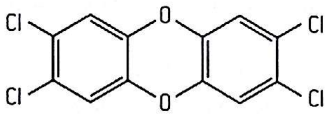
XENOBIOTICS

Chemicals found in organisms, which are not normally present or produced by the organism, or are present in organisms in abnormally high amounts, are known as xenobiotics. Examples of xenobiotics include drugs in animals. Antibiotics, for example, are not produced by animals nor are they part of a normal diet. The use of antibiotics in animal feed and in sewage plants has meant that they pass through into the human food chain and increase resistant strains of bacteria. Some xenobiotics may be natural compounds but most are pollutants. Two classic xenobiotics are dioxins and polychlorinated biphenyls (PCBs).

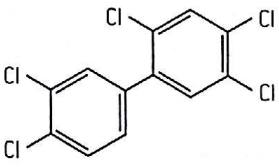
Dioxins can be formed when polymers are combusted, unless the temperature is extremely high. They do not decompose in the environment and can be passed on in the food chain. Many dioxins, particularly chlorinated dioxins, are highly carcinogenic as they can disrupt the endocrine system (hormone action) and lead to cellular and genetic damage. Examples of dioxins and dioxin-like substances include 1,4-dioxin, polychlorinated dibenzodioxins (PCCDs) and polychlorinated biphenyls (PCBs). The general formulas of PCCDs and PCBs are given in Section 31 of the IB data booklet. Some specific examples are:



1, 4-dioxin



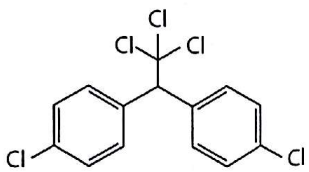
2, 3, 7, 8-tetrachlorodibenzodioxin (an example of a PCCD)



2, 3′, 4, 4′, 5-pentachlorobiphenyl (an example of a PCB)

PCBs contain from one to ten chlorine atoms attached to a biphenyl molecule. They are chemically stable and have high electrical resistance so were used in transformers and capacitors. Although not strictly dioxins (as they contain no oxygen atoms) they also persist in the environment and have carcinogenic properties.

One of the problems associated with xenobiotics is biomagnification. As the xenobiotic passes through the food chain its concentration increases in higher species. DDT (dichlorodiphenyltrichloroethane) is an effective insecticide particularly against the malaria mosquito but its use is now banned as it accumulates at high levels in birds of prey, which threatens their survival.



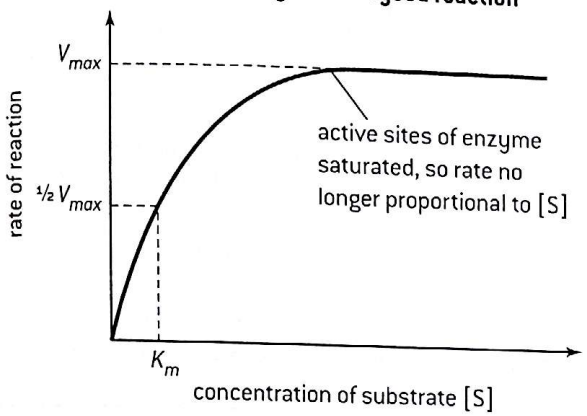
DDT dichlorodiphenyltrichlorethane

HL Proteins and enzymes (1)

V_{MAX} AND THE MICHAELIS-MENTEN CONSTANT, K_M

We have seen already that at low substrate concentrations the rate of an enzyme-catalysed reaction is proportional to the concentration of the substrate but it reaches a maximum at higher substrate concentrations. This maximum is known as V_{max}.

Effect of concentration of substrate on rate of enzyme-catalysed reaction

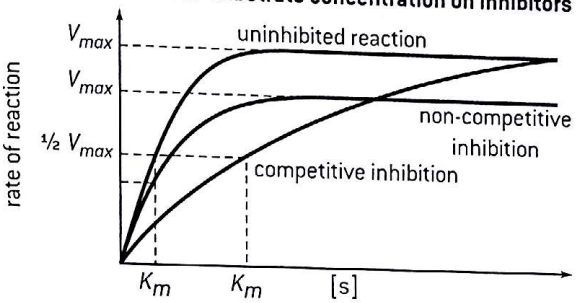


The Michaelis-Menten constant, K_m is the substrate concentration when the rate of the reaction is ½ V_{max}. A particular enzyme with the same substrate will always have the same value for K_m. It indicates whether the enzyme functions efficiently at low substrate concentrations, or whether high substrate concentrations are necessary for efficient catalysis.

COMPETITIVE AND NON-COMPETITIVE INHIBITION

Inhibitors are substances that slow down the rate of enzyme-catalysed reactions. Competitive inhibitors resemble the substrate in shape, but cannot react. They slow down the reaction because they can occupy the active site on the enzyme thus making it less accessible to the substrate. Non-competitive inhibitors also bind to the enzyme, but not on the active site. They bind at the allosteric site, which causes the enzyme to change its shape so that the substrate cannot bind. As the substrate concentration is increased the effect of competitive inhibitors lessens, as there is increased competition for the active sites by the substrate. With non-competitive inhibitors increasing the substrate concentration has no effect, as the enzyme's shape still remains altered.

Effect of substrate concentration on inhibitors



For non-competitive inhibitors, V_{max} is lower but K_m is the same.
For competitive inhibitors, V_{max} is the same but K_m is increased.

BUFFER SOLUTIONS

Enzymes only function efficiently within a narrow pH region. Outside of this region the structure is altered and the enzyme becomes denatured, hence the need for buffering. A buffer solution resists changes in pH when small amounts of acid or alkali are added to it.

An acidic buffer solution can be made by mixing a weak acid together with the salt of that acid and a strong base. An example is a solution of ethanoic acid and sodium ethanoate. The weak acid is only slightly dissociated in solution, but the salt is fully dissociated into its ions, so the concentration of ethanoate ions is high.

$$NaCH_3COO(aq) \rightarrow Na^+(aq) + CH_3COO^-(aq)$$

$$CH_3COOH(aq) \rightleftharpoons CH_3COO^-(aq) + H^+(aq)$$

If an acid is added the extra H^+ ions coming from the acid are removed as they combine with ethanoate ions to form undissociated ethanoic acid, so the concentration of H^+ ions remains unaltered.

$$CH_3COO^-(aq) + H^+(aq) \rightleftharpoons CH_3COOH(aq)$$

If an alkali is added the hydroxide ions from the alkali are removed by their reaction with the undissociated acid to form water, so again the H^+ ion concentration stays constant.

$$CH_3COOH(aq) + OH^-(aq) \rightarrow CH_3COO^-(aq) + H_2O(l)$$

In practice acidic buffers are often made by taking a solution of a strong base and adding excess weak acid to it, so that the solution contains the salt and the unreacted weak acid.

$$NaOH(aq) + CH_3COOH(aq) \rightarrow NaCH_3COO(aq) + H_2O(l) + CH_3COOH(aq)$$

limiting reagent — salt — excess weak acid

Buffer solution

An alkali buffer with a fixed pH greater than 7 can be made from a weak base together with the salt of that base with a strong acid. An example is ammonia with ammonium chloride.

$$NH_4Cl(aq) \rightarrow NH_4^+(aq) + Cl^-(aq)$$

$$NH_3(aq) + H_2O(l) \rightleftharpoons NH_4^+(aq) + OH^-(aq)$$

If H^+ ions are added they will combine with OH^- ions to form water and more of the ammonia will dissociate to replace them. If more OH^- ions are added they will combine with ammonium ions to form undissociated ammonia. In both cases the hydroxide ion concentration and the hydrogen ion concentration remain constant.

HL Proteins and enzymes (2)

BUFFER CALCULATIONS

The equilibrium expression for weak acids also applies to acidic buffer solutions,

e.g. ethanoic acid/sodium ethanoate solution.

$$K_a = \frac{[H^+] \times [CH_3COO^-]}{[CH_3COOH]}$$

The essential difference is that now the concentrations of the two ions from the acid will not be equal.

Since the sodium ethanoate is completely dissociated the concentration of the ethanoate ions in solution will be almost the same as the concentration of the sodium ethanoate, as very little will come from the acid.

If logarithms are taken and the equation is rearranged then:

$$pH = pK_a + \log_{10} \frac{[CH_3COO^-]}{[CH_3COOH]}$$

This is known as the Henderson–Hasselbalch equation (the general formula can be found in Section 1 of the IB data booklet).

Two facts can be deduced from this expression. Firstly the pH of the buffer does not change on dilution, as the concentration of the ethanoate ions and the acid will be affected equally.

Secondly the buffer will be most efficient when $[CH_3COO^-] = [CH_3COOH]$. At this point, which equates to the half equivalence point when ethanoic acid is titrated with sodium hydroxide, the pH of the solution will equal the pK_a value of the acid.

Calculate the pH of a buffer containing 0.200 mol of sodium ethanoate in 500 cm^3 of 0.100 mol dm^{-3} ethanoic acid (given that K_a for ethanoic acid = 1.8×10^{-5} mol dm^{-3}).

$[CH_3COO^-] = 0.400$ mol dm^{-3}; $[CH_3COOH] = 0.100$ mol dm^{-3}

$$K_a \approx \frac{[H^+] \times 0.400}{0.100} = 1.8 \times 10^{-5} \text{ mol dm}^{-3}$$

$[H^+] = 4.5 \times 10^{-6}$ mol dm^{-3}

pH = 5.35

Calculate what mass of sodium propanoate must be dissolved in 1.00 dm^3 of 1.00 mol dm^{-3} propanoic acid ($pK_a = 4.87$) to give a buffer solution with a pH of 4.5.

$$[C_2H_5COO^-] = \frac{K_a \times [C_2H_5COOH]}{[H^+]} = \frac{10^{-4.87} \times 1.00}{10^{-4.5}}$$

$$= 0.427 \text{ mol dm}^{-3}$$

Mass of NaC_2H_5COO required = $0.427 \times 96.07 = 41.0$ g

BLOOD

An important buffer is blood, which only functions correctly within a very narrow pH range. Blood is a complex buffering system, which is responsible for carrying oxygen around the body. One of the components of the system is that the oxygen adds on reversibly to the haemoglobin in the blood.

$$HHb + O_2 \rightleftharpoons H^+ + HbO_2^-$$

If the pH increases ($[H^+]$ falls) the equilibrium will move to the right and the oxygen will tend to be bound to the haemoglobin more tightly. If the pH decreases ($[H^+]$ increases) the oxygen will tend to be displaced from the haemoglobin. Both of these processes are potentially life threatening.

PROTEIN ASSAY BY UV-VIS SPECTROSCOPY

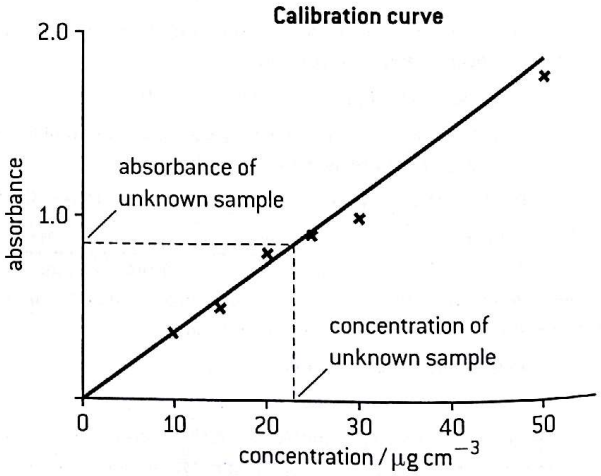
Determining the concentration of a protein in solution by UV-VIS spectroscopy depends essentially on two relationships. The first is that the protein needs to be made into a coloured compound such that the intensity of the colour depends upon the concentration of the protein in the solution. One way in which this can be done is to add a dye called Coomassie Brilliant Blue. The coloured complex with the dye absorbs light at a particular wavelength. In the case of Coomassie Brilliant Blue this wavelength is 595 nm. This can be shown by running a spectrum of the solution containing the complex and seeing that the maximum absorption (known as λ_{max}) occurs at 595 nm. The second relationship required involves the Beer–Lambert Law. This states that for dilute solutions at a fixed wavelength

$$\log_{10} \frac{I_o}{I} = \varepsilon l c$$

where: I_o is the intensity of the incident radiation and I is the intensity of the transmitted radiation.
ε is the molar absorption coefficient (a constant for each absorbing substance).
l is the path length of the absorbing solution (usually 1.0 cm) and c is the concentration.

Most spectrometers measure $\log_{10} I_o/I$ 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 λ_{max} then it is easy to see that the measured absorbance is directly proportional to the concentration. Using Coomassie Brilliant Blue the Beer–Lambert Law holds true for solutions of protein covering the range from 0 to approximately 1500 μg cm^{-3}.

To find the concentration of the solution of the protein with unknown concentration, it is therefore necessary to first obtain a calibration curve by using a range of known concentrations of protein and measuring the associated absorbance. A line of best fit is obtained and once the absorbance of the unknown sample has been measured its concentration can be determined by interpolation of the graph.



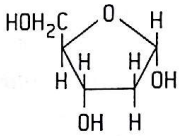
Nucleic acids

STRUCTURE OF NUCLEOTIDES AND NUCLEIC ACIDS

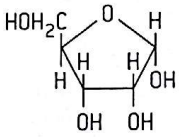
Almost all cells in the human body contain DNA (deoxyribonucleic acid). DNA and a related material RNA (ribonucleic acid) are macromolecules with relative molar masses of up to several million. Both nucleic acids are made up of repeating base-sugar-phosphate units called nucleotides. A nucleotide of DNA contains the condensation products of deoxyribose (a pentose sugar), phosphoric acid, and one of four nitrogen-containing bases, adenine (A), guanine (G), cytosine (C) or thymine (T). RNA contains a different sugar, ribose, but also contains a phosphate group and four nitrogen-containing bases. Three of the bases are the same as those in DNA but the fourth, uracil (U), replaces thymine.

In DNA, the polynucleotide units are wound into a helical shape with about 10 nucleotide units per complete turn. Two helices are then held together by hydrogen bonds between the bases to give the characteristic double helix structure. The stability of this double helix structure is due to the base-stacking interactions between the hydrophilic and hydrophobic components as well as hydrogen bonding between the nucleotides. The hydrogen bonds are very specific. Cytosine can only hydrogen bond with guanine and adenine can only hydrogen bond with thymine (uracil in RNA). Unlike DNA, RNA normally exists as single polynucleotide chains.

sugars

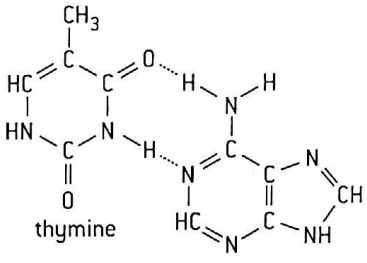


deoxyribose (used in DNA)

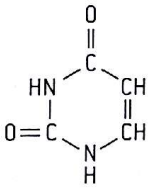


ribose (used in RNA)

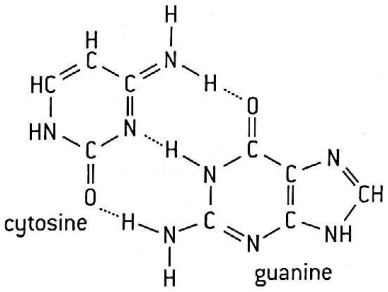
bases (showing complementary hydrogen bonding)



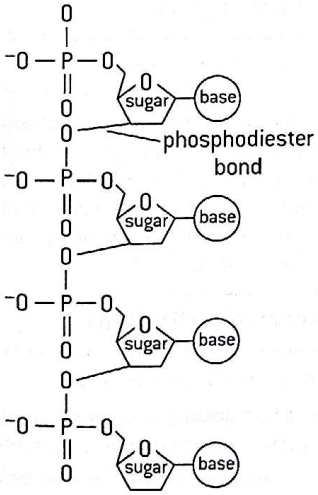
thymine adenine



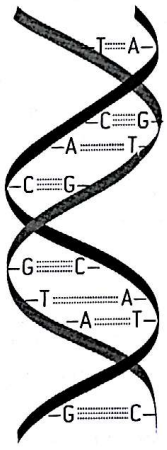
uracil (replaces thymine in RNA)



cytosine guanine



Nucleotides condense to form a polynucleotide. Each nucleotide is joined by a phosphodiester bond between C_3 of the sugar and the neighbouring phosphate group.



The double helix structure of DNA is shown here. Note the hydrogen bonds between the two different strands of polynucleotides.

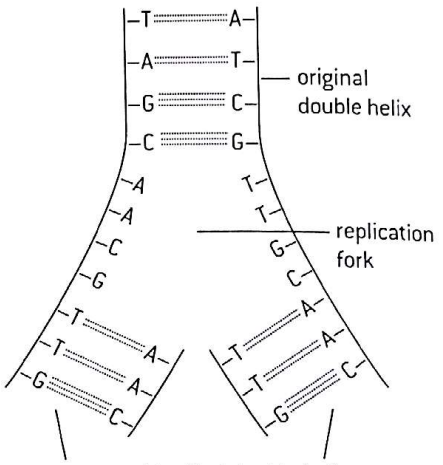
The genetic code

THE GENETIC CODE

When cells divide, the genetic information has to be replicated intact. The genetic information is stored in chromosomes found inside the nucleus. In humans there are 23 pairs of chromosomes. Chromosomes are effectively a very long DNA sequence. The DNA is compacted efficiently in the eukaryotic nucleus by forming DNA-protein complexes with histones. Histones are positively charged proteins that bond tightly to the negatively charged phosphate groups in the DNA's phosphate-sugar backbone. The DNA in the cell starts to partly unzip as hydrogen bonds between the bases break. Sugar-base units will be picked up from the aqueous solution to form a complementary new strand. Because adenosine can only hydrogen bond with thymine (A–T) and cytosine can only hydrogen bond with guanine (C–G) the new strand formed will be identical to the original. Thus if the sequence of bases in one strand is -C-G-A-T-T-A- the complementary strand will have the sequence -G-C-T-A-A-T-.

The information required to make complex proteins is passed from the DNA to messenger RNA by a similar unzipping process, known as transcription, except that the new strand of mRNA contains a different sugar and uracil in place of thymine.

The coded information held in the mRNA is then used to direct protein synthesis using a triplet code by a process known as translation. Each sequence of three bases represents one amino acid and is known as the triplet code. The triplet code allows for up to 64 permutations known as codons. This is more than sufficient to represent the 20 amino acids and several different codons may represent the same amino acid. Consecutive DNA codons of AAA, TAA, AGA, GTG, and CTT will transcribe to RNA codons of UUU, AUU, UCU, CAC, and GAA which will cause part of a strand of a protein to be formed that contains the amino acid residues –Phe-Ile-Ser-His-Glu-.



Replication of DNA

UUU	Phe	UCU	Ser	UAU	Tyr	UGU	Cys
UUC	Phe	UCC	Ser	UAC	Tyr	UGC	Cys
UUA	Leu	UCA	Ser	UAA	Terminator	UGA	Terminator
UUG	Leu	UCG	Ser	UAG	Terminator	UGG	Trp
CUU	Leu	CCU	Pro	CAU	His	CGU	Arg
CUC	Leu	CCC	Pro	CAC	His	CGC	Arg
CUA	Leu	CCA	Pro	CAA	Gln	CGA	Arg
CUG	Leu	CCG	Pro	CAG	Gln	CGG	Arg
AUU	Ile	ACU	Thr	AAU	Asn	AGU	Ser
AUC	Ile	ACC	Thr	AAC	Asn	AGC	Ser
AUA	Ile	ACA	Thr	AAA	Lys	AGA	Arg
AUG	Met	ACG	Thr	AAG	Lys	AGG	Arg
GUU	Val	GCU	Ala	GAU	Asp	GGU	Gly
GUC	Val	GCC	Ala	GAC	Asp	GGC	Gly
GUA	Val	GCA	Ala	GAA	Glu	GGA	Gly
GUG	Val	GCG	Ala	GAG	Glu	GGG	Gly

The genetic code carried by RNA

GENETICALLY MODIFIED FOODS

Genetic engineering involves the process of selecting a single gene for a single characteristic and transferring that sequence of DNA from one organism to another. Thus a genetically modified (GM) food can be defined as one derived or produced from a genetically modified organism. The GM food can be substantially different or essentially the same in composition, nutrition, taste, smell, texture and functional characteristics to the conventional food. An example of genetically modified food is the FlavrSavr tomato. In normal tomatoes, a gene is triggered when they ripen to produce a substance that makes the fruit go soft and eventually rot. In the FlavrSavr tomato the gene has been inhibited to produce a tomato with a fuller taste and a longer shelf life.

Benefits of GM foods

- With crops, it can enhance the taste, flavour, texture and nutritional value and also increase the maturation time.
- Plants can be made more resistant to disease, herbicides and insect attack.
- With animals, GM foods can increase resistance to disease, increase productivity and feed efficiency to give higher yields of milk and eggs.
- Anti-cancer substances and increased amounts of vitamins (such as vitamin A in rice) could be incorporated and exposure to less healthy fats reduced.
- Environmentally 'friendly' bio-herbicides and bio-insecticides can be formed. GM foods can lead to soil, water and energy conservation and improve natural waste management.

Potential concerns of GM foods

- The outcome of alterations is uncertain as not enough is known about how genes operate.
- They may cause disease as antibiotic-resistant genes could be passed to harmful microorganisms.
- Genetically engineered genes may escape to contaminate normal crops with unknown effects.
- They may alter the balance of delicate ecosystems as food chains become damaged.
- There are possible links to an increase in allergic reactions (particularly with those involved in food processing).

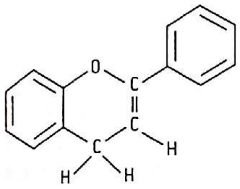
HL Biological pigments (1)

ULTRAVIOLET AND VISIBLE ABSORPTION IN ORGANIC MOLECULES

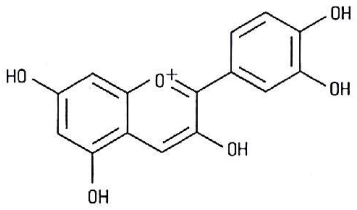
Organic compounds containing unsaturated groups such as C=C, C=O, –N=N–, $–NO_2$ and the benzene ring can absorb in the ultraviolet or visible part of the spectrum. Such groups are known as chromophores and the precise energy of absorption is affected by the other groups attached to the chromophore. The absorption is due to electrons in the bond being excited to an empty orbital of higher energy, usually an anti-bonding orbital. The energy involved in this process is relatively high and most organic compounds absorb in the ultraviolet region and thus appear colourless. For example ethene absorbs at 185 nm. However, if there is extensive conjugation of double bonds (i.e. many alternate C–C single bonds and C=C double bonds) in the molecule involving the delocalization of pi electrons then less energy is required to excite the electrons and the absorption occurs in the visible region. Biological pigments are coloured compounds produced by metabolism. Good examples include anthocyanins, carotenoids, chlorophyll and haem. One other obvious example is the pigment melanin, which is responsible for different tones of skin, eye and hair colour.

ANTHOCYANINS

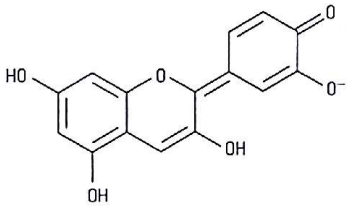
Anthocyanins are aromatic, water-soluble pigments widely distributed in plants. They contain the flavonoid $C_6C_3C_6$ skeleton.



The flavonoid $C_6C_3C_6$ backbone



Structure of cyanidin in acidic solution. Less conjugation so absorbs in blue-green region and transmits red light.



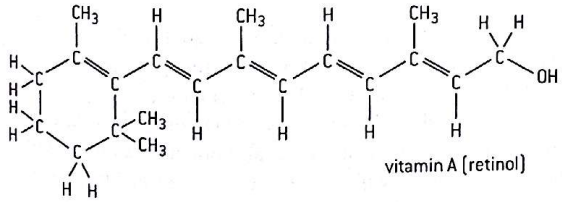
Structure of cyanidin in alkaline solution. More conjugation so absorbs in the orange region of the spectrum and transmits blue light.

It is the conjugation of the pi electrons contained in this structure that accounts for the colour of anthocyanins. The more extensive the conjugation, the lower the energy (longer the wavelength) of the light absorbed. This can be exemplified using cyanidin. In acidic solution it forms a positive ion and there is less conjugation than in alkaline solution where the pi electrons in the extra double bond between the carbon and oxygen atom are also delocalized.

This difference in colour depending on pH explains why poppies that have acidic sap are red whereas cornflowers, which also contain cyanidin but have alkaline sap, are blue. Other anthocyanins differ in the number and types of other groups such as hydroxyl or methoxy groups, which affect the precise wavelength of the light absorbed and hence the colour transmitted. Because their precise colour is so sensitive to pH changes anthocyanins can be used as indicators in acid–base titrations. The addition of other groups also affects other properties of anthocyanins. The basic flavonoid $C_6C_3C_6$ backbone is essentially non-polar. As more polar hydroxyl groups are added the potential for them to form hydrogen bonds with water molecules increases and many anthocyanins, such as cyanidin with several –OH groups, are appreciably soluble in water for this reason.

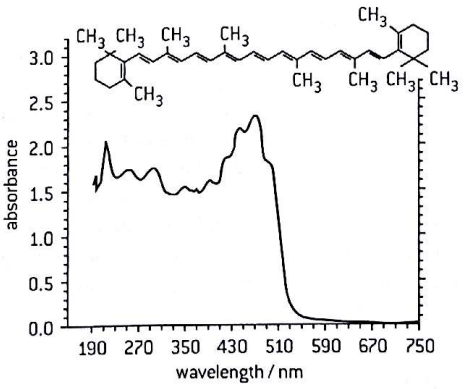
CAROTENOIDS

Carotenoids are lipid-soluble pigments, and are involved in harvesting light in photosynthesis. The conjugation in carotenoids is mainly due to a long hydrocarbon chain (as opposed to the ring system in anthocyanins) consisting of alternate single and double carbon to carbon bonds.



vitamin A (retinol)

The majority of carotenoids are derived from a (poly)ene chain containing forty carbon atoms, which may be terminated by cyclic end groups and may also be complemented with oxygen-containing functional groups. The hydrocarbon carotenoids are known as xanthophylls. Examples include α-carotene, β-carotene and vitamin A. α- and β-carotene and vitamin A are all lipid-soluble and not water-soluble. Although vitamin A does contain one polar hydroxyl group the rest of the molecule is a large non-polar hydrocarbon. Because of the unsaturation in the double bond carotenoids are susceptible to oxidation. This oxidation process can be catalysed by light, metals and hydroperoxides. It results in a change of colour, loss of activity in vitamin A and is the cause of bad smells.



β-carotene is found in carrots and has a characteristic orange colour. It contains eleven conjugated double bonds and absorbs strongly in the violet-blue (400–510 nm) region.

HL Biological pigments (2)

CHLOROPHYLL AND HAEM

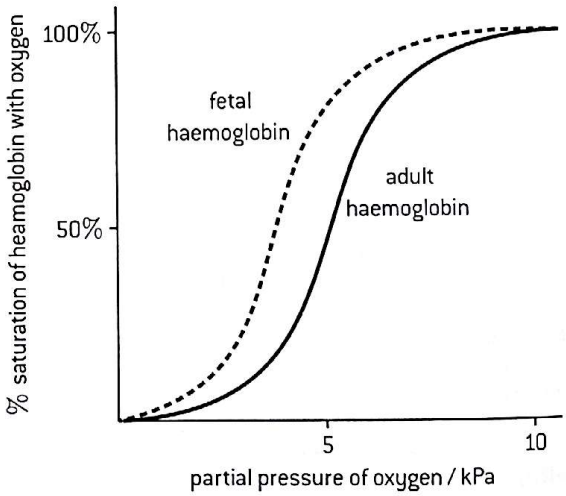
Porphyrin compounds, such as haemoglobin, myoglobin, chlorophyll and many cytochromes are chelates of metals with large nitrogen-containing macrocyclic ligands. Porphyrins contain a cyclic system in which all the carbon atoms are sp^2 hybridized. This results in a planar structure with extensive pi conjugation. The non-bonding pairs of electrons on the four nitrogen atoms enable the porphyrin to form coordinate bonds with metal ions. Chlorophyll contains a magnesium ion and its structure is given in Section 35 of the IB data booklet. It is found in two closely related forms. In chlorophyll a, the -R group is a methyl group, $-CH_3$, and in chlorophyll b the -R group is an aldehyde group, $-CHO$. Chlorophyll is essential for photosynthesis. Its function is to absorb light energy and undergo a redox reaction to donate an electron through a series of intermediates in an electron transport chain. Cytochromes in the electron transport chain contain haem groups in which the iron ion interconverts between iron(II) and iron(III) during redox reactions.

Haemoglobin, which is found in the blood, carries oxygen from the respiratory organs to the rest of the body. Like myoglobin (which is found in muscles) and some of the cytochromes, it contains haem (also spelt heme) groups with the porphyrin group bound to an iron(II) ion. When oxygen binds to one of the iron atoms in the complex to form HbO_2 it causes the iron atom to move towards the centre of the porphyrin ring and at the same time the imidazole side-chain of a histidine residue is pulled towards the porphyrin ring. This produces a strain that is transmitted to the remaining three monomers in the quaternary haemoglobin. This brings about a similar conformational change in the other haem sites so that it is easier for a second oxygen molecule to bind to a second iron atom. Each time the haem group's affinity to attract oxygen increases as the remaining sites become filled. Haem becomes fully saturated with oxygen when all four iron atoms have been utilized forming HbO_8. The binding of oxygen is, thus, a cooperative process. Various factors affect the amount of oxygen that binds. Low pH and a relatively high pressure of carbon dioxide (i.e. during exhalation) cause oxygen to be released from the haemoglobin into the tissues. Conversely at lower carbon dioxide pressure (which causes the pH to rise) more oxygen is taken up by the haemoglobin. Temperature also has an effect. At higher temperatures more oxygen is released from haemoglobin. When muscles are metabolically active they emit energy (heat) and the haemoglobin provides them with the increased oxygen required.

The partial pressure also affects the oxygen uptake. Because of the cooperative process achieved through the induced changes of the haemoglobin protein complex the oxygen binding curve of saturation of haemoglobin with oxygen against partial pressure is sigmoidal in shape compared with the normal hyperbolic curve expected if no cooperative binding takes place.

Haemoglobin exists in a slightly different form in fetal blood. It has a greater affinity for oxygen than normal haemoglobin so more oxygen is bound to the haemoglobin at lower partial pressures. This enables the fetal blood in the placenta to take up oxygen from the mother's blood.

Carbon monoxide is a dangerous poison as carbon monoxide is a stronger ligand than oxygen and forms an irreversible complex with the iron in haemoglobin. It thus acts as a competitive inhibitor and prevents the haemoglobin from binding with oxygen.



PAPER AND THIN LAYER CHROMATOGRAPHY

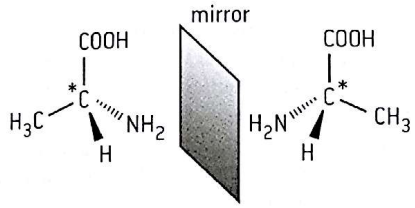
The technique of paper chromatography used to identify amino acids has already been discussed on page 126. It is ideal to use to separate and identify biological pigments by measuring R_f values as no dye or stain is needed to see the spots. In paper chromatography the stationary phase is the water contained in the cellulose fibres in the paper. Thin layer chromatography can also be used. This is similar to paper chromatography but uses a thin layer of a solid, such as alumina, Al_2O_3, or silica, SiO_2, on an inert support such as glass. When absolutely dry it works by adsorption but, like paper, silica and alumina have a high affinity for water, therefore the separation occurs more by partition with water as the stationary phase. The choice of a suitable solvent depends on the polarity or otherwise of the particular pigments. One real advantage of thin layer chromatography over paper chromatography is that each of the separated components can be recovered pure. The section containing the component is scraped off the glass and then dissolved in a suitable solvent. The solution is then filtered to remove the solid support and the solvent can then be evaporated to leave just the pure component.

Stereochemistry in biomolecules

CHIRALITY

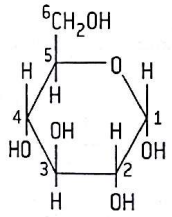
A chiral carbon atom is asymmetric, i.e. contains four different atoms or groups attached to it. Many important biological molecules are chiral and only one of the particular enantiomers is normally active in nature, although sometimes a different enantiomer may have a detrimental effect. This was the case with the drug thalidomide, which was prescribed in the 1950s and 1960s. One enantiomer alleviated the effects of morning sickness in pregnant women, the other enantiomer caused severe defects in the fetus.

All amino acids apart from glycine, $H_2N–CH_2–COOH$, exist as enantiomers but only the L configuration is found in proteins.

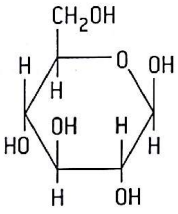


enantiomers of alanine
(* asymmetric carbon/chiral carbon)

Sugars contain several chiral carbon atoms. The D and L stereoisomers of sugars refer to the configuration of the chiral carbon atom furthest from the aldehyde or ketone group. The D forms occur most frequently in nature. The stereochemistry of sugars is further complicated by the position of the hydroxyl groups. The 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).

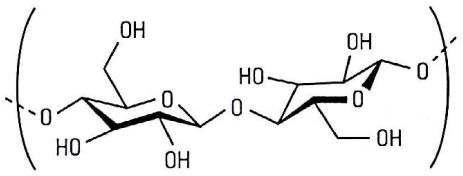


α-D-glucose



β-D-glucose

Polysaccharides formed from glucose can be very different depending upon whether the α- or β- form is involved. Starch, which can be digested by humans, is formed from polymerizing α-D-glucose. Amylose is a straight-chain polymer of α-D-glucose with α-1,4 glycosidic bonds and amylopectin is also derived from α-D-glucose with both α-1,4 and α-1,6 glycosidic bonds. Cellulose is also a polymer of glucose but it is formed from β-D-glucose with β-1,4 linkages.

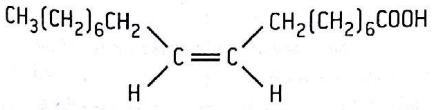


A repeating unit of cellulose showing the β-1,4-linkage

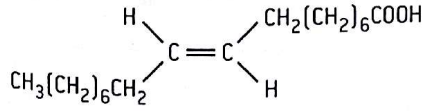
Cellulose, together with lignin, provides the structure to the cell walls of green plants. Most animals, including all mammals, do not have the enzyme cellulose so are unable to digest cellulose. Known as 'roughage', dietary fibre does play an important role in the diet as it aids digestion and makes defecation easier.

CIS- AND TRANS- ISOMERISM

Fatty acids occur naturally as the cis-isomers but as described on page 129 trans-isomers, which can increase the risk of heart disease, can be formed during the partial hydrogenation of unsaturated fats. Like saturated fatty acids, the trans- acids are straighter than their bent cis- isomers. This means that they can pack together more easily and so have higher melting points. For example elaidic acid (trans-9-octadecenoic acid) melts at 45 °C whereas oleic acid (cis-9-octadecenoic acid) melts at 13 °C.

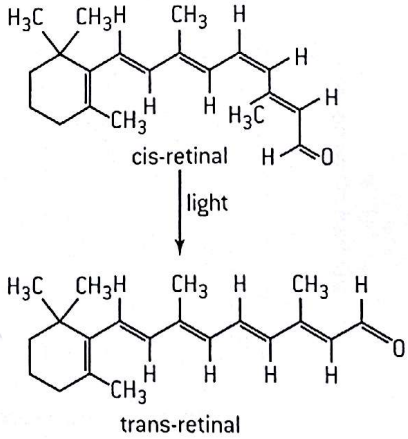


oleic acid (melting point 13°C)



elaidic acid (melting point 45°C)

The process whereby photons of light are converted into electrical signals in the retina at the back of the eye is called the visual cycle. Rhodopsin in the retina consists of a protein, opsin and a covalently bonded co-factor retinal, which is produced in the retina from vitamin A. When light falls on the retina it converts the carotenoid retinal from the cis- to the trans- form and as this happens a nerve impulse is transmitted via an interaction, which causes a conformational change in the structure of opsin to send a signal along the optic nerve to the brain.

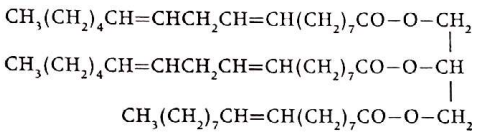


The effect of light on cis-retinal

SHORT ANSWER QUESTIONS – OPTION B – BIOCHEMISTRY

1. a) Deduce the structure of methionine in
   (i) acid solution (pH < 4)
   (ii) at the isoelectric point (pH = 5.7)
   (iii) alkaline solution (pH > 9) [3]
   b) Draw the two dipeptides that can be formed when one molecule of methionine condenses with one molecule of alanine. [2]
   c) Determine the number of different tripeptides that could be formed from methionine, alanine and cysteine if each tripeptide contains one residue from each of the three amino acids. [1]
   d) Design an experiment you could use in a school laboratory to determine whether a given protein contains a methionine residue. [4]

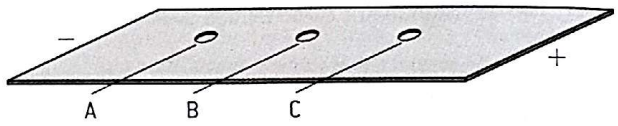
2. The diagram below shows a triacylglycerol (triglyceride).



   a) State the main dietary group this compound belongs to. [1]
   b) Deduce whether this compound is likely to have come from an animal or vegetable source. [2]
   c) Deduce whether this compound is likely to be a solid or a liquid at room temperature. [2]
   d) List two major functions of this class of compounds in the body. [2]

3. The structures of vitamin A (retinol) and vitamin C (ascorbic acid) are given in Section 35 of the IB data booklet.
   a) (i) Identify two functional groups present in retinol. [2]
   (ii) Classify vitamin A and vitamin C as water or fat soluble and justify the difference on the molecular level. [3]
   (iii) State one physical symptom for each of vitamin A and vitamin C deficiency and state the common name given to vitamin C deficiency. [3]
   b) Interpret the information that 0.014 moles of a particular oil was found to react exactly with 14.2 g of iodine. [3]

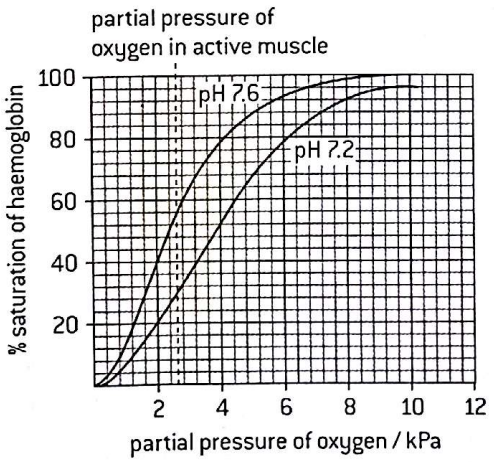
4. a) A mixture of the amino acids serine (Ser), glutamic acid (Glu) and lysine (Lys) was separated using electrophoresis and a buffer of pH 5.7. A drop containing the mixture was placed in the centre of the paper and a potential difference was applied. The amino acids were developed and the following results were obtained.



   (i) Describe how the amino acid spots may have been developed. [1]
   (ii) Predict which amino acid is present at spot C. Explain your answer. [3]
   (iii) The amino acid at spot B is at its isoelectric point. Describe one characteristic of an amino acid at its isoelectric point. [1]
   b) Explain, using equations, how the amino acid glycine (Gly) can act as a buffer. [2]

HL

5. a) Iron combines reversibly with oxygen in haemoglobin. Other than variable oxidation states, state two typical characteristics of transition metals that are shown by iron in haemoglobin. [2]
   b) The ability of haemoglobin to carry oxygen at body temperature depends on the concentration of oxygen, the concentration of carbon dioxide and on the pH. The graph shows how the percentage saturation of haemoglobin with oxygen changes with pH at different partial pressures of oxygen.



   (i) The partial pressure of oxygen in active muscle is shown by the dotted line at 2.8 kPa. Calculate the difference in the percentage saturation of haemoglobin with oxygen in active muscle when the pH changes from 7.6 to 7.2. [1]
   (ii) When the cells in muscles respire they excrete carbon dioxide and sometimes lactic acid as waste products. Explain how this affects the ability of haemoglobin to carry oxygen. [2]

6. The acid dissociation constant, K_a, for lactic acid, $CH(CH_3)(OH)COOH$, is 1.38×10^{-4} at 298 K.
   a) Explain why lactic acid can exist in two different enantiomeric forms. [1]
   b) Calculate the pH of 0.100 mol dm^{-3} lactic acid solution at 298 K. [3]
   c) Determine the mass of lactic acid that must be added together with 2.00 g of sodium hydroxide to make 500 cm^3 of a buffer solution at 298 K with a pH of 4.00. [4]