EP2.1

Investigating amines and amino acids

In this activity you will compare the properties of amines and amino acids, using butylamine as an example of an amine and glycine as an example of an amino acid.

Requirements

- Universal Indicator paper
- test-tubes (8) and rack
- butylamine (2 cm^3)
- glycine (aminoethanoic acid) (2 g)
- concentrated hydrochloric acid (a few drops)
- sodium hydroxide solution, 2 mol dm^{-3} (10 cm^3)
- hydrochloric acid, 0.01 mol dm^{-3} (1 cm^3)
- sodium hydroxide solution, 0.01 mol dm^{-3} (2 cm^3)
- copper(II) sulphate solution, 0.1 mol dm^{-3} (2 cm^3)
- ethanoyl chloride (2 cm^3)

butylamine  IRRITANT  HIGHLY FLAMMABLE

ethanoyl chloride  CORROSIVE  HIGHLY FLAMMABLE

hydrochloric acid  CORROSIVE

sodium hydroxide solution  CORROSIVE

CARE Butylamine and ethanoyl chloride are volatile and have unpleasant, highly flammable vapours. Use the bottles in a fume cupboard and avoid inhaling the vapours.

CARE Eye protection must be worn.  WEAR EYE PROTECTION

What you do

Carry out the tests in Part 1 on butylamine, an example of an amine. Then carry out a similar series of tests in Part 2 on glycine, an example of an amino acid. Before you start, read through the tests and draw up a suitable table in which to record your observations. Your table should allow you to compare the behaviour of the two compounds.

Part 1: Reactions of an amine

1 Solubility Add a few drops of butylamine (**CARE** Highly flammable; irritant vapour) to 1 cm depth of water in a test-tube.

a Is butylamine soluble in water? Explain any solubility in terms of interactions between the particles concerned.

b Record the pH of any solution which has been formed. Write an equation to explain any change to the pH of the water.

2 Adding acid and alkali Add a few drops of concentrated hydrochloric acid (**CARE** Corrosive) to the butylamine solution from test **1**. Make a note of any changes, including smell, before and after addition of the acid (**CARE** Take very great care when smelling the vapours. Just gently waft your hand over the mouth of the test-tube towards your nose. Keep your head well away from the tube. Do this experiment *very* cautiously.). Then add about 2 cm depth of 2 mol dm^{-3} sodium hydroxide solution (**CARE** Corrosive) and shake the tube gently; again, note any changes.

c Write equation(s) for any changes you have noted.

3 Reaction with copper(II) sulphate Add a few drops of butylamine to 1 cm depth of copper(II) sulphate solution in a test-tube. Make a note of any changes which occur.

d Write down the formula of any new copper-containing particles which may have been formed.

4 Adding ethanoyl chloride Place 10 drops of butylamine in a dry test-tube. Add 10 drops, *one drop at a time*, of ethanoyl chloride (**CARE** Highly flammable and corrosive. **Can react violently**).

e Make a note of the results, and write an equation for any reaction which occurs.

Add 1 cm depth of water to the tube and carefully stir the mixture. Then add 3 cm depth of 2 mol dm^{-3} sodium hydroxide solution. Warm the mixture, and hold a piece of moistened pH paper at the mouth of the test-tube.

f Explain any change to the pH paper in terms of the process occurring in the test-tube.

Part 2: Reactions of an amino acid

5 Solubility Add a few crystals of glycine to 1 cm depth of water in a test-tube.

g Is glycine soluble in water? Explain any solubility in terms of interactions between the particles concerned.
h Record the pH of any solution which has been formed.

6 Adding acid and alkali Add 1 cm depth of 0.01 mol dm^{-3} hydrochloric acid, in two separate 0.5 cm depth portions, to the glycine solution from test **5**. Then add 2 cm depth of 0.01 mol dm^{-3} sodium hydroxide in four separate 0.5 cm depth portions. Record the pH after each addition.

i Use your knowledge of the acid-base properties of an amino acid like glycine to explain how the pH of the solution behaves during the addition of acid or alkali.

7 Reaction with copper(II) sulphate Add a few crystals of glycine to 1 cm depth of copper(II) sulphate solution in a test-tube. Make a note of any changes which occur.

j Compare the behaviour of butylamine and glycine with copper(II) sulphate solution.

8 Adding ethanoyl chloride Place a few crystals of glycine into a dry test-tube, and repeat the procedure in test **4**.

k Compare the behaviour of butylamine and glycine with ethanoyl chloride.

Comparison

l Make a note of the similarities between the reactions of butylamine (an amine) and glycine (an amino acid).

m Explain any differences in the chemical properties of the two types of compound.

n Suggest why butylamine is a low-boiling liquid but glycine is a high-melting crystalline solid.

EP2.2

What's in aspartame?

Is this activity you will apply your skills at chromatography to help you investigate the conditions needed for the hydrolysis of peptide bonds. You will be looking at a simple example: aspartame, a dipeptide used as an artificial sweetener. You will be investigating what concentration of acid, and what reflux time, are needed to hydrolyse the dipeptide.

Requirements

- aspartame, eg Searle 'Canderel' tablets (1–3 tablets)
- hydrochloric acid: various concentrations, eg 4 mol dm^{-3}, 2 mol dm^{-3}, 1 mol dm^{-3} (25 cm^3 each)
- 10 cm^3 measuring cylinder
- apparatus for heating under reflux, eg 50 cm^3 pear-shaped flask and water condenser
- anti-bumping granules
- apparatus for paper chromatography
- 250 cm^3 beaker
- butan-1-ol (20 cm^3)
- glacial ethanoic acid (4 cm^3)
- separating funnel
- aspartic acid solution, 0.01 mol dm^{-3}
- phenylalanine solution, 0.01 mol dm^{-3}

} small amounts for chromatography

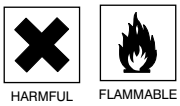
- ninhydrin in butan-1-ol, 0.5% solution in spray bottle
- mortar and pestle
- hair dryer
- protective gloves

butan-1-ol

ethanoic acid

hydrochloric acid

ninhydrin solution



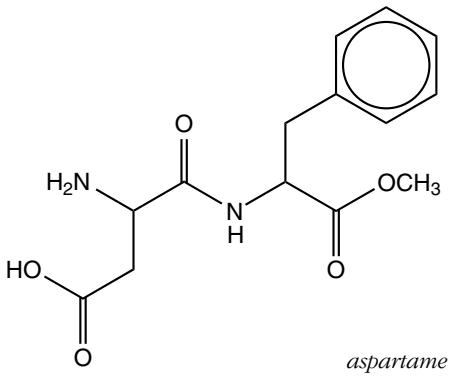
CARE Eye protection must be worn.

CARE Ninhydrin solution must be used in a fume cupboard. Gloves must be worn. Wash the solution off with lots of soap and water if it contacts your skin.

Introduction

Peptide bonds in proteins can be hydrolysed by refluxing with hydrochloric acid. You will use the artificial sweetener aspartame as a substitute for a protein in this activity. Aspartame is the methyl ester of the dipeptide Asp Phe. The ester link in aspartame will also be hydrolysed under these conditions, so you will get aspartic acid and phenylalanine just as you would from the dipeptide itself.

The skeletal formula for aspartame is shown below.



aspartame

What you do

You are to investigate what concentration of acid and what reflux time are sufficient to hydrolyse the aspartame in one tablet.

You can use your skill at chromatography to analyse your reaction products to find out whether the free amino acids are present in reasonable quantities. (Some tablets contain phenylalanine with the aspartame so you must detect *both* phenylalanine *and* aspartic acid to be sure hydrolysis has occurred.)

The chromatography solvent can be made by shaking together butan-1-ol (**CARE** Harmful and flammable), glacial ethanoic acid (**CARE** Corrosive; avoid inhaling the vapour) and water in the ratio 5:1:4 by volume in a separating funnel. When the mixture settles, two layers will form. Use the upper, non-aqueous layer as your solvent.

Amino acids can be detected by spraying the paper with ninhydrin solution (**CARE** Harmful, flammable; observe the safety warnings above) and then warming the paper, for example using a hair dryer. **Do not use a flame**. The spots may be slow to develop. Circle them with pencil when they appear, as they will fade.

QUESTION

Write an equation for the hydrolysis of aspartame, showing the skeletal formulae of the three products formed.

EP2.3

Using nuclear magnetic resonance (n.m.r.) spectroscopy for structure determination

In this activity you will have practice in analysing n.m.r. spectra of organic compounds.

Introduction

In Part 1 of this activity you will analyse the n.m.r spectra of a range of simple organic compounds. In Part 2, the n.m.r spectra of three different nitrogen-containing compounds, one of which is the analgesic paracetamol, will be considered. You will then be asked to make predictions about the infrared spectrum of paracetamol.

The typical resonance positions for various hydrogen atoms, expressed as chemical shifts, are given in the **Data Sheets** (Table 23). Some of the compounds in this activity contain hydrogen atoms attatched to oxygen and nitrogen atoms. The chemical shifts for both N–**H** and O–**H** can be variable, the value depending on several factors. They are usually broad peaks.

The *shape* of the signal given by any particular hydrogen atom is related to the number of neighbouring hydrogen atoms on adjacent carbons, as summarised in Table 1. This rule does not always apply to hydrogen atoms on oxygen or nitrogen.

Number of H atoms on adjacent C atoms	Shape of signal
0	single peak – singlet
1	two close peaks – doublet
2	three close peaks – triplet
3	four close peaks – quartet

Table 1 Shape of n.m.r. signal in relation to the number of neighbouring hydrogen atoms on adjacent carbon atoms. These splittings are only seen clearly in high resolution spectra.

What you do

Part 1: N.m.r. spectra of simple organic compounds

a Figures 1, 2 and 3 show the ^{1}H (proton) n.m.r. spectra of ethanol, ethanal and ethanoic acid respectively.

i Draw the full structural formula of each of these molecules.

ii Identify the hydrogen atoms responsible for each of the signals in the spectra.

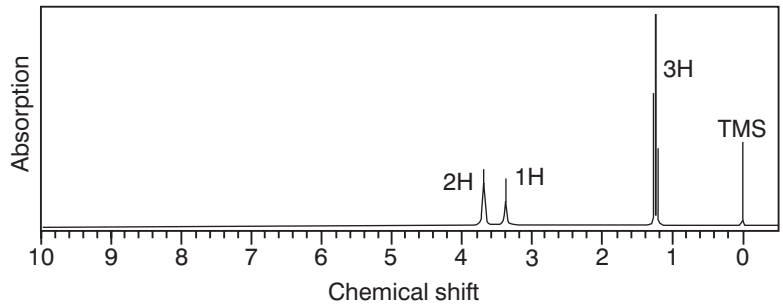


Figure 1 The n.m.r. spectrum of ethanol

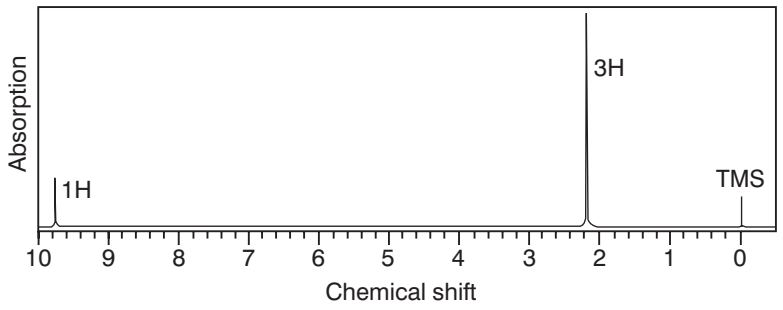


Figure 2 The n.m.r. spectrum of ethanal

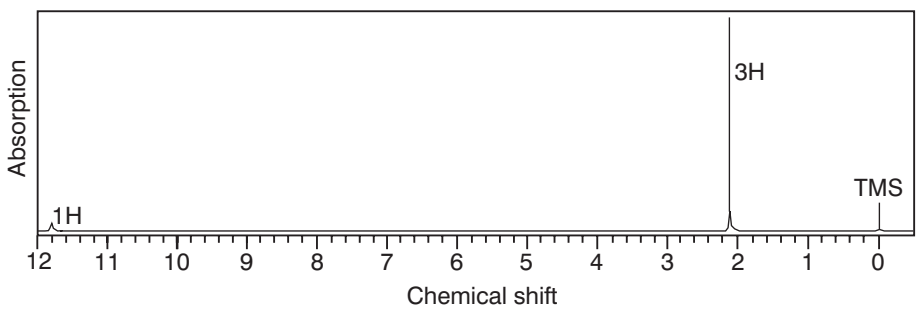


Figure 3 The n.m.r. spectrum of ethanoic acid

b Figures 4–7 show the n.m.r. spectra of propan-1-ol, propanal, propanone and propanoic acid, but not necessarily in this order.

i Draw the full structural formula of each of these molecules.

ii Identify, with reasons, which of the spectra, labelled A, B, C, D in Figures 4–7, corresponds to each of the structures you have drawn.

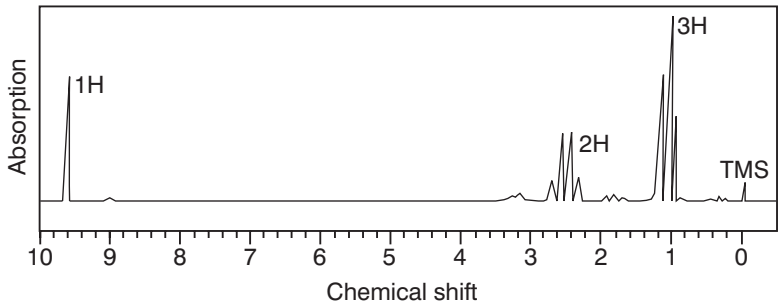


Figure 4 The n.m.r. spectrum A

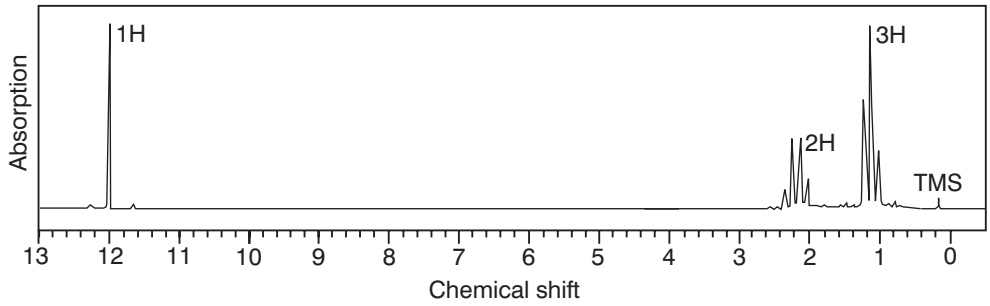


Figure 5 The n.m.r. spectrum B

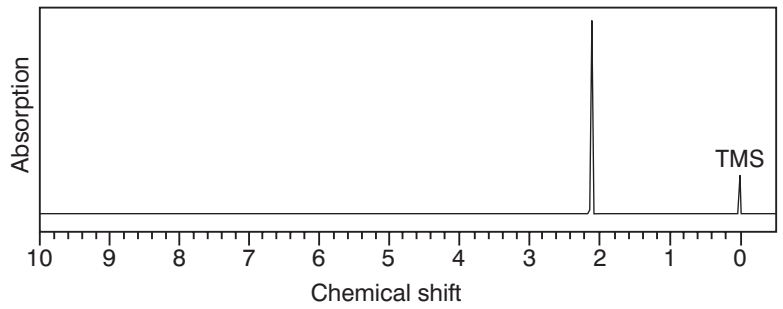


Figure 6 The n.m.r. spectrum C

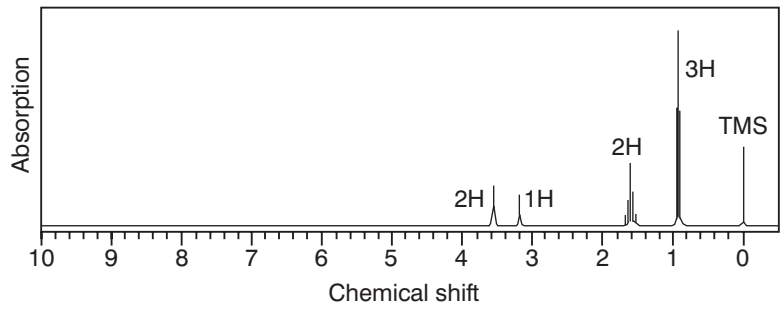


Figure 7 The n.m.r. spectrum D

c Figures 8, 9 and 10 show the n.m.r. spectra of aminoethane, 1-aminopropane and propanamide respectively.

i Draw the full structural formula of each of these molecules.

ii Identify the hydrogen atoms responsible for each of the signals in the n.m.r. spectra and, where possible, comment on the shape of these signals.

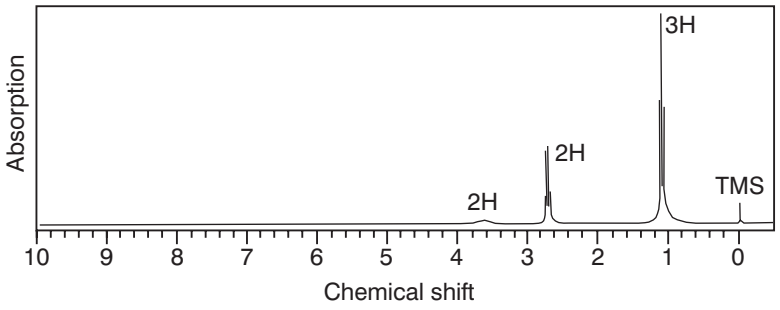


Figure 8 The n.m.r. spectrum of aminoethane

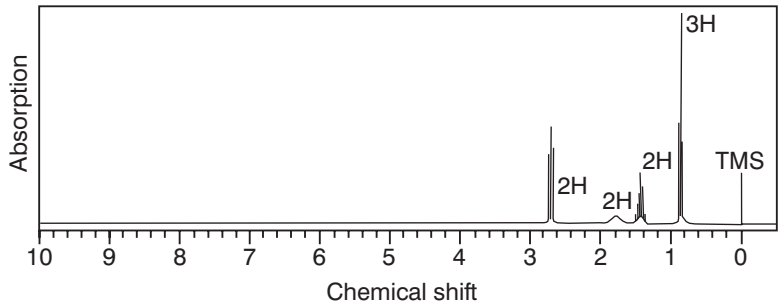


Figure 9 The n.m.r. spectrum of 1-aminopropane

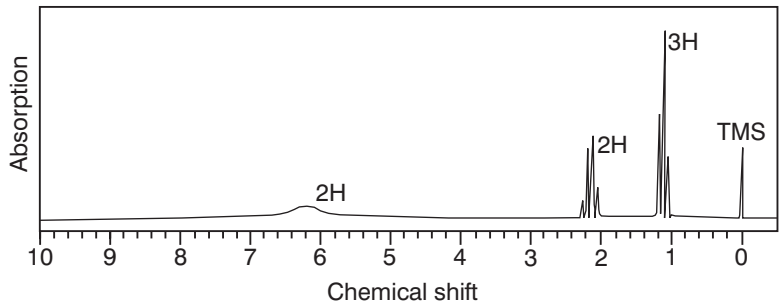
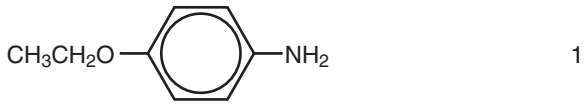


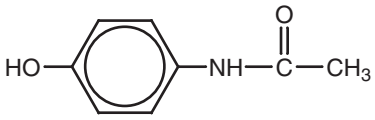
Figure 10 The n.m.r. spectrum of propanamide

Part 2: N.m.r. spectra of other nitrogen-containing compounds

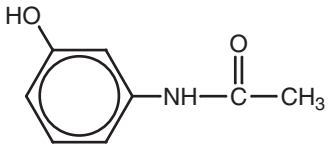
d Use the information provided in the **Data Sheets**, together with your knowledge of n.m.r. spectra in Part 1 to match the following structures 1–3 with the n.m.r. spectra E–G in Figures 11–13.



1



2



3

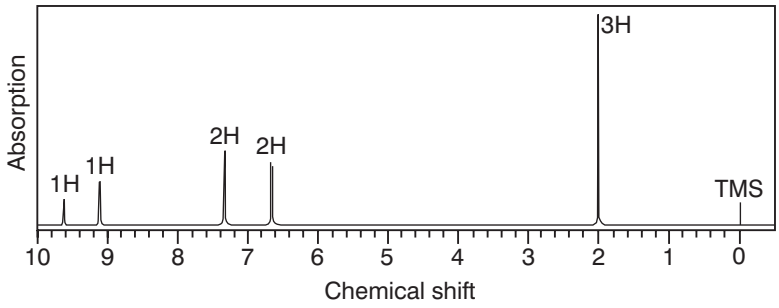


Figure 11 The n.m.r. spectrum E

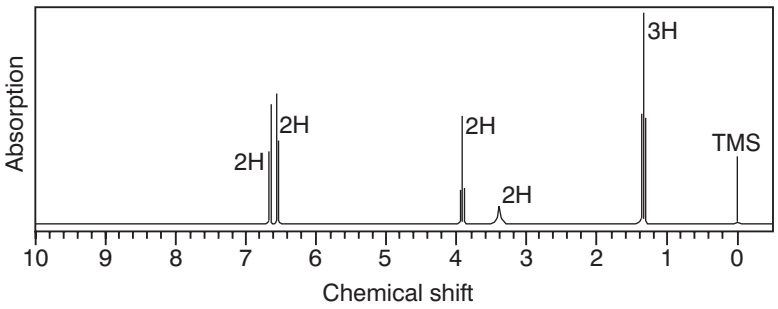


Figure 12 The n.m.r. spectrum F

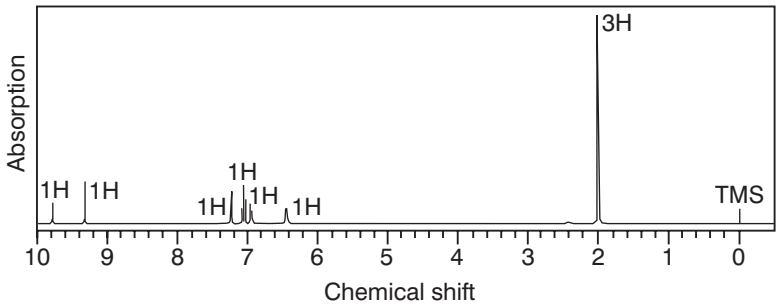


Figure 13 The n.m.r. spectrum G

e For the analgesic paracetamol, structure 2, use the information in the **Data Sheets** to predict the main features of its i.r. spectrum.

EP2.4

The shapes of α-amino acids

This activity reinforces your reading of* Chemical Ideas 3.3 *and* 3.6 *on the shapes of molecules and optical isomerism.

Requirements

- molecular model kit
- molecular modelling software (optional)

What you do

1 Draw a full structural formula for glycine. Mark on it the values you would expect for the bond angles.

2 Draw full structural formulae for the following amino acid –R groups, showing clearly any lone pairs. Mark on each structure the values you would expect for the bond angles.
a valine **b** serine **c** methionine **d** aspartic acid.

3 Build models of the structures in steps **1** and **2**. Check that the bond angles you predicted were correct, and see how the representations of the structures on flat pieces of paper compare with their three-dimensional shapes.

4 Build a simplified model of a general α-amino acid by using:
- a hydrogen atom for hydrogen
- a carbon atom for the –R group
- an oxygen atom for the –COOH group
- a nitrogen atom for the $-NH_2$ group.

You now have a central carbon atom surrounded by four different groups. Each group (–H, –R, –COOH and $-NH_2$) is represented by a different coloured atom. (This removes some of the 'clutter' of bulky groups so that you can see the arrangement round the central carbon atom clearly.)

Build a second model which is the mirror image of the model you have just built. Confirm to yourself that the two structures represent **enantiomers** (optical isomers).

5 Stand your two models from step **4** so that the H atoms point upwards. Look down each one from the H atom towards the central carbon atom. One enantiomer will have the sequence
COOH, **R**, **N**H_2 (CORN)
in a clockwise direction. This is the **L-amino acid**, the configuration that occurs in proteins. The other structure corresponds to the **D-amino acid**; D-amino acids occur in some bacterial peptides.

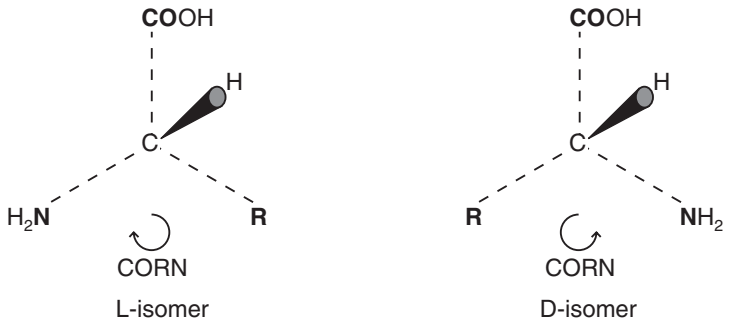


Figure 1 Looking down the H–C bond from hydrogen towards the central carbon atom

6 Replace the –R groups of your amino acid structures with H atoms, so that you have two models of glycine. Confirm that optical isomerism is no longer possible without four different groups around the central atom.

EP2.5

A testing smell

This activity makes use of a naturally occurring pair of enantiomers to illustrate how optical isomers can have different properties.

Requirements

- spearmint chewing gum (a half of a piece)
- caraway seeds, crushed (about 20)
- test-tubes wrapped in foil or paper to obscure their contents
- stoppers or clingfilm

What you do

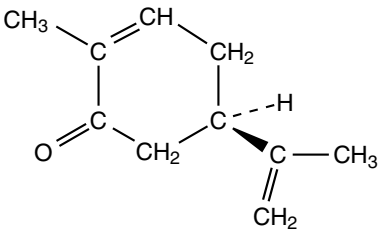
1 Label the test-tubes and their stoppers X, Y and Z. Place about a quarter of a piece of chewing gum into one of the tubes, and about 10 caraway seeds into another. Make sure you keep a record of which material goes into which tube.

2 Then place either another piece of chewing gum or another 10 caraway seeds into the third tube. Seal all three tubes with the correct stoppers.

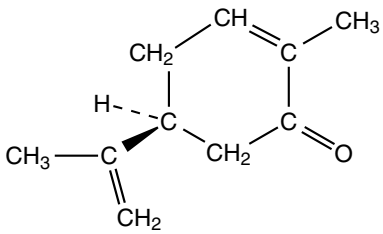
3 Get other members of your group to close their eyes and smell the contents of each tube in turn, telling you which tubes smell the same and which is different.

What it means

The principal smell of chewing gum is due to L-carvone which smells of spearmint. Caraway seeds smell of the enantiomer, D-carvone. It is claimed that about 20% of people cannot distinguish between these two smells.



L-carvone



D-carvone

QUESTIONS

a Which two functional groups are present in carvone?

b **i** Draw skeletal formulae for D- and L-carvone.
ii On your skeletal formulae, use an asterisk (*) to denote the chiral carbon atom.

c **i** What is the molecular formula of carvone?
ii Write down the molecular formula of the product of the reaction of D-carvone with bromine molecules, $Br_2(l)$.
iii Would you expect L-carvone to react in the same way with bromine? Explain your answer.
iv Draw a skeletal formula for the product of the reaction in **c ii**.
v How many chiral carbon atoms are there in the structure in **c iv**? Mark each one with an asterisk.

d D- and L-carvone are different in the way they smell to the majority of people. Suggest a reason why the enantiomers produce different responses from the smell receptors in the body.

e Work out the percentage of people in your class survey who fail to detect a difference between the two forms of carvone. How does your result compare with the figure of 20% quoted earlier in this activity? Comment on the fairness of the comparison.

EP2.6

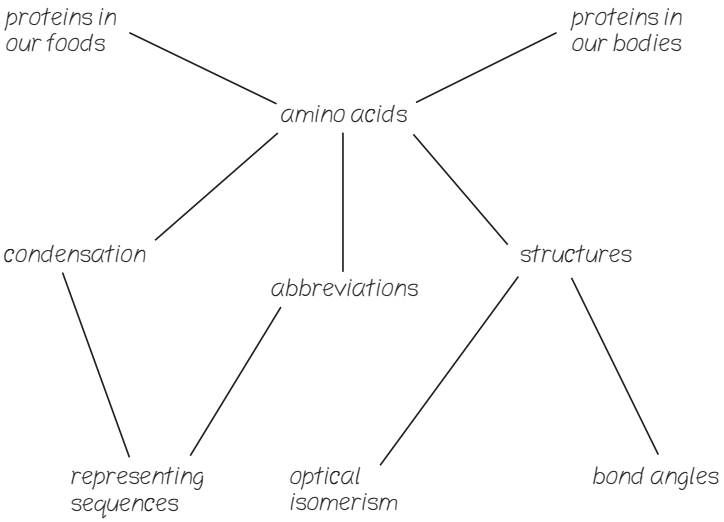
Taking note of proteins

***This activity will help you summarise the information in the first part of* Storyline EP2.**

Making a summary

Very early in this course, in the unit **The Elements of Life**, you probably carried out an activity – 'Making the most of your study of chemistry' (**Activity EL2.2**) – about recording information. This introduced the idea of writing 'branched notes'.

Below is the basis of some branched notes on amino acids and proteins. Each of the points needs to be branched out further with more information. Add the branches you think are necessary, and so build up a summary of the work you have done so far in **Storyline EP2**.



EP2.7
Modelling DNA

The double-helix arrangement of DNA can be explained in terms of intermolecular forces. This activity helps you work out these explanations, and become more familiar with the DNA structure.

Requirements

- molecular model kit
- plastic-coated wire, e.g. RS30 × 0.25 mm strand (1 m)
- plastic pegs, eg Cochrane's 'Minit' peg, type a (30)
- plastic straws to fit pegs, 10 cm (15)
- RASMOL molecular visualisation application and files of nucleic acid structures (optional)

Base pairing

1 Refer to **Storyline EP2** and use a molecular model kit to build models of the structures of the four bases in DNA. Leave spare bonds to indicate the connections to the sugar–phosphate 'backbone'.

2 Try different combinations of pairs of bases to investigate which molecules form strong hydrogen bonds with one another. Remember:
- the bonds to the sugar–phosphate 'backbone' must be at opposite sides of the bases
- the hydrogen bonds should be about 50% longer than the covalent bonds
- the bases interact in a flat arrangement.

3 Draw diagrams for the structures of the base pairs which fit well together.

The double helix

4 Use the plastic-coated wire, plastic straws and pegs supplied to make a ladder like the one shown in Figure 1. Use about 15 pegs on each side.

a What feature of DNA is represented by:
- **i** the plastic-coated wire?
- **ii** the straws?

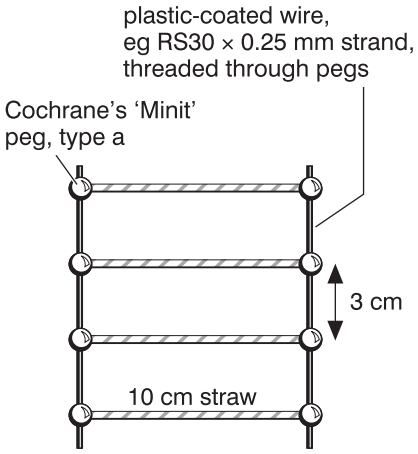


Figure 1 Building a double helix

5 Twist your model to form a double helix with ten straws to a turn – that is the *eleventh* straw should lie directly over the *first* straw. This represents the extent to which DNA is twisted.

6 Compare your model with the space-filling representation of DNA shown in Figure 2.

7 You might take the opportunity to investigate the DNA structure further by using a molecular visualisation package (eg RASMOL).

b What do you notice about the region in the centre of the double helix – the region occupied by the bases? (These are shown as unshaded atoms in the figure.)

c Explain why the DNA double helix could not be twisted more tightly than it is.

d As you have seen, hydrogen bonding is responsible for the interactions between the bases in a direction *across* the axis of the double helix – in other words, horizontally in Figure 2. Explain what type of intermolecular bonding is responsible for the interactions between the bases *along* the axis of the double helix – in other words, vertically in Figure 2.

e Your answer to **d** should help you to understand why the DNA double helix is twisted to the extent shown by your model. Explain why a *less* tightly twisted DNA double helix would be unlikely to form.

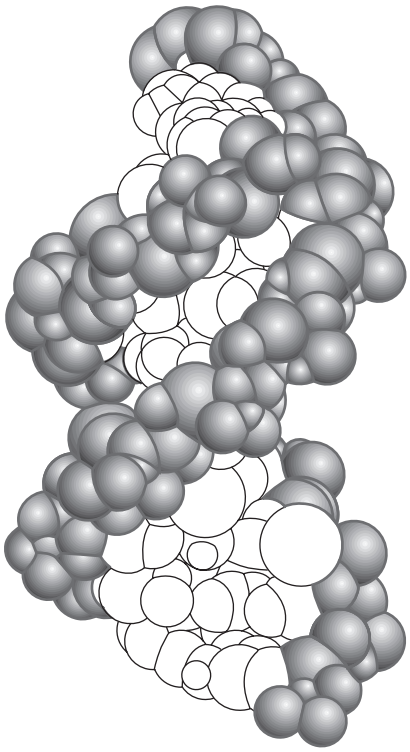


Figure 2 Space-filling model of DNA

EP2.8

Life reveals its twisted secret

This activity will give you practice at composing a piece of scientific writing from a range of source materials.

What you do

Imagine that it is 1953. Francis Crick and James Watson have just announced a momentous discovery about the structure of DNA. You have been commissioned to write an article of 400–500 words for a science magazine. Your article should review the various structures which have been proposed for DNA, and explain why the Crick-Watson structure seems most appropriate.

To help you, you have collected together some clippings from books and magazines, and some brief details about the principal research scientists in the field. You may include illustrations in your article. Remember – your audience will have some understanding of science, though they will not be experts in this particular field.

When you have finished, write a short abstract (no more than 50 words) which summarises the main points of your article.

Setting the scene

By the early 1950s, protein structure had been well worked out. Several groups were turning their attention to DNA, the one remaining cell polymer with an unknown structure.

The following people were foremost among those involved.

Maurice Wilkins

He was a respected physicist working at King's College, London. He had decided to tackle the DNA structure using X-ray diffraction as his research technique. At a conference in Naples in Spring 1951 he showed a slide of the X-ray diffraction pattern of DNA which, in spite of Wilkins' dry delivery, excited James Watson to the possibilities of X-ray study of the molecule.

Rosalind Franklin

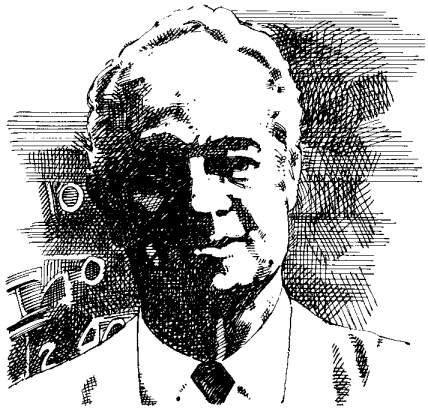
She was a young, brilliant X-ray crystallographer, and an ardent feminist, who also worked at King's College, London. Called in by Wilkins to assist with his DNA work, she soon became an equal partner in the research.

James Watson

A young fun-loving American biologist who came to Cambridge in 1951 to pursue his hunch that X-ray diffraction was the clue to understanding the structure of macromolecules. He joined the Cavendish laboratory in a group working on protein structure, but his thoughts were always turning to DNA.

Linus Pauling

He was a very successful and established chemist working at the California Institute of Science and Technology (Cal Tech). He had recently discovered (with Robert Corey) the α-helical structure for proteins. This he revealed in a lecture with a distinct 'show business' flair, proudly unveiling his model with a flourish near the end of the lecture.



Francis Crick

A maverick English physicist who had worked on magnetic mines in the Second World War. He was supposed to be researching for a PhD in the Cavendish group which Watson joined. In practice, though, he was constantly picking up and attempting to improve the ideas of others, and he too had his sights set on the DNA structure.

The clippings

Rosalind Franklin's early ideas about DNA (November 1951)

The general characteristics of the diagram suggest that the DNA chains are in a helical form.

… The results suggest a helical structure (which must be very closely packed) containing probably 2, 3 or 4 co-axial nucleic acid chains per helical unit, *and having the phosphate groups near the outside*.

A. Sayre 1975. *Rosalind Franklin and DNA* (New York: Norton, pp. 125–6). Reprinted 1978.

Crick and Watson's 3-chain model (1951/1952)

Decisions had to be made about the number of polynucleotide chains within the DNA molecule. Superficially, the X-ray data were compatible with two, three, or four strands. It was all a question of the angle and radii at which the DNA strands twisted about the central axis.

… we had decided upon models in which the sugar-phosphate backbone was in the center of the molecule.

… we looked at the pros and cons of one, two, three, and four chains, quickly dismissing one-chain helices as incompatible with the evidence in our hands. As to the forces that held the chains together, the best guess seemed to be salt bridges in which divalent cations like Mg^{++} held together two or more phosphate groups. Admittedly there was no evidence that Rosy's samples contained any divalent ions, and so we might be sticking our necks out. … with luck, the addition of magnesium or possibly calcium ions to the sugar-phosphate backbone would quickly generate an elegant structure, the correctness of which would not be debatable.

… a shape began to emerge which brought back our spirits. Three chains twisted about each other in a way that gave rise to crystallographic repeat every 28 Å along the helical axis.

James D. Watson 1968. *The Double Helix* (London: Weidenfeld & Nicolson, pp. 77–89). With new introduction, 1999 (London: Penguin Books).

Note: An ångström (Å) is 1×10^{-10} m, so 28 Å is 2.8 nm.

Franklin's response to the Crick-Watson 3-chain model (1952)

Wilkins was invited to Cambridge to witness the triumph; William Seeds, who worked with Wilkins, came along, and Rosalind and Gosling as well. The session was opened by Crick with an exposition of helical diffraction theory, a subject upon which he was very expert, and went on to a description of the model, of which Rosalind plainly did not think much. Her disdain of it Watson accounts for on the grounds that what was proposed was a helical structure, while Rosalind did not admit that a shred of evidence existed to indicate that DNA was helical – a curious statement, considering that very shortly before she had presented a good deal of evidence suggesting that the B form of DNA was exactly that. What she did object to in the proposed structure – and aggressively, we are told – was that the three-chain model had its phosphate groups held together by Mg^{++} ions in a way she thought unlikely, considering that by her calculations the Mg^{++} ions would be surrounded by tight shells of water molecules.

And as Watson was required to confess, her objections, though very annoying, were not mere perversity.

A. Sayre 1975. *Rosalind Franklin and DNA* (New York: Norton, pp. 135–6). Reprinted 1978.

Pauling's 3-helix model (1952/1953)

We have formulated a structure for the nucleic acids which is compatible with the main features of the X-ray diagram and with the general principles of molecular structure, and which accounts satisfactorily for some of the chemical properties of the substances. The structure involves three intertwined helical polynucleotide chains. Each chain, which is formed by phosphate di-ester groups and linking β-D-ribofuranose [D-ribose] or β-D-deoxyribofuranose [D-deoxyribose] residues with 3', 5' linkages, has approximately twenty-four nucleotide residues in seven turns of the helix. The helixes have the sense of a right handed screw. The phosphate groups are closely packed about the axis of the molecule, with the pentose residues surrounding them, and the purine and pyrimidine groups projecting radially, their planes being approximately perpendicular to the molecular axis. The operation that converts one residue to the next residue in the polynucleotide chain is rotation by about 105° and translation by 3.4 Å.

A detailed description of the structure is appearing in the February 1953 issue of the *Proceedings of the National Academy of Sciences of the United States of America*.

Linus Pauling, Robert B. Corey 1953. Structure of the Nucleic Acids. In *Nature*, February 21, vol. 171, p.346.

Watson's response to Pauling's ideas (1953)

At once I felt something was not right. I could not pinpoint the mistake, however, until I looked at the illustrations for several minutes. Then I realized that the phosphate groups in Linus' model were not ionized, but that each group contained a bound hydrogen atom and so had no net charge. Pauling's nucleic acid in a sense was not an acid at all. Moreover, the uncharged phosphate groups were not incidental features. The hydrogens were part of the hydrogen bonds that held together the three intertwined chains. Without the hydrogen atoms, the chains would immediately fly apart and the structure vanish.

Everything I knew about nucleic-acid chemistry indicated that phosphate groups never contained bound hydrogen atoms.

James D. Watson 1968. *The Double Helix* (London: Weidenfeld & Nicolson, p. 160). With new introduction, 1999 (London: Penguin Books).

Pauling's later comments

I calculated the number of polynucleotide chains per unit to be exactly three. This result surprised me, because I had expected the value 2 if the nucleic acid fibres really represented genes … During the next month I strove to find a way of arranging the polynucleotide chains in a triple helix, and was successful, although the structure was described as "an extraordinarily tight one, with little opportunity for change in positions of the atoms" …

In hindsight, it is evident that I made a mistake … in having decided to study the triple helix rather than the double helix. … I am now astonished that I began work on the triple helix structure, rather than on the double helix. I had not forgotten … that the gene might consist of two complementary molecules, but for some reason, not clear to me now, the triple chain structure apparently appealed to me, possibly because the assumption of a three-fold axis simplified the search for an acceptable structure.

Linus Pauling 1974. Molecular Basis of Biological Specificity. In *Nature*, vol. 248, p. 771.

Crick and Watson's crucial paper

They acknowledge the contribution of Rosalind Franklin and Maurice Wilkins at the end of the paper. (See next sheet.)

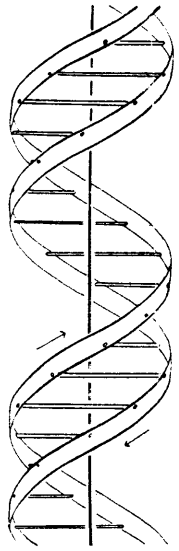
MOLECULAR STRUCTURE OF NUCLEIC ACIDS

A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three intertwined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.



This figure is purely diagrammatic. The two ribbons symbolize the two phosphate—sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate diester groups joining ß-D-deoxyribofuranose residues with 3',5' linkages. The two chains (but not their bases) are related by a dyad perpendicular to the fibre axis. Both chains follow right-handed helices, but owing to the dyad the sequences of the atoms in the two chains run in opposite directions. Each chain loosely resembles Furberg's[2] model No. 1; that is, the bases are on the inside of the helix and the phosphates on the outside. The configuration of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There is a residue on each chain every 3·4 A. in the z-direction. We have assumed an angle of 36° between adjacent residues in the same chain, so that the structure repeats after 10 residues on each chain, that is, after 34 A. The distance of a phosphorus atom from the fibre axis is 10 A. As the phosphates are on the outside, cations have easy access to them.

The structure is an open one, and its water content is rather high. At lower water contents we would expect the bases to tilt so that the structure could become more compact.

The novel feature of the structure is the manner in which the two chains are held together by the purine and pyrimidine bases. The planes of the bases are perpendicular to the fibre axis. They are joined together in pairs, a single base from one chain being hydrogen-bonded to a single base from the other chain, so that the two lie side by side with identical z-co-ordinates. One of the pair must be a purine and the other a pyrimidine for bonding to occur. The hydrogen bonds are made as follows: purine position 1 to pyrimidine position 1; purine position 6 to pyramidine position 6.

If it is assumed that the bases only occur in the structure in the most plausible tautomeric forms (that is, with the keto rather than the enol configurations) it is found that only specific pairs of bases can bond together. These pairs are: adenine (purine) with thymine (pyrimidine), and guanine (purine) with cytosine (pyrimidine).

In other words, if an adenine forms one member of a pair, on either chain, then on these assumptions the other member must be thymine; similarly for guanine and cytosine. The sequence of bases on a single chain does not appear to be restricted in any way. However, if only specific pairs of bases can be formed, it follows that if the sequence of bases on one chain is given, then the sequence on the other chain is automatically determined.

It has been found experimentally[3,4] that the ratio of the amounts of adenine to thymine, and the ratio of guanine to cytosine, are always very close to unity for deoxyribose nucleic acid.

It is probably impossible to build this structure with a ribose sugar in place of the deoxyribose, as the extra oxygen atom would make too close a van der Waals contact.

The previously published X-ray data[5,6] on deoxyribose nucleic acid are insufficient for a rigorous test of our structure. So far as we can tell, it is roughly compatible with the experimental data, but it must be regarded as unproved until it has been checked against more exact results. Some of these are given in the following communications. We were not aware of the details of the results presented there when we devised our structure, which rests mainly though not entirely on published experimental data and stereochemical arguments.

It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.

Full details of the structure, including the conditions assumed in building it, together with a set of co-ordinates for the atoms, will be published elsewhere.

We are much indebted to Dr. Jerry Donohue for constant advice and criticism, especially on interatomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. Watson
F. H. C. Crick

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems.
Cavendish Laboratory, Cambridge.
April 2.

[1] Pauling, L., and Corey, R. B.. *Nature*, **171**, 346 (1953); *Proc. U.S. Nat. Acad. Sci*., **39**, 84 (1953).
[2] Furberg, S., *Acta Chem. Scand*., **6**, 634 (1952).
[3] Chargaff, E., for references see Zamenhof, S., Brawerman, G. and Chargaff, E., *Biochim. et Biophys. Acta*, **9**, 402 (1952).
[4] Wyatt, G. R., *J. Gen. Physiol*., 36, 201 (1952).
[5] Astbury. W. T., Symp. Soc. Exp. Biol. 1, Nucleic acid, 66 (Camb. Univ. Press, 1947).
[6] Wilkins, M. H. F., and Randell, J. T., *Biochim. et Biophys. Acta*, **10**, 192 (1953).

NATURE

NO. 4356 April 25, 1953

EP6.1

Testing for glucose

Diabetes can be diagnosed by detecting glucose in the urine. The test must be specific to avoid confusion with other compounds which may be present. The specificity of an enzyme in its reaction with a particular substrate is therefore a useful basis for such a test. In this activity you can find out about the specificity of the enzyme glucose oxidase and investigate some of its other properties.

Requirements

- glucose test strips (such as Clinistix™ or Diastix™)
- hydrochloric acid, 1 mol dm^{-3}
- sodium hydroxide solution, 1 mol dm^{-3}
- glucose solution, 0.02 mol dm^{-3}
- solutions or samples of other sugars

sodium hydroxide solution

IRRITANT

CARE Eye protection must be worn.

WEAR EYE PROTECTION

What you do

Carefully read the instructions which come with the glucose test strips, and make sure you know how they work (see also **Storyline EP6**).

1 The glucose solution you are supplied with has a similar concentration to the glucose in the urine of someone who is diabetic. Check that the test strips work with the glucose solution.

2 Investigate how the activity of the enzyme *glucose oxidase* is affected by changing:

- pH
- temperature
- substrate (the sugar used).

Record your results in the form of a table.

3 You might like to go on to refine your experiments in order to discover in more detail how the enzyme's activity depends on pH and temperature.

4 Summarise your findings and suggest explanations for the effects you have noted.

EP6.2

Succinate dehydrogenase (Optional extension)

In this activity you will use models to investigate the binding of a substrate to the active site of an enzyme. This will help you to understand why enzymes are so specific, and how some compounds can act as enzyme inhibitors.

Requirements

- molecular model kit

Introduction

Butanedioic acid (succinic acid) is oxidised to *trans*-butenedioic acid by removal of hydrogen. This reaction is catalysed by the enzyme *succinate dehydrogenase*.

$$\underset{\text{butanedioic acid}}{HOOC{-}CH_2{-}CH_2{-}COOH} \rightarrow \underset{\textit{trans}\text{-butenedioic acid}}{HOOC{-}CH{=}CH{-}COOH}$$

The enzyme is *inhibited* by propanedioic acid: $HOOC{-}CH_2{-}COOH$; in other words, its catalytic activity is less in the presence of propanedioic acid. This, together with other evidence, suggests that *two* –COOH groups are involved in binding the substrate to the active site.

The action of the enzyme is summarised in Figure 1.

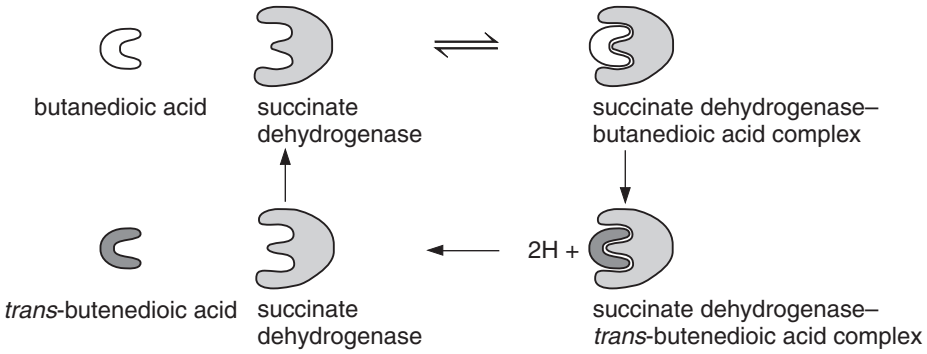


Figure 1 The action of succinate dehydrogenase

What you do

1 Make molecular models of the three acids (butanedioic acid, *trans*-butenedioic acid, and propanedioic acid).

2 Place the molecule of *trans*-butenedioic acid on a piece of paper so that all the atoms are touching the paper. Draw circles on the paper to mark the positions of the oxygen and hydrogen atoms of the –COOH groups. Note the position of the other hydrogen atoms. (Remember, this is the product of the oxidation reaction.)

3 Now manipulate your structure of butanedioic acid so that the hydrogens and oxygens of its –COOH groups can be placed on the same marks. One H atom of each $-CH_2-$ group should also be touching the paper. Note the positions of the other two H atoms – the ones which are removed by the enzyme.

4 Leave the model of butanedioic acid in place and superimpose your model of propanedioic acid on it so that the –COOH groups coincide. (You will need to 'close up' the butanedioic acid structure a little to achieve this.) Using a different colour, mark the new positions of the oxygen and hydrogen atoms of the –COOH groups.

This should show you that both butanedioic acid and propanedioic acid can be bound by their –COOH groups to the same site on the enzyme. The product of the oxidation reaction, *trans*-butenedioic acid, binds to this site less well and is released from the enzyme.

The oxidation (removal of hydrogen) occurs at another part of the active site. This must be where the C–C bond between the two central carbons of butanedioic acid naturally comes when it is placed as above.

Propanedioic acid is an inhibitor because it has no C–C bond to oxidise, but it can bind onto the site and block it.

5 Now consider pentanedioic acid: $HOOC{-}CH_2{-}CH_2{-}CH_2{-}COOH$.

Make a model of its structure. Try to fit it onto the marks you made in **4**. Decide whether you would expect it to:

- bind to the enzyme or not
- be oxidised by the enzyme to $HOOC{-}CH_2{-}CH{=}CH{-}COOH$
- be an inhibitor.

EP6.3

The effect of enzyme and substrate concentrations on the rate of a reaction

In this activity you will follow the progress of a catalysed reaction by measuring the volume of gas produced as the reaction proceeds. You will use the initial rates of a series of experiments to find the orders of the reaction with respect to enzyme and substrate.

Requirements

- burette (50 cm^3)
- trough or bowl
- boiling tube with bung and delivery tube
- graduated pipette (5 cm^3) and safety filler
- measuring cylinder (10 cm^3)
- hydrogen peroxide solution, 5 vol (25 cm^3)
- yeast suspension (20 cm^3), made from 2 g dried yeast in 160 cm^3 water aerated for several hours
- stopwatch

CARE Eye protection must be worn.

Introduction

In this activity, the substrate is *hydrogen peroxide* (H_2O_2) and the enzyme is *catalase*. You will use yeast as a source of catalase.

Hydrogen peroxide is formed as a waste product of metabolism by many organisms. It is toxic and must be rapidly removed from the cells. The enzyme catalase catalyses the decomposition of hydrogen peroxide to produce water and oxygen.

$$2H_2O_2(aq) \rightarrow 2H_2O(l) + O_2(g)$$

The reaction can be monitored by measuring the volume of oxygen produced as the reaction proceeds, and plotting a graph of the volume of oxygen produced against time. You can find the rate of the reaction (in terms of the volume of oxygen produced per second) at any time by measuring the gradient of the curve.

You may like to practise your IT skills and make use of graph-plotting computer software to plot your results. It is usually best, however, to draw the best-fitting line or curve by hand.

It is important when investigating rates of reaction to vary one factor at a time. All other factors which could affect the rate should be kept constant.

What you do

It will be best to work in groups. One group should tackle Part 1 while another group does Part 2. Combine your results at the end.

Take the opportunity to use a spreadsheet to collect your data. The graph-plotting function will help you to find the initial rate of each reaction.

Part 1: Varying the concentration of hydrogen peroxide

1 Fill a burette with water and invert it in a trough of water. Hold it in place with a clamp and check that the burette is leak-proof. Make sure you leave enough room in the trough for water which will be displaced from the burette.

2 Place 2.5 cm^3 of well-stirred yeast suspension in a boiling tube and set up the apparatus in Figure 1. *Carefully* open the tap on the burette until the meniscus falls to the 50 cm^3 mark (i.e. zero for this experiment as the burette is upside down).

3 Measure out 5 cm^3 of hydrogen peroxide in a 10 cm^3 measuring cylinder. Organise yourselves for taking and recording readings of volume at 10-second intervals for 4 minutes.

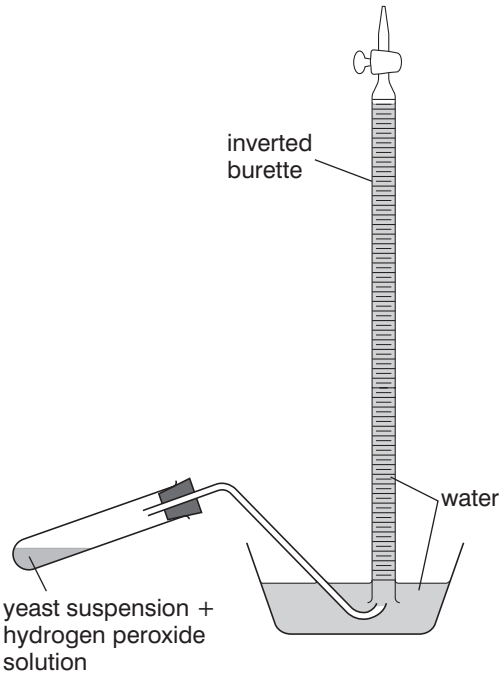


Figure 1 Measuring the volume of oxygen produced

4 Add 5 cm^3 of hydrogen peroxide to the yeast suspension and *quickly* replace the bung. Zero time is counted as the time the first bubble appears in the burette. Take a reading of the volume of gas in the burette every 10 seconds for 4 minutes.

5 Wash out the boiling tube, refill the burette, and repeat steps **1**–**4** four more times, using:

4 cm^3 H_2O_2 + 1 cm^3 distilled H_2O
3 cm^3 H_2O_2 + 2 cm^3 distilled H_2O
2 cm^3 H_2O_2 + 3 cm^3 distilled H_2O
1 cm^3 H_2O_2 + 4 cm^3 distilled H_2O

in the measuring cylinder. Everything else should be the same in each experiment.

6 Plot the volume of O_2 given off (vertical axis) against time (horizontal axis) for each experiment, drawing all the curves on the same axes.

QUESTIONS

a How does the rate of the reaction change as the reaction proceeds? Explain why the rate changes in this way.

b Draw a tangent to each curve at $t = 0$. This represents the *initial rate* of the reaction: its rate at the start. How does the initial rate of the reaction vary with the starting concentration of hydrogen peroxide?

c Measure the gradient of each tangent. Plot the initial rate for each experiment against the volume of hydrogen peroxide used. (The volume of hydrogen peroxide is proportional to its concentration since the total volume is kept constant.) What is the order of the reaction with respect to hydrogen peroxide?

Part 2: Varying the concentration of enzyme

7 Follow the procedure in steps **1**–**4** in Part 1.

8 Wash out the boiling tube, refill the burette, and repeat steps **1**–**4** four more times, using:

2.0 cm^3 yeast + 0.5 cm^3 distilled H_2O
1.5 cm^3 yeast + 1.0 cm^3 distilled H_2O
1.0 cm^3 yeast + 1.5 cm^3 distilled H_2O
0.5 cm^3 yeast + 2.0 cm^3 distilled H_2O

in the boiling tube. Everything else should be the same in each experiment.

9 Plot the volume of O_2 given off (vertical axis) against time (horizontal axis) for each experiment, drawing all the curves on the same axes.

QUESTIONS

d How does the rate of the reaction change as the reaction proceeds? Explain why the rate changes in this way.

e Draw a tangent to each curve at $t = 0$. This represents the *initial rate* of the reaction: its rate at the start. How does the initial rate of the reaction vary with the starting concentration of the enzyme? You can assume that the starting concentration of the enzyme is proportional to the volume of yeast used, since the total volume was kept constant.

f Measure the gradient of each tangent. Plot the initial rate for each experiment against the volume of yeast used. What is the order of the reaction with respect to the enzyme?

EP6.4

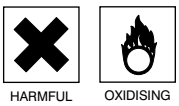
Using the iodine clock method to find the order of a reaction

This activity illustrates another way in which the initial rate method can be used to determine the order of a reaction with respect to one of the reactants.

Requirements

- 0–110 °C thermometer
- boiling tubes (5)
- test-tubes
- burettes (or graduated pipettes 1 cm^3, 2 cm^3 and 5 cm^3)
- potassium iodide solution, 1.00 mol dm^{-3} (15 cm^3)
- potassium peroxodisulphate(VI) ($K_2S_2O_8$) solution, 0.0400 mol dm^{-3} (10 cm^3)
- sodium thiosulphate ($Na_2S_2O_3$) solution, 0.0100 mol dm^{-3} (10 cm^3)
- freshly made starch solution (5 cm^3)
- stopwatch

potassium peroxodisulphate(VI) solution



CARE Eye protection must be worn.

About the reaction

Peroxodisulphate(VI) ions and iodide ions react together in solution to form sulphate(VI) ions and iodine.

$$S_2O_8^{2-}(aq) + 2I^-(aq) \rightarrow 2SO_4^{2-}(aq) + I_2(aq)$$

Both reactants and the sulphate ions are colourless, so the progress of the reaction can be measured by following the colour of the iodine produced. The iodine can be detected even more clearly by placing some starch in the reaction mixture: iodine forms an intense blue-black complex with starch.

One way of measuring the *initial rate* of the reaction is to measure how long the reaction takes to produce a small, fixed amount of iodine. By answering the questions which accompany this activity, you can work out what amount of iodine has been chosen here, and what fraction of the extent of reaction this represents.

You can make the time taken to produce a particular amount of iodine really obvious if you add thiosulphate ions to the reaction mixture at the start. Thiosulphate ions turn iodine back to iodide ions.

$$2S_2O_3^{2-}(aq) + I_2(aq) \rightarrow S_4O_6^{2-}(aq) + 2I^-(aq)$$

So, no starch-iodine colour will appear until all the thiosulphate has been used up. What you see is a colourless reaction mixture sitting there as though nothing is happening; then, suddenly, it turns blue. If you measure how long that takes, you know how long it took to use up all the thiosulphate and, therefore, how long it took to produce the equivalent amount of iodine.

This method of studying reaction rates is sometimes called the *clock method*, and this experiment is an example of an *iodine clock* experiment. You are going to use it to investigate how the reaction rate depends on the concentration of iodide ions in the reaction mixture.

You may like to practise your IT skills and make use of graph-plotting computer software to plot your results. It is usually best, however, to draw the best-fitting line or curve by hand.

What you do

1 First you are going to make up reaction mixture 1 from Table 1, and measure how long it takes for the blue iodine-starch colour to appear.

Look carefully at Table 1. The five mixtures differ only in the concentration of iodide ions. Water is added to keep the total volume of solution constant, so the concentration of everything else is the same in each mixture.

Reaction rates depend upon temperature. Measure the temperature of each experiment. Make sure that all your results are taken at approximately the same temperature and record an average value.

You can place the potassium iodide, sodium thiosulphate, starch and (for later mixtures) water straight into a boiling tube. You must measure out the potassium peroxodisulphate(VI) solution (**CARE** Harmful. Oxidiser) into a separate container, such as a test-tube.

2 When you are ready to start the experiment, pour the potassium peroxodisulphate(VI) solution into the mixture in the boiling tube. Immediately start timing, and carefully stir the reaction mixture with a thermometer to ensure that everything is properly mixed. Record the time taken for the blue colour to appear.

Mixture	Volume of KI(aq) /cm^3	Volume of water /cm^3	Volume of $Na_2S_2O_3$(aq) /cm^3	Volume of starch solution/cm^3	Volume of $K_2S_2O_8$(aq) /cm^3
1	5	0	2	1	2
2	4	1	2	1	2
3	3	2	2	1	2
4	2	3	2	1	2
5	1	4	2	1	2

Table 1 Mixtures for the iodine clock experiments

3 Repeat the procedure with mixtures 2–5, and record the time taken for the blue colour to appear in each case.

Find the order of reaction

You could use a spreadsheet to collect your results and to perform the calulations.

To find the order of the reaction with respect to iodide ions, you need to know how the initial rate of the reaction varies with iodide ion concentration. The steps in the calculation are summarised below:

- Work out [I^-] in each mixture.
- Work out the amount of I_2 produced before the blue-black colour appears.
- Use this amount of I_2 and the clock time to work out the initial rate of reaction for each mixture.
- Plot a graph of the initial rate of reaction against [I^-].
- Use the graph to determine the order of the reaction with respect to I^- ions.

The questions on the next sheet will help you work through these steps.

QUESTIONS

a Draw up a results table using the headings below:

Mixture	Concentration of $I^-(aq)$/mol dm^{-3}	Clock time /s	Rate /mol dm^{-3} s^{-1}	Temp /°C

Table 2 Results table

b Calculate values for the concentrations of iodide ions in the five reaction mixtures. Record these in the table, together with the corresponding times for the blue-black colour to appear (the clock times).

c Which reactant, I^- or $S_2O_8^{2-}$, is in excess in the reaction mixtures? The reactant *not* in excess will be used up in the reaction. It determines the total amount of iodine which can be produced.

d What is the total amount in moles of iodine which can be produced by each of the reaction mixtures?

e **i** What amount in moles of thiosulphate ions is added to each reaction mixture?
ii What amount of iodine will be used up by thiosulphate ions during the course of each experiment?
iii What percentage of the extent of reaction is studied during the experiments? (For the clock method to work well, the extent of reaction studied should be no more than 10–15% of the total extent of the reaction.)

f The initial rate of the reaction can be measured in mol dm^{-3} I_2 produced per second.
i For each mixture, divide your answer to **e ii** by the time taken for the appearance of the blue colour, and so calculate the initial rate (in mol dm^{-3} s^{-1}) at which each mixture reacts.
ii Record the reaction rates in the table.

g **i** Plot a graph of rate against concentration of iodide ions (horizontal axis).
ii What is the order of reaction with respect to iodide ions?

h A similar series of experiments shows that the reaction is first order with respect to $S_2O_8^{2-}$ ions.
i Write a rate equation for the reaction of peroxodisulphate(VI) ions and iodide ions.
ii What is the *overall* order of the reaction?
iii Calculate the rate constant for the reaction using the gradient of the graph you obtained in **g i**. Make sure you give the correct units and the temperature at which your measurements were made.

EP6.5

Enzyme kinetics

Accurate results in experiments involving enzymes can be hard to obtain. This activity provides you with two exercises, based on accurate data, which should help to reinforce your work about the rates of enzyme-catalysed reactions. Use* Storyline EP6 *to help you explain your results.

Introduction

The enzyme *urease* catalyses the hydrolysis of urea.

$$H_2N–CO–NH_2(aq) + H_2O(l) \rightarrow CO_2(aq) + 2NH_3(aq)$$

In the exercises which follow, the initial rate of reaction was measured by finding the number of moles of urea which had been hydrolysed during the first three minutes of the reaction. The average rate over the first three minutes, in units of mol dm^{-3} min^{-1}, was then found.

Exercise 1

The results described in Table 1 represent a series of experiments in which the concentration of urea (the substrate) was varied but the concentration of urease (the enzyme) was kept constant.

Concentration of urea/mol dm^{-3}	Rate/mol dm^{-3} min^{-1}
0	0
0.005	1.7×10^{-6}
0.010	2.3×10^{-6}
0.020	3.2×10^{-6}
0.050	4.4×10^{-6}
0.100	5.9×10^{-6}
0.200	7.2×10^{-6}
0.300	7.7×10^{-6}
0.400	8.0×10^{-6}
0.500	8.1×10^{-6}

Table 1

Plot a graph of the reaction rate against the substrate concentration (horizontal axis).

a When the substrate concentration is high, what is the approximate order of reaction with respect to substrate?

b The following mechanism has been proposed for enzyme-catalysed reactions (E = enzyme, S = substrate, P = product):

$$E + S \rightarrow ES \rightarrow EP \rightarrow E + P$$

Explain why your answer to **a** tells you that the first step in this mechanism is not the rate-determining step at high substrate concentration.

c At high substrate concentrations, all the enzyme active sites are occupied. This is known as active site *saturation*. Explain how this idea supports your answers to **a** and **b**.

d At lower substrate concentrations, there is a change in the way the rate depends on concentration. At very low substrate concentrations, the reaction is approximately first order with respect to the substrate. Suggest a reason for this.

Exercise 2

The results in Table 2 come from an experiment in which the concentration of urea (the substrate) was kept fixed and the concentration of urease (the enzyme) was varied. This was achieved by adding different volumes of urease solution to the reaction mixture, and keeping the total volume constant by making up with the appropriate volume of water.

Volume of urease solution/cm^3	Rate/mol dm^{-3} min^{-1}
0	0
0.050	0.6×10^{-6}
0.100	0.8×10^{-6}
0.200	1.8×10^{-6}
0.300	3.2×10^{-6}
0.500	4.8×10^{-6}
1.000	10.4×10^{-6}
1.500	14.9×10^{-6}
2.000	19.5×10^{-6}

Table 2

Plot a graph of the reaction rate against the volume of urease solution, which is a measure of the urease concentration (horizontal axis).

e What is the order of reaction with respect to the enzyme?

f Describe how your answer to **e** can be explained in terms of the mechanism proposed in **b**.

EP7

Check your notes on *Engineering Proteins*

This activity helps you get your notes in order at the end of this unit.

Use this list as the basis of a summary of the unit by collecting together the related points and arranging them in groups. Check that your notes cover the points and are organised in appropriate ways. Remember that you will be coming back to some of the ideas in later units.

Most of the points are covered in the **Chemical Ideas**, with supporting information in the **Storyline** or **Activities**. However, if the *main* source of information is the Storyline or an Activity, this is indicated.

- Proteins are condensation polymers formed from amino acid monomers.
- The general structure of amino acids.
- The acid–base properties of amino acids and the formation of zwitterions.
- The formation and hydrolysis of the peptide link between amino acid residues in proteins (**Storyline EP2**; **Activity EP2.2**).
- The importance of amino acid sequence in determining the properties of proteins, and the diversity of proteins in living things (**Storyline EP2**).
- Stereo-isomers: *cis-trans* and optical isomers (enantiomers).
- The use of the term *chiral* as applied to a molecule.
- How nuclear magnetic resonance (n.m.r.) spectroscopy can be used for the elucidation of molecular structure.
- The interpretation of n.m.r. spectra for simple compounds given relevant information (**Activity EP2.3**).
- The expression for the equilibrium constant, K_c, for a given reaction.
- The way in which changes of temperature and pressure affect the magnitude of the equilibrium constant.
- The use of values of K_c, together with given data on equilibrium concentrations, to calculate the composition of equilibrium mixtures.
- The primary, secondary and tertiary structures of proteins (**Storyline EP4**).
- The role of hydrogen bonds and other intermolecular forces in determining the structure and properties of proteins (**Storyline EP4**).
- The double helix structure of DNA in terms of a sugar–phosphate backbone and attached bases (**Storyline EP2**).
- The significance of hydrogen bonding in the pairing of bases in DNA, and the replication of genetic information (**Storyline EP2**; **Activities EP2.7** and **EP2.8**).
- How DNA encodes for the amino acid sequence in a protein.
- The use of empirical rate equations of the form: rate = $k[A]^m[B]^n$ where m and n are integers.
- The meaning of the terms: *rate of reaction*, *rate constant*, *order of reaction* (both overall and with respect to a given reagent).
- Experimental methods for measuring the rate of reactions.
- How to use experimental data to find the order of a reaction (zero, first or second order).
- How to use given data to calculate half-lives for a reaction.
- The industrial importance of enzymes (**Storyline EP6**).
- The characteristics of enzyme catalysis, including: specificity, temperature and pH sensitivity, and inhibition (**Storyline EP6**).
- The specificity of enzymes in terms of a simple 'lock and key' model of enzyme action.
- The technique of 'genetic engineering' and its applications (**Storyline EP3** and **EP5**).