Nuffield Advanced Chemistry Special Study

BIOCHEMISTRY

Teachers' and Technicians' guide

Contents

Experiment 1 **The effect of pH on enzyme activity** (Chapter 2)

Experiment 2 **How does the rate of an enzyme-catalysed reaction vary with the concentration of the substrate?** (Chapter 2)

Experiment 3 **Making whey syrup using an immobilized enzyme** (Chapter 2)

Experiment 4 **Investigating the synthesis of starch by a metabolic reaction** (Chapter 3)

Experiment 5 **Isolation of DNA from cress** (Chapter 4)

Experiment 6 **Isolation of DNA from peas** *alternative to Experiment 5* (Chapter 4)

This is the teachers' and techicians' guide to the January 2005 edition of the *Biochemistry* students' book and experiments, downloadable from www.chemistry-react.org

ISBN 0 904956 31 8

Nuffield Advanced Chemistry Co-ordinator: Alan Furse
Editors of this special study: Alan Furse and Andrew Hunt
Contributor: Dean Madden (NCBE) Adviser: Philip Jackson
Safety adviser: Peter Borrows

Based on the 3rd edition published by Longman in 1998
Editor of the 3rd edition: Michael Vokins
Author: Roger Barnes
Contributors and advisers:
Jeanette Bartholomew
Sue Crickmore
Ray Matthias (John Innes Centre)
Richard Price (SAPS)
Michael Reiss

Drawings by Hugh Neill and Oxford Illustrators Limited

First published by Longman in 1971 This edition downloaded from www.nuffieldchemistry.org

ISBN 0 904956 31 8

Health and Safety

See the safety notes given with each experiment.

Health and safety in school and college science affects all concerned: teachers and technicians, their employers, students, their parents or guardians, as well as authors and publishers.

As part of the reviewing process, these publications have been checked for health and safety. In particular, we have attempted to ensure that:
• all recognized hazards have been identified,
• suitable precautions are suggested,
• where possible, the procedures are in accordance with commonly adopted model (general) risk assessments,
• if a special risk assessment is likely to be necessary this has been pointed out
• where model (general) risk assessments are not available, we have done our best to judge the procedures to be satisfactory and of an equivalent standard.

It is assumed that:

• practical work is conducted in a properly equipped and maintained laboratory,
• rules for student behaviour are strictly enforced,
• mains-operated equipment is regularly inspected, properly maintained and appropriate records are kept,
• care is taken with normal laboratory operations such as heating substances and handling heavy objects,
• good laboratory practice is observed when chemicals are handled,
• eye protection is worn whenever risk assessments require it,
• any fume cupboard required operates at least to the standard of Building Bulletin 88,
• students are taught safe techniques for such activities as heating chemicals, smelling them, or pouring from bottles,
• hand-washing facilities are readily available in the laboratory.

Under the COSSH and the Management of Health and Safety at Work regulations, employers are responsible for carrying out risk assessments before hazardous procedures are undertaken or hazardous chemicals used or made. Teachers are required to co-operate with their employers by complying with such risk assessments.

However, teachers should be aware that mistakes can be made and, in any case, different employers adopt different standards. Therefore, before carrying out any practical activity, teachers should always check that what they are proposing is compatible with their employer's risk assessments and does not need modification for their particular circumstances. Any local rules issued by the employer must always be followed, whatever is recommended here.

Model (general) risk assessments have been taken from, or are compatible with:
CLEAPSS *Hazcards* (see annually updated CD-ROM)
CLEAPSS *Laboratory handbook* (see annually updated CD-ROM)
CLEAPSS *Recipe cards* (see annually updated CD-ROM)
ASE *Safeguards in the school laboratory* 10th edition 1996
ASE *Topics in Safety* 3rd edition, 2001
ASE *Safety reprints*, 2000 or later

Clearly, you must follow whatever procedures for risk assessment your employers have laid down. As far as we know, all the practical work and demonstrations in this course are covered by the model (general) risk assessments detailed in the above publications, and so, in most schools and colleges, you will not need to take further action.

If you or your students decide to try some procedure with hazardous substances beyond what is in this course, and you cannot find it in these or other model (general) assessments, then your employer will have to make a special risk assessment. If your employer is a member, then CLEAPSS will act for them. Otherwise the ASE may be able to help.

Only you can know when your school or college needs a special risk assessment. But thereafter, the responsibility for taking all the steps demanded by the regulations lies with your employer.

Investigations will involve independent action by the student. Our notes on investigations warn students to carry out a risk assessment; students should be responsible for safety in the first instance and credited in any assessment for making safe plans. Nevertheless, proposals must be seen by you the teacher and you must ensure that you make an appropriate check, particularly with respect to safety, on what will go on. You will need to take particular care if students consult library books published before modern safety standards came into force or get ideas from the internet.

INTRODUCTION BIOCHEMISTRY

Biochemistry helps students to understand how our knowledge of DNA has developed and how it may alter our lives in the future. Material on DNA is set in the context of enzyme chemistry and metabolism.

There is a useful summary of the chemistry needed for biochemistry in the Biochemical Society booklet 1:'Essential chemistry for biochemistry'. This can be downloaded from their website (see the weblinks on the Biochemistry page of www.nuffieldchemistry.org)

Aims

1 To demonstrate the fundamental chemical nature of living processes.

2 To study the structure and behaviour of some of the major chemical compounds found in living systems.

3 To provide experimental work to illustrate how practical work in biochemistry both depends on and differs from chemical methods.

4 To support an appreciation of the applications of biochemical knowledge

Contents and timing

The Special Study is designed to occupy four weeks, assuming about 4.5 hours of contact time are available each week. Chapter 2 will take two weeks, and Chapters 3 and 4 one week each. Chapters 1 and 5 are assumed to be covered mostly during private study time.

The Study Guides for each chapter on the *Re:act* website will help students with their work on this Special Study.

What is examinable?

The examinable content of this Special Study is defined by the Edexcel specification. Clarification of the depth of treatment can be gauged by looking at the downloadable student chapters and instructions for experiments, published in the Special Study section of the *Re:act* website http:/www.chemistry-react.org

The content of the comment and case study boxes in the students' text does *not* have to be learnt.

Experiment 1 The effect of pH on enzyme activity *(see Chapter 2)*

Each group of students will need:

Beaker, 100-cm^3

Graduated pipette, 5-cm^3, and safety filler (for use with saliva)

6 test-tubes

7 teat pipettes

5 glass rods

4 spotting tiles

Stop clock

Access to:

7 labelled burettes to contain starch, sodium chloride and buffer solutions

1% fresh starch solution (10 g dm^3), 40 cm^3 per group; see below

0.2 M sodium chloride (g dm^{-3}), 10 cm^3 per group

0.067 M phosphate buffer solutions at pH 4.5, 5.9, 7.0, 8.0, and 8.8, 15 cm^3 of each solution per group; see below

0.001 M iodine, I_2 (0.25 g dm^{-3} in potassium iodide solution, 1 g dm^{-3})

Bucket with freshly-diluted 1% sodium chlorate(I) solution (undiluted solution is CORROSIVE) for used apparatus contaminated with saliva

Notes

Starch solution is prepared by making 10 g of dry starch into a thin cream with cold water and pouring into 1 dm^3 of boiling water. Boil briefly and allow to cool.

The buffer solutions are made by mixing 0.067 M potassium dihydrogenphosphate (KH_2PO_4, 9.1 g dm^{-3}) and 0.067 M disodium hydrogenphosphate ($Na_2HPO_4.2H_2O$, 11.9 g dm^{-3}).

Check the pH of the solution with a pH meter.

⚠ HAZARDS

Saliva can transmit certain infections. Students must be instructed to follow exactly the instructions about how to use saliva. Students must handle only their own saliva, and must rinse their apparatus at the end, before putting in the disinfectant. Technicians should wear gloves when dealing with the apparatus in the disinfectant. Students should not rinse their mouths out with lab tap water. Use e.g. bottled water, and drink from disposable cups.

Procedure

Full details are given on the students' sheet. Students should study the instructions thoroughly in advance. The experiment gives good results, but it is recommended that students work in pairs because of the organization required.

Purified amylases are available, but it should be noted that most amylases extracted from plants or micro-organisms have a pH optimum of about 4.5 to 5.0, and are therefore not suitable for this experiment. Pancreatic amylase has similar properties to those of the salivary enzyme, but the cost of the enzyme is likely to preclude its use in school experiments. Powdered amylases, as they are enzymes, may well be sensitizers in some individuals. Hence, if used, gloves must be worn and care taken to avoid raising dust.

The amylase activity in saliva will vary from person to person, and it is therefore necessary to carry out a preliminary experiment (part 2) to determine the amount of enzyme solution required in the subsequent experiments.

The first 2 minutes of the main experiment (part 3) require a high degree of organization. Every 30 seconds, the calculated volume of saliva solution is added to one of the test-tubes A–E, the mixture is shaken, and 3 drops are transferred to the iodine test solution in the correct cavity of the dropping tile. One student should concentrate on pipetting the saliva solution while the second student shakes and tests the starch saliva mixture. The experiment should continue for at least 17 minutes; during this time, seven samples will have been removed from each test-tube, A–E.

Since the samples in a particular row have all been removed after the same incubation time, the effect of pH on amylase activity is readily apparent. Hydrolysis of starch in the presence of NaCl should be most rapid in tube C, pH 7.0, and slowest in tubes A and E. The colours tend to fade slowly on standing, and results should therefore be recorded as soon as the experiment is concluded.

Students should devise a suitable way of recording their results. One method is to measure the time taken for a particular colour to develop in each experiment, for example a shade of brown. This will require some subjective estimation, since the identical colour will probably not be present in all five columns. The reciprocal of the time taken is a measure of the amount of starch converted per minute (that is, the activity of the enzyme). A plot of these reciprocals against pH allows the pH optimum to be determined.

Students should be able to explain the effect of pH on enzyme activity in general terms. Alterations in the bonding between side chains, due to changes in their charges, modify the essential three-dimensional shape of the enzyme. Also, alterations in the charges on critical groups may affect the catalytic mechanism.

Continued on the next page

Experiment 1 The effect of pH on enzyme activity *continued* *(see Chapter 2)*

Sample results

These results (see below) refer to the first appearance of a chestnut brown colour. The rate of reaction, the activity of the enzyme, is proportional to 1/time.

It can be seen from the graph on the right that the optimum pH for amylase activity is just below pH 7.0.

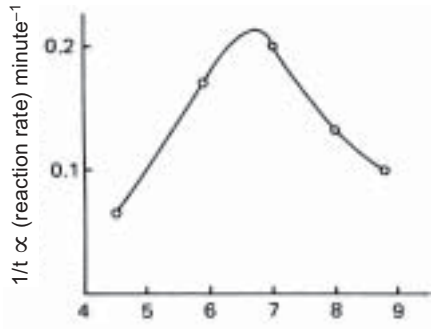
The chloride ions present in the reaction mixture act as a cofactor. It is possible that a chloride ion may stabilize a positively charged intermediate, for example, a carbocation, formed during the hydrolysis. The presence of a bound chloride ion may also modify the three-dimensional structure of the enzyme, and critical amino acids may be better aligned for catalysis.

Composition of buffer mixtures

pH (approx)	0.067 M KH_2PO_4 /cm^3	0.067 M $Na_2HPO_4 . 2H_2O$ /cm^3
4.5	100	0
5.9	90	10
7.0	40	60
8.0	5	95
8.8	0	100

Sample results

pH of buffer solution /pH	Time taken for colour to appear, *t* /minutes	Rate of reaction, 1/*t* /minute^{-1}
4.5	15.0	0.067
5.9	6.5	0.15
-		
7.0	5.0	0.20
8.0	7.5	0.13
8.8	10.0	0.10



The effect of pH on amylase activity in the presence of sodium chloride. Notice that the graph is not symmetrical.

Answers to questions

Question 1

Iodine inhibits amylase activity. If this did not occur the reaction would continue on the dropping tile and the colours would change significantly while the experiment was being completed.

Question 2

The N-terminal amino group, the C-terminal carboxylic acid group, and many of the amino acid side chains are able to act as acids or bases. As the pH alters, different groups will lose or gain protons and the overall charge of the protein will alter. Modification of the three-dimensional shape of the protein, which will result from changes in the intramolecular bonds, may result in decreased enzyme activity. In addition, the protonation or dissociation of one of the critical amino acid side chains required for catalysis may lead to the complete loss of enzyme activity. For example, a group which brings about acid catalysis will be ineffective once it has lost a proton.

Experiment 2 How does the rate of an enzyme-catalysed reaction vary with the concentration of the substrate? *(see Chapter 2)*

Each group of students will need:

Mortar and pestle
Scissors
Square of double thickness muslin cloth (12 x 12 cm)
2 measuring cylinders, 10-cm^3
Measuring cylinder, 50- cm^3
Graduated pipette, 10-cm^3, and safety filler
Beaker, 100-cm^3
Beaker, 250-cm^3
Side-arm flask, 250-cm^3
Bung with glass tube through it to fit side-arm flask; see notes
Suba-seal self-sealing cap
2 hypodermic needles and syringe, 2-cm^3
Gas syringe with rubber tubing to connect it to flask
Bunsen burner, tripod, gauze
Boiling-tube
Stop clock
Glass rod
Fresh liver, 10 g per group; see notes
Fine white sand, 3 g per group
Graph paper

Access to:

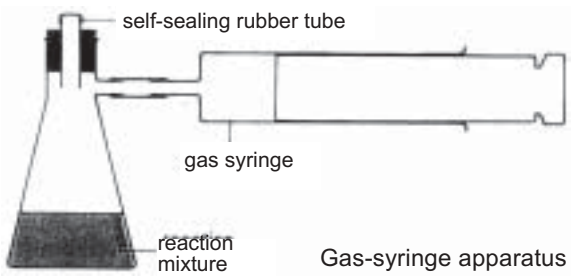
3 labelled burettes to contain phosphate buffer solution, pure water, and hydrogen peroxide
0.067 M phosphate buffer solution, pH 7 (see experiment 1), 300 cm^3 per group
'20 volume' hydrogen peroxide, 100 cm^3 per group; see notes
Centrifuge and centrifuge tubes
Balance

Notes

1 A glass tube approximately 0.8 cm in diameter is put through the centre of the bung as shown below.

2 Fresh or frozen lamb's liver or pig's liver from local shops is suitable.

3 Fresh 20 volume hydrogen peroxide, which has not partially decomposed, should be used. 20 volume hydrogen peroxide has a concentration of 1.74 mol dm^{-3}.



Gas-syringe apparatus

⚠ HAZARDS

20 volume hydrogen peroxide is an irritant. Students must wear eye protection when doing this experiment. Hydrogen peroxide must always be kept in a cool place away from direct sunlight, to prevent its decomposition.

Because of the potential for theft and drug abuse, great care must be taken with the syringes and especially the needles, even in schools where there is no known drug problem. Bring into the lab just before use and remove immediately after use. Count them out and count them in.

Care should be taken over the disposal of used liver, so as not to block sinks etc. Wipe down benches with disinfectant.

Procedure

If the experiment is not to be carried out immediately after extraction of the catalase, the enzyme solution should be stored in a refrigerator as the undiluted extract. It is worth stressing that it is the *diluted* extract which should be boiled!

Different flasks may be used for each experiment where a large number of side arm flasks are available. However, re-using a single flask, after washing and without drying, does not affect the concentration of hydrogen peroxide significantly because of the large volumes of solution involved.

It is suggested that one student injects the enzyme solution, keeps the flask shaken, and notes the time, while another reads the volume of oxygen on the gas syringe. The flasks must be continuously shaken in order that the oxygen may be released quickly from the frothy solution. If the gas syringe plunger sticks during the experiment, it should be eased out slightly by hand. A fresh hypodermic syringe is used in run number 9 to avoid contamination of boiled enzyme with 'active' enzyme.

After measuring the enzyme activity in run number 1, it may be necessary to adjust the concentration of enzyme so that a suitable volume of oxygen (between 40 and 60 cm^3) is measured. This procedure enables sufficiently accurate syringe readings to be made in runs 6 to 9 where the volumes of oxygen produced after 15 seconds are much lower.

For an accurate assessment of enzyme activity, the initial rate of reaction should be calculated by measuring the volumes of oxygen produced at various time intervals. Since it is not possible to measure this accurately, the volume of oxygen released after 15 seconds is measured. This volume is approximately proportional to the initial rate of the reaction. A 15-second time interval is chosen because the volume of oxygen released may be read with reasonable accuracy and the hydrogen peroxide has not yet been completely decomposed.

Continued on the next page

Experiment 2 How does the rate of an enzyme-catalysed reaction vary with the concentration of the substrate? *continued*

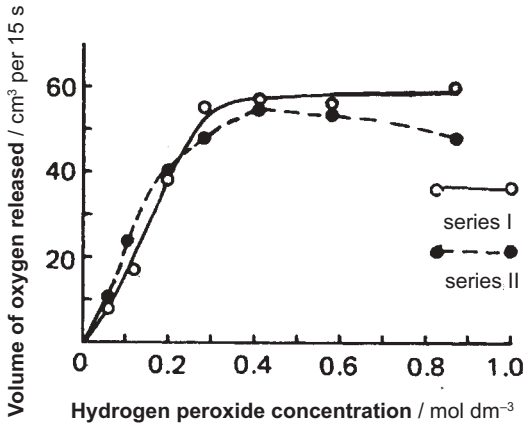
(see Chapter 2)

Sample results

The table and graph show the results for two complete series of runs. The volumes of oxygen in the table have been corrected for the volume of enzyme added and the volume of oxygen produced in the control run, number 8. The rate of reaction, enzyme activity, is proportional to the volume of oxygen released in 15 seconds.

Flask number	Concentration of aqueous H_2O_2 /M	Volume of O_2 released (series I) /cm^3	Volume of O_2 released, (series II) /cm^3
1	0.87	60	49
2	0.58	56	54
3	0.41	57	54
4	0.29	55	49
5	0.20	38	39
6	0.12	17	23
7	0.06	8	10
8	0.00	0	0
9	0.87	0	0

It can be seen from the graph below that the catalase activity increases with substrate concentration up to about 0.4 M, but then remains constant or falls slightly. Students should be very puzzled by this result, which is not the sort of result obtained when investigating inorganic catalysts.



The effect of hydrogen peroxide concentration on the volume of oxygen to be released.

Answers to questions

Question 1

A volume of 2 cm^3 was subtracted from each reading of the gas syringe because the added 2 cm^3 of enzyme displaces this volume of air from the flask.

Question 2

Run number 8 was a control, which allowed the measurement of gas production by the extract in the absence of added substrate. It is included to quantify any other reactions which might produce a gas.

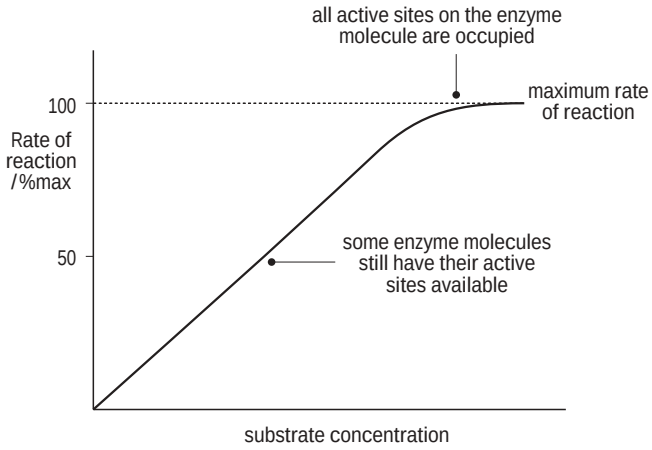
Question 3

Boiled catalase solution exhibits little or no activity. The thermal inactivation of enzymes can be explained in terms of the breaking of hydrogen bonds, ionic bonds, and van der Waals bonds, resulting in the disorganization of the active site of the enzyme. On cooling, bonds are reformed both within individual chains and between adjacent molecules. The probability of any chain having the shape required for catalysis is very small.

Question 4

i At low substrate concentrations the rate of the enzyme-catalysed reaction is roughly proportional to the concentration of the substrate. The reaction appears to be first order with respect to hydrogen peroxide (see graph below).

ii At high substrate concentrations, the rate is independent of substrate concentration. The reaction now appears to be zero order with respect to hydrogen peroxide (see graph below).



Variation in the rate of reaction with substrate concentration

Continued on the next page

Experiment 2 How does the rate of an enzyme-catalysed reaction vary with the concentration of the substrate? *continued* *(see Chapter 2)*

The graphs obtained by the students may exhibit a curve in the form of a rectangular hyperbola. However, in some runs the curve may be sharper (compare series I and II in the graph of effect of hydrogen peroxide on the previous page). In most runs there is likely to be a significant reduction in the amount of oxygen evolved in run 1 which contains the highest concentration of hydrogen peroxide (and there may even be a slight reduction in flask 2).

High concentrations of hydrogen peroxide disrupt the hydrogen bonding within the protein. The altered shape of the protein results in reduced catalase activity. At lower concentrations, denaturing does not affect the results significantly if the volume of oxygen released is measured over a very short period of time.

Question 5

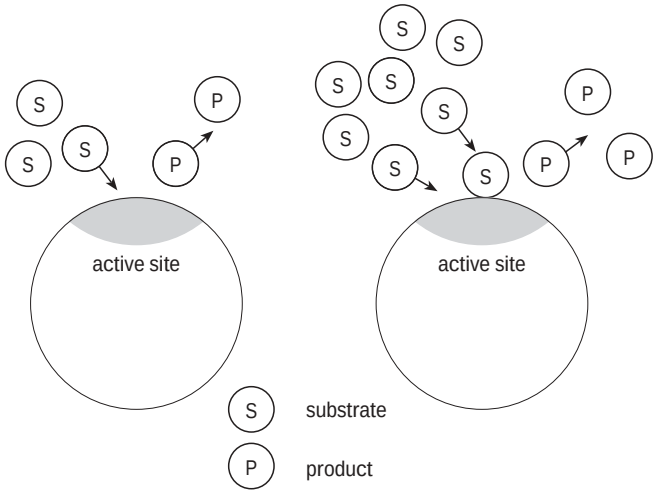
At low substrate concentrations not all the enzyme is combined with the substrate at any instant. The enzyme thus fails to show its maximal catalytic activity because at any particular time there are a number of enzyme molecules which have an active site that is still free to accept a substrate molecule (see the diagram opposite).

At this stage, the concentration of substrate is rate limiting. The slowest step in the mechanism is the binding of substrate to enzyme. The rate therefore depends on the concentration of both enzyme and substrate (although the dependence on enzyme concentration is not demonstrated in this experiment).

Question 6

At high substrate concentrations, maximum enzyme activity is reached when the concentration of the substrate is such that all the enzyme molecules are combined with substrate at any particular time. Further increase in substrate concentration has no effect because the enzyme is completely saturated with substrate (see opposite).

The concentration of enzyme limits the rate of the reaction; the rate of breakdown of the enzyme–substrate complex is now the rate-determining step. The rate could be increased by increasing the amount of enzyme.



Low substrate concentration
The length of time for which the enzyme is bound to the substrate does not limit the rate of reaction. Increasing the substrate concentration increases the velocity.

High substrate concentration
The length of time for which the enzyme is bound to the substrate does limit the rate of reaction. Increasing the substrate concentration now has little effect on velocity.

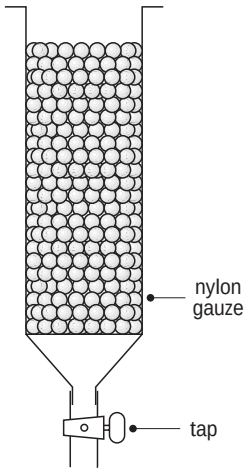
The effect of low and high concentrations of substrate on enzyme activity.

Experiment 3 Making whey syrup using an immobilized enzyme

(see Chapter 2)

Each group of students will need:

2 plastic syringes, 10-cm^3, one fitted with filter and stop tap (see diagram)
Beaker, 250-cm^3
2 beakers, 100-cm^3
Strainer (plastic, for tea)
Lactase enzyme, 2-cm^3
2% sodium alginate solution, 8-cm^3
0.1 M calcium chloride, 100-cm^3
8% whey solution, 50-cm^3
Glucose test strips, semi-quantitative



column for enzyme beads

Procedure

This experiment is also included in the *Food Science* special study.

Whey powder and Lactozyme® can be bought from the National Centre for Biotechnology Education (see below right) and semi-quantitative diabetic glucose test strips can be bought from pharmacists. When stored in a refrigerator the enzyme will retain its activity for at least 12 months. Milk (non-UHT) can be used instead of whey solution.

Question 1

The concentration of glucose rises to a max and then declines as the whey syrup passes through the column. Students should be able to make an estimate of the amount of glucose in their whey syrup.

Question 2

The column contains an enzyme so should be reusable until the enzyme eventually becomes so denatured that the process becomes inefficient.

Question 3

The enzyme has been trapped within a gel.

National Centre for Biotechnology Education (NCBE) supply suitable materials.
See 'Enzymes for education' on their website.
NCBE, School of Food Biosciences, University of Reading, Whiteknights, PO Box 226 Reading RG6 6AP
tel 0118 987 37 43
web www.ncbe.reading.ac.uk

Experiment 4 Investigating the synthesis of starch by a metabolic reaction

(see Chapter 3)

The experiments in the chapter on enzymes were concerned with reactions that broke down molecules; this experiment provides a simple example of the importance of enzymes in lowering the energy requirement of a synthetic reaction.

Each group of students will need:

Pestle and mortar
Fine sand
Fluted filter paper, qualitative grade
Test-tubes
200-cm^3 beaker, as an ice bath
Spotting tile
Dropping pipette
Small potato, cut into a 2-cm cube
0.005 M iodine, I_2, 1.3 g dm^{-3} in potassium iodide, 1 g dm^{-3}, 1 cm^3
0.05 M glucose, 9 g dm^{-3}, 1 cm^3
0.05 M sucrose, 17 g dm^{-3}, 1 cm^3
0.05 M glucose–1–phosphate (*see note*), 19 g dm^{-3}, 1 cm^3, freshly prepared and stored in an ice bath
Ice

Note

Glucose–1–phosphate is sold as glucose–1–phosphate, disodium tetrahydrate (molar mass 304). It is relatively expensive, but 5 g should be enough for 100 students. It must be stored in a freezer at about –20 °C, and the recommended shelf-life is 3 years (Sigma cat.no. G7000 – see below right).

Procedure

Full details are given on the students' sheet. Medium-sized potatoes should be used, not new or very large potatoes. A good grade of filter paper is neeeded for the filtration, or muslin can be used.

Only glucose–1–phosphate reacts to form starch – a strong blue colour should be obvious after 10–20 minutes. The spotting tiles in particular need to have been well cleaned before use.

Sigma-Aldrich Company Ltd
The Old Brickyard, New Road, Gillingham, Dorset SP8 4XT
freephone 0800 717 181 freefax 0800 378 785
www.sigmaaldrich.com

Experiment 5 Isolation of DNA from cress

(see Chapter 4, and the pea alternative given in Experiment 6)

DNA can be extracted quite easily from a range of sources, but its purity will be variable. As with protein purification, the isolation of biologically active nucleic acids from cells requires gentle techniques of extraction, and some degree of pH and temperature control throughout the purification procedure.

Supermarkets sell punnets of 'salad cress': this consists mostly of oil seed rape seedlings (*Brassica napus*) with a few seedlings of cress (*Lepidium sativum*). These give a good extract of DNA from the chloroplasts and nuclei of the cells in their cotyledons. Rather than the whole group working on cress, students could also try extracting DNA from other fresh plants.

The buffer solution (see notes on the experiment) needs to be prepared in advance.

⚠ HAZARD
To comply with current safety requirements power should cut off automatically from the centrifuge motor when the lid is opened. Older centrifuges may not comply with this requirement.

Each group of students will need:
Cress, about 3–5 g of fresh green tops
Pestle and mortar
Silver sand, a few grams
Measuring cylinder, 10 cm^3
Conical flask, 100 cm^3, and cap (Al foil)
Centrifuge tubes, 4
Dropping pipette

Access to:
Scissors
Water bath, set to 65°C
SDS extraction buffer, 10 cm^3 each, warmed to 65 °C
Centrifuge
Ethanol, 10 cm^3 each HIGHLY FLAMMABLE
Paper towel

Optional:
Access to refrigerator below 5 °C (for storage)

Notes on buffer solution

⚠ Tris hydrochloride (solid) is IRRITANT.
SDS extraction buffer is 0.01 M Tris. HCl containing 0.001 M EDTA, pH 7.6, and 0.25% SDS, which has to be added *just before use*.
TE buffer can be purchased from NCBE (see below right) or Sigma (T9285) as a concentrated solution and this is recommended.

Procedure
Centrifuges are usually calibrated in rpm so the following approximate formula may prove useful for converting to gravitational force (g) for the cress DNA extraction:

$$g = 10\,R\,(n/1000)^2$$

where R = radius of the rotor (cm)
n = rotor speed in rpm

Answers to questions

Question 1
Reasonable answers would list enzymes, inorganic salts, degradation products from the plant pigments.

Question 2
To break open the cells to release the DNA.

Question 3
Reasonable suggestions would refer to greater hydration forces of attraction and hydrogen bonding in water than in ethanol.

National Centre for Biotechnology Education (NCBE)
For TE buffer see 'Replacement items' on their website.
NCBE, School of Food Biosciences, University of Reading, Whiteknights, PO Box 226 Reading RG6 6AP
tel 0118 987 37 43
www.ncbe.reading.ac.uk

Sigma-Aldrich Company Ltd
The Old Brickyard, New Road, Gillingham, Dorset SP8 4XT
freephone 0800 717 181 freefax 0800 378 785
www.sigmaaldrich.com

Adapted from an experiment © SAPS.

Experiment 6 Isolation of DNA from peas

(see Chapter 4, and the cress alternative given in Experiment 5)

The protocol described here uses frozen peas. This has several advantages over other methods. Firstly, no equipment such as blenders or centrifuges are needed. Secondly, supplies of peas can be stored easily in the freezer and taken out in suitable amounts when required. It is easier and more reliable than the cress experiment. And last, but not least, unlike the onion DNA extraction used in many schools and colleges, the peas don't smell!

Variations of this extraction procedure can be used for other food items, such as fish sperm (milt or soft roe) or fish eggs. Several publications refer to the use of calf thymus tissue, but its use in schools is no longer recommended.

Isolating the DNA (and RNA) takes about 35 minutes, including an incubation period of 15 minutes.

Advance preparation

The ethanol must be ice cold. Place it in a plastic bottle in a freezer at least 24 hours before you attempt this activity. See Hazards.

Each group of students will need:

Peas, about 50 g (frozen ones are suitable, but thaw them first)
Washing-up liquid, 10 cm^3 (use a watery type, not the thicker, concentrated variety)
Table salt, 3 g
Distilled water, 90 cm^3
Ethanol, very cold, about 10 cm^3, straight from the freezer (industrial methylated spirit, IMS, is suitable, but see the Hazard note)
Novozymes Neutrase (a protease), 2–3 drops
Ice, in a jug with cold water
Coffee filter paper (do not use laboratory filter paper, as liquid takes too long to pass through it)
Plastic syringe, 1 cm^3, without a needle
Plastic funnel, large
250-cm^3 beakers, 2
Boiling tube or plastic graduated tube
Glass rod with a flattened end or a spoon for stirring the mixture

Access to:

Water bath, maintained at 60 °C

Note on suppliers

Most items required for this procedure can be obtained from a supermarket.
Novozymes Neutrase can be bought in small volumes from the National Centre for Biotechnology Education (see box).

⚠ HAZARDS

Ethanol in freezers
Ethanol is highly flammable. Most freezers are not spark-proof. Consequently, you must ensure that any ethanol placed in a freezer is in a sealed, vapour-tight container. Use a screw-top plastic bottle and check it really is sealed by tipping it upside down.
An alternative to using a freezer is to stand the sealed bottle of ethanol in ice for several hours before use.

Procedure

Full details are given on the students' sheet.

Answers to questions

Question 1

Reasonable answers would list soluble proteins (including enzymes), inorganic salts, and degradation products from the plant pigments.

Question 2

To break open the cells to release the DNA.

Question 3

Nucleic acids are insoluble in cold ethanol. Reasonable explanations would refer to greater hydration forces of attraction and hydrogen bonding in water than in ethanol.

National Centre for Biotechnology Education (NCBE)
For TG buffer see 'Replacement items' on their website.
NCBE, School of Food Biosciences, University of Reading, Whiteknights, PO Box 226 Reading RG6 6AP
tel 0118 987 37 43
www.ncbe.reading.ac.uk