INVESTIGATING PHOTOSYNTHESIS

Purpose

- To investigate experimentally the link between the light-dependent and light-independent reactions.

SAFETY

DCPIP solution can stain, so avoid skin contact.

Wear eye protection.

Ensure the centrifuge has stopped before inserting or removing the tubes.

Write a risk assessment including any safety precautions. Discuss this with your teacher before starting.

YOU NEED

- Fresh green spinach, lettuce or cabbage leaves
- Scissors
- Cold pestle and mortar (or blender or food mixer) which has been kept in a freezer compartment for 15–30 minutes (if left too long the extract may freeze)
- Muslin or fine nylon mesh
- Filter funnel
- Centrifuge and centrifuge tubes
- Ice-water-salt bath
- Glass rod or Pasteur pipette
- Measuring cylinder, 20 cm^3
- Beaker, 100 cm^3
- Pipettes, 5 cm^3 and 1 cm^3
- Bench lamp with 100 W bulb
- 0.05 M phosphate buffer solution, pH 7.0
- Isolation medium (sucrose and KCl in phosphate buffer)
- DCPIP solution

Using isolated chloroplasts

It is fairly easy to show that plants produce oxygen and starch in photosynthesis. At KS4 you may have collected the gas given off by Canadian pondweed (*Elodea*) and tested leaves for starch. It is not quite so easy to demonstrate the other reactions in photosynthesis. In this experiment, DCPIP (2,6-dichlorophenolindophenol), a blue dye, acts as an electron acceptor and becomes colourless when reduced. DCPIP solution is added to isolated chloroplasts allowing any reducing agent produced by the chloroplasts to be detected. In the cell, NADP is the electron acceptor that is reduced in the light-dependent reactions and provides electrons and hydrogen for the light-independent reactions. This reaction was first demonstrated in 1938 by Robert (known as Robin) Hill and is often called the Hill Reaction.

1 Scientific questions and information research

Before you start the experiment you should research the background science relevant to the light-dependent and light-independent reactions of photosynthesis. You should think about the role of DCPIP in the experiment. It may help to look back at Activity 1.25 which used DCPIP to investigate the content of vitamin C in fruit juice.

Read through the procedure and predict what will happen in each of the five tubes, and give a reason to explain your prediction.

2 Planning and experimental design

Go through the procedure below and consider the following points. Explain your answers in each case.

- Is the apparatus and procedure appropriate for the investigation?
- What observations or measurements will you make and how will they be made?
- Are there any safety issues in the use of the apparatus?
- How would you reduce any risks identified?

Procedure

Isolating chloroplasts

Follow the instructions below to isolate chloroplasts from leaves. This may have already been done for you.

NB Keep solutions and apparatus constantly cold during the extraction procedure, steps 1–8, to preserve enzyme activity. The extraction should also be carried out as quickly as possible.

1 Cut three small green spinach, lettuce or cabbage leaves into small pieces with scissors, but discard the tough midribs and leaf stalks. Place in a cold mortar or blender containing 20 cm^3 of cold isolation medium (scale up quantities for blender if necessary).

2 Grind vigorously and rapidly (or blend for about 10 s).

3 Place four layers of muslin or nylon in a funnel and wet with cold isolation medium.

4 Filter the mixture through the funnel into the beaker and pour the filtrate into pre-cooled centrifuge tubes supported in an ice-water-salt bath. Gather the edges of the muslin, wring thoroughly into the beaker and add filtrate to the tubes.

5 Check that each centrifuge tube contains about the same volume of filtrate.

6 Centrifuge the tubes for sufficient time to get a small pellet of chloroplasts (10 minutes at high speed should be sufficient).

7 Pour off the liquid (supernatant) into a boiling tube being careful not to lose the pellet. Re-suspend the pellet with about 2 cm^3 of isolation medium, using a glass rod. Squirting in and out of a Pasteur pipette five or six times gives a uniform suspension.

8 Store this leaf extract in an ice-water-salt bath and use as soon as possible.

Using the chloroplasts

9 Read all the instructions before you start.

Note: The DCPIP solution should be used at room temperature.

10 Set up five labelled tubes as follows:

Tube	Leaf extract/ cm^3	Supernatant/cm^3	Isolation medium/cm^3	Distilled water/cm^3	DCPIP solution/cm^3
1	0.5	–	–	–	5
2	–	–	0.5	–	5
3	0.5	–	–	–	5
4	0.5	–	–	5	–
5	–	0.5	–	–	5

11 When the DCPIP is added to the extract, shake the tube and note the time. Place tubes 1, 2 and 4 about 12–15 cm from a bright light (100 W). Place tube 3 in darkness. Note any changes observed in the five tubes. Make any appropriate measurements to allow comparisons between the tubes.

12 Describe any unexpected safety issues that arose in carrying out the practical work. Explain how you dealt with them, including any advice you sought in dealing with them.

Analysis and interpretation of data

Present all your results in the most suitable format.

Conclusion and evaluation

In the write-up of your experiment, make sure your report includes:

- a clear conclusion to your work which is supported by evidence from the data and your own biological knowledge
- comments on how valid your conclusion is
- comments on the accuracy and precision of the results obtained in this experiment
- comments on whether or not the outcome of your work was as you expected – and if it wasn't, try to explain why not
- discussion about any safety precautions you took during the experiment
- descriptions of any modifications you made to the procedure and how the experiment could be improved.

Questions

Q1 The rate of photosynthesis in intact leaves can be limited by several factors, including light, temperature and carbon dioxide. Which of these factors will have little effect on the reducing capacity of the leaf extract?

Q2 Describe how you might extend this practical to investigate the effect of light intensity on the light-dependent reactions of photosynthesis.

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SAFETY

Review the students' risk assessments and discuss any safety considerations.

DCPIP solution can stain, so avoid skin contact.

Ensure eye protection is worn.

Ensure centrifuge is used safely and appropriately.

Notes on the procedure

Traditionally the production of oxygen and starch are used as evidence for photosynthesis. The Student Sheet for this activity describes the experiment used to investigate how the light-dependent reactions produce a reducing agent. This normally reduces NADP, but in this experiment the electrons are accepted by the blue dye DCPIP. Reduced DCPIP is colourless. Using extracted chloroplasts, this confirms the reduction that occurs in the light-dependent reactions. This experiment was originally completed by Robert (known as Robin) Hill in 1938; he concluded that water had been split into hydrogen and oxygen. This is now known as the Hill reaction.

Students must develop a clear understanding of the link between the light-dependent and light-independent reactions to be able to decide what observations/measurements are appropriate for the investigation, and to interpret the results. If colorimeters are not available, a qualitative colour assessment of the tubes could be done after 5–10 minutes.

This is a core practical, the focus is on correctly following instruction, selecting the appropriate measurements to make and interpreting the findings. On completion of the practical students could use the Developing Practical Skills Self-evaluation Sheet to reflect on what they have done in this practical. This can be found in the Practical Skills Support section of the online resources.

The use of carbon dioxide by *Elodea* can be demonstrated using hydrogen carbonate indicator; in the light the indicator turns purple as carbon dioxide concentration decreases. There is a good SAPS photosynthesis practical in which algae are immobilised, placed in hydrogen carbonate indicator and used to investigate the rate of photosynthesis. A detailed worksheet can be downloaded from the SAPS website. The URL is in the weblinks that accompany this activity.

Sample results

Colour change and inferences that can be made from the results:

Tube 1 (leaf extract + DCPIP): colour changes until it is the same colour as tube 4 (leaf extract + distilled water).

Tube 2 (isolation medium + DCPIP): no colour change. This shows that the DCPIP does not decolourise when exposed to light.

Tube 3 (leaf extract + DCPIP in the dark): no colour change. It can therefore be inferred that the loss of colour in tube 1 is due to the effect of light on the extract.

Tube 4 (leaf extract + distilled water): no colour change. This shows that the extract does not change colour in the light. It acts as a colour standard for the extract without DCPIP.

Tube 5 (supernatant + DCPIP): no colour change if the supernatant is clear; if it is slightly green there may be decolouring, see results on page 2.

The results should indicate that the light-dependent reactions of photosynthesis are restricted to the chloroplasts that have been extracted.

Using a bench centrifuge

The experimental procedure was followed. A standard lab centrifuge was used to spin down the chloroplasts (Clifton NE 010GT/I) at 2650 RPM, 95× *g* for 10 minutes.

The experiment was started within 5 minutes of preparing the chloroplasts. The reaction was followed using an EEL colorimeter with a red filter – readings taken every minute. Results for tube 1 and 5 are presented in Figure 1.

Time/min	Absorption	
	Tube 1	Tube 5
2	5.0	5.0
3	4.6	4.6
4	4.3	4.0
5	4.0	3.7
6	3.8	3.4
7	3.4	3.0
8	3.0	2.7
9	2.6	2.6
10	2.2	2.3
11	1.9	2.0
12	1.4	1.7
13	0.9	1.6
14	0.6	1.3
15	0.5	1.0
16	0.5	0.8
17	0.4	0.5
18	0.5	0.3
19	0.4	0.3
20	0.3	0.3

Tube 3 (incubated in the dark) gave a reading of 5.4 absorption units after 20 minutes. A colour change easily discernible by eye is obtained in about 10 minutes. If the colour change is too slow the DCPIP can be diluted a little.

Tube 2 (DCPIP with no leaf extract) was 6.2 absorption units.

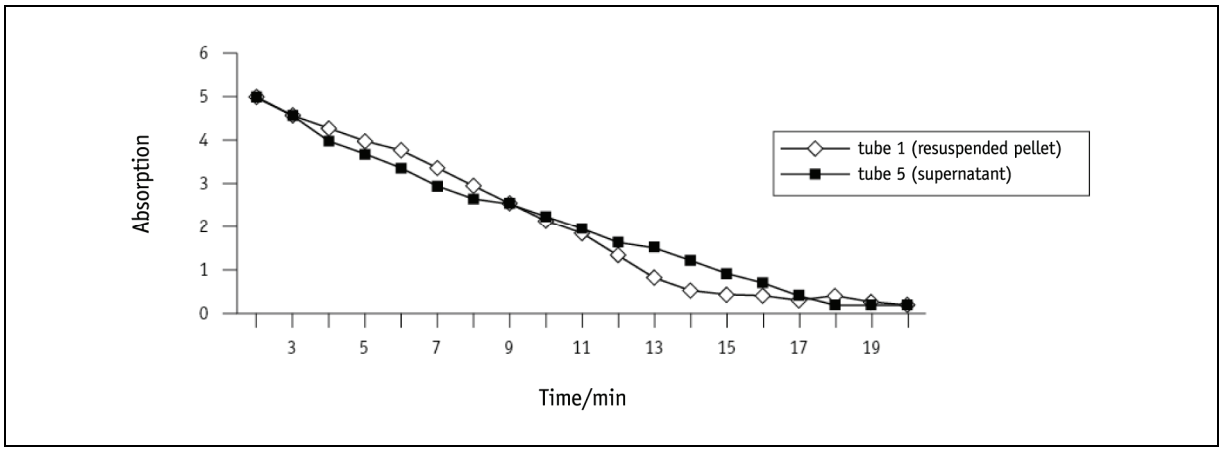

Figure 1 Results using bench centrifuge. DCPIP chloroplast extract spun for 10 minutes at 95× *g*.

Using a micro-centrifuge

The experiment was repeated using a micro-centrifuge. Results for tubes 1 and 5 are presented in Figure 2.

Time/min	Absorption	
	Tube 1	Tube 5
1	3.8	3.5
2	2.9	3.1
3	2.3	2.8
4	1.8	2.5
5	1.3	2.3
6	0.9	2.1
7	0.7	1.8
8	0.6	1.6
9	0.6	1.3
10	0.6	1.1

Tube 3 (incubated in the dark) gave a reading of 4.9 absorption units after 10 minutes.

Tube 2 (DCPIP with no leaf extract) was 6.4 absorption units.

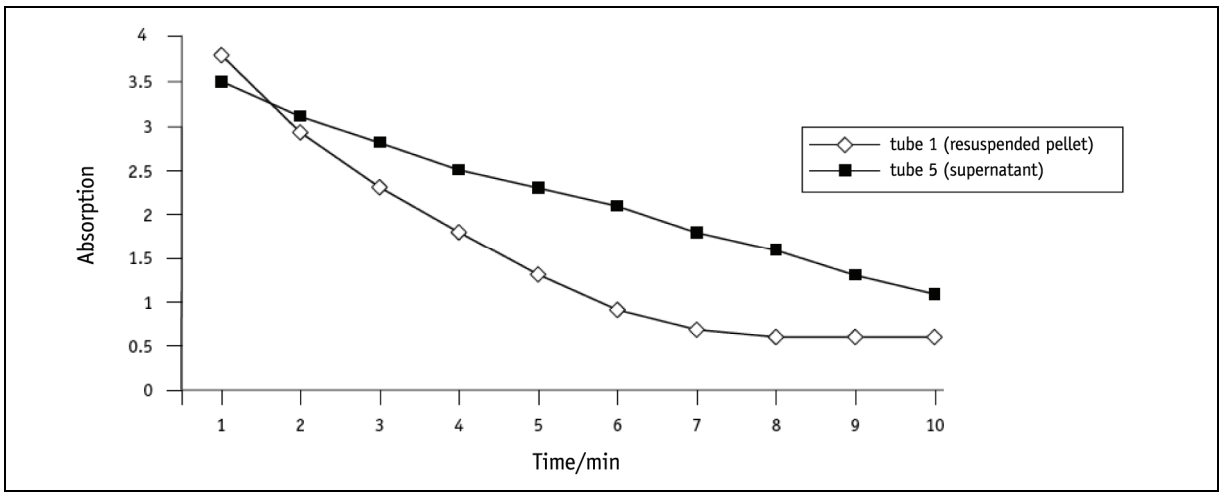

Figure 2 Results using micro-centrifuge. DCPIP chloroplast extract spun for 5 minutes at 2000× *g*.

The relative activity of the pellet was higher than when the bench centrifuge was used.

The micro-centrifuge tubes were only 1.5 cm^3 capacity – not ideal for this practical. A higher speed bench centrifuge would be better.

In order to check for loss of chloroplast activity, the experiment was repeated using the same chloroplast suspension 1 and 2 hours after preparation. Chloroplast suspension was kept in an ice-water-salt bath. There was no loss of activity when the extract was kept in ice for up to 2 hours.

Answers

Q1 Carbon dioxide will have no effect, because it is not involved in the light-dependent reactions.

Q2 Students should describe a procedure using isolated chloroplasts and DCPIP in which light intensity is varied, but temperature is controlled.

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See the Teacher Sheet for sample results and information on loss of activity of chloroplast suspension with time.

SAFETY

Wear eye protection and disposable gloves.

Although DCPIP solution presents minimal hazard apart from staining, it is best to avoid skin contact in case prolonged contact with the dye causes sensitisation. See CLEAPSS Hazcard 32 'Dyes, indicators and stains' for further information.

Ensure that the centrifuges are safe and suitable for the intended purpose.

Do not handle light bulbs with wet hands.

Requirements per student or group of students	Notes
3 small fresh green spinach, lettuce or cabbage leaves	Discard the midribs.
Scissors	
Cold pestle and mortar (or blender or food mixer)	Keep in a freezer compartment for at least 15–30 minutes before use.
Measuring cylinder, 20 cm^3	
Beaker, 100 cm^3	
Muslin or fine nylon mesh	
Filter funnel	
Centrifuge and centrifuge tubes	
Ice-water-salt bath	
Glass rod or Pasteur pipette	
0.05 M phosphate buffer solution, pH 7.0	$Na_2HPO_4.12H_2O$ 4.48 g (0.025 M) KH_2PO_4 1.70 g (0.025 M) Make up to 500 cm^3 with distilled water and store in a refrigerator at 0–4 °C.
Isolation medium (sucrose and KCl in phosphate buffer)	Sucrose 34.23 g (0.4 M) KCl 0.19 g (0.01 M) Dissolve in phosphate buffer solution (pH 7.0) at room temperature and make up to 250 cm^3 with the buffer solution. Store in a refrigerator at 0–4 °C.
DCPIP solution	DCPIP 0.007–0.01 g (10^{-4} M approx.) KCl 0.93 g (0.05 M) Dissolve in phosphate buffer solution at room temperature and make up to 250 cm^3. Store in a refrigerator at 0–4 °C. Use at room temperature. (NB KCl is a cofactor for the Hill reaction.)
5 test tubes	
Boiling tube	
Pipette for 5 cm^3 and 0.5 cm^3	
Pipette filler	
Waterproof pen	To label tubes.
Colorimeter and tubes or light sensor and datalogger	If students are following decolourisation over time.
Bench lamp with 100 W bulb	

For the experiment using immobilised algae and hydrogen carbonate indicator see the SAPS worksheets that can be downloaded from their website (see weblinks that accompany this activity.)