

Lesson Element

The Hill Reaction

Instructions and answers for teachers

These instructions should accompany the OCR resource 'The Hill Reaction' activity which supports OCR A Level Biology A and Biology B.



The Activity:

This experiment allows the teacher to introduce several topics of the Biology A specification, particularly section 5.2.1 Photosynthesis, and the Biology B specification, particularly section 4.3.1 Photosynthesis, food production and management of the environment.

It also gives students opportunities to demonstrate several of the skills and techniques required by the A Level Practical Endorsement in both Biology A and Biology B:

1.2.1 (b) safely and correctly use a range of practical equipment and materials

1.2.1 (c) follow written instructions

1.2.1 (j) use a wide range of experimental and practical instruments, equipment and techniques appropriate to the knowledge and understanding included in the specification

1.2.2 (f) use of qualitative reagents to identify biological molecules

CPAC 1 and 3

Associated materials:

The Hill Reaction Lesson Element learner activity sheet.



The Theory behind the Hill Reaction

This experiment investigates the light dependent reactions of photosynthesis which take place in the thylakoid membranes of chloroplasts. Chloroplasts are isolated from plant cells and exposed to light. The name 'Hill' comes from the scientist Robert Hill who developed the procedure and showed that isolated chloroplasts would continue to perform some of the reactions of photosynthesis if provided with the correct conditions *in vitro*.

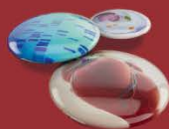
The Hill Reaction depends on electrons released during the light dependent stage of photosynthesis being picked up by the blue electron acceptor DCPIP. The reaction can only occur if the thylakoid membranes are illuminated as the light dependent stage stops in the dark (tube B in the procedure).

DCPIP is blue when oxidised (at pH 7.0) and colourless when reduced, so it is possible to monitor the loss of blue colour as an indication that DCPIP has accepted electrons. It can be used to participate in, and monitor, redox reactions.

In this experiment the DCPIP takes the place of NADP, allowing photolysis to continue even when the supply of NADP has been exhausted because the DCPIP can continue to accept the electrons from the electron transport chain.

The process of isolating the chloroplasts will inevitably cause some damage to the chloroplasts. Students will in fact be using a mixture of intact chloroplasts and thylakoid membranes without the surrounding stroma and outer membranes. This is actually an advantage because it means that the DCPIP can access the thylakoid membranes directly, without having to pass through the outer membranes, to accept electrons directly from the electron transport chain.

The effect of heat is a good discussion point. Assuming that tube C fails to decolourise DCPIP we can conclude that the light dependent reactions have ceased. The 'easy' answer to explain this is to talk about denaturing enzymes. Note that the enzymes of the light independent reactions will indeed be denatured by boiling, but this is irrelevant in this case as we are using DCPIP to 'decouple' the light dependent reactions from the light independent reactions. Therefore it is the denaturing of proteins involved in the light dependent reactions which will affect this experiment. Much of the light dependent stage is based on electron transfer rather than enzymatic reactions so it is less sensitive to temperature.



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However if students can picture the devastating consequences of boiling for the thylakoid membrane (catastrophic destruction of the bilayer as well as gross structural changes to integral and extrinsic membrane proteins) they should appreciate that it is unlikely that this exquisite, intricate biological marvel will still be functioning well!

Using ice cold phosphate buffer solution and ice cold sucrose solution is necessary to slow enzyme action and prevent damage to the chloroplasts before the experiment begins.

There are no lysosomes in the chloroplast solution since the chloroplasts are isolated from the rest of the plant cell.

The theory of centrifugation and differential centrifuging can be discussed if an ultra-centrifuge is not available and an alternative method of extracting the chloroplasts can be used:

- Use a blender to blend 25 g of leaves with 100 cm³ of ice cold 0.5M sucrose solution for one minute.
- Filter through a nylon stocking or muslin or a double layer of cheesecloth.
- Use 5 cm³ of this solution in each of the tubes A, B and C.

This will not produce a pure extract of chloroplasts since all the cell debris removed during the ultra-centrifuge process will remain apart from the largest of the cell wall fractions, however, this can still be used for the experiment.

Other suggested activities

This experiment allows the teacher to introduce several topics of the Biology A specification, particularly section 5.2.1 Photosynthesis, and the Biology B specification, particularly section 4.3.1 Photosynthesis, food production and management of the environment.

The chloroplast extract from this experiment could be filtered using fine muslin and used to spot chromatography paper and run as a chromatograph to identify the various pigments found in chloroplasts. The extract could also be used in a spectroscope to illustrate the absorbance spectrum of chloroplasts and so the wavelengths used in photosynthesis. The red fluorescence that can be observed demonstrates the electrons emitted during the light dependent stage of photosynthesis.



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Light intensity could be varied by moving the lamp used for illumination various distances away from the tubes of chloroplasts and DCPIP. The distance of the lamp from the reacting tube could be measured and the actual value of the light intensity can then be calculated using the calculation of $1/\text{distance squared}$.

The effect of changing the pH could be investigated, using a buffer and increasing the number of tubes with a tube of chloroplast extract and DCPIP for each pH buffer used.

The use of DCPIP and other electron acceptors in redox reactions can be discussed. Redox reactions are reactions where **reduction** and **oxidation** take place. This will allow discussion of the electron transport chain in respiration, as well as the electron carriers in the light dependent reaction of photosynthesis.

Materials

- 5 g of freshly chopped leaves, such as lettuce, nettles or spinach leaves
- 12 cm^3 of ice cold 0.5 M sucrose solution
- 20 cm^3 of ice cold phosphate buffer pH 7.0
- 30 cm^3 0.1% DCPIP

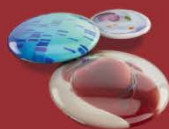
Apparatus

- pestle and mortar
- 3 cm^3 , 5 cm^3 and 10 cm^3 graduated pipettes
- teat pipettes (for collecting the supernatants)
- 3 x test tubes, labelled A, B and C
- Bunsen burner, tripod and gauze
- small beaker approximately 25 cm^3
- centrifuge tubes
- bench lamp
- an ultra-centrifuge, which allows varying times for centrifuging and varying speeds. The medium speed should be approximately 1500 G, whilst the high speed should be approximately 3000 G.

Or for the alternative method of extracting chloroplasts;

- a blender
- muslin, cheesecloth or nylon stocking for filtering
- 250 cm^3 beaker for filtering

Version 2



Health and Safety

Teachers are advised to try out all experiments prior to using them in class.

A full risk assessment should always be carried out prior to practical work.

Procedure

1. Grind 5 g of freshly chopped leaves with 10 cm³ of ice cold 0.5 M sucrose solution using a pestle and mortar
2. Strain the resulting liquid into a cooled centrifuge tube and centrifuge for 5 minutes at medium speed
3. Decant the supernatant into another cooled centrifuge tube and centrifuge for a further 10 minutes at high speed
4. Carefully pipette off the supernatant of this tube and discard. Now, using the sediment, add 2 cm³ of the ice cold sucrose solution and mix gently
5. Add 20 cm³ of ice cold pH 7.0 phosphate buffer solution and mix well
6. Add 5 cm³ of the chloroplast/ice-cold phosphate buffer solution into each of the test tubes labelled A, B and C
7. Take tube C and boil for five minutes using a water bath made of a small beaker over a Bunsen burner and then cool the tube under a running tap
8. Add 10 cm³ DCPIP to each of tubes A and B and C
9. Leave tubes A and C under a bench lamp to make sure they are well lit
10. Put tube B in a dark cupboard
11. Keep observing tube A until the blue colour of the DCPIP has completely disappeared
12. At this stage remove tube B from the cupboard and compare all three tubes
13. Extension: cover tube A and B in aluminium foil to exclude light and see whether over time the DCPIP in tube A returns to its oxidised state.

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