The Hill Reaction

Biology Summer School

June, 2013

***Prior learning****:*

Learners will already understand that in the presence of light energy green leaves will synthesise carbohydrate and that this process requires the presence of water and carbon dioxide.

In simple terms, this can be represented as a summary word equation,

light energy (absorbed by chlorophyll)

 Carbon dioxide + water  **→** sugar + oxygen

Or,

light energy (absorbed by chlorophyll)

 6CO**2** + 6H**2**O **→** C**6**H**12**O**6** + 6O**2**

Learners will have participated in practical investigations which illustrate the uptake of carbon dioxide and the evolution of oxygen. They will understand that the simple sugars produced in the process of photosynthesis are further synthesised to starch, and they will have tested leaves kept in different conditions for the presence of starch.

From the study of photosynthesis at ***National 5*** learners will understand:

*a. The chemistry of photosynthesis, as a series of enzyme-controlled reactions, in a two-stage process.*

***Light reactions****: the light energy from the sun is trapped by chlorophyll in the chloroplasts and is converted into chemical energy in the form of ATP. Water is split to produce hydrogen and oxygen. Excess oxygen diffuses from the cell.*

***Carbon fixation****: hydrogen and ATP produced by the light reaction are used with carbon dioxide to produce carbohydrate.*

*b. The chemical energy in carbohydrate is available for respiration or can be converted into plant products such as starch and cellulose.*

*c. Limiting factors: carbon dioxide concentration, light intensity and temperature and their impact on photosynthesis and cell growth.*

***The Hill Reaction Curriculum links, Revised Higher Biology***

***Unit 3 Sustainability and Interdependence***

*(b) Plant growth and productivity (i) Photosynthesis*

*Absorbed energy excites electrons in the pigment molecule. Transfer of these high-energy electrons through electron transport chains releases energy to generate ATP by ATP synthase. Energy is also used for photolysis, in which water is split into oxygen, which is evolved, and hydrogen, which is transferred to the coenzyme NADP*+*.*

***Background***

The Hill reaction provides an excellent context in which learners can begin to engage with the more complex biochemistry of photosynthesis.

Working in the 1930s, Robert Hill was able to demonstrate that isolated chloroplasts will generate oxygen in the presence of light and a suitable electron acceptor. He also demonstrated that this will happen even if no carbon dioxide is present thus confirming that the oxygen which is generated comes from water rather than from carbon dioxide.

2H20 + 2A 2AH2 + O2

‘A’ represents an artificial electron acceptor, for example, 2,6-dichlorophenol-indophenol (DCPIP) which is blue in its oxidised form and colourless when it is reduced (DCPIPH2).

 DCPIP + 2H+ + 2e- DCPIPH2

***Hill’s conclusions and findings***

* When leaf extract was illuminated, a non-biological electron acceptor (dye) became colourless and oxygen was evolved
* In the dark neither reduction of the dye nor evolution of oxygentook place
* Oxygen was neither required nor was it reduced under these conditions i.e. oxygen evolution did not involve carbon dioxide

It was later shown that NADP+ is the biological electron acceptor in chloroplasts.

 light

2H2O + 2NADP+ 2NADPH + 2H+ + O2

***Light reactions of photosynthesis***

* Light energy is absorbed by chlorophyll and other pigments creating excited electrons
* High energy electrons are transferred through an electron transfer chain releasing energy and generating ATP
* Energy is also used to split water (photolysis) into oxygen which is released and hydrogen which is accepted by NADP+ to form NADPH

 ***Carbon fixation – Calvin cycle***

Carbon is fixed when carbon dioxide enters the Calvin cycle by attaching to ribulose bisphosphate (RuBP). The enzyme that catalyses this reaction is rubisco (ribulose bisphosphate carboylase / oxygenase). ATP and NADPH from the light reactions provide energy and hydrogen for the subsequent synthesis of sugars.

***Demonstrating the Hill reaction***

***Equipment***

* Fresh spinach leaves
* Scissors
* 5 x test tubes and parafilm covers
* 1 x 100 cm3 beaker
* 1 x large beaker
* 1 x microcentrifuge tube
* 1 x foam tube holder
* 1 x 10 cm3 syringe
* 5 x 1 cm3 disposable pipettes
* Mortar and pestle
* 1 x filter funnel
* 1 x bijou bottle
* Muslin
* Metal foil
* Stopwatch
* Bench lamp
* Crushed ice
* Centrifuge
* Ice bath
* Water bath set at 800 C
* DCPIP solution
* Buffer solution

***Method***

***NB It is essential that all solutions, tubes and other equipment are chilled beforehand and kept in melting ice until the DCPIP reduction stage.***

1. Chop 3 fresh spinach leaves (excluding stems) into a mortar. Add 10 cm3 of buffer solution and grind with the pestle.
2. Quickly strain the mixture through muslin held in a filter funnel over a small beaker. Squeeze the liquid through the muslin by gathering the corners and twisting them. Discard the solid contents.
3. Fill the microcentrifuge tube with the suspension. Label the tube with your initials. Centrifuge the suspension for 10 minutes.
4. While the centrifuge is running, prepare 5 test tubes for the next stage as follows:
* Label the test tubes 1 – 5
* Cover test tube 2 with tin foil to exclude light
* Place all 5 tubes in a large beaker of iced water
* Using the syringe, add 3 cm3 buffer solution to tubes 1, 2, 4 and 5. Add 4 cm3 buffer solution to tube 3.
* Using a clean pipette, add 2 cm3 DCPIP to tubes 1, 2 and 3.
* Using a clean pipette add 2 cm3 distilled water to tube 4.
1. Once the 10 minute centrifuge run is complete, pour off the supernatant taking care not to lose the pellet. Discard the supernatant.
2. Place 5 cm3 buffer solution in the bijou bottle. Using a pipette add a little of this buffer solution to the pellet in the microcentrifuge tube and mix to re-suspend the pellet. Add this mixture to the buffer solution in the bijou bottle and mix well to produce an appropriate volume of chloroplast suspension.
3. Using a fresh pipette, add 1 cm3 chloroplast suspension to tube 5. Cover the tube with parafilm and place it in the water bath for 2 minutes. Allow to cool. Add 2 cm3 DCPIP. Place the tube in the beaker of iced water.
4. Now add 1 cm3 chloroplast suspension to tubes 1, 2 and 4.
5. Cover all 5 tubes with parafilm, shake each gently and note the starting colour of the contents.
6. Replace the tubes in the beaker of iced water ensuring that they are lying against the inner surface of the beaker.
7. Place the beaker in bright light and examine the contents of the tubes for disappearance of the blue colour after 5 minutes, 10 minutes and 20 minutes.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Contents | Tube1 | Tube2 | Tube3 | Tube4 | Tube5 |
| Covered with tin foil | No | **Yes** | No | No | No |
| Buffer solution | **3 cm3** | **3 cm3** | **4 cm3** | **3 cm3** | **3 cm3** |
| DCPIP | **2 cm3** | **2 cm3** | **2 cm3** |  | **2 cm3 \*** |
| Distilled water |  |  |  | **2cm3** |  |
| Chloroplast suspension | **1 cm3** | **1 cm3** |  | **1 cm3** | **1 cm3** |
| Heated to 800C for 2 minutes \*\* | No | No | No | No | **Yes** |

**\***Add DCPIP to tube 5 after heating buffer and chloroplast suspension mixture.

\*\*Heat tube 5 before adding chloroplast suspension to tubes 1, 2 and 4.

***Results***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | Tube 1 | Tube 2 | Tube 3 | Tube 4 | Tube 5 |
| Starting colour |  |  |  |  |  |
| Colour after 5 minutes |  |  |  |  |  |
| Colour after 10 minutes |  |  |  |  |  |
| Colour after 20 minutes |  |  |  |  |  |

 **DCPIP** (dichlorophenolindophenol) **solution**: 0.01 g DCPIP in 100 cm3 of buffer solution.

 **Buffer solution:** 2.8 g anhydrous disodium hydrogen phosphate (Na2HPO4),

* 1. g potassium dihydrogen phosphate (KH2PO4), 102.8 g sucrose, 37g potassium chloride, 1 litre water.