Investigation of the hill reaction

Science, Biology

Theory: Photosynthesis is a process carried out in plants, in the photosynthetic organ which is the leaf.

$CO_2 + H_2O \rightarrow (CH_2O)_n + O_2$

A plant takes in CO_2 and H_2O and absorbs sunlight in order for photosynthesis to take place. These reactants then travel to the chloroplasts in the palisade cells of the leaf, where the actual process takes place. In photosynthesis there are two reactions; the light dependant reaction and the light independent reaction. The light dependant reaction takes place in thylakoids of the grana of the chloroplasts whereas the light independat takes place in the stroma.

The thylakoids of the chloroplasts contain pigments which can be divided into accessory and primary pigments. These pigments form light harvesting clusters which can then be divided into different photosystems, those being photosystem I and photosystem II.

In photosystem I, the accessory pigments trap energy from the sunlight and funnel it to the primary pigment or chlorophyll a. This then becomes excited and releases an electron which is accepted by an electron acceptor. The electron then moves through a series of electron carriers arranged in order of their redox potentials. Meanwhile, the photosystem is said to be unstable as a result of chlorophyll a losing an electron. The electron then moves back down the series of electron carriers to return to stability producing energy thus A. T. P.

Photosystem II differs in that the process is non cyclic (i. e. the electron emitted from chlorophyll a does not return to chlorophyll a), it produces NADPH and O2 as a by-product in addition to A. T. P. and photosystem I is in fact a component of photosystem II. In this case the electron emitted from chlorophyll a travels through a series of electron carriers and then is accepted by a hydrogen ion (which is then picked up by NADP+) and this upsets the equilibrium for the splitting of water, causing more water to split. However, the electrons harvested from the splitting of water goes to photosystem II(which would have been energized to release an electron simultaneously with photosystem I), which would have been unstable. Also, the electron that left chlorophyll a from photosystem I would have passed through a series of electron carriers, arranged in redox potentials, to stabilize photosystem I producing A. T. P.

The NADPH and A. T. P are then transported to the Calvin cycle.

Apparatus:

* Filter funnel

* Muslin

* Mortar/pestle

* Ice/salt water bath

* Lamp

* Test tubes

* Foil

Materials:

* Lettuce

* DCPIP (blue)

* Isolation Medium

* Chloroplast suspension

Method:

1. The chloroplast suspension was prepared and placed in test tubes covered with aluminium, then placed in salt/ice water.

2. 0. 5 ml of the suspension was then placed in another test tube followed by 5 ml of DCPIP.

3. The foil covering that tube was then removed and the exposed solution in the test tube was placed next to the light source of the lamp at a measured distance of 10 cm.

4. The time for taken for the blue DCPIP to turn from blue to colourless was then observed and recorded.

5. 0. 5 ml of the isolation medium was placed in a test tube followed by 5 ml of DCPIP solution.

6. This solution was then placed at a distance of 10 cm away from the light source and the time taken for a colour change was observed and recorded.

7. 0. 5 ml of the chloroplast suspension was placed in a test tube followed by 5 ml of DCPIP. This was then placed in the dark and the aluminium foil was not removed.

8. The time taken for a colour change from blue to green was then observed and recorded.

Diagram:

Diagram 1. 0 shows setup of apparatus to check time taken for colour changes in tubes.

Results:

Tube

Time taken for colour change from blue to colourless/min

10 cm away from light source

1. 53

100 cm away from light source

15. 55

Isolation in medium only

No change

Tube in dark

No change

Table shows different times for colour changes to be observed.

Discussion: Photosystem ll as mentioned in the theory, NADPH and 02. In this process light strikes both photosystem l and ll, the energy is funneled down by the accessory pigments to the primary pigment and this causes the primary pigment from PS l and PS ll to release an electron. The electron from PS l is accepted by an electron acceptor and then passes through a series of electron carriers arranged in their redox potentials. The electron then is accepted by another electron acceptor and then neutralizes the H+ in the splitting of water.

H2O - 2H+ + 2e- + O2

This H is then picked up by NADP and forms NAPH. NADP is therefore an electron acceptor. As the H is picked up, this disturbs the equilibrium causing more water to split.

However, Robert Hill discovered, that isolated chloroplasts can in fact, liberate oxygen, from the equation above, in the presence of an oxidizing agent or an electron acceptor. In photosynthesis this is NADP but another one, that being DCPIP can be used in isolated conditions. In this process, the DCPIP which is blue becomes reduced DCPIP which is colourless. This is known as the Hill reaction.

In the experiment, the mortar/pestle was used to grind the lettuce and the muslin is used to collect the isolation medium. The isolation medium is a solution made up of sucrose and potassium chloride dissolved in a phosphate buffer. The isolation medium provides a bufferedenvironmentwhich does not allow the chlorophyll to leave the lettuce leaves.

In this experiment, the light source used was a lamp. In this case, the light intensity was varied by changing the distance from the light to the tube. The relationship between the amount of light and the distance is said to be one that obeys the inverse square law. For example if 10 cm were 100 units then 20 cm would be 10 units. With all other factors being constant and optimum, the light intensity is said to be a limiting factor, i. e. a factor that would the control the rate of photosynthesis. Therefore as the light intensity increases, so should the rate of photosynthesis thus, as the distance of the tube from the light source decreased, the time taken for the colour change to take place should have taken longer, which was the case. It can be observed that as the distance increased to 100 cm away from the lamp, the time taken for the colour change at 10 cm, 1. 53 minutes increased to 15. 55 minutes.

The investigation then goes on to show that in the presence of the isolation medium only, in the tube there was no colour change at all. This is because the isolation medium contained no chlorophyll for photosynthesis to take place. However, when the tube with the chloroplast suspension which includes the isolation medium was placed in a tube, but in the dark, there was no colour change either. This shows that light is needed for photosynthesis to take place.

Precautions:

* The amount of chloroplast suspension and DCPIP placed in the tube was standardized.

* The distance of the light source from the tube was ensured with a centimeter ruler.

* The salt/ice water was used to preserve the samples so that no biological activity would have taken place before the experiment had taken place.

* The aluminium foil was used so that no light would have entered the sample before the experiment started.

Limitations:

* The DCPIP was blue in colour and the chloroplast suspension was blue in colour. Therefore, the colour change was not from blue to colourless and this could have affected one'sobservationof the colour change.

Sources of error:

* The experiment was done during the day and sunlight entering the room could have affected the results in some way of it had any exposure to the test tubes.

Improvements:

* It could have been ensured that the room was completely dark or sunlight was not reaching the tubes in any way.

* The amount of light could have been reduced by putting layers of tissue paper between the light and the test tube to produce a range of light intensities.