Investigating the Effect of Temperature on the Permeability of Membranes

The permeability of membranes can be altered in several ways. From previous preliminary work I know that reagents, for example detergents and alcohol alter the arrangement of the phospholipids in the membrane allowing substances to leak out of the cell. Temperature also affects the membrane. As membranes relay on protein molecules to allow molecules to enter and leave the cell through facilitated diffusion the temperature must not exceed the approximate temperature of 50°C as at roughly this temperature proteins are denatured. This is due to an increase in kinetic energy making atoms in the protein to vibrate more; this breaks hydrogen and ionic bonds. These proteins will then not be able to function correctly within the membrane and permeability will increase, as substances are able to move more freely out of the cell. The temperature at which cell membranes are placed at also affects the phospholipids bilayer in the cell; this is due to the fluidity of the membrane. A cell membrane will remain fluid as the temperature around it decreases until it reaches a critical temperature at which a membrane solidifies, due to its fatty acid composition. This means that at temperatures below this critical point no dye in the beetroot cell would be able to leak from the cell and would not change the colour of the surrounding water. The phospholipids also undergo phase changes at higher temperatures causing the membrane to be more permeable thus allowing the red pigment to move more freely into the water surrounding the beetroot.

Sources of Background Information

New Understanding Biology for Advanced Level Fourth addition By Glenn and Susan Toole

http://telstar.ote.cmu.edu/Hughes/tutorial/cellmembranes/

To investigate the effects of temperature I will be carrying out the following experiment on beetroot tissues to observe the movement of the red pigment out of the cells at different temperatures to see is the above theory is correct of temperature, and that at higher temperatures the red pigment will leak into the area surrounding the beetroot.

Equipment

1.Beetroot, to use the cells to investigate how temperature alters the permeability of the cell membrane. Beetroot will be used, other then another cell sample as the red pigment contained in the cells can be observed moving form the cells if the membrane is altered.

2.Cork Borer, to cut sections of the beetroot out, this will be used to ensure the cylinders of beetroot are of equal diameter as I know from my pilot study that if they are not of the same size it will not be a fair test.

3.Ruler, to measure the lengths of the beetroot to ensure they are of equal length as well as diameter, as by making the lengths of beetroot the same length it will improve the accuracy of my results as it will be a fair test.

4.Scalpel, to cut the beetroot cylinders to the desired length.

5.Cutting mat, to safely cut the lengths of beetroot to ensure no damage is done to other work surfaces.

6.15 test tubes, these will be used to put the beetroot in whilst in the water bath, 15 will be needed as three samples of beetroot are put into 5 different temperature water baths in order for an average to be taken at each temperature to gain more accurate results. These will be used instead of boiling tubes or any other apparatus, as they are able to fit into the colorimeter to measure they colour transmission of the water.

7.Water baths set at five different temperatures, to place the three samples of beetroot into to test the affect of temperature. These will be used over any other methods, as they are able to keep a constant temperature.

8.Thermometers, to check the water baths are at the desired temperatures before the beetroot samples are placed in the water.

9. Stop clock, to time how long each sample of beetroot has been in the water so they are in for the same length of time also to ensure a fair test.

10.Colorimeter, to test the colour of the water the beetroot has been placed in after the experiment to see how much dye has escaped the cell. This will be used, as it will provide me with a set of figures that a mean and standard deviation can be taken from in order to produce more accurate results.

11.Samples to set the colorimeter, a sample of clear liquid, water and a liquid containing a deep colour will be needed to set the scale of the colorimeter, the water will have a high transmission and set it to 100% and the deep colour will give a low transmission and set it to zero (0%)

12.Syringe, to measure out volumes of water, this will be used as it provides a more accurate measurement than a beaker.

13.Test tube rack, to store the samples of beetroot when they are taken from the water baths to ensure nothing is spilled.

14.Tongs, to remove the test tubes from the water baths to minimise the risk of burns from hot water.

Risk Assessment

Care will need to be taken when cutting lengths of beetroot with a scalpel. Beetroot should be cut in a direction away from the body to minimise the risk of cutting yourself. A cutting mat should be used as not to damage surfaces underneath by cutting or stained by any of the leaked pigment as a result of cutting the beetroot. Plastic gloves and apron

may be used to avoid the staining of hands and clothes with beetroot pigment. Care will also need to be taken when placing and removing samples of beetroot in hot water, tongs should be used to avoid burns.

Diagram

Method

Firstly the equipment needed should be gathered in order to carry out the experiment. The beetroot will then be placed on the cutting mat and using the cork borer several lengths of beetroot should be cut out. The lengths should then be placed on the mat and measured to the length of 6mm; they should then be cut with the scalpel so there are 15 samples all measuring 6mm and of the same diameter, All the lengths should be equal so it is a fair test as the same amount of beetroot is used in each experiment.

Before placing each length of beetroot into the test tubes they should be rinsed with water so that any excess dye that may have leaked out due to damage caused by cutting with the scalpel will not move into the surrounding water and alter the accuracy of the results as then the experiment will not be fair and the test would then not be able to support a conclusion.

The lengths of beetroot will then be placed into a test tube containing 8cm3 of water. The water will be measured out previously with a syringe to ensure equal accurate amounts are used in each test. The test tubes containing the beetroot samples will be placed in five different water baths at the temperature 30°C, 40°C, 50°C, 60°C and 70°C.

The temperature of the water will be the independent variable as I am able to control what temperatures are used; the dependent variable in this experiment will be the leakage of pigment that will be measured by the colorimeter to enable me to find out a relationship between the two. Other independent variables that could be investigated to find the affects they have on membrane permeability are the pH of the solution the beetroot is kept in whilst keeping the temperature constant or an experiment to investigate whether the surface area has an affect of the leakage of pigment in beetroot.

The water baths will be checked using a thermometer before the samples are placed in the water to ensure they are desired temperature. After this the first three samples should be placed into the water bath at 30°C, the stop clock should then be started. When leaving the beetroot in the water baths it must be ensured that they are in the water for exactly the same amount of time, 10 minutes, to ensure a fair test. This will make sure that the same period of time is given for pigment leakage to occur. To control this, putting the samples of beetroot into each water bath at two minutes intervals will ensure enough time for them to be removed before the next test tube is ready to have its contents removed. When the stop clock reads two minutes the second set of three test tubes should be placed into the water bath at 40°C. When the clock reads four minutes the next set of three should be placed into the 50°C water bath. When the clock reads six minutes the next set should be placed into the 60°C water bath and at eight minutes the final set of test tubes should be

added to the final water bath. After a further two minutes, when the clock reads ten minutes the first set of test tubes should be removed from the water and placed in the test tubes rack, the other samples should then be removed after further two minute intervals.

The beetroot should then be removed from the test tubes of water and then placed in the colorimeter they will obstruct the amount of light that can flow through the samples.

The colorimeter should then be set up in order to test the transmission of light that can pass thought the solution. The colourimeter measures the amount of light that passes through the solution, if much light can pass through the sample taken it has a high transmission and if a little can pass through it has a low transmission. In order to set this up the sample of water should be placed into the colorimeter and the scale set to 100%. The second sample of high colour or a cloudy solution should be placed inside and the scale set to 0%. This will then be used to see what transmission of light can pass through the samples of water taken from the water bath and thus how much pigment has been able to leak from the beetroot cells to see if the permeability of the membrane has changed at all.

The results taken from the different samples will then be recorded into a table. To improve the accuracy of my results three samples were placed in each temperature water bath. To gain an average the three readings will be added together and divided by three to produce a more reliable result, a more reliable results are needed so they are able to support a conclusion. I will also work out the standard deviation of the results to find out the spread of the data from which I calculated a mean.

For example 7,8 and 9 give a mean of 24 and a low standard deviation that shows a normal distribution of data of results with no anomalous results. However 1,19 and 4 also give a mean of 24 but a high standard deviation this shows the results may not be as accurate as anomalies are present. This will show me that results taken are accurate if they show a low standard deviation, in order to reach a conclusion.

Due to afore mentioned background information and observations made of the colour of water in my pilot study I predict that as the temperature increases to 50°C and above the proteins in the membrane will be denatured and the permeability of the membrane changed. Therefore I predict that more pigment will leak into the water as the temperatures rise, therefore the less light will pass through the sample and will give a low colour transmission.