

Plasmolysis and
membrane
permeability of
plasma biology essay



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Plasma membranes are bi-layered membranes made up of amphiphilic molecules (having charged polar heads tending to be hydrophilic and uncharged fatty acid tails tending to be hydrophobic) that selectively allow entrance of certain large molecules into the cell's cytosol and through which water and small non-polar molecules may freely diffuse.

This experiment seeks to understand limited aspects of the permeability of the plasma membrane using the Elodea leaf membrane as model organism. Some of the factors upon which permeability of the plasma membranes of biological organisms depend are differences in pH on opposite sides of the membrane, temperature, osmolarity, expression of certain membrane receptors and the concentration gradients of various molecules.

This experiment is very limited in scope and seeks to answer only the question of what is the time dependence for permeability of glycerol through the cell membrane. Other experiments have answered many of our questions regarding this and have resulted in mathematical equations describing these results[i]. This experiment will use one of the formula derived from these prior experiments, the Ether: Water partition coefficient for alcoholsⁱⁱⁱ as a means of hypothesizing what the outcome of this present experiment will be.

I have hypothesized that within seconds of exposure to a 0.3M (molar) hyper-tonic solution of glycerol, dissolved in an isotonic deionized water (dH₂O)/sucrose solution, the Elodea leaf will plasmolyze irreversibly-an assumption I believe is supported by the fact that glycerol's ether: water partition coefficient is only 0.00066ⁱⁱⁱ. Further support for this supposition is

the fact that glycerol has a relatively bulky chemical structure^{viii}-owing to its three large, highly polar hydroxyl groups-and a large molecular weight of 92.0938 grams per mole. Alternatively, it may be hypothesized that the glycerol-being an aliphatic alcohol (see diagram in section IV(i) *infra*) which, itself makes up a part of the plasma membrane^{vi}-will be capable of more easily diffusing across the plasma membrane as compared to the sucrose, which cannot diffuse across the membrane, in which case not only will there be no severe plasmolysis but there may, instead, be a build up of turgor pressure inside the cell due to the inward movement of the alcohol and its confinement in the central vacuole.

Methodsⁱⁱ

In order to discover what molar concentration of sucrose will be needed in an aqueous solution to create a solution that is isotonic to the leaf's cytosol I shall perform a bifurcated experiment in which the first part shall be to determine this concentration. Part two of this experiment will be to determine the period of time it takes for glycerol to diffuse across the plasma membrane.

In order to determine which molar solution of sucrose is isotonic to the cytosol of the Elodea cell I labeled 6 micro-centrifuge tubes with the markings: 0.2M, 0.3M, 0.4M, 0.5M, 0.6M and "isotonic" respectively and using an adjustable pipette placed 1000 $\hat{1}$ /₄L of premixed sucrose solution of each of the indicated molarities into the respective tubes. In each of these tubes I placed an Elodea leaf and allowed them to sit for approximately five minutes [my observations of plasmolysis along with photographs of leaves in similar states to what I observed are provided in table 2 of the "Table of <https://assignbuster.com/plasmolysis-and-membrane-permeability-of-plasma-biology-essay/>

observations of plasmolysis” and photographs #2-#6 in the “ Photograph table” which can be found in sections III(A) & (B) respectively.]

While awaiting the leaves to finish soaking I viewed a dry mounted Elodea leaf under a microscope using 20X and 40X objectives with 10X ocular so as to have a better idea of what a normal Elodea leaf looks like for comparison to the viewing of the wet mounts [photo of a leaf in similar state to what I observed is provided as photo #1 in the “ Photograph table” of section III(B).] I then labeled 6 microscope slides using the same concentrations I used when labeling the micro-centrifuge tubes.

After five minutes I prepared an individual wet mount of an Elodea leaf by placing a leaf from a micro-centrifuge tube onto a microscope slide, bearing its respective molarity, with the upper surface of the leaf face up. I placed a cover slip over the leaf and gently tapped the cover slip so as to seat it onto the slide and to remove any excess solution. I then viewed the wet mount- searching for indications of plasmolysis-under a microscope using the same 20X and 40X objective lenses and the 10X ocular lens I had viewed the dry mount and recorded my observations then repeated this process for each of the leaves in the remaining tubes.

I was unable to obtain photos of my observations but I have included photos downloaded from the internet which were similar to what I had observed and provided them in tables 1-6 of section III(B).

Having established which molarity of sucrose solution was isotonic with the cytosol of the cell (see table in section III(A)) I calculated the quantities of sucrose, glycerol (test solution) and 1-Propanol (counter test solution) I
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would need for the second part of this study. In those calculations I used the data presented in table 1 below. My calculations are presented in the Table of Calculations, table 3 of section III(C) infra.

Solution

Condensed structure formula

Partition Coefficient

Molecular Wt.

Sucrose

$\text{OHCH}_2[\text{CH}(\text{OH})]_3\text{CHOHCH}_2[\text{CH}(\text{OH})]_3\text{COCH}_2\text{OH}$

N/A

342. 3g

Glycerol

$\text{OHCH}_2\text{CH}(\text{OH})\text{CH}_2\text{OH}$

0. 00066

92. 0938g

1-Propanol

$\text{CH}_3\text{CH}_2\text{CH}_2\text{OH}$

1. 9

60. 0950g

Table 1[iii]: Molecular weights of chemical solutions used.

I plugged the results I obtained from table 3 into the formula $C_1 \times V_1 = C_2 \times V_2$ so that I may calculate the volumetric quantity of each of these chemicals I would need to add to each of my two 1 x 10³ ¼L test solutions, my calculations for each may be found in Table 4 of section III(C).

Using those calculation I then added the quantities of sucrose to each of the other two chemicals and subtracted the sum from the final volume of solution (1000 ¼L) I would be creating so that I will know the volume of deionized water (dH₂O) I would need. Those calculations are shown in table 5 of section III(C).

Using these calculations I then prepared 5 new micro-centrifuge tubes as follows: 3 tubes each containing a 1000 ¼L isotonic (0. 4M) sucrose solution (one of which is to be used as a negative control); the fourth containing an aqueous solution of isotonic (0. 4M) sucrose and 0. 3M glycerol mixtures; and the fifth containing an aqueous solution of isotonic (0. 4M) sucrose and 0. 3M 1-Propanol mixtures (counter control). I placed one Elodea leaf into each of the 3 isotonic solutions and allowed them to soak for approximately five minutes. After five minutes I prepared a wet mount of the first of the 3 leaves as previously described. After viewing the first leaf (the negative control) I placed the second leaf on a slide and added 2 drops of the 0. 3M glycerol/Sucrose solution to the slide then viewed and recorded my observations. I then prepared the third leaf using 2 drops of the 0. 3M

glycerol/Sucrose solution and viewed to be certain I obtained the same result
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as the last slide then after approximately 30 seconds added 2 drops of 1-Propanol/Sucrose solution (the counter test solution) to see if this would have an effect opposing that of the glycerol/Sucrose solution and recorded my observations which I describe next.

Results

A. Table of observations of Plasmolysis.

Table 2[iv]: Plasmolysis observations within five minutes of Elodea using different sucrose solutions.

Sucrose concentrations

Plasmolysis observed (Y/N)

Sucrose concentrations

Plasmolysis observed (Y/N)

0. 2M

N

0. 5M

N

0. 3M

N

0. 6M

Y

0.4M

N

Isotonic

N

B. Photograph tables (Photographs[v]of Elodea leaves in various solutions):

1. Normal leaf

(similar observation as prior to placing in solution)

2. Hypo-tonic solution

(similar to observation as seen in <0.4M sucrose solutions)

3. Isotonic solution[vi]

(similar observation as in the “ isotonic” solution and the ~0.4M-0.5M sucrose solutions)

4. Hyper-tonic solution

(similar observation as seen in the 0.6M sucrose solution)

5. Plasmolysed leaf

(similar observation as would have been seen in hyper-tonic solutions)

6. Plasmolysis & Recovery

(did not observe any recovery events but this is what I would also have been looking for had plasmolysis & recovery taken place)

C. Tables of Calculations:

Sucrose

Total needed

0.4 M

342.3 g

1.0 L

0.13692 g

L

mole

1000 mL

Glycerol

Total needed

0.3 M

92.0938 g

1.0 L

0.02763g

L

mole

1000 mL

1-Propanol

Total needed

0.3M

60.0950g

1.0 L

0.01802g

L

mole

1000 mL

Table 3: Calculations for concentration of 0.3M glycerol/Sucrose solution.

Amount of sucrose needed:

$$[.137\text{g}] \times V = 0.4\text{M} \times .001\text{ L}$$

$$V = (.0004\text{g/L}) / (0.137\text{g}) = 0.002919\text{ L or } 2.91 \times 10^3\text{ mL}$$

Amount of glycerol needed:

$$[0.028\text{g}] \times V = 0.3\text{M} \times 0.001\text{L}$$

$$V = (0.0003\text{g/L}) / (0.028\text{g}) = 0.01071\text{L} \text{ or } 10.7 \times 10^3\text{ mL}$$

Amount of 1-Propanol needed:

$$[0.018\text{g}] \times V = 0.3\text{M} \times 0.001\text{L}$$

$$V = (0.0003\text{g/L}) / (0.018\text{g}) = 0.01667\text{L} \text{ or } 16.7 \times 10^3\text{ mL}$$

Table 4: Calculations of volumetric quantities of each chemical needed to make 1000 $\hat{1}$ /₄L of each solution.

$$2.92 \hat{1}/_4\text{L sucrose} + 10.7 \hat{1}/_4\text{L glycerol} + x(\text{dH}_2\text{O}) = 1000 \hat{1}/_4\text{L}$$

$$13.62 \hat{1}/_4\text{L} + x(\text{dH}_2\text{O}) = 1000 \hat{1}/_4\text{L}$$

$$x(\text{dH}_2\text{O}) = 1000 \hat{1}/_4\text{L} - 13.62 \hat{1}/_4\text{L}$$

$$x(\text{dH}_2\text{O}) = 986.38 \hat{1}/_4\text{L}$$

$$2.92 \hat{1}/_4\text{L sucrose} + 16.7 \hat{1}/_4\text{L 1-Propanol} + x(\text{dH}_2\text{O}) = 1000 \hat{1}/_4\text{L}$$

$$19.62 \hat{1}/_4\text{L} + x(\text{dH}_2\text{O}) = 1000 \hat{1}/_4\text{L}$$

$$x(\text{dH}_2\text{O}) = 1000 \hat{1}/_4\text{L} - 19.62 \hat{1}/_4\text{L}$$

$$x(\text{dH}_2\text{O}) = 980.38 \hat{1}/_4\text{L}$$

Table 5: Calculations of amounts to add to each solution.

Discussion

At first viewing I did not quite understand what was happening as I had not previously seen an Elodea leaf that presented without its large central vacuole let alone one that presented with chlorophyll throughout the entire cytosolic space. Having consulted with my fellow researchers (one of which obtained findings similar to mine in her experiment), none of whom had explanations for this result, I shall instead provide a summary of what I observed and what I had expected to observe.

I had expected my first hypothesis to be borne out regarding the outward movement of water across the membrane and toward the hyper-tonic glycerol solution providing a sighting as in photograph 5, however what I discovered was an Elodea leaf showing absolutely no sign of plasmolysis. Instead of the expected I saw what was a leaf that appeared to be in a state of iso-osmolarity with its environment which would have been expected only in an isotonic solution as in photograph 3. There, also, was no turgor pressure as would have been seen in photograph 2 had the alternative hypothesis of inward movement of glycerol across the plasma membrane been borne out.

Finally, had there been a plasmolysed cell the addition of the counter test solution of 1-propanol should have caused recovery as seen in photograph 6 but being I was unable to obtain a plasmolysed cell I was also unable to observe recovery of such cell.

The results of this experiment has left me unable to either accept or reject either of the two hypotheses provided above.

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Diagrams:

i. Phospholipid bilayer[vii]:

The following structures and coefficient information was obtained from The Royal Society (Publish) of London[viii].

ii. Structure of glycerol:

iii. Structure of 1-Propanol:

References cited: