Effect of Cytochalasin on Average Pseudopodia Length in *Amoeba Proteus*

Alex Waciega

Independent Research Project Report

Cell Biology 219

December 3, 2008

Introduction

In our experiment we studied the effects of the drug cytochalasin on *Amoeba Proteus*. Cytochalasin is a fungal toxin that acts within the cell by binding to the barbed end of the actin filaments in the actin cortex and prohibiting their elongation. (Cooper, 2007) The barbed ends of the actin filaments are where the ATP bound actin monomers bind to the growing polymer. If this end of the filament is capped by cytochalasin, this prevents the cell from polymerizing any new actin filaments and from extending the filaments it already has. (Cooper, 2007) As such, in this study we tested the hypothesis that if cytochalasin is introduced into the environment of *Amoeba Proteus* at a concentration of 40μg/mL, then the average length of the pseudopodia would decrease. Pseudopodia in *Amoeba Proteus* are formed by the polymerization of actin filaments within the areas of the plasma membrane that must be extended. Therefore, if cytochalasin is preventing the polymerization of actin filaments then the pseudopodia would not be able to form. (Cooper, 2007) This, we believe, will lead to an overall shortening of the pseudopodia. This experiment sheds light onto how cells move within their environments and the importance of actin in other cell processes. By preventing the cell from polymerizing its actin we can observe what effects this has on the cells movement, and the movement and function of its internal organelles.

The drug solution that we are using is a mixture of both cytochalasin and DMSO, or dimethyl sulfoxide. DMSO is a solvent molecule that is amphipathic and used extensively in biochemistry because of its ability to cross the plasma membrane so easily. It dissolves both polar and non-polar compounds and it is this attribute that makes it such a useful solvent. DMSO also has the unique ability to carry with it other molecules across the plasma membrane as well. This property of DMSO is used extensively in biochemistry and the pharmaceutical industry as a vector to administer drugs directly into cells. (Vignes, 2000) In this case, DMSO is carrying the large cytochalasin molecules across the plasma membrane directly into the cell. This makes the importation process much more efficient such that results are seen
sooner.

*Amoeba Proteus* is a single celled protist that uses pseudopodia to move around its environment and to envelope smaller organisms and food particles. (Durr, 2002) The process of extending and retracting pseudopodia is a complex process completed in several steps. The cell must first develop an internal polarity of the plasma membrane and actin cortex. Then the pseudopodium must extend outward and latch onto the surface which the organism is moving upon. Then the trailing edge of the cell must retract, thus moving the cell forward. (Cooper, 2007) We are studying this particular organism because of its mode of cellular movement. The pseudopodia are dependent of the polymerization of actin filaments in the cortex of the cell, which provides us with an easy way to measure the effectiveness of the cytochalasin. (Cooper, 2007) Also these cells are easy to observe and measure because of their massive size; the average size of an *Amoeba Proteus* is around 750μm across depending on the spot of measurement. (Burr, 2002) Both of these factors make this organism the ideal test subject for this experiment and give quantitative and decipherable results. By measuring the average length of the pseudopodia on the cell before the drug is added and comparing it to the average pseudopodial length after the drug is introduced, we can derive whether or not the cytochalasin had any effect on pseudopodial length. By averaging the lengths of the many pseudopodia on the cell we also get results that are more statistically significant.

**Materials and Methods**

In this experiment we used the organism *Amoeba Proteus* and studied them by constructing flow cells out of a slide, cover slip, cover slip chips, and VALAP. To construct the flow cells we placed cover slip chips on the slide using forceps and placed a cover slip on top of the chips, sealing it with the VALAP on the long sides of the slide. We used a solution of cytochalasin and DMSO (dimethyl sulfoxide) at a concentration of 40μg/mL as our experimental reagent. As our control reagent we used a solution of pond water and DMSO at the same concentration. To get the drug solution into the flow cell we used transfer pipettes and Kimwipes. We observed them under a Nikon Eclipse E200 and using a Sony DFWX700 we took digital images of the organisms using the program BTV. After the images were gathered we used Photoshop to generate a scale bar on the images.

For our experiment we constructed two flow cells, one as the experimental slide and the other as the control. Each were constructed the same way using cover slip chips under a cover slip and sealed by VALAP on two edges. The organisms were observed under 100x magnification for a few minutes to collect initial data. We then took digital images of one organism in each of the flow cells every fifteen seconds for two minutes. The drugs were then applied to...
the experimental slide using a transfer pipette to add two drops of the cytochalasin and DMSO solution to the edge of
the cover slip. We then used a Kimwipe on the opposite end of the cover slip to wick through the solution. This process
was repeated three times with the experimental slide, and also repeated three times in the control slide, but using the
pond water and DMSO solution instead. This was repeated three times so as to ensure a complete transfer of fluid and a
uniform concentration throughout the flow cell. The slides were allowed to sit for approximately twenty minutes each
to allow the cells to take up the drug. After the waiting period we put the experimental slide back onto the microscope
and again took digital images every fifteen seconds for two minutes. We then gave the cells another twenty minute
waiting period each, after which we again took digital images every fifteen seconds for two minutes each.

For data collection we used the scale bar we created in Photoshop to measure the lengths of the pseudopodia on
the cells in micrometers. We then averaged together the lengths of all these pseudopodia to get an average pseudopodial
length for that time in the experiment. These averages were then compared to each other to determine the effectiveness
of the drug. We then counted up the number of pseudopodia on each cell at the twenty minute intervals. We defined a
pseudopodium to be a projection of the plasma membrane that is 0.04mm in length or higher.

Our experimental conditions were an environment of DMSO and cytochalasin at 40\(\mu\)g/mL, and our control
conditions were an environment of simply pond water and DMSO. This control was the best possible one because it
shows that any results that were gathered we a product of the change in concentration of cytochalasin and not DMSO.
The pond water is simply the natural environment of this particular organism and thus is conducive to their health.

Results

![Average Pseudopodial Length vs. Time](image)

Figure 1.1: The average length of the pseudopodia of the cell at each time interval.
Figure 1.2: The number of pseudopodia that the cell had at each time interval. As time passed the number of pseudopodia of the cell in the cytochalasin dropped significantly and consistently while the control stayed fairly level.

Figure 1.3: (Scale bar=0.1mm) Each black line represents an example of where a particular pseudopodium was measured from. This picture is of the control group after 40 minutes with six pseudopodia over 0.04mm clearly visible.

Discussion

The results that we gathered refuted our hypothesis that the average pseudopodial length decrease after the cytochalasin was introduced to the environment. As can be clearly seen in Figure 1.1 the data actually showed a significant increase in the average pseudopodial length in the experimental group. However, as is shown in Figure 1.2 there was a significant and consistent decrease in the overall number of pseudopodia that the cell extended in the experimental group over time. The control group showed a fairly stable and slight increase in the number of pseudopodia extended. While these graphs clearly refute our original hypothesis, there seems to be a correlation between the cytochalasin and the actual number of pseudopodia extended rather than affecting their length.
If this is in fact the case, then it would seem that instead of limiting the reach of the cells pseudopodia, the cytochalasin slowly disables the cell from extending any pseudopodia at all. This would mean that once that part of the actin cortex in the cells cytoskeleton is inhibited by cytochalasin then there can be no more pseudopodial growth from that particular region. This would be a much different scenario than if it simply hindered the extension of pseudopodia; instead it appears to be instantly halting the process. For future experiments I would run this same procedure, but give the cells more time to react to the drug. In other words I would take measurements at 20, 40, 60, 80, and 100 minutes to make sure the drug had taken full effect. I would also do at least two more runs of this experiment so that our data were statistically significant.

The most significant source of error in this experiment is the vague nature of a pseudopodium itself. There is no definitive starting point, which makes the measuring process much more vague and inaccurate. Two peoples measurement of the same pseudopodium could be different by almost 10% based simply on where they judge the “beginning” of the pseudopodium to be. The other significant source of error is what the definition of a pseudopodium is, and how many micrometers long one requires a pseudopodium to be. This can also vary from person to person, making a clear and concrete number hard to achieve. These judgment calls can significantly alter the results from person to person, even within the same group. To refine this experiment I would make the total number of observations higher and would take digital images every ten minutes instead of every twenty. This would give a more gradual slope to the graphs while also allowing the drug to fully take effect.

References

