

The Effect of Cytochalasin B on Pseudopod Formation Rate in *Amoeba proteus*

Robert Manguso
Independent Research Project
Bio 219: Cell Biology
December 3, 2008

Introduction

One of the primary mechanisms by which cells exhibit motility is through the dynamic assembly and disassembly of cytoskeletal actin filaments (Cooper & Hausman, 2007). G actin monomers polymerize onto the ends of preexisting F actin filaments, elongating the F actin and ultimately providing cells with an essential source of tension (Cooper & Hausman, 2007). In addition to its important role in linking the cytoskeleton to the extracellular matrix (ECM), actin also plays an invaluable role in the processes of cell motility due to its uncanny ability to rapidly polymerize and depolymerize through a process known as treadmilling (Cooper & Hausman, 2007). The dynamic polymerization and depolymerization reactions that take place during treadmilling are the underlying cause of the phenomenon referred to as amoeboid movement. This type of movement is literally a type of cell crawling during which actin polymerization coordinated in one direction causes a protrusion in the cell membrane referred to as a leading edge. The extension of the leading edge and subsequent contraction of the trailing edge on the other side of the cell leads to the migration of the cell in one direction (Cooper & Hausman, 2007). The specific type of cell membrane protrusion on which this experiment will focus is the pseudopodium or pseudopod, which is a cell membrane extension of moderate width made up of actin filaments cross-linked into a three-dimensional network (Cooper & Hausman, 2007). This type of cell migration is exciting to study because of the vast number of cell types that utilize it. For instance, embryonic cells, metastasizing cancer cells, white blood cells and nerve cells all travel by way of amoeboid movement (Cooper & Hausman, 2007).

For this experiment the protozoan *Amoeba proteus* has been chosen as the model organism to study amoeboid movement. This choice was made due to the fact that *Amoeba proteus* is constantly displaying this behavior and is a very large cell and thus fairly easy to image. Despite the fact that the amoeba can move freely, the reorganization of the actin cytoskeleton is influenced by a number of environmental factors as well as protein interactions, many of which are not well understood at this time (Pomorski, Krzeminski, Wasik, Wierzbicka, Baranska & Klopocka, 2007).

However, it is known that the active locomotion of the amoeba involves the same reorganization of cytoskeletal actin that takes place in other cell types, where actin filaments run parallel to the plasma membrane and longitudinal axis of the cell (Pomorski et al. 2008).

Due to the fact that actin is known to play the primary role in amoeboid movement in the *Amoeba proteus* model, it can be inferred that an interference with the cell's ability to polymerize actin would result in a severe debilitation of pseudopod formation that would eventually lead to the inability of the cell to freely migrate. The drug cytochalasin B is a potent inhibitor of both actin polymerization and the interaction of actin filaments in solution (MacLean-Fletcher & Pollard, 1980). Specifically, the drug inhibits the addition of actin monomers to the barbed end of the polar actin filaments, where the G actin monomers normally have a higher binding affinity (MacLean-Fletcher & Pollard, 1980). The drug also inhibits actin filament interactions which would normally lead to the formation of a filamentous actin network (MacLean-Fletcher & Pollard, 1980). This experiment was designed to specifically test the effect of cytochalasin B on the rate of amoeba pseudopodium expansion. During the procedure, *Amoeba proteus* specimens were observed under normal conditions in a flow cell and measurements of pseudopod formation rate were recorded under those control conditions. The data were compared with measurements of pseudopod formation 5 minutes and 30 minutes after the addition of cytochalasin B to the flow cells. The expected result was that the addition of 150 μL of cytochalasin B to flow cells containing *Amoeba proteus* would result in a decrease in the average pseudopod formation rate of the specimen over time.

Materials and Methods

Professor Bob Morris of the Wheaton College Biology department provided the *Amoeba proteus* specimens used in this experiment. The samples were viewed under a Nikon Eclipse E200 microscope. Images were captured using a Sony DFW-X700 camera through the BTV imaging program. The captured images were manipulated using Adobe Photoshop CS and analyzed using an image analysis program called ImageJ. The cytochalasin B used in the experiment was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 40 $\mu\text{g}/\text{mL}$. Professor Morris also provided the Drug solution, and the laboratory equipment used (pipettes, slides, cover slips, gloves, etc) was provided by the Wheaton College Biology Department.

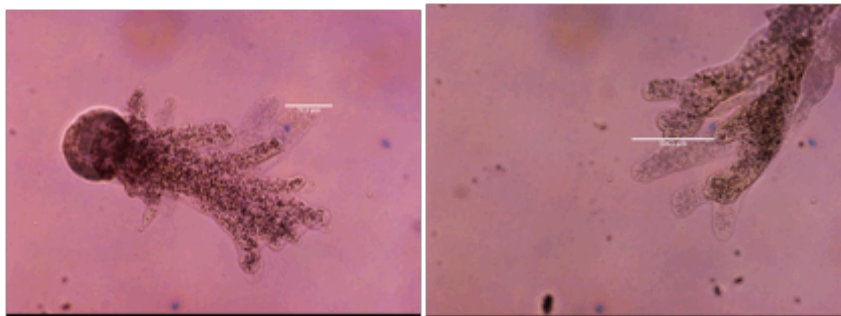
To conduct this experiment, two flow cell slides (labeled A and B) were created by placing four fragmented cover slip chips on each slide with the goal of supporting the cover slips to prevent the specimens on the slides from being crushed. Amoebae were then extracted from a container by the Professor and placed onto the flow cell slides. A cover slip was applied to the slides and the edges of the cover slip facing the long edge of the slides were sealed shut

with VALAP. The other two opposite edges of the cover slip were left open so that cytochalasin B could later be flowed under the cover slips. When the amoebae were located on the slide with a microscope, data collection could begin. For the control measurements, amoebae were observed in an environment somewhat similar to their natural habitat; pond water filled with paramecia. Some fungus was also observed growing in the pond water. To measure pseudopod formation rate a series of 6 images were taken in 5 second intervals for 30 seconds. For the purposes of this procedure, a pseudopod was defined as a temporary projection of the cytoplasm where two sections of the plasma membrane become parallel at some point. Once a series of images was collected, they were examined for any cytoplasmic projections which fit this definition of pseudopod. For an image series containing more than one cytoplasmic projection fitting this definition of pseudopod, the projection which displayed the most linear growth pattern was selected for analysis. This selection criterion was used because the actual length of the pseudopod extension could be measured more accurately if the extension occurred in a linear fashion. One amoeba was successfully located on each of the flow cell slides, and 3 image series were taken on each amoeba for a total of 6 control image sequences. After the completion of control data collection, cytochalasin B was obtained and applied to slide A. To apply the cytochalasin B to the flow cells, three 50 μL aliquots of drug solution were applied to one open side of the cover slip and sucked through the flow cell from the other side by absorbing some water with a Kimwipe. The approximate volume of liquid under the cover slip is 150 μL , so this procedure was designed to replace the entire volume of pond water under the cover slip with cytochalasin B solution. Five minutes after the application of drug solution to slide A, three image sequences were recorded using the same process described for the control conditions. Next, the drug solution was applied to slide B using the same process as described for slide A. Once again, three image sequences were recorded after 5 minutes for slide B using the same procedure as described previously. Finally, three image sequences were collected for both slide A and B 30 minutes after cytochalasin B solution had been applied to each slide.

After the data was collected and the pseudopodium for each image sequence was chosen, the first and last images from each sequence were superimposed onto one another using Adobe Photoshop CS. Once layered on top of one another, the foreground image was tinted with magenta and the opacity of the background image was decreased such that both images were visible at the same time. Figure 1 shows an example of two of the superimposed images. The superimposed images allowed for the exact distance between the pseudopodial membrane boundaries to be measured using ImageJ. Using the line analysis tool of the ImageJ program, the distance between the pseudopodial boundaries of the superimposed images was measured in pixels. With the help of a stage micrometer, it was determined that 1.63 pixels equal 1 μm . This conversion factor was used to convert the distances of pseudopod growth in pixels to

distances in micrometers. The distance covered by the expanding pseudopod in μm was then divided by the total time of the interval (30 seconds for all trials) to yield a growth rate in $\mu\text{m}/\text{second}$. The data from the three conditions for both amoebae observed were combined and thus the data reported in the results section to follow reflects a compilation of the 3 observations from each condition, yielding a data set with 6 observations for each condition.

Figure 1. Superimposed Time Lapse Images Created In Adobe Photoshop.

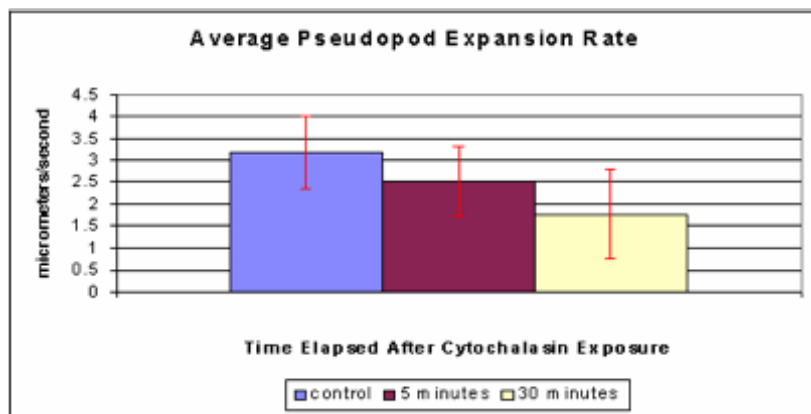


These images were created so that the exact length of pseudopod extension could be measured. The first image shows the pseudopod extension of a specimen after 5 minutes of cytochalasin exposure. The second image shows the pseudopod extension of a specimen during the control condition. Notice that the far plasma membrane boundaries of both the background and foreground images are clearly visible. Scale: Image 1: $72.2\mu\text{m}$ Image 2: $124.5\mu\text{m}$

Results

The results of the pseudopod formation rate analysis indicate that the addition of cytochalasin B to the environment of the amoebae did significantly hinder the rate of outward pseudopod expansion. Figure 2 shows the average decrease in formation rate after 5 minutes and 30 minutes versus the control formation rate. A two sample assuming unequal variances t-Test was used to statistically analyze the observed data. The pseudopod formation rate 5 minutes after cytochalasin exposure ($M= 2.516$, $SD= .787$) did not differ significantly from the control pseudopod formation rate ($M= 3.178$, $SD= .825$), $t(10) = 2.23$, $p = .18$. However, pseudopod expansion rate 30 minutes after drug exposure ($M= 1.783$, $SD= 1.01$) did differ significantly from the control expansion rate, $t(10) = 2.23$, $p = .025$. The rate of pseudopod expansion after 5 minutes did not differ significantly from the expansion rate after 30 minutes, $t(10) = 2.26$, $p = .194$, but Figure 2 shows that an overall trend of steady decrease was observed throughout the three conditions. The pseudopod expansion rate experienced a 20.8% decrease from the control condition to the 5 minute drug exposure condition. A 29.1% decrease was then observed from 5 minute to 30 minute drug exposure.

Figure 2. The Average Rate of Outward Pseudopod Expansion

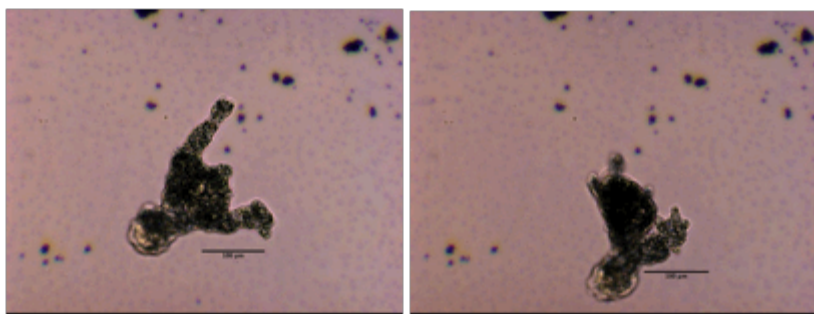


Each of the above columns represents one of the three conditions under which pseudopod formation rate was measured. The blue column represents the control measurements, the red column represents measurements taken 5 minutes after cytochalasin exposure and the yellow column represents measurements taken 30 minutes after drug exposure. The red error bars on each column represent the standard deviation of the data in each condition. All measurements are reported in micrometers/second and $n=6$ for all conditions.

The standard deviation of the data values from each condition are represented by the red bars located at the top of each column. The standard deviations from the control and 5 minute exposure conditions were relatively similar, but the data from the 30 minute exposure condition yielded a larger standard deviation of ± 1.01 micrometers/second.

Figure 3 illustrates pseudopod expansion following 30 minutes of cytochalasin B exposure. While the specimen does appear to have suffered structural damage, the only quantifiable data that can be drawn from these images is a measurement of pseudopod expansion length. This specimen extended its pseudopod a distance of $38.67 \mu\text{m}$ over a 30 second interval. This corresponds to a pseudopod extension speed of $1.289 \mu\text{m}/\text{second}$. A round formation was also observed protruding from the body of the specimen. The possible implications of this observation will be addressed in the discussion.

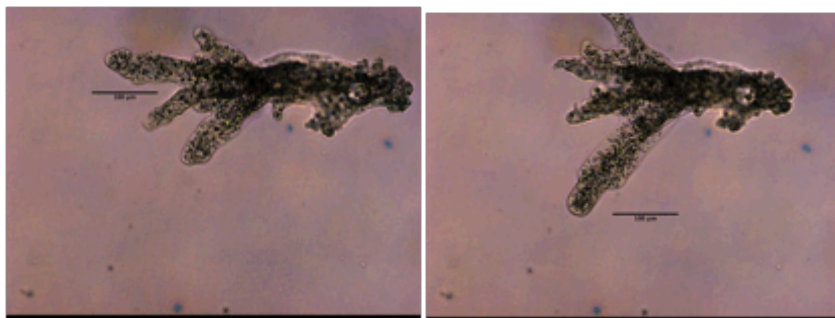
Figure 3. Amoeba After 30 minute Cytochalasin B Exposure.



The above images depict the change in behavior of an amoeba over a 30 second interval after 30 minutes of cytochalasin B exposure. The growth of a pseudopod can be seen out of the top of the specimen. Scale: Image 1 and 2: $100 \mu\text{m}$

The discrepancy between the organisms after 30 minutes of Cytochalasin B exposure and the control organisms is highlighted in figure 4. The specimen in figure 4 is the same specimen from figure 3, but has not yet been exposed to cytochalasin B. It is much larger, and its pseudopod extends over a greater distance in 30 seconds than it does after exposure to cytochalasin. The large pseudopod measured on the specimen in figure 4 covers 78.801 μm in 30 seconds, at a growth rate of 2.627 $\mu\text{m}/\text{sec}$.

Figure 4. Control Condition Amoeba.



The above images depict the change in behavior of the same amoeba over a 30 second interval during the control condition. The growth of a pseudopod can be seen out of the bottom of the specimen. Scale: Image 1 and 2: 100 μm

Discussion

The results obtained from this procedure support the original prediction that exposure to cytochalasin B would significantly decrease the rate of pseudopod growth in *Amoeba proteus*. This result is consistent with the findings reported in Cooper and Hausman (2008), but is inconsistent with the findings of a study by Prusch (1981). Prusch reports that while cytochalasin B does cause an initial decrease in locomotion and the formation of randomly distributed pseudopodia, *Amoeba proteus* will resume its normal shape and pattern of locomotion after 30-45 minutes of sustained cytochalasin B exposure (Prusch, 1981). No such result was observed during this procedure and the data obtained generally indicate that the conditions of the amoebae worsened with time. It should be noted that data collection and observation for this procedure ended after the 30 minute cytochalasin exposure interval and it is unclear whether the specimen would have recovered if observed for an additional 20 minutes.

Nevertheless, these data lead to some general conclusions regarding the mechanism by which amoebae mobilize. The results clearly demonstrate the key role of actin in pseudopodial growth and function. Exposure to an actin polymerization inhibitor had a profound negative effect on the rate at which amoebae were able to extend pseudopodia and thus mobilize themselves. The results also indicate that these effects worsen with

time if exposure to the drug is maintained.

Despite the statistical significance of the experimental results, some major sources of potential error do exist and must be considered when analyzing the data. The most obvious source of error is the lack of a proper control for the experimental conditions. The DMSO solvent in which the cytochalasin B was dissolved was not controlled for and thus it cannot be assumed with confidence that all the negative effects suffered by the amoebae assumed to be caused by cytochalasin B were in fact caused solely by cytochalasin B. For this experiment to be properly controlled it would have required a control group for which only DMSO was added to the flow chamber. An additional source of error arose from the observation that the technique used for applying the drug to the amoebae may have been problematic. It is possible that the cytochalasin B solution did not distribute evenly throughout the flow cell in each trial. The drug may have only filled a part of the flow cell or may have been absorbed by the Kimwipe to reduce its concentration. An error of this type may explain the relatively large standard deviation observed in the 30 minute drug exposure condition. If the drug was less concentrated in one of the cells, there is a possibility that the actin polymerization inhibiting effect of the cytochalasin B dissipated before the 30 minute exposure measurements were recorded, as was reported by Prusch (1981). Even if the drug solution did distribute itself evenly in the flow cell, there is a chance that the measurement of the 50 μ L aliquots was inaccurate and a differing volume of drug was added to each slide. To control for these potential sources of error in the future an automatic pipette should be used with a fixed volume. Also, the selected volume of drug should be placed on an empty slide and the amoebae specimens should be added to the drug to ensure that each specimen is exposed to the same volume of drug solution.

The results of this experiment leave a multitude of questions unanswered and thus there are many possible avenues that future research could take. It would be interesting to attempt to replicate the results reported by Prusch (1981) and analyze pseudopod formation rate at different concentrations and different time intervals. It would also be interesting to conduct a similar experiment using a drug such as nocodazole that inhibits the polymerization of microtubules as opposed to actin filaments.

References

Cooper, G. M., & Hausman, R. E. (2007). *The Cell: A molecular Approach*. Washington D.C.: ASM Press.

MacLean-Fletcher, S., & Pollard, T. D. (1980). Mechanism of action of cytochalasin B on actin. *Cell*. 2, 329-341.

Pomorski, P., Krzemiński, P., Wasik, A., Wierzbicka, K., Baráńska, J., & Klopocka, W. (2007). *Protoplasma*. Actin dynamics in *Amoeba proteus* motility. 231, 31-41.

Prusch, R. D. (1981). The Influence of Concanavalin A and Cytochalasin B on Pinocytotic Activity in *Amoeba proteus*. *Protoplasma*, [106(3-4)], 230-230.

This report was created in collaboration with Ian Greenstein, my lab partner. We used the same data that we generated during class time. We also collaborated on ways to analyze and represent data and created

many of the figures and statistics together.

Due to the similarity of our data, some ideas for graphs and analysis were also created with Zaheer Faizi.