Evidence that the number of pseudopodia in Amoeba proteus is lower after exposure to the drug Cytochalasin B

Kyle Glass
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Introduction:

In this study, we examined the cellular process of pseudopodia formation. Pseudopodia are a specific type of cellular extension involved in cell movement. The formation and deformation of pseudopodia is dependent on the organization of the actin, an important cytoskeletal protein, into rapidly rearranging bundles or three dimensional networks. Unicellular organisms such as Amoeba proteus demonstrate pseudopodia growth while carry out cell movement.

The fundamental unit of the protein actin is a monomer called globular actin (G actin). G actin monomers polymerize in a head to tail reaction to form dimers. These dimers assemble into long polymers called filamentous actin (F actin). Actin filaments have polarity and are characterized by a barbed plus end and a pointed minus end. The formation of an actin trimer initiates actin polymerization and actin filaments are able to grow by the reversible addition of monomers to both ends. The affinity is such that the barbed end grows significantly faster than the pointed end (Cooper and Hausman, 2007, pg. 474-475). Actin monomers also can associate with ATP, which hydrolyzes following filament assembly to form ADP. Actin polymerization is reversible and filaments can depolymerize by the dissociation of actin filaments and or subunits. ATP-actin dissociates less readily than ADP-actin which can create a difference in the critical concentration of monomers needed for polymerization at the two ends (Cooper and Hausman, 2007, pg. 475-476). The dynamic behavior of actin filaments results in a phenomenon known as treadmilling, which is important in the formation of cell processes such as pseudopodia formation and in cell movement (Cooper and Hausman, 2007, pg. 476).

The drug Cytochlasin acts as an inhibitor of actin polymerization. Cytochalasins bind to the barbed ends of actin filaments and block their elongation, resulting in change in cell shape and the inhibition of some types of cell movement (Cooper, Hausman 2007, pg 476). The drug is useful in cell biology to test for the dependence of certain cellular processes on actin polymerization. In one study, mouse peritoneal macrophages were exposed to Cytocholasin B. The results suggest that cytochalasin B blocks phagocytosis of large particles by affecting the distances over which any putative actomyosin-mediated forces are generated (Painter, Whisen and McIntosh, 1981). In another more recent study, Archeospores of *Porphyra pulchella* were exposed to cytochalasins B and F. The results showed that actin and myosin

have a role in generating force for pseudopodial activity (Ackland, West and Pickett-Heaps, 2006).

In this study, we tested the hypothesis that the number of pseudopodia in Amoeba proteus will be lower after exposure to the drug Cytochalasin B. For the study of pseudopodia growth, Amoeba proteus is a convenient test organism. Amoeba proteus is a relatively large unicellular organism allowing for increased visibility of many cellular processes and behaviors including pseudopodia growth. Amoeba proteus is also relatively inexpensive to buy and is easily maintained. We assessed pseudopodia formation by counting the number pseudopodia in Amoeba proteus before and after exposure to Cytochalasin B. In testing our hypothesis we created two slides with Amoeba proteus, one experimental and one control, and captured images from which we counted the number of pseudopodia. We then administered Cytochalasin B to slide A, once again capturing images and counting the number of pseudopodia. We presented our data in the form of a bar graph comparing the number of pseudopodia before and after exposure to Cytochalasin B.

Materials:

One Sharpie marker

Two slides

Two cover slips

One forceps

One large petri dish

One dish cover slip chips

Two transfer pipettes

VALAP

One Hotplate

One paintbrush

One box Kimwipes and paper towels

One tube of Cytochalasin B (Concentration = 40 micrograms per milliliter)

One test tube rack

Amoeba proteus

Nikon Eclipse E200 microscope with 1.0X Diagnostic Instruments lens

Sony DFW-X700 Camera

BTV 6.0b1

Adobe Photoshop Cs2

Microsoft Excel 2008

One pair latex gloves

Trash container

Glass disposal container

Methods:

Preparing the slides:

Two glass slides were obtained. A black Sharpie marker was used to label one slide A (the experimental slide) and the other slide B (the control slide). Cover slip chips were placed on slides A and B using a pair of forceps. Cover slip chips were arranged to form a chip chamber in order to prevent the test organism, Amoeba proteus from being harmed by the pressure of the cover slip. Amoeba proteus was added to slides A and B using a transfer pipette. At least one Amoeba was placed on each slide. Cover slips were added to slides A and B to create chip chambers containing Amoebae suspended in water. VALAP was set on a hotplate and heated to a liquid form. For both slides A and B, a thin layer of VALAP was applied to the two long sides of the slide using a paintbrush, sealing the two sides of the cover slip to the top of the slide and creating a flow cell. Caution was used while applying the VALAP because VALAP can interfere with microscopic visibility. The drug Cytochalasin B was prepared at a concentration of 40 micrograms per milliliter and placed in a test tube with a separate transfer pipette. The test tube containing Cytochalasin was set in a test tube rack.

Observing Amoeba proteus under the microscope and counting pseudopodia:

A Nikon Eclipse E200 microscope with 1.0X Diagnostic Instruments lens was aligned for proper Koehler illumination. A Sony DFW-X700 Camera was assembled with the microscope and the program BTV 6.0b1 was used for microscopic imaging. A Slide A was observed under the microscope at 10X magnification. An Amoeba was located in the view field and an initial image was captured. A second image was captured one minute later. Then Slide B was observed under the microscope using the 10X objective lens and Phase 1 optics. An Amoeba was located in the view field and an initial image was captured. A second image was captured one minute later. Then the number of pseudopodia was manually counted in each image. For this study, a pseudopod was defined as a finger-like cytoplasmic extension with opposite parallel sides of plasma membrane that projected from a region of the cell that demonstrated cytoplasmic streaming. The base of the pseudopod was determined at the point at which the two sides of the plasma membrane diverged from parallel. All cell structures that met these criteria were counted as pseudopodia.

*Water was added to slides A and B using a transfer pipette to prevent the environment inside the chip chamber from drying out.

*When slides were not being observed under the microscope, they were kept in a large petri dish

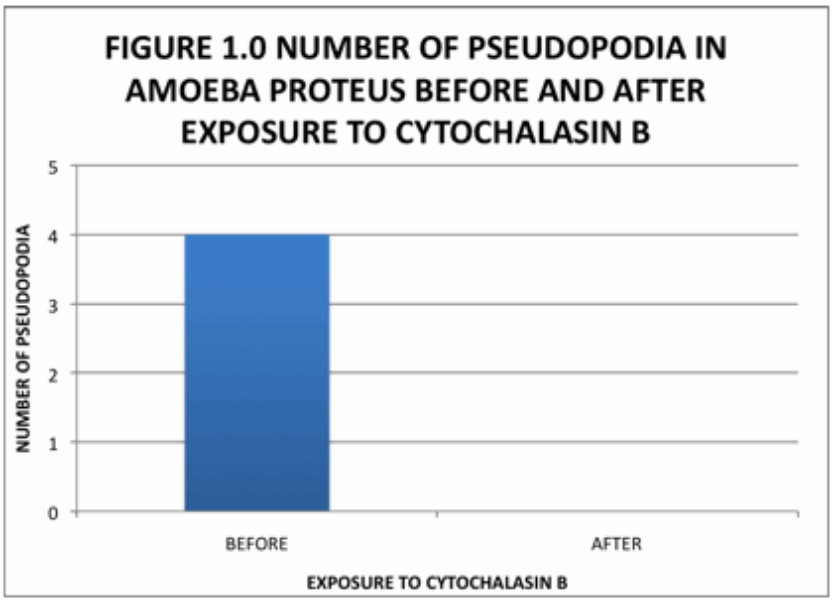
Adding Cytochalasin B to slide A and observing behavioral change:

Latex gloves were worn during this part of the procedure as a safety precaution. Slide A was placed on top of a paper towel. The drug Cytochalasin B was added to slide A using a transfer pipette. 50 micro liters of the drug was drawn into the transfer pipette and then administered to one side of the cover slip. As the drug was being administered, a Kimwipe was then touched to the other side of the cover slip and used to wick the drug under the cover slip. Three applications of the 50 micro liters of the drug were necessary to ensure that the concentration of the drug under the coverslip was approximately 40 micrograms per milliliter. After the drug was administered, slide A was observed under the microscope using the 10X objective lens and Phase 1 optics. An Amoeba was located in the view field and an image was captured immediately. A second image was captured one minute later. Then the number of pseudopodia was manually counted in each image. A period of approximately 15 minutes was waited before capturing a third image. A fourth image was captured one minute later. Then the number of pseudopodia was manually counted in each image.

Data Analysis:

Scale bars of 100 microns were added to all of the images using Adobe Photoshop Cs2. The number of pseudopodia was averaged in the two images of the Amoeba captured in slide A before exposure to Cytochalasin B. The number of pseudopodia was also averaged in the two images captured 15 minutes after exposure to Cytochalasin B. These data were presented in a bar graph created in Microsoft Excel 2008 showing the number of pseudopodia in Amoeba proteus before and after exposure to Cytochalasin B.

Results:



Let n = the number of measured data points averaged for the graph. For Exposure to Cytochalasin B (BEFORE), n=2. For Exposure to Cytochalasin B (After), n=2. This Figure shows that the Amoeba observed in slide A had an average of four pseudopodia before being exposed to Cytochalasin B and an average of zero pseudopodia after being exposed to Cytochalasin B.

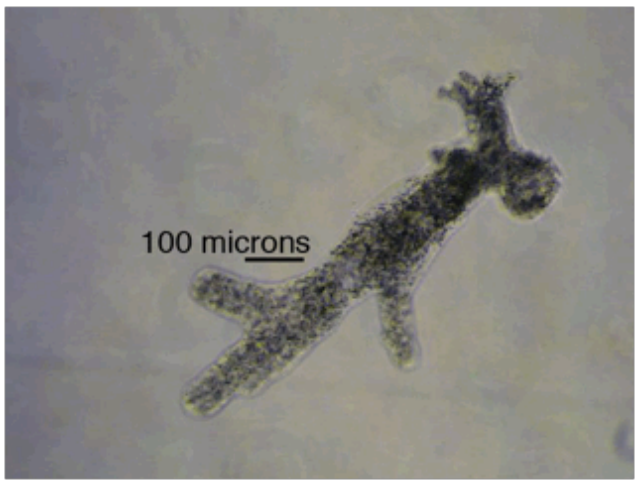


FIGURE 1.1 AMOEBA PROTEUS BEFORE EXPOSURE TO CYTOCHALASIN B

Scale bar = 100 microns. This is an image of an Amoeba from slide A before being exposed to Cytochalasin B. The image was captured using Phase 1 optics and the 10X objective lens on 11/19/2008 at 3:14:00 PM. Five distinct pseudopodia were counted on this particular image of the Amoeba.

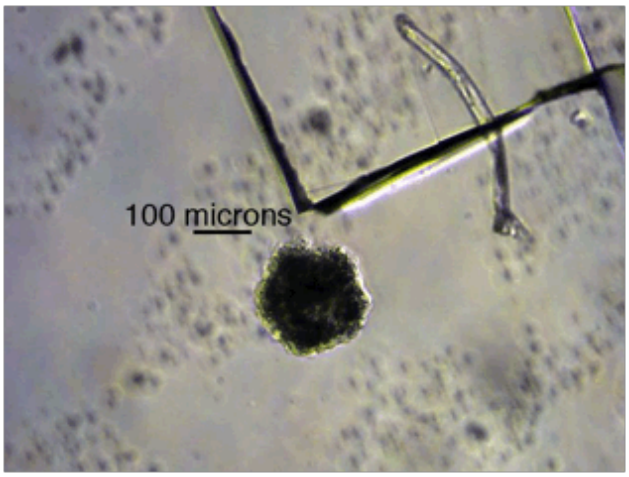


FIGURE 1.2 AMOEBA PROTEUS AFTER EXPOSURE TO CYTOCHALASIN B

Scale bar = 100 microns. This is an image of an Amoeba from slide A after exposure to Cytochalasin B. The image was captured using Phase 1 optics and the 10X objective lens on 11/19/2008 at 4:07:00 PM. Zero pseudopodia were counted on this particular image of the Amoeba.

Discussion:

Our results supported our hypothesis and the number of pseudopodia in Amoeba proteus was lower after exposure to the drug Cytochalasin B. Figure 1.0 shows that the Amoeba observed in slide A averaged a total of four pseudopodia before being exposed to Cytochalasin B. The Amoeba then averaged zero pseudopodia after being exposed to Cytochalasin B. These results indicate that the number of pseudopodia is lower after exposure to Cytochalasin B due to the inhibition and depolymerization of actin.

There were some sources of error in our procedure. During the first application of Cytochalasin B to slide A, the Amoeba was lost and we had to create a new experimental slide. As a result, the data for the number of pseudopodia before exposure to Cytochalasin B and the data for the number of pseudopodia after exposure to Cytochalasin B were collected from different slides. In a second trial of the same experiment, we could refine our procedure and apply the drug more carefully and more gradually to prevent this error from reoccurring. Another source of error to consider is that we did not control for the amount of oxygen in our control and experimental slides. The amount of oxygen in the environment under the coverslip on our slides may decrease over time as the Amoebae respire. We added water to slides A and B using a transfer pipette to prevent the environment inside the chip chamber from drying out. This may have partially contributed to replenishing the oxygen source for the Amoebae. However, we did not treat both the experimental and control slides the same in this regard. In a second trial of the experiment, we could account for this by applying the same amount of water to both the experimental and control slides. For this study, we captured images of Amoebae in experimental slide at three different times: before exposure to the drug, immediately after administration of

the drug, and 15 minutes after administration of the drug. We only captured images of the Amoebae in the control slide during the first time interval. It may be beneficial to capture images of Amoebae in both the experimental slide and the control slide at approximately the same 3 times intervals. We then would have more data to average and our control data would be much stronger.

In a recent study, the actin inhibitors Cytochalasin D (CD) and latrunculin B (Lat B), were found to disrupted the actin filament network and reversibly inhibited pseudopodial activity in Archeospores of *Porphyra pulchella*, resulting in the rounding and immobilization of spores (Ackland, West and Pickett-Heaps, 2006). In our study we qualitatively observed that the Amoebae that were exposed to Cytochalasin B demonstrated similar rounding, were immobilized and had no new pseudopodia forming. In a related future experiment, we could investigate how various concentrations of Cytochalasin B influence cell shape and the ability to form new pseudopodia in Amoeba proteus. Cell shape can be measured by calculating the projected area of the Amoeba using the program BTV 6.0b1. The number of pseudopodia would be counted using the same criteria as before. One testable hypothesis is that higher concentrations of Cytochalasin B will cause a greater decrease in the projected area of Amoeba proteus and fewer new pseudopodia will form. We can test this hypothesis by creating a control slide, similar to the control we used in our first experiment, and 3 additional experimental slides. Three different concentrations of the drug Cytochalasin B would be applied to the three different experimental slides. One slide would receive the drug in a concentration of 40 micrograms per milliliter. The other two slides would receive concentrations of the drug either lower or higher than 40 micrograms per milliliter. Microscopic images would be captured before and after exposure to the drug. Then the projected areas would be calculated and the number of pseudopodia would be counted. The data could be represented in the form of a bar graph.

Sources Cited:

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