Reduction of Lamellipodial Region in Cytochalasin B Treated Amoebae

Farrah Laliberte

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Abstract:

The lamellipodial region is an area of clear gel-like plasma that doesn't appear to have organelles within it. However, within the lamellipodial region is the actin cortext which is responsible for structural support of a cell. Cytochalasin B is a toxin that depolymerizes actin filaments, the main component of the actin cortext, making the cell swell. Therefore, the theory was lamellipodial region in Cytochalasin B treated amoebae is reduced. The width of lamellipodial region in *Amoeba proteus* pseudopodia was measured in amoebae exposed to Cytochalasin B. Two flow cells were made and control and experimental amoebae were exposed to fresh water (control) and Cytochalasin B (experimental). The results were recorded using Spot Advanced and analyzed using Adobe Photoshop and Image J. The width of the lamellipodial region in the control amoeba was 2.43 ± 0.80 µM and the width in the experimental amoeba was 1.64 ± 0.74 µM. These data indicate that the width of lamellipodial region in control amoeba is wider than the lamellipodial region in Cytochalasin B treated amoebae.

Introduction:

Amoeba proteus is a unicellular eukaryote that is remarkable because it has the ability to change shape rapidly (Durr, S., 2002). It's a transparent organism that is easily viewed with a standard light microscope, making this organism easy to study in undergraduate Cellular Biology courses. It can be up to 1mm long and it extends long finger-like processes called pseudopodia (Leidy, J., 1878). Pseudopodia assist in amoeboid migration and in engulfing prey (Leidy, J., 1878). Pseudopodia also determine the direction amoeba will move and it could be stimulated by the fluid pressure from the constriction of the plasma membrane starting from the back and moving towards the front (Bershadsky, A. *et al.,* 2001). *A. proteus* are found in wet locations such as in freshwater ponds, hot springs, sewage, and moist soil where it consumes algae such as *Chilomonas paramecium* (Durr, S., 2002). Normal behavior has been characterized as exhibiting amoeboid movement, cytoplasmic streaming, extending pseudopodia, extend or contract pseudopodia, channels of streaming organelles between regions of stationary organelles, unidirectional cytoplasmic streaming, and multiple pseudopodia that kept moving (Morris, R., 2008).

Within the pseudopodia of *A. proteus* is a region known as the lamellipodial region. The lamellipodial region, also known as the marginal clear zone, is located immediately adjacent to the plasma membrane within an advancing pseudopod and contains the actin cortex (Domon, T. *et al.*, 2006: Cooper, G. M. *et al.*, 2007). This region is also known as the ectoplasm and the organelle- containing cytoplasm that is seen streaming into pseudopodia is known as the endoplasm (University of Edinburgh, 2003). Endoplasm is in a liquid state and ectoplasm is in a gelatin state (University of Edinburgh, 2003). It has been reported that endoplasm can convert into ectoplasm and vice versa in a process known as Sol-Gel interconversion (Stebbings,

H., 2005). According to previous studies, Sol-Gel interconversions may stimulate cytoplasmic streaming (Stebbings, H., 2005). The process of cytoplasmic streaming is very common in living cells including green algae *Nitella* and it assists in nutrient, metabolite and genetic information delivery to all parts of the cell (Takahashi, T., 2007: Stebbings, H., 2005).

Cytochalasin B is a cell-permeable toxin that binds to the barbed end of actin filaments in the actin cortex and depolymerizes them (BIOMOL, 2006). The actin cortex is important for cell shape and motility, so depolymerization of actin filaments will reduce cell motility and change the cell shape from an organism with a clearly defined shape to an amorphous blob (Cooper, G. M. *et al.*, 2007). Since the lamellipodial region contains the actin cortext, the effect of Cytochalasin B on the lamellipodial region is interesting because it provides greater insight on the role of the actin cortex in cell shape and motility. In this study, the tested hypothesis was that amoebae treated with Cytochalasin B will have a reduced lamellipodial regional width. Spot Advanced will be used to record the lamellipodial region in a series of photographs while Adobe Photoshop and Image J will be used to measure the width of the lamellipodial region.

Materials and Methods:

Amoeba proteus *and Cytochalasin B*

A. proteus was obtained from WARDs Natural Science and they were kept in distilled water with rice grain and *Chilomonas paramecium* at room temperature. Cytochalasin B was obtained from SIGMA Chemicals at a stock concentration of 4 mg/ml in DMSO. It is kept refrigerated and it was diluted 1:200 at a concentration of 40 µg/ml.

Creating a Flow Cell

Cover slips were broken into small chips which were arranged in a circle around the center of a glass slide. Amoeba was collected with a glass pipette using a dissection microscope to locate *A. proteus*. Water containing the detached amoebae was pipetted in the center of the aforementioned cover slip chips. The droplet was covered with a cover slip and sealed with VALAP (an amalgamation of vaseline, lanoline and paraffin mixed together in a 1:1:1 weight ratio, from the Morris Lab at Wheaton College) on the top and bottom sides of the cover slip. For this experiment, two flow cells were made.

Measuring the Lamellipodial Region

Two flow cells containing control and experimental *A. proteus* were observed using Spot Advanced, a Spot Insight camera, and a Nikon E700 microscope. The amoebae were viewed on the Pisces computer in the Imaging Center for Undergraduate Collaboration (ICUC). The microscope was aligned for Koehler Illumination and a series of 4 sequential images of the control and experimental lamellipodial region was taken at 40X magnification at a rate of one picture per second before and after they were exposed to fresh water (control) and Cytochalasin B (experimental). Cytochalasin B (40 µg/ml) and fresh water was

injected into individual flow cells using a pipette and a Kim Wipe. Also, a photograph of a stage micrometer at 10X magnification was taken. A 100 µM scale bar was added to all photographs using Adobe Photoshop, the stage micrometer photograph, and the outline tool. Also, the number of pixels within the scale bar was measured and recorded. Each photograph was labeled identifying important physical features of control and experimental amoeba. Photographs of the lamellipodial region in control and experimental amoebae were opened in Image J. The width of the lamellipodial region was measured in pixels using the "Line" tool from the most distal region of the plasma membrane to the most distal organelle. The measurements obtained before exposure to fresh water or Cytochalasin B and the control values obtained after exposure to fresh water were averaged together. The measurements obtained after the experimental amoeba was exposed to Cytochalasin B were averaged together and graphed in Microsoft Excel 2007. The width of the lamellipodial region in µM was calculated by converting pixels to µM through a conversion factor where 100 µM = 139 pixels.

Results:

Healthy amoebae were exposed to fresh water (control) and Cytochalasin B (experimental) in flow cells and the lamellipodial region was observed. Before exposing amoebae to water or Cytochalasin B, the pseudopodia of both amoebae exhibited cytoplasmic streaming, channels of cytoplasmic streaming between regions where there were stationary organelles, and pseudopodial extension and contraction. A few seconds after introducing Cytochalasin B to the experimental amoeba, there appeared to be a reduction in cytoplasmic streaming and a decrease in the width of the lamellipodial region. The control pseudopodia didn't exhibit a change in cytoplasmic streaming velocity nor did the width of the lamellipodial region decrease.

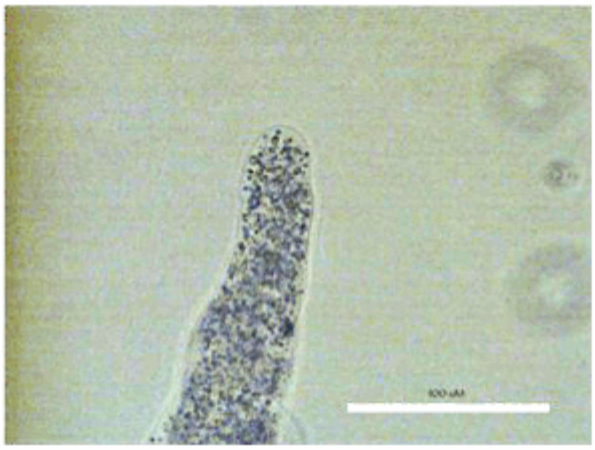

Figure 1: Pseudopod from control amoeba after exposure to fresh water. Width of the lamellipodial region was measured using Image J and Adobe Photoshop by measuring from the distal region of the pseudopod's plasma membrane to the most distal organelle. Scale bar = 100 µM

I observed a noticeable difference between the width of the control and experimental lamellipodial region. The average width of the control lamellipodial region was 2.43 ± 0.80 µM (n=10 lamellipodia). The average experimental width of the lamellipodial region was 1.64 ± 0.74 µM (n=4 lamellipodia). The average width of the lamellipodial region in both control and

experimental amoeba is shown in figure 2.

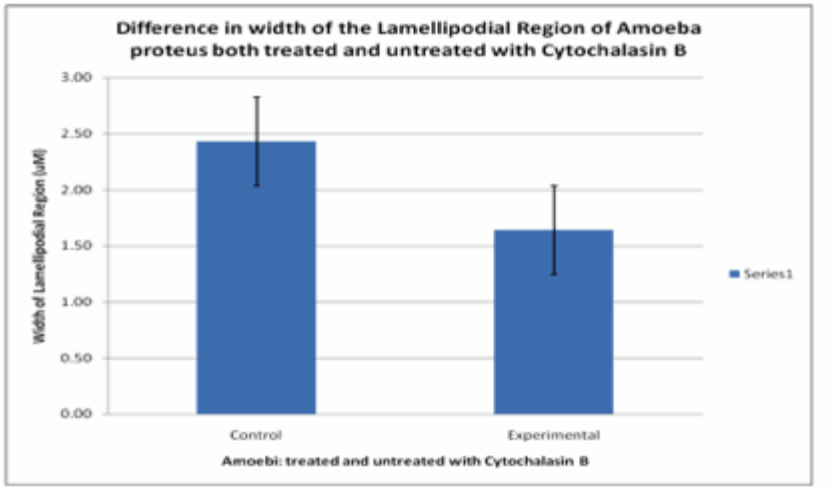

Figure 2: Difference in width of Lamellipodial region between Amoeba proteus treated and untreated with Cytochalasin B (40 µg/ml). The control amoeba was exposed to fresh water while the experimental amoeba was exposed to Cytochalasin B in a flow cell. Photographs taken in Spot Advanced were used to record lamellipodial regional width. Image J was used to analyze the width of the control and experimental lamellipodial regions. Control data represents 2.43 ± 0.80 (n=10 lamellipodia) and experimental data represents 1.64 ± 0.74 (n=4 lamellipodia).

Discussion:

In this study, the hypothesis was supported. I exposed experimental amoeba to Cytochalasin B while exposing control amoeba to fresh water. The lamellipodial region of control and experimental amoebae were measured using Spot Advanced to take photographs and Image J to measure the width. The amoeba exposed to Cytochalasin B responded instantly by reducing cytoplasmic streaming and decreasing the lamellipodial region width. This happened because Cytochalasin B depolymerizes actin filaments by binding to its barbed end (Domon, T. *et al.*, 2006: Cooper, G. M. *et al.*, 2007). When actin filaments, a component of the lamellipodial region, depolymerizes osmotic pressure is changed and water flows into the cell, making the cell extend and distend (Morris, R., 2008). At the same time, this depolymerization of actin filaments is reducing the width of the lamellipodial region. There was a noticeable difference between the widths of the lamellipodial region where the control amoeba had a width of 2.43 ± 0.80 µM and the experimental had a width of 1.64 ± 0.74 µM.

A potential source of error for this study is the flow cells became a little dry over the course of the study since it was very dry that day. If the amoebae I recording were exposed to a dry environment, they would have become sick and died. I didn't have a partner for this study so my results cannot be compared to a collaborator. However, my results do support what past researchers have observed about Cytochalasin B depolymerizing the actin cortex. For future studies, I recorded the lamellipodial region width shortly after I exposed the control and experimental amoebae to fresh water or Cytochalasin B. If I were to have waited a few minutes longer I might have seen a greater difference between their widths because I would have given Cytochalasin B time to depolymerize more actin filaments, exhibiting a thinner lamellipodial region. Also, the flow cells

should be checked more often to ensure that they don't dry out. Future experiments could be directed towards studying the possible effect Cytochalasin B could have on organelles involved in cytoplasmic streaming.

Literature Cited:

1. Durr, S. Microbial Inhabitants of Freshwater – *Amoeba proteus*. [Internet]. 2002 Jan 1. [cited 2008 Dec 1]. American Society of Microbiology. Available from http://www.microbelibrary.org/asmonly/details.asp?id=508&Lang=
2. Leidy, J. 1878. Amoeba Proteus. *The American Naturalist.* 12 (4): 235-238.
3. Bershadsky, A.D. and Elbaum, M. Cell Locomotion. [Internet]. 2001 [cited 2008 Dec 1] *Encyclopedia of Life Sciences.* Available from http://mrw.interscience.wiley.com/emrw/9780470015902/els/article/a0001257/current/pdf
4. N/A. Cell Locomotion. [Internet]. 2003 Jan 20 [cited 2008 Dec 1] The University of Edinburgh. Available from http://www.bms.ed.ac.uk/research/others/smaciver/Cell%20biol.topics/Cell_Locomotion.htm
5. Stebbings, H. Cell Motility. [Internet]. 2008 [cited 2008 Dec 1] *Encyclopedia of Life Sciences.* Available from http://mrw.interscience.wiley.com/emrw/9780470015902/els/article/a0001250/current/pdf
6. Domon, T., Taniguchi, Y. Fukui, A., Suzuki, R., Takahashi, S., Yamamoto, T., and Wakita, M. 2006. Features of the clear zone of odontoclasts in the Chinook salmon (Oncorhynchus tshawytscha). *Anatomy and Embryology.* 211: 87-93.
7. Morris, R. 2008. Cellular Biology lecture. 10/28/08.
8. Morris, R. 2008. Cellular Biology laboratory lecture. 11/12/08.
9. Cooper, G. M. and Hausman, R.E. 2007. *The Cell: A Molecular Approach*, 4th ed. ASM Press, D.C, pg 482.