Evidence That Treatment with Cytochalasin B Increases the Average Velocity of Cytoplasmic Streaming in Amoebae Proteus

Amanda Rawson

Independent Research Project Report

Bio219 – Cell Biology

December 3, 2008

Introduction

The streaming and movement of cytoplasmic particles is a phenomenon that occurs in a number of plant and animal cells. One of the best examples of cytoplasmic streaming occurs in *Amoeba proteus*, which are large complex cells that are highly mobile. Amoeboid movement is a fundamental cellular process in which cytoplasm and cytoplasmic particles stream within pseudopodia (Allen and Allen, 1978). Since these particles do not have the ability to create this movement on their own, it is believed that the forces responsible for this action are generated from external cytoplasmic forces (Bradley, 1973).

The streaming of cytoplasmic particles is an irreversible deformation of whole cytoplasm that is reliant upon the activity of contractile proteins (Allen and Allen, 1978). Both unorganized streaming activity and bulk contractility have been observed in the cytoplasm of *Amoeba proteus* (Taylor *et. al*, 1973). The occurrence of bulk contractility has been verified by experiments completed by Simard-Duquesne and Couillard, which demonstrated that the thick an think filaments present in the cytoplasm of *Amoeba proteus* are capable of movement with the addition of ATP (Taylor *et. al*, 1973). An additional investigation conducted by Simard-Duquesne and Couillard indicated that the cytoplasm of *Amoeba proteus* contains calcium-activated ATPase activity that resembles the ATPase found in striated muscle found in vertebrae (Taylor *et. al*, 1973). Together, these two studies established that the motility of *Amoeba proteus* is dependent on the activity of contractile proteins.

Research on cytoplasmic streaming has rapidly progressed more recently. This is because the broad biochemistry of contractile proteins has a wide range of applications in cellular motility studies (Allen and Allen, 1978). Progress in motility research has also been stimulated by publications that have pointed out the potential for motility control systems (Allen and Allen, 1978).

The goal of the present investigation is to test the hypothesis that treatment with Cytochalasin B will inversely inhibit the velocity of cytoplasmic streaming in *Amoeba proteus*. Cytochalasin B has been shown to suppress cytoplasmic streaming in both *Nitella* and *Avena* cells without causing structural changes (Bradley, 1973). For this reason it is hypothesized that Cytochalasin B will also suppress the streaming of cytoplasmic particles in *Amoeba proteus*.

In this study, the cytoplasmic particles of untreated *Amoeba proteus* and Cytochalasin B treated *Amoeba proteus* were observed over the period of a half hour. The average velocity of streaming cytoplasmic particles was then determined using microscopic imaging and image analysis software.

Specimens *of Amoeba proteus* were chosen to carry out this study because they are easy to maintain, easy to image and they contain a large number of cytoplasmic particles. Furthermore, Amoeba proteus are excellent model organisms because their lack of complexity allows specific systems to be studied in them.

Materials and Methods

Materials

Materials were obtained as follows: 22 mm sq glass cover slides, 75x25mm Gold Seal Micro Slides, Cytochalasin B, VALAP sealant, glass test tubes, disposable glass pipettes, disposable plastic pipettes, rubber gloves, and forceps

Methods

CULTURES: Specimens of *Amoeba proteus* were obtained from Ward's Biological Supply Company and were grown in distilled water. Rice grain was included in the distilled water in order to supply nutrients to the *Amoeba proteus* that remained in culture. The amoebae were collected using a glass Pasteur pipette, and were transferred onto a glass micro slide. A few droplets of distilled water from the amoeba culture were also placed on the slide. The addition of distilled water was carried out in order to ensure that the amoeba would not dry out.

CHIP CHAMBERS: Chip chambers were constructed to prevent the amoeba from being crushed under a cover slip. These chambers were made using the 22 mm sq glass cover slides and the 75x25mm micro slides. Small glass fragments were created by carefully crushing 2 or 3 glass cover slips. The resulting chip fragments were set aside in a tiny glass Petri dish. About four or five fragments were placed on the glass cover slide, so that they surrounded the amoeba. Once the glass chips were appropriately positioned, a glass cover slip was placed over

the amoeba. The top and bottom edges of the coverslip were sealed off using VALAP sealant. This left two edges exposed, providing a site for drug treatment.

DRUGS: All solutions were prepared by Dr. Robert Morris and were provided to us by Wheaton College. Cytochalasin B was used at a concentration of 40mg/ml. Additions of Cytochalasin B were made using a glass Pasteur pipette. 1-2 droplets of Cytochalasin B were placed at one open end of the cover slip. This allowed the droplet to be pulled under the coverslip by capillary action. Cytochalasin B treatment was applied to a single slide, labeled slide A. Additions of Cytochalasin B were made at 3 minutes, 10 minutes, 20 minutes and 30 minutes. This allowed the slide to be treated at 10-minute intervals over the course of a half hour. An additional slide which was treated with additions of distilled water served as a control. This slide was marked as slide B.

MICROSCOPY AND IMAGING: All images and movies were obtained using a Nikon Optiphot-2 microscope and a Sony DFW – X700 camera. All images were taken using the Hoffman Modulation Contrast HMC10 100 x objective. The images were recorded using BTV Pro software and analyzed using ImageJ software. Movies were taken for duration of 10 seconds. Imaging and Image analysis were completed in the Imaging Center for Undergraduate Research and Collaboration at Wheaton College.

ANALYSIS: Images and movies were opened in ImageJ. Once the image was opened, a 50mm scale bar was placed in the bottom right corner and a cytoplasmic particle was selected. The path of the cytoplasmic particle was traced using the trace function in ImageJ. Once the path was traced, the measuring function was used to determine the distance that the particle traveled. This path was made permanent by selecting the fill option in the main menu of ImageJ. This distance was then recorded in an excel file for convenience and this process was repeated until three cytoplasmic particles were analyzed for each image. Once the distances for three particles were gathered, the average distance and the standard deviation of the distances were calculated. In total 4 averages (one for each time interval) were gathered for the both the control slide and the Cytochalasin B treated slide. This meant there were 4 data points gathered for each slide.

Results

The cytoplasmic particles in the *Amoeba proteus* treated with Cytochalasin B, had an increased average velocity. The average velocity of particles found on Slide A (treated slide) had an overall increase of 5.4 mm/s. The average velocity of these particles prior to treatment was 9.06 mm/s. Following a half hour of treatment,

their velocity increased to 14.46 mm/s. The opposite affect was shown by particles found on Slide B. These particles went from an average velocity of 7.98 mm/s to 1.26 mm/s. This was an overall decrease in velocity by 6.72 mm/s.

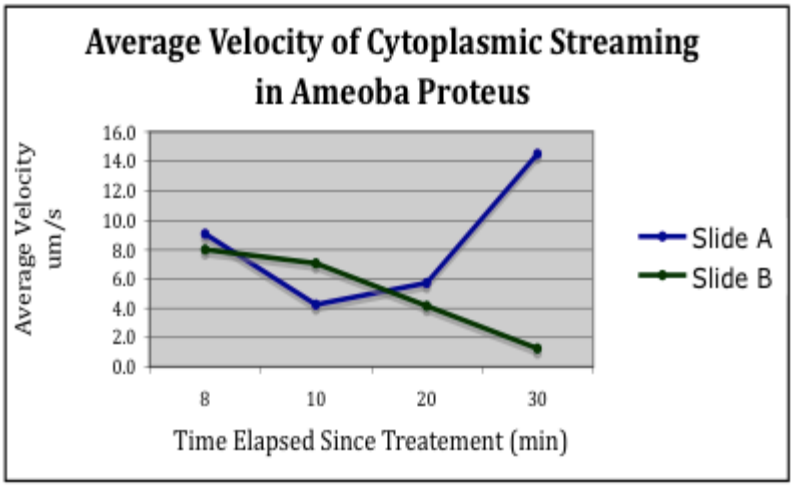

Figure 1. This is a graph of the Average Velocity of Cytoplasmic Streaming in Amoeba Proteus, where Slide A was treated with Cytochalasin B and Slide B was left untreated.

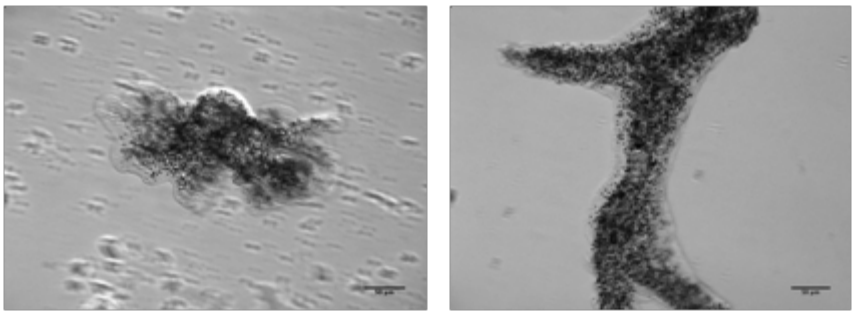

Image 1. These are both images of the *Amoeba proteus* on Slide A . The image to the left shows the *Amoeba proteus* before treatment while the image on the right shows the amoeba proteus after 10 minutes of treatment with Cytochalasin B.

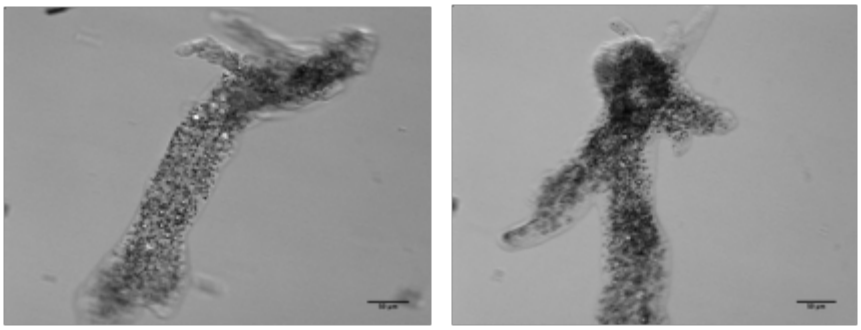

Image 2. This is an image of the Amoeba proteus on Slide B. The image on the left displays the *Amoeba proteus* before treatment, while the image on the right shows the *Amoeba proteus* after treatment with distilled water after 20 minutes.

Discussion

The results of this experiment did not support our original hypothesis. Based on previous studies, we believed that the addition of Cytochalasin B would decrease the velocity of cytoplasmic streaming. This however, was not the case and our results showed an overall increase in the velocity of particles following treatment with Cytochalasin B. These results suggests that Cytochalasin B does not interfere with cytoplasmic streaming, however we must take into consideration the fact that this was observed in only one sample. In order for these results to be statistically significant, the experiment would have to be repeated with a much larger sample size.

The small sample size is one source of error that has been identified in this experiment. An additional source of error was in the number of particles observed. We observed three particles for each time interval. This number seems quite small in reference to the number of cytoplasmic particles present in *Amoeba proteus*.

If we were to accept the results of this experiment, we could conclude that the contractile proteins involved with amoeboid movement were not impaired by the presence of Cytochalasin B. We could also conclude that the cellular processes involving these proteins and the thick and thin filaments of amoeboid cytoplasm remained unaffected.

If this experiment were to be repeated, a larger sample size and additional measurements would be gathered. The time interval at which the measurements were taken would also be increased. For example, instead of calculating the average velocity of particles every ten minutes, it may be more accurate to calculate the velocity for two-minute intervals.

In the future, experiments that used alternative drugs may provide information that would be useful to the field. The effects of Cytochalasin B could also be tested on other organisms. The experiment could also be repeated under varying conditions, in which light and temperature were altered. This would provide evidence as to weather or not environmental changes have an effect on the cytoplasmic streaming of *Amoeba Proteus*.

References

Bradley, M.O. 1973. Microfilaments And Cytoplasmic Streaming: Inhibition Of Streaming With Cytochalasin. J. Cell Science. 12: 327-343

Cooper, Geoffrey M, The Cell A Molecular Approach. Sinauer Associated Inc. 1997

Allen, Robert Day, Allen, Nina Stromgren. 1978. Cytoplamsic Streaming In Amoeboid Movement. Ann. Rev. Biophys. Bioeng. 7:469-495

Taylor, D.L., Condeelis, J.L., Moore, P.L., Allen, R.D. 1973 The Contractile Baisis of Amoeboid Movement. J. of Cell Biology 59: 378-394

Acknowledgements

The author of this paper would like to thank Blair Rossetti for help with gathering and analyzing data. The author would also like to thank Dr. Robert Morris for assistance with experimental techniques, materials, and for providing background information. In addition special thanks go out to Ian Greenstein and the rest of 230 and 110 for their assistance with editing.