

Role of Calcium in the Control of Cytoplasmic Streaming in Permeabilized Nitella Cell

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Abstract: *The most plausible factor responsible for controlling the Cytoplasmic Streaming is the Ca^{++} concentration in the Cytoplasm. Many reports have indicated that various kinds of movements in Eukaryotic cells are regulated by Ca^{++} . It has also been found that red and far red light affect Ca^{++} movement across the cell membrane (Dreyer and Weisenseel, 1979; Hale and Roux, 1980). In Nitella inter nodal Cell Cytoplasmic Streaming is stopped more rapidly in the presence of Ca^{++} when the cells were irradiated with far-red light and the effect of the light was completely removed by EGTA (Ethylene Glycol-bis, N-N-tetra acetic acid). These facts may be interpreted as showing that Calcium accumulates in the Cytoplasm during irradiation and that an increase in Ca^{++} Concentration in the Cytoplasm inhibits Cytoplasmic Streaming.*

Keywords: Cytoplasmic Streaming, Calcium, Permeabilized, Nitella

1. Introduction

Cytoplasmic Streaming is one of the model of the mechanism of movements occurring at cellular level generated by the actin-myosin System. This presents a unique phenomenon to be observed under the microscope perpetually moving within the cylindrical cells as Cytoplasm coming from one side and rotating to the opposite direction. Although it occurs in small Eukaryotic cells like Mesophyll Cells of the leaves of many Plants and even the larger protozoan cells, at the most vividly observable is found in the Characean algal Cells viz. inter nodal cells in Chara, Nitella etc, which are very big giant sized ranging from 3 to 4 cm to 15 to 16 cm in length and up to 20 to 50 μ m in diameter, cylindrical in shape and are multinucleated like striated muscle cells, their coenocytic state must have been the result of the fusion of many cells to form such huge giant sized cell. The Cytoplasm keeps moving continuously as an unending process like rotating belt along a more or less helical path. The rate of this movement is fairly good and found to occur up to 100 μ m/second. The rate and flow pattern of the Streaming are always constant and directional. The interaction of actin and myosin Proteins generate the required force to drag the Cytoplasm. The example of giant Algal cell such as Nitella internodal Cell, is very interesting in this respect as here only actin exists in filamentous form and the myosin exists in free molecular form and their interaction causes Cytoplasmic Streaming instead of Contractile movement. This study is concerned with the role of Ca^{++} in the control of Cytoplasmic Streaming in Permeabilized Nitella Cell. It is found that Ca^{++} causes change in membrane potential due to its transient high permeability hence whenever there is increase in Ca^{++} permeability of membrane, the Cytoplasmic Streaming would stop. This suggests that Ca^{++} transport System is linked to the machinery of Cytoplasmic Streaming.

2. Materials and Methods

Nitella is obtained from local seasonal pond. There is requirement of Nitella round the year as experimental material and hence both outdoor and indoor culture is desirable. It can be grown outdoor in a small tank, 1 metre

deep containing fine mud, rotting leaves, some compost of cow dung etc. Initially natural pond water is used to grow Nitella since it contains all the biota. Plants are removed and cultured indoor under constant fluorescent light for acclimatization to laboratory condition several weeks prior to the experiment. For indoor culture, Nitella were planted in big (18" high x 9" diameter) Jars containing soil (mixed with hummus and manure) at the bottom. Even in the laboratory the most suitable season for its flourishing growth is from the month of July to September. Internodal cells of Nitella are cut out from the plant measuring 1 mm diameter and 4 to 6 cm long and kept in Artificial Pond Water (APW) for a day or two before the experiment.

Permeabilization of Nitella Internodal Cell

In Nitella, there is no cortical layer outside the plasmalemma of Internodal Cell. Hence it can be done by simple method that is Electrical Pulse as adopted by Shimmen and Tazawa (1983). In Permeabilized cells, large molecules can not enter the Cytoplasm due to the presence of cell wall but the Cytoplasm maintains its integrity since it is sandwiched between the tonoplast and the cell wall.

The present study on Cytoplasmic Streaming has been taken up in two Systems i.e. the Internodal "intact cell" and the "Permeabilized cell" of Nitella to show the role of Calcium in controlling the Cytoplasmic Streaming. I examined the effect of external Ca^{++} on cells irradiated with far-red light. First the specimens were treated with EGTA (Ethylene Glycol-bis N-N-tetra acetic acid) in the dark to induce streaming in all cells. Some were then transferred to Calcium containing APW and irradiated continuously with far-red light. Other specimens irradiated in Calcium free APW. The time-course of the Streaming in total cells were observed in the presence and absence of Ca^{++} . Similar process is adopted for the Permeabilized Nitella Cell.

Calcium in the Cytoplasm

To demonstrate the increase in Ca^{++} concentration visually, specimens were fixed in the presence of Potassium pyroantimonate, which is known to be a fairly specific precipitant of Calcium. First, I fixed cells just after the pretreatment. In these the Cytoplasm didn't show any sign

of streaming. Precipitates are formed in the Cytoplasm and the middle lamella of the cell wall. Precipitates are also abundant in the Vacuole.

Next, I used cells treated with 10 mM EGTA solution for 25 to 40 minutes to induce streaming. Only a small amount of precipitate is seen in the Cytoplasm.

3. Result and Discussion

The Ca^{++} Concentration of 10 to 45 μ M stops streaming which shows that Ca^{++} sensitizing Components are present in the intact endoplasm. The streaming is completely inhibited at 7 μ M Ca^{++} Concentration. The induced streaming was markedly inhibited only by the combined action of Ca^{++} and irradiation with far-red light. In some cells streaming continued after 2 hours irradiation. In the absence of Calcium, the inhibition was about the same as that in the dark. The time course in the dark was almost the same in the presence or absence of Ca^{++} . In the presence of Ca^{++} , streaming stopped within 80 minutes on each irradiation. However, in the absence of Ca^{++} it took longer, about 160 minutes before streaming came to a complete stand still. Reactivation of streaming by EGTA could be repeated but Streaming didn't stop completely on the second irradiation, even after 3 hours.

Precipitates are abundantly visible in the Cytoplasm in cells treated previously with EGTA and subsequently irradiated with far-red light in APW. Precipitates were accumulated in the Chloroplast, Mitochondria and Endoplasmicreticulum. The middle lamella was heavily stained.

These observations reveal that the Intracellular Calcium Concentration is much lower when the Cytoplasm is involved in streaming than when it is immobile. Forde and Steer (1976) observed that active Streaming was inhibited by external application of Ca^{++} . Yamaguchi and Nagai (1981) reported that streaming in Vallisneria epidermal Cells could be induced by the application of 5 to 10 mM EGTA.

The rapid stimulating effect of EGTA on Streaming is understandable if changes in a low Ca^{++} Concentration in a small Cytoplasmic compartment and a low rate of Calcium transport from the Vacuole are involved. Though vacuolar Calcium Concentration is supposed to be rather high during treatment with EGTA, precipitates were observed in the cells. This was due to Calcium chelation into the Vacuole during the treatment. In Nitella Cells, Ca^{++} concentration in the Streaming of Cytoplasm has been found to be about 10^{-7} M or less. Ca^{++} accumulation in cells of Mougeotia has been reported to be accelerated by irradiation with red light and this effect is cancelled by subsequent irradiation with far-red light (Dreyer and Weisenseel, 1979). Efflux of Ca^{++} from Oat Coleoptiles and their Protoplast is enhanced by irradiation with red light and subsequent irradiation with far-red light induces a decrease to levels near the dark control (Hale and Roux, 1980).

The precipitant, pyroantimonate, can penetrate the biological membrane and hence can be used to fix Ca^{++} where it forms a dense precipitate with it (Caswell, 1979).

Role Of Ca^{++} In Permeabilized Nitella Cell

In Permeabilized cells the rate of Cytoplasmic Streaming was lowered by about 40% of that of intact cell at the same level of concentration of Ca^{++} and the Streaming stops completely at only 1 μ M Ca^{++} concentration.

4. Conclusion

On the basis of these observations it is concluded that rotational Cytoplasmic Streaming can be induced when the Ca^{++} concentration in the Cytoplasm decreases and the induced streaming stops when Ca^{++} concentration in the Cytoplasm increases.

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