Protoplasma 115, 18–24 (1983)

PROTOPLASMA

Control of Cytoplasmic Streaming by ATP, Mg^{2+} and Cytochalasin B in Permeabilized *Characeae* Cell

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Received June 7, 1982
Accepted August 4, 1982

Summary

A method to permeabilize the *Nitella* cell without using detergent was developed. The cell was pretreated with an EGTA-containing medium and subsequently plasmolysed at low temperature. The streaming which had been stopped in a medium containing no ATP could be reactivated by the Mg·ATP-containing medium and continued for more than one hour. The rate of the reactivated streaming for the first 30 minutes was almost the same as that of the normal streaming. The reactivated streaming was inhibited irreversibly by Mg^{2+} depletion and reversibly by cytochalasin B. Thus, the present model is superior to the previous detergent model in which the streaming lasted shorter time and was inhibited irreversibly by cytochalasin B (SHIMMEN and TAZAWA 1982 a).

Keywords: *Nitella*; Cytoplasmic streaming; Cytochalasin B; Demembranated cell model.

Abbreviations: ATP, adenosine-5′-triphosphoric acid; APW, artificial pond water; CB, cytochalasin B; CyDTA, 1,2-cyclohexane diamine N,N′-tetraacetic acid; DMSO, dimethylsulfoxide; EGTA, ethyleneglycol-bis(β-aminoethyl ether)-N,N′-tetraacetic acid.

1. Introduction

Our previous paper (SHIMMEN and TAZAWA 1982 a) reported that the *Nitella* cell membrane can be permeabilized by treating it with an ice-cooled medium containing EGTA and a detergent (saponin or Triton X-100). In the permeabilized cell model, we can control the cytoplasmic streaming with extracellular ATP or Mg^{2+}. However, the detergent cell model was not ideal for the following reasons. The streaming reactivated by Mg·ATP continued only for about 10 minutes. Inhibition of the reactivated streaming by cytochalasin B was irreversible, whereas it is reversible in normal cells.

In the present study, we tried to improve the cell model by prolonging streaming which can be reactivated by Mg·ATP and inhibited reversibly by CB. As found previously, plamolysis is essential for cell permeabilization (SHIMMEN and TAZAWA 1982 a). In the new cell model, the reactivated streaming continued for more than one hour. Thus, we were able to develop a method for obtaining high yields of a good cell model without the use of detergent.

2. Materials and Methods

Internodal cells of *Nitella axilliformis* and *N. expansa* were cultured in plastic buckets or glass vessels filled with tap water containing extracts of soil and rotten leaves. In the case of *N. axilliformis*, the buckets were placed near the window of our laboratory and illuminated by sunlight coming through the window. Therefore, the light intensity and the temperature varied during the culture. *N. expansa* was cultured in an airconditioned room (25° ± 2 °C) and illuminated with fluorescent lamps (20 W × 2) from above for 16 hours a day.

Young internodal cells were isolated. Cell fragments 1 cm long were secured by ligation with either polyester thread (*N. axilliformis*) or silk thread (*N. expansa*) and were kept in artificial pond water (APW) containing 0.1 mM each of KCl, NaCl, and $CaCl_2$ before use. The thread served later for transfer of the permeabilized cells, which had completely lost their turgor.

To observe the cytoplasmic streaming under continuous perfusion of the cell exterior, the perfusion chamber described previously (SHIMMEN and TAZAWA 1982 a) was used. The medium around the cell was exchanged within a few seconds after the start of perfusion.

The compositions of the media are shown in Table 1. Their

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0033-183X/83/0115/0018/$ 01.40

Table 1. *Compositions of media (mM)*

	EGTA	Mg	Mg·ATP	CyDTA
EGTA	5	5	5	0
CyDTA	0	0	0	5
ATP·2Na	0	0	1	1
$MgCl_2$	0	6	6	0
PIPES	30	30	30	30
K^+	65	67	69	68
Sorbitol	280	0-250	220-250	280

All were adjusted to pH 7.0.

osmolarities were controlled by modifying the sorbitol concentration. In the text, the sorbitol concentration is shown in parenthesis immediately after the name of the medium. For example, when the Mg medium contained 220 mM sorbitol, it is given as Mg(220) medium.

The turgor pressure of cells was measured by the turgor balance method (TAZAWA 1957) and the osmolality of solutions was measured on the vapor pressure osmometer (Wescor Inc. 5100C).

Experiments were carried out at room temperature (20-23 °C).

3. Results

3.1. Permeabilization of Nitella Cells

3.1.1. *Nitella axilliformis*

Experiments to find how to permeabilize cells without using detergent were mostly carried out on *N. axilliformis*. As the criterion of permeabilization, cessation of the cytoplasmic streaming under continuous perfusion with the Mg medium was adopted, since loss of Mg · ATP from the permeabilized cell should stop the streaming (WILLIAMSON 1975, TAZAWA *et al.* 1976, SHIMMEN 1978). Five different treatments were tried.

In treatment A, cells were externally perfused with Mg(0) medium at room temperature (18 °C). As shown in Fig. 1, the cytoplasmic streaming stopped suddenly just after the start of perfusion, probably because of depolarization of the plasmalemma due to increase in the extracellular K^+ concentration (HILL and OSTERHOUT 1938). The streaming recovered with time and continued for more than 120 minutes. Thus, this treatment proved to be ineffective for permeabilization of the cell.

In treatment B, cells were externally perfused with Mg(220) medium at room temperature. Fig. 2 shows changes in the rate of streaming in four different cells. Just after the start of perfusion, plasmolysis occurred and the streaming suddenly stopped and then a gradual

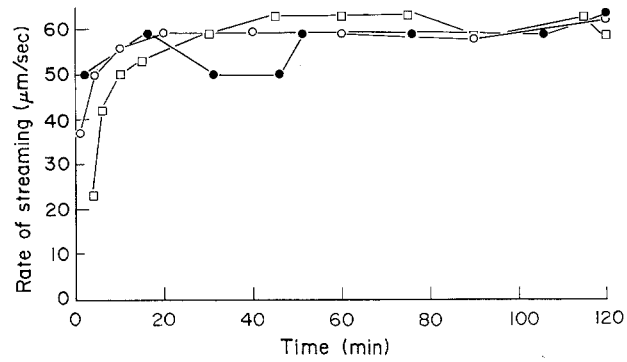

Fig. 1. Effect of Mg(0) medium on the streaming of *N. axilliformis*. Cells were first bathed in APW. At time zero perfusion with Mg(0) medium of room temperature was started. Different symbols show different cells

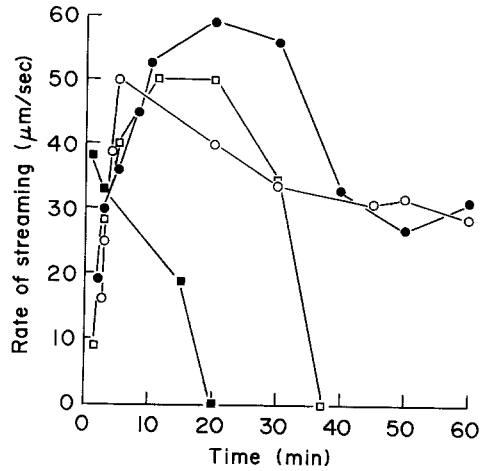

Fig. 2. Effect of plasmolysis at room temperature on the streaming of *N. axilliformis*. Cells were first bathed in APW. At time zero, perfusion with Mg(220) medium of room temperature was started. Different symbols show different cells

recovery began. Only one cell (closed square) showed quick recovery of the streaming. In two cells (closed and open squares), the streaming stopped at 20 and 37 minutes after the start of perfusion. This stoppage must be caused by leakage of ATP through the permeabilized plasmalemma, since the streaming was reactivated by perfusion with Mg·ATP(220) medium. In two cells (closed and open circles), the streaming continued under continuous perfusion with the Mg(220) medium for more than 60 minutes, evidence that the plasmalemma retained its function as a diffusion barrier. Thus, treatment B was not quite satisfactory for obtaining permeabilized cells.

In treatment C, cells were treated with ice-cooled (0.5 °C) Mg(220) medium for 15 minutes and then transferred into the perfusion chamber. At time zero of

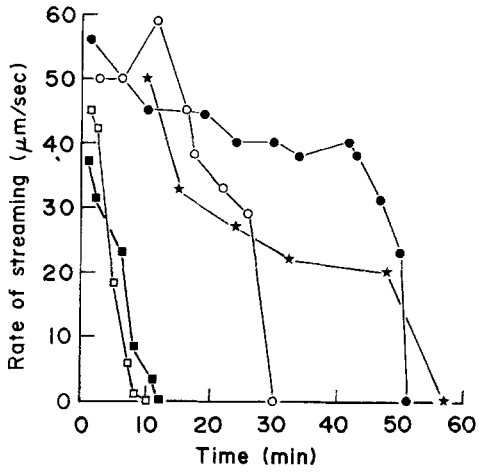

Fig. 3. Effect of plasmolysis at low temperature on the streaming of *N. axilliformis*. Cells were treated with ice-cooled Mg(220) medium for 15 minutes and then transferred into the perfusion chamber. At time zero, perfusion with Mg(220) medium of room temperature was started. Different symbols show different cells

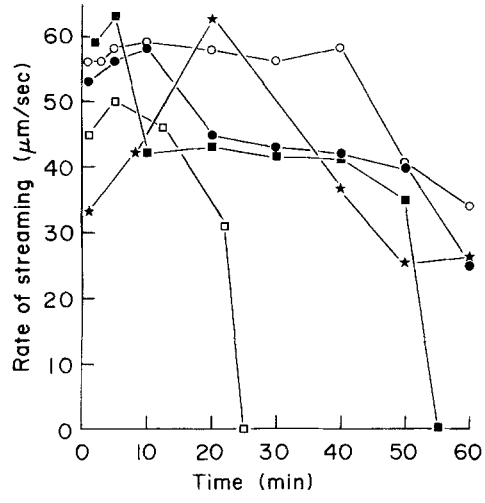

Fig. 4. Effect of pretreatment of cells with Mg(0) medium and subsequent plasmolysis at room temperature on the streaming of *N. axilliformis*. Cells were treated with Mg(0) medium of room temperature for 5 minutes and then transferred into the perfusion chamber containing Mg(0) medium. At time zero, perfusion with Mg(220) medium of room temperature was started. Different symbols show different cells

Fig. 3, perfusion with Mg(220) medium of room temperature was started. The streaming stopped within 60 minutes in all cells. The time needed to stop the streaming varied widely among the cells. The streaming could be reactivated by perfusion with the Mg · ATP(220) medium. Thus, this treatment was effective for permeabilization of the cell.

In treatment D, cells were treated with Mg(0) medium

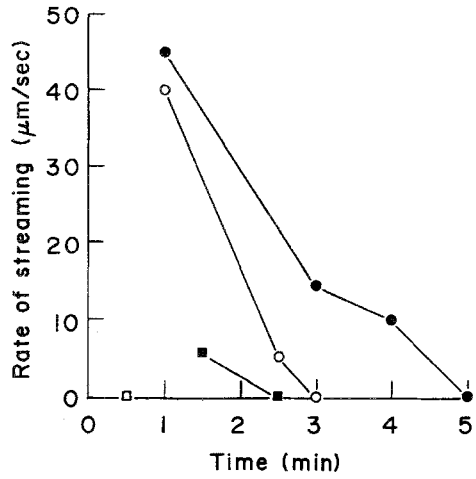

Fig. 5. Effects of pretreatment with Mg(0) medium and subsequent plasmolysis at low temperature on the streaming of *N. axilliformis*. Cells were treated with Mg(0) medium for 5 minutes and incubated in ice-cooled Mg(220) medium for 5 minutes. They were transferred into the perfusion chamber and at time zero, the cell exterior was perfused with Mg(220) medium of room temperature. Different symbols show different cells

of room temperature for 5 minutes and then transferred into the perfusion chamber containing Mg(0) medium. At time zero of Fig. 4, perfusion with Mg(220) medium was started. Just after that plasmolysis occurred but sudden cessation of the streaming was not observed. In two cells (closed and open squares), the streaming stopped within 60 minutes. The streaming was reactivated by perfusion with the Mg · ATP medium. In the other three cells, streaming continued under perfusion with the Mg(220) medium for more than 60 minutes. Thus, this treatment like treatment D was not satisfactory for permeabilization of the cell.

In treatment E, cells were treated with Mg(0) medium of room temperature for 5 minutes and then with ice-cooled Mg(220) medium for 5 minutes. They were transferred into the perfusion chamber. At time zero of Fig. 5, perfusion with Mg(220) medium was started at room temperature. In one cell (open square), the streaming had already stopped when microscopic observation was started. In other cells the streaming stopped within 5 minutes. The streaming was reactivated by perfusion with the Mg · ATP(220) medium in all cells.

In other series of experiments, cells were treated with Mg(0) medium of room temperature for 30 minutes and then with ice-cooled Mg(220) medium for 5 minutes. The streaming of all cells had already stopped when they were transferred into the perfusion chamber containing Mg(220) medium of room temperature. The

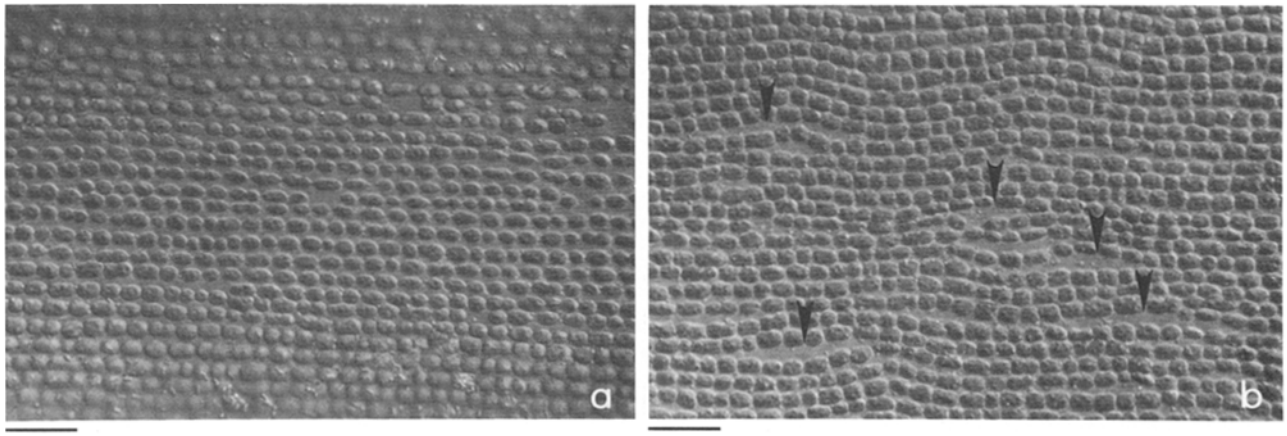

Fig. 6. Micrographs of chloroplast arrangement of *N. expansa* before (*a*) and after (*b*) permeabilization treatment. The cell was treated with ice-cooled Mg(0) medium for 30 minutes and then with ice-cooled Mg(220) medium for 5 minutes. Arrows show clefts formed between chloroplasts files. Scales show 20 μm

streaming was reactivated with Mg·ATP(220) medium (results on five cells). Thus, treatment E was very effective for obtaining permeabilized cells; *i.e.*, by pretreatment of the cells with Mg(0) medium of room temperature and subsequent treatment with Mg(220) medium at low temperature.

These permeabilization experiments with *N. axilliformis* were carried out in December, 1981 and the reactivated streaming continued for about 60 minutes. However, when these experiments were done in January, 1982, the reactivated streaming stopped within 10-20 minutes. Since *N. axilliformis* was cultured under uncontrolled light and temperature conditions, we thought some property of the cell might have been affected. We examined another batch of *N. axilliformis* cultured in the airconditioned room, but the reactivated streaming also continued only for 10-20 minutes.

3.1.2. *Nitella expansa*

The alga was cultured under controlled conditions and was permeabilized best by treatment with ice-cooled Mg(0) medium for 30 minutes and then with ice-cooled Mg(250) medium for 5 minutes. The reactivated streaming continued for more than 60 minutes.

Micrographs of *N. expansa* cells before and after the permeabilization are shown in Fig. 6. Before the treatment, chloroplasts attached to the cortical gel showed a regular arrangement (Fig. 6 *a*). When the cell was permeabilized, this regularity was disturbed more or less by plasmolysis. Many small clefts formed between files of chloroplasts (Fig. 6 *b*).

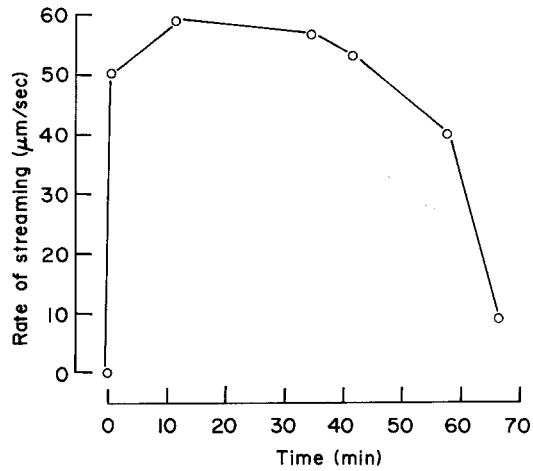

Fig. 7. Time course of the reactivated streaming of *N. expansa*. The cell was treated with ice-cooled Mg(0) medium for 30 minutes and then with ice-cooled Mg(250) medium for 5 minutes. It was transferred into the perfusion chamber containing Mg(250) medium of room temperature. At time zero, perfusion with the Mg·ATP(250) medium was started

3.2. *Effects of ATP and Mg^{2+} on Streaming in Permeabilized Cell Models*

A permeabilized cell of *N. expansa* was bathed in Mg(250) medium of room temperature and perfusion with Mg·ATP(250) medium was started at time zero (Fig. 7). Within 10 to 20 seconds after the start of the perfusion, the streaming was reactivated. The reactivated streaming continued for more than 60 minutes under continuous perfusion with the Mg·ATP(250) medium.

The reversibility of the inhibition of reactivated

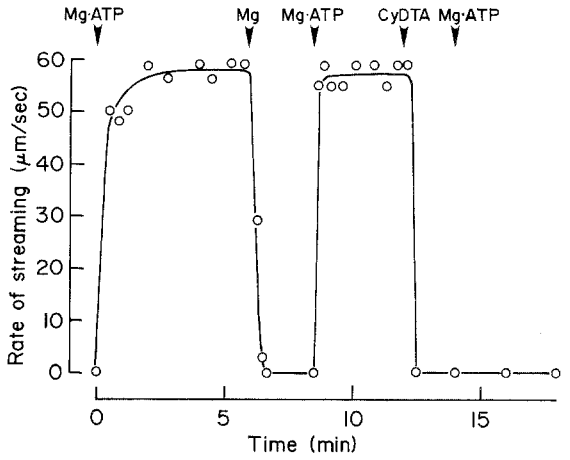

Fig. 8. Inhibition of reactivated streaming of *N. expansa* by ATP and Mg^{2+} depletion. The cell was treated with ice-cooled Mg(0) medium for 30 minutes and then with ice-cooled Mg(250) medium for

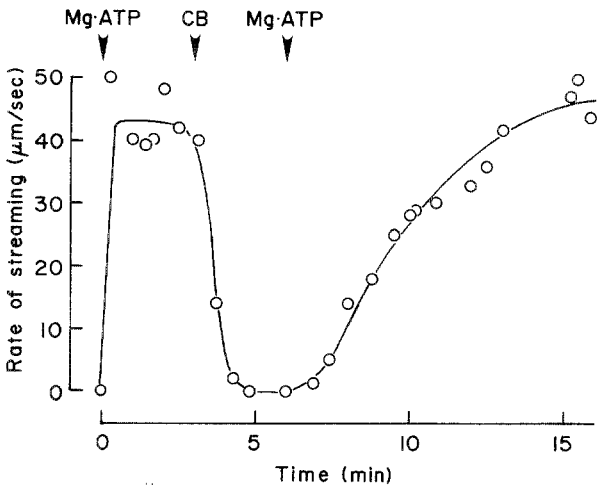

Fig. 10. Reversible inhibition of reactivated streaming with cytochalasin B. A cell of *N. expansa* was treated with ice-cooled

In summary, the present cell model prepared without detergent proved to be superior to the one prepared with detergent (SHIMMEN and TAZAWA 1982a) in that the streaming of the former model lasted longer and was inhibited reversibly by CB. The present cell model is expected to be useful for the study of cytoplasmic streaming.

In preliminary experiments, we found that the reactivated streaming is controlled by Ca^{2+} of physiological concentration (10^{-6} M). Quantitative studies on the Ca^{2+} effect are now in progress.

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gradually and attained its original rate after 10 minutes. In four other cells, the same qualitative result was obtained. CB of 5 μg/ml reduced the rate of the reactivated streaming to half the original rate within 3–4 minutes. The reduced rate was maintained for a longer period. The rate recovered to its original level when CB was washed away (results with three cells, data not shown).

4. Discussion

4.1. Treatment of the Cell for Permeabilization of the Plasmalemma

The present study clarified the conditions necessary for permeabilization of *Nitella* cells. Treatment with Mg(0) medium at room temperature did not cause permeabilization and the streaming continued at a constant rate for more than 120 minutes (Fig. 1). The streaming stopped or its rate decreased within 60 minutes when the cells were incubated in the Mg(220) medium at room temperature (Fig. 2). These results suggest that the plasmolysis in the presence of EGTA permeabilizes the plasmalemma to some extent. The suitable concentration of sorbitol in the Mg medium for preparation of successful cell models which quickly responded to changes of ATP concentration was empirically found by testing Mg media of various sorbitol concentrations. The osmolalities of the Mg(220) and Mg(250) media were 334 and 367 mosM, respectively. The turgor pressures of *N. axilliformis* and *N. expansa* in APW were 258 ± 2 (n = 5) and 331 ± 7 (n = 6) mosM (± SEM, n = number of cells used), respectively. Thus, the difference between osmolalities of the cell and the Mg medium suitable for preparation of the cell model varied among the species.

When the plasmolysis was induced in ice-cooled Mg(220) medium, the streaming stopped within 60 minutes in all cells (Fig. 3), suggesting that plasmolysis at low temperature makes the membrane more fragile. Decrease of membrane fluidity at low temperature (SCHECHTER *et al.* 1974) may make the membrane susceptible to plasmolysis. Pretreatment of the cell with Mg(0) medium before the plasmolysis (Fig. 5) further increases the extent of permeabilization. Removal of Ca^{2+} from the outer surface of the plasmalemma with EGTA may make the plasmalemma more fragile at the moment of plasmolysis. Only partial permeabilization occurred when the plasmolysis was induced at room temperature after pretreatment with the Mg(0) medium (Fig. 4). This again shows the importance of low temperature at the moment of plasmolysis. When the plasmolysis was induced at room temperature, large convex plasmolysis and large clefts between files of chloroplasts were observed (data not shown). Such a cell was proved to be unsuitable as the model, because depletion of ATP from the cytoplasm in the Mg medium took a long time (Figs. 2, 3, and 4). On the other hand, when the model was prepared at low temperature, small clefts between files of chloroplasts formed uniformly (Fig. 6 *b*). In this case, very little and sometimes no plasmolysis was observed. This shows that the plasmalemma quickly lost semipermeability. In this successful cell model, the streaming responded quickly to changes in the ATP concentration (Figs. 8 and 9). The procedures for permeabilization of the plasmalemma can be summarized as follows: removal of calcium from the outer surface of the plasmalemma and subsequent plasmolysis under low temperature.

4.2. Irreversible Inhibition of the Reactivated Streaming by Mg^{2+} Depletion

The CyDTA medium irreversibly inhibited the reactivated streaming (Fig. 8), which is consistent with previous reports (SHIMMEN 1978, SHIMMEN and TAZAWA 1982 a, b) discussing the dual roles of Mg^{2+} in the cytoplasmic streaming, one to act as a cofactor of the ATPase reaction and the other to maintain some structure necessary for the streaming. Inhibition of the reactivated streaming by perfusion with EGTA medium containing no Mg · ATP was dependent on the duration of the perfusion (Fig. 9). When the perfusion lasted for 1 minute or less, the inhibition was completely reversible. When the perfusion was prolonged to 7 minutes, the inhibition became irreversible (SHIMMEN and TAZAWA 1982 b). Many works have suggested localization of myosin in the flowing endoplasm (CHEN and KAMIYA 1975, KURODA and KAMIYA 1975, KAMITSUBO 1981, SHIMMEN and TAZAWA 1982 b). In smooth muscle, it was reported that the structure of the myosin filament is destroyed by Mg^{2+} depletion (SAIDA and NONOMURA 1978, NONOMURA 1979). Further biochemical and ultrastructural studies are awaited.

The reactivated streaming in permeabilized cells was reversibly inhibited by CB as in normal cells. The time needed to stop the streaming on application of 50 μg/ml CB was about 2 minutes, which is the same order of magnitude as the inhibition time caused by 100 μM CB applied internally in tonoplast-free cells (NOTHNAGEL *et al.* 1981).

In summary, the present cell model prepared without detergent proved to be superior to the one prepared with detergent (SHIMMEN and TAZAWA 1982a) in that the streaming of the former model lasted longer and was inhibited reversibly by CB. The present cell model is expected to be useful for the study of cytoplasmic streaming.

In preliminary experiments, we found that the reactivated streaming is controlled by Ca^{2+} of physiological concentration (10^{-6} M). Quantitative studies on the Ca^{2+} effect are now in progress.

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