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Active Sliding Movement of Latex Beads Coated with Skeletal Muscle Myosin on *Chara* Actin Bundles

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Summary

Latex beads coated with rabbit skeletal muscle myosin were introduced by intracellular perfusion into *Chara* cells from which the tonoplasts had been removed. Mg · ATP dependent movement of the beads along files of *Chara* chloroplast layers was observed. The movement was in opposite directions on the two sides of the indifferent line, indicating that the movement was dependent on the polarity of the actin bundles. This suggests that the unknown factor responsible for generating the motive force for cytoplasmic streaming in *Chara* endoplasm is myosin. The advantages of the present experimental system for studying the sliding mechanism of actomyosin are discussed.

Keywords: Actin; *Chara*; Cytoplasmic streaming; Myosin.

Abbreviations: APW, artificial pond water; ATP, adenosine 5'-triphosphoric acid; DTT, dithiothreitol; EDTA, ethylenediamine tetraacetic acid; EGTA, ethyleneglycol-bis(β-aminoethyl ether) *N, N, N', N'*-tetraacetic acid; HMM, heavy meromyosin; LMM, light meromyosin; NEM, N-ethylmaleimide; PIPES, piperazine-*N, N'*-bis(2-ethanesulfonic acid).

1. Introduction

Sliding between myosin and actin as a result of ATP dissipation is one of the most fundamental molecular processes for cell motility (WESSELLS *et al.* 1971). YANO (1978) and YANO *et al.* (1978) first succeeded in constructing a "streaming cell", in which active streaming of a solution containing heavy meromyosin (HMM) relative to fixed actin filaments could be visualized. Recently, an "actomyosin motor" was constructed, in which a blade with attached F-actin filaments moves in a solution containing HMM (YANO *et al.* 1982).

In *Characeae*, KAMIYA and KURODA (1956) found that the motive force for cytoplasmic streaming was generated by the shearing between a stagnant gel layer and the flowing endoplasm. Accumulated data show that actin bundles are anchored on the inner surface of the gel layer (KAMITSUBO 1966, NAGAI and REBHUN 1966, PALEVITZ and HEPLER 1975). The presence of actin in *Characeae* has suggested that myosin is involved in the cytoplasmic streaming. Physiological studies have suggested that myosin is present in the endoplasm of algae (CHEN and KAMIYA 1975, 1981, KAMITSUBO 1981). KATO and TONOMURA (1977) isolated myosin form *Nitella*. Thus, it is reasonable to assume that cytoplasmic streaming in *Characeae* is a manifestation of sliding between myosin in the endoplasm and actin bundles attached to the inner surface of the gel layer. Since heavy meromyosin from rabbit muscle can bind to *Characeae* actin (WILLIAMSON 1974), it is probable that active movement may be inducible between *Characeae* actin and muscle myosin. KURODA and KAMIYA (1975) found that HMM from rabbit muscle can induce active rotation of *Nitella* chloroplasts which should be covered with actin bundle(s).

In the present study, we designed an *in situ* experiment to produce "streaming" of myosin from rabbit skeletal muscle along *Chara* actin bundles. The internodal cell

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was first made tonoplast-free by perfusing the vacuole with a medium containing EGTA, a Ca^{2+}-chelator (TAZAWA *et al.* 1976). In tonoplast-free cells, the chemical composition and also the composition of organelles in the original cytoplasmic space can easily be controlled (TAZAWA and SHIMMEN 1980, SHIMMEN and TAZAWA 1982b). The tonoplast-free cell was perfused with the medium containing EDTA. Depletion of Mg^{2+} irreversibly inactivated the endoplasm but not the actin bundles with respect to their ability to sustain cytoplasmic streaming (SHIMMEN 1978, SHIMMEN and TAZAWA 1982a, b. 1983). Latex beads coated with muscle myosin were introduced into the tonoplast-free cell in order to induce sliding movement of muscle myosin on *Chara* actin bundles.

2. Materials and Methods

2.1. Preparation of Chara

Chara australis was cultured in a plastic bucket filled with tap water. The bottom of the bucket was covered with soil and rotten leaves. The bucket was illuminated from above with two fluorescent lamps (Toshiba 20 W) in a 14-hour light, 10-hour dark cycle. The culture was kept in an air-conditioned room (25 °C). Before the experiments, internodal cells were isolated from neighboring cells and stored in artificial pond water (APW) containing 0.1 mM each of KCl, NaCl and $CaCl_2$ (pH about 5.6)). The three types of intracellular perfusion media used are described in Tab. 1.

Table 1. *Media composition (mM)*

	Media		
	Mg	Mg·ATP	EDTA
ATP	0	1	1
$MgCl_2$	6	6	0
EGTA	5	5	0
EDTA	0	0	5
PIPES	30	30	30
K^+	67	71	67
Sorbitol	200	200	200
pH	7.0	7.0	7.0

Preparation of the tonoplast-free cell was carried out as reported previously (Fig. 1) (TAZAWA *et al.* 1976). Both ends of the internodal cell were cut open and the cell sap was replaced with the Mg·ATP medium by vacuolar perfusion. After the end of perfusion, both cell ends were ligated with strips of polyester thread and the cell was kept in APW for at least 30 minutes during which the tonoplasts disintegrated. The tonoplast-free cell was again perfused with the EDTA medium which drastically lowered the Mg^{2+} concentration. The cell ends were kept open for 5 minutes on the perfusion bench. After the EDTA medium had been replaced by perfusing the cell with

Perfusion with Mg·ATP medium

↓ Incubation in APW for 30–60 minutes

Perfusion with EDTA medium

↓ Incubation on perfusion bench for 5 minutes

Perfusion with Mg·ATP medium

↓

Perfusion with myosin-coated latex beads suspended in Mg·ATP medium

Fig. 1. Procedure for inducing sliding movement of myosin-coated latex beads along *Chara* actin bundles. For details, see text

the Mg·ATP medium myosin-coated latex beads suspended in the Mg·ATP medium were introduced into the cell by the perfusion and both cell ends were again ligated. The sliding movement of the beads was recorded by taking photographs at intervals of 30 or 60 seconds. The perfusion experiments were carried out at room temperature (20–25 °C). Movement of the beads in the *Chara* cell was observed with a Nomarski microscope (Zeiss, Photomicroscope III).

2.2. Preparation of Latex Beads Coated with Rabbit Muscle Myosin

Myosin was prepared from white muscle of the leg and the back of rabbit according to PERRY (1955). Light meromyosin (LMM) was obtained by the method of SZENT-GYÖRGYI (1953). Latex beads (2 μm in diameter, Dow chemicals) were placed in a poly(L-lysine) solution (5 mg/ml, 0.4 N KOH) at 0 °C for 3 hours and washed three times by centrifugation (4,000 × g for 20 minutes) with a solution containing 100 mM KCl, 0.5 mM $MgCl_2$ and 2 mM imidazole-HCl buffer (pH 7.6). They were then suspended in a LMM solution (5 mg/ml) containing 500 mM KCl and 10 mM Tris-HCl buffer (pH 7.6). After washing several times by centrifugation with a buffer solution [300 mM KCl, 10 mM Tris-HCl buffer (pH 7.6)], the beads were suspended in a solution of soluble myosin containing 5 mg/ml myosin, 400 mM KCl, 50 mM PIPES (pH 7.0). The ionic concentration of the solution was gradually decreased by dialysis in a Mg medium (Tab. 1).

This caused the filamentous myosin to bind to the beads. NEM-treated myosin beads were obtained by incubating the beads for 1 hour in Mg medium containing 1 mM NEM, followed by addition of DTT to 2 mM. The ATPase activity was determined by measuring the time course of phosphate liberation according to MARTIN and DOTY (1949).

3. Results

3.1. Characterization of Myosin-Coated Latex Beads

Beads coated with muscle myosin suspended in Mg·ATP medium are shown in Fig. 2. The beads were covered with filamentous aggregates of myosin. Myosin aggregates not associated with the beads were also present. The beads did not move actively, other than showing Brownian motion. ATPase activity of the myosin-coated beads in suspension was 13.5 nmol·P_i/mg protein·minute.

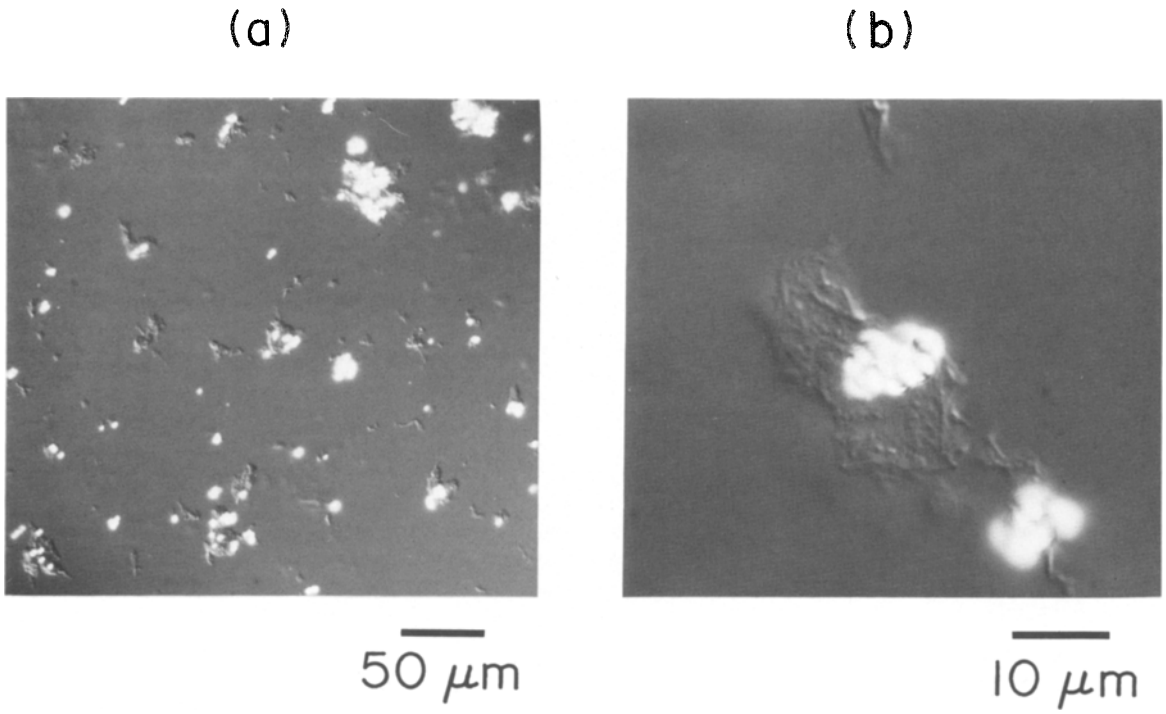

Fig. 2. Nomarski microphotograph of myosin-coated beads suspended in Mg·ATP medium. Objective was × 40 (*a*) and × 100 (*b*)

3.2. Sliding Movement of Myosin-Coated Latex Beads in Chara Cells

When the tonoplast-free cell was perfused with Mg·ATP medium after pretreatment with EDTA medium, no active movement was observed. Since the cell was perfused twice after disintegration of the tonoplast, endoplasmic granules were almost completely effused out and very few granules remained in the cell. Actin bundles were observed on the inner surface of chloroplasts after EDTA treatment.

After the tonoplast-free cell had been successively perfused with EDTA and Mg·ATP media, myosin-coated latex beads suspended in Mg·ATP medium were introduced into the cell by perfusion. Immediately after perfusion, the cell ends were closed by ligation and observed within 2–3 minutes in a microscope. Most of the latex beads located near the chloroplast layer moved along the files of the chloroplast layer. Beads located in the central area of the cell displayed only Brownian motion, indicating that the motive force was generated at the inner surface of the chloroplasts. Bead movement was in opposite directions on the two sides of the indifferent line (Fig. 3). This implies that the direction of bead movements is determined by the polarity of the actin bundles, which is reversed at the indifferent line in *Chara* (KERSEY *et al.* 1976). Tab. 2 shows the sliding velocities of beads obtained in two series of experiments. Active sliding was observed in all cells tested. The velocities of three to seven beads or bead aggregates were measured in the cell and the highest velocity is shown in Tab. 2. Myosin aggregates free of latex beads (Fig. 2) also moved along the chloroplasts files. In the cell, however, we could scarecely observe the aggregates themselves because of the poor optical conditions due to the presence of large amounts of latex beads.

Many kinds of control experiments were carried out. When the tonoplast-free cell was successively treated with 10 mM NEM and 1 mM DTT to inactivate native *Chara* endoplasm (CHEN and KAMIYA 1975, SHIMMEN and TAZAWA 1982 b), which might remain active after treatment with EDTA, active movement could be induced by introducing myosin-coated beads. Myosin-free beads or myosin-coated beads pretreated successibely with NEM and DTT did not move actively in the tonoplast-free cell. Myosin-coated beads suspended in Mg medium did not move, indicating that the movement was fueled by ATP.

4. Discussion

Sliding movement of latex beads coated with rabbit skeletal muscle myosin occurred along files of the chloroplasts layer of *Chara*. The movement was in opposite directions on the two sides of the indifferent

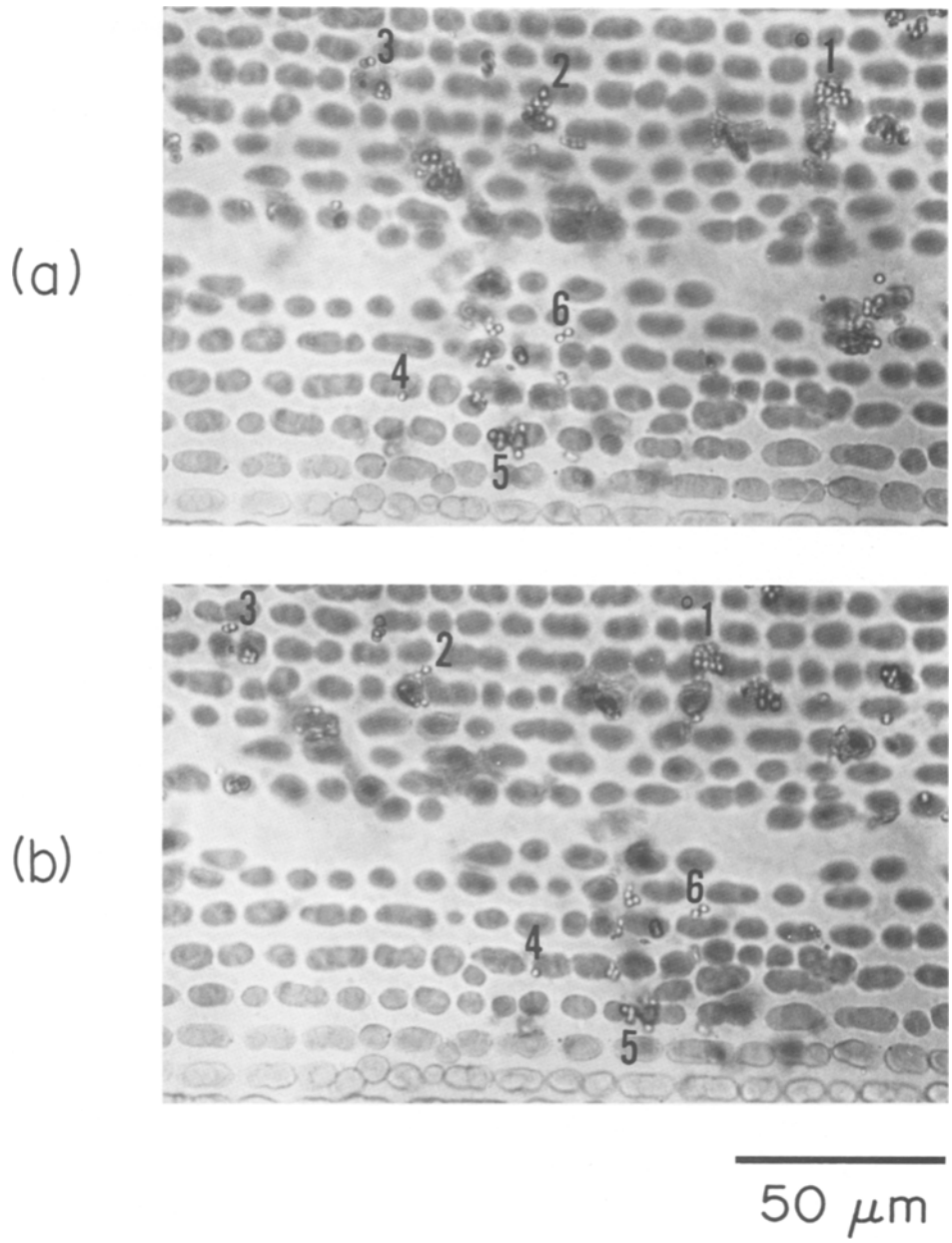

Fig. 3. Time-lapse microphotographs showing latex beads movement in *Chara* cell. Myosin-coated beads suspended in Mg·ATP medium were introduced into a tonoplast-free *Chara* cell according to the procedure shown in Fig. 1. Time interval between (*a*) and (*b*) was 30 seconds. Numbers in the photograph identify beads or bead aggregates

line (Fig. 3). Since the polarity of the actin bundles is reversed at the indifferent line (KERSEY *et al.* 1976), this suggests the involvement of actin bundles in bead movement. Also, myosin-free beads and myosin-coated beads pretreated with NEM did not move, indicating that myosin is necessary for the movement. Thus, the motive force for bead movement is thought to be generated by *Chara* actin bundles and skeletal muscle myosin. Furthermore, absence of movement in the Mg medium shows that the movement is ATP dependent.

These results strongly support the hypothesis that the component in the endoplasm of *Chara* responsible for generating the motive force of cytoplasmic streaming is myosin (CHEN and KAMIYA 1975, 1981, KAMITSUBO 1981). The sliding velocity of the latex beads was significantly lower than that of normal cytoplasmic streaming of *Chara* (60 μm/sec) (HAYAMA *et al.* 1979). When cytoplasmic streaming was reconstituted from *Nitella* actin bundles and isolated *Chara* endoplasmic granules, the rate of streaming was 16–50 μm/sec

Table 2. *Sliding velocity of myosin-coated beads in Chara (μm/sec)*

	Experiment 1	Experiment 2
	1.0	2.1
	0.7	2.5
	0.6	2.1
	0.7	2.0
	1.1	2.4
	0.9	2.2
	0.7	1.5
	0.4	2.1
	0.5	1.6
	1.0	2.4
	0.6	1.9
	0.8	
	0.8	
	0.9	
	1.2	
Average	0.8	2.1

(SHIMMEN and TAZAWA 1982b), which is of the same order of magnitude as that of the original streaming velocity. The orientation of myosin heads around latex beads is probably random, and the direction of movement can be determined by the polarity of the *Chara* actin bundles (Fig. 3). On the other hand, the "myosin" bound to granules of native *Characeae* endoplasm might be arranged with the orientation optimal for generation of the motive force along the actin bundles. NAGAI and HAYAMA (1979) observed granules with filamentous structures which attached to the actin bundles. WILLIAMSON (1979) also reported filaments associated with the endoplasmic reticulum in the cytoplasm. During the preparation of this manuscript, SHEETZ and SPUDICH (1983) reported the movement of HMM-coated fluorescent beads on *Nitella* actin bundles. They used internodal cells cut open longitudinally. The velocity observed was almost the same as that observed in our study. KURODA (1983) recently reported that HMM restored cytoplasmic streaming which had previously been inactivated by NEM treatment. Myosin is thought to exist in filamentous and bipolar forms in living cells. In the present study, we used filamentous myosin in place of HMM and observed active movement. This means that the direction of movement is dependent only on the polarity of the actin bundles and not on the polarity of the myosin filaments.

Since skeletal muscle myosin can interact with *Chara* actin bundles to generate a sliding force, sliding between myosin and actin molecules can be easily studied by the present method. The arrangement of actin bundles with a uniform polarity (KERSY *et al.* 1976) simplifies analysis of the movement. In a tonoplast-free cell, exogeneous molecules can easily be introduced so as to interact with the actin bundles. We conclude that the present method is advantageous for studying the sliding mechanism of actomyosin.

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