Effect of Lateral Forces on the Movement of Myosin-Coated Beads on Actin Cables Studied Using a Centrifuge Microscope

Takakazu KOBAYASHI, Thomas GROSS, Shigeru CHAEN,
Seiryō SUGIURA, Hiromitsu TANAKA, and Haruo SUGI

*Department of Physiology, School of Medicine, Teikyo University,
Itabashi-ku, Tokyo, 173 Japan*

Abstract We developed an *in vitro* motility assay system, in which myosin-coated polystyrene beads were made to slide on actin filament arrays (actin cables) in giant algal cells and subjected to centrifugal forces, which were parallel to the direction of bead movement to serve as external loads on actin-myosin sliding (Oiwa *et al.* (1990) Proc Natl Acad Sci USA 87: 7893–7897), and succeeded in determining the steady-state force-velocity relation of ATP-dependent actin-myosin sliding. To give further information about the properties of actin-myosin sliding, we have applied centrifugal forces, in parallel with the plane of actin-myosin sliding but at right angles with the direction of bead movement, and have found that such “lateral” centrifugal forces reduced the velocity of bead movement. In addition, we have also found that the velocity of bead movement is reduced more markedly with lateral forces applied from the left side of the bead (“left” lateral forces) than those applied from the right side of the bead (“right” lateral forces). These results are discussed in connection with the direction of sliding force generated by the myosin heads on the bead which interact with the right-handed double helix of actin monomers constituting actin filaments.

Key words: actin-myosin sliding, *in vitro* motility assay, lateral forces, centrifuge microscope.

The recent development of the *in vitro* motility assay systems has made it possible to visualize ATP-dependent sliding between actin and myosin under a light microscope [1, 2], though these systems are not suitable for obtaining the relation between force and velocity of actin-myosin sliding. To eliminate the gap between muscle physiology and muscle biochemistry, we constructed an *in vitro* assay system combined with a centrifuge microscope, in which myosin-coated polystyrene beads were made to slide on well organized actin filament arrays (actin cables) [3, 4]

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within an internodal cell segment of an alga *Nitellopsis*, and subjected to centrifugal forces directed opposite to the bead movement to serve as positive loads. With this system, we determined the steady-state force-velocity relation of ATP-dependent actin-myosin sliding essentially similar to that of contracting muscle fibers [5]. In addition, it was found that, when the beads were pushed forward by centrifugal forces, the bead movement was slowed down, an unexpected phenomenon on the basis of the contraction model of A. F. Huxley [6]. Thus, the force-movement assay system with the centrifuge microscope not only provides experimental results comparable with those in contracting muscle fibers, but also reveals new "mysteries" in the properties of ATP-dependent actin-myosin sliding.

The present experiments were undertaken to study the effect of centrifugal forces, directed parallel to the plane of the rotor but at right angles with the actin cables, so that the myosin-coated beads moving on actin cables were subjected to "lateral" centrifugal forces. The experimental situation is compared to the situation in which an "actomyosin train," moving on the actin cable rail, is subjected to a strong lateral wind. It is shown that the movement of the myosin-coated beads is slowed down by such lateral centrifugal forces, and that the above effect of lateral forces is more prominent when they are applied from the left side than from the right side of the beads.

METHODS

Internodal cell preparation containing myosin-coated polystyrene beads. The methods of preparing myosin-coated polystyrene beads (Dynabeads; diameter 2.8 μm ; specific gravity, 1.3) and the internodal cell preparation from the green alga *Nitellopsis obtusa* were the same as those described previously [5, 7]. In brief, an internodal cell (diameter, 0.5 mm; length, 12–17 cm) was cut open at both ends, and the cell sap was replaced by Mg-ATP solution containing 5 mM EGTA, 6 mM MgCl_2 , 1 mM ATP, 200 mM sorbitol, 50 mM KOH, and 20 mM PIPES (pH 7.0) by perfusing the cell interior with the solution several times the cell volume. At the late stage of perfusion, polystyrene beads coated with rabbit skeletal muscle myosin were introduced into the cell, and both ends of the cell were ligated. The internodal cell preparation (length, 1 cm) was put into the centrifuge cuvette to be used for the experiment.

Centrifuge microscope and video recording system. The centrifuge microscope and the video recording system have also been described elsewhere [5, 8]. A stationary image of the specimen mounted on the rotor of the microscope (rate of rotation, 250–5,000 rpm) could be observed and video-recorded during the course of centrifugation by means of a stroboscopic light source.

Experimental procedures. The internodal cell preparation containing the myosin-coated beads were placed in the cuvette filled with artificial pond water [5] in such a way that its chloroplast rows, along which the actin cables extended straight [9], were at right angles with the direction of centrifugal force. The

unidirectional bead movement along the actin cables was observed with a Nikon $40\times$ dry objective (n.a., 0.55). Attention was only focused on the beads that moved smoothly along actin cables running straight on the rows of chloroplasts (Fig. 1A). Care was also taken to choose microscopic field in which all chloroplasts were equally in focus, indicating that the plane of actin-myosin sliding was parallel to the plane of centrifugation. Though the actin cables are not exactly in parallel with the long axis of the internodal cells, their arrangement within the microscopic field (diameter, $450\mu\text{m}$) was reasonably straight. Thus, it was possible to apply "lateral" centrifugal forces directed parallel to the plane of actin-myosin sliding but at right angles with the direction of bead movement, i.e. that of actin-myosin sliding, as illustrated in Fig. 1A. Since the polarity of actin cables that determines the direction of actin-myosin sliding is reversed at both sides of the indifferent zone

Fig. 1. Diagrams showing the application of lateral centrifugal forces to myosin-coated polystyrene beads moving on actin cables on the rows of chloroplasts. (A) Reversal in the direction of bead movement across the indifferent zone as a result of reversal of actin filament polarity, while lateral centrifugal forces can be applied to the moving beads from one side of the internodal cell preparation. (B) "Left" and "right" lateral forces with respect to a bead moving on actin cables.

[10], where chloroplasts are sparsely distributed, the lateral centrifugal forces can be directed either from the left side or from the right side of a bead moving forward. Such lateral centrifugal forces will therefore be classified as "left" or "right" lateral forces according to their relation to the beads (Fig. 1B). The amount of centrifugal force F on the bead is given by:

$$F = \Delta\rho \cdot V r \omega^2,$$

where $\Delta\rho$ is the difference in specific gravity between the bead and the surrounding medium (0.3), V is the bead volume ($12 \mu\text{m}^3$), r is the effective radius of centrifugation (4.5–7 cm), and ω is the angular velocity of the rotor. The velocity of bead movement under a given lateral force was determined on replay from the video cassette recorder (Sony, VO-9600) on the monitor screen (30×20 cm) with an accuracy of $0.5 \mu\text{m}$ by a video microscaler (For-A).

All experiments were made at room temperature (21–24°C). The statistical significance of the difference between mean values was determined by Student's *t*-test.

RESULTS

Reduction of the velocity of bead movement in response to lateral centrifugal forces

Without application of centrifugal forces, the beads moved on actin cables with a constant unloaded velocity V_{max} ($2.5 \pm 0.9 \mu\text{m/s}$, mean $\pm$ SD, $n = 21$). When lateral centrifugal forces were applied to the moving beads, the velocity of bead movement was found to be reduced, though they continued to move with constant velocities. Two selected frames from a videotape of the beads moving on actin cables under a lateral centrifugal force are presented in Fig. 2. Figure 3 shows typical examples of bead movement under various "left" (A) and "right" (B) lateral forces. In both "left" and "right" lateral forces, the velocity of bead movement was reduced with increasing lateral forces from zero to a certain value. With further increase of lateral forces, the velocity of bead movement either increased slightly (A) or stayed nearly constant (B), and the bead eventually stopped moving for a few seconds, and was then suddenly detached from actin cables to flow away in the direction of applied centrifugal force. The critical lateral force required to detach a bead from actin cables will be designated as F_{max} . The value of F_{max} did not differ significantly between "left" and "right" lateral forces, being 3.7 ± 1.0 pN ($n = 10$) and 3.6 ± 1.8 pN ($n = 11$), respectively. The largest value of F_{max} observed was 4.0 pN for "left" lateral forces and 6.2 pN for "right" lateral forces.

Relation between the amount of lateral force and the velocity of bead movement

Figure 4 shows typical relations between the amount of lateral centrifugal force and the velocity of bead movement for "left" (A) and "right" (B) lateral forces. The velocity of bead movement is expressed relative to V_{max} , while the

A

B

Fig. 2. Selected frames from a videotape of a myosin-coated bead (arrows) moving on actin cables (from right to left) under a “right” lateral centrifugal force of $0.50 F_{\max}$. The interval between the frames is 20 s.

Fig. 3. Constant-velocity movements of myosin-coated beads on actin cables under different lateral centrifugal forces. In A, a bead was subjected to four different "left" lateral forces, while another bead was subjected to four different "right" lateral forces in B. The amount of lateral forces is expressed relative to F_{max} , i.e. the force required to detach the bead from actin cables.

Fig. 4. Typical examples of the relation between the amount of lateral centrifugal force and the velocity of bead movement. (A) Relation between "left" lateral forces and the velocity of bead movement. (B) Relation between "right" lateral forces and the velocity of bead movement. The force and the velocity are expressed relative to V_{max} and F_{max} , respectively.

Fig. 5. Lateral force-velocity relations for "left" lateral forces (open circles) and for "right" lateral forces (filled circles), constructed from ten and eleven different data sets, respectively. Data points represent mean values with vertical and horizontal bars indicating SD for velocity and force values ($n=5-11$). Asterisks indicate force ranges where the velocities for "left" lateral forces are significantly lower than those for "right" lateral forces.

amount of lateral centrifugal force is expressed relative to $F_{\max}$. It was noticed that the extent of reduction of velocity of bead movement tended to be more marked with "left" lateral forces than with "right" lateral forces. The above tendency is evident in Fig. 5, in which the lateral force-velocity relations for "left" (open circles) and "right" (filled circles) lateral forces are constructed from ten and eleven different force-velocity data sets, respectively. For the sake of comparison, both "left" and "right" lateral forces were classified into ten different force ranges relative to $F_{\max}$, and the average velocity of bead movement is shown for each force range. Despite a large scatter of the velocity values and a limited number of experiments, the average velocities for four different force ranges above $0.3 F_{\max}$ (marked with asterisks) were significantly lower for "left" lateral forces than for "right" lateral forces ($p < 0.05$). These results may be taken to indicate that "left" lateral forces are more effective in reducing the velocity of bead movement than "right" lateral forces.

DISCUSSION

The present experiments have shown that, when myosin-coated beads moving along actin cables were subjected to constant lateral centrifugal forces, they still moved along actin cables with reduced constant velocities (Fig. 3). The velocity of bead movement was reduced with increasing lateral force from zero to a certain value, and the beads were eventually detached from actin cables when lateral forces reached a critical value $F_{\max}$ (Fig. 4).

The above effect of lateral centrifugal forces are analogous to that of centrif-

ugal forces serving as negative loads [5]. In both cases, the velocity of bead movement is reduced with increasing centrifugal forces from zero to a certain value. As both kinds of centrifugal forces exert forces on the myosin heads interacting with actin cables in directions never experienced by the myosin heads in muscle, these results indicate that, if the myosin heads sliding along actin cables are subjected to forces with "abnormal" directions, they slow down their cycling rate with actin, resulting in a reduction of the velocity of sliding on actin cables. In other words, the cycling rate between actin and myosin is reduced if myosin heads are subjected to forces with abnormal directions. The maximum amount of negative load required to detach the beads from actin cables was 25 pN [5], being much larger than the maximum values of $F_{\max}$ in the present study (4.0 and 6.2 pN for "left" and "right" lateral forces, respectively), indicating that the beads can be detached from actin cables more readily with lateral forces than with forces serving as negative loads.

By comparing the relation between the amount of lateral centrifugal force and the velocity of bead movement (Fig. 5), it has been found that "left" lateral forces are more effective in reducing the velocity of bead movement than "right" lateral forces. In the internodal cells of green algae, the appearance of actin filaments constituting actin cables is very similar to actin filaments in muscle, and also form arrowhead structures similar to those of actin filaments in muscle [3, 4, 9, 10] with heavy meromyosin or myosin subfragment-1. These results indicate structural similarities between actin filaments constituting actin cables and those in muscle. Since actin cables are fixed to the cell inner surface, only one side of actin filaments facing the cytoplasm can interact with the myosin heads on the bead. If it is further taken into consideration that actin filaments consist of right-handed double helixes of actin monomers [11], the myosin heads would mostly interact with actin monomers nearest the bead surface. In this geometrical situation, the direction of the myosin head sliding force is not parallel to the actin filament axis, but inclines to the right, reflecting the right-handed nature of the actin monomer helix. Then, if the direction of sliding force is also assumed to be in parallel with that of the myosin head movement on the actin filament, the present results can be accounted for in the following way. When a myosin head generating a sliding force is subjected to a given amount of lateral centrifugal force, the magnitude and direction of the resultant force are different depending on the direction of lateral force; the magnitude of resultant force is larger with "left" lateral forces than with "right" lateral forces, while the direction of the resultant force vector deviates from the direction of actin-myosin sliding more markedly with "left" lateral forces than with "right" lateral forces (Fig. 6). Therefore, the present finding that "left" lateral forces are more effective in reducing the velocity of bead movement than "right" lateral forces might be accounted for, at least in part, by the difference of resultant force between "left" and "right" lateral force.

It should also be noted that the myosin head sliding along one actin monomer strand should move to another strand in every pitch of the double helix. This

Fig. 6. Diagram illustrating the geometrical situation of interaction between the actin monomers constituting right-handed double helix of actin filament and the myosin head on the bead surface. It is assumed that only the actin monomers nearest the bead surface (shaded ones) can interact with the myosin heads on the bead. The direction of sliding force is shown together with the resultant force during the application of "left" or "right" lateral forces.

situation may enhance the different effect of "left" and "right" lateral forces stated above, since the myosin heads should move from right to left in order to "change" the actin monomer strand, i.e. nearly in the direction of "right" lateral force (Fig. 6).

On the other hand, the result that the force required to detach the bead from actin cables $F_{\max}$ did not differ significantly between "left" and "right" lateral forces might be associated with local distortion of actin filament helical structure with large lateral forces; if the distortion takes place in such a way that the direction of sliding force becomes nearly parallel to the actin filament axis, then there would be little difference in the magnitude and duration of the resultant force vector between "left" and "right" lateral forces. Another possibility is that $F_{\max}$ only depends on the strength of actin-myosin linkages, but not on the direction of centrifugal force.

If an actin filament axis is surrounded by the myosin heads interacting with it, the sliding forces around the filament would tend to rotate the filament. It has been demonstrated that, if an actin filament is fixed in position at its front part, while the

rear part is allowed to slide past a track of heavy meromyosin, the filament tends to twist to form a left-handed superhelix as a result of right-handed rotation [12].

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