Movement of actin away from the center of reconstituted rabbit myosin filament is slower than in the opposite direction

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ABSTRACT By decreasing ionic strength slowly, thick filaments of several micrometers in length were obtained from purified rabbit skeletal muscle myosin. Dark-field observation showed these filaments with their center scattering light extensively. Active movement of actin filaments complexed with tetramethyl rhodamine-phalloidin along the reconstituted myosin filaments was observed. Actin filaments moved towards the center of myosin filaments at a speed of $3.9 \pm 1.6\ \mu m\ s^{-1}$ (mean ± SD, $n = 40$) and often continued to move beyond the center towards the tip of the opposite side at a lower speed. The speed of the movement away from the center was $1.0 \pm 0.6\ \mu m\ s^{-1}$ ($n = 59$). Thus, the functional bipolarity in terms of the movement speed which was first found in native thick filaments of molluscan smooth muscle is also seen in reconstituted filaments from purified rabbit skeletal muscle myosin. The difference of the speed between the two directions is considered to be due to properties of myosin molecules themselves.

INTRODUCTION

Many kinds of movements in life, such as muscle contraction, are based on the interaction between actin and myosin with the use of chemical energy from ATP hydrolysis. In living cells and under the physiological conditions in vitro, the myosin molecules normally form thick filaments by side-by-side interactions occurring at their long tails. The thick filament has a bipolar structure with a bare zone near its center. Actin also forms filaments having a specific polarity. In striated muscles, these two kinds of filaments are packed in sarcomeres so that the pointed ends of actin filaments point towards the center of bipolar myosin filaments (Huxley, 1963). Thus the actin filaments normally interact with parts of myosin filaments of a particular polarity. Active sliding movement occurs only unidirectionally so that the sarcomeres shorten, and the movement of the opposite direction is a passive process.

Recently, many in vitro motility experiments have shown that the direction of movement of the actomyosin system is determined by the polarity of actin filaments (Sheetz and Spudich, 1983; Shimmen and Yano, 1984; Spudich et al., 1985; Kron and Spudich, 1986; Toyoshima et al., 1989; Sellers and Kachar, 1990; Yamada et al., 1990). The polarity of myosin filaments does not determine the direction. However, there is a controversy on the problem of whether the polarity of myosin filaments affects the movement. Toyoshima et al. (1989) have shown that actin filaments can move in both directions at the same speed along tracks of myosin heads formed when actin filaments decorated with heavy meromyosin (HMM) are placed on a nitrocellulose film. Contrastingly, along oriented myosin molecules on native thick filaments isolated from molluscan smooth muscles, actin filaments move towards the center of the thick filaments much faster than when they move in the opposite direction (Sellers and Kachar, 1990; Yamada et al., 1990). So far, the reason for the difference between the results of the two experiments is unknown. The myosin heads in the tracks of HMM may not be orderly arranged to show the difference between the speeds in the two directions. Otherwise, it is possible that some proteins other than myosin affect the movement of actin filaments in the case of the molluscan thick filaments. As reported previously (Yamada et al., 1989), native thick filaments from a molluscan smooth muscle contain a large amount of paramyosin and several other proteins in addition to myosin. Particularly, it is known that paramyosin forms a bipolar core of the thick filament (Szent-Györgyi et al., 1971), and its polarity may affect the movement. In this study, we focused on the problem of whether the functional bipolarity shown in the movement of actin filaments along native thick filaments of molluscan smooth muscles originates from properties of myosin molecules themselves or from other factors. A preliminary report of this work has already appeared in the abstract of the Annual Meeting of the Biophysical Society of Japan (Yamada et al., 1991).

MATERIALS AND METHODS

Myosin was extracted from rabbit back muscle and purified, essentially as described by Szent-Györgyi (1947). Myosin (~10 μg protein ml^{-1}) was dissolved in 0.4 M KCl in buffer I containing 1 mM $MgCl_2$, 1 mM ethyleneglycol-bis-(2-ethylether)-*N,N,N',N'*-tetraacetic acid (EGTA),

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Abbreviations used: HMM, heavy meromyosin; SDS-PAGE, sodium dodecylsulfate-polyacrylamide gel electrophoresis; EGTA, ethyleneglycol-bis-(2-ethylether)-*N,N,N',N'*-tetraacetic acid.

0006-3495/93/02/565/05 $2.00

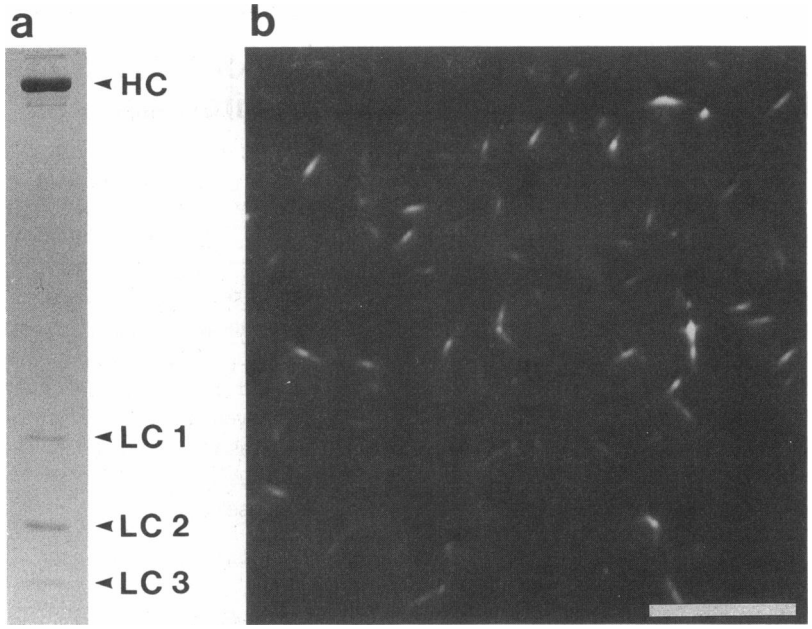

FIGURE 1 (*a*) Myosin used for the reconstituted filaments was analyzed by the 12.5% SDS-PAGE. The gel was stained with Coomassie Brilliant Blue R-250. HC; myosin heavy chain, LC 1, LC 2, LC 3; myosin light chains 1, 2 and 3, respectively. Note that the amount of proteins other than myosin components is very small. (*b*) Reconstituted myosin filaments placed on a Formvar-coated coverslip observed with a dark-field light microscope. Each filament shows its image with its center being brightest. Bar indicates 10 μm.

2.5 mM dithiothreitol (DTT) and 10 mM piperazine-*N,N'*-bis-(2-ethanesulfonic acid) (PIPES)-KOH (pH 7.0) and was dialyzed against the same solution. Buffer I without KCl was slowly added to the external solution using peristaltic pump so that the KCl concentration decreased to 0.12 M after 10 h. The external solution was extensively stirred with a magnetic stirrer during the dilution process. The dialysate contained myosin filaments as shown in Fig. 1 *b* and was used for the further experiment of motility.

The in vitro motility assay was performed essentially as described by Kron and Spudich (1986). In brief, reconstituted myosin filaments were placed on a Formvar-coated coverslip (Fig. 1 *b*) and actin filaments complexed with tetramethyl rhodamine-phalloidin were applied under the condition of 40 mM KCl, 3 mM $MgCl_2$, 2 mM Na_2ATP, 2 mM EGTA, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES)-KOH (pH 7.5), 1% (vol/vol) 2-mercaptoethanol, 5 mg ml^{-1} glucose, 0.1 mg ml^{-1} glucose oxidase and 0.02 mg ml^{-1} catalase at room temperature (24–25°C). Before applying the fluorescent actin filaments, they were mechanically torn to short pieces by pumping the suspension many times with a micropipette. By doing so, it became easy to observe the movement of actin filaments along relatively short tracks of myosin filaments. Movement of fluorescent actin filaments was observed with a fluorescence microscope (Olympus BH-2) with an oil immersion objective lens (Olympus UVFL100, ×100, numerical aperture = 1.30) and high sensitive video camera (Hamamatsu Photonics C2400-08), and was recorded on video tapes using an S-VHS video tape recorder (Sharp VC-D100X). The fluorescence image of a torn actin filament was seen as a spot rather than a long "filament" (see Fig. 2 *c*), and we determined the position of its center by eye on the video monitor. We determined at least three points in succession with intervals of 0.1–0.5 s in almost all cases and the speed was calculated by the least square methods. In a few cases, speed of very slow movement was determined with intervals of 1 s or more.

RESULTS AND DISCUSSION

We used myosin prepared from rabbit skeletal muscles (Szent-Györgyi, 1951). Its protein composition detected by sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) is shown in Fig. 1 *a*. It consists of the heavy chain, three kinds of the light chains and very small amount of other components. By decreasing slowly the KCl concentration of the solution (see Materials and Methods for details), myosin molecules form filaments of several micrometers in length (Nagashima, 1986; Davis, 1988). Figure 1 *b* shows the images of these reconstituted filaments placed on a Formvar-coated coverslip prepared for an in vitro movement assay experiment observed with a dark-field microscope. As was reported by Nagashima (1986), each image of the myosin filament is brightest in the central part and becomes dimmer towards both ends. Katsura and Noda (1971) reported that the myosin filaments formed under the condition similar to the present one were very heterogeneous in length. It is considered that the length distribution of the myosin filaments in this study is similar to theirs. Although the length of the central bare zone of the filaments of purified myosin is considered to be similar to the length of the myosin rod and is ~0.2 μm, the brightest region is longer (see Fig. 2, *a* and *b*). This was considered to be due to the optical system used here. However, by the dark-field observation, we could identify the position of each filament, its orientation in the view and the position of its center, although the exact positions of both ends of the filament could not be identified. We observed movement of actin filaments complexed with tetramethyl rhodamine-phalloidin along these myosin filaments. The fluorescence images of actin filaments recorded on video tapes were compared with

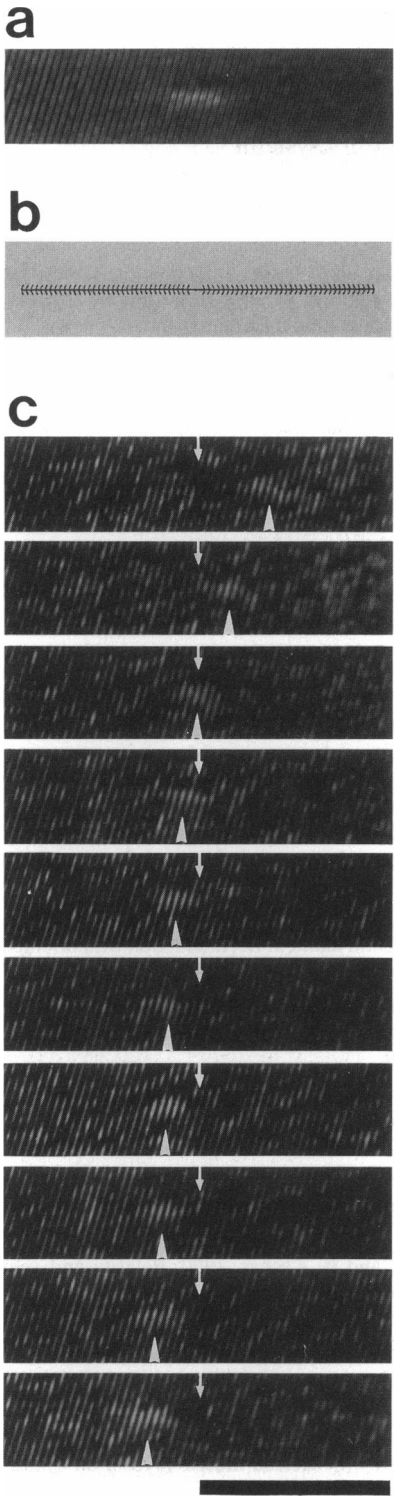

FIGURE 2 Movement of an actin filament along a reconstituted myosin filament. (*a*) A myosin filament observed with a dark-field microscope as shown in Fig. 1. The central part extensively scatters light. (*b*) A diagrammatic representation of the interpretation of the reconstituted myosin filament shown in *a*. Note that the length of the central bare zone is shorter than the bright region of the dark-field image. (*c*) Movement of an actin filament complexed with tetramethyl rhodamine-phalloidin observed by fluorescence microscopy; frames are taken at 0.2 s intervals. The position of the center of the myosin filament is indicated by arrows and the position of the actin filament is indicated by arrowheads. Note that the speed of the actin filament slows down when the filament passes beyond the center of the myosin filament. The bar indicates 5 μm.

dark-field images of the same field of view in order to identify a myosin filament along which each actin filament moved. Fig. 2 shows an example of movement of an actin filament observed with a fluorescence microscope (Fig. 2 *c*) along a myosin filament which could be observed with dark-field illumination (Fig. 2 *a*). As shown, actin moved towards the center of the myosin filament and continued to move beyond the center. The length of the central bare zone is considered to be ~0.2 μm, and hence actin filaments ~ 1 μm in length can easily pass the bare zone. However, the movement away from the center was much slower than that towards the center. We examined the speeds of the two kinds of movement, i.e., towards and away from the center of the myosin filaments (Fig. 3). The speed of movement towards the center was 3.9 ± 1.6 μm s^{-1} (mean $\pm$ SD, $n = 40$), and that of the movement away from the center was 1.0 ± 0.6 μm s^{-1} ($n = 59$). Since the length of the half of a myosin filament was ~3 μm, the distance of each movement was no more than 3 μm, and each measurement was performed within this. Consequently, fluctuations in the speed of the movement and the errors in measurements were considered to contribute significantly to broaden the speed distributions. This seems to be the reason why the speed distributions of the movements shown in Fig. 3 are relatively broad.

This result indicates that the functional bipolarity of myosin filaments in terms of the movement speed, which was first found in native thick filaments of molluscan smooth muscles (Sellers and Kachar, 1990; Yamada et al., 1990), is due to myosin molecules themselves and not due to other proteins contained in the native molluscan thick filaments such as paramyosin. Also, this result suggests that this property is not limited to myosin of molluscan muscles but is common to myosin molecules of other sources. Furthermore, it is possible that other movement systems in life, such as dynein- and kinesin-microtubule systems, have a similar property. So far many in vitro motility experiments have been carried out under the condition that orientation of motor proteins (myosin, dynein and kinesin) is random relative to the orientation of the moving filaments (actin and microtubule). Recently, Yamada and Takahashi (1992) found that when the two different interactions are imposed on one single actin filament moving along native molluscan thick filaments, the actin filament moves in the fast speed mode. This means that myosin cross-bridges of reverse orientation do not make a significant load for cross-bridges of normal orientation, and that if randomly oriented monomeric myosin molecules are attached to the coverslip, actin filaments must move at the faster speed. In addition, assuming the theoretical model

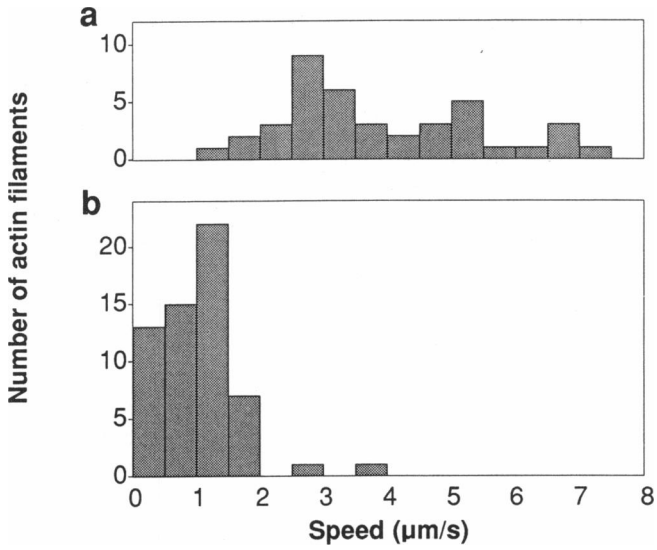

FIGURE 3 Distributions of the speed of movement of actin filaments. (*a*) Speed distribution of movement towards the center of the myosin filament. (*b*) Speed distribution of movement away from the center. The mean speeds ± SD's are 3.9 ± 1.6 $\mu m\ s^{-1}$ (n = 40) and 1.0 ± 0.6 $\mu m\ s^{-1}$ (n = 59) for *a* and *b*, respectively.

presented by Tawada and Sekimoto (1991), it is suggested that the magnitude of the force development by the former cross-bridges is much less than that by the latter cross-bridges (Yamada and Takahashi, 1992). Also, in the case of rabbit skeletal muscle myosin filament, the extent of the force development is possibly different between the two modes.

The ratio of speeds between the two movement modes was about four, and was less than in the case of native thick filaments of molluscan smooth muscles. This fact may be because the arrangement of myosin molecules on reconstituted filaments is not complete. In this sense, native molluscan thick filaments are more useful for studying the properties of oriented myosin molecules. Also, since they are up to no less than 50 μm in length (Szent-Györgyi et al., 1971), very long regions where myosin molecules are arranged in the same orientation can be obtained.

During our present work, Ishijima and Yanagida (1991) also reported that along rabbit skeletal muscle myosin filaments co-polymerized with fluorescence-labelled myosin rods, actin filaments can move away from their center but much slower than in the opposite direction. Their result is consistent to ours and also supports the present discussions.

Now we know that the direction of movement of the actomyosin system is determined by the polarity of actin filaments. The orientation of myosin molecules does not determine the direction but does modulate the speed. Therefore, we have to pay attention to this effect when we plan in vitro motility experiments and consider the results. Also, the theoretical model of the movement must be able to explain this phenomenon in addition to other known many properties of muscles. In other words, the phenomenon presented here is considered to be important for clarifying the mechanism of the actomyosin motility system.

We thank Professor H. Hotani (Teikyo University) for a gift of the dark-field condenser lens used for the observation of myosin filaments. We are also grateful to Ms. K. Saeki and Mr. T. Yasunaga for technical assistances throughout the work and to Dr. M. Tokunaga for beneficial discussions and reading the manuscript.

This work was supported by a grant-in-aid to A. Yamada from the Japan Society for the Promotion of Science, by a grant-in-aid for Specially Promoted Project to T. Wakabayashi from the Ministry of Education, Science and Culture of Japan, and by a grant for "Biodesign Research Program" from RIKEN also to T. Wakabayashi.

Received for publication 14 January 1992 and in final form 6 June 1992.

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