

Review

Active Movement of Actin and Myosin Filaments *In Vitro*

Sugie HIGASHI-FUJIME and Kingo TAKIGUCHI

Received November 1, 1988

We demonstrated for the first time that F-actin bundles isolated from *Nitella* moved actively in vitro, and also purified muscle actomyosin moved in a well defined medium consuming energy liberated by ATP hydrolysis. These observations stimulated the recent progress in research of in vitro motility by using video microscopy.

A single myosin filaments could slide along an F-actin filament, and a single F-actin filaments slid on the myosincoated glass surface unidirectionally at the velocity of $\sim 6 \mu\text{m/s}$ which corresponded to the maximum shortening speed of muscle. The unidirectionality of sliding is specified by the structural polarity of F-actin. Furthermore, F-actin was slid by interacting with proteolytic fragments of myosin, heavy meromyosin, or even subfragment-1 which is a head portion of myosin containing ATPase and actin binding sites whereas intact myosin has two headed structure. Only a myosin head assumes the responsibility for force generation in sliding F-actin.

Accumulating evidence has been providing new information and brings serious questions against the prevalent idea of rotational movement of myosin heads as the molecular mechanism of sliding for muscle contraction.

KEY WORDS: Actin/ Cytoplasmic streaming/ *In vitro* movement/ Myosin subfragment-1/ *Nitella*/ Sliding mechanism/

Various kinds of cell motility are based on the interaction between actin and myosin. Amoeboid movement, cytoplasmic streaming, cell division as well as muscle contraction are those examples. The typical one is contraction of striated muscle which has a well organized crystal like structure of parallel arrangement of thin (F-actin) and thick (myosin) filaments. Relative sliding between these two kinds of filaments causes contraction.^{1,2)} One of the most intriguing questions is how chemical energy liberated through ATP hydrolysis by myosin can be converted into mechanical energy for contraction. The molecular mechanism proposed so far for sliding is that relative sliding movement between myosin and F-actin filaments is evoked by repetitive rotational motion of myosin heads on F-actin accompanied by ATP hydrolysis.^{3,4)} The ATP hydrolysis site and the actin binding site reside in the myosin head projected from the thick filament whose shaft is composed of the myosin tails. Enormous efforts have been dedicated to confirm the rotational motion of myosin heads during contraction by means of biochemical and structural analyses.^{5,6,7,8)} This question is yet unsettled.

We have been attempting to reconstitute the in vitro movement of F-actin and myosin filaments for more than a decade and observed it at the molecular level, in

order to gain deep insights of the molecular mechanism of sliding. We, first, observed travelling of F-actin bundles isolated from green algae *Nitella* in an artificial medium,⁹⁾ and successively observed the active movement of actin and myosin filaments purified from muscle.^{10,11)} These observations triggered the recent progress of the in vitro motility assay by light microscopy.

Dark field microscopy enabled us to see directly very thin filaments such as bacterial flagella,¹²⁾ single myosin filaments, F-actin bundles, and so forth. Fluorescence microscopy visualized single F-actin filaments by labelling with fluorescent dyes in a solution.^{13,14)} Images of moving filaments were recorded by using a highly sensitive video camera on video tape, and processed with a image processor.

Reconstitution of in vitro movements is now becoming a promising technique, and giving us new information, for example, on the occurrence of sliding between single F-actin and myosin filaments in a solution, identification of loci responsible for force generation in a myosin molecule, and so forth.

I. IN VITRO MOVEMENTS WITH PURIFIED MUSCLE PROTEINS, ACTIN AND MYOSIN

I-1. *Superprecipitation*

Actin polymerizes into double stranded helical polymer with polarity. Myosin is an ATP hydrolysing enzyme which is activated remarkably by F-actin, and forms bipolar filaments at low ionic strength. Upon addition of ATP at low ionic strength, actin and myosin filaments give rise to superprecipitation which is a process of large aggregates formation and has been thought to present muscle contraction in vivo. After the first step of superprecipitation, active movements of fibers composed of myosin and actin filaments were observed by dark field microscopy. Large precipitates dispersed gradually into networks of moving fibrils, repeating formation and disintegration of fibrils in the presence of ATP, owing to interaction between actin and myosin. Movements continued for about 30 min at room temperature and after consumption of ATP moving fibrils disappeared.

This observation demonstrated for the first time that purified muscle proteins can really move in a solution by consuming energy of ATP hydrolysis.¹⁰⁾

I-2. *Unidirectional sliding of myosin filaments along F-actin bundles*

Under the condition of superprecipitation at a high concentration of $MgCl_2$ (~ 20 mM), a new type of F-actin bundles having a polarity were spontaneously formed. Short myosin filaments ($\sim 0.6 \mu m$ long) slid unidirectionally along these bundles one after another at the average velocity of $\sim 6.0 \mu m/s$ at room temperature for ~ 30 min as far as ATP remained in the medium (Fig. 1).¹¹⁾ This velocity corresponded to the maximum velocity of muscle shortening under no load.

Electron micrographs exhibited F-actin bundles to which myosin filaments were attached (Fig. 2a). It provided no knowledge about the direction of movement of myosin filaments, since profiles of myosin filaments attached were structurally quite similar in both sides of the filament. The F-actin bundles, however, had polarity: When F-actin filaments in a bundle were decorated with heavy meromyosin (HMM)

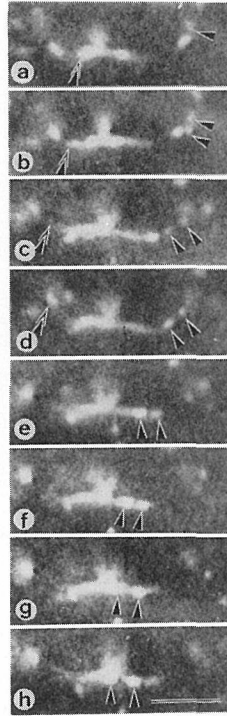


Fig. 1. Unidirectional sliding of myosin filaments on an F-actin bundle. Two myosin filaments (arrowheads) started sliding before coming onto the bundle. A myosin filament is leaving the bundle at the other end (double arrowhead). Myosin filaments slid usually at a constant speed along the bundle and continued sliding before coming onto the bundle and after leaving the bundle for a while. This indicated that F-actin bundles splayed out at both ends into single F-actin filaments which were not visible by dark field microscopy. Time interval between successive photographs is $1/4$ s. The bar indicates $5 \mu\text{m}$.

which is a polypeptide fragment of myosin head portion cleaved by protease, all arrowhead structures of acto-HMM pointed to the same direction in a bundle (Fig. 2b). This indicated that the unidirectionality of sliding movement of myosin filaments was substantially specified by the polarity of F-actin filament, as myosin filament has a bipolar structure.

By dark field microscopy, bundles of F-actin filaments and individual myosin filaments were observable, however, single F-actin filaments were not. There must exist a lot of single F-actin filaments in a dark background of a microscopic field. Along these dispersed single F-actin filaments myosin filaments also exhibited active sliding movement, which was fast and had long straight spans distinct from Brownian movement.^{11,15)} The myosin filament could change the direction by changing the track of the F-actin filament, according to the polarity of the F-actin filament. These observations showed clearly that muscle contraction occurs actually by mutual sliding between actin and myosin filaments.

The sliding velocity of myosin filaments along F-actin filaments is constant and

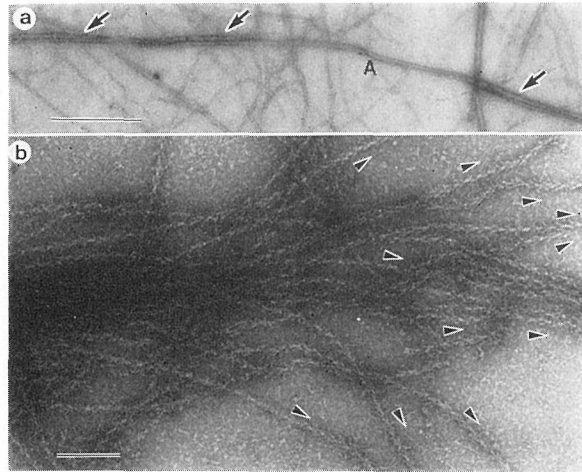


Fig. 2. Electron micrographs of the F-actin bundle associated with myosin filaments. (a) At low magnification, many bundles as well as dispersed single F-actin filaments were observed. Some myosin filaments (arrows) attached to a bundle (A) evenly in their both sides of the bare zone which is the central portion of a bipolar myosin filaments. Electron micrographs did not give us evidence about the direction of movement, even at high magnification. The bar indicates $0.5\ \mu\text{m}$. (b) The bundle decorated with HMM. In the absence of ATP, HMM tightly attached to F-actin forming the arrowhead structure, which shows us the structural polarity of F-actin filaments. In a bundle all arrowheads point to the same direction, indicating that bundles have polarity. Arrowheads in the photograph point to the same direction in those of acto-HMM filaments. The bar indicates $0.2\ \mu\text{m}$.

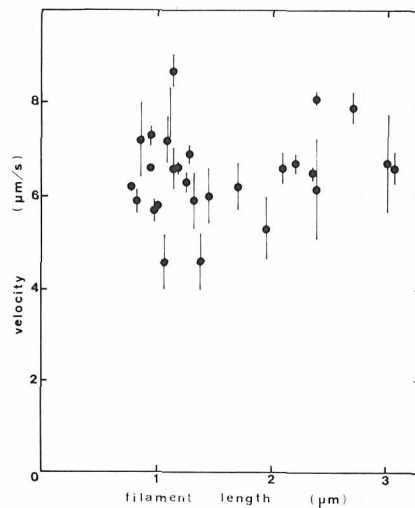


Fig. 3. Sliding velocity independent of filament lengths. The sliding velocity of each myosin filament was measured and was plotted against its filament length. The bar associated with each point is the standard deviation of the velocity of individual filaments. The average velocity of this sample was $6.5\ \mu\text{m/s}$.

independent of the length of myosin filaments (Fig. 3).¹⁶⁾

II. FLUORESCENCE MICROSCOPY

In order to investigate the motility of myosin monomers or its proteolytic fragments, and the motility under various conditions far from that of superprecipitation, observing the movement of single F-actin filaments is one of the feasible strategies. Fluorescence microscopy made it possible to observe them labelled with fluorescent dye. In a solution, HMM and ATP augmented flexible motion of F-actin but failed to cause sliding like movement.¹³⁾ Pursuing the molecular events and more precise loci in a molecule requisit for force generation to slide, we found,¹⁷⁾ independently of Toyoshima and her coworkers,¹⁸⁾ that F-actin could be moved by proteolytic fragments of myosin, HMM which lost the tail portion and has two-headed structure as in intact myosin molecule, and subfragment-1 (S-1) which has only one head.

II-1. Movement of single F-actin filaments

To observe sliding movements of single filaments, either of interacting proteins, actin or myosin, should be fixed on a large substratum like the cover slip. Myosin filaments attached directly to the glass surface, or to silicone coated, or nitrocellulose

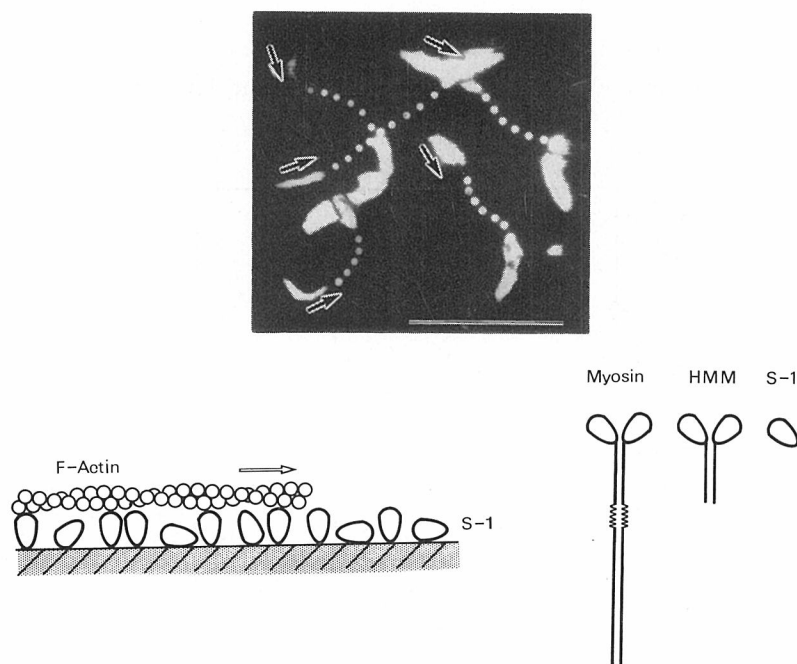


Fig. 4. Sliding of single F-actin filaments labelled with fluorescent dye on the S-1 coated surface. (a) Images of moving F-actin filaments were superimposed at a certain time and after 6 sec. The track and direction of movement are indicated by the dotted line and the arrow, respectively. Bar, 10 μ m. (b) Schematic illustration of sliding of F-actin on an S-1 coated film, and the molecular shape of myosin, HMM and S-1.

coated glass surface produced sliding movement of F-actin filaments at 4 to 6 $\mu\text{m/s}$ at room temperature. Filaments composed of single headed myosin retained the sliding ability.¹⁹⁾ Heavy meromyosin and S-1 attached properly on silicone coated or nitrocellulose coated surface moved F-actin (Fig. 4). Although the sliding velocities were slower than that with myosin ($\sim 3 \mu\text{m/s}$ with HMM, 1–2 $\mu\text{m/s}$ with S-1), this fact clearly showed that neither tail portion of myosin molecule and filamentous form, nor the hinge region which located in the area connecting HMM and the tail, are required for sliding movement. Only the single head of S-1 is sufficient for sliding.

We extensively surveyed motility of F-actin with myosin, HMM and S-1, by varying KCl concentration.²⁰⁾ Sliding of F-actin occurred at low concentration of KCl ($< 60 \text{ mM}$ with myosin, $\leq 40 \text{ mM}$ with HMM, and $\leq 100 \text{ mM}$ with S-1). At around those upper limits of KCl concentrations for movements, fragmentation of F-actin rarely happened and most F-actin filaments were very long ($\sim 10 \mu\text{m}$ long), but they attached at only one or a few points on the glass surface. The moving F-actin filament passed exactly this attached point from the anterior to the posterior end of the F-actin filament at a constant velocity, though free ends of the filament swayed markedly in Brownian movement. This point had to be the site of myosin head interacting with F-actin and generating force for sliding F-actin.

II-2. Biochemical properties

In case of myosin and HMM, loss of motility at the KCl concentrations over

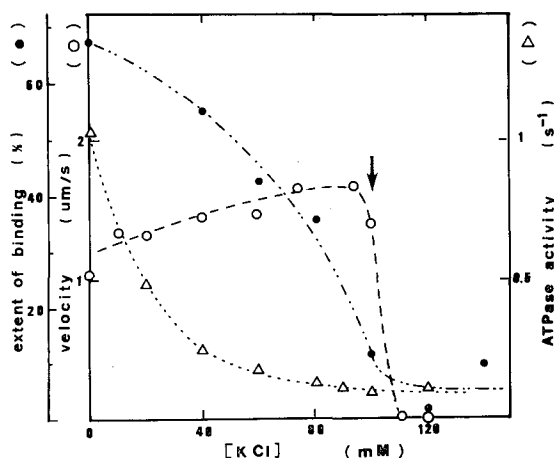


Fig. 5. Mechanochemical properties of acto-S-1. We examined motility, ATPase activity and binding of S-1 to F-actin in the presence of ATP, by varying the KCl concentration. ATPase activity decreased steeply with increasing KCl concentration, whereas the velocity of sliding was almost constant. The upper limit of KCl concentration for sliding was 100 mM (arrow), but the ATPase activity was the same at KCl concentrations over 80 mM and had no correlation with motility. Motility, however, correlated well with binding.

the limit correlated well with both decreasing ATPase activity and loss of binding activity of myosin heads to F-actin in the presence of ATP.²⁰⁾ Subfragment-1, however, behaved differently: Acto-S-1 ATPase activity in a solution steeply decreased with increasing KCl concentration from 0 to 60 mM. At the concentration over 80 mM, ATPase activity did not change up to 140 mM at low level, while no movement was observed at the concentrations higher than 100 mM (Fig. 5). What is involved in this switching of motility?

As shown in Fig. 5, motility of S-1 correlated well with the binding of S-1 to F-actin in the presence of ATP, but not with the ATPase activity. Emergence of motility thus coincided with the binding of heads of myosin, HMM or S-1 to F-actin in the presence of ATP. The binding was quite strong at low ionic strength. This would explain why the *in vitro* movement occurred at low ionic strength, but not at the physiological ionic strength.

III. ENDOPLASMIC STREAMING IN NITELLA CELLS

In *Nitella* cells, endoplasm streams continuously and rotationally along the array of chloroplasts which reside beneath the cell wall. Chloroplasts are linked by F-actin bundles²¹⁾ which are located in the interface between endoplasm (sol-phase) and ectoplasm (gel-phase) and responsible for endoplasmic streaming.²²⁾

III-1. Travelling of F-actin bundles isolated from *Nitella*

F-actin bundles squeezed out of the cell of *Nitella* could move in an artificial medium in an ATP-dependent manner. F-actin bundles continued travelling for 5–10 min in the vicinity of the surface of glass slide (Fig. 6).⁹⁾ The vigorous flow of the medium to wash out chloroplasts which interfered with observation did not affect the movements of the bundles, and the bundles continued moving. The average velocity of travelling of $\sim 10 \mu\text{m/s}$ was similar to that of endoplasmic streaming in the living cell ($\sim 40 \mu\text{m/s}$). F-actin bundles would travel by interacting with *Nitella* myosin attached to the glass surface. No filamentous structures like myosin filaments, however, were found on the glass surface by dark field microscopy. Putative *Nitella* myosin might be soluble protein like myosin I from *Acanthamoeba*.²³⁾

F-actin bundles detached from the array of chloroplasts often formed rings which rotated at the similar velocity. Direction of the travelling or ring rotation was never reversed. This unidirectionality was again specified by the structural polarity of the F-actin bundle, which was confirmed by electron microscopy of the bundle decorated with muscle HMM.

Travelling of F-actin bundles required removal of Ca^{2+} . Endoplasmic streaming in the living cell was also inhibited by Ca^{2+} , and regulated by phosphorylation of some regulatory protein(s).^{24,25)} At present, *Nitella* myosin has not yet been confirmed. It may be quite different from muscle myosin and streaming may be regulated in a different manner from that in skeletal and smooth muscle contraction. *In vitro* motility assay methods can be useful for further investigation on the regulation of streaming.

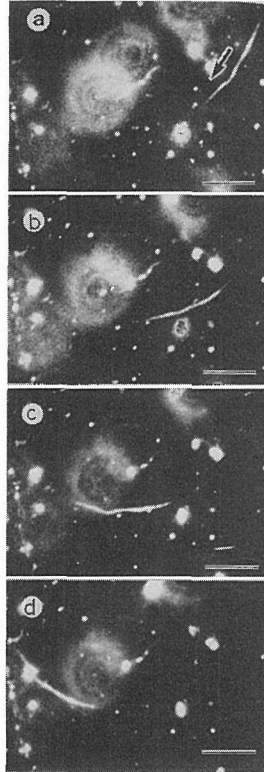


Fig. 6. Travelling of F-actin bundles isolated from *Nitella*. A bundle of F-actin filaments (upper right corner) moved in the direction pointed by the arrow in (a). Movement was observed by dark field microscopy and recorded directly on film. Bar, 10 μ m.

III-2. Elongation of endoplasmic reticulum

Endoplasmic reticulum (E.R.) is a ubiquitous structure found in various kinds of cells and is postulated to be implicated in protein syntheses. But little is known about its function. In *Nitella* cells, E.R. forms the network structure composed of membrane tubes and moves along the actin bundles.²⁶⁾

When the cell contents were squeezed out of the cell, network structures of E.R. spread and attached on the glass surface, and were stationary even in the presence of ATP. By the addition of muscle actin (~ 0.1 mg/ml) to the medium containing Mg-ATP and sucrose the network fibers of E.R. tube formed many branches which began to elongate at the maximum speed of about 30 μ m/s.²⁷⁾ Fibrous tube of E.R. in the network branched, elongated and fused with another E.R. tube, and the network pattern changed at every moment (Fig. 7).

A plausible explanation of this phenomena is that a presumptive *Nitella* myosin or motor protein associated with E.R. membrane causes elongation by interacting with added F-actin which might attach on the glass surface. So far as reported, in cultured cells from animal sources, E.R. movement was microtubule-dependent^{28,29)} and in plant cells, such as *Nitella*,^{26,27)} or onion cells,³⁰⁾ it is actin-based movement.

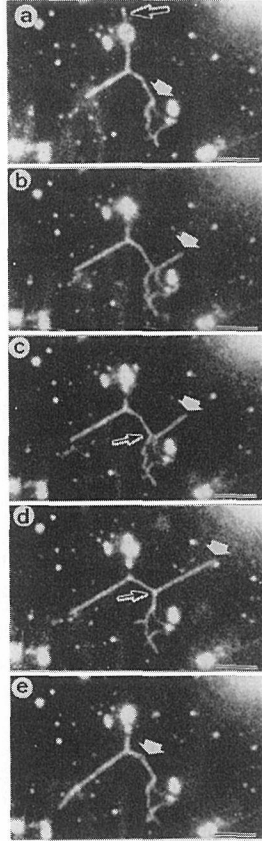


Fig. 7. Elongation of E.R. induced by muscle actin. A small network comprising of tubular membrane of E.R. isolated from *Nitella* was attached on the surface of a glass slide. These network fibers formed branches, and elongated (white arrow) or retracted (black and white arrow in (a)), upon addition of muscle actin in the presence of ATP. The point where the branch started elongation often moved along E.R. as indicated by the black and white arrows in (c) and (d). Bar, 5 μ m.

In the cell, the cytoplasm contains various subcellular structures such as mitochondria, vesicles, E.R. and also soluble proteins and small molecules. It seems to be in chaos. Some structures, however, move and change their localization according to their functions during cell cycle. As suggested by our observation on the movement *in vitro*, these movements may be well organized by submicroscopic structures of cytoskeleton composed of microtubules or microfilaments (F-actin). Some motor proteins as well as myosin and kinesin³¹⁾ or cytoplasmic dynein³²⁾ may be involved in such intracellular movements.

IV. APPLICATION OF *IN VITRO* MOTILITY ASSAY

In vitro motility assay techniques by using video microscopy are still developing and very informative, such as tension measurement.³³⁾ *Nitella*-based motility assay

methods are also useful.^{34,35)} After washing out endoplasm with care from the longitudinally opened *Nitella* cell, myosin-coated beads were applied on it. The beads were attached to and moved along the F-actin bundles. As long term observation is possible without interference by light, e.g., bleaching of fluorescent dye, this method is useful to assay slow movement of proteins, such as non-muscle myosins.^{36,37,38)}

V. SLIDING MECHANISM

Evidences that we have obtained so far from the experiments on in vitro movements, bring us to a new step for understanding the molecular mechanism of muscle contraction and non-muscle cell motility.

Muscle contraction is in fact the sliding between two kinds of filaments, actin and myosin. Polarity of the F-actin filament specifies the direction of the sliding movement and the head portion of myosin is sufficient for sliding. The head, S-1, where actin binding site and ATPase site are located, assumes responsibility for molecular events of sliding. Since, as described in the text, the sliding velocity is independent of the length of both myosin and F-actin filaments, interactions of myosin heads with an F-actin filament do not increase the velocity in an additive manner. In other words, the velocity obtained by extrapolating to zero length of the filament is the same as that of the maximum sliding velocity. It is very likely that one or a few heads are sufficient for sliding at the maximum velocity. This is also suggested by observations at KCl concentrations close to the upper limit: One point (probably one myosin head) attached to F-actin caused continuous sliding of the filament at the same speed as the average. In addition, a head of myosin once attached to an F-actin can slide keeping interaction with F-actin from one end to the other end.

The phenomena described above show that the sliding movement is not simply due to the cyclic reaction of association and dissociation of myosin head concomitant to ATP hydrolysis. In vitro movements occur at low KCl concentrations where the binding of myosin heads to F-actin in the presence of ATP is strong.³⁹⁾ The activity of this binding correlated well with motility of F-actin (or myosin) in vitro. During sliding, a myosin head keeps binding to an F-actin, but at the same time it has to change the actin molecule interacting with it, consecutively along the F-actin filament. It must be a “dynamic state of binding” of actomyosin.

According to a scheme of actin activated ATPase of myosin, there are two routes (Fig. 8). One of those is the ATP hydrolysis cycle of myosin without

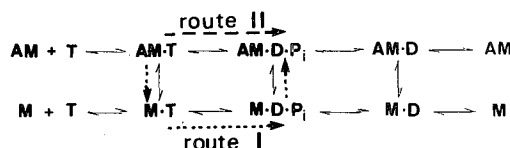


Fig. 8. A scheme of myosin ATPase reaction activated by F-actin. Myosin hydrolyses ATP without dissociation from F-actin through route II (upper row)^{38,39} or repeating association and dissociation from F-actin through route I⁴⁰. A, F-actin; M, myosin; T, ATP; D, ADP; P_i, inorganic phosphate.

dissociation from F-actin: route II in Fig. 8.^{40,41)} Through this route sliding between F-actin and myosin filaments can occur.

The ATPase activity does not directly determine the sliding velocity. The ATPase activity of acto-S-1 does not change at the KCl concentrations higher than 80 mM, but motility is lost over 100 mM. Subfragment-1 cleaved by trypsin into three polypeptides which associated non-covalently can slide F-actin faster than myosin.⁴²⁾ The dynamic state of binding must require the flexibility in myosin heads and F-actin. A specific sequence of molecular motions in myosin heads and F-actin must be important to produce "sliding".

The velocity in vitro sliding ($\sim 6 \mu\text{m/s}$) is quite similar to the maximum shortening velocity of muscle. One or a few myosin heads may produce the sliding movement, as described above. If the sliding is due to the head rotation coupled with the ATP hydrolysis,⁴³⁾ the ATPase activity of 200 s^{-1} should be required. From biochemical data, the maximum ATPase rate is $20\text{--}30 \text{ s}^{-1}$ in a solution. According to this value, the myosin head has to advance more than 60 nm by the hydrolysis of one ATP molecule.^{11,44)} This distance corresponds to more than 10 actin molecules on an F-actin. Does a myosin molecule really take out stored energy in small quantity during sliding?

We are now gaining insights into the molecular mechanism of sliding through the experiments of in vitro movements. Advancing knowledge intensifies our curiosity.

ACKNOWLEDGEMENT

We thank Prof. F. Oosawa for his stimulating discussion and critical reading of this manuscript.

REFERENCES

- (1) J. Hanson and H.E. Huxley, *Nature*, **172**, 530 (1953).
- (2) A.F. Huxley and R. Niedergerke, *Nature*, **173**, 971 (1954).
- (3) A.F. Huxley, *Prog. Biophys.*, **7**, 255 (1957).
- (4) R.W. Lyman and E.W. Taylor, *Biochemistry*, **10**, 4617 (1971).
- (5) R. Cooke, M.S. Crowder and D.D. Thomas, *Nature*, **300**, 776 (1982).
- (6) T. Yanagida, *J. Mol. Biol.*, **146**, 539 (1981).
- (7) Y. Amemiya, T. Tameyasu, H. Tanaka, H. Hashizume and H. Sugi, *Proc. Japan. Acad.*, **56B**, 235 (1980).
- (8) H.E. Huxley, R.E. Simmons, A.R. Faruqi, M. Kress, J. Bordas and M.H. Koch, *Proc. Natl. Acad. Sci. USA*, **78**, 2297 (1981).
- (9) S. Higashi-Fujime, *J. Cell Biol.*, **87**, 569 (1980).
- (10) S. Higashi-Fujime, *Cold Spring Harbor Symp. Quant. Biol.*, **46**, 69 (1982).
- (11) S. Higashi-Fujime, *J. Cell Biol.*, **101**, 2335 (1985).
- (12) H. Hotani, *J. Mol. Biol.*, **106**, 151 (1976).
- (13) T. Yanagida, M. Nakase, K. Nishiyama and F. Oosawa, *Nature*, **307**, 58 (1984).
- (14) H. Honda, H. Nagashima and S. Asakura, *J. Mol. Biol.*, **101**, 131 (1986).
- (15) H. Nagashima, *J. Biochem. (Tokyo)*, **100**, 1023 (1986).
- (16) S. Higashi-Fujime, *Cell Motil. Cytoskel.*, **6**, 159 (1986).
- (17) K. Takiguchi and S. Higashi-Fujime, *Cell Motil. Cytoskel.*, **10**, 347 (1988).
- (18) Y.Y. Toyoshima, S.J. Kron, E.M. McNally, K.R. Niebling, C. Toyoshima and J.A. Spudich, *Nature*, **328**, 536 (1987).

- (19) Y. Harada, A. Noguchi, A. Kishimoto and T. Yanagida, *Nature*, **326**, 805 (1987).
- (20) K. Takiguchi and S. Higashi-Fujime, Submitted to *J. Cell Biol.*
- (21) B.A. Palevitz, J.F. Ash, P.K. Hepler, *Proc. Natl. Acad. Sci. USA*, **71**, 363 (1974).
- (22) N. Kamiya and K. Kuroda, *Bot. Mag. (Tokyo)*, **69**, 544 (1956).
- (23) T.D. Pollard and E.D. Korn, *J. Biol. Chem.*, **248**, 4682 (1973).
- (24) Y. Hayama, T. Shimmen and M. Tazawa, *Protoplasma*, **99**, 305 (1979).
- (25) Y. Tominaga, R. Wayne, H.Y.L. Tung and M. Tazawa, *Muscle Res. Cell Motil.*, **8**, 285 (1987).
- (26) B. Kachar and T.S. Reese, *J. Cell Biol.*, **106**, 1545 (1988).
- (27) S. Higashi-Fujime, *Protoplasma*, Suppl. 2, 27 (1988).
- (28) S.L. Dabora and M.P. Sheetz, *Cell*, **54**, 27 (1988).
- (29) C. Lee and L.B. Chen, *Cell*, **54**, 37 (1988).
- (30) H. Quader, A. Hofmann and E. Schnepf, *Eur. J. Cell Biol.*, **44**, 17 (1987).
- (31) R.D. Vale, T.S. Reese and M.P. Sheetz, *Cell*, **42**, 39 (1985).
- (32) B.M. Paschal and R.B. Vallee, *Nature*, **330**, 181 (1987).
- (33) A. Kishino and T. Yanagida, *Nature*, **334**, 74 (1988).
- (34) T. Shimmen and M. Yano, *Protoplasma*, **121**, 131 (1984).
- (35) M.P. Sheetz and J.A. Spudich, *Nature*, **303**, 31 (1983).
- (36) J.R. Sellers, J.A. Spudich and M.P. Sheetz, *J. Cell Biol.*, **101**, 1897 (1985).
- (37) J.P. Albanesi, H. Fujisaki, J.A. Hammer III, E.D. Korn, R. Jones and M.P. Sheetz, *J. Biol. Chem.*, **260**, 8649 (1985).
- (38) S. Umemoto, A.R. Bengur and J.A. Sellers, *Biophys. J.* **53**, 579a (1988).
- (39) J.M. Chalovich, L.A. Stein, L.E. Greene and E. Eisenberg, *Biochemistry*, **23**, 4885 (1984).
- (40) A. Inoue, M. Ikebe and Y. Tonomura, *J. Biochem. (Tokyo)*, **88**, 1663 (1980).
- (41) L.A. Stein, R.P. Schwarz Jr., P.B. Chock and E. Eisenberg, *Biochemistry*, **18**, 3895 (1979).
- (42) Y.Y. Toyoshima, S.J. Kron, K.R. Niebling and J.A. Spudich, *Biophys. J.*, **53**, 238a (1988).
- (43) A.F. Huxley and R.M. Simmons, *Nature*, **233**, 533 (1971).
- (44) T. Yanagida, T. Arata and F. Oosawa, *Nature*, **316**, 336 (1985).