Subtilisin Cleavage of Actin Inhibits In Vitro Sliding Movement of Actin Filaments Over Myosin

Deborah H. Schwyter,* Stephen J. Kron,‡ Yoko Y. Toyoshima,‡ James A. Spudich,‡ and Emil Reisler*

*Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles, California 90024; and ‡Department of Cell Biology, Stanford University School of Medicine, Stanford, California 94305

Abstract. Subtilisin cleaved actin was shown to retain several properties of intact actin including the binding of heavy meromyosin (HMM), the dissociation from HMM by ATP, and the activation of HMM ATPase activity. Similar V_{max} but different K_m values were obtained for acto-HMM ATPase with the cleaved and intact actins. The ATPase activity of HMM stimulated by copolymers of intact and cleaved actin showed a linear dependence on the fraction of intact actin in the copolymer. The most important difference between the intact and cleaved actin was observed in an in vitro motility assay for actin sliding movement over an HMM coated surface. Only 30% of the cleaved actin filaments appeared mobile in this assay and moreover, the velocity of the mobile filaments was ~30% that of intact actin filaments. These results suggest that the motility of actin filaments can be uncoupled from the activation of myosin ATPase activity and is dependent on the structural integrity of actin and perhaps, dynamic changes in the actin molecule.

I T is generally accepted that muscle contraction occurs when ATP hydrolysis by the myosin head drives actin and myosin filaments to slide past each other. However, the mechanism by which ATP hydrolysis is coupled to the movement of the filaments remains unclear. It has been suggested that ATP hydrolysis results in conformational changes in the myosin head (Huxley, 1969) or in a "hinge" region of the myosin rod (Harrington, 1971, 1979) and that these changes generate the contractile force. The in vitro motility assays of Hynes et al. (1987) and Kron and Spudich (1986) have provided an experimental tool for determining the minimal structural unit of myosin that is necessary for the sliding of actin filaments. It is remarkable that the myosin head alone (myosin subfragment 1; S-1)[1] is able to support sliding movements of actin filaments (Toyoshima et al., 1987). It might be suggested from these studies that the head portion of myosin is the essential element for the motility of actin filaments.

Considerable effort has been invested in the search for the elusive conformational changes taking place in S-1 that could power the sliding of filaments in muscle (Cooke, 1986). Less work has been devoted to understanding the role of actin in this process. For the most part, the study of actin has been focused on mapping its important structural and functional sites by chemical cross-linking (Mornet et al., 1981; Sutoh, 1982; Sutoh and Lin, 1989; Vandekerckhove et al., 1989),

specific modification reactions (Miki et al., 1987 and references therein), immunochemical techniques (Mejean et al., 1987; Miller et al., 1987; Mejean et al., 1988; DasGupta and Reisler, 1989), and nuclear magnetic resonance methods (Barden, 1987; Levine et al., 1988). In several of these studies it has been shown that the NH$_2$-terminal region of actin may be important for its interactions with myosin (Mornet et al., 1981; Sutoh, 1982; Levine et al., 1988; Chaussepied and Morales, 1988; DasGupta and Reisler, 1989). Recently, another acto-S-1 interaction site spanning the region between amino acids 40 and 113 has been identified by chemical cross-linking reactions (Bertrand et al., 1988).

. The role of actin in facilitating motility has been examined in several studies. In models put forward by Oosawa (1983) and Schutt et al. (1989), among others, conformational change within actin filament has been proposed to drive muscle contraction. Supporting such models, spectroscopic evidence for a rotation or distortion of actin monomers during contraction has been obtained by Yanagida and Oosawa (1978), Yanagida (1984), and Thomas et al. (1979). Miki et al., (1987) have collected data consistent with the idea that actin monomers undergo conformational changes upon binding to S-1.

The work described in this paper indicates that the integrity of actin monomers within the actin filament plays an important role in filament sliding. Using the protease subtilisin, monomeric actin is cleaved between Met-47 and Gly-48 (Schwyter et al., 1989). Filaments of this "cleaved actin" appear morphologically normal, bind heavy meromyosin (HMM) in the absence of nucleotide, induce HMM to hydrolyze ATP at a maximal rate similar to that induced by normal

---

Y. Toyoshima's present address is Department of Biology, Ochanomizu University, Ohtsuka, Tokyo, Japan.

---

1. *Abbreviations used in this paper*: HMM, heavy meromyosin; S-1, myosin subfragment 1.

actin filaments, and release HMM rapidly in the presence of ATP. However, despite the apparent retention of many actin functions, filaments of cleaved actin show severely impaired motility. It is possible that the uncoupling between actin's motility and the activation of myosin ATPase is due to inhibition of essential conformational transitions in actin by its subtilisin cleavage.

Materials and Methods

Reagents

Subtilisin Carlsberg (type VIII bacterial protease), α-chymotrypsin, phenylmethylsulfonylfluoride, β-mercaptoethanol, and ATP were purchased from the Sigma Chemical Co. (St. Louis, MO). Phalloidin was obtained from Boehringer-Mannheim Diagnostics, Inc. (Indianapolis, IN). Rhodamine-phalloidin was obtained from Molecular Probes (Eugene, OR).

Protein Preparations and Concentration Determinations

Actin was isolated by the method of Spudich and Watt (1971). G-actin was stored in 2 mM Tris pH 7.6, 0.2 mM $CaCl_2$, 0.2 mM ATP, and 0.5 mM β-mercaptoethanol (G-actin buffer). HMM was prepared by digesting myosin with α-chymotrypsin in 0.12 M KCl, 2 mM $CaCl_2$, 10 mM $NaPO_4$ pH 7.0 for ∼10 min at 25°C (Weeds and Pope, 1977). For motility experiments, HMM was prepared as described by Toyoshima et al. (1987).

The concentrations of actin and HMM were determined spectrophotometrically by using the following extinction coefficients and molecular weights: actin $E_{280}^{1\%}$ = 11.0 cm^{-1}, 42,000; HMM, $E_{280}^{1\%}$ = 6.5 cm^{-1}, 340,000.

Actin Cleavage by Subtilisin

Actin was cleaved with subtilisin as described by Schwyter et al. (1989). Essentially, G-actin at 4 and 9 mg/ml was digested at a 1:1,500 and 1:3,500 weight ratio of subtilisin to actin, respectively, over 40–50 min at room temperature (23°C). The reaction was stopped with the addition of 2 mM PMSF. SDS-PAGE analysis was performed using 15% wt/wt polyacrylamide gels (Laemmli, 1970).

Light Scattering Measurements of Acto-HMM

HMM (3 μM) was added to intact actin or cleaved actin filaments at 3 μM in G-buffer containing 4 mM $MgCl_2$ and 5 μg/ml phalloidin. The formation of the acto-HMM complex was monitored by light scattering. Once complex formation was completed, ATP (1 mM) was added to the mixture and the dissociation of HMM from the intact and cleaved actin filaments was detected by a corresponding decrease in light scattering intensity. All light scattering intensity measurements were collected at 90°, at λ = 660 nm, and at 25°C in a spectrophotometer (Spex Fluorolog; Spex Industries, Inc., Edison, NJ) as described by Miller et al. (1988).

ATPase Activity Measurements

The ATPase activities of HMM activated by both intact and cleaved actin filaments were measured at 30°C in 4 mM $MgCl_2$, 2.5 mM KCl, and 10 mM imidazole (pH 7.0) as previously described (Reisler, 1980). No difference in ATPase activity was noted when the reaction buffer included 5 μg/ml phalloidin. Enzyme activities were calculated as turnover rates (micromoles phosphate) (micromoles myosin heads)$^{-1}$ (s)$^{-1}$.

Motility Assays

The motility of actin and cleaved actin filaments was assayed as described by Toyoshima et al. (1987). In short, HMM diluted in assay buffer (10 mM imidazole pH 7.4, 2.5 mM KCl, 4 mM $MgCl_2$, and 1 mM DTT) was allowed to bind to nitrocellulose coated coverslips in a flow cell. Unbound HMM was washed from the cell with a solution of 0.5 mg/ml bovine serum albumin dissolved in the assay buffer. Rhodamine-phalloidin-labeled actin (RP-actin, 10 nM) was infused into the flow cell and allowed to bind to the HMM-coated surfaces. After the addition of 1 mM ATP in assay buffer, the motility of actin filaments was observed by fluorescence microscopy as described by Kron and Spudich (1986). Rates of filament movement were determined from video records using a microprocessor-based analysis system (Sheetz et al., 1986). Percent motility was determined from traces of single video frames taken 10 s apart.

Electron Microscopy

Rhodamine-phalloidin-labeled actin (0.78 μM) was prepared as described before (Kron and Spudich, 1986). Decoration of actin filaments by HMM was performed by mixing 20 μl RP-actin, 4 μl HMM (0.7 mg/ml), and 2 μl 10 mM potassium phosphate buffer (pH 7.2), and incubating for 20 min at room temperature. The sample was diluted with an equal volume of 10 mM potassium phosphate buffer, applied to a carbon-coated formvar EM grid, and stained with 1.0% uranyl acetate. The grids were examined with a Phillips EM 201 at 45,000×.

Results

Subtilisin Cleaved Actin

The preparation and the properties of subtilisin cleaved actin including its polymerization and interaction with S-1 were described in earlier work (Schwyter et al., 1989). It was found that subtilisin nicks monomeric actin between Met-47 and Gly-48. This cleaved actin is composed of an NH$_2$-terminal peptide (with an apparent mobility of 9 kD) and a COOH-terminal fragment (36 kD) separable upon SDS denaturation (Schwyter et al., 1989). A typical preparation of subtilisin cleaved actin used in this work is shown in Fig. 1. The 9-kD peptide is often poorly stained by Coomassie blue and not always apparent in SDS gels. Similar properties were ascribed to chymotryptically cut actin (Konno, 1987).

Morphology of Cleaved Actin and Acto-HMM Complexes

With the addition of 2 mM $MgCl_2$, cleaved G-actin monomers assemble into filaments (Schwyter et al., 1989). Fig. 2 A shows an electron micrograph of these filaments that appear morphologically indistinguishable from intact actin filaments. As shown in Fig. 2 B, HMM decorates cleaved actin filaments and forms the distinctive arrowhead structures observed in intact acto-HMM complexes. This confirms the results of earlier observations of S-1 complexes with intact and cleaved actins (Schwyter et al., 1989).

Interactions of Cleaved Actin with HMM

Earlier work has established that while the affinity of S-1 for cleaved actin is somewhat lower than that for intact protein, the maximum velocities of the activated ATPase reactions are virtually the same for both actins (Schwyter et al., 1989). The hydrolysis of ATP by HMM in the presence of both cleaved and intact actins follows the same pattern. Lineweaver-Burk plots of actin-activated ATPase activities of HMM measured at 2.5 mM KCl yield K_m values of ∼200 and 33 μM for cleaved and intact actins, respectively (Fig. 3); the V_{max} values for both reactions are estimated to be 12.5 s^{-1}.
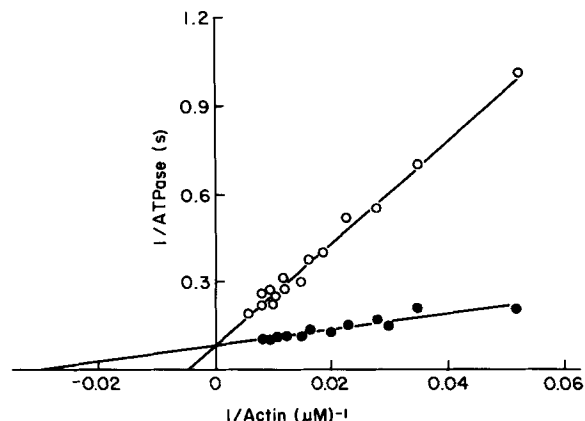
It has been shown before that mixtures of cleaved and intact actin can form copolymers (Schwyter et al., 1989). The ATPase activity of HMM in the presence of copolymers of intact and cleaved actin is shown in Fig. 4. Under conditions of low KCl concentration, a linear relationship is found between the ATPase activity of any given copolymer and the fraction of intact actin that it contains. In other words, intact and cleaved actin units contribute independently and addi-
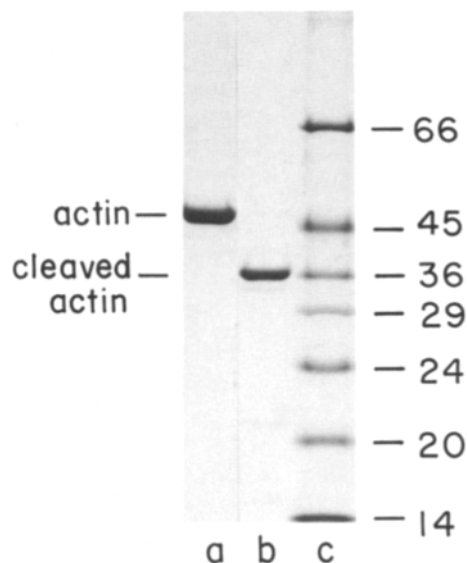
Figure 1. Subtilisin-cleaved actin. G-actin (4 mg/ml) was digested with subtilisin (at 1:1,500 wt/wt ratio) at 23°C for 40 min as described in Materials and Methods. Lane a shows the intact molecule, lane b shows the cleaved species, and lane c contains the molecular mass standards. The carboxyl-terminal fragment of cleaved actin runs with a molecular mass of 36 kD. The amino-terminal fragment is not resolved by this gel system.

Figure 3. Lineweaver-Burk plots for the activation of HMM ATPase activity by intact and cleaved actin. ATPase activities of HMM (0.6 μM S-1 sites) were measured in the presence of intact or cleaved actin in 10 mM imidazole, pH 7.0, 4 mM $MgCl_2$, and 2.5 mM KCl. Actin concentration ranged from 0.8 to 7.1 mg/ml. The K_m values were 33 and 200 μM for intact and cleaved actin, respectively. V_{max} was 12.5 s^{-1}.

tively to the activation of HMM ATPase reaction by a co-polymer.

The binding and dissociation of HMM from cleaved and intact actins were compared directly in light scattering experiments (not shown). The binding of HMM to either actin or cleaved actin filaments resulted in similar, fast increases in light scattering intensity. With the addition of ATP, the intensity of scattered light decreased immediately and re-

mained low during ATP hydrolysis. Once the ATP was hydrolyzed, the two proteins readily reassociated. While the hydrolysis time was significantly longer for cleaved actin than for intact actin, the dissociation of HMM from the two forms of actin occurred to a similar extent (not shown).

Motility of Cleaved Actin

Intact actin filaments labeled with rhodamine-phalloidin will bind to HMM adsorbed to a nitrocellulose coated glass surface. With the addition of ATP, the filaments move over the HMM covered surface. This movement is easily observed by fluorescence microscopy and has been described in detail (Kron and Spudich, 1986).
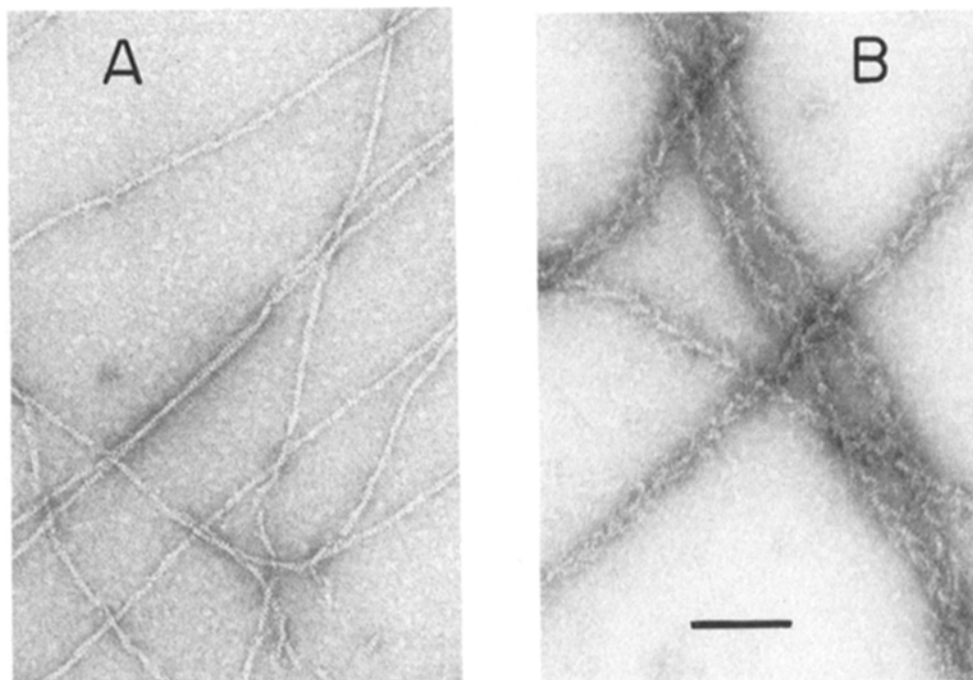
Figure 2. Electron micrographs of actin filaments and Acto-HMM complexes. (A) Filaments of cleaved actin; (B) complexes of HMM and cleaved actin filaments, demonstrating the characteristic decoration pattern.
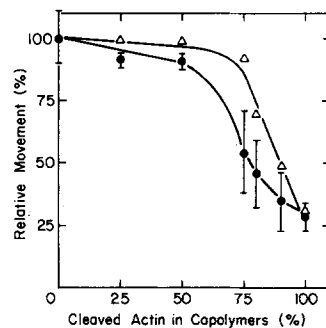
Schwyter et al. In Vitro Movement of Actin Filaments

467
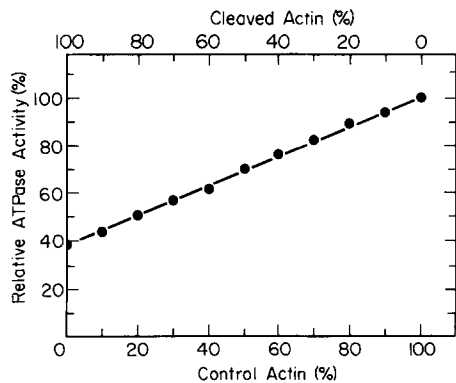
Figure 4. Activation of HMM ATPase activity by copolymers of intact and cleaved actin. The activation of HMM (0.6 μM S-1 sites) ATPase activity was measured for copolymer of intact and cleaved actin (36 μM) in a buffer of 10 mM imidazole, pH 7.0, 4 mM MgCl$_2$, and 2.5 mM KCl. Relative ATPase activities were expressed as a percent of ATPase activity measured for control actin. The copolymers were prepared by mixing monomers of intact and cleaved actin and polymerizing them with 4 mM MgCl$_2$. The total actin concentration was 36 μM.

Figure 5. In vitro motility of cleaved actin and copolymers of intact and cleaved actin. Intact, cleaved, and copolymers of intact and cleaved actin were labeled with rhodamine-phalloidin and allowed to bind HMM adsorbed to microscope coverslips. Upon the addition of 1 mM ATP, the motility of the filaments in 10 mM imidazole pH 7.0, 2.5 mM KCl, and 4 mM MgCl$_2$ was observed by fluorescence microscopy. The proportion (n = 71–198 filaments) (Δ) and velocities (n = 10 for each preparation) ($\bullet$) of the mobile filaments in the copolymer preparations are normalized to those observed for filaments of intact actin.

In the present work, the movement of intact actin filaments is compared with that of filaments of cleaved actin and copolymers containing different fractions of cleaved and intact proteins. The difference in motility between intact and cleaved actin is striking. While virtually all the intact filaments are motile, only 30% of the cleaved actin filaments undergo translational movement (Fig. 5). In the presence of ATP, many of the cleaved actin filaments undergo characteristic "wiggling" motions. Such filaments slowly change shape while remaining bound to the surface. These movements, however, do not result in any net displacement across the field of the microscope.

The number of copolymer filaments that show motility depends on the fraction of cleaved actin in the filament. When cleaved actin constitutes 60% or more of the copolymer filament, the number of motile filaments is proportional to the amount of intact actin present. When cleaved actin makes up <40% of a copolymer, the number of filaments moving is greater than that expected from additive contributions of intact and cleaved actins. The relationship between the percentage of motile filaments and the fractions of cleaved actin units within those filaments is shown by the upper curve in Fig. 5. This graph shows that a high intact actin content appears to compensate partially for the inhibition of motility due to the cleaved actin units.

The velocities of moving filaments of intact and cleaved actin and copolymers of these proteins are also compared in Fig. 5. Intact actin moves at an average rate of ~5.3 ± 0.5 μm/s. Those cleaved actin filaments that move do so with a velocity ~30% that of intact actin (1.5 ± 0.3 μm/s). When the copolymer filaments contain 75% of cleaved actin, the measured velocity of the filaments is slower than that expected from the respective contributions of intact and cleaved actins. This decreased velocity may be a result of intrafilament interactions in the copolymers.

The interaction of actin with myosin is sensitive to ionic strength conditions. Thus, the motility assays for intact, cleaved, and copolymers of intact and cleaved actin have been conducted in the presence of both 2.5 as well as 25 mM

KCl. No difference in the motility properties of any actin preparation was observed between the two salt conditions.

Discussion

Monomeric actin nicked by subtilisin has provided an interesting tool with which to study the role of actin in muscle contraction and cell motility. While cleaved actin monomers can assemble into F-actin filaments, these filaments have lost a significant part of their ability to undergo translational motions as assessed by in vitro motility assays. These assays show that only ~30% of the cleaved actin filaments move, and their movement proceeds at velocities that are at best 30% of those observed for the intact actin. To the extent that it would be justified to represent the general motile properties of the population of filaments by a product of the number of the moving filaments and their velocities (i.e., by crude measurement of a total distance traveled by all filaments), the cleaved actin appears to retain at most 10% of the motile activity of its parent molecule.

The nonlinear dependence of the motility of actin on the composition of its copolymers could be explained by the presence within the filaments of intermolecular interactions between the intact and cleaved protein. Earlier evidence of actin-based cooperativity and intrafilament interactions is consistent with such a possibility (Loscalzo et al., 1975; Fujime and Ishiwata, 1971; Ando, 1989).

Despite the fact that a large majority of the cleaved actin filaments do not display translational motility, the presence of ATP does seem to induce the wiggling of these molecules. Similar wiggling motion has been also observed for regulated actin filaments in the absence of Ca^{2+} (Toyoshima, Y. Y., S. J. Kron, and J. A. Spudich, unpublished results; Honda and Asakura, 1989). In the latter case the wiggling motion could be ascribed to incomplete regulation or perhaps viewed as the movement of catalytically "switched off" regulated actin (Chalovich et al., 1981). This explanation would not apply to cleaved actin filaments which can activate myosin ATPase to the same V_{max} values as intact actin.

The cyclic nature of the association and dissociation of myosin heads from thin filaments is essential to muscle contraction. These events are coupled to ATP hydrolysis. The higher K_m value measured for the ATPase reaction activated by cleaved actin suggests a weaker binding of S-1 to the modified protein than that to intact actin in the presence of ATP.

However, the observation that intact and tryptically cleaved S-1 support equally well the movement of actin filaments (Toyoshima, Y. Y., S. J. Kron, K. R. Niebling, and J. A. Spudich. 1988. *Biophys. J.* 53[2, Pt. 2]:238a[Abstr.]) in spite of their different affinities for actin in the presence of ATP (Botts et al., 1982) suggests that the motility assay is relatively insensitive to differences in K_m values. Furthermore, it appears from light scattering experiments that HMM dissociates from cleaved actin filaments in the presence of ATP to the same degree as it does from normal actin filaments. Therefore, it would be difficult to ascribe the inhibition of sliding movement by subtilisin cleavage of actin to either poor association or dissociation from HMM during ATP hydrolysis. However, it should be noted that light scattering measurements would be unable to detect the presence of a small fraction of "damaged" actin (1–2%) that binds HMM irreversibly in the otherwise homogeneous preparations of cleaved actin. In the case of such an unlikely scenario, the movement of actin filaments would be slowed by the damaged protein.

The observations reported in this work cannot be easily accounted for by invoking changes in the kinetics of actomyosin interaction caused by subtilisin cleavage. Perhaps, the slow filament sliding and the wiggling motions of the filaments represent a partial uncoupling of ATP hydrolysis from motility. An obvious question is whether these data can be reconciled with cross-bridge theories which invoke conformational change within myosin as the conformational engine driving muscle contraction. In one form of such theories, the actin filament serves as a rigid cable on which the head of myosin can form a loaded "spring-like" complex. The "spring" would relax with release of the products of ATP hydrolysis, driving the actin filament forward. If cleavage with subtilisin results in a loss of rigidity in the NH$_2$-terminal region of the actin monomer (a site of actomyosin interaction), the action of the proposed myosin spring might be partially uncoupled from filament sliding and the spring energy might be dissipated among internal motions in actin filaments.

Alternatively, if conformational change within a spring-like domain on actin is the mechanochemical transducer of muscle contraction (Oosawa, 1983; Schutt et al., 1989), cleavage of actin and the presumed loss of some of its rigidity might interfere with the loading of the putative actin spring during actomyosin interaction. The loaded and unloaded states might correspond to two conformations of actin. Transitions between these conformations may be inhibited by subtilisin cleavage. Further work is necessary to test these and other models for the role of actin in motility.

References

Ando, T. 1989. Propagation of acto-S-1 ATPase reaction-coupled conformational change in actin along the filament. *J. Biochem.* 105:818–822.

Barden, J. A. 1987. Conformation of the ATP binding peptide in actin revealed by proton NMR spectroscopy. *Biochemistry.* 26:6023–6030.

Bertrand, R., P. Chaussepied, R. Kassab, M. Boyer, C. Roustan, and Y. Benyamin. 1988. Crosslinking of the skeletal myosin subfragment-1 heavy chain to the N-terminal actin segment of residues 40-113. *Biochemistry.* 27: 5728–5736.

Botts, J. A., A. Muhlrad, R. Tokashi, and M. F. Morales. 1982. Effects of tryptic digestion of myosin subfragment 1 on its actin-activated adenosinetriphosphatase. *Biochemistry.* 21:6903–6905.

Chalovich, J. M., P. Boon Chock, and E. Eisenberg. 1981. Mechanism of actin of troponin-tropomyosin. *J. Biol. Chem.* 256:575–578.

Chaussepied, P., and M. F. Morales. 1988. Modifying preselected sites on proteins: the stretch of residues 633–642 of the myosin heavy chain is part of the actin-binding site. *Proc. Natl. Acad. Sci. USA.* 85:7471–7475.

Cooke, R. 1986. The mechanism of muscle contraction. *CRC Crit. Rev. Biochem.* 21:53–118.

DasGupta, G., and E. Reisler. 1989. Antibody against the amino terminus of α-actin inhibits actomyosin interactions in the presence of ATP. *J. Mol. Biol.* 207:833–836.

Fujime, S., and S. Ishiwata. 1971. Dynamic study of F-actin by quasielastic scattering of laser light. *J. Mol. Biol.* 62:251–265.

Harrington, W. F. 1971. A mechanochemical mechanism for muscle contraction. *Proc. Natl. Acad. Sci. USA.* 68:685–689.

Harrington, W. F. 1979. On the origin of the contractile force in skeletal muscle. *Proc. Natl. Acad. Sci. USA.* 76:5066–5070.

Honda, H., and S. Asakura. 1989. Calcium-triggered movement of regulated actin in vitro. *J. Mol. Biol.* 205:677–683.

Huxley, H. 1969. The mechanism of muscle contraction. *Science (Wash. DC).* 164:507–520.

Hynes, T. R., S. M. Block, B. T. White, J. A. Spudich. 1987. Movement of myosin fragments in vitro: domains involved in force production. *Cell.* 48:953–963.

Konno, K. 1987. Functional chymotryptically split actin and its interaction with myosin subfragment 1. *Biochemistry.* 26:3582–3589.

Kron, S. J., and J. A. Spudich. 1986. Fluorescent actin filaments move on myosin fixed to a glass surface. *Proc. Natl. Acad. Sci. USA.* 83:6272–6276.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T$_4$. *Nature (Lond.).* 227:680–685.

Levine, B. A., A. J. G. Moir, and S. V. Perry. 1988. The interaction of troponin-I with the N-terminal region of actin. *Eur. J. Biochem.* 172:389–397.

Loscalzo, J., G. H. Reed, and A. Weber. 1975. Conformational change and cooperativity in actin filaments free of tropomyosin. *Proc. Natl. Acad. Sci. USA.* 72:3412–3415.

Mejean, C., M. Boyer, J. P. Labbe, L. Marlier, Y. Benyamin, and C. Roustan. 1987. Anti-actin antibodies. An immunological approach to the myosin-actin and the tropomyosin-actin interfaces. *Biochem. J.* 244:571–577.

Mejean, C., H. K. Hue, C. Rouston, and Y. Benyamin. 1988. Cation binding site on actin: a structural relationship between antigenic epitopes and cation exchange. *Biochem. Biophys. Res. Commun.* 152:368–375.

Miki, M., C. G. dos Remedios, and J. A. Barden. 1987. Spatial relationship between the nucleotide-binding site, Lys-61 and Cys-374 in actin and a conformational change induced by myosin subfragment-1 binding. *Eur. J. Biochem.* 168:339–345.

Miller, L., M. Kalnoski, Z. Yunossi, J. C. Bulinski, and E. Reisler. 1987. Antibodies directed against N-terminal residues on actin do not block actomyosin binding. *Biochemistry.* 26:6064–6070.

Miller, L., M. Phillips, and E. Reisler. 1988. Polymerization of G-actin by myosin subfragment 1. *J. Biol. Chem.* 263:1996–2002.

Mornet, D. R., R. Bertrand, P. Pantel, E. Audemard, and R. Kassab. 1981. Structure of the actin-myosin interface. *Nature (Lond.).* 292:301–306.

Oosawa, F. 1983. Macromolecular assembly of actin. *Muscle Non-Muscle Motil.* 1:151–216.

Reisler, E., C. Smith, and G. Seegan. 1980. Myosin minifilaments. *J. Mol. Biol.* 143:129–145.

Schutt, C. E., U. Lindberg, J. Myslik, and N. Strauss. 1989. Molecular packing in profilin: actin crystals and its implications. *J. Mol. Biol.* 209:735–746.

Schwyter, D. H., M. Phillips, and E. Reisler. 1989. Subtilisin-cleaved actin: polymerization and interaction with myosin subfragment 1. *Biochemistry.* 28:5889–5895.

Sheetz, M. P., S. M. Block, and J. A. Spudich. 1986. Myosin movement in vitro: a quantitative assay using oriented actin cables from Nitella. *Methods Enzymol.* 134:531–544.

Spudich, J. A., and S. Watt. 1971. The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. *J. Biol. Chem.* 246:4866–4871.

Sutoh, K. 1982. Identification of myosin-binding sites on the actin sequence. *Biochemistry.* 21:3654–3661.

Sutoh, K., and H. L. Lin. 1989. End-label fingerprintings show that the N- and C-Termini of actin are in the contact-site with gelsolin. *Biochemistry.* 28:5269–5275.

Thomas, D. D., J. C. Seidel, and J. Gergely. 1979. Rotational dynamics of spin-labeled F-actin in the sub-millisecond time range. *J. Mol. Biol.* 132: 257–273.

Toyoshima, Y. Y., S. Kron, E. M. McNally, K. R. Niebling, C. Toyoshima,

and J. A. Spudich. 1987. Myosin subfragment-1 is sufficient to move actin filaments *in vitro*. *Nature (Lond.)*. 328:536–539.

Vandekerckhove, J. S., D. A. Kaiser, and T. D. Pollard. 1989. *Acanthamoeba* actin and profilin can be cross-linked between glutamic acid 365 of actin and lysine 115 of profilin. *J. Cell Biol.* 109:619–626.

Weeds, A. S., and B. Pope. 1977. Studies on the chymotryptic digestion of myosin. Effects of divalent cations on proteolytic susceptibility. *J. Mol. Biol.* 111:129–157.

Yanagida, T. 1984. Angles of fluorescently labeled myosin heads and actin monomers in contracting and rigor stained muscle fibers. *In* Contractile Mechanism in Muscle. G. H. Pollack and H. Sugi, editors. Plenum Publishing Corp., New York. 397–411.

Yanagida, T., and F. Oosawa. 1978. Polarized fluorescence from G-ADP incorporated into F-actin in a myosin free single fiber. Conformation of F-actin and changes induced by heavy meromyosin. *J. Mol. Biol.* 126:507–524.