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Dual role of tropomyosin on chemically modified actin filaments from skeletal muscle

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Abstract Actin filaments copolymerized with both intact and chemically modified actin monomers restored their sliding activity when they were supplemented with tropomyosin extracted from skeletal muscle. In contrast, the ATPase activation of the copolymers was decreased when supplemented with tropomyosin. The results indicate that tropomyosin along with actin monomers may facilitate sliding activity of the entire actin filament but suppress ATPase activation of intact actin monomers themselves. Accordingly, tropomyosin molecules could be viewed as playing a dual role of both mechanical and chemical regulation of actin monomers.

Key words: Tropomyosin; Actin; Motility; Chemical modification; ATPase; 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

1. Introduction

Tropomyosin is one of the component molecules assuming major roles in calcium signal transduction in vertebrate skeletal muscle [1–3]. Tropomyosin, along with troponin, forms a calcium-sensitive switch [4] which involves changes in position relative to the actin helix [5–11]. This positional change may lead to changes in the binding site of an actin relative to tropomyosin as accompanied by conformational changes of either one or both of those proteins. Noting that 98% of the tropomyosin content in weight participate in constituting a helical structure [12], it may be argued that the conformational change of troponin due to calcium binding could initiate some changes in actin molecules through tropomyosin, although the role of tropomyosin in mechano-chemical activity in actomyosin complex remains unidentified [13,14]. In this report, we examined the contribution of conformational changes in actin molecules by artificially controlling the extent of EDC modification applied to those molecules. We then observed that, as the ratio of EDC-modified actin monomers in the actin filament increased, both the ATPase activation of myosin and the sliding velocity of the filaments exhibited totally different behaviors, especially with regard to their cooperativities. Tropomyosin was found to enhance mechano-chemical transduction, while suppressing ATPase activation, with such modified actin filaments.

2. Materials and methods

2.1. Reagents and buffers

EDC and HEPES were purchased from Dojin Chemicals (Kumamoto), and Tris and DTT from Wako Pure Chemicals (Osaka). These and other reagents used were of special reagent grade.

HEPES buffer adjusted to the level of pH 7.5 at 25°C was used for preparation of microscopic specimens.

2.2. Proteins

Rabbit skeletal muscle proteins were used. Actins were purified according to Spudich and Watt [15], and myosins were prepared by the method of Perry [16]. Tropomyosins were obtained by the method of Bailey et al. [10] with slight modification.

Purified myosins were dissolved at a concentration of 20 mg/ml in 0.6 M KCl, 10 mM DTT, 10 mM potassium phosphate buffer (pH 7.0), and dropped into liquid nitrogen. The frozen droplet sample was stored in a deep-freeze at −80°C, and thawed before use.

Tropomyosins were added to actin filament at a molar ratio of 1:1 which ensures the binding of tropomyosins to actin filament [17].

2.3. EDC modification

1 mg/ml of G-actin was suspended with 5 mM HEPES (pH 7.0), 0.3 mM ATP and 0.1 mM $CaCl_2$. EDC was added to the specimen at a concentration of 15.0 mM. The solution was then incubated for about 30 min at 25°C. The reaction was terminated by the addition of 2-mercaptoethanol at a final concentration of about 1%. The cross-linked actins were then dialyzed against the following medium: 5 mM Tris, 0.1 mM ATP and 0.05 mM $CaCl_2$ for 24 h.

2.4. Microscopic observation

In order to make the glass slide hydrophobic, we introduced the siliconizer L-25 (Fuji System Co. Ltd.). Tropomyosin-complexed actin filaments were prepared by mixing G-actin solution and tropomyosin solution at a molar ratio of 1:1 in order to ensure the complete binding of tropomyosin to the actin filament. The in vitro motility assay we used was performed as described previously [18,19] under the conditions: 25 mM KCl, 20 mM HEPES (pH 7.8), 2 mM ATP, 2 mM $MgCl_2$ and 5 mM DTT.

2.5. ATPase assay

We assayed actin-activated myosin ATPase in the presence of 0.35 mg/ml tropomyosin under the following conditions: 0.1 mg/ml myosin, 0.2 mg/ml actin, 25 mM KCl, 20 mM HEPES, 2 mM $MgCl_2$, 2 mM ATP and 5 mM DTT. ATP hydrolysis was monitored by measuring the concentration of inorganic phosphate using the Malachite Green method [20] after terminating the reaction with perchloric acid at final concentration of 0.3 M.

2.6. Binding and polymerization assay

Both the binding ability to tropomyosin and polymerization of EDC-modified actin were assayed by ultracentrifugation at 60000 rpm for 60 min followed by densitometric analysis with an image scanner (GT-9000, Epson Co. Ltd.). The extent of polymerization was also monitored by viscometric measurement on an Ostwald viscometer.

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Abbreviations: ATP, adenosine 5′-triphosphate; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HEPES, *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid; DTT, dithiothreitol

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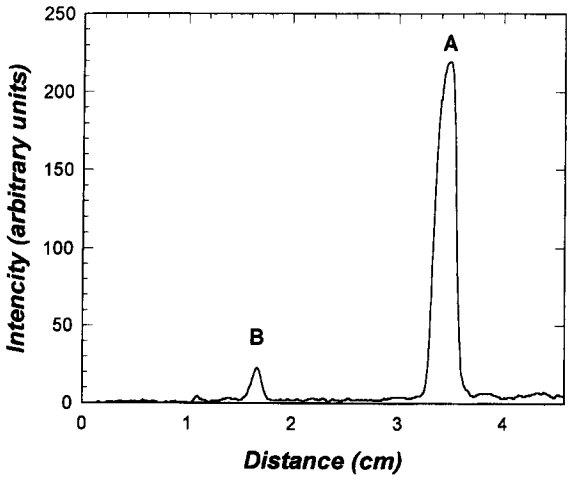

Fig. 1. SDS-polyacrylamide gel electrophoretic patterns of actin. Bands marked with A correspond to modified monomers with no apparent change of molecular weight, and B to dimers of EDC modified actins. See text for detailed experimental conditions.

3. Results

3.1. EDC modification of G-actin

In order to obtain partially modified actin monomers, we treated globular actin (G-actin) with EDC. The experimental conditions described in section 2 were determined so as to prevent the formation of actin trimers (Fig. 1). The prepared actins could polymerize into filamentous actins (F-actin) as measured by densitometry of the solution. They were able to bind to intact tropomyosin as shown in Table 1. More than 95% of actins were modified by EDC, as estimated by isoelectric focusing (data not shown). Although the explicit manner of modification with EDC is still unknown, those modified groups within actin molecules remained unaltered during the course of the experiments reported here. The ratio of dimeric actins to monomeric actins was fixed and reproducible over more than 20 independent experiments.

3.2. ATPase activity of copolymers

We have examined the ATPase activation of EDC-modified actins in the form of filamentous copolymers of both modified and intact actins. Fig. 2 demonstrates the ATPase activation of the copolymers both with and without tropomyosins. Although the copolymers without tropomyosins exhibited a gradual decrease in ATPase activation with increasing ratio of EDC-actin, the similar activation for the copolymers with tropomyosins abruptly vanished as the ratio of EDC-actin rose above 13% (Fig. 2). A sudden disappearance of ATPase activity was thus highly cooperative.

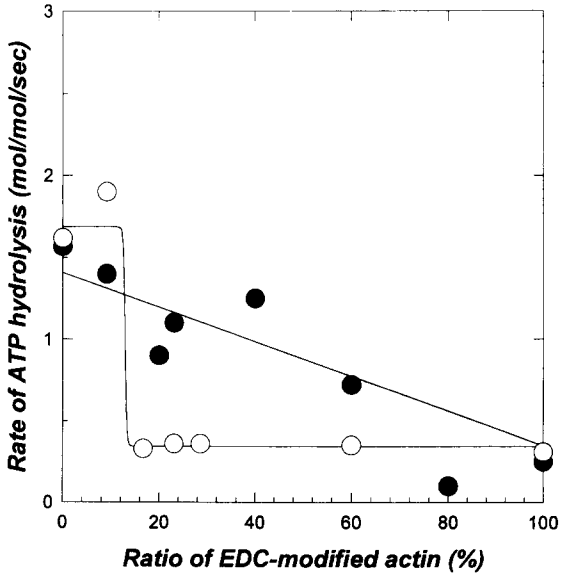

Fig. 2. The rate of ATP hydrolysis with respect to the ratio of EDC-modified actins within the actin filaments. Filled symbols represent the rate without tropomyosins, and the line was drawn by a linear-least-mean-square-fit. Open symbols correspond to the rate in the presence of tropomyosins. The curve was fitted with a Hill plot.

3.3. Increase of the sliding velocity of actin filaments complexed with tropomyosin

To examine the effect of tropomyosin on the cooperativity as observed in ATPase activation, we measured the sliding velocity of intact actin filaments both with and without tropomyosins as a function of the Mg-ATP concentration (Fig. 3). The results indicate two aspects: (1) the sliding velocity increased about 2-fold on addition of tropomyosin; (2) the Mg-ATP concentrations reaching half-maximal velocity were about 0.1 mM. This value was independent of the concentration of tropomyosins.

3.4. Sliding movement of copolymers

We have also investigated the sliding velocity of the copolymerized filaments as a function of the molar ratio of EDC-modified actins. As the ratio reached about 70%, the sliding velocity decreased abruptly to zero in the absence of tropomyosin (Fig. 4, −TM). In contrast, the sliding velocity of the copolymerized filaments in the presence of tropomyosin remained rather constant even with the increase of the ratio of EDC-modified actins (Fig. 4, +TM).

4. Discussion

We have examined the role of tropomyosin in the ATPase

Table 1
Physiological capacities of EDC-modified actin

Capacities	Binding to tropomyosin[a]	Specific viscosity[b]	Sedimentation (%)[c]
EDC modified actin	1.5×10^5	2.12	98
Intact actin	2.2×10^5	2.11	100

[a]Binding of each actin to tropomyosin was performed as follows: a solution of 1 ml of 0.5 mg/ml actin, 0.03–0.21 mg/ml tropomyosin, 25 mM KCl, 20 mM HEPES (pH 7.8), 2 mM ATP, 2 mM $MgCl_2$ and 5 mM DTT was incubated for 8 h at 4°C, and then centrifuged at 40000 rpm for 40 min. The relative amounts of proteins within supernatants and pellets were determined by SDS-PAGE and densitometry.
[b]Specific viscosity was measured by using an Ostwald viscometer under the following conditions: 1 mg/ml actin, 0.2 mM ATP, 2 mM Tris-HCl (pH 7.8), 0.2 mM $CaCl_2$, 0.5 mM DTT and 0.15 M KCl.
[c]Sedimentation analysis was performed under the similar conditions with those for the measurement of viscosity, then centrifuged at 60000 rpm for 40 min.

activation of actin filaments copolymerized with EDC-modified monomers (EDC-AF). As shown in Fig. 2, ATPase activation gradually decreased with increase in the ratio of EDC-actin. In contrast, in the absence of tropomyosin, the activation suddenly vanished as the ratio of EDC-actin reached and surpassed 13%. This value of 13% admittedly corresponds to the case of one tropomyosin molecule allotted to 7 actin monomers, suggesting that ATPase activation of the entire actin monomers in a filament may decrease in a cooperative manner [21–23] to a minimum level while contacting with EDC-actins intermediated by tropomyosins. The extent of cooperativity reported here is consistent with that expected from myosin subfragment-1 binding onto tropomyosin-actin complexes [24].

The sliding velocity of EDC-AF without tropomyosin was also examined. In the absence of tropomyosin, adding EDC-modified G-actin to the intact actin filament caused a further decrease in activation of myosin ATPase (Fig. 2, −TM). However, the sliding velocity of the filaments did not decrease proportionally (Fig. 4, −TM). When the ratio of modified monomers reached the level of 65–75%, the sliding velocity abruptly decreased. This abruptness was consistent with that observed by Prochiniewicz and Yanagida [25] and should be an indication of the cooperativity reported by Butters et al. [26]. The ratio of such modified monomers at the onset of exhibiting an abrupt decrease in sliding velocity was 65–75% in our experiments, while that reported previously was about 50% [25]. The difference may be due to EDC modification of actin monomers. How actin monomers could be modified especially with regard to those modified residues remains to be seen.

The sliding velocity of intact actin filaments in the presence of tropomyosin was compared with that in its absence. Fig. 3 shows that the association of tropomyosins onto actin filaments increased the sliding velocity of the filaments. This demonstrates a cooperativity of sliding motion with respect to Mg-ATP concentration. That Mg-ATP concentrations resulting in half-maximum velocity did not change upon addition of tropomyosins indicates that the enhancement of cooperativity due to the addition of tropomyosins did not affect the ATPase activation but definitely influenced the sliding motion itself. The fact that tropomyosin-actin filaments can slide roughly twice as fast as F-actin without tropomyosin on the myosin-coated glass slide similarly prepared indicates that tropomyosins without troponin complexes may possess a unique capacity for influencing the sliding movement that could be plastic enough [27] on their own.

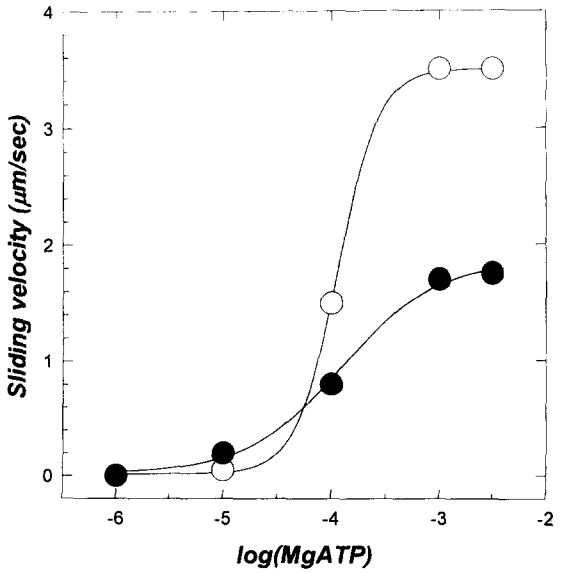

Fig. 3. ATP concentration-dependent velocity of the actin filament with and without tropomyosin. All the actin molecules used in this experiment were intact, i.e. 0% modification of EDC. Filled symbols represent the sliding velocity without tropomyosin and open symbols with tropomyosin. Curves were drawn by following a Hill plot.

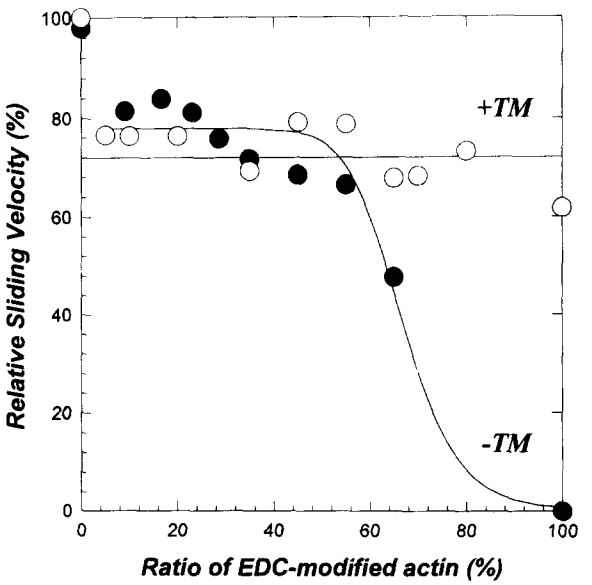

Fig. 4. Sliding velocities of copolymerized filaments with and without tropomyosin. Sliding velocities are shown as relative values normalized to the averaged sliding velocity of intact actin filament with or without tropomyosins. Filled symbols correspond to the sliding velocity without tropomyosin noted as '−TM'. Open symbols correspond to that with tropomyosin (+TM) and the straight line denotes the value of 72%.

An increase in the ratio of EDC-modified actins within EDC-AF in the presence of tropomyosin was associated with a decrease in ATPase activation in a cooperative manner (Fig. 2, +TM). On the other hand, tropomyosin may seem to compensate those defects within single filaments caused by EDC-modified molecules (Fig. 4, +TM) and may not influence the sliding velocity even for the case of 100% modified actins. Accordingly, the association of tropomyosins along actin filaments seems to enhance the cooperativity of the filaments in a dual manner, suppressing ATPase activation while enhancing sliding motility. Put differently, tropomyosin molecules may have a capacity for influencing the entire actin filaments incorporating EDC-modified actins positively for sliding activity, but negatively for ATPase activation.

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