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Caldesmon restricts the movement of both C- and N-termini of tropomyosin on F-actin in ghost fibers during the actomyosin ATPase cycle ☆

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Abstract

New data on the movements of tropomyosin singly labeled at α- or β-chain during the ATP hydrolysis cycle in reconstituted ghost fibers have been obtained by using the polarized fluorescence technique which allowed us following the azimuthal movements of tropomyosin on actin filaments. Pronounced structural changes in tropomyosin evoked by myosin heads suggested the ''rolling'' of the tropomyosin molecule on F-actin surface during the ATP hydrolysis cycle. The movements of actin-bound tropomyosin correlated to the strength of S1 to actin binding. Weak binding of myosin to actin led to an increase in the affinity of the tropomyosin N-terminus to actin with simultaneous decrease in the affinity of the C-terminus. On the contrary, strong binding of myosin to actin resulted in the opposite changes of the affinity to actin of both ends of the tropomyosin molecule. Caldesmon inhibited the ''rolling'' of tropomyosin on the surface of the thin filament during the ATP hydrolysis cycle, drastically decreased the affinity of the whole tropomyosin molecule to actin, and ''freezed'' tropomyosin in the position characteristic of the weak binding of myosin to actin.

Keywords: Tropomyosin; Caldesmon; F-actin; Myosin subfragment S1; Ghost muscle fibers; Fluorescence polarization

Tropomyosin is a constituent of thin filaments in all muscle types. Its polymers, formed by coiled-coil dimers joined end-to-end, are located in the grooves of the F-actin double helix. It is generally accepted that the movement of tropomyosin polymers on actin filaments plays an important role in the thin filament-based regulatory process of muscle contraction (for review, see [1]). The first structural support for tropomyosin movement came from the studies of low angle X-ray diffraction of vertebrate skeletal muscle fibers [2,3] and was later confirmed by the 3D helical electron microscopic image reconstruction of negatively stained individual thin filaments [4,5]. Another line of evidence for the description of tropomyosin position on regulated skeletal muscle actin filaments was supplied by biochemical and kinetic analysis [6,7] by using the atomic models of actin, tropomyosin, myosin subfragment 1 complex [8,9], and cryoelectron microscopy [10]. It was demonstrated that the position of tropomyosin on regulated skeletal muscle thin filaments controls the interaction of actin with myosin heads which depicts the three functional states of the thin filament in terms of the tropomyosin position described by the so-called ''steric blocking model'': (a) blocked position, at low Ca^{2+} concentration, when tropomyosin covers the strong- and weak-binding sites of the myosin heads on actin, (b) closed position, at low Ca^{2+} concentration, when only the strong-binding sites for myosin heads are covered by tropomyosin, and (c) open, at high Ca^{2+} concentration, when all binding sites on actin are available for myosin

☆ *Abbreviations:* S1, myosin subfragment 1; IAEDANS, *N*-iodoacetyl-*N*′-(5-sulfo-1-naphthylo)ethylenediamine; 5-IAF, 5-iodoacetamide fluorescein.

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heads. As shown by Pirani et al. [11], the position of tropomyosin on actin is not fixed in either state but remains in dynamic equilibrium. The inhibitory states (blocked and closed, termed also the OFF states) of skeletal muscle thin filament are evoked by another component of thin filament—troponin, the molecules of which are distributed periodically along the F-actin-tropomyosin threads; this protein, together with tropomyosin, inhibits the actomyosin ATPase activity in vitro.

Much less information is available on the mechanism of thin filament-based regulatory system in smooth muscle. Smooth muscle thin filaments do not contain troponin but their major component besides F-actin and tropomyosin is caldesmon. Caldesmon molecules are located along the thin filament and inhibit in vitro the actomyosin ATPase activity in a manner similar to troponin; this inhibition is enhanced in cooperative way by tropomyosin (for reviews, see [12,13]).

Although the early studies of X-ray diffraction on "living" smooth muscle indicated that activation of contraction results in movement of tropomyosin [14], interpretation of the results of the 3D helical image reconstruction studies was more complicated. For example, in thin filaments, both native and devoid of caldesmon, in the OFF state (in terms of the actomyosin ATPase) tropomyosin covers only part of potential strong binding sites of actin for myosin heads whereas in the ON state (in the absence of caldesmon) tropomyosin is positioned over all strong binding sites on actin [15,16]. These results suggested that the structural basis of caldesmon inhibition is different from that of troponin.

Recent evidence suggests that the myosin-induced movement of tropomyosin plays a key role in regulation. Caldesmon interacts with and alters the position of tropomyosin in a reconstituted actin thin filament and thereby limits the ability of myosin heads to move tropomyosin. Caldesmon interacts with the Cys190 region in the C-terminus of tropomyosin, resulting in the movement of this part of tropomyosin to a new position on actin. Additionally, this constrains the myosin-induced movement of this region of tropomyosin. On the other hand, caldesmon does not appear to interact with the Cys36 region in the N-terminus of tropomyosin and neither alters the position of nor significantly constrains the myosin-induced movement of this part of tropomyosin. The ability of caldesmon to limit the myosin-induced movement of tropomyosin provides a possible molecular basis for the inhibitory function of caldesmon. The different movements of the two halves of tropomyosin indicate that actin-bound tropomyosin moves as a flexible molecule and not as a rigid rod [17].

Recently with the help of FRET technique [18,19] two opposite positions of tropomyosin on F-actin, induced by binding of myosin heads and caldesmon, were revealed in solution. These positions are correlated, respectively, with activation and inhibition of actomyosin ATPase.

The aim of the present work was the investigation by the polarized fluorescence method of the mutual effect of myosin heads and caldesmon on the movement of the C- and N-terminal parts of tropomyosin molecules during the sequential steps of the actomyosin ATPase cycle in model system of reconstituted ghost fibers. The actin to myosin binding strength during each step was determined by addition of respective nucleotide.

Materials and methods

Preparation of proteins. Duck gizzard caldesmon was prepared according to the procedure of Bretscher [20]. Chicken gizzard tropomyosin was purified as described in [21,30]. Rabbit skeletal muscle myosin was prepared according to the method of Kielley and Bradley [22]. Myosin subfragment 1 was obtained by digestion of myosin with α-chymotrypsin [23]. In some experiments S1 was chemically modified with *N*-ethylmaleimide (NEM-S1) or with *N*,*N*′-*p*-phenylenedimaleimide (pPDM-S1) to produce the analogs of the "strong" or the "weak" binding state of actomyosin complex. In agreement with the data published earlier, NEM and pPDM modified S1 lost its ATPase activity but retained its ability to bind to actin [24,25]. Purity of protein preparations, as well as the composition of the fibers after washing out of the unbound proteins, was monitored by SDS–PAGE in 7–20% gradient slab gels [26]. Protein concentrations were determined by measuring UV absorbance using the following absorption coefficients and molecular mass values: caldesmon, $A^{1\%}_{278} = 4.0$ [27], 87 kDa; tropomyosin, $A^{1\%}_{280} = 1.9$ [28], 68 kDa; G-actin, $A^{1\%}_{290} = 6.3$, 42 kDa [29], and rabbit skeletal myosin S1, $A^{1\%}_{280} = 7.5$, 115,000 [30].

Preparation and labeling of ghost fibers. Ghost fibers were prepared from single glycerinated fibers of rabbit *psoas* muscle by extraction of myosin and regulatory proteins with a solution containing 800 mM KCl, 1 mM $MgCl_2$, 10 mM ATP, and 67 mM phosphate buffer (pH 7.0) as described previously by Borovikov and Gusev [31]. Thin filaments were reconstituted in ghost fibers by supplementing them with chicken gizzard tropomyosin and duck gizzard caldesmon.

Labeling of tropomyosin with 1,5-IAEDANS at Cys36 and 5-IAF at Cys190 was performed without dissociation to α- and β-chains essentially as described [32] at probe to protein ratio 0.8:1. The specificity of labeling by AEDANS at β-chain and by IAF at α-chain was checked by visualization of tropomyosin bands at polyacrylamide gels (Fig. 1, inset). The sequence of the incorporation of proteins into the ghost fibers was as follows: tropomyosin, S1, caldesmon.

Fluorescence polarization measurement. The polarized fluorescence from 5-IAF-labeled and 1,5-IAEDANS-labeled tropomyosin was excited at 479 ± 5 and 365 ± 5 nm, respectively, and recorded at 550–650 nm. The intensities of the four components of polarized fluorescence were measured in parallel ($_{\parallel}I_{\parallel}$, $_{\parallel}I_{\perp}$) and in perpendicular ($_{\perp}I_{\perp}$, $_{\perp}I_{\parallel}$) orientation of the fiber axis to the polarization plane of the exciting light. From these four components, the degrees of fluorescence polarization, $P_{\parallel}$ and $P_{\perp}$ were calculated. The ratios of fluorescence intensities were considered as functions of angles Φ_A, Φ_E, and N, where Φ_A, Φ_E are the angles between the fiber axis and the absorption and emission dipoles of fluorophores, respectively; N is the relative number of randomly oriented fluorophores. Changes in the parameters of fluorescence (Φ_A, Φ_E, and N) were interpreted in terms of structural alterations of tropomyosin. The data were further analyzed assuming that a fraction of probe molecules describe a perfect helical array, while the remaining fraction represents probes randomly oriented (the helix plus isotropic model) [33–35].

The significance of the data differences observed was determined by Student's *t*-test.

Results and discussion

To access the myosin-induced movement of tropomyosin on thin filaments during the sequential steps of the actomyosin ATPase cycle, we used a well-organized model