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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

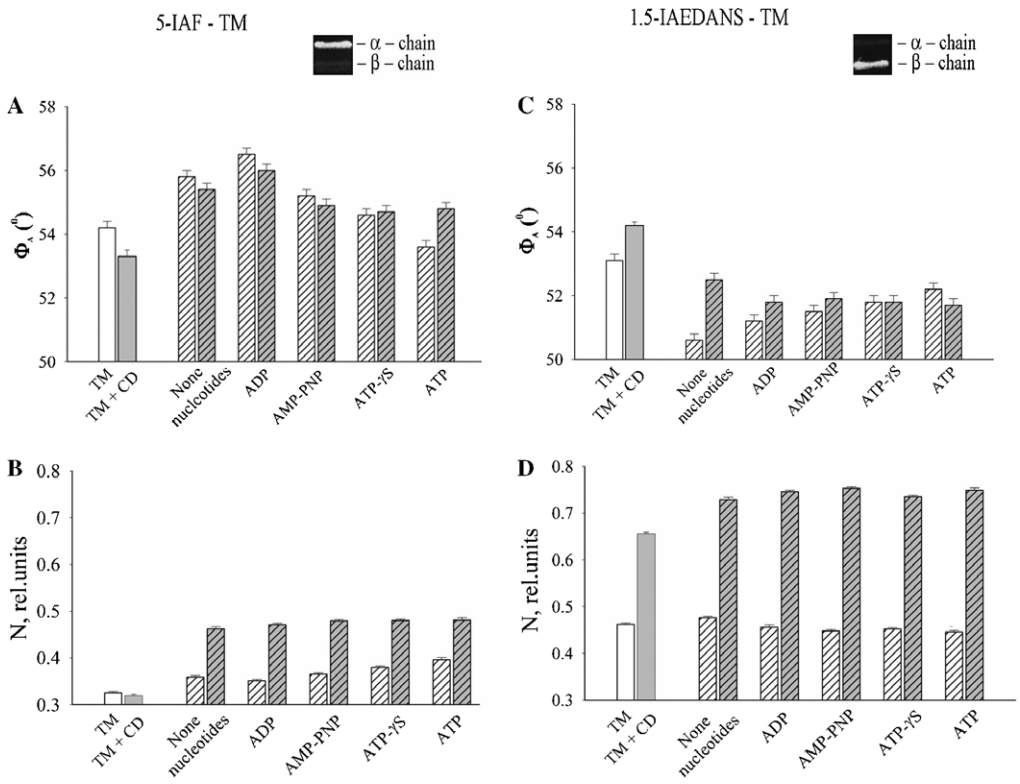

Fig. 1. Orientational distribution of the angles of probe absorption dipoles Φ_A (A,C) and the fraction, N (B,D), of unordered probes of the fluorescence polarization of 5-IAF bound to Cys190 (A,B) or 1,5-IAEDANS bound to Cys36 (C,D) of smooth TM in ghost fibers during the sequential steps of the acto-S1 ATPase cycle in the absence or presence of caldesmon (CD) by the helix plus isotropic model fitting. Empty, gray, hatched, and hatched gray bars denote the thin filaments in the presence of tropomyosin; tropomyosin and caldesmon; tropomyosin and subfragment-1, and tropomyosin, subfragment-1, and caldesmon, respectively. Calculations of the angles of excitation Φ_A dipoles of the dye relative to the muscle fiber axis and the fraction, N, of disordered probes, as well as the preparation of the fibers, their composition, and the conditions of the experiments are described in Materials and methods. The data represent means of the results obtained for 15–80 ghost fibers (see Table). The Φ_A and N values in the absence and in the presence of nucleotides are significantly altered by caldesmon ($P < 0.05$). Error bars indicate ±SEM. TM, tropomyosin; CD, caldesmon. Insets: specificity of tropomyosin α- and β-chain labeling as checked by visualization of tropomyosin bands at polyacrylamide gels.

system of thin filaments reconstituted in skeletal muscle ghost fibers from endogenous F-actin and fluorescently labeled smooth muscle tropomyosin in the absence or presence of caldesmon (see Fig.1 in [36]). To follow all steps of ATP hydrolysis with mimicking the intermediate states, we have used ADP, ATP, the non-hydrolyzable ATP derivative ATPγS, and ATP analog, AMP-PNP.

In our work, we have used tropomyosin specifically labeled at Cys36 or Cys190 by a fluorescent dye, 1,5-AEDANS or 5-IAF (Fig. 1). In accordance with the previously published data [32] the extent of the effect of AEDANS-TM or IAF-TM on the acto-S1 ATPase activity was similar to that of the unlabeled TM, indicating that labeling does not greatly affect the structure and function of TM.

Obtaining of tropomyosin heterodimers singly labeled at β-chain Cys36 or α-chain Cys190 allowed us to perform the studies of the orientation and mobility of the probe attached to tropomyosin bound to actin by measurements of polarized fluorescence at addition of myosin S1 and caldesmon. The Table summarizes the effect of caldesmon on S1-induced changes in the parameters of polarized fluorescence $P_{\|}$ and $P_{\perp}$ of tropomyosin labeled with 5-IAF on Cys190 and with 1,5-AEDANS on Cys36, respectively, in the presence of various nucleotides.

In the experiments with tropomyosin labeled with 5-IAF on Cys190 or with 1,5-AEDANS on Cys36, the degree of fluorescence polarization was higher when the fiber was oriented perpendicular to the polarization plane of the exiting light ($P_{\perp}$). These indicated that the 5-IAF and 1,5-AEDANS dipoles were oriented predominantly perpendicular to the fiber axis. When the helix plus isotropic model (see Materials and methods) was fitted to the fluorescence polarization of 5-IAF and 1,5-AEDANS in the absence of caldesmon, S1, and nucleotides, the angles of absorption and emission dipoles of probe Φ_A and Φ_E were close to 54°, and the relative quantity of unordered fluorophores did not exceed 50% (Fig. 1). Taking into account the rigid binding of the dye this indicated a well-ordered orientational distribution of tropomyosin in thin filaments. Myosin subfragment 1 in the absence or presence of nucleotides as well as caldesmon has a prominent effect on the parameters of the polarized fluorescence ($P_{\|}$, $P_{\perp}$ Φ_A, Φ_E, and N) (Table 1 and Fig. 1).

Table 1
The effect of nucleotides on polarization ratios of 5-IAF bound to Cys190 or 1,5-IAEDANS bound to Cys36 of smooth muscle tropomyosin incorporated in ghost fibers in the absence or presence of caldesmon (CD)

Nucleotide	CD	S1	Labeled Cys190			Labeled Cys36		
			n	$P_{\parallel} \pm$ SEM	$P_{\perp} \pm$ SEM	n	$P_{\parallel} \pm$ SEM	$P_{\perp} \pm$ SEM
None	–	+	80	0.147 ± 0.001	0.274 ± 0.001	18	0.239 ± 0.001	0.245 ± 0.001
	+	+	30	0.200 ± 0.002	0.295 ± 0.001	15	0.347 ± 0.002	0.323 ± 0.001
ADP	–	+	62	0.143 ± 0.002	0.283 ± 0.001	20	0.233 ± 0.002	0.237 ± 0.001
	+	+	30	0.207 ± 0.002	0.302 ± 0.001	15	0.348 ± 0.002	0.333 ± 0.001
AMP-PNP	–	+	58	0.163 ± 0.002	0.255 ± 0.001	22	0.233 ± 0.002	0.227 ± 0.001
	+	+	31	0.218 ± 0.002	0.279 ± 0.001	15	0.339 ± 0.002	0.333 ± 0.001
ATPγS	–	+	71	0.171 ± 0.002	0.259 ± 0.001	18	0.230 ± 0.002	0.237 ± 0.001
	+	+	30	0.219 ± 0.002	0.274 ± 0.001	15	0.332 ± 0.002	0.338 ± 0.001
ATP	–	+	38	0.181 ± 0.002	0.257 ± 0.001	19	0.234 ± 0.002	0.243 ± 0.001
	+	+	28	0.212 ± 0.002	0.288 ± 0.001	15	0.347 ± 0.002	0.337 ± 0.001

$P_{\parallel}$ and $P_{\perp}$ measurements were performed as described in Materials and methods; n denotes the number of fibers used in experiments. Error indicates ±SEM. The difference between actin-TM-S1, actin-TM-S1-ADP, actin-TM-S1-AMP-PNP, actin-TM-S1-ATPγS, and actin-TM-S1-ATP complexes in the values of $P_{\parallel}$ and $P_{\perp}$ is statistically significant, and these values are altered significantly by caldesmon ($P < 0.05$).

The changes of the polarization parameters were reversible in all experiments. Exchange of MgATP-containing buffer for a solution devoid of nucleotide resulted in nearly full reversion of polarization parameters to the initial level. On the contrary, exchange of S1 preparations capable of conformational changes under the influence of nucleotides for S1 preparations uncapable of conformational changes (NEM-S1 and pPDM-S1, see Materials and methods) led to full loss of the dependence of polarized fluorescence parameters on the nucleotides. Thus, the changes on the polarized fluorescence of the probe were conditioned presumably by conformational changes in tropomyosin in the F-actin-S1-tropomyosin complex, the conformational changes of tropomyosin being possible only under the condition that the myosin head is capable of conformational changes under the influence of nucleotides.

Since in all experiments the pattern of the changes in Φ_A was similar to those in Φ_E, only Φ_A changes are presented in Fig. 1.

Binding of S1 to IAF-tropomyosin-actin complex in the absence of caldesmon and nucleotides as well as in the presence of MgADP, MgAMP-PNP, and Mg-ATPγS evoked a statistically significant ($P < 0.05$) increase in Φ_A, which indicated the tilting of the 5-IAF dipoles away from the fiber axis and increased the probe mobility as judged by the increase of N values (Fig. 1A and B). The changes in the orientation of the 5-IAF dipoles in the C-terminus of tropomyosin relative to the fiber axis were maximal at strong binding of S1 to actin (in the presence of MgADP) and sequentially decreased (by approximately 5%) during the subsequent steps of the ATPase cycle (caused by transition from S1-ADP to S1-ATP) (Fig. 1A).

Caldesmon significantly inhibited the dependence of the parameters of polarized fluorescence of the probe on the presence of nucleotides. At transition from S1-ADP to S1-ATP, Φ_A decreased by only 1%, being close to the corresponding values of this parameter characteristic of S1-AMP-PNP complex in the absence of caldesmon (Fig. 1A), i.e., to the values observed in the absence of caldesmon at weak binding of myosin to actin.

Fig. 1C and D represents the changes in the fluorescence parameters for tropomyosin labeled in the N-terminus (Cys36) with 1,5-IAEDANS. The changes presented of Φ_A at this figure look like a mirror-reflection of the graphs for tropomyosin labeled at C-terminal cysteine (compare Fig. 1A and C) which indicates that the changes were directed oppositely. The changes in Φ_A for the N-terminus of tropomyosin were smaller ($P < 0.05$) and sequentially increased (by approximately 3%) during the following steps of the ATPase cycle (caused by transition from S1 to S1-ATP). Caldesmon exerts practically full inhibition of any changes of Φ_A in the N-terminus of tropomyosin at transition from S1-ADP to S1-ATP. The Φ_A values approach the corresponding values observed in the absence of caldesmon in the presence of ATPγS (Fig. 1C), i.e., the values typical to the weak binding of myosin to actin.

While the probes are rigidly bound to C- and N-termini of tropomyosin, respectively, and at transition from S1 to S1-ATP the changes in the spatial orientation of the dye dipoles located at different ends of the tropomyosin molecule essentially differ (the dipoles located at the C-terminus rotate away from the fiber axis while at the N-terminus, on the contrary, they rotate towards the fiber axis), it may be suggested that the C- and N-termini of tropomyosin behave differently during the ATP hydrolysis cycle: the C-terminus of tropomyosin moves towards the periphery of the fiber while the N-terminus moves towards the long fiber axis.

It is not the single possible interpretation of the results. The differences in the dipole movement may be explained also by suggesting that during the ATP hydrolysis cycle tropomyosin rolls on the surface of thin filament. Similar interpretation of experimental results has been suggested by Bacchiocchi et al. [37] who used multisite FRET

technique to observe the myosin-induced movement of smooth muscle tropomyosin on actin in solution.

In accordance with such interpretation the results presented in Fig. 1 may be explained by a suggestion that during the ATP hydrolysis cycle the changes in myosin conformation induce the rolling of the tropomyosin molecule on the surface of actin filament. During such changes a certain structural state and spatial orientation of tropomyosin corresponds to each intermediate state of the actomyosin. Caldesmon inhibits the rolling of tropomyosin during the ATP hydrolysis cycle by ''freezing'' tropomyosin in a position characteristic of the weak binding of myosin to actin.

S1, caldesmon, and nucleotides have a prominent effect on the mobility of the C- and N-termini of tropomyosin in the actin-S1-tropomyosin complex (Fig. 1B and D). Decoration of the thin filaments with S1 in the absence of caldesmon and nucleotides visibly increased the relative amount of the unoriented dye dipoles located at the C-terminus of tropomyosin molecules (the *N* value increased by 11%), which is the evidence of the increased mobility of this part of tropomyosin on the surface of actin. The nucleotides also significantly increase the fraction of the unoriented dipoles in the C-terminus of tropomyosin. According to the increase of this fraction, the nucleotides may be arranged as follows: MgAMP-PNP, MgATPγS, and MgATP. The smallest fraction of the unoriented dipoles has been observed in the presence of MgADP.

The increase in parameter *N* values by S1, caldesmon, and nucleotides reflects, in our opinion, the decrease in the affinity of tropomyosin to actin. Indeed, if the tropomyosin at binding to actin involves ('fix') some superficial areas of the tropomyosin ([1] and references therein), then the decrease in the *N* value would reflect the decrease in the character or time of the contact of tropomyosin with actin induced by the decrease in affinity of these proteins. Thus, the *N* parameter may be used as an indicator of the character of actin-tropomyosin interaction [38].

It means that the weak binding of myosin to actin is accompanied by the decrease of the affinity of the C-terminus of tropomyosin to actin in the actin-S1-tropomyosin complex. On the contrary, the smallest fraction of the unoriented dipoles in the N-terminus of tropomyosin has been observed in the presence of MgATP. This indicates that the weak binding of myosin to actin is accompanied by the increase of the affinity of the N-terminus of tropomyosin to actin in the actin-S1-tropomyosin complex.

Caldesmon, in both absence and presence of the nucleotides, drastically increased the fraction of unoriented dipoles of the fluorescent dyes (the values of *N* increased by 30–50%, see Fig. 1B and D) located in both N- and C-termini of the tropomyosin molecule implicating a sharp decrease in the affinity of the whole tropomyosin molecule to actin in the presence of caldesmon.

Thus, the rolling of tropomyosin on actin surface during the ATP hydrolysis cycle may be accompanied by different pattern of changes in the affinity of the N- and C-terminal parts of the tropomyosin molecule to actin. Weak binding of myosin to actin leads to an increase in the affinity of the N-terminus of tropomyosin to actin with simultaneous decrease in the affinity of the C-terminus. On the contrary, strong binding of myosin to actin results in the opposite changes of the affinity to actin of both ends of the tropomyosin molecule. Caldesmon drastically decreases the affinity of the whole tropomyosin molecule to actin.

Our data concerning the movement of the C-terminus of tropomyosin are consistent with the data by Graceffa [19] who demonstrated that the changes in distance between tropomyosin Cys190 and actin measured by FRET technique upon addition of S1 in solution were at least 0.2–0.6 nm. In our experiments carried out in muscle fibers, we also observed the rotation of tropomyosin by changes in the angles of absorption dipoles of the fluorescent probes induced by S1 during sequential steps of the ATPase cycle. Thus, our results additionally demonstrate that the movement of actin-bound tropomyosin correlates to the strength of S1 to actin binding.

Recently Graceffa and Mazurkie [18] reported that caldesmon interacts with the Cys190 region of tropomyosin, thereby moving this part of tropomyosin to a new position on actin and inhibiting myosin heads ability to move this region. For Cys190 the maximum S1-induced change in the donor to acceptor distance in the absence of caldesmon was 0.5 nm. In the presence of caldesmon this distance decreased by 0.33 nm. In our experiments, caldesmon induced the diminishing of the angles of absorption dipoles of the fluorescent probes which means the restriction of the S1-induced movement of the C-terminus of tropomyosin by caldesmon. Tropomyosin occupied the position characteristic of weak binding of myosin to actin.

On the other hand, Graceffa and Mazurkie [18] showed that caldesmon does not bind, or weakly binds, to the Cys36 region of tropomyosin and thus does not move this part of tropomyosin nor inhibit the movement of this region of the molecule by myosin heads [18]. Conversely, in our work we demonstrate that the myosin heads move the N-terminus of tropomyosin while caldesmon fully inhibits this movement. It is probable that we were able to observe these changes since our technique of measurements allows us to follow the azimuthal movements of tropomyosin on actin filament whereas the FRET technique permits to measure the distance between the donor and acceptor along F-actin axis.

Taken together, the data presented above suggest that in smooth muscle caldesmon inhibits the ATPase activity by both reversing the S1-evoked tropomyosin potentiation by some allosteric mechanism inhibiting a step in the actomyosin ATPase cycle (see Fig. 1) and by blocking the binding of myosin to actin [36,39].

Moreover, the changes in the spatial orientation of the fluorescent probes attached to the N- or C-terminal cysteines in tropomyosin are directed oppositely, thus indicating the flexibility of the tropomyosin molecule. During the whole ATPase cycle the myosin heads move tropomyosin

in one direction, while caldesmon moves it in the opposite direction, which is also consistent with the data by Graceffa and Mazurkie [18] who observed this effect in the presence and absence of ATP. As distinct from the data by these authors, we demonstrate that this concerns not only the C-terminal half of tropomyosin, but also the N-terminal part.

Our present results, together with the previously obtained data [35,36,38], allowed us to follow the rearrangement of the proteins in the thin filament (F-actin, tropomyosin, and caldesmon) under the influence of the myosin heads during the ATPase cycle. In this work, the polarized fluorescence studies provide evidence that caldesmon alters the position of tropomyosin in thin filaments reconstituted in ghost fibers and thereby affects the ability of myosin heads to move tropomyosin. Recently, the first direct experimental evidence for Ca^{2+}-induced tropomyosin rolling in skeletal muscle thin filament has been presented [40]; these data favor an active role for troponin in positioning the tropomyosin molecule. It is quite possible that in smooth muscle the similar role in positioning tropomyosin is played by caldesmon, and our data favor its role in this process.

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