Acta Biochimica Polonica

Vol. 47 No. 4/2000

1007–1017

QUARTERLY

Order-disorder structural transitions in synthetic filaments of fast and slow skeletal muscle myosins under relaxing and activating conditions✪

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Received: 26 September, 2000; revised: 7 November, 2000; accepted: 9 November, 2000

Key words: fast and slow skeletal muscle myosin, myosin filaments, Ca^{2+}-induced structural transitions, method of slow skeletal muscle myosin preparation.

In the previous study (Podlubnaya *et al.*, 1999, *J. Struc. Biol.* 127, 1–15) Ca^{2+}-induced reversible structural transitions in synthetic filaments of pure fast skeletal and cardiac muscle myosins were observed under rigor conditions (–Ca^{2+}/+ Ca^{2+}). In the present work these studies have been extended to new more order-producing conditions (presence of ATP in the absence of Ca^{2+}) aimed at arresting the relaxed structure in synthetic filaments of both fast and slow skeletal muscle myosin. Filaments were formed from column-purified myosins (rabbit fast skeletal muscle and rabbit slow skeletal *semimebranosus proprius* muscle). In the presence of 0.1 mM free Ca^{2+}, 3 mM Mg^{2+} and 2 mM ATP (activating conditions) these filaments had a spread structure with a random arrangement of myosin heads and subfragments 2 protruding from the filament backbone. Such a structure is indistinguishable from the filament structures observed previously for fast skeletal, cardiac (see reference cited above) and smooth (Podlubnaya *et al.*, 1999, *J. Muscle Res. Cell Motil.* 20, 547–554) muscle myosins in the presence of 0.1 mM free Ca^{2+}. In the absence of Ca^{2+} and in the presence of ATP (relaxing conditions) the filaments of both studied myosins revealed a compact ordered structure. The fast skeletal muscle myosin filaments exhibited an ax-

✪This work was partially supported by a grant to the Nencki Institute from the State Committee for Scientific Research (KBN, Poland) and grant RFBR 00-04-48200.

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Abbreviations: DTT, dithiothreitol; EM, electron microscopy; PMSF, phenylmethanesulphonyl fluoride; RLC, myosin regulatory light chain; TNC, troponin C.

ial periodicity of about 14.5 nm and which was much more pronounced than under rigor conditions in the absence of Ca^{2+} (see the first reference cited). The slow skeletal muscle myosin filaments differ slightly in their appearance from those of fast muscle as they exhibit mainly an axial repeat of about 43 nm while the 14.5 nm repeat is visible only in some regions. This may be a result of a slightly different structural properties of slow skeletal muscle myosin. We conclude that, like other filaments of vertebrate myosins, slow skeletal muscle myosin filaments also undergo the Ca^{2+}-induced structural order-disorder transitions. It is very likely that all vertebrate muscle myosins possess such a property.

The role of Ca^{2+} ions in activation of all types of muscles is well known. In the vertebrate striated muscles the binding of Ca^{2+} to the thin filament component – troponin C (TNC), triggers the contraction. The role of another divalent cation, Mg^{2+}, present in the sarcoplasm at a concentration much higher than that of Ca^{2+}, is less understood (Kerrick & Donaldson, 1975; Donaldson *et al.*, 1978; Kurihara, 1994). In skeletal muscle the concentration of Mg^{2+} ions is almost constant and is considered to be about 2.5 mM (Dawson *et al.*, 1978). Therefore, unlike Ca^{2+}, Mg^{2+} is not recognized as a regulatory cation. In vertebrate striated muscle there are several proteins containing EF-hand domains binding competitively Ca^{2+} and Mg^{2+}. Among them, the TNC's physiological regulatory (triggering) sites are specific for Ca^{2+}, and high non-physiological concentrations of Mg^{2+} are required for its competition with Ca^{2+} binding. Two TNC's nonspecific Mg^{2+}-, Ca^{2+}- binding sites (so called structural sites) are considered to be saturated with Mg^{2+} during the relaxation – contraction cycle due to high Mg^{2+} concentration in muscle cell. Another Me^{2+}-binding site is located on the myosin regulatory light chain (RLC). This is a true Mg^{2+} for Ca^{2+} replaceable site during Ca^{2+} fluctuations within the physiological level. It has been calculated by Holroyde *et al.* (1979) that, during full activation of skeletal muscle, about 35% of binding sites on the RLC are occupied by Ca^{2+}. Thus, Mg^{2+} bound to the RLC in relaxed state can be partially replaced by Ca^{2+} during prolonged activation of muscle, and this replacement might play a regulatory role. However, this is not sufficiently documented. Metzger & Moss (1990) described the influence of such a divalent cation replacement in the RLC on the tension development in skinned skeletal muscle fibers. According to their data, during submaximal Ca^{2+} activation the RLC is repressive to crossbridge transitions from non-force producing state to the force producing state and this repression is relieved by increasing the concentration of Ca^{2+}. One has to consider also another set of binding sites on myosin, so called low affinity binding sites (Borejdo & Weber, 1982). These are probably located in the rod portion of myosin molecule. The saturation of the low affinity sites with Mg^{2+} can lead to association of crossbridges with the filament backbone (Reisler *et al.*, 1983). By the use of electron microscopy such an association was observed for synthetic filaments of fast skeletal, cardiac (Podlubnaya *et al.*, 1999a) and smooth (Podlubnaya *et al.*, 1999b) muscle myosins in the absence of Ca^{2+} but in the presence of millimolar concentrations of free Mg^{2+}. In the same solution in the presence of free 0.1 mM Ca^{2+} the compact ordered filament structure with 14.5 nm axial repeat, characteristic for myosin heads (and subfragments 2, S2) associated with the filament backbone, was transformed into the disordered "spread" structure with randomly arranged heads and S2s. Since both in the absence and in the presence of Ca^{2+} the level of free Mg^{2+} was constant, the most probable target of Ca^{2+} action is the RLC of myosin but not the low affinity sites.

The main problem in arresting the Ca^{2+}-induced structural transformation in vertebrate striated native and synthetic myosin filaments is the difficulty to preserve the ordered ("relaxing") state (Ménétret *et al.*, 1990, Podlubnaya *et al.*, 1999a). Although, accord-

ing to our EM-observations, the ordered structure with 14.5 nm-periodicity predominated in the majority of the synthetic filaments of fast skeletal and cardiac muscle myosins in the absence of Ca^{2+}, however some filaments or their regions remained partially disordered. In our recent work (Podlubnaya *et al.*, 1999b) it has been demonstrated that conditions of Frado & Craig (1989) appear to be optimal for arresting the ordered structural state of synthetic filaments of vertebrate smooth muscle myosin. The compact ordered filament structure was distinct under relaxing conditions (2 mM ATP/absence of Ca^{2+}) and therefore the Ca^{2+}-induced disordering effect on crossbridges under activating conditions (2 mM ATP/presence of Ca^{2+}) was very pronounced.

In the present work we have applied the conditions of Frado & Craig (1989) to synthetic filaments of fast skeletal muscle myosin. To elucidate whether the observed Ca^{2+}-induced structural transitions are characteristic for filaments of myosins other than those previously studied, we have also tested synthetic filaments of column-purified myosin from slow skeletal muscle. Additionally, we have attempted to correlate those Ca^{2+}-dependent structural transitions with the Ca^{2+} sensitivity of actin-activated ATPase activity of both fast and slow skeletal muscle myosins. Some of the results were presented previously (Malyshev *et al.*, 2000).

MATERIALS AND METHODS

Protein preparation. Fast skeletal muscle myosin was obtained from rabbit back muscle, as described previously (Stępkowski *et al.*, 1985) and further purified by column chromatography on DEAE-Sephadex A-50. Slow skeletal muscle myosin was isolated from myofibrils prepared from rabbit *semimembranosus proprius* muscle by the modified methods described by Herrmann *et al.* (1993) and Tartakowski (1978). This muscle expresses 100% of slow muscle isoforms of myosin (Bacou *et al.*, 1996). About 10 g of chopped muscles was homogenized in 10 vol. of buffer A (5 mM EDTA, 0.5% Triton X-100, 0.1 M sodium acetate, 5 mM KCl, 2 mM dithiothreitol (DTT), 50 mM Tris, pH 7.4, 0.5 mM NaN_3, 0.2 mM PMSF) using Polytron homogenizer, and pelleted (2806 × ***g***, 10 min). The obtained pellet was homogenized in 10 vol. of buffer B containing: 0.1 M $(CH_3COO)Na$, 5 mM KCl, 2 mM DTT, 50 mM Tris, pH 7.4, 2 mM $(CH_3COO)_2Mg$, 0.5 mM NaN_3 and 0.2 mM PMSF. After centrifugation the pelleted myofibrils were washed 4 times in buffer B (without homogenization). Myosin was purified from the obtained myofibrillar preparation. About 8 g of the myofibrillar pellet was extracted for 20 min with 5 vol. of Tartakowski buffer: 0.3 M KCl, 0.1 M KH_2PO_4, 0.05 M K_2HPO_4, 10 mM $Na_4P_2O_7$, 1 mM $MgCl_2$, and centrifuged at 2806 × ***g*** for 15 min. The obtained supernatant was precipitated with 13 vol. of cold H_2O. After centrifugation (4989 × ***g***, 20 min) the precipitate was resuspended and incubated for 20 min in 0.5 M KCl, 20 mM Tris/acetate, pH 8.0, 5 mM EDTA, at room temperature. The suspension was then ultracentrifuged at 113700 × ***g*** for 1.5 h. The supernatant was precipitated with 13 vol. of H_2O and collected by centrifugation. The precipitate was resuspended in 0.5 M KCl and dialyzed overnight against 20 mM Tris/HCl, pH 7.4, 1 mM $MgCl_2$, and next pelleted by centrifugation at 2806 × ***g*** for 15 min. The obtained pellet was again resuspended in 0.5 M KCl and ultracentrifuged at 113700 × ***g*** for 1.5 h. The obtained supernatant, 5 ml, was dialyzed overnight against buffer E: 0.15 M phosphate buffer, pH 7.5, 0.01 M EDTA, 1 mM DTT, and clarified by centrifugation at 4989 × ***g*** for 20 min. The obtained supernatant (about 10 ml) was loaded onto DEAE-Sephacel (Pharmacia) column. The column was washed with buffer E and eluted with a linear gradient produced by buffer E and buffer E plus 0.5 M KCl. Myosin containing fractions were precipitated by

overnight dialysis against: 40 mM KCl, 20 mM imidazole pH 6.5, 0.5 mM DTT, 0.05 mM PMSF. An example of the myosin preparation from *semimembranosus proprius* muscle is shown in Fig. 1.

Figure 1. Electrophoregram of column purified slow skeletal muscle myosin.

SDS/PAGE was carried out in 5%/15% gel. MHC-I, myosin heavy chain; MLC-1S and MLC-1V, myosin essential light chains; MLC-2S, myosin regulatory light chain.

F-actin was prepared by the method of Strzelecka-Gołaszewska *et al.* (1975). Concentrations of fast muscle myosin, slow muscle myosin and actin were determined from their absorbance at 280 nm, using absorption coefficients of $A^{1\%}_{280}$ equal to 5.6, 5.7 and 11 cm^{-1}, respectively.

Filament formation and electron microscopy. Synthetic myosin filaments were formed from column-purified fast and slow skeletal muscle myosins (0.2 mg/ml in 0.5 M KCl) by overnight dialysis against solutions containing 10 mM imidazole, pH 7.0, 0.1 M $(CH_3COO)Na$, 3 mM $(CH_3COO)_2Mg$, 1 mM EGTA, 1 mM NaN_3, 2 mM ATP (relaxing solution); and relaxing solution plus 1.1 mM $CaCl_2$ (activating solution) according to Frado & Craig (1989). All procedures were carried out at 4°C. The suspension of the filaments was placed on collodion and carbon coated grids and negatively stained with 1% aqueous (w/v) uranyl acetate. The samples were examined in JEM-1200EX electron microscope at an accelerating voltage of 80 kV with a 50 μm objective aperture. The magnification was calibrated with negatively stained paramyosin paracrystals, characterized by 14.5 nm repeat.

Electrophoresis. All myosin and actin preparations were examined by electrophoresis on SDS/PAGE 5%/15% gels according to Laemmli (1970). The purity of slow skeletal muscle myosin was additionaly checked on 2–15% gradient gels. Only preparations with intact myosin subunits were used for EM experiments and ATPase measurements.

ATPase activity measurements. Actin-activated Mg^{2+}-ATPase activities of myosins were measured under conditions described in the Figure legends. The determinations of liberated phosphate were performed as described by Fiske & SubbaRow (1925).

RESULTS

In our previous works (Podlubnaya *et al.*, 1999a; 1999b) we have observed Ca^{2+} induced structural transitions in synthetic cardiac and fast skeletal muscle myosin filaments in the absence of ATP. When Podlubnaya *et al.* (1999b) applied the conditions of Frado & Craig (1989) (the presence of ATP, –/+ Ca^{2+}) to synthetic filaments of unphosphorylated smooth muscle myosin, significantly more pronounced striations (14.5 nm repeat) were observed in filaments under relaxing conditions (2 mM ATP, the absence of Ca^{2+}). In contrast, under activating conditions (2 mM ATP, the presence of Ca^{2+}) the release of myosin heads with S2 portion of myosin molecules from the filament surface was observed. Therefore, in the present study we have also applied conditions of Frado & Craig (1989) to check whether the fast and slow skeletal muscle myosin filaments reveal the structural behavior similar to that of the filaments of

smooth muscle myosin. The results are presented in Fig. 2. One can observe that the cross-striation in synthetic fast skeletal muscle myosin filaments (14.5 nm repeat) is very pronounced in the presence of 3 mM Mg^{2+} and in the absence of Ca^{2+} (Fig. 2a, b). Fig. 2c represents the image of fast skeletal muscle myosin filaments in the presence of 3 mM Mg^{2+} and 0.1 mM excess of Ca^{2+} over EGTA. Random disposition of myosin heads with S2s protruding from the filament shaft is distinctly seen. The results of measurements of actin-activated Mg^{2+}-ATPase in purified fast skeletal muscle myosin preparations revealing such Ca^{2+}-induced structural transitions, are presented in Table 1. In all preparations exhibiting Ca^{2+}-induced structural transitions the ATPase activity level was higher in the presence of Ca^{2+}. The extent of activation varied from 17% to 61% depending on the preparation. It was interesting whether such a behavior was also characteristic of the preparations of column-purified slow skeletal muscle myosin obtained from rabbit *semimebranosus proprius* muscle. Figure 3 represents the electron micrographs of synthetic slow muscle myosin filaments formed in the presence of EGTA (Fig. 3a and Fig. 3b) and at 0.1 mM excess of Ca^{2+} over EGTA (Fig. 3c and 3d). The electron micrographs of slow skeletal muscle myosin filaments formed in the absence of Ca^{2+} differ from those of fast skeletal muscle myosin filaments as they show mainly the repeats of about 43 nm and only in some places more fine striation with 14.5 nm repeat. The axial periodicity of about 43 nm might have resulted from a different array of myosin heads on the filament backbone or/and from the decoration of the filaments with some other proteins. However, electrophoretic experiments designed to check this supposition did not show the presence of other proteins in the myosin preparations (not shown). Since the fast skeletal muscle column-purified myosin

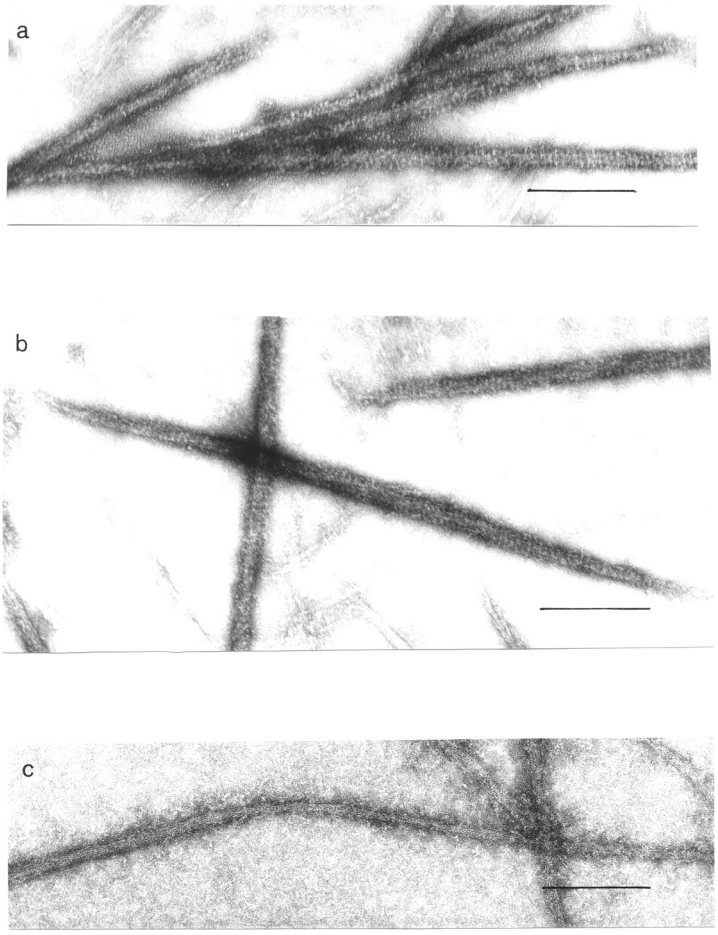

Figure 2. Electron micrographs of synthetic filaments formed by rabbit fast skeletal muscle myosin.

(a, b) In relaxing solution (in the absence of Ca^{2+} and in the presence of ATP, see Materials and Methods). The filaments show compact ordered structure. The cross-striation with an interval of 14.5 nm formed of myosin heads is very distinctly seen along the entire length of the filaments. Bar in all micrographs corresponds to 250 nm. (c) In activating solution (in the presence of Ca^{2+} and ATP). The filaments exhibit disordered, spread structure and axial periodicity of about 14.5 nm is absent. Myosin heads are randomly arranged at a large distance from the filament backbone, due to the release of S2s from the surface of the filament.

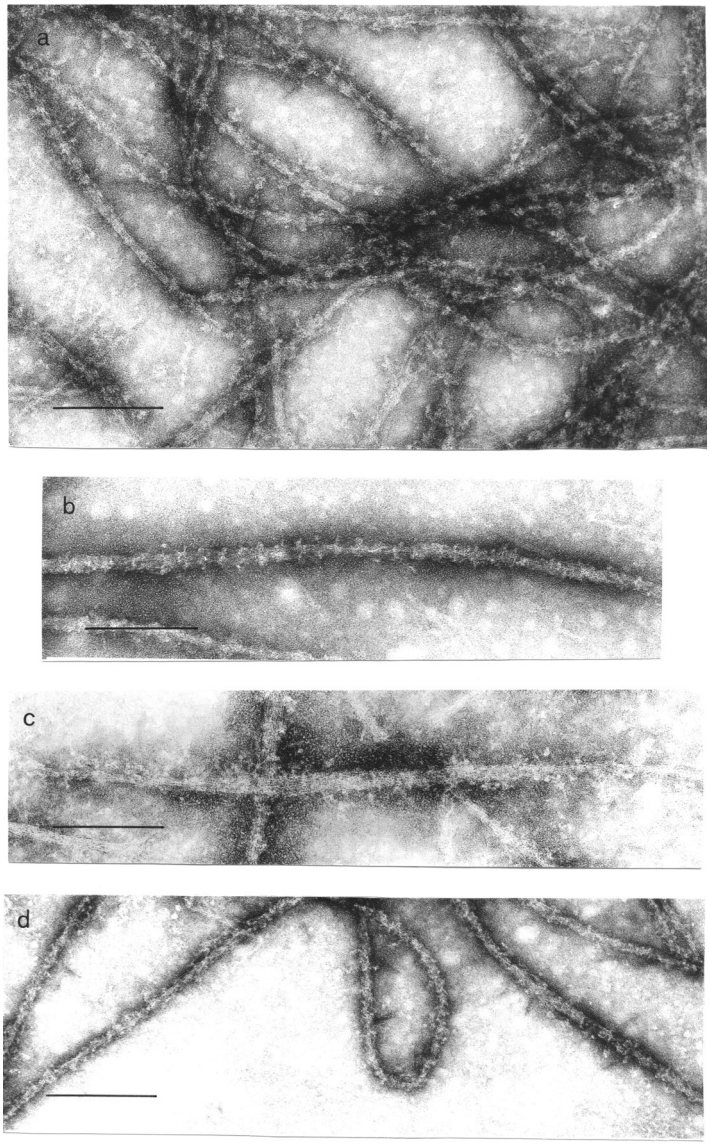

Figure 3. Electron micrographs of synthetic filaments formed by rabbit slow (*semimembranosus proprius*) skeletal muscle myosin.

(a, b) In relaxing solution. There is an axial periodicity of about 43 nm along the entire length of the compact filaments. Such a periodicity is very distinctly seen in the center of the filaments in (b). (c, d) In activating solution. The filaments are disordered with randomly oriented myosin heads and S2s around the filaments.

preparations do not show such a repeat in synthetic filaments, and the procedure of purification on the DEAE-Sephacel column was in both cases the same, we assume that the 43 nm repeat might be a structural property of slow skeletal muscle myosin filaments. In the presence of 0.1 mM excess of Ca^{2+} over EGTA the structure of slow skeletal muscle myosin filaments is indistinguishable from that of fast skeletal, cardiac (Podlubnaya *et al.*, 1999a) and smooth (Podlubnaya *et al.*, 1999b) muscle myosin filaments (Fig. 3c and d). The myosin heads are located randomly around the filament shaft at a distance up to 50 nm from the backbone. It is clear that S2 fragments of myosin molecule are also released from the surface of filaments. The actin-activated Mg^{2+}-ATPase activities of slow skeletal muscle myosin preparations are slightly higher in the presence of Ca^{2+} (see Table 2).

DISCUSSION

The Ca^{2+}-induced structural transition in myosin synthetic filaments

Using electron microscopy Frado & Craig (1989) have demonstrated that under relaxing conditions (ATP/low Ca^{2+}), the synthetic filaments of Ca^{2+}-regulated scallop striated muscle myosin exhibit compact ordered structure with axial periodicity of about 14.5 nm corresponding to the regular arrangement of the

crossbridge shelves close to the filament surface. Both rigor (removal of ATP) and activating conditions (ATP/high Ca^{2+}) resulted in the loss of structural order and induced the transition to disordered spread structure of the filaments due to the movement of the myosin crossbridges away from the filament backbone. Although the authors noted that filaments were not as disordered upon removal of ATP in the absence of Ca^{2+} as in the activated state, they have concluded that only the presence of ATP coupled with the absence of Ca^{2+} is an obligatory requirement for the achievement of the ordered filament structure (relaxed state).

Since the presence of ATP at millimolar concentrations was reported to interfere with the formation of synthetic filaments of skeletal muscle myosin (Pinset-Härström & Truffy, 1979), in our previous electron microscopic studies we have analyzed the effects of Ca^{2+} on the structure of vertebrate striated muscle myosin filaments only under rigor conditions (Podlubnaya *et al.*, 1999a). Nevertheless, we did manage to reveal the extended regions with the compact ordered structure in the synthetic filaments of rabbit fast skeletal and cardiac muscle myosins in the absence of Ca^{2+}. No ordered filaments were seen in the presence of Ca^{2+}. All filaments exhibited a spread

Table 1. Actin-activated ATPase activity of rabbit fast skeletal muscle myosin.

ATPase assay solution: 0.1 M KCl, 10 mM imidazole/HCl (pH 7.0), 2 mM $MgCl_2$, 0.5 mM ATP-Na_2, 50 μg/ml each of actin and myosin and 1 mM EGTA or 0.1 mM $CaCl_2$, respectively. Myosin filaments were formed by dilution. Actin was preincubated with ATP, the reaction was started by mixing equal volumes of actin and myosin-containing solutions. ATPase activity is presented in nmoles $P_i \times mg^{-1} \times min^{-1}$ ± S.E., n = 3–4.

Myosin preparation		ATPase activity		Ca^{2+}-sensitivity (%)
		1 mM EGTA	0.1 mM $CaCl_2$	
I	M	35±3	89±4	61
	M_{col}	72±6	164±14	56
II	M	80±10	166±11	52
	M_{col}	185±12	248±16	25
III	M	102±8	247±24	59
	M_{col}	63±4	90±7	30
IV	M	50±5	113±10	56
	M_{col}	81±7	168±13	52
V	M	107±9	175±6	38
VI	M	90	140	36
	M_{col}	113±15	201±20	44
VII	M_{col}	108±8	151±11	28
VIII	M_{col}	40±6	76±7	47
IX	M_{col}	122±10	208±14	41
X	M_{col}	169±15	226±7	25
XI	M_{col}	53±3	65±4	18
	M_{dial}	71±4	86±4	17

M, Myosin with minor proteins; $M_{col,}$ column-purified myosin; $M_{dial,}$ myosin filaments formed by dialysis; Ca^{2+}-sensitivity = $100\ [(ATPase_{EGTA})/(ATPase_{Ca^{2+}})] \times 100$.

disordered structure and, in this way, the first evidence of a structural Ca^{2+}-sensitivity of the synthetic filaments of vertebrate striated muscle myosins was obtained.

Podlubnaya *et al.* (1999b) have shown that the relaxing and activating conditions of Frado and Craig are optimal for demonstrating the Ca^{2+}-induced structural transition in synthetic filaments of vertebrate smooth muscle myosin, therefore in the present work we have tried to use these conditions for synthetic filaments of fast skeletal muscle myosin. In contrast to the previous data (Pinset-Härström & Truffy, 1979), we have not found any dissociating effects of 2 mM ATP on the filaments. As expected, more ordered structure of the filaments in relaxing solution was revealed. The cross-striation with an interval of 14.5 nm was very distinctly seen along the entire length of the filaments and this striation was much more pronounced than under rigor condition in the absence of Ca^{2+}. Our results confirm the observation of Frado & Craig (1989) concerning the additional ordering effect of ATP on the structure of myosin filaments in the absence of Ca^{2+}. Under activating conditions the filament structure is indistinguishable from that observed previously for fast skeletal and cardiac muscle myosins in rigor in the presence of Ca^{2+}. It is characterized by a irregular arrangement of myosin heads and S2s protruding from the filament backbone. Thus, the conditions of Frado & Craig (1989) appeared to be applicable for EM-studies on the synthetic filaments of vertebrate striated myosin and proved to be optimal for the manifestation of the structural Ca^{2+} sensitivity.

As noted above, the Ca^{2+}-dependent order-disorder transition was observed previously for synthetic filaments of three different types of myosin. In this work we have tested the fourth type of myosin – the slow skeletal muscle myosin. Synthetic filaments of this myosin were found to show a similar pronounced Ca^{2+}-induced structural behavior. There is a clear transition from the compact ordered structure in the absence of Ca^{2+} to the disordered spread structure in the presence of Ca^{2+}. However, the ordered structure of slow skeletal muscle myosin filaments differs from that typical for cardiac, fast skeletal and smooth muscle myosins. Synthetic slow skeletal muscle myosin filaments in relaxed state exhibit mainly 43 nm axial repeats with a rare periodicity of 14.5 nm. It is quite possible that this type of myosin differs in its mode of packing in the synthetic filaments from the other studied myosins. In the presence of Ca^{2+} a disordered structure of slow skeletal muscle myosin filaments looks similar to that of other myosins filaments. This might be explained by the irregular arrangement of the myosin heads and S2s masking the inner filament structure.

We conclude that, like other myosin filaments, the slow skeletal muscle myosin filaments also reveal reversible structural transitions induced by Ca^{2+} ions. This additional evidence confirms our hypothesis that such an

Table 2. Actin-activated ATPase activities of rabbit slow skeletal muscle myosin.

The ATPase activities were determined under the conditions of Frado & Craig (1989) for myosin filaments formed by dilution or dialysis. The activity is presented in nmoles $P_i \times mg^{-1} \times min^{-1}$ ± S.E., n = 3. Ca^{2+}-sensitivity was calculated as in Table 1.

Filament formation	ATPase activity		Ca^{2+}-sensitivity (%)
	EGTA	$CaCl_2$	
Diluted filaments	45±9	53±3	15
Dialyzed filaments	100±8	110±3	9

order-disorder transition is a common property of all vertebrate striated muscle myosins. A schematic representation of this structural transition is presented in Fig. 4.

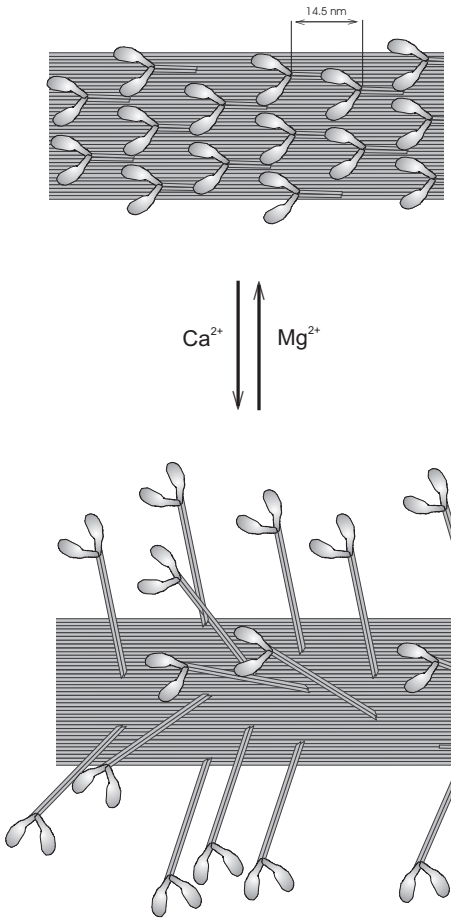

Figure 4. Schematic representation of structural transition in synthetic filaments occurring due to Ca^{2+} for Mg^{2+} exchange in regulatory light chains of myosin.

Orientation of myosin heads relative to S2 is not taken into consideration.

The possible Ca^{2+} target

In the pure myosin system Ca^{2+} ions can bind to two kinds of binding sites. The first is the high affinity nonspecific binding site located on the RLC. This site can be saturated either by Mg^{2+} or Ca^{2+}. As mentioned in the introduction, the saturation of RLC by Ca^{2+} is less than a half during full activation of muscle cell (Holroyde *et al.*, 1979). However, one can assume that owing to intermolecular cooperativity within thick filaments (Moss, 1992; Martin & Barsotti, 1994; Stępkowski *et al.*, 1997) even partial saturation of Me^{2+}-binding sites with Ca^{2+} might be sufficient for switching the structure of the whole filament. In our studies we have applied conditions (0.1 mM free Ca^{2+}) under which almost complete saturation of the RLC binding site with Ca^{2+} was assured.

The other possible Ca^{2+} binding sites are located on the myosin rod as reported by Borejdo & Weber (1982). However, these sites have association constants around 10^3 M^{-1}, and it is unlikely that Ca^{2+} at a concentration by one order of magnitude lower would saturate those sites in the presence of millimolar concentrations of Mg^{2+}. Therefore, the RLC binding site is the most probable target for Ca^{2+} binding inducing the reversible structural transition observed by us. This conclusion is strongly substantiated by our other studies revealing that the compact-to-spread structural transition in synthetic filaments of both cardiac and fast skeletal muscle column-purified myosins (Podlubnaya *et al.*, 2000) is suppressed by truncation of the N-terminus of the RLC.

The functional consequences of myosin filament order-disorder transitions

The question arises whether the attachment and detachment of myosin heads to and from the filament surface may influence enzymatic properties of the actomyosin system. In our hands, both fast and slow skeletal muscle myosin filaments exhibited higher actin-activated Mg^{2+}-ATPase activity in the presence of Ca^{2+}. This enzymatic Ca^{2+} sensitivity was observed both for the filaments formed by dialysis (long unipolar structures) and by dilution from high salt solution (bipolar short filaments). In the EM experiments we used long filaments formed by dialysis because they are more convenient for observation of periodic striations. The Ca^{2+}-induced activation is not very strong and varies from 10% to 60%. In the

light of our microscopic observations this activation may be interpreted as a result of increased mobility of the myosin heads and S2s in the presence of Ca^{2+} providing better accessibility of heads for interaction with actin. Since actin filaments in solution interact with myosin filaments in a stochastic way, the activating effect cannot be expected to be high. However in muscle, where the filament positions are fixed within the hexagonal lattice, better accessibility of myosin heads to actin could significantly accelerate the contractile response to increasing Ca^{2+} concentration. A similar explanation was suggested for the increase in tension development at low Ca^{2+} level due to RLC phosphorylation accompanied by an analogous order-disorder transition in isolated skeletal muscle thick filaments (Levine *et al.*, 1996). In this connection, the results obtained by Metzger & Moss (1990) should be noted. In their experiments on skinned fast skeletal fibers, the RLCs saturated with Mg^{2+} were repressive to the fiber tension and to the rate of force development. The observed effects were relieved by increasing Ca^{2+} concentration. These results are consistent with our conception of RLC-mediated attachment-detachment behavior of the myosin heads and S2s on the thick filament surface, depending on the free Ca^{2+} level.

To summarize, we propose, that the Ca^{2+}-induced structural transitions observed by us may contribute to regulatory properties of the corresponding muscle systems.

The authors appreciate the contribution of Prof. Irena Kąkol, Dr. Anna Moczarska and Dr. Sergey N. Udaltsov to this paper.

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