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Effect of nucleotide on interaction of the 567–578 segment of myosin heavy chain with actin

Brian M. Martin [a], Emilia Karczewska [b], Barbara Pliszka [b,*]

[a] NIMH, National Institutes of Health, Bethesda, MD 20892, USA
[b] Department of Muscle Biochemistry, Nencki Institute of Experimental Biology, 3 Pasteur St., PL-02-093 Warsaw, Poland

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Abstract

To probe the effect of nucleotide on the formation of ionic contacts between actin and the 567–578 residue loop of the heavy chain of rabbit skeletal muscle myosin subfragment 1 (S1), the complexes between F-actin and proteolytic derivatives of S1 were submitted to chemical cross-linking with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide. We have shown that in the absence of nucleotide both 45 kDa and 5 kDa tryptic derivatives of the central 50 kDa heavy chain fragment of S1 can be cross-linked to actin, whereas in the presence of MgADP.AlF_4, only the 5 kDa fragment is involved in cross-linking reaction. By the identification of the N-terminal sequence of the 5-kDa fragment, we have found that trypsin splits the 50 kDa heavy chain fragment between Lys-572 and Gly-573, the residues located within the 567–578 loop. Using S1 preparations cleaved with elastase, we could show that the residue of 567–578 loop that can be cross-linked to actin in the presence of MgADP.AlF_4 is Lys-574. The observed nucleotide-dependent changes of the actin-subfragment 1 interface indicate that the 567–578 residue loop of skeletal muscle myosin participates in the communication between the nucleotide and actin binding sites.

Keywords: Actin–myosin interaction; Subfragment 1; Chemical cross-linking

1. Introduction

Cyclic interaction of myosin with actin coupled with hydrolysis of ATP transforms the chemical energy of ATP into movement. The powerstroke is closely linked to a modification of the contact area between the head part of the myosin molecule—subfragment 1 (S1)—and actin. During the ATPase cycle, the initial weak-binding complex, where myosin mostly interacts with actin by ionic contacts, undergoes the multistep transition into the strong-binding complex, where the contacts are both of hydrophobic and ionic nature. A model of the actomyosin interface has been built using X-ray structures of individual proteins and electron micrographs of actin filaments saturated by S1 [1]. Since crystallized actin–S1 complexes are not available, it is necessary to study the changes of these proteins interaction during the ATPase cycle by non-crystallographic methods. Many studies on ionic contacts between actin and myosin using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC), a zero-length cross-linking reagent which couples carboxyl and amino groups in proteins, indicate that there are two lysine-rich S1 subsites that can interact with negatively charged residues of the N-terminus of actin. It is well documented that one of them is located within loop 2, comprising residues 626–647 of the S1 heavy chain. It is situated between the upper and lower part of the central subdomain of the myosin head [2]; in the heavy chain sequence, it lies between the central 50 kDa and C-terminal 20 kDa fragments that are produced (besides the N-terminal 25 kDa fragment) by limited tryptic digestion of skeletal muscle myosin S1 [3]. The other subsite is located on the lower part of the central subdomain of the myosin head [2]. This subsite has been shown to lie between Trp-510 and Trp-595 [4] where the only positively charged cluster that can interact with actin is the sequence Lys–Pro–Lys–Pro–Ala–Lys–Gly–Lys–Ala–Glu–Ala–His (residues 567–578). This sequence, located in

Abbreviations: S1, myosin subfragment 1; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; IAEDANS, N-iodoacetyl-N′-(5-sulfo-1-naphthyl)ethylenediamine

* Corresponding author. Fax: +48 22 822 53 42.
E-mail address: b.pliszka@nencki.gov.pl (B. Pliszka).

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the 50 kDa tryptic heavy chain fragment of S1 [5], has been called loop 3 [6]. Both loops are flexible, and although they are not visualized in the crystal structure of skeletal muscle myosin S1, their position is known, and the distance between them is about 5 nm [2]. It has been shown by chemical cross-linking that when F-actin is in excess over S1, loop 2 and loop 3 of skeletal muscle myosin contribute to the interaction of a single myosin head with two adjacent actin monomers [7,8]. Such interaction is predominant in the weak-binding state populated with MgADP.P_i analogs (9).

Loop 2 modulates the actin affinity and actin-activated ATPase activity of myosin [10–13]. It has been shown that the presence of ATP modifies the contribution of lysine residues of loop 2 in the formation of the interface between S1 and actin [14]. Until now, the area of loop 3 involved in contacts with actin in different actomyosin states has not been determined. It is known that in the presence of nucleotide the 50 kDa heavy chain fragment of myosin becomes susceptible to trypsin at a site that is located about 5 kDa from its C-terminus [15]. Although this site has not been precisely identified, it was clear that it must lie close to loop 3. Since the change in proteolytic susceptibility probably reflects a structural rearrangement of the polypeptide chain in this region, one can suppose that the formation of ionic contacts between myosin loop 3 and actin might be affected by nucleotide.

In this work, we applied cross-linking with EDC to probe the effect of nucleotide on the interaction of myosin loop 3 with actin. We have found that the peptide bond cleaved by trypsin in the presence of nucleotide is located within loop 3, between Lys-572 and Gly-573. The results of cross-linking between actin and S1 preparations split at this bond indicate that in the presence of Mg.ADP.P_i analog–MgADP.AlF_4–the area of loop 3 involved in the formation of the actin–myosin interface is smaller than in the absence of nucleotide.

2. Materials and methods

2.1. Materials

ATP, ADP, chymotrypsin, trypsin, elastase, soybean trypsin inhibitor, EDC and N-iodoacetyl-N′-(5-sulfo-1-naphtyl)ethylenediamine (IAEDANS) were obtained from Sigma, molecular weight markers from Bio-Rad.

2.2. Proteins

Myosin was prepared from rabbit back muscles, and S1 was obtained by digestion of myosin with chymotrypsin [16]. Actin was obtained as described in [17].

2.3. Labeling of F-actin

Actin was labeled with the fluorescent dye 1,5-IAEDANS according to [18].

2.4. Preparation of S1 derivatives

The (25–50–20 kDa)S1 derivative was obtained by digestion of S1 with trypsin at the enzyme to substrate ratio of 1:25 (w/w) in buffer A (25 mM NaCl, 10 mM HEPES, pH 7.5, 2 mM $MgCl_2$), for 15 min at 25 °C, then the reaction was stopped by the addition of soybean trypsin inhibitor at 1.5:1 (w/w) ratio to trypsin. To obtain the (22–50/45–20 kDa)S1 derivative, S1 was digested with trypsin in buffer B (100 mM NaCl, 10 mM HEPES, pH 7.9, 1 mM $MgCl_2$, 10 mM ATP), for 40 min at 25 °C; after addition of soybean trypsin inhibitor the samples were exhaustively dialyzed for 24 h at 0 °C against buffer A. To obtain the (28–48–22 kDa) S1 derivative, S1 was cleaved with elastase at the enzyme to substrate ratio of 1:10 (w/w) in buffer A for 20 min at 25 °C, then the reaction was stopped by the addition of 1 mM PMSF.

2.5. Preparation of S1–nucleotide–aluminium fluoride complexes

The complexes of S1 derivatives with MgADP.AlF_4 were obtained by incubation of each S1 derivative (25 μM) for 10 min at 25 °C in buffer A with the addition of 2 mM ADP, 9 mM NaF and 2 mM $AlCl_3$.

2.6. Cross-linking of S1 derivatives to F-actin

For cross-linking reaction S1 derivatives (13 μM) and actin (28 μM) were kept for 30 min at 25 °C in buffer A, and then incubated with 20 mM EDC for 15 or 30 min at 25 °C. The reaction was terminated by addition of 70 mM 2-mercaptoethanol.

2.7. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

The cross-linking products were analyzed by glycine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis (glycine-SDS-PAGE) according to [19]. Low-molecular weight peptides were separated by tricine-SDS-PAGE as described in [20], using 16.5% separating gels.

2.8. Densitometric measurement

To estimate relative fluorescence intensities of cross-linked proteins, the gels were scanned under UV light in a Fluor-S Multimager BioRad Scanner. The relationship between the concentration of actin labeled with IAEDANS and fluorescence intensity was standardized by loading the gel with increasing amounts of labeled actin. This relationship was linear within the range of fluorescence intensities observed in our experiments.

2.9. Amino acid sequence analysis

The 5 kDa peptide separated by tricine-SDS-PAGE was electrophoretically transferred to a polyvinylidene fluoride (Immobilon P^{SQ}) transfer membrane [21]. The blotted band was sequenced on an ABI Procise Model 492 using the pulsed liquid protocol and PTH-amino acids identified by on-line analysis based on a 10-pmol PTH standard.

3. Results

Preparations of the (22–50/45–20 kDa)S1 derivative used in our experiments contained a large fraction of S1 molecules in which the 50-kDa fragment of the heavy chain was converted by trypsin into the 45 kDa one (and the 25-kDa fragment into the 22 kDa one). The mixture of the (22–50/45–20 kDa)S1 derivative and fluorescent actin was treated with EDC in the absence of nucleotide and then submitted to SDS-PAGE. As shown in Fig. 1, besides the earlier observed 60 kDa, 90 kDa, and 95 kDa cross-linking products, composed of actin and the 20 kDa, 45 kDa and 50 kDa fragment, respectively [22], a fluorescent band located above actin could also be seen on the gels. Since this fluorescent band was absent when actin was crosslinked to the (25–50–20 kDa)S1 derivative (compare lanes a′ and b′ in Fig. 1), this product was concluded to be composed of actin and a 5 kDa C-terminal part of the 50-kDa fragment of the S1 heavy chain. Cross-

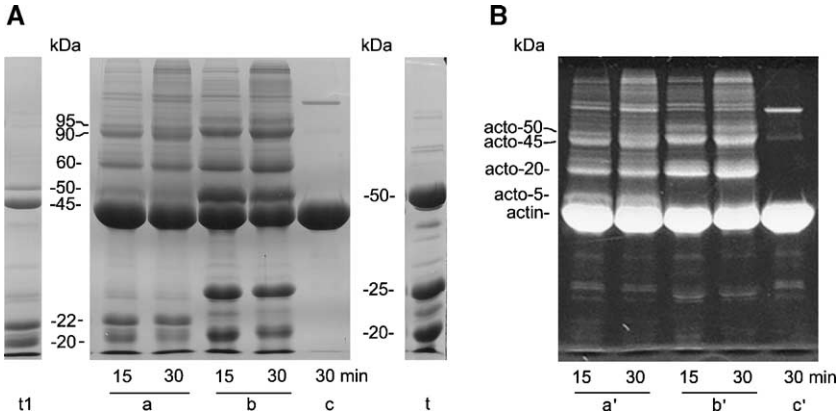

Fig. 1. Cross-linking between actin and tryptic S1 derivatives with EDC. Complexes of IAEDANS-labeled actin and (22–50/45–20 kDa)S1 (lanes a,a′) or (25–50–20 kDa)S1 (lanes b, b′) were cross-linked in the absence of nucleotide as described in Materials and methods. Acto-5, acto-20, acto-45 and acto-50 bands represent products of cross-linking between actin and 5 kDa, 20 kDa, 45 kDa and 50 kDa S1 heavy chain fragments, respectively. Lanes c, c′ represent cross-linked actin control. The times of cross-linking are indicated in the figure. Lanes t1 and t, noncross-linked (22–50/45–20 kDa)S1 and (25–50–20 kDa) S1, respectively. (A) Coomassie blue stained, (B) fluorescent gel. Glycine–SDS-PAGE was performed on 12% separating gels.

linking of the (22–50/45–20 kDa)S1 to actin in the presence of ADP generated similar products to those obtained in the absence of nucleotide (not shown).

Fig. 2 shows the effect of MgADP.AlF_4, an analog of MgADP.P_i, on cross-linking between the (22–50/45–20 kDa)S1 and actin. The experiment was performed on two preparations that differed in 50 kDa : 45 kDa ratios. The electrophoretic separation of the EDC-treated proteins shows that in samples cross-linked in the medium containing MgADP.AlF_4, the acto-5 kDa fluorescent product was also present. Besides this product, the samples contained the acto-50 kDa product, but the band of actin cross-linked to the 45 kDa S1 fragment was practically absent (lanes b,b′ and e,e′). Similar results of cross-linking between the (22–50/45–20 kDa)S1 and actin were obtained in the presence of MgADP.-BeF_x that also induces the weak-binding state of the actomyosin complex (not shown).

The above results show that in all tested conditions the C-terminal 5 kDa part of the 50-kDa heavy chain fragment participates in the formation of cross-links between S1 and actin, whereas cross-linking of the 45-kDa part to actin in the weakly bound state is inhibited. To determine the participation of particular lysine residues in the interaction of loop 3 with actin, it was necessary to identify the peptide bond where the 50-kDa fragment can be split by trypsin into the 45-kDa and 5-kDa parts.

It has been previously shown using the tricine-SDS PAGE system that tryptic treatment of S1 in the presence of Mg.ADP.AlF_4 generated a peptide material of about 5 kDa, whereas in the presence of MgADP this peptide(s) was degraded into smaller fragments [23]. In this work, the 5-kDa peptide(s) was obtained by tryptic digestion of S1 in the medium containing ATP (see Materials and methods), separated by tricine-SDS PAGE system (see Fig. 3), and then submitted to the sequencing procedure. The 5 kDa band, besides minor contaminants, contained the major peptide beginning with Gly–Lys–Val–Glu–Ala–His–Phe–Ser–Leu–Val–His–Tyr. This sequence is identical with that of the 50 kDa S1 heavy chain region comprising residues 573–584 [5]. Thus, trypsin splits S1 heavy chain between Lys-572

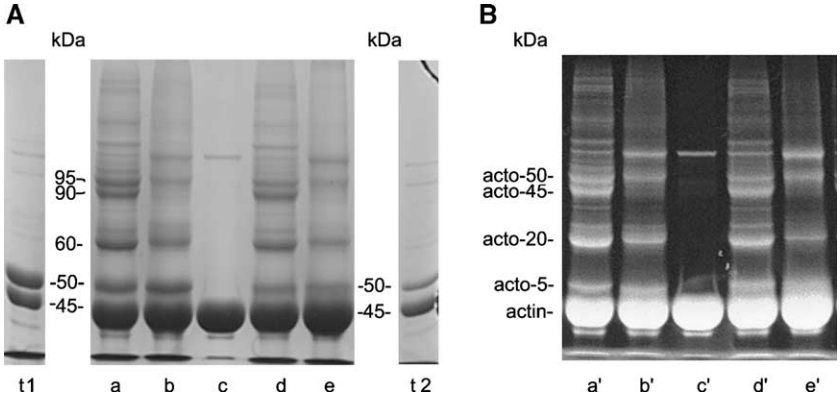

Fig. 2. Effect of MgADP.AlF_4 on cross-linking between actin and trypsin-split S1. Actin and (22–50/45–20 kDa)S1 preparations obtained by digestion of S1 with trypsin for 40 min (lanes a,a′ and b,b′) or 90 min (lanes d,d′ and e,e′) as described in Materials and methods, were cross-linked with EDC for 15 min, in the absence (lanes a,a′ and d,d′) or presence (lanes b,b′ and e,e′) of 2 mM MgADP.AlF_4. Lanes c,c′, cross-linked actin control; lanes t1 and t2 represent noncross-linked (22–50/45–20 kDa)S1 preparations obtained by 40 min or 90 min digestion of S1, respectively. Glycine–SDS-PAGE was performed on 9% separating gels. (A) Coomassie blue stained, (B) fluorescent gels.

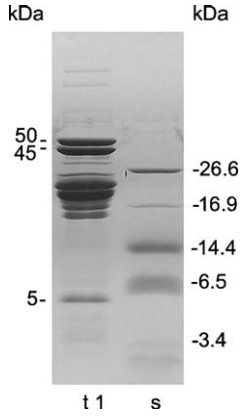

Fig. 3. Resolution of low-molecular weight components of (22–50/45–20 kDa)S1 by tricine–SDS-PAGE. Lane t1, (22–50/45–20 kDa)S1 tryptic derivative, lane s, molecular weight markers.

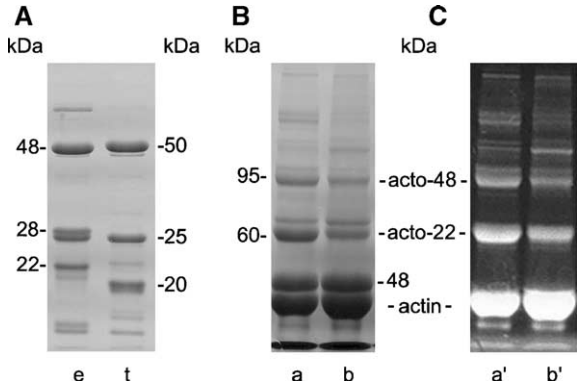

Fig. 5. Cross-linking between actin and elastase-split S1. (A) elastase-split S1 (lane e) and tryptic (25–50–20 kDa)S1 derivative (lane t) are compared by glycine–SDS-PAGE on 15% gel. (B, C) complex of actin and elastase-split S1 was cross-linked with EDC in the absence (lanes a,a′) or presence (lanes b,b′) of 2 mM MgADP.AlF_4 for 15 min and then separated by glycine-SDS-PAGE on 9% gels. (A, B) Coomassie blue stained, (C) fluorescent gels.

and Gly-573. The above localization of the cleavage site indicates that besides Lys-636 of loop 2 (the C-terminal residue of the 50-kDa and its 5-kDa derivative) contributing to the interaction with actin [14], the 5-kDa peptide also contains a part of loop 3 with Lys-574, a residue that can potentially be in contact with negatively charged actin residues, and that is situated close to the N-terminus of this peptide.

To test the participation of Lys-574 in the formation of cross-links between S1 and actin in the weakly bound state, S1 preparations were (before cross-linking) treated with elastase that splits the heavy chain into N-terminal 28 kDa, central 48 kDa, and C-terminal 22 kDa fragment [24]. The 48 kDa–22 kDa junction is cleaved between Glu-630 and Glu-631 [14], thus elastase cut off all the lysine residues of loop 2 from the central heavy chain fragment. The distribution of lysine residues of two loops on proteolytic fragments of S1 is demonstrated in Fig. 4. As shown in Fig. 5, elastase-split S1 can be crosslinked to actin via its 48 kDa and 22 kDa fragments yielding products of about 95 kDa and 60 kDa, respectively, not only in the absence of nucleotide but also in the presence of MgADP.AlF_4. Densitometric analysis of the fluorescent bands showed that the yield of both acto-48 kDa and acto-22 kDa complexes was decreased by about 50% by the presence of the nucleotide analog. However, the formation of the acto-48 kDa product in the presence of MgADP.AlF_4 indicates that Lys-574 is responsible for ionic contacts between S1 loop 3 and actin in the weak-binding state of the actomyosin complex. The

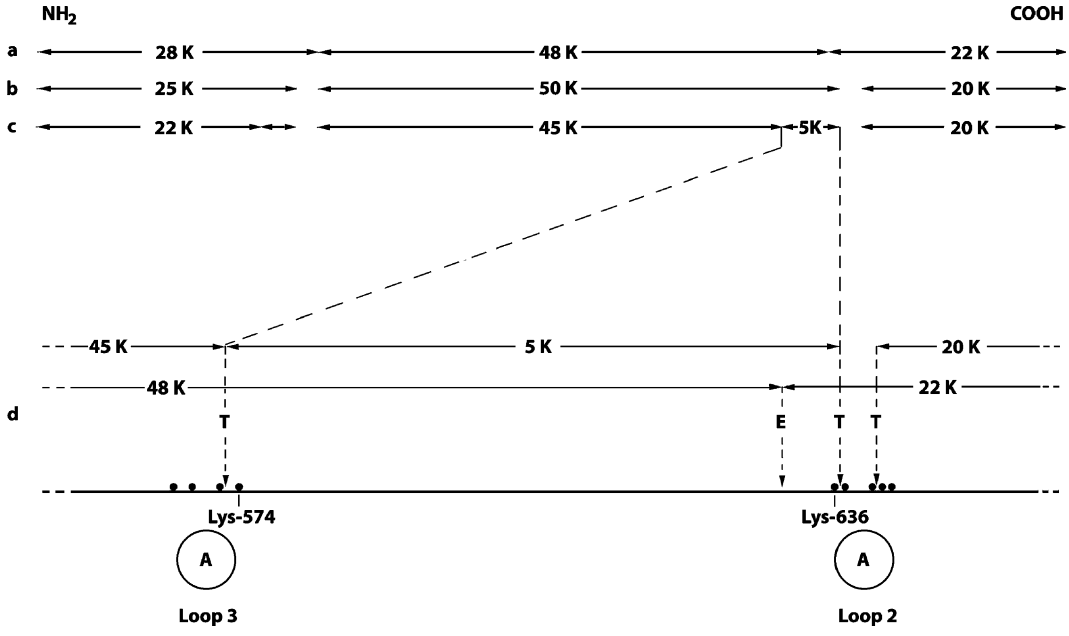

Fig. 4. Schematic diagram illustrating the localization of lysine residues of loop 2 and loop 3 in proteolytic fragments of the S1 heavy chain. a, b, and c show S1 derivatives: (a) is (28–48–22 kDa)S1 generated by elastase, (b) and (c) are (25–50–20 kDa)S1 and (22–50/45–20 kDa)S1 generated by trypsin, respectively. Localization of lysines of loop 2 and loop 3 in the S1 heavy chain is shown in (d). Arrows indicate sites accessible for trypsin (T) and elastase (E). Distribution of cleavage sites within the 50 kDa–20 kDa junction is shown according to [25] for trypsin and to [14] for elastase. A in circle represents actin; lysine residues are marked with black circles.

observed reduction of this product could be expected, since in the presence of MgADP.AlF_4, the interaction of S1 loop 3 with actin is supposed to involve fewer lysine residues than in the absence of nucleotide.

4. Discussion

Chemical cross-linking applied to the complexes of actin with S1 and/or its proteolytic derivatives have provided important information about actin–myosin interaction (e.g. [8,22,26–28]). It has been earlier shown that the splitting of the 50-kDa heavy chain fragment of S1 into a 45 kDa one (and the 25-kDa fragment into a 22-kDa one) does not impair the enzymatic site of myosin [29]. The results of this work indicate that the (22–50/45–20 kDa)S1 derivative can be useful in studies on nucleotide-dependent changes of the actomyosin interface.

The identification of the peptide bond between Lys-572 and Gly-573 as a site that in the presence of nucleotide becomes susceptible to trypsin was very helpful in the interpretation of our results. It is known that loop 3 contains four lysine residues. As suggested by the fitting of the atomic structure of S1 to the model of F-actin, most likely two of them, Lys-572 and Lys-574, can form ionic contacts with negatively charged residues of actin [1]. We have shown that Lys-574, located in the tryptic 5 kDa fragment of the heavy chain, contributes to interaction of S1 with actin both in the presence and absence of nucleotide. On the other hand, the formation of cross-links between the 45 kDa heavy chain fragment and actin in the absence, but not in the presence of MgADP.AlF_4, indicates that nucleotide affects the contact area between S1 loop 3 and actin. One cannot exclude that this area might be affected by tryptic cleavage of the S1 heavy chain at Lys-572. It is, however, little probable that this cleavage is responsible for the observed effect of the nucleotide analog on cross-linking, because in each experiment the treatment of proteins with EDC, both in the presence and absence of MgADP.AlF_4, was performed with the same (22–50/45–20 kDa)S1 preparation.

As we have indicated in the Introduction, a nucleotide-dependent structural rearrangement of loop 3 has been suggested based on the observation that the presence of nucleotide makes this loop susceptible to trypsin. Our present results confirm this view. Although it is not clear how nucleotide affects the structure of loop 3, the above data indicate that besides widely described changes in the width of the cleft extending from the catalytic site to the actin-binding site, nucleotide also induces less prominent distortions within the actin-binding area of S1.

The lack of formation of the acto-45 kDa cross-linking product not only in the presence of MgADP.AlF_4 but also with MgADP.BeF_x is in agreement with the observation that in the case of these two nucleotide analogs the actin–S1 interface does not seem to be distinct [9]. It has been shown by X-ray crystallography that S1.MgADP.BeF_x can adopt a conformation different from (data for Dictyostelium discoideum myosin, Ref. [30]) or the same as that of S1.MgADP.AlF_4 complex (data for smooth muscle myosin, Ref. [31]). These conflicting results have been interpreted as indicating that S1 in complex with beryllium fluoride can exist in more than one conformation. We can suppose that under conditions of our experiments, a form similar to that of S1. MgADP.AlF_4 prevails. It is also possible that, as suggested in [9], two sets of structure, induced by these two nucleotide analogs, are not related to two different classes of actin–S1 interfaces.

The above effect of nucleotide on S1 loop 3 is similar to that described for loop 2, where in the presence of ATP mostly one lysine residue is involved in contacts with actin, whereas in the rigor state all five lysines can contribute to the S1–actin interaction [14]. Although we do not know how many lysines of loop 3 are involved in contact with actin in rigor, it must be at least one residue more than in the weak binding state. Thus, one can conclude that in addition to loop 2, also loop 3 of the head of skeletal muscle myosin participates in communication between the nucleotide and actin binding sites.

It is known that both loops are highly divergent in length and in charge contents. The importance of loop 2 for the actomyosin function has been widely established. The role of loop 3 is less clear. It has been observed that an antipeptide antibody directed against loop 3 of skeletal muscle myosin had a strong inhibitory effect both on the movement of actin filaments in an in vitro motility assay and on actin-activated ATPase activity of myosin [32]. On the other hand, the substitution of loop 3 region in Dictyostelium discoideum motor domain by that of skeletal muscle myosin did not affect the actomyosin ATPase activity of the chimera construct [6]. It has been also shown that the formation of cross-links between loop 3 and actin, highly efficient in skeletal [7,8] and cardiac myosin [33], in the case of other tested myosin isoforms was poor or none [6]. It has been suggested that the myosin loop 3 region has a modulating effect on the interaction of S1 with actin only in the case of striated muscle myosin. Taking into account the above suggestion, we would like to point out that contraction of both skeletal and cardiac muscle is regulated via tropomyosin–troponin system. One cannot exclude that in these types of muscle the additional contact of S1 with actin—between loop 3 and the second actin monomer—facilitates transition between closed and open states of the thin filament, a process that is crucial for the regulation of the actin–myosin interaction [34].

It is noteworthy that in loop 3 of smooth muscle myosin S1, which shows very poor cross-linking to actin [6], there are 3 net positive charges (like in skeletal muscle myosin). However, Gly-573 present in skeletal muscle myosin is replaced in smooth muscle myosin by negatively charged aspartate. In view of the contribution of Lys-574 to the interaction of S1 with actin, the inhibitory effect of this substitution on the chemical cross-linking of two proteins can be better understood.

The described results reflect γ-phosphate mediated structural changes of the actin–myosin loop 3 interface. Loop 3 is located in the vicinity of 529–558 residue cluster that is thought to be involved in the interaction of S1 with actin in the tightly bound state [1]. The structural changes within loop 3 region of myosin may play a role in the transition between the weak and strong binding states of the actomyosin complex.

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References

[1] I. Rayment, H.M. Holden, M. Whittaker, C.B. Yohn, M. Lorenz, K.C. Holmes, R.A. Milligan, Structure of the actin–myosin complex and its implications for muscle contraction, Science 261 (1993) 58–65.
[2] I. Rayment, W.R. Rypniewski, K. Schmidt-Base, R. Smith, D.R. Tomchick, M.M. Benning, D.A. Winkelman, G. Wesenberg, H.M. Holden, Three-dimensional structure of myosin subfragment-1: a molecular motor, Science 261 (1993) 50–58.
[3] D. Mornet, P. Pantel, E. Audemard, R. Kassab, The limited tryptic cleavage of chymotryptic S-1: an approach to the characterization of the actin site in myosin heads, Biochem. Biophys. Res. Commun. 89 (1979) 925–932.
[4] K. Sutoh, Mapping of actin-binding sites on the heavy chain of myosin subfragment 1, Biochemistry 22 (1983) 1579–1585.
[5] S.W. Tong, M. Elzinga, Amino acid sequence of rabbit skeletal muscle myosin, J. Biol. Chem. 265 (1990) 4893–4901.
[6] J. Van Dijk, M. Furch, C. Lafont, D.J. Manstein, P. Chaussepied, Functional characterization of the secondary actin binding site of myosin II, Biochemistry 38 (1999) 15078–15085.
[7] A.L. Andreeva, O.A. Andreev, J. Borejdo, Structure of the 265-kilodalton complex formed upon EDC cross-linking of subfragment 1 to F-actin, Biochemistry 32 (1993) 13956–13960.
[8] N. Bonafe, P. Chaussepied, A single myosin head can be cross-linked to the N-termini of two adjacent actin monomers, Biophys. J. 68 (1995) 35s–43s.
[9] J. Van Dijk, C. Fernandez, P. Chaussepied, Effect of ATP analogues on the actin–myosin interface, Biochemistry 37 (1998) 8385–8394.
[10] T.Q.P. Uyeda, K.M. Ruppel, J.A. Spudich, Enzymatic activities correlate with chimaeric substitutions at the actin-binding face of myosin, Nature 368 (1994) 567–569.
[11] M. Furch, M.A. Geeves, D.J. Manstein, Modulation of actin affinity and actomyosin adenosine triphosphatase by charge changes in the myosin motor domain, Biochemistry 37 (1998) 6317–6326.
[12] C.T. Murphy, J.A. Spudich, The sequence of the myosin 50–20 K loop affects myosin's affinity for actin throughout the actin–myosin ATPase cycle and its maximum ATPase activity, Biochemistry 38 (1999) 3785–3792.
[13] P.B. Joel, K.M. Trybus, H.L. Sweeney, Two conserved lysines at the 50/20-kDa junction of myosin are necessary for triggering actin activation, J. Biol. Chem. 276 (2001) 2998–3003.
[14] K. Yamamoto, Binding manner of actin to the lysine-rich sequence of myosin subfragment 1 in the presence and absence of ATP, Biochemistry 28 (1989) 5573–5577.
[15] T. Hozumi, Structure and function of myosin subfragment 1 as studied by tryptic digestion, Biochemistry 22 (1983) 799–804.
[16] A.G. Weeds, B. Pope, Studies on chymotryptic digestion of myosin. Effect of divalent cations on proteolytic susceptibility, J. Mol. Biol. 111 (1977) 129–137.
[17] J.A. Spudich, S. Watt, The regulation of rabbit skeletal muscle contraction, J. Biol. Chem. 246 (1971) 4866–4871.
[18] K. Tawada, P. Wahl, J.C. Auchet, Study of actin and its interaction with heavy meromyosin and regulatory proteins by the pulse fluorimetry in polarized light of a fluorescent probe attached to an actin cysteine, Eur. J. Biochem. 88 (1978) 411–419.
[19] U.K. Laemmli, Cleavage of structural proteins during the assembly of the head of bacteriophage T4, Nature 227 (1970) 680–685.
[20] H. Schagger, G. von Jagow, Tricine-sodium dodecyl sulfate-polyacrylamide gel electrophoresis for the separation of proteins in the range from 1 to 100 kDa, Anal. Biochem. 166 (1987) 368–379.
[21] P. Matsudaira, Sequence from picomole quantities of proteins electroblotted onto polyvinylidene difluoride membranes, J. Biol. Chem. 262 (1987) 10035–10038.
[22] J.-P. Labbe, R. Bertrand, E. Audemard, R. Kassab, D. Walzthony, T. Walliman, The interaction of skeletal myosin subfragment 1 with the polyanion, heparin, Eur. J. Biochem. 143 (1984) 315–322.
[23] B. Pliszka, E. Karczewska, Inhibitory effect of ATP analogs and actin on the modification of myosin subfragment 1 wiyh 9-anthroylnitrile, Biochim. Biophys. Acta 1699 (2004) 201–205.
[24] D. Applegate, E. Reisler, Protease-sensitive regions in myosin subfragment 1, Proc. Natl. Acad. Sci. U. S. A. 80 (1983) 7109–7112.
[25] K. Yamamoto, Identification of the site important for the actin-activated MgATPase activity of myosin subfragment-1, J. Mol. Biol. 217 (1991) 229–233.
[26] T. Chen, D. Applegate, E. Reisler, Cross-linking of actin to myosin subfragment 1 in the presence of nucleotide, Biochemistry 24 (1985) 5620–5625.
[27] R. Bertrand, P. Chaussepied, R. Kassab, Cross-linking of the skeletal myosin subfragment 1 heavy chain to the N-terminal actin segment of residues 40–113, Biochemistry 27 (1988) 5728–5736.
[28] R. Bertrand, J. Derancourt, R. Kassab, Probing the hydrophobic interactions in the skeletal actomyosin subfragment 1 and its nucleotide complexes by zero-length cross-linking with a nickel-peptide chelate, Biochemistry 36 (1997) 9703–9714.
[29] G. Mocz, L. Szylagyi, R. Chen Lu, F. Fabian, M. Balint, J. Gergely, Effect of nucleotides, divalent cations and temperature on the tryptic susceptibility of myosin subfragment 1, Eur. J. Biochem. 145 (1984) 221–229.
[30] A.J. Fisher, C.A. Smith, J. Thoden, R. Smith, K. Sutoh, H.M. Holden, I. Rayment, X-ray structures of the myosin motor domain of Dictyostelium discoideum complexed with MgADP. BeF_x and MgADP.AlF_4, Biochemistry 34 (1995) 8960–8972.
[31] R. Dominguez, Y. Freyzon, K.M. Trybus, C. Cohen, Crystal structure of a vertebrate smooth muscle myosin motor domain and its complex with the essentials light chain: visualization of the pre-power stroke state, Cell 94 (1998) 559–571.
[32] E. Blotnick, C. Miller, U. Groschel-Stewart, A. Muhlrad, Immunochemical probing of the functional role of the 238–246 and 567–574 sequences of myosin heavy chain, Eur. J. Biochem. 232 (1995) 235–240.
[33] O.A. Andreev, J. Borejdo, Interaction of the heavy and light chains of cardiac myosin subfragment-1 with F-actin, Circ. Res. 81 (1997) 688–693.
[34] D.F.A. Mc Killop, M.A. Geeves, Regulation of the interaction between actin and myosin subfragment 1: evidence for three states of the thin filament, Biophys. J. 65 (1993) 693–701.