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

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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

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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-