Biochemical and Biophysical Research Communications 495 (2018) 163–167

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Transient interaction between the N-terminal extension of the essential light chain-1 and motor domain of the myosin head during the ATPase cycle

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ARTICLE INFO

Article history:
Received 13 October 2017
Accepted 30 October 2017
Available online 7 November 2017

Keywords:
Myosin head
Myosin subfragment 1
Essential light chain-1
N-terminal extension
Fluorescence resonance energy transfer

ABSTRACT

The molecular mechanism of muscle contraction is based on the ATP-dependent cyclic interaction of myosin heads with actin filaments. Myosin head (myosin subfragment-1, S1) consists of two major domains, the motor domain responsible for ATP hydrolysis and actin binding, and the regulatory domain stabilized by light chains. Essential light chain-1 (LC1) is of particular interest since it comprises a unique N-terminal extension (NTE) which can bind to actin thus forming an additional actin-binding site on the myosin head and modulating its motor activity. However, it remains unknown what happens to the NTE of LC1 when the head binds ATP during ATPase cycle and dissociates from actin. We assume that in this state of the head, when it undergoes global ATP-induced conformational changes, the NTE of LC1 can interact with the motor domain. To test this hypothesis, we applied fluorescence resonance energy transfer (FRET) to measure the distances from various sites on the NTE of LC1 to S1 active site in the motor domain and changes in these distances upon formation of S1-ADP-BeF_x complex (stable analog of $S1^*$-ATP state). For this, we produced recombinant LC1 cysteine mutants, which were first fluorescently labeled with 1,5-IAEDANS (donor) at different positions in their NTE and then introduced into S1; the ADP analog (TNP-ADP) bound to the S1 active site was used as an acceptor. The results show that formation of S1-ADP-BeF_x complex significantly decreases the distances from Cys residues in the NTE of LC1 to TNP-ADP in the S1 active site; this effect was the most pronounced for Cys residues located near the LC1 N-terminus. These results support the concept of the ATP-induced transient interaction of the LC1 N-terminus with the S1 motor domain.

1. Introduction

The molecular mechanism of muscle contraction is based on the ATP-dependent cyclic interaction of myosin heads with actin filaments. An important feature of the myosin head, named subfragment-1, or S1, is the presence of two major structural domains: a globular motor (or catalytic) domain which is responsible for the ATP hydrolysis and actin binding, and a regulatory domain which is a long α-helix stabilized by two light chains [1]. According to the present concept of the myosin motor function, the regulatory domain acts as a lever arm which rotates relative to the motor domain and amplifies global conformational changes occurring in the motor domain during the ATPase cycle [2–4]. The previous works indicated that this rotation can be accompanied by an interaction between the motor domain and the C-terminal part of the essential light chain (ELC) associated with the regulatory domain [5–7].

The ELCs are highly conserved, and are expressed as two isoforms, originally named alkali 1 (A1) and alkali 2 (A2). Myosin from

Abbreviations: S1, myosin subfragment 1; ELCs, myosin essential light chains; LC1, essential light chain 1; NTE, N-terminal extension of LC1; BeF_x, beryllium fluoride; FRET, fluorescence resonance energy transfer; 1,5-IAEDANS, 5-(iodoacetamidoethyl)amino-naphtalene-1-sulfonic acid; TNP-ADP, 2′,3′*O*-Trinitrophenyl-adenosine-5′-diphosphate.

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https://doi.org/10.1016/j.bbrc.2017.10.172
0006-291X/

cardiac and slow skeletal muscles contains only A1-like ELCs, whereas in smooth muscle myosin only A2-like ELCs are present; both these ELC isoforms are simultaneously expressed only in fast skeletal muscle. The principal difference between them is that A1 (also designated LC1) contains an N-terminal extension (NTE) of 40–45 additional amino acid residues including multiple Ala-Pro repeats and two pairs of lysine residues near the N-terminus [8]. These positively charged Lys residues can interact with the C-terminal acidic residues of actin monomer [9,10] different from that to which the motor domain of the head is bound [11–13], thus forming an additional actin-binding site on the myosin head and making stronger its binding to actin. It is also proposed from structural modeling that another important part of the NTE of LC1 containing multiple Ala-Pro repeats due to its semi-rigid antenna-like structure can function as a 'bridge' between the LC1 core associated with the regulatory domain of the myosin head and the binding site of the highly charged LC1 N-terminus on an actin filament [14]. The functional importance of the NTE of LC1 in maintaining the myosin motor function during force generation was demonstrated in experiments on cardiac muscle preparations from transgenic mice expressing an ELC mutant with truncation of its N-terminal 43 amino acid residues [15].

When the myosin head binds ATP during the ATPase cycle, it undergoes global conformational changes and dissociates from actin filament; after ATP hydrolysis the head binds to another actin monomer of the filament and produces force. The question arises: what happens to the extended and rather rigid NTE of LC1 when the myosin head dissociates from actin in the relaxed phase of the cycle? Obviously, the highly charged N-terminus of LC1 should also detach from actin, and any its contacts with actin should be prevented during ATP binding and hydrolysis. In each ATPase cycle, the NTE of LC1 should detach from actin and then quickly attach back together with the motor domain of the head. Therefore it seems unlikely that the NTE is located randomly when the head is unbound. It is reasonable to assume that in this state of the head, the NTE of LC1 in the presence of ATP should be located rather close to actin filament, to be ready to interact quickly with actin together with the motor domain. Most likely that for this, the NTE of LC1 should be not far away from the motor domain or interact with it. The best way to verify this assumption could be direct observation of the NTE in the crystal structure of this ELC region of myosin head, which unfortunately does not exist yet. In the crystal structure of nucleotide-free skeletal S1 [1] this region was digested by papain [16], whereas the A2-like ELC in smooth muscle [5] and non-muscle (scallop) myosin S1 [4] do not contain the NTE.

To answer the question and to test this assumption, we applied the fluorescence resonance energy transfer (FRET) to measure the distances from various sites on the NTE of ELC (LC1 isoform) associated with the S1 regulatory domain to the nucleotide-binding site in the S1 motor domain, and to examine the changes in these distances upon formation of S1-ADP-BeF_x complex (stable analog of the S1 ATPase intermediate state $S1^*$-ATP [17,18]). For this, we exploited a series of engineered cysteines in the LC1 (in the NTE or in its C-terminal part) in order to label them with 1,5-IAEDANS (FRET donor) and then introduced these labeled mutant LC1 into S1. The trinitrophenylated ADP analog (TNP-ADP) bound in the S1 nucleotide-binding site was used as an acceptor.

2. Materials and methods

2.1. Protein preparations

The previously described DNA construct of human skeletal LC1 containing the His tag at the C-terminus and a single cysteine residue (Cys-180) near the C-terminus [7] was used to generate several mutant constructs containing single cysteine residues at different positions in the C- and N-terminal parts of the protein. First, a cysteine-free sequence was produced in which the wild-type Cys-180 was changed to Ala. Using this sequence, two constructs were obtained by mutating Ser-99 and Glu-160 in the C-terminal part of LC1 to cysteines as described earlier [7]. The residues Ser-40, Ala-15 and Val-6 in the NTE of LC1 were replaced by Cys in a similar way. The C180A cDNA was also used to produce LC1 with an N-terminal tag containing cysteine (ACGI) followed by the native LC1 sequence starting at Pro-2 instead of Ala [6,19]. Here we denote this LC1 mutant with Cys residue introduced at the N-terminus as Cys-N.

The LC1 was then overexpressed in M15(pREP4) *E. coli* cells and purified using affinity chromatography on a HisTrap HP 5 ml column (Amersham Pharmacia). The concentration of LC1 was determined spectrophotometrically using extinction coefficient $A^{1\%}$ at 280 nm of 2.0 cm^{-1}. Each isolated LC1 was labeled by incubation with a 5-fold molar excess of 1,5-IAEDANS for 4 h at 4 °C in a 10 mM Na-phosphate buffer (pH 7.0) containing 100 mM NaCl. The unreacted dye was removed by exhaustive dialysis against 50 mM imidazole-HCl buffer (pH 7.0) containing 100 mM NaCl and 5 mM DTT. The labeling efficiency was assessed spectrophotometrically using the extinction coefficients of 1,5-IAEDANS equal to 5.7 10^3 mol^{-1} cm^{-1} at 337 nm and 1.03 10^3 mol^{-1} cm^{-1} at 280 nm. Typically, more than 85% of the LC1 was labeled.

S1 was prepared by digestion of rabbit skeletal myosin filaments with TLCK-treated α-chymotrypsin (Sigma) as described earlier [7,20]. The concentration of S1 was estimated spectrophotometrically using extinction coefficient $A^{1\%}$ at 280 nm of 7.5 cm^{-1}.

2.2. Exchange of LC1 in S1

Fluorescently labeled LC1 was introduced into S1 according to exchange procedure described by Zaager and Burke [21]. The exchange was performed by incubation of S1 with an 8-fold molar excess of free LC1 in 50 mM imidazole-HCl buffer (pH 7.0) containing 5 mM DTT, 10 mM MgATP and 100 mM NaCl at 37 °C for 30 min. The reaction was stopped by cooling on ice. S1 was purified on a SP-trisacryl column to separate exchanged S1(LC1) from free LC1 and unexchanged S1 [22] and after that the S1 was purified using affinity chromatography on a HisTrap HP 1 ml column (Amersham Pharmacia) to obtain a preparation with completely exchanged LC1.

2.3. Preparation of stable ternary S1 complexes with TNP-ADP and BeF_x

Trapping of TNP-ADP by phosphate analog BeF_x was performed by the same method as was described for stable ternary complexes S1-ADP-BeF_x [17]. S1 was incubated with 10 μM Mg-TNP-ADP and 5 mM NaF for 10 min at 25 °C prior to addition of $BeSO_4$ to a final concentration of 0.5 mM. Then the solution was incubated overnight on ice. Formation of the complexes was controlled by measuring the NH_4^+-EDTA-ATPase activity of S1. The ATPase activity of S1 in the S1–TNP-ADP–BeF_x complexes did not exceed 5–7% of the activity measured in the absence of the P_i analog.

2.4. FRET measurements

Fluorescence studies were performed on a Cary Eclipse spectrofluorimeter (Varian). All measurements were performed at 25 °C in a 50 mM Tris-HCl buffer (pH 7.5) containing 2 mM $MgCl_2$ at a protein concentration of 0.025 mg/ml [23]. Emission spectra of the donor (AEDANS) attached to LC1 from 360 nm to 630 nm (slit width 2.5 nm) were recorded at an excitation wavelength of 337 nm (slit width 5 nm).