ELSEVIER

Biophysical Chemistry 106 (2003) 57–66

Biophysical Chemistry

www.elsevier.com/locate/bpc

The functional domains of human ventricular myosin light chain 1

Baotong Xie, Renjian Huang, Li Huang, Guoying Zhou, Zuxun Gong*

Key Laboratory of Proteomics, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yue-Yang Road, Shanghai 200031, PR China

Received 5 March 2003; received in revised form 11 June 2003; accepted 11 June 2003

Abstract

The biological functions of the myosin light chain 1 (LC1) have not been clearly elucidated yet. In this work we cloned and expressed N- and C- terminal fragments of human ventricular LC1 (HVLC1) containing amino acid residues 1–98 and 99–195 and two parts, NN and NC of N fragment in GST-fusion forms, respectively. Using GST pull-down assay, the direct binding experiments of LC1 with rat cardiac *G*-actin, *F*-actin and thin filaments, as well as rat cardiac myosin heavy chain (RCMHC) have been performed. Furthermore, the recombinant complexes of rat myosin S1 with N- and C-fragments, as well as the whole molecular of HVLC1 were generated. The results suggested that both binding sites of HVLC1 for actin and myosin heavy chain are positioned in its N-terminal fragment, which may contain several actin-binding sites in tandem. The polymerization of *G*-actin, the tropomyosin and troponin molecules located in the thin filaments do not hinder the binding of N-terminal fragment of HVLC1 with actin and thin filaments in vitro. The recombinant complex of rat cardiac myosin S1 (RCMS1) with N fragment of HVLC1 greatly decreased actin-actived Mg^{2+}-ATPase activity for lack of C fragment. We conclude that the N-fragment is the binding domain of human ventricular LC1, whereas the C-fragment serves as a functional domain, which may be more involved in the modulation of the actin-activated ATPase activity of myosin.

Keywords: Actin; ATPase activity; Binding; Human ventricular myosin light chain 1; Rat myosin S1

1. Introduction

The motor molecule in skeletal muscle, myosin, is composed of two heavy chains (MHC) and three types of light chains with different molecular mass of 21, 19 and 16.5 kDa. These light chains are named alkali-1 (LC1) or essential light chain, DTNB or regulatory (LC2) light chain and alkali-2 (LC3) light chain, respectively. The two cardiac light chains, CLC1 and CLC2, are thought to be similar to LC1 and LC2. The MHC has two domains, a rod-shaped tail involved in filament assembly and a globular head containing the sites of both ATP and actin binding, as well as the light chain binding sites [1–4]. The site of light chain binding in MHC has been located at the head–rod junction [5–7]. The light chains bound in tandem with LC1 more proximal to the head [8]. In skeletal muscle, LC2 may be removed by treatment with DTNB (5,5′-dithiobis-12-nitrobenzoic acid) without significantly affecting ATPase and actin

*Corresponding author. Tel.: +86-21-54921220; fax: +86-21-54921125.

E-mail address: gongzx@sunm.shcnc.ac.cn (Z. Gong).

0301-4622/03/$ - see front matter © 2003 Elsevier Science B.V. All rights reserved.
doi:10.1016/S0301-4622(03)00172-8

binding activities [9,10]. In smooth muscle and non-muscle systems, phosphorylation and dephosphorylation of LC2 appear to control the actin-activated ATPase activity [2,11]. Mutagenesis of LC2 of *Drosophila* cytoplasmic myosin, spaghetti-squash, results in defect in cytokinesis and is lethal during the larval stage of development [12].

The role of LC1 in myosin function is less clear and the reports appear to be rather controversial. An apparent function of LC1 is to wrap around, and thereby stabilize, a α-helix, which extends from the C-terminus of the myosin head. It is thus believed to play a structural role in the myosin neck region [13]. Early studies of skeletal muscle myosin showed that LC1 could be removed by alkali treatment [14,15], leading to the loss of actin binding and the ATPase activities of myosin [9,16]. However, later experiments showed that skeletal myosin stripped of the LC1 was not affected in the actin-activated ATPase activity under mild and non-denaturing conditions of MHC [17].

On the other hand, in the non-muscle system, myosin isolated from LC1-deficient *Dictyostelium* cell lines does not exhibit significant actin-activated ATPase activity [18,19]. It was confirmed by experiments of mutations in LC1 of *Dictyostelium* myosin leading to reduced actin-activated ATPase activities [20].

With regard to actin binding of LC1, the evidence presented so far is also somewhat confusing. Experiments using papain digested S1, of which LC1 was found to lose the basic 13 N-terminal amino acid residues and those using recombinant LC1 lacking the first 45 or 11 amino acid residues, indicated that the N-terminal region and most likely the N-terminal 11-residue segment, binds to the actin molecule and affects the mechanism of actin-activated ATPase [21,22]. In contrast, studies using a monoclonal antibody against the N-terminus of LC1 suggested that the N-terminal region of LC1 is not essential for actin binding, although it is involved in modulating actin-activated ATPase activity [23].

In the present study, purified N-terminal recombinant fragment containing the amino acid residues 1–98 and the C-terminal fragment containing the residues 99–195 of human ventricular LC1 (HVLC1) were used for the GST pull-down assays with rat cardiac *G*-actin, *F*-actin and thin filaments, as well as with rat cardiac myosin heavy chain (RCMHC) using Glutathione Sepharose 4B beads. Furthermore, we used these two fragments of HVLC1 for recombination experiments with the myosin S1 generated by chymotrypsin digestion. Our results suggested that both binding sites of HVLC1 for actin and RCMHC are positioned in its N-terminal region and the N-fragment of HVLC1 may contain several actin-binding sites in tandem. Our experiments also showed that when the N-fragment was recombined with rat cardiac myosin S1 (RCMS1) that lacks LC1 (RCLC1), the resulting complex exhibited only slightly effected Ca^{2+}-ATPase and $EDTA^{+}$-ATPase activities, but a greatly decreased actin-activated Mg^{2+}-ATPase activity. We, therefore, conclude that the N-fragment is the binding domain of human cardiac LC1, whereas the C-fragment serves as a functional domain, which may be more involved in the modulation of the actin-activited ATPase activity of S1.

2. Experimental

2.1. Reagents

The enzymes pepsin and chymotrypsin were purchased from Sigma (St. Louis, USA). The pGEX-3X expression vector was purchased from Promega (Madison, USA). The DEAE-Cellulose was from Whatman (Maidstone, UK). Glutathione Sepharose 4B was purchased from Am-Pharmacia (Uppsala, Sweden).

2.2. Isolation of rat cardiac myosin heavy chain and myosin S1

Isolation and purification of rat cardiac myosin and rat cardiac myosin heavy chain were performed according to the method of Ebashi [24]. The S1 preparation of rat cardiac myosin (RCMS1) was performed according to the procedures described by Wagner and Weeds [10].

2.3. Purification of rat cardiac actin and thin filaments

Rat cardiac actin was purified according to Spudich and Watt [25]. The polymerization of *G*-

actin into *F*-actin was induced by resolving the actin in high salt buffer with ATP (5 mM imidazole, 50 mM KCl, 2 mM $MgCl_2$, 0.4 mM ATP; 0.2 mM $CaCl_2$, pH 7.0) instead of PBS. Rat cardiac thin filaments were isolated from rat heart muscle according to the protocol of Martin [26]. The concentration of rat thin filament preparation was determined with BCA Protein Assay Kit (Pierce).

2.4. Expression and purification of human cardiac myosin light chain 1 (HVLC1) and its N and C fragments

Expression and purification of human cardiac myosin light chain 1 (HVLC1) were described previously by Huang et al. [27]. For constructing glutathione *S*-transferase (GST) fusion vectors of N and C fragments, the HVLC1 gene obtained in our lab and reported in previous paper [27] was used as the template. Two primers designed from the 294 bp of the coding region of the HVLC1 gene in opposite directions were synthesized. The two pairs of primers used for the expression of the N-fragment and the C-fragment of HVLC1 are as follows:

N-fragment:

(+) 5′-CGGGATCCCGATGGCCCCAAAAAGCC -3′

(−) 5′-CCGGAATTCGGCTTCCCCAGGACACGGAGC -3′

C-fragment

(+) 5′-CCGGGATCCCACCAAGACAGGAAGAGCTC -3′

(−) 5′-GGAATTCCTTAGCTGGACATGATGT -3′

The sequences underlined represent the *BamH* I and *EcoR* I restriction sites, respectively. The PCR products were digested by *BamH* I and *EcoR* I and then inserted into pGEX-3X vectors. Both vectors pGEX-3X-N and pGEX-3X-C were sequenced by the dideoxy chain termination method [28]. Both purification and factor Xa digestion of the expressed products were described previously [27].

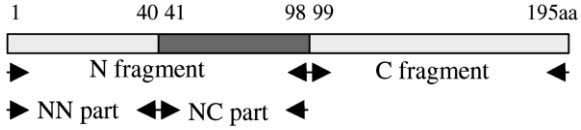

Fig. 1. Schematic diagram showing N fragment, C fragment, NN part and NC part of HVLC1 involved in the article.

2.5. Expressions of NN and NC parts of the N-fragment of HVLC1

The HVLC1 gene was again used as the template. Two primers used for the expression of NN and NC parts of N fragment of HVLC1 were designed from the coding region of HVLC1 gene in opposite directions (Fig. 1) as follows:

NN part:

(+) 5′-CGGGATCCCGATGGCCCCAAAAAGCCAG-3′

(−) 5′-CGCGGAATTCCTTGGAAGCATCAAACTCGA-3′

NC part:

(+) 5′-CGCGGGATCCCGATCAAGATTGAGTTCACACCT-3′

(−) 5′-CCGGAATTCGGCTTCCCCAGGACACGGAGC-3′

The procedures of inserting the PCR products into the expression vector pGEX-3X, expression and purification of NN and NC parts were similar as described above.

2.6. Bindings of N- or C-fragment of HVLC1 to rat cardiac myosin heavy chain (RCMHC)

The GST fusion fragment binds to the substrate glutathione linked to Sepharose 4B beads (G-beads). G-beads were first equilibrated with PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.4) overnight and then washed with the same buffer. It was then centrifuged at $500\times g$ for 30 s. The pellets were collected and the washing was repeated for five times. An equal volume of PBS was added to each pellet. 20 μg of N- or C-fragment of HVLC1 dissolved in 0.5 ml of PBS-T was mixed separately with 50 μl of G-beads. The mixture was incubated at 4 °C

with end-over-end mixing overnight. After being washed with PBS-T for five times, 20 μg of RCMHC dissolved in 0.5 ml of PBS-T was added to the G-beads pellets and mixed gently. The mixture was incubated at 4 °C with end-over-end mixing overnight and washed again for five times. After high speed centrifugation, an equal volume of SDS-PAGE sample buffer was added to each pellet. RCMHC was added to G-beads without prebinding of N- or C-fragments and the preparation was used as controls. The resulting mixtures were examined by SDS-PAGE.

2.7. Bindings of N and C fragments of HVLC1 with G-actin, F-actin and thin filament and bindings of NN and NC parts of N fragment to G-actin

The procedures of bindings of the NN and NC parts of the N-fragment, as well as the N- and C-fragments of HVLC1 to *G*-actin were exactly same as described for RCMHC. In the case of bindings of GST fusion proteins with *F*-actin and rat cardiac thin filaments, the high salt binding buffer (5 mM imidazole, 50 mM KCl, 2 mM $MgCl_2$, 0.4 mM ATP; 0.2 mM $CaCl_2$, pH 7.0, 0.1% Triton X-100), instead of PBS−T buffer for bindings with G-actin and RCMHC, were used.

2.8. Recombination and hybridization of HVLC1, N- and C-fragments with RCMS1

Recombination and hybridization experiments of HVLC1 and its N- and C-fragments with RCMS1 heavy chain were performed using the method described by Wagner and Weeds [10].

2.9. Enzyme assays

The Mg^{2+}, Ca^{2+}, $EDTA^{+}$ ATPase activities of RCMS1 and recombined RCMS1 preparations were determined by the method of Pollard and Korn [29]. The second peaks (peak B) of elution profiles of RCMS1 recombined with expressed HVLC1 and its N-fragments were concentrated and used for ATPase activity assay. In the case of C-fragment the peak A and peak B, which were eluted at low and high salt concentration, respectively, were used for ATPase activity assay.

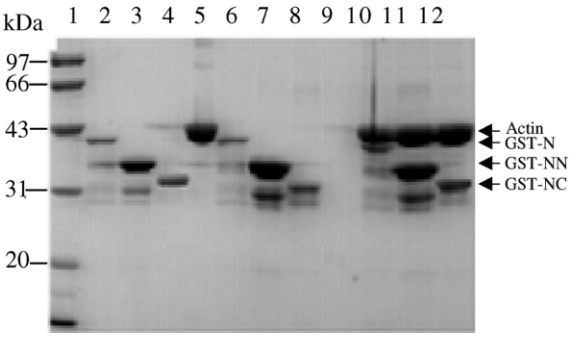

Fig. 2. SDS-PAGE of bindings of N fragment, NN and NC parts of N fragment of HVLC1 with actin. (1) Molecular mass markers, (2) Purified GST-N fragment, (3) Purified GST-NN part, (4) Purified GST-NC part, (5) Actin, (6) GST-N fragment bound with G-beads, (7) GST-NN part bound with G-bead, (8) GST-NC part bound with G-beads, (9) Actin with G-beads, (10) Binding of actin with GST-N fragment bound with G-beads, (11) Binding of actin with GST-NN part bound with G-beads, (12) Binding of actin with GST-NC part bound with G-beads.

The ATPase activity unit represents Pi nmol/mg (myosin)·min.

3. Results

3.1. Expression of N- and C-fragments, as well as the NN and NC parts of N-fragment of HVLC1

The schematic diagram demonstrating the N and C fragments, NN and NC parts of HVLC1 involved in this study is shown in Fig. 1.

Using 588 bp ORF sequence of cDNA of HVLC1 gene as the template, two 294 bp cDNA fragments expressing the N- and C-fragments of HVLC1 were obtained by PCR. After being inserted into pGEX-3X vectors and sequenced, the 5′ and 3′ fragments of the HVLC1 gene were expressed in *E. coli* DE-3 and purified. SDS-PAGE showed that the molecular mass of expressed fusion product of the N-fragment is ∼39 kDa, whereas the molecular mass of the C-fragment is only ∼33 kDa. According to the deduced amino acid sequence the molecular masses of both fragments containing 26 kDa of the GST fusion part should be approximately 37 kDa (Figs. 2 and 3)

Similarly, the NN and NC parts of the N-fragment of HVLC1 were expressed and purified

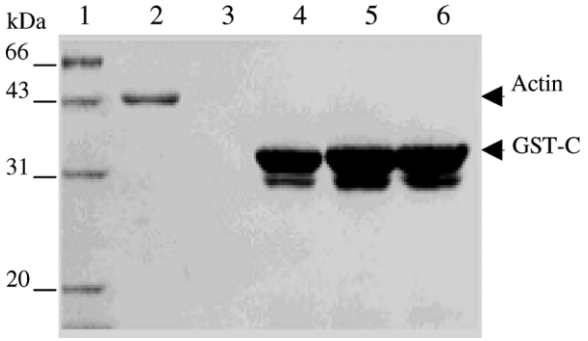

Fig. 3. SDS-PAGE of binding of C fragment of HVLC1 with actin. (1) Molecular mass markers, (2) Actin, (3) Actin with G-beads, (4) Purified GST-C fragment, (5) GST-C fragment bound with G-beads, (6) Binding of actin with GST-C fragment bound with G-beads.

in the fusion form with GST. The molecular mass of the NN part of the N-fragment of HVLC1 is approximately 36 kDa on SDS-PAGE and is higher than that of the NC part with a molecular mass approximately 31 kDa, although the NC part has 16 amino acid residues more than the NN part (Fig. 2).

3.2. Bindings of the NN and NC Parts of the N-fragment, as well as the N- and C-fragments of HVLC1 to RCMHC and G-actin

The results of binding experiments showed that only the N-fragment of HVLC1 binds RCMHC and *G*-actin. The C-fragment is unable to bind either RCMHC or *G*-actin (Figs. 2–4), but both the NN and NC parts of HVLC1 could bind *G*-actin (Fig. 2). In the case when *G*-actin was added first and MHC was added later, the amount of bound RCMHC increased apparently (Fig. 5).

3.3. Bindings of N-and C-fragments of HVLC1 to F-actin and rat cardiac thin filaments

The polymerization of *G*-actin into *F*-actin could be induced by high salt (50 mM KCl, 2 mM $MgCl_2$) and ATP. SDS-PAGE of purified rat cardiac thin filaments reveals three major bands at 43 kDa (actin), 37 kDa (tropomyosin), 31 kDa (troponin I) and a weak band of 41 kDa (troponin T),

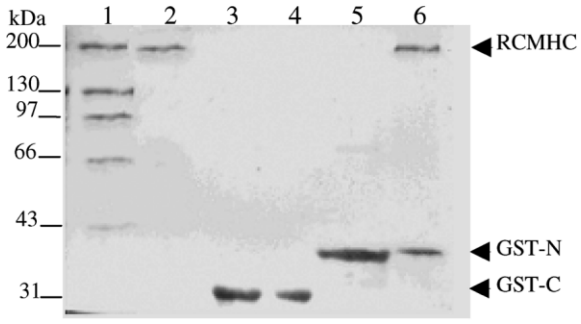

Fig. 4. SDS-PAGE of bindings of N and C fragments of HVLC1 with rat cardiac myosin heavy chain (RCMHC). (1) Molecular mass markers, (2) RCMHC, (3) GST-C fragment with G-beads, (4) Binding of RCMHC with GST-C fragment bound with G-beads, (5) GST-N fragment bound with G-beads, (6) Binding of RCMHC with GST-N fragment bound with G-beads.

as already shown by Matin S et al. [26] (Fig. 6). Only N-fragment, not C-fragment, of HVLC1 could bind to both of *F*-actin and native thin filaments (Fig. 6).

3.4. Recombination of S1 of rat cardiac myosin (RCMS1) with the N- and C-fragments of HVLC1

After dialysis against 50 mM imidazole buffer that contains 0.1 mM DTT to remove NH_4Cl, the

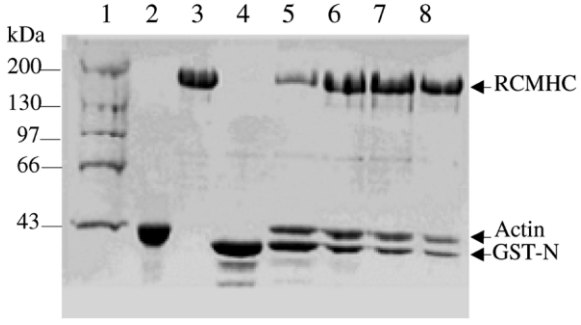

Fig. 5. SDS-PAGE of bindings of N fragment of HVLC1 with actin and rat cardiac myosin heavy chain (RCMHC). (1) Molecular mass markers, (2) Actin, (3) RCMHC, (4) GST-N fragment, (5) Bindings of GST-N fragment with RCMHC then with actin, (6) Bindings of GST-N fragment with actin then with RCMHC, (7) Bindings of GST-N fragment with RCMHC and subsequently with actin and with RCMHC, (8) Bindings of GST-N fragment with actin and subsequently with RCMHC and with actin.

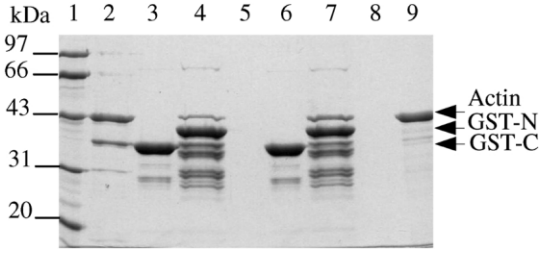

Fig. 6. SDS-PAGE of bindings of N-and C-fragments of HVLC1 to *F*-actin and rat cardiac thin filaments. (1) Molecular mass markers, (2) purified thin filament, (3) Binding of GST-C fragment with thin filament, (4) Binding of GST-N fragment with thin filament, (5) Binding of G-beads with thin filament, (6) Binding of GST-C fragment with *F*-actin, (7) Binding of GST-N fragment with *F*-actin, (8) Binding of G-beads with *F*-actin, (9) *F*-actin.

mixtures of S1 and S1 recombined with intact HVLC1, the N- and C-fragments of HVLC1 were applied to a DEAE-cellulose column (Whatman DE-52, 40×1.5 cm). The mixture of S1 and S1 plus HVLC1 was fractionated as shown in Fig. 7a.

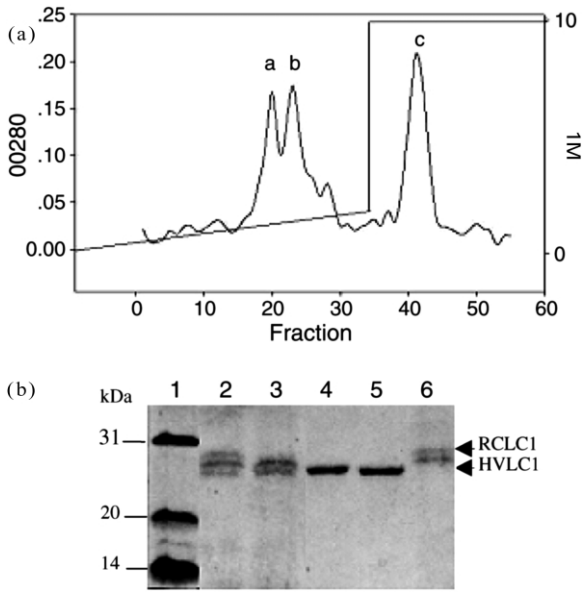

Fig. 7. (a) Elution profile of RCMS1 recombined with HVLC1 from DEAE-cellulose 4B (DE52) column. (b) SDS-PAGE of collected fractions of RCMS1HC+HVLC1 from column. (a) Molecular mass markers, (b) Peak A, (c) Peak B, (d) Peak C, (e) HVLC1, (f) RCMS1.

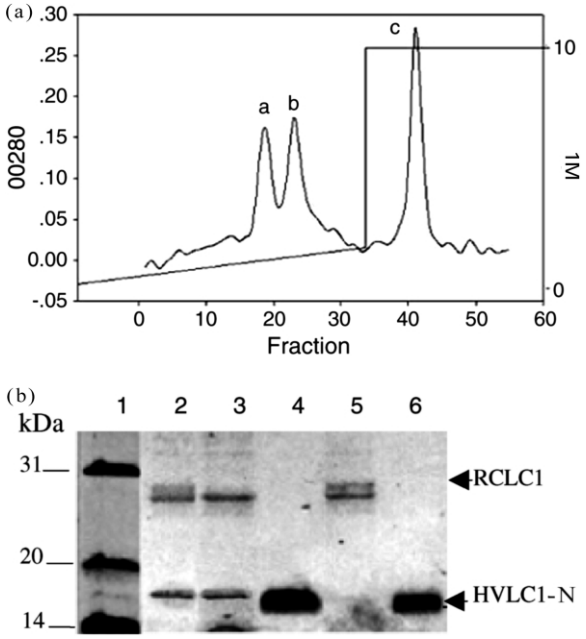

Fig. 8. (a) Elution profile of RCMS1 recombined with HVLC1 N from DEAE-cellulose 4B (DE52) column. (b) SDS-PAGE of collected fractions of RCMS1HC+HVLC1 N from column. (a) Molecular mass markers, (b) Peak A, (c) Peak B, (d). Peak C, (e) RCMS1, (f) HVLC1 N.

Two peaks (A and B) were obtained in low salt elution and one peak (C) was obtained when the column was washed with 1 M NaCl. The elution profile from DEAE-cellulose column is exactly same as the profile showed by Wagner and Weeds [10] and the results are accordingly repeated. SDS-PAGE of these peaks showed that peak A contained mixture of both of rat cardiac myosin S1 and added HVLC1. The second peak (B) contained the recombined S1 generated by recombination of S1 heavy chain (S1HC) with added HVLC1 and S1 stripped of the rat cardiac LC1 (RCLC1) which is bound originally with S1 heavy chain. Most of the added HVLC1 remained in peak C (Fig. 7b). When using the N-fragment of HVLC1 instead of the intact molecule, a similar elution profile from DEAE-cellulose column was observed (Fig. 8a). Peak A contained mixture of S1 preparation and added N fragment, but the S1 eluted in the peak B was generated by recombination of S1HC with added N-fragment (Fig. 8b).

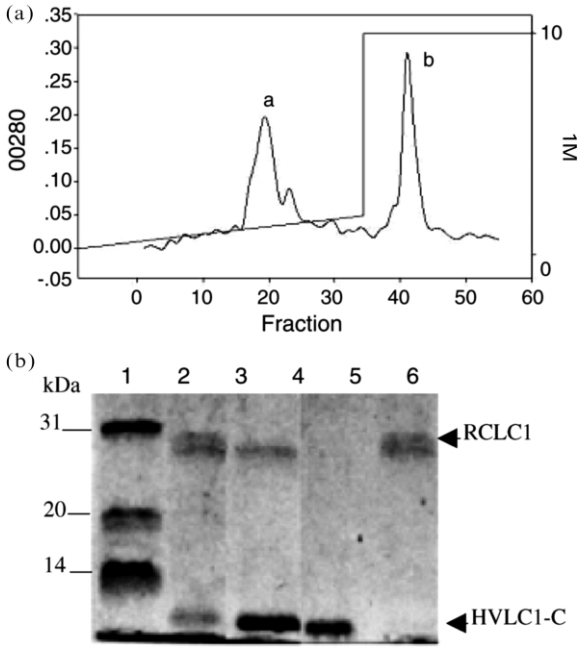

Fig. 9. (a) Elution profile of RCMS1 recombined with HVLC1C from DEAE-cellulose 4B (DE52) column. (b) SDS-PAGE of collected fractions of RCMS1HC+HVLC1C from column. (a) Molecular mass markers, (b) Peak A, (c) Peak B, (d) HVLC1C, (e) RCMS1.

However, when C-fragment was used in the recombination experiment, the elution profile showed that there was only one peak (peak A) and the second peak almost disappeared (Fig. 9a). Peak A contained the mixture of S1 and of a trace of the added C-fragment, but most of the C-fragment and free S1HC were washed by high salt buffer (peak B) (Fig. 9b). There was no recombination of S1 with the added C-fragment. As a control, the recombined S1 with dissociated RCLC1 was obtained by this method too.

3.5. ATPase activities of recombined rat cardiac myosin S1 (RCMS1)

The Mg^{2+}, Ca^{2+}, $EDTA^{+}$ ATPase activities of rat cardiac myosin S1 formed by recombination with expressed HVLC1 and its N- and C-fragments were measured in the presence of actin and compared with that of corresponding measurements made on positive controls of intact rat cardiac myosin S1 and S1 recombination with dissociated RCLC1. As shown in Table 1, the actin-activated ATPase activities of myosin S1 recombined with dissociated RCLC1 and expressed HVLC1 were a little lower, but still comparable to that of controls with the intact S1 preparation. When the myosin S1 lacking LC1 was recombined with added N-fragment of HVLC1, there was no effect on Ca^{2+} ATPase and $EDTA^{+}$ ATPase activities, but the actin activited Mg^{2+} ATPase activity was drastically decreased. Not surprisingly, the mixture of S1 and the C fragment of HVLC1 showed comparable values of ATPase activities with positive controls, but the mixture of S1HC and C-fragment demonstrated no ATPase activity, as in the negative control-S1HC alone.

4. Discussion

It has been reported that the molecular mass of HVLC1 isolated from human cardiac muscle is

Table 1
Mg^{2+}ATPase, Ca^{2+}ATPase and K^{+} ($EDTA^{+}$)ATPase activities of recombined RCMS1 HC with HVLC1, HVLC1 N, HVLC1C and with RCLC1

	Mg^{2+} ATPase	Ca^{2+} ATPase	K^{+} ($EDTA^{+}$) ATPase
Positive control: RCMS1	32.90	83.04	50.33
Recombined RCMS1HC–HVLC1	28.39	74.19	42.58
Recombined RCMS1HC–RCLC1	26.45	73.55	45.16
Recombined RCMS1HC–HVLC1N	11.61	72.39	45.81
Mixture RCMS1 and HVLC1C (low salt)	25.81	71.61	44.90
Mixture RCMS1 and HVLC1C (high salt)	1.94	−1.68	1.81
Negative control: RCMS1HC	1.55	−1.42	0.64
Blank (without RCMS1)	0	0	0

Unit: Pi nmol/mg (myosin) min.

estimated to be 25 m~27 kDa on SDS-PAGE. It is higher than the calculated molecular mass (22 kDa) based on the HVLC1 amino acid sequence [30]. It was inferred that abnormality in HVLC1 mobility on SDS-PAGE might be caused by the existence of a proline and phenylalanine hydrophobic domain as well as by the presence of many lysine residues at the N-terminus. The mobility of the NN and NC parts on SDS-PAGE showed in the present study clearly provided evidence that the abnormality existed in the stretch of 1–41 amino acid residues in the NN part of HVLC1.

Using GST pull-down assays we found that only the N-fragment of HVLC1 could bind the rat cardiac myosin heavy chain and the rat cardiac actin. In the present study we have used the rat cardiac myosin heavy chain and actin instead of the human cardiac myosin and actin, because the experimental materials from human source are difficult to obtain. On the basis of sequence comparison it is clear that the rat ventricular essential LC is most close to the human one in a proposed evolutionary tree of myosin essential light chains (ELC) [31]. With regard to the actin-binding site there are some confusion in literatures. Most of evidence suggested that the N-terminal region of myosin ELC binds to the actin molecule [21]. The studies of human atrial ELC showed that the wild-type ELC could be cross-linked to *F*-actin, whereas the truncated mutant lacking the first 11 amino acid linked poorly with actin under the same conditions [22]. However, in the present studies the NC part (residue 42 to 98) lacking the first 41 amino acid residues alone is clearly able to bind actin. This result could interpret the finding, which suggested that the N-terminal region of LC1 is not essential for actin binding based on the experiments of blocking N-terminal region of LC1 with monoclonal antibody (2H2) raised directly against this region. Thus, the N-fragment of HVLC1 may contain several actin-binding sites. The prebinding with actin failed to decrease the binding of MHC to N-fragment implies that the N-fragment of HVLC1 has different binding sites specific for actin and MHC. The increased amount of bound MHC in the case when actin was added first and MHC was added later could be explained by that MHC not only binds the N-fragment of HVLC1, but also binds actin, which already is linked, to the N-fragment.

We also showed in present study that only N-fragment, not C-fragment of HVLC1 could bind to *F*-actin and native cardiac thin filaments. The results mean that the polymerization of *G*-actin into *F*-actin and the tropomyosin and troponin molecules located in the thin filaments do not hinder the bindings of HVLC1 to *F*-actin and native thin filament or block the binding sites of actin for HVLC1. The physiological significance of the binding of HVLC1 with actin or thin filaments is not clear now, because upon the present knowledge the HVLC1 does not directly contact with thin filaments during muscle contraction.

The interaction between myosin heavy chain and LC1 is well known from the crystal structural models of different myosin S1 isoforms. The model of myosin S1 from chicken skeletal muscle was first proposed by Rayment et al. in 1993 [8]. In this model the authors indicated that the essential light chain (LC1) interacts with the long α helix of the heavy chain by that the second (B) and third (C) helix in the N-terminal domain of LCI abut the heavy chain with their external surfaces. The hydrophobicity analysis of the amino acid sequence of HVLC1 shows that the most hydrophobic region in the HVLC1 molecule is in the fragment from residue 41 to residue 98 (NC fragment). Therefore, the hydrophobic interaction between the α-helix of MHC and the fragment ranging from residues 41 to 98 may be one of the major factors for the interaction between the myosin heavy chain and light chains. Not surprisingly, light chain 3, the isoform of the skeletal myosin ELC, which lacks the first approximately 40 amino acid residues, naturally binds to the heavy chain without difficulty. The authors did not describe the contacts between C-terminal domain of LC1 and the heavy chain in details. The results in current paper demonstrated that the C-fragment of LC1 was unable to bind heavy chain in vitro when GST pull-down assays were used, but the crystal structure of a vertebrate smooth muscle myosin motor domain S1 [32] demonstrated the contacting interface between the C-terminal lobe of LC1 and the motor domain despite the overall arrangement of

the LC1 and the heavy chain helix to which it is bound is quite similar in both structures of the smooth muscle and skeletal muscle S1. The explanation may be that the interactions between C-fragment and heavy chain are weaker so they are unable to overcome the entropic barrier of a partially unfolded isolated fragment. A more likely explanation for this result is that the LC1 used in present paper and used in three-dimensional structure studies were from different myosin isoforms. In the structure of smooth S1 residues Asp96 to Ile138 of LC1 (which includes the *F* and *G* helixes) are in contact with the loop segments from the motor domain. In particular, as authors indicated, the *G* helixes of LC1 which has a number of negative charged residues (Glu121, Glu122, Glu123, Glu125) is in contact with the 25/50 kDa loop of the motor domain that is mainly positive charged. If we compare the amino acid sequences of human cardiac LC1 (used in current paper) and of smooth muscle (used in crystal structure), these two sequences totally differ. For example, the comparison of the sequences between *G* helix of smooth LC1 and human cardiac LC1 from residues 121 to 138 is showed as following:

121 EEEVEQLVAG HEDSNGCI 138 (smooth)
121 ISKNKDTGTY EDFVEGLR 138 (cardiac)

The loop of motor domains is also one of the most variable regions in the myosin superfamily. Thus, the difference in their sequences could influence the contacts and interactions of C fragments of LC1 to heavy chain in different muscle systems. Further studies on the binding of the NN, NC parts and the fragment (41–195 aa) of HVLC1 with the heavy chain were performed in our lab and the results will be reported elsewhere.

The SDS-PAGE date and the actin-activated ATPase activities of the recombined S1 with HVLC1 confirmed that the method of dissociation and recombination, established by Wagner and Weeds, works well. But the recombination of S1 heavy chain and LC1 from different sources may affect the hybridization efficiency and the ATPase activity values. We had success in reassociation or recombination of the S1 generated by chymotrypsin digestion of rat cardiac myosin with the N-fragment of HVLC1, but recombined rat cardiac myosin S1 with the C-fragment could not be obtained by the same procedure. We report that the recombined rat cardiac myosin S1 (RCMS1) with the N-fragment of HVLC1 slightly affected the Ca^{2+} and $EDTA^{+}$-ATP activities, but without the C-fragment it results in significant changes of actin activated Mg^{2+}-ATP activity. We inferred that the N-fragment is the binding domain of human cardiac LC1, whereas its C-fragment near the nucleotide binding site of the heavy chain revealed in the crystal structure is a functional domain, which may be more involved in the modulation of actin-activited ATPase activity of myosin.

Acknowledgments

We wish to thank Prof. S. Ebashi in Okazaki, Japan for his encouragement and Dr Albert L. Wang in Boston Biomedical Research Institute, USA for his comments and revising the English version of the manuscript.

References

[1] H.E. Huxley, Electron microscope studies on the structure of natural and synthetic protein filaments from striated muscle, J. Mol. Biol. 7 (1963) 281–308.
[2] R.S Adelstein, E. Eisenberg, Regulation and kinetics of the actin–myosin–ATP interaction, Annu. Rev. Biochem. 49 (1980) 921–956.
[3] A. Delozanne, J.A. Spudich, Disruption of the *Dictyostelium* myosin heavy chain gene by homologus recombination, Science 236 (1987) 1086–1091.
[4] P. Vibert, C. Cohen, Domains, motions and regulation in the myosin head, J. Muscle Res. Cell Motil. 9 (1988) 296–305.
[5] T. Katoh, S. Lowey, Mapping myosin light chains by immnoelectron microscopy, use of anti-fluorescyl antibodies as structural probes, J. Cell. Biol. 109 (1989) 1549–1560.
[6] E.J. Mitchell, R. Jakes, J. Kendrick-Jones, Localization of the light chain and actin binding sites on myosin, Eur. J. Biochem. 161 (1986) 25–35.
[7] E.J. Mitchell, J. Karn, D.M. Browm, R. Jakes, J. Kendrick-Jones, Regulatory and essential light chain binding sites in myosin heavy chain subfragment 1 mapped by site-directed mutagenesis, J. Mol. Biol. 208 (1989) 199–205.
[8] I. Rayment, W.R. Rypniewski, K. Schmidt-Base, R. Smith, D.R. Tomchick, M.M. Benning, et al., Three-

dimensional structure of myosin subfragment-1: a molecular motor, Science 261 (1993) 50–58.
[9] A.G. Weeds, S. Lowey, Structure of the myosin molecule II. The light chains of myosin, J. Mol. Biol. 61 (1971) 701–725.
[10] P.D. Wagner, A.G. Weeds, Studies on the role of myosin alkali light chains, J. Mol. Biol. 109 (1977) 455–472.
[11] K.M. Trybus, G.S. Waller, T.A Chatman, Coupling of ATPase activity and motility in smooth muscle myosin is mediated by the regulatory light chain, J. Cell Biol. 113 (1994) 963–969.
[12] R.E. Karess, X.J. Chang, K.A. Edwards, S. Kulkarni, I. Aguilera, D.P. Kiehart, The regulatory light chain of nonmuscle myosin is encoded by spaghetti-squash, a gene required for cytokinesis in *Drosophila*, Cell 65 (1991) 1177–1189.
[13] X. Xie, D.H. Harrison, I. Schlichting, R.M. Sweet, V.N. Kalabokis, Structure of the regulatory domain of scallop myosin at 2.8 Å resolution, Nature 368 (1994) 306–312.
[14] A.G. Weeds, Small subunits of myosin, Biochem, J. 105 (1967) 25c–27c.
[15] L.C. Gershman, A. Stracher, P. Dreizen, Subunit structure of myosin, J. Mol. Biol. 44 (1969) 2726–2763.
[16] L.C. Gershman, P. Dreizen, Relationship of structure to function in myosin 1 subunit dissociation in concentrated salt solutions, Biochemistry 9 (1970) 1677–1687.
[17] P.D. Wagner, E. Giniger, Hydrolysis of ATP and reversible binding to *F*-actin by myosin heavy chains free of all light chains, Nature 292 (1981) 560–562.
[18] R.S. Pollenz, T-L. Chen, L. Trivinos-Lagos, R.L. Chisholm, The dictyostelium essential light chain is required for myosin function, Cell 69 (1992) 951–962.
[19] T.-L. Chen, P.A. Kowalczyk, G. Ho, R.L. Chisholm, Targeted disruption of the *Dictyostelium* myosin essential light chain gene produces cells defective in cytokinesis and morphogenesis, J. Cell Sci. 108 (1995) 3207–3218.
[20] G. Ho, R.L. Chisholm, Substitution mutations in the myosin essential light chain lead to reduced acting-activated ATPase activity despite stoichiometric binding to the heavy chain, J. Biol. Chem. 272 (1997) 4522–4527.
[21] T. Hayashibara, T. Miyanishi, Binding of the amino-terminal region of myosin alkali 1 chain to actin and its effect on actin–myosin interaction, Biochemistry 33 (1994) 12 821–12.
[22] D.J. Timson, H.R. Trayer, I.P. Trayer, The N-terminus of A1-type myosin essential light chains binds actin and modulates myosin motor function, Eur. J. Biochem. 255 (1998) 654–662.
[23] W. Boey, A.W. Everett, J. Sleep, J. Kendrick-Jones, C.G. Remedios, Uncoupling of actin-activated myosin ATPase activity from actin binding by a monoclonal antibody directed against the N-terminus of myosin light chain 1, Biochemistry 31 (1992) 4090–4095.
[24] S. Ebashi, Calcium binding activity of vesicular relaxing factor, J. Biochem. 50 (1961) 236–244.
[25] J.A. Spudich, S. Watt, The regulation of rabbit skeletal muscle contraction, J. Biol. Chem. 246 (1971) 4866–4871.
[26] S. Martin, O.S. Michel, M. Anna, W. Bettina, A. Ueli, A Dan, Isolation, electron microscopic imaging and 3-D visualization of native cardiac thin myofilaments, J. Structural Biol. 126 (1999) 98–104.
[27] R.J. Huang, G.Y. Zhou, J.Y. Shen, Z.X. Gong, The sequence analysis and expression of cDNA of human cardiac myosin light chain 1 and the preparation of monoclonal antibody to the expressed product, Acta Biochim. Biophys. Sin. 3 (2000) 158–162.
[28] F. Sanger, S. Nicklen, A.R. Coulson, DNA Sequencing with chain terminating inhibitors, Proc. Natl. Acad. Sci. 74 (1977) 5463–5467.
[29] T.D. Pollard, E.D. Korn, *Acanthamoebia* myosin I. Isolation from *Acanthamoebia Castellanii* of an enzyme similar to muscle myosin, J. Biol. Chem. 248 (1973) 4682–4690.
[30] M. Kurabayashi, I. Komuro, H. Tsuchimochi, F. Takaku, Y. Yazaki, Molecular cloning and characterization of human atrial and ventricular myosin alkali light chain cDNA clones, J. Biol. Chem. 263 (1998) 13930–13936.
[31] J.H. Collins, Myosin light chains and troponin C: structural and evolutionary relationships revealed by amino acid sequence comparisons, J. Muscle Res. Cell Motil. 12 (1991) 3–25.
[32] R. Dominguez, Y. Freyzon, K.M. Trybus, C. Cohen, Crystal structure of a vertebrate smooth muscle myosin motor domain and its complex with the essential light chain: visualization of the pre-power stroke state, Cell 94 (1998) 559–571.