ISSN 1672-9145 **Acta Biochimica et Biophysica Sinica** 2006, **38**(9): 625–632 **CN** 31-1940/Q

Preparation of Monoclonal Antibodies against Human Ventricular Myosin Light Chain 1 (HVMLC1) for Functional Studies

Zhen-Yan FU[1], Bao-Tong XIE[2], Yi-Tong MA[1]*, and Zu-Xun GONG[2]*

[1] *Cardiovascular Research Center, First Clinical College of Xinjiang Medical University, Urumqi 830054, China;*
[2] *Key Laboratory of Proteomics, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China*

Abstract Using purified recombinant human ventricular myosin light chain 1 (HVMLC1) as the antigen, three monoclonal antibodies, designated C8, C9 and B12, were prepared. Immunoblot experiments demonstrated that all monoclonal antibodies could react with the ventricular myosin light chain 1 isolated from different sources, such as human, rat or pig. It was also demonstrated that C8 was directed against the NN part of the N-fragment (amino acid 1–40) of HVMLC1, and both C9 and B12 against the C-fragment (amino acid 99–195). The affinity constants of C8, C9 and B12 were 3.20×10^8, 8.60×10^7 and 1.77×10^8 M^{-1}, respectively, determined by non-competitive ELISA. The isotype of B12 was determined as IgG2a, whereas the isotype of both C8 and C9 were IgG1. In the presence of C9 or B12, the actin-activated Mg^{2+}ATPase activity of myosin was greatly inhibited, but there was almost no effect on the Mg^{2+}ATPase activity for C8. B12 and C9 also inhibited the superprecipitation of porcine cardiac native actomyosin (myosin B) and reconstituted actomyosin, but C8 did not. The results indicate that all three monoclonal antibodies could bind the intact myosin molecule, but B12 and C9 might more easily react with epitopes located in the C-fragment of HVMLC1. The inhibitory effects of B12 and C9 on ATPase activity and superprecipitation assays show that light chain 1, particularly the C-fragment domain, is involved in the modulation of the actin-activated Mg^{2+}ATPase activity of myosin and, as a consequence, plays an essential role in the interaction of actin and myosin.

Key words human ventricular myosin light chain 1 (HVMLC1); monoclonal antibody; Mg^{2+}ATPase activity; superprecipitation

Cardiac myosin contains two kinds of light chains, cardiac light chain (CLC) 1 and CLC2, which are associated with the neck region of myosin heavy chain. They were thought to be similar to LC1 and LC2 of skeletal muscle myosin. In skeletal muscle, the removal of LC2 from myosin molecules by treating with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) did not significantly affect ATPase and actin binding activities of myosin [1,2]. However, in smooth muscle and non-muscle systems, phosphorylation and dephosphorylation of LC2 appear to control the actin-activated ATPase activity and play essential roles in muscle contraction with myosin-linked regulation [3]. Mutagenesis of LC2 of *Drosophila* cytoplasmic myosin results in a defect in cytokinesis and is lethal during the larval stage of development [4].

The role of LC1 in myosin function is less clear. X-ray crystallographic studies have demonstrated a functional structure of LC1, which wrap around and thereby stabilize a long α-helix, extending from the C-terminus of the myosin head [5]. Early studies of skeletal muscle myosin showed that LC1 could be removed by alkali treatment, leading to the loss of actin binding and ATPase activities of myosin [6,7]. In the non-muscle system, myosin isolated from LC1-deficient *Dictyostelium* cells does not show significant actin-activated ATPase activity [8,9]. It was

Received: March 20, 2006 Accepted: June 2, 2006
This research was supported by a grant from the Natural Science Foundation of Xinjiang (No. 97832)
*Corresponding authors:
Yi-Tong MA: Tel, 86-991-4362611; Fax, 86-991-4365330; E-mail, myt-xj@163.com
Zu-Xun GONG: Tel, 86-21-54921220; Fax, 86-21-54921125; E-mail, zxgong@sibs.ac.cn

DOI: 10.1111/j.1745-7270.2006.00203.x

confirmed by experiments that mutations in LC1 of *Dictyostelium* myosin leads to reduced actin-activated ATPase activities [10]. However, controversial results were also reported that skeletal myosin stripped of LC1 was not affected in the ATPase activity under wild and non-denaturing conditions of MHC [11].

Regarding CLC1, several studies on the physiological roles of ventricular and atrial LC1 in the regulation of human heart contractility were reported [2,12,15]. The diagnostic value of CLC1 in acute myocardial infarction and necrosis was suggested in many reports [16–19]. Recently, we have shown that only the N-fragment (amino acid residue 1–98) of HVMLC1 binds to S1, and the recombinant complex of rat cardiac myosin S1 and the N-fragment greatly decreased the actin-activated Mg^{2+}ATPase activity for the lack of the C-fragment (amino acid residue 99–195). We concluded that the N-fragment was the binding domain of HVMLC1, whereas the C-fragment served as a functional domain, which may be more involved in the modulation of the actin-activated ATPase activity [20].

In this study, three monoclonal antibodies against the N- and C-fragments of HVMLC1 were prepared and the roles of N- and C-fragments of ventricular LC1 (VLC1) in the actin-activated Mg^{2+}ATPase activity and superprecipitation of myosin were examined using these monoclonal antibodies. Both B12 and C9 monoclonal antibodies reacted with the C-fragment of LC1 and greatly inhibited the actin-activated Mg^{2+}ATPase activity and superprecipitation of myosin. C8 reacted with the N-fragment of LC1 but did not show any effect. Therefore, the data provided further evidence to confirm the previously postulated importance of the C-domain of LC1 in the regulation of actin-activated ATPase activity of myosin and in the interaction between actin and myosin.

Materials and Methods

Protein preparations

The isolation and purification of human and porcine ventricular myosin was carried out as follows. Human tissue was obtained from hospital patients with permission, the porcine heart and leg skeletal muscle were purchased from the slaughterhouse, and the porcine ventricular native actomyosin (myosin B) was prepared according to the protocol of Ebashi [21]. Actin was prepared from acetone powder of rat cardiac muscle according to Spudich and Watt [22]. The expression and purification of the GST-NN and GST-NC parts of the N-fragment, GST N-fragment and GST C-fragment of HMVCL1 (**Fig. 1**) were previously described [20].

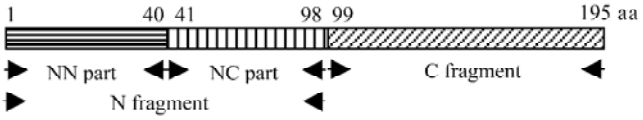

Fig. 1 Schematic diagram showing the N-fragment, C-fragment, NN part and NC part of human ventricular myosin light chain 1 involved in the article

Construction of recombinant vector of intein-HVMLC1

pTXB1 is a vector designed for in-frame fusion of an intein tag into the C-terminus of the target protein (New England Biolabs, Beverly, USA). The fusion protein consisting of the target protein and the intein tag can be bound to chitin beads, and the intein tag induced by thiol-reagents, such as DTT, can undergo an autocatalytic cleavage to release the purified target protein from the chitin beads.

For constructing pTXB1-HVMLC1, the full-length HVMLC1 cDNA previously obtained in our laboratory was used as a template [23], and a pair of primers, forward primer 5′-GGGAATTCCATATGGCCCCCAAAAAGC-CAG-3′ (*Nde*I site underlined) and reverse primer 5′-CCG-GAATTCGCTGGACATGATGTGCTT-3′ (*Eco*RI site underlined), were designed. PCR product was digested with *Nde*I and *Eco*RI and ligated into *Nde*I/*Eco*RI-digested pTXB1. The resulting vector pTXB1-HVMLC1 encodes an intein-HVMLC1 fusion protein.

Expression and purification of HVMLC1

Escherichia coli DE3(BL21) cells with the pTXB1-HVMLC1 plasmid were grown at 37 ºC overnight. The culture was diluted at 1:100 with fresh LB medium then grown at 37 ºC for 2 h. After adding IPTG to a final concentration of 0.3 mM, the cells were grown at 37 ºC for another 4 h. The cells were centrifuged and the pellet was resuspended in PBS, lysed by sonication and centrifuged at 20,000 *g* for 15 min. The supernatant was loaded onto a chitin bead column at 4 ºC. The column was washed with 20 bed volumes of washing buffer (20 mM Tris-HCl, pH 8.0, 50 mM NaCl, 0.1 mM EDTA, 0.1% Triton X-100) and flushed with 3 bed volumes of cleavage buffer (20 mM Tris-HCl, pH 8.0, 50 mM NaCl, 0.1 mM EDTA) containing 50 mM DTT. The column was kept at 4 ºC for 24 h. The HVMLC1 was eluted using 3 bed volumes of

cleavage buffer. The protein solution was dialyzed against PBS then concentrated by Centricon YM-30 (Millipore, Bedford, USA). The concentration of HVMLC1 was determined by BCA protein assay kit (Pierce, Rockford, USA).

Preparation of monoclonal antibodies

BALB/c mice (female, 6 weeks old) were immunized by intraperitoneal (i.p.) injection of 250 μl HVMLC1 (0.8 mg/ml) emulsified in an equal volume of Freund's complete adjuvant for primary immunization. Four weeks later, the first booster immunization was performed. The same volume of HVMLC1 (0.8 mg/ml) emulsified in an equal volume of Freund's incomplete adjuvant was injected i.p. at an interval of two weeks. After 2–3 booster immunizations, approximately 50 μl of blood sample was obtained from the tail vein and detected by two-dimensional immunodiffusion. Three days prior to cell fusion, each mouse was hyperimmunized with 200 μg HVMLC1 i.p. in 500 μl PBS (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.76 mM KH_2PO_4, pH 7.5). A653 myeloma cells were grown in RPMI 1640 medium (Gibco, Grand Island, USA) containing 20% fetal calf serum. The fusion between myeloma cells and isolated spleen cells of BALB/c mouse was carried out using a routine procedure [24], and the supernatants from wells were screened for hybridoma growth by indirect ELISA using HVMLC1-coated microplates in the presence of anti-HVMLC1 antibody. The positive wells were expanded and subcloned twice by limiting dilution to ensure monoclonality.

For the production of ascites in BALB/c mouse and purification of monoclonal antibody, the positive cell lines were expanded and propagated in 2,6,10,14-tetramethylpentadecane (pristine) primed mouse. Five hundred microliters of pristine was injected i.p. into each mouse. After two weeks, 1.0×10^6 hybridoma cells were injected i.p. into each primed mouse. The ascites fluid was harvested at the time of maximum ascites production (10–15 d after injection). The cells were removed by centrifugation at 5000 *g* for 10 min.

The monoclonal antibodies from ascites fluid were purified by ammonium sulfate precipitation followed by anion exchange chromatography. The ammonium sulfate was slowly added to 45% saturation concentration to ascites fluid with gentle stirring on an ice bath. After stirring the mixture for at least 6 h at 4 ºC, the pellet was collected by centrifugation at 10,000 *g* for 15 min, resuspended in 10 mM sodium phosphate (pH 7.5) and dialyzed against the same buffer to remove the ammonium sulfate. The solution was applied to a DEAE-52 column equilibrated with 10 mM sodium phosphate (pH 7.5). The antibodies could be eluted by 50 mM sodium phosphate buffer (pH 7.5) containing 40 mM NaCl. The purity and the specificity of antibodies were further evaluated by SDS-PAGE and Western blot.

Immunoblotting

The proteins were separated by SDS-PAGE on 12% gels. The protein bands were transferred to the nitrocellulose membrane using a Bio-Rad mini-transfer apparatus (Bio-Rad, Hercules, USA). After blocking with 5% non-fat milk in TBS-T (20 mM Tris-HCl, 137 mM NaCl, 0.1% Tween-20, pH 7.6) for 1 h at room temperature, the membrane was first incubated with various monoclonal antibodies against HVMLC1, and then incubated with secondary peroxidase-conjugated polyclonal antibody (goat anti-mouse; Rockland, Gilbertsville, USA) for 1 h. The detection was carried out using enhanced chemiluminescence reagents (ECL kit; Amersham, Arlington Heights, USA) according to the protocols.

Measurement of binding affinity of monoclonal antibody

The binding affinity of monoclonal antibody was measured according to the method of Beatty *et al.* [25]. HVMLC1 (1 μg/ml or 0.25 μg/ml and plus 0.75 μg/ml BSA) dissolved in coating buffer (15 mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.5) were coated onto the Maxisorp microplate (Nunc, Roskilde, Denmark) overnight at 4 ºC in a humid atmosphere. The liquid was removed and the plate was washed four times with washing buffer (20 mM Tris-HCl, 0.1% Tween-20, pH 7.5). The plate was blocked with 5% non-fat milk in PBS for 1 h at 37 ºC. After another washing, serial dilutions of each monoclonal antibody in PBS-T (PBS, 0.1% Tween-20, pH 7.5) were added to the wells and the plate was then incubated for 1 h at 37 ºC. After washing again, the HRP-labeled secondary antibody (1:8000 in PBS-T, 1% BSA, goat anti-mouse IgG-HRP; Rockland) was added. The plate was incubated at 37 ºC for 1 h then washed five times. One hundred microliters of substrate solution (0.0625 mg/ml TMB, 0.003% H_2O_2 in 50 mM Na_2HPO_4 and 25 mM citric acid, pH 5.0) was added to each well and the plate was incubated in the dark at 37 ºC for 20 min and 100 μl of 2 M H_2SO_4 was added to each well to stop the reaction. The absorbance was read at 450 nm in a multiplate reader (Model 550; Bio-Rad).

Assay of monoclonal antibody isotype

The isotypes of monoclonal antibodies B12, C9 and C8

were determined by the Mouse monoclonal antibody isotyping kit according to manufacturer's protocol (Zymed, San Francisco, USA).

ATPase assay

The Mg^{2+}ATPase activity of porcine ventricular myosin was determined by the method of Pollard and Korn [26]. B12, C9, C8 and BSA (each 0.05 mg) with 0.1 mg myosin were separately dissolved in reaction buffer (50 mM Tris-HCl, pH 8.0, 5 mM $MgCl_2$, 0.25 mM $CaCl_2$). The final volume was 1 ml. The mixture was incubated at 25 ºC for 30 min. ATP (3 mM) and 0.05 mg actin were added to the mixture, and it was incubated again at 25 ºC for 30 min. The reaction was terminated by adding 250 μl TCA (50%). After placement in an ice bath for another 30 min, the solution was centrifuged at 10,000 *g* for 1 min to precipitate denatured proteins. For quantitative evaluation of the amount of released inorganic phosphate, an equal volume of colorimetric reagent (one volume of 3 M sulfuric acid, two volumes of ddH_2O, one volume of 0.25% ammonium molybdate and one volume of 10% ascorbic acid) was added to the supernatant and the mixture was incubated at 25 ºC for 1 h. Absorbance was measured at 660 nm.

The amount of released inorganic phosphate was calculated and compared with the standard curve. All assays were repeated three times.

Superprecipitation assay

The superprecipitation assays were performed according to the method described previously [27]. To observe the effects of monoclonal antibodies on superprecipitation, B12, C9, C8 and BSA (each 0.5 mg) were separately added to 0.15 mg of either porcine cardiac native actomyosin or cardiac reconstituted actomyosin dissolved in 950 μl of superprecipitation buffer (10 mM Tris-HCl, pH 6.8, 1 mM $MgCl_2$, 50 mM KCl). The mixtures were first incubated at 37 ºC for 30 min and then cooled to 20 ºC. ATP (50 μl of 1 mM) was added to each mixture. Different concentrations of ATP (0.3 mM, 1 mM, 10 mM and 40 mM) were used for observing the clear phase of superprecipitation. The turbidity changes at 660 nm were measured at 30 s intervals using a spectrophotometer (UV-2100; Shimadzu, Kyoto, Japan). All assays were repeated at least twice.

Results

Expression and purification of recombinant HVMLC1

The fusion protein intein-HVMLC1 was expressed and accumulated in the induced *E. coli* cells. HVMLC1 protein without intein was collected after self-cleavage by DTT and showed a single major band in SDS-PAGE (**Fig. 2**). The molecular mass of expressed HVMLC1 estimated in SDS-PAGE is approximately 25–27 kDa and is higher than the calculated molecular mass (22 kDa) based on the HVMLC1 amino acid sequence.

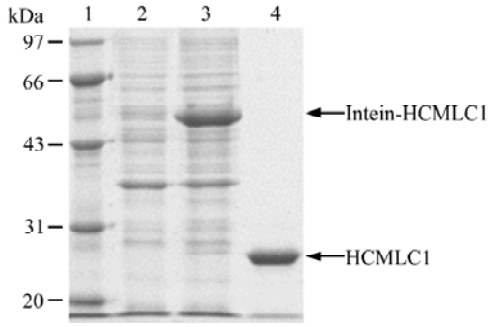

Fig. 2 Expression and purification of human ventricular myosin light chain 1 (HVMLC1) in *Escherichia coli*

1, molecular weight standard; 2, uninduced cell extract; 3, induced cell extract showing expressed fusion protein intein-HVMLC1; 4, purified HVMLC1.

Characterizations of monoclonal antibodies

The reactivity of C8, C9 and B12 with the expressed HVMLC1 and the expressed N- and C-fragment of HVMLC1, as well as myosin isolated from human and porcine tissues, were analyzed by Western blot, as shown in **Fig. 3**. The results showed that C8, C9 and B12 could all bind LC1, either expressed or dissociated from the isolated myosin by SDS-PAGE. However, C8 only reacts with the N-fragment, whereas both C9 and B12 could bind with the C-fragment of LC1. Although the epitopes of C8, C9 and B12 have not yet been determined, the Western blot analysis of the NN and NC parts of the N-fragment with C8 indicated that the antigenic determinant of C8 is located at the NN part within amino acid residue 1–40 of LC1 (**Fig. 4**).

The binding constants of C8, C9 and B12 were measured using ELISA. The sigmoid curve was plotted to represent the relationship of *A* versus lg(DM). DM represents the dilution multiples of antibody and *A* is the optical absorbance at 450 nm. **Equation 1** was used for calculation, as follows:

$$K_{aff}=(n-1)/[2(n[Ab']_t-[Ab]_t)] \quad 1$$

where $n=(1.00\ \mu g/ml/0.25\ \mu g/ml)=4$. $[Ab']_t$ and $[Ab]_t$ are the antibody concentrations at *A*50 (50% of *A*100, the

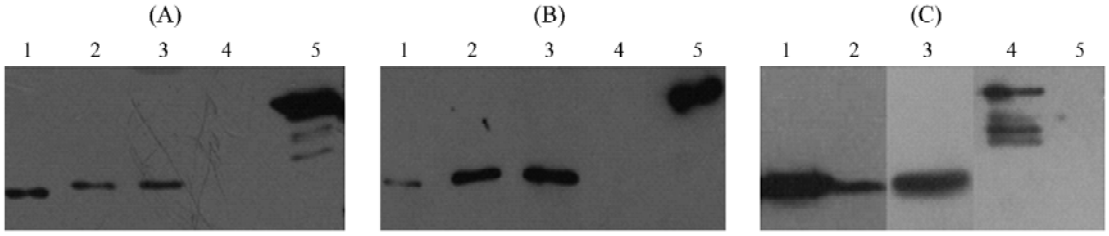

Fig. 3 Interactions of monoclonal antibodies B12, C9 and C8 with expressed human ventricular myosin light chain 1 (HVMLC1) and its N- and C-fragments, and MLC1 isolated from human or porcine cardiac myosin analyzed by Western blot

(A) Monoclonal antibody B12. (B) Monoclonal antibody C9. (C) Monoclonal antibody C8. 1, expressed HVMLC1; 2, HVMLC1 isolated from human ventricular myosin; 3, porcine ventricular myosin light chain 1 isolated from porcine ventricular myosin; 4, expressed GST-HVMLC1-N; 5, expressed GST-HVMLC1-C.

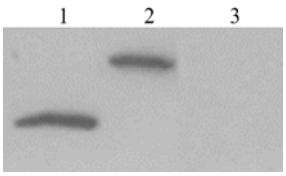

Fig. 4 Interactions of monoclonal antibodies C8 with expressed NN and NC parts of human ventricular myosin light chain 1 (HVMLC1) analyzed by Western blot

1, expressed HVMLC1; 2, expressed GST-NN part of HVMLC1; 3, expressed GST-NC part of HVMLC1.

upper plateau) for 1 μg/ml and 0.25 μg/ml of antigen coating solution, respectively. $[Ab]_t$ and $[Ab']_t$ can be determined according to the initial concentration of monoclonal antibody and DM value at *A*50. The binding constants of C8, C9 and B12 are summarized in **Table 1**.

Table 1 Binding affinity constants of monoclonal antibodies B12, C9 and C8

Antibody	$[Ab]_t$ (μg/ml)	$[Ab']_t$ (μg/ml)	K_{aff} (M^{-1})
B12	0.167	0.381	1.77×10^8
C9	0.340	0.780	8.60×10^7
C8	0.037	0.199	3.20×10^8

Using the Mouse monoclonal antibody isotyping kit, the isotype of B12 was determined as IgG2a, whereas, for both C8 and C9, the isotype was determined as IgG1.

Inhibition of actin-activated Mg^{2+}ATPase activity of myosin by B12 and C9

The presence of C9 and B12 inhibited the actin-activated Mg^{2+}ATPase activity of porcine cardiac myosin. It was shown that after adding the same amount of B12 and C9, the actin-activated Mg^{2+}ATPase activity of myosin dramatically decreased to 53.8% and 57.5%, respectively, compared with the control set containing myosin alone. BSA had no effect on actin-activated Mg^{2+}ATPase activity. Interestingly, C8 did not change myosin actin-activated Mg^{2+}ATPase activity either (**Table 2**).

Table 2 Influence of monoclonal antibodies B12, C9 and C8 on the actin-activated Mg^{2+}ATPase activities of porcine cardiac myosin

Group	Mg^{2+} ATPase activity [nmol Pi·(mg protein)$^{-1}$·min^{-1}]
Myosin	30.72
Myosin+B12	16.53
Myosin+C9	17.67
Myosin+C8	30.84
Myosin+BSA	31.21

BSA, bovine serum albumin.

Inhibition of superprecipitation of native actomyosin (myosin B) by B12 and C9

The time-course of superprecipitation of porcine ventricular native actomyosin is similar to that of skeletal muscle native actomyosin, except that the clear phase did not appear even after the addition of a relatively high concentration of ATP. After adding the antibodies B12, C9 and C8, as well as BSA to the actomyosin, the interaction between antigen and monoclonal antibodies prior to incubation did not change the superprecipitation profile very much, but B12 and C9 clearly had inhibitory effects on superprecipitation of both native and reconstituted

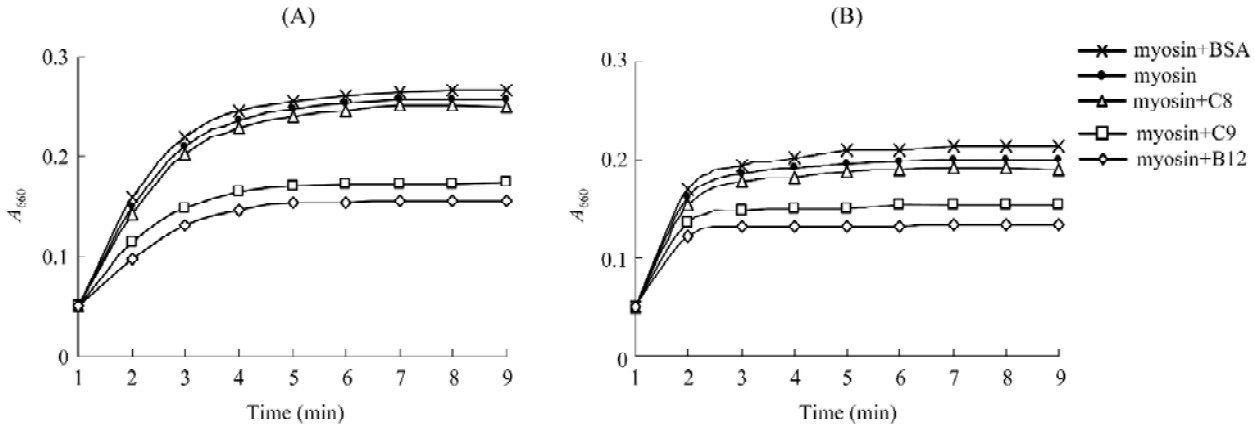

Fig. 5 Effects of monoclonal antibodies C8, C9 and B12 and BSA on superprecipitation of porcine ventricular reconstituted (A) and native (B) actomyosin

actomyosin. The maximum level decreased 40% and 34% by B12, and 35% and 26% by C9 in superprecipitations of native and reconstituted actomyosin, respectively (**Fig. 5**), compared with actomyosin alone, whereas C8 and BSA did not show any inhibitory effects on superprecipitation of native or reconstituted actomyosin.

Discussion

It has been commonly accepted that LC1 has a structural role in the stabilization of the myosin neck region [5], but the function of LC1 in muscle contraction has been controversial in published reports. Some researchers reported that the removal of LC1 from myosin molecules led to loss of ATPase activity, whereas others believed that removal of LC1 did not affect the actin-activated ATPase activity [6,7,11]. With regard to CLC1, several reports described the difference in the physiological roles of atrial and ventricular LC1 isoforms, and concluded that myosin molecules containing ventricular type LCs had a longer duration and higher average force compared to atrial type LCs. The ATPase activity, however, was not different between these two types of myosin [12–15,28]. In general, the physiological role of LC1 in the modulation of ATPase activity and muscle contraction is less understood than that of LC2, the regulatory light chain in smooth muscle contraction. We have demonstrated in our previous studies that only the N-fragment of HVMLC1 contains actin and myosin heavy chain binding sites and the recombined rat cardiac myosin S1 binding with the N-fragment of HVMLC1 showed a dramatic decrease in actin-activated Mg^{2+}ATPase activity. We inferred that the N-fragment is the binding domain of human CLC1, whereas its C-fragment near the nucleotide binding site of the heavy chain revealed in the crystal structure is a functional domain, which might be more involved in the modulation of actin-activated ATPase activity [20].

Using the conventional method to prepare monoclonal antibody and express recombinant HVMLC1, we have obtained three monoclonal antibodies (C8, C9 and B12) against HVMLC1. Western blot analysis showed that all three monoclonal antibodies reacted with HVMLC1 of myosin either isolated from human cardiac tissue or expressed in *E. coli*. Moreover, the monoclonal antibodies also react with VMLC1 isolated from rat (data not shown) and porcine ventricular myosin. This is not surprising, because the rat ventricular LC1 is most closely related to the human one in a proposed evolutionary tree of myosin LC1 [29]. There was no report about the amino acid sequence of porcine VMLC1, but the sequence of porcine skeletal muscle myosin LC3 (PSMLC3) showed a great homology with HVMLC1, except that PSMLC3 lacks 47 amino acid residues at the N-terminal of PSMLC1 [30]. Western blot analysis demonstrated that C8 specifically reacted with the NN part of the N-fragment, whereas both C9 and C12 reacted only with the C-fragment of HVMLC1. Therefore, these monoclonal antibodies can be used to clarify the physiological importance of the N-fragment and C-fragment domains of HVMLC1 and HVMLC1 as a whole, instead of recombined S1 with the N-fragment domains of HVMLC1 under more severe conditions, as previously reported [20].

The results in this study demonstrated that the monoclonal antibodies C9 and B12 greatly inhibited the actin-activated Mg^{2+}ATPase activity of porcine cardiac

myosin to 53.8% and 57.5% for C9 and B12, respectively, but there was almost no effect for C8. On the other hand, the superprecipitation reaction could be used as an *in vitro* model to examine the interaction between myosin and actin under ATP consumption. The superprecipitation assays were performed for reconstituted actomyosin and native actomyosin (myosin B) isolated from different types of muscles, such as skeletal, smooth and adductor catch muscles [27,31–34]. Here we describe for the first time the kinetic behavior of superprecipitation of the porcine ventricular reconstituted and native actomyosin. The time-courses of superprecipitation of both types of actomyosin were similar to that of skeletal muscle actomyosin. The major difference in the kinetics of superprecipitation of cardiac and skeletal actomyosin is the appearance of a clear phase immediately after adding ATP. At proper levels of ATP concentration, the typical clear phase could be easily observed for skeletal actomyosin, but the clear phase did not appear at the same concentration of ATP in the case of cardiac actomyosin. At a very high concentration of ATP, the superprecipitation reaction for cardiac actomyosin was delayed for a long time. This might be one of the characteristics of superprecipitation of cardiac actomyosin. Since it was shown that the superprecipitation kinetics kept their profiles at a very wide range of ATP concentration, not surprisingly, the inhibition of ATPase activity by C9 and B12 did not change the superprecipitation profile very much, except that the initial increasing phase was slower compared with that for C8 or BSA. C9 and B12 also showed inhibitory effects on superprecipitation of porcine ventricular native actomyosin, but C8 did not. The results in both ATPase activity and superprecipitation are in good agreement with our previous data [20]. B12 showed maximum inhibitory effect in both experiments, because B12 has a higher binding affinity than C9. These results suggest again that the C-fragment domain of VLC1 is more involved in the modulation of ATPase activity and consequently more involved in the interaction of myosin and actin, whereas the N-fragment domain plays a structural role in binding the myosin heavy chain at the neck region. These results also clearly indicate that the ventricular myosin LC1 is actively involved in the regulation of muscle contraction.

References

1 Wagner PD, Weeds AG. Studies on the role of myosin alkali light chains. Recombination and hybridization of light chains and heavy chains in subfragment-1 preparations. J Mol Biol 1977, 109: 455–470

2 Weeds AG, Lowey S. Substructure of the myosin molecule. II. The light chains of myosin. J Mol Biol 1971, 61: 701–725

3 Trybus KM, Waller GS, Chatman TA. Coupling of ATPase activity and motility in smooth muscle myosin is mediated by the regulatory light chain. J Cell Biol 1994, 124: 963–969

4 Karess RE, Chang XJ, Edwards KA, Kulkarni S, Aguilera I, Kiehart DP. The regulatory light chain of nonmuscle myosin is encoded by spaghetti-squash, a gene required for cytokinesis in *Drosophila*. Cell 1991, 65: 1177–1189

5 Rayment I, Rypniewski WR, Schmidt-Base K, Smith R, Tomchick DR, Benning MM, Winkelmann DA *et al*. Three-dimensional structure of myosin subfragment-1: A molecular motor. Science 1993, 261: 50–58

6 Gershman LC, Stracher A, Dreizen P. Subunit structure of myosin. III. A proposed model for rabbit skeletal myosin. J Biol Chem 1969, 244: 2726–2736

7 Gershman LC, Dreizen P. Relationship of structure to function in myosin. I. Subunit dissociation in concentrated salt solutions. Biochemistry 1970, 9: 1677–1687

8 Pollenz RS, Chen TL, Trivinos-Lagos L, Chisholm RL. The *Dictyostelium* essential light chain is required for myosin function. Cell 1992, 9: 951–962

9 Chen TL, Kowalczyk PA, Ho G, Chisholm RL. Targeted disruption of the *Dictyostelium* myosin essential light chain gene produces cells defective in cytokinesis and morphogenesis. J Cell Sci 1995, 108: 3207–3218

10 Ho G, Chisholm RL. Substitution mutations in the myosin essential light chain lead to reduced actin-activated ATPase activity despite stoichiometric binding to the heavy chain. J Biol Chem 1997, 272: 4522–4527

11 Wagner PD, Giniger E. Hydrolysis of ATP and reversible binding to F-actin by myosin heavy chains free of all light chains. Nature 1981, 292: 560–562

12 Morano I, Hadicke K, Haase H, Bohm M, Erdmann E, Schaub MC. Changes in essential myosin light chain isoform expression provide a molecular basis for isometric force regulation in the failing human heart. J Mol Cell Cardiol 1997, 29: 1177–1187

13 Morano M, Zacharzowski U, Maier M, Lange PE, Alexi-Meskishvili V, Haase H, Morano I. Regulation of human heart contractility by essential myosin light chain isoforms. J Clin Invest 1996, 98: 467–473

14 Yamashita H, Sugiura S, Fujita H, Yasuda S, Nagai R, Saeki Y, Sunagawa K *et al*. Myosin light chain isoforms modify force-generating ability of cardiac myosin by changing the kinetics of actin-myosin interaction. Cardiovasc Res 2003, 60: 580–588

15 Sanbe A, Gulick J, Fewell J, Robbins J. Examining the *in vivo* role of the amino terminus of the essential myosin light chain. J Biol Chem 2001, 276: 32682–32686

16 Miyazaki S, Hamaoki M, Nagata A. Development of enzyme-linked immunosorbent assay for human cardiac myosin light chain. Methods Find Exp Clin Pharmacol 2005, 27: 323–326

17 Aikawa R, Yazaki Y. Biochemical markers for acute coronary syndromes. Nippon Rinsho 1998, 56: 2569–2675

18 Katoh H, Sugi M, Chino S, Ishige M, Kuroda M, Fujimoto M, Nagai R *et al*. Development of an immunoradiometric assay kit for ventricular myosin light chain 1 with monoclonal antibodies. Clin Chem 1992, 38: 170–171

19 Nakai K, Nakai K, Nagane Y, Obara W, Sato M, Ohi K, Matsumoto N *et al*. Serum levels of cardiac troponin I and other marker proteins in patients with chronic renal failure. Clin Exp Nephrol 2004, 8: 43–47

20 Xie B, Huang R, Huang L, Zhou G, Gong Z. The functional domains of human ventricular myosin light chain 1. Biophys Chem 2003, 106: 57–66

21 Ebashi S. Calcium binding activity of vesicular relaxing factor. J Chir 1961, 50: 236–244

22 Spudich JA, Watt S. The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. J Biol Chem 1971, 246: 4866–4871

23 Huang RJ, Peng BZ, Zhou GY, Gong ZX. 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 2000, 32: 158–162

24 Harlow E, Lane D. Antibodies: A Laboratory Manual. New York: Cold Spring Harbor Laboratory Press 1988

25 Beatty JD, Beatty BG, Vlahos WG. Measurement of monoclonal antibody affinity by non-competitive enzyme immunoassay. J Immunol Methods 1987, 100: 173–179

26 Pollard TD, Korn ED. *Acanthamoeba* myosin I. Isolation from *Acanthamoeba castellanii* of an enzyme similar to muscle myosin. J Biol Chem 1973, 248: 4682–4690

27 Nonomura Y, Ebashi S. Electron microscopic studies of superprecipitation with special reference to its optical properties. J Mechanochem Cell Motil 1974, 3: 1–8

28 Abdelaziz AI, Segaric J, Bartsch H, Petzhold D, Schlegel WP, Kott M, Seefeldt I *et al*. Functional characterization of the human atrial essential myosin light chain (hALC-1) in a transgenic rat model. J Mol Med 2004, 82: 265–274

29 Collins JH. Myosin light chains and troponin C: Structural and evolutionary relationships revealed by amino acid sequence comparisons. J Muscle Res Cell Motil 1991, 12: 3–25

30 Hasegawa Y, Ueda Y, Watanabe M, Morita F. Studies on amino acid sequences of two isoforms of 17-kDa essential light chain of smooth muscle myosin from porcine aorta media. J Biochem 1992, 111: 798–803

31 Shelud'ko NS, Tikunov BA, Kropacheva IV, Permiakova TV, Iudin IuK. A mechanism for dual-stage kinetics of actomyosin superprecipitation. Biofizika 1994, 39: 418–422

32 Ikebe M, Reardon S, Mitani Y, Kamisoyama H, Matsuura M, Ikebe R. Involvement of the C-terminal residues of the 20,000-dalton light chain of myosin on the regulation of smooth muscle actomyosin. Proc Natl Acad Sci USA 1994, 91: 9096–9100

33 Labyntseva RD, Ul'ianenko TV, Kosterin SA. Effect of heavy metal ions on superprecipitation and ATPase activity of uterine smooth muscle actomyosin activity. Ukr Biokhim Zh 1998 70: 71–77

34 Shen L, Gong ZX, Cao TQ. Purification and some properties of a 20 kDa regulatory protein obtained from catch musch. Acta Biochim Biophys Sin 1989, 21: 12–21

Edited by
Zu-Chuan ZHANG