DIFFERENCES IN ATRIAL AND VENTRICULAR MYOSIN LIGHT CHAIN LC_1

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1. Introduction

Atrial myosin is an isozyme of myosin, having a greater Ca^{2+}-stimulated ATPase activity [1,2], variances in the electrophoretic mobility, and immunological properties of the light chains [1], as well as differences in the primary structure of the heavy chains [2].

We wish to report here that there are structural differences in beef atrial and ventricular myosin light chains, and although the LC_1 light chain, as well as the heavy chain, of atrial myosin are structurally different from that of the ventricle, the ventricular myosin light chains, nevertheless, still recombine stoichiometrically with light chain-deficient atrial myosin. The studies on light chains of atrial and ventricular myosin show that:

(i) There are not only differences in electrophoretic mobility between atrial and ventricular LC_1 light chain, but also that there are variances in amino acid composition.
(ii) There are several differences in their tryptic peptide maps.

2. Materials and methods

2.1. *Myosin and light chain purification*

Myosin was purified as in [1] then precipitated twice with 9 vol. (v/w) H_2O at pH 6.5 [1]. The myosin light chains were purified from the myosin oligomer with 8 M urea [1]. The individual light chains were isolated by column chromatography using DEAE–Sephadex A-25 [3]. After a 1 g sample of light chains was applied to a Sephadex A-25 column (30 × 1.5 cm) the column was washed with 0.05 M Tris·HCl (pH 7.5) containing 1 mM dithiothreitol (DTT), and the different myosin light chains were eluted with a salt gradient (0–0.5 M KCl), using the same buffer. Protein was monitored at 230 nm against the gradient buffer containing a high salt concentration.

2.2. *Reduction and carboxymethylation of light chain LC_1*

The isotope iodo-[^{14}C]acetamide (9.4 mCi/mM) was purchased from New England Nuclear. The myosin light chains were dialyzed against 0.05 M Tris·HCl (pH 8.2) and 8 M urea containing a 2.5 molar excess of DTT over SH groups, then incubated at 37°C for 2 h. At the end of the incubation period a 5 molar excess of iodo-[^{14}C]acetamide over SH groups was added and incubated at 37°C for an additional 2 h. The reaction was terminated with a large excess of β-mercaptoethanol. After keeping for 30 min at room temperature, myosin light chain LC_1 of atrial and ventricular myosin (10 mg/ml) was dialyzed against 0.001 M HCl and freeze-dried. In the cases where the native myosin was reduced and carboxymethylated conditions were the same except that 0.5 M KCl was present during the carboxymethylation. Also, at the end of the incubation native myosin was precipitated with 9 vol. H_2O instead of the lyophilization, and solubilized in 0.05 M Tris·HCl (pH 7.5) and 0.5 M KCl. After myosin light chains were freeze-dried and dissolved in 0.1 M ammonium carbonate (pH 8.2), trypsin (Sigma Chem. Co., DCC-treated) was added in the ratio of 1:50, and the mixture was incubated at room temperature overnight. The reaction was terminated by lyophilization.

2.3. *Amino acid composition*

Protein hydrolysis and amino acid composition was performed by AAA Lab. (Seattle Washington). The protein was hydrolyzed for varying lengths of time in 6 N HCl as shown in table 1.

2.4. *Peptide mapping*

The lyophilized tryptic digest was dissolved in 0.05 M NH_4OH and applied to 3 MM Whatman paper (24 × 24 in) and electrophoresis was carried out using pyridine:acetate:water (100:4:896, by vol.) (pH 6.5) at 3000 V for 30 min. After electrophoresis the paper was dried and electrophoresis was carried out in the second dimension using formate:acetate:water (20:80:900, by vol.) (pH 1.9) until the standards lysine and ε-DNP-lysine moved to known positions. The paper was again dried and peptides were made visible with ninhydrin. For identification of the radioactive cysteine groups the peptide map was exposed to Kodak non-screen X-ray film for 72 h. In further studies 24 of the main peptides were eluted from ventricular LC_1 peptide map and 21 main spots were eluted from atrial LC_1 tryptic peptide map. Samples of all peptides were acid hydrolyzed as described above for identification of compositional amino acids. In other studies samples were taken for identification of the COOH-terminal amino acid by treating with carboxypeptidase A and B. The method in [4] was used for identification of compositional amino acids and the released COOH-terminal amino acid.

2.5. *Dissociation of myosin light chains*

Procedures from [6] were used for dissociation of light chains as well as both recovery of light chain-deficient myosin and the released light chains. For reassociation, light chains were recombined with light chain-deficient myosin in the presence of 5 mM $CaCl_2$ and incubated at 4°C overnight. Reassociated myosin was then purified from the free light chains by bringing the solution to 45% $(NH_4)_2SO_4$, then centrifuged and solubilized in 0.05 M Tris-HCl (pH 7.5) containing 0.5 M KCl.

2.6. *Polyacrylamide gradient slab-gel electrophoresis*

Polyacrylamide gradient slab gels containing sodium dodecylsulfate (SDS) were prepared as in [1]. For determination of protein concentration present in the Coomassie blue-stained gels, the dye was eluted with 25% pyridine as in [7]. For analyses of specific activity, ghost gel and eluted dye in vials were dried together. After drying 1 ml 30% H_2O_2 was added to the vials, then sealed and kept overnight at 50°C. The radioactivity was measured using liquid scintillation fluid in a Beckman liquid scintillation counter.

Fig.1. A polyacrylamide gradient (SDS) electrophoretogram of (1) purified LC_1 and (2) native myosin from beef ventricle. Details of purification are in section 2.

3. Results

A pattern of purified LC_1 light chain is shown in fig.1 and electrophoretograms of light chain, LC_1, from atrial and ventricular myosin is shown in fig.2. Similar variances in the electrophoretic mobility of LC_1 light chain between atrial and ventricular myosin were observed in the sheep and dog [1]. Differences were also observed in the content of aspartate, cysteine, lysine, methionine and proline (table 1), as well as some other amino acid residues. The presence of 2 cysteine groups in LC_1 of atrial myosin and 3 in that of the ventricle, was further substantiated by isotopic labeling of the cysteine groups. A representative profile of atrial (A) and ventricular (B) myosin after labeling with iodo-[^{14}C]acetamide is shown in fig.3. Specific activity of the isotopically labeled protein bands was carried out as in section 2. Results for the heavy chains were similar to those in [2]; ~14 mol cysteine were present in atrial myosin heavy chain and 16 mol in ventricular myosin heavy chains. Tryptic peptide maps of atrial and ventricular myosin light chain LC_1 also revealed differences not only in number, but also, in the mobilities of various peptides (fig.4). More specifically, two of the cysteine peptides were the same between the two light chains, however, the third was absent in atrial myosin light chain LC_1. The two peptides containing histidine, as well as several other peptides, were also different. The peptide containing isoleucine as the C-terminal amino acid residue was the same between the two LC_1 light chains.

Table 1

	Beef atria[a] C_1	Beef ventricle[d] C_1
Ala	16.0	18.0
Arg	5.2	5.0
Asp	13.9	17.7
Cys/2	2.0[b]	3.0
Glu	26.6	27.6
Gly	10.7	13.7
His	2.5	3.0
Isoleu	5.9[f]	7.0
Leu	13.4	13.7
Lys	15.7	19.9
Meth	3.9	6.8
Phen	8.4	9.7
Pro	14.4	17.3
Ser	6.6[e]	5.1
Thr	7.8[e]	10.8
Tryp	0[c]	0
Tyr	2.3[f]	3.0
Val	9.0	7.8
Mol wt	(21 000)	

[a] Acid hydrolyses were in 6 N HCl at 110°C for 24, 48, 72, 96 and 120 h, and average hydrolyses values taken except where indicated; values are mean of 2 different preparations carried out by AAA Labs, using a Durrum Model D500
[b] Performic acid oxidized prior to acid hydrolysis. Calculated from cysteine/alanine ratio
[c] 48 h alkaline hydrolysis at 135°C method in [12]
[d] According to [11]
[e] Extrapolated to zero time
[f] Extrapolated to infinite time

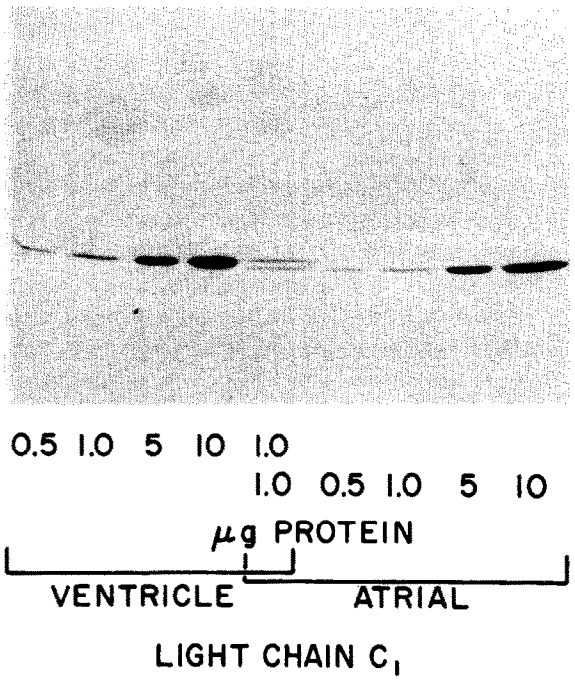

Fig.2. Purified light chain LC_1 from beef ventricle and atria were examined on 5–20% polyacrylamide gradient gel electrophoresis at varying concentrations.

Despite a large number of differences between the LC_1 light chains and heavy chains of atrial and ventricular myosin, reassociation of ventricular myosin light chains with atrial myosin heavy chains occurred (fig.5). A partial release of atrial myosin light chains was achieved by subjecting myosin to a brief incubation

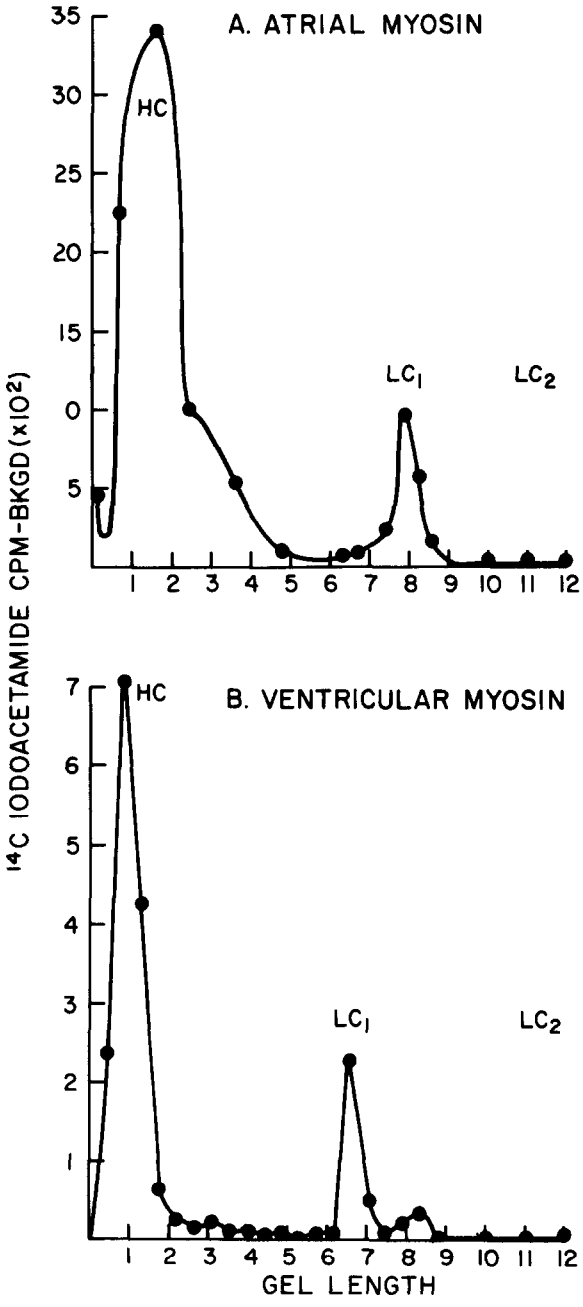

Fig.3. Myosin was reduced and carboxymethylated in the presence of iodo-[^{14}C]acetamide as in section 2. The isotopically labeled myosin was analyzed on a slab of 5–20% polyacrylamide as in section 2. A representative profile of iodo-[^{14}C]acetamide-labeled (A) atrial and (B) ventricular myosin is shown. The gel was sliced, digested with H_2O_2 and analyzed for isotope incorporation.

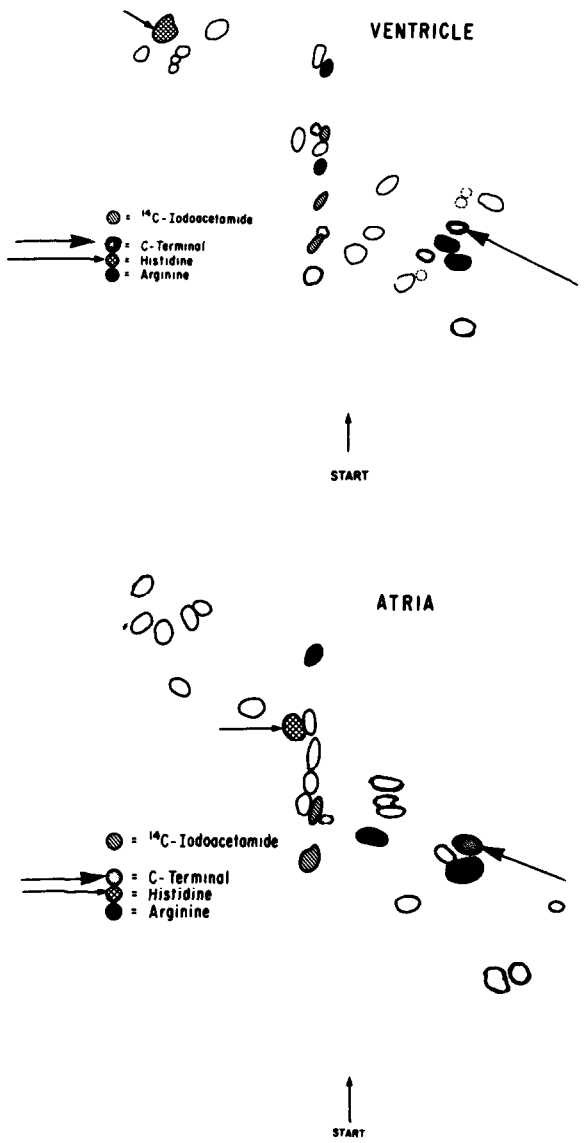

Fig.4. Two-dimensional paper chromatography patterns of ventricular and atrial myosin light chain LC_1, following complete trypsin digestion is shown. Although all of the ninhydrin-stained spots are shown, there were 24 main spots in ventricular LC_1 light chain and 21 in that of atrial LC_1 light chain. All of these contained either arginine or lysine as the COOH-terminal, except for the C-terminal amino acid as indicated, which contained isoleucine. Isoleucine was shown to be the C-terminal amino acid of other alkali light chains [10]. Additional spots, ninhydrin-stained, which could not be accounted for by the number of arginyl and lysyl residues, may have occurred through chymotrypsin contamination although trypsin used in these studies was DCC-treated.

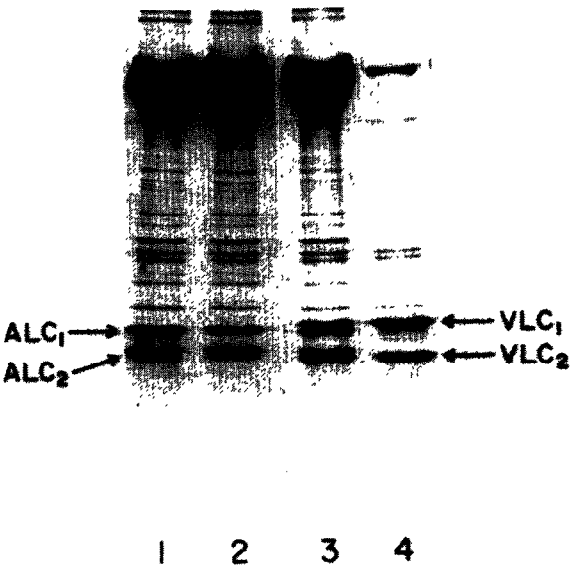

Fig.5. Gel electrophoretic patterns of: (1) atrial myosin; (2) light chain-deficient atrial myosin; (3) ventricular myosin light chains recombined with light chain-deficient atrial myosin; (4) ventricular myosin light chains.

in the K^+/EDTA kinetic system as in [6]. The purified light chain-deficient atrial myosin (table 2) was shown to have released 24% of the LC_1 light chain and 60% of the Ca^{2+} binding light chain LC_2. A release of light chains from ventricular myosin was prepared in the same way, and the light chains were recovered in the 80% $(NH_4)_2SO_4$ fraction as in [6]. The ventricular myosin light chains reassociated with light chain-deficient atrial myosin in the presence of 5 mM $CaCl_2$ at 4°C (fig.5). However, the light chains prepared from rabbit skeletal muscle myosin did not complex with light chain-deficient atrial myosin.

4. Discussion

The present results indicate that there are differences in the primary structure between the LC_1 light chain of atrial and ventricular myosin, as reported for heavy chains [2]. Despite various differences in structure, ventricular light chains cross-hybridize with light chain-deficient atrial myosin. This indicates certain functional similarities (association with heavy chains of atrial and ventricular myosin with light chains of both types) between the light chains of atrial and ventricular myosin.

The maximal velocity of shortening of atrial muscle, which is several-fold greater than that of ventricle [8,9] is at least in part due to the presence of an isozyme of myosin which has a higher actin-activated myosin ATPase activity. Since 25% of myosin light chain LC_1 and 50% of LC_2 light chain can be dissociated from the myosin oligomer with no corresponding decrease in myosin ATPase activity, it appears that differences we observe between atrial and ventricular myosin ATPase activity are due to alterations in the heavy chains [2]. However, the structural variances

Table 2

Light chains complexed to heavy chains (% control)		ATPase activity (% control)		
LC_1	LC_2	K^+-activated	Ca^{2+}-activated	Actin + Mg^{2+} activated
76	40	103	98	76

The K^+ and Ca^{2+} stimulated ATPase activities were analyzed as in [1]. Actin + Mg^{2+}-stimulated ATPase activity was measured in a mixture containing 0.3×10^{-4} M $CaCl_2$, 0.02 M imidazole (pH 7.0) 1 mM $MgCl_2$, 0.03 M KCl and 0.8 mM ATP. All other conditions were as in [1,6]. Using these conditions we obtained 0.10 μmol P_i/mg.min for ventricular myosin and 0.21 μmol P_i/mg.min for atrial myosin. Assays were for 5 min at 35°C

Light chain dissociation and interchange was carried out with sheep myosin because of the higher ATPase activity

reported here for the LC_1 light chain, may partially account for the differences in actin-activated myosin ATPase activity. These aspects are presently being studied, along with the role of light chains in relation to force generation during muscle contraction.

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