ATRIAL AND VENTRICULAR CARDIAC MYOSINS CONTAIN DIFFERENT HEAVY CHAIN SPECIES

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1. Introduction

Myosin isolated from the atria of the heart has been reported to have greater ATPase activity and a different light chain composition than myosin prepared from the ventricles [1]. However, no differences between the heavy chains of these myosins were found by immunodiffusion techniques.

We wish to report here that atrial and ventricular myosins isolated from calf heart possess heavy chains with different primary structures. We have compared cyanogen bromide digests of atrial and ventricular myosins in a two-dimensional gel electrophoresis system which resolves the myosin heavy chain peptides quite well. The results show firstly that there are differences in the patterns of heavy chain peptides from these myosins. Secondly, they demonstrate a difference in the distribution of [^{14}C]carboxymethylated cysteine containing heavy chain peptides. To our knowledge, this is the first demonstration of a structural difference between cardiac atrial and ventricular myosin heavy chains.

2. Materials and methods

2.1. *Preparation and assay of myosins*

Myosin was isolated from the atria and left ventricles of ~3–4 month Holstein calves as in [2], and purified by chromatography in DEAE–Sephadex A-50 by the methods in [3]. Fractions from the major peak of protein were pooled and precipitated by addition of saturated $(NH_4)_2SO_4$ to final conc. 50% and collected by low speed centrifugation.

The Ca^{2+}-ATPase activity of myosin was measured as in [2] at 30°C in medium containing 0.05 M Tris/HCl, pH 7.6, 0.05 M KCl, 0.005 M ATP and 0.01 M $CaCl_2$. To determine the K^+(EDTA)-ATPase activity, $CaCl_2$ was replaced by 0.01 M EDTA and KCl was increased to 0.5 M. Inorganic phosphate liberated in the reactions was measured by the method in [4].

2.2. S-*carboxymethylation*

$(NH_4)_2SO_4$-precipitated myosin was dissolved by dialysis against 0.5 M KCl, 0.01 M Tris/HCl, at pH 7.5. Dry urea and pH 8.0 Tris/HCl were added to final conc. 8 M and 0.1 M, respectively. Dithioerythritol was added in 100-fold excess over the number of cysteine residues in myosin and the mixture was stirred under a stream of dry nitrogen for 4 h at room temperature (22–24°C) to insure that all thiol groups were completely reduced. An amount of iodo-[^{14}C]acetate (0.14 mCi/mmol) was added, equivalent to a 5% excess over the total number of thiol groups present. After 30 min, glacial acetic acid was added to pH 4.0 to stop the reaction and the solution was dialyzed exhaustively against 25% acetic acid.

2.3. *Cyanogen bromide cleavage*

The lyophilized proteins were dissolved in 70% formic acid and treated for 17 h with a 200-fold excess of cyanogen bromide over the no. mol

Abbreviations: bis–Tris, bis-(2-hydroxyethyl)-imino-tris-(hydroxymethyl) methane; MES, 2(*N*-morpholino)ethane sulfonic acid; PPO, 2,5-diphenyloxazole

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methionine residues. Amino acid analysis revealed a 92–94% decrease in methionine content.

2.4. *Two dimensional polyacrylamide gel electrophoresis*

The cyanogen bromide peptides of myosin heavy chains were resolved by two-dimensional polyacrylamide gel electrophoresis using a modification of the method in [5]. The slab gel apparatus used for the second dimension separations permitted two samples to be run simultaneously.

The protein digests were dissolved in 0.1 M Tris–glycine/HCl buffer, pH 8.6, containing 8 M urea, 1% β-mercaptoethanol and 10% glycerol and incubated for 1 h at room temperature (22–24°C). For first-dimensional separations, ~150 μg myosin digests were applied to 0.5 × 9.5 cm disk gels containing 10% polyacrylamide, 0.5% *N,N'*-diallyltartardiamide (w/v), 8 M urea, 0.02 M Tris–glycine/HCl buffer, pH 8.6. A solution of 0.02 M Tris–glycine/HCl, pH 8.6, was used in the upper and lower electrode chambers. Electrophoresis was carried out at 20°C either toward the anode for 3.5 h at 2 mA/gel tube or toward the cathode for 4 h at 3.0 mA/gel tube.

Immediately after electrophoresis in the first dimension, gels were removed from their tubes and placed along the top of the second dimensional gel slabs. The slab gels were 10 × 10 × 0.5 cm and contained 0.143 M bis–Tris/HCl, pH 6.75, 9.5% acrylamide, 1.1% *N,N'*-diallyltartardiamide, 7 M urea and 0.02% SDS. A spacer gel solution comprised of 0.57 M bis–Tris/HCl buffer, pH 5.0, 7.5% acrylamide and 0.9% methylene bisacrylamide then was poured over the disk gel. The upper electrode solution contained 0.2% SDS, 0.07 M MES, 0.07 M bis–Tris/HCl, pH 6.75, and the lower electrode solution was 0.028 M bis–Tris/HCl, pH 6.75. Electrophoresis was performed at 25 mA/slab for 4–5 h at 20°C. Actin (42 000 daltons), beef cardiac myosin light chain 1 (22 000 daltons) and the cyanogen bromide peptides of myoglobin (see [6] for molecular masses) were run simultaneously in the second dimension as a guide to the approximate molecular masses of the peptides. The slab gels were stained with Coomassie brilliant blue by the Weber and Osborn method [7]. After destaining, gels were photographed using Kodak high-contrast copy film with a Tiffen no. 12 yellow filter.

For fluorography, gels were equilibrated with dimethyl sulfoxide and impregnated with PPO by immersion in 4 vol. 22% (w/v) PPO in dimethyl sulfoxide as in [8]. After soaking in water to remove the dimethyl sulfoxide, the polyacrylamide gels were dried down, supported on Whatman 3 MM paper using a commercial gel drying apparatus (Bio-Rad Labs, Model 224). When dry, the gels were placed in cassettes with Kodak XR-5 film and exposed at −70°C for 4–6 weeks.

3. Results and discussion

The ATPase activities of atrial and ventricular cardiac myosins are compared in table 1. Although the Ca^{2+}-ATPase activity of atrial myosin was nearly 2-fold greater than the value obtained for ventricular myosin, the K^+(EDTA)-ATPase activities of these proteins were about the same. These results are similar to those in [1] using atrial and ventricular myosins isolated from dog heart.

The amino acid composition of the two cardiac myosin isozymes is given in table 2. The cysteine content of atrial myosin is significantly smaller than that of ventricular myosin, that is, 6 cysteine residues/10^5 g versus 8 cysteinyls/10^5 g. Otherwise, the amino acid composition of atrial and ventricular cardiac myosins appeared to be similar.

The patterns obtained upon two-dimensional gel electrophoresis of atrial and ventricular myosin are compared in fig.1,2. These patterns represent myosin heavy chain peptides, since under the conditions used in these experiments, the small peptides arising from myosin light chains migrate off the first-dimensional gels. As shown in fig.1, when first-dimensional gels

Table 1
Comparison of the ATPase activities of atrial and ventricular cardiac myosins

Myosin source	Ca^{2+}-ATPase (μmol P_i/mg/min)	K^+(EDTA)-ATPase (μmol P_i/mg/min)
Atria	0.56 ± .05	0.93 ± .08
Ventricle	0.30 ± .03	1.00 ± .10

Assays were performed as in section 2. Values are means ± SE for 4 experiments

Table 2
Amino acid composition of atrial and ventricular cardiac myosins expressed as residues/10^5 g

	Atrial	Ventricular
Cysteine	6.4	8.4
Aspartic acid	91.7	88.8
Threonine	34.9	36.2
Serine	34.3	32.0
Glutamic acid	166.8	166.2
Proline	21.2	21.5
Glycine	37.4	35.5
Alanine	75.0	76.5
Valine	38.4	37.2
Methionine	22.7	23.2
Isoleucine	38.0	37.2
Leucine	100.2	95.4
Tyrosine	16.0	15.7
Phenylalanine	29.7	31.9
Histidine	20.0	20.6
Lysine	87.1	90.3
Arginine	52.9	51.3

Each value is the average of 3 or 4 determinations. The cysteine content was determined as *S*-carboxymethyl cysteine. Samples were hydrolyzed for 22 h in 6 N HCl in evacuated, sealed tubes. Analyses were performed using a single column system with Beckman AA-15 resin and buffers prepared as described in the Beckman manual

were run toward the anode about 44–47 distinct spots could be identified in atrial myosin (fig.1A) and 44–46 distinct spots in ventricular myosin (fig.1B). Comparison of the gels revealed that there were at least 9 peptides in atrial myosin which were not present in ventricular myosin. Also there were 11 peptides in ventricular myosin which were not present in atrial myosin. Essentially identical results were obtained with digests of two preparations of each type of myosin.

Atrial and ventricular myosins (~470 000 daltons) both contain about 108 methionine residues/mol (table 2). There were about 12 methionine residues in the two light chains of ventricular myosin [9]. Assuming that the methionine content of atrial light chains are similar, the heavy chains of both myosins should contain about 96 methionine residues/mol (~210 000 daltons). If the two heavy chains in each type of myosin are identical, they should give rise to about 49 cyanogen bromide peptides. By this reasoning, the number of spots in these 'maps' is somewhat smaller than would be anticipated.

To detect additional basic peptides which might not migrate toward the anode at pH 8.6, first-dimensional separations also were run toward the cathode

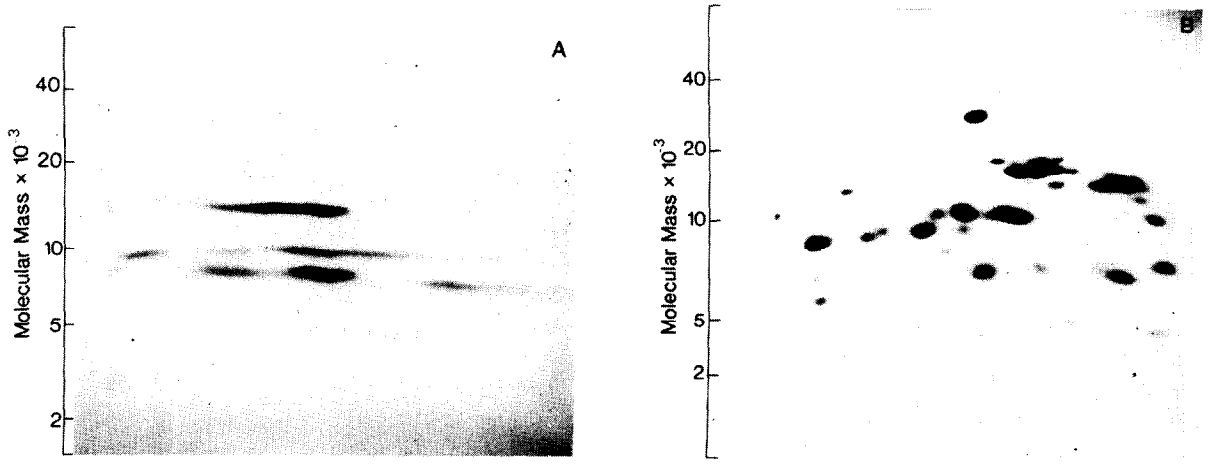

Fig.1. Two-dimensional electrophoresis of cyanogen bromide digests of myosins. First-dimensional separations were performed in polyacrylamide disk gels containing 8 M urea and 0.02 M Tris–glycine/HCl buffer, pH 8.6, and run toward the anode. Second-dimensional separations were performed in SDS–urea polyacrylamide slab gels which were prepared and run as in section 2. Under these running conditions, the patterns obtained represented peptides arising from myosin heavy chains. The direction of the first-dimensional separation was from left to right and the second-dimensional separation was from top to bottom. (A) Digest of atrial myosin. (B) Digest of ventricular myosin.

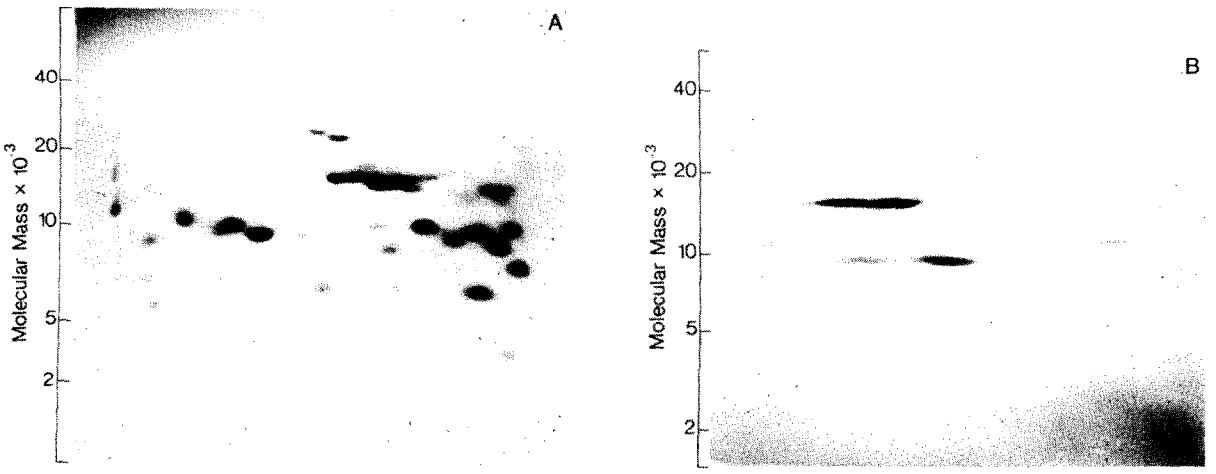

Fig.2. Two-dimensional electrophoresis of cyanogen bromide digests of myosins. The electrophoretic techniques used were the same as in fig.1, except that first-dimensional separations were performed toward the cathode. The SH_1–SH_2 thiol peptide migrates as two components in first-dimensional gels; the reason for this charge heterogeneity is unknown. The direction of the first-dimensional separation was from left to right and the second-dimensional separation was from top to bottom. (A) Digest of atrial myosin. (B) Digest of ventricular myosin.

(fig.2). This revealed about 12–14 spots in both proteins, most of which stained faintly even when the amount of material applied was increased 3-fold. It should be noted that these spots are grouped into 'rows' corresponding to molecular masses of about 16 000, 10 000, 8000 and 5000 daltons, suggesting that there might actually be only 4 or 5 cathodically migrating peptides which exhibit charge heterogeneity. This possibility was supported by the observation that the two major spots at ~8000 daltons in atrial and ventricular myosins co-electrophoresed with a peptide isolated in this laboratory from beef ventricular cardiac myosin which contains two essential thiol groups, the so-called SH_1 and SH_2 thiols [10]. The reason for the charge heterogeneity for this peptide is unclear, but it may arise as a result of partial deamination of asparagine or glutamine residues or other alterations during isolation.

Figure 3 schematically summarizes the results obtained from two-dimensional electrophoretic studies of atrial and ventricular myosin heavy chains. When the first-dimensional separations were performed toward the anode (fig.3A) about 29 of the peptides appeared to be common to both proteins. In addition, 18 peptides were found only in ventricular myosin and 16 were present only in atrial myosin. When the first-dimensional separations were performed toward the cathode (fig.3B) about eight of the peptides appeared common to both proteins. Six additional peptides were detected only in ventricular myosin and 6 were unique to atrial myosin.

The positions of radiolabeled thiol containing heavy chain peptides also are indicated in fig.3. In atrial myosin there were a total of 23 labeled spots, 15 of which migrated toward the anode in the first-dimensional separation and 8 migrated toward the cathode. In ventricular myosin heavy chains, there were 27 labeled spots, 18 of which migrated toward the anode in the first-dimensional separation and nine migrated toward the cathode. Interestingly, 12 of the labeled peptides were common to both proteins. The smaller number of thiol peptides in digests of atrial myosin is consistent with its lower cysteine content (table 2).

The present results demonstrate that there are differences in primary structure between the heavy chains of atrial and ventricular myosins. The presence of myosin isozymes in atrium and ventricle may be related to differences in the contractile properties of these chambers. It has been observed frequently that atrial contraction appears to be more rapid and shorter in duration than that of

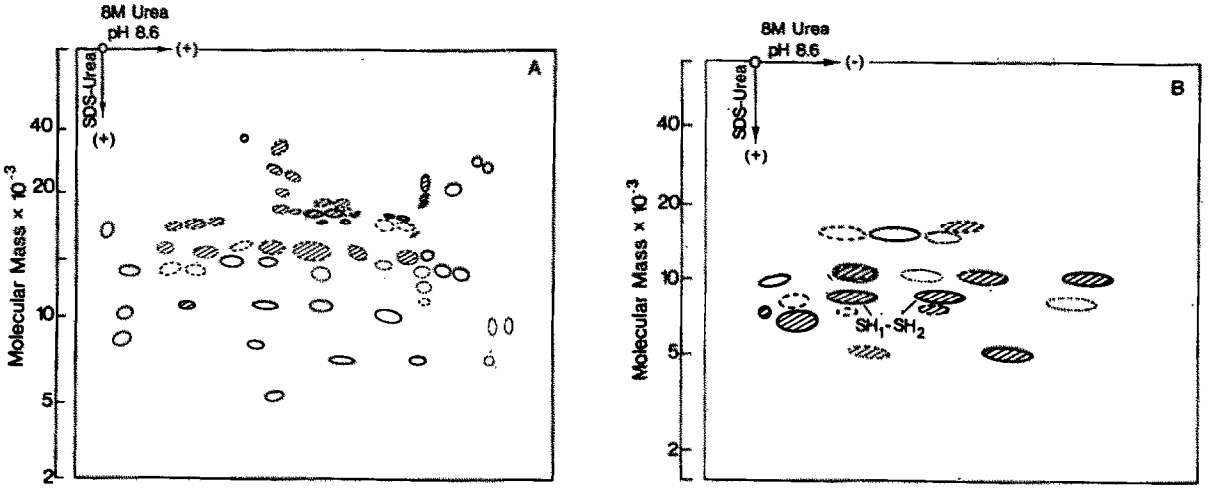

Fig.3. Schematic representation of the patterns obtained upon two-dimensional polyacrylamide gel electrophoresis of cyanogen bromide digests of atrial and ventricular myosins in which all the thiol groups were blocked using iodo-[^{14}C]acetate. (A) First-dimensional separations performed toward the anode. (B) First-dimensional separations performed toward the cathode. Peptides common to atrial and ventricular myosins (——); peptides found only in atrial myosin (· · ·); peptides found only in ventricular myosin (– – –); radiolabeled thiol peptides (/ / /).

the ventricles in the same spontaneously beating heart [11,12]. This phenomena has been verified recently by direct comparison of atrial and ventricular mechanical performance under similar experimental conditions [13–15]. These studies indicate that atrial muscle achieves the same developed and total tensions but in a shorter time than ventricular muscle. At any given muscle length, the rate of tension rise in isolated atrial strips or trabeculae is at least twice that for ventricular trabeculae or papillary muscles. Also, at any given load up to 1.5 g/mm^2, the maximal velocity of shortening of atrial muscle is about three to four times greater than ventricular muscle.

Since there appears to be a close relationship between the speed of muscular contraction and the myosin ATPase activity among various muscle types [16], the greater velocity of atrial contraction may be related directly to the higher Ca^{2+}-ATPase activity of myosin and the greater Mg^{2+}-ATPase of myofibrils [13] isolated from atrial muscle. The finding that the K^+(EDTA)-ATPase activity of atrial and ventricular myosins is similar probably can be explained by the fact that the mechanism of ATP hydrolysis by myosin is different depending upon whether monovalent or divalent metal cations are present [17]. In the presence of calcium or magnesium, hydrolysis of ATP proceeds via formation of a series of reaction intermediates that do not occur in the presence of potassium and a divalent metal cation chelator such as EDTA. Thus it seems likely that the ATPase activities of atrial and ventricular myosins are different only with regard to the reaction pathway involving divalent metal cations. The latter is thought to be the physiologically important catalytic mechanism, and therefore, is more likely to parallel the mechanical performance of the muscle.

Acknowledgements

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