Characteristics of ATPase activity and the subunit composition of myosin in the conduction system of bovine heart

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The ATPase activity, light chains and isoenzymes of myosin from specialized myocardial tissue (the A-V node, bundle of His, and right and left bundle branches) of bovine heart were compared with those of atrial and ventricular myosins. The order of Ca^{2+}-activated ATPase activity was atrial > specialized myocardial tissue > ventricular myosin. SDS-polyacrylamide gel electrophoresis showed that myosin from the specialized myocardial tissue contained the light chains of both atrial and ventricular myosins. On the other hand, the specialized myocardial tissue contained one V_3 isomyosin and showed no difference from ventricular myocardial tissue on pyrophosphate gel.

Cardiac conduction system *Specialized myocardial tissue* *Myosin* *ATPase* *Myosin light chain* *Myosin isoenzyme*

1. INTRODUCTION

The cardiac conduction system consists of a specialized type of myocardial tissue serving as a conduction system. Previously [1] we reported that the compositions of myosin light chains in the specialized and ventricular myocardial tissues of bovine heart are quite different. In addition to the two light chains present in ventricular myocardial tissue (LC_1 and LC_2), myosin from the specialized myocardial tissue has another light chain of slightly lower molecular mass (22.5 kDa) than LC_1 (25 kDa). Here we compared the ATPase activities, light chain compositions and isoenzymes of myosin from the specialized myocardial tissue with those of atrial and ventricular myosins.

2. MATERIALS AND METHODS

2.1. *Isolation of the conduction system*

The conduction system (specialized myocardial tissue) and left atrial and ventricular myocardial tissues were isolated from bovine heart. The conduction system consisted of the atrioventricular node, the bundle of His and the right and left bundle branches. Rat ventricular myocardial tissue was obtained from adult Wistar rats.

2.2. *Preparation of myosin*

Purified myosin was prepared from the specialized, atrial and ventricular myocardial tissues. All procedures for purification of myosin were carried out at 4°C. The tissues were minced, extracted for 14.5 min with 10 vols of 0.6 M KCl, 1 mM EDTA, 1 mM ATP and 20 mM histidine buffer (pH 7.0) with stirring, and purified by the dilution technique in [1]. The myosin was purified further by column chromatography on DEAE–Sephadex A-50 [2].

2.3. *Determination of ATPase activity*

Assays were performed for 5 min at pH 7.5 and 25°C in a final volume of 1 ml. Reactions were started by adding ATP and stopped by adding 1 ml

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of 10% perchloric acid, and the precipitated protein was removed by centrifugation. ATPase activity was determined by measuring liberated inorganic phosphate as in [3]. The reaction mixture contained 50 mM Tris and 3 mM Na_2ATP and myosin at 0.2 mg/ml. K^+, EDTA-activated and Ca^{2+}-activated ATPase activities were assayed in the presence of 1 mM EDTA with 0.6 M KCl and 10 mM $CaCl_2$ plus 50 mM KCl, respectively.

2.4. *SDS-polyacrylamide gel electrophoresis*

SDS-polyacrylamide gel electrophoresis of myosin preparations was carried out on 10% cross-linked gel containing 0.1% SDS under the electrophoretic conditions of [4]. Gels were prepared in 5 mm (i.d.) × 6.0 cm tubes. Electrophoresis was carried out in 0.1 M sodium phosphate buffer, pH 7.0, containing 0.1% SDS, at a constant current of 6 mA/gel for approx. 5 h. Gels were stained as in [1]. Molecular mass was determined from the electrophoretic mobility on SDS gel with ovalbumin, carbonic anhydrase, soybean trypsin inhibitor and α-lactalbumin as markers. The proportions of protein in the various subunits of myosin were measured as in [5].

2.5. *Pyrophosphate gel electrophoresis*

Pyrophosphate gel electrophoresis of myosin preparations was performed as in [6]. Cylindrical polyacrylamide gels [5 mm (i.d.) × 6.0 cm] were prepared from 3.88% (w/v) acrylamide and 0.12% (w/v) *N,N'*-methylenebisacrylamide in buffer consisting of 20 mM pyrophosphate (pH 8.8), 10% (v/v) glycerol and 0.15% (v/v) *N,N,N',N'*-tetramethylethylenediamine. Electrophoresis was carried out in a Pharmacia GE4 electrophoresis chamber with buffer containing 20 mM pyrophosphate (pH 8.8) and 10% (v/v) glycerol recirculated between the lower and upper chambers. The buffer was maintained at 2°C, and electrophoresis was carried out at a constant voltage of 14 V/cm for 18 h. The gels were stained as in [7].

3. RESULTS AND DISCUSSION

Table 1 shows the myosin ATPase activities of the specialized, ventricular and atrial myocardial tissues of bovine heart. The Ca^{2+}-activated ATPase activity of atrial myosin was approximately twice that of ventricular myosin, as reported [6,8–11], and the activity of myosin from the specialized myocardial tissue was intermediate between those of atrial and ventricular myosins. The K^+,EDTA-activated ATPase activities of the 3 myocardial tissues were approximately the same.

Table 1

ATPase activities of myosins from the specialized, ventricular and atrial myocardial tissues

Myocardial tissue	K^+,EDTA-ATPase (μmol P_i/mg per min)	Ca^{2+}-ATPase (μmol P_i/mg per min)	
Specialized	0.323 ± 0.026	0.248 ± 0.009	*
Ventricular	0.355 ± 0.020	0.185 ± 0.006	* **
Atrial	0.412 ± 0.048	0.377 ± 0.033	*

The mixtures for assay of K^+,EDTA- and Ca^{2+}-activated ATPases are described in section 2. Values are means ± SE for 5 experiments. * $p < 0.001$, ** $p < 0.01$

On SDS-polyacrylamide gel electrophoresis, myosins gave bands of light chains: SL_1, SL_2 and SL_3 for myosin from the specialized myocardial tissue, VL_1 and VL_2 for ventricular myosin, and AL_1 and AL_2 for atrial myosin, as shown in fig.1. On electrophoresis of mixtures of these myosins, the bands of SL_1 and VL_1, SL_2 and AL_1, and SL_3, VL_2 and AL_2, respectively, were superimposed. The molecular masses of the light chains calculated from the calibration curve were 25 kDa for SL_1 and VL_1, 22.5 kDa for SL_2 and AL_1 and 18 kDa for SL_3, VL_2 and AL_2.

The ratio of the amounts of protein present in myosin heavy and light chains was determined by measuring the amounts of dye eluted from bands in SDS-polyacrylamide gel (6%). The percentage of heavy chain in myosin protein was about 84% in all 3 myocardial tissues. Table 2 shows the percentage proportions of the light chains of myosin. The percentage proportions of 25-, 22.5- and 18-kDa subunits from the specialized, ventricular and atrial myocardial tissues were about 25:32:43, 55:0:45 and 0:63:37, respectively.

It has been demonstrated that the electrophoretic mobility and immunological and structural properties of the light chains in atrial and ventricular myosins are different [6,8,12]. Interestingly, authors in [13] observed that a myosin

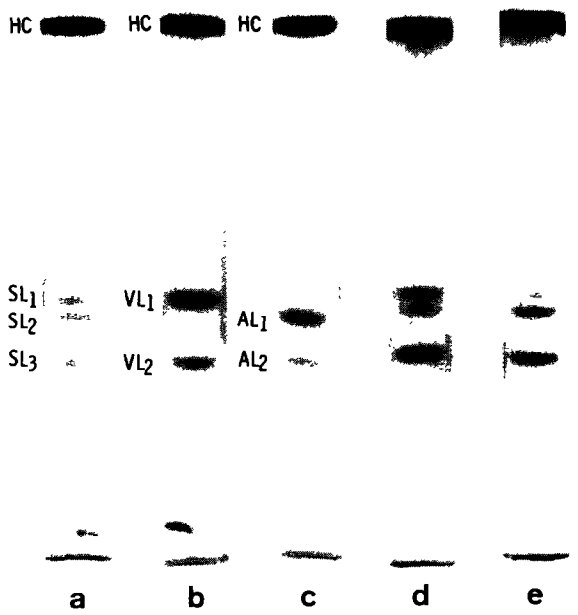

Fig.1. SDS-polyacrylamide gel (10%) electrophoretic patterns of myosins from the specialized, ventricular and atrial myocardial tissues. (a) Myosin from specialized myocardial tissue (6 μg), (b) ventricular myosin (6 μg), (c) atrial myosin (6 μg), (d) mixed sample (3 μg myosin from specialized myocardial tissue and 3 μg ventricular myosin), (e) mixed sample (3 μg myosin from specialized myocardial tissue and 3 μg atrial myosin). HC, heavy chain; L_1, L_2 and L_3, light chain 1, 2 and 3, respectively; S, V and A, specialized, ventricular and atrial myocardial tissue, respectively.

Table 2

Percentage proportions of the light chains of myosin obtained from SDS-polyacrylamide gels

Myocardial tissue	Light chain (Da)		
	25000	22500	18000
Specialized	25 (SL_1)	32 (SL_2)	43 (SL_3)
Ventricular	55 (VL_1)	0	45 (VL_2)
Atrial	0	63 (AL_1)	37 (AL_2)

The ratio of the amounts of protein present in light chains was determined by measuring the amounts of dye eluted from bands in SDS-polyacrylamide gel (6%)

light chain present in fetal but not adult ventricular myosin was very similar, and possibly identical, to the light chain found in fetal or adult atrial and adult Purkinje fiber (false tendon) myosins of bovine heart on two-dimensional gel electrophoresis. In our experiment, the false tendon was not included in the preparation of specialized myocardial tissue, but SL_2 appeared identical to AL_1 on SDS-polyacrylamide gel and this light chain was not found in ventricular myosin. We are now studying whether SL_2 (or AL_1) is identical to the light chain of atrial and Purkinje fiber myosins demonstrated in [13].

On pyrophosphate gel electrophoresis, atrial and ventricular myosins of bovine heart gave single major bands that had very similar mobilities to rat V_1 and V_3 isomyosins, respectively, as shown in fig.2. Previous studies using pyrophosphate gel electrophoresis showed that bovine left ventricle contained one V_3 isomyosin [14,15] and authors in

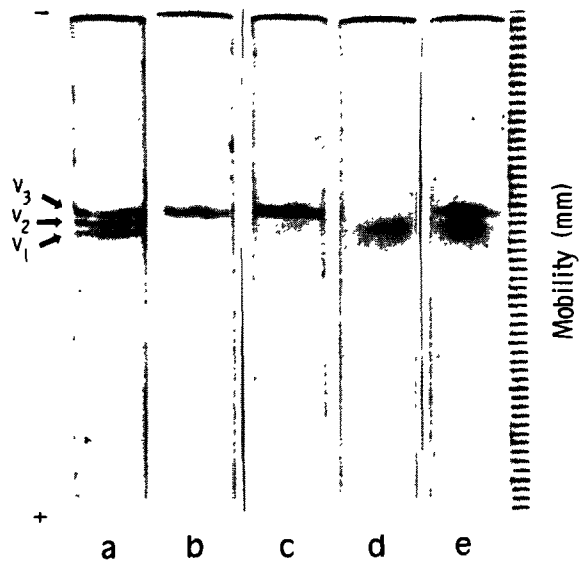

Fig.2. Pyrophosphate gel electrophoresis of myosin from various rat and bovine myocardial tissues. Electrophoresis was carried out in 4% polyacrylamide gels with a voltage gradient of 14 V/cm at 2°C for 18 h. (a) Myosin from rat ventricular myocardial tissue (2 μg), (b) myosin from specialized myocardial tissue of bovine heart (2 μg), (c) bovine ventricular myosin (2 μg), (d) bovine atrial myosin (2 μg), (e) mixed sample (1 μg bovine ventricular myosin and 1 μg bovine atrial myosin).

[15] found that this material had scarcely any reactivity with an anti-V_1 monoclonal antibody. Atrial myosin was reported to contain V_1-type epitopes [15]. Furthermore, it was demonstrated that the primary structures of the heavy chains of atrial and ventricular myosins are different [9,10]. The specialized myocardial tissue of bovine heart contains one V_3 isomyosin and no difference was found on pyrophosphate gel between the specialized and ventricular myocardial tissues (fig.2). Moreover, authors in [13] found no difference in the one- and two-dimensional peptide maps of myosin heavy chains of the left ventricular tissue and the conduction tissue, strongly suggesting that the conduction tissue contains a V_3 isomyosin.

Since the isoenzyme compositions of myosins from the specialized and ventricular myocardial tissues are the same, it seems probable that the higher Ca^{2+}-activated ATPase activity of myosin from the specialized myocardial tissue than that of ventricular myosin is related to the difference in the light chain compositions of the two myosins.

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