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THE ATPase ACTIVITY OF SUBFRAGMENT-1 FROM THE HYPERTROPHIED HEART

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Summary

Myosin and subfragment-1 were prepared from rabbit hearts hypertrophied secondary to pulmonary artery constriction. The Ca^{2+}-stimulated ATPase activity was reduced while the potassium/EDTA-stimulated ATPase activity was unchanged in both the myosin and subfragment 1 (S-1) from hypertrophied hearts. When hypertrophy myosin was mixed with an equal quantity of control myosin, the ATPase activity of the mixed protein fell halfway between control and hypertrophy values. Similar results were obtained with control and hypertrophy S-1. The actin-stimulated ATPase activity of hypertrophy S-1 was slightly depressed but unlike hypertrophy myosin this depression was not significant when compared to normal S-1. This suggests that papain cleavage may have removed part of the conformational difference that exists between control and hypertrophy myosins.

Introduction

Cardiac hypertrophy induced by chronic pressure overload leads to mechanical and biochemical alterations of the heart. Several investigators have demonstrated a depression in cardiac contractility [1—3] and a decrease in the cardiac myofibrillar [4—6], actomyosin [7,8], and myosin [9—11] ATPase activities. As reported recently in this laboratory [12], the Ca^{2+}- and actin-stimulated ATPase activities of hypertrophy myosin are reduced, whereas the potassium/EDTA- and *N*-ethylmaleimide-stimulated ATPase activities are not changed. The depressed activities have been correlated with depressed mechanical properties in papillary muscles taken from the same hearts [13].

The above observations suggest that a structural alteration of myosin is

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produced in response to the hypertrophic stress. It was postulated that these changes occur near the reactive first class sulfhydryl (SH_1) group [12]. This conformational change appeared to allow the SH_1 group of myosin from hypertrophied hearts to be functionally intact with regard to its role in potassium/EDTA- and *N*-ethylmaleimide-stimulated ATPase activities but functionally deficient with regard to Ca^{2+}- and actin-stimulated ATPase activities.

Since the globular head region of the myosin molecule contains the enzymatic reactive sites, an investigation was carried out to cleave the myosin with papain and observe if this conformational changes persists in myosin subfragment-1 (S-1) isolated from hypertrophied hearts.

Methods

Animal model. Right ventricular hypertrophy was induced in male albino rabbits (1.7—2.0 kg) by a 66% reduction in the external diameter of the pulmonary artery [1]. Hemodynamic evaluation of this model indicated that hypertrophy develops without evidence of failure [9]. Animals were killed by a blow to the head 35—45 days postoperatively. Since it was shown previously that dry to wet weight values for control and hypertrophied hearts were identical [1], hypertrophy was verified by the ratio of the right ventricular weight to total ventricular weight.

Unoperated weight- and sex- matched rabbits were used as controls. To obtain sufficient tissue (approx. 4.0 g) for control and hypertrophy preparations, 4 control right ventricles and 2 hypertrophied right ventricles were pooled in each experiment.

Myosin preparation. Myosin was prepared by the method of Shiverick et al. [14]. This method gives a yield of 4—6 mg myosin/g starting material for both control and hypertrophy myosins. RNA content was less than 1% in all cases as determined by the A_{280nm}/A_{260nm} ratio of greater than 1.4 for the final myosin preparations. Dithiothreitol was removed prior to use of myosin samples in ATPase assays by dialysis at room temperature for 4 h under N_2 against 0.5 M KCl/0.05 M Tris · HCl (pH 7.6).

S-1 preparation. S-1 was prepared by papain digestion of insoluble myosin by the method of Balint et al. [15]. Myosin was prepared for cleavage following the final 100 000 × *g* centrifugation by overnight dialysis against 2 mM KCl/3 mM EDTA/20 mM sodium phosphate buffer (pH 6.5), 2 mM dithiothreitol at 4°C. Crystalline papain was activated before use by incubating with cysteine (50 mM) for 1 h at 35°C. Papain activity was usually between 20 and 30 *N*-benzoyl-L-arginine-ethyl (Bz-Arg-OEt) units/ml. Myosin (2—3 mg/ml) was digested for 20 min at 23°C and constant pH 6.5 (using pH-stat apparatus) in a mixture containing 2 mM KCl/3 mM EDTA/20 mM sodium phosphate buffer/2 mM dithiothreitol/0.055 Bz-Arg-OEt units papain per mg myosin. The cleavage reaction was stopped by addition of freshly neutralized 1 M iodoacetic acid giving a final concentration of 2 mM. Light meromyosin, undigested myosin, and the insoluble rod portion of myosin were then removed by centrifugation at 100 000 × *g* for 1 h. The supernatant, containing S-1 and subfragment-2 (S-2), was dialyzed overnight at 4°C against 2 mM KCl, 0.03 M Tris · HCl (pH 7.7), and 1 mM iodoacetic acid. The protein mixture was then

applied to a 13 cm × 0.9 cm DE-52 cellulose ion exchange column which had been pre-equilibrated with 0.03 M Tris · HCl (pH 7.7) at 4°C. After washing the column with 0.03 M Tris · HCl, S-1 was eluted with 0.13 M KCl/0.03 M Tris · HCl (pH 7.7). The yield of S-1 from both control and hypertrophied hearts was approximately 1.2 mg/10 mg myosin cleaved.

Actin preparation. Actin was extracted from the back and leg muscles of rabbits by the following modification of the method of Rees and Young [16]. After the first polymerization step, KCl was adjusted to 0.6 M and the viscous mixture was stirred gently for 1.5 h [17]. After the final polymerization (0.05 M KCl/0.5 mM $MgCl_2$), F-actin was collected by centrifugation for 2 h at 105 000 × *g* and resuspended in 0.5 M KCl/2 mM imidazole (pH 7.0) at a concentration range of 4.5—6.5 mg/ml. Actin was freshly prepared for each experiment and appeared to be free of any significant contamination (Fig. 1).

ATPase assay. Ca^{2+}-stimulated ATPase activity was assayed in 0.05 M KCl/9 mM $CaCl_2$/4 mM ATP/0.05 M Tris · HCl (pH 7.6). The assay mixture for potassium/EDTA-activated ATPase contained 0.6 M KCl/1 mM EDTA/5 mM ATP/0.05 M Tris · HCl (pH 7.6). The actin-stimulated ATPase activity was assayed in 0.03 M KCl/2.5 mM ATP/2.5 mM $MgCl_2$/0.02 M imidazole (pH 7.0). Protein concentrations of myosin and S-1 for all assays were 0.1 mg/ml in a final volume of 1.0 ml. Reactions were started by addition of the protein and stopped at a time such that no more than 10% of the substrate was hydrolyzed. All assays were carried out at 25°C and were stopped by addition of 0.5 ml cold 20% $HClO_4$. Precipitated protein was removed by centrifugation and inorganic phosphate determined by a modified Fiske-Subbarow method [18] using a Technicon autoanalyzer.

Protein determination. Actin, Myosin and S-1 concentrations were measured by the Biuret method [19] and the latter two using molar extinction coefficients, $E^{280}_{1\%}$, of 5.60 and 9.20.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis. All protein preparations were subjected to gel electrophoresis in 7.5% polyacrylamide gels containing 0.1% SDS and either 25 mM Tris/192 mM glycine buffer (pH 8.3) or 200 mM sodium phosphate buffer (pH 6.9) [20]. Gels were stained with Coomassie Brilliant Blue. Molecular weights were determined from standard curves with proteins of known molecular weights.

Material. Dithiothreitol was obtained from Calbiochem, and ATP, iodoacetic acid, and Bz-Arg-OEt from Sigma Chemical Co. Electrophoresis grade reagents were obtained from Bio Rad Laboratories. All other reagents were analytical grade. Double glass-distilled water was used throughout this study.

Results

Cardiac S-1 subunit structure. The banding patterns of S-1 indicate the presence of three major fragments (Fig. 1). The molecular weights of these fragments depend upon the buffer used in the SDS electrophoresis (Table I). With Tris · glycine buffer the molecular weights of the three major components of S-1 were 79 900, 69 900 and 21 900, whereas the phosphate buffer produced an increase of 16, 9 and 5%, respectively, for the S-1 components. Balint et al. [15] also observed three major components of cardiac S-1 from

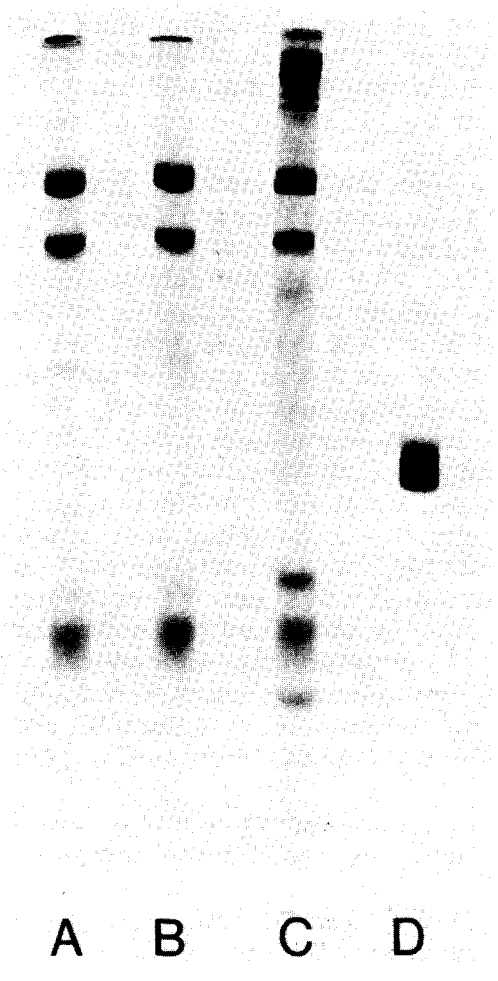

Fig. 1. SDS polyacrylamide gels (7.5%) of various protein preparations. (A) Cardiac control S-1 (20 μg); (B) Cardiac hypertrophy S-1 (20 μg); (C) Cardiac control myosin (35 μg) + cardiac control S-1 (18 μg) (D) Skeletal actin (27 μg).

SDS-6% polyacrylamide gels. Using a sodium phosphate buffer (pH 7.0), they reported molecular weights of major fragment 1 and 2 intermediate between our values observed with Tris · glycine and phosphate buffers (Table I). Major fragment 3 was similar to that observed in our studies. The myosin light chain 1 was 7% heavier using the phosphate buffer, whereas the myosin light chain 2 was approximately the same for the 2 buffers. There were no significant differences between the molecular weights of control and hypertrophy myosin light chains or S-1 fragments using the Tris · glycine or sodium phosphate buffers.

The cardiac S-1 light chains from control and hypertrophied hearts appear to be broken down (Fig. 1). There is only a faint band at the region of the cardiac light chain 1, whereas the cardiac light chain 2 disappears. This observation is similar to the results of Balint et al. [15]. They demonstrated that both light chains of cardiac myosin completely disappear after 2 min of papain digestion

TABLE I

MOLECULAR WEIGHTS OF SUBUNIT STRUCTURES OF CARDIAC MYOSIN AND S-1

The molecular weights were determined from SDS 7.5% polyacrylamide using either the Tris · glycine or sodium phosphate buffers. Values are mean ± S.E. for ( ) number of gels. There were no sigificant differences between control and hypertrophy myosin light chains.

	Tris/glycine buffer (pH 8.4)	Sodium phosphate buffer (pH 6.9)	Results of Balint et al. [15] (using sodium phophate buffer pH 7.0, SDS/6% polyacrylamide)
Control myosin			
Light chain 1	24 900 ± 380 (8)	27 500 ± 300 (11)	
Light chain 2	19 000 ± 110 (9)	19 400 ± 380 (12)	
Hypertrophy myosin			
Light Chain 1	25 100 ± 300 (9)	27 000 ± 290 (9)	
Light Chain 2	18 900 ± 200 (10)	19 300 ± 340 (9)	
Control S-1			
Major fragment 1	79 900 ± 560 (21)	95 200 ± 1580 (13)	89 900
Major fragment 2	69 900 ± 630 (18)	77 000 ± 1270 (13)	72 000—76 000
Major fragment 3	21 900 ± 360 (16)	23 000 ± 410 (13)	23 000—24 000
Hypertrophy S-1			
Major fragment 1	79 700 ± 730 (21)	95 600 ± 1610 (9)	
Major fragment 2	68 900 ± 570 (21)	77 300 ± 1050 (9)	
Major fragment 3	21 700 ± 390 (14)	23 300 ± 930 (9)	

with a gradual accumulation of material between the two cardiac light chains. In our studies it is not known if the light chain 1 cleavage is part of the 23 000-dalton component. Since the cardiac S-1 is enzymatically active (Table II), this suggests that either one or several of the S-1 fragments are components of the enzymatic complex.

Ca^{2+}- and potassium/EDTA-stimulated ATPase activities. Consistent with previous studies in this laboratory [12], the Ca^{2+}-stimulated ATPase of myosin from hypertrophied hearts was significantly depressed ($P < 0.001$) whereas there was no observed difference in potassium/EDTA-stimulated ATPase activity between control and hypertrophy myosins (Table II). Similarly, the

TABLE II

Ca^{2+}-STIMULATED AND POTASSIUM/EDTA-STIMULATED ATPase ACTIVITIES

Activities were determined according to the conditions described in Methods. Values are mean ± S.E. for N number of preparations. The differences in Ca^{2+}-stimulated ATPase activities are statistically significant ($P < 0.001$) by the paired Student's t-test. No significant differences were observed for the potassium/EDTA-stimulated ATPase activities.

	ATPase activity (P_i/mg · min)			
	Myosin		S-1	
Control	0.327 ± 0.015	(n = 13)	1.221 ± 0.060	(n = 13)
Hypertrophy	0.207 ± 0.014		0.869 ± 0.035	
Potassium/EDTA-stimulated ATPase activity				
Control	0.495 ± 0.028	(n = 11)	1.409 ± 0.149	(n = 8)
Hypertrophy	0.447 ± 0.038		1.479 ± 0.142	

TABLE III

Ca^{2+}-STIMULATED ATPase ACTIVITY OF MIXED CONTROL AND HYPERTROPHY MYOSINS AND CONTROL AND HYPERTROPHY S-1

Activities were determined according to the conditions described in Methods. Values are means ± S.E. for N number of preparations. Final protein concentration in pooled samples was 0.05 mg/ml control myosin or S-1 plus 0.05 mg/ml hypertrophy myosin or S-1.

	Ca^{2+}-stimulated ATPase activity (μmol P_i/mg · min)	
	Myosin (N = 7)	S-1 (N = 4)
Control	0.314 ± 0.016	1.271 ± 0.093
Hypertrophy	0.185 ± 0.237	0.925 ± 0.051
Control + hypertrophy	0.237 ± 0.016	1.079 ± 0.089

Ca^{2+}-stimulated ATPase activity of S-1 from hypertrophied hearts was significantly depressed ($P < 0.001$) and there was no difference in potassium/EDTA-stimulated ATPase activity.

In several experiments Ca^{2+}-stimulated ATPase activity was determined in an assay solution containing a mixture of control and hypertrophy myosins (Table III). In these preparations the mixed sample activity falls close to the value obtained when the separate control and hypertrophy ATPase values were averaged. Similar results were obtained with a mixed control and hypertrophy S-1 (Table III).

Actin activation of myosin and S-1. Recently, this laboratory documented a decreased actin-stimulated ATPase activity of myosin from hypertrophied hearts. The actin-stimulated myosin ATPase activities were measured at an actin to myosin weight ratio of 2 : 1 in order to verify this observation in the myosin samples used for preparation of S-1. It has been determined that this weight ratio produces near maximal actin-stimulated ATPase of myosin [12]. In the present studies, control myosin specific activity was 0.136 ± 0.010 μmol P_i $mg^{-1} \cdot min^{-1}$ compared to hypertrophy value of 0.094 ± 0.007 μmol P_i $mg^{-1} \cdot min^{-1}$. These values are significantly different ($P < 0.05$).

The actin-stimulated ATPase activities of S-1 from control and hypertrophied hearts were measured at a series of actin to S-1 weight ratios (2 : 1 to 16 : 1). These weight ratios are in the same range of molar ratios that was used previously for actin : myosin [12]. There was no significant difference in activation of the control and hypertrophy S-1 at any of the actin to S-1 weight ratios, although the hypertrophy S-1 activity was slightly depressed at all ratios except 2 : 1. From the Lineweaver-Burk plot the extrapolated V values are 1.41 and 1.03 μmol P_i $mg^{-1} \cdot min^{-1}$ and apparent K_m values for actin are 57.5 and 40.0 μM for control and hypertrophy S-1, respectively. The variation of actin-atimulated ATPase activities among different S-1 preparations were such that a significant difference between control and hypertrophy extrapolated V could not be determined.

Discussion

The Ca^{2+}-stimulated ATPase activity was reduced in hypertrophy S-1 as was previously reported hypertrophy myosin [12]. The existence of cardiac myosin

isozyme may be responsible for this enzymatic change. Recently, there has been indirect evidence to substantiate this hypothesis. During the development of rabbit heart muscle, there are structural changes in both the heavy and light chains of myosin [21]. Reported increases in the rate of synthesis and degradation of rabbit cardiac myosin in pressure-induced hypertrophy have also been observed [22,23]. Recently, Raszkowski et al. [24] have demonstrated a decrease in the 1/2-cystine residues in the amino acid composition of myosins from failing hearts.

An alternate explanation for the observed differences between control and hypertrophy myosins is changes in the extent of myosin phosphorylation. Recently, Frearson et al. [25] reported changes in the phosphate content of cardiac myosin light chain 2 during a positive inotropic response. It has already been demonstrated in smooth muscle that phosphorylation of myosin may be involved in the Ca^{2+} regulation of contractility [26—28]. Thus it is conceivable that the covalent binding of phosphate to the hydroxyl group of serine in cardiac myosin may cause a shift in the molecular structure around the enzymatic site. However, since the cardiac light chain 2 was removed in our studies, it is unlikely that the difference in phosphorylation is the cause for the depressed Ca^{2+}-stimulated ATPase activity in S-1 from hypertrophied hearts.

Interestingly, there was no significant difference in the actin-stimulated ATPase activity of control and hypertrophy S-1, whereas there was a difference with control and hypertrophy myosins. The following explanations may account for these experimental results: (1) the rod portion of the myosin (S-2 and light meromyosin) may be involved in the conformational difference between control and hypertrophy myosins. (2) Papain cleavage of cardiac light chains may have removed part of the structural differences. Since Frearson et al. [25] have shown that phosphorylation occurs on the cardiac light chain 2 which was removed in our preparation of S-1, this could account for the lack of difference between actin-stimulated ATPase of control and hypertrophy S-1. (3) Papain cleavage of cardiac heavy chain from the globular myosin head may have removed this enzymatic difference. (4) Head-head interaction may account for part of the structural differences between control and hypertrophy myosins. Recently, Schaub et al. [29] presented evidence for cooperativity existing between the heads of cardiac myosin.

Thus the data presented in this paper suggest that there is a structural difference between control and hypertrophy myosins and this difference is at least partially retained in the cardiac S-1. Further studies are required to determine the molecular nature of this difference.

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