An Additional Low Molecular Weight Subunit in Purified Preparations of Myosin from Hypertrophied Myocardium (37404)

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Hypertrophied compared to normal myocardium exhibits diminished shortening velocity (1) and decreased ATPase activity of isolated contractile proteins (2). In skeletal muscle from different species shortening velocity correlates with myosin ATPase activity (3) and the ATPase activity of myosin from skeletal muscle appears to depend on its complement of light chain components (4, 5). Accordingly, diminished shortening velocity and altered myosin ATPase in hypertrophied myocardium might reflect changes in the complement of light chain constituents in cardiac myosin.

Methods. Animal preparations. Right ventricular hypertrophy (at least 2-fold) was produced in 30 male New Zealand rabbits weighing between 2.5 and 3.0 kg by constriction of the pulmonary artery for 1 wk (6). Sham operated animals served as controls.

Preparation of myosin. Myosin was prepared at 0–4° from free right ventricular walls from 4 to 6 rabbits (10 g wet weight). Myocardium was minced finely, extracted for 30 min in solution A (KCl 0.3 *M*; potassium phosphate buffer 0.15 *M*, pH 6.5; $MgCl_2$, 5 m*M*; K_2ATP 3 m*M*; and Na_2EDTA, 0.1 m*M*), and centrifuged for 10 min at 1500*g*. Myosin was precipitated from the supernatant fraction by dilution with 12 vol of distilled water. After centrifugation at 600*g*, the precipitate was dissolved in 15 ml of solution B (NaCl, 0.5 *M*; Na_2EDTA, 1 m*M*; Tris–HCl, 10 m*M*, pH 7.5). The dilution–precipitation procedure was repeated and the final precipitate redissolved in solution B. For purification by gel filtration the myosin extract was dialyzed for 24 hr in 3 exchanges of solution C (sodium pyrophosphate, 40 m*M*; Na_2EDTA, 0.1 m*M*, pH 7.5). For purification by ultracentrifugation Na_2ATP, 5 m*M* and $MgCl_2$, 3 m*M* were added to the high ionic strength extract prior to centrifugation at 150,000*g* for 180 min. After centrifugation the supernatant fractions in the upper two-thirds of each tube were pooled, and dialyzed against solution D (KCl, 10 m*M*; Na_2EDTA, 0.1 m*M*, pH 7.0) for 24 hr. The precipitate was recovered from the dialysis bag, dissolved in 6 ml of solution B, centrifuged again for 120 min at 150,000*g* and the supernatant fractions from the upper two-thirds of each tube were pooled (7).

Purification of myosin by column chromatography. Myosin was purified by chromatography by DEAF-Sephadex A50 (8). An extract containing 50 mg of myosin in 20 ml of solution C was applied to a 2.5 × 35 cm DEAE-Sephadex A50 column previously equilibrated with the same buffer. The column was then washed with 3 column volumes of solution C and myosin was eluted with a linear gradient (0.0 to 0.5 *M* KCl in solution C). Myosin appeared in fractions containing 0.13 to 0.15 *M* KCl. Those fractions containing myosin were pooled, dialyzed for 24 hr against solution D and the precipitate was redissolved in solution B to provide a final concentration of 3 mg/ml.

Separation of the subunits of myosin by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. The purified myosin extracts were incubated overnight in urea, 5 *M*; mercaptoethanol, 1%; sodium phosphate pH 7.0, 0.01 *M*; and SDS, 1%

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(9). Samples containing up to 50 μg protein in less than 150 μl were mixed with 3 μl of 0.05% bromphenol blue and 1 drop of glycerol, and applied to SDS gels (10% acrylamide, 0.15% bisacrylamide). Electrophoresis was performed at 1 mA/tube for 1 hr followed by 8 mA/tube for 4 hr. Fixation and staining were performed conventionally (9).

ATPase activity of myosin. ATPase activity was assayed colorimetrically (6). Calcium activated ATPase activity was determined with $CaCl_2$, 10 m*M*, KCl, 200 m*M*; Tris–HCl, pH 7.4, 50 m*M*; Na_2ATP, 3 m*M*; and myosin, 0.5 mg/ml. KCl-EDTA activated ATPase activity was determined with KCl, 500 m*M*; Na_2EDTA, 1 m*M*; Tris–HCl, pH 7.4, 50 m*M*; Na_2ATP 3 m*M*; and myosin 0.5 mg/ml. Protein was determined by the Lowry *et al.* method (10).

Results. The composition of myosin subunits analyzed by SDS-gel electrophoresis. a. Crude preparations of myosin. The pattern of protein bands visualized after SDS-gel electrophoresis of crude preparations of myosin resembled the pattern seen in myofibrillar extracts (11, 12). Although high molecular weight components (heavy chains of myosin and actinins) penetrated the gels only slightly, low molecular weight components were resolved clearly. Normal and hypertrophied hearts exhibited three bands with MW between 45,000 and 35,000 corresponding to actin (43,000), the heavy fraction of troponin (37,000) and tropomyosin (35,000) (12). Both exhibited intense bands corresponding to molecular weights of 27,000 and 20,000 representing light subunits of cardiac myosin (13, 14) (Fig. 1).

An additional faint band (MW approx 23,000) was present in all gels, probably representing the inhibitor fraction of troponin (12). The 18,000 MW Ca^{2+} sensitive troponin component was not resolved consistently.

Myosin from normal myocardium exhibited no components with MW less than 19,000. However, corresponding preparations from hypertrophied hearts exhibited a distinct band with MW approximately 16,000 (Fig. 1).

b. Purified preparations of myosin. After purification by chromatography and/or ultracentrifugation myosin from normal hearts exhibited two components with MW of 27,000 and 20,000 (Fig. 1) while that from hypertrophied hearts showed an additional band with MW of 16,000. The additional band was as striking in purified as in crude preparations.

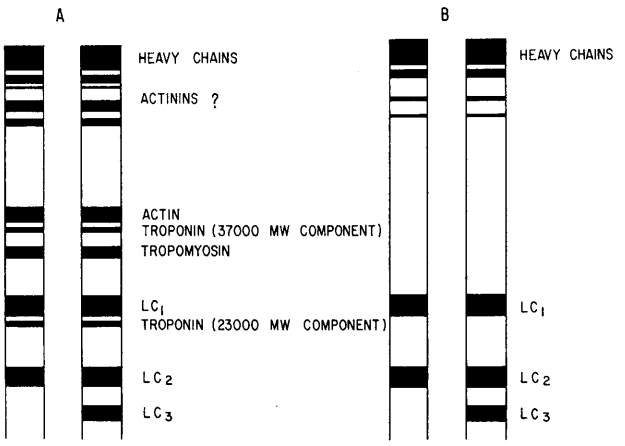

FIG. 1. SDS-polyacrylamide gel electrophoresis of crude myosin preparations (A) and preparations purified with DEAE-Sephadex A50 chromatography (B). Myosin from control hearts exhibited the pattern shown on the left in both preparations compared to myosin from hypertrophied hearts shown on the right. The diagrams were obtained from the original gels by tracing the bands visualized. LC_1, LC_2, and LC_3 refer to the 3 low molecular weight components visualized.

TABLE I. ATPase Activity of Myosin Preparations.[a]

Preparation	Ca^{2+}-activated	EDTA-activated
Control	0.139 ± 0.004	0.280 ± 0.007
Hypertrophied	0.122 ± 0.006[b]	0.240 ± 0.010[b]

[a] Values expressed are μmoles of inorganic phosphate liberated/mg protein/min (mean ± SE) at 25°.

[b] $p < 0.05$ compared to control.

c. ATPase activity of myosin. Myofibrillar and actomyosin ATPase activities decrease in hypertrophied rabbit right ventricles (6). ATPase activity of myosin from normal and hypertrophied myocardium differed significantly as well (Table I) even after incubation for 1 hr with dithiothretitol, 2 m*M* (15).

Discussion. Our results indicate that preparations of myosin from hypertrophied rabbit myocardium exhibit not only diminished ATPase activity but also a low molecular weight component not seen in myosin from normal hearts. Myosin consists of two large helical polypeptide chains (MW approx 200,000), each terminating in a globular structure (16) comprising several small subunits or light chains which are essential for ATPase activity and binding of actin (4, 5). In the present study, myosin from normal rabbit myocardium was found to contain two light chains similar to those observed by others in myosin from chicken, sheep and beef myocardium (13, 14, 17). However, myosin from hypertrophied hearts was associated with an additional component of low molecular weight. Since purification of the myosin preparations did not alter the relative intensity of the three low molecular weight components observed, the extra component appears to be as tightly bound to myosin as the two normal light chains. Ample precedent indicates that myocardium undergoing hypertrophy may exhibit alterations in subunit compositions of proteins, perhaps reflecting differential rates of synthesis of constituents in response to physiological stress (18).

In skeletal muscle myosin ATPase activity correlates with the intrinsic speed of muscle shortening (3, 19) and corresponding differences in the complement of light chains of myosin are seen. Myosin from white muscle (fast) contains three light subunits with molecular weights of 25,000, 18,000, and 15,000 compared to only two light subunits (MW = 25,000 and 18,000) in red muscle (slow) (13, 14). Cross-innervated fast and slow skeletal muscles exhibit myosin ATPase activity corresponding to the new nerve supply (20). Thus, functional characteristics of skeletal muscle may be related to the subunit composition of myosin.

Myocardium undergoing hypertrophy secondary to increased afterload exhibits decreased maximal shortening velocity (1) as well as decreased activity of myofibrillar, actomyosin, and myosin ATPase (2, 6). The present results suggest that these changes are related to alterations in the complement of low molecular weight subunits in myosin.

Summary. In order to determine whether the altered contractility of hypertrophied hearts is associated with changes in the low molecular weight (MW) components of myosin, we examined rabbit heart myosin subunit composition and ATPase activity. Myosin was purified by ultracentrifugation and gel chromatography. Preparations from normal hearts exhibited two low MW components (27,000 and 20,000) on SDS-gels, compared to those from hypertrophied hearts which showed an additional low MW component (16,000). Ca^{2+} and EDTA-activated myosin ATPase activities were diminished in preparations from hypertrophied hearts. Thus, myosin from hypertrophied myocardium contains an additional component which may contribute to diminished contractility.

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