Isolation and Characterization of Myosin from Subjects with Asymmetric Septal Hypertrophy

BARRY J. MARON, VICTOR J. FERRANS, AND ROBERT S. ADELSTEIN

SUMMARY Human cardiac myosin isolated from operatively obtained samples of ventricular septum and left ventricular free wall of subjects with asymmetric septal hypertrophy (ASH) was compared, with respect to structural and enzymatic properties, to myosin isolated from hearts of subjects without heart disease. The following parameters were studied: (1) activation of myosin ATPase activity by K⁺-EDTA and Ca²⁺, (2) molecular weight of the heavy and light chains of myosin as determined by electrophoretic migration in polyacrylamide-sodium dodecyl sulfate (SDS) gels, and (3) ability to form bipolar aggregates at low ionic strength, as examined by electron microscopy. No difference was present in any of these parameters between human cardiac myosin from subjects with ASH and from subjects without heart disease. Thus, the genetic defect present in subjects with ASH is not expressed in the particular structural and functional characteristics of myosin evaluated in this study.

ASYMMETRIC septal hypertrophy (ASH) is a genetically determined cardiac disease that is transmitted as an autosomal dominant trait and is characterized by disproportionate thickening of the ventricular septum with respect to the left ventricular free wall. Hypertrophied and bizarrely shaped cardiac muscle cells arranged in a disorganized fashion are the characteristic histological feature of the ventricular septum of virtually all patients with ASH. These disorganized cardiac muscle cells presumably are a morphological expression of the genetic defect in ASH. Disorganized cardiac muscle cells are distributed widely in the left ventricular free wall of patients without outflow obstruction. With these morphological observations in mind, the present study was undertaken to determine whether the biochemical and structural characteristics of myosin isolated from the ventricular septum of subjects with obstructive ASH differ from the characteristics of myosin isolated from the left ventricular free wall of the same subjects or of subjects without heart disease.

Methods

PATIENT SELECTION AND CLINICAL DATA

The biochemical and ultrastructural studies reported in this communication are based on analyses of myocardium obtained from 14 patients with obstructive ASH and four patients without heart disease. The clinical diagnosis of ASH was confirmed in each of the 14 patients by cardiac catheterization, by echocardiography, and at operation. Each of the four patients used as controls in this study died of noncardiac diseases and had no evidence of heart disease at necropsy.

Patients with ASH were selected for operation if they had cardiac symptoms sufficient to produce severe functional limitation (New York Heart Association functional classes III or IV) and did not respond adequately to propranolol. The patients ranged in age from 20 to 71 years (average, 42); seven were men and seven were women. Thirteen patients had a significant obstruction to left ventricular outflow under basal conditions, with peak systolic pressure gradients between left ventricle and systemic arteries in these patients ranging from 20 to 120 mm Hg. The other patient had only a 6-mm Hg gradient under basal conditions but a 70-mm Hg gradient after provocation with isoproterenol infusion.

SELECTION OF TISSUE

In each patient with ASH, myocardium was taken at the time of left ventricular myotomy-myectomy. The tissues studied are removed routinely at our institution from patients with ASH to determine the extent of the myopathic process. In keeping with institutional policy, informed consent was not required for performance of the additional studies done on these tissues. This tissue (0.5–2.7 g) was resected from the cephalad portion of the left side of the ventricular septum (12 patients) after 20-40 minutes of cardiopulmonary bypass and after a period of 10–12 minutes of cardiac arrest. During the latter period the
aorta was clamped and the heart was cooled to approxi-
mately 30°C; the coronary arteries were not perfused. Biopsy
s (0.2-0.5 g) of the left ventricular apex were taken in five patients after 2-5 minutes of cardiopulmo-
nary bypass but before institution of cardiac arrest. Poste-
rior papillary muscle (1.2 g) was obtained in one patient at
the time of mitral valve excision and after 15 minutes of cardiopulmonary bypass. Tissue from the four patients
without heart disease was obtained from the left ventricu-
lar free wall. 6-20 hours after death.

PREPARATION OF ACTOMYOSIN
Cardiac muscle was obtained from the sources outlined
above and immediately cut into small pieces with scis-
sors. Each gram of muscle was suspended in 2 ml of an
ice-cold extracting buffer containing 15 mM tris(hydroxy-
methyl)aminomethane hydrochloride (Tris-HCl) (pH 7.5), 0.5 M KCl, 1 mM disodium ethylenediamine-
tetraacetate (EDTA), and 5 mM dithiothreitol (DTT), and
was homogenized five times for 15 seconds each in a Virtis Omnimix. This and all subsequent proce-
dures were performed at 2-4°C; deionized water was used
throughout. The homogenized muscle was suspended in the same extracting buffer for an additional 10 minutes.
The time elapsed from removal of muscle until completion of
the actomyosin extraction was about 45 minutes. The
extract was sedimented at 24,000 g for 10 minutes. The
supernatant was decanted, the concentration of KCl was
lowered to 0.1 M by dilution with 5 vol of an ice-cold
solution of 1 mM EDTA, and the pH was adjusted to 6.3.
The solution was sedimented at 24,000 g for 10 minutes. The
resulting (“low salt”) precipitate of cardiac acto-
myosin was resuspended in a volume of the extracting
buffer sufficient to provide a protein concentration of 8-
12 mg/ml. The pH of this solution was adjusted to 7.3 and
the precipitate was allowed to dissolve overnight.

PURIFICATION OF MYOSIN
MgCl2 and ATP were added to preparations of cardiac
actomyosin to a final concentration of 5 mM each, at pH
7.3. Two fractions were obtained from the acto-
myosin by the addition of saturated ammonium sulfate (0-
30% and 30-50% saturation, respectively). Final puri-
fication of myosin (i.e., separation of myosin from actin
and aggregated myosin) was obtained by chromatography of
the 30-50% ammonium sulfate fraction on a column (1.9
X 82 cm) of Sepharose 4B equilibrated with a solution
containing 15 mM Tris - HCl buffer (pH 7.5), 0.5 M KCl, 1
mM EDTA, and 5 mM DTT. The resulting fractions of the
effluent volume were assayed for ATPase activity. The
fractions with peak ATPase activity were pooled for char-
gerization of myosin ATPase activity (in the presence of
EDTA). Ca2+, or Mg2+) and for gel electrophoresis (Fig.
1), as described below. In some preparations the fractions
found to have the peak of ATPase activity (after chroma-
tography on Sepharose 4B) were concentrated by the
addition of saturated ammonium sulfate to 50% satu-
ration. The resulting precipitate (i.e., myosin) was solubi-
}zized in a solution containing 15 mM Tris - HCl buffer (pH
7.5), 0.5 M KCl, 1 mM EDTA, and 5 mM DTT and characte-
ized by the methods outlined above.

GEL ELECTROPHORESIS
Polyacrylamide-sodium dodecyl sulfate (SDS) gel elec-
trophoresis was performed by the method of Fairbanks et
al.6 Gels (6.0 cm in length and 0.5 cm in width) were
prepared from solutions containing either 5%, 7.5%, or
10% polyacrylamide and 1% SDS. 0.4 M Tris·HCl (pH 7.4), 0.2 M sodium acetate, and 0.02 M EDTA. Protein
samples were dried in a vacuum desicator and then dis-
solved in a solution containing 1% SDS, 10 mM Tris·HCl
(pH 8.0), and 50 mM DTT by boiling for 2 minutes. Elec
trophoresis was performed on 20- to 40-μl samples
(containing 40-80 μg of protein) for about 90 minutes at
6 mA per gel. The gels were stained for about 14 hours in a
solution containing 300 mg of Coomassie brilliant blue,
300 ml of methanol, 85 ml of acetic acid, and 615 ml of
water and destained in the same solution without dye. The
gels were photographed and stored in 5% acetic acid.

DETERMINATION OF ATPase ACTIVITY OF MYOSIN
The ATPase activity of myosin was determined in theour patients without heart disease and in 12 of 14 patients

Figure 1 Profile of Sepharose 4B gel filtration using a 30-50%
ammonium sulfate fraction of actomyosin prepared from ventricu-
lar septal myocardium of a 62-year-old man with obstructive asym-
metric septal hypertrophy (ASH). The sample was made 5 mM with
respect to MgCl2 and ATP just prior to column chromatography.
The ordinate indicates the A 280 of the ATPase assay; the abscissa
indicates the effluent volume. A 2.5-ml sample (4 mg/ml) was
applied to a column (1.9 X 82 cm) of Sepharose 4B equilibrated
with a solution containing 15 mM Tris·HCl buffer (pH 7.5), 0.5 M
KCl, 1 mM EDTA, and 5 mM dithiothreitol (DTT). For this
column the void volume was at 45 ml of the effluent volume and the
salt boundary was at 145 ml of the effluent volume. The sample
was eluted with the same buffer at a flow rate of 9 ml/hour into
fractions of 3 ml each. The peak of ATPase activity at 55-75 ml of
the effluent volume represents purified myosin. A 7.5% polyacryl-
amide-1% sodium dodecyl sulfate (SDS) gel, after electrophoresis
of myosin obtained from the peak of ATPase activity, is shown at
the right. Electrophoretic migration was from top to bottom. HC =
heavy chain of myosin; LC-1 = light chain of myosin having a
molecular weight of 25,000 daltons; LC-2 = light chain of myosin
having a molecular weight of 20,000 daltons, and d = tracking
dye.
with ASH (ventricular septum in 12 patients and also left ventricular free wall in four of these 12 patients). Tissue from the remaining two patients with ASH were used only for polyacrylamide-SDS gel electrophoresis and for ultrastructural studies. ATPase assays were performed at 37°C in 0.2 M Tris-HCl buffer in the presence of 0.5 mM KCl and 2 mM ATP, in addition to either 2 mM EDTA, 10 mM CaCl₂, or 5 mM MgCl₂. For each assay three samples (0.9 ml) of the incubation mixture were removed (the first sample immediately after the addition of ATP and the other two 15–90 minutes later) for the assay, by a modification of the method of Martin and Doty.¹¹ Inorganic phosphate production. Phosphate production was linear with time during the duration of the assay. Measurements of ATPase activity were made within 3 days after the isolation of myosin. Myosin ATPase activities were compared statistically using Student's t-test. ATPase activity of fractions of the effluent volume obtained by Sepharose chromatography was assayed by methods that have been previously described.¹³ Protein concentrations were determined by the procedure of Lowry et al.¹² Molecular weights of proteins were determined by polyacrylamide-SDS gel electrophoresis according to the method of Weber and Osborn.¹⁴ with rabbit skeletal muscle myosin and actin as standards.

PREPARATION OF BIPOLAR AGGREGATES OF MYOSIN MOLECULES

Samples (about 0.1 mg) of purified myosin (in 0.5 mM KCl) were diluted either slowly by dialysis (12–24 hours) against a buffer containing 10 mM Tris (pH 7.0), 0.1 mM KCl, 1 mM MgCl₂, and 2.5 mM DTT, or diluted rapidly by addition of 4 vol of water (to a final KCl concentration of 0.1 mM). After dialysis a drop of the protein solution was placed on a 300-mesh. Formvar-coated copper grid, negatively stained with 1% uranyl acetate by the method of Huxley and examined with a Jeol 100B electron microscope.

Results

MYOSIN ATPase ACTIVITY

Data on the myosin ATPase activities of specimens analyzed in this study are summarized in Table 1. The K⁺-EDTA-activated myosin ATPase activity of left ventricular myocardium from subjects without heart disease ranged from 1.3 to 1.6 (mean, 1.4 ± 0.1) μmol of inorganic phosphate released per mg of protein per minute.

The K⁺-EDTA-activated ATPase activity in the 12 specimens of ventricular septum from subjects with ASH ranged from 1.2 to 2.1 (mean, 1.5 ± 0.3) μmol of inorganic phosphate released per mg of protein per minute and did not differ significantly from that obtained in subjects without heart disease. Furthermore, K⁺-EDTA-activated myosin ATPase activity in four specimens of left ventricular free wall (left ventricular apex or papillary muscle) from subjects with ASH (range, 1.2–2.1; mean, 1.4 ± 0.6 μmol of inorganic phosphate released per mg of protein per minute) was slightly lower but not significantly different from that of myosin from the ventricular septum of the same subjects (range, 0.7–1.9; mean, 1.6 ± 0.4 μmol of inorganic phosphate released per mg of protein per minute).

In all specimens of myosin analyzed the Ca²⁺-activated ATPase activity was significantly lower (range, 0.1–0.6; mean, 0.3 μmol of inorganic phosphate released per mg of protein per minute) than the K⁺-EDTA-activated ATPase activity. Ratios of K⁺-EDTA-activated to Ca²⁺-activated myosin ATPase activities did not differ significantly in specimens from subjects with ASH or subjects without heart disease (Table 1). Myosin ATPase activity in the presence of Mg²⁺ ranged from 0.005 to 0.01 μmol of inorganic phosphate released per mg of protein per minute in each specimen. Protein concentrations of samples used in the ATPase assays ranged from 0.2 to 4.1 mg/ml.

In subjects with ASH there was no relation between the myosin ATPase activity obtained from specimens of ventricular septum and the subject’s age, clinical condition, magnitude of left ventricular outflow tract obstruction, left ventricular end-diastolic pressure, or cardiac index.

POLYACRYLAMIDE-SDS GEL ELECTROPHORESIS

Polyacrylamide-SDS gel electrophoresis was performed on preparations of cardiac actomyosin. purified myosin, or both, from subjects with ASH and from subjects without heart disease (Figs. 2–4). Preparations of actomyosin showed a characteristic pattern of bands (Fig. 2) that included: (1) a band at 200,000 daltons, representing the heavy chain of myosin, (2) a band at 25,000 daltons and a band at 20,000 daltons representing the two different light chains of myosin, and (3) bands representing other contractile proteins including M-protein (molecular weight of 165,000 daltons), actin (molecular weight of 44,000 daltons), and troponomyosin (subunit molecular weight of

### Table 1

**ATPase Activity of Myosin from Subjects with Asymmetric Septal Hypertrophy (ASH) and from Subjects without Heart Disease**

<table>
<thead>
<tr>
<th>Condition</th>
<th>n</th>
<th>K⁺-EDTA-ATPase (mean ± sd)</th>
<th>Ca²⁺-ATPase (mean ± sd)</th>
<th>Mg²⁺-ATPase (mean ± sd)</th>
<th>Ratio, K⁺-EDTA-ATPase/Ca²⁺-ATPase (mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ventricular septum</td>
<td>12</td>
<td>1.5 ± 0.3</td>
<td>0.3 ± 0.1</td>
<td>&lt;0.01</td>
<td>5.0</td>
</tr>
<tr>
<td>LV free wall†</td>
<td>4</td>
<td>1.4 ± 0.6</td>
<td>0.3 ± 0.3</td>
<td>&lt;0.01</td>
<td>5.0</td>
</tr>
<tr>
<td>Normal</td>
<td>4</td>
<td>1.4 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>&lt;0.01</td>
<td>4.5</td>
</tr>
</tbody>
</table>

LV = left ventricular; n = number of subjects.

* Specific activity is expressed in μmol of inorganic phosphate released per mg of protein per minute.

† Includes left ventricular apex in three subjects and the left ventricular posterior papillary muscle in one subject.
<FIGURE 2> Pattern of peptide bands obtained from actomyosin prepared from the ventricular septum of a 71-year-old woman with obstructive asymmetric septal hypertrophy (ASH); 5% polyacrylamide-1% SDS gel. Electrophoretic migration was from top to bottom. HC = heavy chain of myosin; M = M-protein; A = actin; T = tropomyosin; LC-1 = light chain of myosin (molecular weight of 25,000 daltons); LC-2 = light chain of myosin (molecular weight of 20,000 dalton), and d = tracking dye.

35,000 daltons). Other relatively indistinct bands, which were not definitively identified, also were present in these preparations; the protein represented by these bands did not constitute more than 5% of the total protein on any gel. Polyacrylamide-SDS gel electrophoresis of purified preparations of myosin showed the heavy and light chains of myosin and only trace amounts of other proteins (Figs. 1 and 3).

The patterns of bands in polyacrylamide-SDS gels (using preparations of actomyosin or purified myosin) were similar for the ventricular septum and the left ventricular free wall of subjects with ASH (Figs. 4 and 5) and for the left ventricular free wall of subjects without heart disease (Fig. 3). By scanning the absorbance of protein bands at 584 nm in a Beckman ACTA no. 2 gel scanner (and calculating the area under each peak by planimetry), we determined from representative gels of actomyosin and purified myosin preparations that the relative amounts of light chain of myosin are similar in the ventricular septum and left ventricular free wall of subjects with ASH and the left ventricular free wall of subjects without heart disease. For example, the light chain with molecular weight of

<FIGURE 3> Patterns of peptide bands of purified human cardiac myosin after electrophoresis in 7.5% polyacrylamide-1% SDS gels. Left: preparation from ventricular septum of a 62-year-old man with obstructive ASH. Right: preparation from left ventricular free wall of a subject without heart disease; 50 μg of protein were applied to each gel. Electrophoretic migration was from top to bottom. Abbreviations as in Figure 2. Trace amounts of other proteins (that were not identified) also are present in both gels.

<FIGURE 4> Patterns of peptide bands obtained from actomyosin prepared from a 55-year-old man with obstructive ASH; 7.5% polyacrylamide-1% SDS gels. Left: preparation from ventricular septum. Right: preparation from left ventricular apex; 75 μg of protein were applied to each gel. Electrophoretic migration was from top to bottom. Abbreviations as in Figure 2.
formed after either rapid or slow dilution in preparations from subjects with ASH compared to subjects without heart disease. Furthermore, these aggregates of myosin molecules were similar in appearance to those described for skeletal or canine cardiac myosin.

Discussion

The results of this study show that myosin obtained from the ventricular septum of subjects with obstructive ASH does not differ from myosin obtained from the left ventricular free wall of subjects with ASH or subjects without heart disease with respect to (1) ATPase activity, (2) molecular weights of the myosin subunits as determined by electrophoresis in polyacrylamide-SDS gels, and (3) morphological features of bipolar aggregates of myosin molecules. Numerous previous studies have reported ATPase activities in hypertrophied or failing ventricular muscle from subjects with various types of heart disease as well as from animals with experimentally produced pressure or volume overload. However, there is no general agreement among these investigators as to whether the ATPase activity of myosin is increased, decreased, or unchanged in ventricular hypertrophy or failure. Furthermore, it is difficult to compare the results of these studies to ours, because different methods for the preparation of myosin and for the assay of myosin ATPase activity were used in such studies.

Our investigation represents the first detailed biochemical study of purified human cardiac myosin from patients with ASH. In agreement with the findings of others, we have shown that purified myosin, with preservation of ATPase activity, can be obtained from normal human cardiac muscle for at least 20 hours after death. Other studies have demonstrated that myosin ATPase activity does not change appreciably during this period of time. It was of concern to us initially that myosin isolated from hearts obtained post mortem would be degraded and oxidized to such an extent that it would be unsuitable for comparison with myosin isolated from operatively obtained specimens. However, with the procedures outlined in this study, myosin which showed no evidence of proteolysis or oxidation of sulfhydryl groups was purified from postmortem tissue. The absence of significant proteolysis was confirmed by the fact that little or no degraded myosin was evident after electrophoresis of myosin in polyacrylamide-SDS gels (Figs. 1–4). Furthermore, the ratio of the K+-EDTA-activated myosin ATPase activity to the Ca2+-activated myosin ATPase activity was the same for myosin isolated from postmortem tissue of subjects without heart disease and from operatively obtained specimens from subjects with ASH. This finding indicated that oxidation of sulfhydryl groups did not occur in our preparations, since oxidation usually results in an increase in the Ca2+-activated myosin ATPase activity and a decrease in the K+-EDTA-activated myosin ATPase activity. Further confirmation of this point was obtained by the study of myosin isolated from the left ventricle of a dog immediately after it was killed and again from the same heart after it had stood for 24 hours at 4°C. No difference in the K+-EDTA-activated or Ca2+-activated myosin ATPase activities, in the ratio of these ATPase activities, or in the
patterns of bands on polyacrylamide-SDS gels. was noted between the myosin isolated immediately after death and that isolated 24 hours later.

References

6. Ferrans VJ, Morrow AG, Roberts WC: Myocardial ultrastructure in idiopathic hypertrophic subaortic stenosis; a study of operatively excised left ventricular outflow tract muscle in 14 patients. Circulation 45: 769-792, 1972
Isolation and characterization of myosin from subjects with asymmetric septal hypertrophy.

B J Maron, V J Ferrans and R S Adelstein

Circ Res. 1977;40:468-473
doi: 10.1161/01.RES.40.5.468

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1977 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/40/5/468