Adenosinetriphosphatase Activity of Cardiac Myosin

COMPARISON OF THE ENZYMATIC ACTIVITIES AND ACTIVATION BY ACTIN OF DOG CARDIAC, RABBIT CARDIAC, RABBIT WHITE SKELETAL AND RABBIT RED SKELETAL MUSCLE MYOSINS

By Arnold M. Katz, M.D., Doris I. Repke, C.T., and Bonnie B. Rubin, B.A.

ABSTRACT

Examination of the ATPase activities of rabbit cardiac, rabbit red skeletal and rabbit white skeletal muscle myosins has demonstrated the existence of two types of myosin. One type, characterized by the higher ATPase activity, is present in white skeletal muscle; the other is found in red skeletal and cardiac muscle. The differences between myosin preparations could not be attributed to varying degrees of actin contamination nor to changes occurring in the myosins during the preparative procedure. Chromatography of dog cardiac myosin on DEAE-cellulose showed a single component. No differences were observed between the cardiac myosins of the dog and rabbit.

Activation of the ATPase activity of cardiac myosin by Ca" was less than that of white skeletal myosin while the extent of inhibition by Mg" was similar for both. The stoichiometry of the interaction with actin of both cardiac and white skeletal myosins was similar but actin caused less activation of the cardiac myosin ATPase. Cardiac and white skeletal actins showed no differences in their abilities to activate myosin ATPase activity, which is in accord with previously documented similarities in the structures of these actins.

Comparison of the present findings with previously reported mechanical measurements made with intact muscles does not indicate that the intrinsic level of myosin ATPase activity determines the maximal tension developed during the active state. On the other hand, there may be a direct relationship between shortening velocity and myosin ATPase activity.

ADDITIONAL KEY WORDS Mg" Ca" cardiac actomyosin skeletal actomyosin mechanics of cardiac muscle

Differences between the ATPase activities of myosins prepared from cardiac and skeletal muscle have been reported by a number of investigators1-6 who compared rabbit skeletal muscle myosin with myosin prepared from the hearts of dogs,3,5 cattle,6 and pigs.1 In these studies the rate of ATP hydrolysis by the cardiac myosin preparations was found to be less than that of the rabbit skeletal muscle myosin. The possibility that the observed differences reflected variations between the myosins of the different species was raised by Mueller et al.,7 who found the ATPase activities of dog cardiac and dog skeletal muscle myosins to be similar under a variety of conditions, while both had activities less than that of rabbit skeletal muscle myosin. However, this interpretation is not consistent with the pioneering work of Bailey,8 who found that the ATPase activity of acto-
myosin from rabbit hearts was less than that of rabbit skeletal muscle actomyosin, nor with a more recent comparison of cardiac and skeletal muscle myosins obtained from the rabbit. An alternative explanation for the observation that the ATPase activities of dog cardiac and skeletal muscle myosins are similar to each other but less than that of rabbit skeletal muscle myosin has been suggested by the recent finding that rabbit red skeletal muscle, which has many biochemical and physiological features in common with cardiac muscle, contains myosin and actomyosin having lower ATPase activities than the corresponding proteins of rabbit white skeletal muscle. It thus appeared likely that the similarity between ATPase activities of dog cardiac and skeletal muscle myosins reflected the fact that the dog skeletal myosin was obtained from muscle that contained significant amounts of red fibers (H. Mueller, personal communication), whereas the higher ATPase activity of the rabbit skeletal muscle was due to its high content of white muscle fibers.

In the present investigation, myosins from dog cardiac muscle and rabbit red skeletal, white skeletal, and cardiac muscle were prepared in order to clarify the role of differences in striated muscle type, as opposed to species variation, in causing differences in myosin ATPase activity. The possibility that impurities or changes occurring in the myosin during the purification procedure influenced the enzymatic activity of the various myosin preparations was examined. It is concluded that at least two types of myosin exist in mammalian striated muscle; one of these is found in white skeletal muscle while the other, characterized by lower ATPase activity and a lesser degree of activation by actin, is present in both red skeletal and cardiac muscle. In contrast to these two types of myosin, actins from the different muscle types caused the same degree of activation of myosin ATPase activity.

Methods

Considerable difficulty was encountered in developing a method for obtaining actomyosin-free myosin, therefore a full description of the preparative procedure is presented. Fresh muscle tissue was obtained from dogs anesthetized with pentobarbital, or rabbits killed by a sharp blow to the base of the skull or intravenous pentobarbital. The muscle was immediately chilled in crushed ice, then ground in a small meat grinder or an Arbor Tissue Press (Harvard Apparatus Co.). The myosin prepared from coarsely ground muscle contained less actomyosin than that from more finely minced tissue (see below). For each 10 g of muscle 33 ml of Guba-Straub solution was added and the mixture was stirred gently for 15 min at 4°C. Stirring was continued and 130 ml of cold water was added and the muscle residue removed by straining through gauze (this residue can be used for preparing actin or tropomyosin). The myosin was precipitated by adding 240 ml of cold water while stirring constantly and the pellet, collected by centrifugation at 30,000 rpm for 20 min in a Spino Model L Ultracentrifuge and the myosin was precipitated by adding 9 volumes of cold water with constant stirring. The myosin, collected by centrifugation in the PR-2 for 20 min at 2,000 rpm, was suspended in 0.5 M Tris* acetate at pH 6.8. After the larger particles were dispersed by brief, gentle homogenization in a loosely-fitting glass teflon homogenizer, 3 M KCl was added to a final concentration of 0.27 M. Actomyosin and solid debris were removed by centrifugation at 30,000 rpm for 20 min in a Spino Model L Ultracentrifuge and the myosin was precipitated by adding 9 volumes of cold water with constant stirring. The myosin, collected by centrifugation in the PR-2 for 20 min at 2,000 rpm, was suspended in cold water, then dissolved by adding 1/10 volume of 3 M KCl. Actin was prepared from dog cardiac and rabbit skeletal muscle according to the method of Straub, as modified by Katz and Hall. To ensure exclusion of tropomyosin, the acetone-dried muscle powder was extracted at 2 to 4°C and the second polymerization of G-actin was induced by adding MgCl₂ to a final concentration of 0.6 mM. MgATP was added to final concentration of 10 mM. Traces of actin were removed by centrifugation for 24 hours at 40,000 rpm and the upper two thirds of the supernatant myosin solution was dialyzed against two changes of approximately 50 volumes of 0.6 M KCl and 20 mM Tris acetate at pH 6.8.

Actin was prepared from dog cardiac and rabbit skeletal muscle according to the method of Straub, as modified by Katz and Hall. To ensure exclusion of tropomyosin, the acetone-dried muscle powder was extracted at 2 to 4°C and the second polymerization of G-actin was induced by adding MgCl₂ to a final concentration of 0.6 mM. Reconstituted actomyosin was made by adding 4 parts of myosin (by weight) to 1 part

*Abbreviation: Tris = tris (hydroxymethyl) aminomethane.
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of F-actin according to the procedure previously described.\textsuperscript{14}

Inorganic phosphate ($P_i$) was determined by the method of Taussky and Shorr.\textsuperscript{16} In some experiments the time course of $P_i$ liberation by actomyosin was measured in the pH-Stat (Radiometer, Inc.) using the methods described elsewhere.\textsuperscript{14} The release of $P_i$ by both myosin and actomyosin usually showed an initial burst,\textsuperscript{16,17} so that values for ATPase activity were calculated from the slope of the curves of $P_i$ liberation beginning 1 to 2 min after initiation of the reaction. A detailed study of the initial rapid phase of ATP hydrolysis was not carried out. All values for ATPase activity are expressed in terms of the myosin concentration to facilitate comparison between results obtained with myosin and actomyosin.

Protein concentrations were determined by the Biuret reaction standardized by Kjeldahl nitrogen determinations. The calibration factor previously determined for rabbit white skeletal myosin was used for all of the myosin preparations examined in this report.

All chemicals used were reagent grade; 1,2-bis-(2-dicarboxymethylaminoethoxy) ethane (EGTA) was obtained from LaMont Laboratories, Dallas, Texas.

Results

PREPARATION OF A HIGHLY PURIFIED, ACTOMYOSIN-FREE CARDIAC MYOSIN

At the outset of this study, myosin was prepared from frozen muscle by the KCl method of Bárány et al.\textsuperscript{9} In spite of several washings of extensively homogenized myofibrils prior to extraction of actomyosin, and repeated reprecipitation of actomyosin prior to removal of actin, this method yielded a cardiac myosin with a reddish tinge not seen in white skeletal myosin preparations. Furthermore, the myosins made from frozen muscle were not completely free of natural actomyosin, as evidenced by sensitivity to EGTA (Fig. 1). This actomyosin contamination generally was greater in the cardiac myosin than that in concurrently prepared white skeletal myosin. To prepare an actomyosin-free cardiac myosin preparation it was necessary to employ fresh muscle as the starting material.

Following the procedure outlined in Methods, the yields of cardiac myosin were one half to one third those of white skeletal muscle. Chromatography of the cardiac myosin showed a single asymmetrical peak like that previously found in the case of skeletal myosin (Fig. 2). It was noted that the cardiac myosin precipitated at low ionic strength formed less dense pellets after low speed centrifugation than did white skeletal myosin. White skeletal myosin prepared from rabbits anesthetized with pentobarbital showed no significant differences from preparations from rabbits killed by a blow to the head.

To determine whether the nonmyosin constituents of cardiac muscle could have caused the low specific ATPase activity of cardiac myosin, myosin was prepared from samples of cardiac muscle, white skeletal muscle, and a mixture where equal weights of the two types of muscle were combined immediately after grinding, prior to extraction and purification of the myosins. The ATPase activity of the myosin prepared from the mixture of cardiac and white skeletal muscle was high (Table 1) demonstrating that substances such as iron, which are present in higher concentrations in ground cardiac muscle, did not re-
Chromatographic pattern of cardiac myosin. Approximately 10 mg of cardiac myosin was chromatographed on 2 gm of DEAE-cellulose in a 1 x 8 cm column. The elution was effected by raising the concentration of KCl (x—x, right-hand ordinate) in a 5 mm tri nitrate buffer containing 5 mm ATP, following the procedure of Asai. Samples of 1.7 ml were collected and protein concentration was determined by the method of Lowry at an OD of 770 mülü (O—O, left-hand ordinate).

FIGURE 2

Initial Steady State, ATPase Activities of Myosins prepared from Dog Cardiac Muscle, Rabbit White Skeletal Muscle, and a Mixture of Equal Portions of These Two Types of Muscle

<table>
<thead>
<tr>
<th>Activating ion</th>
<th>Skeletal</th>
<th>Cardiac</th>
<th>Mixed</th>
<th>Mixed, predicted*</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0 mm Mg**</td>
<td>.0076</td>
<td>.0014</td>
<td>.0066</td>
<td>.0067</td>
</tr>
<tr>
<td>1.0 mm Ca**</td>
<td>.630</td>
<td>.025</td>
<td>.444</td>
<td>.548</td>
</tr>
</tbody>
</table>

Reactions were carried out in 1.0 mm ATP, 0.10 M KCl, and 10 mm Tris acetate at pH 6.8.

*These values are approximations based on the estimated yields of cardiac and skeletal myosins in the mixture.

duce the ATPase activity of the white skeletal myosin in the mixture.

The cardiac myosin preparations obtained by the procedure described above (see Methods) were free of the reddish color seen when myosin was prepared from exhaustively washed frozen heart muscle. In view of this finding and the absence of detectable changes resulting from contact of white skeletal myosin with ground cardiac muscle, the extensive washing procedure advocated by Bárány et al. was omitted.

COMPARISON OF THE ATPase ACTIVITIES OF DOG CARDIAC AND RABBIT WHITE SKELETAL MYOSINS

The rates of ATP hydrolysis by cardiac and white skeletal myosins were examined at several ionic strengths in the presence of Ca** or Mg**. Data from a number of preparations are illustrated to indicate the extent of variation seen in this series of experiments. The Mg**-activated ATPase activities of both cardiac and skeletal myosins were moderately inhibited at higher concentrations of KCl (Fig. 3). At all ionic strengths skeletal myosin was approximately three times as active as the cardiac myosin. When the Ca**-activated ATPase activities were compared, white skeletal myosin was approximately five times as active (Fig. 4).

Comparison of these activities with that measured in 0.25 mm EDTA indicates that the extent of Mg** inhibition of both cardiac and white skeletal myosins was similar.
whereas activation by Ca** was greater in the case of the skeletal myosin (Table 2).

**Comparison of the ATPase Activities of Cardiac, White Skeletal, and Red Skeletal Myosins of the Rabbit**

Myosin prepared from rabbit cardiac muscle, like that from dog cardiac muscle, had a lower ATPase activity than rabbit white skeletal myosin (Table 3). On the other hand, neither the Ca**- nor Mg**-activated ATPase activities of myosin from red skeletal muscle (semitendinosus, gluteus, and soleus) differed significantly from that of cardiac muscle. Comparison of the ATPase activities of rabbit cardiac myosin with those of dog cardiac myosin showed no significant differences (compare Table 3 with Figs. 2 and 3).

**Activation of the ATPase Activities of Dog Cardiac and Rabbit White Skeletal Myosins by Actin**

Both the rate of liberation of Pi during the clearing phase and the more rapid hydrolysis of ATP after superprecipitation were less in the case of actomyosin made from cardiac myosin (Fig. 5).

**Table 2**

<table>
<thead>
<tr>
<th>Cation</th>
<th>ATPase activity (amoles Pi/min/mg myosin)</th>
<th>Me**: EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>None (0.25 mM EDTA)</td>
<td>.0276</td>
<td>.0864</td>
</tr>
<tr>
<td>1.0 mM Ca**</td>
<td>.119</td>
<td>.528</td>
</tr>
<tr>
<td>2.0 mM Mg**</td>
<td>.0040</td>
<td>.0116</td>
</tr>
</tbody>
</table>

Reactions were carried out in 1.0 mM ATP, 0.1 M KCl, and 10 mM Tris acetate at pH 6.8.
Comparison of the ATPase Activities of Myosins prepared from White Skeletal, Red Skeletal and Cardiac Muscle of the Rabbit

<table>
<thead>
<tr>
<th>Cation</th>
<th>White</th>
<th>Red</th>
<th>Cardiac</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.0 mM Mg** (±0.25 mM EGTA)</td>
<td>.0097 ± .0017*</td>
<td>.0023 ± .0012</td>
<td>.0024 ± .0009</td>
</tr>
<tr>
<td>1.0 mM Ca**</td>
<td>.600 ± .085</td>
<td>.130 ± .011</td>
<td>.106 ± .033</td>
</tr>
</tbody>
</table>

Reactions were carried out in 1.0 mM ATP, 0.1 M KCl, and 10 mM Tris acetate at pH 6.8. *Standard deviation.

The extent of activation by actin of the initial steady state, Mg**-activated ATPase activity of white skeletal myosin was greater than that of cardiac myosin (Fig 6). The possibility that the lesser degree of activation of cardiac myosin ATPase activity reflected a different stoichiometry of interaction of actin with the different types of myosin could be excluded by comparing the ATPase activities of actomyosins made with varying amounts of actin. Maximal stimulation of both cardiac and white skeletal myosin ATPase activities was seen when actin was present in an amount equal to approximately one third the weight of myosin (Fig. 7). The extent of activation of myosin ATPase activity by cardiac and skeletal actins was identical (Table 4).

Discussion

In view of the different chemical compositions of cardiac and white skeletal muscle, the possibilities that the low cardiac myosin ATPase activity resulted from simultaneous extraction of an inhibitor present in cardiac, but not in white skeletal muscle, and that cardiac myosin was damaged during the purification procedure were examined. The high iron content of red and cardiac muscle, which may...
Comparison of the extent of activation by rabbit skeletal actin of myosins made from rabbit white skeletal muscle (a) and dog cardiac muscle (b). The initial steady state, ATPase activities of actomyosins in 2.0 mM Mg\(^{2+}\) and 1.0 mM ATP (●) at several concentrations of KCl are plotted along with the ATPase activities of the myosin alone (— — —). The ratios between the actomyosin and myosin ATPase activities (x) are also plotted.

### Table 4

Comparison of the Activation of the Initial Steady State, Mg\(^{2+}\)-activated ATPase Activity of Dog Cardiac and Rabbit White Skeletal Myosin by Actins from Dog Cardiac and Rabbit White Skeletal Muscle

<table>
<thead>
<tr>
<th>Myosin</th>
<th>Actin</th>
<th>ATPase activity (nmoles Pi/min/mg myosin)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac</td>
<td>None</td>
<td>0.041</td>
</tr>
<tr>
<td></td>
<td>0.16 mg per ml cardiac</td>
<td>0.078</td>
</tr>
<tr>
<td></td>
<td>0.16 mg per ml skeletal</td>
<td>0.078</td>
</tr>
<tr>
<td>Skeletal</td>
<td>None</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>0.16 mg per ml cardiac</td>
<td>0.120</td>
</tr>
<tr>
<td></td>
<td>0.16 mg per ml skeletal</td>
<td>0.118</td>
</tr>
</tbody>
</table>

Results with 0.64 mg per ml myosin in 2.0 mM Mg\(^{2+}\), 1.0 mM ATP, 0.10 M KCl, and 10 mM Tris acetate at pH 6.8.

be partly responsible for the lability and low activity of cardiac relaxing granules,\(^\text{19}\) could modify the myosin ATPase activity by catalyzing reactions that alter specific amino acids. Examination of the myosin mixture, prepared when white skeletal and cardiac muscle minces were combined at the beginning of the purification procedure, failed to demonstrate significant reduction of the white skeletal myosin ATPase activity (Table 1); therefore, the observed differences in ATPase activity did not arise from damage to the cardiac myosin during purification. While the possibility that a tightly bound inhibitor was extracted with
Comparison of the stoichiometry of the interaction of rabbit white skeletal actin with myosin from rabbit white skeletal and dog cardiac muscle. The left-hand graph shows the initial steady state, ATPase activities of 0.84 mg per ml cardiac (■) and skeletal (■) myosins at different concentrations of actin. Reactions were carried out in 2.0 mM Mg**, 1.0 mM ATP, 0.10 M KCl and 50 mM Tris acetate at pH 6.8. The right-hand graph illustrates the ratios of actomyosin to myosin ATPase activities for cardiac (○) and skeletal (●) myosins containing varying amounts of actin.

The present findings demonstrate that within a single mammalian species the ATPase activity of cardiac myosin is less than that of white skeletal myosin but similar to that of red skeletal myosin (Table 3). Furthermore, no differences between dog and rabbit cardiac myosins were observed. Initial studies that were carried out with dog cardiac and dog quadriceps femoris muscle showed a slightly greater ATPase activity for the latter, as was previously reported by Mueller et al.7 This skeletal muscle appeared to be more like red muscle than white muscle but further characterization of the canine skeletal muscle was not attempted because homogeneous red
ATPase ACTIVITY OF CARDIAC MYOSIN

and white muscle tissue could readily be obtained from the rabbit. The findings with the various rabbit myosins are generally similar to those reported by Bárány et al.8,10 and Seidel et al.11 However the Mg\(^{2+}\)-activated ATPase activity of actomyosin-free cardiac myosin was less than that of actomyosin-free white skeletal myosin at all KCl concentrations between 0.05 and 0.6 M (Fig. 3) and no difference between red and cardiac myosins was noted in the present investigation. The ratio of Ca\(^{2+}\)- to Mg\(^{2+}\)-activated myosin ATPase activities was higher in the case of white skeletal myosin (Table 2) suggesting that Ca\(^{2+}\) leads to greater activation of the ATPase activity of white skeletal myosin than of cardiac myosin, while the extent of inhibition by Mg\(^{2+}\) is similar for both (Table 2).

Actin, like Ca\(^{2+}\), activates the ATPase activity of white skeletal myosin more than it does cardiac myosin (Fig. 6). Examination of the liberation of Pi throughout the entire course of the reactions with ATP demonstrates that this difference is present both during the clearing phase and after superprecipitation (Fig. 5). Comparison of the stoichiometry of interaction between white skeletal actin, and cardiac or white skeletal myosin showed no differences (Fig. 7). Therefore, the lower ATPase activity of cardiac actomyosin does not reflect a lesser binding of actin by cardiac myosin.9 The similar abilities of actins made from cardiac and skeletal muscle to activate myosin ATPase9 (Table 4) are in accord with the identity of a number of structural features of red and white skeletal and cardiac actins.12,22-28

The enzymatic differences between cardiac and skeletal myosins are not reflected in different hydrodynamic properties of cardiac and white skeletal myosins. The shapes, sizes, and molecular weights of both myosins are the same.9,7,24 several aspects of the secondary and tertiary structures are alike,25 and the amino acid compositions appear generally similar.26 However, differences in the content of cysteine have been noted,9 the sensitivities of cardiac and skeletal myosins to proteolytic enzymes differ,4,27,28 and immunological dissimilarities have been reported.29

A relationship appears to exist between shortening velocity and myosin ATPase activity when red and white skeletal muscle are compared. The maximal rate of tension development30 and maximal velocity of shortening per unit of muscle length31,32 in red skeletal muscle are approximately one third those of white skeletal muscle while red skeletal myosin is one fifth to one third as active as white skeletal myosin. In the heart, where the mechanical parameters are more variable and less accessible to measurement than in white skeletal muscle, the shortening velocity is also slow.33,34 These correlations raise the possibility that the low ATPase activities of red skeletal and cardiac myosins are the cause of the lesser maximal shortening velocity, but it should be emphasized that the ratios between the ATPase activities of the different myosins and actomyosins depend upon the conditions under which the comparison is made.

The relative weakness of cardiac muscle25,35 and glycerinated cardiac fibers37 cannot be attributed to the low ATPase activities of cardiac myosin and actomyosin because the maximal tetanic tension (P\(_{o}\)) of red skeletal muscle, which also has a myosin with low ATPase activity, does not differ from that developed by the same cross-sectional area of white skeletal muscle.31 The lower tension developed by the myocardium more likely results from the greater number of mitochondria and, therefore, fewer contractile elements per unit of cross-sectional area. This interpretation is supported by the lower yields of myosin from cardiac muscle.

Acknowledgment

The authors thank Mr. Harvey Schneier, who participated in the earlier phases of this study, and Miss Barbara Cohen for technical assistance.

References

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Circ Res. 1966;19:611-621
doi: 10.1161/01.RES.19.3.611

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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