Cardiac Myosin Adenosinetriphosphatase Activity

MODIFYING FACTORS AND COMPARISON WITH SKELETAL MUSCLE MYOSIN ADENOSINETRIPHOSPHATASE ACTIVITY

By Robert J. Luchi, M.D., and Eve Marie Kritcher, A.B.

ABSTRACT

Cardiac myosin prepared by any one of a number of modifications of the basic Szent-Gyorgyi method and cardiac myosin prepared by the lithium chloride-ammonium sulfate technique differ in two important respects: 1) Szent-Gyorgyi-prepared myosin solutions are inhomogeneous by both chemical and immunologic criteria; 2) the ATPase activity of Szent-Gyorgyi-prepared myosin is low in comparison to lithium chloride-ammonium sulfate prepared cardiac myosin. Evidence is presented in this paper showing that the reduced ATPase activity of Szent-Gyorgyi-prepared cardiac myosin is the result of inhibition of the enzyme, possibly by a nucleotide contaminant, and a "diluting" effect of other nitrogen-containing contaminants of low specific activity.

Many earlier studies indicate that cardiac and skeletal muscle myosin prepared by the Szent-Gyorgyi method differed strikingly in their ATPase activities. Comparison of lithium chloride-ammonium sulfate prepared cardiac and skeletal muscle myosin shows that skeletal muscle myosin has a slightly but significantly higher ATPase activity than canine cardiac myosin. A methodologic error was not excluded as the cause of this discrepancy. In a number of other characteristics, canine cardiac and skeletal muscle myosin ATPase activity was virtually identical.

ADDITIONAL KEY WORDS purification of canine cardiac myosin characterization of cardiac and skeletal myosin myosin enzyme activity kinetics of myosin ATPase contractile protein myosin contamination adenosinetriphosphatase systems fractionation of myosin biochemistry of myosin

Cardiac myosin adenosinetriphosphatase activity undoubtedly plays an important role in the contraction cycle of the heart, and for this reason it has been the object of study by a number of investigators for more than 20 years. There is a consensus among investigators in this field that cardiac myosin ATPase activity is significantly, i.e., one-half to two-thirds lower than the ATPase activity of skeletal myosin. In 1965, we reported that conventionally prepared cardiac myosin solutions* treated with ammonium sulfate in the presence of 2 M lithium chloride can be separated into three components. The evidence indicated that the major fraction so obtained (by 45% saturation with ammonium sulfate) possessed the physicochemical

*The terms "conventionally prepared myosin," "prefractionation myosin" and "prefractionation protein" refer to myosin obtained by extraction with phosphate salts, precipitation three times by dilution of solutions to low ionic strength and preparative ultracentrifugation. "Postfractionation myosin" and "45% fraction" refer to myosin extracted with phosphate salts, precipitated one or more times by dilution of the myosin solution to low ionic strength and precipitation of the myosin by ammonium sulfate in the presence of 2 M lithium chloride as described in a previous publication. Prior to fractionation, preparative ultracentrifugation may or may not be done.
characteristics usually ascribed to myosin. The two other components, minor in terms of their percentage of the total prefractionation nitrogen, are heterogenous and not simply denatured or aggregated myosin. The second important finding of this study was that fractionation with lithium chloride and ammonium sulfate increased the ATPase activity of myosin approximately twofold. There are at least two possible explanations for this increase. First, it could result from a "denaturing" effect of lithium chloride-ammonium sulfate treatment, analogous to the stimulating effect of p-chloromercuribenzoate (PCMB), ethylene glycol or DNP. Second, the increased enzyme activity could result from removal of tightly adhering contaminants which, in one way or another, interfere with cardiac myosin enzyme activity. If this latter explanation is correct, a re-examination of the characteristics of cardiac myosin ATPase activity would be in order. The first part of this report is devoted to the cause of the increased ATPase activity of cardiac myosin prepared by the lithium chloride-ammonium sulfate technique.

It is generally agreed that cardiac and skeletal myosin are similar in most of their physicochemical properties, differing greatly only in ATPase activity. The lower ATPase activity of cardiac myosin has been thought to explain, at least partly, certain physiological differences between these two types of striated muscle, particularly the difference in maximum tension generation. The ATPase activity of postfractionation cardiac myosin is nearly as great as postfractionation or conventionally prepared skeletal myosin, suggesting that the difference between cardiac and skeletal myosin is not so great as heretofore believed. The second objective of this study, therefore, is to compare further cardiac and skeletal myosin ATPase activity.

The results indicate that the increase in ATPase activity noted after lithium chloride-ammonium sulfate fractionation is the result of 1) removal of contaminating material of low specific activity, and 2) removal of material that inhibits myosin ATPase activity. Further, the results suggest that lithium chloride-ammonium sulfate treatment does not act directly to stimulate cardiac myosin ATPase activity as does PCMB, DNP, EDTA and ethylene glycol, because the ATPase activity of postfractionation cardiac myosin can be increased by each of these substances. The $K_m$ for the myosin-ATP interaction, calcium activation of enzyme activity, and the effect of monovalent cations on enzyme activity are similar for both cardiac and skeletal myosin ATPase. However, cardiac myosin ATPase activity is slightly, although significantly, lower than skeletal myosin ATPase activity.

**Methods**

The preparation and fractionation of canine cardiac myosin have been described previously. Prefractionation canine cardiac myosin was prepared by the various published modifications of the basic Szent-Györgyi method. Skeletal myosin was obtained from the hind limb of dogs. Prior to excision of the muscle, the extremity was cooled in ice water for two hours. Skeletal myosin was extracted and fractionated by the same method as outlined for cardiac myosin, i.e., fractionation with ammonium sulfate in the presence of 2M lithium chloride. Three fractions were obtained from both skeletal and cardiac myosin and were designated according to the percent saturation of ammonium sulfate at which they were collected. The "45% fraction" was the largest of the three and possessed the physicochemical properties of myosin. The 45% fraction and, where indicated, the 38% and 90% fractions were subjected to the fractionation procedure two additional times.

Protein concentration was determined by the micro-Kjeldahl method, assuming a nitrogen content of 16% for myosin.

The enzymatic activity of the prefractionation protein, postfractionation myosin and the various protein fractions was determined as described in a previous paper. The standard assay mixture contained 0.2 M tris-0.025 M maleic acid buffer (pH 6.5), 1.0 $\times$ 10$^{-2}$ M CaCl$_2$, 1.6 $\times$ 10$^{-3}$ M ATP and 200 to 500 $\mu$g of protein in a total volume of 6.1 ml. ATPase activity was expressed as $\mu$moles phosphorus liberated/mg of protein/5 min. Approximately 8% of the ATP was hydrolyzed during the five-minute incubation period. Duplicate determinations of myosin ATPase activity on a given day did not vary by more than 3%.
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TABLE 1

Effects of Lithium Chloride and Ammonium Sulfate on Prefractionation Cardiac Myosin ATPase Activity

<table>
<thead>
<tr>
<th>Protein preparation</th>
<th>Recovery</th>
<th>ATPase activity</th>
<th>Comparison between</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of total protein</td>
<td>μmoles P/mg/5 min</td>
<td>prefractionation and</td>
</tr>
<tr>
<td>A. LiCl-(NH₄)₂SO₄</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>38% (6)*</td>
<td>32.8 ± 1.9†</td>
<td>1.49 ± 0.16</td>
<td>0.49</td>
</tr>
<tr>
<td>45% (6)</td>
<td>63.2 ± 3.8</td>
<td>4.44 ± 0.16</td>
<td>2.81</td>
</tr>
<tr>
<td>90% (6)</td>
<td>2.0 ± 1.0</td>
<td>0.66 ± 0.32</td>
<td>0.01</td>
</tr>
<tr>
<td>Prefractionation myosin (6)</td>
<td>100.</td>
<td>2.21 ± 0.11</td>
<td>—</td>
</tr>
<tr>
<td>B. LiCl alone (2)</td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Prefractionation myosin (2)</td>
<td>100.</td>
<td>2.23</td>
<td>—</td>
</tr>
<tr>
<td>C. (NH₄)₂SO₄ alone</td>
<td></td>
<td></td>
<td>—</td>
</tr>
<tr>
<td>38% (5)</td>
<td>76.5 ± 3.4</td>
<td>2.26 ± 0.45</td>
<td>1.73</td>
</tr>
<tr>
<td>45% (5)</td>
<td>19.2 ± 2.5</td>
<td>3.31 ± 0.44</td>
<td>0.64</td>
</tr>
<tr>
<td>90% (5)</td>
<td>1.2 ± 1.2</td>
<td>0.72 ± 0.89</td>
<td>0.01</td>
</tr>
<tr>
<td>Prefractionation myosin (5)</td>
<td>100.</td>
<td>2.39 ± 0.22</td>
<td>—</td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate number of experiments.
†Mean values and, when indicated, ±1 SD.

of ATPase activity on different preparations of cardiac or skeletal myosin done weeks or months apart did not vary by more than 12%.

The spectrophotometric procedure of Boyer was used to measure the concentration of PCMB and to follow the reaction of myosin SH groups with PCMB. The change in optical density at 255 mμ was measured in a Zeiss PMQ II spectrophotometer. Solutions of PCMB, two times recrystallized, were made up before each experiment and their concentration determined at 232 mμ, using 1.69 × 10⁻⁴ as the molar absorbancy index. PCMB was added to the myosin solutions at 0°C. ATPase activity was measured immediately or at intervals in a reaction mixture containing 0.25 M KCl-0.05 M tris buffer (pH 7.6), 5 × 10⁻⁴ M CaCl₂ and 1.6 × 10⁻⁸ M ATP.

Table 1: Effects of Lithium Chloride and Ammonium Sulfate on Prefractionation Cardiac Myosin ATPase Activity

<table>
<thead>
<tr>
<th>Protein preparation</th>
<th>Recovery</th>
<th>ATPase activity</th>
<th>Comparison between</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prefractionation myosin</td>
<td>100.</td>
<td>2.21 ± 0.11</td>
<td>—</td>
</tr>
</tbody>
</table>

The effect on prefractionation myosin ATPase activity of lithium chloride and ammonium sulfate added separately is also shown (table 1B). Lithium chloride was added to the prefractionation myosin as indicated under “Methods,” without subsequent fractionation by ammonium sulfate. The ATPase activity prior to the addition of the lithium chloride was 2.23 μmoles P/mg protein/5 min. Following exposure to 2 M lithium chloride the ATPase...
activity was 2.51 μmoles P/mg protein/5 min, a difference of less than 1%.

Five preparations of conventionally prepared myosin were fractionated with ammonium sulfate alone (table 1C). In two, only two precipitates were obtained, a larger one at 38% saturation and a smaller one at 45% saturation. In the other three 38%, 45%, and 90% fractions were obtained. The precipitates were dissolved in and dialyzed against 0.4 M KCl-borate buffer, and the ATPase activity of each fraction was determined. In these five comparisons the sum of the "weighted" ATPase activities was, on the average, less than 1% different from the ATPase activity of the pre-fractionation protein.

**B. Effect of the 38% and 90% Fractions on the ATPase Activity of Postfractionation Cardiac Myosin**

Inhibition of postfractionation myosin ATPase activity by the 38%, or 90% fractions could not be demonstrated directly. Arbitrary mixtures of these recycled fractions and postfractionation myosin were tested for ATPase activity. Experimentally determined ATPase activities were not different from those calculated from the sum of the individual "weighted" ATPase activities.

Comparison of the ATPase activity of pre-fractionation myosin, postfractionation myosin (45% fraction), the combined 38% and 45% fractions and the combined 45% and 90% fractions for six protein samples is shown in table 2A. The combined 38% and 45% fractions were obtained by rapidly raising the ammonium sulfate concentration to 45%, collecting the precipitate, dissolving it in 0.4 M KCl-borate buffer and dialyzing it against repeated changes of this solvent. The 45% and 90% fractions were obtained by first removing the 38% fraction and then rapidly raising the ammonium sulfate concentration of the supernatant solution to "90%" saturation. The ATPase activity of postfractionation myosin is taken to be 100%. Each of the combined fractions has an ATPase activity lower than that of postfractionation myosin. The combined percentage decrease in ATPase activity of the 38% and 45% fractions and of the 45% and 90% fractions totalled 52.8%. The percentage difference between pre- and postfractionation myosin was 50.5%.

Comparison is also made between the observed ATPase activity of the combined 38% and 45% fractions, and the calculated ATPase

### Table 2

**Effects of the 38% and 90% Fractions on the ATPase Activity of Postfractionation Myosin**

<table>
<thead>
<tr>
<th>Protein preparation</th>
<th>Per cent of total protein</th>
<th>ATPase activity μmoles P/mg/5 min</th>
<th>&quot;Weighted&quot; (％ total protein X ATPase activity)</th>
<th>Comparison with 45% fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Postfractionation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>myosin, 45% (6)*</td>
<td></td>
<td>4.44 ± 0.10*</td>
<td>0.51</td>
<td>100%</td>
</tr>
<tr>
<td>Prefractionation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>myosin (6)</td>
<td>100</td>
<td>2.21 ± 0.11</td>
<td>-50.5 ± 3.2*</td>
<td></td>
</tr>
<tr>
<td>38-45% (6)</td>
<td>34-66</td>
<td>2.94 ± 0.11</td>
<td>-35.2 ± 3.4*</td>
<td></td>
</tr>
<tr>
<td>45-90% (6)</td>
<td>97-3</td>
<td>3.711 ± 0.14</td>
<td>-17.6 ± 2.6*</td>
<td></td>
</tr>
<tr>
<td>B. 38% (6)</td>
<td>34</td>
<td>1.49 ± 0.16</td>
<td>0.51</td>
<td>-15.0* (P &lt; 0.001)</td>
</tr>
<tr>
<td>45% (6)</td>
<td>66</td>
<td>4.47 ± 0.23</td>
<td>2.95</td>
<td>-15.0% (P &lt; 0.001)</td>
</tr>
<tr>
<td>38-45% (6)</td>
<td>34-66</td>
<td>2.94 ± 0.11</td>
<td>4.34</td>
<td>-14.9% (P &lt; 0.001)</td>
</tr>
<tr>
<td>C. 45% (6)</td>
<td>97</td>
<td>4.47 ± 0.23</td>
<td>3.49</td>
<td></td>
</tr>
<tr>
<td>90% (6)</td>
<td>3</td>
<td>0.66 ± 0.32</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>45-90% (6)</td>
<td>97-3</td>
<td>3.71 ± 0.14</td>
<td>-14.9% (P &lt; 0.001)</td>
<td></td>
</tr>
</tbody>
</table>

*Numbers in parentheses indicate number of experiments.
†Mean values and, when indicated, ±1 sd.

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activity based on the amount and individual ATPase activity of each fraction present in the mixture (table 2B and 2C). An example of the calculation follows. The observed ATPase activity of the combined 38% and 45% fractions averaged 2.94 μmoles P/mg protein/5 min. In this mixture, the 45% fraction contributed 66% of the total nitrogen and the 38% fraction, 34% of the total nitrogen. ATPase activities of these fractions averaged 4.47 μmoles P/mg protein/5 min and 1.49 μmoles P/mg protein/5 min, respectively. One would have expected a contribution of ATPase activity of 0.51 μmoles P/mg protein/5 min from the 38% fraction and 2.95 μmoles P/mg protein/5 min from the 45% fraction, a total of 3.46 μmoles P/mg protein/5 min from both. The difference between the observed value and the theoretically calculated one is 0.52 μmoles P/mg protein/5 min, a decline of 15.0%. This value is statistically significant at the 0.1% level.

Similar calculations may be made between the observed and expected ATPase activity of the combined 45% and 90% fractions. In this mixture the 45% fraction contributed 97% of the total protein. The discrepancy between the observed and the theoretically calculated ATPase activity for this combined fraction averaged 14.9%. If the 90% fraction were totally inactive in splitting ATP (which is not the case) only a 2% decline in ATPase activity would be anticipated.

Both the 38% and 90% fractions were extracted with hot 10% NaCl as described by Mihalyi et al. Each fraction contained a substance with an absorption peak at 260 μm.

The reciprocal of the Michaelis constant for the interaction between pre- and postfractionation myosin and ATP was derived from the Lineweaver-Burk plot shown in figure 1. The $K_m$ for the prefractionation protein was $1.53 \times 10^4$; the $K_m$ for postfractionation myosin was $2.02 \times 10^4$. When ATPase activity was measured at a lower ionic strength and lower pH (fig. 2), $K_m$ values were $1.23 \times 10^4$ for prefractionation protein and $3.83 \times 10^5$ for postfractionation myosin.

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II. COMPARISON OF CARDIAC AND SKELETAL MYOSIN ATPase ACTIVITY

The mean value for postfractionation skeletal myosin ATPase activity was 5.41 μmoles P/mg protein/5 min. The mean value for cardiac myosin ATPase activity was 4.44 μmoles P/mg protein/5 min (table 3). Thus, there was a slight but consistent and statistically significant difference ($P < 0.001$) of approximately 22% between the ATPase activity of postfractionation canine skeletal and cardiac myosin. The SH groups of both postfractionation car-

![Figure 1](http://circres.ahajournals.org/)

Lineweaver-Burk plot of prefractionation and postfractionation cardiac myosin. All measurements were performed in 0.5 M KCl-0.05 M tris buffer (pH 8.0) and $5 \times 10^{-4}$ M CaCl$_2$. Broken line: prefractionation cardiac myosin ($K_m = 1.53 \times 10^4$). Solid line: postfractionation cardiac myosin ($K_m = 2.02 \times 10^4$).

![Figure 2](http://circres.ahajournals.org/)

Lineweaver-Burk plot of prefractionation and postfractionation cardiac myosin. All measurements were performed in 0.2 M tris-maleate buffer (pH 6.5) and $1 \times 10^{-4}$ M CaCl$_2$. Broken line: prefractionation cardiac myosin ($K_m = 1.23 \times 10^4$). Solid line: postfractionation cardiac myosin ($K_m = 3.83 \times 10^5$).
diac and skeletal myosin were determined by PCMB titration. The individual values for six different protein preparations are listed in table 4. The average value is 8.3 half cystine residues per $10^5$ g of protein. Using a molecular weight of 500,000 for cardiac myosin, a value of 41.5 SH groups/mole of protein was calculated. This figure is in agreement with those obtained by direct amino acid analyses. The molecular weight of canine skeletal myosin determined in our laboratory is $493,000 \pm 40,000$; hence the SH groups/mole of protein is the same for both skeletal and cardiac myosin.

The reciprocal of the Michaelis constant ($K_m$) for the interaction between ATP and postfractionation skeletal myosin was derived from the Lineweaver-Burk plots illustrated in figure 3. The $K_m$ for skeletal myosin was $2.92 \times 10^4$, and $4.40 \times 10^8$ when measured in a reaction mixture of lower ionic strength and pH.

Figures 4 and 5 illustrate the change in ATPase activity for both proteins with an increase in sodium and potassium ion concentration. Significant depression of cardiac myosin ATPase activity does not occur until the concentration of sodium exceeds $10^{-1}$ M.
Skeletal myosin ATPase activity is slightly less sensitive to increases in sodium concentration. Increasing the concentration of potassium ions has an effect similar to that of sodium on cardiac myosin ATPase activity. Again, skeletal myosin ATPase appears somewhat less sensitive to increases in the concentration of the potassium ion. Dog skeletal myosin ATPase is activated by calcium. Maximum ATPase activity is achieved with a calcium concentration of $10^{-2}$ M, in agreement with the results for cardiac myosin previously reported by us.7 Figure 6 shows the change in ATPase activity for both cardiac and skeletal myosin ATPase activity as a function of pH. The pH optima are the same for both proteins.

Table 5 shows the stimulatory effect on cardiac myosin ATPase activity of PCMB, ethylene glycol, DNP and EDTA. The stimulation of cardiac myosin ATPase activity by PCMB and ethylene glycol was found to be dependent on the ionic concentration in which ATPase activity is measured. This is illustrated for PCMB in figures 7 and 8. In all experiments in this group PCMB was added to the myosin solution at 0°C and the ATPase activity was measured immediately thereafter. The stimulation of ATPase activity by PCMB was qualitatively similar for skeletal and cardiac myosin when ATPase activity was measured in a reaction mixture containing 0.25 M KCl-0.05 M tris buffer (pH 7.6) and $5 \times 10^{-3}$ M CaCl$_2$.

### Table 5

<table>
<thead>
<tr>
<th>Buffer</th>
<th>ATPase activity (µmoles P/mg protein/5 min)</th>
<th>Per cent increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25 M KCl-0.05 M tris, pH 7.6</td>
<td>Control 2.57</td>
<td>56%</td>
</tr>
<tr>
<td>0.5 M KCl-0.1 M tris, pH 8.0</td>
<td>0.04 µmoles PCMB/mg, Control 1.34</td>
<td>138%</td>
</tr>
<tr>
<td>0.2 M tris-maleate, pH 6.5</td>
<td>45% ethylene glycol 3.16, Control 4.46</td>
<td>128%</td>
</tr>
<tr>
<td>0.5 M KCl-0.1 M tris, pH 8.0</td>
<td>5 $\times 10^{-5}$ M DNP 6.88, Control 1.38</td>
<td>54%</td>
</tr>
<tr>
<td></td>
<td>1 $\times 10^{-3}$ M EDTA 3.14</td>
<td>128%</td>
</tr>
</tbody>
</table>

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However, no stimulation of ATPase activity of cardiac or skeletal muscle by PCMB was noted when the reaction was carried out in 0.2 M tris-0.025 M maleic acid buffer (pH 6.5).

This observation is in accord with the work of Perry and Cotterill, indicating that potassium was required for the stimulation of skeletal myosin ATPase activity by mercurials. Inhibition began with concentrations of PCMB exceeding 0.04 μmoles/mg of protein in the case of skeletal myosin, and with all concentrations of PCMB when cardiac myosin was tested.

PCMB stimulation of cardiac myosin ATPase activity is time dependent. Figure 9 illustrates the decline in PCMB-stimulated cardiac myosin ATPase activity as a function of time. In this study 0.04 μmoles PCMB was added per mg of myosin and the mixture was kept at 0°C. Aliquots of this mixture were analyzed for ATPase activity immediately and at 30, 90, 120, 180, and 240 minutes after addition of PCMB. Aliquots of the control solution of myosin were analyzed for ATPase activity at identical time intervals. PCMB-stimulated myosin ATPase activity decreased to approximately the control value at 90 minutes. Thereafter, ATPase activity decreased slightly but progressively so that at the end of four hours the PCMB-myosin mixture had an ATPase activity 22% below that of the control.

Cardiac myosin was equilibrated by dialysis with 45% ethylene glycol in 0.5 M KCl-0.1 M
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tris buffer (pH 8.0). ATPase activity was increased 136% above the control. However, neither 10%, 30% nor 45% ethylene glycol increased cardiac myosin ATPase activity when the reaction was measured in 0.2 M tris-maleate buffer (pH 6.5). In this buffer system the intrinsic viscosity of cardiac myosin is markedly increased by 45% ethylene glycol indicating gross denaturation of the protein.

Both DNP and EDTA stimulated the ATPase activity of cardiac myosin. DNP in the final concentration of 5 x 10^{-3} M increased cardiac myosin ATPase activity by 54%. The reaction mixture contained, in addition to DNP, 0.2 M tris-maleate buffer (pH 6.5) and 1.6 x 10^{-3} M ATP. EDTA increased cardiac myosin ATPase activity 128%. The reaction mixture contained 0.5 M KCl-0.1 M tris buffer (pH 8.0), 1.6 x 10^{-3} M ATP, and 1 x 10^{-8} M EDTA.

discussion

RELATIONSHIP BETWEEN THE ATPASE ACTIVITY OF PRE- AND POSTFRACTIONATION CARDIAC MYOSIN

Our conclusion from these studies is that the higher ATPase activity of the 45% fraction is “masked” in the mixture of components in the prefectionation protein. This “masking” is the result of both direct inhibition of the 45% fraction ATPase activity and dilution of its effect by the presence of enzymatically inactive protein.

It is clear that the increase in ATPase activity of postfractionation myosin is related to removal of the 38% and 90% components. Some but not all of the difference between the ATPase activity of pre- and postfractionation myosin can be attributed to removal from the prefectionation protein mixture of material containing enzymatically inactive nitrogen. If one ignores the amount of 45% protein in the 38% and 90% fractions (presumably responsible for the ATPase activity of these fractions) and calculates the increase in ATPase activity of postfractionation myosin due solely to removal of these two fractions, the increase in ATPase activity is 59% (2.21 to 3.50). Clearly, this is a maximum figure. The experimentally obtained percentage difference in ATPase activity of pre- and postfractionation myosin is 100% (2.21 to 4.44). The discrepancy between the calculated and experimentally derived values could be explained either by assuming alteration of the myosin molecule by lithium chloride or ammonium sulfate in such a manner as to increase the rate of ATP splitting, or by assuming inhibition of myosin by components of the 38% and 90% fractions.

Neither lithium chloride nor ammonium sulfate alone altered myosin ATPase activity. The $K_m$ of the postfractionation myosin is virtually the same as the $K_m$ of the prefectionation protein when ATPase activity is measured at $1/2 = 0.68$ (excluding ATP). The similarity of $K_m$ for the pre- and postfractionation protein also supports the conclusion that lithium chloride-ammonium sulfate fractionation does not directly alter the enzyme site on the myosin molecule. The $K_m$ of prefectionation protein of 1.53 x 10^4 is very close to the value of 1 x 10^4 reported by Brahms and Kay for their preparation of cardiac myosin as determined from ATPase activities measured in an environment identical with that used by us. All these findings suggest that the enzyme site on postfractionation myosin is not changed significantly by the fractionation procedure.

Another point may be made. Blum has reported that the ATPase stimulating effects of PCMB, EDTA and DNP are not additive. If the fractionation procedure increases the ATPase activity of cardiac myosin in a manner analogous to that of DNP, PCMB or EDTA, depression rather than an augmentation of ATPase activity would be expected when the ATPase activity of postfractionation cardiac myosin is measured in the presence of one of these reagents. Under these conditions an increase in ATPase activity is noted, indicating clearly that the fractionation procedure does not affect the myosin molecule in the manner described by others for this group of compounds.

Inhibition of postfractionation myosin (45% fraction) by components of the 38% and 90% fractions could not be demonstrated directly. However, an indirect demonstration of their effect on the ATPase activity of ammonium sulfate-lithium chloride fractionated myosin...
(45% fraction) may be inferred. The experimentally determined value for ATPase activity of the combined 38% and 45% fractions was 2.94 μmoles P/mg protein/5 min. The percentages of 38% and 45% material found in this combined fraction and the individual ATPase activities of the 38% and 45% components were determined. The sum of the ATPase activities of these individual fractions, weighted according to their per cent contribution to total nitrogen in the combined fraction, was 3.46 μmoles P/mg of protein/5 min. The difference between the calculated “weighted” ATPase activity and the experimentally determined value is 15%. Thus, one may infer inhibition of the ATPase activity of the 45% fraction by a component or components in the 38% fraction. Similarly, inhibition by a component or components of the 90% fraction can be determined. The inhibition of 45% fraction ATPase activity by the 90% fraction is approximately 15%. However, the 90% fraction is present in far smaller concentration than the 38% fraction. Hence, a more potent inhibitory effect may be attributed to the 90% fraction.

Schwartz isolated a histone from heart muscle which, in a concentration as low as 10^{-9} M, inhibited both cardiac and skeletal actomyosin ATPase activity. Mihalyi found evidence of a nucleic acid contaminant in conventionally prepared skeletal myosin solutions. In our study an ethanol-precipitable subtraction of both the 38% and 90% fractions with a peak absorbancy of 260 mμ was obtained. This finding is not inconsistent with the presence of a nucleotide component in these two fractions. No further attempts were made to establish the chemical nature of the myosin ATPase inhibitor. It remains possible that the inhibiting action of both fractions may be related to a nucleohistone component similar to that isolated by Schwartz and shown to be capable of inhibiting cardiac and skeletal actomyosin ATPase activity. However, on the basis of the evidence presented, one cannot exclude the possibility that the inhibitor removed by the ammonium sulfate-lithium chloride fractionation procedure may be a heavy metal, perhaps magnesium.

In a solution of tris-maleate buffer, pH 6.5 (ionic strength 0.18 excluding ATP) the value for K_m of postfractionation cardiac myosin falls and the velocity of ATP hydrolysis rises, suggesting a conformational change at or near the enzyme site induced by the altered ionic environment. The K_m of prefractionation myosin does not change appreciably in tris-maleate buffer, pH 6.5 (although the velocity of ATP splitting increases somewhat), suggesting that a conformational change at the enzyme site does not occur. It appears that one or more of the components of the 38% and 90% fractions prevents this conformational change (and the expected increase in velocity of ATP hydrolysis) from occurring. Thus, prefractionation cardiac myosin ATPase activity is “inhibited” in tris-maleate buffer, pH 6.5. Whether the conformational change under discussion results in a cardiac myosin molecule more or less like that occurring in vivo cannot be determined at present and is, therefore, irrelevant to this discussion. It is, however, pertinent to state that removal of components comprising the 38% and 90% fractions result in a preparation of cardiac myosin permitting study of the in vitro characteristics of the myosin molecule unhampered by complicating artifacts.

COMPARISON OF THE ATPASE ACTIVITIES OF POST-FRACTIONATION CARDIAC AND SKELETAL MYOSINS

In a previous publication we reported that postfractionation canine cardiac myosin and postfractionation chicken skeletal myosin had similar sedimentation coefficients, molecular weights and intrinsic viscosities. The ATPase activity of postfractionation canine cardiac myosin was about 20% below that of postfractionation chicken skeletal myosin. Mueller et al. have reported that conventional preparations of canine skeletal and canine cardiac myosin are virtually identical but ATPase activities for both skeletal and cardiac myosins were very low. Brahms and Kay, in their comparison of canine skeletal and cardiac myosin, indicate a molecular weight for cardiac myosin of 758,000, higher than that reported for skeletal myosin by Lowey and Cohen or Kielley and Harrington. Brahms
and Kay also found a much lower ATPase activity for cardiac myosin compared to skeletal myosin. Barany et al., comparing rabbit cardiac and skeletal myosin, found a lower ATPase activity and a decreased number of SH groups of cardiac myosin as compared to skeletal myosin.

The studies reported in this communication indicate that the molecular weights of postfractionation canine cardiac and skeletal myosin are not significantly different. However, the ATPase activity of postfractionation cardiac myosin is consistently lower than that of canine skeletal myosin by approximately 20%. The reason for this discrepancy in ATPase activity is not known. Although random error is unlikely, a systematic difference in the process of excision and in the extraction and purification procedures has not been excluded. The heart was excised while still beating and was then cooled in ice water; the extremity from which skeletal muscle was obtained was cooled before the muscle was excised. It is likely that neither this procedural difference nor the type of muscle examined, i.e., red vs. white is related to the discrepancy in ATPase activity. Measurement of rabbit heart (red muscle) and psoas (white) myosin ATPase activity, excised as described above for the dog, is the same for both proteins, i.e., 5.58 μmoles P/mg protein/5 min. Cooling of the dog heart in situ with ice for one-half hour before excision did not result in an increase in ATPase activity of the extracted myosin.

Except for this relatively minor discrepancy in ATPase activities, comparison of postfractionation canine cardiac and skeletal myosin is striking indeed. The Kₐ of both proteins, though not identical, are very close. Both proteins undergo a conformational change in tris buffer, pH 6.5, resulting in a decrease in Kₐ values and an increase in the rate of ATP splitting. The pH optima and calcium activation of postfractionation skeletal myosin ATPase activity are the same as those previously reported for postfractionation cardiac myosin. The decrease in ATPase activity with increasing sodium and potassium ion concentration is similar for cardiac and skeletal myosin. The increase in ATPase activity of skeletal myosin with PCMB, DNP, and EDTA is well known. The ATPase activity of postfractionation cardiac myosin also is increased by these substances. Under appropriate conditions, ethylene glycol increases the ATPase activity of both cardiac and skeletal myosin. The total number of SH groups measured by PCMB titration is the same for both proteins. Amino acid analysis of cardiac and skeletal myosin is similar, as reported by Iyengar and Olson.

It is difficult to impute a major difference between postfractionation cardiac and skeletal myosin only on the basis of a 20% discrepancy in the rate of ATPase hydrolysis. Even if this difference is not due to the methods used, the evidence suggests a much closer relationship between cardiac and skeletal muscle myosin than has been recognized heretofore. Cardiac and skeletal actin are indistinguishable insofar as amino acid analysis, sedimentation coefficient, intrinsic viscosity, SH group content and molecular weight are concerned. If, then, the two major proteins of cardiac and skeletal muscle are so similar in the physicochemical characteristics so far studied, another explanation for the physiological differences between these two types of muscle should be sought.

References


Cardiac Myosin Adenosinetriphosphatase Activity: Modifying Factors and Comparison with Skeletal Muscle Myosin Adenosinetriphosphatase ACTIVITY
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