Comparison of Tropomyosins from Cardiac and Skeletal Muscle

By Arnold M. Katz, M.D., and Ronald P. Converse, B.A.

Characterization of the myocardial contractile proteins has been undertaken in an attempt to define biochemical mechanisms responsible for the physiological dissimilarities between cardiac and skeletal muscle. These functional differences include the lesser tension generated by cardiac muscle, \(^1,^2\) different actions of inotropic agents, \(^3,^4\) and the relative slowness of onset of the active state of cardiac muscle when compared to its skeletal counterpart. \(^5\) The relative weakness of cardiac muscle is manifested in the behavior of glycerol-extracted cardiac fibers \(^6\) and may be related to the lower adenosinetriphosphatase activities of cardiac actomyosin \(^7\) and cardiac myosin. \(^8,^9,^{10}\) Physicochemical studies of cardiac myosin indicate a molecular weight similar to that of skeletal myosin \(^8,^9,^{10}\) although different amino acid sequences are suggested by immunological dissimilarities \(^12\) and different effects of trypsin. \(^8\) Comparison of cardiac and skeletal actins has failed to reveal detectable differences in physicochemical properties, \(^13\) amino acid compositions, \(^14\) and the number and reactions of sulfhydryl (SH) groups. \(^15\)

Tropomyosin, \(^*\) the third of the more abundant myofibrillar proteins, is present in cardiac as well as skeletal muscle. \(^16\) Several reports suggesting differences between cardiac and skeletal tropomyosins have been published but no systematic comparison has been made. In the present study sedimentation behavior, intrinsic viscosities, molecular weight estimates, quantitative amino acid compositions, and peptide patterns after tryptic digestion of cardiac and skeletal tropomyosins have been examined. These failed to reveal significant differences between the two proteins. Furthermore, tropomyosin A was not found in heart muscle.

Methods

Tropomyosin was prepared from the back and leg muscles of freshly killed rabbits and from frozen rabbit hearts. The procedures outlined by Bailey \(^10\) were followed except that the tropomyosin precipitate in 5.30 M ammonium sulfate was collected by ultracentrifugation at 35,000 \(\times\) g for one hour. For each preparation the ammonium sulfate purification was repeated three times and all tropomyosin preparations appeared as a single symmetrical peak during ultracentrifugation.

Sedimentation velocities and intrinsic viscosities were measured using the techniques previously described. \(^13\) All physicochemical studies, unless otherwise specified, were carried out in 0.01 M HCl, 0.3 M KCl, a solvent which minimizes aggregation of the protein. \(^17\) A value of 0.71 g/ml \(^18\) for the partial specific volume of both cardiac and skeletal tropomyosins was used in all calculations.

Amino acid analyses were done using a Spinco model MS automatic amino acid analyzer. Samples of cardiac and skeletal tropomyosins were hydrolyzed in evacuated sealed tubes with constantly boiling HCl for 20 hours at 106 to 107°C; corrections for hydrolytic losses of 2% for threonine and 4% for serine were applied. \(^19\)

Peptide patterns of tryptic digests of \(\beta\)-mercuribenzoate-treated, urea-denatured tropomyosins were prepared as previously described. \(^14\) Tryptic digestions were done in 0.03 M ammonium carbonate, pH 8.0, at room temperature for 18 hours.

Protein concentration was determined by the

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From the Department of Medicine, Los Angeles County Heart Association Cardiovascular Research Laboratory, University of California, Los Angeles, California.

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Mr. Converse was a summer student Research Fellow of the University of California School of Medicine, San Francisco, California.

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* In this report, the term tropomyosin designates tropomyosin \(\beta\), the vertebrate tropomyosin; tropomyosin \(\alpha\) designates the tropomyosin associated with invertebrate smooth muscle (paramyosin).
biuret reaction, calibrated against Kjeldahl nitrogen determinations. In the latter, a nitrogen content of 16.7% for both cardiac and skeletal tropomyosins was used.16

Results

The yields of tropomyosin from cardiac and skeletal muscles appeared comparable; because of losses in the purification procedure no attempt was made to quantify the yields. In the case of a single frozen dog heart, the fraction obtained between 1.97 and 2.72 M ammonium sulfate was examined because this fraction would be expected to contain tropomyosin A.20 No precipitate appeared and centrifugation at 35,000 × g for one hour failed to bring down a pellet. A large yield of tropomyosin B was subsequently obtained when the ammonium sulfate concentration was raised to 5.30 M.19

The concentration dependence of the sedimentation velocities of cardiac and skeletal tropomyosins was similar (fig. 1); the value for S₂₀,ω, calculated from the combined data by the method of least squares, was 2.53 S.

Concentration dependence of reduced viscosities, expressed as deciliters per gram, of tropomyosins from cardiac (o) and skeletal (•) muscle. All determinations were made in 0.3 M KCl, 0.01 M HCl.

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The sedimentation velocity of skeletal tropomyosin in 0.3 M KCl, 0.06 M phosphate buffer at pH 6.8, corrected for the densities and viscosities of the solvents, was 11% higher than that of the same concentration of the protein in the acid solvent.

The reduced viscosities of cardiac and skeletal tropomyosins were similar (fig. 2) and the intrinsic viscosity, calculated by the method of least squares, was 0.43 deciliter per gram for each. Axial ratios of cardiac and skeletal tropomyosins, estimated from the viscosity increment, were 27 assuming that the protein behaved as an anhydrous prolate ellipsoid of revolution. Molecular weights calculated from sedimentation and viscosity data using the Scheraga-Mandelkern equation were 54,800 for the tropomyosins from both tissues. This estimate requires the assumption that the tropomyosin behaves as a rigid prolate ellipsoid of revolution and does not consider the hydration of the protein (see reference 13 for details of these calculations).

The amino acid compositions of cardiac and skeletal tropomyosins did not differ significantly (table 1). Failure to detect proline in these analyses probably reflects the low concentration of this amino acid in the protein rather than its absence.

Peptide patterns of cardiac and skeletal tropomyosins revealed 33 identical spots (fig. 3); 9 additional fainter peptide spots also appeared similar and no consistent differences between the tropomyosins could be detected upon examination of several chromatograms made from two digests of each protein.

### Discussion

The present results fail to demonstrate chemical differences between the tropomyosins from cardiac and skeletal muscles. Furthermore, no free tropomyosin \( \alpha \) was obtained from heart muscle, in accord with similar findings in the case of mammalian skeletal muscle. Previous reports indicate that the crystalline configurations of cardiac and skeletal tropomyosins are also identical while differing significantly from crystals of tropomyosin prepared from uterine or bladder smooth muscle.

The present estimate of 54,800 for the molecular weights of rabbit cardiac and skeletal tropomyosins is similar to most published values for the latter. These include 53,000 from osmotic pressure measurements, 59,000 from sedimentation and diffusion data, 60,000 to 62,000 from amino acid composition, 52,000 from polarization of fluorescence measurements, and 53,000 to 55,000 from analysis of light-scattering. A molecular weight of 89,000 for porcine cardiac tropomyosin was determined by osmotic pressure measurements at pH 6.8. This apparent difference may reflect aggregation of the protein at the neutral pH employed because high values for the molecular weight of rabbit skeletal tropomyosin have been obtained in such solvents. From present findings it appears unlikely that the molecular weights of cardiac and skeletal tropomyosins differ significantly.

The value of 0.43 deciliter per gram for the intrinsic viscosities of both rabbit cardiac and skeletal tropomyosins agrees well with 0.45 deciliter per gram reported for porcine cardiac tropomyosin, but is slightly less

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**TABLE 1**

<p>| Amino Acid Compositions of Hydrolysates of Tropomyosin Preparations from Heart and Skeletal Muscle of Rabbits |
|-------------------------------------------------|-------------------------------------------------|</p>
<table>
<thead>
<tr>
<th>Residue</th>
<th>Cardiac</th>
<th>Skeletal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysine</td>
<td>62.5</td>
<td>62.7</td>
</tr>
<tr>
<td>Histidine</td>
<td>3.5</td>
<td>3.2</td>
</tr>
<tr>
<td>Arginine</td>
<td>23.8</td>
<td>23.3</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>46.7</td>
<td>46.6</td>
</tr>
<tr>
<td>Threonine</td>
<td>12.5</td>
<td>12.3</td>
</tr>
<tr>
<td>Serine</td>
<td>19.0</td>
<td>19.8</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>116.7</td>
<td>118.1</td>
</tr>
<tr>
<td>Proline</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Glycine</td>
<td>6.4</td>
<td>6.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>57.9</td>
<td>57.3</td>
</tr>
<tr>
<td>Valine</td>
<td>13.8</td>
<td>14.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>8.8</td>
<td>9.0</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>17.3</td>
<td>17.1</td>
</tr>
<tr>
<td>Leucine</td>
<td>53.1</td>
<td>52.2</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>9.2</td>
<td>9.4</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Total</td>
<td>453.6</td>
<td>453.0</td>
</tr>
</tbody>
</table>

* Values were arbitrarily corrected to a recovery of 95%.
than the value of 0.523 deciliter per gram determined in the case of rabbit skeletal tropomyosin. The latter difference may be a result of the higher protein concentrations used in the previous study.

The present values of 2.53 S for the sedimentation coefficients of rabbit cardiac and skeletal tropomyosins in an acid solvent are lower than the reported values of 2.95 S for rabbit skeletal tropomyosin and 2.85 S for fish tropomyosin at neutral pH. This discrepancy appears to be due to the pH of the solvents because at pH 6.8 the sedimentation velocity of one of the skeletal tropomyosin preparations used in this study was 11% higher than in the acid solvent.

The amino acid content of bovine cardiac tropomyosin has been reported to differ significantly from that of rabbit skeletal tropomyosin with respect to glycine. No such difference was seen in the present study of tropomyosins from the two tissues of the rabbit. Thus, the reported difference may reflect a species variation rather than a difference between the tropomyosins of cardiac and skeletal muscle. The amino acid analyses reported here agree well with those of Kominz et al. for rabbit skeletal tropomyosin.

The close similarities of all of the parameters of cardiac and skeletal tropomyosins examined provides strong evidence that the two proteins are identical. However, minor differences may have been overlooked and the establishment of identity must await determination of the complete amino acid sequence. The results of the present report, coupled with those of previous studies with cardiac actin, indicate that, of the three major contractile proteins, only the myosins from heart and skeletal muscle can now be considered to be chemically dissimilar.

Summary

No differences were found between the sedimentation coefficients, intrinsic viscosities, amino acid contents, and peptide patterns re-
sulting from tryptic digestion of rabbit cardiac and skeletal tropomyosins. From sedimentation and viscosity data, the molecular weights of cardiac and skeletal tropomyosins were estimated to be 54,800 for both. This value is similar to previous estimates of the molecular weight of rabbit skeletal tropomyosin.

No evidence was obtained for the existence in heart muscle of tropomyosin A. Of the three more abundant contractile proteins of the heart, myosin, actin, and tropomyosin, only myosin appears to differ from its skeletal counterpart.

Acknowledgment

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References
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