Regulatory Proteins in Hamster Cardiomyopathy

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We have shown in genetic myopathic hamsters that cardiac myofibrillar ATPase regulation by calcium is altered and that there are shifts in myosin isozyme distribution (V1→V3) suggesting abnormalities in multiple components of the contractile apparatus. To focus more on the regulatory proteins (troponin and tropomyosin), individual proteins of the skeletal and cardiac actomyosin system were reconstituted under controlled conditions. In this way, myosin plus actin and troponin-tropomyosin from the normal and myopathic animals could be studied enzymatically. The proteins were isolated from the skeletal or cardiac muscle of random-bred control and cardiomyopathic hamsters (BIO 53:58) at 7 months of age. Sodium dodecyl sulfate gel electrophoretic patterns indicated differences in the troponin I and troponin C regions of myopathic skeletal muscle, but cardiac samples from control and myopathic hamsters showed similarities in their mobilities. This suggests the possibility of different cardiac isozymes in the regulatory protein complex, as reported in our previous studies of cardiac myosin in cardiomyopathy. Calcium sensitivity was markedly decreased in the actomyosin reconstituted with troponin-tropomyosin from skeletal as well as cardiac muscle from myopathic animals. In summary, our data show that the regulatory proteins in skeletal and cardiac muscle of the myopathic hamsters have decreased inhibitory action on Mg2+-actomyosin ATPase activity. This loss of calcium regulation along with shifts in cardiac myosin heavy chain may be partially responsible for the impaired cardiac function in the hearts of myopathic hamsters. (*Circulation Research* 1990;66:1302–1309)

In vertebrate striated muscle, regulatory components of the thin filaments (troponin [Tn] and tropomyosin [Tm]) are responsible, in part, for transducing the effect of free calcium in contractile protein activation and for inhibiting this activity when calcium is absent. Tn-Tm (also called relaxing factor1,2) forms a key protein complex for the control of muscular contraction. Under relaxing conditions, these proteins inhibit the substrate (ATP)-driven actomyosin cycle either by blocking the attachment of myosin cross bridges to actin3,4 or by inhibiting subsequent reactions of the bound cross bridge.5 The whole Ca2+-sensitive complex is made up of three nonidentical Tn subunits: 1) troponin T (TnT; tropomyosin-binding unit), 2) troponin I (TnI; inhibitory unit), 3) troponin C (TnC; Ca2+-binding subunit) plus tropomyosin. The properties of Tn subunits that cause binding with Tm to form an actin-linked calcium-sensitive regulatory system in skeletal and cardiac muscle contraction in different pathologic and physiological models are quite unclear. The relative properties of fast and slow skeletal TnT are subject to neural, hormonal, and developmental influences.6–8 To date, three major isoforms of TnI that exist in cardiac tissue and in fast and slow skeletal muscle have been detected.9,10 In thyroidectomy, for example, it has been suggested that thyroid hormones can directly alter the expression of the genes controlling the synthesis of the components of the Tn complex in the absence of an active nerve.11 Differences in fast and slow skeletal and cardiac TnT have also been described under various conditions.12–16 Immunochemical studies on the polymorphic forms of TnT and TnC and their localization in striated muscle cell types have demonstrated that TnC in fast skeletal muscle differs from TnC present in slow skeletal and cardiac muscle cells.12 Besides the presence of multiple forms of Tn subunits in different muscles, the existence of different isomorphs of tropomyosin (α and β) is well established. In skeletal muscle, two different types of Tm have been reported, whereas α-Tm predominantly exists

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Supported by US Public Health Service grants HL-15498 and HL-37412.

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Received February 7, 1989; accepted December 19, 1989.
in the cardiac muscle of mammalian species. Tm from different tissues or species exhibits similarities in a number of physical, chemical, and functional properties.\textsuperscript{17,18} In a study on the regulatory proteins of the myocardium, Humphreys and Cummins\textsuperscript{10} suggested that developmental transitions were associated with altering Tm subunit expression. Recently, an extensive review\textsuperscript{19} of the role of Tm and Tn in the regulation of skeletal muscle contraction has been published describing the molecular mechanisms involved in the contractile regulation. Little is known about the role of the Tn-Tm in the regulation of skeletal and cardiac muscle in various pathological and physiological states.

Using electrophoretic and immunoelectrophoretic techniques, Obinata et al\textsuperscript{20} demonstrated that the types of TnI vary in developing normal and dystrophic chicken skeletal muscle. In another study Toyooka and Ross\textsuperscript{21} reported that the loss of Ca\textsuperscript{2+} sensitivity of natural actomyosin, which was prepared from myocardium undergoing infarction after coronary occlusion, could be attributed to the destruction of regulatory proteins. Alterations in Tn subunits were also observed by the analysis of the Tn-Tm complex during myocardial infarction in the dog heart.\textsuperscript{22} Studies from our laboratory\textsuperscript{23} on isolated purified cardiac myofibrils from cardiomyopathic Syrian hamsters suggest that the regulatory control of contractile proteins may be altered in these animals. To further elaborate on the contributory role of Tn-Tm in the cardiomyopathic hamsters, hybridization studies were performed in the present study by cross-reacting skeletal or cardiac myosin with skeletal or cardiac relaxing factor (Tn-Tm) from control or myopathic animals at 7 months of age. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) profiles indicated differences in the Tn-Tm complex isolated from control compared with myopathic skeletal muscle, but the electrophoretic mobilities of cardiac Tn-Tm from the control and myopathic hamsters were similar. Tn-Tm of cardiac or skeletal muscle from myopathic hamsters showed a loss in Ca\textsuperscript{2+} sensitivity when compared with skeletal or cardiac actomyosin from normal animals.

**Materials and Methods**

ATP, dithiothreitol (DTT), EDTA, EGTA, and proteolytic enzyme inhibitors (phenylmethylsulfonyl fluoride [PMSF], leupeptin, \textit{Nα-p-tosyl-L-lysine chloromethyl ketone} [TLCK], and pepstatin) were purchased from Sigma Chemical, St. Louis, Missouri. Genetically myopathic male Syrian hamsters (BIO 53:58 or TOs) were obtained from Dr. Michael J. Sole, Professor of Medicine, University of Toronto, Toronto General Hospital, Canada. The time point selected for study was 7–8 months of age after birth. For controls, random-bred animals of the same age were used. Control and myopathic animals were killed at the same time, and their skeletal or heart tissue was processed and analyzed simultaneously. Animals were anesthetized with ether, and their hearts and samples of mixed skeletal (fast and slow) muscle were removed. After washing the hearts, the atria and great vessels were removed, and a homogenizer (Tekmar, Cincinnati, Ohio) was used for homogenization of the whole ventricular portions. Twenty-five to 30 hearts (10 g) were pooled in each group for purification of cardiac regulatory proteins. Muscle tissue (50 g) from the back and leg was used for skeletal regulatory proteins. All the tissues were frozen and stored at \textdegree{}70\textdegree{} C in 50% glycerol containing (mM) KCl 30, KPO\textsubscript{4} 10 (pH 7.0), and β-mercaptoethanol 5 before the preparation of different proteins. There was no difference in actomyosin-ATPase activity if hearts were prepared immediately or after a period of storage.

**Preparation of Proteins**

Cardiac myosin from normal mongrel dogs, Wistar rats, and random-bred control and myopathic hamsters was prepared by methods previously described.\textsuperscript{24} Actin was extracted and purified from rabbit skeletal muscle acetone powder.\textsuperscript{25}

**Tropomin and Tropomyosin**

The Tn-Tm complex was prepared by a slight modification of the methods of Eisenberg and Kielley\textsuperscript{26} and Potter.\textsuperscript{27} In all the preparations, various proteolytic enzyme inhibitors, such as PMSF (0.1–0.2 mM), leupeptin (1 µg/ml), pepstatin (1 µg/ml), and TLCK (0.1 mM), were used.

Briefly, skeletal muscle (50 g) or cardiac tissue (10 g) was minced and homogenized with 10 vol of 0.05 M KCl and 0.01 M KPO\textsubscript{4} (pH 7.0) with 1 mM DTT and centrifuged. The pellets, which consisted of crude myofibrils, were further treated with 0.05 M KCl, 0.01 M KPO\textsubscript{4} (pH 7.0), and 2 mM EGTA (pH 7.0), followed by washing with buffer containing 0.1% Triton X-100. Myofibrils washed with Triton X-100 were extracted briefly with KCl-pyrophosphate buffer containing (M) KCl 0.47, sodium pyrophosphate 0.02, and KH\textsubscript{2}PO\textsubscript{4} 0.01 (pH 6.8) for 15 minutes to isolate and purify cardiac or skeletal myosin. Myosin was purified from the high salt–pyrophosphate extract as reported earlier.\textsuperscript{24} The remaining pellet after the extraction of myosin was washed with KHCO\textsubscript{3}, repeatedly suspended in ethanol (four times), and subsequently resuspended in diethyl ether (four times) to obtain the muscle ether powder. The ether powder was further dried under vacuum overnight to completely eliminate the solvent. The cardiac or skeletal muscle ether powder (0.5–1 g) was extracted overnight with 10 ml of 1 M KCl, 50 mM Tris buffer (pH 8.0), 1 mM DTT, 0.1 mM PMSF, and 3 mM Na\textsubscript{2}N\textsubscript{2} and centrifuged at 12,000 rpm for 30 minutes. The supernatant was fractionated with saturated ammonium sulfate, and a partially purified cardiac or skeletal Tn-Tm pellet was collected in the 40–65% fraction. This pellet was dissolved and then dialyzed against low ionic strength buffer (50 mM KCl [pH 7.0] containing 0.1 mM DTT and 0.1 mM PMSF). The Tn-Tm fraction was checked by SDS-
PAGE and was subsequently used for reassociated actomyosin-ATPase measurements within 24–48 hours of the isolation period to avoid proteolysis.

**Methods**

**Analytical Measurements.** ATPase activity of recombined actomyosin was assessed at a final volume of 1 ml at pH 7.0 and 25°C. Mg$^{2+}$-ATPase activity was measured in 20 mM imidazole buffer (pH 7.0), 3 mM Mg$^{2+}$, 2 mM ATP, and myosin:actin:Tn-Tm complex in a molar ratio of 2:1:1 or 4:3:3. Incubations were carried out as reported previously. The microphosphate method of estimating P$_i$ was used.

**Protein determination.** Protein estimation was made by the colorimetric biuret method or Bio-Rad protein assay (Bio-Rad Laboratories, Richmond, California).

**SDS-PAGE.** Different protein preparations were monitored on SDS-PAGE (5–16.5%) gradient slab gels. \cite{30} Scans of slab gels of the relaxing factor (Tn-Tm) from control and myopathic muscles were performed on an EC apparatus recorded at 605 nm, attached to an integrator (model 3390A, Hewlett-Packard, Palo Alto, California). For coelectrophoresis of Tn-Tm, samples of beef cardiac TnT, TnC, and Tm and rabbit skeletal Tn-T, TnI, TnC, and skeletal Tm were kindly supplied by Dr. Larry Tobacman, The University of Iowa, Iowa City, Iowa, and Dr. James Potter, University of Miami, Coral Gables, Florida, respectively.

**Ca$^{2+}$ Dose-Response.** For determining the Ca$^{2+}$ dependence of activation of actomyosin-ATPase in the presence of Tn-Tm, Ca$^{2+}$-EGTA buffers were prepared according to the procedure of Hathaway and coworkers. \cite{31}

**Statistics.** Comparisons of reconstituted actomyosin-ATPase inhibition were made. The reconstituted proteins were obtained by mixing 1) myosin from control hamsters plus Tn-Tm complex from control hamsters, 2) myosin from control hamsters plus Tn-Tm complex from myopathic hamsters, 3) myosin from myopathic hamsters plus Tn-Tm complex from control hamsters, and 4) myosin from myopathic hamsters plus Tn-Tm complex from myopathic hamsters. The reconstituted proteins were subjected to analysis of variance, followed by the Newman-Keuls multiple-comparison test. \cite{32} In group A in Tables 1 and 2, statistical comparisons were made for the Ca$^{2+}$-ATPase, EGTA-ATPase, and percent inhibition data individually; values of p<0.05 were considered significant. Group B in Table 1 was subjected to Student's t test; values of p<0.05 were considered statistically significant. In Figures 4 and 5, Mg$^{2+}$-ATPase values were compared between control and myopathic hamsters at 0.1 mM Ca$^{2+}$ concentration and in the presence of 2.5 mM EGTA only.

**Results**

Figure 1 shows the representative SDS gradient slab gel electrophoretic patterns and the densitometric scans of the Tn-Tm complex isolated from the skeletal muscle of control and myopathic hamsters. Coelectrophoresis and scans of the Tn-Tm complex,
with individually purified skeletal Tn subunits and Tm, demonstrate a reduction in the TnI and TnC subunits (indicated by arrows in Figure 1) in the myopathic sample when compared with the control sample. SDS-PAGE and densitometric scans of cardiac Tn-Tm complex are shown in Figure 2; similar patterns occur in control and myopathic samples of cardiac regulatory protein preparations with no evidence of any other additional bands. However, very small changes were visible in the region of TnI and TnC in the cardiac Tn-Tm complex.

To check the viability of the Tn-Tm complex in regulating the actomyosin-ATPase activity in the hamster muscle study, initial studies were focused on the regulatory complex isolated from the myocardium of two different normal mammalian species (dog and rat). Figure 3 shows the regulated actomyosin sigmoid curves for normal dog and rat cardiac actomyosin, which was reconstituted with normal dog and rat cardiac regulatory proteins at increasing free Ca\(^{2+}\) ion concentrations. The ATPase activity, with and without calcium present, demonstrated the viability and regulation of actomyosin-ATPase by the Tn-Tm complex in the cardiac muscles. Table 1 presents the Ca\(^{2+}\),Mg\(^{2+}\)-ATPase activity of reconstituted skeletal actomyosin from control and myopathic hamsters at 7 months of age. As shown in the table, actomyosin-ATPase was also studied at higher molar ratios of actin: the Tn-Tm complex with myosin. Results were similar in the two sets of studies. The data demonstrate a loss of the inhibitory effect of EGTA (removal of Ca\(^{2+}\)) in the presence of relaxing factor (Tn-Tm) from myopathic hamsters mixed with myosin from either control or myopathic hamsters. Actomyosin from control hamsters that was recombined with regulatory proteins (Tn-Tm) from myopathic hamsters had a 23–29% depression of Ca\(^{2+}\), Mg\(^{2+}\)-ATPase activity when compared with the Ca\(^{2+}\),Mg\(^{2+}\)-ATPase activity of actomyosin from control hamsters measured in the presence of relaxing factor (Tn-Tm) from control hamsters. Table 2 shows similar Ca\(^{2+}\),Mg\(^{2+}\)-ATPase activity of reconstituted cardiac actomyosin regulatory proteins. Myosin, from control or myopathic hamsters, that was hybridized with regulatory complex (Tn-Tm) from control hamsters showed 67–71% inhibition, whereas the inhibi-
tion was diminished to 44–51% when myosin from control hamsters was reassociated with cardiac relaxing factor extracted from myopathic hamsters. Figure 4 shows the Ca\(^{2+}\)-dependent skeletal actomyosin-ATPase activity using control myosin in the presence of control or myopathic skeletal regulatory complex. The basal Mg\(^{2+}\)-ATPase (EGTA-ATPase) of actomyosin was significantly elevated with myopathic relaxing factor, but the overall activation by free calcium in the range of 1 μM–0.1 mM was slightly decreased when control myosin was reconstituted with myopathic relaxing factor. Figure 5 shows Ca\(^{2+}\) dose-response curves of hamster cardiac myosin recombined with control or myopathic cardiac regulatory complex. As with skeletal proteins, cardiac proteins exhibited a basal EGTA-ATPase significantly higher in myopathic samples than in control samples. The overall activation of cardiac actomyosin-ATPase activity using myopathic cardiac Tn-Tm complex was minimally depressed in the range of free Ca\(^{2+}\) ion concentration (1 μM–0.1 mM).

Mg\(^{2+}\)-inhibitory ATPase activity by EGTA in actomyosin reconstituted with regulatory protein complex from different tissues is summarized in Figure 6. Data from dog and rat cardiac muscles (III and IV, respectively, in Figure 6) are shown as normal controls for comparisons. Random-bred control hamster (skeletal or cardiac) actomyosin combined with matched regulatory proteins (Tn-Tm) showed 72–75% inhibition in the presence of calcium chelator (EGTA). Skeletal or cardiac myopathic relaxing factors reconstituted with cardiac or skeletal myosin showed only 33–44% inhibition in the presence of EGTA.

### Table 1. Ca\(^{2+}\),Mg\(^{2+}\)-ATPase Activity of Actomyosin Reconstituted With Skeletal Myosin and Skeletal Regulatory Proteins in Control and Myopathic Hamsters

<table>
<thead>
<tr>
<th>Reconstituted proteins</th>
<th>n</th>
<th>Ca(^{2+}) (10 μM)</th>
<th>EGTA (2.5 mM)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 My[RB]+Tn-Tm[RB]</td>
<td>11</td>
<td>0.284±0.012*</td>
<td>0.071±0.007*</td>
<td>75±3*</td>
</tr>
<tr>
<td>2 My[RB]+Tn-Tm[M]</td>
<td>7</td>
<td>0.218±0.022</td>
<td>0.145±0.009</td>
<td>33±4</td>
</tr>
<tr>
<td>3 My[M]+Tn-Tm[RB]</td>
<td>3</td>
<td>0.210±0.014</td>
<td>0.048±0.007*</td>
<td>77±4*</td>
</tr>
<tr>
<td>4 My[M]+Tn-Tm[M]</td>
<td>3</td>
<td>0.210±0.015</td>
<td>0.123±0.020</td>
<td>41±10</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 My[RB]+Tn-Tm[RB]</td>
<td>3</td>
<td>0.368±0.007*</td>
<td>0.084±0.019*</td>
<td>77±5*</td>
</tr>
<tr>
<td>6 My[RB]+Tn-Tm[M]</td>
<td>3</td>
<td>0.261±0.010</td>
<td>0.174±0.024</td>
<td>34±7</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n, Number of different preparations; My[RB], myosin from control hamsters; My[M], myosin from myopathic hamsters; Tn-Tm[RB], troponin-tropomyosin complex from control hamsters; Tn-Tm[M], troponin-tropomyosin complex from myopathic hamsters. In group A, the weight ratio of reconstituted actomyosin with Tn-Tm was 2:1:1 (myosin:actin:Tn-Tm); in group B, the ratio was 4:3:3 (myosin:actin:Tn-Tm). Tn-Tm content for assays was calculated on the basis of its approximate 90% purity by gels. Specific activity is expressed as micromoles P, per minute per milligram at 25°C. Percent inhibition=100–(EGTA-ATPase/Ca\(^{2+}\),Mg\(^{2+}\)-ATPase).

*p<0.05 when comparing groups 1–4 for Ca\(^{2+}\)-ATPase, EGTA-ATPase, and percent inhibition in group A and when comparing groups 5 and 6 for Ca\(^{2+}\)-ATPase, EGTA-ATPase, and percent inhibition in group B.

### Table 2. Ca\(^{2+}\),Mg\(^{2+}\)-ATPase Activity of Regulated Actomyosin by Reconstituting Cardiac Myosin and Cardiac Regulatory Proteins in Control and Myopathic Hamsters

<table>
<thead>
<tr>
<th>Reconstituted proteins</th>
<th>n</th>
<th>Ca(^{2+}) (10 μM)</th>
<th>EGTA (2.5 mM)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 My[RB]+Tn-Tm[RB]</td>
<td>8</td>
<td>0.229±0.010</td>
<td>0.066±0.006*</td>
<td>71±3*</td>
</tr>
<tr>
<td>2 My[RB]+Tn-Tm[M]</td>
<td>7</td>
<td>0.204±0.014</td>
<td>0.115±0.012</td>
<td>44±5</td>
</tr>
<tr>
<td>3 My[M]+Tn-Tm[RB]</td>
<td>4</td>
<td>0.219±0.014</td>
<td>0.072±0.014*</td>
<td>67±2*</td>
</tr>
<tr>
<td>4 My[M]+Tn-Tm[M]</td>
<td>4</td>
<td>0.224±0.021</td>
<td>0.109±0.010</td>
<td>51±6</td>
</tr>
<tr>
<td>Group B</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 My[RB]+Tn-Tm[RB]</td>
<td>2</td>
<td>0.307</td>
<td>0.123</td>
<td>60*</td>
</tr>
<tr>
<td>6 My[RB]+Tn-Tm[M]</td>
<td>2</td>
<td>0.301</td>
<td>0.151</td>
<td>35*</td>
</tr>
</tbody>
</table>

Values are mean±SEM. n, Number of different preparations of the various proteins used for reconstitution and assays; My[RB], myosin from control hamsters; My[M], myosin from myopathic hamsters; Tn-Tm[RB], troponin-tropomyosin complex from control hamsters; Tn-Tm[M], troponin-tropomyosin complex from myopathic hamsters. Specific activity is expressed as micromoles P, per minute per milligram at 25°C. Percent inhibition is calculated as described in Table 1. In group A, the weight ratio of reconstituted actomyosin with Tn-Tm was 2:1:1 (myosin:actin:Tn-Tm); in group B, the ratio was 4:3:3 (myosin:actin:Tn-Tm). Tn-Tm complex was calculated on the basis of its 90% purity by gels.

*p<0.05 when comparing groups 1–4 for Ca\(^{2+}\)-ATPase, EGTA-ATPase, or percent inhibition, respectively.
**Discussion**

It is well established that the Tn-Tm complex is essential to confer calcium responsiveness on myofibrillar assembly.\textsuperscript{3,5} Also, in vertebrate striated muscle, actomyosin-ATPase is activated by calcium ion in the presence of regulatory complex, but the regulatory response is inhibited in the absence of this metal ion.\textsuperscript{2} In genetically myopathic hamsters, we have shown that cardiac myofibrillar ATPase revealed a change in the regulation of Ca\textsuperscript{2+} along with shifts in myosin isoforms, suggesting abnormalities in several components of the contractile protein apparatus.\textsuperscript{23} Because of the complex nature and protein isoform heterogeneity of myofibrillar contractile machinery, the present study was designed to compare the regulated actomyosin system by isolating and purifying the different proteins and then recombining them under controlled conditions. In this way, the role of myosin and the Tn-Tm complex in control and experimental animals could be studied independently. We report alterations in the regulatory proteins based on the enzymatic analysis of actomyosin conducted in the presence of the Tn-Tm complex isolated from the skeletal or cardiac muscle of cardiomyopathic hamsters at 7 months of age. To our knowledge, this is the first study in which actomyosin has been hybridized with different regulatory proteins from random-bred control and myopathic muscle in a genetic myopathic model of cardiac hypertrophy. The data indicate that regulatory proteins in myopathic hamsters have decreased inhibitory action (33–44%) on Mg\textsuperscript{2+}-actomyosin ATPase activity and, thus, have diminished response to Ca\textsuperscript{2+}. In skeletal muscle, Ca\textsuperscript{2+} sensitivity remained markedly decreased in the actomyosin when reconstituted with different molar ratios of regulatory complex from myopathic animals. Data reported earlier from our laboratory revealed that cardiac myofibrils from myopathic animals also demonstrated a loss in Ca\textsuperscript{2+} sensitivity, suggesting possible changes in the regulatory proteins.\textsuperscript{23} The present studies are in parallel with our earlier observations with respect to de-
increased Ca\(^{2+}\) sensitivity, but those studies were conducted only in the intact complex myofibrillar proteins from myopathic hamsters. In an earlier study, Bailin et al\(^{23}\) compared and characterized the affinities between the contractile proteins specifically with reference to the regulatory complex of bovine cardiac and fast skeletal muscle. Toyo-oka and Ross\(^{21}\) reported the loss of Ca\(^{2+}\) sensitivity in natural actomyosin extracted from cardiac tissue after corneal occlusion; they attribute this loss to the breakdown of the regulatory proteins especially in the TnI region. Obinata and coworkers\(^{20}\) have also shown changes in the TnI subunit in dystrophic chickens. In the present study, SDS gradient gel slab gels and densitometric scans of the skeletal Tn-Tm complex from myopathic hamsters when compared with the regulatory protein complex from the skeletal muscle of random-bred control hamsters demonstrate the differential loss in TnI and TnC regions. Coelelectrophoresis of this skeletal Tn-Tm complex with column-purified individual Tn subunits isolated from other skeletal muscle species confirms these findings (Figure 1).

The phosphorylation of TnI appears to influence the regulation of cardiac myofibrils; it has been proposed by Robertson et al\(^{34}\) that TnI phosphorylation reduces the amount of myofibrillar bound Ca\(^{2+}\) over the same pCa range at which myofibrillar ATPase is activated by Ca\(^{2+}\). This suggests that TnI phosphorylation most likely alters the Ca\(^{2+}\) binding properties of TnC. The conditions of our extraction procedure make it highly unlikely for the different proteins isolated to be in a phosphorylated state. In the present study, the Tn-Tm complex analyzed by slab gel electrophoresis showed that Tn subunits on gradient gels were more visibly reduced in skeletal myopathic muscle than in random-bred control muscle. One of the possible explanations in our study could be that TnI and TnC are lower in content in myopathic skeletal muscle than in control skeletal muscle, as seen on SDS gradient slab gels; another explanation could be that TnI and TnC are degraded by one or multiple proteases in vivo or in vitro. We and others have previously reported the presence of an alkaline protease\(^{24}\) and Ca\(^{2+}\) activated protease\(^{25}\) present in the myopathic hamster muscle. To our knowledge, the breakdown of one or more of the subunits of the regulatory protein complex in myopathic muscles during isolation and purification in the present study would be unlikely because several protease inhibitors were present throughout our preparative procedures. To support the above explanation, Tn-Tm was prepared from the random-bred control and myopathic muscles at the same time under the same experimental conditions.

Regulatory proteins from the cardiac muscle of myopathic animals and random-bred controls had similar mobility on SDS slab gels except in the TnI and TnC regions, where very small changes may be present. Thus, the loss in sensitivity in the myopathic hamster hearts may be explained by either different contents of Tn-Tm subunits present or different isoymic forms of these regulatory protein subunits. Quantitative estimation of the Tn-Tm complex contents ruled out any differences in regulatory protein subunit content (data not shown) from control and myopathic hearts. Thus, the different makeup of isoymic forms in the regulatory complex may be a more likely explanation.

In conclusion, SDS-PAGE patterns of skeletal Tn-Tm complex from control and experimental muscles in dystrophic hamsters at 7 months of age were indicative of differences in the TnI and TnC regions. However, in cardiac muscle, SDS-PAGE of the Tn-Tm complex from control and myopathic hamsters showed similar mobilities. This may be due to an alteration or to the presence of different isozymes of the regulatory proteins, as shown earlier in cardiomyopathic studies of cardiac myosin.\(^{23}\) Ca\(^{2+}\) sensitivity was markedly decreased in the actomyosin reconstituted with Tn-Tm from myopathic animals in the skeletal as well as in the cardiac muscles. These data suggest that Ca\(^{2+}\) sensitivity of regulatory proteins in skeletal and cardiac muscle of the myopathic hamsters has decreased inhibitory action on Mg\(^{2+}\)-actomyosin ATPase activity.

The present data cannot provide conclusive evidence as to which specific subunits of the regulatory protein complex may be altered in cardiac muscle in cardiomyopathy. However, in myopathic skeletal muscle, visual inspection of SDS gradient gels and the densitometric analyses of the thin-filament complex demonstrated reduction in TnI and TnC bands. One of the conclusions that can be drawn is that the mechanism involved in the loss of Ca\(^{2+}\) sensitivity in the two different muscles (cardiac and skeletal) may not have a common pathway. To resolve this, future studies would involve isolation and purification of the different subunits of the regulatory proteins (Tn-Tm) individually from the cardiac and skeletal muscle of control and myopathic hamsters, which could then be hybridized to study the Ca\(^{2+}\) sensitivity of the reconstituted actomyosin system enzymatically. Alternately, immunological approaches could be employed to localize the different components of Tn-Tm subunits. Since those experiments at present are not within the scope of this manuscript, we can only identify with the possible abnormalities in the regulatory protein machinery in the skeletal as well as in the cardiac muscle of the cardiomyopathic hamster model.

Acknowledgments

I wish to thank Dr. James Scheuer for his encouragement and advice and for reviewing the manuscript critically; I would also like to acknowledge the helpful suggestions of Dr. Peter Buttrick during the preparation of this manuscript. I would like to acknowledge the excellent technical assistance provided by Ms. Cecilia M. Lopez and Mr. Kirit Patel. Secretarial help by Ms. Janice Brewton is deeply appreciated. Sincere thanks are due to Dr. James Potter, University of Miami, Coral Gables, Florida, and Dr. Larry Tobacman, The University of Iowa, Iowa City, Iowa, for the

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generous gifts of individually purified samples of troponin and tropomyosin, respectively, for sodium dodecyl sulfate electrophoresis.

References


KEY WORDS • hamster cardiomyopathy • regulatory proteins • troponin-tropomyosin • actomyosin ATPase • myosin
Regulatory proteins in hamster cardiomyopathy.
A Malhotra

doi: 10.1161/01.RES.66.5.1302

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