Troponin–Tropomyosin Abnormalities in Hamster Cardiomyopathy

Ashwani Malhotra and James Scheuer
With the technical assistance of **Kirit Patel and M. Cecilia Lopez**
Division of Cardiology, Department of Medicine, Montefiore Medical Center, and Albert Einstein College of Medicine, Bronx, New York 10467

Abstract

Cardiac myofibrils from cardiomyopathic hamsters exhibit elevated Mg^{2+} ATPase activity and a parallel upward shift of the calcium ATPase dose response curve. To explore the mechanism, myofibrils from control and cardiomyopathic hamster hearts were incubated with isolated troponin–tropomyosin complex (Tn.Tm) from cardiomyopathic and control hamster or from dog hearts. Tn.Tm from control hamster or dog hearts restored normal Mg^{2+} ATPase activities to myofibrils from myopathic hearts. However, the maximum ATPase response to calcium stimulation was less in cardiomyopathic myofibrils compared to controls, even when control Tn.Tm was included. Electrophoretic patterns of Tn.Tm from myopathic and control hearts were similar. Electrophoresis of the hamster myofibrils mixed with dog cardiac Tn.Tm and then washed demonstrated binding of this complex to myopathic myofibrils.

To further confirm that the incubation experiments resulted in binding, ^{125}I troponin–tropomyosin was cross-hybridized with myofibrils, extensively washed, and then analyzed enzymatically and autoradiographically. Autoradiograms demonstrated similar percent binding of ^{125}I Tn.Tm to all myofibrillar preparations and enzymatic effects like those found using cold Tn.Tm.

These studies suggest that Tn.Tm from cardiomyopathic hearts inhibits Mg^{2+} myofibrillar ATPase activity to a lesser degree than Tn.Tm from control hearts. Decreased stimulation by calcium in myopathic preparations may be due to abnormalities in troponin–tropomyosin and/or to the decreased myosin ATPase activity observed previously. (*J. Clin. Invest.* 1990. 86:286–292.) Key words: cardiomyopathy • troponin–tropomyosin • myofibrils • Ca^{2+} Mg^{2+} ATPase • actomyosin

Introduction

Previous studies from our laboratory have demonstrated that cardiac myofibrils from myopathic hamsters have multiple abnormalities in the contractile machinery (1). Besides the shifts in myosin isoenzymes and diminished myosin ATPase activity, studies conducted on reconstituted actomyosin or in purified cardiac myofibrils suggest that the regulatory control by the proteins, troponin–tropomyosin (Tn.Tm)[1] may be altered. The role of troponin–tropomyosin (Tn.Tm) in physiological and pathological states of skeletal and cardiac muscle have only been explored in a limited way (2, 3). Myofibrillar proteins from fast and slow striated muscles contain multiple forms of fast and slow troponin isoforms (4–6).

Exchange studies using hamster skinned skeletal muscle fibers demonstrate that myocardial troponin C is more sensitive to tension control than skeletal muscle troponin C (7). Cardiac myofibrils isolated from neonatal and adult rat hearts contain different isoforms of troponin I (6). Additionally, it has been suggested that the changes in troponin T isoforms differentially modulate Ca^{2+} binding and activation of force generation in the developing myocardium (4). Varying types of troponin I have been demonstrated in developing normal and dystrophic chicken skeletal muscle. The loss of Ca^{2+} sensitivity of myocardial actomyosin in infarcted myocardium has been attributed to alterations in the regulatory protein complex (8, 9). In a recent study from our laboratory, regulatory proteins from skeletal and cardiac muscle of myopathic hamsters were shown to have decreased inhibitory action on Mg^{2+} ATPase activity of reconstituted actomyosin (10). The present investigation was undertaken to explore the role of the cardiac troponin–tropomyosin complex in the regulation of cardiac myofibrils isolated from cardiomyopathic hamsters. Cross hybridization experiments were conducted under soluble conditions by mixing the cardiac myofibrils from myopathic hamsters with cardiac troponin–tropomyosin complex from control hamsters to focus on Mg^{2+} ATPase and responses to adding and deleting Ca^{2+}. The results suggest an abnormality in the Tn.Tm complex which increases Mg^{2+} ATPase and elevates the free Ca^{2+} dose response curves of cardiac myofibrils from myopathic hamsters.

Methods

ATP, DTT, EDTA, EGTA, and proteolytic enzyme inhibitors: PMSF, Leupeptin, *N*-α-*P*-Tosyl-L-lysine chloromethyl ketone (TLCK), and Pepstatin were obtained from Sigma Chemical Co. (St. Louis, MO). Genetically myopathic male hamsters (TO's) were bred at the University of Toronto. The time point selected for study was 6–7 mo of age. For controls, random bred control (C) animals of the same age were used. Control and myopathic animals were sacrificed at the same time and their heart tissue processed and analyzed simultaneously. Animals were anesthetized with ether and their hearts removed. After washing the hearts, the atria and great vessels were removed and the whole ventricular portions were homogenized with a homogenizer (Tekmar Co., Cincinnati, OH). For the preparation of cardiac myofibrils, two to three hearts were pooled. 25–30 hearts (10 g muscle) were pooled in each group for purification of the regulatory proteins. For cardiac

Parts of the manuscript were presented at the Biophysical Society Meeting, 1989.

Address reprint requests to Ashwani Malhotra, Ph.D, Division of Cardiology, Department of Medicine, Montefiore Medical Center, 111 E. 210th St., Bronx, NY 10467.

Received for publication 29 June 1989 and in revised form 17 January 1990.

J. Clin. Invest.

0021-9738/90/07/0286/07 $2.00
Volume 86, July 1990, 286–292

1. *Abbreviation used in this paper:* Tn.Tm, troponin–tropomyosin.

studies on hybridization of myofibrils from myopathic hamsters with canine regulatory complex; cardiac dog Tn.Tm was isolated from the ether powder of normal Mongrel dog tissue (50 g). All the tissues were stored at −70°C in 50% glycerol containing 30 mM KCl, 10 mM KPO_4 (pH 7.0), 5 mM β-MSH (β-mercaptoethanol) before the preparation of different proteins. There was no difference in actomyosin or myofibrillar ATPase activity if hearts were prepared immediately or after a period of storage.

ATPase measurements. Cardiac ATPase activity of myofibrils or actomyosin was assayed in a final volume of 1 ml at pH 7.0 at 25°C or 30°C using 0.2–0.25 mg of protein in each assay. The mixture contained 50 mM KCl, 20 mM imidazole buffer (pH 7.0), 3 mM Mg^{2+}, 2 mM ATP, 6 mM NaN_3, 0.1 mM $CaCl_2$, or 2.5 mM EGTA. Mg^{2+} ATPase was measured in the absence of free Ca^{2+} but in the presence of 3 mM Mg^{2+} and 2.5 mM EGTA. Mg^{2+} Ca^{2+} ATPase is defined as Ca^{2+} stimulated Mg^{2+} ATPase and was assayed in the presence of Mg^{2+} (3 mM) using different concentrations of Ca^{2+} buffered by EGTA. Ca^{2+}–EGTA buffers were prepared according to the procedure of Hathaway et al. (11).

Incubations and microphosphate determinations were carried out as reported previously (12). Protein content was determined by the colorimetric Biuret or Biorad methods (13, 14).

SDS-PAGE. SDS-PAGE was carried out on all protein preparations using 5.0–16.5% and 10–15% gradient slab gels (15). Scans of the gels were performed on an EC-apparatus recorded at 605 nM, attached to an integrator (model 3390A; Hewlett Packard Co., Palo Alto, CA).

Preparation of proteins. Myofibrils were isolated and purified with Triton X-100 by the modified technique of Solaro et al. (16). Proteolytic enzyme inhibitors PMSF (0.1–0.2 mM), Pepstatin (1 μg/ml), Leupeptin (1 μg/ml), and TLCK (0.1 mM) were included in the isolation mixture (1). Cardiac myosin from normal random bred control (C) and myopathic (M) hamsters was prepared by methods described previously (12, 17). Actin was purified from rabbit skeletal muscle (18). When pure actin and myosin were studied together the ratio was 2:1 (wt/wt) and this mixture is denoted as actomyosin throughout the paper.

Troponin and tropomyosin (Tn.Tm). Tn.Tm complex was prepared by a slight modification of the methods of Eisenberg and Kielley (19) and Potter (20). Triton X-100–washed myofibrils were extracted briefly with KCl-pyrophosphate buffer (pH 7.0), 1 mM DTT, 0.1 mM PMSF, and TLCK (0.1 mM) for 15 min to isolate and further purify cardiac or skeletal myosin. The remaining pellet was washed with $KHCO_3$, repeatedly suspended in absolute ethanol, and subsequently resuspended in diethyl ether to obtain the muscle ether powder. The ether powder was further dried overnight under vacuum to completely eliminate the solvent. The cardiac muscle ether powder (0.5–1.0 g) was extracted overnight with 1 M KCl, tris buffer, pH 8.0, 1 mM DTT, 0.1 mM PMSF, 3 mM NaN_3, and centrifuged at 12,000 rpm for 30 min. The supernatant was fractionated with saturated ammonium sulfate, and the partially purified cardiac troponin–tropomyosin pellet was collected in the 35–65% fraction. This pellet was dissolved and then dialyzed against low ionic strength buffer (50 mM KCl; pH 7.0 containing 0.1 mM DTT, 0.1 mM PMSF). This Tn.Tm fraction was checked on SDS-PAGE and subsequently used for iodination and hybridization experiments with myofibrils or in reassociated actomyosin ATPase measurements.

Iodination of regulatory protein (Tn.Tm) complex. The regulatory complex (5 mg/ml) was dialyzed against 0.1 M KCl, 20 mM Imidazole, pH 7.0, 1 mM DTT, 0.1 mM PMSF. It was mixed with 0.1 M sodium borate (pH 8.5) buffer (1:1 [vol/vol]) and iodinated with Bolten-Hunter Reagent (0.25 mCi; ^{125}I: Monoiodo Bolton Hunter Reagent; specific activity 2,200 Ci/mmol) at 0°C for 45 min (21). The mixture was then subjected to Sephadex G50 column (1 × 30 cm) pre-equilibrated with 0.1 M KCl, 20 mM Imidazole, pH 7.0, and eluted with the same buffer. ^{125}I Tn.Tm complex was eluted in the void volume and checked on SDS gels and counted for ^{125}I incorporation.

Hybridization of myofibrils with cold or ^{125}I Tn.Tm. Cardiac myofibrils (2 ml; 3 mg/ml) were mixed with ^{125}I Tn.Tm or cold Tn.Tm (2 mg/ml) under soluble conditions (0.4 M KCl, 20 mM, Imidazole, pH 7.0, 2 mM $MgCl_2$, 1 mM DTT, 0.2 mM PMSF) for 4–5 h at 0°C and dialyzed overnight against low ionic strength buffer (60 mM KCl, 30 mM Imidazole, pH 7.0, 2 mM $MgCl_2$, 1 mM DTT, 0.2 mM PMSF) at 4°C. The mixture was centrifuged at 2,500 rpm for 15 min, and the supernatant was saved for ^{125}I counting and SDS gel electrophoresis. The pellet was washed extensively (three to four times) with the same buffer (60 mM KCl; pH 7.0) and finally suspended in 2 ml of Solaro's

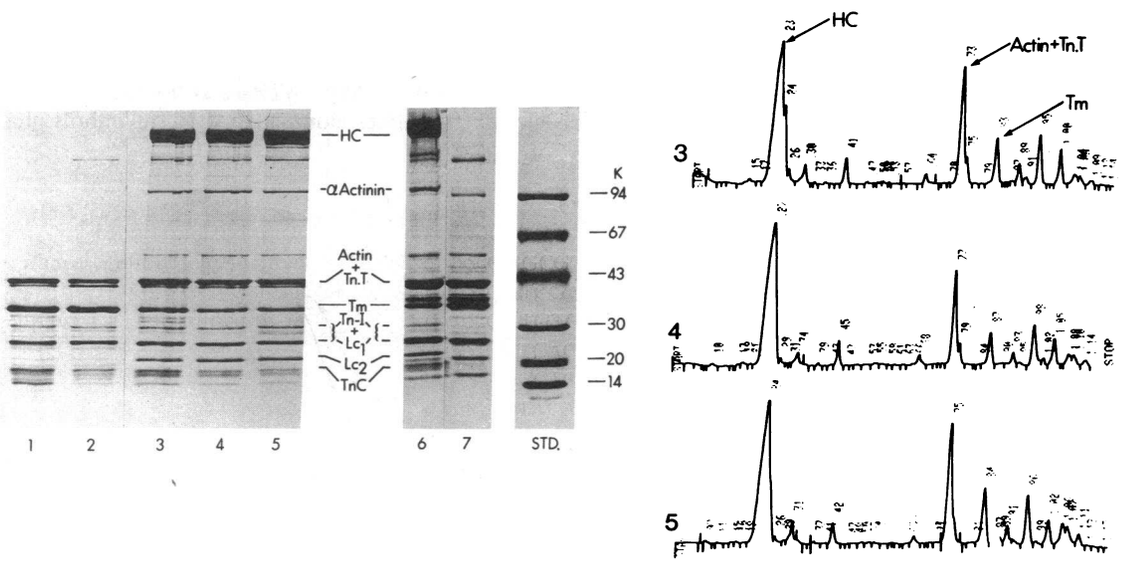

Figure 1. SDS gradient slab gel (5.0–16.5%) and densitometric scans of cardiac myofibrils (MF) hybridized with the cold cardiac Tn.Tm complex from normal dog (D), control (C) and myopathic (M) hamsters. Lane *1*, Tn.Tm (C); lane *2*, Tn.Tm (M); lane *3*, MF (C); lane *4*, MF(M); lane *5*, MF(M) plus Tn.Tm (C); lane *6*, MF [M] plus Tn.Tm (D); lane *7*, Tn.Tm (D) complex; and lane *8*, standard molecular weight protein markers. On the right are the densitometric scans of preparations from lanes *3*, *4*, and *5*. *HC*, heavy chains; LC_1, light chain-1; LC_2, light chain-2; *Tm*, tropomyosin; *Tn-I*, troponin-I; *Tn.T*, troponin T; and *TnC* = troponin-C.

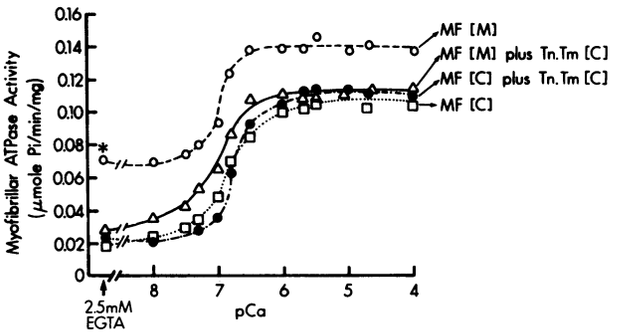

Figure 2. Cardiac Ca^{2+} Mg^{2+} activated myofibrillar ATPase vs. free calcium concentration. Myofibrils from control (*MF[C]*); myopathic (*MF[M]*); control myofibrils mixed with control troponin–tropomyosin complex (*MF[C] plus Tn.Tm [C]*); and myopathic myofibrils plus control Tn.Tm (*MF [M] plus Tn.Tm [C]*). Mean results are for four to seven different preparations; two experiments each for every preparation. * indicates $P < 0.05$ when Mg^{2+} ATPase for MF[M] is compared with all other myofibrillar Mg^{2+} ATPase(s).

low ionic strength buffer (16) for enzymatic analysis, SDS gels, and autoradiographs.

Statistics. Statistical comparisons between multiple data points among the various preparations were subjected to analysis of variance followed by Newman Keuls multiple comparison test to determine significance at the $P < 0.05$ level (22).

Results

Fig. 1 shows the SDS gradient slab gel patterns and densitometric scans of cardiac myofibrils incubated with the cold troponin–tropomyosin (Tn.Tm) complex. The electrophoretic patterns of Tn.Tm isolated from control and myopathic hearts look the same (lanes *1* and *2*). The myofibrillar proteins from control and myopathic hamsters and from the hybridized myofibrillar pellets obtained after incubating myopathic myofibrils with the regulatory complex isolated from control hearts also look similar (lanes *3*, *4*, and *5*). The incubation of the control Tn.Tm complex with the virgin samples 3 and 4 does not change the pattern. Lane *6* shows the SDS electrophoretic

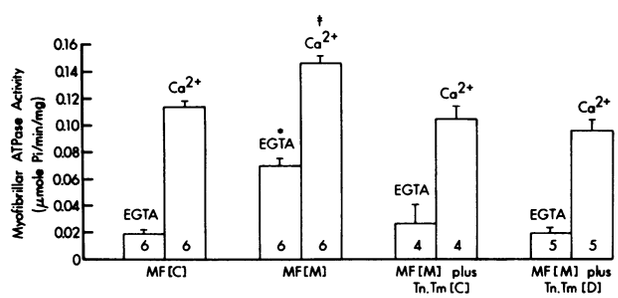

Figure 3. Cardiac myofibrillar ATPase activity plotted in the absence of Ca^{2+} (Mg^{2+} ATPase) and in the presence of 0.1 mM Ca^{2+} ion (Ca^{2+} Mg^{2+} ATPase). Results are mean±SE. The number of experiments are indicated in each bar. * indicates $P < 0.05$ when comparing Mg^{2+} ATPase for MF[M] with all other groups. ‡ indicates $P < 0.05$ while comparing Ca^{2+} Mg^{2+} ATPase of MF(M) against all other preparations.

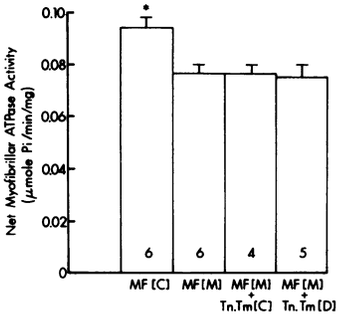

Figure 4. Net Ca^{2+} myofibrillar ATPase activity (Mg^{2+} ATPase subtracted from total Ca^{2+} Mg^{2+} myofibrillar ATPase activity; 0.1 mM Ca^{2+}) of myofibrils. Designations for columns 1, 2, 3, and 4 are the same as in Fig. 3.

pattern of the myofibrillar pellet obtained after treating cardiac myopathic myofibrils with dog cardiac Tn.Tm. Lane *7* shows the dog cardiac Tn.Tm complex alone. Several bands from the dog Tn.Tm complex (lane *7*) are found in the washed hybridized sample (lane *6*), but not in the nonhybridized hamster preparations (lanes *3* and *4*). These include a doublet in the Tm region and an additional band that migrates in the TnC vicinity of the dog cardiac preparation. That this complex corrected abnormal ATPase activity of myopathic myofibrils is shown as discussed below (see Figs. 2 and 3).

Fig. 2 shows the myofibrillar ATPase and its response to calcium. The native myofibrils from myopathic hearts exhibited the parallel upward shift of the curve compared to controls observed previously (1). The addition of control Tn.Tm did not have any affect on the Mg^{2+} or the Ca^{2+} Mg^{2+} ATPase activity of control myofibrils, but when myopathic myofibrils were incubated with control Tn.Tm, the curve appeared to be normal.

Fig. 3 shows the myofibrillar Mg^{2+} and Mg^{2+} Ca^{2+} ATPase activities (in the presence of 0.1 mM Ca^{2+}). The abnormal elevations of both ATPases in myopathic myofibrils were reversed to the control values when Tn.Tm from either control hamster or dog cardiac tissue were complexed with the myopathic myofibrils. The columns in Fig. 3 correspond to lanes *3*, *4*, *5*, and *6* in Fig. 1.

Fig. 4 shows the net Ca^{2+} myofibrillar ATPase (Mg^{2+} ATPase subtracted from Ca^{2+} Mg^{2+} ATPase at 0.1 mM Ca^{2+}) in controls alone, myopathics alone, myopathic myofibrils plus

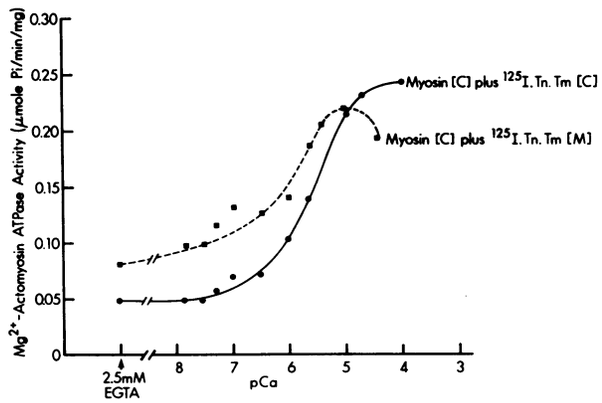

Figure 5. Mg^{2+} ATPase of reconstituted actomyosin as a function of free Ca^{2+} ion in the presence of cardiac ^{125}I-Tn.Tm complex isolated from control (*C*) and myopathic (*M*) hamsters. The number of preparations for each curve is two.

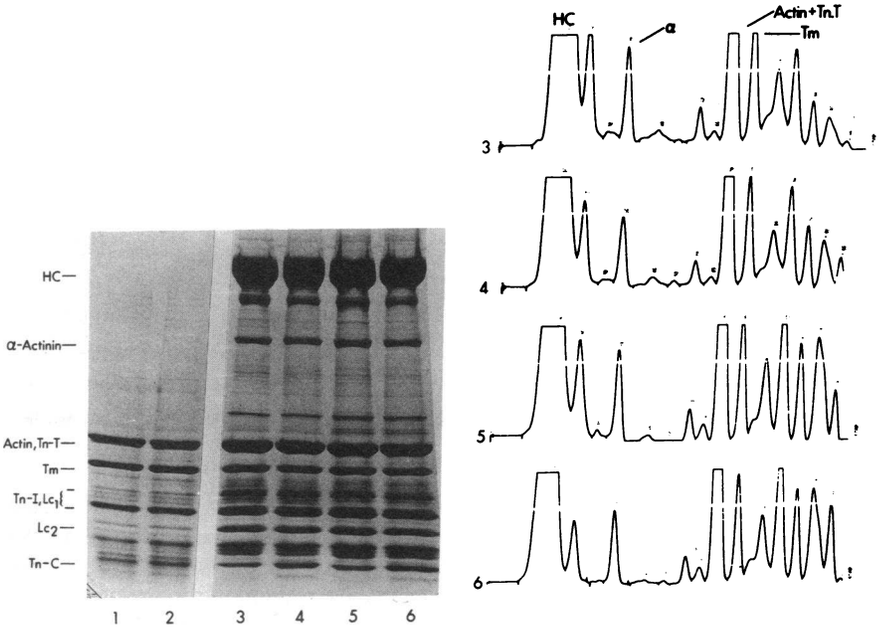

Figure 6. SDS gradient slab gel (5.0–16.5%) and densitometric scans of the cardiac myofibrils hybridized with ^{125}I-Tn.Tm in control (*C*) and myopathic (*M*) hamster hearts and vice versa. Lane *1*, ^{125}I-Tn.Tm (C); lane *2*, ^{125}I-Tn.Tm(M); lane *3*, MF (C) plus ^{125}I-Tn.Tm (C); lane *4*, MF (C) plus ^{125}I-Tn.Tm(M); lane *5*, MF (M) plus ^{125}I-Tn.Tm (C); and lane *6*, MF(M) plus ^{125}I-Tn.Tm(M).

Tn.Tm from control hamsters, and myopathic myofibrils plus dog cardiac Tn.Tm. Net Ca^{2+} ATPase was lowered ~ 20–22% in all the myopathic myofibrillar preparations compared to controls.

To confirm the binding of the control Tn.Tm to the myofibrils in hybridization studies, ^{125}I-iodinated Tn.Tm was complexed to myofibrillar preparations from controls and myopathic hamster hearts. To assure that the biological activity in the ^{125}I Tn.Tm was retained during radioactive hybridization studies of cardiac myofibrils, the complex was first studied with reconstituted purified actin and myosin. The results are shown in Fig. 5. Mg^{2+} actomyosin ATPase activity and the responses to calcium were similar to the curves seen earlier with the cold Tn.Tm complex (not shown here) (10). Control actomyosin recombined with myopathic ^{125}I-Tn.Tm was shifted upwards and showed lesser absolute rise with Ca^{2+} when compared to control ^{125}I Tn.Tm plus control myosin. Fig. 6 shows heavily loaded gradient SDS slab gels and the densitometric scans of the myofibrils hybridized with ^{125}I-Tn.Tm. The gels and their scans show similar profiles in all the myofibrillar preparations suggesting that the proteins cross-reacted to similar degree. Fig. 7 shows the autoradiographs of SDS slab gels of the four different hybridized cardiac myofibrillar preparations from Fig. 6. Similar distributions of ^{125}I-Tn.Tm in control and myopathic myofibrils are demonstrated. This is confirmed in Table I which presents the percent ^{125}I-Tn.Tm incorporated into cardiac myofibrils isolated from control and myopathic hamster hearts. Incorporation of ^{125}I-Tn.Tm into the extensively washed myofibrillar pellets ranged from 25 to 38%. Recovery of counts in the pellets, the supernatant, and the washings totaled 100%.

Table II exhibits the Mg^{2+} ATPase, total Ca^{2+} Mg^{2+} ATPase, and the net Ca^{2+} ATPase (Mg^{2+} ATPase subtracted form the maximum Ca^{2+} Mg^{2+} ATPase) enzymatic activity of the myofibrils incubated with or without cold cardiac Tn.Tm and also with ^{125}I-Tn.Tm. The last two preparations (group IV) shown in the table are the control experiments conducted for reassociated actomyosin with ^{125}I-Tn.Tm. ^{125}I-hybridized myofibrillar preparations qualitatively had similar enzymatic activity profiles as the cold preparations. Because binding of ^{125}I-Tn.Tm to myofibrils required solubilization in high salt and reprecipitating the hybridized myofibrils over a 48-h time period, the overall activity of the iodinated preparations was slightly lower than with the cold myofibrillar enzymatic activity.

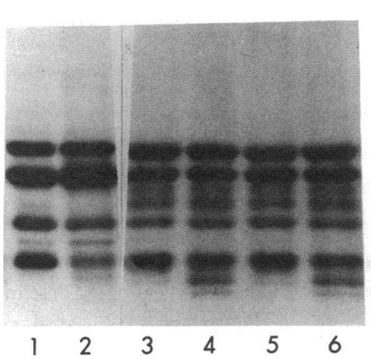

Figure 7. Autoradiographs of SDS gels of the four different myofibrillar preparations from C and M hamster hearts hybridized with ^{125}I-Tn.Tm from C and M hearts. Numbers *1–6* are the autoradiographs of lanes *1–6* in Fig. 6.

Discussion

The purpose of this research was to explore the role of the cardiac regulatory proteins, troponin–tropomyosin, in the abnormal enzymatic activity observed in myofibrils from cardiomyopathic hamster hearts (1). Previous reports have demonstrated a number of biochemical changes that could partially explain diminished function in these hearts. These include alterations of the sarcoplasmic reticulum (23, 24), cardiac myosin and myosin heavy chain isoenzymes (1). The observations that ATPase activity of myopathic myofibrils was elevated suggested the possibility that the troponin–tropomyosin complex inhibits Mg^{2+} ATPase activity less completely in hearts from myopathic hamsters than in hearts from controls. The responsiveness of the myofibrils to calcium appeared to be close to normal. The present study demonstrates that the ab-

Table I. Incorporation of ^{125}I-Tn.Tm in Myofibrils (MF) from Control (C) and Myopathic (M) Hamsters

Proteins mixed	Percent ^{125}I-Tn.Tm				
	Incorporated in pellet [MF]	Recovered in supernatant	Washings		
			W_1	W_2	W_3
(1) MF[C] + ^{125}I-Tn.Tm[C]	33.1	56.8	6.0	2.7	1.4
(2) MF[C] + ^{125}I-Tn.Tm[M]	38.0	51.2	7.2	2.2	1.4
(3) MF[M] + ^{125}I-Tn.Tm[C]	28.7	58.2	8.2	2.8	2.1
(4) MF[M] + ^{125}I-Tn.Tm[M]	36.3	52.0	7.8	2.4	1.5
(5) MF[C] + ^{125}I-Tn.Tm[D]	29.0	63.0	5.0	2.0	1.0
(6) MF[M] + ^{125}I-Tn.Tm[D]	25.7	67.2	4.1	1.8	1.2

Results are mean of two different preparations. *^{125}I-Tn.Tm [D]*, iodinated Tn.Tm from normal dog cardiac muscle. *W_1*, *W_2*, and *W_3* are washings obtained after suspending the pellet in low ionic strength buffer.

normalities in Mg^{2+} ATPase of the myopathic myofibrils can be reversed by the addition of troponin–tropomyosin complex from control hearts. We had previously shown that adding myopathic Tn.Tm to a mixture of control hamster myosin and actin elevated Mg^{2+} ATPase similar to that seen in myopathic myofibrils (1, 10). To demonstrate that the troponin–tropomyosin from myopathic hearts is directly responsible, actomyosin reconstituted from pure control myosin and from skeletal actin was complexed with cold Tn.Tm or ^{125}I-troponin–tropomyosin from myopathic hearts, and this produced a calcium response curve (Fig. 5), quite similar to that seen in myopathic myofibrils (Fig. 2).

The effect of the troponin–tropomyosin appears not to be solely due to mixing excess regulatory complex with the myofibrils, but results from actual binding. This is strongly supported by the studies using ^{125}I-labeled troponin–tropomyosin which was incubated with myofibrils and were then washed extensively. Binding was demonstrated to be similar in the various preparations (Fig. 7 and Table I). These myofibrils incubated with the radioactive troponin–tropomyosin (Table II) acted similarly to preparations mixed with cold troponin–tropomyosin. This provides strong support that binding of troponin–tropomyosin took place and that it is the bound complex which is responsible for restoring normal basal activity to the myopathic myofibrils. Further, the studies demonstrating that cold troponin–tropomyosin from dog cardiac muscle, which has a different electrophoretic mobility on SDS gels than hamster cardiac troponin–tropomyosin (Fig. 1), could restore normal Mg^{2+} ATPase activity to myofibrils from myopathic hearts is confirmatory evidence that the abnormality resided in the troponin–tropomyosin complex from the myopathic hearts.

Table II. ATPase Activity of Cardiac Actomyosin and Myofibrils [MF] in Control [C] and Myopathic [M] Hamsters at 30°C

	Number	Proteins mixed	Mg^{2+} ATPase	Total Ca^{2+} Mg^{2+} ATPase	Net Ca^{2+} ATPase
				μmol P_i/min · mg	
Group I	1. (7)	C [MF] + 0	0.020	0.114	0.094*
	2. (7)	M [MF] + 0	0.069*	0.146*	0.077
	3. (4)	M [MF] + Tn.Tm[C]	0.028	0.105	0.077
	4. (4)	M [MF] + Tn.Tm[D]	0.020	0.096	0.076
Group II	5. (2)	C [MF] plus ^{125}I-Tn.Tm[C]	0.017	0.091	0.074
	6. (2)	M [MF] plus ^{125}I-Tn.Tm[M]	0.036	0.084	0.048
	7. (2)	M [MF] plus ^{125}I-Tn.Tm[C]	0.020	0.078	0.058
Group III	8. (2)	C [MF] plus ^{125}I-Tn.Tm[D]	0.016	0.105	0.089
	9. (2)	M [MF] plus ^{125}I-Tn.Tm[D]	0.022	0.085	0.063
Group IV	10. (2)	C [Acto.] plus ^{125}I-Tn.Tm[C]	0.057	0.233	0.176
	11. (2)	C [Acto.] plus ^{125}I-Tn.Tm[M]	0.097	0.193	0.096

* < 0.05 against all other values in each column 1 → 4 in group I. *C*, control; *M*, myopathic; *D*, dog; *MF*, myofibrils; *Acto.*, actomyosin; and *Tn.Tm*, troponin–tropomyosin complex. *Mg^{2+} ATPase*, Mg^{2+} ATPase in the absence of Ca^{2+}; *Total Ca^{2+} Mg^{2+} ATPase*, Ca^{2+} Mg^{2+} ATPase in the presence of 0.1 mM Ca^{2+}; and *Net Ca^{2+} ATPase*, Mg^{2+} ATPase subtracted from total maximum Ca^{2+} Mg^{2+} ATPase activity. Number of preparations are indicated in parenthesis.

The fact that complexing control Tn.Tm to myopathic myofibrils did not restore the maximal Ca^{2+} response to normal may reflect either the depressed cardiac myosin ATPase activity in these hearts (1), or only partial binding of Tn.Tm or a decrease in the calcium responsiveness of the Tn.Tm per se.

The nature of the changes in regulatory proteins occurring in cardiomyopathy has not been elucidated. It is possible that alterations in the tropomyosin or troponin subunit isoenzymes are responsible, and this needs further exploration. The small amount of Tn.Tm complex isolated from the tiny hamster hearts precluded isolation of pure subunits and recombining them for biochemical enzymatic studies. Proteolytic degradation of Tn.Tm must be considered since the myocardial content of neutral protease is increased in cardiomyopathy (25), and the possibility exists that the complex from myopathic hearts is more susceptible to proteolysis in vitro than Tn.Tm from normal hearts. However, the inclusion of protease inhibitors in the extraction medium makes this less likely. The similarity of the electrophoretic SDS gel patterns is evidence against differential in vitro proteolysis.

The current studies were conducted using myopathic animals at 7 mo of age. We have previously demonstrated alterations in the myofibrillar calcium dose response curve to occur in younger animals (1), before clear evidence of histologic cardiomyopathy (26), so it is possible that the abnormalities in the complex occur before the onset of heart failure. Whether this abnormality has physiological implications and is responsible in part for cardiac dysfunction as the animal matures cannot be determined from these studies, but it is possible that an increased resting level of ATPase activity would cause greater resting ATP use, and oxygen demand in a heart where coronary microvascular spasm could decrease focal oxygen supply (26).

The abnormality in troponin–tropomyosin observed in these studies might also be translated to pathophysiologic states in other experimental animals and humans. Abnormalities in myofibrillar dose response curves have been observed in experimental diabetic cardiomyopathy (24, 27), pressure induced overload without and with congestive heart failure (28) and in humans with hypertensive congestive heart failure, ischemic cardiomyopathy and idiopathic cardiomyopathy (29, 30). The possibility that the myofibrillar calcium control proteins are partially responsible for myocardial failure is in concert with the observation that there is ample or even increased myoplasmic calcium in cardiomyopathic states (31) and agrees with the hypothesis of Blinks and Endoh (32) that a major abnormality may occur in the conversion of calcium binding to mechanical function in abnormal cardiac muscles.

Acknowledgments

The authors would like to acknowledge the excellent secretarial help provided by Ms. Janice Brewton.

This work was supported by U. S. Public Health Service grants HL-15498 and HL-37412.

References

1. Malhotra, A., M. Karell, and J. Scheuer. 1985. Multiple cardiac contractile protein abnormalities in myopathic Syrian hamsters (Bio 53:58). *J. Mol. Cell. Cardiol.* 17:95–107.

2. Schachat, F. H., D. D. Bronson, and O. B. McDonald. 1985. Heterogeneity of contractile proteins: a continuum of troponin-tropomyosin expression in mammalian skeletal muscle. *J. Biol. Chem.* 260:1108–1113.

3. Humphreys, J. E., and P. Cummings. 1984. Regulatory proteins of the myocardium. *J. Mol. Cell. Cardiol.* 16:643–657.

4. McAuliffe, J. J., and R. J. Solaro. 1989. Changes in troponin T isoforms modulate Ca^{2+} binding and activate force generation in the developing myocardium. *Biophys. J.* 55:591a. (Abstr.)

5. Solaro, R. J., P. Kumar, E. M. Blanchard, and Ann F. Martin. 1986. Differential effects of pH on calcium activation of myofilaments of adult and perinatal dog hearts: evidence for developmental differences in thin filament regulation. *Circ. Res.* 58:721–729.

6. Kumar, P., A. F. Martin, and R. J. Solaro. 1989. Identification of troponin-I isoforms in developing rat hearts. *Biophys. J.* 55:592a. (Abstr.)

7. Babu, A., S. S. Scordilis, E. H., Sonnenblick, and J. Gulati. 1987. The control of myocardial contraction with skeletal fast muscle troponin C. *J. Biol. Chem.* 262:5815–5822.

8. Katagari, T., Y. Kobayashi, Y. Sasai, K. Toba, and H. Niitani. 1981. Alterations in cardiac myosin subunits in myocardial infarction. *Jpn. Heart J.* 22:653–664.

9. Toyo-oka, T., and J. Ross, Jr. 1981. Ca2+ sensitive change and troponin loss in cardiac natural actomyosin after coronary occlusion. *Am. J. Physiol.* 240:H704–708.

10. Malhotra, A. 1990. Regulatory proteins in hamster cardiomyopathy. *Circ. Res.* 66:1302–1309.

11. Hathaway, D. R., D. K. Werth, and J. R. Haeberla. 1982. Limited autolysis reduces the Ca^{2+}-activated protease. *J. Biol. Chem.* 257:9072–9077.

12. Malhotra, A., S. Huang, and A. Bhan. 1979. Subunit function in cardiac myosin: effects of removal of LC_2 (18,000 M.W.) on enzymatic properties. *Biochemistry.* 18:461–467.

13. Gernall, A. G., C. J. Barawill, and M. M. David. 1949. Determination of serum proteins by means of the Biuret reaction. *J. Biol. Chem.* 177:751–766.

14. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Chem.* 72:248–254.

15. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. *Nature (Lond.).* 27:680–685.

16. Solaro, J. R., D. Pang, and N. Briggs. 1971. The purification of cardiac myofibrils with Triton X-100. *Biochim. Biophys. Acta.* 245:259–262.

17. Malhotra, A., S. Penpargkul, F. S. Fein, E. H. Sonnenblick, and J. Scheuer. 1981. The effect of streptozotocin-induced diabetes in rats on cardiac contractile proteins. *Circ. Res.* 49:1243–1250.

18. Spudich, J. A., and S. Watt. 1971. The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. *J. Biol. Chem.* 246:4866–4871.

19. Eisenberg, E., and E. W. Kielly. 1974. Column chromatographic separation and activity of the three active troponin components with and without tropomyosin present. *J. Biol. Chem.* 249:4742–4748.

20. Potter, J. D. 1982. Preparation of troponin and its subunits. *Methods Enzymol.* 85:241–263.

21. Bolton, A. E., and W. M. Hunter. 1973. The labelling of proteins to high specific radioactivities by conjugation to a ^{125}I-containing acylating agent. *Biochem. J.* 133:529–539.

22. Zar, J. H. 1974. Biostatistical Analysis. Prentice Hall, Englewood Cliffs, NJ.

23. Gertz, E. W., A. L. Stam, Jr., and E. H. Sonnenblick. 1970. Quantitative and qualitative defect in the sarcoplasmic reticulum in

the hereditary cardiomyopathy of the Syrian Hamster. *Biochem. Biophys. Res. Commun.* 40:746–753.

24. Dhalla, N. S., P. V. Sulakhe, M. Fedelesova, and J. C. Yates. 1974. Molecular abnormalities in cardiomyopathy; Comparative pathology of the hearts. *Adv. Cardiol.* 13:282–300.

25. Kuo, T. H., F. Giacomelli, K. Kithier, and A. Malhotra. 1981. Biochemical characterization and cellular localization of serine protease in myopathic hamster. *J. Mol. Cell. Cardiol.* 13:1035–1049.

26. Factor, S. M., T. Minase, S. Cho, R. Dominitz, and E. H. Sonnenblick. 1982. Microvascular spasm in the cardiomyopathic Syrian hamster: a preventable cause of focal myocardial necrosis. *Circulation.* 66:342–354.

27. Pierce, G. N., and N. S. Dhalla. 1981. Cardiac myofibrillar ATPase activity in diabetic rats. *J. Mol. Cell. Cardiol.* 13:1063–1069.

28. Chandler, B. M., E. H. Sonnenblick, J. R. Spann, and P. E. Pool. 1967. Association of depressed myofibrillar adenosine triphosphatase and reduced contractility in experimental heart failure. *Circ. Res.* 21:717–725.

29. Alpert, N. R., and M. S. Gordon. 1962. Myofibrillar adenosine triphosphatase activity in congestive heart failure. *Am. J. Physiol.* 20:940–946.

30. Pagani, E. D., A. A. Alonsi, A. M. Grant, T. M. Older, S. W. Dziuban, and P. D. Allen. 1988. Changes in myofibrillar content and Mg^{2+}-ATPase activity in ventricular tissues from patients with heart failure caused by coronary artery disease, cardiomyopathy, or mitral valve insufficiency. *Circ. Res.* 63:380–385.

31. Gwathmey, J. K., L. Copelas, R. MacKinnon, and F. J. Schoen. 1987. Abnormal intracellular calcium handling in myocardium from patients with end-stage heart failure. *Circ. Res.* 61:70–76.

32. Blinks, J. R., and M. Endoh. 1986. Modification of myofibrillar responsiveness to Ca^{2+} as an inotropic mechanism. *Circulation.* 73(Suppl. III):85.