Tropomyosin Modulates pH Dependence of Isometric Tension

Hideaki Fujita* and Shin'ichi Ishiwata*#§¶

*Department of Physics, School of Science and Engineering, #Advanced Research Institute for Science and Engineering, and §Materials Research Laboratory for Bioscience and Photonics, Waseda University, Tokyo 169-8555, and ¶Core Research for Evolutional Science and Technology, "Genetic Programming" Team 13, Kawasaki 216-0001 Japan

ABSTRACT We investigated the effect of pH on isometric tension in actin filament-reconstituted and thin filament-reconstituted bovine cardiac muscle fibers in the pH range of 6.0–7.4. Thin filament was reconstituted from purified G-actin with either bovine cardiac tropomyosin (Tm) or rabbit skeletal Tm in conjunction with cardiac or skeletal troponin (Tn). Results showed that isometric tension decreased linearly with a decrease in pH. The slope of the pH-tension relation, $\Delta F/\Delta$pH (Δrelative tension/Δunit pH), was 0.28 and 0.44 in control cardiac fibers and skeletal fibers, respectively. In actin filament-reconstituted fibers without regulatory proteins, $\Delta F/\Delta$pH was 0.62, namely larger than that in cardiac or skeletal fibers. When reconstituted with cardiac Tm-Tn complex (nTm), $\Delta F/\Delta$pH recovered to 0.32, close to the value obtained in control cardiac fibers. When reconstituted with skeletal nTm, $\Delta F/\Delta$pH recovered to 0.48, close to the value for control skeletal fibers. To determine whether Tm or Tn is responsible for the inhibitory effects of nTm on the tension decrease caused by reduced pH, thin filament was reconstituted with cardiac Tm and skeletal Tn, or with skeletal Tm and cardiac Tn. When cardiac Tm was used, pH dependence of isometric tension coincided with that of control cardiac fibers. When skeletal Tm was used, the pH dependence coincided with that of control skeletal fibers. Furthermore, closely similar results were obtained in fibers reconstituted with actin and either cardiac or skeletal Tm without Tn. These results demonstrate that Tm but not Tn modulates the pH dependence of active tension.

INTRODUCTION

It is known that intracellular pH decreases when muscle fatigues, and in the onset of ischemia and hypoxia. A decrease in pH causes decreases in active tension, shortening velocity, and Ca^{2+} sensitivity in skinned muscle fibers (Dawson et al., 1978; Edman and Matiazzi, 1981; Donaldson and Hermansen, 1978; Fabiato and Fabiato, 1978; Robertson and Kerrick, 1979; Chase and Kushmerick, 1988; Cooke et al., 1988; Metzger and Moss, 1987). Moreover, ATPase activity increases when pH is reduced (Curtin et al., 1988), resulting in an increase in tension cost (Godt and Kentish, 1989; Potma et al., 1994).

The degree of the effect of reduced pH depends on the muscle type. For example, the rightward shift in the pCa-tension relationship is greater in cardiac than in skeletal muscle (Donaldson and Hermansen, 1978; Metzger et al., 1993). Furthermore, the decrease in maximum isometric tension is greater in skeletal muscle with predominantly fast twitch fibers than in soleus muscle, which has predominantly slow twitch fibers (Metzger and Moss, 1987; Potma et al., 1994). Because different isoforms of myofilament proteins are expressed in these fiber types (Nadal-Ginard and Mahdavi, 1989), this difference in pH dependence among different fiber types may be caused by a difference in their protein isoforms. It is known that different Tn isoforms are involved in the different pH effects on Ca^{2+} sensitivity (Solaro et al., 1986, 1989; Palmer and Kentish, 1994; Kawashima et al., 1995, Parsons et al., 1997). However, it is not known whether different Tm isoforms are involved in the difference in pH dependence on maximum isometric tension. It may be possible to examine this by using muscle fibers prepared from transgenic animals expressing Tm isoforms (Palmiter et al., 1996). In the present study, we investigated this possibility through the use of thin filament-reconstituted cardiac muscle fibers (Fujita et al., 1996; Fujita and Ishiwata, 1998).

The thin filament in skeletal or cardiac muscle fibers can be selectively removed using calf plasma gelsolin, an actin filament-severing protein (Funatsu et al., 1990, 1993; Yasuda et al., 1995). Actin filaments in such thin filament-removed cardiac muscle fibers can be fully reconstituted by adding exogenous actin (Fujita et al., 1996; Fujita and Ishiwata, 1998; Ishiwata et al., 1998). Furthermore, thin filament possessing full Ca^{2+} sensitivity can then be reconstituted by adding regulatory proteins Tm and Tn to the actin filament-reconstituted fibers. We found that, as in control muscle fibers, pH dependence of tension development in actin filament-reconstituted bovine cardiac muscle fibers in the absence of regulatory proteins was nearly linear in the pH range 6.0–7.4, but that the slope was steeper. The original slope was regained by reconstitution with bovine cardiac nTm (Tm and Tn). Furthermore, fibers reconstituted with rabbit skeletal nTm showed a pH dependence resembling that of rabbit skeletal muscle. Similar modulation of pH dependence of isometric tension was also observed in fibers reconstituted with actin and Tm but without Tn. These results demonstrate that $\Delta F/\Delta$pH depends on the type of Tm isoform, such that Tm modifies the pH dependence of isometric tension.

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Address reprint requests to Dr. Shin'ichi Ishiwata, Department of Physics, School of Science and Engineering, Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan. Tel.: +81-3-5286-3437; Fax: +81-3-3200-2567; E-mail: ishiwata@mn.waseda.ac.jp.

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MATERIALS AND METHODS

Solutions

The solutions used were as follows: rigor solution, 170 mM KCl, 1.0 mM $MgCl_2$, 1.0 mM EGTA, and 10 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) (pH 7.0); relaxing solution, 117 mM KCl, 5.0 mM $MgCl_2$, 4.0 mM ATP, 1.0 mM EGTA, 10 mM MOPS (pH 7.0), and 20 mM 2,3-butanedione 2-monoxime (BDM); contracting solution, 117 mM KCl, 4.25 mM $MgCl_2$ (2.2 mM free Mg^{2+}), 2.2 mM ATP (2.0 mM $MgATP^{2-}$), 2.0 mM EGTA, 20 mM MOPS (pH 6.6–7.4) or 2-(*N*-morpholino)ethanesulfonic acid (MES) (pH 6.0–6.4), and 2.0 mM $CaCl_2$. ATP (disodium salt) was purchased from Boehringer Mannheim (Mannheim, Germany); EGTA, MOPS, and MES were from Dojindo Laboratories (Kumamoto, Japan). Tetramethyl rhodamine-5-iodoacetamide (Rh-IA) and fluorescein phalloidin (Fl-Ph) were purchased from Molecular Probes (Eugene, OR). Other chemicals were of reagent grade.

Muscle fibers and proteins

Glycerinated bovine cardiac muscle fibers and rabbit white skeletal muscle fibers were prepared in a solution composed of 50% (v/v) glycerol, 0.5 mM $NaHCO_3$, 5.0 mM EGTA, and 2.0 mM leupeptin. Glycerinated fibers were stored at −20°C and used within 2–8 weeks. Bovine plasma gelsolin was prepared according to the method of Kurokawa et al. (1990). Actin was extracted from acetone powder (Kondo and Ishiwata, 1976) of bovine cardiac muscle according to the method of Spudich and Watt (1971). Purified G-actin was stored at 0°C and used within 1 week. Tm, Tn, and nTm were prepared from bovine cardiac muscle and from rabbit white skeletal muscle according to the method of Ebashi et al. (1968) and purified using DEAE Sephadex A-25 (Pharmacia, Sweden). Tm labeled with Rh-IA (9% labeled) was prepared according to the method of Ishii and Lehrer (1990).

Tension measurement

A thin muscle bundle (~1 mm in length, ≤60 μm in diameter) was carefully stripped from a glycerinated bovine cardiac muscle fiber with a pair of forceps under a stereomicroscope just before experiments. To prepare a suitably thin bundle, dissection was carried out in glycerol solution at about −10°C (Fukuda et al., 1996). Both ends of the bundle were fixed to thin tungsten wires with enamel (commercial nail polish for cosmetic use), one of which was attached to a tension transducer (AE-801; SensoNor a.s, Holten, Norway). The muscle bundle was then immersed in rigor solution containing 1% Triton X-100 for 20 min to remove residual portions of the membrane system. After Triton X-100 was washed out with rigor solution, the bundle was immersed in the relaxing solution. Active tension was measured with a pen recorder (VP-6533A; National, Japan) by immersing the muscle bundle in the contracting solution. Active tension of control cardiac muscle fibers were 60–80 kN/m^2 (cf. Fujita et al., 1996). The maximum activated tension in control and actin filament-reconstituted fibers did not change with the addition of creatine kinase (1.0 mg/ml)/creatine phosphate (10 mM), suggesting that ATP depletion within the muscle bundles did not occur, probably because the diameter of the muscle bundle was small (≤60 μm) and the solution was continuously stirred (Yamaguchi, 1998). The measurement chamber used was a silicon-coated aluminum block (10 cm × 10 cm × 1 cm) with several small wells (7 mm in diameter) filled with ~0.4 ml each of experimental solutions (Horiuti, 1986). The bundle was immersed just below the surface at the deepest part of the solution droplet in the well such that only 1–2 s was required for the transfer of the bundle from one solution to another. Measurements were carried out at 25°C.

Removal and reconstitution of actin filaments

Removal and reconstitution of the actin filament were performed as previously described (Fujita et al., 1996; Fujita and Ishiwata, 1998; Ishiwata et al., 1998). In brief, thin cardiac muscle bundles were immersed in contracting solution containing 20 mM BDM (to suppress tension development during gelsolin treatment, which requires Ca^{2+}) and 0.3 mg/ml gelsolin at 2°C for 80 min to selectively remove thin filament. We confirmed that no active tension developed after gelsolin treatment. The bundles were then immersed in actin-polymerizing solution (80 mM KI, 4.0 mM $MgCl_2$, 4.0 mM ATP, 4.0 mM EGTA, 20 mM BDM, and 20 mM K-phosphate, pH 7.0) containing 1 mg/ml purified G-actin, which was mixed just before use. G-actin in the polymerizing solution was freshly prepared just before use and exchanged every 7 min to avoid nucleation. Actin polymerization was performed for a total of 28 min (7 min × 4). Removal and reconstitution procedures were carried out at 2°C. Relaxation of the actin filament-reconstituted fibers was achieved by immersing the fibers in relaxing solution containing 20 mM BDM. Activation of actin filament-reconstituted fibers was achieved by washing out BDM relaxing solution with contracting solution. There was no Ca^{2+} sensitivity in actin filament-reconstituted fibers.

Reconstitution of thin filaments

To reconstitute the thin filament, actin filament-reconstituted fibers were immersed in relaxing solution containing 3 mg/ml nTm and 20 mM BDM at 2°C for 12 h. To reconstitute fibers with skeletal Tm and cardiac Tn, or with cardiac Tm and skeletal Tn (chimera nTm fibers), the fibers were first immersed in relaxing solution containing 1.5 mg/ml Tm and 20 mM BDM at 2°C for 12 h. The fibers were then immersed in relaxing solution containing 1.5 mg/ml Tn and 20 mM BDM at 2°C for 5 h. These thin filament-reconstituted fibers showed no active tension development in the absence of Ca^{2+}, but did develop tension when Ca^{2+} was added in the absence of BDM. Fibers that developed less than 70% of the tension obtained before reconstitution with regulatory proteins were not used.

To confirm the binding of Tm to the reconstituted actin filaments in the fibers, thin filaments were reconstituted with Rh-IA-labeled Tm instead of unlabeled Tm according to the same procedure as described above. The fibers were then fixed with relaxing solution containing 1% formaldehyde for 30 min and stained with 6.6 μM Fl-Ph in relaxing solution for 5 h at 2°C to visualize actin filaments. They were then mounted on a coverslip and washed with relaxing solution containing 4.5 mg/ml glucose, 0.22 mg/ml glucose oxidase, 0.036 mg/ml catalase, and 10 mM dithiothreitol. Preparations were observed under a laser scanning confocal microscope equipped with a 25-mW Ar laser at 488 nm (Fluoview-IX/AR; Olympus Co., Tokyo). No crossover between fluorescence images of rhodamine (>610 nm; red) and fluorescein (510–540 nm; green) was detectable. As shown in Fig. 1, *A* and *B*, the distribution of Rh-IA was identical to that of Fl-Ph except for the weak fluorescence at the Z line, showing that incorporated Tm was bound to actin filaments except at the Z line. Control cardiac muscle fibers were also incubated with Rh-IA-labeled Tm for 12 h at 2°C in BDM relaxing solution and then stained with Fl-Ph after formaldehyde fixation. As shown in Fig. 1, *C* and *D*, fluorescence of Rh-IA was not detectable, indicating that Tm was not incorporated into the thin filaments in control fibers.

RESULTS

pH dependence of isometric tension in actin filament-reconstituted and thin filament-reconstituted fibers

Fig. 2 shows pen traces of isometric tension measured at various pH values in actin filament-reconstituted fibers. First, glycerinated cardiac muscle fibers were treated with gelsolin (*arrow G* in Fig. 2). Removal of thin filaments was verified by measuring active tension, which was negligible after gelsolin treatment. The muscle bundles were then treated with actin-polymerizing solution (*arrow A* in Fig. 2),

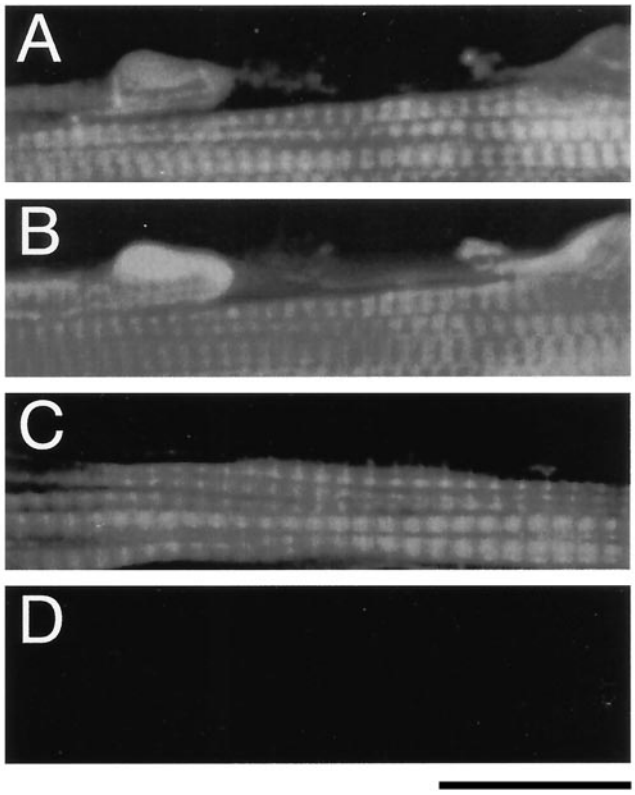

FIGURE 1 Confocal fluorescence micrographs showing the distribution of actin filaments labeled with Fl-Ph (*A, C*) and Tm labeled with Rh-IA (*B, D*) in thin filament-reconstituted (*A, B*) and control (*C, D*) cardiac muscle fibers. Bar, 20 μm.

and the pH dependence of active tension was measured. pH was increased stepwise from 6.0 to 7.0 at a 0.2 interval, and decreased again stepwise to 6.0. Active tension increased in proportion to the increase in pH and decreased in proportion to the decrease in pH. Relative tension was calculated by dividing average tension by that at pH 7.0. The stability and durability of the reconstituted muscle fibers with respect to contractile properties were indistinguishable from those of control fibers (Fig. 2).

The pH dependence of isometric tension in the actin filament- and thin filament-reconstituted fibers is shown in Fig. 3. In control bovine cardiac and rabbit skeletal muscle fibers, the pH-tension relation was nearly linear in the pH range 6.0–7.4. The slope of the pH-tension relation was greater in skeletal than in cardiac muscle fibers, with $\Delta F/\Delta$pH of 0.28 for cardiac (Fig. 3, *empty circles, thick line*) and 0.44 for skeletal muscle fibers (Fig. 3, *empty triangles, thin line*). $\Delta F/\Delta$pH in actin filament-reconstituted fibers without regulatory proteins was 0.62, the greatest value of all combinations tested (Fig. 3, *empty squares, dotted line*). When thin filament was reconstituted with bovine cardiac nTm, $\Delta F/\Delta$pH decreased to 0.32, close to the control value (Fig. 3, *filled circles, thick broken line*). The thin filament-reconstituted fibers did not develop active tension in the absence of Ca^{2+}. These results indicate that the regulatory proteins suppressed the decrease in tension caused by decreasing pH. In fibers reconstituted with rabbit skeletal nTm, $\Delta F/\Delta$pH was 0.48, close to that of the control skeletal fibers (Fig. 3, *filled triangles, thin broken line*).

pH-tension relation in fibers reconstituted with actin and chimera nTm

To determine whether Tm or Tn is responsible for the pH dependence, the pH-tension relation was examined in fibers reconstituted with chimera nTm, i.e., either cardiac Tm (cTm) and skeletal Tn (sTn), or skeletal Tm (sTm) and cardiac Tn (cTn). In fibers reconstituted with cTm + sTn, the relation resembled that in cardiac muscle fibers (Fig. 4, *half-filled circle*), with a $\Delta F/\Delta$pH value of 0.29, whereas in those reconstituted with sTm + cTn it resembled that in skeletal muscle fibers (Fig. 4, *half-filled triangle*), with a $\Delta F/\Delta$pH value of 0.44. These results indicate that the pH-tension relation is modified by Tm but not by Tn.

To confirm that the chimera nTm fibers were constructed successfully, the pCa-tension relation was examined. Fibers reconstituted with sTm + cTn showed a pCa-tension relation indistinguishable from that of control cardiac muscle fibers at pH 7.0 (Fig. 5 *A*) and pH 6.4 (Fig. 5 *B*). At pH 7.0, the Hill coefficient (n_H) for control cardiac and chimera nTm fibers was 1.9 and 2.0, respectively, and the pCa_{50} value, an indicator of Ca^{2+} sensitivity, was 6.05 and 6.1, respectively. The respective values at pH 6.4 were 2.0 and 2.0, and 5.6 and 5.55. A rightward shift in the pCa-tension relation by a decrease in pH was observed in control and chimera fibers to the same degree. The Hill coefficient did not change significantly with a decrease in pH from 7.0 to 6.4.

pH-tension relation in fibers reconstituted with actin and Tm

Next, we examined the pH-tension relation of fibers reconstituted with actin and Tm. Tm-reconstituted fibers generated active tension in a Ca^{2+}-insensitive manner because of the lack of Tn. In cTm-reconstituted fibers, $\Delta F/\Delta$pH was 0.33 (Fig. 6, *empty circle*), similar to that of control cardiac fibers, whereas in sTm-reconstituted fibers it was 0.49 (Fig. 6, *filled circle*), similar to that of control skeletal fibers. $\Delta F/\Delta$pH values for various types of muscle models thus obtained are summarized in Table 1. The difference in the pH-tension relation between different Tm isoforms was statistically significant, whereas there was no statistically significant difference between different Tn isoforms when the same Tm isoform was used.

DISCUSSION

Experiments using thin filament-reconstituted fibers

Actin polymerization was performed for only 28 min (7 min $\times$ 4) instead of the 42 min (7 min $\times$ 6) used in the

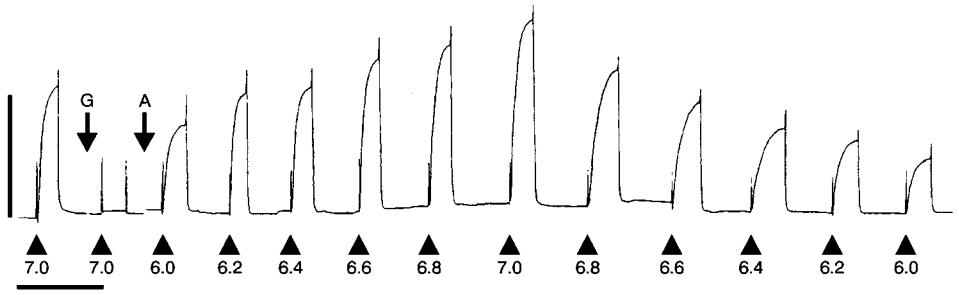

FIGURE 2 Recordings of isometric tension at varying pH in actin filament-reconstituted cardiac muscle fibers. After measurement of control tension, fibers were immersed in contracting solution containing 0.3 mg/ml gelsolin and 20 mM BDM for 80 min at 2°C (*arrow G*). After confirmation that fibers developed no active tension after gelsolin treatment, they were immersed in actin polymerizing solution containing 1 mg/ml G-actin for a total of 28 min (7 min × 4) at 2°C (*arrow A*). Arrowheads indicate solution change. pH values of contracting solutions are indicated below the arrowheads. Spikes are artifacts due to solution change. Active tension at varying pH was measured at 25°C. Relaxation was obtained by immersing the fiber in relaxing solution containing 20 mM BDM at 2°C. Vertical and horizontal bars, 2×10^{-4} N and 2 min, respectively.

original work (Fujita et al., 1996) to minimize fiber damage due to repeated high-tension developments. Consequently, the average tension developed in the actin filament-reconstituted fibers was closely similar to that in the control fibers. The only problem with this actin filament reconstitution method is that it was difficult to control the extent of tension recovery after reconstitution, which varied between preparations (Fujita et al., 1996). Because this variability makes it difficult to quantitatively analyze the difference between active tension of control fibers and actin filament-reconstituted fibers, experiments should have been designed to compare the properties of reconstituted and control fibers, using the same actual fiber. Here, the decrease in active tension with decreasing pH was compared between actin (thin) filament-reconstituted fibers and their controls. These properties were reproducible and reliable, as reported here.

In the present study, we succeeded in constructing chimera nTm fibers composed of either skeletal Tm and cardiac Tn or vice versa. To construct chimera nTm-reconstituted fibers, actin filament-reconstituted fibers were first

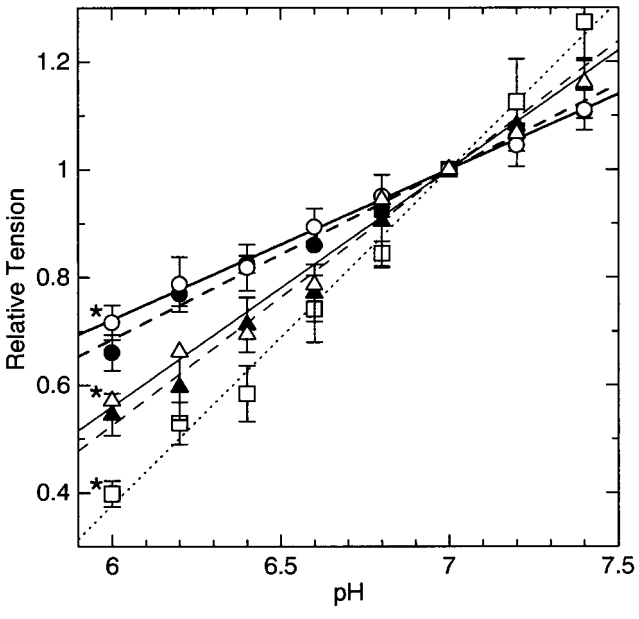

FIGURE 3 Effect of pH on isometric tension in control cardiac muscle fibers (*empty circles, thick line*), control skeletal muscle fibers (*empty triangles, thin line*), actin filament-reconstituted muscle fibers (*empty squares, dotted line*), thin filament-reconstituted fibers with cardiac nTm (*filled circles, thick broken line*), and thin filament-reconstituted fibers with skeletal nTm (*filled triangles, thin broken line*). Relative tension was normalized to that at pH 7.0. Lines are fitted by least-squares fit. Vertical bars show SD calculated from three to five data points. Active tension was measured at 25°C. The difference between any two data points marked with * at pH 6.0 was statistically significant ($p < 0.001$ by Student's *t*-test).

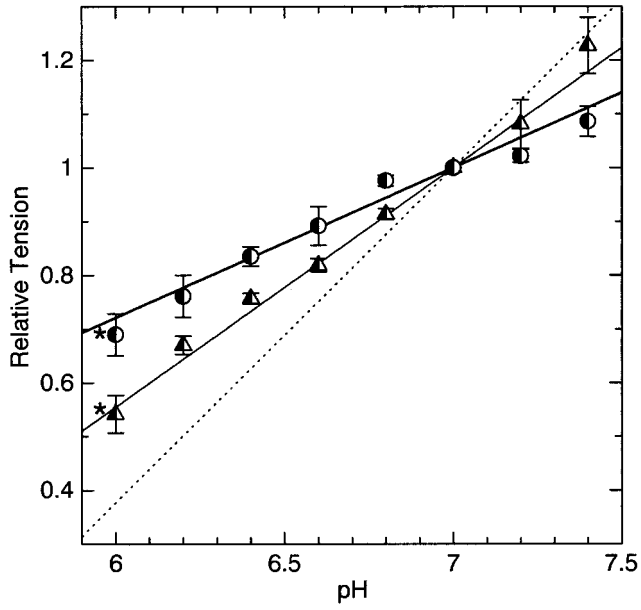

FIGURE 4 Effect of pH on isometric tension in fibers reconstituted with cTm and sTn (*half-filled circles*), and with sTm and cTn (*half-filled triangles*). Thick, thin, and dotted lines show, respectively, the fitted lines for control cardiac, control skeletal, and actin filament-reconstituted fibers obtained in Fig. 3. Relative tension was normalized to that at pH 7.0. Vertical bars show SD calculated from three to five data points. Active tension was measured at 25°C. The difference between two data points marked with * at pH 6.0 was statistically significant ($p < 0.01$ by Student's *t*-test).

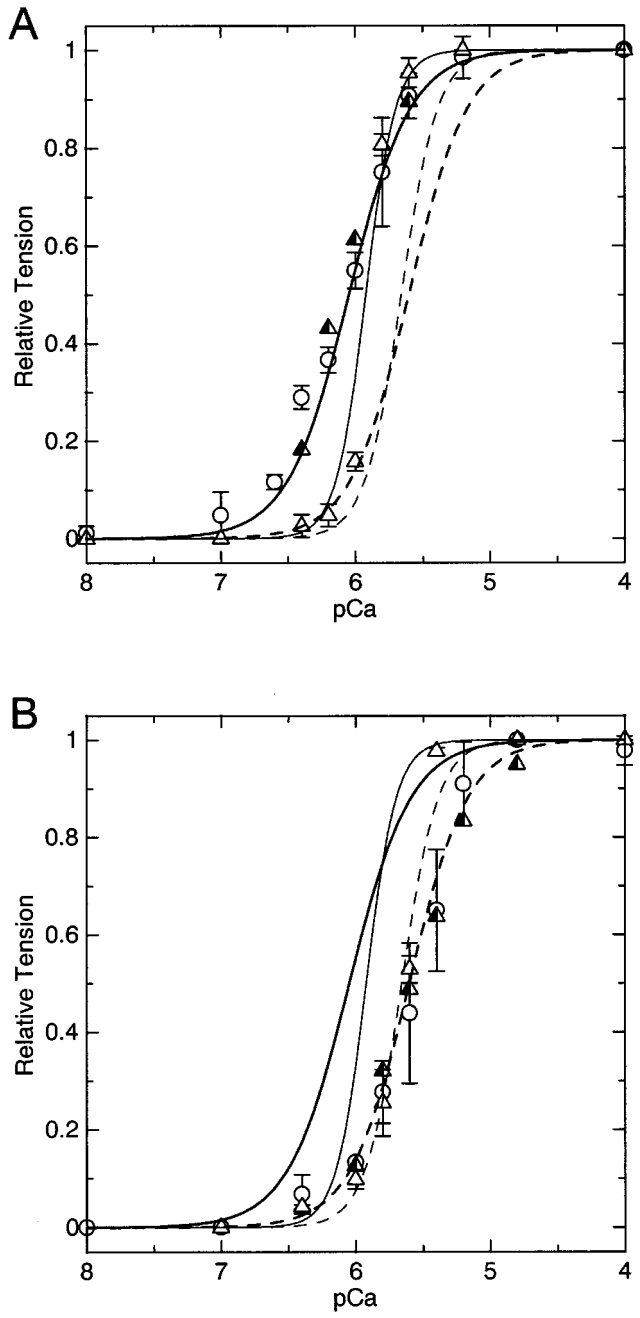

FIGURE 5 pCa-tension relation of control cardiac (*empty circles*), control skeletal (*empty triangles*), and (sTm + cTn)-reconstituted fibers (*half-filled triangles*) at pH 7.0 (*A*) and pH 6.4 (*B*). Thick and thin lines are the curves fitted for control cardiac and skeletal fibers (*solid line*, pH 7.0; *broken line*, pH 6.4), respectively. Relative tension was normalized to that at pCa 4.0. Vertical bars show SD calculated from three to five data points. Active tension was measured at 25°C.

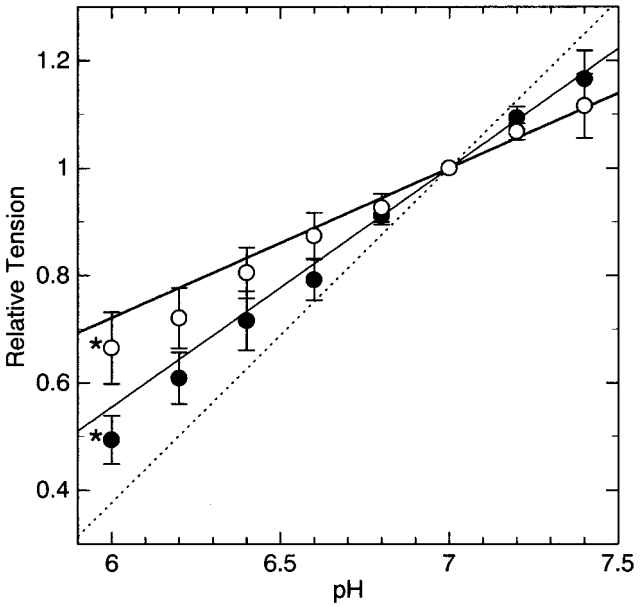

FIGURE 6 Effect of pH on isometric tension in fibers reconstituted with cTm (*empty circles*) and sTm (*filled circles*). Thick, thin, and dotted lines show, respectively, fitted lines for control cardiac, control skeletal, and actin filament-reconstituted fibers obtained in Fig. 3. Relative tension was normalized to that at pH 7.0. Vertical bars show SD calculated from three to five data points. Active tension was measured at 25°C. The difference between two data points marked with * at pH 6.0 was statistically significant ($p < 0.01$ by Student's *t*-test).

reconstituted with Tm and then with Tn so that nonspecific binding of Tn to actin filaments was avoided (Ishiwata and Kondo, 1978). The purity of added proteins was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis before reconstitution (data not shown), but the quality of reconstitution (e.g., the stoichiometry of actin, Tm, and Tn) in the reconstituted fibers could not be evaluated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis because of the small amount of proteins. To confirm the reconstitution of thin filaments, the pCa-tension relation was therefore examined. On the basis that both the pCa_{50} value and the Hill coefficient in the chimera nTm fibers were determined by the type of Tn (Fig. 5), we conclude that the function of thin filaments was fully recovered.

Maximum decrease in active tension by decreased pH in actin filament-reconstituted fibers

In control cardiac muscle fibers, active tension decreased linearly with a decrease in pH in the range of pH 6.0–7.4 (Fig. 3, *empty circles*), which is consistent with the previous results (Metzger and Moss, 1987; Godt and Kentish, 1989; Solaro et al., 1989). Although a linear decrease in active

TABLE 1 $\Delta F/\Delta pH$ values for various types of muscle model

Control			Thin filament-reconstituted		
			cTn	sTn	no Tn
Cardiac	0.28*	cTm	0.32*	0.29[#]	0.33[§]
Skeletal	0.44*	sTm	0.44[#]	0.48*	0.49[§]
Actin reconstituted	0.62*				

*Taken from Fig. 3.
[#]Taken from Fig. 4.
[§]Taken from Fig. 6.

tension by reduced pH was also observed in actin filament-reconstituted fibers (Fig. 3, *empty squares*), $\Delta F/\Delta$pH was more than twice that of control cardiac fibers. Because the only difference between control cardiac muscle fibers and actin filament-reconstituted fibers is the presence of regulatory proteins (Fujita et al., 1996), the difference in $\Delta F/\Delta$pH should be related to regulatory proteins. Given that isometric tension is thought to be proportional to the population of attached cross-bridges, the decrease in active tension with reduced pH may be the result of cross-bridge detachment.

Several factors can be considered in the regulation of the number of attached cross-bridges. Myosin has a positively charged loop (called loop 2) in the upper 50-kDa domain, and actin has four corresponding negatively charged acidic residues located at the N-terminus in subdomain 1. These are assumed to make the initial contact in the actin-myosin interaction (Chaussepied and Van Dijk, 1999). Additionally, there are many histidine residues that have neutral pK values. In theory, a decrease in pH from 7.0 to 6.0 should decrease the proportion of negatively charged carboxyl groups (assume pK 4.7) by ~5% and increase that of positively charged imido groups (assume pK 6.5) by ~70%. The large decrease in active tension by reduced pH in the actin filament-reconstituted fibers may be primarily attributed to the effect of these changes in surface charge on the actin-myosin interaction. Changes in the charge distribution of actin, myosin, or both may also modulate secondary factors that affect the actin-myosin interaction, such as the distance between thick and thin filaments.

Modulation of the pH dependence of active tension by regulatory proteins

Given that the protein composition of skeletal nTm-reconstituted fibers is considered to be the same as that of cardiac muscle fibers with the exception of regulatory proteins, the results shown in Fig. 3 indicate that the differing pH dependency among muscle types is attributable to regulatory protein isoforms. We therefore conclude that the type of regulatory proteins modulates the pH dependence of active tension: the pH dependence of fibers reconstituted with sTm and cTn resembled that of the control skeletal muscle fibers (Fig. 4, *half-filled triangle*), whereas fibers reconstituted with cTm and sTn resembled that of control cardiac muscle fibers (Fig. 4, *half-filled circle*). These findings demonstrate that the pH dependence of active tension is modulated by Tm, and that Tn has no effect on this variable.

When Tm alone (cardiac or skeletal) was added to actin filament-reconstituted fibers, $\Delta F/\Delta$pH decreased in both cases (Fig. 6). The effect was similar with nTm. These results support the conclusion that Tm alone modulates the pH dependence of active tension. It has been reported that the binding of Tm to actin is stabilized by lowering pH (Tanaka, 1972). The difference among Tm isoforms in modulating the pH dependence of active tension may be due to differences in the stability of Tm isoforms binding to actin filaments.

Three-dimensional modeling of thin filament showed that Tm does not cover the charged group on actin's surface in the presence or absence of Ca^{2+} (Lehman et al., 1995). However, because negative staining was used to visualize Tm, which may alter its location, the possibility remains that Tm is closer to the charged residues in vivo. In addition, the effect of Tm on the pH-tension relation may be attributable to allosteric regulation of charge distribution on actin filaments. That is, the presence of Tm may affect the accessibility of charged groups on actin, even though they are distant from the area covered by Tm.

Mammalian Tm has α and β subunits, which can be arranged as two isomers, $\alpha\alpha$ and $\alpha\beta$ (Eisenberg and Kielley, 1974). The amino acid sequence of these α and β subunits in rabbit skeletal and cardiac muscle is identical (Stone and Smillie, 1977; Lewis and Smillie, 1980). The subunits differ by 39 residues, including two residues, giving a more negative net charge on the β (Mak et al., 1979). Because the α:β ratio depends on muscle type (in general, the ratio of α to β in cardiac muscle is larger than that in skeletal muscle), the modulation of pH dependence of isometric tension in actin filament-reconstituted fibers may also be due to differences in charge distribution on Tm isoforms.

The present study used rabbit fast skeletal Tm and bovine cardiac Tm, both of which are a mixture of α and β Tm. It is not clear whether the difference in pH dependence of active tension between skeletal and cardiac Tm (Figs. 3 and 4) results from a difference in skeletal and cardiac Tm or in rabbit and bovine Tm. However, this ambiguity does not change our major conclusion that Tm modulates the pH dependence of active tension. The molecular basis of Tm's modulation of pH dependence of active tension may be clarified by reconstitution of thin filaments by the use of purified α and β Tm or mutant Tm with different charge distribution. This awaits further investigation.

The smaller decrease in active tension in an acidic environment in cardiac muscle than in skeletal muscle may have developed during the process of evolution. The ability to survive an acidic environment is of particular advantage for cardiac muscle cells, which are often subject to lowered pH induced by cardiac hypoxia and ischemia.

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