

Effects of Partial Extraction of Troponin Complex upon the Tension-pCa Relation in Rabbit Skeletal Muscle

Further Evidence That Tension Development Involves Cooperative Effects within the Thin Filament

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ABSTRACT Partial extraction of troponin C (TnC) decreases the Ca^{2+} sensitivity of tension development in mammalian skinned muscle fibers (Moss, R. L., G. G. Giulian, and M. L. Greaser. 1985. *Journal of General Physiology*. 86:585), which suggests that Ca^{2+} -activated tension development involves molecular cooperativity within the thin filament. This idea has been investigated further in the present study, in which Ca^{2+} -insensitive activation of skinned fibers from rabbit psoas muscles was achieved by removing a small proportion of total troponin (Tn) complexes. Ca^{2+} -activated isometric tension was measured at pCa values (i.e., $-\log[\text{Ca}^{2+}]$) between 6.7 and 4.5: (a) in control fiber segments, (b) in the same fibers after partial removal of Tn, and (c) after recombination of Tn. Tn removal was accomplished using contaminant protease activity found in preparations of LC_2 from rabbit soleus muscle, and was quantitated using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and scanning densitometry. Partial Tn removal resulted in the development of a Ca^{2+} -insensitive active tension, which varied in amount depending on the duration of the extraction, and concomitant decreases in maximal Ca^{2+} -activated tensions. In addition, the tension-pCa relation was shifted to higher pCa values by as much as 0.3 pCa unit after Tn extraction. Readdition of Tn to the fiber segments resulted in the reduction of tension in the relaxing solution to control values and in the return of the tension-pCa relation to its original position. Thus, continuous Ca^{2+} -insensitive activation of randomly spaced functional groups increased the Ca^{2+} sensitivity of tension development in the remaining functional groups along the thin filament. In addition, the variation in Ca^{2+} -insensitive active tension as a function of Tn content after extraction

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suggests that only one-third to one-half of the functional groups within a thin filament need to be activated for complete disinhibition of that filament to be achieved.

INTRODUCTION

The regulation of the interaction of myosin and actin in vertebrate skeletal muscles involves the binding of Ca^{2+} to two low-affinity sites on the troponin C (TnC) subunit of troponin (Tn) (Potter and Gergely, 1975), a regulatory protein bound at regular intervals to the actin-containing thin filaments. This switch-like action of Ca^{2+} to disinhibit the thin filament and allow cross-bridge attachments is widely recognized; however, several recent reports suggest that additional factors may modify the apparent affinity of TnC for Ca^{2+} (Bremel and Weber, 1972; Fuchs, 1977; Brandt et al., 1980, 1982; Grabarek et al., 1983; Moss et al., 1985*b*), as well as the number of actin monomers that are disinhibited per TnC at low levels of Ca^{2+} activation (Murray and Weber, 1980). Bremel and Weber (1972) were the first to suggest that binding of myosin cross-bridges within a functional group (i.e., seven actin monomers, one troponin, and one tropomyosin) resulted in an increased affinity for Ca^{2+} at the low-affinity sites on TnC. A similar effect has been seen in skinned muscle fibers, where reductions in the levels of MgATP resulted in an increase in the Ca^{2+} sensitivity of tension development (Reuben et al., 1971). Bremel and Weber also found that binding of rigor bridges within a functional group allowed cyclic interactions of nucleotide-bound cross-bridges with other actin monomers within that functional group. Cross-bridge activation of functional groups was evident in a recent study (Moss and Haworth, 1984) in which skinned single fibers were found to actively shorten at low levels of MgATP in the absence of Ca^{2+} . Moreover, Brandt and his colleagues (1980, 1982) suggested that after the initial activation of functional groups with Ca^{2+} , a substantial fraction of the functional groups were kept active because of cross-bridge attachments, since it is likely that Ca^{2+} bound to the low-affinity sites on TnC would dissociate much more rapidly than would cross-bridges attached to actin. This cooperativity in thin-filament activation was suggested as a possible explanation for the steepness of the tension-pCa relation, which otherwise could not be modeled solely on the basis of two low-affinity Ca^{2+} -binding sites (Brandt et al., 1980, 1982). More recently, Grabarek et al. (1983) suggested that the steepness of the relation between actomyosin ATPase and pCa might be due to long-range interactions along the thin filaments, such that binding of Ca^{2+} to the low-affinity sites associated with one functional group would actually enhance Ca^{2+} binding within adjacent or remote functional groups within the same thin filament. These investigators believed that the molecular mechanism of the cooperativity in Ca^{2+} binding could involve tropomyosin (Tm) as a transducing element. Studies in this laboratory (Moss et al., 1985*b*) and in others (Brandt et al., 1984) supported this hypothesis in that partial extraction of TnC from skinned fibers resulted in a decrease in the Ca^{2+} sensitivity of tension development by the functional groups with TnC still bound. Presumably, the groups from which TnC was extracted would be permanently inactivated,

resulting in an immobilization of the associated Tm and disrupting the cooperativity in Ca^{2+} binding.

The present study is a further investigation of the modulation of tension development caused by cooperative mechanisms within the thin filament. A method was developed for partial removal of whole troponin complex from skinned single fibers from psoas muscles of the rabbit. This was done in an attempt to achieve Ca^{2+} -insensitive activation of a portion of the functional groups within any given thin filament and subsequently to measure the effects of activation upon the Ca^{2+} sensitivity of tension development involving the remaining functional groups. A preliminary report of the results of this study was made at the Annual Meeting of the Biophysical Society (Moss et al., 1985a).

METHODS

Psoas muscles were obtained from adult male New Zealand rabbits. Bundles of ~50 fibers were stored at -22°C in relaxing solution containing 50% (vol/vol) glycerol for up to 21 d before use (Moss et al., 1983). Individual fibers were then pulled from each bundle and mounted in the experimental chamber containing the relaxing solution. The sarcomere length in the relaxed fiber segments was adjusted to 2.5–2.6 μm by changing the overall segment length. The force-transducer, motor, and solution-changing device were as previously described (Moss et al., 1983). The fiber segments were activated in solutions containing various concentrations of free calcium between pCa (i.e., $-\log[\text{Ca}^{2+}]$) 6.70 and 4.5. The solutions contained 7 mM EGTA, 1 mM free Mg^{2+} , 20 mM imidazole (pH 7.00), 6.28 mM total ATP, 14.5 mM creatine phosphate, various free Ca^{2+} concentrations, and sufficient KCl to yield an ionic strength of 180 mM. The pCa of the relaxing solution was 9.0. The computer program of Fabiato and Fabiato (1979) was used to calculate the final concentrations of each metal, ligand, and metal-ligand complex, based on the stability constants listed by Godt and Lindley (1982). The apparent stability constant for Ca-EGTA was corrected to 15°C (Fabiato and Fabiato, 1979) and for the effects of ionic strength (Martell and Smith, 1974). At any given pCa, a steady tension was allowed to develop, at which time the segment was rapidly (i.e., within 1 ms) slackened, and the segment was subsequently relaxed. The difference between the steady developed tension and the tension baseline obtained immediately after the slack step was measured as total tension. Active tension was calculated as the difference between total tension and the resting tension (usually <1 mg-wt in untreated fiber segments) measured in the same segment while in relaxing solution. Tensions (P) at submaximally activating levels of calcium were expressed as a fraction of P_0 ; the Ca^{2+} -activated tension developed at pCa 4.50. Every third or fourth contraction was performed at pCa 4.50 in order to assess any decline in fiber performance (Moss, 1979). In the experiments in which alterations in the tension-pCa relation after partial extraction of Tn were measured, P_0 after extraction was found to decline substantially. Submaximal tensions measured after the extraction of Tn were expressed as a fraction of the P_0 obtained under the same conditions.

The relationship between relative isometric tension and pCa was measured in any one fiber segment first before treatment (i.e., control), then in the same segment after partial removal of Tn, and finally, in some of the segments, after recombination of Tn into the segment. Although methods for the removal of Tn using endogenous Ca^{2+} -activated proteases have been described (Nakayama et al., 1983), we have been able to partially remove Tn from psoas fibers by using contaminant protease activity found in preparations of myosin LC_2 from bovine masseter muscle. The partial removal of Tn was done by

bathing the fiber segments in a solution containing 50 mM KCl, 10 mM EDTA, 5 mM PO_4 , pH 7.00, and 1 mg/ml slow LC_2 at 15°C for a period of 120 min, unless otherwise indicated (see Fig. 6). Otherwise identical solutions that did not contain the LC_2 preparation were ineffective in removing Tn. To obtain LC_2 , myosin was extracted from bovine

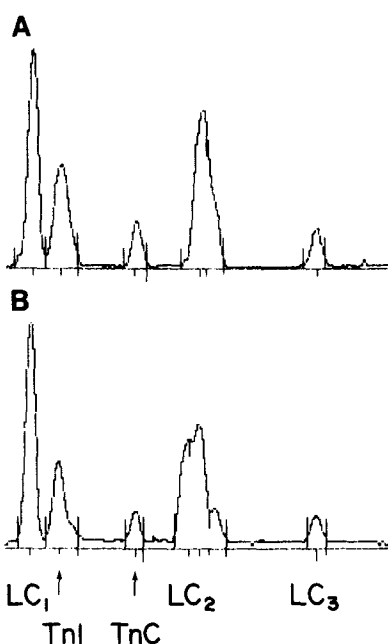


FIGURE 1. Densitometric scans of a polyacrylamide gel on which were run segments obtained from the same single fiber before treatment (A) and after partial Tn removal (B). The scan is shown for only the low molecular weight portion of the gel, containing the myosin light chains (LC_1 , LC_2 , and LC_3), troponin I (TnI), and troponin C (TnC). The Tn content of this particular fiber after 300 min of incubation was 76% of control as determined from the ratios of the sum of the areas of the TnI and TnC peaks to actin or LC_1 in each segment.

	Areas under peaks (area units)	
	Control	Treated
LC_1	722	707
TnI	501	367
TnC	151	119
LC_2	883	851
LC_3	130	131

The Ca^{2+} -insensitive tension generated by this fiber segment after incubation was 67% P_o .

masseter muscle and treated with 5 M guanidine HCl to dissociate the light chains (Perrie and Perry, 1970). After dilution with an equal volume of water, the solution was fractionated by addition of ethanol and the 66–90% cut was retained. The protein was applied to a DEAE-cellulose column in 6 M urea, 10 mM potassium phosphate (pH 6.0), and 15 mM β -mercaptoethanol, and eluted with a linear phosphate gradient to 0.1 M.

The LC₂ fraction was dialyzed vs. 0.1 M KCl/10 mM imidazole (pH 7.0) and stored frozen at -70°C before use.

Tn was recombined into the fiber segments by bathing the segments in relaxing solution containing ~ 1 mg/ml purified Tn (prepared by the method of Greaser and Gergely, 1971). This soak was continued for 40 min at 15°C and was followed by two successive 5-min washes in relaxing solution in order to remove excess Tn. In most fiber preparations, Tn removal and recombination were verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), as described previously (Giulian et al., 1983). In instances in which SDS-PAGE was done, the fiber segment was divided into three pieces of approximately equal lengths. The first of these was dissolved in an SDS-containing sample buffer (Giulian et al., 1983). The other two pieces were tied into the experimental chamber. One was tied between the force-transducer and motor (used for physiological measurements), and the other was tied at both ends to the motor arm so that it was exposed to the same solutions as the experimental segment. After the treatment to partially remove Tn, the segment tied only to the motor arm was detached and then dissolved in sample buffer. Finally, after the recombination of Tn and the subsequent determination of the relative tension-pCa relation, the experimental segment was dissolved in sample buffer. Thus, the Tn content could be determined in pieces of the same fiber segment under all three experimental conditions, i.e., before treatment, after partial removal of Tn, and after recombination of Tn into the segment.

After the staining and gel-drying procedures, the gels were scanned using a laser-light scanning densitometer, as described previously (Giulian et al., 1983). Since we had no means to measure absolute amounts of Tn within the fiber segments, it was necessary to express Tn as a fraction of another myofibrillar protein thought to be unaffected by the extraction conditions. The relative amount of Tn present in any given fiber segment was taken as the ratio of the sum of the areas of the peaks corresponding to the Tn subunits to the area of the peak corresponding to actin (Fig. 1). Actin was selected for normalization because there was virtually no difference in P_0 after recombination of Tn into the fiber segments relative to control values, which indicates that actin was not removed during the extraction procedure. Additional evidence that the content was unaffected by the extraction procedure was obtained by SDS-PAGE of the extracting solution that bathed several fiber segments during the extraction procedure. There was no evidence of actin in the extractant after the extraction procedure. Similar results regarding changes in Tn content were obtained when LC₁ rather than actin was used for normalization.

RESULTS

Original tension traces obtained during maximal (pCa 4.5) and low (pCa 6.4) levels of Ca^{2+} activation under various experimental conditions are shown for a single fiber segment in Fig. 2. In the untreated (control) fiber segment, pCa 6.4 was subthreshold for active tension development (Fig. 2B) and tension in the relaxing solution was < 1 mg-wt. After treatment for 120 min with the solution containing the putative protease activity, the Tn content of the fiber segments was found to decrease by $\sim 14\%$ relative to control, as determined by SDS-PAGE (see Fig. 1 for example of densitometric scans). Tension in the relaxing solution increased and there was a concomitant decrease in the maximal Ca^{2+} -activated tension (Fig. 2C). In the several fibers that have been studied, the sum of the Ca^{2+} -insensitive (i.e., in relaxing solution) and maximum Ca^{2+} -activated (pCa 4.5) tensions after 120 min of protease treatment was approximately equal ($0.94 \pm 0.08 P_0$; $n = 27$) to the tension at pCa 4.5 in the control segments (see also Fig.

6*B*). Bathing the fiber in TnC-containing relaxing solution, as described previously (Moss et al., 1983), did not appreciably alter the total tension developed by the treated fibers, which suggests that the Tn remaining within the fiber segments was not significantly TnC deficient. A further finding was that Ca^{2+} -

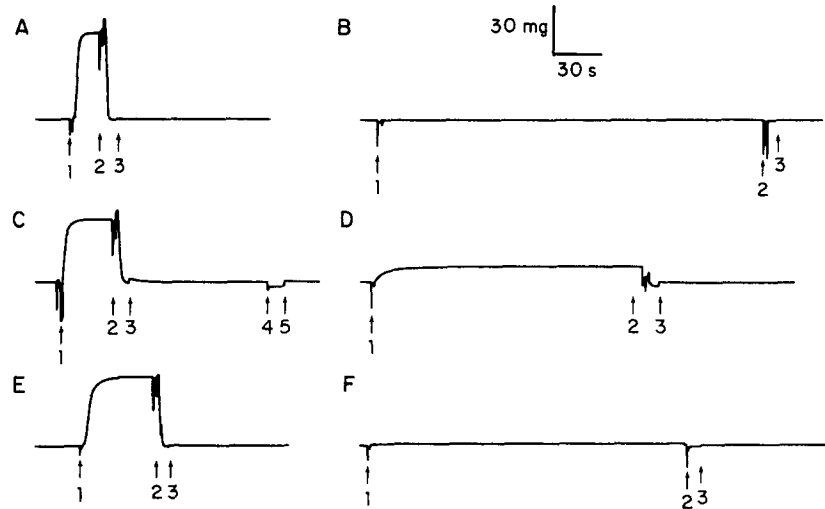


FIGURE 2. Records of isometric tension development in one fiber segment before treatment (i.e., control, *A* and *B*), after partial extraction of Tn (*C* and *D*), and after recombination of Tn (*E* and *F*). The activations in *A*, *C*, and *E* were performed at pCa 4.50; those in *B*, *D*, and *F* were done at pCa 6.40. In each frame, the fiber was transferred from relaxing solution (pCa 9.0) to activating solution at the time point indicated as "1." At time point 2, the fiber was slackened (length step complete in ≤ 1 ms) to obtain a zero-tension baseline that is not apparent on the slow-timebase recording (see Fig. 4). The fiber was subsequently relaxed and at time point 3 was re-extended to its original length. The record in *C* also indicates the method of measurement of resting tension, which involved rapidly slackening the fiber at pCa 9.0 (time point 4) and subsequent re-extension (time point 5). The following steady tensions were measured:

pCa	Tension (mg-wt)		
	Control	After Tn removal	With Tn
4.50 (corrected for tension at 9.0)	56.7	44.8	49.0
6.4 (corrected for tension at 9.0)	0.2	9.7	0.4
9.0 (relaxing)	0.2	5.7	0.8

activated tension development at pCa 6.4 increased substantially (Fig. 2*D*). These effects were for the most part reversed by bathing the fiber segment in relaxing solution containing whole Tn (Fig. 2, *E* and *F*). The decline in Ca^{2+} -insensitive tension during the soak in the Tn-containing relaxing solution occurred with a

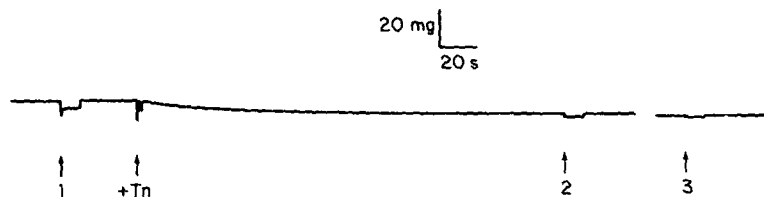


FIGURE 3. Time course of the recovery of Ca^{2+} -insensitive tension upon exposure to Tn-containing relaxing solution. The fiber had previously been bathed in Tn-extracting solution for 120 min. Tension was subsequently measured in relaxing solution (time point 1) and was 11.6 mg-wt, compared with 0.5 mg-wt before treatment. After addition of Tn to the relaxing solution (+ Tn), tension declined, reaching 2.1 mg-wt at 4 min (time point 2) and 0.7 mg-wt at 20 min (time point 3). For comparison, P_o developed by this same fiber segment before treatment was 65.5 mg-wt.

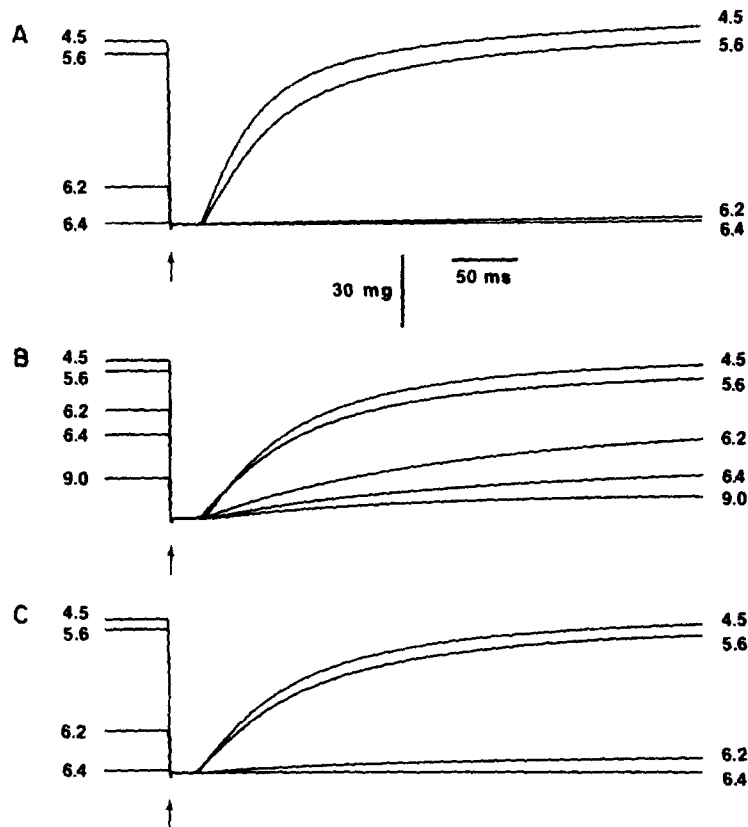


FIGURE 4. High-speed tension records obtained during the measurement of tension at a number of pCa values, indicated next to each tension record. The time point marked by the arrows in each frame corresponds to time point 2 in Fig. 2, and indicates the time at which the slack step was introduced (length step not shown). These records were from the same single fiber segment before treatment (A), after extraction for 120 min (B), and after recombination of Tn (C).

halftime of ~ 60 s and was virtually complete within 4 min (Fig. 3). The recombination of Tn into the fiber segment was verified by SDS-PAGE.

High-speed tension records obtained from one fiber segment at a number of pCa values are shown in Fig. 4 for each stage of the experimental protocol. Again, after Tn removal, the fiber was found to develop a substantial Ca^{2+} -insensitive active tension in relaxing solution, and this was accompanied by a reduction in the maximum Ca^{2+} -activated tension. At pCa 6.2 and 6.4, Ca^{2+} -activated tensions increased relative to control tensions at the same pCa values, both in absolute terms and when scaled to P_o measured under the same condition. At pCa 5.6, the absolute Ca^{2+} -activated tension decreased after Tn removal; however, when referenced to the maximum Ca^{2+} -activated tension developed

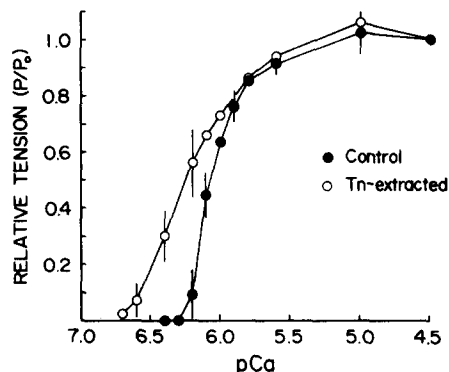


FIGURE 5. Plot of relative tension vs. pCa for control (●) and extracted (○) fiber segments ($n = 8$). Control tensions were scaled to the tension developed at pCa 4.5. Tensions plotted for the Tn-extracted fibers include only the Ca^{2+} -activated tensions, which were scaled to the Ca^{2+} -activated tension at pCa 4.5 after extraction. Error bars indicate ± 1 SD.

after Tn removal, the tension was unchanged when compared with similarly scaled control values. An additional observation from Fig. 4 is that after Tn extraction, the fiber segments took up the slack that was imposed during the measurement of isometric tension, even at pCa 9.0. This indicates that the cross-bridges involved in the generation of Ca^{2+} -insensitive tension undergo cyclic interactions with actin. A summary plot of tension-pCa data from eight fiber segments is shown in Fig. 5. There are two main effects on this relationship as a result of partial extraction of the whole Tn complex. First, the tension-pCa relation at $P/P_o = 0.50$ shifted to the left by 0.25 pCa unit. Second, there was a decrease in the steepness of the relationship for $P/P_o < 0.7$. The Hill coefficient (n) after the partial extraction of Tn was 4.02, as compared with 8.55 in the same fibers before extraction. The steepness of the relationship for $P/P_o \geq 0.7$ was unaffected by Tn removal in that n was 1.81 after extraction vs. 1.86 in the untreated segments.

The tension-pCa relation obtained in untreated skinned fibers was quite steep at low levels of Ca^{2+} (Fig. 5). One possible explanation of this finding is that activation of a single Tn complex on the thin filament might activate (or partially

activate) adjacent functional groups with unoccupied low-affinity Ca^{2+} -binding sites. To test this possibility, Ca^{2+} -insensitive tension was measured as a function of Tn content in 23 additional fiber segments (Fig. 6A). The proportion of Tn removed from individual fibers was varied by altering the duration of the incubation. Prolonged incubations increased the amplitude of Ca^{2+} -insensitive

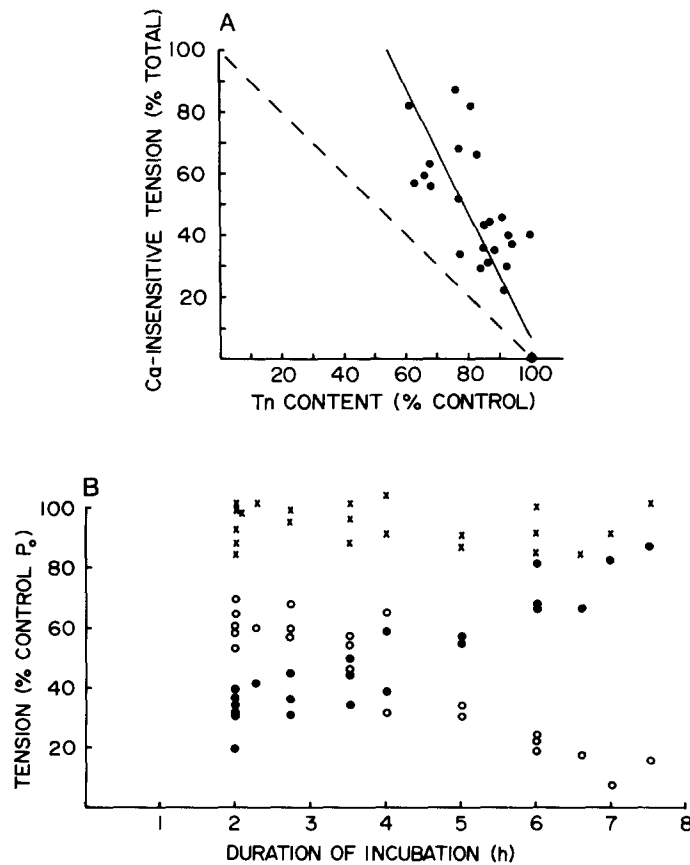


FIGURE 6. (A) Plot of Ca^{2+} -insensitive tension vs. Tn content. Tension at pCa 4.5 was first measured in untreated control segments. Various amounts of Tn were then removed by varying the duration of incubation and the tensions developed at pCa 9.0 and 4.5 were subsequently measured. Ca^{2+} -insensitive tension (measured at pCa 9.0) is expressed as a percent of the total tension (i.e., Ca^{2+} -sensitive plus Ca^{2+} -insensitive components) measured at pCa 4.5. The Tn content of these same fibers was determined by SDS-PAGE (Fig. 1), and is expressed relative to control values measured in segments from the same fibers. The solid line was fitted by least squares to include data from 23 control fibers (coordinates 100,0); all other points represent single determinations. (B) Ca^{2+} -sensitive (○) and -insensitive (●) tensions vs. the duration of protease treatment. The sum of these tensions for each fiber is also plotted (×). All tensions are scaled to the maximum Ca^{2+} -activated tension (i.e., P_0) measured in the same fibers before Tn removal. Data are from the same fibers as in A.

tension development and decreased the maximum Ca^{2+} -activated tension, with the sum of these two tensions being approximately equal to P_o in the untreated fiber (Fig. 6B). The long soaks used in this series of experiments resulted in a loss of some TnC from Tn complexes that were not removed during the incubation. For this reason, these fiber segments were bathed for 40 min in relaxing solution containing 1 mg/ml TnC (Moss et al., 1982) after the incubation and before measurement of the Ca^{2+} -insensitive active tension. (In the instances in which this tension was very high, the tension developed during TnC recombination could be very much reduced by substituting ATP γ S for ATP in the relaxing solution.) As the Tn content was lowered, the corresponding increase in Ca^{2+} -insensitive tension was disproportionately high in relation to the amount of Tn that was removed (Fig. 6A). Analysis of these data using linear regression yielded a correlation coefficient of 0.87. The largest Ca^{2+} -insensitive tension that we were able to achieve was 87% P_o and this only after an incubation period of 7.5 h. Nonetheless, extrapolation of the fitted line indicated that the Tn content would have to be lowered to ~54% of the control level in order to attain a Ca^{2+} -insensitive active tension equivalent to P_o .

DISCUSSION

The main finding of this study is that the removal of a small proportion of the whole Tn complexes from skinned skeletal muscle fibers results in a reversible increase in the Ca^{2+} sensitivity of tension development. On the basis of the results of studies involving preparations of isolated contractile and regulatory proteins, it is likely that extraction of a given Tn results in the permanent disinhibition of at least some of the actin monomers underlying the associated Tm molecule(s). The development of active tension in relaxing solution after Tn extraction (Figs. 2 and 4) is indicative of cross-bridge interactions with actin independent of Ca^{2+} activation. This view is corroborated by the finding that the sum of the Ca^{2+} -insensitive and maximal Ca^{2+} -activated tensions developed after partial Tn removal was in each fiber approximately equivalent to P_o measured before extraction (Fig. 6B). Thus, activation of some of the functional groups within the thin filament enhanced the Ca^{2+} sensitivity of tension development involving functional groups with Tn still bound.

The binding of Ca^{2+} is clearly not required to initiate this cooperative mechanism, since Tn removal was the method used to activate the first functional groups and these subsequently altered the Ca^{2+} sensitivity of the remaining groups. The detailed mechanism of this cooperativity has yet to be worked out, but it seems likely that it would involve movements of Tm that have been inferred from x-ray diffraction patterns obtained during tension development in living muscle (Huxley and Faruqi, 1983). As shown in Fig. 7, regions of interaction at the ends of adjacent Tm molecules (Smillie, 1979) could provide a means of communication through which the state of activation of one Tn-Tm (tropomyosin) complex could influence the Ca^{2+} sensitivity of neighboring complexes. In addition, it appears that there may be direct interactions between the TnT of one functional group and the Tm of the adjacent functional group (Fig. 7; Brisson et al., 1984).

Recently, Grabarek et al. (1983) showed that Ca^{2+} binding to the low-affinity sites of TnC in solution occurred with virtually no cooperativity; however, the incorporation of Tn into the thin filament resulted in cooperativity of Ca^{2+} binding at the low-affinity sites. A further enhancement of the apparent cooperativity of Ca^{2+} binding was observed in the presence of myosin, yielding a Hill coefficient (n) of 2.4. Such high values for n were used as a basis for suggesting the presence of cooperativity between Tn complexes in the binding of Ca^{2+} . Such an effect may be indeed mediated by the intervening Tm molecules, although Walsh et al. (1984) recently found no change in the cooperativity apparent in the Ca^{2+} dependence of the acto·subfragment-1 (S-1) ATPase when

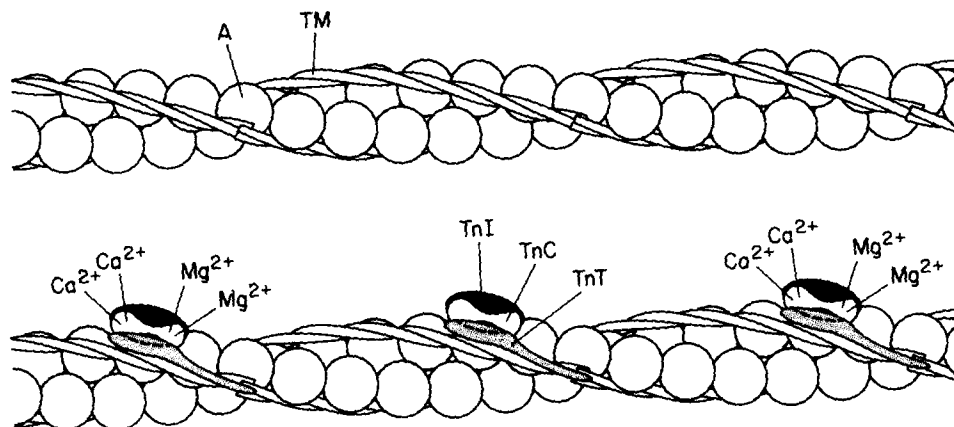


FIGURE 7. Diagrams of thin-filament structure illustrating the overlap of adjacent Tm molecules (upper) and the interaction of TnT with the neighboring Tm (lower). Modified from Ebashi et al. (1969).

communication between adjacent Tm molecules was interrupted. With regard to the present study, these results may suggest that the activation-induced enhancement of the Ca^{2+} sensitivity of tension development is due in part to cross-bridge binding within the first functional groups that were activated. Alternatively, the molecular mechanisms of cooperativity in the responses of tension and actomyosin ATPase activity to Ca^{2+} may very well be different because of the different ionic strengths used in the two types of measurements. Also, tension measurements are performed on fiber preparations in which the thick- and thin-filament lattices are intact, and this structure is absent from the solution biochemical measurements involving S-1 and regulated thin filaments.

The suggestion from our results that the regulation of tension development in skinned fibers involves cooperativity within the thin filament is consistent with recent biochemical studies in which strong cooperativity in the binding of myosin·S-1·ADP to regulated thin filaments was observed (Greene and Eisenberg, 1980). This cooperativity, while still clearly apparent, decreased in the presence of Ca^{2+} and when the concentration of S-1 was reduced. Good agreement with the binding data was obtained in molecular models that incorporated

nearest-neighbor interactions between Tm molecules (Greene and Eisenberg, 1980; Hill et al., 1983). These models suppose that Tm exists in either of two states, one characterized by weak and the other by strong binding of S-1 to actin. Interactions between adjacent tropomyosins vary with Ca^{2+} binding to the associated Tn complex. The strong binding state is favored by increasing the concentration of Ca^{2+} or, in in vitro biochemical systems, by increasing the concentration of S-1. The strong cooperativity that is apparent in the tension-pCa relation of fast-twitch skeletal muscle has been simulated in a kinetic model that includes a term to account for end-to-end Tm interactions (Hill, 1983).

In addition to the effects on Ca^{2+} sensitivity of tension development, partial Tn extraction was found to decrease the steepness of the tension-pCa relation for P/P_0 less than ~ 0.7 . Such an effect could be interpreted as reflecting a decrease in thin-filament cooperativity in the corresponding range of free Ca^{2+} concentrations. However, a decrease in the steepness of the relative tension-pCa relation is an expected result of such an experiment since the activation of a significant fraction of the functional groups within these fibers occurred independently of Ca^{2+} concentration. Thus, the lowered threshold of Ca^{2+} -activated tension development is presumably due to prior activation of a number of functional groups as a result of Tn removal.

The amplitude of the Ca^{2+} -insensitive active tension was found to increase as the Tn content of the fiber segments was reduced by increasing the duration of extraction (Fig. 6). The approximate linearity of this relationship suggests that an equal number of actin monomers are disinhibited for each Tn complex that is removed. Murray and Weber (1980) had previously proposed that the number of actin monomers disinhibited per functional group was smaller at low Ca^{2+} concentrations than at high in order to explain the relatively shallow slope of the relationship between myofibrillar ATPase and Ca^{2+} in the low- Ca^{2+} range. Although differences in the experimental variables measured may preclude direct comparison between our study and theirs, the disparity in results may derive from substantial differences in the ionic compositions of the solutions that were used, as discussed earlier. Also, disproportionately greater ATPase activities, relative to isometric tension, have been noted previously (Levy et al., 1977; Murray and Weber, 1980; Grabarek et al., 1983) and may indicate the existence of additional cooperative mechanisms that modulate the interaction of actin and myosin, but which do not involve the Ca^{2+} -binding proteins on the thin filament.

Extrapolation of the relationship between Ca^{2+} -insensitive active tension and Tn content indicates that only one-third to one-half of the functional groups along the thin filament need to be activated in order to activate the entire thin filament (Fig. 6). Brandt et al. (1984) recently proposed that the thin filament activated as a unit, based on their finding that a maximum decrease in the Ca^{2+} sensitivity of tension development could be achieved by extracting only 5% of the total TnC. Our results suggest that removal of a single Tn complex activates the associated functional group and perhaps the one or two nearest neighbors on either side. Longer-range interactions of functional groups activated by the removal of Tn (and presumably by the binding of Ca^{2+} in the intact thin filament) with other functional groups are certainly implicit in our finding that the Ca^{2+} sensitivity of tension development is enhanced by such activations.

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