NH$_2$-terminal Truncation of Skeletal Muscle Troponin T Does Not Alter the Ca$^{2+}$ Sensitivity of Thin Filament Assembly*

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To investigate how Ca$^{2+}$ binding to troponin C regulates muscle contraction, the Ca$^{2+}$-sensitive properties of thin filament assembly were studied as the tropomyosin binding, NH$_2$-terminal region of troponin T was progressively shortened. Troponin complexes were prepared that contained skeletal muscle troponin C, troponin I, and either intact troponin T (TnT) (residues 1–259) or fragment TnT-(70–259), TnT-(151–259), or TnT-(159–259). In the absence of Ca$^{2+}$ their respective affinities for pyrene-labeled tropomyosin were 2.3 × $10^{-7}$ m$^{-1}$, 1.2 × $10^{-7}$ m$^{-1}$, 1.9 × $10^{-7}$ m$^{-1}$, and 1.9 × $10^{-7}$ m$^{-1}$. Ca$^{2+}$ had only a small effect on these affinities: 1.1 × $10^{-7}$ m$^{-1}$ for whole troponin, 2 × $10^{-7}$ m$^{-1}$ for troponin-(151–259), and 2.8 × $10^{-7}$ m$^{-1}$ for troponin-(159–259). Forms of troponin that bound weakly to tropomyosin in the absence of actin increased the actin affinity of tropomyosin only 2–3-fold, even in the absence of Ca$^{2+}$; weak binding of troponin to tropomyosin correlated with weak effects on tropomyosin-actin binding. In contrast, whole troponin had an approximately 500-fold effect on tropomyosin binding to actin, regardless of whether Ca$^{2+}$ was present. The small effect of Ca$^{2+}$ on the energetics of thin filament assembly is not attributable to the amino-terminal region of troponin T. The results suggest that Ca$^{2+}$ causes the interaction between actin and the globular region of troponin to switch between two energetically similar states.

The regulation of muscle contraction is accomplished by the reversible binding of Ca$^{2+}$ to the thin filament protein troponin. Regulation is proposed to involve a Ca$^{2+}$-induced dissociation of one region of troponin from actin and from tropomyosin (Hitchcock, et al., 1973; Margossian and Cohen, 1973; Potter and Gergely, 1974; Pearlstone and Smillie, 1983; Tanokura and Ohtsuki, 1984; Ishii and Lehrer, 1991), thereby facilitating repositioning of tropomyosin on the actin filament (Lehman, et al., 1994). In particular, Ca$^{2+}$ weakens the actin binding of the troponin subunit, TnI, and the tropomyosin binding of the carboxyl-terminal region of TnT. In this widely held scheme of thin filament function, troponin remains anchored to the thin filament by relatively Ca$^{2+}$-insensitive binding of the amino-terminal region of TnT to the carboxy-terminal region of tropomyosin (Pato, et al., 1981; Mak and Smillie, 1981; Tanokura, et al., 1983; Cho and Hitchcock-DeGregori, 1990; Ishii and Lehrer, 1991). Indeed, rotary-shadowed electron micrographs of troponin and of TnT (Flicker, et al., 1982) show a highly extended molecule with a narrow tail mostly attributable to the amino-terminal portion of TnT (White, et al., 1987) and a distinctly more globular head region that is likely to include portions of all three subunits.

Consistent with the existence of a Ca$^{2+}$-insensitive anchor, we recently reported that the affinity of troponin for actin-tropomyosin remains tight (>$10^{-5}$ m$^{-1}$) regardless of whether Ca$^{2+}$ is present (Dahiya, et al., 1994). Perhaps more surprisingly, the troponin-tropomyosin complex bound much more tightly to actin than did tropomyosin alone, even in the presence of Ca$^{2+}$ or in the absence of TnI. Parallel results were found for the binding of troponin, tropolin-Ca$^{2+}$, or TnT to actin-tropomyosin. These data would appear to complicate models of regulation in which troponin has two sites of interaction with the thin filament: a Ca$^{2+}$-insensitive site primarily between tropomyosin and the elongated subunit TnT and a Ca$^{2+}$-reversible site that primarily involves actin-TnI binding and tropomyosin-TnT binding. Rather, although Ca$^{2+}$ may cause conformational changes in troponin that result in substantially changed binding to the thin filament, the net energetics of this binding are not significantly altered by Ca$^{2+}$.

This recent report was not the first to examine the effect of Ca$^{2+}$ on troponin binding to the thin filament. The new aspects included an attempt to exclude the contributions of cooperative effects in thin filament assembly by the application of a linear lattice model. Also, equilibrium linkage relationships were used to calculate the affinity of troponin (±Ca$^{2+}$) for actin-tropomyosin. In general this affinity has been too tight to directly measure, with or without a linear lattice approach. Thus, the conclusions of this report are dependent upon two caveats, that the assumptions of the linear lattice model are valid for calculating and excluding cooperative aspects of assembly and that the described equilibrium linkage relationships are correct.

An alternative approach is to examine the properties of the Ca$^{2+}$-sensitive region of troponin (TnI, TnC, and the carboxyl terminus of TnT), in the absence of the apparently more Ca$^{2+}$-insensitive tail region (i.e. the elongated amino-terminal portion of TnT). Because it lacks the Ca$^{2+}$-insensitive, anchoring portion of TnT, the effect of this truncated troponin complex on thin filament assembly may be very Ca$^{2+}$-sensitive. This can be tested by measuring the Ca$^{2+}$ dependence of the affinity constants involved in assembling thin filaments containing either troponin or truncated troponin. To accomplish this, troponin molecules were prepared containing intact TnT or one of several COOH-terminal fragments of TnT. Tropomyosin binding to pyrene-modified tropomyosin was weaker when TnT was truncated but was no more sensitive to Ca$^{2+}$. Whereas whole troponin promoted tropomyosin binding to actin about 500-fold, regardless of whether Ca$^{2+}$ was present, truncated troponin...
had a minimal effect on tropomyosin-actin binding, again regardless of whether Ca$^{2+}$ was present. These results have significance for how Ca$^{2+}$ regulates muscle contraction and for the participation of different regions of tropinin thin filament assembly and regulation.

**EXPERIMENTAL PROCEDURES**

Protein Preparation—Rabbit fast skeletal muscle actin, tropinin and troponin subunits were prepared as described previously (Hill et al., 1992). Tropinin was also reconstituted, and the ternary complex was purified (Tobacman and Lee, 1987) from purified rabbit Tnl, TnC, and rat fast skeletal muscle TnT-(151–259) or TnT-(70–259). These TnT fragments are numbered according to the rabbit sequence, and reconstitutions include either Met-151 to the COOH terminus or Met-70 to the COOH terminus. They were purified from DE3 (BL21) cells transformed with the pET8c expression vector (Studier et al., 1990) with the Tnl cDNA inserted at the NcoI/BamHI site (Hill et al., 1992). Construction of the TnT-70–259 expression plasmid, expression of the protein, and the protein purification procedure were described previously (Hill et al., 1992). To insert the cDNA encoding TnT residues 151–259 into the pET3d vector, a polymerase chain reaction fragment was generated using a vector-sequence primer and a primer overlapping the codon for Met-151 but including an NcoI site at this position. The restricted fragment was inserted into pET8c at the NcoI/BamHI sites. The complete coding sequence was confirmed by dideoxynucleotide sequencing (Sanger et al., 1977). Another form of truncated tropinin was obtained by controlled chymotryptic digestion of whole rabbit tropinin followed by purification of the ternary complex of TnC, Tnl and TnT-(159–259) (Morris and Lehrer, 1984; Hill et al., 1992). Cardiac tropominisin purified as described previously (Tobacman and Adelstein, 1986) and stoichiometrically labeled at Cys-190 either by carboxymethylation with [3H]iodoacetic acid (Amersham Corp.) (Hill et al., 1992) or by reaction with N-(1-pyrene)iodoacetamide (Molecular Probes) (Morris and Lehrer, 1994; Daihya et al., 1994). Bovine cardiac tropominisin approximately 90% of the $\alpha$ isoform, diminishing the heterogeneity that results when $\alpha/\beta$ skeletal muscle tropominisin is denatured for labeling and then reconstituted to a mixture of $\alpha/\beta$, $\alpha/\alpha$, and $\beta/\beta$ forms. However, we cannot exclude the possibility that slightly different results would have been obtained using skeletal muscle ($\alpha/\beta$) tropominisin.

Binding Assays—Binding of tropinin or truncated tropinin to pyrene-tropominisin was performed using an SLM 8000 spectrofluorometer. Water jacketed 2-ml samples were incubated in silenized cuvettes at 25°C in the presence of 10 mM Tris-HCl (pH 7.5), 3 mM MgCl$_2$, 0.1 mM dithiothreitol, either 0.5 mM EGTA or 0.1 mM CaCl$_2$, and either 60 mM or 300 mM KCl. Sequential aliquots of tropinin (or truncated tropinin) in the same buffer were added (maximum no more than 15% of initial volume) and the fluorescence intensity was monitored. Excitation was at 340 nm, and emission was at 405 nm. The effects of dilution on all concentrations were calculated. The best fit of each data set to simple 1:1 equilibrium binding was calculated as described previously (Daihya et al., 1994) using the curve-fitting program MINiSQ (MicroMath).

The effects of tropominisin or truncated tropinin on the binding of tropominisin to actin were measured by cosedimentation of radiolabeled tropominisin with actin (Hill et al., 1992). Conditions were as follows: 25°C, 10 mM Tris-HCl (pH 7.5), 3 mM MgCl$_2$, 0.1 mM dithiothreitol, 3–5 $\mu$M F-actin, 60 or 300 mM KCl, and either 0.5 mM EGTA or 0.1 mM CaCl$_2$. The concentrations of tropominisin and tropominisin varied for each experiment as indicated below. The actin-bound tropominisin was calculated from the difference between the total and the supernatant $^3$H-tropominisin after sedimentation for 30 min at 35,000 rpm using a TLA100 rotor in the TL100 ultracentrifuge. (In the absence of actin, there was no sedimentation.) Tropominisin was added in a constant molar excess compared with the tropominisin concentration, and the concentration of the tropominisin-tropominisin complex was calculated as described (Daihya et al., 1994). Using the affinity constants for the tropominisin-tropominisin complex calculated under the same conditions in the fluorescence experiments below. The binding of tropominisin-tropominisin to actin, as a function of the tropinin-tropominisin concentration, was analyzed as a linear lattice problem in which the ligand spans seven equivalent sites on the lattice (McGhee and Von Hippel, 1974; Tsuchiya and Szabo, 1982; Willadsen et al., 1992). Binding data are fit to obtain three parameters: the affinity, the lattice size, and the length. These parameters are calculated when binding involves cooperative interactions with one adjacent bound ligand, and the concentration of bound ligand at saturation.
designated troponin-(70–259), weakened its affinity for tropomyosin by approximately one-half. This modest effect suggests that these residues make little contribution to troponin-tropomyosin binding. This is consistent with evidence that CB3 (comprised of troponin T residues 1-70) does not bind to tropomyosin (Pearlstone and Smillie, 1982; White et al., 1987). In fact, deletion of TnT residues 1–45 causes troponin binding to immobilized tropomyosin to be slightly stronger rather than weaker (Pan et al., 1993).

On the other hand, the present data demonstrate much weaker troponin binding by a further truncation of troponin, troponin-(151–259), which includes rabbit TnC, rabbit TnI, and the carboxyl-terminal portion of (recombinant rat) TnT. The affinity of troponin-(151–259) for tropomyosin was 2 orders of magnitude less than the affinity of whole troponin for tropomyosin (Table I, Fig. 2). Because the binding was so weak, it could not be determined whether the fluorescence transition was attributable to a 1:1 association between troponin and troponin-(151–259). This weak binding (in comparison to whole, nontruncated troponin) was not due to the few amino acid differences between rat and rabbit TnT, nor to the presence versus the absence of reconstitution from subunits: indistinguishable results (Table I) were obtained using troponin-(159–259), produced by controlled chemotryptic digestion of rabbit whole troponin, which selectively removes TnT residues 1–158 from the ternary troponin complex.

One possibility from these data is that the interaction between troponin and the region of tropomyosin near Cys-190 (Ohtsuki, 1979; Pearlstone and Smillie, 1982; Chong and Hodges, 1982; Morris and Lehrer, 1984) is quite weak when whole troponin is present. Several pertinent observations support this interpretation. Electron microscopic images of tropomyosin-troponin polymers show that the globular head region of troponin is often distinctly separated from the tropomyosin filament (Flicker et al., 1982). Co-crystals of tropomyosin and troponin show that troponin is most ordered near the carboxyl terminus of tropomyosin and less ordered near tropomyosin residues 190–235 (White et al., 1987; White, 1988). Also, troponin has little effect on the local thermal unfolding of tropomyosin near Cys-190 (Ishii and Lehrer, 1991). Furthermore, although troponin causes prolongation of the fluorescence decay of IAEDANS attached to tropomyosin Cys-190, this prolonged component is only 20% of the total amplitude (Lamkin et al., 1983). Finally, the binding of the TnI-TnT-(159–259) complex to immobilized tropomyosin is weakened substantially by the addition of TnC, even in the absence of Ca$^{2+}$ (Pearlstone and Smillie, 1983).

Comparisons among the progressively truncated forms of troponin (Table I) show that removal of TnT residues 70–150 had a 60-fold effect on the binding constant, whereas removal of TnT residues 1–69 or 151–158 had little effect. This suggests that TnT residues 70–150 include the major region within the TnT “tail” that interacts with tropomyosin. These data show that ternary troponin complexes behave similarly to isolated troponin T fragments. The CB1 fragment of TnT (residues 71–151) binds readily to immobilized tropomyosin (Pearlstone and Smillie, 1977) and also binds to glutaraldehyde-fixed Bailey crystals of tropomyosin (White et al., 1987).

Notably, there is no detectable Ca$^{2+}$ sensitivity to the relatively weak binding of troponin-(159–259) to tropomyosin (151–259) (Table I). In the absence of the extended amino-terminal region of TnT, which binds to tropomyosin in a largely Ca$^{2+}$-insensitive manner, the binding of the remaining portion of troponin to tropomyosin is much reduced, yet it does not become more dependent upon the presence or absence of Ca$^{2+}$. This does not prove that Ca$^{2+}$ has no effect on troponin-tropomyosin interactions. However, it does imply that the net energetics of this interaction are not significantly altered by Ca$^{2+}$ even when the elongated tail region of TnT is absent. A possible explanation for this result is that, whether Ca$^{2+}$ is present or not, there is only a weak interaction between troponin and this region of tropomyosin.

Effect of Truncated Troponin on the Binding of Tropomyosin to Actin—Although truncated troponin binds weakly to tropomyosin, regardless of the Ca$^{2+}$ concentration, this interaction might be important when actin is also present. For whole cardiac troponin, we have recently shown there is only a 2-fold effect of Ca$^{2+}$ on troponin binding to actin-tropomyosin. To see if this effect is much larger for truncated troponin, which lacks the region believed to act as a Ca$^{2+}$-insensitive anchor, we investigated the coincident binding of tropomyosin and truncated troponin to actin. SDS-polyacrylamide gel electrophoresis analysis of sedimentation data obtained under the same conditions as in Fig. 1 (not shown) indicated that the amount of truncated troponin that cosedimented with actin-tropomyosin did not saturate at a 1:1:7 ratio of truncated troponin:tropomyosin:actin. Instead, the amount of pelleted truncated tropo-
Actin, even in the absence of Ca\(^{2+}\), cated troponin had little effect on the binding of tropomyosin demonstrated in Fig. 2. Furthermore (Fig. 3), cated troponin and is consistent with the weak tropomyosin-truncated troponin demonstrated in Fig. 2. Furthermore (Fig. 3), the thin filaments with the same stoichiometry as whole troponin was radioactively tagged, permitting measurement of its

Because of the weak affinity of tropomyosin-(159–259) for troponin (Table I), it was necessary to determine whether the results in Fig. 3A were attributable to inadequate concentrations of truncated troponin. Fig. 3B shows that this was not the case, since little change was seen at higher concentrations of truncated troponin. Fig. 3, A and B, shows a trend for truncated troponin to have a greater effect on tropomyosin-actin binding in the absence of Ca\(^{2+}\). Since the free actin concentration is nearly constant, one can estimate the effect of Ca\(^{2+}\) on the affinity of tropomyosin-truncated troponin for actin from the Ca\(^{2+}\)-induced change in the ratio of bound to free tropomyosin in the presence of 6 μM troponin-(151–259) or troponin-(159–259). (This calculation uses the last data point of each curve in Fig. 3B.) Removal of Ca\(^{2+}\) increased this ratio by a factor of three in each case, suggesting a 3-fold effect of Ca\(^{2+}\) on tropomyosin-truncated troponin binding to actin.

Equilibrium Linkage Analysis of Troponin and Tropomyosin Binding to Actin—The relevant comparisons for the effects of truncated troponin in Fig. 3 are the analogues of whole skeletal muscle troponin. More specifically, we sought to compare the effects of whole troponin and truncated troponin on thin filament assembly and to compare the effects of Ca\(^{2+}\) in each case. However, the properties of the two forms of troponin could not be studied under identical conditions. Truncated troponin has little interaction with actin or tropomyosin unless the ionic strength is relatively low (e.g. 60 mM KCl as in Fig. 2). The effects of whole troponin on thin filament assembly, on the other hand, require experimental conditions of higher ionic strength (300 mM KCl) to prevent troponin-tropomyosin polymerization (Hill et al., 1992). Fig. 4 shows a representative experiment to determine the affinity of troponin for pyrene-tropomyosin under these higher ionic strength conditions. In comparison to these data with relatively weak binding, the association constant is 25–30-fold higher in the presence of 60 mM KCl (summarized in Table I). The effect of Ca\(^{2+}\) is similar at both KCl concentrations.

Fig. 5 shows a representative experiment of troponin-tropo- myosin binding to actin. Troponin was added in a constant molar excess of the troponin concentration. The tropomyosin was radioactively tagged, permitting measurement of its

FIG. 3. Effect of truncated troponin on binding to tropomyosin to actin. The binding of \(^3\)H-tropomyosin to 3.5 mM F-actin was studied by cosedimentation using a tabletop ultracentrifuge, and conditions were as in Fig. 1. A, the concentrations of tropomyosin and troponin-(159–259) were varied in parallel so that the total concentration of truncated troponin was always 2 mM in excess of the total tropomyosin concentration. Open symbols, EGTA; filled symbols, CaCl\(_2\); circles, tropomyosin alone; triangles, tropomyosin plus troponin-(159–259). In the absence of truncated troponin, there is no effect of CaCl\(_2\) on the free (i.e. non-actin-bound) tropomyosin concentration required for half saturation of the actin. Truncated troponin had a small effect on the apparent affinity, most notably in the absence of CaCl\(_2\), when the apparent \(K_a\) was shifted to about 0.1 mM, as compared with 0.2–0.3 mM for tropomyosin under these higher ionic strength conditions. In other words, require experimental conditions of higher ionic strength (300 mM KCl) to prevent troponin-tropomyosin polymerization (Hill et al., 1992). Fig. 4 shows a representative experiment to determine the affinity of troponin for pyrene-tropomyosin under these higher ionic strength conditions. In comparison to these data with relatively weak binding, the association constant is 25–30-fold higher in the presence of 60 mM KCl (summarized in Table I). The effect of Ca\(^{2+}\) is similar at both KCl concentrations.

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free and actin-bound concentrations. Comparing the filled and open symbols in Fig. 5, it can be seen that removal of Ca\(^{2+}\) strengthens binding of troponin-tropomyosin to actin about 2-fold, similar to the results with truncated troponin (Fig. 3). The Fig. 3 results can only be analyzed qualitatively because of uncertainty in the stoichiometry of truncated troponin concentration. Two representative data sets are shown, either in the presence of EGTA (C) or in the presence of CaCl\(_2\) (●). The concentration of free troponin-tropomyosin was calculated from the measured, non-actin-bound concentration of \(^{3}H\) tropomyosin and the binding constants in Table I. The theoretical curves correspond to \(K_0 = 1.8 \times 10^8\) M\(^{-1}\) and \(y = 29\) in the presence of CaCl\(_2\) and to \(K_0 = 2.2 \times 10^8\) M\(^{-1}\) and \(y = 44\) in the presence of EGTA. \(K_0\) is the affinity of troponin-tropomyosin (or troponin-tropomyosin-Ca\(^{2+}\)) for an isolated site on F-actin in the absence of any interaction with other troponin-tropomyosin complexes. \(y\) is a cooperativity parameter, and the product \(K_0y\) is approximately equal to the apparent overall binding constant.

The advantage of this analysis is that it permits calculation of the affinity of troponin for actin-tropomyosin, using the thermodynamic linkage relationships schematically shown in Fig. 6. It should be noted that there is a potential error in this calculation, because pyrene-tropomyosin may have different properties than \(^{3}H\) tropomyosin. Fluorescence competition studies (Dahiya et al., 1994) suggest there are no major differences between them, at least at low ionic strength.

Significantly, whereas truncated troponin has a weak, difficult to quantify effect on the binding of tropomyosin to actin, whole skeletal muscle troponin has a very large effect (Fig. 6). The figure shows obligate equilibrium linkage relationships in the associations of tropomyosin and troponin for an isolated site on F-actin. Note that the actin binding constants do not depend upon cooperative effects (this is required for this scheme to be valid and is one of the measurements that results from the linear lattice analysis of actin binding data) and therefore differ from apparent binding constants (1/\(K_0\)) that partially depend upon cooperative binding of tropomyosin or troponin-tropomyosin to actin. The average affinity of troponin-tropomyosin for an isolated site on F-actin (determined from several experiments similar to those in Fig. 5) is \(3.4 \pm 1.0 \times 10^5\) M\(^{-1}\) in the absence of Ca\(^{2+}\) and \(2.6 \pm 0.8 \times 10^5\) M\(^{-1}\) in the presence of Ca\(^{2+}\). Regardless of whether Ca\(^{2+}\) is present, troponin binding to tropomyosin causes its affinity for actin to increase about 500-fold. This is very different from the small effects of truncated troponin on tropomyosin-actin binding seen in Fig. 3, despite concentrations of truncated troponin that would be expected to produce, based upon Table I and in the absence of actin, significant formation of the tropomyosin-truncated troponin complex. Fig. 6 also shows the affinity of troponin for an isolated actin-tropomyosin site, as indirectly calculated from the equilibrium linkage relationships. This affinity is too tight to measure directly, whether Ca\(^{2+}\) is present (2 \(\times 10^5\) M\(^{-1}\)) or absent (4 \(\times 10^5\) M\(^{-1}\)).

**Discussion**

The data in this report suggest the following. 1) Troponin greatly promotes the binding of individual tropomyosin molecules to actin, regardless of the Ca\(^{2+}\) concentration. This effect depends upon the presence of TnT residues 1–150. More narrowly, the important region for this effect consists of residues 70–150, since tropomin-(70–259) promotes tropomyosin-actin binding almost as well as does whole tropomin (Hill et al., 1992). 2) The amino-terminal region of TnT, especially residues 70–150, is required for tight association of tropomin to tropomyosin. These results are consistent with observations involving TnT fragments, as opposed to the ternary tropomin complexes in the present work. Also, Morris and Lehrer (1984) found that tropomin-(159–259) bound more weakly to tropomyosin than did intact troponin. In a separate study of TnT fragments, Ishii and Lehrer (1991) concluded that it was the NH\(_2\)-terminal rather than the COOH-terminal region of TnT that binds most...
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tightly to actin. Our results more narrowly suggest the importance of the TnT-(70–150) region but do not by themselves allow any conclusion comparing this region with the tropinin (159–259) region. 3) There is a direct relationship between the strength of tropinin binding to tropomyosin (as the amino-terminal region of TnT is progressively deleted or included), and the ability of tropinin to enhance tropomyosin-actin binding. Binding of tropinin-(151–259) or tropinin-(159–259) to tropomyosin had only a small effect on the affinity of the resultant complex for actin, regardless of the Ca$^{2+}$ concentration. One possible explanation is that, unlike whole tropinin, these truncated tropinons do not interact simultaneously with both actin and tropomyosin; tropinin-(159–259) is primarily an actin-binding portion of tropinin and TnT-(1–150) is the primary tropomyosin-binding portion. Alternatively, it is possible that the weak effect of truncated tropinin on thin filament assembly occurs because deletion of TnT residues 1–150 has a detrimental effect on the stability and function of the remainder of the tropinin complex.

Ca$^{2+}$ binding to the thin filament results in a structural change generally considered to include a repositioning of tropinin on actin (Haselgrove, 1972; Huxley, 1972; Parry and Squire, 1973; Lehman et al., 1994). This event rapidly follows Ca$^{2+}$ release, even in the absence of cross-bridge binding (Kress et al., 1986). Although cross-bridges also affect thin filament conformation and cooperative cross-bridge effects may be important in muscle activation (Lehrer, 1994), it is clear that Ca$^{2+}$ alone has a major effect on thin filament structure. Despite progress on the Ca$^{2+}$-induced conformational change in TnC at high resolution (Herzberg et al., 1986; Gagné et al., 1994), the absence of an atomic structure for whole tropinin has hampered determination of how this change in TnC is transmitted to the remainder of the thin filament. At present, the best supported idea (reviewed in Leavis and Gerety, 1984; Zott and Potter, 1987; Chalovich, 1992) is that TnI interacts with actin in the presence of low Ca$^{2+}$ concentrations and, because TnI is part of a ternary tropinin complex that is bound to tropomyosin, the position of tropomyosin on the actin filament is thereby constrained. Ca$^{2+}$ binding to TnC releases this constraint by facilitating the interaction between TnI and TnC, which disrupts the TnI-actin binding. Indeed, fluorescence resonance energy transfer data support a Ca$^{2+}$-induced movement of TnI relative to actin within the thin filament (Tao et al., 1990). Furthermore, a specific TnI peptide has been shown to interact with either Ca$^{2+}$-TnI or with actin and to inhibit actin-myosin interactions (Syska et al., 1976).

It is worthwhile to consider how the present work relates to this model for regulation. Simply put, the small effect of Ca$^{2+}$ summarized in Fig. 6 is inconsistent with the idea that the action of Ca$^{2+}$ can be understood as a release of TnI from actin. This small energetic change in tropinin-thin filament binding is not plausibly consistent with disruption of an actin-TnI protein-protein interface. Rather, a Ca$^{2+}$-induced release of TnI-actin binding can only be occurring if some other interactions (between tropinin and the thin filament) are strengthened by Ca$^{2+}$. A recent publication (Dahiya et al., 1994) reached a similar conclusion regarding cardiac thin filament assembly and suggested TnT as one possibility for a Ca$^{2+}$-strenthened interaction with the thin filament, perhaps via the direct TnI-actin binding that was demonstrated by Heeley and Smillie (1988). Also, it was shown that an increased cooperative interaction between adjacent cardiac tropinin-tropomyosin complexes did not occur on Ca$^{2+}$ binding, so one should look primarily within each tropinin-tropomyosin-7 actin region for the thermodynamic changes accompanying Ca$^{2+}$ binding to TnC. This present work shows similar behavior for skeletal muscle tropinin, which is the proteing of interest for the data supporting the model of TnI-mediated regulation. More significantly, the present data suggest that the small effect of Ca$^{2+}$ on thin filament assembly (Fig. 6) is probably not attributable to compensatory effects involving the amino-terminal region of TnT. Removal of this region weakened tropinin’s interaction with tropomyosin, but did not increase the Ca$^{2+}$ sensitivity of thin filament assembly.

The above arguments suggest that, within the intact thin filament, Ca$^{2+}$ has little effect on the energetics of the interaction between actin-tropomyosin and the TnC-TnI-TnT-(159–259) region of tropinin. However, it is hard to imagine how regulation could be accomplished without Ca$^{2+}$ causing major alterations in the interface between this region of tropinin and actin-tropomyosin. It is likely that many specific interactions are altered, even if the net energetics are little changed, and that different actin and tropinin and tropomyosin are involved in these interactions in the presence as opposed to the absence of Ca$^{2+}$. The identification of these residues by mutagenesis of actin and tropinin may prove a useful avenue for future studies.

REFERENCES

Chalovich, J. M. (1992) Pharmacol. & Ther. 55, 95–148
Chao, Y. J., Liu, J., and Hitchcock-DeGregori, S. E. (1990) J. Biol. Chem. 265, 538–545
Chong, P. C. S., and Hodges, R. S. (1982) J. Biol. Chem. 257, 9152–9160
Dahiya, R., Butters, C. A., and Tobacman, L. S. (1994) J. Biol. Chem. 269, 24957–24961
Flicker, P. F., Phillips, G. N., and Cohen, C. (1982) J. Mol. Biol. 162, 495–501
Gagné, S. M., Tsuda, S., Li, M. X., Chandra, M., Smillie, L. B., and Sykes, B. D. (1994) Protein Sci. 3, 1903–1913
Haselgrove, J. C. (1972) Cold Spring Harbor Symp. Quant. Biol. 37, 225–234
Heeley, D. H., and Smillie, L. B. (1988) Biochemistry 27, 8227–8232
Herzberg, O., Moult, J., and James, M. N. G. (1986) J. Biol. Chem. 261, 2638–2644
Hill, L. E., Mehegan, J. P., Butters, C. A., and Tobacman, L. S. (1992) J. Biol. Chem. 267, 16106–16113
Hitchcock, S. E., Huxley, H. E., Szent-Gyorgyi, A. G. (1973) J. Mol. Biol. 80, 825–836
Huxley, H. E. (1972) Cold Spring Harbor Symp. Quant. Biol. 37, 361–367
Ingram, R. H., and Swenson, C. A. (1985) Biochemistry 24, 5221–5225
Ishii, Y., and Lehrer, S. S. (1991) Biopolymers 26, 6894–6903
Kress, M., Huxley, H. E., and Faruq, A. R. (1986) J. Mol. Biol. 188, 325–342
Lamkin, M., Tao, T., and Lehrer, S. S. (1983) Biochemistry 22, 3053–3058
Leavis, P. C., and Gerety, J. (1984) CRC Crit. Rev. Biochem. 16, 235–305
Lehrer, C. S. (1990) J. Muscle Res. Cell Motil. 15, 232–236
Mak, A. S., and Smillie, L. B. (1981) J. Biol. Chem. 256, 543–550
Margeolian, S. S., and Cohen, C. (1973) Can. J. Biochem. 51, 409–413
McGhee, J. D., and von Hippel, P. H. (1974) J. Mol. Biol. 86, 469–489
Morris, E. P., and Lehrer, S. S. (1984) Biochemistry 23, 2214–2220
Ohtsuki, I. (1984) J. Biol. Chem. 259, 491–497
Parry, D. A., and Squire, J. M. (1973) J. Biol. Chem. 248, 6955–6960
Pearlstone, J. R., and Smillie, L. B. (1983) Biochemistry 22, 469–489
Pearlstone, J. R., and Smillie, L. B. (1981) J. Biol. Chem. 256, 602–607
Pearlstone, J. R., and Smillie, L. B. (1977) J. Biol. Chem. 252, 354–355
Pearlstone, J. R., and Smillie, L. B. (1982) J. Biol. Chem. 257, 331–337
Tsuchiya, T., and Szabo, A. (1982) Biopolymers 21, 979–994
White, S. P., Cohen, C., and Phillips, G. N. (1987) Biopolymers 26, 185–190
Annu. Rev. Biophys. Chem. 23, 535–559
Biochemistry 24, 5221–5225
Nature 266, 2327–2342
Biochemistry 25, 798–802
J. Biol. Chem. 260, 4694–4694
Tocris, T., and Szabo, A. (1982) Biopolymers 21, 979–994
White, S. P., Cohen, C., and Phillips, G. N. (1987) Nature 325, 826–828
White, S. P. (1989) Structure of Co-crystals of Troponin Tropomyosin and Phd, dissertation, University of Illinois at Urbana-Champaign
Willadsen, K. A., Butters, C. A., Hill, L. E., and Tobacman, L. S. (1992) J. Biol. Chem. 267, 23746–23752
Zot, H. G., and Potter, J. D. (1987) Annu. Rev. Biophys. Chem. 16, 535–559