Modulation of Contractile Activation in Skeletal Muscle by a Calcium-insensitive Troponin C Mutant*

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Calcium controls the level of muscle activation via interactions with the troponin complex. Replacement of the native, skeletal calcium-binding subunit of troponin, troponin C, with mixtures of functional cardiac and mutant cardiac troponin C insensitive to calcium and permanently inactive provides a novel method to alter the number of myosin cross-bridges capable of binding to the actin filament. Extraction of skeletal troponin C and replacement with functional and mutant cardiac troponin C were used to evaluate the relationship between the extent of thin filament activation (fractional calcium binding), isometric force, and the rate of force generation in muscle fibers independent of the calcium concentration. The experiments showed a direct, linear relationship between force and the number of cross-bridges attaching to the thin filament. Further, above 35% maximal isometric activation, following partial replacement with mixtures of cardiac and mutant troponin C, the rate of force generation was independent of the number of actin sites available for cross-bridge interaction at saturating calcium concentrations. This contrast with the marked decrease in the rate of force generation when force was reduced by decreasing the calcium concentration. The results are consistent with hypotheses proposing that calcium controls the transition between weakly and strongly bound cross-bridge states.

The cyclic interaction of myosin and actin produces force and shortening in contractile cells. In muscle fibers, actin and myosin interaction is regulated by the intracellular calcium concentration acting through the thin filament regulatory proteins, the troponin complex, and tropomyosin. Until calcium binds to the troponin complex, the muscle fiber remains relaxed with >95% of the myosin cross-bridges detached (1, 2). The influx of calcium into the filament lattice of muscle fibers stimulates the association of actin and myosin enabling the production of force or shortening and accelerating the actomyosin ATPase rate by >100-fold during isometric contraction.

Several models have been postulated to account for this control. The steric-blocking model of cross-bridge regulation asserts that tropomyosin/troponin (Tm/Tn) prevents cross-bridge attachment in the absence of calcium by “blocking” cross-bridge access to binding sites on the thin filament (3, 4). Alternatively, the kinetic regulation model assumes that the cross-bridges can, under all conditions, bind weakly to the thin filament, and calcium controls the kinetics of cross-bridge turnover via changes in the weakly bound to strongly bound cross-bridge transition (5, 6). More recently, three-dimensional reconstructions of electron micrographs have identified three distinct structural states of the thin filament (7–9). In the absence of calcium, tropomyosin blocks strong myosin binding sites on actin. Following Ca2+ binding to the troponin complex, the tropomyosin shifts away from the myosin binding sites but does not completely expose all the putative strong binding sites on actin. Further movement of the tropomyosin requires strong cross-bridge binding to fully expose the myosin binding sites. Full activation of the thin filament requires Ca2+ binding to the troponin complex and subsequent strong cross-bridge binding to the thin filament.

Brenner and Eisenberg (10) developed a method to measure the kinetics of cross-bridge transitions from weakly bound to force-producing states in activated muscle fibers and found the rate of tension development (ktr) to be calcium-sensitive (6). This result is inconsistent with the steric-blocking mechanism, and Brenner (6) suggested that Tm/Tn controlled the rate of P1 release. However, subsequent work showed that the kinetics of P1 release are independent of [Ca2+] (11–14). Further, others have found the kinetics of cross-bridge cycling to be unaffected by compounds that affect thin filament dynamics (15). Taken together, these studies indicate that calcium is regulating muscle activation by control of cross-bridge access. To reconcile these observations with Brenner’s data and hypothesis it was proposed that [Ca2+] controls the transition from weak to strong cross-bridge binding preceding the generation of force (11, 16).

To this point, studies have investigated calcium regulation of muscle contraction by adding various compounds, removing proteins, or adjusting the free calcium concentration. These investigations have left several issues unresolved. In particular, when the free calcium concentration rises, it is unclear how this increases the rate of force generation. Does the effect require a relatively high density of myosin binding to actin, which tends to activate the thin filament, or does calcium binding to troponin have a more direct effect on ktr?

In the present study we describe a method to control the fraction of troponin complexes to which calcium is bound, thereby also controlling the fraction of the thin filament available for myosin binding while maintaining the free calcium troponin C; CBMII, cardiac binding mutant (site II); REL, relaxing solution; ktr, rate of tension redevelopment; PAGE, polyacrylamide gel electrophoresis; kN, kilonewton; cTnC, cardiac TnC; sTnC, skeletal TnC; a, actin; m, myosin.

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§ The abbreviations used are: Tn, tropomyosin; Tn, troponin; TnC, troponin C; CBMII, cardiac binding mutant (site II); REL, relaxing solution; ktr, rate of tension redevelopment; PAGE, polyacrylamide gel electrophoresis; kN, kilonewton; cTnC, cardiac TnC; sTnC, skeletal TnC; a, actin; m, myosin.
concentration constant at a high level. The native skeletal TnC was extracted from thin filaments of skinned muscle fibers and replaced with variable combinations of cardiac TnC and an inactive cardiac mutant TnC, CBMII TnC (17, 18). Cardiac TnC has a single regulatory calcium binding site (site II) as site I was held at a level determined by the CBMII TnC content.

Isometric tension and the rate of force redevelopment were measured in muscle fibers following TnC replacement with ratios of cTnC and CBMII TnC. The data show that isometric force is directly proportional to the number of active thin filament units (A<sub>TnTn</sub>) at saturating calcium, and the rate of force redevelopment is unaffected by a reduction in crossbridge number. The results suggest that k<sub>0</sub> is primarily controlled by calcium binding to troponin rather than the density of cross-bridges binding to the actin filament. Together, the results suggest that calcium controls cross-bridge access to the thin filament by regulating an equilibrium between weakly (non-productively) and strongly (productively) bound, but non-force-bearing cross-bridges. The present work accounts for the discrepancies between the opposing kinetic and steric-blocking models of thin filament regulation. A preliminary report of this work was published previously (22).

**EXPERIMENTAL PROCEDURES**

**Solutions**—All fiber solutions contained 100 mM N,N-bis[2-hydroxyethyl]-2-aminoethanesulfonic acid (BES), pH 7.1, at 15 °C, 5 mM MgATP, 1 mM magnesium acetate, 20 mM potassium acetate, 15 mM creatine phosphate, 200 units/ml creatine phosphokinase, and 1 mM dithiothreitol with an ionic strength of 200 mM. Relaxing (REL) and activating solutions also contained 20 mM EGTA. Calcium was added as Ca<sup>2+</sup>-EGTA and Ca<sup>2+</sup>-EDTA, 20 mM Tris-HCl (pH 7.2), and 0.5 mM trifluoperazine dihydrochloride at 15–17 °C (24). The fibers were incubated until the Ca<sup>2+</sup>-activated isometric tension fell below 10% P<sub>0</sub>, generally within 10 min. The fibers were reconstituted by incubation in REL containing 0.5 mg/ml TnC for 1 min followed by a wash in REL for 30 s. This was repeated until Ca<sup>2+</sup>-dependent tension reached a maximal, constant value. Skeletal TnC was kindly provided by Marion Greaser (University of Wisconsin) and purified as described by Greaser and Gergely (25). Cardiac TnC (cTnC and CBMII TnC) were isolated as described (18).

**SDS-PAGE**—The extraction and reconstitution of the troponin C was quantified using SDS-PAGE. Muscle fibers containing sTnC, cTnC, or ratios of CBMII TnC/cTnC were placed in sample buffer, heated to 95 °C for 3 min, and sonicated to denature and solubilize the muscle fibers. The samples were loaded onto a 12% Tris-HCl separating gel. Following electrophoresis, the gels were stained using the silver stain technique of Guilian et al. (26) with minor modifications. After staining, the gels were dried and scanned. The apparent molecular mass of CBMII TnC is 1 kDa <em>&lt;</em> purified cTnC, allowing quantitative separation of the two proteins by their mobilities. Gels were analyzed using a GS-700 scanning densitometer (Bio-Rad) normalizing bands to the area under the myosin light chain 1 peak. The relative content of cTnC and CBMII TnC was determined from the ratio of cTnC/cTnC + CBMII TnC) after accounting for differences in staining intensity and a background peak at the same position as CBMII TnC.

**RESULTS**

**Extraction/Replacement of TnC**—To investigate the effects of cardiac and CBMII TnC replacement on muscle function, it was necessary to effectively remove the native sTnC from the muscle fibers. Extraction reduced the Ca<sup>2+</sup>-dependent force to 8.1% (±1.1) of the maximal force obtained at pCa 4.5 (P<sub>0</sub> = 146.7 (±3.8) kN/m<sup>2</sup>, n = 45). Extravention of the TnC and subsequent reconstitution with purified sTnC returned maximal Ca<sup>2+</sup>-dependent force to 125.5 ± 10.3 kN/m<sup>2</sup> or 86 ± 3% P<sub>0</sub> (n = 5). Reconstitution with cTnC returned maximal isometric force to 96.9 (±7.4) kN/m<sup>2</sup> (n = 17) or 65.4% (±5.0) of that observed prior to extraction.

It was important to determine whether the cardiac TnC or...
CBMII TnC preferentially binds to the fiber thin filaments under the extraction/replacement procedure. The difference in the apparent molecular weights of cTnC and CBMII TnC made quantification possible. Fig. 1 shows two lanes (A) and the associated profiles (B) of a gel containing fiber segments reconstituted with 100% cTnC and 50% cTnC:50% CBMII TnC. Determination of the relative proportion of cTnC in fibers reconstituted with various ratios of cTnC and CBMII TnC as a function of the relative amount of cTnC added to the reconstitution solution is shown in Fig. 2. The results demonstrate that the binding of cTnC and CBMII TnC to the thin filaments of fibers is similar under the reconstitution conditions used (pCa 9.0), which is consistent with the binding studies performed using cTn and CBMII Tn complexes (18).

**Steady-state Isometric Tension**—As CBMII TnC is a cardiac TnC mutant, it was necessary to determine the effects on the isometric tension and the calcium sensitivity in fibers after extraction of native TnC and replacement with cTnC. The difference in calcium sensitivity between the control fibers containing native sTnC and those after cTnC replacement is shown in Fig. 3. Following replacement of sTnC with cTnC the Hill coefficient was reduced from 2.83 (±0.11) to 1.95 (±0.13) and the pCa50 shifted from 6.72 (±0.01) to 6.64 (±0.02); both changes were significant (p<0.05). The reduction in the Hill coefficient and shift in pCa50 after replacement with cTnC have been reported previously (27, 28). Extraction of endogenous sTnC and replacement with purified sTnC produced no significant differences (p<0.01) in the Ca2+ sensitivity (nH = 2.87 (±0.43); pCa50 = 6.74 (±0.03); n = 5).

Isometric force is believed to be dependent on the number of cross-bridges attached to the thin filament (29–31). To determine whether reduction in the level of thin filament activation directly correlates with a decrease in the number of thin filament sites, fibers were reconstituted with various ratios of CBMII:cTnC, and the isometric tension was measured. Fig. 4 demonstrates that tension fell in direct proportion to the reduction in cTnC content of the fiber. Because the cTnC content added correlated well with the amount of cTnC bound to the thin filaments (Fig. 2), the cTnC content is given as the ratio of cTnC added. The tension measured after replacement with CBMII:cTnC was normalized against the average maximal force produced after replacement with 100% cTnC (65.4% of Po50%; p<0.01). The direct proportionality of the force reduction to the fractional content of cTnC suggests that there is little cooperativity between the steady-state isometric force and the number of attached cross-bridges at saturating [Ca2+].

**The Rate of Tension Redevelopment (ktr)—**The rate of tension redevelopment (6) is controlled by [Ca2+]i, which implies regulation of a cross-bridge transition involving force generation (11, 12, 32). Representative traces of ktr, as a function of pCa, are shown in Fig. 5A. It is evident that the Ca2+ sensitivity of force redevelopment is affected by the troponin C isoform and the number of attached cross-bridges at saturating [Ca2+].

![Fig. 2. Relative cTnC content of fibers reconstituted with various ratios of cTnC:CBMII TnC. Each point represents a single fiber segment analyzed as shown in Fig. 1 and described under “Experimental Procedures.” Analysis was performed on three separate gels. The solid line indicates the linear regression through the data and the dashed lines represent the 95% confidence levels. Linear regression of the data yields a line (R^2 = 0.93) with a slope of 0.887 ± 0.1 and a y intercept of 0.076 ± 0.069.](image-url)
Hill equation (see “Experimental Procedures”) with $k_v$ obtained were normalized against the maximum force produced by the cardiac TnC.

Prior studies have reduced the fraction of the thin filament in skinned muscle fibers. The most straightforward, although not the only, interpretation of Fig. 4 is that under conditions of saturating calcium ($pCa$ 4.5), CBMII TnC replacement of cTnC limits the number of cross-bridges capable of binding to the thin filament, and the isometric force decreases in direct proportion to the reduction in active thin filament regulatory units. The force-$pCa$ relationship in skinned fibers is more cooperative than can be explained by Ca$^{2+}$ binding to individual troponin subunits. If this were the only cooperative mechanism involved, the maximal $n_{H}$ would be 1.0 for cardiac and 2.0 for skeletal TnC (19). Adjacent troponin subunits are connected by troponyosin, and enhancement of Ca$^{2+}$ binding via cooperative strong cross-bridge binding may influence the degree of thin filament activation and therefore the mechanism of regulation (21, 38, 39).

The results obtained here present a clearer picture of what occurs during force generation in isometrically contracting skinned muscle fibers. The most straightforward, although not the only, interpretation of Fig. 4 is that under conditions of saturating calcium ($pCa$ 4.5), CBMII TnC replacement of cTnC limits the number of cross-bridges capable of binding to the thin filament, and the isometric force decreases in direct proportion to the reduction in active thin filament regulatory units. Isometric force, a steady-state measurement, is linearly dependent on the number of thin filament actin monomers available for cross-bridge interaction. This suggests that reduction in the number of active thin filament units, by either lowering [Ca$^{2+}$] or increasing the proportion of CBMII/TnC, directly limits the number of cross-bridges capable of binding to the thin filament.

The relationship between force and Ca$^{2+}$ binding is linear (Fig. 4) and therefore seems unaffected by cooperative interac-
tions between adjacent regulatory units along the length of the thin filament. Either a concave or a convex relationship between the fractional occupancy and the isometric force would be evidence for such cooperativity but was not observed. The absence of this behavior does not prove, however, that force generation is unaffected by longitudinal cooperativity along the thin filament. One reason for this is that the linear behavior in Fig. 4 may reflect a balance of compensating cooperative effects in which regulatory units with calcium induce myosin binding on adjacent units and units without calcium restrict myosin binding on adjacent units. Each phenomenon has been reported in other types of experiments with partial extraction of either whole troponin (40) or TnC (35). Further, any non-linearity between the fractional Ca\(^{2+}\) binding and isometric force would be difficult to detect for thin filament occupancies less than 25% and therefore cannot be excluded with the present data. Finally, the use of CBMII TnC precludes a possible source of cooperativity that can occur in normal thin filaments; cross-bridge binding to a regulatory unit where Ca\(^{2+}\) is bound may induce Ca\(^{2+}\) binding on adjacent regulatory units (21). Despite these caveats about an underlying complexity in the system, the linear results in Fig. 4 imply that Ca\(^{2+}\) is controlling the steady-state isometric force by limiting cross-bridge access to the thin filament.

Is a similar mechanism at work during transient events (e.g. \(k_{tr}\)) in the muscle fiber? In this study, we tested whether \(k_{tr}\) is dependent on [Ca\(^{2+}\)] or the number of cross-bridges attached to the thin filament. Brenner (6) showed that the rate of tension redevelopment is highly dependent on the [Ca\(^{2+}\)] with a non-linear decline in \(k_{tr}\) as calcium levels were reduced and suggested that regulation occurred during a weak to strong transition. If Ca\(^{2+}\) specifically controls the transition from a weakly bound to a strongly bound, force-generating state, then \(k_{tr}\) and \(k_{Pi}\), the rate of the tension decline following photogeneration of Pi from caged-Pi, would be the same. However, measurements of \(k_{tr}\) revealed little or no Ca\(^{2+}\) dependence (11, 12, 14, 16) even though \(k_{tr}\) measured in the same preparation exhibited a strong dependence on [Ca\(^{2+}\)]. In the present study, decreasing the number of force-generating cross-bridges reduced steady-state tension but did not greatly affect the rate...
of force generation until the level of thin filament activation was less than \(-35\%\). These results are consistent with the hypothesis that \([\text{Ca}^{2+}]\) controls a cross-bridge transition preceding force generation, proposed to be a transition from a weakly bound to a strongly bound, non-force-bearing cross-bridge state (11, 14, 16, 41).

If \(k_{tr}\) actually measures a two-step process as suggested, (i.e. a weakly to strongly bound transition followed by the force-generating isomerization or \(P_i\) release), the differing effects of CBMII or decreasing the calcium concentration on cross-bridge function can be explained by the model described below.

In this model, cross-bridges are detached or weakly attached (W), strongly attached but not generating force (S), or strongly attached and generating force (F). The weakly attached states (M-ATP, M-ADP-P_i, or A-M-ADP-P_i) attach and detach from the actin filament rapidly and do not sustain significant force (the hyphen indicates a weakly attached state). The strongly attached state (AM-ADP-P_i) does not generate force. Entry into the strongly bound state (S) involves a thin filament isomerization controlled by troponin and tropomyosin with the forward rate \(k_{-1}\) (increased by elevations in \([\text{Ca}^{2+}]\)) and the reverse rate \(k_{-1}\). The strongly attached and force-exerting state (F), AM-ADP (and its isomers), are generated by an isomerization and/or the release of \(P_i\) from the strongly bound AM-ADP-P_i state controlled by \(k_{-2}\) and \(k_{-2}\). The rigor cross-bridge, AM, is also a strongly bound, force-exerting cross-bridge. The rate of force-generating cross-bridge detachment to the detached/weakly attached cross-bridges (W) is defined by \(k_{tr}\) and is slow (2–4 s\(^{-1}\)) under isometric conditions as estimated from the steady-state isometric ATPase rate (6). At low [P_i] (~1 mm), \(k_{-2}\) and \(k_{-2}\) are \(-20\) s\(^{-1}\) and \(3\) s\(^{-1}\), respectively (11, 12, 16). We assume that the TnC isoform and pCa have no direct effect on \(k_{-2}\), \(k_{-2}\), or \(k_{tr}\) as these rates should only depend on the myosin and actin present, neither of which changed during our experiments. We also assume that addition of calcium and/or replacement of regulatory proteins changes only \(k_{-1}\) and/or \(k_{-1}\). Assuming that \(k_{-1}\), \(k_{-2}\), \(k_{-2}\), and \(k_{tr}\) are constant in skeletal muscle and that \(k_{-1}\) is \(4\) s\(^{-1}\), then steady-state isometric force, \(F_s\), is a hyperbolic function of \(k_{-1}\) defined by

\[
F_s = k_{tr}/(3 + 1.25 k_{tr})
\] (Eq. 5)

The analytical expressions used to determine the steady-state isometric force, \(F_s\) (assumed to be proportional to the fraction of cross-bridges attached in the force-generating states) and the time course of force production, \(F(t)\), either from rest or after a period of rapid shortening and re-stretch are given under “Experimental Procedures.” The time course of force generation is dominated by the \(A\exp(\lambda t)\) term as \(B\) is insignificant compared with \(A\) at relative forces of ~90%. At larger values of \(k_{-1}\) (>15 s\(^{-1}\) when \(F_s\) > 90%), the difference in magnitude of \(\lambda_1\) and \(\lambda_2\) produces a slowing of \(k_{tr}\) to a value <15% different from that predicted by \(\lambda_1\) alone. The consequence of this behavior is that the rate of force subsequent to rapid shortening (during which \(k_{tr}\) is ~100 s\(^{-1}\)) is well fit by a single exponential term \((R^2 > 0.95)\).

The overall cross-bridge cycling rate is slow and limited by an irreversible isomerization step preceding ADP release defined by \(k_{tr}\) (42). The measurement of the rate of tension redevelopment isolates the force-generating step (\(k_{tr}\)) and the preceding equilibrium \((k_{tr}/k_{-1}\)) from the overall cross-bridge cycle. Thus when \(k_{tr}\) and \(k_{-1}\) are both small, \(k_{tr}\) approaches \(k_{-1}\) as a limit. Modeling of this mechanism suggests that \([\text{Ca}^{2+}]\) activates the muscle by increasing \(k_{-1}\) while not affecting \(k_{tr}\). By varying \(k_{-1}\) from 0 to 20 s\(^{-1}\) to simulate changes in the free calcium concentration, the model correctly defines the observed non-linear behavior of \(k_{tr}\) as a function of relative isometric force in fibers containing sTnC (Fig. 5B, solid line labeled sTnC). The changes in \(k_{tr}\), produced by replacing sTnC with cTnC (Figs. 5B and 6B, solid lines labeled cTnC) can be produced by raising \(k_{tr}\) from 4 s\(^{-1}\) to 10 s\(^{-1}\) and allowing \(k_{tr}\) to vary from 0 to 13 s\(^{-1}\) as the calcium concentration is raised. Therefore, the model suggests that the rate detached or weakly bound cross-bridges productively bind to the thin filament determines the rate of force generation.

In the present experiments, as \([\text{Ca}^{2+}]\) was reduced in fibers containing sTnC, \(k_{tr}\) fell from ~18 to 2 s\(^{-1}\). After extraction of endogenous sTnC and replacement with cTnC, the \(Ca^{2+}\)-induced reduction in \(k_{tr}\) was smaller, from ~11 to 3 s\(^{-1}\). Cardiac muscle exhibits a smaller, 3–6-fold increase in \(k_{tr}\) as \([\text{Ca}^{2+}]\) is raised from submaximal to maximal levels (43, 44). Although \(k_{tr}\) depends on \([Ca^{2+}]\) and the myosin isoform (6, 32), it is significant that substitution of TnC alone causes large changes in the sensitivity and rates associated with force generation. Because incorporation of different TnC isoforms should not alter the cross-bridge structure or the intrinsic cross-bridge cycling rate of myosin, the changes must be caused by TnC-dependent effects. The proposed model correctly accounts for the observed differences of fibers containing sTnC or cTnC in \(k_{tr}\) as a function of relative force. As shown in Fig. 5B, the cTnC data are well fit by simply increasing \(k_{-1}\) by 4 s\(^{-1}\) to 10 s\(^{-1}\) and reducing the maximal rate of \(k_{tr}\) to 13 s\(^{-1}\) leaving the other rate constants unchanged.

Why is \(k_{tr}\) reduced in the presence of cTnC compared with sTnC? The most likely reason is that the TnC interaction with \([\text{Ca}^{2+}]\) and signaling to the other Tn subunits, Tm and actin, play a role in controlling the state of the thin filament activation, which is a complex and incompletely understood process (45–47). Although differences in the \([\text{Ca}^{2+}]\) affinity between the two TnC isoforms may contribute to this behavior, it is more likely that the changes are due to the markedly different structure of the cardiac TnC stalk and regulatory domain from that of skeletal TnC. The structural differences may alter the ability of the TnC to interact with TnI and effect sequential changes in tropomyosin position that influence the rate of cross-bridge attachment. Also, NMR studies reveal that the cTnC structure shows a more closed conformation than sTnC and that the \([\text{Ca}^{2+}]\) binding and dissociation produces slower conformational changes in cTnC (48, 49). Thus, the rate at which TnC can undergo the required conformational changes to affect the inherent properties of the thin filament are TnC isoform-dependent and therefore alter fiber function. Such changes have physiological implications because cardiac muscle does not require the rapid and complete activation necessary for normal physiological function in skeletal muscle.

The presence of CBMII TnC affects only the regulatory units containing the mutant TnC while the other regulatory units are all potentially fully active. If the \([\text{Ca}^{2+}]\) is saturating, most of the native TnC molecules will be in their calcium-bound state and the tropomyosin will be oscillating primarily over the \(Ca^{2+}\)-induced position providing cross-bridge access to the actin binding sites. Thus, \(k_{tr}\) will not be limited by calcium binding and the weak to strong cross-bridge transition. The mechanism described in Scheme 1 successfully predicts the behavior shown in Fig. 5B in which \(k_{tr}\) changes little until the steady-state isometric force rises to values greater than ~50% maximal. This way of thinking about the regulatory mechanism indicates that regulation involves kinetic regula-
tion of the transition from a weakly bound to a strongly bound state as first suggested by Brenner (6). It also suggests that kinetic and steric mechanisms are not truly separate because steric effects from tropomyosin positioning on the thin filament affect the weak to strong cross-bridge transition.

Evidence for a potential role of strongly bound cross-bridges contributing to thin filament activation at lower thin filament occupancies is given by the data in Fig. 6. \( k_{\text{tr}} \) is plotted as a function of the isometric force at pH 4.5 in fibers containing various fractions of CBMII TnC. At forces >25% of maximal, \( k_{\text{tr}} \) is independent of isometric force. However, at 25% isometric force \( k_{\text{tr}} \) is markedly reduced even though \([Ca^{2+}]\) is saturating. This could occur if reduction in the level of thin filament activation is accelerated by the decline in productively attached cross-bridges. The results imply that \([Ca^{2+}]\) controls the steady-state isometric force over the range of 25–100% force by limiting cross-bridge access to the thin filament.

How do these results relate to biochemical and structural mechanisms thought to underlie the regulation of muscle contraction? Biochemical investigations have revealed the presence of three thin filament states: blocked, closed, and open, (45) while more recent cryo-electron microscopy studies have identified three structural states of the thin filament: off, Ca\(^{2+}\)-induced, and myosin-induced (7–9) that may correspond to the blocked, closed, and open states of skeletal troponin C and to Will Silverman (UCLA) for his assistance with gel analysis.

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