Calcium Binding to the Low Affinity Sites in Troponin C Induces Conformational Changes in the High Affinity Domain

A POSSIBLE ROUTE OF INFORMATION TRANSFER IN ACTIVATION OF MUSCLE CONTRACTION*

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Residues 89–100 of troponin C (C89–100) and 96–116 of troponin I (I96–116) interact with each other in the troponin complex (Dalgarno, D. C., Grand, R. J. A., Levine, B. A. Moir, A. J. G., Scott, G. M. M., and Perry, S. V. (1982) FEBS Lett. 150, 54–58) and are necessary for the Ca2+ sensitivity of actomyosin ATPase (Syska, H., Wilkinson, J. M., Grand, R. J. A., and Perry, S. V. (1976) Biochem. J. 153, 373–387 and Grabarek, Z., Drabikowski, W., Leavis, P. C., Rosenfeld, S. S., and Gergely, J. (1981) J. Biol. Chem. 256, 13121–13127). We have studied Ca2+-induced changes in the region C89–100 by monitoring the fluorescence of troponin C (TnC) labeled at Cys-98 with 5-(iodoacetamidoethy)aminonaphthalene-1-sulfonic acid. Equilibrium titration of the labeled TnC with Ca2+ indicates that the probe is sensitive to binding to both classes of sites in free TnC as well as in its complex with TnI. When Mg2+-TnC is mixed with Ca2+ in a stopped flow apparatus, there is a rapid fluorescence increase related to Ca2+ binding to the unoccupied sites I and II followed by a slower increase ($k = 9.9 s^{-1}$) that represents Mg2+-Ca2+ exchange at sites III and IV. In the TnC-TnI complex, the fast phase is much larger and the Mg2+-Ca2+ exchange at sites III and IV results in a small decrease rather than an increase in the fluorescence of the probe. The possibility is discussed that the fast change in the environment of Cys-98 upon Ca2+ binding to sites I and II is instrumental in triggering activation of the thin filament by facilitating a contact between C89–100 and I96–116.

Regulation of contraction in striated muscle involves the actin-bound troponin-tropomyosin complex (for recent review see Leavis and Gergely (1)). Calcium ions released from the sarcoplasmic reticulum upon stimulation of the actin tail the Ca2+-binding component of troponin (TnC). Effects of subsequent changes in the conformation of TnC are transmitted to other components of the thin filament and eventually lead to force generation by the actin-myosin complex. Studies on the isometric twitch in fast skeletal muscles show that the development of tension is very rapid. In the rat extensor digitorum longus, for example, maximum tension is reached in 10–13 ms and it decays within 40–50 ms after excitation (2). The Ca2+ binding and dissociation at the triggering sites and the related conformational changes must, therefore, be faster than the time limits established by physiological experiments (3, 4).

Among the four Ca2+-binding sites in troponin C, sites I and II bind Ca2+ specifically, whereas Ca2+ and Mg2+ compete for sites III and IV (5). Various studies have implicated the sites of lower affinity as the triggering sites for activation (6, 7). Perhaps the most conclusive are kinetic studies showing that only the low affinity sites bind and release Ca2+ fast enough to account for the rapid rise and fall of tension in muscle fibers (3, 4). In the resting muscle, sites III and IV are occupied by Mg2+, therefore Ca2+ binding to these sites is limited by the Mg2+ off-rate ($k = 8 s^{-1}$ (3)) which is too slow to account for the onset of tension (4).

In our studies on proteolytic fragments of TnC we found that a fragment containing the two low affinity sites (residues 9–84) was unable to regulate actomyosin ATPase activity in a reconstituted system in spite of its ability to form a complex with the other troponin components (7). Another peptide (residues 1–100), however, containing the same two sites plus a portion of Ca2+-binding region III, was active, as was a peptide containing the C-terminal half of TnC (TRzC, residues 89–159). Since the common segment of these active peptides was an amino acid stretch comprising residues 89–116, several studies suggest that C89–116 interacts with the stretch of amino acid residues 96–116 in TnI (I96–116) (8–10). A region also recognized as being able to bind to actin and to inhibit actomyosin ATPase (11–14). Although intersubunit interactions in the complete regulated thin filament may be different from those inferred from peptide studies, these results imply that region 96–116 of TnI may alternate between binding to actin at low free Ca2+ and binding to region C89–100 at high Ca2+ concentration.

In this work we have used TnC labeled at Cys-98 with the 3-ethyl-u-amino naphthalene-1-sulfonic acid (AEDANS when covalently bound to a peptide).
fluorescent probe 1,5-IAEDANS to study the kinetics of Ca2+-induced conformational changes in region C98-106. We found that, in addition to the slow fluorescence change corresponding to Mg2+-Ca2+ exchange, there is a fast transition indicative of conformational changes in the environment of Cys-98 upon Ca2+ binding to sites I and II. The amplitude of the fast transition is increased in the TnCARDANS-TnI complex. No fast transitions were detected in analogous experiments utilizing peptide TR-C, the tryptic fragment of TnC containing only the two high affinity sites and carrying the same fluorescent label. We conclude that Ca2+ binding to the triggering sites I and II induces a change in conformation of the region C98-106 which may trigger its interaction with region 96-116 of TnI.

MATERIALS AND METHODS

Troponin was isolated from rabbit back and leg muscles and separated into subunits on DEAE-Sephadex A-50 in the presence of 6 M guanidinium chloride-EGTA (15, 16). The TRC fragment was obtained by digestion of TnC with trypsin in the presence of Ca2+ (17) and purified by preparative gel electrophoresis (18).

Fluorescent Labeling—The single SH group of TnC or its tryptic fragment containing the high affinity sites (peptide TR-C) was labeled with 7-carboxy-6-carboxyfluorescein (15). Typically, a protein sample (2 mg/ml) in a solution containing 0.1 M KCl, 50 mM Heps, pH 7.5, and 1 mM EGTA was incubated with the label for 5 h at room temperature at a 2:1 molar ratio of label to protein followed by overnight dialysis. The extent of labeling (typically 0.75-0.85 mol/mol) was calculated using a value of 7000 ± 85 cm−1 for the absorption coefficient of AEDANS at 330 nm (19). The protein concentration of the AEDANS derivative was measured using Bradford’s reagent (20) (Bio-Rad) calibrated at 288 nm using $\Delta\lambda = 1986$ (21) and taking into account that TnC contains two tyrosyl residues.

Ca2+ Titrations—Equilibrium Ca2+ titrations were performed using an EGTA-NTA Ca2+-buffering system; the fluorescence was monitored with a Perkin-Elmer MPF 4 spectrofluorometer. Small portions of a 0.1 M CaCl2 solution were added directly to the protein solution in a quartz cuvette using a Hamilton microsyringe. In a control experiment we determined that at pH 7.5 approximately one H+ is released upon the complexation of one Ca2+. Therefore appropriate volumes of 0.1 M KOH were added with each addition of CaCl2 permitting maintenance of constant pH during the titration (±0.005 pH). Free Ca2+ concentrations were calculated using published equilibrium constants (22) and an iterative computer program (23). The data were fitted with the Hill equation assuming two independent classes of Ca2+-binding sites:

$$F = F_0 + \sum_{i=1}^{2} A_i K_i [\text{Ca}^2+]^{n_i}$$

where $F_0$ is the fluorescence of the apoprotein, $A_i$ the fluorescence increases, and $K_i$ and $n_i$ are the binding constants and Hill coefficients, respectively, for the high affinity ($i = 1$) and low affinity ($i = 2$) binding sites in TnC. A numerical value of $K$ is related to the transition midpoint by: $pC_{Ca} = \log (K/n)$. The effect of cooperativity in ligand binding on the fluorescence probe in a model system we have discussed previously (24).

Stopped flow experiments were performed in a Dionex (Model 130000) stopped flow spectrophotometer interfaced with a Digital PDP 11/03 computer. Typically, a solution containing 5 uM TnCARDANS or TnCARDANS, 0.1 M KCl, and 50 mM Pipes, pH 6.8, was mixed with an equal volume of a solution containing EDTA or CaCl2 in addition to KCl and Pipes (for details see figure legends). All experiments were performed at 25 °C. The fluorescent probe was excited at 355 nm and the emitted light was passed through a 470 nm interference filter. The sampling rate was adjusted so as to acquire 200–300 data points during the total acquisition time. Typically, a 2-ms sampling rate was employed. The time course of the digitized fluorescence signal was fitted with a single or double exponential equation using a nonlinear regression method.

RESULTS

Ca2+ Binding under Equilibrium Conditions—We have previously reported (25, 26) that Ca2+ binding to sites III and IV of TnCARDANS causes a 1.25-fold increase in the fluorescence of the probe. Analysis of the fluorescence data reveals a best fit when two transitions are assumed, about 80% of the fluorescence change occurring at $pC_{Ca} = 7.5$ and 20% at $pC_{Ca} = 6.2$ (Fig. 1). The first transition represents changes in the environment of the probe induced by Ca2+ binding to the high affinity sites III and IV and is presumably related to the well-known coil-helix transition in the region adjacent to Cys-98 (27). The second transition may reflect changes in the environment of the probe upon Ca2+ binding to the distant low affinity sites or it may result from some low-affinity-site labeling, e.g. at Met-25 (28). If, however, the probe binds to additional site(s), an increase in the label-to-protein ratio during the labeling procedure should result in an increase in the extent of labeling and in an increase in the relative contribution of the second transition to the total fluorescence change. No change in the relative contributions of the two transitions could be detected at IAEADANS/TnI ratios varying between 0.2 and 10 (Table I). More importantly, the sum of the number of bound labels and unreacted protein thiol groups remained constant at ~0.9 per TnC molecule. Based

![FIG. 1. Ca2+ titration of TnCARDANS and its complex with TnI. Fluorescence changes of TnCARDANS (●) and TnCARDANS, TnI complex (▲) were monitored in a solution containing 0.1 M KCl, 0.05 M Pipes, pH 7.5, 1 mM EGTA, 1 mM NTA, 5 uM TnCARDANS, and 20 uM TnI when present. The excitation and emission wavelengths were 330 and 470 nm, respectively. The curves represent the best fit to the Hill equation assuming two independent classes of sites. For the purpose of comparison, transition midpoints $pC_{Ca}$ are given rather than the cumulative binding constant $K$.](http://www.jbc.org/)
on these observations, the fluorescence transition at high Ca$^{2+}$ concentrations reflects changes in the environment of the probe located at Cys-98 upon Ca$^{2+}$ binding to sites I and II.

In the presence of excess TnI there is an approximate 10-fold increase in the Ca$^{2+}$ affinity of both classes of sites in TnC as previously reported (5) and the fluorescence change corresponding to binding to the low affinity sites is increased to 25% of the total change (Fig. 1). Ca$^{2+}$ binding to sites III and IV in both the free TnC and the TnC-TnI complex shows apparent cooperativity with Hill coefficients ($n$) of 1.7 and 2.0, respectively. The cooperative binding to sites III and IV in the absence of Mg$^{2+}$ is in agreement with previous studies (25, 29).

**Kinetics of the Ca$^{2+}$-induced Changes in the Environment of Cys-98**—Rapid mixing of a solution of apo-TnC$^{	ext{AEDANS}}$ with 0.5 mM CaCl$_2$ in the stopped flow apparatus results in a fluorescence increase ($F/F_0 = 1.25$) completed within the mixing time of the apparatus (viz. 2.6 ms). This observation is consistent with reported fast on-rates of Ca$^{2+}$ to both classes of sites in apo-TnC (3, 30, 31). The magnitude of the fluorescence increase is similar to that calculated from the equilibrium Ca$^{2+}$-titration curve. Removal of Ca$^{2+}$ from TnC$^{	ext{AEDANS}}$ with EDTA results in a biphasic fluorescence decrease (Fig. 2). The fast phase that occurs within the dead time of the apparatus amounts to about 19% of the total, and it is followed by a slow monoexponential decrease, $k = 2.2$ s$^{-1}$. The magnitude of the fluorescence change in the fast phase corresponds to that of the second transition in Fig. 1 and most probably represents the dissociation of Ca$^{2+}$ from sites I and II. The apparent rate of the slower phase agrees well with published values for the off-rate of Ca$^{2+}$ from sites III and IV in TnC (3).

The time course of the fluorescence changes induced by Ca$^{2+}$ in TnC$^{	ext{AEDANS}}$ and the relative magnitude of the changes corresponding to the two classes of sites are altered when Mg$^{2+}$ is bound to sites III and IV (Fig. 3). Upon addition of excess Ca$^{2+}$ to the Mg$^+$-TnC$^{	ext{AEDANS}}$, 32% of the total fluorescence increase occurs within the first 2.6 ms of the mixing time. The remaining change can be fitted with a biexponential equation. The slower change ($k = 9.9$ s$^{-1}$) most probably represents Mg$^{2+}$-Ca$^{2+}$ exchange at sites III and IV and is slightly faster than the Mg$^{2+}$ off-rate ($k = 6.8$ s$^{-1}$) measured by mixing Mg$^+$-TnC$^{	ext{AEDANS}}$ with excess of EDTA (Fig. 3A). The faster rate constant (146 s$^{-1}$) must represent an additional process occurring upon Ca$^{2+}$ binding to sites I and II which, however, is slower than any fluorescence change occurring during Ca$^{2+}$ binding to apo-TnC. This process is apparently characteristic for Mg$^+$-TnC, suggesting that the response of the probe of Ca$^{2+}$ binding to sites I and II depends on whether Mg$^{2+}$ or Ca$^{2+}$ is bound to sites III and IV.

In the case of the TnC$^{	ext{AEDANS}}$, TnI complex addition of Ca$^{2+}$ causes a large increase in fluorescence, again within the instrumental dead time (2.6 ms), followed by a small decrease that can be fitted with a single exponential, $k = 7.5$ s$^{-1}$, probably representing the Mg$^{2+}$-Ca$^{2+}$ exchange at sites III and IV (Fig. 3B). The highly fluorescent species that is formed within the first 2.6 ms is probably the Ca$^2+$-TnC$^{	ext{AEDANS}}$-TnI complex. Apparently the high fluorescence is a result of a combined action of Ca$^{2+}$ binding at sites I and II plus the presence of TnI. Since the probe reflects the environment of Cys-98, the high fluorescence in the TnI complex seems to reflect the interaction of TnI with the region Cys$^{98}$-100 of TnC in response to Ca$^{2+}$ binding at sites I and II.

The above interpretation of the kinetic data is based on the assumption that any fast fluorescence change is related to Ca$^{2+}$ binding to sites I and II. To test this assumption we have performed similar experiments using the peptide TR$_C$, a proteolytic fragment of TnC that contains only the high affinity sites. Mixing Mg$^{2+}$-saturated TR$_C$AEDANS with Ca$^{2+}$ or with EDTA yields fluorescence changes that can be fitted with single exponential equations (Fig. 4A). Similar results were obtained for the complex of TR$_C$ with TnI (Fig. 4B). No rapid change within the mixing time could be detected with or without TnI. The apparent rate constants range from 8.2 to 20 s$^{-1}$ (Table II). These data support the view that the rapid fluorescence changes observed for TnC$^{	ext{AEDANS}}$ and its complex with TnI are related to Ca$^{2+}$ binding to the Ca$^{2+}$-specific sites, while the slow change ($k \sim 10$ s$^{-1}$) reflects Mg$^{2+}$-Ca$^{2+}$ exchange at sites III and IV.

**DISCUSSION**

Calcium binding to both classes of sites in TnC affects the environment of the AEDANS probe attached to Cys-98. This
permits us to monitor changes specifically in that region of Cys-98 is close to Ca2+-binding site I in primary structure, as and with low affinity site binding comes from the kinetic studies. Displacement by Ca2+ of the Mg2+ initially bound to the higher it is not surprising that the probe responds to Ca2+ binding to the thin filament (4). The location of the probe at Cys-98 the Ca2+-induced Conformational Changes Rate constants have been detected using a number of spectroscopic methods in the high affinity sites. Conformational changes in this region induced by Ca2+ binding to the unoccupied low affinity sites, I and II, When Ca2+ is rapidly mixed with Mg2+ TnC, there is a rapid allows us to observe a transient form of the protein; fluorescence enhancements as Ca2+ binds first to the high induced structural changes in the region of Cys-98 associated with AEDANS at Cys-98 starting from the Mg2+-bound state the AEDANS label attributable to Ca2+ binding to the low affinity sites; however, they did observe such changes when Ca2+- binding to sites I1 and IV. Further evidence for Ca2+- induced fluorescence changes (k > 600 s⁻¹), corresponding to the binding of Ca2+ to the unoccupied low affinity sites, I and II, followed by a slower increase (k = 9.9 s⁻¹) reflecting the displacement by Ca2+ of the Mg2+ initially bound to the higher affinity sites III and IV. Thus, the use of kinetic methods allows us to observe a transient form of the protein; viz. Ca2+- Mg2+-TnC, that has been suggested to be capable of activating the thin filament (4). The location of the probe at Cys-98 permits us to monitor changes specifically in that region of TnC that has been shown to be involved in Ca2+-dependent binding to TnI (7, 8) and to be essential for the ability of TnC to confer Ca2+ sensitivity upon actomyosin ATPase activity (7).

Relation between Fluorescence Changes of AEDANS and the Ca2+-induced Conformational Changes in TnC—Since Cys-98 is close to Ca2+-binding site II in primary structure, it is not surprising that the probe responds to Ca2+ binding to the high affinity sites. Conformational changes in this region have been detected using a number of spectroscopic methods (6, 18, 32, 23) and were interpreted as a coil-helix transition induced by Ca2+ binding to sites III and IV (27). The fluorescence transition at pCa = 6.2 in TnCaEDANS indicates that Ca2+ binding to sites I and II also affects this region of the molecule. Interactions between binding regions in the two halffs of the molecule are particularly intriguing in view of the recent x-ray diffraction studies showing that the two pairs of binding domains are connected by a 29-residue a-helical segment without direct interactions between them (34, 35).

Both the work reported here and that of Rosenfeld and Taylor (31) show that Ca2+ binding to the low affinity sites brings about changes in the environment of probes bound to Cys-98. Although in these two studies Mg2+-TnC and apo-TnC, respectively, and different labels on Cys-98, IAEDANS, and n-(N-iodoacetoyl)ethyl-N-methyl)amino-7-nitrobenz-2-oxa-1,3-diazole, respectively, were used, a fast transition within the mixing time of the instrument was observed. A similar transient attributable to binding to the low affinity sites was observed by Johnson et al. (3) and Iio and Kondo (30) using a DANZ label bound in the vicinity of these sites (for a detailed review see Leavis and Gergely (1)). Cheung et al. (36) were unable to detect changes in the fluorescence of the AEDANS label attributable to Ca2+ binding to the low affinity sites; however, they observed such changes when Cys-98 was labeled with 5-(iodoacetamido)eosin. Thus, it appears that Ca2+ binding in the N-terminal binding sites induces not only local changes but also alters the structure in more distant parts of the molecule. Such long range effects are also suggested by the influence of metals bound in one class of sites on the dissociation rates for those in the other class (37). In the case of the TnC-TnI complex, a fluorescence change may reflect a change in disposition of the probe relative to the TnI molecule, indicative of a Ca2+-induced interaction between the two subunits in the region C08-100. The highly fluorescent transient state seen in Fig. 3B most probably reflects a higher degree of hydrophobicity in the probe environment in the Ca2+-Mg2+-TnC-TnI complex as compared to the Ca2+ complex. Wang et al. (38) have recently shown that the mobility of a spin probe attached to Cys-98 in TnC is decreased when Ca2+ binds to sites III and IV but is not affected by Ca2+ binding to sites I and II. However, when TnC is complexed to TnI or TnT, a further immobilization of the spin probe associated with Ca2+ binding to sites I and II can be observed. These results and our present data complement each other insofar as they both point to the importance of TnI (as well TnT in the spin label studies) in transferring the effect of Ca2+ binding at sites I and II to the region of the TnC molecule close to Cys-98. Several lines of evidence indicate that the free energy of Ca2+ binding to TnC and the free energy of Mg2+ binding to TnC are coupled. The Ca2+-binding constant for both classes of sites in the TnC-TnI complex is approximately 10 times higher than in the free TnC (5). Conversely, the equilibrium binding constant between TnC and TnI is higher in the presence of Ca2+ (26, 39-41). There seems to be no positive coupling, on the other hand, between TnI and Mg2+ binding to TnC. The Mg2+ off-rates measured in this paper are slightly but consistently faster in complexes with TnI both for the intact TnC and the TnC peptide. These observations suggest that the interaction between TnI and the C-terminal part of TnC is different depending on whether Ca2+ or Mg2+ is bound in sites III and IV. This in turn implies that the interactions between troponin components during a single twitch may be different from the interactions during prolonged stimulation, i.e. after Mg2+ is replaced by Ca2+ in sites III and IV.

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TABLE II

<table>
<thead>
<tr>
<th>Addition of CaCl2</th>
<th>Addition of EDTA</th>
</tr>
</thead>
<tbody>
<tr>
<td>TnC</td>
<td>&gt;600 s⁻¹ 146 9.95 6.77</td>
</tr>
<tr>
<td>TnC:TnI</td>
<td>&gt;600 s⁻¹ 7.52 13.4</td>
</tr>
<tr>
<td>TR2C</td>
<td>10.3 8.19</td>
</tr>
<tr>
<td>TR2C:TnI</td>
<td>16.5 20.9</td>
</tr>
</tbody>
</table>

*Estimated on the assumption that 80% of the change is completed within 2.6 ms of the mixing time.*
The above studies are consistent with TnCs being a dynamic protein able to undergo fast conformational changes and to transmit these changes to distant regions of the molecule and ultimately to the other troponin components. The recently published crystal structure of TnC (34, 35) does not offer obvious clues as to the mechanism of this transmission. The presence of a 29 residue α-helical segment interconnecting the high and low affinity domains of the molecule renders it difficult to envision how changes initiated by Ca²⁺ binding in one domain induce changes in the other as is suggested by this work and others (37, 42). It is important to point out that the crystalline form of TnC contains Ca⁺⁺ ions only at its two high affinity sites. Other Ca⁺⁺ and Mg⁺⁺ complexes of the protein may exhibit different conformations in solution.

**Implications for the Mechanism of Thin Filament Regulation**—Our present findings that Ca⁺⁺ binding to sites I and II in TnC has a rapid effect (within less than 2.6 ms) on the fluorescent probe located in region C₈₉₋₁₀₀ in the TnC-TnI complex is relevant to the mechanism of information transfer in the troponin complex and to thin filament regulation. It is generally held that the role of troponin in regulation is to lock the thin filament in the inhibitory state in the absence of Ca⁺⁺. This interpretation arises from the two-pronged attachment of troponin to the thin filament, the TnC component being bound to actin and the TnT component to tropomyosin. TnI and TnT bind to each other and to TnC. In the presence of Ca⁺⁺, TnC added to a complex of actin-tropomyosin-TnI binds to the TnI component causing its dissociation from the actin-tropomyosin filament (43, 44). These studies indicated that in the absence of TnT, TnI could bind either to TnC or to actin but not to both simultaneously. Later work employing proteolytic fragments of TnI (11) identified two segments of the amino acid sequence (residues 1–21 and 96–116) that were able to bind to TnI. One of these, I₈₉₋₁₁₆, was also able to bind to actin and to mimic intact TnI in the inhibition of actomyosin ATPase activity (11). Subsequent studies using synthetic analogs of I₈₉₋₁₁₆ (10, 12, 13) confirmed its ability to bind to either TnC or actin, and recent 'H NMR studies (14) have shown that certain residues (Arg-103, -108, -112, -115, and -115) are perturbed when I₈₉₋₁₁₆ binds to actin while others (Lys-98, Leu-99, Phe-100, Leu-102, Lys-105, Phe-106, Lys-107, and Leu-111) are affected by binding to TnC.

Complementary studies using fragments of TnC have also established the existence of multiple sites of interaction with TnI located in Ca⁺⁺-binding regions II, III, and IV (7, 8). Among these, the stretch of residues 89–100 (C₈₉₋₁₀₀) is of special interest because it has been shown to be indispensable for TnC role in the modulation of actomyosin ATPase activity (7). Furthermore, a number of studies (8, 10, 14, 45) have shown that C₈₉₋₁₀₀ binds directly to I₈₉₋₁₁₆. Although the data are still not unequivocal regarding the Ca⁺⁺ dependence of this interaction, the kinetic data reported here suggest that it is modulated specifically by Ca⁺⁺ binding to sites I and II.

A model of the role of TnC and TnI emerges from a consideration of the earlier observations together with the conclusions reached in this paper. In the relaxed state, one F-actin monomer in each hexameric unit along the thin filament interacts with region 96–116 of TnI. This interaction is probably instrumental in maintaining the thin filament in an inhibitory state that prevents actin from activating myosin ATPase activity. In this state Mg⁺⁺ is bound at sites III and IV of TnC whereas sites I and II are empty. Ca⁺⁺ binding to sites I and II causes a fast change in the conformation of region C₈₉₋₁₀₀ or in its disposition relative to TnI, facilitating interaction between C₈₉₋₁₀₀ and I₈₉₋₁₁₆. This interaction releases the TnI-actin bond, allowing the transition of the filament to the active state. The key feature of this scheme is that the transfer of information in the troponin complex occurs from the low affinity sites in TnC, to region C₈₉₋₁₀₀, to TnI, and ultimately to the other thin filament components.

The above scheme does not include a role for the other interaction sites between TnC and TnI. The existence of another, Ca⁺⁺-dependent, TnI binding site in the region of the low affinity sites of TnC (7, 11) suggests an alternative or complementary route of information transfer: Ca⁺⁺-induced conformational changes in the N-terminal half of TnC would result in its interaction with the N-terminal region of TnI (I₁₋₂₁) and subsequent conformational changes within TnI would lead to dissociation of I₈₉₋₁₁₆ from actin and to its interaction with C₈₉₋₁₀₀. Although a switch of from I₈₉₋₁₁₆ from its interaction site on actin to the site on TnC would still be included, the propagation of the conformational changes originating in sites I and II would involve TnI rather than solely TnC as described above. Clearly, more work is required to provide a more detailed picture of the pathways involved.

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Calcium binding to the low affinity sites in troponin C induces conformational changes in the high affinity domain. A possible route of information transfer in activation of muscle contraction.

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