A New Model of Cooperative Myosin-Thin Filament Binding

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Cooperative myosin binding to the thin filament is critical to regulation of cardiac and skeletal muscle contraction. This report delineates and fits to experimental data a new model of this process, in which specific tropomyosin-actin interactions are important, the tropomyosin-tropomyosin polymer is continuous rather than disjointed, and tropomyosin affects myosin-actin binding by shifting among three positions as in recent structural studies. A myosin- and tropomyosin-induced conformational change in actin is proposed, rationalizing the ~10,000-fold strengthening effect of myosin on tropomyosin-actin binding. Also, myosin S1 binding to regulated filaments containing mutant tropomyosins with internal deletions exhibited exaggerated cooperativity, implying an allosteric effect of tropomyosin on actin and allowing the effect’s measurement. Comparisons among the mutants suggest the change in actin is promoted much more strongly by the middle of tropomyosin than by its ends. Regardless of calcium binding to troponin, this change in actin facilitates the shift in tropomyosin position to the actin inner domain, which is required for tight myosin-actin association. It also increases myosin-actin affinity 7-fold compared with the absence of tropin-tropomyosin. Finally, initiation of a shift in tropomyosin position is 100-fold more difficult than is its extension from one actin to the next, producing the myosin binding cooperativity that underlies cooperative activation of muscle contraction.

Deciphering the regulatory mechanism of cardiac and skeletal muscle contraction is a long standing problem in physiology. Contraction is activated very cooperatively by Ca\(^{2+}\) binding to the thin filament protein troponin, and the binding of myosin S1 to actin on the thin filament is also very cooperative, especially in the absence of Ca\(^{2+}\) (reviewed in Refs. 1 and 2). The sharpness of these transitions suggests that the thin filament undergoes abrupt conformational change(s) that are promoted by Ca\(^{2+}\) and myosin. Experimental and theoretical efforts have been made to understand this for several decades (3–9), and recently these efforts have been aided by atomic structural information for myosin and actin (10, 11) and improved data on thin filament conformational states (12–14).

Tropomyosin internal deletion mutants that span four or five rather than the usual seven actins have provided unexpected insights into thin filament-mediated regulation (15). Ca\(^{2+}\) binding to troponin induces normal repositioning of tropomyosin on actin in such mutant filaments. Nevertheless, myosin cycling is inhibited in the presence of Ca\(^{2+}\), and regulatory protein binding to actin is destabilized by the mutations when and only when myosin is present (16, 17). In the present report, myosin S1-thin filament binding is examined and found to exhibit very exaggerated cooperativity in the presence of these mutant tropomyosins. Based upon these data and work in the literature, a new model of myosin-thin filament binding is proposed. Finally, the model and experimental results are used to elucidate the regulatory behavior of normal thin filaments.

In 1997 three conformations of the thin filament were identified using electron microscopy and three-dimensional reconstructions of actin-myosin S1 filaments in the presence of tropomyosin and of actin-tropomyosin-tropomyosins \(\pm \text{Ca}^{2+}\) (14). As Ca\(^{2+}\) and then myosin bind to regulated thin filaments, the azimuthal position of tropomyosin on the actin filament shifts in two steps, with increasing exposure of the myosin-binding site on actin. These data suggested that any analysis of cooperative myosin-thin filament binding should include three tropomyosin positions as a critical component. Geeves and coworkers (8) presciently described many features of such a model in 1993 and used kinetic and equilibrium binding data to measure transitions among three thin filament states, termed blocked, closed, and open with respect to myosin binding.

The current report presents a new model that includes three positions for tropomyosin but otherwise is a significant departure from previous analyses. Myosin and tropomyosin strongly promote each other’s binding to actin (18–21), indicating either direct interactions between them or indirect interactions mediated by a conformational change in actin. A conformational change in actin is a central feature of the new analysis, with implications regarding different interactions of myosin with unregulated actin, regulated actin, and regulated actin that is partially saturated with myosin (7, 9, 20, 22, 23). Specific tropomyosin-actin interactions are identified experimentally and then emphasized in the model, in contrast to previous treatments. Also, the polymerized tropomyosin strand is properly analyzed as continuous, rather than as functionally affecting discrete units of seven actins. Detailed predictions are derived by the statistical weight matrix method (24) for obtaining the thin filament partition function. The results offer a new perspective on the regulation of striated muscle contraction.

**EXPERIMENTAL PROCEDURES**

**Protein Purification and Modification**—Rabbit fast skeletal muscle myosin S1 (25) and actin (26) and bovine cardiac troponin (27) were obtained as previously reported. A panel of six recombinant rat striated muscle α-tropomyosins were expressed in DE3(BL21) cells using the vector pET3d and purified to homogeneity (15, 17). Each of these previously described (15, 17) tropomyosins includes an Ala-Ser NH\(_2\)-terminal dipeptide that corrects the poor ability of unacylated tropomyosin expressed in bacteria to polymerize (28). The panel includes full-length tropomyosin (Ala-Ser Tm)\(_1\) and five internal deletion mu-

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1 The abbreviations used are: Tm, tropomyosin; Tn, troponin; Ap\(_5\)A, P\(_5\)P\(_5\)-di(adenosine 5\'-)pentaphosphate.
FIG. 1. Schematic model of the effects of Ca\(^{2+}\) binding and myosin binding on thin filament conformational states. Top, tropomyosin (line segments overlaying bi-lobed actin monomers) shifts among three positions on actin depending upon Ca\(^{2+}\) and myosin (S1) binding to actin-tropomyosin-troponin (Tm Tn Tn) (14). The lower lobe corresponds to the outer domain of actin filaments and the upper lobe to the inner domain. Strong myosin-actin binding is proposed to involve a change in the actin monomer (hatched lines on the surface of subdomain 3). Alternatively, this change can be promoted by tropomyosin binding to the actin inner domain. Equilibrium constants are expressed per actin for whole filament transitions. Weak myosin binding is small at equilibrium, and only strong binding is shown. Bottom, strong myosin binding also changes actin conformation in the absence of tropomyosin-troponin (Tm Tn). Weak myosin binding (not shown) may precede the indicated change in actin, but such a kinetic path would not alter the illustrated equilibrium pathway.

**RESULTS AND DISCUSSION**

**Description of a Thin Filament Model**—Fig. 1 schematically illustrates three tropomyosin positions on actin as follows: (i) on the outer domain in the absence of Ca\(^{2+}\), a position which blocks strong myosin binding; (ii) near the boundary between the inner and outer domains in the presence of Ca\(^{2+}\); (iii) on the inner domain when myosin is bound strongly to actin, regardless whether Ca\(^{2+}\) is present. Additionally, the scheme includes a novel feature designed to account for positively cooperative interactions between myosin and tropomyosin. Myosin S1 binds to actin-tropomyosin filaments, or to actin-troponin-tropomyosin filaments, with an affinity 4–7-fold higher than to bare actin (20, 22). Correspondingly, myosin S1 must increase the actin affinity of tropomyosin-troponin binding by 4–7-fold, i.e., 10,000-fold or more. This very high affinity binding has experimental support (18, 19), and any convincing model should be consistent with this evidence that myosin and tropomyosin interact, either directly or indirectly. To explain this, the model invokes a conformational change in actin that is promoted both by myosin and by specific interactions between tropomyosin and the actin inner domain (15, 16). This change in actin is schematically indicated by a shading of the surface of subdomain 3 in the figure, because thin filament reconstructions indicate that myosin S1 contacts the edge of this subdomain, where tropomyosin is also bound (14). Some effect of myosin on actin atomic structure must occur and has been spectroscopically detected (29–31), but high resolution data to define this effect are lacking. The assumption in the model is that myosin causes the same effects on actin, regardless of the presence of the regulatory proteins. When tropomyosin is located on the actin inner domain, it is proposed to promote all or part of the same changes in actin. For example, tropomyosin-induced movements of actin subdomain 3 surface residues might increase myosin binding to actin by 4–7-fold. Fig. 2 provides qualitative support for a conformational change in actin, as discussed below.

The bottom of Fig. 1 shows a two-step energetic path for myosin S1 binding to unregulated actin. (This is not a proposed kinetic pathway.) \(K_T\) describes the (unfavorable) equilibrium for bare actin to undergo the same conformational change that tropomyosin induces when present. Myosin S1 binding then follows with the same affinity, \(K_{S1}\), as it has for the active state of a regulated filament. The observed binding constant of myosin S1 for bare actin can be considered the product \(K_{T} K_{S1}\).

The equilibrium constants illustrated in Fig. 1 are defined as per actin constants for transitions of entire filaments from one state to another. Definitions therefore include contributions due to changes in site-site interaction strength that may accompany such transitions. Actual filaments are assumed to undergo partial transitions. \(K_T\) describes the average equilibrium for activation of the filament, from the Ca\(^{2+}\)-state to the active state, including the conformational change in actin. \(L_C\) concerns a shift in tropomyosin position (away from the inner domain) in the absence of a conformational change in actin. Larger \(L_C\) corresponds to a smaller population of the illustrated intermediate state. The product \(K_T L_C\) describes the equilibrium constant for the activating conformational change in actin, once tropomyosin is located on the inner domain.

Ca\(^{2+}\) binding to troponin is critical to muscle regulation, and this event is proposed to affect selected equilibria. In the absence of Ca\(^{2+}\) tropomyosin is in a position that blocks strong...
myosin binding to actin, and $L_B$ describes the equilibrium constant holding tropomyosin away from the inner domain in the absence of Ca$^{2+}$. Because of troponin I-actin interactions, $L_B$ is expected to be larger than $L_C$ when the model is fit to experiment. On the other hand, once tropomyosin is on the inner domain the conformational change in actin is attributed to tropomyosin-actin interactions, and so this equilibrium constant ($K_{PLC}$) is assumed to be unaffected by Ca$^{2+}$, as is the final step of myosin binding, $K_{STL}$. Finally, the schematic shows no interconversion between the blocked and Ca$^{2+}$-induced states (unless Ca$^{2+}$ binds or dissociates). This indicates the simplifying assumption that the distribution between these two states is strongly biased in one direction or the other. This simplification is very useful analytically but could be relaxed in a future treatment. It has strong support in the presence of Ca$^{2+}$. (i) Rapid myosin S1 binding to thin filaments in the presence of Ca$^{2+}$ implies that the blocked state is sparsely populated (8, 9). (ii) Thin filament helical reconstructions indicate tropomyosin is not in a blocking, outer domain location under these conditions (13). In the absence of Ca$^{2+}$ the simplification is more uncertain (see below) but has the following support. (i) Specific interactions between troponin I and actin are believed to hold tropomyosin on the outer domain in the absence of Ca$^{2+}$ (reviewed in Ref. 1). (ii) Thin filament helical reconstructions indicate tropomyosin is on the outer domain under these conditions (13).

**Cooperativity in Thin Filament Activation**—Several transitions are defined by Fig. 1, but few of them are plausible sources of cooperativity. The mechanism proposed in the model is that multiple shifts of tropomyosin on actin cause an energetic penalty or strain relative to fewer shifts, of magnitude $RT \ln(Y)$ for each shift back and forth. This differs from Y in Hill et al. (3) in that shifts can occur for segments of the tropomyosin strand spanning any number of actins, rather than multiples of seven actins. Y includes any effect of strain within the tropomyosin itself. The magnitude of this component is inversely affected by the degree of tropomyosin flexibility. The other, perhaps larger contribution to Y, is strain on tropomyosin-actin interactions, particularly strain on the specific interactions between tropomyosin and active-state actin.

The only other possible sources of cooperativity are myosin-myosin interactions (transition $K_{LLC}$) and/or actin-actin interactions (transition $K_{TLC}$). These are rejected because myosin S1 binding to bare actin is not cooperative (22, 32). If either of these processes contributed to cooperative myosin S1 binding to the regulated thin filament, then they would also be expected to produce cooperativity in myosin S1 binding to bare actin.

Troponin is located at every seventh actin along the thin filament, and influential previous models of activation represented thin filament transitions as occurring simultaneously in units of seven actins. However, this premise is not in agreement with significant aspects of thin filament structure. Tropomyosin forms a continuous strand along the actin filament, with end-to-end overlaps of successive tropomyosins (5) that are further strengthened by the tail region of troponin (33). There is no evidence that end-to-end interactions are interrupted as tropomyosin moves on the surface of actin, particularly in the presence of Ca$^{2+}$, a condition in which myosin causes only a small shift in tropomyosin position. Therefore, the current model treats the thin filament as an actin monomer chain, with conformational change propagated by the continuous tropomyosin strand from one actin to the next. The critical distinction of this new approach is that myosin binding to an actin monomer affects the likelihood of myosin binding to any nearby monomer, rather than selectively to monomers that happen to contact the same tropomyosin molecule or that happen to be in the same “apparent cooperative unit” (34).

**Mathematical Solution of the Model by the Statistical Weight Matrix Method**—In the presence of Ca$^{2+}$, each actin on the thin filament can be in any of four states illustrated in Fig. 1, i.e. any state except the blocked state. For a filament N actins long, there are $4^N$ possible thin filament configurations, existing in proportions defined by the equilibrium constants described above and the myosin S1 concentration. The partition function, $\xi$, is a summation of all these configurations, weighted according to their relative proportions. Average properties such as binding saturation are straightforward to calculate from $\xi$, in the same manner that a binding polynomial is used for a small system. The frequency of any filament configuration depends upon the equilibrium defined in Fig. 1 but also depends upon the number of times tropomyosin shifts back and forth on actin. By using the matrix method (24), the probability-weighted states at an index actin are multiplied repetitively by the similarly weighted states at successive actins, keeping track of statistical penalties due to changes in tropomyosin position. The parti-
tion function is shown in Equation 1:

$$\xi = T \left( \frac{1}{1/L_C + 2/K_T(1 + K_T S_1)} \right) \left( \frac{1}{1/L_C + 2/K_T(1 + K_T S_1)} \right)^N$$  \hspace{1cm} (Eq. 1)

For large $N$, $\xi = \lambda_{\text{max}}^N$, where $\lambda_{\text{max}}$ is the larger of the two eigen values of the matrix. Solving the characteristic equation of the matrix,

$$2\lambda_{\text{max}} = 1 + 1/L_C + 2/K_T(1 + K_T S_1) + \sqrt{\gamma},$$  \hspace{1cm} (Eq. 2)

where

$$\gamma = ([1 - 1/L_C - K_T(1 + K_T S_1)]^2 + 4[1/L_C + K_T(1 + K_T S_1)]^2)^{1/2}$$

From the partition function one can immediately derive fractional saturation with myosin $S_1(\theta_{S_1})$ as shown in Equation 3,

$$\theta_{S_1} = 1/S_1 \cdot \frac{\partial \xi}{\partial S_1} = \lambda_{\text{max}} \cdot \frac{\partial \lambda_{\text{max}}}{\partial S_1} = \frac{K_T K_T S_1(1 + 2Y - 1 + 1/L_C + K_T(1 + K_T S_1))}{1 + 1/L_C + K_T(1 + K_T S_1) + \sqrt{\gamma}}$$  \hspace{1cm} (Eq. 3)

The partition function is an $N^{th}$ order polynomial not only in $S_1$ but also in $K_T$ and in $1/L_C$. This allows the derivation of the fraction of sites in the active state ($\theta_{\text{active}}$), the intermediate state ($\theta_{\text{intermediate}}$), and the Ca$^{2+}$ state ($\theta_{\text{Ca}^{2+}}$) (see Equations 4–6),

$$\theta_{\text{active}} = \frac{K_T \cdot \lambda_{\text{max}}}{1/L_C + 2/K_T(1 + K_T S_1)}$$  \hspace{1cm} (Eq. 4)

$$\theta_{\text{intermediate}} = \frac{1}{L_C \cdot \lambda_{\text{max}}} \cdot \frac{1 + [1/L_C - 1 + K_T(1 + K_T S_1) + 2Y]/\sqrt{\gamma}}{1 + 1/L_C + K_T(1 + K_T S_1) + \sqrt{\gamma}}$$  \hspace{1cm} (Eq. 5)

$$\theta_{\text{Ca}^{2+}} = 1 - \theta_{\text{active}} - \theta_{\text{intermediate}}$$  \hspace{1cm} (Eq. 6)

Equations 3–5 remain applicable in the absence of Ca$^{2+}$, but with $L_B$ and $K_T L_C/L_B$ substituted for $L_C$ and $K_T$, respectively. Equation 6 then yields $\theta_{\text{blocked}}$ rather than $\theta_{\text{Ca}^{2+}}$.

**Binding of Myosin-S1-ADP to Actin-Troponin-Ca$^{2+}$-Tropomyosin Thin Filaments Containing Deletion Mutant Tropomyosins**—Binding of myosin S1 to the thin filament was determined for actin-troponin-Ca$^{2+}$-tropomyosin filaments containing either full-length tropomyosin (Ala-Ser Tm) or tropomyosin internal deletion mutants (Fig. 2). These internal deletion mutants selectively and profoundly inhibit tropomyosin binding to myosin S1-decorated actin (17), so correspondingly they were expected to alter myosin binding to regulated actin. This prediction was confirmed previously for mutant $\Delta 234$Tm (16), and Fig. 2 shows that it is true generally for the deletion mutant tropomyosins. These mutants lack either two or three of the seven quasi-repeating regions of tropomyosin (5), due to deletions of either $\delta$ or $\nu$ amino acids. They span five or four actins instead of seven and are designated according to which repeating regions are missing. For example, residues 47–165 are deleted in $\Delta 234$Tm, which lacks regions 2–4. The deletions greatly weakened the initial portions of the binding curves, had little effect on the upper portions of the curves, and dramatically increased cooperativity. This is particularly worth noting for filaments with deletion mutant $\Delta 234$Tm, because electron microscopy and three-dimensional reconstructions have been performed using these filaments and indicate that tropomyosin is in the Ca$^{2+}$-induced position (16). Therefore, the S-shaped curves imply abrupt transitions from the Ca$^{2+}$-induced state to the active state.

As noted above, either myosin and tropomyosin interact directly when bound to actin to strengthen each other’s binding or else they indirectly interact via a change in actin. Data using the deletion mutants strongly imply that the latter is the case. In Fig. 2, myosin binding to filaments containing full-length tropomyosin is 7-fold tighter than to bare actin filaments. If this were caused by direct tropomyosin-myosin interactions, then the deletions (which impair tropomyosin binding to actomyosin by 1–3 orders of magnitude (17)) would simply weaken myosin S1 binding to the thin filament, producing patterns more closely resembling that for bare actin. Instead the deletions increased cooperativity, and more importantly, in the presence of sufficient myosin S1 concentrations the mutant filaments exhibited much tighter myosin binding than did bare actin (Fig. 2). This behavior cannot be explained by deletion of direct interaction sites. Rather, it implies an allosteric mechanism involving a conformational change in actin as proposed in the model. Functional differences have long been noted among actin alone, regulated actin in the presence of Ca$^{2+}$, and regulated actin that is partially saturated with myosin (7, 20, 22, 23). The present model provides a framework for understanding these differences, a conformational change in actin that alters myosin binding.

Data for six types of actin-troponin-tropomyosin-Ca$^{2+}$ complexes are shown in Fig. 2. The curves fall into three patterns, closely paralleling the published effects of the same tropomyosin deletions on the binding of tropomyosin to actin-myosin S1 (17). Mutations $\Delta 34$Tm, $\Delta 234$Tm, and $\Delta 456$Tm have large and very similar effects on myosin S1 binding (all are shown by $\Delta$s), and they also produce results very similar to each other on tropomyosin binding to actin-S1 (17). $\Delta 456$Tm has even larger effects in both types of experiments, and $\Delta 23$Tm has much smaller effects in both experiments. It is significant that $\Delta 23$Tm is the only mutant that is similar to full-length tropomyosin in Fig. 2. This explains why all of the mutants except $\Delta 23$Tm inhibit thin filament-myosin S1 MgATPase activity and heavy meromyosin-propelled in vitro motility (17).

**Global Fit of the Model to Myosin-S1-Thin Filament Binding Data Obtained in the Presence of Tropomyosin Deletion Mutants**—Because the deletion mutations selectively weaken tropomyosin binding to the active state of the thin filament (17), they are expected to alter, selectively, transitions into that state. Therefore, Equation 3 can be globally fit to data for all six tropomyosins in Fig. 2, assuming that all curves have identical $K_T$ (all are shown by $\Delta$s), and they also produce results very similar to each other on tropomyosin binding to actin-S1 (17). $\Delta 456$Tm has even larger effects in both types of experiments, and $\Delta 23$Tm has much smaller effects in both experiments. It is significant that $\Delta 23$Tm is the only mutant that is similar to full-length tropomyosin in Fig. 2. This explains why all of the mutants except $\Delta 23$Tm inhibit thin filament-myosin S1 MgATPase activity and heavy meromyosin-propelled in vitro motility (17).
free energy of tropomyosin binding to actin-S1; ΔΔG is altered in relation to how many of these particular regions are missing. Comparisons among the curves in Fig. 2 suggest a similar pattern. Therefore, one can make the approximation that \( K_3 \approx K_4 \approx K_5 \). Also, \( K_2 \approx K_6 \), since \( \Delta \Delta G_{n} \) and \( \Delta \Delta G_{o} \) behave similarly here and in Ref. 17. All of the tropomyosins examined contain end regions 1 and 7, so the product \( K_1 K_7 \) contributes to each \( K_T \). Global curve-fitting was performed to determine values for \( L_C, K_{S1}, Y, K_2, K_3, \) and \( (K_1 K_7)^{1/2} \) that simultaneously describe all six quasi-repeating-tropomyosin curves in Fig. 2, calculated according to Equation 3. To determine \( K_{app} \) from the unregulated actin data (medium dashes), a non-cooperative model was used, with \( K_{app} = K_{S1} K_T \).

Significance of the Measured Parameters—Cooperativity parameter \( Y \) is a measure of how much more likely it is that a shift in tropomyosin position will be extended by one actin than that a new shift will be initiated. It is \( 97 \pm 21 \)-fold preferred to extend an activated region rather than to initiate one. \( RT \ln 97 = 2.8 \text{ kcal/mol} \), so the energetic penalty for an extra shift of tropomyosin is similar to loss of one or two hydrogen bonds. In a large system such as the thin filament, a relatively small energetic effect can result in a tendency to propagate a conformational change over a very sizable region.

In the presence of \( \text{Ca}^{2+} \) the equilibrium for tropomyosin to shift away from the inner domain of actin is \( L_C = 3.41 \pm 0.47 \) in the absence of a conformational change in actin. However, once the tropomyosin is located on the inner domain, it is stabilized in that position by a favorable equilibrium for the activating conformational change in actin, \( K_T L_C = 2.5 \). The overall equilibrium constant for activation, \( K_T \), is equal to 0.73 in the presence of \( \text{Ca}^{2+} \), indicating as expected a relatively small energetic barrier to activation.

From Table I and the above discussion, the fitting was successful in defining the equilibrium constants in the model, with a precision of 10–20%. This would not have been possible from control thin filament data alone and indicates the importance of the mutant thin filament experiments. On the other hand, the data were less successful in measuring the base-line condition of control filaments, i.e. the distribution among conformational states prior to myosin addition. By using Equations 4–6 and the values in Table I, 44% of the filament is in the \( \text{Ca}^{2+} \)-induced state in the absence of myosin; 40% is in the active state, and the remaining 16% is in the intermediate state in which tropomyosin has shifted but the actin has not changed conformation. However, if \( K_T \) equals 0.66 rather than 0.73 (a 1 S.D. change), then 62% of the filament is in the \( \text{Ca}^{2+} \)-induced state rather than 44%. Given this uncertainty, there is no inconsistency between the present data and thin filament reconstructions that show tropomyosin localized to one predominant position (13, 35).

\[ K_1 K_2 K_3 K_4 K_5 K_6 = K_T^7 \]

**Tropomyosin Quasi-Repeating Region:**

**Fig. 2.** Schematic model of unequal contributions of tropomyosin regions to thin filament activation and of cooperativity parameter \( Y \). Very tight tropomyosin binding to the active state (18, 19) implies specific interactions between tropomyosin and actin. Since the quasi-repeating regions of tropomyosin are not identical, their actin interactions can differ, resulting in equilibrium constants, \( K_1, K_2, \ldots, K_6, K_T \) is the equilibrium constant, per actin, for a whole filament transition. Single region equilibria are decreased by 1/\( Y \) (note \( Y \gg 1 \)) if and only if they introduce an additional back and forth shift in tropomyosin position on actin.

**Table I**

Equilibrium constants for thin filament transitions

| \( K_{S1} \) | \( 1.34 \pm 0.10 \times 10^{6} \text{ M}^{-1} \) |
| \( K_T \) | \( 0.731 \pm 0.068 \) |
| \( L_C \) | \( 3.41 \pm 0.47 \) |
| \( K_{TLC} \) | \( 2.50 \pm 0.56 \) |
| \( Y \) | \( 97 \pm 21 \) |
| \( (K_1 K_7)^{1/2} \) | \( 0.16 \pm 0.02 \) |
| \( K_S \) | \( 0.46 \pm 0.07 \) |
| \( K_P \) | \( 2.76 \pm 0.33 \) |
| \( K_T \) | \( 0.147 \pm 0.012 \) |
| \( L_R \) | \( 4.43 \pm 0.06 \) |

By using \( K_{S1} \) from the global fit, and the relatively weak \( K_{app} \) for unregulated actin, the “activation” equilibrium constant for unregulated actin is \( K_{T0} = 0.15 \). This is 17-fold less than \( K_{TLC} \), the equilibrium constant for the conformational change in actin once tropomyosin is on the inner domain. Myosin binding can be considered to involve the work of producing change in actin, and tropomyosin reduces this energetic cost. However, tropomyosin also has negative effects. Strong myosin binding to regulated actin is sterically inhibited unless tropomyosin shifts onto the actin inner domain. If tropomyosin promotes the change in actin poorly, as do the deletion mutants, then tropomyosin does not shift and sterically interferes with myosin binding.

Comparisons among the deletion mutants indicate that the seven quasi-repeating regions do not interact equally with actin. The data strongly suggest that the central portions of muscle tropomyosin are particularly important for cooperative myosin-tropomyosin interactions. The unfavorable value of \( K_{T0} \) for actin alone (0.15) is increased to \( K_T = 2.76 \pm 0.33 \) when only tropomyosin regions 3–5 are considered, 0.459 ± 0.065 when only regions 2 and 6 are considered, and is unchanged at 0.159 ± 0.019 when the combined effects of regions 1 and 7 are considered. Once the tropomyosin is on the actin inner domain, all tropomyosin regions promote the change in actin much more than occurs for actin alone: \( K_T L_C = 9.4 \) for actins contacting the middle three tropomyosin regions, \( K_T L_C = 1.57 \) for actins contacting tropomyosin regions 2 or 6, and \( (K_1 K_7)^{1/2} L_C = 0.54 \) for actins contacting the end segments of tropomyosin, in each case greater than \( K_T = 0.15 \). These measurements could be confounded by unknown aspects of protein structure and will require validation in the future with other tropomyosin constructs. However, they offer the first opportunity to make such discriminations systematically among the seven quasi-repeating regions of tropomyosin.
FIG. 4. Myosin S1 binding to thin filaments in the absence of Ca\textsuperscript{2+}. Myosin S1 binding to the thin filament was monitored as in Fig. 2, except in the absence of Ca\textsuperscript{2+}. Removal of Ca\textsuperscript{2+} caused each of the curves the shift to the right, i.e. to become more cooperative. However, the upper portions of the curves are similar to those in Fig. 2. Lines are best fit calculated curves as described in the text, using parameters $K_{s1}$, $K_{yLC}$, and $Y$ from Table I, and individually fit $L_B$ values are as listed in Table II.

Binding of Myosin-S1-ADP to Regulated Thin Filaments Containing Deletion Mutant Tropomyosins in the Absence of Ca\textsuperscript{2+}—Removal of Ca\textsuperscript{2+} markedly increases the cooperativity of myosin S1 binding to regulated actin containing muscle tropomyosin (21). Ca\textsuperscript{2+} removal also increased cooperativity for filaments containing recombinant tropomyosin (squares, Fig. 4 versus Fig. 2) but not as much as with muscle tropomyosin (see Refs. 8 and 21, data not shown). This presumably reflects differences in tropomyosin NH\textsubscript{2}-terminal structure (28). Nevertheless, binding curves for all the recombinant filaments exhibited increased cooperativity when Ca\textsuperscript{2+} was removed (Fig. 4). These data were analyzed by assuming that troponin I-actin interactions cause the blocked state to be more stable than the Ca\textsuperscript{2+}-induced state, resulting in a value for $L_B$ that is greater than $L_C$. Steps governed by $K_{s1}$ and $K_{yLC}$ were constrained to be unaffected by removal of Ca\textsuperscript{2+}. $Y$ was also held constant, although it may increase in the absence of Ca\textsuperscript{2+} because tropomyosin shifts further on the actin.

By using the above assumptions, a good fit was obtained for full-length tropomyosin (squares), suggesting that each TnI increased the equilibria away from the inner domain by a factor ($L_B/L_C$)\textsuperscript{2} = 6.2. Similar calculations for the deletion mutant tropomyosins gave factors ranging from 2.1 to 18 (Table II). Each of these factors is greater than 1, indicating that Ca\textsuperscript{2+} removal had qualitatively similar effects for all tropomyosins, but the reasons for the quantitative discrepancies are not clear. One possibility is that the deletions remove sites of specific tropomyosin-troponin interactions. However, it is also possible that the model’s treatment of activation as smoothly continuous is a poor approximation in the absence of Ca\textsuperscript{2+}, due to TnI-actin tethering of the mid-portion of tropomyosin on the actin outer domain.

By using the full-length recombinant tropomyosin data in the absence of Ca\textsuperscript{2+}, the fraction of sites in the blocked state is 88% (Equations 4–6). By using tropomyosin isolated from bovine hearts, this value increased to 95–98% (data not shown). As expected, strong myosin binding is blocked in the absence of Ca\textsuperscript{2+}. Interestingly, kinetic data imply that the rate of myosin binding is inhibited by only 75% in the absence of Ca\textsuperscript{2+} (8, 9). An oscillation of tropomyosin between the blocked state position and the calcium state positions has been suggested to explain this (8). This transition could be appended to the present model, should future kinetic experiments require it and facilitate its analysis. However, other explanations for the kinetic data have been suggested (36).

In summary, the present report describes a new, statistical mechanical model of cooperative thin filament activation that rationalizes a wide variety of structural and biochemical data. The analysis suggests that tropomyosin promotes a conformational change in actin that is energetically unfavorable in the absence of tropomyosin. The mid-portion of tropomyosin promotes the conformational change in actin much more strongly than do the ends of tropomyosin. This change in actin permits tropomyosin to shift its position on actin, relieves steric blocking of myosin-actin binding, and causes myosin-actin binding to occur with higher affinity than for actin in the absence of troponin-tropomyosin. Initiation of this tropomyosin shift is 100-fold more difficult than is its extension from one actin to the next in the presence of Ca\textsuperscript{2+}, producing cooperative behavior in solution and presumably underlying cooperative activation of muscle contraction.

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TABLE II
Comparisons among deletion mutant tropomyosins

| $K_r$ | $L_B$ | $L_B/L_C$ | $m$ |
|-------|-------|-----------|-----|
| Ala-Ser-Tm ($K_rK_yK_zK_{s1}$) \textsuperscript{1/4} | 0.73 ± 0.07 | 4.43 ± 0.06 | 6.2 |
| Δ23Tm ($K_rK_yK_zK_{s1}$) \textsuperscript{1/5} | 0.62 ± 0.05 | 6.07 ± 0.11 | 18 |
| Δ34Tm ($K_rK_yK_zK_{s1}$) \textsuperscript{1/6} | 0.43 ± 0.04 | 3.96 ± 0.05 | 2.1 |
| Δ234Tm ($K_rK_yK_zK_{s1}$) \textsuperscript{1/7} | 0.42 ± 0.04 | 5.17 ± 0.09 | 5.3 |
| Δ345Tm ($K_rK_yK_zK_{s1}$) \textsuperscript{1/8} | 0.270 ± 0.025 | 4.26 ± 0.70 | 2.4 |
| Δ456Tm ($K_rK_yK_zK_{s1}$) \textsuperscript{1/9} | 0.42 ± 0.04 | 4.66 ± 0.03 | 3.5 |

*Note: Elements of $K_r$ are determined in the presence of Ca\textsuperscript{2+} (8, 192, 193, 351). $L_B$ values were determined in the absence of Ca\textsuperscript{2+} (Fig. 4), by analyzing each curve separately and using values for $K_{s1}$, $Y$, $L_B$, $K_r$, and $K_y$ from Fig. 2. ($L_B/L_C$)\textsuperscript{m} is the effect of Ca\textsuperscript{2+} removal on the equilibrium away from the active state of the filament, expressed per troponin. $m$ is the number of actins spanned by each tropomyosin: either 7, 5, or 4. *
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