Opposite Effects of Myosin Subfragment 1 on Binding of Cardiac Troponin and Tropomyosin to the Thin Filament*  

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Michael Cassell and Larry S. Tobacman†  
From the Departments of Internal Medicine and Biochemistry, University of Iowa, Iowa City, Iowa 52246  

To better understand the regulation of striated muscle contraction, the effects of myosin subfragment 1 (S-1) on the actin binding of cardiac troponin and tropomyosin were investigated. Troponin’s affinity for actin-tropomyosin was 4-fold stronger in the absence than in the presence of myosin S-1. CaCl2 had no effect on troponin binding to the thin filament in the presence of myosin S-1. The binding curve was weakly cooperative, implying interactions between adjacent troponin molecules. Myosin S-1 increased (40-200-fold) the affinity of tropomyosin for the thin filament, an effect opposite to the effect of myosin on troponin. This effect was highly cooperative and occurred in the presence of ADP or in the absence of nucleotide. Myosin altered the effect of ionic conditions on tropomyosin-actin binding, consistent with tropomyosin binding to a different site on F-actin in the presence of myosin. The results indicate that troponin-tropomyosin and strongly binding myosin cross-bridges do not compete for an F-actin binding site. Although repositioning of troponin-tropomyosin on the actin filament may be sterically required for tight myosin-actin binding, a myosin-induced conformational change in actin provides a better explanation for the complex effects of myosin on thin filament assembly.

Troponin and tropomyosin regulate striated muscle contraction by causing complex, Ca2+-sensitive effects on the interactions between myosin and actin (1-5). Thermodynamic linkage requires that effects of the regulatory proteins on myosin-actin binding should correspond to effects of myosin on regulatory protein-actin binding; investigation of either process provides information about the other. Also, important models for the mechanism of regulation imply that muscle activation may be accompanied by specific alterations in actin-regulatory protein binding. Therefore, such models can be tested and modified by measuring the process of thin filament assembly. For example, we have recently shown by several methods that the binding of Ca2+ to the regulatory sites on troponin C produces little change in the affinity of troponin or of troponin-tropomyosin for the thin filament (6-8). These observations must somehow be accounted for if Ca2+ binding to troponin C disrupts troponin I-actin binding.

In the present report we describe how thin filament binding by cardiac tropomyosin and troponin is altered by the other ligand believed important in muscle activation: strongly binding myosin cross-bridges. Reminiscent of the classical sterical blocking model (9-11), recent models of regulation (4, 12) suggest that the troponin-tropomyosin complex blocks crucial portions of the actin-myosin interface. Myosin movement of the regulatory proteins away from such a position could be caused by their displacement from the lowest energy (most preferable) binding position or by a conformational change in actin that results in alternate binding sites of the same or even lower free energy (stronger binding). The mechanistic significance of this issue has long been recognized, and a 1976 report by Eaton (13) indicated that myosin increased rather than decreased the affinity of tropomyosin for the thin filament, at least in the absence of troponin. In part because of the enduring interest in variations on the sterical blocking model, the present report returns to this problem for a more detailed study of myosin’s effect on thin filament assembly. The results, which are similar in the absence of nucleotide and in the presence of ADP, indicate that myosin S-1 has opposite effects on the affinities of the two regulatory proteins for the thin filament. Troponin binding to actin-tropomyosin is weakened by myosin S-1, but tropomyosin-actin binding (as previously reported) is profoundly strengthened. Furthermore, the effects of ionic conditions on tropomyosin-actin binding are different in the presence and in the absence of myosin, consistent with binding to a different site on the actin filament.

**EXPERIMENTAL PROCEDURES**

Protein Preparation—Rabbit fast skeletal muscle actin (14) and myosin S-1 (15) were prepared as described previously. Bovine cardiac troponin and tropomyosin were purified as in Tobacman and Adelstein (16). Troponin subunits were purified, troponin T was stochiometrically labeled on Cys190 with [3H]iodoacetic acid (8, 17) (Amersham Corp.; 2 × 108 cpm/μmol), and the ternary troponin complex was then reconstituted from denatured subunits (18). For some of the experiments, the troponin was denatured with guanidine hydrochloride and then labeled on Cys236 (19) with the same isotope as above.

Measurement of Binding to Actin—Radiolabeled troponin or tropomyosin binding to the thin filament was measured by comparing the radioactivity before and after centrifugation at 35,000 rpm in an TLA100 rotor for 30 min at 25 °C. In a control experiment, this method was compared with SDS-PAGE analysis of identical samples, which contained 5 μM F-actin, 0.5 mM EGTA, 10 mM Tris-HCl (pH 7.5), 0.3 mg/ml bovine serum albumin, 0.2 mM dithiothreitol, 5 μM myosin S-1, 3 mM MgCl2, 300 mM KCl, and either 1.5 or 1.2 μM [3H]tropomyosin. As measured by loss of radioactivity in the supernatant, 45 or 52% of the tropomyosin co-sedimented with the actin, depending upon the total concentration of tropomyosin added. This was compared with SDS-PAGE analysis of Coomassie Blue-stained total and supernatant aliquots, quantified with a Bio-Rad two-dimensional gel scanner and intercalated using a troposin standard curve. Duplicate lanes on the gel were analyzed together after background subtraction. The volume loaded in each lane was adjusted to ensure that the amount of tropomyosin fell within the standard curve, between 0.3 and 2.0 μg/lane. By gel scanning, 37% of the tropomyosin pelleted in the sample, for which 45% pelleted by radioactivity, and 43% pelleted by gel scanning for the sample that demonstrated 52% sedimentation by radioactivity. These data exclude a major discrepancy between actual sedimentation of tropomyosin and tropomyosin sedimentation as monitored by PAGE, polyacrylamide gel electrophoresis.

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† To whom correspondence should be addressed.
radiolabeled on troponin T to permit assessment of the association of troponin with the thin filament by co-sedimentation. As previously (8, 19), high ionic strength (0.3 M KCl) was used to prevent both tropomyosin-tropomyosin polymization and nonspecific (i.e. tropomyosin-independent) binding of troponin to actin. Fig. 1 shows that the binding of troponin to actin-tropomyosin was relatively tight, despite the presence of myosin on the actin. Control experiments (not shown) assessed by SDS-PAGE confirmed that the myosin S-1, added in a 1:1 ratio with F-actin, bound tightly to actin in the absence of the regulatory proteins. An excess of myosin S-1 relative to actin was not necessary to saturate the effect of myosin S-1 under these conditions. The tropomyosin, actin, and myosin S-1 concentrations in Fig. 1 resulted in saturation of the actin with tropomyosin in the absence of troponin (see below). Therefore, the figure represents the binding of troponin to a thin filament already saturated with tropomyosin and myosin S-1.

The experiment in Fig. 1 was designed so that the free and actin-bound concentrations of tropomyosin were equal. Despite this, most of the added troponin cosedimented with the thin filament, except in those samples that contained an excess of troponin relative to the sites on the thin filament. This indicates that troponin binds more tightly to actin-tropomyosin-myosin S-1 than to tropomyosin alone. (By equilibrium linkage
Myosin S-1 alters troponin and tropomyosin binding to F-actin

The increase in overall tropomyosin-actin affinity (of the thin filament when myosin S-1 is present. This myosin S-1 should increase the affinity of tropomyosin for actin, and this experimental result has been reported by Eaton et al. (27) (using heavy meromyosin instead of myosin S-1). We obtained similar results (Fig. 2), using conditions identical to those employed in our troponin-binding experiments, described above. Comparison between panels A and B indicates a major change in affinity, with 40-fold higher tropomyosin concentrations required to reach 50% saturation of the thin filament when no myosin S-1 is present. This myosin S-1-induced increase in overall tropomyosin-actin affinity ($y K_1$) is not due to increased end-to-end interactions between adjacent tropomyosins. These interactions are weakened by myosin: 5-fold higher cooperativity in binding is observed in the absence ($y = 178$) rather than in the presence ($y = 34$) of myosin S-1. The increased overall affinity of tropomyosin for the thin filament is attributable to a very large increase in tropomyosin's affinity for an isolated site on the actin filament ($K_o$). Myosin S-1 increases $K_o$ about 200-fold, from $2.3 \times 10^3$ in the absence of cross-bridges to $4.6 \times 10^5$ M$^{-1}$ in the presence of rigor cross-bridges.

Unfortunately, this type of experiment does not permit determination of how much change in $K_o$ occurs when, for example, there is only one myosin S-1 bound to the seven actins to which a tropomyosin is also associating. This is because, when myosin is added in a constant substoichiometric amount, biphasic tropomyosin-actin binding curves are found (27), presumably due to cooperative clustering of the myosin on the thin filament. This prevents assessment of tropomyosin binding to sites with a low density of myosin. However, if the 200-fold effect in Fig. 2 is in fact “spread out” over seven actin-myosin binding events, there would be about a $200^{1/7} = 2.1$-fold effect of each successive myosin S-1 on the gradually increasing affinity of tropomyosin for the thin filament. This compares reasonably well with reported values of an approximately 3-fold effect (26) on what should be a thermodynamically equivalent process: the effect of tropomyosin on myosin S-1 binding to actin.

Highly Cooperative Effect of Myosin S-1 on Tropomyosin-Actin Binding—High ionic strength inhibits the binding to actin of myosin S-1 (26), tropomyosin (19), and troponin-tropomyosin (28, 29). For example, the affinity of tropomyosin for actin is 100-fold stronger in the presence of $60$ mM KCl than in the presence of $300$ mM KCl (19). Accordingly, we speculated that tropomyosin binding to actin-myosin S-1 might be weakened by raising the salt concentration. As shown in Fig. 3, however, even in the presence of $0.5$ M KCl tropomyosin binds tightly to the thin filament if sufficient myosin S-1 is included. At least 75% of added tropomyosin (at a substoichiometric concentration of 0.4 mM) binds to actin in the presence of saturating myosin S-1 concentrations. This corresponds to tropomyosin binding to actin-myosin S-1 that is as tight as is evidenced in the rising portion of the curve in Fig. 2A, performed at $0.3$ mM KCl.

A prominent feature of Fig. 3 is the large amount of cooperativity implied by the very S-shaped curve. Raising the ionic strength by the addition of $0.5$ M KCl allows this degree of cooperativity to become evident, apparently by weakening the separate binding of tropomyosin and myosin S-1 to actin. Not only does each of them bind cooperatively to the thin filament; myosin S-1 very cooperatively influences the cooperative binding of tropomyosin to actin.

Effects of ADP and Tropomyosin on Tropomyosin Binding to Actin-Myosin S-1—ADP significantly weakens myosin S-1 binding to the thin filament (30). Therefore, myosin's effect on tropomyosin-actin binding could be altered by ADP. Control experiments similar to Fig. 3 (not shown) indicated that a 5 mM excess of myosin S-1-ADP relative to F-actin is sufficient to saturate the effect of myosin S-1-ADP on tropomyosin-actin binding to the thin filament (30). Varying concentrations of tropomyosin radiolabeled on Cys$^{350}$ were added to constant concentrations of F-actin in the presence or absence of myosin S-1. Panel A, 4 mM F-actin, no myosin S-1; panel B, 5 mM F-actin plus 5 mM myosin S-1. Ionic conditions were the same as in Fig. 1. Myosin S-1 caused the tropomyosin to bind much more tightly to F-actin. The solid lines are best fit curves with isolated site binding constants ($K_o$) equaling $2.3 \pm 0.4 \times 10^3$ M$^{-1}$ and $4.6 \pm 0.7 \times 10^5$ M$^{-1}$ in the absence and presence of myosin S-1, respectively. The corresponding cooperativity parameters ($y$) for these two conditions were $178 \pm 34$ and $34 \pm 5$. The overall binding constant is given by the product $y K_o$ in each case: 0.4 mM$^{-1}$ in the absence of myosin S-1 and 16 mM$^{-1}$ in the presence of myosin S-1.

Fig. 2. Effect of myosin S-1 on tropomyosin binding to actin in the absence of tropo- myosin. Varying concentrations of tropomyosin radiolabeled on Cys$^{350}$ were added to constant concentrations of F-actin in the absence or presence of myosin S-1. Panel A, 4 mM F-actin, no myosin S-1; panel B, 5 mM F-actin plus 5 mM myosin S-1. Ionic conditions were the same as in Fig. 1. Myosin S-1 caused the tropomyosin to bind much more tightly to F-actin. The solid lines are best fit curves with isolated site binding constants ($K_o$) equaling $2.3 \pm 0.4 \times 10^3$ M$^{-1}$ and $4.6 \pm 0.7 \times 10^5$ M$^{-1}$ in the absence and presence of myosin S-1, respectively. The corresponding cooperativity parameters ($y$) for these two conditions were $178 \pm 34$ and $34 \pm 5$. The overall binding constant is given by the product $y K_o$ in each case: 0.4 mM$^{-1}$ in the absence of myosin S-1 and 16 mM$^{-1}$ in the presence of myosin S-1.
binding. Fig. 4 indicates that the affinity of tropomyosin for acto-S-1 is not significantly decreased by the addition of ADP. The theoretical curve corresponds to a $K_o$ of $6 \times 10^8 \text{M}^{-1}$ and $y$ of 25, values similar to those shown in Fig. 2B in the absence of ADP. According to recent three-dimensional reconstructions of decorated actin filaments (31, 32), the most prominent effect of ADP on actin-myosin S-1 is in the position of the "tail" region of the cross-bridge. This may explain the lack of an effect of ADP on tropomyosin binding to actin-myosin S-1.

The figure also shows that troponin has little effect on binding of tropomyosin to actin-myosin S-1-ADP (solid versus open symbols). Similarly, no significant effect of troponin on tropomyosin binding to actin-myosin S-1 was observed in the absence of ADP (data not shown; conditions as in Fig. 2). This sharply contrasts with results found in the absence of myosin, in which case troponin-tropomyosin has an affinity for actin 2 orders of magnitude greater than that of troponin-myosin alone (6, 7, 19). However, as discussed above in the context of Fig. 1, troponin binds more tightly to actin-tropomyosin-myosin S-1 than to tropomyosin alone. This is consistent, because of equilibrium linkage relationships (6, 7), with an absence of an effect of troponin on tropomyosin binding to actin-myosin S-1. Therefore, the lack of an effect of troponin in Fig. 4 may indicate a limitation in the measurement; tropomyosin binding to actin-myosin S-1 is sufficiently tight and so close to stoichiometric that a further increase in affinity due to troponin is not detected. Additionally, the inability to reliably measure the effect of troponin made it impossible to calculate indirectly troponin’s affinity for actin-tropomyosin-myosin S-1 using equilibrium linkage relationships as was done previously in the absence of myosin S-1 (6). This is not a major limitation, because this affinity is measured directly in Fig. 1. However, it prevented use of the direct measurements (available only in the presence of myosin S-1) to check the validity of the indirect, equilibrium linkage calculations (available only in the absence of myosin S-1).

**Fig. 3.** The effect of myosin S-1 on tropomyosin-actin binding is cooperative and is present at relatively high ionic strength. Ionic conditions were as in Fig. 1, except the KCl concentration was 500 mM. The fraction of added tropomyosin ($Tm$) (total concentration constant at 0.43 mM) that bound to the thin filament ($5 \mu M$ F-actin) is plotted as a function of the variable total concentration of added myosin S-1. Myosin S-1 had little effect on tropomyosin-actin binding until a threshold concentration was reached. However, at least 75% of the tropomyosin bound to the thin filament in the presence of high concentrations of myosin S-1. Since the free tropomyosin concentration was approximately 0.1 mM for the sample containing 10 mM myosin S-1, and since the actin lattice was about 50% saturated with tropomyosin in this sample, this implies an overall affinity of about $10^{13} \mu M^{-1}$. This is similar to the overall affinity ($yK_o$) found in the presence of 300 mM KCl (Fig. 2B).

**Fig. 4.** Binding of tropomyosin to actin-myosin S-1-ADP in the presence and absence of troponin. Ionic conditions were as in Fig. 1 except for the addition of 3 mM ATP. To ensure saturation of the effect of myosin S-1, an excess relative to actin was used: $3 \mu M$ F-actin and 10.5 mM myosin S-1. Open symbols, no troponin; filled symbols, troponin also included, with a concentration that exceeded the total tropomyosin concentration in each sample by 0.4 mM. For comparison with results obtained in the absence of ADP, the solid line shows the calculated binding curve corresponding to $K_o = 6 \times 10^8 \text{M}^{-1}$ and $y = 25, yK_o = 15 \mu M^{-1}$, values similar to those obtained in the presence of added nucleotide.

**DISCUSSION**

The effects of myosin S-1 on troponin and tropomyosin binding to the thin filament are summarized in Fig. 5. Overall binding constants are shown, including any contributions of cooperativity to the overall affinity. To avoid any exaggeration of the effects of myosin, the values shown provide the most conservative estimates for comparison. Myosin has a large effect on tropomyosin’s affinity for the thin filament. The addition of myosin S-1 increases tropomyosin’s affinity for the thin filament at least 40-fold. As discussed under "Results," this is a 200-fold effect if the contribution of cooperativity to the binding is excluded and binding to an isolated site is considered. Perhaps more significantly, Fig. 4 suggests that tropomyosin’s overall affinity of $16 \mu M^{-1}$ may be an underestimate, so the effect of myosin S-1 may be greater than implied by this value.

Fig. 5 also indicates that myosin S-1, in contrast to its effects on tropomyosin, weakens rather than strengthens troponin’s affinity for actin-tropomyosin. The figure compares results from Fig. 1 with experiments performed previously in the absence of myosin S-1 (6). These latter experiments measured troponin’s affinity for an isolated tropomyosin on the actin filament to be $500 \mu M^{-1}$ using an indirect method to obtain this result. However, a correction must be made before comparing isolated site binding from Ref. 6 with overall binding from Fig. 1 of the present work. When troponin’s effects on nearest neighbor cooperativity are taken into consideration ($y = 178$ in the absence of troponin, from Fig. 2A; $y = 43$ in the presence of troponin, data not shown) then troponin’s overall affinity for the thin filament equals $500 \times 43^{178} = 130 \mu M^{-1}$ in the absence of myosin S-1, and myosin S-1 has only the 4-fold effect shown in Fig. 5.

The 4-fold effect of myosin S-1 is based upon a comparison of values determined by different methods, but there is an additional test that can be used for checking the comparison’s validity. In studies of skeletal muscle proteins, troponin-Ca$^{2+}$ has no effect on the binding of myosin S-1 to actin-tropomyosin (26), so myosin S-1 must have no effect on troponin-Ca$^{2+}$ binding to actin-tropomyosin. A similar finding (i.e. little effect of...
fig. 5. Schematic representation of opposite effects of myosin S-1 on the affinities of tropomyosin and troponin for the thin filament. The values shown correspond to overall binding constants, including any contributions or energetic penalty from cooperative interactions with adjacent regions of the thin filament (which are not seen in this simplified diagram). The addition of myosin S-1 increases the affinity of tropomyosin for actin by at least 40-fold. In contrast, myosin S-1 decreases the affinity of troponin for the thin filament, a 4-fold effect. In the case of tropomyosin binding to actin or actin-myosin S-1, the figure shows the product $k_2^*$ from Fig. 2. For troponin binding to a tropomyosin-saturated, myosin-saturated thin filament, the result is from Fig. 1. For tropomyosin binding to a tropomyosin-saturated thin filament in the absence of myosin, the value of 130 mM$^{-1}$ is calculated by correcting the affinity constant of troponin for an isolated tropomyosin on F-actin (500 mM$^{-1}$) found in the presence (27) is less than the effect of myosin S-1 in Fig. 2, determined in the presence of 300 mM KCl. Finally, unlike the very Mg$^{2+}$- dependent tropomyosin-actin binding observed in the absence of myosin S-1 (35), we found no effect of lowering the MgCl$_2$ concentration to 0.2 mM when myosin S-1 was present (data not shown), and Eaton (13) found tight tropomyosin binding to actin-myosin S-1 even in the presence of EDTA. These results suggest that the specific protein-protein contacts involved in the interaction of tropomyosin-actin binding may be different in the presence and in the absence of myosin, with ionic interactions less important when myosin is present.

The present observation that myosin S-1 weakens troponin binding to the thin filament is arguably consistent with a steric displacement of troponin from its optimal position for binding. In comparison with the effect of troponin C regulatory site Ca$^{2+}$-, which weakens troponin-thin filament binding with an effect that is only 2-fold (6–8), the effect of myosin is not much larger, just 4-fold. Furthermore, troponin’s affinity for actin-tropomyosin-myosin independently implies tight troponin binding to actin-tropomyosin-myosin S-1, since most of the added troponin cosediments despite significant free concentrations of tropomyosin. These data suggest that thin filament-bound troponin may directly interact with actin as well as with tropomyosin, even in the presence of rigor cross-bridges. In summary, tight binding myosin cross-bridges have complex effects on the affinities of troponin and tropomyosin for the thin filament. Tropomyosin binding is promoted by myosin S-1, but troponin binding occurs with lower affinity. The results are most easily explained by suggesting that myosin causes a complex change in the quaternary structure of the thin filament, including opposite effects on the binding of the two regulatory proteins. We suggest that allosteric as well as steric effects of myosin are involved in thin filament activation and in the regulation of muscle contraction.

REFERENCES
1. Ebashi, S., Endo, M., and Ohtsuki, I. (1969) Q. Rev. Biophys. 2, 351–384
2. Leavis, P. C., and Gergely, J. (1984) CRC Crit. Rev. Biochem. 16, 235–305
3. Chalovich, J. M. (1992) Pharmacol. Ther. 55, 95–148
4. Geeves, M. A., and Conibear, P. B. (1995) Biophys. J. 68, 194a-205s
5. Tobacman, L. S. (1996) Annu. Rev. Physiol. 58, 477–481
6. Dahlia, R., Butters, C. A., and Tobacman, L. S. (1994) J. Biol. Chem. 269, 29457–29461
7. Fisher, D., Wang, G., and Tobacman, L. S. (1995) J. Biol. Chem. 270, 25455–25460
8. Huynh, Q., Butters, C. A., Leiden, J. M., and Tobacman, L. S. (1996) Biophys. J. 70, 1447–1455
9. Ebashi, S., Endo, M., and Ohtsuki, I. (1969) Q. Rev. Biophys. 2, 351–384
10. Leavis, P. C., and Gergely, J. (1984) CRC Crit. Rev. Biochem. 16, 235–305
11. Chalovich, J. M. (1992) Pharmacol. Ther. 55, 95–148
12. Geeves, M. A., and Conibear, P. B. (1995) Biophys. J. 68, 194a-205s
13. Tobacman, L. S. (1996) Annu. Rev. Physiol. 58, 477–481
14. Dahlia, R., Butters, C. A., and Tobacman, L. S. (1994) J. Biol. Chem. 269, 29457–29461
15. Fisher, D., Wang, G., and Tobacman, L. S. (1995) J. Biol. Chem. 270, 25455–25460
16. Huynh, Q., Butters, C. A., Leiden, J. M., and Tobacman, L. S. (1996) Biophys. J. 70, 1447–1455

Myosin S-1 Alters Troponin and Tropomyosin Binding to F-actin

Myosin S-1 binding to myosin-actin binding by myosin implies a myosin-induced conformational change in actin. Therefore, although any myosin-induced repositioning of tropomyosin also fulfills steric requirements, it is an allosteric effect that facilitates any repositioning of tropomyosin. We suggest that a combination of both steric and allosteric effects is most consistent with available structural and biochemical data.

A prominent feature of both tropomyosin and tropomyosin-troponin binding to actin in the presence of myosin S-1 is the inhibition of these processes by salt (7, 17, 19, 28). Therefore, it is interesting that tropomyosin binding to actin-myosin S-1 is very tight even in the presence of 0.5 mM KCl. Although these data do not by themselves exclude a significant sensitivity to salt, other evidence also supports the interpretation that thin filament assembly is less influenced by ionic strength in the presence of myosin. For example, when the ionic strength is much lower than in the present study this has a greater effect in the absence than in the presence of myosin, so that myosin becomes less significant in thin filament assembly.

In the presence of 60 mM KCl, myosin S-1 has only a 4-fold effect promoting unacylated tropomyosin-troponin binding to an isolated site on F-actin (28). Also, the reported effect of heavy meromyosin C on tropomyosin-actin binding in the presence of 60 mM KCl (27) is less than the effect of myosin S-1 in Fig. 2, determined in the presence of 300 mM KCl. Finally, unlike the very Mg$^{2+}$-dependent tropomyosin-actin binding observed in the absence of myosin S-1 (35), we found no effect of lowering the MgCl$_2$ concentration to 0.2 mM when myosin S-1 was present (data not shown), and Eaton (13) found tight tropomyosin binding to actin-myosin S-1 even in the presence of EDTA. These results suggest that the specific protein-protein contacts involved in tropomyosin-actin binding may be different in the presence and in the absence of myosin, with ionic interactions less important when myosin is present.

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9. Huxley, H. E. (1972) Cold Spring Harbor Symp. Quant. Biol. 37, 361–376
10. Parry, D. A. D., and Squire, J. M. (1973) J. Mol. Biol. 75, 33–55
11. Haselgrove, J. C. (1972) Cold Spring Harbor Symp. Quant. Biol. 37, 341–352
12. Holmes, K. C. (1995) Biophys. J. 68, 25–75
13. Eaton, B. L. (1976) Science 192, 1337–1339
14. Spudich, J. A., and Watt, S. (1971) J. Biol. Chem. 246, 4866–4871
15. Weeds, A. G., and Taylor, R. S. (1975) Nature 257, 54–56
16. Tobacman, L. S., and Adelstein, R. S. (1986) Biochemistry 25, 798–802
17. Mehegan, J. P., and Tobacman, L. S. (1991) J. Biol. Chem. 266, 966–972
18. Tobacman, L. S., and Lee, R. (1987) J. Biol. Chem. 262, 4059–4064
19. Hill, L. E., Mehegan, J. P., Butters, C. A., and Tobacman, L. S. (1992) J. Biol. Chem. 267, 16106–16113
20. McGhee, J. D., and von Hippel, P. H. (1974) J. Mol. Biol. 86, 469–489
21. Tobacman, L. S., and Sawyer, D. (1990) J. Biol. Chem. 265, 933–939
22. Hill, T. L. (1985) Cooperativity Theory in Biochemistry. Springer-Verlag New York, Inc., New York
23. Trybus, K. M., and Taylor, E. W. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 7209–7213
24. Greene, L. E., and Eisenberg, E. (1980) Proc. Natl. Acad. Sci. U. S. A. 77, 2616–2620
25. Nagashima, H., and Asakura, S. (1982) J. Mol. Biol. 155, 409–428
26. Williams, D. L., Jr., and Greene, L. E. (1983) Biochemistry 22, 2770–2774
27. Szczesna, D., Borovikov, Y. S., and Sobieszak, A. (1989) Biol. Chem. Hoppe-Seyler 370, 399–407
28. Willadsen, K. A., Butters, C. A., Hill, L. E., and Tobacman, L. S. (1992) J. Biol. Chem. 267, 23746–23752
29. Butters, C. A., Willadsen, K. A., and Tobacman, L. S. (1993) J. Biol. Chem. 268, 15565–15570
30. Greene, L. E. (1981) Biochemistry 20, 2120–2126
31. Whittaker, M., Wilson-Kubalek, E. M., Smith, J. E., Faust, L., Milligan, R. A., and Sweeney, H. L. (1995) Nature 378, 748–751
32. Jontes, J. D., Wilson-Kubalek, E. M., and Milligan, R. A. (1995) Nature 378, 751–753
33. Lorenz, M., Poole, K. J. V., Popp, D., Rosenbaum, G., and Holmes, K. C. (1995) J. Mol. Biol. 246, 108–119
34. Rayment, I., Holden, H. M., Whittaker, M., Yohn, C. B., Lorenz, M., Holmes, K. C., and Milligan, R. A. (1993) Science 261, 58–66
35. Yang, Y.-Z., Korn, E. D., and Eisenberg, E. (1979) J. Biol. Chem. 254, 2084–2088
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