Comparison of the Effects of Tropomyosin and Troponin–Tropomyosin on the Binding of Myosin Subfragment 1 to Actin†

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ABSTRACT: The effect of tropomyosin alone on the binding of myosin subfragment 1 (S-1) to actin was compared to that of troponin–tropomyosin, with and without Ca^{2+}. Over a range of ionic strength and with different ligands (adenyl-5′-yl imidodiphosphate, pyrophosphate, and adenosine 5′-diphosphate), tropomyosin confers slight cooperativity to the binding of S-1 to actin. In the presence of Ca^{2+}, troponin does not affect the cooperative action of tropomyosin alone. In addition, troponin–tropomyosin and tropomyosin alone are also identical in their ability to strengthen the binding of S-1·ligand to actin 3-fold and the binding of S-1 alone to actin 7-fold, at high levels of saturation of the actin with S-1. Although troponin does not significantly affect the cooperative action of tropomyosin alone in the presence of Ca^{2+}, it does markedly enhance the cooperativity in the binding of S-1 to actin in the absence of Ca^{2+}.

The troponin–tropomyosin complex confers positive cooperativity to the binding of myosin subfragment 1[1] to actin in the absence of nucleotide, in the presence of ADP, and in the presence of AMP-PNP (Greene & Eisenberg, 1980a; Trybus & Taylor, 1980; Greene, 1982): in the presence and absence of Ca^{2+}, S-1 binds more weakly to the troponin–tropomyosin–actin complex (regulated actin) at low levels of S-1 binding site saturation than at high levels of saturation. These results were accounted for by the model of Hill et al. (1980) in which the tropomyosin–actin units of regulated actin exist in two states: one state which binds S-1 weakly (weak state) and another state which binds S-1 strongly (strong state). Furthermore, it was observed that the transition from the weak to the strong state requires more S-1 to be bound to actin in the absence than in the presence of Ca^{2+}. The model of Hill et al. therefore proposed that the equilibrium between the weak and strong states is shifted toward the strong state both by Ca^{2+} and by the binding of S-1.

Both tropomyosin alone and troponin–tropomyosin (with and without Ca^{2+}) also confer positive cooperativity on the acto·S-1 ATPase activity (Bremel & Weber, 1972; Bremel et al., 1972). The effect of tropomyosin on this activity has been reported to be identical with that of troponin–tropomyosin with Ca^{2+} (Murray et al., 1980a,b). From these observations, Murray et al. (1982) assumed that the effects of tropomyosin and troponin–tropomyosin with Ca^{2+} on acto·S-1 binding are also identical. However, other workers found that the effect of tropomyosin on the actin-activated ATPase activity was different from that of troponin–tropomyosin with Ca^{2+}; tropomyosin had an effect intermediate between that of troponin–tropomyosin with Ca^{2+} and that of troponin–tropomyosin in the absence of Ca^{2+} (Lehrer & Morris, 1982; Nagashima & Asakura, 1982; Williams & Swenson, 1982). These later observations imply that tropomyosin may have different effects on acto·S-1 binding than troponin–tropomyosin with Ca^{2+}.

In the present study, we have compared the effect of tropomyosin alone on the binding of S-1 to actin in the presence of AMP-PNP, PP_i, and ADP with that of troponin–tropomyosin both with and without Ca^{2+} over a wide range of S-1 to actin ratios. We find that tropomyosin confers a slightly cooperative 3-fold strengthening to the binding of S-1 to actin in the presence of AMP-PNP, PP_i, and ADP, an effect which is virtally identical with the effect of troponin–tropomyosin plus Ca^{2+}. This indicates that troponin has no significant effect on the binding of S-1 to regulated actin in the presence of Ca^{2+}. However, in the absence of Ca^{2+}, troponin–tropomyosin confers pronounced cooperativity to acto·S-1 binding; i.e., a much greater occupancy of the actin with S-1 is required for the actin filament to undergo the transition to the strong state. Thus, the major effect of troponin on the binding of S-1 to tropomyosin–actin complex is to increase the cooperativity of the binding in the absence of Ca^{2+}.

Materials and Methods

Proteins. Myosin was prepared from rabbit back and leg muscle according to the method of Kielley & Harrington (1960) and was radioactively labeled by covalent modification of SH-1 by iodo[^{14}C]acetamide (Amersham) according to the method of Greene & Eisenberg (1980b). The average extent of labeling of 13 preparations was 0.99 ± 0.09 label per myosin head. Myosin subfragment 1 (S-1) was prepared from radioactively labeled myosin by the method of Weeds & Taylor (1975) except that 1 mM DTT was included in our solutions. F-Actin was prepared by the method of Spudich & Watt (1971) with an additional centrifugation at 3.3 M KCl to separate α-actinin from the actin. The F-actin was treated with Dowex 1-X2Cl$^-$ to eliminate unbound nucleotide. Troponin–tropomyosin was prepared according to the method of Eisenberg & Kielley (1974). Tropomyosin was separated from the troponin–tropomyosin complex by hydroxylapatite chromatography according to the same method. Protein concentrations were determined spectrophotometrically at 280 nm by using the following absorbances and molecular weights: myosin, $E^{0.1\%} = 0.56$ cm^2/mg, M_r 480 000; S-1, $E^{0.1\%} = 0.75$ cm^2/mg, M_r 120 000; actin, $E^{0.1\%} = 1.15$ cm^2/mg, M_r 42 000; troponin–tropomyosin, $E^{0.1\%} = 0.38$ cm^2/mg, M_r 150 000; tropomyosin, $E^{0.1\%} = 0.33$ cm^2/mg, M_r 68 000.

Ligands. AMP-PNP and Ap_5A were obtained from Sigma. ADP was obtained from P-L Biochemicals. All lots used were

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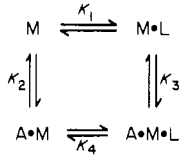
[1] Abbreviations: ADP, adenosine 5′-diphosphate; AMP-PNP, adenyl-5′-yl imidodiphosphate; Ap_5A, P^1,P^5-di(adenosine-5′) pentaphosphate; DTT, dithiothreitol; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; HMM, heavy meromyosin; PP_i, inorganic pyrophosphate; S-1, myosin subfragment 1.

Scheme I



assayed for purity by poly(ethylenimine)–cellulose thin-layer chromatography in 0.75 M KH_2PO_4 (pH 3.4) and acid-labile phosphate (Yount et al., 1971). The AMP-PNP was judged to be better than 90% pure; ADP was better than 95% pure. Sodium pyrophosphate was obtained from Fisher.

Binding Studies. Acto·S-1 binding was measured with either a Beckman preparative ultracentrifuge or a Beckman Airfuge. In both cases, sample solutions were prepared by adding the actin, ^{14}C-labeled S-1, and tropomyosin or troponin–tropomyosin last. After incubation at 25 °C for 30 min, samples were centrifuged at 80000*g* for 1 h in a type 40 rotor in a Beckman L2-65B preparative ultracentrifuge at 25 °C or at 178000*g* for 20 min in the Beckman Airfuge. A portion of the sample before centrifugation was analyzed for ^{14}C in a Beckman LS-250 to determine the total S-1 concentration. A portion of the supernatant after centrifugation was also analyzed to determine free S-1 concentration. The difference between total and free gives the bound S-1 concentration, which divided by the actin concentration gives θ, the moles of S-1 bound per actin monomer. The association constant is $\theta/[(1 - \theta)[S\text{-}1]_{free}]$. Initial sample volumes were 4.0 mL for the preparative ultracentrifugation and 1.0 mL for the Airfuge. The Airfuge rotor was maintained at 25 °C prior to centrifugation, and the rotor temperature was measured with a small thermistor after each run. Room temperature was adjusted to maintain rotor temperatures at 25 ± 1 °C.

The effect of pure tropomyosin on S-1 binding to actin was compared in 0.5 mM Ca^{2+} and 1 mM EGTA at low actin site saturations to ensure that no contamination from troponin was present. No difference in binding was observed between the two conditions for pure tropomyosin. The presence of contaminating myokinase could create problems for measuring S-1 binding, as ATP could be produced from ADP, which would weaken the acto·S-1 binding. We therefore checked for myokinase activity at each solution condition by using the method described in Greene (1982). We routinely used ~300 μM Ap_5A in our studies and found no effect of myokinase contamination. To ensure the S-1 sedimented when bound to actin and stayed in the supernatant in the absence of actin, controls were run in the absence of nucleotide. When actin was not present, 90–95% of the S-1 remained in the supernatant after centrifugation, while in the presence of actin 95% of the S-1 sedimented.

Treatment of Data. The binding of S-1 to actin in the presence of ligand fits Scheme I for the formation of acto·S-1·ligand ternary complex where A = a monomer in an F-actin filament, M = S-1, L = ligand, and K = association constants. We used the following equation derived from this scheme (Greene & Eisenberg, 1978, 1980a) to analyze the data in Figure 5:

$$K_{app} = K_3/(K_4[L]) + K_3 \quad (1)$$

When the data are plotted as K_{app} vs. $1/[L]$, the ordinate intercept is then equal to K_3, the binding constant of actin to the S-1·ligand complex, and the abscissa intercept equals $-K_4$, the binding constant of ligand to acto·S-1.

Theoretical curves for cooperative binding were fitted to the data by using the procedure described in Hill et al. (1980).

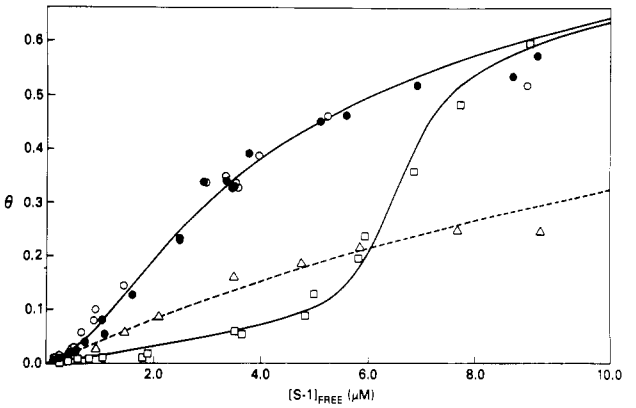


FIGURE 1: Binding of S-1 to actin in the presence of AMP-PNP. The conditions were 4 mM AMP-PNP, 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM $CaCl_2$ or 1 mM EGTA, and 10 mM imidazole at pH 7.0, 25 °C (μ = 86 mM). The binding was examined in the Airfuge using 10 μM actin with additions of 2.9 μM pure tropomyosin (two tropomyosins per seven actins), or 2.9 μM troponin–tropomyosin (two troponin–tropomyosins per seven actins). The dashed theoretical curve for the independent binding of S-1 to unregulated actin (open triangles) was fitted to the data by using $K_{unreg} = 4.5 \times 10^4$ M^{-1}. The theoretical curves for the cooperative binding of S-1 to tropomyosin–actin or troponin–tropomyosin–actin were fitted to the data points by using the model of Hill et al. (1980) with the following parameters: tropomyosin–actin (solid circles) and troponin–tropomyosin–actin with Ca^{2+} (open circles), $K_{strong} = 1.6 \times 10^5$ M^{-1}, $K_{weak} = 1.5 \times 10^4$ M^{-1}, $L' = 3$, $Y = 10$; troponin–tropomyosin–actin without Ca^{2+} (open squares), $K_{strong} = 1.7 \times 10^5$ M^{-1}, $K_{weak} = 1.7 \times 10^4$ M^{-1}, $L' = 88$, $Y = 20$. Data points for tropomyosin–actin include conditions of 0.5 mM Ca^{2+} and 1 mM EGTA.

Results

We investigated the binding of S-1 to actin–regulatory protein complexes in the presence of 4 mM AMP-PNP, 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM DTT, and 10 mM imidazole at pH 7.0, 25 °C (μ = 86 mM). As a control, the binding of S-1·AMP-PNP to unregulated actin was first examined. As shown in Figure 1 (open triangles), the binding of S-1·AMP-PNP to unregulated actin results in a hyperbolic curve ($K_{unreg} = 4.5 \times 10^4$ M^{-1}) when the data are plotted as fraction of actin sites occupied by S-1 (θ) vs. free S-1 concentration. Therefore, as was shown previously (Greene & Eisenberg, 1978), S-1 binds independently along the actin filament. As expected, identical results were obtained in the presence of 0.5 mM Ca^{2+} and in the presence of 1 mM EGTA.

Effect of Tropomyosin on the Binding of S-1·AMP-PNP to Actin. Addition of tropomyosin to the actin (two tropomyosins per seven actins) results in a binding isotherm with a sigmoid shape (closed circles), indicating cooperative binding of S-1·AMP-PNP to actin in the presence of tropomyosin. There is a 3-fold strengthening of S-1·AMP-PNP binding to tropomyosin–actin when the S-1 binding sites on actin are more than 25% filled ($K = 1.6 \times 10^5$ M^{-1}), whereas when the S-1 binding sites are less than about 2% filled, the binding of S-1·AMP-PNP to tropomyosin–actin appears the same as that of S-1·AMP-PNP to actin alone. In the context of the model of Hill et al. (1980), this does not necessarily indicate that S-1 binds to the weak state with the same affinity as it does to unregulated actin, rather the actin–tropomyosin filament may be partially shifted to the strong state even in the absence of S-1. The slight cooperativity conferred by tropomyosin (a 3-fold increase in the binding constant between the lowest and highest S-1 binding site saturations) is shown more clearly in Figure 2, which is an expansion of a portion of Figure 1. At low binding site saturation, the data always fall below the theoretical curve (dashed line) for independent binding. Since identical results were obtained in the presence of 0.5 mM Ca^{2+}

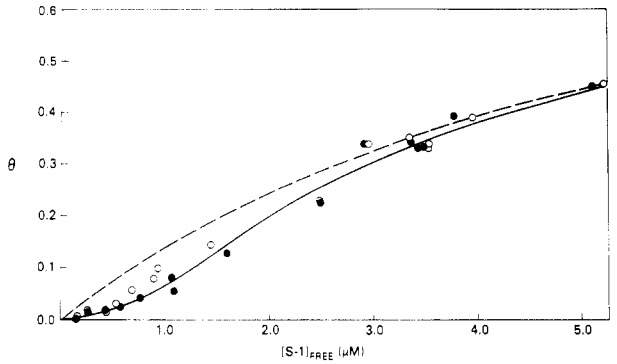


FIGURE 2: Binding of S-1·AMP-PNP to tropomyosin–actin and troponin–tropomyosin–actin with Ca^{2+}: comparison of cooperative and independent theoretical binding curves. This figure is an expansion of a portion of Figure 1. The data points and solid theoretical curve are the same as in Figure 1. The dashed theoretical curve is for independent binding of S-1 to actin with a binding constant of 1.6×10^5 M^{-1}.

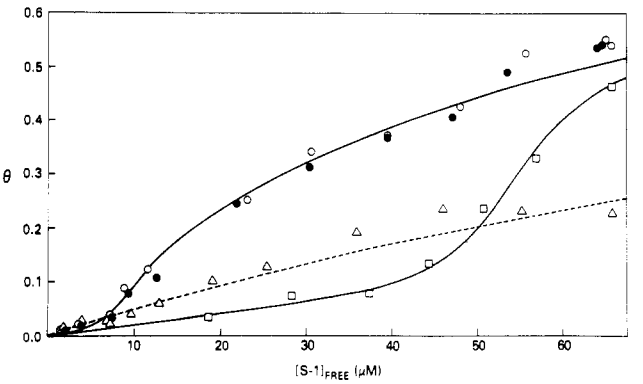


FIGURE 3: Binding of S-1·AMP-PNP to actin at 186 mM ionic strength. Solution conditions were identical with those in Figures 1 and 2 except [KCl] = 150 mM. The binding was examined in the Airfuge with 40–50 μM actin with additions of tropomyosin or troponin–tropomyosin to give a 2/7 mole ratio with actin. The dashed theoretical curve for the independent binding of S-1 to unregulated actin (open triangles) was fitted to the data by using $K_{unreg} = 5.1 \times 10^3$ M^{-1}. The theoretical curves for cooperative binding were fitted to the data by using the model of Hill et al. (1980) with the following parameters: tropomyosin–actin (closed circles) and troponin–tropomyosin–actin with Ca^{2+} (open circles), $K_{strong} = 1.6 \times 10^4$ M^{-1}, $K_{weak} = 2.0 \times 10^3$ M^{-1}, $L' = 2.3$, $Y = 20$; troponin–tropomyosin–actin without Ca^{2+} (open squares), $K_{strong} = 1.6 \times 10^4$ M^{-1}, $K_{weak} = 2.0 \times 10^3$ M^{-1}, $L' = 38$, $Y = 20$. Data points for tropomyosin–actin include conditions of 0.5 mM Ca^{2+} and 1 mM EGTA.

and in the presence of 1 mM EGTA, this cooperativity is not due to contamination by troponin. A test for the presence of myokinase activity, which could weaken binding at low S-1 concentrations, was performed in every binding experiment (see Materials and Methods). In the absence of Ap_5A, myokinase activity was never detected in the pure tropomyosin, although it was present in the troponin–tropomyosin complex. This suggests that the additional steps used to purify the tropomyosin (hydroxylapatite column) separated the myokinase activity from the tropomyosin.

Comparison of Effects of Tropomyosin with Effects of Troponin–Tropomyosin. The binding of S-1·AMP-PNP to the troponin–tropomyosin–actin complex in the presence of Ca^{2+} (open circles, Figures 1 and 2) is virtually identical with that observed for binding to the tropomyosin–actin complex. In both cases, there is slight cooperativity in the binding isotherm, with 3-fold strengthening of S-1·AMP-PNP binding to actin as the S-1 binding sites on actin become filled. Therefore, troponin has no detectable effect on S-1·AMP-PNP binding to the tropomyosin–actin complex in the presence of Ca^{2+}.

On the other hand, in the absence of Ca^{2+} (open squares, Figure 1) troponin–tropomyosin causes pronounced cooperativity in S-1·AMP-PNP binding to actin, as reported previously (Greene, 1982). When the sites on actin are mostly empty, there is marked inhibition of the binding of S-1·AMP-PNP to actin. When the S-1 binding sites on actin become filled, the S-1·AMP-PNP binding affinity then increases to 3 times the value observed for actin alone. In order to reach a given S-1 binding site occupancy below 50%, a much greater concentration of free S-1 is required for troponin–tropomyosin–actin in the absence of Ca^{2+} than is required for tropomyosin–actin or troponin–tropomyosin–actin plus Ca^{2+}. On the other hand, when the S-1 binding site occupancy is greater than 60%, the effects of tropomyosin, troponin–tropomyosin plus Ca^{2+}, and troponin–tropomyosin without Ca^{2+} are indistinguishable from each other; in all cases the binding strength is increased about 3-fold over the level obtained with unregulated actin.

Effect of Ionic Strength and Different Ligands. To determine whether ionic strength affected our results, we increased the ionic strength from 86 to 186 mM, thereby weakening the binding of S-1·AMP-PNP to actin about 10-fold. Figure 3 shows the binding isotherms of S-1·AMP-PNP to actin at μ = 186 mM in the presence of both tropomyosin and troponin–tropomyosin (with and without Ca^{2+}), as well as in the absence of regulatory proteins. The results are very similar to those obtained at lower ionic strength: (1) tropomyosin again confers a cooperative 3-fold strengthening effect to S-1·AMP-PNP binding to actin ($K = 1.6 \times 10^4$ M^{-1} at high S-1 binding site saturation compared to $K_{unreg} = 5.1 \times 10^3$ M^{-1} for the binding constant obtained with unregulated actin), and (2) the effect of troponin–tropomyosin in the presence of Ca^{2+} is virtually identical with that of tropomyosin alone and very different from that of troponin–tropomyosin in the absence of Ca^{2+}.

To show that these results apply to the equilibrium binding of S-1 to actin in the presence of other ligands, we also examined the effect of tropomyosin and troponin–tropomyosin on the binding of S-1 to actin in the presence of PP_i and ADP. PP_i weakens the acto·S-1 binding to a similar extent as AMP-PNP, whereas ADP weakens this binding to a lesser extent (Greene & Eisenberg, 1980b). The binding isotherms of S-1 to tropomyosin–actin, troponin–tropomyosin–actin, and unregulated actin obtained in the presence of PP_i and ADP are shown in parts A and B of Figure 4, respectively. Again, the results with both PP_i and ADP are clearly similar to those obtained with AMP-PNP. Therefore, over a wide range of ionic strength and with several different ligands, tropomyosin has the same effect on S-1 binding as troponin–tropomyosin plus Ca^{2+}.

Strengthening of S-1 Binding to Actin by Tropomyosin and Troponin–Tropomyosin. Recently, Murray et al. (1982) reported that, in the presence of Ca^{2+}, troponin–tropomyosin does not strengthen the binding of S-1·AMP-PNP to actin when the acto·S-1 complex is saturated with nucleotide. However, as shown in Figures 1 and 3, at 4 mM AMP-PNP, we observe that both tropomyosin and troponin–tropomyosin strengthen the binding of S-1·ligand to actin about 3-fold when the sites on actin are mostly occupied with S-1. Since at 4 mM AMP-PNP the acto·S-1 complex may not be fully saturated with ligand, we next examined whether this strengthening also occurs at full saturation.

The method which was used has been described previously (Greene & Eisenberg, 1978; Murray et al., 1982; Greene,

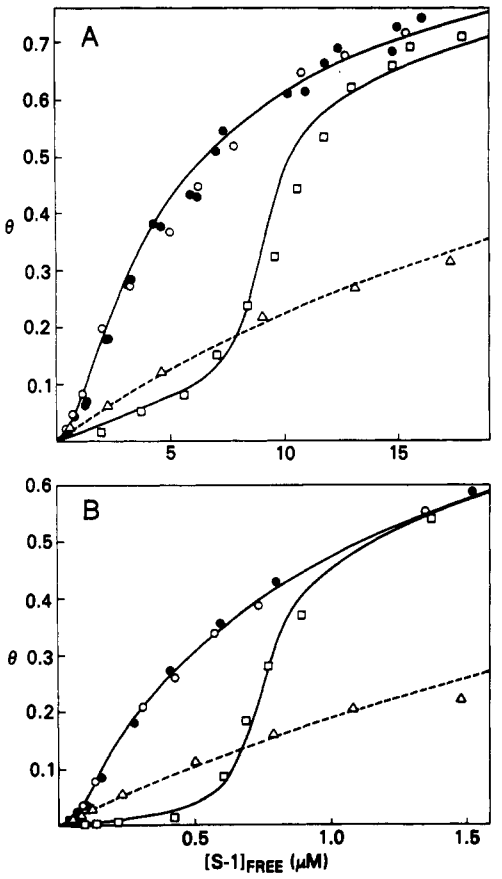


FIGURE 4: Binding of S-1 to actin in the presence of either PP_i or ADP. (A) The conditions were 4 mM PP_i, 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM DTT, 1 mM EGTA with or without 1 mM $CaCl_2$, and 10 mM imidazole at pH 7.0, 25 °C. The binding was examined in the preparative ultracentrifuge using 10 μM actin with additions of 2.9 μM tropomyosin or troponin–tropomyosin (2/7 mole ratio with actin). The dashed theoretical curve for the independent binding of S-1 to unregulated actin (open triangles) was fitted to the data by using $K_{unreg} = 2.9 \times 10^4\ M^{-1}$. The theoretical curves were fitted to the data by using the model of Hill et al. (1980) with the following parameters: tropomyosin–actin (solid circles) and troponin–tropomyosin–actin with Ca^{2+} (open circles), $K_{strong} = 1.5 \times 10^5\ M^{-1}$, $K_{weak} = 1.7 \times 10^4\ M^{-1}$, $L' = 3$, $Y = 10$; troponin–tropomyosin–actin without Ca^{2+} (open squares), $K_{strong} = 1.3 \times 10^5\ M^{-1}$, $K_{weak} = 1.6 \times 10^4\ M^{-1}$, $L' = 88$, $Y = 20$. Data points for tropomyosin–actin include conditions of 1 mM EGTA with and without 1 mM Ca^{2+}. (B) The conditions were 3 mM ADP, 200 mM KCl, 5 mM $MgCl_2$, 0.5 mM DTT, 1 mM EGTA or 0.5 mM Ca^{2+}, and 10 mM imidazole at pH 7.0, 25 °C. The binding was examined in the preparative ultracentrifuge using 4 μM actin with additions of 1.1 μM tropomyosin or troponin–tropomyosin (2/7 mole ratio with actin). The dashed theoretical curve for the independent binding of S-1 to unregulated actin (open triangles) was fitted to the data by using $K_{unreg} = 2.3 \times 10^5\ M^{-1}$. The theoretical curves were fitted to the data by using the model of Hill et al. (1980) with the following parameters: tropomyosin–actin (solid circles) and troponin–tropomyosin–actin with Ca^{2+} (open circles), $K_{strong} = 9 \times 10^5\ M^{-1}$, $K_{weak} = 4.5 \times 10^4\ M^{-1}$, $L' = 1.7$, $Y = 20$; troponin–tropomyosin–actin without Ca^{2+} (open squares), $K_{strong} = 9 \times 10^5\ M^{-1}$, $K_{weak} = 4.5 \times 10^4\ M^{-1}$, $L' = 27$, $Y = 20$. Data points for tropomyosin–actin include conditions of 0.5 mM Ca^{2+} and 1 mM EGTA.

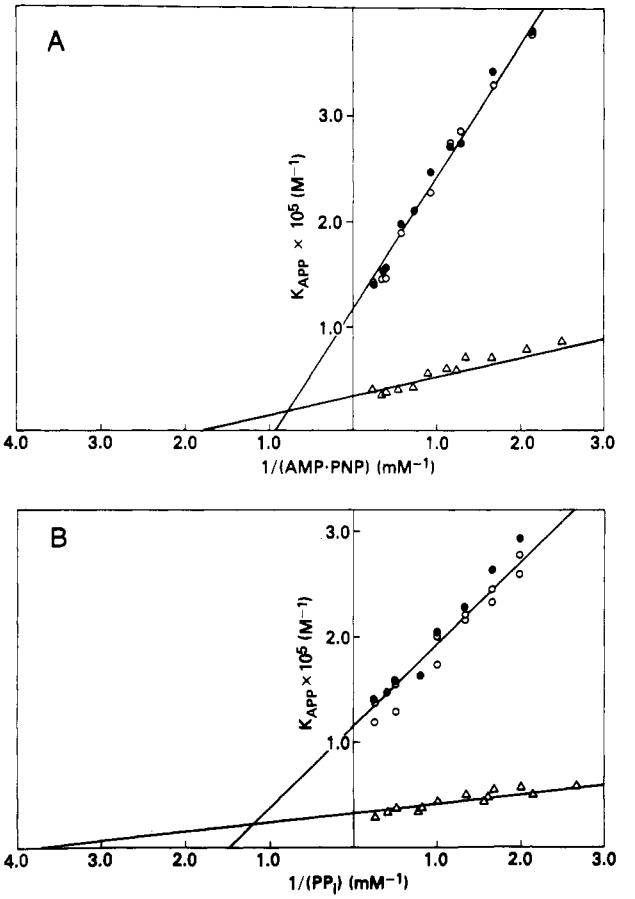


FIGURE 5: Binding of S-1 to actin in the presence of regulatory proteins as a function of [ligand]. In (A), the binding of S-1·AMP-PNP to actin (open triangles), tropomyosin–actin (solid circles), and troponin–tropomyosin–actin with Ca^{2+} (open circles) was examined in the Airfuge under the following conditions: 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM $CaCl_2$, 10 mM imidazole at pH 7.0, 25 °C, and varying AMP-PNP concentrations (0.4–4 mM). Protein concentrations were 10 μM actin and 8 μM S-1, with additions of 2.9 μM tropomyosin or troponin–tropomyosin (2/7 mole ratio to actin). In (B), the binding of S-1·PP_i to actin (open triangles), tropomyosin–actin (solid circles), and troponin–tropomyosin–actin with Ca^{2+} (open circles) was examined in the preparative ultracentrifuge under the following conditions: 50 mM KCl, 5 mM $MgCl_2$, 0.5 mM DTT, 1 mM Ca-EGTA, 10 mM imidazole at pH 7.0, 25 °C, and varying PP_i concentrations (0.4–4 mM). Protein concentrations were 10 μM actin and 8 μM S-1, with additions of 2.9 μM tropomyosin or troponin–tropomyosin (2/7 mole ratio to actin). The actin was always at least one-fourth saturated with S-1 ($\theta > 0.25$) to ensure that the S-1 was binding in the noncooperative strong binding region. Linear regression analysis was used to analyze these data, and the data for tropomyosin–actin and troponin–tropomyosin–actin with Ca^{2+} were treated together.

1982). The binding constant of S-1 to actin is measured as a function of ligand concentration and is plotted as a function of 1/[ligand] (see Materials and Methods). The data for AMP-PNP and PP_i are shown in parts A and B of Figure 5, respectively. In the presence of AMP-PNP and PP_i, the binding of S-1 to tropomyosin–actin or troponin–tropomyosin–actin with Ca^{2+} is about 3.5-fold stronger than it is to unregulated actin when the data are extrapolated to infinite ligand concentration (y intercept). Therefore, the strengthening effect conferred by tropomyosin and troponin–tropomyosin with Ca^{2+} appears to occur at all ligand concentrations.

Although Murray et al. did not observe a strengthening of the binding of S-1 to actin at saturating concentrations of AMP-PNP, they did observe a 3–6-fold strengthening of the binding of S-1 to actin by troponin–tropomyosin with Ca^{2+} in the absence of nucleotide. To determine whether we observe a similar strengthening, we applied the principle of detailed balance to the data in Figure 5 (see Scheme I under Materials and Methods). The extent to which the binding of S-1 alone to actin is strengthened by tropomyosin or troponin–tropomyosin is determined by the ratio of the y intercepts obtained in the presence and absence of regulatory proteins ($K_{3,reg}/K_{3,unreg}$) divided by the ratio of the x intercepts ($K_{4,reg}/K_{4,unreg}$). The x intercepts give the binding constants of ligand to acto·S-1 complexes, and as shown in Figure 5, both tropomyosin and troponin–tropomyosin weaken ligand binding

Table I: Effect of Tropomyosin or Troponin-Tropomyosin with Ca^{2+} on Acto·S-1·Ligand Complex Formation[a]

	$K_{3,reg}/K_{3,unreg}$	$K_{4,reg}/K_{4,unreg}$	$K_{2,reg}/K_{2,unreg}$
AMP-PNP	3.5	0.53	6.6
PP_i	3.6	0.41	8.8

[a] K_{reg} refers to equilibrium constants determined in the presence of tropomyosin or troponin-tropomyosin with Ca^{2+}. The ordinate intercepts in Figure 5 give K_3, and the abscissa intercepts give $-K_4$. The values in the third column are determined by using the principle of detailed balance on Scheme I: $K_{2,reg}/K_{2,unreg} = (K_{3,reg}/K_{3,unreg})/(K_{4,reg}/K_{4,unreg})$.

about 2-fold. Therefore, as shown in column 3 of Table I, the binding constant of S-1 to actin is strengthened about 7-fold by tropomyosin and troponin–tropomyosin, in agreement with the results of Murray et al.

Discussion

In this study we have shown that over a range of ionic strength for a variety of ligands (AMP-PNP, PP_i, and ADP), tropomyosin strengthens the binding of S-1 to actin in a cooperative manner which is indistinguishable from the effect of troponin–tropomyosin with Ca^{2+}. Therefore, troponin does not appear to affect the binding of S-1·ligand to regulated actin in the presence of Ca^{2+}. Troponin does, however, confer pronounced cooperativity on the binding of S-1·ligand to the tropomyosin–actin complex in the absence of Ca^{2+}, as reported previously (Greene & Eisenberg, 1980a; Trybus & Taylor, 1980; Greene, 1982).

The cooperativity obtained in the presence of both tropomyosin and troponin–tropomyosin can be analyzed with the model of Hill et al. (1980). In this model, each group of seven actins and one tropomyosin (or troponin–tropomyosin) is assumed to exist in one of two states, weak or strong, depending upon the strength of its interaction with myosin. The cooperativity is expressed by using four parameters: K_{strong} and K_{weak} (the binding constant of S-1·ligand to the strong and weak states of the actin–regulatory protein complex, respectively), L' (the equilibrium constant per tropomyosin–actin unit for the transition of a filament with all its units in the weak state to one with all its units in the strong state in the absence of S-1), and Y (an interaction parameter arising from end-to-end interactions of the tropomyosin along the actin filament).

It was previously shown (Greene, 1982) that the major difference in the binding isotherms of S-1 to regulated actin in the presence and absence of Ca^{2+} is in the value of L'. The value of L' is much less in the presence of Ca^{2+} ($L' = 1.5$–3) than in the absence of Ca^{2+} ($L' = 33$ at $\mu = 0.18$ M, 25 °C). K_{weak} may also be different in the presence and absence of Ca^{2+}. In the absence of Ca^{2+}, K_{weak} is at most 40% of K_{unreg} and may even be considerably less, while K_{weak} could be equal to K_{unreg} in the presence of Ca^{2+}. In contrast, the value of K_{strong} is not affected by Ca^{2+}; in the presence of AMP-PNP or ADP, K_{strong} is 3-fold greater than K_{unreg} both with and without Ca^{2+}. Finally, in the absence of Ca^{2+}, Y could range from 10 to 50, while in the presence of Ca^{2+}, the cooperativity is too small to determine a value for Y.

The data obtained in the presence of tropomyosin are experimentally indistinguishable from the data obtained in the presence of troponin–tropomyosin with Ca^{2+}. Therefore, both sets of data were fitted with the same parameters, determined previously for troponin–tropomyosin in the presence of Ca^{2+}. While the value of L' is essentially the same for tropomyosin alone and troponin–tropomyosin with Ca^{2+}, it is important to recognize that the value of Y may be different for these two cases, since the shapes of the binding curves are insensitive to changes in Y.

Although we have found that tropomyosin alone and troponin–tropomyosin with Ca^{2+} have the same effect on the binding of S-1 to actin, they do not always have the same effect on the acto·S-1 ATPase activity (Lehrer & Morris, 1982; Nagashima & Asakura, 1982; Williams & Swenson, 1982). Differences in the value of Y which do not manifest themselves in the binding data could be responsible for the different effects of tropomyosin and troponin–tropomyosin on the acto·S-1 ATPase activity. However, much more work will be necessary to determine if this is the case.

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Registry No. Ca, 7440-70-2; AMP-PNP, 25612-73-1; PP_i, 14000-31-8; ADP, 58-64-0.

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