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Comparison of Effects of Smooth and Skeletal Muscle Tropomyosins on Interactions of Actin and Myosin Subfragment 1†

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ABSTRACT: The ATPase activity of acto-myosin subfragment 1 (S-1) was measured in the presence of smooth and skeletal muscle tropomyosins over a wide range of ionic strengths (20–120 mM). In contrast to the 60% inhibitory effect caused by skeletal muscle tropomyosin at all ionic strengths, the effect of smooth muscle tropomyosin was found to be dependent on ionic strength. At low ionic strength (20 mM), smooth muscle tropomyosin inhibits the ATPase activity by 60%, while at high ionic strength (120 mM), it potentiates the ATPase activity 3-fold. All of these ATPase activities were measured at very low ratios of S-1 to actin, under conditions at which a 4-fold increase in S-1 concentration did not change the specific activity of the tropomyosin–acto·S-1 ATPase. Therefore, the potentiation of the ATPase activity by smooth muscle tropomyosin at high ionic strength cannot be explained by bound S-1 heads cooperatively turning on the tropomyosin–actin complex. To determine whether the fully potentiated rates are different in the presence of smooth muscle and skeletal muscle tropomyosins, S-1 which was extensively modified by *N*-ethylmaleimide was added to the ATPase assay to attain high ratios of S-1 to actin. The results showed that, under all conditions, the fully potentiated rates are the same for both tropomyosins. Therefore, the difference in the effect of smooth muscle and skeletal muscle tropomyosins on the acto·S-1 ATPase activity at low ratios of S-1 to actin appears to be due to a greater fraction of the tropomyosin–actin complex being turned on in the absence of S-1 with smooth muscle tropomyosin than with skeletal muscle tropomyosin. This interpretation was supported by the equilibrium binding studies of S-1 to actin at 120 mM ionic strength in the presence of the ATP analogue adenyl-5′-yl imidodiphosphate. These studies indicate that in the absence of S-1, a greater fraction of the tropomyosin–actin complex is in the strong S-1-binding form with smooth muscle tropomyosin than with skeletal muscle tropomyosin. Hence, our data provide evidence that the fraction of tropomyosin–actin complex in the strong S-1-binding form correlates with the effect of tropomyosin on the actin-activated ATPase rate.

Skeletal muscle contraction is controlled by troponin–tropomyosin, a complex of proteins which lies along the actin filament (Ebashi et al., 1969). In the absence of Ca^{2+}, troponin–tropomyosin markedly inhibits the actomyosin ATPase activity, thus causing muscle relaxation. Although Ca^{2+} sensitivity is conferred on the actomyosin system only by the complete troponin–tropomyosin complex, tropomyosin alone has interesting effects on the actomyosin ATPase activity (Bremel et al., 1972; Weber & Murray, 1973; Eaton et al., 1975; Murray et al., 1975, 1980a,b; Lehrer & Morris, 1982). The effect of tropomyosin itself may be physiologically relevant since in certain contractile systems, such as smooth muscle, tropomyosin is bound to the actin filaments but no troponin is present. Furthermore, as with other complex allosteric systems, studying a simpler version of the system can often be informative.

We previously showed that the effect of skeletal muscle tropomyosin on the binding of myosin subfragment 1 (S-1)[1] to actin in the absence of ATP is very much like that of troponin–tropomyosin in the presence of Ca^{2+} (Williams & Greene, 1983). Tropomyosin strengthens the binding of S-1 to actin in a cooperative manner; that is, at low ratios of S-1 to actin, tropomyosin has little effect on the binding, while at high ratios of S-1 to actin, tropomyosin increases the strength of binding 3-fold. This cooperative effect of tropomyosin is explained by the model of Hill et al. (1980) which assumes

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[1] Abbreviations: AMP-PNP, adenyl-5′-yl imidodiphosphate; Ap_5A, P^1,P^5-di(adenosine-5′) pentaphosphate; ATP, adenosine 5′-triphosphate; DTT, dithiothreitol; NEM, *N*-ethylmaleimide; NEM-S-1, myosin subfragment 1 extensively modified with NEM; S-1, myosin subfragment 1.

that the tropomyosin–actin complex can exist in two forms, a weak S-1-binding form and a strong S-1-binding form; the binding of S-1 to actin shifts the equilibrium toward the strong-binding form.

Skeletal muscle tropomyosin not only affects the binding of S-1 to actin but also affects the acto·S-1 ATPase activity. Like its effect on S-1 binding, the effect of tropomyosin on the acto·S-1 ATPase activity appears to depend cooperatively on the amount of S-1 bound to actin (Bremel et al., 1972; Weber & Murray, 1973; Eaton et al., 1975; Murray et al., 1975, 1980a,b; Lehrer & Morris, 1982). At low ratios of S-1 to actin, skeletal muscle tropomyosin inhibits the acto·S-1 ATPase activity, while at high ratios of S-1 to actin, it potentiates the ATPase activity to a value greater than that obtained with unregulated acto·S-1. On the other hand, Sobieszek (1982) has reported that smooth muscle tropomyosin always potentiates the acto·S-1 ATPase activity.

In the present study, we investigated the difference between the effects of smooth muscle and skeletal muscle tropomyosins on the acto·S-1 ATPase activity. We examined this difference over a wide range of conditions, both under conditions at which the tropomyosin complex maximally inhibits the acto·S-1 ATPase activity and under conditions at which it maximally potentiates it. Our results suggest that the effect of both smooth muscle and skeletal muscle tropomyosins on the acto·S-1 ATPase activity is correlated with the amount of the tropomyosin–actin complex which is present in the strong S-1-binding form.

Materials and Methods

Proteins. Myosin was prepared from rabbit back and leg muscles according to the method of Kielley & Harrington (1960). For the binding studies, the myosin was radioactively labeled on SH-1 by iodo[^{14}C]acetamide (Amersham) according to the method of Greene & Eisenberg (1980). The extent of labeling was approximately 0.95 label per myosin head. Myosin subfragment 1 (S-1) was prepared by the method of Weeds & Taylor (1975) except that 1 mM DTT was included in our solutions. NEM-S-1 was prepared from the [^{14}C]acetamide-labeled S-1 by the method of Nagashima & Asakura (1982) using nonradioactive *N*-ethylmaleimide. Rabbit skeletal muscle 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 actin. The F-actin was treated with Dowex 1-X8Cl$^-$ to eliminate unbound nucleotide. Rabbit skeletal muscle tropomyosin was prepared according to the method of Eisenberg & Kielley (1974). Turkey gizzard tropomyosin was obtained from residual material from a gizzard myosin preparation (Sellers et al., 1981). After the 42–65%-saturated $(NH_4)_2SO_4$ fraction was dialyzed to low ionic strength, the gizzard myosin was removed by centrifugation. The supernatant was adjusted to pH 4.6, and the gizzard tropomyosin was precipitated. The gizzard tropomyosin was then resolubilized at pH 7.0 in 1 M KCl and 1 mM DTT. This material was then passed over a hydroxylapatite column according to the method of Eisenberg & Kielley (1974).

The NEM-S-1 concentration was determined from ^{14}C specific activity, and the concentrations of the other proteins were determined spectrophotometrically at 280 nm by using the following absorbances and molecular weights: myosin, $E^{0.1\%}_{280nm}$ = 0.56 cm^2/mg, M_r 480 000; S-1, $E^{0.1\%}_{280nm}$ = 0.75 cm^2/mg, M_r 120 000; actin, $E^{0.1\%}_{280nm}$ = 1.15 cm^2/mg, M_r 42 000; skeletal muscle tropomyosin, $E^{0.1\%}_{278nm}$ = 0.33 cm^2/mg, M_r 68 000; smooth muscle tropomyosin, $E^{0.1\%}_{278nm}$ = 0.22 cm^2/mg, M_r 68 000 (Clive Sanders, personal communication).

Actin-Activated ATPase Assays. Actin-activated S-1 ATPase activities were measured at 25 °C by measuring the rate of liberation of [^{32}P]P_i from [γ-^{32}P]ATP (Chock & Eisenberg, 1979). Assays were carried out in 1.5 mL of solution under conditions described in the figure legends. A single assay usually consisted of five time points. In all figures, the rate for S-1 alone (and where appropriate, NEM-S-1) has been subtracted from the measured rates. Vanadate-free ATP was obtained from Sigma. [γ-^{32}P]ATP was obtained from New England Nuclear.

Ligands. AMP-PNP and Ap_5A were obtained from Sigma and assayed for purity by poly(ethylenimine)–cellulose thin-layer chromatography in 0.75 M KH_2PO_4 (pH 3.4) and for acid-labile phosphate (Yount et al., 1971). The AMP-PNP was judged to be more than 90% pure.

Binding Studies. Binding of ^{14}C-labeled S-1 or NEM-S-1 to actin was measured with a Beckman preparative ultracentrifuge as described previously (Williams & Greene, 1983). There was no significant difference in the binding of S-1 to the tropomyosin–actin complex in the presence or absence of Ca^{2+}, indicating that there was no troponin in the tropomyosin preparation. We routinely used approximately 300 μM Ap_5A in our studies. When we assayed for the presence of contaminating myokinase using the method described by Greene (1982), we found no effect of myokinase contamination. In the absence of actin, 90–95% of the S-1 remained in the supernatant after centrifugation, while in the presence of actin but without AMP-PNP, 95% of the S-1 sedimented. Centrifugation of the actin alone showed that >96% sedimented as determined by the absorbance. NEM-S-1 was clarified by centrifugation at 80000*g* for 1 h in a type 40 rotor immediately before use in binding studies.

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

Results

Effect of Skeletal and Smooth Muscle Tropomyosins at Low S-1 to Actin Ratios. We first compared the effects of smooth muscle and skeletal muscle tropomyosins on the skeletal muscle acto·S-1 ATPase activity at very low ionic strength, as in most of our previous kinetic studies. These experiments were carried out at low ratios of S-1 to actin under conditions at which skeletal muscle tropomyosin would be expected to maximally inhibit the acto·S-1 ATPase activity. Surprisingly, we found that at low ionic strength, smooth muscle tropomyosin inhibits the acto·S-1 ATPase activity to about the same extent as does skeletal muscle tropomyosin (Figure 1). This result contrasts with the previous reports which indicated that smooth muscle tropomyosin has only a potentiating effect on the acto·S-1 ATPase activity. Figure 1 also shows that, as expected, the maximal effect of both skeletal and smooth muscle tropomyosins occurs at a ratio of about one tropomyosin per seven actin monomers. In all subsequent studies, we used at least a 2/7 mole ratio of tropomyosin to actin to ensure saturation.

The previous studies showing the potentiating effect of smooth muscle tropomyosin on the acto·S-1 ATPase activity were performed at relatively high ionic strength and relatively low [Mg^{2+}]. To determine whether the inhibitory effect of smooth muscle tropomyosin shown in Figure 1 was due to the solution conditions of this experiment, we compared the effects of smooth and skeletal muscle tropomyosins over a wide range of [Mg^{2+}] and ionic strengths. As shown in Figure 2, a higher free Mg^{2+} concentration is required for the maximum effect of smooth muscle tropomyosin on the acto·S-1 ATPase activity than is the case for skeletal muscle tropomyosin. Therefore,

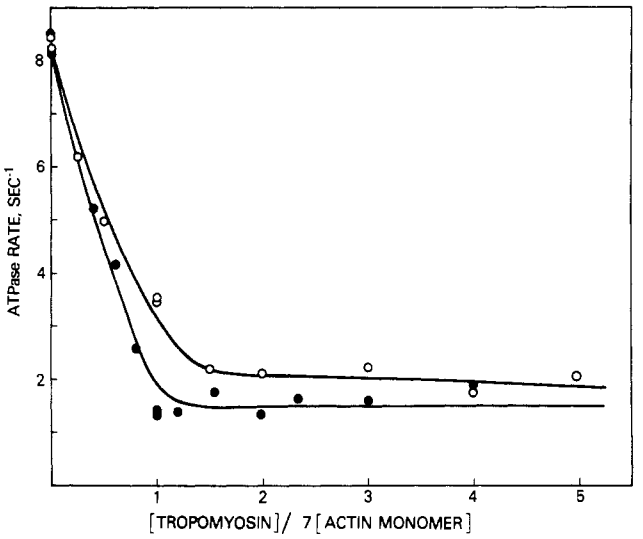

FIGURE 1: Inhibition of acto-S-1 ATPase activity by skeletal muscle and gizzard tropomyosins at low ionic strength. The solution contained 1 mM ATP, 4.5 mM $MgCl_2$, 0.5 mM DTT, and 10 mM imidazole, pH 7.0 at 25 °C (I = 20.5 mM). Protein concentrations were 20 μM actin and 0.033 μM S-1. Skeletal muscle tropomyosin (closed circles) and gizzard tropomyosin (open circles) concentrations were varied.

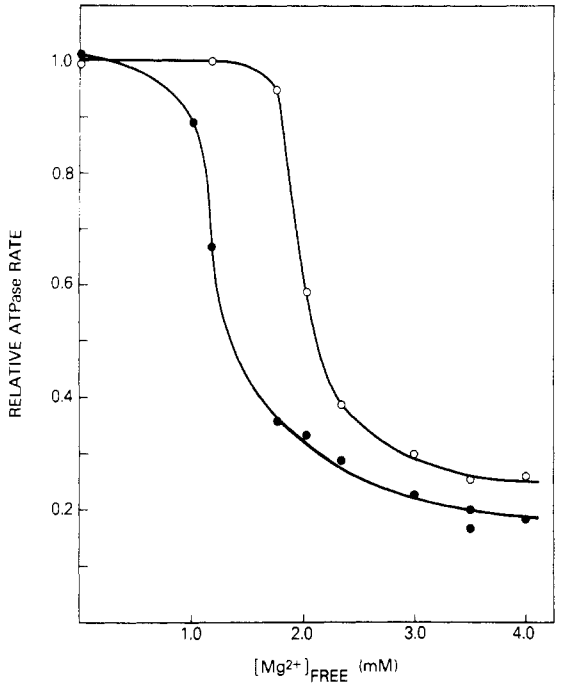

FIGURE 2: Mg^{2+} concentration dependence of the effect of skeletal muscle tropomyosin and gizzard tropomyosin on the acto-S-1 ATPase activity at low ionic strength. The solution conditions were 1 mM ATP, 0.5 mM DTT, and 7 or 10 mM imidazole at pH 7.0, 25 °C. The ionic strength was brought to 20.5 mM by additions of $MgCl_2$ and/or KCl. The free $[Mg^{2+}]$ is the $[Mg^{2+}]$ in excess of ATP. Protein concentrations were 20 μM actin, 0.033 μM S-1, and 5.7 μM skeletal muscle tropomyosin (closed circles) or smooth muscle tropomyosin (open circles).

in all of the experiments reported in this paper, we used a free Mg^{2+} concentration of 3.5 mM to ensure the tropomyosin remained bound to actin under all conditions examined. We next varied the ionic strength from 20.5 to 120.5 mM. The ATPase activity obtained in the absence of tropomyosin (dashed line, open triangles), in the presence of smooth muscle tropomyosin (open circles), and in the presence of skeletal muscle tropomyosin (closed circles) is plotted in Figure 3 as ATPase activity vs. actin concentration. As can be seen, skeletal muscle tropomyosin has about a 60% inhibitory effect

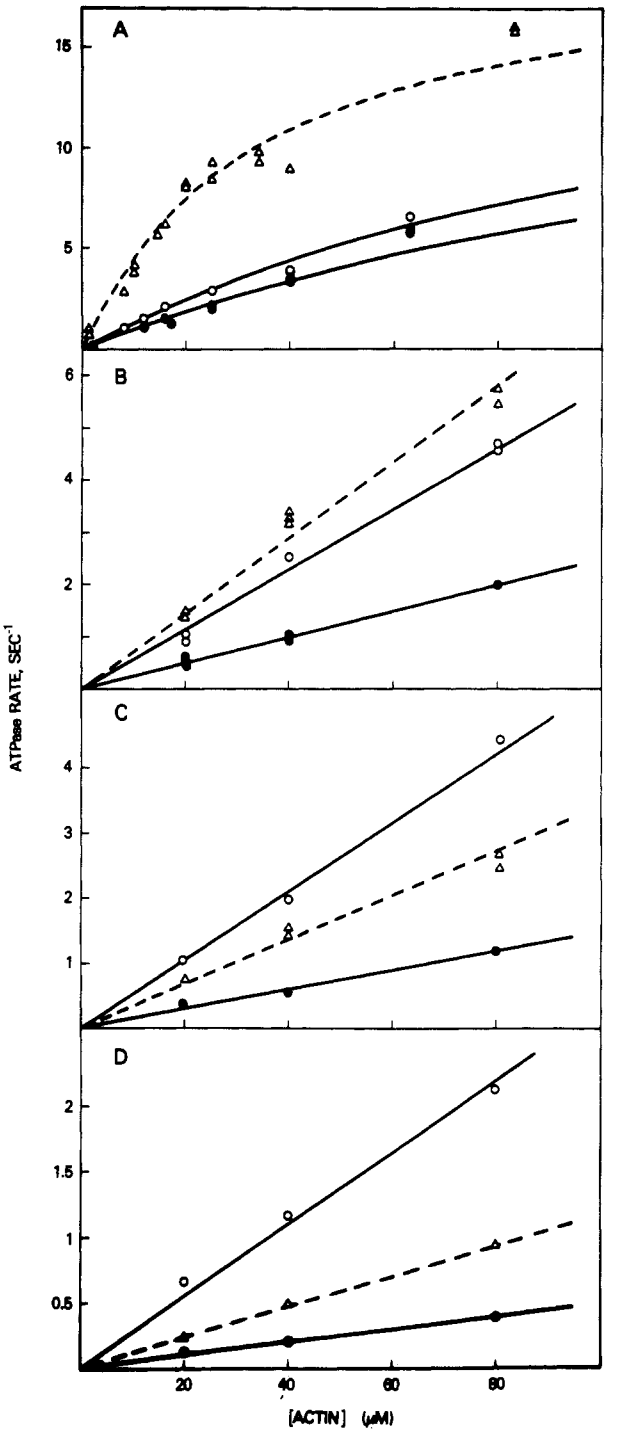

FIGURE 3: Effect of skeletal muscle tropomyosin and gizzard tropomyosin on the acto-S-1 ATPase activity at low S-1 to actin ratios. Solutions contained 1 mM ATP, 4.5 mM $MgCl_2$, 0.5 mM DTT, and 10 mM imidazole at pH 7.0, 25 °C. Ionic strength was adjusted with KCl. The acto-S-1 ATPase activity in the presence of skeletal muscle tropomyosin (closed circles) or gizzard tropomyosin (open circles) is compared to the acto-S-1 ATPase activity in the absence of tropomyosin (dashed lines, open triangles). (A) No KCl (I = 20.5 mM), [S-1] = 0.02–0.07 μM; (B) 22 mM KCl (I = 42.5 mM), [S-1] = 0.025–0.1 μM; (C) 54.5 mM KCl (I = 75 mM), [S-1] = 0.05–0.25 μM; (D) 100 mM KCl (I = 120.5 mM), [S-1] = 0.06–1 μM.

on the acto-S-1 ATPase activity over this entire range of ionic strengths. In contrast, the 60% inhibitory effect of smooth muscle tropomyosin at I = 20.5 mM is lessened at I = 42.5 mM, becomes a 1.5-fold potentiating effect at I = 75 mM, and then becomes a 3-fold potentiating effect at I = 120.5 mM. Therefore, in contrast to the effect of skeletal muscle tropomyosin, the effect of smooth muscle tropomyosin on the

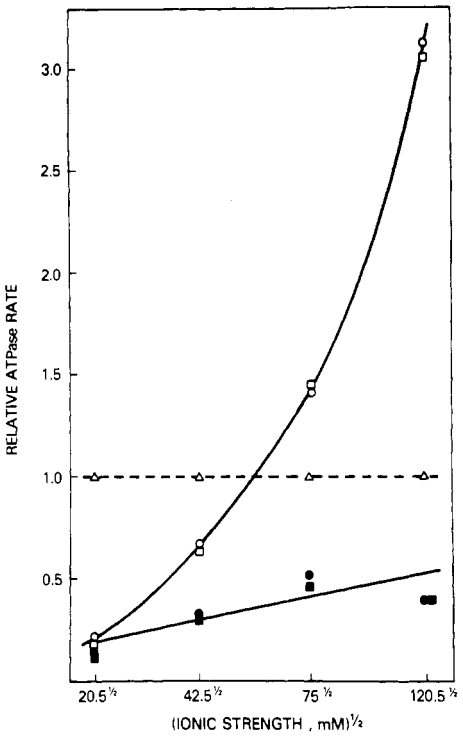

FIGURE 4: Effect of skeletal muscle tropomyosin and gizzard tropomyosin on the acto-S-1 ATPase activity at low S-1 to actin ratios. Each measurement was performed at two S-1 concentrations. Acto-S-1 ATPase rates in the presence of skeletal muscle tropomyosin (closed circles and squares) or gizzard tropomyosin (open circles and squares) are given relative to the rate in the absence of tropomyosin (dashed lines, open triangles) for a given set of conditions. For a given ionic strength, solution conditions were the same as those given in Figure 3. Protein concentrations were the following: at I = 20.5 mM, 12 μM actin, 3.4 μM tropomyosin, and 0.017 (circles) or 0.067 (squares) μM S-1; at I = 42.5 mM, 20 μM actin, 5.7 μM tropomyosin, and 0.025 (circles) or 0.1 (squares) μM S-1; at I = 75 mM, 20 μM actin, 5.7 μM tropomyosin, and 0.1 (circles) or 0.25 (squares) μM S-1; at I = 120.5 mM, 20 μM actin, 5.7 μM tropomyosin, and 0.1 (circles) or 0.5 (squares) μM S-1.

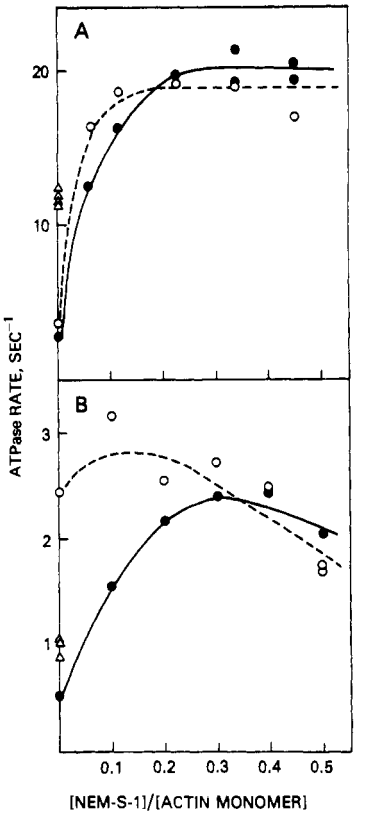

FIGURE 5: Effect of NEM-S-1 on the tropomyosin–acto-S-1 ATPase activity. The NEM-S-1/actin mole ratio is based on the 70% of NEM-S-1 which binds tightly to actin in the presence of ATP. The slight NEM-S-1 ATPase activity (0.014 s^{-1}) has been subtracted from the data. (A) Conditions were 1 mM ATP, 4.5 mM $MgCl_2$, 0.5 mM DTT, and 10 mM imidazole at pH 7.0, 25 °C (I = 20.5 mM). Protein concentrations were 20 μM actin, 0.015 μM S-1, and 5.7 μM skeletal muscle tropomyosin (closed circles, solid line) or 5.7 μM gizzard tropomyosin (open circles, dashed line). Data for actin in the absence of tropomyosin and NEM-S-1 (open triangles) are also shown. (B) Conditions were 5 mM ATP, 8.5 mM $MgCl_2$, 80 mM KCl, 0.5 mM DTT, and 10 mM imidazole at pH 7.0, 25 °C (I = 120.5 mM). Protein concentrations were 40 μM actin, 1 μM S-1, and 11.4 μM skeletal muscle tropomyosin (closed circles, solid line) or 11.4 μM gizzard tropomyosin (open circles, dashed line). Data for actin in the absence of tropomyosin and NEM-S-1 (open triangles) are also shown. The linear portions of the curves for smooth muscle tropomyosin at [NEM-S-1]/[actin monomer] $\geq$ 0.1 and for skeletal muscle tropomyosin at [NEM-S-1]/[actin monomer] $\geq$ 0.3 were determined by linear regression analysis and fitted as straight lines. The curves were then fit to the remainder of the data, with most weight being given to points on the ordinate (which do not require correction for NEM-S-1 ATPase activity). The error for a given point is less than 10%.

acto-S-1 ATPase activity is very dependent on ionic strength.

These experiments were carried out at relatively low ratios of S-1 to actin in the presence of ATP, conditions in which a significant amount of actin would not be complexed with S-1. Therefore, the actin–tropomyosin complex would not be expected to be cooperatively turned on under these conditions. To be certain that this is the case, we tested whether an increase in S-1 concentration had any effect on the ATPase rate. Figure 4 shows that over a 4-fold range of S-1 concentrations, the measured ATPase rates were essentially constant in the presence of both skeletal muscle tropomyosin and smooth muscle tropomyosin. Hence, in these experiments, the potentiation of the ATPase activity observed with smooth muscle tropomyosin was not due to S-1 cooperatively turning on the actin–tropomyosin complex.

Effect of Skeletal and Smooth Muscle Tropomyosins in a Fully Turned-On System. Having observed a difference in the effect of skeletal muscle and smooth muscle tropomyosins at low ratios of S-1 to actin, we were interested in determining whether a similar difference occurs under conditions where the actin–tropomyosin complex is completely turned on. To turn on the tropomyosin–actin complex, we used S-1 which had been extensively modified with NEM (Pemrick & Weber, 1976; Nagashima & Asakura, 1982). Binding studies indicate that 70% of this NEM-S-1 binds strongly to the tropomyosin–actin complex even in the presence of ATP. At the same time, NEM-S-1 has a very low actin-activated ATPase rate (approximately 0.014 s^{-1}). Therefore, it can be used to turn on the tropomyosin–actin complex without interfering with measurement of the actin-activated ATPase rate of the unmodified S-1.

The effect of NEM-S-1 on the tropomyosin–actin-activated S-1 ATPase rates at low ionic strength (I = 20.5 mM) is shown in Figure 5A. The ATPase activity of NEM-S-1 has been subtracted from the reported rates. As increasing amounts of NEM-S-1 are added, the ATPase rate in the presence of skeletal muscle tropomyosin (closed circles, solid line) and smooth muscle tropomyosin (open circles, dashed line) increases to a rate about twice that of unregulated acto-S-1 and then levels off. Nagashima & Asakura (1982) previously reported that addition of NEM-S-1 to unregulated acto-S-1 caused the ATPase rate to decrease by reducing the free actin concentration. However, since decreasing the free actin concentration does not significantly decrease the ATPase rate,

under the conditions of Figure 5A, the potentiated actin-activated ATPase rate is probably close to its V_{max}. It therefore appears that at low ionic strength the maximally potentiated acto·S-1 ATPase rate is about the same in the presence of skeletal muscle and smooth muscle tropomyosins.

Figure 5B shows the effect of NEM-S-1 on the tropomyosin–actin-activated ATPase rate at high ionic strength (I = 120.5 mM). Here again, the ATPase rate initially increases for both skeletal muscle tropomyosin (closed circles, solid line) and smooth muscle tropomyosin (open circles, dashed line). However, since under these conditions the system is far from V_{max}, further binding of NEM-S-1 to actin decreases the potentiated ATPase rate by decreasing the free actin concentration. Nevertheless, just at at low ionic strength, the acto·S-1 ATPase rate appears to be about the same for both skeletal muscle and smooth muscle tropomyosins at an NEM-S-1 to actin ratio of 0.3 or greater. Furthermore, the two ATPase plots seem to extrapolate to similar points on the ordinate. Therefore, the difference in the effect of skeletal muscle and smooth muscle tropomyosins on the acto·S-1 ATPase rates at high ionic strength does not appear to arise from a difference in the maximally potentiated ATPase rates for the two systems.

Effect of Skeletal and Smooth Muscle Tropomyosins on the Binding of S-1 to Actin. At high ionic strength, the above ATPase studies show that increasing the NEM-S-1 concentration cooperatively turns on the skeletal muscle tropomyosin–actin complex but has almost no effect on the smooth muscle tropomyosin–actin complex. This may be because the smooth muscle tropomyosin–actin complex is already completely turned on before the S-1 is added. This suggests that almost all of the smooth muscle tropomyosin–actin complex may be in the strong S-1-binding form (Hill et al., 1980) in the absence of S-1. We would therefore expect the binding of S-1 to the tropomyosin–actin complex to be less cooperative with smooth muscle tropomyosin than with skeletal muscle tropomyosin.

Figure 6 shows the binding of S-1 to actin at high ionic strength with skeletal muscle tropomyosin, with smooth muscle tropomyosin, and in the absence of tropomyosin. These binding studies were performed in the presence of AMP-PNP as in our previous work. The binding of S-1·AMP-PNP to actin in the absence of tropomyosin (dashed line, open triangles) has an association constant of 2.3×10^4 M^{-1} under these conditions and, as expected, shows no evidence of cooperativity. As we reported previously (Williams & Greene, 1983), the binding in the presence of skeletal muscle tropomyosin (closed circles) is slightly cooperative with the binding constant increasing from 2.3×10^4 to 7.0×10^4 M^{-1} as the S-1 concentration is increased. In contrast, with smooth muscle tropomyosin, there is little evidence of any cooperativity; the binding constant is about 6.6×10^4 M^{-1} throughout the whole titration curve. The binding data indicate that, in the absence of S-1 at high ionic strength, more tropomyosin–actin units are in the strong S-1-binding form with smooth muscle tropomyosin than with skeletal muscle tropomyosin. The ATPase studies suggest that, in the absence of S-1 at high ionic strength, there are more turned-on tropomyosin–actin units with smooth muscle tropomyosin than with skeletal muscle tropomyosin. These results are consistent with turned-on tropomyosin–actin units being equivalent to the strong S-1-binding tropomyosin–actin units.

It would be of interest to compare the effects of the two tropomyosins on the binding of S-1 to actin at low (20.5 mM) ionic strength, conditions at which both inhibit the actin-activated ATPase activity. However, it is not possible to saturate

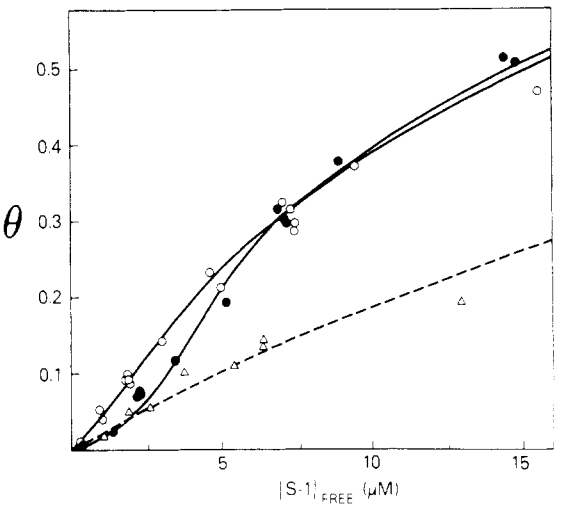

FIGURE 6: Effect of skeletal muscle tropomyosin and gizzard tropomyosin on the binding of S-1·AMP-PNP to actin. The conditions were 4 mM AMP-PNP, 7.5 mM $MgCl_2$, 89 mM KCl, 0.5 mM DTT, and 10 mM imidazole at pH 7.0, 25 °C (I = 120.5 mM). The binding was examined by using 20 μM actin with additions of 5.7 μM skeletal muscle or gizzard tropomyosin (2/7 mole ratio with actin). The fraction of actin sites occupied by S-1 (θ) is measured as a function of $[S\text{-}1]_{FREE}$. The measured binding constant (K) is $K = \theta/[(1 - \theta)[S\text{-}1]_{FREE}]$. The dashed theoretical curve for the independent binding of S-1 to actin in the absence of tropomyosin (open triangles) was fitted to the data by using a binding constant of 2.3×10^4 M^{-1}. The theoretical curve was fitted to the skeletal muscle and smooth muscle tropomyosin data by using the model of Hill et al. (1980). This model describes the binding of S-1 to tropomyosin–actin by using binding constants for each of the strong- and weak-binding forms of tropomyosin–actin, K_{strong} and K_{weak}, respectively. The cooperativity is described by the parameter L' which is affected by a tropomyosin end to end interaction parameter Y. The following parameters were used: skeletal muscle tropomyosin–actin (closed circles), $K_{strong} = 7.0 \times 10^4$ M^{-1}, $K_{weak} = 7.0 \times 10^3$ M^{-1}, $L' = 3$, $Y = 5$; gizzard tropomyosin–skeletal muscle actin (open circles), $K_{strong} = 6.6 \times 10^4$ M^{-1}, $K_{weak} = 1.1 \times 10^4$ M^{-1}, $L' = 1.05$, $Y = 5$.

S-1 with AMP-PNP and still keep 3.5 mM free Mg^{2+} at this ionic strength. At the lowest ionic strength we could achieve (41.5 mM), the shapes of the binding curves were similar to those obtained at 120.5 mM ionic strength (data not shown). However, there is still a 2.5-fold difference in the effects of the two tropomyosins on the acto·S-1 ATPase activity at this ionic strength, so that any change in the binding curve which occurred with smooth muscle tropomyosin may have been too small to detect.

Discussion

In this study, we have shown that the effect of smooth muscle tropomyosin is dependent on ionic strength. At low ratios of S-1 to actin, smooth muscle tropomyosin has an inhibitory effect on the acto·S-1 ATPase activity at low ionic strength and a potentiating effect at high ionic strength. Previous workers only observed the potentiating effect because they did not work at a low enough ionic strength for the inhibitory effect to occur. In contrast to the effect of smooth muscle tropomyosin, skeletal muscle tropomyosin has an inhibitory effect on the ATPase activity at both high and low ionic strengths. However, the actin-activated ATPase activity is *not* completely inhibited with either of the tropomyosins at low ionic strength (unlike troponin–tropomyosin in the absence of Ca^{2+}) as a significant residual ATPase activity occurs with both tropomyosins. Since this residual ATPase specific activity remains constant over a 4-fold range of S-1 concentrations, it cannot be due to the binding of S-1 to actin turning on the tropomyosin–actin complex. Therefore, our results do not

support the conclusions of Lehrer & Morris (1982), who argued that all of the actin-activated ATPase activity observed in the presence of skeletal muscle tropomyosin is due to a cooperative turning-on effect of S-1. Instead, our results suggest that the amount of actin-activated ATPase activity observed in the presence of tropomyosin is an intrinsic property of the actin–tropomyosin complex itself.

Our results also indicate that at both low and high ionic strengths when the actin–tropomyosin complex is fully turned on by the binding of NEM-S-1, the actin-activated ATPase rate is the same with skeletal muscle and smooth muscle tropomyosins. This shows that the difference in the effect of smooth muscle and skeletal muscle tropomyosins at low S-1 concentration is not due to a difference in the ability of the turned-on tropomyosin–actin complex to potentiate the acto·S-1 ATPase activity. Rather, the difference observed at low S-1 concentration may occur because, in the absence of S-1, there are more turned-on tropomyosin–actin units present with smooth muscle tropomyosin than with skeletal muscle tropomyosin.

This view is supported by experiments on the binding of S-1 to the tropomyosin–actin complex in the absence of ATP. These experiments show that at high ionic strength, a larger fraction of the tropomyosin–actin complex is in the strong S-1-binding form with smooth muscle tropomyosin than with skeletal muscle tropomyosin. Thus, there is a correlation between the amount of actin-activated ATPase activity observed with tropomyosin and the fraction of tropomyosin–actin units in the strong S-1-binding form.

The simplest way to interpret these data is to assume that the strong S-1-binding form of the tropomyosin–actin complex is equivalent to the turned-on form of this complex, while the weak S-1-binding form is unable to activate the S-1 ATPase activity. On this basis, if 12–15% of the skeletal muscle tropomyosin–actin complex were in the strong-binding form in the absence of S-1, an assumption which is consistent with our binding studies, it would explain the residual ATPase activity which occurs at both low and high ionic strengths at low ratios of S-1 to actin. Similarly, with smooth muscle tropomyosin at high ionic strength, if almost all of the tropomyosin–actin complex were in the strong S-1-binding form in the absence of S-1, it would explain the potentiation of the actin-activated ATPase activity which is observed even at very low ratios of S-1 to actin.

At the present time, we do not know the physiological significance of the tropomyosin effects described in this paper. In terms of its effect on S-1 binding to actin, skeletal muscle troponin–tropomyosin in the presence of Ca^{2+} appears to act like tropomyosin alone. It is therefore possible that, in skeletal muscle, the binding of force-producing cross-bridges to the thin filament in the presence of Ca^{2+} modulates the ATP turnover rate in the same way as the binding of S-1 to the tropomyosin–actin complex modulates the ATPase rate in vitro. If fewer force-producing cross-bridges were bound to the thin filament when the muscle was shortening at high velocity, it could cause the ATP turnover rate to decrease, an effect which is observed experimentally. In smooth muscle, it is possible that this type of modulation does not occur and the tropomyosin–actin complex is always fully turned on at physiological ionic strength (which we have called "high" ionic strength in this paper). However, it is also possible that smooth muscle tropomyosin acts differently when it is bound to smooth muscle actin rather than skeletal muscle actin. Clearly, more work will be necessary to understand the physiological role of tropomyosin in smooth muscle.

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