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Binding of Gizzard Smooth Muscle Myosin Subfragment 1 to Actin in the Presence and Absence of Adenosine 5′-Triphosphate†

Lois E. Greene,* James R. Sellers, Evan Eisenberg, and Robert S. Adelstein

ABSTRACT: The binding of gizzard smooth muscle subfragment 1 (S-1) and skeletal muscle S-1 to skeletal muscle actin was compared at varying ionic strengths in the presence and absence of nucleotide. The nucleotides, AMP-PNP and ATP, weaken the binding of smooth muscle S-1 and skeletal muscle S-1 to actin to a similar extent. In both cases, the acto·S-1 association constant was reduced about 500-fold and 50 000-fold in the presence of AMP-PNP and ATP, respectively. On the other hand, the effect of ionic strength on the binding of S-1 to actin was found to be markedly different for smooth muscle S-1 and skeletal muscle S-1. Increasing ionic strength only slightly weakened the binding of smooth muscle S-1 to actin, whereas it greatly weakened the binding of skeletal muscle S-1. At low ionic strength ($\mu < 0.05$ M), skeletal muscle S-1 bound more strongly to actin than did smooth muscle S-1 both in the presence and in the absence of nucleotide, whereas at high ionic strength ($\mu > 0.05$ M), the reverse was true. The steady-state kinetics of the actomyosin ATPase were also examined with smooth muscle S-1. At low ionic strength ($\mu = 0.012$ M), the apparent binding constant obtained from the double-reciprocal plot of ATPase activity vs. actin concentration ($K_{ATPase} = 3.2 \times 10^4$ M^{-1}) was 4-fold larger than the binding constant obtained from direct measurement of the binding of smooth muscle S-1 to actin in the presence of ATP ($K_{binding} = 8.5 \times 10^3$ M^{-1}). This is similar to the difference between K_{ATPase} and $K_{binding}$ observed for skeletal muscle S-1. Therefore, at physiological ionic strength, the cross-bridges in smooth muscle may undergo a similar kinetic cycle as in skeletal muscle but may bind more tightly to the actin filaments.

The fundamental process which drives muscle contraction is the interaction of actin, myosin, and ATP. In skeletal muscle, these interactions have been well characterized by using the single-headed soluble fragment of myosin, subfragment 1. S-1[1] binds to actin with a binding constant of about 10^7 M^{-1} at physiological ionic strength (Marston & Weber, 1975; Highsmith, 1977; Margossian & Lowey, 1978; Greene & Eisenberg, 1980a), but this binding becomes considerably weaker in the presence of nucleotide. ADP weakens this binding about 30-fold, AMP-PNP weakens it about 500-fold, and ATP weakens it more than 5000-fold (Greene & Eisenberg, 1980a; Marston et al., 1979; Hofmann & Goody, 1978; Stein et al., 1979). In addition, with all of these nucleotides, as well as in the absence of nucleotide, increasing ionic strength weakens the binding of S-1 to actin (Highsmith, 1977; Margossian & Lowey, 1978; Greene & Eisenberg, 1978).

Studies have suggested that skeletal and smooth muscle myosins may differ in their strength of binding to actin in the presence of nucleotide. Krisanda & Murphy (1980) found the K^+-EDTA ATPase activity of smooth muscle (gizzard) myosin was inhibited by skeletal muscle actin at much lower concentrations than was the ATPase activity of skeletal muscle myosin. On the basis of this observation, they suggested that, at least at high ionic strength ($\mu \simeq 0.5$ M), smooth muscle myosin binds to actin more strongly than does skeletal muscle myosin. On the other hand, two studies conducted at much lower ionic strength suggest that smooth muscle myosin might bind to actin more weakly than does skeletal muscle myosin. Marston & Taylor (1980) measured the ATPase activity of both smooth muscle S-1 and skeletal muscle S-1 as a function of actin concentration. The values they obtained for the apparent binding constant (K_{ATPase}) suggested that in the presence of ATP, smooth muscle S-1 bound to actin more weakly than did skeletal muscle S-1. Similarly, Ikebe et al. (1981) found that, at low ionic strength in the presence of AMP-PNP, smooth muscle HMM bound more weakly to actin than did skeletal muscle HMM. To determine exactly how smooth and

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[1] Abbreviations: S-1, subfragment 1; acto·S-1, a complex of actin with S-1; AMP-PNP, adenyl-5′-yl imidodiphosphate; DTT, dithiothreitol; Mops, 4-morpholinepropanesulfonic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N′,N′*-tetraacetic acid; PEI, poly(ethylenimine); Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride.

skeletal muscle myosins differ in their binding to actin, we conducted a detailed investigation of the binding of smooth muscle S-1 to skeletal actin in the presence of different nucleotides at varied ionic strengths.

Materials and Methods

Chemicals. AMP-PNP and diadenosine pentaphosphate were from Sigma, and [γ-^{32}P]ATP was from New England Nuclear. The AMP-PNP was >90% pure, analyzed by PEI–cellulose chromatography in 0.75 M KH_2PO_4 (pH 3.4) and phosphate analysis (Yount et al., 1971). The iodo-[^{14}C]acetamide was from Amersham/Searle.

Preparation of Proteins. Myosin was prepared from fresh turkey gizzard muscle as previously described (Sellers et al., 1981) with omission of the Sepharose 4B chromatography. Subfragment one was prepared by papain (Worthington) digestion of myosin (20 mg/mL) in 50 mM NaCl, 1 mM $MgCl_2$, 0.1 mM EGTA, 10 mM Mops, and 1 mM DTT (pH 7.0, 2.5 °C) for 7.5 min with 0.008 unit of papain/mg of myosin. Papain was activated according to Lowey et al. (1969). The reaction was terminated by addition of 100 mM iodoacetic acid (pH 7.3) to a final concentration of 5 mM. The digested myosin was then centrifuged at 40000*g* for 20 min and the supernatant dialyzed against 20 mM NaCl, 10 mM $MgCl_2$, 0.1 mM EGTA, 10 mM Mops, and 1 mM DTT, pH 7.0, for 4 h to precipitate the remaining intact myosin and rods. The insoluble material was removed by centrifugation at 40000*g* for 20 min, and the supernatant-containing S-1 was dialyzed against 10 mM imidazole hydrochloride, 0.1 mM EGTA, and 1 mM DTT, pH 7.0. The S-1 was clarified by centrifugation at 10000*g* for 20 min just prior to use. The sodium dodecyl sulfate gel of the smooth muscle S-1 resembled that of Marston & Taylor (1980). Protein concentration was determined from the absorbance at 280 nm with $E^{1\%}_{280}$ equal to 7.3.

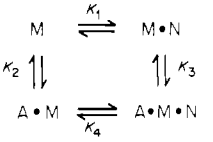
Smooth muscle actin was prepared from fresh turkey gizzard muscle by the "high salt/EDTA" procedure as described by Strzelecka-Golaszewska et al. (1980).

Skeletal muscle myosin was purified from rabbit back and leg muscles according to the method of Kielley & Harrington (1960). Skeletal muscle F-actin was prepared by using a modified method of Spudich & Watt (1971) (Eisenberg & Kielley, 1974). Chymotryptic S-1 was prepared by the method of Weeds & Taylor (1975). The procedure described previously by Greene & Eisenberg (1980b) was used for labeling to block the SH_1 groups of myosin with iodo[^{14}C]acetamide. After the myosin was labeled (1 ± 0.1 mol of iodoacetamide/mol of heads), chymotryptic S-1 was made. Protein concentrations were determined from the absorbance at 280 nm with $E^{1\%}_{280}$ equal to 5.6, 7.5, and 11.5 for myosin, S-1, and F-actin, respectively. The molecular weights used for myosin, S-1, and F-actin were 470 000, 120 000, and 42 000, respectively.

Actin-Activated Assays. The actin-activated Mg-ATPase activity was determined by using [γ-^{32}P]ATP as described by Pollard & Korn (1973). The conditions are given in the figure legend.

Binding Experiments. The binding of smooth muscle S-1 to actin was measured as described previously (Greene & Eisenberg, 1978) in a Beckman Model E analytical ultracentrifuge equipped with a photoelectric scanner. The binding of smooth muscle S-1 to actin in the presence of ATP was measured in a Beckman airfuge according to the procedure of Chalovich & Eisenberg (1982) as modified in Sellers et al. (1982). The amount of S-1 remaining in the supernatant was determined by measuring the $(NH_4)^+$-EDTA ATPase activity in 0.4 M NH_4Cl, 35 mM EDTA (Tris salt), 25 mM Tris-HCl (pH 8.0), 5 mM ATP, and 1 mg/mL bovine serum albumin at 25 °C. Details of this assay are described in Sellers et al. (1982).

Scheme I



Treatment of Data. The binding of smooth muscle S-1 to actin in the presence of AMP-PNP was assumed to fit Scheme I for the formation of the acto·S-1·nucleotide ternary complex where A = a monomer in an F-actin filament, M = S-1, N = nucleotide, and K = association constants. This scheme, which has been shown applicable for the binding of skeletal S-1 to actin (Greene & Eisenberg, 1980a), assumes smooth muscle S-1 binds independently to F-actin with a stoichiometry of one S-1 per F-actin monomer both in the presence and in the absence of nucleotide. As in our previous studies (Greene & Eisenberg, 1978, 1980a), we used the following equation derived from this scheme to analyze the data:

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

Plotting the data as K_{app} vs. $1/[N]$ the ordinate intercept is then equal to K_3, the binding constant of actin to the S-1·AMP-PNP complex, and the abscissa intercept gives K_4, the binding constant of AMP-PNP to acto·S-1.

Results

The ability of smooth muscle S-1 to bind to skeletal muscle actin was first examined in the absence of nucleotide by having skeletal muscle S-1 and smooth muscle S-1 compete for binding sites on skeletal F-actin. The skeletal muscle S-1 was modified with iodo[^{14}C]acetamide to enable the concentration of unbound skeletal muscle S-1 to be determined from the radioactivity in the supernatant after the actin was sedimented. These competition experiments were conducted over a range of ionic strengths, from 0.01 to 0.21 M with 10 μM smooth muscle S-1, 11 μM skeletal muscle S-1, and 10 μM actin. The order of addition of the smooth muscle S-1 and skeletal muscle S-1 to actin did not significantly affect the results, indicating the proteins were in equilibrium.

The results in Table I show that ionic strength has a very different effect on the binding of smooth muscle S-1 and skeletal muscle S-1 to actin. As the ionic strength was increased, the total amount of S-1 bound to actin remained the same, but the fraction of smooth muscle S-1 bound increased, and the fraction of skeletal muscle S-1 bound decreased. From these data, the ratio of the smooth muscle S-1–actin association constant to the skeletal muscle S-1–actin association constant was calculated (last column). This ratio shows that at μ = 0.01 M, smooth muscle S-1 binds about 2-fold more weakly to actin than does skeletal muscle S-1, whereas at μ = 0.21 M, smooth muscle S-1 binds about 14-fold more strongly to actin than does skeletal muscle S-1. Thus there is a reversal in the affinity of smooth and skeletal muscle S-1 for actin when the ionic strength is varied from 0.01 to 0.21 M. Similar results were obtained when smooth muscle actin, instead of skeletal muscle actin, was used in these competition experiments (data not shown).

An approximate value for the binding constant of smooth muscle S-1 to actin can be obtained by using the ratio given

Table I: Competition of Smooth S-1 (10 μM) and Skeletal S-1 (11 μM) for Sites on Skeletal F-Actin (10 μM)[a]

ionic strength (M)	[skeletal S-1]$_{bound}$[b] (μM)	[smooth S-1]$_{bound}$[c] (μM)	ratio of assoc const K_{sm}:K_{skel}[d]
0.01	6.1 ± 0.4	3.4 ± 0.4	0.4 ± 0.2 (n = 4)
0.05	3.8 ± 0.7	5.7 ± 0.5	2.5 ± 0.8 (n = 6)
0.21	2.0 ± 0.3	7.5 ± 0.2	13.9 ± 1.6 (n = 4)

[a] Conditions were 18 mM imidazole, 0.2 mM $MgCl_2$, and 1 mM DTT, pH 7.0 at 25 °C. At μ = 0.05 M, 40 mM KCl was also added, and at μ = 0.21 M, 200 mM KCl was added. The proteins were incubated 1 h at 4 °C and then 30 min at 25 °C before centrifugation in a Beckman airfuge for 20 min at 178000g at 25 °C. [b] The concentration of skeletal muscle S-1 bound to actin was determined by measuring the amount of skeletal S-1 added to the actin and the amount remaining in the supernatant after centrifugation. Skeletal muscle S-1 was modified with iodo[^{14}C]acetamide. [c] The total concentration of actin sites available for S-1 binding (9.5 μM) was determined by centrifuging actin with an excess of skeletal muscle S-1. The concentration of smooth muscle S-1 bound to actin was then calculated from the difference in the total concentration of actin sites and the concentration of bound skeletal muscle S-1. Under all conditions, the actin was completely saturated with S-1. [d] K_{sm} is the binding constant of smooth muscle S-1 to actin. K_{skel} is the binding constant of skeletal muscle S-1 to actin.

in Table I and the values previously determined for the binding constant of skeletal muscle S-1 to actin. At μ = 0.23 M, the binding constant of skeletal muscle S-1 to actin is about 5 × 10^6 M^{-1} (Greene & Eisenberg, 1980a), which gives a calculated value of 7 × 10^7 M^{-1} for the binding constant of smooth muscle S-1 to actin. Similarly, at μ = 0.05 M, the binding constant of smooth muscle S-1 to actin is calculated to be 3 × 10^8 M^{-1} by using 10^8 M^{-1} as the binding constant of skeletal muscle S-1 to actin (Marston & Weber, 1975). Therefore, these data indicate that increasing ionic strength weakens the binding of smooth muscle S-1 to actin to a lesser degree than it weakens the binding of skeletal muscle S-1.

The effect of ionic strength on the binding of S-1 to actin was next examined in the presence of AMP-PNP. The binding constant of skeletal muscle S-1 to actin has been measured over a wide range of ionic strengths in the presence of this nucleotide (Greene & Eisenberg, 1978; Greene, 1981). The binding constant of smooth muscle S-1 to actin in the presence of AMP-PNP was first determined over a range of ionic strengths (μ = 0.023–0.43 M). This was done by measuring the binding of S-1 to actin at varying concentrations of AMP-PNP and then plotting the data according to eq 1 (see Treatment of Data). From the ordinate intercepts in Figure 1, the values for the binding constant of smooth muscle S-1·AMP-PNP to actin are 4.3 × 10^5 M^{-1} and 1.0 × 10^5 M^{-1} at μ = 0.023 M and μ = 0.43 M, respectively. This shows that there is less than a 5-fold difference in the value of this binding constant over this very wide range of ionic strength. In addition, Figure 1 shows that the binding constant of AMP-PNP to acto·S-1, obtained from the abscissa intercept, is about 7 × 10^2 M^{-1}, regardless of the ionic strength.

It is important to recognize that the plots in Figure 1 are based on the assumption that S-1 binds independently along the actin filament (see Scheme I). This assumption is shown to be valid by the linear Scatchard plots shown in Figure 2. These plots were obtained by measuring the binding of smooth muscle S-1 to actin in the presence of 2 mM AMP-PNP at μ = 0.023 M and μ = 0.43 M. From the slopes of the lines in Figure 2, the binding constants of smooth muscle S-1 to actin in the presence of AMP-PNP are 4 × 10^5 M^{-1} and 1 × 10^5 M^{-1} at μ = 0.023 M and μ = 0.43 M, respectively, in good

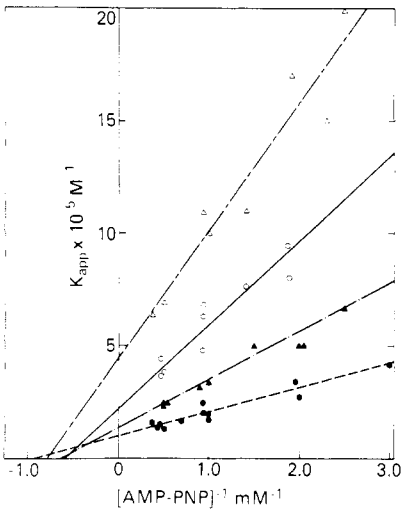


FIGURE 1: Binding of smooth muscle S-1 to actin in the presence of AMP-PNP at varying ionic strengths. At μ = 0.023 M (△), conditions were 10 mM imidazole, 5 mM $MgCl_2$, 1.5 mM KP_i, 25 μM diadenosine pentaphosphate, and 1 mM DTT, at pH 7.0, 25 °C, using 10 μM S-1, 10 μM actin, and varying AMP-PNP concentrations (0.4–2.0 mM). At μ = 0.063 M (○), conditions were the same except that 40 mM KCl was also added. At μ = 0.15 M (▲), conditions were the same except that 130 mM KCl was added. At μ = 0.43 M (●), conditions were the same except that 400 mM KCl and 5 mM KP_i were added. The data were fitted by using linear regression analysis.

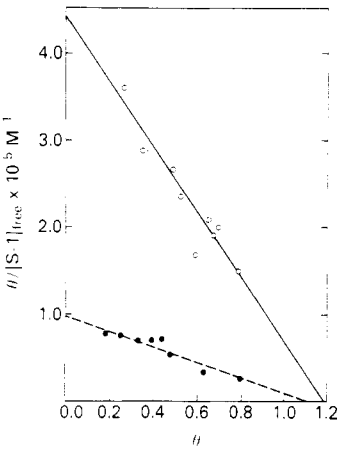


FIGURE 2: Scatchard plots of smooth muscle S-1 binding to actin in the presence of 2 mM AMP-PNP. Conditions are given in Figure 1. At μ = 0.23 M (○), 2–10 μM S-1 was added to 7.1 μM actin, and at μ = 0.43 M (●), 3–25 μM S-1 was added to 10 μM actin. The data were fitted by using linear regression analysis.

agreement with the results in Figure 1. Since the binding constant of smooth muscle S-1 to actin in the absence of nucleotide was calculated above to be on the order of 10^8 M^{-1}, these results also show that AMP-PNP reduces the binding constant of smooth muscle S-1 to actin about 1000-fold; the degree of this weakening in binding is similar to that found with skeletal muscle S-1 (Greene & Eisenberg, 1978, 1980a; Hofmann & Goody, 1978; Marston et al., 1979).

After the binding constant of smooth muscle S-1 to skeletal actin was determined at different ionic strengths (Figure 1), the data were then plotted in Figure 3 on a semilog plot as K vs. $\mu^{1/2}$. For comparison, data obtained at varying ionic strengths for the binding of skeletal muscle S-1 to actin in the presence of AMP-PNP are also shown in Figure 3 (open circles). Neither of these plots are linear, which is not surprising since actin and S-1 are not uniformly charged spheres. As can be seen from Figure 3, ionic strength clearly affects

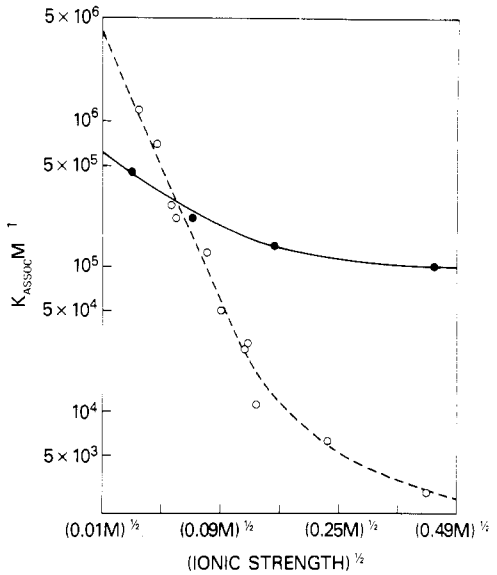


FIGURE 3: Ionic strength dependency of the binding of smooth muscle S-1 and skeletal muscle S-1 to actin in the presence of AMP-PNP. The binding constants of smooth muscle S-1 (●) to actin in the presence of AMP-PNP were obtained from the ordinate intercepts in Figure 1. The binding constants of skeletal muscle S-1 (○) to actin in the presence of AMP-PNP were obtained at the varying ionic strengths by measuring the binding of SH_1-blocked S-1 to actin in the presence of 2 mM AMP-PNP (Greene, 1981). At this concentration of AMP-PNP, more than 85% of the acto-S-1 complex has bound AMP-PNP (Greene & Eisenberg, 1978). At least five measurements using different concentrations of S-1 were made in determining the binding constant at each ionic strength.

the binding of skeletal muscle S-1 to a much greater extent than the binding of smooth muscle S-1. For example, from μ = 0.023 M ($\mu^{1/2}$ = 0.15) to μ = 0.15 M ($\mu^{1/2}$ = 0.39), the skeletal muscle S-1 binding constant decreases 100-fold, whereas the smooth muscle S-1 binding constant decreases only about 3-fold. Similar to the results obtained in the absence of nucleotide, these results show that skeletal muscle S-1 binds to actin with greater affinity than smooth muscle S-1 at low ionic strength, whereas at high ionic strength, the reverse is true. Therefore, ionic strength has the same effect on the binding of smooth muscle S-1 and skeletal muscle S-1 to actin in the presence of AMP-PNP and in the absence of nucleotide.

The binding of gizzard S-1 to actin was next examined in the presence of ATP to determine first how strongly smooth muscle S-1 binds to actin in the presence of ATP at low ionic strength. Previously, Stein et al. (1979) found that skeletal muscle S-1 binds to actin in the presence of ATP with $K_{binding}$ equal to 3×10^4 M^{-1} at μ = 0.012 M, 15 °C. This was done by measuring turbidity in the stopped-flow apparatus. The binding of smooth muscle S-1 to actin in the presence of ATP was initially measured at μ = 0.012 M, 25 °C, with the same method, but the binding was significantly weaker than with skeletal muscle S-1, making it difficult to obtain an accurate binding constant. We therefore used a different method, previously used by Chalovich & Eisenberg (1982), which measures more accurately values of $K_{binding} < 10^4$ M^{-1}. The binding of smooth muscle S-1 to actin in the presence of ATP was measured by sedimenting the bound S-1 in an airfuge and determining the concentration of unbound S-1 with the NH_4^+-EDTA ATPase assay (see Materials and Methods). Since actin is a potent inhibitor of this ATPase activity (Krisanda & Murphy, 1980), controls were run to establish that no significant inhibition was occurring during the assay. This restricted the actin concentration to an upper limit of ~300 μM.

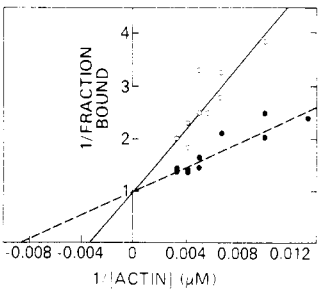


FIGURE 4: Binding of smooth muscle S-1 to actin in the presence of ATP. At μ = 0.012 M (●), the conditions were 10 mM imidazole, 1.8 mM $MgCl_2$, 1.0 mM ATP, and 0.1 mM DTT (pH 7.0, 25 °C). At μ = 0.042 M (○), the conditions were the same except that 30 mM KCl was also added. In both cases, the S-1 concentration was 0.5 μM. The binding experiment at μ = 0.072 M and μ = 0.112 M, which is discussed in the text, is also under the same conditions except that 60 mM KCl and 100 mM KCl were added, respectively. The data were computer fitted by using the Marquardt compromise (Bevington, 1969).

The data from the binding studies were plotted on a double-reciprocal plot of fraction S-1 bound vs. actin concentration. As shown in Figure 4, the binding of smooth muscle S-1 to actin fits a linear double-reciprocal plot which intercepts the ordinate at one, indicating that, at infinite actin concentration, the S-1 is all bound to actin. At μ = 0.012 M the binding constant ($K_{binding}$) of smooth muscle S-1 to actin in the presence of ATP is determined from the abscissa intercept to be 8.5×10^3 M^{-1}. Therefore, smooth muscle S-1, like skeletal muscle S-1, binds weakly to actin in the presence of ATP. The binding was next measured at μ = 0.042 M (open circles) to determine the effect of ionic strength on the steady-state binding of smooth muscle S-1 to actin. Figure 4 shows that at μ = 0.042 M, $K_{binding}$ is 3.3×10^3 M^{-1}. At higher ionic strength (μ = 0.072 M and μ = 0.112 M), the binding was too weak to measure over a wide enough range of actin concentration to enable the data to be plotted on a double-reciprocal plot. Instead, the binding was repeatedly measured by using 100, 200, and 300 μM actin. The binding constants were determined to be $(3.0 \pm 0.34) \times 10^3$ M^{-1} and $(1.6 \pm 0.2) \times 10^3$ M^{-1} at μ = 0.072 M and μ = 0.112 M, respectively. Therefore, even at physiological ionic strength, it is possible to observe binding of smooth muscle S-1 to actin in the presence of ATP.

The salt dependency of $K_{binding}$ is shown in Figure 5 where the data obtained with smooth muscle S-1 are plotted as K vs. $\mu^{1/2}$ on a semilog plot (closed triangles). For comparison, values of $K_{binding}$ obtained with skeletal muscle S-1 are also plotted (open triangles). Figure 5 clearly shows that the binding of skeletal muscle S-1 to actin in the presence of ATP is weakened to a greater degree than the binding of smooth muscle S-1 by changing ionic strength. At μ = 0.012 M, skeletal muscle S-1 binds about 4-fold more strongly to actin in the presence of ATP than smooth muscle S-1. As the ionic strength is raised to 0.05 M, there is a reversal in affinity, and smooth muscle S-1 now binds about 3-fold more strongly than skeletal muscle S-1. Unfortunately, this comparison between the steady-state binding of smooth and skeletal muscle S-1 to actin cannot be extended to higher ionic strengths because the binding of skeletal muscle S-1 to actin becomes too weak to measure. In any case, the effect of ionic strength on the binding of smooth muscle S-1 and skeletal muscle S-1 to actin in the presence of ATP shows the same pattern which has been observed both in the presence of AMP-PNP (Figure 3) and in the absence of nucleotide (Table I).

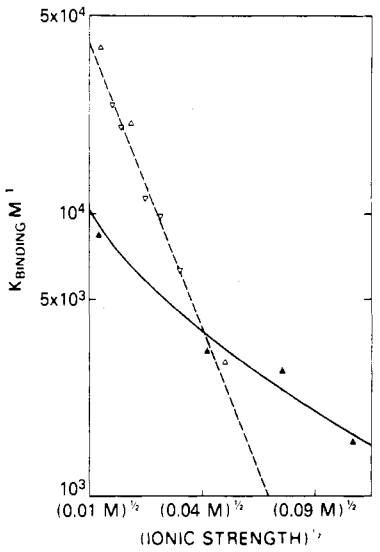


FIGURE 5: Ionic strength dependency of the binding of smooth muscle S-1 and skeletal muscle S-1 to actin in the presence of ATP. The binding constants of smooth muscle S-1 (▲) to actin in the presence of ATP were obtained from Figure 4 at $\mu = 0.012$ M and $\mu = 0.042$ M. See text for determination of binding constants at $\mu = 0.072$ M and $\mu = 0.112$ M. The binding constants of skeletal S-1 to actin in the presence of ATP (△, ▽) were determined by measuring turbidity on the stopped-flow (▽) (L. Stein, unpublished data) or by ATPase assays after centrifugation in the airfuge (△) (Chalovich & Eisenberg, 1982).

Table II: Interaction of Smooth S-1 and Actin in the Presence of ATP[a]

ionic strength (M)	$K_{binding}$ (M^{-1})	K_{ATPase} (M^{-1})
0.012	$8.5 \pm 0.56 \times 10^3$	$3.2 \pm 0.21 \times 10^4$
0.042	$3.3 \pm 0.25 \times 10^3$	$1.4 \pm 0.03 \times 10^4$
0.072	$3.0 \pm 0.34 \times 10^3$	$2.5 \pm 1.12 \times 10^3$
0.112	$1.6 \pm 0.20 \times 10^3$	$1.7 \pm 0.65 \times 10^3$

[a] The conditions for the determination of $K_{binding}$ and K_{ATPase} are given in Figures 4 and 6, respectively.

In the presence of ATP, the interaction of actin and myosin can also be measured by the kinetic binding constant, K_{ATPase}, obtained from the double-reciprocal plot of ATPase activity vs. actin concentration. The values of K_{ATPase} for smooth muscle S-1 were obtained over the same range of ionic strength that was used in measuring $K_{binding}$. The double-reciprocal plots at these different ionic strengths are shown in Figure 6. At the higher ionic strengths, it is not technically possible to work at very high actin concentrations which makes it necessary to extrapolate the value of K_{ATPase}. The values of K_{ATPase} range from 3.2×10^4 M^{-1} at $\mu = 0.012$ M to about 1.7×10^3 M^{-1} at $\mu = 0.112$ M. Figure 6 also shows that the values of V_{max} do not change significantly over this range of ionic strength.

Table II compares the values of $K_{binding}$ and K_{ATPase} obtained with smooth muscle S-1 at the different ionic strengths. At $\mu = 0.012$ M, K_{ATPase} is about 4-fold larger than $K_{binding}$, similar to the results obtained with skeletal muscle S-1 (Stein et al., 1979). This difference between K_{ATPase} and $K_{binding}$ has been explained by a special rate-limiting step occurring before the release of P_i and after the ATP hydrolysis step (Stein et al., 1979). Table II also shows that as the ionic strength is raised, the difference between K_{ATPase} and $K_{binding}$ decreases until these binding constants have about the same value at $\mu = 0.112$ M. It appears that this pattern may also occur with skeletal muscle S-1. Therefore, the interactions of smooth muscle S-1 and skeletal muscle S-1 with actin in the presence of ATP appear to be similar, except that the binding of smooth muscle S-1 is considerably less affected by ionic strength.

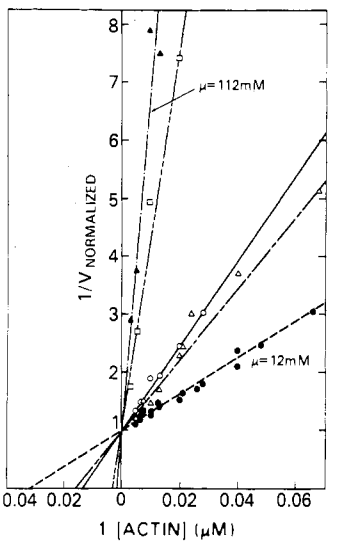


FIGURE 6: Actin-activated MgATPase activity of smooth muscle S-1. The assay conditions were the same as in Figure 3 with the ionic strength being varied by increasing the KCl concentration. The following ionic strengths were examined: $\mu = 0.012$ M (●), $\mu = 0.027$ M (△), $\mu = 0.042$ M (○), $\mu = 0.072$ M (□), and $\mu = 0.112$ M (▲). Phosphate was analyzed as described under Materials and Methods. The results are the composite of two experiments. In each case the data for all ionic strengths were normalized to the value for the V_{max} of the S-1 at 0.012 M ionic strength. The actual values for the V_{max} was 0.67 and 0.83 s^{-1} for the two experiments. The normalized V_{max} at the various ionic strengths obtained from the fitting procedure did not vary by more than 10%.

Discussion

This study shows that the effect of ionic strength on the binding of S-1 to actin is very different for smooth muscle and skeletal muscle S-1. Increasing ionic strength weakens the binding of smooth muscle S-1 to actin only slightly, whereas it markedly weakens the binding of skeletal muscle S-1 to actin. At the same time, it is interesting that this ionic strength effect does not depend on the nucleotide bound to S-1; i.e., the same trend was observed in the absence of nucleotide, in the presence of ATP, and in the presence of AMP-PNP. The differential effect of ionic strength on skeletal and smooth muscle S-1 probably accounts for the difference in the results of Krisanda & Murphy (1980) on the one hand and Marston & Taylor (1980) on the other. Krisanda and Murphy worked at relatively high ionic strength which accounts for their finding that smooth muscle myosin binds more tightly to actin than skeletal muscle myosin. On the other hand, Marston and Taylor worked at very low ionic strength where smooth muscle myosin binds more weakly than skeletal muscle myosin.

In contrast to the effect of ionic strength, the effect of nucleotide on the binding of S-1 to actin is similar for smooth muscle and skeletal muscle S-1. Both AMP-PNP and ATP reduce the affinity of smooth muscle and skeletal muscle S-1 to actin to about the same extent. For example, at $\mu = 0.04$ M, smooth muscle S-1 binds to actin with a binding constant of about 10^8 M^{-1} in the absence of nucleotide, whereas the binding constant is 3×10^5 M^{-1} and 3×10^3 M^{-1} in the presence of AMP-PNP and ATP, respectively. Skeletal muscle S-1 and smooth muscle S-1 are also similar in that the binding constant of AMP-PNP to acto-S-1 is insensitive to ionic strength (Greene & Eisenberg, 1978; Greene, 1981).

This study also examined the kinetics of the actomyosin ATPase under steady-state conditions with smooth muscle S-1. Marston & Taylor (1980) previously found that, qualitatively,

the kinetics of the actomyosin ATPase were similar with smooth and skeletal muscle S-1, although certain rates were slower with smooth muscle S-1 (i.e., the maximum ATPase rate, the rate of dissociation of acto·S-1 by ATP and the rate of ADP release from the acto·S-1·ADP complex). However, they did not measure the binding of the smooth muscle S-1 to actin in the presence of ATP. The present study shows that, similar to skeletal muscle S-1 at very low ionic strength, $K_{binding}$ (determined by directly measuring the binding of S-1 to actin in the presence of ATP) is significantly less than K_{ATPase} (determined from the double-reciprocal plot of ATPase activity vs. actin concentration). Interestingly, with smooth muscle HMM, this difference was not observed (unpublished results). It has been suggested that the difference between K_{ATPase} and $K_{binding}$ depends on the ratio of two rate constants in the kinetic model of Stein et al. (1979). It may be that shifts in this ratio explain why $K_{ATPase} \simeq K_{binding}$ both at higher ionic strength and with smooth muscle HMM. In any case, it appears that the actin-activated ATPase activity of smooth muscle S-1 and skeletal muscle S-1 has a similar kinetic cycle.

In conclusion, at physiological strength, smooth muscle S-1 binds more strongly to actin than skeletal muscle S-1 both in the presence and in the absence of nucleotide. It is possible that this stronger binding is related to the high force generation per myosin molecule observed in smooth muscle. To determine whether this is indeed the case, we will have to learn more about the structural organization of smooth muscle and the role it plays in generating isometric force.

Registry No. ATP, 56-65-5; AMP-PNP, 25612-73-1; ATPase, 9000-83-3.

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