Interaction of Isozymes of Myosin Subfragment 1 with Actin: Effect of Ionic Strength and Nucleotide†

Joseph M. Chalovich,‡ Leonard A. Stein,§ Lois E. Greene, and Evan Eisenberg*

ABSTRACT: Myosin subfragment 1 (S-1) can be fractionated into two isozymes, (A1)S-1 containing alkali light chain 1 and (A2)S-1 containing alkali light chain 2. The predominant difference in the behavior of the two isozymes of S-1 is that, at low ionic strength, the actin concentration required for half-maximal ATPase activity is considerably lower for (A1)S-1 than for (A2)S-1; that is, the apparent binding constant K_{ATPase} for (A1)S-1 is greater than K_{ATPase} for (A2)S-1 [Weeds, A. G., & Taylor, R. S. (1975) *Nature (London) 257*, 54–56]. This difference disappears at high ionic strength [Wagner, P. D., Slater, C. S., Pope, B., & Weeds, A. G. (1979) *Eur. J. Biochem. 99*, 385–394]. In the present study we investigated whether the difference in the K_{ATPase} values of (A1)S-1 and (A2)S-1 is due to a difference in the actual affinity of these S-1 isozymes for actin. Binding was measured in the presence of ATP and AMP-PNP and in the absence of nucleotide at varied ionic strengths. We found that at low ionic strength where K_{ATPase} is several times stronger for (A1)S-1 than for (A2)S-1, the binding of (A1)S-1 to actin is correspondingly stronger than that of (A2)S-1 irrespective of the nucleotide present. Furthermore, as the ionic strength is increased, just as the difference between the K_{ATPase} values for (A1)S-1 and (A2)S-1 disappears so too does the difference in the affinity of the two isozymes for actin. We conclude, first, that the difference in K_{ATPase} between (A1)S-1 and (A2)S-1 is due to a difference in the binding affinity of these isozymes for actin, a difference that is maintained even in the absence of nucleotide. Second, we conclude that, although it is relatively weak, the binding of S-1 to actin observed in the presence of ATP is a specific reaction because it reflects the difference in the affinity of (A1)S-1 and (A2)S-1 for actin.

Skeletal muscle myosin is a hexamer composed of two heavy chains, two phosphorylatable light chains, and two alkali light chains that can be removed by alkali treatment. Functionally each myosin molecule has a dimeric structure containing two active sites or heads. Most kinetic studies on the myosin ATPase activity have been carried out with the soluble single headed proteolytic fragment of myosin subfragment 1 (S-1).[1] S-1 prepared by chymotryptic digestion lacks the phosphorylatable light chain but does contain an alkali light chain (Yagi & Otani, 1974; Weeds & Taylor, 1975). Since there are two kinds of alkali light chains, A1 and A2, there are two isozymes of S-1, (A1)S-1 and (A2)S-1. These two isozymes can be separated by using DEAE-cellulose chromatography.

For many years it was thought that the alkali light chain was essential to the activity of myosin. However, recently it has been demonstrated that the alkali light chain can be removed from S-1 without elimination of the ATPase activity (Wagner & Giniger, 1981; Sivaramakrishnan & Burke, 1982). Nevertheless, the alkali light chain does appear to modulate the actin-activated ATPase activity of S-1 (Weeds & Taylor, 1975). At low ionic strength the concentration of actin required to reach half-maximal ATPase activity is considerably lower for (A1)S-1 than for (A2)S-1; i.e., the apparent binding constant, K_{ATPase}, is greater for (A1)S-1 than for (A2)S-1. In addition, the maximal ATPase activity (V_{max}) is greater for (A2)S-1 than for (A1)S-1. Both of these differences decrease markedly as the ionic strength is increased (Wagner et al., 1979).

Since K_{ATPase} is a kinetic constant rather than a true binding constant, the difference in the values of K_{ATPase} for (A1)S-1 and (A2)S-1 could be due to a difference in rate constants for the two isozymes rather than to a real difference in their affinity for actin. In the present study, we investigated this question by directly measuring the binding of (A1)S-1 and (A2)S-1 to actin in the presence of ATP and AMP-PNP and in the absence of nucleotide at varied ionic strengths. Our results show that the difference between K_{ATPase} for (A1)S-1 and (A2)S-1 reflects a real difference in their affinity for actin in the presence of ATP. Furthermore, this difference in binding affinity is maintained in the presence of AMP-PNP and in the absence of nucleotide, although the affinity increases by 100- and 1000-fold, respectively, under these conditions. These results show that the effect of the alkali light chains on the binding of S-1 to actin is unrelated either to the strength of this binding or to the presence or absence of nucleotide at the active site. They also show that, although the binding observed between S-1 and actin in the presence of ATP is relatively weak, it is a highly specific interaction.

Materials and Methods

Proteins. Myosin was isolated from the back and leg muscles of rabbits by standard procedures (Kielley & Harrington, 1960). S-1 was prepared by chymotryptic digestion of myosin (Weeds & Taylor, 1975). S-1 was fractionated into the two isozymes (A1)S-1 and (A2)S-1 by chromatography on Whatman DE-52 (Weeds & Taylor, 1975). Iodo[^{14}C]-acetamide-labeled (A1)S-1 and (A2)S-1 were prepared by fractionation of labeled S-1 (Greene & Eisenberg, 1980a) on the same DE-52 column. All solutions used in the preparation of S-1, (A1)S-1, and (A2)S-1 contained 1 mM dithiothreitol.

† From the Laboratory of Cell Biology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20205. *Received January 9, 1984.*

‡ Present address: Department of Biochemistry, School of Medicine, East Carolina University, Greenville, NC 27834.

§ Present address: Department of Cardiology, State University of New York at Stony Brook, Health Sciences Center, Stony Brook, NY 11794.

[1] Abbreviations: S-1, myosin subfragment 1; (A1)S-1, S-1 enriched in the M_r 21 000 light chain; (A2)S-1, S-1 enriched in the M_r 17 000 light chain; AMP-PNP, 5′-adenylyl imidodiphosphate; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; K_{ATPase}, apparent association constant determined from the actin dependence of ATPase rates; $K_{binding}$, association constant of S-1 to actin; SDS, sodium dodecyl sulfate.

This article not subject to U.S. Copyright. Published 1984 by the American Chemical Society

Actin was prepared by the procedure of Spudich & Watt (1971) as modified by Eisenberg & Kielley (1974). The molecular weights used for actin and S-1 were 42000 and 120000, respectively. Protein concentrations were determined spectrophotometrically at 280 nm by using absorption coefficients of 750 cm^2/g for S-1 and 1150 cm^2/g for actin.

ATPase Assays. Actin-activated S-1 ATPase activities were measured in 4 mL of solution containing 1 mM ATP, 1.8 mM $MgCl_2$, 2 mM imidazole hydrochloride, pH 7.0, 1 mM dithiothreitol, and variable KCl with a pH stat as previously described (Eisenberg & Moos, 1967). The NH_4^+-EDTA ATPase activity was measured at 25 °C by the rate of liberation of $[^{32}P]P_i$ from $[\alpha\text{-}^{32}P]ATP$ as described previously (Chalovich & Eisenberg, 1982). Four or five time points were taken to define each rate.

Binding Assays. The binding of (A1)S-1 and (A2)S-1 to actin in the presence of ATP was usually measured by sedimenting the acto-S-1 in a Beckman airfuge and determining the free S-1 concentration by the NH_4^+-EDTA assay (Chalovich & Eisenberg, 1982). Binding assays were carried out using 1.5-mL volumes of a solution whose composition is defined in the figure legends. Binding in the presence of ATP was generally measured with 0.05 μM S-1. In some cases, binding in the presence of ATP was determined by the stopped-flow turbidity method (Stein et al., 1979). Binding in the presence of AMP-PNP was determined by measuring the iodo[^{14}C]acetamide-labeled (A1)S-1 or (A2)S-1 in the supernatant after centrifugation in a preparative ultracentrifuge (Greene & Eisenberg, 1980b). The total volume was 4 mL, and the S-1 concentration was 2.5–4 μM.

In the absence of nucleotide, the binding was too strong to measure directly at low ionic strength. The relative affinities were determined by a competition experiment. Equimolar amounts of (A1)S-1, (A2)S-1, and actin were combined in a buffer composed of 10 mM imidazole hydrochloride, 2 mM $MgCl_2$, 1.0 mM DTT, and 0–100 mM KCl. Following a 30-min incubation at 25 °C, the mixture was centrifuged to sediment actin and acto-S-1. The ratio of (A2)S-1 to (A1)S-1 in the supernatant was determined by SDS–polyacrylamide gel electrophoresis according to Weber & Osborn (1972). Gels were stained with Coomassie Blue R and scanned at 595 nm with a Helena Quick Scan R & D scanner equipped with an integrator. It can be shown that, in this competition experiment, the ratio of the association constant for (A1)S-1 to the association constant for (A2)S-1 is the equal to the square of the ratio of free (A2)S-1 to free (A1)S-1. This is because the initial concentrations of (A1)S-1, (A2)S-1, and actin are equal and (A1)S-1 bound = $(A2)S\text{-}1_{free}$ and $(A1)S\text{-}1_{free} = (A2)S\text{-}1_{bound}$.

Results

We began our study by investigating whether the difference between K_{ATPase} for (A1)S-1 and (A2)S-1 is accompanied by a parallel difference in the affinity of the two isozymes for actin in the presence of ATP. Figure 1A shows double-reciprocal plots of ATPase activity vs. actin concentration for (A1)S-1 and (A2)S-1 under the same conditions as our previous kinetic studies (Stein et al., 1984). One kinetic difference between (A1)S-1 and (A2)S-1 is that, as we reported previously, at high actin concentration, (A1)S-1 shows about 30% inhibition of the actin-activated ATPase activity whereas under the same conditions (A2)S-1 shows no evidence of such inhibition. Marston observed a similar inhibition with unfractionated S-1 (Marston, 1978). The major difference in the kinetic behavior of the two isozymes lies in their values for K_{ATPase}. Extrapolation of the double-reciprocal plots shows that the value of

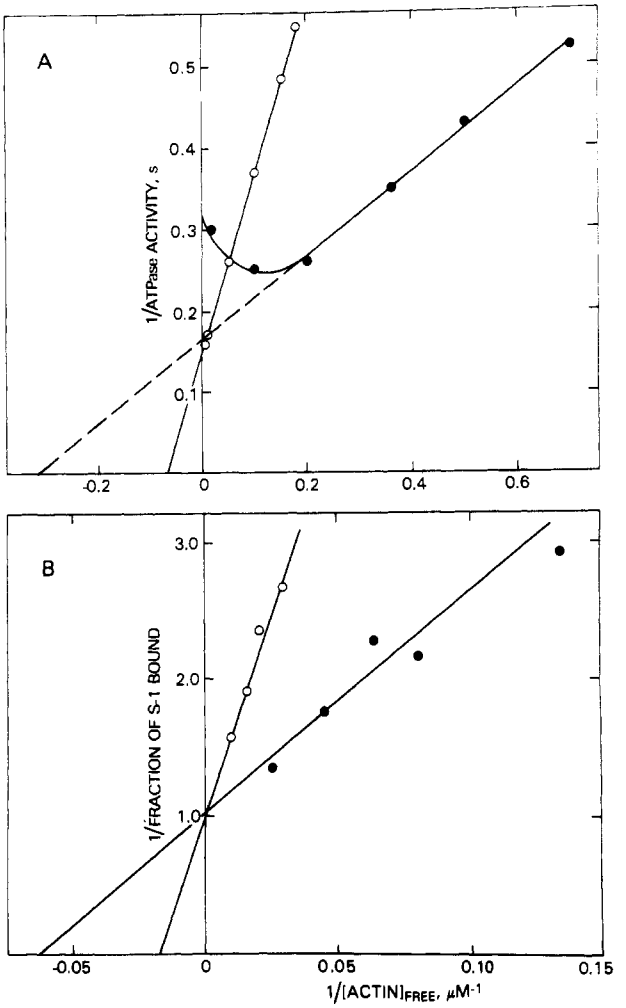

FIGURE 1: ATPase rates and binding of S-1 as a function of actin concentration at 15 °C, 12.4 mM ionic strength. (●) (A1)S-1; (○) (A2)S-1. (A) Actin-activated ATPase rates corrected for the rate of S-1 alone. (A1)S-1: $V_m = 6.0 \pm 0.4\ s^{-1}$; $K_{ATPase} = (3.4 \pm 0.3) \times 10^5\ M^{-1}$. (A2)S-1: $V_m = 6.7 \pm 0.3\ s^{-1}$; $K_{ATPase} = (6.9 \pm 0.3) \times 10^4\ M^{-1}$. (B) Binding to actin. (A1)S-1: $K_{binding} = (6.3 \pm 0.3) \times 10^4\ M^{-1}$. (A2)S-1: $K_{binding} = (1.7 \pm 0.1) \times 10^4\ M^{-1}$. Conditions: 1 mM ATP, 1.8 mM $MgCl_2$, 2 or 10 mM imidazole, pH 7.0, and 1 mM dithiothreitol. All values are given ± the approximate 95% confidence interval.

K_{ATPase} is about 5.6-fold greater for (A1)S-1 and (A2)S-1. Note that whereas in Figure 1A the values of V_{max} for (A1)S-1 and (A2)S-1 are equal, we sometimes observe a 50% increase in the value for V_{max} of (A2)S-1, in qualitative agreement with the results of Wagner et al. (1979).

Binding studies, in the presence of ATP, were performed by using stopped-flow turbidity measurements under conditions identical with those used for kinetic studies. The results are given in Figure 1B, which is a double-reciprocal plot of S-1 bound vs. actin concentration for both (A1)S-1 and (A2)S-1. Comparison of parts A and B of Figure 1 shows that the difference in K_{ATPase} for (A1)S-1 and (A2)S-1 is paralleled by a difference in $K_{binding}$ for the two isozymes; $K_{binding}$ is about 4-fold stronger for (A1)S-1 than for (A2)S-1. Therefore, the difference in K_{ATPase} for A1(S-1) and (A2)S-1 appears to be caused by a real difference in the affinity of the two isozymes for actin.

Note that, although the difference between K_{ATPase} for (A1)S-1 and that for (A2)S-1 is paralleled by a difference in $K_{binding}$, with neither of the isozymes does K_{ATPase} equal $K_{binding}$. As we reported previously for (A1)S-1, K_{ATPase} is 6-fold stronger than $K_{binding}$ at 15 °C (Stein et al., 1984), and sim-

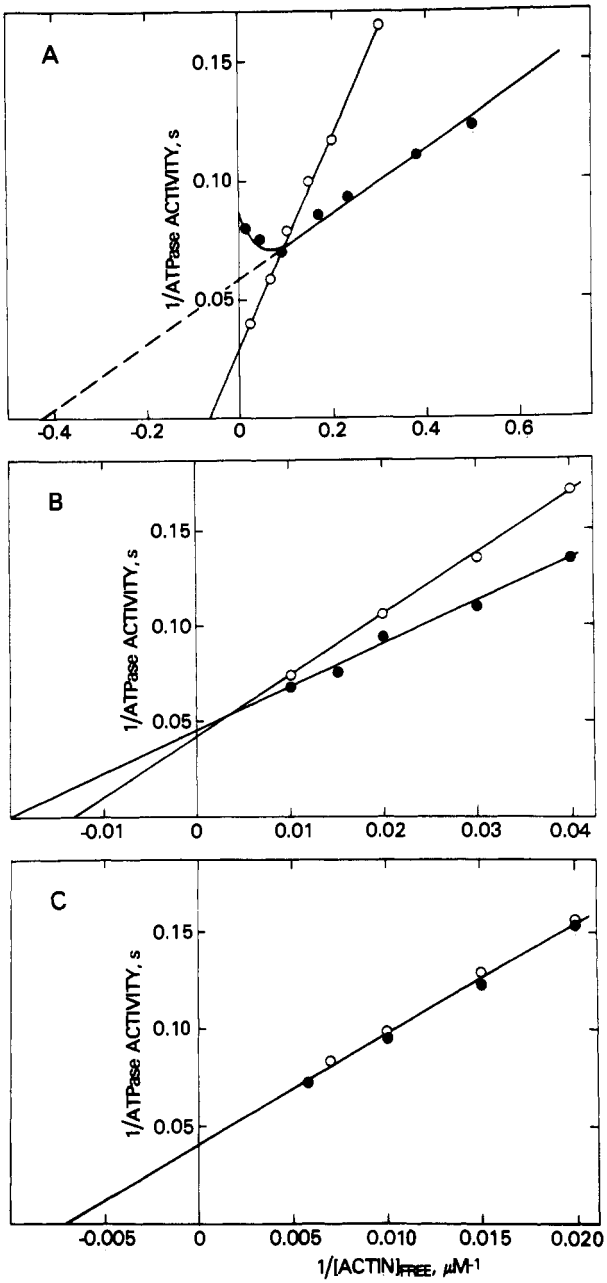

FIGURE 2: Ionic strength dependence of the actin-activated ATPase activity of (A1)S-1 and (A2)S-1 at 25 °C. (A) (●) (A1)S-1; (○) (A2)S-1. 12.4 mM ionic strength. (A1)S-1: $V_m = 17 \pm 0.6\ s^{-1}$; $K_{ATPase} = (4.3 \pm 0.4) \times 10^5\ M^{-1}$. (A2)S-1: $V_m = 32 \pm 6\ s^{-1}$; $K_{ATPase} = (6.7 \pm 0.3) \times 10^4\ M^{-1}$. (B) 35 mM ionic strength. (A1)S-1: $V_m = 22 \pm 2\ s^{-1}$; $K_{ATPase} = (2.0 \pm 0.3) \times 10^4\ M^{-1}$. (A2)S-1: $V_m = 24 \pm 2\ s^{-1}$; $K_{ATPase} = (1.3 \pm 0.1) \times 10^4\ M^{-1}$. (C) 50 mM ionic strength. Both (A1)S-1 and (A2)S-1: $V_m = 24 \pm 1\ s^{-1}$; $K_{ATPase} = (7.3 \pm 0.7) \times 10^3\ M^{-1}$. The conditions are the same as in Figure 1 except for the addition of KCl to vary the ionic strength. All values are given ± the approximate 95% confidence interval.

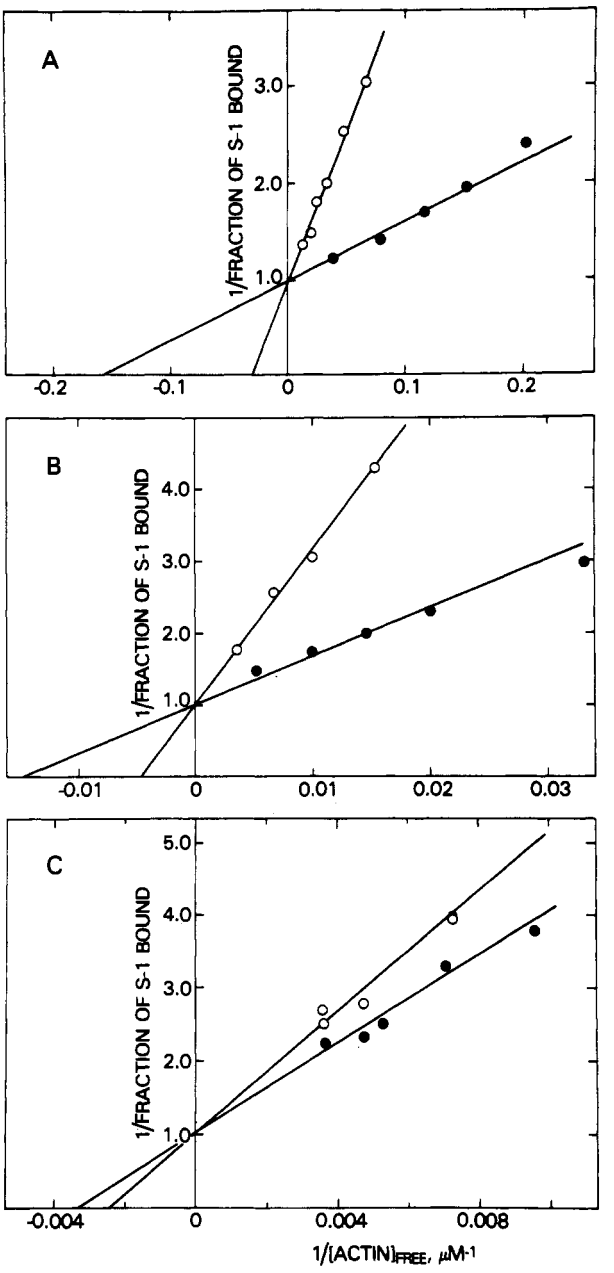

FIGURE 3: Ionic strength dependence of the binding of (A1)S-1 and (A2)S-1 to actin in the presence of ATP at 25 °C. (●) (A1)S-1; (○) (A2)S-1. (A) 12.4 mM ionic strength. (A1)S-1: $K_{binding} = (1.60 \pm 0.08) \times 10^5\ M^{-1}$. (A2)S-1: $K_{binding} = (3.1 \pm 0.2) \times 10^4\ M^{-1}$. (B) 35 mM ionic strength. (A1)S-1: $K_{binding} = (1.50 \pm 0.08) \times 10^4\ M^{-1}$. (A2)S-1: $K_{binding} = (4.6 \pm 0.2) \times 10^3\ M^{-1}$. (C) 50 mM ionic strength. (A1)S-1: $K_{binding} = (3 \pm 1) \times 10^3\ M^{-1}$. (A2)S-1: $K_{binding} = (2.0 \pm 0.8) \times 10^3\ M^{-1}$. The conditions are the same as in Figure 1 except for the addition of KCl to vary the ionic strength. All values are given ± the approximate 95% confidence interval. The difference between values of $K_{binding}$ and the corresponding values of K_{ATPase} are statistically significant ($p < 0.05$) for all cases except (A1)S-1 at 35 mM ionic strength.

ilarly for (A2)S-1, K_{ATPase} is 4-fold stronger than $K_{binding}$.

If the difference in K_{ATPase} for (A1)S-1 and (A2)S-2 is indeed due to a difference in the affinity of the two isozymes for actin, then, like the difference in the value of K_{ATPase} (Wagner et al., 1979), the difference in $K_{binding}$ for the two isozymes should decrease with increasing ionic strength. We used sedimentation in the airfuge to measure binding in the presence of ATP at varied ionic strength. The weak binding that occurs at high ionic strength can be measured more accurately with the airfuge method than with the stopped-flow turbidity method. These experiments were done at 25 °C where it was most convenient to operate the airfuge. Figure 2 shows the effect of increasing ionic strength on the difference between K_{ATPase} for (A1)S-1 and (A2)S-1. As has been reported previously, the kinetic differences between (A1)S-1 and (A2)S-1 decrease with increasing ionic strength, disappearing almost completely at $\mu = 0.05$ M. Figure 3 shows that the difference between $K_{binding}$ for (A1)S-1 and (A2)S-1 also decreases with increasing ionic strength. At $\mu = 0.012$ M, $K_{binding}$ for (A1)S-1 is 5-fold greater than for (A2)S-1; in contrast at $\mu = 0.05$ M, there is only a 50% difference between the $K_{binding}$ values for the two isozymes. The parallel effect of ionic strength on $K_{binding}$ and K_{ATPase} confirms that the difference between K_{ATPase} for (A1)S-1 and (A2)S-1 is due to a real

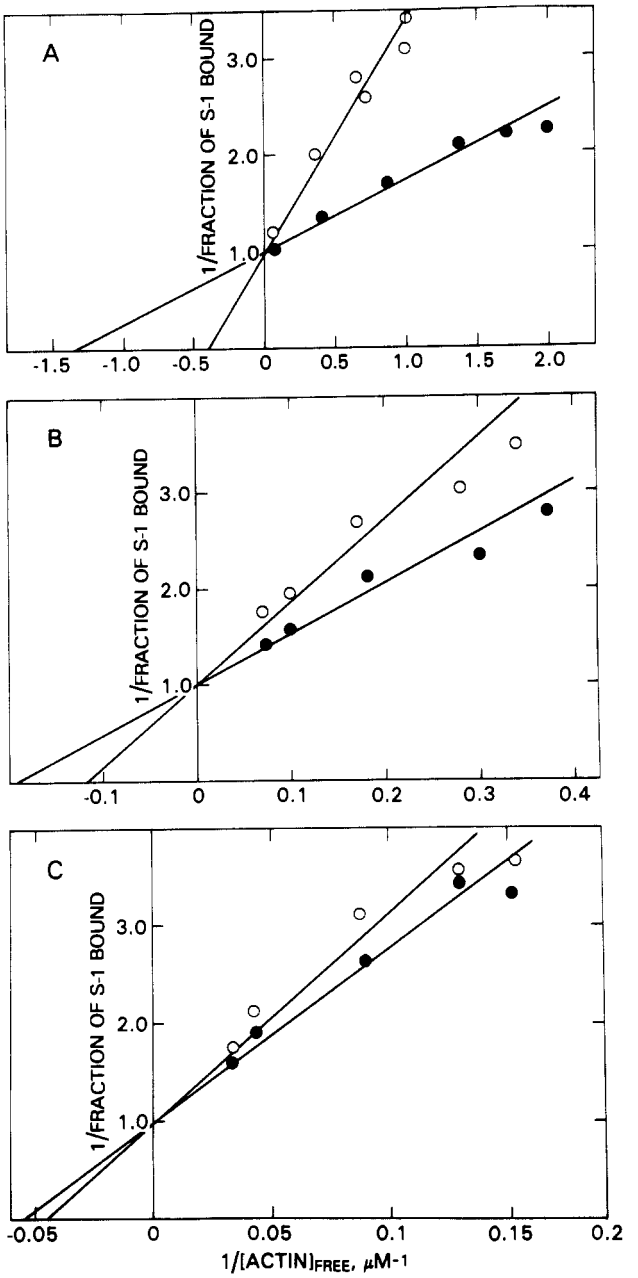

FIGURE 4: Binding of S-1 to actin in the presence of AMP-PNP at 25 °C as a function of ionic strength. (●) (A1)S-1; (○) (A2)S-1. (A) 20 mM ionic strength. $K(A1) = (1.40 \pm 0.07) \times 10^6\ M^{-1}$; $K(A2) = (4.0 \pm 0.2) \times 10^5\ M^{-1}$. (B) 60 mM ionic strength. $K(A1) = (1.9 \pm 0.1) \times 10^5\ M^{-1}$; $K(A2) = (1.1 \pm 0.1) \times 10^5\ M^{-1}$. (C) 120 mM ionic strength. $K(A1) = (5.5 \pm 0.3) \times 10^4\ M^{-1}$; $K(A2) = (4.4 \pm 0.3) \times 10^4\ M^{-1}$. Conditions: 2 mM AMP-PNP, 4 mM $MgCl_2$, 10 mM imidazole, 1 mM DTT, 7 μM diadenosine pentaphosphate, pH 7.0, and 0, 35, or 95 mM KCl. All values are given ± the approximate 95% confidence interval.

difference in the affinity of the two isozymes for actin.

The next question we addressed in this study was whether (A1)S-1 and (A2)S-1 only differ in their affinity for actin in the presence of ATP or whether, in fact, this is a more general phenomenon. It is known that the interaction of S-1 with actin and actin–troponin–tropomyosin is markedly different in the presence of ATP than in the presence of AMP-PNP and ADP or in the absence of nucleotide (Chalovich et al., 1983). The binding of (A1)S-1 and (A2)S-1 to actin in the presence of AMP-PNP is shown in Figure 4 as a function of ionic strength. It is clear that at 20 mM ionic strength (A1)S-1 has a stronger affinity for actin than does (A2)S-1; the association constant for (A1)S-1 is 3.5 times that of (A2)S-1. Like the difference observed in the presence of ATP, the difference between

Table I: Relative Affinities of (A1)S-1 and (A2)S-1 to Actin in the Absence of Nucleotide As Determined by a Competition Method

ionic strength[a] (M)	n[b]	(A2)S-1_{free}[c]/(A1)S-1_{free}	$K(A1)$[d]/$K(A2)$
0.011	3	1.97	3.9 ± 0.6
0.021	4	1.85	3.4 ± 0.7
0.031	3	1.53	2.3 ± 0.4
0.061	3	1.27	1.6 ± 0.5
0.111	3	1.07	1.1 ± 0.3

[a] 10 mM imidazole hydrochloride, pH 7.0, 2 mM $MgCl_2$, 1.0 mM DTT, and 0–100 mM KCl, 25 °C. [b] Number of determinations. [c] 10 μM actin, 10 μM (A1)S-1, and 10 μM (A2)S-1 in the original mixture. The ratio of free (A1)S-1 to (A2)S-1 was determined by electrophoresis of the total free S-1 and integration of the light chain intensities of the gels stained with Coomassie Blue. [d] $K(A1)/K(A2) = (A2_{free}/A1_{free})^2$, where the K's are association constants. Values are given with the standard deviation.

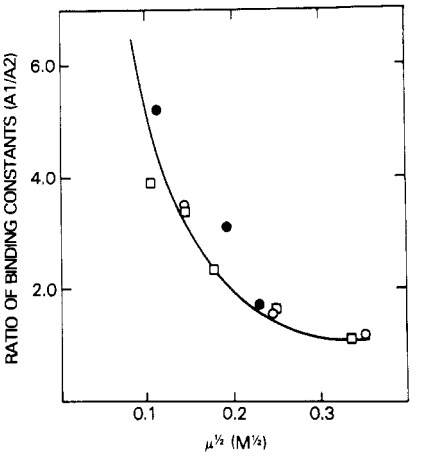

FIGURE 5: Effect of ionic strength on the ratio of association constants of (A1)S-1 and (A2)S-1 to actin. (●) Ratio of association constants in ATP; (○) ratio of association constants in AMP-PNP; (□) ratio of association constants in the absence of nucleotide.

(A1)S-1 and (A2)S-1 in the presence of AMP-PNP is reduced as the ionic strength is increased. At 60 mM ionic strength the association constant for (A1)S-1 is only 70% greater than that of (A2)S-1 (Figure 4B), and there is virtually no difference between the two association constants at 120 mM ionic strength (Figure 4C).

A similar phenomenon appears to occur even in the absence of nucleotide. Table I shows a competition experiment between (A1)S-1 and (A2)S-1 for actin, in the absence of nucleotide. In this experiment an equimolar mixture of (A1)S-1 and (A2)S-1 was combined with actin at several ionic strengths. After removal of the free actin and acto-S-1 by centrifugation, the relative amounts of (A1)S-1 and (A2)S-1 in the supernatants were determined by SDS–polyacrylamide gel electrophoresis. As can be seen at $\mu = 0.011$ M, (A1)S-1 clearly binds in preference to (A2)S-1 just as occurs in the presence of ATP or AMP-PNP. Furthermore, this difference between the two isozymes decreases with increasing ionic strength until at $\mu = 0.11$ M there is no significant difference between them. Therefore, the difference in affinity of (A1)S-1 and (A2)S-1 for actin at low ionic strength is maintained even in the absence of nucleotide. Wadzinski et al. (1979) reported that, in the absence of nucleotide, (A1)S-1 and (A2)S-1 bind to actin with similar affinities. However, their measurements were all done at ionic strengths greater than 100 mM under which conditions we also found no difference in affinity between the two species.

Discussion

Figure 5 summarizes the difference in affinity observed between (A1)S-1 and (A2)S-1 for actin as a function of ionic

strength in the presence of ATP and AMP-PNP and in the absence of nucleotide. These data show that the increased affinity for actin caused by the presence of the A1 light chain is essentially unaffected by the nucleotide bound at the active site and hence is unaffected by the strength of binding of the S-1 to actin. S-1 binds about 10 000 times more strongly to actin in the absence of ATP than in the presence of ATP. Yet in both cases the A1 light chain causes about a 4-fold increase in binding at low ionic strength.

These data are consistent with the NMR studies (Prince et al., 1981) and the affinity chromatography studies (Winstanley & Trayer, 1979; Winstanley et al., 1979), which suggest that the A1 light chain, but not the A2 light chain, interacts with actin. Also, it has been recently demonstrated that the A1 light chain can be chemically cross-linked to the C-terminal region of actin (Sutoh, 1982). The only difference between the A1 light chain and the A2 light chain is that the A1 light chain contains an extra 41 amino acid segment at its N-terminal end (Frank & Weeds, 1974). Therefore, it is probably this portion of the A1 light chain that interacts with actin. Our data are consistent with a model in which, at low ionic strength, this portion of the A1 light chain binds to actin with an association constant of about 4 and in which the binding is independent of the relatively strong interaction of actin with the major actin binding site of S-1. Presumably it is this latter interaction that is affected by the binding of nucleotide to S-1. On this basis, no matter how strongly or weakly the major actin binding site interacts with actin, the binding of the A1 light chain to actin will always increase the overall binding constant by a factor of 4 (Jencks, 1981).

Our data also suggest that the interaction of the N-terminal portion of the A1 light chain with actin decreases as the ionic strength is increased. This effect of ionic strength suggests that the interaction of the A1 light chain with actin may not occur at physiologic ionic strength, a view that is supported by studies that show no differences in the actin-activated ATPase activity of A1 and A2 myosin filaments above 40 mM ionic strength (Pope et al., 1981; Pastra-Landis et al., 1983). However, we cannot definitely rule out a physiologic role for the interaction of the A1 and A2 light chains with actin until we understand how the ordered structure of the muscle affects the interaction of the myosin cross bridges with actin.

The observed effect of the A1 light chain provides important information about the interaction of S-1 with actin in the presence of ATP. First, the data presented in this paper suggest that K_{ATPase} is greater than $K_{binding}$ in the case of (A2)S-1 as well as (A1)S-1 and S-1. Therefore, this kinetic behavior does not depend on the nature of the alkali light chain bound to myosin. Second, our data strongly suggest that the binding of S-1 to actin that occurs in the presence of ATP is a specific interaction even though it is relatively weak. It seems unlikely that the A1 light chain would affect the interaction of S-1 with actin in the same way in the absence and presence of ATP, if, in the former case, the interaction were specific but in the latter case were nonspecific. The similar salt dependence of the binding of S-1 to actin in the presence and absence of ATP also supports the view that the weak binding that occurs in the presence of ATP is a specific interaction of the S-1 with actin. In this regard, it is of interest that stiffness measurements have recently demonstrated attachment of myosin to actin in the presence of ATP in single skinned rabbit muscle fibers at low ionic strength (Brenner et al., 1982).

Acknowledgments

We are grateful to Louis Dobkin for his assistance in preparing the proteins and to Dr. Mark Schoenberg for his assistance with the statistical data analysis.

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

References

Brenner, B., Schoenberg, M., Chalovich, J. M., Greene, L. E., & Eisenberg, E. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 7288.

Chalovich, J. M., & Eisenberg, E. (1982) *J. Biol. Chem. 257*, 2432.

Chalovich, J. M., Greene, L. E., & Eisenberg, E. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 4909.

Chalovich, J. M., Stein, L. A., Greene, L. E., & Eisenberg, E. (1984) *Biophys. J. 45*, 221a (Abstr.).

Eisenberg, E., & Moos, C. (1967) *J. Biol. Chem. 242*, 2945.

Eisenberg, E., & Kielley, W. W. (1974) *J. Biol. Chem. 249*, 4742.

Frank, G., & Weeds, A. G. (1974) *Eur. J. Biochem. 44*, 317.

Greene, L. E., & Eisenberg, E. (1980a) *J. Biol. Chem. 255*, 549.

Greene, L. E., & Eisenberg, E. (1980b) *Proc. Natl. Acad. Sci. U.S.A. 77*, 2616.

Jencks, W. P. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 4046.

Kielley, W. W., & Harrington, W. F. (1960) *Biochim. Biophys. Acta 41*, 401.

Marston, S. B. (1978) *FEBS Lett. 92*, 147.

Pastra-Landis, S. C., Huiatt, T., & Lowey, S. (1983) *J. Mol. Biol. 170*, 403.

Pope, B., Wagner, P. D., & Weeds, A. G. (1981) *Eur. J. Biochem. 117*, 201.

Prince, H. P., Trayer, H. R., Henry, G. D., Trayer, I. P., Dalgarno, D. C., Levine, B. A., Cary, P. D., & Turner, C. (1981) *Eur. J. Biochem. 121*, 213.

Sivaramakrishnan, M., & Burke, M. (1982) *J. Biol. Chem. 257*, 1102.

Spudich, J. A., & Watt, S. (1971) *J. Biol. Chem. 246*, 4866.

Stein, L. A., Schwarz, R. P., Jr., Chock, P. B., & Eisenberg, E. (1979) *Biochemistry 18*, 3895.

Stein, L. A. Chock, P. B., & Eisenberg, E. (1984) *Biochemistry 23*, 1555.

Sutoh, K. (1982) *Biochemistry 21*, 3654.

Wadzinski, L., Botts, J., Wang, A., Woodward, J., & Highsmith, S. (1979) *Arch. Biochem. Biophys. 198*, 397.

Wagner, P. D., & Giniger, E. (1981) *Nature (London) 292*, 560.

Wagner, P. D., Slater, C. S., Pope, B., & Weeds, A. G. (1979) *Eur. J. Biochem. 99*, 385.

Weber, K., Pringle, J. R., & Osborn, M. (1972) *Methods Enzymol. 26*, 3.

Weeds, A. G., & Taylor, R. S. (1975) *Nature (London) 257*, 54.

Winstanley, M. A., & Trayer, I. P. (1979) *Biochem. Soc. Trans. 7*, 703.

Winstanley, M. A., Small, D. A. P., & Taylor, I. P. (1979) *Eur. J. Biochem. 98*, 441.

Yagi, K., & Otani, F. (1974) *J. Biochem. (Tokyo) 76*, 365.