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Binding of Myosin Subfragment 1 to Actin

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The dissociation constant for the binding of myosin subfragment 1 (S1) and chromatographed actin in the presence and absence of nucleotide was measured at various ionic strengths and various temperatures. The dissociation constant was of nM order in the absence of nucleotide and increased by ~100- and ~100,000-fold in the presence of ADP and ATP, respectively. The dissociation constant also increased with increasing ionic strength, irrespective of the presence of nucleotide, and its dependence on the ionic strength was increased by the presence of ATP but decreased by the presence of ADP. The standard enthalpy change and entropy change for the binding of S1 to actin were both positive, irrespective of the presence of nucleotide, indicating that the binding was entropy-driven. The standard entropy change was essentially unaffected by the presence of ADP but was greatly decreased by ATP, suggesting that the large increase in the dissociation constant in the presence of ATP was due to the decrease of hydrophobic interactions. On the other hand, the increase in the dissociation constant for acto-S1 in the presence of ADP might be induced by the decrease of electrostatic interactions.

Key words: actin, actomyosin, actomyosin ATPase, acto-S1, myosin.

Muscle contraction is driven by the cyclical interaction of myosin heads in the thick filaments with actin monomers in the thin filaments. The interaction between myosin heads and actin is coupled by the activation of ATP hydrolysis, supplying the energy required for the contraction. The interaction is very strong in the absence of nucleotide and in the presence of ADP, a product of the ATPase reaction (strong binding state), but is markedly weakened by the presence of ATP (weak binding state) (*1*). In order to understand the mechanism of muscle contraction, it is important to study the change in properties of the interaction during the actomyosin ATPase reaction. We previously reported the binding of myosin subfragment 1 (S1) and unchromatographed actin at various ionic strengths (*2*). However, we noticed afterwards that our unchromatographed actin preparation contained denatured actin that could not bind S1. The denatured actin could be removed by gel filtration chromatography of G-actin. Therefore, using the chromatographed actin, we restudied the binding of S1 and actin in the presence and absence of nucleotides at various ionic strengths. We also examined, as reported here, the temperature-dependences of their binding. The binding was found to be entropy-driven, irrespective of the presence of nucleotide, suggesting that hydrophobic interaction is a main determinant. In addition, the change of acto-S1 from the strong binding state to the weak binding state might be induced by the decrease of hydrophobic interactions.

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Abbreviations: Mops, 3-(*N*-morpholino)propanesulfonic acid; S1, myosin subfragment 1.

EXPERIMENTAL PROCEDURES

Proteins—Myosin was prepared from rabbit skeletal muscle by the method of Perry (*3*). S1 was prepared by chymotryptic digestion of myosin as described previously (*4*). Actin was prepared from rabbit skeletal muscle according to the method of Spudich and Watt (*5*) and purified by Sephacryl S-300 HR (Pharmacia LKB) gel filtration in 0.2 mM $CaCl_2$, 0.5 mM DTT, 0.2 mM ATP, 0.1 mM NaN_3, 2 mM Tris (pH 7.5) as described previously (*6*).

The concentrations of S1 and actin were determined from the absorbance at 280 nm using the absorption coefficients of 0.72 and 1.10 $(mg/ml)^{-1} \cdot cm^{-1}$, respectively. The molecular weights used for S1 and actin were 115,000 and 42,000, respectively.

Binding of S1 to Actin—In the absence of nucleotide, S1 (0.2-0.9 μM) was incubated for 30 min with 1.0 μM actin in 1 mM $MgCl_2$, 1 mM DTT, 0.2 mg/ml BSA, 10 mM 3-(*N*-morpholino)propanesulfonic acid (Mops)-NaOH (pH 7.0), and various concentrations of NaCl. In the presence of ADP, S1 (1.0 μM) was incubated for 30 min with 0.2-5.0 μM actin in 3 mM $MgCl_2$, 2 mM ADP, 1 mM DTT, 0.2 mg/ml BSA, 10 mM Mops-NaOH (pH 7.0), and various concentrations of NaCl. In the presence of ATP, S1 (0.10 μM) was incubated for 30 min with 6.0-200 μM actin in 1 mM $MgCl_2$, 1 mM DTT, 0.2 mg/ml BSA, 10 mM Mops-NaOH (pH 7.0), and various concentrations of NaCl. Just prior to ultracentrifugation, 2 mM MgATP was added to the mixture. The ionic strengths of these mixtures were adjusted with NaCl. BSA was added to the mixture to protect S1 and actin at low concentrations from the nonspecific adsorption to the surface of vessel and from denaturation (*7*). After the incubation, the mixture was

ultracentrifuged at 400,000×g for 10 min (Hitachi CP100H ultracentrifuge) to pellet F-actin and acto-S1. The concentration of free S1 remaining in the supernatant was determined by NH_4^+-EDTA-ATPase assay as described previously (*8*). For the determination, NH_4^+-EDTA-ATPase activities of S1 at various concentrations were measured in the actin under the same conditions. The activity was proportional to the concentration of S1 under the conditions used. Phosphate released was determined by colorimetric methods (*9-12*). The concentration of S1 pelleted in the absence of actin was negligible. The concentration of actin remaining in the supernatant was assumed to be negligible based on the previous reports (*7, 13*). The concentration of S1 bound to actin was obtained by subtraction of the concentration of free S1 from the total concentration of S1. The stoichiometry of the binding of actin with S1 was obtained as 0.95-1.0 mol S1/mol actin in the absence of nucleotide (Fig. 1). Therefore, assuming the binding stoichiometry of 1 mol S1/mol actin in the presence of nucleotide, the binding of S1 to actin was analyzed on the basis of first-order binding. To confirm that the actin preparation does not contain denatured actin and binds to S1 stoichiometrically, the binding of S1 to actin in the absence of nucleotide was measured with various concentrations of S1. In the presence of nucleotide, on the other hand, the binding was measured by varying the concentration of actin to minimize the consumption of ATP in its presence and to keep the concentration of free S1 within an appropriate range for precise determination of the NH_4^+-EDTA-ATPase activity.

The dependence of the dissociation constant of acto-S1 on ionic strength was analyzed by use of the equation $\log K_d = -\log K_d^0 - Z \cdot I^{1/2}$, where I is ionic strength, K_d^0 is the dissociation constant at $I=0$, and Z is a constant that is dependent on charges involved in the interaction of actin and S1. Therefore, the decrease in the slope (Z) indicates the increase of electrostatic interactions between S1 and actin.

The standard free energy change (ΔG^0) for the binding of S1 with actin was calculated according to the equation, $\Delta G^0 = RT \ln K_d$, where R is the gas constant, T, temperature, and K_d, the dissociation constant for acto-S1 complex. The standard entropy change (ΔS^0) and standard enthalpy change (ΔH^0) were estimated, on the basis of $\Delta G^0 = \Delta H^0 - T\Delta S^0$, from plots of $-\Delta G^0$ *vs.* T and $\Delta G^0/T$ *vs.* $1/T$, respectively, assuming that ΔS^0 and ΔH^0 were constant and independent of temperature.

RESULTS AND DISCUSSION

Binding of S1 to actin purified by gel filtration was examined at various ionic strengths by separating free S1 from S1 bound to F-actin by sedimentation. In the absence of nucleotide, S1 bound to actin according to first-order binding with the stoichiometry of 0.95-1.0 mol S1/mol actin, independently of the ionic strength (Fig. 1). The dissociation constant, however, increased with an increase in the ionic strength. The dissociation constants were in the range of 0.5-10 nM at 20°C. This value is more than 100-fold lower than that which we reported previously (*2*). Such a difference could be explained if ~20% of actin in the actin preparation was denatured and did not bind S1. Calculation based on this assumption also accounts for the insufficient fitting of the previous binding data with the first-order binding (*2*). The binding of S1 to actin was also measured in the presence of ADP (Fig. 2) or ATP (Fig. 3). The data were fitted well with the first-order binding curve with the maximum binding of 1 mol actin/mol S1, suggesting a binding stoichiometry of 1 mol actin/mol S1 in the presence of ADP or ATP, as well as in the absence of nucleotide. The dissociation constant of acto-S1 was increased more than 100-fold by the presence of ADP and 80,000-fold by the presence of ATP at ionic strength of 28 mM. The dissociation constant in the presence of ADP or ATP also increased with an increase in the ionic strength.

The dissociation constants of acto-S1, which were obtained as shown in Figs. 1-3, were plotted against the

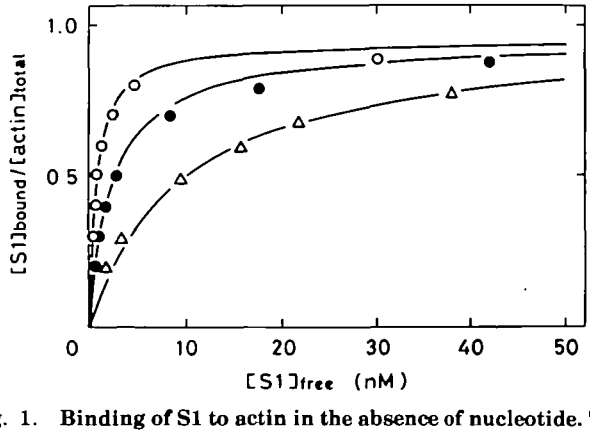

Fig. 1. **Binding of S1 to actin in the absence of nucleotide.** The binding was measured at 20°C using 0.2-0.9 μM S1 and 1.0 μM actin in 1 mM $MgCl_2$, 1 mM DTT, 0.2 mg/ml BSA, 10 mM Mops-NaOH (pH 7.0), and various concentrations of NaCl. Ionic strength of the assay mixture was adjusted with NaCl to 28 (○), 68 (●), or 108 mM (△). The lines represent the first-order binding curves with K_d and the maximum binding of 0.7 nM and 0.95 mol S1/mol actin (28 mM ionic strength), 2.2 nM and 0.95 mol S1/mol actin (68 mM ionic strength), and 9.5 nM and 0.97 mol S1/mol actin (108 mM ionic strength), respectively.

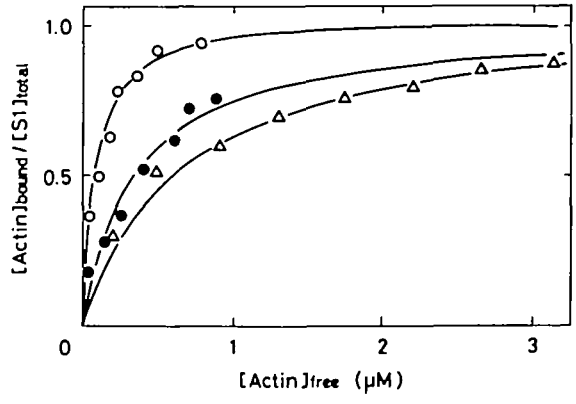

Fig. 2. **Binding of S1 to actin in the presence of ADP.** The binding was measured at 20°C using 1.0 μM S1 and 0.2-5.0 μM actin in 3 mM $MgCl_2$, 2 mM ADP, 1 mM DTT, 0.2 mg/ml BSA, 10 mM Mops-NaOH (pH 7.0), and various concentrations of NaCl. Ionic strength of the assay mixture was adjusted with NaCl to 28 (○), 74 (●), or 114 mM (△). The lines represent the first-order binding curves with K_d of 0.086 μM (28 mM ionic strength), 0.34 μM (74 mM ionic strength), and 0.67 μM (114 mM ionic strength). The maximum extent of binding was assumed to be 1.0 mol actin/mol S1.

square root of ionic strength (Fig. 4). The dissociation constant was found to show in a linear relationship with the square root of ionic strength, irrespective of the presence of nucleotide. The slopes of fitted lines were unaffected by the temperature, but considerably decreased by the presence of ADP and increased by the presence of ATP. These results indicate the decrease and increase of electrostatic interactions between actin and S1 in the presence of ADP and ATP, respectively. On the other hand, when the temperature was lowered, an increase in the dissociation constant was observed in the absence or presence of ADP, indicating the contribution of hydrophobic interactions to the binding of S1 and actin.

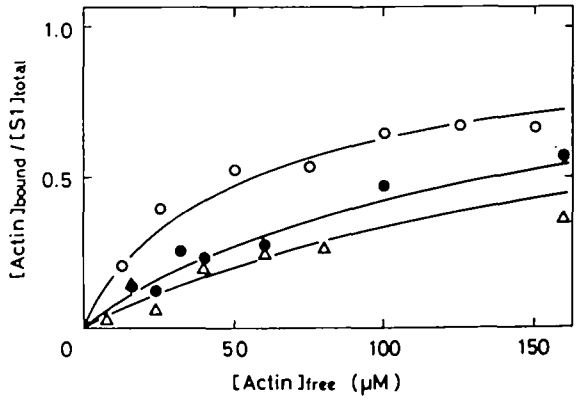

Fig. 3. **Binding of S1 to actin in the presence of ATP.** The binding was measured at 20°C using 0.10 μM S1 and 6.0-200 μM actin in 3 mM $MgCl_2$, 2 mM ATP, 1 mM DTT, 0.2 mg/ml BSA, 10 mM Mops-NaOH (pH 7.0), and various concentrations of NaCl. Ionic strength of the assay mixture was adjusted with NaCl to 28 (○), 38 (●), or 48 mM (△). The lines represent the first-order binding curves with K_d of 59 μM (28 mM ionic strength), 138 μM (38 mM ionic strength), and 200 μM (48 mM ionic strength). The maximum extent of binding was assumed to be 1.0 mol actin/mol S1.

To examine the contribution of hydrophobic interactions more in detail, the bindings of S1 to actin were measured at various temperatures. ΔG^0 for the binding of acto-S1 was calculated from the dissociation constant, and $-\Delta G^0$ and $\Delta G^0/T$ were plotted against T and $1/T$, respectively (Fig.

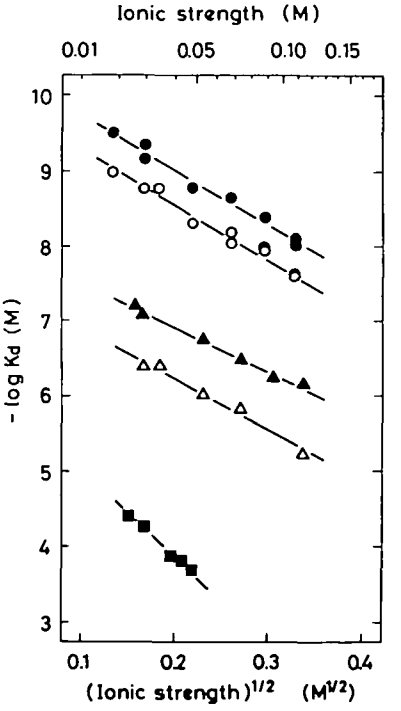

Fig. 4. **Ionic strength dependence of the binding of S1 to actin in the absence or presence of nucleotide.** The binding was measured at 4°C (open symbol) or 20°C (closed symbol) in the absence of nucleotide (○, ●) or in the presence of ADP (△, ▲) or ATP (■). The other conditions were as described in the legends of Figs. 1-3.

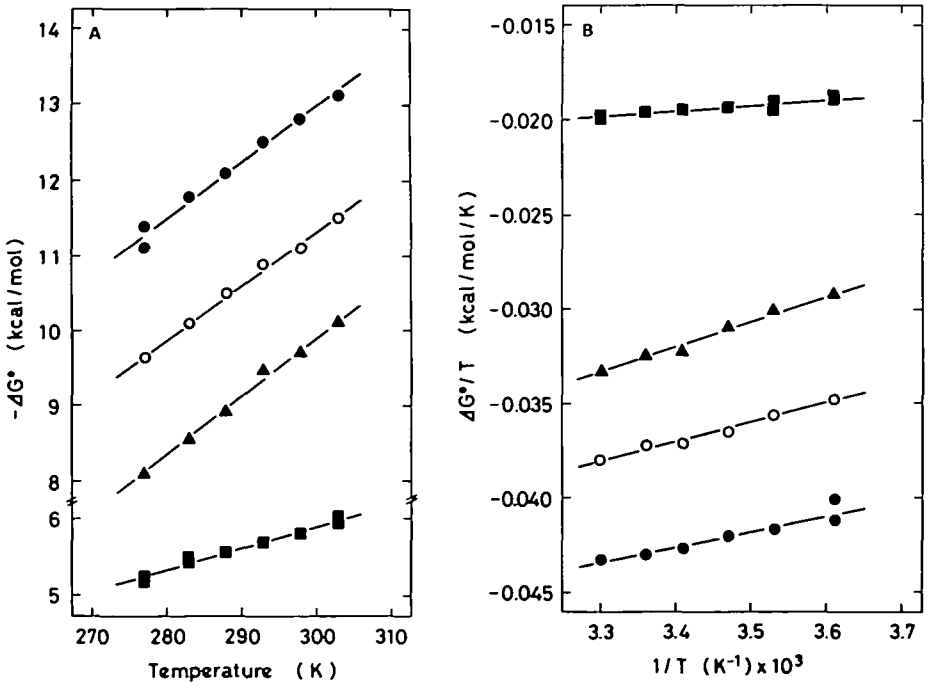

Fig. 5. **Temperature dependence of ΔG^0 for the binding of S1 to actin.** A, $-\Delta G^0$ *vs.* temperature; B, $\Delta G^0/$temperature *vs.* 1/temperature. Binding was measured at 4-30°C at 28 mM (closed symbol) or 108 mM (open symbol) in the absence of nucleotide (○, ●) or in the presence of ADP (▲) or ATP (■). The other conditions were as described in the legends of Figs. 1-3.

TABLE I. **ΔH^0 and ΔS^0 for the binding of S1 to actin.** ΔH^0 and ΔS^0 values were obtained from Fig. 5, B and A, respectively.

Nucleotide	Ionic strength (mM)	ΔH^0 (kcal/mol)	ΔS^0 (cal/mol/K)
None	28	8	70
	108	10	70
ADP	28	13	72
ATP	28	3	29

5). All the series of data were fitted well with linear lines. ΔS^0 and ΔH^0 for the binding of S1 to actin were estimated from the slopes of the former (Fig. 5A) and the latter (Fig. 5B) plots, respectively, and were shown in Table I. The ΔH^0 and ΔS^0 values were positive in the absence and presence of nucleotide. Yasui *et al.* (*14*) have also reported positive ΔH^0 and ΔS^0 in the absence of nucleotide. These results indicate that the S1-actin binding is entropy-driven, suggesting that hydrophobic interaction is a main determinant of the binding between S1 and actin, irrespective of the presence of nucleotide (*15*). The ΔS^0 value was unaffected by the presence of ADP but greatly decreased by the presence of ATP. These results suggest that hydrophobic interactions between S1 and actin were decreased by ATP but not by ADP. On the other hand, the ΔH^0 value in the absence of nucleotide increased with an increase in the ionic strength. The ΔH^0 value was increased by the presence of ADP but decreased by the presence of ATP. These results were consistent with the decrease and increase of electrostatic interactions between S1 and actin by ADP and ATP, respectively, in agreement with the reports of Highsmith (*16*) and Highsmith and Murphy (*17*). Consequently, the increase in the dissociation constant of acto-S1 by ADP may be due to the decrease of electrostatic interactions between S1 and actin, but the large increase in the dissociation constant by ATP may be due to the decrease of hydrophobic interactions. Therefore, a certain conformational transition that increases hydrophobic interactions and decreases electrostatic interactions between S1 and actin may occur accompanying the step from acto-S1-ADP-P_i (weak binding state) to acto-S1-ADP (strong binding state), corresponding to the force-generating step (*1*).

Taylor (*18*) suggests that the binding of S1 to actin occurs in a two-step process: the first step involves the acto-S1 complex formation as a result of their electrostatic and hydrophobic attractions, and the second step involves an isomerization of the complex as a result of hydrophobic interactions. ATP affects both steps, the second step particularly strongly, leading to the large decrease in the affinity of S1 for actin. Accordingly, the increase of hydrophobic interactions between S1 and actin accompanying the release of products may be induced by the isomerization of actin and/or S1.

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