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# Actomyosin Interactions in the Presence of ATP and the N-Terminal Segment of Actin†

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ABSTRACT: The binding of myosin subfragment 1 (S-1) to actin in the presence of ATP and the acto–S-1 ATPase activities of acto–S-1 complexes were determined at 5 °C under conditions of partial saturation of actin, up to 90%, by antibodies against the first seven N-terminal residues on actin. The antibodies [$F_{ab}(1-7)$] inhibited strongly the acto–S-1 ATPase and the binding of S-1 to actin in the presence of ATP at low concentrations of S-1, up to 25 $\mu$M. Further increases in S-1 concentration resulted in a partial and cooperative recovery of both the binding of S-1 to actin and the acto–S-1 ATPase while causing only limited displacement of $F_{ab}(1-7)$ from actin. The extent to which the binding and the ATPase activity were recovered depended on the saturation of actin by $F_{ab}(1-7)$. The combined amounts of S-1 and $F_{ab}$ binding to actin suggested that the activation of the myosin ATPase activity was due to actin free of $F_{ab}$. Examination of the acto–S-1 ATPase activities as a function of S-1 bound to actin at different levels of actin saturation by $F_{ab}(1-7)$ revealed that the antibodies inhibited the activation of the bound myosin. Thus, the binding of antibodies to the N-terminal segment of actin can act to inhibit both the binding of S-1 to actin in the presence of ATP and a catalytic step in ATP hydrolysis by actomyosin. The implications of these results to the regulation of actomyosin interaction are discussed.

Kinetic schemes for the hydrolysis of ATP by actomyosin predict the existence of two groups of states for the binding of myosin cross-bridges to actin. The "weak-binding" states are identified with M·ATP and M·ADP·$P_i$ complexes, while the "strong-binding" states describe the interactions of myosin and actin in the absence of nucleotides or in the presence of ADP, $PP_i$, adenyl-5′-yl imidodiphosphate and (AMP·PNP)[1] (Brenner, 1990). Stiffness measurements on muscle fibers confirm the results of kinetic studies in solution and provide evidence for "weak-binding" (Schoenberg, 1988) and "strong-binding" (Brenner et al., 1986) cross-bridge states in muscle. It is expected that the large differences between these states in the affinities of myosin for actin have a structural basis and that the transition between the different structures is important for the power stroke in muscle. Indeed, X-ray diffraction studies (Yu & Brenner, 1987; Harford & Squire, 1990), electron microscopy (Craig et al., 1985; Applegate & Flicker, 1987; Frado & Craig, 1991), and solution studies (Bernett & Thomas, 1989; Duong & Reisler, 1989; Trayer & Trayer, 1988) attest to significant conformational differences between myosin heads (S-1) bound strongly and weakly to actin.

Further progress in understanding the structure and function of different cross-bridge states should be facilitated by a recent determination of the atomic resolution structure of G-actin (Kabsch et al., 1990), the refined models of F-actin (Holmes et al., 1990; Milligan et al., 1990), and advances in the mapping of actomyosin binding sites (Labbe et al., 1990). Two binding sites, one on S-1 and one on actin, merit special attention in the context of state-specific actomyosin interactions. On the S-1, several residues around the reactive $SH_1$ cysteine have been implicated in the formation of the strong actomyosin bond (Suzuki et al., 1987; Keane et al., 1990). However,

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[1] Abbreviations: S-1, myosin subfragment 1; S-1·T, S-1 in the presence of MgATP; $F_{ab}(1-7)$, affinity-purified $F_{ab}$ fragment of polyclonal peptide antibodies raised against the first seven N-terminal residues of α-skeletal actin; AMP·PNP, adenyl-5′-yl imidodiphosphate; ELISA, enzyme-linked immunosorbent assay; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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neither the corresponding site on actin nor even the actual presence of the $SH_1$ peptide at the actomyosin interface has been established satisfactorily (Chase et al., 1991). On the other hand, the actin site which is comprised of up to 10 N-terminal residues (Sutoh, 1982) has been shown convincingly to bind to residues 632–642 from the N-terminus of S-1 (Mornet et al., 1981; Sutoh, 1982; Chaussepied & Morales, 1988; Yamamoto, 1991). Interestingly, also, while the interaction between the N-terminal segment of actin and S-1 seemed to contribute little to the rigor (strong) bond between these proteins (Mejean et al., 1986, 1987; Miller et al., 1987), this segment appeared important for acto–S-1 interactions in the presence of ATP (Chaussepied & Morales, 1988; DasGupta & Reisler, 1989; Bertrand et al., 1989). ATP introduced a shift in the cross-linking of S-1 lysine residues in the sequence 632–642 to actin (Yamamoto, 1990), and antibodies to the first seven N-terminal residues of actin, $F_{ab}(1-7)$, inhibited the binding of S-1 to actin in the presence of ATP (DasGupta & Reisler, 1989). This raised the possibility that the N-terminal segment of actin could be an important contact site in the weakly bound acto–S-1 complexes. Related to such a scenario are the findings that the regulatory proteins troponin I (Grabarek & Gergely, 1987; Levine et al., 1988) and caldesmon (Patchell et al., 1989; Bartegi et al., 1990; Levine et al., 1990; Adams et al., 1990) interact also with the N-terminal segment of actin. Thus, in principle, these proteins could regulate actomyosin function by competing with myosin·ATP for the same binding site on actin.

In this work, we have examined in greater detail the binding of S-1·ATP to actin in the presence of $F_{ab}(1-7)$ over a range of S-1 and $F_{ab}$ concentrations. The present experiments were prompted in part by the original observations (DasGupta & Reisler, 1989) on almost complete inhibition of acto–S-1 ATPase and S-1·T (short for S-1 in the presence of MgATP) binding to actin by subsaturating concentrations of $F_{ab}$. We were stimulated also by the more recent finding that $F_{ab}(1-7)$ inhibited strongly the binding of all S-1–nucleotide complexes to actin but that this inhibition could be overcome in a cooperative manner with increasing S-1 concentrations (DasGupta & Reisler, 1991). The implication of these results was that even partial saturation of actin with $F_{ab}(1-7)$ could suppress actin's affinity for S-1–nucleotide complexes. It is shown in this paper that in analogy to complexes of S-1 with other nucleotides, the inhibited (by $F_{ab}$) binding of S-1·T to actin is activated cooperatively at high molar ratios of S-1 to actin. It is documented also that the activation of the ATPase activity of S-1 bound to actin can be blocked or greatly reduced by the binding of $F_{ab}(1-7)$. Thus, the inhibitory effects of $F_{ab}(1-7)$ on S-1·T binding to actin and the actin-activated ATPase activity of S-1 can be uncoupled. The implications of this result to the role of the N-terminal segment of actin in actomyosin interactions are discussed.

## Materials and Methods

*Reagents.* Distilled and Millipore-filtered water and analytical reagents were used in all experiments. ATP and TLCK-treated chymotrypsin were purchased from Sigma Chemical Co. (St. Louis, MO). ELISA plates (Dynatech Immulon I) were obtained from Fisher Scientific Co.

*Preparation of Proteins.* Rabbit skeletal muscle actin was prepared in G-actin buffer (0.5 mM $\beta$-mercaptoethanol, 0.2 mM ATP, 0.2 mM $CaCl_2$, and 5 mM Tris, pH 7.6) by the procedure of Spudich and Watt (1971). G-Actin was polymerized by the addition of 2 mM $MgCl_2$. Myosin was obtained as described by Godfrey and Harrington (1970). Subfragment 1 (S-1) was prepared by chymotryptic digestion of myosin according to the method of Weeds and Pope (1977). Antibodies against residues 1–7 from the N-terminus of $\alpha$-skeletal actin and their $F_{ab}$ fragments, $F_{ab}(1-7)$, were prepared as described previously (Miller et al., 1987). Antibody titers were routinely checked by ELISA using actin as the coating antigen (DasGupta et al., 1990).

*Measurements of Actin-Activated ATPase Activity of S-1.* Actin-activated ATPase activities of S-1 in the presence and absence of $F_{ab}(1-7)$ were determined by calorimetric analysis of liberated $P_i$ in a solvent containing 3 mM ATP, 3 mM $MgCl_2$, 10 mM KCl, and 10 mM imidazole (pH 7.0) at 5 and 25 °C as described previously (DasGupta & Reisler, 1989). The employed protein concentrations were 3.0 $\mu$M for F-actin, 0–9.0 $\mu$M for $F_{ab}$, and between 3.0 and 60 $\mu$M for S-1. The concentration of S-1 rather than that of actin was varied in the acto–S-1 ATPase measurements. This was done for $F_{ab}$ economy reasons and in order to simplify the preparation of solutions at constant molar ratios of $F_{ab}$ to actin. The ATPase reaction mixtures were incubated with MgATP for variable amounts of time (between 30 s and 15 min) depending on the S-1 concentration and the temperature, so that ≤35% of the total ATP content would be hydrolyzed during each ATPase reaction. The reported ATPase activities are corrected for the ATPase of isolated S-1 and are given in micromoles of $P_i$ released per micromole of S-1 per minute.

*Airfuge Binding Experiments.* F-Actin (3.0 $\mu$M) was incubated for 20 min at 5 °C with various amounts of S-1 (between 0 and 60 $\mu$M) in the presence of $F_{ab}$ (between 0 and 9.0 $\mu$M) in the same solvent as that employed in the ATPase activity assays; 3.0 mM ATP was added to these solutions immediately prior to their centrifugation at 140000*g* for 10 min at 5 °C in an air-driven ultracentrifuge (Beckman Instruments). As explained earlier (DasGupta & Reisler, 1989), the choice of experimental conditions, including the range of protein concentrations, temperature, and time of centrifugation, was limited in these experiments by two conflicting demands. The first one was the need to retain high levels of unhydrolyzed ATP throughout the experiment, and the second was to have protein concentrations sufficient for significant actomyosin binding in the presence of ATP. We verified that for each set of binding experiments the hydrolysis of ATP during the centrifugation time did not exceed 35% of its total initial content. We verified also that in each centrifugation experiment ≥95% of F-actin present in the solution was pelleted. Occasional samples or experiments in which these conditions were not satisfied were rejected.

The pelleted proteins were resolubilized in the original solvent (30 $\mu$L), denatured, and run on 15% SDS–polyacrylamide gels (Laemmli, 1970). The original uncentrifuged protein mixtures and the supernatants of the pelleted samples were run also on SDS gels. Coomassie Blue R stained protein bands were scanned with a Biomed Instruments (Fullerton, CA) Model SLR 2D/1D soft laser scanning densitometer interfaced to a DTK computer. The densitometric traces of protein bands were analyzed to determine the molar ratios of S-1 and $F_{ab}$ pelleted with actin in the presence of MgATP. Molar stain ratios for S-1, $F_{ab}$, and actin were obtained from appropriate calibration gels. The binding of $F_{ab}$ to actin was checked also by using radioiodinated ($^{125}I$) $F_{ab}$ (DasGupta & Reisler, 1989). The labeling stoichiometry was 0.012 $^{125}I/F_{ab}$. The binding of $F_{ab}$ to actin was calculated by radioactive counting of the binding assay solutions before and after their ultracentrifugation.

*Concentration Determinations.* Protein concentrations were determined spectrophotometrically by using the following

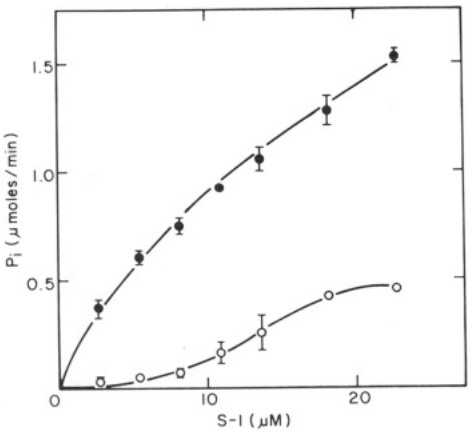


FIGURE 1: Dependence of acto–S-1 ATPase activity on S-1 concentration at 25 °C in the presence and absence of $F_{ab}(1–7)$. The ATPase activities were measured in 10 mM KCl, 3 mM MgATP, and 10 mM imidazole (pH 7.0) in the presence (○) or absence (●) of antibodies. The protein concentrations were 3.0 μM for actin and $F_{ab}(1–7)$ and between 0 and 22 μM for S-1. All measurements were corrected for the ATPase activity of S-1 alone. The error bars represent experimental deviation between separate sets of measurements (between three and five).

extinction coefficients at 280 nm: actin, $E^{1\%} = 11.0\ cm^{-1}$; S-1, $E^{1\%} = 7.50\ cm^{-1}$; $F_{ab}$, $E^{1\%} = 16.0\ cm^{-1}$.

## RESULTS

*Actomyosin ATPase Activity in the Presence of $F_{ab}(1–7)$.* Previous study showed that at less than 50% saturation of actin by antibodies against its N-terminal segment, $F_{ab}(1–7)$, the acto–S-1 ATPase activity and the binding of S-1·T to actin were almost completely inhibited (DasGupta & Reisler, 1989). Figure 1 confirms this observation. At low S-1 concentrations (≤5.0 μM) and at 25 °C, the addition of 3.0 μM $F_{ab}(1–7)$ to 3.0 μM F-actin is sufficient to inhibit strongly the acto–S-1 ATPase activity. However, the actin-activated ATP hydrolysis in the presence of $F_{ab}$ is "switched-on" in a cooperative manner with increasing concentrations of S-1. The sigmoidal dependence of the ATPase reaction on S-1 concentrations is strikingly similar to the recently reported relationship between the binding of S-1·AMP·PNP, S-1·ADP, and S-1·$PP_i$ to actin–$F_{ab}$ complexes and the concentration of S-1 (DasGupta & Reisler, 1991).

The interpretation of the results shown in Figure 1 depends on information on the binding of S-1 to actin in the presence of ATP and antibodies. Because of experimental constraints including airfuge centrifugation time, ATPase reaction, and the affinities of actin and S-1 for each other, such binding experiments had to be done at 5 °C (DasGupta & Reisler, 1989). Thus, the actin-activated ATPase reaction of S-1 was reinvestigated at 5 °C over a range of $F_{ab}$ and S-1 concentrations. Figure 2 reveals that with the exception of experiments done at a low $F_{ab}$ concentration (1.5 μM), the ATPase activities measured in the presence of $F_{ab}$ (3.0, 6.0, and 9.0 μM) show the same S-1 concentration-dependent profile at 5 °C as that observed at 25 °C. At the lowest concentration (1.5 μM), $F_{ab}$ inhibits the acto–S-1 ATPase activity but does not saturate actin sufficiently to induce cooperative effects. At higher $F_{ab}(1–7)$ concentrations, the acto–S-1 ATPase activities are strongly inhibited at low S-1 levels and are "switched-on" with an increase in S-1 concentration. (Figure 2). It may be noted that at each level of $F_{ab}$ (between 3.0 and 9.0 μM), the ATPase activities appear to saturate and that the maximal activities decrease with increasing $F_{ab}$ concentrations. The levels of S-1 required to "switch-on" the acto–S-1 ATPase in the presence of $F_{ab}$ are higher at 5 °C than at 25 °C (Figures 1 and 2) in accordance with the known effect of temperature on actomyosin binding (Geeves & Gutfreund, 1982). Also, predictably, the rates of ATP hydrolysis are much slower at 5 °C than at 25 °C.

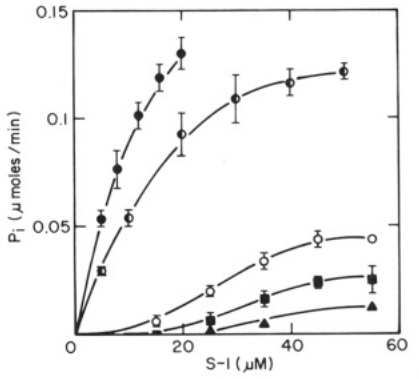


FIGURE 2: Dependence of acto–S-1 ATPase activity on S-1 concentration at 5 °C in the presence and absence of $F_{ab}(1–7)$. The ATPase activities in the absence of $F_{ab}$ (●) and at various molar ratios of $F_{ab}$ added to actin (◐, ○, ■, ▲) were measured in the same solvent as in the legend to Figure 1. The concentrations of $F_{ab}$ were 1.5 (◐), 3.0 (○), 6.0 (■), and 9.0 μM (▲). Actin concentration was set at 3.0 μM, and the concentrations of S-1 varied between 0 and 55 μM. All measurements are corrected for the ATPase activity of S-1 alone.

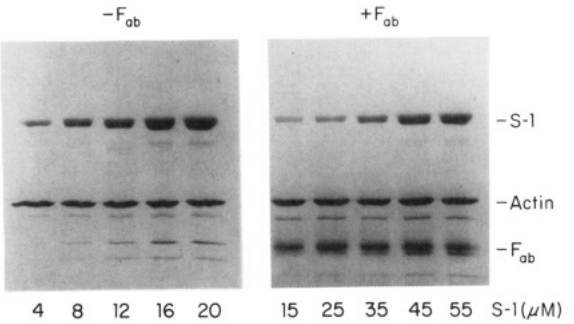


FIGURE 3: Representative Coomassie blue stained SDS gel electrophoretic patterns of pellet fractions obtained by airfuge centrifugation of actin, S-1, and $F_{ab}(1–7)$ in the presence of 3 mM MgATP at 5 °C. F-Actin (3 μM) was incubated with different amounts of S-1 in the absence (A) and presence (B) of $F_{ab}$ (3 μM) at 5 °C in a solvent containing 10 mM imidazole (pH 7.0), 10 mM KCl, and 3 mM $MgCl_2$. 3 mM ATP was added to these mixtures immediately prior to their centrifugation. The concentrations of S-1 present in the mixtures are indicated under each lane.

*Binding of S-1 and $F_{ab}$ to Actin in the Presence of ATP.* The binding of S-1 and $F_{ab}(1–7)$ to actin in the presence of ATP was measured by pelleting the mixtures of these proteins under conditions identical to those used in ATPase measurements at 5 °C. The range of S-1 concentrations employed in these experiments was constrained by the requirement to limit the ATP hydrolysis to ≤35% of the total nucleotide concentration. Figure 3 shows representative SDS gels of pelleted acto–S-1 complexes. As expected, visual inspection of Figure 3 reveals that the binding of S-1·T to actin increases progressively with the increase in S-1 concentration (between 4 and 20 μM). By way of contrast, Figure 3 shows also the strong effect of $F_{ab}$ on the binding of S-1·T to actin. The inhibition of S-1 binding to actin is striking at low S-1 concentrations but is less pronounced at higher S-1 concentrations (≥30 μM).

The results of acto–S-1 binding experiments in the presence of ATP and different amounts of $F_{ab}$ are presented in Figure 4. In agreement with the ATPase activity measurements (Figure 2), low concentrations of $F_{ab}$ (1.5 μM, i.e., added at a 0.5:1 molar ratio to actin) inhibit the binding of S-1·T to

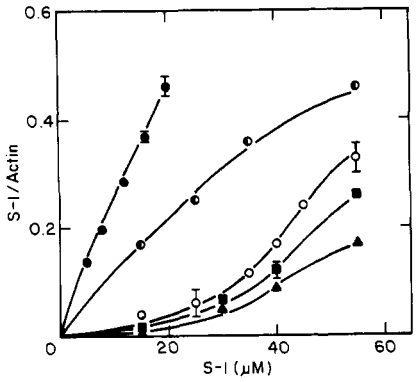


FIGURE 4: Binding of S-1 to actin in the presence of $F_{ab}$ and 3.0 mM MgATP at 5 °C. The fractions of actin occupied by S-1 in the absence (●) and presence of $F_{ab}$ were calculated from densitometric tracings of S-1 and actin bands on SDS gels similar to that shown in Figure 3. The concentration of actin was 3.0 μM. The molar ratios of $F_{ab}$ added to actin were (◐) 0.5:1, (○) 1:1, (■) 2:1, and (▲) 3:1, i.e., the respective concentrations of $F_{ab}$ were 1.5, 3.0, 6.0, and 9.0 μM. Solvent and incubation conditions were the same as in legend to Figure 3. The calculated binding constants of S-1 to actin in the presence of ATP were $K_a = 2.5 \times 10^4$ $M^{-1}$ in the absence of $F_{ab}$ and $K_a = 3.6 \times 10^3$ $M^{-1}$ in the presence of 1.5 μM $F_{ab}$.

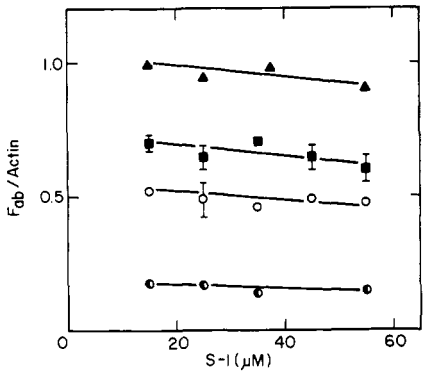


FIGURE 5: Binding of $F_{ab}$ to actin in the presence of S-1 and 3.0 mM MgATP at 5 °C. The fractions of actin occupied by $F_{ab}$ were calculated from densitometric tracings of actin and $F_{ab}$ bands on the same SDS gels as those used for the calculation of S-1 binding to actin in Figure 4. Solvent conditions and symbols are the same as in the legend to Figure 4.

actin but do not change its hyperbolic dependence on S-1 concentration. At higher levels of $F_{ab}$, and in analogy to the ATPase activity results, the binding of S-1·T to actin is strongly inhibited at low S-1 concentrations. This binding inhibition, as indicated also by Figure 3, is cooperatively overcome with increasing S-1 concentration. Recently, a similar pattern of S-1 binding to actin in the presence of $F_{ab}(1-7)$ was observed also in the presence of AMP·PNP, ADP, and $PP_i$, but not under rigor conditions (DasGupta & Reisler, 1991).

Measurements of $F_{ab}(1-7)$ binding to actin over the same range of S-1 concentrations as employed above were carried out to facilitate the interpretation of the effect of antibodies on actomyosin interactions in the presence of ATP. Examination of Figure 5 reveals very limited displacement of $F_{ab}$ bound to actin by S-1·T. The mole ratios of $F_{ab}$ bound to actin decrease between 10 and 20% with an increase in S-1 concentrations at all levels of saturation of the antibody binding to actin (between 0.17 and 0.95 $F_{ab}$/actin). Most notably, the cooperative upswing in the binding of S-1·T to actin (Figure 4) is not correlated with any significant shift in the binding of $F_{ab}(1-7)$ to actin (Figure 5).

*Relationship between the Binding of S-1·T to Actin and the Acto–S-1 ATPase in the Presence of $F_{ab}(1-7)$.* The results of ATPase activity measurements and the information on the

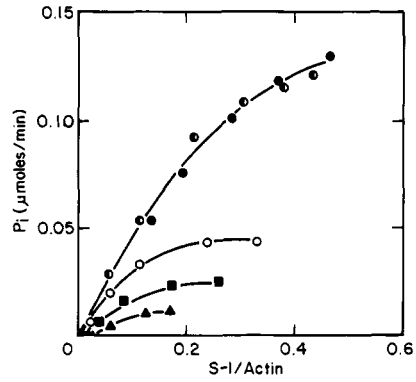


FIGURE 6: Relationship between the acto–S-1 ATPase activity at 5 °C and the binding of S-1 to actin at various levels of actin saturation by $F_{ab}(1-7)$. Acto–S-1 ATPase activities at different concentrations of S-1 and in the presence of $F_{ab}$ were taken from Figure 2. The amounts of S-1 bound to actin at the same S-1 and $F_{ab}$ concentrations were taken from Figure 4. The molar ratios of $F_{ab}$ added to actin were as follows: (●) no $F_{ab}$; (◐) 0.5:1; (○) 1:1; (■) 2:1; (▲) 3:1.

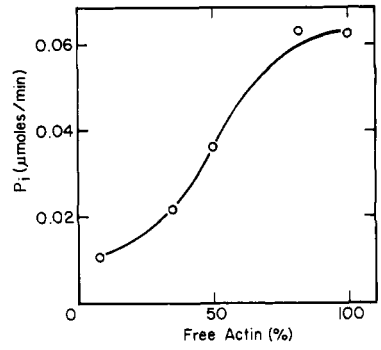


FIGURE 7: Dependence of the acto–S-1 ATPase activity of the bound S-1 (0.15 S-1/actin) on the binding of $F_{ab}$ to actin. The ATPase activities at 0.15 molar ratio of S-1 bound to actin were taken from Figure 6. The amount of $F_{ab}$ bound to actin and thus the fraction of actin free of $F_{ab}$ were calculated from the data shown in Figure 5.

binding of S-1·T to actin are combined in Figure 6 to show the dependence of the activities on the mole ratio of S-1 bound to actin. In this form, the plot assesses the activating efficiency of actin at any particular level of acto–S-1 binding. Clearly, in the absence of antibody and in the presence of low concentrations of $F_{ab}$ (≤0.2 mole ratio of $F_{ab}$ bound to actin), the rates of $P_i$ release increase identically and hyperbolically with the increase in S-1 binding to actin (Figure 6). Thus, at low saturation levels, $F_{ab}(1-7)$ does not alter the activating function of actin. At higher ratios of $F_{ab}$ bound to actin (≈0.5:1, 0.7:1, and 1.0:1), the dependencies of the ATPase activities on S-1 concentration split into separate curves, distinct from the one established for the ATPase activity in the absence of $F_{ab}$. These curves reach saturating values for the rates of ATP hydrolysis at relatively low occupancy of actin by S-1. Most significantly, different rates of ATP hydrolysis are measured for the same amounts of S-1 bound to actin depending on the extent of saturation of actin by $F_{ab}$. This conclusion can be reached by examining the ATPase rates along any vertical line crossing the curves shown in Figure 6, i.e., at a constant mole ratio of S-1 bound to actin.

For illustration purposes, the rates of actin-activated ATP hydrolysis at a constant 0.15 mole ratio of S-1 bound to actin are replotted in Figure 7 as a function of "free actin", i.e., actin which has no $F_{ab}$ bound to it. (The data shown in Figure 7 are derived from Figures 5 and 6.) A 6-fold decrease in the rates of ATP hydrolysis occurs on transition from approximately 80% to 10% $F_{ab}$-free actin, i.e., between actin with 0.2

and 0.9 mole ratio of $F_{ab}(1-7)$ bound to it. The inhibition of the acto–S-1 ATPase by $F_{ab}$ becomes more cooperative at higher levels of the bound S-1. At 0.20 mole ratio of S-1 bound to actin, the ATPase rate decreases 9-fold upon a decrease from 80% to 15% $F_{ab}$-free actin. The obvious conclusion from these results is that $F_{ab}(1-7)$ inhibits acto–S-1 ATPase activity not only by inhibiting the binding of S-1·T to actin but also by "switching-off" the ability of actin to activate the bound myosin.

## Discussion

A particularly interesting feature of F-actin complexes with $F_{ab}(1-7)$ is the recently documented difference in their binding of S-1 under rigor conditions and in the presence of AMP·PNP, ADP, or $PP_i$ (DasGupta & Reisler, 1991). In the presence of these nucleotides, the antibodies appeared to "switch-off" the binding of S-1 to actin at low S-1 concentrations but allowed for a cooperative enhancement of S-1 binding at high S-1 levels. Yet, even at high concentrations of S-1, its binding to actin was most likely restricted to actin molecules which were not occupied by $F_{ab}$. These results called for a closer scrutiny of the competition between S-1·T and $F_{ab}(1-7)$ for actin (DasGupta & Reisler, 1989) and the implied involvement of the N-terminal residues of actin in the binding of S-1·T.

The results of this study confirm the original observation on the inhibition by $F_{ab}(1-7)$ of the acto-S-1 ATPase and the binding of S-1·T to actin at low S-1 concentrations ($\leq 25$ $\mu$M; DasGupta & Reisler, 1989). However, when the dependence of the ATPase activity and S-1·T binding to actin on S-1 concentration is examined over a range of actin saturations by $F_{ab}$, a more detailed and interesting picture of the $F_{ab}$–actin–S-1 system emerges. In analogy to the binding of S-1 to actin in the presence of other nucleotides and $F_{ab}$ (DasGupta & Reisler, 1991), the ATPase and the binding of S-1·T to actin show a sigmoidal, cooperative dependence on the concentration of S-1. This pattern is not observed at the lowest level of actin saturation by $F_{ab}$, between 0.15 and 0.20 mole ratio of $F_{ab}$ bound to actin, even though both the binding of S-1·T and acto–S-1 ATPase are significantly inhibited by the bound antibody. Thus, the "switching-off" of actin's affinity for S-1·T (and acto–S-1 ATPase activity) occurs upon binding of between 0.20 and 0.45 mol of $F_{ab}$ per mole of actin (Figures 2 and 4). Apparently, the $F_{ab}$-induced change in actin spreads through intrafilament interactions to affect actin molecules which are not bound to the antibody.

The possibility of cooperative interactions within the actin filament even in the absence of regulatory proteins has been recognized before (Loscalzo et al., 1975). The binding of S-1 or HMM to actin has been associated with spectral transitions of probes (Loscalzo et al., 1975; Oosawa et al., 1973) or the actin-bound nucleotide (Harvey et al., 1977), changes in the flexibility of actin filaments (Fujime & Ishiwata, 1971), and the bundling of filaments (Ando, 1987), all of which indicated intrafilament cooperativity. In the present case, the initial changes in actin are caused by the binding of $F_{ab}$ to the N-terminal residues on actin. Steric effects cannot be excluded but appear to be an unlikely cause for the observed pattern of S-1·T binding to actin. Although such effects can lead to the inhibition of binding, they cannot account for the cooperative increase in the binding of S-1·T to actin at high S-1 concentrations. Also, the fact that similar $F_{ab}$-induced changes in the binding of S-1 to actin have been observed in the presence of other nucleotides (DasGupta & Reisler, 1991), i.e., at higher binding affinities between S-1 and actin than in the presence of ATP, supports the claim for a specific perturbation of the acto–S-1 interface by $F_{ab}(1-7)$.

The cooperative increases in acto–S-1 ATPase activities and the binding of S-1·T to actin result in only partial restoration of these functions relative to their values in the absence of $F_{ab}$. With increasing binding of $F_{ab}$ to actin, decreasing maximum levels of acto–S-1 ATPase are reached at high S-1 concentrations. Although similar saturation levels have not been reached for the binding of S-1·T to actin in the presence of $F_{ab}$ (because of ATP hydrolysis related constrains on S-1 concentrations), the trend of the data is the same as in the ATPase measurements. The binding of S-1·T to actin decreases with an increase in $F_{ab}$ binding to actin. In all cases, the combined occupancy of actin by $F_{ab}$ and S-1, $\theta_{Fab+S-1}$, does not exceed 1 mol of $(F_{ab} + S\text{-}1)/1$ mol of actin. This suggests, albeit does not prove, that S-1 binds primarily to the $F_{ab}$-free actin. The same conclusion has been reached with respect to complexes of S-1 with other nucleotides, for which clear binding saturation levels consistent with $\theta_{Fab+S-1} \leq 1.0$ could be reached (DasGupta & Reisler, 1991). In contrast to this, the rigor binding of S-1 to actin frequently results in $\theta_{Fab+S-1} > 1.0$. Thus, as suggested before (DasGupta & Reisler, 1989; Chaussepied & Morales, 1989; Bertrand et al., 1989), the N-terminal residues of actin play a more important role in the binding of S-1·nucleotide complexes than S-1 alone.

Additional insight into the acto–S-1 system in the presence of $F_{ab}(1-7)$ is gained by representing the acto–S-1 ATPase activities as a function of S-1 bound to actin. As shown in Figure 6, the partially and cooperatively restored functions of actin (at high S-1 concentrations) do not reproduce the original dependence of ATPase activities on S-1 binding to actin. The normal relationship between the binding of S-1·T and the activation of the ATPase reaction by actin is uncoupled by $F_{ab}(1-7)$. If indeed S-1·T binds mainly to the $F_{ab}$-free actin, as it appears to be, then the activating function of individual actin molecules must be altered through changes on adjacent, $F_{ab}$-occupied actins. It is striking that different acto–S-1 ATPase activities are measured for the same amounts of S-1 bound to actin depending on the level of actin saturation by $F_{ab}$. The inhibition of acto–S-1 ATPase does not require the displacement of S-1 from actin; the bound S-1 can be inhibited as well through the binding of $F_{ab}$ to the N-terminal residues of actin (Figure 7). The catalytic "switching-off" of actin by $F_{ab}(1-7)$ may involve conformational changes either in the N-terminal segment or elsewhere on the $F_{ab}$-free actin. It is also possible that the nonactivated S-1·T is linked to actin sites other than its N-terminal segment (Labbe et al., 1990) and, thus, lacks the catalytically important contact.

It is pertinent to note that a similar situation exists in a regulated actin system containing troponin and tropomyosin. The acto–S-1 ATPase of that complex can be turned on and off by $Ca^{2+}$ ions without comparable changes in actomyosin binding (Chalovich & Eisenberg, 1982). Thus, troponin–tropomyosin inhibits a catalytic step in the ATPase reaction of the bound acto–S-1 complex (Chalovich & Eisenberg, 1982). Other lines of evidence have implicated already the regulatory proteins in the interactions with the N-terminal segment of actin (Grabarek & Gergely, 1987, 1990; Levine et al., 1988, 1990; Bartegi et al., 1990; Adams et al., 1990). The fact that antibodies to the first seven residues on actin mimic to some extent the effect of regulatory proteins, i.e., induce cooperative interactions between actin and S-1·nucleotide complexes, and inhibit the acto–S-1 ATPase by inhibiting both the binding and the catalytic steps of this reaction, supports the hypothesis that the regulation of actomyosin ATPase activity involves controlled changes in the

contact between the N-terminal segment of actin and myosin (Levine et al., 1990; Grabarek & Gergely, 1987).

After submission of this work for publication, new evidence has been presented by Sutoh et al. (1991) which confirms the importance of the N-terminal residues of actin for actomyosin interactions in the presence of ATP. *Dictyostelium* actin mutants with two N-terminal aspartic residues replaced by histidine showed sharply diminished maximum turnover rates ($V_{max}$) for actin-activated ATPase of myosin without a significant change in the apparent affinity of actin for myosin ($K_m$). The mutant work (Sutoh et al., 1991) and our antibody results indicate that the contact between myosin and the N-terminal segment of actin is crucial for the activation step of the myosin ATPas activity. A more drastic alteration of the N-terminal segment of actin by replacement of Asp-3 and Asp-4 residues on $\beta$-actin with lysines was reported to interfere even with the rigor actomyosin binding (Aspenstrom & Karlsson, 1991). However, in this latter case, it remains to be verified that the charge reversal on the N-terminus of actin does not introduce secondary structural changes in actin and modifies in such way actomyosin interactions.

**Registry No.** ATP, 56-65-5.

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