Interactions between G-Actin and Myosin Subfragment 1: Immunochemical Probing of the NH_2-Terminal Segment on Actin†

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ABSTRACT: The role of the N-terminal segment of actin in myosin-induced polymerization of G-actin was studied by using peptide antibodies directed against the first seven N-terminal residues of α-skeletal actin. Light scattering, fluorescence, and analytical ultracentrifugation experiments showed that the F_{ab} fragments of these antibodies inhibited the polymerization of G-actin by myosin subfragment 1 (S-1) by inhibiting the binding of these proteins to each other. Fluorescence measurements using actin labeled with pyrenyliodoacetamide revealed that F_{ab} inhibited the initial step in the binding of S-1 to G-actin. It is deduced from these results and from other literature data that the initial contact between G-actin and S-1 involves residues 1–7 on actin and residues 633–642 on the S-1 heavy chain. This interaction appears to be of major importance for the binding of S-1 and G-actin. The presence of additional myosin contact sites on G-actin was indicated by concentration-dependent recovery of S-1 binding to G-actin without displacement of F_{ab}. The reduced F_{ab} inhibition of S-1 binding to polymerizing and polymerized actin is consistent with the tightening of acto–S-1 binding at these sites or the creation of new sites upon formation of F-actin.

Cells control the polymerization of cytoplasmic actin into filaments and subsequent movement or further organization of these filaments into higher order structures by employing specialized actin-binding proteins [see reviews by Stossel (1989), Pollard and Cooper (1986), and Korn (1982)]. Myosin and its proteolytic fragments, heavy meromyosin (HMM)[1] and the globular heads (S-1), were among the first proteins recognized to polymerize G- into F-actin (Martonosi & Gouvea, 1961; Yagi et al., 1965; Tawada & Oosawa, 1969; Cook & Morales, 1971; Yazawa & Yagi, 1973; Grazi et al., 1980). The assembly of actin by S-1 is of particular interest. First, it is more amenable to experimental analysis than the reactions involving the double-headed myosin and HMM. Second, the clarification of the mechanism of actin assembly by S-1 may shed light not only on protein-regulated polymerization of G-actin but also on some yet unrecognized aspects of actomyosin interactions.

Although detailed studies of S-1 interaction with G-actin have been obstructed by the polymerization reaction, extensive information is available on the binding of S-1 to F-actin. The 1:1 actin and S-1 stoichiometry in F-actin–S-1 complexes appears well established (Sutoh, 1983; Greene, 1984; Heaphy & Treager, 1984; Chen et al., 1985, 1987) and is suggestive of a 1:1 binding of S-1 to G-actin. Indeed, fluorescence polarization experiments of Chaussepied and Kasprzak (1989a) support this possibility. Also, consistent with the 1:1 binding of S-1 and G-actin are the light-scattering measurements of actin polymerization by S-1 and the conclusion that only stoichiometric amounts of actin are incorporated into filamentous acto–S-1 complexes (Miller et al., 1988a). These findings imply that the binding of S-1 to G-actin changes the conformation of the latter and lowers its critical concentration for polymerization (Detmers et al., 1981). The different roles of S-1 (A1) and S-1 (A2) isozymes in this process (Chaussepied & Kasprzak, 1989a; Chen & Reisler, 1990) are yet to be fully explored.

The mapping of acto–S-1 sites which might be involved in the polymerization of actin has been pursued by chemical modification methods. These experiments revealed that labeling of Lys-61 on actin by fluorescein isothiocyanate inhibits the S-1-induced G to F transformation (Miki et al., 1987; Miller et al., 1988b). The S-1-driven assembly of actin is also inhibited by subtilisin cleavage of the latter between Met-47 and Gly-48 (Schwyter et al., 1989). A complete inhibition of G-actin polymerization by S-1, related to the inhibition of their binding to each other, resulted from covalent coupling of "antipeptide" (containing oppositely charged amino acids) to the stretch of residues 633–642 on the S-1 heavy chain (Chaussepied & Kasprzak, 1989b). The positively charged myosin sequence 633–642 is known to interact with the negatively charged N-terminal actin site on F-actin (Sutoh, 1982; Yamamoto, 1989; Duong & Reisler, 1989). Several studies suggest that this interaction may be particularly important in the presence of ATP. The labeling of actin's N-terminus (Bertrand et al., 1989), the attachment of antipeptide to S-1 (Chaussepied & Morales, 1988), and antibodies against the N-terminal residues 1–7 on actin (DasGupta & Reisler, 1989) greatly inhibit actomyosin binding in the presence of ATP. Yet, the same SαN antibodies (i.e., against residues 1–7 of α-actin) do not interfere much with actomyosin binding under rigor conditions (Mejean et al., 1986, 1987; Miller et al., 1987), which must be dominated by contact sites other than residues 633–642 on S-1 and residues 1–7 on actin.

In this work, we have probed the involvement of the N-terminal segment of actin in myosin-induced polymerization

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[1] Abbreviations: pyrene-labeled actin, actin modified at Cys-374 with *N*-(1-pyrenyl)iodoacetamide; S-1, myosin subfragment 1; fluorescein-labeled S-1, S-1 modified at SH_1 residues with 5-(iodoacetamido)-fluorescein; SαN antibodies, affinity-purified peptide antibodies raised against the first seven N-terminal residues of α-skeletal actin; ELISA, enzyme-linked immunosorbent assay.

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of G-actin by using the specific SαN antibodies. We show that the antibodies inhibit the S-1-induced polymerization of G-actin by inhibiting the binding of these proteins to each other. The recovery of G-actin binding to S-1 at high concentrations of myosin heads occurs without a significant displacement of antibodies from actin. These results complement a recent work with an antipeptide S-1 complex (Chaussepied & Kasprzak, 1989b) and show that the contact between the N-terminal segment of actin and its binding sites on S-1 is an important but probably not essential component of the G-actin–S-1 interaction.

Materials and Methods

Reagents. Distilled and Millipore-filtered water and analytical-grade reagents were used in all experiments. TLCK-treated chymotrypsin and ATP were purchased from Sigma Chemical Co. (St. Louis, MO). *N*-(1-Pyrenyl)iodoacetamide and 5-(iodoacetamido)fluorescein were obtained from Molecular Probes (Junction City, OR). Bradford protein assay solution was from Bio-Rad (Richmond, CA). ELISA plates (Dynatech Immulon I) were purchased from Fisher Scientific Co.

Preparation of Proteins. Skeletal muscle actin was prepared in G-actin buffer (0.5 mM 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). Unlabeled and labeled gel-filtered actin were obtained as described by MacLean-Fletcher and Pollard (1980). Myosin was prepared 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 directed against residues 1–7 from the N-terminus of α-skeletal actin, SαN, and the F_{ab} fragments derived from them were prepared and purified as described previously (Miller et al., 1987). The titer of these antibodies was checked routinely in ELISA.

Labeling of Proteins. Pyrene-labeled actin was prepared by the method of Cooper et al. (1983). The extent of labeling was measured by using a molar extinction coefficient of $E_{344} = 2.2 \times 10^4\ M^{-1}\ cm^{-1}$ for the protein–dye complex (Kouyama & Mihashi, 1981). The concentration of pyrene-labeled actin was measured in Bradford assays (1976). The labeling stoichiometry ranged between 0.7 and 0.9 pyrene per actin.

Fluorescein-labeled S-1 was obtained by modifying the reactive SH_1 groups on S-1 (4.0 mg/mL) with a 20-fold molar excess of 5-(iodoacetamido)fluorescein. The reaction was carried out for 1 h at 4 °C in 0.1 M NaCl, 10 mM imidazole (pH 7.0), and 1 mM MgADP. The reaction was terminated with β-mercaptoethanol and the excess reagent removed on two Penefsky (1977) columns and by subsequent dialysis of the modified protein. The labeling stoichiometry was determined by using the molar extinction coefficient of fluorescein, $E^{M}_{492} = 68\,000$, and was between 0.85 and 0.95 fluorescein/S-1. CaATPase activities of fluorescein-labeled S-1 were elevated by about 150% compared to unmodified S-1 while the K^+-(EDTA)-ATPases of these samples were inhibited by 95–97% relative to controls. These activities are consistent with the properties of SH_1-modified S-1.

Immunochemical Assays. Enzyme-linked immunosorbent assays (ELISA) were performed according to the method of Atherton and Hynes (1981) and as described by DasGupta et al. (1990). The ELISA plates were coated with G-actin and blocked with BSA. Different amounts of S-1 (between 0 and 128 μg/well) were then added to triplicate wells along with affinity-purified F_{ab} (0.4 μg/well) and incubated for 2 h at 37 °C. After appropriate washings and the reaction with secondary antibodies, the developed color was measured at 410 nm in a Dynatech MR 600 microplate reader.

Fluorescence and Light-Scattering Measurements. Fluorescence intensities for pyrene-labeled actin were measured in the Spex Fluorolog spectrophotometer (Spex Industries, Inc., Edison, NJ) as previously described (Miller et al., 1988a). The excitation monochromator was set at 368 nm, and the emission monochromator was at 407 nm. A small amount of pyrene-labeled actin (between 0.4 and 0.5 μM) was combined with G-actin in G-actin buffer containing no ATP at the beginning of polymerization experiments. After the fluorescence intensity of this sample was recorded, either S-1 or first F_{ab} and then S-1 were added to the actin solution. Subsequent changes in fluorescence were monitored as a function of time. Prior to fluorescence measurements, F_{ab} was dialyzed against G-actin buffer (free of ATP) and centrifuged for 10 min in an Eppendorf 5414 table-top centrifuge. S-1 was transferred to G-actin buffer (free of ATP) on Penefsky (1977) columns (Miller et al., 1988a).

Rapid-mixing experiments were carried out in the Spex Fluorolog spectrophotometer by using a Hi-Tech Scientific SF11 (Salisbury, England) rapid kinetics accessory.

Light-scattering measurements at 90° were conducted in the same Spex spectrophotometer at 310 nm (Miller et al., 1988a). Fluorescence intensity and light-scattering data are presented in arbitrary units.

Analytical Ultracentrifugation. Analytical ultracentrifugation experiments were carried out at 25 °C in a Beckman Model E analytical ultracentrifuge equipped with a photoelectric scanning system. On-line data collection and analysis was carried out with a VAX 11/780 computer. In order to selectively monitor the sedimentation of S-1 in the presence of actin and F_{ab}, fluorescein-labeled S-1 was employed in these experiments, and the monochromator was set at 492 nm. This rendered the sedimentation boundaries of G-actin, F_{ab}, and actin–F_{ab} "invisible" and simplified the sedimentation analysis of free and complexed S-1. The runs were started at 20 000 rpm to enable the pelleting of polymerized acto–S-1 and then continued at 44 000 rpm to observe the S-1 remaining in solution. In each experiment, three samples were run simultaneously and compared: free labeled S-1 (6.5 μM); actin (4.5 μM) and labeled S-1 (6.5 μM); and actin (4.5 μM), F_{ab} (9 μM), and labeled S-1 (6.5 μM). All solutions were mixed in G-actin buffer (free of ATP) immediately before the centrifugation. S-1 and F_{ab} were transferred to this buffer as described for fluorescence measurements.

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

SαN F_{ab} Inhibits the Polymerization of G-Actin by S-1. The addition of S-1 to G-actin leads to a rapid increase in the light scattering of actin solutions (Figure 1). The change in light scattering reflects the polymerization of G-actin by S-1 in the G-actin buffer (Miller et al., 1988a; Chaussepied & Kasprzak, 1989b). When the same experiment is done in the presence of SαN F_{ab}, the light-scattering changes are greatly inhibited. At equimolar ratios of F_{ab}, actin, and S-1 (4 μM each), very slow but detectable changes in the light scattering of actin can still be observed. At higher molar ratios of F_{ab} to actin (2:1), the light-scattering data do not reveal any polymerization of G-actin by S-1.

Additional information on the interaction between G-actin with S-1 can be derived from fluorescence measurements using actin labeled with pyreneyliodoacetamide (pyrene–actin).

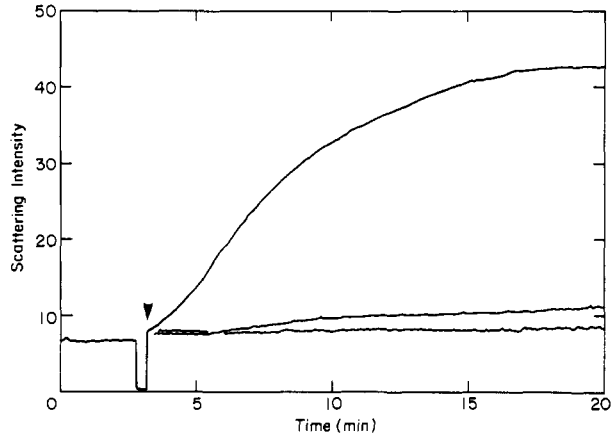

FIGURE 1: Time course of actin polymerization by S-1 in G-actin buffer. The polymerization was monitored by the increase in light-scattering intensity (λ = 310 nm) in the presence and absence of SαN F_{ab} at 25 °C. The polymerization was induced by the addition of S-1 (4 μM) to G-actin (4 μM). The scattering curves correspond in descending order to the polymerization of actin by S-1 in the absence of F_{ab} and in the presence of 4 and 8 μM F_{ab} (i.e., 1:1 and 2:1 molar ratios of F_{ab} to actin). The arrowhead indicates the time of S-1 addition to G-actin. Scattering intensities are given in arbitrary units.

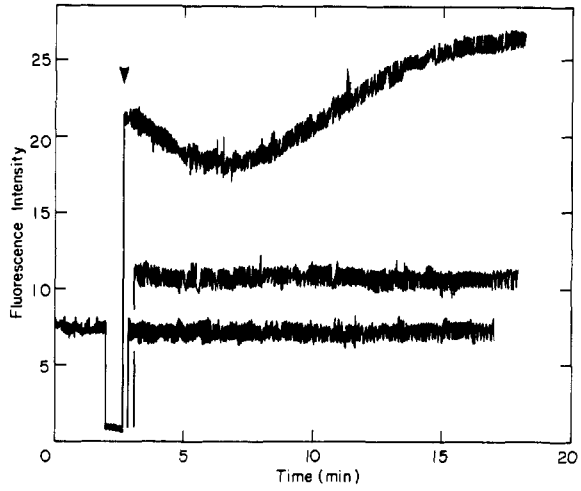

FIGURE 2: Changes in pyrene fluorescence of G-actin following the addition of S-1 in the presence and absence of SαN F_{ab}. S-1 (4.5 μM) was added to G-actin (final concentration 3 μM) containing a small amount of pyrene-labeled actin and preequilibrated with F_{ab} (when present). The fluorescence curves correspond in descending order to solutions containing no F_{ab} and 2.1 and 6 μM F_{ab} (i.e., 0.7:1 and 2:1 molar ratios of F_{ab} to actin). The initial fluorescence jump, notable in the upper and middle curves, reflects the initial binding of S-1 to G-actin. The time-dependent fluorescence changes shown in the upper curve monitor the subsequent polymerization of G-actin by S-1. The arrowhead indicates the time of S-1 addition to G-actin. Fluorescence intensities are given in arbitrary units.

Although the overall change in pyrene fluorescence due to the polymerization of G-actin is rather small in this case, due to the quenching of pyrene–F-actin fluorescence by S-1 (Criddle et al., 1985), the initial events in the S-1-induced reaction are readily monitored (Miller et al., 1988a). As shown by the upper tracing in Figure 2, the early pyrene fluorescence jump, associated with the binding of S-1 to G-actin, is followed by polymerization-linked changes in the fluorescence signal (Miller et al., 1988a). In agreement with light-scattering results, these S-1-induced changes in pyrene fluorescence are completely inhibited in the presence of a 2-fold molar excess of F_{ab} over actin (the bottom tracing in Figure 2).

The link between the binding of S-1 to pyrene–G-actin and the fluorescence jump is supported by several lines of evidence. Most important, the jump occurs at a fast time scale, before

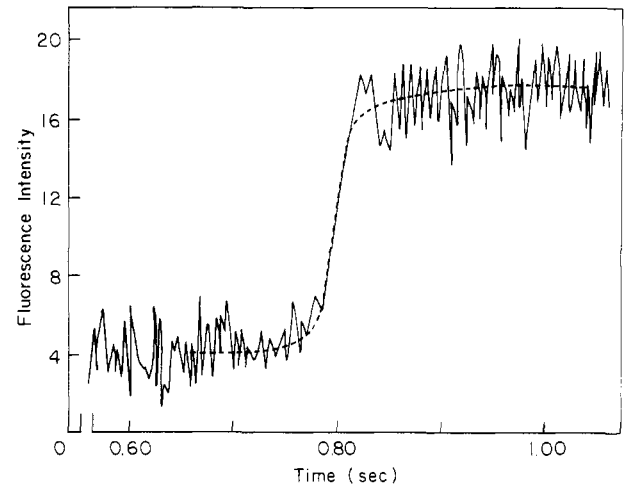

FIGURE 3: Fluorescence jump observed upon binding of S-1 to G-actin. S-1 (4.5 μM) was rapidly mixed with G-actin (3 μM) containing a small amount of pyrene–actin in the rapid kinetics accessory (concentrations after mixing). Fluorescence intensities are given in arbitrary units. Since the rapid mixing experiments required different instrumental settings, the fluorescence values in this figure cannot be compared with those in Figures 2 and 4.

the onset of any detectable increase in the light scattering of G-actin and S-1 solutions. Figure 3 shows that the fluorescence jump is completed within a fraction of a second from the rapid mixing of the proteins. No polymerization-related change in light scattering of G-actin and S-1 can be detected within the same or significantly longer time. The separation between the acto–S-1-binding step and the polymerization reaction was particularly pronounced at low protein concentrations. With G-actin and S-1 set at or below 1 μM levels, small and slow light-scattering changes could be detected only after prolonged incubations (between 5 and 20 min) while the fluorescence jump still occurred on a fast time scale. The polymerization-related changes in pyrene fluorescence, which follow the fluorescence jump (Figure 2; Miller et al., 1988a), are less pertinent to the present study and will be presented in greater detail elsewhere.

It is also noted here that the jump and the subsequent fluorescence changes are observed with unpurified proteins as well as with mixtures of column-purified pyrene–actin and actin.

SαN F_{ab} Inhibits the Binding of S-1 to G-Actin. Figure 4A shows the effect of F_{ab} on the fluorescence jump, which is normally observed upon binding of S-1 to G-actin. The inhibition of the fluorescence jump by F_{ab} is suggestive of an inhibition of S-1 binding to G-actin. The inhibition is reversible, and can be countered by increasing concentrations of S-1 (Figure 4B). Thus, at 8:1:1 molar ratio of S-1, actin, and F_{ab} (i.e., in the presence of 24 μM S-1), about 50% of the original fluorescence jump observed in the absence of F_{ab} is restored. Light-scattering data (not shown) also reveal partial recovery of G-actin polymerization by S-1 (24 μM) under these conditions. In the absence of F_{ab}, the magnitude of the initial fluorescence change does not vary with S-1 concentration. This result is consistent with the expected saturation of G-actin-binding sites at the employed concentrations of S-1 and actin (Chaussepied & Kasprzak, 1989a).

The partial recovery of the binding signal (fluorescence jump) and the polymerization of G-actin at high molar excesses of S-1 over F_{ab} raise a question about a possible displacement of antibodies from G-actin by S-1. However, very little if any competition between F_{ab} and S-1 was detected in ELISAs containing up to 0.5 mg/mL S-1 and a fixed amount

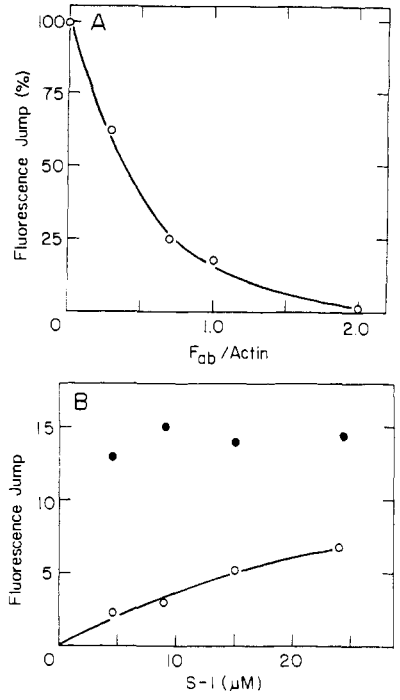

FIGURE 4: (A) Inhibition of fluorescence jump (due to S-1 binding to G-actin) by F_{ab}. Small amounts of pyrene-labeled actin were mixed with unlabeled G-actin (final concentration 3 μM) and F_{ab}. The fluorescence jump was triggered by the addition of S-1 (4.5 μM) to solutions of G-actin and F_{ab}. (B) Dependence of the fluorescence jump in the presence of SαN F_{ab} on S-1 concentration. S-1 was added to G-actin (3 μM) containing a small amount of pyrene–actin. The fluorescence jump was recorded for solutions of G-actin preequilibrated with 3 μM F_{ab} (○) and those in the absence of antibodies (●). Fluorescence jump values are given in arbitrary units.

of F_{ab}. As judged by constant OD_{410} readings in ELISAs, virtually the same amounts of F_{ab} could bind to the coated G-actin in the presence and absence of S-1 (not shown). This result is similar to the previous findings of limited displacement of SαN antibodies from F-actin's N-terminus by S-1 in the absence of nucleotides (Mejean et al., 1986, 1987; DasGupta et al., 1990).

Conclusive evidence for the inhibition of S-1 binding to actin by SαN F_{ab} has been obtained in sedimentation velocity experiments. In order to simplify the observation of S-1 sedimentation in the presence of F_{ab} and actin, and bypass the need for resolving between the boundaries of S-1, F_{ab}, and actin–F_{ab} at 280 nm, myosin heads labeled at their SH_1 groups with (iodoacetamido)fluorescein have been used in these experiments. In each experiment, the sedimentation of free fluorescein-labeled S-1 was compared to the sedimentation of this protein in the presence of either G-actin or G-actin and F_{ab}. (As judged by light-scattering results, the SH_1-labeled S-1 polymerized G-actin, albeit slower than unmodified S-1, and this polymerizing effect was inhibited by F_{ab}.)

Figure 5 presents the main results of these experiments. The two sets of virtually identical sedimentation boundaries (solid and dashed curves) taken 36 and 108 min after reaching the speed (42000 rpm) correspond to 6.5 μM free S-1 (solid curve) and S-1 (6.5 μM) incubated and then run in the presence of G-actin (4.5 μM) and F_{ab} (9 μM) (dashed curve). The sedimentation coefficients of free S-1 and that in the presence of G-actin and F_{ab} are s_{20} = 6.2 ± 0.2 S and 6.5 ± 0.3 S, respectively. The identical sedimentation boundaries of fluorescein–S-1 in both cells prove that very little if any S-1 binds to actin in the presence of SαN F_{ab}. On the other hand, when the same amounts of fluorescein–S-1 are incubated with G-actin in the absence of F_{ab} and then sedimented in the

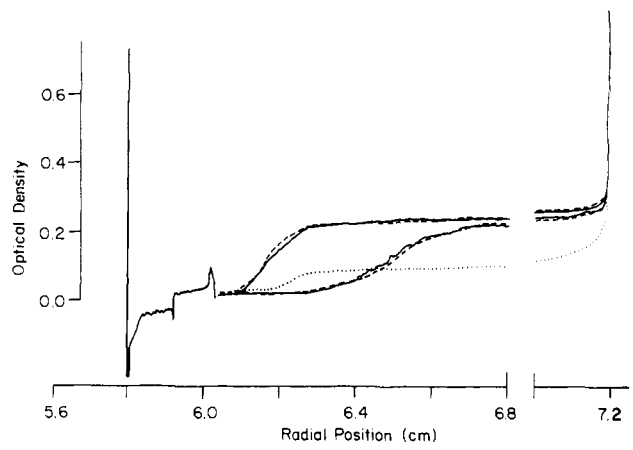

FIGURE 5: Sedimentation velocity boundaries of fluorescein S-1 as monitored at λ = 492 nm. 6.5 μM labeled S-1 (—), 6.5 μM labeled S-1 and 4.5 μM actin (···), and 6.5 μM labeled S-1, 4.5 μM actin, and 9 μM F_{ab} (- - -) were sedimented at 44000 rpm. The two sets of boundary scans were taken 36 and 108 min after reaching the final run speed.

ultracentrifuge, most of the S-1 is pelleted (dotted curve in Figure 5). The small amount of remaining, unpelleted S-1 (because of molar excess of S-1 over actin) has a sedimentation coefficient of s_{20} = 5.9 ± 0.3 S. The contrast between pelleting of S-1 in the absence of F_{ab} (dotted curve) and the lack of any S-1 pelleting in the presence of F_{ab} (dashed curve) shows again that F_{ab} inhibits the polymerization of actin by S-1.

S-1 Binds to Oligomeric Actin in the Presence of F_{ab}. It has been shown in earlier work that the SαN antibodies do not inhibit greatly the binding of S-1 to F-actin in the absence of nucleotides (Mejean et al., 1986, 1987; Miller et al., 1987). The inhibition of the G-actin–S-1 interaction by the same antibodies raises the possibility that actomyosin interactions change upon the G to F transition in actin (Chaussepied & Kasprzak, 1989b). To test whether such changes occur in the early stages of actin polymerization, the effects of S-1 and F_{ab} on G-actin "prenucleated" with $MgCl_2$ have been examined by light-scattering measurements. Figure 6 shows that the addition of 3 μM S-1 to a solution of 3 μM G-actin preincubated (at an initial concentration of 6 μM) with 2 mM $MgCl_2$ dramatically increases the rate of G-actin polymerization. The small increase in the light scattering of actin during its incubation with $MgCl_2$, and prior to the addition of S-1, verifies the formation of oligomeric actin species. These oligomers may be stabilized by S-1, leading to a rapid assembly reaction (Chaussepied & Kasprzak, 1989b). When S-1 is introduced to similarly preincubated actin but after the addition of SαN F_{ab}, the polymerization of actin is still accelerated but much less than in the absence of F_{ab}. Yet, for proper contrast, the dashed curve in Figure 6 shows again that S-1 does not polymerize G-actin bound to F_{ab} in the absence of preformed nucleai. Thus, the accelerated assembly of prenucleated actin by S-1 in the presence of F_{ab} indicates that the antibodies inhibit the binding of S-1 to actin oligomers less effectively than that to G-actin.

Discussion

The main observation of this work is that blocking the N-terminal segment of actin with antibodies inhibits the binding of S-1 to G-actin and the polymerization of actin by S-1. The inhibition of binding is documented in sedimentation velocity experiments and indicated by the loss of the pyrene fluorescence jump. Since the fluorescence jump occurs at millisecond time scale and reports on initial events in the

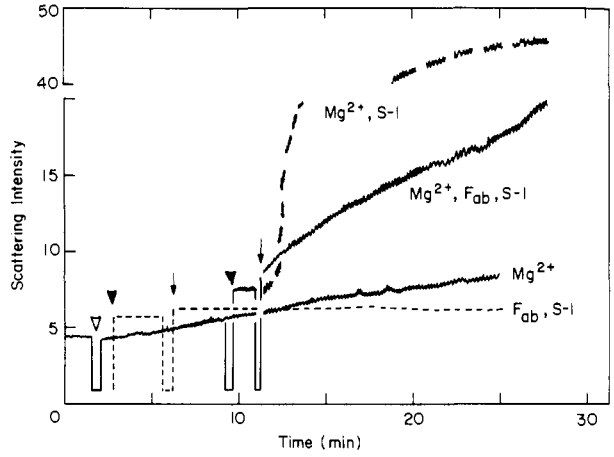

FIGURE 6: Effect of SαN F_{ab} on the polymerization of "prenucleated" actin by S-1. Prenucleated actin was prepared by the addition of 2 mM $MgCl_2$ (▽) to G-actin (6 μM) and subsequent incubation of this solution until initial changes in scattering intensity (at 310 nm) could be easily detected (usually between 5 and 7 min). At that time, the sample of actin was diluted to 3 μM by the addition of either S-1 (↓) or F_{ab} (▼). All proteins (actin, F_{ab}, and S-1) were at 3 μM final concentration during this second phase of polymerization. The Mg^{2+} curve shows the slow polymerization of undiluted G-actin (6 μM) by 2 mM $MgCl_2$. The F_{ab}, S-1 curve corresponds to actin solution diluted (to 3 μM) by the addition of F_{ab} (▼) and then supplemented with S-1 (↓). When G-actin is incubated with 2 mM $MgCl_2$ to generate polymerization nuclei, similar procedures and additions of F_{ab} (▼) and S-1 (↓) lead to accelerated increases in light scattering (Mg^{2+}, F_{ab}, S-1 curve). The top curve represents the rapid polymerization of prenucleated actin upon addition of S-1 (▼). All scattering data are given in arbitrary units.

binding of S-1 to G-actin, the inhibition of this jump by F_{ab} implicates the N-terminus of G-actin in the formation of initial contact(s) with S-1. The inhibition of S-1-induced polymerization of actin by SαN F_{ab}, which stems from the inhibition of binding, is evident from light-scattering and pyrene fluorescence measurements, as well as the absence of pelleted acto-S-1 in ultracentrifugation experiments.

The immunochemical evidence for a contribution of the N-terminal segment (residues 1–7 on actin) to the binding of G-actin to S-1 complements and confirms the findings of antipeptide S-1 experiments of Chaussepied and Kasprzak (1989b). These authors concluded that residues 633–642 on the S-1 heavy chain are essential for the binding of S-1 to G-actin. The N-terminal segment of actin is the most likely binding site for the above sequence of S-1 (Sutoh, 1982). The results of this work, when taken together with the antipeptide S-1 studies (Chaussepied & Kasprzak, 1989b), suggest strongly that the interaction between residues 1–7 on actin and residues 633–642 on the S-1 heavy chain is important for the binding of S-1 to G-actin.

A possible concern about the size of F_{ab} and, thus, indirect effects of antibodies on S-1 binding to G-actin is decreased by several observations. First, SαN F_{ab} shows the same binding affinity for G- and F-actins and has very little if any effect on the kinetics of actin polymerization by $MgCl_2$ (DasGupta et al., 1990). This result indicates that the N-terminal segment of actin is distant from actin–actin interface in the filament. Second, SαN F_{ab} does not inhibit greatly the rigor binding of S-1 to F-actin (Mejean et al., 1987; Miller et al., 1987), suggesting the absence of any serious steric exclusion of S-1 by F_{ab}. The fact that the same antibodies do inhibit acto-S-1 binding in the presence of ATP (DasGupta & Reisler, 1989) reveals the sensitivity of the antibody probe to changes in the acto-S-1 interface. Finally, the agreement with the antipeptide S-1 work (Chaussepied & Kasprzak, 1989b) suggests that SαN F_{ab} and S-1 bind to the same rather than to adjacent sites on actin.

In analogy to antibodies to the N-terminus of actin, the antipeptide to S-1 does not block the binding of S-1 to F-actin (Chaussepied & Morales, 1988). The different effects of antipeptide on the binding of S-1 to G- and F-actin suggested the creation of new myosin binding sites upon polymerization of actin (Chaussepied & Kasprzak, 1989b). The effects of SαN F_{ab} on S-1 binding to G- and F-actin appear consistent with such a possibility. However, closer examination of G-actin, F_{ab}, and S-1 interactions has revealed a concentration-dependent recovery of G-actin binding to S-1 (and actin polymerization). This effect can be explained at least in two ways. First, F_{ab} may be displaced from actin by high concentrations of S-1. Second, a weak acto–S-1 contact (intrinsically weak or weakened by F_{ab} or antipeptide) could be detected only at appropriate protein concentrations. It is possible that such weak contacts were not detected by antipeptide S-1 because protein concentrations were not varied in that work (Chaussepied & Kasprzak, 1989b).

Solution measurements of competitive binding or displacement of F_{ab} by S-1 are feasible with F-actin, but rather difficult with G-actin. ELISAs provide a valid alternative to such measurements of actin–antibody–S-1 interactions (Mejean et al., 1986, 1987). The poor displacement of F_{ab} by S-1 in ELISAs indicates that the affinity of S-1 for the N-terminus of G-actin cannot match that of F_{ab} ($\sim 10^6$ M^{-1}; Miller et al., 1987; Das Gupta et al., 1990). The strong overall binding of S-1 to G-actin (Chaussepied & Kasprzak, 1989a) may result then from combined, and perhaps cooperative, contributions of the N-terminal segment and other sites on actin to the binding interface. Thus, although the N-terminal segment on actin plays a major role in the G-actin–S-1 interaction, it is not essential for their binding.

In agreement with antipeptide S-1 results, S-1 accelerates the polymerization of G-actin by $MgCl_2$ in the presence of F_{ab}. Since F_{ab} itself does not affect the kinetics of actin polymerization by $MgCl_2$ (DasGupta et al., 1990), the increase in the rate of this reaction must be ascribed to S-1. The acceleration, although small, shows that S-1 binds somewhat better to actin oligomers than to G-actin in the presence of F_{ab}. Yet, the much bigger acceleration of actin assembly by S-1 alone indicates that the antibodies still inhibit significantly the binding of S-1 to actin oligomers. Although these results are not inconsistent with the creation of new myosin-binding sites, they can be equally well explained by tightening of acto–S-1 binding at weak contact sites during the early stages of actin polymerization. The "binding transitions" appear to continue beyond the step of oligomer formation.

In conclusion, this study shows that the N-terminal region of actin plays a major, though not necessarily essential, role in the binding of S-1 to G-actin. The weak G-actin–S-1 binding interactions, which are detected with the F_{ab}–actin complexes, appear to be augmented during the polymerization of actin, and perhaps new contact sites for S-1 are created as well. From comparisons with the F-actin, S-1, and F_{ab} system, and the limited but reciprocal competition between F_{ab} and S-1 for F-actin, it may be deduced that the binding interactions of S-1 with F-actin and G-actin are different.

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