Interactions of Myosin Subfragment 1 Isozymes with G-Actin†

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ABSTRACT: The polymerization of G-actin by myosin subfragment 1 (S-1) isozymes, S-1(A1) and S-1(A2), and their proteolytically cleaved forms was studied by light-scattering, fluorescence, and analytical ultracentrifugation techniques. As reported previously, S-1(A1) polymerized G-actin rapidly while S-1(A2) could hardly promote the assembly reaction (Chaussepied & Kasprzak, 1989a; Chen and Reisler, 1990). This difference between the isozymes of S-1 was traced to the very poor, if any, ability of G-actin–S-1(A2) complexes to nucleate the assembly of actin filaments. The formation of G-actin–S-1(A2) complexes was verified in sedimentation velocity experiments and by fluorescence measurements using pyrene-labeled actin. The G-actin–S-1(A2) complexes supported the growth of actin filaments and accelerated the polymerization of actin in solutions seeded with $MgCl_2$-, KCl-, and S-1(A1)-generated nuclei. The growth rates of actin–S-1(A2) filaments were markedly slower than those for actin–S-1(A1) filaments. Proteolytic cleavage of S-1 isozymes at the 50/20-kDa junction of the heavy chain greatly decreased their binding to G-actin and thus inhibited the polymerization of actin by S-1(A1). These results are discussed in the context of G-actin–S-1 interactions.

The interaction between myosin subfragment 1 and G-actin is of considerable interest to students of cell motility and muscle contraction. In order to support motile and contractile processes in cells, actin must accelerate the rate of adenosine 5′-triphosphate (ATP)[1] hydrolysis by myosin. The activation of myosin ATPase can be achieved only by the polymerized F-actin and not the monomeric G-actin (Offer et al., 1972). Thus, it is important to define the differences between the interactions of G-actin and F-actin with S-1. The understanding of these differences may bring new insights into the molecular mechanism by which actin activates the myosin ATPase activity.

Equally important is the task of unraveling the mechanism of cellular assembly of G-actin into filaments. In this context, S-1 can be considered as a model actin-polymerizing protein. The analysis of actin assembly by S-1 should shed light on other actin polymerization reactions controlled by various acting-binding proteins (Pollard & Cooper, 1986; Stossel, 1989). The interaction between S-1 and G-actin leads invariably to a rapid polymerization of actin (Cooke & Morales, 1971; Yazawa & Yagi, 1973; Detmers et al., 1981). In the course of such a polymerization reaction, only stoichiometric amounts of actin are incorporated into 1:1 acto–S-1 complexes, and the binding of S-1 to G-actin is assumed to change the conformation of the latter (Miller et al., 1988). Successful probing and better understanding of such conformational changes depend on the ability to stabilize a monomeric complex of G-actin and S-1 and are facilitated by a detailed structural description of actin and preliminary information on actomyosin contact sites. The last condition has been satisfied by mapping some of the S-1 contact sites on F-actin (Sutoh, 1982; Mejean et al., 1987; Miller et al., 1987; Moir et al., 1987; Bertrand et al., 1988). The recent publication of the atomic resolution structure of G-actin (Kabsch et al., 1990) and the preparation of monomeric G-actin–S-1 complexes (Bettache et al., 1989; Chaussepied & Kasprzak, 1989a) set the stage for more detailed conformational and assembly studies on the actin–S-1 system.

Two recent complementary studies have implicated the N-terminal segment of actin (DasGupta et al., 1990) and the corresponding site on S-1, the 50/20-kDa junction on the S-1 heavy chain (Chaussepied & Kasprzak, 1989b), as essential components of the G-actin–S-1 interaction. The first work employed specific antibodies against the first seven N-terminal residues on actin; the latter study involved the blocking of the 50/20-kDa junction on S-1 with an antipeptide. Interestingly, the same structural probes have suggested that the same sites on actin (Mejean et al., 1878; Miller et al., 1987) and S-1 (Chaussepied & Morales, 1988) do not play a crucial role in rigor binding of myosin to F-actin.

The goal of this work was to assess the importance of different structural elements of S-1 to its binding and polymerization of G-actin. The initial approach was to employ various proteolytically cleaved preparations of S-1 to gain further insight into the role of the 25/50- and 50/20-kDa junctions on S-1 in G-actin–S-1 interactions. The analysis of such experiments was hampered by the need to resolve between the consequences of the S-1 heavy chain and light chain (A1) cleavage. To bypass this difficulty, S-1 was separated into the S-1(A1) and S-1(A2) isozymes in all subsequent experiments. The immediate result of the first actin polymerization experiments with the isozymes was the finding of the dominant, if not exclusive, contribution of A1 light chains on S-1 to the

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[1] Abbreviations: HMM, heavy meromyosin; S-1, myosin subfragment 1; S-1(A1), S-1 isozyme with alkaline light chain; S-1(A2), S-1 isozyme with alkaline light chain 2; DTT, dithiothreitol; ATP, adenosine 5′-triphosphate; NEM, *N*-ethylmaleimide; pyrene-actin, actin labeled at Cys-374 with *N*-(1-pyrenyl)iodoacetamide; DE52, (diethylaminoethyl)-cellulose; CM52, (carboxymethyl)cellulose; pPDM, *N*′,*N*′-*p*-phenylenedimaleimide; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetate.

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nucleation of actin polymerization (Chen & Reisler, 1990). The same observation was made by Chaussepied and Kasprzak (1989a), who reported also on the formation of a stable monomeric G-actin–S-1(A2) complex. Thus, for logical (but not chronological) reasons, the Results section is centered around the effects of S-1(A1) and S-1(A2) on G-actin. Light-scattering, fluorescence, and sedimentation velocity measurements are presented to document the efficient nucleation of G-actin polymerization by S-1(A1) and the inability of S-1(A2) to induce the same transitions in G-actin. S-1(A2) can, however, propagate the growth of actin filaments. By using proteolytically cleaved preparations of S-1(A1) and S-1(A2), it is also verified that the 50/20-kDa junction on S-1 is indeed important for G-actin–S-1 interactions. These results are discussed in relation to the G to F transition in actin.

Materials and Methods

Reagents. α-Chymotrypsin, trypsin, soybean trypsin inhibitor, thermolysin, catalase, dithiothreitol, ATP, and *N*-ethylmaleimide were purchased from Sigma Chemical Co. (St. Louis, MO). *N*-(1-Pyrenyl)iodoacetamide was obtained from Molecular Probes (Junction City, OR). Arg-C protease was purchased from Boehringer Manheim. DE52 and CM52 resins were purchased from Whatman (Hillsboro, OR). Sephacryl S-200 was from Pharmacia (Piscataway, NJ). All other reagents used were analytical grade.

Proteins. Myosin and actin from rabbit psoas muscle were prepared according to Godfrey and Harrington (1970) and Spudich and Watt (1971), respectively. Actin was purified further by passing it through a Sephacryl S-200 column (2.5 × 40 cm) (MacLean-Fletcher & Pollard, 1980). Fractionated G-actin was stored in G-actin solution (5 mM Tris, 0.5 mM DTT, 0.2 mM $CaCl_2$, and 0.2 mM ATP). S-1 was prepared by digestion of myosin with α-chymotrypsin at 4 °C as described previously (Weeds & Pope, 1977). The isozymes of S-1, S-1(A1), and S-1(A2), were separated by ion-exchange chromatography. S-1(A1) was obtained by collecting the fractions of the first peak of a DE52 column eluted according to Weeds and Taylor (1975). The fractions of the second DE52 peak, which contained S-1(A2) and a small amount of S-1(A1), were also pulled and concentrated. S-1(A2) was then separated from the contaminating S-1(A1) by CM52 ion-exchange column chromatography (Bechet et al., 1982).

Proteolytic Digestions of S-1. In order to generate S-1 cleaved at the 50/20-kDa junction, S-1 (2 mg/mL) was digested with trypsin (1:100 w/w) at room temperature for 15 min in the presence of 10 mM MgATP and 0.6 NaCl (Mathern & Burke, 1986; Chen et al., 1987) or with 20 units of Arg-C protease for 2 h (Bertrand et al., 1989). To generate the 25/50-kDa-cleaved S-1, S-1 (2 mg/mL) was digested by trypsin (1:10 w/w) in the presence of actin (2 mg/mL) (Chen et al., 1987).

Pyrene Labeling of Actin. Pyrene-labeled actin was prepared according to Cooper et al. (1983). Modifications to the published procedure included short (3 h) dialysis of actin into G-actin buffer devoid of DTT or mercaptoethanol, polymerization of this actin (at 1 mg/mL) by 0.1 M KCl and 2 mM $MgCl_2$, and its final labeling with a 3-fold molar excess of *N*-(1-pyrenyl)iodoacetamide for 17 h. The extent of labeling was determined by using a molar extinction coefficient of E_{344} = 22000 M^{-1} cm^{-1} for the pyrene–protein complex (Kouyama & Mihashi, 1981). Protein concentrations were determined by Bio-Rad protein assay (Bradford, 1976). The labeling efficiency was usually between 0.7 and 1.0 pyrene per actin. Pyrene-labeled G-actin was purified further by passing it through a Sephacryl S-200 column (1.2 × 40 cm).

Cross-Linking of Actin with p-Phenylenedimaleimide (pPDM). pPDM cross-linking of actin was carried out according to Mockrin and Korn (1981). F-Actin at 1 mg/mL was reacted with a 1.4 molar excess of pPDM dissolved in dimethylformamide for 1 h. The reaction was quenched with 10 mM DTT. Labeled F-actin was isolated by centrifugation at 100000*g* for 1 h. The pellet was homogenized and dialyzed against G-actin buffer (5 mM Tris, 0.2 mM $CaCl_2$, 0.5 mM DTT, and 0.2 mM ATP, pH 7.6) overnight. Depolymerized pPDM–actin was centrifuged at 100000*g* for 2 h. The supernatant (about 20 mg of actin) was chromatographed on a Sephacryl S-200 column (1.2 × 40 cm). The fractions containing most of the dimers were used in the polymerization experiments.

Light-Scattering Measurements. Light-scattering measurements were conducted at 25 °C in a Spex Fluorolog spectrophotometer (Spex Industries, Inc., Edison, NJ). All solutions were degassed and filtered through 0.45-μm Millipore filters. The proteins were filtered through 3.0-μm Millipore filters. Prior to measurements, the proteins were centrifuged also at 100000*g* for 1 h in a Beckman TL-100 centrifuge. S-1 and S-1 isozymes were equilibrated with G-actin buffer (5 mM Tris/0.2 mM $CaCl_2$, pH 7.6) by centrifugation on Penefsky columns (Penefsky, 1977) and were used immediately in the experiments. Light-scattering measurements were taken at a 90 °C angle at 325 or 600 nm for a minimum of 20 min. The light-scattering signal at 325 nm was much more sensitive to the polymerization of actin than that at 600 nm. Because of dilutions with G-actin buffer devoid of ATP, the amount of ATP present in the final mixtures was normally between 15 and 20 μM and had very little, if any, effect on G-acto–S-1 binding.

Fluorescence Measurements. Fluorescence measurements were carried out in the Spex instrument described above. The excitation wavelength was set at 368 nm, and the emission was monitored at 408 nm. All solutions and proteins were handled in the same way as for light-scattering measurements.

Analytical Ultracentrifugation. Analytical ultracentrifugation experiments were carried out at 44000 rpm and 25 °C in a Beckman Model E analytical ultracentrifuge equipped with a photoelectric scanning system. On-line data collection and analysis were carried out with a Vax 11/780 computer. Because of the high absorbance of proteins, the sedimentation was monitored at 292 nm. The sedimentation of G-acto–S-1(A2) complexes in the G-actin buffer (5 mM Tris/0.2 mM $CaCl_2$, pH 7.6) was carried out at protein concentrations of 7 μM each for actin and S-1. The concentration of ATP in these solutions was 20 μM. The scans were taken at 4-min intervals.

SDS–Polyacrylamide Gel Electrophoresis. Gel electrophoresis was carried out according to the procedure of Laemmli (1970) using a two-phase gel of 10% (upper) and 15% (lower) acrylamide (w/w). The optical densities of Coomassie Blue stained protein bands and the respective mass distributions were determined with a Zeineh soft laser scanning densitometer Model SLR-1D/2D (Biomed Instruments, Inc., Fullerton, CA). To account for gel loading variations, the intensities of all protein bands in each lane were normalized to the intensity of the catalase band in the same lane. The differences in dye absorption were corrected by dividing the normalized intensity by the molecular weight of the respective protein band.

Results

Interactions of G-Actin with S-1(A1) and S-1(A2). Figure 1 shows the great difference in the abilities of S-1(A1) and

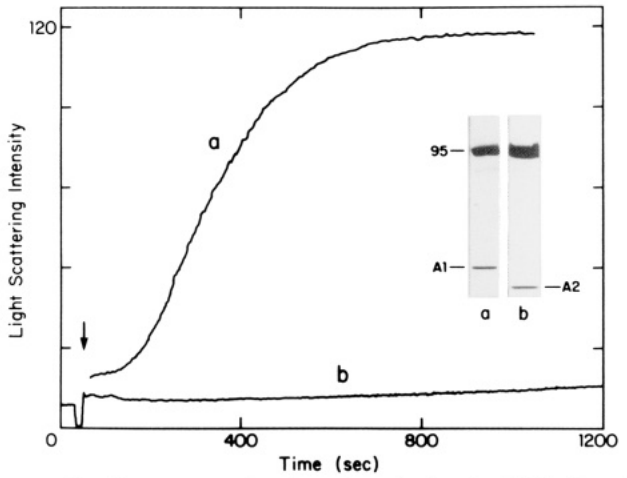

FIGURE 1: Time course of actin polymerization by S-1(A1) and S-1(A2). S-1(A1) and S-1(A2) (inset: lanes a and b, respectively) were separated and purified as described under Materials and Methods. The polymerization of G-actin by S-1(A1) (curve a) and S-1(A2) (curve b) in G-actin buffer was monitored by light-scattering measurements at 325 nm as described under Materials and Methods. Unless specified, the protein concentrations in all experiments were 5 μM for actin and S-1. The arrow indicates the addition of S-1 to the solution. Both isozymes were stored in 0.1 M KCl/50 mM Tris, pH 8.0 at 4 °C. Prior to experiments, the isozymes were centrifuged at 100000*g* for a minimum of 1 h and then passed through Sephadex G-50-80 Penefsky columns equilibrated with the G-actin solution.

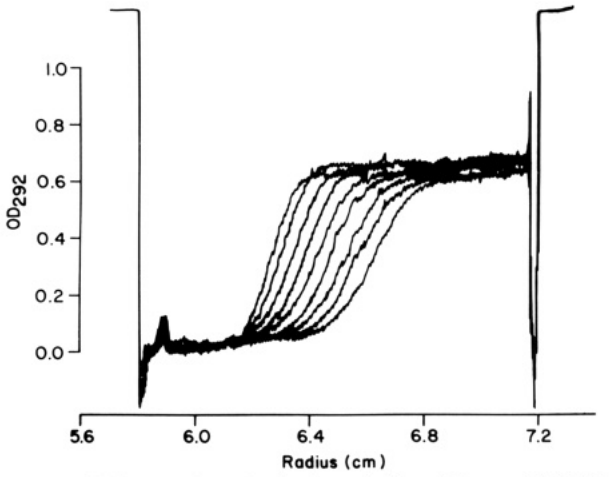

FIGURE 2: Sedimentation velocity boundaries of G-acto–S-1(A2) complexes as monitored at 292 nm. G-Actin and S-1(A2), both at 7 μM, were mixed and sedimented first at 30000 rpm and then at 44000 rpm. The time interval between successive scans was 4 min. The first scan shown here was taken at about 30 min after reaching the final run speed. A small amount of higher molecular weight materials sedimented to the bottom of the cell at the beginning of the run.

S-1(A2) to polymerize actin. The light-scattering profile of G-actin polymerization by S-1(A1) was similar to that observed with unfractionated S-1(A1+A2) (not shown). The initial lag phase (nucleation) was followed by a rapid growth of actin filaments (Figure 1). At equal protein concentrations, the final light-scattering intensities of polymerized acto–S-1(A1) and acto–S-1(A1+A2) were the same (±10%).

In the presence of S-1(A2), the light-scattering intensity of the actin and S-1 mixture increased very slowly (Figure 1). Yet, when incubated at 4 °C overnight, all the actin was polymerized. The polymerized complex was ATP-sensitive, indicating that it did not result from destabilized actin. When the same polymerization reaction was monitored by light scattering at a less sensitive wavelength (λ = 600 nm; Chaussepied & Kasprzak, 1989a), no assembly of actin was detected, in agreement with Chaussepied and Kasprzak (1989a).

In order to verify that the poor, if any, nucleation of actin was not due to the lack of binding of S-1(A2), mixtures of these proteins were examined in sedimentation velocity experiments. Except for a small amount (<5%) of higher molecular weight material which was pelleted at the beginning of the centrifugation run, the sedimentation boundary of the proteins was hypersharp and characteristic of a homogeneous species with a sedimentation coefficient of $s_{20,w}$ = 7.0 ± 0.4 S (Figure 2). This value agreed well with the *s* value of 7.35 S calculated for a globular G-acto–S-1(A2) complex. Free, uncomplexed G-actin and S-1(A2) were not detected in the sedimenting solutions. This result verified the formation of a stable complex of G-actin and S-1(A2) (Chaussepied & Kasprzak, 1989a). Yet, it should be noted that the supernatant containing the G-acto–S-1(A2) complex, when removed from the ultracentrifuge cell, polymerized completely after overnight incubation at 4 °C.

The results of analytical ultracentrifugations showed that the difference in the polymerization of actin by S-1 isozymes could not be attributed to a lack of binding of S-1(A2) to G-actin. In fact, fluorescence measurements using pyrene-labeled G-actin suggested that the two isozymes were binding with similar but not identical strength to G-actin. Both S-1(A1) and S-1(A2) triggered an initial jump in the fluorescence of pyrene-labeled G-actin (Table I). Such fluorescence change is indicative of the binding of S-1 to actin (DasGupta et al., 1990). The small difference in the S-1(A1)- and S-1(A2)-induced jumps is consistent with somewhat different affinities of S-1 isozymes for actin.

Table I Initial Increases in the Fluorescence of Pyrene-Labeled G-Actin upon Addition of S-1 Isozymes[a]

S-1 species	concn (μM)	ΔF (%)
S-1(A1)	5	340
S-1(A2)	5	280
Arg-C-cleaved S-1(A1)	5	65
Arg-C-cleaved S-1(A1)	10	110
Arg-C-cleaved S-1(A2)	5	37

[a] The fluorescence jump, ΔF (%), observed upon addition of various S-1 species to G-actin (5 μM) in G-actin buffer was calculated according to the expression ΔF (%) = $100[(F - F_0)/F_0]$ where F is the fluorescence intensity immediately after the addition of S-1 to pyrene-labeled G-actin and F_0 is the initial fluorescence intensity of pyrene-labeled actin. All measurements were repeated at least 3 times on separate protein preparations. ΔF (%) values varied between ±10 and ±20% in repeat experiments.

Polymerization of Actin by S-1(A2). As mentioned above, despite the fact that unfractionated S-1(A1+A2) contained about 66% S-1(A1), the final scattering intensities for polymerized complexes of acto–S-1(A1) and acto–S-1(A1+A2) were virtually the same. To gain further insight into the significance of this observation, the polymerization of actin by mixtures of S-1(A1) and S-1(A2) was examined by light-scattering measurements. By varying the molar ratios of S-1(A1) and S-1(A2) between 1 and 0.1, the lag phase in actin polymerization was increased while the overall rate of the polymerization reaction was decreased (Figure 3). Yet, the final light-scattering intensities (115 ± 6 in arbitrary units after 2-h incubation) suggested that regardless of the isozyme ratios the same amounts of polymers were formed in all mixtures. Pelleting these mixtures revealed that both S-1(A1) and S-1(A2) cosedimented with the polymerized actin (not shown). These results demonstrate that although S-1(A2) is

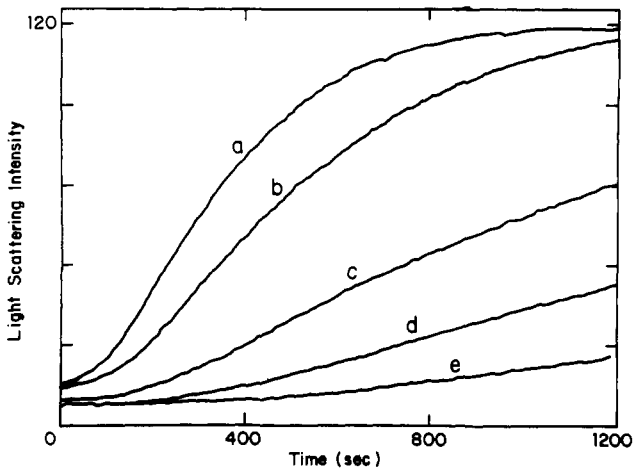

FIGURE 3: Light-scattering measurements of the polymerization of G-actin by mixtures of S-1(A1) and S-1(A2). The respective concentrations of S-1(A1) and S-1(A2) were (a) 4 and 1 μM, (b) 3 and 2 μM, (c) 2 and 3 μM, (d) 1 and 4 μM, and (e) 0.5 and 4.5 μM. The concentration of actin was 5 μM.

a poor, if at all, nucleating agent for actin polymerization, the S-1(A2) isozyme contributes to the growth and stability of actin filaments. The binding of S-1(A2) to G-actin results in the incorporation of the G-acto-S-1(A2) units into actin filaments.

That S-1(A2), independently of S-1(A1), can polymerize actin has been documented by seeding the assembly of actin with pPDM-cross-linked actin oligomers (Mockrin & Korn, 1981). The seeded solutions did not polymerize on their own. However, the addition of 5 μM S-1(A2) to these solutions triggered rapid polymerization of actin with no detectable lag phase. For comparison with S-1(A1), Figure 4A shows polymerization experiments carried out at 1 μM concentrations of S-1 and actin. Clearly, in the absence of S-1(A1), S-1(A2) induced a rapid polymerization of seeded actin (Figure 4A, curve b). The polymerization of seeded actin by S-1(A1) was faster than that by S-1(A2) and did not reveal a lag phase even at low protein concentrations (Figure 4A, curve a). Similar differences in actin polymerization by S-1(A1) and S-1(A2) were also observed when actin oligomers were formed in solutions of actin preincubated with $MgCl_2$. Polymerization of actin by 2 mM $MgCl_2$ alone was very slow (Figure 4B, solid curve). The addition of S-1(A2) to actin preincubated with $MgCl_2$ resulted in a rapid polymerization reaction (Figure 4B, dashed curve). These results show, as noted by Chaussepied and Kasprzak (1989a), that actin nuclei accelerate the polymerization of G-actin by S-1(A2). The fact that the rate of actin polymerization by S-1(A1) is faster than that by S-1(A2) also for seeded actin can be attributed to different elongation rates of actin filaments by these isozymes. Yet, the possible contribution to the assembly process of additional nucleation sites generated by S-1(A1) cannot be ruled out.

Polymerization of Actin by S-1 Isozymes in 0.1 M KCl. Striking differences between the effects of S-1(A1) and S-1(A2) on the polymerization of G-actin under low ionic strength conditions (G buffer) were found by Chaussepied and Kasprzak (1989a) and in this work. The other known differences between S-1(A1) and S-1(A2), such as in actin-activated ATPase activities and the binding to F-actin in rigor conditions and in the presence of MgATP, are salt-dependent and decrease or disappear at higher ionic strength conditions (Chalovich et al., 1984; Wagner et al., 1979; Reisler, 1980). Thus, the polymerization of actin by S-1 isozymes was tested

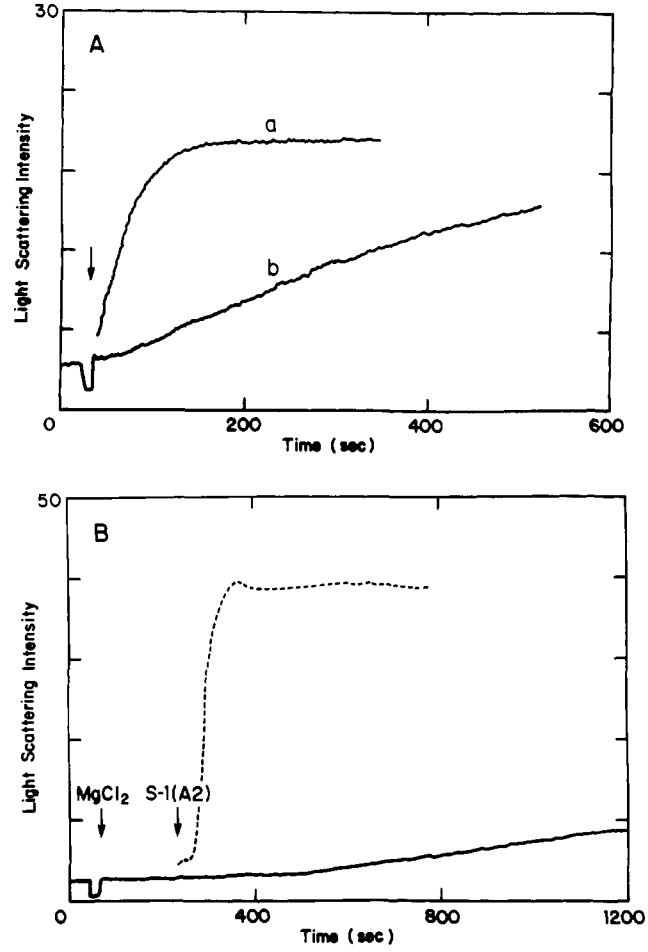

FIGURE 4: (A) S-1-induced polymerization of G-actin seeded with pPDM-cross-linked actin. To obtain measurable polymerization rates, the concentrations of G-actin, S-1(A1) (curve a), and S-1(A2) (curve b) were reduced to 1 μM. G-Actin was mixed with 50% pPDM-actin to a final concentration of 1 μM. Such seeded actin alone did not polymerize in G-actin buffer (data not shown). The arrow indicates the addition of S-1 to the solution. (B) Acceleration of actin polymerization by S-1(A2) in the presence of 2 mM $MgCl_2$. The final G-actin concentration was 5 μM, and that of S-1(A2) was 1 μM. S-1(A2) was added to actin at about 150 s after the addition of $MgCl_2$, at which time only a slight increase could be detected in the light scattering of the solution. The solid curve monitors the polymerization of G-actin by $MgCl_2$; the dashed curve follows the polymerization accelerated by S-1(A2).

also in the presence of 0.1 M KCl. At low G-actin concentrations (1 μM), as judged by both light-scattering (Figure 5A) and pyrene fluorescence measurements (not shown), KCl did not induce a detectable actin polymerization within the standard 20-min observation time. The addition of S-1(A2) to actin solutions containing 0.1 M KCl led to much faster polymerization of actin by S-1(A2) than in the absence of KCl (Figures 5A and 1). This is most likely due to the presence of KCl-generated actin nuclei in the 0.1 M KCl solutions. Yet, even in the presence of KCl, S-1(A1) polymerized actin better than S-1(A2) (Figure 5A). It is unlikely that the differences in the rates of polymerization reactions induced by S-1(A1) and S-1(A2) over a 10-fold range of S-1 concentrations (Figure 5B) could be explained by differences in the binding strength of the isozymes to actin. The differences in the polymerization rates and their dependencies on isozyme concentrations suggest that the rates of elongation of actin filaments by S-1(A1) and S-1(A2) are different also in the presence of 0.1 M KCl.

Effect of S-1 Cleavage at the 50/20-kDa Junction on G-

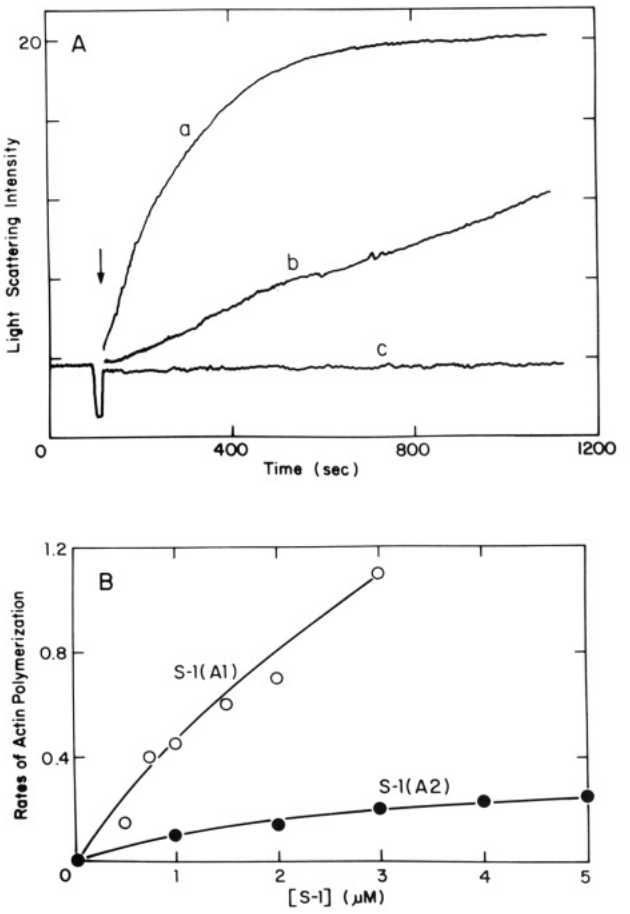

FIGURE 5: (A) Polymerization of G-actin by S-1(A1) and S-1(A2) in 0.1 M KCl as monitored by light-scattering measurements. The polymerization was monitored after the addition of S-1(A1) (curve a), S-1(A2) (curve b), and for G-actin alone (curve c). Protein concentrations were 5 and 1 μM for G-actin and S-1 isozymes, respectively. The arrow indicates the addition of S-1 to actin. (B) Comparison of the rates of actin polymerization by S-1(A1) and S-1(A2) in 0.1 M KCl as a function of S-1 concentration. To obtain measurable polymerization rates, the actin concentration was fixed at 1 μM. The concentrations of S-1 isozymes were varied from 0.5 to 5 μM. The rates, in arbitrary units, were calculated from the initial changes in the light scattering of acto–S-1 solutions per minute.

Acto–S-1 Interactions. S-1(A1) cleaved specifically at its 50/20-kDa junction, i.e., consisting of the 75- and 20-kDa fragments, was obtained by limited proteolysis of this isozyme with Arg-C protease. The resulting S-1 is visualized in the inset to Figure 6. As shown in the same figure, the cleavage of the 50/20-kDa junction greatly inhibited the ability of S-1(A1) to polymerize G-actin. The contribution of the second junction on S-1, between the 25- and 50-kDa fragments of the heavy chain, to the polymerization of actin could not be assessed directly. The reason for that was that specific tryptic cleavage of S-1(A1) at the 25/50-kDa junction (in the presence of actin) was accompanied by a degradation of A1 to species with an electrophoretic mobility close to that of the A2 light chains.

The relationship between the polymerization of actin and the binding of cleaved S-1 to pyrene-labeled G-actin was established by monitoring the fluorescence jump associated with the binding of these proteins to each other (DasGupta et al., 1990). As reported in Table I, S-1(A1) and S-1(A2) produced rather similar fluorescence changes upon their binding to G-actin. Arg-C-cleaved or tryptically cleaved S-1(A1) elicited

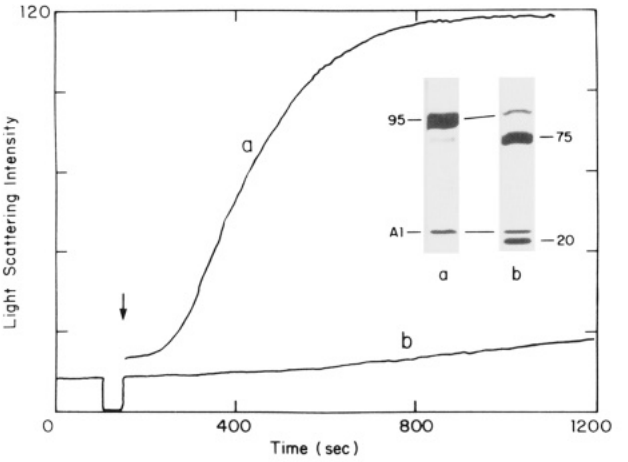

FIGURE 6: Light-scattering measurements of actin polymerization by S-1(A1) cleaved at the 50/20-kDa junction. Curve a, the polymerization of G-actin by intact S-1(A1). Curve b shows the polymerization of G-actin by Arg-C-cleaved S-1(A1). The inset shows SDS gels of intact S-1(A1) (lane a) and S-1(A2) cleaved by Arg-C at the 50/20-kDa junction to yield the 75- and 20-kDa bands (lane b). The conditions of light-scattering measurements were the same as in Figure 1. The arrow indicates the addition of S-1(A1) to actin.

much smaller fluorescence jumps upon addition to G-actin. Since the fluorescence jump could be increased by increasing the concentration of the Arg-C-cleaved S-1(A1) added to actin (Table I), the small fluorescence changes reflected reduced binding (about hundredfold) of the cleaved protein to G-actin. A similar decrease in S-1 binding to actin was observed upon blocking the 50/20-kDa junction with an antipeptide (Chaussepied & Kasprzak, 1989b). As judged by fluorescence data (Table I), the cleavage of the 50/20-kDa junction in S-1(A2) by Arg-C or by trypsin (not shown) decreased the binding of this isozyme to G-actin similarly to the binding decrease measured for the cleaved S-1(A1). These results suggest that the inhibition of the actin-polymerizing function of S-1(A1) upon cleavage of its 50/20-kDa junction is caused by the inhibition of S-1(A1) binding to G-actin.

Discussion

The main observation of this work, on the different abilities of the S-1(A1) and S-1(A2) isozymes of myosin to polymerize G-actin, is in agreement with the recent findings of Chaussepied and Kasprzak (1989a). The present study, which was carried out independently of that work, confirms that S-1(A2) is a very poor polymerizing agent of G-actin. As shown by Chaussepied and Kasprzak (1989a) and also by our fluorescence and sedimentation velocity data, the unexpected lack of actin polymerization by S-1(A2) cannot be attributed to a deficient binding of these proteins to each other. It is documented also in this work that G-actin–S-1(A2) complexes polymerize into filaments when seeded with actin nuclei or oligomers and copolymerize with G-actin–S-1(A1) over a wide range of molar ratios of these isozymes. Thus, G-actin–S-1-(A2) complexes contribute to and support the growth of actin filaments. It may be concluded then that the poor, if any, ability of isolated S-1(A2) to polymerize G-actin reflects the incompetence of this isozyme to generate actin nuclei. The fact that overnight incubations of G-actin and S-1(A2) yield polymerized actin could stem from the very slow nucleation rate by this isozyme or, perhaps more likely, from artifactual aggregation of proteins or even trace contamination of S-1(A2) by S-1(A1). Whatever the reason, the long-term stability of

G-actin–S-1(A2) complexes may be difficult to achieve.

At present, there is no experimental evidence for the linking of two actin molecules into a stable dimer through their respective binding to the S-1 heavy chain and A1 subunits. Of course, such double-site contacts of S-1(A1) and actin need not be preserved in F-actin and would be difficult to detect in the rapidly polymerizing G-actin–S-1(A1) system. Nevertheless, it is possible that earlier suggestions on the binding of S-1 to two actin molecules (Mornet et al., 1981; Amos et al., 1982) may be explained by such putative heavy chain and A1 light chain interactions with actin. Alternatively, if both subunits contact the same actin monomer (Milligan et al., 1990), S-1(A1) may nucleate G-actin by imposing a specific conformational change on that protein. Whether, and in what way, the S-1(A1)-induced change is related to the documented interaction of the A1 chains with the C-terminal region on actin (Sutoh, 1982; Trayer et al., 1987) is yet to be explored. Such S-1(A1) specific change, if indeed induced, does not extend to the Cys-374 site on actin. This is deduced from the fact that changes in the fluorescence of the pyrene probe (located at Cys-374) associated with the binding of S-1 to F- and G-actin are similar for both isozymes. Other known differences in the interactions of S-1 isozymes with actin can be explained by different binding affinities of the isozymes for actin (Wagner et al., 1979; Reisler, 1980; Chalovich et al., 1984). As discussed above, binding considerations cannot account for the different effects of S-1(A1) and S-1(A2) on actin polymerization.

It is interesting to note also that S-1 isozymes retain some of their different effects on actin even in the presence of 0.1 M KCl, i.e., conditions under which acto-S-1 ATPase and acto-S-1 binding are similar for both isozymes (Chalovich et al., 1984). S-1(A1) promotes the elongation of actin filaments better than S-1(A2) irrespective of the origin of the seeded nuclei (i.e., obtained by incubation of actin with $MgCl_2$, cross-linking with pPDM, and incubation with 0.1 M KCl). The growth rate of actin–S-1(A1) filaments appears to be severalfold faster than that of actin–S-1(A2) filaments. However, the possibility that additional nucleation of actin by S-1(A1) contributes to the very rapid growth of seeded actin filaments by this isozyme cannot be excluded. This, if true, would lead to an overestimation of the difference in the elongation rates of actin filaments by S-1(A1) and S-1(A2).

Another important observation of this work is that selective proteolytic cleavage of S-1(A1) at the 50/20-kDa junction greatly inhibits the ability of S-1 to polymerize actin. This inhibition is caused by a decrease in the binding between G-actin and S-1(A1). This observation confirms the findings of two recent reports which have ascribed an important role in the G-actin–S-1 interaction to the N-terminal segment of actin (DasGupta et al., 1990) and residues 633–642 on S-1 (Chaussepied & Kasprzak, 1989b). In one study, the binding of S-1 to G-actin was strongly inhibited by antibodies against the N-terminal segment of actin (DasGupta et al., 1990) while in the other work the binding of G-actin to S-1 was blocked by coupling an antipeptide to residues 633–642 on S-1 (Chaussepied & Kasprzak, 1989b). In both cases, the size of the employed probes left open the possibility that steric or indirect effects are responsible for the observed loss of G-actin–S-1 binding. Such a possibility can be discounted now since similar inhibition of G-actin–S-1 binding is observed upon cleavage of the 50/20-kDa junction (comprised of residues 633–642) on S-1.

Interestingly, the contact between the N-terminal segment on actin and residues 633–642 on S-1 is important for G-actin–S-1 and F-actin–S-1·ATP (DasGupta & Reisler, 1989; Chaussepied & Morales, 1988) but not for rigor F-actin–S-1 binding (Miller et al., 1987; Mejean et al., 1987). This variability suggests that the actomyosin interface may be composed of multiple contact sites. Which of these are utilized will depend on the interaction conditions and will determine the conformational changes in both proteins.

In conclusion, this work confirmed the different effects of S-1 isozymes on the polymerization of G-actin. These differences were traced to the virtual inability of G-actin–S-1(A2) complexes to nucleate the polymerization of actin and a markedly slower elongation of actin filaments by G-actin–S-1(A2) than by G-actin–S-1(A1) complexes. The important role of the 50/20-kDa junction on S-1 in the binding of S-1 to G-actin was demonstrated by using myosin heads selectively cleaved at that junction.

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Protein Secondary Structure of the Isolated Photosystem II Reaction Center and Conformational Changes Studied by Fourier Transform Infrared Spectroscopy†

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ABSTRACT: The secondary structure of the photosystem II (PSII) reaction center isolated from pea chloroplasts has been characterized by Fourier transform infrared (FTIR) spectroscopy. Spectra were recorded in aqueous buffers containing H_2O or D_2O; the detergent present for most measurements was dodecyl maltoside. The broad amide I and amide II bands were analyzed by using second-derivative and deconvolution procedures. Absorption bands were assigned to the presence of α-helices, β-sheets, turns, or random structure. Quantitative analysis revealed that this complex contained a high proportion of α-helices (67%) and some antiparallel β-sheets (9%) and turns (11%). An irreversible decrease in the intensity of the band associated with the α-helices occurs upon exposure of the isolated PSII reaction center to bright illumination. This loss of α-helical content gave rise to an increase in other secondary structures, particularly β-sheets. After similar pretreatment with light, sodium dodecyl sulfate polyacrylamide gel electrophoresis reveals lower mobility and solubility of constituent D1 and D2 polypeptides of the PSII reaction center. Some degradation of these polypeptides also occurs. In contrast, there is no change in the mobility of the two subunits of cytochrome b_{559}. In the absence of illumination, the PSII reaction center exchanged into dodecyl maltoside shows good thermal stability as compared with samples in Triton X-100. Only at a temperature of about 60 °C do spectral changes take place that are indicative of denaturation.

Crystallization and X-ray analyses of reaction centers from the purple photosynthetic bacteria *Rhodopseudomonas viridis* (Deisenhofer et al., 1984, 1985) and *Rhodobacter sphaeroides* (Allen et al., 1987a,b) have given detailed information about the organization of protein subunits and pigment molecules at atomic resolution. The core of the bacterial reaction center is made up of the L/M heterodimer. Both the L and M subunits possess a high level of α-helical secondary structure due to five transmembrane helices and some nontransmembrane helical regions. A third subunit, H, contains a single transmembrane helix. The remaining residues of the H subunit exist as predominantly parallel or antiparallel β-sheets.

Because of the sequence homologies between the bacterial reaction center L/M subunits and the higher plant D1/D2 polypeptides, it was proposed that the latter also form a heterodimer and constitute the reaction center of photosystem II (PSII)[1] (Trebst, 1986; Michel & Deisenhofer, 1986). This notion was supported by the isolation of a complex consisting of the D1 and D2 polypeptides but free of other chloro-

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[1] Abbreviations: FTIR, Fourier transform infrared; Hepes, *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid; PSII, photosystem II; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; Tris, tris(hydroxymethyl)aminomethane.

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