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Myosin Subfragment 1 and Structural Elements of G-Actin: Effects of S-1(A2) on Sequences 39–52 and 61–69 in Subdomain 2 of G-Actin†

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ABSTRACT: The effect of myosin on the structure of two sequences on G-actin, a loop between residues 39 and 52 and a segment between residues 61 and 69 from the NH_2-terminus, was probed by limited proteolytic digestions of G-actin in the presence of the myosin subfragment 1 isozyme S-1(A2). Under the experimental conditions of this work, no polymerization of actin was induced by S-1(A2) [Chen & Reisler (1991) *Biochemistry 30*, 4546–4552]. S-1(A2) did not change the rates of subtilisin and chymotryptic digestion of G-actin at loop 39–52. In contrast to this, the second protease-sensitive region on G-actin, segment 61–69, was protected strongly by S-1(A2) from tryptic cleavage. The minor if any involvement of loop 39–52 in S-1 binding was confirmed by determining the binding constants of S-1(A2) for pyrene-labeled G-actin (1.2×10^6 M^{-1}), subtilisin-cleaved pyrenyl G-actin (0.3×10^6 M^{-1}), and DNase I–pyrenyl G-actin complexes (0.3×10^6 M^{-1}). Consistent with this, the activity of DNase I, which binds to actin loop 39–52 [Kabsch et al. (1990) *Nature 347*, 37–44], was inhibited almost equally well by actin in the presence and absence of S-1(A2). These results confirm the observation that DNase I and S-1(A2) bind to distinct sites on actin [Bettache et al. (1990) *Biochemistry 29*, 9085–9091] and demonstrate myosin-induced changes in segment 61–69 of G-actin.

An intriguing feature of actin is its ability to interact with a large and ever-increasing number of proteins. In muscles, actin is organized into thin filaments within the myofibrils. The adenosine 5′-triphosphate (ATP)[1]-dependent interaction between filaments of actin and myosin is the basis of muscle contraction and any actomyosin-based cell motility. In non-muscle cells, actin is present in polymerized and depolymerized forms, and the equilibrium between these states is regulated

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[1] Abbreviations: ATP, adenosine 5′-triphosphate; DTT, dithiothreitol; FITC, fluorescein 5-isothiocyanate; MBS, *O*-(*m*-maleimidobenzoyl)-*N*-hydroxysuccinimide ester; PFPITC, pentafluorophenyl isothiocyanate; PMSF, phenylmethanesulfonyl fluoride; pyrenyl actin, actin modified at Cys-374 with *N*-(1-pyrenyl)iodoacetamide; S-1, myosin subfragment 1; S-1(A2), S-1 isozyme with alkaline light chain 2; SDS, sodium dodecyl sulfate.

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by a host of actin binding proteins. These proteins can be classified according to their mode of action on actin into groups of nucleating, elongating, bundling, cross-linking, severing, etc. agents [for recent reviews, see Pollard (1990), Vandekerchove (1990), and Stossel (1989)].

Recently, an atomic structure of G-actin complexed to DNase I has been determined (Kabsch et al., 1990). This important achievement enables now the examination of the three-dimensional relationships between divalent cation, nucleotide, and protein binding sites on actin. The so-called "large" and "small" domains of actin have turned out to be similar in mass, each one consisting of two subdomains. The domains are separated by a cleft in which divalent cations and adenine nucleotides bind. The major DNase I binding sites on actin are located between residues 36 and 69 in subdomain 2 with a couple of contacts in subdomain 4. Thus, DNase I binds to the "top" of the G-actin molecule, bridging over the two sides of the cleft (Kabsch et al., 1990). It appears from the atomic model of F-actin structure (Holmes et al., 1990) that DNase I inhibits actin polymerization by blocking inter-actin interactions (Holmes et al., 1990; Tellam et al., 1989).

Several lines of evidence implicated sequences 1–28, 40–113 (particularly 92–100), and 360–363 on actin in the interaction with myosin (Sutoh, 1982, 1983; Mejean et al., 1986, 1987; Bertrand et al., 1988; DasGupta & Reisler, 1989, 1991; Labbe et al., 1990). Thus, the presently known myosin binding sites on actin, all of them located in subdomain 1, are distinct from the DNase I site on G-actin. Such a conclusion is supported by the recent work of Bettache et al. (1990). These authors employed an intramolecularly cross-linked, nonpolymerizable actin derivative, MBS–G-actin, to generate a stable acto–DNase I–S-1 ternary complex. Yet, on the other hand, earlier studies suggested that DNase I and myosin competed for the binding to actin. For example, the depolymerization of F-actin by DNase I is inhibited by S-1 (Hitchcock et al., 1976; Mannherz et al., 1975; Chen, unpublished observations). Also, FITC modification of Lys-61 on actin, a residue which forms a direct contact with Glu-69 on DNase I, has been shown to affect acto–S-1 binding (Miki et al., 1987). In the same vein, a smaller but similar probe, PEPITC, attached to Lys-61, was strongly perturbed by the binding of S-1 to actin (Barden & Phillip, 1990). Finally, subtilisin cleavage of G-actin (between Met-47 and Gly-48) impairs both the binding of S-1 to F-actin and the S-1-induced polymerization of actin (Schwyter et al., 1989).

In this work, we explored the question of whether the S-1 binding sites or the S-1 sphere of influence on actin extends to the 39–69 sequence by employing proteolytic enzymes and DNase I as markers. Myosin isozyme S-1(A2) was used to form a stable complex with native, un-cross-linked G-actin which did not polymerize under our experimental conditions (Chaussepied & Kasprzak, 1989; Chen & Reisler, 1990, 1991). The results presented here show that S-1 induces specific conformational changes in segment 61–69 of G-actin and that DNase I and S-1 bind to distinct sites on G-actin.

Materials and Methods

Reagents. TLCK-treated α-chymotrypsin, trypsin, subtilisin, soybean trypsin inhibitor, PMSF, bovine pancreatic DNase I, and salmon sperm DNA were purchased from Sigma Chemical Co. (St. Louis, MO). Leupeptin was purchased from Boehringer Mannheim. *N*-(1-Pyrenyl)iodoacetamide was obtained from Molecular Probes. DE52 and CM52 ion-exchange resins were purchased from Whatman. Sephacryl S-200 was from Pharmacia (Piscataway, NJ). All other reagents used in experiments were of analytical grade.

Proteins. Myosin and actin from rabbit psoas muscle were prepared according to Godfrey and Harrington (1970) and Spudich and Watt (1971), respectively. S-1 was prepared by digesting myosin with α-chymotrypsin as described by Weeds and Pope (1977). The S-1 isozyme, S-1(A2), was isolated and purified as described before (Chen & Reisler, 1991). Protein purity was examined by SDS–PAGE. Pyrene modification of Cys-374 of actin was carried out according to Cooper et al. (1983) and as described in a previous paper (Chen & Reisler, 1991); 25–30 mg of G-actin, modified or unmodified, was purified further by passing it through a Sephacryl S-200 column (1.5 × 40 cm) (Maclean-Fletcher & Pollard, 1980). Fractionated G-actin was stored in actin depolymerizing solution (5 mM Tris, 0.5 mM DTT, 0.2 mM $CaCl_2$, and 0.2 mM ATP) at 0 °C. Immediately before each experiment, S-1(A2) was transferred into the actin depolymerizing solution free of ATP by passing it through a Sephadex G-50-80 Penefsky column (Penefsky, 1977). Such S-1(A2) was stored at 0 °C and used within 8 h. DNase I was dissolved in the actin depolymerizing solution with 1 mM PMSF and 20 μg/mL leupeptin added as protease inhibitors at a stock concentration of 100 μM and then dialyzed overnight against the same G-actin buffer (Burtnick & Chan, 1980). Subtilisin-cleaved actin was obtained by digesting G-actin (4.0 mg/mL) with subtilisin (0.004 mg/mL) for 30 min at 22 °C (Schwyter et al., 1989).

Protein Concentrations. Protein concentrations were determined by using the following extinction coefficients: S-1, $E^{1\%}_{280nm} = 7.5$ cm^{-1}; actin, $E^{1\%}_{280nm} = 11$ cm^{-1}; DNase I, $E^{1\%}_{280nm} = 12$ cm^{-1}. Pyrenyl G-actin concentration was determined by a Bio-Rad protein assay (Bradford, 1976). The extent of actin labeling was determined by using a molar extinction coefficient $E_{344nm} = 22000$ M^{-1} cm^{-1}.

Proteolytic Digestions of G-Actin. Digestions of G-actin (5.0 or 10 μM) were carried out with subtilisin (0.002 mg/mL), chymotrypsin (0.1 mg/mL), and trypsin (0.05 mg/mL). Subtilisin and chymotrypsin digestions were stopped with PMSF (final concentration = 1.0 mM); tryptic digestions were stopped by 0.1 mg/mL soybean trypsin inhibitor. All digestions were done in the actin depolymerizing solution at 22 °C. Digestions of G-actin in the presence of S-1(A2) were carried out at 5.0 μM concentrations of S-1(A2) and G-actin. These low concentrations and short digestion times (up to 4 min) were chosen to avoid any polymerization of actin by S-1(A2) (Chen & Reisler, 1991; Valentin-Ranc et al., 1991).

Fluorescence Measurements. Pyrene fluorescence measurements were carried out in a Spex Fluorolog spectrophotometer (Spex Industries, Inc., Edison, NJ) at 25 °C. The excitation wavelength was 368 nm, and the emission was monitored at 408 nm.

SDS–Polyacrylamide Gel Electrophoresis. Gel electrophoresis was carried out according to the procedure of Laemmli (1970) using a 12.5% gel or a two-phase gel of 10% (upper) and 15% (lower) acrylamide (w/w). The gels were stained with Coomassie brilliant blue, and the optical densities of protein bands were determined with a Zeineh soft laser scanning densitometer Model SLR-1D/2D (Biomed Instruments, Inc., Fullerton, CA).

DNase I Assays. The standard hydrolytic activity of DNase I was tested at 22 °C according to Blikstad et al. (1978). In short, 10 μL of DNase I was added to 1 mL of DNA (40 μg/mL) to a final concentration of 10.8 nM or as specified. In the assays of DNase I inhibition by G-actin, actin either was first incubated with DNase I for 15 min or was added directly to the DNA sample before the addition of DNase I.

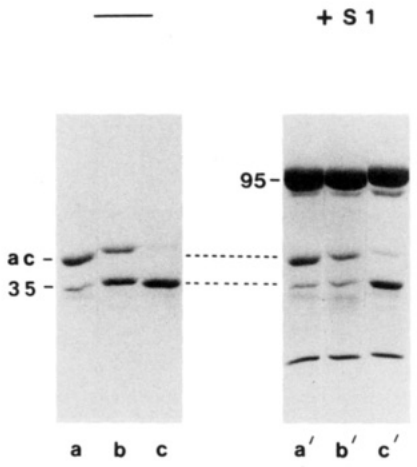


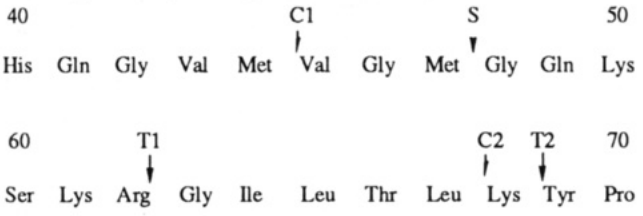
FIGURE 1: SDS–PAGE showing the time course of subtilisin digestion of G-actin in the presence and absence of S-1(A2). G-Actin (5.0 μM) was digested by subtilisin (0.002 mg/mL) in the presence and absence of S-1(A2) (5.0 μM) in the actin depolymerizing solution (5.0 mM Tris, 0.2 mM DTT, 0.2 mM $CaCl_2$, and 0.2 mM ATP, pH 7.6) at 22 °C. ac, 35, and 95 symbols denote the intact actin, the 35-kDa actin fragment, and S-1 heavy-chain bands, respectively. Digestion times in minutes were 0 (lanes a, a′), 1.5 (lanes b, b′), and 3.0 (lanes c, c′). The rates of cleavage at the S site in the presence and absence of S-1(A2) were obtained from semilogrithmic plots of the relative intensities of the 42-kDa bands versus digestion times (similar to those shown in Figure 2) and were 0.50 and 0.54 min^{-1}, respectively.

The hyperchromicity of DNA was monitored at 260 nm in an HP 8452A diode array spectrophotometer.

Binding Experiments. The binding of S-1(A2) to intact and subtilisin-cleaved G-actin was carried out in depolymerizing solution at 25 °C with protein concentrations set at 10 μM for pyrenyl G-actin (80–90% modified) and between 0.5 and 20 μM for S-1(A2). The binding was measured by monitoring the increase in pyrene fluorescence upon the addition of S-1-(A2). In binding experiments carried out in the presence of DNase I, the concentration of pyrenyl G-actin (80–90% modified) was fixed at 3 or 5 μM, S-1(A2) concentration ranged between 0.2 and 30 μM, and DNase I concentration varied between 0 and 50 μM.

Results

The segments spanning residues 39–52 and 61–69 in subdomain 2 of G-actin (Kabsch et al., 1990) are known to be highly susceptible to various proteases (Mornet & Ue, 1984). Three proteases, subtilisin, α-chymotrypsin, and trypsin, with well-defined cleavage sites in that region of actin (Jacobson & Rosenbusch, 1976; Mornet & Ue, 1984; Konno, 1987; Schwyter et al., 1989), were selected for probing the effects of S-1(A2) binding on the structure of G-actin. The sites of proteolytic cleavage, C, S, and T, for chymotrypsin, subtilisin, and trypsin, respectively, are shown:



Proteolytic Digestion of G-Actin by Subtilisin. As reported before (Schwyter et al., 1989), subtilisin cleaves G-actin between Met-47 and Gly-48, generating the 35-kDa and the N-terminal 10-kDa fragments (Figure 1; the smaller fragment migrated out of the SDS gels). Figure 1 shows that S-1(A2) does not change the pattern of G-actin digestion by subtilisin. Thus, the rates of subtilisin cleavage of G-actin in the presence and absence of S-1(A2) could be determined by monitoring the disappearance of the 42-kDa actin band on SDS gels (due to the digestion reaction) in semilogarithmic plots similar to those shown in Figure 2B. Such an analysis confirmed the visual impression from Figure 1 that the presence of S-1(A2) did not change significantly the S site on G-actin. In this (Figure 1) and other experiments, the rates (slopes) of subtilisin cleavage of G-actin were very similar in the presence (0.50 min^{-1}) and absence (0.54 min^{-1}) of S-1(A2). The lack of S-1(A2)-induced changes at the S site could not be attributed to the lack of acto–S-1 binding or to proteolytic damage of S-1. As determined by pyrene fluorescence measurements (Chen & Reisler, 1991), about 70% (or more) of actin and S-1 were bound to each other at the protein concentrations employed in this study. Also, under present conditions, S-1(A2) was not cleaved by subtilisin (Figure 1).

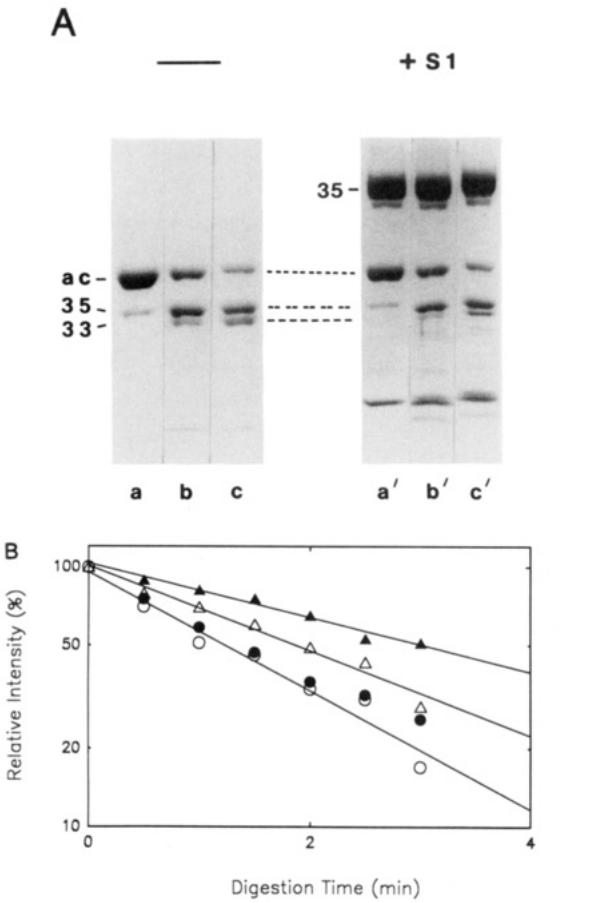


FIGURE 2: Chymotryptic digestion of G-actin in the presence and absence of S-1(A2). The conditions of digestion reactions were the same as in the legend to Figure 1 except that the chymotrypsin concentration was 0.1 mg/mL. (A) SDS–PAGE showing the digestion pattern of G-actin in the presence and absence of S-1(A2). The symbols ac, 35, 33, and 95 denote intact actin, its 35- and 33-kDa fragments, and S-1 heavy-chain bands, respectively. The digestion time points are 0 (a, a′), 1.5 (b, b′), and 3.0 (c, c′) min. (B) Time course of the disappearance of the intact actin band (○, ●) or that of the total actin, i.e., intact actin and the 35-kDa fragment (△, ▲). The decay of the intact actin band measures the rate of chymotryptic cleavage at the C1 site (Met-44/Val-45), whereas the decay of total actin measures the rate of chymotryptic cleavage at the C2 site (Leu-67/Lys-68). (●, ▲) Digestions in the presence of S-1(A2); (○, △) digestions in the absence of S-1(A2). The cleavage rates at C1 site were 0.50 and 0.54 min^{-1} in the presence and absence of S-1(A2), respectively, and those at the C2 site were 0.20 and 0.34 min^{-1}, respectively.

Chymotryptic Digestion of G-Actin. Chymotrypsin cleaves G-actin mainly at two sites, C1 and C2, between Met-44 and Val-45 and between Leu-67 and Lys-68, respectively (Jacobson

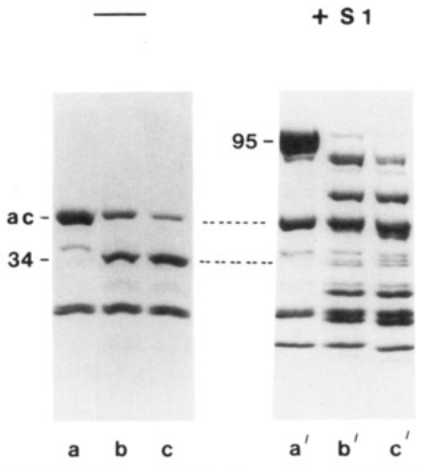


FIGURE 3: SDS–PAGE showing the time course of tryptic digestion of G-actin in the presence and absence of S-1(A2). Digestion conditions were the same as above except that the trypsin concentration was 0.05 mg/mL. The digestions were stopped with 0.1 mg/mL soybean trypsin inhibitor. The digestion times were 0 (a, a′), 1.5 (b, b′), and 3.0 (c, c′) min digestions. The symbols ac, 34, and 95 represent intact actin, its 34-kDa fragment, and S-1 bands, respectively. The band of approximately 22 kDa in molecular mass visible in all lanes corresponds to trypsin inhibitor. The rates of cleavage of T1 site were 0.55 min^{-1} in the absence of S-1(A2) and 0 in the presence of S-1(A2).

& Rosenbusch, 1976; Konno, 1987). Konno (1987) showed that the two cleavages occurred sequentially. The first chymotryptic attack at the C1 site generates a 35-kDa fragment which is further cleaved at the C2 site to form the 33-kDa fragment (Figure 2A). The chymotryptic 35–10-kDa actin, though normally contaminated by the intact G-actin, showed polymerization and S-1 binding properties similar to these of the subtilisin 35–10-kDa G-actin (Konno, 1987; Schwyter et al., 1989). However, the two-site (C1 and C2) cleavage of G-actin to the 33–10-kDa product impaired almost all the essential properties of actin with the exception of nucleotide binding (Jacobson & Rosenbusch, 1976; Konno, 1987). Chymotryptic digestion of G-actin in the presence of S-1(A2) produced the same 35- and 33-kDa fragments as those generated in the absence of S-1(A2) (Figure 2A). No chymotryptic cleavage of S-1(A2) was observed under the employed reaction conditions.

The rate of chymotryptic cleavage at the C1 site was determined by following the decay of the 42-kDa band with time. The rate of cleavage at the C2 site was obtained from the decay of the sum of the 42- and 35-kDa bands which can be easily shown to yield the rate of formation of the 33-kDa fragment. Figure 2B shows that S-1(A2) did not affect significantly the cleavage rate at the C1 site but slowed the reaction rate at the C2 site by 40%. The protection of the C2 site by S-1(A2) warranted the examination of the tryptic digestion of G-actin in the presence of S-1(A2).

Tryptic Digestion of G-Actin. Trypsin cleaves G-actin exclusively in the segment spanning residues 61–69 in subdomain 2. Two successive cleavages in this region were first reported by Jacobson and Rosenbusch (1976). The first cleavage takes place between Arg-62 and Gly-63 (T1), and the second occurs between Lys-68 and Tyr-69 (T2) (see the cleavage scheme). In most cases, SDS gels revealed only a single 34-kDa fragment (Figure 3), which sometimes converted into a doublet at the end of the digestion. The properties of the tryptic 34–10-kDa G-actin were shown by the above authors to be similar to those of the chymotryptic 33–10-kDa actin; i.e., it could not be polymerized by salt or S-1 and did not bind calcium. As shown in Figure 3, both T1 and T2 sites were completely protected from tryptic attack in the presence of S-1(A2). Although the 95-kDa heavy chain of S-1 is cleaved in this case, the lack of cleavage on G-actin cannot be attributed to a selective trypsin preference for S-1 and a consequent decrease in the trypsin:actin ratio for the following reasons: (1) The digestion of the 95-kDa S-1 heavy chain occurred very fast, with the 95-kDa band disappearing almost completely by the first minute. Subsequently, the composition of the generated S-1 fragments (75, 50, 25, and 20 kDa) and their relative mass distribution remained relatively unchanged (less than 20% loss in individual bands even after 4 min of digestion). Inspite of almost no further cleavage of S-1 after the first minute, the 42-kDa band of actin remained intact and was only slowly converted into a 41-kDa band at later time points (lane c′ in Figure 3). This latter conversion is most likely due to tryptic cleavage at the C-terminus of actin (Mornet & Ue, 1984). (2) In the presence of MgATP, when S-1 and G-actin were dissociated, both actin and S-1 heavy chain were cleaved by trypsin (data not shown).

It is interesting to note that very little digestion of actin occurred even after the 95-kDa heavy chain of S-1 was converted into its fragments. This suggests that intact and cleaved S-1(A2) protect segment 61–69 of G-actin from trypsin.

Taken together, the results of digestion experiments suggest that S-1(A2) protects segment 61–69 but not loop 39–52 of G-actin from proteolytic cleavages. The difference in the extent of actin protection by S-1 noted in the chymotryptic and tryptic digestions of segment 61–69 may be ascribed, at least partially, to the weaker binding of S-1 to the 35–10-kDa G-actin generated in the C1 cleavage than to intact actin (see below).

Binding of S-1(A2) to Subtilisin-Cleaved (Met-47/Gly-48) G-Actin. Schwyter et al. (1989) showed that subtilisin cleavage of G-actin inhibited its polymerization and weakened the binding of DNase I. In addition, they found that F-actin formed from such actin had reduced affinity for S-1. However, it was not clear from that study whether the last change was due to an altered F-actin structure or to changes at the S-1 binding site of actin. To address this question, the binding of subtilisin-cleaved pyrenyl G-actin to S-1(A2) was measured in this work by monitoring the fluorescence enhancement of the pyrene probe on actin (Miller et al., 1988; DasGupta et al., 1990). Scatchard plots of these data (not shown) yielded the association constant of intact pyrenyl G-actin to S-1(A2), $K_a = 1.2 \times 10^6$ M^{-1}, which agreed well with other K_a values reported for modified forms of G-actin (Bettache et al., 1990; Chaussepied & Kasprzak, 1989). A somewhat higher binding constant of S-1 for G-actin ($K > 10^7$ M^{-1}) in the absence of free ATP was reported recently by Valentin-Ranc et al. (1991). The binding constant of subtilisin-cleaved pyrenyl G-actin to S-1(A2) was reduced 3–4-fold relative to intact actin, to about 0.3×10^6 M^{-1}.

Binding of S-1(A2) to G-Actin in the Presence of DNase I. The binding of S-1(A2) to pyrenyl G-actin in the presence of DNase I was measured by fluorescence titrations of actin with S-1(A2) at different fixed levels of DNase I (Figure 4). The binding of S-1(A2) to G-actin was reduced slightly but reproducibly in the presence of DNase I. The K_{app} values were plotted against 1/[DNase I], yielding a binding constant of 0.3×10^6 M^{-1} for S-1(A2) and G-actin saturated with DNase I (Figure 4, inset). These data suggested that the binding of S-1(A2) and DNase I to G-actin appeared to be only slightly competitive. In order to estimate the binding of the actin–DNase I complex in the presence of S-1(A2), the inhibition of DNase I activity by G-actin was monitored in parallel experiments. At estimated 50% saturation of actin by S-1,

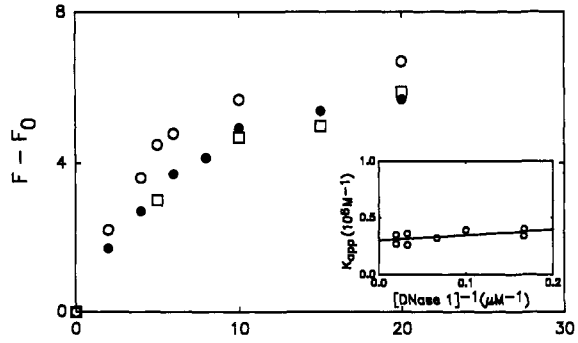


FIGURE 4: Binding of S-1(A2) to pyrenyl G-actin in the presence and absence of DNase I. The binding measurements were carried out with 5.0 μM actin, between 0 and 30 μM S-1(A2), and between 0 and 50 μM DNase I. Fluorescence enhancement of pyrenyl G-actin as a function of S-1(A2) concentration: (○) in the absence of DNase I; (●) in the presence of 10 μM DNase I; (□) with 50 μM DNase I. F_0 and F are the fluorescence intensities of pyrenyl G-actin prior and after the addition of S-1(A2), respectively. Inset: Apparent association constants of S-1(A2) to G-actin at various DNase I concentrations were plotted versus reciprocal DNase I concentrations. The intercept represents the true association constant of S-1(A2) for G-actin at infinite DNase I concentration.

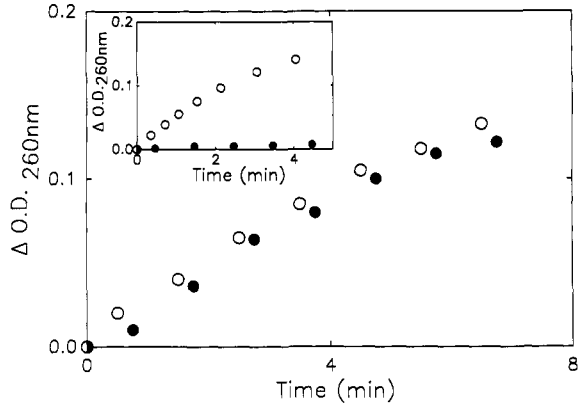


FIGURE 5: Inhibition of DNase I activity by G-actin in the presence of S-1(A2). DNase I binding to G-actin in the absence (●) and presence (○) of S-1(A2) was monitored by measuring the DNase I activities using conditions similar to those used in the S-1 binding experiments. The protein concentrations were 1.7 μM for G-actin and DNase I and 4.0 μM for S-1(A2). Inset: Standard DNase I assays carried out as described under Materials and Methods. (○) DNase I activity in the absence and (●) in the presence of actin.

the activity of DNase I was only slightly higher than in the absence of S-1 (Figure 5). Thus, the binding of DNase I to G-actin appeared to be also not affected significantly by S-1(A2).

Discussion

The main objective of this work was to test for a possible interaction between myosin and subdomain 2 of actin by probing the effects of S-1 on selected structural elements of G-actin. Until recently, such experiments were not feasible because of a complication inherent to these proteins: the rapid polymerization of G-actin by S-1 (Cooke & Morales, 1971; Yazawa & Yagi, 1973; Detmers et al., 1981; Miller et al., 1988). By using the myosin S-1 isozyme S-1(A2), which nucleates slowly actin polymerization (Chaussepied & Kasprzak, 1989; Chen & Reisler, 1991), it is now possible to test the effects of S-1 binding on the structure of G-actin.

The first conclusion of this study is that S-1 protects segment 61–69 but not loop 39–52 of G-actin from proteolytic cleavages. A similar protection of region 61–69 of actin from proteolysis was also observed for acto–DNase I complexes (Burtnick & Chan, 1980). In tryptic digestions, the cleavage of actin was almost completely blocked in the presence of S-1(A2). The lesser protection afforded by S-1(A2) to the C2 site in chymotryptic digestions of the same segment may be attributed to either one or both of the following reasons: (1) cleavage at the C2 site occurs only after cleavage at the C1 site, which as shown by subtilisin-cleaved G-actin reduces the affinity of actin for S-1. (2) The C2 site is separated by six residues from the T1 site and, thus, may show different sensitivity to S-1 binding.

The protection of segment 61–69 from proteolysis suggests either direct or indirect interaction or communication between S-1 and this part of actin. In this regard, it is interesting to note that Konno (1987) has reported that the chymotryptic 33–10-kDa form of actin (i.e., cleaved at the C1 and C2 sites) could not bind S-1. In addition to this, and as mentioned before, spectroscopic studies on Lys-61-labeled actin have implicated this residue in the interactions with S-1 (Miki et al., 1987; Barden & Phillip 1990). Taken together, these results place the 61–69 region of actin at the crossroad between the DNase I binding site and the S-1 interaction domain. This conclusion accounts at least in part for the observed reduction in the rate of DNase I induced depolymerization of actin by S-1 (Hitchcock et al., 1976; Mannherz et al., 1975; Chen and Reisler, unpublished results).

Loop 39–52, located less than 20 residues away from the 61–69 region of actin, was also protected by DNase I from proteolytic cleavages (Burtnick & Chan, 1980). The crystal structure of the acto–DNase I complex showed that indeed both regions of actin contained contact sites with DNase I (Kabsch et al., 1990). However, in contrast to DNase I and the effects of S-1 on segment 61–69, S-1(A2) had no effect on loop 39–52 of G-actin both in subtilisin and in chymotryptic digestions. Nevertheless, the binding of S-1(A2) to G-actin was reduced somewhat by the cleavage in that region. The relatively small change in acto–S-1 binding may be caused by a destabilizing effect of cleavage in loop 39–52 on vicinal regions and/or the loss of communication along the protein backbone to induce the "perfect fit" for S-1 binding.

The second conclusion of this work is that S-1 and DNase I have little effect on the binding of each other to G-actin (Figures 4 and 5). S-1 and DNase I appear to bind to distinct sites on G-actin with little overlap between the two proteins. Yet, as mentioned above, segment 61–69 on G-actin is impacted by both S-1(A2) and DNase I. In the course of this work, Bettache et al. (1990) reported the formation of a ternary complex of G-acto–DNase I–S-1 by using an intramolecularly cross-linked G-actin (MBS–G-actin). Since that modification affected several lysine residues and altered some of the intrinsic properties of actin, it is important that similar ternary complexes of G-acto–DNase I–S-1 were formed in this work with intact, unmodified actin. Clearly, the results obtained with DNase I and proteolytic probes of G-actin suggest that the S-1 site on actin does not extend to loop 39–52 but that S-1 affects or communicates with the adjacent segment 61–69.

In previous work, we have shown that the G-acto–S-1(A2) complexes exhibited pre-"F"-actin-state characteristics (Chen & Reisler, 1991); i.e., they could be polymerized more readily than free G-actin. In this regard, it is interesting to note the S-1(A2)-induced change in G-actin detected here affects residues 61–69 which are located at or close to the mouth of the cleft, between the "large" and "small" domains of G-actin. It is known that the G- to F-actin transition in actin involves a large change in the exchange rates of nucleotides trapped in the cleft (Mannherz et al., 1980). Such a change could

result, for example, from polymerization-related closing or blocking of the cleft. Although S-1(A2) did not polymerize G-actin, its effect on segment 61–69 was consistent with the onset of the pre-F-actin state. Further work on S-1(A2) and S-1(A1)-induced changes in G-actin (now in progress) should provide more insight into the mechanism of S-1-induced polymerization of actin.

Registry No. DNase I, 9003-98-9.

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