Evidence for Functional Homology in the F-actin Binding Domains of Gelsolin and α-Actinin: Implications for the Requirements of Severing and Capping

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Abstract. The F-actin binding domains of gelsolin and α-actinin compete for the same site on actin filaments with similar binding affinities. Both contain tandem repeats of ~125 amino acids, the first of which is shown to contain the actin-binding site. We have replaced the F-actin binding domain in the NH2-terminal half of gelsolin by that of α-actinin. The hybrid severs filaments almost as efficiently as does gelsolin or its NH2-terminal half, but unlike the latter, requires calcium ions. The hybrid binds two actin monomers and caps the barbed ends of filaments in the presence or absence of calcium. The cap produced by the hybrid binds with lower affinity than that of gelsolin and is not stable: It dissociates from filament ends with a half life of ~15 min. Although there is no extended sequence homology between these two different F-actin binding domains, our experiments show that they are functionally equivalent and provide new insights into the mechanism of microfilament severing.

A large number of actin-binding proteins, such as α-actinin, filamin, and fimbrin, are able to interact with filamentous but not monomeric actin (Matsudaira, 1991). By contrast, proteins like gelsolin or severin that sever filaments and cap their barbed ends, require both F- and G-actin binding sites for their activities (Yin, 1988; Bryan, 1988; Matsudaira and Janmey, 1988). Gelsolin contains three distinct actin-binding domains that are unevenly distributed within the sixfold segmental repeat (S1-6) in the amino acid sequence (Kwiatkowski et al., 1986, Way and Weeds, 1988). Two of these, S1 and S4-6, bind G-actin while the third, S2-3 binds strongly only to filaments (Yin et al., 1988; Bryan, 1988) and is responsible for targeting gelsolin to F-actin. S2-3 binds very weakly to G-actin and its high affinity for F-actin may depend on binding at the interface between adjacent subunits (Pope et al., 1991). Of the two constructs containing the F-actin binding site, S1-3 and S2-6, only the former severed filaments, showing that S1 is essential for severing activity (Way et al., 1989).

S1 is the smallest of the three domains, containing 150 residues in human plasma gelsolin (Kwiatkowski et al., 1986) and has the highest affinity for actin (Bryan, 1988). It has been suggested that the first 10 amino acid residues of S2-3 play a crucial role in severing, because COOH-terminal truncation of gelsolin to just 160 residues yields a protein with calcium-dependent severing activity (Kwiatkowski et al., 1989). However, viscometric analysis of severing by this protein expressed in E. coli (here termed N160) showed that its activity was quantitatively much weaker than that of gelsolin (Weeds et al., 1991). Furthermore, there was a time-dependent recovery of viscosity, which was not seen with gelsolin; this indicates that the severed filaments are not stably capped by N160 (Weeds et al., 1991). The weaker severing activity of N160 has been confirmed by Yu et al. (1991), who showed that additional features of S2-3 are required for F-actin binding, since deletion of residues 150-160 in S2-3 reduced but did not abolish its association with filaments. Elsewhere it has been shown that a COOH terminally deleted mutant of severin containing the first domain plus 26 residues of domain 2 (i.e., the homologous sequence to the first 10 residues of S2-3) retained severing activity that was absent in the first domain, but this activity was only 2% of that of intact severin (Eichinger et al., 1991). Thus, while this NH2-terminal part of S2 confers some severing activity on S1, its effect is weak and the exact location of the F-actin binding site in S2-3 has yet to be defined.

The position of the F-actin binding domain in α-actinin is better characterized (Blanchard et al., 1989). This domain is highly homologous to similar sized domains in other cross-linking proteins including dystrophin (Koenig et al., 1988), spectrin (Byers et al., 1989), 120 kD gelation factor from Dictyostelium discoideum (Noegel et al., 1989), fimbrin (de Arruda et al., 1990) and filamin (Hartwig and Kwiatkowski, 1991). All these domains are ~255 residues in length and each can be subdivided into two ~125-amino acid repeats (de Arruda et al., 1990) (see Fig. 1). Limited proteolysis of red cell β-spectrin showed that F-actin binding is associated with the first repeat (Karinch et al., 1990). More recently, the F-actin binding site of chick smooth mus-
cule α-actinin has been localized to residues 108-189 (Hemnings et al., 1992), and a 27-residue peptide from Dictyostelium 120k gelation factor, located at residues 108-134, was shown to bind actin (Bresnick et al., 1990; Bresnick et al., 1991). Thus, the actin-binding activity of these cross-linking proteins is associated with the COOH-terminal part of the first repeat.

In this paper we have investigated the properties and relationships of the F-actin binding domains of gelsolin and α-actinin. The F-actin binding site is localized in the first repeat of both proteins and they share a common binding site on actin. We further show that a hybrid containing S1 and the filament binding domain of α-actinin severs and caps actin filaments. Although there is no overall sequence relationship between the filament binding sites of gelsolin and α-actinin, these domains may therefore have a similar structure that recognizes a common binding site.

Materials and Methods

Construction of Expression Vectors

Gelsolin. The construction of S2-3 has been described previously (Pope et al., 1991). S2 was defined as amino acid residues 151-266 and S3 as residues 267-407 based on Way and Weeds (1988) (Fig. 1). S2 and S3 were engineered by PCR using the reverse S2 stop primer in conjunction with an upstream forward primer against pMW172.

Alpha-Actinin. The clone C17 containing the chick smooth muscle α-actinin gene was a kind gift of Dr. D. Critchley. An NcoI and HincII digest corresponding to the F-actin binding domain αA1-2, amino acid residues 1-269, was cloned into the NcoI and Stul sites of pMW172 (Fig. 1). An NcoI and EcoRV digest of C17 was used to generate the first repeat of αA1-2, residues 1-140 (αA1) based on the sequence alignments of deArruda et al. (1991). The second repeat, αA2 (residues 141-269), was generated by PCR using a forward primer to insert a BamHI site adjacent to residue 141 in conjunction with a reverse primer against pMW172. All gelsolin and alpha actinin PCR products were cloned into M13mpl9 and fully sequenced.

Construction of Expression Vectors

Expression and Purification of Mutants

All pMW172 constructs were expressed in high yield in the E. coli strain BL21 (DE3) and purified as described previously for S1 and dystrophin (Way et al., 1990, 1992a,b). All gelsolin constructs, αA1 and αA2, were isolated from inclusion bodies; hybrids were purified from the soluble fraction as was αA1-2, although this was also isolated in active form from inclusion bodies. Gel filtration on Sephacryl S200 confirmed that the proteins were monomeric and SDS-PAGE showed that all were >98% pure. Protein concentrations were calculated from A280 values using extinction coefficients for tryptophan and tyrosine (Gill and von Hippel, 1989). The micromolar concentrations corresponding to A280 = 1.0 cm⁻¹ are as follows: αA1-2 = 23.2; αA1 = 39.5; αA2 = 56.2; S2-3 = 36.4; S2 = 79.0; S3 = 65.7;
8.0, 0.2 mM ATP, 0.2 mM DTT, and either 0.2 mM CaCl₂ or 0.1 mM MgCl₂ and 0.2 mM EGTA and the control rate of depolymerization measured from the reduction in fluorescence. Severing proteins were added after ~60 s and rate constants for depolymerization determined by nonlinear least squares fitting of the exponential decrease in fluorescence (Way et al., 1992a). The F-actin concentration decreases according to the equation: $A(t) = A(0) e^{-kt}$, where $A(t)$ is the concentration of F-actin at time $t$, $A(0) = 200$ nM F-actin, $k_b$ is the off-rate constant for monomers from the barbed end and $k_c$ is the off-rate constant for monomers from the pointed end of filaments. In experiments with gelsolin, $N$ is assumed to be the gelsolin concentration and, since the barbed ends are capped, $k_c = 0$. Thus, a plot of $k_b$ as a function of gelsolin concentration gives a slope $= k_b/N_c$.

Capping activity was assessed from the inhibition of rate of actin polymerization. 6 μM G-actin containing 15% PI-actin in 10 mM Tris-HCl, pH 8.0, 0.2 mM ATP, 0.2 mM DTT, and 0.2 mM CaCl₂ was polymerized by addition of 1 mM MgCl₂, 50 mM NaCl, and 0.5 mM EGTA and the rate of polymerization measured from the fluorescence increase. Either N153/αA1-2 or ternary complexes of N153/αA1-2 with two actin monomers was added after ~200 s, when ~1 μM actin had polymerized and the fluorescence was rising linearly. The difference between the control polymerization rate and that in the presence of N153/αA1-2 ($V_o-V$) is a measure of the extent of capping at the barbed ends of growing filaments. Using the formulation of Selve and Wegner (1986) it is possible to estimate the dissociation constant $K_{sp}$ based on the equation: $V_o - V = \frac{[A]^2[B]^2K_a^2[C]^2}{([C]^2 + K_{sp})}$, where $[A]^4$, $[B]^4$, and $[C]^2$ are the total concentrations of actin monomers, barbed ends and capping protein, respectively. Since hybrids or complexes are added when the fluorescence has reached the same point in each experiment and the slopes are identical, $[A]^4$ and $[B]^4$ are the same for each value of $[C]^2$. Thus a plot of $(V_o - V)^2$ against $(V_o - V)/[C]^2$ gives a slope $= K_{sp}$.

Assays for Interaction with G-Actin

Complex formation was determined both from the percent inhibition of actin polymerization using PI-actin and from fluorescence enhancement of 100 nM NBD-actin (actin reacted with N-ethylmaleimide on cys 374 then on Lys 373 with 7-chloro-4-nitrobenzeno-2-oxa-1,3-diazole) in the presence of increasing concentrations of SI-2 or N153/αA1-2 as described previously (Way et al., 1990, 1992a). The polymerization inhibition experiments were carried out in the presence and absence of gelsolin as a nucleator. Assays in the absence of gelsolin, although slower to reach equilibrium, have the advantage of showing whether or not capping occurs, because of the effects of low concentrations of capping proteins on the actin critical concentration (Way et al., 1992a).

Results

Interaction of S2-3, αA1-2, and Individual Repeats with F-actin

All proteins were readily purified from E. coli as soluble monomeric species in milligram quantities (data not shown). Analysis of the binding properties of S2-3 and αA1-2 by cosedimentation with F-actin confirmed that both bound actin filaments in a calcium-independent manner (Fig. 2 A). Densitometry of SDS gels showed maximum binding at 1 mol per actin subunit and $K_c$ values, calculated from nonlinear least squares fitting or Scatchard analysis, were virtually identical for the two proteins (Fig. 2 B, Table I).

When supernatant fractions obtained after sedimentation of S2-3 or αA1-2 with actin were again added to 20 μM F-actin and repelleted, the majority of the protein was found in the pellet, confirming that unbound S2-3 or αA1-2 from the original assay was not denatured. Because S2-3 and αA1-2 are readily resolved by SDS-PAGE (Fig. 3 B), it is possible to quantitate the binding of each component in the presence of the other (Fig. 2 C). Such competition experiments showed that the two proteins compete for the same sites on filaments. The exact reciprocity of binding by S2-3 and αA1-2 confirms the similarity of $K_c$ values (Table I).
Analysis of the binding of individual repeats of both S2–3 and αAI–2 showed that only the first repeat bound F-actin (Fig. 2 A). S2 showed only marginally weaker binding than S2–3, but αAI gave a $K_v$ value an order of magnitude higher than that of αAI–2 (Table I). Thus the F-actin binding site of gelsolin appears to be exclusively contained within S2.

**Interactions of S1–2 with F- and G-Actin**

Viscometric assays showed that S1–2 severs actin filaments almost as efficiently as does gelsolin but, like S1–3, in a calcium-insensitive manner (Fig. 4 A). There was no time-dependent recovery in viscosity with S1–2 confirming the presence of a stable cap (not shown). Comparison of the slopes of the depolymerization rate constants as a function of S1–2 concentration (Fig. 5) shows that the monomer dissociation rate is 67% of that of gelsolin. Since severed filaments are stably capped at their barbed ends, this suggests that the severing activity is close to that of gelsolin.

Polymerization inhibition experiments showed that S1–2 binds two actin monomers with high affinity both in calcium (Fig. 6 A) and EGTA (data not shown). In the absence of gelsolin as a nucleator, the inhibition profile was similar to that shown for N153/αAI–2 in Fig. 6 A, extrapolating to give an apparent increase in unpolymerized actin of 0.68 μM at zero S1–2 concentration (data not shown). This is evidence for barbed end capping by S1–2, which causes a rise in critical concentration.

**Interactions of Hybrids with F- and G-Actin**

N153/αAI–2 reduced the specific viscosity of actin in a calcium-dependent manner similar to gelsolin (Fig. 4 A). The effects of N162/αAI–2 were much less marked. At molar ratios to actin subunits of 0.015 and 0.03, the specific viscosity was reduced to 0.28 and 0.25 compared to values of 0.17 and 0.13 for N153/αAI–2. In contrast to the effects of gelsolin or S1–2, there was a slow recovery of viscosity with time (Fig. 4 B). Semi-log plots showed that the half-times for recovery were 15 and 19 min for N153/αAI–2 and N162/αAI–2, respectively. Ternary complexes of N153/αAI–2 and actin had no effect on the viscosity of F-actin, but when filaments were sonicated in the presence of complexes, there was a marked fall in viscosity followed by the same slow recovery seen with the hybrid alone. By comparison, sonicated actin in the absence of complexes showed almost complete recovery of viscosity within 2 min.

Severing by N153/αAI–2 was also shown to be calcium-dependent in the depolymerization assay (Fig. 5 A). The severing activity was quantitated from the kinetics of depolymerization (Fig. 5 B). The slope of the plot was 2.68 μM$^{-1}$s$^{-1}$ compared to 0.923 μM$^{-1}$s$^{-1}$ for gelsolin. The higher dissociation rate of N153/αAI–2 shows that the severed filaments depolymerize from both ends, as expected if capping is not stable.

NBD-actin titrations in the presence or absence of calcium showed maximal fluorescence enhancement at a stoichiometry of 1.90 actin: N153/αAI–2 (Fig. 6 B) and the sharp transition indicated high affinity for both sites. Polymerization inhibition experiments gave a similar stoichiometry and showed evidence for barbed end capping from the high levels of inhibition of polymerization at very low concentrations of N153/αAI–2 (Fig. 6 A).
Figure 4. (A) Effects of gelsolin (●), S1–2 (●), and N153/αA1–2 (▲) on the viscosity of 15 μM F-actin in calcium (closed symbols) and EGTA (open symbols). (B) Time-dependent recovery of viscosity after mixing 14 μM F-actin with 0.21 μM N153/αA1–2 (▲) and 0.3 μM N162/αA1–2 (+). The initial viscosity is shown on the ordinate in the former case. The insert shows Ln (~150 – ~t) against time, where ~150 is the specific viscosity at 150 min and ~t the specific viscosity at the times shown on the abscissa.

Figure 5. (A) Time courses of decrease in fluorescence of PI-actin in the severing assay. Depolymerization initiated by dilution of F-actin to 200 nM in the absence of calcium. The unmarked arrow indicates addition of 10 nM N153/αA1–2. In the upper trace (open symbols) no further additions were made, but in the lower, 0.2 mM excess calcium was added, giving an exponential decrease in fluorescence with rate constant 0.0278/s. (B) Effects of gelsolin (●), S1–2 (●), and N153/αA1–2 (▲) on the rate constant of F-actin depolymerization in the severing assay in the presence of calcium.

Capping activity was measured directly from the inhibitory effects of N153/αA1–2 on actin polymerization in the absence of calcium (Fig. 7). Addition of 95 nM N153/αA1–2 inhibited the elongation rate by >80%. (Similar experiments in calcium showed a fivefold increase in polymerization rate compared to controls due to severing and the production of new filaments.) Quantitation is difficult because N153/αA1–2 may bind either to actin monomers or to filament ends. Assays were therefore carried out in which complexes containing N153/αA1–2 and two actin monomers were added to the polymerizing actin (Fig. 7). The extent of inhibition increased with concentration of complex added, giving a value for Kc of 0.47 nM.

Discussion

Interactions of αA1–2, S2–3, and SI–2 with Actin
We have previously proposed that the F-actin binding domains of gelsolin and α-actinin might share common structural features based on their similar sizes, tandem sequence repeats, and calcium independent F-actin binding properties, even though they have only limited local sequence similarities (Way et al., 1991). We therefore sought to test this hypothesis by examining whether the F-actin binding domain of α-actinin would functionally replace that of gelsolin through the construction of hybrids. However, before addressing this question it was necessary to characterize the actin binding properties of S2–3 and αA1–2 produced in E. coli. Kd values for αA1–2 of ~4 μM are similar to the ~3 μM value obtained for the related actin-binding domain of filamin (Gorlin et al., 1990). The binding of αA1–2 to filaments is weaker than that of α-actinin itself due to cross-linking by the intact molecule: chicken gizzard α-actinin gave half saturation of binding at 0.4 μM (Meyer and Aeberi, 1990).

The Kd values for S2–3 are consistent with observations of Bryan (1988) using the proteolytic fragment CT28N corresponding to S2–3. There was ~80% saturation of binding when 5 μM F-actin was mixed with 9.5 μM CT28N: this suggests a Kd of ~2 μM. By contrast, Yin et al. (1988) reported 1:1 stoichiometry and a Kd value of 0.2 μM for the same chymotryptic fragment, a value that cannot be reconciled with our data. The exact reciprocity in the actin binding profiles in competition sedimentation experiments shows that S2–3 and αA1–2 bind to the same or overlapping sites on the filament and confirm their similar binding affinities.

Analysis of the binding properties of the individual repeats of S2–3 and αA1–2 showed that S2 has only marginally weaker binding than S2–3, but αA1 binds an order of magnitude weaker than αA1–2 (Fig. 2 A and Table I). In both cases the second repeat shows no binding. Our results with αA1

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Figure 6. (A) Complex formation determined from the percent inhibition of actin polymerization with increasing concentrations of S1-2 (○) and N153/αAI-2 (▲). In the former case, gelsolin was present as a nucleator in all samples; thus, the percent inhibition (=[complex]) is compared to controls in which the critical concentration is elevated due to barbed end capping by gelsolin. In the latter case, no gelsolin was present, so the percent inhibition is compared to controls in which there is no barbed end capping. Extrapolation to zero concentration of measurements in the presence of N153/αAI-2 shows elevation of the percent inhibition demonstrating barbed end capping by N153/αAI-2. (B) Fluorescence enhancement of 100 nM NBD-actin in the presence of N153/αAI-2. Closed symbols calcium and open symbols EGTA.

confirm localization of the actin-binding site in the first repeat (Karinch et al., 1990; Bresnick et al., 1990, 1991). Although we cannot rule out the possibility that the reduced affinity of αAI compared to αAI-2 reflects incorrect folding after solubilization from inclusion bodies, αAI-2 purified from inclusion bodies showed similar affinity to αAI-2 from the soluble fraction. This suggests that refolding from urea is not the cause of weaker binding but that elements in αAI-2 may be required for high affinity association. In support of this, the actin binding site has been localized to amino acid residues 108–189 of α-actinin (Hemings et al., 1992) suggesting involvement of the NH₂-terminal part of αA2. We attempted to test this by expressing the first 191 of the 269 residues of αAI-2, but were unable to obtain soluble protein.

F-actin binding by S2 with $K_{d}$ similar to S2-3 unequivocally demonstrates that S2 alone contains the F-actin binding site. Furthermore, analysis of the severing activity by S1-2 demonstrates that segments 1 and 2 together are sufficient for efficient severing and stable capping (Figs. 4–6). Recent analysis of severin has localized an F-actin binding site to the second repeat of this protein, but unlike S2, this binding is calcium-dependent (Eichinger and Schleicher, 1992). A further distinction between severin and S2-3 is the presence of an additional site in the third repeat of severin.

Calcium-dependent Severing by S1-αActinin Hybrids

If αAI-2 is able to substitute for S2-3 in S1-3, it must retain the same spatial proximity to S1 as shown by S2-3. The splice junction was selected on the basis of the local sequence homology (Fig. 1 A). The two splice positions allow residues 151–160 of gelsolin to be either included (N162/αAI-2) or excluded (N153/αAI-2) in the hybrid. The other major difference is that N153/αAI-2 contains an additional pentapeptide (DWDRD), derived from α-actinin. These additional residues may account for the differences observed in the properties of the hybrids by providing additional interdomain flexibility.

Both hybrids severed actin filaments but were quantitatively different in their activities. N162/αAI-2 reduced the actin viscosity to a significantly smaller extent than N153/αAI-2; its severing activity was quantitatively similar to that of N160 (Weeds et al., 1991). This suggests that either the F-actin binding site of αAI-2 does not contribute to the severing by this hybrid or that deletion of the nine NH₂-terminal residues of αAI significantly diminishes filament binding. The latter is unlikely because the binding site is
elsewhere in the molecule (Hemnings et al., 1992). F-actin binding by this hybrid was demonstrated by sedimentation analysis in the absence of calcium (data not shown). It is probable therefore that the lack of efficient severing occurs because the actin binding domains in S1 and αAl-2 are not optimally oriented in the hybrid and act independently of each other. Because the severing behavior of N162/αAl-2 was little different from that of N160, no further work was done with this hybrid.

N153/αAl-2 reduces the viscosity of actin filaments in a calcium-sensitive manner comparable to that of gelsolin (Fig. 4). However, there was a slow time-dependent recovery of viscosity (t½ = 15 min), not seen with gelsolin, which suggests that the cap formed by N153/αAl-2 is not stable. Instability of capping was confirmed from the similar time dependence in the recovery of viscosity after sonicating F-actin in the presence of ternary complexes of N153/αAl-2 with actin. By contrast, the recovery of viscosity was virtually instantaneous after sonication in the absence of hybrid, consistent with observations of Murphy et al. (1988). The ternary complexes do not affect the actin viscosity in the absence of sonication, which shows that they do not sever filaments, nor is there detectable shearing of filaments in the viscometer.

Unstable Capping by SI-αActinin Hybrids

Direct evidence for capping was obtained from the inhibitory effects of N153/αAl-2 on the rate of actin polymerization in the absence of calcium (Fig. 7). Because N153/αAl-2 binds both G- and F-actin, capping activity was also measured using the ternary complex of N153/αAl-2 with two actin monomers. Even at concentrations as low as 2.5 nM, the complex inhibited the polymerization rate of 6 μM actin by ~70%. A value of 0.47 nM was estimated for Kcap (Fig. 7). This compares with values for the gelsolin complex with actin of <0.07 nM in the absence of calcium (Selvège and Wegner, 1986) and 20–40 nM for SI (Weber et al., 1991). In both cases, capping in calcium was tighter (for the gelsolin complex Kcap is <10 pM). Thus, the capping affinity of the actin complex of N153/αAl-2, although much greater than that of SI-actin, is much weaker than the cap produced by gelsolin.

Further evidence for the instability of the cap came from experiments to quantitate severing activity. N153/αAl-2 accelerates the rate of depolymerization of F-actin 2.9 times greater than does gelsolin (Fig. 5). Because gelsolin is assumed to be close to 100% efficient in its severing activity, the depolymerization rate in the presence of hybrid cannot exceed that with gelsolin unless the severed filaments depolymerize from both ends. However, if there was no capping, based on the off-rate constants for ADP-actin subunits of Pollard (1986), the depolymerization rate would be over 20 times that observed for capped filaments, which is over 6 times the rate observed with N153/αAl-2. Thus, because viscometric analysis indicates that the severing efficiency of N153/αAl-2 is close to that of gelsolin, the slow rate of depolymerization of filaments capped with N153/αAl-2 compared to that of uncapped filaments indicates that the off-rate of the cap is relatively slow.

The Severing Mechanism of Gelsolin and SI-αActinin Hybrid

Both SI-2 and SI-3 sever actin filaments in a calcium-insensitive manner. However, it has been shown that calcium increases the affinity of SI for actin ~1,000-fold and that calcium is trapped in the complex (Bryan, 1988; Way et al., 1990). Recent analysis of the X-ray structure of the actin/SI complex suggests that the calcium site contains structural elements of both proteins, i.e., calcium acts as a bridge at the interface. Furthermore, the location of SI in the complex is such that it would disrupt actin–actin interactions in the two-start actin helix (McLaughlin, P. J., unpublished work). Severing by SI-2 and SI-3 occurs because the F-actin binding site of S2 targets SI to its optimal position, where it binds with an affinity that is at least 100-fold greater than the actin–actin affinity, even in the absence of calcium. Hence, filaments are severed and their barbed ends irreversibly capped because a minimum of two adjacent subunits are tightly bound by the severing protein.

We suggest that the calcium requirement for severing by N153/αAl-2 occurs in the following manner: although targeting to F-actin occurs in the absence of calcium, the position or orientation of SI in the complex does not produce optimal binding, hence no severing occurs. Calcium considerably enhances the affinity of SI for actin by increasing the bonding at the interface, which thereby facilitates severing. However, the binding sites in SI and αAl-2 do not produce a capping affinity as high as that of SI-2 or SI-3. This means that the cap dissociates slowly even when calcium is present. Capping of barbed ends also occurs in the absence of calcium (Fig. 7). Differences in the calcium requirement for capping and severing have also been observed for villin (Northrop et al., 1986).

These results provide valuable insights into the mechanism by which gelsolin severs actin filaments. It is clear that the properties of SI in N153/αAl-2 are significantly modified by the presence of αAl-2. The F-actin binding domain of α-actinin targets SI to the filament and facilitates efficient severing, suggesting that the F-actin binding sites of gelsolin and α-actinin are functionally equivalent. Differences in severing activities between N153/αAl-2 and N162/αAl-2 show the importance of maintaining the correct spatial proximity and orientation of the filament and monomer binding sites. Although N153/αAl-2 shows severing activity comparable to SI-2 or SI-3, the SI moiety does not display its full binding potential in the hybrid: severing requires calcium and the stability of the cap is reduced. Efficient severing and capping require very precise orientation of both the F-actin binding domain and SI on the filament, but the latter is more important. This is currently being elucidated from crystals of the SI/actin complex.

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