Definition of the EGTA-independent interface involved in the serum gelsolin–actin complex

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The gelsolin–actin complex in the presence of Ca^{2+} revealed at least three interacting sites on the gelsolin molecule located in the S1, S2–3, and S4–6 domains. In the presence of EGTA, the N-terminal domain of gelsolin is known to be involved. However, the corresponding site on the surface of actin is poorly defined. The present result locates the Ca^{2+} -independent plasma gelsolin-binding site on the actin surface. Natural and synthetic actin peptides were tested for their possible interaction with gelsolin

and monitored by fluorescence anisotropy measurements and e.l.i.s.a. The interface was thus located within the 360–372 actin sequence near the C-terminal extremity. In addition, we used a chymotryptic digest of gelsolin and determined that its N-terminal domain (S1) was implicated in this interface. We conclude that the interaction of the 41–126 region of plasma gelsolin is the counterpart of the 360–372 sequence in subdomain 1 of actin.

INTRODUCTION

A great deal of studies have shown the importance of specific actin-binding proteins in the modulation of actin ultrastructure. Among these proteins, gelsolin, which is a member of a superfamily that also includes villin, fragmin and severin, is involved in the equilibrium between G- and F-actins (André et al., 1988). These proteins seem to have evolved from a 15000 Da repeating subdomain (Way and Weeds, 1988) and therefore have multifunctional activities.

The basic activities of gelsolin, which is present in vertebrate cytoplasm and extracellular fluid (Kwiatkowski et al., 1986), have been well documented. Indeed, gelsolin binds to the barbed end of actin filaments, severs them and nucleates actin filament growth (Yin and Stossel, 1980). All these activities are supported by at least three functional domains distributed along the six segment repeats in the gelsolin sequence (Bryan, 1988; Way et al., 1989). It has also been reported that the gelsolin molecule has regulatory sites specific to Ca^{2+} or phospholipids (PtdInsP_2 and PtdInsP) (Yin, 1988; Jamney and Stossel, 1987; Jamney et al., 1987) and possesses a high affinity site for nucleotide triphosphate (Yamamoto et al., 1990). Binding of G-actin to gelsolin thus requires the presence of Ca^{2+} in the medium. Upon Ca^{2+} interaction, gelsolin undergoes large conformational changes, as demonstrated in hydrodynamic, phosphorescence and immunological studies (Hwo and Bryan, 1986; Patkowski et al., 1990; Tellam, 1991). In particular, hydrodynamic parameters determined in laser-light-scattering studies showed a significant increase in the hydrodynamic volume of gelsolin leading to a more spherical form (Patkowski et al., 1990). The large structural changes would cause exposure of the actin-binding regions outside the molecule, thus allowing the interaction of two actins per gelsolin molecule.

Several investigations on the topology of the interacting domains have reported the specific role of gelsolin segments obtained either by limited proteolysis or by deletion mutants in *Escherichia coli* (Bryan, 1988; Kwiatkowski et al., 1989; Way et al., 1989). These studies have demonstrated the occurrence of

three actin/gelsolin interfaces, distributed along the gelsolin polypeptide chain, characterized by their Ca^{2+} -dependence.

The isolated N-terminal segment residues (1–150) (S-1) includes a high-affinity Ca^{2+} -independent actin site and is therefore implicated in the 1:1 actin–gelsolin complex which is not dissociated by EGTA (Bryan, 1988; Coué et al., 1986; Kwiatkowski et al., 1985). Occupation of the corresponding actin site by this gelsolin domain is corroborated by total inhibition of salt-induced actin polymerization (Way et al., 1989).

The central part of the gelsolin molecule (residues 151–406) (S2–3) contains a Ca^{2+} -sensitive site whose dependency is lost after cleavage and isolation of the sequence (Bryan, 1988).

Finally, a Ca^{2+} -dependent site in the C-terminal half of gelsolin (S4–6) interacts with an actin segment located within residues 305–326 (Bryan, 1988; Chaponnier et al., 1986; Houmeida et al., 1991).

Although these interfaces have been extensively studied, relatively little information is available on the actin sequence involved in the interaction of the N-terminal domain (S-1) of gelsolin. Cross-linking experiments showed the proximity between the N-terminal segment of gelsolin and the two (N- and C-) extremities of the actin polypeptidic chain (Sutoh and Yin, 1989; Doi et al., 1991), both located at the bottom of actin subdomain 1 (Kabsch et al., 1990).

In the present study, we obtained topological information on the structure of the Ca^{2+} -independent actin–gelsolin complex. We investigated the binding properties of several natural or synthetic fragments and determined, by two independent technical approaches (e.l.i.s.a. and fluorescence-anisotropy measurements), a limited region in the C-terminal part of actin that is required for interaction with the N-terminal domain of gelsolin.

MATERIALS AND METHODS

Proteins

Rabbit skeletal-muscle actin was purified from acetone-dried powder (Spudich and Watt, 1971). Actin was specifically labelled at Lys³⁷³ with 7-chloro-4-nitrobenzo-2-oxa-1,3-diazole (Nbd-Cl)

(Coué et al., 1986) or at Cys³⁷⁴ with *N*-iodoacetyl-*N*-(5-sulpho-1-naphthyl)ethylenediamine (1,5-I-AEDANS) (Takashi, 1979). To obtain the Nbd-Cl or dansylated fragments corresponding to positions 285–375 and 356–375, labelled actins were cleaved by 4-nitro-5-thiocyanobenzoic acid or CNBr respectively (Boyer et al., 1985; Lebart et al., 1990; Mejean et al., 1992). The 359–372 synthetic peptide, in which Lys³⁵⁹ was replaced with Cys residue, was obtained by a solid-phase synthesis using the 9-fluorenylmethoxycarbonyl/*t*-butyl system with a Milligen Pep-Synthetizer TM 9050 (Milligen Bioresearch Division, Watford, Herts., U.K.) and was subsequently labelled with 1,5-I-AEDANS (Houmeida et al., 1992).

Bovine plasma gelsolin was purified as described by Soua et al. (1985). Its digestion by chymotrypsin (gelsolin/chymotrypsin = 100:1, w/w) was performed for 90 min (Bryan, 1988; Houmeida et al., 1991). Purification of the major chymotryptic gelsolin fragments was performed as reported in a previous paper (Houmeida et al., 1991) using the method of Bryan (1988).

Scallop arginine kinase was prepared as previously described (Reddy et al., 1991). Thyroglobulin was purchased from Sigma Chemical Co. Antiserum specific for serum gelsolin was elicited in rabbits as described by Soua et al. (1985). Anti-IgG antibodies labelled with alkaline phosphatase were purchased from Biosys (Compiègne, France). Protein concentrations were determined by u.v. absorbance (Porte and Harricane, 1986; Labbé et al., 1992).

Fluorescence measurements

Fluorescence experiments were performed using a Perkin–Elmer Luminescence Spectrometer LS 50. Fluorescence enhancement experiments were carried out using Nbd-Cl-labelled peptides of actin in 10 mM Tris buffer, pH 7.6 (buffer A), supplemented with 4 mM CaCl₂ at 25 °C. The excitation and emission wavelengths were set at 465 nm and 535 nm respectively. By measuring fluorescence polarization of a chromophore label (dansyl) attached to small peptides derived from the actin sequence, we could detect changes in the hydrodynamic volume of the peptides occurring during the formation of peptide–gelsolin or peptide–gelsolin fragment complexes. The fluorescence polarization experiments were performed as previously described (Houmeida et al., 1992). Dansylated peptides were incubated in buffer A supplemented with 4 mM CaCl₂ at 25 °C, before the addition of increasing concentrations of gelsolin or gelsolin fragments. The excitation and emission wavelengths were set at 340 nm and 500 nm respectively. Polarization (*p*) was subsequently converted into anisotropy (*a*):

$$a = 2p/(3 - p)$$

Anisotropy was then used to describe ligand-binding equilibria.

E.I.s.a.

The interaction between gelsolin and actin fragments was studied by direct e.l.i.s.a. as previously described (Mejean et al., 1987, 1992). Briefly, actin peptides and fragments were coated at 5 µg/ml to the plastic of microtitration plate wells by incubation in 50 mM NaHCO₃/Na₂CO₃, pH 9.5, overnight at 4 °C. Unoccupied protein-binding sites were saturated in 140 mM NaCl/50 mM Tris buffer, pH 7.8, (buffer B) supplemented with 0.5% gelatin/3% gelatin hydrolysate and 2 mM CaCl₂. Gelsolin and antibody dilutions were also carried out in buffer B supplemented with 4 mM CaCl₂, gelatin and gelatin hydrolysate. Binding was revealed using alkaline-phosphatase-labelled anti-IgG diluted to 1:3000. After washing, a solution of *p*-nitrophenyl phosphate (1 mg/ml) was added and staining was read at 405 nm.

Non-specific absorption was determined for each sample under the same conditions using uncoated wells. Each assay was conducted in triplicate and the mean value was plotted after subtraction of non-specific absorption. The binding parameters were determined by non-linear fitting. Arginine kinase, which does not interact with the C-terminal sequence of actin (Reddy et al., 1992), was used as a negative reference for studying the 356–375-peptide–gelsolin complex formation.

In indirect e.l.i.s.a. experiments performed under similar conditions, and after coating of the actin peptide (at 5 µg/ml), a fixed concentration of gelsolin (usually 30 µg/ml) was reacted in the presence of increasing concentrations of actin to shift the equilibrium between gelsolin and the coated actin peptide.

The specific conditions are described in the Figure legends.

Affinity chromatography

Affinity chromatography with the 285–375 actin fragment was carried out as follows. The actin fragment was first coupled to CNBr-activated Sepharose 4B (Pharmacia). The coupled resin was equilibrated with buffer B supplemented with 4 mM CaCl₂. The chymotryptic digest of gelsolin was put on the column (1 cm × 3 cm) and, after washing in the same buffer, the retained material was successively eluted with buffer B supplemented with 10 mM EGTA and with 20 mM phosphate buffer, pH 12, supplemented with 10% dioxan. The eluted material was analysed by SDS/PAGE (Laemmli, 1970).

RESULTS

Interaction of gelsolin with the C-terminal actin fragment (residues 285–375)

Interaction of gelsolin with a large C-terminal actin fragment (285–375) was tested both in heterogeneous (e.l.i.s.a. experiments) and homogeneous (fluorescence experiments) phases. The interaction pattern, in the presence or absence of Ca²⁺, between gelsolin and the 285–375 actin fragment coated on the plastic of microtitre plates, is shown in Figure 1(a). The reactivity of gelsolin with the fragment was of the same order of magnitude (apparent dissociation constant of about 0.06 µM) as observed with coated monomeric actin (Houmeida et al., 1991).

This interaction was also noted in solution using the same fragment labelled with Nbd-Cl at Lys 373 (Figure 1b). Fluorescence enhancement (about 1.1-fold) was observed and appeared very low in comparison with the effect reported for G-actin (about 2-fold enhancement) (Coué et al., 1986). Differences in the orientation and the environment of the chromophore in the fragment and in the entire actin molecule could explain these results. However, as the interaction of the gelsolin S4–6 domains with the 305–326 segment of actin did not affect fluorescence of Nbd-actin, the observed fluorescence enhancement suggests the presence of another site for gelsolin which could be located near the C-terminal end of actin (Houmeida et al., 1991; Way et al., 1989).

To characterize further the gelsolin domains implicated in these interfaces, we then studied the interaction of a limited proteolytic digest of gelsolin with an insolubilized 285–375 actin fragment. Plasma gelsolin can be cleaved by chymotrypsin into three functional domains and other minor products. These domains corresponded to an N-terminal domain (CT15N), a C-terminal fragment (CT47C) and an additional fragment (CT31N) spanning CT15N to CT47C (Kwiatkowski et al., 1989; Way et al., 1989). Interactions between G-actin and CT15N or CT31N are known to be EGTA-independent, in contrast with the C-terminal half of gelsolin (Way et al., 1989).

Figure 1 Interaction of serum gelsolin with actin fragments

(a) Gelsolin binding to the 285–375 actin sequence monitored by e.i.s.a. 285–375 fragment (5 $\mu\text{g/ml}$) was coated on plastic plates and gelsolin was added at increasing concentrations (0–50 $\mu\text{g/ml}$) in buffer B containing 4 mM CaCl_2 , 0.5% gelatin and 3% gelatin hydrolysate (●) or buffer B supplemented with 10 mM EGTA (○), 0.5% gelatin and 3% gelatin hydrolysate. The interaction was monitored at 405 nm. (b) Fluorescence enhancement of Nbd-labelled 285–375 or 356–375 actin sequence upon binding to gelsolin. Labelled 285–375 fragment (20 $\mu\text{g/ml}$) (●) or 356–375 peptide (5 $\mu\text{g/ml}$) (○) was mixed in buffer A containing 4 mM CaCl_2 with increasing amounts of gelsolin (0–430 $\mu\text{g/ml}$). After incubation for 15 min, the fluorescence intensity of each sample was measured.

Figure 2 Interaction of gelsolin chymotryptic fragments with 285–375 actin fragment coupled to resin

Lane 1 shows the pattern of a chymotryptic digest of gelsolin. Lanes 2 and 3 show the material obtained after successive elutions from the affinity chromatography column by 10 mM EGTA and by 10% dioxan/10 mM phosphate buffer, pH 12, respectively. Proteins on SDS/PAGE were revealed by Coomassie Blue staining. Abbreviation: *M*, molecular mass.

The whole chymotryptic digest was passed through an affinity column containing the 285–375 actin fragment coupled to Sepharose 4B (see the Materials and methods section). The bound material was successively eluted with 10 mM EGTA, then with 10% dioxan at pH 12. The EGTA-eluted material was subjected to SDS/PAGE (Figure 2) and contained a polypeptidic fragment with the same electrophoretic mobility as CT47C and some bands of higher molecular mass, in agreement with previously obtained findings (Houmeida et al., 1991). When the material was eluted at pH 12, we observed a major band with high electrophoretic mobility similar to the CT15N domain (Figure 2).

On the basis of the fluorescence and affinity chromatography results, we concluded that two gelsolin-binding sites were located in the 285–375 actin sequence. The precise location of the

Figure 3 Interaction of gelsolin with the coated actin 356–375 peptide (CB9) monitored by e.i.s.a.

356–375 peptide (5 $\mu\text{g/ml}$) was coated on plastic plates and gelsolin was added at increasing concentrations (0–23 $\mu\text{g/ml}$) in buffer B supplemented with 0.5% gelatin and 3% gelatin hydrolysate containing either 4 mM CaCl_2 (●) or 10 mM EGTA (□). Binding of scallop arginine kinase was depicted negatively (○). The inset shows competitive binding of gelsolin to the 356–375 actin sequence and to G-actin studied by indirect e.i.s.a. Gelsolin (30 $\mu\text{g/ml}$) and increasing concentrations of G-actin (0–75 $\mu\text{g/ml}$) were simultaneously incubated in the presence of the coated 356–375 actin sequence. The interactions were monitored at 405 nm.

binding site situated near the C-terminal of actin was then determined by studying the reactivity of small peptides from this actin region.

Involvement of the 360–372 actin sequence

The gelsolin binding site located near the C-terminal side of actin was more precisely defined by studying the interaction of gelsolin with the CNBr 356–375 peptide (CB9). The peptide was modified either with 1,5-I-AEDANS at Cys³⁷⁴ or with Nbd-Cl at Lys³⁷³ (see the Materials and methods section). The gelsolin interaction with the coated 356–375 peptide was followed using a specific anti-gelsolin antiserum (Soua et al., 1985). The saturation curve of the association reaction is shown in Figure 3. The interaction appeared to be Ca^{2+} -dependent, as shown by the lack of binding when 10 mM EGTA was added to the coated peptide before incubation with gelsolin. The Ca^{2+} -dependence precluded the possibility of a large non-specific absorption of native gelsolin to the 356–375 peptide. The absence of aspecific binding was also checked using arginine kinase, a muscular enzyme whose interactions with actin in the 285–375 segment were limited to the 305–326 sequence (Reddy et al., 1992) and therefore did not bind to the 356–375 peptide (Figure 3). Finally, we substantiated the specificity of these interactions by using free actin to inhibit the binding activity of gelsolin with the 356–375 peptide (competitive e.i.s.a.). As shown in Figure 3 inset, the interaction of gelsolin with the coated 356–375 peptide was strongly altered by G-actin.

These results were confirmed by fluorescence-anisotropy measurements in solution. Before anisotropy experiments, we performed direct fluorimetry determinations. We did not observe any significant fluorescence enhancement when gelsolin was added to the 356–375 actin peptide labelled with Nbd-Cl (Figure 1b) in contrast with results using native actin (Coué et al., 1986) or the derived 285–375 fragment (Figure 1b). Similar negative results were observed with the dansylated peptide (results not shown). Since the fluorescence intensity of the dansylated peptide was not altered in the presence of gelsolin, fluorescence polarization can be used to monitor such interactions easily. A large anisotropy enhancement which is Ca^{2+} -dependent was observed

upon the addition of gelsolin to the labelled peptide and confirmed the occurrence of an interaction site in the last 20 actin residues (Figure 4).

This location was precised by the positive results obtained with the smaller synthetic peptide of sequence 359–372 in which Lys³⁵⁹ was substituted by Cys and subsequently dansylated. An interaction was observed by e.l.i.s.a., as shown in Figure 5, and then confirmed by fluorescence-polarization experiments (results not shown).

To define this actin/gelsolin interface completely, we then attempted to determine the region of the gelsolin molecule involved in the EGTA-resistant interaction.

Figure 4 Interaction of gelsolin with dansylated (356–375) peptide studied by fluorescence polarization

Fluorescence measurements were performed as described in the Materials and methods section. In a first experiment, increasing concentrations of gelsolin (0–110 $\mu\text{g/ml}$) were incubated in the presence of the labelled peptide (0.1 $\mu\text{g/ml}$) in buffer A either in the presence of 4 mM CaCl_2 (○) or 7 mM EGTA (■). The effect of thyroglobulin was depicted negatively (□).

Figure 5 Interaction of gelsolin with dansylated synthetic (360–372) peptide

Gelsolin was incubated with coated synthetic peptide (5 $\mu\text{g/ml}$) in buffer B supplemented with 0.5% gelatin and 3% gelatin hydrolysate, containing either 4 mM CaCl_2 (▲) or 10 mM EGTA (●). The interaction was studied by e.l.i.s.a. at 405 nm.

Figure 6 Interaction of chymotryptic fragments S1 and S2–3 of gelsolin with the dansylated (356–375) fragment of actin monitored by fluorescence polarization

Increasing concentrations of the gelsolin fragments, S1 (●) or S2–3 (○) (0–5 $\mu\text{g/ml}$), were incubated with the labelled peptide (0.1 $\mu\text{g/ml}$) in buffer A containing 4 mM CaCl_2 , as described in the legend to Figure 4.

Gelsolin counterpart of the C-terminal part of actin

To identify the gelsolin domain in the interaction with the C-terminal sequence of actin, reactivity of the purified chymotryptic gelsolin fragment CT15N was tested. We observed an increase in the fluorescence polarization of the dansylated peptide (356–375 sequence) upon the addition of the CT15N fragment. The corresponding polarization values increased from 0.020 for the peptide alone to an estimated 0.095 for the complex, in agreement with the molecular mass of the domain (Chaussepied and Kasprzak, 1989). A saturation curve expressed as fluorescence anisotropy is shown in Figure 6. We estimated an apparent dissociation constant of about 0.5 μM . In the same experiment, EGTA (7 mM), added after interaction of the gelsolin fragment (at 5 $\mu\text{g/ml}$) with the actin 356–375 peptide, did not dissociate the peptide–gelsolin complex. Lastly, the other Ca^{2+} -independent fragment (CT31N) did not significantly interact with the actin peptide (Figure 6).

DISCUSSION

Recent studies describing the interface of associated proteins on actin provided evidence of the occurrence of numerous functional domains. In the present study, we have defined a gelsolin–actin interface by using direct and competitive e.l.i.s.a. This highly sensitive method, which involved immunological and enzymic amplifications, was previously developed to describe interfaces between actin and α -actinin (Lebart et al., 1990), filamin (Mejean et al., 1992), myosin-head (Labbé et al., 1992), vitamin D-binding protein (Houmeida et al., 1992) and arginine kinase (Reddy et al., 1992). In addition, the results obtained here were substantiated by fluorescence anisotropy determinations in solution as reported in previous papers (Reddy et al., 1992; Houmeida et al., 1992).

In the presence of Ca^{2+} , it is well known that the gelsolin molecule interacts with at least 2 mol of actin. The first actin is slowly incorporated, whereas the second quickly interacts (Schoepper and Wegner, 1991). In addition, only an EGTA stable 1:1 gelsolin–actin complex remains after removal of Ca^{2+} (Coué and Korn, 1985; Porte and Harricane, 1986). These results are structurally supported by the presence of one Ca^{2+} -insensitive site in the N-terminal domain (S1) of gelsolin.

With regard to the counterpart in actin, we showed that gelsolin presented activity towards a large C-terminal fragment of actin sequence 285–375. One site has been already defined as the 305–326 sequence in the subdomain 3 of actin (Houmeida et al., 1991). In contrast, the cross-linking experiments of Doi et al. (1992) suggested that the N-terminal part of gelsolin lies about 1 nm from Cys³⁷⁴ in subdomain 1. Another interface should therefore occur near the actin C-terminal extremity. Accordingly, binding of the S-1 domain and gelsolin induces changes in the environment of the Nbd when coupled to Lys³⁷⁸ of actin (Coué et al., 1986; Way et al., 1989), suggesting that the interface is located near this residue.

To test this hypothesis, we firstly performed affinity chromatography with the insolubilized 285–375 actin fragment and examined the interaction of two different domains of cleaved gelsolin. A first, eluted with EGTA, corresponded to the S4–6 gelsolin domains interacting with the 305–326 sequence (Houmeida et al., 1991). The second, EGTA-resistant was identified with the N-terminal S1 gelsolin domain. This identification was also supported by the apparent molecular mass of the eluted fragment.

All of these results prompted us to purify a small fragment including the C-terminal end of actin (residues 356–375). Direct interaction between gelsolin and the sequence was characterized

Figure 7 Schematic representation of gelsolin/actin interfaces in the C-terminal region of actin.

▨, Location of the interaction sites for S1 and S4-6 gelsolin domains in the actin sequence.

both in solution and in the heterogeneous phase. The interaction required the presence of Ca^{2+} , thus pointing out the specificity of the binding. This new binding site was more precisely located as follows. Chemical modification of Lys^{373} and Cys^{374} did not prevent the gelsolin interaction. The C-terminal extremity can therefore be excluded from the interface. A smaller actin fragment, lacking the three C-terminal amino acids, reacted well with gelsolin, narrowing the actin sequence implicated to positions 360–372.

Our data on actin-binding domains of gelsolin allowed us to compare the binding properties of gelsolin and vitamin D-binding (DBP). Thus, in plasma, gelsolin can cause severing of actin filaments and DBP sequester actin monomers, since these two proteins are directly involved in clearing actin from the circulation (Coué et al., 1986; Harper et al., 1987; Dueland et al., 1990). With regard to DBP binding (Houmeida et al., 1992), the gelsolin site seems to be located within a common or proximal actin sequence. Competition between these two proteins (Coué et al., 1986) would be related to this interface, located in actin subdomain 1. Accordingly, Coué et al. (1986) have reported that this competition concerns the 1:1 EGTA-resistant actin-gelsolin complex. Furthermore, when DBP and gelsolin were simultaneously added to G-actin, a DBP-actin complex was preferentially formed. This could be explained by differences in the rates of interaction of DBP and the first mole of gelsolin with G-actin (Coué et al., 1986; Schoepper and Wegner, 1991). Moreover, gelsolin S-1 and DBP prevented actin polymerization. Interaction of both of these proteins with Nbd-labelled actin altered fluorescence of the chromophore, but to different extents (Coué et al., 1986). However, in contrast with gelsolin, DBP only interacted with G-actin. Its binding site appeared to be located near Cys^{374} , since antibodies directed against dansylated cysteine impede the sequestering of actin monomers (Houmeida et al., 1992). All of these observations substantiate the presence of two proximal, but different, binding sites for these proteins.

To determine accurately the part of gelsolin involved, we studied direct binding of the purified N-terminal chymotryptic fragment (S1) and showed that this domain interacted with the 356–375 actin sequence.

We conclude that part of the N-terminal domain of gelsolin, more specifically some amino acids in the 41–126 sequence as determined by mutagenesis experiments (Way et al., 1992), is in contact with the sequence containing amino acids 360–372 of actin (Figure 7). Analysis of deletions in the N-terminal part of S1 have thus shown that actin binding occurs until amino acid 41

is removed, indicating that a N-terminal sequence is critical in these interactions (Way et al., 1992). However, some charged residues in sequence 119–123, which is conserved in some other severing proteins such as severin and villin, are probably directly involved in the interface (Way et al., 1992). In summary, two gelsolin-binding sites (Figure 7) were located within the 285–375 sequence in subdomains 1 and 3 of actin at the barbed end of the microfilament.

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