Conformational Changes in Actin Induced by Its Interaction with Gelsolin

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ABSTRACT Actin cleaved by the protease from *Escherichia coli* A2 strain between Gly^{42} and Val^{43} (ECP-actin) is no longer polymerizable when it contains Ca^{2+} as a tightly bound cation, but polymerizes when Mg^{2+} is bound. We have investigated the interactions of gelsolin with this actin with regard to conformational changes in the actin molecule induced by the binding of gelsolin. ECP-(Ca)actin interacts with gelsolin in a manner similar to that in which it reacts with intact actin, and forms a stoichiometric 2:1 complex. Despite the nonpolymerizabilty of ECP-(Ca)actin, this complex can act as a nucleus for the polymerization of intact actin, thus indicating that upon interaction with gelsolin, ECP-(Ca)actin undergoes a conformational change that enables its interaction with another actin monomer. By gel filtration and fluorometry it was shown that the binding of at least one of the ECP-cleaved actins to gelsolin is considerably weaker than of intact actin, suggesting that conformational changes in subdomain 2 of actin monomer may directly or allosterically affect actin-gelsolin interactions. On the other hand, interaction with gelsolin changes the conformation of actin within the DNase I-binding loop, as indicated by inhibition of limited proteolysis of actin by ECP and subtilisin. Cross-linking experiments with gelsolin-nucleated actin filaments using *N,N*-phenylene-bismaleimide (which cross-links adjacent actin monomers between Cys^{374} and Lys^{191}) reveal that gelsolin causes a signficant increase in the yield of the 115-kDa cross-linking product, confirming the evidence that gelsolin stabilizes or changes the conformation of the C-terminal region of the actin molecule, and these changes are propagated from the capped end along the filament. These results allow us to conclude that nucleation of actin polymerization by gelsolin is promoted by conformational changes within subdomain 2 and at the C-terminus of the actin monomer.

INTRODUCTION

Gelsolin-related actin-associated proteins seem to be ubiquitously expressed in eukaryotes. They occur in the form of several variants, with a molecular mass of either ~80 kDa (e.g., mammalian gelsolin) or ~40 kDa (e.g., fragmin from *Physarum*) and comprising either six or three structurally homologous but functionally distinct domains (for a review, see Stossel, 1994). The most characteristic property of gelsolin and related proteins is the efficient severing of actin filaments, but they also promote the nucleation of actin polymerization and cap the fast polymerizing end of the actin filament. In the case of mammalian gelsolin, two actin monomers associate in a Ca-dependent manner with one molecule of gelsolin to form a ternary complex (Bryan and Kurth, 1984). It is this stoichiometric complex that forms both the nucleus and the capping factor. Upon the removal of Ca^{2+}, one actin dissociates from the ternary complex. The resulting binary complex is very stable and EGTA resistant, but it can be dissociated by phospholipids like PIP_2 (Yin, 1989). With regard to the nucleation process, it is not fully clear whether gelsolin simply stabilizes the actin nucleus by bringing two actin monomers in close contact with each other, or nucleation is promoted by conformational changes within the actin molecule upon its interaction with gelsolin.

Received for publication 19 December 1996 and in final form 21 April 1997.

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0006-3495/97/08/929/09 $2.00

Actin cleaved between Gly^{42} and Val^{43} by the protease from *Escherichia coli* A2 strain (ECP-actin) (Khaitlina et al., 1991) retains the principal structural properties of intact actin, but loses its ability to polymerize and to interact with DNase I. Both properties can be restored when the tightly bound Ca^{2+} is replaced by Mg^{2+} (Khaitlina et al., 1993), apparently as a consequence of conformational changes induced by the Ca/Mg exchange (Mejean et al., 1988; Strzelecka-Golaszewska et al., 1993). Therefore ECP-actin is a suitable model for the study of the mechanisms of nucleated actin polymerization, provided that the nucleating factor is capable of stimulating polymerization of ECP-(Ca)actin.

In this work, polymerization of ECP-(Ca)actin in the presence of gelsolin was studied by using the fluorescence of pyrenyl-labeled actin as a tool. We show here that the complex of ECP-cleaved (Ca) actin with gelsolin nucleates polymerization of intact actin, indicating a restored polymerizability of ECP-actin. Inhibition of proteolytic cleavage of actin within its DNase I-binding loop was observed in actin/gelsolin complexes. These data, together with the results of cross-linking of actin filaments nucleated by substoichiometric concentrations of gelsolin, allowed us to assume that the interaction with gelsolin induces conformational changes, both at the C-terminus and within the DNase I-binding loop of the actin molecule.

MATERIALS AND METHODS

Protein preparations

Rabbit skeletal muscle actin was purified by the procedure of Spudich and Watt (1971), with an additional gel filtration step on Sephadex G-150 to

remove traces of actin-binding proteins. G-actin in buffer G (0.2 mM ATP, 0.1 mM $CaCl_2$, 0.4 mM β-mercaptoethanol, 5 mM Tris-HCl, pH 8.2, 1 mM NaN_3) was stored on ice and used within a week.

Actin was labeled with *N*-(1-pyrenyl)iodoacetamide as described by Kouyama and Mihashi (1981). Pyrenyl-labeled actin was lyophilized in the presence of 2 mM sucrose and stored at −70°C. Before use, the lyophilized pyrenyl-actin was dissolved in buffer G and dialyzed overnight against the same buffer.

Gelsolin was isolated from pig stomach smooth muscle (Hinssen et al., 1984). The C-terminal and N-terminal domains of gelsolin were obtained by cleavage of gelsolin with thermolysin (Chaponnier et al., 1986) and purifying the fragments by chromatography on a Mono Q anionic exchanger column (Pharmacia).

Actin cleaved with *E. coli* A2 protease (ECP-actin) was obtained by using partially purified preparations of *E. coli* protease (Usmanova and Khaitlina, 1989). G-actin (2–3 mg/ml) was digested at an enzyme/protein mass ratio of 1:100 for 2–3 h at 20°C and used within 1 day. Because ECP-actin is resistant to further degradation by the protease (Khaitlina et al., 1991) and gelsolin was not a substrate for ECP, no protease inhibitor was used. Integrity of the cleaved actin molecule was checked as described previously (Khaitlina et al., 1991, 1993). The cleaved actin contains two fragments of 36 kDa and 5 kDa, but under the electrophoretic conditions used in this work, the small fragment was not always seen on gels.

Fluorescence measurements

Fluorescence of pyrenyl-labeled actin was measured at 407 nm after excitation at 365 nm in a Shimadzu PC 5000 fluorometer.

Viscometry

The specific viscosity of polymerized actin was measured with an Ostwald-type capillary viscometer (Cannon Instruments), with a sample volume of 1 ml and an outflow time for water of 25 s at 20°C.

Gel chromatography

Gelsolin and gelsolin-actin complexes were chromatographed on a 1.6 cm × 60 cm column of Superdex G-200 (Pharmacia) with a flow rate of 0.5 ml/min. Samples containing 0.5–1 mg protein were applied in concentrations of ~1 mg/ml protein.

SDS-PAGE

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed using 15% acrylamide, 0.1% bisacrylamide slab gels in the Laemmli buffer system (Laemmli, 1970).

Limited proteolysis

G-actin (1 mg/ml), either alone or as a complex with gelsolin or its proteolytic fragments (1 mg/ml), was digested with the *E. coli* A2 protease at an enzyme/protein mass ratio of 1:100 at 25°C. At different time points, the digestion was stopped by the addition of SDS buffer, and the samples were analyzed by SDS-PAGE. Digestion of both G-actin and actin-gelsolin complexes (1 mg/ml) with subtilisin was carried out at an enzyme/protein mass ratio of 1:200 at 25°C. Reaction was stopped by 1 mM phenylmethylsulfonyl fluoride, and samples were analyzed by SDS-PAGE.

Cross-linking experiments

For cross-linking of actin/gelsolin complexes with 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide (EDC), the 2:1 complexes of intact or ECP-cleaved actin (24 μM) with gelsolin obtained in the presence of 0.2 mM $CaCl_2$ were incubated with 1/5 volume of 20 mM EDC in water for 1 h at room temperature (Sutoh and Yin, 1989). The reaction was stopped by the addition of the SDS-PAGE buffer, followed by boiling for 3 min, and cross-linking products were analyzed by SDS-PAGE.

Cross-linking of F-actin with *N,N'*-1,2-phenylenebismaleimide ($PhMal_2$) was carried out as described by Millonig et al. (1988). Actin (2–3 mg/ml) was mixed with either gelsolin or its fragments at different actin/gelsolin ratios, and the concentration of calcium in the mixtures was adjusted to 0.2 mM. Actin was polymerized with 0.1 M KCl and left at room temperature for 1 h. F-actin was incubated with $PhMal_2$ at a cross-linker/actin molar ratio of 1:1 for 15 min at room temperature. The reaction was stopped by boiling in SDS sample buffer.

RESULTS

Interaction of gelsolin with ECP-cleaved Mg-actin

As shown previously (Khaitlina et al., 1991), cleavage of the actin polypeptide chain between Gly^{42} and Val^{43} results in a complete loss of actin polymerizability if actin contains calcium as the tightly bound cation (ECP-Ca-actin). One way to restore polymerizability of this actin is transformation into the magnesium form (ECP-(Mg)actin) by exchanging Ca^{2+} for Mg^{2+} (Khaitlina et al., 1993). Therefore ECP-F-(Mg)actin was used to test whether the actin cleavage by the *E. coli* A2 protease modifies the interaction of actin with gelsolin. As shown in Fig. 1 *A*, KCl-polymerized filamentous ECP-(Mg)actin was immediately disassembled by gelsolin when added at a 1:100 molar ratio. The same result was obtained when only the N-terminal domain of gelsolin was used. The gelsolin-induced viscosity decrease of ECP-cleaved actin was much larger than that of intact actin, and the level of the resulting steady-state viscosity corresponded rather to G-actin than to the short polymers expected at this molar ratio of gelsolin to actin (Hinssen et al., 1984). This difference may be attributed to the very high critical concentration for polymerization of ECP-(Mg)actin in the presence of gelsolin (Fig. 1 *B*). The measured value of 22 μM represents the critical concentration for the slow polymerizing end of ECP-actin because the fast polymerizing end is capped by gelsolin. An increase in critical concentration for uncapped actin from 0.05 μM to 2.7 μM has been reported previously for KCl-induced polymerization of ECP-(Mg)actin versus intact actin (Khaitlina et al., 1993). This means that although the initial phase of the reaction as shown in Fig. 1 *A* is very likely a severing process, as it is for intact actin, the subsequent slow decrease in viscosity represents a depolymerization of the gelsolin-capped actin fragments.

Moreover, the data suggest that with the high critical concentration of actin polymerization, like that of ECP-(Ca)actin, gelsolin-nucleated polymerization of the latter can hardly be detected under the conditions used. Therefore, to reveal possible effects of gelsolin on the nucleation capability of ECP-cleaved (Ca)actin, polymerization of intact actin in the presence of ECP-(Ca)actin/gelsolin complexes was studied.

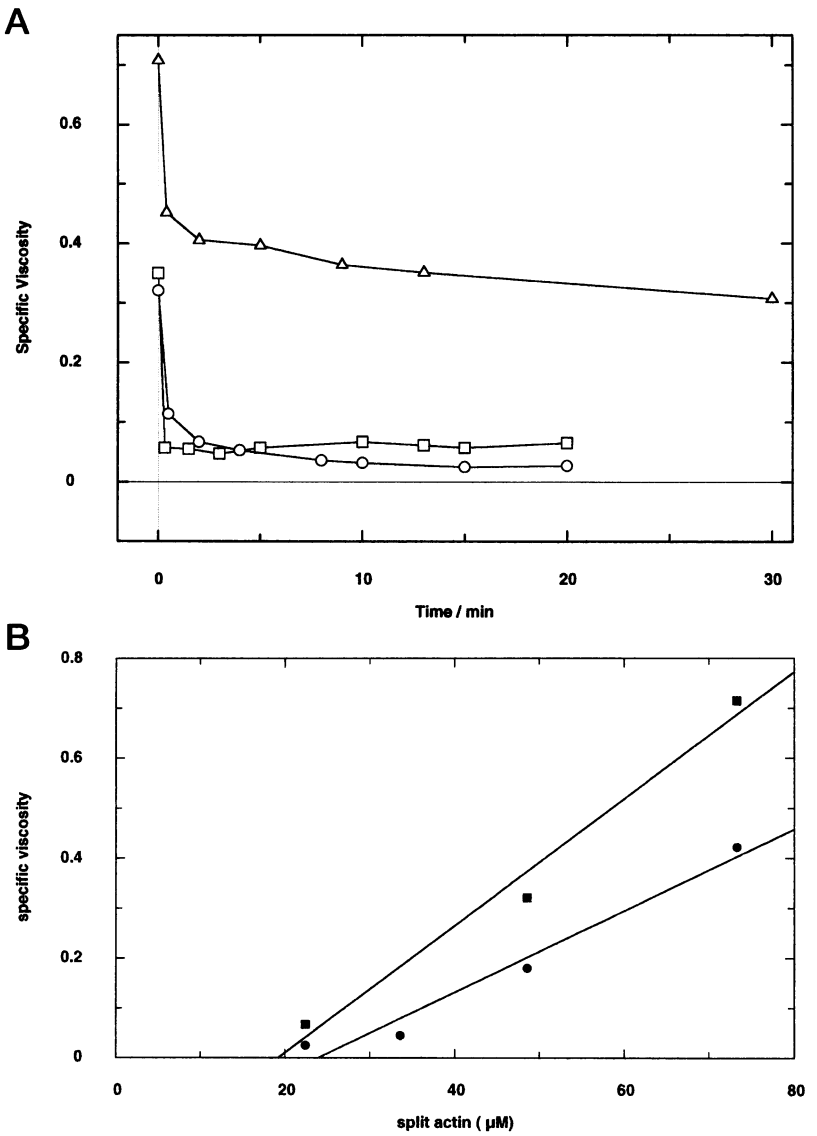

FIGURE 1 Interaction of ECP-cleaved F-actin with gelsolin. (*A*) Severing of ECP-cleaved F-(Mg) actin by gelsolin as revealed by Ostwald viscometry. At zero time, gelsolin (○, △) or its 40-kDa N-terminal fragment (□) was added to ECP-cleaved (○, △) or intact (□) F-actin (24 μM) in 1.2 mM ATP, 0.1 mM $CaCl_2$, 1 mM $MgCl_2$, 50 mM KCl, 0.4 mM β-mercaptoethanol, 0.4 mM NaN_3, 10 mM imidazole, pH 7.2. (*B*) Critical concentration for ECP cleaved (Mg) actin polymerization in the presence of gelsolin. ECP-(Ca)actin (24–72 μM) in 0.2 mM ATP, 0.04 mM $CaCl_2$, 0.04 mM β-mercaptoethanol, 0.4 mM NaN_3, 5 mM Tris-HCl, pH 8.2 (buffer G) was converted to Mg-actin by incubation with 0.4 mM $MgCl_2$ for 10 min. Then gelsolin (●) or its 40-kDa N-terminal fragment (■) was added to the samples at a 1:100 molar ratio, and polymerization was initiated by the addition of 50 mM KCl, 1 mM ATP, 10 mM imidazole, pH 7.2, complemented by $CaCl_2$ and $MgCl_2$ to adjust their concentrations to 0.1 mM and 1 mM, respectively.

Nucleation of actin polymerization by actin/gelsolin complexes

As shown by Khaitlina et al. (1993), ECP-cleaved (Ca)actin does not polymerize spontaneously, and its polymerization is not promoted by intact F-actin. In agreement with these data and with the results described above, no polymerization of ECP cleaved (Ca) actin was observed when gelsolin was added as a promotor of nucleation (data not shown). However, a fast increase in the fluorescence of pyrenyl-labeled intact actin was induced by stoichiometric complexes of ECP-cleaved (Ca) actin with gelsolin, indicating an enhancement of nucleation under conditions in which intact actin alone would polymerize very slowly (Fig. 2 *A*). Corresponding results were obtained when polymerization of unlabeled intact actin was initiated in the presence of the complexes of pyrenyl-labeled cleaved (Ca) actin with gelsolin (Fig. 2 *B*). In this case the increase in fluorescence could result only from the formation of actin filaments on the cleaved pyrenyl-actin/gelsolin nuclei. We concluded, therefore, that gelsolin can restore the actin-binding capability of ECP-cleaved (Ca) actin.

Both the rate and the extent of cleaved actin-gelsolin nucleated polymerization were lower than those of polymerization nucleated by intact actin-gelsolin complexes. This difference may be explained by a lower affinity of ECP-cleaved actin for gelsolin, resulting in a reduced amount of pyrenyl-labeled nuclei involved in the polymerization. This assumption was proved by gel filtration and exchange experiments under nonpolymerizing conditions.

Gel filtration of cleaved actin-gelsolin complexes

Under nonpolymerizing conditions and at $[Ca^{2+}] > 10^{-6}$ M, gelsolin and actin form a ternary (1:2) complex that dissociates into a binary (1:1) complex and actin upon addition of EGTA. Correspondingly, the existence of a 1:1

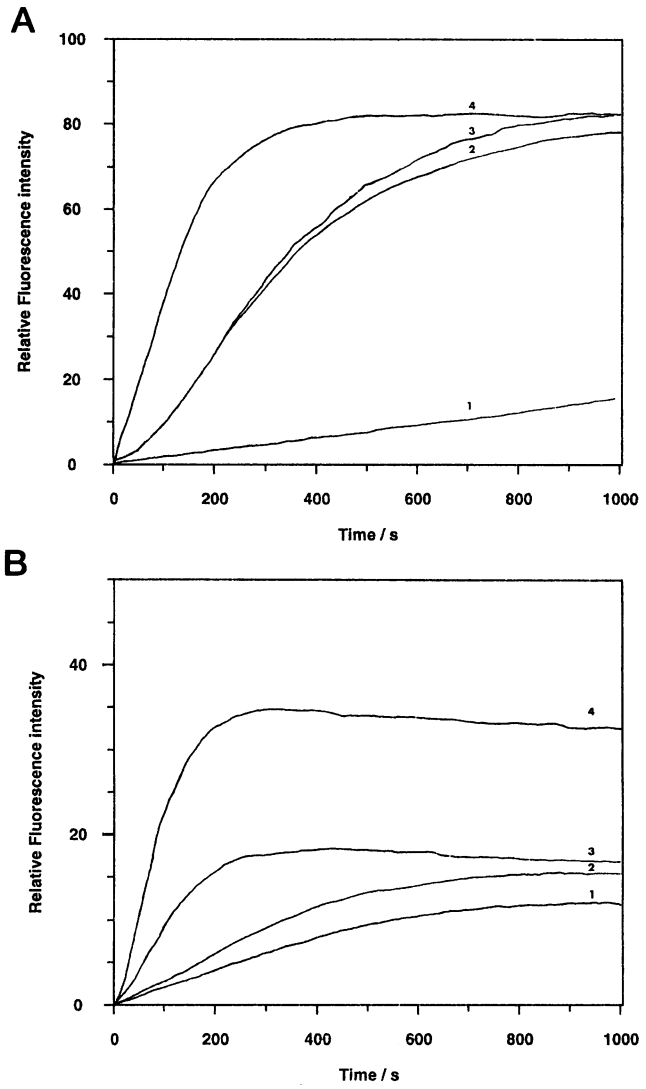

FIGURE 2 Nucleation of actin polymerization by ECP-cleaved (Ca)actin-gelsolin complexes. (*A*) 12 μM actin in buffer G containing 10% pyrenyl-actin was polymerized alone (*curve 1*), in the presence of preformed ECP:actin-gelsolin 2:1 complexes at 1:30 (*curve 2*) or 1:100 (*curve 3*) molar ratios, and in the presence of the ternary (2:1) complex of intact actin-gelsolin at a molar ratio of 1:100 (*curve 4*). Complexes were prepared by mixing gelsolin (in 0.2 mM EGTA, 0.5 mM dithiothreitol (DTT), 10 mM imidazole, pH 7.0) with ECP-cleaved or intact actin at a molar ratio of 1:2, and the concentration of $CaCl_2$ in the mixtures was adjusted to 0.2 mM in excess EGTA. Polymerization was initiated by the addition of 50 mM KCl, 1 mM ATP, 10 mM imidazole, pH 7.2. (*B*) 12 μM unlabeled actin was polymerized in the presence of the ECP-cleaved (*curves 1* and *2*) or intact (*curves 3* and *4*) pyrenyl-actin:gelsolin complexes at molar ratios of 1:100 (*curves 1* and *3*) or 1:30 (*curves 2* and *4*). The final amount of pyrenyl-actin during polymerization was ~1% in curves 1 and 3 and ~3% in curves 2 and 4. All other conditions were as in *A*.

complex of gelsolin with ECP-actin was revealed when a (2:1) mixture of cleaved actin with gelsolin prepared in the presence of 0.2 mM Ca^{2+} was subjected to gel filtration in 0.5 mM EGTA. Elution volumes of the binary complex with ECP actin and intact actin were identical (data not shown). However, when the same mixture of ECP-actin and gelsolin was chromatographed in the presence of 0.5 mM $CaCl_2$, the major peak eluted slightly later than the ternary complex of intact actin with gelsolin. Densitometry of SDS-PAGE samples taken from the peak fractions of the gel filtration runs showed that the molar ratio of cleaved actin/gelsolin was 1.6:1, whereas in the peak sample from gel filtration of the ternary complex with intact actin it was clearly 2:1 (as expected). The lower amount of actin in the gel filtration peak sample containing cleaved actin may be due to a gradual release of one of the actin monomers from the preformed 2:1 complex during chromatography, indicating that one monomer is weakly bound to gelsolin.

Exchange of pyrenyl-labeled actin monomers in the actin/gelsolin complexes

To further characterize binding of gelsolin to ECP-cleaved actin, the gelsolin-induced increase in the fluorescence of pyrenyl-labeled actin (Bryan, 1988; Weeds et al., 1986) was measured. In the presence of calcium ions, interaction of both intact and cleaved pyrenyl-actin with gelsolin was followed by a two- to threefold increase in fluorescence intensity (Fig. 3 *A*). This increase was also observed in the presence of DNase I, independent of whether DNase I was mixed with actin before or after gelsolin (not shown). The 2.5-fold fluorescence intensity increase was also registered upon interaction of pyrenyl-actin with the N-terminal gelsolin fragment, whereas the C-terminal gelsolin fragment produced no effect (Table 1).

Addition of unlabeled intact actin to the preformed complex of pyrenyl-actin with both the whole gelsolin and the N-terminal gelsolin fragment resulted in a rapid decrease in fluorescence intensity, likely to be caused by the replacement of one pyrenyl actin monomer by unlabeled actin (Fig.

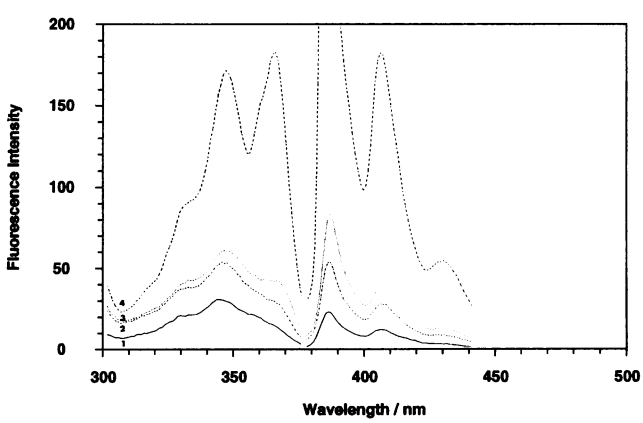

FIGURE 3 Fluorescence increase after complex formation of intact and ECP-cleaved pyrenyl-actin with gelsolin under nonpolymerizing conditions. Fluorescence spectra of cleaved and intact pyrenyl-actins were recorded before mixing with gelsolin (*curve 1*) and 10 min after mixing (*curves 2* and *3* for cleaved and intact actin-gelsolin, respectively). The excitation spectra (*left*) were recorded at 407 nm, and the emission spectra (*right*) were recorded after excitation at 365 nm. Curve 4 represents fluorescence spectra of polymerized intact actin.

TABLE 1 Increase in the fluorescence intensity of pyrenyl-labeled actin upon interaction with gelsolin and gelsolin fragments

Actin	Gelsolin	Increase in the fluorescence intensity (F/F_0)*
Intact	Whole gelsolin	2.62
ECP cleaved	Whole gelsolin	2.29
Intact	N-terminal gelsolin fragment	2.54
Intact	C-terminal gelsolin fragment	1.1

*F_0, Fluorescence intensity of pyrenyl-labeled G-actin; F, intensity of pyrenyl fluorescence in the actin-gelsolin complexes; λ_{ex} = 365 nm, λ_{em} = 407 nm.

4). ECP-cleaved pyrenyl-actin was displaced from the complex faster than intact actin (Fig. 4). In Fig. 4 (*inset*), the final values of the fluorescence were plotted versus increasing concentrations of added unlabeled actin. The figure shows that ~80% of the fluorescence was lost, and the ratio of unlabeled to labeled actin reached 0.5. At higher concentrations of unlabeled actin, no significant further decrease in the fluorescence was observed, indicating that one of the two gelsolins bound is easily exchanged, whereas the other monomer exchanges very slowly, if at all. This result confirms the conclusion about the weak binding of this monomer drawn from the gel filtration experiments.

Limited proteolysis of actin in the presence of gelsolin

To localize conformational changes that may accompany the interaction of actin with gelsolin, limited proteolysis of actin and actin/gelsolin complexes with the *E. coli* protease ECP 32 and subtilisin within the DNase I-binding loop (Khaitlina et al., 1991) was employed. In the 2:1 actin-gelsolin complex, specific cleavage of actin by ECP was inhibited by ~50% (Fig. 5, *A* and *B*). Gelsolin also protected actin against proteolysis with subtilisin (not shown). According to the model of F-actin (Holmes et al., 1990; Lorenz et al., 1993), the DNase I-binding loop is involved in extensive interactions with the neighboring subunits within the same and the opposite strands. Therefore burying of the DNase I-binding loop in the ternary actin/gelsolin complex might also be due to actin-actin interaction. In contrast to such an interpretation, cleavage of actin between Gly^{42} and Val^{43} with ECP 32 was also inhibited in the 1:1 complex of actin with gelsolin formed in the presence of EGTA and purified from the released actin monomer by gel filtration (Fig. 5 *C*). Because gelsolin segments 1, 2–3, and 4–6 are shown to bind subdomain 1 of actin (Pope et al., 1991) and because DNase I does not prevent binding of gelsolin to actin (Pope et al., 1991; see also Fig. 4 in this work), this inhibition does not seem to be sterical. Therefore we conclude that interaction of actin with gelsolin induces conformational changes within the DNase I-binding loop at the top of subdomain 2 of the actin molecule.

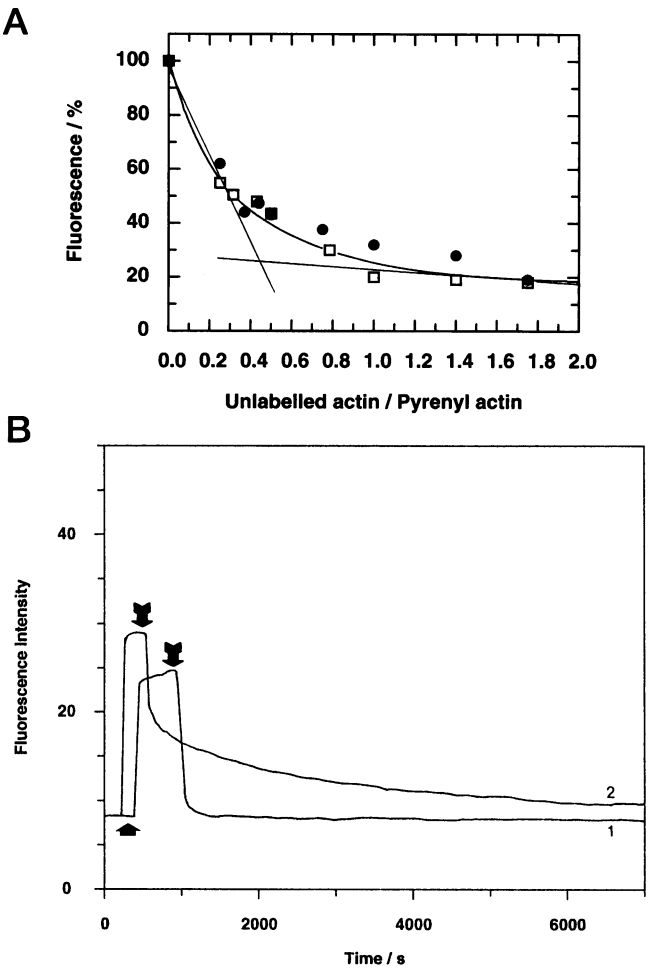

FIGURE 4 Exchange of actin subunits in the actin-gelsolin 2:1 complex. ECP-cleaved or intact pyrenyl-actin (2.4 μM) in buffer G was mixed with 1.2 μM gelsolin in 0.2 mM EGTA, 0.5 mM DTT, 10 mM imidazole, pH 7.0, and the concentration of $CaCl_2$ in the mixture was adjusted to 0.2 mM (*arrowhead*). After fluorescence reached the plateau, unlabeled intact actin (12 μM) was added (*arrows*). The inset shows titration of intact (□) or ECP-cleaved (●) pyrenyl actin/gelsolin complexes with unlabeled intact actin. Fluorescence of pyrenyl-labeled actin was measured at 407 nm after excitation at 365 nm. Decrease in the fluorescence intensity was calculated as F (%) = ($F_{final} - F_o/F_{max} - F_o$) 100%, where F_0 and F_{max} are intensities of pyrenyl fluorescence in G-actin and in actin/gelsolin complexes, respectively; F_{final} is the steady-state fluorescence intensity after the addition of unlabeled actin.

Protection of the DNase I-binding loop was also observed in the complex of actin with the N-terminal fragment of gelsolin (Fig. 5 *D*). Cleavage of actin complexed with the C-terminal fragment of gelsolin was not inhibited, and was even slightly accelerated (not shown).

Chemical cross-linking of ECP-cleaved actin with gelsolin

As shown previously (Sutoh and Yin, 1989), two N-terminal gelsolin fragments (17 kDa and 28 kDa) are cross-linked to the N-terminal segment of actin by 1-ethyl-3-[3-(dimeth-

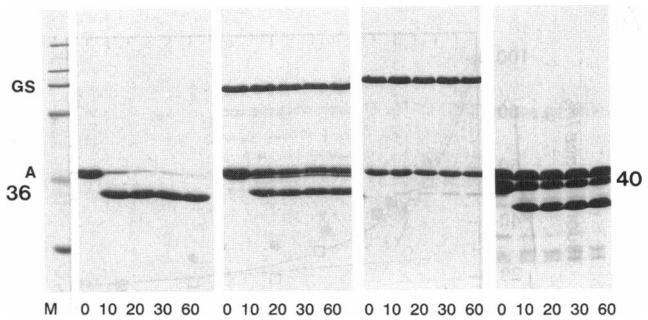

FIGURE 5 Cleavage of actin by ECP in the presence of gelsolin, SDS-PAGE. (*A*) Actin (24 μM) in buffer G. (*B*) Actin (24 μM) in buffer G was mixed with 12 μM gelsolin in 0.2 mM EGTA, 0.5 mM DTT, 10 mM imidazole, pH 7.0, and the concentration of $CaCl_2$ in the mixture was adjusted to 0.2 mM (in excess EGTA). (*C*) The 1:1 actin-gelsolin complex formed in the presence of 0.5 mM EGTA and purified from the second actin subunit by gel filtration on Superdex G 200. (*D*) Actin (12 μM) was mixed with 6 μM N-terminal fragment of gelsolin; cleavage was performed in the presence of 0.2 mM $CaCl_2$. M, High molecular mass markers (Sigma), myosin heavy chain (205 kDa), galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), and carbonic anhydrase (29 kDa). GS, A, 40, and 36 denote positions of gelsolin, actin, the N-terminal 40 kDa gelsolin fragment, and the 36 kDa ECP-cleaved actin fragment, respectively.

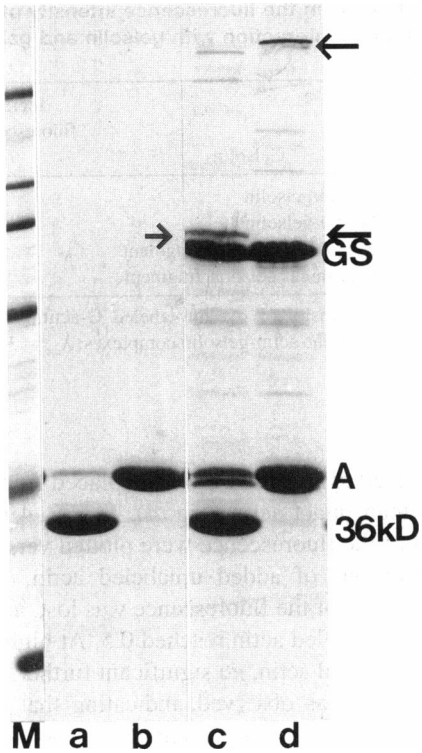

FIGURE 6 Cross-linking of ECP-cleaved actin with gelsolin. Intact actin or ECP-actin (24 μM) in buffer G was mixed with 12 μM gelsolin in 0.2 mM EGTA, 0.5 mM DTT, 10 mM imidazole, pH 7.0, and the concentration of $CaCl_2$ was adjusted to 0.2 mM over EGTA. Cross-linking with EDC was performed as described in Materials and Methods. (*Lane a*) ECP-cleaved actin; (*lane b*) intact actin; (*lanes c* and *d*) complexes of gelsolin with ECP-cleaved and intact actin, respectively. Arrows show positions of the cross-linked products. M, Molecular weight markers as in Fig. 5. GS, A, and 36 kD denote positions of gelsolin, actin, and of the 36-kDa ECP-cleaved actin fragment, respectively.

ylamino)propyl]-carbodiimide (EDC). In the complex of actin with the whole gelsolin molecule, a high-molecular-mass product with an apparent molecular mass of ~250 kDa is formed after EDC cross-linking (Fig. 6, *lane d*). If we take into account that on SDS-PAGE, dimers of actin cross-linked by either *N,N'*-1,2-phenylenebismaleimide or glutaraldehyde move with an apparent molecular mass of 115 kDa (Millonig et al., 1988), this product probably corresponds to the ternary complex of gelsolin with two actin monomers. Cross-linking of ECP-cleaved actin generates a product with a molecular mass of 95 kDa (Fig. 6, *lane c*), which seems to result from cross-linking of gelsolin with the N-terminal 5-kDa fragment of the cleaved actin. These data confirm that the N-terminal part of actin is tightly bound to gelsolin, and that this binding is not disturbed by the cleavage within the DNase I-binding loop.

Chemical cross-linking of actin monomers in gelsolin-nucleated F-actin

It has recently been shown that binding of gelsolin to the barbed end of the actin filament affects the orientation of the Cys^{374}-bound erythrosin, and changes both the torsional flexibility and the helical structure of the whole filament (Prochniewicz et al., 1996; Orlova et al., 1995). These authors suggested that capping of actin filaments by gelsolin induces conformational changes at the C-terminus of the directly bound actin subunits, and that these changes are propagated from the terminal molecules throughout the filament. To further analyze the correlation of the gelsolin-induced effects with structural transitions at the C-terminus of the actin monomer, we probed actin filaments polymerized in the presence of gelsolin and its fragments by cross-linking with the sulfhydryl bifunctional reagent *N,N'*-1,2-phenylenebismaleimide. In F-actin a dimer of 115 kDa resulting from cross-linking C-terminal Cys^{374} of one actin monomer with Lys^{191} of the monomer on the adjacent subunit is a main product of the reaction; and, furthermore, a small amount of the 90-kDa dimer is generated (Elzinga and Phelam, 1984; Millonig et al., 1988). In the ternary actin-gelsolin complex, the 115-kDa dimer is not formed, whereas the generation of the 90-kDa dimers is enhanced (Hesterkamp et al., 1993). In our experiments, where before cross-linking actin was polymerized in the presence of gelsolin or its N-terminal 40 kDa-fragment at substoichiometric gelsolin/actin mass ratios from 1:200 to 1:50, the yield of the 115-kDa product increased (Fig. 7). Consistent with the inability of the C-terminal 45-kDa gelsolin fragment to nucleate actin polymerization, addition of this fragment to actin did not influence the amount of the 115-kDa dimers produced. These data confirm that gelsolin may indeed change or stabilize the position of the C-terminal segment of actin monomer.

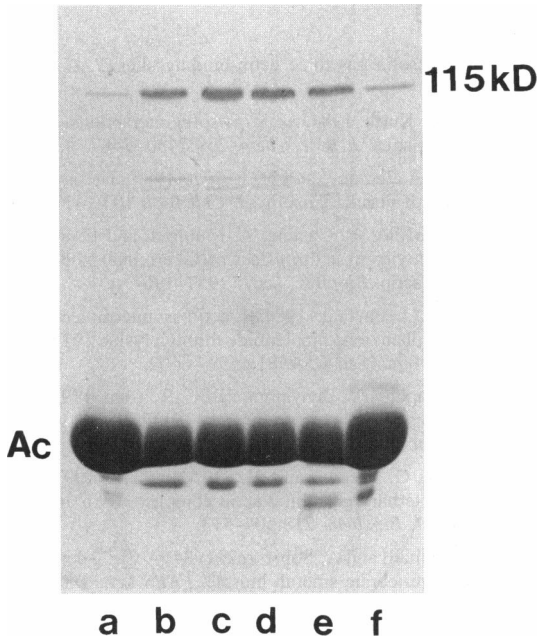

FIGURE 7 Cross-linking of actin polymerized in the presence of substoichiometric concentrations of gelsolin or its fragments. Actin (75 μM) was polymerized with 0.1 M KCl, and subsequently cross-linked either alone (*lane a*) or in the presence of gelsolin at gelsolin/actin mass ratios of 1:50 (*lane b*), 1:100 (*lane c*), and 1:200 (*lane d*), as well as in the presence of the N-terminal 40-kDa (*lane e*) and the C-terminal 45-kDa (*lane f*) gelsolin fragments added to actin at a 1:100 mass ratio (SDS-PAGE). M, Molecular mass markers. Ac and 115 kD show positions of actin and the 115-kDa dimer, respectively.

DISCUSSION

ECP (Ca) actin fails to polymerize because of the conformational changes of DNase I-binding loop on the top of subdomain 2, which is one of the sites of monomer-monomer interaction along the actin polymer (Holmes et al., 1990; Lorenz et al., 1993). Moreover, the structural differences between intact and ECP-cleaved F-actin, involving the C-terminal Cys^{374} (Khaitlina et al., 1993; Orlova and Egelman, 1995), suggests cleavage-induced conformational changes in the vicinity of the actin C-terminus, where an additional actin-actin interaction site is located (Holmes et al., 1990; Lorenz et al., 1993).

To restore polymerizability of the cleaved actin, a conformation equivalent to the native conformation must be restored. Therefore, if some factor appears to promote polymerization of ECP-cleaved actin, this factor likely induces conformational changes in the actin molecule. By the same token, a factor that would induce conformational changes within the DNase I-binding loop and/or the C-terminus is likely to affect actin polymerization. It was the aim of this investigation to provoke polymerization of ECP-(Ca)actin by gelsolin, and subsequently to analyze the gelsolin-induced conformational changes within subdomain 2 (the DNAse-binding loop) by limited proteolysis. Our results demonstrate that polymerizability of ECP-(Ca)actin is restored, at least in some aspects, when it is in a complex with gelsolin, because it is incorporated into filaments at their fast polymerizing end. As was shown recently, polymerization of ECP-(Ca) actin can also be induced by myosin subfragment I (Wawro et al., 1996).

In the ternary (2:1) complex of actin and gelsolin, the gelsolin-induced 50% inhibition of actin proteolysis by ECP can be interpreted as specific cleavage of only one of the two actins associated with gelsolin. Presumably, the second actin undergoes conformational changes that render it inaccessible for the protease. Consequently, a full inhibition of ECP cleavage was observed with the EGTA-resistant binary gelsolin-actin complex. Because this complex has been shown to involve the N-terminus of gelsolin (Feinberg et al., 1993; McLaughlin et al., 1993), the cleavage data permit the conclusion that the interaction of ECP-actin with gelsolin strongly influences the conformation of the monomer associated with the N-terminal segment of gelsolin. These conformational changes of the actin molecule enable binding of another actin and thereby restore, at least in part, the polymerizability of ECP-actin.

The data obtained previously by fluorescence studies (Bryan and Kurth, 1984) as well as by exchange of actin-bound ATP (Bryan, 1988) indicate that in the ternary gelsolin-actin complex, the actin monomer associated with the C-terminal half of gelsolin is exchangeable. Our experiments also revealed that under nonpolymerizing conditions, one of the actins in the ternary complex is easily exchanged. If such an exchange also takes place under polymerizing conditions, the complex we used in nucleation experiments may therefore contain intact actin at the C-terminal half and ECP-actin at the N-terminal half of gelsolin. Thus our results show that at least the actin monomer that is bound to the N-terminal domain of gelsolin undergoes conformational changes that enable binding of another actin monomer.

On the other hand, because cleavage of actin within the DNase I-binding loop weakened its binding to the C-terminal domain of gelsolin, it may be concluded that conformational changes in subdomain 2 of actin can directly or allosterically modify actin-gelsolin interaction. One the other hand, because the DNase I binding loop is intimately involved in actin-actin contacts in the filament (Holmes et al., 1990; Lorenz et al., 1993), binding of gelsolin to the actin C-terminus may allosterically influence the actin-binding properties of the DNase I-binding loop.

Another part of the actin monomer affected by gelsolin is apparently the C-terminus, because interaction with gelsolin changes the environment of Cys^{374}, where the fluorescent probe is attached. An increase in pyrenyl fluorescence upon the formation of ternary complexes of actin and gelsolin has been reported before (Bryan and Kurth, 1984; Weeds et al., 1986). We have observed this effect with both gelsolin and with the N-terminal 40-kDa gelsolin fragment, but not with the C-terminal gelsolin fragment, which appears to bind one actin monomer (Chaponnier et al., 1986; Pope et al., 1995). The appearance of a peak at 365 nm in the excitation spectrum of pyrenyl-actin is also characteristic of actin-actin association (Kouyama and Mihashi, 1981). Therefore

the observed gelsolin-induced increase in the pyrenyl fluorescence might be explained by direct interaction between the two actin monomers involved in the complex rather than by actin-gelsolin interaction.

However, the fluorescence increase created by complex formation with gelsolin was almost as high for ECP-actin as for intact actin, whereas the polymerization-induced increase in pyrenyl fluorescence of ECP-actin was only about one-fifth of that in intact actin (Khaitlina et al., 1993). Moreover, gelsolin induced nearly the same increase in *N*-7-nitrobenz-2-oxa-1,3-diazol-4-yl-amino- and pyrenyl-fluorescence intensities, whereas the polymerization-dependent changes in fluorescence intensities differ for the two by one order of magnitude. It is also of importance that 20% of the fluorescence was preserved after replacing one pyrenyl actin by an unlabeled one. We may therefore assume that the conformational change in the C-terminal region of actin upon binding of gelsolin results from both the formation of dimers and the effect of gelsolin on the actin conformation.

This conclusion is consistent with the atomic model of the gelsolin segment I-actin complex (McLaughlin et al., 1993), as well as with the observation of C-terminus-associated conformational changes in gelsolin-nucleated filaments (Orlova et al., 1995; Prochniewicz et al., 1996). The clear enhancement of actin cross-linking between Cys^{374} and Lys^{191} that we observed in gelsolin-nucleated actin filaments as compared to normal F-actin, directly revealed an effect on either the position or the stability of the C-terminal segment of actin. In addition, because the increased cross-linking was evident, even at low gelsolin:actin ratios, it indicated a propagation of the conformational changes along the actin filament. This finding confirms the conclusions of other authors (Orlova et al., 1995; Prochniewicz et al., 1996) obtained by different methods.

It has been shown recently that removal of the three C-terminal amino acids of actin causes an inhibition of subtilisin cleavage within the DNase I-binding loop (Strzelecka-Golaszewska et al., 1995). On the other hand, modification of the DNase I-binding loop upon binding of DNase I (Crosbie et al., 1994) or by proteolysis (Kuznetsova et al., 1996) resulted in structural alterations within the subdomain 1 of actin. It was suggested in these works that the conformational coupling between the C-terminus and the DNase I-binding loop is the basis for regulation of actin by several actin-binding proteins. Because gelsolin is shown to directly interact with subdomains 1 and 3 of the actin monomer (Pope at al., 1991; McLaughlin et al., 1993), our observation of gelsolin-induced changes within the DNase I-binding loop, which restore the ability of ECP-(Ca)actin to be incorporated into filaments, strongly supports this idea.

The authors thank Stefanie Gehner for her excellent technical assistance.

This work was supported by the Deutsche Forschungsgemeinschaft and in part by the Russian Foundation for Fundamental Investigations.

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