Interactions of Gelsolin and Gelsolin–Actin Complexes with Actin. Effects of Calcium on Actin Nucleation, Filament Severing, and End Blocking†

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ABSTRACT: Gelsolin is a calcium binding protein that shortens actin filaments. This effect occurs in the presence but not in the absence of micromolar calcium ion concentrations and is partially reversed following removal of calcium ions. Once two actin molecules have bound to gelsolin in solutions containing Ca^{2+}, one of the actins remains bound following chelation of calcium, so that the reversal of gelsolin's effect cannot be accounted for simply by its dissociation from the ends of the shortened filaments to allow for elongation. In this paper, the interactions with actin of the ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA) stable 1:1 gelsolin–actin complexes are compared with those of free gelsolin. The abilities of free or complexed gelsolin to sever actin filaments, nucleate filament assembly, bind to the fast growing (+) filament ends, and lower the filament size distribution in the presence of either Ca^{2+} or EGTA were examined. The results show that both free gelsolin and gelsolin–actin complexes are highly dependent on Ca^{2+} concentration when present in a molar ratio to actin less than 1:50. The gelsolin–actin complexes, however, differ from free gelsolin in that they have a higher affinity for (+) filament ends in EGTA and they cannot sever filaments in calcium. The limited reversal of actin–gelsolin binding following removal of calcium and the calcium sensitivity of nucleation by complexes suggest an alternative to reannealing of shortened filaments that involves redistribution of actin monomers and may account for the calcium-sensitive functional reversibility of the solation of actin by gelsolin.

Gelsolin is an actin binding protein of mammalian cell cytoplasm (Yin et al., 1981a) and of plasma (Norberg et al., 1979; Chaponnier et al., 1979; Harris et al., 1980; Harris & Schwartz, 1981; Yin et al., 1984). It was first isolated from macrophages (Yin & Stossel, 1979) and found responsible for inhibiting the gelation of actin in macrophage extracts in the presence but not in the absence of micromolar free Ca^{2+} concentrations. Macrophage gelsolin was subsequently determined to bind two calcium ions with high affinity and to interact with actin in a calcium-sensitive manner (Yin & Stossel, 1980a,b; Yin et al. 1980, 1981b). In a Ca^{2+}-containing medium it rapidly shortens preformed actin filaments, serves as a nucleus for actin polymerization, and binds to the fast-growing (+) ends of actin filaments. The effect of gelsolin–calcium in inhibiting actin gelation is adequately explained by its shortening of the actin fiber length distribution (Yin et al., 1980; Zaner & Stossel, 1983). The rapidity of gelsolin's action suggested that it actively severs actin filaments. Furthermore, gelsolin can also nucleate filament assembly, and as new filaments formed by nucleation of the monomers, further shortening would result from the redistribution of monomers to a larger number of polymers.

In the absence of calcium ions and at low gelsolin to actin ratios, these effects on actin are not observed. Moreover, the decrease in solution viscosity that results from the shortening of actin filaments by gelsolin in the presence of Ca^{2+} is reversed following chelation of free calcium ions by ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), although the rate of viscosity increase is slow compared to the rate of fiber shortening (Yin & Stossel, 1979; Yin et al., 1980). These results suggested that gelsolin could solate an actin filament network following a rise in Ca^{2+} concentration and that when the Ca^{2+} concentration returned to a submicromolar level, the gelsolin would dissociate from the severed filament ends, allowing them to reassemble and reanneal to form an elastic network.

However, several laboratories have recently presented findings inconsistent with this simple interpretation. Harris & Weeds (1983) concluded that although the rate of shortening of actin filaments in 30% glycerol by pig plasma gelsolin depended on the calcium ion concentration, the final extent of depolymerization did not. Bryan & Kurth (1984) and Kurth et al. (1983) pursued the finding of several groups that it was difficult to separate gelsolin–actin complexes from platelets (Wang & Bryan, 1981; Lind et al., 1982; Markey et al., 1982) or other sources (Petrucci et al., 1983). They showed that free platelet gelsolin does not form a tight complex with actin in EGTA as it does in Ca^{2+}. However, once the complex, containing two actins and one gelsolin, forms in Ca^{2+}, only one of the actin monomers dissociates when free Ca^{2+} is chelated by EGTA. The 1:1 complex that remains is stable in EGTA and retains a calcium ion. The difficulty in obtaining free platelet gelsolin was explained by partial activation of the platelets which caused the cytoplasmic gelsolin to be exposed to Ca^{2+} before cell disruption (Kurth & Bryan, 1984). The equimolar complex of actin–platelet gelsolin alters the rate of polymerization of actin in both EGTA and Ca^{2+}, suggesting that it binds to the (+) end of actin filaments with high affinity irrespective of the Ca^{2+} concentration.

In light of the evidence that the binding of platelet gelsolin to actin is not completely reversed following the removal of calcium ions, it became necessary to examine carefully the properties of macrophage gelsolin to determine if they resemble those of platelet gelsolin. In this paper we report that this is the case. We also describe the interaction of free macrophage gelsolin and of EGTA-stable gelsolin–actin complexes with

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G- and F-actin in solutions containing both high or low free Ca^{2+} concentrations. The abilities of free gelsolin and of gelsolin–actin complexes to nucleate actin filament assembly, to block the (+) ends, and to sever actin filaments were compared. In all cases, these interactions were strongly affected by the presence of calcium ion, and with the exception of severing, gelsolin–actin complexes had functional properties similar to those of free gelsolin. Although the gelsolin–actin 1:1 complex is stable in low Ca^{2+} concentrations, actin filaments shortened by gelsolin in the presence of Ca^{2+} elongate when Ca^{2+} is removed, and less gelsolin is bound to a given mass of long actin fibers. A mechanism involving disassembly of blocked actin filaments and the subsequent assembly of new filaments is proposed to account for the calcium-dependent reversibility of gelsolin's effects on actin filaments.

Experimental Procedures

Proteins. Cytoplasmic gelsolin was purified from rabbit alveolar macrophages by the method of Yin & Stossel (1980a,b). Actin was purified from rabbit skeletal muscle by the method of Spudich & Watt (1971) and stored as G-actin in 2 mM tris(hydroxymethyl)aminomethane, 0.2 mM $CaCl_2$, 0.2 mM ATP, and 0.1 mM β-mercaptoethanol, pH 7.6 (buffer A). Vitamin D binding protein (DBP) was purified from citrated human plasma by actin affinity chromatography (Vandekerckhove & Sandoval, 1983). Actin was fluorescently labeled at Cys-374 with *N*-(1-pyrenyl)iodoacetamide (Molecular Probes) as previously described (Kouyama & Mihashi, 1981). Solutions containing labeled and unlabeled G-actin were further purified by gel filtration on Sepharose G-150 to remove contaminating actin oligomers and used within 2 days thereafter. EGTA-stable actin–gelsolin complexes were prepared by incubating actin and gelsolin at a molar ratio of two actin and one gelsolin in 0.2 mM $CaCl_2$ for 10 min and then adding 1 mM EGTA. For some experiments these complexes were further purified by affinity chromatography using immobilized deoxyribonuclease (DNase) (Bretscher & Weber, 1980). For other experiments actin–gelsolin complexes were separated from uncomplexed actin by affinity chromatography using a recently developed monoclonal antibody to gelsolin (Chaponnier & Yin, 1984) and complexes separated from free gelsolin by ion-exchange chromatography as will be described elsewhere (C. Chaponnier et al., unpublished results). Protein concentrations were determined by absorbance assuming an extinction coefficient of 0.62 for 1 mg/mL at 290 nm for unlabeled actin (Gordon et al., 1976) and by the protein–dye binding method of Bradford (1976) for pyrene-labeled actin and all other proteins with bovine serum albumin as standard. All experiments were done at 23 °C.

Actin Polymerization and Depolymerization Measured by Changes in Fluorescence Intensity. The fluorescence intensity of pyrene-labeled actin increases approximately 25-fold when the actin monomer containing the label becomes incorporated into a polymer (Kuoyama & Mihashi, 1981). This large increase in fluorescence intensity allows monitoring of the kinetics of processes that result in changes in the amount of polymerized actin as well as quantification of the proportion of actin in the G and F states.

(*1*) *Nucleation of Actin Assembly.* Pyrene-labeled actin was added to solutions containing gelsolin or complexes of gelsolin and unlabeled actin, and polymerization was initiated by addition of 2 mM $MgCl_2$ and 150 mM KCl. All solutions contained 0.2 mM ATP and 0.2 mM $CaCl_2$, and some contained 1 mM EGTA. Fluorescence was monitored continuously for the first few minutes and periodically thereafter by using an excitation wavelength of 365 nm and monitoring emission at 386 nm with a slit width of 3 nm. The rate of change of fluorescence was measured at a time when 15% of the maximum fluorescence had been attained and the rate of increase was constant. The measurements were done on a Perkin-Elmer 44-E spectrofluorometer.

(*2*) *Blocking of Actin Filament Ends.* The ability of either gelsolin or gelsolin–actin complexes to bind to the (+) end of actin filaments was investigated by two different methods. One method was used by Walsh et al. (1984) to study the binding of villin to F-actin. Pyrene-labeled actin was polymerized in buffer A with 2 mM $MgCl_2$ and 150 mM KCl (buffer B) at a concentration of 10 μM. The F-actin was then diluted to 80 nM in the same buffer containing either 3 nM gelsolin or 3 nM gelsolin–actin complexes purified by DNase affinity chromatography in either 0.2 mM $CaCl_2$ or 0.8 mM EGTA. Since 80 nM actin is well below the critical monomer concentration, the actin filaments will depolymerize at a rate that depends on the number of free filament ends. Proteins such as villin that bind to the (+) end can retard the rate of depolymerization nearly 10-fold (Walsh et al., 1984). The rate of decrease of fluorescence was calculated from the slope of the plot of fluorescence vs. time after 1 min of depolymerization.

The second method measures the change in actin monomer critical concentration after addition of gelsolin or gelsolin–actin complexes to F-actin. Since the critical concentration of the (−) end of actin filaments in buffer B is higher than that of the (+) end (Wegner, 1976; Pollard & Mooseker, 1981; Bonder et al., 1983), the amount of free actin monomers increases as the (+) ends of the filaments become capped.

(*3*) *Severing and Depolymerization of Actin Filaments.* Solutions of F-actin containing labeled and unlabeled actin monomers were formed by incubation of actin in 2 mM $MgCl_2$ and 150 mM KCl for several hours prior to use. The F-actin was then mixed with solutions containing gelsolin, DBP, or unlabeled actin–gelsolin complexes and 200 μL of the mixture immediately placed into fluorescence cuvettes. The delay between mixing and the first fluorescence measurement was 15 s.

(*4*) *Quantification of F-Actin.* The fraction of filamentous actin can be determined by comparing the fluorescence of equal amounts of pyrene-labeled G- and F-actin. The fluorescence of labeled G-actin was determined from a solution containing 1 μM pyrene–actin in buffer A. The fluorescence of F-actin was determined by mixing 1 μM labeled with 30 μM unlabeled actin and incubating in $MgCl_2$ and KCl for 4 h to maximize incorporation of labeled monomers into filaments. No correction was made for the small fraction (<3%) of pyrene–actin that would be expected to remain unpolymerized under these conditions. The fraction of filamentous actin under various conditions was calculated assuming that the sample fluorescence was an average of the fluorescence of pyrene-labeled actin monomers in the G and F states:

$$\text{fraction of F-actin} = (f - G)/(F - G) \qquad (1)$$

where F and G are the fluorescence intensities expected if all of the actin were in the F and G states, respectively, and f is the sample fluorescence. The background fluorescence of identical samples not containing pyrene-labeled actin was less than 3% of the fluorescence of pyrene-labeled F-actin. For experiments in which the amount of F-actin changes, during either polymerization or depolymerization, the rate of change of fluorescence can be used to calculate the rate of polymerization or depolymerization by multiplying the total actin concentration by the time derivative of eq 1.

The data of Harris & Weeds (1983) suggest that the fluorescence of a pyrene-labeled actin monomer at the end of a gelsolin-blocked filament may be low compared to that of a monomer in the interior of a filament, presumably due to a difference in their conformations. The experiments presented in this paper confirm such a difference. Therefore, a decrease in fluorescence not only may be due to an increase in monomeric actin but may also reflect formation of filament ends or complexes with gelsolin. For most experiments in which pyrene–actin has been used, the number of filament ends is negligible compared to the number of actin monomers in filaments. However, for experiments in which the gelsolin:actin ratio is high and the average filament length is short, the low fluorescence of the ends becomes significant. We have used this phenomenon to study the mechanism of gelsolin's interaction with actin.

(5) *Reversibility of Gelsolin–Actin Interactions.* The dissociation of actin from gelsolin following gelation of calcium by EGTA was followed by measuring the fluorescence increase accompanying repolymerization of the released actin monomers. EGTA was added directly to the fluorescence cuvette, and the solution was gently mixed by drawing it up several times into a 200-μL glass micropipet. In some experiments, unlabeled actin at high concentrations was also added in order to polymerize free labeled actin monomers in equilibrium with polymers.

Effects of Gelsolin and Actin–Gelsolin Complexes on Actin Viscosity. F-Actin, formed by polymerizing 24 μM actin in either buffer B or buffer B with 1 mM EGTA, was mixed with various amounts of either free gelsolin or gelsolin–actin complexes to obtain a final actin concentration of 12 μM. The mixtures were immediately introduced into 100-μL glass micropipets, and the time needed for a steel ball of a diameter of 0.025 in. to roll down the tube inclined at 15 deg was measured in separate tubes following 45- and 105-min incubation (MacLean-Fletcher & Pollard, 1980).

Measurement of Actin Filament Length by Electron Microscopy. A solution of 10 μM F-actin identical with samples used for fluorescence measurements of depolymerization rates was diluted to 5 μM in buffer B and immediately applied to 200 mesh formvar-coated copper grids by using a large diameter pipet tip to minimize filament breakage. After being washed with 50 mM Tris and 150 mM NaCl, pH 7.4, the grids were stained with 1% uranyl acetate and rotary shadowed with platinum at 10°. Photographs were taken at three different magnifications from areas selected at random from three different grids. The length of every filament from each image was measured by a computer graphics method (Niederman et al., 1983). A total of 180 filaments was measured.

Sucrose Density Gradient Sedimentation. Linear 5–15% or 5–20% sucrose density gradients were prepared and analyzed as previously described (Yin et al., 1981a,b); 45 fractions were collected from each gradient, and every third fraction was analyzed by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS–PAGE) (Laemmli, 1970). The gels were stained with Coomassie blue dye. In some samples, 50 μg each of catalase, bovine alkaline phosphatase, ovalbumin, and hemoglobin was mixed with the gelsolin samples prior to centrifugation to serve as internal standards.

RESULTS

Acceleration of Actin Assembly. In the presence of 0.2 mM $CaCl_2$, gelsolin increases the initial polymerization rate of 3.2 μM actin at a molar ratio to actin greater than 1:3300 (Figure 1a), whereas in EGTA, an appreciable acceleration of polymerization is seen only at a gelsolin:actin molar ratio greater than 1:10. EGTA itself accelerated actin polymerization approximately 2-fold, far less than the enhancement by gelsolin in calcium at molar ratios above 1:1000. These data are consistent with our previous conclusions based on viscosity measurements that gelsolin promotes actin nucleation more efficiently in calcium (Yin et al., 1981b). Actin–gelsolin complexes, made by incubating unlabeled actin monomers and gelsolin at a molar ratio of 2:1 actin:gelsolin in the presence of 0.2 mM calcium, also accelerates the polymerization of pyrene–actin (Figure 1b). Complexes purified by DNAse affinity chromatography gave similar results (data not shown). As with gelsolin alone, far more actin–gelsolin complex is needed to nucleate actin polymerization in EGTA than in calcium. An appreciable acceleration is seen at molar ratios greater than 1:33. These data indicate that the gelsolin–actin complexes have a slightly less stringent requirement for calcium than free gelsolin, although the extent of nucleation is still very much larger in Ca^{2+} than in EGTA. In contrast to gelsolin alone, however, very low concentrations of actin–gelsolin complex decrease the initial rate of actin polymerization by as much as 50% in EGTA-containing solutions. This effect was first reported by Kurth et al. (1983) and was attributed to the binding of actin–gelsolin complexes to filament ends in the absence of calcium. Such binding must also occur in Ca^{2+}, but a decrease in polymerization rate is not seen, suggesting that there is sufficient nucleus formation to offset the decrease in polymerization rate due to blocking of (+) filament ends.

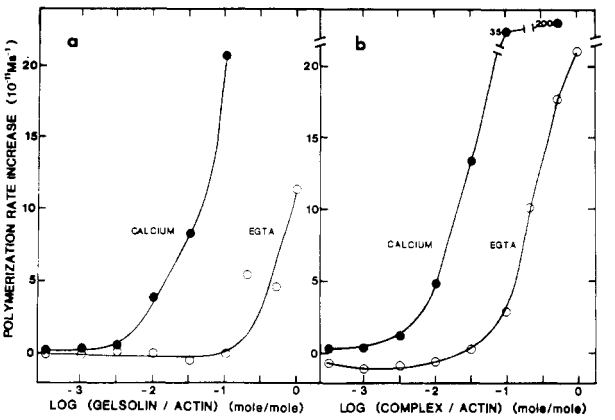

FIGURE 1: Nucleation of actin filament assembly by gelsolin and gelsolin–actin complexes. Pyrene-labeled G-actin at 3.2 μM was incubated with either gelsolin (a) or EGTA-stable gelsolin–actin complexes (b) in either 0.2 mM $CaCl_2$ or 1 mM EGTA immediately prior to addition of 2 mM $MgCl_2$ and 150 mM KCl to initiate polymerization. The rate of polymerization was calculated from the time derivative of eq 1 as described in the text. The greatest polymerization rates, lying off the scale of the ordinate, are given next to their respective points. The rate of assembly of actin alone was 8.8×10^{-10} M/s in calcium and 17.8×10^{-10} M/s in EGTA. These values were subtracted from the rates of nucleated assembly.

Blocking of Actin Filament Ends. The effect of gelsolin and actin–gelsolin complexes on the amount of F-actin at steady state in a 3.2 μM pyrene–actin solution is shown in Figure 2. In the presence of Ca^{2+}, gelsolin decreased the amount of F-actin. This decrease occurs in two phases. The first is maximal at a gelsolin:actin ratio of 1:1000. The amount of actin depolymerized in this phase (0.8 μM) exceeds greatly the amount of added gelsolin, consistent with an increase in critical concentration of actin due to gelsolin's ability to block the fast-growing (+) filament ends. At much higher gelsolin:actin ratios the amount of F-actin fluorescence decreases further in proportion to the amount of added gelsolin, until

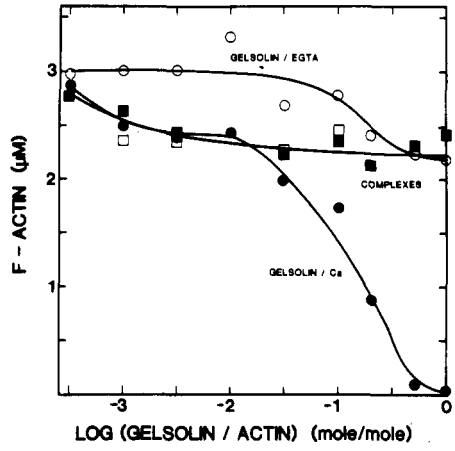

FIGURE 2: Blocking of filament ends as measured by critical monomer concentration. The steady-state fluorescence levels of pyrene–actin–gelsolin solutions were measured 2 h after completion of copolymerization with gelsolin in calcium (●) or EGTA (○) or with gelsolin–actin complexes in calcium (■) or EGTA (□). Conditions are those given in Figure 1.

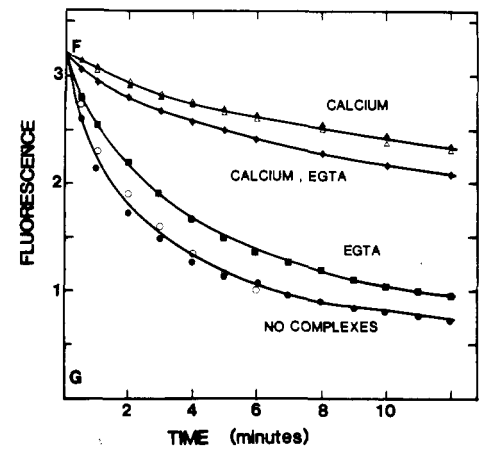

FIGURE 3: Depolymerization of blocked and unblocked filaments diluted below the critical concentration. F-Actin at 10 μM was diluted to 80 nM in buffer B containing either 3 nM gelsolin (△) or 3 nM EGTA-stable actin–gelsolin complexes (▲) in 0.2 mM $CaCl_2$ or actin–gelsolin complexes in 1 mM EGTA (■). The rate of depolymerization was compared with that of F-actin alone in calcium (●) or EGTA (○). In addition, F-actin was diluted into gelsolin–actin complexes in calcium and the EGTA was added 30 s later (♦).

almost no F-actin is measured at a gelsolin:actin ratio of 1:2. This second and continuous decrease is most easily explained by the formation of a large number of complexes containing gelsolin and actin monomers or oligomers in which the pyrene fluorescence is low compared to that of a monomer within an actin filament.

With gelsolin–actin complexes, the initial decrease in F-actin fluorescence at low complex to actin ratios is the same as with gelsolin, but the further decrease at higher ratios is eliminated. Even with equimolar mixtures of complex with actin, the amount of fluorescence decrease is no greater than can be accounted for by an increase in the critical concentration caused by blocking of the fast-growing ends of filaments. The difference between the effects of gelsolin and complexes at high concentration on F-actin fluorescence cannot be attributed to gross differences in the quantity of polymerized actin or in the number of filament ends. As shown below (Figure 7), actin–gelsolin complexes decrease F-actin viscosity to the same extent as free gelsolin, implying that the average filament length and therefore the number of filament ends created by the addition of complexes is comparable to that formed by gelsolin alone. Rather, these data strongly suggest that when pyrene-labeled actin monomers are bound directly to gelsolin following polymerization of labeled actin in the presence of free gelsolin, their fluorescence is low. When unlabeled actin–gelsolin complexes copolymerize with labeled actin, the labeled actin monomers are at least one monomer removed from the filament and have higher fluorescence.[1] In the presence of EGTA, free gelsolin does not decrease the amount of F-actin unless it is present at molar ratios to actin greater than 1:10, ratios that are also sufficient to accelerate actin polymerization (Figure 1a). Even equimolar to actin, gelsolin in EGTA decreases F-actin fluorescence only to an extent consistent with blocking of the (+) end and does not cause the further decrease seen in Ca^{2+}. The gelsolin–actin complexes in EGTA decrease the amount of F-actin at low molar ratios to the same extent as observed in Ca^{2+}, suggesting that the complexes can bind and block (+) ends of actin filaments even in EGTA.

Determination of Binding Affinities of Gelsolin to Actin Filament (+) Ends. The critical monomer concentration is increased nearly maximally by gelsolin–actin complexes or gelsolin–calcium at a concentration of 5×10^{-9} M, suggesting that the affinity of these species for the (+) filament ends is high. An equivalent increase in critical concentration by gelsolin in EGTA requires a concentration above 10^{-6} M, suggesting that gelsolin has at least a 200-fold lower affinity for (+) ends in EGTA compared to Ca^{2+}. The affinity of gelsolin–actin complexes for the (+) end in either Ca^{2+} or EGTA was sufficiently high so that a difference under these two conditions, which is demonstrated below, could not be determined at these relatively high protein concentrations.

The binding of gelsolin and of gelsolin–actin complexes to actin filament ends was further studied by observing the kinetics of depolymerization of dilute actin solutions as shown in Figure 3. Pyrene-labeled F-actin diluted into solutions containing 3×10^{-9} M gelsolin–actin complexes in Ca^{2+} depolymerizes slowly compared to actin alone. In EGTA, gelsolin or gelsolin–actin has little effect on the rate of depolymerization. The depolymerization rate of F-actin added to either free gelsolin or gelsolin–actin complexes in Ca^{2+} is 7% of that of actin alone. This result is similar to the effect seen by Walsh et al. 1984) for actin depolymerization in the presence of villin. This 15-fold difference in depolymerization rate between uncapped and gelsolin-capped actin filaments suggests that the rates of monomer exchange are much slower at the (−) end than the (+) end under these conditions. The binding affinities of gelsolin and gelsolin–actin complexes to filament ends can be estimated from the above experiments. The association constant of gelsolin binding to the (+) end of actin filaments is given by

$$K_a = [\text{bound (+) end}]/([\text{free (+) end}][\text{free gelsolin}]) \quad (2)$$

Assuming that the 93% decrease in depolymerization rate caused by gelsolin or by gelsolin–actin complexes in Ca^{2+} (Figure 3) represents the blocking of at least 93% of the (+)

[1] The constant level of fluorescence as the average filament length is increasingly shortened by gelsolin–actin complexes is in contrast to the recent results of Pantaloni et al. (1984), who have shown, using a similar pyrene–actin fluorescence assay, that the critical monomer concentration of sonically fragmented unblocked actin filaments in ATP depends on the number of filament ends and can become as high as 8 μM, more than twice the total actin concentration in the experiments shown in Figure 2. The lack of increased monomer concentration as the number of filaments ends is increased by gelsolin–actin confirms the conclusion that the mechanism for increasing the critical monomer concentration demonstrated by Pantaloni et al. (1984) involves events at the fast growing (+) filament end, and these events are eliminated when gelsolin binds to this end.

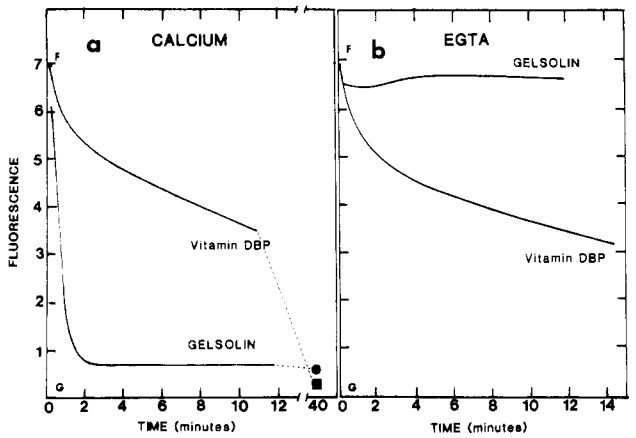

FIGURE 4: Depolymerization of F-actin by gelsolin and vitamin D binding protein (DBP). Fluorescence decrease following addition of either 2 μM gelsolin or 4 μM DBP to 4 μM F actin in buffer B with either 0.2 mM $CaCl_2$ (a) or 1 mM EGTA (b).

filament ends and that the 30% decrease in the depolymerization rate in EGTA represents binding to 30% of the (+) ends, the binding affinity can be quantified if the number of filament ends is known. The number of ends can be calculated from the initial depolymerization rate of pure actin shown in Figure 3. The rate of depolymerization of actin filaments is a function of the number of filament ends and the rate constants for dissociation of monomers from the filament ends:

$$d[\text{actin monomer}]/dt = k_-[\text{actin filaments}] \quad (3)$$

where k_- is the rate constant for dissociation of monomers from both ends of an actin filament. These rate constants have been measured by Bonder et al. (1983) and by Pollard & Mooseker (1981). In the presence of both Mg^{2+} and KCl, the rate constant for dissociation of monomers from both ends of an actin filament is $k_- = 3/s$. The rate of depolymerization was determined by measurement of the initial rate of fluorescence decrease and eq 1. From these measurements, the calculated concentration of filaments is 9.4×10^{-11} M, consistent with the estimate of 1000 monomers per filament for a more concentrated sample based on direct measurements of filament length in the electron microscope as described in the next section. From these quantities, the binding affinity of gelsolin or gelsolin–actin complexes in Ca^{2+} for the (+) ends of actin filaments calculated from eq 2 is at least 4×10^9 M^{-1} in calcium and 40-fold less (1×10^8 M^{-1}) for the complexes in EGTA. On the basis of the difference in affinities seen in Figure 2, the affinity of free gelsolin for the (+) filament end is estimated to be 1×10^7 M^{-1} in the absence of calcium.

Fragmentation of F-Actin. When equimolar amounts of macrophage gelsolin were added to pyrene-labeled F-actin in Ca^{2+}, the fluorescence decreased to levels comparable to that of G-actin as shown in Figure 2. The time course of this depolymerization is very rapid, as shown in Figure 4a. In EGTA, however, very little decrease in fluorescence is seen (Figure 4b), and even after several hours, the fluorescence level remains almost unchanged. Both the kinetics and the calcium sensitivity of the depolymerization of actin by gelsolin differ markedly from depolymerization of actin by DBP which acts by sequestering actin monomers (Coue et al., 1983; Lees et al., 1984). The rate of depolymerization by DBP in calcium (Figure 4a) is much slower than by gelsolin and is insensitive to calcium concentration as shown by comparison with Figure 4b. Thus, gelsolin and DBP are shown by this assay to cause depolymerization by different mechanisms, in agreement with the results of Lees et al. (1984), who compared plasma gelsolin and DBP.

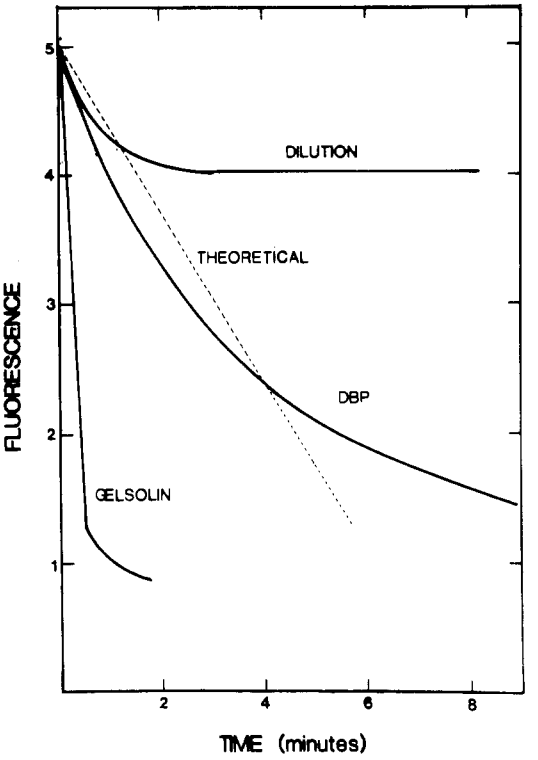

FIGURE 5: Relative rates of depolymerization of F-actin by gelsolin and DBP. Fluorescence decrease following dilution of 10 μM pyrene-labeled F-actin to 5 μM in buffer B alone or with 4 μM DBP or 2 μM gelsolin. The theoretical rate is calculated from average filament length and rate constants for monomer exchange at filament ends as described in the text.

The mechanisms of depolymerization by gelsolin and DBP in Ca^{2+} were further investigated by comparison with the rate of depolymerization of F-actin when it is diluted and partially depolymerizes to restore the critical monomer concentration (Figure 5). The initial rate of depolymerization of actin alone following dilution is the same as that seen when actin filaments are added to DBP, although the final levels are different. This rate may be compared with the theoretical rate of depolymerization calculated from eq 3.

$$d[\text{actin monomer}]/dt = k_-[\text{actin filaments}] = k_-[\text{total actin}]/(\text{monomers per filament}) \quad (4)$$

For this calculation, the number of filament ends is estimated from the average filament length determined by electron microscopy. Measurements of filament length for a sample identical with the sample immediately after dilution gave a value of 3.6 μm for the number average length. Assuming 370 actin monomers per micron (Hanson & Lowy, 1963), the concentration of such filaments in 5 μM F-actin is 3.7×10^{-9} M. The dotted line shown in Figure 5 is the theoretical rate of depolymerization from both ends of 3.7×10^{-9} M actin filaments. This theoretical rate of depolymerization agrees well with the rates of depolymerization obtained either by diluting the actin solution or by adding nearly equimolar DBP concentrations.

However, the rate of depolymerization in the presence of an equimolar amount of gelsolin, also shown in Figure 5, is at least 10 times faster than that induced by dilution or by DBP. Since the more rapidly exchanging (+) filament end is blocked by gelsolin, depolymerization, if it were caused by removal of monomers from the end, would be expected to be even slower than in the presence of DBP. Therefore, the large acceleration in fluorescence decrease caused by gelsolin could result entirely from severing of filaments by gelsolin or from

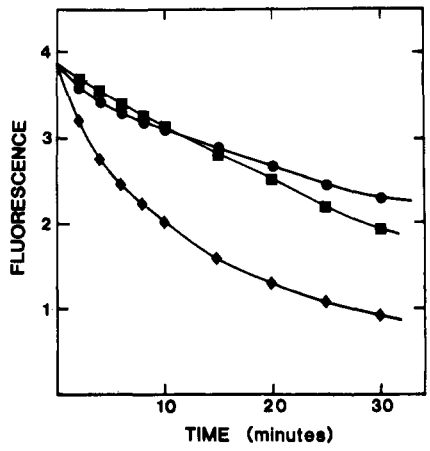

FIGURE 6: Severing of actin filaments by gelsolin but not gelsolin–actin complexes. F-Actin at 20 μM (30% pyrene labeled) was mixed with buffer B (●), free gelsolin (♦), or purified 1:1 gelsolin–actin complexes (■) and immediately diluted to 200 nM actin and either 23 nM gelsolin or 57 nM complexes.

a combination of filament fragmentation and depolymerization from an increased number of filament ends following filament breakage.

Since the fluorescence of F-actin is not decreased following addition of an equimolar amount of unlabeled actin–gelsolin complexes (Figure 2), the assay described above cannot be used to investigate the severing activity of actin–gelsolin complexes. In order to determine if actin–gelsolin complexes can sever actin filaments in calcium, thereby increasing the number of filament ends, F-actin was mixed with either free gelsolin or 1:1 actin–gelsolin complexes. The mixture was immediately diluted to 200 nM, well below the critical monomer concentration of the (−) filament end but near the critical concentration of the (+) end so that depolymerization would occur primarily at the (−) end at a rate reflecting the number of filaments and would be minimally retarded by blocking of (+) ends by gelsolin. Addition of free gelsolin to F-actin increased the rate of depolymerization of filaments (Figure 6), consistent with its ability to sever the filaments to form more ends for depolymerization. Gelsolin–actin complexes, however, did not increase the rate of depolymerization, suggesting that these complexes cannot sever actin filaments.

Although gelsolin–actin complexes are unable to sever actin filaments, they do lower the final viscosity of F-actin in a calcium-sensitive manner. Figure 7 shows the relative apparent viscosities of F-actin incubated in various concentrations of either free gelsolin or gelsolin–actin complexes in calcium or EGTA. As reported previously (Yin et al., 1981b), both free gelsolin and the complexes lower the viscosity far more effectively in calcium than in EGTA. The complexes are as effective in this respect as free gelsolin. Since the complexes do not sever actin filaments, the decrease in viscosity must occur by a different mechanism such as through redistribution of monomers onto nucleating gelsolin–actin complexes. Although the latter mechanism would be slower than direct severing, it can easily occur in the relatively long time needed to make the viscosity measurements.

Reversibility of Gelsolin–Actin Binding. The reversibility of the interaction of gelsolin with actin in Ca^{2+} following chelation of free Ca^{2+} by EGTA was investigated by three different studies. EGTA was added to gelsolin-blocked actin filaments after dilution below their critical concentration to detect increased depolymerization due to unblocking of filament (+) ends. In addition, polymerization of actin monomers dissociated from complexes with gelsolin was studied by addition of EGTA and unlabeled actin monomers to gelsolin–pyrene–actin complexes in calcium. Changes in the amount of gelsolin cosedimenting with F-actin and in the migration of gelsolin in sucrose density gradients following addition of EGTA to gelsolin–actin mixtures in calcium were also examined.

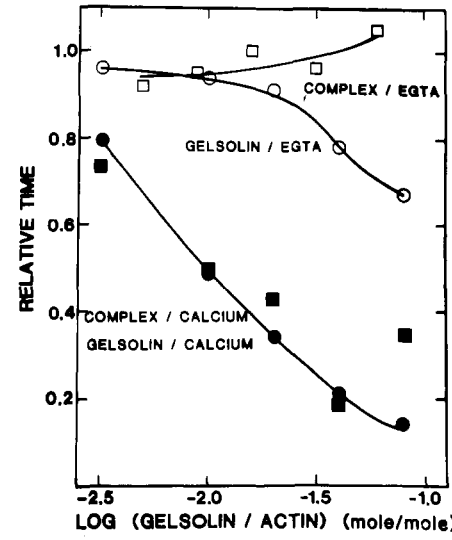

FIGURE 7: Effects of gelsolin and gelsolin–actin complexes on low shear viscosity of F-actin. Relative falling ball time of 12 μM F-actin incubated with gelsolin (●, ○) or EGTA-stable gelsolin–actin complexes (■, □) in buffer B (closed symbols) or buffer B with 1 mM EGTA (open symbols).

(*1*) *Depolymerization of Gelsolin-Capped Actin Filaments following Dilution.* Addition of EGTA to F-actin incubated in calcium with either gelsolin or gelsolin–actin complexes and diluted to below the critical monomer concentration did not cause the large increase in depolymerization rate following dilution that would be expected if the gelsolin had dissociated from the filament ends following chelation of calcium. The lack of substantial reversal, shown in Figure 3, suggests either that the gelsolin does not dissociate from the filament end or that the rate of release is slow compared to the rate of exchange of actin monomers.

(*2*) *Repolymerization of Actin Complexed with Gelsolin.* The extent of reversal of the calcium-dependent interaction of gelsolin with actin following addition of EGTA was investigated further in the experiment shown in Figure 8. When gelsolin (7 μM) was added to actin (9 μM) in the presence of calcium (0.2 mM), the fluorescence dropped quickly to a level near that for monomeric actin. When calcium was chelated by addition of 1 mM EGTA, the fluorescence immediately began to increase and reached a maximum after 5 min which was considerably lower than that of the F-actin control. To estimate the amount of free labeled actin monomers released by addition of EGTA, excess unlabeled actin was added to polymerize labeled actin maximally. From the total increase in fluorescence, we estimate that 60% of the actin was dissociated from gelsolin following removal of free calcium from actin–gelsolin mixtures. This result is consistent with the finding (Bryan et al., 1984) that half of the actin monomers bound to gelsolin can be eluted by EGTA.

In the experiment shown in Figure 9, pyrene–actin and macrophage gelsolin at a 2:1 actin:gelsolin ratio, incubated with either calcium or EGTA, were mixed with a 6-fold excess of unlabeled actin under polymerizing conditions. The fluorescence of the sample containing Ca^{2+} changed very little following addition of unlabeled actin. Since the actin–gelsolin complexes present in calcium can nucleate filament assembly,

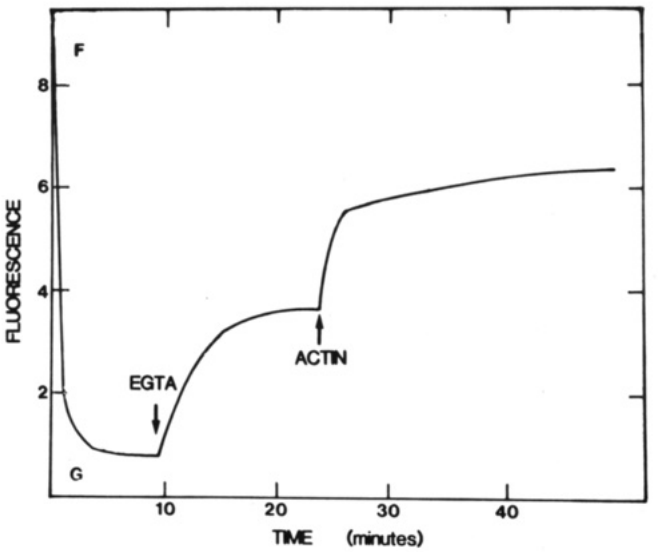

FIGURE 8: Limited polymerization of pyrene–actin following addition of EGTA to actin–gelsolin complexes. Fluorescence intentisy of 9 μM pyrene-labeled F-actin following successive addition of 7 μM gelsolin in buffer B, 2 mM EGTA, and 11 μM unlabeled actin. The letters G and F near the ordinate denote the fluorescence levels of 9 μM pyrene-labeled G-actin and F-actin, respectively. Similar results were also obtained at 37 °C (data not shown).

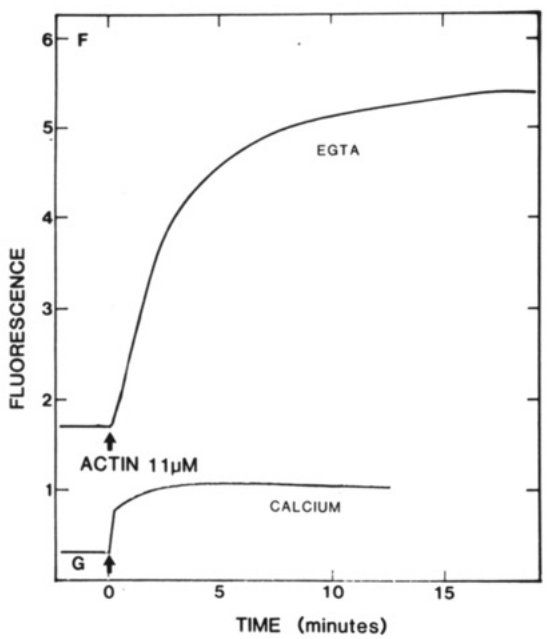

FIGURE 9: Lowered fluorescence and irreversibility of actin–gelsolin complexes in calcium. Fluorescence increase following addition of 11 μM unlabeled actin monomers to a mixture of 2 μM pyrene-labeled F-actin and 1 μM gelsolin in buffer B (lower curve) or buffer B with 1 mM EGTA (upper curve).

the labeled actin in these complexes should be located at the ends of filaments following addition of unlabeled actin. The lack of a fluorescence increase clearly demonstrates that pyrene-labeled actin monomers at the (+) end of a filament do not undergo the large fluorescence enhancement seen for monomers in the filament interior. The finding that the fluorescence does not increase after long times is evidence that the labeled actin at the (+) end cannot exchange with the unlabeled monomers in solution. The fluorescence of the EGTA-containing sample increased immediately after the addition of unlabeled actin to a level very nearly that of completely polymerized pyrene–actin. This result suggests either that, in EGTA, the fluorescence decrease caused by

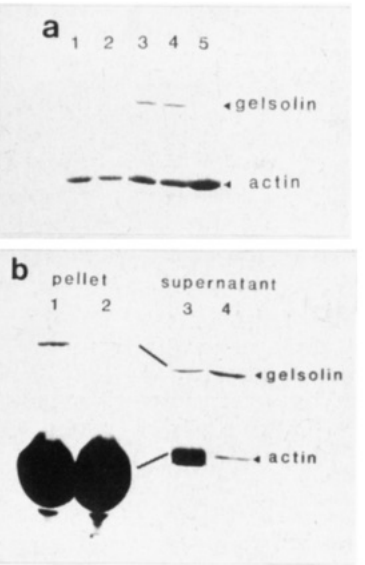

FIGURE 10: Binding of gelsolin to actin filaments. (a) Depletion of gelsolin from supernatants by cosedimentation with F-actin. F-Actin (20 μM) was incubated for 30 min at 25 °C with gelsolin (0.23 μM) in 100-μL solutions containing 0.15 M NaCl, 5 mM ATP, 1 mM $MgCl_2$, 0.5 mM $CaCl_2$, and 20 mM Tris-HCl, pH 7.4 (solution C), with or without 1 mM EGTA. EGTA was then added to one of the calcium-containing samples to 1 mM, and all samples were immediately centrifuged for 30 min at 30 psi in a Beckman airfuge. The supernatants were analyzed by SDS–PAGE. Control actin in calcium (lane 1); control actin in EGTA (lane 2); gelsolin and actin in calcium and then EGTA (lane 3); gelsolin and actin in EGTA (lane 4); gelsolin and actin in calcium (lane 5). (b) Release of gelsolin from sedimentable actin after incubation of F-actin–gelsolin complexes in EGTA. A gelsolin–F-actin pellet formed in calcium by centrifuging (2 h, 93000g at 4 °C) 1.4 μM gelsolin and 104.6 μM actin in solution C was resuspended in the above buffer. After 1 h, EGTA was added to half of the sample to 1 mM, and the samples were centrifuged for 30 min at 20 psi in an airfuge. The pellets formed were analyzed by SDS–PAGE. Pellets and supernatants in calcium (lanes 1 and 3) or in EGTA (lanes 2 and 4). The large amount of nonsedimentable actin in the calcium sample (lane 3) is probably due to incomplete pelleting of actin filaments shortened by gelsolin during the second spin which was at lower force than the initial pelleting.

gelsolin is due to the production of a high concentration of actin monomers, which can polymerize when the total actin concentration is raised by the addition of unlabeled actin, or that the pyrene–actin bound to gelsolin rapidly exchanges with unlabeled monomers under these conditions. The ability of unlabeled actin monomers to compete with gelsolin for binding to labeled actin in EGTA is consistent with the affinity (10^7 M^{-1}) of gelsolin for the filament (+) end in EGTA estimated above.

(3) Shift in Sedimentation of Actin Complexed with Gelsolin. Gelsolin cosediments with F-actin in a calcium-sensitive manner (Yin & Stossel, 1979). We used cosedimentation to determine if the calcium-dependent interaction of gelsolin with F-actin is affected by subsequent addition of EGTA. Gelsolin was added to F-actin at a molar ratio of 1:100 in the presence of either Ca^{2+} or EGTA. A portion of the Ca^{2+}-containing sample was subsequently incubated with excess EGTA, and the solutions were centrifuged at high speed. Figure 10a shows that gelsolin was depleted from the supernatant in the presence of calcium by cosedimentation with actin (lane 5). Nonsedimentable actin increased, presumably because the actin oligomers generated by gelsolin were too small to sediment and/or there was an increase in the critical concentration for actin polymerization. In EGTA, less gelsolin was depleted from the supernatant (lane 4). There are two explanations for the decrease in gelsolin depletion. First, the affinity of

gelsolin for filaments is lower in EGTA than in calcium. In addition, there are fewer ends for binding because the filaments are not fragmented by gelsolin in EGTA. Gelsolin did not increase the amount of nonsedimentable actin in EGTA, consistent with a lack of cutting and a decreased blocking of actin filaments. EGTA alone did not affect the sedimentability of actin under the centrifugation conditions used here (compare lanes 1 and 2). Lane 3 shows that when the gelsolin–actin solution was incubated first with 0.2 mM $CaCl_2$ and then with 1 mM EGTA before sedimentation, the amounts of actin and gelsolin recovered in the supernatant were comparable to those in lane 4 (not treated with calcium), suggesting that a large proportion of gelsolin bound to actin filaments in Ca^{2+} was no longer associated with sedimentable actin filaments after addition of EGTA. To quantitate the amount of gelsolin released from filaments by EGTA, a gelsolin–F-actin pellet formed in Ca^{2+} was resuspended and incubated in buffer B containing either EGTA or Ca^{2+} centrifuged, and the gelsolin remaining in the pellets was measured by densitometry after SDS–polyacrylamide gel electrophoresis. Figure 10b shows that about 70% of the gelsolin associated with the sedimentable F-actin in Ca^{2+} became nonsedimentable after a 20-min incubation with EGTA.

A shift in the sedimentability of gelsolin bound to actin in calcium followed by exposure to EGTA is demonstrated by sucrose density gradient centrifugation. Figure 11a shows that, in the continuous presence of calcium, gelsolin was detected in a number of fractions but was particularly concentrated in the bottom half of the sucrose gradient, where it was presumably bound to actin polymers of various lengths. In contrast, after subsequent incubation with EGTA, only a small fraction of the gelsolin sedimented to the bottom of the gradient, while the bulk of it remained in the upper half of the gradient (Figure 11b), indicating that it was no longer associated with the more highly sedimentable actin polymers. The sedimentability of actin itself was not affected by EGTA.

To determine whether gelsolin eluted from sedimentable actin filaments in EGTA exists free or as a complex with actin, its sedimentation was compared with that of free gelsolin. High-speed supernatants containing gelsolin that did not cosediment with actin were centrifuged in sucrose density gradients (Figure 11c). Internal standards were added to estimate sedimentation constants. Free gelsolin, which has a sedimentation constant of 4.9 S (Yin & Stossel, 1980a,b), migrated between bovine intestinal alkaline phosphatase (M_r 136 K; s = 6.9 S) and human hemoglobin (s = 1.6 S) on the sucrose density gradient (Figure 11c). The bulk of the gelsolin recovered in the supernatant of the gelsolin–calcium–F-actin sample with EGTA migrated in the sucrose gradient close to alkaline phosphatase, suggesting that it was bound in an equimolar complex with actin. A small amount of gelsolin may exist free of actin under these conditions, since there was a small trailing peak coinciding with the sedimentability of free gelsolin. In contrast, gelsolin that was recovered from the supernatant of the gelsolin–F-actin in calcium migrated ahead of alkaline phosphatase (data not shown), consistent with complexes of two or more actin molecules per gelsolin. These data confirm that once gelsolin binds two actin molecules in calcium, it remains complexed with one actin after the calcium is removed by EGTA. Furthermore, despite the limited reversibility of gelsolin binding to monomers and actin filament ends, gelsolin–actin complexes are dissociated from actin *filaments* when the calcium level decreases.

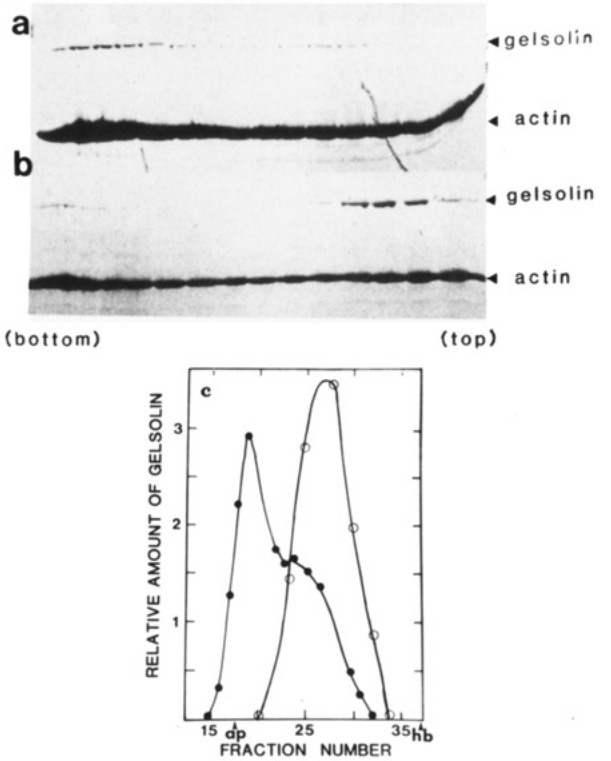

FIGURE 11: Sedimentation profiles of gelsolin. Gelsolin (1.4 μM) was added to F-actin (104.6 μM) in 1 mL of solution C. The actin was centrifuged for 2 h at 93000g at 4 °C and the pellet resuspended in 0.6 mL of the same buffer. After equilibration for 1 h, EGTA was added to half of the sample to 1 mM. Aliquots were analyzed directly (a, b) by sucrose density gradient centrifugation. The remainder was centrifuged for 2 h at 93000g to collect nonsedimentable actin and gelsolin which were then separated in a sucrose gradient (c). (a) Gelsolin–actin solution maintained in 0.2 mM $CaCl_2$ throughout. (b) Gelsolin–calcium–actin solution as above, analyzed after free calcium was chelated by EGTA. 5–20% sucrose density gradient. (c) A comparison of the distribution profiles of gelsolin in a high-speed supernatant derived from treating gelsolin–calcium–F-actin with EGTA (●) and free gelsolin which did not interact with actin (○). EGTA-containing gradients (5–15%) were used to facilitate separation of free gelsolin with 1:1 gelsolin–actin complexes. The profiles were aligned by superimposing the absorbance peaks of internal standards incorporated into each sample, as indicated by arrows. ap, bovine alkaline phosphatase; hb, hemoglobin.

DISCUSSION

Macrophage gelsolin, like platelet gelsolin, binds two actin monomers in the presence of calcium ions, but only one of the two bound actin molecules dissociates after chelation of calcium with EGTA (Kurth et al., 1983; Bryan & Kurth, 1984). The existence of an EGTA-stable 1:1 gelsolin–actin complex was documented directly in sucrose density gradients and indirectly in experiments examining the blocking of actin filament ends and the exchange of fluorescent actin monomers with unlabeled actin in EGTA. Therefore, the elongation of actin filaments shortened by gelsolin–Ca^{2+} described previously (Yin & Stossel, 1979; Yin et al., 1980) and in this paper cannot be explained simply by the dissociation of gelsolin from actin filament ends.

The basis of the functional reversibility of gelsolin's effects on actin filament length was sought in a survey of the actin-modulating effects of gelsolin and of gelsolin–actin. It is recognized that in experiments in which free gelsolin is mixed with F-actin, gelsolin may either bind directly to F-actin or bind to actin monomers in equilibrium with the filaments to form complexes. Nevertheless, results of such experiments differ from those in which preformed 2:1 actin–gelsolin complexes are added directly to F-actin, suggesting that direct gelsolin–actin filament interaction predominates when free gelsolin is added to F-actin. These results are summarized

Table I: Comparison of Gelsolin and Gelsolin–Actin Complexes

		gelsolin		1:1 complexes	
		calcium	EGTA	calcium	EGTA
(1) nucleation	acceleration of actin polymerization at molar ratio to actin of 1:100	5.5	1.5	6.7	0.70
(2) blocking	binding affinity for filament (+) end, K_a (M^{-1})	$>4 \times 10^9$	10^7	$>4 \times 10^9$	1×10^8
(3) viscosity decrease	relative falling ball time at molar ratio of 1:100	0.55	0.95	0.5	0.96
(4) severing	ability to fragment actin filaments	yes	no	no	no

in Table I. The most important difference between the effects of free gelsolin and preformed actin–gelsolin complexes is the unique ability of free gelsolin to sever F-actin in micromolar Ca^{2+} as evidenced by a rapid decrease in pyrene–actin fluorescence (Figure 4).

The summary table emphasizes that, except for blocking of the (+) end of actin filaments, the functions of both free gelsolin and gelsolin–actin complexes are strongly influenced by calcium. Although, as was recognized earlier (Yin & Stossel, 1979; Yin et al., 1981b; Bryan & Kurth, 1984), gelsolin does interact weakly with actin in the absence of calcium, Ca^{2+} increases the affinity of gelsolin for actin filament ends at least 200-fold. This effect is reflected in the much greater potency of gelsolin for nucleating actin assembly and severing F-actin in the presence, relative to the absence, of free Ca^{2+}. Of these interactions, F-actin severing by gelsolin has the most stringent requirement for calcium.

Calcium also increases the affinity of gelsolin–actin complexes for actin. However, the affinity of the 1:1 complex for actin in the absence of Ca^{2+} is much higher than that of free gelsolin. This difference is consistent with the greater ability of the complex, compared with free gelsolin, to block the (+) end of actin filaments and to nucleate actin assembly at high complex to actin ratios in the absence of Ca^{2+}. The nucleating ability of the complexes at all ratios to actin, however, is clearly enhanced by Ca^{2+}. A predominance of actin filament end-blocking activity over the promotion of nucleation by gelsolin–actin complexes at low complex to actin ratios in the presence of EGTA was first described for platelet gelsolin (Kurth et al., 1983).

The findings reported here provide some explanations of the variable calcium sensitivity reported by others for the actin-modulating effects of gelsolin and gelsolin–actin complexes. Our results with macrophage gelsolin and gelsolin–actin complexes are entirely consistent with those reported for platelet gelsolin (Kurth et al., 1983; Bryan & Kurth, 1984), and we conclude that the interactions of both macrophage and platelet gelsolin with actin are regulated by calcium. On the basis of limited direct comparison of macrophage and human plasma gelsolin (data not shown), we conclude that the latter, contrary to the results of Harris & Weeds (1983), is also highly calcium sensitive. An apparent lack of calcium sensitivity of the effect of gelsolin–actin complexes on actin viscosity and nucleation may be ascribable to the use of high complex to actin ratios where the calcium-independent effects of the complexes are maximally expressed. Observations of an apparently high activity of free gelsolin in EGTA can often be explained by the fact that gelsolin–Ca^{2+} was added directly to actin in EGTA and not treated with EGTA prior to addition to actin in EGTA. It is our experience that in the former case there is residual activity which suggests that the gelsolin-bound calcium ion cannot be chelated rapidly enough by EGTA to stop the interaction of gelsolin–Ca^{2+} with actin. Although the relevance of calcium-independent effects of gelsolin on actin in vivo cannot presently be resolved, it is of interest that the estimated cytoplasmic gelsolin concentration relative to actin is sufficiently low [approximately 1:100, assuming a uniform distribution of both components (C. Chaponnier, unpublished experiments)] so that the calcium-independent effects of gelsolin on actin should be small, and accordingly, Ca^{2+} would effect a highly significant activation of both free and complexed gelsolin function.

Our data confirm that following binding to actin filament ends in Ca^{2+} gelsolin does not dissociate readily when treated with EGTA. Therefore, the functional reversal of actin shortening by EGTA cannot be ascribed to dissociation of gelsolin from filament ends, to allow filament growth by elongation from the uncapped (+) end or annealing of filament fragments. Instead, the calcium sensitivity of gelsolin–actin complexes in nucleating filament growth in conjunction with the calcium insensitivity of blocking of (+) filament ends provides a reasonable alternative explanation as to how removal of Ca^{2+} permits lengthening of actin filaments shortened by gelsolin–calcium.

Carlier et al. (1984) have recently provided evidence that the stability of actin nuclei can influence the length distribution of actin filaments. According to their formulation, the continuous reversible exchange of monomers from unblocked filaments will lead to the eventual disappearance of some of the filaments. Because the rate of nucleus formation is significantly slower than the rate of addition of actin monomers to filament ends, there is a drift of monomers from short filaments to longer ones, and this process is balanced by filament fragmentation. In the presence of Ca^{2+} the promotion of actin nucleation by gelsolin or 2:1 actin–gelsolin complexes will stabilize nuclei so that the number of filaments remains constant. On subsequent removal of Ca^{2+}, however, 1:1 actin–gelsolin complexes are formed that do not stabilize actin nuclei, although they can bind to (+) filament ends. We have recently demonstrated such a gradual elongation of gelsolin-capped actin filaments following chelation of Ca^{2+}, and the kinetics of disappearance of filaments is in agreement with the predictions of Carlier et al. (1984) (unpublished results). In fact, this binding will further favor the redistribution of actin monomers to longer filaments, because the gelsolin-containing filaments will only have exposed (−) ends that will tend to lose monomers to other filaments with both (−) and (+) ends free. Such unblocked filaments may arise by spontaneous nucleation following the net rise in actin monomer concentration that accompanies blocking of actin filaments at their (+) ends. Therefore, although macrophage gelsolin resembles platelet gelsolin in forming an apparently EGTA-irreversible complex with an actin monomer following exposure to calcium, its ability to shorten actin filaments can still be reversed following removal of calcium. Nevertheless, since the property of severing actin filaments is unique to free gelsolin, the kinetics of actin filament shortening in response to a rise in cytosolic calcium would be very different depending on whether free gelsolin or the gelsolin–actin complex predominates.

The finding that macrophage gelsolin activated by Ca^{2+} to bind actin remains tightly complexed with actin after subsequent chelation of free Ca^{2+} must be reconciled with the observation that the bulk of cytoplasmic gelsolin is easily purified from macrophages (Yin & Stossel, 1980a,b; C. Chaponnier, unpublished experiments) and from smooth muscles (Hinssen et al., 1984; H. L. Yin, unpublished experiments) and cardiac

muscles (Rouayrenc et al., 1984) in a free state, despite the fact that these are motile cells in vivo, and that this motility is believed to depend on episodic increases in cytoplasmic free Ca^{2+}. Therefore, either gelsolin turnover is sufficient to clear from the cytoplasm gelsolin-actin complexes formed during movement or as yet unidentified mechanisms exist for dissociating the complex in vivo.

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