Severin is a gelsolin prototype

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A number of Ca^{2+}-activated actin filament severing proteins have been identified in eukaryotic cells of diverse lineages. Gelsolin and villin, with molecular mass of about 80–90 kDa, and severin and fragmin, with molecular mass of about 40 kDa, have been isolated from vertebrates and invertebrates, respectively. We report here a direct comparison of the functional properties of gelsolin and severin, and the finding that the actin filament severing activity of severin, like that of gelsolin, is inhibited by polyphosphoinositides. However, severin does not nucleate actin filament assembly as well as gelsolin. These characteristics are very similar to those ascribed to the NH_2-terminal half of gelsolin, supporting the idea that they are evolutionarily related. Regulation of severin by polyphospholipids raises the possibility that it may participate in agonist-stimulated regulation of the actin cytoskeleton in *Dictyostelium discoideum*.

Calcium ion; Polyphosphoinositide severing protein; Gelsolin; Severin

1. INTRODUCTION

Eukaryotic cells from diverse lineages contain dynamic actin microfilament systems which may be similarly regulated in response to agonist stimulation. A large family of Ca^{2+}-activated actin-severing protein have been identified. These include gelsolin [1] and villin [2], found in vertebrates, and severin and fragmin which are approximately half as large as gelsolin and have hitherto only been found in *Physarum* [3] and *Dictyostelium* [4]. Although there are speculations that the severing proteins are related evolutionarily [5–7], the functional properties of the small severing proteins have not been compared directly with those of the large severing proteins. Of particular interest is the question whether they are, like gelsolin and villin, regulated by phosphatidyl inositol 4-monophosphate (PIP) and 4,5-bisphosphate (PIP2) [8,9]. This regulation may contribute to the rapid actin assembly observed following chemotactic stimulation of *D. discoideum* [10].

2. MATERIAL PROCEDURES

2.1. *Proteins*

Human plasma gelsolin and severin were purified as described in [11,12]. Gelsolin and severin concentrations were estimated using extinction coefficients E_{280} of 117 580 $M^{-1} \cdot cm^{-1}$ [11] and 41 270 $M^{-1} \cdot cm^{-1}$, respectively, calculated from the number of tyrosine and tryptophan residues present in the proteins [6,13].

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Abbreviations: PPIs, polyphosphoinositides; PIP, phosphatidyl inositol 4-monophosphate; PIP_2, phosphatidyl inositol 4,5-bisphosphate

2.2. *Interaction with pyrene-labeled actin*

G-actin was labeled with *N*-(1-pyrenyl)iodoacetamide [14]. Actin nucleation and severing activities were measured by following the rate of polymerization and depolymerization, respectively, of pyrene-labeled actin, as described previously [15]. For severing assays, solutions containing severing proteins were placed in cuvettes and the total volume adjusted to 300 μl with buffer B (2 mM $MgCl_2$, 0.5 mM ATP, 1 mM EGTA, 1.1 mM $CaCl_2$, 150 mM KCl, 20 mM Tris-HCl, pH 7.5). 12 μM pyrene-labeled F-actin, polymerized with 0.15 M KCl and 2 mM $MgCl_2$, was diluted into this mixture to a final concentration of 0.2 μM, and the fluorescence was monitored in an I.S.S. Greg PC fluorescence spectrophotometer. For nucleation assays, pyrene-labeled G-actin was added to 300 μl of severing proteins in buffer B to a final concentration of 2.3 μM. PIP_2 (Sigma) was suspended at a concentration of 1 mg/ml in water by sonication with a Heat Systems (Farmington, NY) W185 apparatus operating at maximum intensity until an optically clear solution was formed.

3. RESULTS

Fig. 1 shows the Coomassie blue staining pattern of severin and gelsolin used in these studies. In each case, 10 μl of the protein solution was analyzed, which would correspond to 1.42 and 1.13 μg of gelsolin and severin, respectively, based on estimates using the calculated extinction coefficients. The relative intensities of the gelsolin and severin bands are consistent with such estimates, validating our quantitation of protein concentrations.

The severing activities of gelsolin and severin were determined by adding various amounts of each protein

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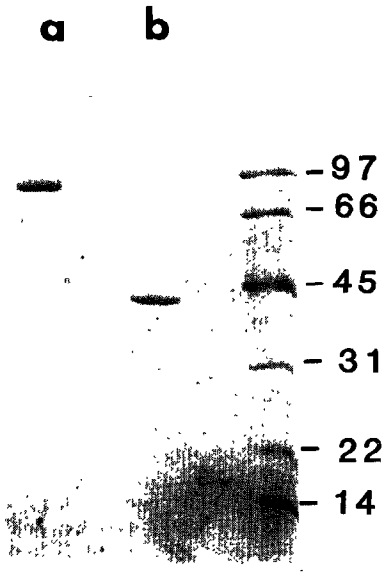

Fig. 1. Coomasie blue staining of gelsolin and severin. 1.42 μg of gelsolin (lane a) and 1.13 μg of severin (lane b) were analyzed. The molecular mass standards are indicated on the right in in kilodaltons.

to pyrene-labeled F-actin prior to dilution and quantifying the number of filament ends from the initial rate of fluorescence decrease. Fig. 2 shows a plot of depolymerization rate as a function of the amount of gelsolin or severin added. Linear least-squares fits to the data are shown, calculated separately for severin and gelsolin. The calculated slopes of these plots are within 9% of each other, demonstrating that these two proteins have approximately equal severing activities.

The severing activity of severin, like that of gelsolin, is inhibited by PIP_2. Fig. 3 shows the relative severing activity of severin in the presence of a range of PIP_2 concentrations. Half-maximal inhibition of 0.1 μM severin is observed at a PIP_2 concentration of 1.6 μM, a molar ratio similar to that observed with gelsolin and PIP_2 [8,16].

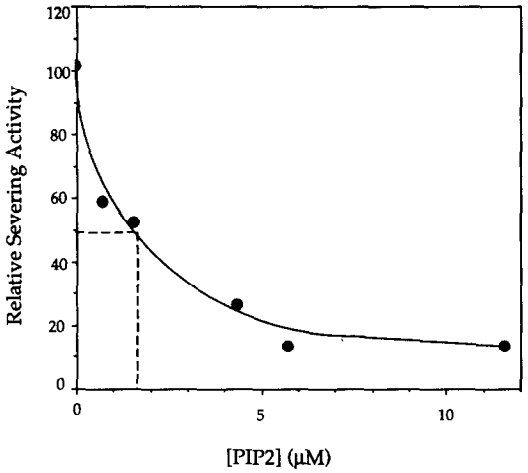

Fig. 3. Effect of PIP_2 on actin filament severing by severin. PIP_2 was added to 104 nM severin, and the effect on actin severing determined.

In contrast to the equal severing activities shown in Fig. 2, the ability of severin to accelerate actin assembly from monomers is much less than that of gelsolin. Fig. 4 shows that 75 nM gelsolin eliminated the lag phase and increased the rate of polymerization of 2.3 μM actin by 9-fold, while 125 nM severin increased the rate by 2-fold. These data suggest that severin does not efficiently form complexes with monomeric actin that can act as nuclei for actin polymerization. Similar low actin nucleating activity accompanying potent severing activity has been reported previously for the NH_2-terminal severing half of gelsolin [17,18] and villin [19].

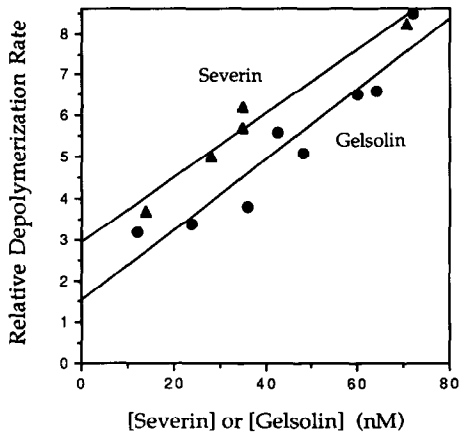

Fig. 2. The rate of actin severing as a function of severing protein concentrations. Actin severing was determined from the rate of depolymerization of pyrene iodoacetamide-labeled actin, and severin, gelsolin concentrations determined from their extinction coefficients. The linear least-squares fit to the data are shown, and the slopes for gelsolin and severin are 0.085 and 0.078, respectively.

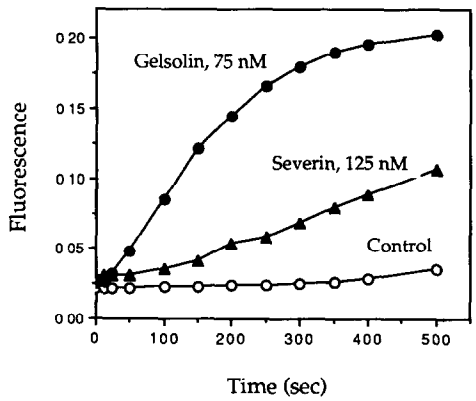

Fig. 4. Effects of severin and gelsolin on actin polymerization. Gelsolin or severin was added to 2.3 μM G-actin to final concentrations of 75 nM and 125 nM, respectively, and the increase in fluorescence monitored. Control, actin alone with no additional protein. The fluorescence of completely polymerized pyrene-actin is approximately 0.3 on this arbitrary scale.

4. DISCUSSION

Direct comparisons of gelsolin and severin activity show that severin severs actin filaments as efficiently as gelsolin, and severing is inhibited by PPI to a similar extent. However, severin does not promote actin nucleation as effectively as gelsolin. These data together with the finding that severin forms an EGTA-resistant 1:1 complex with actin [20] describe characteristics similar to those reported previously for the NH_2-terminal half of gelsolin, which contains the PPI-regulated actin severing domain [18,21]. Therfore, it is reasonable to postulate that gelsolin, which is twice as large as severin, and contains homologous COOH- and NH_2-terminal domains [13], may have evolved from a severin-like molecule through a gene duplication event.

The chemotactic response of developing *D. discoideum* cells is based on two distinct signal transduction pathways: a signal relay system which is mediated by adenylate cyclase, and a signal transmission system towards cytoskeletal elements which is responsible for oriented movement [22–24]. Upon stimulation of *D. discoideum* cells with chemoattractant, a transient and sharp increase of F-actin and IP_3 can be detected within 3–10 s [10,23,25,26]. The finding that severin activity is regulated by PPI suggests that slime molds possess an actin regulatory system similar to that found in vertebrates which is able to mediate actin polymerization in response to chemotactic trigger. Although severin per se does not nucleate actin filament assembly as well as gelsolin, rapid actin polymerization from the 'barbed' end of actin filaments can be achieved through dissociation of severin from the barbed end of short actin oligomers. If this is the mechanism of action [27], we would suggest that the initial decrease in PIP_2 and transient increase in Ca^{2+} following agonist stimulation will promote severing and capping of barbed actin filament ends. A subsequent increase in PIP or PIP_2 would dissociate capping proteins and allow rapid polymerization of filaments from the barbed end. This burst of actin polymerization is facilitated further by the release of polymerizable actin from profilin by PPi [28]. Our data do not rule out that additional nucleation activities such as described by [29] for *D. discoideum* and by [30] for white blood cells are also present. These activitities may compensate for the loss of severin in a *D. discoideum* mutant recently described [31].

In conclusion, based on the similarities and differences in gelsolin and severin, we suggest that the Ca^{2+}-, PPi-regulated actin filament severing function of the severin/gelsolin family has evolved first, and enhanced Ca^{2+}-regulated actin nucleation was acquired through an apparent gene duplication event.

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