Plasma gelsolin caps and severs actin filaments

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Plasma gelsolin caps actin filaments at their 'barbed' ends and severs them along their length. Capping has been demonstrated both by direct visualization using gold-labeled gelsolin and by inhibition of actin polymerization onto the barbed ends of fragments of the acrosomal process of *Limulus* sperm. Severing activity is demonstrated by the fact that actin filaments nucleated off acrosomal fragments are shortened or removed within a few seconds by added plasma gelsolin without any obvious disruption of the actin bundles in the acrosomal processes themselves.

Actin capping protein *Actin polymerization* *Gold labeling*

1. INTRODUCTION

Plasma gelsolin (formerly called actin depolymerizing factor) is a protein of 92 kDa which has been isolated from pig [1], rabbit [2] and human plasma [3]. It rapidly shortens actin filaments, promotes filament assembly from G-actin and increases the critical concentration of monomeric actin when F-actin and gelsolin are mixed together [4]. These observations suggest that plasma gelsolin caps the 'barbed' ends of filaments. Selective capping of this kind has been demonstrated for macrophage and platelet gelsolins [5,6], villin [7,8] and a number of other proteins [9–13], in most cases by inhibition of actin monomer assembly onto actin filaments decorated with myosin subfragment-1 (S-1). This paper describes two types of experiment to demonstrate barbed end capping by plasma gelsolin. The first uses fragments of *Limulus* sperm acrosomal processes to show inhibition of actin assembly on one end [8]. The second method is novel and uses gold-labeled gelsolin to nucleate actin assembly, followed by S-1 decoration to determine polarity. It is further shown that plasma gelsolin shortens actin filaments which have been nucleated from *Limulus* acrosomal processes.

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2. MATERIALS AND METHODS

Pig plasma gelsolin was prepared as in [4] but with two modifications. The first was to substitute polyethylene glycol precipitation at pH 4.6 for the initial ammonium sulphate fractionation and to collect the material that precipitated between 5 and 15%. The second modification was to replace the hydroxyapatite column with an actin-Sepharose affinity column [14] (details to be published elsewhere). The plasma gelsolin produced in this way shows less proteolytic degradation and fewer contamination products as evidenced by polyacrylamide gel electrophoresis. Gelsolin concentration was measured using the DNase inhibition assay [15].

Limulus sperm acrosomal processes (false discharges), prepared as in [16] were a gift from Dr Paul Matsudaira. They were stored in 75 mM KCl, 1 mM $MgCl_2$, 0.2 mM $CaCl_2$, 10 mM Tris–HCl, 1 mM azide (pH 8.0) and broken into fragments approx. 1 μm long by sonication before use.

Actin and myosin subfragment-1 (S-1) were prepared from rabbit skeletal muscle as in [17,18].

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G-actin was prepared from F-actin by dialysis against 0.2 mM ATP, 2 mM Tris–HCl (pH 8.0), 0.2 mM dithiothreitol, 0.1 mM $CaCl_2$, and 1 mM sodium azide.

Colloidal gold with a particle diameter of about 15 nm [19] was prepared by reduction of 0.01% chloroauric acid with trisodium citrate [20] and adjusted to pH 6.2 with 0.2 M K_2CO_3. Five ml of colloidal gold was complexed with a 10% excess of plasma gelsolin by addition of 0.275 ml gelsolin in 2 mM sodium succinate (pH 6.2), 0.1 mM $CaCl_2$, after determining the minimal amount of protein needed to stabilize the gold [21]. Polyethylene glycol (20 kDa) was added at 0.01% (w/v) and the complex was sedimented at 15000 × *g* for 20 min. The complex was resuspended in 10 ml of 0.02% polyethylene glycol and sedimented again as described. Finally the complex was resuspended in 0.5 ml of 100 mM KCl, 20 mM Tris–HCl (pH 7.5), 0.02% polyethylene glycol, 2 mM sodium azide and 0.1 mM $CaCl_2$.

Gold-labeled gelsolin was used to nucleate actin filament assembly by mixing the complex (containing about 80 nM gelsolin) with 2.5 μM G-actin and adding 100 mM KCl and 1 mM $MgCl_2$.

For electron microscopy, 5 μl samples were placed on colloidion and carbon-coated grids washed with 5–10 drops of 20 mM NaCl, 1 mM $MgCl_2$, 5 mM sodium phosphate buffer (pH 7.0) and stained with 1% uranyl acetate. S-1 decoration

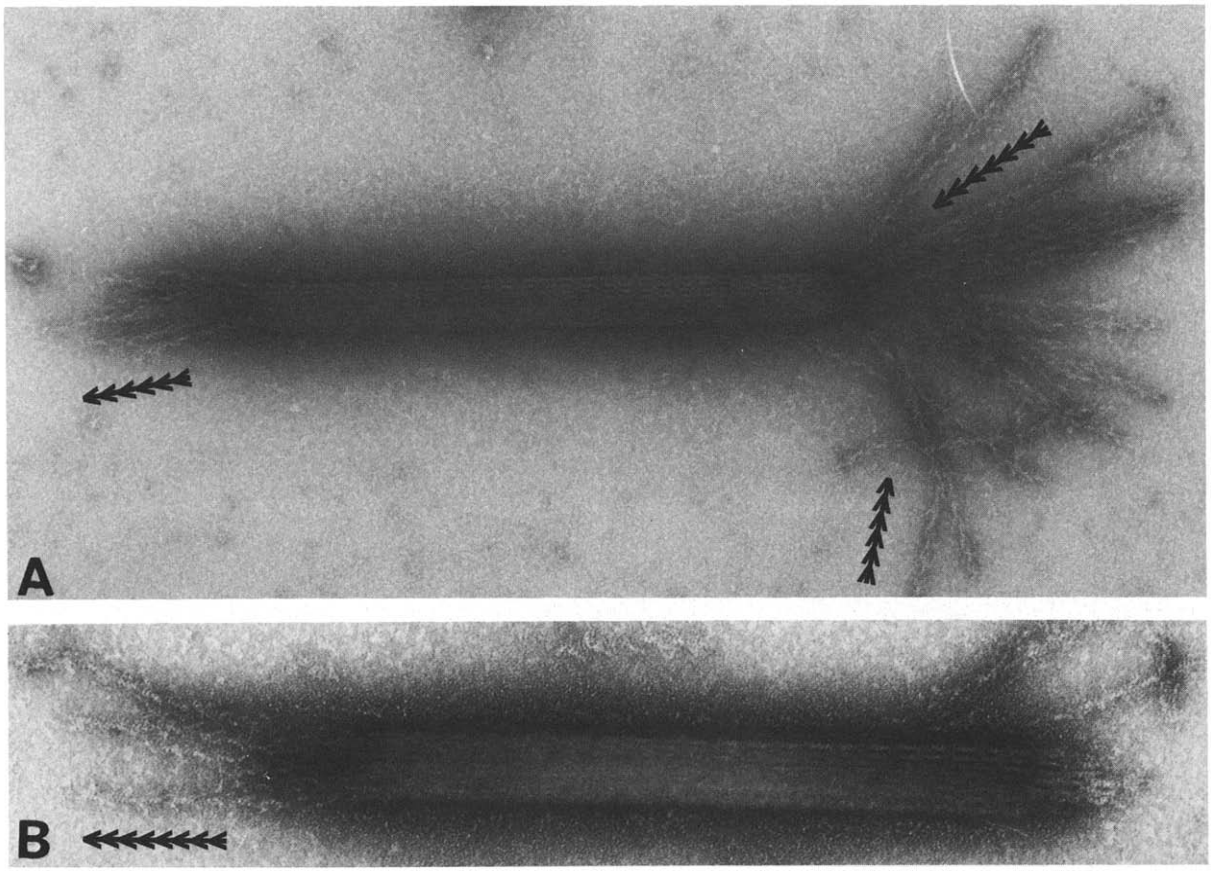

Fig.1. Gelsolin binds to the barbed end of actin filaments. (A) Fragment of a *Limulus* acrosomal bundle which was incubated at room temperature with 3.4 μM G-actin in 10 mM Tris–HCl (pH 8), 75 mM KCl, 70 μM ATP, 1 mM $MgCl_2$, 0.2 mM $CaCl_2$, 1 mM azide, for 30 s, before being placed on a grid and decorated with S-1. (Arrows have been added to indicate the polarity of the S-1 decoration.) (B) Acrosomal bundle preincubated with 0.09 μM gelsolin before treatment with G-actin and subsequent S-1 decoration as in A.

was carried out by washing grids, after application of sample, with 2–5 drops of subfragment-1 at 0.2 mg·ml^{-1} in 30 mM NaCl, 5 mM imidazole–Cl (pH 7.0) and then washing and staining as usual. Grids were examined in a Philips 300 or 301 electron microscope at 80 kV.

3. RESULTS

3.1. *Gelsolin binds to the barbed ends of actin filaments*

The acrosomal processes from *Limulus* sperm are bundles of actin filaments, complexed with two other proteins, which render them highly stable [16]. Sonicated bundles readily nucleate assembly of actin at both ends [8]; much longer filaments extend at the barbed ends (identified by S-1 decoration) than at the 'pointed' ends (fig.1A; [8]). Preincubation of sonicated bundles with plasma gelsolin, before G-actin addition, completely blocks growth of filaments at the barbed end (fig.1B). This was found to be the case for all bundles observed for which polarity could be determined unambiguously. Thus plasma gelsolin binds to the barbed ends of filaments.

The location of gelsolin on actin filaments can also be visualized directly. Complexes of colloidal

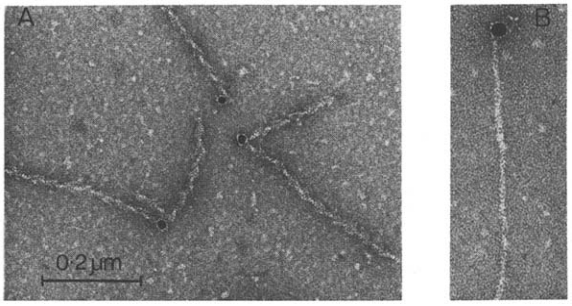

Fig.2. Gold-labeled gelsolin caps the barbed end of actin filaments. (A) Gold-labeled gelsolin used to nucleate the assembly of actin filaments, followed by S-1 decoration (see section 2 for details). (B) Gold-labeled gelsolin attached to undecorated actin filament (at higher magnification, the diameter of the colloidal gold is about 15 nm).

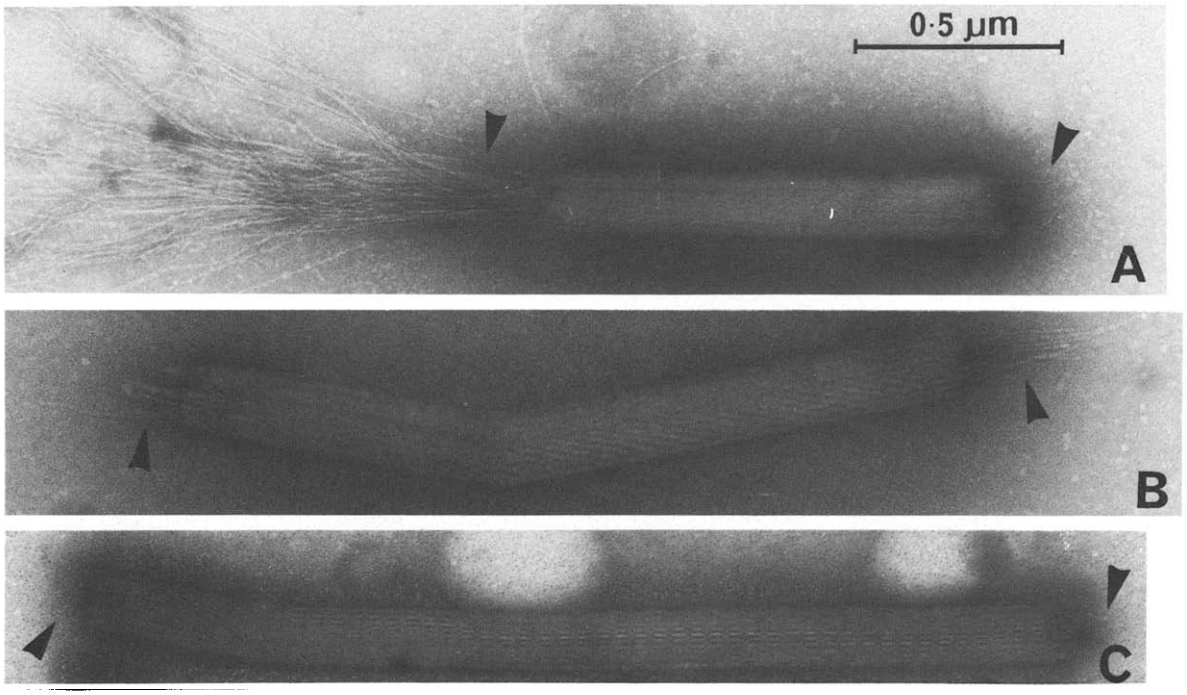

Fig.3. Gelsolin severs actin filaments. (A) Fragment of an acrosomal actin bundle incubated with 4.1 μM G-actin before being placed on a grid (conditions as in fig.1). Arrowheads indicate filaments at end of bundles. (B) Actin bundle incubated with G-actin as in A, and then washed on the grid with 0.23 μM gelsolin before staining. (C) Actin bundle incubated with G-actin as in A, and then washed on the grid with 2.3 μM gelsolin before staining.

Table 1

Effect of gelsolin on actin filaments grown from *Limulus* acrosomal bundles

Conditions	Bundles exhibiting filament growth				
	No filaments	One end	Both equal length	Ends unequal length	Annealed bundles
No gelsolin	2	5	0	19	1
Gelsolin:actin					
1:50	2	4	0	2	0
1:18	8	0	1	0	0
1:6	19	2	0	0	0

A suspension of sonicated bundles was incubated with 4.1 μM G-actin (other conditions as in fig.1) and gelsolin or gelsolin buffer was added to the suspension, to a final mole ratio of gelsolin:G-actin as tabulated. Samples were immediately (within a few seconds) placed on grids, washed and stained. All bundles within a single grid square were scored for growth of filaments from the ends

gold and gelsolin are used to nucleate actin filament assembly and the structures so formed are decorated with S-1 to show their polarity. The filaments are all much shorter than samples without gelsolin and many have gold particles at one end. Fig.2 shows examples: the dense gold spheres are always attached to the barbed ends of filaments. In some cases several filaments are seen attached to the same gold particle, suggesting that several gelsolin molecules have associated with a single gold particle.

3.2. *Gelsolin removes actin from both ends of acrosomal actin bundles*

Sonicated actin bundles from *Limulus* acrosomal processes have clean, unfrayed ends. Under the conditions of fig.3, actin filaments grow to about 2 μm in length at one end of the bundle and 0.1–0.2 μm at the other end. Washing such bundles on the grid with plasma gelsolin removes filaments from both ends of the bundles. At low concentrations, the gelsolin partially removes filaments, so that the two ends are often indistinguishable (fig.3B). The ends of such bundles may also have fewer filaments protruding from them, and these filaments are of unequal lengths. Gelsolin at higher concentrations removes all the filaments down to the unfrayed ends of the bundles (fig.3C). (The bundles themselves are resistant to gelsolin.) Some quantitative data are shown in table 1.

4. DISCUSSION

It is shown here by two different methods that plasma gelsolin caps the barbed ends of actin filaments. The direct attachment of colloidal gold to the capping protein is novel and we have similarly demonstrated the presence of gelsolin at the ends of filaments using a rabbit antibody to plasma gelsolin together with goat anti-rabbit IgG to which gold particles have been complexed (not shown).

Many intracellular proteins with similar capping activity have been described [5–13], though some of these have no severing activity [9–11]. Thus these two activities need not be linked. The mechanism of filament severing remains unclear. Since plasma gelsolin caps the barbed ends of actin filaments producing an increase in the critical concentration, shorter filaments could be produced simply by dissociation of monomers from the free pointed ends. However, shortening by this mechanism cannot explain disruption of filaments whose pointed ends are attached to acrosomal fragments. As shown in fig.3, gelsolin removes actin filaments growing from both ends of *Limulus* acrosomal processes within seconds. This rapid rate of severing (up to 2 μm removed from the barbed end and 0.1–0.2 from the pointed end within seconds) is inconsistent with measured disassembly rates [22,23]. It seems likely that gelsolin, like villin [7,8], severs actin filaments

along their length, leaving the newly generated barbed ends blocked to prevent re-annealing. In addition, actin monomers may dissociate from the free pointed ends [4,24,25]. This seems the only satisfactory explanation for the complete, extremely rapid removal of all labile monomers from the acrosomal bundles.

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