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The control of cellular motility and the role of gelsolin

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Solation of actin gel by gelsolin is much less efficient in the presence of a high concentration of macromolecular solutes. The rigidity of the gel formed by 12 μM actin is lowered from 4 to 0.33 dynes/cm² by 15 nM gelsolin, while in 6% (w/v) polyethylene glycol, rigidity is lowered only from 20 to 11 dynes/cm² by 64 nM gelsolin. Owing to the large concentration of protein, transitions in the fluid- and gel-like properties of the cytoplasm are expected to be problematic when promoted by gelsolin alone.

Gelsolin; Cell motility

1. INTRODUCTION

Suzuki et al. [1] have shown that polyethylene glycol 6000 or a protein, ovalbumin, can make bundles of actin filaments. Starting from this finding, we have undertaken a systematic investigation on the effect of macromolecular solutes on the architecture of the cytoskeleton. We have shown that, in the presence of a large concentration of macromolecular solutes, filamin, tropomyosin and caldesmon modulate the reversible conversion of F-actin into bundles of filaments [2,3], that macromolecular solutes potentiate the actin gelling activity of chicken gizzard α-actinin at physiological temperature [4] and allow smooth muscle actomyosin retraction even in the presence of unphosphorylated smooth muscle myosin [5].

We show here that solation of actin gel by gelsolin is much less efficient in the presence of high concentration of macromolecular solutes and prospect the hypothesis that, in vivo, gel–sol conversion may require the combined action of gelsolin with proteins of the fragmin/severin family.

2. MATERIALS AND METHODS

G-Actin from rabbit muscle was prepared and assayed as previously described [6]. Gelsolin was purified from bovine plasma [7] and tropomyosin from chicken gizzard [8]. Molar concentrations were calculated on the basis of molecular masses of 86 kDa for gelsolin [7] and 65 kDa for tropomyosin [9]. The absorption coefficients used were $A^{1\%}_{280nm}$ = 15.38 for gelsolin [10] and $A^{1\%}_{276nm}$ = 3.85 for tropomyosin [11].

The incubation mixtures were prepared by mildly mixing 6 vols. of the F-actin–tropomyosin salt-buffer solution with 4 vols. of polyethylene glycol–water solutions of appropriate concentration. Gelsolin was added last, 10 min after mixing. Final electrolytes concentration was the following: 104 mM K^+, 16 mM Na^+, 6 mM Mg^{2+} 5 mM orthophosphate, 7 mM ATP, 37 mM Cl^- and 70 mM propionate. pH was 7.0 and temperature 37°C.

To measure the rigidity of the samples, the gels were allowed to form by incubating for 60 min at 37°C the actin–tropomyosin–gelsolin solutions in graduated glass tubes (inner diameter 6 mm). The measurements were performed by delivering, under the meniscus of the solutions, by means of a Terumo microsyringe, 0.003 ml droplets of toluene–carbon tetrachloride mixtures of different density. Each tube was utilized for only one determination. The trials were continued until the density was found that allowed the droplets to remain stationary in the tube. The rigidity (T_o) was calculated according to the equation [12]:

$$T_o = 0.76 \times r \times \alpha \, (\rho_d - \rho_m)$$

where r is the radius of the droplets (0.09 cm), α is the acceleration due to gravity (980 cm·s^{-2}) and ρ_m and ρ_d are the densities of the medium and of the droplets, respectively, in g/ml.

For electron microscopic observation the samples were diluted 5 times with the salt-buffer solution, applied to carbon-coated 400 mesh grids, washed once with one drop of water and stained with 5 drops of a 1% (w/v) uranyl acetate solution, pH 4.25. The samples were transferred by Pasteur pipette to avoid mechanical damage. Electron microscopy was performed on a Hitachi H-800 electron microscope.

3. RESULTS

As is shown in Fig. 1a, the rigidity of the gel formed by 12 μM actin is almost completely abolished by 15 nM gelsolin. The gel formed by 12 μM actin, decorated with 1.5 μM tropomyosin, is more resistant to the action of gelsolin, a feature already reported [13].

More relevant is the finding that, in 6% (w/v) polyethylene glycol, the gels formed by F-actin and by tropomyosin-decorated F-actin are both fully protected against the action of 15 nM gelsolin, their rigidity being 20, 20 and 11–12 dynes/cm² at 0 nM, 15 nM and 64 nM gelsolin, respectively (Fig. 1b).

The possibility that the protection was due to a weaker binding of gelsolin to F-actin, in polyethylene gly-

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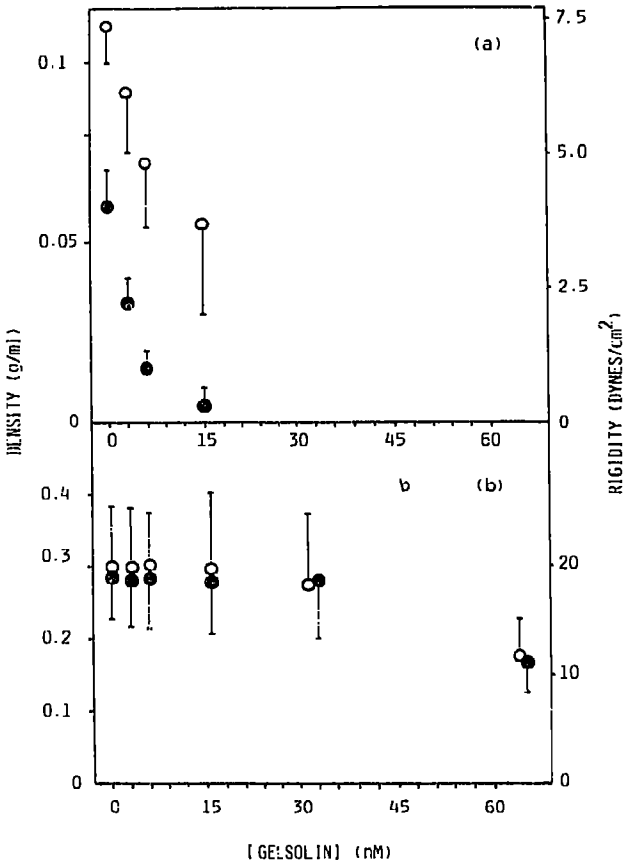

Fig. 1. Effect of increasing concentrations of gelsolin on the rigidity of the network formed by F-actin and by tropomyosin-decorated F-actin, in the presence and in the absence of polyethylene glycol. The mixtures contained F-actin (12 μM as monomer) with (○) or without (●) 1.5 μM tropomyosin and the salt-buffer solution described in section 2. In (b) the mixtures were supplemented by 6% (w/v) polyethylene glycol 6000. Concentration of gelsolin was as indicated in the figure. After 60 min of incubation at 37°C and pH 7.0, rigidity measurements were performed as described in section 2. ⊿Density represents the density at which the droplets remained stationary in the complete system and in the salt solution without protein. Points represent means ± range for quadruplicate experiments.

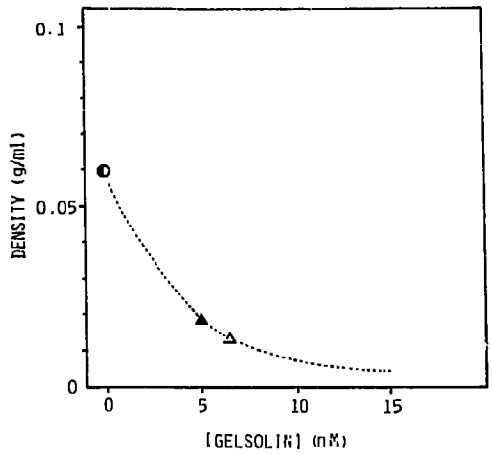

Fig. 2. Estimate of the binding of gelsolin to F-actin, in the presence and in the absence of polyethylene glycol. Either 45 nM (circles) or 120 nM (triangles) gelsolin was added to F-actin (12 μM as monomer) in the presence (filled symbols) or in the absence (empty symbols) of 6% (w/v) polyethylene glycol 6000. After 10 min of incubation at 37°C, F-actin was sedimented by centrifugation at 366,000 rpm for 30 min at 37°C. The supernatant solutions, containing unbound gelsolin, were diluted 6 times with the same salt buffer solution. To the diluted samples F-actin (12 μM as monomer) was added and, after 60 min of incubation at 37°C, the rigidity of the resulting gels was measured. These values were plotted on a calibration curve (dotted line) obtained by measuring the rigidity of F-actin (12 μM as monomer) treated with increasing concentrations of gelsolin.

col, was tested as described in Fig. 2. It was found that polyethylene glycol did not significantly decrease the capability of gelsolin to bind to F-actin.

Electron microscope observation showed the expected fragmentation of F-actin promoted by gelsolin (compare Fig. 3a and b). In the presence of 6% (w/v) polyethylene glycol, the general shape of the bundles of actin filaments did not differ significantly in the presence and in the absence of gelsolin (compare Fig. 3c and d). In the presence of 6% (w/v) polyethylene glycol, tropomyosin-decorated F-actin did not form bundles [2,3]. The addition of gelsolin produced some fragmentation of the filaments (compare Fig. 3e and f).

4. DISCUSSION

In the cell the molar ratio of gelsolin:actin is believed to be about 1:[illegible] value adequate, in vitro, to support nucleation, capping and cutting of actin filaments. Furthermore, since these functions are regulated by Ca^{2+} and by phosphatidylinositol 4,5-bisphosphate [15], gelsolin becomes an attractive candidate to promote gel–sol conversion in the cell.

In the in vitro studies it is usually overlooked that the concentration of macromolecules in the cell is high: a condition that makes bundles of actin filaments [1]. When this is taken into account it is found that solation of actin gel by gelsolin is much less pronounced than in the absence of macromolecular solutes. When the gel is formed by F-actin, the effects of cutting by gelsolin is, very likely, neutralized by the strong latero-lateral association, which takes place in the bundles of filaments. Additional factors must be operating, however, since the presence of a high concentration of macromolecules makes solation by gelsolin less efficient also when the gel is formed by tropomyosin-decorated F-actin, which does not form bundles of filaments. Perhaps, also in this case, the extent of the latero-lateral association increases, even though not to such a level to be detected by the electron microscope. It is also known that the presence of high concentrations of macromolecules decreases substantially the critical concentration of actin [16]; that is to say that it strengthens monomer–monomer interaction, thus hampering the action of gelsolin.

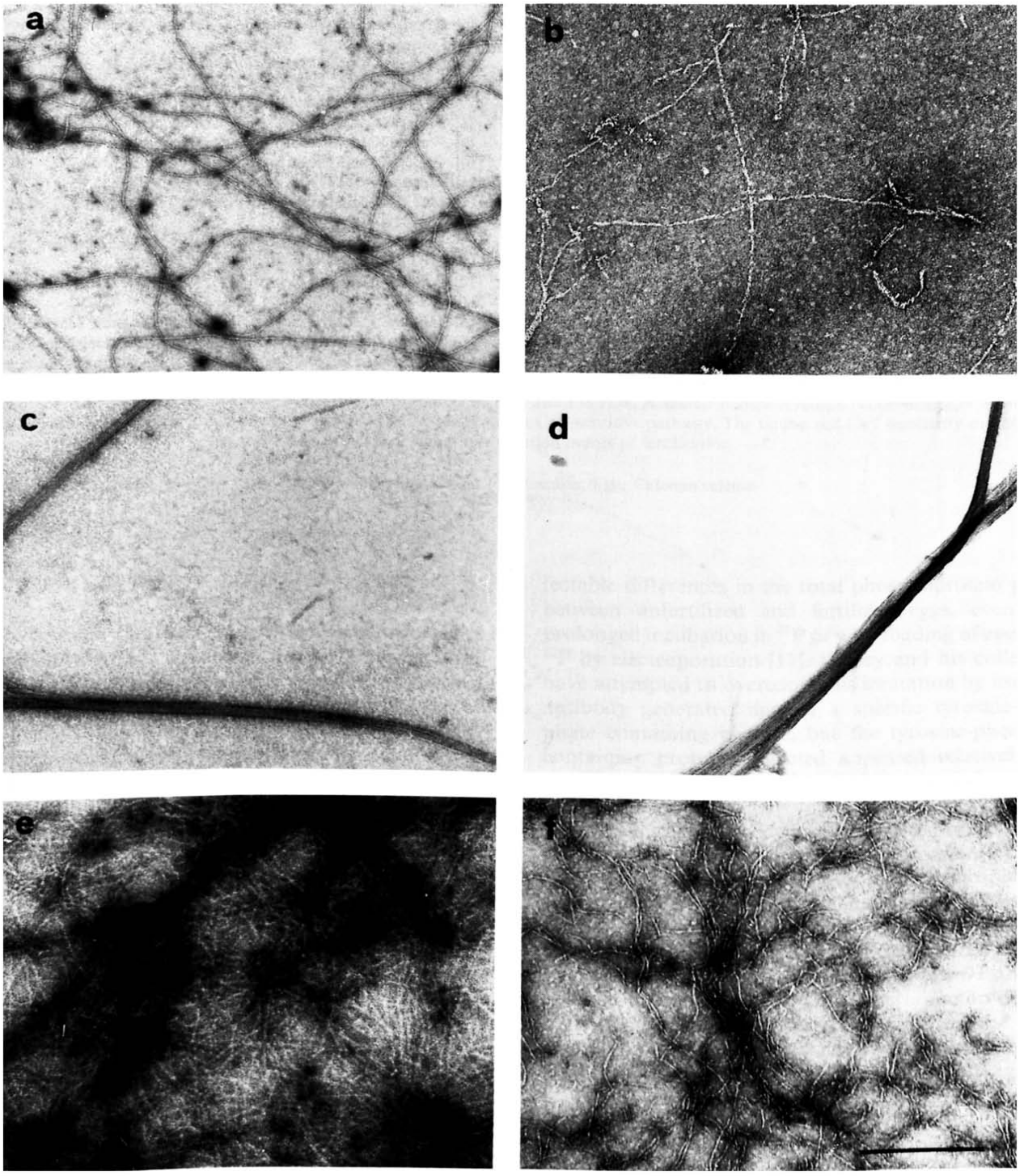

Fig. 3. Electron micrographs of the structures formed by F-actin (12 μM as monomer) (a); F-actin (12 μM as monomer) plus 75 nM gelsolin (b); F-actin (12 μM as monomer) plus 6% polyethylene glycol (c); F-actin (12 μM as monomer) plus 6% polyethylene glycol plus 75 nM gelsolin (d); F-actin (12 μM as monomer) plus 1.5 μM tropomyosin plus 6% polyethylene glycol (e); F-actin (12 μM as monomer) plus tropomyosin 1.5 μM plus 6% polyethylene glycol plus 75 nM gelsolin (f). Bar = 330 nm.

We have previously reported that high concentrations of macromolecular solutes potentiate the effect of the actin gelling proteins [4] and we show now that, under the same conditions, solation of actin gel by gel-

solin is strongly hindered. These two pieces of data support the idea that, in vivo, gelsolin alone may fail to support gel– sol transition and that the contribution of other severing proteins may be required.

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