FILAMIN, A HIGH RELATIVE MOLECULAR MASS ACTIN-BINDING PROTEIN FROM SMOOTH MUSCLES, PROMOTES ACTIN POLYMERIZATION

V. E. KOTELIANSKY, V. P. SHIRINSKY, G. N. GNEUSHEV and V. N. SMIRNOV
National Cardiology Research Center, USSR Academy of Medical Sciences, Petroverigsky lane 10, Moscow, USSR

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1. Introduction

High-M_r actin-binding proteins play an important role in the structural organization and functioning of different muscle types and in cell motility [1–3]. Filamin, a high-M_r actin-binding protein was first isolated from smooth muscles and then identified in skeletal and cardiac muscles [4–8]. Filamin is a dimer with a monomer M_r of 250 000–270 000 [5,6]. Elongated and asymmetric filamin molecules contain several domains formed by a β-structure and interconnected by flexible, mobile segments of the polypeptide chain [9–11]. Indirect immunofluorescence staining has shown the presence of filamin in stress fibers, microspikes and membrane ruffles of cultured cells [12]. In skeletal and cardiac muscles filamin has been localized on the border of the sarcomers in the *Z*-line region [7,8].

The intracellular distribution of filamin and also its ability to crosslink actin filaments causing the formation of a gel resemble the properties of another actin-binding protein that of α-actinin. However, α-actinin stimulates actin polymerization in vitro [13]. A macrophage high-M_r actin-binding protein also accelerates the onset of actin polymerization [14]. In the presence of this protein, bipolar and perpendicular branching of actin filaments is observed [14].

The results of our studies on the effect of filamin on actin polymerization are presented here. We have found that sub-stoichiometric concentrations of filamin stimulate actin polymerization.

2. Materials and methods

Actin was isolated from the skeletal muscles of rabbits as in [15]. Just before the experiment, actin was gel-filtered on a Sephadex G-150 column (2.6 × 90) in 2 mM Tris–HCl buffer ($pH_{20°C}$ 7.5) containing 0.2 mM $CaCl_2$, 0.2 mM ATP and 0.2 mM dithiothreitol (buffer A). The fractions containing G-actin were pooled, passed through a 0.45 μm Millipore filter and stored at +4°C in buffer A. Actin preparations were used within two days after the purification. The concentration of G-actin was determined spectrophotometrically using an extinction coefficient of 620 cm^2/g at 290 nm [16].

Filamin was isolated from chicken gizzards as in [11] and based on the methods in [5,6]. Before the experiment filamin was dialyzed against buffer A and clarified by centrifugation at 10 000 × *g* for 5 min. The concentration of filamin was determined spectrophotometrically using the extinction coefficient of 820 cm^2/g at 278 nm [11].

α-Actinin was isolated from chicken gizzards according to [17].

Actin, filamin and α-actinin were ≥98% pure as determined by SDS–polyacrylamide gel electrophoresis. More than 90% of the protein in the filamin and α-actinin preparations were able to interact with F-actin.

The kinematic viscosity was measured in Cannon-Manning semimicroviscometers (size 100, 150) at 23°C. The sample volume was 0.5 ml. Specific viscosities were calculated as: (sample flow time – buffer flow time)/buffer flow time. The buffer flow time was 60–70 s.

3. Results and discussion

A monomer actin (G-actin) in the presence of

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appropriate concentrations of salts (0.5–2 mM Mg^{2+}, 20–100 mM KCl) polymerizes, forming long filaments (F-actin). The polymerization process includes at least 3 steps: nucleation, elongation and, probably, annealing [18]. The first step is a condensation of actin monomers into oligomers (nuclei) which act as the growing points for filament elongation. The nucleation is a rate-limiting step of the process and depends on the monomer concentration and the conditions of polymerization [18]. If [salts] are decreased below 1 mM Mg^{2+} or 100 mM KCl, the rate of polymerization falls, as the formation of actin oligomers slows down. The second step is the addition of monomers to the ends of actin oligomers. The rapid increase of the Ostwald viscosity is a result of filament elongation. The annealing of the short filaments probably accompanies elongation [18].

Fig.1,2 show the time course of actin polymerization measured as the increase of the Ostwald viscosity. In the presence of 2 mM Mg^{2+} (fig.1a) or 100 mM KCl (fig.2b) the polymerization is rapid and begins in a few minutes. However, no significant raise of specific viscosity was observed within 1 h in 0.5 mM Mg^{2+} (fig.1e) or even within 2 h in 40 mM KCl (fig.2e). The main difference in the time course of actin polymerization in the presence of a high and low [Mg^{2+}] or [KCl] is the length of the lag phase, reflecting the time necessary for the formation of the short actin oligomers, i.e., nucleation.

Thus, in the experimental conditions nucleation was slow (addition of a small amount of F-actin caused rapid polymerization). If actin was polymerized in the presence of filamin, stimulation of the polymerization process was observed. The stimulation depended on the filamin/actin ratio, increasing with the increase of the filamin concentration. The effect was significant if the filamin/G-actin molar ratio was 1:800 (fig.2d) or 1:350 (fig.1c). Thus, sub–stoichiometric concentrations of filamin are effective for

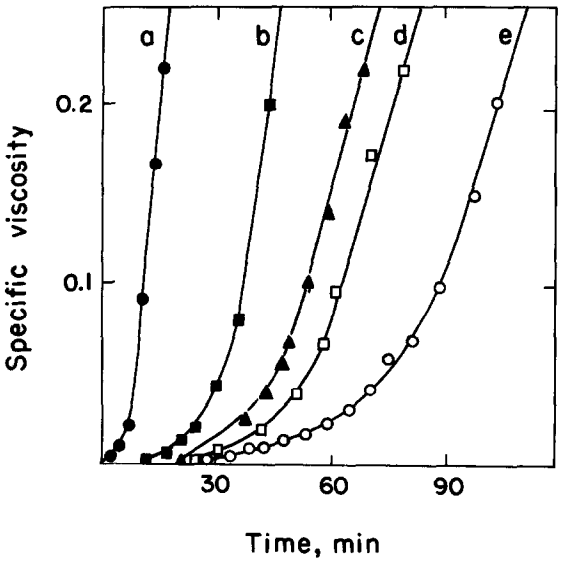

Fig.1. Effect of filamin on Mg^{2+}-stimulated actin polymerization. G-actin (0.5 mg/ml) was preincubated with filamin in buffer A at 0°C for 5 min and then $MgCl_2$ was added. The sample (0.5 ml) was immediately transferred into a viscometer and the flow time was measured at 23°C. The specific viscosity of G-actin, filamin and α-actinin solutions was <0.015; (a) actin, polymerized in the presence of 2 mM $MgCl_2$ (●——●); (b) actin, polymerized in the presence of filamin (molar ratio to G-actin 1:170) and 0.5 mM $MgCl_2$ (■——■); (c) the same as (b), but the filamin/G-actin molar ratio was 1:350 (▲——▲); (d) actin, polymerized in the presence of α-actinin (molar ratio to actin 1:170) and 0.5 mM $MgCl_2$ (□——□); (e) actin, polymerized in the presence of 0.5 mM $MgCl_2$ (○——○).

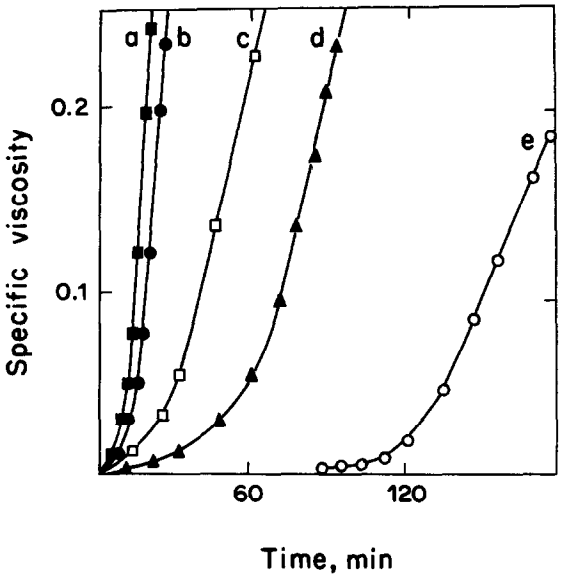

Fig.2. Effect of filamin on KCl-stimulated actin polymerization. The conditions are described in fig.1, but KCl was added instead of $MgCl_2$ to start the polymerization: (a) actin, polymerized in the presence of filamin and 100 mM KCl (■——■); (b) actin, polymerized in the presence of 100 mM KCl (●——●); (c) actin, polymerized in the presence of filamin and 40 mM KCl (□——□), filamin/G-actin molar ratio 1:170; (d) the same as (c), but filamin/G-actin molar ratio 1:800 (▲——▲); (e) actin, polymerized in the presence of 40 mM KCl (○——○).

stimulating actin polymerization (a filamin · F-actin complex in vitro contains 1 filamin molecule/10–12 actin monomers) [19]. The main effect of filamin is to decrease the lag phase. This means that the nucleation step is promoted. It is essential that filamin acts in a dose-dependent manner. If actin polymerization proceeds rapidly, filamin does not affect the process significantly (fig.2a,b). This result also suggests that filamin promotes the nucleation step.

It has been shown earlier that α-actinin, which has many common features with filamin, stimulates actin polymerization [13]. In our hands α-actinin also stimulated actin polymerization (fig.1d), but the stimulation required a higher concentration of α-actinin in comparison to filamin (fig.1b,d). Thus, filamin is a more effective stimulator of actin polymerization than is α-actinin.

The results obtained demonstrate the ability of filamin to stimulate actin polymerization, by promoting the nucleation step. The effect of filamin on the time course of actin polymerization resembles that of another high-M_r actin-binding protein from macrophages [14]. However, it is not clear at present whether filamin and macrophage actin-binding protein affect microfilament formation in the same manner or not. In a forthcoming study we shall test the ability of filamin to stimulate the bipolar and perpendicular branching of actin filaments that is characteristic of macrophage actin-binding protein [14]. In any case, the ability of sub-stoichiometric concentrations of filamin to stimulate actin polymerization by promoting nucleation may be very important in myogenesis and in microfilament formation in non-muscle cells.

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