The effects of phalloidin on actin gel–sol transformation

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1. INTRODUCTION

Phalloidin is toxic bicyclic peptide from poisonous mushrooms *Amanita phalloides* whose major pharmacological action seems to be the stabilization of actin filaments in liver parenchymal cells [1]. Phalloidin binds to actin with high affinity ($K_d = 3.6 \times 10^{-8}$ M) [2]. In vitro phalloidin stabilizes F-actin against depolymerization, heat and alkali denaturation, action of cytochalasins and proteolytic digestion [3–7]. Major effects of phalloidin on actin polymerization are the following: phalloidin accelerates actin polymerization, decreases the critical concentration, stabilizes the short polymers formation during the nucleation step [3–5]. The effects of phalloidin on actin polymerization could be explained by peptide ability to decrease the values of k_- rather than affecting the values of k_+ [5]. Thus, in vivo phalloidin seems to inhibit the mechanisms regulating actin depolymerization. If that is the case, phalloidin should also disturb the mechanisms regulating actin gel–sol transformation in the cell. The only experimental fact, showing the effect of phalloidin on actin gel–sol transformation is the phalloidin inhibition of cytochalasin B action on actin gelation [8].

Here, we present the results of our studies of the effect of phalloidin on the actin filaments network formation in the presence and absence of actin crosslinking proteins, filamin and α-actinin. Phalloidin in stoichiometric concentrations increases low shear viscosity of F-actin and decreases critical concentrations of filamin and α-actinin necessary for F-actin gelation.

2. MATERIALS AND METHODS

Phalloidin and all other reagents were purchased from Sigma. Muscle G-actin was extracted from rabbit acetone powder [9] and purified further by gel-filtration on Sephacril S-200 [10]. Actin preparations were used within 3 days. α-Actinin was isolated as in [11] and filamin by Wang's method [12] according to our modification [13]. All proteins were at least 98% pure as judged by SDS–polyacrylamide gel electrophoresis.

2.1. *Analytical procedures*

The viscosity of F-actin solutions was measured at low shear rates in falling ball viscometers [14]. Solution of actin drawn up into 100 μl pipettes and sealed at the bottom. The pipettes mounted at a 60° angle, and the time required for a stainless steel ball (0.64 mm) to fall 7 cm down the micropipettes was determined. In all experiments actin was 11.6 μM. To initiate actin polymerization $MgCl_2$ (final conc. 2 mM) or KCl (final conc. 0.1 M) were added to 11.6 μM G-actin solution in 3 mM Tris–HCl buffer (pH 7.5), with 0.2 mM ATP, 0.2 mM $CaCl_2$, 0.5 mM dithiothreitol. After 3 h incubation at 22°C the low shear viscosity of F-actin was measured. The critical crosslinking concentration of filamin and α-actinin defined as con-

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centration of protein which causes an abrupt decrease in the rate at which the stainless steel ball falls. The weight average filament length, L_w was calculated from [15,16]:

L_w = [Actin]/(2[Filamin or α-actinin] × 370)

where concentration of actin, filamin and α-actinin are in mol/l and numerical factor of 370 is the no. actin monomers/μm filament [17].

3. RESULTS AND DISCUSSION

The effect of phalloidin on F-actin low shear viscosity is demonstrated in fig.1 and 2. Phalloidin increases low shear viscosity of F-actin. The addition of actin oligomers for stimulating the actin polymerization nucleation step does not affect actin viscosity in the presence and absence of phalloidin. Stimulating effect of phalloidin strictly depends on the peptide concentration reaching the maximal level (9-times higher if compared to control) at ~30–35 μM (fig.2) while the phalloidin concentration increases further the stimulating effect goes down and exceeds a plateau of 80–100 μM phalloidin (not shown). This plateau corresponds to 3–4-times increase of F-actin low shear viscosity. After the addition of the salts to initiate polymerization of F-actin apparent viscosity increases monotonically and reaches a constant value during the third hour in the presence of phalloidin as well as in control (fig.1). Thus, in stoichiometric concentrations phalloidin significantly stimulates actin filament network formation. The effect of the peptide is time and concentration dependent. The dose-dependence of the phalloidin action is complicated and we cannot yet explain the shape of the curve. However, all the concentrations of phalloidin tested increased F-actin low shear viscosity.

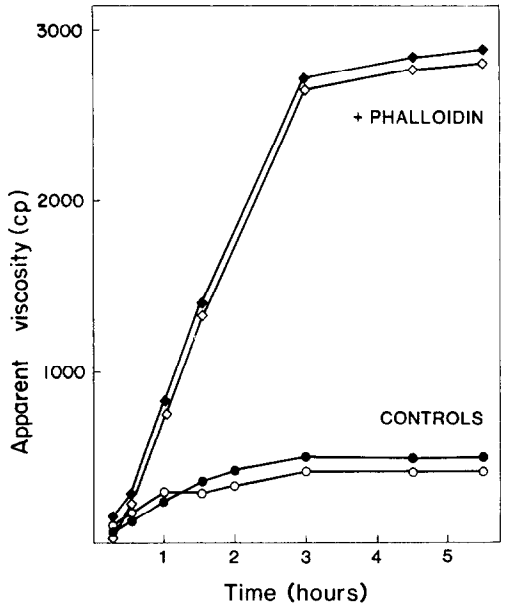

Fig.1. Effect of phalloidin on apparent viscosity of F-actin. Phalloidin was 30–35 μM. Polymerization of 11.6 μM G-actin was initiated by the addition of 0.05 M $MgCl_2$ stock solution to a final conc. 2 mM: (○—○) G-actin alone; (◇—◇) G-actin plus phalloidin; (●—●) G-actin plus 0.6 μM actin fully polymerized and sonicated for 20 s immediately before use; (◆—◆) G-actin plus phalloidin and 0.6 μM sonicated F-actin.

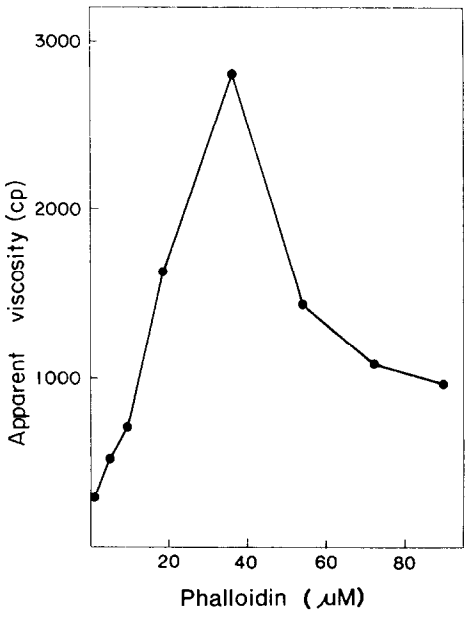

Fig.2. F-actin apparent viscosity dependence on phalloidin concentration.

Fig.3 and 4 demonstrate the effect of phalloidin on F-actin sol–gel transition in the presence of crosslinking proteins filamin and α-actinin. In the presence of phalloidin, critical concentrations of filamin and α-actinin necessary for actin gelation are 3.5–4-times lower. The effect of phalloidin does not depend on the salts added to initiate the actin polymerization ($MgCl_2$ or KCl). Phalloidin

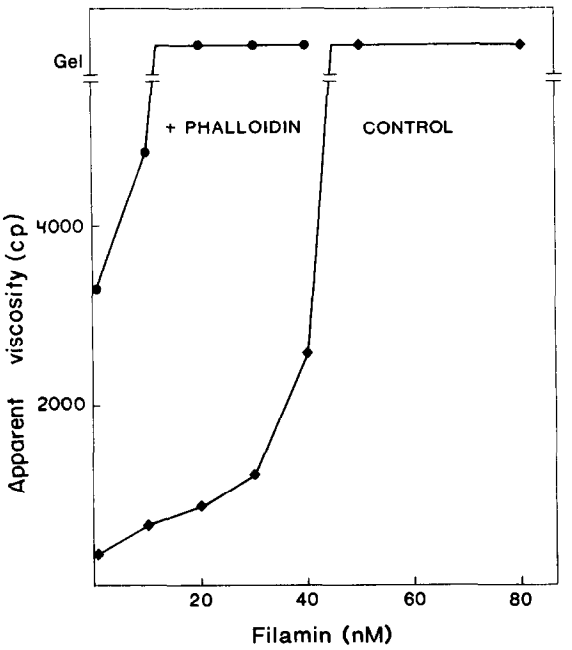

Fig.3. Effect of phalloidin on filamin-induced actin gelation. Concentration of phalloidin was 30–35 μM. Actin polymerization was initiated by 0.1 M KCl and 2 mM $MgCl_2$: (◆——◆) actin plus filamin; (●——●) actin plus filamin and phalloidin.

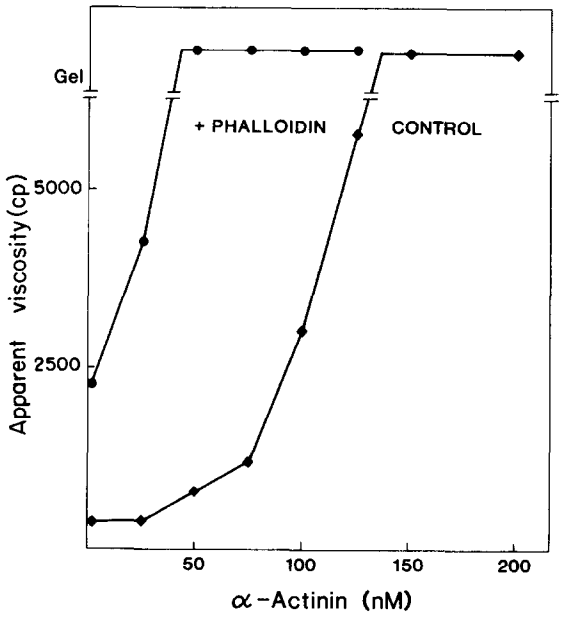

Fig.4. Effect of phalloidin on α-actinin-induced actin gelation. Actin polymerization was initiated by 2 mM $MgCl_2$: (◆——◆) actin plus α-actinin; (●——●) actin plus α-actinin and phalloidin.

also does not influence filamin and α-actinin binding to F-actin as judged by sedimentation analysis (not shown).

According to [8,16,18] F-actin in physiological solutions is an entangled viscoelastic sol and to form the gel it is necessary to crosslink filaments. According to the network theory of gel formation [19] the critical concentration of the crosslinker proteins required for incipient gelation of F-actin is inversely proportional to the weight average filament length [8,16,18]. All the factors shortening F-actin increase the critical concentration of the crosslinking proteins [8,16,20,21]. Here, phalloidin increased low shear viscosity of F-actin and decreased the critical concentrations of crosslinker proteins. The simplest explanation of stimulating effect of phalloidin on F-actin gel formation is:

phalloidin increases F-actin filament length inhibiting spontaneous fragmentation of actin filaments.

Under physiological conditions, actin filaments break spontaneously [22,23]. The stabilization of F-actin by phalloidin against various depolymerizing or denaturing agents was described in [3–7]. A decrease of 3–4-times the critical concentration of filamin and α-actinin necessary for actin gelation in the presence of phalloidin corresponds to the 6–8-times increase of the weight average filament length (calculations are based on the equation in section 2). In the presence of a constant pool of actin molecules, an increase of the filament length necessitates a decrease in the number of filaments. According to the network theory of gel formation the amount of crosslinker proteins required for actin gelation in this case decreases. These results for the first time document the existence of physiological agent which can regulate gel–sol transformation increasing actin filament length.

5. CONCLUSION

The toxic heptapeptide phalloidin stimulates actin network formation. Phalloidin also decreases the critical concentration of crosslinker proteins required for F-actin gelation. The stimulating effect of phalloidin is probably due to its ability to increase the actin filament length.

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