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Reannealing of phalloidin-treated actin filaments during recovery after sonication and its inhibition by β-actinin

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Phalloidin (2 mol per mol actin)-treated pyrenyl F-actin showed a critical concentration of 1.8 μM in the presence of 10 mM KCl, 0.2 mM ADP, and 5 mM Tris-HCl buffer, pH 8.0 at 25°C. The filament weight concentration did not change at all during and after sonication, yet degrees of flow birefringence increased and the filament number concentration decreased after the termination of sonication. The latter changes were not affected by EDTA, but inhibited by β-actinin. These observations suggest that reannealing of short pieces of phalloidin-treated actin filaments fragmented during sonication takes place during recovery after sonication.

Actin; Phalloidin; Reanncaling; Actin filament; β-Actinin

1. INTRODUCTION

In 1961, Asakura [1] introduced sonication techniques into the field of actin research showing an ATPase action of F-actin during sonication. Subsequently, formation of short F-actin filaments during sonication and elongation of actin filaments after sonication were demonstrated [2,3]. Both Oosawa's [2] and our group [3] postulated reassociation (or reannealing) of sonicated F-actin fragments after sonication. Therefore, it was assumed that β-actinin, now known as a pointed end-capping protein [4,5], inhibited the reassociation process following sonication [3,6].

In 1984, however, Korn and his associates [7] invented an elegant device to observe directly the changes in the G–F transformations during and after sonication using *N*-pyrenyliodoacetamide labeled (pyrenyl) actin, and reached the conclusion that a diffusion-like random walk mechanism for length redistribution accounts for apparent elongation of fragmented F-actin after sonication [8]. Therefore, we re-examined this problem using phalloidin-treated F-actin and obtained evidence that reannealing of F-actin pieces could occur under specified conditions. Phalloidin, a toadstool mushroom toxin, is known to stabilize actin filaments even under conditions where F-actin is depolymerized (cf. [9]).

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2. MATERIALS AND METHODS

Actin was prepared from rabbit skeletal muscle [10] and purified by Sephadex G-150 chromatography. β-Actinin was prepared from rabbit skeletal muscle [4] and purified according to Funatsu and Ishiwata [5]. Pyrenyl G-actin [11] was treated with Dowex-1 to remove free ATP. G-actin was polymerized in a solution containing 0.2 mM ADP, 10 mM KCl, 0.1 mM $CaCl_2$, 0.01% NaN_3, 5 mM Tris-HCl, pH 8.0, and phalloidin (molar ratio of 2 to actin monomers) at 25°C. Phalloidin was a gift from Professor Wieland. The resultant F-actin solution was subjected to sonication three times (each time 1 min) to exhaust actin-bound ATP, if any [8]. The G–F transformations during

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and after sonication were directly observed in a Hitachi 650-60 fluorescence spectrophotometer. Sonic vibration was applied to the optical cell in the thermostatted jacket of the fluorometer, constructed according to Pantaloni et al. [7]. A sonicator, Tomy UR-20P, was used. Degrees of flow birefringence were measured in a Waken Micro FBR Mark-II apparatus. Negatively stained actin filaments were observed under a JEM 100S electron microscope.

3. RESULTS

Recently, Kuroda and his associates [12] have shown that one mol of phalloidin per two mol of actin monomers protects actin filaments from depolymerization even at very low ionic strength. In the present study, G-actin freed from ATP was polymerized overnight in the presence of phalloidin (2 mol per mol of actin monomers) and 10 mM KCl. The critical concentration of phalloidin-treated F-actin in the presence of 0.2 mM ADP was estimated to be only 1.8 μM, as shown in fig.1. This value was much smaller than that of intact F-actin in the presence of ADP.

Fluorescence intensities of phalloidin-treated F-actin hardly changed during and after sonication (fig.2a). On the other hand, in intact F-actin in the presence of ATP, the fluorescence intensity greatly dropped on the application of sonication (fig.3a) and rapidly recovered after the termination of sonication, as already shown by Korn's group [8]. In both cases, the degrees of flow birefringence were decreased by sonication and increased after sonication (fig.2b; fig.3b). It should be noted that the values of the extinction angle markedly increased during sonication and gradually decreased after sonication. Thus, the elongation of actin filaments took place after sonication [2,3]. Addition of 1 mM EDTA did not affect the changes in the fluorescence intensity or the degrees of flow birefringence at all (fig.2a,b), in contrast to the case with intact F-actin in the presence of 0.1 M KCl and 0.2 mM ADP (fig.3b). In the latter, the increase in the degrees of flow birefringence was

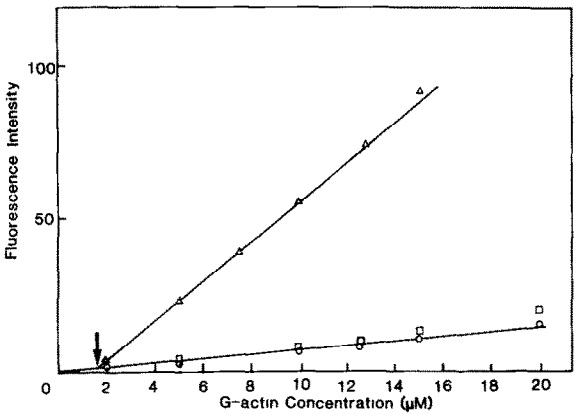

Fig.1. Measurement of the critical concentration of phalloidin-treated F-actin. Various concentrations of G-actin were incubated for 24 h at 25°C in the presence of 10 mM KCl, 0.2 mM ADP, 0.1 mM $CaCl_2$, 0.01% NaN_3, and 5 mM Tris-HCl, pH 8.0. Fluorescence intensity is given as relative value. (□) Control; (△) incubated with phalloidin (2 mol/mol of actin); (○) G-actin without addition of KCl. The arrow indicates the critical concentration.

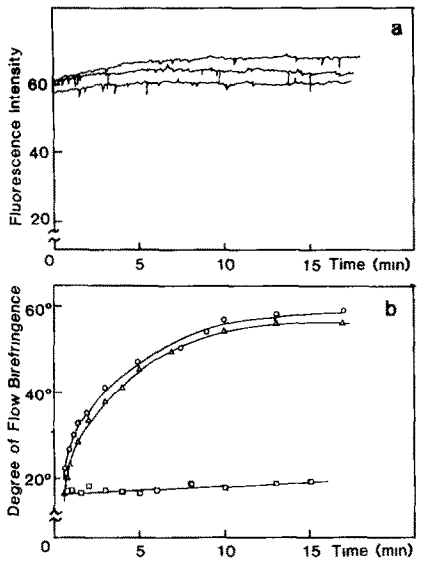

Fig.2. Effects of EDTA and β-actinin on the changes in the fluorescence intensity (a) and the degrees of flow birefringence (b) of phalloidin-treated F-actin after sonication. Dowex-1-treated pyrenyl G-actin, 13.8 μM, was polymerized overnight in the presence of phalloidin (2 mol per one mol actin), 10 mM KCl, 0.2 mM ADP, 0.1 mM $CaCl_2$, 0.01% NaN_3, and 5 mM Tris-HCl buffer, pH 8.0, at 25°C. Each sample was sonicated for 40 s, and then, immediately before the termination of the sonication, EDTA or β-actinin was added to the concentrations of 1 mM and 0.5 μM, respectively. (a) Curves: upper, control; middle, + EDTA; lower, + β-actinin. (b) Control (○); + EDTA (△); + β-actinin (□).

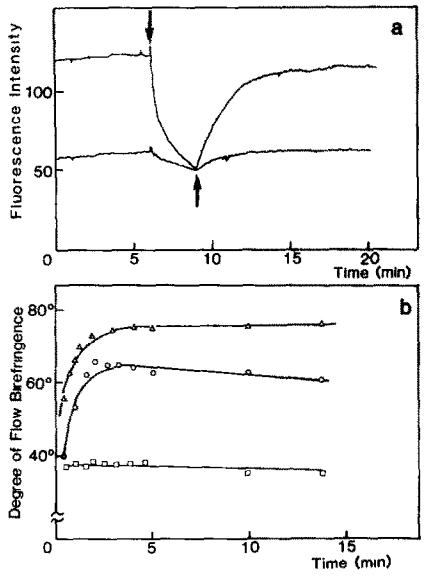

Fig.3. Effects of sonication on the fluorescence intensity (a) and the degrees of flow birefringence (b) of F-actin. (a) Curves: upper, F-actin (13.8 μM) in 0.2 mM ATP, 0.1 M KCl, 0.1 mM $CaCl_2$, 0.01% NaN_3, and 5 mM Tris-HCl buffer, pH 8.0, at 25°C; lower, F-actin in 0.2 mM ADP, 0.1 M KCl, and 5 mM Tris-HCl buffer. Arrows indicate the application and the termination of sonication. (b) F-actin (13.8 μM), in 0.2 mM ADP, 0.1 M KCl, and 5 mM Tris-HCl buffer, was sonicated for 40 s. After the termination of the sonication, the degrees of flow birefringence were measured at a velocity gradient of 10 s^{-1}. (△) Control; (○) 1 mM EDTA added; (□) β-actinin, 0.5 μM added.

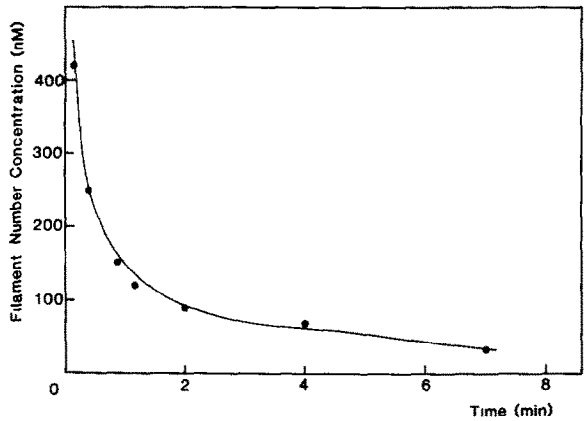

Fig.4. Changes in filament number concentrations of phalloidin-treated F-actin after sonication. The conditions were the same as in fig.2. After the termination of the sonication, samples were taken for measurements of filament number concentrations according to Pantaloni et al. [7].

significantly lowered by EDTA (fig.3b). It is known that EDTA quickly inactivates G-actin in the absence of ATP [13,14]. Here it was observed that the half-life time of the polymerizable G-actin was approximately 20 s in the presence of 10 mM KCl and 0.2 mM ADP, and 3 min in the presence of 0.1 mM KCl and 0.2 mM ADP. On the other hand, β-actinin stopped the recovery process in the degrees of flow birefringence but did not affect the fluorescence intensity profile (fig.2a,b).

Number concentrations of phalloidin-treated actin filaments determined by the method of Pantaloni et al. [7] rapidly decreased after the termination of sonication (fig.4). The changes in number concentrations after sonication were in good agreement with those of the intact F-actin reported [8]. However, in the presence of β-actinin, a high level of number concentration remained almost constant, although exact estimations were not possible due to capping the pointed ends of actin filaments (not shown).

The presence of short pieces of phalloidin-treated F-actin right after sonication and their elongation thereafter are shown in the electron micrographs of fig.5a,b. In the presence of β-actinin, elongation did not occur at all (fig.5c,d). These situations were the same as intact F-actin in 0.1 M KCl [3].

4. DISCUSSION

It has been reported that the critical concentration of phalloidin-treated F-actin eventually became zero in the presence of 0.1 M KCl, 1 mM $MgCl_2$ and 0.5 mM ATP at pH 7.5, since the dissociation rate constants were reduced to nearly zero, while the association rate constants were not affected [15,16]. We observed a small decrease in the fluorescence intensities of phalloidin-treated F-actin during sonication in the presence of 0.1 M KCl, 1 mM $MgCl_2$ and 0.2 mM ATP at pH 8.0. Furthermore, at this KCl concentration, it took some time to inactivate G-actin with 1 mM EDTA. Therefore, in the present study, conditions (10 mM KCl) were chosen, where EDTA quickly denatured G-actin.

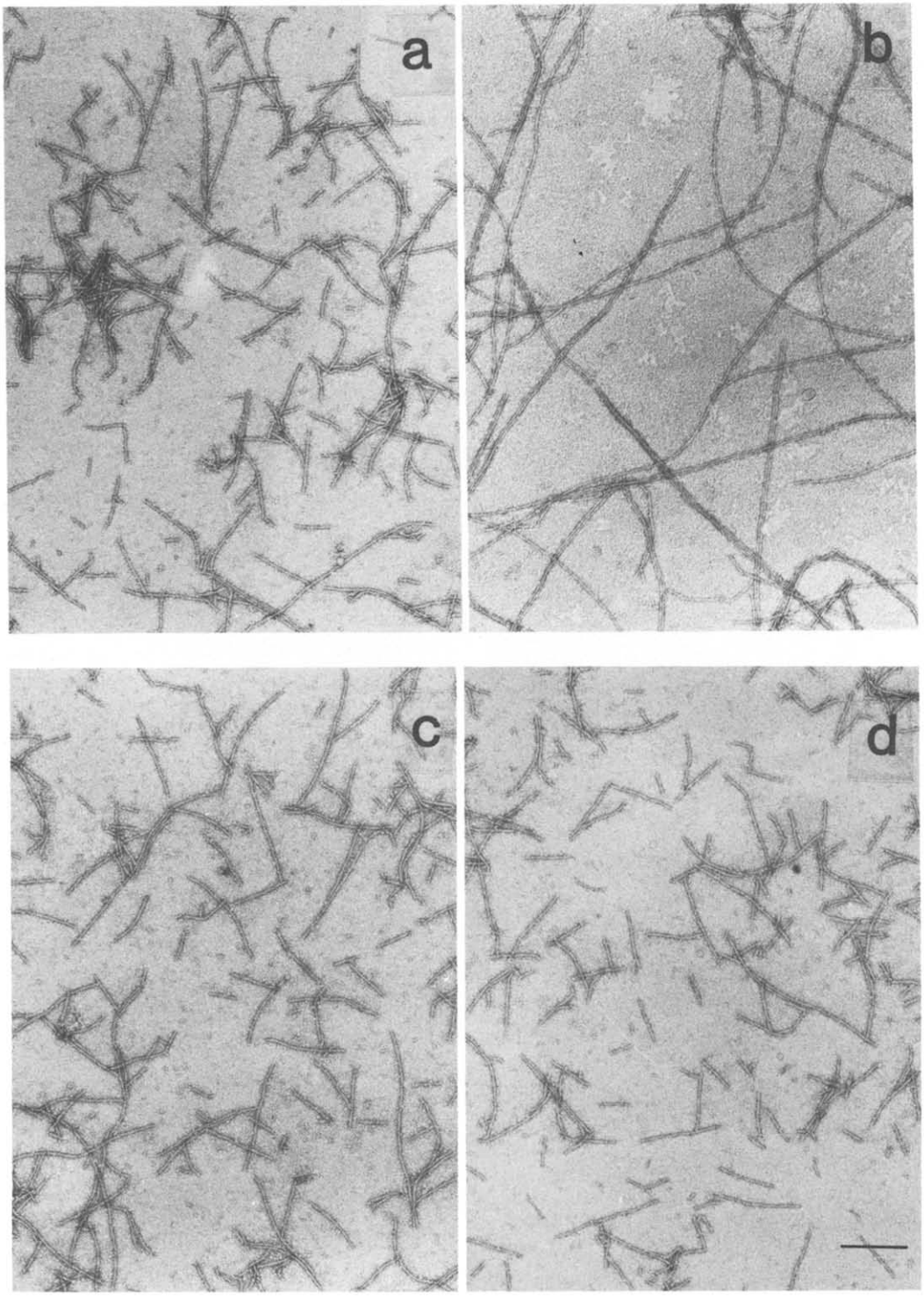

Fig.5. Electron micrographs of phalloidin-treated F-actin after the termination of sonication. The conditions were the same as in fig.2. (a,b) Control; (c,d) + β-actinin. 10 s (a,c) or 15 min (b,d) after the termination of sonication, the samples were negatively stained with 1% uranyl acetate. Bar, 0.2 μm.

The present work with phalloidin-treated actin filaments clearly showed that (i) there were no detectable changes in the amount of F-actin at 10 mM KCl during and after sonication, (ii) yet, elongation of actin filaments took place after sonication, (iii) number concentrations rapidly decreased after sonication, (iv) EDTA, G-actin inactivating agent, did not affect the elongation process at all, and (v) β-actinin inhibited both the elongation and the decrease in filament number concentrations. In conclusion, in phalloidin-treated F-actin reannealing of short pieces of actin filaments fragmented during sonication takes place after the termination of sonication.

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