# Polymorphism of F-Actin Assembly. 2. Effects of Barbed End Capping on F-Actin Assembly†

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ABSTRACT: In the accompanying paper [Suzuki, A., Yamazaki, M., & Ito, T. (1996) *Biochemistry 35*, 5238−5244], we presented a quantitative phase diagram of actin filament (F-actin) described with the F-actin concentration and $\Delta\chi$ value which characterizes the affinity of F-actin with solvent. The phase diagram shows that F-actin changes its assembly structure from an isotropic disordered distribution to a dilute ordered assembly of a lyotropic liquid crystalline with an increase in the concentration and to a concentrated ordered assembly of a crystalline-like bundle with an increase in the $\Delta\chi$ value (i.e., with a decrease in the affinity with the solvent), respectively, in the physiological concentration range. We report here that capping the barbed end of F-actin significantly affects the phase diagram. The F-actin capped by gelsolin (capped F-actin) decreased the $\Delta\chi$ value required for the formation of the concentrated ordered assembly. The time taken for the decrease in the $\Delta\chi$ value to reach a stationary state after the barbed end capping was proportional to the filament length (∼1 h/$\mu$m length). The electron microscopic morphology of the concentrated ordered assembly of the capped F-actin was a wide and loose bundle, which was distinctly different from the crystalline-like bundle of the uncapped F-actin. Fragmin from the acellular slime mould, which has similar functions to gelsolin, showed the same effects. These results suggest that the barbed end capping of F-actin gradually changes the nature of whole filament so as to make the interaction with the solvent more unstable, and the F-actin loses the ability to make a crystalline-like bundle.

Thermodynamically, macromolecule assembly in solution depends on the shape of a macromolecule, its concentration, and affinity with the solvent. From this point of view, Flory (1956) made a theoretical phase diagram of macromolecule assembly in solution, using the $\chi$ parameter which characterizes the affinity of the solute with the solvent as the free energy change on transferring the solute molecule from the pure to the infinite dilution. In the accompanying paper (Suzuki et al., 1996), we developed a novel method to manipulate the $\chi$ parameter of actin filament (F-actin) in the physiological buffered solution using poly(ethylene glycol) of average molecular weight 6000 (PEG 6K) as a cosolvent. As shown by our thermodynamic analysis, the preferential exclusion of the PEG from the region adjacent to the F-actin molecule increases the free energy of F-actin and consequently decreases the affinity with the solvent, in proportion to the PEG concentration. We represented the PEG-induced increase of the free energy (the PEG-induced decrease of the affinity with the solvent) of F-actin per mol of subunit as $\Delta\chi$. Using this method, we made the first quantitative phase diagram of F-actin described with the concentration of F-actin and $\Delta\chi$.

The phase diagram shows that F-actin takes various assembly structures similar to those observed in cells in the physiological concentration range. F-Actin takes isotropic disordered distribution at a concentration below 2 mg/mL. When the concentration exceeds 2 mg/mL, F-actin begins to form a dilute anisotropic ordered assembly of a lyotropic liquid crystalline. On the other hand, when the $\Delta\chi$ value exceeds a critical value, F-actin suddenly forms a concentrated anisotropic ordered assembly of a crystalline-like bundle. These results indicate that F-actin behaves as a rod-like molecule thermodynamically (Suzuki, et al., 1996).

Theoretically, the assembly structure of a rod-like molecule strongly depends on the axial ratio (length to diameter), $x$ (Flory, 1956). A smaller $x$ should make the isotropic disordered structure more stable, because it increases the number of the random configurations of the rod-like molecules in limited space. Consequently, the molecule with a smaller $x$ should require a higher concentration to take the liquid-crystalline phase and a larger $\Delta\chi$ value to take the concentrated assembly. It should be important to investigate the F-actin assembly from this point of view, because there are a lot of actin binding proteins which regulate F-actin length.

Previously, we studied the effect of the length of F-actin on the liquid-crystalline formation, using F-actin whose length was regulated by gelsolin (Suzuki et al., 1991). Gelsolin is a multifunctional actin binding protein which widely exists in the cytoplasm and blood of mammalians (Yin & Stossel, 1979; Yin et al., 1981; Janmey et al., 1985; Janmey & Stossel, 1987). In the presence of a micromolar order of calcium ion, gelsolin severs F-actin and caps the barbed end of the severed filament or forms a complex with G-actin which has nucleation activity of actin polymerization (Janmey et al., 1985). Therefore, the length of F-actin can

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be regulated by adding a certain amount of gelsolin to F-actin solution or polymerizing G-actin at an appropriate molar ratio of G-actin to gelsolin. In either case, F-actin is capped at the barbed end by gelsolin, and its length is proportional to the molar ratio of G-actin to gelsolin (Janmey et al., 1985). Using this length-regulated F-actin, we showed that the minimum concentration of F-actin to bring about the liquid-crystalline phase increases with a decrease in the length as theoretically expected. The concentration is proportional to the inverse of the length of F-actin with the relation that $V_c = 2.5/x$, where $V_c$ is the volume fraction of F-actin and $x$ the axial ratio. The $V_c$ value of F-actin is somehow smaller than $V_c = 8/x$ obtained by Flory (1956) but nearly equal to $V_c = 3.4/x$ by Ishihara (1951) and $V_c = 3.34/x$ by Onsager (1949) in their theoretical analyses.

In the present report, we have investigated the effects of gelsolin on the formation of the concentrated anisotropic ordered assembly of F-actin (the concentrated assembly). According to the theoretical consideration described above, the shortened length of F-actin polymerized in the presence of gelsolin should have increased the $\Delta\chi$ value to induce the concentrated assembly. However, the effects of gelsolin were apparently unexpected ones. That is, a small amount of gelsolin significantly decreased the $\Delta\chi$ value of F-actin required to form the concentrated assembly, and then the $\Delta\chi$ value increased with the increasing amount of gelsolin. Quite the same effect was observed in fragmin from the acellular slime mould *Physarum polycehalum*, which has similar functions to gelsolin. It indicates that capping of the barbed end by gelsolin or fragmin changes the nature of F-actin so as to decrease the affinity with the solvent. In addition, the electron microscopic morphology of the concentrated assembly of F-actin capped by gelsolin was distinctly different from pure F-actin, reflecting the difference in the natures of the individual F-actins. These results indicate a novel effect of the barbed end capping on the properties of F-actin.

## MATERIALS AND METHODS

We used poly(ethylene glycol) with average molecular weight 6000 (PEG 6K) from nakarai tesque (Kyoto, Japan) without further purification. We purified monomer actin (G-actin) from skeletal muscle, gelsolin from swine serum and fragmin from the acellular slime mould *P. polycehalum* by the methods of Spudich and Watt (1971), Doi et al. (1987), and Hasegawa et al. (1980), respectively. We polymerized G-actin into filamentous form (F-actin) in F-buffer (100 mM KCl, 2 mM $MgCl_2$, 0.2 mM $CaCl_2$, 0.2 mM ATP, 10 mM imidazole hydrochloride, pH 7.5).

We quantified the fraction of F-actin in the PEG 6K-induced aggregation (concentrated assembly) by the low-speed centrifugation method of Suzuki et al. (1996). After polymerizing G-actin completely in the presence of various concentrations of PEG 6K, we agitated the solution with a couple of upside down inversions and then centrifuged with 15000*g* for 30 min. We estimated the fraction of F-actin in the pellet by measuring the concentration of F-actin in the supernatant by the Bio-Rad dye-based assay.

We prepared the 1:2 gelsolin/actin complex by mixing purified gelsolin and G-actin in G-buffer (0.2 mM $CaCl_2$, 0.5 mM ATP, 0.5 mM $\beta$-mercaptoethanol, 2 mM Tris-HCl, pH 8.0) at a molar ratio of 1:2 and allowing the mixture at

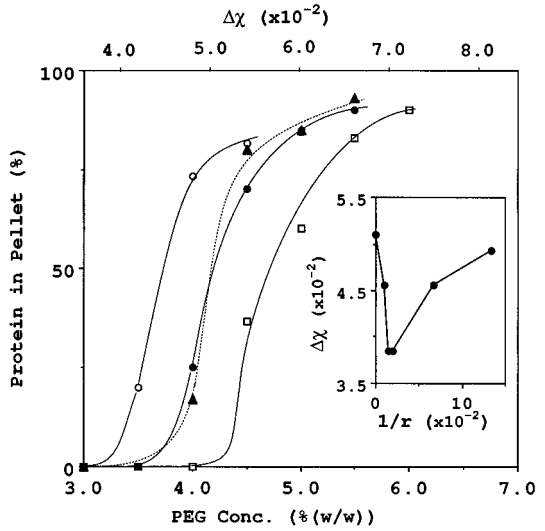


FIGURE 1: Effect of gelsolin on the concentrated assembly formation. G-Actin (0.5 mg/mL) was polymerized in F-buffer containing various concentrations of PEG 6K in the presence of gelsolin at an appropriate molar ratio to G-actin. The fraction of F-actin in the PEG-induced aggregation (concentrated assembly) was quantified by the low-speed centrifugation method of Suzuki et al. (1996). The molar ratios of gelsolin to G-actin were 1:1000 (●), 1:700 (○), 1:150 (▲) and absence of gelsolin as control (□), respectively. In the inset, the $\Delta\chi$ value required to precipitate 10% of total F-actin is plotted against the molar ratio of gelsolin to actin, $1/r$.

4 °C for overnight. We mixed the gelsolin/actin complex with F-actin solution by a few upside down inversions. We confirmed the loss of the severing activity of the complex by the pyrene-labeled F-actin method of the severing assay according to Janmey et al. (1985).

We prepared the electron microscopic samples stained with 4% uranyl acetate by the same way as described in Suzuki et al. (1996). We observed the image of the sample by a JOEL 100 B electron microscope under 80 kV of accelerating voltage.

## RESULTS

In the accompanying paper (Suzuki et al., 1996), we showed that addition of polyethylene glycol with average molecular weight 6000 (PEG 6K) to F-actin solution increases the free energy (the chemical potential) of F-actin with the relation that

$$\Delta\chi = (1.2 \times 10^{-2})C \qquad (1)$$

where $\Delta\chi$ is the increase of the free energy divided by $RT$ per mole of subunit actin and $C$ is the concentration of PEG 6K (% w/w). $RT$ has the usual meaning. It should be noted that the $\Delta\chi$ value relates to the PEG-induced decrease in the affinity of F-actin with the solvent thermodynamically.

Figure 1 shows PEG 6K-induced formation of the concentrated assembly of F-actin whose length was regulated by gelsolin. F-Actin polymerized in the absence of gelsolin (pure F-actin) formed the concentrated assembly at $\Delta\chi$ values larger than 0.05. F-Actin polymerized in the presence of gelsolin (gelsolin-nucleated F-actin) formed it at smaller $\Delta\chi$ values. The $\Delta\chi$ value decreased by ~0.005 at a very small amount of gelsolin, whose molar ratio to subunit actin, $1/r$, is equal to 1/1000. It approached the minimum at $1/r$ = 1/700−1/500, decreasing by ~0.01. Then, the value in-

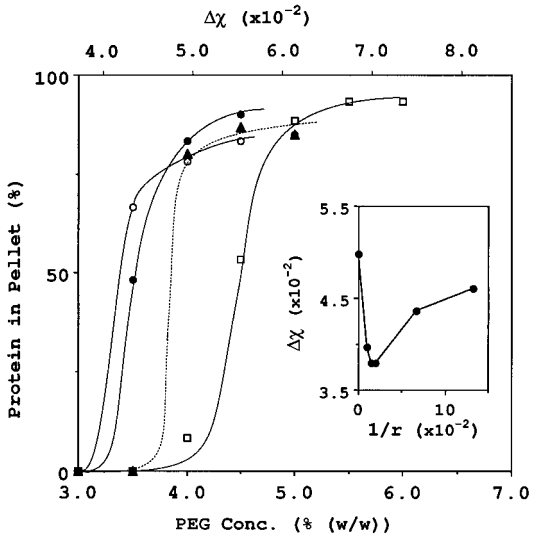


FIGURE 2: Effect of fragmin on the concentrated assembly formation. G-actin was polymerized in the presence of fragmin as described in Figure 1. The molar ratios of fragmin to G-actin were 1:1000 (●), 1:700 (○), 1:150 (▲), and absence of fragmin as control (□), respectively. In the inset, the $\Delta\chi$ value required to precipitate 10% of total F-actin is plotted against the molar ratio of fragmin to actin, $1/r$.

creased with the increase in $1/r$ (see inset of Figure 1). In these experiments, the length of F-actin, $l$, should become shorter with the increase in $1/r$, according to $l = r/370$ $\mu$m (Janmey et al., 1986). Quite the same effects were observed in fragmin from the acellular slime mould *P. polycehalum*, which has similar functions to those of gelsolin (Figure 2).

Figure 3 shows the electron microscopic morphology of the concentrated assembly. The morphology of the assembly structure of the gelsolin-nucleated F-actin evidently differs from the crystalline-like bundle of the pure F-actin (panel a in Figure 3). The gelsolin-nucleated F-actin forms a comparatively loose and wide bundle in which the long axis of the F-actin is ordered almost in parallel (panels b−d in Figure 3). The morphology is considerably irregular in contrast with the crystalline-like regularity of the pure F-actin. In some cases, overlapping of two or more of the bundles gives a network-like appearance. Such morphology did not change with an increase in $\Delta\chi$ value without transforming into a crystalline-like bundle such as observed in the pure F-actin (panel d in Figure 3). The same effect of gelsolin was also observed when gelsolin was added to F-actin solution after completion of polymerization (panel e in Figure 3).

The morphological change was induced by a small amount of gelsolin of $1/r = 1/700$ (panel b in Figure 3), and the characteristic features of the morphology were common to all of the gelsolin-nucleated F-actin irrespective of the difference in the amount of gelsolin. These results indicate that the gelsolin-induced change in the morphology should not be attributed to the change in the length of the F-actin. We directly demonstrated it by using the 1:2 gelsolin/actin complex, which has the activity of barbed end capping of F-actin but does not have the severing activity (Janmey et al., 1985). The F-actin treated by the gelsolin/actin complex after polymerization is capped at the barbed end without changing the length. As shown in panel f in Figure 3, the morphology of the concentrated assembly of the F-actin capped by the gelsolin/actin complex was distinctly different from the pure F-actin, showing the same characteristic features as the gelsolin-nucleated F-actin.

All of the results described above strongly suggest that the barbed end capping of F-actin has significant effects on the formation of the concentrated assembly of F-actin. To further address the capping effect, we have investigated the effects of gelsolin and the gelsolin/actin complex on the time course of formation of the concentrated assembly. After making pure F-actin in the presence of 3.7% (w/w) PEG 6K at which the F-actin could not form the concentrated assembly but the gelsolin-nucleated F-action could (Figure 1), we added gelsolin or the gelsolin/actin complex at various molar ratios to actin subunit, $1/r$. Figure 4 shows the time course of the formation of the concentrated assembly. The rate was not accelerated by agitating the sample with the upside down inversions, which indicates that the rate of the formation is not diffusion-limited. In the case of gelsolin, a higher molar ratio of gelsolin could form the concentrated assembly faster (Figure 4 A). At the molar ratio of $1/r = 1/200$, it took less than 1 h for the formation to reach the stationary state, while at $1/r = 1/700$, it took more than 6 h. In the case of the gelsolin/actin complex, it took more than 6 h, independent of the molar ratio to subunit actin (Figure 4B). The half-time of the concentrated assembly formation decreases nearly in proportion to the molar ratio of subunit actin to gelsolin, $r$, but is constant for the gelsolin/actin complex (see inset of Figure 4B). These results indicate that the effects of the barbed end capping gradually propagate in the whole filament in proportion to its length (see Discussion for detail).

## DISCUSSION

The isotropic disordered distribution of F-actin with a shorter length should be more stable, since the number of random configurations in limited space increases with a decrease in the length. In fact, the critical concentration of F-actin to form the liquid-crystalline assembly increases with the decrease in the length (Suzuki et al., 1991). Therefore, it is reasonable to expect that the $\Delta\chi$ value to induce the concentrated assembly should also increase with the decrease in the length. To investigate such effect of the length on the formation of the concentrated assembly, we used F-actin which was polymerized in the presence of gelsolin (gelsolin-nucleated F-actin) to regulate the length (Janmey et al., 1986). However, the experimental results were a little bit complicated (Figure 1). The $\Delta\chi$ value of the F-actin to induce the concentrated assembly decreased by a small amount of gelsolin, approached the minimum at $1/r = 1/700-1/500$, and then it increased with an increase in $1/r$ (see inset of Figure 1A). Quite the same effects were observed in fragmin from the acellular slime mould, which is structurally unrelated to gelsolin but has similar functions (Figure 2). These results may be attributed to two different effects of gelsolin/fragmin: one is the barbed end capping, and the other is the shortening of the filament. At a small amount of gelsolin/fragmin which does not significantly shorten the length of F-actin, the $\Delta\chi$ value remarkably decreases by the effect of the barbed end capping, and then it increases with the increase in the amount of gelsolin/fragmin by the effect of the length shortening. This conclusion was confirmed by capping pure F-actin directly with the 1:2 gelsolin/actin complex without changing its length (Janmey et al., 1985): the gelsolin/actin complex also decreased the $\Delta\chi$ value.

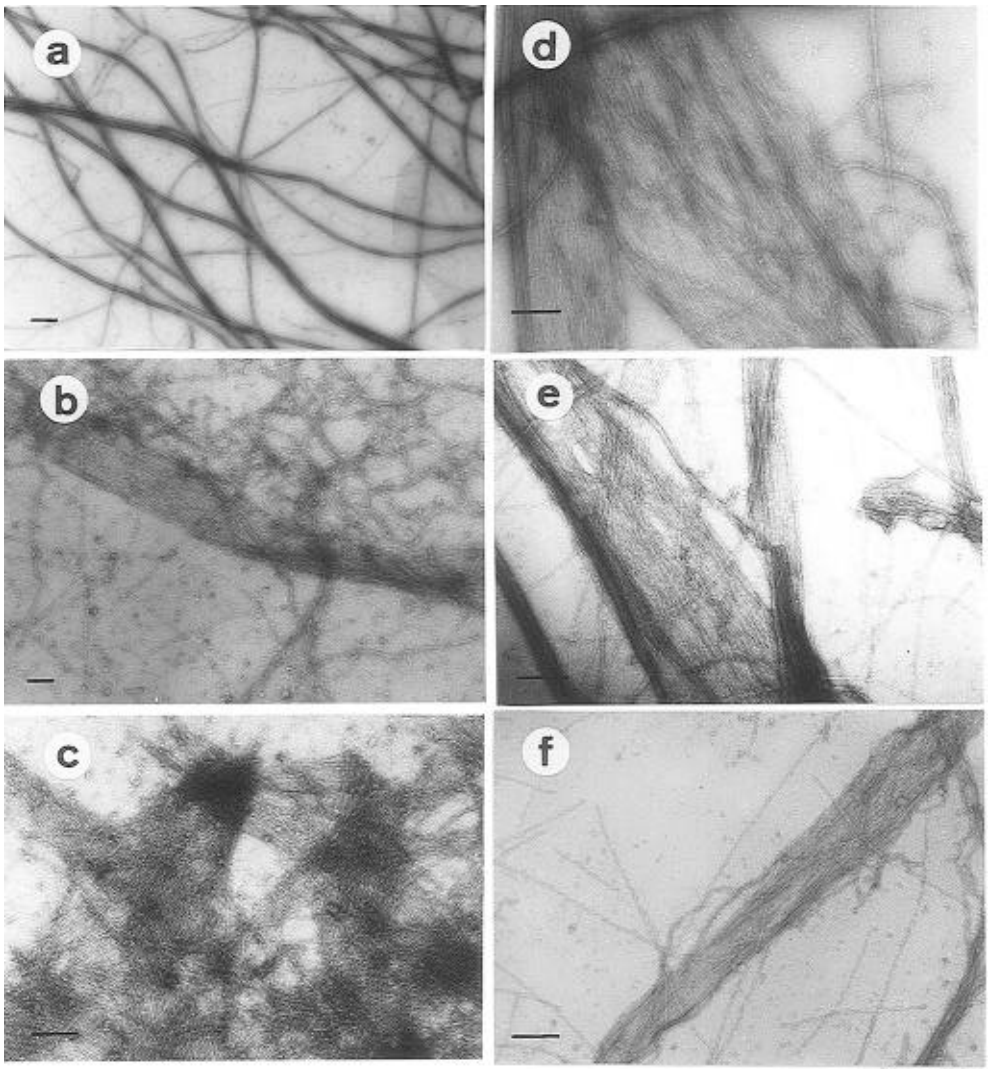


FIGURE 3: Morphology of the concentrated assembly of F-actin capped by gelsolin or the 1:2 gelsolin/actin complex. G-Actin (0.5 mg/mL) was polymerized in F-buffer containing PEG 6K in the presence (b−d) or absence (a) of gelsolin, or after G-actin was polymerized in the absence of gelsolin for 2 h, gelsolin (e) or the 1:2 gelsolin/actin complex (f) was added. Each sample solution was kept at RT for 20 h. The sample for electron microscopy was prepared as described under Materials and Methods. Panels: (a) 4.5% PEG 6K (control), (b) gelsolin:actin = 1:700, 3.5% PEG 6K, (c) gelsolin:actin = 1:150 and 3.7% PEG 6K, (d) gelsolin:actin = 1:700 and 5% PEG 6K, (e) gelsolin:actin = 1:500, 3.5% PEG 6K, and (f) 1:2 gelsolin/actin complex:actin = 1:500 3.5% PEG 6K. Bar, 0.5 $\mu$m.

The decrease in the $\Delta\chi$ value caused by gelsolin/fragmin indicates that capping the barbed end of F-actin should change the nature of F-actin so as to make the interaction of F-actin with the solvent more unfavorable. Energetically, the decreased $\Delta\chi$ value of 0.012 observed in the gelsolin-nucleated F-actin of $1/r = 1/500$ may correspond to ~0.012 *RT*/mol for subunit actin and ~6 *RT*/mol for F-actin with the length of 1.4 $\mu$m, which is estimated by $r/370$ $\mu$m. This result gives an interesting implication: a biological polymer composed of many identical subunits may significantly change its energy state by a negligibly small change induced in a subunit.

The formation of the concentrated assembly caused by the barbed end capping proceeds slowly (Figure 4). The half time decreases in proportion to the molar ratio of subunit actin to gelsolin, *r*, but is constant for the gelsolin/actin complex (see inset of Figure 4B). In addition, the formation is not accelerated by the upside down inversions which cause shear alignment of F-actin. It indicates that the formation should not be the diffusion-limited process, the rate-determining step of which is the alignment of F-actin (Suzuki et al., 1996). Therefore, the results shown in Figure 4 could not be interpreted in terms of a change in the mobility of F-actin due to the shortening of the length by gelsolin. Alternatively, it is suggested that capping of the barbed end gradually changes the nature of the whole actin filament. The time of the capping-effect propagating should be proportional to the F-actin length, because the length of the F-actin treated by gelsolin, *l*, becomes shorter in proportion to *r*, according to $l = r/370$ $\mu$m, while the length of the F-actin treated by the gelsolin/actin complex is unchanged (see inset of Figure 4B). We estimated the rate of propagating as ~1 h/$\mu$m (~10 s/subunit).

The morphology of the assembly structure of the capped F-actin evidently differs from the uncapped one (Figure 3). The difference in the morphologies should reflect the difference in the natures of the individual F-actins as discussed above. The observed crystalline-like structure of the uncapped F-actin (panel a in Figure 3) could not be expected to be produced only by nonspecific force caused by the increase in $\Delta\chi$. Some specific interaction force which is operable only for more highly ordered arrangement should be required for the existence of the crystalline-like regular structure (Flory, 1956). On the other hand, a comparatively

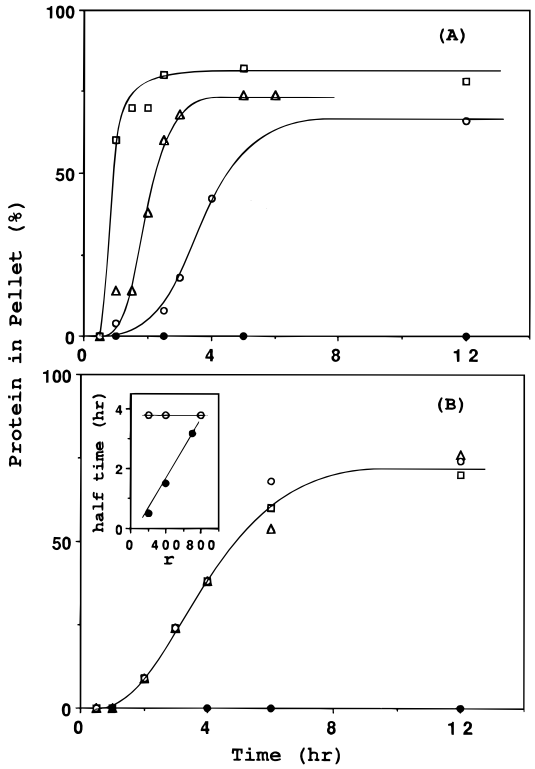


FIGURE 4: Effect of gelsolin or the gelsolin/actin complex on the time course of the formation of the concentrated assembly. G-actin (0.5 mg/mL) was polymerized for 1 h in F-buffer containing 3.7% PEG 6K at which the concentrated assembly was not formed as shown in Figure 1. At time = 0, gelsolin (A) or the 1:2 gelsolin/actin complex (B) was added at various molar ratios to G-actin, and the fraction of F-actin in the concentrated assembly was quantified by the low-speed centrifugation method at an appropriate time. Panel A: (●) control without gelsolin, (○) gelsolin:actin = 1:700, (△) gelsolin:actin = 1:400, and (□) gelsolin:actin = 1:200. Panel B: (●): control without the gelsolin/actin complex, (○) 1:2 gelsolin/actin complex:actin = 1:700, (△) 1:2 gelsolin/actin complex:actin = 1:400, and (□) 1:2 gelsolin/actin complex:actin = 1:200. The inset in (B) represents the half-time of the formation of the concentrated assembly plotted against molar ratios of gelsolin to actin (●) or the 1:2 gelsolin/actin complex to actin (○).

loose and irregular bundle structure of the capped F-actin (panels b−f in Figure 3) suggests that such specific force does not work on the capped F-actin. The difference in the formation of the concentrated assembly between the uncapped and capped F-actins is illustrated in Figure 5. The increasing $\Delta\chi$ value changes the assembly structure of the individual F-actins from the isotropic assembly to the concentrated anisotropic one. However, owing to lack in specific interaction such as operable for the uncapped F-actin, the assembly structure of the capped F-actin could not transformed into a crystalline-like bundle.

The change in the F-actin assembly caused by the barbed end capping reported here was detected only when the $\Delta\chi$ value was just a little smaller than the value on the boundary of the isotropic distribution and the concentrated assembly in the phase diagram of F-actin, which was presented in the accompanying paper (Suzuki et al., 1996). In fact, such a change in the F-actin assembly was not induced below 3% (w/w) of PEG 6K (Figure 1 and 2). The present results may suggest a novel biological function of F-actin capping proteins which has not been able to be recognized in the buffered solution conventionally used for the study of F-actin. In cytoplasm, the $\Delta\chi$ value should be larger than in the

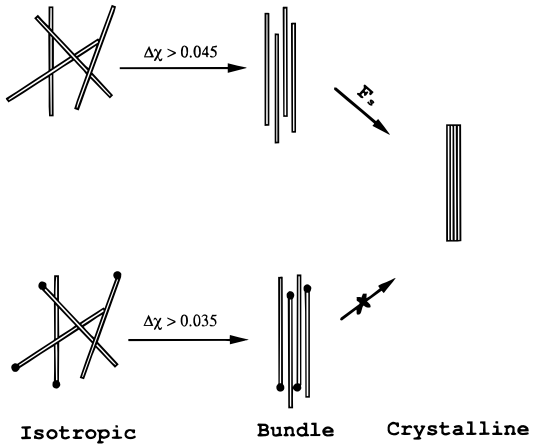


FIGURE 5: Schematic representation of the formation of the concentrated assembly of the uncapped F-actin and the capped F-actin. The rod and the rod with black circle at the end represent the uncapped and the capped F-actin, respectively. $F_s$ means the assumed specific force which may be operable for the uncapped F-actin but not for the capped F-actin. See text for details.

buffered solution without protein, because cytoplasmic proteins should give the same effect as PEG (Suzuki et al., 1989). Therefore, F-actin in cytoplasm may exist near the phase boundary, and consequently the F-actin could change its assembly structure dynamically with a small change of the energy caused by the barbed end capping. Fuchtbauer et al. (1983) and Cooper et al. (1987) reported that microinjection into living cells of capping proteins or the gelsolin fragment which have the activity of the barbed end capping induces remolding of F-actin assembly structure in cytoplasm, although these materials do not show such effects in a permeabilized cell. These results might be attributed to the effect of barbed end capping as discussed here.

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