Mechanism of Regulation of Actin Polymerization by *Physarum* Profilin

KAZUHO OZAKI and SADASHI HATANO
Institute of Molecular Biology, Faculty of Science, Nagoya University, Nagoya 464, Japan

ABSTRACT *Physarum* profilin reduces the rates of nucleation and elongation of F-actin and also reduces the extent of polymerization of actin at the steady state in a concentration-dependent fashion. The apparent critical concentration for polymerization of actin is increased by the addition of profilin. These results can be explained by the idea that *Physarum* profilin forms a 1:1 complex with G-actin and decreases the concentration of actin available for polymerization. The dissociation constant for binding of profilin to G-actin is estimated from the kinetics of polymerization of G-actin and elongation of F-actin nuclei and from the increase of apparent critical concentration in the presence of profilin. The dissociation constants for binding of *Physarum* profilin to *Physarum* and muscle actins under physiological ionic conditions are in the ranges of 1.4–3.7 μM and 11.3–28.5 μM, respectively. When profilin is added to an F-actin solution, profilin binds to G-actin which co-exists with F-actin, and then G-actin is dissociated from F-actin to compensate for the decrease of the concentration of free G-actin and to keep it constant at the critical concentration. At the steady state, free G-actin of the critical concentration is in equilibrium not only with F-actin but also with profilin–G-actin complex. The stoichiometry of 1:1 for the formation of complex between profilin and G-actin is directly shown by means of chemical cross-linking.

Actin is a ubiquitous protein in eucaryotic cells and takes two forms, the unpolymerized and the polymerized forms. In nonmuscle cells the amount of unpolymerized form of actin exceeds 50% of the cytoplasmic actin even though ionic conditions in these cells favor complete polymerization (1). The cells have been expected to contain some regulatory systems to keep actin in the unpolymerized form. Recently, several actin-binding proteins that regulate the conversion between the unpolymerized and the polymerized forms of actin have been found in many nonmuscle cells (7, 21).

An unpolymerized form of actin was first isolated from calf spleen as a 1:1 complex with another protein termed profilin (6). Many mammalian tissues (2, 19, 24), *Acanthamoeba* (30), and sea urchin egg (23) contain similar proteins. Profilins have a molecular weight of 12,000–16,000 daltons and reduce the rate of polymerization of actin in vitro. Tobacman and Korn (34) and Tseng and Pollard (36) have reported that *Acanthamoeba* profilin decreases the elongation rate of actin filaments and increases the apparent critical concentration for polymerization of actin, and have explained these effects of profilin on the basis of the formation of a 1:1 complex with actin.

Physarum plasmodia show cytoplasmic shuttle streaming based on an actin-associating motile system (12, 15). The shuttle streaming, which is caused by a rhythmical contraction–relaxation cycle of cytoplasmic actomyosin biochemically, is associated morphologically with the sol–gel transformation of cytoplasm. Isenberg and Wohlfarth-Bottermann (17) showed that a considerable amount of actin is in the unpolymerized form within flowing endoplasm, and proposed the idea that the cyclic sol–gel transformation of the cytoplasm is based on G–F transformation of actin. Actin-binding proteins may play key roles in such a cytoplasmic sol–gel transformation. *Physarum* profilin isolated from plasmodia is one of the actin-binding proteins that modulate the equilibrium between G- and F-actin. As shown in a previous paper, *Physarum* profilin was similar to profilins isolated from other sources in its molecular weight (13,000) and its effect on polymerization of actin according to the preliminary kinetic analysis, although the amino acid composition of the profilin was notably different from other profilins (29).

In this article, to understand the mechanism of regulation of polymerization of actin by *Physarum* profilin, we have examined the effects of the profilin on the rates of polymerization of actin, elongation of F-actin nuclei, and depolymerization of F-actin, as well as on the extents of polymerization

and depolymerization of actin at steady states. All of the results obtained can be explained by a 1:1 binding of the profilin to G-actin. The dissociation constant for binding of *Physarum* profilin to *Physarum* actin (or muscle actin) was estimated as on the order of 10^{-6} M (or 10^{-5} M) under physiological ionic conditions.

MATERIALS AND METHODS

Plasmodia of *Physarum polycephalum* were cultured by the method of Camp (5) with slight modifications described earlier (12).

Physarum profilin was prepared by the procedure of Ozaki et al. (29). Fragmin was purified by the procedure of Hasegawa et al. (11). *Physarum* actin was purified by gel filtration through Sephadex G-100 (Pharmacia Fine Chemicals, Uppsala, Sweden) according to the method of Hatano and Owaribe (14). Muscle actin was extracted from acetone-dried rabbit skeletal muscle (9) and was purified by the method of Kondo and Ishiwata (20) with gel filtration through Sephadex G-100 as the final step.

Profilin concentration was determined by the absorbance at 280 nm using $\epsilon = 2.3 \times 10^4$ M^{-1} cm^{-1} (29). *Physarum* actin, muscle actin, and fragmin concentrations were determined by the biuret method (10) using $A_{280 nm}^{1\%, 1 cm} = 0.70$ (13). The molecular weight of *Physarum* profilin was determined to be 13,000 by the analysis of the amino acid composition (29). Molecular weights of actin and fragmin were both taken to be 43,000.

Polymerization of actin was monitored with conventional semi-micro Ostwald viscometers. The flow times for a buffer solution were ~30 s. The extent of polymerization of actin at steady states was determined by the flow birefringence, which was measured with a homemade concentric cylinder apparatus (13). For the experiments shown in Figs. 3–7, actin was polymerized in a standard buffer solution consisting of 0.4 mM dithiothreitol (DTT),[1] 0.1 mM CaCl$_2$, 1 mM ATP, 1.5 mM NaN$_3$, 5 mM imidazole-HCl, pH 7.0, and 50 mM KCl (or 50 mM KCl plus 1 mM MgCl$_2$). F-actin passed through a 25-gauge syringe needle four times was used as nuclei for the elongation experiments. The initial rate of polymerization of actin was determined directly from the slope of the viscosity vs. time curves extrapolated to time zero.

The chemical cross-linking of *Physarum* actin to *Physarum* profilin was done by the use of cross-linking reagents, dimethyl suberimidate (DMS) (8) and 1-ethyl-3-(3-[dimethylamino]propyl)-carbodiimide (EDC) (32). Actin (0.26 mg/ml) and profilin (0.32 mg/ml) in 0.1 M triethanolamine hydrochloride, pH 8.5, 0.1 mM ATP, 0.2 mM DTT, and 50 mM KCl were mixed with DMS (3.7 mM) which was dissolved in triethanolamine hydrochloride, pH 8.5, immediately before use. In another experiment, actin and profilin in 10 mM imidazole-HCl, pH 7.0, 0.1 mM ATP, 0.2 mM DTT, and 50 mM KCl were mixed with EDC dissolved in imidazole-HCl, pH 7.0. The concentrations of actin, profilin, and EDC were 0.42 mg/ml, 0.50 mg/ml, and 15 mM, respectively, for the experiments shown in Fig. 1, and 0.26 mg/ml, 0.32 mg/ml, and 5 mM, respectively, for the experiments shown in Fig. 2. The reaction mixtures were left at 20°C for 2 h, and then polypeptide components in the samples were analyzed by PAGE in the presence of SDS.

SDS PAGE was carried out by the method of Hubbard and Lazarides (16) which was a slight modification of the method of Laemmli (22). Gels were stained with Coomassie Brilliant Blue (Sigma Chemical Co., St. Louis, MO) and scanned at 570 nm in a Gilford spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, OH). A silver stain was also used for detection of the components in the samples (25).

RESULTS

Binding of Physarum Profilin to Physarum Actin

When *Physarum* profilin was applied to a DEAE-cellulose (DE-52, Whatman, England) column equilibrated with a buffer solution containing 50 mM KCl, 0.2 mM DTT, 0.1 mM ATP, and 10 mM Tris-HCl, pH 8.2, at 4°C, profilin passed through the column. However, when a mixture of profilin and *Physarum* actin was applied to the same column, part of the profilin was retained in the column and eluted only after applying a gradient of KCl from 50 to 250 mM (Fig. 1A). The molar ratio of profilin to actin in the eluted

---

[1] *Abbreviations used in this paper:* DMS, dimethyl suberimidate; DTT, dithiothreitol; EDC, 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide.
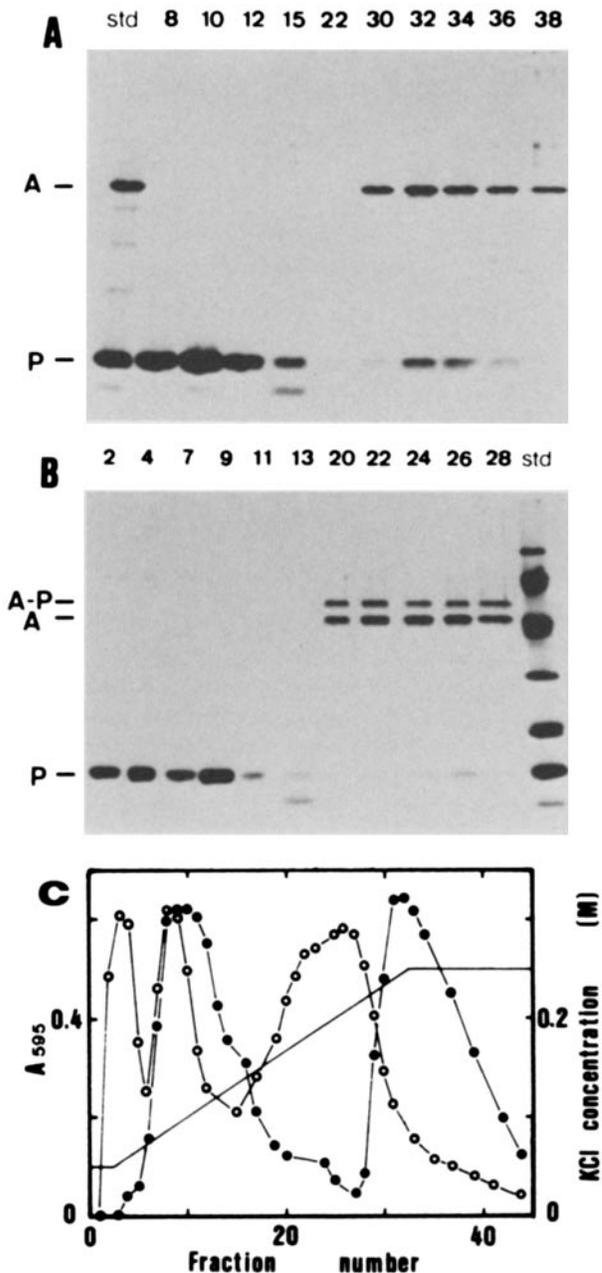
FIGURE 1 Ion-exchange column chromatography of the mixture of *Physarum* actin and profilin. Fractions were analyzed by 15% SDS PAGE in *A* and *B*, and by protein-dye binding assay according to the method of Bradford (4) in *C*; gels were stained with silver after electrophoresis. (*A*) Actin (0.42 mg/ml) and profilin (0.50 mg/ml) in 50 mM KCl, 0.1 mM ATP, 0.2 mM DTT, and 10 mM Tris-HCl, pH 8.2, (3 ml) were applied to a DEAE-cellulose column (1.2 × 5 cm) equilibrated with the same buffer solution. A linear gradient of KCl from 50 to 250 mM (50 ml) was applied, and then 2-ml fractions were collected. Numbers at the top of the lanes on SDS PAGE correspond to the fraction number of the column chromatography. *std.,* molecular weight standards: *A, Physarum* actin (43,000 daltons) and *P, Physarum* profilin (13,000 daltons). (*B*) Actin (0.42 mg/ml) and profilin (0.50 mg/ml) in 10 mM imidazole-HCl, pH 7.0, 0.1 mM ATP, 0.2 mM DTT, and 50 mM KCl (3 ml) were incubated with 15 mM EDC at 20°C for 2 h, and then the reaction was quenched by the addition of excess 2-mercaptoethanol. The sample was dialyzed against 10 mM Tris-HCl, pH 8.2, 0.1 mM ATP, 0.2 mM DTT, and 50 mM KCl at 4°C for 20 h and applied to a DEAE-cellulose column under the same conditions as described in *A*. Numbers at the top of the lanes on SDS PAGE correspond to the fraction number of

sample was ~0.7, which was estimated by scanning gels of SDS PAGE stained with Coomassie Brilliant Blue. When a mixture of profilin and F-actin in 0.1 M KCl was centrifuged at 100,000 g for 90 min at 15°C, profilin remained in the supernatant. These results suggested that *Physarum* profilin interacted with G-actin at a molar ratio of 1:1 but did not apparently interact with F-actin.

To show that profilin did, in fact, form a 1:1 complex with G-actin, proteins were treated with a chemical cross-linking reagent, EDC, which forms zero-length covalent bonds between carboxyl and amino groups in the contact area. When a mixture of profilin and actin treated with EDC was applied to the same column as described above, a 50,000-dalton peptide and actin were observed as major bands in the fractions coming off the column after applying a gradient of KCl, but cross-linked actin oligomers were not observed (Fig. 1 *B*). When the fractions 31–34 of Fig. 1*A* which contained both actin and profilin were collected and treated with EDC, the 50,000-dalton peptide was detected on SDS PAGE (data not shown). Fig. 2 shows that if actin or profilin alone were treated with EDC, cross-linked products were not formed appreciably. However, a major new band at a molecular weight of 50,000 appeared when a mixture of profilin and actin was treated with EDC (Fig. 2). These results show that profilin strongly interacted with G-actin and the new 50,000-dalton peptide was a cross-linked 1:1 complex between profilin and G-actin, although its apparent molecular weight was a little

lower than the expected value (56,000) on SDS PAGE. Thus, the formation of a 1:1 complex between profilin and G-actin expected in the results shown in Fig. 1*A* was proved.

Another cross-linking reagent, dimethyl suberimidate (DMS), which forms 1.1-nm-long covalent bonds between amino groups, was tested (Fig. 2, lanes *d–f*). In this case, two major new bands appeared; apparent molecular weights were 50,000 and 52,000. We supposed that these new polypeptides were the cross-linked products of profilin–G-actin complex, and the variation of motility of the cross-linked products on SDS PAGE was caused by conformational modification due to intramolecular cross-linking between the different sites.

Initial Rate of Polymerization

The effect of *Physarum* profilin on polymerization of *Physarum* and muscle actins was investigated by viscometry (Fig. 3). Profilin strongly inhibited the polymerization of *Physarum* actin in a solution containing 2 mM $MgCl_2$, 0.2 mM DTT, 0.1 mM ATP, and 20 mM imidazole-HCl, pH 7.0, (Fig. 3*A*). The initial rate of polymerization of 10 μM *Physarum* actin was reduced to 1/15 by 6.4 μM profilin and to 1/300 by 13 μM profilin. Profilin also inhibited the polymerization of muscle actin (Fig. 3*B*), although the effect was weaker than on *Physarum* actin. The initial rate of polymerization of 7.4 μM muscle actin was reduced to 1/18 by 15 μM profilin.

Initial Rate of Elongation

Polymerization of actin consists of at least two steps, nucleation and elongation, and nucleation is a rate-limiting step
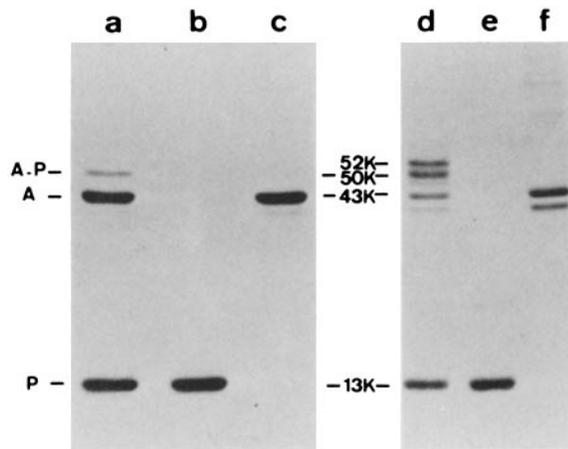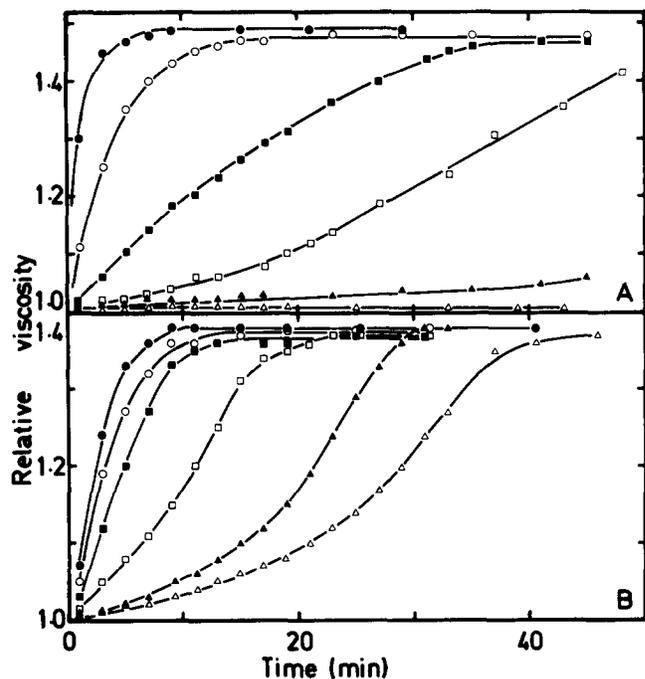

Figure 2 Cross-linking of *Physarum* actin to *Physarum* profilin. Proteins (0.26 mg/ml actin and 0.32 mg/ml profilin) were incubated with 5 mM EDC or 3.7 mM DMS at 20°C for 2 h in 50 mM KCl, 0.1 mM ATP, 0.2 mM DTT, and 10 mM imidazole-HCl, pH 7.0, or 0.1 M triethanolamine hydrochloride, pH 8.5, and the samples were then analyzed by 15% SDS PAGE. (*a–c*) Treated with EDC, (*d–f*) treated with DMS, (*a* and *d*) actin plus profilin, (*b* and *e*) profilin alone, (*c* and *f*) actin alone. Gels were stained with Coomassie Brilliant Blue. The molecular weights (*K*, × 10³) for the major bands are indicated in the figure. *A*, *Physarum* actin; *P*, *Physarum* profilin; and *A-P*, cross-linked actin–profilin complex.

the column chromatography. *std.*, molecular weight standards: phosphorylase *b* (94,000 daltons), albumin (67,000 daltons), ovalbumin (43,000 daltons), carbonic anhydrase (30,000 daltons), trypsin inhibitor (20,100 daltons), and α-lactalbumin (14,400 daltons). *A*, *Physarum* actin; *P*, *Physarum* profilin; and *A-P*, cross-linked actin–profilin complex. (*C*) The elution patterns of the EDC-untreated (●) and the EDC-treated (○) samples on DEAE-cellulose column chromatography.

Figure 3 Time course of polymerization of *Physarum* and muscle actins in the presence of *Physarum* profilin assayed by viscometry. (*A*) *Physarum* G-actin (10 μM) was polymerized in 2 mM $MgCl_2$, 0.2 mM DTT, 0.1 mM ATP, 20 mM imidazole-HCl, pH 7.0, and various concentrations of profilin at 23°C. The actin and profilin were preincubated for 10 min before the addition of 2 mM $MgCl_2$. ●, control (actin alone); ○, profilin of 3.4 μM; ■, 6.4 μM; □, 10 μM; ▲, 13 μM; △, 20 μM. (*B*) Muscle actin (7.4 μM) was polymerized in the same conditions as in *A*. ●, control (actin alone); ○, profilin of 1.9 μM; ■, 3.7 μM; □, 7.5 μM; ▲, 15 μM; △, 23 μM.

in the process (18, 28). It is important to investigate the effect of profilin on the nucleation step and the elongation step separately. The rate of elongation was determined by the addition of fragmented F-actin as a nucleus to a G-actin solution containing profilin.

Equal amount of F-actin nuclei were added to solutions of G-actin containing 50 mM KCl and 0.1 mM CaCl₂ at various concentrations of *Physarum* profilin (Fig. 4). In this experimental condition the polymerization of G-actin was extremely slow without F-actin fragments. Fragmented F-actin markedly increased the rate of polymerization of *Physarum* actin even in the presence of profilin. The rate of elongation decreased with increasing concentration of profilin added. Profilin of 5, 10, and 20 μM reduced the initial rate of polymerization of 10 μM *Physarum* G-actin to 2/3, 1/3, and 1/6, respectively.

Extent of Polymerization

The extent of polymerization or depolymerization of actin in the presence and absence of *Physarum* profilin at steady states was investigated by starting from G- or F-actin as shown in Figs. 5 and 6. Actins were incubated with profilin for 22–24 h at 22°C in the standard buffer solution containing 50 mM KCl (or 50 mM KCl plus 1 mM MgCl₂), and then the flow birefringence was measured.

Fig. 5 shows the degrees of flow birefringence of *Physarum* actin solutions, one of which was polymerized from 19 μM G-actin and the other was depolymerized from 19 μM F-actin at the same concentration of profilin. In these two solutions, polymerization of actin which started from G- and F-actin reached the same extent. The reduction of the degree of flow birefringence by profilin was linearly proportional to the concentration of profilin.

FIGURE 5 Extent of polymerization of *Physarum* actin at steady states as a function of *Physarum* profilin concentration. 19 μM *Physarum* F-actin (or G-actin) was incubated with various concentrations of profilin for 22–24 h at 22°C in 50 mM KCl (or 50 mM KCl plus 1 mM MgCl₂), 0.4 mM DTT, 0.1 mM CaCl₂, 1 mM ATP, 1.5 mM NaN₃, and 5 mM imidazole-HCl, pH 7.0, and the degrees of flow birefringence were measured. Profilin was added to: ●, F-actin in 50 mM KCl plus 1 mM MgCl₂; ▲, G-actin in 50 mM KCl plus 1 mM MgCl₂; ○, F-actin in 50 mM KCl; △, G-actin in 50 mM KCl.
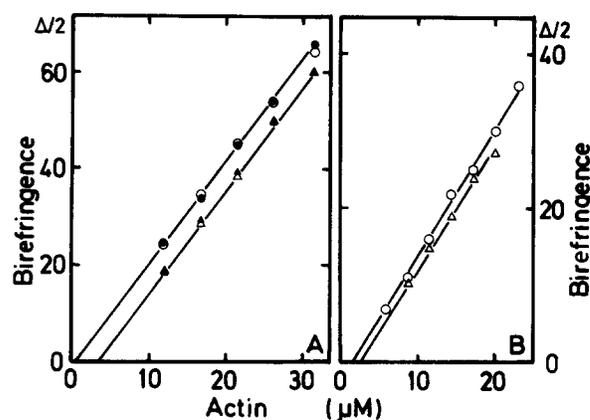
FIGURE 6 Extent of polymerization of *Physarum* and muscle actins at steady states as a function of actin concentration. (A) *Physarum* F-actin (48 μM) was diluted to the concentrations indicated in the figure with a solution of 50 mM KCl (○, △) or 50 mM KCl plus 1 mM MgCl₂ (●, ▲), 0.4 mM DTT, 0.1 mM CaCl₂, 1 mM ATP, 1.5 mM NaN₃, and 5 mM imidazole-HCl, pH 7.0, at 22°C in the presence (△, ▲) and absence (○, ●) of 10 μM profilin. Samples were left standing for 22–24 h at 22°C and then the degrees of flow birefringence were measured. (B) Muscle actin (29 μM) was diluted to the concentrations indicated in the figure with the same buffer solution as described in A containing 50 mM KCl in the presence (△) and absence (○) of 20 μM profilin.
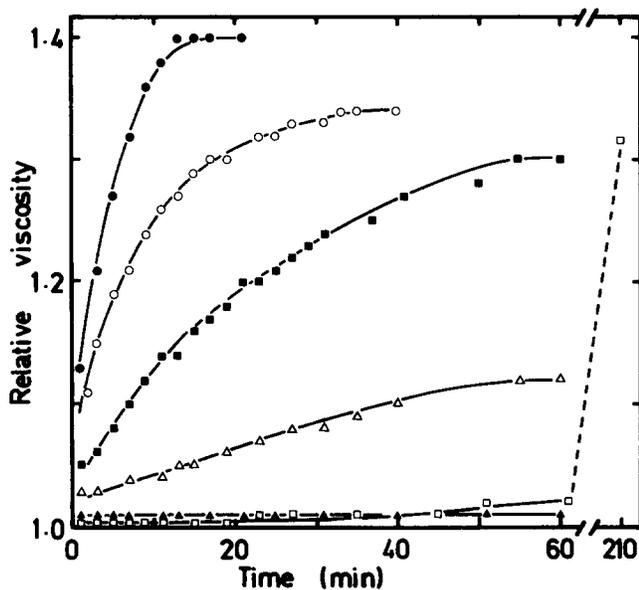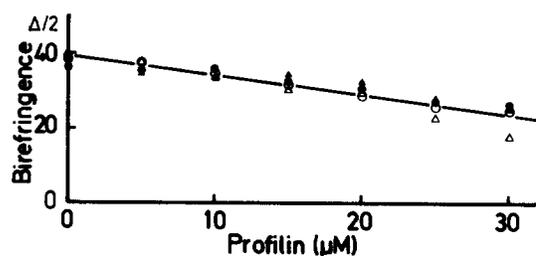

FIGURE 4 Time course of nucleated polymerization of *Physarum* actin in the presence of *Physarum* profilin assayed by viscometry. *Physarum* G-actin (10 μM) was polymerized with 1 μM of fragmented F-actin in 50 mM KCl, 0.4 mM DTT, 0.1 mM CaCl₂, 1 mM ATP, 1.5 mM NaN₃, 5 mM imidazole-HCl, pH 7.0, and various concentrations of profilin at 25°C. The actin and profilin were preincubated for a minute before the addition of F-actin nuclei and KCl. ●, control (10 μM G-actin); ○, profilin of 5 μM; ■, 10 μM; △, 20 μM; ▲, 40 μM; □, 11 μM G-actin and 10 μM profilin without seeds.

This reduction is attributable to the increase of the apparent critical concentration in the presence of profilin. In Fig. 6, the relationship between the extent of polymerization and the total concentration of actin in the presence of profilin was exactly parallel to that in the absence of profilin. The critical concentration for *Physarum* actin was increased from 0.6 to 3.0 μM by the addition of 10 μM profilin both in 50 mM KCl and 50 mM KCl plus 1 mM MgCl₂ (Fig. 6A) and that for muscle actin was increased from 1.5 to 2.5 μM by 20 μM profilin in 50 mM KCl (Fig. 6B).

The decrement in the extent of polymerization, which is the increment of the apparent critical concentration, was linearly proportional to the concentration of profilin. As the concentration of free G-actin in equilibrium with F-actin (actual critical concentration) is independent of the concentration of profilin at any concentration of actin, the increment of apparent critical concentration corresponds to the amount of complex formed. Thus, free G-actin has to be in equilib-

rium not only with F-actin but also with complex of profilin–G-actin at the steady state. Profilin reduces the concentration of F-actin unaffecting the length distribution of filaments unlike the effects of end-blocking proteins; for example, fragmin, villin, and severin sever F-actin filaments and block the association of the fragments by capping their ends (3, 11, 37).

Calculation of the Dissociation Constant for Binding of Physarum Profilin to G-actin

As described in the previous sections, our results strongly suggest that profilin binds to actin monomer at a molar ratio of 1:1 and the concentration of actin available for polymerization is reduced because the complex cannot polymerize. According to this scheme, we can calculate the dissociation constants for binding of *Physarum* profilin to *Physarum* and muscle actins.

(*a*) The dissociation constant was calculated from the reduction of the degree of flow birefringence at steady states (Figs. 5 and 6). As summarized in Table I, the dissociation constants for binding of profilin to *Physarum* and muscle actins are 1.9 μM in 50 mM KCl or 50 mM plus 1 mM $MgCl_2$ and 28.5 μM in 50 mM KCl, respectively. In Fig. 5, the concentration of profilin given by extrapolating the values of flow birefringence to zero is ~80 μM. This value is the minimal concentration required for complete depolymerization of *Physarum* F-actin at a concentration of 19 μM. The dissociation constant calculated from these values is 2.0 μM as the critical concentration is 0.6 μM in this condition.

(*b*) The dissociation constant was calculated from the initial rate of polymerization (Fig. 3). The initial rate of polymerization depends on the third or fourth power of the concentration of actin (18, 28, 35). If the initial rate of polymerization is proportional to the fourth power of the concentration of G-actin, the initial concentration of free G-actin in the presence of profilin can be estimated from the initial rate of polymerization. For example, 6.4 μM profilin reduced the initial concentration of *Physarum* G-actin from 10 to 5 μM; the concentration of complex, therefore, was 5 μM (Table II). The dissociation constants for binding of profilin to *Physarum* and muscle actins in 2 mM $MgCl_2$ range from 1.4 to 3.7 μM and 11.3 to 16.2 μM, respectively, (Table II).

(*c*) The dissociation constant was calculated from the rate of elongation (Fig. 4). We can estimate the decrease in the concentration of free G-actin due to the formation of profilin–

TABLE II
Dissociation Constants for Binding of Physarum Profilin to Actin Calculated from the Initial Rate of Polymerization

Total actin	Total profilin	Initial rate	Free G-actin	Complex	Free profilin	K_D
μM	μM	min^{-1}	μM	μM	μM	μM
			Physarum			
10.0	0	0.3	10.0	—	—	—
	3.4	0.11	7.7	2.3	1.1	3.7
	6.4	0.02	5.0	5.0	1.4	1.4
	10.0	0.004	3.4	6.6	3.4	1.8
	13.0	0.001	2.4	7.6	5.4	1.7
			Muscle			
7.4	0	0.07	7.4	—	—	—
	1.9	0.05	6.7	0.7	1.2	11.5
	3.7	0.04	6.2	1.2	2.5	12.9
	7.5	0.02	5.3	2.1	5.4	13.6
	15.0	0.004	3.7	3.7	11.3	11.3
	23.0	0.003	3.4	4.0	19.0	16.2

The data shown in the first through the third columns were obtained from Fig. 3. Initial rate of polymerization of actin was directly measured on the graphs. The rate is the increment in specific viscosity per one minute. The concentration of free G-actin is calculated as follows: ([free G-actin]/[free G-actin in the absence of profilin])^4 = (initial rate in the presence of profilin)/ (initial rate in the absence of profilin). The concentration of complex is the difference between the total and free G-actin concentrations. The concentration of free profilin is the difference between the total profilin and complex concentrations. The dissociation constant (K_D) is calculated by the relation of [free G-actin] [free profilin]/[complex], assuming stoichiometry of 1:1.

G-actin complex from the initial rate of nucleated polymerization, as the initial rate of elongation is directly proportional to the concentration of G-actin above the critical concentration; $(k_+C - k_-) = k_+(C - k_-/k_+)$, where k_+ and k_- are the rate constants of association of monomers to F-actin and dissociation of monomers from F-actin, respectively, C is the concentration of G-actin, and k_-/k_+ is defined as the critical concentration (28). For example, profilin at 10 μM reduced the initial rate of elongation of F-actin at 10 μM of *Physarum* G-actin to 1/3 of that in the absence of profilin. As the critical concentration is 0.6 μM in this condition (Fig. 6*A*), the initial concentration of free G-actin is estimated as 3.7 μM. Therefore, the equivalent of 6.3 μM G-actin must be in the form of the complex. The dissociation constant for binding of profilin to *Physarum* actin is 2.2 μM in this example. Thus, the values of the dissociation constant for formation of profilin–G-actin complex estimated by different methods are in reasonable agreement (Tables I and II).

Rate of Depolymerization

When various concentrations of *Physarum* profilin were added to *Physarum* F-actin in the standard buffer solution containing 50 mM KCl, the viscosity of each sample decreased to new levels at the steady state (Fig. 7). It took 1–4 h to reach the steady-state levels, where the viscosity depended on the concentration of profilin added. However, the initial rate of decrease in viscosity was independent of the concentration of profilin >10 μM. For 50% of the reduction in viscosity, 5–25 min was required.

When profilin is added to an F-actin solution, profilin rapidly binds to G-actin which co-exists with F-actin in the solution. Profilin reduces the rate of association of monomer to polymer of actin by reducing the concentration of free G-actin. The initial rate of decrease in viscosity is independent of the concentration of profilin because the dissociation of monomer from polymer of actin is rate limiting in this
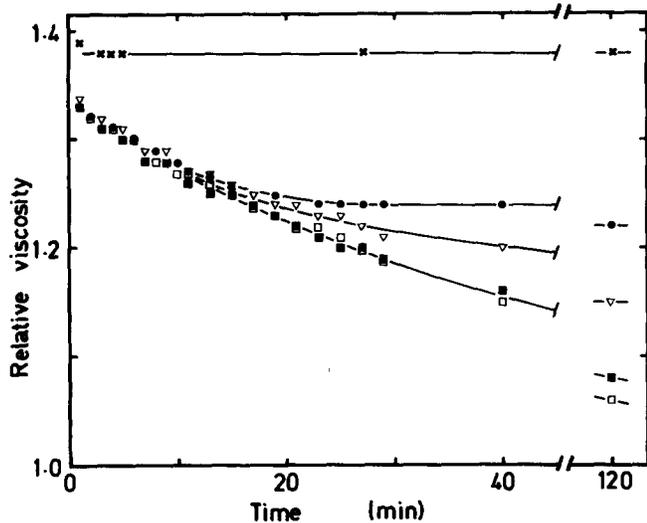
TABLE I
Dissociation Constants for Binding of Physarum Profilin to Actin Calculated from the Critical Concentration at Steady State

Total actin	Total profilin	Critical concentration	Complex	Free profilin	K_D
μM	μM	μM	μM	μM	μM
		Physarum			
10.0	0	0.6	—	—	—
	10.0	3.0	2.4	7.6	1.9
		Muscle			
10.0	0	1.5	—	—	—
	20.0	2.5	1.0	19.0	28.5

The data for the critical concentrations were obtained from Fig. 6. The concentration of complex is calculated as the difference between the critical concentrations in the presence and absence of profilin. The critical concentration in the absence of profilin corresponds to the concentration of free G-actin in the presence of profilin. The dissociation constant (K_D) is calculated by the relation of [free G-actin] [free profilin]/[complex], assuming stoichiometry of 1:1.

Figure 7 Time course of depolymerization of *Physarum* F-actin in the presence of *Physarum* profilin assayed by viscometry. 43 μM F-actin was diluted to 10 μM with a solution of 50 mM KCl, 0.4 mM DTT, 0.1 mM CaCl₂, 1 mM ATP, 1.5 mM NaN₃, and 5 mM imidazole-HCl, pH 7.0, at 25°C. At time zero, various concentrations of profilin were added to the solution. X, control (actin alone); ●, profilin of 12 μM; ∇, 23 μM; ■, 35 μM; □, 47 μM.
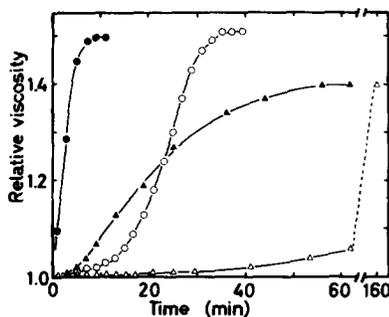

Figure 8 Effect of MgCl₂ on polymerization of *Physarum* actin in the presence of *Physarum* profilin. G-actin (15 μM) and profilin (10 μM) were incubated in 0.4 mM DTT, 0.1 mM CaCl₂, 1 mM ATP, 1.5 mM NaN₃, and 5 mM imidazole-HCl, pH 7.0, for a minute at 25°C, and then 50 mM KCl or 50 mM KCl plus 1 mM MgCl₂ was added. O, actin in 50 mM KCl; ●, actin in 50 mM KCl plus 1 mM MgCl₂; △, actin and profilin in 50 mM KCl; ▲, actin and profilin in 50 mM KCl plus 1 mM MgCl₂.

process. When the concentration of free G-actin reaches the critical concentration, the equilibrium at a new level of steady state is established.

Effect of MgCl₂

The addition of 1 mM MgCl₂ increased the initial rate of polymerization of *Physarum* actin both in the presence and absence of *Physarum* profilin (Fig. 8). The extent of polymerization of *Physarum* actin was unaffected by the additional MgCl₂, however, and the extent of polymerization depended on the concentration of profilin in the same way both in the presence and absence of MgCl₂ (Fig. 5).

Effect of Fragmin

The initial rate of polymerization of *Physarum* actin in the presence of *Physarum* profilin was markedly accelerated by fragmin in the presence of CaCl₂ (Fig. 9) but it was not affected in the absence of CaCl₂ (data not shown). It has been reported that fragmin accelerates the initial stage of polymerization of actin in the presence of micromolar concentra-
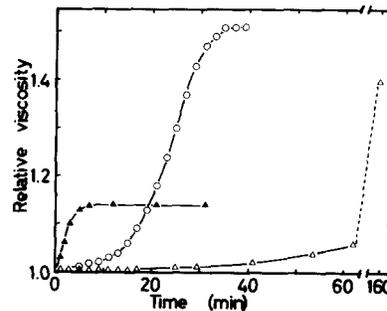

Figure 9 Effect of frag-min on polymerization of *Physarum* actin in the presence of *Physarum* profilin. G-actin (15 μM) and profilin (10 μM) were incubated in the buffer solution as described in Fig. 7, and then 50 mM KCl or 50 mM KCl plus 0.3 μM fragmin was added. O, actin in 50 mM KCl; △, actin and profilin in 50 mM KCl; ▲, actin, profilin, and fragmin in 50 mM KCl.

tions of CaCl₂, and keeps the length of actin filaments short (11, 31). Our results show that fragmin interacts with free G-actin to form nuclei and increases the initial rate of polymerization of actin although the final viscosity is reduced in the presence of profilin. Profilin does not interfere directly with the interaction between fragmin and actin.

DISCUSSION

Polymerization of actin consists of nucleation and the growth process (18, 28). Each of the processes is closely related to the concentration of G-actin. Analysis of the effect of *Physarum* profilin on these two processes that occur during polymerization gave us insight into the stoichiometric binding of actin to profilin. Profilin sequesters G-actin, so that the rates of actin polymerization and F-actin elongation decrease and the apparent critical concentration increases.

When G-actin and profilin are mixed, G-actin binds to profilin with a dissociation constant of 1.4–3.7 μM (for *Physarum* actin) or 11.3–16.2 μM (for muscle actin) (Table II). Upon the addition of salts, only the remaining free G-actin can form nuclei and associate to F-actin. When the polymerization proceeds in the mixture, the dissociation of complex continues until the final steady state is reached. When profilin is added to an F-actin solution, G-actin which co-exists with F-actin (at the critical concentration) binds to profilin, and then G-actin is dissociated from F-actin to compensate for the decrease of the concentration of free G-actin and to keep the concentration of free G-actin constant. At the steady state, an equilibrium is established among the following states:

F-actin ⇌ G-actin (of the critical concentration)

⇌ profilin–G-actin complex.
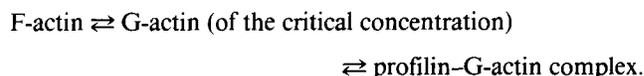
Such a mechanism of regulation of polymerization of actin by *Physarum* profilin is identical to that reported in the case of *Acanthamoeba* profilin (34, 36).

The results from the kinetic analysis of polymerization of actin in the presence of profilin were consistently explained by the idea that profilin forms a 1:1 complex with G-actin. We showed directly the stoichiometry of 1:1 for the formation of complex between profilin and G-actin by means of chemical cross-linking (Figs. 1 and 2). It has been reported that mammalian profilactin is a 1:1 complex of actin and profilin (6), and the stoichiometry for binding of *Acanthamoeba* profilin to muscle actin was calculated to be 1:1 (26).

The effect of *Physarum* profilin on muscle actin is weaker than that on *Physarum* actin (Figs. 3 and 6). The dissociation constant for binding of *Physarum* profilin to muscle actin is

28.5 μM, which is about 15-fold higher than that to *Physarum* actin (1.9 μM) (Table I). Species-specific binding of actin with profilin has also been reported for *Acanthamoeba* (34, 36) and sea urchin egg (23). The dissociation constants for binding of *Acanthamoeba* profilin to *Acanthamoeba* and muscle actins are 4–11 μM and 60–80 μM, respectively, (34). These values are comparable to those for binding of *Physarum* profilin to *Physarum* and muscle actins.

Tseng and Pollard (36) reported that $MgCl_2$ strongly inhibited the formation of complex between *Acanthamoeba* profilin and actin. However, Tobacman and Korn reported that the dissociation constant for binding of *Acanthamoeba* profilin to actin was independent of the conditions of salts including $MgCl_2$ (33, 34). *Physarum* profilin reduced the rate of polymerization of actin in the presence of $MgCl_2$ as well as in the absence of $MgCl_2$ (Figs. 3 and 8), although the addition of $MgCl_2$ accelerated the rate of polymerization of actin in the presence of profilin (Fig. 8). The concentration of complex at the steady state depends on the critical concentration of actin. At the higher critical concentration, the concentration of complex is higher. As the concentration of complex was unaffected by the addition of $MgCl_2$ (Figs. 5 and 6), the extent of polymerization of *Physarum* actin in the presence of profilin was unaffected by the addition of $MgCl_2$ in Fig. 8. Therefore, $MgCl_2$ accelerates nucleation of F-actin, but not affect the binding of profilin to G-actin. Most recently, Tobacman and Korn (35) have shown fluorometrically that $MgCl_2$ increases the rate constant for nucleation in the polymerization of actin.

According to the results, we can estimate that a notable amount of actin in plasmodia is in the unpolymerized state, forming the complex with profilin. For example, if intracellular actin concentration, as estimated previously (27), is 50 μM and the concentration of profilin is assumed to be equal to the concentration of actin, 17 μM actin is calculated to be in the form of complex with profilin by the use of the following values: 1 μM of critical concentration for polymerization of actin and 2 μM of dissociation constant for binding of profilin to G-actin (Table I). Therefore, 36% of the total actin is in the unpolymerized form. If the critical concentration for polymerization of actin shifts from 0.5 to 1.5 μM, the concentration of F-actin shifts from 39.5 μM (79% of the total actin) to 27.5 μM (55% of the total actin). Such a change in critical concentration could occur in vivo, in that fragmin doubles the critical concentration by blocking the barbed end of actin filaments (31). Even in the presence of profilin, fragmin interacted with actin independently of the effect of profilin (Fig. 9). Therefore, the critical actin concentration in plasmodia is regulated not only by intracellular ionic conditions but also by fragmin which is activated by Ca^{2+} above a micromolar concentration. In the presence of profilin, a small change in critical concentration could be amplified into a notable change in F-actin concentration; 24% of the total actin are transformed between the unpolymerized and the polymerized forms of actin. Thus, *Physarum* profilin should be a key factor for cytoplasmic sol–gel transformation in plasmodia.

REFERENCES

1. Blikstad, I., F. Markey, L. Carlsson, T. Persson, and U. Lindberg. 1978. Selective assay of monomeric and filamentous actin in cell extracts, using inhibition of deoxyribonuclease I. *Cell.* 15:935–943.
2. Blikstad, I., I. Sundkvist, and S. Eriksson. 1980. Isolation and characterization of profilactin and profilin from calf thymus and brain. *Eur. J. Biochem.* 105:425–433.
3. Bonder, E. M., and M. S. Mooseker. 1983. Direct electron microscopic visualization of barbed end capping and filament cutting by intestinal microvillar 95-K dalton protein (Villin): a new actin assembly assay using the *Limulus* acrosomal process. *J. Cell Biol.* 96:1097–1107.
4. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.
5. Camp, W. G. 1936. A method of cultivating myxomycete plasmodia. *Bull. Torrey Bot. Club.* 63:205–210.
6. Carlsson, L., L.-E. Nyström, I. Sundkvist, F. Markey, and U. Lindberg. 1977. Actin polymerizability is influenced by profilin, a low molecular weight protein in non-muscle cells. *J. Mol. Biol.* 115:465–483.
7. Craig, S. W., and T. D. Pollard. 1982. Actin-binding proteins. *Trends Biochem. Sci.* 7:88–92.
8. Davies, G. E., and G. R. Stark. 1970. Use of dimethyl suberimidate, a cross-linking reagent, in studying the subunit structure of oligomeric proteins. *Proc. Natl. Acad. Sci. USA* 66:651–656.
9. Ebashi, S., and F. Ebashi. 1964. A new protein component participating in the superprecipitation of myosin B. *J. Biochem. (Tokyo).* 55:604–613.
10. Gornall, A. G., C. S. Bardawill, and M. M. David. 1949. Determination of serum proteins by means of the biuret reaction. *J. Biol. Chem.* 177:751–766.
11. Hasegawa, T., S. Takahashi, H. Hayashi, and S. Hatano. 1980. Fragmin: a calcium ion sensitive regulatory factor on the formation of actin filaments. *Biochemistry.* 19:2677–2683.
12. Hatano, S., and F. Oosawa. 1966. Isolation and characterization of plasmodium actin. *Biochim. Biophys. Acta.* 127:488–598.
13. Hatano, S., and K. Owaribe. 1976. Actin and actinin from myxomycete plasmodia. *In* Cell Motility, Vol. 3, Book B, R. Goldman, T. Pollard, and J. Rosenbaum, editors. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. 499–511.
14. Hatano, S., and K. Owaribe. 1977. A simple method for the isolation of actin from myxomycete plasmodia. *J. Biochem. (Tokyo).* 88:201–205.
15. Hatano, S., and M. Tazawa. 1968. Isolation, purification and characterization of myosin B from myxomycete plasmodium. *Biochim. Biophys. Acta.* 154:507–519.
16. Hubbard, B. D., and E. Lazarides. 1979. Copurification of actin and desmin from chicken smooth muscle and their copolymerization in vitro to intermediate filaments. *J. Cell Biol.* 80:166–182.
17. Isenberg, G., and K. E. Wohlfarth-Bottermann. 1976. Transformation of cytoplasmic actin: importance for the organization of the contractile gel reticulum and the contraction-relaxation cycle of cytoplasmic actomyosin. *Cell Tissue Res.* 173:495–528.
18. Kasai, M., S. Asakura, and F. Oosawa. 1962. The cooperative nature of G-F transformation of actin. *Biochim. Biophys. Acta.* 57:22–31.
19. Kobayashi, R., W. A. Bradley, and J. B. Field. 1982. A simple and rapid method for purification of profilin from bovine thyroid. *Anal. Biochem.* 120:106–110.
20. Kondo, H., and S. Ishiwata. 1976. Uni-directional growth of F-actin. *J. Biochem. (Tokyo).* 79:159–171.
21. Korn, E. D. 1982. Actin polymerization and its regulation by proteins from nonmuscle cells. *Physiol. Rev.* 62:672–737.
22. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.
23. Mabuchi, I., and H. Hosoya. 1982. Actin-modulating proteins in the sea urchin egg. II. Sea urchin egg profilin. *Biomed. Res.* 3:465–476.
24. Markey, F., U. Lindberg, and L. Eriksson. 1978. Human platelets contain profilin, a potential regulator of actin polymerizability. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 88:75–79.
25. Merril, C. R., D. Goldman, S. A. Sedman, and M. H. Ebert. 1981. Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. *Science (Wash. DC).* 211:1437–1438.
26. Mockrin, S. C., and E. D. Korn. 1980. *Acanthamoeba* profilin interacts with G-actin to increase the rate of exchange of actin-bound adenosine 5'-triphosphate. *Biochemistry.* 19:5359–5362.
27. Nachmias, V. T. 1979. The contractile proteins of *Physarum polycephalum* and actin polymerization in plasmodial extracts. *In* Cell Motility: Molecules and Organization. S. Hatano, H. Ishikawa, H. Sato, editors. University of Tokyo Press, Tokyo. 33–57.
28. Oosawa, F., and S. Asakura. 1975. Thermodynamics of the polymerization of protein. Academic Press Inc., London. 10–55.
29. Ozaki, K., H. Sugino, T. Hasegawa, S. Takahashi, and S. Hatano. 1983. Isolation and characterization of *Physarum* profilin. *J. Biochem. (Tokyo).* 93:295–298.
30. Reichstein, E., and E. D. Korn. 1979. *Acanthamoeba* profilin. A protein of low molecular weight from *Acanthamoeba castellanii* that inhibits actin nucleation. *J. Biol. Chem.* 254:6174–6179.
31. Sugino, H., and S. Hatano. 1982. Effect of fragmin on actin polymerization: evidence for enhancement of nucleation and capping on the barbed end. *Cell Motility.* 2:457–470.
32. Sutoh, K. 1982. Identification of myosin-binding sites on actin sequence. *Biochemistry.* 21:3654–3661.
33. Tobacman, L. S., S. L. Brenner, and E. D. Korn. 1983. Effect of *Acanthamoeba* profilin on the pre-steady state kinetics of actin polymerization and on the concentration of F-actin at steady state. *J. Biol. Chem.* 258:8806–8812.
34. Tobacman, L. S., and E. D. Korn. 1982. The regulation of actin polymerizaion and the inhibition of monomeric actin ATPase activity by *Acanthamoeba* profilin. *J. Biol. Chem.* 257:4166–4170.
35. Tobacman, L. S., and E. D. Korn. 1983. The kinetics of actin nucleation and polymerization. *J. Biol. Chem.* 258:3207–3214.
36. Tseng, P. C., and T. D. Pollard. 1982. Mechanism of action of *Acanthamoeba* profilin: demonstration of actin species specificity and regulation by micromolar concentrations of $MgCl_2$. *J. Cell Biol.* 94:213–218.
37. Yamamoto, K., J. D. Pardee, J. Reidler, L. Stryer, and J. A. Spudich. 1982. Mechanism of interaction of *Dictyostelium* severin with actin filaments. *J. Cell Biol.* 95:711–719.