Mechanism of Action of *Acanthamoeba* Profilin: Demonstration of Actin Species Specificity and Regulation by Micromolar Concentrations of $MgCl_2$

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ABSTRACT *Acanthamoeba* profilin strongly inhibits in a concentration-dependent fashion the rate and extent of *Acanthamoeba* actin polymerization in 50 mM KCl. The lag phase is prolonged indicating reduction in the rate of nucleus formation. The elongation rates at both the barbed and pointed ends of growing filaments are inhibited. At steady state, profilin increases the critical concentration for polymerization but has no effect on the reduced viscosity above the critical concentration. Addition of profilin to polymerized actin causes it to depolymerize until a new steady-state, dependent on profilin concentration, is achieved. These effects of profilin can be explained by the formation of a 1:1 complex with actin with a dissociation constant of 1 to 4 μM. $MgCl_2$ strongly inhibits these effects of profilin, most likely by binding to the high-affinity divalent cation site on the actin. *Acanthamoeba* profilin has similar but weaker effects on muscle actin, requiring 5 to 10 times more profilin than with amoeba actin.

Profilin is a small protein which was first isolated from lymphoid tissue in a 1:1 complex with actin (4), but its mechanism of action and its biological function are not established, in part, because profilin purified from both mammals (4, 9; 2) and *Acanthamoeba* (16) did not interact strongly with purified actin. The vertebrate profilins consist of a single polypeptide with a molecular weight, calculated from its sequence, of 15,220 (14), whereas the *Acanthamoeba* profilin had a lower molecular weight (~12,000) as shown by gel electrophoresis. All of the purified profilins prolong the lag phase at the outset of the polymerization of monomeric actin. This led several authors to conclude that profilin inhibits the rate of actin nucleus formation. There is less agreement about the effects of profilin on the extent of polymerization at steady state. It is difficult to compare the four different published studies, because there is no uniformity in the buffer conditions or the concentration or type of actin. In five published experiments using 14 to 18 μM muscle actin in phosphate buffer with 2 mM $MgCl_2$ or $CaCl_2$, 8 to 12 μM profilin from brain, spleen, thymus (2), or platelets (9) inhibited the steady-state viscosity <20%. With 30 μM platelet profilin there was no inhibition in 2 mM $MgCl_2$. However 34 μM spleen, brain, or thymus profilin all inhibited the steady-state viscosity of muscle actin >90% in 2 mM $CaCl_2$ (2). There were no experiments to test whether the buffer composition (especially the divalent cation) might explain these differences. In the single experiment with 12 μM *Acanthamoeba* actin and 34 μM *Acanthamoeba* profilin in phosphate buffer with 2 mM $MgCl_2$, there was no inhibition of the steady-state viscosity (16). Although it was stated in several of these papers that profilin does not inhibit the elongation of actin filaments, none of the experiments actually allow one to evaluate possible effects of profilin on this step.

Perhaps the most definitive information available on the interaction of purified profilin with actin was obtained by Mockrin and Korn (12). They showed that *Acanthamoeba* profilin increases the rate of ATP exchange between muscle actin monomers and the medium. The dependence of the reaction rate on profilin concentration allowed them to calculate that the proteins form a 1:1 complex with $K_d = 4.7 \times 10^{-5}$. Other than this elegant, but indirect, assay there is no published information on the binding of profilin to actin monomers, although there is evidence that neither mammalian (4) nor *Acanthamoeba* (16) profilin binds to actin filaments in pelleting assays.

The conclusion of this brief summary is that we do not know how actin and profilin interact. To make progress towards this goal, we need detailed quantitative information about the binding of profilin to actin and the effects of profilin on each step in the polymerization of actin. It will be necessary to explore how each of the reaction parameters is influenced by the concentration of profilin, the species of actin, and the solution conditions. Here we have examined the effects of *Acanthamoeba* profilin on the nucleation rate, elongation rate and the steady-state extent of polymerization of both *Acantha-*

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moeba and muscle actin. The experiments show that *Acanthamoeba* profilin reacts more strongly with *Acanthamoeba* than muscle actin and that the interaction is affected dramatically by micromolar concentrations of $MgCl_2$.

MATERIALS AND METHODS

Materials

We used the following sources: Sigma Chemical Co. (St. Louis, MO): grade 1 ATP; grade 1 imidazole; dithiothreitol (DTT); ethanolamine; Sephadex G-25. Polysciences, Inc. (Warrington, PA): glutaraldehyde. Pharmacia Fine Chemicals (Piscataway, NJ): Sephacryl S-200. Other chemicals were reagent grade.

Methods

PROTEIN PURIFICATION: *Acanthamoeba* profilin was purified by minor modifications of the method of Reichstein and Korn (16) and its concentration was measured by absorbance at 280 nm using $E = 1.4 \times 10^4\ M^{-1}\ cm^{-1}$ (Tseng et al. Manuscript submitted for publication). *Acanthamoeba* actin was purified by a modification of the method of Gordon et al. (8). Rabbit skeletal muscle actin was purified from acetone powder according to MacLean-Fletcher and Pollard (11), a procedure which includes gel filtration as the final step. Actin concentration was measured by absorbance at 290 nm using $E = 2.7 \times 10^4\ M^{-1}\ cm^{-1}$. Chicken muscle myosin subfragment-1 (S-1) was prepared by α-chymotrypsin digestion according to Weeds and Pope (18) and purified by gel filtration on Sephacryl S-200 in 0.5 M KCl, 20 mM imidazole, pH 7, 1 mM EDTA, 1 mM DTT, 3 mM sodium azide.

POLYMERIZATION ASSAYS: In most cases actin polymerization was followed by measurements of the viscosity of 0.6-ml samples in Ostwald capillary viscometers size 150 from Cannon Instruments (State College, PA). The temperature was 25°C and buffer flow times were 28 to 30 s. As described in detail elsewhere (5), the shearing in the viscometer breaks some of the actin filaments and accelerates the polymerization of bulk samples by increasing the number of ends available for growth. To keep this factor constant, measurements were made at regular intervals, usually 1 or 2 min, whenever possible. In those cases where polymerization was nucleated by preformed actin filaments, the filament concentration was adjusted to give both (*a*) hyperbolic plots of viscosity vs. time and (*b*) initial rates dependent on the monomer concentration. In this way viscometry could be used to give a semiquantitative data on elongation rates. The constituents of the samples were mixed in the following order: 10-fold concentrated buffer, profilin or its buffer, water, nuclei (if used), and actin monomer. The standard buffer contained 50 mM KCl, 10 mM imidazole (1 M stock was pH 7.5 @ 25°C, but pH was 6.9 to 7.0 after dilution), 0.2 mM ATP. The actin buffer contributed, in addition, 0.8 mM Tris-Cl, 0.2 mM DTT, 0.08 mM $CaCl_2$, 0.08 mM ATP. In some experiments $MgCl_2$ was included in the concentrations noted in figure legends.

Elongation rates were also measured directly using a modification of the approach originally described by Woodrum et al. (19). Cross-linked decorated nuclei were prepared as follows: equal concentrations (17 μM) of polymerized *Acanthamoeba* actin and muscle S-1 were mixed in 10 mM imidazole pH 7, 2 mM $MgCl_2$ for 2 h at room temperature to allow the S-1 to hydrolyze all of the free ATP from the actin sample and to combine with the actin filaments. The proteins were then treated with 10 mM glutaraldehyde for 8 min followed by 50 mM ethanolamine for 10 min, both at room temperature. The cross-linked decorated filaments were then separated from the other reactants on a 1.5 × 12 cm column of Sephadex G-25 equilibrated and eluted with 50 mM KCl, 10 mM imidazole pH 7. The fixed decorated filaments were fragmented by 25 passages through a 26-gauge needle just before use. Elongation experiments were carried out by mixing reactants in a small tube in the following order: variable volumes of water, 20 μl of 250 mM KCl, 50 mM imidazole pH 7; decorated nuclei; variable volumes of profilin and, finally, 16 μl of 24 μM actin to bring the volume to 100 μl and start the reaction. The final sample contained 0.45 mg/ml of nuclei (that is ~2.5 μM actin in decorated filaments), 4 μM actin monomer and various concentrations of profilin in 50 mM KCl, 10 mM imidazole, pH 7. Immediately after mixing samples were transferred carefully to parafilm. Glow-discharged, carbon-coated electron microscopy grids were touched to the surface of the droplet. After 15 to 60 s from the addition of monomers, the reaction was stopped by draining the sample from the grid by contact with filter paper and staining the absorbed material with 1% uranyl acetate for 5 s. In another experiment, 1 mM $MgCl_2$ was included in the reaction mixture. Electron micrographs of random, well-stained areas were taken at 10,000 times with a JEOL 100 CX microscope. The length of filaments grown from the decorated nuclei was measured on prints enlarged to 25,000 times. Most samples consisted of 35 to 60 filaments with a range of 5 to 88. The sample of 5 came from a 15-s time point, with the highest concentration (20 μM) of profilin where there was no growth on most of several hundred nuclei photographed.

RESULTS

Polymerization from Monomeric Actin

When purified *Acanthamoeba* (Fig. 1*A* and *B*) or muscle (Fig. 1*C* and *D*) actin monomers are polymerized in 50 mM KCl ± 1 mM $MgCl_2$ the time course of the viscosity change is sigmoidal, because slow steps limit the initial rate of the reaction. The initial lag is shorter in $MgCl_2$ (Fig. 1*B* and *D*), because Mg^{++} increases the rate of nucleation (6) without altering the polymer elongation rate.

Purified *Acanthamoeba* profilin prolongs the initial lag phase for both types of actin with or without $MgCl_2$ (Fig. 1*A–F*). The duration of the lag phase depends on the concentration of profilin, the type of actin and the ionic conditions (Fig. 1*E* and *F*). 10 μM profilin doubles the lag phase for *Acanthamoeba* actin with or without 1 mM $MgCl_2$, but 4 to 5 times more is required to double the lag phase of muscle actin (± $MgCl_2$). Experiments similar to this led previous investigators to conclude that profilin from spleen (2), *Acanthamoeba* (16) and platelets (9) inhibits nucleation, but all of these results are also

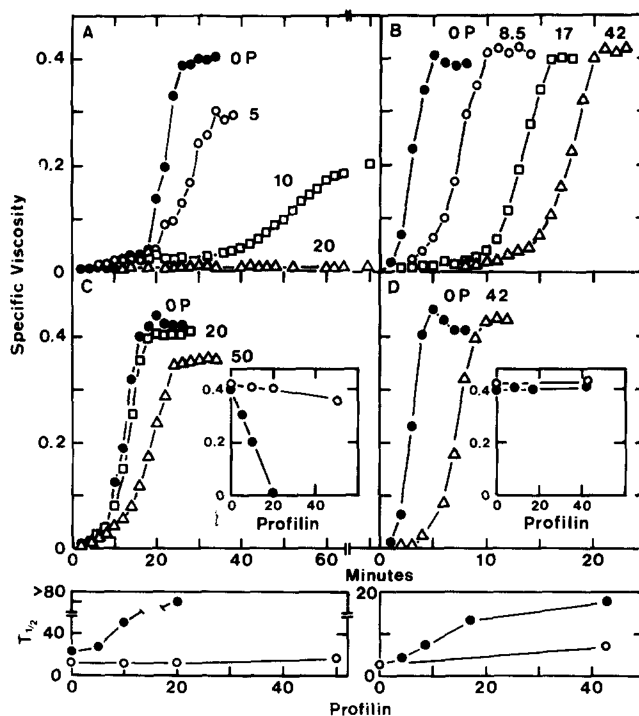

FIGURE 1 Effect of profilin on the time course of gel-filtered actin monomer polymerization assayed by Ostwald capillary viscometry. (*A*) *Acanthamoeba* actin (10 μM) in 50 mM KCl, 0.28 mM ATP, 10 mM imidazole pH 7.0. Profilin concentrations: ●, zero; ○, 5 μM; □, 10 μM; △, 20 μM. (*B*) *Acanthamoeba* actin (9.5 μM) in 50 mM KCl, 1 mM $MgCl_2$, 0.28 mM ATP, 10 mM imidazole pH 7.0. Profilin concentrations: ●, zero; ○, 8.5 μM; □, 17 μM; △, 42 μM. (*C*) Muscle actin (9.5 μM) in 50 mM KCl, 0.28 mM ATP, 10 mM imidazole, pH 7.0. Profilin concentrations: ●, zero; □, 20 μM; △, 40 μM. *Inset*: Plot of steady-state viscosity vs. profilin concentration in 50 mM KCl. ●, *Acanthamoeba* actin; ○, muscle actin. (*D*) Muscle actin (10 μM) in 50 mM KCl, 1 mM $MgCl_2$, 0.28 mM ATP, 10 mM imidazole, pH 7.0. Profilin concentrations: ●, zero; △, 42 μM. *Inset*: Plot of steady state viscosity vs. profilin concentration in 50 mM KCl, 1 mM $MgCl_2$. ●, *Acanthamoeba* actin; ○, muscle actin. (*E*) Dependence of the time required to reach half the steady-state viscosity in 50 mM KCl on the profilin concentration: ●, *Acanthamoeba* actin; ○, muscle actin. (*F*) Dependence on the time required to reach half the steady-state viscosity in 50 mM KCl, 1 mM $MgCl_2$ on the profilin concentration; ●, *Acanthamoeba* actin; ○, muscle actin.

consistent with a more general effect on polymerization, including inhibition of elongation.

Steady State

In 50 mM KCl, purified *Acanthamoeba* profilin strongly inhibits the steady state viscosity of *Acanthamoeba* actin (Fig. 1 *C*, *inset*) and weakly inhibits the steady-state viscosity of muscle actin (Fig. 1 *C*, *inset*). Muscle actin is ~15 times less sensitive to profilin than *Acanthamoeba* actin. In contrast, profilin has no effect at concentrations up to 42 μM on the steady-state viscosity of either actin in 50 mM KCl with 1 mM $MgCl_2$ (Fig. 1 *D*, *inset*). Nearly identical results are obtained when polymerization is nucleated with preformed filaments (see Fig. 3, below).

In 50 mM KCl the steady-state viscosity of 10 μM *Acanthamoeba* actin is inversely proportional to the profilin concentration, with a 50% reduction in viscosity at 10 μM profilin (Fig. 1 *A* and *C*). The viscosity is lower in the presence of profilin, because the critical concentration for *Acanthamoeba* actin polymerization in 50 mM KCl is higher (Fig. 2 *A*). The critical concentration depends on the concentration of profilin (Table I). Above the critical concentration the reduced viscosity (slope of viscosity vs actin concentration) is the same as the control for all profilin concentrations up to at least 10 μM (Fig. 2 *A* and other experiments not illustrated). Since the viscosity depends on filament length (13), we interpret this result to mean that profilin has no effect on the polymer size distribution at steady state, at least when measured in a high-shear viscometer. *Acanthamoeba* profilin has no effect on the critical concentration or the viscosity of any concentration of *Acanthamoeba* actin tested in 50 mM KCl with 1 mM $MgCl_2$ (Fig. 1 *D*, *inset*, and 2 *B*).

Elongation

Two independent assays demonstrate that *Acanthamoeba* profilin is a strong inhibitor of *Acanthamoeba* actin filament elongation in 50 mM KCl. First, in a nucleated polymerization assay where the initial rate of the viscosity change is proportional to the actin monomer concentration above the critical concentration (Fig. 3 *A*), profilin reduced the initial rate of the viscosity change in a concentration-dependent fashion (Fig. 3 *B*, *C*, and *inset*). Preincubation of the actin and profilin for times from 0 to 60 min before adding nuclei and KCl had no effect on the time course of the viscosity change.

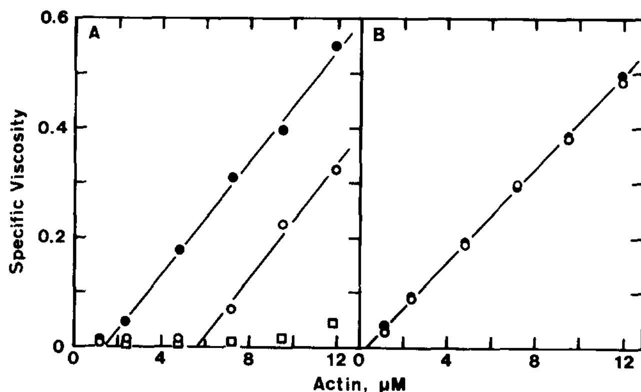

FIGURE 2 Dependence of the extent of *Acanthamoeba* actin polymerization at steady-state on the concentrations of actin, profilin, and $MgCl_2$. (*A*) Viscosity of *Acanthamoeba* actin in 50 mM KCl, 0.28 mM ATP, 10 mM imidazole, pH 7.0 for 15 h. Concentrations of profilin: ●, zero; ○, 8.5 μM, □, 20 μM. (*B*) Viscosity of *Acanthamoeba* actin in 50 mM KCl, 1 mM $MgCl_2$, 0.28 mM ATP, 10 mM imidazole pH 7.0 for 15 h. Concentrations of profilin: ●, zero; ○, 8.5 μM.

TABLE I

Effect of Profilin on the Critical Concentration for Acanthamoeba Actin Polymerization

Conditions	Total profilin	Observed critical concentration	Actin-profilin complex	Free profilin	K_D
	μM	μM	μM	μM	μM
50 mM KCl	0	1.4	0	0	—
	2.5	2.9	1.5	1.0	0.9
	4.0	3.4	2.0	2.0	1.4
	5.0	3.9	2.5	2.5	1.4
	8.5	5.8	4.4	4.1	1.3
50 mM KCl, 1 mM $MgCl_2$	8.5	0.35	0	8.5	∞

The data were obtained from experiments similar to Fig. 2 *A* and *B*. The concentration of complex was calculated from the observed critical concentration minus the critical concentration without profilin assuming a 1:1 complex. Free profilin is total profilin minus complex. $K_D = \frac{(A_1^\infty)(P_f)}{(AP)}$. In 50 mM KCl $A_1^\infty = 1.40$. In 50 mM KCl, 1 mM $MgCl_2$ $A_1^\infty = 0.35$.

In 50 mM KCl, 1 mM $MgCl_2$ high concentrations of profilin inhibit the initial rate of the viscosity change of nucleated samples (Fig. 3 *C*). About five times more profilin is required than in 50 mM KCl without $MgCl_2$.

Profilin also inhibits the rate of the viscosity change when muscle actin monomers are used with the nuclei (Fig. 3 *D*), but the effect is weaker. Compared with the effect on the nucleated polymerization of *Acanthamoeba* actin, five to seven times more profilin is required for an equivalent effect on muscle actin.

Second, we measured the growth of actin from the ends of myosin S-1 decorated nuclei by electron microscopy (Fig. 4). Under the conditions of the assay the length of new polymer at the barbed end was proportional to the time of incubation. In 50 mM KCl, profilin inhibited the rate of barbed end elongation (Fig. 4) even more strongly than in the viscometric assay (Fig. 3 *C*, *inset*)[1]. In another experiment, 10 μM profilin inhibited barbed end elongation of 4 μM actin 85% by electron microscopy. Profilin also inhibited elongation at the pointed end because no growth was seen there on hundreds of nuclei. However, events at the pointed end were impossible to quantitate using these nuclei, because the growth rate there is so slow even in the controls. In 50 mM KCl with 1 mM $MgCl_2$, there was no inhibition of the elongation rate by 8 or 16 μM profilin (Fig. 4).

Depolymerization

When profilin is added to *Acanthamoeba* actin filaments at steady state in 50 mM KCl the filaments will depolymerize (Fig. 5). After an extended period of time required to reach a new equilibrium, the viscosity plateaus at the same value as a

[1] It is not expected that the electron microscopy and viscometric assays of elongation should agree, because the viscometric assay is only semiquantitative due to concurrent nucleation and filament breakage, both of which increase the rate of the viscosity change. An additional factor contributing to the difference is the concentration of actin. When the total actin monomer concentration is 4 μM (Fig. 4) compared with 10 μM (Fig. 2), less than half as much profilin is required to reduce the free monomer concentration to less than the critical concentration.

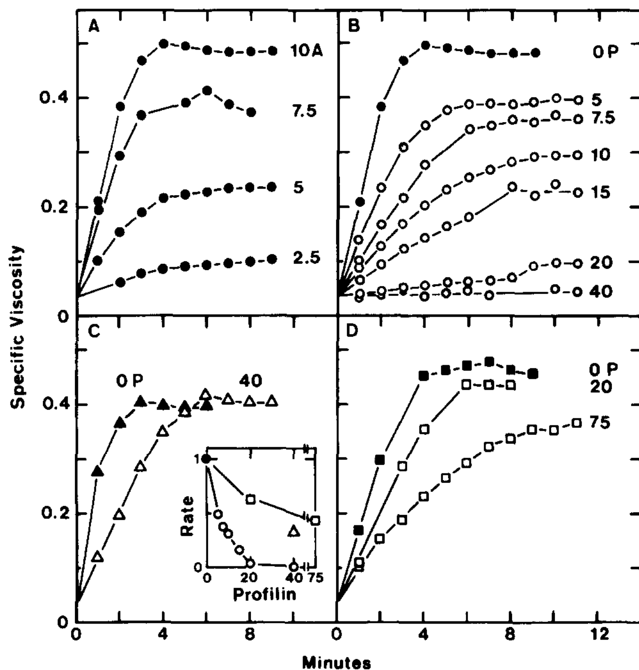

FIGURE 3 Viscometric assay of the dependence of the time course of nucleated actin polymerization on the concentrations of actin and profilin. (*A*) Dependence on the concentration of *Acanthamoeba* actin monomers. Conditions: 50 mM KCl, 0.28 mM ATP, 10 mM imidazole, pH 7.0 with 0.75 μM polymerized actin as nuclei. Concentrations of actin monomers are given beside each curve in micromoles per liter. (*B*) Effect of profilin on the time course of polymerization of 10 μM *Acanthamoeba* actin monomers nucleated with 0.75 μM polymerized actin in 50 mM KCl, as in *A*. The concentrations of profilin in micromoles per liter are given beside each curve. (*C*) Effect of profilin on the time course of polymerization of 10 μM *Acanthamoeba* actin monomers nucleated with 0.75 μM polymerized actin in 50 mM KCl, plus 1 mM $MgCl_2$. The concentrations of profilin in micromoles per liter are given beside each curve. *Inset*: Profilin concentration dependence of the initial rates of nucleated viscosity change. Rates are expressed as fractions of the control without profilin. O, 10 μM *Acanthamoeba* actin in 50 mM KCl (control rate 0.17 cs/min); Δ, 10 μM *Acanthamoeba* actin in 50 mM KCl, 1 mM $MgCl_2$ (control rate 0.23 cs/min); □, 10 μM muscle actin in 50 mM KCl (control rate 0.13 cs/min). (*D*) Effect of profilin on the time course of polymerization of 10 μM muscle actin monomers nucleated with 0.75 μM polymerized actin in 50 mM KCl, as in *A*. The concentrations of profilin in micromoles per liter are given beside each curve.

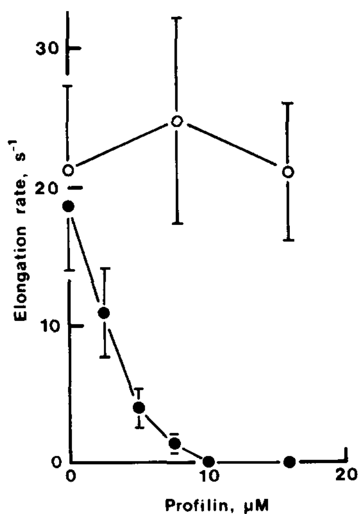

FIGURE 4 Dependence of *Acanthamoeba* actin filament elongation rate on the profilin concentration. Absolute elongation rates (mean number of monomers added /30 s, ± 1 SD) were measured by electron microscopy as described in Materials and Methods using 3.8 μM monomers in 50 mM KCl (●), or 50 mM KCl, 1 mM $MgCl_2$ (O) and a 30-s incubation.

sample polymerized from nuclei and monomers in the presence of the same concentration of profilin (Fig. 5). If 1 mM $MgCl_2$ is present in the buffer, the filaments do not depolymerize when 20 μM profilin is added (Fig. 5), because it has no effect on the extent of polymerization (Figs. 1 *B* and 2 *B*).

Effect of $MgCl_2$

As noted in the previous sections, $MgCl_2$ has a strong effect on the polymerization of mixtures of profilin and *Acanthamoeba* actin. In its absence, profilin inhibits the growth rate and extent of polymerization, but while in 1 mM $MgCl_2$ there is no effect on the steady-state viscosity and a weak effect on the growth rate.

These effects of $MgCl_2$ occur at very low concentrations. In the nucleated polymerization assay, 20 μM profilin reduces the initial rate of the viscosity change to approximately zero in 10 μM $MgCl_2$ (Fig. 6*A*) but has relatively little effect in 1 mM $MgCl_2$. Only 100 μM $MgCl_2$ is required to give half the effect of 1 mM $MgCl_2$ and 25 μM $MgCl_2$ a substantial effect.

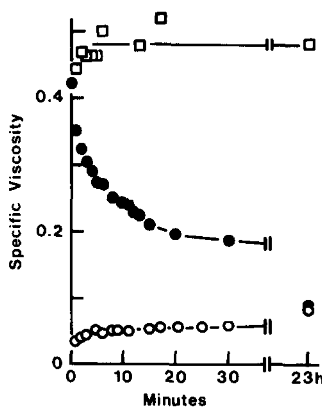

FIGURE 5 Effect of profilin addition on the viscosity of steady state actin filaments. *Acanthamoeba* actin was polymerized to steady state in 50 mM KCl, 0.28 mM ATP, 10 mM imidazole, pH 7.0 either with (□), or without (●), 1 mM $MgCl_2$. At time zero profilin was added to give final concentrations of 20 μM profilin and 10 μM actin and the viscosity followed with time. Another sample consisting of 0.75 μM polymerized actin, 9.25 μM actin monomers and 20 μM profilin was polymerized in 50 mM KCl without $MgCl_2$ (O). Final readings at 23 h are given on the right.

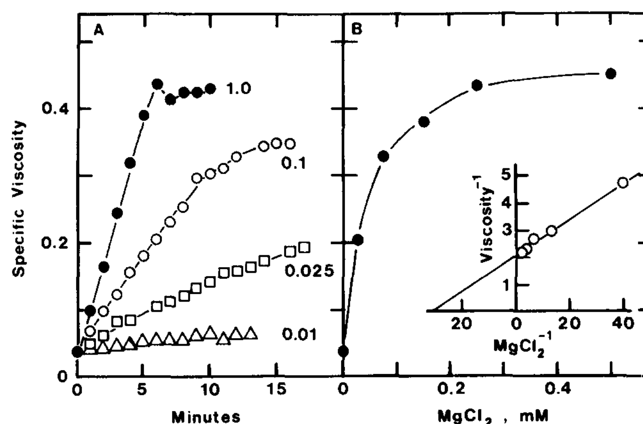

FIGURE 6 Dependence of the effects of profilin on the concentration of $MgCl_2$. (*A*) Time course of nucleated polymerization of 10 μM *Acanthamoeba* actin with 20 μM profilin. Conditions: 0.75 μM actin filaments, 50 mM KCl, 0.1 mM ATP, 10 mM imidazole, pH 7.5. The concentrations of $MgCl_2$ in millimoles per liter are given beside each curve. (*B*) Dependence of the steady-state viscosity of 10.75 μM *Acanthamoeba* actin and 20 μM profilin on the concentration of $MgCl_2$. 10 μM actin monomers, 0.75 μM actin nuclei and 20 μM profilin were incubated for 18 h at 25°C in 50 mM KCl, 0.1 mM ATP, 10 mM imidazole, pH 7.0 with various concentrations of $MgCl_2$. *Inset*: a double reciprocal plot of the viscosity (cs^{-1}) vs. $MgCl_2$ concentration (mM^{-1}). The K_D is 33 μM $MgCl_2$.

The steady-state viscosity of actin-profilin mixtures is even more sensitive to the $MgCl_2$ concentration (Fig. 6*B*). The steady-state viscosity after 18-h incubation has a hyperbolic dependence on $MgCl_2$ concentration. A double reciprocal plot (Fig. 6*B*, *inset*) is linear and gives a K_D of 33 μM $MgCl_2$. In another experiment (Fig. 6*A*) the steady-state viscosities attained in 10 to 30 min for 50, 75, 100, and 1,000 μM $MgCl_2$ fit on the same hyperbola as the 18-h data and give a linear reciprocal plot with a K_D of 38 μM. Note that these samples contained both 100 μM ATP and 100 μM $CaCl_2$ in addition to the $MgCl_2$.

The low concentrations of $MgCl_2$ giving a half-maximal effect on the profilin action are insufficient by themselves to cause the polymerization of the actin, but they do affect the critical concentration in 50 mM KCl. The critical concentration for *Acanthamoeba* actin polymerization in 50 mM KCl is 1.4 μM without $MgCl_2$ and 0.35 μM in 1 mM $MgCl_2$. The dependence of the critical concentration on $MgCl_2$ concentration between 0.025 and 1 mM gives a linear double reciprocal plot ($1/MgCl_2$ vs. 1/Keq) with K_D = 35 μM $MgCl_2$.

DISCUSSION

To summarize our findings, in 50 mM KCl, *Acanthamoeba* profilin prolongs the lag phase at the outset of *Acanthamoeba* actin polymerization, decreases the elongation rate, increases the critical concentration for polymerization, and depolymerizes preformed actin filaments. In 50 mM KCl, 1 mM $MgCl_2$, *Acanthamoeba* profilin has a weak effect on the lag phase and the rate of elongation but no effect on the steady-state extent of *Acanthamoeba* actin polymerization. *Acanthamoeba* profilin affects muscle actin polymerization in a similar way, but the profilin concentration must be five to ten times higher for an equivalent effect.

Our observations on profilin effects on the steady-state polymerization of actin are consistent with previous work reviewed in the Introduction. Reichstein and Korn (16) probably did not observe an effect of 34 μM *Acanthamoeba* profilin on *Acanthamoeba* actin because of the 2 mM $MgCl_2$ in the buffer. Grumet and Lin (9) may not have observed an effect of 30 μM platelet profilin because they used muscle actin in 2 mM $MgCl_2$ for their assay. Blikstad et al. (2) found that high concentrations of mammalian profilin reduce the steady-state viscosity of muscle actin presumably because they used $CaCl_2$ instead of $MgCl_2$ in their buffer.

To explain the mechanism of action of profilin quantitatively it will be necessary to measure directly the stoichiometry and affinity of profilin binding to the two actins under various conditions. However, some estimates of these parameters can be made from the profilin concentration dependence of the critical concentration of steady-state polymerization (Table I) and of the elongation rate (Table II) using Eq. 1.

$$K_D = \frac{(A_1)(P_f)}{(AP)} \quad (1)$$

In the case where polymerization was allowed to go to steady state, the free actin monomer concentration (A_1^∞) is simply the critical concentration for actin polymerization in that buffer without profilin. The concentration of actin-profilin complex (AP) is the critical concentration observed in the presence of profilin minus A_1^∞. Free profilin (P_f) is obtained by difference, assuming a 1:1 complex between actin and profilin. Since we have been unable to detect by a pelleting assay the binding of profilin to actin filaments under the conditions of our experiments, we assume that no profilin is bound to filaments. In the growth rate experiment the concentrations of free actin monomer, complex and free profilin could be estimated directly from the growth rates (Table II). The stoichiometry of actin and profilin in the complex is the major unknown in this analysis and will have to be established by direct binding studies. We chose 1:1 stoichiometry for this analysis because mammalian profilactin consists of a 1:1 ratio of the two proteins (3, 4). Another note of caution is that the analysis in both Tables I and II disregards any binding of profilin to actin which does not alter the polymerization process.

TABLE II

Calculation of Actin-Profilin Affinity in 50 mM KCl from the Polymerization Rate Experiment (Fig. 4)

Total profilin	Polymerization rate	Free actin	Complex	Free profilin	K_D
μM	s^{-1}	μM	μM	μM	μM
0	18.6	3.8	0	—	—
2.5	10.9	2.8	1.0	1.5	4.0
5.0	4.0	1.9	1.9	3.1	3.1
7.5	1.4	1.6	2.2	5.3	3.7

The concentration of free actin monomer was estimated using the knowledge that the polymerization rate is directly proportional to the actin concentration above the critical concentration (1.4 μM) where dl/dt = 0. Using the rate in the absence of profilin the slope of this line is $k_+ = 7.8 \times 10^6\ M^{-1}\ s^{-1}$. The concentration of complex is the difference between the total and free actin concentrations. Free profilin is the difference between the concentrations of total profilin and complex. This assumes a 1:1 stoichiometry. $K_D = \frac{(A_1)(P_f)}{(AP)}$.

The results of these calculations are summarized in Tables I and II. The apparent K_D's for profilin binding to amoeba actin in 50 mM KCl are between 1 and 4 μM. The apparent affinity is strongly dependent on the $MgCl_2$ concentration, with the K_D being unmeasurably large in 1 mM $MgCl_2$. For muscle actin we have less data, but the apparent affinity is much less, with the K_D >30 μM.

Most of our observations can be explained by a simple mechanism of action in which profilin binds to actin monomers at a site or sites which inhibit polymerization. These sites are probably buried in the filament, because profilin does not bind to filaments. Alternatively, profilin might fail to bind to exposed sites on the filament due to the conformation of the actin in the filament. We favor the former model, because binding to a buried site would explain by steric interference why the actin-profilin complex would not polymerize.

A simple monomer sequestering mechanism does not completely explain why the lag phase is so much more sensitive to profilin than elongation rate or steady-state extent of polymerization. This discrepancy is especially clear in $MgCl_2$. Hopefully, this will become clear when we learn more about nucleation and other reactions which occur during the lag phase.

The great difference in the affinity of *Acanthamoeba* profilin for *Acanthamoeba* and muscle actins is fascinating, because of the sequences of the two actins are so similar (15). The NH_2-terminal sequence of *Acanthamoeba* actin has not been completed, but most of the primary structures of the two actins are nearly identical. There are 14 isopolar substitutions and one charge difference, a histidine at position 228 in the amoeba actin where muscle actin has an alanine. We speculate that profilin may bind near this histidine or possibly to the NH_2-terminus. This should eventually become clear from crystallographic studies (3).

The profound effect of $MgCl_2$ on *Acanthamoeba* actin-pro-

filin interaction occurs at low $MgCl_2$ concentrations with a half-maximal effect at about 35 μM Mg^{++} in the presence of 100 μM CaATP. Most likely this is due to Mg^{++} binding to the high-affinity divalent cation site on the actin (7). On the other hand, a direct effect of $MgCl_2$ on profilin cannot be ruled out at the present time. These results suggest that profilin binds weakly or not at all to Mg^{++}-actin, a matter which deserves further study because of the implications for the action of profilin in the cell, where it is generally assumed that there is Mg^{++} bound to actin.

Our new information about profilin makes it clear that it is similar to other actin monomer sequestering proteins such as DNase-I (10), brain depolymerizing protein (1), and vitamin D-binding protein (17) which all affect actin polymerization by simply binding to actin monomers in a way that prevents them from polymerizing. For the most part, the differences in their action are accounted for by the affinity of the sequestering protein for actin relative to the equilibrium constant for the polymerization reaction. Compared with the other proteins, profilin has a lower affinity for actin, especially muscle actin, and is very sensitive to $MgCl_2$.

Until in vivo studies are carried out, the function of profilin in the cell will remain speculative, especially since we know so little about Mg^{++} in the amoeba. However, the available biochemical data indicate that profilin might function as an actin monomer buffer in the cytoplasm.[2]

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[2] Drs. L. Tobacman and E. D. Korn of the National Institutes of Health have carried out complementary experiments with *Acanthamoeba* profilin. They kindly sent us a copy of a paper to be published in *J. Biol. Chem.* In general, their results agree with ours. They also found that *Acanthamoeba* actin was more sensitive than muscle actin to the action of *Acanthamoeba* profilin. One unexplained difference is that they did not find a striking effect of Mg^{++} as we have. Drs. H. Sugino and S. Hatano have purified a 12,000 mol wt profilin from *Physarum* which reacts more strongly with *Physarum* than muscle actin. Their work will appear in *J. Biochem. (Tokyo).*

REFERENCES

1. Bamberg, J. R., H. E. Harris, and A. G. Weeds. 1980. Partial purification and characterization of a depolymerizing factor from brain. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 121:178–182.
2. Blikstad, I., I. Sundkvist, and S. Erickson. 1980. Isolation and characterization of profilactin and profilin from calf thymus and brain. *Eur. J. Biochem.* 105:425–433.
3. Carlsson, L., L-E. Nyström, U. Lindberg, K. K. Kannan, H. Cid-Dresdner, and S. Lovgren. Crystallization of a nonmuscle actin. *J. Mol. Biol.* 105:353–366.
4. 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.* 155:465–483.
5. Cooper, J. A., and T. D. Pollard. 1982. Methods for measuring actin polymerization. *In* Methods in Enzymology. Structural and Contractile Proteins. L. W. Cunningham and D. W. Frederiksen, editors. Academic Press, Inc., New York. 182–210.
6. Cooper, J. A., T. Tsong, and T. D. Pollard. 1982. Magnesium affects the nucleation step in actin polymerization. *Biophys. J.* 37(2, Pt. 2): 191a (Abstr.).
7. Frieden, C., D. Lieberman, and H. R. Gilbert. 1980. A fluorescent probe for conformational change in skeletal muscle G-Actin. *J. Biol. Chem.* 255:8991–8993.
8. Gordon, D. J., E. Eisenberg, and E. D. Korn. 1976. Characterization of cytoplasmic actin isolated from *Acanthamoeba castellanii* by a new method. *J. Biol. Chem.* 251:4778–4786.
9. Grumet, M., and S. Lin. 1980. Reversal of profilin inhibition of actin polymerization in vitro by erythrocyte cytochalasin-binding complexes and cross-linked actin nuclei. *Biochem. Biophys. Res. Commun.* 92:1324–1334.
10. Hitchcock, S. E., L. Carlsson, and U. Lindberg. 1976. Depolymerization of F-actin by deoxyribonuclease I. *Cell.* 7:531–542.
11. MacLean-Fletcher, S., and T. D. Pollard. 1980. Identification of a factor in conventional muscle actin preparation which inhibits actin filament self-association. *Biochem. Biophys. Res. Commun.* 96:18–27.
12. Mockrin, S. C., and E. D. Korn. 1980. *Acanthamoeba* profilin interacts with G-actin to increase the rate of exchange of actin-bound ATP. *Biochemistry.* 19:5359–5362.
13. Nunnally, M. H., L. D. Powell, and S. W. Craig. 1981. Reconstitution and regulation of actin gel-sol transformation with purified filamin and villin. *J. Biol. Chem.* 256:2083–2086.
14. Nyström, L-E., U. Lindberg, J. Kendrick-Jones, and R. Jakes. 1979. The amino acid sequence of profilin from calf spleen. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 101:161–165.
15. Pollard, T. D., U. Aebi, J. A. Cooper, M. Elzinga, W. E. Fowler, L. M. Griffith, I. M. Herman, J. Heuser, G. Isenberg, D. P. Kiehart, J. Levy, S. MacLean-Fletcher, P. Maupin, M. S. Mooseker, M. Runge, P. R. Smith, and P. Tseng. 1981. *In* Cell Motility, Vol. 2, R. Dowben and J. Shay, editors. Plenum Publishing Corp., NY. 15–44.
16. 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.
17. Van Baelen, H., R. Bouillan, and P. De Moor. 1980. Vitamin D-binding protein (Gc-globulin) binds actin. *J. Biol. Chem.* 255:2270–2272.
18. Weeds, A. G., and B. Pope. 1977. Studies on the chymotryptic digestion of myosin. Effects of divalent cations on proteolytic susceptibility. *J. Mol. Biol.* 111:129–157.
19. Woodrum, D. T., S. A. Rich, and T. D. Pollard. 1975. Evidence for biased bidirectional polymerization of actin filaments using heavy meromyosin prepared by an improved method. *J. Vrll Biol.* 67:231–237.