Quantitative Analysis of the Effect of *Acanthamoeba* Profilin on Actin Filament Nucleation and Elongation†

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ABSTRACT: The current view of the mechanism of action of *Acanthamoeba* profilin is that it binds to actin monomers, forming a complex that cannot polymerize [Tobacman, L. S., & Korn, E. D. (1982) *J. Biol. Chem. 257*, 4166–4170; Tseng, P., & Pollard, T. D. (1982) *J. Cell Biol. 94*, 213–218; Tobacman, L. S., Brenner, S. L., & Korn, E. D. (1983) *J. Biol. Chem. 258*, 8806–8812]. This simple model fails to predict two new experimental observations made with *Ancanthamoeba* actin in 50 mM KCl, 1 mM $MgCl_2$, and 1 mM EGTA. First, *Acanthamoeba* profilin inhibits elongation of actin filaments far more at the pointed end than at the barbed end. According to the simple model, the K_d for the profilin–actin complex is less than 5 μM on the basis of observations at the pointed end and greater than 50 μM for the barbed end. Second, profilin inhibits nucleation more strongly than elongation. According to the simple model, the K_d for the profilin–actin complex is 60–140 μM on the basis of two assays of elongation but 2–10 μM on the basis of polymerization kinetics that reflect nucleation. These new findings can be explained by a new and more complex model for the mechanism of action that is related to a proposal of Tilney and co-workers [Tilney, L. G., Bonder, E. M., Coluccio, L. M., & Mooseker, M. S. (1983) *J. Cell Biol. 97*, 113–124]. In this model, profilin can bind both to actin monomers with a K_d of about 5 μM and to the barbed end of actin filaments with a K_d of about 50–100 μM. An actin monomer bound to profilin cannot participate in nucleation or add to the pointed end of an actin filament. It can add to the barbed end of a filament. When profilin is bound to the barbed end of a filament, actin monomers cannot bind to that end, but the terminal actin protomer can dissociate at the usual rate. This model includes two different K_d's—one for profilin bound to actin monomers and one for profilin bound to an actin molecule at the barbed end of a filament. The affinity for the end of the filament is lower by a factor of 10 than the affinity for the monomer, presumably due to the difference in the conformation of the two forms of actin or to steric constraints at the end of the filament.

The profilins are a class of small proteins that can bind to actin monomers and may participate in the regulation of actin polymerization in nonmuscle cells. The original profilin came from vertebrate lymphoid tissues (Carlsson et al., 1976), and molecules with similar properties have been purified from brain (Blikstead et al., 1980), platelets (Grumet & Lin, 1980), *Acanthamoeba* (Reichstein & Korn, 1979), *Physarum* (Ozaki et al., 1983), and *Thyone* sperm (Tilney et al., 1983). All of these profilins can inhibit actin polymerization. Many of the effects can be explained by the formation of a complex of profilin with an actin monomer that cannot participate in any aspect of polymerization. On the other hand, some effects of profilin cannot be explained by this simple model (Tseng & Pollard, 1982; Tilney et al., 1983). It was our purpose here to understand the mechanism of action in enough detail to account for all of the experimental observations. *Acanthamoeba* profilin was a favorable subject for such a study, because large quantities can be obtained in high purity (Reichstein & Korn, 1979) and more was already known about its properties than any other profilin.

Acanthamoeba profilin is a small (M_r 11 700), soluble protein present in very high concentrations, approaching 200 μM, in the amoeba, where it is distributed throughout the cytoplasm (Tseng et al., 1984). Previous studies established that *Acanthamoeba* profilin inhibits both the rate and extent of actin polymerization (Reichstein & Korn, 1979; Tobacman & Korn, 1982; Tseng & Pollard, 1982; Tobacman et al., 1983). These effects are more pronounced with *Acanthamoeba* actin than with muscle actin, suggesting that the amoeba profilin has a higher affinity for its own actin. There is evidence from four separate types of experiments that *Acanthamoeba* profilin binds to actin monomers (Mockrin & Korn, 1980; Tobacman & Korn, 1982; Lee et al., 1982; Runge et al., 1982). Most of the data are consistent with profilin forming a 1:1 complex with the actin monomer. However, the stoichiometry was measured directly only in the experiments described in a preliminary report by Runge et al. (1982). That data suggested that there may actually be two profilin binding sites on one actin molecule, but this remains to be proven. In pelleting assays, no profilin appeared to bind to actin filaments (Reichstein & Korn, 1979; Tseng & Pollard, 1982).

Many of the effects of *Acanthamoeba* profilin on actin polymerization can be explained, at least qualitatively, by a simple mechanism with free profilin and free actin monomers in equilibrium with an actin–profilin complex that cannot participate in actin filament nucleation or elongation reactions (Tobacman & Korn, 1982; Tseng & Pollard, 1982; Tobacman et al., 1983). On the other hand, Tseng & Pollard (1982) found that in $MgCl_2$ low concentrations of *Acanthamoeba* profilin inhibit the initial slow steps in the spontaneous polymerization of actin monomers with little or no effect on the rate of elongation. On a qualitative level, these results might be explained if the actin–profilin complex were inactive in forming nuclei but could still participate in the elongation of the barbed end of filaments.

In the present work we used improved methods to analyze actin polymerization (Cooper et al., 1983a,b; Pollard, 1983) to study in detail the mechanism of action of profilin in 50 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, and 0.1 mM ATP, pH 7. These conditions were chosen to approximate the salt concentrations in cells. We find that *Acanthamoeba* profilin inhibits nucleation and elongation at the pointed end of the

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filament 10–20 times more strongly than elongation at the barbed end. A simple model of monomer sequestration with a single dissociation constant for the actin–profilin complex cannot account quantitatively for the effects of profilin on both elongation and nucleation in the presence of $MgCl_2$. One mechanism that is consistent with most of the available data is that profilin binds to actin monomers with a K_d of about 5 μM and to the barbed end of actin filaments with a K_d of about 100 μM. Only free monomers can form nuclei or add to the pointed end, but the actin–profilin complex can bind at the same rate as free monomers to the barbed end of the filament. This mechanism would allow profilin to suppress spontaneous nucleation in the cell without preventing the growth of filaments from the barbed end.

Experimental Procedures

Materials. Sigma Chemical Co., St. Louis, MO, supplied ATP (grade I), dithiothreitol, imidazole (grade III), ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA), Sephadex G-150, and NaN_3. Pyrenyliodoacetamide came from Molecular Probes, Junction City, OR.

Protein Purification. Profilin was isolated from *Acanthamoeba* by a modification (Tseng et al., 1984) of the method of Reichstein & Korn (1979). Actin was purified from *Acanthamoeba* by a modification (Pollard, 1984) of the method of Gordon et al. (1976) and stored in buffer G (2 mM imidazole, pH 7, 0.5 mM dithiothreitol, 0.2 mM $CaCl_2$, 0.2 mM ATP, 0.5 mM NaN_3). By polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS), the profilin was more than 99% pure as shown by Tseng et al. (1984). The protein contaminants detected on overloaded gels consisted of a number of different polypeptides with molecular weights of 20 000–60 000. The actin was labeled with pyrenyliodoacetamide by using methods described by Cooper et al. (1983b) except that the sample was mixed with a magnetic stirrer during the coupling reaction. The usual ratio of pyrenyliodoacetamide to actin in the reaction was 7 to 1, and the purified pyrene-labeled actin usually contained 0.7–1.0 mol of pyrene per mole of actin depending on the duration of the coupling reaction (5–18 h at 22 °C). This labeled actin was mixed with unlabeled actin to give a final ratio of 0.05 pyrene per actin.

Polymerization Assays and Data Analysis. The actin polymer concentration was measured fluorometrically as described by Cooper et al. (1983b). Under these conditions the fluorescence could be measured continuously without appreciable bleaching during the times employed for the experiments.

Elongation rates were measured at 25 °C with either unlabeled or pyrene-labeled actin filaments as nuclei (Pollard, 1983). Conditions were chosen such that the initial rate of elongation from these nuclei was proportional to the nucleus concentration and proportional to the free actin monomer concentration above the critical concentration (Pollard, 1983). These initial rates were plotted against the actin monomer concentration to obtain the critical concentration (A_1) and the relative values of the association (k_+) and dissociation (k_-) rate constants. In this type of experiment the observed rate constants are the sum of the rate constants at the two ends.

Elongation rates were also measured by electron microscopy using *Limulus* sperm acrosomal processes as nuclei (Tilney et al., 1983). The processes were suspended in 2× buffer (100 mM KCl, 2 mM $MgCl_2$, 2 mM EGTA, 20 mM imidazole, pH 7). The reaction was carried out at 22 °C in a drop of solution on parafilm prepared by adding, in order, 25 μL of acrosomal processes, 10 μL of profilin or profilin buffer (10 mM imidazole, pH 7), and 15 μL of 6.7 μM *Acanthamoeba* actin monomers in buffer G to give a final actin concentration of 2 μM. A formvar- and carbon-coated grid, prepared by glow discharge in a partial vacuum, was floated on the surface of the droplet during the reaction. The reaction was stopped at 30 or 60 s by draining excess sample from the grid by touching the edge to filter paper and inverting the grid onto a droplet of 1% aqueous uranyl acetate for 10 s. Electron micrographs were taken at 12000×. The fields chosen had good negative staining and included one or more acrosomal processes with growth at one or both ends. Hundreds of similar fields were observed but not photographed. The average length of the filaments grown at two ends of each acrosomal process was measured by an independent observer with no prior knowledge of the experiment. For each time point, 6–25 acrosomal processes were measured, each with 10–25 filaments at each end that grew. Elongation rates (molecules per second) were calculated from the mean lengths at 30 s divided by 370 subunits/μm and by 30 s.

Critical concentrations for polymerization at 25 °C were determined by measuring the dependence of the steady-state polymer concentration (from pyrene–actin fluorescence) on the total actin concentration. The steady-state polymer concentration was obtained in two ways. Either various concentrations of actin monomer were polymerized at 25 °C in the presence of 0.1 μM polymer for 14–24 h or a steady-state polymer sample was diluted to a series of different concentrations, sonicated for 5 s in a bath-type sonicator, and incubated at 25 °C for 4–24 h as suggested by Tobacman et al. (1983). The critical concentration is the inflection point in plots of fluorescence vs. total actin.

Nucleation was evaluated by first measuring the time course of polymerization at several different concentrations of actin monomers alone and then analyzing the data with the polymerization model and curve-fitting programs of Cooper et al. (1983a) to obtain the size of the nucleus and the rate constant for nucleation of 500 s^{-1}. For the elongation reaction we used a value of 10^7 M^{-1} s^{-1} for k_+, the association rate constant, and A_1k_+ (where A_1 is the critical concentration) for k_-, the dissociation rate constant. The calculated nucleation rate constant and the nucleus size for actin alone were then used to analyze experimental plots of polymer concentration vs. time at several different profilin concentrations. We evaluated the simple monomer sequestration model and the complex model that are described in detail under Results and under Discussion. In the simple model, profilin forms a 1:1 complex with actin monomers. Using the binding equation and the total concentrations of profilin and actin monomers, the computer calculated the free actin monomer concentration at every interval. An assumption of rapid equilibrium is necessary for this calculation. This free actin monomer concentration was employed in the equations previously described to simulate polymerization. In the complex model, profilin binds to actin monomers as in the simple model, but profilin also binds to the barbed end of actin filaments. There were two binding equations (eq 2 and 6) with different dissociation constants. At each interval, with the total concentrations of profilin, actin monomers, and barbed ends, the computer solved for free concentration by solving the two binding equations 5 times in succession, each time using the previous solutions. We found that this number of repetitions was sufficient to reach the solution given the time interval employed. These calculations also require an assumption of rapid equilibrium. The free actin monomer concentration was used to calculate the rates of the steps in nucleation. For elongation, barbed

and pointed ends were considered separately. The rate constants at the pointed end were assumed to be one-ninth of those at the barbed end. At the barbed end, the filament number concentration was the number of free barbed ends, as determined from the binding equations, and the monomer concentration was the total actin monomer concentration since in this model the actin–profilin complex can add to barbed ends. At the pointed end, the filament number concentration was the total filament number concentration, and the monomer concentration was only the free actin monomer concentration. To achieve the best fit between experimental and theoretical curves, the computer systematically varied the K_d's until the least-squares difference between the curves was minimal. In the simple model, there was only one K_d to vary, but in the complex model, both K_d's were varied.

Results

The results are presented in four sections. In the first two we consider in detail the effects of profilin on the elongation and nucleation of *Acanthamoeba* actin filaments under more or less physiological conditions in 50 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM $CaCl_2$, 0.1 mM ATP, and 10 mM imidazole (pH 7.0). In the third section we consider the influence of the profilin and actin preparations on the observations. In the final section we present additional data on these reactions in the presence of 0.1 mM $CaCl_2$ ± 1 mM $MgCl_2$.

Elongation Reaction in $MgCl_2$ and EGTA. We evaluated the effect of profilin on the elongation reactions by both pre-steady-state and steady-state methods. We calculated the apparent dissociation constant (K_d) of the actin–profilin complex as described by Tseng & Pollard (1982) and Tobacman & Korn (1982). K_d = [free actin monomer][free profilin]/[actin–profilin complex]. We assumed that the change in rate or extent of polymerization is due to the formation of a 1:1 complex of actin with profilin that does not participate in the reaction being measured. At steady state, the critical concentration (A_1) for polymerization of actin alone was taken as the concentration of free actin monomer in the presence of profilin.

(*A*) *Pre-Steady-State Experiments.* We measured the rate of actin filament elongation in bulk samples by mixing pyrene-labeled actin monomers with actin filament nuclei in polymerization buffer and recording the time course of the fluorescence change as a function of actin monomer concentration (Figure 1A). In this assay the rate of polymerization is proportional to the filament concentration (Pollard, 1983) and the filament number concentration is constant, because the loss of monomers is first order throughout the reaction (Figure 1C). A plot of elongation rate vs. actin monomer concentration (Figure 1D) is linear with a slope proportional to the association constant, k_+, a y intercept proportional to the dissociation rate constant, k_-, and an x intercept equal to the critical concentration.

Profilin inhibited the rate of elongation in a concentration-dependent fashion (Figure 1B–D). As in the controls, the reaction was first order throughout the time course (Figure 1C) and plots of initial rate were a linear function of actin monomer concentration above the critical concentration (Figure 1D). The y intercepts were approximately the same with and without profilin. The difference in the y intercept between the control and profilin samples shown in Figure 1D is larger than that in most experiments. In the presence of profilin, the x intercepts of these plots are larger and the slopes are lower than controls without profilin. Theoretical calculations show that plots of elongation rate vs. monomer concentration are linear as observed (Figure 1D) even with profilin, providing that the affinity of profilin for actin is so low that essentially all of the profilin is free at the actin concentrations tested. The apparent K_d of the actin–profilin complex can be calculated from either the slopes or x intercepts. Twenty separate determinations with six different preparations of proteins gave K_d's of 60–140 μM (mean = 98 μM, SD = 37 μM).

Our interpretation of these data is that profilin has little effect on the dissociation of subunits at the ends of the filaments and that it binds to actin in a way that very weakly inhibits elongation. These findings are consistent with both the monomer sequestration and capping models described under Discussion.

We measured actin filament elongation rates directly by electron microscopy using actin filament bundles from *Limulus* sperm as the nuclei (Figures 2 and 3) as an independent method to evaluate the effect of profilin on actin polymerization. This experiment confirmed that *Acanthamoeba* profilin has a weak effect on the growth of actin filaments at the barbed (fast-growing) end and established that it strongly inhibits growth at the pointed (slowly growing) end. This confirms the results of Tseng & Pollard (1982) regarding inhibition of elongation at the two ends. Specifically, 55 μM profilin inhibited the rate of growth at the barbed end by less than 70%, while 5.5 μM profilin prevented any detectable growth at the pointed end over 60 s (Figures 2 and 3). We have not yet tested by electron microscopy the dependence of the elongation rates in the presence of profilin on the concentration of actin to obtain a precise value for the apparent K_d's for the elongation reaction at the two ends. However, using the known critical concentrations at the two ends (A_1^B = 0.15 μM; A_1^P = 0.6 μM) and the elongation rates in 2 μM actin, we first calculated the rate constants for actin alone (k_+^B = 6.7 × 10^6 M^{-1} s^{-1}; k_-^B = 1.0 s^{-1}; k_+^P = 1.1 × 10^6 M^{-1} s^{-1}; k_-^P = 0.6 s^{-1}). Then we calculated theoretical curves for the dependence of the elongation rate at 2 μM total actin on the concentration of profilin at several different K_d's for the actin–profilin complex (Figure 3). This inhibition is consistent with both the monomer sequestration and capping models described under Discussion. The effect of profilin at the barbed end is consistent with a K_d of 50–100 μM, in agreement with the experiments with bulk samples (Figure 1). On the other hand, the apparent K_d at the pointed end is about 2 μM. Since the pointed end contributes so little to the total elongation reaction, this strong inhibition by profilin of elongation at the pointed end was not detected by experiments with bulk samples (Figure 1).

(*B*) *Steady-State Experiments.* We obtained very similar steady-state polymer concentrations by either diluting actin polymers to various final concentrations (Figure 4) or polymerizing various concentrations of actin monomers with 0.1 μM polymerized actin as nuclei (data not shown). In the absence of profilin plots of steady-state polymer concentration vs. total actin were always linear above the critical concentration.

In the KCl–$MgCl_2$–EGTA buffer, low concentrations of profilin (<10 μM) have almost no detectable effect on the steady-state polymer concentration, while high concentrations have two different effects that depend on the actin concentration (Figure 4). At actin concentrations >1.5 μM, profilin shifts plots of polymer concentration vs. total actin concentration to the right in direct proportion to the concentration of profilin (inset, Figure 4). This shift could be due to either monomer sequestration or capping, as described under Discussion. These data gave K_d's of 50 to >100 μM for 19

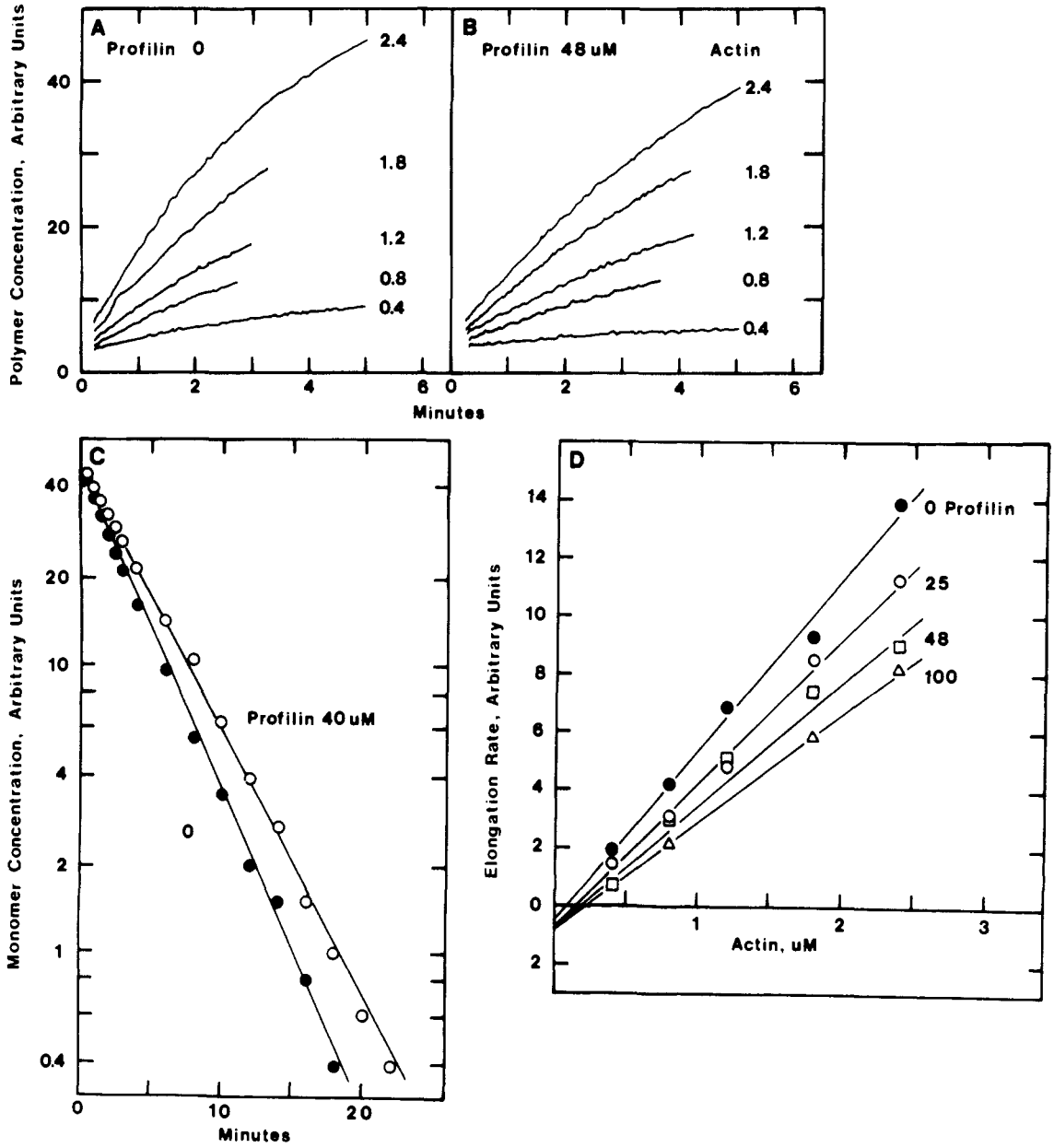

FIGURE 1: Inhibition by *Acanthamoeba* profilin of the elongation of *Acanthamoeba* actin filaments nucleated by polymerized actin. The actin polymer concentration was measured by the enhancement of the fluorescence of pyrene-labeled actin. Conditions: 25 °C; 50 mM KCl, 1 mM $MgCl_2$, 1mM EGTA, 0.1 mM $CaCl_2$, 0.1 mM ATP, 0.25 mM dithiothreitol, 10 mM imidazole, pH 7, 1 μM polymerized unlabeled actin, and 0.4–2.4 μM 5% pyrene-labeled *Acanthamoeba* actin monomer (as indicated to the right of each curve in panels A and B). (A) Time course of polymerization of actin without profilin. (B) Time course of polymerization with 48 μM profilin. (C) Semilog plot of the actin monomer concentration as a function of time with 0 or 40 μM profilin and an inital actin monomer concentration of 2.4 μM. (D) Plots of the initial rate of nucleated polymerization as a function of actin monomer concentration with profilin concentrations of 0, 25, 48, and 100 μM as indicated to the right of each plot. Each linear plot was fit by linear regression. The K_d's calculated from these plots were 96, 102, and 119 μM.

determinations with six different preparations of protein.

When the profilin concentration is high (>15 μM) and the actin concentration is low (<1.5 μM), plots of polymer concentration vs. total actin are not linear (Figure 4). The reasons for this curvature are not yet understood, but it is unlikely to be due to failure to reach steady state. In depolymerization experiments the fluorescence was the same 4 and 30 h after diluting the 5 μM stock solution of fully polymerized actin. Similarly, polymerization and depolymerization experiments had the same sort of curvature in the plots. The change in the critical concentration is directly proportional to the profilin concentration (inset, Figure 4). Again, one can calculate the K_d from the dependence of the apparent critical concentration on the profilin concentration using either the monomer sequestration or capping model, both of which yield identical results. The inset in Figure 4 shows the data (filled circles) and a theoretical plot for K_d = 12.5 μM. In other experiments the effects of profilin were somewhat smaller and the K_d's ranged from 12.5 to 50 μM.

Nucleation Reaction in $MgCl_2$ and EGTA. Acanthamoeba profilin slows the time course of *Acanthamoeba* actin polymerization in a concentration-dependent fashion [Figure 5 and earlier work by Tseng & Pollard (1982), Tobacman & Korn (1982), and Tobacman et al. (1983)], especially prolonging the lag phase at the outset. In agreement with the steady-state experiments (Figure 4), profilin concentrations up to 15 μM had little or no detectable effect on the final polymer concentration at total actin concentrations of 5 or 10 μM when the reaction was carried to completion (not illustrated). This suggested that profilin inhibits nucleation more strongly than elongation.

Since nucleation rates cannot yet be measured directly, we

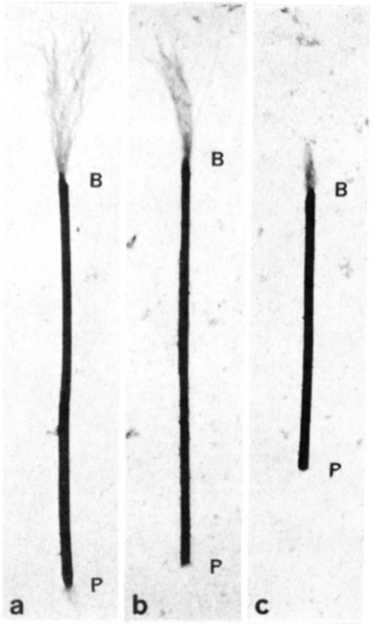

FIGURE 2: Electron microscopic assay of the inhibition by profilin of the elongation of *Acanthamoeba* actin filaments nucleated by *Limulus* acrosomal processes. These samples were taken after 30 s of growth. The experimental conditions are given in the text. Magnification: 27000×. (a) Control without profilin. Note the growth at both the barbed (upper) and pointed (lower) ends. (b) Complete inhibition of pointed end growth by 5.5 μM profilin. (c) Inhibition of growth at both ends by 55 μM profilin.

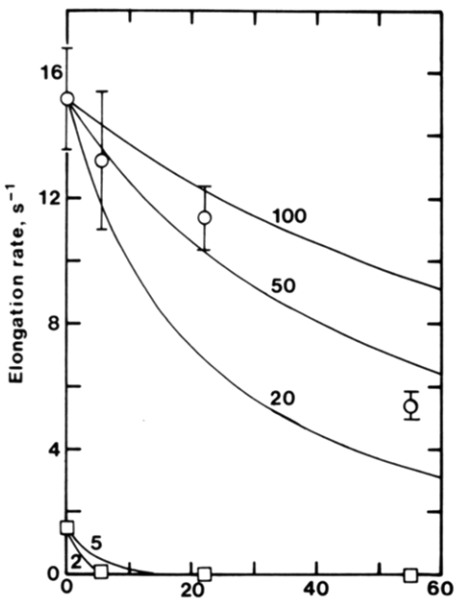

FIGURE 3: Quantitative analysis of the electron microscopic assay for the elongation of *Acanthamoeba* actin filaments at the two ends of *Limulus* acrosomal processes as a function of profilin concentration. The rates were obtained at 30-s time points and are expressed as molecules added per second: circles, mean rates at the barbed end; squares, mean rates at the pointed end; vertical bars are ±1 SD. The solid lines are theoretical curves for the rates expected at several different K_d's (in μM) as indicated by the numbers next to each curve. The curves originating at 15.2 s^{-1} are for the barbed end. The curves originating at 1.5 s^{-1} are for the pointed end.

used a kinetic modeling method (Cooper et al., 1983a) to analyze the effect of profilin on the nucleation reaction. The first step was to select a set of rate constants for differential equations that describe the processes of activation, nucleation, elongation, and fragmentation that best fit the time course of polymerization over a range of actin concentrations (Figure 6). As in the case of muscle actin (Cooper et al., 1983a), there

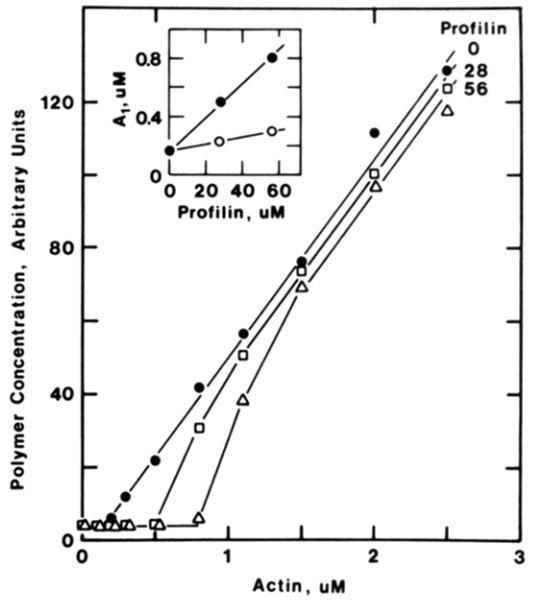

FIGURE 4: Effect of profilin on the steady-state concentration of polymerized *Acanthamoeba* actin measured by the fluorescence of pyrene–actin. Conditions: 25 °C; 50 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM $CaCl_2$, 0.1 mM ATP, 0.25 mM dithiothreitol, and 10 mM imidazole, pH 7. The actin was polymerized at a concentration of 5 μM, diluted to the concentrations indicated into 0, 28.2, or 56.4 μM profilin, sonicated briefly, and incubated for 4 h before measuring the fluorescence. The fluorescence did not change significantly after incubation for an additional 16 h. The inset shows the dependence of the steady-state actin monomer concentration on the concentration of profilin as measured at the critical concentration (filled symbols) or by the shift in the polymer concentration at 2 μM total actin (open symbols). The lines in the inset are theoretical plots for K_d's of 12.5 μM for the filled symbols and 55 μM for the open symbols.

is excellent agreement between the experimental and theoretical curves over the 6-fold range of actin concentrations that we tested. These samples differ by a factor of more than 20 in the time required for full polymerization. A nucleus size of trimer was better than dimer or tetramer, and the nucleation rate constant was 4700 M^{-1} s^{-1}; these findings are the same as for muscle actin. In the interest of simplicity a first-order activation step was not employed to fit the theoretical curves to the experimental data. Plots of log (delay time)$^{-1}$ vs. log (actin concentration) had a slope of 1.8. See Cooper et al. (1983a) for the interpretation of such plots. Briefly, this value implies that either the nucleus size is between dimer and trimer (which is possible) or that a monomer activation step precedes nucleation. Fragmentation was not included in the modeling presented here (Figures 6 and 7) for several reasons. We would have preferred to include fragmentation because the kinetics in the presence of high concentrations of profilin were slow and had a slope reminiscent of samples with substantial fragmentation. We tried to determine a fragmentation rate constant from the controls without profilin. However, in the buffer used here, polymerization was so rapid at all actin concentrations that the fragmentation process was negligible and the fragmentation rate constant was not significantly different from zero. At high profilin concentrations the fit between experimental and theoretical curves was not good. To attempt to improve the fit, fragmentation was included and the fragmentation rate constant was systematically varied, but the fit was not significantly improved.

The second step was to use the nucleus size and nucleation rate constants obtained from the analysis of the polymerization of actin alone to analyze the kinetic curves obtained in the presence of a range of profilin concentrations. We considered

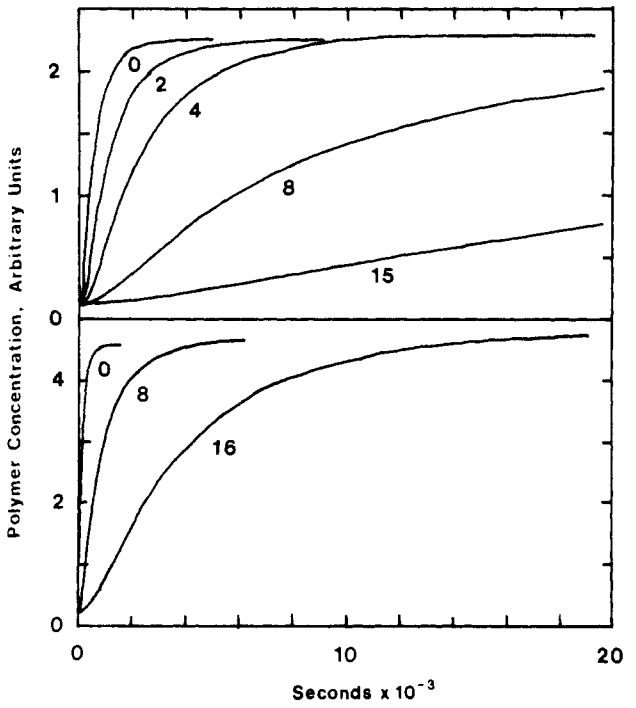

FIGURE 5: Time course of spontaneous polymerization of *Acanthamoeba* actin at several concentrations of profilin. The polymer concentration was measured by the fluorescence of pyrene–actin. Conditions: 25 °C; 50 mM KCl, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM $CaCl_2$, 0.1 mM ATP, 0.25 mM dithiothreitol, and 10 mM imidazole, pH 7. The profilin concentrations in μM are indicated to the right of each curve. Actin monomer concentrations: 5 μM in the upper plots; 10 μM in the lower plots. In the upper plots all of the curves had the same final fluorescence.

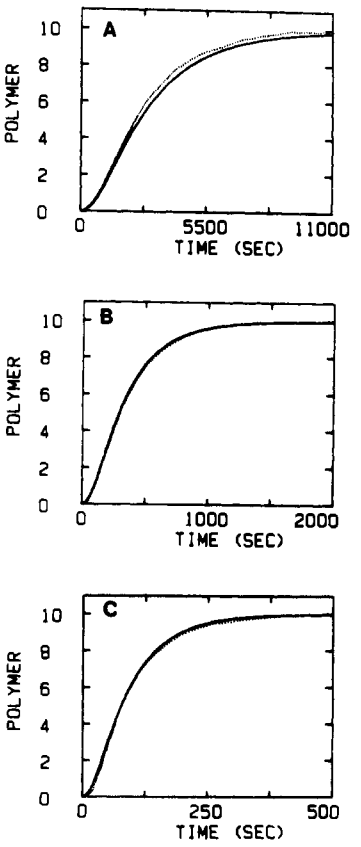

FIGURE 6: Modeling the kinetics of *Acanthamoeba* actin polymerization in the absence of profilin. Polymer concentration in arbitrary units based on pyrene–actin fluorescence is plotted vs. time. The dots are experimental points, and the solid line is calculated from a theoretical model for actin polymerization. The actin concentration is (A) 2.5, (B) 7.5, and (C) 15 μM. Two other concentrations, 5 and 10 μM, are not shown. All five curves were employed to choose the best association rate constant for nucleation, which was necessary for modeling the effect of profilin. That value is 4687 M^{-1} s^{-1}, and the dissociation rate constant for nucleation is 500 s^{-1}. Activation and fragmentation are not included in this model. The nucleus size is a trimer, which is significantly better than dimer or tetramer. The association rate constant for elongation is 10^7 M^{-1} s^{-1}, and the dissociation rate constant for elongation is 1.2 s^{-1}. Conditions are the same as for Figure 5.

two different types of models.

Model 1: The monomer sequestration hypothesis for the mechanism of action of profilin is that profilin binds to actin monomers, but not polymers, preventing the monomers in the complex from participating in all aspects of polymerization (Tobacman & Korn, 1982; Tobacman et al., 1983). We included in the theoretical model for polymerization an equation describing such a binding reaction ($A + P \rightleftarrows AP$) and specified that only free actin monomers are active in nucleation and elongation. The computer systematically varied the K_d of the actin–profilin complex to search for the best fit between the theoretical and experimental curves. Figure 7 shows four of nine time courses from one experiment. A model with a K_d of 8 μM fits the experimental data well at low profilin concentrations but not at high concentrations of profilin. Note especially that the theoretical model predicts a lower final polymer concentration than observed. This implies that the steady-state K_d is higher than 8 μM and confirms that the simple model is inconsistent with the data as also shown above.

Model 2: This complex model with two K_d's combines features of monomer sequestration and capping and is described in detail under Discussion and Experimental Procedures. This model fits the experimental data better than the simple model (Figure 7). A K_d of 2.5 μM for profilin binding to actin monomers gives the best fit. The final polymer concentrations agree in the theoretical and experimental plots due to the use of 100 μM for the K_d for profilin binding to the barbed end of filaments. This K_d in fact yields results that are not significantly different from the case of no binding of profilin to barbed ends. At low profilin concentrations the fit between the experimental and theoretical curves is good. At high concentrations the fit is better than with the simple model, but it is not good.

This analysis shows that the kinetic data can be fit reasonably well with a model where the apparent K_d for the actin–profilin complex is in the range of 2–5 μM when measured by the effect of profilin on nucleation. This is, of course, the same result obtained from the effect of profilin on the elongation of actin filaments at the pointed end. We can be confident that profilin inhibits nucleation and elongation at the pointed end more strongly than elongation at the barbed end. However, the failure of even the complex theoretical model to fit the observations perfectly means that we do not yet fully understand all of the details of the mechanism. There are a number of possible explanations for the discrepancy between the theoretical calculations and the experimental data, including the following: (a) some aspect of the theoretical model for actin polymerization, such as the nucleation or fragmentation steps, is not correct; (b) the trace impurities in the profilin affect the time course of polymerization when present at high concentration; (c) the model for actin–profilin binding is not correct in some detail.

Influence of Profilin and Actin Preparations on Observations. Similar results were obtained in all of the assays with profilin from six different preparations. Fractions from the leading and trailing edges of the protein peak eluting from the G-75 column gave the same results as fractions from the

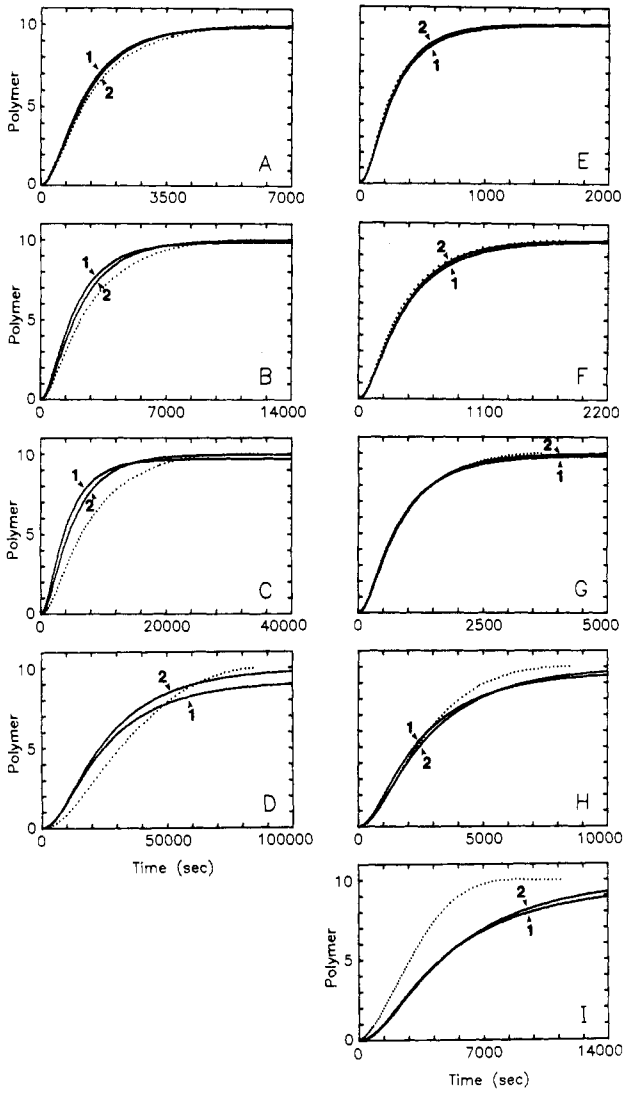

FIGURE 7: Modeling the effect of profilin on the kinetics of *Acanthamoeba* actin polymerization. Polymer concentration in arbitrary units based on pyrene–actin fluorescence is plotted vs. time. The dots are experimental points, and the solid lines are calculated from two different theoretical models for the effect of profilin on actin polymerization. In both models profilin binds to actin monomers. Model 1 is the simple monomer sequestration model, and model 2 is the complex model, which combines features of monomer sequestration and capping. Both are described in the text. For model 1, the K_d is 8 μM. For model 2, the K_d for monomer binding is 2.5 μM and the K_d for barbed end binding is 100 μM. [Actin] is (A–D) 5 and (E–H) 10 μM; [profilin] is (A) 2.5, (B) 5, (C) 10, (D) 28, (E) 2.5, (F) 5, (G) 10, (H) 20, and (I) 28 μM. The fit between the theoretical and experimental curves is good at low profilin concentrations, but at high profilin concentrations, the fit is not so good. Model 2 fits better than model 1. Conditions are the same as for Figure 5.

center. After actin and profilin were incubated together for 36 h at 25 °C during a critical concentration experiment, there was no change in the actin or profilin bands on gel electrophoresis in SDS. Furthermore, it was possible to recover the profilin from these samples by ammonium sulfate precipitation and gel filtration. This repurified profilin behaved just like the original profilin in an actin filament elongation assay similar to Figure 1.

Dr. Steve Brenner of the NIH provided us with two samples of *Acanthamoeba* profilin. Using our actin in KCl–$MgCl_2$–EGTA, we found that at two different concentrations (2.4 and 5.9 μM) his profilin prolonged the time course of *Acanthamoeba* actin polymerization to exactly the same extent as our profilin in an experiment similar to Figure 5. There was no detectable effect on the steady-state polymer concentration. In actin filament elongation assays in KCl–$MgCl_2$–EGTA like Figure 1, his fresh preparation of profilin yielded an apparent dissociation constant of 80 μM. His frozen preparation of profilin gave an apparent dissociation constant of 28 μM.

We also prepared *Acanthamoeba* actin by the method of Tobacman et al. (1983) that includes dialysis against buffer G containing 50 μM $MgCl_2$ instead of 200 μM $CaCl_2$. When tested in a buffer containing 50 mM KCl, 1 mM $MgCl_2$, and no added Ca^{2+} or EGTA (Tobacman et al., 1983), this Mg–actin had polymerization properties similar to conventionally prepared actin in the same buffer with 1 mM EGTA. Profilin had only weak effects on the elongation step. The apparent K_d's were about 75 μM in an elongation rate experiment similar to Figure 1 and greater than 100 μM in a steady-state experiment similar to Figure 4. In this KCl–$MgCl_2$ buffer, low concentrations of profilin prolonged the time course of polymerization of Mg–actin monomers as in Figure 5. We analyzed one set of kinetic curves similar to those in Figure 5 and found that a K_d of about 5 μM gave the best fit for both the simple and complex models, as in our more extensive analysis (Figure 7). The fit was best at low concentrations of profilin, just as in KCl–$MgCl_2$–EGTA.

Storage of *Acanthamoeba* actin in buffer G has a curious and unexplained effect on its interaction with *Acanthamoeba* profilin. Profilin prolongs the time course of spontaneous polymerization more when the actin is freshly prepared than after storage for 1 week or more in buffer G. This is curious, because actin alone polymerizes more slowly after storage. For example, freshly prepared 5 μM *Acanthamoeba* actin in KCl–$MgCl_2$–EGTA buffer required 8 min to polymerize to 50% of the steady-state polymer concentration, and 10 μM profilin prolonged this time to 160 min. After storage in buffer G for 2 weeks at 4 °C, the same actin required 14 min for half-maximal polymerization, but 10 μM profilin increased the time to only 51 min. Two other actin preparations behaved the same way. The profilin did not change its activity during storage at 4 °C for more than 1 month.

Effects of Profilin on Actin Filament Nucleation and Elongation under Other Conditions. (A) KCl–$MgCl_2$–$CaCl_2$. Although *Acanthamoeba* actin has a higher critical concentration (about 0.5 μM) and polymerizes more slowly in KCl–$MgCl_2$ with 0.1 mM $CaCl_2$ than with 1 mM EGTA, profilin has essentially the same effects in both buffers. In KCl–$MgCl_2$–$CaCl_2$, profilin weakly inhibits elongation, judging from both pre-steady-state and steady-state experiments. In bulk elongation rate experiments similar to Figure 1D, high profilin concentrations reduce the slope without changing the y intercept. The apparent K_d's ranged from 35 to 63 μM (mean = 44 μM, SD = 11 μM) in five experiments with three different preparations of profilin and actin. In steady-state experiments like Figure 4, profilin at concentrations up to 30 μM reduced the polymer concentration very little at actin concentrations greater than 1 μM. The apparent K_d's were 40 to >100 μM in 12 experiments with five different preparations of profilin and actin. At high profilin concentrations, plots of fluorescence vs. total actin were curved at low concentrations of actin as in Figure 4. Similar to Figure 5, low concentrations of profilin prolonged the time course of polymerization, although we have not yet analyzed these data quantitatively to determine the apparent K_d based on the inhibition of nucleation. We conclude from these experiments in 50 mM KCl and 1 mM $MgCl_2$ that free Ca^{2+} may strengthen very slightly the effect of profilin on the elongation of actin filaments.

Table I: Comparison of Apparent Dissociation Constants for the Actin–Profilin Complex in KCl and $MgCl_2$ with a Low Concentration of Ca^{2+}

assay	present report	Tobacman et al. (1983)
nucleation (μM)	2–5	5
elongation (μM)		
barbed end	50	5
pointed end	≤2	ND[a]
steady-state polymer concn at high actin concn (μM)	50–100	ND[a]
steady-state critical concn (μM)	12.5–50	8.6

[a] ND is not determined.

(B) KCl–$CaCl_2$. *Acanthamoeba* profilin inhibits the elongation of *Acanthamoeba* actin filaments much more strongly in KCl with $CaCl_2$ than in KCl–$MgCl_2$ with $CaCl_2$. In 11 experiments with four different protein preparations, low concentrations of profilin raised the critical concentration consistent with a K_d of 5 μM for the actin–profilin complex. This confirms earlier experiments with other polymerization assays (Tobacman & Korn, 1982; Tseng & Pollard, 1982). In parallel experiments (similar to Figure 1) low concentrations of profilin strongly inhibited the initial rate of elongation from actin filament nuclei. Plots of elongation rate vs. actin concentration were nearly parallel to the control plots at actin concentrations that gave positive rates of growth. Below the critical concentrations these plots (obtained by using pyrene-labeled nuclei) curved sharply and had approximately the same y intercept as the control. Theoretical calculations show that this is the behavior expected when the K_d for the actin–profilin complex is low. The data were consistent with a K_d of 3–9 μM (mean = 5.5 μM, SD = 3.3 μM, n = 11). Low concentrations of profilin also prolonged the time course of polymerization as in Figure 5, but these data have not yet been analyzed quantitatively. We conclude that $MgCl_2$ greatly weakens the effect of profilin on the elongation process.

Discussion

Comparison of Our Data with Previous Reports. Our most important finding is that, in KCl with 1 mM $MgCl_2$ and a low concentration of Ca^{2+}, *Acanthamoeba* profilin inhibits the formation of nuclei and growth at the pointed end of filaments more strongly than elongation at the barbed end (Table I). As in previous work with these proteins (Tseng & Pollard, 1982; Tobacman & Korn, 1981; Tobacman et al., 1983), we first analyzed the data in terms of a model where a 1:1 complex of profilin is in rapid equilibrium with free actin and profilin and calculated the affinity of binding as an apparent K_d using the assumption that only free actin monomers participate in all of the steps of polymerization. Judging from the effect of profilin on nucleation, the apparent K_d is 2–5 μM. The strong inhibition of elongation at the pointed end of filaments is also consistent with a K_d of about 2 μM, although our data are not yet extensive enough to calculate a precise K_d. In contrast, several new assays confirm our previous conclusion (Tseng & Pollard, 1982) that elongation at the barbed end of *Acanthamoeba* actin filaments in $MgCl_2$ is relatively insensitive to profilin: (a) High concentrations of profilin are required to change the rate of elongation from preformed actin filament nuclei, as measured by the pyrene–actin fluorescence assay (Figure 1). (b) High concentrations of profilin are required to inhibit the growth of *Acanthamoeba* actin filaments from the barbed end of *Limulus* acrosomal processes (Figures 2 and 3). (c) High concentrations of profilin are required to change the steady-state fluorescence of polymerized pyrene–actin (Figures 4, 5, and 7). Together these assays for actin filament elongation at the barbed end suggest that the K_d for the actin–profilin complex is at least 40 μM and may be larger than 100 μM. This K_d is more than an order of magnitude higher than the K_d's calculated from the effects of profilin on nucleation and on elongation at the pointed end of filaments. This difference is the reason why a complex model with two K_d's fits the experimental data on the time course of polymerization better than a simple monomer sequestration model with one K_d.

This conclusion differs from that of Tobacman et al. (1983), who suggested that all of the effects of *Acanthamoeba* profilin on the polymerization of *Acanthamoeba* actin could be explained by a simple model with a single K_d of about 5 μM regulating the concentration of actin available for nucleation and elongation at both ends. The two laboratories agree that the effects of profilin on nucleation are consistent with a K_d of about 5 μM but disagree on the effects of profilin on elongation.

Both laboratories used pre-steady-state and steady-state experiments to evaluate the effect of profilin on elongation. For their pre-steady-state assay, Tobacman et al. used cross-linked actin filaments as nucleation measure elongation rates. In KCl and $MgCl_2$, 4–34 μM profilin strongly inhibited the maximum rate of nucleated polymerization, from which they calculated that the K_d of the actin–profilin complex is 7–8 μM. These data differ from our results presented in Figure 1. Tobacman et al. also reported a steady-state critical concentration experiment using 9.7 μM profilin and 0–1.1 μM actin. From the inhibition of polymerization they calculated a K_d of 8.6 μM. Looking over a much wider range of actin and profilin concentrations, we found that the plots of polymer concentration vs. total actin concentration are nonlinear in the region that they examined (Figure 4). This has been confirmed by Dr. Steve Brenner of the NIH group (personal communication). Although we could estimate a K_d of 12.5–50 μM from our steady-state experiments at low actin concentrations, it may be premature to base any conclusions on these experiments, because the data disagree with two other assays for elongation (Figures 1, 2, and 3) and because the basis of the nonlinearity in the plots is not yet explained. In contrast, at higher actin concentrations, we find that the apparent K_d is much higher than 8.6 μM—in the range of 50–100 μM as indicated in Figures 4, 5, and 7.

In an effort to understand the differences in the data collected by the two laboratories, we exchanged and tested profilin samples. They have also tested one of our actin samples. We find that the differences are attributable to the assay procedures and experimental conditions, not to the profilin preparations. An important difference in the conditions is that we examined a broader range of actin and profilin concentrations. Both in the steady-state polymerization assay (Figure 4) and in the analysis of nucleation (Figure 7), a wide range of actin and profilin concentrations was necessary to reveal that the simple model with only a single K_d of 5 μM fails to fit the experimental data. Where we did examine the same protein concentrations, the most obvious difference in the data is in the pre-steady-state elongation assays. They observed a definite lag after adding monomers to the cross-linked filament nuclei and salts [Figure 5 of Tobacman et al. (1983)], while in our experiments any lag is substantially less than the 10–12 s required to mix the reactants and initiate data collection (Figure 1A,B). A lag at the outset of an elongation experiment could be due to ion binding or ion-induced changes in the monomers or nuclei [as suggested by Tobacman & Korn

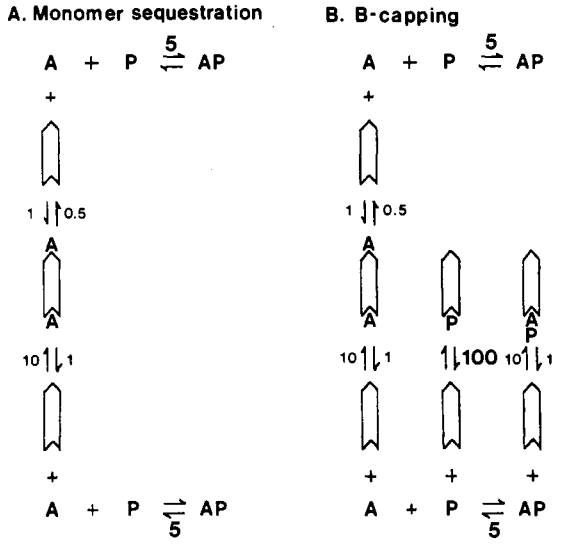

FIGURE 8: Molecular mechanisms for the effect of *Acanthamoeba* profilin on *Acanthamoeba* actin polymerization. (A) The simple model with one K_d and only free actin monomers available for nucleation and elongation. (B) A complex model with high-affinity monomer sequestration, binding of the complex to the barbed but not the pointed end, and low affinity capping of the barbed end.

(1983)], but it could also be due to the formation of nuclei from monomers during the early stages of the reaction. From our experience (Pollard, 1983), substantial endogenous nucleation occurs at the actin concentration (6 μM) used by Tobacman et al. (1983). We used lower monomer concentrations and a concentration of nuclei high enough to measure what appear to be pure elongation rates. This conclusion is supported by the linearity of the plots of initial polymerization rate vs. actin monomer concentration (Figure 1D) and by the fact that the electron microscopic assay of the effects of profilin on elongation (Figures 2 and 3) gave the same results. The existence of some spontaneous nucleation in the nucleated polymerization assay of Tobacman et al. (1983) could explain why profilin inhibited elongation more strongly in their experiments than in ours. Profilin would appear to inhibit elongation, if it inhibited any nucleation that occurred during the experiment.

Mechanism of Action. The essential requirement of a model for the action of profilin is to account for our experimental data, which show that profilin inhibits both elongation at the pointed end and nucleation far more than it inhibits elongation at the barbed end. The simple monomer sequestration model (Figure 8) proposed previously (Tobacman & Korn, 1982; Tseng & Pollard, 1982; Tobacman et al., 1983) does not fulfill these requirements. We propose a complex model of profilin binding to actin monomers and barbed ends that does account for the data. First, we will introduce a new capping model and show that it is not distinguishable from the traditional monomer sequestration model by the use of experiments that measure elongation rates or apparent critical concentrations in solution.

The capping model is that profilin binds to filament ends and prevents monomer binding but not the dissociation of the terminal actin protomer. Note that this model differs from the usual notion of a molecular cap that inhibits both association and dissociation. To compare this model mathematically with the monomer sequestration model, first consider elongation, which is described by

$$dC_p/dt = k_+NA_1 - k_-N \quad (1)$$

where C_p is the concentration of polymerized actin, k_+ is the association rate constant, A_1 is the actin monomer concentration, N is the number concentration of polymer ends, and k_- is the dissociation rate constant. In the monomer sequestration model, actin monomers bind to profilin and form a complex that cannot bind to the end of the filament. The binding is described by

$$K_d = A_{1f}P_f/[AP] \quad (2)$$

where K_d is the dissociation constant, A_{1f} is the concentration of free actin monomers, [AP] is the concentration of the complex, and P_f is the concentration of free profilin. Since

$$[AP] = A_1 - A_{1f} \quad (3)$$

where A_1 is the total concentration of actin monomer, substituting for [AP] in eq 2 and rearranging, one obtains

$$A_{1f} = K_dA_1/(P_f + K_d) \quad (4)$$

Since in this model only free monomer can participate in elongation, one substitutes A_{1f} for A_1 in eq 1 to yield

$$\frac{dC_p}{dt} = k_+NA_1\left(\frac{K_d}{P_f + K_d}\right) - k_-N \quad (5)$$

In the capping model, profilin binds in rapid equilibrium to the end of the filament forming a complex that blocks subunit addition but not dissociation. The binding is described by

$$K_d = N_fP_f/[NP] \quad (6)$$

where K_d is the dissociation constant, N_f is the concentration of free ends, and [NP] is the concentration of capped ends.

$$[NP] = N - N_f \quad (7)$$

Substituting eq 7 into eq 6 and solving for N_f yields

$$N_f = K_dN/(P_f + K_d) \quad (8)$$

Since the complex prevents subunit addition but not dissociation of the terminal protomer, one substitutes N_f from eq 8 for N in the first term but not the second term of the right side of eq 1.

$$\frac{dC_p}{dt} = k_+NA_1\left(\frac{K_d}{P_f + K_d}\right) - k_-N \quad (9)$$

Equations 5 and 9 are the same, illustrating why monomer sequestration and capping are not distinguishable with an experiment measuring elongation, such as Figure 1.

Second, consider an experiment to measure the apparent critical concentration, like the one in Figure 4. At steady state, in both eq 5 and 9, $dC_p/dt = 0$. Solving for A_1, the total monomer concentration, gives eq 10, starting with either eq 5 or 9.

$$A_1 = \frac{k_-(P_f + K_d)}{k_+K_d} = \frac{k_-P_f}{k_+K_d} + \frac{k_-}{k_+} \quad (10)$$

A_1 is the apparent critical concentration measured in the experiment. Again, this experiment cannot distinguish between these two models.

In fact, the two models could be distinguished if either of two simplifications in the above analysis was not included and the experimental data were highly precise. The first simplification involves the free profilin term in eq 5 and 9. The free profilin concentration is, of course, not known. Investigators generally assume that the free profilin is the total profilin. This assumption is a good one in many experiments because the K_d is so high and the actin monomer or filament end concentration is so low that the amount of bound profilin is a small

portion of the total. Without this assumption an extra equation analogous to eq 3 and 7 is included, stating that profilin in the complex is equal to the total profilin minus free profilin. Equations 4 and 8 then become the solution of a quadratic equation, and eq 5 and 9 are in fact not identical because N, the total filament end concentration, is not the same as A_1, the total actin monomer concentration. Using the experimental data to solve for K_d with eq 5 and 9 would then give different results. We calculate, however, that such a difference would be less than 1% given a K_d near 100 μM, a total profilin concentration less than 1 mM, a total actin concentration less than 5 μM, and a total filament number concentration less than 10 μM. The two models could be distinguished if the K_d were lower, especially if it were below the actin concentration.

The second simplification is that the barbed and pointed ends are not considered independently. To extend the analysis to the case where profilin binds to only barbed but not pointed ends, eq 5 and 9 would give

$$\frac{dC_p}{dt} = k_+^B N A_1 \left(\frac{K_d}{P_f + K_d} \right) + k_+^P N A_1 - k_-^B N - k_-^P N \quad (11)$$

where the superscripts B and P indicate barbed and pointed ends, respectively. Since the rate constants for the barbed end are so much greater than those for the pointed end, the results predicted by eq 11 are very similar to those for eq 5 and 9. Upon substituting reasonable values for the rate constants and dissociation constant, we find that the predicted values for apparent critical concentration and for elongation rate differ by less than 10% at profilin values up to 100 μM. Therefore, we conclude that either of these two models can predict our results from solution experiments measuring elongation rates and apparent critical concentrations. However, neither model alone is sufficient to explain all the experimental findings.

We next propose a complex model that does explain these findings. This model combines features of both the monomer sequestration and capping models described above. The features of the model are as follows:

(1) Profilin binds to actin monomers with a K_d of 2–5 μM. This complex cannot participate in nucleation or elongation at the pointed end. The complex can add to barbed ends at a normal rate. This part of the model explains several experimental findings: (a) The apparent K_d for nucleation is 2–5 μM, with good fit between experimental and theoretical time courses for polymerization (Figure 7). The one unexplained feature of these data is that the fit is better at low than at high profilin concentrations. (b) The apparent K_d for elongation at the pointed end is less than or equal to 2 μM (Figure 3). (c) Several previous measurements of the dissociation constant for binding of actin monomers to profilin are in the range of 5–10 μM. Indirect measurement by the effect of profilin on the adenosinetriphosphatase (ATPase) activity of actin monomers in 0.35 mM $MgCl_2$ gave a K_d of 5.5 μM (Tobacman & Korn, 1982). Indirect measurement by enhancement of the fluorescence of pyrene-labeled actin in buffer G gave a K_d of 6–10 μM (Lee et al., 1982; S. Lee and T. D. Pollard, unpublished results). A dialysis-rate method gave a K_d of 10 μM in buffer G (Runge et al., 1982).

(2) Profilin binds to barbed ends of actin filaments with a K_d of 50–100 μM. When profilin is bound to a barbed end, no more actin subunits can add, but the terminal actin subunit (to which the profilin is bound) can dissociate at a normal rate. This portion of the model predicts several other experimental findings: (a) The apparent K_d determined from elongation at the barbed end in the electron microscope assay is about 50 μM (Figure 3). (b) The apparent K_d determined from elongation in solution is 60–140 μM (Figure 1). (c) The apparent K_d determined from critical concentration experiments (Figure 4) and the steady-state fluorescence of higher actin concentrations (Figures 5 and 7) is 50 to >100 μM. The one unexplained feature of these data is the curvature of the plots from critical concentration experiments with high profilin concentrations (Figure 4).

Above we demonstrated that this sort of capping model can explain these types of findings. Profilin does not inhibit the actin dissociation rate, as judged by the extrapolated y intercepts in Figure 1.

Therefore, this model is able to predict our experimental findings. Other models with a similar or greater complexity than this one can also predict the data, but we choose to describe this one because it seems relatively simple and involves reasonable assumptions. One strong feature of this model is that profilin need only bind to one site on an actin molecule, although it binds with different K_d's depending on whether the actin is monomeric or bound to the barbed end of a polymer. That site would be on the barbed side of the molecule. Profilin binds to the site on the monomer with a low K_d, to the same site on the terminal protomer at the barbed end of a filament with a high K_d, and not at all to the same site on the internal subunits in a filament or the terminal protomer at the pointed end of a filament. Consider the pointed end. The barbed side of the terminal protomer is facing inward toward the next protomer, so the site is not available for binding. Elongation at the pointed end involves the joining of the pointed end of a filament with a barbed side of a monomer. The barbed side of the monomer can be blocked by profilin with a low K_d; therefore, elongation is prevented with an apparent low K_d. Consider the barbed end. Elongation at the barbed end involves the joining of the barbed side of the terminal protomer at the barbed end with the pointed side of a monomer. Profilin does not bind to the pointed side of a monomer, but it does bind to the exposed barbed side of the terminal protomer. However, now it binds to that same site with a high K_d. This difference in K_d is reasonable because the conformation of actin in the filament is different than that of the monomer (Higashi & Oosawa, 1965). Formation of the actin–actin bonds might have an allosteric effect on the profilin binding site of the actin molecule. Alternatively, the adjacent actin subunits in the filament may themselves interfere physically with the binding of profilin to the terminal actin subunit and lower the affinity. It is interesting to note that a barbed end can be blocked by either a profilin binding to it or an actin–profilin complex binding to it. Consider the process of nucleation. The first step involves the reaction between the barbed side of one monomer and the pointed side of another monomer. Profilin can bind to its usual site on the barbed side with a low K_d, which results in nucleation being inhibited with a low apparent K_d.

Overall, we feel that this model (Figure 8B) is is the best way to explain the data gathered so far, but we are well aware that other mechanisms might fit the data equally well. For example, each actin molecule could have two profilin binding sites: a high affinity site where profilin binding results in the inhibition of nucleation and pointed end growth and a lower affinity site where profilin binding interferes with barbed end growth.

Our model could be substantially supported with more experimental data. In any system where the hypothesis is that the binding of one molecule to another alters the functional

properties of the other, one ideally wants to measure both the binding of the molecules to each other in a direct physical assay and also the function of the molecules when they are mixed together. These experiments should show that extent of binding correlates with extent of change of function. Our system is quite complicated due to the different physical forms of actin that are often present at once. While there has been relative success in determining the effect of profilin on the functional properties of the different forms, there is little data on the binding of profilin to the different forms. The electron microscope assay for elongation separately measures elongation at the barbed and pointed ends. The solution assays for elongation rate and critical concentration reflect elongation at the barbed end for the most part. The kinetic modeling measures nucleation, a reaction mainly involving monomers with each other. There are no data, however, about the binding of profilin to the barbed or pointed ends of filaments and minimal data about the binding of profilin to actin monomers. The reason for this lack of data is the difficulty in designing experiments to measure binding to these different forms separately, especially since they are all present at quite low concentrations in polymerizing conditions. Future experiments that do measure this binding directly will allow one to correlate the extent of binding with the extent of inhibition of function.

The general features of complex models including both monomer binding and filament capping were originally suggested independently by ourselves for *Acanthamoeba* profilin (Lee et al., 1982) and by Tilney for a profilin-like protein from *Thyone* sperm (Tilney et al., 1982) at the 1982 Annual Meeting of the American Society for Cell Biology and were elaborated upon by Tilney et al. (1983) in a recent paper. The data presented here put the model on a firmer quantitative footing and suggest that different molecules in the profilin class of actin binding proteins may have similar mechanisms of action. It will be very important to extend this analysis to the vertebrate profilins. Calf spleen profilin is isolated as a 1:1 complex with actin. The complex, profilactin, can polymerize. Polymerization of profilactin, as measured by high-shear viscosity, was accelerated by the addition of spectrin–actin–band 4.1 complex but slowed by villin (Markey et al., 1982). The authors' interpretation was that barbed ends, created by spectrin–actin–band 4.1 complex, have a critical concentration lower than that for pointed ends, created by villin. Profilin lowers the monomer concentration, and the barbed end is more sensitive than the pointed end. The C-terminus of calf spleen profilin is not important for its interaction with actin, but the C-terminus of actin is important to that interaction (Malm et al., 1983).

The recognition of the differential effects of profilin on actin filament nucleation and on elongation at the two ends of the filament under physiological conditions has some interesting implications for the role that profilin might play in the cell. It now seems likely that the major roles of profilin are to suppress the spontaneous formation of actin filaments and prevent growth at the pointed ends of the filaments. On the other hand, since profilin has only a weak effect on the elongation process at the barbed end when $MgCl_2$ is present, profilin will not prevent the growth of actin filaments in the barbed direction when nucleating sites are present in the cell. This should allow the cell to specify, with much higher precision than a simple self-assembly mechanism would provide, when and where actin filaments are created in the cell. For example, profilin would increase the probability that all of the new actin filament formation in the cytoplasm occurs from actin nucleating molecules like *Acanthamoeba* capping protein (Isenberg et al., 1980). By the suppression of spontaneous nucleation, the problem of regulating the sites of actin filament formation is reduced to that of simply specifying when and where active nucleating sites become available in the cell.

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