Mechanism of the Interaction of Human Platelet Profilin with Actin

Pascal J. Goldschmidt-Clermont,* Laura M. Machesky, Stephen K. Doberstein, and Thomas D. Pollard
Department of Cell Biology and Anatomy, *Department of Medicine, Cardiology Division, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

Abstract. We have reexamined the interaction of purified platelet profilin with actin and present evidence that simple sequestration of actin monomers in a 1:1 complex with profilin cannot explain many of the effects of profilin on actin assembly. Three different methods to assess binding of profilin to actin show that the complex with platelet actin has a dissociation constant in the range of 1 to 5 μM. The value for muscle actin is similar. When bound to actin, profilin increases the rate constant for dissociation of ATP from actin by 1,000-fold and also increases the rate of dissociation of Ca²⁺ bound to actin. Kinetic simulation showed that the profilin exchanges between actin monomers on a subsecond time scale that allows it to catalyze nucleotide exchange. On the other hand, polymerization assays give disparate results that are inconsistent with the binding assays and each other: profilin has different effects on elongation at the two ends of actin filaments; profilin inhibits the elongation of platelet actin much more strongly than muscle actin; and simple formation of 1:1 complexes of actin with profilin cannot account for the strong inhibition of spontaneous polymerization. We suggest that the in vitro effects on actin polymerization may be explained by a complex mechanism that includes weak capping of filament ends and catalytic poisoning of nucleation. Although platelets contain only 1 profilin for every 5–10 actin molecules, these complex reactions may allow substoichiometric profilin to have an important influence on actin assembly. We also confirm the observation of I. Lassing and U. Lindberg (1985. Nature [Lond.] 318:472–474) that polyphosphoinositides inhibit the effects of profilin on actin polymerization, so lipid metabolism must also be taken into account when considering the functions of profilin in a cell.
tion. These new ideas are more compatible than the original sequestration mechanism with what is known about the interactions of profilin with membrane lipids and the response of the actin system to extracellular agonists.

**Materials and Methods**

**Materials**

Outdated human platelets were obtained from the blood bank of The Johns Hopkins Hospital and used within 10 d after phlebotomy. Poly-L-proline (14,000 mol wt), adenosine triphosphate (ATP), Quina2, L-M-ethenoadenosine 5'-triphosphate (e-ATP), phosphatidylisoproline (PIP), diethiothreitol (DTT), Triton X-100, phosphatidylisoproline 4 phosphate (PIP), and IP} were from Sigma Chemical Co. (St. Louis, MO). PIP2 was from Calbiochem-Behring Corp. (La Jolla, CA). Phosphatidylserine (PS) was from Avanti Polar Lipids (Pelham, AL). 125I-Protein A was from DuPont-New England Nuclear (Boston, MA). Freund's adjuvant (complete and incomplete) were from Gibco Laboratories (Grand Island, NY). Peroxidase-labeled goat antisem to rabbit immunoglobulin was from Hycnote Laboratories (Logan, UT).

**Protein Purification and Modification**

Actin was purified from rabbit skeleton muscle (Spandich and Watt, 1971), monomers were separated from oligomers by gel filtration on Sephacryl S-300 in G-buffer (2 mM Tris, pH 7.5, 0.5 mM DTT, 0.2 mM ATP, 0.1 mM CaCl2, 0.2 mM NaCl), and used within 7 d. Actin was labeled with pyrenylodacetylne (Pollard, 1984) and stored in G-buffer. Samples were gently mixed for 15 min at 22°C. The beads were pelleted and the free actin in the supernatant measured by the Bradford method using profilin standards.

The fractions containing actin were immediately dialyzed against 2 mM Tris, pH 7.5, 0.4 mM ATP, 0.5 M KCl, 5 mM DTT, 0.1 mM CaCl2, 2 mM MgCl2, for 18 h at 4°C. After dialysis, polymerized actin was pelleted by centrifugation at 105 g for 2 h at 2°C and then depolymerized by dialysis against G-buffer. The sample was filtered onto Sephacryl S-300, as described for muscle actin. Approximately 50-75% of the actin retained by the poly-L-proline agarose column, possibly as a complex with profilin, was recovered by this procedure. This represents a yield of 0.2-0.4 mg of actin per gram of packed platelets. This actin was >99% pure (Fig. 1) and had precisely the same critical concentration in a physiological buffer as muscle actin (Fig. 2). A higher yield was obtained by eluting the poly-L-proline agarose column with 1 M KI, but the actin obtained did not polymerize normally. The actin concentration was measured by UV absorbance at 290 nm using an extinction coefficient of 0.026 OD μm cm−1 (Tseng et al., 1984).

After the KI wash and a 4 M urea wash, human platelet profilin was eluted from the poly-L-proline column with 8 M urea and renatured by dialysis for 48 h against G-buffer. The purified profilin was homogeneous by gel electrophoresis (Fig. 1). The concentration of profilin was measured by UV absorbance at 280 nm using an extinction coefficient of 0.015 OD μm cm−1 (Tseng et al., 1984).

**Lipid Preparation**

The purity of the phosphoinositides, PIP2, PIP and PI was confirmed by thin layer chromatography (Goldschmidt-Clermont et al., 1990). Micelles of PIP2 and small vesicles of PIP, PI and phosphatidylserine were obtained by sonication in deionized water (Goldschmidt-Clermont et al., 1990).

**Quantitation of Total Platelet Actin and Profilin**

(a) Gel Electrophoresis. Platelets were pelleted by centrifugation of platelet rich plasma at 1,000 g for 30 min at 4°C. The supernatant was discarded and aliquots from the pellet weighed. The volume occupied by packed platelets was assumed to be 0.9 ml/g. Samples of whole platelets and pure profilin standards were solubilized in boiling SDS and electrophoresed on SDS-polyacrylamide gels. The gels were stained with Coomassie brilliant blue, and the intensity of the band corresponding to actin in the sample was compared with a standard curve (Goldschmidt-Clermont et al., 1986a).

(b) Quantitative Immunoblotting. Dilutions of platelets and purified profilin were run on SDS polyacrylamide gels, blotted onto nitrocellulose, incubated with rabbit antisem to platelet profilin, and then reacted either with peroxidase-labeled goat anti-rabbit immunoglobulin or with 125I-protein A. The antisem was obtained by immunizing rabbits with 0.4 mg of human platelet profilin in CFA and boosted with 0.3 mg in incomplete Freund's adjuvant after 6 and 10 wk. The profilin concentration in packed platelets was estimated by comparison with standards either by eye (peroxi- dase; Young et al., 1987) or by counting (125I-Protein A; Kiehart et al., 1984). Total protein in platelet extracts was measured according to Bradford (1976) using platelet profilin as the standard.

(c) Affinity Chromatography on Poly-L-Proline Agarose. A weighed sample of packed platelets was extracted and chromatographed on the affinity column. All of the immunoreactive profilin in the extract passed through the column (Fig. 1) and eluted with 8 M urea. This profilin was quantitated by UV absorbance. The loss of profilin trapped in the pellet was estimated by weighing the pellet after removal of the clear extract.

**Measurement of the Binding of Profilin to Actin Monomers**

(a) Poly-L-Proline-immobilized Profilin. 40-μl aliquots of poly-L-proline agarose beads with 17.4 μg of purified profilin bound were mixed with various concentrations of actin monomers in a total volume of 200 μl of G-buffer. Samples were gently mixed for 15 min at 22°C. The beads were pelleted and the free actin in the supernatant measured by the Bradford (1976) method using actin standards. In the absence of profilin <2% of the actin pellet was found on poly-L-proline beads.

(b) Ultracentrifugation. Mixture of profilin and actin were centrifuged at 30,000 g for 3 min at 22°C in microconcentrators (Centrifloc from Amicon Corp., Danvers, MA) with a 30-kD cutoff (Goldschmidt-Clermont et al., 1986b). Abut 10% of the starting sample was filtered. This separates free profilin from profilin bound to actin and free actin monomers, since no detectable actin monomers but >95% of free profilin passed through the filters. Free profilin in the filtrate was quantitated by the Bradford (1976) method using profilin standards.

**Polymerization and Depolymerization of Actin**

The concentration of polymerized actin was measured by 90°C light scatter-
ing at 400 nm (Wegner and Engel, 1975) or by the fluorescence of 10% pyrene-labeled actin using an excitation wavelength of 365 nm and emission wavelength of 407 nm (Cooper et al., 1983). As chemical modification of the COOH-terminus of actin has been reported to affect the interaction of the high affinity divalent cation binding site of actin (Gershman et al., 1986). Excitation was at 340 nm, emission at 500 nm. Experiments were performed at 22°C in Q2-buffer (2 mM Tris, pH 7.5, 0.5 mM DTT, 0.2 mM ATP, 0.1 mM CaCl2, 0.2 mM Na++), 50 mM KCl, 1 mM MgCl2, 1 mM EGTA at 22°C. Each point represents the mean of three separate samples. The vertical bars are ± 1 SD. (Inset) Time courses of polymerization of 5 μM platelet actin (P) and 5 μM muscle actin (M).

### Table I. Concentrations of Actin and Profilin in Human Platelets

<table>
<thead>
<tr>
<th>Assay</th>
<th>Profilin (μM)</th>
<th>Actin (μM)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quantitative immunoblot</td>
<td>30–50</td>
<td>–</td>
<td>This report</td>
</tr>
<tr>
<td>Polyproline-beads</td>
<td>40–45</td>
<td>–</td>
<td>This report</td>
</tr>
<tr>
<td>Gel electrophoresis</td>
<td>35–55</td>
<td>–</td>
<td>Lind et al. (1987)</td>
</tr>
<tr>
<td>Ultrafiltration</td>
<td>270–320</td>
<td>–</td>
<td>This report</td>
</tr>
<tr>
<td>DNease I inhibition assay</td>
<td>875</td>
<td>–</td>
<td>Gordon et al. (1977)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>~700</td>
<td>–</td>
<td>Nachmias and Yoshida (1988)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>–</td>
<td>Fox (1986)</td>
</tr>
</tbody>
</table>

In this study the concentration of platelet profilin was measured three times by each of two methods, quantitative immunoblotting and binding to poly-L-proline agarose beads. The actin concentration was estimated by gel electrophoresis of two pooled platelet preparations. The ranges of measured concentrations for each protein are shown. The range of total protein concentration in these pooled platelet pellets was 40–70 mg/ml. The values from the study by Lind et al. were calculated by assuming a platelet protein concentration of 57 mg/ml. The difference in methods used in the various reports to pellet platelets is a likely source of the range of reported values. The extracellular space in the pellets may represent a substantial fraction of the total volume, so the concentrations of profilin and actin in this table are minimal values.

### Results

#### Concentration of Profilin and Actin in Human Platelets

In a pellet of packed platelets the concentration of profilin is 30–50 μmol/liter and the concentration of actin is 230–320 μmol/liter (Table I). The immunological and poly-L-proline binding assays for profilin, which are based on completely different interactions, gave similar results. The assay
for actin assumes no comigrating proteins on the SDS-gel, an obvious oversimplification, but previous work with two-dimensional gels suggests that this assumption does not result in serious overestimation of the plateau actin concentration (Goldschmidt-Clermont, P., unpublished observation). These results are in general agreement with previous reports (Table I), although the absolute concentration of actin varies by threefold, most likely due to differences in the way the platelets were pelleted and in the assay used to quantitate actin.

**Binding of Profilin to Actin Monomers at Steady State**

Two different assays, based on different physical principles, gave dissociation constants of 0.9–50 μM for the complex of actin and platelet profilin in a low ionic strength buffer (Table II). Platelet profilin had a similar affinity for platelet actin and muscle actin in these assays.

**Effect of Profilin on Nucleotide Exchange**

We confirmed that profilin increases the rate of exchange of nucleotide bound to actin in a concentration-dependent fashion (Fig. 3) as originally described by Mockrin and Korn (1980) and by Nishida (1985). Moreover, we now present evidence that the effect of profilin on the nucleotide exchange is catalytic. Thus even when actin is in excess of profilin and the profilin concentration is well below the $K_d$ of the complex, profilin can accelerate the exchange of nucleotide of the whole population of actin monomers (Fig. 3).

Using kinetic simulation, we found a relatively simple mechanism with seven reactions that accounts for the effects of a wide range of profilin concentrations on nucleotide exchange (Table III; Fig. 3). Starting with the rate constants for reactions 1 and 2 at low concentrations of calcium from Waecht and Engel (1975), we varied the values independently until the simulation fit the time course for the control experiments (Fig. 3A). To simplify the analysis, we assumed that the rate constants for the reactions of profilin with the three forms of actin (ATP-actin, eATP-actin, and actin free of nucleotide) are identical. After trial and error, we arrived at values for reactions 5, 6, and 7 that are consistent with the observed $K_d$ of 2.5 μM and a value of 13 s$^{-1}$ for the dissociation rate constant of ATP and eATP from the actin profilin complex. With these constants (Table III), the mechanism fits the experimental data over a range of profilin concentrations of 0.03 to 1.92 μM (Fig. 3). None of these rate constants could be changed by more than twofold without altering substantially the quality of the fit at one or more profilin concentrations. A good fit required different rate constants for reactions 1 and 2, but the fit could not be improved by varying the rate constants of reactions 6 and 7 independently.

Other models might also fit our experimental data, but we did not find a simpler model that worked as well. Since ADP dissociates more rapidly from actin than ATP (Neid, and Engel, 1979), we considered that profilin might accelerate nucleotide exchange by increasing the rate of ATP hydrolysis by actin. However, previous studies demonstrated that profilin, if anything, decreases the rate of ATP hydrolysis by actin (Tobacman and Korn, 1982).

Our analysis shows that profilin stimulates exchange of the actin nucleotide by increasing the rate of nucleotide dissociation by >1,000 fold when it binds transiently to actin monomers. At low concentrations of profilin only a small fraction of actin molecules is associated with profilin (horizontal lines in Fig. 3), but the profilin catalytically accelerates the nucleotide exchange of the whole population of actin molecules by exchanging rapidly between actin molecules.

**Effect of Profilin on Divalent Cation Exchange**

Profilin increases the rate of exchange of Mg$^{2+}$ for Ca$^{2+}$ at the high-affinity site on monomeric platelet actin (Fig. 4). In control experiments, profilin did not alter the fluorescence of Quin2 in either the presence or the absence of Ca$^{2+}$. Thus there is no evidence that profilin itself binds Ca$^{2+}$. 

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Effect of profilin on the time course of the exchange of eATP for ATP bound to platelet actin monomers. At time zero, 50 μM eATP was added to 06 μM platelet actin-ATP in 2 mM Tris, pH 7.2, 1 μM CaCl$_2$, 2 μM ATP, 5 μM DTT at 22°C with the following profilin concentrations: (A) zero; (B) 0.03 μM; (C) 0.24 μM; (D) 1.92 μM. The fluorescence was converted to the concentration of actin with bound eATP (left axis) using the fluorescence of 0.6 μM actin saturated with eATP in the absence of profilin. The vertical bars in A are ± 1 SD from three separate runs. The grey lines are time courses generated by kinetic simulation using the mechanism and rate constants from Table III. The solid lines are the steady state concentrations of complexes of profilin with all actin species (ATP-actin, eATP-actin, and actin free of nucleotide, right axis) calculated by kinetic simulation. (Inset) Dependence of the half-time ($t/2$) of the exchange reaction on profilin concentration.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>Rate Constant ($k_+$)</th>
<th>Rate Constant ($k_-$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T + A = TA$</td>
<td>7</td>
<td>0.012</td>
</tr>
<tr>
<td>$eT + A = eTA$</td>
<td>7</td>
<td>0.026</td>
</tr>
<tr>
<td>$T + AP = TAP$</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>$eT + AP = eTAP$</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>$A + P = AP$</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>$TA + P = TAP$</td>
<td>1</td>
<td>2.5</td>
</tr>
<tr>
<td>$eTA + P = eTAP$</td>
<td>1</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Seven reactions were used to simulate the exchange of nucleotide and the reaction of actin with profilin. For each reaction, the rate constants were varied individually over at least three orders of magnitude in both directions to optimize the fit with the data. Alteration of the constants presented here by more than twofold markedly alters the kinetic curves and reduces the fit of the theoretical curves to the data shown in Fig. 3. A, monomeric platelet actin; $T$, ATP; $eT$, etheno-ATP; and $P$, profilin.
Catalytic Regulation of Actin by Profilin

Like other profilins (Tobacman and Korn, 1982; Pollard and Cooper, 1984; Larsson and Lindberg, 1988), human platelet profilin increases the critical concentration for polymerization measured at steady state (Fig. 5). Using two different profilin concentrations, the $K_c$ for the profilin–platelet actin complex was 1.73 $\mu$M (mean of three experiments, Fig. 5, Table II). Similar results were obtained starting with actin monomers or actin filaments. The $K_c$ for the profilin-muscle actin complex calculated from the steady-state polymerization assay was larger than the $K_c$ measured for this complex in low ionic strength buffer (Table II). A micromolar $K_c$ for the profilin-muscle actin complex was also measured using steady-state viscosity in buffer containing MgCl$_2$ (Larsson and Lindberg, 1988).

In a buffer containing MgCl$_2$, KCl, ATP, and EGTA, profilin inhibits elongation at the barbed and pointed ends of platelet actin more strongly than muscle actin (Fig. 6). Assuming a model where only free actin participates in the elongation reaction, we calculated the equilibrium constants for the profilin–actin complex from the data at the two ends of each type of actin filament (Table II). Profilin concentrations that have relatively small effects on the steady-state polymer concentration (Fig. 5) or elongation at the barbed end (Fig. 6) markedly prolong the lag phase at the onset of spontaneous polymerization (Fig. 7). This strong effect of profilin is far in excess of the reduction in actin monomer concentration expected from the binding of profilin to actin. For example, a mixture of 5 $\mu$M actin with 4.06 $\mu$M profilin is expected to contain $\sim$2.5 $\mu$M free actin monomer, but this mixture (Fig. 7 B) polymerizes much slower than 2.5 $\mu$M actin alone (Fig. 7 A). Moreover, when profilin is added 60–120 s after initiating spontaneous polymerization, its effect on the time course of actin polymerization is markedly reduced (Fig. 7 C). Therefore the inhibition of actin polymerization by profilin is likely to be mediated by the effect of profilin on actin monomers, possibly by altering their interaction with divalent cations and nucleotides.

We cannot exclude the possibility that profilin could interact with small actin oligomers and prevent their elongation (capping of nuclei).

Polyphosphoinositides Inhibit the Effects of Profilin on Actin

We confirmed the observations of Lassing and Lindberg of a range of profilin concentrations on the time course of Ca$^{2+}$ dissociation.

**Effects of Profilin on Actin Polymerization**

Like other profilins (Tobacman and Korn, 1982; Pollard and Cooper, 1984; Larsson and Lindberg, 1988), human platelet profilin increases the critical concentration for polymerization measured at steady state (Fig. 5). Using two different profilin concentrations, the $K_c$ for the profilin–platelet actin complex was 1.73 $\mu$M (mean of three experiments, Fig. 5, Table II). Similar results were obtained starting with actin monomers or actin filaments. The $K_c$ for the profilin-muscle actin complex calculated from the steady-state polymerization assay was larger than the $K_c$ measured for this complex in low ionic strength buffer (Table II). A micromolar $K_c$ for the profilin-muscle actin complex was also measured using steady-state viscosity in buffer containing MgCl$_2$ (Larsson and Lindberg, 1988).

In a buffer containing MgCl$_2$, KCl, ATP, and EGTA, profilin inhibits elongation at the barbed and pointed ends of platelet actin more strongly than muscle actin (Fig. 6). Assuming a model where only free actin participates in the elongation reaction, we calculated the equilibrium constants for the profilin–actin complex from the data at the two ends of each type of actin filament (Table II). Profilin concentrations that have relatively small effects on the steady-state polymer concentration (Fig. 5) or elongation at the barbed end (Fig. 6) markedly prolong the lag phase at the onset of spontaneous polymerization (Fig. 7). This strong effect of profilin is far in excess of the reduction in actin monomer concentration expected from the binding of profilin to actin. For example, a mixture of 5 $\mu$M actin with 4.06 $\mu$M profilin is expected to contain $\sim$2.5 $\mu$M free actin monomer, but this mixture (Fig. 7 B) polymerizes much slower than 2.5 $\mu$M actin alone (Fig. 7 A). Moreover, when profilin is added 60–120 s after initiating spontaneous polymerization, its effect on the time course of actin polymerization is markedly reduced (Fig. 7 C). Therefore the inhibition of actin polymerization by profilin is likely to be mediated by the effect of profilin on actin monomers, possibly by altering their interaction with divalent cations and nucleotides.

We cannot exclude the possibility that profilin could interact with small actin oligomers and prevent their elongation (capping of nuclei).
(1985, 1988) on the effects of polyphosphoinositides on the inhibitory effects of profilin on actin polymerization (Fig. 8). PIP2 has a stronger effect than PI or other acidic phospholipids such as PS have little or no effect (Fig. 8). IP3, the head group of PIP2, by itself had no effect on actin polymerization in the presence of profilin (Fig. 8). The effects of equal concentrations PIP2 in homogeneous micelles or mixed with PS in small vesicles were similar. Micelles of PIP2 also inhibit the effect of profilin on the exchange of actin nucleotide (data not shown), suggesting that polyphosphoinositides prevent all interactions between profilin and actin.

**Discussion**

**Affinity of Vertebrate Profilin for Actin Monomers**

Our results confirm that profilin purified from a vertebrate nonmuscle cell has a micromolar affinity for both cytoplasmic and muscle actin, as originally shown by DiNubille and Southwick (1985). In our reconstituted system, we find no evidence for a high-affinity complex that might explain the ability to isolate (Carlsson et al., 1977) and crystallize (Carlsson et al., 1976; Markey et al., 1981) a stable complex of profilin with actin. This difference remains one of the most intriguing aspects of profilin and deserves additional research.

![Figure 6](image1.png)

**Figure 6.** Effect of profilin on the elongation rate of actin at (A) the barbed end and the (B) pointed end of actin filaments measured by electron microscopy using Limulus acrosomal processes as nuclei. Conditions: 2 mM Tris, pH 7.5, 0.5 mM dithiothreitol, 0.2 mM ATP, 0.1 mM CaCl2, 0.2 mM NaN3, 50 mM KCl, 1 mM MgCl2, 1 mM EGTA, 22°C. Muscle actin, (○, ●) or platelet actin (▲, △) without profilin (filled symbols) or with 7 μM profilin (open symbols). The solid straight lines correspond to the best fit to the data obtained in the absence of profilin. In the absence of profilin the rate constants for the reactions of ATP-actin with the barbed ends (k+: 13.2 μM⁻¹s⁻¹; k⁻: 1.9 s⁻¹) and with the pointed ends (k+: 0.9 μM⁻¹s⁻¹; k⁻: 0.2 s⁻¹) of actin filaments were identical for platelet actin and muscle actin. Theoretical curves for the reactions in the presence of profilin were calculated, assuming that only free actin monomers participate in elongation and using the rate constants measured in the absence of profilin. The best fits were obtained with the following Kc's for the profilin-actin complex: 22.5 μM for the barbed end of muscle actin; 7.0 μM for the barbed end of platelet actin; 33.0 μM for the pointed end of muscle actin; and 1.2 μM for the pointed end of platelet actin.

![Figure 7](image2.png)

**Figure 7.** Effect of profilin on the time course of spontaneous polymerization of platelet actin measured by light scattering. Conditions: 2 mM Tris, pH 7.5, 0.5 mM DTT, 0.2 mM ATP, 0.1 mM CaCl2, 0.2 mM NaN3, 50 mM KCl, 1 mM MgCl2, 1 mM EGTA, 22°C. (A) Dependence of the polymerization without profilin on the concentration of actin: (○) 1.25 μM; (●) 2.50 μM; (△) 3.75 μM; (△) 5.00 μM. (B) Dependence of the polymerization of 5 μM actin on the concentration of profilin: (●) zero; (○) 1.67 μM; (●) 3.15 μM; (△) 4.06 μM; (△) 5.00 μM. (C) Effect of the time of addition of 5.5 μM profilin to 3.75 μM actin on the extent of polymerization at 2,700 s after initiation of polymerization with MgCl2, KCl, and EGTA. At this time samples without profilin have just reached steady state (dotted line). The inhibitory effect of profilin drops off sharply after the first 60 s in polymerizing conditions in spite of the fact that little polymer is detected until 200 s.
the COOH terminus of actin (Larsson and Lindberg, 1988) using an affinity chromatography step should select for actin context, both the phosphorylation of profilin by protein kinase C (Hansson et al., 1988) and proteolytic processing of the nucleotide exchange. We were unable to account for the presence of various concentrations of lipids: (●) micelles of PIP2; (●) small unilamellar vesicles of PIP; (●) small unilamellar vesicles of PI; (○) small unilamellar vesicles of PS; (□) small unilamellar vesicles of PS and PIP; or in the presence of various concentrations of IP3 (●). The concentration of polymer was measured by the pyrene fluorescence: 100% corresponds to the fluorescence of fully polymerized actin in the absence of profilin, which reached steady state in <1,800 s (see Fig. 7 A); 0% corresponds to the fluorescence of actin with 5 μM profilin at 10,800 s, which is nearly the same as unpolymerized actin (see Fig. 7 B). In the absence of profilin the concentrations of phospholipids and IP3 used in these experiments did not affect the fluorescence of pyrene actin.

because it suggests that there could be an unrecognized mechanism that regulates the affinity of profilin for actin. In this context, both the phosphorylation of profilin by protein kinase C (Hansson et al., 1988) and proteolytic processing of the COOH terminus of actin (Larsson and Lindberg, 1988) need further study. Our method to purify actin from platelets using an affinity chromatography step should select for actin with the highest affinity for profilin. However, despite this precaution, the reconstituted complex has a micromolar $K_d$.

**Effects of Profilin Binding on the Properties of Actin Monomers**

Binding of profilin to actin increases dramatically the rate of dissociation of ATP and divalent cation from their high affinity binding sites on actin. We confirm earlier observations by Mockrin and Korn (1980) and Nishida (1985) and provide for the first time a quantitative kinetic analysis for the nucleotide exchange. We were unable to account for the divalent cation exchange data with a simple model. The ATP and divalent cation clearly interact with each other (Frieden, 1982; Nowak et al., 1988; Valentin-Ranc and Carlier, 1989) and bind to sites in a deep cleft in the actin molecule (Kabsch et al., 1990). Profilin can be chemically cross-linked to actin residue 364 (Vandekerckhove et al., 1989), which is located on the surface opposite the cleft. It seems likely that binding of profilin alters the conformation of the actin, perhaps opening the cleft in a way that allows both the nucleotide and the divalent cation to dissociate more freely than from native actin. Binding of DNaseI has the opposite effect on actin (Hitchcock, 1980; Mannherz et al., 1980), most likely because it blocks the exit from the cleft (Kabsch et al., 1990).

Perhaps the most remarkable feature of these effects of profilin on actin monomers is that the profilin acts catalytically to promote the exchange of nucleotide and possibly of divalent cation. This is possible because the rate of exchange of profilin between actin monomers is relatively rapid but slower than the rapid dissociation of the actin ligands that occurs during the transient binding of profilin to each actin molecule (Table III). Thus, substoichiometric profilin causes the rapid exchange of the actin ligands under conditions where only a small fraction of the actin molecules are associated with profilin at any given time.

**Effects of Profilin on the Polymerization of Actin Filaments**

A simple monomer sequestration mechanism can account for some but not all of the effects of profilin on actin polymerization. Assuming that only free actin monomers can elongate actin filaments, the monomer sequestration mechanism and the independently measured affinity of profilin for actin monomers can explain the effects of profilin on the steady-state critical concentration and elongation at the pointed end of platelet actin filaments. Elongation at the barbed end is slightly less sensitive to inhibition by profilin than predicted by the $K_d$, but this anomaly is less striking than with Acanthamoeba profilin (Pollard and Cooper, 1984). The data can be explained by a mechanism where actin–profilin complexes or free profilin can bind to the barbed end of filaments forming a weak cap (Pollard and Cooper, 1984). This mechanism is also consistent with recent structural studies showing that profilin binds to the barbed end of the actin molecule (Vandekerckhove et al., 1989; Kabsch et al., 1990). A monomer sequestration mechanism cannot account for the very strong inhibition of spontaneous polymerization by profilin, where it appears to poison nucleation substoichiometrically, like its effects on nucleotide and divalent cation exchange. Bound ATP and Mg$^{2+}$ both promote the unfavorable step of nucleation. Therefore the effect of profilin on actin affinity for these ligands might represent the mechanism whereby profilin strongly inhibits actin nucleation. Additional research will be necessary to understand the connection between these processes.

**Effects of Profilin on Actin in Cells**

Three factors suggest that profilin accounts for only a small fraction of the unpolymerized actin in platelets and other cells. First, there is general agreement that the concentration of actin is 5–10 times larger than the concentration of profilin in platelets (Table I). Second, the dissociation constant of the complex is in the micromolar range (Table II). Third, at least some of the profilin is bound to membrane lipids (Hartwig et al., 1989; Machesky et al., 1990). Consequently, other sequestering molecules such as the 5-kD protein Fx (Safer et al., 1990) or members of the actophorin/depactin/destrin group of actin monomer binding proteins (reviewed by Pollard and Cooper, 1986) or some other mechanisms are required to account for the unpolymerized fraction of actin in vivo.

We speculate that the predominant effect of profilin on actin in cells might be the catalysis of nucleotide and divalent
cation exchange. Given an excess of ATP over ADP in the cell, profilin may maximize the concentration of ATP-actin monomers, by increasing markedly the rate of exchange of the nucleotide bound to actin.

Since ATP-actin elongates actin filaments faster than ADP-actin (Pollard, 1986), the effect of profilin on nucleotide exchange might actually promote rather than inhibit this aspect of polymerization. For example, upon activation of leukocytes, the concentration of actin polymers oscillates rapidly as a result of cycles of polymerization and depolymerization (Oman et al., 1989), but the general trend is an increase in the concentration of polymers (Fechheimer and Zigmond, 1983; Howard and Oresajo, 1985). The depolymerization cycles will produce monomeric ADP-actin so the rate of reassembly might be limited by the rate of nucleotide exchange. Since substoichiometric profilin also inhibits actin nucleation more strongly than elongation of filaments, it might limit the number of new filaments and enhance the elongation of preformed filaments.

**Connection of Profilin-Actin Interactions to Phosphoinositide Metabolism**

The available evidence suggests that a fraction of the profilin could be bound to PIP2, in the inner leaflet of the platelet plasma membrane and also on the cytoplasmic face of other cellular membranes (Hartwig et al., 1989; Goldschmidt-Clermont et al., 1990). This conclusion is based on the high concentration of PIP2 (140–240 μM; Cohen et al., 1971), the 1:5 stoichiometry and submicromolar affinity of the complex, and the apparent ability of profilin to aggregate PIP2 into small patches (Goldschmidt-Clermont et al., 1990; Machesky et al., 1990). An association of profilin with the cytoplasmic face of the plasma membrane of human platelets and leukocytes has been observed independently by electron microscopy (Hartwig et al., 1989).

Binding of profilin to membrane phosphoinositides provides a possible mechanism for the regulation of the interaction of profilin with actin (Lassing and Lindberg, 1983; Goldschmidt-Clermont et al., 1990). For example, agonist induced stimulation of flux through the phosphoinositide pathway from PI to PI3, and DAG may dissociate profilin from membranes, account for the transient formation of profilin–actin complexes (Lind et al., 1987) and through nucleotide or divalent cation exchange promote the observed assembly of actin filaments (Fox, 1986).

Since profilin also affects the rate of hydrolysis of PIP2 by soluble PLC isozymes (Goldschmidt-Clermont et al., 1990), it stands at an important regulatory crossroads in the cell. Although we have focused on profilin in this report, we understand that many other molecules are likely to act in concert with profilin in regulating the assembly of the actin cytoskeleton (see Stossel, 1989).

This work was supported by National Institutes of Health research grants GM-26338 and GM-35711. P. J. Goldschmidt-Clermont was supported by a fellowship from the American Heart Association. L. M. Machesky was supported by a National Science Foundation predoctoral fellowship.

Received for publication 27 November 1990 and in revised form 13 February 1991.

**References**


