The Actin Released from Profilin–Actin Complexes Is Insufficient to Account for the Increase in F-Actin in Chemoattractant-stimulated Polymorphonuclear Leukocytes

Frederick S. Southwick and Clarence L. Young

Infectious Disease Section, The Department of Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Abstract. Chemoattractant stimulation of polymorphonuclear leukocytes is associated with a nearly twofold rise in actin filament content. We examined the role of the actin monomer sequestering protein, profilin, in the regulation of PMN actin filament assembly during chemoattractant stimulation using a Triton extraction method. Poly-L-proline-conjugated Sepharose beads were used to assess the relative concentration of actin bound to profilin with high enough affinity to withstand dilution (profilin–actin complex) and DNase I-conjugated beads to measure the relative concentration of actin in the Triton-soluble fraction not bound to profilin. Actin associated with the Triton-insoluble fraction (F-actin) was also measured. In unstimulated PMN, the relative concentration of actin bound to profilin was maximum. After FMLP stimulation, profilin released actin monomers within 10 s, with the profilin–actin complex concentration reaching a nadir by 40 s and remaining low as long as the cells were exposed to chemoattractant (up to 30 min). If FMLP was dissociated from PMN membrane receptors using t-BOC, actin reassociated with profilin within 20 s. Quantitative analysis of these reactions, however, revealed that profilin release of and rebinding to actin could account for only a small percentage of the total change in F-actin content. Determination of the total profilin and actin concentrations in PMN revealed that the molar ratio of profilin to actin was 1 to 5.2. When purified actin was polymerized in PMN Triton extract containing EGTA, removal of profilin from the extract minimally affected (12% reduction) the high apparent critical concentration at which actin began to assemble. Although profilin released actin at the appropriate time to stimulate actin assembly during exposure to chemoattractants, the concentration of profilin in PMN was insufficient to explain the high unpolymerized actin content in unstimulated PMN and the quantity of actin released from profilin too small to account for the large shifts from unpolymerized to polymerized actin associated with maximal chemoattractant stimulation.

POLYMORPHONUCLEAR leukocytes undergo rapid changes in morphology in response to various stimuli. Chemoattractants induce polarization and formation of lamellipodia; phagocytic particles induce formation of pseudopods and attachment to surfaces induce spreading and lamellipodia formation. All of these stimuli also induce a nearly twofold rise in actin filament content (11, 16, 36, 40, 45, 46). In unstimulated PMN, a high percentage of the total actin (60–70% or ~200 μM, assuming a uniform distribution) is in an unpolymerized state. After chemoattractant stimulation there is rapid filament assembly, F-actin content reaching a maximum (60–70% of total actin, 200 μM) and G-actin content reaching a nadir (30–40% of total actin, 100 μM) within 30–60 s. These observations indicate that the high concentrations of unpolymerized actin in resting PMNs are capable of being incorporated into filaments following stimulation.

Profilin is an attractive candidate for maintaining the high concentrations of monomeric actin in resting PMN and for regulating the release of actin monomers after stimulation. This protein is a 15,500-kD polypeptide that binds actin in a 1:1 complex and prevents monomers from being incorporated into filaments. Profilin was initially purified from spleen (7). Subsequently a protein of similar molecular weight and function has been purified from brain, thymus (4), platelets (14, 28), and macrophages (10). The human profilin gene has recently been cloned and Northern blot analysis has revealed that this protein is expressed in a wide variety of tissues including liver, uterus, kidney, and heart (18). A protein with a lower molecular weight, 12,800 kD, but similar function, has also been purified from *Acanthamoeba* (35, 43) and *Physarum* (34).

Two major questions concerning the role of profilin in regulating the state of actin in nonmuscle cells have not been

clearly answered by these investigators. First, in stimulated cells is the release of actin monomers from profilin temporally associated with stimulus induced actin filament assembly? The possible role of profilin in regulating the shift from G to F-actin has been recently addressed by Lasing and Lindberg (23) who have found the profilin–actin complexes can be dissociated by phosphoinositol bisphosphate, a phosphatidyl inositol by-product whose turnover increases after chemotactic stimulation. Using platelets two groups of investigators studied the effects of thrombin stimulation, a stimulus that induces rapid actin filament assembly in platelets. Their results were contradictory. Markey et al. (29) found maximal concentrations of actin bound to profilin in resting platelets and observed a rapid dissociation of actin from profilin in response to thrombin stimulation, whereas Lind et al. (25) found low concentrations of actin bound to profilin in resting platelets and during initial thrombin stimulation observed an increase in the concentration of actin bound to profilin. These latter findings suggested that profilin might not play a role in regulating the initial rise in actin filament content induced by thrombin, since profilin was shown to be sequestering monomers at the same time actin filaments were assembling.

A second question to be clarified is whether or not the concentrations of profilin found in the cytoplasm can totally account for the high concentrations of unpolymerized actin found in unstimulated nonmuscle cells. Estimates in nonmuscle cells of the contribution of profilin-actin complexes to the total unpolymerized actin pool have ranged from 14–100% (4, 7, 14, 29, 35, 44).

We have shown that human PMN also contain a protein with physical-chemical and functional characteristics similar to previously purified mammalian profilins. Using poly-L-proline-conjugated Sepharose beads we have examined the effects of chemotactic stimulation on high-affinity profilin–actin complex concentration in human PMN, as well as attempted to determine the contribution of profilin in maintaining the high concentration of monomers found in PMN. In unstimulated PMN, like Markey et al., we have found profilin-actin complex concentration to be maximal. Following stimulation at all time points studied (10 s to 30 min) profilin released actin monomers. The concentrations of profilin found in PMN, however, could account for less than one third of unpolymerized actin in resting PMN. In addition during chemoattractant stimulation the relative concentrations of monomeric actin released by profilin were too small to account for the concomitant large increases in F-actin content.

Materials and Methods

Purification of Profilin from PMN

200 ml of freshly expired pharesis granulocytes ($3\text{–}5 \times 10^7$ cells/ml WBC) were obtained from the American Red Cross. The remaining red cells were removed by dextran sedimentation (one part 4 g/dl dextran T500 to four parts PMN solution) and hypotonic lysis as previously described (39). Wright-stained specimens were shown to contain ≥90% neutrophils. PMNs were pretreated with diisopropylfluorophosphate as previously described (1) and washed with PBS containing 5 mM *N*-ethylmaleimide (NEM) before lysis. We discovered that this condition dissociated profilin–actin complexes (see Results). The cell pellets were next suspended in a 1:1 vol of homogenization buffer containing 0.34 sucrose, 5 mM DTT, 5 mM ATP, 5 mM EGTA, 1 mM PMSF, 0.075 mg/ml benzamidine, 0.01 mg/ml leupeptin, 0.04 mg/ml aprotonin and 20 mM imidizole-HCl, pH 7.5 and broken by a Dounce homogenizer as previously described (40). The mixture was centrifuged at 12,000 *g* for 30 min. The resulting supernatant was incubated overnight at 4°C after the addition of final concentrations of 0.1 M KCl and 2 mM $MgCl_2$. The solution was then subjected to ultracentrifugation at 100,000 *g* for 1 h. This supernatant was applied directly to a 0.5 × 7.0 cm poly-L-proline Sepharose column equilibrated in homogenization buffer, washed with five column volumes of buffer A (10 mM imidazole chloride, pH 7.4, 1 mM DTT, 0.1 M KCl), then eluted with the same buffer containing 5 mg/ml poly-L-proline and washed finally with additional buffer A. The 3.5-ml eluate was concentrated with a microconcentrator (Centricon-10; Amicon Corp., Danvers, MA) to 0.5 cc and applied to a 1.0 × 20 cm ACA-54 ultragel column (IBF Biotechnics, Villeneuve-la-Garenne, France) in buffer A. Protein concentrations were determined by UV absorption using the previously reported extinction coefficient for mammalian profilin (22) or by the Bradford method (5) using an immunoglobulin standard. Simultaneous determination of profilin concentration using both assays resulted in nearly identical values.

Peak fractions, identified by absorbance at 280 nm, were analyzed by SDS-PAGE on 12.5% gels and for their ability to prolong the lag period for the polymerization of gel filtered G-actin containing 6% pyrene-labeled actin. The concentration of pyrenyl-actin was kept below the critical concentration for actin filament assembly to reduce the artifact resulting from the low affinity of profilin for pyrenyl actin (19, 27).

Isoelectric Focusing

Isoelectric focusing was performed as described by O'Farrell (33) using ampholine polyacrylamide gels, pH 3.5–9.5 (PAGplates; LKB, Bromma, Sweden) using a Multiphor II system. 4.5 μg of purified profilin was applied to paper pledgets placed at two locations on the gel, and focused for 3 1/2 h at 10 W.

Purification of Human PMN for Triton Extract Studies

40 ml of whole blood from healthy adult volunteers was drawn into syringes containing 3.2 ml of a 10 g/dl EDTA (Sigma Chemical Co., St. Louis, MO) solution. In the majority of experiments neutrophils were purified by centrifugation through Ficoll/Hypaque solution (36). In some experiments PMN were purified by hypotonic lysis and differential centrifugation as previously described (36). Purified cells were suspended in a modified Hanks' buffer (138 mM NaCl, 6 mM KCl, 1.2 mM $MgSO_4$, 0.64 mM Na_2HPO_4 (pH 7.15), 0.66 mM KH_2PO_4, 5.6 mM glucose, 20 mM Hepes). $CaCl_2$, final concentration of 1 mM, was added to cell suspensions 10 min before cell stimulation in most experiments. For PMN treated with 1 mM EGTA, cells were also preincubated for 10 min before stimulation. PMN were purified and stored at room temperature. All experiments were performed within 3 h of venipuncture. To assess the "resting" state of our PMN on several occasions superoxide production was measured after FMLP stimulation using the cytochrome *c* assay as previously described (12).

Actin Association with the Triton-insoluble Cytoskeleton

A modification of the method described by White et al. (46) was used. Purified human PMN, final concentration of 1.5×10^7 cells per ml, were prewarmed at 37°C for 10 min before stimulation and then stimulated with FMLP (Sigma Chemical Co., St. Louis, MO) (final concentration 5×10^{-8} M). In some experiments FMLP binding was reversed using the antagonist t-butoxycarbonyl-phe-leu-phe-leu-phe (t-BOC).[1] For these experiments, PMN were stimulated with a final concentration of 1×10^{-8} M for 40 s followed by the addition of a final concentration of 1×10^{-4} M tBOC for 20 s. The reaction was stopped by the addition of 1/10 vol of a stock solution containing 10% Triton X-100, 10 mm diisopropylfluorophosphate 0.075 mg/ml benzamidine, 0.04 mg/ml aprotinin, 0.01 mg/ml leupeptin, and 1 mM PMSF in modified Hanks' buffer solution. After mixing by two gentle inversions, the solution was left standing for 2 min at 25°C, and then centrifuged for 2 min at 12,000 *g* in a tabletop centrifuge. The supernatant was decanted and placed on ice (see below). The pellet, representing the Triton-

1. *Abbreviations used in this paper*: PA, profilin–actin complex; PLP, poly-L-proline; tBOC, t-butoxycarbonyl-phe-leu-phe-leu-phe.

insoluble fraction, was solubilized in 100 μl of gel sample buffer (8 M urea, 10 g/liter SDS, 20 g/liter β-mercaptoethanol, 100 g/liter sucrose, 0.06 M Tris-HCl, pH 6.8), sonicated for 30 s using a sonifier (model 200 with a tapered micro tip, output 3; Branson Co., Danbury, CT), boiled for 5 min, and frozen at −70°C.

Determination of Actin and Profilin Content in Triton-treated PMN Supernatants

The Triton-soluble supernatant, described above, was added to 60-90 μl of a 1:1 mixture of poly-L-proline (PLP) beads and modified Hanks' buffer, rotated at 4°C for 3 h, and then centrifuged at 12,000 *g* for 2 min. Previous experiments using purified profilin demonstrated that this volume of beads bound 22–33 μg of profilin, a capacity considerably higher than the total profilin content of our PMN extracts (see Results). In some experiments the supernatant fluid was added to a second set of PLP beads. This step failed to bind significant concentrations of either profilin or actin. The supernatant was next incubated for 3 h with 60 μl of a 1:1 mixture of DNase I-Sepharose beads and modified Hanks' buffer, followed by centrifugation at 12,000 *g*. After removal from the DNase I-beads, the supernatant was dialyzed overnight against 400 vol of H_2O and lyophilized.

After incubation in the supernatants, the beads were mixed with 30 vol of 0.3 M $MgCl_2$ in modified Hanks' buffer inverted twice and centrifuged at 12,000 *g* for 2 min. The supernatant was replaced by 30 vol of modified Hanks' buffer, mixed and centrifuged. Beads were then washed a second time with 30 vol of modified Hanks' solution. $MgCl_2$ was used in our initial wash because this ionic condition has been shown to depolymerize actin filaments associated with Sepharose beads (9, 25). $MgCl_2$ has also been shown to reduce the affinity of spleen profilin for actin (22), therefore PLP beads were exposed to our $MgCl_2$ solution for <3 min. To determine if $MgCl_2$ treatment dissociated PMN profilin-actin complexes, on several occasions duplicate sets of PLP beads incubated in PMN Triton extract were washed with 0.1 M KCl or 0.3 M $MgCl_2$. In the presence of KCl, the percentage of profilin molecules bound to actin was the same as for beads washed in $MgCl_2$, however, KCl-treated beads also contained higher concentrations of other less tightly bound polypeptides. Further controls were also performed using profilin-actin complexes purified as described in Fig. 1. Purified PA (final concentration 12.2 μM) was incubated in 0.1 M KCl, 0.3 M $MgCl_2$ or in low ionic strength buffer for 2 h at 25°C. Purified actin alone (final concentration 12.2 μM) was treated identically. Samples were then centrifuged in an Airfuge (Beckman Instruments Co., Fullerton, CA) at 160,000 *g* for 30 min to sediment any polymerized actin. No actin polymerized in any of the PA solutions while a high percentage of the purified actin incubated in the first two solutions formed sedimentable actin filaments. Purified PA complexes (1.4 and 0.7 μM final concentrations) were also incubated with or without 0.3 M $MgCl_2$ for 2 h at 4°C followed by absorption to poly-L-proline. The ratio of actin to profilin was identical in the presence or absence of $MgCl_2$. These findings all indicate that $MgCl_2$ does not destabilize high-affinity PA complex under the conditions of our experiments.

We also examined the effects of serum on profilin-actin complexes. Incubation of PMN supernatants containing 25% serum did not interfere with binding of profilin to PLP, however the relative amount of actin bound to profilin was reduced by 60%. This finding may be explained by the presence of vitamin D binding protein in serum which can remove actin from profilin-actin complexes (30). All subsequent experiments were performed in serum-free media.

Washed PLP- and DNase I-beads and lyophilized supernatant were combined with gel sample buffer and boiled for 5 min. Triton-insoluble PMN pellets, beads, and supernatant were next electrophoresed into 5-15% polyacrylamide slab gels followed by staining with Coomassie blue. Gels were scanned with a laser densitometer (Zeineh; Biomed Instruments, Inc., Fullerton, CA) and the relative peak areas were determined by integration using an Apple II computer. The relative peak areas of the 42,000-D polypeptide in the various fractions: Triton-insoluble pellet, PLP beads, DNase I-beads, and final supernatant, were determined for each experiment. The sum of these areas was defined as the total actin content. The percent of total PMN actin in each fraction was calculated for each experiment. The sum of the areas of the individual fractions was equivalent to the area of the 42,000-D polypeptide in whole cell extract. The relative increase in F-actin content after chemoattractant stimulation was also calculated as previously described (46).

Using known concentrations of purified actin, we found a linear relationship between the area of the actin peak on Coomassie blue-stained polyacrylamide slab gels and actual protein content in the range of 2.5–30 μg of actin. Using mixtures of purified profilin and skeletal muscle actin, we also determined that under the conditions of our experiments, profilin bound Coomassie blue 90%, as well as actin on a weight basis. This difference was taken into account in calculating actin/profilin molar ratios.

Determination of the Total Content of Profilin and Actin in Unstimulated Human PMN

Total protein concentration of PMN (1×10^6) was determined using the Biuret method (21) and the concentrations of actin and profilin calculated based on scanning densitometry. The denominator for determining the percentage of the total protein represented by actin and profilin was the total integration units found in whole cell extract or the sum of the areas of all the polypeptides found in the four fractions of Triton-extracted PMN. Similar values were derived using either denominator. Total actin content was defined as either the area under the 42 kD polypeptide in whole cell extract or sum of the 42-kD polypeptide areas from the Triton-soluble and -insoluble fractions. Similar values for actin content were determined using either method.

Total profilin content was defined as the amount of profilin bound to PLP beads incubated in the Triton-soluble fraction. To assure that no profilin was trapped in the Triton-insoluble fraction, in several experiments the samples were vigorously sonicated on ice immediately after addition of the Triton solution. No further profilin was released by sonication. The affinity of PLP beads for profilin-actin complexes has been shown to be somewhat lower than for free profilin (42); therefore it was possible that during our wash steps profilin-actin complexes were inadvertently removed. To assure that profilin-actin complexes were not underestimated in our experiments, on several occasions the order of incubation of beads in the Triton-soluble fraction was reversed, samples first being incubated with DNase I-conjugated Sepharose beads to bind all monomeric actin including actin bound to profilin. Samples were subsequently incubated with PLP beads. The total amount of profilin bound to conjugated beads was comparable to that found

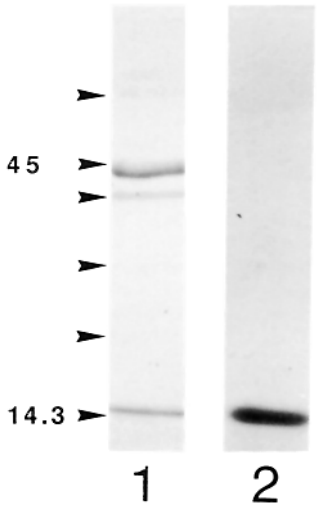

Figure 1. Coomassie blue-stained SDS-PAGE of gel filtration fractions from untreated and NEM-treated human PMN. Lane *1*, representative gel filtration fraction from extract derived from PMN not treated with NEM. The extract was first passed over a PLP-conjugated Sepharose column that was eluted with PLP. The eluted proteins were concentrated by pressure dialysis and loaded on an ACA-54 Sephacryl column. Many fractions contained a 1:1 molar ratio of actin to profilin. Others contained lower molar ratios of actin to profilin (0.5). In the later fractions, very dilute-free profilin were also found. The 34-kD polypeptide probably represents a degradation product of actin. The protein load was ~10 μg, with profilin representing 2.5 μg. Lane *2*, representative gel filtration fraction of PMN treated with NEM as described in Materials and Methods and then treated identically to extract shown in lane *1*. The total protein load was similar to lane *2*; however, since no actin was bound to this preparation of profilin, the 15-kD band appears denser than in lane *1*, since this polypeptide accounts for the total protein added. The yields of profilin in the non-NEM and NEM preparations depicted were nearly identical, 0.57 and 0.6 mg, respectively. Numbers and arrows represent the molecular weights in kilodaltons of known standards: Bovine plasma albumin (66 kD), ovalbumin (45 kD), pepsin (34.7 kD), trypsinogen (24 kD), β-lactoglobulin (18.4 kD), and lysozyme (14.3 kD). Slab gels contained 12.5% acrylamide.

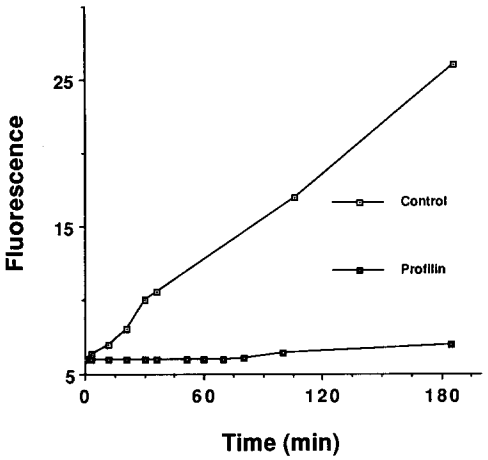

Figure 2. The effects of purified PMN profilin on the rate of actin assembly. A final concentration of 1.5 μM rabbit skeletal muscle actin containing 5% pyrenyl-actin in buffer B (0.5 mM ATP, 1 mM DTT, 0.2 mM $CaCl_2$, 10 mM imidizole, pH 7.4) was polymerized by the addition of a final concentration of 0.1 M KCl and 2 mM $MgCl_2$ in the presence (*solid squares*) or absence (*open squares*) of a final concentration of 4 μM purified human PMN profilin. Actin polymerized in the absence of profilin had a lag phase of $\sim$3 min after which the fluorescence intensity began to increase reaching a steady-state relative fluorescence intensity of 79 (read at 24 h). The same concentration of actin polymerized in the presence of profilin demonstrated a marked prolongation of the lag phase, >70 min. At 24 h, the relative fluorescence intensity was 13. The critical concentration of the actin used in these experiments was 0.36 μM. The K_D of the profilin-actin complex was calculated to be 1.1 μM. In a duplicate experiment the K_D of the PA complex was 0.9 μM.

using our standard method, with the amount of profilin-actin bound to the DNase I beads being offset by a reduction in the amount of profilin-actin complex bound to PLP beads. This finding indicated that the difference in affinity of PLP beads for profilin and profilin-actin complexes did not significantly affect our ability to quantitate the percentage of actin bound to profilin.

In some experiments the DNase I fractions were associated with a small polypeptide or peptides whose molecular weight was <15 kD (see Fig. 3 *B*, lanes *5* and *6*). When DNase I beads preceded PLP beads, two bands were seen, one distinct band at 15 kD and a second more diffuse band clearly migrating below 15 kD. If the Triton-soluble supernatant was first absorbed with PLP beads followed by DNase I beads, the 15-kD band was no longer seen in the DNase fraction, indicating all PA complex was removed by PLP beads.

Pyrenyl-Actin Studies of PMN Triton Extracts

Triton-soluble supernatants from human PMN were prepared as previously described (8). The extract was diluted with suspension buffer containing 0.1% Triton X-100, 138 mM KCl, 2 mM $MgCl_2$, 1 mM EGTA, 1 mM ATP, 10 mM imidizole, pH 7.4 to achieve a final protein concentration of 1-2 mg/ml containing between 5 and 11 μM PMN actin. Extract was combined with varying concentrations of purified skeletal muscle actin containing a subcritical concentration of pyrene-labeled actin (6.6%) and kept at 25°C. The fluorescence intensity of these mixtures were read at 0 and 16 h. Parallel experiments were performed by incubating similar concentrations of actin in the Triton-containing buffer. Skeletal muscle actin and pyrene-conjugated actin were prepared by previously described methods (32, 41). Human PMN actin was purified using DNase I-Sepharose chromatography and formamide as previously reported (47).

Statistical Analysis

The statistical significance of differences for the means of samples were analyzed using the paired and nonpaired *t* test.

Results

Purification of Human PMN Profilin

PMN profilin was purified by PLP affinity chromatography and gel filtration. In the absence of NEM nearly all the profilin copurified as a 1:1 complex with actin (see Fig. 1), however, after PMN were treated with NEM, the major species purified by the same procedure was free profilin. As shown in Fig. 1, PMN profilin could be purified to apparent homogeneity after NEM treatment. 100 mg of PMN extract yielded between 0.6 mg and 1 mg of profilin. The molecular weight of PMN profilin was found to be 15,100 D by SDS polyacrylamide gel electrophoresis on 5-17% gradient gels when compared to known protein standards (see Fig. 1). The protein had a pI of 8.8. PMN profilin isolated by this method prolonged the lag period for actin polymerization (Fig. 2) and reduced the extent of actin filament assembly as observed with other mammalian profilins. The calculated dissociation constant of the PMN profilin-actin complex was 1 μM.

Partitioning of Actin in Unstimulated PMN

Using SDS-PAGE and scanning densitometry (see Figs. 3, *A* and *B*), the relative concentrations of actin associated with the Triton-insoluble cytoskeleton, actin bound with high affinity to profilin, and unpolymerized actin not complexed with profilin were estimated (see Materials and Methods). In unstimulated PMN 42.6% $\pm$ 2.0 (mean, SEM n = 14) of the total actin was associated with the Triton-insoluble cytoskeleton (F-actin), 6.8 $\pm$ 0.05% (n = 14) of the actin was bound to profilin (PA-complex) and 48.9 $\pm$ 2.1% (n = 14) was found in the Triton-soluble fraction not complexed to profilin. Assuming profilin binds with 1:1 stoichiometry, 44 $\pm$ 5% (n = 12) of the profilin molecules collected by PLP bead absorption were complexed with actin. This value is lower than would have been predicted based on the high yield of 1:1 profilin-actin complex during purification of PMN profilin (see Fig. 1, lane *I*). Unlike the experiments described above which used PMN within 3 h of phlebotomy, PMN used for profilin purification had been stored by the Red Cross for 24-48 h before being released for experimental use. The difference in the percentage of profilin complexed to actin in these two experimental conditions suggests that prolonged storage of PMN may increase the formation of high-affinity PA complexes.

To exclude the possibility that PMN had been inadvertently stimulated during Ficoll/Hypaque purification and to more accurately determine the true relative percentage of actin bound to profilin in "resting cells," PMN were rapidly purified by differential centrifugation and hypotonic lysis (see Materials and Methods). Cells were suspended in modified Hanks' solution for 30 min before Triton extraction. All procedures were performed at 4°C. The relative percentage of actin associated with the Triton-insoluble fraction was somewhat lower than Ficoll/Hypaque purified PMN 34.5 $\pm$ 1.3 (n = 4) vs. 42.6 $\pm$ 2.0% (n = 14, P = 0.06), however the percentage of actin bound to profilin did not differ significantly under the two conditions 6.4 $\pm$ 0.8 (n = 4) (Differential centrifugation) vs. 6.8 $\pm$ 0.05% (n = 14) (Ficoll/Hypaque). The lower F-actin content of these more rapidly purified PMN was associated with a significantly higher unpolymerized fraction 59.1 $\pm$ 1.6 vs. 48.9 $\pm$ 2.1%

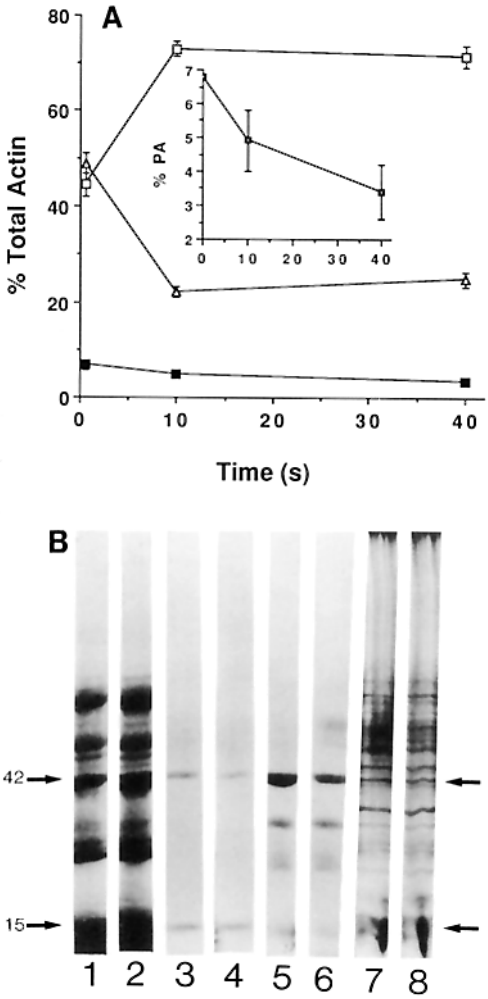

Figure 3. (*A*) Early time course of PMN actin assembly and profilin-actin complex dissociation after exposure to chemoattractant. Human PMN (7.5×10^6 in 500 ul) were exposed to FMLP (5×10^{-8} M final concentration) at time 0 sec. All experiments were performed at 37°C. At the indicated times the reaction was stopped by addition of a Triton buffer solution containing protease inhibitors and EGTA (see Materials and Methods). The cells were centrifuged at 12,000 *g* yielding a Triton-insoluble pellet and a Triton-soluble supernatant. The supernatant was then absorbed with PLP-conjugated beads followed by DNase I-conjugated beads. The supernatant was then dialyzed against water and lyophilized. The beads were washed as described in Materials and Methods. The Triton-insoluble pellet, beads and final supernatant were then subjected to SDS-PAGE and stained with Coomassie blue (see Fig. 3 *B*). For each time point the percentage of actin contained in each of these fractions was determined by laser densitometry. F-actin represented by the open boxes = percent of total actin contained in the Triton insoluble pellet, actin complexed with profilin (PA) represented by the solid boxes and shown in the magnified insert = the percent of total actin bound to profilin removed by the PLP beads. Unpolymerized actin not bound to profilin represented by the open triangles = the percent of total actin bound to the DNase I beads plus actin remaining in the supernatant. The total actin content in resting and stimulated PMN was nearly identical, indicating no loss of actin due to proteolysis. (*B*) Coomassie blue stained SDS-PAGE of the PMN Triton-insoluble and soluble fractions before and after 40 s stimulation with chemoattractant. Example of unstimulated and stimulated PMN prepared as described in *A*. Lanes *1* and *2*, Triton-insoluble pellet, unstimulated PMN (lane *1*), and PMN stimulated for 40 s with FMLP (lane *2*). Lanes *3* and *4*, actin associated with profilin. PLP beads mixed with supernatant from unstimulated cells (lane *3*) and from stimulated PMN (lane *4*). Lanes *5–8*, actin bound to DNase I beads and remaining in the final supernatant, the G-actin fraction not bound to profilin. After absorption with PLP beads, DNase beads were added to supernatant from unstimulated PMN (lane *5*). After removal of the DNase I beads the supernatant was electrophoresed on the same gel (lane *7*). The G-actin fraction in supernatants from stimulated PMN is also shown: the fraction bound to DNase I beads (lane *6*) and the final supernatant (lane *8*). Numbers represent the molecular weights of PMN actin and profilin in kilodaltons. The low molecular weight polypeptide seen in the DNase I bead lanes migrated below profilin. In other experiments in which samples were electrophoresed a greater distance this difference in molecular weights was even more apparent.

($P = 0.04$). In addition PMN were purified using lipopolysaccharide-free reagents as previously described (13). When stimulated with 1×10^{-6} M FMLP, these PMN demonstrated minimal superoxide release (2.7 nmol O_2^-/2.5 $\times$ 10^6 PMN, PMN "primed" by incubation with lipopolysaccharide release 39 nmol/2.5 $\times$ 10^6 PMN in response to the same concentration of FMLP) (13). The percentage of actin bound to profilin and the ratio of actin to profilin did not differ significantly from our other unstimulated PMN preparations.

Partitioning of Actin after Chemoattractant Stimulation

After stimulation with FMLP 5×10^{-8} M for 10 s, F-actin content increased 1.7-fold to 73.0 ± 1.6% ($n = 7$, $P < 0.001$, paired). Comparable values were also seen after 40 s stimulation, 71.5 ± 2.2% ($n = 7$, $P < 0.001$, paired). Actin dissociated from profilin after stimulation, the percentage bound to profilin being significantly lower than unstimulated values at both 10 s, 4.9 ± 0.9 ($n = 7$, $P = 0.03$ paired), and 40 s, 3.4 ± 0.8% ($n = 7$, $P = 0.002$ paired) (see Fig. 3 *A*, *inset*). The percentage of profilin complexed to actin decreased from 44 to 25 ± 4% ($n = 6$, $P = 0.008$ paired) after 10 s and to 27 ± 5% ($n = 6$, $P = 0.005$ paired) at 40 s. The percent of Triton-soluble actin uncomplexed to profilin also decreased upon stimulation with chemoattractant for 10 s, 22.2 ± 1.1% ($n = 7$, $P < 0.001$ paired), remaining at the same level after 40 s, 24.9 ± 1.5% ($n = 7$, $P < 0.001$ paired). The total actin content of stimulated PMN was nearly identical to unstimulated PMN, indicating insignificant loss of actin due to proteolysis.

More prolonged incubation with FMLP, as noted by others, was associated with a gradual decline in F-actin content, the percent actin associated with the Triton-insoluble cytoskeleton decreasing to ~50.0% after 5, 15, and 30 min stimulation with chemoattractant (see Fig. 4). This decrease in F-actin was not associated with a rise in actin bound to profilin, the percent actin bound to profilin remaining constant during this period at 3.2–3.1%. The decrease in F-actin was associated only with a significant rise in the unpolymerized actin pool not bound to profilin, which increased to 46% at all three time points.

To examine further the effects of FMLP receptor occu-

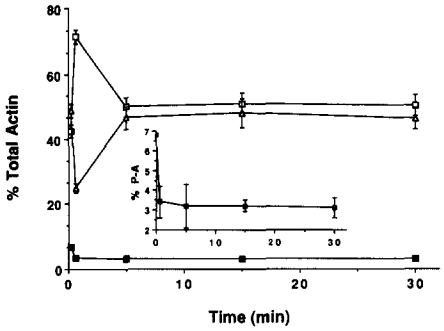

Figure 4. The effects of prolonged chemoattractant stimulation on the percentage of actin assembled into filaments and complexed to profilin. The same methods were used as described in Fig. 3. PMN were exposed to FMLP for the durations shown on the graph before the addition of Triton stop solution. Although F-actin content decreased with prolonged chemoattractant stimulation, the percent of actin bound to profilin remained low, whereas the percent of total actin in the unbound fraction increased.

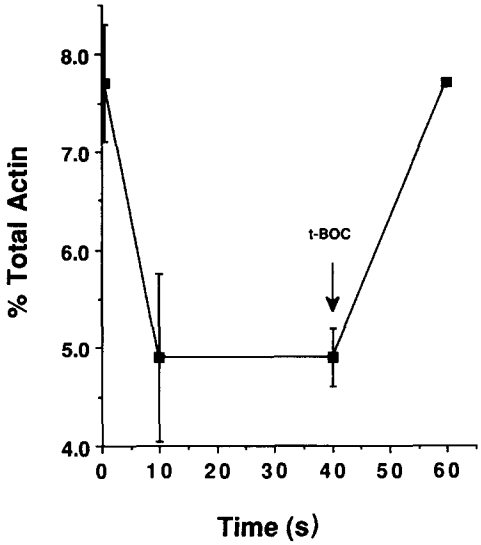

Figure 5. The effects of tBOC on profilin actin-complex formation. PMN were treated as described in Fig. 3 *A* except PMN were stimulate with a final concentration of 1×10^{-8} M for 40 s followed by the addition of final concentration of 1×10^{-4} M tBOC for 20 s. The percentage of actin bound to profilin at each time point is depicted on the graph. Addition of tBOC resulted in an increase in the percent of actin bound to profilin, which was comparable to unstimulated cells. Bars represent the SEM of four to seven experiments. The final point represents the mean of two experiments, values differing by only 0.3% of the total actin content.

pancy on the concentration of actin bound to profilin, t-BOC peptide was used to rapidly displace the chemotactic peptide. As observed by others, the addition of t-BOC was associated with a rapid depolymerization of PMN actin, F-actin content decreasing to resting values within 20 s (data not shown) (17, 37). Unlike the depolymerization associated with continued FMLP occupancy, the displacement of FMLP by t-BOC resulted in a rapid rise in the concentration of actin associated with profilin, values increasing to that of unstimulated PMNs within 20 s (see Fig. 5).

Removal of calcium ions from the media by addition of 1 mM EGTA rather than addition of calcium ions 10 min before stimulation with chemoattractant did not significantly affect the relative concentration of actin bound to profilin in unstimulated or stimulated PMN (data not shown).

Determination of Whole Cell Content of PMN Profilin and Actin

The number of PMN used in our experiments, 7.5×10^6, contained 1.12 ± 0.05 mg (SEM of $n = 6$ separate preparations of PMN) of total protein of which 222.9 ± 4.5 μg ($n = 52$) or 5.3 nmol was actin and 15.8 ± 0.5 μg ($n = 52$) or 1.0 nmol was profilin. Only 44% of profilin molecules were bound to actin in unstimulated PMN. Because the volume of our cell preparations was 500 μl, the concentration of actin in the Triton-soluble fraction was 6.1 μM in resting PMN and 3.2 μM in stimulated PMN; and the concentration of profilin 2 μM. If it was assumed that 100% of the profilin molecules bound actin monomers in resting PMN, profilin could bind to and prevent polymerization of 19–20% of the total actin or 33–35% of the unpolymerized actin in unstimulated PMN. Vigorous sonication of the Triton-insoluble fraction before centrifugation failed to increase the concentration of profilin in the Triton-soluble fraction. Repeated absorption with PLP beads also failed to increase the profilin yield.

Polymerization of Purified Skeletal Muscle Actin in Triton-solubilized PMN Extracts

High concentrations of actin remained in the supernatants of Triton-solubilized extracts of unstimulated PMN. To determine the apparent critical concentration (true critical concentration plus unpolymerized actin) of monomeric actin required to initiate assembly of the Triton-soluble PMN actin, increasing amounts of purified actin containing a low concentration of pyrene-labeled actin (6.6%) and physiologic concentrations of salt (0.138 M KCl, 2 mM $MgCl_2$) were added to the extracts. We found that the Triton buffer alone increased the critical concentration of purified actin from 0.2 μM to 0.37 ± 0.05 μM (SEM, $n = 7$), however when purified actin was combined with PMN extract, a 1 mg/ml concentration increased the concentration at which actin monomers began to polymerize to 5.8 μM. Higher concentrations of extract caused a proportionally greater increase in apparent critical concentration (see Fig. 6, *inset*). As shown in a representative experiment in Fig. 6, at extract concentrations of 1 mg/ml or higher in many of the experiments the slope of the line relating fluorescence (F-actin concentration) to the total starting actin concentration was flatter in extract, than in buffer alone. More prolonged incubation of the samples did not result in a change in the steady-state fluorescence of extract samples.

To determine to what extent profilin in PMN extracts contributed to the marked inhibition of actin assembly, extract was absorbed with an excess concentration of PLP beads to remove profilin. PLP treatment of extract was associated with a small reduction (~12% decrease) in the concentration at which actin began to assemble, reflecting the concentration of PMN actin removed as profilin-actin complex. (Example: 1 mg/ml untreated PMN extract, unpolymerized actin = 5.75 μM; after absorption with PLP beads the same concentration of extract, unpolymerized actin = 5.05 μM; actin polymerized in buffer, unpolymerized actin = 0.2 μm.)

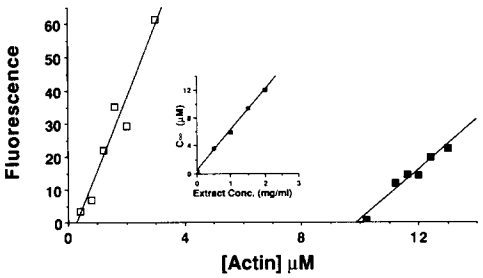

Figure 6. Determination of the concentration of unpolymerized actin (C_∞) in the Triton-soluble fractions from PMN. Increasing concentrations of purified rabbit skeletal muscle actin containing 6.6% pyrenyl actin were added to 1.8 mg/ml of Triton-soluble PMN extract (*closed squares*) prepared as described in the methods or to the Triton buffer solution alone (*open squares*). Samples were incubated at 25°C and fluorescence intensities read after 12 h. Previous experiments revealed that fluorescence intensity values reach a steady state by 6 h. The buffer contained final concentrations of 0.138 M KCl and 2 mM $MgCl_2$. For clarity, the G-actin fluorescence intensities have not been included. The critical concentration of actin polymerized in the Triton buffer was 0.4 μM, whereas the concentration of unpolymerized actin in the extract was nearly 10 μM. The insert shows the effect of increasing concentrations of extract on the concentration of unpolymerized actin (C_∞). The slope of the line is 5.8 μM/mg/ml extract.

To exclude the possibility that the Triton-soluble PMN actin had been denatured, the Triton-soluble fraction from PMN was subjected to DNase I chromatography followed by elution with formamide and dialysis (see Materials and Methods). As compared with the native actin in a 1 mg/ml PMN extract, apparent critical concentration = 5.8, purified PMN actin had a critical concentration of 0.6 μM (two separate purifications).

Discussion

Profilin is thought to play a major role in maintaining the high concentrations of monomeric actin in unstimulated cells and in regulating the assembly of these monomers into filaments. For profilin to fulfill these roles in the PMN, the profilin–actin complex must dissociate at the same time as F-actin content is increasing and secondly, profilin must be present in sufficient quantities to bind the high concentrations of monomers found in the resting PMN. Our investigations indicate that PMN profilin fulfills the first but not the second of these requirements.

Methods similar to those used by Lind et al. (25) were used to examine profilin function in human PMN. Data were analyzed by defining three pools of actin. The first pool consisted of actin associated with the Triton-insoluble cytoskeleton. This actin was assumed to be primarily F-actin. The other two pools of actin were derived from the Triton-soluble fraction. The profilin–actin complex (PA complex) pool was measured by absorbing the Triton-soluble fraction with PLP beads followed by extensive washing. Profilin–actin complex as measured by our methods represented a high-affinity complex since the complex remained intact after incubation for 7 min in very dilute solutions (see Materials and Methods). The final pool of unpolymerized actin not bound to profilin was defined as the percentage of the total actin absorbed by DNase I-beads incubated in the PMN Triton-soluble solution previously absorbed with PLP beads plus any 42,000-D peptide (generally <3% of the total actin, see Fig. 3 *B*, lanes 7 and 8) remaining in the final supernatant.

In unstimulated granulocytes the highest percentage of total actin was found in the third pool, unpolymerized actin not bound to profilin, whereas 30–40% was associated with the Triton-insoluble cytoskeleton and only 6.8% was represented by high affinity PA complex. Unstimulated PMN contained the highest observed concentrations of PA complex, 44% of the profilin molecules being complexed with actin. After stimulation with chemoattractant the relative concentration of actin bound to profilin decreased to 3.4 and only 27% of the profilin molecules were bound to actin. The relative PA-complex concentration remained low with continued stimulation. However if FMLP was displaced by t-BOC, PA complex rapidly increased to unstimulated values, indicating that chemoattractant receptor occupancy was required to maintain the dissociation of actin from profilin.

Our findings were similar to platelet studies of Markey et al. (29), but differed from those of Lind et al. (25) who found very low concentrations of PA complex (only 3–10% of profilin was complexed to actin) in unstimulated platelets. Upon thrombin stimulation Lind et al. observed a rapid increase in high-affinity complex (70% of profilin bound to actin) which with continued thrombin stimulation was then followed by a second decline in PA complex concentration. There are several possible reasons for the differences between Lind et al. and our findings: (*a*) Variations in buffer conditions could have altered PA complex concentration; (*b*) PMN may not have been in a true resting state; and (*c*) Platelet and PMN profilin function differently.

Our buffers and Triton solution were nearly identical to those used by Lind et al. with the exception that platelets were incubated in plasma in some experiments. Since serum and plasma contain vitamin D binding protein which can dissociate actin monomers from profilin (30), this condition could falsely lower the concentration of actin bound to profilin (see Materials and Methods) and might explain the low resting concentrations of profilin–actin complex in some of their experiments. We performed all of our experiments in serum free buffers. To address the second possibility that PMN were not in a true resting state, PMN were purified using minimal perturbation and prolonged incubation on ice to minimize inadvertent stimulation. Also PMN were purified in lipopolysaccharide-free media to eliminate inadvertent priming. Under these conditions high-affinity PA complex concentrations were nearly identical to Ficoll/Hypaque-purified PMN. In addition, extracts derived from human PMN stored in the cold and subjected to PLP affinity chromatography and gel filtration, yielded primarily profilin complexed to actin. Only by using NEM treatment, a chemical that binds to cysteine 374 in actin and weakens profilin's affinity for actin (20, 27), were we able to purify higher concentrations of free profilin. Finally as discussed above, when FMLP was displaced from its PMN membrane receptors, an increase in actin associated with profilin was observed. These findings all suggest that PA complex concentration is maximal in unstimulated PMN.

Although PMN profilin–actin complexes dissociated at the appropriate time during stimulation, a condition that would be expected to enhance actin filament assembly, the concentration of profilin present in human PMN relative to the total

actin concentration (molar ratio profilin to actin 1:5.3) was too low to account for a major portion of the actin found in the Triton-soluble fraction, both before and after stimulation. A similarly low molar ratio of profilin to actin (1:6) was also recently reported in platelets (25). In PMN there was sufficient PA complex to account for only 12–14% of the actin found in the Triton-soluble fraction of unstimulated as well as PMN stimulated with chemoattractant (assuming PLP beads bound all the profilin molecules, see below). If the contribution of low-affinity profilin–actin complex were included in our calculations, assuming a K_D of 1 μM (see Fig. 2), these complexes could account for only an additional 2.5–5% of the unpolymerized actin. If it were assumed that each molecule of profilin bound an actin molecule in unstimulated PMN (i.e., the percent of profilin bound to actin with high affinity was 100%, rather than 44%), actin bound to profilin could still only account for a minority (approximately one-third) of the Triton soluble pool. However, in this last circumstance, the profilin–actin pool could theoretically contribute nearly half of the actin monomers for chemoattractant stimulated actin filament assembly.

Addition of purified actin to the Triton-soluble PMN extract also suggested that profilin alone could not account for the high concentrations of unpolymerized PMN actin. The apparent critical concentration under the conditions of our experiments, physiologic salt and low Ca^{2+} was 5.8 μM for each mg/ml concentration of extract. Preabsorption of extract with PLP beads had only a small affect on this value, decreasing the concentration of unpolymerized actin by only a small fraction (12%). Purification of polymerization competent actin from Triton-soluble extract excluded the possibility that PMN actin had been denatured during extract preparation.

It is possible that our methods underestimated the true profilin content; however, attempts to increase the yield of profilin by repeated absorption with PLP beads, by initially mixing supernatant with DNase I beads to enhance recovery of profilin–actin complexes, and by vigorously sonicating the samples in Triton before addition of PLP beads all failed to increase the profilin yield. It remains possible that there is a large population of profilin in PMN that fails to bind to PLP beads. If, however, it is assumed this affinity method binds the majority of PMN profilin molecules, our findings suggest the presence of an additional activity or activities that serve to inhibit actin filament assembly in EGTA treated PMN cytoplasmic extracts. A number of such activities have recently been discovered in other nonmuscle cells including cofilin (31), actobindin (20) and actin-depolymerizing factor (2, 3, 26). Actin phosphorylation could also play a role in maintaining the high concentrations of unpolymerized actin found in unstimulated PMN (38).

Although profilin may not entirely account for the large generalized shifts from unpolymerized to filamentous actin seen when PMN are maximally stimulated with chemoattractant, this protein does release actin at the appropriate time during stimulation and therefore may serve to regulate localized assembly of cytoplasmic actin. Profilin has been shown in artificial systems to bind tightly to lipid (24). It has recently been shown that this protein is more highly concentrated at the cytoplasm–membrane interface (15) and therefore could play a major role in regulating actin assembly at the leading edge of the cytoplasm during pseudopod and lamellipodia formation.

We would like to thank Mary Schloff and Edwin Smith for their technical assistance, Dr. J. R. Forehand for performing PMN superoxide studies, and Dr. Paul Janmey for his advice concerning the profilin assay. In addition we appreciated the helpful advice from Drs. Annemarie Weber and Sally Zigmond.

These studies were funded by National Institute of Health grant RO1 AI123262 and the Robert Wood Johnson Foundation.

Received for publication 10 October 1989 and in revised form 16 January 1990.

References

1. Amrein, P. C., and T. P. Stossel. 1980. Prevention of proteolysis of contractile proteins in human polymorphonuclear leukocytes by diisopropylfluorophosphate. *Blood.* 56:442–447.
2. Bamburg, J. R., and D. Bray. 1987. Distribution and cellular localization of actin depolymerizing factor. *J. Cell Biol.* 105:2817–2825.
3. Bamburg, J. R., H. E. Harris, and A. G. Weeds. 1980. Partial purification and characterization of an actin depolymerizing factor from brain. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 121:178–182.
4. Blikstad, I., I. Sundkvist, and S. Eriksson. 1980. Isolation and characterization of profilactin and profilin from calf thymus and brain. *Eur. J. Biochem.* 105:425–433.
5. Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.
6. Deleted in proof.
7. Carlsson, L., L.-E. Nystrom, I. Sundkvist, F. Markey, and U. Lindberg. 1977. Actin polymerizability is influenced by profilin, a low molecular weight protein in non-muscle cells. *J. Mol. Biol.* 115:465–483.
8. Carson, M., A. Weber, and S. H. Zigmond. 1986. An actin-nucleating activity in polymorphonuclear leukocytes is modulated by chemotactic peptides. *J. Cell Biol.* 103:2707–2714.
9. Chaponnier, C., H. L. Yin, and T. P. Stossel. 1987. Reversibility of gelsolin:actin interaction in macrophages: evidence for Ca^{2+}-dependent and Ca^{2+}-independent pathways. *J. Exp. Med.* 165:97–106.
10. DiNubile, M. J., and F. S. Southwick. 1985. Effects of macrophage profilin on actin in the presence and absence of acumentin and gelsolin, proteins which block the opposite ends of actin filaments. *J. Biol. Chem.* 260: 7402–7409.
11. Fechheimer, M., and S. H. Zigmond. 1983. Changes in cytoskeletal proteins of polymorphonuclear leukocytes induced by chemotactic peptides. *Cell Motil.* 3:349–361.
12. Forehand, J. R., M. J. Pabst, W. A. Phillips, and R. B. Johnston, Jr. 1989. Lipopolysaccharide priming of human neutrophils for an enhanced respiratory burst. *J. Clin. Invest.* 83:74–83.
13. Guthrie, L. A., L. C. McPhail, P. M. Henson, and R. B. Johnston, Jr. 1984. Priming of neutrophils for enhanced release of oxygen metabolites by bacterial lipopolysaccharide. *J. Exp. Med.* 160:1656–1671.
14. Harris, H. E., and A. G. Weeds. 1978. Platelet actin: sub-cellular distribution and association with profilin. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 90:84–88.
15. Hartwig, J. H., K. A. Chambers, K. L. Hopcia, and D. J. Kwiatkowski. 1989. Association of profilin with filament-free regions of human leukocyte and platelet membranes and reversible membrane binding during platelet activation. *J. Cell Biol.* 109:1571–1579.
16. Howard, T. H., and C. O. Oresajo. 1985. The kinetics of chemotactic peptide-induced change in F-actin content, F-actin distribution and the shape of neutrophils. *J. Cell Biol.* 101:1078–1085.
17. Howard, T. H., and C. O. Oresajo. 1985. A method for quantifying F-actin in chemotactic peptide activated neutrophils:study of the effect of tBOC peptide. *Cell Motil.* 5:545–557.
18. Kwiatkowski, D. J., and G. A. P. Brune. 1988. Human profilin. *J. Biol. Chem.* 263:5910–5915.
19. Lal, A. A., and E. D. Korn. 1985. Reinvestigation of the inhibition of actin polymerization by profilin. *J. Biol. Chem.* 260:10132–10138.
20. Lambooy, P. K., and E. D. Korn. 1986. Purification and characterization of actobindin, a new actin monomer-binding protein from *Acanthamoeba castellanii*. *J. Biol. Chem.* 261:17150–17155.
21. Lane, E. 1957. Spectrophotometric and turbidimetric methods for measuring proteins. *Methods Enzymol.* 3:447–454.
22. Larsson, H., and U. Lindberg. 1988. The effect of divalent cations on the interaction between calf spleen profilin and different actins. *Biochim. Biophys. Acta.* 953:95–105.
23. Lassing, I., and U. Lindberg. 1985. Specific interaction between phosphatidylinositol 4,5-bisphosphate and profilactin. *Nature (Lond.).* 314:472–474.
24. Lassing, I., and U. Lindberg. 1988. Evidence that the phosphatidylinositol cycle is linked to cell motility. *Exp. Cell Res.* 174:1–15.
25. Lind, S. E., P. A. Janmey, C. Chaponnier, T. J. Herbert, and T. P. Stossel. 1987. Reversible binding of actin to gelsolin and profilin in human platelet extracts. *J. Cell Biol.* 105:833–842.
26. Makekawa, S., E. Nishida, Y. Ohta, and H. Sakai. 1984. Isolation of low

molecular weight actin-binding proteins from porcine brain. *J. Biochem. (Tokyo).* 95:377–385.
27. Malm, B. 1984. Chemical modification of Cys-374 of actin interferes with the formation of the profilactin complex. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 173:399–402.
28. Markey, F., U. Lindberg, and L. Eriksson. 1978. Human platelets contain profilin, a potential regulator of actin polymerisability. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 88:75–79.
29. Markey, F., T. Persson, and U. Lindberg. 1981. Characterization of platelet extracts before and after stimulation with respect to the possible role of profilactin as microfilament precursor. *Cell.* 23:145–153.
30. McLeod, J. F., M. A. Kowalski, and J. G. Haddad, Jr. 1989. Interactions among serum vitamin D binding protein, monomeric actin, profilin, profilactin. *J. Biol. Chem.* 264:1260–1267.
31. Nishida, E., S. Maekawa, and H. Sakai. 1984. Cofilin, a protein in porcine brain that binds to actin filaments and inhibits their interactions with myosin and tropomyosin. *Biochemistry.* 23:5307–5313.
32. Northrop, J., A. Weber, M. S. Mooseker, C. Armstrong, M. F. Bishop, G. R. Dubyak, M. Tucker, and T. P. Walsh. 1986. Different calcium dependence of the capping and cutting activities of villin. *J. Biol. Chem.* 261:9274–9281.
33. O'Farrell, P. H. 1974. High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.* 250:4007–4021.
34. Ozaki, K., H. Sugino, T. Hasegawa, S. Takahashi, S. Hatano. 1983. Isolation and characterization of *Physarum* profilin. *J. Biochem. (Tokyo).* 93:295–298.
35. Reichstein, E., and E. D. Korn. 1979. *Acanthamoeba* profilin. *J. Biol. Chem.* 254:6174–6179.
36. Shalit, M., G. A. Dabiri, and F. S. Southwick. 1987. Platelet-activating factor both stimulates and "primes" human polymorphonuclear leukocyte actin filament assembly. *Blood.* 70:1921–1927.
37. Sklar, L. A., G. M. Omann, and R. G. Painter. 1985. Relationship of actin polymerization and depolymerization to light scattering in human neutrophils: dependence on receptor occupancy and intracellular Ca^{++}. *J. Cell Biol.* 101:1161–1166.
38. Sonobe, S., S. Takahashi, S. Hatano, and K. Kuroda. 1986. Phosphorylation of Amoeba G-actin and its effect on actin polymerization. *J. Biol. Chem.* 261:14837–14843.
39. Southwick, F. S., and T. P. Stossel. 1981. Isolation of an inhibitor of actin polymerization from human polymorphonuclear leukocytes. *J. Biol. Chem.* 256:3030–3036.
40. Southwick, F. S., G. A. Dabiri, M. Paschetto, and S. H. Zigmond. 1989. Polymorphonuclear leukocyte adherence induces actin polymerization by a transduction pathway which differs from that used by chemoattractants. *J. Cell Biol.* 109:1561–1569.
41. Spudich, J. A., and S. Watt. 1971. The regulation of rabbit muscle contraction: biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. *J. Biol. Chem.* 246:4866–4871.
42. Tanaka, M., and H. Shibata. 1985. Poly(L-proline)-binding proteins from chick embryos are a profilin and a profilactin. *Eur. J. Biochem.* 151: 291–297.
43. Tseng, P. C-H., and T. D. Pollard. 1982. Mechanism of action of *Acanthamoeba* profilin: demonstration of actin species specificity and regulation by micromolar concentrations of $MgCl_2$. *J. Cell Biol.* 94:213–218.
44. Tseng, P. C-H., M. S. Runge, J. A. Cooper, R. C. Williams Jr., and T. D. Pollard. 1984. Physical, immunochemical, and functional properties of *Acanthamoeba* profilin. *J. Cell Biol.* 98:214–221.
45. Wallace, P. J., R. P. Westo, C. H. Packman, and M. A. Lichtman. 1984. Chemotactic peptide-induced changes in neutrophil actin conformation. *J. Cell Biol.* 99:1060–1065.
46. White, J. R., P. H. Naccache, and R. I. Sha'afi. 1983. Stimulation by chemotactic factor of actin association with the cytoskeleton in rabbit neutrophils. *J. Biol. Chem.* 258:14041–14047.
47. Zechel, K. 1980. Isolation of polymerization-competent cytoplasmic actin by affinity chromatography on immobilized DNAse I using formamide as eluant. *Eur. J. Biochem.* 110:343–348.