Chemoattractant-stimulated Polymorphonuclear Leukocytes Contain Two Populations of Actin Filaments that Differ in Their Spatial Distributions and Relative Stabilities

Lynne Cassimeris, Helen McNeill,* and Sally H. Zigmond
Biology Department and *Physiology Department, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6018

Abstract. Chemoattractants stimulate actin polymerization in lamellipodia of polymorphonuclear leukocytes. We find that removal of chemoattractant results in rapid (within 10 s at 37°C) and selective depolymerization of the F-actin located in lamellipodia. Addition of 10 μM cytochalasin B, in the presence of chemoattractant, also resulted in rapid and selective depolymerization of lamellar F-actin. The elevated F-actin level induced by chemoattractant rapidly returns to the level present in unstimulated cells after (a) a 10-fold decrease in chemoattractant concentration; (b) the addition of 10 μM cytochalasin B; or (c) cooling to 4°C. The F-actin levels of unstimulated cells are only slightly affected by these treatments. Based on the similar effects of cytochalasin addition and chemoattractant dilution, it is likely that both treatments result in actin depolymerization from the pointed ends of filaments.

Based on our results we propose that chemoattractant-stimulated polymorphonuclear leukocytes contain two distinct populations of actin filaments. The actin filaments within the lamellipodia are highly labile and in the continued presence of chemoattractant these filaments are rapidly turning over, continually polymerizing at their plus (barbed) ends, and depolymerizing at their minus ends. In contrast, the cortical F-actin filaments of both stimulated and unstimulated cells are differentially stable.

The formation and retraction of lamellipodia is critical for the directional migration of polymorphonuclear leukocytes (PMNs).1 A PMN orients its locomotion along a gradient of a chemoattractant by preferentially extending lamellipodia in the direction of the higher concentration of chemoattractant (Zigmond, 1974; Zigmond et al., 1981). A cell moving perpendicular to the direction of the gradient has a high probability of making its next turn up the gradient. To turn, a cell extends a lamellipodium up the gradient and retracts lamellipodia extending down the gradient. These changes occur rapidly as the PMN translocates at rates of ∼10 μm/min.

In many systems the formation of lamellipodia or filopodia correlates spatially and temporally with actin polymerization (Tilney, 1973; Fox and Phillips, 1981; Fechheimer and Zigmond, 1983; Hall et al., 1988). In PMN, lamellipodia formation and increased actin polymerization occur concomitantly upon exposure of cells to a rapid increase in the concentration of chemoattractant (Zigmond and Sullivan, 1979; Fechheimer and Zigmond, 1983; Hall et al., 1988; Howard and Oresajo, 1985a; Rao and Varani, 1983). A resting PMN has ∼30% of its actin in the filamentous form. Within 30 s after addition of chemoattractant, the F-actin level doubles with the newly assembled actin localized in the newly extended lamellipodia. Actin assembly is required for pseudopod extension: the presence of cytochalasin, which in vitro blocks the plus (high affinity or barbed) end of the actin filament, inhibits both pseudopod extension and actin polymerization.

Continued presence of chemoattractant is necessary for continued lamellipodia extension and actin polymerization. Removal of chemoattractants causes lamellipodia withdrawal (Zigmond et al., 1981; Zigmond and Sullivan, 1979) and a decrease in F-actin (Fechheimer and Zigmond, 1983; Sklar et al., 1985; Howard and Oresajo, 1985b). These studies did not determine the location of F-actin depolymerization and could not determine whether chemoattractant removal stimulates further actin depolymerization or merely inhibits actin polymerization.

In this paper, we show that removal of chemoattractant results in rapid depolymerization of F-actin, and that actin depolymerization occurs selectively in the lamellipodia. Cortical F-actin and F-actin in the tail remain under these conditions. The addition of cytochalasin B also results in rapid F-actin depolymerization, with morphological characteristics similar to those observed after chemoattractant removal. The combination of cytochalasin addition and chemoattractant removal does not cause a greater loss of F-actin than that maximally lost after either treatment alone.

1. Abbreviations used in this paper: FNLLP, formyl-norleucyl-lysyl-l-leucyl-phenylalanine; mHBSS, Hank's balanced salt solution without bicarbonate and with 10 mM Hepes, pH 7.2; PMN, polymorphonuclear leukocyte.
We propose that the presence of chemoattractant allows the persistence of elevated F-actin levels by maintaining free F-actin plus ends that are actively elongating. At steady state there must be equal depolymerization from the pointed ends of filaments. Based on the cytochalasin results, we suggest that when chemoattractant is removed, the plus ends become capped and the F-actin in the lamellipodia rapidly depolymerizes from the minus end. The rapid polymerization and depolymerization of actin filaments within lamellipodia allow these organelles to be highly responsive to changes in concentrations of chemoattractants.

Materials and Methods

Materials

Rhodamine phalloidin (TRITC labeled), cytochalasin B, N-formylnor-leucylleucylphenylalanine (FNLLP), NP-40, and Hepes were all obtained from Sigma Chemical Co. (St. Louis, MO). Glutaraldehyde (8% solution) was from Polysciences, Inc. (Warrington, PA). HBSS was obtained from Grand Island Biological Co., Grand Island, NY.

Morphological Studies

A drop of blood from a finger prick was allowed to clot on a coverslip incubated at 37°C in a moist chamber. After 45 min the retracted clot was rinsed off with saline leaving a monolayer of primarily PMN with a few monocytes. These attached cells were incubated in a drop of 10^8 M FNLLP, in modified Hank's Balanced Salt Solution (Hank's without bicarbonate and with 10 mM Hepes, pH 7.2, mHBSS) 10-30 min at 37°C. The coverslips were then immerssed 0-30 s in beakers of 37°C mHBSS before fixing with 1% glutaraldehyde in mHBSS. Cells were also incubated in 1 or 10 µM cytochalasin B, either in the continued presence of chemoattractant or simultaneously with chemoattractant dilution. Cells were permeabilized with 1% NP-40 and stained with 2 × 10^-7 M rhodamine phalloidin in mHBSS for 1 h. The coverslips were mounted on slides and the cells were examined and photographed using a 100×/1.25 NA objective on a microscope equipped for epifluorescence (Carl Zeiss. Inc., Thornwood, NY). For quantitation, cells were examined using a 100×/1.4NA planapo objective (Carl Zeiss, Inc.) on a microscope equipped for epifluorescence (Olympus Corporation of America, New Hyde Park, NY). Images were projected to a SIT video camera (Dage MTI 65) set for manual gain and offset. Images were acquired by summing 32 video frames using an Image-1/AT image processor (Interactive Video Systems, Inc., Concord, MA). The Image-1/AT system was used to measure the staining intensity at three locations within each cell. For each cell the intensity within the lamella, the body of the cell, and the tail was measured by summing the intensity within a 7 × 7 pixel area. The brightest areas of the lamella, the body of the cell, and the tail were selected for measurement for each cell. Approximately 45-70 cells were analyzed at each time point for each experiment. For each time point, images were collected from 3-4 regions of the coverslip. Cells were excluded from analysis if they overlapped other cells or if they were polarized perpendicular to the coverslip. To pool data from several experiments the data was normalized by setting the mean intensity of control lamella equal to 100.

Scanning Electron Microscopy

Cells were fixed in 1% glutaraldehyde in mHBSS for at least 30 min, washed with 50 mM phosphate buffer, pH 6.2 for 5 min at room temperature, postfixed in 1% OsO₄ in phosphate buffer, pH 6.2, dehydrated in a graded series of acetone (50, 70, 90, 100, 100%), 10 min in each, critical point dried, mounted on stubs, and sputter coated. Cells were examined and photographed with a scanning electron microscope (model 1000; Amray, Inc., Bedford, MA).

Quantitative Measure of F-Actin in Cell Populations

F-actin was quantitated by a rhodamine phalloidin--binding assay originally developed by Howard and Oresajo (1985a). Rabbit peritoneal exudate PMNs obtained as described previously (Zigmond and Sullivan, 1979) were suspended (4 × 10⁶ cells/ml) in mHBSS (without calcium) at room temperature. Typically, 100 µl of cells was mixed with 100 µl of 4 × 10⁻⁸ M FNLLP for 90 s at room temperature before the cells were either fixed by addition of 8% glutaraldehyde to yield a final concentration of 1% glutaraldehyde, or were diluted with a 2-10-fold (1.8 µl) vol of mHBSS without chemoattractant for 15, 30, or 60 s before fixation. Controls were diluted to the same final volume with mHBSS containing FNLLP. Cells were permeabilized by addition of NP-40 (final concentration of 1%) and rhodamine phalloidin was added (final concentration 2 × 10⁻⁹ M). Non-saturable binding of the rhodamine phalloidin was determined by incubating samples in rhodamine phalloidin plus an excess of unlabeled phalloidin (2 × 10⁻⁸ M) (Sigma Chemical Co.). The cells were stained for at least 1 h before pelleting at 2,000 rpm for 9 min. The supernate was discarded, the rhodamine phalloidin in the pellet was extracted with 1 ml of methanol, and the rhodamine fluorescence was measured at 540(ex)/575(em) in a fluorimeter (model LS-5; Perkin Elmer Corp. Instrument Div., Norwalk, CT). The data are presented as "phalloidin bound" which is the fluorescence reading, in arbitrary units, after subtraction of the fluorescence due to unsaturable binding (usually ñ25% of the fluorescence reading of stimulated cells).

Results

Chemotactant Removal Results in Altered Cell Morphology and Selective Loss of F-Actin Staining in Lamellipodia

Cell Morphology after Chemotactant Removal. More than 80% of the cells incubated in chemotactant had a polarized morphology with a leading ruffling lamellipod and a knob-like tail (Fig. 1 a). These lamellipodia exclude cytoplasmic granules (Fig. 1 a, arrowhead). About 50% of the lamellipodia contained phase-dense regions that project radially to the edge of the lamellipodia (Fig. 1 a, long arrows). By shifting the plane of focus, these densities can be recognized as lamellipodia extending perpendicular to the substrate. The three-dimensional nature of lamellipodia is apparent in scanning electron micrographs (Fig. 2 a).

Within 3-5 s after chemotactant removal, the granule-free lamellipodia were shorter (Fig. 1 b). By 10 s few granule-free lamellipodia were present (Fig. 1 e, small row) and there were no radial phase dense projections. When viewed by scanning microscopy, the lamellipodia appeared collapsed onto the body of the cell (Fig. 2 b). By 30 s the cells had multiple blebs from their surface (not shown).

F-Actin Localization after Chemotactant Removal.

The majority of cells (~95%) fixed in the presence of chemotactant contained lamella that stained brightly with rhodamine phalloidin (phalloidin binds specifically to F-actin; Cooper, 1987) (Fig. 1 b). Both the thin regions of lamellipodia, and particularly the phase dense radial projections in the lamellae, stained brightly (Fig. 1 b). Bright staining was also present in both the cortex and in the tails of many cells. The rhodamine staining was due to a saturable binding of phalloidin (to F-actin) since it could be effectively dis-

Figure 1. Cell morphology and actin distribution in human PMNs fixed at various time points after chemotactant removal at 37°C. Phase contrast (a, c, e) and fluorescence (b, d, f) micrographs of cells incubated 15-20 min in 2 × 10⁻⁸ M FNLLP and fixed at 0 (a, b), 3 (c, d), or 10 s (e, f) after removal of FNLLP. Phase contrast (g) and fluorescence (h) micrographs of cells incubated as in a and then stained with 2 × 10⁻⁸ M rhodamine phalloidin and 2 × 10⁻⁹ M unlabeled phalloidin. Bar, 10 µm.
Cassimeris et al. Two Populations of Actin Filaments in PMN

[Images of cells with actin filaments highlighted]
be retraction fibers that had collected on the surface of the tail (Fig. 2, a and b). Cortical staining appeared similar to cells before dilution of chemoattractant.

The micrographs suggested that F-actin selectively depolymerized from the lamella. We confirmed this qualitative impression by quantifying the rhodamine phalloidin fluorescence intensity in three locations within each cell (see Materials and Methods). As shown in Fig. 3, lamellar staining decreased after chemoattractant removal. In contrast, the bodies and tails of the cells showed little change in fluorescent staining. The slight increase in body and tail staining may be caused by the rounding of cells observed after FNLP removal.

Localized Actin Depolymerization Occurs in Rabbit and Human PMNs and Is Not Altered by Adhesion to a Substrate. Rabbit and human PMNs showed similar changes in F-actin-staining intensity after chemoattractant removal. Cells from both sources showed maximal loss of lamellar F-actin staining 10 s after peptide removal at 37°C, and 30 s after peptide removal at 23°C (not shown).

Examination of cells on coverslips allows convenient analysis of changes in cell morphology and actin localization, but cell adhesion to a substrate can promote actin polymerization, apparently through a separate pathway (Southwick et al., 1989). To examine specifically the effects of chemoattractant dilution, the above experiments were repeated with rabbit cells in suspension. Cells were stimulated for 90 s at room temperature and then diluted 10-fold (2 × 10⁶ M–2 × 10⁹ M FNLLP). Both the time course and selective depolymerization of F-actin were qualitatively indistinguishable from those observed with adherent cells (data not shown). Again, at room temperature, maximal loss of lamellar staining occurred 30 s after peptide dilution.

Dose Dependence of F-Actin Depolymerization after Chemoattractant Dilution. The above results suggested that F-actin was depolymerizing and not merely rearranging after peptide removal. We examined this issue further by quantitating F-actin levels after peptide dilution using an as-

Figure 2. Scanning electron micrographs of human PMNs incubated in chemoattractant and (a) fixed without FNLP dilution; (b) 10 s after chemoattractant removal; and (c) 10 s after addition of 10 μM cytochalasin B. Bar, 1 μm.

placed by inclusion of a 10-fold excess of unlabeled phalloidin (Fig. 1, g and h).

Upon removal of chemoattractant, the rhodamine phalloidin staining of the lamellipodia decreased in brightness. The staining continued to extend to the edge of the lamellipodia, but this brightly staining region was often narrower (Fig. 1 d). By 10 s few cells retained brightly staining lamella (Fig. 1 f). By 30 s bright staining of the cell front was virtually nonexistent (not shown).

During the same time that the lamellar staining was decreasing, staining of the cortex and tail remained. The rhodamine phalloidin staining of the tail occurred both in the constriction at the base of the tail and on what appeared to
The percent saturable staining of rhodamine phalloidin fluorescence staining after dilution of chemoattractant. (a) Time course. The percent saturable staining of rhodamine phalloidin (relative to that present in cells fixed after 90 s in 2 × 10^{-8} M FNLLP at room temperature) was determined in cells fixed at 15, 30, or 60 s (at room temperature) after a 10-fold dilution of FNLLP as described in Materials and Methods. The data plotted are the mean rhodamine fluorescence ± SEM of seven experiments, each experiment with duplicate determinations. The filled symbol shows the staining of cells before stimulation.

Addition of 10 μM cytochalasin B, in the continued presence of chemoattractant, caused a rapid loss of rhodamine phalloidin staining of the lamella (Figs. 5 and 6). After 10 s some cortical staining remained and a few bright spikes persisted at the cell front (Fig. 5f). As shown in Fig. 6, incubation in cytochalasin B resulted in selective depolymerization of lamellar F-actin. The staining intensity within the bodies and tails of the cells showed little change in staining intensity over this time course. Cells fixed after a 10-s treatment with a combination of chemoattractant removal and cytochalasin B addition appeared similar to cells fixed at 10 s after either treatment alone (compare figs. 1, c and f, 2, b and c, 5, c and g with 5, d and h).

Addition of Cytochalasin B Results in Actin Depolymerization in Stimulated Cells, but not in Unstimulated Cells. Addition of 10 μM cytochalasin B to cells incubated for 90 s in chemoattractant caused a rapid depolymerization of F-actin to basal levels within 30 s (Fig. 7a). The addition of cytochalasin B to unstimulated cells had little effect on their F-actin levels over this same time course. The maximal extent of depolymerization observed by either a 10-fold chemoattractant dilution or addition of 10 (or 20) μM cytochalasin B was similar to that observed with a combination of both treatments (Fig. 7a). Each treatment resulted in depolymerization of chemoattractant-stimulated F-actin back to approximately the level of unstimulated cells (Fig. 7a). Combinations of suboptimal dilutions of chemoattractant, e.g., threefold, and suboptimal concentrations of cytochalasin B, e.g., 1 μM, resulted in a slightly faster depolymerization compared to either treatment alone. The final level of actin polymer decreased to approximately the level in unstimulated cells (Fig. 7b). In addition, the presence of cytochalasin inhibited actin repolymerization observed between 30 and 60 s after chemoattractant dilution (Figs. 4a and 7a).

The effects of cytochalasin B were not due to its blockage of glucose transport since dihydroyctochalasin B, which does not block glucose transport, gave identical results (data not shown). Furthermore, no loss of lamellar staining was seen in cells switched from medium containing 1 mg/ml glucose to glucose-free medium.

We also examined the effects of cytochalasin B in amounts substoichiometric relative to actin. In these experiments 100 μl of 1.8 × 10^6 cells (~2.3 × 10^{-7} mol actin) was incubated in 3 μM cytochalasin B (~3 × 10^{-4} mol cytochalasin B). Cells were fixed 30 s later and total F-actin levels determined for unstimulated cells, stimulated cells, and stimulated cells plus cytochalasin B. Again, actin levels in stimulated cells treated with cytochalasin B decreased to the level in unstimulated cells (not shown).
Cassimeris et al. Two Populations of Actin Filaments in PMN

Figure 6. Changes in relative rhodamine phalloidin staining intensity after incubation in 10 μM cytochalasin B with and without chemoattractant dilution. Cells were fixed and stained as in Fig. 5. The intensity of rhodamine phalloidin staining was measured in 7 × 7 pixel areas in the lamella (L), the body of the cell (B), and the tail (T). Plot of a representative experiment of relative fluorescence intensity ± SEM (see Materials and Methods). Approximately 50 cells were analyzed at each time point.

Chemotactant-stimulated F-Actin Has Increased Sensitivity to Low Temperature. To determine if the F-actin induced by chemoattractant was also selectively sensitive to cold, cells incubated in 2 × 10⁻⁸ M FNLLP for 90 s were mixed with a 20-fold vol of 2 × 10⁻⁸ M FNLLP at room temperature or at 4°C. Cells were fixed after 0.3, 1, or 3 min. Unstimulated cells were incubated in mHBSS either at room temperature or at 4°C and fixed at the various time points. As shown in Fig. 8, the chemoattractant-stimulated F-actin decreased upon incubation at 4°C while the F-actin in unstimulated cells was stable. In both stimulated and unstimulated cells, the F-actin levels were stable at 25°C for 3 min.

Discussion

In this paper we show that removal of chemoattractant results in a very rapid loss of F-actin selectively from lamellipodia (Figs. 1 and 3). The level of F-actin rapidly decreases (within 10 s at 37°C) to approximately that present in unstimulated cells (Fig. 4, a and b). Our demonstration that continued presence of chemoattractant is required to maintain elevated levels of F-actin is consistent with previous reports that F-actin levels decrease rapidly in human PMN upon addition of a chemoattractant antagonist (Sklar et al., 1985; Howard and Oresajo, 1985b).

We also found that 2-10-fold dilutions of chemoattractant rapidly decreased the level of F-actin (see Figs. 4 a and b). When the concentration of chemoattractant present after dilution was sufficient to stimulate actin polymerization (e.g., 2 × 10⁻⁸ M FNLLP) the level of F-actin 60 s after peptide dilution (at room temperature) was frequently greater than that present after 30 s. The depolymerization and repolymerization of F-actin after 2-10-fold dilutions of FNLLP correlate with observations of cell behavior under similar conditions. Zigmond and Sullivan (1979) observed that upon FNLLP dilution, previously locomoting cells transiently ceased locomotion, formed blebs over their surface, and then recovered and resumed locomotion (see also Deverotes and Zigmond, 1988). This recovery apparently requires repolymerization, and thus like the initial actin polymerization (White et al., 1983; Howard and Oresajo, 1985b), can be inhibited by cytochalasin.

We also observed rapid depolymerization of F-actin after incubation in cytochalasin B (Figs. 5-7). As with chemoattractant removal, cytochalasin B addition results in rapid and selective depolymerization of lamellar F-actin. Addition of cytochalasin B resulted in actin depolymerization to approximately the level present in unstimulated cells. Over this same time course, the actin in unstimulated cells remained relatively unchanged.

In vitro, cytochalasin B caps the plus ends of actin filaments and inhibits actin assembly at this end (Cooper, 1987). Attempts to detect monomer binding with cytochalasin B have been unsuccessful (Brenner and Korn, 1979, 1981; Lin et al., 1980), suggesting that if cytochalasin B binds monomer, its affinity must be very low. We did several controls to confirm that cytochalasin B was likely acting by capping filament plus ends in vivo. First, the observed effects were not due to blockage of glucose transport since dihydrocytochalasin B and cytochalasin B gave similar results. Cellular F-actin was also unaffected by incubation in glucose-free medium. Second, addition of cytochalasin B at a stoichiome-

Figure 5. Cell morphology and actin distribution in human PMNs fixed at various times after incubation in 10 μM cytochalasin B. Phase contrast (a,c,e,g) and fluorescence (b,d,f,h) micrographs of cells incubated in FNLLP, and fixed 0 (a,b), 3 (c,d) or 10 s (e,f) after addition of 10 μM cytochalasin B. (g,h) Cells fixed 10 s after the combination of cytochalasin B addition and peptide removal. Bar, 10 μm.

Figure 7. Effect of cytochalasin B with and without peptide dilution on rhodamine phalloidin binding. (a) Cells incubated in 2 × 10⁻⁸ M FNLLP were either diluted 10-fold into 2 × 10⁻⁸ M FNLLP (open box), into 2 × 10⁻⁸ M FNLLP plus 10 μM cytochalasin B (open circles), into buffer (open triangles), or into buffer plus 10 μM cytochalasin B (closed circles) for 15, 30, or 60 s before fixation, staining with rhodamine phalloidin, and extraction as described in Materials and Methods. Cells fixed before stimulation with FNLLP are shown with the closed box. The y-axis is mean rhodamine fluorescence (after subtraction of the nonsaturable fluorescence (see Materials and Methods)) of duplicate samples from a representative experiment. (b) The experimental protocol was the same as in a except that the chemoattractant was diluted threefold and the cytochalasin B concentration was 1 μM. Symbols as in a.
Figure 8. Effect of temperature on the chemoattractant induced actin polymerization. Cells incubated in buffer (filled symbols) or peptide (open boxes) were diluted 20-fold with buffer or peptide, respectively, at room temperature or 4°C. After 0.3, 1, or 3 min, the cells were fixed and stained with rhodamine phalloidin and extracted as described. The data shown are the mean ± SEM, n = 4.

try one-tenth that of the total actin concentration gave similar results. Based on the similar initial effects of chemoattractant dilution and cytochalasin B addition and the fact that cytochalasin acts primarily by capping filament plus ends, it is likely that the depolymerization observed after either treatment occurs from the minus end of the actin filaments. If actin depolymerization occurring after chemoattractant removal were due to loss of subunits from the plus end of the actin filament, cytochalasin B (by capping the plus end) should block this loss. However, the presence of cytochalasin did not inhibit depolymerization under these conditions (Figs. 6 and 7). In fact, the combination of peptide removal and cytochalasin B addition resulted in a small increase in the initial rate of actin depolymerization compared to that produced by either treatment alone (Fig. 7).

Actin Depolymerization Is Rapid In Vivo

The rates of minus end actin depolymerization, measured in vitro, appear too slow to account for the depolymerization seen in vivo. The total actin in a PMN has been estimated to be 1.3 × 10^{-10} mol, (∼200 μM) (White et al., 1983). In a stimulated cell ∼60%, and in an unstimulated cell ∼30%, of the actin is in the filamentous form. At room temperature, F-actin levels in stimulated cells decreased to the level found in unstimulated cells 30 s after peptide removal (Fig. 3). This is equivalent to depolymerization of 30% of the total cellular actin or 2.3 × 10^7 molecules of actin. Published off rates determined for the minus ends of filaments in vitro (at 22–25°C) range from 0.1 to 0.8 s^{-1} depending on experimental methods and the nucleotide composition of the dissociating species (Weber et al., 1987; Pollard, 1986; Korn et al., 1987; Carlier and Pantaloni, 1988). For the extent of actin depolymerization determined here, these off rates would require 1–8 × 10^9 minus ends. In this case, the average filament would contain somewhere between 3 and 24 monomers and would be 0.008–0.07 μm long. This is in contrast to estimates that F-actin filaments in the lamellipodia of macrophages are 0.5–0.6 μm long (Hartwig and Yin, 1988). If the F-actin were distributed in filaments of this mean length, there would be ∼1 × 10^7 filaments and an off rate of 8 s^{-1} would be required to account for the observed depolymerization (at 37°C an off rate of 24 s^{-1} would be required). This is 10–80 times greater than the off rate measured in vitro. Currently it is not known if PMNs contain many short filaments or if cytoplasmic factors actively increase the rate of depolymerization from the minus end.

F-Actin Is Differentially Stable in Unstimulated PMNs

The level of F-actin present in unstimulated cells is stable under various conditions that cause depolymerization of the chemoattractant stimulated F-actin. Removal of chemotactant, addition of cytochalasin B, the combination of both treatments, or incubation at 4°C reduced F-actin levels in stimulated cells only to the level of unstimulated cells. None of these treatments significantly decreased the basal F-actin levels. These results confirm previous observations that addition of cytochalasin B to either unstimulated or chemoattractant-stimulated cells reduces the amount of actin pelleting with a Triton insoluble cytoskeleton to approximately basal levels (maximally 20% below the resting level) (White et al., 1983). Similar effects of cytochalasin have also been obtained in platelets (Casella et al., 1981; Fox and Phillips, 1981). The stability of the basal F-actin may be due to its being in equilibrium with free G-actin or to its binding accessory proteins.

Stimulated PMNs Contain Two Populations of Actin Filaments

We propose that stimulated PMNs contain two populations of actin filaments. One is a rapidly turning over population, induced by chemoattractants and located primarily in the lamellipodia. The second is a relatively stable population of actin filaments located in the cortical region and present in both stimulated and unstimulated cells. Two populations of actin filaments that differ in their turnover rates have also been observed recently in the giant axon of the squid (Fath and Lasek, 1988).

Our model of rapidly turning over F-actin in PMN lamellipodia is similar to other models of actin dynamics in fibroblasts and neurons (Bray and White, 1988; Svitkina et al., 1986). The F-actin in lamellipodia of fibroblasts and neurons is also selectively sensitive to cytochalasin. Continuous F-actin polymerization at the distal edges of the lamellipodia followed by rearward movement of the filaments is suggested by the observation that in Aplysia neurons, the cytochalasin B–induced actin loss first appears at the edge of the lamellipodia and then moves back toward the cell body (Forscher and Smith, 1988). Furthermore, injected fluorescent actin is rapidly incorporated at the front of a lamellipodia (Wang, 1985; Glacy, 1983). A photobleached area of fluorescent actin at the front of a fibroblast moves rearward (Wang, 1985). The photobleaching results as well as the morphological observations support the concept of rearward movement of F-actin (Heath, 1983). Therefore, to maintain steady state, actin filaments grow proximal to the membrane (at their plus ends) and depolymerize distal to the membrane (from their minus ends) (Small et al., 1978).

In summary, based on information in the literature as well as the data presented in here, we propose: (a) chemoattractants mediate a rapid increase in the free plus ends of actin filaments (Carson et al., 1986). A modest increase in available plus ends is maintained by the continued presence of chemoattractants. These high-affinity ends allow a persistent
increased rate (over resting cells) of actin polymerization. 

(b) The F-actin induced by chemoattractant is located in the lamellipodia and is labile, continually and rapidly turning over. This allows the lamellipodia to be highly responsive to local and temporal changes in the concentration of chemoattractants.

We are very grateful to Molly Tilney for help with the scanning microscopy; to Michael Joyce for excellent laboratory assistance; to Richard Saad and to Manuel Cano for helpful discussions. We are indebted to Jean and Joe Sanger for help with quantitation of fluorescence in individual cells.

This work was supported by National Institutes of Health (NIH) grant AI 19883 to S. H. Zigmond and NIH Training Grant CA 09140 to L. Cassimeris.

Received for publication 13 July 1989 and in revised form 27 November 1989.

References

Bray, D., and J. G. White. 1988. Cortical flow in animal cells. Science (Wash. DC). 239:883-887.

Brenner, S. L., and E. D. Korn. 1979. Substoichiometric concentrations of cytochalasin D inhibit actin polymerization. J. Biol. Chem. 254:9982-9985.

Brenner, S. L., and E. D. Korn. 1981. Stimulation of actin ATPase activity by cytochalasins provides evidence for a new species of monomeric actin. J. Biol. Chem. 256:8663-8670.

Carlier, M.-F., and D. Pantaloni. 1988. Binding of phosphate to F-ADP-actin and role of F-ADP-ATP-actin in ATP-actin polymerization. J. Biol. Chem. 263:817-825.

Carson, M., A. Weber, and S. H. Zigmond. 1986. An actin-nucleating activity in PMNs is modulated by chemotactic peptides. J. Cell Biol. 103:2707-2714.

Casella, J. F., M. D. Flannagan, and S. Lin. 1981. Cytochalasin D inhibits actin polymerization and induces depolymerization of actin filaments formed during platelet shape change. Nature (Lond.). 293:302-305.

Cooper, J. A. 1987. Effects of cytochalasin and phalloidin on actin. J. Cell Biol. 105:1473-1478.

Devreotes, P. N., and S. H. Zigmond. 1988. Chemotaxis in eukaryotic cells: a focus on leukocytes and Dictyostelium. Annu. Rev. Cell Biol. 4:649-686.

Fath, K. R., and R. J. Lasek. 1988. Two classes of actin microfilaments are associated with the inner cytoskeleton of axons. J. Cell Biol. 107:613-621.

Fechheimer, M., and S. H. Zigmond. 1983. Changes in cytoskeletal proteins of polymorphonuclear leukocytes induced by chemotactic peptides. Cell Motil. 3:349-361.

Forscher, P., and S. J. Smith. 1988. Actions of cytochalasins on the organization of actin filaments and microtubules in a neuronal growth cone. J. Cell Biol. 107:1505-1516.

Fox, J. E. B., and D. R. Phillips. 1981. Inhibition of actin polymerization in blood platelets by cytochalasins. Nature (Lond.). 310:691-693.

Glacy, S. D. 1983. Subcellular distribution of rhodamine-actin microinjected into living fibroblastic cells. J. Cell Biol. 97:1207-1213.

Hall, A., A. Schlein, and J. Condeelis. 1988. Relationship of pseudopod extension to chemotaxis and actin polymerization in amoeboid cells. J. Cell Biol. 107:285-300.

Hartwig, J. H., and H. L. Yin. 1988. The organization and regulation of the macrophage actin skeleton. Cell Motil. Cytoskel. 10:117-125.

Heath, J. P. 1983. Direct evidence for microfilament-mediated capping of surface receptors on crawling fibroblasts. Nature (Lond.). 302:532-534.

Howard, T. H., and C. O. Oresajo. 1985a. 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.

Howard, T. H., and C. O. Oresajo. 1985b. A method for quantifying F-actin in chemotactic peptide activated neutrophils: study of the effect of floc peptide. Cell Motil. 5:545-557.

Korn, E. D., M.-F. Carlier, and D. Pantaloni. 1987. Actin polymerization and ATP hydrolysis. Science (Wash. DC). 238:638-644.

Lin, D. C., K. D. Tokin, M. Grumet, and S. Lin. 1980. Cytochalasins inhibit nuclei-induced actin polymerization by blocking filament elongation. J. Cell Biol. 84:455-460.

Pollard, T. D. 1986. Rate constants for the reactions of ATP- and ADP-actin with the ends of actin filaments. J. Cell Biol. 103:2747-2754.

Rao, K. M. K., and J. Varani. 1982. Actin polymerization induced by chemotactic peptide and concanavalin A in neutrophils. J. Immunol. 129:1605-1607.

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.

Small, J. V., G. Isenberg, and J. E. Celis. 1978. Polarity of actin at the leading edge of cultured cells. Nature (Lond.). 272:638-639.

Southwick, F., 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 chemotax attractants. J. Cell Biol. 109:1561-1569.

Svitkina, T. M., A. A. Neyfakh, and A. D. Bershadsky. 1986. Actin cytoskeleton of spread fibroblasts appears to assemble at the cell edges. J. Cell Sci. 82:235-248.

Tilney, L. G. 1973. The polymerization of actin: its role in the generation of the acrosomal process of echinoderm sperm. J. Cell Biol. 59:109-126.

Wallace, J. P., R. P. Wersto, C. H. Packman, and M. A. Lichtman. 1984. Chemotactic peptide-induced changes in neutrophil actin conformation. J. Cell Biol. 99:1060-1065.

Wang, Y.-L. 1985. Exchange of actin subunits at the leading edge of living fibroblasts: possible role of treading. J. Cell Biol. 101:597-602.

Weber, A., J. Northrop, M. F. Bishop, F. A. Ferrone, and M. S. Mooskier. 1987. Kinetics of actin elongation and depolymerization at the pointed end. Biochemistry. 26:2528-2536.

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: effect of calcium and cytochalasin B. J. Biol. Chem. 258:14041-14047.

Zigmond, S. H. 1974. Mechanisms of sensing chemical gradients by polymorphonuclear leukocytes. Nature (Lond.). 249:450-452.

Zigmond, S. H., and S. J. Sullivan. 1979. Sensory adaptation of leukocytes to chemotactic peptides. J. Cell Biol. 82:517-527.

Zigmond, S. H., T. H. Levinsky, and B. J. Kroel. 1981. Cell polarity: an examination of its behavioral expression and its consequences for polymorphonuclear chemotaxis. J. Cell Biol. 89:585-592.