Rapid Modulation of *N*-Formyl Chemotactic Peptide Receptors on the Surface of Human Granulocytes: Formation of High-affinity Ligand-Receptor Complexes in Transient Association with Cytoskeleton

A. J. JESAITIS, J. R. NAEMURA, L. A. SKLAR, C. G. COCHRANE, and R. G. PAINTER
Department of Immunology, Scripps Clinic and Research Foundation, La Jolla, California 92037

ABSTRACT When human granulocytes were exposed to 50 nM *N*-formyl-Met-Leu-[^{3}H]Phe at 37°C they rapidly formed ligand-receptor complexes that dissociated 50–100 times more slowly than those on cells initially exposed to the peptide at 4°C. These complexes of apparent higher affinity were stable after detergent solubilization of the cells with Triton X-100. The complexes co-isolated with the detergent insoluble cytoskeletal residues and were free of the cytosolic and Golgi markers, lactate dehydrogenase and galactosyl transferase, respectively. After 5 s of exposure to f-Met-Leu-Phe, 2,000–3,000 molecules of ligand per cell were trapped in such complexes. Continued exposure resulted in capture of a maximum of 14,000 molecules per cell by 5 min. Exposure at 15°C, a temperature at which endocytosis of the receptor is prevented, resulted in complex formation at a linear rate for at least 20 min to levels twice those measured at 37°C. At 4°C, complex formation was ~10% of the maximum amount formed at 37°C. Pulse-chase experiments revealed that the complex was in transient association with the cytoskeleton with a half life ranging between 30 s to 4 min depending on the length of the original incubation. Electron microscopic autoradiography indicated that after 1 min of incubation at 37°C, the majority of the specific autoradiographic grains were localized to the outer circumference of the cellular cytoskeleton. After 4 min of incubation, the grains were less frequent at the cytoskeleton periphery but still threefold enriched over a random cellular distribution. We conclude that a metabolically controlled modulation of the state of the *N*-formyl chemotactic peptide receptor occurs in the plasma membrane which may be the result of transient association of ligand-receptor complex and the cell cytoskeleton.

Granulocytes (polymorphonuclear leukocytes) are major cellular mediators of host defense (29, 64) and inflammation (9, 46). They, furthermore, exhibit a variety of biochemical and morphological responses to a class of *N*-formylated peptides termed *N*-formyl chemotactic peptides (3, 44, 63). Consequently, these cells can serve as a model system well suited for the study of such basic cellular processes as sensory transduction, motility, and membrane processing while permitting direct investigation of the role of these processes in inflammatory disease.

The interplay between events occurring at the cell surface and those in the cytoplasm forms the basis for sensory transduction in granulocytes, as well as many other cell types. These surface events, such as receptor-hormone binding, are causally related to cellular morphological responses such as motility, exocytosis, endocytosis, and polarization, all of which probably involve the cell's cytoskeleton in varying degrees. There are numerous examples of various forms of association of cell surface proteins with the cell cytoskeleton (5, 14, 19, 31, 36, 38, 40, 54, 70) including one in human neutrophils (59). In a number of systems, such associations are inducible by the binding of multivalent ligands, such as lectins or antibodies (10, 17, 48, 59, 68), and include the induction of linkage between hormone receptors and cytoskeleton structures (67). The measurement of restricted lateral mobilities of cell surface proteins and hormone receptors

THE JOURNAL OF CELL BIOLOGY · VOLUME 98 APRIL 1984 1378–1387

supports the hypothesis of the control of lateral organization by a cytoskeletal substructure (25, 50, 56, 58, 69). Indeed, in cases where cytoskeleton can be disrupted, the same proteins gain increased mobility (15, 39, 58, 66, 69) consistent with their theoretical maxima (51).

Recently, Vale and Shooter (67) have observed that the lectin induced association of nerve growth factor receptors with the cytoskeleton of PC-12 cells, also results in a fivefold decrease in the dissociation rates of the receptors. They proposed that such changes in receptor-hormone dynamics, also accounted for in other hormone systems by the mobile receptor hypothesis (7, 24), are consistent with interaction of the receptors with cytoskeletally linked effector molecules.

We have recently obtained evidence suggesting that similar interactions between *N*-formyl peptides and their receptor systems might be occurring in human granulocytes without the participation of a cross-linking lectin or antibody. After binding to its receptor, but before endocytosis of the ligand receptor complex (27), the association between f-Met-Leu-Phe stabilizes exhibiting a much lower dissociation rate. This stabilization permitted isolation of a high molecular weight form of the complex in detergent extracts of purified membranes (28). Because insolubility of membranes in Triton X-100 is often assumed to be indicative of the association of cell membrane components with the cell cytoskeleton or its submembranous counterpart (6, 30, 36, 38, 48, 70), our results could be interpreted as a manifestation of complex formation between cell cytoskeleton and an altered form of the *N*-formyl peptide receptor. Moreover, since we have also shown that such a complex is undetectable in resting membranes (49) then we hypothesize that formation of the complex may have relevance to sensory transduction as well as receptor-ligand processing in these cells.

The work described here reports the transient formation of f-Met-Leu-Phe receptor complexes at the granulocyte surface. These complexes co-isolate with Triton X-100 extracted cell cytoskeletons and are under cellular control. The extent and kinetics of formation as well as the disappearance of this species of receptor is consistent with its involvement in the transduction process. Complex formation, moreover, is probably a manifestation of receptor modulation culminating in its endocytosis.

MATERIALS AND METHODS

The materials used, methods of granulocyte isolation, their incubation with *N*-formyl Met-Leu-Phe, and use of subcellular organelle markers have been described previously (28). Dulbecco's phosphate buffered saline with 0.1 percent bovine serum albumin and 0.1 percent glucose (DPBS)[1] was used to incubate, wash, and store the cells.

Determination of f-Met-Leu-Phe Uptake

In this study the nondissociable component of f-Met-Leu-Phe absorption by cells was measured. We define this component as uptake. The determination of the specific and nonspecific contributions to the cellular uptake was accomplished by measuring the tritium content in cells that had been incubated with 50 nM f-Met-Leu-[^{3}H]Phe ± 2.5 μM f-Met-Leu-Phe under various conditions and then thoroughly washed. The radioactivity co-isolating with the Triton X-100 insoluble (cytoskeletal) fraction of the cell was also determined.

Isolation of cytoskeleton-enriched cell fractions, based on a modification of the method of Koch (30), was carried out in three ways. Each used the lysis buffer of Vale and Shooter (67) with 0.5% Triton X-100 in buffer containing 3 mM $MgCl_2$, 20 mM Tris-HCl, pH 7.4, and varying amounts of sucrose. Washed cells (2 ×) were layered and centrifuged on three types of step gradients containing sucrose and Triton-containing lysis buffer.

METHOD I: LINEAR GRADIENT: 0.5 ml of DPBS containing 1 × 10^7 cells; 0.5 ml of DPBS containing 8% sucrose (wt/wt); 4.0 ml of lysis buffer and a linear gradient of sucrose from 20–60%. Centrifugation was for 3 h at 256,000 *g* in a Beckman SW 50.1 rotor (Beckman Instruments, Inc., Palo Alto, Inc.).

METHOD II: STEP GRADIENT: 0.2 ml of DPBS containing 1 × 10^7 cells; 0.2 ml of DPBS containing 4% sucrose (wt/wt); 1 ml of lysis buffer containing 8% sucrose ± 0.5% Triton X-100. Centrifugation was for 1 min at 8,000 *g* in a Beckman microfuge (Beckman Instruments, Inc.).

METHOD III: STEP GRADIENT: 0.2 ml of cell lysis buffer containing 10^7 cells, 1.2 ml of lysis buffer or 0.5% Triton X-100 and 8% sucrose. Centrifugation was for 1 min at 8,000 *g* in a Beckman microfuge.

Pellets were resuspended either in 1 N NaOH (10^7 cell equivalents per 0.2 ml) or in the same volume of 20 mM Tris-HCl, 3 mM $MgCl_2$ pH 7.4. For kinetic studies the content of f-Met-Leu-Phe per microgram protein was determined. The number of molecules per cell equivalent was calculated assuming 50 pg protein per intact cell and 30 pg per cytoskeletal residue (Methods II and III) or 20 pg (Method I).

Electron Microscopic Autoradiography

Cell suspensions or cytoskeletons were fixed after treatments described in the text, by adding 0.25 vol of 8% glutaraldehyde, 0.1 M Na phosphate buffer (pH 7.4) and incubating for 30 min on ice. After postfixation with O_sO_4 under conditions that minimize actin filament damage (37), the cell or cytoskeleton pellets were dehydrated, embedded, and sectioned as described previously (47). The gold sections, stained with uranyl acetate and lead citrate, were coated with ~50 Å carbon film, and Ilford L4 nuclear track emulsion applied by the loop method (8). After 1–4 wk, the autoradiograms were developed with Microdol-X (diluted 1:2), fixed, and viewed in a Hitachi Model 12A electron microscope at 70 kV.

SDS PAGE

One dimensional, 10% polyacrylamide slab gels were prepared and run as described by Laemmli (33) using a modification (45). Pellets or samples in 20 mM Tris-HCl, 3 mM $MgCl_2$, 15% sucrose pH 7.4 were solubilized by dilution 1:1 in 150 mM Tris pH 6.8, 3.0% SDS, 30 percent glycerol, 3% β-mercaptoethanol, and 0.004% bromophenol blue, addition of urea to 8 M, and heating for 10 min at 100°C. The slabs were 3.5 cm high, 7 cm wide, and 1 mm thick. A 1 cm, 4% stacking gel was used. Electrophoresis was carried out at a constant current: 10 mA through the stacking gel and 20 mA through the separating gel (20 and 25 min), respectively. The gels were fixed and stained in 0.05 percent Coomassie Blue G-250 in 25% isopropanol and 10% acetic acid and destained in 10% acetic acid.

RESULTS

To directly isolate detergent stable complexes of f-Met-Leu-Phe and chemotactic receptors, washed human granulocytes that had been exposed to 50 nM f-Met-Leu-[^{3}H]Phe for 4 min at 37°C were sedimented into a linear sucrose gradient devoid of or containing 0.5% Triton X-100. The distribution of radioactivity in the fractions of the gradients is shown in Fig. 1. The dense region of the gradient is on the right (see Fig. 2 for sucrose data) and contains 60–70% of the radioactivity of the washed cells loaded onto the Triton-free gradient (*top*). Remaining on top of the gradient is peptide that was loosely bound and had dissociated from the cell before and during sedimentation. In the lower panel, the distribution of radioactivity in the gradient containing Triton X-100 is shown. Approximately 50% of the radioactivity sedimenting with intact cells (Fig. 2, *top*) co-sediments with the cell fraction remaining insoluble in Triton X-100. The specificity of uptake by cells or by the Triton-insoluble fraction can be calculated by comparing the distribution of radioactivity in the gradients obtained from cells exposed to 50 nM f-Met-Leu-[^{3}H]Phe in the presence of 2.5 μM nonradioactive peptide. In general, the uptake into the Triton insoluble fraction was 87 ± 5% specific after 4 min of incubation at 37°C and occurred with

[1] *Abbreviation used in this paper:* DPBS, Dulbecco's phosphate buffered saline.

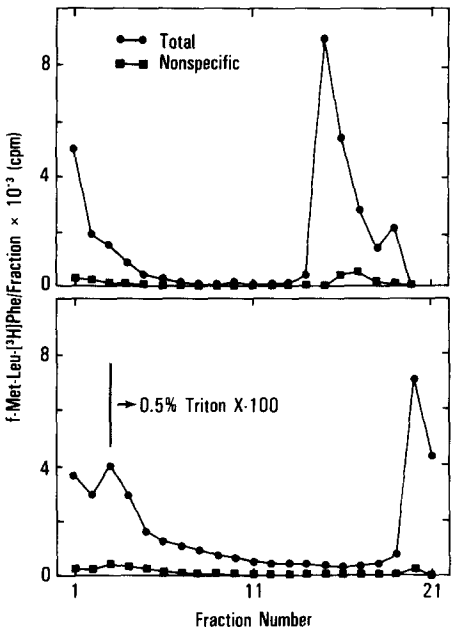

FIGURE 1 Distribution of granulocyte associated f-Met-Leu-[^{3}H]-Phe in sucrose gradients devoid of (*top*) or containing 0.5% Triton X-100 (*bottom*). 1 × 10^8 cells were loaded with f-Met-Leu-[^{3}H]Phe at 37°C for 4 min in the presence and absence of excess nonradioactive peptide, layered onto a sucrose gradient, centrifuged as described in Method I of the Materials and Methods section. Tritium content per fraction was determined and plotted as a function of fraction number. The sucrose densities and other markers are shown in Fig. 2.

the same dose dependency (data not shown) as uptake of f-Met-Leu-[^{3}H]Phe by cells (28).

To determine the efficiency of the solubilization, a marker analysis of the Triton X-100 gradients was performed. The analysis indicated that the cytosolic marker, lactate dehydrogenase, and the Golgi marker, uridine diphosphate-galactose galactosyl transferase, were quantitatively solubilized and remained near the interface of the detergent free cushion and the Triton X-100 layer (Fig. 2). This result demonstrates that little cytosol was trapped in the Triton insoluble residues and that an internal membrane protein was completely solubilized. The plasma membrane marker, ^{125}I-conjugated wheat germ agglutinin (28) used in trace amounts as a reversible surface label, remained 75–80% sedimentable in these gradients. Such insolubility of plasma membrane proteins is also characteristic of other cytoskeletal preparations (6). In the control gradients (no detergent) all the corresponding markers sedimented to the highest density regions containing the sedimentable radioactivity (Fig. 1, *top*, fractions 14–19).

Approximately 40% of the total cell protein (20 pg) remained Triton insoluble in these gradients. To demonstrate that the sedimentable protein contained cytoskeleton, gradient fractions were further solubilized in SDS and analyzed by polyacrylamide gel electrophoresis under reducing conditions. The banding pattern shown in Fig. 3 indicates a prominent band (~44,000 daltons) which co-migrates with actin. These fractions also bound nitrobenzoxadiazole-phallacidin (not shown) confirming the presence of F-actin (2), the structural unit of the cytoskeleton. These results suggest that actin remains a significant constituent of the cytoskeletal fraction in spite of its major presence in the soluble pool of the cell (59).

The morphology of the Triton-insoluble residues was investigated by electron microscopy to confirm isolation of cytoskeletal structures. Fig. 4 shows a micrograph of a thin section of the high density fraction. These structures contained the remains of nuclei and granules without their boundary membranes and resemble Triton-insoluble structures isolated from other nucleated cell types (6, 35) and granulocytes (59). A network of filamentous actin-like structures lay under the remnants of a deformed cell surface which appeared amorphous in some regions and bilaminar in other regions (Fig. 4 *inset*). Thus, by morphology and their enrichment in F-actin, these structures appear to be cell "cytoskeletons" as defined by Lenk et al. (35).

We define the nondissociable component of f-Met-Leu-Phe binding to intact cells as uptake. It is comprised of the fraction trapped within the cell by internalization and the fraction

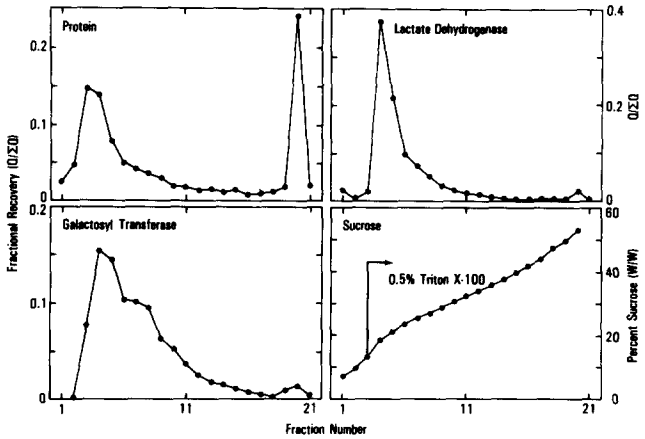

FIGURE 2 Distribution of granulocyte derived protein, lactate dehydrogenase, galactosyl transferase, and sucrose in a Triton X-100 containing linear sucrose gradient. The distribution of markers from the Triton X-100-containing gradient of Fig. 2 which was run with cells exposed to f-Met-Leu-[^{3}H]Phe only.

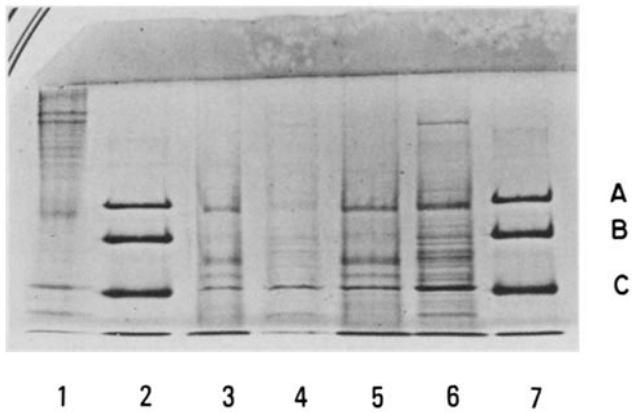

FIGURE 3 SDS PAGE of granulocyte cell fractions obtained from gradients of Method I and II in the Materials and Methods section. Lane *1*, red cell ghosts prepared after Dodge et al. (13); lanes *2* and *7*, molecular weight standards: *A*, phosphorylase B (94,000 daltons), *B*, bovine serum albumin (68,000 daltons); *C*, ovalbumin (43,000 daltons); lane *3*, peak sedimentible protein and f-Met-Leu-[^{3}H]Phe fraction, Method I gradient; lane *4*, Triton X-100 interface, Method I gradient; lane *5*, cytoskeletons, Method II gradient; lane *6*, solubilized whole cells. Lane *4* contained 4.5 μg protein. All other lanes contained 9 μg protein.

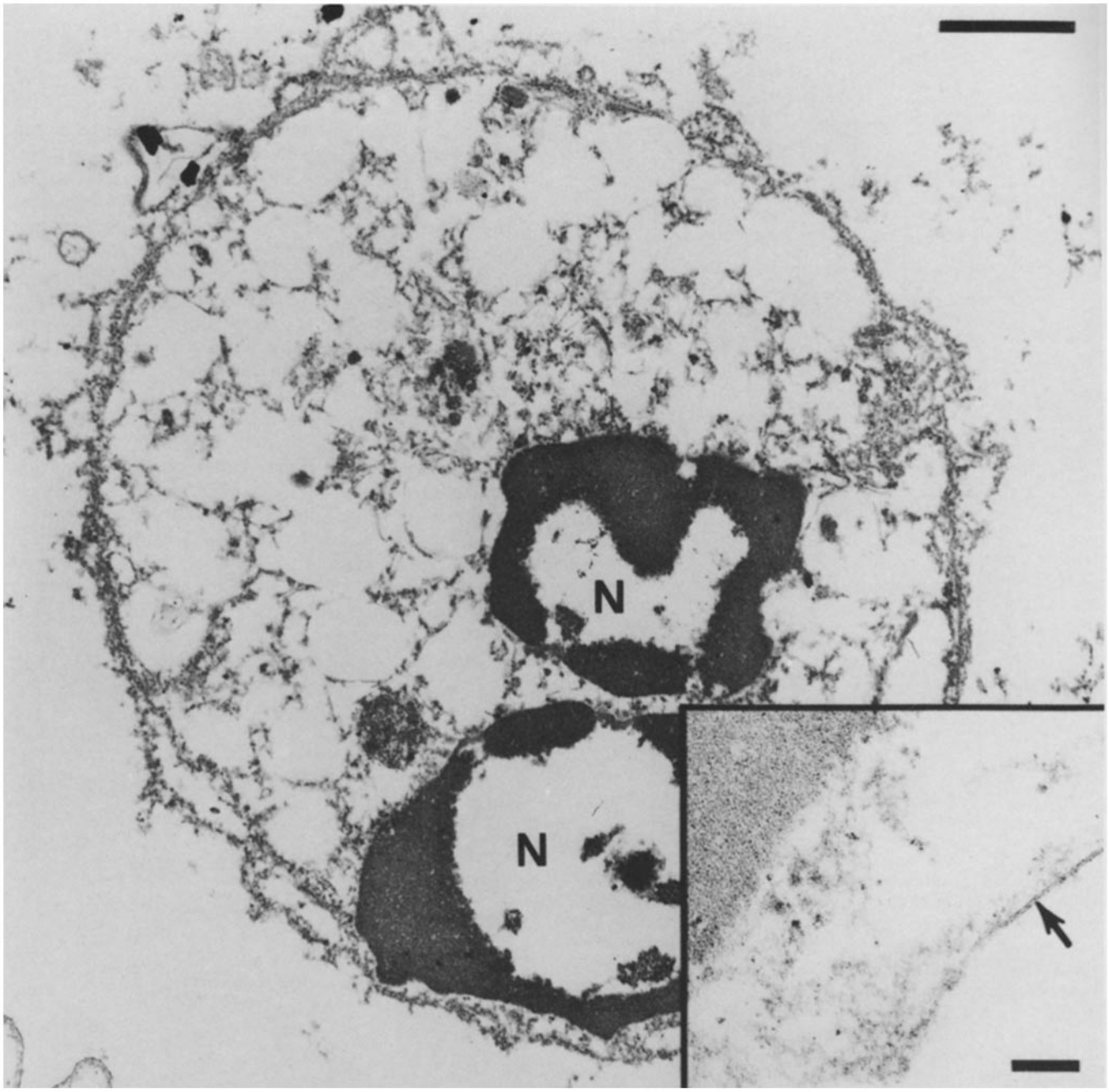

FIGURE 4 Morphology of Triton X-100 insoluble fraction prepared as in Fig. 1. Characteristic cytoskeletal structures are observed. Nuclear (*N*), granule, and plasma membrane remnants and filaments are apparent. Bar, 1 μm. × 24,019. Inset shows a detail of the surface structure showing some bilaminar structures. Bar, 0.1 μm. × 117,400.

trapped at the surface, presumably in very slowly dissociating receptors. To probe this compartmentation further, a kinetic analysis of the uptake was performed. Fig. 5 compares the kinetics of uptake in whole cells and the cytoskeletal cell fraction. In these experiments, a simplified protocol using a step gradient was substituted for the linear gradient. The upper panels of Fig. 5 show the kinetics of total and nonspecific uptake of f-Met-Leu-[^{3}H]Phe in cells and their cytoskeletal subfraction obtained by pelleting washed cells through an 8% sucrose cushion devoid of or containing 0.5% Triton X-100, respectively. The bottom of Fig. 5 shows the calculated specific uptake (i.e., total − nonspecific) expressed as molecules of peptide remaining associated per cell or cytoskeletal equivalent. A slightly more rapid rise of cell-associated peptide in the first minute is indicative of the binding phase which is followed by a continued increase resulting from binding and endocytosis. In contrast, the uptake by the Triton-insoluble fraction saturates by 5–6 min at a level of radioactivity equivalent to $(14 \pm 7) \times 10^3$ molecules per cell ($n = 27$). Of importance is the observation that uptake is detected as early as 5 s and continues for ~1 min at the same rate as in the whole cell. During this time internalization of the ligand has not occurred (16, 27, 28, 60).

After internalization begins, part of the cell-associated peptide is stored in the cytosol (28). The absence of lactate dehydrogenase in the Triton-insoluble pellet is indicative of

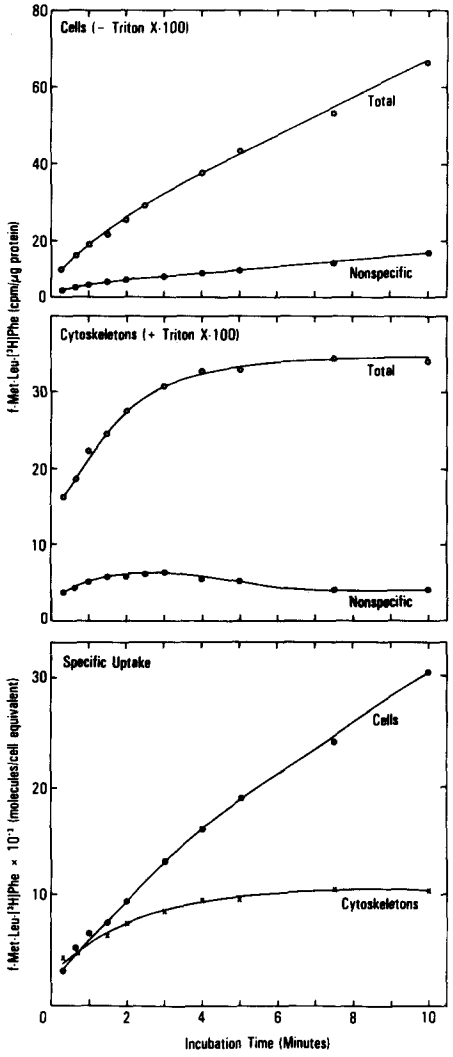

FIGURE 5 Kinetics of uptake at 37°C of f-Met-Leu-Phe by whole cells and their Triton X-100 insoluble fraction. Cells were incubated with 50 nM f-Met-Leu-[^{3}H]Phe at 37°C as is described in the Materials and Methods section. Incubation was terminated at all the specified times by fivefold dilution in ice cold buffer followed by washing (2 times) and sedimentation in a gradient by Method II as the final step. Gradients without Triton X-100 were used to prepare washed cells and those with Triton X-100 to prepare the Triton X-100 insoluble fractions. Total and nonspecific (in presence of 2.5 μM f-Met-Leu-Phe) uptake in cells and the Triton insoluble fraction is shown in the upper and middle panels, respectively, and is expressed as radioactivity per microgram protein of the pellets containing the equivalent of 10^7 cells (i.e., ~500 μg). Specific uptake is shown in the lower panel and is expressed as molecules of f-Met-Leu-[^{3}H]Phe per cell equivalent. Pellets were 95% free of the cytoplasmic marker lactate dehydrogenase, contained 50–60% of the cell protein (250–300 μg), 75% of surface accessible wheat germ agglutinin sites, and also contained actin (see Fig. 3).

cell lysis and loss of cytosol. Thus, a significant reduction in the total cell-associated chemotactic peptide after exposure to detergent is expected. Fig. 5 also shows that the compartmentation of the cell associated f-Met-Leu-Phe depends on the length of exposure at 37°C. Thus at 1.5 min 93 ± 14% (n = 7) of the peptide is trapped with the cytoskeleton, whereas at 4 min this figure is 61 ± 11%. The specificity of uptake at these two times was 81 ± 6% and 87 ± 5%, respectively. These values are also indistinguishable from those obtained using Method I (linear gradients), thus substantiating the validity of the step gradient sedimentation.

To measure the stability of the association of f-Met-Leu-Phe with cells and their cytoskeletal fractions, the dissociation kinetics of the ligand from washed cells and their Triton-insoluble residues at 4°C was measured. Fig. 6 compares the exchange of radioligand from the pellet fractions (± Triton X-100) of cells loaded with 50 nM f-Met-Leu-[^{3}H]Phe for 20 min at 15°C (a temperature at which endocytosis is prevented; see below) and then washed. If the cells are lysed with Triton X-100 in the presence of 2.5 μM nonradioactive peptide and then centrifuged at different times after lysis (Method III) then radioactivity is lost from the pellet with a $t_{1/2}$ of ~100 min. This dissociation is probably not the result of proteolysis from hydrolytic enzymes released from granules because diisopropylfluorphosphate treatment of the cells, which fully protects actin binding protein and other protease sensitive proteins (1), results in the similar dissociation kinetics. In addition, since the cytoskeletal pellets retain >90% of their original radioactivity after 1 h of incubation in lysis buffer at 4°C (without added nonradioactive peptide) the instability noted above is probably due to ligand exchange at the receptor binding site rather than dissociation of the ligand-receptor complex from the Triton X-100 insoluble matrix. Incubation of the cytoskeletal pellets at 15°C or higher for 1 h destabilized the complex so that <10 percent of the original radioactivity remained sedimentable. Intact cells preloaded with f-Met-Leu-[^{3}H]Phe show a more stable interaction at 4°C than cytoskeletons. A 1-h incubation in the presence of the nonradioactive ligand resulted in a reduction of only 20% in the level of sedimentable radioactivity.

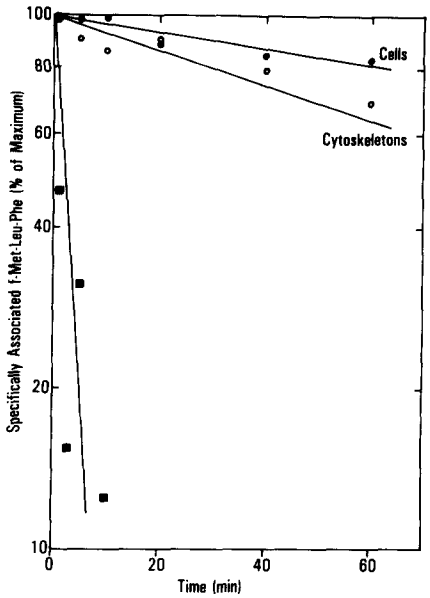

FIGURE 6 Stability of f-Met-Leu-Phe association at 4°C. Twice washed cells were prepared by incubation at 15°C for 20 min with 20 mM f-Met-Leu-[^{3}H]Phe as is described in the Materials and Methods section. These cells were then exposed at 4°C to 2.5 μM nonradioactive f-Met-Leu-Phe in the presence (○) and absence (●) of 0.5 percent Triton X-100 for the times indicated before centrifugation through a Method III gradient. The percentage of the initial specifically associated f-Met-Leu-[^{3}H]Phe remaining in the pellets is displayed on a semilogarithmic scale. For comparison, the dissociation kinetics of f-Met-Leu-[^{3}H]Phe bound to cells at 4°C for 30 min is also shown (■). 2.5 μM f-Met-Leu-Phe was added at time zero to the binding mixture which was then directly centrifuged at the times shown through a sucrose cushion without Triton.

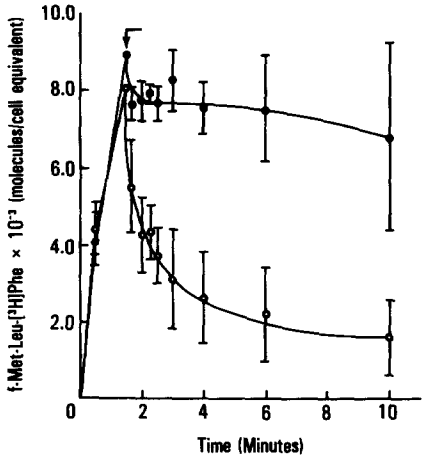

FIGURE 7 Transient association of f-Met-Leu-[^{3}H]-Phe with granulocyte cytoskeletons. After incubating cells with f-Met-Leu-[^{3}H]Phe at 37°C for 1.5 min, 2.5 μM nonradioactive peptide was added as a chase (arrow). At the indicated subsequent times, aliquots were removed and washed cells (●) or cytoskeletons (○) were prepared by Method II. Specific radioactive uptake and protein content of pellets were determined. (n = 6).

The dissociation of ligand from the bulk receptor population (i.e., 4°C incubation with the radioligand) is also shown in Fig. 6 for comparison. Cells were allowed to bind ligand at 4°C for 30 min (to levels approximately twice those of the 15°C/washed cells above), exposed to 2.5 μM nonradioactive ligand, and then sedimented into the sucrose cushion at the different times shown. The $t_{1/2}$ for dissociation from prebound receptor was observed to be ~2 min, demonstrating that at 15°C and at 37°C a receptor conversion and transformation occurred.

To determine if the temporal saturation of f-Met-Leu-Phe uptake in the cytoskeletal fraction at 37°C (Fig. 5) was simply the static accumulation of the occupied receptors in the cytoskeletal compartment, a pulse-chase experiment was performed. Fig. 7 shows that the association of f-Met-Leu-Phe with the Triton insoluble cell fraction, on the contrary, appears to be transient in the metabolically active cell. After 90 s of exposure to 50 nM f-Met-Leu-[^{3}H]Phe to cells at 37°C, a chase of 2.5 μM nonradioactive peptide causes the specifically associated radioactivity in this fraction to decrease rapidly to control levels with a $t_{1/2}$ of ~50 ± 25 s (n = 6), whereas that associated with the intact cell remains high. This experiment also demonstrates that the association of the peptide with the cytoskeletal fraction is not a result of processes occurring after cell lysis such as nonspecific binding of peptide released from the cell interior to the pellet residue. If the incubation time is increased to 4 min then the loss of radioactivity from the cytoskeletal fraction is lengthened with a $t_{1/2}$ of 2 ± 1.5 min (n = 6). Reducing the level of the chase to 500 nM does not significantly change the kinetics of decay (data not shown).

During the initial phase of f-Met-Leu-Phe uptake virtually all of the cell-associated ligand sedimented with the Triton insoluble fraction (see Fig. 5). This result is suggestive of a small population of preformed or very rapid formation of the complex in the plasma membrane. Support for this hypothesis was found in a study of the effects of temperature on the kinetics of uptake of f-Met-Leu-Phe into cells and their Triton insoluble fractions and is shown in Fig. 8. When incubations were performed under conditions that block internalization (i.e., 15°C; 60), the uptake of ligand into the cytoskeletal fraction was quantitatively identical to that seen in the whole cell. In contrast, saturation in the Triton insoluble fraction occurred by 5 min when cells were incubated at 37°C, while continued association of ligand with the cytoskeletal fraction was observed to occur at 15°C. At 4°C, much lower levels of this "high-affinity" complex were observed.

To confirm localization of the formyl peptide to the cytoskeletal periphery, an electron microscopic autoradiographic study was carried out. In two experiments, the Triton insoluble cytoskeletal residues were prepared in type I linear gradients. Cells were incubated for 1 and 4 min at 37°C with 10 nM N-formyl-Nle-Leu-Phe-Nle [^{125}I]Tyr-Lys to permit covalent cross-linking to the receptor (41) during fixation for electron microscopy. More than 70% of the initial cellular uptake was measured in the final Epon block. Fig. 9 shows a representative autoradiograph obtained following a 1 min incubation in which ~66% of the specific radiographic grains (see Table I) were localized to within 1,500 Å (HD_{50} for ^{125}I = 800 Å; 50) of the cytoskeletal periphery. Since a 3,000 Å spherical shell including the surface of a spherical cytoskeleton of 4.5 μm radius represents 20% of the total cell volume, the grain distribution is >3.2-fold enriched at the surface over what would be expected for a uniform volumetric distribution. A slightly less enriched (~2.4-fold) distribution of grains was seen when intact cells were examined (not shown) indicating that the pattern observed after Triton lysis was not due to redistribution of peptide label after Triton X-100 lysis. In addition, in evaluating this data, it must be remembered that they represent an underestimate of the surface localization since they were not corrected for the surface-derived grains falling outside the 2-times HD_{50} counting limits (53), the additional washing of the samples prior to fixation, or the inability to distinguish between topographically external peptide in cell-invaginations (22, 23) and internally trapped peptide.

Surface grain enrichment in cytoskeleton was preserved, but to a smaller degree, at longer incubation times. When cells were incubated with saturating doses of peptide for 4 min at 37°C there is significant down regulation of receptor and accumulation of peptide and receptor within the cell (27, 28, 42, 65). The results in Table I indicate that, in intact cells, the peptide was internalized to a greater degree, i.e., with a

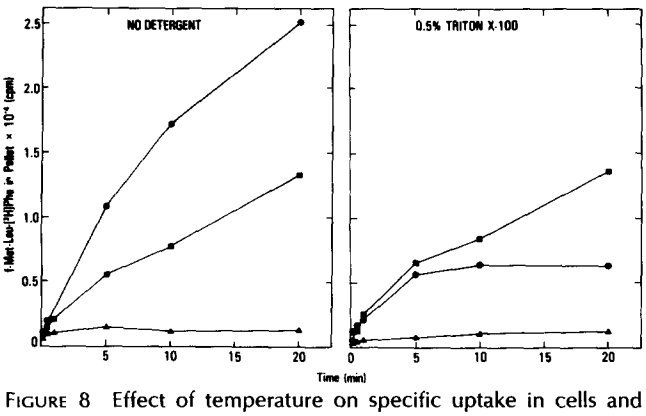

FIGURE 8 Effect of temperature on specific uptake in cells and cytoskeletons. Granulocytes were incubated at the indicated times and temperatures before treatment by Method II to prepare cells and cytoskeletons. Specific content of f-Met-Leu-[^{3}H]Phe and protein in each pellet was determined and specifically associated radioactively calculated on a per microgram protein basis. ●, 37°C; ■, 15°C; ▲, 4°C.

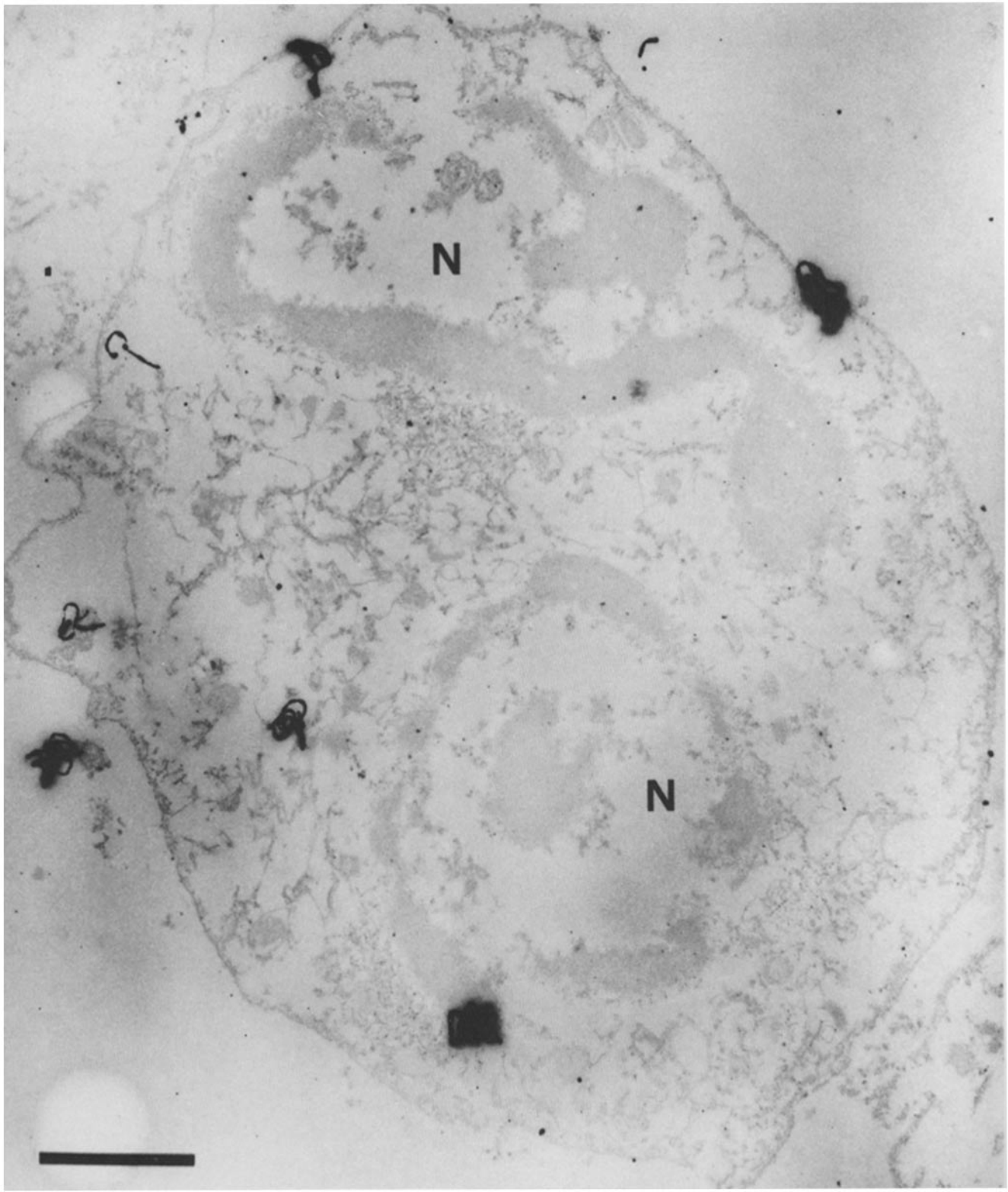

FIGURE 9 Localization of *N*-formyl chemotactic peptide in granulocyte cytoskeletons by electron microscopic autoradiography. Electron microscopic autoradiogram of a typical Triton "cytoskeleton" prepared from granulocytes exposed to 4 nM f-Nle-Leu-Phe-Nle-[^{125}I]Tyr-Lys (42) for 1 min at 37°C. After washing at 4°C, the cell pellet was applied to a Triton-containing sucrose gradient (Method I). Fractions containing the sedimentible radioactivity were fixed with 2% glutaraldehyde, and processed for electron microscopic autoradiography as described in the Materials and Methods section. 4-wk exposure, developer Kodak Microdol-X (1:2). Bar, 1.0 μM; × 29,000.

smaller fraction remaining at the cell surface. This observation is in agreement with the extent of internalization in this system (>60%; 16, 28, 60). The distribution of grains in the cytoskeleton obtained from such cells reflects a higher degree of surface localization than in the intact cells (1.6-fold vs. 0.7-fold; respectively).

TABLE I

*Autoradiographic Localization of N-Formyl Chemotactic Peptide in Human Granulocyte Cytoskeletons**

	Incubation time	Total grains counted		Specific grain distribution‡		Surface enrichment§
		Surface	Internal	Surface	Internal	
	min			%	%	
Intact Cells	1	147 (12)	163 (13)	48.4 (5)‖	51.6 (5)	2.4 (0.2)
Cytoskeletons	1	184 (14)	116 (11)	65.8 (6)	32.2 (4)	3.3 (0.3)
Intact Cells	4	81 (9)	219 (15)	14.5 (2)	85.5 (6)	0.7 (0.1)
Cytoskeletons	4	124 (11)	200 (14)	32.0 (3)	68.0 (6)	1.6 (0.2)

* Cells and cytoskeletons were prepared as described in legend to Fig. 9. Grains were counted at random and scored as surface localized if an individual grain fell within 1,500 Å of either side of the cell. The standard deviation (SD) of total grains counted in each compartment is equal to the square root of the latter.

‡ Calculated by determining the percent of grains that were surface localized in blocked (1 μM nonradioactive peptide) controls together with the measured percentage of nonspecifically bound ^{125}I-peptide determined by directly counting the Epon blocks prior to sectioning. The latter was found to be 19–25% of that bound in the absence of radioactive peptide. Since the nonspecific background was low and the blocked and unblocked samples were not drastically different with respect to percent of surface localized grains, these corrections were minor.

§ Surface enrichment corrects for the relatively small volume of the surface shell (±1,500 Å) relative to the total cell volume. Assuming a spherical shape of 4.5-μM radius, the surface shell volume is 20% of the total cell volume. The radius of intact cells and cytoskeletons was approximately equal, i.e., 4–4.5 μm.

DISCUSSION

Our investigations have uncovered a number of novel features of the processing of *N*-formyl chemotactic peptides by human granulocytes that may be significant for leukocyte receptor mediated endocytosis and activation. In particular our results suggest that a significant percentage of the plasma membrane f-Met-Leu-Phe receptors rapidly form a transient, virtually nondissociating and noncovalent complex with the peptide at 37° and 15°C and not at 4°C. Since the association rate of the formyl peptide does not greatly vary with temperature (61), the greatly decreased rate of dissociation at 15°C and 37°C as compared with 4°C suggests that the receptor undergoes a true affinity change. The complex co-isolates with a cytoskeletal-like cell fraction with kinetics that overlap the time scale for leukocyte activation and endocytosis.

Specific Interaction with the Cytoskeleton

Solubilization of the cells by Triton X-100 permits removal of the cytosolic stored peptide (28). The remaining peptide demonstrates slow exchange rates with properties suggesting that, after isolation, the occupied receptor continues to be associated with the cytoskeletal structure in an altered, slowly dissociating form. There is ample evidence of cytoskeletal linkage of cell surface proteins and receptors in many cell types (5, 19, 30, 70) including neutrophils (59). An important special case of cytoskeletal-membrane interactions is that observed between microfilaments and coated pits (11, 52) resulting in endocytosis of surface proteins. Since we have not observed a 170,000-dalton band characteristic of clathrin in electrophoretic analyses (Fig. 3) or correlation of frequency of autoradiographic grain enrichment around coated pits (R. Painter, R. Allen, L. Sklar, M. Schmitt, C. Cochrane, and A. Jesaitis, unpublished results), participation of a coated pit mechanism seems unlikely. Other linkages induced by plant lectins or antibodies have, however, been demonstrated in a number of systems (11, 17, 30, 31, 38, 48, 67, 68). The chemotactic peptide receptor system is unique in that the observed association with cytoskeleton involves a monovalent ligand.

At this juncture, we cannot unequivocally state that there is a specific association of the occupied, surface receptor with the cell cytoskeleton in the sense of protein-protein interactions between actin or a series of undefined actin linkage proteins. However, a number of results are compatible with this hypothesis. First, our previous work has shown that a Triton X-100-insoluble, high-molecular-weight complex containing receptor can be isolated from the purified plasma membranes of f-Met-Leu-Phe stimulated cells (28). This insolubility cannot be due to inherent insolubility of the receptor in Triton as reported in the case for t-RNA aminoacyl transferase (12), since we have shown that affinity-photolabeled receptor can be completely extracted from plasma membrane of unstimulated cells in a form having a Stokes' Radius of <50 Å (49). Second, most of the unoccupied receptors must either be solubilized or inactivated since f-Met-Leu-Phe binding activity of the cytoskeletal fractions showed no detectable decrease after significant depletion of the surface pool of receptors by conversion to their high affinity occupied form at 15°C (A. J. Jesaitis, unpublished results). Third, in studies now in progress we have also shown that in the presence of the cytoskeletal disrupting agent, cytochalasin B, there was 80% inhibition of receptor uptake as well as formation of the cytoskeletally-associated, high-affinity, receptor-ligand complex. Fourth, since there was transient complex formation at 37°C and minimal formation at 4°C, then the regulation of the complex must be under cellular control. Finally, the surface nature of the complex can be inferred from the autoradiographic results (Fig. 9) and from its formation under conditions in which internalization did not occur, such as at 15°C or up to 1 min after exposure to peptide at 37°C (Figs. 8 and 5, respectively). Although these observations favor a specific interaction between the cytoskeleton and the chemotactic peptide receptor at the plasma membrane, we cannot, however, exclude the possibility that the Triton solubility of the receptor is dependent on another membrane constituent, possibly a critical lipid not extracted by our procedure, but still under cellular control and able to modulate membrane protein interactions.

Significance of High Affinity Receptor Complexes Anchored to the Cytoskeleton

In PC12 cells a lectin-induced linkage between cytoskeleton and nerve growth factor receptor is associated with a large decrease in off rate of the nerve growth factor (67). An analogous effect is also seen with the insulin receptor system (55). One interpretation of the changes experienced by surface receptors involves an extension of the mobile receptor hypothesis (7, 24) which states that receptors interact with "effector" molecules in the plane of the membrane to alter their own physical and functional state (affinity change and

activation). The effector molecules in this case would be a cytoskeletal or cytoskeletal-associated protein as proposed by Vale and Shooter (67).

The functional significance of the high affinity "anchored" form of the receptor is still obscure and currently under investigation in our laboratory. Becker, however, (4) has emphasized that the receptor is multifunctional with a sensitivity to chemotactic peptide that can vary in order of magnitude depending on the response measured. From the relative dissociation rates of the receptor ligand complex formed in resting (4°C) and stimulated cells (37°C) we estimate an affinity increase of ~100-fold. In addition, formation of the complex between 5 and 60 s at various temperatures occurs at an approximately linear rate and, when extrapolated to zero time, would suggest an upper limit of ~2,000–3,000 high affinity preanchored receptors in the resting cell, ~5% of the total. Such receptors might be a manifestation of lateral surface compartmentation (34, 62) required to regulate functional diversity and may have been responsible for the nonlinear Scatchard plots observed by others (21, 57). In particular, they could correspond to the small population (10% of the total) of chemotactic peptide receptors having an ~50-fold higher affinity than that of the bulk receptors reported by Koo et al. (32) in isolated membranes from human granulocytes. Since the affinity of these receptors appears to be under guanyl nucleotide control, participation of the high affinity receptors in sensory transduction, such as in the visual or β-adrenergic systems, seems plausible. The fact that these receptors may be directly linked to the motile apparatus, reduces the necessity of having multiple intermediate transduction steps or second messengers intervene between the receptor and effector. This fact, together with the stimulation of chemokinesis demonstrating the highest sensitivity (4) to formyl peptides (i.e., highest affinity receptors), makes this hypothesis more credible.

The high affinity complex itself could play a role as an intermediate in the sensory transduction process. Sklar et al. (60) have shown that only a 10% occupancy of receptor is required to achieve a maximum response for several neutrophil responses. The number of high affinity, anchored complexes observed after 5 s of incubation with 50 nM peptide at 37°C is ~2,000–3,000, which represents ~10–20% of the number occupied at that time. Therefore, a 10% occupancy requirement would imply a conversion of 1–2% (i.e., 500–1,000) of the total receptors population to the complexed high affinity form. Since Zigmond (71) has calculated that the sensitivity limit of neutrophil for chemotaxis is an occupancy differential of only 10 receptors, it is clear that the neutrophil is exquisitely sensitive to extremely low levels of receptor occupancy, and by extrapolation, small numbers of ligand-receptor-effector complexes.

Probably the most apparent role for the high affinity receptor-cytoskeletal complex is to be a vehicle for endocytosis and removal of occupied receptors (and functioning receptor-effector complexes) from the cell surface, as has been suggested by Condeelis (11) and Salisbury et al. (52) in *Dictyostelium* and cultured B lymphoblastoid cells. Therefore, we hypothesize that the "saturating level" of peptide co-isolated with cytoskeleton (at 5 min) represents a steady state in which the complex is removed from the surface (Fig. 7) by endocytosis (27, 43) and replenished (Fig. 8) by receptor occupancy after expression of additional surface receptors during recycling processes (72) or expression of the latent or internal receptor pool (18, 20, 26). Thus, whether the high affinity receptor-cytoskeletal complex participates in sensory transduction, response regulation, or only as the carrier for surface clearance of occupied receptors is still an open question. We are currently investigating the molecular organization and functional composition of these complexes and thus expect answers to some of these questions to be forthcoming.

The authors wish to thank Mrs. Monica Bartlett and Mrs. Dian Caudebec for typing the manuscript and James Tolley for technical assistance on parts of this study.

This research was supported by National Institutes of Health Grants AI-17354 and HL-16411. R. G. Painter is the recipient of the National Institutes of Health Research Career Development Award AM-00437.

Received for publication 20 July 1983, and in revised form 28 November 1983.

REFERENCES

1. Amreim, P. C., and T. P. Stossel. 1980. Prevention of degradation of human polymorphonuclear leukocytes proteins by diisopropylfluorophosphate. *Blood.* 56:442–447.
2. Barak, L. S., R. R. Yocum, E. A. Nothnagel, and W. W. Webb. 1980. Fluorescence staining of the actin cytoskeleton in living cells with 7-nitrobenz-2-oxa-1,3-diazole-phallacidin. *Proc. Natl. Acad. Sci. USA.* 77:980–984.
3. Becker, E. L., P. H. Naccache, H. L. Showell, and R. W. Walenger. 1981. Early events in neutrophil activation: receptor stimulation, ionic fluxes, and arachidonic acid metabolism. *In* Lymphokines. E. Pick, editor. 4:297–334.
4. Becker, E. L. 1979. A multifunctional receptor on the neutrophil for synthetic chemotactic oligopeptides. *J. Reticuloendothel. Soc.* 26:701–709.
5. Bennett, V., and P. J. Stenbuck. 1979. The membrane attachment protein for spectrin is associated with band 3 in human erythrocyte membranes. *Nature (Lond.).* 280:468–473.
6. Ben-Ze'ev, A., A. Duerr, F. Solomon, and S. Perlman. 1979. The outer boundary of the cytoskeleton: a lamina derived from plasma membrane proteins. *Cell.* 17:859–865.
7. Boeynaems, J. M., and J. E. Dumont. 1975. Quantitative analysis of the binding of ligands to their receptors. *J. Cyclic Nucleotide Res.* 1:123–142.
8. Caro, L. G., and R. P. VanTubergen. 1962. High resolution autoradiography. I. Methods. *J. Cell Biol.* 15:173–188.
9. Cochrane, C. G. 1977. Role of granulocytes in immune complex induced tissue injuries. *Inflammation.* 2:319–333.
10. Condeelis, J. 1979. Isolation of convanavalin A caps during various stages of formation and their association with actin and myosin. *J. Cell Biol.* 80:751–758.
11. Condeelis, J. 1981. Microfilament-membrane interactions in cell shape and surface architecture. *In* Symposium of 2nd International Congress on Cell Biology Berlin. H. G. Schweiger, editor. Springer Verlag, Berlin. 306–320.
12. Dang, C. V., D. C. H. Yang, and T. D. Pollard. 1983. Association of methionyl-t RNA synthetase with detergent-insoluble components of rough endoplasmic reticulum. *J. Cell Biol.* 96:1138–1147.
13. Dodge, J. T., C. Mitchell, and D. J. Hanahan. 1963. The preparation and chemical characterization of haemoglobin-free ghosts of human erythrocytes. *Arch. Biochem. Biophys.* 100:119–130.
14. Edelman, G. M. 1976. Surface modulation in cell recognition and cell growth. *Science (Wash. DC).* 192:218–226.
15. Elgsaeter, A., and D. Branton. 1976. Intramembrane particle aggregation in erythrocyte ghosts. I. The effects of protein removal. *J. Cell Biol.* 63:1018–1036.
16. Finney, D. A., and L. A. Sklar. 1983. Ligand/receptor internalization: A kinetic, flow cytometric analysis of the internalization of N-formyl peptides by human neutrophils. Cytometry. 4:54–60.
17. Flanagan, J., and G. L. E. Koch. 1978. Cross-linked surface Ig attaches to actin. *Nature (Lond.).* 273:278–281.
18. Fletcher, M. P., B. E. Seligmann, and J. I. Gallin. 1982. Correlation of human neutrophil secretion, chemoattractant receptor mobilization, and enhanced functional capacity. *J. Immunol.* 128:941–948.
19. Fowler, V. M., E. J. Luna, W. R. Hargreaves, D. L. Taylor, and D. Branton. 1981. Spectrin promotes the association of F-actin with the cytoplasmic surface of the human erythrocyte membrane. *J. Cell Biol.* 88:388–395.
20. Gallin, J. E., E. K. Gallin, and E. Schiffmann. 1979. Mechanism of leukocyte chemotaxis. *In* Advances in Inflammation Research. G. Weissmann, editor. Raven Press, New York. 123–138.
21. Goetzl, E. J., D. W. Foster, and D. W. Goldman. 1981. Isolation and partial characterization of human neutrophil receptors for chemotactic formyl methionyl peptides. *Biochemistry.* 20:5717–5722.
22. Henson, P. M. 1980. Mechanisms of exocytosis in phagocytic inflammatory cells. *Am. J. Pathol.* 101:494–514.
23. Hoffstein, S. T., R. S. Friedman, and G. Weissmann. 1982. Degranulation, membrane addition and shape change during chemotactic factor-induced aggregation of human neutrophils. *J. Cell Biol.* 95:234–241.
24. Jacobs, S., and P. Cuatrecasas. 1976. The mobile receptor hypothesis and "cooperativity" of hormone binding. Application to insulin. *Biochem. Biophys. Acta.* 433:482–495.
25. Jesaitis, A. J., and J. Yguerabide. 1980. Lateral mobility of plasma membrane lipids and Na^+K^+ATPase in cultured canine kidney cells. *Fed. Proc.* 39:1703.
26. Jesaitis, A. J., J. R. Naemura, R. G. Painter, L. A. Sklar, and C. G. Cochrane. 1982. Intracellular localization of N-formyl chemotactic receptor and Mg^{2+} dependent ATPase in human granulocytes. *Biochem. Biophys. Acta.* 719:556–568.
27. Jesaitis, A. J., J. R. Naemura, R. G. Painter, L. A. Sklar, and C. G. Cochrane. 1982. The fate of the *N*-formyl chemotactic peptide receptor in human granulocytes. *J. Cell Biochem.* 20:177–191.

28. Jesaitis, A. J., J. R. Naemura, R. G. Painter, L. A. Sklar, and C. G. Cochrane. 1983. The fate of an N-formyl chemotactic peptide in stimulated human granulocytes. *J. Biol. Chem.* 258:1968–1977.
29. Klebanoff, S. J. 1975. Antimicrobial mechanisms in neutrophilic polymorphonuclear leukocytes. *Semin. Hematol.* 12:117–141.
30. Koch, G. L. E. 1981. The anchorage of cell surface receptors to the cytoskeleton. *In* Symposium of 2nd International Congress on Cell Biology. Berlin. H. G. Schweiger, editor. Springer Verlag. 321–330.
31. Koch, G. L. E., and M. J. Smith. 1978. An association between actin and the major histocompatability antigen H-2. *Nature (Lond.).* 273:274–278.
32. Koo, C., R. J. Lefkowitz, and R. Snyderman. 1983. Guanine nucleotides modulate the binding affinity of oligopeptide chemoattractant receptor on human polymorphonuclear leukocytes. *J. Clin. Invest.* 72:748–753.
33. Laemmli, U. K. 1970. Cleavage of structural protein during the assembly of the head of bacteriophage T_4. *Nature (Lond.).* 222:680–685.
34. Lazarides, E., and W. J. Nelson. 1983. Erythrocytes and brain forms of spectrin in cerebellum: Distinct membrane-cytoskeletal domains in neurons. *Science (Wash. DC)* 220:1295–1296.
35. Lenk, R., L. Ransom, Y. Kaufmann, and S. Penman. 1977. A cytoskeletal structure with associated polyribosomes obtained from HeLa cells. *Cell.* 10:67–78.
36. Luna, E. J., V. M. Fowler, J. Swanson, D. Branton, and D. L. Taylor. 1981. A membrane cytoskeleton from *Dictyostelium discoideum*. I. Identification and particle characterization of an actin-binding activity. *J. Cell Biol.* 88:396–409.
37. Maupin-Szamier, P., and T. D. Pollard. 1978. Actin filament destruction by osmium tetroxide. *J. Cell Biol.* 77:837–852.
38. Mescher, M. F., M. J. L. Jose, and S. P. Balk. 1981. Actin containing matrix associated with the plasma membrane of murine tumor and lymphoid cells. *Nature (Lond.).* 289:139–144.
39. Nicholson, G. L., and R. G. Painter. 1973. Anionic sites of human erythrocyte membranes. II. Antispectrin-induced transmembrane aggregation of the binding sites for positively charged colloidal particles. *J. Cell Biol.* 59:395–406.
40. Nicholson, G. L., and G. Poste. 1976. The cancer cell: dynamic aspects and modifications in cell surface organization. *New Eng. J. Med.* 295:197–258.
41. Niedel, J., J. Davis, and P. Cuatrecasas. 1980. Covalent affinity labeling of the formyl peptide chemotactic receptor. *J. Biol. Chem.* 255:7063–7066.
42. Niedel, J. E., S. Wilkinson, and P. Cuatrecasas. 1979. Receptor mediated uptake and degradation of ^{125}I-chemotactic peptide by human neutrophils. *J. Biol. Chem.* 254:10700–10706.
43. Niedel, J. E., I. Kahane, and P. Cuatrecasas. 1979. Receptor-mediated internalization of fluorescent chemotactic peptide by human neutrophils. *Science (Wash. DC).* 205:1412–1414.
44. Niedel, J., and P. Cuatrecasas. 1980. Formyl peptide chemotactic receptors of leukocytes and macrophages. *Curr. Top. Cell. Regul.* 17:137–170.
45. Nochumson, S., and S. G. Gibson. 1983. Oven drying of high percentage polyacrylamide slab gels using a new cross-linking agent. *BioTechniques.* 1:18–19.
46. Olsson, I., and P. Venge. 1980. The role of the human neutrophil in the inflammatory reaction. *Allergy.* 35:1–13.
47. Painter, R. G., and A. T. McIntosh. 1979. The regional association of actin and myosin with sites of particle phagocytosis. *J. Supramol. Struct.* 12:369–384.
48. Painter, R. G., and M. Ginsberg. 1982. Concanavalin A induces interactions between surface glycoproteins and the platelet cytoskeleton. *J. Cell Biol.* 92:565–573.
49. Painter, R. G., M. Schmitt, A. J. Jesaitis, L. A. Sklar, K. Preissner, and C. G. Cochrane. 1982. Photoaffinity labeling of the N-formyl peptide receptor of human polymorphonuclear leukocytes. *J. Cell. Biochem.* 20:203–214.
50. Peters, R. 1981. Translation diffusion in the plasma membrane of single cells as studied by fluorescence microphotolysis. *Cell Biology International Reports.* 5:733–760.
51. Saffman, P. G., and M. Delbrück. 1975. Brownian motion in biological membranes. *Proc. Natl. Acad. Sci. USA.* 72:3111–3113.
52. Salisbury, J. L., J. S. Condeelis, and P. Satir. 1980. Role of coated vesicles, microfilaments, an calmodulin in receptor-mediated endocytosis by cultured B lymphoblastoid cells. *J. Cell Biol.* 87:132–141.
53. Salpeter, M. M., H. C. Fertuck, and E. E. Salpeter. 1977. Resolution in electron microscope autoradiography. III. Iodine-125, the effect of heavy metal staining and reassessment of critical parameters. *J. Cell Biol.* 72:161–173.
54. Schecter, A. L., and M. A. Bothwell. 1981. Nerve growth factor receptors on PC12 cells: evidence for two receptor classes with differing cytoskeletal association. *Cell.* 24:867–874.
55. Schecter, Y., K.-J. Chang, S. Jacobs, and P. Cuatrecasas. 1979. Modulation of binding and bioactivity of insulin by antiinsulin antibody: relation to possible role of receptor self-aggregation in hormone action. *Proc. Natl. Acad. Sci. USA.* 76:2720–2724.
56. Schlessinger, J., Y. Schechter, P. Cuatrecasas, M. C. Willingham, and I. Pastan. 1978. Quantitative determination of the lateral diffusion coefficients of the hormone-receptor complexes of insulin and epidermal growth factor on the plasma membrane of cultured fibroblasts. *Proc. Natl. Acad. Sci. USA.* 75:5356–5357.
57. Seligmann, B. E., M. P. Fletcher, and J. I. Gallin. 1982. Adaptation of human neutrophil responsiveness to the chemoattractant N-formyl methionylleucyulphenylalanine. *J. Biol Chem.* 257:6280–6286.
58. Sheetz, M. P., M. Schindler, and D. E. Koppel. 1980. Lateral mobility of integral membrane proteins is increased in sperocytic erythrocytes. *Nature (Lond.).* 285:510–512.
59. Shelterline, P., and C. R. Hopkins. 1981. Transmembrane linkage between surface glycoproteins and components of the cytoplasm in neutrophil leukocytes. *J. Cell Biol.* 90:743–754.
60. Sklar, L. A., A. J. Jesaitis, R. G. Painter, and C. G. Cochrane. 1982. Ligand/receptor internalization: a spectroscopic analysis and a comparison of ligand binding, cellular response, and internalization by human neutrophils. *J. Cell Biochem.* 20:193–202.
61. Sklar, L. A., D. A. Finney, Z. G. Oades, A. J. Jesaitis, R. G. Painter, and C. G. Cochrane. 1984. The dynamics of ligand-receptor interactions. Real time analyses of association, dissociation and internalization of an N-formyl peptide and its receptors on the human neutrophil. *J. Biol. Chem.* In press.
62. Simionescu, N., M. Simionescu, and G. E. Palade. 1981. Differentiated microdomains on the luminal surface of capillary endothelium. I. Preferential distribution of anionic sties. *J. Cell Biol.* 90:605–613.
63. Synderman, R., and E. J. Goetzl. 1981. Molecular and cellular mechanisms of leukocyte chemotaxis. *Science (Wash. DC).* 213:830–837.
64. Spitznagel, J. D. 1977. Bactericidal mechanisms of the granulocyte. *In* The Granulocyte: Function and Clinical Utilization. T. J. Greenwalt and G. A. Jamieson, editors. Alan R. Liss, Inc., New York. 103–131.
65. Sullivan, S. J., and S. H. Zigmond. 1980. Chemotactic peptide receptor modulation in polymorphonuclear leukocytes. *J. Cell Biol.* 85:703–711.
66. Tank, D. W., E. S. Wu, and W. W. Webb. 1981. Enhanced molecular diffusibility in muscle membrane blebs: release of lateral constraints. *J. Cell Biol.* 92:207–212.
67. Vale, R. D., and E. M. Shooter. 1982. Alteration of binding properties and cytoskeletal attachment of nerve growth factor receptors in PC12 cells by wheat germ agglutinin. *J. Cell Biol.* 94:710–717.
68. Woda, B. A., and M. L. McFadden. 1983. Association of lymphocyte membrane proteins with the detergent insoluble cytoskeletal matrix. *Fed. Proc.* 42:321a. (Abstr.)
69. Woda, B. A., J. Yguerabide, and J. D. Feldman. 1980. The effect of local anesthetics on the lateral mobility of lymphocyte membrane proteins. *Exp. Cell Res.* 126:327–331.
70. Yu, J., D. A. Fischman, and T. L. Steck. 1973. Selective solubilization of proteins and phospholipids from red blood cell membranes by nonionic detergents. *J. Supramol. Struct.* 1:233–248.
71. Zigmond, S. H. 1981. Consequences of chemotactic peptide receptor modulation for leukocyte orientation. *J. Cell Biol.* 88:644–647.
72. Zigmond, S. H., S. J. Sullivan, and D. A. Lauffenburger. 1982. Kinetic analysis of chemotactic peptide receptor modulation. *J. Cell Biol.* 92:34–43.