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Covalent Affinity Labeling, Detergent Solubilization, and Fluid-Phase Characterization of the Rabbit Neutrophil Formyl Peptide Chemotaxis Receptor†

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ABSTRACT: The formyl peptide chemotaxis receptor of rabbit neutrophils and purified rabbit neutrophil plasma membranes has been identified by several affinity labeling techniques: (1) covalent affinity cross-linking of *N*-formyl-Nle-Leu-Phe-Nle-^{125}I-Tyr-Lys (^{125}I-hexapeptide) to the membrane-bound receptor with either dimethyl suberimidate or ethylene glycol bis(succinimidyl succinate) and (2) photoactivation of *N*-formyl-Nle-Leu-Phe-Nle-^{125}I-Tyr-N^{ϵ}-[6-[(4-azido-2-nitrophenyl)amino]hexanoyl]Lys (^{125}I-PAL). These techniques specifically identify the receptor as a polypeptide that migrates as a broad band on sodium dodecyl sulfate–polyacrylamide electrophoresis, with M_r 50 000–65 000. The receptor has been solubilized in active form from rabbit neutrophil membranes with the detergents 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) and digitonin and from whole cells with CHAPS. Chemotaxis receptor activity was measured by the ability of the solubilized membrane material to bind ^{125}I-hexapeptide or fMet-Leu-[^{3}H]Phe with gel filtration or rapid filtration through poly(ethylenimine)- (PEI) treated filters as assay systems. ^{125}I-PAL was specifically cross-linked to the same molecular weight material in the CHAPS and digitonin solubilized extract, but no specific labeling of the receptor was seen when membranes were extracted with Nonidet P-40 and Triton X-100. Therefore, although a large number of detergents are able to solubilize the receptor, it appears that some release the receptor in an inactive form. The ligand binding characteristics of fMet-Leu-[^{3}H]Phe to the CHAPS-solubilized receptor shared properties with the membrane-bound formyl peptide receptor, both of which showed curvilinear, concave-upward Scatchard plots. Computer curve fitting with NONLIN and statistical analyses of the binding data indicated that for both the membrane-bound and solubilized receptors a two saturable sites model fitted the data significantly better ($p < 0.01$) than did a one saturable site model. The characteristics of the two saturable sites model for the soluble receptor were a high-affinity site with a K_D value of 1.25 ± 0.45 nM and a low-affinity site with a K_D value of 19.77 ± 3.28 nM. A total of 35% of the two sites detected was of the higher affinity. In addition, a Hill coefficient of 0.61 ± 0.12 was observed.

Structural identification and detergent solubilization of specific membrane receptors is required to understand ligand–receptor interactions and the subsequent activation pathways that result in a multitude of effector responses. Since the initial discovery of the formyl peptide receptors (Showell et al., 1976; Aswanikumar et al., 1977; Williams et al., 1977) and the numerous biological responses that are mediated through them [reviewed in Becker (1979), Snyderman & Goetzl (1981), and Becker & Marasco (1985)], structural information has lagged behind the advances made in our understanding of the activation pathways and other functional properties of the receptor. Only recently has limited structural information been obtained. Niedel et al. (1980) first showed that the receptor can be covalently affinity labeled and also extracted from the neutrophil in a soluble active form (Niedel, 1980), findings that have been abundantly confirmed (Dolmatch & Niedel, 1983; Schmitt et al., 1983; Baldwin et al., 1983). The receptor on the human neutrophil is a glycoprotein

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of M_r 55 000–70 000 as judged by SDS–PAGE.[1] Goetzl et al. (1981) have shown specific elution of three major adsorbed proteins (apparent M_r 94 000, 68 000, and 40 000) following detergent solubilization of human neutrophil membranes and affinity absorption with *N*-formyl-Met-Leu-Phe–Sepharose. Papain treatment of intact human neutrophils converts the receptor from an M_r of 55 000–70 000 to an M_r of 28 000–35 000 with no change in its ability to bind *N*-formyl peptides or in its biological activity (Dolmatch & Niedel, 1983). In addition, solubilized partially purified receptor from the HL-60 myeloid leukemia cell line has been reconstituted into liposomes with apparently unchanged *N*-formyl peptide binding characteristics (Hoyle & Freer, 1984).

We have begun a systematic study aimed at obtaining detailed structural information on the rabbit neutrophil formyl peptide receptor. In the present paper, we report on the identification, solubilization, and detailed binding characteristics of the fluid-phase receptor. Analysis of ligand binding demonstrated Hill coefficients <0.7 and curvilinear Scatchard plots similar to those obtained for whole cells suggesting (1) the presence of negatively cooperative interactions among solubilized receptors and/or (2) the presence of multiple classes of receptors or (3) the solubilization of a complex involving the interaction of the receptor with an additional membrane component(s).

Experimental Procedures

Materials

Sodium azide, 2-deoxyglucose, oyster glycogen (type II), Me_2SO, poly(ethylenimine), Triton X-100, and fMet-Leu-Phe were purchased from Sigma Chemical Co., St. Louis, MO. Nonidet P-40 and digitonin were from BDH Chemicals Ltd., Poole, England. fMet-Leu-[^{3}H]Phe (sp act. 47.6–60.0 Ci/mmol) was purchased from New England Nuclear, Boston, MA. fNle-Leu-Phe-Nle-Tyr-Lys was purchased from Peninsula Laboratories, Inc., Belmont, CA. Ethylene glycol bis(succinimidyl succinate), *N*-hydroxysuccinimidyl 6-[(4-azido-2-nitrophenyl)amino]hexanoate, dimethyl suberimidate hydrochloride, and CHAPS detergent were obtained from Pierce Chemical Co., Rockford, IL.

Methods

Cells. Rabbit were injected with 400 mL of 0.1% oyster glycogen in sterile saline, and the cells were recovered 18 h later. Morphologic analysis showed greater then 96% polymorphonuclear leukocytes as determined by differential Wright's stain. To obtain a sufficient yield of cells for the preparation of plasma membranes, cells from four to six rabbits were pooled.

Preparation of Neutrophil Plasma Membranes. Rabbit neutrophil plasma membranes were prepared by a modification of the method of Woodin & Wienecke (1966). In brief, (2.5–5) × 10^9 cells were collected and washed in Hank's buffer. The cells were then resuspended to 50 mL in 11.6% (w/v) sucrose (10 mM HEPES, 1 mM EDTA, 10 μM PMSF), incubated at 37 °C for 15 min, and centrifuged for 2500*g*·min. Resuspension, incubation, and centrifugation were repeated twice. The cells were resuspended to 15 mL in the 11.6% (w/v) sucrose solution and subjected to 600 psi of nitrogen in a pressure chamber (Artisan Industries, Inc., Waltham, MA) for 30 min at 4 °C with continuous stirring. The pressure was released over a 2-min period. The disrupted cells were centrifuged for 1000*g*·min, yielding a pellet, supernatant, and foam. The combined pellet and foam were resuspended to 7.5 mL in the 11.6% (w/v) sucrose solution and homogenized, for an additional 3 min with a Fischer Dyna-Mix (setting 6).

The resulting supernatant, along with the original low-speed supernatant, was layered onto discontinuous sucrose gradients constructed from 10.8 mL of 50% (w/v) sucrose, 8.4 mL of 40% (w/v) sucrose, and 10.8 mL of 30% (w/v) sucrose. All sucrose solutions contained 10 mM HEPES, 1 mM EGTA, and 10 μM PMSF (pH 7.0–7.2). The supernatants were centrifuged for 10^7*g*·min at 4 °C (Beckman L5-65B ultracentrifuge, SW28 rotor). Membrane proteins from the 11.6%–30% (band 1), 30%–40% (band 2), and 40%–50% (band 3) sucrose interfaces were separately collected, diluted in phosphate-buffered saline (2.14 mM $NaH_2PO_4H_2O$, 9.15 mM Na_2HPO_4, 268 mM NaCl, pH 7.2), and pelleted by centrifugation for 1.8 × 10^5*g*·min. Band 1 and band 2 pellets were resuspended to a final volume of 1 mL each, while the band 3 pellet was resuspended to 2 mL. Protein concentration was determined with a Bio-Rad protein assay kit (*Bio-Rad Protein Assay Instruction Manual*, 1979), with bovine serum albumin as the standard.

In experiments where more membrane material was required than could be prepared as band 2, a crude membrane preparation was used (i.e., the experiments shown in Table I, Table II, and Figure 6). In brief, rabbit neutrophils, collected as described above, were resuspended in 10 mM Tris-HCl buffer (pH 7.4), washed twice, and resuspended to 3 × 10^7 cells/mL in 0.34 M sucrose in 10 mM Tris-HCl buffer, pH 7.4. The cells were then sonicated on ice in four 15-s, 30-W bursts with a Model W-255R sonicator from Heat-Systems-Ultrasonics Inc., Plainview, NY. The cells were then centrifuged at 4 °C for 2000*g*·min. The supernatant was then centrifuged for 2.5 × 10^6*g*·min. The pelleted material was resuspended in phosphate-buffered saline (PBS) and assayed for protein as described above.

Synthesis, Purification, and Characterization of the Photoactivatable Hexapeptide Derivative. The PAL derivatized formyl peptide was synthesized according to the method described by Schmitt et al. (1983) with several modifications. In brief, formyl-Nle-Leu-Phe-Nle-Tyr-Lys (1.2 μmol), *N*-hydroxysuccinimidyl 6-[(4-azido-2-nitrophenyl)amino]hexanoate (2.4 μmol), and triethylamine (2.5 μmol) were reacted in 0.5 mL of dry dimethyl formamide for 16 h at 24 °C in the dark. The crude orange-red reaction product was separated and purified from free *N*-hydroxysuccinimidyl 6-[(4-azido-2-nitrophenyl)amino]hexanoate and free formyl-Nle-Leu-Phe-Nle-Tyr-Lys by HPLC as described below.

High-Performance Liquid Chromatography of Formyl Peptides. All formyl peptides used in this study were analyzed for purity by HPLC as previously described (Marasco et al., 1984). In brief, the liquid chromatography system consisted of a Varian LC-5020 and Varichrom variable-wavelength detector. The sample was run through a reverse-phase C_{18} 5-μm (MCH5) particle size column (Varian Instruments). Chromatographic conditions were as follows: flow rate 1.0 mL/min, temperature 30 °C, gradient elution from 20% (v/v)

[1] Abbreviations: fMet-Leu-Phe, formylmethionylleucylphenylalanine; Nle, norleucine; hexapeptide, *N*-formyl-Nle-Leu-Phe-Nle-Tyr-Lys; ^{125}I-PAL, *N*-formyl-Nle-Leu-Phe-Nle-^{125}I-Tyr-N^{ϵ}-[6-[(4-azido-2-nitrophenyl)amino]hexanoyl]Lys; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; Me_2SO, dimethyl sulfoxide; PMSF, phenylmethanesulfonyl fluoride; CHAPS, 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate; PEI, poly(ethylenimine); TFA, trifluoroacetic acid; EGS, ethylene glycol bis(succinimidyl succinate); HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; EDTA, ethylenediaminetetraacetic acid; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; Tris-HCl, tris(hydroxymethyl)-aminomethane hydrochloride.

to 45% (v/v) acetonitrile in H_2O contaning 0.1% (v/v) TFA throughout, over 60 min. Spectrophotometric detection was set at 210 nm. Under these conditions, column pressure never exceeded 250 atm. The tripeptide fMet-Leu-[^{3}H]Phe appeared as a single peak at 45 min, corresponding exactly to the retention time of unlabeled fMet-Leu-Phe (Marasco et al., 1984). Formyl-Nle-Leu-Phe-Nle-Tyr-Lys eluted as a single peak at 24 min under the following conditions: isocratic 25% acetonitrile in H_2O plus 0.1% TFA for 10 min followed by a gradient of 40%–55% CH_3CN over 32 min. When the PAL derivative was being purified, elution of the material was carried out under isocratic conditions (10% acetonitrile in H_2O containing 0.1% TFA throughout) for 5 min followed by a gradient of 10%–65% CH_3CN over 80 min. Under these conditions, two major orange zones were observed, one at 62 min corresponding to free *N*-hydroxysuccinimidyl 6-[(4-azido-2-nitrophenyl)amino]hexanoate and one at 74 min that was biologically active and represented the PAL-derivatized peptide. Throughout these procedures, the product was protected from light.

For analysis of both biological activity and percent recovery of products, 1-min fractions were collected, dried in a Speed Vac (Savant Instruments) rotary evaporator, resuspended to the original volume in dimethyl formamide, and assayed for lysosomal enzyme releasing activity as previously described (Marasco et al., 1984). Activity recovered was 50%–70% of starting activity.

Radioiodination of Chemotactic Peptides. Radioiodination of formyl-Nle-Leu-Phe-Nle-Tyr-Lys was achieved as described (Niedel et al., 1979) with several modifications. Formyl-Nle-Leu-Phe-Nle-Tyr-Lys (2 mM) freshly dissolved in Me_2SO was diluted to 0.2 mM with methanol for use in the iodination procedure. To 10 μL of formyl-Nle-Leu-Phe-Nle-Tyr-Lys (2 nmol) was added 40 μL of 0.5 M KPO_4, pH 7.4, 2–3 mCi of carrier-free Na^{125}I, and 30 μL of 0.35 mM chloramine T (10 nmol) in water, and the reaction was allowed to proceed for 10 min at room temperature before being quenched with 15 μL of 0.52 mM sodium metabisulfite. The identical procedure was used for the radioiodination of the PAL-derivatized peptide.

For purification of the radioiodinated chemotactic peptides, one of two methods was used. In our earlier experiments, the reaction mixture was fractionated on a 35-mL Bio-Gel P-2 column equilibrated with 25 mM NaOH. The reaction mixture was counted for total ^{125}I added with a calibrated external source. A small aliquot (5 μL) of each 1-mL fraction was subsequently counted, and the excluded-volume and retained-volume ^{125}I peaks of the P-2 column were integrated to measure percent incorporation into the peptide. In our more recent experiments, purification by HPLC was used. The HPLC system was a Spectrophysics SP-8700. The sample was run through a reverse-phase C_{18} 10-μm particle size column (Altech 600-RPA). Chromatographic conditions were as follows: flow rate 1.0 mL/min, temperature 30 °C, isocratic elution [20% (v/v) acetonitrile in H_2O containing 0.05% (v/v) TFA throughout] for 5 min followed by a gradient of 20%–60% acetonitrile over 40 min and then isocratic elution with 60% acetonitrile for an additional 35 min. ^{125}I-Hexapeptide eluted as two peaks at 47 and 53 min. These peaks contained 35% and 65% of the incorporated radiolabel, respectively, and corresponded to mono- and diiodinated derivatives of the peptide (Dr. Gerald Nordblom, unpublished observations). Similarly, monoiodinated ^{125}I-PAL was eluted at 56 min (60% of incorporated label) and diiodinated ^{125}I-PAL eluted at 62 min (40% of incorporated label). The samples were neutralized with 250 mM K_2HPO_4 and stored at 4 °C. The specific activities of these various peptides ranged from 500 to 1500 Ci/mmol.

Measurement of fMet-Leu-[^{3}H]Phe and fNle-Leu-Phe-Nle-^{125}I-Tyr-Lys Binding to Viable Neutrophils and Neutrophil Plasma Membranes. Binding of fMet-Leu-[^{3}H]Phe and fNle-Leu-Phe-Nle-^{125}I-Tyr-Lys to whole cells was measured with the silicone oil centrifugation assay [Mackin et al., 1982; as modified by Marasco et al. (1985)]. Specifically, (2.0–2.5) × 10^6 rabbit neutrophils in 0.1 mL of Hank's buffer was gently layered onto 0.5 mL of SF1250 silicone fluid (density 1.05 g/cm^3) (General Electric, Silicone Products Department, Waterford, NY) contained in a 1.5-mL plastic microcentrifuge tube (Walter Sarstedt, Inc., Princeton, NJ). Five microliters of an appropriate dilution of labeled peptide was then added to the cells. A single concentration of the appropriate unlabeled peptide (80 μM fMet-Leu-Phe or 200 nM hexapeptide) was used to determine nonspecific binding. The cells were then incubated for 25 min at 4 °C, conditions found to be sufficient to establish equilibrium. The studies were done at 4 °C with 10 mM 2-deoxyglucose and 10 mM sodium azide in the incubating buffer to minimize internalization of the labeled peptide (Marasco et al., 1983, 1985). After incubation, the tubes were centrifuged for 2 min in an Eppendorf microcentrifuge (Brinkmann Model S412), a time sufficient to pellet the cells. After aspiration of the buffer and oil, the bottom of the microfuge tube was cut off, and the cell pellet was solubilized with 0.75 mL of a 1:1 Protosol–toluene mixture. The scintillation vials were capped, vortexed, and allowed to sit overnight at room temperature. After 18 h, 5.0 mL of scintillation fluid [Omnifluor (16 g/gal) in toluene] was added, the tubes were again vortexed, and their radioactivity was determined. For ^{125}I-hexapeptide binding, the cell pellet was counted directly in a gamma counter (Beckman Model LS7000). Nonspecific binding was generally 5%–10% of total fMet-Leu-[^{3}H]Phe binding and 20%–30% of total ^{125}I-hexapeptide binding. Data points were determined in triplicate and averaged.

^{125}I-Hexapeptide binding to purified rabbit neutrophil plasma membranes (band 2) was also measured. Fifty microliters of a 600 μg/mL solution of band 2 membrane in phosphate-buffered saline was layered onto 75 μL of a mixture (151:49) of F50 and F96 silicone fluids (General Electric, Silicone Products Department, Waterford, NY) in a 500-μL plastic microcentrifuge tube. The F50 and F96 oils were mixed to attain a density that would allow the membranes to pellet. Bound radioactivity was quantitated and analyzed as described for whole cells. Data points were determined in duplicate.

Analysis of Binding Data. The binding data were analyzed with NONLIN, a weighted nonlinear least-squares regression analysis computer program (Metzler et al., 1974). Nonspecific binding was first subtracted from total binding either as individual points or as a parameter. Similar results were obtained with both methods. The binding data were fit to one or more of the appropriate equations for a one saturable site model, a two saturable site model, and the Hill equation as previously described (Fischel & Medzikradsky, 1981; Marasco et al., 1985). Initial estimates of apparent dissociation constants (K_D) and binding capacities (nM) for use with the NONLIN program were determined by linear regression analysis of data plotted according to the method of Scatchard (1949). An initial estimate of 0.75 was used for the Hill coefficient when fitting the Hill equation. When a good fit was obtained, higher and lower values (0.5 and 1.0) were tested to assure that convergence had not occurred upon a local minimum

(Boxenbaum et al., 1974). The weighting factor, $1/y_{obsd}$, was selected only after determining that the variance was a linear function of the dose of fMet-Leu-[^{3}H]Phe added to the reaction (Finney & Phillips, 1977). The weighted sums of squared deviations (WSSDs) generated by NONLIN for a one saturable site model, a two saturable sites model, and the Hill equation were compared by the F test (Boxenbaum et al., 1974). In addition, the data were subjected to a Runs test (Freund, 1971; Beyer, 1966) to further demonstrate the fit to the theoretical curve.

Covalent Affinity Labeling of the Formyl Peptide Chemotaxis Receptor. Dimethyl suberimidate cross-linking was performed as described by Niedel et al. (1980). In brief, 5 μg of membrane protein and 5 nM ^{125}I-hexapeptide in 50 μL of Hank's buffer, pH 6.75, were incubated for 30 min at 24 °C. Nonsaturable cross-linking was determined in the presence of 500 nM unlabeled formyl peptide. Following the 30-min binding incubation, 10 μL of dimethyl suberimidate (10 mg/mL) in 75 mM sodium phosphate, pH 9.0, was added, and the incubation was continued for 30 min at 37 °C. Cross-linking was quenched by the addition of 10 μL of 5 mM glycine in water, and the reaction mixture was cooled to 4 °C.

Ethylene glycol bis(succinimidyl succinate) cross-linking was performed by a modification of the method of Niedel (1981). In brief, 5 μg of membrane protein and 5 nmol of ^{125}I-hexapeptide in 50 μL of 10 mM potassium phosphate, pH 6.75, were incubated for 30 min at 24 °C. Nonsaturable cross-linking was determined in the presence of 500 nM unlabeled formyl peptide. Ten microliters of ethylene glycol bis(succinimidyl succinate), which was diluted 1:4 in 10 mM HEPES from a stock solution in Me_2SO of 20 mg/mL, was immediately added to the binding incubation and the reaction continued for 15 min at 22 °C. Cross-linking was quenched by the addition of 10 μL of 5 mM glycine in water and the reaction mixture was cooled to 4 °C. In preliminary experiments, with ethylene glycol bis(succinimidyl succinate) final concentrations from 0.05 to 1.0 mg/mL, 0.5 mg/mL was shown to give the highest efficiency of affinity labeling, with acceptable protein–protein cross-links (Figure 1). The apparent molecular weight of the receptor was unaffected by concentration of EGS, although some aggregation appeared at the highest concentration tested.

Photoaffinity labeling with the ^{125}I-PAL-derivatized peptide was performed with modifications of methods previously described (Dolmatch & Niedel, 1983; Schmitt et al., 1983). Specifically, 10 μg of purified rabbit plasma membrane was incubated with 8 nM ^{125}I-PAL in Hank's buffer (pH 6.75) at 24 °C for 30 min in the absence of light. Photolysis was performed for 5 min at 24 °C (12 cm from a 360-nm ultraviolet light source, Gelman Sci. Inc., Model 51438). Nonspecific binding was determined in the presence of either 8 μM unlabeled PAL or unlabeled hexapeptide. After photolysis, the labeled membranes were immediately run on SDS–PAGE.

Electrophoresis. SDS–PAGE was performed with a modification of the method of Laemmli (1970) in a minigel slab (1.5 mm) system with 8 M urea incorporated into the gel matrix. The entire reaction mixture was layered on the gel without washing away unbound ^{125}I-hexapeptide. The samples were run at 30 mA/side, stained in 0.05% Coomassie brilliant blue R-250 in 10% acetic acid and 30% methanol, destained in 10% acetic acid, and dried in filter paper under vacuum. Kodak X-Omat AR film (XAR-5) was exposed to the dried gels for 6 h to 7 days with a Cronex Lightning-Plus enhancing screen. Analysis of the autoradiographs by scanning densitometry [Zeinek (soft laser) scanning densitometer, Biomed Instruments, Inc.] was used to determine the extent of solubilization of the membrane receptor labeled with ^{125}I-PAL. In some cases, the gels were silver stained with a Bio-Rad silver staining kit. Molecular weight standards were run on every gel with either an electrophoresis calibration kit (Pharmacia Fine Chemicals, Piscataway, NJ) or ^{14}C-labeled standards (Bethesda Research Laboratoreis, Inc., Rockville, MD).

Detergent Solubilization of the Formyl Peptide Chemotaxis Receptor. (A) Rabbit Neutrophil Membranes. Membrane fractions were solubilized with 10 mM CHAPS in Hank's buffer. CHAPS at 10 mM was used only after determining that this was the optimum detergent concentration for solubilization of ^{125}I-hexapeptide binding activity (see Results). Rabbit membranes (5–10 mg/mL) in 10 mM CHAPS were placed in a glass–glass homogenizer and homogenized with 20 manual strokes during a 30-min incubation at room temperature. The homogenizer was then centrifuged at 250g for 5 min in a Beckman (Model TJ-6) table-top centrifuge. The resulting supernatant was centrifuged for $10^7 g$·min at 4 °C to remove any contaminating membrane residue. The final supernatant was then assayed for fMet-Leu-[^{3}H]Phe or ^{125}I-hexapeptide binding activity or stored at −20 °C.

(B) Whole Rabbit Neutrophils. One-milliliter aliquots of cells (5×10^7 cells) in Hank's buffer with the various detergent combinations were homogenized at 24 °C for 30 min with 20 strokes of a glass–glass homogenizer. The homogenate was then transferred to an Eppendorf tube and centrifuged for 15 min at 4 °C. Triplicate 100-μL aliquots of the resulting supernatant were assayed for soluble receptor activity by analyzing ^{125}I-hexapeptide binding in the PEI-treated filter assay (see below).

Solubilized Receptor Assays. (A) Gel Filtration. Receptor-bound and free radiolabeled formyl peptides were separated by gel filtration through 2.3-mL columns (AcA-44) (LKB Instruments, Inc., Gaitherburg, MD) that were equilibrated at 4 °C in the appropriate buffer and detergent to be tested. The columns were constructed out of 5-mL siliconized glass pipets. Solubilized membrane extracts (50–120 μg) were incubated with either of the radiolabeled formyl peptides for 30 min at 24 °C. The entire 100-μL reaction mixture was then cooled to 4 °C, applied to the column, and washed through with the equilibrating buffer and detergent. Three-drop fractions were collected at a flow rate of approximately 4.5 mL/h. Duplicate determinations were performed for total binding and nonspecific binding and the results reported as specific binding (total binding vs. nonspecific binding). Nonspecific binding was determined in the presence of an excess of the corresponding unlabeled formyl peptide.

(B) Poly(ethylenimine)-Treated Filters. Solubilized receptor preparations were also assayed for binding activity with poly(ethylenimine)- (PEI-) treated glass fiber filters in a rapid filtration assay (Bruns et al., 1983). The 10% stock PEI solutions were first prepared by diluting commercial 50% PEI free base 1:5 with distilled water. Stock solutions of 10% PEI were kept for up to 2 weeks at 4 °C. Whatman GF/C glass fiber filters were soaked in a fresh 0.3% PEI solution (pH 10) (1:33 dilution of 10% stock PEI in distilled water) for 1–24 h and were not washed before use.

In brief, 50 μL of solubilized membrane in 10 mM CHAPS containing 10–30 μg of protein was added to 50 μL of Hank's buffer containing various concentrations of ^{125}I-hexapeptide or fMet-Leu-[^{3}H]Phe in siliconized 12 × 75 mm test tubes. After 30 min, 2 mL of Hank's buffer (4 °C) was added, and the tubes were vortexed. Binding was then terminated by rapid filtration through the PEI-soaked filters. The filters were

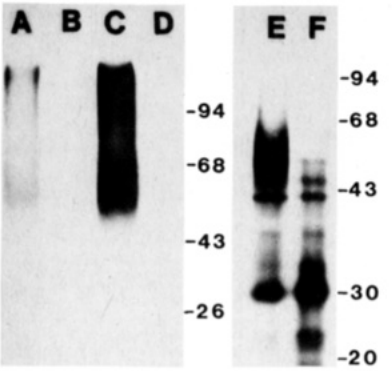

FIGURE 1: Autoradiographs demonstrating covalent affinity radiolabeling of the M_r 50 000–65 000 receptor polypeptide. ^{125}I-Labeled formyl peptide at 5 nM was incubated with 5 μg of band 2 membrane in the absence (lanes A and C) and presence (lanes B and D) of 500 nM unlabeled formyl peptide. (Lanes A and B) Dimethyl suberimidate cross-linked ^{125}I-formyl peptide; (lanes C and D) ethylene glycol bis(succinimidyl succinate) cross-linked ^{125}I-formyl peptide; (lanes E and F) 8 nM ^{125}I-PAL was incubated with 10 μg of band 2 membrane in the absence (lane E) or presence (lane F) of 800 nM unlabeled formyl peptide. Lanes A–D ^{14}C-Labeled molecular weight standards: phosphorylase *b*, 94 000; bovine serum albumin, 68 000; ovalbumin, 43 000; α-chymotrypsinogen, 25 700. Lanes E and F Molecular weight standards: phosphorylase *b*, 94 000; bovine serum albumin, 68 000; ovalbumin, 43 000; carbonic anhydrase, 30 000; soybean trypsin inhibitor, 20 100.

washed with an additional 8 mL of buffer and analyzed for bound radioactivity as already described. Nonsaturable binding was defined as the amount of binding in the presence of a 1000-fold excess of the corresponding unlabeled formyl peptide and was typically 5–15% of total binding. In addition, filter binding was determined in the absence of solubilized membranes and was compared with filter binding in the presence of unlabeled ligand. The difference was used to correct the amount of nonsaturable binding subtracted from total binding. All measurements were made in triplicate and averaged.

RESULTS

Covalent Affinity Labeling of Rabbit Neutrophils and Plasma Membranes with the Various Peptide Derivatives. The dimethyl suberimidate and the ethylene glycol bis(succinimidyl succinate) cross-linked ^{125}I-hexapeptide and the photoactivatable ^{125}I-PAL were found to covalently radiolabel a limited number of polypeptides in rabbit neutrophil membranes (Figure 1). A polypeptide with an apparent M_r of between 50 000 and 65 000 was specifically labeled by all three techniques (Figure 1, lanes A, C, and E). Specificity is demonstrated since radiolabeling of only this polypeptide was abolished in the presence of a 1000-fold excess of nonradiolabeled formyl peptide, whereas cross-linking of the other polypeptides was unaffected (Figure 1, lanes B, D, and F). The pattern of nonspecific labeling was different with each method, perhaps reflecting differences in the chemical requirements for cross-linking or differences in the low-affinity interactions of the various derivatives. However, with the ^{125}I-PAL, we often observed a darker band in the nonspecific control lanes of M_r 25 000 (Figure 1, lane F; Figure 3, lanes B, D, F, and H; Figure 4, lanes B, D, and F). The significance of this observation and its relation, if any, to the active proteolytic fragment of the receptor described by Dolmatch & Niedel (1983) is not yet known. With both chemical cross-linkers, the addition of small amounts [0.025% (w/v)] of either BSA or α-lactalbumin to the stock solutions of ^{125}I-hexapeptide resulted in high nonspecific cross-linking of the carrier proteins.

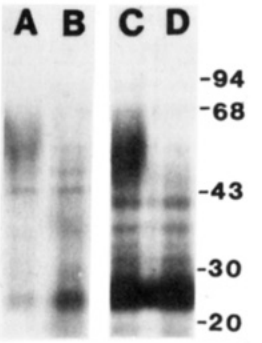

FIGURE 2: Autoradiograph demonstrating radiolabeling of the formyl peptide receptor on whole cells and purified plasma membranes. Photoaffinity labeling was performed with 10 μg of whole cells (2.5 × 10^5 cells) (lanes A and B) and 10 μg of membrane protein (lanes C and D) and 8 nM ^{125}I-PAL in the absence (lanes A and C) and presence (lanes B and D) of 800 nM unlabeled hexapeptide. Molecular weight standards are the same as those used in Figure 1 (lanes E and F).

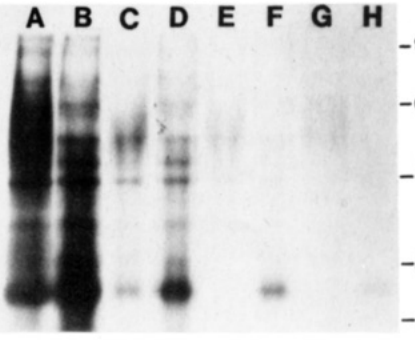

FIGURE 3: Efficiency of ^{125}I-PAL photo-affinity labeling of the formyl peptide receptor as a function of ^{125}I-PAL concentration. Aliquots of 10 μg of band 2 membrane were incubated with various concentrations of ^{125}I-PAL in the absence (lanes A, C, E, and G) and presence (lanes B, D, F, and H) of 200 nM unlabeled hexapeptide for 25 min at 24 °C in the dark. The membranes were then irradiated at 360 nm for 5 min at 4 °C and then run on SDS–urea–PAGE. (Lanes A and B) 10 nM ^{125}I-PAL; (lanes C and D) 5 nM ^{125}I-PAL; (lanes E and F) 2.5 nM ^{125}I-PAL; (lanes G and H) 1.25 nM ^{125}I-PAL. For molecular weight standards, see Figure 1, lanes E and F.

In some cases, this obscured the identification of the receptor; therefore, protein was not added in subsequent studies.

The preparation of plasma membranes did not alter the molecular weight of the receptor on whole cells (Figure 2). As can be seen, the ^{125}I-PAL cross-linking procedure identifies the same molecular weight material from whole cells (lanes A and B) and plasma membranes (lanes C and D).

The incorporation of ^{125}I-PAL into the M_r 50 000–65 000 band was concentration dependent (Figure 3) and approached saturation at approximately 20 nM ^{125}I-PAL (data not shown). Efficiency of covalent labeling was greatest for ^{125}I-PAL, compared to the two chemical cross-linkers used with the ^{125}I-hexapeptide, and was as high as 30% of the bound peptide at 10 nM ^{125}I-PAL. Because concentrations of between 5 and 10 nM ^{125}I-PAL gave high efficiency of receptor labeling with acceptably low nonspecific labeling, subsequent studies investigating the effects of detergents on the ^{125}I-PAL-labeled membrane-bound receptor were performed within this concentration range.

Detergent Solubilization of the Rabbit Neutrophil Formyl Peptide Receptor. (A) Loss of fMet-Leu-[^{3}H]Phe Binding to Plasma Membranes after Treatment with Various Detergents. The ability of various detergents to cause loss of fMet-Leu-[^{3}H]Phe binding from neutrophil membranes is shown in Table I. As can be seen, all seven detergents were

Table I: Loss of fMet-Leu-[^{3}H]Phe Binding to Neutrophil Plasma Membranes after Treatment with Various Detergents[a]

% concn of detergent	detergent added	residual fMet-Leu-[^{3}H]Phe binding activity (% total)[b]	detergent added	residual fMet-Leu-[^{3}H]Phe binding activity (% total)[b]
1	SDS	1.0 ± 1.0[c]	Tween 80	44.1 ± 4.4
0.1		1.0 ± 1.0		54.3 ± 7.2
0.01		34.5 ± 14.0		74.8 ± 8.4
1	Nonidet P-40	1.3 ± 1.3	CHAPS	14.0 ± 1.2
0.1		2.0 ± 2.0		149.3 ± 42.4
0.01		88.2 ± 5.8		104.5 ± 4.3
1	Triton X-100	6.5 ± 5.3	CHAPSO	5.5 ± 1.1
0.1		19.7 ± 18.7		131.3 ± 15.7
0.01		104 ± 15.1		102.2 ± 3.3
1	digitonin	26.8 ± 11.3		
0.1		36.3 ± 6.3		
0.01		78.7 ± 13.3		

[a] A total of 20 μg of neutrophil membranes were incubated at 24 °C for 30 min with the various concentrations of detergents before the addition of 10 mM fMet-Leu-[^{3}H]Phe. After an additional 20-min incubation, the specific fMet-Leu-[^{3}H]Phe binding to the residual unsolubilized membrane was determined by the glass fiber filtration assay. [b] Total binding refers to the amount of fMet-Leu-[^{3}H]Phe bound under the same assay conditions but in the absence of detergents. [c] Mean of three individual experiments, each performed in triplicate, ± SEM.

effective, in a dose-dependent manner, in causing a decrease in receptor binding in concentrations between 0.01% and 1%. At the lower concentrations, SDS, Nonidet P-40, and Triton X-100 were particularly effective in causing loss of binding activity. Digitonin and Tween 80 required higher concentrations to cause equivalent loss of binding activity, whereas CHAPS and CHAPSO caused loss of binding at only the highest concentrations tested (>0.5%) and caused augmentation of binding at 0.1%. However, since this loss of residual fMet-Leu-[^{3}H]Phe binding activity in detergent-treated membranes may be due either to the solubilization of the receptor or to the inactivation/inhibition of the receptor by the solubilization procedure, these possibilities were investigated.

(*B*) *Fate of Affinity-Labeled Receptor after Treatment with Various Detergents.* The ^{125}I-PAL-labeled receptor in the detergent supernatant and membrane residue from band 2 membranes treated with 10 mM CHAPS is shown in Figure 4. Analysis of the autoradiograph by scanning denistometry showed that approximately 60% of the receptor is solubilized (lanes C and D) as compared to the starting material (lanes A and B) and residual pellet (lanes E and F). Solubilization ranged in several experiments from 30%–70%. Virtually identical results were obtained with digitonin-solubilized membrane proteins (data not shown), thus confirming the observations made by Niedel (1981) on human neutrophils. In addition, the loss of fMet-Leu-[^{3}H]Phe binding with increasing concentrations of the several detergents that were examined (Table I) correlated closely with the concentrations of detergents used to solubilize the ^{125}I-PAL-labeled receptor. However, although treatment of membranes with the detergents Triton X-100 and Nonidet P-40 resulted in a significant decrease in the ^{125}I-PAL-labeled receptor in the membrane residue (data not shown), when the solubilized membrane proteins were later cross-linked with ^{125}I-PAL, the protein of M_r 50 000–65 000 seen with the CHAPS- and digitonin-solubilized membrane proteins was not detected. In addition, no specific binding (i.e., chaseable binding) of any membrane protein was apparent. Therefore, although a large number of detergents are able to solubilize the receptor, it appears that

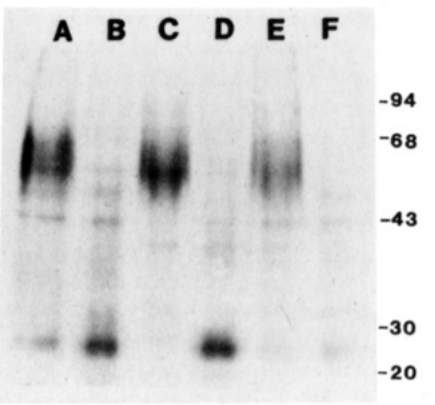

FIGURE 4: Autoradiograph demonstrating radiolabeling of the formyl peptide receptor from band 2 membranes. Affinity labeling was performed with 10 μg of membrane protein and 8 nM ^{125}I-PAL in the absence (lanes A, C, and E) and presence (lanes B, D, and F) of 800 nM unlabeled hexapeptide. (Lanes A and B) Intact membranes; (lanes C and D) membrane proteins cross-linked after CHAPS solubilization; (lanes E and F) residual membrane proteins in pellet after CHAPS solubilization. For molecular weight standards, see Figure 1, lanes E and F.

many may release the receptor in inactive form.

Soluble Receptor Assays. Many techniques have been devised for separating free ligand from bound ligand. Poly(ethylene glycol) (Cuatrecasas, 1972), ammonium sulfate (Rossi et al., 1977), and dilution below the critical micellar concentration of detergent (Schneider et al., 1979) have been used to precipitate the receptor–ligand complex, which can then be retained on a filter and washed to remove unbound ligand. However, because of the hydrophobic natue of the ^{125}I-hexapeptide, the addition of exogenous protein, which is required in all of these assays, caused high nonspecific binding, thus obscuring detection of any saturable receptor binding. As a result, none of these techniques could be used with the formyl peptide receptor. We have investigated the following methods:

(*A*) *Gel Filtration.* Niedel (1981) has previously demonstrated that gel filtration of digitonin-solubilized human neutrophil membranes through an acrylamide cross-linked agarose matrix (AcA-44) will separate receptor-bound and free ^{125}I-hexapeptide. Baldwin et al. (1983) have recently used a method involving centrifugation through columns of Sephadex G-50 to separate receptor-bound and free fMet-Leu-[^{3}H]Phe from digitonin-solubilized rabbit neutrophil membranes. We have obtained results similar to their observations, as shown in Figure 5. When aliquots of 10 mM CHAPS membrane extract were incubated with either ^{125}I-hexapeptide (panel A) or fMet-Leu-[^{3}H]Phe (panel B) and fractionated on a 2.3-mL column (AcA-44), saturable binding of ligand to a soluble component was observed. In addition, with equal amounts of CHAPS membrane extract, a greater percentage of ^{125}I-hexapeptide was bound compared to fMet-Leu-[^{3}H]Phe at all concentrations. This may be due to the higher affinity of the ^{125}I-hexapeptide for the receptor. Similar results as shown in Figure 5 were obtained with digitonin extract; however, no binding was observed when either 0.1% Triton X-100 or 0.1% Nonidet P-40 extracts were analyzed. With this gel filtration assay, an optimum CHAPS concentration for recovery of the soluble receptor was found to be 10 mM; however, the extract could be diluted to 1 mM CHAPS without any loss in binding activity (see below).

The receptor present in the CHAPS and digitonin extracts did not sediment during centrifugation for $10^7 g$·min, nor was it retained on a 0.2-μm pore size Millipore filter.

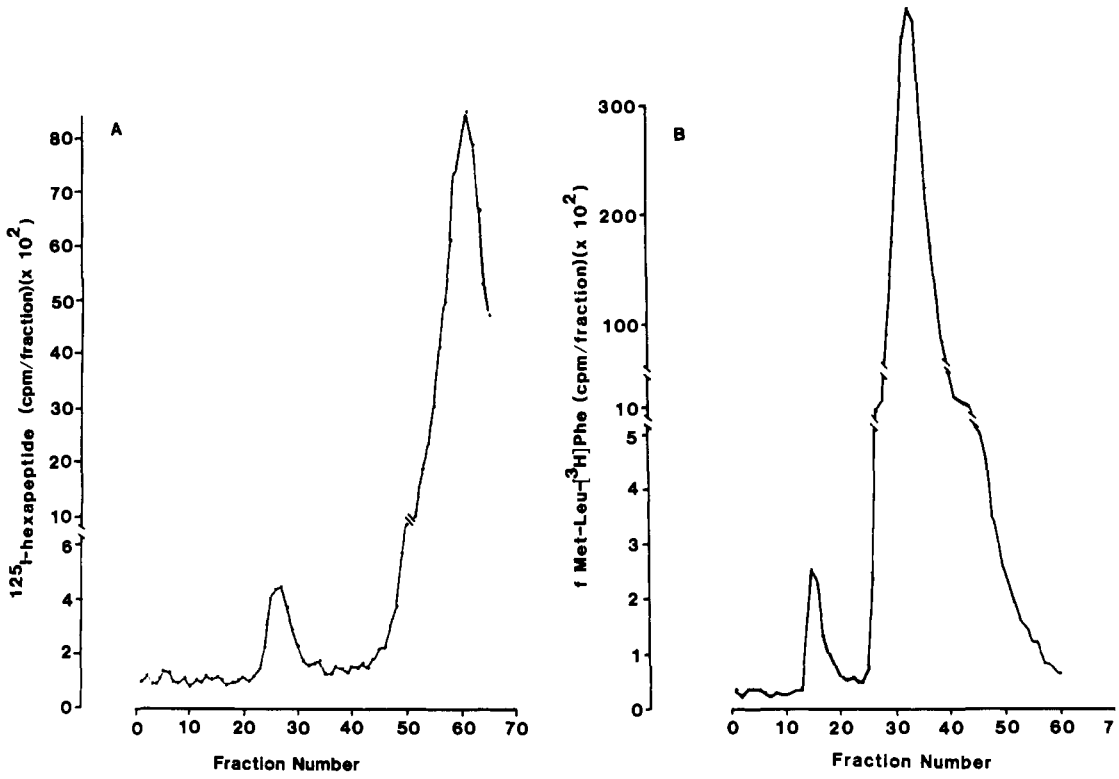

FIGURE 5: Separation of receptor-bound and free radiolabeled formyl peptides by gel filtration. The 10 mM CHAPS extract from band 2 neutrophil membranes was incubated with either of the radiolabeled formyl peptides for 30 min at 24 °C (●●). Nonspecific binding (not shown) was determined in the presence of an excess of the corresponding unlabeled formyl peptide. The 0.1-mL reaction mixture was then fractionated at 4 °C on a 2.3-mL AcA-44 column as described under Methods. Each 50-μL fraction was assayed for radioactivity. (A) The 77 μg of CHAPS extract was incubated with 6.6 nM ^{125}I-hexapeptide. Nonspecific binding, determined in the presence of 200 nM unlabeled hexapeptide, represented approximately 20% of total binding. (B) The 110 μg of CHAPS extract was incubated with 40 nM fMet-Leu-[^{3}H]Phe. Nonspecific binding, determined in the presence of 40 μM unlabeled fMet-Leu-Phe, represented less than 5% of total binding.

(B) Rapid Filtration through Poly(ethylenimine)-Treated Filters. Separation of free from receptor-bound formyl peptide in CHAPS-solubilized membrane extracts was also accomplished by filtration through PEI-treated glass fiber filters. This assay is simple and rapid, and it combines a high yield with low nonspecific binding. The major mechanism of retention of soluble receptors is thought to be ionic, and since integral membrane proteins tend to be acidic, the polycationic PEI-coated filters bind these proteins strongly (Bruns et al., 1983). Furthermore, using two-dimensional isoelectrofocusing/SDS–urea–PAGE, Schmitt et al. (1983) have shown that the ^{125}I-PAL labels two major acidic forms of the receptor from whole human neutrophils (isoelectric points of 6.0 and 6.5).

With the PEI-treated filter technique, a high degree of specific receptor binding was obtained with the CHAPS-solubilized receptors (see below). In order to determine the efficiency of the PEI-treated filters in retaining solubilized receptors during the filtration step, results from the PEI-filter technique were compared to results from the gel filtration method. For example, when 10 μg of CHAPS-solubilized membrane extract was incubated with 8 nM ^{125}I-hexapeptide for 30 min at 24 °C before the initiation of either assay, results from the PEI filter technique for specific binding [104 260 ± 3880 (SEM)] were identical in both specific counts bound and sample variance compared to the gel filtration technique [99 720 ± 3410 (SEM)]. The percentage of nonspecific binding determined in the presence of 1.6 μM unlabeled hexapeptide was equivalent in both assays [16 680 ± 2350 (SEM) and 15 500 ± 1530 (SEM) for the PEI filter technique and gel filtration technique, respectively] and accounted for approximately 15% of total binding. The data obtained for fMet-Leu-[^{3}H]Phe binding gave similar results (data not shown). Apparently, some component of the CHAPS extract (protein, lipid, or detergent) coats the filter and renders it unable to bind the free radiolabeled formyl peptides. The PEI filter assay is more rapid than gel filtration and just as accurate. It is therefore the method of choice for the assessment of solubilized formyl peptide receptor activity.

Binding Characteristics of Soluble Receptor. Initial experiments using the PEI filter technique demonstrated that binding equilibrium for fMet-Leu-[^{3}H]Phe was reached in 30 min at 24 °C (data not shown). The saturable binding of fMet-Leu-[^{3}H]Phe to the CHAPS-solubilized receptor (Figure 6; panel A) shared properties with the saturable binding of fMet-Leu-[^{3}H]Phe to the membrane-bound formyl peptide receptor. The equilibrium binding data were subjected to statistical analysis and to computer curve fitting with NONLIN, a weighted nonlinear least-squares regression analysis. As can be seen in the representative experiment shown in Figure 6 (panel B), curvilinear, concave-upward Scatchard plots were obtained. NONLIN and statistical analyses of the binding data indicated that a two saturable site model was preferable to a one saturable site model. The assumption of additional parameters reduced the weighted sums of squared deviations sufficiently to justify the more complex model and was shown to be statistically valid by the F test ($p < 0.01$). The characteristics of the two-site model for the soluble receptor were a high-affinity site with a K_D value of 0.80 ± 0.19 nM and a low-affinity site with a K_D value of 23.04 ± 4.33 nM. A total of 36% of the two sites detected was of the higher affinity. The data, when applied to the Hill equation, were characterized by a Hill coefficient of 0.48 ± 0.04. Data from two experiments each on CHAPS-solubilized membranes and in-

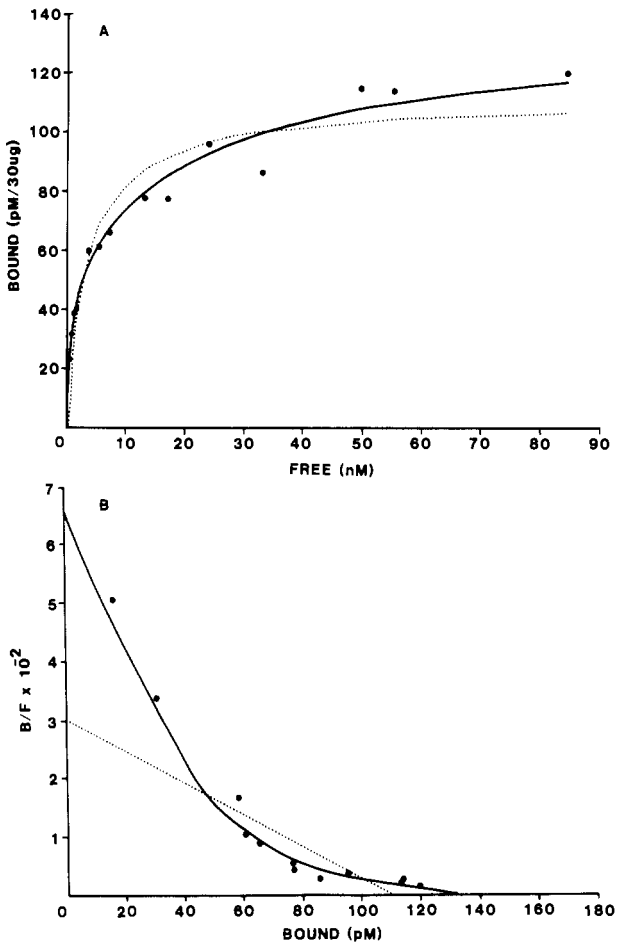

FIGURE 6: (A) Binding isotherm of specific fMet-Leu-[^{3}H]Phe bound to CHAPS-solubilized neutrophil plasma membranes (30 μg). The assay conditions for the PEI-treated filter technique are described under Methods. The data were corrected for nonspecific binding and analyzed with NONLIN. The dotted line represents a one-site fit to the data, and the solid line represents a two-site fit to the data. The data points of specific fMet-Leu-[^{3}H]Phe binding are also shown (●). The fit derived from the two-site model was significantly better ($p < 0.01$) than that derived from the one site model. (B) The same data plotted according to the method of Scatchard. The computer-generated curves for the one saturable site model (dotted line) and the two saturable site model (solid line) are shown. Each point is the average of triplicate determinations.

tact viable neutrophils gave similar results (Table II).

Scatchard analysis of ^{125}I-hexapeptide binding to the CHAPS-solubilized receptor was not performed. However, one class of binding sites was evident from the analysis of both cells and membrane preparations. A K_D value of 2.25 nM was obtained.

Detergent Solubilization of the Formyl Peptide Receptor from Whole Cells. Experiments were conducted to determine whether active receptors could be solubilized from whole cells. For example, in one experiment a total of 372 415 ± 3827 (SEM) counts of ^{125}I-hexapeptide was added to the extracts from 5 × 10^6 cells treated with various detergents (see Methods). Of the three detergents tested, CHAPS, Triton X-100, and Nonidet P-40, only CHAPS solubilized the receptor in active from as determined by the ability of the detergent extracts to bind ^{125}I-hexapeptide in the PEI-treated filter assay. As was found with plasma membranes, 10 mM CHAPS was the most effective concentration for solubilization with a total of 143 073 counts bound when only labeled peptide was included in the reaction, and 32 772 counts were bound nonspecifically in the presence of 1 μM unlabeled hexapeptide. The 110 301 counts specifically bound (29.6% of total counts)

Table II: Analysis of Steady-State Binding of fMet-Leu-[^{3}H]Phe

binding parameters	binding site models[a] one saturable	two saturable	Hill eq
CHAPS-Solubilized Rabbit Neutrophil Membranes			
K_{D1} (nM)	5.38 ± 1.74	1.25 ± 0.45	4.54 ± 0.38[c]
binding capacity (pmol/mg)	0.280 ± 0.11	0.116 ± 0.58	0.389 ± 0.21
K_{D2} (nM)	NA[d]	19.77 ± 3.28	NA
binding capacity (pmol/mg)	NA	0.211 ± 0.09	NA
significance (p)[b]	NA	<0.01	<0.01
Hill coeff	NA	NA	0.613 ± 0.128
Whole Rabbit Neutrophils			
K_{D1} (nM)	6.78 ± 0.07	0.975 ± 0.046	12.36 ± 1.91
sites/cell	32 200 ± 1 676	7 702 ± 107	58 188 ± 3 729
K_{D2} (nM)	NA	40.75 ± 10.36	NA
sites/cell	NA	44 461 ± 3,001	NA
significant (p)[b]	NA	<0.01	<0.01
Hill coeff	NA	NA	0.690 ± 0.054

[a] Shown are the computer-estimated mean values and the corresponding standard deviations. The estimations were based on two experiments each for the CHAPS-solubilized receptors and for the whole rabbit neutrophils. [b] Significance, indicated by results of F ratio tests, pertains to the statistical difference of the data relative to those for the model with one saturable site. [c] The Hill equation for the computer modeling (Marasco et al., 1984b) can be simplified such that at half-saturation $K_D = [L]^{n_H}$, where [L] equals the molar concentration of free fMet-Leu-[^{3}H]Phe and n_H equals the Hill coefficient. Here, we report the K_D at half-saturation raised to the Hill coefficient. [d] NA, not applicable.

were used to compare the other detergent combinations that are expressed as a percentage of the specific binding observed with 10 mM CHAPS (100%). A concentration of 5 mM CHAPS was found to be only 60% as effective in solubilization. In addition, it was demonstrated that combinations of detergents such as 10 mM CHAPS plus 0.1% Triton X-100 or 0.1% Nonidet P-40 resulted in significant loss of biological activity, 53.5% and 50% of maximal binding, respectively. Complete loss of specific ^{125}I-hexapeptide binding activity was seen with the extracts of cells treated with 1% Nonidet P-40 alone, 1% Triton X-100 alone, 10 mM CHAPS plus 1% Nonidet P-40 or 1% Triton X-100, and 5 mM CHAPS plus 1.0% or 0.1% Nonidet P-40 or 1% Triton X-100. Approximately 75% of the activity in 5 mM CHAPS extracts was lost with 5 mM CHAPS plus 0.1% Triton X-100 (15.4% maximal). Thus, of the three detergents tested, only CHAPS can release an active, soluble receptor from whole cells.

DISCUSSION

In the studies described in this paper, covalent affinity labeling of the rabbit neutrophil formyl peptide receptor was achieved with two different techniques. The first approach, previously used to identify the human neutrophil formyl peptide receptor (Niedel et al., 1980; Niedel, 1981), involved chemical cross-linking of the ^{125}I-hexapeptide to the receptor with either dimethyl suberimidate or with ethylene glycol bis(succinimidyl succinate). Both reagents resulted in the labeling of a polypeptide that migrated as a broad zone with an apparent M_r between 50 000 and 65 000 on SDS–PAGE. This suggests that the receptor may be heterogeneous in size, perhaps due to variable glycosylation or proteolysis. Furthermore, in support of its glycoprotein nature, the digitonin-solubilized (Dolmatch & Niedel, 1983) and Triton X-100 solubilized (Painter et al., 1982) human neutrophil formyl peptide receptor and the CHAPS-solubilized rabbit neutrophil formyl peptide receptor bind to wheat germ agglutinin and can be specifically eluted with *N*-acetylglucosamine (unpublished results).

The second technique involved the derivatization of the N-formylated hexapeptide to yield the highly reactive photoaffinity label ^{125}I-PAL (Dolmatch & Niedel, 1983; Schmitt et a., 1983). The efficiency of specific labeling was generally 20%–30%. In addition, the ^{125}I-PAL proved to be a suitable and convenient probe with which to analyze the efficiency of detergent solubilization of the formyl peptide chemotaxis receptor. While our results demonstrate that all of the detergents examined were capable of solubilizing the ^{125}I-PAL affinity-labeled, membrane-bound receptor, it appears that many of the detergents (except CHAPS and digitonin) release the receptor in inactive form. In particular, extracts from membranes treated with 0.1%–1.0% Triton X-100 or Nonidet P-40 that were subjected to affinity labeling in solution failed to demonstrate the specific labeling of the 50 000–65 000 band or of any other membrane protein. Furthermore, no specific binding of fMet-Leu-[^{3}H]Phe or ^{125}I-hexapeptide could be demonstrated by either the gel filtration or the PEI-filter technique with both plasma membrane and whole cell solubilized extracts.

The solubilization of the receptor in "active form" has been attempted by several laboratories with various detergents, but unfortunately, these studies have yielded conflicting results. In agreement with our observations that Triton X-100 inactivates the receptor are the reports by Niedel (1981) and Baldwin et al. (1983). Niedel (1981) found that either no or very little specific labeling of the receptor, as determined by covalent cross-linking of ^{125}I-hexapeptide, could be demonstrated with Triton X-100 extracts of human neutrophil membranes whereas specific labeling was observed with digitonin extracts. Baldwin et al. (1983) reported that digitonin was the only detergent (out of 24 tested) capable of solubilizing the receptor from rabbit neutrophil membranes in a form that retained fMet-Leu-[^{3}H]Phe binding activity. In these studies, Triton X-100 was found to inactivate the receptor. However, these authors also reported that treatment with CHAPS, which did not cause inactivation, failed to solubilize binding activity. In our studies, 30%–70% of the receptor binding activity was solubilized with CHAPS.

In contrast to our findings that Nonidet P-40 inactivates the rabbit receptor, Goetzl et al. (1981), observed the specific elution of three proteins (M_r 94 000, 68 000, and 40 000) after fMet-Leu-Phe–Sepharose affinity chromatography of an Nonidet P-40 soluble fraction of human neutrophil membranes. The proteins of M_r 68 000 and 40 000 retained high-affinity binding of fMet-Leu-[^{3}H]Phe as shown by equilibrium analysis. Hoyle & Freer (1984) have found the active human receptor can be purified from Nonidet P-40 solubilized human promyelocytic leukemia cell (HL-60) membranes. Also, Jesaitis et al. (1983) reported with Triton X-100 solubilized human neutrophil membranes that fMet-Leu-[^{3}H]Phe eluted in the void volume following Sepharose 4B column chromatography. This suggested to these authors the formation of a "high-affinity complex" of the solubilized receptor. Further work will be required to resolve these differences.

As shown in Figure 6, specific fMet-Leu-[^{3}H]Phe binding in the CHAPS-solubilized preparations was saturable and Scatchard plots were curvilinear. Similar observations have been previously reported on whole rabbit neutrophils and their purified plasma membranes (Mackin et al., 1982). NONLIN analysis of the equilibrium binding data indicated that the two saturable site model fits the data significantly better ($p < 0.01$) than the one saturable site model. The Hill coefficients of <0.65 were repeatedly obtained. However, it is well-known that curvilinear concave-upward Scatchard plots can be due to a variety of causes, the best known being (1) the presence of multiple classes of binding sites that have different but fixed affinities, (2) the existence of site–site interactions of the type defined as "negative cooperativity", or the simultaneous presence of both [reviewed by DeMeyts (1976)]. Since steady-state binding data alone do not discriminate between these curvilinear models, studies of the kinetics of dissociation can be used to differentiate between the two (De Meyts et al., 1973, 1976). This has recently been shown for the rat neutrophil formyl peptide receptor (Marasco et al., 1985). A third cause is the formation of a soluble ternary complex (DeLean et al., 1980) involving the interaction of the receptor (R) with an additional membrane component (X) leading to the agonist-(H-) promoted formation of a high-affinity ternary complex (HRX).

The demonstration of curvilinear Scatchard plots and Hill coefficients <1.0 for solubilized receptors is not unique to this chemotaxis receptor system. Among numerous examples, the retention of negatively cooperative interactions among receptor sites after solubilization has been suggested for the curvilinear binding of thyrotropin-stimulating hormone to its receptor in bovine thyroid (Tate et al., 1975) and the binding of dihydroalprenolol to β-adrenergic receptors from frog erythrocytes (Caron & Lefkowitz, 1976). However, direct kinetic techniques similar to those applied to their respective particulate preparations are still required to confidently assess the presence of negative cooperativity among the solubilized receptor sites. Retention of negative cooperativity among detergent-solubilized avian insulin receptors (Ginsberg et al., 1976) has been proposed to explain the insulin-induced decrease in molecular weight of the receptor in gel filtration experiments where a tetrameric form of the receptor can dissociate to a monomeric form with increased insulin concentration. Moreover, glucagon induces a decrease in molecular weight of the myocardial glucogon receptor of at least 4-fold (Klein et al., 1983).

Although the formation of a soluble ternary complex may also explain the curvilinear Scatchard plots of the CHAPS-solubilized receptor, the existence of such effector molecules and the formation of a soluble formyl peptide receptor ternary complex have not been determined. However, preliminary observations from other laboratories (Huang et al., 1984; Lane & Snyderman, 1984) suggest that such data is forthcoming. Further work will be required to investigate these several possibilities.

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