Detergent Solubilization of the Formyl Peptide Chemotactic Receptor

STRATEGY BASED ON COVALENT AFFINITY LABELING

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The formyl peptide chemotactic receptor has been solubilized by digitonin treatment of purified human neutrophil membranes. Of several potential assay methods tested for their ability to separate receptor-bonded from free ligand, only gel filtration through an acrylamide cross-linked agarose matrix yielded satisfactory results. Approximately 70% of the receptor initially present in the membranes was recovered in the digitonin extract. Binding of $^{125}$I-labeled N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys to the soluble receptor was rapid ($t_{1/2}$ at 22 °C <5 min), of high affinity ($K_d$ = 2.2 nm) and saturable. The relative potencies of a small series of peptides as inhibitors of binding to the soluble receptor paralleled their potencies as inhibitors of the membrane-bound receptor. N-Formylation of the peptides was required for high affinity binding. Binding was maximal at pH 6.5 and was sulphhydryl-dependent; 20 nM $p$-chloromercuriphenylsulfonic acid decreased binding by 50%. $^{125}$I-labeled N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys was specifically cross-linked to the soluble receptor with ethylene glycol bis(succinimidyl succinate) and an apparent molecular weight of 55,000 to 70,000 was determined for the soluble receptor by sodium dodecyl sulfate polyacrylamide gel electrophoresis. A strategy for obtaining an active, detergent-soluble receptor preparation based on covalent affinity labeling is presented.

Human phagocytic cells (neutrophils, monocytes, and macrophages) will exhibit in vitro chemotaxis, lysosomal enzyme release, superoxide production, and cell aggregation in response to several inflammatory stimuli. Of these stimuli, the formylated oligopeptides represent the most experimentally accessible system for the study of these important cellular functions (1). A specific cell surface receptor for the oligopeptides has been demonstrated, as has receptor-mediated peptide endocytosis and peptide-induced receptor regulation (2-9). Although the functional characteristics of the formyl peptide receptor have been studied in detail, the inability to obtain a soluble receptor preparation which retains binding specificity for the formyl peptides has limited studies of its physical properties. The apparent molecular weight of the receptor has been determined by covalent affinity labeling (10), but no other information is available regarding its biochemical characteristics.

The present study reports the detergent solubilization of the formyl peptide receptor in an active form from human neutrophil membranes. The binding characteristics of the soluble receptor are compared with the known characteristics of the membrane-bound receptor.

EXPERIMENTAL PROCEDURES

Materials
Ethylene glycol bis(succinimidyl succinate) was obtained from Pierce Chemical Co., Rockford, IL; $p$-chloromercuriphenyl sulfonic acid was from Sigma, St. Louis, MO; and digitonin was from BDH Chemicals Ltd., Poole, England (a kind gift from Dr. Marc Caron, Duke University). Ammonyx-Lo was from ONYX Chemical Corp., Roboken, NJ; Triton X-100, deoxycholate, cholate, Nonidet P-40, and octyl-$p$-glucoside were from Sigma, St. Louis, MO. LKB, Durham, NC, supplied the A: A 44; polyacrylamide gel reagents were from Bio-Rad Laboratories, Richmond, CA; Cronex Lightening Plus enhancing screens were from DuPont, Wilmington, DE; and Royal-X-Omat film was supplied by Eastman Kodak, Rochester, NY.

Methods
Preparation of Purified Membranes—Neutrophils were prepared by the method of Boyum (10) from 1800 ml of human blood obtained from four healthy donors. A suspension of 10° cells/ml in 0.25 M sucrose, 10 mM potassium phosphate, 2 mM EDTA, 2 mM EGTA, and 1 mM phenylmethylsulfonyl fluoride, pH 6.75, was subjected to nitrogen cavitation following equilibration at 800 p.s.i. for 30 min at 4 °C. The supernatant obtained by centrifugation at 2500 × g for 10 min was layered on a discontinuous sucrose gradient (15%, 35%, 40%) and centrifuged at 100,000 × g for 2 h. The material banding at the 15 to 35% interface, which displayed high formyl peptide binding and absent $p$-glucuronidase activity (11), was collected, pelleted, resuspended with gentle glass-Teflon homogenization to approximately 500 μg/ml in 20 mM potassium phosphate, pH 6.75, and stored frozen at −20 °C. Membrane protein was determined by the method of Lowry (12).

Covalent Affinity Radiolabeling—N-Formyl-Nle-Leu-Phe-Nle-Tyr-Lys was radiiodinated as previously reported (4). Ethylene glycol bis(succinimidyl succinate) cross-linking was performed as follows. Approximately 50 μg of membrane protein and 2.5 pmol (5 nm) of $^{125}$I-labeled INLPNTL in 500 μl of 10 mM potassium phosphate, pH 6.75, were incubated for 30 min at 22 °C. Ethylene glycol bis(succinimidyl succinate), 20 μg/ml in dimethyl sulfoxide, was diluted to 0.4 μg/ml in 50 mM dibasic potassium phosphate. Five hundred μl were immediately added to the 500-μl binding incubation and the reaction continued for 15 min at 22 °C. The cross-linked membranes were pelleted, resuspended in 200 μl of 20 mM potassium phosphate, pH 6.75, and stored frozen at −20 °C. In preliminary experiments, using ethylene glycol bis(succinimidyl succinate) final concentrations from 0.05 to 1.0 mg/ml, 0.2 mg/ml was shown to give the highest efficiency of affinity labeling, with acceptable protein-protein cross-links (Fig. 1). The apparent molecular weight of the receptor was unaffected by cross-linker concentration, although a trace of receptor dimer appears at the highest concentration (lane i).

Affinity labeling of the soluble receptor in detergent extracts of

1 The abbreviations used are: EGTA, ethylene glycol bis(β-aminoethyl ether) $N,N,N',N'$-tetraacetic acid; $^{125}$I-labeled INLPNTL, N-formyl-Nle-Leu-Phe-Nle-$^{125}$I-Tyr-Lys; INLPNTL, N-formyl-Nle-Leu-Phe-Nle-Tyr-Lys.
membranes was performed as follows. Fifty µl of the detergent extract and 200 fmol (4 nM) of 125I-labeled MLPNTL were incubated at 22 °C for 30 min. Ethylene glycol bis(succinimidy1 succinate) was prepared as above, 50 µl was added to the completed binding incubation to a final concentration of 0.2 µg/ml, the reaction was continued for 15 min at 22 °C and quenched by the addition of 10 µl of 1 M glycine. Nonsaturable binding and cross-linking were determined in the presence of 200 nM unlabeled MLPNTL. Sodium dodecyl sulfate gel electrophoresis and autoradiography were performed as previously described (10).

Digitonin Receptor Solubilization and Assay — To 200 µg of membrane protein in 500 µl of 100 mM potassium phosphate, pH 6.75, containing 0.1 M dithiothreitol was added digitonin to a final concentration of 0.3% (w/v). The mixture was homogenized manually with 10 strokes of a glass-Teflon homogenizer and incubated at 22 °C for 30 min. Membrane residue was pelleted in a Beckman airfuge at 180,000 × g (30 p.s.i.) for 30 min, the supernatant collected, diluted 2-to-10-fold with 50 mM potassium phosphate, pH 6.75, 0.1% digitonin (w/v) and stored frozen at −20 °C until use. Receptor solubilization with Triton X-100, Ammonyx-Lo, Nonidet P-40, and octyl-β-D-glucoside were performed under identical conditions with the indicated detergent concentrations. Cysteate and deoxycholate solubilizations were done at pH 7.6.

For the soluble receptor-binding assay, the digitonin extract from 0.5 to 5 µg of membrane protein was incubated with 400 fmol (4 nM) of 125I-labeled MLPNTL in 100 µl of 50 mM potassium phosphate, pH 6.75, 0.1% digitonin (w/v) for 30 min at 22 °C. Nonsaturable binding was determined in the presence of 20 pmol (200 nM) of unlabeled MLPNTL. Receptor-bound and free 125I-labeled MLPNTL were separated by gel filtration through a 1.6-ml ACA 44 column equilibrated with 50 mM potassium phosphate, pH 6.75, 0.1% digitonin (w/v) at 4 °C. The entire 106-µl reaction mixture was applied to the column and washed sequentially with a 0.9-ml and 1.6-ml aliquot of buffer. The first 1 ml was collected within approximately 2 min and contained the receptor-bound 125I-labeled MLPNTL. The second 1.6 ml contained the free 125I-labeled MLPNTL. Duplicate determinations were performed for total binding and nonsaturable binding and the results reported as saturable binding (total binding minus nonsaturable binding). The range of the duplicate values never exceeded 16% of the mean value. Binding to the membrane-bound receptor was done as previously described (10).

RESULTS

Affinity Labeled Receptor—The inability to demonstrate an active, soluble formyl peptide receptor could result from 1) an inability to solubilize the receptor from the membrane, 2) inactivation or inhibition of the receptor by the solubilization procedure, or 3) lack of a suitable assay for the soluble receptor. The technique of covalent affinity labeling of the receptor provided an experimental approach by which these possibilities could be investigated sequentially.

The first possibility was eliminated because the affinity-labeled receptor was solubilized by a wide variety of detergents, including Triton X-100, deoxycholate, cholate, Nonidet P-40, octyl-β-D-glucoside, Ammonyx-Lo, and digitonin. The labeled receptor in the detergent supernatant and membrane residue is shown in Fig. 2 for membranes treated with digitonin, Ammonyx-Lo, deoxycholate, and Triton X-100. All four detergents were effective in concentrations between 0.2% and 1.0%. Ammonyx-Lo and deoxycholate in the supernatants caused artificial changes in the mobility of the labeled receptor (Fig. 2, lanes c and e). Similar concentrations of digitonin, Ammonyx-Lo, deoxycholate, and Triton X-100 also decreased the amount of receptor present in membrane preparations which had not been affinity-labeled, suggesting that the receptor could also be extracted from native membranes. Reagents which extract peripheral membrane proteins, 1.0 M potassium iodide, 1.0 M acetic acid, or divalent ion chelators, did not solubilize the affinity-labeled receptor.

Detergent Solubilization of the affinity-labeled formyl peptide receptor. Approximately 5 µg of affinity-labeled neutrophil membrane protein were treated with the indicated reagents for 30 min at 4 °C. The supernatants and pellets, separated by centrifugation at 180,000 × g for 30 min, were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and autoradiography as described under "Methods." The affinity-labeled formyl peptide receptor is designated (> R). Lane a, 0.5% digitonin supernatant; lane b, 0.5% digitonin residual pellet. Lane c, 0.5% Ammonyx-Lo supernatant; lane d, 0.5% Ammonyx-Lo residual pellet. Lane e, 0.5% deoxycholate supernatant; lane f, 0.5% deoxycholate residual pellet. Lane g, 0.5% Triton X-100 supernatant; lane h, 0.5% Triton X-100 residual pellet. Lane i, 1.0 M potassium iodide supernatant; lane j, 1.0 M potassium iodide pellet. Lane k, 1.0 M acetic acid supernatant; lane l, 1.0 M acetic acid pellet.
Because of the large number of detergents which were able to solubilize the affinity-labeled receptor, affinity labeling of detergent extracts of native membranes was attempted to determine whether any of the detergents released an active, soluble receptor. As seen in Fig. 3, a protein with the appropriate apparent molecular weight by sodium dodecyl sulfate polyacrylamide gel electrophoresis was specifically affinity labeled in the 0.2% digitonin supernatant (Fig. 3, lane 7). No specific labeling was seen in the 0.5% deoxycholate or 0.2% Triton X-100 supernatants, while a trace of specific labeling may have been present in the 0.5% Triton X-100 supernatant (Fig. 3, lane 4). The pattern of nonspecific labeling was different with each of the detergents tested. These studies indicated that Triton X-100 and deoxycholate either did not release the receptor or released an inactivated or inhibited form of the receptor, whereas digitonin treatment released an active, soluble receptor, and therefore, a suitable assay for the digitonin-solubilized receptor was sought.

**Soluble Receptor Assay**—Many techniques have been devised for separating free ligand from receptor-bound ligand. Polyethylene glycol (13), ammonium sulfate (14), and dilution below the critical micellar concentration of detergent (15) have been used to precipitate the receptor-ligand complex, which can then be retained on a filter and washed to remove unbound ligand. These assays depend on relatively concentrated protein solutions or the addition of exogenous carrier proteins to insure receptor precipitation. Because of the hydrophobic nature of 125I-labeled fNLPNTL, the addition of exogenous protein caused high nonsaturable binding which obscured any saturable binding component. As a result, these techniques could not be used with the formyl peptide receptor. Lectin-agarose immobilization has been used to assay the soluble epidermal growth factor receptor (16). However, high nonspecific adsorption of 125I-labeled fNLPNTL to the lectin-agarose matrix precluded the use of this technique, as well.

Gel filtration through an acrylamide cross-linked agarose matrix (AcA 44) proved to be a satisfactory method of assay. The optimal conditions for separation were determined using the digitonin-solubilized, affinity-labeled receptor. Sodium dodecyl sulfate polyacrylamide electrophoresis performed on each column fraction demonstrated that the labeled receptor was quantitatively recovered in a single peak (data not shown). When aliquots of a 0.3% digitonin extract of native membranes, incubated with 4 ml of 125I-labeled fNLPNTL in the presence or absence of 200 nm unlabeled fNLPNTL, were fractionated on a 1.6-ml AcA 44 column, saturable binding of the ligand to a soluble component was observed (Fig. 4). Each one-drop fraction contained 50 μl, so that the nadir at fraction 22 corresponded to the first 1.1 ml elution volume. When an identical experiment was performed with a 0.3% digitonin extract of human erythrocyte membranes, no saturable binding was detected. Using this assay, an optimal digitonin concentration for recovery of the soluble receptor was found between 0.2 and 0.5% (Fig. 5). Using 0.3% digitonin, the maximal recovery was increased from 40 to 70% by gentle glass-Teflon homogenization and inclusion of 0.1 mM dithiothreitol in the extraction buffer. Inclusion of protease inhibitors, divalent ion chelators, or altering the pH between 6 and 8 did not increase soluble receptor recovery.

**Fig. 3.** Affinity-labeling of the detergent solubilized formyl peptide receptor. 125I-labeled fNLPNTL was crosslinked with ethylene glycol bis(succinimidyI succinate) to the various detergent extracts in the absence or presence of 200 nm unlabeled fNLPNTL as described under "Methods." Lane 1, 0.5% deoxycholate, total labeling; lane 2, 0.5% deoxycholate, labeling in the presence of 200 nm unlabeled fNLPNTL (nonsaturable). Lane 3, 0.2% Triton X-100, total labeling; lane 4, 0.5% Triton X-100, total labeling; lane 5, 0.5% Triton X-100, nonsaturable labeling. Lane 6, (standard) ethylene glycol bis(succinimidyI succinate) affinity labeling of the membrane-bound receptor (>R). Lane 7, 0.2% digitonin, total labeling; lane 8, 0.2% digitonin, nonsaturable labeling. A modified Fairbanks electrophoresis system with a 10% acrylamide gel was used (18). Lanes 1 to 6 were run in one experiment, lanes 7 to 8 were run in another.

**Fig. 4.** Separation of receptor-bound and free 125I-labeled fNLPNTL by gel filtration. The 0.3% digitonin extract of neutrophil membranes was incubated with 4 nM 125I-labeled fNLPNTL in the absence (○) or presence (■) of 200 nm unlabeled fNLPNTL and fractionated at 4°C on a 1.6-ml AcA 44 column as described under "Methods." Each 50-μl fraction was assayed for 125I-labeled fNLPNTL.

**Fig. 5.** Receptor recovery as a function of digitonin concentration. Approximately 50 μg of membrane protein was treated with the indicated concentration of digitonin for 15 min at 22°C. The supernatants (○) and residual pellets (■) were separated by centrifugation at 180,000 × g for 30 min and assayed for saturable 125I-labeled fNLPNTL binding in triplicate. Data points are presented as per cent receptor recovery relative to the 350 fmol of 125I-labeled fNLPNTL bound to the untreated membranes.
The receptor present in the digitonin supernatant did not sediment during centrifugation at 100,000 × g for 2 h, nor was it retained on a 0.2-μm pore size Millipore filter.

**Characteristics of the Soluble Receptor**—The saturable binding of 125I-labeled fNLPNTL to a component of the digitonin extract (Fig. 4) shared properties with the saturable binding of 125I-labeled fNLPNTL to the membrane-bound formyl peptide receptor. Binding equilibrium was reached in 15 min at 22 °C and 60 min at 4 °C (Fig. 6). Binding to the soluble receptor was saturable, with a $K_d = 2.2$ nM and only one class of sites evident by Scatchard analysis (Fig. 7, a and b). The membrane-bound receptor, prior to detergent extraction, demonstrated a $K_d = 0.75$ nM. Nonsaturable binding was approximately 25% of total binding at 125I-labeled fNLPNTL concentrations below the $K_d$ and exceeded 50% of total binding at 8 nM 125I-labeled fNLPNTL. The relative potencies of a small series of formylated and unformylated peptides as inhibitors of saturable binding in the digitonin extract paralleled the known potencies of these peptides as inhibitors of binding to the membrane-bound receptor (4) (Fig. 8). N-Formyl-Nle-Leu-Phe-Nle-Tyr-Lys was at least 4 orders of magnitude more potent than the unformylated peptide. A pH optimum between 6.5 and 7.5 was evident, with a maximum at pH 6.5 (Fig. 9).

The binding was sulfhydryl-dependent. Parallel inhibition by p-chloromercuriphenylsulfonic acid of both the membrane- and soluble receptor was demonstrated; a concentration of 20 μM produced approximately 50% inhibition (Table I). This inhibition was partially reversed by subsequent incubation with dithiothreitol. When the membrane-bound receptor was partially inactivated with increasing concentrations of p-chloromercuriphenylsulfonic acid, a parallel decrease in the

![Fig. 6. Time course of 125I-labeled fNLPNTL binding to the soluble receptor](image)

**Fig. 6.** Time course of 125I-labeled fNLPNTL binding to the soluble receptor. Incubations for total and nonsaturable binding were performed under standard assay conditions as described under "Methods," except that the incubation volumes were increased to 2.0 ml and the incubation was done at both 4 °C (●) and 22 °C (○). At the times indicated, 100-μl duplicate aliquots were removed and fractionated by AcA 44 chromatography.

![Fig. 7. Binding of 125I-labeled fNLPNTL as a function of increasing concentration](image)

**Fig. 7.** Binding of 125I-labeled fNLPNTL as a function of increasing concentration. a, the assay conditions were as described under "Methods" with the indicated concentrations of 125I-labeled fNLPNTL. Saturable binding (●) was the difference between total binding (data not shown) and nonsaturable binding (○). b, Scatchard analysis of the data shown in a.

![Fig. 8 (left). Inhibition of 125I-labeled fNLPNTL binding by other synthetic peptides](image)

**Fig. 8 (left).** Inhibition of 125I-labeled fNLPNTL binding by other synthetic peptides. Inhibition of 125I-labeled fNLPNTL binding by the various peptides was determined using the standard binding assay to which the indicated concentration of peptide was added prior to the addition of the detergent extract. Formyl-Met-Leu-Phe-Met-Tyr (●), formyl-Nle-Leu-Phe-Nle-Tyr-Lys (○), formyl-Met-Leu-Phe (●), and unformylated Nle-Leu-Phe-Nle-Tyr-Lys (▲—▲).

**Fig. 9 (right).** Effect of pH on binding. The standard binding assay as described under "Methods" was used except that the pH was varied from 5.5 to 8.5 as indicated. The AcA 44 columns used to fractionate receptor-bound and free ligand were equilibrated at the pH of the corresponding binding incubation.

### Table I

| p-Chloromercuriphenyl sulfonic acid inhibition of soluble and membrane-bound formyl peptide receptor |
|---------------------------------------------------------------|
| Approximate 10 μg of membrane protein or the 0.3% digitonin extract from 10 μg of membrane protein were incubated for 15 min at 22 °C with the indicated concentrations of p-chloromercuriphenyl sulfonic acid and then assayed for 125I-labeled fNLPNTL binding as described under "Methods." |

| μM   | Soluble receptor % inhibition | Membrane receptor % inhibition |
|------|-----------------------------|-------------------------------|
| 0    | 0                           | 0                             |
| 10   | 22                          | 17                            |
| 50   | 35                          | 71                            |
| 200  | 92                          | 82                            |
| 500  | 100                         | 87                            |
| 2000 | 100                         | 100                           |

### Table II

**Effect of treatment of membrane-bound receptor with p-chloromercuriphenyl sulfonic acid on subsequent recovery of soluble receptor**

Approximately 20 μg of membrane protein were incubated with the indicated concentrations of p-chloromercuriphenyl sulfonic acid for 15 min at 22 °C, pelleted at 180,000 × g for 15 min and resuspended in 200 μl of 50 mm potassium phosphate, pH 6.75. A 100-μl aliquot was assayed in duplicate for residual membrane-bound receptor. The other 100-μl aliquot was solubilized with 0.3% digitonin and the supernatant assayed in duplicate for soluble receptor as described under "Methods." The percentage recovery of the soluble receptor relative to the receptor present in untreated membranes is presented.

| μM   | Membrane receptor binding % control | Membrane receptor binding % recovery |
|------|------------------------------------|-------------------------------------|
| 0    | 100                                | 62 (100)                            |
| 10   | 84                                 | 43 (69)                             |
| 20   | 62                                 | 38 (61)                             |
| 50   | 14                                 | 12 (19)                             |
| 200  | 8                                  | 0 (0)                               |
yield of soluble receptor prepared from these treated membranes was observed (Table II).

**DISCUSSION**

In the studies described in this manuscript, covalent affinity labeling was used to develop a method for detergent solubilization of the formyl peptide chemotactic receptor. First, the efficiency of solubilization of the affinity-labeled, membrane-bound receptor was determined with various detergents. Detergent concentrations which solubilized approximately 50% of the labeled receptor were then used to prepare extracts of native membranes. The soluble receptors in these extracts were then subjected to affinity labeling in solution in an attempt to determine which detergent-extraction procedure resulted in an active receptor preparation. In the case of the formyl peptide receptor, many detergents solubilized the affinity-labeled receptor, but digitonin extracts contained the native membranes. The soluble receptors in these extracts of the labeled receptor were then used to prepare extracts of the membrane-bound receptor.

Several lines of evidence supported the presence of a soluble, active formyl peptide receptor in supernatants prepared by digitonin treatment of human neutrophil membranes. The receptor preparation met the two generally accepted criteria of solubility. It was not sedimented during centrifugation at 100,000 x g for 2 h, nor was it retained by a 0.2-μm pore size filter.

The soluble receptor exhibited many of the important physical and functional properties which had been determined for the membrane-bound receptor. A polypeptide in the digitonin extract with an apparent molecular weight by sodium dodecyl sulfate polyacrylamide gel electrophoresis between 55,000 and 70,000 was covalently affinity labeled with 125I-labeled fNLPNTL and co-migrated with the affinity-labeled, membrane-bound formyl peptide receptor (10). This demonstrated not only that the receptor was present in the extracts, but also that it had not been degraded during the solubilization.

Binding of the 125I-labeled fNLPNTL to the soluble receptor was saturable, with a single class of sites demonstrating a Kd = 2.2 nM. The Kd for the soluble receptor was slightly higher than the Kd = 0.75 nM seen with the same batch of membranes prior to detergent treatment. This may represent a true change in the binding constants of the receptor upon alteration of its lipid environment, or alternatively, may simply reflect the differences in assay conditions used for the soluble and membrane-bound receptor. N-Formyl-Nle-Leu-Phe-Neu125I-Tyr-Lys is quite hydrophobic and a considerable proportion may partition into the digitonin micelles and be unavailable for receptor binding. If such were the case, the concentration of peptide free in solution would be considerably less than the total peptide in solution. The Kd = 2.2 nM was based on the assumption that all of the radiolabeled peptide in solution was available for binding; the true Kd may be lower.

Both the membrane-bound and soluble receptor show a marked preference for formylated peptides. Whereas N-formyl-NLPNTL inhibited binding to the soluble receptor by 50% at 10 nM, unformylated NLPNTL showed an insignificant effect at 10 μM. N-Formyl-Met-Leu-Phe-Met-Tyr and N-formyl-Met-Leu-Phe demonstrated a Kd of 1 nM and 200 nM, respectively. These values are higher than the values found for inhibition of binding to the receptor on whole cells (4), but again partitioning of the unlabeled peptides into the digitonin micelles may explain these quantitative differences. No saturable binding of 125I-labeled fNLPNTL to digitonin extracts of erythrocyte membranes was evident.

The pH optimum for binding to whole cells (4) isolated membranes (17) and the soluble receptor were identical. Likewise, both the membrane-bound (17) and soluble receptor were sensitive to inhibition by sulfhydryl reagents and inactivation of the membrane-bound receptor led to a parallel decrease in the yield of soluble receptor.

The soluble receptor will withstand several cycles of freeze-thaw over a 5- to 10-day period without loss of activity. No rigorous data regarding requirements for longer term stability are available, but dithiothreitol may be of benefit. The extraction procedure and assay method detailed here represent the first steps in an attempt to purify and characterize this immunologically important receptor.

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