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MEASUREMENT OF ENVIRONMENTAL FORMYLMETHIONYL-PEPTIDES

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Formylmethionyl-peptides are naturally occurring, biologically active ligands produced by bacteria. They produce a variety of biological effects including neutrophil chemotaxis, cellular degranulation, oxygen-free radical production, and smooth muscle contraction. Our studies have demonstrated that oxidized and reduced forms of formylmethionyl-leucyl-phenylalanine (fMLP) can be detected in bulk environmental organic dust samples. Organic dust fMLP content may not reflect total formylmethionyl-peptide content and pathological sequelae. Attempts to develop a total formylmethionyl-peptide assay that would reflect its pathological potential have thus far been unsuccessful. Information has been derived concerning the biology of formylmethionyl-peptides from these studies. Chromatographic, radioenzymatic, and radioreceptor-ligand binding studies were performed. High-performance liquid chromatography (HPLC) analysis of synthetic and environmental fMLP demonstrated that fMLP is labile, forming three oxidation products. HPLC is limited by inadequate sensitivity for air sample analysis and the probability of the presence of multiple formylmethionyl-peptides. Deformylases were isolated from Escherichia coli, *but their usefulness in a competitive assay to detect formylmethionyl-peptides was limited by specificity differences from that for biological receptors. Receptor binding studies were conducted in an attempt to replace the deformylase with a biological receptor. The receptor binding patterns noted were consistent with the existence of three distinct formylmethionyl-peptide receptor subsets in neutrophils and alveolar macrophages. The plurality of fMLP receptor subtypes interfered with formylmethionyl-peptide measurement in a competitive assay. Formylmethionyl-peptides may contribute to organic dust-induced disease, but better techniques for the assessment of exposure to these agents are needed to properly assess their health impact.*

N-Formylated methionyl-peptides of bacterial origin are potent chemotactic agents for granulocytes at femtomolar concentrations (Showell et al., 1976). They can also cause granulocyte degranulation, superoxide production, and mast-cell histamine release at nanomolar levels (Korchak et al., 1984; Lehmeyer et al., 1979; Simchowitz & Spilberg, 1979; Siraganian & Hook, 1977). There has been controversy over the number of formyl-peptide receptor subtypes. Receptor binding studies have thus far failed to demonstrate more than one receptor (Freer et al., 1982; Aswanikumar et al., 1977;

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Tennenberg et al., 1988). However, receptor-formylmethionyl-peptide affinity studies employing association and dissociation rates have suggested the existence of two binding sites with different affinities (Mackin et al., 1982; Koo et al., 1982; Mehta & Spilberg, 1983).

N-Formylmethionyl-leucyl-phenylalanine (fMLP) is the most commonly studied peptide in this group. Marasco et al. (1984) identified fMLP as the major chemotactic peptide from *Escherichia coli*. They were able to isolate several formylated peptides of varying chemotactic potencies from culture filtrates. While fMLP is the most abundant chemotactic peptide from *E. coli*, it is probably not the most potent. The tetrapeptides from this group have been shown to be considerably more potent (Freer et al., 1982).

fMLP is subject to oxidation by the granulocyte myeloperoxidase (Tsan & Denison, 1981). Oxidation of fMLP causes a partial inactivation and a decrease in its potency on granulocytes (Van Dyke et al., 1984). Fedan et al. (1990) noted that both fMLP and oxidized fMLP (ox-fMLP) could be found in cotton dust extracts. Since inhalation of fMLP causes pulmonary responses in a guinea pig animal model that are similar to those resulting from exposure to cotton dust, it has been suggested that fMLP may play a role in pulmonary diseases such as byssinosis (Frazer et al., 1992).

We have examined several alternatives for measuring bacterial formylated peptides from environmental samples. These include high-performance liquid chromatography (HPLC), competitive radioenzymatic studies, and receptor binding assays. Each of these methods has drawbacks that hinder its use in quantitation of environmental formylated peptides levels for correlation with occupational/environmental lung diseases.

MATERIALS AND METHODS

HPLC

Two HPLC methods were employed. The first was essentially the same as that described by Fedan et al. (1990). The fMLP was eluted from a μBondapak C_{18} reverse-phase column (3.9 mm x 300 mm, Waters Chromatography, Milford, Mass.), using 60% methanol/40% 0.1 *M* acetic acid and detected at 420 nm. The second method employed a 3.9 x 150 mm cyanopropyl column, 25% acetonitrile/75% 0.1 *M* phosphate buffer (pH 3) and a 190-nm detection wavelength. Tailing was noted when larger injections of fMLP/methanol were made (>50 μl). Acidification of the sample with the mobile phase phosphate buffer alleviated this problem.

Culture of Bacteria for fMLP Analysis

Bacteria isolated from environmental and clinical sources were revived from lyophilized preparations, and a fresh agar slant of each organism was used to prepare inoculum on tryptic soy agar (TSA, Difco, Detroit, Mich.). Fifty milliliters of each culture was added to a 125-ml Erlenmeyer flask and

incubated for 24 h at 24°C in susceptibility test medium (STM) in a shaker-incubator at 150 revolutions/min. After incubation, the contents of each flask were centrifuged in a 50-ml conical centrifuge tube (1000 x g, 30 min, 4°C). The supernatant fluid was filtered through a 0.45-μm cellulose acetate filter (Millipore Corp., Bedford, Mass.). The filtrate was stored at –80°C until analysis.

Oxidation of fMLP

Twenty-five milligrams fMLP (Sigma Chemical, St. Louis, Mo.) was added to a 0.75% H_2O_2/methanol (total volume 4 ml). This preparation was incubated at room temperature for 12 h and evaporated to dryness under nitrogen. The ox-fMLP was stored dry at –20°C.

Fluorescamine Derivatization

Various concentrations of fMLP were added to 0.067 ml fluorescamine (3 mg/ml dioxane, Sigma Chemical, St. Louis, Mo.) in a 0.05 *M* phosphate buffer (pH 8, final volume 2 ml). Fluorescent intensity was monitored at 476 nm using an excitation wavelength of 390 nm. A secondary emission peak was noted at 780 nm.

Radioenzymatic Assay

Escherichia coli (*E. coli*), from a clinical isolate, was revived from a lyophilized preparation and used as a source of bacterial deformylase. Fresh tryptic soy agar slant (TSA, Difco, Detroit, Mich.) was used to prepare inoculum. The slant was then used to inoculate 1 L of tryptic soy broth, and the bacteria was grown on an orbital shaker at 37°C for 24 h. The bacteria were washed three times with phosphate-buffered saline (PBS), resuspended in PBS, and the cell membranes were cracked using a Carver-French press to free intracellular enzymes, including deformylases. This preparation was lyophilized and stored at –70°C. The lyophilized bacterial preparation was resuspended in HEPES buffer (145 m*M* NaCl, 5 m*M* KCl, 1 m*M* $CaCl_2$, 5.5 m*M* glucose, and 10 m*M* HEPES; pH = 7.4) and centrifuged (1000 x g, 30 min, 4°C). One hundred microliters of the bacterial supernatant fluid was added to a tube containing 50 μl ^{14}C-fMLP (final concentration 8.1 μ*M*, 16 mCi/mmol, ^{14}C on formyl group; Sigma Chemical, St. Louis, Mo.) and 50 μl of cold peptide. The reaction was carried out at 37°C for 60 min and stopped by the addition of 20 μl of 2 *M* H_3PO_4. The reaction mixture was centrifuged and 200 μl was applied to a C_{18} solid-phase separation medium (350 mg, Waters Chromatography). The C_{18} column had been prewashed sequentially with 3 ml each of methanol, water, and 0.2 *M* H_3PO_4. The free formyl groups were eluted from the solid phase into a scintillation vial using 2 ml 0.2 *M* H_3PO_4. Ten milliliters of scintillant was added and the radioactivity was determined using a liquid scintillation counter.

The bacterial enzyme preparation was partially purified by adding 10 mg of the lyophilized bacterial preparation to 0.25 ml dimethyl sulfoxide and

applied to a column containing 0.5 g carboxymethylcellulose. The loaded column was washed with 4 ml of 0.05 *M* LiCl, and six 1-ml fractions with 0.25 *M* LiCl were eluted. Each fraction, as well as a 2 mg/ml lyophil-bacteria/0.25 *M* LiCl control, was then concentrated approximately fivefold in Centricon 10 microconcentrators (Amicon, Beverly, Mass.) precoated with bovine serum albumin (5 mg/ml PBS).

Receptor Binding Assays

Three different cell types, guinea pig pulmonary macrophages, rabbit peritoneal neutrophils, and human peripheral neutrophils, were studied. Guinea pig pulmonary macrophages were obtained by bronchoalveolar lavage. The purity was assessed and cell numbers were quantitated using an electronic cell counter equipped with a cell sizing attachment as previously described (Castranova et al., 1987). All cells were kept on ice throughout the procedure. Varying amounts (0–709 n*M* final concentration) of ^{3}H-fMLP (New England Nuclear, Boston) were added to 10^6 macrophages in PBS (145 m*M* NaCl, 5 m*M* KCl, 1.9 m*M* NaH_2PO_4, and 9.35 m*M* Na_2HPO_4; pH = 7.4) and incubated on ice for 60 min. The cells were rapidly filtered onto glass fiber filters and washed twice with 5 ml PBS. The filters were transferred to scintillation vials containing Scintiverse (New England Nuclear). The vials were vortexed and allowed to sit overnight (to allow for complete dissociation of the label from the glass fiber filters) prior to counting on a liquid scintillation counter. Human neutrophils were isolated using Mono-Poly Resolving Media (Flow Laboratories, McLean, Va.). Excess red cells were hypotonically lysed and neutrophil fMLP receptor binding was assayed in a similar manner. Ten million neutrophils/tube were used. Rabbit neutrophils were obtained by peritoneal lavage 1 d after intraperitoneal injection of 50 ml of 0.1% glycogen. The cells were pretreated with tosyl-1-phenylalanyl chloromethane (Sigma Chemical) to inhibit enzymatic degradation. Receptor binding assays were performed on 5×10^6 neutrophils/tube as described earlier.

RESULTS

Initial measurement of fMLP and ox-fMLP was performed using the published HPLC method, which employs ultraviolet (UV) detection at 420 nm (Fedan et al., 1990). A single peak for fMLP and one for ox-fMLP was observed. This method was not very sensitive (limit of detection 850 ng fMLP). The second HPLC method employed a variable-wavelength detector set at 190 nm. This necessitated changing the mobile phase to one with an acceptable ultraviolet cutoff. The acetonitrile-phosphate buffer mobile phase proved acceptable for this purpose. The fMLP and ox-fMLP could be separated. However, three ox-fMLP peaks were now separated and detected (Figure 1). The limit of detection for fMLP using the new HPLC method with 190 nm detection was 6.5 ng. Stock fMLP (reduced form) was also found to

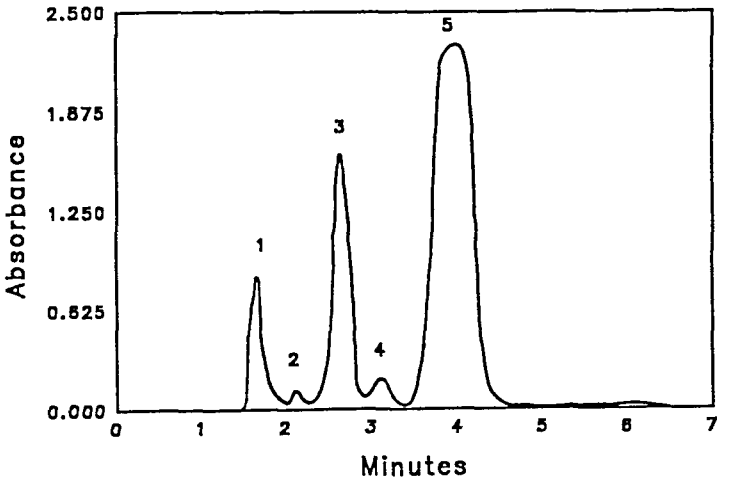

FIGURE 1. Chromatogram of fMLP and ox-fMLP. Peak 1, solvent; peaks 2, 3, and 4, fMLP oxidation products; peak 5, fMLP (reduced form). Peaks were eluted from a 3.9 × 150 mm cyanopropyl column using 25% acetonitrile/75% 0.1 *M* phosphate buffer (pH 3) at 1 ml/min and detected at 190 n*M*.

have some ox-fMLP (0.02% ox-fMLP). The same cotton dust extracted and assayed by Fedan et al. (1990) was extracted by the same protocol and reassayed by both HPLC methods. The oxidized form of fMLP was detected at levels similar to what was reported previously. Fedan et al. (1990) reported cotton dust extract contained 103.2 and 649.0 μg/g fMLP and ox-fMLP, respectively. Using our new HPLC method, ox-fMLP was measured at 595 μg/g, while no reduced fMLP was detected (Table 1). These differences reflect degradation during storage (this second dust analysis was performed on dust samples that had been stored for >2 yr in a sealed glass vial at room temperature).

Bulk sorghum samples, collected as part of a study of pulmonary reactions in grain handlers, have also been assayed using the more sensitive HPLC method, and both ox-fMLP and fMLP have been detected (Table 1). The sorghum samples contained varying amounts of seed, leaf, and dust. The predominant form in these samples was again ox-fMLP. All three oxidized peaks were observed in the sorghum extracts, yet in different area ratios than seen in the laboratory H_2O_2 oxidized standards. The sum of the areas of the three ox-fMLP peak areas was used for quantitative purposes.

Various bacterial isolates from both clinical and environmental sources were cultured and the culture supernatant was assayed directly by HPLC (190 nm UV detection). Table 2 indicates the bacterial cultures tested and the source of each. Ox-fMLP and fMLP were detected in two of the *E. coli* cultures and in the *Pseudomonas aeruginosa* culture.

We have also examined qualitatively fMLP solubility in different solvents. Methanol was used for extraction, as it proved to be the best solvent in terms of fMLP solubility (>10 mg/ml). Tetrahydrofuran and 0.05 *M* phos-

TABLE 1. fMLP and ox-fMLP Content of Environmental Samples

Samples	fMLP $X \pm SD$ (range)	ox-fMLP $X \pm SD$ (range)
Cotton dust ($n = 1$)	ND	595
White milo sorghum[a] ($n = 4$)	36.48 ± 25.45 (ND to 55.9)	75.44 ± 53.97 (ND to 137.9)
Red milo sorghum[a] ($n = 5$)	44.48 ± 24.53 (ND to 71.8)	69.33 ± 74.44 (ND to 171.1)

Note. All values are μg ligand/g sample. ND, not detected.
[a]Sorghum samples contain seed, leaf, and dust in varying proportions.

phate buffer (pH 7) were also good solvents (>1 mg/ml). Solubility in acetone was poor, and only very limited solubility was seen with acetonitrile and water (<0.1 mg/ml).

It was noted that stock fMLP was fluorescent in a concentration-dependent manner when reacted with fluorescamine (Figure 2). Unfortunately, this could not be chromatographed. Reverse-phase chromatography employing mobile phase modifiers, including octane sulfonic acid, phosphate buffer (pH 8), dibutylamine, butanol-saturated water, and tetraethyl-ammonium-formate, failed to elute a fluorescamine-fMLP peak. Normal-phase chromatography and ion-exchange chromatography were also unsuccessful.

TABLE 2. Bacterial Culture Supernatant Fluids Assayed for fMLP and ox-fMLP

Bacteria	Source
Cultures with detectable fMLP production	
Escherichia coli	Sick building air sample
Escherichia coli	Urinary tract infection
Pseudomonas aeruginosa	Tracheal washing of cystic fibrosis patient
Cultures in which fMLP could not be detected	
Yersinia pseudotuberculin	Oil
Pseudomonas cepecia	Sick building air sample
Staphylococcus aureus	Cow feces
Psuedomonas mallei	Oil
Bacillus sp.	Hay
Staphylococcus sp.	Human blood
Streptomyces sp.	Hay
Enterococcus fecalis	River water
Micrococcus sp.	Clinical isolate
Aeromonas hydrophylus	Oil
Clavibacter michiganese	Chicken barn air sample
Escherichia coli	Porcine intestine
Streptococcus sp.	Urinary tract infection

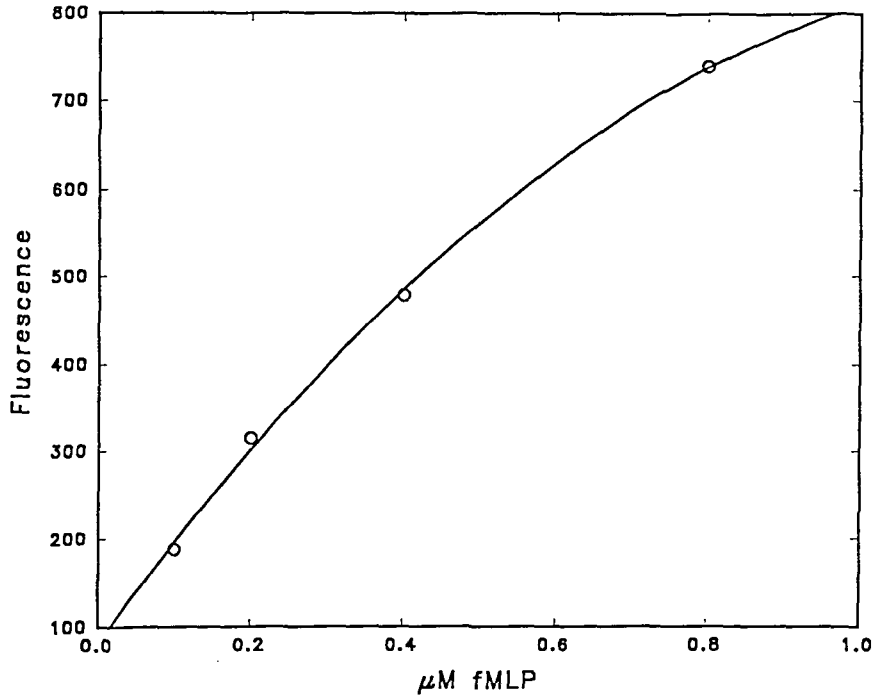

FIGURE 2. Dose-dependent fluorescence after reacting stock fMLP with fluorescamine. Fluorescence measured at λ_{em} = 476 mm with λ_{ex} = 390.

Deformylases obtained from *E. coli* were used in a competitive assay in an attempt to measure total formyl chemotactic peptide. The deformylases liberate formic acid from the peptide. A competitive assay employing fMLP and ^{14}C-fMLP was developed. Solid-phase C_{18} extraction was employed to separate the free formic acid from the parent peptide. This method was easy, quick, and produced little radioactive solvent waste. The addition of cold fMLP to a crude *E. coli* deformylase preparation competitively inhibited [^{14}C]formate liberation from ^{14}C-fMLP (Figure 3). An attempt was made to refine the enzyme preparation. Three of the six fractions collected displayed measurable deformylase activity (fractions 1, 3, and 6). The crude deformylase preparation was tested for specificity. Formyl-leucyl-leucyl-phenylalanine, formylmethionyl-leucyl-tyrosine, and acetyl-methionyl-leucyl-phenylalanine all inhibited the deformylation of fMLP (data not shown).

We examined the fMLP receptors on guinea pig pulmonary macrophages, rabbit neutrophils, and human neutrophils (Figure 4). The data are normalized to the highest ^{3}H-fMLP concentration used, 709 n*M*. Three distinct receptor subpopulations were consistently seen in the guinea pig and rabbit cells. The highest affinity receptor became saturated between 50 and 100 n*M*, and the next at approximately 140 n*M* fMLP. The lower affinity receptor was saturated at 350 n*M* fMLP with guinea pig pulmonary macro-

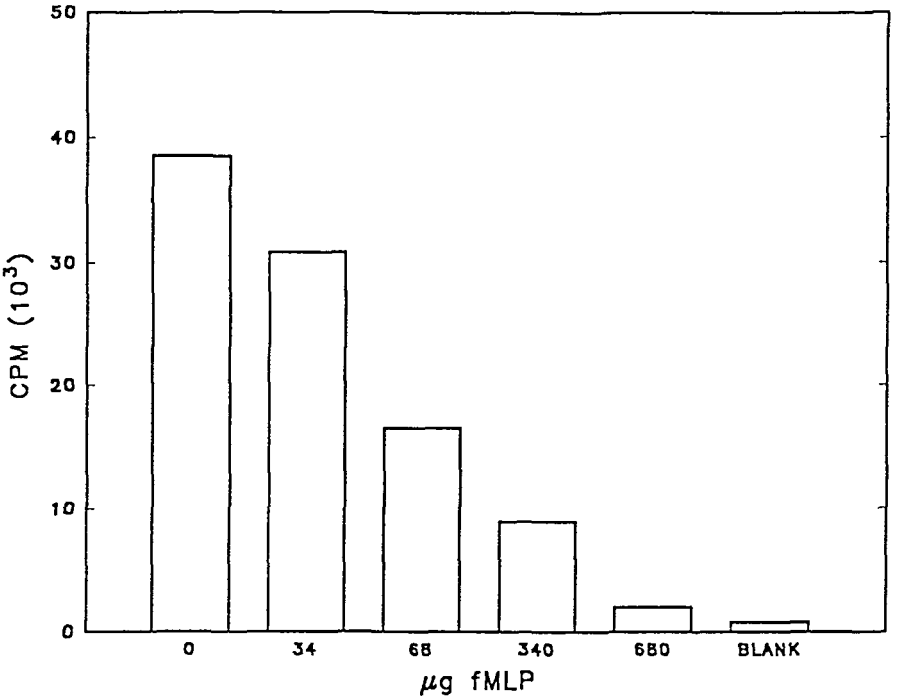

FIGURE 3. Competitive inhibition of *E. coli* deformylase activity on ^{14}C-fMLP (8.1 μM) by the addition of cold fMLP. Blank, no deformylase. Data from a single experiment shown.

phages and rabbit neutrophils, but saturation was not apparent with human neutrophils. The higher affinity fMLP receptor subpopulations were less distinct in the human cells and were not always discernable. Two radioreceptor studies were run on neutrophils isolated from the same individual 4 mo apart. The receptor subpopulations were distinct at one time but not the next. Receptor binding analysis at 37°C for 12 min provided lower binding than incubation on ice for 1 h, suggesting metabolism of fMLP by macrophage and neutrophil proteases. However, preincubation with soybean trypsin inhibitor or cytoclasin B decreased, rather than increased, fMLP binding to guinea pig macrophages. The coefficient of variation (CV) for the assay was 14.2%. The average error in receptor binding within a given species was 27.4%. The dependent axis on Figure 4 has been normalized to the binding resulting from the highest concentration of ^{3}H-fMLP used to allow for comparison between different species/cell types of the trends noted.

Nonspecific binding was not assessed in these preparations. It was found, in agreement with Tennenberg et al. (1988), that high concentrations of fMLP are required to fully saturate all the receptor sights. Millimolar concentrations of cold fMLP are required to fully saturate and competitively dis-

place the ^{3}H-fMLP. Solubility of fMLP in physiological buffers is a problem at these concentrations, with peptide precipitation interfering with analysis. The various subpopulations and the high concentrations of fMLP required to compete with the label precluded the use of a receptor inhibition assay for analysis of environmental formyl-chemotactic peptide.

DISCUSSION

HPLC analysis for the quantitation of fMLP can be a useful tool. We have used HPLC to identify the presence of and quantitate fMLP in environmental samples, bacterial culture supernatant fluids, and chamber samples from animal exposure studies (Frazer et al., 1992). HPLC analysis also pointed out the concern over stability of formylmethionyl-peptides. The methionine group is subject to oxidation. This was noted in stock fMLP standards stored in methanol at −20°C and environmental samples (cotton dust and sorghum). The inability to detect the reduced form of fMLP in extracts of the same cotton dust used by Fedan et al. (1990) could be explained by oxidation and degradation during storage. The fluorescamine derivatization studies may also have indicated product degradation or contamination in stock fMLP. Fluores-

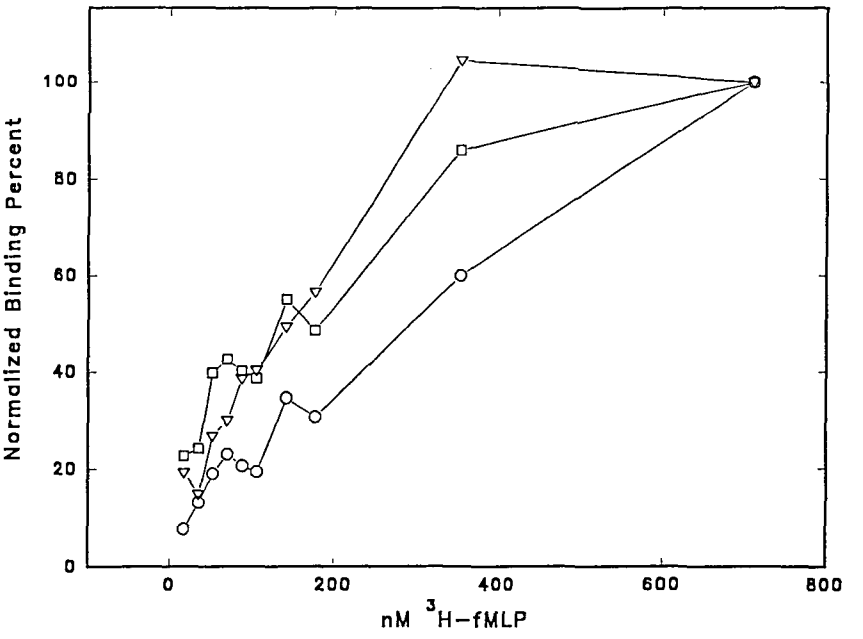

FIGURE 4. Human, guinea pig, and rabbit receptor binding of fMLP. ∇, Human peripheral neutrophils (n = 2); □, guinea pig alveolar macrophages (n = 3); ○, rabbit neutrophils from peritoneal exudate (n = 2 pooled). Receptor binding normalized to highest ^{3}H-fMLP concentration used (709 nM fMLP ≈ 100%) to allow for comparison between species. The standard errors averaged 27.4% of the mean between animals. Assay CV = 14.2%.

camine usually fluoresces following binding to primary amines, of which there are none in fMLP. The fluorescence noted in fMLP samples treated with fluorescamine was probably due to contamination. Oxidation of fMLP greatly reduces its potency (Van Dyke et al., 1984), which may confound laboratory studies if the samples and standards are not properly handled. The present work demonstrates that sampling and storage methodology is critical for work involving such peptides.

There are several drawbacks to the use of HPLC for the measurement of formylmethionyl-chemotactic peptides in environmental samples, including inadequate sensitivity for quantitation of fMLP from typical environmental air samples, which usually contain only a few milligrams of total dust. Interference peaks were also a problem in some of the dirtier bulk environmental samples. Quantitation of the ox-fMLP by HPLC assumes that the extinction coefficient at 190 nm of each oxidation product is the same and that each ox-fMLP form has a similar biological potency. At present, the composition of the three oxidized peaks and their relative biologic activities are not known. Since several different formylmethionyl-chemotactic peptides are produced by bacteria (Marasco et al., 1984), it was also felt that quantitation of fMLP and ox-fMLP alone may not reflect the inflammatory potential of organic dusts containing formylmethionyl-peptides.

It is of interest that fMLP and/or ox-fMLP were produced by bacteria associated with respiratory disease (Table 2). The contribution of formyl-chemotactic peptides to the pathological sequelae during these diseases (cystic fibrosis, urinary tract infection, and sick building syndrome) is unknown. The potency of fMLP does suggest that it may at least contribute to, or exacerbate, a respiratory disease condition. The bacterial cultures that were assayed but did not exhibit fMLP or ox-fMLP are also listed in Table 2. The lack of detectable fMLP does not demonstrate the lack of production by these organisms. Although these cultures had visible growth, the sampling time may not have been optimum. It is also possible that the major formyl-peptide produced by these bacteria is not fMLP, or that these cultures had greater protease activity, which quickly digested the fMLP that was produced.

The probability that fMLP is not the only bacteria-derived formyl-methionyl-chemotactic peptide and that the relative amounts of formyl-methionyl peptides may depend on the type of bacteria contaminating the environmental samples suggests that fMLP may not reflect the total formyl-chemotactic peptide activity in a sample. Because of this possibility, our direction was then shifted to the development of an assay for the measurement of total formyl-chemotactic peptides. To this end, we looked at the bacteria's ability to posttranscriptionally modify formylmethionyl-peptides. It has been suggested that one such process would entail deformylation of the peptide. The deformylase would liberate formic acid, which could then be measured. A competitive assay between fMLP and ^{14}C-fMLP was developed.

The enzyme specificity was found not to be comparable to that reported for the biological receptor (Freer et al., 1982). Therefore, this radioenzymatic assay was determined to be inappropriate for environmental formylmethionine-peptide analysis because of lack of specificity and sensitivity. Microgram quantities of fMLP are required for this assay. Industrial hygiene personal air samples usually contain only a few milligrams of total dust. Ideally, a limit of detection in the picogram range is needed. The method must also have a specificity similar to the receptors located on the target tissue to appropriately reflect the potency of the formylmethionyl peptides in the environmental sample. Identification of bacterial metabolic enzyme activity on formylmethionyl-peptides again points out that handling and storage of environmental samples may affect quantitative analytical measurement. Environmental samples containing live bacteria that produce deformylases may digest fMLP-like peptides and cause underestimation of the quantity in a specific environment and their contribution to disease.

The key to measuring total formylmethionyl-chemotactic peptide with the specificity of the biological response seemed to lie in the use of the biological receptor itself as a detection tool. Many studies concerning fMLP receptors have been published. Freer et al. (1982) reported rabbit neutrophil lysosomal release ED50 values for formylmethionyl-peptides in the 10^{-11} *M* range. They also reported binding inhibition ID50 values formyl-*n*-leucyl-leucyl-phenylalanine on various formyl peptides in the range of 10^{-7} to 10^{-10} *M*. Tennenberg et al. (1988) found that $>5 \times 10^{-5}$ *M* fMLP was required to saturate human neutrophil receptors, suggesting a much lower receptor affinity than that reported by Freer et al. (1982). Tennenberg et al. (1988) found only this one low-affinity fMLP receptor in their study. However, the chemotactic ability of fMLP at much lower concentrations and kinetic studies argue for at least one other higher affinity fMLP receptor.

Our receptor binding studies suggest that at least three fMLP receptors can be found on alveolar macrophages and neutrophils. This is the first time that evidence suggesting the existence of three distinct fMLP receptor subpopulations has been reported. Receptor subpopulation affinities are overlapping and easily smoothed over during analysis of receptor binding studies. The intraspecies variation in binding of fMLP to cellular receptors was great (SE = 27.4%). Only a small part of this error can be attributed to methodology (CV = 14.2%). This intraspecies variation in receptor binding (i.e., density) also may contribute to a masking of receptor subpopulations in binding studies. The variation between individuals or in one individual at different times reflects the dynamic nature of receptors. Receptor expression is subject to up- or down-regulation. This may greatly affect the ability to identify receptor subpopulations in binding studies. The consistent appearance of breaks in the receptor binding curves, as well as known biological activities that correspond to two of the subpopulations seen, such as neutrophil chemotaxis at 10^{-10} *M* fMLP and respiratory burst at 10^{-6} *M* (Van Dyke et al.,

1984), argues to support the premise of distinct fMLP receptor subpopulations. The low-affinity receptor was noted here, but has not been reported in the kinetic studies. However, the kinetic studies did not employ high enough fMLP concentrations to examine this receptor subpopulation.

As noted in the results section, the three receptor subpopulations were also found at times in human neutrophils, but they were not always discernable as displayed by the average of the binding studies (Figure 4). Environmental influences on the host could possibly influence receptor expression. The isolation technique for human neutrophils employed hypotonic lysis of residual red blood cells. The extent of hypotonic stress may have also influenced receptor expression.

Reported Scatchard analyses of fMLP receptors are flawed because of the overestimation of nonspecific binding. The number of receptors is not saturated and the labeled ligand is not fully displaced at concentrations of cold fMLP employed in reported studies. The concentration of fMLP required to fully saturate and compete with a radiolabeled ligand (i.e., 100 × saturation) was beyond the solubility of fMLP in physiological buffers and caused a pronounced precipitant.

Other possibilities for the measurement of formylmethionyl-chemotactic peptide include radioimmunoassay (RIA) or use of an isolated single receptor or receptor binding fragment. Marasco et al. (1982) examined the specificity of various anti-fMLP antibodies raised in rabbits and rats. He noted significant differences in specificity between neutrophil receptors and antibodies directed against fMLP for the carboxyl terminus end of phenylalanine and amino acids beyond phenylalanine. The formyl-tetrapeptides, which have greater potency than their formyl-tripeptide counterparts, are included in this group, which are poorly bound by specific anti-fMLP antibodies. Radioimmunoassay (RIA) may provide the sensitivity required for analysis of environmental air samples, but differences in specificity from the biological receptor may cause it to greatly underestimate the pathological potential of environmental dusts due to formyl-chemotactic peptide activity. Five extracellular domains have been identified for a human fMLP receptor (Radel et al., 1991). The second of these regions, containing 18 amino acids, has been reported to have the highest affinity for fMLP. It may be feasible to use the region two peptide to detect environmental formylmethionyl-peptides. However, the specificity or susceptibility to binding interferences of this peptide region is not known.

Although our attempts to develop an assay that would reflect the formyl-chemotactic peptide biological activity in an environmental sample have not been successful thus far, considerable information regarding the biology and detection of fMLP has evolved from these studies, including (1) the suggestion of three distinct fMLP receptors on alveolar macrophages and neutrophils, (2) the development of a new, more sensitive HPLC method for the detection of fMLP, (3) the development of a method for the measurement of bacterial deformylase activity, (4) elucidation of the problems associated

with formyl-peptide instability, and (5) demonstration of the association of fMLP with environmental samples known to produce disease and with bacteria associated with disease.

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