RESPONSE OF HUMAN NEUTROPHILS TO FORMYL-PEPTIDE MODIFIED AT THE TERMINAL AMINO AND CARBOXYL GROUPS

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Abstract—Two analogs of chemotactic peptide N-formyl-L-methionyl-L-leucyl-L-phenylalanine were examined for their capacity to activate several functions of human neutrophils. The C-terminus methyl ester derivative of the chemotactic peptide was found to possess strong biological activity and was able to induce levels of chemotaxis, enzyme secretion, and superoxide generation comparable to those observed with the same concentrations of N-formyl-L-methionyl-L-leucyl-L-phenylalanine. The analog containing a tert-butyloxycarbonyl group at the N-terminus, as well as the C-terminal methyl ester, was completely devoid of activity towards neutrophils. From these results, it appears that the free carboxyl group is not necessary for biological function. In contrast, the substituent at the N-terminus may play a critical role in the induction of the cellular response.

INTRODUCTION

The tripeptide N-formyl-L-methionyl-L-leucyl-L-phenylalanine (f-Met-Leu-Phe) can elicit a chemotactic response in polymorphonuclear leukocytes (PMNs). In addition, other cellular responses such as aggregation, granule enzyme secretion, and superoxide generation are triggered by this peptide (1, 2). Structure-activity studies have shown a strict correlation between the composition of this peptide and its biological potency. Although the structural requirements for activity are generally well established, the effects of substitutions at the amino and carboxyl termini are still controversial (3–6).

The aim of the present study was to determine the biological activity, towards human PMNs, of two derivatives of f-Met-Leu-Phe: one analog was methylated at the C-terminus, while the other contained a tert-butyloxycarbonyl...
group (Boc) at the N-terminus, in addition to the methyl ester block at the carboxyl end. Each analog was tested for its ability to affect random and oriented locomotion, activate chemotaxis, induce release of granule enzymes, and stimulate superoxide anion (O$_2^-$) production.

**MATERIALS AND METHODS**

**Cells.** Human PMNs were purified employing the standard techniques of dextran (Pharmacia, Uppsala, Sweden) sedimentation, centrifugation on Ficoll-Paque (Pharmacia), and hypotonic lysis (7). The cells were washed twice and were resuspended in KRPG (Krebs-Ringer-phosphate containing 0.1% w/v glucose, pH 7.4) at a concentration of 5–10 x 10$^6$ cells/ml. The percentage of PMNs was 99–100%.

**Chemotactic Factors.** f-Met-Leu-Phe was obtained from Sigma Chemical Co., St. Louis, Missouri. The two analogs, the methyl ester of f-Met-Leu-Phe (f-Met-Leu-Phe-OMe), and the Boc derivative of this analog (Boc-Met-Leu-Phe-OMe) were prepared essentially as described by Tognolo et al. (8). Briefly, the Boc amino acid was synthesized by using di-tert-butyl dicarbonate as the tert-butyloxycarbonylating agent; the amino acid methyl ester hydrochloride was prepared by reaction of the free amino acid with methanol in the presence of thionyl chloride. Removal of the Boc group was carried out by the treatment with a mixture of TEA-CHC1$_3$. Peptide coupling was achieved by the mixed-anhydride method. The formyl group was introduced through a conventional anhydride coupling. Thin-layer chromatography was carried out on precoated silica gel (F$_{254}$ Merck, BRD), using two solvent systems: system A = CHC1$_3$-MeOH-C$_6$H$_6$ (85:10:5) and system B = AcOET-pyridine-AcOH-H$_2$O (60:20:6:11). Peptides, considered homogeneous when a single spot was observed, were detected by spraying the chromatograms with either ninhydrin or modified chlorine reagent. $R_f$ values for Boc-Met-Leu-Phe-OMe were 0.83 in system A and 0.93 in system B, and for f-Met-Leu-Phe 0.62 in system A and 0.86 in system B, respectively. Stock solutions of the peptides (10$^{-2}$ M in dimethylsulfoxide) were dissolved in KRPG prior to the experiments. The chemotactic factor casein “Hammarsten” (Merck), was suspended in KRPG containing 1 mg/ml human serum albumin (Orth Beringwerke, BRD), as previously described (9).

**Random Locomotion.** Random locomotion was evaluated using modified Boyden chambers by estimating the distance in micrometers which the leading-front of the cells migrated, using the method of Zigmond and Hirsch (10). To test the chemokinetic effect, the peptides were added to both the top and the bottom compartment at a final concentration of 10$^{-5}$–10$^{-7}$ M.

**Chemotaxis.** To study its potential chemoactive activity, each peptide was added to the lower compartment of the Boyden chamber. The effect of peptides on casein-induced chemotaxis was evaluated by addition of the selected peptide (10$^{-9}$–10$^{-5}$ M) to both the top and the bottom compartments. A precise distinction between the effects of the peptides on the chemokinetics and chemotaxis of PMNs was made by using different concentration gradients of chemoattractant in the absence or in the presence of peptide. Results were analyzed, and true chemotaxis was taken as the difference between the observed values for positive gradients and the estimated values based on chemokinesis (10).

**Deactivation.** Neutrophils were incubated at 37°C for 30 min in the presence of different concentrations of each peptide (3) and subsequently tested for chemotaxis towards varying concentrations (10$^{-5}$–10$^{-6}$ M) of f-Met-Leu-Phe and its analogs.

**Superoxide Anion Production.** O$_2^-$ release was monitored continuously in a thermostatted spectrophotometer as superoxide dismutase-inhibitable reduction of ferricytochrome c, as described elsewhere (11). At zero time, different amounts (10$^{-9}$–10$^{-5}$ M) of each peptide were added and absorbance change accompanying cytochrome c reduction was monitored at 550 nm.