Bioorganic Chemistry **28,** 252–259 (2000)
doi:10.1006/bioo.2000.1175, available online at http://www.idealibrary.com on IDEAL®

Differences Arising in Human Neutrophil Activation Passing from *N*-Formyl to *N*-Acetyl-oligopeptides

Susanna Spisani* and Giorgio Cavicchioni†,[1]

**Department of Biochemistry and Molecular Biology, and*
†Department of Pharmaceutical Sciences, University of Ferrara, 44100 Ferrara, Italy

Received December 15, 1999

N-formyl- and *N*-acetyl-peptides were synthesized and compared in order to understand which features can best elicit biological responses. The behavior of *N*-formyl-peptides confirms the previously found sequential obligations in the residues, while acetyl-derivatives do not seem suitable for an efficacious stimulation of human neutrophils.

Key Words: human neutrophils; formyl-peptides; acetyl-peptides; chemotactic activity; superoxide anion production; lysozyme release.

INTRODUCTION

Regulation of the level of circulating neutrophils is very important because they control microbial infection by removing and killing pathogens. Neutrophils detect invading agents by means of signal molecules called chemoattractants, generated by bacterial infection and tissue damage (*1*); they express specific G-protein coupled receptors (*2*) for chemoattractants, which enable them to sense invading foreign particles and to approach the site of infection by triggering cytoskeletal reorganization and cell shape change (*3*,*4*).

N-formyl-Met-Leu-Phe-OH (fMLP), which derives from bacterial sources or disrupted mitochondria, is the reference chemotactic peptide together with its synthetic methylester derivative for-Met-Leu-Phe-OMe (fMLP-OMe).

Neutrophil chemotaxis toward the site of invasion is activated by low concentrations of the ligand (*5*); in contrast, its high concentration activates an array of other responses, including the release of hydrolytic enzymes from the granules into the extracellular fluid, and the triggering of a "respiratory burst," which is characterized by the generation of superoxide anion (O_2^-) (*6*).

Although it seems well-established that the formyl as amino-terminal blocking group is crucial for both binding and biological activity (*7*), a number of studies have

[1] To whom correspondence and reprint requests should be addressed. Fax: +39-532-291296. E-mail: g5z@dns.unife.it.

0045-2068/00 $35.00

nevertheless been performed on acetyl-oligopeptides, but the picture is still unclear. While it seems ascertained that acetyl-Met-Leu-Phe-OMe (Ac-MLP-OMe) is devoid of any activity, biological responses have been found for acetyl-peptides containing a varied and/or longer sequence: e.g., acetyl-peptides containing five or more residues elicit a potent chemotactic activity for human neutrophils (*8,9*).

These observations prompted us to try and give a final answer to the question whether a biological response is present (and if so, which) when the sequence in the prototype peptide is changed, and/or when the formyl group is replaced by an acetyl group in short peptides.

For this purpose, formyl- and acetyl-tri and tetra-peptides were synthetized to test their efficiency in triggering biological responses in human neutrophils. Chemotaxis, superoxide anion production, and lysozyme release of Ac-Met-Leu-Phe-OMe **1**, for-Met-Phe-Leu-OMe **2**, Ac-Met-Phe-Leu-OMe **3**, for-Met-Phe-Leu-Val-OMe **4**, Ac-Met-Phe-Leu-Val-OMe **5**, for-Met-Leu-Phe-Val-OMe **6**, and Ac-Met-Leu-Phe-Val-OMe **7** were compared with those observed for the reference peptide fMLP-OMe.

These sequences have been chosen because they are present in the N-terminal sequence of the calpain small subunit and exhibit chemotactic activity (*8*).

MATERIALS AND METHODS

Chemistry

The ^{1}H-NMR spectra were recorded in deuterated chloroform ($CDCl_3$) and dimethylsulphoxide (DMSO-d6) on a Bruker AC200 spectrometer at 200 MHz. Chemical shifts are expressed as δ (ppm) related to the TMS signal.

Optical rotations were determined in MeOH at 20°C with a Perkin–Elmer Model 241 polarimeter.

Melting points were determined on a Reichert–Kofler block and are uncorrected.

Thin layer chromatography was performed on precoated silica gel F_{254} (Merck) with the solvent system: methylene choloride/toluene/methanol 17/1/2.

Satisfactory microanalyses were obtained for all compounds, analytical results being within $\pm 0.4\%$ of the theoretical values.

Amino acids hydrochlorides as well as their Boc and acetyl derivatives were purchased from Fluka. Removal of the Boc group was performed by treatment with a 1:1 mixture of trifluoroacetic acid (TFA)-$CHCl_3$. Peptide coupling was achieved (i) by the racemization-free mixed-anhydride method with isobutylchloroformate (IBCF) and triethylamine (TEA) (*10*), and (ii) by the 1-hydroxy-benzotriazole (HOBt)–*N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDC) method (*11*), while the formyl group was introduced according the *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) method (*12*).

Purification of all the final products were achieved by reverse phase HPLC analysis on a Waters Delta Prep 3000 and revelation with UV spectrometer Waters 484 at 220 nm using, as stationary less polar phase a Delta Pack C 18-300 A column (30 mm × 30 cm, particles 15 μm) with a proper eluting system.

Ac-Met-Leu-Phe-OMe **1**

The peptide was synthesized following standard procedures in solution (Fig. 1). Solid (mp.185–187°C; R_f 0.53; $[\alpha] = -15.5°$, $c = 1$). ^{1}H-NMR ($CDCl_3$): 0.88 and

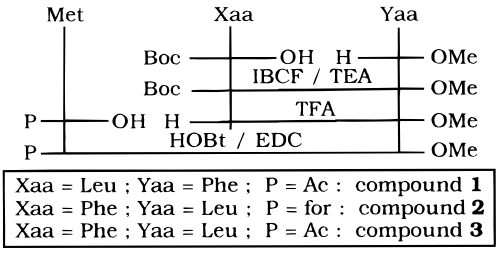

FIG. 1. Scheme of synthesis of compounds **1, 2**, and **3**. All the abbreviations are reported under Materials and Methods.

0.91 (6H; $2CH_3$; 2d; $J = 5.22$ Hz); 1.35–1.60 (2H; CH_2; m); 1.90–2.05 (3H; CH_2+CH; m); 2.00 (3H; CH_3CO; s); 2.08 (3H; SCH_3; s); 2.49 (2H; CH_2; m); 3.06 and 3.14 (2H; CH_2; 2dd; AB of ABX; $J_{AB} = 14.41$ Hz; $J_{AX} = 5.60$ Hz; $J_{BX} = 6.27$ Hz); 3.70 (3H; OCH_3; s); 4.45–4.90 (3H; 3CH; m); 6.72 (1H; NH; d; $J = 8.14$ Hz); 6.93 (1H; NH; d; $J = 7.83$ Hz); 7.07–7.13 (3H; C_6H_5+NH; m); 7.23–7.28 (3H; C_6H_5; m).

For-Met-Phe-Leu-OMe **2**

The peptide was synthesized following standard procedures in solution (Fig. 1). Solid (mp. 176–178°C; R_f 0.49; $[\alpha] = -70.3°$, $c = 1$). ^{1}H-NMR ($CDCl_3$/DMSO-d6 1:1): 0.84 and 0.87 (6H; $2CH_3$; 2d; $J = 7.08$ Hz); 1.45–1.95 (5H; $2CH_2$+CH; m); 1.97 (3H; SCH_3; s); 2.31 (2H; CH_2; t; $J = 7.68$ Hz); 2.80 and 3.05 (2H; CH_2; 2dd; AB of ABX; $J_{AB} = 13.02$ Hz; $J_{AX} = 4.38$ Hz; $J_{BX} = 8.59$ Hz); 3.60 (3H; OCH_3; s); 4.25–4.45 (2H; 2CH; m); 4.55 (1H; CH; m); 6.72 (1H; NH; d; $J = 8.14$ Hz); 7.17–7.13 (5H; C_6H_5; s); 7.97 (1H; HCO; s); 8.02–8.16 (2H; 2NH; m).

Ac-Met-Phe-Leu-OMe **3**

The peptide was synthesized following standard procedures in solution (Fig. 1). Solid (mp.168–170°C; R_f 0.45; $[\alpha] = -42.7°$, $c = 1$). ^{1}H-NMR ($CDCl_3$): 0.87 and 0.90 (6H; $2CH_3$; 2d; $J = 5.62$ Hz); 1.45–1.65 (2H; CH_2; m); 1.80–2.02 (3H; CH_2+CH; m); 1.96 (3H; CH_3CO; s); 2.05 (3H; SCH_3; s); 2.47 (2H; CH_2; m); 3.06 (2H; CH_2; m); 3.69 (3H; OCH_3; s); 4.43–4.89 (3H; 3CH; m); 6.62 (1H; NH; d broad); 6.72 (1H; NH; d broad); 6.95 (1H; NH; d broad); 7.10–7.40 (5H; C_6H_5; m).

For-Met-Phe-Leu-Val-OMe **4**

The peptide was synthesized following standard procedures in solution (Fig. 2). Solid (mp.215–218°C; R_f 0.65; $[\alpha] = -9.1°$, $c = 1$). ^{1}H-NMR ($CDCl_3$/DMSO-d6 1:1): 0.85–0.87 (12H; $4CH_3$; 2d); 1.42–1.53 (2H; CH_2; m); 1.54–1.90 (3H; CH_2+CH; m); 1.96–2.09 (1H; CH; m); 2.03 (3H; SCH_3; s); 2.29–2.36 (2H; CH_2; m); 2.81 and 3.02 (2H; CH_2; 2dd); 3.61 (3H; OCH_3; s); 4.18 (1H; CH; m); 4.35 (1H; CH; m); 4.53 (1H; CH; m); 7.17 (5H; C_6H_5; m); 7.69 (1H; NH; d; $J = 8.27$ Hz); 7.83 (1H; NH; d; $J = 6.99$ Hz); 7.85 (1H; HCO; s); 7.90 (1H; NH; d; J=8.17 Hz); 8.07 (1H; NH; d; $J = 8.31$ Hz).

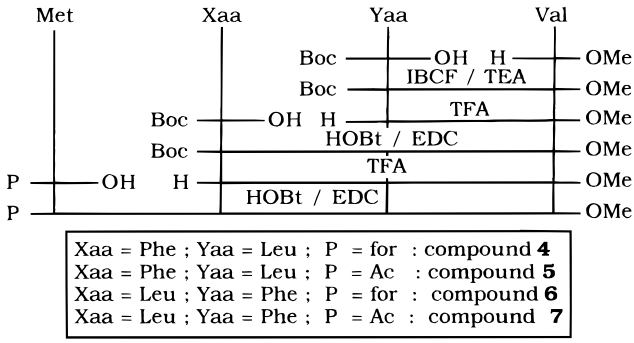

FIG. 2. Scheme of synthesis of compounds **4, 5, 6**, and **7**. All the abbreviations are reported under Materials and Methods.

Ac-Met-Phe-Leu-Val-OMe **5**

The peptide was synthesized following standard procedures in solution (Fig. 2). Solid (mp.>220°C; R_f 0.56; $[\alpha] = -27.1°$, $c = 1$). ^{1}H-NMR (DMSO); 0.89–0.96 (12H; 4CH_3; 2d); 1.47–1.85 (3H; CH_2+CH; m); 1.92 (3H; SCH_3; s); 2.07 (3H; CH_3; s); 2.15 (1H; CH; m); 2.49 (2H; CH_2; t; $J = 7.0$ Hz); 3.12 (2H; CH_2; AB of ABX; $J_{AB} = 6.50$ Hz; $J_{AX} = 3.17$ Hz; $J_{BB} = 2.54$ Hz); 3.74 (3H; OCH_3; s); 4.41–4.58 (3H; 3CH; m); 4.75 (1H; CH; m); 6.31 (1H; NH; d; $J = 6.63$ Hz); 6.95 (1H; NH; d; $J = 10.00$ Hz); 7.02 (1H; NH; d; $J = 9.12$ Hz); 7.10–7.40 (7H; C_6H_5+NH; m).

For-Met-Leu-Phe-Val-OMe **6**

The peptide was synthesized following standard procedures in solution (Fig. 2). Solid (mp.218–220°C; R_f 0.61; $[\alpha] = -48.7°$, $c = 1$). ^{1}H-NMR ($CDCl_3$): 0.85–0.88 (12H; 4CH_3; 2d); 1.42–1.60 (2H; CH_2; m); 1.90–2.20 (5H; 2CH_2+CH; m); 2.04 (3H; SCH_3; s); 2.47 (2H; CH_2; m); 3.05 (2H; CH_2; d; $J = 6.82$ Hz); 3.70 (3H; OCH_3; s); 4.44 (1H; CH; m); 4.71 (1H; CH; m); 4.96 (1H; CH; m); 7.18 (1H; NH; d broad); 7.21 (5H; C_6H_5; m); 7.37 (1H; NH; d broad); 7.57 (1H; NH; d broad); 7.79 (1H;NH; d broad); 8.21 (1H; HCO; s).

Ac-Met-Leu-Phe-Val-OMe **7**

The peptide was synthesized following standard procedures in solution (Fig. 2). Solid (mp.>220°C; R_f 0.69; $[\alpha] = -51.4°$, $c = 1$). ^{1}H-NMR ($CDCl_3$); 0.83–0.89 (12H; 4CH_3; 2d); 1.48 (2H; CH_2; m); 1.93–2.17 (4H; CH_2+2CH; m); 2.03 (3H; SCH_3; s); 2.08 (3H; CH_3; s); 2.49 (2H; CH_2; m); 3.08 (2H; CH_2; m); 3.70 (3H; OCH_3; s); 4.42 (2H; 2CH; m); 4.61–4.80 (2H; 2CH; m); 6.59 (1H; NH; d; $J = 7.03$ Hz); 6.68 (1H; NH; d; $J = 7.14$ Hz); 7.00 (1H; NH; d; $J = 7.78$ Hz); 7.10–7.40 (6H; C_6H_5+NH; m).

Biological Essay (13)

Human neutrophils were purified employing the standard techniques of dextran sedimentation of heparinized blood, followed by centrifugation on Ficoll–Paque and