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Conformational studies of chemotactic HCO-Met-Leu-Phe-OMe analogues

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Summary. In order to investigate the proper peptide backbone conformation able to elicit a biological activity, HCO-Met-Pro-Phe-OMe, HCO-Met-Ψ[COO]Leu-Phe-OMe, and HCO-Met-OLeu-Phe-OMe, analogues of the prototypical chemotactic peptide HCO-Met-Leu-Phe-OMe, were studied by CD and IR techniques. The results obtained comparing biological and conformational data evidence the critical presence of (i) the NH group at position 2, (ii) a rather flexible backbone, (iii) the chemical structure of the central residue which can affect the stability of a possible active conformer.

Keywords: Amino acids – Chemotactic peptides – Structure/activity relationship – CD – IR – Conformation

Introduction

Chemotaxis is defined as a reaction by which the direction of locomotion of cells is determined by substances, called chemotactic agents, in their environment. These substances of different origin, chemical nature and molecular size, are responsible for the accumulation of leukocytes, particularly neutrophils in areas of inflammation. The discovery that N-formyl peptides are chemoattractants for these cells (Schiffmann, 1975) has led to the investigation of structural requirements for peptide-receptor interaction (Showell, 1976; Freer, 1980; Freer, 1982; Toniolo, 1984). HCO-Met-Leu-Phe-OH (f-MLF-OH), produced by *Escherichia coli* (Marasco, 1984), and its synthetic analogue HCO-Met-Leu-Phe-OMe (f-MLF-OMe) emerged as the prototypic chemotactic tripeptides.

A variety of conformational studies in solution indicate that f-MLF-OH would adopt an extended conformation (Becker, 1979; Bismara, 1985; Valensin, 1986; Toniolo, 1989) while the peptide backbone of the f-MLF-OMe was found to be folded at the Leu residue in solution (Vertuani, 1987) as well as in the crystal state (Gavuzzo, 1989). As a consequence, since both

cited formyl peptides show biological activity, the flexibility of the peptide backbone could be an important factor for the chemotactic activity, allowing the folded peptides to extend at the receptor site or "vice versa". Moreover recent studies have shown that the peptide HCO-Met-Pro-Phe-OMe (f-MPF-OMe) adopts a γ-turn conformation characterized by a hydrogen bond between the NH group of Phe and the CO group of Met. The Pro residue, increasing the rigidity of the peptide backbone, seems to stabilize the folded structure. This peptide is not active (Dugas, 1993).

In order to explore the influence of the conformation on biological activity, we report the results of a conformational investigation in solution, carried out by CD and IR spectroscopy, of three synthetic analogues of prototypical tripeptide f-MLF-OMe to which they are referred. HCO-Met-Pro-Phe-OMe was selected both for its relatively rigid peptide backbone and for the absence of the amide proton at position 2 which can have a possible effect on the conformation-activity relationship. HCO-Met-Ψ[COO]-Leu-Phe-OMe (f-MΨ[COO]LF-OMe) was investigated as a more flexible analogue without the NH group at the same position 2. HCO-Met-OLeu-Phe-OMe (f-M(O)LF-OMe) was selected as a flexible analogue too, in which the amide bond is substituted by an amidoxy bond (-CO-NH-O-), leaving the NH group to be protic but introducing a possible hydrogen bond acceptor.

Experimental

Standard abbreviations for amino acids residues, derivatives and peptides are used according to the IUPAC-IUB Joint Commission on Biochemical Nomenclature, Eur. J. Biochem. 138, 9–37; 1984 .

Materials

The pure amino acids were purchased from Fluka and are in L configuration. Spectrograd solvents were purchased from Merck. All materials were of reagent grade.

Analyses

CD spectra were carried out at room temperature using a Jasco model J-500 A automatic recording circular dichrograph interfaced (IF-500II) with an IBM AT computer. The instrument was calibrated with an aqueous solution of d-10-camphorsulfonic acid and a dioxane solution of epiandrosterone (Schippers and Dekkers, 1991). Cylindrical fused quartz cells of 0.2 and 0.5 mm pathlengths were used for CD measurements. The usual instrumental precautions were taken to avoid artefacts. The values for tripeptide CD spectra are given in $[\theta]_M$ molar ellipticity (deg cm^2 dmol^{-1}) using the molecular weight of the compounds. The peptide concentrations ranged from 1.8×10^{-3} to 9.0×10^{-4}M.

IR spectra in solution were determined with a Perkin-Elmer model 58OB spectrophotometer. The band position are accurate to $\pm 1\,cm^{-1}$. A cell of 0.1 cm pathlength was employed at low concentrations, whereas a cell with pathlength 0.01 cm was used at high concentrations.

Purification of all the final products were achieved by reverse phase HPLC analysis on a Brucker LC-21-B equipped with Rheodine injection valve, revelation with UV spectrometer LC 313 at 220 nm, using as stationary less polar phase a Spherisorb (2.5 $\times$

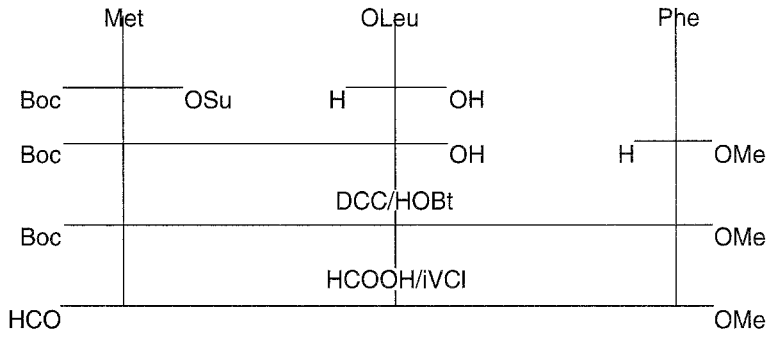
4.6mm, particles 5.0μm) column with the eluting system 10% MeCN in water containing 0.1% TFA.

Melting points were determined on a Reichert-Kofler block and are uncorrected. Thin-layer chromatography was performed on precoated silica gel F_{254} (Merck).

Strategy

Synthesis and characterization of tripeptides f-MLF-OMe, f-MPF-OMe and f-MΨ[COO]LF-OMe have been described earlier (Vertuani, 1987, Dugas, 1993; Cavicchioni, 1994).

The peptide f-M(O)LF-OMe was synthesized following the traditional methodology in solution according to Scheme 1.



Scheme 1. The required aminoxy-acid were prepared according to literature (Briggs and Morley, 1979). *iVCl* isovaleroyl chloride

Synthesis of HCO-Met-(O)Leu-Phe-OMe

Boc-Met-OLeu-OH: TEA (20mmol; 2.8ml) was added at 0°C to a stirred solution of HCl·H-OLeu-OH (20mmol; 3.66g) in pyridine (40ml). After 10min Boc-Met-OSu (18mmol; 6.22g) was added in small portions at 0°C. The reaction mixture was allowed to stir at room temperature overnight, concentrated to dryness, dissolved 3 times in ethyl acetate (3 × 30ml) and concentrated. The residue was dissolved in ethyl acetate (60ml) and washed with 1N HCl (3 × 100ml), saturated aqueous $NaHCO_3$ (3 × 100ml) and brine (3 × 100ml). The organic phase was dried (Na_2SO_4), filtered and concentrated to dryness. Colourless solid; 4.79g; 70.5%; mp 97–99°C; $[\alpha]_D^{20}$ −106° (DMF, C 1.04). CHN Found C, 50.9; H, 8.0; N, 7.6%. Calcd.for $C_{16}H_{30}N_2O_6S$ C, 50.7; H, 7.9; N, 7.4%

Boc-Met-OLeu-Phe-OMe: HOBt (11mmol; 1.48g) in DMF (15ml) and DCC (10mmol; 2.06g) were added at 0°C to a stirred solution of Boc-Met-OLeu-OH (10mmol; 3.78g) in ethyl acetate (30ml). A solution of HCl·H-Phe-OMe (11mmol; 2.36g) and TEA (11.5mmol; 1.6ml) in DMF (15ml), cooled at 0°C, was added. The reaction mixture was allowed to stir at room temperature overnight and concentrated to dryness. The residue was dissolved in ethyl acetate (50ml) and washed with 1N HCl (3 × 100ml), saturated aqueous $NaHCO_3$ (3 × 100ml) and brine (3 × 100ml). The organic phase was dried (Na_2SO_4), filtered and concentrated to dryness. Colourless solid (from ethyl acetate-petroleum ether); 4.30g; 79.7%; mp 116–118°C; $[\alpha]_D^{20}$ −73.75° (DMF, C 1.02). CHN Found C, 57.7; H, 7.6; N, 7.7%. Calcd. for $C_{26}H_{41}N_3O_7S$ C, 57.8; H 7.6; N, 7.7%.

HCO-Met-OLeu-Phe-OMe: TFA (10ml) was added at 0°C to a stirred solution of Boc-Met-OLeu-Phe-OMe (5mmol; 2.7g) in $CHCl_3$ (10ml). After 5min at 0°C and 90min at room temperature, the reaction mixture was concentrated to dryness, diluted 3 times

with toluene (10 ml) and concentrated. The residue was finally diluted with ethyl ether (10 ml) and concentrated to dryness to give TFA·H-Met-OLeu-Phe-OMe.

N-Methylmorpholine (20 mmol; 2.2 ml) and isovaleroyl chloride (18 mmol; 2.2 ml) were added to a solution of HCOOH (20 mmol; 0.75 ml) in THF (25 ml) at −1.5°C. The whole reaction product TFA·H-Met-OLeu-Phe-OMe (5 mmol; 2.77 g) and N-methylmorpholine (5 mmol; 0.55 ml) in DMF (10 ml) was cooled to 0°C and added. The reaction mixture was stirred 2 hours at room temperature and 3-dimethylamino-1-propylamina (20 mmol; 2.5 ml) was added at 0°C. After 30 min at room temperature the mixture was concentrated to dryness. The residue was dissolved in ethyl acetate (250 ml) and washed with 1N HCl (3 × 100 ml), saturated aqueous $NaHCO_3$ (3 × 100 ml) and brine (3 × 100 ml). The organic phase was dried (Na_2SO_4), filtered and concentrated to dryness. Colourless solid (from methanol-water); 2.02 g; 85.4%; mp 140–142°C; $[\alpha]_D^{20}$ −78.56° (DMF, C 1.04). CHN Found C, 56.2; H 7.2; N 8.9%. Calcd.for $C_{22}H_{33}N_3O_6S$ C, 56.5; H, 7.1; N, 8.9%.

Results and discussion

The CD profiles of prototypical active peptide f-MLF-OMe indicated that the peptide seems to prefer a folded conformation in diluted solution of some organic solvents which are often used to mimic the hydrophobic environment

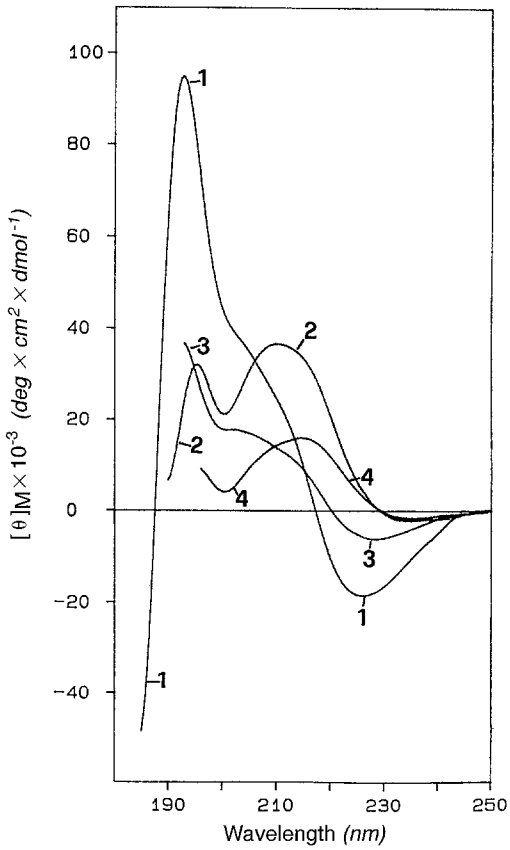


Fig. 1. CD spectra of f-MPF-OMe in TFE (*1*) and MeOH (*2*) compared with spectra of f-MLF-OMe in TFE (*3*) and MeOH (*4*)

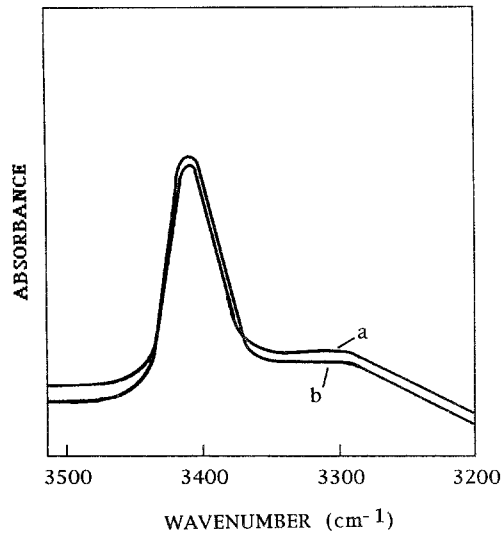


Fig. 2. IR absorption spectra of f-MPF-OMe in the N-H stretching region in $CDCl_3$ solution at 1.2×10^{-3}M (*a*) and 1.3×10^{-4}M (*b*)

of the lipidic membrane (Urry, 1971). However this conformation is not very stable as shown by the low intensity, at hight dilution ($CDCL_3$ 10^{-4}M) of the IR band at 3310 cm^{-1} which usually is attributed to the intramolecular hydrogen bonded NH vibrators (Vertuani, 1987).

According to NMR and molecular modeling researches, which demonstrated that the inactive f-MPF-OMe assumes a folded γ-turn structure in solution (Dugas, 1993), the CD spectra (Fig. 1) evidence, if compared with f-MLF-OMe, a higher intensity of the bands and a better resolution of the positive maxima in TFE and MeOH. This result indicates a higher stability of the folded structure which probably does not favour the formation of an extended conformation in the binding site, as confirmed by IR spectra (Fig. 2) where the ratio between the intensity of the band at 3421 cm^{-1} (non hydrogen bonded NH vibrators) and the intensity of the band at 3320 cm^{-1} (hydrogen bonded NH vibrators) is lower than the ratio obtained for f-MLF-OMe.

The peptide f-MΨ[COO]LF-OMe, in which the amide linkage is replaced by an ester bond, is also not active (Cavicchioni, 1994). The CD spectra in TFE and MeOH (Fig. 3), characterized by a negative band at ~235 nm and a positive shoulder at 215–218 nm, show a profile similar to that obtained for the active f-MLF-OMe which does not present a very stable folded conformation. Moreover the IR spectra at high dilution (Fig. 4), showing a band of very low intensity at 3320 cm^{-1} (hydrogen bonded NH vibrators), confirm that the presence of a flexible ester bond does not favour a folded structure. It must be stressed that in this peptide the NH function at position 2 is absent while in the folded analogue containing the Pro residue is present but aprotic. This finding suggests that both the protic NH linkage and the peptide backbone flexibility are important for the biological activity.

The CD curves of f-M(O)LF-OMe (Fig. 5) are characterized by a high ratio between the ellipticities of the positive (215–218 nm) and negative bands

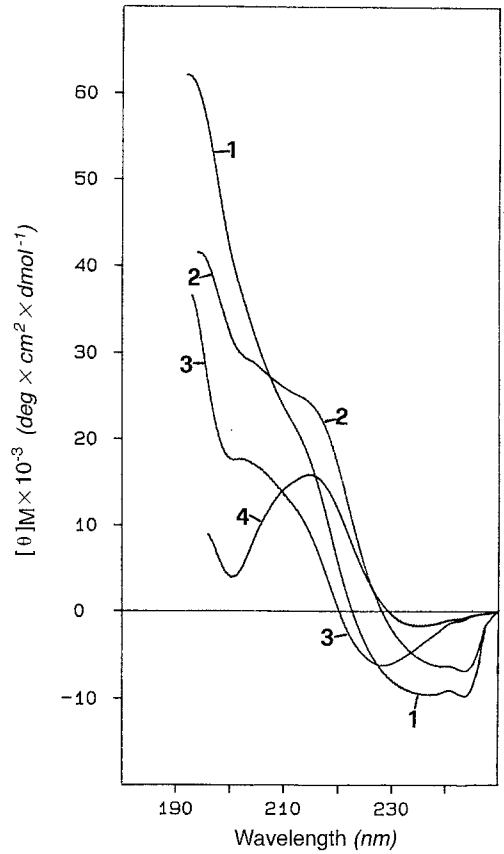


Fig. 3. CD spectra of f-MΨ[COO]LF-OMe in TFE (*1*) and MeOH (*2*) compared with spectra of f-MLF-OMe in TFE (*3*) and MeOH (*4*)

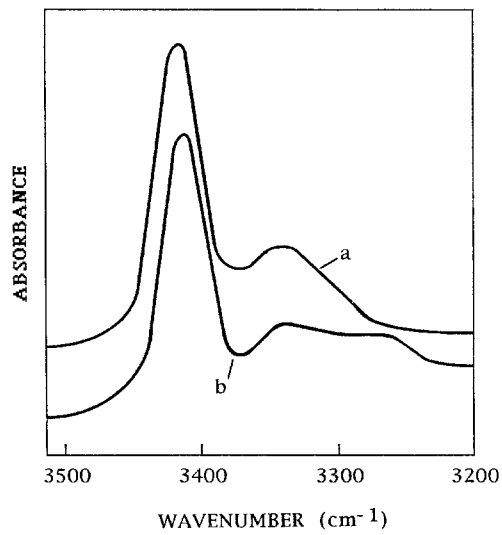


Fig. 4. IR absorption spectra of f-MΨ[COO]LF-OMe in the N-H stretching region in $CDCl_3$ solution at 1.9×10^{-2}M (*a*) and 1.6×10^{-3}M (*b*)

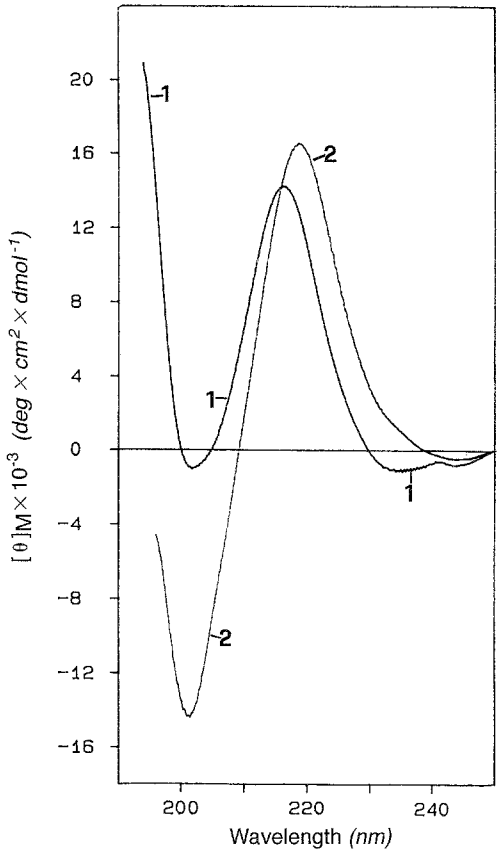


Fig. 5. CD spectra of f-M(O)LF-OMe in TFE (*1*) and MeOH (*2*)

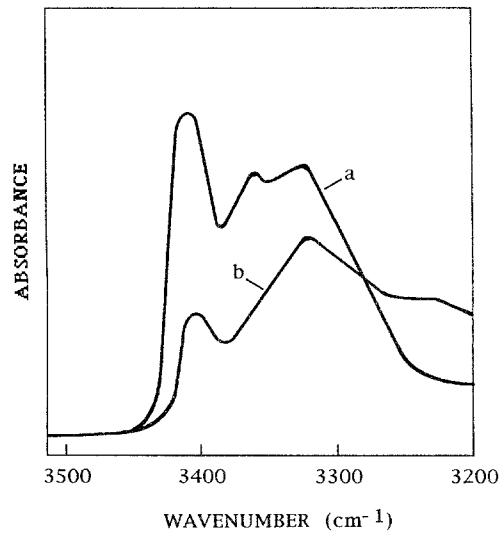


Fig. 6. IR absorption spectra of f-M(O)LF-OMe in the N-H stretching region in $CDCl_3$ solution at 5.8×10^{-2}M (*a*) and 6.0×10^{-4}M (*b*)

(235–242 nm) in the used solvents. The IR spectra (Fig. 6) show a very intense band at 3310 cm^{-1} at high dilution. These data indicate that the peptide, which is inactive (manuscript in preparation), assumes a stable folded structure, probably the most stable among the peptides previously examined. In the analogue the insertion of an oxygen atom which lengthens the peptide backbone, reasonably modifies the hydrogen bond properties of the amide group at position 2, favouring the formation of a folded structure that probably involves the formyl CO and the NH group of the Leu residue, which thus are not available to interact with the proper receptor area.

In summary, the conformational data described in this paper and a careful examination of the recent literature, reasonably indicate that (a) chemotactic tripeptides must have rather flexible backbone in order to elicit the highest biological activity; (b) the structural environment of the central amino acid is quite important since it can affect the stability of a possible active conformer; (c) the NH group at position 2 seems to be a critical determinant for binding with the receptor.

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