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Water Induced β-Turn Modification in a Chemotactic Tetrapeptide. Synthesis, Crystal Conformation, and Activity of HCO-Met-Leu-Δ^ZPhe-Phe-OMe

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Abstract: In order to study the influence of the conformation on the activity and bioselectivity, the new tetrapeptide ligand of the chemotactic formylpeptide receptors HCO-Met-Leu-Δ^ZPhe-Phe-OMe (**2**) has been studied. Compound **2** has been designed so as to induce a preferential β-turn conformation with the *N*-formyl group located outside the backbone loop. The crystallographic analysis reveals that **2** adopts only in part the expected conformation due to the presence of a water molecule inside the turn. Details on the H-bonding network stabilizing the "open-turn" are given. The tetrapeptide **2** is active towards human neutrophils, stimulating directed migration, superoxide anion generation and lysozyme release. The influence of the backbone conformation on the bioselectivity is discussed.

Introduction

The increasing attention dedicated to chemotactic *N*-formylpeptides is due to their involvement in defence mechanisms against acute inflammation processes, through binding with specific receptors located on the neutrophil membranes.[1] *N*-formylmethionyl-leucyl-phenylalanine (fMLP) is the most extensively studied ligand of this class of bioactive peptides and its structure-activity relationships have been well documented.[2-6] Recent efforts in this field are focused on the design of conformationally constrained models in order to acquire a better understanding of the bioactive conformation features[7,8] as well as to develop selective receptor agonists.[9,10]

We reported recently on *N*-formyl-methionyl-Δ^zleucyl-phenylalanine methyl ester [Δ^zLeu^2]fMLP-OMe, a fMLP analog containing the (*Z*)-α,β-didehydroleucine (Δ^zLeu) residue in place of the central leucine.[11] It was established that this unsaturated ligand adopts a type II β-bend conformation in which the carbonyl oxygen of the *N*-terminal formyl group is engaged in an intramolecular H-bond with the NH of the last residue. Whereas [Δ^zLeu^2]fMLP-OMe is practically inactive in the stimulation of human neutrophil directed migration, it is active in eliciting both superoxide generation and enzyme release. The conformational restrictions imposed on the peptide backbone by the presence of the α,β-unsaturated residue may be responsible for the observed selective bioactivity.

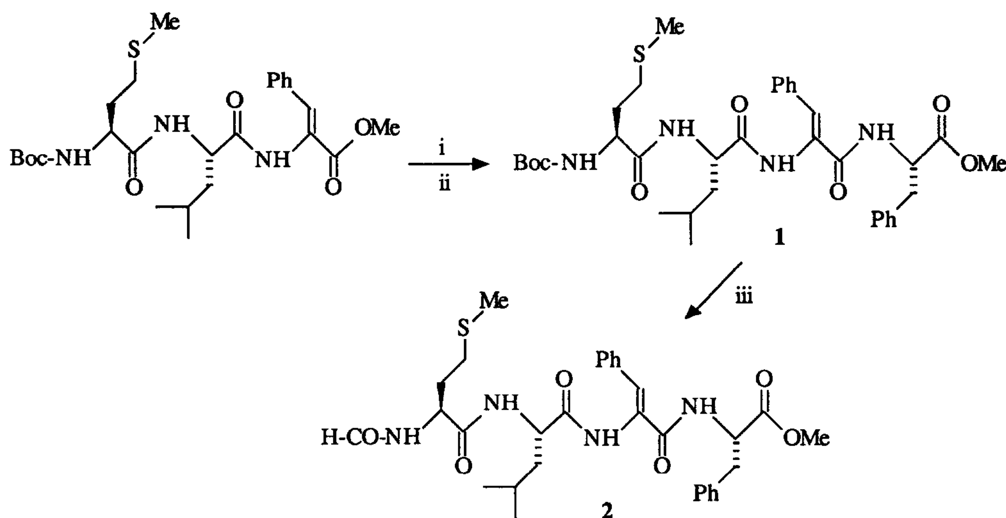
Since the presence of the *N*-terminal formyl group is essential for the biological activity of chemotactic peptides and folded conformations analogous to that adopted by [Δ^zLeu^2]fMLP-OMe are likely to play a role in the interaction with the receptors, it seemed interesting to continue our studies in this field by examining the

activity of β-folded models in which the formylic group is not engaged in the intramolecular H-bonding. Thus, the new ligand HCO-Met-Leu-Δ^zPhe-Phe-OMe (2), containing an α,β-unsaturated residue at the carboxy penultimate position, has been designed. The known tendency of the Δ^zPhe residue to occupy the (i+1) or (i+2) corner positions of β-turn structures, should induce a folding with an intramolecular Met CO···Phe NH H-bond which leaves apart the HCO group. In this report we describe the synthesis, crystal conformation and biological activity of the *N*-formyltetrapeptide **2**.

RESULTS

Synthesis

The synthesis of **2** has been performed as reported in the Scheme 1.



i: OH^-, H^+; ii: *i*-BuOCOCl, H-Phe-OMe·HCl, *N*-methylmorpholine; iii: HCOOH, EEDQ

Scheme 1

Starting Boc-Met-Leu-Δ^zPhe-OMe has been hydrolysed and coupled with Phe-OMe to give *N*-Boc protected tetrapeptide **1**; simultaneous deprotection and formylation was performed by using formic acid and then *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), following the Lajoie and Kraus procedure.[12]

Crystal Conformation

In order to define the solid state conformation adopted by **2**, an X-ray analysis has been performed. Suitable crystals have been obtained from methanol-water solution. The perspective view of the conformation of

2, reported in Fig. 1, shows that the tetrapeptide derivative crystallizes in a monohydrate form. The backbone is folded with the Δ^zPhe residue occupying the (i+2) corner position and the *N*-terminal formyl group not participating to the folding. However, the expectation of a β-turn conformation for **2** is only in part fulfilled due to the unusual presence of a water molecule located inside the backbone loop. There is at the present considerable interest in clarifying the specific interactions between peptide secondary structures and water and more in general in the understanding the hydration mechanisms of peptides and proteins;[13-15] thus, the analysis of the present structure appears useful beyond our initial objective.

All three peptide bonds of **2** are involved in the β-turn and are of the *trans*-planar type with the largest deviation from planarity shown by the central Leu-Δ^zPhe bond (ω_2 = 168.2°). The formamido group is completely flat (ω_0 = 0°) and presents the formylic hydrogen *cis* to the Met NH. An unusual conformational feature is presented by the relative spatial arrangement of the two hydrogens of the formamido group and the hydrogen on Met C^α: these three atoms lie on the same plane and are eclipsed each other (ω_0 = 0 and ϕ_1 = 57.2°). This feature is forbidden to a formylic group involved in β-turn structures and has not been encountered in solid state conformations of formylpeptides studied before. Due to the presence of the C_3^α-C_3^β double bond the aromatic side chain takes the characteristic orientation in which the backbone nitrogen N_3 is eclipsed with the aromatic C_3^γ carbon atom (χ_3^1 = 3.4°). The alteration of the bond lengths induced by the electronic effects of the C^α-C^β unsaturation is practically limited to the C_3^α-C_3' and C_3'-O_3 bonds which are shorter (1.464 Å) and longer (1.248 Å), respectively, than the standard values in saturated models (1.54 and 1.22 Å, respectively). The C_3^β-C_3^γ bond length (1.498 Å) is not significantly altered and this in accordance with the high deviation of the plane of the aromatic ring from that of the double bond ($\chi_3^{2,1}$ = -44°). These data indicate an electron conjugation from the double bond toward the carbonylic oxygen and a reduced involvement of the aromatic ring and the enamidic nitrogen N_3. Analogous results have been recently reported by studying (*Z*)-*N*-acetyl-α,β-didehydrophenylalanine methylamide.[16]

As can be deduced by the values reported in the Table, the torsion angles φ and ψ for the Leu (i+1) and Δ^zPhe (i+2) residues of -81.1, 161.2, 65.2, 33.4° deviate significantly from those predicted for a conventional type II β-turn (-60, 120, 80, 0°),[17,18] the largest deviation from the ideal values, induced by the presence of the water molecule, being 41° for the ψ_{i+1} torsion angle. Helical and extended conformations are found at the Met (i) and Phe (i+3) positions with φ and ψ of 57.2, 44.7 and -141.6, 148.3°, respectively.

As it is shown in Fig. 1 a water molecule is located inside the turn and is completely surrounded by polar groups of the peptide backbone. It forms in fact three H-bonds which are of 2.827 Å with the Met CO and of 3.034 and 2.966 Å with the Phe NH and CO groups, respectively. The water hydrogens not detected in the final Fourier map should lie not too far from the two $O \cdots O_w$ directions since the $O_1 \cdots O_w \cdots O_4$ angle is 128.4°. Additional favourable interaction may take place between the dipoles of the water and that of the Leu-Δ^zPhe amide junction (the angle between the plane of the amide and that containing the O_1, O_4, O_w atoms is 39.1°).

As a consequence of the water inclusion, the usual (i+3) ---> (i) intraturn H-bond, closing a 10-membered ring, is not found and the distance between the Phe nitrogen N_4 and the Met carbonyl oxygen O_1 increases from a β-turn mean value of 3 Å to 4.65 Å.[18] Furthermore, the distance between the C^α atoms of the two external residues, *i.e.* Met C_1^α and Phe C_4^α , becomes 7.02 Å. Since the accepted definition classifies a backbone portion as a turn when the C_i^α --- C_{i+3}^α distance is less than 7 Å,[18,19] the value exhibited by **2** indicates that, although the backbone maintains an overall folded conformation, the water inclusion results in a profound

Table. Relevant torsion angles (°) of **2** with e.s.d.'s in parentheses.

Backbone			Side-chain			
O_0-C_0'-N_1-C_1^{α}	ω_0	0(1)	Met	N_1-C_1^{α}-C_1^{β}-C_1^{γ}	χ_1	-65.2(9)
C_0'-N_1-C_1^{α}-C_1'	φ_1	+57.2(8)		C_1^{α}-C_1^{β}-C_1^{γ}-S_1^{δ}	χ_1^2	+173.1(7)
N_1-C_1^{α}-C_1'-N_2	ψ_1	+44.7(7)		C_1^{β}-C_1^{γ}-S_1^{δ}-C_1^{ϵ}	χ_1^3	+83(2)
C_1^{α}-C_1'-N_2-C_2^{α}	ω_1	-177.2(5)	Leu	N_2-C_2^{α}-C_2^{β}-C_2^{γ}	χ_2^1	-54.5(7)
C_1'-N_2-C_2^{α}-C_2'	φ_2	-81.1(6)		C_2^{α}-C_2^{β}-C_2^{γ}-$C_2^{\delta 1}$	$\chi_2^{2,1}$	-56.1(9)
N_2-C_2^{α}-C_2'-N_3	ψ_2	+161.2(5)		C_2^{α}-C_2^{β}-C_2^{γ}-$C_2^{\delta 2}$	$\chi_2^{2,2}$	-179.5(7)
C_2^{α}-C_2'-N_3-C_3^{α}	ω_2	+168.2(5)	Δ^ZPhe	N_3-C_3^{α}-C_3^{β}-C_3^{γ}	χ_3^1	+3.4(9)
C_2'-N_3-C_3^{α}-C_3'	φ_3	+65.2(7)		C_3^{α}-C_3^{β}-C_3^{γ}-$C_3^{\delta 1}$	$\chi_3^{2,1}$	-44.1(9)
N_3-C_3^{α}-C_3'-N_4	ψ_3	+33.4(8)		C_3^{α}-C_3^{β}-C_3^{γ}-$C_3^{\delta 2}$	$\chi_3^{2,2}$	+135.8(7)
C_3^{α}-C_3'-N_4-C_4^{α}	ω_3	-178.3(6)		C_2'-N_3-C_3^{α}-C_3^{β}		-112.7(6)
C_3'-N_4-C_4^{α}-C_4'	φ_4	-141.6(7)		O_3-C_3'-C_3^{α}-C_3^{β}		+27.9(9)
N_4-C_4^{α}-C_4'-O_5	ψ_4	+148.3(8)	Phe	N_4-C_4^{α}-C_4^{β}-C_4^{γ}	χ_4^1	-165.4(7)
C_4^{α}-C_4'-O_4'-C_5		-179(1)		C_4^{α}-C_4^{β}-C_4^{γ}-$C_4^{\delta 1}$	$\chi_4^{2,1}$	-103(1)
O_4-C_4'-O_4'-C_5		3(1)		C_4^{α}-C_4^{β}-C_4^{γ}-$C_4^{\delta 2}$	$\chi_4^{2,2}$	+82(1)

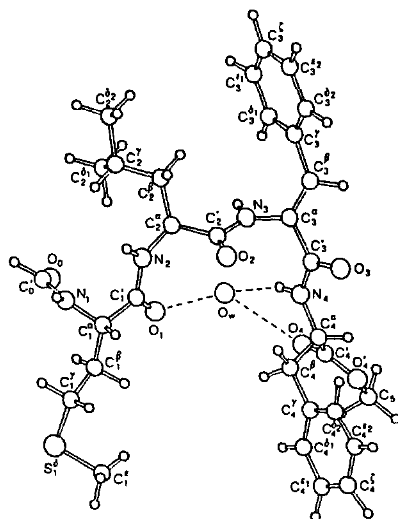


Fig 1. A perspective view of crystal conformation of formyltetrapeptide **2**

alteration of the classical β-turn structure. The factors which cooperate in stabilizing the "open-turn" conformation[18] found in 2 can be identified in the β-turn inducing effect of the Δ^zPhe residue located at (i+2) position and in the peptide-water H-bonding network.

Both the NH and CO groups of the central Leu-Δ^zPhe junction are involved in intermolecular H-bonds with a formylic oxygen ($N_3H \cdots O_0$ = 2.892 Å) and a Met NH ($O_2 \cdots HN_1$ = 2.881 Å), respectively; an additional intermolecular H-bond is found between Leu NH and Δ^zPhe CO (2.934 Å).

Biological Activity

The biological activity of the formyltetrapeptide 2 has been determined on human neutrophils and compared to that of the parent tripeptide fMLP-OMe. The directed migration (chemotaxis), superoxide anion production and lysozyme release have been measured. As shown in Fig 2A, 2 is a chemotactic agent ($p < 0.05$) over a broad range of concentration, 10^{-11}-10^{-7} *M* , even if in a lesser extent than the parent. In the case of superoxide anion production (Fig.2B), 2 shows a behaviour similar to the parent fMLP-OMe at high concentrations, both having a peak at 10^{-7} *M* ; compound 2, however, maintains the efficacy at lower concentrations (10^{-9}-10^{-8} *M*) ($p < 0.01$). As shown in Fig. 2C, 2 shows a behaviour analogous to the parent as a secretagogue agent in the concentration range of 10^{-8}-10^{-5} *M* .

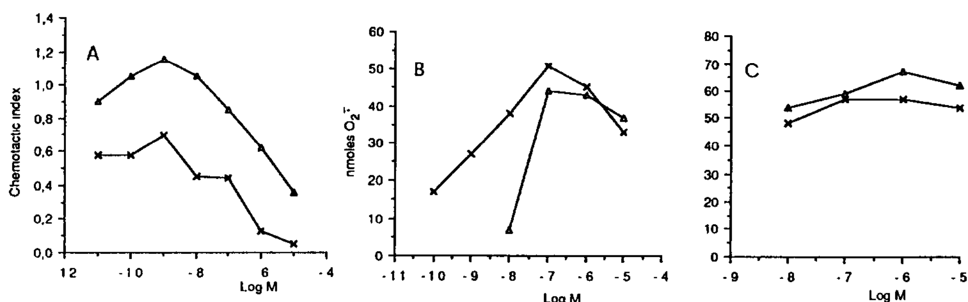


Fig. 2. Biological activities of fMLP-OMe (Δ) and tetrapeptide 2 (X) determined on human neutrophils. The points are mean of six separate experiments done in duplicate. A: chemotactic activity; standard errors are in the 0.02-0.09 chemotactic index range. B: superoxide anion production; standard errors are in the 0.1-4% range. C: release of neutrophil granule enzymes evaluated by determining lysozyme activity; standard errors are in 1-6% range.

DISCUSSION

We reported recently that the conformationally constrained analogs of fMLP-OMe containing 4-amino-tetrahydrothiopyran-4-carboxylic acid (Thp) in place of methionine, [Thp[1]]fMLP-OMe and [Thp[1],Ain[3]]fMLP-OMe (Ain = 2-aminoindane-2-carboxylic acid), although highly active as chemoactractants, induce neither superoxide production nor lysozyme release.[9] Furthermore, the previously cited tripeptide [Δ^zLeu[2]]fMLP-OMe shows an analogous although opposite bioselectivity.[11]

It is worth noting that, unlike the above cited ligands, the tetrapeptide 2 is not able to select the different biological responses related to the chemotaxis. As interpretation of these first results it could be suggested that the ability of a formylpeptide ligand to discriminate receptor variants and biological responses is strongly

dependent upon the direct involvement of the *N*-terminal residue, bearing the formyl group, into a conformational constraint. Both the [Thp[1]] and [Δ^zLeu[2]] analogs meet this condition: the *N*-formyl group of the [Thp[1]] analogs is bound in fact to a conformationally restricted amino acid and in the [Δ^zLeu[2]] peptide the formyl carbonyl is engaged in an intramolecular H-bond with the Phe[3] NH. The conformational space available to the *N*-formylmethionine moiety of **2**, on the other hand, is not significantly limited since the β-turn structure involves, as expected, the last three residues. This interpretation is in accordance with the findings of extensive studies on chemotactic formylpeptides which emphasize the crucial role of the formylic group both for the binding and the biological activity.

As regards peptide-water interaction, although several structures involving water molecules bound to external polar sites of folded peptide backbones have been reported,[14,20-22] a situation analogous to that found in **2** has been only observed in the case of the dipeptide derivative *t*-Bu-CO-Pro-Me-D-Ala-NHMe.[23] Here the X-ray analysis revealed that the water molecule connects, through two H-bonds, the *t*-butyl carbonyl group with the *C*-terminal methylamide NH, thus preventing the intraturn H-bonding characteristic of β-turn structures. Unlike the type of water-peptide interaction found in the case of the tetrapeptide **2**, the water molecule in *t*-Bu-CO-Pro-Me-D-Ala-NHMe makes the third H-bond with the Me-D-Ala CO group of a neighbouring molecule thus giving rise to a H-bonding scheme which involves intra- and inter-turn interactions.

The present structural investigation gives a further insight into the influence of water on the peptide conformation, a problem which cannot be easily determined by spectroscopic techniques. An interesting aspect concerning the specific interaction between the β-turn and water comes from the observation that in globular proteins β-turns are in general located on the surface which is exposed to the solvent. Furthermore, on the basis of an investigation of 938 β-turns extracted from the X-ray structure of 58 proteins at resolution$\leq$ 2Å, Wilmot and Thornton[19] have shown that about 60% of the β-turns examined had deformations of ±30° around the ideal values with a further flexibility of one angle to deviate by as much as 45°. These deformations are comparable to those found in the hydrated crystal conformation of peptide **2**. Therefore the present structure could serve as a static model of solvation of a β-turn backbone containing apolar side-chains.

EXPERIMENTAL

Peptide Synthesis

Melting points were determined with a Büchi oil bath apparatus and are uncorrected. Optical rotations were taken at 20 °C with a Schmidt-Haensch Polartronic D polarimeter in a 1 dm cell. IR spectra (KBr disks) were recorded with a Perkin-Elmer 983 spectrophotometer. ^{1}H NMR spectra were measured with a Varian XL-300 spectrometer in $CDCl_3$ (tetramethylsilane as internal standard).

Boc-Met-Leu-Δ^zPhe-Phe-OMe (1)

Isobutyl chloroformate (0.175 mL, 1.18 mmol) was added at -15°C to a stirred solution of the tripeptide Boc-Met-Leu-Δ^zPhe-OH (0.598 g, 1.18 mmol), obtained by alkaline hydrolysis[9] for one day of the corresponding methyl ester,[24] in dry DMF (2.4 mL) containing *N*-methylmorpholine (0.137 mL, 1.41 mmol). The temperature was kept at -15 °C for 15 min, then a solution of H-Phe-OMe·HCl (0.254 g, 1.18 mmol) and *N*-methylmorpholine (0.115 mL, 1.18 mmol) in dry DMF (2.4 mL) was added. The mixture was stirred at room temperature for 24 h, and then evaporated under *vacuum*. The residue was dissolved in ethyl acetate and washed with 5% aqueous $KHSO_4$, water, saturated aqueous $NaHCO_3$ and brine. The organic phase was dried (Na_2SO_4) and evaporated to give a residue which was chromatographed on a column of Merck silica gel 60 (230-400 mesh, 1:40). Elution with dichloromethane-ether (9:1) afforded 0.595 g (76%) of the title compound **1**, mp 84-87 °C (dec) (from dichloromethane-*n*-hexane); $[\alpha]_D$ = -27° ($CHCl_3$, *c* 1.0); IR ν_{max}: 3303, 1745,

1659, and 1514 cm^{-1}; ^1H NMR: δ 0.90 and 0.95 (6H, two d, J = 6 Hz, $(CH_3)_2CH$), 1.38 (9H, s, $C(CH_3)_3$), 1.50-2.00 (5H, two m, $(CH_3)_2CH$-CH_2 and CH_2-CH_2-S), 2.03 (3H, s, S-CH_3), 2.52 (2H, t, J = 7 Hz, CH_2-S), 3.18 (2H, sharp m, Phe β-CH_2), 3.70 (3H, s, $COOCH_3$), 4.14 (1H, m, Met α-CH), 4.36 (1H, m, Leu α-CH), 4.92 (1H, m, Phe α-CH), 5.26 (1H, d, J = 5.5 Hz, Met NH), 6.82 (1H, unresolved d, Leu NH), 7.12 (1H, d, J = 7.5 Hz, Phe NH), 7.14-7.42 (11H, m, aromatic and Δ^zPhe β-CH), 7.99 (1H, s, Δ^zPhe NH). Anal. calcd for $C_{35}H_{48}N_4O_7S$: C, 62.85; H, 7.23; N, 8.38%. Found: C, 62.60; H, 7.22; N, 8.18%.

For-Met-Leu-Δ^zPhe-Phe-OMe (2)

Treatment of Boc-protected tetrapeptide **1** (0.301 g, 0.45 mmol) with formic acid and EEDQ, as described in a previous paper[12] afforded the title *N*-formylpeptide **2** in 97% yield, mp 153°C (from dichloromethane-*n*-hexane), $[\alpha]_D$ = -34° ($CHCl_3$, *c* 1.0); IR ν_{max}: 3278, 1745, 1642, and 1518 cm^{-1}; ^1H NMR: δ 0.88 and 0.93 (6H, two d, J = 6 Hz, $(CH_3)_2CH$), 1.56-1.79 (3H, m, $(CH_3)_2CH$-CH_2), 1.85-2.14 (2H, m, CH_2-CH_2-S), 2.00 (3H, s, S-CH_3), 2.49 (2H, t, J = 7 Hz, CH_2-S), 3.14 (2H, d, J = 6.5 Hz, Phe β-CH_2), 3.67 (3H, s, $COOCH_3$), 4.38 (1H, m, Leu α-CH), 4.66 (1H, m, Met α-CH), 4.87 (1H, m, Phe α-CH), 6.99 (1H, d, J = 8 Hz, Met NH), 7.10-7.46 (13H, m, aromatic, Phe and Leu NH, Δ^zPhe β-CH), 7.95 (1H, s, CHO), 8.33 (1H, s, Δ^zPhe NH). Anal. calcd for $C_{31}H_{40}N_4O_6S$: C, 62.39; H, 6.75; N, 9.39%. Found: C, 62.22; H, 6.69; N, 9.60%.

X-ray Structural Analysis

Crystals of **2**, suitable for X-ray analysis, were obtained from a methanol-water solution. The compound crystallizes in the monohydrate form ($C_{31}H_{40}N_4O_6S{\cdot}H_2O$; M.W. = 614.7) in the orthorhombic system, space group $P\,2_1\,2_1\,2_1$ with a = 9.447(6), b = 12.636(8), c = 28.454(14) Å, V = 3397(3) $Å^3$, Z = 4, d_c = 1.20 g cm^{-3}. Intensity data were collected up to 2θ = 138° on a Syntex $P2_1$, four-circle diffractometer equipped with graphite-monochromatized Cu-Kα radiation (λ = 1.5418 Å) using the θ - 2θ scan technique. Out of a total of 3469 collected reflections, 2409 had $I > 2.5\,\sigma(I)$ and were used in the refinement. The structure was solved by direct methods using the program SHELXS86[25] and refined anisotropically by full-matrix least-squares method to R = 0.069.[26] Tables containing final atomic fractional coordinates together with thermal parameters, valence bond distances and angles have been deposited at the Cambridge Crystallographic Data Centre.

Biological Assay

Cells. Human neutrophils were purified employing the standard tecniques of dextran (Pharmacia) sedimentation, centrifugation on lymphocyte separation medium (Flow S.p.A.) and hypotonic lysis of red cells. The cells were washed twice and resuspended in KRPG (Krebs-Ringer-phosphate containing 0.1% w/v glucose, pH 7.4) at a concentration of 50×10^6 cells/mL. The percentage of neutrophils was 98-100% pure.

Random locomotion. Random locomotion was performed with 48-well microchemotaxis chamber (Bio Probe, Italy) and the migration into the filter was evaluated by the method of leading-front.[27]

Chemotaxis. In order to study the potential chemotactic activity, each peptide was added to the lower compartment of the chemotaxis chamber. Peptides were diluted from a stock solution ($10^{-2}M$ in dimethylsulfoxide) with KRPG containing 1 mg/mL of bovine serum albumin (Orha Beringwerke, BRD) and used at concentrations ranging from $10^{-11}M$ to $10^{-5}M$. Data were expressed in term of chemotactic index, which is the ratio: (migration towards test attractant minus migration towards the buffer)/migration towards the buffer.

Superoxide anion (O_2^-) production. O_2^- release was monitored continuously in a thermostatted spectrophotometer as superoxide dismutase-inhibitable reduction of ferricytochrome c (Sigma, USA), as described elsewhere.[28] At zero time, different amounts (10^{-8} - $10^{-5}M$) of each peptide were added and absorbance change accompanying cytochrome c reduction was monitored at 550 nm. Results were expressed as net nmoles of $O_2^-/2\times10^6$ cells/5 min. Neutrophils were incubated with 5 μg/mL cytochalasin B (Sigma) for 5 min prior to activation by peptides.

Enzyme assay. Release of neutrophil granule enzymes was evaluated by determining lysozyme activity;[28] this was quantified nephelometrically by the rate of lysis of cell wall suspension of *Micrococcus lysodeikticus* (Sigma). Enzyme release was expressed as a net percentage of total enzyme content released by 0.1% Triton X-100. Total enzyme activity was 85 ±1 μg/1x10^7 cells/min. To study the degranulation-inducing activity of each peptide, neutrophils were first incubated with cytochalasin B for 15 min at 37°C and then in the presence of each peptide in a final concentration of 10^{-8} - 10^{-5} *M* for a further 5 min.

Statistical analysis. The non parametric Wilcoxon test was used in the statistical evaluation of differences between groups.

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REFERENCES

1. Harvath, L. *Experientia, Suppl.* **1991**, *59*, 35-52.
2. Showell, H. J.; Freer, R. J.; Zigmond, S. H.; Schiffmann, E.; Aswanikumar, S.; Corcoran, B.; Becker, E.L. *J. Exp. Med.* **1976**, *143*, 1154-1169.
3. Freer, R. J.; Day, A. R.; Radding, J.A.; Schiffmann, E.; Aswanikumar, S.; Showell, H. J.; Becker, E. L. *Biochemistry* **1980**, *19*, 2404-2410.
4. Freer, R. J.; Day, A. R.; Muthukumaraswamy, N.; Pinon, D.; Wu, A.; Showell, H. J.; Becker, E. L. *Biochemistry* **1982**, *21*, 257-263.
5. Toniolo, C; Bonora, G. M.; Showell, H. J.; Freer, R. J.; Becker, E. L. *Biochemistry* **1984**, *23*, 698-704.
6. Rot, A.; Henderson, L. E.; Copeland, T. D.; Leonard, E. J. *Proc. Natl. Acad.Sci. USA* **1987**,*84*, 7967-7971.
7. Toniolo, C.; Crisma, M.; Valle, G.; Bonora, G. M.; Polinelli, S.; Becker, E. L.; Freer, R. J.; Sudhanand; Balaji Rao, R.; Balaram, P.; Sukumar, M. *Pept. Res.* **1989**, *2*, 275-281.
8. Dentino, A. R.; Raj, P. A.; Bhandary, K. K.; Wilson, M. E.; Levine, M. J. *J. Biol. Chem.* **1991**, *266*, 18460-18468, and references cited therein.
9. Torrini, I.; Pagani Zecchini, G.; Paglialunga Paradisi, M.; Lucente, G.; Gavuzzo, E.; Mazza, F.; Pochetti, G.; Spisani, S.; Giuliani, A.L. *Int. J. Peptide Protein Res.* **1991**, *38*, 495-504.
10. Kodama, H.; Miyazaki, M.; Kondo, M.; Sakaguchi, K.; Stammer, C. H.; Chen, H. C. In *Peptides. Chemistry and Biology* ; Smith, J. A.; Rivier, J. E. Eds.; ESCOM: Leiden, 1992; pp. 423-424.
11. Pagani Zecchini, G.; Paglialunga Paradisi, M.; Torrini, I.; Lucente, G.; Gavuzzo, E.; Mazza, F.; Pochetti, G.; Spisani, S. *Tetrahedron Lett.* **1991**, *32*, 4375-4376.
12. Lajoie, G.; Kraus, J.L. *Peptides* **1984**, *5*, 653-654.
13. Sundaralingam, M.; Sekharudu, Y. C. *Science* **1989**, *244*, 1333-1337.
14. Perczel, A.; Hollosi, M.; Foxman, M. B.; Fasman, G. D. *J. Am. Chem. Soc.* **1991**, *113*, 9772-9784.
15. Soman, K. V.; Karimi, A.; Case, D.A. *Biopolymers* **1991**, *31*, 1351-1361
16. Souhassou, M.; Lecomte, C.; Ghermani, N. E.; Rohmer, M. M.; Wiest, R.; Benard, M.; Blessing, R. H. *J. Am. Chem. Soc.* **1992**, *114*, 2371-2382.
17. Venkatachalam, C. M. *Biopolymers* **1968**, *6*, 1425-1436.
18. Rose, G. D.; Gierasch, L. M.; Smith, J. A. *Adv. Protein Chem.* **1985**, *37*, 1-109.
19. Wilmot, C. M.; Thornton, J. M. *Protein Eng.* **1990**, *3* , 479-493.
20. Karle, I. L. *J. Am. Chem. Soc.* **1977**, *99*, 5152-5157.
21. Karle, I. L.; Duesler, E. *Proc. Natl. Acad. Sci. USA* **1977**, *74*, 2602-2606.
22. Brown, J. N.; Teller, R. G. *J. Am. Chem. Soc.* **1976**, *98*, 7565-7569.
23. Aubry, A.; Vitoux, B.; Boussard, G.; Marraud, M. *Int. J. Peptide Protein Res.* **1981**, *18*, 195-202.
24. Chauhan, V. S.; Kaur, P.; Sen, N.; Uma, K.; Jacob, J.; Balaram, P. *Tetrahedron* **1988**, *44*, 2359-2366.
25. Sheldrick, G. M. SHELXS86 *Program for the solution of crystal structures*, University of Göttingen, Germany 1986.
26. Camalli, M.; Capitani, D.; Cascarano, G.; Cerrini, S.; Giacovazzo, C.; Spagna, R.; SIR-CAOS (It. Pat. n. 35403c/86): *User Guide* 1986, Istituto di Strutturistica Chimica CNR, C.P. n. 10, 00016 Monterotondo Stazione, Rome
27. Zigmond, S.H.; Hirsch, J.G. *J. Exp. Med.* **1973**, *137*, 387-410.
28. Spisani, S.; Cavalletti, T.; Gavioli, R.; Scatturin, A.; Vertuani, G.; Traniello, S. *Inflammation* **1986**, *10*, 363-369.