

CONFORMATIONAL ANALYSIS OF AN ACTIVE CHEMOTACTIC PEPTIDE ANALOG CONTAINING
Z-DEHYDROPHENYLALANINE AT POSITION 3

V.S. Chauhan^a, Paramjeet Kaur^a, Nirupa Senb,

K. Uma^b, Jose Jacob^b and P. Balaram^{b*}

^a Department of Chemistry, Delhi University,
Delhi 110 007, India

^b Molecular Biophysics Unit, Indian Institute of Science,
Bangalore 560 012, India

Abstract: Formyl-Met-Leu- Δ^Z -Phe-OMe, an analog of the chemotactic tripeptide Formyl-Met-Leu-Phe has been synthesized to evaluate the effect of substitution of α,β -dehydrophenylalanine on activity and conformation. The analog peptide shows high biological activity in stimulating superoxide production by rabbit neutrophils. An NMR analysis of the solution conformation of the Δ^Z -Phe analog, using nuclear Overhauser effects and comparisons with the corresponding saturated peptides, favours a significant population of extended backbone conformations.

Formyl-methionyl-leucyl-phenylalanine (Formyl-Met-Leu-Phe-OH) and several synthetic analogs have been shown to act as chemoattractants for neutrophils^{1,2}. These peptides induce a selective release of lysosomal enzymes in neutrophils² and also stimulate a burst of oxidative metabolism³. Formyl-Met-Leu-Phe-OH and its analogs bind to a specific membrane bound receptor on the neutrophil cell surface. Several studies have attempted to correlate structure-activity data in order to establish the structural features necessary for biological activity^{4,5} and to delineate the nature of the receptor bound conformation of the peptide effector⁶⁻¹⁰. Studies with conformationally constrained analogs have suggested that folded, 8-turn structures centred at residues 1 and 2 may be responsible for receptor recognition. The high biological activity of position 2 analogs containing α -aminoisobutryl (Aib), 1-aminocycloalkane-1-carboxyl (Acc⁵ or Acc⁶, where superscript denotes number of carbon atoms in the cycloalkane ring) residues supports this point of view^{6,7}. Other reports have favoured fully extended solution conformations for the parent sequence⁸ and several unconstrained analogs^{8,9}, suggesting that extended conformations are capable of interacting with biological receptors¹⁰. In this report we describe the synthesis, solution conformations and biological activity of an analog containing Z-dehydrophenylalanine (Δ^Z -Phe) at position 3. Formyl-Met-Leu- Δ^Z -Phe-OMe (1). The results are compared with those obtained for the analog Boc-Met-Leu- Δ^Z -Phe-OMe (2) and the saturated peptide Formyl-Met-Leu-Phe-OMe (3).

RESULTS AND DISCUSSION

Biological Activity

The binding of active chemotactic peptide analogs to neutrophil receptors triggers superoxide production¹¹, which can be conveniently assayed spectrophotometrically by redox reactions of cytochrome C¹³ or ascorbic acid¹⁴. Figure 1a shows the effect of addition of the peptide Formyl-Met-Leu-Phe-OMe (3) to neutrophil suspensions containing cytochrome C. Appreciable reduction is detectable at a concentration of 10^{-8} M. This analog has been shown to be highly active in inducing lysosomal enzyme release⁷. Figure 1b shows the results of a comparable experiment with the analog Formyl-Met-Leu- Δ^Z -Phe-OMe (1),

which establishes almost the same level of activity at 10^{-8} M. Earlier studies have shown that the amino terminal formyl group is essential for activity, with Boc protected peptides acting as **antagonists**^{4,5}. Figure 1c demonstrates that addition of $\sim 30\mu$ M Boc-Met-Leu-Phe-OMe dramatically reduces the activity of **3**. Figure 1d shows that Eoc-Met-Leu- Δ^Z -Phe-OMe (**2**) also functions as an antagonist. The results establish that replacement of Phe(3) by Δ^Z -Phe(3) does not have an appreciable influence on biological activity.

Conformational studies

^1H NMR (270 MHz) studies were carried out in CDCl_3 and $(\text{CD}_3)_2\text{SO}$. Assignment of NH resonances was straightforward in **2**, with the Leu and Met NH resonances identified by spin decoupling. In **1**, the Met and Leu C^αH resonances overlap in 23.8% CDCl_3 - $(\text{CD}_3)_2\text{SO}$ and $(\text{CD}_3)_2\text{SO}$ (Fig.2a shows the ^1H NMR spectrum of **1** in $(\text{CD}_3)_2\text{SO}$). The Met NH doublet was recognized by virtue of a strong NOE to the formyl proton. The degree of solvent exposure of the NH groups was probed by means of temperature dependence of NH chemical shifts in $(\text{CD}_3)_2\text{SO}$ and by comparing changes in chemical shifts between a poorly hydrogen bonding solvent like CDCl_3 and a good hydrogen bond acceptor, $(\text{CD}_3)_2\text{SO}$ ¹⁵. The NMR parameters for the various NH resonances in **1** and **2** are listed in Table 1 and compared with the values obtained for Formyl-Met-Leu-Phe-OMe (**3**). It is clearly seen that in all the peptides there is no evidence for inaccessibility of any NH group to the solvent, suggesting the absence of intramolecularly hydrogen bonded conformations, in solution.

TABLE 1
 ^1H NMR Parameters for NH Resonances in Peptides **1**, **2** and **3**

	Peptide 1 (3) ^a			Peptide 2		
	Met	Leu	Δ^Z -Phe (Phe)	Met	Leu	Δ^Z -Phe
$\delta_{\text{NH}}(\text{CDCl}_3)^{\text{b}}$ (ppm)	8.01 (6.95)	7.86 (7.12)	9.31 (6.92)	5.22	6.73	7.91
$\delta_{\text{NH}}[(\text{CD}_3)_2\text{SO}]$ (ppm)	8.33 (8.29)	8.23 (8.05)	9.78 (8.33)	7.04	7.97	9.76
$d\delta/dT$ (ppm $\text{K}^{-1}\times 10^3$)	4.50 (6.20)	5.67 (5.10)	6.33 (4.30)	5.80	4.60	5.80
$J_{\text{NHC}^\alpha\text{H}}(\text{CDCl}_3)^{\text{b}}$ (Hz)	7.3 (9.0)	8.0 (8.7)	- (7.8)	7.8	8.9	-
$J_{\text{HNC}^\alpha\text{H}}[(\text{CD}_3)_2\text{SO}]$ (Hz)	8.3 (7.4)	7.8 (8.1)	- (7.4)	5.4	7.1	-

^a Values in parentheses are the NMR parameters for peptide For-Met-Leu-Phe-OMe, **3**.

^b Peptide **1** does not dissolve in CDCl_3 . Hence δ_{NH} and $J_{\text{HNC}^\alpha\text{H}}$ for this peptide are those obtained in a solvent mixture of 76.2% CDCl_3 and 23.8% $(\text{CD}_3)_2\text{SO}$.

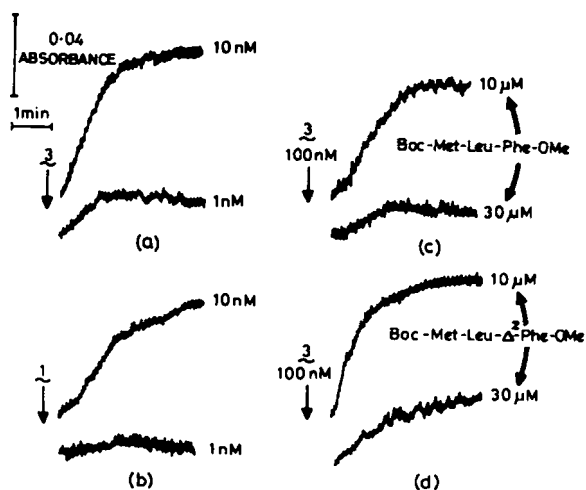


Fig.1: Superoxide generation from rabbit peritoneal neutrophils. Responses by a) 1nM and 10nM Formyl-Met-Leu-Phe-OMe 3 and b) 1nM and 10nM Formyl-Met-Leu-A'-Phe-OMe 1. Inhibition of the 100nM Formyl-Met-Leu-Phe-OMe response on preincubation of cells for 2min with c) 10μM and 30μM Boc-Met-Leu-Phe-OMe and d) 10μM and 30μM Boc-Met-Leu-Δ^Z-Phe-OMe 2.

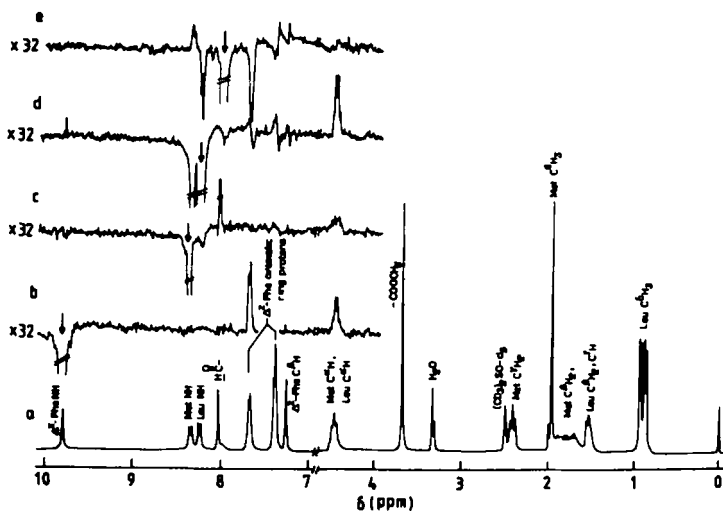


Fig.2: a) 270 MHz ¹H NMR spectrum of Formyl-Met-Leu-Δ^Z-Phe-OMe 1 in (CD₃)₂SO. b-e) Difference NOE spectra obtained by irradiation of b) Δ^Z-Phe NH c) Met NH d) Leu NH and e) formyl proton.

Figure 2 shows the results of typical difference NOE experiments designed to probe short ($< 3\text{\AA}$) interproton distances¹⁷. The magnitudes of the NOEs observed on irradiation of specific peptide NH resonances are summarized in Tables 2 and 3. In CDCl_3 , both $\text{C}_i^{\alpha}\text{H} \leftrightarrow \text{N}_i\text{H}$ (intraresidue) and $\text{C}_i^{\alpha}\text{H} \leftrightarrow \text{N}_{i+1}\text{H}$ (interresidue) NOEs were observed in all cases, with the latter being appreciably more intense than the former. For peptides 2 and 3 the following strong NOEs were observed: $\Delta^2\text{-Phe NH} \leftrightarrow \text{Leu C}^{\alpha}\text{H}$ and $\text{Leu NH} \leftrightarrow \text{Met C}^{\alpha}\text{H}$. These successive interresidue NOEs are compatible with extended backbone conformations having $\psi_{\text{Met}} \approx \psi_{\text{Leu}} \sim 120^\circ \pm 30^\circ$ ^{17,18}. The relatively large $J_{\text{HNC}^{\alpha}\text{H}}$ values for Met and Leu ($> 8\text{Hz}$) are consistent with large [negative] ϕ values characteristic of extended peptide chains¹⁷. For 3 in $(\text{CD}_3)_2\text{SO}$, the NOEs are suggestive of a population of extended structures, although the lower $J_{\text{HNC}^{\alpha}\text{H}}$ values may indicate a greater degree of conformational averaging. The observation of a weak $\text{N}_i\text{H} \leftrightarrow \text{N}_{i+1}\text{H}$ NOE in CDCl_3 [between Leu NH and $\Delta^2\text{-Phe NH}$] for peptide 1 is indicative of a small population of folded conformers having $\text{Leu } \phi \sim -50^\circ, \psi \sim -50^\circ$. This corresponds to the helical region of ϕ, ψ space. This NOE is relatively weaker in the more polar solvent $(\text{CD}_3)_2\text{SO}$.

The insolubility of peptide 1 precluded a study in CDCl_3 . In both 23.8% $(\text{CD}_3)_2\text{SO}-\text{CDCl}_3$ and pure $(\text{CD}_3)_2\text{SO}$ the Met $\text{C}^{\alpha}\text{H}$ and Leu $\text{C}^{\alpha}\text{H}$ resonances overlap, thus preventing a definitive analysis of the interresidue NOEs. An appreciable NOE is indeed observed between $\Delta^2\text{-Phe NH}$ and the composite $\text{C}^{\alpha}\text{H}$ resonance at 4.62δ , which may be assigned to a short interproton distance $\Delta^2\text{-Phe NH} \cdots \text{Leu C}^{\alpha}\text{H}$. The NOEs observed on the $\text{C}^{\alpha}\text{H}$ resonances when the Leu NH group is saturated could contain contributions from both inter- and intraresidue effects. An NOE between $\Delta^2\text{-Phe NH}$ and Leu $\text{C}^{\alpha}\text{H}$ is also observed for peptide 2 in $(\text{CD}_3)_2\text{SO}$. These NMR results support the presence of largely extended peptide conformations in the chemotactic peptide analogs 1 to 3 in organic solvents (Fig.3). The results are in agreement with earlier reports on Formyl-Met-Leu-Phe-OH¹¹ and Formyl-Met-Leu-Phe-OMe²⁰, which favoured extended conformations in dimethylsulfoxide solutions.

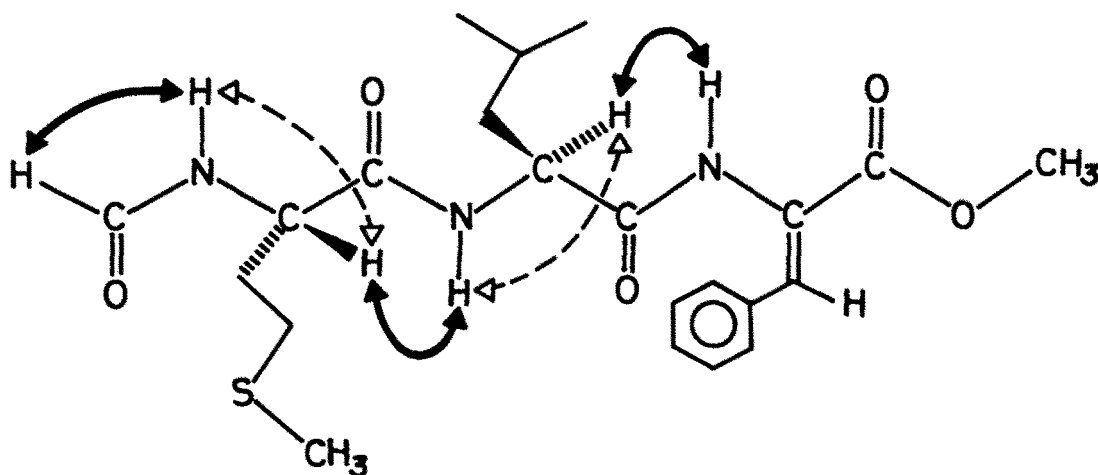


Fig.3: Proposed extended conformation for Formyl-Met-Leu- Δ^2 -Phe-OMe 1. Dark arrows and broken arrows indicate short interproton distances which result in diagnostic interresidue and intraresidue NOEs, respectively.

Despite considerable effort and notwithstanding their apparent structural simplicity, the nature of the "biologically active" conformation of chemotactic peptides remains unclear at present. Analogs which are necessarily constrained to adopt folded conformations by virtue of incorporation of stereochemically rigid amino acid residues have proved to be highly active^{6,7}. However, analogs which show a strong propensity to favour extended

TABLE 2

Nuclear Overhauser Effects (%) Observed on Irradiation of NH Resonances in CDCl_3

Reeonance Irradiated	Peptide 1 ^a	Peptide 2	Peptide 3
Met NH	Formyl proton (13.9) Met C ^{α} H (7.6) ^b	Met C ^{α} H (2.5)	Met C ^{α} H (4.2)
Leu NH	δ^Z -Phe NH (2.7) Met C ^{α} H, Leu C ^{α} H ^c (7.88)	Met NH (1.1) Leu C ^{α} H (3.8) Met C ^{α} H (12.0)	Leu C ^{α} H (3.9) Met C ^{α} H (14.2)
δ^Z -Phe NH or Phe NH	Leu C ^{α} H (12.0) ^b	Leu C ^{α} H (8.7)	Phe C ^{α} H (2.9) Leu C ^{α} H (7.0)

^a Peptide 1 was studied in a solvent composition of 76.2% CDCl_3 and 23.8% $(\text{CD}_3)_2\text{SO}$.^{b,c} Met C ^{α} H and Leu C ^{α} H resonances overlap in peptide 1. For NOB calculations for the C ^{α} H resonances. in cases (b), half the intensity of C ^{α} H resonance and in (c), the complete intensity has been used.

TABLE 3

Nuclear Overhauser Effects (%) Observed on Irradiation of NH Resonances in $(\text{CD}_3)_2\text{SO}$

Resonance Irradiated	Peptide 1	Peptide 2	Peptide 3
Met NH	Formyl Proton (13.9) Met C ^{α} H (2.8) ^a	Met C ^{α} H (1.3)	Met C ^{α} H (1.7)
Leu NH	δ^Z -Phe NH (1.6) Met C ^{α} H, Leu C ^{α} H (7.9) ^b	Leu C ^{α} H (2.2)	Met C ^{α} H (5.1)
δ^Z -Phe NH or Phe NH	Leu C ^{α} H (6.0) ^a	Leu C ^{α} H (4.0)	Phe C ^{α} H (4.5) Leu C ^{α} H (5.5)

^{a,b} In peptide 1, the Met C ^{α} H and Leu C ^{α} H resonances overlap in $(\text{CD}_3)_2\text{SO}$. Hence for NOB calculations for C ^{α} H resonances, in (a) half the intensity and in (b) complete intensity of C ^{α} H resonace has been used.

conformations in solution are also highly active^{8,11}. The peptide **1** discussed in this report falls into this category. Stereochemically unconstrained analogs can, in principle, undergo a conformational change on receptor binding. Alternatively, multiple receptor sites could recognize discrete peptide conformations as in the well documented case of the **enkephalins**²¹. The possibility of a gross three dimensional similarity in the disposition of the atoms interacting with the receptor, for both folded and extended structures may also be a factor as noted recently for **enkephalins**²².

The present study establishes that substitution of L-Phe by Δ^Z -Phe occurs without major perturbation of backbone conformations and indeed results in a biologically active analog. The introduction of the α,β -double bond does not appreciably limit the accessible backbone conformations, a feature which has been emphasized in recent studies on the conformation of Δ^Z -Phe containing **peptides**²³⁻²⁵. The orientation of the aromatic ring in the sidechain restricted Δ^Z -Phe analog **1** appears suitable for receptor binding. It will be of particular interest to examine the biological activity of the Δ^E -Phe isomer and studies in this direction are in progress.

EXPERIMENTAL

Boc amino acids and amino acid methyl esters were prepared by standard procedures. TLC was carried out on silica gel G in the following solvent systems: A) CHCl_3 :MeOH (9:1) and B) $n\text{-BuOH}$:AcOH: H_2O (4:1:1). The final peptides were each shown to yield a single peak by HPLC on a Lichrosorb RP-18 column (4mmx250mm, particle size 10 μm), using a methanol/water gradient (60-80% MeOH in 20min, 226nm detection). All ^1H NMR spectra were recorded on a Bruker WH-270 FT NMR spectrometer equipped with an Aspect 2000 computer, at the Sophisticated Instruments Facility, Indian Institute of Science. Difference NOE experiments were carried out as described earlier.¹⁶

Peptide Synthesis

Boc-Leu-DL-Phe (β -OH)-OH 4:

A solution of Boc-Leu-OH (5.4g, 21.6mmol) and N-methylmorpholine (2.4ml, 21.6mmol) in THF (50ml) was cooled to -5 to -10°C and isobutylchloroformate (2.8ml, 21.6mmol) was added and the mixture stirred for 20min. A precooled solution of DL- β -phenylserine (5.9g, 32.5mmol) in aqueous NaOH (1M, 32.5ml) was then added and the mixture stirred for 2h at 0°C and overnight at room temperature. The reaction mixture was concentrated ~~in vacuo~~ and aqueous solution was washed with ethyl acetate (1x25ml), acidified with solid citric acid to pH 3 and extracted with ethyl acetate (3x30ml), dried over anhydrous Na_2SO_4 and evaporated to give **4** as an amorphous solid. Yield, 8.5g (100%); m.p., 75-78°C; $R_f(\text{A})$, 0.62; $R_f(\text{B})$, 0.71; $[\alpha]_{\text{D}}^{27}$, -23.4' (c 1.0, MeOH).

Boc-Leu- Δ^Z -Phe azlactone 5:

A solution of the dipeptide **4** (8.5g, 21.6mmol) in acetic anhydride (65ml) and anhydrous sodium acetate (2.3g, 28.1 mmol) was stirred for 24h at room temperature. The reaction mixture was poured over crushed ice, stirred and resultant precipitate filtered, washed with 5% NaHCO_3 solution, water and dried to give **5**. Yield, 7.8g (100%); m.p., 118-120°C; $R_f(\text{A})$, 0.77; $R_f(\text{B})$, 0.88; $[\alpha]_{\text{D}}^{27}$, -113.2' (c 1.0, MeOH).

Boc-Leu- Δ^Z -Phe-OMe 6:

To a solution of **5** (2.0g, 5.5mmol) in absolute methanol (50ml) was added 4-dimethylamino pyridine (0.68g, 5.5mmol) and the mixture stirred overnight at room temperature. A saturated solution of citric acid was added till pH was 4, methanol evaporated in vacuo and extracted with ether (3x25ml). The ether extracts were combined, washed with 59 citric acid (2x15ml),

saturated NaCl (1x15ml) and dried over anhydrous Na_2SO_4 . Ether was evaporated in vacuo to give **6**. Yield, 1.75g (**81%**); $R_f(\text{A})$, 0.79; $[\alpha]_{\text{D}}^{27}$, 38.8° (c 1.0, MeOH).

Boc-Met-Leu- Δ^Z -Phe-OMe 2:

The dipeptide **6** (1.75g, 4.5mmol) was treated with a 1:1 mixture of TFA and CH_2Cl_2 for 1h at room temperature. Excess TFA was removed, residue washed with dry ether and dried over KOH in vacuo, to give TFA,H-Leu- Δ^Z -Phe-OMe. Yield, 1.3g (72.2%).

A solution of Boc-Met-OH (0.76g, 3.1mmol) and N-methylmorpholine (0.34ml, 3.1mmol) in THF (15ml) was chilled and isobutyl chloroformate (0.41ml, 3.1mmol) was added and mixture stirred for 15 min. A precooled solution of TFA salt and N-methylmorpholine (0.36ml, 3.3mmol) in THF (10ml) was added and mixture stirred for 2h at 0°C and overnight at room temperature. The reaction mixture was evaporated in vacuo, residue dissolved in ethyl acetate, washed successively with saturated NaHCO_3 solution, water, 5% citric acid solution, water and dried over anhydrous Na_2SO_4 . Solvent was evaporated in vacuo to yield a solid which upon trituration with dry ether gave pure tripeptide **2**. Yield, 1.2g (75%); m.p., 148-149°C; $R_f(\text{A})$, 0.82; $R_f(\text{B})$, 0.71; HPLC retention time, 18.4min. Anal. Calc. for $\text{C}_{26}\text{H}_{39}\text{O}_6\text{N}_3$, C 59.88, H 7.48, N 8.06. Found C 59.67, H 7.50, N 7.95. 270 MHz ^1H NMR, δ , ppm [$(\text{CD}_3)_2\text{SO}$]: 9.76(Δ^Z -Phe NH, 1H, s); 7.97(Leu NH, 1H, d); 7.65, 7.39(Δ^Z -Phe aromatic protons, 5H, m); 7.26(Δ^Z -Phe C^{β}H , 1H, s); 7.04(Met NH, 1H, d); 4.45(Leu $\text{C}^{\alpha}\text{H}$, 1H, m); 4.03(Met $\text{C}^{\alpha}\text{H}$, 1H, m); 3.69(-COOCH₃, 3H, s); 2.44(Met $\text{C}^{\gamma}\text{H}_2$, 2H, t); 1.99(Met $\text{C}^{\epsilon}\text{H}_3$, 3H, s); 1.76(Met $\text{C}^{\beta}\text{H}_2$, 2H, m); 1.52(Leu $\text{C}^{\beta}\text{H}_2$ and Leu $\text{C}^{\gamma}\text{H}$, 3H, m); 1.37(Boc CH₃, 9H, s); 0.91(Leu $\text{C}^{\delta}\text{H}_3$, 6H, m).

Formyl-Met-Leu- Δ^Z -Phe-OMe 1:

98% Formic acid (2ml) was added to **2** (100mg, 0.2mmol) kept under nitrogen. Reaction was stopped after 3.5h when TLC showed absence of starting material and reaction mixture evaporated to dryness, to give the formate salt of **2**.

Acetoformic acid reagent was prepared by adding 1ml of 98% formic acid to 2ml of freshly distilled acetic anhydride, which was precooled to 0°, and mixing thoroughly. This mixture was then heated to 50°C for 15min and cooled immediately in an ice bath. 1.1ml of this reagent was added to the formate salt of **2**, stoppered and maintained for 1h in an ice bath, and at room temperature for 1.5h. Reaction mixture was then evaporated to dryness, and water added, to give a white precipitate. Solid was extracted with ethyl acetate (3x10ml). Combined organic extracts were washed with water, 1M NaHCO_3 and again, water. Ethyl acetate layer was dried over anhydrous Na_2SO_4 and evaporated in vacuo. The crude product was purified by HPLC (retention time, 10.5min). 270-MHz ^1H NMR, δ , ppm [$(\text{CD}_3)_2\text{SO}$]: 9.78(Δ^Z -Phe NH, 1H, s); 8.33(Met NH, 1H, d); 8.23(Leu NH, 1H, d); 8.03(Formyl proton, 1H, s), 7.69, 7.42(Δ^Z -Phe aromatic protons, 5H, m); 7.26(Δ^Z -Phe C^{β}H , 1H, s); 4.46(Met and Leu $\text{C}^{\alpha}\text{H}$, 2H, m); 3.70(-COOCH₃, 3H, s); 2.42(Met $\text{C}^{\gamma}\text{H}_2$, 2H, t); 1.97(Met $\text{C}^{\epsilon}\text{H}_3$, 3H, s); 1.78(Met $\text{C}^{\beta}\text{H}_2$, 2H, m); 1.54(Leu $\text{C}^{\beta}\text{H}_2$ and Leu $\text{C}^{\gamma}\text{H}$, 3H, m); 0.92(Leu $\text{C}^{\delta}\text{H}_3$, 6H, m) (see Fig.2a for a representative spectrum).

Biological Assay

Rabbit peritoneal neutrophils were prepared as described²⁶. Briefly, 0.1% glycogen in normal saline was injected intraperitoneally. The exudate formed was tapped after 4h. Cells were washed by centrifugation and suspended in buffer containing 17mM HEPES, 120mM NaCl, 5mM KCl, 1mM MgSO_4 and 5mM glucose. Superoxide generation was assayed as described¹³ with minor modifications. 1.5×10^6 cells were stirred and incubated at 37°C in 2ml of buffer containing 1mM CaCl_2 and 0.625mg/ml cytochrome C. Cells were pretreated with 4 $\mu\text{g/ml}$ of cytochalasin B before addition of the test samples. Changes in absorbance at 550nm were measured.

Acknowledgements

This research was supported by a grant from the Department of Science and Technology, Government of India. KU and JJ acknowledge the receipt of a research fellowship and research associateship, respectively, from the Council of Scientific and Industrial Research.

References

1. E. Schilffmann, B.A. Corcoran and S.M. Wahl. Proc. Natl. Acad. Sci. USA, **72**, 1059 (1975).
2. H.J. Showell, R.J. Freer, S.H. Zigmond, E. Schiffmann, S. Aswanikumar, B.A. Corcoran and E.L. Becker. J. Exp. Med., **143**, 1154 (1976).
3. B. Dewald, T.G. Payne and M. Baggiolini, Biochem. Biophys. Res. Commun., **125**, 367 (1984).
4. R.J. Freer, A.R. Day, J.A. Radding, E. Schiffmann, S. Aswanikumar, H.J. Showell and E.L. Becker. Biochemistry, **19**, 2404 (1980).
5. R.J. Freer, A.R. Day, N. Muthukumaraswamy, D. Pinon, A. Wu, H.J. Showell and E.L. Becker. Biochemistry, **21**, 257 (1982).
6. M. Iqbal, P. Balaram, H.J. Showell, R.J. Freer and E.L. Becker, FEBS Lett., **165**, 171 (1984).
7. M. Sukumar, P.A. Raj, P. Balaram and E.L. Becker, Biochem. Biophys. Res. Commun., **128**, 339 (1985).
8. C. Toniolo, G.M. Bonora, H. Showell, R.J. Freer and E.L. Becker, Biochemistry, **23**, 698 (1984).
9. G.M. Bonora, C. Toniolo, R.J. Freer and E.L. Becker. Biochim. Biophys. Acta, **884**, 545 (1986).
10. E.L. Becker, R.J. Freer, C. Toniolo and P. Balaram. Membrane Receptors and Cellular Regulation. M.P. Czech and C.R. Kahn (eds.), A.R. Liss, New York, pp.129-134 (1985).
11. E.L. Becker, H.E. Bleich, A.R. Day, R.J. Freer, J.A. Glasel and J. Visintainer Biochemistry, **18**, 4656 (1979).
12. E.L. Becker, J. Reticuloendothel. Soc., **26** (Suppl.), 701 (1979).
13. C.A. Parkos, C.G. Cochrane, M. Schmitt and A.J. Jesaitis, J. Biol. Chem., **260**, 6514 (1985).
14. H. Hemilia, P. Roberts and M. Wikstrom, FEBS Lett., **178**, 25 (1984).
15. K. Wüthrich, NMR in Biological Research: Peptides and Proteins. North Holland, Elsevier, Amsterdam, (1976).
16. B.N.N. Rao, A. Kumar, H. Balaram, A. Ravi and P. Balaram, J. Am. Chem. Soc., **105**, 7423 (1983).
17. M. Billeter, W. Braun and K. Wüthrich, J. Mol. Biol., **155**, 321 (1982).
18. M.D. Shenderovich, G.V. Nikiforovich and G.I. Chipens, J. Magn. Resonance, **59**, 1 (1984).
19. A. Pardi, M. Billeter and K. Wüthrich, J. Mol. Biol., **180**, 741 (1986).
20. P.A. Raj and P. Balaram, Biopolymers, **24**, 1131 (1985).
21. P.W. Schiller. The Peptides: Analysis, Synthesis, Biology. Vol VI, S. Udenfriend and J. Meienhofer (eds.), Academic Press, Inc., Florida, pp. 219-268 (1984).
22. M. Doi, M. Tanaka, T. Ishida and M. Inoue, FEBS Lett., **213**, 265 (1987).
23. A.C. Bach, II and L.M. Gierasch. J. Am. Chem. Soc., **107**, 3349 (1985).
24. V.S. Chauhan, A.K. Sharma, K. Uma, P.K.C. Paul and P. Balaram. Int. J. Peptide Protein Research, **29**, 126 (1987).
25. A.C. Bach, II and L.M. Gierasch, Biopolymers, **25**, 5175 (1986).
26. J.G. Hirsch, J. Exp. Med., **103**, 589 (1956).