

# Conformationally constrained chemotactic peptide analogs of high biological activity

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The stereochemically constrained chemotactic peptide analogs, formylmethionyl- $\alpha$ -aminoisobutyryl-phenylalanine (formyl-Met-Aib-Phe-OH) and formylmethionylcycloleucylphenylalanine (formyl-Met-Cyl-Phe-OH) are highly effective in inducing lysosomal enzyme release from rabbit neutrophils. NMR studies of the Aib<sup>2</sup> analog in (CD<sub>3</sub>)<sub>2</sub>SO favor a folded conformation in which the Phe NH group is inaccessible to solvent. Intramolecularly hydrogen-bonded conformations involving a Met-Aib- $\beta$ -turn or a  $\gamma$ -turn centered at Aib<sup>2</sup> are considered. The results suggest that folded conformations may allow highly active interactions with the neutrophil formylpeptide receptor.

*Chemotactic peptide      Neutrophil      Chemotaxis      Formylpeptide*

## 1. INTRODUCTION

The finding that low molecular mass tripeptides are chemoattractants for neutrophils which also induce selective release of lysosomal enzymes [1,2] has stimulated considerable interest in developing models for the biologically active conformation of these peptides [3-5]. Extensive <sup>1</sup>H and <sup>13</sup>C NMR relaxation studies of the most active member of this class of peptides *N*-formyl-Met-Leu-Phe-OH (FMLP) have suggested an extended, monomeric  $\beta$ -sheet conformation in dimethyl sulfoxide solution [3]. The observation of a low temperature coefficient ( $d\delta/dT$ ) value for the Phe NH proton in the anionic form of FMLP in (CD<sub>3</sub>)<sub>2</sub>SO did provide some evidence, albeit weak, for the possibility of a folded structure in which the Phe NH group was shielded from the solvent. However, this con-

formation was ruled out as it was incompatible with the analysis of relaxation data in terms of interproton distances [3]. On the basis of structure-activity data involving analysis of results from a large number of analog peptides, an extended  $\beta$ -sheet conformation has been tentatively suggested as the biologically active form of the chemotactic peptide, FMLP [6]. In order to test this hypothesis, we report studies on conformationally constrained analogs, in which the central Leu residue is replaced by an  $\alpha$ ,  $\alpha$ -dialkyl amino acid,  $\alpha$ -aminoisobutyric acid (Aib) or cycloleucine (1-aminocyclopentane-1-carboxylic acid, Cyl). The results suggest that 'active' conformations may be folded at the receptor site.

## 2. MATERIALS AND METHODS

Peptides were synthesized by conventional solution phase procedures [7]. All peptides were chromatographically pure as judged by thin layer chromatography in three solvent systems (*n*-

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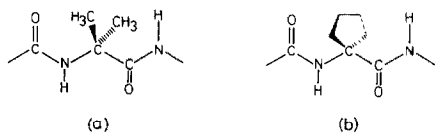


Fig. 1. The Aib (a) and Cyl (b) residues in a peptide chain.

butanol-acetic acid-water, 4:1:1; chloroform-methanol-acetic acid-water, 65:30:4:1; benzene-acetic acid-water, 9:9:1) and yielded fully consistent 270 MHz  $^1\text{H}$  NMR spectra.

The peptides were tested for their ability to induce release of the lysosomal enzymes,  $\beta$ -glucuronidase and lysozyme from cytochalasin B-treated rabbit neutrophils, as in [2].

270 MHz  $^1\text{H}$  NMR spectra were recorded on a Bruker WH-270 FT NMR spectrometer at the Sophisticated Instruments Facility, Bangalore. NMR samples were prepared by directly dissolving solid peptide in  $(\text{CD}_3)_2\text{SO}$ , at a concentration of 10 mg/ml.

### 3. RESULTS AND DISCUSSION

The analogs formyl-Met-Aib-Phe and formyl-Met-Cyl-Phe were synthesized with the view of imposing stereochemical restrictions on backbone folding.  $\alpha$ ,  $\alpha$ -Dialkyl  $\alpha$ -amino acids preferentially adopt only a limited range of conformations due to unfavorable steric contacts involving atoms on the two flanking peptide units (fig.1), with the geminal substituents at  $\text{C}^\alpha$  [8,9]. Extensive crystallo-

graphic, spectroscopic and theoretical studies on Aib peptides [10-13] have provided strong support for the overwhelming preference of this residue for  $\phi$ ,  $\psi$  values in the right- and left-handed  $3_{10}/\alpha$ -helical regions of the Ramachandran map ( $\phi \pm 60^\circ \pm 20^\circ$ ,  $\psi \pm 30^\circ \pm 20^\circ$ ) [14]. In small peptides, 4 $\rightarrow$ 1 hydrogen-bonded  $\beta$ -turns have been almost exclusively found for Aib-X and X-Aib sequences [11,12]. This residue occurs with considerably lower frequency in the  $\gamma$ -turn (intramolecular 3 $\rightarrow$ 1 hydrogen bond) conformation ( $\phi \pm 80^\circ$ ,  $\psi \mp 80^\circ$ ) although this is sterically allowed and only slightly higher in energy than structures in the helical region [11]. Cyl is expected to behave in a similar fashion since it also possesses two alkyl substituents at the  $\text{C}^\alpha$  atom.

Table 1 summarizes the results of the neutrophil lysosomal enzyme release assay on FMLP and a few analogs. It is clearly seen that both the analogs incorporating Aib or Cyl at position 2 are highly active. The data in table 1 also provide additional support for the view that the C-terminal carboxylic acid group is not essential for activity [4,15], as demonstrated by the low  $ED_{50}$  values for the ester and amide analogs of FMLP. Replacement of the formyl group by a *t*-butyloxycarbonyl (Boc) function leads to a dramatic attenuation of activity in the position 2 analogs [5]. This is confirmed by the low activity of Boc-Met-Cyl-Phe-OH.

270 MHz  $^1\text{H}$  NMR studies were carried out on the peptides formyl-Met-Leu-Phe-OH and formyl-Met-Aib-Phe-OH, in order to compare spectroscopic properties in solution. The chemical

Table 1

$ED_{50}$  (M) values for peptide-induced lysosomal enzyme release

Peptide	Lysozyme	$\beta$ -Glucuronidase
Formyl-Met-Leu-Phe-OH	$(1.5 \pm 0.5) \times 10^{-10}$	$(2.0 \pm 1.0) \times 10^{-10}$
Formyl-Met-Aib-Phe-OH	$(1.9 \pm 0.8) \times 10^{-9}$	$(2.9 \pm 1.4) \times 10^{-9}$
Formyl-Met-Cyl-Phe-OH	$(3.9 \pm 0.7) \times 10^{-10}$	$(6.1 \pm 1.9) \times 10^{-10}$
Formyl-Met-Leu-Phe-OMe	$(3.2 \pm 1.5) \times 10^{-11}$	$(3.6 \pm 2.0) \times 10^{-11}$
Formyl-Met-Leu-Phe-NH <sub>2</sub>	$(1.2 \pm 0.7) \times 10^{-11}$	<sup>a</sup> $(4.2 \pm 1.0) \times 10^{-11}$
Boc-Met-Cyl-Phe-OH	$(5.5 \pm 0.8) \times 10^{-7}$	$(6.7 \pm 0.9) \times 10^{-7}$

$ED_{50}$ , concentration of peptide required to produce 50% of the maximum effect determined by the concentration effect curve. Each value is the average  $\pm$ SE of 3 determinations

<sup>a</sup> Averaged over 2 determinations

Table 2  
Chemical shifts and temperature coefficients for NH groups<sup>a</sup>

Peptide		Met	Leu/Aib	Phe
Formyl-Met-Leu-Phe-OH <sup>b</sup>	$\delta$	8.08	8.01	8.25
	$d\delta/dT$	0.0052 (0.0041) (0.0085)	0.0044 (0.0045) (0.0085)	0.0039 (0.0061) (0.0026)
Formyl-Met-Aib-Phe-OH	$\delta$	8.36	8.11	7.48
	$d\delta/dT$	0.0050	0.0045	0.0027

<sup>a</sup> Parameters determined in (CD<sub>3</sub>)<sub>2</sub>SO.  $d\delta/dT$  values expressed as ppm/°C.

<sup>b</sup>  $d\delta/dT$  values in parentheses are from [3]. Upper values are for a sample lyophilized from aqueous solution at pH 2. Lower values correspond to pH 8. Values determined in (CD<sub>3</sub>)<sub>2</sub>SO

shifts ( $\delta$ , ppm) and temperature coefficient ( $d\delta/dT$ ) values for the various NH groups in (CD<sub>3</sub>)<sub>2</sub>SO are summarized in table 2. The values obtained in an earlier study of FMLP [3] are provided for comparison. In both peptides the Phe NH resonance has a significantly lower  $d\delta/dT$  value as compared to the NH resonances of residues 1 and 2. Low  $d\delta/dT$  values (<0.003 ppm/°C) in (CD<sub>3</sub>)<sub>2</sub>SO are generally indicative of solvent shielded (presumably intramolecularly hydrogen-bonded) NH groups [16]. The value of 0.0027 ppm/°C obtained for Phe NH in formyl-Met-Aib-Phe is low enough to argue for its inaccessibility to solvent. Together with the known propensity of Aib residues for folded, hydrogen-bonded conformations, the NMR data support the existence of folded structures, involving Phe NH in an intramolecular hydrogen bond.

Two structures may be considered as feasible. These are the 4→1 hydrogen-bonded Met-Aib  $\beta$ -turn and the 3→1 hydrogen bonded  $\gamma$ -turn centered at Aib (fig.2). In both structures the Phe

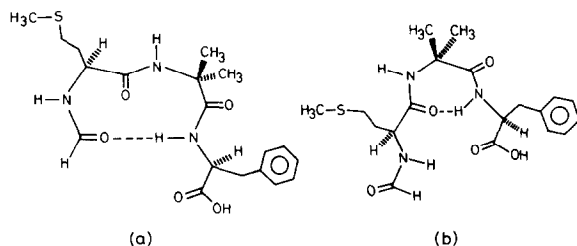


Fig. 2. Hydrogen-bonded conformations of formyl-Met-Aib-Phe-OH involving Phe NH. (a) Met-Aib  $\beta$ -turn. (b)  $\gamma$ -turn centered at Aib.

NH is hydrogen-bonded and the two possibilities differ only in the nature of the acceptor C=O group. Theoretical analysis of Aib residues suggests that the differences in energies for the  $\beta$ -turn ( $\phi \sim \pm 60^\circ$ ,  $\pm 20^\circ$ ,  $\psi \sim \pm 30^\circ \pm 20^\circ$ ;  $i+2$  residue in type III(I) or III'(I') and type II or II'  $\beta$ -turns) and a  $\gamma$ -turn conformation ( $\phi \pm \sim 80^\circ$ ,  $\psi \sim \pm 80^\circ$ ) is 3 kcal · mol<sup>-1</sup> [11]. This is small enough to be offset by other interactions. While several examples of  $\beta$ -turn structures are documented in the solid state [10-13], the  $\beta$ -turn has been observed crystallographically in the cyclic peptide dihydrochlamydocin [17]. C<sub>7</sub> conformations have also been proposed for solution IR studies of small Aib peptides [18] and considered for (Aib-Pro)<sub>n</sub> sequences [19,20]. The earlier proposal of an 'active'  $\beta$ -sheet conformation [6] requires the conformational angles at residue 2 to have values of  $\phi_2 = -140^\circ$ ,  $\psi_2 = 140^\circ$ . In fact, the NMR relaxation analysis for FMLP suggest families of solution conformations with  $\phi_2 = -120^\circ$ ,  $\psi_2 = 161^\circ$  or  $\phi_2 = -135^\circ$ ,  $\psi_2 = 120^\circ$  [3]. These values are incompatible with the known stereochemical preferences of Aib residues [10-13]. Theoretical estimates suggest that the ideal antiparallel  $\beta$ -sheet conformation is 10 kcal · mol<sup>-1</sup> less stable than a helical conformation for Aib residues [11]. The observation of high biological activity for the Aib<sup>2</sup> and Cyl<sup>2</sup> analogs implies that extended structures may not be essential in mediating receptor interactions. The higher activity of the Cyl<sup>2</sup> analog as compared to the Aib<sup>2</sup> peptide may reflect favorable interactions between a bulky hydrocarbon side chain at residue 2 and a hydrophobic receptor site. The Aib<sup>2</sup> is

about 10-times, the Cyl<sup>2</sup> derivative 2.5-3-times, less active than FMLP. This suggests that a folded conformation although capable of giving high activity is not compatible with the optimal activity given by the extended conformation presumably present in FMLP. Alternatively, it is possible that the lower activities reflect the less than optimal side chain interactions of the Aib<sup>2</sup> and Cyl<sup>2</sup> derivatives with the receptor site compared to the corresponding side chain of FMLP.

It is interesting to note that the Phe NH in FMLP also has a relatively low  $d\delta/dT$  value, which suggests a measure of shielding from the solvent. The value is, however, not low enough to favor definitively involvement in an intramolecular hydrogen bond. The  $d\delta/dT$  values obtained for Phe NH in FMLP, in the present study, differ from those reported earlier (table 2). However, the mode of sample preparation in the earlier study was distinctly different, involving dissolving the peptide in aqueous media at pH 2 or 8, followed by lyophilization and redissolution in (CD<sub>3</sub>)<sub>2</sub>SO [3]. It is likely that FMLP does favor an extended conformation in (CD<sub>3</sub>)<sub>2</sub>SO, although populations of folded structures could be considered.

Our results establish that chemotactic peptide analogs which favor folded backbone conformations are biologically highly active. It would thus appear that the nature of the receptor bound conformation of the chemotactic peptide remains to be established. Further speculative hypotheses may stimulate additional experimental investigations.

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