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A HIGHLY ACTIVE CHEMOTACTIC PEPTIDE ANALOG INCORPORATING THE UNUSUAL RESIDUE 1-AMINOCYCLOHEXANECARBOXYLIC ACID AT POSITION 2

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SUMMARY: Analogs of chemotactic peptides (Formyl-Met-X-Phe-OMe) containing the stereochemically constrained residues α-aminoisobutyric acid (Aib), 1-aminocyclopentanecarboxylic acid (Acc^5) and 1-aminocyclohexanecarboxylic acid (Acc^6) at position 2 are compared with the parent sequence (X=Leu) for their ability to induce lysozyme release in rabbit neutrophils. The Acc^6 analog is about 78 times more active than the parent peptide, For-Met-Leu-Phe-OH, whereas Aib and Acc^5 analogs are approximately 3 and 2 times, respectively, less active than the parent peptide. NMR and model building studies clearly favour a Met-Acc^6 β-turn solution conformation in the Acc^6 analog, suggesting that the neutrophil receptor is capable of recognizing a folded peptide structure. The significant differences in the activities of the Acc^5 and Acc^6 analogs suggest an important role for the residue 2 sidechain in receptor interactions.

The tripeptide Formyl-methionyl-leucyl-phenylalanine (For-Met-Leu-Phe-OH)$^+$ induces neutrophil chemotaxis and release of lysosomal enzymes (1). These effects are undoubtedly mediated by specific interactions of the peptide with receptors on the neutrophil cell surface (1). Structure-activity correlations using a wide variety of chemotactic peptide analogs (1-3) have been interpreted in terms of extended β-sheet structures (4), as the 'biologically active' (receptor bound conformation) of the peptide (5). Recent studies using stereochemically constrained analogs, incorporating α-aminoisobutyric acid (Aib) or 1-aminocyclopentane-carboxylic acid (Acc^5, earlier referred to as cycloleucine, Cyl) however suggest that folded conformations are indeed biologically active (6). The folded analogs were found to be marginally less active than the parent peptide. This could be due to less effective receptor recognition of the folded backbone conformation or a consequence of less than optimal interactions of the residue 2 sidechain

Abbreviations: Acc^n, 1-aminocycloalkanecarboxylic acid residue, where n is the number of atoms in the cycloalkane ring; Aib, α -amino-isobutyric acid; Boc, t-butyloxycarbonyl; For, formyl; TEMPO, 2, 2, 6, 6-tetramethyl-piperidine-1-oxyl.

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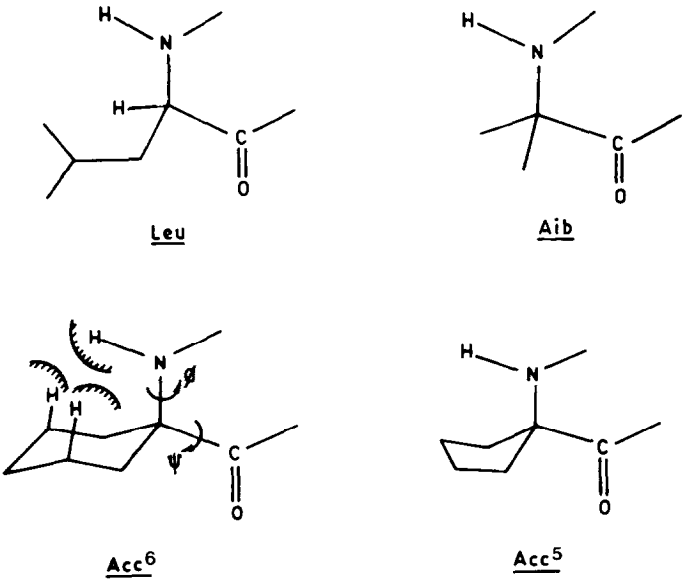

Figure 1 Structures of sidechains at position 2. Note 1, 3-diaxial interactions in Acc^6 restrict Ø values.

at the receptor site. In order to distinguish between these possibilities we examined the peptide For-Met-Acc^6-Phe-OMe, in which the residue 2 sidechain more closely approximates the Leu sidechain (Figure 1). This report establishes that the Acc^6 analog is significantly more active than the parent peptide and presents evidence for a folded β-turn solution conformation.

MATERIALS AND METHODS

1-aminocyclohexanecarboxylic acid (H-Acc^6-OH) was synthesized from cyclohexanone, using a procedure analogous to that described for α-aminoisobutyric acid (7). All peptides were synthesized by conventional racemization free procedures, using Boc and methyl ester groups for amino and carboxyl protection. Peptides were purified by column chromatography on silica gel and shown to be homogeneous by reverse phase HPLC on a Lichrosorb RP-18 column using methanol-water gradient elution (40-70% methanol in 30 min, flow rate 0.8 ml min^{-1}). The sample of For-Met-Acc^6-Phe-OMe was also purified by HPLC. The peptides were fully characterized by 270 MHz ^1H NMR. All NMR studies were carried out on a Varian FT-80A spectrometer or a Bruker WH-270 spectrometer at the Sophisticated Instruments Facility, Bangalore. Lysozyme release from cytochalasin B treated rabbit neutrophils was measured as described earlier (1).

RESULTS AND DISCUSSION

Table 1 summarizes the granule enzyme releasing activity of four chemotactic peptide analogs, For-Met-X-Phe-OMe (X = Leu 1, Aib 2, Acc^5 3 and Acc^6 4). The peptides 2 and 3 possess significant activity, whereas the Acc^6 analog 4 is approximately 12.5 fold more

Table 1

Lysozyme Releasing Activity and NH group NMR Parameters in Chemotactic Peptide Analogs Formyl-Met-X-Phe-OMe

Peptide	ED_{50}(M)[a]	Relative Activity [b]	NH NMR Parameters [b] Met NH		X - NH		Phe NH	
			dδ/dT	Δδ	dδ/dT	Δδ	dδ/dT	Δδ
X = Leu 1	$7.4 \pm 0.5 \times 10^{-11}$ (7)	6.2	6.2	1.38	5.1	0.92	4.3	1.36
X = Aib 2	$7.4 \pm 0.6 \times 10^{-10}$ (4)	0.32	5.0	1.65	4.7	1.1	3.5	0.93
X = Acc^5 3	$4.7 \pm 0.6 \times 10^{-10}$ (4)	0.51	5.2	2.15	4.7	1.55	3.2	0.65
X = Acc^6 4	$5.0 \pm 0.6 \times 10^{-12}$ (4)	7.8	5.3	2.2	4.8	1.47.	3.3	0.73

a) ED50 is the peptide concentration required to produce 50% of the maximum effect determined by the concentration-effect curve. Each value is the average ± SE. The figure in the parentheses is the number of independent observations.

b) Relative to For-Met-Leu-Phe-OH. The activities of all of the peptides were not measured at the same time and in the same experiments. To take account of possible differences in the sensitivity of the cells from experiment to experiment, the ED50 of the reference peptide For-Met-Leu-Phe-OH was determined. The mean ED50 ± S.E. of For-Met-Leu-Phe-OH determined in the same experiment as the given peptide methyl ester (figure in parenthesis following the ED50) are: $4.6 \pm 0.5 \times 10^{-10}$M (1); $2.4 \pm 0.5 \times 10^{-10}$ (2, 3); $3.9 \pm 0.7 \times 10^{-10}$M (4).

c) dδ/dT is the temperature coefficient (ppm/K x 10^3) measured in $(CD_3)_2$ SO. $\Delta\delta = \delta[(CD_3)_2SO] - \delta(CDCl_3)$ ppm.

active than the Leu analog 1. The peptide esters 1, 2, 3 have higher activity than the corresponding peptide acids (6, see also ref. 3). For-Met-Acc^6-Phe-OMe is the first tripeptide analog exhibiting substantially greater activity than the parent peptide, although enhanced activity has been very recently reported for tetrameric analogs having four peptide molecules covalently linked to a macrocyclic tetraazacycloalkane (8).

The high biological activity of For-Met-Acc^6-Phe-OMe provides a strong stimulus for determining its solution conformation. Solvent shielded or intramolecularly hydrogen bonded NH groups in the peptide were delineated by a comparison of the temperature dependence of chemical shifts in $(CD_3)SO$, solvent dependence of NH chemical shifts in $CDCl_3$-$(CD_3)_2SO$ mixtures and paramagnetic radical induced line broadening in $CDCl_3$ (9). The temperature coefficient ($d\delta/dT$) and solvent shift ($\Delta\delta$) values for the four peptides are compared in Table 1. It is clearly seen that the Met and Leu NH groups are significantly more sensitive to changes in solvent composition or temperature, as compared to the Phe NH group in peptides 2 - 4. In 1, all three NH groups show comparable sensitivity. A similar observation is made in the free radical induced line broadening experiment. Addition of 2, 2, 6, 6-tetramethylpiperidine-1-oxyl (TEMPO) to a $CDCl_3$ solution of 1 resulted in almost equal broadening of all three NH groups. On the contrary, in peptides 2 - 4 the order of line broadening is Met NH $\geqslant$ X NH >> Phe NH. The NMR results thus strongly suggest that the Phe NH is appreciably shielded from the solvent in peptides 2, 4. A detailed comparison of peptides 1 and 2 over a wide range of concentrations has led to a similar conclusion (10). It is therefore likely that the Phe NH is involved in an intramolecular hydrogen bond, since steric shielding of the NH group is unlikely in a small peptide. Supporting evidence for the presence of intramolecularly hydrogen bonded conformations comes from IR studies in $CHCl_3$, where an NH stretching band is seen at 3380-3390 cm^{-1} even in very dilute solutions.

An examination of molecular models and preliminary conformational energy calculations suggests that the Acc^6 residue is stereochemically restricted almost exclusively to the right or left handed helical ($\phi = \mp\ 60 \pm 20°$ $\psi = \mp\ 30° \pm 20°$) regions of the conformational map. This conformational preference is also shown by the Aib and Acc^5 residues (11). However, in the Acc^6 residue, 1-3 diaxial interactions between the axial substituent at C-1 and the axial hydrogens at C-3 and C-5 further restricts the

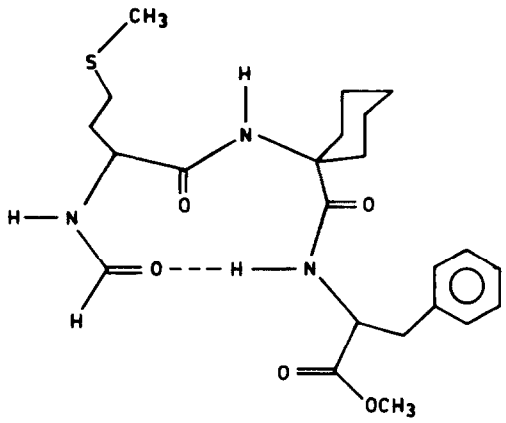

Figure 2. β-turn conformation proposed for For-Met-Acc^6 - Phe-OMe.

accessible values of ϕ. It should be noted that the amino group in Acc^6 necessarily adopts the axial position (12), a feature observed in two recent crystal structures of model Acc^6 peptides (13). In the crystal conformations of Boc-Aib-Acc^6-OMe and Boc-Aib-Acc^6-NHMe the conformational angles observed for the Acc^6 residue are $\phi = \pm 48°$ $\psi = \pm 42.6°$ and $\phi = \pm 68.4°$ $\psi = \pm 15°$, respectively (13).

These observations suggest that either a Type I or Type II-Met-Acc^6- β -turn conformation (Figure 2) is consistent with the NMR data for peptide 4 and the known stereochemical preferences for the Acc^6 residue. Although a distinction between these structures is not possible on the basis of the available data, the results provide compelling evidence for the high biological activity of folded, conformationally constrained chemotactic peptide analogs. The dramatic enhancement in activity observed by introduction of a single methylene group in the position 2 sidechain in the Acc^6 peptide as compared to the Acc^5 analog, strongly suggests that peptide-receptor interactions involving this site are an important determinant of biological effects.

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