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Conformational Investigations on Analogs of Inflammation Response Inducing Chemotactic Tri-peptide, fMLP

Abstract: Conformations of three analogs of for-L-Met-L-Leu-L-Phe-**OH** (fMLP), which initiates inflammatory response by interaction with formyl peptide receptor (FPR-1), have been investigated by the application of X-ray crystallographic technique. The investigated analogs of fMLP peptides are: for-L-Met-1-Amino-1-cyclooctane-carbonyl(Ac₈c)-L-Phe-OMe (**1**); for-L-Met-L-Leu-L-p-iodo-Phe-OH (**2**); and for-L-Met-Di-n-propylglycyl(Dpg)-L-Phe-OMe (**3**). Peptide backbone in **1** and **3** is constrained at position-2 of fMLP by the introduction of C^{α,α}-di-substituted glycines. In peptide **2**, Phe-OMe is substituted by p-iodo-Phe-OH. Crystal structures reveal an over-all folded conformation adopted by **1** and **2**. The former is folded in type-II β-turn, which is stabilized by an intramolecular 1←4 (formyl) C=O···H-N (Phe) hydrogen bond, whereas the latter is folded in an open-turn without any intramolecular hydrogen bond. On the other hand, peptide **3** has an extended conformation and two different molecules (**3a**, **3b**) in a crystallographic asymmetric unit form an anti-parallel β-sheet like structure. In **1** and **3**, residues Ac₈c and Dpg adopt left-handed helical and fully-extended (C₅) conformations, respectively. Cyclooctane ring in Ac₈c acquires boat-chair conformation. Crystal packing of **1** is characterized by the association of aliphatic-aromatic rings via C-H···π interaction. In the crystal of **2**, contrary to the usual observations, peptides are interlinked *via* networks of head-to-tail hydrogen bond and π···π interactions, which are generally observed to be mutually exclusive. The structure-function mechanism of ligand-receptor interaction is discussed.

Keywords: fMLP/fMLF analogs; **Formyl Peptide Receptor**; chemotaxis; inflammation; **aromatic** interactions; crystal structures; GPCR; X-ray crystallography

INTRODUCTION

Pathogenic microorganisms and mitochondria on metabolism produce for-L-Met-L-Leu-L-Phe-**OH** (fMLP or fMLF) and other formyl peptides. When formylated peptide binds to a glycoprotein receptor on the neutrophil surface, known as formyl peptide receptor (FPR-1), it induces an assembly of ligand-receptor-G protein complex. The formation of ternary complex triggers several intra-cellular signals through G-coupled protein pathway and series of biological actions such as: directed migration of cells (chemotaxis) along the concentration gradient of fMLP, to the site of injury, resulting in inflammation; superoxide anion (O_2^-) productions to kill pathogens; and enzyme secretion.^{1,2}

Numerous structure-function studies have been performed to elucidate receptor-bound conformation(s) of fMLP. The prime motivation of such studies is to understand the mechanism of chemo-attractant receptors and to design anti-inflammatory drugs. Towards this end, different approaches have been adopted, which can broadly be classified in two different categories: One is the receptor-based approach, which aims to understand the protein structure by biophysical techniques.³⁻⁵ The experimental efforts to determine structure of this G-protein coupled receptor (GPCR) of rhodopsin family (family-A)⁶ have not been fruitful yet. The elucidation of three-dimensional structure of Rhodopsin in recent times, however, has opened the gate for modeling other homologous receptors.⁷ With the application of homology modeling technique, partial models^{3,8} using automated procedure defined for GPCRs as well as complete three-dimensional structure (Rathore, private communication) have been developed for FPR-1. Various experimental studies performed on ligand-binding domain of FPR-1 and homologous receptors in conjunction with theoretical modeling studies provide an estimation of binding pocket defined by residues in the inner trans-membrane regions and possibly at extra-cellular loop, E2 (Fig. 1).

The other approach is directed towards structure-activity studies of peptide ligands. In this ligand-based examination, many different mutants of fMLP are designed, followed by structural examinations and test of biological activities. This methodology seeks to correlate ligand structure with its function and is intended to understand the structural requirement for ligand-binding. The studies performed by Toniolo, Freer, Becker, Balaram and coworkers, collectively reveal that an over-all folded or extended conformation of ligand is essential for high biological activities.^{2,9-22} In continuation of peptide structure-function study, in this ligand-based approach, the three different crystal structures of fMLP analogs (Fig. 2) and the relevance of their conformation with respect to the receptor-binding have been examined.

MATERIALS AND METHODS

Materials

Peptides **1** and **3** were obtained from Dr. S. Prasad and Prof. P. Balaram.¹² Compound **2** was purchased from *SIGMA Chemicals*, USA. Coordinates of previously reported fMLP analogs, which were used for comparisons, were collected from Cambridge Structural Database²³ or Journals. In some cases, they were derived from reported torsion-angle values. The coordinates of a polymorph crystal of **3** (numbered as **4**) were supplied by Prof. A. R. Dentino, New York.

X-ray Diffraction

All the three crystals were grown by slow evaporation of methanol-water mixtures. Data were collected on an *Enraf-Nonius CAD-4* diffractometer.²⁴ The over-all intensity variation during the data collection period was within 5%. Absorption and extinction corrections were applied only to the crystal **2**, which was severely affected by that. Initial trial structure of **1** was found by the random phase extension, using *MULTAN-87*.²⁵ Structure solutions of **2** and **3** were obtained by the application of *Patterson* and *direct phase determination* techniques, respectively, using *SHELX-86* program.²⁶ Partial fragments, obtained from structure solutions, were expanded and structures were refined by successive computation of difference electron density map using *SHELX-97*.²⁷ Anisotropic displacement parameters were added to the refinement gradually, starting first from the sulfur and iodine atoms. Due to the disorder, the phenyl rings in **3** were idealized to a regular hexagon. The statistical disorders, present in the structures were treated in the following manner:- The terminal methyl group attached to sulfur in **1** and **3** occupied two positions. The partial occupancies were refined as: 0.68(2), 0.32(2) in **1**; 0.63(7), 0.37(7) in **3a**; and 0.53(2), 0.47(2) in **3b**. Bond distances S^δ-C^ε were restrained to 1.79 Å in **3**. Additionally, in **1**, distance S^δ-C^γ and angle C^γ-S^δ-C^ε were restrained to 1.80 Å and 100.9°. ²⁸ The same isotropic displacement parameters were assigned to both the disordered positions of C^ε. The partial occupancies for disordered iodine, in **2**, were refined as 0.80(2), 0.20(2).

The function minimized during the refinement was $\Sigma w(F_o^2 - F_c^2)^2$, where $w = 1 / [\sigma^2(F_o^2) + (aP)^2 + bP]$ and $P = (\text{Max}(F_o^2, 0) + 2 F_c^2) / 3$. The parameters a and b were 0.0758, 0.0; 0.1292, 0.0; and 0.1200, 0.0, for **1**, **2** and **3**, respectively. Reflection (2, 2, 32) in **1** was omitted in the last stage of refinement since it was severely affected by extinction or absorption. The H-atoms were refined with fixed geometry, riding on their carrier atoms with U_{iso} set at 1.2 (1.5 for the methyl H-atoms) U_{eq} of the parent atom. Crystal data and refinement parameters are given in Table I. All calculations, analyses and diagrams were made with *Win-Gx*²⁹ and *INSIGHT-II*³⁰ programs. Atomic numbering scheme and torsion angle conventions follow the *IUPAC-IUB* recommendations.³¹ The geometrical parameters agree well with the average values reported for peptides.²⁸ Crystallographic data have been deposited at the Cambridge Crystallographic Data Center with entry numbers, CCDC210161(**for 1**), CCDC210162(**for 3**) and CCDC210163(**for 2**).

RESULTS

Peptide Conformation

Conformational angles and hydrogen bond parameters are described in Table II and III. Peptides **1** and **2** adopt conformations which can be described as folded, while **3** assumes an extended conformation (Fig. 3).

Folded Conformation of Peptide 1 and 2: Peptide **1** is folded in type-II β -turn. The β -turn is stabilized by an intramolecular 1 $\leftarrow$ 4 hydrogen bond between phenyl-NH and formyl-oxygen (Fig. 3a). Central residue Ac₈c adopts the lowest energy left-handed helical conformation.³⁵ Met and Phe on either side are partially-extended. Cyclooctane ring has close to ideal boat-chair conformation^{35,36} (Fig. 3c). The asymmetric parameter, ΔC_s , about the mirror plane passing through C2B2-C2D1, is 2.02°. The Cremer and Pople puckering parameters of cyclooctane ring,³⁷ are following: puckering amplitudes: $q_2 = 0.991(9)\text{\AA}$, $q_3 = 0.609(10)\text{\AA}$, $q_4 = -0.358(9)\text{\AA}$; phase angles: $\Phi_2 = -88.1(5)^\circ$, $\Phi_3 = -46.6(8)^\circ$; polar angles, $\theta_2 = 58.4(5)^\circ$, $\theta_3 = 120.5(6)^\circ$; and total puckering amplitude $Q = 1.217(9)\text{\AA}$.

The over-all conformation of **2** is also described as folded with central Leu residue adopts right-handed helical conformation (Fig. 3b). However in contrast to **1**, the molecular structure is not stabilized by any intramolecular hydrogen bond, similar to the one observed in the crystal structure of parent peptide, fMLP-OMe.¹⁴ This is because flanking residues, Met and p-iodo-Phe assume nearly fully-extended conformations. The r.m.s.d. of backbone atoms between **2** and fMLP-OMe is 0.17Å.

Extended Conformation of 3: The structure of peptide **3**, as opposed to **1** and **2**, is described by an extended anti-parallel β -sheet conformation. There are two different conformers, **3a** and **3b**, in the crystallographic asymmetric unit. The r.m.s.d. for all but disordered methyl-atoms, between molecules **3a** and **3b**, is 0.29 Å. These molecules are joined together via (Met of **3b**) C=O $\cdots$ H-N (Met of **3a**) and (Met of **3a**) C=O $\cdots$ H-N (Met of **3b**) hydrogen bonds, forming an anti-parallel β -sheet (Fig. 3d). However, unlike canonical β -sheet structure, here the two strands are connected by Met residues only. The central Dpg residues in both the conformers adopt fully-extended conformation. Met residues adopt (ϕ , ψ) values of anti-parallel β -sheet conformation with maximum deviation of $\sim 20^\circ$ from the standard values (-139° , 135°). The extended backbone structure of peptide is distorted at C-terminus since the Phe residues adopt left-handed helical conformation. Crystal structure conformations of **1** and **3** are similar to the solution state conformation examined earlier by NMR.^{12,13}

The fully-extended conformations of p-iodo-Phe and Met in **2**, and Dpg residues in **3**, form planar C₅ structures (Fig. 3b, 3d and Table II, III). The C₅ conformation is characterized by torsion angle ($\pm 180 \pm 20^\circ$, $\pm 180 \pm 20^\circ$) and C=O of the same residue

hydrogen-bonded to H-N, forming a planar pentagonal ring containing H, N, C^α, C and O atoms.³⁸⁻⁴¹ The endocyclic $\tau(\text{N-C}^\alpha\text{-C})$ angle in C₅ conformation, which is generally smaller than the ideal tetrahedral value, are 104.4(6)°, 104.7(6)°, 107.0(6)° and 108.0(6)°, in Dpg (**3a**), Dpg(**3b**), Met (**2**) and p-iodo-Phe (**2**), respectively. The average computational and experimental values of τ -angle have been reported to be 100° and 103.2°.^{40,42} The crystal structure of molecule **3** has been reported earlier in different space group, I222 (**4**).¹³ Conformations of **3** and **4** are similar *i.e.* anti-parallel β -sheet structure except notable differences in Met- ψ and Phe- ϕ angles at N- and C- termini. The r.m.s.d. of C^α-atoms between molecules (**3a**, **4**) and (**3b**, **4**) are 0.03 and 0.02 Å, respectively.

Intermolecular Interactions

The parameters characterizing hydrogen bond and aromatic interactions are described in Table III and IV.

Peptide 1: C-H $\cdots\pi$ Interaction: The crystal packing of **1** is characterized by clustering of aromatic-aliphatic rings (Fig. 4a). These rings, as generally observed in this type of associations, are joined together by C-H $\cdots\pi$ interactions.^{43,44} The contact is formed by, C2E-H2E2 $\cdots\pi$ (Phe of x, **y-1**, z).

Peptide 2: Networks of Head-to-tail Hydrogen-bond and $\pi\cdots\pi$ Interactions: The packing of **2** is the most spectacular among all. Here, the peptide units are linked head-to-tail along crystal b-direction by a strong, (formyl)C=O $\cdots$ H-OT(p-iodo-Phe) hydrogen bond (Fig. 4b). The O-H $\cdots$ O contact is remarkably very short (1.76 Å). The head-to-tail arrangement is of *zigzag* type, as previously classified.⁴⁵ Additionally, these molecules are linked together by $\pi(\text{p-iodo-Phe})\cdots\pi(\text{p-iodo-Phe})$ interactions,^{46,47} giving rise to a network of $\pi\cdots\pi$ interactions **similar to herring-bone type** along a-axis^{46,48} (Fig. 4c). The calculated parameters, characterizing $\pi\cdots\pi$ interactions are provided in Table IV. The coexisting networks of (i) strong head-to-tail hydrogen bonds along b-axis and (ii) **aromatic $\pi\cdots\pi$** interactions along a-axis is an exceptional feature since the hydrogen bond and $\pi\cdots\pi$ interactions have frequently been observed to have insulating nature *i.e.*, presence of one tend to exclude the other.^{46,48}

Peptide 3: Three-center Hydrogen Bond: In the molecular assembly of **3**, peptides **3a** and **3b** are related by *pseudo* 2-fold symmetry. The fully-extended propyl chains ($\chi^2\sim 180^\circ$) of Dpg, which are symmetrically disposed on either side of the peptide, form planar side chain groups. The amino-groups of Dpg residues are involved in three-center hydrogen bonds. The three-center interactions are formed with the carbonyl group of (i) the same residue (in C₅-ring structure), and (ii) Phe of symmetry related molecule (Fig. 4d and Table III). The three-center configurations are planar,⁴⁶ and the sum of angles about proton (N-H) is 353.7° and 354.2°, in **3a** and **3b**, respectively.

DISCUSSION

Biological Implications of Crystal Structure Conformations

The introduction of constraints in the peptide-backbone to dictate the desired range of conformation, in *de novo* design strategy, has been quite successful for protein design. This methodology has also been employed for the development of peptide ligands as new and improved pharmaceutical agents.^{38,39,49,50} In peptide **1** and **3**, linear and cyclic constraints³⁹ at the central position of fMLP-OMe have been introduced, while **2** has halogenated phenyl substituent at position-3. Theoretical and experimental studies have revealed conformational preferences of many constrained residues.^{38,39} In particular, Ac₈c residue adopts the lowest energy left- or right-handed helical conformation.^{35,36} On the other hand, both the helical and extended conformations of Dpg, corresponding to the minima of comparable energy,^{34,38,39,41} have been observed. The folded and extended conformations of **1** and **3** were realized in conformity with the folding propensities of Ac₈c and Dpg residues. Peptide **2**, similar to the parent fMLP-OMe,¹⁴ is also in folded conformation. In small peptides, the over-all folded and extended conformations, in addition to the backbone (ϕ , ψ) values, are unambiguously defined by C $^{\alpha}$ -C $^{\alpha}$ distances between the first and the last residues.⁵¹ The C $^{\alpha}$ (1)-C $^{\alpha}$ (3) distances, in **1**, **2**, **3a** and **3b** are 5.6Å, 5.3Å, 7.1Å and 7.2Å, respectively, as compared to 5.3-5.6Å in α /3₁₀-helices and 6.7-7.2Å in β -strands. These peptides were selected to probe the effect of different steric and hydrophobic character of side chains on peptide function. Peptide **1** and **3** exhibit high activity, similar to the parent peptide in inducing enzyme secretion.^{12,13} With the exception of hydrophobicity as proposed earlier,^{2,19} the less stringent steric and conformational requirements at position-2 of fMLP, in agreement with the previous findings (Table V), are evident from the present study.

An Analysis on Conformational Studies of fMLP Analogs

The discovery of formyl peptides as chemo-attractants for neutrophils by Schiffmann *et al.* (1975),^{21,22} led to series of investigations and tri-peptide fMLP emerged as prototypic chemotactic peptide. The formylated peptide can be viewed as composed of 5-different functional groups as previously classified,² namely, N-term formyl group, Met side chain with a region of positive charge around electron-rich sulfur, hydrophobic Leu and Phe side chains, and carbonyl group at C-terminus. To probe the structural requirement, these groups were mutated with residues having different steric, charge and hydrophobic groups, and constraints were introduced to achieve the desired conformation. A large number of analogs of fMLP have been designed and synthesized, examined for their biological activities and quite a few have also been studied for their conformation using X-ray crystallography and NMR techniques.^{2,9-22,52-95} Results of conformational and activity studies on these peptides are summarized in Table V. The structures of biologically active peptides can broadly be classified in two different categories, *i.e.*, folded and extended conformations, based on C $^{\alpha}$ (1)-C $^{\alpha}$ (3) distances. Interestingly, several peptide analogs with different side groups and

residue lengths have demonstrated significant biological activities. As elucidated by homology modeling, the binding pocket, which resides in the inner trans-membrane region, has an approximate dimension of 15Å×10Å×10Å in length, breadth and depth (Rathore, private communication). This is an estimate of the volume, available to the peptide fMLP, during the “inactive state” of the receptor. The cavity dimension suggests that up to pentapeptide can be easily accommodated in this pocket and this could possibly rationalize the findings which indicate substantial activity for many peptide analogs with 3-5 residue lengths.

The data presented in the table suggest that the folded conformations of formyl peptides are largely populated with the β -turn, whereas for extended structures, the anti-parallel β -sheet conformation with twisted backbone of peptide at C-terminus is more common. The stereo diagrams of differences among analog structures, clustered in two aforementioned categories, are shown in Fig. 5. Evidently, the peptide backbone is more converged in the folded category. The large number of rotamers observed at Met- ψ and Phe- ϕ angles, at N- and C- termini, indicate the possibility of conformational transition in the peptide after receptor binding. The very few reported structures of inactive compounds however have limited the scope of such analysis.

Receptor-bound Conformation: Folded or Extended?

Although experimental studies clearly demonstrate folded and extended conformation required for biological activity, the exact receptor-bound conformation remains unanswered. To delineate receptor-bound conformation i.e. folded vs. extended, many explanations were sought.^{2,9-22} Computational energetic analysis^{16,96} and dynamics studies⁹¹ on analogs as well as experimental work on Bence-Jonce dimer⁹⁷ suggest folded structure of peptide as receptor-bound, on the other hand, the early work by Freer *et al.*,¹⁹ favors for extended conformation. To reconcile the existing dilemma, the hypothesis of multiple binding sites at the receptor and induced-fit mechanism have been proposed.^{2,12} The model structure of FPR-1 does not support the assumption of multiple binding sites at the receptor. The conformational transition in the ligand-receptor complex is arguably a more likely event, which is also prominent among other GPCR-proteins.

SUMMARY AND CONCLUSION

In conclusion, the structures of three analogs of fMLP, mutated at position-2 and 3 have been characterized in this report. By introduction of constraints, the folded conformation of **1** and extended conformation of **3** were realized. Peptide **2** is in folded conformation similar to the parent peptide. Peptide **1** and **3**, with higher steric and hydrophobic bulk at position-2, in two different conformations evoke similar biological response. The increased flexibility of fMLP at this position should prove useful for the development of new peptide ligands. In order to delineate the receptor-bound conformation of ligand, a survey of previous structure-function investigations on fMLP-analogs has been presented. Argument in support of induced-fit explanation has been emphasized. It will be of particular interest to study the structure of ligand-receptor complex using modeling approaches and studies in this direction are in progress. Coexistence of strong hydrogen bond and aromatic interactions in the crystal of **2** is an interesting feature, which has implications for supramolecular assembly and design.

The present work had formed the part of author's PhD thesis. I am grateful to Prof. N. Shamala for guidance, and allowing this work to publish, and Prof. P. Balam for support. The fellowship of University Grants Commission, India is acknowledged. Thanks to Dr. S. Prasad who provided two peptide samples, Prof. A. R. Dentino supplied coordinates of a peptide (**4**), Mr. T. Narasimhamurthy provided computational resources and Dr. Stefan Suresh shared a data reduction program. I am grateful to all the referees for thorough reading of the manuscript and valuable suggestions.

Table I

Crystallization, data collection and structure refinement details

	1	2	3
Crystal Data			
Empirical formula	C ₂₅ H ₃₇ N ₃ O ₅ S ₁	C ₂₁ H ₃₀ I ₁ N ₃ O ₅ S ₁	C ₂₄ H ₃₇ N ₃ O ₅ S ₁
Molecular weight	491.64	563.44	479.63
Morphology	colorless, needle	colorless, thin-needle	colorless, thin-plate
Crystallizing solvent	methanol/water	methanol/water	methanol/water
Crystal size (mm)	0.74 x 0.08 x 0.06	1.82 x 0.05 x 0.05	1.0 x 0.44 x 0.1
Cell Parameters			
a(Å)	5.822(3)	5.461(2)	13.116(2)
b(Å)	13.559(2)	19.028(3)	10.062(3)
c(Å)	35.221(6)	23.775(3)	21.894(5)
β(°)			98.01(2)
Volume(Å ³)	2780(2)	2471(1)	2861(1)
Cell determination from reflections	25	25	25 ^a
θ-range (°)	7-34	10-30	10-20
Z	4	4	4
Crystal system	orthorhombic	orthorhombic	monoclinic
Space group	P2 ₁ 2 ₁ 2 ₁	P2 ₁ 2 ₁ 2 ₁	P2 ₁
Molecule/asym. unit	1	1	2
Density(cal.) (g/cm ³)	1.174	1.515	1.113
μ (mm ⁻¹)	1.335	11.272	1.285
Absorption correction	none	psi-scan	none
F(000)	1056	1144	1032
Data Collection			
Radiation	CuK _α (λ=1.5418 Å)	CuK _α (λ=1.5418 Å)	CuK _α (λ=1.5418 Å)
Temperature (°K)	293(2)	293(2)	293(2)
θ-range (°)	2.5 – 58.0	2.97 – 60.0	2.0 - 60.0
Index ranges	0<=h<=6	0<=h<=5	-14<=h<=14
	0<=k<=14	0<=k<=21	-10<=k<=11
	0<=l<=38	0<=l<=26	-8<=l<=24
Scan type	ω-2θ	ω-2θ	ω-2θ
Scan speed	variable	variable	variable
Independent reflections	2293	2132	4457
Observed [F > 4 σ (F)]	1047	1814	2063
Refinement			
Final R (%)	0.0543	0.0627	0.0843
wR2_obs(%)	0.1252	0.1533	0.2019
wR2_all(%)	0.1472	0.1596	0.2334
Goodness-of-fit (S)	0.926	1.092	1.201
Absolute structure parameter	-.07(10)	.03(1)	-.02(11)
(Δ/σ) _{max}	0.017	0.006	0.014
Δρ _{max} and Δρ _{min} (e Å ⁻³)	0.27 and -0.18	.99 and -1.61	.29 and -.40
Data/restraints/ parameter ratio	2293 /2/306	2132 / 0/291	4457 /11/ 579

^a The presence of polymorph crystal was established by *Oscillation/ Weissenberg* photographs.

Table II
Relevant torsion angles(°) ^a

Residue	Backbone			Side chain			
	ϕ	ψ	ω	χ^1	χ^2	χ^3	
1							
Met	-55.2(9)	129.5(6)	174.2(6)	-178.0(8)	177.9(6)	89(1) ^d	
Ac ₈ c	56.4(8)	35.9(7)	171.8(5)	-73.4(7), -174.8(6)	-	-	
Phe	-63.8(7)	138.4(6) ^b	177.1(6) ^c	175.6(6)	70.1(9), -108.3(8)	-	
2							
Met	-148.7(8)	162.3(6)	169.2(6)	-61(1)	-165.1(6)	-60.1(9)	
Leu	-63.8(8)	-43.4(8)	-177.6(6)	-62.3(9)	-64(1), 172.7(9)	-	
p-iodo-Phe	-171.5(6)	174.4(7) ^b	-	42.7(9)	67(1), -113.5(9)	-	
3							
Met	3a	-135(1)	155.1(8)	175.1(9)	-64(1)	176(1)	-29(4) ^d
	3b	-149(1)	152.6(9)	173.8(9)	-70(1)	-174.0(9)	-76(2) ^d
Dpg	3a	-172.8(8)	177.2(7)	-178.5(7)	57(1), -57(1)	180(1), 175.6(9)	-
	3b	-177.5(9)	-175.5(8)	-179.8(8)	54(1), -56(1)	179(1), 175(1)	-
Phe	3a	49(1)	52.0(9) ^b	178(1) ^c	-55(1)	-40(1), 137.5(7)	-
	3b	54(1)	52(1) ^b	176(1) ^c	-58(1)	-37(1), 141.8(8)	-

^aFormyl-amido bond is described by torsion angle, ω_0 (H0-C0-N1-C1A): -175.1(7)° in **1**, 173.3(8)° in **2**, -171.9(9)° and -178.9(9)° in **3**. Side chain conformations of Met, Leu, Phe and Dpg agree well with the low-energy rotamer values, commonly observed in peptides and proteins.³²⁻³⁴ The predominantly observed (g⁻, t) conformation³⁵ in Ac_gc is also observed in the present structure.

^b ψ_{3T} = N3 - C3A - C3 - O32;

^c ω_{3T} = C3A - C3 - O32 - C32;

^dTorsion angles correspond to the higher occupancy position of terminal methyl group in Met.

Table III
Parameters for possible hydrogen bond and C-H... π interactions

Type	Donor Residue	Atom	Acceptor Residue	Atom	D...A (Å)	H...A (Å)	D-H...A (°)
1							
<i>Intramolecular</i> 1 $\leftarrow$ 4	Phe	N3	For	O0	3.140(7)	2.396	145.0(4)
<i>Intermolecular</i>	Met	N1	Met	O1 ^a	2.956(7)	2.137	159.2(4)
	Ac ₈ c	N2	Ac ₈ c	O2 ^b	2.884(7)	2.060	160.1(4)
	Phe	C3A	For	O0 ^c	3.325(8)	2.345	178.8(4)
C-H... π	Ac ₈ c	C2E	Phe	π ^g	4.03(1)	3.21	145.4(5)
2							
<i>Intramolecular</i> Fully-extended (C ₅)	Met	N1	Met	O1	2.641(9)	2.287	104.8(5)
	p-iodo-Phe	N3	p-iodo-Phe	O31	2.646(9)	2.258	107.4(4)
<i>Intermolecular</i>	Leu	N2	Leu	O2 ^c	2.820(8)	2.059	147.1(4)
	Met	C1A	Met	O1 ^c	3.287(9)	2.406	149.2(5)
Head-to-tail	p-iodo-Phe	O32	For	O0 ^e	2.56(1)	1.76	165.0(5)
3							
<i>Intramolecular</i> Fully-extended (C ₅)	Dpg	N2(3a)	Dpg	O2(3a)	2.60(1)	2.16	111.2(5)
	Dpg	N2(3b)	Dpg	O2(3b)	2.60(1)	2.17	110.3(5)
<i>Intermolecular</i> Anti-parallel β -sheet	Met	N1(3a)	Met	O1(3b)	2.83(1)	2.06	147.2(6)
	Met	N1(3b)	Met	O1(3a)	2.87(1)	2.10	149.0(5)
Others	Dpg	N2(3a)	Phe	O31(3a) ^f	3.06(1)	2.26	154.6(5)
	Met	C1A(3a)	Phe	O31(3a) ^f	3.26(1)	2.42	143.5(6)
	Phe	N3(3a)	For	O0(3a) ^g	2.80(1)	1.99	157.3(5)
	Dpg	N2(3b)	Phe	O31(3b) ^h	3.07(1)	2.27	154.9(5)
	Met	C1A(3b)	Phe	O31(3b) ^h	3.25(1)	2.38	147.5(7)
	Phe	N3(3b)	For	O0(3b) ^d	2.75(1)	1.91	163.6(5)

Symmetry codes: ^ax+1/2, -y+1/2, -z+2; ^bx+1, y, z; ^cx-1, y, z; ^dx, y+1, z; ^e-x, y-1/2, -z+3/2; ^f-x+2, y+1/2, -z+1; ^gx, y-1, z; ^h-x+1, y-1/2, -z+2.

Table IV
Geometrical parameters characterizing possible $\pi\cdots\pi$ interactions in **2**

Ring-1	Ring-2	Center-to-center distance (Å)	Inter-planar angle (°)	Minimum distance of approach (Å)
π (p-iodo-Phe)	π (p-iodo-Phe) ^a	5.087(6)	66.7	3.78(1)
π (p-iodo-Phe)	π (p-iodo-Phe) ^b	5.461(7)	0.0	3.55(1)

Symmetry codes: ^ax+1/2, -y-1/2, -z+2; ^bx+1, y, z.

Table V Conformations and biological activities of analogs of fMLP tri-peptide.^a The design of majority of the enlisted analogs follow the existing paradigm that N-formyl methionyl at position-1 and phenylalanyl at position-3 are crucial for optimal activity, whereas alteration to the leucyl at position-2 is well tolerated.^{2, 19,20}

N-formyl tri-peptide analogs ^b	Peptide conformations		Biological activity ^c	References
	Over-all conformation	Backbone (ϕ_n, ψ_n) ^d (°)		
Folded ^e				
for-Met-Leu-Phe-OMe (parent)	folded	(-146, 151), (-68, -49) (-155, 174)	active	Gavuzzo <i>et al.</i> [14]
for-Met-Leu-p-iodo-Phe-OH	folded	(-149, 162), (-64, -43), (-172, 174)	nt	Present work
for-Met-Ac ₅ c-Phe-OMe	β -turn (type-II)	(-55, 130), (56, 36), (-64, 138)	active ^f	Present work
for-Met- Δ^7 Leu-Phe-OMe	β -turn (type-II)	(-55, 130), (72, 14) (-50, 137)	active	Pagani Zecchini <i>et al.</i> [52]
for-Thp-Ac ₆ c-Phe-OMe	folded	(-63, -34), (52, 51) , (-63, 149)	active	Torrini <i>et al.</i> [53]
for-Thp-Leu-Ain-OMe	folded	(63, 31), (-66, -48), (54, 40)	active	Torrini <i>et al.</i> [54]
for-Thp-Leu- Δ^7 Phe-OMe	β -turn (type-I)	(-53, -33), (-74, -10), (64, -170)	in-active	Torrini <i>et al.</i> [55]
Boc-Met-Ac ₅ c-Phe-OMe	β -turn (type-II)	(-53, 140), (64, 32) , (-59, 132)	antagonist	Toniolo <i>et al.</i> [15]
Boc-Met-Aib-Phe-OMe	β -turn (type-II)	(-52, 140), (58, 37), (-59, 137)	antagonist	Bardi <i>et al.</i> [56]
for-Met-Aib-Phe-OH	folded	nmr	active	Toniolo/ Iqbal <i>et al.</i> [15,18]
for-Met-Adt-Phe-OMe	β -turn	nmr	active	Morera <i>et al.</i> [57]
for-Met-azaPro-Phe-OMe	folded	nmr	active	Pagani Zecchini <i>et al.</i> [58]
for-Met-(Ac ₃ c to Ac ₇ c)-Phe-OMe	β -turn	nmr	active	Spisani/Prasad/Toniolo/ Sukumar <i>et al.</i> [10,12,15,59]
for-Met-(Ac ₉ c to Ac ₁₂ c)-Phe-OMe	β -turn	nmr	active	Spisani <i>et al.</i> [10]
Extended ^e				
for-Met-Dpg-Phe-OMe Mol A	anti-parallel β -sheet	(-135,155), (-173,177), (49,52)	active ^f	Present Work
Mol B		(-149,153), (-178,-176), (54,52)		
for-Met-Leu-D-(α Me)Phe-OMe	extended	(-105, 136), (-141, 111), (45, 49)	nt	Toniolo <i>et al.</i> [60]
for-Met-Leu-(α Me)Phe-OMe	extended	(-139, 126), (-107, 122), (-47, -49)	active	Toniolo <i>et al.</i> [61]
for-Met-Dmt-Phe-OMe	extended	(-162, 155), (-179, 172), (-74, -32)	active	Torrini <i>et al.</i> [11]
for-Met-Leu- Δ^7 Phe-Phe-OMe	extended	(57,45),(-81,161),(65,33),(-142,148)	active	Torrini <i>et al.</i> [62]
for-Hse(Me)-Leu-Phe-OMe	anti-parallel β -sheet	(-144, 122), (-93, 117), (-95, 29)	active	Torrini <i>et al.</i> [63]
for-Met-Leu-Phe-OtBu	anti-parallel β -sheet	(-128, 118), (-99, 114), (-91, -176)	nt	Michel <i>et al.</i> [64]
for-Met ⁺ -Leu-Phe-OMe	anti-parallel β -sheet	(-137, 105), (-83, 160), (-141, 174)	nt	Michel <i>et al.</i> [64]
for-Met-Dpg-Phe-OMe	anti-parallel β -sheet	(-76, -30), (173,179), (-97,34)	active	Dentino <i>et al.</i> [13]
for-Met-Leu-Ain-OMe Mol A	extended	(-115, 130), (-127, 125), (61, 27)	active	Gavuzzo <i>et al.</i> [65]
Mol B		(-137, 145), (-132, 111), (-62, -33)		
for-Met-Leu-D[sic]-Phe-OH ^g	extended	(-157,156), (-103,129), (-127,-179)	nt	Morffew <i>et al.</i> [66]
for-Met-Leu-Phe-OH	anti-parallel β -sheet	nmr	active	Becker <i>et al.</i> [67]
for-Met-Leu- Δ^7 Phe-OMe	extended	nmr	active	Chauhan <i>et al.</i> [68]
for-(S,R)-HmMet-Leu-Phe-OMe	extended	nmr	active	Witkowska <i>et al.</i> [9]
for-Met-(Deg, Dbg)-Phe-OMe	extended	nmr	active	Prasad <i>et al.</i> [12]
for-Met(so)-Leu-Phe-OMe	extended	nmr	active	Toniolo <i>et al.</i> [69]
for-Met-(γ -lactam)-Phe-OMe	extended	nmr	active	Pagani Zecchini <i>et al.</i> [58]
for-Dmt-Leu-Phe-OMe	extended	nmr	in-active	Torrini <i>et al.</i> [11]

^aConformational parameters for N-formyl di-peptides have been compiled and presented by Srikrishnan *et al.*⁷⁰ Couple of cyclic analogs of fMLP were also studied. Conformation of an antagonist, cyclo(Boc-Lys-Phe) has extended β -sheet conformation at Lys and Phe. The another cyclic analogue cyclo(for-Met-Lys-Phe) is a biologically active.⁷¹ Studies on analogs modified by 2-azetidinecarboxylic acid, 2-piperidinecarboxylic acid and norvaline at position-2, and cyclohexylalanine at position-3, using IR/UV method, suggest folded conformations of these analogs with significant biological activities.^{72, 73} The retroisomer of fMLP i.e., CHO-Phe-Leu-Met-NH₂ and its D-analogs were found to be 100 to 10,000 times less active.⁷⁴ Many other formyl peptides were also examined recently, for biological activities;⁷⁵⁻⁹⁵

^bAbbreviations (alphabetically):- Ac_nc: 1-Amino cycloalkane-1-carboxylic acid (n, number of carbon atoms in the cycloalkane ring)/ Adt: 4-Amino-1,2-dithiolane-4-carboxylic acid/ Aib: α -Aminoisobutyric acid/ Ain: 2-Aminoindane-2-carboxylic acid/ Δ^7 Phe: Z-dehydrophenylalanine/ Δ^7 Leu: Z-dehydroleucine/ Dmt: 2-[2'-(Methylthio)ethyl] methionine/ Hse: Homo serine/ Met⁺-Leu: Met⁺[CSNH]-Leu/ HmMet: C^α-hydroxymethyl methionine/ Met(so): Oxidized sulphur derivatives (diastereomeric sulfoxides and the sulphone)/ nt: Biological activities not tested/ nmr: Conformation determined using nmr (and also supported by allied spectroscopic methods)/ Thp: 4-Aminotetrahydro thiopyran-4-carboxylic acid;

^cThe term "active" refers to any of the experimentally detected biological activities of fMLP analogs, namely, chemotactic/superoxide anion production/enzyme secretion, and observed activities either high or comparable to the parent peptide or statistically significant;

^dThe conformational parameters provided in column-3 are those which are derived from X-ray crystallographic studies. Accompanying NMR studies, which were done for some of the peptides, were comparable within the same category i.e. folded or extended;

^eThe classification folded and extended is based on C^α(1)-C^α(3) distances. However, in terms of torsion angles, the term, folded and extended refers only to the first two residues in the sequence;

^fThe activity of these peptides were studied by Prasad *et al.*;¹²

^gStructure reported for LLD isomer instead of LLL isomer.

FIGURE 1

Schematic diagram of Formyl peptide receptor (FPR-1). The cartoon diagram is based on a homology model (Rathore, private communication) using the four different crystal structures, available for *Bovine* Rhodopsin⁷ in Protein Data Bank. Helices are numbered from N- to C-terminus and putative binding-pocket residues are represented with small circles. The straight cylinders represent trans-membrane helices (although GPCR-specific tilts and kinks are present) and curved lines represent loops. The binding pocket is formed at inner trans-membrane zone. It is not clear if the cavity is covered by the extra-cellular loop, E2, which has been observed in Rhodopsin structure. This topology also applies to all other GPCR structures with the exception of displayed cavity residues of FPR-1.

FIGURE 2

Chemical structure of fMLP-OMe and its analogs. In **1** and **3**, Leu is substituted by Ac₈c and Dpg, respectively, whereas in **2**, Phe is replaced by p-iodo-Phe and C-terminus is OH.

FIGURE 3

Perspective views of molecular conformations with the adopted *IUPAC-IUB* peptide nomenclature scheme of atoms.³¹ Dashed lines indicate hydrogen bonds. The **statistically** disordered atoms, i.e., iodine, and methyl group attached to sulfur correspond to the higher occupancy position. Folded conformations of peptides:- (a) **1** (type-II β -turn), and (b) **2** (an open-turn); (c) Boat-chair conformation of cyclooctane ring in Ac₈c; (d) Dimers of **3**, in the asymmetric unit, forming an anti-parallel β -sheet.

FIGURE 4

Crystal packing diagrams. The dashed lines represent hydrogen bonds and dotted ones indicate aromatic C-H $\cdots\pi$ and $\pi\cdots\pi$ interactions. (a) Crystal packing of **1**, illustrating the association of aromatic-aliphatic rings through C-H $\cdots\pi$ interactions; (b) Head-to-tail hydrogen bond of *zig-zag* type and $\pi\cdots\pi$ interactions in the packing of **2**; (c) A network of $\pi\cdots\pi$ interactions **similar to herring-bone type**, observed in **2**. Only aromatic rings have been shown for clarity; and (d) Crystal packing in **3**. The molecular identity is shown with letters A (for **3a**) and B (for **3b**) at phenyl ring.

FIGURE 5

Stereo view of superposition of structures of fMLP-analogs. (a) Folded structures. The $C^{\alpha}(1)-C^{\alpha}(3)$ distances varies from 5.3Å to 5.7Å among folded conformers. The r.m.s.d. of C^{α} -atoms is in 0.01-0.16Å range. (b) Extended structures. The $C^{\alpha}(1)-C^{\alpha}(3)$ distances are in 6.2-7.2Å range and r.m.s.d. of C^{α} -atoms amongst extended structures varies from 0.02 Å to 0.60Å.

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