COMPARATIVE CONFORMATIONAL STUDY OF CHEMOTACTIC PEPTIDES formyl-Met-Leu-Phe-OMe AND formyl-Met-Acc5-Phe-OMe.

Youssef Wazady
Laboratoire de recherche, Ecole Supérieur de Technologie, BP 8012 Oasis, Route d'El Jadida, Km 7, Casablanca-Maroc

Chakib Ameziane Hassani
Département de chimie, faculté des sciences et techniques Fes sais, Université Sidi Mohamed Ben Abdellah BP 2202, Fes-Maroc

Mahjoub Lakhdar, Aziz Ezzamarty
Département de chimie, Faculté des Sciences BP 5366 Maârif / Casablanca-Maroc

Received 16-05-2001

Abstract

The chemotactic peptides formyl-Met-Leu-Phe-OMe and formyl-Met-Acc5-Phe-OMe (Acc5 is the α-α disubstituted amino acid l-aminocyclopentane-1-carboxylic acid) were studied by the theoretical method PEPSEA in order to investigate the proper peptide backbone conformation that is biologically active. This study shows that the parent peptide formyl-Met-Leu-Phe-OMe has a flexible structure, and that the other conformationally constrained peptide has a tendency to form the β turn structure. It also gives evidence against the hypothesis proposing the importance of formyl group in the interaction with the receptor.

Introduction

Chemotaxis is defined as a reaction by which the direction of locomotion of cells is determined by substances, called chemotactic agents, in their environment. These substances of different origin are responsible for the accumulation of leukocytes, particularly neutrophils in areas of inflammation. The discovery that *N*-formyl peptides are chemoattractants for these cells[1] has led to the investigation of structural requirements for peptide-receptor interaction.[2,3] The formyl-Met-Leu-Phe-OH and its synthetic analogue formyl-Met-Leu-Phe-OMe emerged as the prototypic chemotactic tripeptides.

Several studies have been carried out in order to better understand this tripeptide. The influence of terminal groups has been studied, and it has been demonstrated that the esterification of the C-terminal carboxylic acid group does not result in loss of biological activity of molecule.[4] However, the replacement of the *N*-terminal formyl group by tert-butyloxycarbonyl group (Boc) induces a dramatic loss of activity.[5]

In an effort to produce synthetic agents that are more active and more resistant to enzymatic hydrolysis, several modifications have been undertaken.[6-10] The replacement of the Met by the thiomethionine residue (Met^S) induces a dramatic loss of activity.[6] The comparative study of the formyl-Met-Leu-Phe-OMe and formyl-Met^S-Leu-Phe-OMe has shown that an active chemotactic peptide must have the formyl group free of any intramolecular interaction in order to be available for the formation of the complex with the receptor.[7]

Among the modifications made on the tripeptide formyl-Met-Leu-Phe-OMe, we can quote the substitution of the Leu residue by the α,α-disubstituted amino acids such as Acc5 (l- aminocyclopentane-1-carboxylic acid).

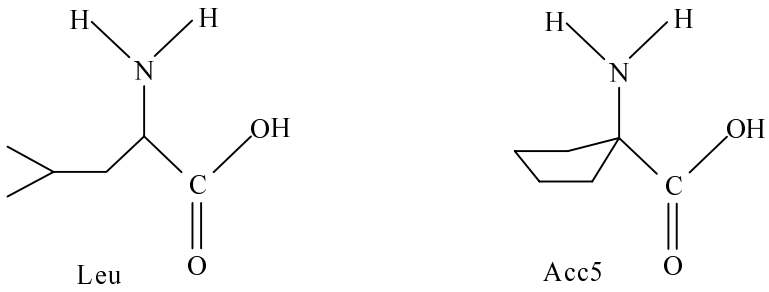

Figure 1. Structures of amino acids Leu and Acc5.

This amino acid was the subject of a considerable number of conformational studies.[11,12] The presence of a α,α-disubstituted carbon atom in this amino acid confers upon it the property to have a considerable steric effect, which can then impose significant constraints on the orientation of the peptide backbone. The use of this residue in a given peptide will facilitate the determination of the conformation adopted during the interaction with the receptor, as well as the search of pharmacophore groups.

To measure the activity of formyl-Met-Leu-Phe-OMe and formyl-Met-Acc5-Phe-OMe, their ability to induce the release of lysosomal enzymes in rabbit neutrophils was used. These measurements, which were undertaken by Sukumar's group,[13] showed that these two peptides are active (chemotactic peptides).

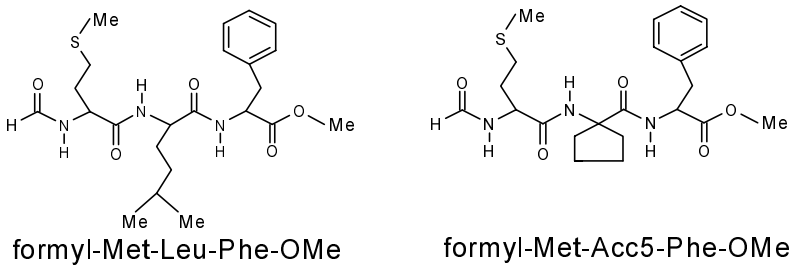

Figure. 2. Structures of formyl-Met-Leu-Phe-OMe and formyl-Met-Acc5-Phe-OMe.

The conformational study, that was carried out by ^{1}H-NMR, IR, and X-rays on these two peptides and other formyl-Met-Leu-Phe-OMe analogs containing other α,α-disubstituted amino acids, has not been able to give a common structure that can explain the biological activity of the chemotactic peptides.[14-16] This can be explained by the fact that the analyses by the various experimental methods are carried out in media and environments that are different to those in which these peptides exert their biological function. Consequently, the resulting structures are not necessarily the active structures.

Using a theoretical method, the present article is interested in the comparative conformational analysis of formyl-Met-Leu-Phe-OMe and formyl-Met-Acc5-Phe-OMe. The main objective of this study is to find the active conformation of chemotactic peptides.

Method

The method used in this study is called PEPSEA (PEPtidic SEArch). It was developed in the structural chemistry laboratory of the Sherbrooke University.[17] This approach is based on the fact that the structural, thermodynamic and statistical properties of a molecular system can be deduced only from a population presenting its conformational space. The principle of PEPSEA consists of generating a population of conformations that characterize a particular peptidic sequence. Rather than striving for global minima, populations of conformers are randomly generated, and their energy is minimized. A statistical analysis can be applied upon these populations to deduce the thermodynamic and structural properties of the peptide under investigation. This new approach is applied with the PEPSEA program.

The force field used by the PEPSEA program to compute the conformational energy is ECEPP/2 "Empirical Calculation Energy Program for Peptide".[18] This force field uses rigid geometry to represent the amino acid residues of a polypeptidic chain. The conformational energy function is given by the sum of the electrostatic term E_{ele}, 12-6 Lennard-Jones term E_{LJ}, and hydrogen-bond term E_{hb} for all pairs of atoms in the molecule together with the torsion term E_{tor} for all torsion angles.

$$\mathbf{E_{conf} = E_{ele} + E_{LJ} + E_{hb} + E_{tor}}$$

The PEPSEA program uses the specific parameters of each residue (atomic coordinates, geometrical and energy parameters...) to describe the geometry of a peptidic molecule. The force field ECEPP/2 possesses the parameters of 26 amino acid residues and of terminal protecting groups commonly found in proteins. However, the Acc5 residue is not included in the database, so it is necessary to calculate its parameters and integrate them in the force field ECEPP/2. The atomic partial charges for this particular residue are computed by CNDO calculation.[18]

It is worth noting that the dielectric constant used by PEPSEA is D= 2 (different of that in vacuous). According to Scheraga and al. this effective dielectric constant D = 2 is equivalent to the experimental dielectric constant (set between 4 and 8) similar to that of proteins in polar medium.[19]

As all endogenous peptides, the tripeptides under investigation in this study are constituted by the sequence of amino acids, all in L configuration.

Experimental

The PEPSEA program described above carried out the conformational search and the localization of the most stable minima. For each of the two considered peptides, 6000 conformers were randomly generated and energy minimized to the closest minima. During this generation process, all torsion angles are allowed to vary except those of the amide bonds; ω (Met), ω (Leu) or ω (Acc5) which are fixed at 180°. For each peptide, the first 100 conformers of lower conformational energies were submitted to a second energy minimization allowing all dihedral angles to be modified. The hessian matrix was calculated and the free energy was evaluated.[20] The resulting population of conformers was sorted by increasing value of the free energies.

The calculations of energy and minimization were performed on HP Apollo 9000 series 700, model 715 workstation at the higher school of technology of Casablanca.

Results

For the evaluation of the minimization efficiency, we have studied the energy distribution of 6000 minimized conformers of tripeptides formy-Met-Leu-Phe-OMe and formyl-Met-Acc5-Phe-OMe. The next figure represents these distributions.

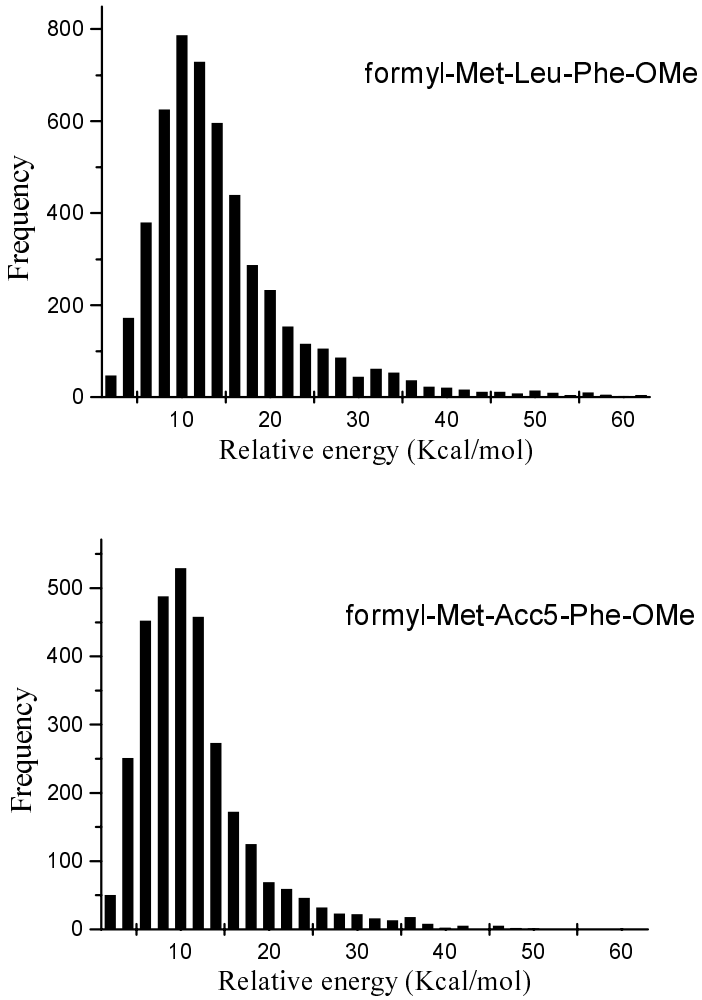

Figure 3. Energy distribution of minimized conformers of tripeptides formy-Met-Leu-Phe-OMe and formyl-Met-Acc5-Phe-OMe.

Table 1. Conformational characteristics of formyl-Met-Leu-Phe-OMe [a]

Conf.	Relative free energy ΔG [b] (kcal/mol)	Relative conformational energy ΔE [c] (kcal/mol)	formyl	Met	Leu	Phe
1	0.00	0.00		CO		NH
2	0.34	0.07		CO		NH
3	0.54	0.38	CO			NH
4	0.69	0.60		CO		NH
5	1.07	0.39		CO		NH
6	1.11	1.41	CO		NH	
7	1.23	1.00	CO		NH	
8	1.58	1.37				
9	1.69	2.37				
10	1.70	0.92	CO			NH
11	1.71	1.40	CO			NH
12	1.73	1.73	CO			NH
13	1.80	0.99		CO		NH
14	1.83	1.53	CO		NH	
15	1.84	1.53	CO		NH	
16	1.87	1.43	CO		NH	
17	1.89	1.83				
18	1.90	0.66				
19	1.92	2.52	CO	CO	NH	NH
20	1.93	2.11	CO	CO	NH	NH

a. First 20 minimum energy conformations are listed.
b. $\Delta G = G - G_0$. G_0 is the free energy of the conformation in order that $E = E_0$.
c. $\Delta E = E - E_0$. E_0 (formyl-Met-Leu-Phe-OMe) = - 4.22 kcal/mol.

Table 2. Conformational characteristics of formyl-Met-Acc5-Phe-OMe [a]

Conf.	Relative free energy ΔG [b] (kcal/mol)	Relative conformational energy ΔE [c] (kcal/mol)	formyl	Met	Acc5	Phe
1	-0,47	2,1				
2	-0,43	1,46	CO			NH
3	-0,3	1,09	CO			NH
4	-0,17	1,8	CO			NH
5	-0,14	1,86	CO			NH
6	-0,13	0,31	CO			NH
7	-0,13	1,86	CO			NH
8	-0,13	1,86	CO			NH
9	0.00	0.00	CO			NH
10	0,37	2,39		CO		NH
11	0,46	2,13	CO			NH
12	0,46	2,13				
13	0,47	2,1	CO			NH
14	0,52	1,9	CO			NH
15	0,63	1,95	CO			NH
16	0,67	3,07				
17	0,68	2,66	CO		NH	
18	0,69	2,19	CO		NH	
19	0,72	2,32				
20	0,76	1,14	CO			NH

a. First 20 minimum energy conformations are listed.
b. $\Delta G = G - G_0$. G_0 is the free energy of the conformation in order that $E = E_0$.
c. $\Delta E = E - E_0$. E_0 (formyl-Met-Acc5-Phe-OMe) = - 4.48 kcal/mol.

We can observe that these graphs have an alone distribution in form of bell "gaussian form". This remark confirms the good progress of the minimization and that the variable torsion angles have been well chosen.

Table 3. List of torsion angles for formyl-Met-Leu-Phe-OMe.

		formyl	Met							Leu							Phe					OMe
	ΔG	θ	ϕ	ψ	ω	χ^1	χ^2	χ^3	χ^4	ϕ	ψ	ω	χ^1	χ^2	χ^3	χ^4	ϕ	ψ	ω	χ^1	χ^2	θ
1	0.00	179	-157	131	177	179	171	173	-60	-79	87	177	-179	63	-68	59	-70	144	-175	-63	107	58
2	0.34	179	-156	131	180	179	171	173	-60	-81	89	180	179	61	-68	58	-72	-25	173	-63	107	-57
3	0.54	-178	-66	-32	178	-171	173	179	60	-62	-38	-176	176	62	-67	-61	-100	25	-170	-52	104	-63
4	0.69	-178	-66	-34	177	-172	174	179	-60	-78	81	178	-176	65	51	179	-75	147	-176	-61	-70	178
5	1.07	179	-157	134	-179	179	170	172	59	-82	79	178	-57	173	-178	70	-63	136	-174	-65	-71	58
6	1.11	-178	-79	75	178	-66	-178	-179	-59	-79	-27	179	-58	172	-59	70	-143	159	-178	-57	-77	179
7	1.23	-179	-73	133	-175	-167	175	179	-60	-67	-53	-175	175	61	172	177	-89	131	-174	-55	-71	-62
8	1.58	-179	-76	-30	179	-67	-179	179	-179	-148	128	-177	179	67	-66	-60	-160	-15	-175	56	-89	58
9	1.69	179	-155	111	180	-175	177	176	60	-79	-49	-180	177	63	-67	-60	-161	-25	-169	177	-102	-63
10	1.70	-179	-68	-39	-175	-173	175	179	179	-82	-38	-177	-55	175	61	-49	-146	-39	-177	-59	-74	-60
11	1.71	-175	-71	-17	174	-68	-179	-179	-179	-60	-36	-173	176	63	173	178	-98	147	-176	-52	106	58
12	1.73	-177	-68	-28	174	-69	-179	-179	59	-133	44	-178	-162	78	-60	-178	-147	-22	-179	-58	105	179
13	1.80	179	-156	138	177	-170	178	175	59	-90	120	174	178	63	172	-60	-156	165	177	57	-92	60
14	1.83	-179	-76	96	176	-172	177	-86	-59	-77	-33	-179	-58	173	61	70	-145	145	-179	-58	103	-60
15	1.84	-179	-76	97	176	-173	176	-86	-59	-77	-33	-179	-58	173	-178	70	-145	146	-179	-58	103	-60
16	1.87	-179	-75	93	172	-172	178	176	61	-77	-32	177	-57	173	61	70	-158	-28	-171	178	-101	-63
17	1.89	-178	-80	-28	-177	-66	-178	-179	-59	-153	143	176	-176	69	174	-59	-139	-20	177	-57	-73	-179
18	1.90	-177	-61	-41	-179	-173	176	-84	61	-109	37	-177	-53	171	60	-52	-148	-1	-173	-58	101	177
19	1.92	-178	-78	77	178	-67	-178	-179	60	-76	80	179	-175	66	51	-60	-157	-26	-177	178	77	59
20	1.93	-179	-69	124	180	-173	69	-174	60	-82	75	180	-55	175	-178	-49	-156	-26	-178	-178	-100	59

Table 4. List of torsion angles for formyl-Met-Acc5-Phe-OMe.

		formyl	Met							Acc5			Phe					OMe
	ΔG	θ	ϕ	ψ	ω	χ^1	χ^2	χ^3	χ^4	ϕ	ψ	ω	ϕ	ψ	ω	χ^1	χ^2	θ
1	-.467	-178	-73	-30	180	-68	-179	-179	-60	58	36	180	-158	151	174	177	78	-177
2	-.433	-179	-60	108	180	-174	173	81	177	58	36	180	-156	148	174	177	-101	-58
3	-.299	-179	-60	108	180	-174	67	-179	179	58	36	180	-156	148	175	177	-100	-178
4	-.165	-179	-64	104	180	-71	-73	179	-60	58	36	180	-157	-30	-177	177	-101	59
5	-.140	-179	-66	102	-175	-71	177	82	177	56	39	-177	-157	-30	-177	177	-101	59
6	-.132	-179	-66	102	-175	-71	177	82	177	56	39	-177	-157	-29	-178	177	78	-60
7	-.130	-179	-66	102	-175	-71	177	82	177	56	39	-177	-157	-30	-177	177	-101	179
8	-.127	-179	-63	108	-176	-174	174	81	176	59	40	170	-138	-26	-179	-62	-62	-60
9	.000	-179	-63	110	-176	-174	67	-178	-59	59	39	170	-138	-26	-179	-63	-62	59
10	.374	-179	-67	-38	-180	-173	177	83	57	64	-86	180	-160	-15	178	52	83	60
11	.457	-179	-61	105	180	-72	-74	179	59	58	35	180	-157	146	174	177	-101	-58
12	.463	-178	-159	132	-177	-175	171	179	-59	55	57	179	-65	138	-174	-62	109	57
13	.466	-178	-65	103	-174	-71	177	82	57	56	38	-177	-158	145	174	176	-100	-178
14	.521	-178	-62	109	-179	-174	68	-179	-60	58	35	177	-70	-33	177	179	-100	-179
15	.629	-178	-62	-33	178	-172	174	179	-60	-51	-40	-177	-83	-35	166	-56	-70	64
16	.674	-178	-73	-30	-179	-69	-73	179	59	57	37	-177	-159	151	174	177	-101	61
17	.681	-179	-70	126	-176	-169	176	-175	-179	-52	-54	176	-77	122	-168	-58	108	55
18	.694	-178	-76	82	175	-68	-177	179	179	-50	-35	176	-153	-9	177	56	-91	-58
19	.718	0	-69	-33	179	-165	-176	178	-59	59	46	178	-156	151	174	179	79	-178
20	.761	-178	-65	104	-174	-70	-178	-82	-57	59	41	171	-138	-30	179	-62	-61	-59

Tables 1 and 2 give the conformational characteristics of the twenty most stable conformers obtained after the second minimization for each peptide. These conformers are classified by order of increasing relative free energy. For each conformation, we find the relative free energy ΔG calculated for T = 300 K, and the relative conformational energy ΔE. The structural characteristics of each conformer are given by indicating the presence or not of intramolecular hydrogen bonds between the different donors and acceptors. The torsion angles for the parent peptide as well as the constrained one are listed in table 3 and table 4, respectively.

The conformational analysis of the twenty most stable conformers of the parent peptide formyl-Met-Leu-Phe-OMe (table 1) shows that it can adopt varied conformational structures, which can be distributed into four classes:

The first class is that of the conformers characterized by the presence of the β turn structure centered on Met and Leu, and it can be represented by four conformers (conformers 3, 10, 11 and 12). Such a structure is stabilized by an intramolecular hydrogen bond including the CO group of the formyl and NH group of Phe. Figure 4-a gives a stereoscopic superposition view of the four conformers belonging to this group.

The second class includes five conformers characterized by the presence of a γ turn centered on Met, and stabilized by an intramolecular hydrogen bond involving the CO group of the formyl and NH group of Leu. The stereoscopic superposition view of these five conformers is given in figure 4-b.

The third class, which includes five conformers, is characterized by conformations adopting a γ turn centered on Leu, and stabilized by an intramolecular hydrogen bond, implying the CO group of Met and NH group of Phe. Figure 4-c gives the stereoscopic superposition of these five conformers.

The fourth class gathers structures in a double γ turn (a γ turn centered on Met and a γ turn centered on Leu at the same time), and includes two conformers of formyl-Met-Leu-Phe-OMe. The stereoscopic superposition view of both conformers of this class is presented on the figure 4-d.

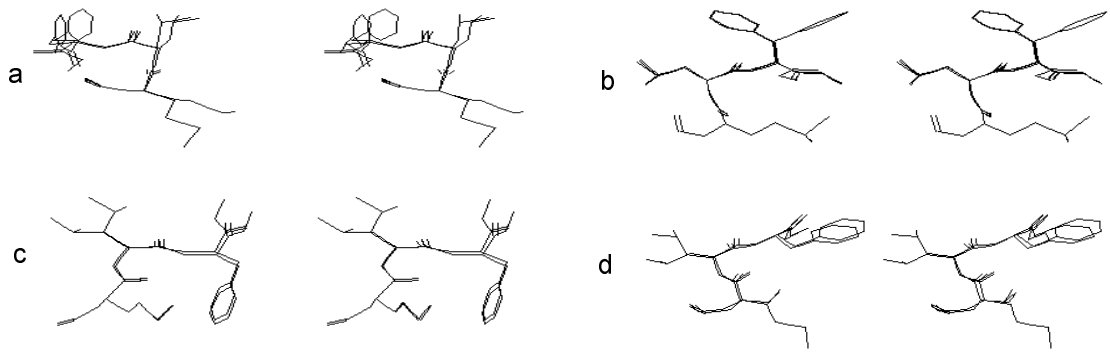

Figure 4. Stereoscopic superposition view of conformers of different classes obtained in the case of formyl-Met-Leu-Phe-OMe.

Concerning the constrained tripeptide formyl-Met-Acc5-Phe-OMe, the conformational analysis of the most twenty stable conformers (table 2) shows a great tendency toward the β turn structure. Indeed, 13 conformers over the 20 most stable conformers, represent this structure. The stereoscopic superposition view of these conformers is given in figure 5.

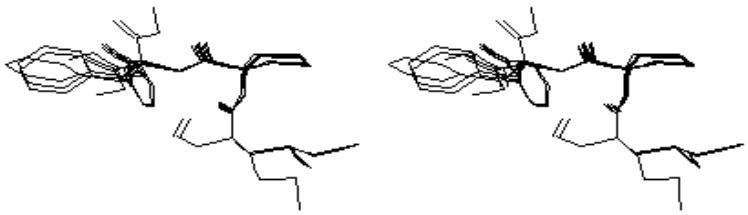

Figure 5. Stereoscopic superposition view of the 13 conformers of formyl-Met-Acc5-Phe-OMe in β turn structure.

Discussion

From these results, it appears clear that the parent peptide formyl-Met-Leu-Phe-OMe can adopt several types of structures in such way that we can not favor a precise structure, as compared to other structures. Therefore, it is not easy to extract the conformational characteristics of the chemotactic peptides using only the parent peptide formyl-Met-Leu-Phe-OMe.

The conformational analysis results of the geometrically constrained peptides formyl-Met-Acc5-Phe-OMe in which the α,α-disubstituted amino acid Acc5 gives it a certain rigidity, shows the preference of this tripeptide to adopt β turn conformation. This result is in perfect agreement with the conformational analysis results of the geometrically constrained peptides formyl-Met-Acc6-Phe-OMe that we have carried out with the same method,[21].

Taking into account the findings above and other studies,[21-22] we can propose that the active structure of chemotactic peptide is the β turn structure preferred by the geometrically constrained peptide formyl-Met-Acc5-Phe-OMe. However, in the case of the parent peptide formyl-Met-Leu-Phe-OMe, we can suppose that its activity is due to its flexibility. This flexibility allows the molecule to fit the convenient structure (β turn)

during the interaction with the receptor. This result is in perfect agreement with the "Zipper" model of Burgen,[23].

Finally, the comparison of results in table 1 and table 2 enables us to reject the proposal that the formyl group must be free of any intramolecular hydrogen bond in order to be available for the formation of the complex with the receptor. Indeed, among the 20 most stable structures of formyl-Met-Acc5-Phe-OMe, 15 conformers have the formyl group implicated in intramolecular hydrogen bonds, even though this peptide is six times more active than the parent peptide formyl-Met-Leu-Phe-OMe.

Conclusion

In conclusion, the conformational analysis described in this study and a careful examination of the recent literature enables us to suggest: a) The active structure of chemotactic peptides is the β turn structure. b) The parent peptide formyl-Met-Leu-Phe-OMe adopts a so flexible structure that can adopt the conformation of the β turn active structure during the interaction with the receptor. c) A rejection of the importance of the formyl group in the interaction with the receptor. This means that this group is not the pharmacophor contrarily to the result found by a recent study.[7]

References and Notes

1. Schiffmann, E.; Corcoran, B. A.; Wahl, S. M. Proc. Nathl. Sci. USA **1975**, *72*, 1059-1062.
2. Showell, H. J.; Freer, R. J.; Zigmond, S. H.; Schiffmann, E.; Aswanikumar, S.; Corcoran, B. A.; Becker, E. L. J. Exp. Med. **1976**, *143*; 1154-1169.
3. Toniolo, C.; Bonora, G. M.; Schowell, H. J.; Becker, E. L. Biochemistry **1984**, *23*, 698-704.
4. Iqbal, M.; Balaram, P.; Showell, H. J.; Freer, R. J.; Becker, E. L. FEBS Letters, **1984**, *165*, 171-174.
5. Bardi, R.; Piazzesi, A. M.; Toniolo, C.; Ry, P. A.; Ragothama, S.; Balaram, P. Int. J. Peptide Protein Res. **1986**, *27*, 229-238.
6. Lajoie, G.; Sauve, G.; Rao, V. S.; Di Paola, A.; Belleau, B. Int. J. Immunopharmac. **1989**, *11*, 467-474.
7. Michel, A. G.; Lajoie G.; Ameziane, C. H. Int. J. Peptide Protein Res. **1990**, *36*, 489-498.
8. Dugas, H.; Laroche, M.; Ptak, M.; Labbé, H. Int. J. Peptide Protein Res. **1993**, *41*, 595-605.
9. Vertuani, G.; Boggian, M.; Breveglieri, A.; Cavicchioni, G.; Spisani, S.; Scatturin, A. Amino Acids, **1995**, *9*, 375-383.
10. Cavicchioni, G.; Breveglieri, A.; Boggian, M.; Vertuani, G.; Reali, E.; Spisani, S. J. Pept. Sci. **1996**, *2* , 135-140.
11. Santini, A.; Barone, V.; Bavoso, A.; Benedetti, E.; Di Blasio, B.; Fraternali, F.; Lelj, F.; Pavone, V.; Pedone, C.; Crisma, M.; Bonora, G. M.; Toniolo, C. Int. J. Biol. Macromol., **1988**, *10*, 292-299.
12. Valle, G.; Crisma, M.; Toniolo, C. Can. J. Chem. **1988**, *66*, 2575-2582.
13. Becker, L.; Bleich, H. E.; Day, A.; Freer, R. J.; Glasel, J. A.; Visintainer, J. Biochemistry **1979**, *18*, 4656-4668.
14. Morfew, A. J.; Tickle, I. Cryst. Struct. Commun., **1981**, *10*, 781-788.

15. Bakir, M.; Stevens, E. S. Int. J. Peptide Protein Res. **1982**, *19*, 133-136.
16. Sukumar, M.; Raj, P. A.; Balaram, P.; Becker, E. L. Biochem. Biophys. Res. Commun. **1985**, *128*, 339-344.
17. Michel, A. G.; Ameziane, C. H.; Bredin, N. Can. J. Chem. **1992**, *70*, 596-603.
18. Nemethy, G.; Pottle, M. S.; Scheraga, H. A. J. Phys. Chem. **1983**, *87*, 1883-1887.
19. Momany, F. A.; McGuire, R. F.; Burgess, A. W.; Scheraga, H. A. J. Phys. Chem. **1975**, *79*, 2361-2381.
20. Zimmerman, S. S.; Pottle, M. S.; Nemethy, G.; Scheraga, H. A. Macromolecules **1977**, *10*, 1-9.
21. Wazady, Y.; Ameziane, C. H.; Lakhdar, M. Ezamarty, A. Int. J. Mol. Sci. **2001**, *2*, 1-9.
22. Toniolo, C.; Crisma, M.; Valle, G.; Bonora, G. M.; Polinelli, S.; Becker, E. L.; Freer, R. J.; Rao, R. B.; Balaram, P.; Sukumar, M. Pept. Res., **1989**, *2*, 275-281.
23. Nogrady, T. Medicinal Chemistry: A biochemical approach; Oxford University Press 1988, pp 47-49.

Povzetek

Predstavljamo študijo povezave med konformacijo glavne verige in biološko aktivnostjo kemotaktičnih peptidov formil-Met-Leu-Phe-OMe in formil-Met-Acc5-Phe-OMe s teoretsko metodo PEPSEA. Dokazali smo, da ima osnovni peptid formil-Met-Leu-Phe-OMe fleksibilno strukturo in da ima konformacijsko oviran peptid tendenco tvorbe β zavoja. Podan je tudi dokaz proti veljavnosti hipoteze, da je za interakcijo z receptorjem potrebna prisotnost formilne skupine.