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CONFORMATIONAL SEARCH OF IMMUNOSTIMULATING TRIPEPTIDE GLY-LEU-PHE STRUCTURE BY MOLECULAR MECHANICS METHOD.

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By molecular mechanics method have been investigated the conformational properties of immunostimulating tripeptide Gly-Leu-Phe-NH_2. It is shown that the spatial structure of this tripeptide can be described by set of low-energy conformations. Calculations produced the values of all dihedral angles of the backbone and side chains of the optimal conformations as well as intra- and inter-residue interaction energies.

Keywords: *immunostimulating tripeptide, conformation, molecular mechanics method*

INTRODUCTION

The knowledge of the spatial structure and conformational behaviour of the bioactive peptides allow more rational investigation of the function-structure relationships of these molecules. The presence of some further biologically active short peptides has been detected in human as well as in cow milk caseins. The tripeptide Gly-Leu-Phe-NH_2 (glycyl-leucyl-phenylalanine amide) was isolated from human milk proteins by enzymatic digestion. This immunostimulating peptide Gly-Leu-Phe-NH_2 (GLF) increased phagocytosis by human and murine macrophages and protected mice against Klebsiella pneumoniae infection [1]. Such fragments retain the biological properties of the parent molecule and may often even possess improved or novel characteristics. Specific binding sites on human polymorphonuclear leukocytes (PMNs) have been demonstrated recently. GLF is also the clear example of a food-derived peptide that exerts an anti-alopecia effect. It is an interesting problem whether other immunostimulating or histamine releasing peptides show anti-alopecia effects [2]. The antialopecia effect of GLF was inhibited by pyrilamine, a histamine H1 receptor antagonist, suggesting that the anti-alopecia effect is mediated by histamine release. In order to correlate activity and structure X-ray analysis in the work [1] has been applIed to Gly-Leu-Phe tripeptide. As a result of this study was received one averaged conformation in crystallic state. This circumstance provokes interest in examining details of the conformational features of this peptide and searcing for its efficient and selective analogs.

In this work was studied the spatial structure and conformational flexibity of Gly-Leu-Phe-NH_2 tripeptide molecule by molecular mechanics method. For understanding of how tripeptides interact with their receptors is required the knowledge of the conformational specificity and dynamics of the native molecule allowing a rational design of compounds acting selectively at their receptor level.

METHOD

This investigation were carried out using molecular mechanics method as described in Refs.[3,4]. Computations were carried out on the computer using universal programs complex [5]. This program calculates the conformational energy of a peptide as a sum of nonbonded,

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hydrogen-bonded and electrostatic energies for pairwise atomic interactions and torsional potential energies for rotation about bonds. Bond lengths and bond angles are fixed at standard values, and only dihedral angles are allowed to vary. For a stable conformation, the φ, ψ,ω, χ dihedral angles of backbone chain are located in a low energy regions: R (φ, ψ=-180°-0°) ,B (φ =-180°-0°, ψ=0°-180°), L (φ, ψ=0°-180°) and P (φ =0°-180°, ψ =-180°-0°). The conformational state of each amino acid residue is conveniently described by backbone φ, ψ,ω and side chain χ_n dihedral angles. All backbone forms of a dipeptide can be classified into two types, referred to as shapes: folded (*f*) and extend (*e*) . For a tripeptide, all possible backbone forms may be specified by four shapes, i.e. *ff, fe, ef* and *ee*. The number of forms in each shape depends on possible combinations of R,B,L and P forms are possible for glycine, R,B, and L forms occur with alanine-type residues. The dihedral angle values corresponding to the lowest energy states of monopeptides were used as starting conformations. The conventions used for torsion angles are those of IUPAC-IUB Commission [6].

RESULTS

Three-dimensional structure of the Gly-Leu-Phe-NH_2 have been investigated basing on the low-energy conformations of monopeptides. The structure of all possible backbone forms may be specified by four shapes, i.e. ff, fe, ef and ee for tripeptide shown in Fig. 1. The values of dihedral angles were taken from the B,R and L conformational areas when the starting structural approximations were determined.

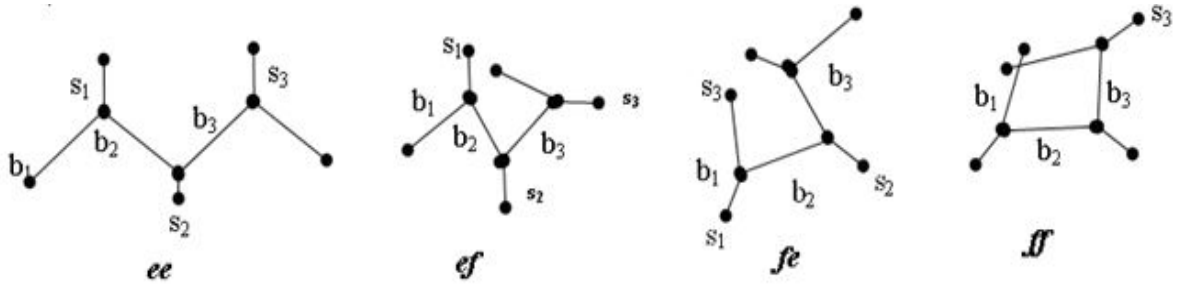

Fig.1. The four possible backbone shapes of the tripeptide molecule (b_1,b_2 and b_3-backbone segments, s_1, s_2 and s_3- side chains segments)

The starting conformations of the tripeptide Gly-Leu-Phe NH_2 were obtained by means of combining of the low-energy structures of each residues. It should be noted that the N-terminal residue of this similar tripeptide has not a side chain. In this tripeptide the second residue Leu has large side chain structure and thefore its side chain is relatively flexible, but third residue Phe has side chain with phenyl ring. For Leu residue the values of χ_1 and χ_2 dihedral angles of the side chain were taken to be 60, 180, and -60^0. But for Phe residue only χ_1 dihedral angle were taken to be 60, 180, and -60^0. The χ_2 angle of side chain of Phe were taken to be equal to 90^0. It was composed 216 structural variants of 4 backbone shapes for conformational analysis of tripeptide Gly-Leu-Phe NH_2. Therefore our investigation of conformational posibilities of these tripeptide is started with conformational analysis of their 216 variants. After energy minimization had been performed a rather limited number of conformations lay in the 0-5 kcal/mol ΔE energy interval. The energy parameters of low-energy conformations of four shapes are presented in the Table1. The energy minimization of the obtained set of the structural variations for these tripeptides, revealed a remarkable energy differentiation among the optimal conformations. The conformation of the

ISSN 1512-1461

RRB folded form belonging to the *ff* shape appeared to be more energetically preferable for these tripeptides. The effective interactions of the opposite charged atom groups of the terminal groups was possible in these conformations. This contacts has the electrostatic nature.

Tables 1. The energy parameters of the optimal conformations of the Gly-Leu-PheNH_2 tripeptide.

№	Conformation	Shape	Energy contributions of the intermolecular interactions (kcal/mol)				
			E_{nb}	E_{elst}	E_{tors}	E_{total}	E_{rel}
1	R R_{21} B_3	ff	-13.4	2.4	2.2	-8.9	0
2	P R_{22} B_3	ff	-10.7	2.7	1.1	-6.9	2.0
3	R R_{12} B_1	ff	-12.9	2.4	3.8	-6.8	2.1
4	R R_{32} B_1	ff	-10.9	2.4	1.7	-6.7	2.2
5	P R_{31} B_1	ff	-11.0	2.4	2.1	-6.5	2.4
6	B R_{21} B_3	ef	-11.1	2.9	1.3	-6.9	2.0
7	L R_{22} B_3	ef	-10.9	2.8	1.3	-6.8	2.1
8	B R_{32} B_1	ef	-10.9	2.9	1.5	-6.5	2.4
9	L R_{31} B_1	ef	-10.8	2.9	1.5	-6.5	2.4
10	L R_{13} B_3	ef	-11.0	2.9	3.8	-3.9	5.0
11	P B_{22} B_1	fe	-10.1	2.7	1.6	-6.8	2.1
12	R B_{22} B_1	fe	-10.7	2.6	1.6	-6.4	2.5
13	P B_{32} B_1	fe	-10.5	2.7	1.8	-6.0	2.9
14	R B_{32} B_1	fe	-10.2	2.6	1.8	-6.8	3.1
15	P B_{13} B_3	fe	-8.7	2.6	1.5	-4.7	4.2
16	B B_{21} B_1	ee	-10.7	2.7	1.6	-6.4	2.5
17	L B_{22} B_1	ee	-10.4	2.7	1.6	-6.1	2.8
18	B B_{32} B_1	ee	-10.6	2.8	1.9	-6.0	2.9
19	L B_{32} B_3	ee	-10.1	2.7	1.5	-5.9	3.0
20	L B_{13} B_3	ee	-9.3	2.6	1.8	-4.8	4.1

Calculations have shown that the lowest-energy conformation of the *ff* shape are considerably more stable than these other shapes, gaining energy more than 2.0 kcal/mol. The low-energy conformations of the *ff* shape enjoy the most efficient interactions between the Gly and Phe residues in the tripeptide. Table 2 demonstated the energy contributions (kcal/mol) of the intra- and interresidues interactions in the stable conformations of the *ff* shape of tripeptide molecule.

Table 2. Energy contributions (kcal/mol) of the intra- and inter-residues interactions in the stable conformations of the *ff* shape of Gly-Leu-PheNH_2 tripeptide molecule.

ISSN 1512-1461

Conformation	Gly	Leu	Phe	
$RR_{21}B_3$	3.4	-1.7	-4.2	Gly
$RR_{12}B_1$	3.3	-1.6	-4.8	
$PR_{22}B_3$	3.2	-1.3	-3.1	
$PR_{31}B_1$	2.9	-2.0	-3.5	
		-0.9	-4.9	Leu
		-1.0	-2.1	
		-1.2	-3.4	
		-0.8	-3.4	
			-2.7	Phe
			-3.0	
			-2.9	
			-3.0	

The interactions of side chains is less effective in the extended *BBB* and *LBB* forms of this tripeptide. In the global conformation of tripeptide is formed the quasicyclic structure by hydrogen bond between the N-terminal amino group with C-terminal carbonyle group C=O (Fig.2). The presence of such a hydrogen bond in lowest-energy conformation testifies to the formation of quasicyclic structure. Stable enough in energy terms, that gives the tripeptide the form necessary for the ligand-receptor interaction to take place. Tables 3 show the values of the dihedral angles of low-energy structures of four diferent backbone form of the obtained after energy minimization for tripeptide Gly-Leu-PheNH_2. The spatial structure of global conformation $RR_{21}B_3$ is presented in Fig.2.This investigation demonstrated a definite similarity in the conformational preference of Gly-Leu-Phe with data of its crytalli state investigation by X-ray analysis. The similar spatial structure of crystallic conformation of tripeptide is presented in Fig.3.

Tables 3. The values of the dihedral angles of low-energy structures of four diferent backbone form of the Gly-Leu-PheNH_2 molecule.

Amino acid	Forma	Backbone angles			Side chain angles			
		φ	ψ	ω	χ_1	χ_2	χ_3	χ_4
Gly	$RR_{21}B_3$	-35	-65	170	-	-	-	-
	$PB_{21}B_1$	53	-70	182	-	-	-	-
	$BB_{21}B_1$	-53	67	181	-	-	-	-
	$BR_{21}B_3$	-77	83	180	-	-	-	-
Leu	$RR_{21}B_3$	-69	-55	175	171	61	178	176
	$PB_{21}B_3$	-96	108	179	175	60	179	175
	$BB_{21}B_3$	-95	99	181	176	60	179	175
	$BR_{21}B_3$	-95	-64	178	173	59	176	175
PheNH_2	$RR_{21}B_3$	-104	152	180	-54	95	-	-
	$PB_{21}B_1$	-155	160	180	54	90	-	-
	$BB_{21}B_1$	-159	162	180	53	88	-	-
	$BR_{21}B_3$	-100	146	180	-57	92	-	-

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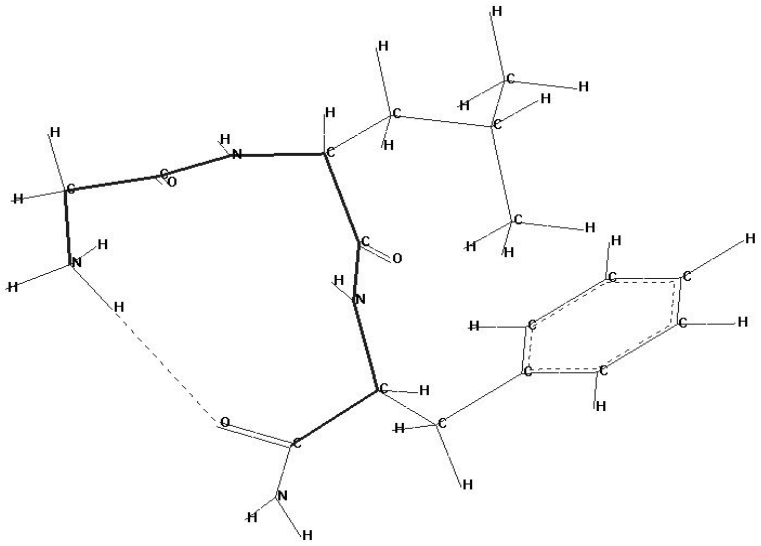

Fig.2. The spatial structure of the preferred calculated conformation of the Gly-Leu-PheNH_2 tripeptide (hydrogen bond is presented by dotted line).

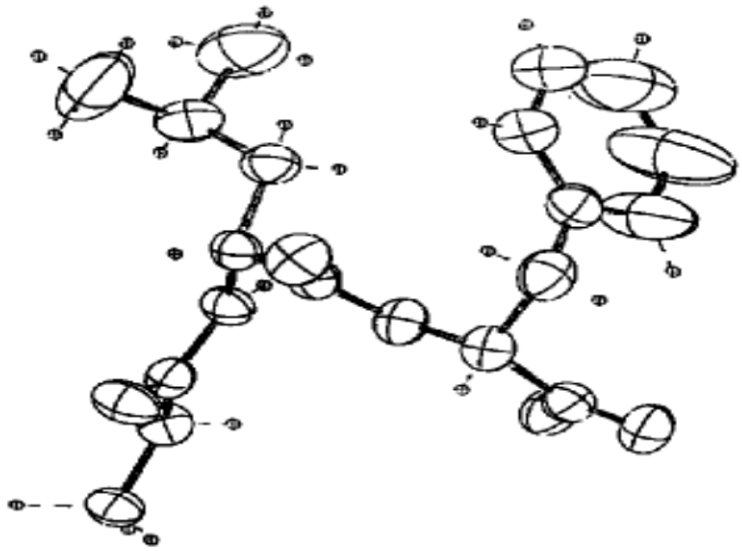

Fig.3. The molecular conformation of Gly-Leu-Phe tripeptide in the crystallic state, obtained by X-ray analysis [1]

CONCLUSIONS

Our calculations of the spatial structure of immunostimulating tripeptide Gly-Leu-PheNH_2 demonstrated this molecule has a limited set of the stable structures that are characterized by the folded backbone form. The conformational analysis helped reveal a number of special features of spatial arrangement of this drug-based tripeptide, which may be useful as a base for a directed search and synthesis of their more effective structural analogs.

ISSN 1512-1461

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In this article are presented three figure and three tables.

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