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Conformational Analysis of Pol-Rfamide II (Glu^1-Trp^2-Leu^3-Lys^4-Gly^5-Arg^6-Phe^7-NH_2) Heptapeptide

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The geometrical structure of the sea anemone and sea pansies neuropeptide Pol-RFamide II Glu^1-Trp^2-Leu^3-Lys^4-Gly^5-Arg^6-Phe^7-NH_2 was carried out by molecular mechanics (MM). The linkage bonds are characterised by the torsional angles ϕ, ψ and ω and the side groups characterised by the torsional angles χ_1, χ_2, χ_3,... subsequently. The energy-map for each monopeptide of the Pol-RFamide II was drawn in the range of -180° to 180° with increments of 20°. Conformation facilities for monopeptides were decided from these maps. These results were used in the analysis of the dipeptide Glu^1-Trp^2. Then, the Glu^1-Trp^2-Leu^3 tripeptide was examined by using the calculated results for dipeptide. Conformational analysis of the Glu^1-Trp^2-Leu^3-Lys^4 tetrapeptide was performed using the low-energy values of the tripeptide. The geometrical structure of Glu^1-Trp^2-Leu^3-Lys^4-Gly^5-Arg^6-Phe^7-NH_2 neuropeptide was determined by rotating the tetrapeptide Glu^1-Trp^2-Leu^3-Lys^4 and the dipeptide Arg^6-Phe^7-NH_2 about the monopeptide Gly^5 due to the minimisation of energy.

Introduction

Studying different physico-chemical properties of physiologically active compounds by theoretical methods gives us the possibility of modelling processes for the living organism. One of the stages in studying the functional mechanism of neuropeptides is to investigate the spatial structure and conformational possibilities of the molecule, necessary for the study of molecular aspects of biological activity and receptor selectivity.

The neuropeptide Pol-RFamide II Glu^1-Trp^2-Leu^3-Lys^4-Gly^5-Arg^6-Phe^7-NH_2 was isolated from sea anemones and sea pansies by Grimmelikhuijzen et al.[1] The conformational state of each residue in neuropeptide is classified as short-, medium- and long-range. Conformational energy computation on polypeptides and proteins require reliable parameters to describe molecular structure and interaction energies. There are no experimental studies on the thermodynamic or other phenomenological properties of the neuropeptide Glu^1-Trp^2-Leu^3-Lys^4-Gly^5-Arg^6-Phe^7-NH_2 Pol-RFamide II. Therefore, the microscopic structure of the neuropeptide is not well known. Because of fluctuations in size and shape, it is difficult to establish detailed

accurate structure from experimental studies alone. The molecular mechanics (MM) simulation method is well suited for investigating many particle systems microscopically, and so it fills the gap between the theoretical and the experimental.

On the theoretical side, ab initio molecular orbital calculations and molecular mechanics calculations have been employed to study the conformational structures and the related energy states of the various molecules[2−9]. Waterhouse[10] computed the conformational energies for models of the disaccharide β-D-fructofuranosyl (2→ 6) -β-D-glucopyranoside by molecular mechanics. To investigate the local interactions in tripeptide sequences composed of amino acids having aromatic side chains, Oka et al.[11] theoretically carried out the conformational analysis of N-acetyl-N$\prime$-methylamide of the Phe-Phe-Phe tripeptide using the conformational energy-minimisation procedure. Subramanian et al.[12] determined the crystal structure of the dipeptides tert ($C_{10}H_{18}N_2O_5$; H_2O).

In the present study, we modelled the isolated molecule to obtain information about the most possible conformations of this neuropeptide Pol-RFamide II by computing its steric energies at different torsional angles of the central linkage bonds, namely, the ϕ, ψ and ω are torsion angles (clarified in the Figure) as well as at the staggered angles of the side groups.

Theoretical

Conformational energy calculations of the Glu[1]-Trp[2]-Leu[3]-Lys[4]-Gly[5]-Arg[6]-Phe[7]-NH_2 neuropeptide Pol-RFamide II were carried out with an empirical conformational energy program for peptides (ECEPP)[13]. The main point of the model concerns the consistency of all types of intra- and inter-molecular interactions in the stable low-energy structures of peptides and proteins. During minimisation all the backbone angles ϕ, ψ and ω and side chain dihedral angles χ_1,χ_2, χ_3,... were allowed to vary. All the lowest energy combinations of single-residues were used as starting conformations. Details of conformational procedure as well as energy functions and semi-empirical parameters used to evaluate nonbonded and electrostatic interactions, hydrogen bonding and torsional components have already been described using a semi-empirical method[14−16]. The simulation of the neuropeptide Pol-RFamide II was carried out at an average temperature of 293 K. The hydrogen bond length and the bond energy were determined in the present conformation analysis and the results are given below.

Atomic groups in H bond	Bond length A°	kcal/mol Energy
NH (Glu[1]), CO (Lys[4])	2.37	-0.50
CO (Glu[1]), NH (Arg[6])	2.22	-0.50
CO (Glu[1]), NH (Phe[7])	1.91	-1.39

From each shape of these energy functions, the 32 lowest energy forms were extracted (Table 1). Table 2 lists the lowest 37 energies (E_{rel}) of 784 possible structural variants for the Pol-RFamide II. These structures exhibit 371 backbone forms belonging to 32 shapes, possible in principle for the neuropeptide Pol-RFamide II. In addition, the calculated values of the elements of the triangular matrices of energy components for the most preferable structure of Pol-RFamide II are given in Table 3. These matrices provide a good illustration for all inter- and intra-residue interaction, as well as for an efficiency and energy distribution of the contacts.

Numerical values of dihedral angles of rotation about the backbond and side chain bonds in lowest-energy structures of the neuropeptide Pol-RFamide II are given in Table 4.

Table 1. Distribution of conformations of the Glu[1]-Trp[2]-Leu[3]-Lys[4]-Gly[5]-Arg[6]-Phe[7]-NH_2 neuropeptide Pol-RFamide II due to the relative energies.

Shape	Energy interval (kcal.mol^{-1})						
	0-1	1-2	2-3	3-4	4-5	5-10	> 10
e e e e e e					1	5	41
e e e e e f				1		3	13
e e e e f e						4	31
e e e f e e							48
e e f e e e							44
e f e e e e						2	26
e e e e f f						4	20
e e e f e f						4	12
e e f e e f						1	13
e e e f f e						7	67
e e f e f e						1	28
e f e e f e						4	25
e e f f e e						2	36
e f e f e e						2	34
e f f e e e						1	7
e e e f f f						4	21
e e f e f f						5	11
e f e f f e						5	29
e e f f f e						4	45
e f f f e e							9
e f f e f e							12
e e f f e f						5	15
e f f f f e						1	6
e e f f f e						1	2
e f e e e f	1						6
e f f e f f							5
e f f e e f							3
e f f f e f						1	4
e f f f f f							4
e f e e f f						1	9
e f e f e f							10
e f e f f f						1	14

Results and Discussion

The structure of neuropeptide Pol-RFamide II (Glu[1]-Trp[2]-Leu[3]-Lys[4]-Gly[5]-Arg[6]-Phe[7]-NH_2) was investigated by the semi-empirical conformational analysis method. The geometry and energy parameters of the stabilized states available in the polarized environment were determined and then the best form of the relevant interaction energies was calculated (Tables 1 and 2).

Table 2. The energy parameters (in terms of kcal.mol^{-1})of the low-energy conformations of the Glu^1-Trp^2-Leu^3-Lys^4-Gly^5-Arg^6-Phe^7-NH_2 neuropeptide Pol-Rfamide II.

Form	E_{nb}	E_{el}	E_{tors}	E_{rel}
$B_{323}B_{32}B_{3233}R_{33332}LB_{2333333}B_{32}$	-40.67	1.87	12.42	5.99
$B_{233}B_{32}R_{3233}L_{33332}BR_{3333133}B_{22}$	-40.48	0.67	13.59	6.14
$B_{233}B_{32}R_{3233}L_{33332}RB_{3333333}R_{32}$	-39.48	1.95	12.34	7.17
$B_{332}B_{22}B_{3133}R_{33332}BR_{2333333}R_{32}$	-28.98	3.21	4.87	11.46
$B_{313}B_{33}R_{3233}B_{13332}BB_{3333333}B_{12}$	-32.69	1.54	9.42	10.64
$R_{332}L_{32}L_{3133}B_{13322}BB_{3333333}B_{12}$	-36.86	2.82	9.92	8.24
$B_{332}B_{22}B_{3133}R_{33332}PR_{3333133}B_{22}$	-32.34	1.13	4.83	5.98
$B_{33}B_{22}B_{3233}B_{23323}LR_{3333133}B_{23}$	-32.00	1.24	6.54	8.13
$B_{323}B_{32}L_{3233}R_{32332}LR_{3333133}B_{22}$	-43.88	1.48	10.05	0.00
$B_{332}B_{22}B_{3233}B_{23323}PB_{3333333}R_{32}$	-31.69	2.36	7.05	10.07
$B_{323}B_{32}R_{3233}R_{32333}PB_{3333333}R_{32}$	-33.22	1.62	5.76	6.52
$R_{332}L_{32}L_{3133}B_{13322}RB_{3333333}B_{12}$	-32.06	2.04	6.91	9.26
$B_{332}R_{32}R_{3233}B_{23232}BB_{2333333}R_{32}$	-39.42	5.62	10.10	8.65
$B_{323}B_{32}B_{3233}R_{33332}RR_{3233333}R_{32}$	-33.54	1.46	8.60	8.89
$B_{323}B_{32}L_{3233}R_{33332}PR_{3333133}B_{22}$	-38.47	1.77	9.57	5.22
$B_{323}B_{32}R_{3233}B_{33322}LR_{3333133}B_{22}$	-35.72	1.84	8.43	6.91
$R_{332}L_{32}L_{3133}B_{13332}LR_{3333133}B_{22}$	-34.63	2.64	9.94	10.30
$B_{323}B_{32}R_{3233}R_{32332}RB_{3133333}B_{32}$	-30.83	1.19	6.93	9.66
$B_{323}B_{32}L_{3233}R_{33332}RB_{3333333}B_{12}$	-37.62	2.00	8.08	4.82
$B_{313}B_{33}R_{3233}B_{13333}PR_{3333133}B_{22}$	-33.23	2.35	7.81	9.29
$B_{332}R_{32}R_{3233}B_{23233}PB_{2333333}R_{32}$	-36.64	4.05	9.65	9.42
$R_{332}R_{32}B_{3233}L_{13233}PR_{3333133}B_{22}$	-35.86	1.29	11.44	9.23
$R_{332}R_{32}R_{3233}R_{33332}RB_{3333333}B_{12}$	-35.44	3.15	9.70	9.78
$R_{332}R_{32}R_{3233}B_{33323}RR_{3333133}B_{22}$	-37.29	2.71	11.04	8.82
$B_{332}R_{32}B_{3133}B_{13332}PR_{3333133}B_{22}$	-35.28	2.41	8.22	7.71
$R_{332}B_{33}R_{3233}B_{23332}PB_{3333133}B_{12}$	-36.30	3.38	7.44	6.88
$R_{332}B_{33}L_{3233}R_{33333}PR_{3333133}B_{22}$	-38.68	1.63	9.30	4.60
$B_{332}L_{32}B_{3233}B_{13232}PR_{3333133}B_{22}$	-35.80	2.42	8.86	7.84
$R_{332}B_{33}R_{3233}B_{23333}RR_{3333133}B_{22}$	-35.53	2.69	9.02	8.54
$B_{332}L_{32}B_{3233}B_{13333}RB_{3133333}B_{32}$	-38.32	2.20	8.07	4.31
$R_{332}B_{33}R_{3233}R_{33332}PB_{3333133}R_{32}$	-36.23	2.26	7.21	5.59
$B_{332}L_{32}B_{3233}B_{13232}LR_{3333133}B_{22}$	-40.19	3.55	10.16	5.88
$B_{332}L_{32}B_{3133}R_{13232}BR_{3333133}B_{22}$	-40.02	2.82	9.63	4.79
$R_{323}B_{32}R_{3233}R_{33232}PB_{3333333}R_{32}$	-34.20	2.19	6.60	6.95
$B_{333}L_{32}B_{3233}B_{13132}RB_{3133333}B_{32}$	-34.88	1.04	8.97	7.50
$B_{333}B_{22}B_{3133}R_{31232}LR_{3332333}B_{22}$	-37.31	1.34	6.68	3.08
$L_{333}L_{33}R_{3233}L_{33333}BB_{2333333}R_{32}$	-38.21	1.99	13.80	9.94
$B_{333}L_{32}B_{3233}B_{13132}BR_{3333333}B_{22}$	-36.99	1.47	10.14	6.98

Conformation analyses of the Pol-RFamide II molecule were performed based on energy minimisation:

First, the minimum energy states of all the monopeptides were determined and the first two of these were combined to give the Glu^1-Trp^2. Then, the minimum energy state of the dipeptide was determined and combined with the third monopeptide to give the (Glu^1-Trp^2-Leu^3) threepeptide. Similar procedures were followed to obtain the (Glu^1-Trp^2-Leu^3-Lys^4) tetrapeptides.

Second, minimum energy states of Arg^6-Phe^7-NH_2 were combined to give the Arg^6-Phe^7-NH_2 dipeptides.

Third, the above tetrapeptide was combined to the dipeptide in terms of Gly^5 monopeptide.

Table 3. The intra- and inter-residue interaction energies (kcal.mol^{-1}) in the conformation (e f e e e f) with E_{rel} = 0.00 kcal.mol^{-1} of the neuropeptide Pol-RFamide II (Glu^1-Trp^2-Leu^3-Lys^4-Gly^5-Arg^6-Phe^7-NH_2)

Glu^1	Trp^2	Leu^3	Lys^4	Gly^5	Arg^6	Phe^7	NH_2
Glu^1	-0.55	-2.92	-0.27	-1.24	-1.16	0.47	-5.91
Trp^2		-1.40	-4.41	-2.14	-1.64	-6.07	-0.76
Leu^3			0.19	-1.70	-1.25	-0.45	0.01
Lys^4				0.43	-1.89	0.67	-0.11
Gly^5					1.22	-0.71	-0.41
Arg^6						-3.94	-3.58
Phe^7NH_2							-2.90

Table 4. Numerical values of dihedral angles of rotation about the backbond and side chain bonds in lowest-energy structures of the neuropeptide Pol-RFamide II.

ϕ_1	χ_{11}	χ_{12}	χ_{13}	ψ_1	ω_1	ϕ_2	χ_{21}
-75.42	-179.69	61.28	-124.	127.67	-168.21	-125.16	174.82
χ_{22}	ψ_2	ω_2	ϕ_3	χ_{31}	χ_{32}	χ_{33}	χ_{34}
90.93	135.16	-176.12	50.39	-171.19	64.00	178.53	172.58
ψ_3	ω_3	ϕ_4	χ_{41}	χ_{42}	χ_{43}	χ_{44}	χ_{45}
55.69	159.97	-86.39	-177.27	177.58	179.56	179.33	-118.52
ψ_4	ω_4	ϕ_5	ψ_5	ω_5	ϕ_6	χ_{61}	χ_{62}
-52.65	157.84	99.03	103.87	174.09	-157.87	-173.25	179.56
χ_{63}	χ_{64}	χ_{65}	χ_{66}	χ_{67}	ψ_6	ω_6	ϕ_7
179.12	-179.45	-0.11	179.66	179.75	-56.44	177.49	-148.76
χ_{71}	χ_{72}	ψ_7	ω_7				
65.406	-89.85	165.27	179.76				

Fourth, the minimum state of the whole molecule was obtained from the rotation of firmed tetrapeptide and firmed dipeptide around Gly^5. Then, the minimum energy states were calculated with respect to all the angles, but no changes were obtained. This confirms the reliability of the followed method.

In this work 784 possible isomers of the molecule were investigated, as explained above. As can be seen in Table 1, only the e f e e e f, e e e e e f and e e e e e e shapes were present in the [0-5] kcal/mol energy range.

The van der Waals' (nonvalent) interaction energy was relatively more effective in stabilisation than the torsional and electrostatic energy (Table 2). The low level of the torsional energy indicates that the molecular structure was unstressed when the van der Waals' contacts were present.

Energy parameters of the inter-monopeptide and among monopeptide-interactions are given in Table 3. As can be seen from the table, the electrostatic repulsion between the side chains of Lys^4 and Arg^6 and strong van der Waals' interaction between monopeptides Glu^1 and Phe^7 are the main properties of this conformation. Another property is the existence of the destructive interactions in the Leu^3-Lys^4 and Gly^5. The second and third important interactions are electrostatic and torsional interactions, respectively, beside the most important van der Waals' interaction in the stabilisation of the molecule. The structure of the molecule is weakly affected by environmental interactions because of the relatively small contribution of the electrostatic interactions and hydrogen bond energies. Consequently, the biological properties and activities of the molecule are conserved in various media with different physical and chemical properties.

There is a 3.08 kcal. mol^{-1}difference between the minimum energy and the next higher one. Therefore, there will be a favourable structural state of the molecule. Consequently, we can say that the molecule is a single functional one.

Explanation of the torsion angles

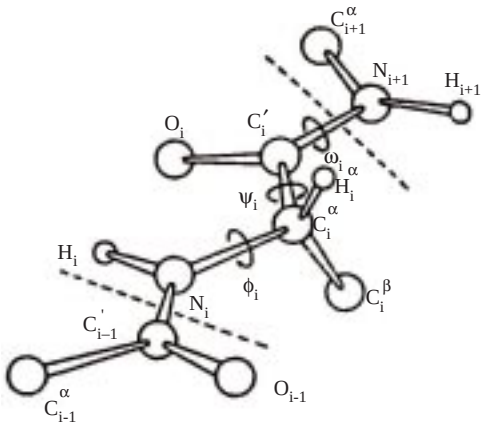

Figure A1 Perspective drawing of a section of polypeptide chain representing two peptide units.

The principal torsion angle describing rotation about N_i-C_i^{α} and C_i^{α}-$C_i^{'}$ are denoted by $\phi_i$and ψ_i, respectively, in a residue (such as *i.*). The rotation about $C_i^{'}$ - N_{i+1} is denoted by ω_i, where C and N are belong to the different residues (such as *i.*and i+1. residue.)

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