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Conformational Analysis of Linear Peptide (Glu^1-Leu^2-Leu^3-Gly^4-Gly^5-Arg^6-Phe^7NH_2)

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Conformational energy-minimization of the Sea Anemone and Sea Pansy neuropeptide Pol-RFamide (Glu^1-Leu^2-Leu^3-Gly^4-Gly^5-Arg^6-Phe^7NH_2) was carried out by molecular mechanics (MM). The linkage bonds were characterized by the torsion angles θ, ψ and ω and the side groups were characterized by the torsion angles $\chi_1, \chi_2, \chi_3 \ldots$ The energy-map for each monopeptide of the Pol-RFamide I was drawn in the range of -180° to 180° with increments of 20°. Conformation facilities for monopeptides were determined from these maps. These results were used in the analysis of the dipeptide (Glu^1-Leu^2). Then, the (Glu^1-Leu^2-Leu^3) tripeptide was examined using the calculated results for the dipeptide. Conformational analysis of the (Glu^1-Leu^2-Leu^3-Gly^4) tetrapeptide was performed using the low-energy values for the tripeptide. The space structure of the (Glu^1-Leu^2-Leu^3-Gly^4-Gly^5-Arg^6-Phe^7NH_2) neuropeptide was found as a result of minimization of energies by rotating the tetrapeptide (Glu^1-Leu^2-Leu^3-Gly^4) and the dipeptide (Arg^6-Phe^7NH_2) about the monopeptide (Gly^5).

Introduction

The neuropeptide Pol-RFamide I (Glu^1-Leu^2-Leu^3-Gly^4-Gly^5- Arg^6-Phe^7NH_2) was isolated from sea anemones and sea pansies by C. J. P. Grimmelikhuijzen, K. L. Rinehart and A. N. Spencer.[1] The conformational state of each residue in a neuropeptide is categorised as short-, medium- or long-range. Conformational energy computation on polypeptides and proteins requires reliable parameters to describe molecular structure and interaction energies. There are no experimental studies on thermodynamic or other phenomenological properties of the neuropeptide (Glu^1-Leu^2-Leu^3-Gly^4-Gly^5- Arg^6-Phe^7NH_2 : Pol-RFamide I). Therefore, the microscopic structure of the neuropeptide is not well known. Because of fluctuations in size and shape, it is difficult to establish the detailed structure from experimental studies alone. The molecular mechanics (MM) simulation method is well suited to investigating many particle systems microscopically, and so it fills the gap between theory and experiment.

Andrew et al.[11] computed conformational enegies for models of the disaccharide β-D-fructofuranosyl-(2→6)-β-D-glucopyranoside by molecular mechanics. On the theoretical side, ab initio molecular orbital calculations and molecular mechanics calculations have been employed to study the conformational structures

and related energy states of various molecules.[2−10] To investigate the local interactions in tripeptide sequences composed of amino acids having aromatic side chains, Oka et al.[12] carried out a theoretical conformational analysis of N-acetyl-N′-methylamide of the Phe-Phe-Phe tripeptide using a conformational energy-minimization procedure. Subramanian et al.[13] determined the crystal structure of the dipeptides of the dipeptides tert ($C_{10}H_{18}N_2O_5$; H_2O).

In the present study, we modeled the isolated molecule to obtain information about the most possible conformations of this neuropeptide Pol-RFamide I by computing the steric energies at different torsion angles of the central linkage bonds, namely, the θ, ψ and ω angles, as well as at the staggered angles of the side groups.

Theoretical

Conformational energy calculations of the (Glu^1-Leu^2-Leu^3-Gly^4-Gly^5-Arg^6-Phe^7NH_2) neuropeptide Pol-RFamide I were performed with an Empirical Conformational Energy Program for Peptides (ECEPP)[14]. 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 minimization, all the backbone angles θ, ψ and ω and side chain dihedral angles $\chi_1, \chi_2, \chi_3 \ldots$ were allowed to vary. All the best combinations of single-residues were used as starting conformations. Details of the conformational procedure as well as energy functions and semiempirical parameters used to evaluate nonbonded and electrostatic interactions, hydrogen bonding and torsional components have already been described using a semiempirical method.[15,16] The simulation of the neuropeptide Pol-RFamide I was carried out at an average temperature of 293 K. The hydrogen bond length and bond energy were determined in the conformational analysis and the results are given below.

Atomic groups in H bond	Bond length, Å	Energy, kcal/mol
NH(Glu^1), O^1(Gly^5)	2.51	-0.33
NH_2(Glu^1), O^1(Gly^1)	2.37	-0.50
O^1(Leu^2), NH(Gly^5)	2.04	-1.09
O^1(Leu^3), NH(Phe^7)	2.40	-0.46
O^1(Gly^4), NH(Arg^6)	2.51	-0.33
NH(Phe^7), CO(Phe^7)	2.30	-0.59
CO(Phe^7), NH_2	2.52	-0.33

These structures for Pol-RFamide I exhibit 732 possible backbone forms in principle for the neuropeptide. Only the lowest energy values relevant to the shapes are given in Table 1. In addition, the calculated values of the elements of the triangular matrices of the energy components for the three most preferable structures of Pol-RFamide I are given in Tables 2 and 3. These matrices provide a good illustration of all the inter- and intra-residue interaction, as well as the efficiency and energy distribution of the contacts. The numerical values of the dihedral angles of rotation about the backbone and side chain bonds in the lowest-energy structures of the neuropeptide Pol-RFamide I are given in Table 4.

Table 1. Distribution of conformations of the (Glu^1-Leu^2-Leu^3-Gly^4-Gly^5- Arg^6-Phe^7NH_2) neuropeptide Pol-RFamide I according to relative energies.

Shape*	Energy interval (kcal.mol^{-1})						
	0-1	1-2	2-3	3-4	4-5	5-10	> 10
ffffff						2	5
fffffe						4	10
ffffef						1	8
ffefff						1	13
ffffee						1	13
ffeffe						2	31
fefffe						5	20
fefeff							19
feffef						1	10
ffefef						1	7
fffeee							16
ffefee							27
feffee						9	34
feefee						3	27
feeeee							41
effffe							8
effffe						2	32
eeffff							4
effffe						1	1
eeefff							2
eefffe							5
eeeffe						2	18
eeefef	1					4	3
eeefee					3	4	21
eeeefe							42
eeeeee					1	3	39
eeeeff						2	16

*explained in the appendix.

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

	Glu^1	Leu^2	Leu^3	Gly^4	Gly^5	Arg^3	Phe^4NH_2	
Glu^1	-0.54	-2.90	-0.23	0.05	-1.87		0.42	-1.9
Leu^2		-0.83	-3.21	-0.90	-2.67		-2.82	-0.20
Leu^3			-0.83	-1.32	-1.11		-3.06	-4.03
Gly^4				1.21	0.17		-1.32	-1.04
Gly^5					1.24		-1.24	-0.39
Arg^6							-3.94	-3.58
Phe^7NH_2								-2.90

Table 3. The intra- and inter-residue interaction energies (kcal.mol^{-1}) in the conformation (fefeef) with E_{rel} =3.93 kcal.mol^{-1} of the neuropeptide Pol-RFamide I (Glu^1-Leu^2-Leu^3-Gly^4-Gly^5- Arg^6-Phe^7NH_2).

	Glu^1	Leu^2	Leu^3	Gly^4	Gly^5	Arg^3	Phe^4NH_2
Glu^1	-0.49	-2.87	-2.91	-1.70	-0.04	1.49	-2.29
Leu^2		-0.79	-1.87	-0.15	-0.01	-2.64	-0.30
Leu^3			-0.91	-0.85	-3.08	-3.51	-0.97
Gly^4				1.21	0.00	-1.31	-0.50
Gly^5					1.19	-1.11	-0.39
Arg^6						-3.95	-3.53
Phe^7NH_2							-2.91

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

ϕ_1	χ_{11}	χ_{12}	χ_{13}	ψ_1	ω_1	ϕ_2	χ_{21}
-75.16	-178.09	175.84	82.11	135.69	-162.79	-118.37	173.25
χ_{22}	χ_{23}	χ_{24}	ψ_2	ω_2	ϕ_3	χ_{31}	χ_{32}
62.59	179.23	177.04	135.22	-163.93	-134.57	174.46	61.59
χ_{33}	χ_{34}	ψ_3	ω_3	ϕ_4	ψ_4	ω_4	ϕ_5
179.14	175.87	-63.84	-161.14	92.07	-76.79	173.05	-94.57
ψ_5	ω_5	ϕ_6	χ_{61}	χ_{62}	χ_{63}	χ_{64}	χ_{65}
59.48	-176.36	-157.25	-173.25	179.56	179.12	-179.45	-0.11
χ_{66}	χ_{67}	ψ_6	ω_6	ϕ_7	χ_{71}	χ_{72}	ψ_7
179.66	179.75	-56.44	177.49	-148.76	65.40	-89.85	165.27
ω_7							
179.76							

Results and discussion

The structure of the neuropeptide Pol-RFamide I (Glu^1-Leu^2-Leu^3-Gly^4-Gly^5-Arg^6-Phe^7NH_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 (Table 1).

Conformation analysis of the Pol-RFamide I molecule was performed based on the minimization principle of energy:

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

Second, the minimum energy states of the Arg and $PheNH_2$ were combined to give the Arg-$PheNH_2$ dipeptides.

Third, the above tetrapeptide was combined with the dipeptide in terms of the Gly^5 monopeptide. Fourth, the minimum energy 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 angles, but no changes were detected. This confirms the reliability of the method used.

In this study, 1920 possible isomers of the molecule were investigated as explained above. As can be seen in Table 1, only the eeefef shape was present in the [0-4] kcal/mol energy range.

The Van der Waals interaction energy was relatively more effective in stabilization than torsional and electrostatic energy. The low level of torsional energy indicates that the molecular structure was unstressed when the Van der Waals contacts were present.

The energy parameters for the inter monopeptide- and among monopeptide-interactions are given in Table 2. As can be seen from this table, there was very weak electrostatic interaction between Glu^1 and Arg^6 due to their opposite charges. The second and third important interactions were the electrostatic and torsional interactions respectively, in addition to the most important Van der Waals interaction in the stabilization of the molecule. The structure of the molecule was mildly 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 was a large difference between the lowest energy level and the next highest. Therefore, the minimum energy state is greatly favoured. Consequently, it is possible to conclude that the molecule is a single functional one.

Appendix

Explanation of the Shapes

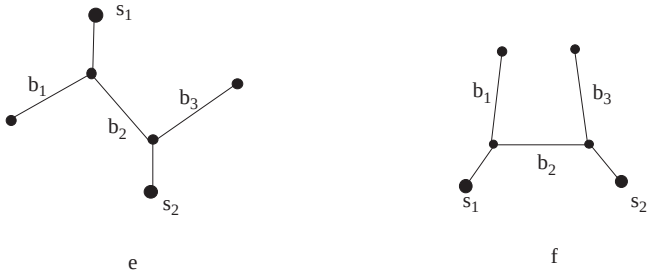

Figure A1

All backbone forms of a dipeptide can be classified into two types, referred to as shapes: folded (f) and extended (e). Two dipeptide backbone forms, B-B and R-R, are shown in Fig. A1. The s_1 and s_2 side chains are located at opposite sides of the axis and of the average plane defined by the main axis and these side chains. In practice, the side chains s_1 and s_2 cannot interact with each other, whatever the φ, ψ and $\chi_1, \chi_2 \ldots$ values. However, depending on the nature of the residue and conformational state, s_1 and s_2 may enter into strong stabilizing contacts with b_1, b_2 and b_3 elements. The R-R form, however, has the potential for effective $b_1 - b_3$ and $s_1 - s_2$ interactions.

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