THE INFLUENCE OF PRIMARY STRUCTURE OF *LEU*-CALLATOSTATINES 1 AND 2 ON FORMATION OF THEIR SPATIAL ORGANIZATION AND CONFORMATION MOBILITY

L.I. VELIYEVA, E.Z. ALIYEV
Baku State University, Az-1148, Z.Khalilov str., 23
Lala_Veliyeva@rambler.ru

The spatial structure of *Leu*-callatostatines 1 and 2 is studied by the method of theoretic conformation analysis. The stability quantitative evaluation of possible molecule conformation states based on calculation of intramolecular conformation energy value in conditions of dipolar medium is carried out.

Keywords: neuropeptides; structure; conformation analysis.
PACS: 87.80.-y

INTRODUCTION

The search and purposeful synthesis of compounds used for regulation of pest number is the one of actual problems in modern science. The neuropeptides synthesized by neurosecretory cerebrum cells of insect different types, in particular, *Calliphora Vomitoria* [1-3] belong to such compounds. The neuropeptides inhibit the synthesis and extraction of juvenile hormones in process of insect ontogenesis; take participation in neurotransmission and regulation of nervous system functions. The study of molecular mechanism basis of neuropeptide influence and formation of effective analogues of these compounds with prolonged action effect is the important aspect of investigations of neuropeptide functional activity. The study of spatial structure and conformation properties of *Leu*-callatostatines 1 and 2 is the aim of the present investigation. The neuropeptide chemical structure and calculation scheme of conformation states grown gradually di-, three- and penta-peptide fragments of molecules is given in fig.1.

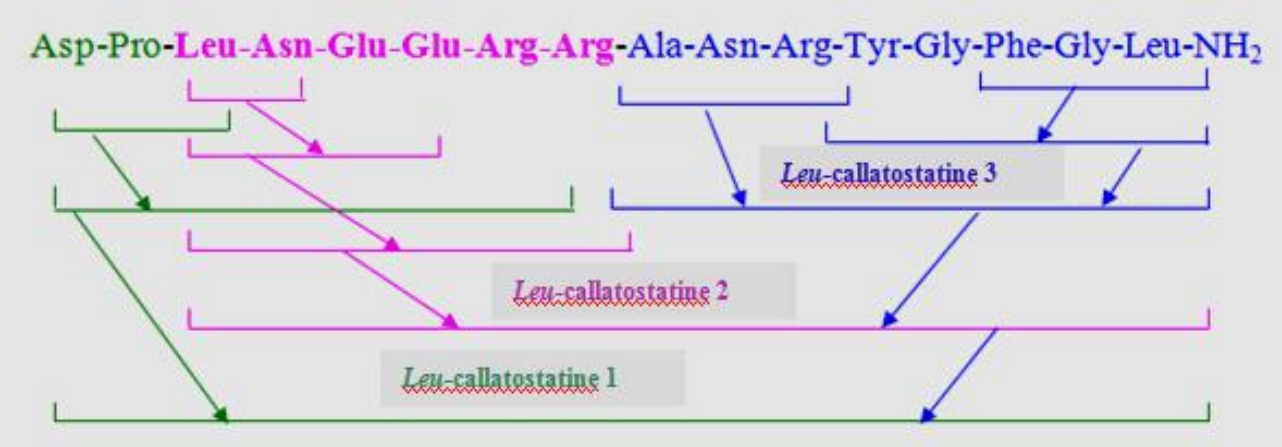


Fig.1. The calculation scheme of *Leu*-callatostatine molecule.

As it is seen from the figure, *Leu*-callatostatine 1 and *Leu*-callatostatine 2 are hexa- and tetra-decapeptide compounds including the eight amino-acid residuals of *Leu*-callatostatine 3. That's why the investigation of their spatial structure is carried out on the base of calculation results obtained for *Leu*-callatostatine 3 and molecule fragments according to scheme given in fig.1. The number decrease of initial structural approximations at conformation analysis of more complex fragments and whole molecule is the aim of the calculations of low-energy conformation states of di-, three- and other peptide sections and their overlapping fragments according to this scheme.

THE CALCULATION METHOD

The low-energy conformation molecule states are established by the way of minimization of total conformation energy in force field of atom-atom potential functions. At energy calculations the non-valence (E_{nv}) and electrostatic (E_{el}) atom interactions, hydrogen bonds (E_h) and torsion contributions (E_{tors}) for description of which the semiemperical potential functions given in works [4,5] are used. The calculations are carried out within framework of strong valence scheme, i.e. at fixed values of valence bond lengths and valence angles of amino-acid residual including in molecule chemical structure. The used system of potential functions and

131 H.Javid ave, AZ-1143, Baku
ANAS, G.M.Abdullayev Institute of Physics
E-mail: jophphysics@gmail.com

computational programs are approved for the big number of peptides and proteins by the authors of the given work and other investigators [7-10].

The parameterization given in works [4-6] is used for water surrounding modeling. The hydrogen bond energy is estimated with the help of Morse potential at dissociation energy of hydrogen bond equal to 1,5 kcal/mol corresponding to distance of bond NH…OC r=1.8Å for water solutions. The dielectric constant value is takes as 10 [4,5]. The conventional classification of peptide structures [6] is used at discussion of calculation results. The choice of structural variants at calculation of separate peptide conformations is carried out on the base of known values of dihedral angles (φ и ψ) corresponding to low-energy ranges of conformation card of R,B,L and P for each monopeptide. The count of dihedral angles corresponds to international nomenclature [6].

THE CALCULATION RESULTS

LEU-CALLATOSTANIE 2.

The general number of structural variants for *Leu*-callatostatine 2 is made up on the base of combination of 38 structures of *Leu*-callatostatine 3 and 17 low-energy conformation states of N-end hexapeptide overlapping by Ala^7 residual. The calculation results of hexapeptide fragments are given in tables 1 and 2 (the calculation intermediate stages aren't given because of the procedure uniformity of calculative experiment). They include 10 low-energy conformations from 31 acceptable ones for such shape fragments the relative energy of which satisfies to condition $E_{rel.} \leq 6$ kcal/mol.

Table 1.

The energy distribution of optimal conformations in shapes of Leu^1-Arg^6 fragment of *Leu*-callatostatine 2.

№	shape	Energy interval $E_{rel.}$ (kcal/mol)				
		0 ÷ 1	1 ÷ 2	2 ÷ 3	3 ÷ 4	4 ÷ 5
1.	*ffffee*	-	-	1	-	1
2.	*ffffef*	1	-	-	-	-
3.	*ffefee*	-	-	-	1	-
4.	*ffefef*	-	-	-	-	1
5.	*ffeffe*	1	-	1	-	3
6.	*ffefff*	-	-	1	-	1
7.	*ffeeff*	-	-	-	1	-
8.	*efefef*	-	-	-	-	1
9.	*efeffe*	-	-	1	-	-
10.	*feffff*	-	-	-	-	1
11.	*fefffe*	-	-	-	-	1
12.	*feffef*	-	-	-	-	1

Table 2.

The low-energy conformational states of *N*-end hexapeptide fragment of *Leu*-callatostatine 2.

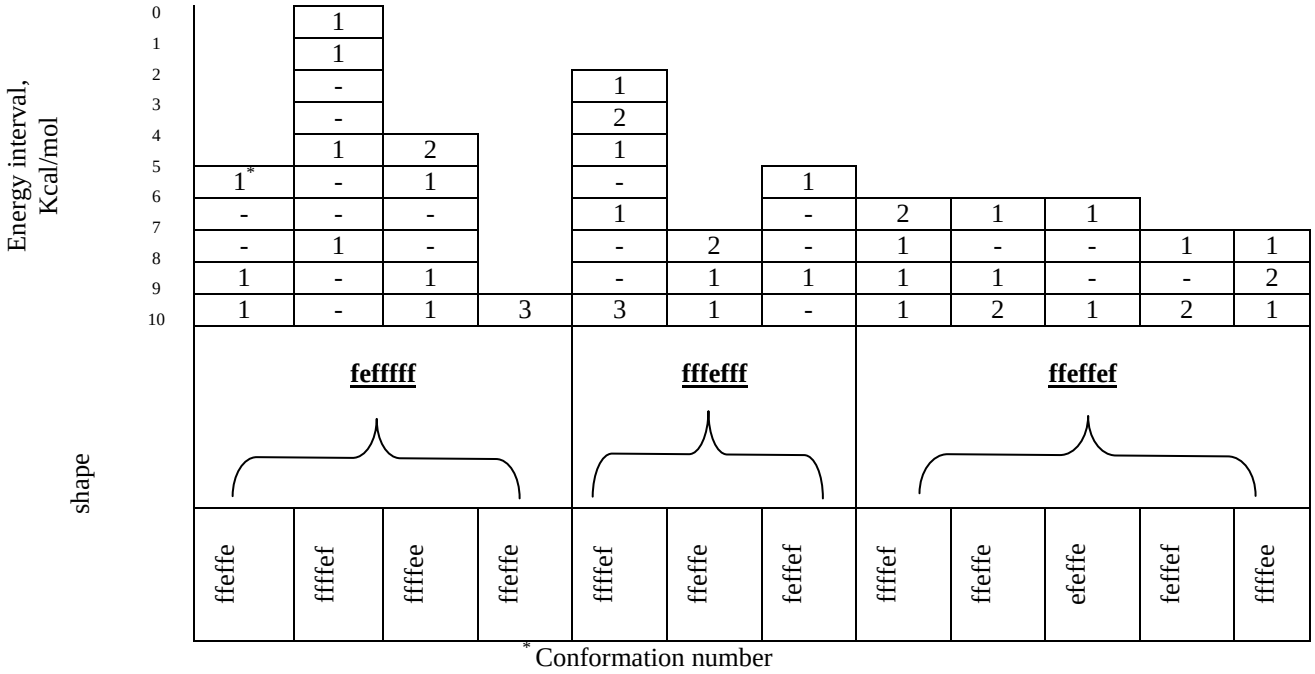
shape	conformation	Energy contributions (kcal/mol)				
		E_{nv}	E_{el}	E_{tors}	E_t	E_{rel}
ffeffe	$R_2R_{11}B_1R_2B_1L_3B$	-33.97	-2.62	5.79	-30.80	**0.00**
	$R_2R_{11}B_1R_2B_1L_3R$	-32.12	-2.34	5.68	-28.78	2.02
ffffef	$R_2R_{11}R_3R_2R_3L_2L$	-37.90	2.17	5.54	-30.19	0.61
ffffee	$R_2R_{11}R_3R_2R_3L_2B$	-36.97	2.90	5.83	-28.24	2.56
	$R_2R_{11}R_3R_2R_3L_2R$	-35.27	2.71	6.10	-26.46	4.34
ffefef	$R_2R_{11}B_1R_2R_3L_2L$	-31.39	0.73	4.74	-25.93	4.87
ffefff	$R_2R_{11}B_1R_2B_1L_2L$	-29.75	-1.07	4.58	-26.24	4.56
	$R_2R_{11}B_1R_2B_1L_3L$	-31.99	-2.22	5.79	-28.42	2.38
efefef	$B_2R_{31}B_3R_2R_3L_2L$	-31.20	1.46	3.53	-26.21	4.59
efeffe	$B_2R_{31}B_3R_2B_3L_3R$	-26.64	-2.14	5.30	-28.47	2.33
fefffe	$R_2B_{11}R_3R_2B_3L_3B$	-32.23	1.91	4.44	-25.88	4.92
feffff	$R_2B_{11}R_3R_2B_3L_3L$	-32/23	1.99	4.27	-25.97	4.83
feffef	$R_2B_{11}R_3R_3B_2R_2R$	-33.00	2.18	9.05	-26.13	4.67

The minimization of conformational energy of whole neuropeptides is carried out at variation of 81 dihedral angles in main and side chains of residuals for different type structures of molecule peptide composition. Only 47 conformations have the energy the value of which satisfies to condition $\Delta E_{rel.} \leq 10$ kcal/mol from 285 calculated conformations.

All of them present the combination of more beneficial states of Leu^1-Arg^6 and Ala^7-Leu^{14} fragments of *Leu*-callatostatine 2 molecule (see tables 2 and 3) in spite of the strong difference in energies of obtained structures.

Table 3.

The distribution of low-energy conformations on shapes of neuropetide *Leu*-callatostatine 2



The formation of far interactions [8] which don't destroy the interresidual interactions formed on free fragments of calculated molecule is the important factor in formation of neuropeptides spatial organization. Thus, the necessity in coincidence of near, middle and far interactions [7,8] promoting to step-by-step oligopeptide packing into native conformation is confirmed. The side chains of amino-acid residuals, from orientation of which depends the rapid convergence of conformational energy in minimization procedure and finding of its global minimum. That's why the finding of local minimums in neighborhood of obtained values of conformational energy by the way of construction of conformational card series φ-ψ and χ_i - χ_{i+1} is the important stage.

They allow us to eliminate the appearing destabilizing contacts at fragment integration in some cases and make the energy minimization at variation of variable limited number.

The investigation results of *Leu*-callatostatine 2 spatial construction are given in tables 3 and 4. The distribution of neuropeptide low-energy conformations in dependence on peptide framework structural type, i.e. the conformation classifications by shapes is given in first table. As it is seen from this table not all combinations of low-energy fragments Leu^1-Arg^6 and Ala^7-Leu^{14} lead to formation of energy profitable and steric accessible states of neuropeptide.

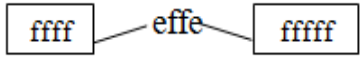
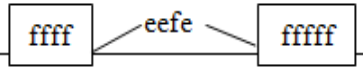
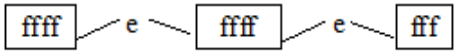
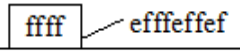
The stability of calculated structures in dependence on stereochemical disposition of amino-acid residuals and their side chains in linear sequence of neuropeptide is determined by the presence of ordered regions in its spatial organization. All neuropeptide conformations can be classified in four groups due to such character elements.

I group consists in the conformations having two regular sections placed on neuropeptide opposite ends. They are characterized by formation of two turns of α-spiral divided by Arg^6 - Arg^9 section having the charged side chains of arginine residuals. The analysis of interresidual interactions in such conformations shows that side chains locate on the surface of neuropeptide compact structure and don't take participation in stabilization of their spatial structure. The four low-energy conformations of *ffffeffeffff* and *ffffeefeffff* shapes in group I differ by the peptide chain state in *Ala* and different orientations of Arg^6 and Arg^9 side chains. The best neuropeptide conformation ($E_{rel.}$= 0 kcal/mol, table 4) consists in α-spiral conformation of *Leu*-callatostatine 3 and one from low-energy conformations of Leu^1-Arg^6 hexapeptide.

The low-energy conformations of second group contain three α-spiral sections in neuropeptide structure and differ by conformational state of residuals in sixth (Arg^6) and eleventh (Gly^{11}) positions of amino-acid linear sequence. The glycine doesn't contain the side chain in difference from rest residuals, that's why *C*-end α-spiral fragment is presented by two possible orientations of relatively less mobile *N*-end section. The difference on conformation relative energy of this group varies in limits 0-1 kcal/mol. The surface of compact structures of this neuropeptide group is represented by only one positively charged side chain of Arg^6 residual. That's why such structures should have the less reactivity in comparison with I group conformations.

Table 4.

The low-energy conformational states of *Leu*-callatostatine 2 neuropeptide and contributions from non-valence, electrostatic and torsion interactions.

Classifi-cation by groups	IIIShape	Conformation	Energy contributions (kkal/mol)				
			$E_{nv.}$	$E_{el.}$	$E_{tors.}$	E_t	$E_{tel.}$
I		$R_2R_{11}R_3R_2B_3R_2RB_{11}R_2B_3PB_2PB_{21}$ $R_2R_{11}R_3R_3B_1R_2RB_{11}R_2B_3PB_2PB_{21}$	-65.60 -65.29	4.15 3.12	24.51 26.43	-36.94 -35.74	**0.0** 1.2
		$R_2R_{11}R_3R_2R_3L_2BL_{11}R_2B_3PB_2PB_{21}$ $R_2R_{11}R_3R_2R_3L_2RB_{21}R_2B_3PR_2RR_{21}$	-65.45 -63.47	4.21 2.98	29.10 28.75	-32.14 -31.74	4.6 5.2
II		$R_2R_{11}R_3R_2B_1R_2RR_{11}R_2R_3PB_2PB_{32}$ $R_2R_{11}R_3R_2B_1R_2RR_{11}R_2R_3PB_2PB_{31}$ $R_2R_{31}R_3R_3B_3R_2RR_{11}R_2R_3PB_2PB_{32}$ $R_2R_{31}R_3R_3B_3R_2RR_{11}R_2R_3PB_2PB_{31}$	-65.42 -65.42 -65.71 -64.37	3.77 3.12 3.56 2.11	27.21 28.96 29.01 29.52	-34.44 -33.34 -33.14 -32.74	2.3 3.6 3.8 4.2
III		$R_2R_{11}R_3R_2B_3R_2RR_{22}B_3B_1PR_1PR_{32}$ $R_2R_{11}R_3R_3B_1R_2RR_{21}B_3B_1PR_1PR_{32}$	-60.84 -64.63	2.60 4.27	28.00 30.62	-30.24 -29.74	6.7 7.2
IV	ffeffeffeffef	$R_2R_{11}B_1R_2B_1L_3BL_{22}L_1B_1PR_1PR_{31}$ $R_2R_{11}B_1R_2B_1L_3RR_{22}B_3B_1PR_1PB_{21}$ $R_2R_{11}B_1R_2B_1L_3RR_{32}B_3B_1PR_1PR_{32}$	-60.08 -59.99 -59.67	2.70 2.74 3.05	28.64 29.41 29.28	-28.74 -27.84 27.34	8.2 9.1 9.6
	efeffeffeffef	$B_2R_{31}B_3R_2B_3L_3RR_{22}B_3B_1PR_1PB_{21}$ $R_2R_{11}B_1R_2B_1L_3RR_{32}B_1B_1PR_1PB_{32}$	-61.25 -60.54	2.98 3.11	31.13 30.39	-27.14 -27.04	9.8 9.9

* Note: The energy from formation of hydrogen bonds is included in E_{nv}.

The conformations, characterizing by α-spiral conformation of *N*-end penta-peptide fragment are combined in group III. They present by big number of conformational states on *C*-end fragment, but only two of them have the energy the value of which doesn't exceed the energy of neuropeptides global conformation more than 10 kcal/mol.

Those conformations which don't consist in the strong nucleations or stable regular elements in their spatial organization form the fourth group. The low-energy representatives of such structures are given in table 4.

LEU-CALLATOSTATINE 1.

As the difference in primary structure of *Leu*-callatostatine 1 is caused by the presence of two Asp^1 and Pro^2 residuals on *N*-end fragment of the last neuropeptides, then the investigation of its spatial organization leads to step-by-step residual generation of linear chain of *Leu*-callatostatine 2. The serially attachable residuals *Pro* and *Asp* are considered in field of all structures of four types obtained by us for *Leu*-callatostatine 2. Moreover, it is taken into consideration that the proline residual not only limits the region of acceptable conformational states for Asp^1 (only *B* and *L* regions of conformational space are possible for it), but it itself has the limited conformational mobility in *B* and *R* regions. The total energy minimization of *Leu*-callatostatine 2 neuropeptides is carried out for 15 conformational states of whole molecule. The calculated results are summarized and the energy characteristics of 6 conformations of *Leu*-callatostatine 1 neuropeptide are given in table 5.

Table 5.

The low-energy conformational states of *Leu*-callatostatine 1 molecule.

shape	conformation	Energy contributions (kcal/mol)				
		E_{nv}	E_{el}	E_{tors}	E_t	E_{rel}
eeffffeffffff	$B_1BR_2R_{11}R_3R_2B_3R_2RB_{11}R_2B_3PB_2PB_{21}$	-75.93	11.22	16.90	-47.82	0.00
eefffeefffff	$B_3BR_2R_{11}R_3R_2R_3L_2BL_{11}R_2B_3PB_2PB_{21}$	-75.42	15.76	12.15	-47.51	0.31
eefffeffffefff	$B_1BR_2R_{11}R_3R_2B_1R_2RR_{11}R_2R_3PB_2PB_{32}$	-74.34	15.11	11.72	-47.50	0.32
effffeffffff	$B_2RR_2R_{11}R_3R_2B_3R_2RB_{11}R_2B_3PB_2PB_{21}$	-72.40	15.32	12.32	-44.71	3.11
effffeefffff	$B_2RR_2R_{11}R_3R_2R_3L_2BL_{11}R_2B_3PB_2PB_{21}$	-79.20	19.63	12.23	-47.34	0.48
effffeffffefff	$B_2RR_2R_{11}R_3R_2B_1R_2RR_{11}R_2R_3PB_2PB_{32}$	-76.16	20.17	12.27	-43.73	4.09

They reveal the succession of structural types of *Leu*-callatostatine 2. The conformational stability is mainly caused by dispersion contacts between residuals. The low-energy and interconnected changes of side chain conformations are possible in narrow limits their values that confirm the fact that their optimal positions are defined not by steric limits but by interactions with each other.

[1] *H. Duve, A.H. Johnsen, A.G. Scott, C.G. Yu, K.J. Yagi, S.S. Tobe, and A. Thorpe.* "Callatostatins: Neuropeptides from the blowfly Calliphora vomitoria with sequence homology to cockroach allatostatins" (1993) Proc. Natl. Acad. Sci. USA, **90**, 2456- 2460.

[2] *H. Duve, A.H. Johnsen, J.L. Maestro, A.G. Scott, P.D. East, A. Thorpe.* "Identification of the dipteran Leu-callatostatin peptide family: the pattern of precursor processing revealed by isolation studies in Calliphora vomitoria" (1996) Regul.Pept., **67**, 11-19.

[3] *H. Duve, A. Thorpe.* "Distribution and functional significance of Leu-callatostatins in the blowfly Calliphora vomitoria" (1994) Cell Tissue Res., **276**, 367-379.

[4] *F.A. Momany, R.F. McGuire, A.W. Burgess, H.A. Scheraga.* "Energy parameters in polypeptides: VII. Geometric parameters partial atomic charges, nonbonded interaction for naturally occurring amino acid" (1975) J.Phys.Chem., **79**, 2361-2381.

[5] *E.M. Popov.* The Structural Organization of Proteins (in Russian), Nauka, Moscow, 1989, 352 pp.

[6] IUPAC-IUB Commision on Biochemical Nomenclature Abbreviations and symbols for description of conformation of polypeptide chains (1974) Pure Appl. Chem., **40**, 291-308.

[7] *T.K. Hayes, X.C. Guan, V. Johnson, A. Strey, S.S. Tobe.* "Juvenile-hormone binding components of locust fat-body". ***Arch. Insect Biochem. and Phys.,*** 1995, v.28, N.3, p.291-309.

[8] *D. Veelaert, L. Schoofs, S.S. Tobe, C.G. Yu, H.G.B. Vullings, F. Couillaud, A. Deloof.* "Biosynthesis and release of juvenile-hormone during the reproductive cycle of the ring-legged earwing". ***Comparative Biochem.and Phys. (pharmocology, toxicology and endocrinology),*** 1995, v.110, N.3, p.241-251.

Recevied: 12.04.2016