SIMULATION SPATIAL STRUCTURE OF AMYLOID BETA-PEPTIDE (31-35) DETERMINED BY MOLECULAR MECHANIC METHOD

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The spatial structure and conformational flexibility of Amyloid β -peptide (31-35) have been investigated by molecular mechanic method. It is revealed that this molecule can exist in several stable conformational states. The energy and geometrical parameters for each of low-energy conformations are obtained. The conformationally rigid and labile segments of this molecule were revealed.

Keywords: Amyloid β- peptide (31-35), Alzheimer's disease, spatial structure, function, conformation, pentapeptide
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1. INTRODUCTION

The amyloid β- peptide (AβP) is known to cause the activation of apoptotic cascades, leading to neuronal death in Alzheimer's disease (AD) [1-4]. Amyloid β- peptide (AβP), a major protein component of the plaques, is a 39–43 amino acid peptide derived from a larger transmembrane protein, amyloid precursor protein (APP). It is well established that AβP possesses neurotoxic activity. AβP neurotoxicity has been associated to peptide self-aggregation, which leads to the formation of amyloid-like fibrils and eventually to neuronal cell death through apoptosis. Some recent studies confirmed that Aβ (31–35), i.e. IIGLM, (Ile–Ile–Gly–Leu–Met), which is a shorter sequence of AβP, can also induce apoptosis in the cortical and hippocampal neurons as Aβ (25–35) does has also been demonstrated in neuronal PC 12 cells and rat cerebellar granule cells [1-4]. In work [2] are used *X*- ray diffraction and electron microscopy to investigate the structure of the assemblies formed by Aβ (25-35) peptides and of various length sequences. In this article it is proposed that the tachykinin- like Aβ (31-35) peptide is a turn exposed at the Aβ oligomer surface where it could interact with the ligand- binding site of the tachykinin *G*- protein-coupled receptor [2].

A synthetic fragment (31–35) of β- amyloid peptide was used in cultured cortical neurons to examine whether this smaller sequence could trigger apoptotic degeneration *in vitro* by using morphological, biochemical and flow- cytometric examinations. On the other hand, it is known that this Ab derived pentapeptide although not exhibiting aggregation phenomena, is able to determine a large number of toxic effects, including the activation of apoptotic pathways in cultured cortical neurons [4]. Here, in order to gain more insight into the mechanism of spatial formation of Aβ (31-35) peptide in nonpolar solution, which particularly stabilizes α-helical conformation, we studied the secondary-structural elements of the Aβ (31-35) peptide in vacuum and polar solution by molecular mechanics simulations. The α -helical environment around Met35 was completely abolished as indicated by circular dichroism (CD) -spectroscopy [5]. The study of the conformational properties of Aβ peptides in their soluble form constitutes a basic approach to design of molecules with "anti-amyloid" activity and to develop anti- Alzheimer therapies. The diversity of biological functions of peptide molecule is undoubtedly connected to its conformational properties. The determination of conformational particularities of biologically active peptide molecules is a necessary stage in the study structured-functional relationships of these molecules. It is difficult to predict the active peptide conformation, which realized in complex with the receptor, since most of small linear molecules exist in aqueous and other environments as set of low-energy conformations with comparative stability. In order to elucidate the mechanism of action of the peptide the investigation of the native three dimensional structure is necessary that first of all requires the information about of the full set of low energy and consequently the potentially and physiologically active conformations of this molecule. The major aim of the present article is the investigation of the three-dimensional structure and conformational flexibility for Aβ (31-35), with the purpose of getting insight into basic structural requirement that determine ligand-receptor interaction. The conformational properties of Aβ (31-35) peptide molecule have been investigated by molecular mechanic method, which allow to determine a whole set of energetically preferred conformers of peptide molecule.

2. METHOD

Molecular mechanics (MM) study of Aβ (31-35) conformation involves multistaged extensive computations of even-increasing fraqments, with a set stable forms of each preceding step used as a starting set in the next step. Only those conformations are

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retained whose energies are smaller than some cut-off values. The sequential method was used, combining all low-energy conformations of constitutive residues [5]. The conformational potential energy of a molecule is given as the sum of the independent contributions of nonbonded, electrostatic, torsional interactions and hydrogen bonds energies. The first term was described by the Lennard-Jones 6-12 potential with the parameters proposed by Scott and Scheraga. The electrostatic energy was calculated in a monopole approximation corresponding to Coulomb's law with partial charges of atoms as suggested by Scott and Scheraga. An effective dielectric constant value ε=1 for vaccum, ε=4 for membrane environment and ε=80 for water surrounding is typically used for calculations with peptides and proteins, which create the effects of various solutions on the conformations of peptides by MM method [5]. The hydrogen bond energy is calculated based on Morse potential Bonding lengths and angles are those given by Corey and Pauling [6] and are kept invariable; the ω angle of the peptide bond was fixed at 180°. The torsional energy was calculated using the value of internal rotation barriers given by Momany et al [7]. Computations were carried out on the computer using universal programs complex [8]. The dihedral rotation angles were counted according to the IUPAC-IUB [9]. Symbols are used to represent the regions of conformational space situated around backbone dihedral angles values (φ, ψ) are: 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°). Rotamer 1, 2, 3: side chain bonds χ_1= 60°, 180°, -60° respectively. Each conformational state of a residue is characterized by $X^{n}_{i,j}$…, where *X* characterizes the backbone φ, ψ angle regions (*B*, *R*, *L* or I), n is the number of a residue in the sequence and subscripts i, j ,… specify the position of the side chain χ_1, χ_2…, respectively, so that *i* or *j* =1 corresponds to the angle χ (χ_1 or χ_2) in the range 0° to 120°; a value of two corresponds to the angle 120° to -120° and three to -120° to 0°.

3. RESULTS AND DISCUSSION

Conformational study of the pentapeptide Aβ (31-35) was carried out in three stages, in each of which were used results of preceding stage. In turn, stages are divided on consecutively decided structured problems. The first stage of calculation included consideration of conformational possibilities of dipeptide fragments Ile-Ile and Leu -Met according to the calculation scheme. Calculations of fragments were conducted for the reason reducing a number of possible initial variants for pentapeptide. But the initial variants of the small fragments were formed on the base of low-energy conformations of the corresponding monopeptides. The optimal backbone forms in the relative 0-5 kcal/mole energy interval of the pentapeptide Ile-Ile-Gly-Leu-Met-NH_2 are presented in Table 1. Then on the base low-energy conformations of the fragments were calculated the stable conformations of the pentapeptide with the *C*-terminal amide group NH_2. The variants for pentapeptide were taken into account conformational particularities of both dipeptides and Gly monopeptıde. Besides, in initial variants of the pentapeptide were taken different orientations of the side chains in peptide chain depending on the type of 20 backbone forms. Only α-helical conformations of these forms with different intermolecular interactions are entered in the interval of relative energy 0-3 kcal/mole. Other low-energy conformations belong to the 11 backbone forms (table1).

The energy contributions of all calculated types of interactions of preferred conformations of the pentapeptide Ile-Ile-Gly-Leu-Met-NH_2 are shown in table2 [11].

In the α- helical global conformation carbonyl group of backbone of Ile1 is very approach with NH group of Met 5 that favours a forming the efficient interactions between them, which promotes shaping the hydrogen bond. It is necessary to note that the essential energy contributions of specific interactions between residues Ile1 and Leu4 are stabilize factor of the conformational stability of these conformations. The molecular model of the preferred conformation of pentapeptıde Aβ (31-35) is represented in the fig. 1.

The spatial model of pentapeptide were built in HyperChem 8.01 [10]. The electrostatic interactions are eliminated between *N*- and *C*-terminal groups that is reflected on the value of energy contribution of the interresidue interactions of the residues. As a result of the calculation for this pentapeptide most favored turns out to be only one conformation with completely turned form of chains. In this conformation C-terminal pentapeptide does not prevent a formation stable intermolecular hydrogen relationship between side chains of the therminal residues. The energy of intra- and interresidues interactions of three stable conformations of the Ile-Ile-Gly-Leu-MetNH_2 pentapeptide is presented in Table 3.

Other optimal conformations are not important for the collecting with the receptor. The values of dihedral angles of the lowest energy conformations of the Aβ (31-35) pentapeptide are presented in table 4.

Then possible expect that the most probable biologically active conformation for Aβ (31-35) pentapeptide is conformation with the form of the frame RRRRR, stabilized by intramolecular hydrogen bond between backbone atoms of Ile1 and Met5.

Thus, the conformational study of Aβ (31-35) pentapeptide enabled the estimation of the role of each of the substituted residues in the structure formation have been carried out. The comparison of specific features of low-energy conformations of pentapeptide revealed general structural criteria of this molecule, which may be necessary for their biological activity.

To summarize, the results of the theoretical conformational analysis of Aβ (31-35) combined with the data on their biological activity indirectly supported by physicochemical data allow an assumption that, when binding to receptor, this pentapeptide prefer the conformation with *a* alpha-helical structure.

Table 1.

The optimal backbone forms in the relative energy interval of the pentapeptide Ile-Ile-Gly-Leu-Met-NH_2.

№	Backbone form	The relative energy interval (kcal/mole)					
		0-1	1-2	2-3	3-4	4-5	>5
1.	BBBBB	-	-	-	-	2	79
2.	BBRBB	-	-	-	-	2	79
3.	BBRRR	-	-	-	-	3	78
4.	BBRBR	-	-	-	-	5	76
5.	BRBBB	-	-	-	2	8	71
6.	BRRBB	-	-	-	-	1	80
7.	BRBRR	-	-	-	2	2	77
8.	BRRRR	-	-	-	-	1	80
9.	RBBBB	-	-	-	1	2	78
10.	RBRRR	-	-	-	-	1	80
11.	RBBRR	-	-	-	2	2	77
12.	RBBBR	-	-	-	1	2	78
13.	RRBBB	-	-	-	-	2	79
14.	RRRBB	-	-	-	2	2	77
15.	RRBRR	-	-	-	2	1	77
16.	RRRRR	7	2	2	-	1	71
17.	BBPBR	-	-	-	1	-	79
18.	RBLBB	-	-	-	1	1	78
19.	RRPRR	-	-	-	1	-	79
20.	RBPBR	-	-	-	1	2	77

Table 2.

Energy contributions of all calculated types of interactions of preferred conformations of the pentapeptide Ile-Ile-Gly-Leu-Met-NH_2.

№	Conformation	Energy contributions (kcal/mole)				E_{rel}
		E_{nb}	E_{el}	$E_{tor.}$	$E_{abs.}$	
1.	$R_{32}R_{32}RR_{22}R_{32}$	-22.1	4.1	3.0	-15.0	0
2.	$R_{32}R_{32}BR_{32}R_{32}$	-18.2	4.3	1.8	-12.0	3.0
3.	$R_{32}B_{22}BR_{32}R_{32}$	-18.0	4.3	1.8	-11.9	3.1
4.	$B_{22}R_{32}BB_{22}B_{32}$	-19.2	4.0	3.4	-11.9	3.1
5.	$B_{12}B_{12}PB_{22}R_{32}$	-19.0	3.8	3.4	-11.8	3.2
6.	$R_{22}B_{12}LB_{32}B_{32}$	-19.3	4.2	3.4	-11.7	3.3
7.	$B_{22}R_{32}BR_{32}R_{32}$	-18.5	4.2	2.9	-11.4	3.6
8.	$R_{32}B_{22}BB_{32}B_{32}$	-17.0	4.0	1.8	-11.3	3.7
9.	$R_{22}R_{22}PR_{32}R_{32}$	-17.2	4.4	1.6	-11.2	3.8
10.	$R_{22}B_{12}BR_{32}R_{22}$	-17.1	4.3	1.8	-11.1	3.9
11.	$B_{12}R_{22}BR_{22}R_{22}$	-17.2	4.2	2.1	-11.0	4.0
12.	$R_{22}B_{12}PB_{22}R_{32}$	-17.6	3.8	2.7	-11.0	4.0
13.	$R_{32}R_{32}BB_{32}B_{32}$	-16.5	4.2	2.0	-10.3	4.6
14.	$B_{22}B_{22}BB_{32}B_{32}$	-16.2	3.8	2.0	-10.4	4.6

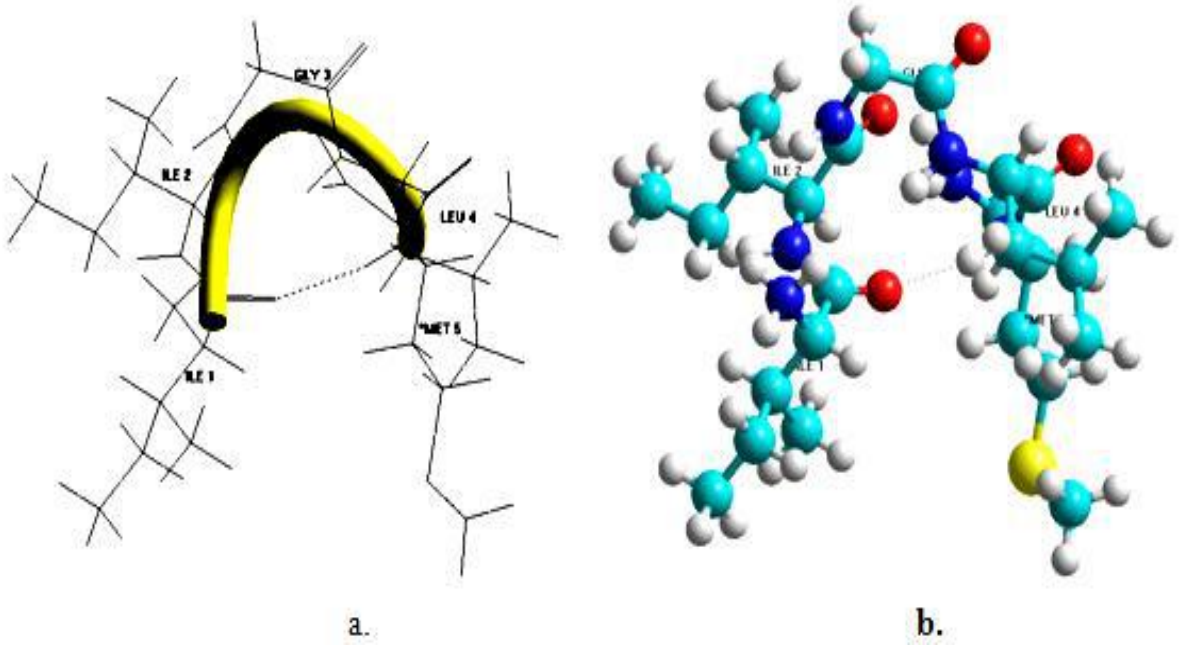

Fig. 1. The molecular model of lowest conformation of Ile-Ile-Gly-Leu-MetNH$_2$ Aβ (31-35) pentapeptide: a) secondary structure – α -helix turn shown in yellow thick line, b) in balls and cylinders. Hydrogen bond is shown dashed line.

Table 3.

The energy of intra- and interresidues interactions of the stable conformations of the Ile-Ile-Gly-Leu-MetNH$_2$ pentapeptide. I- $R_{22}R_{32}RR_{22}R_{32}$; 2- $B_{22}R_{32}BB_{32}B_{22}$; 3- $B_{22}R_{32}BB_{32}B_{22}$

	Ile^1	Ile^2	Gly^3	Leu^4	Met^5	
1 2 3	0.8 -0.3 -0.2	-2.8 -2.7 -2.5	-2.0 -2.2 -1.3	-3.7 -2.8 -3.0	-2.8 -0.1 0.0	
		1.6 0.3 0.3	0.7 -0.8 -0.7	-1.1 -1.3 -1.7	-2.5 0.0 -0.1	Ile^2
			1.2 1.2 1.2	0.7 -0.5 -1.2	-0.7 -0.9 -0.5	Gly^3
				-1.0 -0.7 -0.7	-3.7 -2.2 -1.6	Leu^4
					-2.1 -2.5 -2.4	Phe^5

Table 4.

The values of dihedral angles of the lowest energy conformations of the Aβ (31-35) pentapeptide.

Aminoacid	Dihedral angle	Lowest-energy conformations		
		$R_{22}R_{32}RR_{22}R_{32}$	$B_{22}R_{32}BB_{32}B_{22}$	$B_{22}R_{32}BR_{32}R_{32}$
Ile	φ	-71	-148	-146
	ψ	-52	147	139
	ω	177	179	179
	χ1	-61	185	178
	χ2	184	186	185
	χ3	177	169	168
	χ4	184	187	186
Ile	φ	-70	-92	-106
	ψ	-34	-59	-58
	ω	-181	179	184
	χ1	-61	-59	-58
	χ2	183	186	187
	χ3	177	173	174
	χ4	184	189	189
Gly	φ	-59	-80	-82
	ψ	-39	91	95
	ω	-177	177	189
Leu	φ	-81	-108	-98
	ψ	-63	107	-60
	ω	-173	182	174
	χ1	175	197	-56
	χ2	64	172	176
	χ3	60	191	186
	χ4	58	180	180
Nle	φ	-92	-121	-120
	ψ	-52	141	-60
	ω	-181	181	181
	χ1	-60	-60	-61
	χ2	180	181	180
	χ3	180	181	180
	χ4	181	180	180
E_{total}(kcal/mole)		-15.7	-11.87	-11.39

4. CONCLUSION

Thus, on the basis of conformational studies of amiloid β-peptide (31-35) molecule it has been suggested that the biologically active conformation of this peptide at its receptor is turned structure in solution. The obtained data allow one conclude that, in structures, where is formed a beta-turn at the *N*-terminal segment and residues have only one local minimum. The investigation results therefore indicate that a concrete type of the β-peptide (31-35) structure will essentially depend on the conditions under which the given molecule functions.

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