CONFORMATIONAL STUDY OF THE *N*-TERMINAL PENTAPEPTIDE FROM GUANYLYL CYCLASE *A*

N.T. SULEYMANOVA, I.N. ALIEVA, D.I. ALIEV, N.M. GODJAYEV
Baku State University
Z. Khalilov str.23, Baku, 370148

The conformational peculiarities of the Arg-Thr-Tyr-Trp-Leu *N*-terminal pentapeptide from catalytic domain of the guanylyl cyclase *A*, the atrial natriuretic peptide receptor have been investigated by theoretical conformational analysis method. The energy and geometrical parameters corresponding to the optimal conformers of the fragment are obtained.

INTRODUCTION

Guanylyl cyclase (GC-*A*) is the receptor for the atrial natriuretic peptide (ANP) [1-3]. ANP binding to GC-*A* has been demonstrated by both ligand binding analysis and affinity cross-linking studies [4,5]. ANP directly activates GC-A purified from mammalian tissues and increases cGMP level in a concentration- and time- depended fashion in a variety of cells [6-9].

The deduced primary sequences of the natriuretic peptide receptors predicted a protein with a single transmembrane domain that divides an extracellular ligand- binding domain from an intracellular domain. Deletion mutagenesis studies have demonstrated that the intracellular domain serves regulatory, dimerization, and catalytic functions [6]. This regulatory domain has sequence similarity with protein kinases, particularly the protein tyrosine kinases, which are also single transmembrane domain receptors [10]. The sequences of the *C* - terminal catalytic domains are highly homologous to those of the α - and β - subunits of soluble GC (sGC) and have limited identity with the two catalytic domains of adenylyl cyclase [11]. We report here on the conformational study of the Arg-Thr-Tyr-Trp-Leu *N*-terminal pentapeptide fragment from catalytic domains of the GC-*A*. A molecular model and calculation scheme are illustrated in Figure 1.

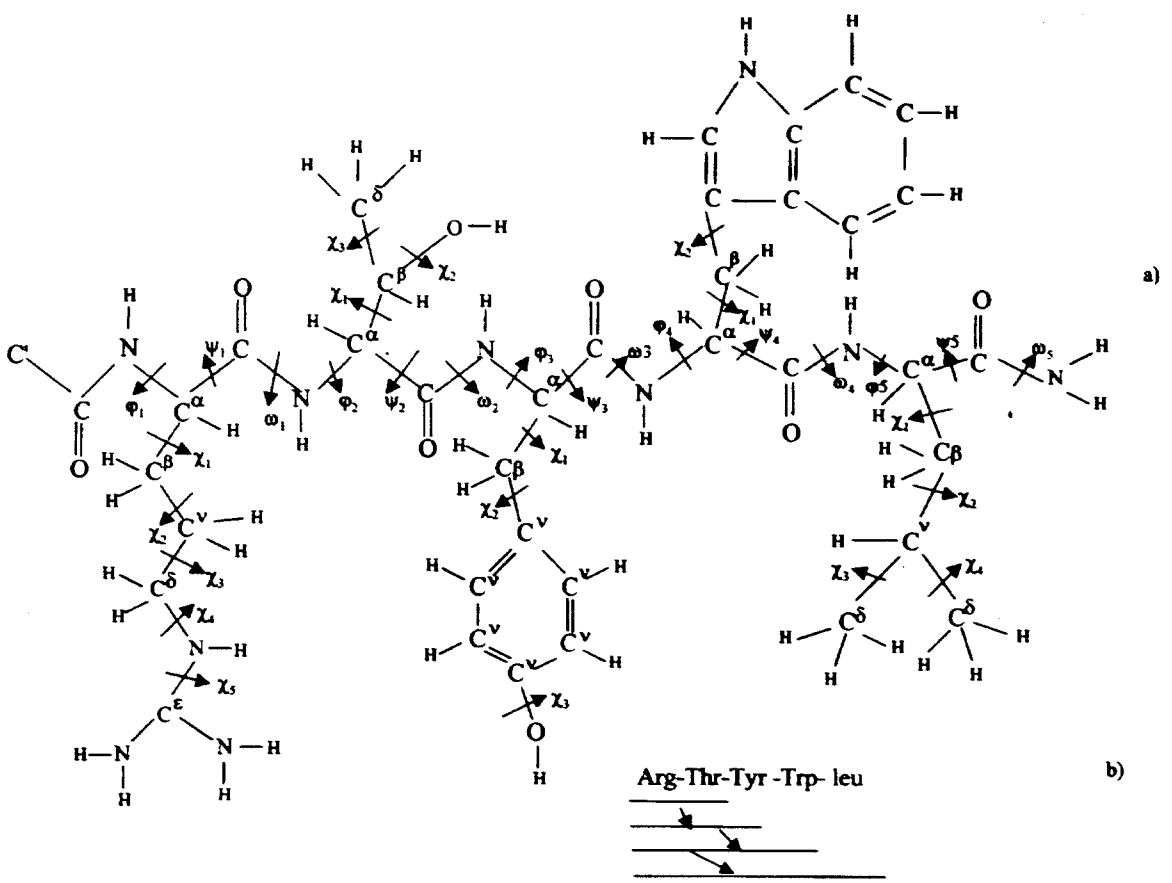

Fig.1. A molecular model (a) and calculation scheme (b) of pentapeptide from catalytic domain.

METHOD AND MODEL FOR CALCULATION

Theoretical conformational analysis was used to study the low-energy conformations available to *N*-terminal pentapeptide fragment of the GC-A. The calculations were carried out on the basis of the approach as described in Ref [12]. The conformational potential energy is considered as the sum of independent contributions of nonbonded (E_{nb}), electrostatic (E_{el}), torsional (E_{tor}) interactions and intramolecular hydrogen

bonding energies (E_{hb}).The energy of nonbonded interactions has been described by Lennard-Jones potential with the parameters proposed by Momany et.al. [13]. A contribution of electrostatic interactions has been taken into account in a monopole approximation according to Coulomb's law, with partial charges of atoms as described in Ref [14]. The effective dielectric constant was taken as equal to ten as described by Lipkind et.al. [14]. The intrinsic energy of a molecule includes also the torsion potentials, describing the barriers of the inner rotation between atoms that have a 1-4 relationship, the values of the torsional barriers heights are taken from work [13]. The energy of hydrogen bond formation is calculated based on Morse potential [13] and dissociation energy of hydrogen bond is taken to be -1.5 kcal·mol^{-1} at an NH... OC distance r0=1.8 Å.

The bond distances and the values of valence angles were regarded as invariable and correspond to [13]. Only dihedral angles were taken to be the intrinsic degrees of freedom. The conventions used for rotational angles correspond to the IUPAC-IUB nomenclature [15]. The identifier system will be used to describe all structures at the intermediate stages of the calculation experiment, with the numerical values of geometry parameters produced only at the final stage. *B*, *R*, *L* and *P* symbols were used to represent the regions of conformational space situated around φ, ψ values as following: $R(\varphi, \psi$=-180-0°), $B(\varphi$=-180-0°, ψ=0-180°), $L(\varphi, \psi$=0-180°) and $P(\varphi$=0-180°, ψ=-180-0°).

The backbone is conventionally described by the "shape" symbols e and f referred to as respectively the extended and folded configuration of peptide chain. Pentapeptide stable conformations were found by the total conformational energy minimization. The minimization procedure has several steps. A final conformation obtained in a preliminary optimization step is taken as an initial one for the next step. A procedure for the pentapeptide global energy was repeated until the minimal values of the global energy retained constant level. Based on the above considerations and calculation program [16] the detailed conformational analysis of the Arg-Thr-Tyr-Trp-Leu pentapeptide was carried out.

RESULTS AND DISCUSSION

According to the calculation scheme (fig.1) the conformations of consequently lengthened di-, tri-, tetra-and finally pentapeptide fragment were calculated (due to ref.[12] the final results are independent on the way of the peptide backbone dividing into fragments). The initial conformations for the pentapeptide energy minimization were obtained by combining the of lowest energy structures of Arg, Thr, Tyr, Trp, and Leu amino acid residues. Initial monopeptide conformations are chosen as given in Ref. [12]. The backbone chains of the amino acid residues, that construct the pentapeptide, can be in *R*, *B* and *L*- forms. The side chain dihedral angle values χ_1=±60 and 180° were taken into account for threonine, tyrosine and tryptohan.

For *N*- terminal of the fragments, however only χ_1=60 and 180° and for the *C*-terminal only χ_1=60 and –60° were considered. Since the variation of χ_2 angle of the threonine has not altered the conformational energy significantly, only one of the equal probably conformations (χ_2=±60, 180°), i.e. χ= 180^0 was taken into account for this residue. For tyrosine, the value of 90° for χ_2 and 180° for χ_3, which correspond to stable states, were used. Thus, for the first approach to Arg-Thr-Tyr-Trp-Leu pentapeptide 24 low-energetic conformers of Arg and 9 rotomers of Leu, Thr, Tyr, Trp were considered. The pentapeptide fragment involves 108 atoms and 33 dihedral angles as indicated in fig 1. All available conformations were classified into 16 shapes. About 324 initial conformations were used for the global energy minimization. Only six of them consist of low energy conformers the relative energy of which is within 0-5kcal·mol^{-1}.

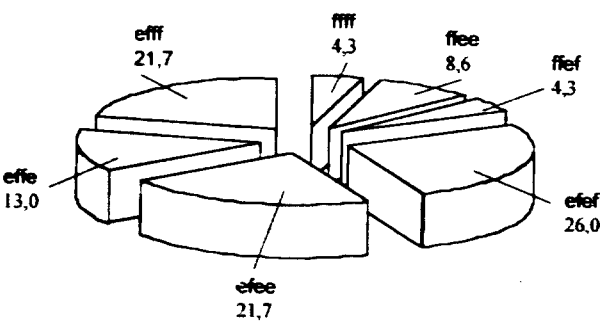

Fig.2. The low-energetical conformations distribution (in percent) for the pentapeptide favourable shapes within E_{rel}=0÷5kcal·mol^{-1}.

The energy distribution of the shapes is illustrated in fig.2. Several helical structures with the folded backbone shape, including the α- helix (RRRR shape), are the most preferred one.

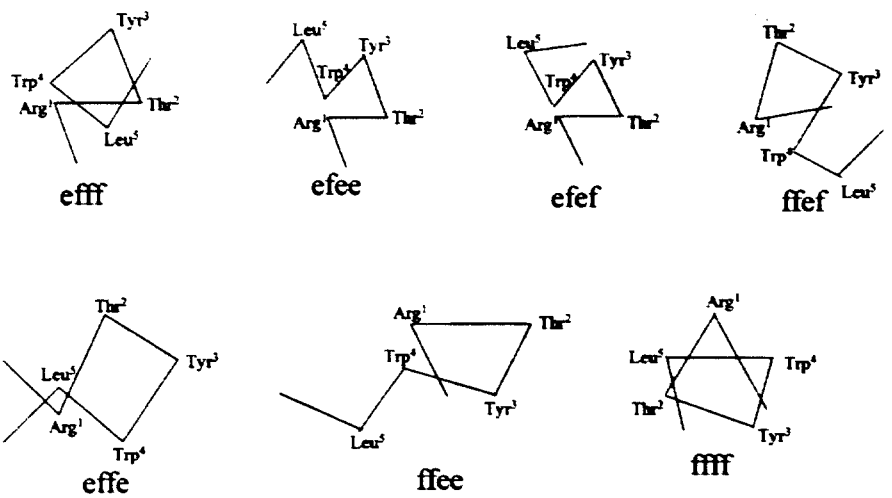

Fig. 3. The schematic representations of the shapes of the low energetical conformations of pentapeptide, based on the coordinates of C^{α} atoms.

Table 1. The energies (kcal· mol^{-1}) and shapes of favourable conformations of the Arg-Thr-Tyr-Trp-Leu fragment

№	SHAPE	Conformation	Inter residue interaction energies										Energy contribution			E_n
			Arg Thr	Thr Tyr	Tyr Trp	Trp Leu	Arg Tyr	Thr Trp	Tyr Leu	Arg Trp	Thr Leu	Arg Leu	E_{vv}	E_{el}	E_{nv}	
1	efff	BRRRB	-1.71	-2.93	-1.18	-3.49	-5.39	-0.45	-0.62	-4.88	-0.90	-2.31	-26.9	2.1	1.4	0.(
2	efee	BRRLB	-1.90	-1.14	-1.55	-0.61	-5.58	-2.94	-4.99	-4.11	-0.16	-0.09	-28.9	2.7	3.0	1.:
3	efef	BRBRR	-1.94	-0.95	-4.41	-1.05	-2.79	-1.19	-2.54	-7.64	0.00	-0.58	-27.7	3.6	4.2	3.(
4	ffef	RRBRR	-2.41	-2.43	-2.48	-4.62	-1.34	-0.20	-2.00	-0.24	-0.01	-0.03	-25.8	4.9	1.6	4.:
5	ffee	RRBBR	-2.44	-1.83	-2.61	-3.50	-1.39	-0.20	-2.82	-0.22	0.00	-0.02	-25.5	4.8	1.6	4..
6	effe	BRBLR	-1.48	-0.55	-1.67	-3.11	-7.42	-0.30	-1.60	-5.85	-1.28	-1.71	-25.5	3.2	3.6	4.:
7	ffff	RRRRR	-2.66	-1.12	-1.92	-5.25	-1.73	-1.03	-0.54	-1.60	-0.62	-3.11	-28.2	5.6	4.1	4.9

Table 2. The geometrical parameters (in degree) of the pentapeptide fragment for low-energetical conformations

Amino Acid	CONFORMATIONS*						
	1	2	3	4	5	6	7
Arg	φ=-118 ψ=120 ω=179 χ_1=181 χ_2=177 χ_3=178 χ_4=179 χ_5=179	φ=-118 ψ=137 ω=179 χ_1=182 χ_2=176 χ_3=179 χ_4=179 χ_5=170	φ=-144 ψ=157 ω=179 χ_1=60 χ_2=178 χ_3=183 χ_4=179 χ_5=180	φ=-91 ψ=-55 ω=179 χ_1=179 χ_2=177 χ_3=179 χ_4=179 χ_5=179	φ=-94 ψ=-53 ω=180 χ_1=179 χ_2=178 χ_3=179 χ_4=179 χ_5=179	φ=-140 ψ=159 ω=179 χ_1=55 χ_2=176 χ_3=181 χ_4=179 χ_5=179	φ=-109 ψ=-58 ω=180 χ_1=178 χ_2=177 χ_3=179 χ_4=179 χ_5=179
Thr	φ=-86 ψ=-52 ω=178 χ_1=56 χ_2=180 χ_3=176	φ=-101 ψ=-68 ω=179 χ_1=57 χ_2=180 χ_3=175	φ=-93 ψ=-57 ω=177 χ_1=57 χ_2=180 χ_3=175	φ=-86 ψ=-60 ω=178 χ_1=57 χ_2=180 χ_3=173	φ=-87 ψ=-56 ω=181 χ_1=57 χ_2=180 χ_3=173	φ=-85 ψ=-30 ω=185 χ_1=195 χ_2=181 χ_3=176	φ=-76 ψ=-45 ω=185 χ_1=57 χ_2=180 χ_3=174
Tyr	φ=-141 ψ=-60 ω=179 χ_1=-59 χ_2=90 χ_3=179	φ=-140 ψ=-69 ω=170 χ_1=168 χ_2=71 χ_3=170	φ=177 ψ=-108 ω=177 χ_1=188 χ_2=84 χ_3=179	φ=-127 ψ=164 ω=174 χ_1=58 χ_2=84 χ_3=179	φ=-122 ψ=171 ω=175 χ_1=66 χ_2=87 χ_3=179	φ=-105 ψ=160 ω=193 χ_1=65 χ_2=84 χ_3=179	φ=-80 ψ=-21 ω=184 χ_1=64 χ_2=81 χ_3=179
Trp	φ=-100 ψ=-63 ω=180 χ_1=189 χ_2=195	φ=43 ψ=57 ω=168 χ_1=-57 χ_2=130	φ=-68 ψ=-61 ω=194 χ_1=-56 χ_2=167	φ=-94 ψ=-56 ω=178 χ_1=175 χ_2=-132	φ=-139 ψ=141 ω=178 χ_1=177 χ_2=-121	φ=53 ψ=69 ω=177 χ_1=186 χ_2=188	φ=-92 ψ=-59 ω=185 χ_1=176 χ_2=-146
Leu	φ=-119 ψ=140 ω=181 χ_1=-59 χ_2=179 χ_3=180 χ_4=179	φ=-125 ψ=113 ω=178 χ_1=192 χ_2=189 χ_3=180 χ_4=179	φ=-100 ψ=-72 ω=185 χ_1=171 χ_2=192 χ_3=180 χ_4=179	φ=-98 ψ=-58 ω=180 χ_1=179 χ_2=184 χ_3=180 χ_4=179	φ=-103 ψ=-59 ω=179 χ_1=177 χ_2=185 χ_3=180 χ_4=178	φ=-100 ψ=-59 ω=179 χ_1=-60 χ_2=180 χ_3=180 χ_4=179	φ=-97 ψ=-66 ω=188 χ_1=-179 χ_2=182 χ_3=180 χ_4=179
E_{rel}·kcal· mol^{-1}	0.0	1.2	3.6	4.2	4.3	4.7	4.9

* Conformation numbering is the same as given in Table 1.

The lowest energy conformer, namely the global conformer of the pentapeptide has efff shape. The large side chains of terminal residues in all helical- types conformers approach to each other and form effective tetra-and pentapeptide interactions (Table 1). The stable structures of the two shapes ffff and efff form a hydrogen bond NH(Leu)...OC (Arg). Specific interaction between the pairs of the Trp and Leu residues (-4.4 kcal-mol.$^{-1}$ in average) also made an important contribution to the stabilization of these conformations. It is found that the strong interaction between Arg and Trp (- 6.2 kcal· mol $^{-1}$, in average) is characteristic for all low energy structures of the pentapeptide from the efff, efef, efee and

effe shapes. As seen in Table 1, both Tyr and Trp make an important contribution towards the dispersion interactions to the stabilization of the low-energetic structures of the pentapeptide from efef shape (~4.4kcal·mol^{-1}) The geometrical parameters of low-energetical conformations are given in Table 2. In figure 3, the main chain shapes of the pentapeptide corresponding to the low- energetical conformations are illustrated.

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N.T. Süleymanova, İ.N. Əliyeva, C.İ. Əliyev, N.M. Qocayev

QUANİLATSUKLAZA *A* PENTAPEPTİDİNİN *N*-SONLUQLU KONFORMASİYA ANALİZİ

Nəzəri konformasiya analizi metodu ilə natriuretik peptidin reseptoru quanilatsiklaza *A*-Arg-Thr-Tyr-Trp-Leu katalitik domenin *N*-sonluqlu pentapeptid fraqmentinin konformasiya xüsusiyyətləri öyrənilmişdir. Fraqmentin optimal konformasiya vəziyyətlərinin enerji və həndəsi parametrləri alınmışdır.

Н.Т. Сулейманова, И.Н. Алиева, Д.И. Алиев, Н.М. Годжаев

КОНФОРМАЦИОННЫЙ АНАЛИЗ *N*-КОНЦЕВОГО ПЕНТАПЕПТИДА ГУАНИЛАТЦИКЛАЗЫ *A*

Методом теоретического конформационного анализа изучены конформационные свойства N-концевого пентапептидного фрагмента Arg-Thr-Tyr-Trp-Leu каталитического домена гуанилатциклазы *A*-рецептора предсердного натрийуретического пептида. Установлены энергетические и геометрические параметры оптимальных конформационных состояний фрагмента.

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