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Conformational particularities of antibacterial, anticancer and antioxidant cyclic dipeptide Cyclo(D-Tyr-D-Phe)

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Abstract

By molecular mechanics method and quantum chemical calculation was applied to examine the most stable conformations and flexible properties of side chains of diketopiperazine cyclic dipeptides with antibacterial, anticancer and antioxidant activity. By calculations on diketopiperazine dipeptide cyclo(D-Tyr-D-Phe) and its analogs cyclo(L-Tyr-L-Phe), cyclo(L-Tyr-D-Phe), cyclo(D-Tyr-L-Phe) as a function of the side-chain torsion angles were determined their energetically preferred conformations. The obtained results of conformational analysis were used to found the relative position of the side chains of residues in the stable conformations of the dipeptides. Our calculations bring out for each cyclic dipeptides formed the two types of conformations as the most favorable. One of them conformation formed asymmetric arrangement of side chains with the tyrosine ring folded over the diketopiperazine ring, but second dipeptide conformation formed with extended arrangement of side chains. Quantum chemical calculation shed light on the structural modifications between the different cyclic dipeptides.

***Keywords**: antibacterial, anticancer, antioxidant activities, cyclic dipeptide, conformation, molecular mechanics method*
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1. Introduction

It is known that antioxidant molecules play an important role in the later stages of an cancer development. The antioxidants may be able to cause the regression of

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premalignant lesions and inhibit their development into cancer. A new microbial diketopiperazine cyclic dipeptide, Cyclo(DTyr-DPhe) based on *tyrosine* (Tyr) and *phenylalanine* (Phe) was isolated from *Bacillus* bacteria and shows an antibacterial, anticancer and *antioxidant activity*. The activity of cyclo(D-Tyr-D-Phe) against Staphylococcus epidermis is better than chloramphenicol, the standard antibiotics. Cyclo(D-Tyr-D-Phe) recorded significant antitumor activity against A549 cells (IC50 value: 10 μM) and this compound recorded no cytotoxicity against factor signaling normal fibroblast cells up to 100 μM. It was compared the biological activity of natural cyclo(D-Tyr-D-Phe) with cyclo(L-Tyr-L-Phe). It was determined that synthetic cyclo(L-Tyr-L-Phe) recorded lower biological activity.

In this investigation have been investigated the conformational particularities of the cyclic dipeptide cyclo(D-Tyr-D-Phe). For this purpose, have been studied and compared the conformational properties of cyclo(L-Tyr-L-Phe) with cyclo(D-Tyr-D-Phe) and their two other derivatives - cyclo(L-Tyr-D-Phe) and cyclo(D-Tyr-L-Phe). The major aim of the present work is the investigation of effect of chirality on three-dimensional structure of cyclo(D-Tyr-D-Phe) and its analogs. It is also studied the conformational dynamics of side chains of these cyclic dipeptides relatively of their diketopiperazine (DKP) ring, called diketopiperazine ring. To determine the chemical reactivity of cyclic dipeptides have been calculated the charge distributions and dipole moments of their most stable conformations by quantum chemical method.

2. Methods

The spatial structure of cyclic dipeptides have been investigated by molecular mechanics method (MM). Molecular mechanics study of cyclo (D-Tyr-D-Phe) and its analogs involves extensive computations of starting structural approximations with different orientations of side chains of residues. This approach involves quantitative estimation of all nonbonded interactions. This investigation were carried out using potentials with energy and geometry parameters as described in Refs. [3, 4]. The nonbonded and electrostatic interactions, intramolecular hydrogen bonds, and restricted rotation about all single bonds of side chains are taken into account. For investigation of the behavior of the side chains within the energy preferred conformations were obtained the conformational maps around dihedral angles χ_1,χ_2,χ_3 for Tyr and χ_1, χ_2 for Phe residues. The conformational energy is considered the sum of independent contributions of nonbonded E_{nb}, electrostatics E_{el}, torsional interactions E_{tor} and hydrogen bonding E_{hb} 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. The dielectric constant is assumed to equal ten. The torsional energy was calculated using the value of internal rotation barriers given by Momany et al. The hydrogen bond energy is calculated based on Morse potential Bonding lengths and angles are those given by Corey and Pauling and are kept invariable; the ω angle of the peptide bond was fixed at 180°. Computations were carried out on the computer using universal programs complex [5]. This program calculates the conformational energy of a peptide as a sum of nonbonded, hydrogen-bonded and electrostatic energies for pairwise atomic interactions and torsional potential energies for rotation about bonds. The dihedral rotation angles were counted according to the IUPAC-IUB [7]. An atomic partial charge distribution is an essential element of any force field for peptide ligands. The peptides electronic structure was investigated, the active site, the way of action and structure-activity relationship were discussed. We uses AM1 calculated method for the determination of electronic charge density for the cyclic molecules [8, 9]. We calculate charge density as a sum of molecular orbital densities, each the square of the orbital wave function. In this study we investigate how the charge distribution of a molecule may be related with their reactionary ability. An atomic partial charge distribution of the stable conformation of molecules allow to predict the relationships of their reactionary ability with separate molecule areas. Here we will only be considering the charge distribution itself, leaving aside for the moment a discussion of intramolecular electrostatic and polarization energies and their relation to intermolecular interaction energies.

3. Results and discussion

In our investigation of conformational possibilities of cyclo(D-Tyr-D-Phe), cyclo(L-Tyr-L-Phe), cyclo(L-Tyr-D-Phe) and cyclo(D-Tyr-L-Phe) we have assumed the diketopiperazin ring is planar, according to crystallographic studies of cyclic dipeptides [10]. Therefore, we have calculated the energy of conformations only as a function of side chain dihedral angles for cyclic dipeptides. The starting conformations were taken by the values of dihedral angle $\chi 1$ of side chain of Tyr residue and $\chi 1$ of Phe at interval 10^0. The characteristics of the three optimal conformations of cyclo(D-Tyr-D-Phe) after energy minimization are showed in Table 1. The most stable conformation is characterized by near $\chi 1 = -60^0$ for Tyr and $\chi 1 = 60^0$ for Phe.. There are two other local minima which are both less stable than the global minimum by about 0.3 and 1.3 kcal/mol respectively. At the second stage of this investigation were studied the conformational dynamics and mobility of the side chains of the cyclo(L-Tyr-L-Phe). For investigation of the behavior of the side chains within the energy preferred conformations were obtained the conformational maps around dihedral angles χ_1 for Tyr and Phe residues. Then obtained values of the

dihedral angles were used as a starting parameters for minimization. After minimization were received the other low-energy structures, showing the dynamics of the side chains into preferred conformation. The results of the energy minimization, obtained by varying of the side chain of cyclo(L-Tyr-L-Phe) are summarized in Table 2. The region corresponding to the most stable conformation marked as (χ_1=60^0 for Tyr, χ_1= 180^0 for Phe) differ from other two conformations by relative energy with 0,2 and 0,9 kcal/mol, respectively. At the third stage of this investigation were studied the mobility of the side chain of cyclo(L-Tyr-D-Phe) and cyclo(D-Tyr-L-Phe). The energy and geometry parameters of the three optimal conformations of both dipeptides cyclo(L-Tyr-D-Phe) and cyclo(D-Tyr-L-Phe) after minimization are presented in Table 3 and Table 4 respectively. The obtained data allow one to conclude that, the Tyr residue are comparatively more dynamic and are realized in two or three local minimums within the identical conformations of all cyclic dipeptides. The comparison of results of theoretical conformational analysis of cyclic dipeptides enable us to support the proposal that cyclic dipeptides are free of any intramolecular hydrogen bond in order to be available for the formation of the complex with the receptor. Our calculations bring out for cyclic dipeptides two different conformations as the most favorable. One of them conformation formed asymmetric arrangement of side chains with the tyrosine ring folded over the diketopiperazine ring, but second dipeptide conformation formed with extended arrangement of side chains. Figure 1 shows the most stable conformations of cyclic dipeptides cyclo(D-Tyr-D-Phe), cyclo(L-Tyr-D-Phe), cyclo(D-Tyr-L-Phe) and cyclo(L-Tyr-L-Phe). These can be described with reference to the two essentially planar units, the diketopiperazine ring and tyrosine and phenylalanine side chain respectively. The energy parameters and summary dipole moments of electronic structure of the lowest energy conformation of cyclic dipeptides cyclo(D-Tyr-D-Phe) and its analogs are shown in Table 5. The partial charge distributions for cyclo(D-Tyr-D-Phe), cyclo(L-Tyr-D-Phe), cyclo(D-Tyr-L-Phe) and cyclo(L-Tyr-L-Phe) in their lowest-energy conformations shown in Figure 2 respectively.

Table 1. Energy and geometry parameters of the stable calculated conformations of Cyclo(D-Tyr-D-Phe)

№	Energy contributions, kcal/mol					Side chain dihedral angles				
						D-Tyr			D-Phe	
	E_{NB}	E_{EL}	E_{TOR}	E_{ABS}	E_{REL}	$\chi1$	$\chi2$	$\chi3$	$\chi1$	$\chi2$
1	-1.8	1.6	0.8	0.6	0.0	-60	90	180	60	90
2	-1.5	1.6	0.8	0.9	0.3	-60	90	180	-60	90
3	-1.8	1.7	2.2	1.9	1.3	180	90	180	180	90

Table 2. Energy and geometry parameters of the stable calculated conformations of Cyclo(L-Tyr-L-Phe)

№	Energy contributions, kcal/mol					Side chain dihedral angles				
						L-Tyr			L-Phe	
	E_{NB}	E_{EL}	E_{TOR}	E_{ABS}	E_{REL}	$\chi1$	$\chi2$	$\chi3$	$\chi1$	$\chi2$
1	-4.0	1.7	0.2	-2.1	0.0	60	90	180	180	90
2	-3.7	1.6	0.2	-1.9	0.2	-60	90	180	60	90
3	-3.3	1.6	0.5	-1.2	0.9	60	90	180	60	90

Table 3. Energy and geometry parameters of the stable calculated conformations of Cyclo(D-Tyr-L-Phe)

№	Energy contributions, kcal/mol					Side chain dihedral angles				
						D-Tyr			L-Phe	
	E_{NB}	E_{EL}	E_{TOR}	E_{ABS}	E_{REL}	$\chi1$	$\chi2$	$\chi3$	$\chi1$	$\chi2$
1	-1.8	1.6	0.8	0.6	0.0	-60	90	180	60	90
2	-1.5	1.6	0.8	0.9	0.3	-60	90	180	-60	90
3	-1.8	1.7	2.1	2.0	1.4	180	90	180	180	90

Table 4. Energy and geometry parameters of the stable calculated conformations of Cyclo(L-Tyr-D-Phe)

№	Energy contributions, kcal/mol					Side chain dihedral angles				
						L-Tyr			D-Phe	
	E_{NB}	E_{EL}	E_{TOR}	E_{ABS}	E_{REL}	$\chi1$	$\chi2$	$\chi3$	$\chi1$	$\chi2$
1	-5.1	1.8	0.2	-3.0	0.0	60	90	180	180	90
2	-2.3	1.6	01	-0.6	2.4	-60	90	180	180	90
3	-2.2	1.6	0.0	-0.5	2.5	180	90	180	180	90

Table 5. The energy parameters and summary dipole moments of electronic structure of the lowest energy conformation of cyclic dipeptides cyclo(D-Tyr-D-Phe) and its analogs.

Electronic parameters of most stable conformations of cyclic dipeptides				
Electronic parameters	**Cyclo (DTyr-DPhe)**	**Cyclo (LTyr-LPhe)**	**Cyclo (LTyr-DPhe)**	**Cyclo (DTyr-LPhe)**
Total energy, kcal/mol	-90555	-90556	-90555	-90558
Binding energy, kcal/mol	-4470	-4471	-4470	-4473
Isolated atomic energy, kcal/mol	-86085	-86085	-86085	-86085
Electronic energy, kcal/mol	-670009	-617845	-664883	-528611
Core-Core interaction energy, kcal/mol	579447	587889	574327	537953
Heat of formation, kcal/mol	-51	-52	-52	-54
Dipole moment, debyes, D	1.5	1.1	1.3	2.1

4. Conclusions

Thus, on the basis of conformational particularities study of diketopiperazine dipeptide cyclo(D-Tyr-D-Phe) and its analogs as a function of the side-chain torsion angles were determined their energetically preferred conformations. The obtained data allow one conclude that, each cyclic dipeptides formed the two types of conformations as the most favorable. One of them conformation formed asymmetric arrangement of side chains with the tyrosine ring folded over the diketopiperazine ring, but second dipeptide conformation formed with extended arrangement of side chains. The investigation results therefore indicate that a concrete type of the diketopiperazine dipeptide cyclo(D-Tyr-D-Phe) and its analogs will essentially depend on the conditions under which the given molecule functions.

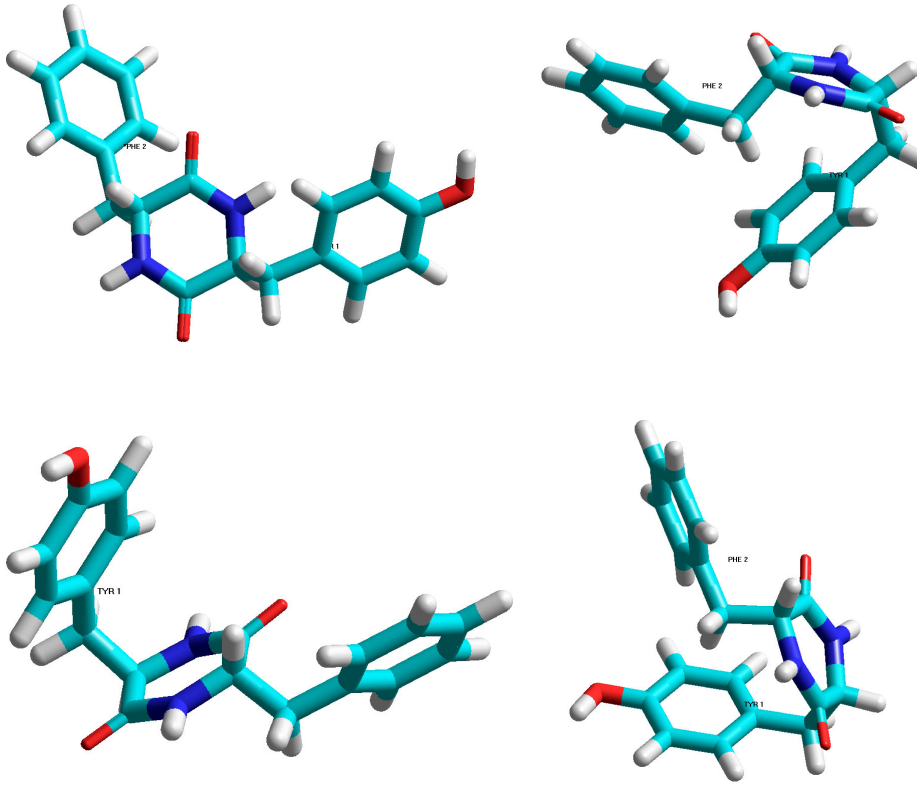

Fig. 1. The most stable conformations of the cyclic dipeptides: cyclo(D-Tyr-D-Phe), cyclo(L-Tyr-D-Phe), cyclo(D-Tyr-L-Phe) and cyclo(L-Tyr-L-Phe).

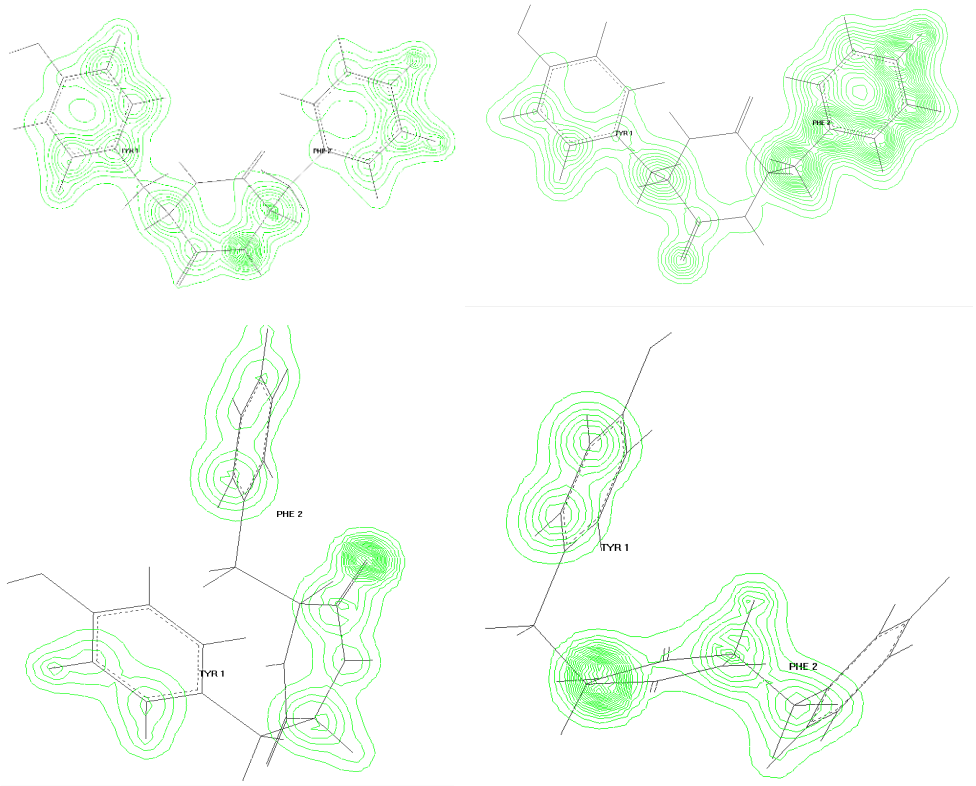

Fig. 2. The charge distributions of most stable conformations of cyclic dipeptides cyclo(D-Tyr-D-Phe), b) cyclo(L-Tyr-D-Phe), c) cyclo(D-Tyr-L-Phe) and d) cyclo(L-Tyr-L-Phe).

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