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SPATIAL STRUCTURES OF [ILE3]-AND [PHE3]-RUBISCOLIN-5 MOLECULE ANALOGUES

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ABSTRACT

Rubiscolin molecules belong to the class of opioids derived from food substances. To understand the various physiological functions they perform, to target them purposefully, and to synthesize artificial analogs that perform specific functions of the natural molecule, it is necessary to study their three-dimensional spatial structures. The spatial structures of rubiscolin molecules and their analogs were investigated using theoretical conformational analysis methods. The potential energy of the molecule was chosen as the sum of non-valent, electrostatic, torsion interaction energies and hydrogen bond energies. The spatial structures of [Ile3]- and [Phe3]-rubiskolin-5 molecules were studied in the context of the low-energy conformations of the natural rubiscolin molecule. The calculations revealed that the solid structure of both analogs is represented by eight low-energy conformations, similar to those of the natural rubiskolin-5 molecule. It has been shown that the energy and geometric parameters of the molecules in both analogues are the same as in the natural rubiscolin-5 molecule, therefore it is not advisable to propose to synthesize both artificial analogues.

Introduction

Regulatory peptides, first discovered in the second half of the 20th century, are actively studied by both physiologists and pharmacologists, since the area of biological activity of peptides is extremely wide. They are one of the main links that unite the three regulatory systems of the body - the nervous system, endocrine and immune into a single whole. At present, more than 9000 physiologically active peptides have been characterized in various animal species and in humans. These are short chains of amino acids (2-70 residues) that act as signal molecules. Most of these peptides cannot be confidently attributed to either neurotransmitters or hormones, since they are synthesized both by neurons (transmitting a signal at the synapse level) and by cells of peripheral tissues (transmitting a signal over longer distances, like hormones). Regulatory peptides are characterized by the impact on many systems of the body at once. Opioid peptides are currently considered the most studied group of peptides signaling substances. Opium causes pain relief, sedation and falling asleep, as well as a euphoric state and a number of vegetative reactions. Opioid peptides are of animal and plant origin. A number of exogenous peptides obtained from food have opiate-like properties. These peptides were called exorphins. The discovery of the opioid activity of the peptide components of food led to the assumption that certain types of food can act on the central nervous system like opiate drugs. Exorphins have been isolated from various plant species. Rubiscolins -5 and -6 are also of

great interest to scientists. These peptides have affinity mainly for β-receptors. Rubiscolins were first isolated from spinach leaves. However, the degree of homology of the large RUBISCO subunit in different species of higher plants is extremely high (more than 90%), and the content of RUBISCO in green leaves is up to 50% of the total protein. Therefore, rubiscolins in significant amounts can enter the body when consuming not only spinach, but also lettuce, sorrel, pars-ley, etc. The green leaves of these plants are an important component of a balanced diet, so studies of the possible neurotropic effect of rubiscolins are of practical value [1-6].

Materials and methods

We have studied the structural and functional organizations of opioid peptides enkephalins, endorphins, endomorphins, dynorphins, neoendorphins, adrenorphin, and are currently studying the spatial structure of exorphins, casomorphins, lactoferroxins, casoxins, soymorphins, rubiscolins. This work is a continuation of our previous studies [7-15].

The molecule was calculated using the method of theoretical conformational analysis. The potential function of the system is chosen as the sum of nonvalent, electrostatic and torsional interactions and the energy of hydrogen bonds. Non-valent interactions were assessed using the Lennard-Jones potential. Electrostatic interactions were calculated in the monopole approximation according to Coulomb's law using partial charges on atoms. The conformational capabilities of the rubiscolin molecule were studied in an aqueous environment, and therefore the dielectric constant was taken to be 10. The energy of hydrogen bonds was estimated using the Morse potential. Our above-mentioned works detail the potential features used. When presenting the calculation results, a classification of peptide structures according to conformations, main chain shapes, and peptide backbone shapes was used. Conformational states are completely determined by the values of the dihedral angles of the main and side chains of all amino acid residues included in a given molecule. The backbone forms of a fragment are formed by combinations of the forms of R, B, L residues in a given sequence. The forms of the dipeptide backbone can be divided into two classes - folded (f) and unfolded (e) forms, which are called shapes. All conformations are grouped by main chain shapes, and shapes by shapes. To designate the conformational states of residues, identifiers of the X_{ij} type are used, where X defines the low-energy regions of the conformational map and ij...=11...,12...,13...,21... determines the position of the side chain, with index 1 corresponding to the angle value ranging from 0 to 120°, 2 – from 120° to -120°, and 3 – from -120° to 0°. Designations and readings of rotation angles correspond to the IUPAC-IUB nomenclature [16]. To calculate the spatial structure of peptide molecules was used a program developed by N.M.Gojayev and his staff [17].

Results and Discussion

In [18] we studied the three-dimensional structure of the rubiscolin-5 molecule (Tyr1-Pro2-Leu3-Asp4-Leu5-NH2) based on low-energy conformations of the corresponding amino acid residues and determined its stable conformations. The calculation results showed that there is an energy differentiation between the conformations, forms of the main chains and shapes. The conformations of eight shapes fall within a wide energy range of 0-5.0 kcal/mol. The most stable conformations were selected from each shape and are presented in Table 1. The energy contributions of non-valent (U_{nv}), electrostatic (U_{el}), torsional (U_{tors}) interactions and the relative (U_{rel}) energy of the optimal conformations of the rubiscolin-5 molecule are indicated here. The energy of non-valent interactions in low-energy conformations changes in the energy range (-22.3) - (-18.9) kcal/mol, electrostatic interactions (-4.6) - (0.9) kcal/mol, torsional interactions (2.9-

5.6) kcal/mol. The geometric parameters of four conformations whose relative energy is less than 4.0 kcal/mol are presented in Table 2.

Table 1. Optimum conformations of the rubiscoline-5 molecule, their shapes, the shapes of their main chains, the energy contribution of non-valent, electrostatic, torsion interactions and relative energies.

№	Shapes	CONFORMATION	Energy contribution			U_{rel}
			U_{nv}	U_{el}	U_{tors}	
1	efee	$B_3RB_{21}B_3B_{32}$	-20.0	-4.6	3.7	0
2	efff	$B_3RR_{12}R_1R_{21}$	-22.3	- 3.8	5.6	0.4
3	efef	$B_3RB_{31}R_3R_{21}$	-21.3	-3.7	4.3	0.2
4	eeef	$B_2BB_{21}R_1R_{21}$	-20.3	0.5	3.0	4.0
5	effe	$B_3RR_{21}B_3B_{32}$	-18.9	-0.7	3.0	4.3
6	eefe	$B_1BR_{32}B_1B_{32}$	-21.7	1.0	4.8	4.9
7	eeff	$B_1BR_{23}R_3R_{32}$	-19.8	0.9	2.9	5.0
8	eeee	$B_1BB_{21}B_1B_{32}$	-19.8	0.9	3.1	5.0

Table 2. Geometric parameters (in degrees) of low energy conformations of the molecule rubiscolin-5 (the values of the dihedral angles are given in the sequence φ, ψ, ω, χ1, χ2...)

Residue	$B_3RB_{21}B_3B_{32}$	$B_3RR_{12}R_1R_{21}$	$B_3RB_{31}R_3R_{21}$	$B_2BB_{21}R_1R_{21}$
Tyr1	-95 159 168 -71 104 0	-89 158 176 -73 113 0	-65 155 171 -70 107 0	-71 115 170 171 79 0
Pro2	-60 -46 167	-60 -33 -179	-60 -63 169	-60 120 -173
Leu3	-122 118 174 173 61 180 177	-66 -41 -177 65 98 179 -179	-106 62 180 -75 65 179 180	-108 103 175 173 62 179 -172
Asp4	-107 144 -175 60 90	-81 -31 176 61 85	-76 -49 179 -56 89	-99 -46 180 55 107
Leu5	-118 119 180 -54 175 -175 180	-63 -45 179 176 63 179 176	-77 -52 180 176 62 179 175	-116 -68 180 175 62 179 176
ΔU	0	0.4	0.2	4.0

Rubiscolin-5 and various analogues of Rubiscolin-5 molecules were synthesized by Sobolczyk M., Perlikowska R. The spatial structure of the [Ile3]-rubiscolin-5 and [Phe3]-rubiscolin-5 molecules synthesized by them was studied. The spatial structure of the [Ile3]-rubiscolin-5 molecule was calculated based on the low-energy conformations of the natural rubiscolin-5 molecule shown in Table 1. At this time, nine conformations of the side chain of the Ile amino acid residue in each low-energy form of the natural molecule, which are possible according to the torsion potential, were considered. The results of the calculations show that there is differentiation according to the energies of the conformations, but there is no differentiation according to the shapes of the main chain forms. Among the calculated conformations, the lowest-energy conformation of the side chain of the Ile amino acid residue in the eight low-energy forms of the main chain was selected. These conformations, their contributions to them by non-valent, electrostatic, and torsional interaction energies, and their total and relative energies are shown in Table 3. As can be seen from Table 3, their relative energies vary in the range of (0 – 6.4) kcal/mol. A comparison of Tables 1 and 3 shows that all low-energy structures of the natural molecule remain the same as low-energy for the [Ile3] analogue.

Table 3. Optimum conformations of the [Ile3]- rubiscoline-5 molecule, their shapes, the shapes of their main chains, the energy contribution of non-valent, electrostatic, torsion interactions, the total and relative energies.

№	Shapes	CONFORMATION	Energy contribution			U_{tot}	U_{rel}
			U_{nv}	U_{el}	U_{tors}		
1	efee	$B_3 R B_{21} B_3 B_{32}$	-18.9	4.4	4.2	-19.2	0.3
2	efff	$B_3 R R_{32} R_1 R_{12}$	-19.2	-3.7	3.3	-19.5	0
3	efef	$B_3 R B_{21} R_3 R_{21}$	-19.6	-3.9	4.4	-19.1	0.4
4	eeef	$B_2 B B_{32} R_1 R_{21}$	-21.5	0.4	4.4	-16.7	2.8
5	effe	$B_3 R R_{32} B_3 B_{32}$	-17.8	-0.3	3.1	-15.0	4.5
6	eefe	$B_1 B R_{33} B_1 B_{32}$	-19.1	1.2	4.8	-13.1	6.4
7	eeff	$B_1 B R_{32} R_3 R_{32}$	-19.3	1.2	2.8	-15.3	4.2
8	eeee	$B_1 B B_{22} B_1 B_{32}$	-20.3	1.1	3.9	-15.4	4.1

Table 4. Energy inside and between residual interactions in the conformations of the molecule [Ile3]- rubiscoline-5 $B_3 R B_{21} B_3 B_{32}$ (U_{rel}=0.3 kcal/mol, first line), $B_3 R R_{32} R_1 R_{12}$ (U_{rel}=0 kcal/mol, second line), $B_3 R B_{21} R_3 R_{21}$ (U_{rel}=0.4 kcal/mol, third line), $B_2 B B_{32} R_1 R_{21}$ (U_{rel}=2.8 kcal/mol, fourth line)

Tyr1	**Pro2**	**Ile3**	**Asp4**	**Leu5**	
3.3	-3.8	-1.8	-10.9	-2.4	**Tyr1**
3.7	-4.2	-2.9	-12.3	0.3	
2.1	-4.4	-2.4	-12.3	-0.8	
2.6	-5.7	-4.1	-4.7	-0.7	
	0.1	-0.8	-1.0	-2.2	**Pro2**
	0.2	-1.8	-0.5	0	
	0.3	-1.9	-0.5	0	
	0.3	-1.1	-0.6	-0.1	
		1.4	-0.6	-1.3	**Ile3**
		1.6	-1.8	-2.7	
		2.4	-1.6	-2.7	
		0.4	-0.9	-2.5	
			2.0	-1.1	**Asp4**
			2.5	-1.6	
			2.3	-1.3	
			1.8	-1.4	
				-3.6	**Leu5**
				-3.6	
				-3.4	
				-4.3	

The relative energies of the first four low-energy conformations in Table 1 vary in the range of (0 – 2.8) kcal/mol. In the eight low-energy conformations shown in Table 3, the contributions to the non-valent interaction energy vary in the range of (-21.5) – (-17.8) kcal/mol, the electrostatic interaction energy in the range of (-4.4) – (1.2) kcal/mol, and the torsion interaction energy in the range of (2.8) – (4.8) kcal/mol (Table 3). The interaction forces between and within amino acid residues in these conformations are shown in Table 4, the values of their dihedral angles are shown in Table 5, and the spatial arrangement of atoms in these conformations is shown in Figure 1. The most stable conformation of the [Ile3]-rubiscolin-5 analogue is $B_3 R R_{32} R_1 R_{12}$, which belongs to the efff shape. The relative energy of this conformation in the natural rubiscolin-5 molecule is 0.4 kcal/mol. The conformation is favorable in terms of both non-valent and electrostatic interaction energies. In this conformation, the N- and C- sides of the molecule approach each other, and a hydrogen bond is formed between the H-atom on the N-side of Tyr1

and the C=O atom in the side chain of Asp4 and the N – H atom of the main chain of Leu5, contributing (-2.0) kcal/mol to the total energy. An effective interaction occurs between the positively charged N-side of the molecule and the negatively charged side chain of Asp4, contributing (-12.3) kcal/mol to the total energy (table 4). Tyr1 interacts favorably with the following Pro2-Ile3 dipeptide fragment, contributing (-7.1) kcal/mol to the total energy. Ile3 interacts effectively with the C-side dipeptide fragment, contributing (-4.5) kcal/mol to the total energy (table 4). A comparison of the energy and geometric parameters of the B_3 R R_{32} R_1 R_{12} conformation in the [Ile3]-rubiscolin-5 analogue and the native molecule shows that the contribution of different interaction energies to the stabilization of both molecules, the interaction energies between amino acid residues, and the values of the dihedral rotation angles almost coincide (tables 1 and 3, tables 2 and 5).

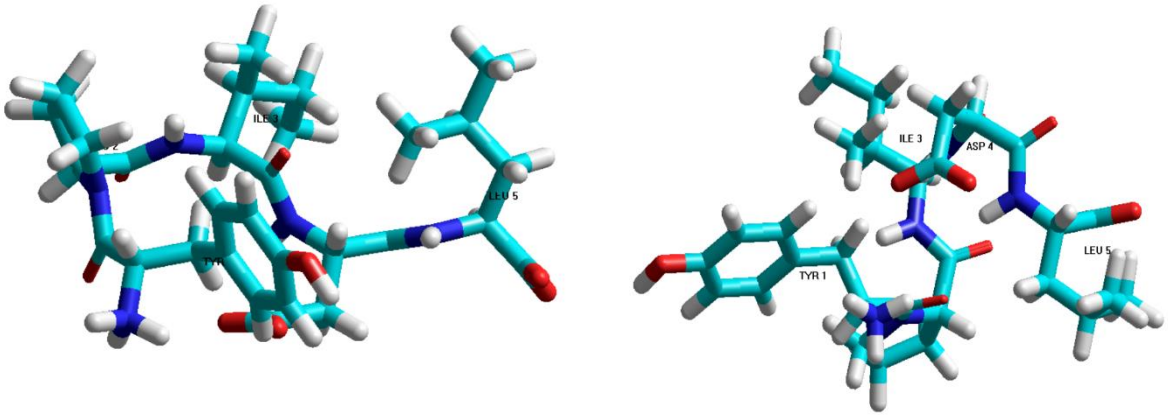
Table 5. Geometric parameters (in degrees) of low energy conformations of the molecule [Ile3]-rubiscolin-5 (the values of the dihedral angles are given in the sequence φ, ψ, ω, χ1, χ2...)

Residue	B_3 R B_{21} B_3 B_{32}	B_2 R R_{32} R_1 R_{21}	B_3 R B_{21} R_3 R_{21}	B_2 B B_{32} R_1 R_{21}
Tyr1	-93 162 167 -73 106 0	-86 157 176 -72 112 0	-63 158 168 -68 108 0	-67 107 165 165 73 0
Pro2	-60 -44 167	-60 -30 -172	-60 -57 169	-60 130 -175
Ile3	-125 119 175 173 179 62 -174	-66 -45 -176 -61 -176 177 -174	-118 101 178 171 174 60 169	-96 104 174 -56 -174 175 -169
Asp4	-104 142 -175 -60 89	-87 -35 -179 61 85	-75 -47 -178 -56 89	-89 -41 178 57 108
Leu5	-118 119 180 -54 172 -175 180	-63 -46 179 176 63 179 176	-40 -56 180 172 62 180 175	-112 -66 179 175 62 179 176
ΔU	**0.3**	**0**	**0.4**	**2.8**

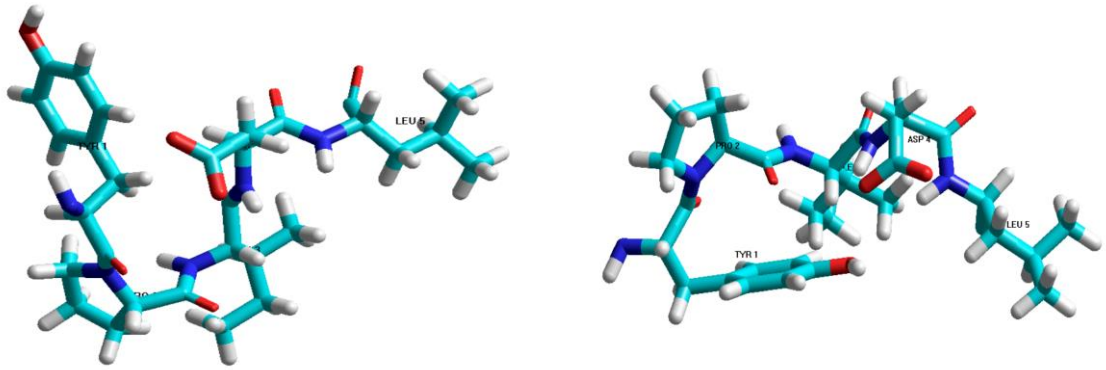
The second low-energy conformation of the [Ile3]-rubiscolin-5 analog is the B_3 R B_{21} B_3 B_{32} of the efee shape, with a relative energy of 0.3 kcal/mol. In the native molecule, its relative energy is 0 kcal/mol (Tables 1 and 3). In this conformation, Pro2, being in the R form of the main chain, turns the tripeptide fragment following it towards the N-side of the molecule. As a result, Tyr1 forms an effective interaction with the tetrapeptide fragment following it Pro2-Ile3-Asp4-Leu5, contributing (-18.9) kcal/mol to the total energy. Electrostatic and non-valent interaction forces play an important role in stabilizing this conformation. The contribution of the electrostatic interaction energy is (-4.4) kcal/mol, which is the largest. The non-valent interaction contributes (-18.9) kcal/mol. Comparisons of Tables 1 and 3, Tables 2 and 5 show that the energy and geometric parameters of the conformations are in perfect agreement.

The third low-energy conformation of the [Ile3]-rubiscolin-5 analog belongs to the B_3 R B_{21} R_3 R_{21} effef shape and its relative energy is 0.4 kcal/mol. The relative energy of this conformation in the native molecule is 0.2 kcal/mol. The contribution of the non-valent interaction energy to its stabilization is (-19.6) kcal/mol, and the contribution of the electrostatic interaction energy is (-3.9) kcal/mol. As can be seen, their contributions to the total energy approximately correspond to those of the 1st and 2nd conformations. Here, the unfolded and folded forms of the amino acid residues alternate, and Tyr1 can form effective electrostatic and dispersion interactions with the

tripeptide fragment Pro2-Ile3-Asp4 that follows it. Effective electrostatic interactions form hydrogen bonds between the positively charged N-side of the molecule and the negatively charged side chain of the Asp4 amino acid residue (Table 4). The geometric and energy parameters of the B_3 R B_{21} R_3 R_{21} conformation in the analog and in the native molecule are consistent (Tables 1 and 3, Tables 2 and 5)



a) B_3 R B_{21} B_3 B_{32} (0.3 kcal/mol) b) B_3 R R_{32} R_1 R_{12} (0 kcal/mol)



c) B_3 R B_{21} R_3 R_{21} (0.4 kcal/mol) d) B_2 B B_{32} R_1 R_{21} (2.8 kcal/mol)

Figure 1. Atomic model of spatial structure of the [Ile3]- rubiscoline-5 molecule a), b), c) and d) corresponded to the structures with the relative energies 0.3 kcal/mol, 0 kcal/mol, 0.4 kcal/mol and 2.8 kcal/mol, respectively.

The relative energy of the B_2 B B_{32} R_1 R_{21} conformation of the eeef shape of the [Ile3]-rubiscolin-5 molecule is (2.8) kcal/mol, while its relative energy in the native molecule is 4.0 kcal/mol. The contribution of non-valent interaction energy to this conformation is (-21.5) kcal/mol and is the largest. It is not favorable due to the electrostatic interaction energy, repulsive forces dominate, and their contribution to the total energy is (0.4) kcal/mol (Table 3). The N-side tripeptide fragment of the molecule is in the fully unfolded B B B form of the main chain and distances the positively charged N-side of the molecule from the negatively charged side chain of aspartic acid, and the interaction force between them contributes only (-4.7) kcal/mol to the total energy (Table 4, Figure 1-d). The energy and geometric parameters of this conformation also do not differ from the parameters of the corresponding conformation of the natural molecule.

The relative energies of the low-energy conformations of the effe, eefe, eeff and eeee shapes of the [Ile3]-rubiscolin-5 molecule vary in the energy interval (4.1) – (6.4) kcal/mol. The energies of the low-energy conformations of these shapes vary in the interval (4.3) – (5.0) kcal/mol. In these shapes, the energy and geometric parameters of the natural molecule and the [Ile3]-analogue also correspond.

Based on the above, it can be concluded that the low-energy conformations of the natural Rubiscolin molecule and its [Ile3]-analog are identical. In low-energy conformations, the interactions between amino acid residues, the contributions of different interaction energies to the stabilization of conformations, and the values of dihedral rotation angles are completely consistent. In the natural molecule, the third amino acid residue leucine is replaced by the isoleucine amino acid residue. Both amino acid residues are non-polar. The only difference between them is that the possible region of the isoleucine amino acid residue due to the φ – ψ angles is slightly smaller than the possible region of the leucine amino acid residue.

It is known that biomolecules perform their physiological functions in certain spatial structures. Therefore, since the spatial structures of both molecules whose spatial structure is studied are identical, the physiological functions they perform will also be identical. Therefore, it is not appropriate to select the [Ile3]-rubiscolin-5 molecule to synthesize an analog that will perform only certain physiological functions of the natural molecule. In [19], the selection of synthesized analogs of the rubiscolin-5 molecule was based on the spatial structure of the natural molecule.

Table 6. Optimum conformations of the [Phe3]- rubiscoline-5 molecule, their shapes, the shapes of their main chains, the energy contribution of non-valent, electrostatic, torsion interactions, the total and relative energies.

№	Shapes	Conformation	Energy contribution			U_{tot}	U_{rel}
			U_{nv}	U_{el}	U_{tor}		
1	efee	$B_3 R B_1 B_3 B_{32}$	-19.6	-4.5	4.1	-19.9	1.0
2	efff	$B_2 R R_1 R_1 R_{21}$	-21.6	- 3.5	4.3	-20.9	0
3	efef	$B_3 R B_3 R_3 R_{21}$	-20.7	-3.7	3.6	-20.9	0
4	eeef	$B_2 B B_3 R_1 R_{21}$	-23.1	0.1	4.3	-18.7	2.2
5	effe	$B_3 R R_2 B_3 B_{32}$	-20.4	1.0	2.5	-16.9	4.0
6	eefe	$B_1 B R_2 B_1 B_{32}$	-19.4	0.9	3.1	-15.4	5.5
7	eeff	$B_1 B R_3 R_3 R_{32}$	-20.7	1.0	4.6	-15.1	5.8
8	eeee	$B_1 B B_2 B_1 B_{32}$	-17.9	-0.6	2.8	-15.6	5.3

The [Phe3]-rubiscolin-5 analogue of the rubiscolin-5 molecule was also selected based on the article [19]. The spatial structure of the [Phe3]-rubiscolin-5 analogue was studied based on the low-energy conformations of the natural rubiscolin-5 molecule shown in Table 1. In each low-energy conformation, the χ angle of the Phe side chain, which exists in the 60°, 180° and -60° states according to the torsion potential, was considered. The results of the calculations show that differentiation occurs only according to the conformations of the phenylalanine side chain. The most stable conformation of the Phe side chain was selected, and these conformations, the forms and shapes of the main chain, the contribution of their various interaction energies, total and relative energies are shown in Table 6. The amino acid residues and internal interaction energies in four conformations with relative energies less than 4.0 kcal/mol are shown in Table 7, the

values of their dihedral rotation angles are shown in Table 8, and the spatial arrangement of atoms in these conformations is shown in Figure 2, a, b, c, d.

Table 7. Energy inside and between residual interactions in the conformations of the molecule [Phe3]- rubiscoline-5 B_3 R B_1 B_3 B_{32} (U_{rel}=1.0 kcal/mol, first line), B_3 R R_1 R_1 R_{21} (U_{rel}=0 kcal/mol,second line), B_3 R B_3 R_3 R_{21} (U_{rel}=0.0 kcal/mol, third line), B_2 B B_3 R_1 R_{21} (U_{rel}=2.2 kcal/mol, fourth line)

Tyr1	Pro2	Phe3	Asp4	Leu5	
-3.8	-4.3	-2.9	-12.2	-0.3	**Tyr1**
3.4	-2.7	-3.2	-9.6	-2.3	
2.1	-4.4	-2.0	-11.8	-0.2	
2.4	-5.6	-5.2	-5.9	-2.4	
	0.2	-2.2	-0.4	0	**Pro2**
	0.2	-1.7	-1.1	-2.3	
	0.3	-2.4	-0.6	0	
	0.3	-1.2	-1.1	-0.1	
		0.3	-0.7	-2.6	**Phe3**
		0.2	-2.2	-1.4	
		-0.1	-0.6	-1.7	
		0.2	-0.4	-0.9	
			2.5	-1.7	**Asp4**
			2.0	-1.1	
			2.3	-1.7	
			1.8	-1.3	
				-3.6	**Leu5**
				-3.6	
				-3.8	
				-3.6	

Table 8. Geometric parameters (in degrees) of low energy conformations of the molecule [Phe3]-rubiscolin-5 (the values of the dihedral angles are given in the sequence φ, ψ, ω, χ1, χ2...)

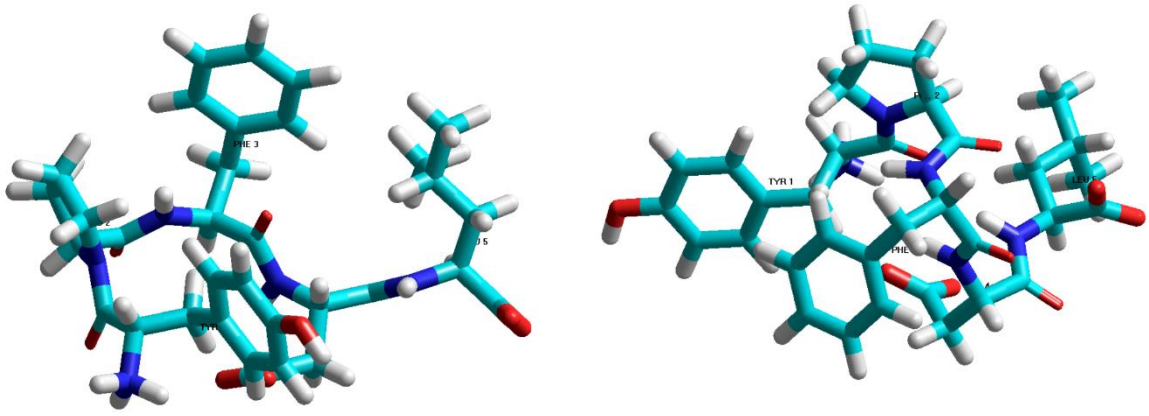
Residue	B_3R B_1 B_3 B_{32}	B_3R R_1 R_1 R_{21}	B_3R B_3 R_3 R_{21}	B_2B B_3 R_1 R_{21}
	-93 161 167	-88 157 -173	-65 155 170	-66 110 165
Tyr1	-72 106 0	-74 112 0	-70 107 0	165 75 0
Pro2	-60 -46 165	-60 -41 -166	-60 -62 169	-60 129 -171
	-127 121 178	-68 -38 -178	-107 98 180	-89 89 -174
Phe3	60 94	65 84	-60 90	-62 94
	-103 143 -173	-85 -34 -179	-76 -49 179	-96 -43 180
Asp4	-60 90	62 85	-56 89	57 109
	-117 119 180	-63 -45 179	-77 -52 180	-114 -79 180
Leu5	-53 173 -175	176 63 179	177 63 179	-178 62 179
	180	179	175	174
ΔU	**1.0**	**0**	**0**	**2.2**

The relative energies of the low-energy conformations of the [Phe3]-rubiscolin-5 analogue vary in the energy range (0-6.0) kcal/mol (Table 7). The contribution of non-valent interaction energy to these conformations varies in the range (-23.1) - (-17.9) kcal/mol, the contribution of electrostatic interaction energy varies in the range (-4.5) - (1.0) kcal/mol, and the torsional interaction energy varies in the range (2.5) - (4.6) kcal/mol. As can be seen from Table 6, the sequence of low-energy structures is almost the same as in the natural molecule. The first four low-energy conformations are the same as in the natural molecule. The relative energy of the B_3R B_1 B_3 B_{32} conformation of the efee shape in the [Phe3]-rubiscolin molecule is 1.0 kcal/mol. This

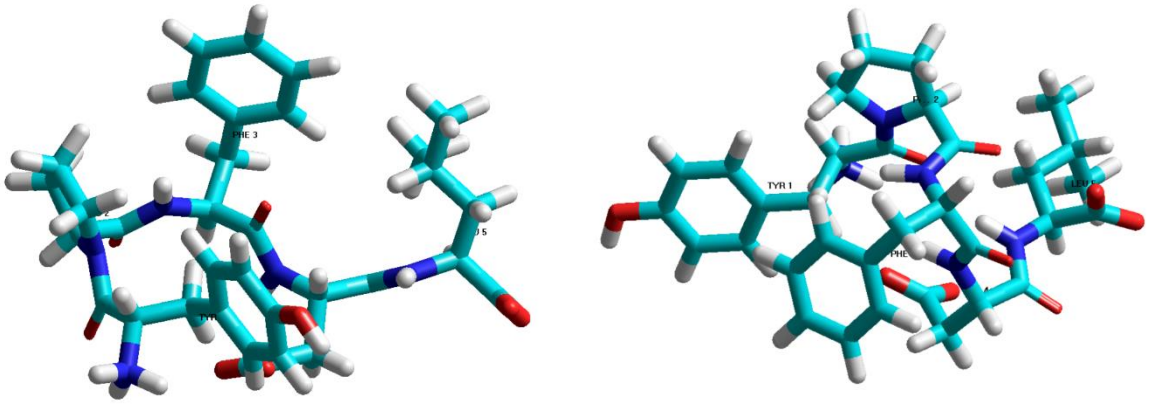
conformation is the global conformation of the natural molecule. The contribution of electrostatic interaction energy to the stabilization of the conformation is the largest. In the conformation, the Pro2 amino acid residue, being in the R form of the main chain, rotates the following Phe3-Asp4-Leu5 tripeptide fragment in such a way that the positively charged N-side of the molecule and the negatively charged side chain of aspartic acid approach each other in space, and an effective interaction occurs between them, contributing up to (-12.2) kcal/mol to the total energy. In addition, the interaction energy between Pro2-Phe3 of Tpr1 contributes up to (-7.2) kcal/mol to the total energy, Pro2-Phe3 to (-2.2) kcal/mol, and Phe3-Asp4-Leu5 to (3.3) kcal/mol to the total energy (table 7). A comparison of tables 6, 7, and 8 with tables 1 and 2 shows that the values of the interaction forces stabilizing the molecules and the geometric parameters of the conformations are the same in both molecules.

The relative energy of the B_2 R R_1 R_1 R_{21} conformation of the efff shape in the [Phe3]-analog is 0 kcal/mol, while in the natural rubiscolin-5 molecule it is 0.4 kcal/mol. The interaction of Tyr-1 with the subsequent Pro2-Phe3-Asp4-Leu5 tetrapeptide fragment contributes to the stabilization of the conformation by (-17.8) kcal/mol, the interaction of Pro2 with the subsequent tripeptide fragment contributes to the total energy by (-5.1) kcal/mol, and the interaction of Phe3 with the subsequent dipeptide fragment contributes to the total energy by (-3.7) kcal/mol. Again, a comparison of Table 1 with Table 6, and Table 2 with Table 8 shows that the interaction forces and the values of the dihedral rotation angles of the molecules stabilizing this conformation in the natural rubiscolin-5 molecule and its [Phe3] analog are almost the same.

Another low-energy structure of the [Phe3]-rubiscolin molecule is the B_3 R B_3 B_3 R_{21} conformation belonging to the efef shape, whose relative energy in the native molecule was 0.2 kcal/mol. The non-valent interaction energy contributed up to (-20.7) kcal/mol to the stabilization of the conformation, the electrostatic interaction energy contributed up to (-3.7) kcal/mol, and the torsional interaction energy contributed up to (3.6) kcal/mol. In this conformation, the positively charged N-side of the molecule and the negatively charged side chain of Asp4 are spatially close to each other, contributing up to (-11.8) kcal/mol to the total energy, and a hydrogen bond is formed between the hydrogen atom of the main chain and the oxygen atom of the aspartic acid side chain. The Tyr1 amino acid residue and Pro2-Phe3 form an effective interaction, contributing (-5.9) kcal/mol to the total energy, while the Pro2 amino acid residue Phe3-Asp4 dipeptide fragment contributes (-3.0) kcal/mol to the total energy. Again, a comparison of Table 1 and Table 6, and Table 3 with Table 8, shows that the energy and geometric parameters of the analog and the native molecule are almost completely consistent.



a) $B_3 R B_1 B_3 B_{32}$ (1.0 kcal/mol) b) $B_2 R R_1 R_1 R_{21}$ (0 kcal/mol)



a) $B_3 R B_1 B_3 B_{32}$ (1.0 kcal/mol) b) $B_2 R R_1 R_1 R_{21}$ (0 kcal/mol)

Figure 2. Atomic model of spatial structure of the [Phe3]- rubiscoline-5 molecule a), b), c) and d) corresponded to the structures with the relative energies 1.0 kcal/mol, 0 kcal/mol, 0 kcal/mol and 2.2 kcal/mol, respectively.

The relative energy of the $B_2 B B_3 R_1 R_{21}$ conformation of the eeef shape is (2.2) kcal/mol. In the natural molecule, its relative energy was (4.0) kcal/mol. A comparison of the values of the energy and geometric parameters of these conformations in the corresponding tables shows that they agree very well.

The next four low-energy conformations of the [Phe3]-rubiscolin5 molecule, presented in Table 6, are similar to the conformations of the natural rubiscolin-5 molecule, shown in Table 1. The total contribution of the various interaction energies in all of the conformations, the interaction energies between the various amino acid residues, and the values of their dihedral rotation angles agree very well in the [Phe3]-rubiscolin-5 analog and the natural rubiscolin molecule.

Calculation of the spatial structure of the [Phe3]-rubiscolin-5 molecule showed that the values of its energy and geometric parameters given in Tables 6, 7, and 8, and the spatial arrangement of atoms in Figure 2 a,b,c,d, correspond to the values and figures given for the natural rubiscolin-5 molecule in the article [18]. Therefore, it can be concluded that the [Phe3] analog is also not a suitable analog for synthesis, and in principle it will retain all the functions of the natural molecule.

Conclusion

1. The spatial structure of the [Ile3]-rubiscolin-5 molecule was studied and it was shown that it is represented by eight low-energy conformations.

2. The roles of different interaction energies and interactions between different amino acid residues in the formation of low-energy conformations of the [Ile3]-rubiscolin-5 molecule were shown.

3. The low-energy conformations of the natural rubiscolin molecule and its [Ile3[analog were compared and it was shown that the energy and geometric parameters of both molecules were the same, and it was shown that the [Ile3]-analog was not a suitable analog for synthesis.

4. The three-dimensional spatial structure of the [Phe3]-rubiscolin analog was studied by the method of theoretical conformational analysis, the assembly of its low-energy conformations, the roles of various types of interactions in stabilizing them, and the values of dihedral rotation angles in low-energy conformations were determined.

5. Comparison of the energy and geometric parameters of the [Phe3]-rubiscolin-5 analogue and the natural rubiscolin-5 molecules showed that they are completely identical. Therefore, it cannot be assumed that this analogue will differ from the functions performed by the natural rubiscolin-5 molecule, and it is not advisable to synthesize the [Phe3]-analogue.

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