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Structure-functional organization of the pentapeptide ARG-PRO-PRO-GLY-PHE

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Abstract

Regulatory peptides include the family of glyprolines, short peptides whose amino acid sequences contain glycine and proline residues. Currently, the mechanisms of action of glyprolines are poorly understood. It is possible to understand the mechanisms of their action if we solve the problem of their structural and functional organization. Therefore, the study of the spatial structure of peptides, its ability to change its conformation when interacting with other molecules is an important and actual problem of molecular biophysics. It should be noted that the action of glyprolines is highly stable, fast and efficient. It seems relevant to carry out structural and functional studies of synthetic analogues of glyprolines on model systems using theoretical research methods. Using the method of molecular mechanics, the spatial structure and conformational properties of the glyproline pentapeptide molecule Arg-Pro-Pro-Gly-Phe were determined. The potential energy of this molecule was estimated as the sum of nonvalent, electrostatic, torsion interactions and the energy of hydrogen bonds. 10 low-energy conformations of this molecule and the values of the dihedral angles of the main and side chains were found. The energy of the intra- and inter-residue interactions was estimated. The calculation showed that semi-folded forms of the main chain are low-energy for the pentapeptide. The side chains of amino acids Arg and Phe in low-energy conformations carry out effective interactions and are conformationally labile amino acids; they bring together the main chain and side chains of the amino acids included in the pentapeptide.

Keywords: pentapeptide, conformation, molecule, spatial structure;

1. Introduction

The functioning of peptide molecules determines all biochemical processes occurring in living organisms. The peptide molecules and thear biological functions in

living systems are related with their specific spatial structures. Therefore, to understand the mechanism by witch the peptides function it is necessary to know their three dimensional structural and functional organization. The study of the spatial structure of peptide molecules, its ability to change its conformation when interacting with other molecules is an actual problem of molecular biophysics. It is important to know the full complement of low-energy conformational states.

Peptides regulate all functions of a living organism. Currently, the role of regulatory peptides in the life of organisms is being actively studied all over the world. Regulatory peptides are a key link in the mechanism of regulation of the functions of the human body. Elucidation of the structural and functional organization of these peptides is of great practical importance in medicine and pharmacology. When creating new drugs, scientists are increasingly turning to the use of the human body's own resources.

Currently, new families of peptides are being discovered and their properties are being studied. One of these families is glyprolines, short linear peptides whose amino acid sequences include glycine (Gly) and proline (Pro) residues. They have a wide range of physiological properties. Glyprolines affect the blood coagulation system, modulate the functioning of the immune and nervous systems, and have an antiulcer effect [1–3]. Glyprolines have neuroprotective properties ensure the preservation of the normal functioning of the insular and anticoagulant blood systems against the background of the development of diabetes. A number of amino acids (Arg, Phe, Leu) are involved in the normalization of triglyceride levels, prevent the risk of diabetes and atherosclerosis.

Computer modeling based on the use of the method of theoretical conformational analysis and programs that allow obtaining a graphical representation of the spatial structures of the molecule was performed for the glyproline pentapeptide molecule Arg-Pro-Pro-Gly-Phe (RPPGF). This pentapeptide being the N-terminal fragment of bradykinin, inhibits alpha-thrombin-induced platelet activation [4-6]. The mechanism by which this pentapeptide inhibits thrombin-induced platelet activation is that Arg-Pro-Pro-Gly-Phe binds to the thrombin active site, forming a parallel β-chain with the thrombin site [6, 7]. The aim of our research is to study the structural and functional organization of the Arg-Pro-Pro-Gly-Phe petapeptide molecule.

Calculation of the glyproline pentapeptide molecule Arg-Pro-Pro-Gly-Phe has been carried out by the method of theoretical conformational analysis with regard to nonvalent, electrostatic and torsional interactions and energy of the hydrogen bonds. Nonvalent interactions are estimated by Lennard-Jones potential. Electrostatic interactions are calculated in the monopole approximation by Coulombs law using partial charges on the atoms. Hydrogen bonds are evaluated by Morze potential. Torsional potentials and values of rotation barriers of amino acid main and side chain dihedral angles are taken from work [8]. For this molecule, low-energy conformations and the dihedral angles of the main and side chains of the amino acids

included in it were found. In this case, the energy of intra- and interresidual interactions in each low-energy structure was estimated.

2. Research method

The method of theoretical conformational analysis makes it possible to calculate the three-dimensional structure of peptide molecules based on a known amino acid sequence. In presenting the results of the calculation of the spatial structure of the molecules we used the spatial classification. According to it all structural versions break down into shapes including certain forms of the main chain and each form is represented by a set of conformations. The conformations are determined by the number of rotational degrees of freedom of the side chains of the residues being included in the molecule.

The developed special classification (conformation, form of the main chain, shape) was used in the calculations. The shapes of the residues were determined by the regions B, R, L and P of the dihedral angles of the main chain ϕ-ψ. In the calculation, extended forms of the dipeptide molecule (BB, BR, LB, LR, RL, PL, PP-shape e) and folded forms of the main chain (RB, RR, BL, LL, PR, PB-shape f) were considered. To designate conformational states of the residues there have been used X (*i, j*) - typed identifiers, where X defines low-energy regions of the conformational map ϕ-ψ : 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°), *i, j*…=11…,12…,13…,21…, and etc. conform to the positions of the side chain (χ_1, χ_2…), subscript 1 corresponds to the angle χ = 0-120°; 2 to χ = 120-(-120 °), 3 to χ = (-120)-0°.

For glycine, the initial approximations were formed from low-energy conformations (R form - ϕ= -90°; ψ= -90°; B form - ϕ= -90°; ψ= 100°; L form - ϕ, ψ = 90° and P form of the main chain - 90°; ψ= -90°), the boundary regions were also taken into account for it. For the amino acid proline 2, only one position was taken into account (B form - ψ=130°), since the R form of the amino acid before Pro is high-energy. For proline3, two forms of the main chain were taken into account (B form ϕ= -60°; ψ=130° and R form ϕ= -60°; ψ= -50°). For the Arg amino acid residue, only the B form was considered, since this amino acid also precedes proline, so the R form is high-energy for it. For the Phe residue, B and R forms of the main chain are possible, but both of these conformations differ in the position of the last three COO atoms. The positions of the side chain of arginine were determined by four dihedral angles χ_1^1, χ_1^2, χ_1^3, χ_1^4, and for phenylalanine - by two angles χ_4^1, χ_4^2.

The calculation was carried out within the framework of the mechanical model of molecules, taking into account non-valent (U_{nv}), electrostatic (U_{el}), torsion interactions (U_{tors}) and energy of hydrogen bond. The designations and readings of the rotation angles correspond to the accepted international nomenclature [9]. A spe-

cially developed program was used to find the spatial structure of this peptide glyproline molecule [10]. This scientific work is a continuation of our calculations of the structure of peptide molecules [11-14].

3. Results and discussion

The amino acid sequence of the studied pentapeptide contains two conformationally rigid residues proline (Pro) and phenylalanine (Phe), as well as a positively charged and conformationally flexible amino acid arginine (Arg). The Gly residue is devoid of a side chain (it has only one hydrogen atom in the side chain).

The conformational possibilities of the glyproline molecule Arg-Pro-Pro-Gly-Phe were studied on the basis of stable conformations of the monopeptides N-acetyl-L-arginine, N-acetyl-L-proline, N-acetyl-L-glycine, and N-acetyl-L-phenylalanine. For this pentapeptide containing 68 atoms and 18 variable dihedral angles, 4 shapes are possible, represented by 16 forms of the main chain. For the Arg amino acid, only one B form of the main chain was considered, since the residue precedes Pro, for which all conformations of the R form of the main chain are high-energy. The same applies to the remainder of Pro2, which comes before Pro3. For the residue Gly, all possible forms of the main chain B, R, L, and P were considered, as well as the boundary values of the angles ϕ and ψ of the main chain. For the Phe residue, the B and R forms of the backbone were considered.

The side chains of arginine and phenylalanine are labile, and the side chain of arginine is bulky and carries a positive charge. Proline has a rigid side chain, while glycine has only one hydrogen atom as its side chain. The specificity of the side chains of all amino acids of the pentapeptide molecule determined the number of initial approximations. In total, more than 250 conformations were calculated, belonging to 16 forms of the main chain and 4 possible shapes for this molecule. All of them were minimized in terms of energy, their geometric and energy parameters were estimated. Low-energy conformations of the Arg-Pro-Pro-Gly-Phe molecule are presented in Table 1.

Of the 16 calculated main chains, the lowest energy one is BBRBB (eefe shape), which has a semi-folded main chain course. In the global conformation $B_{1222}BRBB_{11}$ (U_{rel}=0 kcal/mol), the energy of non-valent interactions is -18.5 kcal/mol, electrostatic -0.6 kcal/mol and torsion 2.9 kcal/mol. The main stabilizing contribution is made by dipeptide (-11.4 kcal/mol), tripeptide (-4.5 kcal/mol), tetrapeptide (-1.7 kcal/mol) and petapeptide (-9.6 kcal/mol) interactions. The $B_{1222}BRBR_{11}$ conformation (U_{rel}=1.4 kcal/mol) loses only 1.4 kcal/mol, in which the terminal phenylalanine is in the R form. Thus, the performed calculation revealed the presence of a sharp energy differentiation in the shapes and forms of the main chain. Of the four shapes, two are low-energy, and the course of the main chain is identical: first, the pentapeptide molecule has an extended main chain, and the end of the molecule

is folded. Such a semi-folded form can easily adapt to the β-chain of the thrombin site. At the same time, at the turn, the side chain of phenylalanine is turned into the solvent and is conformationally free for interactions.

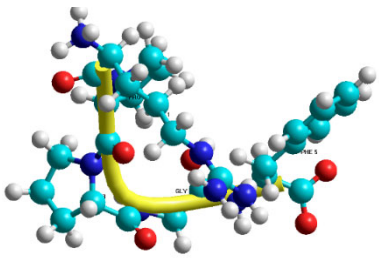
Table 1. Energy parameters (kJ/mol): contributions of nonvalent, electrostatic, torsion interactions and relative energy of low-energy conformations of the pentapeptide molecule Arg-Pro-Pro-Gly-Phe

№	Conformation(shape)	U nv	U el	U tors	Utot	U rel
1	B1222BRBB11 (eefe)	-77.7	-2.5	12.2	-67.6	0.0
2.	B1222BRBR11 (eefe)	-71.4	-2.5	11.8	-61.7	5.9
3	B3222BRBB21 (eefe)	-69.3	2.1	15.5	-51.2	16.4
4	B3322BBPB31 (eeff)	-61.7	-6.3	9.2	-58.8	8.8
5	B2122BRRB21 (eeff)	-47.0	-8.4	5.9	-50.0	17.6
6	B3322BBPR31 (eeff)	-56.3	-6.3	9.2	-53.3	14.3
7.	B1222BBBB31 (eeee)	-68.0	0.4	19.3	-48.7	18.9
8.	B1222BBBR31 (eeee)	-66.4	0.4	18.5	-47.5	20.1
9.	B1222BBRB11 (eeef)	-58.0	0.4	17.2	-40.3	27.3
10.	B1222BBRR11 (eeef)	-60.5	-2.1	17.6	-44.9	22.7

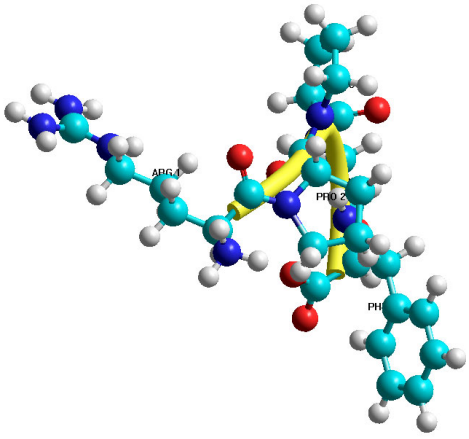
Table 1 presents the low energy conformations of the Arg-Pro-Pro-Gly-Phe pentapeptide molecule. The eefe and eeff shape conformations have the lowest energy; they fall into the energy range of 0–5 kcal/mol. More than 5 kcal/mol lose the fully extended eeee shape and eeef shape conformations. Table 1 lists 10 low energy structures and the corresponding energy parameters: contributions of nonvalent, electrostatic, torsion interactions and relative energy. The main contribution to the low energy of conformations is made by nonvalent interactions. Further, the geometric parameters of low-energy conformations were determined - the dihedral angles of the main and side chains of amino acid residues included in the pentapeptide molecule. Geometrical parameters (in degrees) for four low-energy conformations are shown in Table 2. Location of amino acids in four low-energy conformations $B_{1222}BRBB_{11}$, $B_{3232}BBPB_{31}$,$B_{1222}BRBR_{11}$ and $B_{1222}BBBB_{31}$ are shown in Figures 1 (a), b), c) and d). From the figures, one can see the proximity of the main chain and side chain sections of the amino acids included in this pentapeptide molecule Arg-Pro-Pro-Gly-Phe. The results obtained can be used to study the spatial structure of other analogues of the bradykinin molecule.

Table 2. Geometric parameters (degree) of low-energy conformations of the pentapeptide molecule Arg-Pro-Pro-Gly-Phe

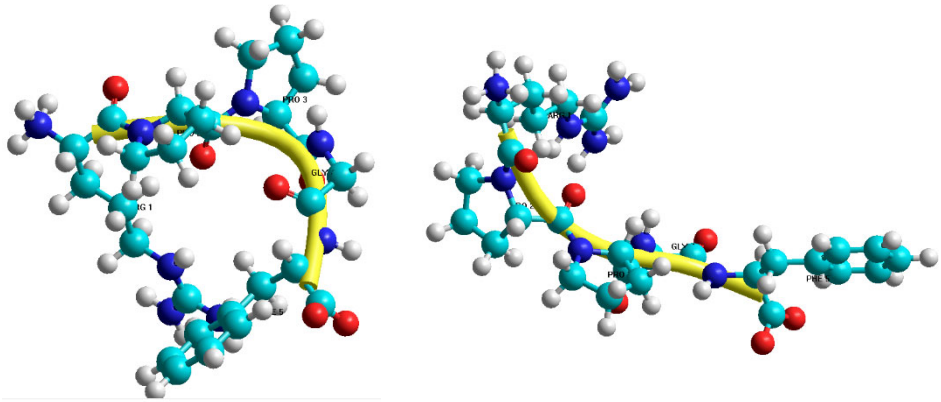
	Dihedral angles	B1222BRBB11	B3322BBPB31	B1222BRBR11	B1222BBBB31
Arg 1	φ1	-82	-131	-81	-74
	χ11	60	-70	60	60
	χ12	179	-76	178	164
	χ13	-175	-177	-175	-171
	χ14	-177	177	-178	175
	ψ1	155	83	154	159
	ω1	172	172	172	159
Pro 2	φ2	-60	-60	-60	-60
	ψ2	167	161	168	160
	ω2	178	178	177	-177
Pro3	φ3	-60	-60	-60	-60
	ψ3	-50	112	-48	114
	ω3	172	178	170	180
Gly4	φ4	-88	102	-89	-116
	ψ4	158	-20	159	49
	ω4	180	-176	-179	-174
Phe5	φ5	-167	-107	-165	-113
	χ51	49	-60	48	-55
	χ52	87	84	90	87
	ψ5	151	119	-45	127
Urel (kJ/mol)		0.0	8.8	5.9	18.9



a) $B_{1222}BRBB_{11}$



b) $B_{3322}BBPB_{31}$



c) $B_{1222}BRBR_{11}$ d) $B_{1222}BBBB_{31}$

Fig. 1. The spatial arrangement of amino acids in low energy conformations

4. Conclusions

Using the method of molecular mechanics, the spatial structure and conformational properties of the glyproline pentapeptide molecule Arg-Pro-Pro-Gly-Phe were determined. The results of the calculation showed that there is an energy differentiation between the shapes, forms of the main chain and conformations. 10 low-energy conformations for the glyproline pentapeptide were found, the dihedral angles of the main and side chains were found, and the energy of intra- and interresidual interactions was estimated. The calculation showed that semi-folded forms of the main chain are low-energy for the pentapeptide. The side chains of amino acids Arg and Phe in low-energy conformations carry out effective interactions and are conformationally labile amino acids; they bring together the main chain and side chains of the amino acids included in the pentapeptide. The calculation of the three-dimensional structure of the pentapeptide molecule made it possible to determine the geometric and energy parameters of the peptide, the dihedral angles of the main chain and side chains of the amino acids included in the molecule, as well as the energy contributions of intramolecular interactions.

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