IOSR Journal of Applied Physics (IOSR-JAP)
e-ISSN: 2278-4861.Volume 13, Issue 5 Ser. II (Sep. – Oct. 2021), PP 62-67
www.iosrjournals.org

Spatial Structure Of The β-Casomorphin-7 Molecule

N.A.Akhmedov[1], L.N.Agayeva, S.R.Akhmedova, R.M.Abbasli, L.I.Ismailova
Baku State University, Institute for Physical Problems
Z.Khalilov 23 Str., Baku, AZ-1148, Azerbaijan

Abstract. *A number of exogenous peptides obtained from food have opioid-like properties. These peptides were called exorphins. The first known exorphins were obtained by in vitro pepsin hydrolysis of α-casein and wheat glutone. The conformational capabilities of the β-casomorphin-7 molecule (Tyr1-Pro2-Phe3-Pro4-Gly5-Pro6-İle7-NH2) have been studied by the method of theoretical conformational analysis. The potential function of the system is chosen as the sum of non-bonded, electrostatic, torsion interactions and the energy of hydrogen bonds. Low-energy conformations of the β-casomorphin-7 molecule were found, the dihedral angles of the main and side chains of amino acid residues included in the molecule were found, and the energy of intra- and intersubstance interactions was estimated. It has been shown that the spatial structure of the β-casomorphin-7 molecule is represented by nine structural types. It can be assumed that the molecule performs its physiological functions in these structures. These three-dimensional structures make it possible to propose synthetic analogs for a given molecule. The results obtained can be used to elucidate the structural and structural-functional organization of casomorphin molecules.*

Keywords: *exorphin, β-casomorphin, opioid, structure, conformation.*

Date of Submission: 08-10-2021 Date of Acceptance: 22-10-2021

I. Introduction

The regulatory peptides first discovered in the second half of the twentieth century, are being actively studied by both physiologists and pharmacologists, since the area of biological activity peptides is extremely wide. They are one of the main links that unite the three regulatory systems of the body - nervous, 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 function as signaling molecules. Most of these peptides cannot be reliably 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 their immediate effect on many systems of the body. Opioid peptides are currently considered the most studied group of signaling substances of peptide nature. Opium causes pain relief, sedation and sleep, as well as a euphoric state and a number of autonomic reactions. Opioid peptides are of animal and plant origin. A number of exogenous peptides obtained from food have opioid-like properties. These peptides were called exorphins. The discovery of the opioid activity of peptide components of food has led to the assumption that certain types of food can act on the central nervous system like opiate drugs.

The first known exorphins were obtained by in vitro pepsin hydrolysis of α-casein and wheat gluten. The resistance of exorphins to the action of pancreatic enzymes has been proven, and the opioid activity of these peptides in vivo has been confirmed. It turned out that opioid-like derivatives of casein and gluten inhibit the activity of adenylate cyclase in cell culture, inhibit contractions of the mouse vas deferens, and also displace radiolabeled opioid receptor agonists from the binding sites on rat brain slices. Among the exorphins of animal origin, the derivatives of milk proteins are best studied. The most famous are milk exorphins (β-casomorphins-4, -5, -6, -7) - products of enzymatic hydrolysis of β-casein in cow's milk. Similar peptides in the β-casein molecule were later found in the milk of other mammalian species, including humans. Exorphins have also been isolated from other milk proteins, but they are much less studied than derivatives of β-casein, they mainly have an affinity for opioid receptors. The α-S1-casomorphin pentapeptide was isolated from α-casein in human milk. Interestingly, this peptide has the ability to suppress the proliferation of breast tumor cells. The physiological effect of antagonistic exorphins is still poorly understood. Some milk exorphins can not only be formed during the digestion of milk in the gastrointestinal tract, but also be contained in cheeses in a "ready-made" form, since the technology of making cheese is associated with enzymatic processing. Special attention of researchers to casomorphins is due to the fact that milk is the only food for young children, and it is known that some changes

[1] Corresponding author: Namiq.49@bk.ru

in the composition of milk can significantly affect the physical and mental development of infants, including causing long-term effects [1-3].

We have investigated the structural and functional organizations of the opioid peptides enkephalins, endorphins, endomorphins, dynorphins, neoendorphins and adrenorphins, and we are currently investigating the spatial structure of molecules of rubiscolins, soymorphins and casomorphins. This work is a continuation of our previous research The development of the ideas about the mechanism of action of the peptide molecules is possible due to the structural studies on the molecular level that can not be achieved solely on the basis of the experimental methods. Molecular modelling can fundamentally advance our ability to gain insights and detailed information on biomolecules. Currently, using the different theoretical calculation methods, the recent advances in computer technology allow researchers to construct the various models of the peptide molecules [4-14].

The molecule was calculated using the method of theoretical conformational analysis. The potential function of the system is chosen as the sum of non-bonded, electrostatic and torsion interactions and the energy of hydrogen bonds. Nonvalent interactions were assessed by Lennard-Jones potential. Electrostatic interactions were calculated in a monopole approximation according to the Coulomb's law using partial charges on atoms. The conformational possibilities of the casomorphin molecule were studied under the conditions of the water environment, in connection with which the value of the dielectric constant was assumed to be 10. The energy of hydrogen bonds was estimated using the Morse potential. Our aforementioned works describe in detail the potential functions used.

In presenting the calculation results, we used the classification of peptide structures by conformations, forms of the main chain, and shapes of the peptide backbone. 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. Forms of the main chain of a fragment are formed by combinations of forms of R, B, L residues in a given sequence. Forms of the main chain of a dipeptide can be divided into two classes - folded (f) and unfolded (e) forms, which are called shapes. All conformations are grouped by backbone shape, and shapes are grouped by shape. To designate the conformational states of the residues, identifiers of the X_{ij} type are used, where X defines the low-energy regions of the conformational map $\varphi - \psi$: $R(\varphi, \psi = -180° - 0°)$, $B(\varphi = -180° - 0°, \psi = 0° - 180°)$, $L(\varphi, \psi = 0° - 180°)$ and $P(\varphi = 0° - 180°, \psi = -180° - 0°)$; ij ... = 11 ..., 12 ..., 13 ..., 21 ... define the position of the side chain (χ_1, χ_2 ...), with the index 1 corresponding to the angle value in the range from 0 to 120 °, 2 - from 120 ° to -120 °, and 3 - from -120 ° to 0 °. The designations and readings of the angles of rotation correspond to the IUPAC-IUB nomenclature [15].

II. Results and discussion

The spatial structure of the β-casomorphin-7 molecule (Tyr1-Pro2-Phe3-Pro4-Gly5-Pro6-Ile7-NH2) was calculated based on the low-energy conformations of β-casomorphin-6 (Pro-Phe-Pro-Gly-Pro-İle-NH2, preferred conformations of the casomorphin-6 molecule are shown in Table 1) and N-acetyl-tyrosine methyl amide. The results of the calculation of the β-casomorphin-7 molecule showed that there is an energetic differentiation between shapes, forms of the main chain and conformations. The energy range of 0-7.0 kcal/mol contains the conformations of nine forms of the main chain of nine shapes. The lowest-energy conformation of each form, the energy contributions of non-valence, electrostatic, torsion interactions and the relative energy are shown in Table 2. In low-energy conformations, the energy of non-valence interactions varies in the range (-20.8) - (-16.7) kcal / mol, electrostatic interactions (-3.5) - (- 2.3) kcal/mol, torsion interactions (2.0) - (6.2) kcal/mol. For the four low-energy conformations, the energies of intra- and inter-residual interactions (in kcal / mol) are shown in Table 3, and the geometric parameters (in degrees) are shown in Table 4. Figure 1 shows the spatial arrangement of atoms in these conformations.

The global conformation of the β-casomorphin-7 molecule is efeeee shape $B_3RB_1RLBB_{32}$. It is simultaneously beneficial for non-valence and electrostatic interactions, the contribution of which is (-20.8) kcal/mol and (-3.5) kcal/mol, respectively. Due to these interactions, the conformation is the lowest in energy (Table 2). In this conformation, the second Pro2 residue is in the R form of the main chain and rotates the peptide chain, while the remaining residues are in the unfolded form of the main chain. The side chains of amino acid residues are directed so that effective interactions arise between the atoms of the main chain between themselves and the atoms of the main and side chains, the value of which is (-11.0) kcal/mol. Phe3 effectively interacts with the dipeptide site Pro4-Gly5, the contribution of interactions is (-5.5) kcal/mol (Table 3). There are no effective interactions between other amino acid residues.

In the $B_1BB_2BLRB_{32}$ conformation of the eefef shape with a relative energy of 0.2 kcal/mol, the energy contributions of non-bonded, electrostatic, and torsion interactions are almost the same as in the global conformation, despite the fact that it differs in the shape of the main chain and the arrangement of side chains of amino acid residues. Tyr1 effectively interacts with the residues Pro2 and Phe3, whose contribution is (-7.8) kcal/mol, Phe3 effectively interacts with the tripeptide site Pro4-Gly5-Pro6, the

contribution of which is (-9.3) kcal/mol, and still effective interactions arise between amino acid residues of the C-terminal tripeptide section (table 3).

The $B_2BL_3RRBB_{32}$ conformation of the efeffe shape has a relative energy of 0.7 kcal/mol. Here, the first Tyr1 residue effectively interacts with the next tripeptide fragment Pro2-Phe3-Pro4, whose contribution is (-9.4) kcal/mol, Phe3 effectively interacts with the tripeptide site Pro4-Gly5-Pro6, whose contribution is (-7.7) kcal/mol, and Gly5 interacts effectively with the Pro6-Ile7 dipeptide (Table 3). The $B_3RB_2BLBB_{32}$ conformation of the efefee shape with a relative energy (1.9) kcal/mol differs from the global conformation in the shape of the Pro4 backbone and the location of the Phe3 side chain. Because of this, the contribution of non-valence interactions loses (2.1) kcal/mol, and loses (0.6) kcal/mol in electrostatic interactions. Tyr1 interacts effectively with Pro2, Phe3 and Pro6. Phe3 effectively interacts with the tetrapeptide site Pro4-Gly5-Pro6-Ile7, the contribution of which is (-10.5) kcal/mol (Table 3). In this conformation, effective di- and tripeptide interactions arise between the amino acid residues of the C-terminal tripeptide region.

Table 1.

Energy contributions of non-valent (U_{nv}), electrostatic (U_{el}), torsional (U_{tors}) interactions and the relative energy (U_{rel}) of the optimal conformations of the molecule casomorphine-6

№	Shapes	Conformation	U_{nv}	U_{el}	U_{tors}	U_{rel}
1	fefee	RB_2BLBB_{32}	-18.9	-4.5	2.8	0
2	fefef	RB_2BLRB_{32}	-18.4	-4.5	2.3	0.1
3	feeef	RB_1RLRB_{32}	-16.4	-4.3	1.5	1.3
4	feeee	RB_1BLBB_{32}	-15.1	-4.4	1.7	2.8
5	eeeef	BB_1RLRB_{32}	-14.8	-4.3	1.6	3.1
6	eeeee	BB_1RLBB_{32}	-13.5	-4.4	1.8	4.4
7	Feffe	BL_3RRBB_{32}	-17.3	-3.9	2.5	1.9
8	eefef	BB_2BLRB_{32}	-16.2	-4.5	2.3	2.1
9	eefee	BB_2BLBB_{32}	-16.4	-4.6	2.7	2.6

Table 2.

Energy contributions of non-valent (U_{nv}), electrostatic (U_{el}), torsional (U_{tors}) interactions and the relative energy (U_{rel}) of the optimal conformations of the molecule casomorphine-7

№	Shapes	Conformation	U_{nv}	U_{el}	U_{tors}	U_{total}	U_{rel}
1	efeeee	$B_3RB_1RLBB_{32}$	-20.8	-3.5	3.5	-20.8	0
2	efeeef	$B_1RB_1RLRB_{32}$	-18.9	-3.2	2.0	-20.1	0.7
3	efeffe	$B_2BL_3RRBB_{32}$	-20.9	-2.3	3.2	-20.1	0.7
4	efefee	$B_3RB_2BLBB_{32}$	-18.7	-2.9	2.8	-18.9	1.9
5	efefef	$B_3RB_2BLRB_{32}$	-18.4	-2.8	2.3	-18.9	1.9
6	eeefef	$B_1BB_2BLRB_{32}$	-21.0	-3.2	3.5	-20.6	0.2
7	eeefee	$B_1BB_2BLBB_{32}$	-18.0	-3.2	6.2	-15.0	5.8
8	eeeeef	$B_1BB_1RLRB_{32}$	-17.9	-3.0	2.4	-18.5	2.3
9	eeeeee	$B_1BB_1RLBB_{32}$	-16.7	-3.0	2.6	-17.2	3.6

Table 3.

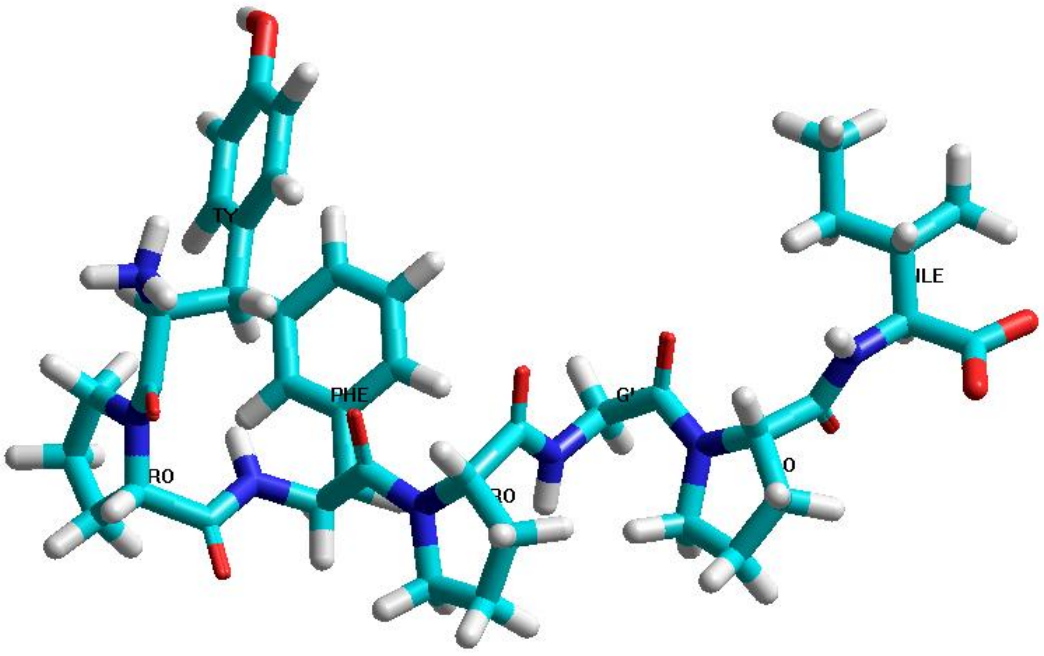
Energy inside and between residual interactions in the conformations of the molecule casomorphine-7: $B_3RB_1RLBB_{32}$ (U_{rel}=0 kcal/mol, first line), $B_1BB_2BLRB_{32}$ (U_{rel}=0.2 kcal/mol, second line), $B_2BL_3RRBB_{32}$ (U_{rel}=0.7 kcal/mol, third line), $B_3RB_2BLBB_{32}$ (U_{rel}=1.9 kcal/mol, fourth line)

Tyr1	Pro2	Phe3	Pro4	Gly5	Pro6	Ile7	
2.3	-4.3	-6.7	-0.5	-0.2	-0.1	-0.1	Tyr1
2.2	-4.6	-3.2	-0.5	0	0	-0.1	
3.9	-5.2	-2.1	-2.4	-0.1	-0.2	-0.2	
5.6	-3.5	-2.1	-0.3	0	-2.2	-0.2	
	0.2	-1.7	-0.5	0	0	0	Pro2
	0.3	-0.4	-0.8	0	-0.1	0	
	0.3	-0.9	-1.2	0	-0.1	0	
	0.3	-1.4	-0.8	0	-0.1	0	
		0.8	-3.7	-1.8	-0.1	0	Phe3
		-0.3	-4.7	-2.0	-2.6	0	
		0.5	-3.4	-0.8	-3.5	-0.3	
		-0.3	-4.7	-2.0	-2.2	-1.7	
			0.3	-0.6	-1.6	-0.1	Pro4
			0.3	0.4	-1.0	0	
			0.3	-0.7	-1.7	-0.1	
			0.3	0.4	-0.8	-0.1	

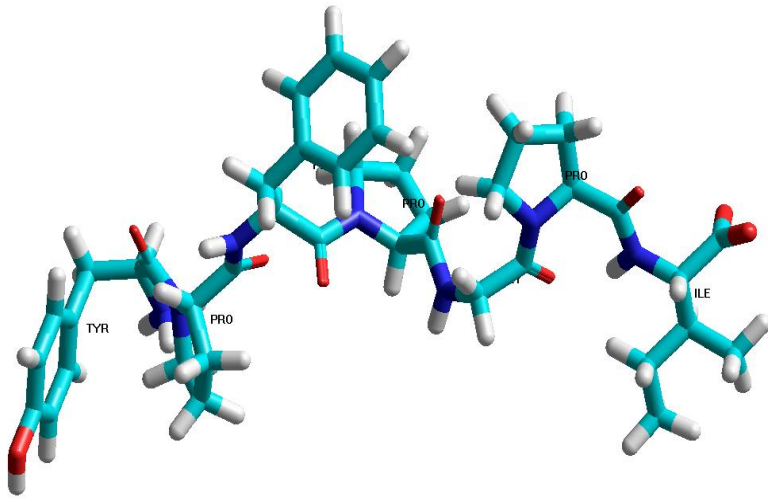
				1.4 1.4 1.4 1.4	-3.3 -3.0 -2.9 -3.3	-0.9 -1.3 -1.0 -1.0	Gly5
					0.4 0.3 0.3 0.3	-1.5 -2.7 -1.4 -1.5	Pro6
						-1.8 -1.7 -1.6 -1.5	Ile7

Table 4.
Geometric parameters (in degrees) of low energy conformations of the molecule casomorphine-7(the values of the dihedral angles are given in the sequence φ, ψ, w, χ1, χ2 …...)

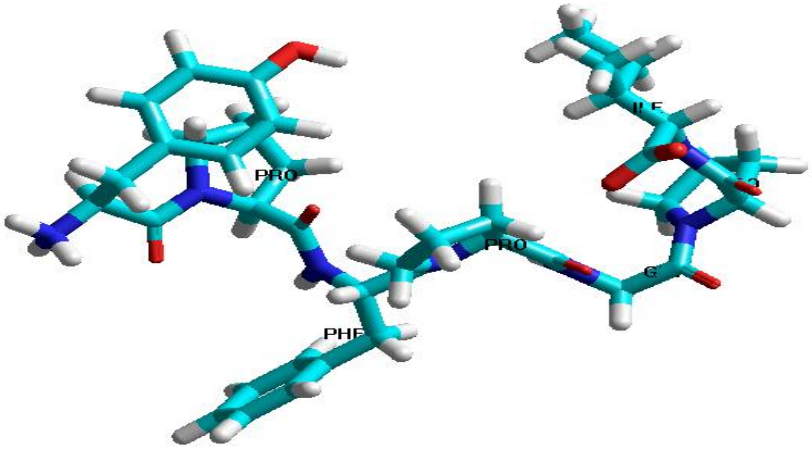
Residue	$B_3RB_1RLBB_{32}$	$B_1BB_2BLRB_{32}$	$B_2BL_3RRBB_{32}$	$B_3RB_2BLBB_{32}$
Tyr1	-70 153 170 -72 106 0	-82 148 177 65 90 0	-97 128 180 180 86 0	-99 141 180 63 94 0
Pro2	-60 -33 -179	-60 119 -172	-60 130 -174	-60 -54 179
Phe3	-96 149 178 57 86	-98 128 177 -178 86	52 70 -179 -53 97	-101 126 176 -177 84
Pro2	-60 -57 -178	-60 117 -173	-60 -60 177	-60 117 -175
Gly5	65 70 179	63 70 -176	-69 -67 -173	68 73 -174
Pro6	-60 138 -178	-60 -52 179	-60 131 -179	-60 131 -177
Ile7	-102 132 180 -57 -175 173 -171	-100 136 180 -58 -176 173 -171	-99 137 180 -58 -175 175 -171	-99 139 180 -57 -175 175 -173
U_{rel}	0	0.2	0.7	1.9



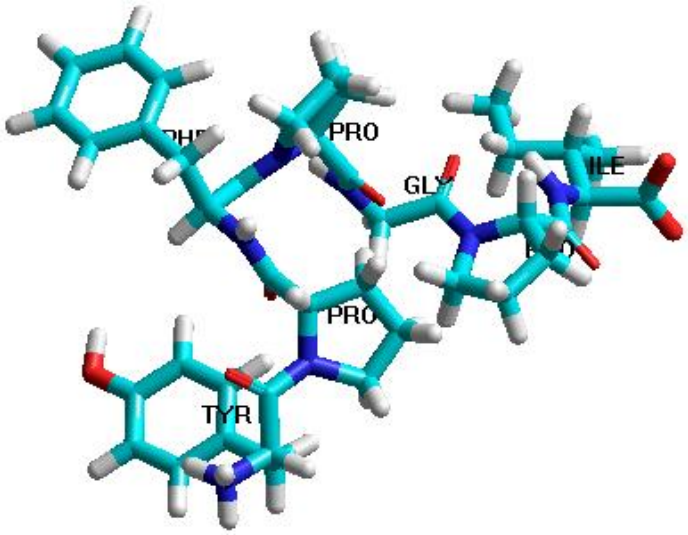
a)



b)



c)



d)

Figure 1. Atomic model of spatial structure of the casomorphine-7 molecule a), b), c) and d) corresponded to the structures with the relative energies 0 kcal/mol, 0.2 kcal/mol, 0.7 kcal/mol and 1.9 kcal/mol, respectively.

III. Conclusion

Thus, the spatial structure of the β-casomorphin-7 molecule can be represented by nine structural types. It can be suggested that the molecule performs its physiological functions in these structures. Based on these structures, synthetic analogs of the molecule can be proposed. The theoretical conformational analysis of the β-casomorphin-7 heptapeptide has led to such a structural organization of the molecule that does not exclude the implementation by the molecule of a number of functions that require strictly specific interactions with various receptors.

References

[1]. K.V.Shevchenko, S.Dulov, L.A.Andreeva, I.Yu.Nagaev, V.P.Shevchenko, A.S.Radilov, N.F.Myasoedov Stability of His-Phe-Arg-Trp-Pro-Gly-Pro to LeucineAminopeptidase, Carboxypeptidase Y, Nasal Slime, Blood and Plasma of Rats. *J. of Bioorg.Chem*,.**42**, №2, 171-181 (2016), https://doi.org/10.1134/S1068162016020126

[2]. H.Meisel, R.J.FitzGerald .Opioid peptides encryptrd in intact milk protein sequences.British J.Nutrition.**84**,S27-S31(2000).

[3]. E.Sienkiewicz-Szlapka, B.Jarmolowska, S.Krawczuk,E.Kostyra.Contents of agonistic and antagonistic opioid peptides in different cheese varieties.Int.Dairy J.**19,** №4, 258-263 (2009).

[4]. G.A. Akverdieva., N.M. Godjaev, S. Akyuz. A. Molecular Mechanics Conformational Study of Peptide T. *Journal of Molecular Structure*, **403**, 95-110 (1997), https://doi.org/10.1016/S0022-2860(96)09410-0

[5]. G.A. Akverdieva., N.M. Godjaev, S. Akyuz. Conformational dynamics of peptide T molecule, *Journal of Molecular Structure*, **609**,115-128 (2002),https://doi.org/10.1016/S0022-2860(01)00974-7

[6]. G.A. Akverdieva., N.M. Godjaev, S. Akyuz. Comparative conformational analysis of peptide T analogs.*Journal of Molecular Structure*, **917**, 21-26 (2009), https://doi.org/10.1016/j.molstruc.2008.06.028

[7]. W. Qin, X.Li, W.-W.Bian, X.-J.Fan, J.-Y.Qi.Density functional theory calculations and molecular dynamics simulations of the adsorption of biomolecules on graphene surfaces.*Biomaterials*,**31**, Issue 5, 1007-1016 (2010), https://doi.org/10.1016/j.biomaterials.2009.10.013

[8]. S.Kecel-Gunduz, B.Bicak, S.Celik, S.Akyuz, A.E.Ozel,Structural and spectroscopic investigation on antioxidant dipeptide, L-Methionyl-L-Serine: A combined experimental and DFT study. *Journal of Molecular Structure*, **1137,** 756-770 (2017),https://doi.org/10.1016/j.molstruc.2017.02.075

[9]. S. Celik, S. Kecel-Gunduz, S.Akyuz&A. E. Ozel.Structural analysis, spectroscopic characterization and molecular docking studies of the cyclic heptapeptide.*Journal of Biomolecular Structure and Dynamics*, **36**, Issue 9,2407-2423 (2018), https://doi.org/10.1080/07391102.2017.1356240

[10]. H.Geng,F.Chen, J. Ye, F.JiangApplications of Molecular Dynamics Simulation in Structure Prediction of Peptides and Proteins, *Computational and Structural Biotechnology Journal*, **17,** 1162-1170 (2019),https://doi.org/10.1016/j.csbj.2019.07.010

[11]. S. Celik, A.E. Ozel,V. Durak &S.Akyuz Vibrational spectroscopic characterization, quantum chemical and molecular docking studies of Valyl-Methionine dipeptide. *An International Journal for Rapid Communication*, **53**, Issue 9, 648-663 (2020), https://doi.org/10.1080/00387010.2020.1821062

[12]. S.V.Efimov, M.V.Dubinin, P.P.Kobchikova. Yu.O.Zgadzay, I.A.Khodov, K.N.Belosludtsev,V.V.Klochkov. Comparison of cyclosporin variants B–E based on their structural properties and activity in mitochondrial membranes, *Biochemical and Biophysical Research Communications*, **526**, Issue 4, 1054-1060 (2020), https://doi.org/10.1016/j.bbrc.2020.03.184

[13]. V.P.Barannikov, M.S.Kurbatova, D.L.Gurina, N. .Giricheva, Quantum chemical and molecular dynamics modeling of interaction of isomolecular dipeptides of α-L-alanyl-α-L-alanine and β-alanyl-β-alanine with sodium dodecyl sulfate micelles, *Computational and Theoretical Chemistry*, **1182**, 112844 (2020), https://doi.org/10.1016/j.comptc.2020.112844

[14]. A.V.Gurbanov., M. L. Kuznetsov, S. D. Demukhamedova, I. N. Alieva, N.M. Godjaev, F. I. Zubkov, K.T. Mahmudov and A. J. L. Pombeiro. Role of substituents on resonance assisted hydrogen bonding vs. intermolecular hydrogen bonding. *CrystEngComm* **22**, 628-633 (2020), https://doi.org/10.1039/c9ce01744e

[15]. IUPAC-IUB. Quantities, Units and Symbols in Physical Chemistry, *Blackwell Scientific, Oxford* (1993).

N.A.Akhmedov, et. al. "Spatial Structure Of The β-Casomorphin-7 Molecule." *IOSR Journal of Applied Physics (IOSR-JAP)*, 13(5), 2021, pp. 62-67.