INSIGHTS INTO BIOACTIVE CONFORMATION OF DERMORPHIN

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The conformational profiles of opioid peptide dermorphin and its active analog have been investigated within molecular mechanics framework and detailed by molecular dynamics simulations and quantum-chemical calculations. On the basis received results and data of SAR studies the bioactive conformation of dermorphin was assessed and the model of pharmacophore for the interaction of this molecule with the opioid receptors was proposed.

Keywords: dermorphin, conformational analysis, molecular dynamics, electronic structure, pharmacophore.
PACS: 36.20.Ey; 36.20.Fz; 36.20.Hb

1. INTRODUCTION

Dermorphin is linear peptide, isolated from a skin of the Southern-American frogs belonging to family Phyllomedusa, has high relationship and selectivity to μ-opioid binding places, but possess certain affinity to receptors δ- type [1-5]. Dermorphin shows strong analgetic influence as on central and peripheral nervous systems that is reflected in various physiological functions of an organism: cardiovascular, immune, anti-inflammatory, thermoregulatory and etc. This peptide is about 30-40 times more potent than morphine [6]. Dermorphin has been illegally used in horse racing as a performance-enhancing drug [7]. Many works are devoted to the structure-functional investigations of peptides of this family [8-12]. In this work using molecular modeling the comparative conformational analysis of dermorphin and its active tetrapeptide analog (1-4)dermorphin [11] was performed and the optimal conformations common to these molecules were found, on the basis received results and data of SAR studies the bioactive conformation of dermorphin was assessed and the model of pharmacophore for its interaction with the opioid receptors was proposed.

2. METHODS

Conformational energy calculations were made with an IBM computer using version of ECEPP (Empirical Conformational Energy Program for Peptides) written in FOTRAN [13,14]. The program was developed from the matrix method principle of Hermans and Ferro [15]. The investigations were carried out within molecular mechanics framework as described in [16,17]. The backbone was described by the "shape" symbols *e* and *f* corresponding to extended and folded configuration of virtual bonds C^{α}_{i}- C^{α}_{i+1}- C^{α}_{i+2}- C^{α}_{i+3}, respectively. The nomenclature and conventions adopted are those recommended by IUPAC-IUB [18].

The molecular dynamics of dermorphin was spent with use of force field AMBER in the temperature interval 293-313K with step 5K during 10 nanoseconds by means [19]. Procedure solvation with application of model of water in the set spherical volume TIP4P [20] has been used. The quantum- chemical calculations of these molecule were conducted by method CNDO [21], used the demonstration version of software package HyperChem (http:// www.hyper.com).

3. RESULTS AND DISCUSSION

3.1. Conformational profiles of dermorphin and (1-4)dermorphin

In first step the conformational possibilities of (1-4) dermorphin have been investigated on the basis of low-energy states of monopeptides. It is establishment, that the conformations of 8 shapes of backbone fall within the 0 - 5 kcal/mol interval. The energy parameters of these conformations are listed in Table1.

As may be inferred from these data, the structures of tetrapeptide with the folded backbone shapes of the N-terminal dipeptide part are the most preferred ones and correspond to stabile states of this sequence. They are representatives of *fee, ffe, fff, fef*-shapes with relative energies in within 0-2 kcal/mol. The mono- and dipeptide interactions are more effective in these structures. The nonvalent interactions of the side chains of Tyr and Phe residues are the major contribution to the conformational energy of this peptide. These interactions are most fully realized in the conformations of mentioned four shapes. Global conformation $L_{21}LB_{11}R$ of this sequense belongs to *fee* shape. This conformation is more favorable among preferred structures on nonvalent interactions. In addition, in this conformation the most successful compromise between the electrostatic and torsion interactions is achieved. Second structure $L_{21}PB_{11}R$ has *ffe* shape of backbone. There is a strong tripeptide interaction between the amino acid residues Tyr1 and Phe3 (approximately -5.7 kcal/mol) is realised in this structure. The effective tetrapeptide contributions (-4.7 kcal/mol) are characteristic for $B_{21}PB_{31}L$ conformation with spiral structure of backbone. The electrostatic interactions play an important role in its stabilization. The distance between the ends of the molecule is equal to 3.9 Å in this structure. Note that all structures of *fff* -shape are compact due folded forms of the main chain of residues. The relative energy of the other four shapes, namely, *eff, efe, eee, eff* changes in the interval 2-5 kcal/mol. The conformations of these shapes are losing in energy as the non-bonded and dispersion interactions, they are favorable only by torsion contributions. Although the form of the main chain of shapes *eee, eef* promotes the spatial separations of the of ends of this fragment (~ 12.5 Å), the rings of the residues Tyr and Phe are close together in space (3,4 Å) there. Table 2 shows the geometrical parameters of the preferred conformations of all shapes of peptide backbone of (1-4) dermorphin.

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Table 1. Energies of the favorable conformations of (1-4) dermorphin

Number	Shape	Backbone form	Energies of interactions										
			$\sum E_{inter.}$	Tyr1 DAla2	DAla2 Phe3	Phe3 Gly4	Tyr1 Phe3	DAla2 Gly4	Tyr1 Gly4	$E_{tot.}$	$E_{rel.}$	$E_{conf.}$	$E_{corr.}$
1	*fee*	LLBR	2.6	-2.9	-1.9	-2.6	-3.1	-0.2	-0.5	-12.5	3.9	1.8	0
2	*ffe*	LPBR	4.1	-2.0	-1.0	-2.7	-5.7	-0.2	-1.6	-11.4	2.4	3.0	0.7
3	*fff*	BPBL	3.4	-3.2	-1.9	-0.6	-2.6	-0.5	-4.7	-12.2	2.3	3.9	0.7
4	*fef*	LLBL	2.5	-3.0	-1.9	-1.2	-3.2	-0.3	-0.4	-7.2	4.1	2.4	1.0
5	*eff*	RPRR	6.2	-2.6	-2.2	0.0	-3.5	-0.4	-3.2	-10.1	4.4	1.3	2.3
6	*efe*	RPBR	6.3	-2.3	-0.9	-1.8	-3.5	-0.2	-2.6	-9.4	4.3	1.8	3.4
7	*eee*	RLBR	5.8	-2.1	-1.3	-2.0	-3.4	-0.3	-0.5	-8.4	4.7	0.8	3.8
8	*eef*	RLBL	5.8	-2.1	-1.3	-1.1	-3.4	-0.4	-0.5	-7.6	4.7	0.8	4.7

Table 2. Geometry parameters (in degrees) of the favorable conformations of (1-4) dermorphin

Residue	Dihedral angles	Conformation							
		$E_{rel.}$=0.0 xcal/mol	$E_{rel.}$=0.7 xcal/mol	$E_{rel.}$=0.7 xcal/mol	$E_{rel.}$=1.0 xcal/mol	$E_{rel.}$=2.3 xcal/mol	$E_{rel.}$=3.4 xcal/mol	$E_{rel.}$=3.8 xcal/mol	$E_{rel.}$=4.7 xcal/mol
Tyr	φ	58	63	-72	58	-110	-110	-114	-113
	χ_1	177	179	177	178	179	176	181	-179
	χ_2	78	78	83	75	87	86	91	91
	χ_3	180	180	180	180	180	179	180	180
	Ψ	140	124	149	143	-64	-69	-59	-59
	ω	178	-172	-175	180	175	171	178	178
D-Ala	φ	95	78	77	94	80	73	101	101
	χ_1	180	178	179	-179	180	180	-179	-179
	Ψ	54	-60	-66	52	-80	-80	65	66
	ω	-179	170	166	180	-179	175	175	175
Phe	φ	-145	-107	-129	-144	-143	-119	-111	-111
	χ_1	60	66	-59	55	-60	60	63	62
	χ_2	94	89	88	90	90	90	88	88
	Ψ	168	170	130	161	-61	160	162	160
	ω	177	180	190	179	180	179	179	179
Gly	φ	-78	-77	71	83	-91	-91	-89	89
	Ψ	-72	-79	76	72	-90	-90	88	88
	ω	180	180	180	180	180	180	180	180

Table 3. Low-energy conformations of dermorphin

Number	Shape	Backbone form	$E_{tot.}$	$E_{rel.}$	$E_{conf.}$	$E_{corr.}$
1	*fffffef*	LPBLLRR	-24.6	1.3	4.2	0
2	*feefee*	LLBRBBR	-24.5	3.1	2.7	0.4
3	*feefef*	LLBRBRR	-24.2	3.2	2.4	0.5
4	*fffffee*	LPRRBBR	-23.8	2.4	3.0	0.7
5	*feeeef*	BLBRBRR	-23.7	3.2	2.3	0.9
6	*effffef*	RPRRBRR	-24.0	3.8	2.2	1.0
7	*fefeee*	LLBLBBR	-24.1	3.0	3.1	1.1
8	*effffee*	RPRRBBR	-24.3	3.9	2.6	1.2
9	*ffefef*	LPBRBRR	-23.1	1.8	3.4	1.3
10	*fffeee*	BPBLBBR	-23.7	1.5	4.5	1.4
11	*ffefee*	LPBRBBR	-22.8	1.5	4.0	1.7
12	*feffef*	LLRRBRR	-23.8	3.6	2.8	1.7

*The energy parameters are given in kcal/mol

Table 4. Geometry parameters (in degrees) of the optimal conformations of dermorphin*

Residue	Conformation				
	$E_{rel.}$=0.0 xcal/mol	$E_{rel.}$=0.4 xcal/mol	$E_{rel.}$=0.5 xcal/mol	$E_{rel.}$=0.7 xcal/mol	$E_{rel.}$=0.9 xcal/mol
Tyr1	66, 179, 80, 180, 128,-173	58, 177, 78, 180, 141, 179,	58, 177, 78, 180, 141,179,	58, 180, 75, 180, 112, 180	-73, 63, 87, 180, 164, 178
DAla2	76, 178, -64, 167	95, 179, 54, -179	95, 179, 54, -179	80, 179, -70, 178	82, 179, 48, 179
Phe3	-114, 63, 86, 162, 175	-146, 59, 94, 169, 179	-146, 59, 94, 167, 177	-138, -61, 90, -63, -178	-142, 61, 87, 168, 179
Gly4	73, 76, 178	-77, -71, -179	-79, -72, 180	-96, -96, -178	-80, -76, 180
Tyr5	51, -172, 71, 180, 71, -178	-132, 65, 91, 180, 151, 173	-117, 55, 83, 180, 142, 174	-134, 64, 91, 180, 155, 175	-115, 54, 83, 180, 142, 175
Pro6	-62, -178	99, -177	-66, -175	100, -177	-67, -175
Ser7	-107, -60, 180, -61, -179	-97, 54, 180, -53, 180	-115, -60, 180, -60, 180	-97, 54, 180, -53, 180	-115, -60, 180, -59,180

*The values of dihedral angles are in the following order: φ, χ_1, χ_2, χ_3, Ψ, ω

In a further step the conformational analysis of dermorphin was carried out through a fragmental calculation on the basis of stable states of monopeptides. The starting structural variants for dermorphin yielded more than 400 conformations belonging to 32 shapes of peptide skeleton, 31 dihedral angles were exposed to

rotation. Despite rather limited conformational possibilities of the N-terminal part of this molecule, thanks to presence residue Gly in its sequence the effective interactions are realized between the terminal segments of molecule in the low-energy conformational states divided by a energy barrier, not exceeding 2 kcal/mol (See Table 3). The presence of proline residue in peptide sequence causes of the bend of peptide chain on the C-terminal tripeptide fragment and reduces the number of sterically allowed structural types for this part of molecule: the conformations only *ee* and *ef* shapes are possible for it.

The optimal conformations of dermorphin were assessed by pairwise cross comparisons of the low energy conformations found for dermorphin and (1-4) dermorphin. These results were compared also with data of two other tetrapeptide analogs of dermorphin - ARPG (H-Tyr-D-Arg-Phe-Gly-OH) and TDAPA (H-Tyr-D-Arg-Phe-beta-Ala-OH), which were studied previously [22,23]. It was found the optimal structures of dermorphin ($E_{rel.}$= 0.0 kcal/mol, 0.4 kcal/mol, 0.5 kcal/mol, 0.7 kcal/mol and 0.9 kcal/mol) are the folded or semifolded conformations stabilized by hydrogen bonds between the atoms of peptide skeleton. These results well coordinate to the data of spectroscopic works and theoretical researches [24-31], that offer as the preferable the semifolded or the folded forms of peptide skeleton of dermorphin molecule. The geometry parameters, the matrix of energy interactions and parameters of hydrogen bonds in the optimal structures of dermorphin molecule are listed in Tables 4, 5 and 6, respectively. These structures are shown in fig.1.

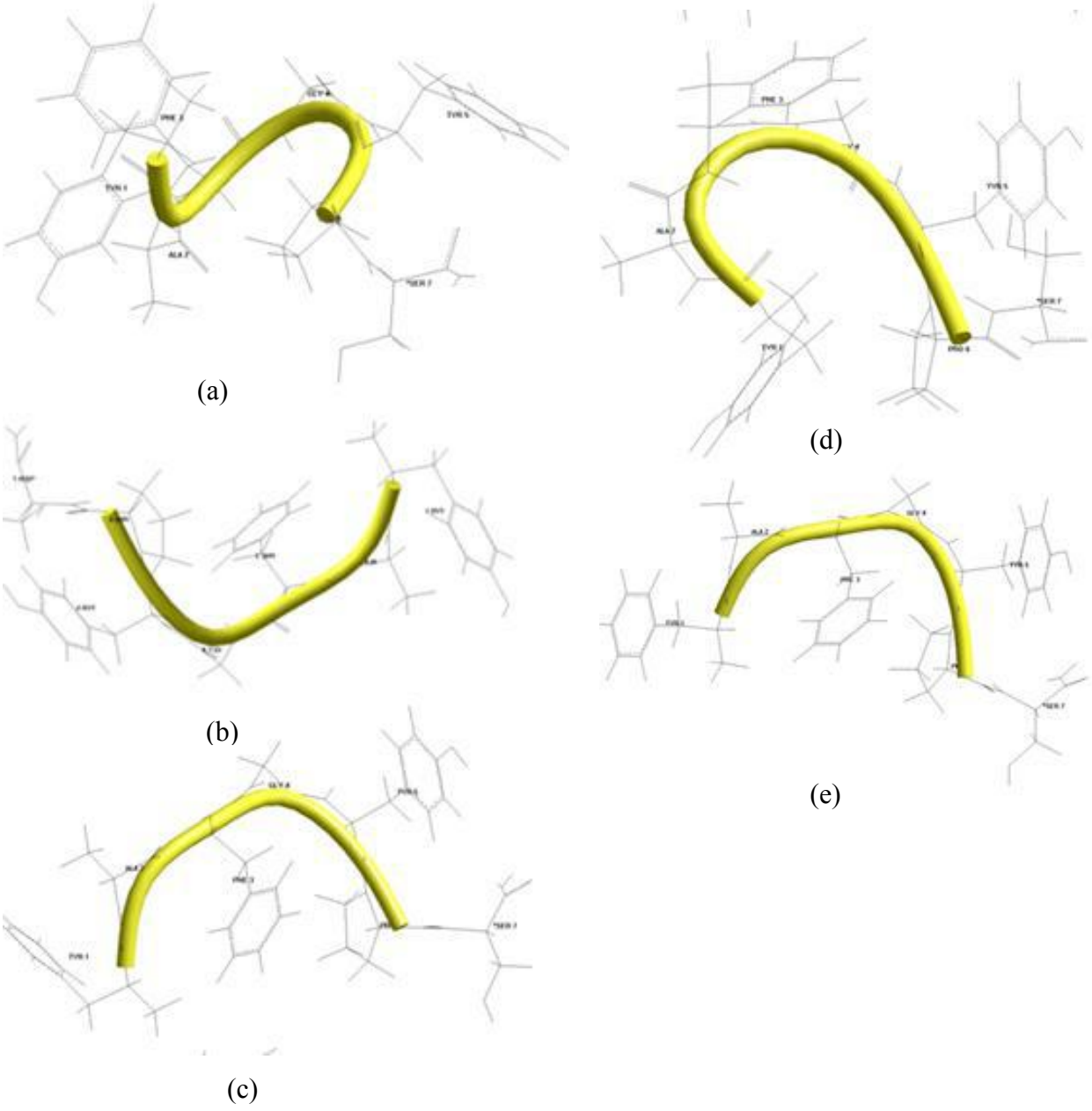

Fig.1. Optimal conformations of dermorphin with $E_{rel.}$= 0.0 kcal/mol (a), $E_{rel.}$= 0.4 kcal/mol (b), $E_{rel.}$= 0.5 kcal/mol (c), $E_{rel.}$= 0.7 kcal/mol (d), $E_{rel.}$= 0.9 kcal/mol (e)

Table 6. Inter- and intra-residue hydrogen bond lengths (in Å) and energies in brackets (in kcal/mol) in the optimal conformations of dermorphin[a]

H-bond	Conformation				
	$E_{rel.}$=0.0 kcal/mol	$E_{rel.}$=0.4 kcal/mol	$E_{rel.}$=0.5 kcal/mol	$E_{rel.}$=0.7 kcal/mol	$E_{rel.}$=0.9 kcal/mol
(Tyr1)NH...OC(Tyr1)	2.69 (-0.20)	2.54 (-0.30)	2.54 (-0.30)	2.74 (-0.17)	2.89 (-0.11)
(Tyr1)NH...OC(Tyr1)					2.40 (-0.46)
(Phe3)NH...OC(Phe3)	2.65 (-0.23)	2.33 (-0.56)	2.33 (-0.56)		2.36 (-0.51)
(Tyr5)NH...OC(Tyr5)		2.49 (-0.35)	2.67 (-0.21)	2.47 (-0.38)	2.69 (-0.20)
(Ser7)NH...O (Ser7)		2.46(-0.38)		2.46 (-0.38)	
(Tyr1)NH...OC(Phe3)	2.10 (-0.98)				
(Tyr1)CO...HN(Phe3)	2.00 (-1.20)			2.01 (-1.18)	
(Phe3)CO...NH (Tyr5)		2.51(-0.23)	2.55(-0.34)		2.52(-0.35)
(Tyr1)NH...OC(Gly4)				2.50 (-0.34)	
(Tyr5)CO...HN(Ser7)		2.49 (-0.35)		2.68 (-0.21)	
(Ser7)CO...H2N	2.52 (-0.33)	2.52 (-0.33)	2.52 (-0.33)	2.52 (-0.33)	2.52 (-0.33)
(Ser7)CO...H2N		2.52 (-0.33)	2.52 (-0.33)	2.52 (-0.33)	

[a] H-bond lengths are given without parentheses and corresponding H-bond energies are given in parentheses

Table 7. Characteristics of electronic structure of the optimal conformations of dermorphin

Conformation	$E_{rel.}$=0.0 kcal/mol	$E_{rel.}$=0.4 kcal/mol	$E_{rel.}$=0.5 kcal/mol	$E_{rel.}$=0.7 kcal/mol	$E_{rel.}$=0.9 kcal/mol
Dipol moment (D)	17.895	5.971	16.160	4.826	17.184
Total energy (kcal/mol)	-373340.976	-375078.471	-374842.040	-368144.928	-376271.080
Binding energy (kcal/mol)	-29080.191	-30817.686	-30581.254	-23884.143	-32010.295
Izolation atomic energy (kcal/mol)	-344260.786	-344260.785	-344260.785	-344260.785	-344260.785
Electron energy (kcal/mol)	-3349486.325	-3230127.022	-3210814.623	-3388005.513	-3179314.148
Energy of nuclear interaction(kcal/mol)	2976145.349	2855048.550	2835972.583	3019860.584	2803043.068
Energy of formation (kcal/mol)	-18087.799	-19825.294	-19588.862	-12891.751	-21017.903
E_{HOMO}	-9.252524	-8.109617	-10.558869	-9.044190	-10.916412
E_{LUMO}	0.991924	0.735477	0.732555	0.297698	1.948923
$\Delta E = E_{HOMO} - E_{LUMO}$	10.24	8.85	11.29	9.34	12.87

Table 8. The respective geometrical arrangement of pharmacophore elements of dermorphin

Distances	In Å	Angles	In degrees	Dihedral angles	In degrees
R_{12}	7.8	α_{121}	6.1	β_{2211}	-101.3
R_{13}	5.9	α_{131}	7.5	β_{3211}	50.4
R_{16}	6.2	α_{161}	9.3	β_{6611}	-140.3
R_{15}	8.4	α_{151}	4.9	β_{5511}	-136.3
R_{23}	2.4	α_{232}	10.2	β_{3322}	180.0
R_{26}	10.4	α_{262}	2.9	β_{6622}	-134.8
R_{25}	13.6	α_{252}	4.0	β_{5522}	-7.5
R_{36}	8.2	α_{363}	7.6	β_{6633}	64.0
R_{35}	12.9	α_{353}	5.0	β_{5533}	143.5
R_{45}	13.7	α_{454}	5.4	B_{5544}	-79.4

The physiologically active N-terminal tetrapeptid of dermorphin which defines the specificity of its interactions with opiate receptor has regular structure - α-spiral in two optimal conformations and semifolded structure in others optimal structures of dermorphin. As in this segment of the molecule the majority of the residues have folded backbone structure, the interactions of Tyr1 residue with the subsequent residues Ala2 and Phe3 by the contributions from -1.9 to -2.9 kcal/mol and from -2.2 to -5.2 kcal/mol, respectively and also the interactions of Tyr5 residue with the subsequent residues Pro6 and Ser7 by the contributions from -3.7 to -4.7 kcal/mol and from -2.9 to -3.8 kcal/mol, respectively, are effective. In the global conformation, belonging to *ffffef* shape of peptide skeleton, the residues Tyr1 and Phe3 with the aromatic side chains approach in space and form quasicyclic

structure with formation of two hydrogen bonds between amino and carboxyl groups of these residues. Therefore in this conformation the interactions of mentioned residues are stronger, than in other structures of molecule. This structure is characterized also by more effective contribution of interactions of residues D-Ala2 and Pro6 (-2.1 kcal/mol). In the conformations 2, 3 and 5 the dispersion contacts of residue Phe3 both with Tyr5 and with Pro6 are effective owing to their spatial approach and the interactions between atoms of aromatic side chains. These structures are characterized by existence of hydrogen bond (Phe3)CO…HN(Tyr5). For conformation 2 the hydrogen bond between the atoms of the main chain of the residues in fifth and seventh positions, namely (Tyr5)CO... HN(Ser7) is characteristic too, but thus HN group of Ser7 participates also in formation of hydrogen bond with O atom of own side chain. Conformation 4 (*fffffee*-shape) represents the particular interest. In this conformation the form of peptide chain of the N-terminal tetrapeptide part is similar to global conformations and the form of peptide chain of C -terminal tripeptide part is similar to conformation 2. Therefore the hydrogen bonds, characteristic for both specified conformations are realized in this structure. But distinctive features of conformation 4 are effective tetrapeptide interaction and formation of hydrogen bond (Tyr1)NH...OC(Gly4). As seen from the results, this conformation is distinguished from others by saturation of hydrogen bonds. In this structure the phenolic ring of the residue Tyr1 hangs over a conformationally rigid ring of the proline residue in the parallel position to it, and as result the interaction between these residues is found to be -4.1 kcal/mol. Here, α-amino group and the carbonyl residue Tyr5 are spatially close together, forming the salt bridge. The interactions of residue Tyr1 with Gly4 (-2.3 kcal/mol) and also with Tyr5 (-2.8 kcal/mol) bring the appreciable contributions to stabilization of this structure. Conformation 5 (*feeeef*-shape) is characterized by folded form of the N- and C-terminal dipeptide parts and by completely extended form of central part of the molecule. For this structure the intraresidual hydrogen bonds for residues Tyr1, Phe3, Tyr5 and also hydrogen bond between carboxyl group of main chain of Ser7 and H-atoms of the terminal amid group NH_2 are characteristic. All optimal structures of dermorphin are characterized by folded of conformation of the dipeptide segment Tyr1-DAla2 that is reason of strong dipeptide interaction. Apparently, the specified minimum structural requirement is important for physiological activity of dermorphin molecule, as it is necessary for protection of this peptide bond from splitting action by enzymes in the process of metabolism of peptide. Though, the specified spatial structures of this molecule are favourable as by energy and by entropic factors, the conformational changes with overcoming of a small energy barrier leading to realization of structures with the extended form of the N-terminal dipeptide segment are possible. Let's notice that structures 1, 9, 11 with L form of the main chain of Tyr1 can be realized also for its B form, slightly conceding in energy, accordingly, on 1.0, 0.7, 0.3 kcal/mol, as these forms of residue in first position differ in angle φ that determines the spatial arrangement of three identical hydrogen atoms with respect to the rest part of molecule. Apparently, from the Table 4, thanks to presence DAla and Pro in peptide chain a number of preferable structures have turns on the terminal parts of molecule. The particular interest is represented by the structures belonging *feefee* and *feefef* shapes of peptide skeleton with the relative energy, equal 0.4 and 0.5 kcal/mol. In these conformations β - turn is formed on the Gly4-Pro5 segment, therefore in them the aromatic side chain of residue Phe effectively interacts with atoms of the subsequent residues and the residue is shaden from solvent. amid proton of Gly4.

It was found that despite the conformational features of investigated optimal structures of dermorphin an α - amino group and the side chains of the tyrosine and phenylalanine residues are in the identical positions in space from each other in all of them. Dermorphin molecule forms compact structures, in which the residues Tyr1 and Ser7 point away from the core surface of molecule, that explains abilities of its OH-groups to interact with the environment. As these residues are capable to participate also in effective interresidual interactions, it is possible to assume that they should possess defined conformational dynamics.

3.2. Dynamic properties of dermorphin

Within the framework of mechanical model, a conformational dynamics of the side chains of the amino acid residues of dermorphin peptide was investigated. For this purpose, a series of conformational maps, or sections of the potential surfaces were constructed over the $\varphi - \chi_1$, $\chi_1 - \chi_2$, $\chi_2 - \chi_3$ or $\chi_1 - \chi_3$ angles for amino acid residues of the molecule [32]. Thus, the dihedral angles around the $N\text{-}C^{\alpha}$, $C^{\alpha}\text{-}C^{\beta}$, $C^{\beta}\text{-}C^{\gamma}$, $C^{\gamma}\text{-}C^{\delta}$ bonds of the side chains were varied, and the backbone of the peptide residues was fixed in accordance with the coordinates of the atoms of optimal structures of the peptide molecule. It has been found that Tyr1 has a considerable conformational mobility. The optimal positions of the Tyr1 side chain are close to the minima of their torsional potential : $\chi_1 = 60^0$, 180^0 , -60^0 and the deviations by $\pm 30^0$ from this values are possible. For χ_2 of the Tyr1 the changes by 30^0 from the values of the torsional minima $\pm 90^0$ are permissible. The angle χ_3 of Tyr1 can take values 0 and 180^0. This fact allows to conclude that such mobiliity of the tyrozin imidozol ring is probably necessary for the complementary binding with specific receptors. This result is very interesting because it correlates with the data from [33]. The investigation of φ-χ_1 conformational map of D-Ala2 revealed the permissible deviation by $+30^0$ from the optimal values of the φ angle. The optimal positions of the Ala1 side chain are close to the minima of their torsional potential: $\chi_1 = 60^0$, 180^0, -60^0 and the deviations by $\pm 30^0$ from these values are possible. The analysis of φ-χ_1 conformational map of Phe3 shows that the mobility of the φ angle is restricted. This angle can take a very fixed position and its variation leads to increase of energy in the whole molecule. Apparently, this fact can be explained due to the important role of this residue in formation of regular structure and of active form of molecule. Despite this, the angle χ_1 which orients the phenolic ring of this residue is mobile. The possible deviations of χ_1 for Phe3 from its torsion minima values 180 and -60^0 by $\pm 30^0$ and from value 60^0 by 30^0 are

allowed. The changes 15^0 are permissible for φ angle of Gly. The side chain of residue Gly4 consists of a single atom, so we can say that it is absent. For the angle φ of these residue the low-energy changes by 15^0 are admissible. Because of the amino acid residue Tyr5 precedes prolin and is located on a bend of the peptide chain conformational mobility of its φ angle is limited. Only minor changes, by 5^0 from optimal value of this angle are possible. The implementation of the positions of the side chain corresponding to the values 180 and -60^0 of χ_1 angle for this residue is possible. The deviations of χ_2 for Tyr5 by $+30^0$ from its optimal values $\pm 90^0$ are allowed. Calculated results indicate that χ_3 for Tyr5 can take two values, 0 and 180^0, corresponding to its stabile states. The deviations of χ_3 angle from these values lead to increase of energy in the whole molecule. Proline residue in the sixth position of the amino acid sequence is a specific residue. Its side chain is rigidly connected with the main chain and forms a cyclic structure. For this reason, the conformational mobility of this residue is impossible. The C-terminal residue Ser7 has large conformational dynamics. This residue is very mobile not only by side chain angles, but also by the φ angle of peptide chain. The changes by 50^0 are permissible for φ angle. The optimal positions of the Ser7 side chain are close to the minima of their torsional potential: $\chi_1 = 60^0$, 180^0, -60^0 and the low-energy barrier corresponding to the conformational transits between them equal 1.9 kcal/mol. The deviations of χ_2 of Ser7 by $+60^0$ from its optimal value are allowed.

Thus, the analysis of the conformational maps has revealed the degree of mobility of the functional residues. It is established that the dynamics of Phe3 and Tyr5 is limited owing to realization of effective stabilizing interactions of its aromatic side chains atoms with atoms of other residues of the molecule. So, the conformational freedom of Phe3 residue is limited by effective interactions with Tyr1, and conformational freedom of Tyr5 residue is limited by effective interaction of its aromatic ring with the atoms of the rigidly fixed prolin residue in sixth position. For this reason the conformational energy is rather sensitive to orientation of the side chains of specified amino acid residues. The side chains of Tyr1 and Ser7 are characterized by the large conformational dynamics. Evidently, such mobility of the terminal residues is necessary for realization of interactions with atoms of environment or receptor. As seen from the presented data, the conformational dynamics of any segment of a molecule is defined by efficiency of its interaction with other segments of molecule. The rotary possibilities of side chains of the residues are various among the considered optimal structures dermorphin, but because of presence of the hydrogen bonds the conformational balance is between them.

At the subsequent investigation step molecular dynamics of dermorphin has been studied and the most probable conformational states of this molecule are defined in vicinities of local minima at physiological temperature. As a result of simulation process, the ranges of change of dihedral angles and distances between atoms of amino acid residues are defined. The quantitative estimation of distances between atoms shows that at modeling the dispersion contacts between the amino acid residues in the first and fourth positions of peptide chain are invariable. The N-terminal tetrapeptide fragment of the molecule preserves the folded structure throughout molecular dynamics simulation, the distance between C^{α}- atoms of the specified residues does not exceed 7Å during simulation. The structure of this part of molecule upon termination of modeling process practically does not differ from the initial stage. It is possible to assume the conformational stability of specified fragment plays important role in functional activity of dermorphin molecule and defines the specificity of its interaction with the receptor. Because of mobility of the C-terminal tripeptide fragment of investigated molecule the distance between the atoms of the side chains of the residues Phe3 and Tyr5 is approximated to 2.8 Å during simulation. As result, the hydrogen bond between atoms of hydroxyl group of residue Tyr5 and the main chain of residue Phe3 is formed. Such conformational behavior may be due presence glycine in fourth position of peptide chain that is characterized by conformational freedom of rotation around C^{α}-$C^{/}$ and $C^{/}$-N bonds, giving certain flexibility to peptide chain. One can conclude, as the system is in an equilibrium state, the possible conformational changes are reversible. The cited data though testifies about stability of optimal conformations of dermorphin, but assumes also the probability of realizations of each of them depending on polarity of environment.

3.3. Electronic structure of dermorphin

As research of the electronic-conformational properties of biologically active molecules can be a basis for additional correlation of the spatial structure and structure-functional relationships, it is interesting to investigate the electronic structure of optimal structures of dermorphin. By optimization of electronic energy as zero states the equilibrium configurations of nuclease corresponding to geometry of the optimal structures of dermorphin have been considered. The half-electron approximation was used. The important parameters characterizing the electronic structure of the molecule have been calculated: the distribution of electronic density, the partial charges on the atoms, molecular orbitales, electric dipole moment, and also a number of energy parameters, such as the total energy, energy of the bind, isolated atomic energy, electronic energy, energy of nuclear interactions, heat of formation, highest occupied and lowest unoccupied molecular orbital energies that provide the information on stability of a molecule (see Table 7). It was established, that the optimal conformations 1-5 of dermorphin has low values of dipole moment and of energy $E_{HOMO,}$ characterizing its electro-donor properties and the energy of activation. It is possible to draw a conclusion that the low-energy structures of dermorphin have the insignificant chemical reactionary ability and the weak electrodonor properties. Apparently from the Table 7, the conformational distinction is reflected first of all in their electronic energy and energy of nuclear interactions. The structure 5 is characterized by folded forms of N- and C- terminal dipeptide sequences, it has lower values of bind energy, core-core interactions, heat of formation that is reflected in value of a total energy, but on value of dipole moment

it yields to structures 2-4. The structure 4, on the contrary, answers the low value of dipole moment (4.826 D) that is connected with its conformational features: the maintenance of the α - spiral on the N-terminal pentapeptide part of the molecule leads to rapprochement of polar groups of atoms of a molecule, but the destabilizing effect of interaction of cores is stronger here, and as a result it considerably concedes to other structures on value of a total energy. The structure 2 is characterized by two β - turns peptide chain answers to the steadiest electronic state of dermorphin. This structure is characterized by small value of dipole moment (5.971D), as well as structure 4, only slightly conceding to it, on value of a total energy it is the following stable structure after structure 5. As we see, the structure 2 is the most compromise for the basic parameters of electronic structure. It was found the conformational transitions between optimal structures of dermorphin are resulted by fluctuations of density of a charge on certain groups of the atoms, not exceeding 18%. The distinctions of charges on the atoms C-terminal dipeptide residues and amid group are revealed.

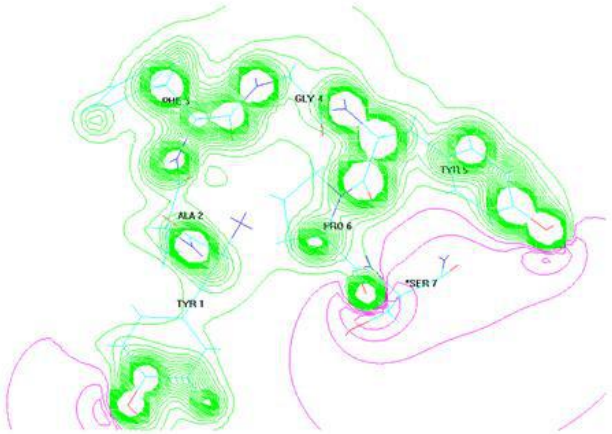

Fig.2. Two-dimensional contours of electrostatic potential in dermorphin molecule.

The distribution of charges on the atoms of the α - amino group and atoms of the side chains of tyrosine and phenylalanine residues are similar for optimal structures, that may be correlate with their biological activity - ability to participate in ligand-receptor interactions. Two-dimensional contour of electrostatic potential for this molecule is shown in fig.2. Green and violet colors represent, accordingly, positive and negative charged segments of the molecule. Apparently from the presented figure, there is the zone of high electronic density subjected to attack of the electrophil reagent.

3.4. Model of pharmacophore for the interaction of dermorphin with the opioid receptors

The review of the works [10-12, 26-27, 33-36] shows that the basic elements defining a way of interaction of opiate peptides with receptors are α - an amino group, the aromatic side chains of residues Tyr1, Phe3 and Tyr5. The spatial arrangement of the structural elements occupying the pharmacophore area and characterizing by specific electronic features may be the important criteria of the biologically active conformation of dermorphin. On the basis of the received results and data of SAR studies the bioactive conformation of dermorphin was assessed and the model of opioid pharmacophore for its interaction with opioid receptors was proposed (see fig.3).

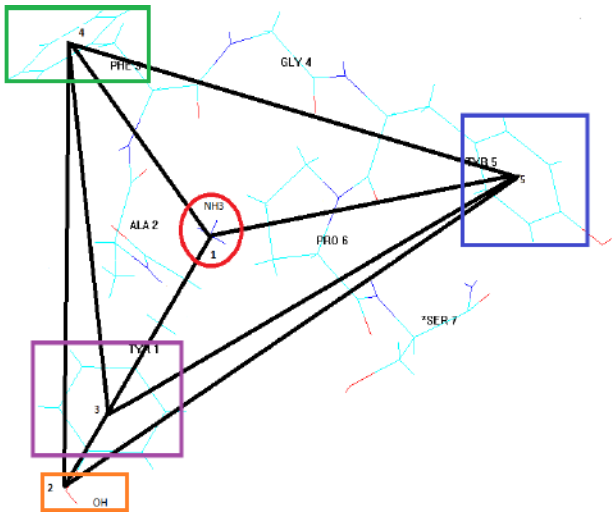

Fig.3. The model of proposed pharmacophore for the recognition of dermorphin to the opioid receptors.

The proposed model contains five areas which are occupied by pharmacophore elements marked by numbers 1-5. The pharmacophore element 1 represents the α - an amino group, namely, the protonated nitrogen atom participating in electrostatic interaction with negatively charged residue of opioid receptor. Thus there is also the formation of hydrogen bond with participation of protonated nitrogen atom. Such interaction in turn can lead to rupture of hydrogen bond between the transmembran spirals of opioid receptor AspIII:7... TyrVII:11 that leads to its activation [36]. The pharmacophore element 2 is presented by hydroxyl group of the side chain of residue Tyr1, it participates in the transit of a charge as the donor of electronic density and in the formation of the hydrogen bond. The pharmacophore elements 3 and 4 represent, accordingly, a phenolic ring of residue Tyr1 and an aromatic ring of residue Phe3, they participate in hydrophob interactions. The pharmacophore element 5 - a phenolic ring of the residue Tyr5 can participate in hydrophob interactions or in the formation of hydrogen bond, that defines the selectivity of this ligand. It should be noted that the pharmacophore elements 1 and 2 represent the affine areas, the pharmacophore elements 3 and 4 – the agonistic areas, the pharmacophore element 5 - antagonistic area of this pharmacophore. In represented model the centres of pharmacophore areas 1 and 2 were taken as protonated nitrogen atom and oxygen atom of hydroxyl group of the side chain of residue Tyr1, respectively; the centres of pharmacophor areas 3-5 were taken as the centres of weights of the structural fragments occupying the corresponding pharmacophore areas. The relative positions of pharmacophore areas of dermorphin molecule

are characterized by a set of distances, angles and dihedral angles (see Table 8):

1) the distances (R_{12}, R_{13}, R_{14}, R_{15}, R_{23}, R_{24}, R_{25}, R_{34}, R_{35}, R_{45}) between the centres of the specified pharmacophore areas;
2) the angles (α_{121}, α_{131}, α_{141}, α_{151}) " centre of pharmacophore area 1- centres of pharmacophore areas 2-5 – hydrogen atom in pharmacophore area 1";
3) the angles (α_{232}, α_{242}, α_{252}) "centre of pharmacophore area 2- centres of pharmacophore areas 3-5 – hydrogen atom in pharmacophore area 2" ;
4) the angles (α_{343}, α_{353}) "centre of pharmacophore area 3- centres of pharmacophore areas 4-5 – nearest atom to the centre of pharmacophore area 3";
5) the angle (α_{454}) "centre of pharmacophore area 4- centre of pharmacophore area 5 – nearest atom to the centre of pharmacophore area 4" ;
6) the dihedral angles (β_{2211}, β_{3311}, β_{4411}, β_{5511}) "centres of pharmacophore areas 2-5 –nearest atom to the centres of pharmacophore areas 2-5 - centre of pharmacophore area 1- hydrogen atom in pharmacophore area 1";
7) the dihedral angles (β_{3322}, β_{4422}, β_{5522}) "centres of pharmacophore areas 3-5 –nearest atom to the centres of pharmacophore areas 3-5 - centre of pharmacophore area 2- hydrogen atom in pharmacophore area 2";
8) the dihedral angles (β_{4433}, β_{5533}) "centres of pharmacophore areas 4-5 – nearest atom to the centres of pharmacophore areas 4-5 - centre of pharmacophore area 3 - nearest atom to the centre of pharmacophore area 3";
9) the dihedral angle β_{5544} "centre of pharmacophore area 5– nearest atom to the centre of pharmacophore area 5 - centre of pharmacophore area 4 - nearest atom to the pharmacophore area 4".

The proposed model defines the presence of the similar structural elements of the bioactive molecules participating in the interaction with opioid receptors. It is expected that received results can be used for search of the ligands of opioid receptors as pharmacological preparations with effective action.

4. CONCLUSION

Generalising results of research of the structure-functional relationship of dermorphin are next:

1. Optimal structures of a molecule dermorphin are characterized by folded conformation of the dipeptide segment Tyr1-DAla2. Apparently, the specified minimum structural requirement is necessary for protection of this peptide bond from splitting action by enzymes in the process of metabolism of peptide;
2. The physiologically active N-terminal tetrapeptide fragment of dermorphin has regular structure - α-spiral in two optimal conformations and semifolded structure in others. Conformational stability of this fragment, apparently, plays important role in functional activity of dermorphin molecule and defines the specificity of its interaction with the opiate receptors;
3. In two optimal structures of dermorphin β-turn is formed on the Gly4-Pro5 segment and the amid proton of Gly4 residue is shaden from solvent;
4. The structure characterizing by folded forms as of the N –terminal and the C-terminal dipeptide segments and by completely extended form of central part of molecule sequence is also answered to equilibrium state of dermorphin;
5. The conformational dynamics of Phe3 and Tyr5 is limited owing to realization of effective stabilizing interactions of its aromatic side chains atoms with the atoms of other residues of the molecule;
6. The residues Tyr1 and Ser7 are localized on a surface of molecule and characterized by large conformational dynamics;
7. The distribution of charges on the atoms of pharmacophore elements in the optimal structures are similar that confirms their identical arrangement in space from each other in all of them and may be correlated with their ability to participate in ligand-receptor interactions;
8. On the basis of the received results and SAR studies the bioactive conformation of dermorphin was assessed and the model of pharmacophore for interaction of this molecule with the opioid receptors was proposed;

The represented results are helpful for the study of the biology active forms of opioid peptides and for the design of opioid peptiomimetics.

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Received: 15.02.2016