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Structural organization of lactoferroxine C

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Abstract

A number of exogenous peptides derived from nutrients have opioid-like properties. Lactoferroxine is a glycoprotein present in milk and small amounts in exocrine fluids such as bile and tears. The conformational capabilities of the lactoferroxine C molecule (H-Lys1-Tyr2-Leu3-Gly4-Pro5-Gly6-Tyr7-OH) 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 lactoferroxine molecule and the dihedral angles of the main and side chains of amino acid residues included in the molecule were found, the energy of intra-and intersubstance interactions was estimated. It has been shown that the spatial structure of the lactoferroxine molecule is represented by eight 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 structure-functional organization of humen casomorphin molecule.

Keywords: *lactoferroxine, opioid, structure, conformation.*
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1. Introduction

A number of exogenous peptides derived from nutrients have opioid-like properties. Lactoferroxine is a glycoprotein present in milk and small amounts in exocrine fluids such as bile and tears. It consists of a single-chain polypeptide and is relatively resistant to proteolysis. Complete DNAs for lactoferroxine from human milk, neutrophils, and cow's milk have been reported, and recombinant proteins have been produced. It has been hypothesized that, due to its iron-binding properties, lactoferroxine plays a role in the absorption of iron by the intestinal mucosa and acts as a bacteriostatic agent by retaining iron in iron-requiring bacteria. In addition, lactoferroxine may act in non-iron-mediated pathways as a growth factor and bactericidal agent [1].

2. Method

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 lactoferroxine 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. The designations and readings of the angles of rotation correspond to the IUPAC-IUB nomenclature [2].

3. Results. Discussion

The spatial structure of the Lactoferroxine-C molecule (H-Lys1-Tyr2-Leu3-Gly4-Pro5-Gln6-Tyr7-OH) was studied in fragments (Figure 1). At the first stage, the spatial structure of the N-terminal tetrapeptide fragment H-Lys1-Tyr2-Leu3-Gly4 was studied based on the low-energy conformations of the corresponding amino acid residues. The calculation results show that sharp energy differentiation occurs between conformations, main chain forms and shapes. For each shape, the lowest energy conformation was selected and they are presented in Table 1. The relative energies of these conformations vary in the energy range of 0-8 kcal/mol. Conformations with an unfolded form of the N-terminal dipeptide fragment are more favorable than conformations with folded forms (Table 1). All conformations presented in Table 1 are selected for the calculation of the Lactoferroxin-C molecule.

Table 1. Low-energy conformations of N-side Lys1-Tyr2-Leu3-Gly4 fragment of lactoferroxin C molecule, share, total and relative energies of non-valent, electrostatic, torsional interaction energies.

№	Shapes	Conformation	Un_v	U_{el}	U_{tors}	U_{tot}	U_{rel}
1	eef	$B_{3122}B_1R_{31}R$	-16.7	8.4	3.6	-4.7	0
2	eee	$B_{1222}B_2B_{22}B$	-15.5	8.5	4.3	-2.7	2.0
3	eff	$B_{1222}R_1R_{21}R$	-12.6	8.6	2.1	-1.8	2.9
4	efe	$B_{1222}R_2B_{21}B$	-13.1	9.9	2.3	-0.9	3.8
5	ffe	$R_{2222}R_1B_{22}B$	-13.4	9.8	2.9	-0.7	4.0
6	fff	$R_{2322}R_1R_{21}R$	-15.4	11.0	4.4	0.0	4.7
7	fef	$R_{2322}B_3R_{32}R$	-12.2	10.0	3.3	1.0	5.7
8	fee	$R_{2322}B_2B_{21}B$	-8.9	9.7	2.6	3.3	8.0

At the second stage, the conformational capabilities of the C-terminal tetrapeptide fragment Gly4-Pro5-Gln6-Tyr7-OH were studied based on the corresponding amino acid residues. The calculation results are shown in Table 2. Here, also for each shape, the lowest energy conformation is selected and presented in Table 2. As can be seen from Table 2, the relative energies of these conformations vary in the energy range of 0-4 kcal/mol (Table 2). All these conformations were chosen as initial approximations for calculating the spatial structure of the entire Lactoferroxin-C molecule.

Table 2. Low-energy conformations of the Gly4-Pro5-Gln6-Tyr7 fragment of the lactoferroxine C molecule, their share, total and relative energies of the non-valent, electrostatic, and torsional interaction energies.

№	Shapes	Conformation	U_{nv}	U_{el}	U_{tors}	U_{tot}	U_{rel}
1.	ffe	R R B_{211} B_3	-13.1	1.2	1.7	-10.3	0
2.	fee	R B B_{311} B_3	-12.5	1.3	2.3	-8.9	1.4
3.	eee	B B B_{311} B_1	-11.2	0.9	2.4	-8.0	2.3
4.	fef	R B R_{211} R_1	-10.3	1.3	1.8	-7.2	3.1
5.	fff	R R R_{311} R_2	-10.8	1.4	1.6	-7.8	2.5
6.	eef	B B R_{211} R_3	-13.6	3.2	2.7	-7.7	2.6
7.	efe	B R B_{331} B_1	-9.9	1.1	1.3	-7.5	2.8
8.	eff	B R R_{231} R_3	-12.0	2.4	3.1	-6.5	3.8

As can be seen, these two fragments are connected by a common amino acid residue Gly4, which has four forms of the main chain R, B, L and P. Therefore, when compiling the initial approximations, all these forms were taken into account and several hundred conformations of the heptapeptide molecule were calculated. The calculation results showed that a sharp energy differentiation occurs between shapes, main chain forms and conformations. It has been shown that the spatial

structure of the lactoferroxin C molecule can be represented by eight forms of the main chain, the relative energy of which varies in the energy range 0-8.0 kcal/mol. In low-energy conformations, the energy of nonvalent interactions changes in the energy range (-31.4) – (-19.5) kcal/mol, the energy of electrostatic interactions in the range (5.3) – (9.3) kcal/mol and torsion interactions in the range (3.3) – (8.2) kcal/mol. The most stable conformation for the lactoferroxin C molecule is $B_{1222}R_1R_{21}BBB_{311}B_3$. It turns out to be advantageous in terms of non-valence and electrostatic interactions. In this structure, the Tyr2-Leu3 residues form a folded form of the main chain, which allocates the first amino acid residue Lys1 and the C-terminal tetrapeptide region -Gly4-Pro5-Gly6-Tyr7.

Table 3 shows the low-energy conformations of the Lactoferroxine-C molecule, the contributions of nonvalent, electrostatic and torsional energies, total and relative energy. For four low-energy conformations, the relative energy of which varies in the energy range 0-4.0 kcal/mol, the energy of intra- and interresidue interactions (in kcal/mol) is shown in Table 4, and their geometric parameters in Table 5. Figure 2 shows the arrangement of atoms in space in these conformations.

Table 3. 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 lactoferroxine C molecule.

№	Shape	Conformation	U_{nv}	U_{el}	U_{tors}	U_{tot}	U_{rel}
1	effeee	$B_{1222}R_1R_{21}BBB_{311}B_3$	-27.0	6.5	3.6	-16.9	0
2	eeffef	$B_{2122}B_1R_{31}RBR_{211}R_1$	-26.6	5.5	4.2	-16.8	0.1
3	feffef	$R_{1222}B_1R_{21}RBR_{211}R_3$	-20.8	5.3	3.3	-12.2	4.7
4	fffeef	$R_{2322}R_1R_{21}BBR_{211}R_1$	-19.5	6.4	3.5	-9.6	7.3
5	eeefee	$B_{1222}B_2B_{22}RBB_{331}B_1$	-31.4	7.3	7.5	-16.6	0.3
6	fffeee	$R_{2222}R_1B_{22}LBB_{311}B_1$	-23.6	6.0	6.0	-11.7	5.2
7	feeffe	$R_{2322}B_2B_{22}RBB_{331}B_1$	-26.7	9.1	6.2	-11.4	5.5
8	effeff	$B_{1222}R_2B_{21}LRR_{231}R_3$	-28.0	9.3	8.2	-10.4	6.5

Table 4. Energy inside and between residual interactions in the conformations of the Lactoferroxine C molecule $B_{1222}R_1R_{21}BBB_{311}B_3$ (U_{rel}=0 kcal/mol, first line), $B_{2122}B_1R_{31}RBR_{211}R_1$ (U_{rel}=0.1 kcal/mol, second line), $B_{1222}B_2B_{22}RBB_{331}B_1$ (U_{rel}=0.3 kcal/mol, third line), $R_{1222}B_1R_{21}RBR_{211}R_3$ (U_{rel}=4.7 kcal/mol, fourth line)

Lys1	Tyr2	Leu3	Gly4	Pro5	Gln6	Tyr7	
8.5	-2.4	-2.2	-0.2	-2.8	-2.6	-4.4	Lys1
8.3	-6.2	-3.4	-0.2	-0.4	0.2	-5.2	
8.8	-6.2	-3.1	-0.2	-0.5	-0.3	-4.3	
10.0	0.7	-0.7	-0.1	-0.3	-0.1	-7.8	
	1.1	-2.7	-1.1	-0.1	-0.8	0.3	Tyr2
	1.2	-2.6	-0.8	-0.3	-3.0	0.2	
	1.0	-3.5	-2.1	-0.1	0	0.3	

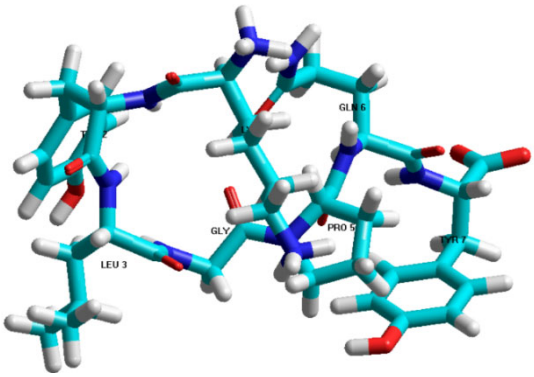
Lys1	Tyr2	Leu3	Gly4	Pro5	Gln6	Tyr7	
	1.4	-2.0	-0.6	0.2	-4.0	-0.4	
		-1.1 -0.6 -0.3 -1.2	-0.8 -0.5 -1.1 -0.9	-0.5 -1.5 -1.8 -1.6	-0.1 0 -0.7 0.1	0 0 -2.2 0.1	Leu3
			1.6 1.3 1.4 1.4	-2.7 -0.5 -2.8 2.9	-1.6 -0.4 -1.0 -0.4	-0.4 0 -0.1 0	Gly4
				0.7 0.5 0.4 0.5	-3.0 -1.1 -1.4 -1.2	-3.6 -1.3 -2.7 -1.3	Pro5
					0.5 -0.7 -0.5 -0.7	-1.4 -4.8 -2.2 -4.7	Gln6
						1.4 1.4 1.1 1.4	Tyr7

Table 5. Geometric parameters (in degrees) of low energy conformations of the molecule lactoferroxine C (the values of the dihedral angles are given in the sequence $\boldsymbol{\varphi}$, ψ, w, χ1, χ2...)

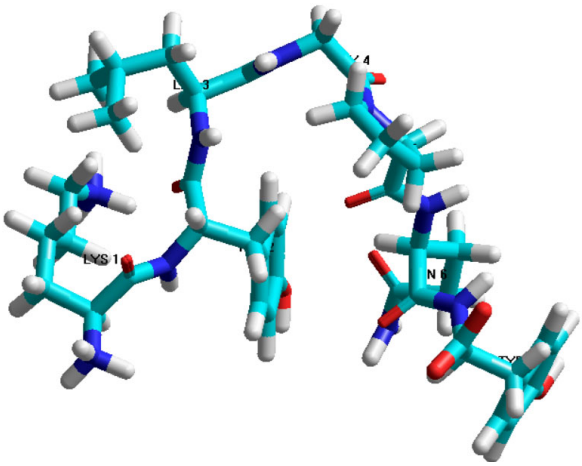
	$B_{1222}R_1R_{21}BBB_{311}B_3$	$B_{2122}B_1R_{31}RBR_{211}R_1$	$B_{2122}B_2B_{22}RBB_{331}B_1$	$R_{1222}B_1R_{21}RBR_{211}R_3$
Lys1	-85 154 179 66 -176 -172 180 178	-175 118 171 -191 59 178 172 178	180 112 164 -197 58 -178 172 178	-83 -53 179 62 -206 180 -176 178
Tyr2	-102 -41 170 67 87 0	-109 149 178 63 80 0	-109 143 -178 178 76 0	-97 148 177 73 86 0
Leu3	-131 -73 176 172 62 179 174	-106 -65 -178 -70 68 179 180	-97 94 -178 -158 175 -172 165	-127 -67 175 172 62 179 175
Gly4	-92 157 177	-80 -60 -172	-75 -71 -172	-71 -70 179
Pro5	-60 157 173	-60 145 179	-60 142 180	-60 150 -179
Gln6	-84 136 -179 -74 60 77	-81 -57 -176 178 63 83	-103 130 -175 -61 -63 107	-78 -56 -176 179 62 83
Tyr7	-115 140 - -58 90 0	-108 -47 - -54 96 0	-164 151 - 49 89 0	-108 -47 - -54 97 0
ΔU	0	0.1	0.3	4.7

In this conformation, the N-terminal Lys1 and the C-terminal tetrapeptide fragment Gly4-Pro5-Gln6-Tyr7-OH are in the unfolded main chain form, folded away from each other by the folded R-R main chain form of the Tyr2-Leu3 dipeptide (Figure 2a). The interactions of Lys1 with subsequent amino acid residues are (-4.6) kcal/mol, and Pro5 (-6.6) kcal/mol and therefore the conformation has become global (Table 4).

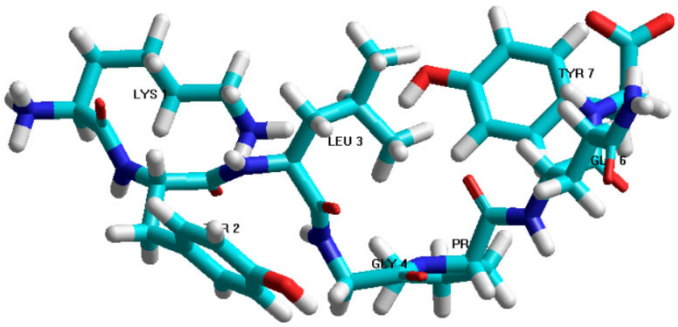
The $B_{2122}B_1R_{31}RBR_{211}R_1$ conformation of the eeffef shape has a relative energy of 0.1 kcal/mol and differs sharply from the global one. In this conformation, the N-terminal dipeptide Lys1-Tyr2 is in the unfolded form of the main chain B-B, then Leu3-Gly4 is in the folded form of the main chain R-R, folds the main chain, Pro5 is in the B form of the main chain, the N-terminal dipeptide fragment is Gln6-Tyr7 is



a) $B_{1222}R_1R_{21}BBB_{311}B_3$



b) $B_{2122}B_1R_{31}RBR_{211}R_1$



c) $B_{2122}B_2B_{22}RBB_{331}B_1$

Fig. 1. Atomic model of spatial structure of the lactoferroxin B molecule a), b) and c) corresponded to the structures with the relative energies 0 kcal/mol, 0.1 kcal/mol and 0.3 kcal/mol, respectively.

in the folded form of the R-R main chain (Figure 2 b). The main role in the stabilization of conformations is played by the interaction of Lys1, the contribution of which with other amino acid residues is (-15.4) kcal/mol. The energy contribution of Tyr2 with subsequent residues is -6.9 kcal/mol, and the contribution of Pro5 with subsequent residues is (-4.1) kcal/mol (Table 4). In the $B_{1222}B_2B_{22}RBB_{331}B_1$ conformation of the eeefee shape, the N-terminal and C-terminal tripeptide regions are in the unfolded form of the BBB main chain, separated from each other by Gly4, which is in the R form of the main chain (Figure 2c). In this conformation, the contribution of nonvalent energies is greatest (Table 3). The contribution of Lys1 with other amino acid residues is (-14.6) kcal/mol, Tyr2 with the following residues is (-5.7) kcal/mol, Leu3 and Pro5 with subsequent amino acid residues is (-5.8) kcal/mol and (-4.1) kcal/mol (Table 4).

4. Conclusion

Thus, the spatial structure of the Lactoferroxine C molecule can be represented by eight 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 Lactoferroxine C 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.

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