THE THEORETICAL MODEL OF THE MET1-ARG16 SEGMENT STRUCTURE FROM N-TERMINUS OF TYROSINE HYDROXYLASE DOMAIN

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The low-energy conformational states of the sequence including 16 amino-acid residues, forming a part of the N-terminus regulator domain of the tyrosine hydroxylase ferment have been established by the method of the force field in the approximation of the semiempirical atom-atom potential functions. It is established, that the state with the global minimum of the conformational energy has the two α-convolutions on the segments of Pro4-Thr7 and Pro9-Lys12 amino-acid residues and a number of several reverse turns on the short segments of the peptide backbone, stabilized by the hydrogen bonds.

Introduction

The structure and the dynamic conformational properties of the sequence from 16 amino-acid residues, forming a part of the *N*-terminus regulator domain of the tyrosine hydroxylase ferment (TH) have been investigated by the method of the semiempirical atom-atom potential functions [1,2]. The calculations, based on the physical theory of the structural organization of the peptides and proteins, include the detail analysis of the conformational possibilities of the big number of segments and their overlapping regions are described in the refs. [3,4]. The sets of the low-energy variants of the structure of monopeptides–molecules of *N*-acetil-α-aminoacids of metilamides, used in the calculations, are universal under the consideration of any amino-acid sequences. The low-energy conformational states of the investigated segments and the whole region Met1-Arg16 of the *N*-terminus regulator domain TH, obtained on the base of the results of the independent calculations, were analyzed with the use of the following parameters: a) relative conformation energy E_{rel}; b) elements of the space structure (α-helix and β-turns), and also c) the hydrogen bonds.

Calculation method

The simulation of the segment structure was carried out by the method of the theoretical conformational analysis with taking under consideration the atom polar surrounding on the base of the parcel of the applied computer programs [5]. They rest on the quantitative calculations of the total conformational energy of the investigated segment and on the searchings of its local minimums by the conjugate gradient method. The conformational energy (E_{conf}) is represented in the form of the additive sum of contributions of the non-valency (E_{nonv}), electrostatic (E_{el}) atom interactions, torsion rotation energy around valency bonds (E_{tors}) and energy of the formation of the hydrogen bonds ($E_{h.b.}$). The semiempirical potential functions and their parametrization, used in the given paper., have been taken from the ref. [6].The polar atom surrounding in the calculate experiment was simulated with the help of the value ε=10 and the *D* parameter, describing the depth of the hydrogen bond, which is equal to 1,5 kcal/mol in the Morse potential [3,6]. The indication of the dihedral angles of the rotation was carried out according to the standard nomenclature [7].

The results ant their discussion

The theoretical conformation analysis of Met1-Arg16 segment was carried out on the base of the fragmentar calculation according to the scheme, given on the fig.1. As the theory of the conformation analysis proceeds from the assumption about co-ordination of all types of the intramolecular interactions, the refinement scheme of the amino acid sequence on the separate segments hasn't the principal meaning and doesn't influence on the final results of the investigation.

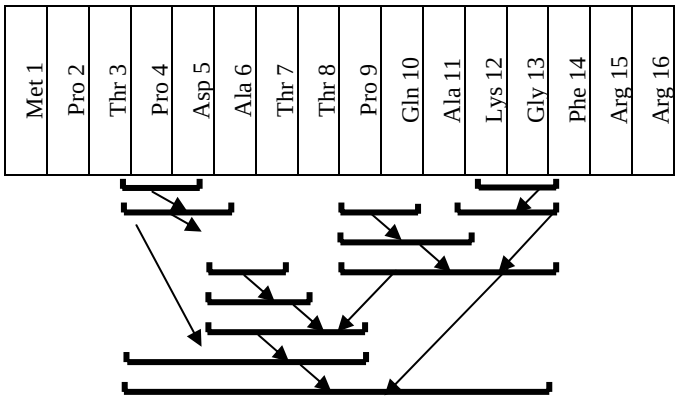


Fig.1. The scheme of the stepwise calculation of Met1-Arg16 segment of *N*-terminus domain from tyrosine hydroxylase.
a)E_{rel}=0.0 kcal/mol.
b) E_{rel} =1.5 kcal/mol.
c) E_{rel} =3.6 kcal/mol.
d) E_{rel} =6.8 kcal/mol.

Nonapeptid Met1-Pro2-Thr3-Pro4-Asp5-Ala6-Thr7-Thr8-Pro9

In the capacity of the initial approximation for the procedure of the minimization of the conformational energy the 684 variants, being combinations of the most profitable forms of the peptide backbone of Met1-Pro4 tetrapeptide and Pro4-Pro9 gexapeptide have been created. The low-energy conformation state contains α-helix region of the peptide chain, including the residues of Pro4-Asp5-Ala6-Thr7-Thr8. The creation of the helix structure is proved by the values of the dihedral angles of the rotation of the main chain and by the hydrogen bonds of the type 1-4, created by oxygen atoms of the carbonyl group and hydrogen atoms of the amid group of the peptide backbone Nh(Pro4)...Oc(Thr8). The energy of such hydrogen bond in the different conformational states of the investigated segment varies in the limits 1,9-2,5 kkal/mol. The side chain of the threonine residuals creates the big number of the intramolecular contacts. Particularly, hydroxyl group in Thr7 and Thr8 begins to participate in the formation of the hydrogen bonds with the side chain of the aspartic. The contribution analysis of the interresidual interactions allows

us to conclude, that the dispersion interactions of the residuals in the positions 4, 8, 9 and 14 of the peptide backbone are the main factors of the stabilization of the global conformation of the nonapeptide segment. The low-energy conformation states of the segment, satisfying to the criterion E_{rel}<8 kcal/mol, have been chosen for the latest calculation.

OktapeptidePro9-Gln10-Ala11-Lys12-Gly13-Phe14-Arg15-Arg16

The peptide backbone Pro9-Arg16 is divided on the simple overlapping two-, three-, and e.t.a. segments for the calculation of the Oktapeptide segment (fig.1). According to the calculation results, the global conformation of the Oktapeptide segment has the short of α-helix on the region Pro9-Gln10-Ala11-Lys12, the turn on the region Gly13-Phe14 (because of the existence conformational mobile glycine, playing the role of the knuckle residue) and the turned compact structure on the region Phe14-Arg15-Arg16. Such structure of the Oktapeptide segment is realized in 70% of the calculated conformations. The rest conformations belong either to the structures of the disorder type, or to the turned ones, among which it is possible to underline the total helix conformation (E_{rel}<2.7 kcal/mol). Let's consider the peculiarities of the space construction of the conformation with the global energy minimum. It is preferred not only by the value of total energy, but on the energy of each separate type of interactions. The existence of such co-ordination between nonvalent, electrostatic and torsion interactions does the energy distribution of the conformations in respect of the parametrization of the potential functions very stable. The residues of the proline (Pro 9) and glytamine (Gln 10), participating in the numerous two-, three-, tetra- and pentapeptide interactions play the significant stabilizing role in the formation of the stable segment structure.

Met1-Arg16 segment

The conformational analysis of Met1-Arg16 segment of *N*-terminus regulator domain of tyrosine hydroxylase included the minimization of the total conformational energy of the segment with taking under consideration the low-energy states of the segments, constituting it. The relative energy of the minimized structures varies in the interval of the values 0-10 kcal/mol. The results of the conformational analysis are given in the tables 1-3 and on the fig.2, where the stereoimages of the most stable conformational states of the segment in the conditions, simulating the water surrounding are given.

According to the investigation results, the state with the global minimum of the conformation energy has two turns of α-helix on the regions Pro4-Thr7 and Pro9-Lys12 and the totally unfolded *N*- and *C*-terminus segments of Met1-Thr3 and Lys12-Arg16. It is need to note, that α-helix on the region Pro9-Lys12 saves in 72% calculated structural variants, whereas it can be subjected to the quick destabilization on the region Pro4-Thr7 in the dependence on the intermolecular interactions with the side chains of the Met1, Thr8, Lys12 and Arg16 residues. The residues of the threonine in the positions of 3 and 8 peptide chain have the maximal number of the energy profitable contacts. The sum effect from their interaction in the global conformation of the segment is 22,4 and 11,5 kcal/mol, correspondingly. The multipeptide chain creates also the several reverse β-turns on the short regions of Thr3-Pro4-Asp5-Ala6, Asp5-ala6-Thr7-Thr8, Thr8-Pro9-Gln10-Ala11 and Gln10-Ala11-Lys12-Gly13. The general criterion, showing the existence of such turns is the distance, which is less, than 7Å between C^{α}-atoms of the residues in the first and fourth positions of the peptide backbone (table 3). The conformations, containing the proline residues in the tops of the reverse turns are the most stable.

Table 1

The energy contributions (kcal/mol) of the different types of the interactions in the optimal conformations of Met1-Arg16 segment

Conformation	$E_{nonv.}$	E_{el}	E_{tors}	E_{conf}	E_{rel}
1	-84.4	15.4	9.3	-68.6	0.0
2	-82.1	5.9	9.0	-67.1	1.5
3	-78.2	4.3	9.0	-65.0	3.6
4	-77.2	6.6	8.8	-61.8	6.8

Table 2

The dihedral angles (grad) in the main and side chains of the amino-acid residues in the global conformation of Met1-Arg16 segment

Residual	Dihedral angles
Met1	-119, 127,177, 185, 181,178,180 *
Pro2	131, 181
Thr3	-99,153,180, 63,182, 179
Pro4	-41,187
Asp5	-77, -35,180, 59, 94
Ala6	-73, -46, 184, 185
Thr7	-74, -60,178, 57,180, 176
Thr8	-123, 84,177, -54, 176, 175
Pro9	-44, 180
Gln10	-56 , -38,185, -79, 64,-107
Ala11	-75, -45,187,180
Lys12	-100,-70,181,180, 180, 179,180,180
Gly13	-91, 79,180
Phe14	-118,157,177, 57,85
Arg15	-106,-58,178,181,178, 180, 179
Arg16	52,63,179,-58,177, 183,179

*Notice. The angles are given in the sequence φ, ψ, ω, χ_1, χ_2, χ_3, χ_4, χ_5 (for Pro2, Pro4 and Pro9 -in the sequence ψ, ω).

The latest segment structures on the energy (E_{rel}=1.5; 3.6 and 6.8 kcal/mol) include the one from the low-energy states of the nonapeptide Met1-Pro9 and save the α-helix on the region Pro9-Lys12, that allows to us to conclude about the existence of the enough close nucleation in the space structure of the segment. From the data comparison, given in the table 1 it is followed, that the nonvalent, i.e. dispersional interactions, i.e. packing density of the amino-acid sequence play the significant role in the stabilization of the low-energy conformational states of the Met1-Arg16 segment. In spite of the differences in the space packing of the polypeptide chain (fig.2), it saves the reverse turns on the regions Thr3-Ala6, Asp5-Thr8, Thr8-Ala11 and Glu10-Gly13 (table 3). Thus, despite on the conformational mobility of the polypeptide

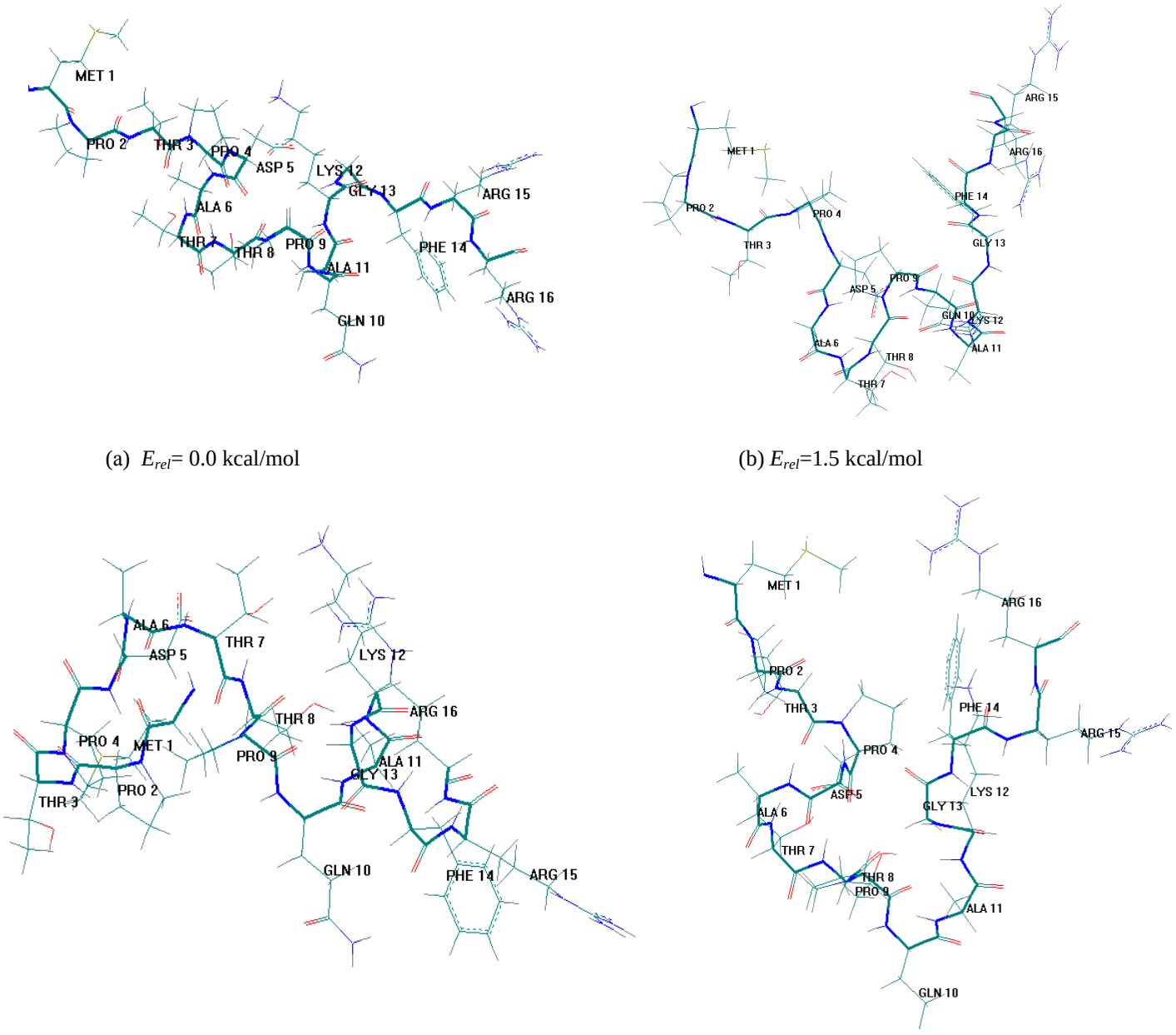
chain, differing by the orientation of the side chains of the separate residues, it is possible to select the character elements in its space structure. It is the existence of two shorts of α-helixes on the regions Pro4-Thr7 and Pro9-Lys12 and several β-rotations, stabilized by the hydrogen bonds between functional groups of the peptide backbone.

Table 3

The energy contributions of the interresidual interactions (kcal/mol) and the distances between C^{α}-atoms of the main chain (Å) in the low-energy conformational states of Met1-Arg16 segment

Residuals	1*		2		3		4	
	Distance	Energy	Distance	Energy	Distance	Energy	Distance	Energy
Met1- Pro2	3.8	-3.4	3.8	-3.2	3.8	-3.1	3.8	-3.4
Met1-Thr3	6.3	-1.2	5.5	-1.9	5.3	-2.3	6.3	-1.2
Met1-Pro4	9.6	-0.2	6.7	-2.4	6.5	-0.4	9.6	0.0
Pro2-Asp5	8.6	-3.2	9.0	-0.1	8.7	-0.1	8.6	-0.2
Pro2-Ala6	6.9	-2.5	9.4	0.0	9.1	0.0	6.9	-0.2
Thr3-Pro4	3.8	-3.1	3.8	-3.3	3.8	-3.3	3.8	-3.0
Thr3-Asp5	5.4	-3.3	5.5	-2.3	5.5	-2.0	5.4	-3.3
Thr3-Ala6	5.2	-2.1	6.7	-0.7	6.7	-0.5	5.3	-2.7
Thr3-Thr7	6.0	-1.3	8.7	-0.2	8.5	-0.2	6.7	-1.7
Thr3-Thr8	8.0	-0.4	8.1	-0.1	8.0	0.0	8.3	-0.1
Thr3-Gln10	12.3	-9.4	11.3	0.0	10.8	0.0	12.1	0.0
Thr3-Ala11	11.6	-2.7	13.0	0.0	13.0	0.0	11.0	0.0
Pro4-Thr7	5.3	-1.2	9.0	0.0	8.9	0.0	5.5	-1.8
Pro4-Thr8	5.4	-2.1	8.0	0.0	8.1	0.0	5.5	-1.1
Pro4-Phe14	11.2	-2.0	8.6	-0.1	13.5	0.0	9.7	0.0
Pro4-Arg15	14.8	-2.4	10.4	0.1	16.2	0.0	11.9	0.1
Asp5-Ala6	3.8	-1.1	3.8	-2.2	3.8	-2.1	3.8	-0.5
Asp5-Thr7	5.5	-1.0	5.7	-2.9	5.6	-2.9	5.5	-0.9
Asp5-Thr8	5.2	-1.1	5.4	-2.6	5.7	-2.9	5.5	-0.9
Asp5-Pro9	4.0	0.0	5.0	-2.1	5.1	-3.1	3.9	-2.2
Asp5-Lys12	5.4	0.0	6.9	-5.7	8.8	-11.7	5.2	-8.9
Asp5-Arg15	11.7	-0.1	11.4	-2.0	15.7	-1.7	9.5	-3.1
Asp5-Arg16	14.4	0.0	11.1	-7.9	15.6	-3.7	11.7	-5.1
Ala6-Thr7	3.8	-1.3	3.8	-1.5	3.8	-1.5	3.8	-1.4
Ala6-Thr8	5.7	-0.7	6.0	-0.5	6.2	-0.4	5.6	-0.8
Ala6-Pro9	5.6	-0.1	7.7	-0.1	7.7	-0.1	5.7	-0.9
Thr7-Thr8	3.8	-1.7	3.8	-2.9	3.8	-2.9	3.8	-1.6
Thr8-Pro9	3.8	-3.8	3.8	-3.1	3.8	-2.8	3.8	-3.7
Thr8-Gln10	5.5	-2.2	5.4	-2.3	5.6	-1.8	5.2	-3.3
Thr8-Ala11	6.0	-1.9	5.8	-2.3	5.8	-2.2	6.0	-1.4
Thr8-Lys12	6.9	-1.2	6.7	-1.5	7.2	-1.4	7.3	-0.6
Thr8-Gly13	7.8	-0.7	8.5	-0.1	8.9	0.0	7.4	-0.2
Thr8-Phe14	9.5	-1.7	8.6	0.0	10.5	0.0	10.6	0.0
Pro9-Gln10	3.8	-2.8	3.8	-0.8	3.8	-3.2	3.8	-2.1
Pro9-Lys12	5.2	-0.5	5.4	-1.6	5.3	-2.9	5.3	-1.5
Pro9-Gly13	4.4	-1.5	5.4	-1.3	5.7	-1.6	3.9	-0.9
Gln10-Gly13	5.8	-1.2	6.4	-0.3	4.8	-1.8	5.8	-0.3
Lys12-Gly13	3.8	-1.6	3.8	-1.8	3.8	-1.7	3.8	1.8
Lys12-Phe14	5.9	-3.0	6.4	-0.4	5.8	-1.4	5.9	-2.9
Gly13-Phe14	3.8	-1.0	3.8	-0.5	3.8	-0.4	3.8	-1.6
Gly13-Arg15	6.9	-0.2	5.9	-0.4	6.1	-0.3	6.3	-0.6
Gly13-Arg16	9.7	0.0	6.0	-3.3	6.5	-2.0	9.1	-0.3
Phe14-Arg15	3.8	-1.5	3.8	-3.2	3.8	-5.4	3.8	-0.2
Phe14-Arg16	6.0	-5.7	6.1	1.6	6.4	-1.0	5.5	-5.0

*Note. Conformation from table1.



(a) E_{rel}= 0.0 kcal/mol

(b) E_{rel}=1.5 kcal/mol

(v) E_{rel} = 3.6 kcal/mol

(g) E_{rel} = 6.8 kcal/mol

Fig.2. The low-energy conformational states of Met1-Arg16 segment from *N*-terminus domain of tyrosine hydroxylase.

[1] *E. Iseki, M. Kato, W. Mauri et al.* J. Neurol. Sci., 2001, v.185(2), p.129-134.

[2] *Nakashima, N. Hayashi, K. Mori et al.* FEBS Lett., 2000, v.465, p.59-63.

[3] *E.M. Popov.* «Strukturnaya organizatsiya belkov», M., «Nauka», 1989, 352p.

[4] *E.M. Popov*. Int. J. Quant. Chem. 1979, v.16, p.707-718.

[5] *I.S. Maksumov, L.I. Ismailova, N.M. Gojayev.* J. Struk. Khimii,1983, v.24, №4, p.147-148.

[6] *F.A. Momany, R.F. Mc.Guire, A.W. Burgess, H.A. Scheraga.* Phys. Chem. 1975, v.79, p. 2361-2381.

[7] IUPAC-IUB Commision on Biochemical Nomenclature Abbreviations and symbols for description of conformation of polypeptide chains. Pure Appl. Chem., 1974, v. 40, p. 291-308.

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TİROZİNHİDROKSİLAZA N-SONLU DOMENİNİN MET1-ARQ16 FRAQMENTİN QURULUŞUNUN NƏZƏRİ MODELİ

Tirozinhidroksilaza N-sonlu tənzimləyici domenin tərkibinə daxil olan 16 amin turşusu qalıqlarının ardıcıllığı aşağı enerjili konformasiya hallarının potensial funksiyaların yarımempirik atom-atom yaxınlaşmasında qüvvə sahəsi metodu ilə təyin edilib.

Hidrogen rabitələrilə sabitləşdirilmiş polipeptid zəncirinin qısa sahələrində yerləşən bir-neçə reversiv dönüşlər və Pro9-Lys12 və Pro4-Thr7 sahələrində α-spiral iki burulmasının olması konformasiya enerjisinin qlobal minimumlu halı təyin edilmişdir.

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ТЕОРЕТИЧЕСКАЯ МОДЕЛЬ СТРУКТУРЫ ФРАГМЕНТА MET1-ARG16 N-КОНЦЕВОГО ДОМЕНА ТИРОЗИНГИДРОКСИЛАЗЫ

Методом силового поля в приближении полуэмпирических атом-атомных потенциальных функций установлены низкоэнергетические конформационные состояния последовательности из 16 аминокислотных остатков, входящих в состав N-концевого регуляторного домена фермента тирозингидроксилазы. Установлено, что состояние с глобальным минимумом конформационной энергии содержит два витка α-спирали на участках Pro4-Thr7 и Pro9-Lys12 и несколько реверсивных поворотов на коротких участках пептидной цепи, стабилизированных водородными связями.

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