CONFORMATIONAL POSSIBILITIES OF THE p21^{ras} PROTEIN FAMILY GTP-BONDING FRAGMENT

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Using a theoretical conformational of method analysis, a three-dimensional structure and conformational properties of Leu53-Ala59 fragment of p21 protein were investigated. The calculations were performed on the basis of the fragmental analysis, using nonvalence, electrostatic and torsional interactions and hydrogen bonds. The obtained data suggest, that this polypeptide can exist only in several low energy conformations. The results of this calculated experiment can be used for the study of structure-functional relationship.

The ras gene family is highly conserved from yeast to humans and ubiquitously expressed in eucaryotic cells. In mammals the gene family consists of three members: H-, K- and N-ras, which code for highly homologous proteins termed p21 according to their molecular weight – 21000 Daltons. The p21 proteins are membrane bound nucleotide binding proteins, which bind GDP and GTP with high affinity and have a low intrinsic GTP activity. They are members of the family of G-binding proteins, which includes the classical heterotrimeric G proteins, the elongation factors and the ras-superfamily. By analogy to the hormone receptor-coupled G proteins, the p21 proteins are believed function as signal switch molecules. In the active GTP conformation, they transmit a signal to an effector molecule that leads to cell proliferation. The p21 effector interaction is switched off by GTP hydrolysis, returning p21 to the inactive GDP bound state [1,2].

The efficiency of the GDP-binding essentially depends on amino acide substitutions in positions 59 and 61 [3,4]. Likeness of 57-63 segment and GDP-binding fragments of another proteins was determinated by Shih, Hattori et.al. [5]. Bolonick, Bollag and McCormick [6] established, that mutations at codone-61 influence on conformation state of Gly60, which determine the dissociation of GTP and GDP from the complex with p21. Therefore 57-63 fragment of ras family is responsible for the guanosine-phosphate binding.

In order to investigate the three-dimensional structure of the address fragment, the next peptide was taken: Leu53-Asp54-Ile55-Leu56-Asp57-Thr58-Ala59.

The search of the optimum conformations was performed by minimization of the energy during variations of the dihedral angles. For the calculations we used the program for semiempirical calculations of conformations of macromolecular components on the computer, developed in the Research Laboratory of Molecular Biophysics of the Baku State University [7]. The conformational energy was determined as the sum of the contributions of nonvalence and electrostatic interactions, hydrogen bonds and torsional barriers. The investigations was carried out on the basis of the step by step approach proposed by Popov [9,10].

At first stage of calculations the conformational possibilities of the 53-55, 55-57 and 57-59 tripeptides were investigated. A total 81 structural models were examined for every fragment.

Leu53-Asp54-Ile55 tripeptide shows high degree of the conformational lability. The representatives of all structural shapes, including the exotic forms BLR, RLR, LRR and LBR, belong to the relative energy interval from 0.0 to 2.2 kcal/mole. A global conformational state of this tripeptide is *ef*, and the most preferable conformations of *ff* and *fe* shapes have E_{rel}=0.3 and 0.6 kcal/mole accordingly.

We have the analogous picture in Ile55-Leu56-Asp57 fragment. The optimal form *ff* outstrips the *ef* shape to 0.1 kcal/mole, and E_{rel}=0.6 kcal/mole corresponds to unfolded *ee* and E_{rel}=1.4 kcal/mole – to *fe* shapes.

A considerable difference of the structural forms was found for Asp57-Thr58-Ala59 sequence. The folded *ff* form yields 1.3 kcal/mole to the global conformation (*ef* shape).

The minimized values of dihedral angles of 53-55 and 55-57 tripeptides were used at the compiling of initial data for the calculation of Leu53-Asp54-Ile55-Leu56-Asp57 pentapeptide. We calculated 339 conformations of this amino acide sequence.

The investigation of this fragment revealed a sharp preference of the folded forms of the main chain. A global form RRRRR corresponds to the helical structure of the polypeptide backborne. Next form BRRRR belong to *efff* shape and outstrip lider only on 0.4 kcal/mole.

Then the calculation of the 345 structural models of 53-59 heptapeptide was realized. The initial conformations were compiled on the basis of the dihedral angles of the most preferable structures of Leu53-Asp57 and Asp57-Ala59 fragments. The main energy and geometrical parameters of the preferable conformations are presented in Table 1.

The conformational analysis data confirms, that folded structures are preferable for this amino acide sequence. The compact packing forms of main and side chains lead to the optimal balance of interatomic contacts, correspond to minimal values of the full energy. The preference of related conformations $B_3R_1R_1R_2R_3R_3R_3$ (*ef*$_5$ shape) and $R_2R_1R_1R_2R_3R_3R_3$ (helical f_6 structure) is obvious. The information, summarized in the Table 1, shows, that the folded shape of the main chain ensures a high intensity of the nonvalence interactions.

Table 1.

Values of the Relative Energy, Nonvalence, Electrostatic and Torsional Contributions for the Most Preferable Molecular Structures of the p21ras 53-59 fragment.

Shapes	Forms	E_{rel}	E_{nonval}	E_{el}	E_{tors}
ef_5	BRRRRRR	0.0	-40.5	13.2	7.7
f_6	RRRRRRR	0.7	-39.8	13.0	7.9
f_3eef	RRRBBRR	3.5	-33.4	11.7	5.7
Effeff	BRRBBRR	3.7	-33.1	11.7	5.5
	BRBLBRR	6.7	-31.1	12.9	5.3
f_4ef	RRRRBRR	4.2	-35.3	13.7	6.2
Effeff	BRBLRRR	4.3	-34.4	14.6	4.5
	BRRBRRR	5.3	-32.3	12.4	5.6
Efefef	BRBRBRR	4.5	-30.9	11.7	4.1
	BBLRBRR	7.2	-28.0	12.2	3.5
ef_3ef	BRRRBRR	4.7	-34.5	14.0	5.6
ffefef	RRBRBRR	5.0	-29.7	11.7	3.3
	RBLRBRR	7.2	-28.0	12.2	3.4
efeffe	BRBRRBR	5.6	-29.7	12.4	3.4
	BBLRRBR	7.1	-28.9	13.1	3.2
f_3eff	RRRBRRR	5.7	-33.3	12.3	7.1
ffeffe	RRBRRBR	5.8	-28.9	12.6	2.6
	RBLRRBR	6.9	-29.0	12.8	5.5
efeeff	BRBBRRR	6.3	-30.7	12.2	5.2

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p21^{ras} ZÜLALLARIN GTP-BAĞLAYICI FRAQMENTİNİN KONFORMASİYA İMKANLARI

Nəzəri konformasiya analizi metodunun köməyi ilə p21ras zülalların Leu53-Ala59 fraqmentinin fəza quruluşu və konformasiya imkanları tədqiq olunmuşdur. Hesablamalar fraqmentar yaxınlaşma əsasında, qeyri-valent, elektrostatik, torsion qarşılıqlı təsiri və hidrogen rabitələri nəzərə almaqla yerinə yetirilmişdir. Tədqiqat nəticələri göstərir ki, bu polipeptid yalnız bir neçə alçaq enerjili konformasiya hallarında ola bilər. Bu hesablama eksperimentin nəticələri struktur-funksional əlaqənin öyrənilməsində istifadə oluna bilər.

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КОНФОРМОЦИОННЫЕ ВОЗМОЖНОСТИ GTP-СВЯЗЫВАЮЩЕГО ФРАГМЕНТА БЕЛКОВ СЕМЕЙСТВА p21^{ras}

С использованием методики теоретического конформационного анализа исследованы трехмерная структура и конформационные свойства фрагмента Leu53-Ala59 белка p21. Рассчеты проводились на основе пофрагментного подхода с учетом невалентных, электростатических и торсионных взаимодействий и водородных связей. Результаты исследований показывают, что данный полипептид может существовать лишь в нескольких низкоэнергетических конформоционных состояниях. Результаты этого вычислительного эксперимента могут быть использованы для изучения структурно функциональной зависимости.

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