DYNAMICAL PROPERTIES OF THE ALLATOSTATIN IV NEUROPEPTIDE SIDE CHAINS IN WATER SOLUTION

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The conformational flexibility of the allatostatin IV neuropeptide side chains in water solution has been investigated by the method of molecular mechanics using atom-atomic potential functions. The series of conformational maps of the potential surfaces were constructed over the angles of the side chains for lowest energy-minimized conformation of the molecule. Permissible deviations of these angles from the optimal values were determined.

1. Introduction

Allatostatins are a family of neuropeptides that inhibit juvenile hormone (JH) synthesis by the corpora allata (CA) in cockroach and related insects [1]. These peptides are produced by cells of the brain and ganglia as well as by midgut endocrine cells [2,3]. Allatostatins have also been identified and purified from muscle tissue, suggesting that these molecules may have other roles apart from regulation of JH production [4]. Here we describe in detail conformational dynamics of the allatostatin IV neuropeptide initially isolated from brains of the virgin female the cockroach *Diploptera punctata* [2,3]. The primary structure of this neuropeptide was assigned as Asp1-Arg2-Leu3-Tyr4-Ser5-Phe6-Gly7-Leu8-NH_2. To clarify the mechanism and specificity of action of the allatostatin IV neuropeptide the permissible deviations of the side chains dihedral angles each amino acid residue from the optimal values for the preferable conformation in water solution was determined. Such study may provide important information regarding the structural and conformational features important for structure-function relationship of the allatostatin IV neuropeptide.

2. Computational method

Calculations have been carried using molecular mechanics method. Molecular mechanics is a computational method designed to give accurate structures and energies of molecules. It treats molecules as collections of masses that are interacting with each other via Van der Waals, electrostatic and torsion forces between non-bonded atoms. Mathematical functions of the atomic coordinates (called potential energy functions) are used to describe these interactions. Various parameters derived from experimental observations are included in the potential energy function. The energy of Van der Waals interaction has been described by Lennard-Jones potential with parameters proposed by Momany et al.[5]. A contribution of electrostatic interactions has been taken into account in a monopole approximation according to Coulomb's law with single atomic charges proposed by Momany et al [5]. The influence of solvent is included in the dielectric constant (ε). Since conformations of neuropeptide have to be analyzed in an aqueous environment, the dielectric constant is assumed to equal 10 as described in [6] and hydrogen bonds calculated from the Morse potential are supposed to be weakened with maximum energy of

1.5kcal/mol at NH...CO distance $r_0=1.8Å$ [6]. Torsion potentials and barriers to rotation about bonds C^{α}-N(φ) and about side chains bonds (χ) were as proposed by Momany et al [5]. The nomenclature and conventions adopted are those recommended by IUPAC-IUB [7]. Low-energy conformational state of the allatostatin IV neuropeptide (fig.1) was found by locating the global minima on a potential energy surface using energy minimization procedure in our previous study [8]. The motions of the amino acid residues side groups are governed by the conformation of the neuropeptide main chain, and it is expected that the studies of the motions of the side groups may provide information on the allatostatin IV neuropeptide molecular structure in detail. By conformational analysis, we estimated the probability distributions of side chain angles (χ_1, χ_2, χ_3...) each amino acid residue of the molecule. The conformational search was confined near the calculated distributions of backbone dihedral angles of each amino acid and includes the following steps:

1. Conformational maps, i.e. energy contour maps as a function of backbone angle φ and side chain angles χ_1, χ_2, χ_3 were constructed for calculated lowest energy conformation (Table 1) of the allatostatin IV neuropeptide keeping all bond lengths, bond angles and peptide bond dihedral angles ψ and ω fixed. The angles φ-χ_1, φ-χ_2, φ-χ_3, and χ_1-χ_2 or χ_2-χ_3 varied at intervals of 30^0.

2 After defining low-energy regions similar conformational maps were produced for the particular φ-χ_1, φ-χ_2, φ-χ_3, and χ_1-χ_2 or χ_2-χ_3 values at a 5^0 step, yielding in this way the detailed potential surfaces for the side chains of the molecule.

3. Structures resulting from step 2 are locally minimized.

3. Results and discussion

The results of conformational maps analysis which reflect the dynamics of functionally important side chains of the allatostatin IV neuropeptide are shown in figs.2-8. The values of dihedral angles corresponding to the optimal energy are marked by crosses; and the energy on equipotential lines is given in kcal/mol. Values for χ_1, χ_2 and χ_3 described low-energy states of the allatostatin IV side chains angles were taken from molecular mechanics calculation described above (Table 1).

Table 1.

The values of the dihedral angles (in degree) of the lowest energy conformation of the allatostatin IV neuropeptide

Residue	Dihedral angles	Fig. 1. Lowest energy conformational state of the allatostatin IV neuropeptide [8].
Asp1	φ=-89, ψ=-44, ω=178, χ_1=54, χ_2=105	
Arg2	φ=-134, ψ=97, ω=179, χ_1=-67, χ_2=174, χ_3=161	
Leu3	φ=-99, ψ=94, ω=183, χ_1=54, χ_2=177, χ_3=182	
Tyr4	φ=-150, ψ=165, ω=177, χ_1=-67, χ_2=95, χ_3=180	
Ser5	φ=-71, ψ=-52, ω=171, χ_1=56, χ_2=177	
Phe6	φ=-60, ψ=-29, ω=178, χ_1=62, χ_2=81	
Gly7	φ=80, ψ=-77, ω=182	
Leu8	φ=-104, ψ=-60, ω=179, χ_1=-53, χ_2=176, χ_3=186	
E_{conf}	-36, 9 kcal/mol	

Three conformational maps of the potential surfaces were constructed over φ-χ_1, φ-χ_2, and χ_1-χ_2 angles of the Asp1 residue in the lowest energy conformational state of the allatostatin IV neuropeptide (fig.2). These maps take into account the low-energy state of the asparagine residue, thereby displaying the minima of the potential functions at all possible values of the χ_1 and χ_2 angles. As can be seen in fig.2, the optimal positions of the Asp1 side chain are close to minima of their torsional potential, i.e.χ_1 may populate ±60, 180^0 and deviations by ±20^0 from minimal values are possible for backbone φ angle. Calculated results indicate that χ_2 for Asp1 can take two values -60 and 100^0, and the low-energy changes of this angle in the range of ±15^0 are allowed (fig.2). The plot of the χ_1 and χ_2 angles distributions is given in fig.3.
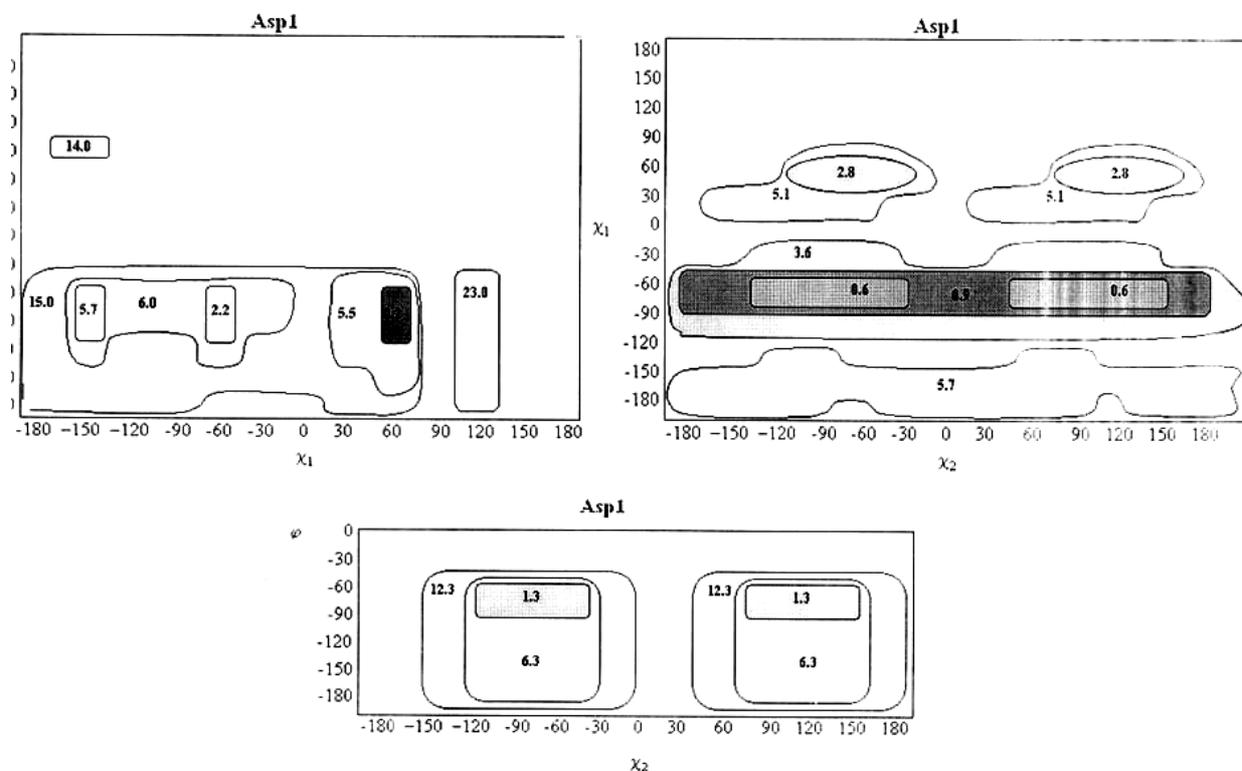
Fig. 2. Energy contours (in kcal/mol) as a function φ-χ_1; φ-χ_2; and χ_1 -χ_2 of the Asp1 for lowest energy conformation of allatostatin IV neuropeptide.

A further calculation scheme included a study of low-energy regions for the Arg2 positively charged side chain. The calculation was made by constructing three conformational maps over the φ-χ_1, φ-χ_2, φ-χ_3 angles, based on low-energy structure of allatostatin IV neuropeptide (fig.4).

As can be seen from fig.4, the energies of the conformations that correspond to the positive value of the φ dihedral angle are approximately 24,1 kcal/mol higher than those with negative values. The low-energy changes from -60^0 to -120^0 of the φ angle and from -120^0 to -60^0 of the χ_1 angle are allowed. Calculated results indicate that χ_2 for Arg2 can take values -60 and 180^0, and low-energy changes of this angle in the range of $\pm15^0$ are allowed. There is very restricted low-energy region for one value of χ_3 for Arg2, i.e. at 180^0. The permissible deviation by $\pm5^0$ from optimal value of this angle is revealed. This result suggest that not only the positive charge of the amino acid residue at position 2, but also the length of the side chain seem to be important for the three-dimensional structure of the neuropeptide. Fig.5 describes distributions of the arginine χ_1 and χ_2 dihedral angles in the lowest energy conformation of the molecule.
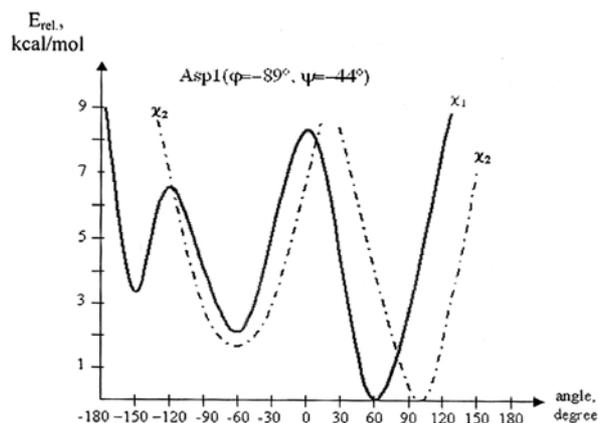
Fig. 3. A plot of the distribution of the Asp1 χ_1 and χ_2 dihedral angles. The x-axis is the value of the dihedral angle, from -180 to +180°, with the distribution in 30° bins. The y-axis is the relative conformational energy of the molecule in kcal/mol.
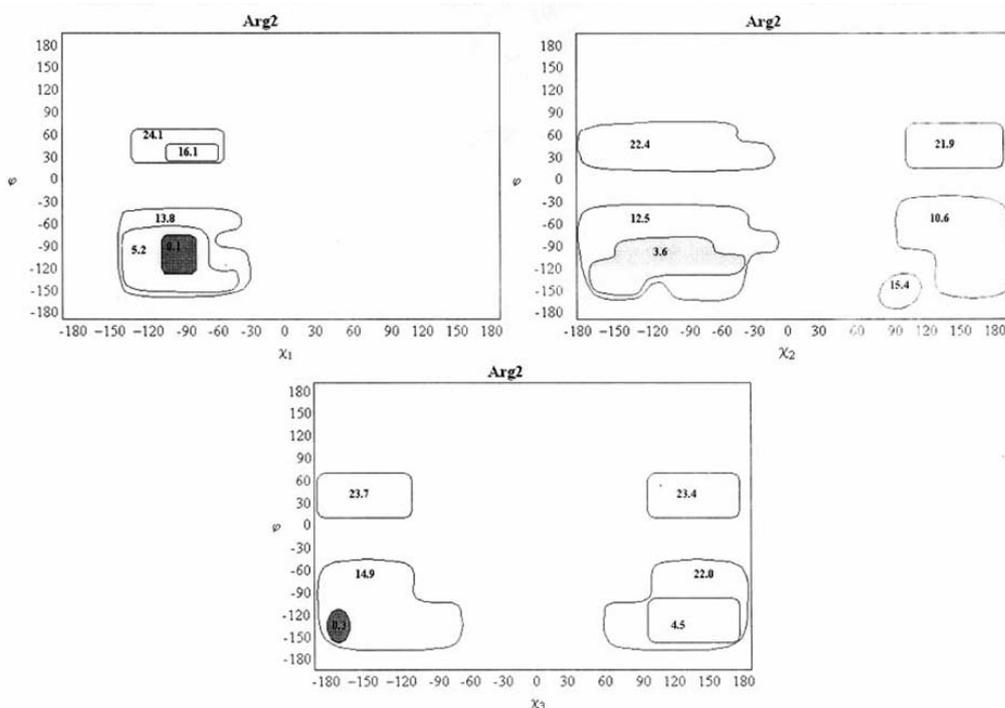
Fig. 4. Energy contours (in kcal/mol) as a function φ-χ_1 ; φ-χ_2 ; and φ-χ_3 of the Arg2 for lowest energy conformation of allatostatin IV neuropeptide.
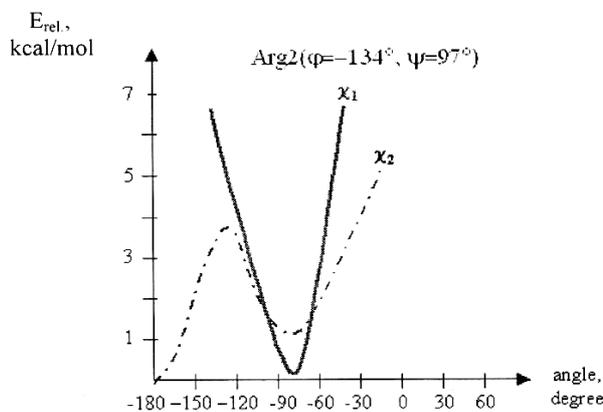
Fig. 5. A plot of the distribution of the Arg2 χ_1 and χ_2 dihedral angles.

For the leucine part of the allatostatin IV neuropeptide, the permissible deviations of the backbone φ and χ_1,χ_2 side chain angles are calculated by constructing φ-χ_1 and φ-χ_2 conformational maps. It must be noted that the dihedral angle χ_3 and χ_4 which determines the spatial state of the end group of the leucine residue, does not contribute significantly to the stabilization of the spatial structure. Conformational maps that indicate the restricted conformational mobility of the Leu3 backbone are given in fig.6. As can be seen in fig.6, the positive values of φ angle form forbidden region in conformational map. There are low-energy regions for χ_2 for Leu3, i.e. at $\pm60^0$ and only one for χ_1, which corresponds to 180^0 (fig.7). Through computer simulation, it was found that the effects caused by variation of χ_3 and χ_4 angles are small compared with those caused by χ_2.
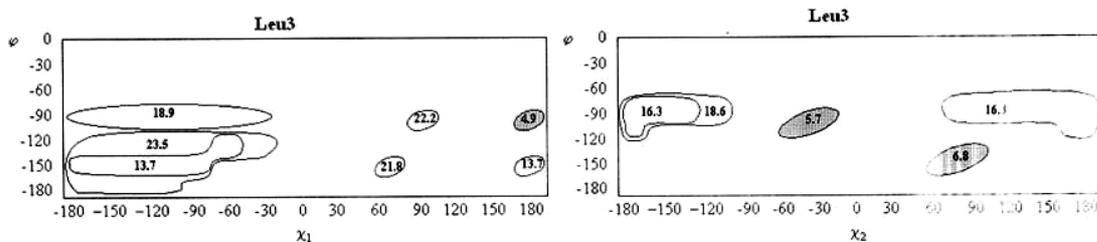
Fig. 6. Energy contours (in kcal/mol) as a function φ-χ_1 and φ-χ_2 of the Leu3 for lowest energy conformation of allatostatin IV neuropeptide.
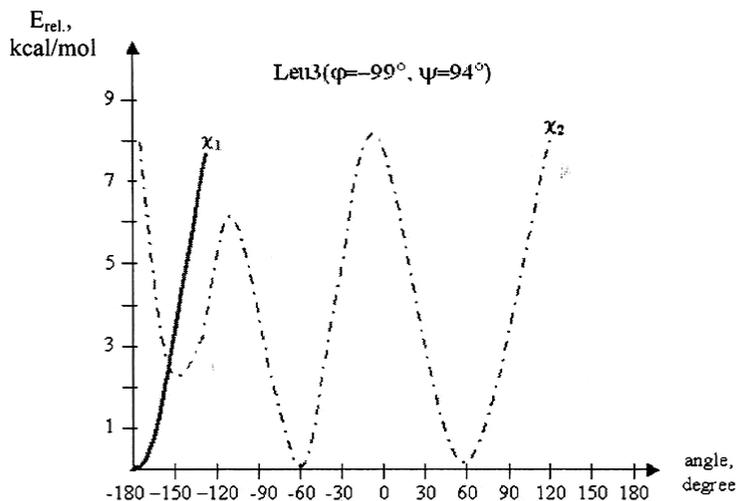
Fig. 7. A plot of the distribution of the Leu3 χ_1 and χ_2 dihedral angles.

Fig.8,a and b show the energy contours of φ, χ_1 and χ_2 angles for Tyr4. The φ angle for Tyr4 has a weak conformational mobility, i.e. its low-energy change by $\pm 5^0$ is possible here. Two minima for χ_2 ($\pm 90^0$) and only one for χ_1 (-60^0) side chain angles are found (fig.9). The angle χ_3 for Tyr4, which defines the orientation of hydroxyl group OH, has a noticeable conformational flexibility.
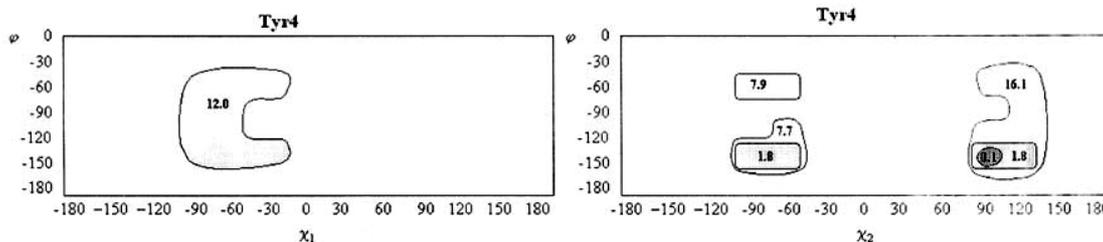
Fig. 8. Energy contours (in kcal/mol) as a function φ-χ_1 and φ-χ_2 of the Tyr4 for lowest energy conformation of allatostatin IV neuropeptide.
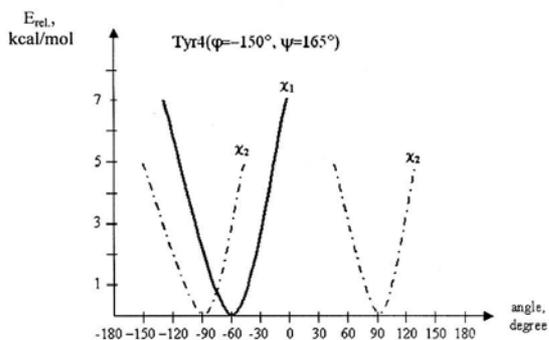
Fig. 9. A plot of the distribution of the Tyr4 χ_1 and χ_2 dihedral angles.

The side chain of the next Ser5 amino acid residue is characterized by significant conformational mobility (fig.10). In spite very fixed backbone, there are two low-energy regions in the φ-χ_2 conformational map. The low-energy changes from -180^0 to 180^0 for χ_1 and it is possible from 90^0 to 180^0 and from -60^0 to 180^0 for χ_2 (fig.11).

A further calculation scheme included a study of low-energy regions for the Phe6 side chain. Calculation was made by constructing two conformational maps over the φ-χ_1 and φ-χ_2 angles, based on low-energy structures of allatostatin IV neuropeptide. The energies of the conformations that correspond to the positive values of the φ dihedral angle are equal approximately 60 kcal/mol that higher than those with negative values. Calculated results indicate that χ_2 angle for Phe6 can take to opposite values $\pm 90^0$, and low-energy

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changes of this angle in the range of $\pm 20^0$ are allowed (fig.12 and fig.13).
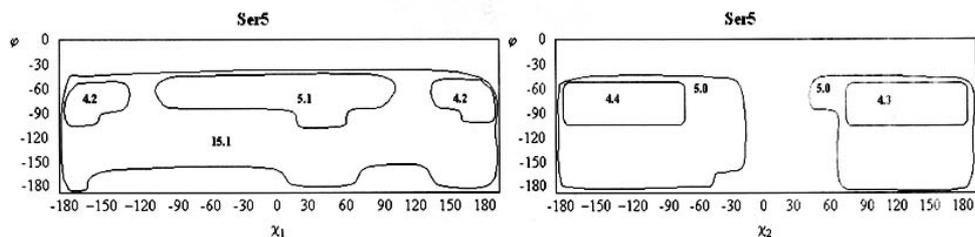
Fig. 10. Energy contours (in kcal/mol) as a function φ-χ_1 and φ-χ_2 of the Ser5 for lowest energy conformation of allatostatin IV neuropeptide.
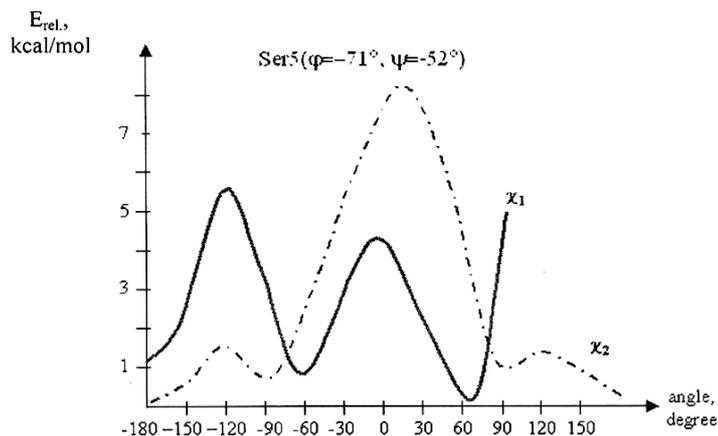
Fig. 11. A plot of the distribution of the Ser5 χ_1 and χ_2 dihedral angles.
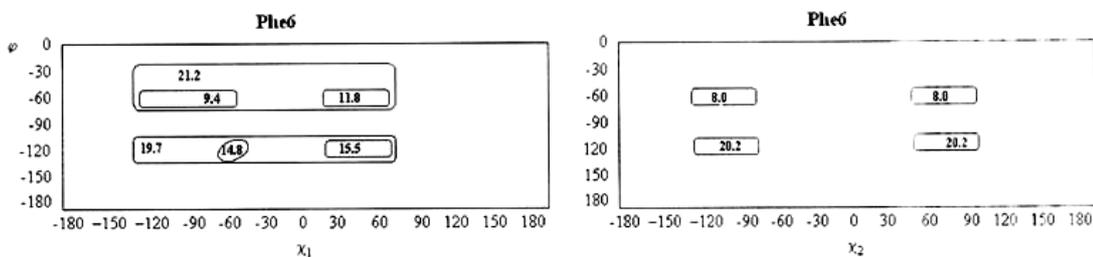
Fig. 12. Energy contours (in kcal/mol) as a function φ-χ_1 and φ-χ_2 of the Phe6 for lowest energy conformation of allatostatin IV neuropeptide.
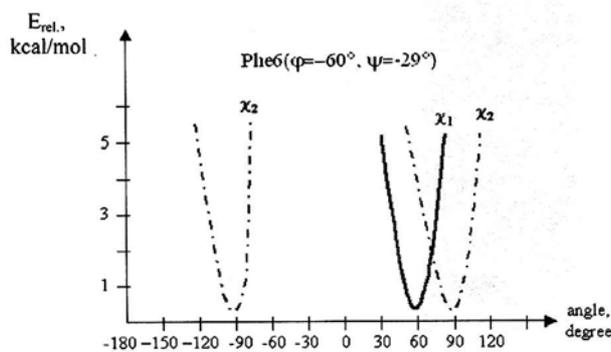
Fig. 13. A plot of the distribution of the Phe6 χ_1 and χ_2 dihedral angles.

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Three conformational maps of the potential surfaces were constructed over the φ-χ_1, φ-χ_2, and φ-χ_3 of the Leu8 for lowest energy conformation of allatostatin IV neuropeptide. The conformational maps, which indicate the forbidden zone that correspond to the positive values of φ backbone angle

are given in fig.14,a and b. χ_1 dihedral angle may populate two states $\pm 60^0$, but there is only one possible state for χ_2 dihedral angle (fig.15).
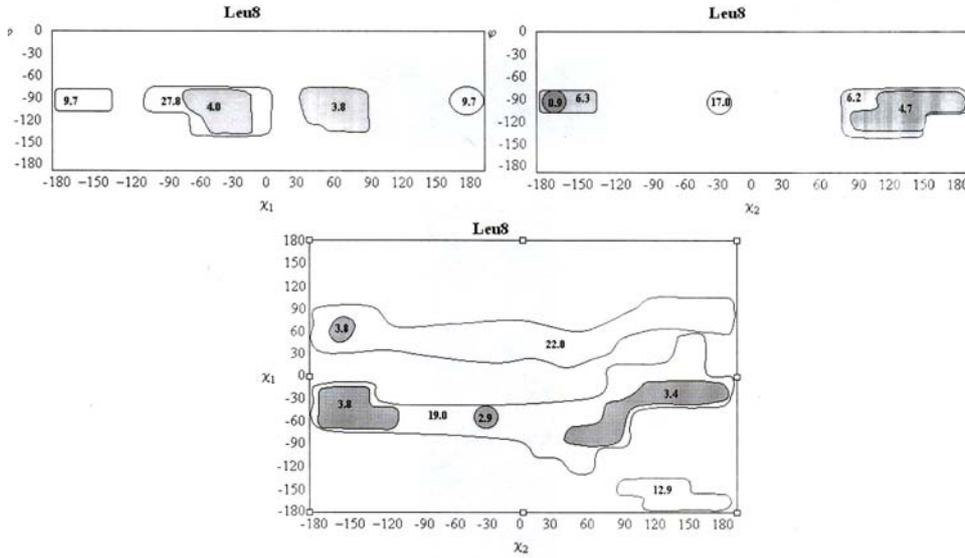
Fig. 14. Energy contours (in kcal/mol) as a function φ-χ_1; φ-χ_2; and φ-χ_3 (c) of the Leu8 for lowest energy conformation of allatostatin IV neuropeptide.
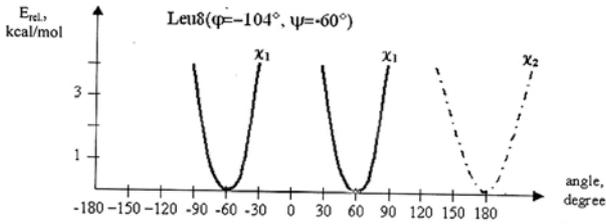
Fig. 15. A plot of the distribution of the Leu8 χ_1 and χ_2 dihedral angles.

The obtained data allow one to conclude that, in spite of considerable conformational mobility of the molecule, only Ser5 side chain has a noticeable conformational mobility. The reported results may serve as the basis for investigations of the structure-function relationship of the allatostatin IV neuropeptide.

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ALLATOSTATİN IV NEYROPEPTİDİNİN SU MÜHİTİNDƏ YAN ZƏNCİRLƏRİNİN DİNAMİK XÜSUSİYYƏTLƏRİ

Atom-atom potensial funksiyalardan istifadə edilməklə molekulyar mexanika üsulu ilə allatostatin IV neyropeptidinin su mühitində amin turşuları qalıqlarının yan zəncirlərinin konformasiya mütəhərrikliyi tədqiq edilmişdir. Neyropeptidin yan zəncirlərlərinin ikiüzlü bucaqlarının fırladılması yolu ilə molekulun qlobal konformasiyası üçün potensial çəpərin hündürlüyünü təsvir edən konformasiya xəritələri qurulmuşdur. İkiüzlü bucaqların öz optimal qiymətlərindən icazə verilən ĸənara çıxmaları müəyyən olunmuşdur.

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ДИНАМИЧЕСКИЕ СВОЙСТВА БОКОВЫХ ЦЕПЕЙ НЕЙРОПЕПТИДА АЛЛАТОСТАТИНА IV В ВОДНОЙ СРЕДЕ

Методом молекулярной механики с помощью атом-атомных потенциальных функций исследована конформационная подвижность боковых цепей аминокислотных остатков нейропептида аллатостатина IV в водной среде. Построены конформационные карты, описывающие сечения потенциальной поверхности для глобального конформационного состояния молекулы путем варьирования двугранных углов в боковых цепях нейропептида. Установлены допустимые отклонения двугранных углов от их оптимальных значений.