# MOLECULAR DYNAMICS SIMULATION FOR SEVEN STRUCTURES OF VISCOTOXIN

ALINA BUŢU[a], STELIANA RODINO[b,c], MARIANA FERDES[a], MARIAN BUŢU [b*]
[a]*University POLITEHNICA of Bucharest, Splaiul Independentei 313, Sector 6, Bucharest, Romania*
[b]*National Institute of Research and Development for Biological Sciences, 0630031, Splaiul Independentei 296, Bucharest, Romania*
[c]*University of Agronomic Sciences and Veterinary Medicine, Mărăşti Blvd. 59, 011464, Bucharest, Romania*

It had been proved by scientific research that in the case of a pathogen attack the antimicrobial peptides are key players of the innate immune system. The antimicrobial peptides have been found in all organisms from plants to humans, and even in the microorganisms. The viscotoxins belong to thionine class of antimicrobial peptides and are produced by leaves and stems of *Viscum album*. In this paper was analyzed the dynamics stability in molecular simulation experiments for seven viscotoxin structures. The conformational structure of the peptide sequence is linked to biological activity and dynamic parameters analysis led to the identification of amino acid residues which show the most important flexibility.

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*Keywords:* Plant antimicrobial peptide, Viscotoxin, "in silico" methods, Molecular dynamics, simulation

## 1. Introduction

The antimicrobial peptides represent a topic of research increasingly addressed because of the biotechnological potential they possess. The research is targeted both in the direction of identification and characterization of antimicrobial peptides and achieving practical application of these peptides. For selective identification of antimicrobial peptides interesting from therapeutic point of view, various strategies have been applied, with varying results. AMPs are generally defined as sequences of less than 100 amino acid residues with a molecular weight less than 10,000 Da, with a total positive electrical charge (usually between +2 and +9), which present as a particularity the presence of multiple lysine and arginine residues and a substantial part (30% or more) of hydrophobic residues. [1].

Antimicrobial peptides have diverse structures and functions and interact with cell membranes of invading cells by disrupting the membrane integrity. This action leads to cell lysis and, later, to their death [2]. Microbes are the cause of many infectious diseases. The increasing microbial resistance to common antibiotics has become a serious threat in maintaining human health and extensive research is conducted in order to find practical solutions to this issue. Due to their characteristics, antimicrobial peptides have become attractive and safe subjects for researchers who are intensively searching for solutions regarding the resistance to antibiotics [3-6]. The knowledge of the way how a peptide sets its conformation represents the first step in determining the mechanisms underlying its activity (eg antimicrobial activity) and in designing of rational treatments. Another important direction of research is the study of antimicrobial peptides

*Corresponding author: marian_butu@yahoo.com

as potential biomarkers of several diseases such as various forms of cancer, AIDS, inflammatory diseases [7-10].

The thermodynamic and interaction parameters, the structure and stability of antimicrobial peptides are issues that require a big volume of research. Exploiting the advances in computing resources allows us to obtain new data on molecular dynamics study of peptides, involving the reducing of the costs and of the time needed for research [11, 12].

The viscotoxins are antimicrobial peptides from plants belonging to the thionine class and are produced by leaves and stems of *Viscum album*. The viscotoxins from the experiments described in the present study consist from 46 residues of aminoacids. In this paper it is aimed to identify the influence corresponding to the modification of a residue from the primary sequence of the viscotoxin on the "in silico" molecular dynamics and on its structural stability. There are analyzed 7 native structures of viscotoxin. The differences from primary structure of all these viscotoxin sequences are shown in table 1 and are labeled in green colour. To be noticed that there are different aminoacids corresponding to the positions 18, 19, 12, 24, 25, 28 and 37 and for 3C8P and for the positions 6.

*Table 1. Sequence alignment of the primary structure of viscotoxin peptides.*

| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A1 | *3C8P* | K | S | C | C | P | S | T | T | G | R | N | I | Y | N | T | C | R | L | T | G | S | S | R | E | T | C | A | K | L | S | G | C | K | I | I | S | A | S | T | C | P | S | N | Y | P | K |
| A2 | *1JMN* | K | S | C | C | P | N | T | T | G | R | N | I | Y | N | T | C | R | F | G | G | G | S | R | Q | V | C | A | S | L | S | G | C | K | I | I | S | A | S | T | C | P | S | D | Y | P | K |
| A3 | *1OKH* | K | S | C | C | P | N | T | T | G | R | N | I | Y | N | A | C | R | L | T | G | A | P | R | P | T | C | A | K | L | S | G | C | K | I | I | S | G | S | T | C | P | S | D | Y | P | K |
| A3 | *1ED0* | K | S | C | C | P | N | T | T | G | R | N | I | Y | N | A | C | R | L | T | G | A | P | R | P | T | C | A | K | L | S | G | C | K | I | I | S | G | S | T | C | P | S | D | Y | P | K |
| B | *1JMP* | K | S | C | C | P | N | T | T | G | R | N | I | Y | N | T | C | R | L | G | G | G | S | R | E | R | C | A | S | L | S | G | C | K | I | I | S | A | S | T | C | P | S | D | Y | P | K |
| B2 | *2V9B* | K | S | C | C | P | N | T | T | G | R | D | I | Y | N | T | C | R | L | G | G | G | S | R | E | R | C | A | S | L | S | G | C | K | I | I | S | A | S | T | C | P | S | D | Y | P | K |
| C1 | *1ORL* | K | S | C | C | P | N | T | T | G | R | N | I | Y | N | T | C | R | F | A | G | G | S | R | E | R | C | A | K | L | S | G | C | K | I | I | S | A | S | T | C | P | S | D | Y | P | K |

A detailed view of the structure of viscotoxin 3C8P sequence, including both the aminoacids sequence (primary structure) and the alpha-helix, beta stand and turns (secondary structure) is shown in figure 1.

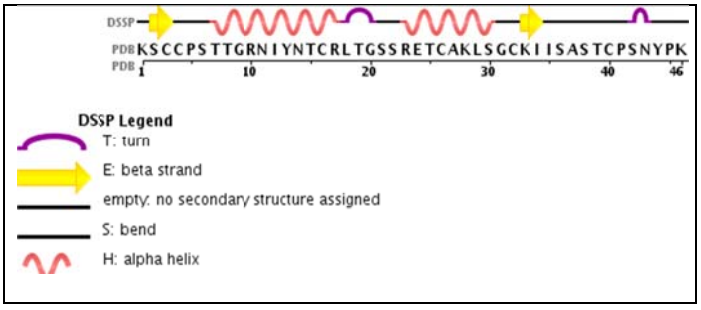
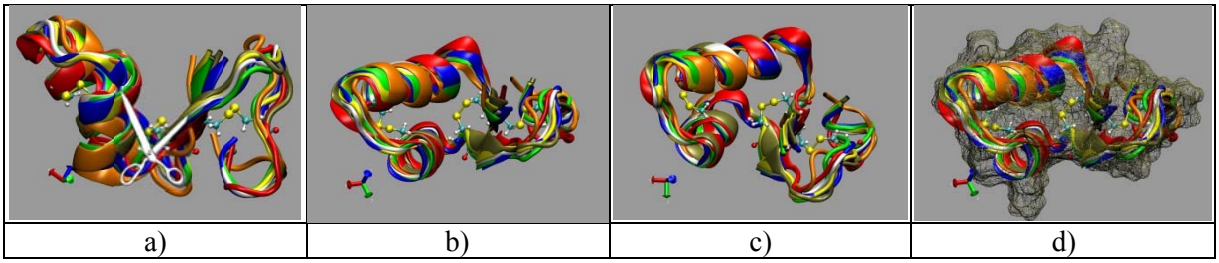



*Fig. 1. Sequence details of Viscotoxin A1 from Viscum album L., 3C8P from PDB [13]*

The 7 structures have a very similar spatial conformation presenting a "scissors" shape (figure 2. a). One of the scissors blades is formed by two alpha-helix and the other one by two beta-stand, and this is making it to be a solid structure. The disulfide bridges are disposed between residues CYS 3 - CYS 40, CYS 4 - CYS 32, CYS 16 – CYS 26 (figure 2). This arrangement strengthens the peptide structure and gives a compact form to the molecular surface. (figure 2.c,d)



a) b) c) d)

*Fig. 2. Newcartoon representation of all seven viscotoxin aligned by the mass center of each residue and CPK representation of the disulfide bridges: a)-b) different views; c) without and d) with molecular surfaces SURF/wireframe representation*

## 2. Materials and methods

The viscotoxin structures used were 3C8P (A1), 1JMN (A2), 1OKH (A3), 1ED0 (A3), 1JMP (B), 2V9B (B2), 1ORL (C1) were taken from PDB [14-18].

The viscotoxins had been analyzed using molecular dynamics simulation method in NPT ensemble and periodic boundary conditions. For the simulation, the peptide sequences were solvated in water. It was produced a trajectory with a length of 100ns for each of the peptides, starting from the structures taken from PDB. There were analyzed the structural and dynamic properties: RMS, accessibility surface area, dihedrals, distances between Cα atoms. For the molecular dynamics simulation it was used the GROMACS package, version 4.5.3 [19], and all atom force field Amber99sb [20]. Water was modeled with SPC-E (Simple Point Charge Extended) method. The minimization was realized using 5000 steps steepest descent method, and 5000 steps conjugate gradient method. The water box had a truncated dodecahedron form.
The number of atoms of viscotoxins from simulation experiments and total number of atoms of the simulated systems are presented in Table 2.

*Table 2. Method of structure determination and number of atoms for the simulated systems.*

| *ID* | *Method of structure determination* | *peptide* | *water* | *ions* | *total* |
|---|---|---|---|---|---|
| **1ED0** | SOLUTION NMR | 667 | 4704 | 6 | 5377 |
| **1JMN** | SOLUTION NMR | 656 | 4719 | 5 | 5380 |
| **1JMP** | SOLUTION NMR | 661 | 4275 | 5 | 4941 |
| **1OKH** | X-RAY DIFFRACTION | 667 | 4518 | 6 | 5191 |
| **1ORL** | SOLUTION NMR | 676 | 5172 | 6 | 5854 |
| **2V9B** | X-RAY DIFFRACTION | 659 | 4542 | 4 | 5205 |
| **3C9P** | X-RAY DIFFRACTION | 672 | 4698 | 6 | 5376 |

Dynamics of heating from 0 to 310K was achieved by rescaling the velocity for 200ps and constraining the links containing hydrogen with LINCS [21]. The step used was of 2fs dynamic equilibration and was performed in 50ps, in NVE ensemble, with periodic boundary conditions and another 50ps in NPT ensemble. Dynamics of production has been achieved for 100ns. For the visualization for primary verification of the systems in dynamics simulation was used VMD program [22, 23].

The simulation was performed in parallel on an HP computer with dual Xeon quad core at 3.2 GHz. The support for parallel simulation was LAM/MP Program (large–scale atomic

molecular massively parallel) [24, 25]. Simulation analysis was performed with GROMACS and VMD programs.

## 3. Results and discussion

The trajectories from molecular dynamics simulation experiments for seven native structures of viscotoxin were analyzed comparatively.

Analyzing the dynamics evolution of RMSD for all protein atoms of viscotoxin can be observed that the peptides 1JMN and 1JMP show some variations of approx. 0.4 nm being stable along the entire trajectory, and 1ORL presents a variation of almost 0.2 nm on intervals of 5-10 ns. The rest of the variations are around 0.1 nm, and the other 4 structures show variations below the value of 0.1 nm along the entire dynamics recorded.(figure 3).

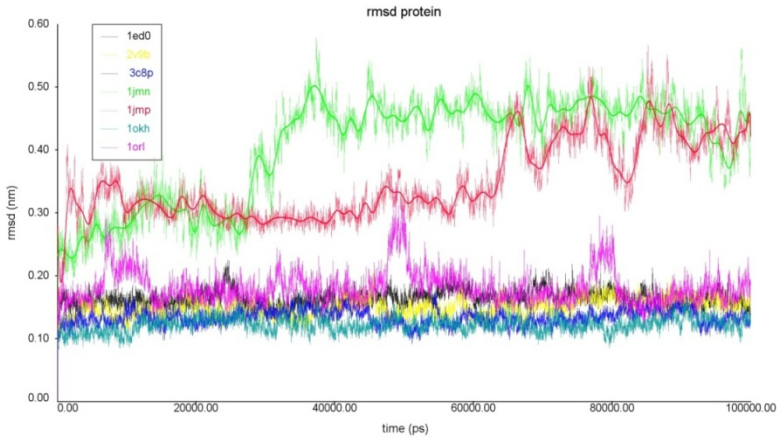



*Fig. 3. RMSD graphic for all protein atoms*

Depending on the comparative evolution of the RMSD of the seven structures, the residues have been classified in four categories as follows:

- very stable residues in all 7 structures – residues 9, 16, 20, 26, 31 present a variation of the RMSD below 0.03 nm;
- stable residues in all 7 structures - residues 2, 5, 7, 8, 12-14, 18, 21, 22, 27, 30, 32, 36-38, 40-42 show a variation of the RMSD between 0.03 nm and 0.1 nm;
- stable residues in some of the structures and unstable in other structures - residues 3, 4, 6, 10, 11, 15, 17, 18, 19, 24, 44 (figure 4);
- slightly unstable residues in some structures and unstable in others - residues 23, 25, 28, 29, 33, 35, 43 (figure 5);
- unstable residues in all 7 structures - residues 34, 39 (figure 6).

Further on, the discussion involves the residues that differ in primary structures of the seven viscotoxins. SER 6 from the structure 3C8P structure shows a high stability, with a variation of the RMSD below 0.02nm, with an evolution different from ASN6 from the 6 other structures that have variations between 0.1 nm and 0.15 nm. The residue ASP11 from the structure 2V9B had a similar behavior with ASN11 from structures 3C8P, 1ED0, 1JMP, 1OKH, with RMSD value of 0.7 nm. ASN11 residues from the structures 1JMN and 1ORL were unstable, with RMSD variations of 0.11nm. PHE18 from the structures 1JMN and 1ORL do not behave differently from LEU18 from the other five structures. Both residues LEU18 and PHE18 showed a high instability and had the value of RMSD variation between 0.1 nm and 0.17 nm. On the position 19 in three of the structures was THR (3C8P, 1OKH and 1ED0), and in the other three structures was GLY (1JMN, 1JMP and 2V9B) and in the structure 1ORL can be found ALA. GLY19 and ALA19 are stable residues with RMSD variations of 0.3 nm, respectively 0.4 for ALA19. THR19 exhibits instability in all structures and presents a variation of RMSD of 0.13 nm. Residue 21 had a high stability in all 7 structures, residue SER21 from the structure 3C8P had a RMSD variation value of 0.07 nm, variation of RMSD of ALA21 from the structures 1OKH and 1ED0 is 0.03 nm, and

GKY21 showed the lowest RMSD variation, namely 0.01 nm. The residue PRO24 from the structures 1OKH and 1ED0 was stable and has a variation of the RMSD value of 0.035 nm. The residues GLN24 from the structures 1JMN and GLU24 from the other four structures behave similarly and showed a variation of RMSD between 0.1nm and 0.16 nm.

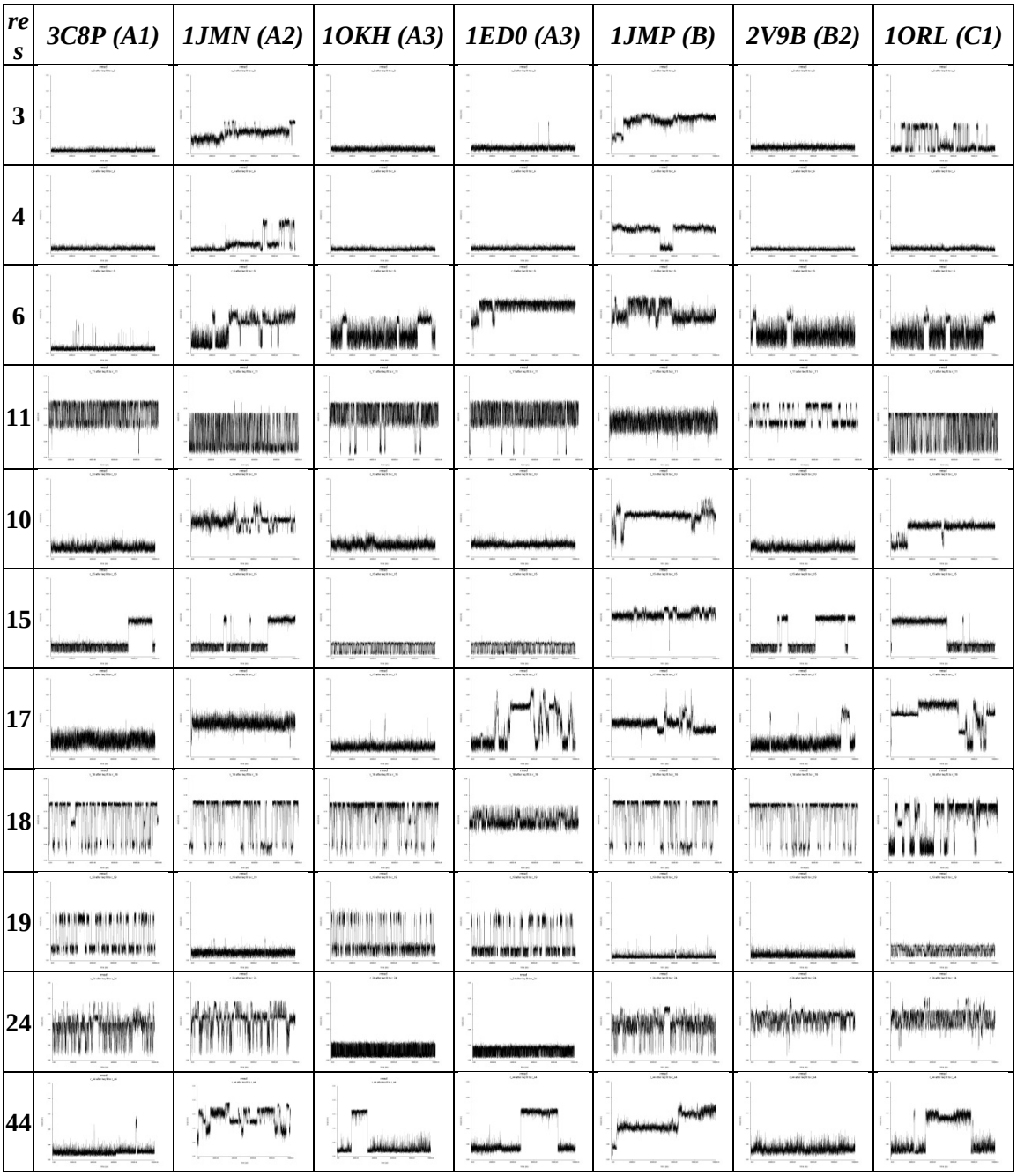



*Fig. 4. The dynamics evolution of RMSD for the residues stable in some structures and unstable in other structures*

The residue THR25 from the structures 3C8P, 1OKH and 1ED0 was stable with a value of RMSD below 0.05 nm. The residues VAL25 and ARG25 were unstable, but ARG25 showed higher instability during the entire dynamics recorded with a RMSD value of 0.2 nm, while VAL25 oscillated between two stable states, and had a variation value of 0.12nm. The residue SER28 from 1JMN, 1JMP and 2V9B was a stable residue, showing an identical behavior in all three structures and had a RMSD value of 0.07 nm. LYS28 from the other four structures was

unstable with variations between 0.1 nm and 0.16 nm. The residues GLU37 and ALA37 were stable in all seven structures and have RMSD values of 0.3nm.

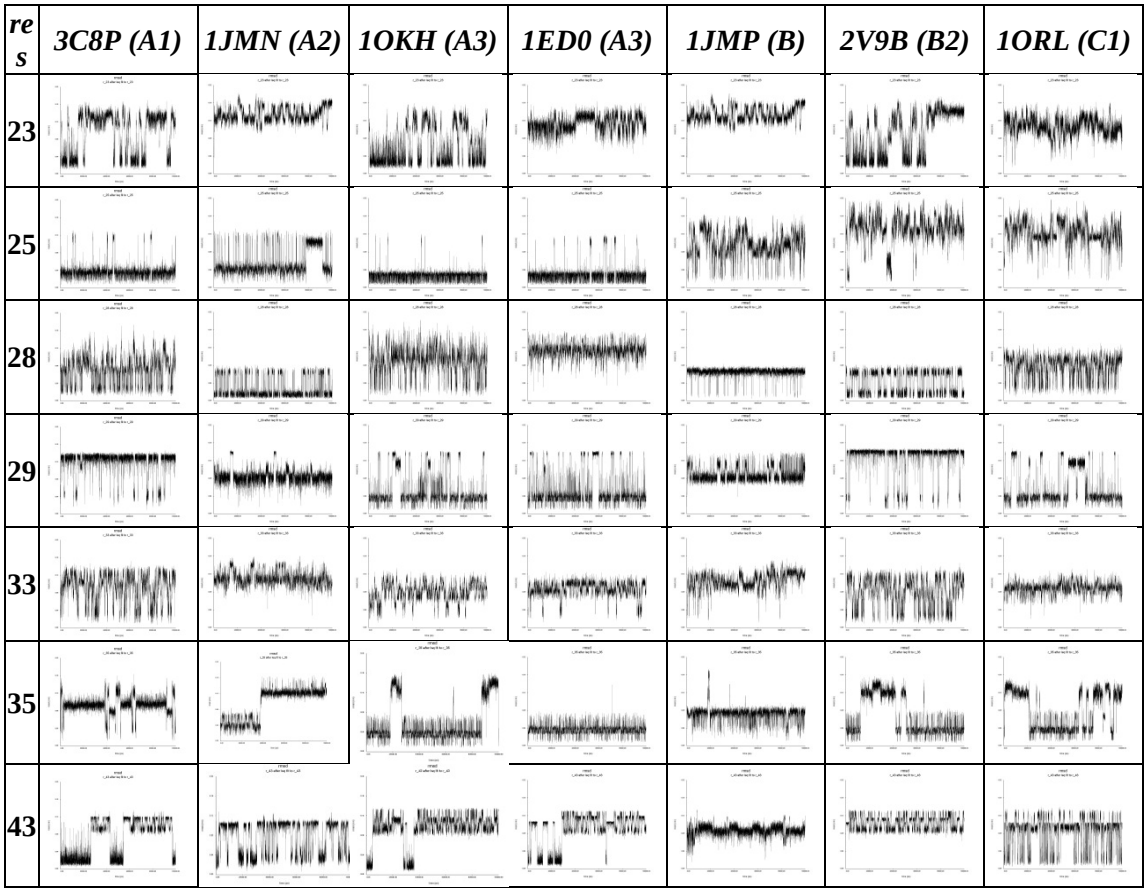



*Fig. 5. The dynamics evolution of RMSD for the residues slightly unstable in some structures and unstable in others*

The residues ILE34 and THR39 from all seven viscotoxin structures showed high instability. ILE34 belongs to the second beta-stand and had RMSD values between 0.14 nm and 0.17 nm. The flexibility of this residue is very important in the movement of the "scissors". This residue is involved in the approach of "scissors blades". THR39 is positioned in the neighborhood of the disulfide bridge CYS3-CYS40, being in the same plane with the residue ILE34, but on the opposite side of the structure (Figure 7). The RMSD value of THR39 for all structures was 0.12 nm. The distance Cα - Cα between the two residues did not present significant variations, which leads to the idea that these two have a coordinated movement.

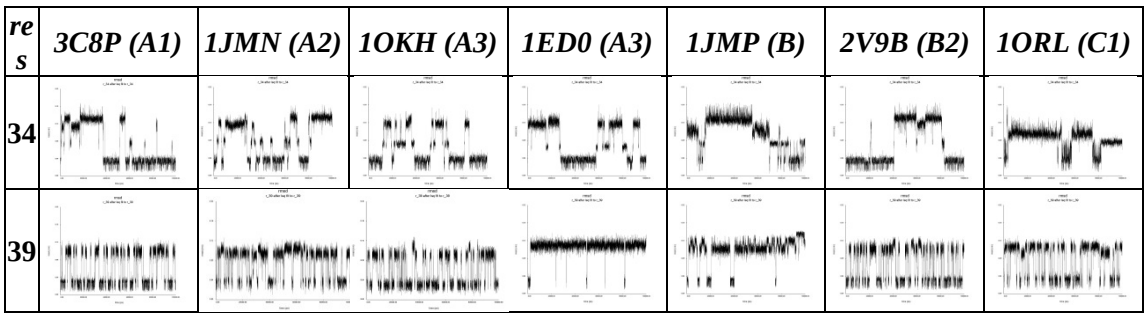



*Fig. 6. The dynamics evolution of RMSD for the unstable residues in all 7 structures*

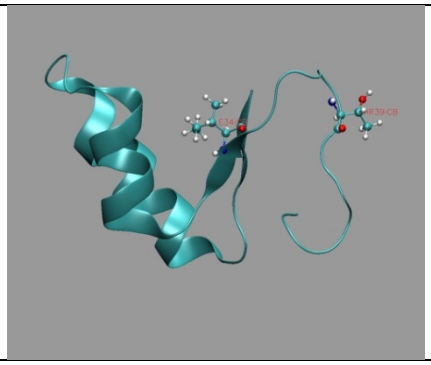


*Fig. 7. CPK representation of residue 34 and 39*

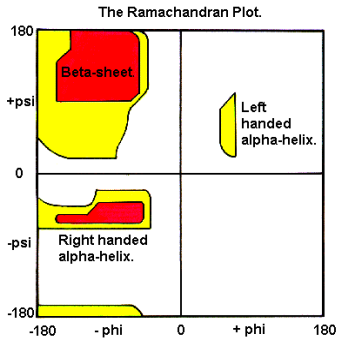



*Fig. 8. Distribution of dihedral angles with respect to the secondary structure elements*

In Figure 9 are compared the variations in distribution of dihedral angle values reported to secondary structure elements as they are described by Ramachandram plot (Figure 8.). The residues that are not found in Figure 9 do not show variations in dynamics nor any differences between the structures. It can be observed that the most important changes were in beta-sheet type structures: residues 2, 3, 4, from the structure 1JMN and 1JMP, residues 40-44 from the structure 1JMN, residues 39, 40, 43 and 45 from the structure 1JMP. Residues 34, 36, 37 and 38 show instability in all structures, in some cases being clearly defined two areas of distribution - residue 36 from the structures 1OKH, 1EDO and the residue 37 in all structures.

| *res* | *3C8P (A1)* | *1JMN (A2)* | *1OKH (A3)* | *1ED0 (A3)* | *1JMP (B)* | *2V9B (B2)* | *1ORL (C1)* |
|---|---|---|---|---|---|---|---|
| **2** | | | | | | | |
| **3** | | | | | | | |
| **4** | | | | | | | |
| **34** | | | | | | | |
| **36** | | | | | | | |
| **37** | | | | | | | |
| **38** | | | | | | | |
| **39** | | | | | | | |

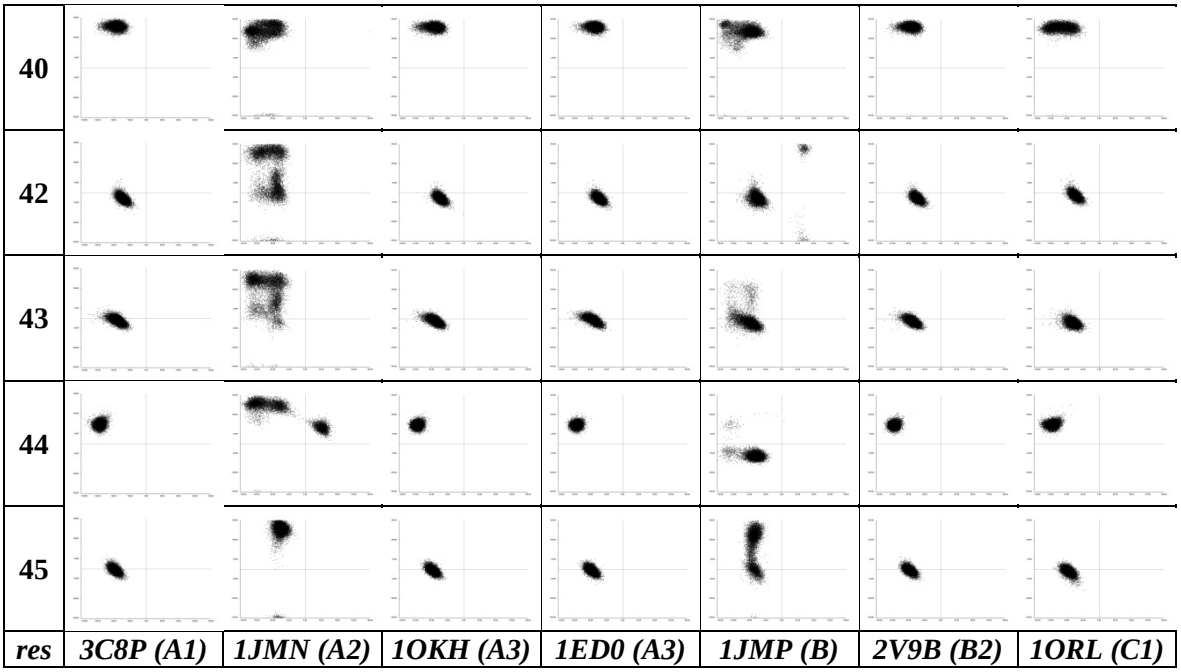



*Fig. 9. Ramachandran plot for viscotoxins*

In Fig. 10 are represented the dynamic structures of the seven viscotoxins on every 10 ns aligned by backbone. It is noted that it started from a compact structure and the area from the beginning of the "scissors" remained compact along the entire dynamics, for all structures. The structure is changed in the “scissors" peak and expanded its volume. The structures 1JMP and 1JMP showed the most visible changes and highest volume.

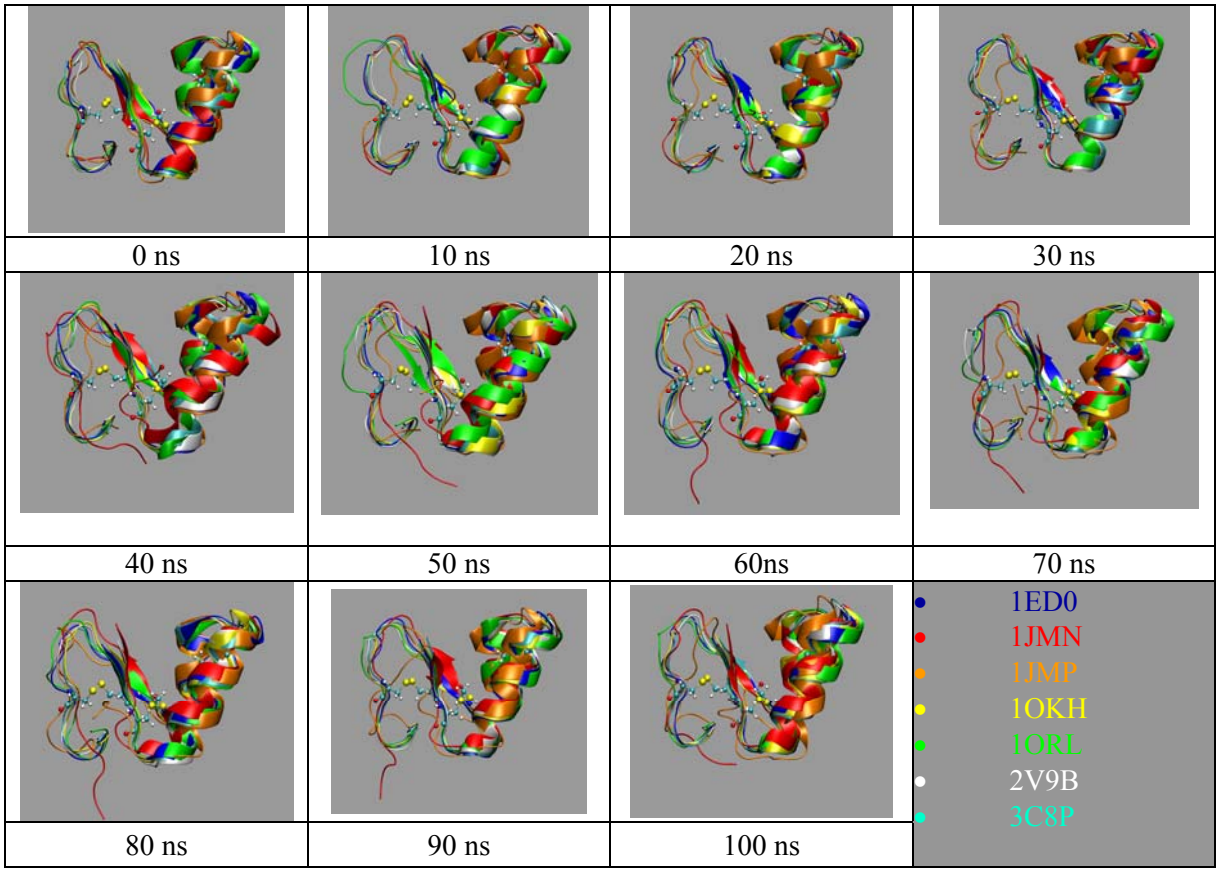



*Fig. 10. Frames from molecular dynamics simulation trajectories of viscotoxins*

## 4. Conclusions

From the analysis of the molecular dynamics simulation of the seven structures of vicotoxins it was concluded that the sequences are preserving the key elements of secondary structure, but the structure volume increases. The largest variations of dynamic parameters appeared in the structures 1JMN and 1JMP. Regarding the differences linked to the amino acids recorded in the primary structure, they do not significantly influence the molecular dynamics.

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