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MOLECULAR DYNAMICS SIMULATION OF THE HUMAN ALPHA-DEFENSIN 5

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The defensins are a family of antimicrobial peptides and contain six cysteine residues that form three characteristic intramolecular disulfide bonds. Human alpha-defensin (HD) 5 is a molecule consisting 32 amino acid residues in three beta-sheet structure and disulfide bridges between Cys 3 – Cys 31, Cys 5 – Cys 20, Cys 10-Cys 30. The HD 5 is mainly expressed in Paneth cells of the small intestine. In this paper was analysed the dynamic stability of the native and mutant structure of the HD 5 by molecular dynamics simulation method. In the mutant structure, arginine amino acid residue 13 is replaced by histidine amino acid residue. From comparative analysis of the native and mutant structure of the HD 5 results that ARG13HIS mutation leads to a structural change at the level of the residue 13 the secondary structure beta-sheet is changes in alpha-helix structure. At global level, in the native structure compaction occur in dynamics, while in the mutant structure there is a volume expansion.

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*Keywords***:** antimicrobial peptide, human alpha-defensin, molecular dynamics, simulation

1. Introduction

The defensins are a family of the antimicrobial peptides and are found in plants, insects, birds, mammals and humans. The antimicrobial peptides represent an ancient weapon of the innate immune system whose role is to defense against bacterial, fungal and viral infection. The antimicrobial peptides act by disrupting the structure or function of the microbial cell membranes [1-3]. Also, the antimicrobial peptides have functions in inflammation, wound repair and regulation of the adaptive immune system [4].

The defensins are small, cationic non-glycosylated peptides with a characteristic β-sheet-rich structure. They contain six cysteine residues that form three intramolecular disulfide bridges. Human defensins are classified into alpha- and beta- subclass, depending on the connectivity of the six cysteine residues. Four alpha-defensins, called Human Neutrophil Peptides (HNP) 1 through 4, are produced by leukocytes, Paneth cells of the small intestine and epithelial cells [4-5]. Two other alpha-defensins, designated Human Defensin (HD) 5 and 6, are mainly expressed in Paneth cells of the small intestine [6-8]. HD may regulate and maintain microbial balance in the intestinal lumen. The properties of the HD 5 are particularly poorly understood [9].

Due to remarkable progress in the computing resources and improvements in accuracy of force fields, in the last years, molecular dynamics simulations with large size and time scales have been used to study the structure of more and more biomolecules. Thus, application of molecular dynamics simulation analysis for refinement of experimental data and even replacing laboratory experiments with *in silico* experiments has become widely used, saving time and financial resources [10-11].

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Investigation of the antimicrobial peptides properties is an area of outstanding interest for the discovery of their mechanisms of action and to develop strategies for new biotechnological applications in medicine and plant protection. The thermodynamic and the interaction parameters, the structure and the stability of antimicrobial peptides have not been studied in detail. The molecular dynamics simulation method is an important tool to detail these studies [12-15].

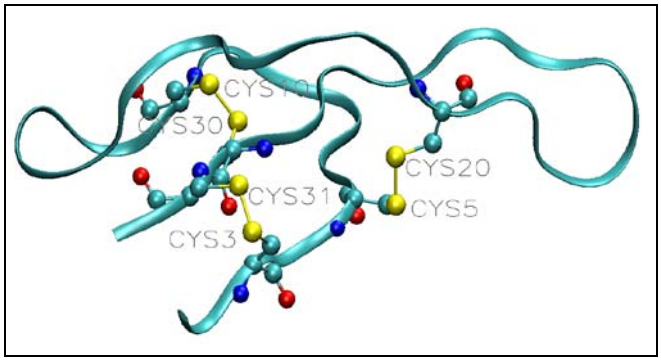


Fig. 1. The disulfide bridges in human alpha-defensin 5

Analysis of the conformational changes of the antimicrobial peptides aimed to determine the effect of various natural and synthetic point mutations on the structural stability and change in the area of accessibility of the peptide. Also, for the use of design "in silico" of peptide sequences with predetermined biological function, is analyzed the dynamics of different types of residues in area of the disulfide bridges.

The main objective of the present work was to analyze stability of human alpha-defensin 5 (HD 5) in experiments of molecular dynamics simulation. HD 5 is a molecule consisting 32 amino acid residues in three beta-sheet structure and disulfide bridges between Cys 3 – Cys 31, Cys 5 – Cys 20, Cys 10-Cys 30 (figure 1). The sequence of amino acid residues is presented in figure 2 [16].

Fig.2. Sequence details of human alpha-defensin 5 (1ZMP from PDB [16])

2. Simulation experiments

Molecular dynamics simulation experiments were carried out for the native structure, 1ZMP of the human alpha-defensin 5 and for its mutant form 3I5W [17, 18]. In the mutant structure the residue of arginine amino acid 13 is replaced with the residue of histidine amino acid (figure 3).

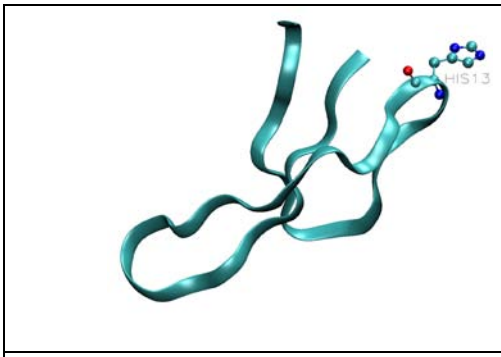


Figure 3. The punctual mutation ARG13HIS on human alpha-defensin 5

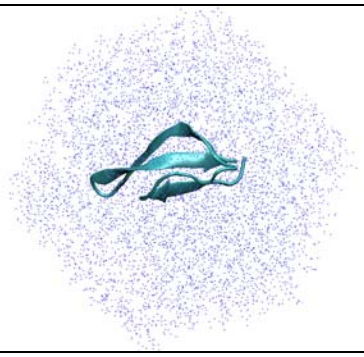


Figure 4. The final model of solvated peptide system for the native human alpha-defensin 5 (6404 atoms)

The simulations took place at a temperature of 500 K and the resulting trajectory length for each structure was 50n. The molecular dynamics simulation used Gromacs versions between 4.0.1 and 4.5.3 [19], force field OPLS (Optimized Potential for Liquid Simulations) [19]. The water model used was TIP3P [19] with the current changes used in Gromacs. The start coordinates of simulation were the structures from Protein Data Bank, PDB [16]. The hydrogen atoms were added to complete the structure, and for neutralizing electric charge chlorine ions were added. For consistency, all such built structures were solvated in water boxes with the same size and shape of the dodecahedron.

Number of atoms of the antimicrobial peptides taken into study (1ZMP and 3I5W) and total number of atoms of the simulated systems are presented in Table 1.

Table 1. Total number of atoms of simulated models

structure	native	mutant
atoms number of peptide	487	480
total number of atoms	6404	5601

The simulations were performed using periodic boundary conditions and the number of molecules N, pressure P and temperature T were kept constant. The peptide was fixed initially and energy of the system was minimized to 700 ps using steepest descent algorithm (SD).

The minimized system was heated from 0 to 500 K by velocity rescaling for 200ps and balanced 100ps in the NVT and 100ps and NPT. During the simulation the length of bonds involving hydrogen was constrained using LINCS algorithm [19].

The NPT simulation of trajectories with a length of 50ns was performed on an IBM X3950 server with the following configuration: processor 32 x Xeon 3,2 GHz, 32GB RAM, HDD SAS 15000rpm 3x72Gb supported by a IBM 10 KVA UPS.

The support for parallel simulation was LAM/MP Program, (large-scale atomic molecular massively parallel) [20]. The simulation analysis was done with Gromacs and VMD programme [21].

3. Results and discussion

The native structure, 1ZMP, of human alpha-defensin 5 was analyzed compared to the mutant structure, 3I5W, in which the arginine residue 13 was replaced by a histidine residue.

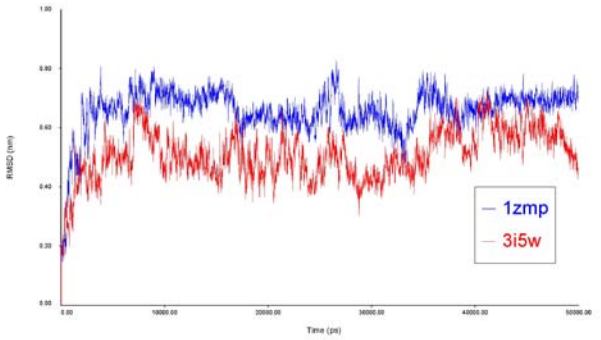


Figure 5. The evolution in dynamics of RMSD for all protein of the native and the mutant structure of human alpha-defensin 5

Analysis of evolution in dynamics of RMSD for all protein of human alpha-defensin 5 showed that both the native and the mutant structure of HD 5 have important variation, but the native structure, 1ZMP is stabilized in the last 10 ns of simulation (figure 5).

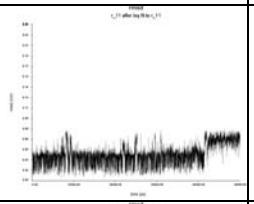
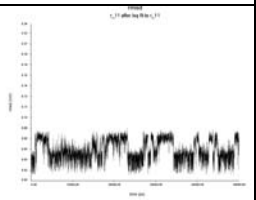
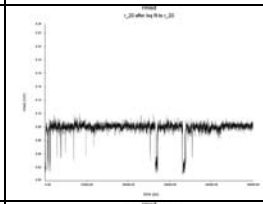
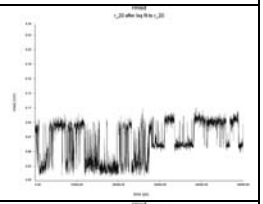
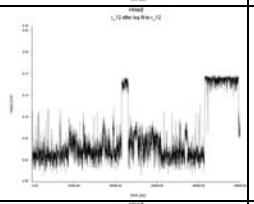
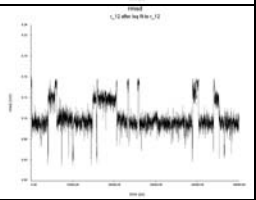
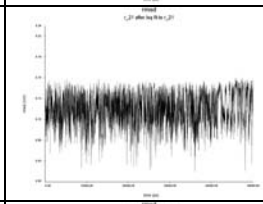
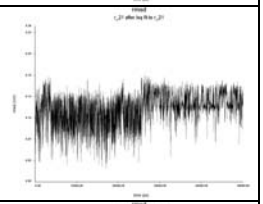
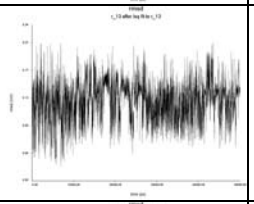
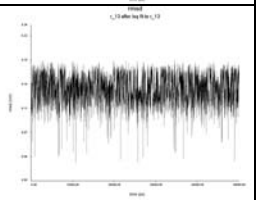
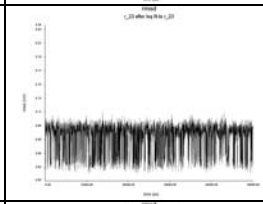
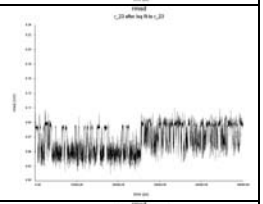
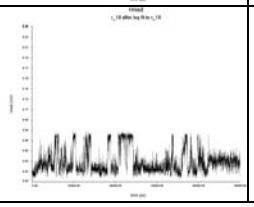
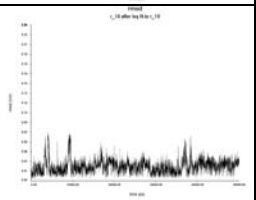
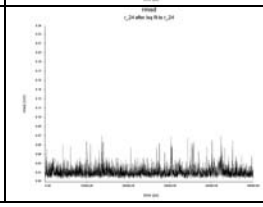
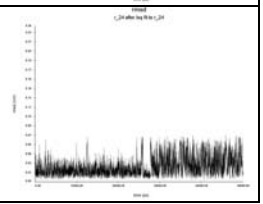
Res no	Native structure	Mutant structure	Res no	Native structure	Mutant structure
10			**19**		
11			**20**		
12			**22**		
17			**23**		

Figure 6. The evolution in dynamics of RMSD per residue for the native and the mutant structure of human alpha-defensin 5

The comparative analysis of the evolution in dynamics of RMSD for each residue of these two structures studied shows differences for residues 10, 11, 12, 17, 19, 20, 22 and 23 (figure 6).

Thus, residue 10 has the same two states in both structures, but in the native structure has only one transition after 40ns of simulation and in the mutant structure has many transitions. The residue 11 also has two states, but they are different for each structure and, again, in the native structure we have fewer transitions. The residue 12 is unstable in both structures, but in the native structure has a greater variation. The residue 17 has one predominate state in both structures, but in the native structure shows more instability then the mutant structure. The residue 19 is very stable in the native structure while in the mutant structure has three states, one is found throughout the entire simulation (RMSD ≈ 9 Å), a second is found in the first 30 ns of simulation (RMSD ≈ 5 Å) and a third is found in the last 20 ns of simulation (RMSD ≈ 2 Å). The residue 20 is very unstable in the native structure and in the first half of simulation of the mutant structure and is something more stable in the last half. The same situation shows the residue 22, but the variation of RMSD is less than of the residue 20. The residue shows a reverse situation, namely, is stable in the native structure and in the first half of simulation of the mutant structure and is unstable in the last half of simulation.

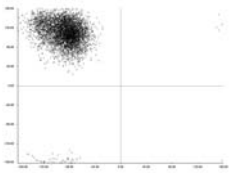
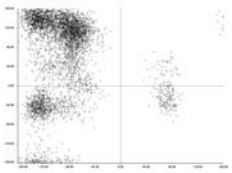
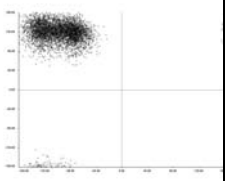
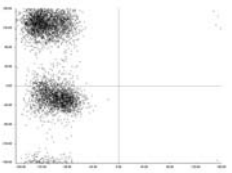
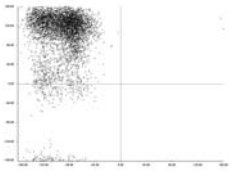
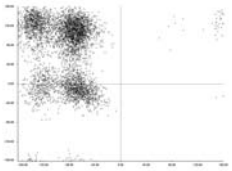
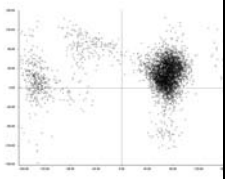
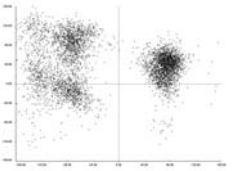
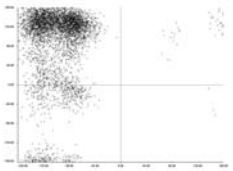
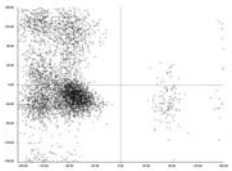
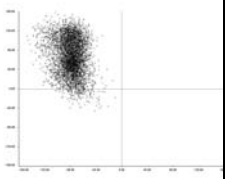
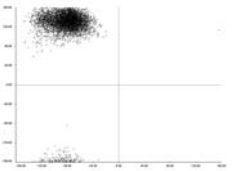
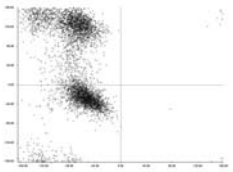
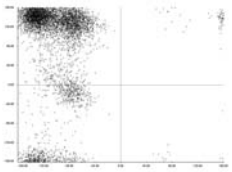
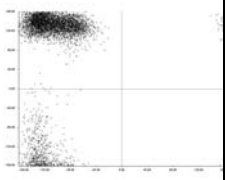
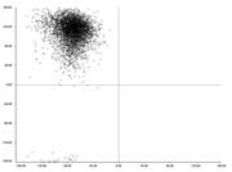
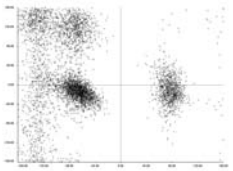
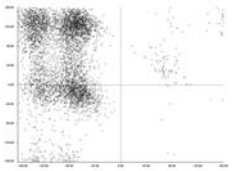
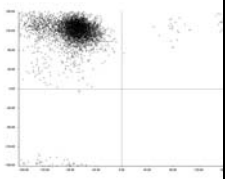
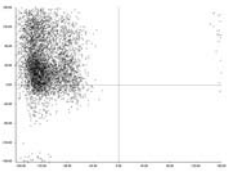
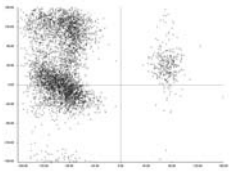
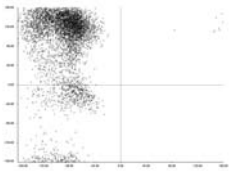
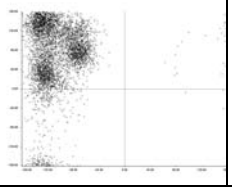
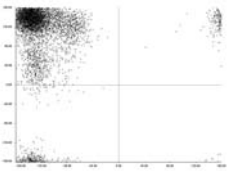
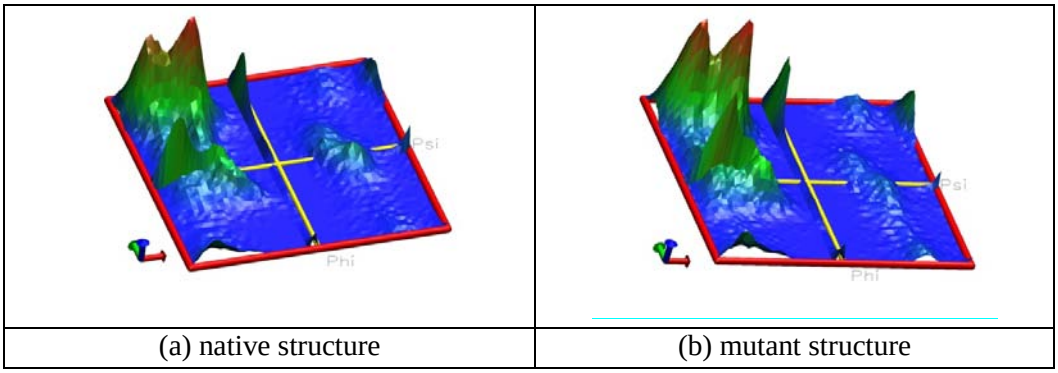
Res no	Native structure	Mutant structure	Res no	Native structure	Mutant structure
4			20		
5			23		
13			28		
14			29		
15			30		
16			31		

Figure 7. Ramachandran plot for native and mutant structure of human alpha-defensin 5

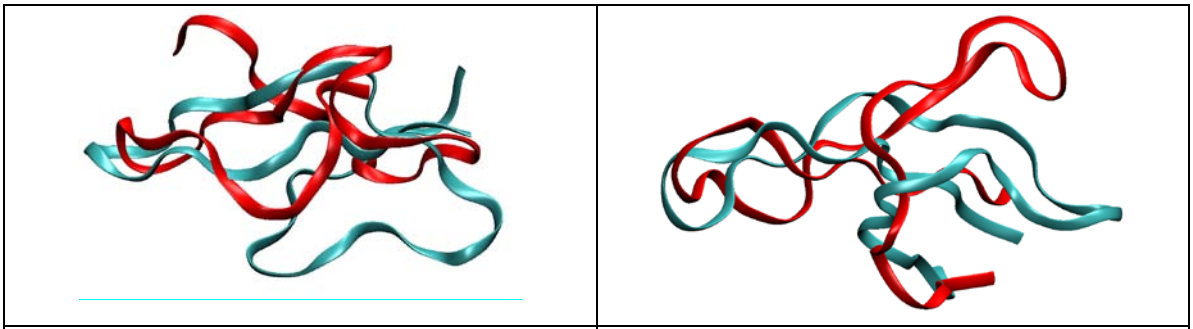
The detailed comparative analysis of the evolution in dynamics of values of phi and psi angles is presented in Ramachandran plot for the native and the mutant structure of HD 5 (figure 7). The residue 4 is concentrated in upper left quadrant in the native structure and is scattered in upper left, lower left (alpha-helix area) and little to other two quadrants. The residue 5 shows a higher density in area of intersection of the upper left and the lower left quadrants, so decreases the density in the beta-sheet area. For the native structure, the residue 13 show values in the beta-sheet area and for the mutant structure, (ARG13HIS) has an evident increase density in the alpha-helix area. The residues 15 and 16 for the mutant structure show in the beta-sheet area an increase density unlike the native structure. The residue 20 has in the mutant structure an increase density in the alpha-helix area and a decrease density of the beta-sheet area unlike the native structure. The residue 23 shows a passage from the upper right quadrant to the upper and lower left quadrants. The residues 28, 29 and 31 have a concentration in the upper left quadrant in the mutant structure unlike the native structure. The residues 30 shows a dispersion in the upper left quadrant in the mutant structure unlike the native structure.



(a) native structure (b) mutant structure

Figure 8. 3D Ramachandran plot of human alpha-defensin 5

3D Ramachandran plot of human alpha-defensin 5 shows in the native structure two peaks with different heights in the beta-sheet area. The mutant structure has two peaks with the same height with highest peak from native structure in beta-sheet area. Also, have a greater dispersion in upper and lower right quadrants and the peak to intersection of upper and lower left quadrants is higher in the mutant structure that the native structure indicating a more residues located in alpha-helix position.



(a) native structure: ■start, ■end of simulation (b) mutant structure: ■start, ■end of simulation

Figure 8. Human alpha-defensin 5 structure to the start and to the end of simulation

In figure 8 is presented the start structure, in blue, and the end of simulation structure, in red, of the native (a) and the mutant (b) structure of the human alpha-defensin 5, aligned by residue 10-20. In this figure the conformational changes are obvious.

4. Conclusion

The native peptide HD5 stabilized in the last 10 ns of the molecular dynamics simulation. From comparative analysis of the native and mutant structure of the human alpha defensin 5 resulted that ARG13HIS mutation lead to a structural change at the level of the residue 13. More exactly, the secondary beta-sheet structure is changed in alpha-helix structure. At global level, in the native structure compaction occur in dynamics, while in the mutant structure there is a volume expansion.

Acknowledgement

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