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IN SILICO STUDY OF MOLECULAR DYNAMICS OF HUMAN TRANSTHYRETIN VARIANTS

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In this paper we presents a research study on the molecular dynamics of human transthyretin. It is already known that the transthyretin, both native and mutant, can forms amyloid deposits. This biostructure causes dysfunction of various organs and lead to disease known as amyloidosis. This research contributes to a better understanding of the mechanism by which amyloid deposit is formed. In this study are conducted, *in silico*, molecular dynamics experiments for 2 native structures and 4 mutant structures of human transthyretin. In mutant structures residue 84 of isoleucine are replaced with residue of serine and alanine respectively. Mutant structures were determined at pH 4.6 and pH 7.5. Variation of RMSD in the simulation experiments performed on transthyretin is between 0.7 Å and 1.1 Å. Comparative analysis performed on residue 84 of the 6 studied structures show that both mutation I84S and I84A mutation induces specific destabilization of protein structure.

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1. Introduction

The amyloid is a very special biostructure which forms in the internal organs by extracellular depositing of protein fibrils. Amyloid causes dysfunction of various organs and the disease it causes is known as amyloidosis [1-3]. It was noted that amyloidosis is a secondary disease that accompanies other diseases deemed essential for the amyloidosis. It was not discovered so far nor the way the amyloidosis is determined by another disease either the mechanism that by which amyloid deposit is formed. Were described over 20 fibril proteins involved in human amyloidoses [4]. One of these proteins is transthyretin [5]. Both the native sequences, and mutant sequences of transthyretin can form amyloid structures. Transthyretin is involved in the amyloidoses that affect the heart, nervous system, gastrointestinal tract, vitreous, lungs and the carpal ligament [6]. The transthyretin transport the thyroxine and the retinol and is mainly synthesized in the liver and choroid plexus of the brain and, to a lesser extent in the retina of the eye, and other tissues. Transthyretin is a tetramer composed of 4 subunits with 127 amino acids each. The transthyretin monomer contains 8 antiparallel domains of β-sheet (figure 1), [7]. In the process of formation of amyloid deposits occur changes of the secondary structure of transthyretin.

In this paper we apply tools developed for statistical physics to study protein conformational changes by molecular dynamics simulation experiments. Thus it is possible to analyze systems that are impossible to study in laboratory experiments *in vitro* or *in vivo*, such as

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protein systems. Using protein system simulation makes it possible to obtain information for accurate and efficient design of laboratory experiments, thus avoiding the development of expensive experiments that would lead to irrelevant conclusions [8]. Also, the results obtained by developed of molecular dynamics simulation experiments to the transthyretin have a high potential for application in medical biotechnology.

Molecular dynamics simulation of protein systems involves solving Newton equations of motion by using integration algorithms. Resulting trajectories are analyzed using statistical mechanics for the conversion of microscopic information to macroscopic observables [9].

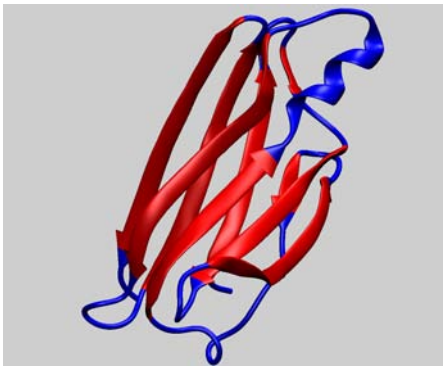


Fig. 1. Antiparallel domains of β-sheet for monomer of transthyretin.

In this paper were done molecular dynamics simulations for 6 transthyretin structures. There were used structures from protein database, PDB [10] the simulation software package GROMACS [11], the visualization program VMD [12].

The human transthyretin structure analysis sought to identify structures with high stability in dynamics, identification of parameters that are risk factors in destabilizing the structure and determination of sequence areas with high potential for the conformational change preceding the formation of amyloid fibrils.

2. Simulation experiments

In the simulation experiments were used two native structures, 1f41 [13] and 2g4g and 4 structures with specific mutants, 2noi, 2g3x, 2g3z, 2g4e, [14]. Mutant structures assume the following replacement of residual 84 of isoleucine with serine residue, alanine respectively (figure 2). Structure determination of mutants was carried out at pH 4.6 and pH = 7.5.

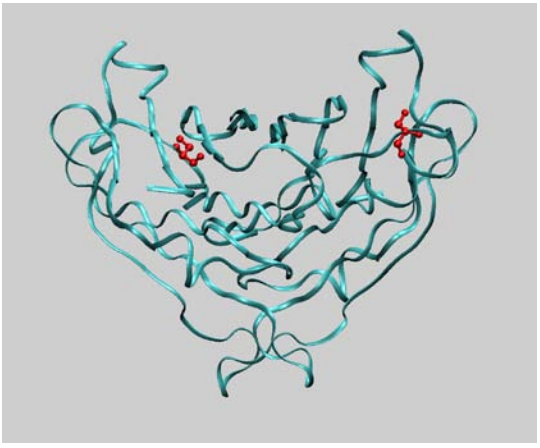


Fig. 2. Human transthyretin structure (blue, NewRibon representation) and position of mutation (red, CPK representation) - residue 84

The temperature in the simulation experiments was 300 K and the resulting trajectory length for each structure was 10 ns. Molecular dynamics simulation program used was Gromacs version 4.0, with force field OPLS (Optimized Potential for Liquid Simulations). The model of water used was TIP3P with current changes used in Gromacs. Starting coordinates of simulations were the structures from Protein Data Bank, PDB [10]. Hydrogen atoms were added, and for neutralizing the electric charge were added sodium ions. For consistency, all proteins constructed were solvated in water boxes with the same size, and shape of the dodecahedron (figure 3).

Table 1. The total number of atoms of the simulated models.

PDB ID of structure	1f41	2g4g	2noy	2g3x	2g3z
Total no. of atoms	35460	36672	35891	35853	35411

Models constructed (figure 4) had between 35411 and 36672 atoms. The total number of atoms, including those of the solvent, for the 6 models is presented in table 1.

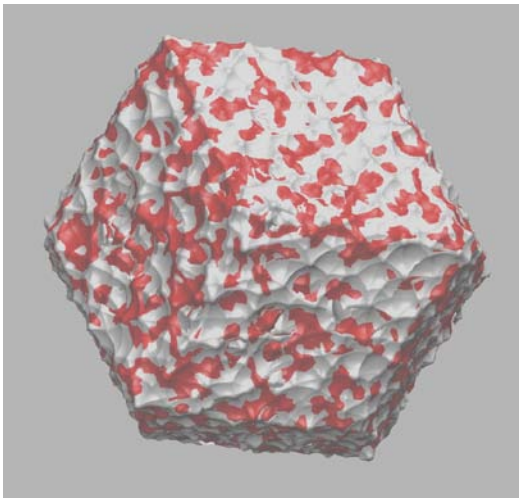


Fig. 3. Representation of the water box used in simulations

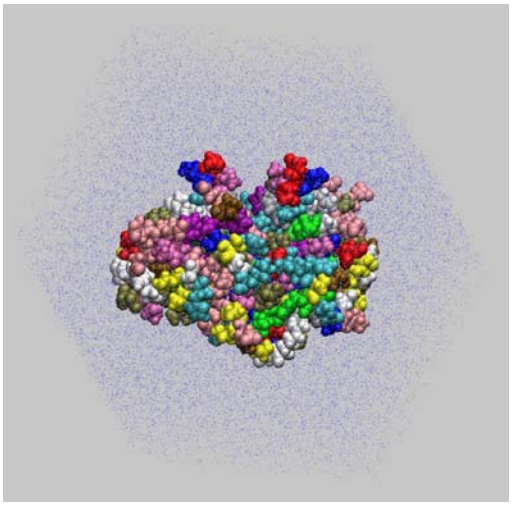


Fig. 4. The final model for protein 2g3x (35853 atoms)

Simulation experiments were performed using periodic boundary conditions and the number of molecules N, pressure P and temperature T were kept constant. The protein was fixed initially and the energy of the system was minimized 5000 steps using steepest descent algorithm (SD) and 2000 steps using conjugate-gradient algorithm (CG). Minimized system was heated from 0 to 300 K by velocity rescaling for 100ps and equilibrated 100ps in the NVT and 100ps in the NPT. During the simulation the length of bonds involving hydrogen was constrained using LINCS algorithm [15].

To achieve simulation experiments was used IBM X3950 server with the following configuration: 32 x Xeon 3, 2 GHz processor, 32GB RAM, HDD SAS 15000rpm 3x72Gb, held by a 10 KVA IBM UPS.

The parallel simulation support was program LAM/MP (large-scale atomic molecular massively parallel) [16].

3. Results and discussion

Primary analysis of molecular dynamics simulations presented in this article showed that for all experiments temperature, energy, pressure, and density in the box and density in dynamics were constant, which showed that the experiments were carried out correctly.

The next stage involved the development of comparative studies on the behavior of native and mutant structures. Were analyzed all 127 aminoacid residues for each of the six structures of transthyretin. To check the occurrence of structural transitions was studied the evolution of phi and psi angles and RMSD's for the whole protein sequence and residues.

Analyzing the dynamics of residue 84 of native structures 1f41, 2g4g and mutant structures 2g3x - I84S (pH 4.6), 2noy - I84S (pH 7.5), 2g3z - I84A (pH 4.6), 2g4e - I84A (pH 7.5), we see that both the mutation I84S and I84A mutation induces specific destabilization of the structure (figure 5). From the standpoint of human transthyretin secondary structure, residue 84 participates in the formation of a "pin-of-hair". Taking into account this fact and considering the evolution of RMSD for this residue (figure 6), we note that I84S mutation leads to an important local destabilization, which may be the cause of the initiation of the formation of amyloid fibrils.

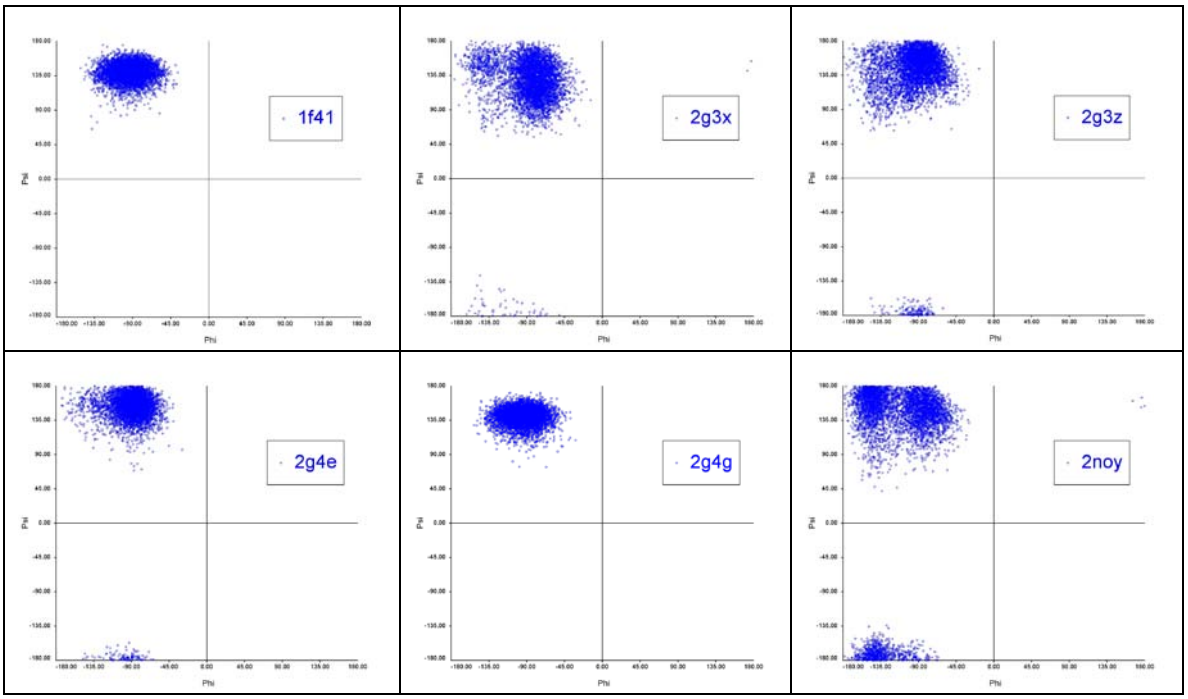


Fig. 5. Ramachandran plot for residue 84

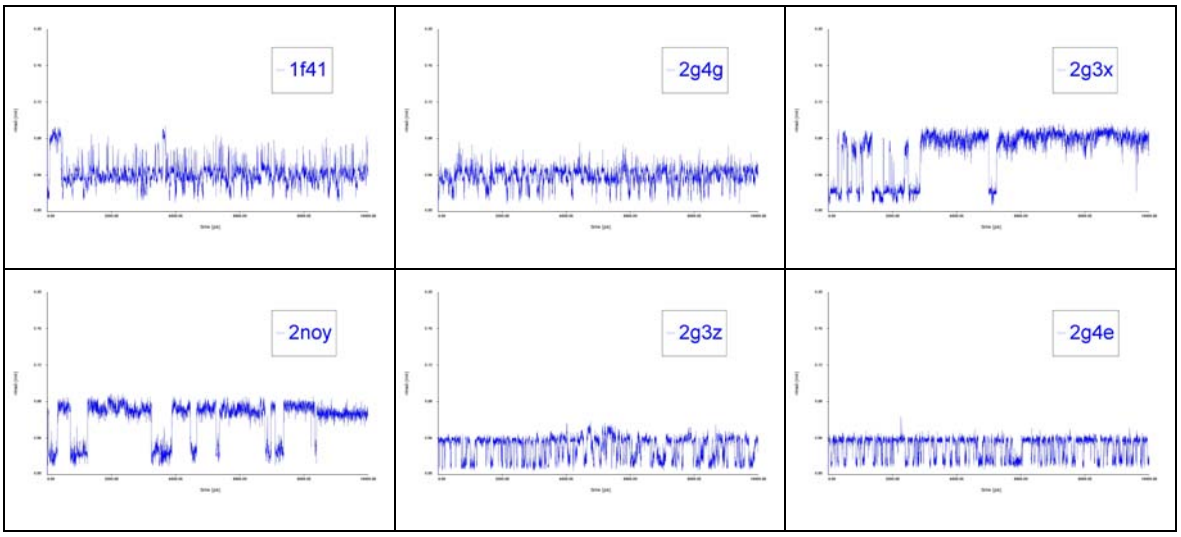


Fig. 6. Evolution of RMSD for residue 84 in production dynamics

The structure of human transthyretin 2g3x are subject to the same punctual mutation I84S, except that the structure determination was carried out at different pH (pH = 4.6 for 2g3x structure and pH = 7.5 for 2noy structure).

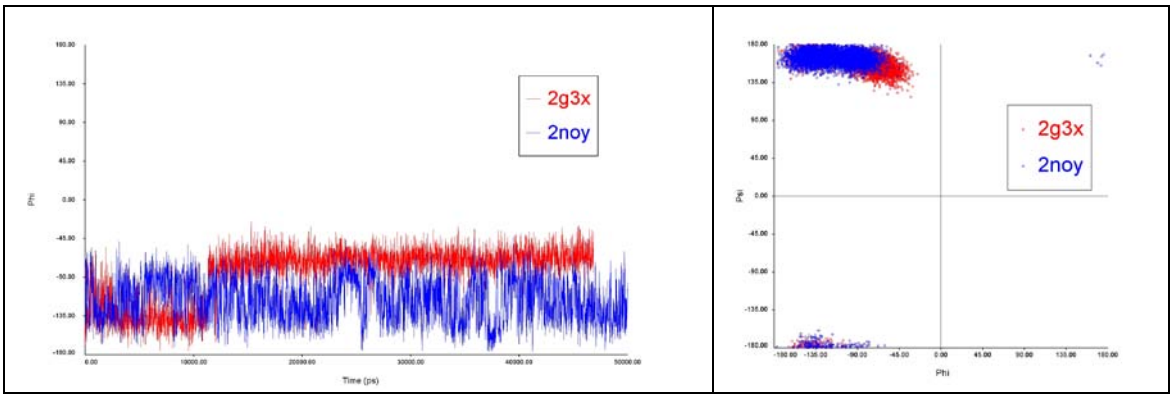


Fig. 1. Evolution of phi and psi angle values for residue 23, the chain A of structures 2noy and 2g3x

Analyzing the evolution of values of phi and psi angles for residue 23 of chain A in the two structures is observed that the structure 2noy is unstable, while 2g3x structure has two distinct states of stability, relatively close, in the last 8 ns of simulation while maintaining the state of stability (figure 7).

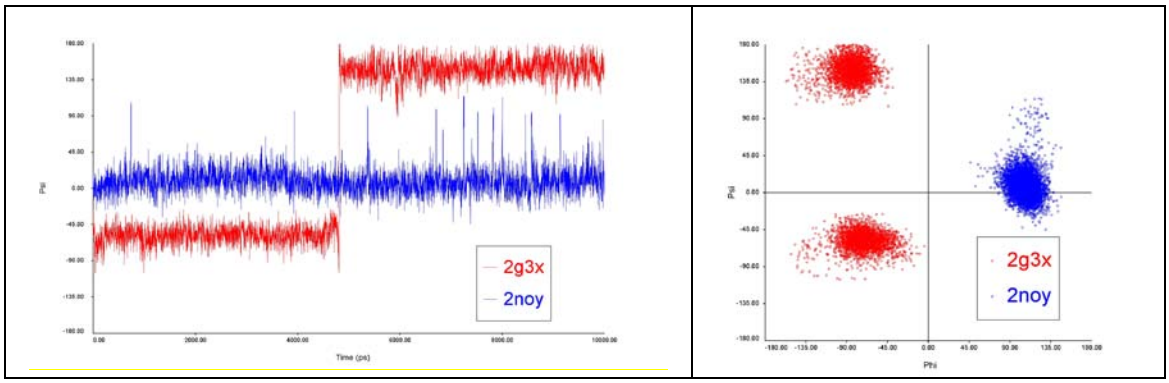


Fig. 2. Evolution of dihedral angle values for residue 57, chain B of structures 2noy and 2g3x

Residue 57 of chain B is punctual stable in 2noy and has two states of stability in 2g3x. Between these two states having a single state transition at about half the duration of the simulation run in the experiments (figure 8). In the Ramachandran diagram for residue 57 of chain B is observed the quadrant change, which indicates that a change in the secondary structure of the protein takes place. The change is in the direction of the transition from area of beta-sheet structure to area of alpha-helix structure.

Residue 75 of chain B has two stable states relatively close in structure 2noy and three states in the structure 2g3x, two of them being in the same quadrant and a third in another quadrant. Ramachandran graphic shows that in 2noz there is no conformational change, while in 2g3x there is a change of secondary structure. From the time evolution of phi and psi angle values and from the Ramachandran graphic is noted that for 2g3x the structure change from the beta-sheet to alpha-helix (figure 9).

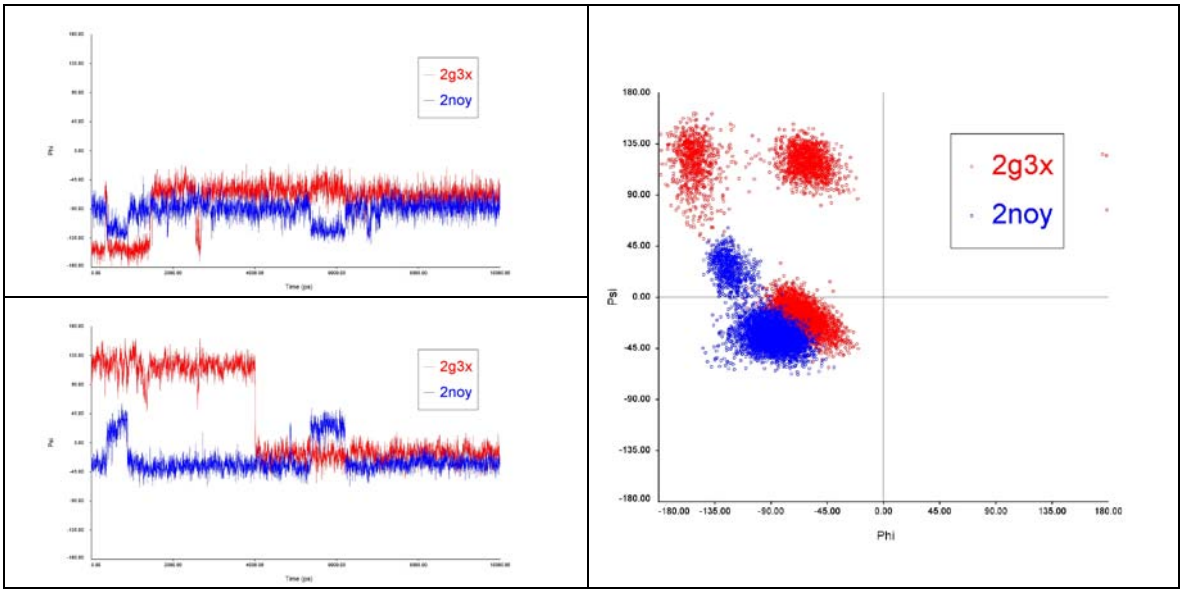


Fig. 3. Evolution of dihedral angle values for residue 75, chain B of structures 2noy and 2g3x

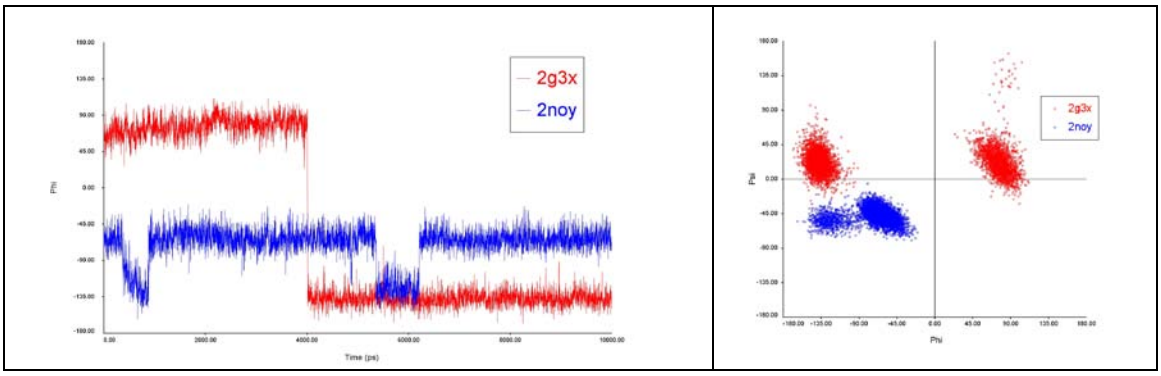


Fig. 4. Evolution of phi and psi angle values for residue 76, chain B of structures 2noy and 2g3x

The dynamics of next residue, 76, from the structure 2g3x confirms the local compacting of secondary structure by transition to beta-sheet structure maintained in the second part of the simulation (figure 10), while the structure 2noy has the same weak punctual destabilization.

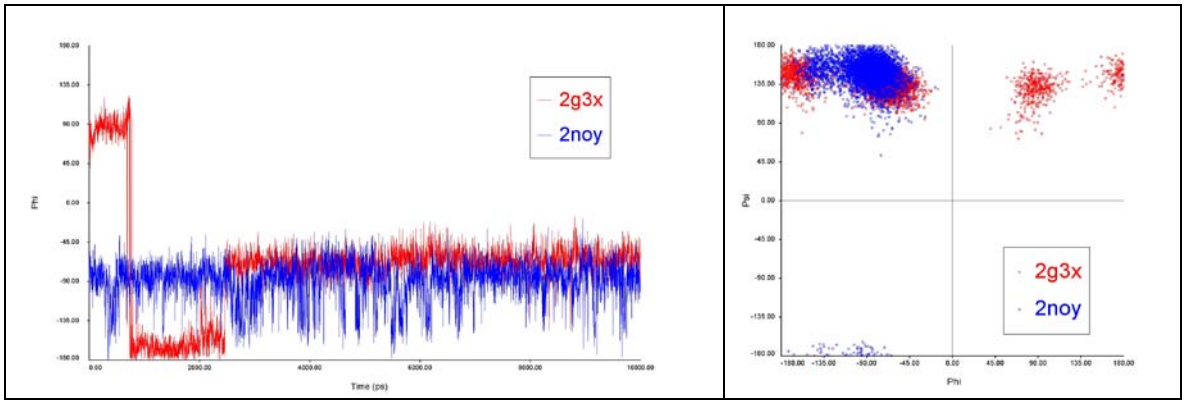


Fig. 5 Evolution of phi and psi angle values for residue 84, chain B of structures 2noy and 2g3x

In the evolution of residue 84 of chain B of the structure 2g3x can be identified two stable states in the first 2.5 ns of simulation, and a third in the next 7.5 ns simulation. This last state is in

the area of beta-sheet structure. As the Ramachandran plot shows, the structure 2noy, the dihedrals angles for residue 84 always maintain the values in the area of beta-sheet structure (figure 11).

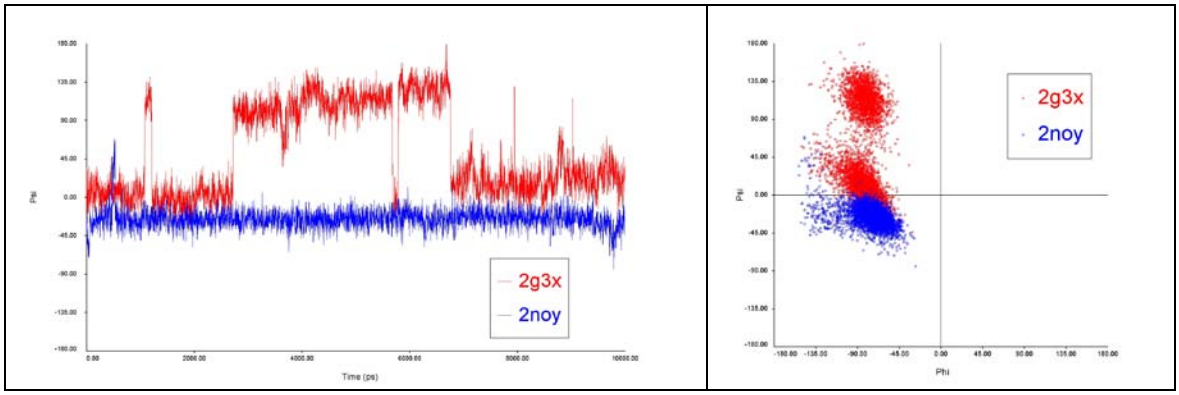


Fig. 6. Evolution of phi and psi angle values for residue 99, chain B of structures 2noy and 2g3x

Residue 99 of chain B of structure 2noy is stable in dynamic, and in the structure 2g3x presents instability, oscillating between two states, one of which is part of area of the beta-sheet structure (figure 12).

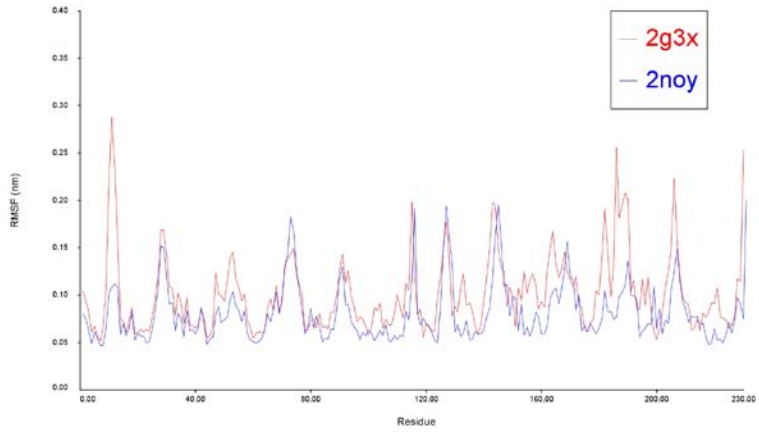
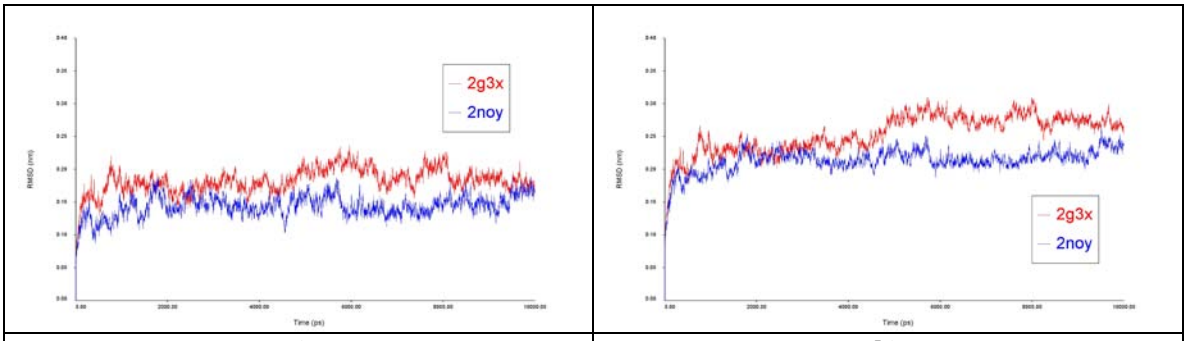


Fig. 7. Evolution of RMS fluctuation for each residue in structures 2noy and 2g3x

From the comparison of RMS's fluctuation for each residue of structures 2noy and 2g3x results that we have a significantly greater fluctuation for residues of the structure 2g3x than for structure 2noy. The largest fluctuations are recorded in the structure 2g3x, for the residues 20 in chain A and 79 and 123 in chain B and have more than 0.25 nm. In the structure 2noy the fluctuations do not exceed 0.20 nm, the higher values having the residue 82 in chain A and residues 20 and 38 in chain B (figure 13).

RMSD's evolution, both for main chain and side chain of human transthyretin shows that the mutant structure 2g3x presents conformation changes greater than mutant structure 2noy. This leads to the idea that acid pH destabilizes the protein structure and induce conformational changes (figure 14).



a) **b)**

Fig. 8. Evolution in dynamics of RMS deviation for structures 2noy and 2g3x; a) main chain, b) side chain

In conclusion, molecular dynamics simulation experiments shows that the mutant at its normal pH, 2noy is more stable than at acidic pH, 2g3x, and mutant at acid pH, 2g3x, although suffering from structural transitions, have high levels of stability.

Table 2. Maximum values of RMSD for all protein.

no	*PDB ID*	*Mutation*	*RMSD max (Å)*
1	**1f41**	**-**	**0,9**
2	**2g4g**	**-**	**1,0**
3	**2noy**	**I84S**	**0,7**
4	**2g3x**	**I84S**	**1,0**
5	**2g3z**	**I84A**	**1,1**
6	**2g4e**	**I84A**	**0,9**

Most RMSD's variation in simulation experiments performed for the 6 structures of transthyretin was 1.1 Å for the mutant structure 2g3z and lowest variation was recorded for mutant structure 2noy and had a value of 0.7 Å (table 2).

4. Conclusions

Variation of RMSD's in the simulation experiments performed for transthyretin is between 0.7 Å and 1.1 Å. The mutant structure 2noy has the lowest variation of RMSD's, namely 0.7 Å. The highest value, 1.1 Å, is recorded for the mutant structure 2g3z. From these data we conclude that the native structure 2noy is more stable than other structures studied, and the mutant structure 2g3z is the most unstable.

A comparative analysis performed on residue 84 of native structures 1f41, 2g4g and mutant structures 2g3x - I84S, 2noy - I84S, 2g3z - I84A, 2g4e - I84A, show that both mutation I84S and mutation I84A induce specific destabilization of the structure. In addition, analysis of RMSD for residue 84, given that this residue is part of a party “pin-of-hair”, we conclude that mutation I84S leads to an important local destabilization. This may be the source of the conformational changes in the formation of amyloid fibrils.

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