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MOLECULAR DYNAMICS SIMULATION OF DNA BINDING DOMAIN OF hTRF2

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ABSTRACT

MD simulations have advanced to a point where the atomic level information of biological macromolecule (protein or DNA-protein or protein-protein) can easily be advantageous to predict the functionality. Human Telomere Binding Protein (hTRF2) which interacts with Rap1 and Mre11 is responsible for the human aging and also helps in the length regulation of telomere. In this study we simulated the protein on GROMACS forcefeild and try to locate the structural changes in it. The N terminal which is mainly interacting with the minor groove was found to be more fluctuating and with the time scale there is a change in the secondary structure. This study can assist to get an idea about the motional properties of hTRF2.

KEYWORDS: GROMACS, Molecular dynamics, DNA binding domain, helix turn helix motif.

INTRODUCTION

Molecular dynamics (MD) [1] is a way to visualize and analyze the structural changes of a protein over a defined time scale. Nowadays Molecular dynamics became an essential tool to calculate the time dependent action of a molecular system. The results of the trajectory files provide the detail information on the fluctuation and conformational changes of the biological system. An average static structure of macromolecule is not enough to predict the functionality as flexibility is an important parameter to its action. As of the reason the independent subunit of protein, domains are simulated in solvent medium to make a connection between three dimensional structure and conformational dynamics. Using Molecular dynamics simulation the interaction and action of DNA binding domains [2] with DNA double stranded helix or with other proteins are analyzed. In this paper we use the GROMACS software [3] for MD simulation of DNA binding domain of human Telomere binding protein (hTRF2) [4].

In human cells, TRF2 are primarily found at chromosome ends where they contribute to the protection and maintenance of telomeric DNA [2]. Human TRF2 (hTRF2) is mainly a DNA

binding protein having Myb-like helix-turn-helix domain in their carboxy terminus and a central conserved domain (TRFH) that includes sequence responsible for the formation of homodimers. The N terminus is basic in hTRF2 [5]. The solution structure of DNA binding domain of hTRF2 consists of 64 amino acid. A well known Helix Turn Helix motif is also cited in this domain (Fig 1). In this article we first predict the secondary structure of hTRF2 and then want to illustrate the changes of secondary structures of hTRF2 during the simulation in solvent condition.

MATERIALS AND METHODS

Retrieval of target structure

The 3D structure file of hTRF2 (PDB: 1vf9) was retrieved from Protein Data Bank (http://www.rcsb.org/pdb/home/home.do) [6]. The length of DNA binding domain part is 64 aminoacid.

Prediction of secondary structure

Secondary structural features were predicted with Self Optimized Prediction Method from Alignment (SOPMA) (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html) [7].

MD simulation

The Molecular Dynamic simulation software GROMACS package (http://www.gromacs.org/) (GROMOS 96 force field) [8] was used to carry out the energy minimization and molecular dynamics simulation. The simulation has four stages:

1. The simulation was run from the NMR structures of hTRF2 (PDB:1vf9) in a cubic box with a 3 A˚ edge length and using 68055 number of solvent molecules the system was solvated (spc216.gro file).
2. The initial structure of hTRF2 was energy minimized to 1000 steps using steepest descents algorithm and then subjected to conjugant gradient algorithm with same number of steps to relax the structure.
3. The position restraint algorithm is used to restrain atoms at a fixed reference position. Here the position restrained simulation run was carried out for 500ps.
4. The MD simulation was run for 500000 steps (1000 ps) at a constant temperature of 300 K and a constant pressure of 1 atm. The temperature and pressure were regulated by weak coupling to an external bath. Electrostatics was dealt with using a 10 A˚ vandarwaal and columbic cut-off. The system was allowed to evolve according to Newton's equations of motion, with the equations being integrated every 2 fs.

RESULT

The DNA binding domain of hTRF2 consists of three helixes and connected with turn and random coils which identify the Helix Turn Helix motif in it. The 64 length of amino acid chain have 46% of Alpha helix and 6.25% of Beta turn respectively, predicted by SOPMA (Fig2). The N-Terminal of the domain is having the random coil which helps in interaction with DNA minor groove [2]. The simulation was carried for total 1000ps and after the run was completed, the trajectory files (*.trr and *.tpr) were generated [9]. There was a significant structural change in the overall conformation of hTRF2, before and after simulation (Fig 3). To locate the exact area of changes we took the snapshots of hTRF2 in different time point within the 1000ps time scale. The N terminal area which is having coiled region seems to be changed to Beta Strand and the other three helixes also changed with respect to their starting secondary structure (Fig 4). To verify the structural changes the fluctuation of N terminal was also predicted. The RMSD graph of N terminal in solvent condition shows the increase of RMSD in the staring time (Fig 5). Among all the three helixes the second helix and third helix shows more amount fluctuation (RMSD 0.1nm-0.2nm for helix 1 and RMSD 0.35nm-0.4nm for helix 2) which justified the secondary structural changes.

DISCUSSION

A **telomere** is a region of repetitive nucleotide sequences at the termini of a chromosome, usually is composed of arrays of guanine-rich, six- to eight-base-pair-long repeats [10]. Telomeres normally terminate with 3′ single-stranded-DNA overhang, which is essential for telomere maintenance and capping [11]. This T loop like structure [12] at the end of the mammalian chromosome is held together by seven known proteins, the most notable ones being TRF2. The different structural conformation leads to get idea on the binding mode of hTRF2 to DNA groove and also help in finding the area where the coil regions is shifting to Beta strand. If the simulation run is carried over for more time then maybe it will give more information regarding structural changes of N terminal as well as full structure. The present study helps in finding the motional changes of hTRF2 in solvent condition which can inspire others to run the simulation for longer timescale.

CONCLUSION

hTRF2 protein and its DNA binding domain is crucial in maintaining the aging problem in human. We hope this study will help the others for better understanding of the simulation of hTRF2 and its action.

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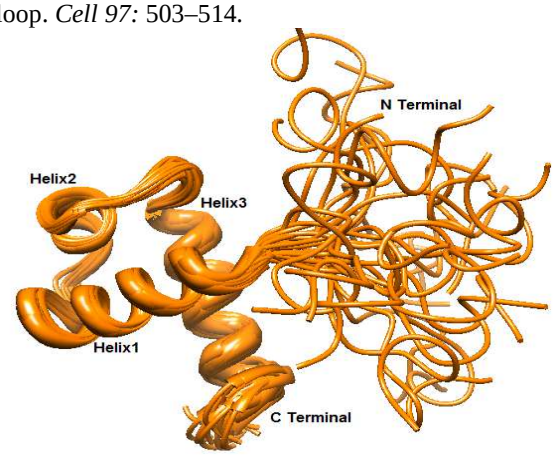

Fig. 1: Stereo view of the superposition of 20 NMR structures of the DNA-binding domain of hTRF2 using CHIMERA.

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        10        20        30        40        50        60
         |         |         |         |         |         |
MEDSTTNITKKQKWTVEESEWVKAGVQKYGEGNWAAISKNYPFVNRTAVMIKDRWRTMKRLGMN
ecccccccccccccchhhhhhhhhhhhttcccchhhhhtcccccccheehhhhhhhhhhhtcc

Sequence length :    64

SOPMA :
   Alpha helix      (Hh) :    30 is  46.88%
   3_10  helix      (Gg) :     0 is   0.00%
   Pi helix         (Ii) :     0 is   0.00%
   Beta bridge      (Bb) :     0 is   0.00%
   Extended strand  (Ee) :     3 is   4.69%
   Beta turn        (Tt) :     4 is   6.25%
   Bend region      (Ss) :     0 is   0.00%
   Random coil      (Cc) :    27 is  42.19%
   Ambigous states  (?)  :     0 is   0.00%
   Other states          :     0 is   0.00%
```

Fig. 2: Secondary structural features of hTRF2 using SOPMA. The blue color 'h' indicates the helix region.

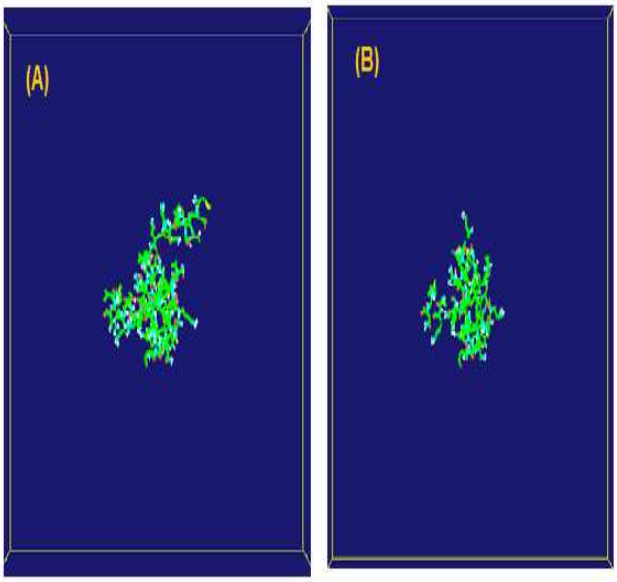

Fig. 3: Different structural conformation of hTRF2 at starting (A) and ending (B) point during molecular dynamics simulation. The green color molecule in the blue cubic box is hTRF2.

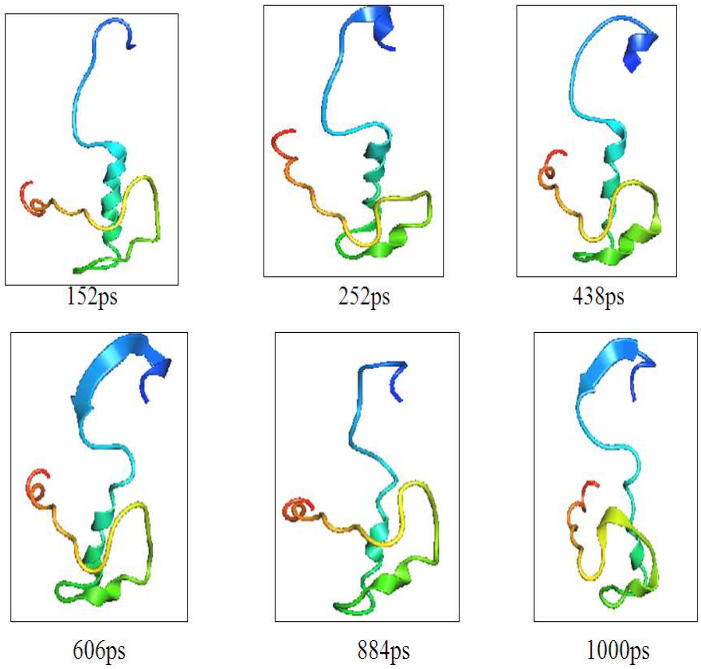

Fig. 4: Snapshots of hTRF2 at different time point (152ps, 252ps, 438ps, 606ps, 884ps and 1000ps) during MD simulation. N Terminal (random coil) of hTRF2 changes with time scale.

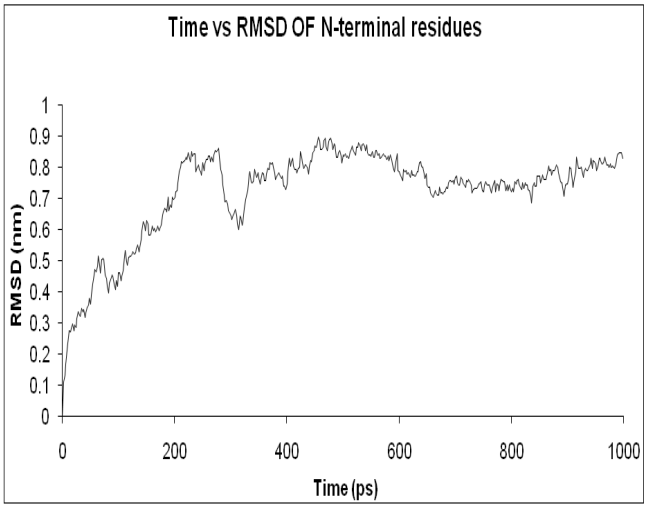

Fig. 5: Showing the Time (ps) vs. RMSD (nm) of N terminal residues of hTRF2 during MD simulation.