Biophysical Chemistry 213 (2016) 32–39

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Use of molecular dynamics simulation to explore structural facets of human prion protein with pathogenic mutations

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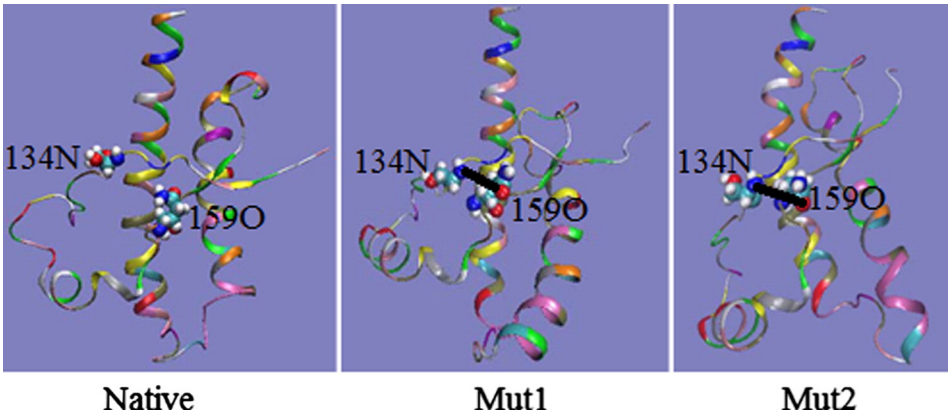
HIGHLIGHTS

- Classical MD simulations have been carried out to examine structural facets of human prion protein.
- It is observed that the mutated form is steadier than the native protein.
- Conformational changes due to relative orientation of the helices show positional movement of H1 and H3 helices.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 25 December 2015
Accepted 17 March 2016
Available online 14 April 2016

Keywords:
Prion diseases
Molecular Dynamics Simulation
Mutation
R148H
T193I

ABSTRACT

Prion diseases are caused by mutations at different positions of the prion protein. A large number of pathogenic mutations are reported in the literature. Two of such point mutations T193I and R148H located at two different helical strands (H2 and H1) of the prion protein associated with fCJD (familial Creutzfeld–Jacob disease) are studied. We have used classical molecular dynamics (MD) simulation technique to understand the conformational changes and dynamics of the protein under the effect of mutation and compared with the native prion protein. The results indicate that: both mutated forms are conformationally steadier than the native prion protein; although there are no major conformational transitions, R148H leads to decreased native β-sheet content, H1 helix becomes less fluctuating, two new turn regions appear and conversion of a 3_{10} region to coil form takes place. Mutation T193I leads to a steady H1 helix, a decreased native β-sheet content and a new 3_{10} region appears in H2 helix. Moreover, mutation R148H results in decreased conformational space with a highly compact and nonfluctuating form.

1. Introduction

A large number of familial neurodegenerative diseases are reported in human such as familial Creutzfeld–Jacob disease (fCJD), Transmissible Spongiform Encephalopathies (TSE), Fatal Familial Insomnia (FFI),

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http://dx.doi.org/10.1016/j.bpc.2016.03.004
0301-4622/

and Gerstmann–Straussler–Scheinker (GSS) which are collectively known as prion diseases [1,2]. These are caused by malfunctioning of cellular prion protein due to point mutations which may arise spontaneously or as a result of infection or inheritance [3]. The factors that induce conformational transitions of prion protein from cellular form to misfolded scrapie form have remained an important unresolved question [4,5]. Once the scrapie form is attained the prion protein induces more nearby prion proteins to acquire misfolded form and finally a dense amyloid fibrillar structure is formed which results into various neurodegenerative pathoforms [6,7]). Chemical difference between the cellular and misfolded form also remains unknown [8]. In most of the reported studies the misfolded form is found to be enriched with β-sheet content [9].

The prion protein (Fig. 1) is a glycoprotein which is attached to the cell membrane via a glycophosphatidylinositol anchor at its C-terminus [3]. It consists of a flexible N-terminal region (residues 23–124) and a globular domain in the C-terminal region (residues 125–228) with three helices, H1 (residues 144–153), H2 (residues 173–194) and H3 (residues 200–228), and two short β-strands, S1 (residues 129–133) and S2 (residues 159–163), that form an antiparallel β-sheet. A disulfide bond bridges between H2 and H3 through residues Cys179 and Cys214 respectively.

T193I [10] and R148H [11] are two important human prion pathogenic mutations (occurring in H2 and H1) causing fCJD [12]. Molecular dynamics simulation studies to explore the structural details for these two point mutations are not found. Hence we have selected these two pathogenic isoforms as our objective for explorations through simulation studies. The flexible N-terminal region is discarded and the globular domain (i.e. C-terminal core) is subjected for studies since N-terminal has negligible effect on the rest of the protein [13,14]. The pathological mechanism lying behind the conformational conversion due to point mutations and disease expression is not known. However, the underlying structural facets evolved due to mutation are expected to shed light on the effect of mutations on conversion of cellular prion to scrapie form.

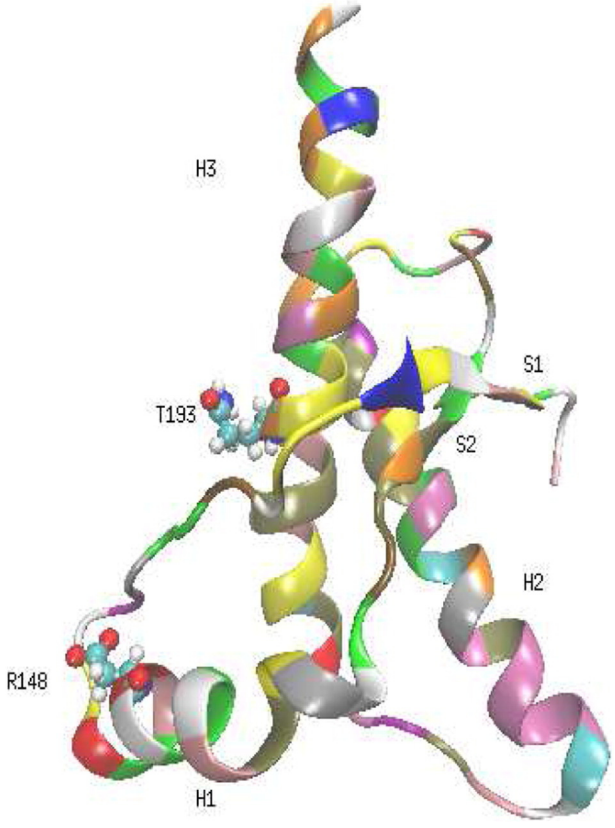

Fig. 1. NMR structure of prion protein (PDB ID: 1HJN) [21] with residue-wise color identity. Secondary structure elements are labeled as H1, H2, H3, S1 and S2.

Experiments provide only limited information regarding misfolded structure of the prion protein and details of conformational conversion mechanism. Hence classical molecular dynamics (MD) simulation is employed to uncover the detailed effect of single amino acid substitution on prion protein conformations and their role in overall conformational behavior of the protein [3,15–20]. The main objective of this study is to bring out the dynamic structural information of prion protein along with the two point mutations.

The rest of the paper is divided into three sections. Section 2 gives a description of models and simulation details, Section 3 presents a discussion of the results and the conclusions are summarized in Section 4.

2. Models and simulation method

The wild type prion protein was retrieved from Protein Data Bank (PDB ID 1HJN, Fig. 1) at pH 7.0 [21]. The two mutated forms (T193I (Mut1) and R148H (Mut2)) were generated using Chimera [22] by changing the corresponding residues of 1HJN (since there are no experimental structures till now). The native prion protein and Mut1 have 11 positively charged side chains and 14 negatively charged side chain. Hence 3 sodium ions were added to neutralize the systems containing these two proteins. In the case of the system containing Mut2, 4 sodium ions were added in order to neutralize 10 positively charged and 14 negatively charged residues. Each of the proteins was solvated separately with 8000 TIP3P water [23] in cubic box to generate three systems so that a minimal distance of 10.0 Å was maintained between the protein and boundary of the box. For the protein AMBERff12SB force field parameters were used for accurate secondary structure propensities [24].

For each of the simulation run the systems were subjected to minimization (steepest descent method followed by conjugate gradient method) and then heated from 0 to 310 K and were subsequently equilibrated at constant pressure (1 atm) and physiological temperature (310 K). The conformations were saved for every 2 ps over the whole production simulation time (in NPT ensembles). AMBER12 [25] molecular dynamics package was used to perform simulation. Temperature was controlled by Langevin dynamics [26] with a collision frequency of 1 ps^{-1}. The non-bonded cutoff distance was 9.0 Å and the long range electrostatic interactions were treated by particle mesh Ewald summation method [27]. All bonds involving hydrogens were constrained by the use of SHAKE algorithm [28]. Berendsen barostat [29] was used to maintain pressure. Periodic boundary conditions were implemented in all three directions.

Trajectories obtained from the MD simulations were analyzed using AMBER ptraj and cpptraj module as well as by VMD [30] (Visual Molecular Dynamics package). All of the systems were run for 50 ns each and for the sake of promoting an equilibrium view, analyses were done on the last 30 ns.

Simulations with three different starting configurations (referred as Run1, Run2 and Run3) were also done for better sampling. The results obtained from the three runs being similar in behavior (as can be seen in Fig. 2(c)), only Run1 is chosen for the calculations of all the parameters. Root mean square deviation (RMSD) plot is shown for all the three runs. The obtained trajectories were analyzed from different structural perspectives. RMSD and RMSFs (root mean square fluctuations) were calculated from initial structure to evaluate the structural fluctuations. To analyze conformational changes, relative orientations of the helices were speculated by calculating the distances and angles among them. In order to measure the effective size of the protein, radius of gyration was calculated. DCCM (dynamic cross correlation map) was calculated to represent correlated and anti-correlated motions. To assign secondary structures DSSP (dictionary of secondary structure of proteins) analysis was carried out. Hydrogen bond occupancy and dPCA analyses were also performed to obtain a detailed comparison of different conformational behaviors adopted by the prion protein and its mutated forms.