Research Article

Molecular dynamics simulation of human prion protein to detect difference in the structures of normal and diseased form

Pooja Mishra*

Centre of Bioinformatics, Institute of Interdisciplinary Studies, University of Allahabad, India

Abstract

The 1997 Nobel Prize was awarded to Stanley B. Prusiner for his discovery of prions and there are now wet lab experimental results available which show the evidence of such kind of molecules. Present studies reveal the role of acidic pH in the conversion of human prion protein to the pathogenic isoform is investigated by means of molecular dynamics simulations, focusing the attention to the effect of protonation of histidine residues on the conformational behavior of human PrPC globular domain. The simulations reveal a significant loss of α-helix content under mildly acidic conditions, due to destructuration of the C-terminal part of HB, there are three helices present in the protein HA, HB, HC, (thus suggesting a possible involvement of HB into the conformational transition leading to the pathogenic isoform) and a transient lengthening of the native β-sheet. Protonation of His-187 and His-155 seems to be crucial for the onset of the conformational rearrangement. This finding can be related to the existence of a pathogenic mutation, H187R, which is associated with GSS (Gerstmann Straussler Syndrome) syndrome.

Keywords: Molecular dynamics simulation, Gromacs, Prion protein, Protation.

Introduction

According to the “Protein only hypothesis” (Griffith, 1967; Prusiner, 1991), the misfolded isoform (PrPSc) of the normal cellular prion protein (PrPC) is recognized as the pathogenic agent responsible for the transmissible spongiform encephalopathies, a class of neurodegenerative diseases including scrapie in sheep; bovine spongiform encephalopathy in cattle; and Kuru, Creutzfeldt-Jakob disease (CJD) Disease, GSS syndrome, and Fatal Familial Insomnia (FFI) in humans. A key event in prion disease pathology appears to be the conformational transition of PrPC to PrPSc. PrPSc is insoluble, partly protease resistant, and its N-terminally truncated form polymerizes into amyloid fibrils. PrPC and PrPSc are chemically indistinguishable but their secondary, tertiary, and quaternary structures differ. PrPSc is more difficult to characterize because of its insolubility and tendency to aggregate. According to circular dichroism and Fourier transform infrared spectroscopic studies, PrPSc has a dramatically higher (43% vs. 3%) β-sheet and lower (30% vs. 42%) α-helix content with respect to PrPC (Pan et al., 1993; Safar et al., 1993). However the precise nature of the key event triggering the conformational transition and the detailed structure of PrPSc are still unknown. Some experimental studies (Gorodinsky and Harris, 1995; Taraboulos et al., 1995; Harris, 2001) indicate that the conversion to the scrapie isoform may occur on the cell surface. On the other hand, the accumulation of PrPSc in endosomes of scrapie-infected cells with prevalent acidic pH values (4.0–6.0) (Lee et al., 1996) has suggested that acidic pH could trigger PrPC conformational transition to PrPSc.

This hypothesis is studied here with the help of molecular dynamics simulations, checking how human PrPC globular domain reacts to mildly acidic conditions, i.e., much less acidic than that investigated by Alonso et al. (Alonso et al., 2001, 2002) and Sekijima et al. (2005), in which protonation involved mainly histidine residues. This goal is achieved by means of molecular dynamic (MD) simulations, which have already been fruitfully used to investigate the dynamical behavior of prion proteins. Particular, the results of several MD simulations of the structured core (125–228 aa) of human prion protein in neutral (hereafter PrPN) and in mildly acidic, a pH ranged
between 3.8 to 4.2, (hereafter PrPH) conditions is presented. In PrPN lysine and arginine are positively charged, glutamic acid and aspartic acid negatively charged, and the four histidines neutral, whereas in PrPH all the histidines are positively charged, but glutamic acid and aspartic acid remain unprotonated. The focus is exclusively on histidine residues because human PrP (90–231) exhibits a conformational change which is complete at pH 4.4 but starts at pH 5.5 (Swietnicki et al., 1997).

Since MD simulations are much more reliable when based on accurate experimental structures, therefore the study is only on the structured part of the prion, the only one for which unambiguous structural data are available. The exclusion of the 90–124 unstructured N-terminal regions avoids errors due to an arbitrary guess for this fragment. This choice, even if preventing me from obtaining relevant conformational changes due to rearrangement of the more flexible N-terminal fragment, should put on a firmer ground all the conclusions drawn from the analysis of my computational results. Furthermore, the structured core of PrPC, even if not infectious, well represents the characteristic behavior of the full length prion protein exhibiting an intrinsic pH-dependence and an affinity for metal cations.

Materials and methods

Both PrPN and PrPH MD simulations started from the NMR structure (Zahn et al., 2000) of human PrP (125–228) (PDB entry 1QLX), determined in mildly acidic conditions (pH = 4.5). Simulations were performed at constant temperature within a fixed-volume box filled with water molecules by using periodic boundary conditions. The net charges of PrPN and PrPH were compensated by adding three Na\(^+\) and one Cl\(^-\) ions, respectively. PrPN simulation includes 20,117 atoms and the PrPH one 20,843 atoms. The temperature was kept constant at 300 K using the isothermal Gaussian temperature coupling.

The particle mesh Ewald method (PME) (Darden et al., 1993, 1999) (grid spacing of 0.12 nm) was used for electrostatic calculations, thus properly accounting for long-range interactions. A non-bonded cutoff of 0.9 nm for Lennard-Jones potential was used. For both systems the solvent was relaxed by energy minimization, followed by 15 ps of MD at 300K, while restraining protein atomic positions with a harmonic potential. The systems were then minimized without restraints and their temperature brought to 300 K in a stepwise manner: 15-ps-long MD runs were carried out at 50, 100, 200, 250 and 300 K before the production runs were started at 300 K. Subsequently both systems were simulated for 10 ns. Both simulations were performed with the GROMACS simulation package (van der Spoel et al., 1995). A modification of GROMOS87 force field (van Gunsteren and Berendsen, 1987; van Buuren et al., 1993; van Gunsteren et al., 1996), widely and successfully applied to the study of different classes of proteins, was used.

Moreover, the force field (GROMOS96) we have used is widely used by the scientific community and several recent studies convincingly show that it delivers reliable results for a number of biological systems, including peptides and proteins. In the particularly difficult field of folding simulations, the GROMOS96 code (based on the GROMOS87 force field) has provided remarkable results for β-peptides. Secondary structure elements were assigned by the DSSP program (Kabsch and Sander, 1983), whereas AgusLab program is used for model manipulation, visual analysis and figure production.

Results

The PDB of the 1QLX prion protein was converted to readable format for GROMACS. The system was solvated in a cubic box of dimension 60 Å where a minimum distance of 5 Å between the protein and the box walls was imposed. Thus the solvated box consists of total of protein molecules of water, and Chlorine ions or Sodium ions whatever may be the case. The PrPN system shows a negative charge of minus three which was neutralized by randomly replacing 3 water molecules with 3 sodium ions Na\(^+\). The PrPH system had a positive charge of plus one, thus this system was neutralized by randomly replacing 1 water molecule with a chlorine ion Cl\(^-\).

The standard notations are used here, these are labels HA, HB, HC, and S1, S2 to indicate the three α-helices and the two strands of the β-sheet, respectively. Regions connecting these secondary structure elements (S1, HA, S2, HB, HC) will be referred to as tA, tB, tC, and tD, respectively. Inspection of above figure reveals that PrPN is stable during the free dynamic run. The C-α root mean-square deviation (RMSD) increases rapidly within 0.5 ns and then it remains stable at 0.18 nm over the whole simulation period. On the other hand, protonation of the four histidine residues gives rise to significant conformational changes. As a matter of fact, PrPH shows a major rearrangement with respect to the starting structure reaching a constant RMSD of 0.28 nm after 6 ns of
simulation.

Fig. : 1

(a) Showing Protein in a solvated box of 60X60X60 (in Å)

(b) Root mean-square deviation of the C-α carbon atoms positions from their positions in the starting NMR structure as a function of time in the PrPN (dashed line) and PrPH (solid line) runs.

Fig. : 2 (a) Energy plot of PrPN protein (b) Energy plot of PrPH protein

The dynamically stabilized structure can also be seen in GROMACS software by using the program ngmx.

The do_dssp command is used to analyze the secondary structure of the models. The program generates the following figure (figure.3 (a), (b)). The result shows the α-helix content, β-sheet content and other secondary structures of the proteins.

Fig. : 3

(a) Secondary structure of PrPN (upper panel) as a function of time, determined with DSSP. (b) Secondary structure PrPH (lower panel) as a function of time, determined with DSSP.
The figure 3(a) for PrPN shows more of blue color i.e., high content of α-helix and lower graph for PrPH shows 3 lines of red in comparison of 2 of the upper graph. This shows that the β-content has increased in the PrPH protein of the model. The positions and lengths of the different secondary structure elements of PrPN were well preserved during the simulation (see figure 3) and similar to the initial NMR structure. Analysis of the secondary structure indicates that HA (residues 144–152) and HC (residues 200–228) are stable throughout the simulation, whereas the C-terminal part (residues 187–194) of HB is highly unstable, undergoing a progressive destructuration during the simulation. Moreover, the β-sheet content increases, reaching nine residues during the 4.3–6.1 ns time interval.

Discussion

This study has shown that protonation of the four histidine residues decreases the conformational stability of human PrP (125–228), leading to a significant structural rearrangement (RMSD 3.0Å) with respect to the starting structure, which involves a decrease in the α-helix and a transient increase in the β-sheet content. The most relevant structural changes involve the destructuration of the C-terminal part of helix B (residues 188–194). It has been assumed that HB and HC constitute the stable core of the protein whereas the conformational transition to the β-rich form involves helix A. A MD simulation (15-ns long) was performed on huPrP (125–228) under strongly acidic conditions (glutamic acid, aspartic acid, and histidine protonated) which did not show any significant conformational rearrangement. Indeed, the average C-α RMSD during the last 5 ns was 2.5 Å and the secondary structure elements are retained during the whole simulation.

The analysis of the behavior of the four histidine residues during the simulations reveals that the protonation of His-155 and His-187 is critical for the onset of the conformational transition. In particular, the key event leading to the destruction process of the C-terminal end of HB is the presence of a positive charge on His-187, providing the driving force for the formation of salt bridge with the Glu-196 side chain. This is a crucial point: His-187 is indeed involved in a pathogenic mutation associated with GSS syndrome in human. On the other hand, experiments show that the prion conformational rearrangement due to pH decrease starts already under mildly acidic conditions, suggesting that histidine can play a relevant role in this phenomenon. This study points out that this is the case: the simple protonation of the histidine residues might trigger a conformational transition involving a significant decrease of α-helical content and the stabilization of extended structures. The analysis of the MD results, showing that the protonation of two histidine residues (His-187 and His-155) could be the main cause of this transition. The effect of mutations could obviously be significant for inter protein interactions.

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References


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