

MOLECULAR DYNAMIC SIMULATION STUDIES OF Q212H, V203G AND
N173K MUTATIONS IN PRION DISEASES

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To my beloved Mohammad, father and mother, sister and brother

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ABSTRACT

The Prion protein (PrP) roots from a collection of diseases identified as transmissible spongiform encephalopathies (TSEs), which are caused by conversion of PrP from its normal cellular form (PrP^C) to a misfolded, oligomeric isoform (PrP^{Sc}). Prion diseases are considered fatal and until now no known treatment has been reported. Prion diseases in humans are grouped based on whether they are sporadic, inherited, or acquired. In the case of inherited prion disease, which is also known as familial prion disease, abnormal prion proteins are produced through a genetic mutation. So far, about 40 point mutations have been discovered. The globular domain (aa125 to 228) of PrP plays a critical role in its folding and stability and many of pathogenic mutations are located on this part. V203G, Q212H and N173K are three PRNP mutations, which were reported recently, but it remained questionable whether the mutations were causal of the disease. In this research, we performed Molecular Dynamic Simulation and structural analysis on the three previously mentioned unknown-disease-related mutations (V203G, Q212H and N173K) and prion disease related mutations V203I and Q212P as positive control for V203G and Q212H respectively and neutral polymorphism N171S as a negative control for N173K. We investigated to see how similar the unknown-disease-related mutations act compared to their controls, to verify whether the mutations were causal of the disease. 50 ns of molecular dynamic simulations were performed for all mutations and wild type, using GROMACS 4.6.3 software and GROMS96 force field. Changes in RMSD, RMSF, salt bridges, secondary structure and Solvent accessible surface area were explored by analyzing the trajectories. The results revealed similar dynamic behavior between Q212H, V203G, N173K and other prion pathogenic mutations; all three under study mutations showed a decrease in the protein's overall stability, an increase in HB and HC region flexibility, a major loss in salt bridges in the HA and HB region, changes in the electrostatic surface of PrP and made the protein more exposed to solvent, which are all common dynamic behaviors among pathogenic mutations.

ABSTRAK

Protein prion (PrP) berasal daripada rangkaian penyakit yang dikenal pasti sebagai ensefalopati spongiform berjangkit (TSEs) akibat penukaran PrP daripada bentuk selular normal (PrP^C) kepada salah lipat, isoform oligomerik (PrP^{Sc}). Penyakit prion membawa kepada maut dan tiada rawatan yang diketahui dilaporkan setakat ini. Penyakit prion pada manusia dikelaskan kepada sporadik, terwaris, atau terperoleh. Dalam kes terwaris atau keturunan, protein prion tidak normal dihasilkan melalui mutasi genetik. Sehingga kini, sebanyak 40 mutasi telah ditemui. Domain globular PrP (aa125 hingga 228) berperanan penting dalam proses pelipatan dan kestabilannya di mana mutasi patogenik banyak tertumpu di bahagian ini. Tiga mutasi PRNP yang baru dilaporkan iaitu V203G, Q212H dan N173K masih tidak diketahui perkaitannya sebagai punca kepada penyakit. Dalam kajian ini, analisis struktur secara simulasi dinamik molekul (MD) telah dijalankan ke atas 3 mutasi yang tidak diketahui perkaitannya dengan penyakit (V203G, Q212H dan N173K), mutasi terkait penyakit prion V203I dan Q212P, masing-masing sebagai kawalan positif bagi V203G dan Q212H serta polimorfisme neutral N171S sebagai kawalan negatif bagi N173K. Mutasi V203G, Q212H dan N173K diselidiki sejauh mana persamaannya berbanding dengan kawalan, bagi mengesahkan sama ada mutasi tersebut menjadi punca kepada penyakit. Kesemua mutan dan jenis liar disimulasi MD selama 50 nanosaat menggunakan medan daya GROMOS96 pada perisian GROMACS versi 4.6.3. Perubahan pada nilai RMSD, RMSF, titian garam, struktur sekunder dan bahagian permukaan terakses pelarut disiasat berdasarkan analisis trajektori. Hasil kajian mendapati perlakuan dinamik adalah hampir sama di antara Q212H, V203G, N173K dan mutasi patogenik prion yang lain. Kesemua 3 mutasi yang dikaji menunjukkan penurunan kestabilan protein secara menyeluruh, peningkatan kelenturan pada kawasan HB dan HC, kehilangan sejumlah besar jambatan garam di kawasan HB dan HC, perubahan pada permukaan elektrostatik PrP yang mendedahkan lagi protein kepada pelarut; di mana kesemua ini merupakan ciri perlakuan dinamik bagi mutasi patogenik

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LIST OF ABBREVIATIONS

PrP	–	Prion Protein
PrP ^c	–	Cellular Prion Protein (Normal Isoform)
PrP ^{Sc}	–	Scrapie Prion Protein (Pathogenic Isoform)
HuPrP	–	Human Prion Protein
GPI	–	Glycophosphatidylinositol
NMR	–	Nuclear Magnetic Resonance
aa	–	Amino Acid
S	–	Sheet
H	–	Helix
PDB	–	Protein Data Bank
TSE	–	Transmissible Spongiform Encephalopathy
MD	–	Molecular Dynamic
WT	–	Wild Type
CJD	–	Creutzfeldt-Jakob Disease
iCJD	–	Iatrogenic Creutzfeldt-Jakob Disease
fCJD	–	Familial Creutzfeldt-Jakob Disease
FFI	–	Fatal Familial Insomnia
GSS	–	Gerstmann-Straussler-Scheinker Disease
sCJD	–	Sporadic Creutzfeldt-Jakob Disease
vCJD	–	Variant Creutzfeldt-Jakob Disease
SB	–	Salt Bridge
RMSD	–	Root-Mean-Square Deviation
RMSF	–	Root-Mean-Square Fluctuation
SASA	–	Solvent Accessible Surface Area
nm	–	Nanometer

CHAPTER 1

INTRODUCTION

1.1 Background of the problem

The prion protein (PrP) roots from a collection of diseases identified as transmissible spongiform encephalopathies (TSEs) (Vila-Viçosa et al. 2012). Neurodegenerative disorders with sporadic, infectious and genetic forms are common diseases found in both human and animals which are caused by conversion of PrP from its normal cellular form (PrP^C) to a misfolded, oligomeric isoform (PrP^{Sc}) (Xu et al. 2012) (De Simone et al. 2013; Thirumalai 2013). In fact, prion diseases are misfolded proteins, which accumulate inside the central nervous system and produce an infectious disease. This has been remained a debate and is considered the main idea of the Prion Hypothesis, because it contrasts compared to other known infectious agents (virus/bacteria/fungus/parasite) which must contain nucleic acids (either DNA, RNA, or both). Prion diseases are considered fatal and until now no known treatment has been reported. Some examples of TSEs include fatal familial insomnia in humans, bovine spongiform encephalopathy in cattle, Scrapie in sheep, and Creutzfeldt– Jakob disease, Gerstmann– Sträussler– Scheinker syndrome and kuru (Vila-Viçosa et al. 2012; Thirumalai 2013).

PrP is monomeric and soluble in its normal cellular form, however its pathogenic form (PrP^{Sc}) contains a high β -structure content which may accumulate and form amyloid fibrils (Vila-Viçosa et al. 2012). PrP^{Sc}, resulting from the misfolding

of PrP^C, gathers extra PrP^C to form deposits of accumulated PrP^{Sc} in the affected individual's brain (Spevacek 2012). Different causes have been proposed for the conversion of PrP^C into PrP^{Sc}, however the mechanism of the conversion still remains unanswered (Vila-Viçosa et al. 2012).

The chemical composition of amino acid sequences of PrP^C and PrP^{Sc} are identical but their difference lies in their conformations specifically in terms of secondary structure (Vila-Viçosa et al. 2012). The structure of PrP^{Sc} has the tendency to assemble into insoluble aggregates, hence NMR spectroscopy and X-ray crystallography are not able to determine the structure of this protein so the structure still remains unresolved. According to limited number of experiments on PrP^{Sc} using the CD spectroscopy and Fourier transform, it was observed that compared to PrP^C, PrP^{Sc} are less helical and consist of more β -structures. Hence, it is logical to conclude that in the transition from PrP^C to PrP^{Sc} there exists a conversion of α -helices to β -structures (Xu et al. 2012). PrP^C's α -helix and β -sheet content is approximately 42% and 3% respectively, while PrP^{Sc} α -helix content decreases to \sim 30% and its β -sheet content about 43% (Mishra 2010; Vila-Viçosa et al. 2012).

PrP is located on chromosome 20 in humans and encoded in the PRNP gene, and it is expressed as a 254 amino acid protein, in which the first 22 amino acids are removed after they signal to translocate the nascent protein to the endoplasmic reticulum (Spevacek 2012). With the addition of a glyco-phosphatidyl-inositol (GPI) anchor to produce a 209 amino acid protein, PrP (amino acids (aa) 23-231), the C-terminal 23 amino acids are cleaved (Spevacek 2012). Several structures of human PrP (huPrP) exist in the Protein Data Bank. Based on these data, the huPrP includes an N-terminal region with no distinct structure (aa 23– 124) and globular domain in the C-terminal region (aa 125– 228) composed of three α -helices (HA: aa 144– 156; HB: aa 174– 194; HC: aa 200– 228) and short two-stranded antiparallel β -structure (S1–S2) (aa 128– 131 + aa 161– 164) (Figure 1.1). It has a disulfide bond between C179 and C214 and two Nglycosylation sites (N181 and N197). The N-terminal region is characterized by octarepeats (aa 51– 91) that seem to develop a structure in the presence of Cu²⁺ or other metals (Vila-Viçosa et al. 2012).

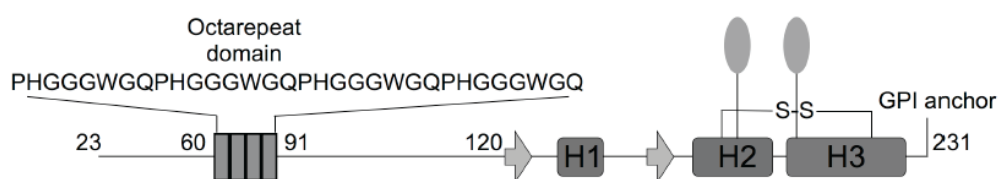


Figure 1.1 Schematic of the prion protein. The flexible N-terminus contains the octarepeat domain. The structured C-terminal domain contains two β -strands (arrows) and three α -helices (boxes). The gray circles stand for the N-linked glycosylations. The C-terminus is with a GPI anchor, which connects the protein to the cell surface (Spevacek 2012)

Currently, Prion diseases in humans are grouped based on whether they are sporadic, inherited, or acquired. In majority of cases prion disease are sporadic; meaning, they develop spontaneously with no known reason. On the other hand, prion disease is rarely inherited due to a faulty gene, and less often acquired through medical procedures, transfusions, or contaminated food.

In the case of inherited prion disease, which is also known as familial prion disease, abnormal prion proteins are produced in the body caused by a genetic mutation (fault in the gene that codes for the prion protein). The abnormal prion proteins are predisposed to undergo the change in shape, which induces the production of rogue proteins.

It is known that conformation transition from PrP^{C} to PrP^{Sc} is induced with the replacement of some amino acids in PrP. Human familial prion diseases are associated with about 40 point mutations of PRNP, and most of these mutations are located in the globular domain of the protein (Guo, Ren, et al. 2012b). This purely structural rearrangement between PrP^{C} and PrP^{Sc} provides an opportunity for investigation from a dynamics point of view. Experimentally, it is unfeasible to distinguish the actual conformational transition or folding/unfolding process between these two states. As mentioned before the hydrophobic nature of PrP^{Sc} prohibit successful structure determination by x-ray crystallography, or NMR spectroscopy, molecular dynamic

(MD) simulation has become one of the few available methods for elucidating PrP^C to PrP^{Sc} conversion (Shamsir et al. 2008).

MD simulation has obvious advantages to study the structural conversion, as it cannot only provide plentiful dynamic structural information but also model the required environment for conversion easily. Actually, this method has been applied successfully to explore how the mutations affect the conversion between PrP^C and PrP^{Sc} (Guo, Ning, et al. 2012a).

1.2 Objectives of the Study

The purpose of this study is to:

- 1) Identify prion mutations as controls for Q212H, V203G and N173K mutations.
- 2) Run molecular dynamics simulation for the mutations and their controls
- 3) Analyse the effects of mutations on the protein structure, stability and intermolecular interactions.

1.3 Scope of the Study

Applying 50 ns of MD simulations in order to investigate three unknown-disease-related PrP mutations (Q212H, V203G, N173K) and an additional MD simulation run for three controls consisting of two pathogenic mutations (Q212P, V203I) taken as positive controls and a neutral polymorphism (N171S) as negative control and a MD simulation of WT as a basis for comparison of all mutations.

1.4 Problem Statement

By running MD simulation for the identified pathogenic mutations (positive controls) and polymorphism (negative control) in human PrP along with the WT PrP we could explore the effect of mutations on WT PrP. By this means, MD simulation could be performed on Q212H, V203G and N173K (the unknown-disease-related mutations) and the results could be compared with the controls. This will help us to explore the dynamic similarity between Q212H, V203G, N173K and prion pathogenic mutations.

1.5 Significance of the Study

Mutations in prion protein gene can produce diverse clinical phenotypes. Hence, diagnosing whether a mutation in prion protein is causal of the disease, becomes difficult and need further examination, which is time consuming and costly. By using MD simulation, present study explores mutations that are not known whether they are pathogenic. This research could serve as a step forward to acquiring a more rapid, economical and reliable approach for a definite distinction of non-pathogenic PrP mutations from pathogenic mutations.

REFERENCES

- Altmepfen, H.C. et al., 2012. Proteolytic processing of the prion protein in health and disease. pp.1–17.
- Apetri, A.C., 2004. The Effect of Disease-associated Mutations on the Folding Pathway of Human Prion Protein. *Journal of Biological Chemistry*, 279(17), pp.18008–18014.
- Barducci, A. et al., 2005. Misfolding Pathways of the Prion Protein Probed by Molecular Dynamics Simulations. *Biophysical Journal*, 88(2), pp.1334–1343.
- Benvegna, S., Poggiolini, I. & Legname, G., 2010. Neurodevelopmental expression and localization of the cellular prion protein in the central nervous system of the mouse. *The Journal of comparative neurology*, 518(11), pp.1879–1891.
- Biljan, I. et al., 2012. Structural basis for the protective effect of the human prion protein carrying the dominant-negative E219K polymorphism. *The Biochemical journal*, 446(2), pp.243–251.
- Brown, K. & Mastrianni, J.A., 2010. The Prion Diseases. *Journal of Geriatric Psychiatry and Neurology*, 23(4), pp.277–298.
- Calzolari, L., 2003. Influence of pH on NMR Structure and Stability of the Human Prion Protein Globular Domain. *Journal of Biological Chemistry*, 278(37), pp.35592–35596.
- Chen, W., van der Kamp, M.W. & Daggett, V., 2010. Diverse Effects on the Native β -Sheet of the Human Prion Protein Due to Disease-Associated Mutations. *Biochemistry*, 49(45), pp.9874–9881.
- Cong, X. et al., 2012. Dominant-negative effects in prion diseases: insights from molecular dynamics simulations on mouse prion protein chimeras. *Journal of Biomolecular Structure and Dynamics*, pp.1–12.
- Dai, S., 2011. Using Computer Modeling to Study Prion Diseases. pp.1–24.
- De Simone, A. et al., 2013. The intrinsic stability of the human prion β -sheet region investigated by molecular dynamics. *Journal of Biomolecular Structure and Dynamics*, 31(5), pp.441–452.
- D'Angelo, P. et al., 2012. Effects of the Pathological Q212P Mutation on Human Prion Protein Non-Octarepeat Copper-Binding Site. *Biochemistry*, 51(31), pp.6068–6079.

- Gambetti, P. et al., 2003. Sporadic and familial CJD- classification and characterisation. pp.1–27.
- Giachin, G. et al., 2013. Probing Early Misfolding Events in Prion Protein Mutants by NMR Spectroscopy. *Molecules*, 18(8), pp.9451–9476.
- Glazer, D.S., Radmer, R.J. & Altman, R.B., 2009. Improving structure-based function prediction using molecular dynamics. *Structure (London, England : 1993)*, 17(7), pp.919–929.
- Guo, J., Ning, L., et al., 2012a. Influence of the pathogenic mutations T188K/R/A on the structural stability and misfolding of human prion protein: Insight from molecular dynamics simulations. *BBA - General Subjects*, 1820(2), pp.116–123.
- Guo, J., Ren, H., et al., 2012b. Exploring structural and thermodynamic stabilities of human prion protein pathogenic mutants D202N, E211Q and Q217R. *Journal of Structural Biology*, 178(3), pp.225–232.
- Imran, M. & Mahmood, S., 2012. An overview of animal prion diseases. *Virology Journal*, pp.1–8.
- Imran, M. & Mahmood, S., 2011. An overview of human prion diseases. *Virology Journal*, pp.1–9.
- Jeong, B.-H. et al., 2010. Creutzfeldt-Jakob disease with the V203I mutation and M129V polymorphism of the prion protein gene (PRNP) and a 17 kDa prion protein fragment. *Neuropathology and Applied Neurobiology*, 36(6), pp.558–563.
- Kabsch, W. & Sander, C., 1983. Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. *Biopolymers*, 22(12), pp.2577–2637.
- Kamp, M.W. & Daggett, V., 2011. Molecular Dynamics as an Approach to Study Prion Protein Misfolding and the Effect of Pathogenic Mutations. In *Topics in Current Chemistry*. Topics in Current Chemistry. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 169–197.
- Klingeborn, M., 2006. The Prion Protein in Normal Cells and Disease. *Doctoral thesis*, pp.1–58.
- Legname, G., 2012. Early structural features in mammalian prion conformation conversion. *Prion*, 6(1), pp.37–39.
- Linden, R. et al., 2008. Physiology of the Prion Protein. *Physiological Reviews*, 88(2), pp.673–728.
- Mead, S., 2006. Prion disease genetics. *European Journal of Human Genetics*, 14(3), pp.273–281.
- Mishra, P., 2010. Molecular dynamics simulation of human prion protein to detect difference in the structures of normal and diseased form. pp.1–5.

- Nunnally, B.K. & Krull, I.S., 2004. Prions and Mad Cow Disease.
- Peoc'h, K. et al., 2000. Identification of three novel mutations (E196K, V203I, E211Q) in the prion protein gene (PRNP) in inherited prion diseases with Creutzfeldt-Jakob disease phenotype. *Human mutation*, 15(5), p.482.
- Prusiner, S.B., 1982. Novel proteinaceous infectious particles cause scrapie. *Science (New York, N.Y.)*, 216(4542), pp.136–144.
- Rossetti, G. et al., 2011. Common Structural Traits across Pathogenic Mutants of the Human Prion Protein and Their Implications for Familial Prion Diseases. *Journal of Molecular Biology*, 411(3), pp.700–712.
- Sandberg, M.K. et al., 2011. Prion propagation and toxicity in vivo occur in two distinct mechanistic phases. *Nature*, 470(7335), pp.540–542.
- Schelzke, G. et al., 2013. Report about Four Novel Mutations in the Prion Protein Gene. *Dementia and Geriatric Cognitive Disorders*, 35(3-4), pp.229–237.
- Shamsir, M.S. & Dalby, A.R., 2005. One gene, two diseases and three conformations: Molecular dynamics simulations of mutants of human prion protein at room temperature and elevated temperatures. *Proteins: Structure, Function, and Bioinformatics*, 59(2), pp.275–290.
- Shamsir, M.S., Hussein, Z.A. & Sharif, J., 2008. Unravelling Prion Diseases using Molecular Dynamics Simulations. In 2008 Second Asia International Conference on Modelling & Simulation (AMS). IEEE, pp. 895–900.
- Spevacek, A., 2012. The Influence of Metal Ions on the Tertiary Structure of the Prion Protein and Inherited Prion Disease. pp.1–114.
- Striebel, J.F. et al., 2011. Strain Specific Resistance to Murine Scrapie Associated with a Naturally Occurring Human Prion Protein Polymorphism at Residue 171 N. A. Mabbott, ed. *PLoS Pathogens*, 7(9), p.e1002275.
- Surewicz, W.K. & Apostol, M.I., 2011. Prion Protein and Its Conformational Conversion: A Structural Perspective. In *Topics in Current Chemistry*. Topics in Current Chemistry. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 135–167.
- Thirumalai, J.C.A.D., 2013. Helices 2 and 3 are the initiation sites in the PrPC → PrPSC transition. pp.1–32.
- Tuite, M.F. & Cox, B.S., 2003. Propagation of yeast prions. *Nature reviews. Molecular cell biology*, 4(11), pp.878–890.
- van der Kamp, M.W. & Daggett, V., 2010. Pathogenic Mutations in the Hydrophobic Core of the Human Prion Protein Can Promote Structural Instability and Misfolding. *Journal of Molecular Biology*, 404(4), pp.732–748.
- van der Kamp, M.W. & Daggett, V., 2009. The consequences of pathogenic mutations to the human prion protein. *Protein engineering, design & selection : PEDS*, 22(8), pp.461–468.

- Vila-Viçosa, D. et al., 2012. Reversibility of Prion Misfolding: Insights from Constant-pH Molecular Dynamics Simulations. *The Journal of Physical Chemistry B*, 116(30), pp.8812–8821.
- Xu, Z. et al., 2012. Stability of the Beta structure in prion protein: A molecular dynamics study based on polarized force field. *Chemical Physics Letters*, 539-540(C), pp.239–244.