MOLECULAR DYNAMIC SIMULATION STUDIES OF THREE NOVEL GERSTMANN-STRAUSSLER-SCHEINKER AND CREUTZFELDT-JACOB PRION MUTATIONS

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A thesis submitted in partial fulfilment of the
requirements for the award of the degree of
Doctor of Philosophy (Bioscience)

Faculty of Biosciences and Medical Engineering
Universiti Teknologi Malaysia

FEBRUARY 2016

To my wonderful parents and my sister, brother

A constant source of inspiration,

The object of my love and admiration,

And the driving force behind my motivation.

No words can express my extreme gratitude

for your unconditional love, unwavering support,

and infinite patience.

Thank you for everything you have done for me,

you are the role models I aspire to be.

ACKNOWLEDGEMENT

Studying with Associate Prof DR MOHD SHAHIR SHAMSIR OMAR has been a true learning experience, and I hope to continue applying the skills I learned from him in my future career. I am deeply grateful DR MOHD SHAHIR that you have introduced me to the world of prions and computational biology, and opened my eyes to the bioinformatic research potential in these fields. Thank you for your continuous encouragement and guidance as I explored different projects and learned new skills. Your patience, dedication, and willingness to support me, scientifically and financially, have helped me grow as a bioinformatician. I am deeply indebted to your mentorship.

I also wish to extend my full appreciation to DR SALEHHUDDIN HAMDAN as co-supervisor for the supporting and motivation.

I am also grateful for the Universiti Teknologi Malaysia for providing the financial support for this research, beside to their supporting by the international doctorate fellowship (IDF) during this research. My thanks are also to the staff, faculty members, and technicians of the faculty of bioscience and biomedical engineering, Universiti Teknologi Malaysia, who contributed in this research.

I am most grateful to my family and close friends for their infinite support, patience, and encouragements during these years.

ABSTRACT

The transformation of normal prion protein into pathogenic variant in transmissible spongiform encephalopathy (TSE) is expedited by mutations. Three novel mutations V176G, E196A and I215V are associated with Creutzfeldt-Jakob disease (CJD) and Gerstmann–Sträussler–Scheinker syndrome (GSS). These mutations are novel but have yet to be characterized. The V176G mutation is exceptional as it showed GSS symptoms but resides in CJD prone segment of the prion structure. Using molecular dynamics simulations; comparative studies were performed between wild type and mutated structures, normal and elevated temperature and neutral and acidic pH to identify the dynamics in structural properties such as salt bridge, solvent accessibility, hydrogen bonds and hydrophobicity. Results indicated that overall effect of the three mutants is destabilization of the native structure, increased hydrophobicity and electrostatic potential change and the gain of new hydrogen bonds but are only restricted to localized effects on the protein such as increased fluctuation of the H1 region, gain of new salt bridge in H3 and abolished salt bridges between H1 and H3 which may be part of the oligomerisation pathways similar to GSS. The two CJD mutations, the E196A and I215V that possess a proximity effect on neighbouring regions of the mutated area. Simulations at elevated temperatures showed that the mutation caused the loss of hydrogen bonds between H3 and H2 with perturbations in the hydrophobic core that induces changes in the overall prion protein structure. In the V176G MD simulations, the mutation biggest effect is on the H1 of the protein where extreme conditions (elevated temperature and low pH) caused early denaturation compared to other segments of the prion protein. These mutations also caused accelerated perturbation in the H1-S2 region and extended the existing S1 and S2. Simulations at different low pH regimes revealed that V176G, E196A and I215V mutated structures denatured earlier in pH 2.5 compared to pH 4.5 with increased fluctuations in H1, S2-H2 loop and H2-H3 loop. In conclusion, the apparent loss of salt bridges and hydrogen bond gains are the main reason for the conformational changes that occur in mutated structure.

ABSTRAK

Transformasi protein prion yang normal ke varian yang patogenik di dalam penyakit berjangkit seperti spongiform encephalopathy (TSE) disebabkan oleh mutasi.Tiga mutasi baru V176G, E196A dan I215V telah dikaitkan dengan penyakit Creutzfeldt-Jakob (CJD) dan sindrom Gerstmann-Sträussler-Scheinker (GSS). Ketiga-tiga mutasi ini adalah unik dan masih belum di perincikan setakat ini. Mutasi V176G adalah luar biasa kerana ia menunjukkan tanda-tanda penyakit GSS tetapi kedudukan mutasi berada di dalam bahagian protin yang menyebabkan penyakit CJD. Menggunakan simulasi molekul dinamik; kajian perbandingan telah dijalankan antara struktur normal dan struktur bermutasi, pada suhu normal dan tinggi dan pada pH neutral dan berasid untuk mengenal pasti perubahan dinamik pada sifat-sifat struktur seperti jambatan garam, akses pelarut, ikatan hidrogen dan sifat kehidrofobikan.Keputusan menunjukkan bahawa kesan tiga mutan tersebut adalah ketidakstabilan struktur asal, meningkat kehidrofobikan dan perubahan potensi elektrostatik dan pertambahan ikatan hidrogen baru. Walau bagaimanapun, mutasi ini tidak menyebabkan perubahan yang ketara kepada struktur secara keseluruhan.Ketidakstabilan yang disebabkan oleh mutasi ini mungkin telah menyebabkan kesan setempat pada struktur protein seperti peningkatan gerakan dinamik di bahagian H1, pertambahan jambatan garam baru di H3 dan pemansuhan jambatan garam antara H1 dan H3 yang mungkin boleh menjadi sebahagian daripada laluan penwujudan oligomer yang menentukan profil patologi yang unik pada penyakit GSS. Kedua- dua mutasi, E196A dan I215V telah menghasilkan manifestasi simtom klinikal yang serupa dengan CJD telah terbukti mempunyai kesan setempat di sekita jujukan asid amino berdekatan kawasan mutasi. Simulasi pada suhu tinggi menunjukkan bahawa mutasi telah menyebabkan kehilangan ikatan hidrogen antara H3 dan H2 beserta gangguan di dalam teras hidrofobik yang mendorong perubahan di dalam struktur protein prion secara keseluruhan. Sebahagian dari S2-H2 dan H2-H3 telah diketahui sebagai sangat mudah dipengaruhi oleh perubahan asid amino, oleh itu mutasi ini telah menyebabkan kesan yang besar ke atas struktur. Jambatan garam baru juga dibentuk di dalam teras hidrofobik. Dalam simulasi V176G, kesan mutasi yang paling besar adalah pada helik H1 di mana keadaan yang melampau (suhu tinggi dan pH rendah) telah menyebabkan penyahaslian struktur lebih awal berbanding segmen lain protein tersebut. Mutasi ini juga menyebabkan gangguan dipermudahkan di bahagian H1-S2 dan memanjangan jujukan S1 dan S2 yang sedia ada. Simulasi pada keadaan pH yang rendah menunjukkan bahawa V176G, E196A dan I215V akan didenaturkan lebih mudah pada pH 2.5 berbanding pH 4.5. Ini akan meningkatkan dinamik H1, bahagian S2-H2 dan gelung H2-H3.Kesimpulannya,kehilangan ketara jambatan garam dan perubahan ikatan hydrogen adalah sebab utama kepada peningkatan dinamik dan perubahan struktur mutasi.

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LIST OF ABBREVIATIONS

BSE	-	Bovine Spongiform Encephalopathy
CJD	-	Creutzfeldt-Jakob disease
CWD	-	Chronic Wasting Disease
DSSP	-	Dictionary of secondary structure of proteins
EM	-	Energy minimization
ER	-	Endoplasmatic Reticulum
fCJD	-	Familial Creutzfeldt-Jakob disease
FFI	-	Fatal familial insomnia
GSS	-	Gerstmann-Sträussler-Scheinker syndrome
H1	-	α-helix 1
H2	-	α-helix 2
H3	-	α-helix 3
HB	-	Hydrogen bond
MD	-	Molecular dynamics
NMR	-	Nuclear Magnetic Resonance
PDB	-	Protein Data Bank
PME	-	Particle mesh Ewald
PRPC	-	Prion protein
PRPSC	-	Prion protein scrapie
RMSD	-	Root mean square deviations
RMSF	-	Root mean square fluctuations
S1	-	β-sheet 1
S2	-	β-sheet 2
SASA	-	Solvent accessible surface area
SB	-	Salt bridge

SC	-	Scrapie
TSE	-	Transmissible Spongiform Encephalopathies
WT	-	Wild type

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CHAPTER 1

INTRODUCTION

1.1 Overview

Prions diseases are diseases that are caused by proteinacious infectious agent (Prusiner, 1998). The term "prion" was originally coined to denote the causative agent for a group of progressive neurodegenerative disorders called transmissible spongiform encephalopathies (TSEs), such as Creutzfeldt-Jakob disease (CJD) in humans, bovine spongiform encephalopathy (BSE) in cattle, chronic wasting disease (CWD) in deer and elk, and scrapie in sheep (Colby and Prusiner, 2011). The majority of human prion diseases are sporadic with undetermined origins while 15% are caused by heritable mutations; the (Gerstmann-Sträussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI) and familial CJD). Prion diseases are unique as they are infectious and can be caused by exposure to contaminated material, such as prion-infected beef. Examples of infectious prion diseases include kuru, variant CJD and iatrogenic CJD (iCJD). Pathological characteristics include relatively long incubation periods, extreme vacuolation in specific regions of the brains and general neuronal degeneration. So far, all prion associated diseases are fatal and no cure has been found.

Prion studies have utilized various techniques, in-vitro, and in-vivo but most importantly, it is studied post-mortem. The delayed onset of the disease and invasive nature of tests have forced researcher to adopt techniques such as molecular dynamics (MD) simulations. MD simulation studies are appropriate as the lack of covalent modification in PrPC to PrPSc conversion. The conformational rearrangement between the two structures provides a chance to study purely from the structural dynamics point of view. The transition from PrPC to PrPSc cannot be studied experimentally, especially the unfolding transition between these two states. The hydrophobic nature of PrPSc also prevents effective and correct structure determination by X-ray crystallography, or even using NMR spectroscopy. Hence, MD simulation techniques have become a viable method for studying PrPC to PrPSc conversion. Amino acid changes that form the body of evidence of destabilizing mutations that predisposes the organism to prion diseases also make studying the dynamics between native and mutant PrPC a promising approach.

Molecular dynamics have utilized extreme condition to mainly study the effect of prion structural stabilities. Two of the main strategies used to achieve structural changes are thermal and low pH denaturation. In thermal denaturation, wild type and mutant PrP are simulated at elevated temperature of 500 K (El-Bastawissy *et al.*, 2001, Sekijima *et al.*, 2003a, Sekijima *et al.*, 2003d). By elevating the temperature, the protein unfolding can be accelerated without changing the pathway of unfolding and this enables the determination of the unfolding pathway at minimal computational expense (Day *et al.*, 2002). In low pH induced denaturation, experimental evidence have showed that acidic pH is capable to induce PrPC to PrPSc (Alonso *et al.*, 2002, Alonso *et al.*, 2001, DeMarco and Daggett, 2004, DeMarco and Daggett, 2007, Gu *et al.*, 2003, Kuwata *et al.*, 2003, Langella *et al.*, 2004, Sekijima *et al.*, 2003a, Sekijima *et al.*, 2003d). The hypothesis of using low pH conditions in MD simulations is that the protein will be stable at the normal pH but will undergo a conformational change at the lower pH due to the altered protonated states. This will disrupt local interactions enabling the peptide to adopt conformations

that are unlikely at neutral pH. The decreased structural stability at acidic pH resulted in increased flexibility and dynamics, which might provide a starting point for the processes that ultimately, leads to conformational transformation. In these two extreme condition MD simulations, the β sheets have been implicated as the changes have included elongation of existing β sheets and formation of β-sheets in the highly disordered region of the N terminus of PrP (Alonso *et al.*, 2002, Alonso *et al.*, 2001, DeMarco and Daggett, 2004). This occurrence may play a seeding role during the initial stages of PrP to PrPSc conversion (Hornemann and Glockshuber, 1998, Jackson *et al.*, 1999a).

1.2 Problem Statement

While the Human familial prion diseases related to PrP originated from 40 points mutations and most of the mutations occur in the protein globular domain (Guo et al., 2012a, Rossetti et al., 2011). The role in protein misfolding which is central to prion disease development has not been explored in full detail these mutations are responsible for spontaneous generation of PrPSc in the brain (Prusiner, 1994). The mutation's effect of the dynamic instability in PrPC enhances the misfolding kinetics of PrP WILD TYPE (Liemann and Glockshuber, 1999, Swietnicki et al., 1998) and the stability of partially folded intermediate species such as PrPSc precursors (Apetri et al., 2004, Horiuchi and Caughey, 1999, Rossetti et al., 2011, Surewicz et al., 2006). The analysis of mutations carrying PrP provides significant insights into factors that increase the likelihood of misfolding and induces different types of spontaneous prion diseases even in the absence of infection from exogenous sources (Biasini et al., 2008, Jeffrey et al., 2009). The aim in this study was to test in vitro, three novel mutations (V176G, E196A and I215V) were studied using molecular dynamics simulations. These mutations are only recently reported and are unusual in their co-occurrence in single patient or within the same family. The (V176G) is a novel

PRNP mutation associated with a neuropathologically condition identified in a single patient. Post mortem analysis revealed unique neuropathological profile consisting of high amyloid plaque encumbers, neurofibrillary degeneration and the brain spongiform alterations similar to Gerstmann-Sträussler-Scheinker (GSS). However, the V176G is located on the area known for CJD mutations (Rossetti et al., 2011). The mutations E196A and I215V are novel PRNP mutations that has been shown to possess distinct clinical phenotypes and overlapping of clinicopathological features related with CJD. The mutations V176G, E196A and I215V as well as their wild type (WILD TYPE) are simulated to study their dynamics and physicochemical properties such as salt bridge, solvent accessibility and hydrophobicity. This study will improve current understanding of the misfolding process and the factors that influence this process with novel mutations.

1.3 Objectives of the Research

1. To study the effect of Creutzfeldt-Jakob disease mutation E196A, I215V on prion protein.
2. To study of effect of V176G Gerstmann-Sträussler-Scheinker mutation that possess Creutzfeldt-Jakob disease symptom.
3. To investigate conformational changes of mutant protein using molecular dynamics.
4. To investigate conformational changes of mutant protein at low pH and elevated temperature.

1.4 Scope of the Research

The scope of the study is purely bioinformatics and computational analysis where all the data are derived from primary data bases and analysed using computational tools.

5.2 Future Works

Currently, there are as many as 40 different mutation associated with prion diseases. The existing limitations such as lack of experimentally determined structure, post-mortem detection, sporadic onset and computing processing have limited majority of the work to be theoretical in nature. Future work should focus on testing the aetiology of the V176G, E196A and I215V mutations and looking to discover the separate symptom pathology form a molecular level using in-vitro method. However, this requires extensive animal model work and genetic screening to detect presence of mutated prion. The long term commitment of resources would limit the focus on established and well documented mutations.

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