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X-ray structural and molecular dynamical studies of the globular domains of cow, deer, elk and Syrian hamster prion proteins

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ABSTRACT

Misfolded prion proteins are the cause of neurodegenerative diseases that affect many mammalian species, including humans. Transmission of the prion diseases poses a considerable public-health risk as a specific prion disease such as bovine spongiform encephalopathy can be transferred to humans and other mammalian species upon contaminant exposure. The underlying mechanism of prion propagation and the species barriers that control cross species transmission has been investigated quite extensively. So far a number of prion strains have been characterized and those have been intimately linked to species-specific infectivity and other pathophysiological manifestations. These strains are encoded by a protein-only agent, and have a high degree of sequence identity across mammalian species. The molecular events that lead to strain differentiation remain elusive. In order to contribute to the understanding of strain differentiation, we have determined the crystal structures of the globular, folded domains of four prion proteins (cow, deer, elk and Syrian hamster) bound to the POM1 antibody fragment Fab. Although the overall structural folds of the mammalian prion proteins remains extremely similar, there are several local structural variations observed in the misfolding-initiator motifs. In additional molecular dynamics simulation studies on these several prion proteins reveal differences in the local fluctuations and imply that these differences have possible roles in the unfolding of the globular domains. These local variations in the structured domains perpetuate diverse patterns of prion misfolding and possibly facilitate the strain selection and adaptation.

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1. Introduction

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are devastating neurological disorders that affect a variety of mammalian species including humans (Aguzzi and Polymenidou, 2004). These are degenerative diseases of the central nervous system that lead to progressive dementia, debilitating motor dysfunction such as ataxia and ultimately to death in 4–6 months from the initial detection of the disease (Collinge, 2001; Heinemann et al., 2007). The sporadic Creutzfeldt–Jakob disease (CJD) is the most common form of the human prion diseases. The clinical and pathological manifestations of human prion diseases can be segregated to in three principal subgroups known as familial Creutzfeldt–Jakob disease (fCJD), Gerstmann–Strauss ler–Scheinker (GSS) syndrome, and Familial Fatal Insomnia (FFI) (Prusiner, 2001; Richardson and Masters, 1995). In 1985, bovine spongiform encephalopathy (BSE) was first detected in cattle (Wells et al., 1987); this became a major public health concern after the emergence of a devastating form of human prion disease, also known as the new variant Creutzfeldt–Jakob disease (nvCJD), that occured upon the consumption of contaminated beef (Bruce et al., 1997; Collinge, 1999; Hill et al., 1997). Like BSE, chronic wasting disease (CWD) is a prion disease in cervids that is found to propagate in captive as well as in free ranging elk, mule deer and white tailed deer (Sigurdson, 2008). The rapid spread of CWD in areas of the western United States and Canada is considered a significant environmental threat as the wild cervids infected with prion disease often come into regular contact with the domesticated animals (Enserink, 2001). Although the prion transmission could occur through the established infection routes of oral, corneal, intraperitoneal, intravenous, intranasal, intramuscular, intralingual, transdermal and interacerebral passage (Aguzzi and Polymenidou, 2004; Heppner et al., 2001; Herzog et al., 2004; Hill et al., 1997; Maignien et al., 1999; Weissmann et al., 2002; Zhang et al., 2004), a recent finding even proposes possible prion spreading as a contaminated aerosol (Haybaeck et al., 2011). A significant amount of contaminant is present in the body

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fluids and excreta of the prion infected animals; these can easily get dispersed in the environment through urine, feces, blood, milk, and placenta thereby harboring potential threats to livestock and other wild animals (Lacroux et al., 2008; Mathiason et al., 2006; Seeger et al., 2005; Tamguney et al., 2009; Vascellari et al., 2007).

The cellular prion protein, PrP^C, has attracted a lot of interest in the scientific community as the misfolded and the aggregated form of this protein is solely responsible for the transmissible spongiform encephalopathies (Prusiner, 1982). A "protein only" hypothesis has been proposed for the prion propagation process (Griffith, 1967; Prusiner, 1982; Prusiner, 1998), and two crucial observations reaffirm this novel principle of disease transmission. First, a transgenic PrP gene knockout strain of mouse, that has lost the capability to express the cellular prion protein, never gets affected by the infectious prion particles (Bueler et al., 1993). Secondly, in the brain tissue of TSE infected animals an aggregated form of the prion protein known as scrapie (PrP^{Sc}) has been isolated (Prusiner, 1998). The ubiquitously present cellular prion protein (PrP^C) undergoes a malicious conformational change into the pathogenic conformation, PrP^{Sc}, and this leads to the onset of these devastating diseases. The non-pathogenic conformer PrP^C is an extra-cellular membrane-anchored glycoprotein that contains two unrelated domains; the N-terminal domain (residues 23–125) that is flexible and unstructured, and the globular C-terminal domain (residues 126–231) comprising two native anti-parallel β-strands, β1 and β2 and three α-helices α1, α2 and α3. On the other hand, it's pathogenic isoform PrP^{Sc} is a multimeric aggregated structure constituted mainly by an elongated stacking of β-sheets. These infectious PrP^{Sc} particles also exhibit atypical physiochemical properties such as protease resistance, relative insolubility, and the propensity to polymerize into amyloid-like fibrillar structures (Prusiner, 1998). In the prion replication and propagation process, the infectious PrP^{Sc} acts as a template that promotes its self-proliferation by recruiting the cellular prion molecules and this vicious cycle continues leading to the formation of long fibril-like structures (Castilla et al., 2005; Sigurdson et al., 2009).

The transmission process of prion diseases across mammalian species has been found to vary widely. BSE shows superior transmission efficiency to humans and to mammalian species such as goats (Eloit, 2005), cats (Wyatt et al., 1991), zoological-kept felidar (Eiden et al., 2010) and to ruminants in comparison to other prion diseases. In the laboratory setting mice (Castilla et al., 2004), pigs (Konold et al., 2009) and guinea pigs (Safar et al., 2011) are also known to transmit BSE quite effectively. The intra-species spreading of prion diseases is quite evident in cases of BSE as well as in cases of CWD. However, the transmission of prion infections between different mammalian species is typically far less efficient and this phenomenon is known as the "species barrier" (Collinge and Clarke, 2007). Unlike BSE, that demonstrates properties to cross species barriers quite efficiently, evidence regarding the CWD suggests limited infectivity towards other mammalian species (Raymond et al., 2000). This anomaly in the inter-species prion infectivity and propagation has been attributed to the prion strain characteristics (Collinge and Clarke, 2007). Until now, a number of strains have been identified from various mammalian species and these are quite different in terms of their constituents, biophysical properties as well as their pathophysiologal manifestations (Beringue et al., 2008). It is quite surprising that these different prion strains are derived from the cellular PrP^C that share a high sequence identity among mammalian species. As the PrP^{Sc} generation is largely dependent on the misfolding of the cellular prion protein there might be a possibility that the emergence of so many strains is rather dependent on differences in the PrP^C misfolding patterns. The C-terminal domains play an important role in this toxic conversion process as the helical nature of the prion protein is substantially lost and it transforms largely into beta-strands and turns which aggregate among themselves and form the long fibril-like structures (Smirnovas et al., 2011). Furthermore, the contribution of the globular domains towards PrP^{Sc} generation is quite evident as many of the pathogenic mutations are present in this region of the molecule (Mead, 2006). Tricyclc anti-prion compounds such as promazine target a unique binding site on the folded domain of PrP^C and stabilize some of the misfolding initiator motifs as well as with regions near its immediate binding site (Baral et al., 2014). Therefore these misfolding initiator motifs are interesting targets for further structural characterization. In the present study, we have determined the crystal structures of the recombinant prion proteins from bovine, deer, elk and Syrian hamster bound to POM1 Fab and have carefully compared the structures of the toxic initiator motifs among them. We have also performed molecular dynamic simulations of the structured domains of these four prion proteins in order to understand the dynamical characteristics of the initiator motifs. Our structural analysis of these mammalian prion proteins should help in understanding the interrelationship of different motifs in prion misfolding behavior and strain selection.

2. Material and methods

2.1. Expression and purification of the boPrP, dePrP, ekPrP and shPrP proteins

We used the pET15b (Novagen) vector for the protein expression purpose which contains a histidine tag at the n-terminal end of the prion proteins. Clones containing the globular domains of recombinant prion proteins, boPrP (103–242), dePrP (123–231), ekPrP(124–231) and shPrP (90–230), were expressed in the bacterial expression strain of BL21–CodonPlus® (DE3)-RIL (Stratagene). The cells were grown in Luria–Bertani (LB) broth containing 0.1 mg/mL ampicillin and 0.34 mg/ml Chloramphenicol at 37 °C, with constant shaking at 200 rpm. The proteins were expressed as inclusion bodies by use of the inducing agent 0.1 M Isopropyl β-D-1-thiogalactopyranoside (IPTG). The cells were then sonicated, pelleted by centrifugation at 18,000 rpm and extensively washed with a 100 mM NaH_2PO_4 buffer. Subsequently, the inclusion bodies were incubated in denaturing buffer G consisting of 8 M Urea, 10 mM Tris–HCl, 100 mM NaH_2PO_4 and 5 mM reduced glutathione pH8.0 for 1 h at room temperature with constant stirring. The extracted, denatured prion proteins were then purified using metal affinity chromatography by loading each one separately onto a Ni-NTA agarose column (Qiagen). The bound prion proteins were refolded on the column by gradient application of buffer G (denaturing buffer) to buffer A (10 mM Tris–HCl, 100 mM NaH2PO4 and 5 mM imidazole, pH 8.0) as described by Yin et al. (Yin et al., 2003). After the refolding, the non-specifically bound impurities were removed by washing with 10 mM Tris–HCl, 100 mM NaH_2PO_4 and 50 mM imidazole, pH 8.0. Finally, the purified prion proteins were eluted with the elution buffer containing 10 mM Tris–HCl, 100 mM NaH_2PO_4 and 400 mM imidazole pH-5.8. The resulting proteins were exchanged into distilled water using Amicon Ultra centrifugal filters (3 kDa molecular weight cutoff, Millipore). The purity of these proteins was confirmed by SDS-PAGE and their protein concentrations were measured by the Bradford method (Bradford, 1976).

2.2. Production and purification of POM1 Fab

The protocol for POM1 hybridoma generation was followed as described previously (Polymenidou et al., 2008). The hybridoma supernatant was loaded on to a protein G Sepharose (PIERCE)

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