Cell Reports
Report

Thermodynamic Stabilization of the Folded Domain of Prion Protein Inhibits Prion Infection in Vivo

Qingzhong Kong,[1,*] Jeffrey L. Mills,[2,3,4] Bishwajit Kundu,[2,3,5] Xinyi Li,[1,3,6] Liuting Qing,[1,3] Krystyna Surewicz,[2] Ignazio Cali,[1] Shenghai Huang,[1] Mengjie Zheng,[1] Wieslaw Swietnicki,[2,7] Frank D. Sönnichsen,[2,8] Pierluigi Gambetti,[1] and Witold K. Surewicz[2,*]

[1]Department of Pathology, Case Western Reserve University, Cleveland, OH 44106, USA
[2]Department of Physiology and Biophysics, Case Western Reserve University, Cleveland, OH 44106, USA
[3]These authors contributed equally to this work
[4]Present address: Department of Chemistry, The State University of New York at Buffalo, Buffalo, NY 14260, USA
[5]Present address: Kusuma School of Biological Sciences, Indian Institute of Technology, Hauz Khas, New Delhi 110016, India
[6]Present address: Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA 92037, USA
[7]Present address: Department of Biotechnology, Wroclaw Research Center EIT+, 54-066 Wroclaw, Poland
[8]Present address: Otto Diels Institute for Organic Chemistry, Christian Albrechts University, 24098 Kiel, Germany
*Correspondence: qxk2@case.edu (Q.K.), witold.surewicz@case.edu (W.K.S.)
http://dx.doi.org/10.1016/j.celrep.2013.06.030

SUMMARY

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are associated with the conformational conversion of the cellular prion protein, PrP^C, into a protease-resistant form, PrP^{Sc}. Here, we show that mutation-induced thermodynamic stabilization of the folded, α-helical domain of PrP^C has a dramatic inhibitory effect on the conformational conversion of prion protein in vitro, as well as on the propagation of TSE disease in vivo. Transgenic mice expressing a human prion protein variant with increased thermodynamic stability were found to be much more resistant to infection with the TSE agent than those expressing wild-type human prion protein, in both the primary passage and three subsequent subpassages. These findings not only provide a line of evidence in support of the protein-only model of TSEs but also yield insight into the molecular nature of the $PrP^C \rightarrow PrP^{Sc}$ conformational transition, and they suggest an approach to the treatment of prion diseases.

INTRODUCTION

Transmissible spongiform encephalopathies (TSEs) are a group of neurodegenerative disorders that include Creutzfeldt-Jakob disease in humans, scrapie in sheep, chronic wasting disease in cervids, and bovine spongiform encephalopathy in cattle (Aguzzi and Polymenidou, 2004; Caughey et al., 2009; Cobb and Surewicz, 2009; Collinge, 2001; Prusiner, 1998; Weissmann, 2004). The prion hypothesis asserts that the transmission of TSEs does not require nucleic acids, and that the infectious TSE agent is proteinaceous in nature, consisting of a misfolded form of prion protein (PrP) (Prusiner, 1982). Once heretical, this protein-only model is now supported by a growing body of evidence, most notably due to the recent success in generating infectious prions in vitro from brain-derived (Castilla et al., 2005; Deleault et al., 2007) or bacterially expressed (Kim et al., 2010; Legname et al., 2004; Makarava et al., 2010; Wang et al., 2010) PrP. However, the mechanisms involved in the conformational conversion of the normal (cellular) PrP (denoted PrP^C) to the misfolded conformer (denoted PrP^{Sc}) remain largely unknown, hindering our understanding of the molecular basis of prion diseases as well as the development of therapeutic approaches.

Cellular human PrP^C is a glycoprotein that consists of an unstructured N-terminal region and a folded C-terminal domain comprised of three α helices and two very short β strands (Zahn et al., 2000). Conversion of this protein to an abnormal PrP^{Sc} isoform occurs by a posttranslational process involving a major conformational change that results in an increased proportion of β structure (Caughey et al., 1991; Pan et al., 1993). Although the three-dimensional structure of PrP^{Sc} remains unknown, a body of evidence suggests that this conformational transition involves at least partial refolding of the helix-rich C-terminal domain (Cobb et al., 2007; Govaerts et al., 2004; Smirnovas et al., 2011). This, together with recent findings that mutations that reduce the thermodynamic stability of PrP^C greatly increase the propensity of PrP^C to undergo a conversion to oligomeric β-sheet forms in vitro (Apetri et al., 2005; Vanik and Surewicz, 2002), prompted us to search for amino acid substitutions that stabilize the native α-helical structure, with the expectation that, if the protein-only hypothesis is correct, such mutations should suppress the $PrP^C \rightarrow PrP^{Sc}$ conversion and thus attenuate replication of the infectious prion agent.

RESULTS AND DISCUSSION

A particularly dramatic increase in thermodynamic stability of PrP^C was found upon replacement of valine (V) at position 209

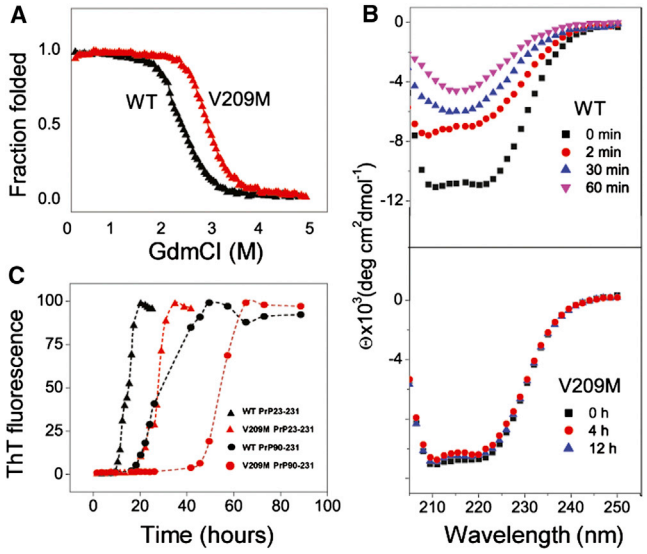

Figure 1. Effect of the V209M Mutation on the Biophysical Properties of Human PrP

(A) GdmCl-induced equilibrium unfolding for wild-type (WT) full-length human PrP (HuPrP23-231) and the V209M variant. The unfolding curves were obtained in 50 mM phosphate buffer, pH 7.

(B) Time-dependent transition of WT HuPrP90-231 and the V209M variant from α-helical structure to β-sheet oligomers as monitored by circular dichroism spectroscopy. Spectra were recorded in 50 mM sodium acetate, pH 4, in the absence of the denaturant and at different time points after the addition of 1 M GdmCl. The spectrum of the α-helical monomer shows a characteristic "double minimum" at ~210 and 220 nm, whereas the spectrum of β-sheet oligomers is characterized by lower intensity and a broad minimum around 215 nm.

(C) Time course of amyloid fibril formation for WT HuPrP and the V209M variant as monitored by thioflavine T fluorescence. Data for both full-length protein (HuPrP23-231) and HuPrP90-231 are shown. The lag phases (mean ± SD) based on three to four experiments are 6.5 ± 0.5 and 16.3 ± 1.4 hr for HuPrP23-231 and V209M HuPrP23-231, respectively, and 18 ± 2 and 42 ± 3 hr for HuPrP90-231 and V209M HuPrP90-231, respectively. See also Figure S1.

with methionine (M). As shown in Figure 1A, equilibrium unfolding of the full-length wild-type recombinant human PrP (HuPrP23-231) in guanidinium chloride (GdmCl) at pH 7 is characterized by a midpoint unfolding GdmCl concentration of 2.1 M and a free-energy difference between the native and unfolded states, ΔG^{o}, of 20.6 kJ/mol. For the V209M variant, the unfolding curve is shifted to much higher denaturant concentrations (midpoint at 2.8 M) and the ΔG^{o} value is increased to 31.9 kJ/mol. A similar effect was observed for N-truncated HuPrP90-231, with ΔG^{O} increasing from 20.9 to 30.0 kJ/mol. The observed increase of ~10 kJ/mol in the free energy of unfolding is remarkably high and, to the best of our knowledge, is among the largest reported for a single residue mutation in any protein. Thermodynamic stabilization of the native PrP structure by the Val209→Met substitution was further confirmed by thermal unfolding experiments using differential scanning calorimetry (Figure S1).

It was previously shown that under mildly acidic conditions in the presence of low concentrations of GdmCl, the N-truncated recombinant HuPrP90-231 undergoes a transition to an oligomeric β-sheet structure mimicking certain properties of PrP^{Sc} (Apetri et al., 2005; Vanik and Surewicz, 2002). Under the present experimental conditions (sodium acetate buffer, pH 4, 1 M GdmCl, protein concentration of 24 μM), the α-helix→β-sheet transition for the wild-type HuPrP90-231 was completed within ~60 min. In contrast, the V209M mutant was highly resistant to this conversion, remaining in a monomeric α-helical form even after 12 hr of incubation under identical conditions (Figure 1B).

Incubation of the recombinant PrP in the presence of GdmCl at neutral pH is known to result in the formation of thioflavine T-positive amyloid structures with fibrillar morphology (Apetri et al., 2005; Baskakov, 2004). Under the present experimental conditions, the conversion to amyloid fibrils for the wild-type HuPrP23-231 was characterized by a lag phase of 6.5 ± 0.5 hr. Again, upon replacement of Val209 with Met, this reaction became much slower, with an increased lag phase of 16.3 ± 1.4 hr (Figure 1C). For the N-truncated HuPrP90-231, the conversion reactions were slower compared with the full-length protein. However, also in this case the V209M mutation reduced the rate of the conversion reaction (lag phase of 18 ± 2 and 42 ± 3 hr for wild-type HuPrP90-231 and V209M HuPrP90-231, respectively; Figure 1C). Collectively, these data demonstrate that the V209M mutation greatly increases the thermodynamic stability of PrP^{C}, resulting in a remarkably reduced propensity of the protein to undergo a conversion to PrP^{Sc}-mimicking, β-sheet-rich aggregates in vitro.

An inspection of nuclear magnetic resonance (NMR) data indicates that the structure of wild-type human PrP (Zahn et al., 2000) is characterized by the presence of three cavities (PDB ID: 1QM0). Two of these cavities (with volumes of 57 and 14 $Å^3$ and surface areas of 76 and 29 $Å^2$) are surrounded by residues of the second and third α-helices and the loops connecting α-helix 1 with β strands 1 and 2 (Figure 2). The third cavity (18 $Å^3$, 34 $Å^2$) is located at the packing interface of helices 1 and 3. The presence of such cavities within the hydrophobic core of proteins is known to have a destabilizing effect (Eriksson et al., 1992). Our initial modeling suggested that the substitution of Met in place of Val 209 should largely eliminate the cavities in PrP, providing a rationale for improved thermodynamic stability. This prediction was verified by the NMR-determined structure (Figures 2 and S2; Table S1). Indeed, as shown in Figure 2, the structure of the V209M variant shows remarkably improved packing within the hydrophobic core, resulting in a complete removal of the two larger cavities near residue 209. This is a consequence of the different side-chain geometry of residue 209 and subtle changes in the packing and geometry of helices 1 and 3. Apart from this minor difference, the overall fold of the mutant protein remains unaltered.

To test whether thermodynamic stabilization of the folded domain of PrP is sufficient to confer resistance to prion replication in vivo, we created two lines of transgenic mice in the Friend Virus B (FVB)/PrP null background: one expressing wild-type human PrP [denoted Tg(HuPrP)] and the other expressing the superstable variant [denoted Tg($HuPrP^{V209M}$)]. Western blot analysis indicates that both lines of transgenic mice express PrP in the brain at a level similar to that observed for wild-type FVB mice, and with comparable electrophoretic profiles dominated by the diglycosylated form (Figures 3A and 3C). Confocal microscopy of primary neurons derived from Tg(HuPrP) and Tg($HuPrP^{V209M}$) mice (Figure 3B), as well as of human