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

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SUMMARY

Prion diseases, or transmissible spongiform encephalopathies (TSEs), are associated with the conformational conversion of the cellular prion protein, PrPC, into a protease-resistant form, PrPSc. Here, we show that mutation-induced thermodynamic stabilization of the folded, α-helical domain of PrPC 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 PrPC → PrPSc 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 PrPC) to the misfolded conformer (denoted PrPSc) remain largely unknown, hindering our understanding of the molecular basis of prion diseases as well as the development of therapeutic approaches.

Cellular human PrPC 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 PrPSc 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 PrPSc 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; Deleault et al., 2007; Smirnovas et al., 2011). This, together with recent findings that mutations that reduce the thermodynamic stability of PrPC greatly increase the propensity of PrPSc 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 PrPC → PrPSc conversion and thus attenuate replication of the infectious prion agent.

RESULTS AND DISCUSSION

A particularly dramatic increase in thermodynamic stability of PrPC was found upon replacement of valine (V) at position 209...
meric recombinant HuPrP90-231 undergoes a transition to an oligo-
the presence of low concentrations of GdmCl, the N-truncated
using differential scanning calorimetry (Figure S1).

tution was further confirmed by thermal unfolding experiments
for HuPrP23-231 and V209M HuPrP23-231, respectively, and 18
for a single residue mutation in any protein. Thermodynamic sta-
and, to the best of our knowledge, is among the largest reported
/10 kJ/mol in the free energy of unfolding is remarkably high
increasing from 20.9 to 30.0 kJ/mol. The observed increase of
effect was observed for N-truncated HuPrP90-231, with
57 and 14 A˚3) is located at the packing interface of helices
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 replica-
ion 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). Con-
 focal microscopy of primary neurons derived from Tg(HuPrP)
and Tg(HuPrP V209M) mice (Figure 3B), as well as of human

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 ob-
tained 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 maximum 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.

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 oligo-
ermic β-sheet structure mimicking certain properties of PrPSc
(Apetri et al., 2005; Vanik and Sureauicz, 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 conver-
sion 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 PrPSc,
resulting in a remarkably reduced propensity of the protein to un-
dergo a conversion to PrPSc-mimicking, β-sheet-rich aggregates
in vitro.

An inspection of nuclear magnetic resonance (NMR) data indi-
cates 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 A˚3
and surface areas of 76 and 29 A˚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 A˚3, 34 A˚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.

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°, 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° value is increased to 31.9 kJ/mol. A similar
effect was observed for N-truncated HuPrP90-231, with ΔG°
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 sta-
bilization of the native PrP structure by the Val209→Met sub-
titution 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 oligo-
meric β-sheet structure mimicking certain properties of PrPSc
(Apetri et al., 2005; Vanik and Sureauicz, 2002). Under the present
neuroblastoma cell line M17 transfected with either wild-type human PrP (HuPrP) or the V209M variant (HuPrP V209M) (data not shown), demonstrates that HuPrP and HuPrP V209M have similar cellular distributions, residing both at the cell surface and in intracellular compartments. Furthermore, treatment of M17 cells expressing PrP V209M with phosphatidylinositol-specific phospholipase C (PI-PLC) released HuPrP V209M into the culture medium, indicating that, like wild-type PrP, PrP V209M is attached to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor (Figure S3). Together, these data indicate that Tg(HuPrP) and Tg(HuPrP V209M) mice express comparable amounts of PrP, and that the cellular localization of PrP V209M is indistinguishable from that of the wild-type protein.

After intracerebral inoculation with brain homogenates derived from subjects with sporadic Creutzfeldt-Jacob disease (sCJD), neuroblastoma cell line M17 transfected with either wild-type human PrP (HuPrP) or the V209M variant (HuPrP V209M) (data not shown), demonstrates that HuPrP and HuPrP V209M have similar cellular distributions, residing both at the cell surface and in intracellular compartments. Furthermore, treatment of M17 cells expressing PrP V209M with phosphatidylinositol-specific phospholipase C (PI-PLC) released HuPrP V209M into the culture medium, indicating that, like wild-type PrP, PrP V209M is attached to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor (Figure S3). Together, these data indicate that Tg(HuPrP) and Tg(HuPrP V209M) mice express comparable amounts of PrP, and that the cellular localization of PrP V209M is indistinguishable from that of the wild-type protein.

After intracerebral inoculation with brain homogenates derived from subjects with sporadic Creutzfeldt-Jacob disease (sCJD),
Intriguingly, second- to fourth-passage Tg(HuPrP) mice inoculated with PrPSc(V209M) were characterized by PrPSc electromorphoretic patterns and lesion profiles that were indistinguishable from those observed in the first experiment with sCJD-inoculated Tg(HuPrP) mice, whereas distinct electromorphetic characteristics of PrPSc(V209M) and lesion profiles were consistently maintained in passages in Tg(HuPrP) mice (Figures S4C–S4E). These data strongly suggest that the PrPSc isoforms are associated with the V209M mutation and can be faithfully propagated only in the host containing this mutation, whereas in Tg(HuPrP) mice they “adapt back” to the characteristics apparently dictated by the wild-type PrP host.

All inoculated Tg(HuPrP) mice fell ill after an incubation time of ~263 days (Table 1), exhibiting classical symptoms of TSE disease. In sharp contrast, only two of six sCJDMM1-inoculated Tg(HuPrP) mice (and none of seven sCJDMM2-inoculated Tg(HuPrP) mice) became symptomatic after a very long incubation time of nearly 700 days (Table 1). As expected, western blot analysis revealed that the brains of all inoculated Tg(HuPrP) mice accumulated relatively large quantities of proteinase K-(PK)-resistant PrP, PrPSc, which electrophoretically reproduced the pattern of the original sCJD PrPSc (Figure 3C). In contrast, PrPSc was found in the brains of only two symptomatic Tg(HuPrPV209M) mice, but not in the remaining 11 symptom-free animals, even after sodium phosphotungstate precipitation treatment of brain homogenate to enrich PrPSc (Safar et al., 2000). The electrophoretic profile of PrPSc from the two infected Tg(HuPrPV209M) mice differed from that of Tg(HuPrP) mice with regard to gel mobility and the ratio of bands representing different PrPSc glycoforms (Figure 3C). Furthermore, compared with the inoculated Tg(HuPrP) mice, the two affected Tg(HuPrPV209M) mice displayed much less severe overall spongiform neurodegeneration, reduced PrP immunostaining, and significantly different lesion profiles (Figures 3D and S4A–S4D).

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The availability of two sCJD-affected Tg(HuPrP) mice provided us with the opportunity to probe this question by performing a second passage experiment. Upon inoculation with the Tg(HuPrP) mice adapted to sCJD preparations (which contained only the newly generated PrPSc(V209M) isoform), all of the Tg(HuPrP) mice (which express wild-type PrP) became infected with an average incubation of 316 days, which is longer than the 263 days of incubation needed after inoculation of the original (not passaged) sCJD preparation (Table 1). This increase in incubation time most likely can be attributed to the mutation barrier now existing between the PrPSc(V209M) of the inoculum and the PrPSc expressed by the recipient Tg(HuPrP) mice. Remarkably, despite the lack of any mutation barrier between the inoculum and Tg(HuPrPV209M) mice, the Tg(HuPrP) mice showed much slower overall spongiform neurodegeneration, reduced PrP immunostaining, and significantly different lesion profiles (Figures 3D and S4A–S4D).

The difference in incubation times between the third and fourth passages in Tg(HuPrV209MPrP) mice is statistically insignificant (p = 0.28).

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**Table 1. Primary and Secondary Transmissions of sCJD in Transgenic Mice**

<table>
<thead>
<tr>
<th>Tg Line</th>
<th>Inoculum</th>
<th>Attack Rate</th>
<th>Incubation Time (Days)</th>
<th>Titors (ID&lt;sub&gt;50&lt;/sub&gt; U/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tg(HuPrP)</td>
<td>sCJDMM1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5/5</td>
<td>316 ± 16</td>
<td>8.3 × 10&lt;sup&gt;5&lt;/sup&gt;</td>
</tr>
<tr>
<td>Tg(HuPrP)</td>
<td>sCJDMM2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5/5</td>
<td>343 ± 30</td>
<td></td>
</tr>
<tr>
<td>Tg(HuPrP)</td>
<td>sCJDMM2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5/5</td>
<td>295 ± 7&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Tg(HuPrP)</td>
<td>sCJDMM2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5/5</td>
<td>399 ± 6&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>Average values and SEMs are shown.

<sup>b</sup>The MM1 and MM2 subtypes of sCJD according to the classification of Parchi et al. (1999).

<sup>c</sup>The difference in incubation times between the third and fourth passages in Tg(HuPrP) mice is statistically insignificant (p = 0.11).

<sup>d</sup>Two mice are still alive with no apparent symptoms (at 366 and 399 days postinoculation).

<sup>e</sup>The difference in incubation times between the third and fourth passages in Tg(HuV209MP) mice is statistically insignificant (p = 0.28).

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See also Figure S4.
PrP<sup>C</sup> as observed in vitro is sufficient to inhibit prion replication in vivo. The answer to this question is clearly yes, providing a line of evidence in support of the protein-only model of TSEs. While the requirement for PrP<sup>C</sup> in TSE pathogenesis has been demonstrated in seminal experiments with PrP-knockout mice (Bueler et al., 1993), the present data show that this requirement is directly related to the ability of PrP<sup>C</sup> to undergo a conversion to the PrP<sup>Sc</sup> state, and that prion propagation in vivo can be effectively inhibited by stabilizing the normal α-helical conformation of PrP<sup>C</sup>. Given the similar electronic and hydrophobic properties of Val and Met residues, it is highly unlikely that the reduced conversion propensity of the mutant protein is due to factors other than thermodynamic stabilization. Furthermore, since residue 209 is buried within the hydrophobic interior of the protein, its substitution should not affect the interaction of PrP<sup>C</sup> with other cellular cofactors in vivo.

The finding that thermodynamic stabilization of the native α-helical domain of PrP<sup>C</sup> inhibits prion propagation in mice impacts the ongoing debate regarding critical yet still unresolved issues in prion research: the mechanism of PrP conversion and the structure of the infectious PrP<sup>Sc</sup> conformer. Although some models postulate that the C-terminal region of PrP<sup>Sc</sup> retains at least partial native α-helical structure of PrP<sup>C</sup> (DeMarco and Daggett, 2004; Govaerts et al., 2004), recent data indicate that PrP conversion involves major refolding of the entire C-terminal region, and that this region in PrP<sup>Sc</sup> consists of β strands and short turns/loops, with no α helices present (Smirnovas et al., 2011). The present finding is consistent with the latter scenario, providing strong support to the notion that PrP conversion in vivo involves a major rearrangement of the α-helical domain of PrP<sup>C</sup>.

Finally, the present data suggest a strategy for pharmacological intervention in prion diseases. Although a number of immuno- and chemotherapeutic approaches have been proposed (Cashman and Caughey, 2004), there is currently no effective treatment for TSEs. A promising new strategy has recently emerged, based on the downregulation of PrP<sup>C</sup> expression using small interfering RNA (Pfeifer et al., 2006). However, this approach may carry significant risks given the reported role of PrP<sup>C</sup> in cell signaling, neuroprotection, and other physiological processes (Westergard et al., 2007). The finding that one can effectively reduce prion replication in vivo by stabilizing the native conformation of PrP<sup>C</sup> without manipulating its expression level offers an alternative and potentially more practical strategy based on screening and/or rational design of small molecules that can thermodynamically stabilize the normal conformation of monomeric PrP<sup>C</sup>. Recently, a generally similar approach was successfully used to develop a clinically effective drug against transthyretin amyloidosis, even though in the latter case the stabilization is kinetic rather than thermodynamic, preventing dissociation of the transthyretin tetramer (Bulawa et al., 2012).

**EXPERIMENTAL PROCEDURES**

**Protein Purification**

Recombinant human PrP 90-231 (HuPrP90-231) and the V209M mutant (both 129M variants) were prepared and purified as described previously (Apetri et al., 2005). Uniformly 15N- and 13C/15N-labeled proteins for NMR spectroscopy were expressed in *Escherichia coli* using a minimal medium containing 13C6 glucose (3 g/l) and/or 15N NH4Cl (1 g/l) as the sole carbon and nitrogen sources, respectively.

**Biophysical Experiments**

Equilibrium unfolding experiments were performed with the use of circular dichroism spectroscopy (50 mM phosphate buffer, pH 7) by monitoring GdmCl-induced changes in ellipticity at 220 nm as described previously (Apetri et al., 2005). The unfolding curves were analyzed using a two-state transition model (Santoro and Bolen, 1988). The conversion of PrP variants to β-sheet oligomers was performed in 50 mM sodium acetate buffer containing 1 M GdmCl, pH 4, and monitored by circular dichroism spectroscopy as described previously (Apetri et al., 2005). The concentration of each protein in these experiments was 24 μM. The conversion of the proteins to amyloid fibrils at neutral pH (50 mM phosphate buffer, 1.5 M GdmCl, pH 7, protein concentration of 30 μM) was performed and monitored by thioflavin T assay as described previously (Apetri et al., 2005).

**Structural Studies**

The structure of V209M human PrP was determined by NMR spectroscopy. Details of these experiments are provided in Extended Experimental Procedures.

**Transgenic Mice**

HuPrP and HuPrP<sup>V209M</sup> transgene constructs were based on the murine half-genomic PrP clone (HGPRP) (Fischer et al., 1996). Tg40, a Tg(HuPrP) line that expresses wild-type human PrP-129M at the same level as the murine PrP in wild-type FVB mice, was described previously (Kong et al., 2005). Tg(HuPrP<sup>V209M</sup>) mice were created in the same fashion and bred with FVB/Pr<sup>mg</sup>120 mice to obtain Tg(HuPrP<sup>V209M</sup>)Pr<sup>mg</sup>120 mice. The transgene expression in brain was examined by western blotting analysis using monoclonal antibody (mAb) 3F4 or 8H4 as described below. Tg20, a Tg(HuPrP<sup>V209M</sup>) line expressing the mutant PrP at the wild-type FVB mouse level, was used for all transmission experiments. All transgenic mice used were in the Prnp<sup>0/0</sup> background.

**Inoculation and Examination of Transgenic Mice**

Frozen brain tissues from human subjects with sCJD or prion-infected transgenic mice were homogenized in PBS and inoculated intracerebrally into the brains of Tg(HuPrP) and Tg(HuPrP<sup>V209M</sup>) mice as previously described (Kong et al., 2005). Thirty microliters of 1% brain homogenate was injected into each animal. Prion symptoms were monitored as previously described (Kong et al., 2005) and the animals were sacrificed within 2–3 days after the appearance of symptoms. Each brain was sliced sagittally; half was frozen for western blot analysis (see below) and half was fixed in formalin for staining with hematoxylin and eosin or anti-PrP antibody 3F4 (Castellani et al., 1996; Kong et al., 2005). The prion titers of the sCJD or mouse brain tissues were calculated according to the incubation time using a previously described method based on endpoint titration of sCJDMM1 in Tg40 mice (Prusiner et al., 1982).

**Western Blotting Analysis and Detection of PK-Resistant PrP**

Total protein concentrations of brain homogenates in the lysis buffer (100 mM Tris, 150 mM NaCl, 0.5% Nonidet P-40, 0.5% sodium deoxycholate, 10 mM EDTA, pH 7.8) were measured with BCA Protein Assay Reagents (Thermo Fisher Scientific). Total PrP and PK-resistant PrP<sup>sc</sup> were examined by western blotting using precast SDS-PAGE gels with 8H4 or 3F4 antibodies in conjunction with horseradish-peroxidase-conjugated goat anti-mouse IgG Fc antibody essentially as described previously (Pan et al., 2001). PK digestion was performed for 1 hr at 37°C using 100 μg/ml of the enzyme.

**Confocal Immunofluorescence Imaging of Primary Neurons**

Primary cortical neuron cultures were prepared from day 14–16 embryos of Tg(HuPrP) and Tg(HuPrP<sup>V209M</sup>) mice (Yuan et al., 2008). Cell surface and intracellular PrP was examined with a Zeiss LSM510 inverted confocal microscope using mAb 3F4 and goat anti-mouse IgG (H+L) conjugated with highly cross-absorbed Alexa Fluor 488 (Invitrogen) as described previously (Mishra et al., 2002).
prion protein 90-231. Biochemistry 129 modulates the conformational conversion of the D178N variant of human

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