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Short Communication

Truncated PrP^c in mammalian brain: interspecies variation and location in membrane rafts

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Abstract

A key molecular event in prion diseases is the conversion of cellular prion protein (PrP^c) into an abnormal misfolded conformer (PrP^{sc}). The PrP^c N-terminal domain plays a central role in PrP^c functions and in prion propagation. Because mammalian PrP^c is found as a full-length and N-terminally truncated form, we examined the presence and amount of PrP^c C-terminal fragment in the brain of different species. We found important variations between primates and rodents. In addition, our data show that the PrP^c fragment is present in detergent-resistant raft domains, a membrane domain of critical importance for PrP^c functions and its conversion into PrP^{sc}.

Keywords: cellular prion protein; cerebral cortex; mammals; raft; truncation.

Prion diseases are characterized by the accumulation of an abnormal misfolded conformer (PrP^{sc}) of the cellular prion protein (PrP^c). PrP^c is a 209-aa (residues 23–231) protein that is highly expressed in the central nervous system (Prusiner, 1998). Although various functions have been proposed for PrP^c, such as copper metabolism (Brown, 2003), synaptic function (Maglio et al., 2004), cell signaling (Mouillet-Richard et al., 2000) and axon regeneration (Moya et al., 2005), its exact physiological role remains elusive. Identified ligands of PrP^c include heat-shock proteins (Edenhofer et al., 1996), membrane-bound receptors (Gauczynski et al., 2001) and heparan sulfate (Shyng et al., 1995). Several PrP^c properties, such as copper binding (Brown et al., 1997), heparan sulfate binding (Chen et al., 1995) and indirect interaction with the 37-/67-kDa laminin receptor (Hundt et al., 2001), have been shown to be mediated through its N-terminal domain. However, in normal metabolism, the N-terminal region of PrP^c can be lost by cleavage. In the human brain, cleavage occurs around amino acid 110 and generates a highly stable membrane-associated fragment called C1 (Shyng et al., 1993; Chen et al., 1995). Thus, cleavage may provide a mechanism for downregulating PrP^c activities mediated by its N-terminal domain at the cell surface. It is therefore important to determine which of the PrP^c forms, either full-length or truncated, is predominant in the mammalian brain. PrP^c possesses a glycosylphosphatidylinositol (GPI)-anchor and, like other GPI proteins, is found in raft-like microdomains of the plasma membrane (Madore et al., 1999). Raft location of PrP^c is a prerequisite for its involvement in signal transduction, as shown by Hugel et al. (2004) in T-cells, and is of critical importance for its conversion into PrP^{sc} (Taraboulos et al., 1995). However, the precise location of the C-terminal fragment of PrP^c at the cell membrane is unknown.

Here, we examined the electrophoretic pattern of PrP^c and looked for the membrane compartment containing the C-terminal PrP^c fragment in several species commonly used as *in vivo* models of prion infections. Two specific anti-PrP antibodies, SAF32 (epitope 59–89) and SAF60 (epitope 157–161), detected several bands representing heterogeneous glycosylation of PrP^c (27–34 kDa; Figure 1A). After enzymatic deglycosylation, SAF32 detected a PrP^c band of 27 kDa corresponding to the full-length form and SAF60 detected an additional band at 18 kDa (N-terminally truncated form of PrP^c). In hamsters, rats, mice, microcebus and marmosets, SAF60 gave a strong signal for the full-length PrP^c and a less intense signal for the C-terminal fragment of PrP^c. In macaques, the intensity of the corresponding signal for truncated PrP^c was greater, while truncated PrP^c was the most abundant of the two PrP^c forms in baboons. All four antibodies used gave very consistent PrP^c patterns, although SAF83 and SAF84 were not able to recognize PrP^c from all species (Figure 1B). The analysis of several individuals in each species yielded consistent results (Figure 1C). We investigated whether the C-terminal fragment observed was the result of *in vitro* lysis. Figure 2 shows that there was no variation in the amount of PrP^c fragment, even after a 4-h incubation at 20°C. These results clearly indicate that there was no detectable *in vitro* formation of PrP^c fragment under our experimental conditions. We also examined whether the C-terminal

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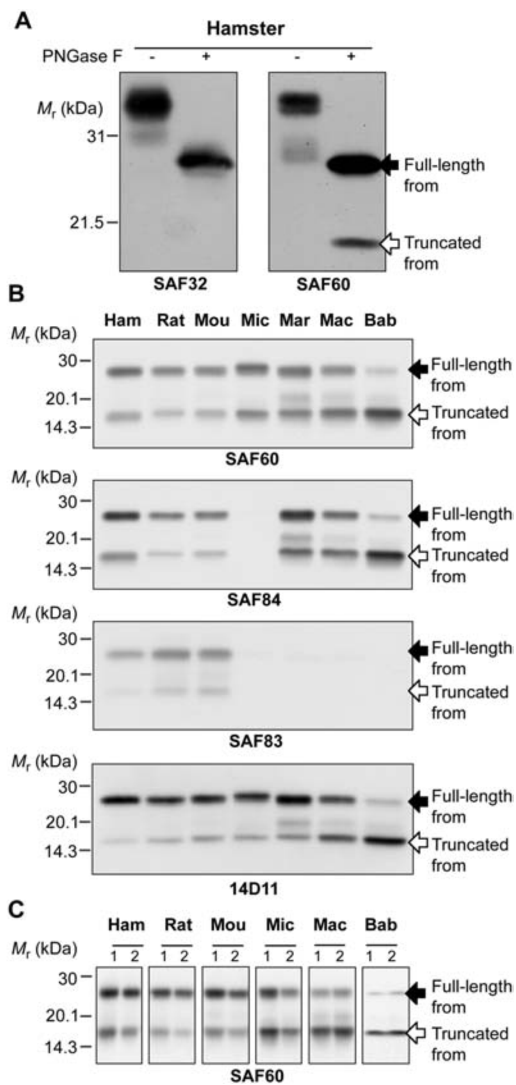

Figure 1 Electrophoretic pattern of PrP^{c} in cerebral isocortex. Brain isocortex samples from adult rodents or primates were homogenized in 10 mM Tris, pH 7.4 containing protease inhibitors (P8340, Sigma, St. Louis, MO, USA). Protein concentration was measured using the Lowry method. Proteins were deglycosylated by incubation with peptide *N*-glycosidase F (PNGase F, P0704L, New England Biolabs, Beverly, MA, USA) and separated on 12% SDS-PAGE. PrP^{c} was detected using specific antibodies: SAF32, SAF60 and SAF84, which recognize an epitope at human PrP residues 59–89, 157–161, and 161–164, respectively; SAF83, which recognizes solid-phase immobilized peptide 126–164, but not peptide 142–160 (CEA/Saclay, Gif-sur-Yvette, France); and mouse monoclonal 14D11 (Roboscreen, Leipzig, Germany) developed against a recombinant human PrP, which is reactive to the aa 180–200. Mouse monoclonal SAF antibodies were raised against scrapie-associated fibrils from Syrian hamster-infected brain (263K; Demart et al., 1999). (A) A sample of 80 μg of hamster homogenate was separated by SDS-PAGE. PrP^{c} was detected using SAF32 or SAF60 antibodies. (B) Electrophoretic pattern of PrP^{c} from cerebral isocortex of various mammals. Samples of 20 μg of homogenate protein from the brain of different animal species were separated by SDS-PAGE. PrP^{c} was detected using SAF60, SAF84, SAF83 or 14D11 antibodies. (C) Comparison of the inter-individual variability in PrP^{c} electrophoretic patterns. Samples of 5 μg of protein homogenate (two specimens per species) were separated by SDS-PAGE. PrP^{c} was detected using SAF60 antibody. Abbreviations: Ham, hamster; Mou, mouse; Mic, microcebus; Mar, marmoset; Mac, macaque; Bab, baboon.

fragment of PrP^{c} was present in raft domains in the isocortex of baboons and hamsters (Figure 3A, B). Cholera toxin B subunit was used to identify the fractions enriched in the lipid raft marker GM1, and anti-transferrin receptor antibody was used to identify non-raft fractions. We observed a distribution of full-length PrP^{c}, in agreement with the data reported by Madore et al. (1999). The distribution of N-truncated PrP^{c} in the different membrane fractions closely followed that of full-length PrP^{c}. The N-truncated PrP^{c} was mainly present in raft fractions in the two species studied.

Our comparison of PrP^{c} patterns from brains of different mammals using four different monoclonal antibodies shows consistent differences in the proportion of the C-terminal fragment, suggesting that PrP^{c} metabolism varies strongly among species. Some proposed cell functions for PrP^{c}, such as the regulation of copper concentration at the synapse (Brown, 2003), the activation of survival signaling pathways (Vassallo et al., 2005), and the modulation of L-type voltage-gated calcium channels (Korte et al., 2003), have been shown to be mediated by its N-terminal domain. Therefore, N-terminal cleavage may result in downregulation of such functions. It thus appears important to take into account the differences of PrP^{c} cleavage between species when using animal experimental models to explore PrP^{c} physiological roles. In addition, it must be stressed that PrP^{c} functions are generally studied using murine models and overexpression strategies that probably lead to underestimation of the potential role of the C-terminal fragment of the protein, the major PrP^{c} form in non-human primates and in some human individuals (Laffont-Proust et al., 2005).

The mammalian disintegrin metalloprotease ADAM10 has been shown to cleave endogenous PrP^{c} in human embryonic kidney 293 stable transfectants (Vincent et al., 2001). In addition, we have shown that in the human brain, high amounts of cleaved PrP^{c} correlated with the presence of detectable active ADAM10 (Laffont-Proust et al., 2005). Thus, we hypothesize that interspecies variations in mature ADAM10 activity may be responsible for the differences in PrP^{c} cleavage described here.

Our results show that the C-terminal fragment, as well as full-length PrP^{c}, was mainly present in light-density raft fractions from brain. Before endocytosis, PrP^{c} quits rafts (Sunyach et al., 2003) and, during the endocytotic cycle, a portion of PrP^{c} is cleaved, leaving a C-terminal fragment at the cell surface (Shyng et al., 1993). Our results suggest that once at the cell surface, cleaved PrP^{c}, without the lipid raft-targeting determinant (aa 23–90; Walmsley et al., 2003), returns to raft domains. This suggests the presence of additional raft determinants within the aa 110–231 PrP^{c} sequence. The presence of truncated PrP^{c} in raft domains is important when considering putative functions of PrP^{c} fragment. Recruitment of PrP^{c} molecules into rafts is important for PrP^{c} engagement in signaling pathways (Hugel et al., 2004). Furthermore, since the presence of PrP^{c} within rafts is required for its conversion into PrP^{sc} (Taraboulos et al., 1995), the presence of the truncated form of the protein in rafts may be of critical importance to this process. The PrP^{c} fragment could alter the interaction between full-length PrP^{sc} and PrP^{c}, and in this regard it is interesting

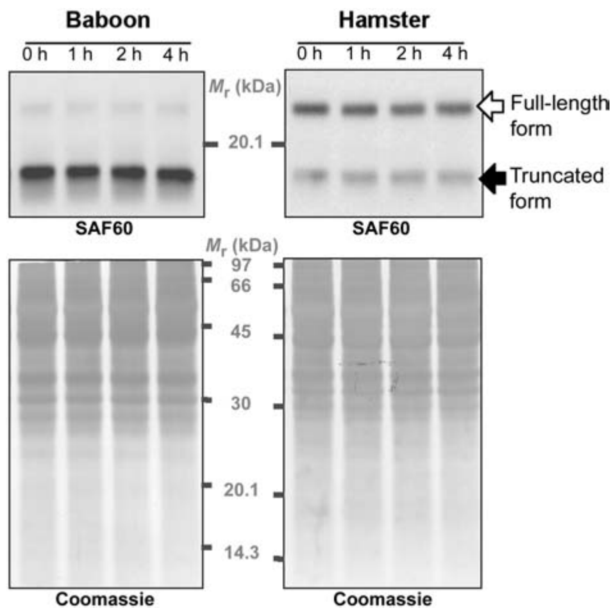

Figure 2 Control of the absence of PrP^c lysis *in vitro*.
Isocortex homogenates from baboons (left panel) and hamsters (right panel) were incubated for 0–4 h at 20°C. Homogenates were treated with PNGase F and samples of 10 μg of protein were separated by SDS-PAGE. PrP^c was detected using SAF60 antibody. Coomassie Brilliant Blue staining of the poly(vinylidene difluoride) filter is shown as a loading control.

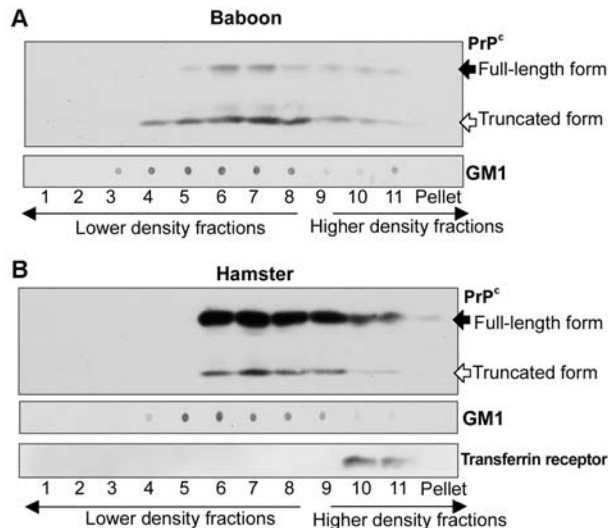

Figure 3 Partitioning into raft-like domains of PrP^c in baboon (A) and hamster (B) cerebral isocortex.
Tissues were homogenized (30 mg/ml) in 10 mM Tris, pH 7.4, 0.01 M EDTA, 0.1 M NaCl and 2% Triton X-100. The homogenates were brought to 40% sucrose, placed in an ultracentrifuge tube, and overlaid by 30–5% sucrose solutions to form a sucrose step gradient. The samples were centrifuged in a Beckman SW41 Ti rotor (Beckman Coulter, Fullerton, CA, USA) for 18 h at 200 000 *g* and 4°C. A total of 11 fractions were collected from the top of the gradient. Western blotting of deglycosylated PrP^c was performed using SAF60 antibody and dot-blotting was performed using peroxidase-conjugated cholera toxin (C4672, Sigma) to detect GM1 ganglioside. A Western blot of transferrin receptor (13-6890, Zymed, San Francisco, CA, USA) is shown as a non-raft control.

to note that the N-terminal region of PrP^c modulates prion propagation (Flechsig et al., 2000; Lawson et al., 2001; Weissmann and Flechsig, 2003).

In summary, our results demonstrate important interspecies variability in the abundance of truncated brain PrP^c among rodents and primates, and that truncated PrP^c is mainly present in detergent-resistant fractions. These results have implications for the study of PrP^c functions and prion propagation.

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