doi:10.1016/j.jmb.2008.08.045 J. Mol. Biol. (2008) 383, 306–312

JMB

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COMMUNICATION

NMR Structure of the Bank Vole Prion Protein at 20 °C Contains a Structured Loop of Residues 165–171

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Received 7 July 2008; received in revised form 18 August 2008; accepted 19 August 2008
Available online
26 August 2008

The recent introduction of bank vole (*Clethrionomys glareolus*) as an additional laboratory animal for research on prion diseases revealed an important difference when compared to the mouse and the Syrian hamster, since bank voles show a high susceptibility to infection by brain homogenates from a wide range of diseased species such as sheep, goats, and humans. In this context, we determined the NMR structure of the C-terminal globular domain of the recombinant bank vole prion protein (bvPrP) [bvPrP(121–231)] at 20 °C. bvPrP(121–231) has the same overall architecture as other mammalian PrPs, with three α-helices and an antiparallel β-sheet, but it differs from PrP of the mouse and most other mammalian species in that the loop connecting the second β-strand and helix α2 is precisely defined at 20 °C. This is similar to the previously described structures of elk PrP and the designed mouse PrP (mPrP) variant mPrP[S170N,N174T](121–231), whereas Syrian hamster PrP displays a structure that is in-between these limiting cases. Studies with the newly designed variant mPrP[S170N](121–231), which contains the same loop sequence as bvPrP, now also showed that the single-amino-acid substitution S170N in mPrP is sufficient for obtaining a well-defined loop, thus providing the rationale for this local structural feature in bvPrP.

Keywords: bank vole; prion protein; NMR structure; transmissible spongiform encephalopathies; species barrier

Edited by M. F. Summers

Introduction

The bank vole (*Clethrionomys glareolus*) has recently attracted interest due to unexpected features revealed by its use as a new laboratory animal for the investigation of prion diseases. When compared to the mouse and the Syrian hamster, the bank vole is found to be highly susceptible to infection by sheep and goat scrapie, and various human strains of transmissible spongiform encephalopathy (TSE) also show a low transmission barrier.[1–3] Responding to the ensuing interest in the three-dimensional (3D) structure of bank vole prion protein (bvPrP), this article describes the NMR structure of the C-terminal globular domain in the healthy cellular form of bvPrP ($bvPrP^C$) at 20 °C. To support the analysis of the structure of bvPrP(121–231), we further solved the structure of a designed mouse PrP (mPrP) variant, mPrP[S170N](121–231) (polypeptide segment of residues 121–231 of the variant of mPrP with Ser170 replaced by Asn; see Schätzl *et al.*[4] for the numeration used), and pursued a detailed comparison of the two structures.

Prion diseases, or TSEs, are a group of neurodegenerative diseases that include scrapie in sheep, Creutzfeldt–Jakob disease in humans, bovine spongiform encephalopathy in cattle, and chronic wasting disease in mule deer and elk.[5–7] Critical events in the development and propagation of TSEs

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Abbreviations used: bvPrP, bank vole prion protein; mPrP, mouse PrP; TSE, transmissible spongiform encephalopathy; 3D, three-dimensional; $bvPrP^C$, cellular form of bvPrP; PrP^C, cellular PrP; ePrP, elk PrP; NOE, nuclear Overhauser enhancement; shPrP, Syrian hamster PrP.

include the conversion of cellular PrP (PrP^C) into a protease-resistant aggregated isoform, PrP^{Sc}, which accumulates most pronouncedly in neurologic tissues of the affected organisms.[5] While TSEs can be transmitted within the same species, "transmission barriers" make interspecies transmission inefficient, as evidenced either by complete absence of detectable transmission or by prolonged incubation periods prior to onset of clinical signs.[8] The occurrence of "new variant Creutzfeldt–Jakob disease" in humans in the 1990s, which was attributed to the prion strain identified in TSE-infected cattle in the 1980s,[9–12] emphasized the critical importance of transmission barriers and the possibility of their breakdown for food management and public health services.

The amino acid sequence homology between PrP of the host and PrP of the donor is the basic determinant of the barrier between different species,[8] whereby single-amino-acid exchanges can cause a significant change in the susceptibility to infection.[8,13] It has also been argued that the efficiency of transmission could further be affected by variation in the PrP^C conformations of the donor and the recipient.[14] The 3D structures of PrP^C from various species, including humans, cattle, sheep, elk, and the widely used laboratory animals hamster and mouse, have been solved by solution NMR.[15–22] The global PrP^C architectures in all mammalian species studied so far are nearly identical, with a flexibly extended 100-residue N-terminal tail and a 100-residue globular C-terminal domain containing three α-helices and an antiparallel β-sheet. Nonetheless, subtle local structure variations between different species were observed in the globular domain, in particular in a loop formed by residues 165–171, which link the second β-strand and helix α2. At 20 °C, the β2–α2 loop is precisely defined in PrP^C from elk (ePrP), but disordered in the NMR structures of PrP^C from a variety of other species, including human, cattle, sheep, and mouse, and is partially ordered in PrP^C from Syrian hamster at 30 °C. This special structural feature in ePrP(121–231) was attributed to the nature of the amino acid residues in positions 170 and 174, since the 3D structure of the designed mouse variant mPrP [S170N,N174T](121–231) also includes a precisely structured loop of residues 165–171 at 20 °C.[21]

Cloning, expression, and purification of bvPrP (121–231) and mPrP[S170N](121–231)

The amino acid sequence of bvPrP(121–231) differs from that of mPrP(121–231) at only four positions (Fig. 1). Therefore, we used site-directed mutagenesis to replace these four residues in mPrP (121–231) in order to obtain the gene encoding bvPrP (121–231). Similarly, we used site-directed mutagenesis with mPrP(121–231) to generate mPrP [S170N](121–231). For the NMR structure determinations of bvPrP(121–231) and mPrP[S170N](121–

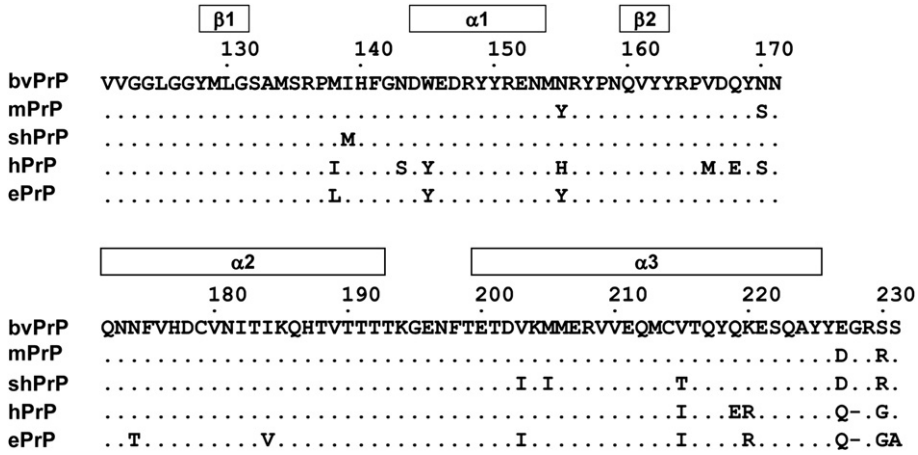

Fig. 1. Amino acid sequence comparison of the C-terminal globular domain, spanning residues 121–231, of bvPrP (*C. glareolus*, GenBank AAL57231) with mPrP (*Mus musculus*, GenBank AAA39997), shPrP (*Mesocricetus auratus*, GenBank AAA37091), human PrP (hPrP, *Homo sapiens*, GenBank AAA60182), and ePrP (*Cervus elaphus elaphus*, GenBank AAU93885). The complete sequence of bvPrP is given, and the numeration of bvPrP and the locations of the regular secondary structures in bvPrP are indicated at the top. For the other species, only amino acids that are different from bvPrP are indicated, with dots indicating the presence of identical amino acids and with dashes indicating deletions. The mPrP variant mPrP[S170N](121–231) was generated from mPrP(121–231) by using the QuikChange® site-directed mutagenesis kit (Stratagene) in accordance with the manufacturer's instructions, with the two primers 5′-CCA GTG GAT CAG TAC AAC AAC CAG AAC ACC-3′ and 5′-GGT GTT CTG GTT GTT GTA CTG ATC CAC TGG-3′. As bvPrP(121–231) differs from mPrP(121–231) by only four amino acid substitutions (Y155N, S170N, D227E, and R230S), its sequence was generated by three subsequent mutagenesis steps, starting from the plasmid encoding mPrP[S170N](121–231). The primers 5′-CGT GAA AAC ATG AAC CGC TAC CCT A-3′ and 5′-T AGG GTA GCG GTT CAT GTT TTC ACG-3′ were used for mutagenesis Y155N; 5′-G GCC TAT TAC GAA GGG CGT CGT TCC-3′ and 5′-GGA ACG ACG CCC TTC GTA ATA GGC C-3′ for D227E; 5′-GCC TAT TAC GAA GGG CGT AGC TCC TAG TAA GAA TTC GAA GCT TGA-3′ and 5′-TCA AGC TTC GAA TTC TTA TCA GGA ACT ACG CCC TTC GTA ATA GGC-3′ for R230S.