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Series A: Life Sciences

Prions and Brain Diseases in Animals and Humans

Edited by

Douglas R. O. Morrison

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transition is likely to result from local rearrangement of the N-terminus around trp-99. The stability of the structure in this region is significantly greater in the PrP^{Sc} isoform than in PrP^C as reflected in the higher concentration of GuHCl for the mid-point of the transition (1.5M as opposed to 0.5M GuHCl).

This implies the N-terminus of our expressed fragment may be a crucial area of sequence involved in early folding events in the conversion of PrP^C to PrP^{Sc}. To further investigate the folding of PrP^C and conversion to PrP^{Sc} we intend to create site-directed mutations in the PrP gene that are known to be implicated in prion disease. The identification of differences in the folding pathways of these mutants should be very revealing as to the primary event involved in the misfolding of PrP^C leading to the formation of PrP^{Sc}.

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AUTONOMOUS FOLDING AND THREE-DIMENSIONAL STRUCTURE OF THE CARBOXY-TERMINAL DOMAIN OF THE MOUSE PRION PROTEIN, PrP(121- 231)

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INTRODUCTION

The "protein-only" hypothesis (Alper et al., 1967; Griffith, 1967; Prusiner, 1982) states that the infectious agent of mammalian transmissible spongiform encephalopathies, such as *Scrapie* in sheep, bovine spongiform encephalopathy (BSE), and the Creutzfeldt-Jakob disease (CJD), the Gerstmann-Sträussler-Scheinker syndrome (GSS), fatal familial insomnia (FFI) and Kuru in man, is mainly, if not entirely, composed of a single protein, the "scrapie" form of the prion protein, PrP^{Sc} (Prusiner, 1982; Prusiner, 1991; Weissmann, 1994; Weissmann, 1996). This infectious form of the prion protein, PrP, is believed to be chemically identical to its monomeric, cellular form, PrP^C (Stahl & Prusiner, 1991), but PrP^{Sc} appears to possess a different three-dimensional structure with increased β -sheet content (Pan et al., 1993) and forms oligomeric, protease-resistant aggregates (Prusiner, 1982; Bolton et al., 1982).

Mammalian PrP^C is a cell surface protein which occurs in neurons and most other tissues. PrP^C has been reported, from observations with certain strains of PrP knock-out mice, to be necessary for normal synaptic function (Collinge et al., 1994), long-term survival of Purkinje neurons (Sakaguchi et al., 1996), and the regulation of circadian activity rhythms and sleep (Tobler et al., 1996). However, knockout mice derived from other strains do not show neurological disorders (Ch. Weissmann, private communication; Lledo et al., 1996). The known amino acid sequences of mammalian prion proteins are highly conserved, and pairs of sequences are generally more than 90 percent identical (Schätzl et al., 1995). PrP^C is a secretory protein and undergoes several posttranslational modifications. The mature form of murine PrP^C consists of 208 amino acids (residues 23 to

231, with deletion of codon 55) (Westaway *et al.*, 1987). The protein has a single disulfide bond between residues 179 and 214, two N-glycosylation sites at residues 181 and 197 and is attached to the cell surface via a glycosyl-phosphatidyl-inositol (GPI) anchor at the carboxy-terminal serine 231 (Stahl & Prusiner, 1991) (amino acid numbering according to hamster PrP, see Schätzl *et al.*, 1995).

The findings that mice devoid of PrP are resistant to infection by prions (Büeler *et al.*, 1993), that inherited prion diseases in humans are associated with mutations in the prion protein gene (Prusiner, 1993), and that a spontaneous prion disease occurs in uninoculated, transgenic mice expressing PrP with a substitution analogous to that observed in an inherited human GSS case (Hsiao *et al.*, 1989) are presumably the most convincing *in vivo* data supporting the protein-only hypothesis. Despite these results, the protein-only hypothesis cannot be considered as proven, since extensive attempts to generate infectious prion material *in vitro* have failed so far. However, excess PrP^{Sc} has recently been shown to convert PrP^C into an insoluble form *in vitro* which exhibits biochemical properties such as protease resistance similar to PrP^{Sc} (Kocisko *et al.*, 1994; Bessen *et al.*, 1995). The failure to produce infectious prions *in vitro* may also be explained by the absence of an essential host factor, for which indirect evidence was obtained recently (Telling *et al.*, 1995).

Two different mechanisms for the generation of an infectious prion particle from PrP^C are presently discussed: A first model proposes that initial transformation of PrP^C to PrP^{Sc} is rate-limiting and that subsequent autocatalytic formation and polymerization of PrP^{Sc} is fast (Prusiner, 1991). In a second model, a rapid equilibrium between monomeric PrP^C and monomeric PrP^{Sc} is proposed, and the rate-limiting step would be the formation of a stable, ordered aggregate of PrP^{Sc} molecules, which would act as a nucleus for growth of the infectious prion (Jarret and Lansbury, 1993).

A prerequisite for understanding the processes underlying the conversion of PrP^C to PrP^{Sc} is the knowledge of folding, thermodynamic stability and the three-dimensional structure of PrP^C. Thermodynamic and structural studies of PrP^C in solution have however been hampered in the past by its low solubility in the absence of detergents and the low yields of PrP^C obtained after purification from its natural source.

In this article, we describe a recombinant carboxy-terminal fragment of residues 121-231 in mouse PrP which represents an intrinsically stable domain of PrP. PrP(121-231) is monomeric, refolds spontaneously and quantitatively after chemical denaturation with guanidinium chloride (GdmCl) and has a free energy of folding of - 22 kJ/mol (Hornemann & Glockshuber, 1996). Its high solubility in water allowed the determination of its three-dimensional structure in solution by nuclear magnetic resonance (NMR) spectroscopy (Wüthrich, 1986). We show that PrP(121-231) possesses a so far unknown protein fold and, in contrast to model predictions of an all-helical structure of PrP^C, contains an antiparallel β -sheet. Implications of the three-dimensional structure of PrP(121-231) will be discussed in conjunction with the formation of PrP^{Sc} from PrP^C, the location of mutated residues

associated with inherited human prion diseases, and the species barrier of prion disease transmission.

A THEORETICAL THREE-DIMENSIONAL MODEL OF PrP^C AND THE IDENTIFICATION OF THE CARBOXY-TERMINAL DOMAIN OF MOUSE PrP, PrP(121-231)

The primary structures of mature, mammalian prion proteins share a rather unusual sequence motif in the amino-terminal part of the protein. It consists of an octapeptide sequence which is repeated five times in most of the cases and has a high glycine content of about 50 percent (Schätzl *et al.*, 1995). The amino-terminal segment of PrP^C may therefore lack a well-defined three-dimensional fold. In any case, attempts failed to predict regular secondary structures within the amino-terminal part of the protein (residues 23-108) including this characteristic, five-fold octapeptide repeat (Huang *et al.*, 1994). However, plausible three-dimensional models for the carboxy-terminal segment of PrP comprising residues 108 to 218 were presented (Huang *et al.*, 1994; Huang *et al.*, 1996). These models predict that the segment 108-218 in PrP^C forms a four-helix bundle.

To study folding and stability of the predicted four-helix bundle domain PrP(108-218), we recombinantly expressed two segments of mouse PrP, comprising residues 95-231 and 107-231, respectively, in *Escherichia coli*. Both segments were fused to the bacterial OmpA sequence for secretory expression to allow formation of the single disulfide bond in the oxidizing environment of the periplasm of *Escherichia coli* under the control of the T7 promoter (Studier & Moffat, 1986). Large amounts of soluble protein were obtained in the periplasmic fraction. However, Edman sequencing of the proteins revealed that both fragments 95-231 and 107-231 were amino-terminally degraded *in vivo*. All cleavage sites were found within the predicted first helix of PrP^C (amino acids 109-122), after residues 112, 118, and 120 (Hornemann & Glockshuber, 1996). This finding was surprising since proteolytic cleavage of folded proteins generally occurs at domain borders or within exposed loop regions, but not within regular secondary structures (Price & Johnson, 1993). We thus assumed that the segment 121-231, which is resistant to degradation in the periplasm of *E. coli*, represents an intrinsically stable domain and that residues 108-120 are not part of this domain.

In order to obtain the presumed carboxy-terminal domain PrP(121-231) with homogeneous amino-terminus, the segment 121-231 was directly fused to the OmpA signal sequence. To allow efficient cleavage of the signal sequence *in vivo* and to avoid a negative charge at the carboxylate to Ser231, recombinant PrP(121-231) was elongated by an additional serine residue at both the amino- and carboxy-terminus.

Periplasmic expression yielded a soluble 13.3 kDa protein. PrP(121-231) could be purified by conventional chromatographic techniques in the absence of any detergents by anion exchange chromatography, hydrophobic chromatography and gel filtration (Hornemann & Glockshuber, 1996). HPLC-analysis before and after reduction of purified

PrP(121-231) with dithiothreitol revealed that the single disulfide bond was formed quantitatively. The mass of the 113-residue protein was verified by electrospray mass spectrometry (error: 1 Da). PrP(121-231) proved to be soluble at concentrations up to 1 mM in distilled water without aggregation between pH 4 and 8.5 (Hornemann & Glockshuber, 1996). Analytical gel filtration on a Superdex 75 HR column revealed that the protein was a monomer in solution, even at high protein concentrations (Figure 1).

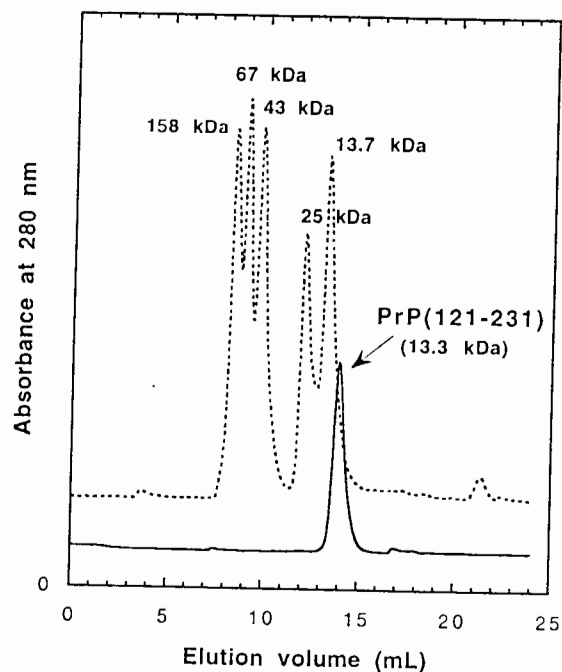


Figure 1. Analytical gel filtration of PrP(121-231) on a Superdex 75 HR column (30 x 1 cm). Gel filtration was performed at room temperature at a flow rate of 0.2 ml/min in 50 mM sodium phosphate, pH 7.0. The protein concentration of the applied sample (50 μ l) was 0.1 mM. The solid line corresponds to the elution profile of purified PrP(121-231). The dashed line represents the elution profile of a mixture of standard proteins with known molecular mass.

Far-UV circular dichroism (CD) spectra of purified PrP(121-231) revealed two minima at 208 and 222 nm and a mean residue ellipticity of $-15600 \text{ deg cm}^2/\text{dmol}$ at 222 nm (Hornemann & Glockshuber, 1996), which demonstrate a relatively high content of α -helices (Johnson Jr., 1994) (Figure 2). Comparison with the far-UV CD spectrum of intact PrP^C from hamster (Pan *et al.*, 1993) indicates that the relative content of regular secondary structure is significantly higher in PrP(121-231). In addition, a characteristic near-UV CD spectrum was obtained for PrP(121-231), which is diagnostic for the presence of tertiary structure (Figure 2, inset).

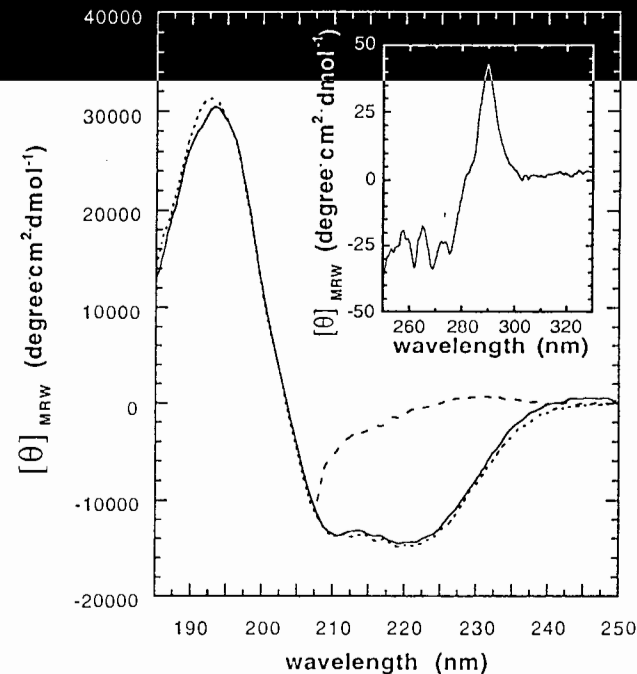


Figure 2. Far-UV circular dichroism spectra of native (solid line), unfolded (dashed line) and refolded (dotted line) PrP(121-231) at pH 7.0 and 22 °C (Inset: near-UV CD spectrum of native PrP(121-231)). Spectra were measured at protein concentrations of 0.5 mg/ml in 20 mM sodium phosphate pH 7.0. The sample of unfolded PrP(121-231) additionally contained 6 M GdmCl. Refolding of PrP(121-231) was achieved by dialysis against 20 mM sodium phosphate pH 7.0. Spectra were corrected for the buffer. The concentration of PrP(121-231) was determined by its absorbance at 280 nm ($A_{280\text{nm}}, 1\text{mg/ml}, 1\text{cm} = 1.55$) (reproduced from Hornemann & Glockshuber (1996) with permission).

In order to prove that PrP(121-231) represents an autonomous folding unit, its guanidinium chloride-dependent unfolding and refolding was measured at pH 7.0 using its far-UV CD signal at 222 nm. PrP(121-231) exhibits a cooperative and completely reversible unfolding transition (Figure 3). The spectroscopic properties of the refolded protein are identical to those of native PrP(121-231) (Figure 2). Evaluation of the data according to a two-state mechanism of folding yielded a free energy of folding of $-21.8 \pm 1.4 \text{ kJ/mol}$ and a midpoint of unfolding at 2.53 M GdmCl (Hornemann & Glockshuber, 1996). The cooperativity of folding (m -value), which is generally proportional to the mass of the protein and the difference in accessible surface area between the unfolded and folded state, has a value of $8.6 \pm 0.5 \text{ kJ mol}^{-1} \text{ M}^{-1} \text{ GdmCl}$ and is in the range expected for a 13.3 kDa protein (Myers *et al.*, 1995).

The biochemical analysis of PrP(121-231) thus gave strong indications that this amino-terminally truncated segment of the mouse prion protein is an isolated domain with defined tertiary structure and high intrinsic stability. Since there is no protein glycosylation in *E. coli*, folding and solubility do not require N-glycosylation at residues 181 and 198. Most importantly, refolding of chemically denatured PrP(121-231) is cooperative and reversible and yields a molecule that is indistinguishable from the native, recombinant protein. It is thus most likely that the three-dimensional structure of recombinant PrP(121-231) expressed in *E. coli* is identical to the structure of the corresponding segment in the cellular prion protein.

Within the framework of the protein-only hypothesis, the reversible unfolding of PrP(121-231) by GdmCl (Figure 3) has an intriguing implication if one assumes that the GdmCl-unfolded, intact cellular prion protein with all its posttranslational modifications also folds reversibly. If PrP^C and PrP^{Sc} indeed have identical covalent structures (Stahl & Prusiner, 1996), the two forms of PrP will yield identical unfolded forms in GdmCl (and probably in other denaturants), so that after reconstitution *in vitro* one would obtain folded PrP^C, in experiments started either with PrP^C or PrP^{Sc}. This would explain why all attempts to reconstitute infectivity after solubilization of infectious PrP^{Sc} with high concentrations of GdmCl or urea have failed (Prusiner et al., 1993).

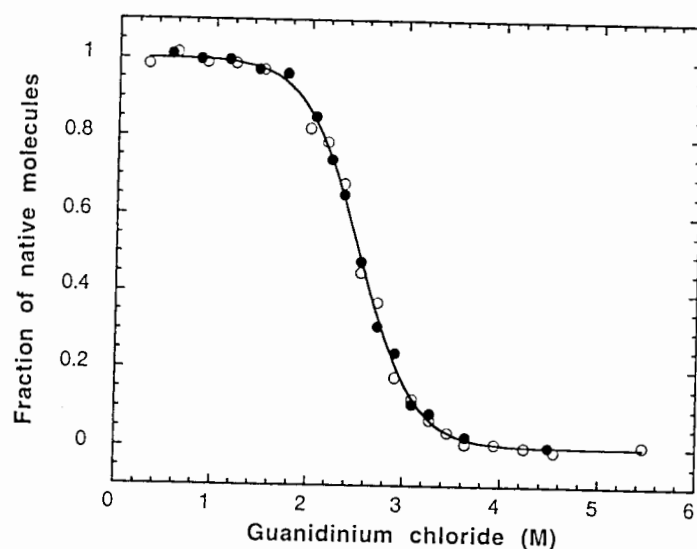


Figure 3. Reversible unfolding (○) and refolding (●) of PrP(121-231) in the presence of guanidinium chloride at pH 7.0 and 22 °C. Native or GdmCl-denatured PrP(121-231) was diluted 1:11 with 20 mM sodium phosphate pH 7.0 containing different GdmCl concentrations (final concentration of PrP(121-231): 38 μM). After incubation for 36 h, the mean residue ellipticity at 222 nm was recorded. The original data were evaluated according to a two-state transition using a six-parameter fit (solid line; Santoro & Bolen, 1988) and normalized (reproduced from Hornemann & Glockshuber (1996) with permission).

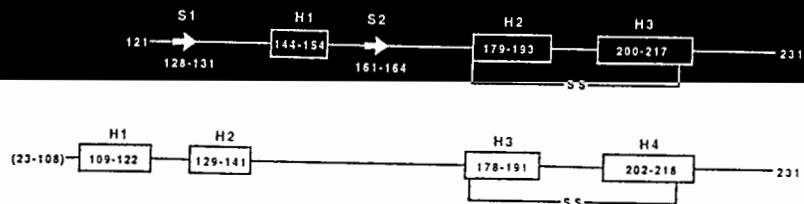
The intrinsically stable folding unit PrP(121-231) is not in accordance with the proposed three-dimensional structure of the PrP^C segment 108-218 (Huang *et al.*, 1994), as it lacks the first helix of residues 109-122 in the predicted four-helix bundle. In the following section we describe the three-dimensional structure of PrP(121-231) in solution and will show that it possesses a novel protein fold. We believe that the high stability and solubility of PrP(121-231) over a wide range of conditions will provide a basis for investigation of its kinetics of folding and unfolding, and for the identification of possible folding intermediates of PrP^C (Baldwin, 1996) that may be involved in the mechanism of transition from PrP^C to PrP^{Sc}.

THE NMR SOLUTION STRUCTURE OF PrP(121-231)

We decided to determine the three-dimensional structure of mouse PrP(121-231) for the following reasons: PrP(121-231) contains all residues which are posttranslationally modified in mature PrP^C, i.e. both glycosylation sites, the single disulfide bond and the residue bearing the GPI anchor. In addition, it contains most of the point-mutation sites in PrP which have been associated with inherited human prion diseases (Prusiner, 1993). Finally, experiments with transgenic mice exclusively expressing the segment 81-231 of mouse PrP proved that this segment is sufficient for generation and propagation of the prion disease *in vivo* (Fischer *et al.*, 1996), which indicated that the carboxy-terminal part of PrP is of special functional importance.

The NMR structure determination of PrP(121-231) was performed at pH 4.5, 20 °C and protein concentrations of 0.8 mM using uniformly ¹⁵N-labeled and ¹⁵N/¹³C doubly labeled samples. The ¹H, ¹³C and ¹⁵N resonances of the backbone were assigned by establishing intraresidual and sequential correlations of the amide ¹H and ¹⁵N resonances with C^α, C^β and H^α signals using three-dimensional (3D) triple resonance experiments and 3D ¹⁵N-resolved [¹H,¹H]-NOESY (nuclear Overhauser enhancement spectroscopy) experiments. The side-chain signals were assigned from 3D through-bond correlation NMR experiments. The initial NMR structure determination was based on sequence-specific resonance assignments at 93 % completeness. It was calculated with the program DIANA (Güntert *et al.*, 1991) using 1368 NOE distance constraints and 227 dihedral angle constraints. The 20 conformers with the lowest DIANA target function values were energy-minimized using the program OPAL (Luginbühl *et al.*, 1996) with the AMBER force field (Cornell *et al.*, 1995). For residues 125-166 and 177-219 of these 20 conformers, the RMSD (root mean square distance) to the mean structure was 1.4 Å for the N, C^α and C' atoms, and 2.0 Å for all heavy atoms (Riek *et al.*, 1996).

The NMR structure of PrP(121-231) revealed a so far unknown protein fold which differs clearly and extensively from the proposed three-dimensional model of PrP^C (Figure 4). Importantly, the relative orientation of the three helices in PrP(121-231) is clearly different from the relative orientation of any three helices in the proposed four-helix-bundle



Structure prediction for PrP^C (Huang et al. (1994), *PNAS* **91**, 7139)

Figure 4. Comparison of the location of regular secondary structures in the NMR solution structure of PrP(121-231) and in the proposed four-helix bundle model by Huang et al. (1994).

model (Huang et al., 1994). A systematic search of the Brookhaven data bank with the program DALI (Holm & Sander, 1994) did not lead to the identification of other proteins with similar folds (Riek et al., 1996).

PrP(121-231) contains three α -helices and a two-stranded antiparallel β -sheet (Figures 5, 6a). The approximate lengths of the helices are from residues 144-154, 179-193 and 200-217, and of the β -strands from residues 128-131 and 161-164. The first turn of the second helix and the last turn of the third helix are linked by the single disulfide bond in the protein. The twisted V-shaped arrangement of these two longest helices forms a scaffold onto which the short β -sheet and the first helix are anchored. At the present stage of refinement all regular secondary structure elements and the connecting loops are well defined with the sole exception of residues 167-176, which appear to lie in a rather flexible loop region. 18 residues contribute to the hydrophobic core of PrP(121-231), which

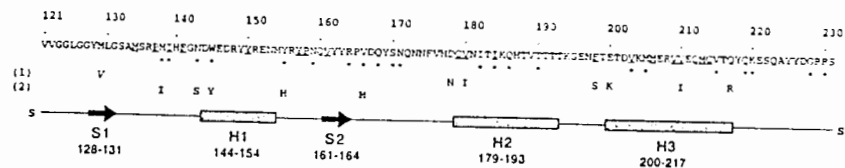


Figure 5. Location in the three-dimensional structure of PrP(121-231) of residues involved in sequence variations among mammalian prion proteins and of residues that have been associated with the species barrier of prion disease transmission and with inherited prion diseases. The primary structure of mouse PrP(121-231) and the location of regular secondary structures is shown. Residues contributing to the hydrophobic core of the domain are underlined, variable residues among mammalian prion proteins (Schätzl et al., 1995) are marked with asterisks. Line (1), mutations in human PrP which have been associated with inherited prion diseases (Prusiner, 1993; a stop codon at residue 145, which has been reported in addition to these point mutations, is not considered here, nor is the Met232Arg mutation, which is not contained in mature PrP^C). All of these residues are identical in wild type human PrP and mouse PrP. The polymorphism at codon 129 in human PrP, where homozygosity appears to increase susceptibility to sporadic CJD, is marked by italics. Line (2), residues in PrP(121-231) for which experimental evidence has been presented that they contribute to the species barrier of prion disease transmission between mice and humans (Telling et al., 1995). (Reproduced with permission from Riek et al., 1996)

includes side chains of the second helix (residues 179, 180, and 184), the third helix (residues 203, 206, 209, 210, 213, and 214), the second strand of the β -sheet (Val161), the first, mostly hydrophilic helix (Tyr150), and three loop regions (residues 134, 137, 139, 141, 157, 158, and 198). The majority of the residues forming the hydrophobic core are invariant in the known mammalian prion protein sequences, with the exceptions of Ile139, Ile184 and Val203 (Figure 5). In contrast, most residues which are variable in mammalian prion proteins are solvent accessible (Riek et al., 1996).

The calculation of the electrostatic surface potential (Honig et al., 1994) of PrP(121-231) revealed that the protein possesses a pronouncedly uneven spatial distribution of acidic and basic residues, which might contribute to direct its orientation relative to the cell membrane (Riek et al., 1996). The positively charged side of PrP, which includes the β -sheet (Figure 6a), would then probably bind to the membrane surface, while the negatively charged side of the protein, which contains both glycosylation sites and the single tryptophan residue, would be exposed to the solvent. The single, invariant disulfide bond of the prion protein is highly shielded from solvent contact and forms an important part of the hydrophobic core. This is in accordance with the observation that the disulfide bond is essential for folding of hamster PrP(90-231) (Mehlhorn et al., 1996). Another remarkable feature of the structure of PrP(121-231) is the fact that the first α -helix

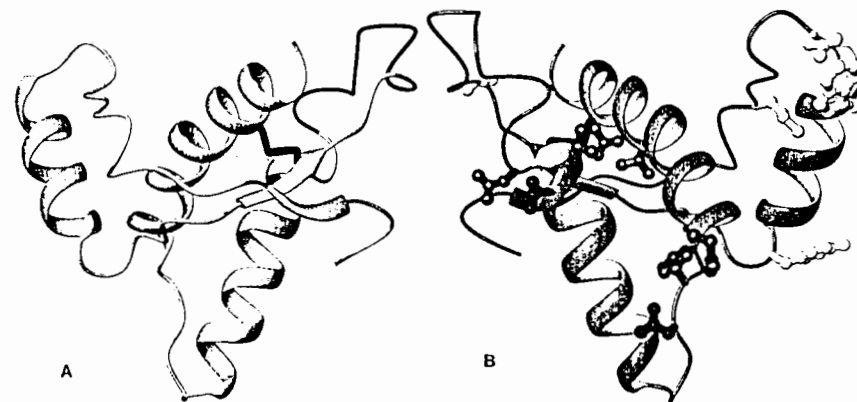


Figure 6. Globular fold and locations of selected residues in the three-dimensional structure of PrP(121-231). **a.** Ribbon diagram of the structure of PrP(121-231), indicating the positions of the three helices and the antiparallel two-stranded β -sheet. The connecting loops are displayed in grey if their structure is well-defined, and in black otherwise. The disulfide bond between Cys179 and Cys214 is shown in black. The N-terminal segment of residues 121-124 and the C-terminal segment 220-231 are disordered and not displayed. **b.** Locations of selected residues in the three-dimensional structure of PrP(121-231). The backbone is shown in grey and compared to a the molecule has been rotated by 180° around a vertical axis. The side chains of residues with mutations that have been associated with inherited prion diseases are highlighted in dark grey (cf. Figure 5, line (1)). The solvent-accessible glycosylation sites at Asn181 and Asn197 and the disulfide bond are shown in black. Five residues which may be involved in the mouse/human species barrier (cf. Figure 5, line (2)), are displayed in light grey (reproduced from Riek et al. (1996) with permission). The Figure was generated with the program MOLMOL (Koradi et al., 1996).

(residues 144-154) is relatively isolated from the rest of the structure. This helix is unusually hydrophilic, contains more than 50 % charged residues and does not show amphipathic character. It only contributes a single residue, Tyr 150, to the hydrophobic core of the carboxy-terminal PrP domain (Riek et al., 1996).

FUNCTIONAL IMPLICATIONS OF THE THREE-DIMENSIONAL STRUCTURE OF PRP(121-231) FOR THE GENERATION OF PRION DISEASES

Regarding previous Fourier-transform infrared data (Pan et al., 1993) and structure predictions for PrP^C (Huang et al., 1994, 1996), the presence of a β -sheet in the NMR structure of PrP(121-231) is an unexpected finding. Since the fraction of β -sheet structure in PrP may increase upon transition from PrP^C to PrP^{Sc} (Pan et al., 1993), it is tempting to speculate that the short antiparallel β -sheet might be a "nucleation site" in PrP^C for a conformational transition to PrP^{Sc}. Such a transition could also include the loops connecting both strands of the β -sheet to the first helix. In this context, it is interesting to note that the polymorphism at codon 129 in human PrP (methionine or valine), which has been linked to predisposition to sporadic CJD (Palmer et al., 1991), lies within the first strand of the β -sheet.

The three-dimensional solution structure of PrP(121-231) allows the spatial localization of residues which have been proposed to be important for the species barrier of prion disease transmission (Scott et al., 1993; Telling et al., 1995) and which have been associated with inherited human prion diseases (Prusiner, 1993) (Figure 6b). The species barrier of prion disease transmission could principally result from an altered PrP^{Sc} binding site in PrP^C. The species barrier between mice and humans appears to be caused by one or more sequence differences between human and mouse PrP within the segment comprising residues 96-167 (Telling et al., 1995). There are a total of eight sequence differences between mouse and human PrP within this segment, of which five are contained in PrP(121-231). Four of these differences are located within or adjacent to the first helix, which might thus be part of a single binding site in PrP^C for PrP^{Sc} (Figure 6b). Regarding the aforementioned possible orientation of PrP^C relative to the cell membrane, the potential binding site for PrP^{Sc} would then be located on the accessible, solvent-exposed side of the cellular prion protein. Importantly, two of the three residues in PrP(121-231) which may determine the species barrier between mouse and hamster (Scott et al., 1993) are also located in the region of the first helix.

PrP(121-231) contains six of the point-mutation sites in mature, human PrP which have been linked with familial human prion diseases (Figure 5, Figure 6b). These six residues are exclusively located within or immediately adjacent to the last two helices which form the scaffold of the structure of PrP(121-231). Three of these six mutations (Val180Ile, Phe198Ser, Val210Ile) affect the hydrophobic core of the protein and three cause changes of charges at exposed residues (Asp178Asn, Glu200Lys, and Gln207Arg). It may be that some of these point mutations destabilize the three-dimensional structure of

PrP^C, which might facilitate its conversion to PrP^{Sc}. Other mutations might affect the self-aggregation of the prion protein and in this way contribute to easier transition to PrP^{Sc}. Replacement of the three exposed residues might however also influence the ligand binding properties of PrP^C. Involvement of a natural ligand that competes with PrP^{Sc} for binding to PrP^C would thus be another explanation why these mutations might favour the generation and propagation of infectious prions.

Besides the fact that homozygosity at codon 129 in human PrP leads to a predisposition to sporadic CJD (Palmer et al., 1991), the Met/Val 129 polymorphism has another striking consequence. In conjunction with the inherited Asp178Asn mutation, it determines the phenotype of this familial prion disease: While the Met129/Asn178 allele segregates with fatal familial insomnia, the Val129/Asn178 allele segregates with inherited CJD (Goldfarb et al., 1992). Analysis of the three-dimensional structure of PrP(121-231) shows that, although there is no close approach of the polypeptide backbone near the residues 129 and Asn 178, side chain/side chain interactions would be sterically possible. More precise information is expected from the ongoing structure refinement.

In conclusion, we believe that the present three-dimensional NMR structure of PrP(121-231) will provide a basis for rational design of *in vitro* and *in vivo* experiments to test the above-mentioned hypotheses on the generation and propagation of prion diseases. For example, if PrP^C did indeed undergo a substantial conformational change during the transition to PrP^{Sc}, it might be possible to generate PrP^{Sc}-specific antibodies using buried segments in the structure of PrP(121-231) as haptens.

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PROTEASE-RESISTANT PRION PROTEIN FORMATION

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INTRODUCTION

In 1981, unique fibrillar structures called scrapie associated fibrils (SAF) were identified in in scrapie-infected, but not normal, brain tissue¹. Biochemical analysis of these fibrils and of other infectious tissue preparations from hosts with different transmissible spongiform encephalopathies (TSE) led to the discovery that they contained an abnormal form of a host protein which is now most widely known as prion protein (PrP)^{2,3}. The abnormal TSE-associated PrP forms insoluble aggregates (e.g. amyloid plaques) and is partially resistant to proteinase K (PK)^{3,4}, which removes approximately 67 amino acid residues (6-7 kDa) from the N-terminus of each molecule in the polymer^{5,6}. Although the PK-resistance of various abnormal TSE-associated PrPs can differ somewhat, we refer to them generically as protease-resistant PrP (PrP-res), but the forms associated with specific TSE diseases are often labelled with a superscript designating that disease, e.g. PrP^{Sc} for scrapie PrP and PrP^{BSE} for bovine spongiform encephalopathy-associated PrP. In contrast, the normal, non-pathogenic form, PrP-sen or PrP^C, is usually soluble in mild detergents and fully degraded by PK^{7,8}.

There are many indications that PrP plays a critical role in TSEs. Thus, it is of interest to understand how PrP-res is made and how it relates to the infectious agents of TSE diseases. One popular idea is that PrP-res might be the self-inducing infectious protein modelled by Griffith⁹. In TSE-infected hosts, PrP-res usually accumulates in the central nervous system and lymphoreticular tissues^{2,3,8,10-12}. Both the normal and abnormal PrP isoforms are encoded by the same host gene¹³ and no TSE associated differences have been observed in either the PrP mRNA levels^{6,14} or the chemical structures of the PrP isoforms^{5,15}. Pulse-chase metabolic labelling studies have shown that PrP-res is derived from mature PrP-sen posttranslationally^{16,17}. These observations led to suggestions that the primary difference between PrP-res and PrP-sen might be conformational or due to interactions with cofactors⁵. Indeed, conformational analyses have provided evidence that PrP-res has a much higher beta sheet secondary structure composition than PrP-sen¹⁸⁻²⁰.