Molecular modelling indicates that the pathological conformations of prion proteins might be β -helical

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Creutzfeldt–Jakob disease, kuru, scrapie and bovine spongiform encephalopathy are diseases of the mammalian central nervous system that involve the conversion of a cellular protein into an insoluble extracellular isoform. Spectroscopic studies have shown that the precursor protein contains mainly α -helical and random-coil conformations, whereas the prion isoform is largely in the β conformation. The pathogenic prion is resistant to denaturation and protease digestion and can promote the conversion of the precursor protein to the pathogenic form. These properties have yet to be explained in terms of the structural conformations of the proteins. In the present study, molecular modelling showed

that prion proteins could adopt the β -helical conformation, which has been established for a number of fibrous proteins and has been suggested previously as the basis of amyloid fibrils. The β -helical conformation provides explanations for the biophysical and biochemical stability of prions, their ability to form templates for the transmission of pathological conformation, and the existence of phenotypical strains of the prion diseases.

Key words: bovine spongiform encephalopathy; Creutzfeldt–Jakob disease; ‘mad cow disease’; scrapie.

INTRODUCTION

Several neurological disorders, including Creutzfeldt–Jakob disease and kuru in humans, scrapie in sheep, and bovine spongiform encephalopathy (‘BSE’, or ‘mad cow disease’) involve the formation of pathological deposits of protein in the brain. The protein responsible, the non-infectious cellular isoform of prion protein (PrP^C), can adopt an aberrant insoluble infectious conformation, PrP^{Sc}, that accumulates extracellularly and is resistant to denaturation and digestion with proteinase [1–3]. Furthermore, PrP^{Sc} is capable of catalysing the conversion of PrP^C to PrP^{Sc}, thereby acting as a transmissible agent termed a prion [4]. It is widely accepted that the properties of PrP^{Sc} result from its folding pattern but the unusual resistance of PrP^{Sc} to denaturation and digestion might not be explained adequately by the conventional cross- β -sheet structure attributed to PrP^{Sc} [5–8] and to amyloid fibrils in general [9]. In addition, it is difficult to reconcile the cross- β conformation with the wide range of amyloid fibril diameters that have been observed [10–15] and the existence of protofibril subunits as small as 2–3 nm in diameter [10]. We have proposed that amyloid fibrils might contain β -helical protofibril subunits [16–18] and we developed graphical conventions to display such structures. In the present study we used three graphical methods to model potential β -helical conformations of prion proteins from several species, demonstrating plausible structures that might help to explain the biological and biophysical properties of these disease-related materials.

EXPERIMENTAL

Graphical conventions for the expression of β -helices

Three different graphical techniques were developed for the investigation of potential β -helical conformations:

First, the one-letter amino-acid code for a polypeptide sequence was arranged so that β -strand residues with side chains assigned to the helix lumen formed one vertical column, as indicated in Figure 1, whereas the β -strand residues with side chains assigned to the exterior of the helix alternated in a parallel column. Residues assigned to intervening sequences that separated the β -strands were then arrayed laterally, without regard to conformation. To assist in the construction and interpretation of the diagrams, each hydrophobic amino acid was represented by a yellow circle; hydrophilic residues were shown as blue circles.

Assignments of conformation were based primarily on analogies with established examples of β -helical conformation [19–23] and on primary factors in protein conformation, especially hydrophobic effects and hydrogen-bonding [24,25]. As in established β -helical structures, residues assigned to the helix lumen were required to be uncharged and predominantly hydrophobic. A second primary criterion was that all β -strands were required to contain the same number of residues, so that strands would coincide, in successive turns of the helix, to form β -sheets. These considerations were best met by a series of five-residue β -strands separated by loops containing various numbers of amino-acid residues in unspecified conformations. Calculations of the percentages of β -helix in each conformation were based on the number of residues assigned to β -strands, divided by the total number of residues in the conformation.

Having constructed a plausible secondary array, the potential for tertiary conformations in the form of anti-parallel associations was explored by using the same linear representations. For representing two-chain anti-parallel β -helical conformations in the linear array, the convention was adopted of placing residues that would be axially juxtaposed (in successive turns of the helix) on the same horizontal line. In this way it was possible to observe the criterion, based on established β -helical structures, that the loops of unspecified conformation would coincide in successive turns of the helix. This arrangement

Abbreviations used: PrP^C, non-infectious cellular isoform of PrP; PrP^{Sc}, infectious isoform of PrP; PrP27–30, mixture of polypeptides produced by proteinase K digestion of PrP^{Sc}.

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Figure 1 Linear expression of β -helical secondary structure

Full-length human (A), sheep (B and C) and bovine (D) prion precursor proteins, and sequences of Syrian hamster (E) and mouse (F) mature prion proteins, with the indicated GenBank Database accession numbers, are shown in the linear expression of β -helices that was developed for the present study. In this convention, the residues belonging to the helix backbone having side chains that are assigned to the helix lumen are shown in one column (a); side chains of residues belonging to the helix backbone that are on the exterior of the helix alternate in a parallel column (b). Loops of residues that are not assigned to the helix backbone are arrayed laterally without regard to conformation. Hydrophobic residues are represented by yellow circles; hydrophilic residues are shown as blue circles. In the mouse sequence (F), red letters or red circles indicate differences in amino acid composition between mouse and hamster prions. Each conformation shown is one of many possible variations on the β -helical theme. In the duplicate sheep sequence (C), red boxes indicate three regions in which plausible alternative conformations are possible. The full conformations shown are considered to exist only in oligomeric forms of scrapie prions, not in monomers or dimers. The direction of each sequence runs from top to bottom. The numbers at the bottom of each panel are GenBank[®] accession numbers.

maximized the number of potential hydrogen bonds between successive turns of the two peptide backbones and provided for additional hydrogen-bonding between residues in the axially juxtaposed loops that extended radially from the helix.

Secondly, the linear arrays representing the two-chain anti-parallel β -helix were translated to axial representations in which each turn of the proposed β -helix was shown as a circle, from which the intervening sequences of unspecified conformation extended radially.

Thirdly, the axial arrays were transcribed to equatorial views of complete β -helices, which showed the residues assigned to the

exterior of the helix and the radially arrayed loops of unspecified conformation.

Space-filling molecular models

Each distinctive molecular conformation that was postulated for the polypeptides investigated was examined for spatial feasibility by construction from space-filling Corey–Pauling–Koltun atomic models (Harvard Apparatus, Natick, MA, U.S.A.). The molecular dimensions of the modelled conformations were measured with graduated callipers.

RESULTS AND DISCUSSION

Potential for β -helix formation by prion proteins

The prion precursor proteins of human, sheep and ox, and the mature prion proteins of the hamster and mouse, are expressed in a novel linear convention in Figure 1. These are β -helical conformations that might be adopted in pathological oligomers and are not intended to represent the conformations of monomers. It should also be understood that these structures represent the maximum possible extent of β -helix formation; it is not implied that the entire molecule necessarily adopts the β -helical conformation. It should also be clear that the 'one sequence: one conformation' dogma does not apply to prions, because the conformation of the normal sequence can transform into the profoundly different pathogenic conformation, either spontaneously (as an event of low probability) or more rapidly by association with pre-existing pathogenic material. Moreover, it is accepted that the occurrence of numerous strains of scrapie in sheep is caused by stable and transmissible variations in conformation. Therefore the construction for each prion sequence shown in Figure 1 is not a unique solution but is one of several plausible β -helical constructions. Folding alternatives for three regions in the sheep prion are shown, where the secondary conformation permits variants with similar structural characteristics and likely thermodynamic stability. Five such sites of alternative conformation could produce a total of 32 possible folding alternatives, approximating the number of known strains of scrapie disease [26]. An additional source of variation in conformation is the differing degrees of offset between the anti-parallel chains, which could also result in different molecular masses in the products of protease digestion.

It is clear that differences in the sequences between species consist mainly of hydrophobic groups replacing other hydrophobic groups, or of hydrophilic groups replacing hydrophilic. The rare interchange of hydrophobic with hydrophilic groups, or the occasional additions or deletions in the sequences, occurred almost exclusively within the loops of unspecified conformation separating the short strands of β conformation. Presumably these conserved features reflect the requirements of the unknown normal biological functions of the prion precursor protein.

Octapeptide repeat region of prion proteins

The octapeptide repeats that have been noted as a feature of prion sequences were first modelled as five-residue β -strands separated by three-to-five-residue loops of random coil (Figure 2). Corey–Pauling–Koltun models indicated that the repeat motif PHGGGWGQ, if wound into a helix, would be stabilized by hydrogen-bonding between the amino proton of each tryptophan indole ring and the carbonyl oxygen in an $n+2$ glutamine side chain.

The octapeptide-repeat sequence of prion proteins is conformationally the most flexible region, as judged from NMR [27,28] and CD [29] spectra. This is consistent with the β -helical conformation proposed for the prion sequences shown in Figures 1 and 2, in which the octapeptide repeat region would be unprotected by external hydrophobic residues from the destabilizing attack of solvent water on hydrogen-bonding between residues in the peptide backbone and between side chains. Published CD spectra indicate that the octapeptide region is not a random conformation but might consist of an unusual type of open helix analogous to the two conformations of poly-(L-proline) [29]. We suggest, as an alternative, that the entire octapeptide region might adopt a configuration consisting of a

Figure 2 Linear expression of an anti-parallel β -helical dimer

The sequence of hamster mature prion protein is shown on the left as an anti-parallel dimer in the linear convention for the β -helical conformation. The structural conventions are the same as those in Figure 1. In an extension of the convention, the two anti-parallel chains shown on the right are displaced axially relative to each other by an amount equivalent to one turn of the helix. Together, the two pairs of chains allow the representation of the residues in one of the chains that are axially juxtaposed above and below each residue in the second chain. Therefore each degree of axial stagger (in which different lateral loops in the two chains are juxtaposed axially) requires two drawings to enable the spatial relationships between all of the residues in the dimer to be visualized. Inspection of the full set of stagger drawings allowed the determination of the most stable dimer (as drawn), decided on by the highest number of hydrogen-bonds that could be formed between the side chains of the backbone residues and between lateral loops. Arrows indicate the sequence direction of each protein chain. Red boxes indicate two locations in each sequence that have been reported to be close to the respective residues in the anti-parallel chain [33]. The partial sequence between the two dimer drawings is an alternative conformation in which the octapeptide region is shown as a concatenation of β -turns.

continuous concatenation of β -turns, as shown in Figure 2. This would explain the CD spectrum of peptide analogues of the region, the principal feature of which is a weak minimum near

Figure 3 Linear expression of human PrP27–30 as a protofibril

PrP27–30 is shown as an anti-parallel β -helical protofibril with the degree of axial stagger of the protein chains that produced the greatest aggregate of hydrogen-bonds between successive turns of the helix. The structural conventions are as indicated in Figures 1 and 2. The red boxes in the right-hand structure indicate the two β -strands in each chain that are reported to be adjacent and anti-parallel to the respective strands in the second chain [32]. Arrows indicate the sequence direction of each peptide chain.

202 nm [29]. A similar CD spectrum is produced by the soluble form of *Bombyx mori* silk fibroin (silk I), for which we recently proposed a continuous β -turn conformation [30]. Published CD spectra of PrP^{Sc} have been interpreted as arising from 20% α -helix, 34% β -sheet and 46% β -turns plus random coil [7], which is compatible with our calculation of 29% β -helix and 33% β -turn based on the numbers of residues in these conformations in our diagrams, assuming β -turn conformation for the octapeptide region, although we postulate no α -helix (see Figure 2). CD spectra of mixture of polypeptides produced by the digestion of PrP^{Sc} by proteinase K (PrP27–30) indicated 43% β -sheet and no α -helix [7], compared with 39% β -helix in our model (Figure 3). Some additional β -sheet conformation could be present in the loops that intervene between β -strands of the helix.

A concatenated β -turn conformation for the octapeptide region of PrP^{Sc} might explain why this material does not form visible fibrils, whereas removal of the octapeptides to give PrP27–30 results in the formation of fibrils that are visible in electron micrographs. Treatment with protease eliminates the octapeptide region, allowing the axial aggregation of PrP27–30 dimers. Computer modelling of concatenated β -turns [30] indicated that strong intermolecular association, involving hydrogen-bonding, can occur between parallel strands of β -turn. In the present instance, the prion octapeptide region might be limited to the formation of dimers because the presence of proline residues prevents intermolecular association, through hydrogen-bond formation, on one side of each β -turn strand. Initial dissociation of aggregated PrP^{Sc} by increasing concentrations of guanidinium

chloride resulted in increased exposure of tryptophan residues to the medium, as evidenced by changes in fluorescence, without affecting the overall secondary structure [7]. Higher concentrations of guanidinium chloride (more than 3 M) resulted in complete dissociation. These results are compatible with the idea that the initial dissociation affects only the intermolecular association of the octapeptide region, suggesting that there is a different conformation in the remainder of the molecule.

β -Helix formation by mature prion proteins

Infrared spectroscopic data indicate an intermolecular anti-parallel β conformation for PrP^{Sc} [8,31,32]. Figure 2 shows a possible two-chain anti-parallel arrangement of hamster mature prion proteins (residues 29–231) in which the axial displacement between the anti-parallel chains was chosen by inspection of each potential degree of stagger. Estimation of the total number of hydrogen bonds that could exist between the amino-acid side chains in successive turns of the helices showed that the stagger in Figure 2 provided the greatest potential for stabilization by side-chain hydrogen-bonding, taking into account that each peptide chain in a two-chain helix is contacted on both sides by the other chain. Support for the resulting degree of stagger between the two hamster PrP^{Sc} chains illustrated in Figure 2 is provided by the knowledge that limited protease action on PrP^{Sc} *in vitro* results in the removal of the protruding octapeptide repeat region, with the cleavage occurring predominantly between positions 90 and 96, producing structures such as those shown in Figure 3. Protection of the remaining PrP27–30 molecule from further protease action might result from the tight β -helical conformation. The structure that we propose for PrP27–30 is stabilized by interchain hydrogen-bonding, and is defended from attack by solvent water by abundant hydrophobic residues on the exterior of the helix. Additional support for the conformation shown in Figure 2 is provided by the observation, based on NMR data, that the β -strands formed by residues 127–130 and 160–163 are in anti-parallel juxtaposition in mouse PrP^C and might act as a nucleation site for the conformational transition to PrP^{Sc} [33].

By inspection of the PrP27–30 product of limited treatment with proteinase K, shown in Figure 3, the total number of residues involved in the β conformation is the sum of the five-residue β -strands, which amounts to 39% of the total number of residues. Some additional β conformation might exist in the loops between β -strands. On the basis of Fourier-transform infrared spectra, PrP27–30 has been reported to contain β conformation totalling 47% [6] or 54% [8] of the overall structure. CD studies of solid-state preparations of PrP27–30 found 43% β -sheet, 57% β -turns plus random coil, and no α -helix [7]. There is therefore reasonable agreement between the experimental values for content of β conformation in PrP27–30 and that deduced from our molecular modelling.

Conformation of synthetic peptide analogues

Four regions of the prion sequence have previously been postulated as potentially α -helical [30], and recent NMR studies indicate that three of these regions do adopt the α -helical conformation in the soluble form of prions [27,34]. However, the same regions can convert to the β conformation in the insoluble form, because synthetic peptides representing three of these regions produced fibrils predominantly in the β conformation [31]. Table 1 compares the proportions of β -helix, estimated from

Table 1 β -Helix content of sections of hamster prion protein

The content of β conformation in synthetic peptides representing sections of hamster prion was previously determined by Fourier-transform infrared spectroscopy (FTIR) [31] and was calculated in the present study from the proportions of β -helix in the structures shown in Figure 4.

Peptide	Sequence	Percentage β conformation	
		FTIR	β -Helix
H1	MKHMAGAAAAGAVV	60	64
H2	MLGSAMSRPMMHF	23	23
H3	DCVNITIKQHTVTT	55	54
H4	DIKIMERVVVEQMCTTQY	70	82

drawings of potential β -helical conformations of these peptides (Figure 4), with the proportions of β -structure deduced from solid-state infrared spectra of fibrils formed by the synthetic peptides [31]. The comparison shows close agreement between the published experimental results and the predicted conformations based on our molecular modelling of the respective peptides, with the same sequence-conformation criteria that we employed for PrP^{Sc} and PrP27–30.

Axial and equatorial drawings of β -helices

After the construction of plausible tertiary conformations of prion proteins by using the linear convention (Figures 2 and 3), the arrangements were transcribed into axial views of successive turns of the postulated two-chain anti-parallel β -helix, as shown in Figure 5 for human PrP27–30. This view provides a clearer impression of a number of significant features of the proposed β -helical conformation. The first is that residues assigned to the helix lumen are unchanged and are either hydrophobic or, if hydrophilic, are in a position to hydrogen-bond with a nearby

residue within the lumen. Figure 5 also emphasizes the axial juxtaposition of loops in successive turns of the β -helix, which is a feature seen in all of the β -helical conformations that have been established experimentally [19–23]. The structural significance of this arrangement seems to result from its maximization of the backbone hydrogen-bonding between successive turns of the double helix, as well as the hydrogen-bonding between adjacent side chains belonging to backbone residues on the exterior of the helix. Backbone and side-chain hydrogen-bonding between residues in the loops sprouting from successive turns of the helix might also be a significant source of conformational stability.

The construction of axial views of the proposed β -helical conformations, as illustrated in Figure 5, made it possible to translate the structures into lateral views of the complete β -helix, as seen from an equatorial perspective. Figure 6 shows one such equatorial view of the human PrP^{Sc} β -helical conformation developed in Figures 1–5. This form of presentation allows the inspection of the spatial proximity of the two cysteine residues in each sequence, which are known to form a disulphide bond both in PrP^C and in PrP^{Sc} [32]. The axial views also make apparent the external location of the two asparagine residues that are known to be glycosylated in prion proteins.

Fibril formation

The formation of prion fibrils can be viewed as an initial axial aggregation of dimer molecules, such as that shown in Figure 6, to form protofibrils. Axial adherence of the dimers would be promoted by backbone hydrogen-bonding, and side-chain hydrogen-bonding, between residues and loops on the ends of the contacting helices, leaving the octapeptide regions excluded. The dimers might be regarded as the fundamental building blocks of the amyloid fibrils and might also constitute the hypothetical intermediate in which the pathological PrP^{Sc} template imparts its conformation to soluble PrP^C molecules [35,36]. The initial protofibril would thus consist of a chain of dimer molecules, and would have a diameter of 2–3 nm. This dimension

Figure 4 Linear and axial views of oligomers of synthetic peptides

Peptides representing four regions of putative α -helix in Syrian hamster prion proteins were synthesized by Gasset et al. [31] and found to assume various degrees of β conformation. In this figure the conformations of the four peptides are drawn as anti-parallel β -helices in both the linear and axial conventions developed in the present study. The proportions of β -helix in the structures were obtained by calculating the ratios of β -strand residues to total residues for each peptide. These proportions of β -helix were compared (Table 1) with the proportions of β conformation measured by solid-state Fourier-transform infrared spectroscopy of the respective peptide fibrils [31].

Figure 5 Axial view of a PrP27–30 protofibril

An axial view of successive turns of a two-chain anti-parallel β -helix, derived from the linear representation of human PrP27–30 in Figure 3. In this convention, helix backbone residues with side chains assigned to the helix lumen are shown within the central circle, whereas helix backbone residues with side chains assigned to the exterior of the helix are arrayed on the exterior of the circle. Residues assigned to the loops of unspecified conformation that sprout from the helix are shown as radial sequences. Blue circles represent the hydrophilic residues belonging to one chain, whereas the purple circles represent the hydrophilic residues in the anti-parallel chain. Hydrophobic residues in both chains are shown as yellow circles but are represented by black letters in the downward chain and by red letters in the upward chain. The spatially uppermost residue (closest to the observer) in each turn of the helix is represented by a larger circle and letter at the 3 o'clock position. The beginning of each chain is indicated by labelling of the sequence position 90; arrows indicate the directions of the respective sequences.

can be compared with the 4–6 nm protofibrils that have been observed in electron micrographs of prion preparations [37] and with the protofibrils 2–3 nm [10] or 3–4 nm in diameter [11,12], measured by atomic force microscopy of amyloid- β preparations. Lateral aggregation of the protofibrils to produce the usual

Figure 6 Lateral view of a PrP27–30 protofibril

A lateral (equatorial) view of the postulated two-chain anti-parallel β -helix formed by human PrP27–30. Each chain begins at the indicated position 96 of the sequence. Blue circles represent the hydrophilic residues belonging to one chain, whereas the purple circles represent the hydrophilic residues in the anti-parallel chain. Hydrophobic residues in both chains are shown as yellow circles but are represented by black letters in the downward chain and by red letters in the upward chain. Arrows show the alternating directions of the chains in successive turns of the helix. Residues that are not assigned to the helix are shown as loops of unspecified conformation radiating from the central helix. Both cysteine residues in each chain are external to the helix and are close enough to form an intrachain disulphide bond between successive turns of the same chain; the intervening turn of the other helix has no loop of residues at that point to interfere with the formation of the disulphide bond. The potential disulphide bonds are shown in black for one chain and in red for the other.

8–12 nm prion rods [13,14,37] is likely to be promoted by the external hydrophobic residues seen in Figure 6. Synthetic peptides that lack hydrophobic groups on the exterior of the putative β -helices form only fibrils 2–3 nm in diameter [18]. The existence of β -helical amyloid and prion protofibrils is supported by the observations that the smallest diameter subunits seem to be round [10–12] and by the association of various numbers of these subunits to form filaments having ribbon and rod structures with a wide range of diameters [10–15].

Transmission of prion diseases

The three methods of graphical expression of prion conformations developed in this study facilitate the exploration of these structures and can result in testable hypotheses about the properties of prions, including the preservation and transmission of pathogenic conformations. Figure 1 shows how several plausible conformations of each PrP^{Sc} sequence are possible; Figure 2 illustrates how these conformations might be preserved in the form of stable dimers. Given the two-chain anti-parallel β -helical conformation that we propose, the transfer of PrP^{Sc} conformation to PrP^C monomers would be somewhat analogous to the transfer of genetic information in nucleic acids, in which the new chain develops anti-parallel to one of two chains that are revealed when the double helix is unwound. The prion conformation could similarly be preserved in an anti-parallel dimer, and one or two additional chains could be folded on the initial conformers as the dimer unwound progressively. The conformation could thus be multiplied and then stored in the form of new dimers or higher oligomers. This general scheme is analogous to the interactions between PrP^{Sc} and PrP^C diagrammed by Prusiner et al. [36]. The present study therefore provides a possible molecular explanation for the existence and transmission of stable prion conformations, using the newly discovered β -helical fold that has been established for a number of natural proteins.

Figure 7 Association of natural mutations with human familial prion diseases

Mutant residues are indicated by red circles or letters where pathogenic, and by green circles or letters where a normal phenotype persists [36,39–42]. Some possible explanations of effects of the substitutions on the conformations of the pathogenic proteins are given in the text. Abbreviations used: GSS, Gerstmann–Sträussler–Scheinker disease; FFI, fatal familial insomnia; fCJD, familial Creutzfeldt–Jakob disease.

Effects of natural mutations on the spontaneous occurrence of prion diseases

Given a normal genotype, prion diseases are rare; however, certain mutations are associated with a much higher incidence [38–42], perhaps because pathogenic folding of the prion molecule is enhanced by the amino-acid substitutions. Figure 7 employs our linear convention to show a number of mutations that have been observed, and the associated effects on phenotype. Some of these substitutions can be correlated with expected effects on the formation or stability of pathogenic conformations, in particular the A117V mutation found in some families with elevated incidence of Gerstmann–Sträussler–Scheinker syndrome. It can be deduced that the increased hydrophobicity resulting from the valine side chain, which we assign to the lumen of a β -helix, would promote the adoption and stability of the pathogenic conformation. On the other hand, an N171R mutation in sheep is associated with resistance to prion transmission when only one allele carries the mutation [38]. We deduce that the incorporation of a charged residue into a hydrophobic environment would strongly resist the formation of a β -helix with an arginine residue in the lumen. The N171S mutation, however, is not pathogenic, perhaps because the alternative serine residue, which we assign to the helix lumen, has inferior hydrogen-bonding capability. The human mutation M129V alone provides a normal phenotype, but in combination with the substitution D178N causes a profound change in the expression of fatal familial insomnia. The molecular effect of the D178N substitution might be to promote and stabilize the conformation of the loop in which it occurs, by hydrogen-bonding between proximal asparagine residues. The same mutation has been found in subjects having

a familial form of Creutzfeldt–Jakob disease. Variations of that syndrome have been found in families having E200K, R208H or Q217R, for which no ready explanation can be made, or V210I, which could result from the increased intraluminal hydrophobicity produced by isoleucine. The pathogenic substitutions P102L and P105L are not easily interpreted because the conformation of the loop to which the residues are assigned has not been decided, but most ordered conformations would be promoted by the elimination of a proline residue. In general, however, it will be difficult to interpret the effects of specific mutations until more insight is available into the precise conformations of prions and the interactions between proximal residues.

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Received 2 June 1999/15 July 1999; accepted 6 August 1999