PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

# Spectroscopic Characterization of Conformational Differences between PrP<latex>$^{\text{C}}$</latex> and PrP<latex>$^{\text{Sc}}$</latex>: An <latex>$\alpha $</latex>-helix to <latex>$\beta $</latex>-sheet Transition

Michael A. Baldwin, Keh-Ming Pan, Jack Nguyen, Ziwei Huang, Darlene Groth, Ana Serban, Maria Gasset, Ingrid Mehlhorn, Robert J. Fletterick, Fred E. Cohen and Stanley B. Prusiner

*Phil. Trans. R. Soc. Lond. B* 1994 **343**, 435-441
doi: 10.1098/rstb.1994.0041

# Spectroscopic characterization of conformational differences between $PrP^{C}$ and $PrP^{Sc}$: an α-helix to β-sheet transition

MICHAEL A. BALDWIN[1], KEH-MING PAN[1], JACK NGUYEN[1], ZIWEI HUANG[1], DARLENE GROTH[1], ANA SERBAN[1], MARIA GASSET[1], INGRID MEHLHORN[1], ROBERT J. FLETTERICK[3,4], FRED E. COHEN[2,3,4] AND STANLEY B. PRUSINER[1,4]*

*Departments of [1]Neurology, [2]Medicine, [3]Pharmaceutical Chemistry and [4]Biochemistry and Biophysics, University of California San Francisco, California 94143-0518, U.S.A.*

## SUMMARY

Although no chemical modifications have been found to distinguish the cellular prion protein $PrP^{C}$ from its infectious analogue $PrP^{Sc}$, spectroscopic methods such as Fourier transform infrared (FTIR) spectroscopy reveal a major conformational difference. $PrP^{C}$ is rich in α-helix but is devoid of β-sheet, whereas $PrP^{Sc}$ is high in β-sheet. N-terminal truncation of $PrP^{Sc}$ by limited proteolysis does not destroy infectivity but it increases the β-sheet content and shifts the FTIR absorption to lower frequencies, typical of the cross β-pleated sheets of amyloids. Thus the formation of $PrP^{Sc}$ from $PrP^{C}$ involves a conformational transition in which one or more α-helical regions of the protein is converted to β-sheet. This transition is mimicked by synthetic peptides, allowing predictions of domains of PrP involved in prion diseases.

## 1. INTRODUCTION

Scrapie is a neurodegenerative disease of sheep and goats (Dickinson *et al.* 1965; Parry 1983). Similar diseases include transmissible mink encephalopathy and bovine spongiform encephalopathy of animals (Marsh *et al.* 1991; Wilesmith *et al.* 1992) as well as kuru, Creutzfeldt–Jakob disease, Gerstmann–Sträussler–Scheinker disease and fatal familial insomnia of humans (Gajdusek 1977; Kirschbaum 1968; Masters *et al.* 1981; Medori *et al.* 1992). For many years the nature of the infectious agent causing these diseases remained enigmatic.

By progressively enriching infectious fractions from the brains of mice and later Syrian hamsters experimentally infected with the scrapie agent, the scrapie prion protein ($PrP^{Sc}$) was discovered (Bolton *et al.* 1982; Prusiner *et al.* 1982). This protein is the major, and possibly the only, component of the infectious agent or prion which causes scrapie and the related fatal neurodegenerative diseases of humans and animals (Prusiner 1991). With the discovery of $PrP^{Sc}$, the mysterious nature of the infectious pathogen causing scrapie began to unravel; in addition, prion diseases were found to be unique in that they can occur in infectious, sporadic and inherited disorders (Prusiner 1991).

* To whom correspondence should be addressed at: Department of Neurology, HSE-781, University of California, San Francisco, California 94143-0518, U.S.A.

The normal cellular form of the prion protein ($PrP^{C}$) which is expressed in neuronal cells is not normally pathogenic. There is no direct evidence that $PrP^{C}$ can spontaneously convert to $PrP^{Sc}$ to cause sporadic disease. However, the development of disease is highly likely if $PrP^{C}$ interacts with $PrP^{Sc}$ in a cellular process prior to its normal turnover. In a laboratory animal such interaction may be induced deliberately by the inoculation of infectious prions; in humans it may occur through cannibalism as in the case of kuru (Gajdusek 1977) or through the administration of infected growth hormone, dura mater grafts, corneal grafts or the use of inadequately sterilized surgical instruments (Brown *et al.* 1992). Numerous mutations in the prion protein (PrP) gene have been identified that also bring about this conversion, thereby causing inherited prion diseases.

Studies of the cell biology of $PrP^{Sc}$ have established that it is formed from $PrP^{C}$ in a post-translational event (Borchelt *et al.* 1990, 1992; Taraboulos *et al.* 1990, 1992; Caughey & Raymond 1991; Caughey *et al.* 1991*a*). Although we have investigated the primary structure and the post-translational covalent modifications of $PrP^{Sc}$ in great detail, we have been unable to identify any chemical differences that would distinguish it from $PrP^{C}$ (Stahl *et al.* 1993). This is despite the very marked physical differences, particularly the highly aggregated and insoluble nature of $PrP^{Sc}$, its protease resistance and the tendency for an infectious truncated form (PrP 27–30) to assemble into amyloid

*Phil. Trans. R. Soc. Lond.* B (1994) **343**, 435–441
*Printed in Great Britain*

rods (McKinley *et al.* 1991; Prusiner *et al.* 1983). These observations lead to the conclusion that the difference between the two forms might be conformational in nature. Thus we sought to compare the secondary, tertiary and quaternary structure of $PrP^{C}$ and $PrP^{Sc}$.

Theoretical algorithms that model protein structure based on the amino acid sequence suggested that PrP might be a four-helix bundle protein and predicted the regions that would give α-helices (Gasset *et al.* 1992). Fourier transform infrared (FTIR) spectroscopy demonstrated that the four peptides synthesized corresponding to these regions were α-helical when dispersed in α-helix-promoting solvents such as hexafluoroisopropanol (HFIP); in contrast, three of the four synthetic peptides displayed high β-sheet content when suspended in aqueous solvents. The β-sheet was observed at a particularly low frequency (LF-β) and the peptides formed fibrils observable by electron microscopy. The fibrils also stained with Congo red and exhibited green–gold birefringence when viewed in polarized light; this is the hallmark of amyloids (Gasset *et al.* 1992). Other workers also demonstrated fibril formation in PrP peptides (Come *et al.* 1993; Forloni *et al.* 1993; Goldfarb *et al.* 1993). Studies of the secondary structure by FTIR established that PrP 27–30 has a higher β-sheet content than predicted (Caughey *et al.* 1991*b*; Gasset *et al.* 1993) but denaturation, which destroys infectivity, also destroys a substantial fraction of the β-sheet, particularly the LF-β (Gasset *et al.* 1993).

These observations led to the hypothesis that $PrP^{C}$ might be a protein with a high α-helical content, some of which is converted into β-sheet during the formation of $PrP^{Sc}$. Recently, $PrP^{C}$ purified by non-denaturing procedures has become available making a comparison of the two isoforms possible. FTIR measurements showed $PrP^{C}$ to be rich in α-helix and low in β-sheet; whereas $PrP^{Sc}$ has a relatively low α-helix and high β-sheet content (Pan *et al.* 1993). The α-helical nature of $PrP^{C}$ was substantiated by measurements of ultraviolet circular dichroism (CD) (Pan *et al.* 1993).

## 2. METHODS FOR ANALYSIS OF SECONDARY STRUCTURE

Studies on proteins of structure known from X-ray diffraction have established that the FTIR amide I vibrational absorption band at 1680–1620 $cm^{-1}$ is influenced by the hydrogen bonding associated with the various secondary structural elements. Absorption at particular frequencies within this range can be used to characterize the secondary structure (Arrondo *et al.* 1993). Coils and α-helices may absorb at the same frequency but deuterium exchange of labile hydrogens shifts the absorption due to random coils and eliminates this overlap (Timasheff & Susi 1966). Computer programs are available for mathematical analysis of the amide I band involving background subtraction, smoothing, deconvolution and iterative least-squares curve fitting. This allows the calculation of the relative contributions of the secondary structural features, which in $D_2O$ are at the following frequencies: 1662–1645, α-helix; 1689–1682 and 1637–1613, β-sheet; 1682–1662.5, turn; 1644.5–1637, coil (Byler & Susi 1986; Goormaghtigh *et al.* 1990). It is assumed that the mean absorption per residue is the same for each of these components. Alternatively the secondary structure can be analysed by factor analysis (Lee *et al.* 1990).

The FTIR spectra of soluble proteins are usually obtained by transmission of radiation through a film of solution, often in $D_2O$ (Arrondo *et al.* 1993). Cells can be made of $CaF_2$ or $BaF_2$ with spacers of up to 50 μm. Insoluble peptides or proteins can be analyzed using attenuated total reflectance (ATR) of thin films deposited on the surface of a crystal (Goormaghtigh *et al.* 1990). It may be difficult to achieve complete hydrogen–deuterium exchange for an insoluble protein such as PrP 27–30 having a strong tendancy to aggregate. Incomplete deuteration can cause considerable overlap between α-helix and random coil, thus the characterization of α-helix may be less reliable than that of β-sheet.

CD is an alternative method that compares the wavelength dependence of absorption of right and left hand polarized radiation by a chromophore such as the amide bonds of a protein. Of the various secondary structural elements in proteins, only α-helices display negative CD curves with strong minima at 222 and 208 nm, thus CD is more definitive than FTIR for α-helices (Yang *et al.* 1986). CD is relatively unaffected by detergents and lipids, a valuable feature for aggregated hydrophobic membrane proteins such as PrP (Park *et al.* 1992). In this study only $PrP^{C}$ was evaluated by CD although spectra have been reported for thin dried films of PrP 27–30 and $PrP^{Sc}$ (Safar *et al.* 1993).

Both FTIR and CD measure bulk phenomena and do not reveal the locations of the secondary structural elements within the molecule. By contrast nuclear magnetic resonance (NMR) determines secondary and higher order structure at the atomic level (Wüthrich 1986). However, for NMR the protein must be soluble in an appropriate solvent and available pure in substantial amounts. As yet neither of these requirements has been met for PrP. Furthermore, to determine the structure of a protein the size of PrP (209 amino acids) it would be necessary to obtain isotopically labelled analogues and to apply three- and four-dimensional NMR techniques.

## 3. RESULTS

### (*a*) *Peptides*

The Syrian hamster (SHa) PrP regions predicted to be α-helical are shown in figure 1. These were synthesized as peptides, designated H1–H4 (Gasset *et al.* 1992). Peptides H1, H3 and H4 proved to have very limited solubility in aqueous media but were readily soluble in HFIP. They were deposited as thin films on germanium crystals and their FTIR spectra were measured by ATR. From HFIP these exhibited the characteristic spectra of α-helices but addition of aqueous media immediately transformed them to β-




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

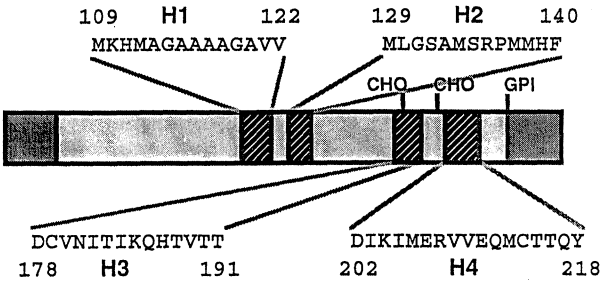


Figure 1. Schematic diagram of PrP showing the putative α-helices H1–H4. Reproduced with permission from Gasset *et al.* (1992).

sheet giving strong LF-β signals. This is illustrated in figure 2 for H1, showing a maximum at 1652 $cm^{-1}$ from HFIP which shifts to 1626 $cm^{-1}$ when treated with $D_2O$ on the crystal. Deposition from $D_2O$/potassium phosphate gave an even lower frequency of 1622 $cm^{-1}$. By contrast, the FTIR spectrum of H2 was typical of an α-helix. Electron microscopy revealed that H1, H3 and H4 formed fibrils. When these were stained with Congo red and viewed by polarization microscopy they showed green–gold birefringence. H2 displayed none of these typical amyloid properties (Gasset *et al.* 1992).

### (*b*) *Denaturation of PrP 27–30*

ATR-FTIR spectra were measured for PrP 27–30. Infectious prion rods deposited on the germanium crystal from aqueous buffer at pH 7 revealed more than 50% β-sheet, two thirds of which was LF-β. Rods were denatured by SDS-PAGE purification, a procedure that also destroys infectivity (Prusiner *et al.* 1993*b*). FTIR spectra of these preparations showed a reduction in β-sheet, particularly the LF-β component (table 1) (Gasset *et al.* 1993).

### (*c*) *Comparison of $PrP^C$ with $PrP^{Sc}$ and PrP 27–30*

The purification of prions was aided by their resistance to proteolysis and their bioactivity, monitored as scrapie infectivity in hamsters. Neither of these is applicable to $PrP^C$ and its purification has been more elusive. In earlier studies, sufficient $PrP^C$ was isolated by denaturing methods to undertake Edman N-terminal sequencing (Turk *et al.* 1988), a preliminary analysis of its GPI anchor (Stahl *et al.* 1992) and to confirm by matrix-assisted laser desorption–ionization mass spectrometry that the molecular mass of the protein was very close to that predicted (Pan *et al.* 1992; M. A. Baldwin *et al.*, unpublished results). A newly developed non-denaturing purification has allowed the study of the secondary structure of $PrP^C$ by FTIR and CD (Pan *et al.* 1993). Figure 3 compares the FTIR amide I spectra of $PrP^C$ (solid line), $PrP^{Sc}$ (dashed line) and PrP 27–30 (dotted line) obtained in $D_2O$/0.15 M NaCl/0.01 M sodium phosphate, pD 7.5 (uncorrected)/0.12% Zwittergent 3–12 in a 50 μm pathlength transmission cell with $CaF_2$ windows. The proteins were H/D equilibrated for at least 18 h before analysis.

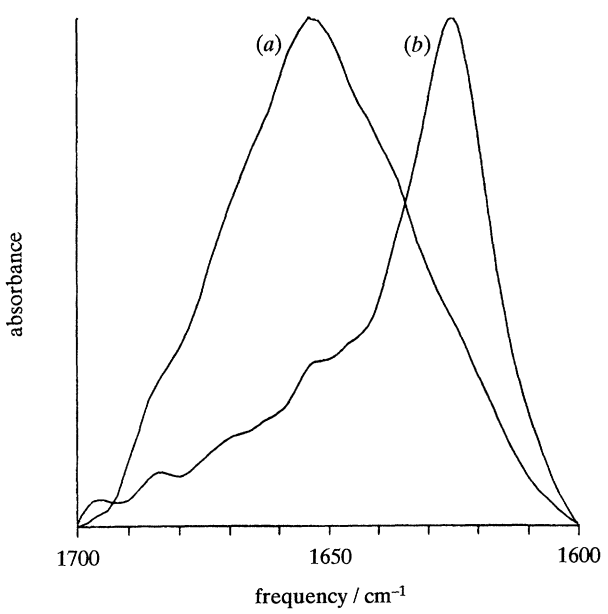


Figure 2. ATR-FTIR amide I spectra of H1 (*a*) deposited from HFIP, and (*b*) treated with $D_2O$.

Table 1. *Percent α-helix and β-sheet content of PrP 27–30, $PrP^C$ and $PrP^{Sc}$ measured by FTIR (Gasset* et al. *1993; Pan* et al. *1993) compared with predictions from a neural network program (Kneller* et al. *1990; Presnell* et al. *1993)*

FTIR

| technique | sample | α-helix | β-sheet |
|---|---|---|---|
| ATR[a] | PrP 27–30[c] | 25 | 54 |
| | SDS-PAGE[d] | 19 | 38 |
| transmission[b] | PrP 27–30[c] | 21 | 54 |
| | $PrP^C$ | 42 | 3 |
| | $PrP^{Sc}$ | 30 | 43 |

predicted

| species | ref.[e] | α-helix | β-sheet |
|---|---|---|---|
| $PrP_{90-231}$ (PrP 27–30) | naive | 20 | 8 |
| | α/α | 45 | 0 |
| | α/β | 39 | 1 |
| | β/β | 0 | 30 |
| $PrP_{23-231}$ ($PrP^C$ & $PrP^{Sc}$) | naive | 14 | 6 |
| | α/α | 31 | 0 |
| | α/β | 27 | 1 |
| | β/β | 0 | 24 |

[a] Samples dried on a Ge crystal from $D_2O$/phosphate and equilibrated with $D_2O$ (Gasset *et al.* 1993).
[b] Transmission FTIR in $D_2O$/0.15 M NaCl/0.01 M sodium phosphate, pD 7.5 (uncorrected)/0.12% Zwittergent 3–12 (Pan *et al.* 1993).
[c] Infectious native prion rods.
[d] Prion rods repurification by elution from an SDS-PAGE gel.
[e] Predominant structure of the reference sets of proteins used as the basis for the predictions.

Deconvolution and curve fitting of the data in figure 3 give the relative proportions of secondary structure listed against 'transmission' in table 1 (Pan *et al.* 1993). These data show a substantial difference in the structure of $PrP^C$, which has low or zero β-sheet content. In contrast, the β-sheet content of both $PrP^{Sc}$ and PrP 27–30 is much higher, i.e. 43 and 54%, respectively. $PrP^C$ is higher in α-helix content (42%)




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

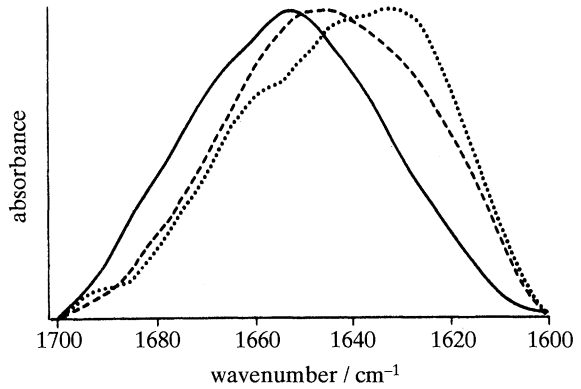


Figure 3. Transmission FTIR amide I spectra of $PrP^{C}$ (solid line), $PrP^{Sc}$ (dashed line) and PrP 27–30 (dotted line) in $D_2O$/0.15 M NaCl/0.01 M sodium phosphate, pD 7.5 (uncorrected)/0.12% Zwittergent 3–12. Reproduced with permission from Pan *et al.* (1993).

than either $PrP^{Sc}$ or PrP 27–30. Denaturation of PrP 27–30 under conditions which diminish infectivity such as SDS-PAGE (Prusiner *et al.* 1993*b*) reduce both the β-sheet (54%→38%) and α-helix (25%→19%) content as measured by ATR-FTIR (Gasset *et al.* 1993). These findings demonstrate profound differences between the secondary structures of $PrP^{C}$ and $PrP^{Sc}$.

Table 1 also gives predicted proportions of secondary structure using a neural network algorithm (Kneller *et al.* 1990; Presnell *et al.* 1993) based on homology with reference sets of naive, α/α, α/β and β/β proteins (Pan *et al.* 1993). Most interestingly, these show substantial differences because predictions for the regions H1, H3 and H4 switch between α-helix and β-sheet, depending on the nature of the reference set, reflecting their observed behaviour.

Figure 4 shows the CD spectrum of $PrP^{C}$ obtained in the same buffer as was used for FTIR except that it contained $H_2O$ rather than $D_2O$. The protein concentration measured by amino acid analysis was used in calculating the ellipticity values on the ordinate axis. Samples from several different preparations of $PrP^{C}$ gave very similar results. The minimum at 208 nm and the shoulder at 222 nm are strongly indicative of an α-helix-containing protein. The proportion of

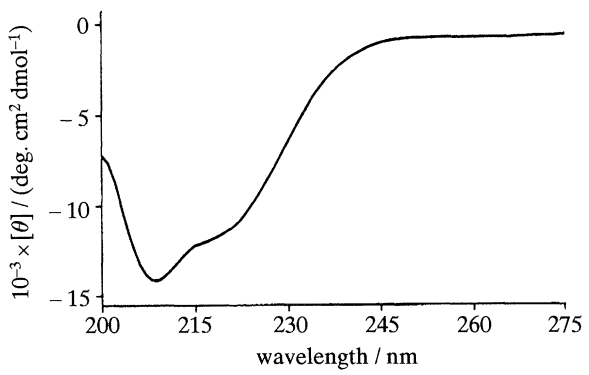


Figure 4. CD spectrum of $PrP^{C}$ in 0.15 M NaCl/0.01 M sodium phosphate, pH 7.5/0.12% Zwittergent 3–12. Reproduced with permission from Pan *et al.* (1993).

α-helix estimated from the ellipticity at 222 nm (Yang *et al.* 1986) is approximately 36% (Pan *et al.* 1993).

## 4. DISCUSSION

The data presented here establish clear differences between the secondary structures of $PrP^{C}$ and $PrP^{Sc}$ which do not seem to arise from covalent modifications as we have been unable to identify any chemical difference between the two PrP isoforms. Thus, scrapie seems to be a disease of protein conformation.

Mature PrP has 209 amino acids corresponding to codons 23–231 (Oesch *et al.* 1985; Basler *et al.* 1986; Stahl *et al.* 1990). It is normally GPI-anchored (Stahl *et al.* 1987) but expression of a non-anchored form still gave low-level conversion to a protease-resistant form in scrapie-infected cells (Rogers *et al.* 1993). A number of findings suggest one domain of PrP may be crucial in the formation of $PrP^{Sc}$: (i) limited proteolysis gives prion rods composed of PrP 27–30 N-terminally truncated at codon 90, which retain the infectivity; (ii) plaques isolated from the brains of patients who died from a familial prion disease contain a truncated protein 58–150 (Tagliavini *et al.* 1991); and (iii) a pathogenic mutation has been identified that stops PrP translation at codon 145 (Kitamoto *et al.* 1993). The PrP domain containing residues 90–145 contains a 21-residue hydrophobic sequence (codons 111–131) that is very highly conserved across all species sequenced to date, including chickens (Gabriel *et al.* 1992). Structure prediction programs contend that this sequence will be α-helical (Garnier *et al.* 1978; Bazan *et al.* 1987) and cell-free translation studies suggest that this region of PrP forms a transmembrane α-helix (Hay *et al.* 1987). However, we observed that H1 (108–121) containing the N-terminal half of this hydrophobic region prefers to form fibrils with LF-β structure (Gasset *et al.* 1992). In common with the βA4 peptide of βAPP which is associated with Alzheimer's disease, the C-terminal portion of the PrP hydrophobic region was identified to contain a repeating motif $(GXXX)_n$ associated with amyloid formation (Jarrett & Lansbury 1992). The corresponding peptide 118–133 AGAVVGGLGGYMLGSA was shown to form LF-β fibrils (Come *et al.* 1993).

Although the region 90–145 is apparently sufficient for the development of a prion disease, other portions of the protein must play some role as many of the pathogenic mutations are located outside this region. It is interesting to note that most of the point mutations are clustered close to H1, H3 or H4, sequences that as peptides have been shown to undergo a conformational switch (Gasset *et al.* 1992).

It remains unproven that amyloid formation is required for the development of prion diseases. The pathology of prion diseases is quite variable and the development of amyloid plaques is not universal. For example, a familial prion disease caused by a 144-base-pair insertion in the prion protein gene gave no characteristic pathology (Collinge *et al.* 1990). That amyloid is not necessary is consistent with irradiation studies which gave the target size of the infectious particle as 55 kDa (Bellinger-Kawahara *et al.* 1988),




PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

suggesting that a prion could be a $PrP^{Sc}$ dimer. The initial event that converts a native $PrP^{C}$ molecule into $PrP^{Sc}$ may be as simple as a geometrical isomerization, such as the conversion of a *trans* amide bond to the energetically less favored *cis* form. There would be a dynamic equilibrium in which the 'normal' conformation would be strongly favoured. Only if two molecules came together in the disease conformation, statistically a rare event, would they dimerize and switch from α-helix to β-sheet, stabilizing the abnormal conformation and preventing destruction and turnover by proteolysis. In addition to the sporadic diseases this could also be the familial mechanism if the mutations stabilize the disease conformation. The presence of β-sheet dimers might accelerate the formation of small polymers of three or four $PrP^{Sc}$ molecules which seem to be responsible for the infectivity. In some cases amyloid might subsequently form as a by-product of the disease through further aggregation, especially if the $PrP^{Sc}$ is proteolytically truncated. We have observed that full-length $PrP^{Sc}$ forms amorphous aggregates whereas PrP 27–30 is highly ordered, displaying a higher proportion of the LF-β structure that is typical of amyloids (Pan *et al.* 1993).

As presented above, the mechanism for $PrP^{Sc}$ production is based on equilibria and mass action. Alternatively, $PrP^{Sc}$ might actually catalyse the conversion of a $PrP^{C}$ molecule, lowering the energy barrier for the conformational transition to give an induced fit. Attempts to form $PrP^{Sc}$ by addition of $PrP^{Sc}$ to a test tube containing $PrP^{C}$ have been unsuccessful (Raeber *et al.* 1992) indicating that the process is complex. Pulse-chase experiments with scrapie-infected cultured cells indicate that continued protein synthesis during the chase period is required for $PrP^{Sc}$ formation (Taraboulos *et al.* 1992). These findings indicate that the synthesis of proteins other than PrP are required for the formation of $PrP^{Sc}$ to occur. Presumably such proteins in concert with a complex containing $PrP^{C}$ and $PrP^{Sc}$ feature in the propagation of prion infectivity.

A *cis* amide bond has been identified between glycine residues 37 and 38 in βA (Spencer *et al.* 1991) but there are a number of possible alternatives to a simple *cis*/*trans* isomerization. These include: (i) the special case of a proline *cis*/*trans* isomerization, which is slow but much more common, ~7% of all proline amide bonds being *cis* (Stewart *et al.* 1990); (ii) stereoisomeric conversion of an L-amino acid to the D form (Mor *et al.* 1992); (iii) a covalent modification that involves no mass change such as conversion of aspartate to isoaspartate, known to occur in βA4 (Roher *et al.* 1993); (iv) a labile modification that is lost in the work-up of the peptides prior to analysis, such as phosphoarginine (Fujitaki & Smith 1984); or (v) a covalent modification to a small fraction of $PrP^{C}$ that initiates the formation of a $PrP^{Sc}$ molecule, which can then recruit unmodified molecules into prions.

Transgenic mice that do not express $PrP^{C}$ cannot be infected with prions (Büeler *et al.* 1993; Prusiner *et al.* 1993*a*). Different 'strains' of prions give characteristically different incubation times and pathology (Hecker *et al.* 1992). Proteins may exhibit different stable conformations (Milner & Medcalf 1991), but experience suggests that only a very limited set of alternative conformations is likely. However, a minor structural change such as is described above could occur at several different sites in the protein. Thus prions from different 'strains' could be subtly different. Only the modification that characterised a particular 'strain' would be perpetuated by inoculation with prions from that 'strain'. Similarly prions would not interact efficiently with $PrP^{C}$ having a foreign sequence, thereby giving rise to the species barrier.

Attempts to create scrapie infectivity *in vivo* based on various treatments of $PrP^{C}$ and denatured $PrP^{Sc}$ have proved unsuccessful (Prusiner *et al.* 1993*b*; Raeber *et al.* 1992). Denatured $PrP^{Sc}$ has now been shown to differ from $PrP^{C}$ in its secondary structure and it is possible that denaturation brings about irreversible changes. Whether $PrP^{C}$ can be converted to $PrP^{Sc}$ *in vitro* remains to be established. Many biological processes can be imitated in a test-tube but at the present time it is not known whether chaperones, enzymes or other intracellular agents play a role in $PrP^{Sc}$ formation.

This work was supported by research grants from the National Institutes of Health as well as by gifts from Sherman Fairchild Foundation, National Medical Enterprises and the Bernard Osher Foundation.

## REFERENCES

Arrondo, J.L.R., Muga, A., Castresana, J. & Goni, F.M. 1993 Quantitative studies of the structure of proteins in solution by Fourier-transform infrared spectroscopy. *Prog. Biophys. molec. Biol.* **59**, 23–56.

Basler, K., Oesch, B., Scott, M. *et al.* 1986 Scrapie and cellular PrP isoforms are encoded by the same chromosomal gene. *Cell* **46**, 417–428.

Bazan, J.F., Fletterick, R.J., McKinley, M.P. & Prusiner, S.B. 1987 Predicted secondary structure and membrane topology of the scrapie prion protein. *Protein Engng.* **1**, 125–135.

Bellinger-Kawahara, C.G., Kempner, E., Groth, D.F., Gabizon, R. & Prusiner, S.B. 1988 Scrapie prion liposomes and rods exhibit target sizes of 55,000 Da. *Virology* **164**, 537–541.

Bolton, D.C., McKinley, M.P. & Prusiner, S.B. 1982 Identification of a protein that purifies with the scrapie prion. *Science, Wash.* **218**, 1309–1311.

Borchelt, D.R., Scott, M., Taraboulos, A., Stahl, N. & Prusiner, S.B. 1990 Scrapie and cellular prion proteins differ in their kinetics of synthesis and topology in cultured cells. *J. Cell Biol.* **110**, 743–752.

Borchelt, D.R., Taraboulos, A. & Prusiner, S.B. 1992 Evidence for synthesis of scrapie prion proteins in the endocytic pathway. *J. biol. Chem.* **267**, 6188–6199.

Brown, P., Preece, M.A. & Will, R.G. 1992 'Friendly fire' in medicine: hormones, homografts, and Creutzfeldt–Jakob disease. *Lancet* **340**, 24–27.

Büeler, H., Aguzzi, A., Sailer, A. *et al.* 1993 Mice devoid of PrP are resistant to scrapie. *Cell* **73**, 1339–1347.

Byler, D.M. & Susi, H. 1986 Examination of the secondary structure of proteins by deconvolved FTIR spectra. *Biopolymers* **25**, 469–487.





PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Caughey, B. & Raymond, G.J. 1991 The scrapie-associated form of PrP is made from a cell surface precursor that is both protease- and phospholipase-sensitive. *J. biol. Chem.* **266**, 18217–18223.

Caughey, B., Raymond, G.J., Ernst, D. & Race, R.E. 1991*a* N-terminal truncation of the scrapie-associated form of PrP by lysosomal protease(s): implications regarding the site of conversion of PrP to the protease-resistant state. *J. Virol.* **65**, 6597–6603.

Caughey, B.W., Dong, A., Bhat, K.S., Ernst, D., Hayes, S.F. & Caughey, W.S. 1991*b* Secondary structure analysis of the scrapie-associated protein PrP 27–30 in water by infrared spectroscopy. *Biochemistry* **30**, 7672–7680.

Collinge, J., Owen, F., Poulter, H. *et al.* 1990 Prion dementia without characteristic pathology. *Lancet* **336**, 7–9.

Come, J.H., Fraser, P.E. & Lansbury, P.T. Jr 1993 A kinetic model for amyloid formation in the prion diseases: importance of seeding. *Proc. natn. Acad. Sci. U.S.A.* **90**, 5959–5963.

Dickinson, A.G., Young, G.B., Stamp, J.T. & Renwick, C.C. 1965 An analysis of natural scrapie in Suffolk sheep. *Heredity* **20**, 485–503.

Forloni, G., Angeretti, N., Chiesa, R. *et al.* 1993 Neurotoxicity of a prion protein fragment. *Nature, Lond.* **362**, 543–546.

Fujitaki, J.M. & Smith, R.A. 1984 Techniques in the detection and characterization of phosphoramidate-containing proteins. In *Methods in enzymology*, vol. 107 (ed. F. Wold & K. Moldave), pp. 23–36. Orlando: Academic Press.

Gabriel, J.-M., Oesch, B., Kretzschmar, H., Scott, M. & Prusiner, S.B. 1992 Molecular cloning of a candidate chicken prion protein. *Proc. natn. Acad. Sci. U.S.A.* **89**, 9097–9101.

Gajdusek, D.C. 1977 Unconventional viruses and the origin and disappearance of kuru. *Science, Wash.* **197**, 943–960.

Garnier, J., Osguthorpe, D.J. & Robson, B. 1978 Analysis of the accuracy and implications of simple methods for predicting the secondary structure of globular proteins. *J. molec. Biol.* **120**, 97–120.

Gasset, M., Baldwin, M.A., Fletterick, R.J. & Prusiner, S.B. 1993 Perturbation of the secondary structure of the scrapie prion protein under conditions associated with changes in infectivity. *Proc. natn. Acad. Sci. U.S.A.* **90**, 1–5.

Gasset, M., Baldwin, M.A., Lloyd, D. *et al.* 1992 Predicted α-helical regions of the prion protein when synthesized as peptides form amyloid. *Proc. natn. Acad. Sci. U.S.A.* **89**, 10940–10944.

Goldfarb, L.G., Brown, P., Haltia, M., Ghiso, J., Frangione, B. & Gajdusek, D.C. 1993 Synthetic peptides corresponding to different mutated regions of the amyloid gene in familial Creutzfeldt–Jakob disease show enhanced *in vitro* formation of morphologically different amyloid fibrils. *Proc. natn. Acad. Sci. U.S.A.* **90**, 4451–4454.

Goormaghtigh, E., Cabiaux, V. & Ruysschaert, J.-M. 1990 Secondary structure and dosage of sobuble membrane proteins by attenuated total reflection Fourier-transform infrared spectroscopy on hydrated films. *Eur. J. Biochem.* **193**, 409–420.

Hay, B., Barry, R.A., Lieberburg, I., Prusiner, S.B. & Lingappa, V.R. 1987 Biogenesis and transmembrane orientation of the cellular isoform of the scrapie prion protein. *Molec. Cell. Biol.* **7**, 914–920.

Hecker, R., Taraboulos, A., Scott, M. *et al.* 1992 Replication of distinct prion isolates in region specific in brains of transgenic mice and hamsters. *Genes Dev.* **6**, 1213–1228.

Jarrett, J.T. & Lansbury, P.T. Jr 1992 Amyloid fibril formation requires a chemically discriminating nucleation event: studies of an amyloidogenic sequence from the bacterial protein OsmB. *Biochemistry* **31**, 12345–12352.

Kirschbaum, W.R. 1968 *Jakob–Creutzfeldt disease.* (251 pages.) New York: American Elsevier.

Kitamoto, T., Iizuka, R. & Tateishi, J. 1993 An amber mutation of prion protein in Gerstmann–Sträussler syndrome with mutant PrP plaques. *Biochem. biophys. Res. Commun.* **192**, 525–531.

Kneller, D.G., Cohen, F.E. & Langridge, R. 1990 Improvement in protein secondary structure prediction by an enhanced neural network. *J. molec. Biol.* **214**, 171–182.

Lee, D.C., Haris, P.I., Chapman, D. & Mitchell, R.C. 1990 Determination of protein structure using factor analysis of infrared spectra. *Biochemistry* **29**, 9185–9193.

Marsh, R.F., Bessen, R.A., Lehmann, S. & Hartsough, G.R. 1991 Epidemiological and experimental studies on a new incident of transmissible mink encephalopathy. *J. gen. Virol.* **72**, 589–594.

Masters, C.L., Gajdusek, D.C. & Gibbs, C.J. Jr 1981 Creutzfeldt–Jakob disease virus isolations from the Gerstmann–Sträussler syndrome. *Brain* **104**, 559–588.

McKinley, M.P., Meyer, R., Kenaga, L. *et al.* 1991 Scrapie prion rod formation *in vitro* requires both detergent extraction and limited proteolysis. *J. Virol.* **65**, 1440–1449.

Medori, R., Tritschler, H.-J., LeBlanc, A. *et al.* 1992 Fatal familial insomnia, a prion disease with a mutation at codon 178 of the prion protein gene. *N. Engl. J. Med.* **326**, 444–449.

Milner, J. & Medcalf, E.A. 1991 Cotranslation of activated mutant p53 with wild type drives the wild-type p53 protein into the mutant conformation. *Cell* **65**, 765–774.

Mor, A., Amiche, M. & Nicolas, P. 1992 Enter a new post-translational modification: D-amino acids in gene-encoded peptides. *TIBS* **17**, 481–485.

Oesch, B., Westaway, D., Wälchli, M. *et al.* 1985 A cellular gene encodes scrapie PrP 27–30 protein. *Cell* **40**, 735–746.

Pan, K.-M., Baldwin, M., Nguyen, J. *et al.* 1993 Conversion of α-helices into β-sheets features in the formation of the scrapie prion proteins. *Proc. natn. Acad. Sci. U.S.A.* **90**, 10962–10966.

Pan, K.-M., Stahl, N. & Prusiner, S.B. 1992 Purification and properties of the cellular prion protein from Syrian hamster brain. *Protein Sci.* **1**, 1343–1352.

Park, K., Perczel, A. & Fasman, G.D. 1992 Differentiation between transmembrane helices and peripheral helices by the deconvolution of circular dichroism spectra of membrane proteins. *Protein Sci.* **1**, 1032.

Parry, H.B. 1993 *Scrapie disease in sheep* (ed. D. R. Oppenheimer) (192 pages.) New York: Academic Press.

Presnell, S.R., Cohen, B.I. & Cohen, F.E. 1993 MacMatch: a tool for pattern-based protein secondary structure predation. *Cabios* **9**, 373–374.

Prusiner, S.B. 1991 Molecular biology of prion diseases. *Science, Wash.* **252**, 1515–1522.

Prusiner, S.B., Bolton, D.C., Groth, D.F., Bowman, K.A., Cochran, S.P. & McKinley, M.P. 1982 Further purification and characterization of scrapie prions. *Biochemistry* **21**, 6942–6950.

Prusiner, S.B., Groth, D., Serban, A. *et al.* 1993*a* Ablation of the prion protein gene in mice prevents scrapie and facilitates production of alpha-PrP antibodies. *Proc. natn. Acad. Sci. U.S.A.* **90**, 10608–10612.

Prusiner, S.B., Groth, D., Serban, A., Stahl, N. & Gabizon, R. 1993*b* Attempts to restore scrapie prion infectivity after exposure to protein denaturants. *Proc. natn. Acad. Sci. U.S.A.* **90**, 2793–2797.

Prusiner, S.B., McKinley, M.P., Bowman, K.A. *et al.* 1983





PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Scrapie prions aggregate to form amyloid-like birefringent rods. *Cell* **35**, 349–358.

Raeber, A.J., Borchelt, D.R., Scott, M. & Prusiner, S.B. 1992 Attempts to convert the cellular prion protein into the scrapie isoform in cell-free systems. *J. Virol.* **66**, 6155–6163.

Rogers, M., Yehiely, F., Scott, M. & Prusiner, S.B. 1993 Conversion of truncated and elongated prion proteins into the scrapie isoform in cultured cells. *Proc. natn. Acad. Sci. U.S.A.* **90**, 3182–3186.

Roher, A.E., Lowenson, J.D., Clarke, S. *et al.* 1993 Structural alterations in the peptide backbone of β-amyloid core protein may account for its deposition and stability in Alzheimer's disease. *J. biol. Chem.* **268**, 3072–3083.

Safar, J., Roller, P.P., Gajdusek, C. & Gibbs, C.J. Jr 1993 *J. biol. Chem.* **268**, 20276–20284.

Spencer, R.G.S., Halverson, K.J., Auger, M., McDermott, A.E., Griffin, R.G. & Lansbury, P.T. Jr 1991 An unusual peptide conformation may precipitate amyloid formation in Alzheimer's disease: application of solid-state NMR to the determination of protein secondary structure. *Biochemistry* **30**, 10382–10387.

Stahl, N., Baldwin, M.A., Burlingame, A.L. & Prusiner, S.B. 1990 Identification of glycoinositol phospholipid-linked and truncated forms of the scrapie prion protein. *Biochemistry* **29**, 8879–8884.

Stahl, N., Baldwin, M.A., Hecker, R., Pan, K.-M., Burlingame, A.L. & Prusiner, S.B. 1992 Glycosylinositol phospholipid anchors of the scrapie and cellular prion proteins contain sialic acid. *Biochemistry* **31**, 5043–5053.

Stahl, N., Baldwin, M.A., Teplow, D.B. *et al.* 1993 Structural analysis of the scrapie prion protein using mass spectrometry and amino acid sequencing. *Biochemistry* **32**, 1991–2002.

Stahl, N., Borchelt, D.R., Hsiao, K. & Prusiner, S.B. 1987 Scrapie prion protein contains a phosphatidylinositol glycolipid. *Cell* **51**, 229–240.

Stewart, D.E., Sarker, A. & Wampler, J.E. 1990 Occurrence and role of *Cis* peptide bonds in protein structures. *J. molec. Biol.* **214**, 253–260.

Tagliavini, F., Prelli, F., Ghisto, J. *et al.* 1991 Amyloid protein of Gerstmann–Sträussler–Scheinker disease (Indiana kindred) is an 11-kd fragment of prion protein with an N-terminal glycine at codon 58. *EMBO J.* **10**, 513–519.

Taraboulos, A., Raeber, A.J., Borchelt, D.R., Serban, D. & Prusiner, S.B. 1992 Synthesis and trafficking of prion proteins in cultured cells. *Molec. Biol. Cell* **3**, 851–863.

Taraboulos, A., Rogers, M., Borchelt, D.R. *et al.* 1990 Acquisition of protease resistance by prion proteins in scrapie-infected cells does not require asparagine-linked glycosylation. *Proc. natn. Acad. Sci. U.S.A.* **87**, 8262–8266.

Timasheff, S.N. & Susi, H. 1986 Infrared investigation of the secondary structure of beta-lactoglobulins. *J. biol. Chem.* **24**, 249–251.

Turk, E., Teplow, D.B., Hood, L.E. & Prusiner, S.B. 1988 Purification and properties of the cellular and scrapie hamster prion proteins. *Eur. J. Biochem.* **176**, 21–30.

Wilesmith, J.W., Ryan, J.B.M., Hueston, W.D. & Hoinville, L.J. 1992 Bovine spongiform encephalopathy: epidemiological features 1985 to 1990. *Vet. Rec.* **130**, 90–94.

Wüthrich, K. 1986 *NMR of proteins and nucleic acids*. New York: Wiley-Interscience.

Yang, J.T., Wu, C.-S.C. & Martinez, H.M. 1986 Calculation of protein conformation from circular dichroism. In *Methods in enzymology*, vol. 130 (*Enzyme structures*, part K) (ed. C. H. W. Hirs & S. N. Timasheff), pp. 208–269. Orlando: Academic Press.



PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES