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Biochemical and conformational variability of human prion strains in sporadic Creutzfeldt–Jakob disease

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Abstract

The pathogenesis of prion (PrP) diseases is thought to be related to conformational changes of a normal cellular protein, PrP^{C}, into a protease resistant protein called PrP^{Sc}, which is infectious by itself. A difficulty with this 'protein only' hypothesis is the existence of numerous PrP strains, that require PrP^{Sc} to have multiple conformations. Sporadic Creutzfeldt–Jakob disease (CJD), which accounts for nearly 80% of human prionoses, was reported to include at least two 'strains' termed types 1 and 2 which differ by electrophoretic patterns of their proteinase K (PK)-resistant fragments (PrP27–30). We have analyzed the biochemical and structural properties of PrP^{Sc} and PrP27–30 isolates from six sporadic CJD patients. Fourier transform-infra-red spectroscopy, PrP27–30 glycosylation patterns and studies of PK sensitivity revealed a striking heterogeneity. Furthermore, one isolate yielded a PrP27–30 fragment with a lower mobility clearly different from previously described sporadic CJD types. Although the average β-sheet content was higher among type 1 isolates, there was overlap between the two types. Our study suggests that human sporadic CJD-related prions display a significant heterogeneity.

Keywords: Prion; Creutzfeldt–Jakob disease; Conformation; PrP; Strains; Fourier transform infra-red spectroscopy

The prion (PrP) related diseases or prionoses are neurodegenerative diseases that affect both humans and animals. The prionoses can be sporadic, infectious or inherited [14,16]. Central to their pathogenesis is thought to be a conformational change of a normal cellular protein called PrP^{C}, into an infectious and protease resistant protein called PrP^{Sc} with higher β-sheet content [14,16]. Although convergent lines of evidence support this 'protein only' hypothesis for prion propagation, a difficulty is the existence of several distinct isolates or 'strains' of prions that can be stably passaged among inbred mice of the same genotype. The existence of strains suggests that PrP^{Sc} could adopt multiple, distinct pathological conformations. Strains are defined by the production of distinct patterns of incubation time, distributions of CNS involvement and the pattern of proteolytic cleavage of PrP^{Sc} following proteinase K (PK) digestion [9,14]. The best studied are the two strains of transmissible mink encephalopathy (TME) called hyper (HY) and drowsy (DY) [2,3]. The truncated DY PrP^{Sc} fragments (PrP27–30) migrate 1–2 kDa faster than similar preparations of HY, due to different sites of PK cleavage and the two strains differ in terms of β-sheet content [2,3]. Parchi et al. [12] defined two distinct types of sporadic Creutzfeldt–Jakob disease (CJD), based on the analysis of PrP codon 129, which encode either a valine or a methionine and by the pattern of SDS 12.5% polyacrylamide gel electrophoresis (SDS-PAGE) migration of the PrP27–30. Type 1 sporadic CJD has a molecular weight of the deglycosylated PrP27–30 of about 21 kDa, while type 2 has a mobility of about 19 kDa. Collinge et al. [5] reported two further types related to infectious CJD. The emergence of nvCJD makes a better biochemical delineation of human CJD strains a more urgent problem. We undertook the biochemical characterization of six cases of human sporadic CJD. The secondary structure was determined using Fourier transform infra-red spectroscopy (FTIR) and the β-sheet content was correlated with PK resistance. In addition, we studied if there are differences in the amino-terminus of PrP27–30 from human CJD types 1 and 2.

Six patients were selected from a group of patients with a

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Table 1
The clinical history of the six sporadic CJD patients studied[a]

Case	Sex	Age at onset (years)	Duration (months)	Signs at onset	Evolution of progressive signs
1	F	64	4	Dementia	Dementia, myoclonus
2	M	49	10	Dementia	Dementia, apraxia, hallucinations
3	M	72	12	Dementia	Dementia, myoclonus
4	F	58	6	Dementia	Dementia, ataxia, dysarthria
5	F	68	8	Ataxia	Ataxia, dysarthria, nystagmus
6	M	63	4	Ataxia	Ataxia, dementia

[a] The selection criteria were: a post-mortem diagnosis of CJD; the availability of frozen brain tissue for biochemical studies, no family history of prion disease, no known exposure to prion contamination and the availability of adequate medical records.

clinical diagnosis of sporadic CJD (Table 1). PrP27–30 was extracted using a modification of a previously published protocol, which achieves a greater than 95% pure preparation [10]. Thirty grams of frontal neocortex were homogenized in 50 mM Tris buffered saline, pH 7.4 (TBS) containing 20% sarcosyl and centrifuged 30 min at $22\,000 \times g$. The supernatant was mixed with 1/3 vol. TBS containing 0.1% SB3–14, centrifuged for 2 h at $250\,000 \times g$ and the pellet was suspended in TBS with 10% NaCl 0.1% SB3–14 and sonicated. This material was centrifuged on 20% sucrose, 10% NaCl, 0.1% SB3–14 TBS 2 h at $200\,000 \times g$. The pellet was suspended in 0.1% SB3–14, TBS and treated with 100 μg/ml PK (Sigma, St Louis, MO) over 2 h at 37°C. After blocking proteolysis with 2 mM PMSF (Sigma), the PK resistant fragments (PrP27–30) were separated by centrifugation for 90 min at $250\,000 \times g$ over a cushion of 20% sucrose in TBS/10% NaCl/0.1% SB3–14. The pellet was suspended in 0.1% SB3–14 TBS, briefly sonicated and analyzed by 12.5% SDS-PAGE, followed by electrotransfer onto PVDF or nitrocellulose membranes. Blots were revealed with anti-PrP antibody 3F4 [10] followed by peroxidase-conjugated anti-mouse IgG F(ab')2 (Amersham, Buckinghamshire, UK) and by enhanced chemiluminescence reaction (ECL, Amersham). Bands were quantitated after spectroscopic scanning using the Quantity One program (BioRad). N-terminal amino acid sequencing was performed by excising the band on PVDF membranes, corresponding to the unglycosylated PrP^{Sc}, on a 477A Microsequencer (Applied Biosystems). For the study of PK resistance, equivalent concentrations (determined by comparison to purified, quantitated PrP^{Sc} on Western blots) of partially purified full-length PrP^{Sc} (just prior to PK digestion as described above) in TBS/0.1% SB3-14 was incubated over 1–24 h at 37°C with 50 μg/ml PK and analyzed by Western blots. The percentage of PrP^{Sc} remaining after 1-, 3-, 6- and 24-h incubation with PK versus the PrP^{Sc} at time 0 was determined, by densitometric analysis of the PrP27–30 bands, using Quantity One software (BioRad). The standard deviation of the PrP27–30 band intensity on at least two independent experiments for each case ranged from 0–10%. The PK resistance was designated from + to +++ (Table 3). The difference between the PK resistance of the three groups of +, ++ to +++ was statistically significant ($P < 0.02$), using a two-way ANOVA analysis (GraphPad Prism software).

For Fourier transform infra-red spectroscopy (FTIR), samples were applied as a dry film after slow evaporation of the buffer and 1 h of deuteration as described [7]; this was repeated on at least three occasions for each PrP^{Sc} preparation in order to assess the reproducibilty of the spectra, with the PrP^{Sc} being extracted twice from each patient to rule out artifactual variation from handling the material. This meth-

Table 2
The results from the six sporadic CJD patients studied[a]

Patient	Mr (kDa)	% Unglyc.	% Monogly.	% Diglyc.	Codon 129
1	21	39 ± 6	45 ± 4	16 ± 3	M/M
2	20.5	24 ± 5	49 ± 3	27 ± 5	V/V
3	21	42 ± 8	46 ± 9	12 ± 2	V/V
4	19	22 ± 5	42 ± 6	36 ± 9	V/V
5	19	30 ± 2	48 ± 3	22 ± 3	M/M
6	22	24	50	26	M/M

[a] The second column shows the Mr (molecular weight in kDa) of the unglycosylated PrP^{sc} band following PK digestion. In the third, fourth and fifth columns the proportions (mean ± standard deviation of three to seven analyses, except for sample six where only one experiment could be performed) of the PrP^{Sc} glycoforms is shown (unglyc., unglycosylated; monogly., monoglycosylated; diglyc., diglycosylated). In the last column the codon 129 genotype is shown for each case, which was done using previously published methods [13].

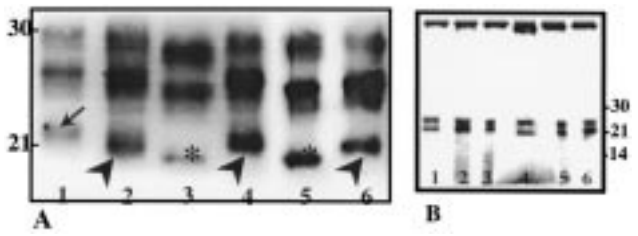

Fig. 1. (A) Shows a Western blot of PrP^{Sc} following Proteinase K digestion, showing the three fragments generated in sporadic CJD in lanes 1–6. On the left, molecular weights are shown in kDa. In lanes 2, 4 and 6 PrP^{Sc} from cases 3, 1 and 2, respectively, is seen: the bottom, deglycosylated band is at 21 kDa (see arrowheads), as is typical for type 1 spordaic CJD [11,12]. In lanes 3 and 5, PrP^{Sc} from cases 5 and 4, respectively, are seen; the bottom, deglycosylated band (see asterix) is at 19 kDa, which is typical for type 2 sporadic CJD [11,12]. In lane 1, PrP^{Sc} from case 6 is seen; the bottom, deglycosyalted band (see arrow) is at 22 kDa, which is higher then typical type 1 or 2 sporadic CJD. (B) Shows a silver stain of PrP^{Sc} from patients 1–6 in lanes 1–6, respectively. The silver stain shows that the PrP^{Sc} preparations are >95% pure (The diglycosylated PrP^{Sc} band does not stain well with silver and is too faint to be seen). At the top of the gel high molecular weight material that does not enter the running gel is present, which is immuoreactive with an anti-PrP and represents aggregated PrP^{Sc}. In some of the lanes a small amount of lower molecular weight material is present, which is immunoreactive with anto-PrP and respresents PrP^{Sc} fragments. Molecular weights are shown in kDa on the right.

odology was also validated by extracting PrP^{Sc} from hamster brains and comparing the obtained spectra to published results [3]. The purity of the samples was evaluated by 12.5% SDS-PAGE stained with Coomassie blue and by silver staining (Fig. 1). The attenuated total reflection (ATR) was recorded at room temperature on a BioRad Infrared FTS 6000 spectrometer equipped with a horizontal Golden Gate diamond ATR cell (Specac), at a resolution of 0.5 cm^{-1}, by co-addition of 1024 scans for each sample and subtraction of D_2O absorption. Due to limited amounts of material, infra-red transmission studies could not be performed in comparison. The efficiency of deuterium exchange was evidenced by low relative absorption of the amide II bands. Fourier self-deconvolution was performed using a Bessel apodization function with the Win-IR Pro System software (BioRad), with a resolution enhancing factor k = 2 and a peak half-width value of 16 cm^{-1}. The amide I peak (1550–1750 cm^{-1}) of the deconvolved spectra was imported into the Grams/32 software (Galactic Industries Co.) and resolved into individual Lorentzian components by iterative curve fitting [6,7].

Based on the apparent molecular weight of the PrP^{Sc} unglycosylated subunits, cases one to three could be assigned to type 1 and cases four and five to type 2 strains [11,12] (Table 2 and Fig. 1). The PrP27–30 unglycosylated band of case six was clearly higher than in type 1 or two cases (Fig. 1) and is also distinct from the reported Western blot appearances of types 3 and 4 CJD [5,12]; hence, this case appears to have a novel Western blot pattern. N-terminal amino acid sequencing of the unglycosylated PrP band from cases one and two revealed a major sequence of GQPHGGG within the octarepeat region of the PrP protein. N-terminal sequence of case five gave a lower yield sequence of SQ(W)N, most likely corresponding to residues 97–100. No sequence data could be obtained from cases three, four and six due to a lack of sufficient material.

The contributions of secondary structure types were calculated from the areas under the curves of individual components of the FTIR spectra after deconvolution [4,7,8] (Table 3 and Fig. 2). In cases two and three, the α-helix absorptions could not be resolved from those assigned to random coil structures and a component with intermediate maximum wavenumber (1648–1652 cm^{-1}) was detected. There was no clear correlation between the percentage of β-sheets and whether the samples corresponded to type 1 or type 2 (Table 3). Although these PrP^{Sc} samples were highly purified it is possible that contaminants may also affect the secondary structure measurements. The relative PK digestion resistance increased with the amount of β-sheet structure (Table 3).

Five of our six sporadic CJD cases are consistent with the

Table 3
The average contribution of each secondary structure type as calculated from at least three ATR-FTIR spectra of purified PrP^{Sc} after PK digestion[a]

Case	Strain type	Turns	α-Helices	Random	β-Sheets	PK resistance
1	1	12 ± 4% 1677 cm^{-1}	22 ± 2% 1661 cm^{-1}	28 ± 2% 1644 cm^{-1}	38 ± 4% 1628 cm^{-1}	++
2	1	8 ± 4% 1668 cm^{-1}	24 ± 4% 1651 cm^{-1}		68 ± 6%1621 and 1635 cm^{-1}	+++
3	1	13 ± 4% 1668 cm^{-1}	38 ± 4% 1649 cm^{-1}		49 ± 6% 1630 cm^{-1}	+++
4	2	6 ± 3% 1675 cm^{-1}	39 ± 3% 1655 cm^{-1}	9 ± 3% 1648 cm^{-1}	47 ± 4% 1628 cm^{-1}	+++
5	2	12 ± 4% 1677 cm^{-1}	27 ± 2% 1659 cm^{-1}	33 ± 2% 1645 cm^{-1}	29 ± 1% 1628 cm^{-1}	+
6	?	20 ± 4% 1668 cm^{-1}	21 ± 7% 1655 cm^{-1}	40 ± 5% 1641 cm^{-1}	19 ± 4% 1625 cm^{-1}	+

[a] Results are the (mean±SD) from each case. Assignments of the different components of the amide I spectra after Fourier self-deconvolution to secondary structure was done according to prior publications [7,8]. The last column shows the relative resistance of the PrP^{Sc} extracted from each patient to PK digestion (+++ indicates a high degree of resistance, while + indicates a relative sensitivity). The difference between the PK resistance of the three groups (+, ++ to +++) was statistically significant ($P < 0.02$) based on a two-way ANOVA analysis (GraphPad Prism software).

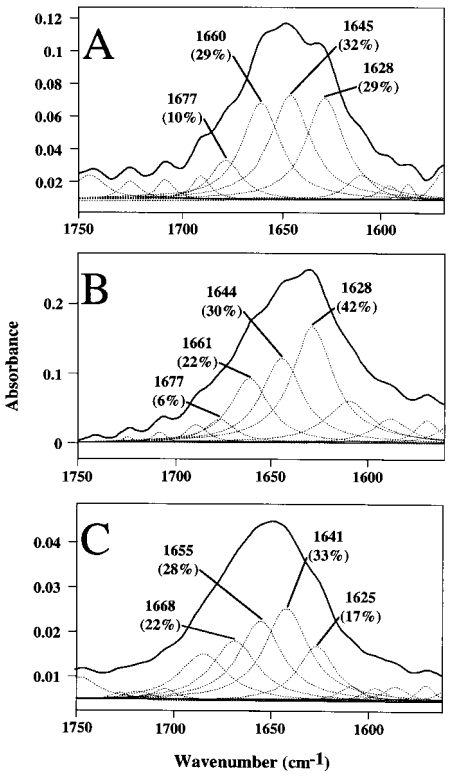

Fig. 2. Shows examples of the FT-IR spectroscopic analysis of the secondary structure of PrP^{Sc} isolated from patient 1 (type 1 sporadic CJD) in panel (A), from patient 5 (type 2, sporadic CJD) in panel (B) and from patient 6 (a novel spordaic CJD type) in panel (C). The deconvolution and curve fitting methods are decribed in the text.

classification of Parchi et al. [11,12]. However, case six does not clearly fit with previously reported CJD types, since the molecular weight of the unglycosylated band is higher (22 kDa; Fig. 1) than that seen in types 1–4 CJD. While this molecular weight is closest to that expected in type 1 patients, this patient's initial clinical presentation of ataxia, as well as the proportion of glycoforms is more typical of type 2 patients (Table 1) [12]. This suggests that further subtypes of sporadic CJD may exist. Our amino-terminal sequencing studies of the type 1 and 2 PrP27–30 has shown that, similar to the DY and HY strains, PK cleavage occurs at distinct sites [1,2].

Our studies using FTIR are consistent with past studies in hamster [6]. Interestingly, the PK resistance of PrP^{Sc} was the greatest in cases two, three and four, which also displayed the highest contribution of β-sheet secondary structure as determined by FTIR. However, this did not correlate with the type 1 and 2 strain distinction. This suggests that the site of PK cleavage is dependent on other factors, in addition to the secondary structure. A recent study by Safar et al. [15], using a conformation-dependent immunoassay, suggested that conformational differences do exist between eight different hamster PrP^{Sc} strains. Our studies of type 1 and 2 sporadic CJD suggest that these isolates show significant heterogeneity and are unlikely to represent pure 'strains' of PrP^{Sc}.

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