Conformational Transitions, Dissociation, and Unfolding of Scrapie Amyloid (Prion) Protein*

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The infectious form of the scrapie amyloid (prion) precursor, PrPSc, is a host-derived protein and a component of the infectious agent causing scrapie. PrPSc and the carboxyl-terminal proteinase K resistant core, PrP27–30, have the potential to form amyloid as a result of a post-translational event or conformational abnormality. We have studied the conformational transitions of both proteins reconstituted into liposomes, associated in solid state in thin films, and dissociated by guanidine HCl. The secondary structure of PrPSc in liposomes deduced from analysis of circular dichroism spectra contained ~34% β-sheets, ~20% α-helix, and ~46% β-turns and random coil. Cleavage of the amino-terminal region of PrPSc resulted in all-β PrP27–30, with an estimated ~43% β-sheet, no α-helix, and ~57% β-turns and random coil. The PrPSc associated in thin films with a tertiary structure perturbation corresponding to unfolding, while the secondary structure was preserved. The PrP27–30 assembled into the solid state with a similar perturbation of tertiary structure but with a large increase in the β-sheet content, probably due to an intermolecular alignment of the external β-sheets, or to a secondary structure transition, or both. The various conformational states had little or no impact on infectivity. Equilibrium dissociation and unfolding demonstrated a greater resistance of PrP27–30 to denaturation. The dissociated monomers unfolded through intermediate(s), suggesting the presence of protein domains with distinct secondary structure stabilities. The results provide experimental evidence for the β-sheet type assembly of scrapie amyloid PrP27–30 in the solid state and demonstrate the importance of amino-terminal cleavage in the stability and alignment of the amyloid-forming monomers.

The high resolution conformation of β-structured fibrous proteins remains largely unknown (1), and the mechanism of assembly is poorly understood (2) because of their intrinsic insolubility and resistance to crystallization. One biologically important protein thought to arise from β-sheet assembly is scrapie amyloid (3) (prion protein, PrP27–30) (4). This host-derived protein is a component of the agent transmitting scrapie and other spongiform encephalopathies. The scrapie amyloid (prion) protein has a critical role in neuronal degeneration and during disease development and serially propagate a three-stage transition as follows: normal form of scrapie amyloid precursor (normal cellular isoform of prion protein, PrPc) → infectious form (scrapie isoform of prion protein, PrPSc) → scrapie amyloid (prion protein, PrP27–30) (3, 4). Such a cascade of events occurs during the development of Creutzfeld-Jakob disease (CJD), kuru, Gerstmann-Sträussler-Sheinker syndrome, fatal familial insomnia in man, scrapie in sheep and goats, encephalopathy in mink, and bovine spongiform encephalopathy in cattle (3, 4).

During experimental scrapie or CJD transmission, PrPc is converted into the protease-resistant PrPSc/CJD, which has different membrane interaction capabilities (5, 6), altered physicochemical properties, and the potential to form amyloid in vitro (4). Additionally, the protein is inseparable from infectivity (7), and several point mutations, insertions, or deletions in the translated region of the PrP gene are linked with disease development in familial cases of Gerstmann-Sträussler-Sheinker syndrome and CJD and also in transgenic mice (for review, see Ref. 4). The apparently genetically transmitted disease in humans is transmitted in experimental animals with no change in the host genome (4). Since both PrPc and PrPSc/CJD protein isoforms are encoded by the same host gene, the differences between these two isoforms must be post-translational or conformational.

The analysis of the tertiary structure of all three participating protein isoforms and of the mechanism of their conversion is critical to the understanding of scrapie (CJD) transmission and development (3, 4, 8). Recent infrared spectroscopy (9, 10) studies confirmed the high β-structure content in PrP27–30 aggregates, anticipated from the transmission electron microscopy (TEM) appearance and green birefringence of Congo red-stained scrapie amyloid fibrils or prion rods (11). However, unknown to date are the secondary and tertiary structures of full-length PrPc, the secondary and tertiary structures of PrPc, the mechanism of PrPSc or PrP27–30 association, the conformational transitions from membrane-bound proteins to amyloid plaques, and the role of proteolytic processing in assembly and amyloid plaque formation.

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1The abbreviations used are: PrP27–30, prion protein; PrPc/CJD, scrapie (Sc) or Creutzfeldt-Jakob disease (CJD) isoform of prion protein; CJD, Creutzfeldt-Jakob disease; PrPc, normal cellular isoform of prion protein; CD, circular dichroism spectroscopy; SE, size-exclusion high pressure liquid chromatography; GdnHCl, guanidine hydrochloride; TEM, transmission electron microscopy; [δ], mean residue ellipticity; PBS, phosphate-buffered saline; DMPC, dimyristoylphosphatidylcholine; PAGE, polyacrylamide gel electrophoresis.
Scrapie Amyloid, Prion Protein, Conformation, Transitions

Purification of Hamster PrPSc and PrP27-30—The purification procedure of PrPSc is described in detail elsewhere (7). To all buffers used for purification, fresh protease-blocking mixture with a final concentration of 3 mM phenylmethylsulfonyl fluoride, 2 µg/ml apro tin, and 1 µg/ml leupeptin was added. The final pellet from the high speed sucrose centrifugation (7) was further purified as described (14) by resuspending in PBS, pH 7.4, containing 2.3 M NaCl and 5% sarcosyl (w/v), and sonicating five times at 10-s bursts at 50 W on ice using a BraunSonic 2000 and microprobe. The resulting detergent-DMPC-PrPSc complexes were centrifuged at 30,000 x g at 23 °C for 30 min. The liposomes were formed by dialysis of the supernatant against PBS, pH 7.4, for 24 h at 23 °C, and small unilamellar vesicles (18) were prepared by sonicating the liposomes in a water bath at 23 °C immediately before CD or electron microscopy analysis. The protein content in PrP27-30 and PrPSc liposomes was calculated according to the DNA-deduced amino acid sequence after subtracting the signal peptide and carboxyl-terminal domains are 109.9 for PrPSc and 109.5 for PrP27-30. The data shown are representative spectra from two to four experiments.

Fluorescence Spectroscopy—Fluorescence spectra were determined with a Perkin-Elmer model MFP-66 spectrofluorometer (Perkin-Elmer Cetus Instruments). The tryptophan fluorescence emission spectra were measured with an excitation wavelength of 295 nm with a 0.5-nm slit. The samples at a protein concentration of 4.3 µM were equilibrated in GdnHCl as described for turbidity and CD and diluted four times with the corresponding buffers immediately before spectrophotometry. Intensity and wavelength maxima were determined after subtracting the background from the corresponding blanks.

Equilibrium Dissociation and Unfolding—Aliquots of PrPSc or PrP27-30 from stock solutions in TBS, pH 7.4, were diluted manually to the final concentration of GdnHCl and the corresponding buffer of TBS and 100 µl was loaded in a 5-nm slit. The excitation and emission wavelengths were 295 and 340 nm, respectively, at the given GdnHCl concentration (23). Absorption and emission spectra were recorded with a Jasco spectropolarimeter, model J-500A/DP-1090 chromatograph interfaced with a Hewlett-Packard 85B computer.

CD Spectroscopy—CD spectra of liposomes were measured at 23 °C in 0.1- or 0.01-cm quartz square sandwich Hellma cells. Spectra are shown as the averages of five to ten scans with the base line of control liposomes subtracted. The PrPSc or PrP27-30 was incorporated into liposomes as described (17) with minor modifications as follows: 4.4 nmol of PrPSc or PrP27-30 were resuspended in 300 µl of TBS, pH 7.4, containing 2% sodium cholate (w/v), transferred to the vial containing a dry film of 5.7 µmol of synthetic dimethylpolysphospho-tidcholesterol (DMPC) (Sigma). They were recrystallized five times at 5-s bursts at 50 W on ice using a BraunSonic 2000 and microprobe. The data shown are the averages of four to ten scans with the base line of control liposomes subtracted. The PrPSc or PrP27-30 was incorporated into liposomes and the pellet after centrifugation by the BCA protein assay (Pierce). For thin films, the PrPSc or PrP27-30 was diluted five times at 5 µg/ml at 50 W on ice using a BraunSonic 2000 and microprobe and dialyzed for 24 h at 4 °C against three changes of H2O. The film was cast onto 0.01-cm path length quartz square sandwich cells by loading 0.9 nmol of the protein in 100 µl of H2O. The films were dried under vacuum in a desiccator for 24 h. The protein content in the film was calculated from the absorbance at 205 nm as described (19). CD spectra remained stable at 4 °C under N2 for several days. The absorbance was measured in the same cells as CD. Measurements were carried out with a Jasco spectropolarimeter, model J-500A/DP-501N, and are expressed as mean residue ellipticity [θ]222 (20, 21). The mean residue weights were calculated according to the DNA-deduced amino acid sequence after subtracting the signal peptide and carboxyl-terminal domains are 109.9 for PrPSc and 109.5 for PrP27-30. The data shown are representative spectra from two to four experiments.

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a TSK 3000 SW (600 × 7.8 mm) column calibrated by a mixture of low molecular weight standard proteins (Bio-Rad). The PrP27–30 was equilibrated for 24 h in TBS, pH 7.4, containing 0.5, 3.0, 4.5, and 6.0 M GdnHCl, protein concentration 4.3 μM, and 100-μl aliquots containing 0.43 nmol of PrPSc were injected into a Beckman 421A HPLC system with UV absorption detection at 280 nm. The PrP27–30 were equilibrated with TBS, pH 7.4, and 0–6 M GdnHCl (w/v) and developed at a flow rate of 0.35 ml/min at 23 °C. The SDS-unfolded monomers of PrP27–30 were purified by SE HPLC as described (7). The glycolipid of unfolded PrP27–30 was cleaved as described (16) with minor modifications; SE HPLC purified, unfolded PrP27–30 was concentrated in the presence of 0.1% (w/v) SDS in a Centricon 30 (Amicon) and then diluted with 15 times with 50 mM triethylamine, pH 7.3, containing 10 mM EDTA, 2 μg/ml aprotinin, and 1 μg/ml leupeptin. The 100-μl samples containing 0.43 nmol of PrPSc were incubated for 4 h at 37 °C with 2.5 units/ml phospholipidylositol-specific phospholipase C from Bacillus cereus (600 units/mg of protein, Boehringer Mannheim). The reaction was stopped by diluting the samples and blanks to 2 ml of 6 M GdnHCl in TBS, pH 7.4, and concentrated using a Centricon 30 at 23 °C. The sample and blanks were reconstituted to the original 2-ml volume by 6 M GdnHCl in TBS, pH 7.4, and reconcentrated. The final 100-μl samples and corresponding blanks were injected into SE HPLC equilibrated with 6 M GdnHCl in TBS, pH 7.4.  

**RESULTS**

**Purification and Properties of PrPSc and PrP27–30**—The TEM of the PrPSc resuspended in H2O and stained with uranylacetate had the appearance of amorphous aggregates previously described after a similar purification process (13, 26). As estimated from the densitometry of silver-stained gels and amino-terminal sequencing of the first 17 amino acids, the purity of PrPSc was ~90%, and the amino-terminal sequence of the protein started at the signal peptide cleavage site (Fig. 1). PrP27–30 resuspended in H2O and stained with uranylacetate demonstrated scrapie amyloid fibrils or prion rods (11) on TEM. The films had green birefringence after Congo red staining (not shown) as previously described with similarly prepared smears (11). The purity of PrP27–30 after proteinase K cleavage was ~95%. The triplet bands on silver-stained gels and Western blots reflect different levels of glycosylation (14). Both aggregated and liposome-incorporated PrPSc were resistant to proteinase K (Fig. 1) as described (27, 28), and the limited amino-terminal cleavage did not diminish the infectivity of the PrPSc samples (Table I). The average size of DMPC liposomes measured by electron microscopy was 40 nm with a range of 20–60 nm; the minimum molar ratio of DMPC/protein, ~1:0.01, corresponded to published data (17). The dispersion of PrP27–30 into liposomes slightly increased and association into solid thin films slightly decreased infectivity levels (Table I). The differences are not statistically significant.  

**CD Spectroscopy of PrPSc**—The CD spectra of PrPSc after dissociation and incorporation into liposomes in TBS at pH 7.4 were recorded from 320 to 205 nm (Fig. 2). They showed a low intensity, broad, negative band centered at 230 nm, a shoulder at 220 nm, a high intensity positive band in the aromatic region with a maximum at 265 nm, and a shallow negative band at 295 nm. The crossover points were at 208, 253, and 287 nm. The CD spectrum of PrPSc in thin film (Fig. 2) had slightly decreased intensity (~20%), and the UV-negative band blue-shifted to 225 nm; the intensity of the positive aromatic peak at 265 nm decreased ≥10x, and the aromatic negative band shifted to 288 nm. The intensity of the negative amide band of the PrPSc spectrum decreased with increasing GdnHCl concentration and, at 7.5 M GdnHCl, changed into a broad negative trough near 197 nm, consistent with a “random coil” spectrum. The near-UV aromatic spectrum of PrPSc responded to increasing GdnHCl concentration by complex perturbations (see below). At 1.5 M, the intensity decreased to a value between liposomes and solid state PrPSc and split into double maxima at 268 and 282 nm. Both peaks disappeared at 3 M GdnHCl, but the 268-nm band reappeared at 7.5 M GdnHCl.  

The CONTIN computer analysis of the spectra (29, 30) (Table II) of dispersed PrPSc gave results consistent with a regular α/β-protein, with a predominantly β-sheet conformation, as well as a large proportion of random structure and β-tURNS.  

**CD Spectroscopy of PrP27–30**—The CD spectrum of PrP27–30, dissociated into liposomes in TBS, pH 7.4 (Fig. 3), differs from that of PrPSc. The positive aromatic peak in the near-UV region appeared as a broad low intensity negative peak, while the negative band in the amide region was blue-shifted to 225 nm, relative to the spectra of PrPSc in liposomes. Additionally, the crossover point of the positive peak, with a maximum at 204 nm, was shifted to 213 nm. After assembly of PrP27–30 into the solid state in thin films, the negative ellipticity signal in the far-UV amide region consistently increased in intensity (≥2 ×) and was blue-shifted to 222.5 nm (Fig. 3). The spectrum of PrP27–30 dissociated at 23 °C by 1.5 M GdnHCl in TBS, pH 7.4, represents the transition from liposome to solid state, as judged by the ellipticity and wavelength maximum at 224 nm. The [θ]225 decreased at 3.5 M GdnHCl and, at 7.5 M GdnHCl, was consistent with an unfolded protein conformation as indicated by the shallow CD spectrum (Fig. 3).  

Computer deconvolution analysis of the spectra of the dissociated PrP27–30 in liposomes indicates an increased β-sheet content (43%), no α-helix, and a slightly higher proportion of random structure and/or turns when compared with the spectra of PrPSc in liposomes (Table II).  

**Equilibrium Dissociation and Unfolding in GdnHCl**—The changes in turbidity (see Fig. 5A) indicate that GdnHCl dissociates aggregated PrPSc and PrP27–30 at pH 7.4 at low denaturant concentrations, with midpoints of the transition in turbidity at 1.6 M GdnHCl for PrP27–30 and at 1.9 M GdnHCl for PrPSc (see Fig. 6A). The sigmoidal curve of relative apparent fractional change Fapp suggests a cooperative two-stage transition from aggregate to monomer (see Fig. 6A).  

The fluorescence emission spectrum of infectious PrPSc (Fig. 4) after excitation at 290 nm has a maximum at 336 nm. The wavelength maximum rapidly shifts (Fig. 5B) upon dissociation/unfolding to 352 nm at 7.5 M GdnHCl (Fig. 4) with the midpoint of the transition at 1.8 M GdnHCl (Fig. 6B). The parallel increase in emission intensity is misleading because of light scattering at low GdnHCl concentration. The Fapp transition in fluorescence maximum wavelength of PrPSc (Fig. 6B) corresponds to the turbidity transition (Fig. 6A) within the experimental error, and both experiments indicate the dissociation of aggregates into monomers and the transfer of Trp residues into a more polar environment.  

Changes in fluorescence wavelength maximum upon dissociation/unfolding of PrP27–30 are more complicated (Fig. 5B) than for PrPSc; the apparently biphasic Fapp transition
FIG. 1. The amino acid sequence, predicted secondary structure and hydrophobicity, SDS-PAGE, and Western blots of purified hamster PrPSc and PrP27-30. The DNA-deduced amino acid sequence of hamster PrPSc and PrP27-30 (15) and the predicted combined hydrophobicity (25) (index ±1.3 as oval shapes, index ≤1.3 as diamond shapes) are shown. α (wave), β (zigzag), and β-turn (turn) secondary structure predictions (24) are shown. SP, signal peptide; A, signal peptide cleavage site; B, glycolipid attachment site; C, amino-terminal of PrP27-30. The inset shows a silver-stained (a and b) SDS-PAGE gel and Western blot (c) of PrPSc and PrP27-30. Inset panel a: lane 1, purified hamster PrPSc in TBS, pH 7.4; lane 2, purified PrP27-30. Inset panel b: lane 1, hamster PrPSc incorporated into DMPC liposomes; lane 2, hamster PrPSc liposomes incubated with proteinase K (*); lane 3, hamster PrPSc liposomes, purined first by SE HPLC in the presence of SDS and then transferred to 6 M GdnHCl; lane 4, hamster PrPSc incorporated into DMPC liposomes. Inset panel c: lanes 1-4, Western blot of samples a1, a2, b1, and b2. D indicates the PrPSc and PrP27-30 dimers; the asterisk shows the proteinase K band.

TABLE I

Infectivity (biologic activity) of hamster PrPSc and PrP27-30 measured by incubation time assay

<table>
<thead>
<tr>
<th>Protein</th>
<th>Exp.</th>
<th>Infectivity</th>
<th>Total protein</th>
<th>Specific infectivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrPSc/PBS</td>
<td>4</td>
<td>9.1 ± 0.2</td>
<td>0.9 ± 0.2</td>
<td>9.1 ± 0.2</td>
</tr>
<tr>
<td>PrP27-30/PBS</td>
<td>4</td>
<td>9.5 ± 0.5</td>
<td>0.8 ± 0.1</td>
<td>9.6 ± 0.1</td>
</tr>
<tr>
<td>PrP27-30/DMPC</td>
<td>3</td>
<td>9.5 ± 0.2</td>
<td>0.17 ± 0.02</td>
<td>10.2 ± 0.2</td>
</tr>
<tr>
<td>PrP27-30/Film/Rec</td>
<td>3</td>
<td>8.7 ± 0.3</td>
<td>1.0 ± 0.0</td>
<td>8.7 ± 0.3</td>
</tr>
</tbody>
</table>

The intrinsic insolubility of PrP27-30 and PrPSc makes it very difficult to directly examine physical properties of these proteins. The apparent midpoint of Fapp transition for PrPSc is at 3.2 M GdnHCl and 4.5-5.5 M GdnHCl for PrP27-30. Both PrPSc and PrP27-30 undergo spectral changes of the aromatic band at 266 nm with increasing GdnHCl concentration (Figs. 5D and 6D). However, the changes in ellipticity in the aromatic region of the aggregated, partially folded, and unfolded protein monomers were all smaller (Fig. 5D) than the large increase in the aromatic CD band after incorporation into liposomes (Figs. 2 and 3).

SE HPLC of PrP27-30—The SE HPLC of PrP27-30 at different GdnHCl concentrations (Fig. 7) demonstrated that the protein is present as a monomer at 1.5 M GdnHCl, with an RT of 56-68 min, and with no dimers, tetramers, or higher oligomers. The Stokes radius Rs calculated from the RT and a calibration curve obtained with standard proteins is 2.4-3.2 nm at 1.5 M GdnHCl. The Stokes radius of PrP27-30 increases with increasing GdnHCl concentration and, paradoxically, at ≥3 M GdnHCl, is that of a tetramer (Fig. 7). However, the existence of higher oligomers cannot be ruled out, since the peak elutes close to the void volume. The SDS-unfolded, monomeric PrP27-30, purified first by SE HPLC in the presence of SDS and then transferred to 6 M GdnHCl, behaves identically. After phosphatidylinositol-specific phospholipase C cleavage of the diacylglycerol moiety from the carboxyl-terminal glycolipid of SDS-unfolded PrP27-30, most of the PrP27-30 is an unfolded monomer with a small residue of dimers and tetramers (Fig. 7).

DISCUSSION

The intrinsic insolubility of PrP27-30 and PrPSc makes it very difficult to directly examine physical properties of these proteins.
proteins, the mechanism of their association, and their secondary and tertiary structures. Both are highly hydrophobic membrane glycoproteins modified by a carboxyl-terminal glycolipid (31). The solvents or detergents able to solubilize secondary and tertiary structures. Both are highly hydrophobic

vent aggregation is to reconstitute the proteins into liposomes or detergent-lipid complexes (32). Although the existence of some lower oligomers cannot be completely dismissed, there is considerable evidence that PrP27-30 is present in liposomes as a monomer or dimer (17, 28, 33). CD spectroscopy allows monitoring of the secondary structure of peptides and proteins incorporated into small liposomes (34), peptides in the solid state (35), and protein monomers in solution (22, 36).

The CD spectrum of PrPSc in liposomes is unusual in that it has a red-shifted amide band at 230 nm, high intensity near-UV positive aromatic band at 265 nm, and an additional shallow negative band at 295 nm. The hamster PrPSc contains 3 Phe, 12 Tyr, and 8 Trp residues; 6 of the 8 Trp residues are clustered at the amino-terminal region within the octapeptide repeats with the sequence: Gly-Gln-Pro-His-Gly-Gly-Gly-Trp (Fig. 1). The positive aromatic band of the PrPSc disappeared after cleavage of 6 of the 8 Trp residues in PrP27-30; the difference CD spectrum (not shown) of [\(\theta\)]PrPSc - [\(\theta\)]PrP27-30 corresponds to that of poly(L-Trp) or Trp-containing peptides (37, 38). Thus, the bands at 265 and 295 nm are apparently induced by Trp interactions in the amino-terminal region. The fine structure of the near-UV spectrum at this temperature is not discernible, but both the ellipticity and pattern closely resemble the near-UV spectra of cyclo(L-His-L-Trp) (39). The intensity of Trp \(\mu\)-\(\mu\) coupling is inversely proportional to the square of distance and is significant only at less than 10–15 Å (40). Thus, the high intensity of aromatic spectra of PrPSc in liposomes is probably induced by the close proximity of Trp and His in the octapeptide repeats as a result of the conformational change induced directly or indirectly by lipid insertion.

The accessibility of charged and moderately hydrophilic amino-terminal octapeptide repeats to proteinase K in liposomes argues against a direct phospholipid partitioning effect. Therefore, the observed aromatic band perturbation could be an indirect, distant conformational effect of a different structure partitioning into phospholipids (41-43). Although the mechanism is unclear, this finding may support the possibility of direct membrane interaction of PrPSc, as suggested by both enzymatic studies (5) and in vitro expression systems (44).

The deconvolution of CD spectra of PrPSc by Provencher and Gloeckner software (30) yielded 34% of \(\beta\)-sheet with less than 20% \(\alpha\)-helix and the rest of the structure random or in \(\beta\)-turns. The method used is particularly insensitive to maxima shifts of the amide band and is reliable for both \(\beta\)-sheet and \(\alpha\)-helix (20, 21). However, if the protein interacts with the phospholipid bilayer, the deconvolution should be considered with caution (45), because the reference data base of this program contains only globular, water-soluble proteins.

When PrPSc protein was associated into the solid state in thin films, the negative amide band of PrPSc was blue-shifted, and the intensity of the aromatic band diminished ~10-fold at 265 nm. These changes could be interpreted either as conformational or as an optical artifact due to differential light scattering and an absorption flattening (46) in liposomes.

**FIG. 2. The UV CD spectra of PrPSc dissociated in liposomes, in solid state in thin films, and GdnHCl-induced dissociation and unfolding.** Shown are: lps (●), the PrPSc in DMPC liposomes in TBS, pH 7.4, at 23 °C, protein concentration 6.5 \(\mu\)M, DMPC 12.3 mm; 1.5–7.5 M GdnHCl (○, ×, □), the PrPSc incubated for 24 h in 1.5, 3.0, and 7.5 M GdnHCl in TBS, pH 7.4, at 23 °C and protein concentration 4.3 \(\mu\)M; f (▲), the PrPSc film cast from 100 μl of 9 \(\mu\)M solution in H2O at 23 °C.

**TABLE II**

<table>
<thead>
<tr>
<th>Protein</th>
<th>(\alpha)-Helix</th>
<th>(\beta)-Sheet</th>
<th>(\beta)-Turns and random structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>PrPSc</td>
<td>20 ± 1.1</td>
<td>34 ± 2.6</td>
<td>46 ± 2.0</td>
</tr>
<tr>
<td>PrP27-30</td>
<td>0</td>
<td>43 ± 1.4</td>
<td>57 ± 1.4</td>
</tr>
</tbody>
</table>

2.5-7.5

20280

43

2.5 46

1.4 51

1.4 51

32). The secondary structure is expressed as percent of the total; the \(\beta\)-turns and random structure are grouped together due to the uncertainty in prediction of \(\beta\)-turns when compared with x-ray data (20, 21).
in the solid state, or in both. However, optical artifacts generated by liposomes are unlikely, since the unilamellar vesicles are <100 nm in size (18, 47, 48); the protein/liposome molar ratio is low (≤2) (48, 49), and the CD spectra of myoglobin and concanavalin A in DMPC liposomes obtained under identical conditions show no distortions, shifts, or band flattening when compared with the solution spectra (not shown). Optical artifacts generated by the solid state are also unlikely, since there are no such changes in the aromatic spectra of the film compared with liposomes in PrP27–30 (see below); the geometry of the protein films (50) indicates a thickness of ~50 nm with rare surface irregularities of 2–40 nm (47, 49), and the undistorted UV CD spectra of homopolymer films obtained previously (35, 38) and the distorted CD spectra of myoglobin, RNase A, concanavalin A, and poly(L-lysine) in films compared with the solution (not shown). However, since the proteins in thin films have not been widely studied, we present additional data on the solid state CD of model proteins and peptides separately (50).

For these reasons, we believe that the difference in the aromatic band intensity and pattern of liposome and film PrPSc CD spectra are real and indicate perturbations of the secondary and tertiary structures. The perturbation in the aromatic band upon assembly in the solid state was similar to the changes induced by GdnHCl and corresponded to an unfolding process (51–53). However, the amide band indicated the presence of a secondary structure with less α-helix, more β-sheets, or both (54). The uncoupling between aromatic and amide bands indicates a disordered or “statistically random” PrPSc tertiary structure in the solid state with a preserved secondary structure (51, 52).

The amino-terminal region of PrPSc, aggregated or dispersed in liposomes, is accessible and sensitive to limited proteinase K cleavage with no change in infectivity. The remaining proteinase K-resistant carboxyl-terminal core, PrP27–30, when incorporated in liposomes, is an all-β-struc-
FIG. 5. PrP<sub>Sc</sub> and PrP<sub>27-30</sub> equilibrium dissociation and unfolding in GdnHCl monitored by turbidimetry at 350 nm (panel A), maximum wavelength of fluorescence emission of Trp (panel B), and mean residue ellipticity [θ] at 230 nm (panel C) and 266 nm (panel D). The PrP<sub>Sc</sub> (○) and PrP<sub>27-30</sub> (▲) were equilibrated with increasing concentrations of GdnHCl in 7TBS, pH 7.4, for 24 h at 23 °C. The protein concentration was 4.3 μM. The curves are polynomial least-square fits of the data.

FIG. 6. Comparison of PrP<sub>Sc</sub> (○) and PrP<sub>27-30</sub> (▲) equilibrium dissociation and unfolding, expressed as apparent fractional change (F<sub>app</sub>) of turbidity (panel A), maximum fluorescence emission wavelength (panel B), mean residue ellipticity [θ] at 230 nm (panel C), and at 266 nm (panel D) during the transition from associated/folded into dissociated/unfolded state. The curves are polynomial least-square fits of the data.

ture (43%) with no α-helix and with 57% of the remainder of the molecule present in β-turns or random structure. The β-sheet content agrees well with the infrared spectroscopy (47 and 54%) data obtained for PrP<sub>27-30</sub> (9, 10). After complete aggregation in thin films, the amplitude of the PrP<sub>27-30</sub> negative amide band in the CD spectrum reproducibly increased 2-3-fold when compared with liposomes, indicating a higher β-sheet content (21). This may be the result of a conversion of secondary structure from random or β-turn elements into β-sheets, or an intermolecular β-sheet alignment in thin films, or both. The data provide direct experimental evidence to explain the β-sheet assembly of PrP<sub>27-30</sub> into the solid state and may simulate the transitions occurring in amyloid plaques. The film retained the infectivity, and, despite difficulties with dispersion of aggregates, most was recovered in a biologically active form by sonication in PBS.
In contrast, the statistically insignificant increase in infectivity of PrP27-30 incorporated into DMPG liposomes probably reflects the efficient dissociation of PrP27-30 aggregates; a similar observation was previously made with different phospholipids (17, 28).

The dissociation/unfolding pathways of biologically active PrPSc and PrP27-30 preparations in GdnHCl could provide essential information about subunits, folding, and stability of the protein conformation (22, 36). With increasing GdnHCl concentration, the turbidity, fluorescence emission maxima, and CD of PrPSc and PrP27-30 underwent complex changes. The nonoverlapping midpoints of transition from an associated/folded to a dissociated/unfolded state suggested stable intermediates with different conformational stability of protein domain(s) or subdomains (1, 36). The aggregated PrPSc dissociated initially into monomers or oligomers with buffer-exposed Trp residues suggested by the wavelength shift in Trp fluorescence (55) and with secondary and tertiary structures close to those found in films as judged by CD. With increasing GdnHCl concentration, the PrPSc unfolded through stable intermediate(s), notably at ~3.5 M GdnHCl. An additional increase in GdnHCl concentration caused complete unfolding of the intermediate into the random coil conformation with little residual CD.

The scrapie amyloid PrP27-30 dissociated into monomers at low GdnHCl concentrations; the Fapp curve fits a two-stage transition (as for PrPSc) and, together with the SE HPLC data, indicates dissociation into monomers (56). The equilibrium unfolding differs for each parameter in the midpoint, and the transition from folded to the unfolded state does not overlap; the Fapp transition curves of maximum Trp fluorescence wavelength and ellipticity at 230 nm are clearly not sigmoidal. The apparent intermediate(s) are more discernible than in PrPSc, and the midpoints are shifted to higher GdnHCl concentrations, suggesting a greater thermodynamic stability for PrP27-30 compared with PrPSc (22, 36). The perturbation of the ellipticity at 266 nm of PrP27-30 during GdnHCl unfolding differs significantly from PrPSc and indicates nonparallel changes in the tertiary structure (57). Because PrP27-30 is a carboxyl-terminal segment of PrPSc, the results could indicate either reshuffling of the tertiary and secondary structures after amino-terminal region cleavage or a different unfolding pattern for the amino- and carboxyl-terminal regions. This finding demonstrates the presence of a protein with domains or subdomains (1). The stages of dissociation and unfolding can be assigned to a four-step pathway composed of (a) aggregates, (b) dissociated folded monomer, (c) partially unfolded intermediate, and (d) unfolded monomer.

SE HPLC of PrP27-30 shows that at low GdnHCl concentration the protein dissociates into heterogeneous but still compact monomers. The Stokes radius is close to the average thickness of the scrapie amyloid protofilaments (2-4 nm) constituting the prion rods (11) and may indicate that they consist of a single chain of monomers. With increasing GdnHCl concentration, PrP27-30 behaves as a tetramer with some dimers. However, the elution pattern changed dramatically after phosphatidylinositol-specific phospholipase C cleavage (16) of the diacylglycerol moiety from the carboxyl-terminal glycolipid of SDS-unfolded PrP27-30. The diacylglycerol of the glycolipid moiety apparently induces oligomerization or, alternatively, causes retardation of the eluted proteins as a result of the hydrophobic interactions. The latter possibility is unlikely because of the existence of dimers and lack of a further decrease in retention time with increasing GdnHCl concentrations. The reason for this paradoxical behavior may be the "salt-out" effect of Cl" counter ions in GdnHCl for long chain fatty acids in the glycolipid (58).

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