Distinct Structures of Scrapie Prion Protein (PrPSc)-seeded Versus Spontaneous Recombinant Prion Protein Fibrils Revealed by Hydrogen/Deuterium Exchange*

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The detailed structures of prion disease-associated, partially protease-resistant forms of prion protein (e.g. PrPSc) are largely unknown. PrPSc appears to propagate itself by autocatalyzing the conformational conversion and oligomerization of normal prion protein (PrPc). One manifestation of PrPSc templating activity is its ability, in protein misfolding cyclic amplification reactions, to seed the conversion of recombinant prion protein (rPrP) into aggregates that more closely resemble PrPSc than spontaneously nucleated rPrP amyloids in terms of proteolytic fragmentation and infrared spectra. The absence of posttranslational modifications makes these rPrP aggregates more amenable to detailed structural analyses than bona fide PrPSc. Here, we compare the structures of PrPSc-seeded and spontaneously nucleated aggregates of hamster rPrP by using H/D exchange coupled with mass spectrometry. In spontaneously formed fibrils, very slow H/D exchange in region 163–223 represents a systematically H-bonded cross-β amyloid core structure. PrPSc-seeded aggregates have a subpopulation of molecules in which this core region extends N-terminally as far as to residue 145, and there is a significant degree of order within residues 117–133. The formation of tightly H-bonded structures by these more N-terminal residues may account partially for the generation of longer protease-resistant regions in the PrPSc-seeded rPrP aggregates; however, part of the added protease resistance is dependent on the presence of SDS during proteolysis, emphasizing the multifactorial influences on proteolytic fragmentation patterns. These results demonstrate that PrPSc has a distinct templating activity that induces ordered, systematically H-bonded structure in regions that are dynamic and poorly defined in spontaneously formed aggregates of rPrP.

Transmissible spongiform encephalopathies (TSEs), or prion diseases, are a group of infectious neurodegenerative disorders that affect many mammalian species and include Creutzfeldt-Jakob disease in humans, scrapie in sheep, chronic wasting disease in cervids, and bovine spongiform encephalopathy ("mad cow" disease) (1–7). All of these diseases appear to be intimately associated with conformational conversion of the normal host-encoded prion protein, termed PrPc, to a pathological isoform, PrPSc (1–5). According to the "protein-only" model, PrPSc itself represents the infectious prion agent (1, 8); it is believed to self-propagate by an autocatalytic mechanism involving binding to PrPc and templating the conversion of the latter protein to the PrPSc state (9, 10). Although molecular details of such a mechanism of disease propagation remain largely unknown, the general principle of protein-based infectivity is supported by a wealth of experimental data (1–7).

PrPc is a monomeric glycosphatidylidyinositol-linked glycoprotein that is highly protease-sensitive and soluble in nonionic detergents. High resolution NMR data show that the recombinant PrP (rPrP), a nonglycosylated model of PrPSc, consists of a flexible N-terminal region and a folded C-terminal domain encompassing three α-helices and two short β-strands (11–13). Conversely, the PrPSc isoform is aggregate in nature, rich in β-sheet structure, insoluble in nonionic detergents, and partially resistant to proteinase K (PK) digestion, with a PK-resistant core encompassing the C-terminal ~140 residues (1–5, 14, 15). Little specific structural information is available, however, for this isoform beyond low resolution biochemical and spectroscopic characterization. Thus, the structure of PrPSc conformer(s) associated with prion infectivity remains one of the best guarded mysteries, hindering efforts to understand the molecular basis of TSE diseases.

Many efforts have been made over the years to recapitulate PrPSc formation and prion propagation in vitro. Early studies have shown that PrPSc can be converted with remarkable species and strain specificities to a PrPSc-like conformation (as judged by PK resistance) simply by incubation with PrPSc from prion-infected animals (16, 17). The yields of these original cell-free conversion experiments were low, and no new infectivity could be attributed to the newly converted material (18). An important more recent study showed that both PrPSc and TSE infectivity can be amplified indefinitely in crude brain homogenates using successive rounds of sonication and incubation (19), a procedure called protein misfolding cyclic amplification (PMCA) (20). Similar amplification of the TSE infectivity was also accomplished by PMCA employing purified PrPc as a substrate, although only in the presence of polyamines such as RNA and copurified lipids (21). Unfortunately, the quantities of...
infectious PrPSc generated by PMCA using purified brain-derived PrPSc are very small, precluding most structural studies.

In contrast to brain-derived PrPSc, large scale purification can be readily accomplished for bacterially expressed rPrP, a form of PrP lacking glycosylation and the glycosphatidylinositol anchor. The latter protein can spontaneously polymerize into amyloid fibrils, and much insight has been gained into mechanistic and structural aspects of this reaction (22–28). However, although rPrP fibrils were shown to cause or accelerate a transmissible neurodegenerative disorder in transgenic mice overexpressing a PrPSc variant encompassing residues 89–231, the infectivity titer of these “synthetic prions” was extremely low (29) or absent altogether (4). This low infectivity coincides with much shorter PK-resistant core of rPrP amyloid fibrils compared with brain-derived PrPSc (26, 30), raising questions regarding the relationship between these fibrils and the authentic TSE agent. In this context, an important recent development was the finding that the PrPSc-seeded PMCA method can be extended to rPrP, yielding protease-resistant recombinant PrP aggregates (rPrP^PMCA or rPrP-res(Sc)) (31). These aggregates display a PK digestion pattern that is much more closely related to PrPSc than that of previously studied spontaneously formed rPrP fibrils, offering a potentially more relevant model for biochemical and biophysical studies. Here, we provide, for the first time, a direct insight into the structure of rPrP fibrils, offering a potentially more relevant model for bio-

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification**—Recombinant Syrian hamster full-length prion protein (rShaPrP-(23–231)) and its fragment (rShaPrP-(90–231)) were expressed and purified as described previously (32); they were stored frozen in 10 mM phosphate buffer, pH 7.4) containing monomeric protein (0.1 mg/ml) as a substrate.

**Peptide Mapping**—Before H/D exchange experiments, peptide masses were calculated from the centroid of the isotopic envelope for the fully protonated species were selected, and the extent of deuterium incorporation was determined from the shift in mass of labeled peptides relative to unlabeled peptides. To correct for the 5% H2O present during the exchange-in and for deuterium back-exchange during proteolysis, HPLC separation, and MS analysis, control experiments were performed by using fully deuterated protein prepared by a 2-h incubation in 6.6 M guanidine hydrochloride in an exchange quench buffer (0.1 M phosphate, pH 2.4) containing a reducing agent (0.1 M tris(2-carboxyethyl)phosphine hydrochloride). After a 5-min incubation on ice, the samples were diluted 10 times with ice-cold 0.05% trifluoroacetic acid in H2O and digested for 5 min with agarase-immobilized pepsin (ThermoFisher Scientific) using 100 µl of the slurry/100 µl of protein solution (~100 µg/ml). The peptic fragments were collected in a peptide microtrap, washed to remove salts, and eluted on a C18 HPLC column using a gradient of 2–35% acetonitrile at a flow rate of 50 µl/min. Peptides separated on the column were analyzed by a Finnigan LTQ mass spectrometer. The trap and the column were immersed in ice.

Peptide masses were calculated from the centroid of the isotopic envelope using MagTran software, and the extent of deuterium incorporation at each time point was determined from the shift in mass of labeled peptides relative to unlabeled peptides. To correct for the 5% H2O present during the exchange-in and for deuterium back-exchange during proteolysis, HPLC separation, and MS analysis, control experiments were performed by using fully deuterated protein prepared by a 2-h incubation in 6.6 M guanidine deuterochloride in D2O/H2O (95:5, v/v) buffered with 10 mM potassium phosphate, pH 7. The extent of deuterium incorporation (corrected for back-exchange) was calculated as %D = (m(t) − m(0%))/(m(100% − m(0%))) × 100, where %D is the relative amount of amide deuterium atoms incorporated in each peptic fragment, m(t) is the observed centroid mass of the peptide at time point t, m(0%) is the measured mass of an undeuterated reference sample, and m(100%) is the observed mass of a fully deuterated reference sample.

Isotopic envelopes showing bimodal-like distribution were analyzed mathematically to estimate the fraction of deuterated species. To this end, the envelopes were fitted as a sum of Gaussian curves corresponding to each isotopic peak. Peaks with the best signal-to-noise ratio that do not overlap with the isotopic envelope for the fully protonated species were selected, and the areas of these peaks were divided by the areas of corresponding peaks in the mass spectrum of the fully deuterated peptide (normalized to the same area).
**RESULTS**

**PMCA Reaction for rShaPrP-(90–231)—**Previous studies have shown that the PMCA procedure of Soto and coworkers (19, 20) can be adapted to convert bacterially expressed recombinant Syrian hamster full-length prion protein to the PK-resistant form, with a 16–17-kDa PK-resistant fragment similar to that found in deglycosylated brain-derived PrPSc (31). This conversion has been accomplished using a buffer containing a mixture of nonionic and acidic detergents (0.1% Triton X-100, 0.1% SDS). Because, for technical reasons, certain aspects of structural studies involving mass spectrometric analysis are more practical using the N-terminally truncated rPrP-(90–231) fragment (see below), here we have adapted the PMCA protocol to rShaPrP-(90–231). Upon completion of the reaction, the product was treated with PK (PK:rPrP ratio of 1:10) and analyzed by gel electrophoresis and Western blotting. As shown in Fig. 1, Western blots probed with 3F4 antibody (which recognizes the epitope at residues 109–112) revealed a single band with a molecular mass of ~17 kDa (31)). However, when probed with silver staining (data not shown) or polyclonal anti-PrP antibody 78295 (which recognizes both the N- and C-terminal epitopes), electrophoretic gels revealed additional PK-resistant fragments with molecular masses between ~10 and 14 kDa (Fig. 1). Importantly, this electrophoretic profile for PMCA-converted rShaPrP-(90–231) is very similar to that of PMCA-converted full-length rShaPrP, indicating essentially identical PK-resistant fragments.

**Peptide Mapping and Coverage—**The first step of HXMS analysis involves identification of peptic fragments that can be separated under the conditions of the rapid HPLC gradient required for hydrogen exchange experiments. Tandem MS experiments with peptic digest of rShaPrP-(90–231) allowed us to identify >60 fragments, 43 of which had a signal-to-noise ratio sufficient for reliable analysis of deuterium incorporation. The number of these fragments (covering ~96% of the entire sequence with multiple overlaps in some regions) compares favorably with peptic coverage in the previous study with human rPrP-(90–231) (26). This improvement is due largely to better MS instrumentation available for the present experiments and the use of resin-immobilized pepsin.

**H/D Exchange Data—**Although the focus of the present study is on structural properties of rShaPrP-PMCA, an important point of reference for interpretation of H/D exchange data is relatively well characterized spontaneously formed recombinant prion protein amyloid fibrils, rPrPSc*®. The extent of deuterium incorporation for peptic fragments derived from rShaPrP-(90–231)*® after different times of isotope exchange (5 min, 2 h, and 24 h) is summarized in Fig. 2A. All fragments corresponding to the C-terminal part of rShaPrP-(90–231) up to residue 161 were found to incorporate deuterium very rapidly (with essentially complete labeling within the first 5 min of the exchange experiment), clearly indicating the lack of any ordered structures within this part of amyloid fibrils. This contrasts sharply with strong protection against deuterium labeling for peptides derived from the C-terminal part of rShaPrP-(90–231)*®. In particular, fragments corresponding to the entire...
170–213 region appear to be especially highly protected, with less than ∼35% deuterium incorporation even after 24 h of exchange. A relatively high, although slightly lower, level of protection (less than ∼50% labeling within 24 h) is also observed for peptic fragments corresponding to residues 161/162–168, 161–174/175, and 218–224. As discussed in the previous study with human rPrP amyloid (26), this highly protected region represents systematically H-bonded cross-β structure, defining the “core” of amyloid fibrils. On the basis of the level of deuterium labeling for the flanking peptides 162–168 and 218–224, we estimate that both of them contain 1–2 unprotected residues. This would place the N- and C-terminal boundaries of the ordered core region at residues ∼163/164 and 222/223, respectively. This H-bonded core region determined herein for rShaPrP-(90–231)Sp is similar to that previously found for human rPrP amyloid fibrils (26). The only significant difference is the N-terminal boundary, which for the latter protein appears to be at residue ∼169 (as indicated by very little protection for the peptic fragments within the 161–168 region).

The “protection map” for peptic fragments derived from rShaPrP-(90–231)PMCA (based on masses calculated from the overall centroids of the isotopic envelopes in mass spectra) is quite different from that for spontaneously formed rShaPrP-(90–231) amyloid fibrils (Fig. 2). The most notable of these differences is the apparent protection against deuteration incorporation for most peptic fragments corresponding to the 117–161 region in rShaPrP-(90–231)PMCA, whereas in rShaPrP-(90–231)Sp, this entire region is highly accessible to deuterium labeling. Furthermore, inspection of mass spectra for peptic fragments derived from rShaPrP-(90–231)PMCA indicates that some of them display at least two partially overlapping isotopic envelopes, indicating an apparently bimodal mass distribution. Spectra of this type are exemplified in Fig. 3 for peptides 120–133, 145–154, and 155–168. Importantly, there is no indication of such a bimodal mass distribution for any of the peptic fragments derived from rShaPrP-(90–231)Sp (Fig. 3).

Conformational Heterogeneity of rShaPrPPMCA Fibrils—The bimodal mass distribution for some peptic fragments derived from rShaPrP-(90–231)PMCA indicates the presence of two different species, one that incorporates deuterium very rapidly and one that is highly protected against H/D exchange. The presence of such two different populations was observed for all peptic fragments within residues 117–133 and 145–168. Thus, rShaPrP-(90–231)PMCA contains at least two conformationally distinct populations of protein molecules: one in which the regions ∼117–133 and ∼145–168 are largely disordered (i.e. highly accessible to deuterium incorporation) and another one in which these regions are involved in ordered structure(s) (i.e. show considerable protection against deuterium labeling). Precise determination of the fractions of these populations is complicated by factors such as back-exchange effects, limited resolution of MS spectra, insufficient signal-to-noise ratio, and likely coexistence within the “exchange accessible” and “exchange-protected” species of multiple subpopulations with slightly different degrees of order. Nevertheless, using the procedure described under “Experimental Procedures,” we were able to estimate the rela-
tive populations of the accessible and protected species for four peptides with the highest signal-to-noise ratio (that cover the entire 117–133 and 145–168 regions) (Fig. 4).

Overall, these data clearly indicate that rShaPrP-(90–231)PMCA fibrils are conformationally heterogeneous, especially with respect to the degree of order within the ~117–133 and ~145–168 regions. Importantly, a significant population of molecules in these aggregates is characterized by an ordered core region that is substantially longer than that in spontaneously formed fibrils. Although in rShaPrP-(90–231)Sp the N terminus of the exchange-protected core region maps to residue ~163/164, in rShaPrP-(90–231)PMCA, there appears to be a population of molecules in which the core extends N-terminally to residue ~155 and another population in which it reaches as far as residue ~145 (Fig. 4). Furthermore, a fraction of molecules in rShaPrP-(90–231)PMCA shows some degree of protection from deuterium labeling within the stretch of residues between ~117 and 133, although the level of this protection is lower compared with that observed for the 145–168 region (as indicated by kinetic data of Fig. 4). The region N-terminal to residue 117 appears to be similar in both types of aggregates, showing no long term protection against deuterium labeling. Large similarities exist also for the region C-terminal to residue 168: as for spontaneously formed fibrils, peptides derived from this part of rShaPrP-(90–231)PMCA generally show a very high degree of protection, although the C-terminus of this protected core region in rShaPrP-(90–231)PMCA appears to be somewhat less ordered, as indicated by data for the peptic fragment 218–224. The color-coded protection map comparing H/D exchange data for rShaPrP-(90–231)Sp and rShaPrP-(90–231)PMCA is shown in Fig. 5.

A very similar pattern of H/D exchange was observed in an experiment with the full-length rShaPrP (i.e. for rShaPrP-(23–231)Sp and rShaPrP-(23–231)PMCA). However, as explained above, in this case, the resolution of exchange data for the N-terminal part of the protein (up to residue 117) was very low.

**Relationship between PK Digestion Pattern and H/D Exchange**

Although PK digestion experiments indicate that the major fraction of rShaPrPPMCA is characterized by an ~16-kDa PK-resistant core starting prior to epitopes of antibodies D13 and 3F4 (residues ~96–106 and ~109–112, respectively) (Fig. 1) (31), HXMS measurements demonstrate that the core region as defined by systematically H-bonded, H/D exchange-protected structure is considerably shorter. This apparent discrepancy could indicate poor correlation between resistance to PK digestion and protection from amide hydrogen exchange. Another possibility, however, is that this is due to the effect of detergents present in the PMCA buffer; whereas these detergents were still...
present in the PK digestion experiments of Atarashi et al. (31), they were removed before the present HXMS measurements (because they interfere with detailed MS analysis).

To address this issue, we repeated PK digestion experiments using same rShaPrP<sup>PMCA</sup> samples as those employed in HXMS studies (i.e., upon washing the samples with the detergent-free buffer). As shown in Fig. 6A, under such conditions, the PK-resistant material gives rise to poorly resolved electrophoretic bands at ~10–12 kDa. According to MS analysis of the PK-digested material, these bands contain at least four C-terminal fragments starting at residues 154, 153, 142, and 140 (molecular masses of 9462, 9577, 11,061 and 11,345 Da, respectively). The sizes of these PK-resistant fragments correlate remarkably well with the structured core region as defined by H/D exchange experiments (Fig. 5). Importantly, there is no evidence for any electrophoretic band at ~16 kDa, a band that is prominent when PK digestion is performed in the detergent-containing buffer used in PMCA experiments (Fig. 6A, lanes 3 and 6). Detailed MS analysis of PK-resistant fragments generated in this buffer is complicated by multiple dodecyl sulfate anions bound to the protein (Fig. 6B). The presence of these strongly bound anions suggests that longer PK-resistant fragments (~16 and 14 kDa) observed when digestion is performed in the presence of detergents may be due to the binding of SDS to rShaPrP<sup>PMCA</sup> aggregates (see “Discussion”). The PK digestion pattern of rShaPrP<sup>SS</sup> is quite different from that of rShaPrP<sup>PMCA</sup>, with major electrophoretic bands at ~8 and 10 kDa in the detergent-free buffer (molecular masses of 8296, 9462, and 9577 Da according to MS analysis) and ~10–11 kDa in the PMCA buffer.

**DISCUSSION**

One of the critical challenges in the TSE field is the determination of the structure of infectious form(s) of the PrP. Detailed insight into the structure of PrP<sup>Sc</sup> conformer(s) is of fundamental importance not only for understanding the molecular basis of prion propagation, but also for deciphering the enigma of prion strains and their role in TSE transmissibility barriers.

Although in the case of fungal prions it is well established that the infectious entity is represented by classical amyloid fibrils (6, 7) and structural data for these amyloid assemblies are rapidly accumulating (36, 37), the picture for mammalian prions is less clear. In fact, little specific structural information is available for brain-derived PrP<sup>Sc</sup> beyond the evidence that it is resistant to proteolytic digestion (with the PK-resistant core usually starting around residue ~90) and low resolution optical spectroscopic data pointing to an increased content of β-sheet structure (14, 15). Nevertheless, several specific models for the structure of PrP<sup>Sc</sup> and/or prion protein oligomers have been recently proposed. One of them, guided by electron micrographic images of two-dimensional crystals sometimes observed in PrP<sup>Sc</sup> isolates, postulates a left-handed β-helical structure involving residues 89–175, with the two C-terminal α-helices largely preserved (38). Another model, largely based on molecular dynamics simulations and docking procedures constrained by the aforementioned electron micrographic images, postulates a very different, “spiral-like” structure, where the “β-core” consists of parallel and antiparallel β-strands within the 116–164 region, with all three α-helices retaining their native conformation (39). Although both of these models may be plausible, they are

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**FIGURE 4.** Fraction of deuterated species for rShaPrP<sup>PMCA</sup>-derived peptic fragments for which mass spectra show biomodal-like mass distribution.

**FIGURE 5.** Graphic illustration of H/D exchange data for rShaPrP-(90–231)<sup>SS</sup> and rShaPrP-(90–231)<sup>PMCA</sup> with color coding of regions showing distinct accessibilities to amide hydrogen exchange. Highly accessible region (red) is defined by more than ~90% deuterium incorporation after a 5-min exchange. Highly protected region (blue) is defined by less than ~40% deuterium incorporation after 24-h exchange.
primarily theoretical, lacking direct higher resolution experimental support.

Opposed to these models, experimentally derived structural constraints were recently obtained for recombinant human PrP-(90–231) amyloid fibrils using H/D exchange and site-directed spin labeling (26, 27). These data indicate that the core of the amyloid maps to the C-terminal part of PrP encompassing residues 110–160 and that residues within this core region form single-molecule layers that stack on top of one another with parallel and in-register alignment of β-strands. Although this parallel, in-register stacking model is supported by residue-specific distance constraints, its obvious limitation is that it is based on experiments with recombinant PrP amyloid fibrils. Compared with their brain-derived counterparts, these fibrils display reduced PK resistance for segment ~90–160 (with PK cleavage sites at residues 152 and ~162) (30) and show very little, if any, infectivity. Thus, the question remains how well this model approximates the structure of infectious PrPSc.

Here, we have extended our structural studies to rPrP aggregates formed in PrPSc-seeded PMCA reactions. As described previously (31), these fibrils differ from spontaneously formed recombinant PrP amyloid fibrils with respect to their physicochemical characteristics such as proteolytic susceptibility and infrared spectra. Importantly, some of these physicochemical characteristics (most notably, PK digestion pattern) were shown to be reminiscent of those of brain-derived PrPSc, suggesting that rPrP^PMCA may provide a useful model for detailed structural studies of PrPSc (31). The principal technique used in the present analysis was H/D exchange coupled with MS. As shown previously, this approach provides a powerful tool for structural characterization of ordered protein aggregates such as amyloid fibrils (40–43); it is especially useful for identification of specific regions involved in the β-sheet core because the amide protons in such regions are exceptionally resistant to the exchange.

Our HXMS data reveal that rShaPrP^PMCA fibrils are conformationally heterogeneous, with subpopulations of molecules differing with respect to the degree of order within the 110–168 and 117–133 regions. In addition to the population in which the exchange-protected region ends at residue ~168, there is a population in which this region extends N-terminally to residue ~155 and another one in which it extends as far as to residue ~145. Although H/D exchange data alone cannot unambiguously differentiate between different types of secondary structure, the long term protection from deuterium labeling as observed for peptides derived from the ~145–220 region of rShaPrP^PMCA is highly characteristic of a cross-β structure (40–45). Furthermore, there appears to be a population of rShaPrP^PMCA in which there is a significant degree of order within residues ~117–133. These features distinguish rShaPrP^PMCA from spontaneously formed recombinant hamster PrP amyloid fibrils, in which case no systematically H-bonded structure could be detected in the entire region N-terminal to

![FIGURE 6. A, PK digestion pattern of different types of rShaPrP-(23–231) and rShaPrP-(90–231) aggregates in the presence of detergents used in the PMCA buffer (0.1% SDS, 0.1% Triton X-100) and after removal of these detergents by washing the samples with the detergent-free buffer. Immunoblots were analyzed using rabbit polyclonal anti-PrP antibody 78295. B, mass spectra for rShaPrP-(90–231)^PMCA in the PMCA buffer (upper panel) and after removal of detergents by washing in a detergent-free buffer (lower panel). Spectra were acquired by transformation of the set of multiple charged states using MagTran software. Masses of prominent peaks in the upper panel correspond to those expected for rShaPrP-(90–231) (with the N- and C-terminal extensions present in our construct) plus one through six dodecyl sulfate (DS) anions. The mass of the main peak in the lower panel corresponds to that expected for detergent-free protein molecule, and peaks corresponding to the detergent-bound protein are greatly diminished.](image-url)
Structure of PrP Aggregates

residue ~163/164. This structural insight clearly demonstrates that PrPSc-seeded PMCA of the rPrP generates highly specific structures, further validating the potential utility of this system for the development of prion diagnostic tests (31).

Molecular Basis of Extended PK-resistant Region in rShaPrP16–17-kDa PK-resistant fragment similar to that observed for nonglycosylated PrPSc, suggesting that it may provide a long sought experimentally accessible model for studying the structure and conversion mechanism of PrPSc. The present analysis reveals, however, that this scrapie-like PK resistance persists only if the digestion is performed in the presence of specific detergents used in the PMCA buffer (0.1% SDS, 0.1% Triton X-100), whereas upon removal of the detergents from preformed rShaPrPPMCA fibrils, the size of the longest PK-resistant fragment is reduced to ~12 kDa (11,345 Da according to MS data). There are two possibilities that could explain this unexpected observation. First, interaction with the detergent molecules could induce formation of an ordered (PK-resistant) structure within the ~96–140 region of rShaPrPPMCA. However, this structure would be stable only in the presence of the detergents, disappearing rapidly upon washing the samples with the detergent-free buffer. Alternatively, rather than having any major structural effects, bound detergent molecules could simply occlude the potential PK cleavage sites within residues ~96–140, resulting in an apparent resistance of this region to proteolytic digestion.

The presence of protein-bound dodecyl sulfate anions in rShaPrPPMCA fibrils strongly suggests that the apparent PK resistance giving rise to an ~16 kDa electrophoretic band is likely due to the interaction of PrP molecules with SDS. This is consistent with the observation that efforts to convert recombinant PrP to PrPSc-mimicking (i.e. PK-resistant) structures using classical PMCA protocol of Soto and co-workers (19) (that employs Triton X-100 as a sole detergent) were not successful, and such structures could be produced only upon addition to the PMCA buffer of an acidic detergent, SDS. Although dodecyl sulfate anions are obviously nonphysiological, it is possible that PrP conversion to PrPSc in vivo may be facilitated by similar interactions with negatively charged molecules present in the cellular environment. This notion seems to be supported by the finding that the PMCA conversion of purified brain-derived PrPSc to bona fide (i.e. infectious) PrPSc could be accomplished only in the presence of polyanions such as RNA (21). The important difference, however, is that if interactions with specific cellular cofactors indeed play a role in the formation of PK-resistant structure(s) associated with PrPSc infectivity, these interactions would have to be very strong (because they persist even after relatively harsh chemical treatment), whereas the interactions with SDS responsible for an apparent PrPSc-like PK resistance of rShaPrPPMCA are intrinsically weak (because they disappear upon washing rShaPrPPMCA fibrils with the detergent-free buffer). Thus, although PrPSc-seeded PMCA of the rPrP supports formation of specific structures that appear to mimic the PK resistance of PrPSc better than amyloid fibrils formed in the absence of the seed, these structures are still distinct from authentic PrPSc conformer(s) isolated from scrapie-infected brain or those formed by PMCA using brain-derived PrPSc as a substrate. Two notable features of natural PrPSc that are lacking in the recombinant PrPSc are N-linked glycans and the glycosphatidylinositol anchor. However, although these posttranslational modifications may strongly influence PrPSc formation under certain circumstances, they do not appear to be essential for the generation of infectious prions in vivo as indicated by studies of scrapie-infected transgenic mice that express PrPSc molecules lacking these modifications (46–48). These observations suggest that conformational details and/or non-PrP molecular cofactors might be critical determinants of prion infectivity.

Methodological Implications—Apart from novel structural insight, the present findings have important methodological implications. The pattern of PK digestion is frequently used as a surrogate marker to draw conclusions regarding the conformation of misfolded PrP aggregates and, especially, to characterize prion strains. The present data clearly indicate that the apparent PK resistance of the PrP can result not only from an ordered conformation (such as β-sheet core of amyloid fibrils), but also from interactions with additional molecules that may occlude potential cleavage sites. Thus, great caution should be exercised when attempting to use a PK digestion pattern as a “structural parameter” to characterize conformational properties of misfolded protein aggregates.

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