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Prion protein (PrP) synthetic peptides induce cellular PrP to acquire properties of the scrapie isoform

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ABSTRACT **Conversion of the cellular isoform of prion protein (PrP^C) into the scrapie isoform (PrP^{Sc}) involves an increase in the β-sheet content, diminished solubility, and resistance to proteolytic digestion. Transgenetic studies argue that PrP^C and PrP^{Sc} form a complex during PrP^{Sc} formation; thus, synthetic PrP peptides, which mimic the conformational pluralism of PrP, were mixed with PrP^C to determine whether its properties were altered. Peptides encompassing two α-helical domains of PrP when mixed with PrP^C produced a complex that displayed many properties of PrP^{Sc}. The PrP^C–peptide complex formed fibrous aggregates and up to 65% of complexed PrP^C sedimented at 100,000 × *g* for 1 h, whereas PrP^C alone did not. These complexes were resistant to proteolytic digestion and displayed a high β-sheet content. Unexpectedly, the peptide in a β-sheet conformation did not form the complex, whereas the random coil did. Addition of 2% Sarkosyl disrupted the complex and rendered PrP^C sensitive to protease digestion. While the pathogenic A117V mutation increased the efficacy of complex formation, anti-PrP monoclonal antibody prevented interaction between PrP^C and peptides. Our findings in concert with transgenetic investigations argue that PrP^C interacts with PrP^{Sc} through a domain that contains the first two putative α-helices. Whether PrP^C–peptide complexes possess prion infectivity as determined by bioassays remains to be established.**

Although many lines of evidence have converged to argue persuasively that prions are composed of the scrapie isoform of prion protein (PrP^{Sc}) (1), identifying conditions for the *in vitro* conversion of the cellular isoform (PrP^C) into PrP^{Sc} wherein scrapie infectivity is generated *de novo* remains to be accomplished. Formation of PrP^{Sc} is a posttranslational process (2) in which PrP^C forms a complex with PrP^{Sc} and is then transformed into a second molecule of PrP^{Sc} (3). While attempts to detect a covalent change that distinguishes PrP^C from PrP^{Sc} were unsuccessful (4), spectroscopic studies demonstrated that PrP^C contains ≈40% α-helix and is devoid of β-sheet (5). In contrast, PrP^{Sc} has a high β-sheet content, which correlates with scrapie infectivity (6–9).

Once studies of mice expressing Syrian hamster (SHa) PrP transgenes indicated that PrP^C and PrP^{Sc} form a complex during the formation of nascent PrP^{Sc} (3), we attempted to demonstrate PrP^{Sc} production through formation of such complexes by mixing purified fractions containing equimolar amounts of the two isoforms (10). Unable to demonstrate conversion of PrP^C into PrP^{Sc} in these mixtures, we pursued the interactions of synthetic PrP peptides that correspond to regions of putative secondary structure and display conformational pluralism (11, 12). In contrast to our earlier findings, other investigators were able to demonstrate an interaction between PrP^{Sc} and PrP^C by mixing a 50-fold excess of PrP^{Sc} with labeled PrP^C (13).

In the current study, PrP peptides encompassing the first two putative α-helical regions and mimicking many structural features of the two PrP isoforms (14, 15) were mixed with PrP^C, which became resistant to proteolytic digestion and sedimented at 100,000 × *g* for 1 h. Mixtures of PrP^C and peptides formed fibrous aggregates and displayed a high β-sheet content. Addition of 2% Sarkosyl disrupted the PrP^C–peptide complex and rendered PrP^C sensitive to protease digestion; anti-PrP monoclonal antibody (mAb) prevented complex formation. Unexpectedly, the peptide in a β-sheet conformation did not bind PrP^C, whereas the random coil did. When the pathogenic A117V mutation causing both the telencephalic and ataxic forms of Gerstmann–Sträussler–Scheinker disease (16–18) was substituted in the peptide, ≈65% of the radiolabeled PrP^C formed sedimentable complexes. Our findings in concert with transgenetic investigations argue that PrP^C interacts with PrP^{Sc} through a domain that contains the first two putative α-helices.

MATERIALS AND METHODS

SHaPrP was subcloned into the glutamine synthetase expression vector pEE 12 (Cell/Tech, Alameda, CA). Chinese hamster ovary (CHO) K1 cells (American Type Culture Collection) were seeded at 10^6 cells per 10-cm dish in GMEM-S medium containing 10% dialyzed fetal calf serum (GIBCO/BRL) (19). Cells were transfected with 10 μg of pEE 12-SHaPrP vector per dish by the $CaPO_4$ method (20). After growing cells in 25 μM methionine sulfoximine (MSX) (Sigma) for 2 weeks, 60 clones were selected and grown in 100, 200, or 400 μM MSX. The clones were analyzed by Western blotting to identify the highest expressors (21). From clone 30C1, phosphatidylinositol-specific phospholipase C digestion released ≈90 ng of $SHaPrP^C$ from 10^6 cells (22).

The CHO cells expressing $SHaPrP^C$ were metabolically radiolabeled with [^{35}S]methionine (100 μCi/ml; 1 Ci = 37 GBq; NEN) (2) and immunoaffinity purified (23) from cell lysates by using the anti-PrP 3F4 mAb (24), which recognizes SHaPrP residues 109–112 (25). $SHaPrP^C$ was eluted from mAb/protein A-Sepharose with 3 M guanidine hydrochloride (Gdn·HCl) and centrifuged at 16,000 × *g* for 2 min at 4°C, and the supernatant was diluted 1:10 in TN buffer composed of 130 mM NaCl and 10 mM Tris·HCl (pH 7.4); in some cases, PrP^C was precipitated with 4 vol of methanol to separate it from the Gdn·HCl and residual detergent. ^{35}S-labeled PrP^C (^{35}S-PrP^C) concentrations were determined by comparison with signals from Western blots with known quantities of PrP^C from SHa brain and by measurements in a scintillation spectrometer.

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Abbreviations: PrP, prion protein; PrP^C, cellular isoform of PrP; PrP^{Sc}, scrapie isoform of PrP; SHa, Syrian hamster; Mo, mouse; mAb, monoclonal antibody.

SHa (Lak:LVG) obtained from Charles River Breeding Laboratories were inoculated with Sc237 prions (26) and sacrificed when they showed signs of central nervous system dysfunction. SHaPrP^{Sc} was purified from the brains of these ill animals (27). Similarly, mouse (Mo) PrP^{Sc} was purified from the brains of ill mice inoculated with RML prions (28). SHaPrP^{C} was purified from the brains of uninoculated adult SHa (5) and radioiodinated with ^{125}I (1 mCi per 100 μg of PrP^{C}; Amersham) using Iodo-Beads (Pierce) (29).

PrP peptides were synthesized and purified as described; conformations were established by Fourier transform infrared spectroscopy and CD (12, 14). Proteinase K (GIBCO/BRL) was used at a concentration of 50 μg/ml and incubated for 1 h at 37°C. Although PrP^{SC} was digested in 3 M Gdn·HCl, in some cases the activity of proteinase K was reduced ≈90%, as measured by a colorimetric assay with carbobenzoxyvalylglycylarginine *p*-nitroanilide (Boehringer Mannheim). Digestions with proteinase K were terminated by addition of 1 mM (4-amidinophenyl) methanesulfonyl fluoride (Boehringer Mannheim). SDS/PAGE was performed according to Laemmli (30) and autoradiograms were obtained. Immunoblots were performed using the ECL system (Amersham) with anti-PrP 3F4 mAb. Using a JEOL 100CX electron microscope, samples were viewed at 80 keV after negative staining.

Fifty nanograms of ^{35}S-SHaPrP^{C} at a concentration ≈10 μg/ml was incubated in Eppendorf microcentrifuge tubes with TN buffer for up to 48 h at 37°C. PrP peptides were mixed with immunopurified, radiolabeled PrP^{C} in 0.3 M Gdn·HCl at molar ratios ranging from 50:1 to 5000:1. Anti-PrP 3F4 and 13A5 mAbs (31) were added to SHaPrP^{C} in molar ratios of 500:1 or 1000:1. SHa- or MoPrP^{Sc} was incubated with SHaPrP^{C} in molar ratios ranging from 1:1 to 50:1. The PrP^{Sc} (≈1 mg/ml) was pretreated with Gdn·HCl at concentrations ranging from 0 to 6 M for 16 h at 37°C. Upon termination of the incubation, an equal volume of TN buffer was added to all samples and analyses were performed immediately.

RESULTS

PrP Peptides Promote Formation of Protease-Resistant PrP. Having found that small PrP peptides can interact and induce conformational changes (12) and that a 56-residue peptide denoted SHa 90–145, which corresponds to the N terminus of PrP 27–30, displayed multiple conformations (14), we asked if synthetic peptides mixed with PrP^{C} could alter its properties. When the random coil peptide was incubated with ^{35}S-PrP^{C} at a ratio of 5000:1, it induced protease resistance (Fig. 1*A*, lanes 1–4). The same peptide in the β-sheet form did not produce resistance to proteolysis (lanes 5–7). When Mo 90–145 in either the random coil or β-sheet form was mixed with SHaPrP^{C}, it did not induce a change in protease resistance (lanes 8 and 9).

To localize the region in which PrP peptides interact with PrP^{C}, we examined shorter peptides. Neither H1 containing residues 109–122 nor a longer version denoted 104H1 composed of residues 104–122 could produce protease resistance in PrP^{C} after mixing (Fig. 1*B*, lanes 2 and 3). In aqueous buffers, H1 rapidly folds into β-sheets and polymerizes (11) while 104H1 is random coil (12). SHa 109–141 and SHa 90–145 both gave protease resistance (lanes 4 and 5). The most efficient formation of protease-resistant radiolabeled PrP^{C} was seen with the SHa 90–145 (A117V) peptide in which V was substituted for A at position 117 (lane 6). Compared with the wild-type peptide (lane 4), only 30–40% of the mutant peptide was needed to produce equivalent amounts of protease-resistant PrP^{C}. Addition of 2% (wt/vol) Sarkosyl disrupted the PrP^{C}–peptide complexes and rendered the PrP^{C} sensitive to protease digestion (data not shown). With SHa 90–145 (A117V), ≈50% of the ^{35}S-PrP^{C}–peptide complex exhibited protease resistance; with SHa 90–145, only 15–20% was resistant.

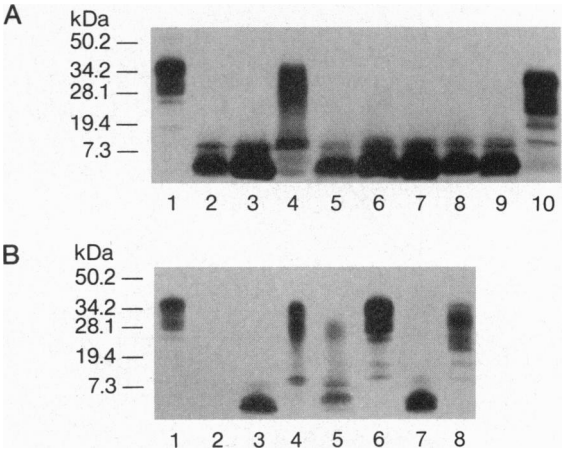

FIG. 1. Incubation of synthetic PrP peptides with PrP^{C}. ^{35}S-SHaPrP^{C} and either synthetic peptides or PrP^{Sc} were incubated in TN buffer containing 0.3 M Gdn·HCl for 48 h at 37°C; samples were digested with proteinase K for 1 h at 37°C followed by SDS/PAGE and autoradiography. (*A*) PrP^{C} without proteinase K digestion (lane 1). SHa 90–145 in a random coil incubated with PrP^{C} with a peptide/PrP^{C} ratio of 50:1 (lane 2), 500:1 (lane 3), or 5000:1 (lane 4). SHa 90–145 in a β-sheet with PrP^{C} at 50:1 (lane 5), 500:1 (lane 6), or 5000:1 (lane 7). Mo 90–145 in a random coil with PrP^{C} at 5000:1 (lane 8). Mo 90–145 in a β-sheet with PrP^{C} at 5000:1 (lane 9). Undenatured SHaPrP^{Sc} with PrP^{C} at 50:1 (lane 10). Lane 1 has 15% of the PrP^{C} in lanes 2–10. (*B*) PrP^{C} without proteinase K digestion (lane 1). Peptides were in a random coil conformation unless otherwise noted and were incubated with PrP^{C} at a ratio of 5000:1. Lane 2, SHa 109–122 (H1) was in a β-sheet conformation. Lane 3, SHa 104–122 (104H1); lane 4, SHa 90–145; lane 5, SHa 109–141; lane 6, SHa 90–145 (A117V); lane 7, Mo 90–145. Undenatured SHaPrP^{Sc} with PrP^{C} 50:1 (lane 8).

Properties of PrP^{C}–Peptide Complexes. Since the protease resistance of the PrP^{C}–peptide complexes resembled that of PrP^{Sc}, we asked if, like PrP^{Sc}, the complexes were insoluble (27, 32, 33). ^{35}S-PrP^{C} was incubated with or without SHa 90–145 (A117V) followed by centrifugation at 100,000 × *g*. Without peptide, <10% of the ^{35}S-PrP^{C} was sedimented, whereas addition of SHa 90–145 (A117V) resulted in ≈65% of the radiolabel in the pellet. Fourier transform infrared spectroscopy of the sedimented PrP^{C}–SHa 90–145 complex showed a substantial increase in β-sheet content compared to pelleted PrP^{C} (Fig. 2*A*). As measured by CD, the supernatant containing primarily unbound SHa 90–145 peptide remained random coil, as did the peptide incubated alone in TN buffer for 48 h (data not shown). It is unknown how much of the increase in β-sheet content was contributed by PrP^{C} and how much was contributed by the peptide. Treating PrP^{C} with 50% acetonitrile for 48 h at 37°C did not produce protease-resistant PrP^{C}. When incubated without peptide, PrP^{C} pellets showed many spherical aggregates up to 20 nm in diameter (Fig. 2*B*). In contrast, numerous large, filamentous polymers were found in the pellets of the PrP^{C}–peptide mixture (Fig. 2*C*).

Anti-PrP mAb Binding to PrP^{C}. Since both anti-PrP 3F4 and 13A5 mAbs bind to SHaPrP^{C} within the region spanned by the SHa 90–145 peptide, we asked if these mAbs could prevent acquisition of protease resistance. Both mAbs prevented formation of protease-resistant PrP^{C}–peptide complexes (Fig. 3*A*, lanes 3 and 4).

Spontaneous Formation of Protease-Resistant PrP. We next asked if PrP^{C} incubated in the absence of PrP peptides could become protease resistant. Immunopurified PrP^{C} (10 μg/ml) from CHO cells (Fig. 3*B*, lanes 1–4) as well as PrP^{C} (1 mg/ml) purified from SHa brain (lanes 5–8) were incubated for 0 min, 2 min, or 48 h at 37°C in the presence of 0.75 M

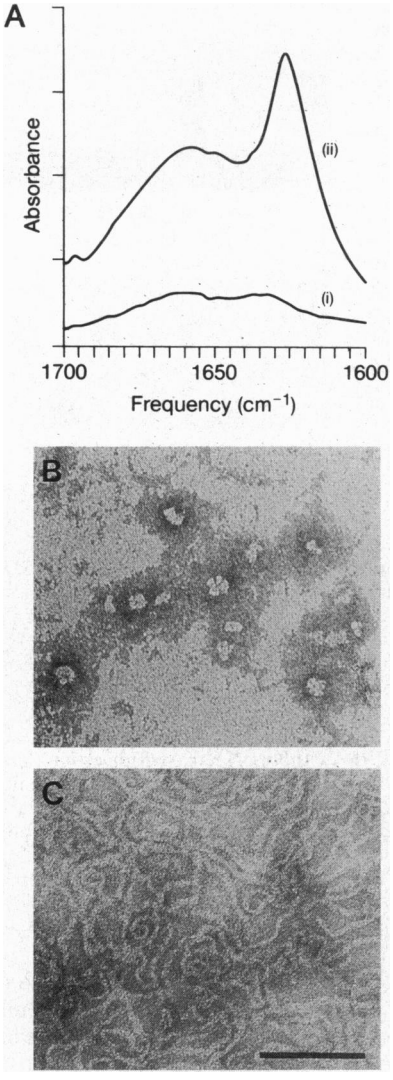

FIG. 2. Physical properties of the PrP^{C}–SHa 90–145 complex. (*A*) Fourier transform infrared spectra of SHa 90–145 (trace i) and the complex (trace ii). SHa 90–145 was incubated alone or with SHaPrP^{C} in TN buffer containing 0.3 M Gdn·HCl for 48 h; samples were centrifuged at 100,000 × *g* for 1 h at 20°C and the pellets were resuspended in TN buffer. (*B*) Ultrastructure of PrP^{C} negatively stained with 2% uranyl acetate. (*C*) PrP^{C}–SHa 90–145 complex negatively stained with 2% ammonium molybdate. (Bar = 100 nm.)

Gdn·HCl. Before digestion with proteinase K for 1 h at 37°C, samples were diluted 1:2 with TN buffer (lanes 2–4 and 6–8). Approximately 1% of the PrP^{C} was found to be protease resistant after 48 h under these conditions (Fig. 3*B*) compared to ≈50% of the PrP^{C} that was rendered protease resistant with the SHa 90–145 (A117V) peptide (Fig. 1). PrP^{C} overexpressed in CHO exhibited a broad size range presumably due to hyperglycosylation (Fig. 3*B*, lane 1) in contrast to PrP^{C} from SHa brain (lane 5). To confirm the identity of the protease-resistant band, the blot was autoradiographed; after 2 weeks of exposure, faint but discrete bands of identical size were detected in lanes containing ^{35}S-PrP^{C} (data not shown). Addition of 0.2% Sarkosyl rendered the protease-resistant PrP^{C} sensitive to proteolytic digestion.

PrP^{Sc} Could Not Be Renatured from Gdn·HCl. Since a small fraction of PrP^{C} acquired protease resistance when incubated alone, and a much larger fraction showed resistance when incubated with synthetic PrP peptides, we revisited the possibility that PrP^{Sc} mixed with PrP^{C} might render it protease resistant. Other investigators reported that PrP^{Sc} denatured in

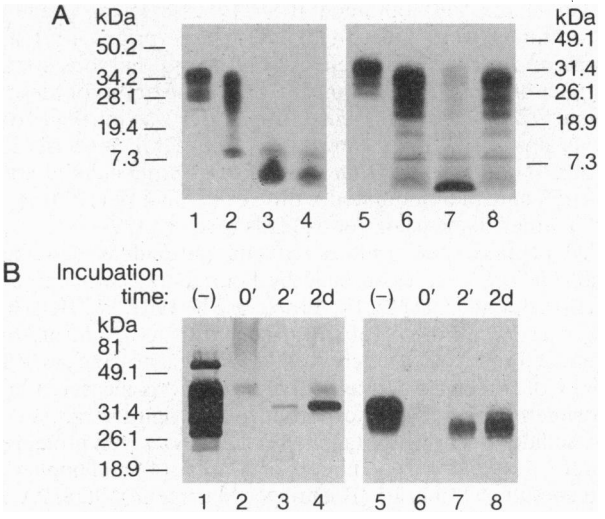

FIG. 3. Anti-PrP mAbs prevent PrP^{C} from acquiring protease resistance; a small fraction of PrP^{C} alone exhibits protease resistance. (*A*) ^{35}S-PrP^{C} was incubated for 48 h with either SHa 90–145 peptide or SHaPrP^{Sc} in the presence or absence of anti-PrP 3F4 or 13A5 mAb. PrP^{C} without proteinase K digestion (lanes 1 and 5). SHa 90–145 peptide incubated with PrP^{C} at a molar ratio of 5000:1 (lane 2); the incubation mixture contained 3F4 (lane 3) or 13A5 mAb (lane 4) in a mAb/PrP^{C} ratio of 500:1. SHaPrP^{Sc} was incubated with PrP^{C} at a ratio of 50:1 (lane 6); the incubation mixture contained 3F4 (lane 7) or 13A5 mAb (lane 8) in a mAb/PrP^{C} ratio of 500:1. Lanes 1 and 5 have 15% of the PrP^{C} in lanes 2–4 and 6–8. (*B*) SHaPrP^{C} was incubated alone for 0 min, 2 min, or 48 h at 37°C followed by digestion with proteinase K. Samples were analyzed by SDS/PAGE and Western blotting using the anti-PrP 3F4 mAb. ^{35}S-SHaPrP^{C} expressed in CHO cells (lanes 1–4) or PrP^{C} purified from SHa brain (lanes 5–8). Lanes 1 and 5 were not digested and have 15% of the PrP^{C} in lanes 2–4 and 6–8.

3 M Gdn·HCl undergoes renaturation and renders PrP^{C} resistant to proteolysis within 2 min of mixing (13). Since numerous attempts to renature prion infectivity from both Gdn and urea had failed (34), we investigated the effect of 3 M Gdn·HCl on PrP^{Sc}. As before, we were unable to demonstrate renaturation of PrP^{Sc} that had been denatured in 3 M Gdn·HCl and then diluted 1:4 to 1:10 before limited protease digestion and SDS/PAGE (Fig. 4*A*). Of note, when the dilution was carried out in the same tube to which the 3 M Gdn·HCl had been added, we did see protease-resistant PrP (data not shown). This was never seen when the tubes were changed, and we surmise that this was due to residual, undenatured PrP^{Sc} bound to the walls of the tube.

When we mixed PrP^{Sc} that had been denatured in 3 M Gdn·HCl and then diluted in buffer to give a final concentration of 0.3–2 M Gdn·HCl with PrP^{C}, no protease-resistant ^{35}S-PrP^{C} could be detected. However, mixing undenatured PrP^{Sc} with PrP^{C} did produce protease-resistant ^{35}S-PrP^{C} (Fig. 3*A*, lane 6). As reported by others (13), a 50-fold excess of PrP^{Sc} was required to produce protease-resistant ^{35}S-PrP^{C}, while a 1:10 excess of PrP^{Sc} did not. The presence of 0.3 M Gdn·HCl in the reaction mixture seems to be essential since its removal by methanol precipitation before mixing prevented complex formation. Although ≈50% of the ^{35}S-PrP^{C} was recovered in complexes sedimented at 100,000 × *g* for 1 h, only 10–15% was protease resistant.

Anti-PrP mAb Prevents Binding of PrP^{Sc} to PrP^{C}. The interaction between PrP^{C} and PrP^{Sc} was found to be inhibited by the anti-PrP 3F4 but not 13A5 mAb (Fig. 3*A*, lanes 7 and 8). This difference between the two mAbs might indicate a critical role for the PrP residues in the vicinity of the 3F4 epitope, which is at the N terminus of the H1 region, or reflect a difference in the avidity of the two mAbs. PrP^{C}-II truncated at the N terminus and lacking the 3F4 epitope did not exhibit

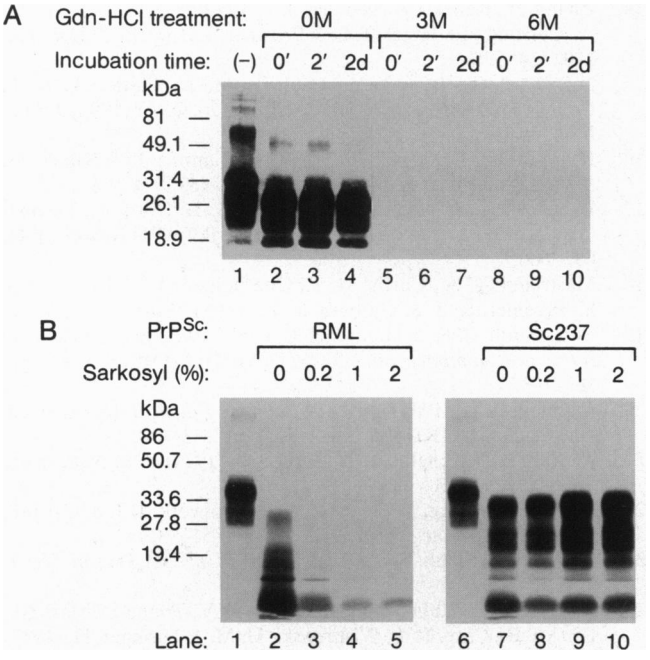

FIG. 4. Incubation of PrP^{Sc} with Gdn·HCl or PrP^{C}. (*A*) $SHaPrP^{Sc}$ was incubated with 0, 3, or 6 M Gdn·HCl for 1 hr, followed by further incubation with a 1:4 dilution of Gdn·HCl for 0 min, 2 min, or 48 h at 37°C. Samples were analyzed by SDS/PAGE and Western blotting using the anti-PrP 3F4 mAb. Lane 1, sample was not digested with proteinase K. Lanes 2, 5, and 8, samples were digested with proteinase K at time 0; lanes 3, 6, and 9, samples were digested at 2 min; lanes 4, 7, and 10, samples were digested at 48 h. Lanes 2–4, samples incubated without Gdn·HCl; lanes 5–7, samples incubated with 3 M Gdn·HCl; lanes 8–10, samples incubated with 6 M Gdn·HCl. Equal amounts of protein were applied to each lane. (*B*) ^{35}S-$SHaPrP^{C}$ incubated for 48 h with unlabeled $MoPrP^{Sc}$ (lanes 1–5) or $SHaPrP^{Sc}$ (lanes 6–10). Lanes 1 and 6, samples were not digested with proteinase K; lanes 2 and 7, samples were digested but in the absence of Sarkosyl. Lanes 3 and 8, samples were exposed to 0.2% Sarkosyl and proteinase K for 1 h; lanes 4 and 9, 1% Sarkosyl; lanes 5 and 10, 2% Sarkosyl. Lanes 1 and 6 have 15% of the PrP^{C} in lanes 2–5 and 7–10.

protease resistance after exposure to PrP^{Sc} (data not shown), supporting the notion that the H1 region, in which the 3F4 epitope lies, is particularly significant (23, 35).

We estimate that 15–20% of PrP^{C} mixed with PrP^{Sc} acquired protease resistance after 48 h in contrast to mixing with the SHa 90–145 (A117V) peptide where ≈50% PrP^{C} demonstrated protease resistance. After incubation with SHa 90–145 for 1 h, ≈35% of the ^{35}S-PrP^{C} that exhibited protease resistance at 48 h was present; by 24 h, ≈75% of the PrP^{C} was protease resistant. Although it has been reported that protease-resistant ^{35}S-PrP^{C} was generated within 2 min after mixing with a 50-fold excess of unlabeled PrP^{Sc}, we were unable to reproduce this finding (13).

Species Specificity of PrP^{Sc} Binding to PrP^{C}. When we mixed $MoPrP^{Sc}$ with $SHaPrP^{C}$, relatively little protease-resistant PrP^{C} was formed (Fig. 4*B*, lanes 1 and 2) and addition of Sarkosyl rendered the complex sensitive to proteolysis (lanes 3–5). In contrast, the ^{35}S-PrP^{C}–$SHaPrP^{Sc}$ complex was resistant to proteolysis (lanes 6–10), even when exposed to up to 2% Sarkosyl for 48 h before digestion. These results are consistent with the finding that SHa 90–145 mixed with $SHaPrP^{C}$ produced protease-resistant protein, whereas Mo 90–145 mixed with $SHaPrP^{C}$ did not (Fig. 1).

Attempts to Disrupt PrP^{C}–PrP^{Sc} Complexes. Although addition of Sarkosyl to the PrP^{C}–peptide complexes or PrP^{C} alone abolished protease resistance, this was not the case for the PrP^{C}–PrP^{Sc} complexes. Additional attempts to disrupt the PrP^{C}–PrP^{Sc} complexes under conditions likely to preserve scrapie prion infectivity utilized detergents such as Nonidet P-40, Tween 20, Zwittergent 3-12, and sodium deoxycholate alone or in combination with phospholipids to form detergent–lipid–protein complexes (36). We also investigated the possibility of disrupting these complexes by using the anti-PrP 3F4 mAb and synthetic peptides containing residues 109–122 or 90–145. Although the mAb and peptides were added to the complexes in molar ratios of 1000:1, they were unable to dissociate the ^{35}S-PrP^{C} from PrP^{Sc}. Addition of a 10-fold excess of unlabeled PrP^{C} from CHO cells also failed to displace the ^{35}S-PrP^{C} from the complex. These results prevented us from determining whether PrP^{C} had acquired protease resistance or displayed this property because it was bound to PrP^{Sc}.

PrP^{C} Purified from SHa Brain. Attempts to render PrP^{C} purified from SHa brain protease resistant by mixing with PrP^{Sc} were unsuccessful. Why ^{35}S-$SHaPrP^{C}$ immunoaffinity purified from CHO cells did bind to $SHaPrP^{Sc}$ and ^{125}I-$SHaPrP^{C}$ purified from brain did not is unclear. Besides iodination, other possible factors to explain this discrepancy include residual anti-PrP mAbs in immunoaffinity-purified preparations of PrP^{C} from the CHO cells, an unidentified factor such as protein X (37) in CHO cell lysates that purifies with PrP^{C}, or the conformation of PrP^{C} purified from brain differs significantly from that of CHO cell-derived PrP^{C}. The presence or absence of the diacylglycerol moiety of the glycosylphosphatidylinositol anchor does not seem to be a significant factor since ^{35}S-PrP^{C} treated with phosphatidylinositol phospholipase C yielded the same degree of protease resistance after mixing with PrP^{Sc} as controls not treated with phosphatidylinositol phospholipase C.

DISCUSSION

Investigations with chimeric transgenes showed that PrP^{C} and PrP^{Sc} are likely to interact within a central domain delimited by codons 96 and 169 (37–40). The investigations reported here using synthetic peptides binding to PrP^{C} provide physical data confirming the conclusions drawn from the results of the transgenetic studies. Furthermore, such studies should permit a detailed assessment of the size of the interactive region as well as defining which residues are critical. Transgenetic studies have also implicated another protein that participates in the formation of PrP^{Sc} by binding to PrP^{C}. This protein, provisionally designated protein X, may function as a molecular chaperone in mediating the transformation of PrP^{C} into PrP^{Sc} (37).

Although other investigators have reported the *in vitro* formation of PrP^{Sc} by mixing a 50-fold excess of PrP^{Sc} with ^{35}S-PrP^{C}, their conclusions assume that protease-resistant PrP^{C} is equivalent to PrP^{Sc} (13). Interestingly, the binding of PrP^{C} to PrP^{Sc} was found to be dependent on the same residues (41) that render transgenic MH2M mice susceptible to SHa prions (39) and it seems to be strain dependent (42). Although we were able to confirm the binding of PrP^{C} to PrP^{Sc} in the presence of a large excess of PrP^{Sc} (Fig. 3*A* and 4*B*), we were unable to reproduce the renaturation of PrP^{Sc} from Gdn·HCl as judged by a restoration of protease resistance (Fig. 4*A*).

Attempts to separate ^{35}S-PrP^{C} from PrP^{Sc} under conditions where scrapie infectivity is preserved were unsuccessful with a variety of detergents, anti-PrP mAbs, detergent–lipid–protein complexes, and synthetic peptides. Until such conditions are identified, we cannot determine whether PrP^{C} has been converted into PrP^{Sc} or is only tightly bound. The experiments presented here with PrP peptides that bind to PrP^{C} and render it protease resistant argue that the latter possibility is more likely to be correct since Sarkosyl disrupted the PrP^{C}–peptide complex and made PrP^{C} sensitive to protease.

Some investigators continue to contend that PrP amyloids participate in PrP^{Sc} formation (13, 41–45) despite much evidence to the contrary. Although PrP amyloid plaques were

found in transgenic SHaPrP mice inoculated with SHa prions, none were detected in the mice inoculated with Mo prions (3). Thus, amyloid deposition in plaque formation is not obligatory for prion propagation. Ultrastructural studies demonstrated that purified PrP^{Sc} molecules exist as amorphous aggregates, which, when partially digested with proteinase K in the presence of detergent, form PrP 27–30 that polymerizes into rod-shaped particles with the properties of amyloid (5, 33).

The SHa 90–145 peptide adopts an α-helical structure in hydrophobic environments created by detergents or lipids while it displays a random coil in H_2O (14). Although 2–3 weeks was required for SHa 90–145 in the presence of 150 mM NaCl to acquire β-sheet conformation and resistance to proteolysis, it displayed these features after 48 h upon mixing with PrP^{C}. While these physical properties of SHa 90–145 resemble those of PrP^{Sc}, this peptide injected intracerebrally into rodents has not produced central nervous system dysfunction to date.

Our investigations offer an additional approach to the study of prions. Synthetic PrP peptides can be used to map regions where PrP molecules interact with each other and to define the degree of homology that facilitates binding. It is likely that this knowledge can be translated to direct the construction of PrP transgenes with predetermined specificities. Since the PrP peptides used in our studies have not exhibited prion infectivity, it will be possible to determine whether the PrP^{C}–peptide complexes that mimic many of the features of PrP^{Sc} are capable of transmitting prion disease in inoculated animals.

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