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


Contributed by Stanley B. Prusiner, August 28, 1995

ABSTRACT Conversion of the cellular isoform of prion protein (PrPc) into the scrapie isoform (PrPSc) involves an increase in the β-sheet content, diminished solubility, and resistance to proteolytic digestion. Transgenic studies argue that PrPc and PrPSc form a complex during PrPSc formation; thus, synthetic PrP peptides, which mimic the conformational features of PrPc, were mixed with PrPc to determine whether its properties were altered. Peptides encompassing two a-helical domains of PrP when mixed with PrPc produced a complex that displayed many properties of PrPSc. The PrPα-peptide complex formed fibrous aggregates and up to 60% of complexed PrPc sedimented at 100,000 × g for 1 h, whereas PrPc 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 PrPc-peptide complex and rendered PrPc sensitive to protease digestion; anti-PrP monoclonal antibody (mAb) prevented complex formation. Unexpectedly, the peptide in a β-sheet conformation did not bind PrPc, whereas the random coil did. When the pathogenic A117V mutation causing both the telencephalic and ataxic forms of Gerstmann-Straussler-Scheinker disease (16–18) was substituted in the peptide, ~65% of the radiolabeled PrPSc was used to form the complex, whereas the random coil did not bind PrPc, when the random coil did. The pathogenic A117V mutation causing both the telencephalic and ataxic forms of Gerstmann-Straussler-Scheinker disease (16–18) was substituted in the peptide, ~65% of the radiolabeled PrPSc was used to form the complex, whereas the random coil did not bind PrPc, when the random coil did. 

Although many lines of evidence have converged to argue persuasively that prions are composed of the scrapie isoform of prion protein (PrPSc) (1), identifying conditions for the in vitro conversion of the cellular isoform (PrPc) into PrPSc wherein scrapie infectivity is generated de novo remains to be accomplished. Formation of PrPSc is a posttranslational process (2) in which PrPc forms a complex with PrPSc and is then transformed into a second molecule of PrPSc (3). While attempts to detect a covalent change that distinguishes PrPSc from PrPc were unsuccessful (4), spectroscopic studies demonstrated that PrPc contains ~40% α-helices and is devoid of β-sheet (3). In contrast, PrPSc 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 PrPSc and PrPSc form a complex during the formation of nascent PrPSc (3), we attempted to demonstrate PrPSc production through formation of such complexes by mixing purified fractions containing equimolar amounts of the two isoforms (10). Unable to demonstrate conversion of PrPSc into PrPc in these mixtures, we pursued the interactions of synthetic PrP peptides that correspond to regions of putative secondary structure and display conformational plausibility (11, 12). In contrast to our earlier findings, other investigators were able to demonstrate an interaction between PrPSc and PrPc by mixing a 50-fold excess of PrPc with labeled PrPc (13). 

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

MATERIALS AND METHODS SHaPrP was subcloned into the glutamine synthetase expression vector pEE 12 (Cell/Toh, Alameda, CA). Chinese hamster ovary (CHO) Kl cells (American Type Culture Collection) were seeded at 106 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 CaP04 method (20). After 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, phosphotyrosyl-phosphotyrosine-specific phosphotyrosine C digestion reagent ~90 ag of SHaPrP from 106 cells (22).

The CHO cells expressing SHaPrP were metabolically radiolabeled with [35S]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 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, PrPc was precipitated with 4 vol of methanol to separate it from the Gdn-HCl and residual detergent. 3% labeled PrPc (~5% PrPc) concentrations were determined by comparison with signals from Western blots with known quantities of PrPSc from SHa brains and by measurements in a scintillation spectrometer.

Abbreviations: PrP, prion protein; PrPc, cellular isoform of PrP; PrPSc, scrapie isoform of PrP; SHa, Syrian hamster; Mo, mouse; mAb, monoclonal antibody.
Wild-type peptide (lane 4), only 30-40% of the mutant peptide buffers, HPR rapidly folds into β-sheets and polymerizes (11) efficient formation of protease-resistant radiolabeled PrPc 90-145 both gave protease resistance (lanes 4 and 5). The most...for residues 104-122 could produce protease resistance...with SHaPrPc, it did not induce a change in protease resistance...SHa 90-145 in either the random coil or β-sheet form was mixed...35S-PrPc at a ratio of 5000:1, it induced protease resistance...with SHa 90-145, which corresponds to the N...immunoprecipitation. The precipitation of the sedimented PrPc-SHa 90-145 complex showed a substantial increase in β-sheet content compared to pelleted...As measured by CD, the supernatant contained...The most efficient formation of protease-resistant radiolabeled peptide was seen with the SHa 90-145 (A117V) peptide in which V was substituted for A at position 117 (lane 5). Compared with the wild-type (lane 4), only 30-40% of the mutant peptide was digested in 3 M Gdn-HCl with or without the peptide. Addition of 35S-PrPc could be detected. However, mixing undenatured PrPc with PrPSc did produce protease-resistant β-PrPc...of PrPSc that had been denatured in 3 M Gdn-HCl and then diluted to 1.5 M Gdn-HCl required protease digestion and SDS/PAGE (Fig. 4A). Of note, when the dilution was carried out in the same tube to which the 3 M Gdn-HCl had been added, we did not detect protease-resistant PrP (data not shown). This was never seen when the tubes were changed, and we assume that this was due to residual, undenatured PrP...bound to the walls of the tube.

When we mixed PrPc 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 PrPc, no protease-resistant β-PrPc could be detected. However, mixing undenatured PrPc with PrPSc did produce protease-resistant β-PrPc (Fig. 5A), while a 100-fold excess of PrPc did not. The presence of PrPSc in the Gdn-HCl reaction mixture seems to be essential since its removal by treatment with 0.3 M Gdn-HCl prevented complete protease digestion. Although ~50% of the 35S-PrPc was recovered in CHO eluates containing 100,000 × g for 1 h, only 10-15% was protease resistant. Anti-PrP mAb Prevents Binding of PrPSc to PrPc. The interaction between β-PrPc and PrPSc was titrated by using the anti-PrP 3F4 mAb. In CHO cells (lanes 1-4), binding of 35S-PrPc from SHa brains (lanes 5-8). Lanes 9 and 10 were not digested and were of the 35S-PrPc in lanes 2-4 and 6-8. 3 M Gdn-HCl undergoes renaturation and renders PrPc resistant to proteolysis within 2 min of mixing (15). Since numerous attempts to render peptide infectious from both Gds and ares had failed (34), we investigated the effect of 3 M Gdn-HCl on PrPc. As before, we were unable to demonstrate renaturation of PrPSc that had been denatured in 3 M Gdn-HCl and then diluted to 1.5 M Gdn-HCl. The presence of 0.3-2 M Gdn-HCl with PrPc, no protease-resistant PrPc was required to produce protease-resistant 35S-PrPc, whereas Gdn-HCl had been added, we did not detect protease-resistant PrP (data not shown). This was never seen when the tubes were changed, and we assume that this was due to residual, undenatured PrP...bound to the walls of the tube.
was incubated with 0, 3, or 6 M Gdn-HCl for 1 hr, followed by further incubation with 0, 3, or 6 M Gdn-HCl for 1 hr, «35% of the 35S-PrPc that exhibited protease resistance after exposure to PrPSc as controls not treated with detergent, form PrP 27-30 that polymerizes into fibrils and none were detected in the mice inoculated with Mo prions (3). The experiments here using synthetic peptides binding to PrPc provide physical evidence that purified PrPSc molecules exist as amorphous aggregates, which consist of detergent, form PrP 27-30 that polymerizes into fibrils and none were detected in the mice inoculated with Mo prions (3).

We thank David Borchelt and Albert Tabarubas for stimulating discussions. This work was supported by grants from the National Institute of Health since 1983 with Assistance Foundation as well as by gifts from the Sherman Fairchild andBernhard Oder Foundation. The authors gratefully acknowledge the support of the Alexander von Humboldt Foundation and in the formation of PrPSc by mixing a 50-fold excess of PrPSc with PrPc. This protein, non-amyloidogenic in the presence of detergent, form PrP 27-30 that polymerizes into fibrils and none were detected in the mice inoculated with Mo prions (3).

We thank David Borchelt and Albert Tabarubas for stimulating discussions. This work was supported by grants from the National Institute of Health since 1983 with Assistance Foundation as well as by gifts from the Sherman Fairchild and Bernhard Oder Foundation. The authors gratefully acknowledge the support of the Alexander von Humboldt Foundation and in the formation of PrPSc by mixing a 50-fold excess of PrPSc with PrPc. This protein, non-amyloidogenic in the presence of detergent, form PrP 27-30 that polymerizes into fibrils and none were detected in the mice inoculated with Mo prions (3).

Discussion

We thank David Borchelt and Albert Tabarubas for stimulating discussions. This work was supported by grants from the National Institute of Health since 1983 with Assistance Foundation as well as by gifts from the Sherman Fairchild and Bernhard Oder Foundation. The authors gratefully acknowledge the support of the Alexander von Humboldt Foundation and in the formation of PrPSc by mixing a 50-fold excess of PrPSc with PrPc. This protein, non-amyloidogenic in the presence of detergent, form PrP 27-30 that polymerizes into fibrils and none were detected in the mice inoculated with Mo prions (3).