Familial Prion Disease Mutation Alters the Secondary Structure of Recombinant Mouse Prion Protein: Implications for the Mechanism of Prion Formation†

Roberto Cappai,*,‡ Leanne Stewart,‡ Michael F. Jobling,‡,§ James M. Thyer,‡ Anthony R. White,‡ Konrad Beyreuther,|| Steven J. Collins,‡ Colin L. Masters,‡ and Colin J. Barrow§

Department of Pathology and The Mental Health Research Institute, and School of Chemistry, The University of Melbourne, Parkville, Victoria 3052, Australia, and ZMBH−Center of Molecular Biology, The University of Heidelberg, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany

Received September 28, 1998; Revised Manuscript Received January 4, 1999

ABSTRACT: A considerable body of data supports the model that the infectious agent (called a prion) which causes the transmissible spongiform encephalopathies is a replicating polypeptide devoid of nucleic acid. Prions are believed to propagate by changing the conformation of the normal cellular prion protein (PrP^c) into an infectious isoform without altering the primary sequence. Proteins equivalent to the mature form of the wild-type mouse prion protein (residues 23−231) or with a mutation equivalent to that associated with Gerstmann−Straüssler−Scheinker disease (proline to leucine at codon 102 in human; 101 in mouse) were expressed in *E. coli*. The mutation did not alter the relative proteinase K susceptibility properties of the mouse prion proteins. The wild-type and mutant proteins were analyzed by circular dichroism under different pH and temperature conditions. The mutation was associated with a decrease in α-helical content, while the *β*-sheet content of the two proteins was unchanged. This suggests the mutation, while altering the secondary structure of PrP, is not sufficient to induce proteinase K resistance and could therefore represent an intermediate isoform along the pathway toward prion formation.

The transmissible spongiform encephalopathies (TSE) constitute a group of neurodegenerative diseases occurring in both humans and animals and include Creutzfeldt−Jakob disease (CJD), Gerstmann−Straüssler−Scheinker disease (GSS),[1] bovine spongiform encephalopathy, and scrapie. A considerable body of data supports the model that the TSE agent (also known as a prion) is a replicating polypeptide devoid of nucleic acid (*1*). Prions are believed to propagate by changing the conformation of the normal cellular prion protein (PrP^c) into an infectious isoform (PrP^{TSE}) without altering the primary sequence. PrP^c is relatively soluble in detergent and is relatively sensitive to proteinase K digestion, while PrP^{TSE} aggregates into rods or filaments and is relatively protease resistant (*2*). The biochemical differences between PrP^c and PrP^{TSE} may be due to altered structures since spectroscopic analyses have shown that PrP^c has a low *β*-sheet content of 3%, while PrP^{TSE} has 43% (*3*).

The majority of human TSE cases are sporadic with only about 10% caused by mutations in the prion protein gene (PRNP). The mutations are believed to promote PrP^c conversion into the infective conformer by destabilizing the protein (*4*). The effects of PRNP mutations have been studied in both transgenic mice and cell culture models. Transgenic mice expressing the PRNP with the GSS (P102L) mutation undergo spontaneous vacuolar neurodegeneration (*5*). Furthermore, the brain extracts from affected mice could transmit disease, indicating the generation of de novo prion disease (*6*). The expression of murine PRNP with the D178N familial mutation in Chinese hamster ovary (CHO) cells resulted in proteinase K resistant and detergent-insoluble PrP (*7*). In contrast, expression of human D178N mutant PrP in the human neuronal M-17 cell line resulted in proteinase K sensitive PrP protein (*8*). This suggests differences in cell types and/or PrP sequence can result in different biochemical properties. Based on the NMR structure of full-length or large fragments of mouse and hamster PrP (*9*−*12*), a number of the mutations are located close to the α-helix and *β*-sheet regions, suggesting they may be critically positioned to alter either the structure or the ligand-binding properties.

To better understand the effect of human TSE-associated mutations on PrP secondary structure and protease resistance, we studied the GSS mutation (proline 102 to leucine) in *E. coli* expressed mouse PrP (residues 23−231). These studies show that the mutation is associated with a decrease in α-helical content but without an appreciable change in protease sensitivity. The loss of some α-helical structure due to the familial mutation may represent an early fundamental transitional change in the stepwise acquisition of altered physicochemical properties by the infectious PrP isoform.

† This work is supported by grants from the National Health and Medical Research Council of Australia to R.C. and C.L.M. and the National Human Pituitary Advisory Council to R.C. and S.J.C.

* Correspondence should be addressed to this author at the Department of Pathology, University of Melbourne, Parkville, Victoria 3052, Australia. Phone: +61-3-9344 5868. Fax: +61-3-9344 4004. E.mail: r.cappai@pathology.unimelb.edu.au.

‡ Department of Pathology and The Mental Health Research Institute, The University of Melbourne.

§ School of Chemistry, The University of Melbourne.

|| ZMBH−Center of Molecular Biology, The University of Heidelberg.

[1] Abbreviations: CD, circular dichroism; GSS, Gerstmann−Straüssler−Scheinker disease; moPrP, mouse prion protein; $moPrP^c$; wild-type mouse prion protein; PRNP, prion protein gene; P101L, proline to leucine at codon 101; PrP^{TSE}, PrP causing transmissible spongiform encephalopathy.

10.1021/bi982328z CCC: $18.00
Published on Web 02/26/1999

MATERIALS AND METHODS

Antibodies. 93/36 is a rabbit polyclonal antibody raised to the mouse PrP peptide 89−103 and used at 1:500. The 3F4 antibody was obtained from Senetek (Missouri) and used at 1:500.

moPrP23-231 Expression Constructs. The mouse PrP (moPrP) gene corresponding to amino acids 23−231 was amplified by the polymerase chain reaction (PCR) from Balb/c mouse genomic DNA with Pwo DNA polymerase (Boehringer Mannheim). The forward and reverse primers used were CCG GAA TTC AAA AAG CGG CCA AAG CCT GGA GGG and CCG GAA TTC CTA GGA TCT TCT CCC GTC GTA ATA GGC. The forward and reverse primers contain an *Eco*RI site. The reaction conditions were 95 °C, 1 min; 50 °C, 1 min; 72 °C, 2 min; 30 cycles. The PCR product was digested with *Eco*RI and ligated to *Eco*RI-digested pRSETB vector (Invitrogen). Recombinant clones were confirmed by sequencing.

Murine PrP amino acid 101 (proline) was converted to a leucine by PCR splice overlap mutagenesis. PCRs were performed using Pwo DNA polymerase on the moPrP23−231 DNA fragment as the template. The N-terminal fragment primers were CCG GAA TTC AAA AAG CGG CCA AAG CCT GGA GGG and GGT TTT TGG TTT GCT TAG CTT GTT CCA C. The C-terminal fragment primers were GTG GAA CAA GCT AAG CAA ACC AAA AAC C and CCG GAA TTC CTA GGA TCT TCT CCC GTC GTA ATA GGC. The PCR conditions were as above. The N- and C-terminal fragments were purified, and an aliquot of each was mixed together and PCR performed with the primers used to amplify moPrP23−231 as above. The PCR fragment was digested with *Eco*RI and ligated to *Eco*RI-digested pRSETB.

Expression of moPrP^c and moPrP-P101L. The recombinant plasmids were transformed into the T7 RNA polymerase containing *E. coli* BL21(DE3). Small-scale expression cultures were performed by inoculating individual colonies into 2 mL of LB media (plus ampicillin 100 μg/mL) and shaken overnight at 37 °C. Then 200 μL of overnight culture was diluted to 2 mL in LB (plus ampicillin 100 μg/mL) and shaken at 37 °C, 1 h. Protein expression was induced by adding isopropyl β-thiogalactopyranoside to 1 mM final concentration and shaken at 37 °C, 2 h. Cells were pelleted by centrifugation, and the pellet was resuspended into 500 μL of SDS−PAGE sample buffer. The insoluble material was removed by centrifuging at 10000*g*, 10 min, and the supernatant was transferred to a fresh tube.

SDS−PAGE and Immunoblotting. Protein samples were separated on a 15% SDS−PAGE gel at 30 mA/gel. Separated proteins were transferred to a PVDF membrane, blocked with 5% skim milk in TBST (10 mM Tris-HCl, 0.9% NaCl, 0.1% Tween-20, pH 7.5) for 3 h at room temperature (RT). A primary antibody was applied to the membrane for 1 h at RT. Blots were washed 3 times (10 min each) between each probing step with TBST (pH 7.5). Enhanced chemiluminescence detection (Amersham) used a secondary anti-mouse or anti-rabbit IgG conjugated to horse peroxidase (1:3000) to immunodetect the bound primary antibody. Chromogenic detection used an alkaline phosphatase conjugated secondary antibody (1:10 000) to visualize the protein by using Fast-Red/Naphthol AS-MX phosphate (Sigma) as substrates.

Large-Scale Expression and Purification Using Ni−NTA under Denaturing Conditions. Expression was scaled up into 100 mL of 2YT media (1.6% bacto-tryptone, 1% bacto-yeast extract, 86 mM NaCl, plus ampicillin 100 μg/mL) in 500 mL flasks as described above. The cells were harvested and resuspended into PBS (plus PMSF 10 μg/mL) and ruptured by one freeze−thaw cycle and then passed through an 18-gauge needle. The mixture was centrifuged at 10000*g* for 10 min and the supernatant discarded. The PrP in the cell pellet was purified on Ni−NTA fast flow matrix (QIAGEN) using conditions based on the metal chelating chromatography protocol described by Zhan et al. (1997) (*13*). The PrP eluted from the Ni−NTA column was desalted with a PD10 column (Pharmacia) into 10 mM potassium phosphate buffer at either pH 5, pH 6, pH 7, or pH 8 and concentrated with a Centricon 10 concentrator into the 10 mM potassium phosphate buffer.

The formation of the disulfide bond was determined by measuring the amount of reduced cysteines with the Ellman test (*14*). Briefly, a 3 mL solution of 0.1 μM purified moPrP^c or moPrP-P101L protein was prepared in 10 mM potassium phosphate of the corresponding pH and mixed with 0.1 mL of 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 4 mg/mL in 10 mM potassium phosphate of the corresponding pH. After 15 min, the absorbance was measured at 410 nm. As a reference, 0.1 mL of DTNB (4 mg/mL in 10 mM potassium phosphate of the corresponding pH) was mixed with 3 mL of 10 mM potassium phosphate of the corresponding pH and the A_{410} measured. The concentration of sulfydryl groups was calculated from the equation: concentration of sulfydryl groups = $[A_{410}(\text{sample}) - A_{410}(\text{reference})]/13650$. This value allowed the percentage of reduced cysteines to be calculated based on moPrP having two cysteine residues.

Proteinase K Digestion. Small-scale expression cultures were harvested, resuspended in PBS, and ruptured by three freeze−thaw cycles. The mixture was centrifuged at 10000*g*, 10 min, and the supernatant discarded. The pellet was resuspended in proteinase K buffer (10 mM Tris, 150 mM NaCl, 1% Triton X-100, and 1 mM deoxycholate, pH 8) using a 19-gauge needle. An aliquot of the lysate (containing 50 μg of protein) was transferred to a fresh Eppendorf tube, proteinase K was added to a final concentration of 5 μg/mL, and the mixture was incubated at 37 °C for 10 min. As a positive control, brain tissue from a normal and a CJD subject were homogenized in proteinase K buffer (10% w/v) and spun at 1600*g* to give a cleared supernatant. A 50 μL aliquot was digested with 50 μg/mL proteinase K as above. The reaction was stopped with an equal volume of SDS−PAGE sample buffer.

Circular Dichroism Analysis. Far-UV circular dichroism spectra were recorded on an Aviv 600 spectropolarimeter at protein concentrations of 0.05−0.2 mg/mL in 10 mM potassium phosphate buffer. The spectra were recorded in a 1 mm cell. Protein concentrations were measured at an absorbance of 280 nm. Specific absorbances ($A_{280\text{nm}}$, 0.1 mg/mL, 1 mm) of 0.024 were used for moPrP(23−231) (*15*). A UV standard concentration curve was calibrated against amino acid analysis results. The thermal denaturation experiments were performed by heating the CD cuvette with a spectroscopic cell jacket connected to a Jasco water bath.

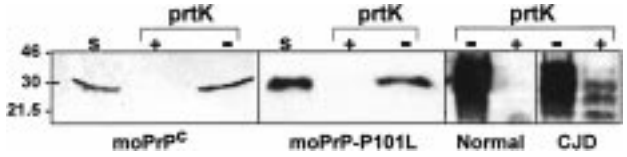

FIGURE 1: Proteinase K resistance properties of moPrP^c, moPrP-P101L, and human brain lysates. *E. coli* cell lysates from moPrP^c and moPrP-P101L were either incubated with (+) or without (−) 5 μg/mL proteinase K (prtK) and then analyzed by Western blotting with the 93/36 antibody. A zero time point (lane s) shows the amount of moPrP^c and moPrP-P101L present in the *E. coli* lysate. The normal and CJD human brain lysates were used to show protease-resistant PrP was detectable in the CJD sample using this assay. The human PrP was detected with the monoclonal antibody 3F4. Note, protease-resistant CJD PrP was present with 5-fold more proteinase K than used for the *E. coli*. The positions of the molecular mass markers in kDa are indicated.

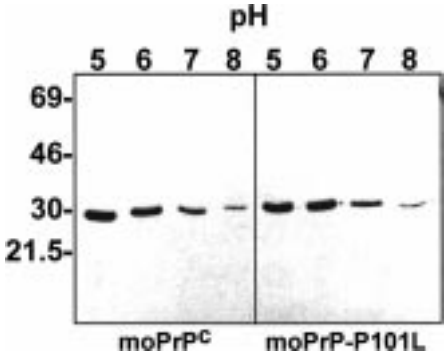

FIGURE 2: Silver stain gel of the purified moPrP^c and moPrP-P101L proteins after desalting and concentrating into 10 mM potassium phosphate at pH 5, 6, 7, and 8. The positions of the molecular mass markers in kDa are indicated.

RESULTS

Protease Sensitivity of Recombinant moPrP-P101L. Wild-type mouse PRNP (moPrP^c) corresponding to amino acids 23−231 (hamster sequence numbering) or with the GSS-associated mutation of proline to leucine at 102 (moPrP-P101L, position 101 in mouse) were cloned into the T7 RNA polymerase based vector pRSETB with an N-terminal hexa-histidine sequence. Western blot analysis of total *E. coli* lysate with the 93/36 antibody identified a major band at the expected size of 30 kDa in both moPrP^c and moPrP-P101L clones but not in *E. coli* transfected with the pRSETB vector alone (data not shown). The solubility of recombinant moPrP was determined by analyzing the supernatant and the pellet fractions following centrifugation of the lysate at 13000*g*. Both moPrP^c and moPrP-P101L were found to be insoluble in aqueous buffer and partitioned into the cell pellet (data not shown). This is consistent with other studies which have shown that 23−231 human PrP (*15*) and the shorter 90−231 hamster PrP (*16*) proteins are insoluble, presumably in the form of inclusion bodies.

We measured the effect of the P101L mutation on the relative proteinase K resistance of *E. coli* expressed moPrP. The moPrP^c- and moPrP-P101L-expressing *E. coli* were lysed into aqueous buffer and then centrifuged. The cell pellet fraction was resuspended into proteinase K buffer and incubated with proteinase K (5 μg/mL). The reaction mixtures were analyzed by Western blotting with the 93/36 antibody (Figure 1). It was found that both moPrP^c and moPrP-P101L were completely digested by 5 μg/mL proteinase K. No digestion of moPrP occurred in the absence of added proteinase K (Figure 1), indicating proteolysis by an endogenous *E. coli* protease was not taking place. There was no detectable difference in the proteinase K sensitivity of moPrP^c compared to mutant PrP incubated with a range of proteinase K concentrations (0.025−5 μg/mL) (data not shown). As a positive control, normal and CJD brain lysates were used, and we found proteinase K resistant PrP was detectable in the CJD sample using this assay. These results indicate that the P101L mutation does not alter the relative proteinase K sensitivity of *E. coli* expressed mutant moPrP.

Circular Dichroism Analysis of moPrP-P101L. There have been a number of reports describing the purification of full-length or large fragments of PrP from *E. coli* (*13*, *15*, *16*). In those cases where PrP was insoluble, it was solubilized with strong denaturants such as 8 M urea (*15*) or 8 M guanidine hydrochloride (*16*) before purification. We utilized the hexa-histidine tag in conjunction with immobilized nickel chelating chromatography to purify moPrP^c and moPrP-P101L in a single step and concentrated it into 10 mM potassium phosphate buffer at different pHs. As judged by silver staining, the final protein preparations contain a single band at 30 kDa (Figure 2) which is immunoreactive with the 93/36 anti-PrP antibody. This indicates no further purification of the protein was required.

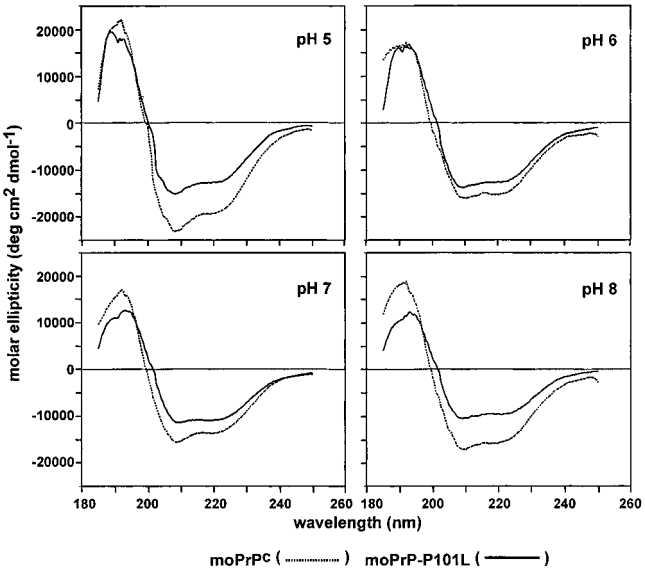

FIGURE 3: Circular dichroism spectra of moPrP^c (dotted line) and moPrP-P101L (solid line) at pH 5, 6, 7, and 8 measured from 185 to 250 nm.

Since the disulfide bond in PrP is important for correctly folded PrP (*13*, *15*, *16*), the formation of the disulfide bond was determined by measuring the amount of reduced cysteine residues by the Ellman test. It was found the purified proteins had very little reduced cysteines (3−10%), indicating the majority of the disulfides had been formed and hence correctly folded protein was expressed. This was confirmed by the secondary structure analysis described below which showed a high α-helical content consistent with other studies.

Circular dichroism (CD) spectroscopy was used to determine the effect of the P101L mutation on the secondary structure of PrP. CD spectra for moPrP^c and moPrP-P101L were acquired at four pH values (Figure 3). Both proteins showed significant α-helical content over the pH range with characteristic double minima at 208 and 222 nm. The shape

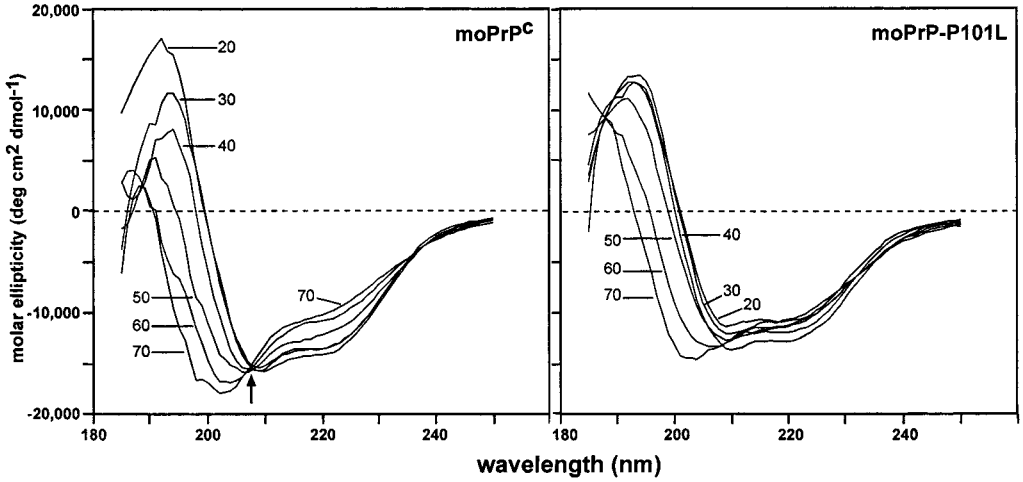

FIGURE 4: Thermal denaturing study of moPrP. Circular dichroism spectra of moPrP^c (left panel) and moPrP-P101L (right panel), pH 7, measured at 20, 30, 40, 50, 60, and 70 °C. The isodichroic point is indicated by the arrow.

Table 1: Calculation of Percentage α-Helix in moPrP^c and moPrP-P101L at Different pHs[a]

	pH 5	pH 6	pH 7	pH 8
mPrP^c	57	45	40	46
mPrP-P101L	38	38	32	29

[a] The values were obtained by measuring the molar ellipticity at 222 nm from the spectra in Figure 3 and assuming 100% α-helix equals −33 000 at 222 nm.

of the CD curve and the mean residue ellipticity at pH 7 for moPrP^c are similar to those reported previously (*15*). For both moPrP^c and moPrP-P101L, there was an overall decrease in the α-helical content with increasing pH, as determined from the 222 nm α-helical minima (Table 1), with moPrP^c exhibiting significantly higher α-helical content at pH 5 than at pH 6, 7, and 8. At each of the four pHs investigated, the mean residue ellipticities of moPrP^c are significantly more negative than those of moPrP-P101L, indicating that moPrP-P101L had a reduction in α-helical structure as compared to the wild-type protein.

The thermal stability of the secondary structure of both moPrP^c and moPrP-P101L was analyzed by obtaining CD spectra at pH 7 at different temperatures (Figure 4). The thermal denaturing of moPrP^c is consistent with a direct α-helix to random coil transition with no β-sheet involvement, as indicated by an isodichroic point at 206 nm. The thermal denaturing of moPrP-P101L appears to involve more than two structure types as no isodichroic point is observed. In addition, the CD thermal denaturing experiments (Figure 4) show that α-helical denaturing occurs more readily for moPrP than for PrP-P101L, although the total α-helical content of moPrP is always higher than that of the mutant at the same temperature.

DISCUSSION

The data in this study show that the well-studied GSS P102L mutation can cause alterations in the secondary structure and thermal properties of PrP. The conformational differences observed in the CD spectra for both moPrP^c and moPrP-P101L at pHs 5, 6, 7, or 8 indicate that the environmental pH has an influence on the final folded structure of both proteins. This may explain the decrease in the yields of purified moPrP at pH 7 and 8 (Figure 2) which suggests a decrease in protein solubility with increasing pH. The pH effect is especially noticeable at pH 5 for moPrP^c, where the α-helical content is significantly higher and there is an obvious difference in the shape of the CD curve from that seen at the other pH values. The increase in the 222 nm minima at pH 5 indicates an increase in α-helical content, while the greater 208/222 ratio also indicates an increased random coil content, probably at the expense of β-structure. It has been shown that acidic pH, together with denaturing agents, can influence the stability and conformation of PrP90−231 and PrP121−231 (*17*, *18*), resulting in unfolding intermediates.

The thermal denaturing of moPrP^c occurs at a lower temperature than that of moPrP-P101L and appears to involve a direct α-helix to random coil transition, as indicated by the isodichroic point at 206 nm. However, the overall α-helical content of moPrP-P101L is significantly lower than that of moPrP^c, even at elevated temperatures. These results indicate that at ambient temperatures moPrP^c contains an α-helical region that is not present in moPrP-P101L. This region has relatively low thermal stability and unravels to random coil at elevated temperatures. However, because this α-helical region is absent in moPrP-P101L even at ambient temperatures, it is possible that the thermally unstable region in moPrP^c is further destabilized by the P101L mutation. This destabilization of an α-helical region by the P101L mutation could decrease the energy required for conversion from PrP^c to PrP^{TSE} and hence provide an explanation for the pathogenicity of this mutation.

The recently obtained NMR structure of moPrP(23−231) shows that under the conditions of the NMR experiment the region containing proline-101 is unstructured (*10*−*12*). Our results indicate that the P101L mutation destabilizes an α-helical region in moPrP^c. A possible explanation is that the mutation may be close in space to an α-helix and consequently alters the global protein structure in a manner that destabilizes an α-helix. An alternative explanation is that the observed CD structure is different from the NMR structure due to such factors as concentration or pH. The unstructured region observed in the NMR tube is probably structured in different solvent environments. An NMR structure of the PrP-P101L mutant protein would help

determine the specific regional effects of this mutation on protein structure.

Interestingly, the alteration in PrP-P101L structure did not result in a change in protease resistance. This suggests that other factors which may be necessary for protease resistance (*19*) are absent in *E. coli*. Protease resistance is not always associated with mutant PrP since the expression of the human fatal familial insomnia D178N mutation in a human neuroblastoma cell line was protease sensitive (*8*), while expression of mutant mouse PrP (including the D178N mutation) in CHO cells and human Q217R-129V in human neuroblastoma cells was protease resistant (*7*, *20*, *21*). Our results are consistent with the suggestion that prion formation is a multistep process (*4*) which requires other events or factors for protease resistance and infectivity to occur. This is supported also by the identification of normal PrP which is detergent insoluble but protease sensitive (*22*, *23*), suggestive of an intermediate form.

The role of mutations in causing prion diseases is believed to involve destabilizing the structure of the protein to favor formation of the infectious form paralleled by an increase in β-sheet with a subsequent decrease in α-helix (*3*, *4*). Our data are consistent with this model, where in *E. coli* the initial change is a decrease in α-helical content and altered thermal stability. The data in this study have provided important insights into how these mutations can influence the structure and possibly the activity of PrP.

REFERENCES

1. Prusiner, S. B. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 13363−13383.
2. Meyer, R. K., McKinley, M. P., Bowman, K. A., Braunfeld, M. B., Barry, R. A., and Prusiner, S. B. (1986) *Proc. Natl. Acad. Sci. U.S.A. 83*, 2310−2314.
3. Pan, K. M., Baldwin, M., Nguyen, J., Gasset, M., Serban, A., Groth, D., Mehlhorn, I., Huang, Z., Fletterick, R. J., Cohen, F. E., and Prusiner, S. B. (1993) *Proc. Natl. Acad. Sci. U.S.A. 90*, 10962−10966.
4. Cohen, F. E., Pan, K. M., Huang, Z., Baldwin, M., Fletterick, R. J., and Prusiner, S. B. (1994) *Science 264*, 530−531.
5. Hsiao, K. K., Groth, D., Scott, M., Yang, S. L., Serban, H., Rapp, D., Foster, D., Torchia, M., Dearmond, S. J., and Prusiner, S. B. (1994) *Proc. Natl. Acad. Sci. U.S.A. 91*, 9126−9130.
6. Telling, G. C., Haga, T., Scott, M., Torchia, M., Tremblay, P., DeArmond, S. J., and Prusiner, S. B. (1996) *Genes Dev. 10*, 1736−1750.
7. Lehmann, S., and Harris, D. A. (1996) *J. Biol. Chem. 271*, 1633−1637.
8. Petersen, R. B., Parchi, P., Richardson, S. L., Urig, C. B., and Gambetti, P. (1996) *J. Biol. Chem. 271*, 12661−12668.
9. Riek, R., Hornemann, S., Korth, C., Wider, G., Billeter, M., Glockshuber, R., and Wuthrich, K. (1996) *Nature 382*, 180−182.
10. James, T. L., Liu, H., Ulyanov, N. B., Farr-Jones, S., Zhang, H., Donne, D. G., Kaneko, K., Groth, D., Mehlhorne, I., Prusiner, S. B., and Cohen, F. E. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 10086−10091.
11. Riek, R., Hornemann, S., Korth, C., Wider, G., Glockshuber, R., and Wuthrich, K. (1997) *FEBS Lett. 413*, 282−288.
12. Donne, D. G., Viles, J. H., Groth, D., Mehlhorne, I., James, T. L., Cohen, F. E., Prusiner, S. B., Wright, P. E., and Dyson, H. J. (1997) *Proc. Natl. Acad. Sci. U.S.A. 94*, 13452−13457.
13. Zhan, R., von Schrotter, C., and Wuthrich, K. (1997) *FEBS Lett. 417*, 400−404.
14. Ellman, G. L. (1959) *Arch. Biochem. Biophys. 82*, 70.
15. Hornemann, S., Korth, C., Oesch, B., Riek, R., Wider, G., Wuthrich, K., and Glockshuber, R. (1997) *FEBS Lett. 413*, 277−281.
16. Mehlhorn, I., Groth, D., Stockel, J., Moffat, B., Reilly, D., Yansura, D., Willett, W. S., Baldwin, M., Fletterick, R., Cohen, F. E., Vandlen, R., Henner, D., and Prusiner, S. B. (1996) *Biochemistry 35*, 5528−5537.
17. Hornemann, S., and Glockshuber, R. (1998) *Proc. Natl. Acad. Sci. U.S.A. 95*, 6010−6014.
18. Swietnicki, W., Petersen, R., Gambetti, P., and Surewicz, W. K. (1997) *J. Biol. Chem. 272*, 27517−27520.
19. Telling, G. C., Scott, M., Mastrianni, J., Gabizon, R., Torchia, M., Cohen, F. E., DeArmond, S. J., and Prusiner, S. B. (1995)-*Cell 83*, 79−90.
20. Lehmann, S., and Harris, D. A. (1996) *Proc Natl Acad Sci U.S.A. 93*, 5610−5614.
21. Singh, N., Zanusso, G., Chen, S. G., Fujioka, H., Richardson, S., Gambetti, P., and Petersen, R. B. (1997) *J. Biol. Chem. 272*, 28461−28470.
22. Priola, S. A., Caughey, B., Wehrly, K., and Chesebro, B. (1995) *J. Biol. Chem. 270*, 3299−3305.
23. Gabizon, R., Telling, G., Meiner, Z., Halimi, M., Kahana, I., and Prusiner, S. B. (1996) *Nature Med. 2*, 59−64.

BI982328Z