On the Mechanism of α-Helix to β-Sheet Transition in the Recombinant Prion Protein†

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ABSTRACT: It is believed that the critical event in the pathogenesis of transmissible spongiform encephalopathies is the conversion of the prion protein from an α-helical form, PrP^C, to a β-sheet-rich conformer, PrP^{Sc}. Recently, we have shown that incubation of the recombinant prion protein under mildly acidic conditions (pH 5 or below) in the presence of low concentrations of guanidine hydrochloride results in a transition to PrP^{Sc}-like β-sheet-rich oligomers that show fibrillar morphology and an increased resistance to proteinase K digestion [Swietnicki, W., Morillas, M, Chen, S., Gambetti, P., and Surewicz, W. K. (2000) *Biochemistry 39*, 424−431]. To gain insight into the mechanism of this transition, in the present study we have characterized the biophysical properties of the recombinant human prion protein (huPrP) at acidic pH in the presence of urea and salt. Urea alone induces unfolding of the protein but does not result in protein self-association or a conversion to β-sheet structure. However, a time-dependent transition to β-sheet structure occurs upon addition of both urea and NaCl to huPrP, even at a sodium chloride concentration as low as 50 mM. This transition occurs concomitantly with oligomerization of the protein. At a given protein and sodium chloride concentration, the rate of monomeric α-helix to oligomeric β-sheet transition is strongly dependent on the concentration of urea. Low and medium concentrations of the denaturant accelerate the reaction, whereas strongly unfolding conditions are not conducive to the conversion of huPrP into an oligomeric β-sheet-rich structure. The present data strongly suggest that partially unfolded intermediates may be involved in the transition of the monomeric recombinant prion protein into the oligomeric scrapie-like form.

Prion diseases, also known as spongiform encephalopathies (TSEs), are fatal neurodegenerative disorders that include scrapie in sheep, bovine spongiform encephalophathy in cattle, and Creutzfeldt−Jakob disease (CJD), Gerstmann−Straussler−Scheinker disease, fatal familial insomnia, and kuru in humans. These diseases may be sporadic, may be inherited, or may be acquired by transmission of an infectious agent (*1*−*4*). The molecular mechanism of TSEs is a matter of controversy (*5*). However, a wealth of experimental data indicates that the main factor responsible for these disorders is a cerebral accumulation of an abnormal (scrapie-like) form of prion protein, PrP^{Sc} [1] (*1*−*4*). According to the "protein only" hypothesis, PrP^{Sc} constitutes the sole component of the infectious prion pathogen (*1*, *6*).

PrP^{Sc} is derived from the normal cellular prion protein, PrP^C. The latter species is a 208−209 residue glycoprotein that has two N-glycosylation sites and a single disulfide bridge (*1*, *2*). PrP^C is transported through the secretory pathway and ultimately tethered, via glycophosphatidylinositol anchor, to the cell surface (*1*, *2*, *7*, *8*) where it preferentially accumulates in cholesterol-rich membrane domains called rafts (*9*). The transition between PrP^C and PrP^{Sc} occurs posttranslationally without any detectable covalent modifications to the protein (*10*). However, the two isoforms of prion protein have dramatically different biophysical properties. PrP^C is monomeric and readily degradable by proteinase K, whereas PrP^{Sc} assembles into insoluble aggregates that show partial resistance to proteinase K digestion (*7*, *11*, *12*). Furthermore, optical spectroscopic studies show that PrP^C is highly α-helical, whereas PrP^{Sc} is rich in β-sheet structure (*13*−*15*). Recently, high-resolution structural data were obtained for a recombinant prion protein expressed in bacterial systems (*16*−*22*). The latter protein, which serves as a structural model of the PrP^C isoform, has also been extensively used to characterize the folding pathway of PrP (*23*−*29*).

Despite great interest in the pathogenesis of prion disorders, the molecular mechanism of the $PrP^C \rightarrow PrP^{Sc}$ conversion in vivo is at present unclear. Recently, we demonstrated that incubation of the recombinant PrP at acidic pH (5 of below) in the presence of low concentrations of an ionic

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[1] Abbreviations: PrP, prion protein; PrP^C, cellular PrP isoform; PrP^{Sc}, scrapie (proteinase-resistant) PrP isoform; huPrP90−231, recombinant human prion protein fragment 90−231; CD, circular dichroism; UV, ultraviolet.

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denaturant guanidine hydrochloride results in a transition to a β-sheet-rich oligomeric form with physicochemical properties very similar to those of PrP^{Sc} (*30*). In the present study, we have focused on the conformational transitions of the recombinant PrP in the presence of urea. The use of a nonionic denaturant allowed us to separately assess the role of "unfolding effects" and "salt effects" in the conversion of the recombinant prion protein to a PrP^{Sc}-like form.

MATERIALS AND METHODS

Materials. Sodium acetate, sodium chloride, acetic acid, and proteinase K were purchased from Fisher. Urea was obtained from Sigma and was deionized using a mixture of anion-exchange (trimethylbenzylammonium) and cation-exchange (Dowex MR-3) resins. The concentration of urea was determined by measuring the refractive index. Uranyl acetate and carbon-coated 600 mesh copper grids were purchased from Electron Microscope Sciences, and thrombin was obtained from Pharmacia.

Plasmid Construction and Protein Purification. The plasmid encoding huPrP90−231 with a N-terminal linker containing a His6 tail and a thrombin cleavage site was described previously (*31*). The protein obtained using this plasmid contains the N-terminal extension Gly-Ser-Asp-Pro. To remove the last two residues of this extension, the plasmid was amplified by PCR using the primers 5′ GCG TGG TTC GGG TCA AGG AG and 5′ CTC CTT GAC CCG AAC CAC GC. The protein was expressed, cleaved with thrombin, and purified as described previously (*30*, *31*).

Spectroscopic Measurements. The far-UV circular dichroism spectra were obtained on a Jasco J-800 spectropolarimeter (Jasco, Easton, MD). The measurements were performed at room temperature in 0.2 or 1 mm path-length cells. Protein concentration was determined spectrophotometrically using the molar extinction coefficient, ϵ_{276}, of 21 640 M^{-1} cm^{-1}.

Size-Exclusion Chromatography. Analytical size-exclusion experiments were performed on a Superdex 200 10/30 HR gel filtration column (Pharmacia) attached to a FPLC system (Pharmacia). Before each run, the column was preequilibrated with at least five column volumes of the elution buffer. Throughout all of the experiments, the flow rate was kept constant at 0.65 mL/min. The elution of the protein was monitored by absorbance at 280 nm. The column was calibrated with molecular weight standards for size-exclusion chromatography (Sigma).

Dynamic Light Scattering. The light scattering experiments were performed at room temperature on a DynoPro-801 dynamic light scattering instrument (Protein Solution Inc). Prior to the measurements, the buffers were filtered through a 0.2 μm membrane filter (Whatman Inc.). The data were analyzed with Dynamics 4.0 software.

Electron Microscopy. Samples for electron microscopy were prepared by incubating huPrP90−231 (1 mg/mL) in a buffer (50 mM sodium acetate, pH 4.0) containing 1 or 3.5 M urea and 150 mM NaCl. Control samples were prepared in the same buffer without sodium chloride. Following incubation for an appropriate period of time, a drop of each sample was placed on a carbon-coated 600 mesh copper grid and negatively stained with 2% aqueous (w/v) uranyl acetate. Grid preparations were visualized using a JEOL 1200 transmission microscope operating at 80 keV.

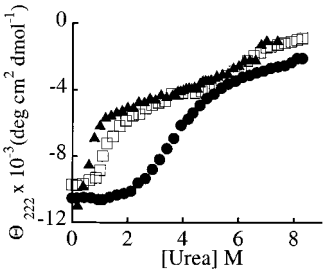

FIGURE 1: Urea-induced unfolding of huPrP90−231 in 50 mM sodium acetate buffer, pH 4.0. Protein (1 mg/mL) unfolded in the absence of NaCl (●) and in the presence of 150 mM NaCl at a concentration of 0.4 mg/mL (□) and 1 mg/mL (▲). The incubation time in each case was 24 h.

RESULTS

Unfolding of huPrP90−231 in Urea. The equilibrium unfolding of the prion protein was followed by changes in ellipticity at 222 nm. Figure 1 shows the urea unfolding curves for huPrP90−231 at pH 4.0 in the absence and presence of sodium chloride. In the absence of NaCl, huPrP90−231 undergoes a highly cooperative unfolding transition that is fully reversible and does not depend on the concentration of the protein. The experimental points could be fitted according to a two-state unfolding model (*32*), yielding an apparent midpoint unfolding urea concentration (C_m) of 3.6 M and an apparent free energy difference between the native and unfolded state ($\Delta G°$) of 12 kJ/M. However, it should be emphasized that this apparently good fit may be fortuitous as the equilibrium unfolding data alone are insufficient to prove the applicability of a two-state unfolding model (*33*) (see also Discussion). Dramatically different urea unfolding profiles for huPrP90−231 were obtained in the presence of sodium chloride. In the latter case, the transition curves show well-defined "plateau" regions and clearly deviate from the two-state unfolding model. Furthermore, these curves are protein concentration-dependent, indicating that unfolding of huPrP90−231 in the presence of urea and NaCl is associated with protein self-association. This precludes analysis of these curves according to a simple thermodynamic model.

In contrast to complex unfolding curves for huPrP90−231 at pH 4, urea unfolding curves at pH 7.0 were essentially identical in the absence and presence of NaCl (data not shown for brevity). Furthermore, even in the presence of sodium chloride, the unfolding profiles at pH 7 showed no dependence on protein concentration. These curves could be analyzed according to the two-state model, yielding a C_m value of 6.2 M and a $\Delta G°$ value of 27 kJ/M.

Salt-Dependent Conformational Transitions of huPrP90−231. The secondary structure of huPrP90−231 was studied by far-UV CD spectroscopy. Figure 2A shows the CD spectra of the protein in 50 mM sodium acetate buffer, pH 4, in the presence of 3.5 M urea and both the absence and presence of 150 mM sodium chloride. Consistent with the previous report (*30*), the spectrum of the protein in the absence of a denaturant exhibits a double minimum at 222 and 208 nm characteristic of α-helical structure (data not shown for brevity). In the presence of 3.5 M urea, the spectrum is typical of a partially denatured α-helical protein. The latter spectrum is independent of protein concentration and does not change over the period of at least 7 days. However, upon

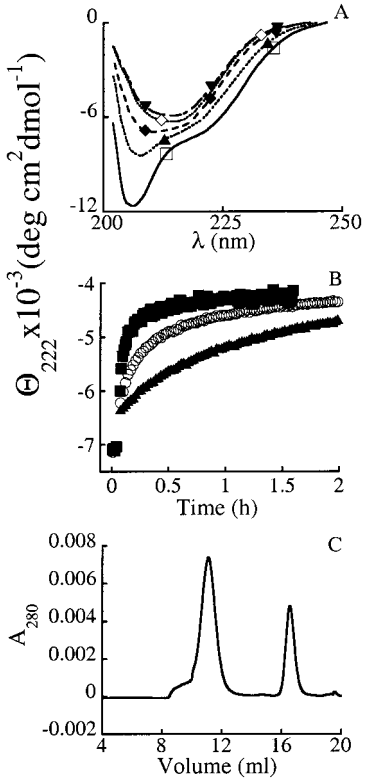

FIGURE 2: Transition to β-sheet structure and oligomerization of huPrP90−231 in the presence of 3.5 M urea and 150 mM NaCl at pH 4.0. (A) Far-UV circular dichroism spectra of the protein (1 mg/mL) in 3.5 M urea in the absence of NaCl (□) and recorded 2 min (▲), 30 min (◆), 1 h (◇), and 120 h (▼) after the addition of 150 mM NaCl. (B) Kinetics of the conformational transition in the presence of 150 mM NaCl (as monitored by changes in ellipticity at 222 nm) at a protein concentration of 0.5 (▲), 1 (○), and 2 (■) mg/mL. (C) Size-exclusion chromatography of huPrP90−231 (1 mg/mL) in 3.5 M urea following 1 h incubation in the presence of 150 mM NaCl. The buffer used in these experiments contained 50 mM sodium acetate and 3.5 M urea, pH 4.0.

addition of 150 mM NaCl to the protein solution in 3.5 M urea, there is a time-dependent decrease in negative ellipticity, concomitant with a change in the shape of the spectrum to one characteristic of β-sheet structure (minimum at 215 nm). The kinetics of the above salt-induced conformational transition could be conveniently monitored by the time course of changes in negative ellipticity at 222 nm. As shown in Figure 2B, the rate of the transition to β-sheet structure strongly depends on protein concentration, becoming faster as the concentration of huPrP90−231 is increased. This suggests that the conformational transition is not a monomolecular reaction and likely involves oligomerization of the protein.

The oligomerization state of huPrP90−231 under different experimental conditions was studied in more detail by a combination of quasi-elastic light scattering and size-exclusion chromatography. In the urea-free sodium acetate buffer, pH 4, quasi-elastic light scattering experiments indicate the presence of a single species with a Stokes radius of 2.0 nm. This is consistent with a monomeric state of huPrP90−231 under these conditions. In the presence of 3.5 M urea, the apparent Stokes radius increases to approximately 2.6 nm. The above increase likely reflects rapid equilibrium between the folded and unfolded monomeric protein (the apparent Stokes radius for fully unfolded huPrP90−231 is 3.1 nm). Upon addition of 150 mM NaCl to huPrP90−231 in 3.5 M urea (sodium acetate buffer, pH 4), light scattering data indicate the presence of large oligomers. Oligomerization of huPrP90−231 under the above experimental conditions was further confirmed by size-exclusion chromatography. As shown in Figure 2C, upon addition of 150 mM NaCl to the protein solution in 3.5 M urea (50 mM sodium acetate buffer, pH 4) huPrP90−231 eluted as a mixture of high molecular weight aggregates with an apparent molecular mass of approximately 400 kDa (first peak) and a monomer (second peak). The proportion of the monomeric species decreased with time: it accounted for approximately 25% and less than 10% of total protein following 1 and 24 h incubation, respectively, in the presence of 150 mM NaCl.

Data obtained in the presence of 3.5 M urea (Figure 2) suggest that the sodium chloride-induced transition of huPrP90−231 to a β-sheet structure is related to self-association of the protein. However, under these experimental conditions the reaction was too fast to allow a quantitative analysis by size-exclusion chromatography. One possible way to reduce the rate of the sodium chloride-induced conformational transition of huPrP90−231 is to lower the concentration of urea in the incubation mixture (see below). Therefore, experiments similar to those illustrated in Figure 2 were also performed in the presence of 1 M urea. Under the latter conditions, the NaCl-induced transition of huPrP90−231 to a β-sheet structure was very slow, occurring over the period of many hours (Figure 3A). Analysis of samples prepared for CD experiments by size-exclusion chromatography revealed a time-dependent loss of the elution peak corresponding to protein monomer, accompanied by the appearance of a peak corresponding to the oligomeric species (Figure 3C). The estimated half-time for the oligomerization reaction was approximately 21 h. This value corresponds closely to the half-time of 22 h for α-helix → β-sheet transition as estimated from the time-course changes in the ellipticity at 222 nm (Figure 3B).

The kinetics of the huPrP90−231 transition to an oligomeric β-sheet structure could also be modulated by the concentration of sodium chloride. The transition (in 3.5 M urea) could be observed even upon reducing the concentration of NaCl to 50 mM. However, the rate of the reaction was 1 order of magnitude slower as compared with that in the presence of 150 mM NaCl (data not shown for brevity).

Morphological Studies. The morphology of the β-sheet-rich oligomers of huPrP90−231 was studied by electron microscopy. The micrographs of the protein preincubated in the presence of urea and sodium chloride showed fibrillar structures (Figure 4). The fibrils were 15−20 nm in diameter and of variable length. The fibrillar filaments were especially abundant when the protein was preincubated in 1 M urea/150 mM NaCl. The fibrils were accompanied by numerous amorphous aggregates of variable shape and size (only the fibril-rich field is shown in Figure 4). Furthermore, the β-sheet-rich aggregates of huPrP90−231 were thioflavin T positive, as indicated by the increased fluorescence intensity of the dye in the presence of the aggregated (but not monomeric) protein (data not shown for brevity).

The Transition of huPrP90−231 to Oligomeric β-Sheet Structure Is Dependent on the Concentration of Urea. To gain insight into the mechanism of the salt-induced transition of huPrP90−231 to a β-sheet structure, the reaction was

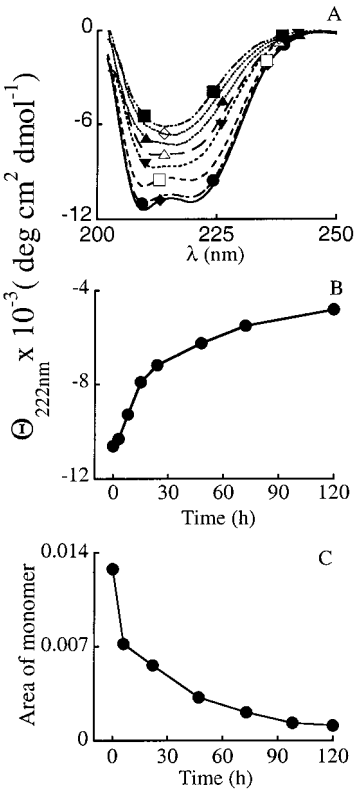

FIGURE 3: Time-dependent transition to β-sheet structure and oligomerization of huPrP90−231 in the presence of 1 M urea and 150 mM NaCl, pH 4.0. (A) Far-UV circular dichroism spectra of the protein incubated for up to 7 days in the absence of sodium chloride (◆) and recorded 3 h (●), 8 h (□), 15 h (▼), 24 h (△), 48 h (▲), 72 h (◇), and 120 h (■) after the addition of 150 mM NaCl. (B) Kinetics of the conformational transition in the presence of 150 mM NaCl as monitored by changes in ellipticity at 222 nm. (C) Kinetics of the huPrP90−231 oligomerization in the presence of 150 mM NaCl as monitored by size-exclusion chromatography. The area of the peak corresponding to the monomeric protein is plotted as a function of incubation time in 150 mM NaCl. The concentration of huPrP90−231 used in these experiments was 1 mg/mL buffer, and the buffer contained 50 mM sodium acetate and 1 M urea, pH 4.0.

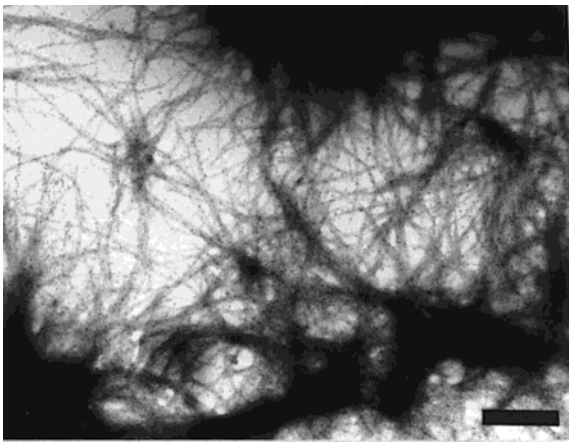

FIGURE 4: Electron micrograph taken following 7 days of incubation of huPrP90−231 (1 mg/mL) in the presence of 1 M urea and 150 mM NaCl (50 mM sodium acetate buffer, pH 4.0). In addition to fibrillar structures shown in the micrograph, numerous amorphous aggregates of highly variable size and morphology were also observed (not shown). The bar corresponds to 200 nm.

initiated by adding 150 mM NaCl to protein samples preincubated for 24 h in the presence of increasing concentrations of urea. As shown in Figure 5, at a low denaturant concentration the conformational transition is very slow. The rate of the reaction increases gradually as the concentration of urea is increased up to approximately 3.5 M (the midpoint of unfolding in the absence of NaCl). Under the present experimental conditions (50 mM sodium acetate buffer, 150 mM NaCl, pH 4; protein concentration of 1 mg/mL), the estimated half-time of the reaction was approximately 21 h, 8 h, 80 min, and 12 min at urea concentrations of 1, 1.5, 2, and 3.5 M, respectively. However, upon further increase in the urea concentration to 4.5 M, the rate of β-sheet structure formation was again decreased (apparent half-time of more than 1 h). In 7.2 M urea, the protein is essentially fully unfolded. Importantly, under the latter conditions the CD spectra indicate no transition to β-sheet structure even after 7 days incubation in the presence of 150 mM NaCl (Figure 5, last panel). This is consistent with size-exclusion data that the protein incubated for 7 days in the presence of 7.2 M urea and 150 mM NaCl remains monomeric (chromatographic profile not shown for brevity).

DISCUSSION

The pathogenesis of transmissible spongiform encephalopathies is associated with the conversion of prion protein from an α-helix-rich form, PrP^C, to a β-sheet-rich conformer, PrP^{Sc} (*1*−*4*, *13*−*15*). The notion that a protein can switch between two different forms characterized by profoundly different secondary structure has spurred great interest in understanding the folding pathway of the prion protein. Biophysical studies of protein folding require large quantities of highly purified protein; therefore, such studies have been largely performed using the recombinant PrP expressed in bacterial systems. The limitation of the latter protein is that, compared to the authentic mammalian prion protein, it lacks glycosylation and the glycosphospatidylinositol anchor. Nonetheless, the recombinant PrP has proven of great value as a model for studying the structure, thermodynamic stability, and conformational transitions of the prion protein (*16*−*30*).

One of the central questions related to the mechanism of conformational conversion of the prion protein is whether both the α-helical and β-sheet-rich forms can exist as monomers or whether the β-sheet conformer is stable only as a polymer. The first scenario appears to be implied by the "template-assistance" (or "heterodimer"') model of PrP conversion (*35*), whereas the second possibility is postulated by the "nucleation-dependent polymerization" model (*36*). Studies with the recombinant prion protein have provided conflicting data regarding the mechanism of α-helix → β-sheet transition. It was reported that, upon reduction of a single disulfide bridge, the recombinant prion protein can reversibly switch between a monomeric α-helical form and a monomeric form rich in β-sheet structure (*34*). However, a more recent study failed to corroborate this claim, finding that β-sheet structure was formed only upon oligomerization of the reduced prion protein (*37*). A conversion of the recombinant human PrP90−231 to an oligomeric (but not monomeric) β-sheet-rich form with characteristics similar to those of PrP^{Sc} could also be induced in the presence of the native disulfide bond (*30*). This transition was observed under mildly acidic conditions (pH 5 or below) in the presence of low concentrations of guanidine hydrochloride.

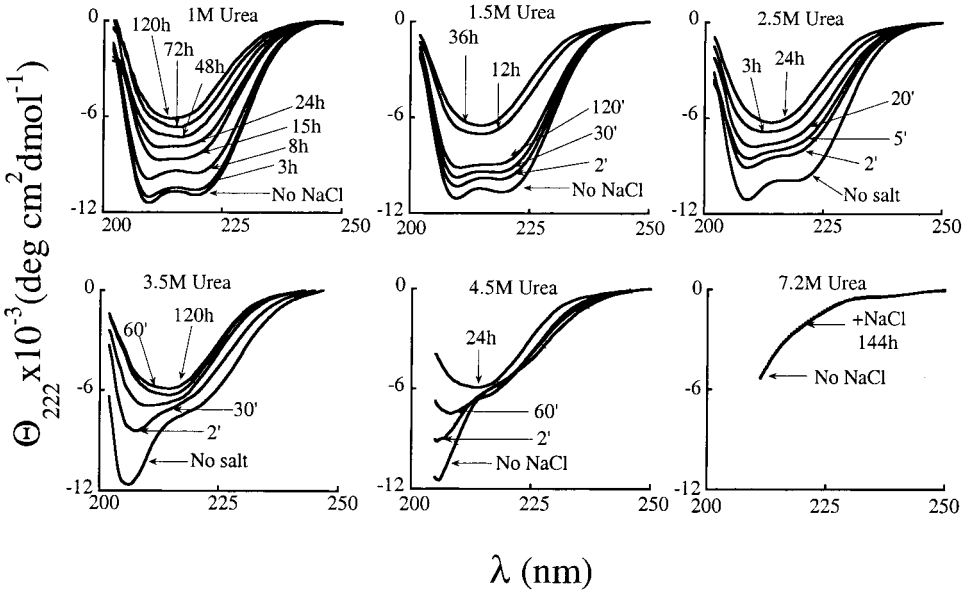

FIGURE 5: Time-dependent changes in circular dichroism spectra of huPrP90−231 after the addition of 150 mM NaCl to the protein preincubated for 24 h in the presence of different concentrations of urea. The number at each spectrum indicates the time after addition of NaCl. The concentration of huPrP90−231 was 1 mg/mL, and the buffer was 50 mM sodium acetate, pH 4.0.

Furthermore, a similar transition was reported for a redacted chimeric mouse−hamster PrP consisting of 106 amino acids (*38*). The above two studies provided no evidence for the presence of a monomeric β-sheet-rich form of the prion protein. However, in a related study with the C-terminal domain 121−231 of mouse PrP, Hornemann and Glockshuber reported that during urea unfolding at acidic pH the protein forms an equilibrium unfolding intermediate with spectral characteristics of a β-sheet (*24*). On the basis of limited experimental data, it was assumed that this "intermediate" represents a monomeric protein. Since the possibility that urea may promote a transition between monomeric α-helical and monomeric β-sheet-rich forms of PrP is highly intriguing, we have undertaken more detailed studies on the unfolding and conformational structure of the recombinant prion protein in this denaturant.

The present data show that urea unfolding of huPrP90−231 at acidic pH is strongly dependent on the presence of salts. The unfolding curve at pH 4 in sodium acetate buffer is highly cooperative and has a midpoint at 3.6 M urea. In contrast to data reported previously (*24*), this curve contains no plateau indicative of the presence of an equilibrium unfolding intermediate. Such a plateau becomes visible, however, if the unfolding experiments are performed in the presence of sodium chloride. The unfolding curves for huPrP90−231 in NaCl-containing buffer at pH 4 are qualitatively similar to those previously reported for mouse PrP121−231 (*24*). However, additional data obtained in the present study suggest a different interpretation of the urea unfolding experiments. In particular, these data argue against the presence of a monomeric β-sheet-rich equilibrium folding intermediate of huPrP, indicating that the β-sheet-rich conformer formed in the presence of urea and NaCl represents an oligomeric species rather than a monomeric protein. Oligomerization of huPrP at low pH in the presence of urea and NaCl is strongly suggested by the protein concentration dependence of the "unfolding curves" (Figure 1). Furthermore, the polymeric nature of the β-sheet conformer is evidenced by the concentration dependence of the rate of β-structure formation (Figure 2) and, more directly, by gel filtration, quasi-elastic light scattering, and electron microscopic data. Very similar rates of protein self-association and β-sheet formation suggest that these two reactions occur concomitantly, although time resolution of the present experiments is insufficient to exclude the possibility that the oligomerization step may be preceded by the formation of a transient monomeric β-sheet-rich species. Such a conformer would be, however, very short-lived and highly prone to aggregation.

The transition of huPrP90−231 to an oligomeric β-sheet structure observed in the presence of urea and NaCl is very similar to that previously reported for the protein incubated with guanidine hydrochloride (*30*). The finding that urea alone is unable to induce a similar transition points to the critical role of ions in the oligomerization of the recombinant prion protein. The nature of the ionic interactions involved is at present unknown. However, preliminary data indicate that the apparent requirement for salt is not simply due to the ionic strength effect but rather due to more specific interactions of the protein with certain anions. The potency of these anions appears to show poor correlation with their ranking in the Hofmeister series: chloride, citrate, and sulfate anions were found to induce a rapid transition of huPrP90−231 to an oligomeric β-sheet structure, whereas anions such as acetate or fluoride appear to be relatively ineffective as promoters of β-structure formation.[2]

An important question related to the mechanism of prion protein conversion into a PrP^{Sc}-like form is: what is the nature of the PrP monomer that self-associates into the oligomeric β-sheet structure? Is it a native α-helical conformer, a fully unfolded form, or a monomeric folding intermediate? The native form is an unlikely candidate since under native conditions the protein shows very little tendency to undergo a transition to a β-sheet-rich structure. The present data demonstrate that, at a given concentration of NaCl, the

[2] D. Vanik and W. K. Surewicz, unpublished data.

rate of the α-helix → oligomeric β-sheet transition increases as the concentration of urea is increased up to approximately 3.5 M. Since urea increases the population of the unfolded protein, this could suggest that the latter species is the one that undergoes a transition to a PrP^{Sc}-like form. However, such a scenario seems unlikely since no protein oligomerization (or β-sheet formation) could be observed under fully unfolding conditions (i.e., in the presence of 7.2 M urea). This leaves us with a possibility that a monomeric folding intermediate(s) of prion protein is (are) involved in the transition to the oligomeric scrapie-like form. It was previously postulated that a stable monomeric β-sheet-rich intermediate is an immediate precursor of the oligomeric PrP^{Sc} conformer (*24*, *25*, *34*). However, our recent results argue against the existence of such a stable monomeric β-sheet-rich folding intermediate of the prion protein (this work and refs *30* and *37*). This does not preclude, however, the involvement in prion protein conversion of a more "conventional" intermediate(s) with a nativelike or partially unfolded secondary structure. Such partially unfolded intermediates are known to promote a conformational conversion in other amyloid-forming proteins (*39*−*41*). It should be noted that previous stopped-flow (*29*) and hydrogen−deuterium exchange experiments (*28*) failed to detect any significantly populated folding intermediates of the recombinant prion protein. However, such studies were performed only at neutral (or close to neutral) pH, whereas the conversion of the recombinant prion protein to scrapie-like form is strongly promoted by acidic conditions. It is possible that the folding pathway of PrP under the latter conditions is significantly different than that reported at neutral pH. pH-dependent conformational transitions of PrP are of considerable interest since the $PrP^{C} \rightarrow PrP^{Sc}$ conversion in vivo may occur in acidic compartments on the endocytic pathway (*42*, *43*). Studies are currently underway to elucidate the effect of pH on the folding pathway of the prion protein.

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