Oligomerization of the Human Prion Protein Proceeds via a Molten Globule Intermediate

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The conformational transition of the human prion protein from an α-helical to a β-sheet-rich structure is believed to be the critical event in prion pathogenesis. The molecular mechanism of misfolding and the role of intermediate states during this transition remain poorly understood. To overcome the obstacle of insolubility of amyloid fibrils, we have studied a β-sheet-rich misfolded isoform of the prion protein, the β-oligomer, which shares some structural properties with amyloid, including partial protease resistance. We demonstrate here that the β-oligomer can be studied by solution-state NMR spectroscopy and obtain insights into the misfolding mechanism via its transient monomeric precursor. It is often assumed that misfolding into β-sheet-rich isoforms proceeds via a compatible precursor with a β-sheet subunit structure. We show here, on the contrary, evidence for an almost natively α-helix-rich monomeric precursor state with molten globule characteristics, converting in vitro into the β-oligomer. We propose a possible mechanism for the formation of the β-oligomer, triggered by intermolecular contacts between constantly rearranging structures. It is concluded that the β-oligomer is not preceded by precursors with β-sheet structure but by a partially unfolded clearly distinguishable α-helical state.

The misfolding of the prion protein is the cause of several fatal neurodegenerative diseases in humans and animals (1, 2). Among these are scrapie in sheep, bovine spongiform encephalopathy in cattle and, in humans, Creutzfeldt-Jakob disease, Gerstmann-Straussler-Scheinker disease, fatal familial insomnia, and kuru. These diseases are associated with the misfolding of the α-helix-rich cellular prion PrPc3 protein to the β-sheet-rich, PrPSc form (1, 3). The native state of PrPc is represented by that of the 19.8-kDa protein (residues 89–231), which consists of three α-helices and a short anti-parallel β-sheet (4) and will be denoted here as α3. The mechanism of conversion of α3 to the pathogenic PrPSc form remains unclear, although it is now known that it occurs post-translationally without detectable covalent modifications (5). The transformation requires a substantial change of conformation from an α-helix-rich monomer to a β-sheet-rich amyloid structure.

In vitro, α3 can be converted into a variety of stable non-native structures with high β-sheet content (6, 7). Different solution conditions can induce different structures, one of which is the β-oligomer, βO, first described by Baskakov et al. (6). The βO state is formed under mildly denaturing and acidic conditions similar to those found in endocytic vesicles in humans. Some of the biophysical properties of βO are similar to PrPSc: high β-sheet content, protease K resistance with slightly different cleavage sites (8), and high binding affinity to 1-anilino naphthalene-8-sulfonate (ANS). In contrast to PrPSc, βO is soluble, monodisperse, and does not bind thioflavin T (6). Importantly, βO is not a template for the formation of PrPSc; on the contrary, it is a very stable long-lived macromolecular assembly, the formation of which proceeds through a pathway that competes with amyloid formation (6). In recent publications (9, 10), the polymorphism at codon 129 was shown to exhibit a measurable effect on the kinetics of formation of βO. It was further shown that βO formed from an equimolar mixture of valine 129 and methionine 129 PrP is very refractory to amyloid formation when compared with homogeneous protein of either allelic form. As heterozygosity at this position is associated epidemiologically with lower rates of prion disease, it was inferred that βO might have an adaptive role by sequestering PrP away from the pathway of amyloid formation.

The key to understanding the mechanism of the conformational transition from α-helical to β-sheet structure is the identification of precursors. Among the hypotheses concerning the nature of the possible precursors of PrPSc, an early model due to Cohen et al. (11) discusses the possibility of a partially unfolded intermediate whose formation and conversion into PrPSc could be promoted by an irreversible reaction with insoluble PrPSc. Similarly, the formation of the off pathway βO involves a dramatic change of secondary structure (6). Others have hypothesized about the existence of weakly populated intermediates during the oligomerization process of βO but could not enrich or stabilize such a species (12). One of the key questions is the structural nature of such an intermediate and whether at an...
early stage it adopts a β-sheet structure or remains α-helical-rich. To this end, we have studied the oligomerization process during the formation of βO where we have identified and partially characterized a precursor state, αi.

**EXPERIMENTAL PROCEDURES**

**Protein Expression and Purification—Escherichia coli**

expression of recombinant human PrP was performed as described previously (9). Briefly, the 90–231 fragment of the PRNP gene was cloned into the pTrcHis2B vector incorporating a C-terminal His tag (Invitrogen, Paisley, UK) and was expressed in the E. coli strain BL21(DE3) (Novagen). Cells were grown in a minimal medium with 13NH4Cl as the sole source of nitrogen for fully 15N-labeled protein. The purification was performed as described previously (9), and stocks of highly purified proteins were kept in a storage buffer (6M guanidine hydrochloride containing 50 mM Tris-HCl, pH 7.2). The protein was fully oxidized as judged by reverse phase chromatography, and the integrity of the samples was further analyzed by mass spectrometry (data not shown). The oxidation state is of particular importance with respect to previously reported and potentially controversial observations of reversible monomeric β-sheet-rich structures (13, 14). The data presented here are solely for the allelomorph HuPrP90–231 Val129. Studies of direct refolding show that the Val129 allelomorph oligomerizes more slowly to βO than Met129 (9). Other than that, the mutation at position 129 has no measurable effect on the folding, dynamics, or stability of PrPC (15).

**Size-exclusion HPLC Preparation of αi and αO**—We used sec-HPLC to refold the protein to either the native state αO or the precursor αi. To form αO and αi, 0.8 mg of protein denatured in 100 µl of storage buffer was injected onto a TSK®-Gel SWXL G3000 HPLC column (7.8 × 300 mm, Phenomenex, Macclesfield, UK), equilibrated with either αO buffer (20 mM sodium acetate, pH 5.5, 150 mM sodium chloride, 1 mM urea, and 0.02% azide) or buffer A (20 mM sodium acetate, pH 4.0, 200 mM sodium chloride, 1 mM urea, and 0.02% azide), as appropriate. The peak corresponding to monomeric proteins at 9.04 min of retention time was manually collected (see also Fig. 1a and b). For NMR studies, up to five runs were collected, pooled, and concentrated (Amicon Ultra-4, 10,000 molecular weight cut-off, Millipore, Carrigtwohill, County Cork, Ireland) to final protein (14.8 mg ml−1) at room temperature with a flow rate of 1 ml min−1 by means of a PerkinElmer Life Sciences HPLC system composed of a Series 200 pump and a diode array detector 235C controlled by Total Chrome software version 6.2 (PerkinElmer Life Sciences, Seer Green, UK), through a PE Nelson 600 series link. The eluent was monitored by UV absorption at 280 nm.

**β-Oligomer (βO) Formation by Dialysis**—As described previously (9), βO was formed by dialyzing 0.5 ml of GuHCl-denatured protein (14.8 mg ml−1) at room temperature for 24 h, twice against 2 liters of 2 M urea, 0.2 M NaCl, 20 mM sodium acetate, pH 3.6, and once against βO buffer (1 M urea, 0.2 M NaCl, 20 mM sodium acetate, pH 3.6, 0.02% sodium azide). We used a Slide-A-Lyser dialysis cassette (Perbio Science UK Ltd., Tattenhall, UK) with a 10-kDa cut-off.

**Circular Dichroism Spectroscopy**—CD spectra were recorded using a Jasco-720 spectrometer at room temperature with the following parameters for far UV and near UV spectra, respectively: cell path 0.1 cm, speed 50 or 100 nm min−1, bandwidth 2.0 or 1.0 nm, resolution 1.0 or 0.5 nm, and a response time of 4 or 1 s, averaged over 4 or 16 scans. Far and near UV spectra were measured in βO buffer (protein concentration 40 and 550 µM), αO buffer (protein concentration 50 and 780 µM), and buffer A (protein concentration 22 and 440 µM) for βO, αO, and αi, respectively (the buffer compositions are specified above). The amount of helical structure was calculated using the Chen algorithm (16).

**Pulsed Field Gradient NMR Diffusion Spectroscopy**—PGF NMR diffusion measurements were performed at 20 °C on a home-built spectrometer with a 1H operating frequency of 600.20 MHz. The pulsed gradient stimulated echo longitudinal encode-decode pulse sequence was used (17), incorporating composite sine gradient pulses (18), as described elsewhere (19). The lengths of all pulses and delays in this sequence were held constant, and 20 spectra were acquired with the strength of the diffusion gradient varying between 5 and 100% of its maximum value. To increase the signal-to-noise ratio and to estimate the experimental error (quoted as ±1 standard deviation), measurements were repeated five, six, and four times for αO, αi, and βO, respectively. The duration of the pulsed gradients (δ) and the stimulated echo delay time (τ) were optimized for each sample to give a total decay in the protein signal of between 80 and 90% for the strongest field gradients. The following values were used for αO, αi, and βO, respectively: δ = 5.5, 5.5, and 6.5 ms and τ = 75, 100, and 150 ms. The protein concentrations were 300, 100, and 150 µM for αO, αi, and βO, respectively. 0.15% dioxan was added as a viscosity probe of known hydrodynamic radius (17). The buffer conditions were αO, A, and βO buffer, as appropriate, except that water was exchanged for D2O. The intensities of the signals (S) from the protein and dioxan as a function of the gradient strength (g) were fitted to the Gaussian function

\[ S(g) = A \exp(-d g^2) \]  

(Eq. 1)
enabling determination of the decay rate d, which is proportional to the diffusion coefficient, D. The hydrodynamic radius of the protein was obtained from that of dioxan, rref as rref (dref/dprot).

**NMR Spectroscopy**—One-dimensional 1H and two-dimensional heteronuclear HSQC NMR experiments were carried out on a Varian Inova 600 spectrometer with a 1H operating frequency of 600 MHz. The buffers used were as described above except that 10% D2O was added without correcting for the volume effect. All 1H-15N HSQC spectra were recorded at
**RESULTS**

**Preparation and Characterization of \( \alpha^N \), \( \beta^O \), and \( \alpha' \)—Recombinant HuPrP\(^{90-231} \) Val\(^{129} \) was metabolically labeled with \(^{15}\)N, purified, and refolded *in vitro*, as described previously for unlabeled protein (Ref. 9 and “Experimental Procedures”). The \( \alpha^N \) and \( \beta^O \) states so formed were identical to those of the unlabeled protein as judged by sec-HPLC, far-UV CD, and ANS binding (Fig. 1a, c, and e, respectively).

The native \( \alpha^N \) and oligomeric \( \beta^O \) states can be clearly differentiated by sec-HPLC with elution times of 9.04 min for \( \alpha^N \) and 6.4 min for \( \beta^O \), respectively (Fig. 1a). The stability of \( \beta^O \) is remarkably high, as judged by reverse phase-HPLC of samples matured for more than a year (9). In an attempt to observe intermediates between \( \alpha^N \) and \( \beta^O \), the oligomerization process was monitored with sec-HPLC by injecting samples previously refolded into buffer A (see “Experimental Procedures”) and incubated at room temperature for different times (Fig. 1b). The transient species appearing in these chromatograms at an elution time of \( \sim 9 \) min is referred to as \( \alpha' \); it elutes with the same retention time as \( \alpha^N \). Although \( \alpha' \) and \( \alpha^N \) cannot be resolved by HPLC, they will be shown by optical and NMR spectroscopy (see below) to be distinct species. Detectable amounts of \( \beta^O \) are formed from \( \alpha' \) after about 2 h. The reaction takes more than 100 days to come to equilibrium under the conditions chosen (buffer A). Reducing pH and/or raising the urea or protein concentrations accelerates the oligomerization. The first and second HPLC chromatograms in Fig. 1b correspond to the initial state for all the experiments on \( \alpha' \); a fresh preparation was used for each experiment.

In the far UV CD measurements (Fig. 1c), the \( \alpha^N \) state exhibits its \( \alpha \)-helical spectrum, whereas \( \beta^O \) shows the expected \( \beta \)-sheet character (22). Interestingly, the \( \alpha' \) state exhibits a clear \( \alpha \)-helix secondary structure, although it was directly refolded in buffer A, which should favor the formation of a \( \beta \)-sheet structure. Judging from the signals at 222 nm, \( \alpha' \) has about 45% \( \alpha \)-helix structure when compared with 48% in \( \alpha^N \) and 28% in \( \beta^O \). A clear difference between \( \alpha^N \) and \( \alpha' \) can be seen in the near-UV CD spectra (Fig. 1d). These spectra, which provide a qualitative measure of tertiary structure, display signal loss for \( \alpha' \) and \( \beta^O \), whereas \( \alpha^N \) is clearly highly organized.

The dye, ANS, preferentially binds to hydrophobic surfaces of proteins with a substantial increase in fluorescence intensity (23). Although its affinity to \( \alpha^N \) is low, it binds strongly to misfolded isomers of PrP, in particular to PrP\(^{263} \) and \( \beta^O \) (6, 24). In addition to a significant increase in ANS fluorescence in the presence of \( \beta^O \), we also found an increase for \( \alpha' \) (Fig. 1e), which probably reflects the accessibility of the hydrophobic core of this partially unfolded state (25).

**Hydrodynamic Radius Measurements with PFG NMR**—To assess the aggregation state and compactness of \( \alpha' \), we performed NMR diffusion experiments to measure the hydrodynamic radii (\( R_h \)) of \( \alpha^N \), \( \alpha' \), and \( \beta^O \) (Fig. 2) as described under “Experimental Procedures.” The obtained radii were 28.3 ± 1.56, 31.9 ± 0.34, and 57.4 ± 3.4 Å for \( \alpha^N \), \( \alpha' \), and \( \beta^O \), respectively. Wilkins et al. (19) established empirical relationships between \( R_h \) and the number of residues in the polypeptide chain (N); for globular folded proteins, \( R_h/\AA = 4.75 N^{0.29} \), whereas for highly denatured states without disulfide bridges, \( R_h/\AA = 2.21 N^{0.57} \). It was shown that predictions based on these equations agree well with data from dynamic light scattering. HuPrP\(^{90-231} \) comprises 169 residues including the unstructured 26-residue histidine tag construct. The overall fold of \( \alpha^N \) comprises a globular domain between residues Tyr\(^{128} \) and Tyr\(^{226} \) and two unstructured segments at the N and C termini comprising 70 residues in total. For \( n = 169 \), the equations above give \( R_h = 21.1 \) and 41.3 Å, respectively, for a fully folded and a fully unfolded state (with the disulfide bond reduced). A very approximate estimate of \( R_h \) can be obtained as follows; considering that 41% of the native protein is naturally...
unstructured, a simple weighted average of these two values of \( R_g \) gives a radius of 29.4 Å, which compares well with the 28.3 Å measured for \( \alpha^N \). A similar analysis can be performed for \( \beta^O \) taking into account its decameric nature (10). Assuming no change in the percentage of the monomer unit that is unstructured (an assumption supported by the HSQC spectra discussed below), the radius of \( \beta^O \) can be estimated as 10\(^{15} \) 29.4 = 63.3 Å, which is reasonably close to the measured value of 57.4 Å. In comparison, flow field-flow fractionation measurements and multiple angle light scattering gave a radius of 56 Å for \( \beta^O \) with a mass of about 200 kDa (10), and a Stokes radius of 65 Å was measured with dynamic light scattering (6). Interestingly, the radius of \( \alpha^i \) is 10% larger than that of \( \alpha^N \) (Fig. 2c). This is in good agreement with the observation of a limited loss of tertiary structure (Fig. 3a–c) and a partial unfolding retaining a high degree of compactness and a monomeric state.

One- and Two-dimensional NMR Spectroscopy—The effects of oligomerization can be studied with NMR spectroscopy. The spectrum in Fig. 3a is of the native state and shows good chemical shift dispersion and narrow line widths (4). Given their very different molecular masses, \( \alpha^i \) and \( \beta^O \) can be clearly distinguished as \( \beta^O \) shows very broad lines, whereas \( \alpha^i \) retains relatively sharp ones. The spectrum of \( \alpha^i \) is clearly distinguishable from the two other states, exhibiting intermediate characteristics between the \( \alpha^N \) and \( \beta^O \) spectra (Fig. 3, a–c). The \( \alpha^i \) and \( \beta^O \) spectra show a loss of high field methyl proton signals when compared with \( \alpha^N \), indicating a loss of tertiary structure (Fig. 3, boxed regions). The loss of these resonances is more pronounced for \( \beta^O \) than for \( \alpha^i \), in agreement with the near UV CD and ANS binding observations above and underlining the intermediate nature of the \( \alpha^i \) state. Downfield shifts of the backbone \( \alpha \)-proton resonances, which appear at around 4.0 ppm, can be observed in both \( \alpha^i \) and \( \beta^O \) spectra. These shifts are most noticeable for \( \beta^O \) and correspond well with the observation of a \( \beta \)-sheet-rich secondary structure (26). Similarly, the amide protons exhibit downfield shifts that are pronounced for \( \beta^O \) and less so for \( \alpha^i \). Chemical shift differences among the three species also exist for the side chain protons, but these are less obvious than for the \( \alpha^i \) and amide protons and somewhat obscured in the spectra by overlap of resonances.

The \( ^1H \)-\( ^15N \) HSQC spectra of \( \alpha^i \) and \( \beta^O \) show a similar set of resonances with almost identical chemical shifts within the random coil region (Fig. 4). Of the 130 resonances expected on the basis of the native \( \alpha^N \) spectrum (Fig. 4a), only about 70 appear in the spectra of \( \alpha^i \) and \( \beta^O \) (Fig. 4, b and c). The dispersed amide cross-peaks from the core region (residues 128–231) of \( \alpha^N \) were not observable for \( \alpha^i \) or \( \beta^O \). The resonances of \( \alpha^i \) and \( \beta^O \) cover a narrow range of \( ^1H \) chemical shifts in the random coil region between 7.8 and 9.0 ppm and overlap with resonances of the disordered N-terminal segment (residues 90–128) of the native state, \( \alpha^N \) (4). Comparison of the spectra of \( \alpha^i \) and \( \beta^O \) with the known chemical shifts of \( \alpha^N \) (15) allows assignment of 30 out of the 35 resonances in the N terminus. The other signals most probably emanate from the C-terminal His tag. The observation of an unstructured highly flexible N terminus is in good agreement with previous observations that \( \beta^O \) and PrPSc have different protease K cleavage sites. The cleavage site in \( \beta^O \) is around residue Ala117, but it is around Met90 in the authentic, infectious PrPSc (8, 9, 27).

The failure to detect a large number of the expected resonances in the HSQC spectra of \( \alpha^i \) and \( \beta^O \) can be attributed in both cases to severe line broadening. The core of the \( \beta^O \) structure tumbles slowly in solution, consistent with the high molec-
ular mass (~200 kDa (10)) of the oligomer, and has broad NMR lines, whereas the N-terminal domain has a high degree of internal mobility yielding sharp resonances. The same argument applies for the N-terminal region of α, but having clearly shown the monomeric nature of this state, we propose (below) an alternative source of line broadening for the core region of this species.

**DISCUSSION**

To understand the molecular mechanisms underlying the misfolding process, it is generally believed that knowledge of intermediate states is essential. Such intermediates may not be stable at equilibrium, but they might be detectable as transient kinetic intermediates with preformed domains. The refolding process of PrP to its native state, αN, is best described as a sequential three-state reaction with the involvement of an intermediate state (28). Moreover, hydrogen exchange experiments (29) together with high pressure NMR (30) and fluorescence spectroscopy (31) also indicate the existence of folding intermediates. Unfortunately, so far, there is very limited information about the transition from PrPC to PrPSc, and the general belief is that a precursor species with a partially unstructured character precedes amyloid formation. Our data clearly identify an intermediate state, αi, that is partially unfolded, monomeric, and on the pathway to form the β-oligomer (Fig. 5). The intermediate we observe is formed quantitatively and rapidly when the protein is transferred into the appropriate buffer and then rearranges rather slowly (hours or days depending on the conditions) as it assembles into the quaternary structure of βO. Furthermore, αi was observed at pH 4.0, which is the lower limit of the range for which βO and PrPSc have been reported to co-exist (6, 34). This is also in the physiological range of lysosomes and endosomes, which is where the onset of prion disease is generally believed to occur (35, 36).

Using sec-HPLC and NMR diffusion measurements, we have revealed the monomeric nature of αi and its slow oligomerization characteristics. Far UV CD shows that refolding of denatured protein into βO-promoting conditions (buffer A) clearly yields a transient state with almost native-like α-helix content. The αi state has reduced tertiary organization as judged from a reduced near-UV CD signal but remains relatively compact with only a 10% increase in hydrodynamic radius when com-

![FIGURE 4. 1H-15N HSQC spectra of αN, αi, and βO. In the spectra of α and βO (b and c), amide cross-peaks from the core region, which are clearly observed for the native state (a), have almost all disappeared. Signals from the N-terminal region (residues 90–128) and the C-terminal histidine tag remain sharp and well resolved with similar chemical shifts in all three states as judged from direct comparison with the αN spectrum. The assigned resonances marked with an asterisk in a correspond to peaks with overlapping chemical shift values in the spectra of α and β. Only a representative subset of resonances has been labeled.](image-url)
pared with the native fold. One- and two-dimensional NMR spectroscopy elucidate the different folds of the monomeric states \( \alpha^N \) and \( \alpha' \). Although \( \alpha^N \) clearly shows the known spectrum of the native, fully oxidized protein, we observe only a subset of peaks for \( \alpha' \) and \( \beta^O \). The resonances in the \(^1\)H-\(^{15}\)N HSQC spectra of \( \alpha' \) and \( \beta^O \) correspond to the highly flexible and natively unstructured parts of the protein. Missing lines in the spectrum of \( \beta^O \) could be due to a molten globule structure, but it would be very difficult to understand how the formation of a decamer could happen without specific side chain interactions. Rapid exchange with solvent is too slow under the conditions of our measurements to be held responsible for the missing H-N resonances (37). Analysis of the one-dimensional NMR data clearly shows downfield resonance shifts for the \( \alpha' \) and amide protons. Depending on their size, such shifts can be attributed to a conformational change to a random coil or to \( \beta \)-sheet secondary structure (26). The spectrum of \( \alpha' \) indicates an intermediate situation between \( \alpha^N \) and \( \beta^O \), whereas \( \beta^O \) exhibits characteristically strong shifts as expected for a \( \beta \)-sheet-rich structure. Furthermore, the hydrodynamic radius of \( \beta^O \) is only increased by \( \sim 2 \)-fold, which makes it a rather close-packed structure presumably with high symmetry. For these reasons, we attribute the loss of NMR peaks for \( \beta^O \) to severe line broadening arising from its slow tumbling, whereas the N termini of the subunits have high mobility and give sharp NMR lines. The CD spectra are not affected by the unstructured N-terminal tail, which contributes only weakly above 210 nm. The length of the unstructured N-terminal region in polypeptide preparations comprising residues 23–231, 81–231, 90–231, and 121–231 influences the far-UV CD spectrum by reducing the relative \( \alpha \)-helix content but not by changing the form of the CD spectrum (38). Therefore we would not expect to observe a superposition of random coil and \( \beta \)-sheet patterns in the CD spectrum of \( \beta^O \) as the number of unstructured residues does not differ significantly between \( \alpha^N \) and \( \beta^O \). The large size of \( \beta^O \) prevents us from obtaining residue-specific structural information on the core region, but far-UV CD clearly indicates the \( \beta \)-sheet characteristics of \( \beta^O \). Fourier transform infrared studies of a similarly prepared oligomer of SHA\( \alpha \)PrP\(^{90–232} \) (Syrian hamster prion protein) strongly suggest intermolecular \( \beta \)-sheet organization (12).

Considering the monomeric state of \( \alpha' \), we attribute our observations to a molten globule like nature with rearranging secondary structure elements on a millisecond to microsecond time scale. Thus, taking the far-UV CD data into consideration,
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we observe with NMR the mobile unstructured and highly flexible regions of an α-helix-rich intermediate. Although very different, the dynamic properties of α°, a molten globule intermediate and β°O, a large slowly tumbling aggregate, lead to very similar two-dimensional NMR spectra. This observation opens the possibility of elucidating at least some structural features of a β-sheet misfolded isoform of the human prion protein using solution NMR spectroscopy.

The spectroscopic data presented above are consistent with an acid-induced (A-state) molten globular structure for the α° species. This hypothesis accounts for the loss of resonances in the HSQC spectrum, which are broadened beyond detectability by millisecond to microsecond rearrangements of secondary structural elements. This property is characteristic of molten globule A-states, such as α-lactalbumin (39, 40). The radius of α-lactalbumin, for example, increases by about 12% when changing to acidic conditions (39), comparable with the increase observed for α° relative to αN. We have shown that α° is α-helix-rich, has reduced tertiary structure, and interacts with ANS. The binding of an α-helix-rich monomer to ANS can be explained by a partial opening of the hydrophobic core, as described previously for molten globules (25). Taken together, these observations are strong indications for a molten globule-like state (25, 41, 42).

We have shown that the α° state is a genuine intermediate, accessed through low pH conditions, on the misfolding pathway to β°O. We have been able to differentiate, using NMR spectroscopy, between different folding domains, one of which is the highly flexible N terminus, which remains almost inert during β°O formation, whereas the core region of the protein rearranges its secondary and tertiary structure. It is often assumed that misfolding into β-sheet-rich isoforms goes via a compatible intermediate with a preformed β-sheet subunit structure. In this study, we have shown, on the contrary, clear evidence of a distinct, almost natively α-helix-rich, monomeric intermediate state, which converts in vitro into the β-oligomeric form, β°O, of PrP. Furthermore, this precursor has a reduced tertiary structure and exists in a molten globule state with high secondary structure organization fluctuating on a micro- to millisecond time scale. Thereby we provide evidence of a possible mechanism for the formation of misfolded isoforms, i.e., through intermolecular contacts between constantly rearranging structures. We conclude that β-oligomer is not preceded by a precursor with β-sheet structure, but on the contrary, by one with almost intact α-helical organization, in a molten globule state.

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Noted Added in Proof—It has come to our attention that the laboratory of Dr. John Viles has published NMR data, while this study was under review, on the flexibility of the N terminus and mass of a β-intermediate, low pH form of PrP (O’Sullivan, D. B., Jones, C. E., Abdelraheem, S. R., Thompsett, A. R., Brazier, M. W., Toms, H., Brown, D. R., and Viles, J. H. (2007) Biochem. J. 401, 533–540).

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PrP Oligomerizes via a Molten Globule Intermediate