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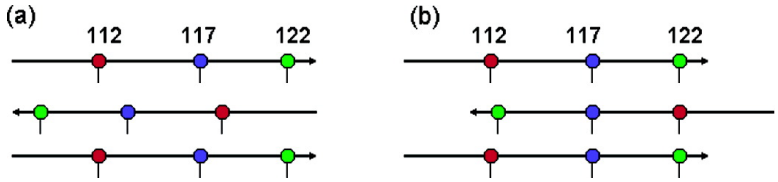

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The Organization and Assembly of a β-Sheet Formed by a Prion Peptide in Solution: An Isotope-Edited FTIR Study

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Protein misfolding leading to the formation of fibrous protein aggregates is the molecular mechanism underlying several diseases, including Alzheimer's and Creutzfeldt−Jakob diseases.[1] While the specific mechanistic details have not yet been elucidated, there is general agreement that fibril formation is preceded by the adoption of extended β-strand structure in a polypeptide; these β-strands assemble to form soluble β-sheet aggregates and eventually complex fibers.[2] In some diseases, the soluble β-sheet aggregates (or protofibrils) may in fact be more cytotoxic than the fibrils themselves. One approach to studying these systems is to focus on model peptides derived from natural sequences, which are capable of undergoing the helix to sheet conformational change, forming aggregates and eventually fibrils. For example, the prion disease scrapie is caused by the misfolded scrapie isoform (PrP^{Sc}) of cellular prion protein (PrP^{C}); the native protein contains only small β-sheet domains, but a conformational change to the PrP^{Sc} results in formation of a large β-sheet domain capable of seeding intermolecular aggregates and eventually the fibrils responsible for the disease.[3] Synthetic peptides derived from the flexible N-terminal region of PrP^{C} can form β-sheets in isolation.[4] H1 is a short peptide with sequence derived from residues 109 through 122 (Ac-MKHMAGAAAAGAVV-NH_2) of Syrian hamster PrP^{C}. This sequence is believed to contain the most amyloidogenic region, with the ability to initiate misfolding of the other regions of the protein as well.[5] While amyloidogenic peptides derived from natural proteins have become standard models for dissecting the mechanism of aggregate formation, there is little detailed structural information about these systems. Isotope-edited infrared (IR) spectroscopy is a valuable technique for obtaining residue-specific information about the conformation and dynamics of peptides. When site-specific ^{13}C labels are introduced into the peptide backbone, the isotope-shifted amide I′ mode couples only weakly with the ^{12}C modes and can be used as a probe for peptide structure at the residue level. This technique has been used to dissect the local details of conformation, stability, and dynamics of α-helices[6] and to map out β-sheet forming regions in dried films of amyloid-forming peptides.[7−9] Here, we report a variable temperature isotope-edited IR study of the peptide H1 in solution, with the goals of using the isotope-edited spectra to identify specific points of interstrand contact within the equilibrium conformation of the β-sheet aggregates and as a probe for the mechanism and dynamics of formation of β-sheet aggregates in solution.

An unlabeled H1 peptide along with a series of 10 peptides containing one or two ^{13}C labels in the backbone were prepared, synthesized, and characterized by FTIR.[10] Before spectra were measured, samples were annealed by a heat/cool cycle to ensure that the peptides were in their equilibrium conformation.[11] The IR spectra in the amide I′ band region of H1 and some singly labeled derivatives are given in Figure 1. The amide I′ band of H1 is a signature of an antiparallel β-sheet, dominated by a weak high frequency and an intense low-frequency component.[12] As the

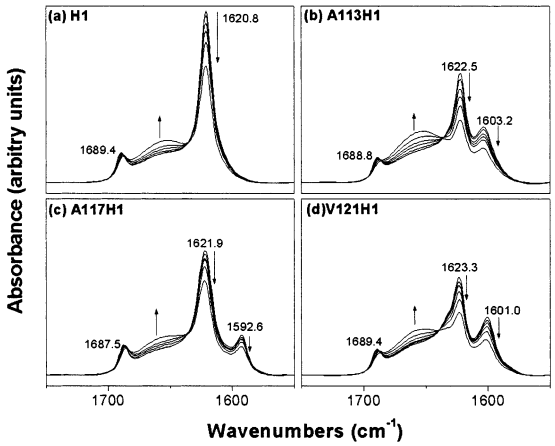

Figure 1. IR absorption amide I′ bands of H1 (a), selected singly labeled derivatives A113 (b), A117 (c), and V121 (d). Peptides were dissolved in a 1:1 mixture of D_2O buffer (20 mM Hepes and 100 mM NaCl in D_2O): acetonitrile (pD = 7.0, corrected). All spectra were recorded from 25 to 75 °C every 10 °C. The frequency positions of the LFβ and HFβ and the ^{13}C component bands are indicated in the each panel. The arrows indicate the direction of the spectral change with increasing temperature. Note that the spectra were rescaled to have the same area from 1750 to 1550 cm^{-1} at 25 °C and the absorbance is given in arbitrary units.

temperature is increased in the range of 25−75 °C, the peptide undergoes a transition to a conformation with significant α-helix or random coil content, giving rise to a broad band centered at ~1655 cm^{-1}.[13] This conformational change is fully reversible; upon cooling back to 25 °C, the initial spectrum is fully reproduced. The lack of a frequency shift in the β-sheet bands upon heating suggests that no detectable intermediate structures occur during unfolding. As the temperature is increased, strands are released from the larger aggregate to form free helix or coil peptides, but leaving the overall structure and organization of the aggregate intact.

Close inspection of the spectra from the labeled peptides allows us to elucidate the details of the conformation and the alignment of residues within the antiparallel β-sheet. When residues of the hydrophobic core of the peptide are labeled, a prominent ^{13}C sideband appears in the spectrum, while it does not appear when residues at the N-terminus of the peptide are labeled. On the basis of the data from the labeled peptides, we propose that the hydrophobic stretch of H1 (residues 112−122) forms a β-sheet, leaving the N-terminal polar residues (109−111) as a dangling end. Peptides labeled at positions A113, A115, A118, A120, and V121 have very similar spectra overall, with ^{13}C amide I′ frequencies within 1600.9−1603.7 cm^{-1}. The peptide labeled at A117, however, gives a ^{13}C amide I′ band at 1592 cm^{-1}, 10 cm^{-1} lower than the others, and with a slightly diminished intensity.

If the β-sheet is formed only by residues 112−122, position A117 will be aligned in all strands, with the carbonyls parallel and in fairly close contact (~4 Å), enabling strong dipole coupling of the

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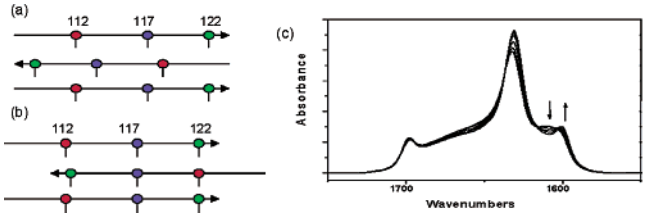

Figure 2. Cartoon schematic of strand alignment in the H1 β-sheet aggregate. (a) Initial (kinetically trapped) alignment of strands, with position of A117 labels indicated. (b) Equilibrium alignment of strands, with the hydrophobic core residues (112−122) forming the core of the β-sheet, resulting in the alignment of the A117 residues in each strand. (c) Amide I′ bands of completely exchanged fresh peptide solution of A117 recorded every 2−3 h after sample preparation at 50 °C. The arrows indicate the direction of spectral changes with increasing time.

^{13}C amide I′ modes and lowering the observed frequency. In the structure proposed in Figure 2b, the arrangement of the A117 residues is unique, because none of the other residues align so closely within the strands; thus, the A117 position is the only site which produces a dipole-coupling shifted ^{13}C amide I′ band. The diminished intensity of the A117 ^{13}C band as compared to other labeled positions is also consistent with this model, because the anamolously large intensity of ^{13}C amide I′ bands in labeled β-sheets has been attributed in part to intensity "borrowing" from ^{12}C modes on adjacent strands.[14] Interestingly, the 117 position is the site of a mutation in the human prion protein (A117V) associated with the inherited human prion disease Gerstmann−Stäussler−Scheinker Syndrome and an increased tendency to form prion fibrils in vivo.[14] The importance of the 117 position may be related to the strand alignment described for the H1 peptide; the β-branched aliphatic valine residue may provide better van der Waals packing between strands than the simple methyl side chain of alanine.[14c]

We have also observed the spectrum of A117 labeled peptide in its initial (nonequilibrium) conformation. The spectrum of a freshly prepared sample also has the general features of an antiparallel β-sheet, but with bandwidths and frequencies slightly different from those of the equilibrium conformation; both the ^{12}C and the ^{13}C amide I′ bands are broader and poorly resolved. If the freshly prepared sample is left at 25 °C, without an annealing cycle, a slow shift of the ^{13}C amide I′ band toward 1592 cm^{-1} occurs over the course of several days. The reaction at 50 °C takes place in about 12 h and can be followed by IR (Figure 2c). The initially formed (kinetically favored) conformation is likely a distribution of strand alignments, including structures in which the polar N-terminal residues are included as a part of the β-sheet (Figure 2a). Without the A117 ^{13}C labeled carbonyls position uniquely aligned, the ^{13}C amide I′ band experiences inhomogeneous broadening and is shifted to higher frequency ($\sim$1602 cm^{-1}). Over time, the aggregates adopt the thermodynamically favored conformation (with the A117 positioned aligned), causing the ^{13}C amide I′ of A117 to shift to 1592 cm^{-1} (Figure 2c). The initial formation of a disordered β-sheet with later rearrangement to a more stable equilibrium conformation may be a general component of the mechanism of β-sheet formation and aggregation. Further studies to characterize this process kinetically and as a function of peptide sequence are currently underway.

The elucidation of details of the structure of the H1 aggregate in solution lends insight into the significance of residue 117 in the formation of prion protein fibrils, and the observation of the transition from a disordered sheet to a well-defined alignment gives new specific details to some of the first steps of aggregate nucleation and propagation. This description of structure and dynamics of prion aggregates in solution at early stages of aggregation begins to fill in an important gap in understanding the mechanism of β-sheet aggregation.

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Supporting Information Available: Spectra of other labeled H1 peptides and a table summarizing the complete set of spectral data (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) Peptides were synthesized using standard Fmoc chemistry on a Perseptive Biosystems Pioneer automated peptide synthesizer, purified using reverse-phase HPLC, and characterized by electrospray mass spectrometry to confirm peptide identity and the presence of the isotope label. The HPLC purified samples were exchanged with $\sim$0.05 mM DCl in D_2O at room temperature and freeze-dried.
(10) For IR samples, 1.5−2 mg of peptides were dissolved in $\sim$100 μL of 20 mM Hepes and 100 mM NaCl in D_2O and loaded into a Wilmad temperature controlled cell attached to an external Neslab RTE temperature controlling unit. Once placed in the cell, all samples were heated to 75 °C, equilibrated for 1 h, recooled to 25 °C, and equilibrated for an additional 1 h. The FTIR spectra were measured on a Bruker Vector 22 spectrometer with 4 cm^{-1} resolution averaging 256 scans.
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