Amyloids

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Infectious and Noninfectious Amyloids of the HET-s(218–289) Prion Have Different NMR Spectra**

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The prion-forming domain comprising residues 218–289 of the fungal prion HET-s has recently been shown to form amyloid fibrils at low pH in vitro which have little or no infectivity.[1] Because a molecular model for the structure of the infectious pH 7 form exists,[2] the study of the non-infectious low-pH fibrils opens an exciting possibility to address, on a molecular level, the differences that distinguish infectious from noninfectious polymorphs of the same protein. Amyloids, in general, and prions, in particular, are known to exist in different polymorphic forms, the formation of which can be controlled in vitro in part by adjusting the pH or stirring the solution.[3,4] Polymorphs can also be inheritable, a phenomenon that is intimately linked to the existence of different strains in prion diseases. Prion strains showing significantly differing biological activity have been described in yeast,[5–7] but for the HET-s prion protein of the filamentous fungus *Podospora anserina* no indications for polymorphism at physiological pH have been found.[1] This finding is reflected in the solid-state NMR spectra of the prion-forming C-terminal domain of HET-s, the fragment HET-s(218–289), for which narrow NMR linewidths for both ^{13}C and ^{15}N resonances have been found, and no indications for peak doubling were detected.[8] Furthermore, no variation of chemical shifts has been found for samples from several different preparations. For the pH 3 fibrils, whose NMR spectra are described in this communication, there is, however, evidence from electron microscopy that several polymorphs indeed coexist, all of which are different from the pH 7 form.[1]

The C-terminal fragment comprising residues 218 to 289 forms the proteinase K-resistant part of the fibrils[9] and has the sequence KIDAIVGRNSAKDIRTEERARVQLGNVVTAAALHGGIRISDQTTNSVETVVGKGESRVLIGNEYGGKGFWDN. This fragment is necessary and sufficient for prion infectivity[10] and forms infectious amyloid fibrils at pH 7 in vitro.[9] The well-resolved NMR spectra of the HET-s(218–289) pH 7 fibrils allowed for an almost complete sequence-specific assignment of the NMR resonances for the rigid parts.[11] The chemical shift information together with additional biophysical data have been used to propose a structural model with four β strands, β_1–β_4. These four strands form a β-solenoid fold with two repeating strand–turn–strand motifs (β_1–β_2 and β_3–β_4) forming two turns of the solenoid. This model is supported by recent electron microscopy data which show a mass-per-length ratio consistent with two layers of β strands per HET-s(218–289) subunit.[12]

M-HET-s(218–289)-H_6 was recombinantly expressed and purified according to a previously described procedure,[9,11] and fibrillization was carried out at pH 3, as described in detail in the Supporting Information. The pH 3 fibrils were found to be more stable than fibrils formed at pH 2, the pH at which most of the experiments in reference [1] were performed. However, the pH 2 and pH 3 fibrils behave very similarly (they have almost identical aggregation kinetics, both induce thioflavin T fluorescence, and they show the same morphology in electron micrographs), and it was confirmed that the pH 3 form is not infectious.[22]

The ^{13}C–^{13}C proton-driven spin diffusion (PDSD) spectrum of the pH 3 fibrils is shown in Figure 1 along with the corresponding spectrum of pH 7 fibrils. After their formation, the pH 3 fibrils were washed in pure water. While fibrillization at neutral pH yields the pH 7 conformational state,[1] the pH 3 form is stable at higher pH and no pH 7 fibrils could be detected in our experiments. The spectra of the pH 3 fibrils are clearly different from those of the pH 7 form, indicating a different molecular structure; the spectral resolution is somewhat lower, indicating higher disorder: The pH 3 fibrils exhibit typical linewidths between 128 Hz and 202 Hz compared to linewidths of less than 100 Hz for the pH 7 fibrils (only well-resolved peaks were analyzed). The reduced resolution made the sequential resonance assignment difficult. Nevertheless, we have been able to identify and tentatively assign 22 spin systems, each corresponding to an amino acid residue, by through-bond ^{13}C–^{13}C TOBSY spectroscopy[13,14] (Figure 2), in combination with the PDSD and HETCOR spectra (Figure 1). The 22 spin systems detected in the rigid parts of the fibril consist of 3 A, 1 D (or N), 2 E, 2 G, 1 H, 2 I, 1 K, 1 L, 1 R, 2 S, 2 T, and 4 V. The complete TOBSY spectrum and the assigned chemical shifts are given in the Supporting Information. For 16 of these spin systems, both the C^α and C^β chemical shifts were assigned and the differences in their secondary chemical shifts, $\delta_{C^\alpha}-\delta_{C^\beta}$, are shown in Figure 3. For all of the spin systems negative values were

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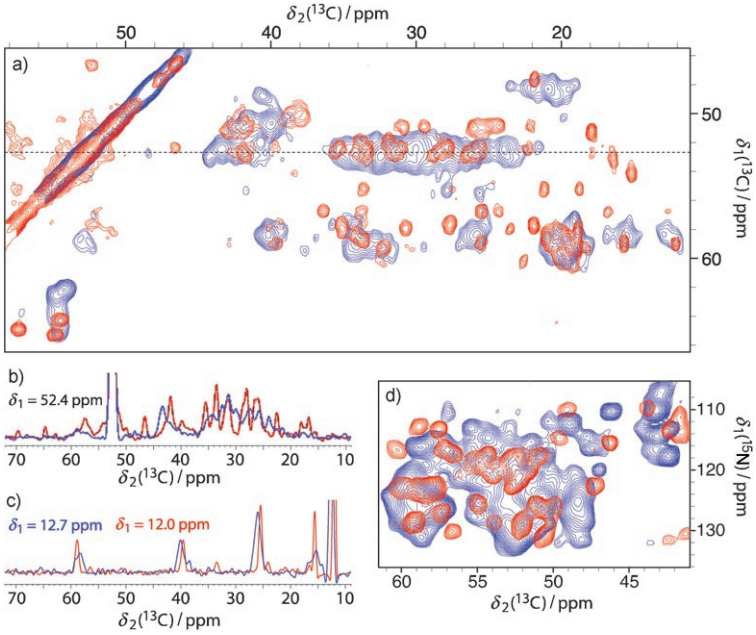

Figure 1. PDSD spectra of HET-s(218–289) fibrils formed at pH 3 (blue) and at pH 7 (red). (The complete spectra are shown in the Supporting Information.) a) Section of the aliphatic region. b,c) Slices through the C^{α} region and at an isoleucine $C^{\delta 1}$ resonance to clarify the differences in observed linewidths. d) Section of the ^{15}N–^{13}C HETCOR spectrum. All spectra were recorded with a mixing period of 50 ms and 90 kHz SPINAL-64 ^{1}H decoupling during t_1 and t_2 at a static magnetic field of 14.09 T.

To test for the presence of flexible residues in the pH 3 fibrils, ^{13}C-detected ^{1}H–^{13}C refocused INEPT and ^{1}H–^{13}C–^{13}C INEPT–TOBSY[16] experiments were performed (Figure 4). Such experiments show only flexible parts of the fibrils.[17] Using the TOBSY connectivities, we identified two histidine residues and one lysine side chain. In contrast to the pH 7 fibrils, no evidence for a flexible loop could be found.[17,18]

Based on the NMR experiments described above, we deduce that the HET-s(218–289) pH 3 fibrils consist of rigid β sheets. In contrast to the infectious pH 7 fibrils, no highly flexible parts (except the H_6 tag) could be detected. A number of additional significant differences indicate that the detailed structure of the pH 3 and pH 7 fibrils must be quite different. As seen in Figure 1, the alanine C^{α}–C^{β} region of the pH 7 fibrils, for example, consists of four strong peaks assigned to A228 (47.4, 21.6 ppm), A237 (51.1, 17.7), A247 (54.1, 14.8), and A248 (53.0, 16.1). Only A228 shows a chemical shift within the region typical for β-sheet structures. For the pH 3 fibrils, in contrast, no alanine resonance is detected outside the β-sheet region, and we suspect that several alanines contribute to the partially resolved signal (48.3, 21.3). Further obvious differences appear in the serine C^{α}–C^{β} region and for valine (e.g. V264 C^{α}–C^{β}

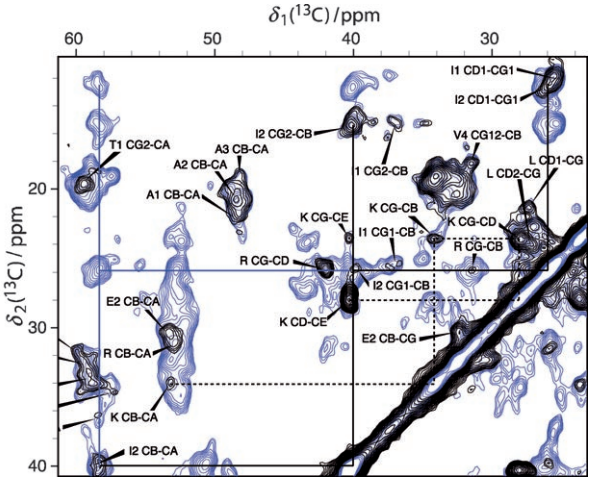

Figure 2. Sections of the ^{13}C–^{13}C TOBSY (black) and 50 ms PDSD (blue) spectra of the pH 3 fibrils. The solid black and blue lines connect cross peaks belonging to I_2 in the TOBSY and the PDSD spectrum, respectively; the dotted black line follows cross peaks of the K spin system. The assignments were obtained by analysis of the TOBSY, PDSD, and N–C HETCOR spectra; the spin systems were numbered arbitrarily. The spectra shown were recorded at 13 kHz MAS with 90 kHz SPINAL-64 ^{1}H decoupling during t_1 and t_2. The mixing times were 5 ms and 50 ms for the TOBSY and the PDSD spectra, respectively.

found, which is indicative of β-sheet structure[15] in accordance with the analysis of FTIR data for pH 2 fibrils.[1]

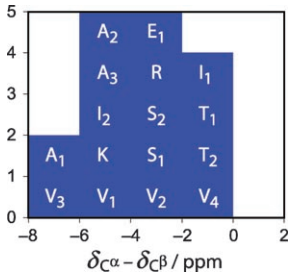

Figure 3. Histogram of observed differences between C^{α} and C^{β} secondary chemical shifts. Negative values indicate β-sheet structure.[15] The corresponding spin systems are given using the same arbitrary numbering as in Figure 2.

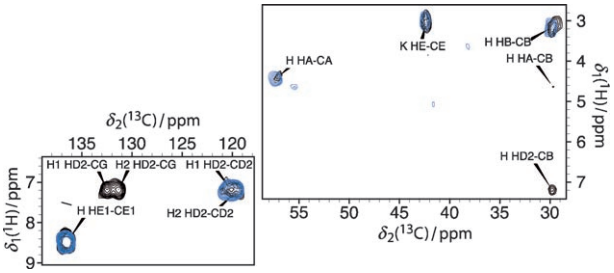

Figure 4. Aliphatic and aromatic regions of the HC-INEPT (blue) and HCC-INEPT-TOBSY (black) spectra with tentative assignments based on the TOBSY cross-peaks and random-coil chemical shifts. Spin systems are numbered arbitrarily. Both spectra were recorded at 13 kHz MAS with 70 kHz SPINAL-64 ^{1}H-decoupling during t_2. The mixing time for the TOBSY spectrum was 4 ms.

and C^{α}–C^{γ}, see the Supporting Information). Also, the overall quality of the CP/MAS spectra is different for the two types of fibrils. The increased linewidths of fibrils formed at pH 3 (Figure 1 b, c) suggest that they are not as well-ordered as the pH 7 fibrils of the same peptide. The mesoscopic structural variability as observed by electron microscopy[1] may be one source of disorder, leading to a different set of signals for each polymorph. In addition, local disorder, which broadens the lines from individual polymorphic forms, could also play a role. Linewidths similar to those for the pH 3 samples have been reported for other amyloids.[19–21]

We conclude that the pH 3 non-prion amyloids of HET-s(218–289) have a rigid part found almost exclusively in β-sheet conformation but—in contrast to the infectious pH 7 form—no flexible residues. Also, the structure of the individual HET-s(218–289) molecule embedded in the fibrils of the non-prion form appears to be quite different from the corresponding one in the prion form while the elementary fibril thickness and mass-per-length are similar.[1,12] Our results suggest that low-pH fibrils are not infectious because their structure differs substantially from that accessible at physiological pH. Also consistent with this view is the fact that low-pH fibrils are poor templates for HET-s in vitro fibrillization at pH 7.[1]

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