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Intermolecular Alignment in β2-Microglobulin Amyloid Fibrils

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Abstract: The deposition of amyloid-like fibrils, composed primarily of the 99-residue protein β2-microglobulin (β2m), is one of the characteristic symptoms of dialysis-related amyloidosis. β2m fibrils formed in vitro at low pH and low salt concentration share many properties with the disease related fibrils and have been extensively studied by a number of biochemical and biophysical methods. These fibrils contain a significant β-sheet core and have a complex cryoEM electron density profile. Here, we investigate the intrasheet arrangement of the fibrils by means of 15N-13C MAS NMR correlation spectroscopy. We utilize a fibril sample grown from a 50:50 mixture of 15N,12C- and 14N,13C-labeled β2m monomers, the latter prepared using 2-13C glycerol as the carbon source. Together with the use of ZF-TEDOR mixing, this sample allowed us to observe intermolecular 15N-13C backbone-to-backbone contacts with excellent resolution and good sensitivity. The results are consistent with a parallel, in-register arrangement of the protein subunits in the fibrils and suggest that a significant structural reorganization occurs from the native to the fibril state.

β2-Microglobulin (β2m) is a 99-residue protein that forms amyloid fibril deposits associated with dialysis-related amyloidosis (DRA).1 Under acidic conditions (pH = 2.5) and low salt concentration, the protein can also form amyloid fibrils in vitro through a nucleation-dependent mechanism.2,3 These fibrils are long, concentration, the protein can also form amyloid fibrils and have been extensively studied by a number of biochemical and biophysical methods. These fibrils contain a significant β-sheet core and have a complex cryoEM electron density profile. Here, we investigate the intrasheet arrangement of the fibrils by means of 15N-13C MAS NMR correlation spectroscopy. We utilize a fibril sample grown from a 50:50 mixture of 15N,12C- and 14N,13C-labeled β2m monomers, the latter prepared using 2-13C glycerol as the carbon source. Together with the use of ZF-TEDOR mixing, this sample allowed us to observe intermolecular 15N-13C backbone-to-backbone contacts with excellent resolution and good sensitivity. The results are consistent with a parallel, in-register arrangement of the protein subunits in the fibrils and suggest that a significant structural reorganization occurs from the native to the fibril state.

Figure 1. (a) 13C CP spectrum of mixed 2-β2m fibrils, 512 scans; (b) ZF-TEDOR spectrum obtained with τmix = 1.76 ms, 512 scans; (c) ZF-TEDOR with τmix = 18 ms, 5120 scans.
Spectrum presents excellent resolution (13C line widths of the 15N shown in Figure 2a (2- have assignments. Conversely, not all of the strong cross-peaks noted, while labels in gray denote cross-peaks that appear only in the 2- a 2D ZF-TEDOR experiment with positions of the observed cross-peaks in this mixed 2- and sufficient sensitivity after a long acquisition period, which was rise to intermolecular contacts in the mixed 2- material (2-). The majority of the cross-peaks in the mixed 2-2 spectrum correspond exactly with the positions of cross-peaks in one-bond (Figure 2a) or two-bond TEDOR spectra (data not shown) of a 2- spectrum. (b) Mixed 2-2, \( \tau_{\text{mix}} = 16 \text{ ms} \), 16 mg of sample, 9 days of experimental time. Labels correspond to intermolecular \( \text{N}_i-\text{C}_\alpha \) or \( \text{N}_i-\text{C} \alpha_{i-1} \) transfer, unless otherwise noted.

Distances of \( \sim 5.0-5.5 \text{ Å} \). The maximum bulk transfer efficiency for the \( \text{C} \alpha \) region is \( \sim 3\% \), which is better than the experimental transfer efficiencies observed for uniformly 13C labeled samples (<1% for similar distances).20

In order to obtain site-specific information regarding the origin of the 15N–13C intermolecular contacts in mixed 2-2, we recorded a 2D ZF-TEDOR experiment with \( \tau_{\text{mix}} = 16 \text{ ms} \) (Figure S3). This spectrum presents excellent resolution (13C line widths \( \sim 50 \) Hz) and sufficient sensitivity after a long acquisition period, which was facilitated by the robustness of the TEDOR sequence. Overall, the positions of the observed cross-peaks in this mixed 2-2 spectrum correspond exactly with the positions of cross-peaks in one-bond (Figure 2a) or two-bond TEDOR spectra (data not shown) of a 2-2 spectrum prepared from 100% 15N, 2-13C glycerol labeled material (2-2). The majority of the cross-peaks in the mixed 2-2 sample could be readily assigned based on known chemical shifts of long, straight 2-2 fibrils, and they correspond exclusively to intermolecular \( \text{N}_i-\text{C}_\alpha \), \( \text{N}_i-\text{C} \alpha_{i-1} \), \( \text{N}_i-\text{C}_{\alpha} \), or \( \text{N}_i-\text{C}o_i \) transfer (Figures 2b and S3). In particular, the following residues giving rise to intermolecular contacts in the mixed 2-2 sample were assigned: H31–S33, N42, G43, R45, I46, V49, H51–F62, P72, T73, and Y78–V82. While P32, S33, G43, F56, S57, K58, and F62 are part of well-ordered loops in the fibrils, the remainder of the residues represent all of the currently assigned fibril \( \beta \)-strands.

Some cross-peaks in Figure 2b (mixed 2-2) do not presently have assignments. Conversely, not all of the strong cross-peaks shown in Figure 2a (2-2) appear in the mixed 2-2 spectrum. This includes G18, G29, E44, H84, and V85 (shown in gray in Figure 2a) among others. This is most likely due to differences in local dynamics and relaxation whose effects are exacerbated at long mixing times, resulting in large variations in the cross-peak intensities.30

The data presented above suggest that long, straight 2-2 fibrils grown at pH 2.5 and low salt concentration form parallel, in-register \( \beta \)-sheets. In such a case the average distances for intermolecular \( \text{N}_i-\text{C}_\alpha \) and \( \text{N}_i-\text{C} \alpha_{i-1} \) contacts are \( \sim 5 \) and \( \sim 5.5 \text{ Å} \) respectively (Figure S4), which is consistent with the bulk ZF-TEDOR buildup (Figure S2). In order to accommodate such an arrangement, substantial reorganization of the native antiparallel \( \beta \)-sheet structure31–33 is required, indicating that the structure of the monomers within the fibrils must be highly non-native. Figure 3 highlights two clear pieces of evidence for the non-native structure of \( \beta \)-m within fibrils: first, residues involved in loops/turms in native \( \beta \)-m (Figure 3a) reorganize to form ordered \( \beta \)-strands in the fibrils (Figures 3b and S5), and second, while all \( \beta \)-strands form antiparallel \( \beta \)-sheet contacts with residues distant in sequence in native \( \beta \)-m, \( \beta \)-strands in the fibrils are parallel and in register.

The parallel arrangement of the \( \beta \)-strands in \( \beta \)-m fibrils was predicted initially by FTIR experiments34,35 and is in agreement with data obtained by site-directed spin labeling and EPR.11 The results described here verify and expand upon the latter, which indicates that spin labels attached to cysteine-substituted residues S33, S55, S61, and T73 among others give EPR spectra indicative of immobile, parallel, and in-register stacked spin labels (Figure S5). Stacks of six \( \beta \)-m monomers arranged in that manner are then required to fulfill the electron density maps obtained by cryoEM.12 The site-specific information regarding the intermolecular arrangement of \( \beta \)-m fibrils presented here provides an important step toward a full molecular model of the fibrils. Additional experiments, particularly aimed at determining the quaternary fold of the fibrils, are in progress and should shed light on how this tertiary fibril arrangement fits into such a complex cryoEM electron density profile.

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Supporting Information Available: Sample and experimental details; EM image of the fibrils; 1D ZF-TEDOR buildup; full 2D ZF-TEDOR spectrum; expected intermolecular distances in a parallel, in-register arrangement; summary of the available sequence-specific structural information for the fibrils. This material is available free of charge via the Internet at http://pubs.acs.org.

References

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