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. 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,13C- 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 fibrillar 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, straight, and unbranched in appearance (Figure S1) and share many properties with the fibrils isolated from tissues of DRA patients, including the same characteristic amide I band in FTIR spectra.4 It has been shown that the fibrils themselves, and not the prefibrillar oligomeric species formed in the lag phase of assembly, can disrupt model membranes and are toxic to cells.5 While an atomic structural model for these fibrils is not yet available, structural details emerged first through methods like limited proteolysis,6,7 hydrogen exchange,8,9 and more recently by magic angle spinning (MAS) NMR,10 electron paramagnetic resonance (EPR),11 and cryo-electron microscopy (cryoEM).12 In particular, analysis of the chemical shifts of 64 assigned residues of β₂m fibrils has shown that the protein contains a rigid fibril core with substantially more β-sheet character than the native protein.10 CryoEM maps revealed a complex picture of the fibrils, where non-native globular β₂m monomers pack in “dimer-of-dimers” building blocks that associate asymmetrically into crescent-shaped units.12 In addition, site-directed EPR spin labeling suggested that the major building block consists of six β₂m polypeptide chains, arranged in a parallel, in-register manner.11

In the experiments described here, we investigate the tertiary structure of β₂m amyloid fibrils with 15N-13C MAS NMR correlations that establish that the protein subunits in long, straight β₂m fibrils formed at pH 2.5 are arranged as parallel, in-register β-sheets.

Our experiments utilize fibrils formed from a 50:50 mixture of 15N,13C- and 14N,13C-labeled β₂m monomers, the latter half being prepared using [2-13C]-glycerol as the carbon source. This sample, referred to as “mixed 2-β₂m”, offers improved resolution in the 13C dimension22–24 (Figure 1a) as well as potential gains in experimental transfer efficiency due to the significantly reduced number of directly bonded 13C atoms.25 The absence of 13C J-couplings and the elimination of strong (intramolecular) dipolar 13N-13C couplings as a result of the mixed nature of the sample improve the efficiency of ZF-TEDOR.20,26,27 In a 100% uniformly 15N,13C-labeled β₂m sample, the experimental one-bond 15N-13C transfer efficiency after 1.76 ms of ZF-TEDOR mixing is typically ~20% of the 13C CP signal. In the mixed 2-β₂m sample, after such a short mixing time, no significant buildup of 13C polarization is observed, as shown in Figure 1b. This is due to the absence of 13C nuclei in the 15N,13C-labeled monomers, which were prepared using 13C-depleted glucose (99.9% purity) to eliminate contributions from natural abundance. In particular, signals from one-bond 15N-13C interactions are not detected. On the other hand, longer ZF-TEDOR mixing times lead to the buildup of 13C intensity, which reaches a maximum at 18 ms (Figure 1c and Figure S2) and is consistent with 15N-13C

Figure 1. (a) 13C CP spectrum of mixed 2-β₂m fibrils, 512 scans; (b) ZF-TEDOR spectrum obtained with τ_C = 1.76 ms, 512 scans; (c) ZF-TEDOR with τ_C = 18 ms, 5120 scans.

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spectrum presents excellent resolution (13C line widths shown 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 $\beta_{2m}$ fibrils grown at pH 2.5 and low salt concentration form parallel, in-register $\beta$-strands. In such a case the average distances for intermolecular $N_i-N_{i-r}$ and $N_i-N_{i-r}$ contacts are $\sim5$ and $\sim5.5$ 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 structure\(^{31-33}\) 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 $\beta_{2m}$ within fibrils: first, residues involved in loops/turns in native $\beta_{2m}$ (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 $\beta_{2m}$, $\beta$-strands in the fibrils are parallel and in register.

The parallel arrangement of the $\beta$-strands in $\beta_{2m}$ fibrils was predicted initially by FTIR experiments\(^{34,35}\) 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 $\beta_{2m}$ 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 $\beta_{2m}$ 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.

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