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The Organization of Aromatic Side Groups in an Amyloid Fibril Probed by Solid-State ^{2}H and ^{19}F NMR Spectroscopy

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At least 25 proteins and peptides are known to assemble into amyloid fibrils associated with human disease. These insoluble protein assemblies contain repeat arrays of β-strands arranged with interstrand hydrogen bonds parallel to the fibril long axis.[1] X-ray analysis of fibril microcrystals has revealed that fibril assembly can involve a complex network of interactions between amino acid side groups, which bring together layers of β-sheets arranged with water-exposed faces and water-excluded cores.[2] It has been proposed that interactions between the side groups of aromatic amino acids may contribute to fibril assembly.[3] Amyloid formation by amylin, the islet amyloid polypeptide (IAPP) implicated in type II diabetes, may involve interactions between the rings of phenylalanine at residue 23, although there is evidence that side-chain size and hydrophobicity at this position may be more important than aromaticity for fibril assembly.[4] Advances in solid-state NMR now allow detailed analysis of the molecular conformation of amyloid proteins in insoluble deposits and for probing fibril architecture.[5] Here, by examining the spatial arrangement of Phe-23 aromatic side groups in fibrils formed by an 11-residue peptide fragment of amylin ($IAPP_{20-29}$) with sequence AcHN-SNNF-GAILSS-$CONH_2$, we demonstrate the utility of ^{2}H and ^{19}F solid-state NMR measurements to refine models for the structure of amyloid fibrils.

$IAPP_{20-29}$ was synthesized with either [2,2′,3,3′,4-$^2H_5$]Phe (i.e., D_5-$IAPP_{20-29}$) or [4-^{19}F]Phe (i.e., F-$IAPP_{20-29}$) incorporated at position 23 (Figure 1). Long, straight fibrils of reproducible morphology in the absence of amorphous material were prepared using published protocols.[7] There was no detectable change in the kinetics of fibril formation, morphology, or yield, suggesting that incorporation of ^{19}F or ^{2}H results in no gross alteration in fibril structure. A ^{2}H NMR spectrum of hydrated, nonfrozen fibrils of D_5-$IAPP_{20-29}$, obtained under magic-angle spinning (MAS) conditions to improve sensitivity (Figure 1), exhibited high intensity at the outer extrema, which indicated that the vast majority of aromatic groups have limited motional freedom. Rings in exposed regions of proteins are likely to have rotational freedom, whereas buried rings are usually more constrained in their motion.[8] The impaired mobility of the aromatic rings is therefore consistent with these residues stacking between layers of adjacent β-sheets.

Constraints on the distances between the Phe rings of adjacent strands were obtained by measuring distance-dependent, intermolecular dipolar couplings between ^{19}F-labeled rings and deuterated rings. Fibrils were prepared from F-$IAPP_{20-29}$ diluted with D_5-$IAPP_{20-29}$ and intermolecular dipolar interactions between ^{19}F and ^{2}H in adjacent rings were measured using REDOR. A dipolar dephasing curve of measured peak intensity ratios (S/S_0) was compared with curves calculated stochastically for a ^{19}F-ring situated at distances r_1 and r_2 from two ^{2}H-ring centers, each ring having a random orientation (Figure 2). The simulations did not reach a unique solution because of the large number of spins and

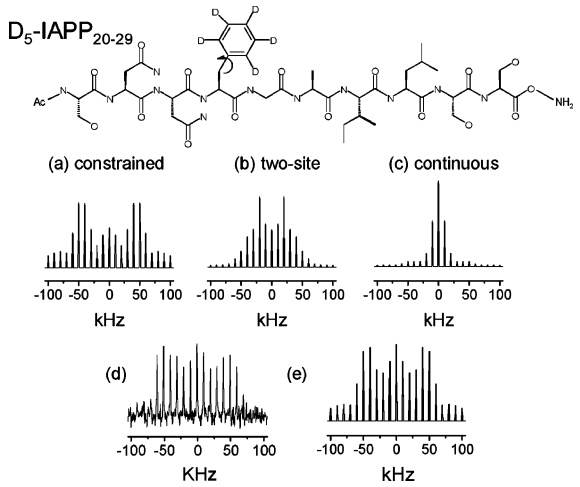

Figure 1. ^{2}H MAS NMR spectra for the $^2H_5$ (D_5)-labeled Phe-23 ring of D_5-$IAPP_{20-29}$. Simulated spectra were calculated[6] for a random dispersion of rigid peptide molecules in which the ring was fixed in space (a) or undergoing rapid two-site jumps (b) or free rotation ($k = 10^{-7}$ s^{-1}) (c) about the $C_\beta-C_\gamma$ bond denoted by the arrow. The experimental spectrum of an aqueous suspension of fibrils (d) was recorded at 4 °C to probe the dynamics of the Phe ring under nonfreezing conditions. A combination of (a), (b), and (c) in a 17:3:1 ratio produced a simulated spectrum (e) in close agreement with the experimental spectrum.

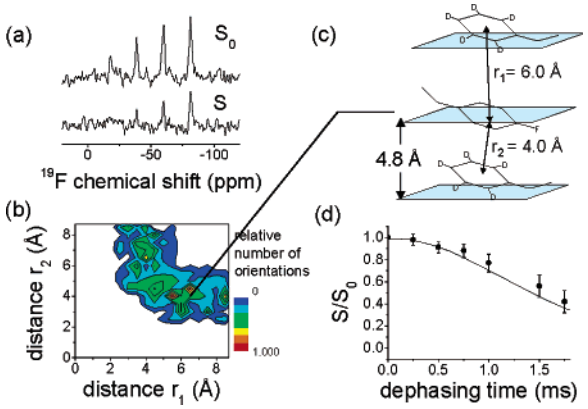

Figure 2. ^{19}F-observed ^{2}H-dephased REDOR experiment on hydrated fibrils of F-$IAPP_{20-29}$/D_5-$IAPP_{20-29}$ (1:5 molar ratio) conducted at −10 °C to reduce the possibility of dipolar scaling by residual ring dynamics. The examples of control (S_0) and ^{2}H-dephased (S) REDOR spectra shown (a), were obtained at a dephasing time of 1.5 ms. The spectra exhibit spinning sidebands separated by the MAS frequency of 10 kHz. A curve of S/S_0 versus dephasing time was compared with simulated curves calculated for 30,000 sets of random coordinates for one ^{19}F-ring separated from two ^{2}H-ring centers by distances r_1 and r_2. The colored regions of the contour plot (b) show r_1/r_2 combinations and the relative number of ring orientations that are consistent with the experimental data. One ring configuration consistent with the data is shown (c). The planes 4.8 Å apart denoting the β-strand repeat distance in the fibrils. The simulated dephasing curve for this configuration is shown (solid line) with the experimental data (d). Error bars indicate the noise level.

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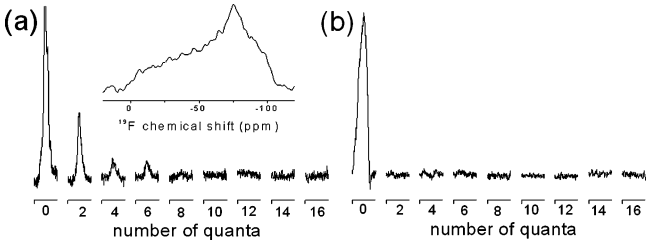

Figure 3. Static ^{19}F MQ excitation spectra at −10 °C of an aqueous suspension of F-IAPP$_{20-29}$ fibrils (a) and an aqueous solution of monomeric F-IAPP$_{20-29}$ (b) at a preparation/mixing time of 0.5 ms. Higher order (n > 8) quanta could not be detected from the fibril sample at longer mixing times, possibly because of pulse imperfections or insufficient proton decoupling. Only zero quantum coherence could be excited from the monomer solution, as expected for a nonaggregated peptide in which the ^{19}F labels lie far apart.

geometric parameters involved but did indicate that the ^{19}F-ring centers are situated <6.5 Å from one or two ^{2}H-rings, perhaps in a π-stacking arrangement. The single-ring fluorine is predicted to reduce the ring binding energy only slightly but may alter the alignment of stacked rings compared to unsubstituted Phe, possibly from an edge-to-face to a face-to-face orientation.[9] It is noted that the calculated distance range is an average over the entire fibril sample and the measured dipolar couplings could be influenced disproportionately by the existence of microdomains in which the ring spacings are unrepresentative of the bulk sample.

The long-range organization of the rings was examined qualitatively in a^{19}F multiple quantum (MQ) NMR experiment[10] on fibrils composed only of F-IAPP$_{20-29}$. The coherence orders represent the number of spins that are correlated through a dipolar-coupling network and can report on the supramolecular structure of fibrils by identifying clusters of groups or molecules.[11] Here, experiments on fibrils of F-IAPP$_{20-29}$ detected up to $n = 8$ quanta and indicated that the spin angular momenta of at least that many ^{19}F nuclei are correlated (Figure 3). Hence, rings from groups of eight or more peptide molecules lie close enough together in space to participate in a coupling network.

These methods provide a useful new constraint that can be combined with existing structural data on fibrils of IAPP$_{20-29}$ to suggest models of the fibril supramolecular structure. In previous studies, FTIR spectra of IAPP$_{20-29}$ were consistent with fibrils composed of antiparallel β-strands,[12] and further NMR constraints suggested that the strands were out of register with overhanging N- and C-terminal residues.[13] In an antiparallel β-sheet composed of in-register strands, the minimum separation of the Phe ring centers is ∼8 Å; this separation is greater still if the strands have the proposed out-of-register configuration (Figure 4, middle). Hence, the observed separation of <6.5 Å between Phe23 rings can only be attained if two or more β-sheet laminae are stacked side-by-side, with the rings facing toward the inter-sheet space within a hydrophobic core. Several such arrangements exist for in-register and out-of-register strands, and two examples are shown in Figure 4 (bottom).

More distance constraints will be needed to discriminate between different models for the organization of β-sheet structure in IAPP$_{20-29}$ fibrils. Nonetheless, the results presented demonstrate the utility of ^{2}H and ^{19}F solid-state NMR for estimating distances between aromatic groups in amyloid fibrils that can be used to refine

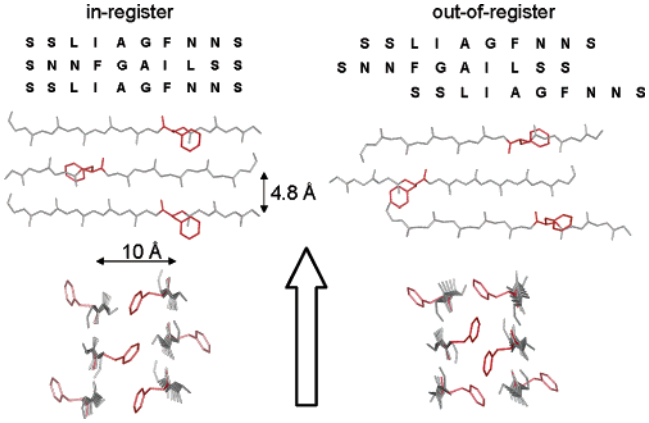

Figure 4. Models of IAPP$_{20-29}$ as antiparallel β-sheets (Phe23 side groups shown only), with strands in full register, according to FTIR measurements,[12] or out of register, according to NMR refinements.[13] The models were created without energy minimization from standard β-sheet geometry with the interstrand distance of 4.8 Å and intersheet distance of ∼9 Å taken from typical X-ray diffraction spacing for IAPP$_{20-29}$ fibrils.[7] In a single, antiparallel sheet, the Phe23 rings are separated by 8 Å or greater. When two of the sheet laminae are aligned side by side (bottom) rings from adjacent sheets can approach each other to <6.5 Å. In the examples shown, the two laminae are antiparallel to each other, with the fibril axis denoted by the arrow.

such models. This combination of nuclei is attractive because the labeled Phe precursors are readily available, there are no technical difficulties associated with spectral assignment or background signal, and ^{19}F is a sensitive nucleus with which to probe long-range distances. Probing the arrangement of aromatic groups will provide new insights into the higher-order stacking of the β-sheets within protofilaments having cross-beta structure.

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Supporting Information Available: Details of REDOR simulations and all NMR experiments. This material is available free of charge via the Internet at http://pubs.acs.org.

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