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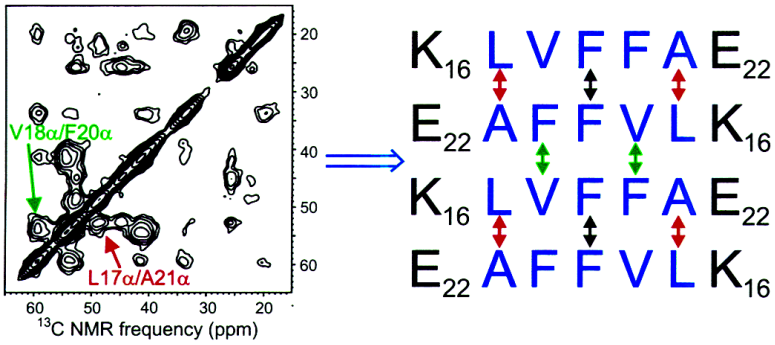

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Constraints on Supramolecular Structure in Amyloid Fibrils from Two-Dimensional Solid-State NMR Spectroscopy with Uniform Isotopic Labeling

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Solid-state nuclear magnetic resonance (NMR) methods permit detailed structural studies of biopolymers in noncrystalline solid forms. Amyloid fibrils, which are filamentous aggregates formed by a diverse class of peptides and proteins, are a particularly important target for solid-state NMR investigations because of their association with amyloid diseases,[1] because of current interest in their physicochemical properties,[2] and because detailed structural information at the molecular level has not been available from other techniques. X-ray fiber diffraction shows that the predominant supramolecular structural motifs in amyloid fibrils are extended β-sheets, arranged with a "cross-β" orientation.[3] Recent solid-state NMR studies show that the amyloid cross-β motif can contain either parallel[4,5] or antiparallel[6,7] β-sheets, depending on the amino acid sequence.

To date, studies of supramolecular structure in amyloid fibrils by solid-state NMR have exclusively employed *selective* isotopic (^{13}C and/or ^{15}N) labeling and dipolar recoupling techniques to measure specific intermolecular carbon−carbon or carbon−nitrogen distances that define the alignment of neighboring peptide chains in the β-sheets.[4−7] Detection of useful supramolecular structural constraints has therefore been a "needle-in-a-haystack" problem, since the likelihood that *selective* isotopic labels end up within the accessible distance range of the recoupling techniques (~0.6 nm) is small in a high-molecular-weight system of unknown structure. On the other hand, high-quality two-dimensional (2D) ^{13}C/^{13}C and ^{15}N/^{13}C spectra of amyloid fibrils with *uniform* ^{15}N and ^{13}C labeling of multiple amino acids have recently been obtained under magic angle spinning (MAS), permitting essentially complete resolution and assignment of isotropic chemical shifts that place strong constraints on molecular conformation.[7−9] Here we demonstrate that constraints on supramolecular structure (specifically, the intermolecular alignment in the cross-β motif) can be obtained from 2D solid-state NMR spectra of amyloid fibrils with *uniform* isotopic labeling of multiple residues. These 2D spectra also provide information about the degree of supramolecular structural order in amyloid fibrils that has not been otherwise available.

Our measurements take advantage of the proton-mediated 2D ^{13}C/^{13}C exchange technique recently introduced by Baldus and coworkers and demonstrated on alanylglyclglycine and ubiquitin.[10] In this technique, exchange of ^{13}C spin polarization between the t_1 and t_2 periods occurs in three steps: (1) ^{13}C → ^{1}H cross-polarization (CP) for time τ_{CP}; (2) ^{1}H ↔ ^{1}H spin diffusion for time τ_{SD}; (3) ^{1}H → ^{13}C CP for time τ_{CP}. Provided that τ_{CP} and τ_{SD} are sufficiently short (~200 μs), strong cross-peaks are observed only between methine or methylene ^{13}C sites with ^{1}H−^{1}H distances less than about 0.3 nm.[10]

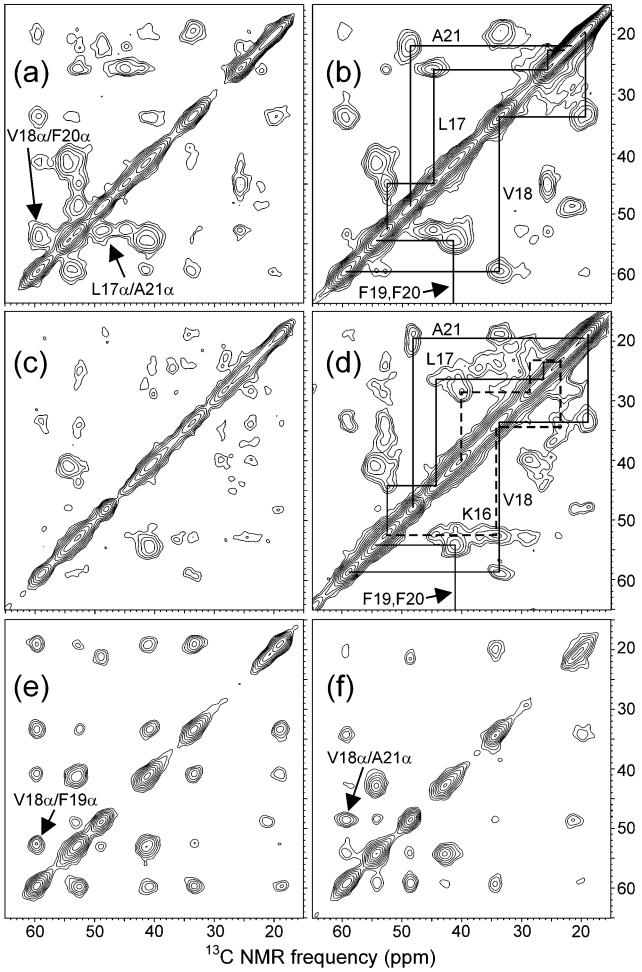

Figure 1. Aliphatic regions of 2D ^{13}C/^{13}C exchange spectra of amyloid fibrils formed by Aβ_{16-22} with uniform labeling of residues 17−21 (a,b), Aβ_{1-40} with uniform labeling of residues 16−21 (c,d), and Aβ_{11-25} with uniform labeling of residues 18−21 at pH 7.4 (e) and pH 2.5 (f). Spectra are obtained at 14.1 T and MAS spinning frequency 21.4 kHz, with proton-mediated exchange (a,c,e,f; τ_{CP} = 150 μs, τ_{SD} = 200 μs) or direct ^{13}C/^{13}C exchange (b,d; τ_{ex} = 10 ms). Assignment pathways through single-bond cross-peaks are shown (b,d). Contour levels increase by factors of 1.6 in all spectra. Strong α/α cross-peaks in a, e, and f indicate antiparallel β-sheet structures for Aβ_{16-22} and Aβ_{11-25} fibrils, but with different intermolecular alignments in each case. Weaker α/α cross-peaks (3−4 times weaker) are due to intramolecular, sequential exchange. The absence of strong α/α cross-peaks in c is consistent with an in-register, parallel β-sheet structure for Aβ_{1-40} fibrils.

Figure 1a,b shows the aliphatic regions of proton-mediated and direct (with irradiation of protons during τ_{ex}, the exchange period[11])

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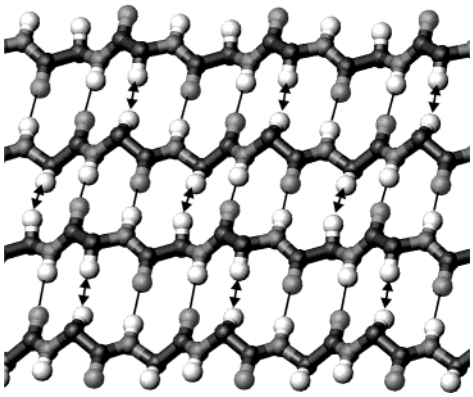

Figure 2. Molecular model of an antiparallel β-sheet, with lines indicating backbone amide−carbonyl hydrogen bonds. Double arrows indicate the short interstrand Hα−Hα distances that give rise to strong α/α cross-peaks as in Figure 1a,e,f, which constrain the intermolecular alignment in the amyloid cross-β motif (figure created with MOLMOL[13]).

2D $^{13}C/^{13}C$ exchange spectra of amyloid fibrils formed by residues 16−22 of the Alzheimer's β-amyloid peptide[7] ($A\beta_{16-22}$, sequence Ac−KLVFFAE-NH_2), with uniform labeling of L17 through A21. The two spectra are markedly different. In particular, the proton-mediated exchange spectrum shows strong cross-peaks between α-carbons of V18 and F20 and between α-carbons of L17 and A21. Given the known β-strand conformation of $A\beta_{16-22}$[7] and the lack of V18α/A21α and F19α/A21α cross-peaks, the strong α/α cross-peaks are necessarily intermolecular. The direct exchange spectrum shows only weak L17α/V18α and V18α/F19α cross-peaks, which arise from intramolecular $^{13}C-^{13}C$ couplings. (Intermolecular α/α cross-peaks are also unidentifiable in direct exchange spectra with $\tau_{ex} = 100$ and 200 ms.)

Figure 2 shows that the shortest distances between α-protons in an antiparallel β-sheet (∼0.21 nm) occur between interstrand residue pairs that are aligned (but not hydrogen bonded). Thus, the spectrum in Figure 1a indicates that $A\beta_{16-22}$ fibrils contain antiparallel β-sheets in which residue 16+k aligns with residue 22−k. This registry agrees with earlier results for selectively labeled $A\beta_{16-22}$ fibrils.[7a]

Under optimized CP conditions, 30 ± 5% of the methine and methylene ^{13}C signal is preserved in a proton-mediated exchange experiment. About 30% of this signal goes to structurally significant cross-peaks at $\tau_{SD} = 200$ μs. Sensitivity is thus comparable to that of a direct exchange experiment (aliphatic cross-peak volumes roughly 10% of diagonal volumes in Figure 1b).

Figure 1c,d shows proton-mediated and direct 2D $^{13}C/^{13}C$ exchange spectra of amyloid fibrils formed by the full-length, 40-residue β-amyloid peptide ($A\beta_{1-40}$), with uniform labeling of K16 through A21.[8] Only weak α/α cross-peaks are observed in Figure 1c, attributable to intramolecular spin diffusion between sequential α-protons (∼0.45 nm distance in a β-strand). Figure 1c is consistent with the parallel β-sheet structure indicated by earlier solid-state NMR data on selectively labeled samples[5] and contained in our recent model for the $A\beta_{1-40}$ fibril structure.[8] Interstrand distances between α-protons in parallel β-sheets are greater than 0.45 nm, precluding strong intermolecular α/α cross-peaks.

Figure 1e,f shows proton-mediated exchange spectra of fibrils formed by residues 11−25 of full-length β-amyloid ($A\beta_{11-25}$, sequence EVHHQKLVFFAEDVG), with uniform labeling of V18 through A21, prepared at pH 7.4 and 2.5, respectively. Isotropic ^{13}C chemical shifts indicate a β-strand conformation for the labeled segment at both pH values. Figure 1e shows strong V18α/F19α cross-peaks, while Figure 1f shows strong V18α/A21α cross-peaks. These spectra indicate an antiparallel β-sheet structure in $A\beta_{11-25}$ fibrils, with alignment of residues 16+k and 21−k at pH 7.4 and alignment of residues 16+k and 23−k at pH 2.5. These data are inconsistent with a recent structural model for $A\beta_{11-25}$ fibrils in which residues 17−20 form a type I β-turn.[12]

The striking differences among proton-mediated 2D exchange spectra in Figure 1 show that the supramolecular structure in amyloid fibrils is not uniquely determined by the sequence, even at the level of 15-residue segments. Multiple side chain−side chain interactions dictate the details of the intermolecular alignment. The absence of detectable 18α/A21α cross-peaks (for example) in Figure 1a,e places an upper bound on "misalignment" defects that might give rise to these cross-peaks (<10% and <4% defect concentrations in $A\beta_{16-22}$ and pH 7.4 $A\beta_{11-25}$ fibrils, respectively). Thus, the intermolecular alignment appears both highly sequence-dependent and highly ordered. The fibril growth process may include an annealing step that maintains a low level of misalignment defects as peptide molecules add to the nascent cross-β motif.

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Supporting Information Available: Pulse sequence diagrams; acquisition parameters and full 2D spectra for Figure 1; sample preparation conditions; 1D spectra of $A\beta_{16-22}$ and $A\beta_{1-40}$ fibrils quantifying ^{13}C NMR signal losses due to double τ_{CP} periods; plot of cross-peak volumes vs τ_{SD} for $A\beta_{16-22}$ fibrils (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Sipe, J. D. *Annu. Rev. Biochem.* **1992**, *61*, 947−975.

(2) (a) MacPhee, C. E.; Dobson, C. M. *J. Am. Chem. Soc.* **2000**, *122*, 12707−12713. (b) Harper, J. D.; Wong, S. S.; Lieber, C. M.; Lansbury, P. T. *Biochemistry* **1999**, *38*, 8972−8980. (c) Lomakin, A.; Teplow, D. B.; Kirschner, D. A.; Benedek, G. B. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 7942−7947.

(3) (a) Sunde, M.; Blake, C. C. F. *Q. Rev. Biophys.* **1998**, *31*, 1−39. (b) Malinchik, S. B.; Inouye, H.; Szumowski, K. E.; Kirschner, D. A. *Biophys. J.* **1998**, *74*, 537−545.

(4) (a) Benzinger, T. L. S.; Gregory, D. M.; Burkoth, T. S.; Miller-Auer, H.; Lynn, D. G.; Botto, R. E.; Meredith, S. C. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 13407−13412. (b) Benzinger, T. L. S.; Gregory, D. M.; Burkoth, T. S.; Miller-Auer, H.; Lynn, D. G.; Botto, R. E.; Meredith, S. C. *Biochemistry* **2000**, *39*, 3491−3499. (c) Burkoth, T. S.; Benzinger, T. L. S.; Urban, V.; Morgan, D. M.; Gregory, D. M.; Thiyagarajan, P.; Botto, R. E.; Meredith, S. C., Lynn, D. G. *J. Am. Chem. Soc.* **2000**, *122*, 7883−7889. (d) Antzutkin, O. N.; Leapman, R. D.; Balbach, J. J.; Tycko, R. *Biochemistry* **2002**, *41*, 15436−15450.

(5) (a) Antzutkin, O. N.; Balbach, J. J.; Leapman, R. D.; Rizzo, N. W.; Reed, J.; Tycko, R. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 13045−13050. (b) Balbach, J. J.; Petkova, A. T.; Oyler, N. A.; Antzutkin, O. N.; Gordon, D. G.; Meredith, S. C.; Tycko, R. *Biophys. J.* **2002**, *83*, 1205−1216.

(6) Lansbury, P. T.; Costa, P. R.; Griffiths, J. M.; Simon, E. J.; Auger, M.; Halverson, K. J.; Kocisko, D. A.; Hendsch, Z. S.; Ashburn, T. T.; Spencer, R. G. S.; Tidor, B.; Griffin, R. G. *Nat. Struct. Biol.* **1995**, *2*, 990−998.

(7) (a) Balbach, J. J.; Ishii, Y.; Antzutkin, O. N.; Leapman, R. D.; Rizzo, N. W.; Dyda, F.; Reed, J.; Tycko, R. *Biochemistry* **2000**, *39*, 13748−13759. (b) Ishii, Y. *J. Chem. Phys.* **2001**, *114*, 8473−8483.

(8) Petkova, A. T.; Ishii, Y.; Balbach, J. J.; Antzutkin, O. N.; Leapman, R. D.; Delaglio, F.; Tycko, R. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 16742−16747.

(9) Jaroniec, C. P.; MacPhee, C. E.; Astrof, N. S.; Dobson, C. M.; Griffin, R. G. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 16748−16753.

(10) Lange, A.; Luca, S.; Baldus, M. *J. Am. Chem. Soc.* **2002**, *124*, 9704−9705.

(11) Takegoshi, K.; Nakamura, S.; Terao, T. *Chem. Phys. Lett.* **2001**, *344*, 631−637.

(12) Serpell, L. C.; Blake, C. C. F.; Fraser, P. E. *Biochemistry* **2000**, *39*, 13269−13275.

(13) Koradi, R.; Billeter, M.; Wuthrich, K. *J. Mol. Graph.* **1996**, *14*, 51−55.

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