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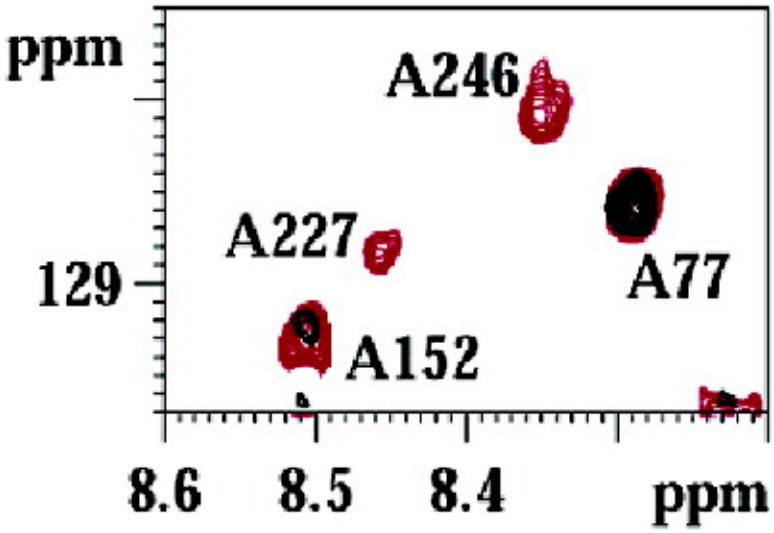

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High-Resolution Magic Angle Spinning NMR of the Neuronal Tau Protein Integrated in Alzheimer's-Like Paired Helical Fragments

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The intraneuronal accumulation of aggregates of the microtubule binding tau protein is together with the extracellular occurrence of β-amyloid plaques the main molecular hallmark of Alzheimer's disease (AD).[1] The in vivo origin of the aggregation process is not precisely known, although Aβ accumulation might trigger caspase-cleavage of tau, leading to hyperphosphorylation in the evolution of AD tangle pathology.[2] Importantly, in vivo aggregated tau is mostly found in its hyperphosphorylated form.[3] In vitro, however, the addition of polyanions such as heparin,[4] heparan sulfate, arachidonic acid,[5] or RNA[6] can aggregate tau into filaments that appear as straight or paired helical filaments (PHFs) under electron microscopy similar to those isolated from diseased brain tissue.[7]

Contrary to the case of the β-amyloid peptide, where a wealth of solid-state NMR data has led to detailed description of the fibers,[8] structural data on tau filaments are scarce. Early on, Pronase treatment and antibody mapping has led to a description in terms of a rigid core and more flexible projection domain,[9] although even here, a precise definition of both domains was not possible. Further structural studies using electron diffraction[10] on both isolated and in vitro formed filaments have led to a description in terms of cross-β structure for the core region, although recent spectroscopic evidence for the presence of α-helix in the fibers isolated post mortem from brain has equally been presented.[11]

Solid-state NMR on the amyloid fibers usually starts with a cross polarization step, where the dipolar interaction allows an effective magnetization transfer between protons and carbon in the rigid fiber.[8] The HRMAS NMR study suggesting a residual degree of motional freedom of the Aβ(1−28) peptide integrated into amyloid fibers therefore came as a surprise.[12] All resonances of the peptide were observed with comparable intensity, and liquid-state TOCSY and NOESY experiments led not only to the full assignment of the peptide but also to a description in terms of a parallel in-register arrangement for the fibrils. However, soluble peptide in exchange with the insoluble form might have been at the origin of the NMR signals, reconciling the HRMAS data with the solid nature of the fibers.[13]

Our recent efforts toward the NMR assignment of the full-length Tau441 protein have confirmed the description of the isolated protein in solution as a largely random coil polymer, with a mere 1 ppm dispersion for the amide proton resonances.[14] To exploit this partial assignment for a HRMAS NMR analysis of the tau filaments, we first formed PHFs by incubation of ^{15}N-labeled Tau441 with heparin at 37 °C. After 24 h of incubation, thioflavin S fluorescence had significantly increased,[15] and electron microscopy indicated the presence of bona fide PHFs (Figure 1). Gel filtration on this sample or UV dosage of protein in the supernatant after 30 min centrifugation at 100000*g* revealed the quasi-absence (<10%) of free tau. The solution containing the fibers was introduced in a plastic tip preinserted in the 4 mm HRMAS rotor, allowing the transfer of the majority of fibers into the rotor by centrifugation for 5 min at 10000*g*.

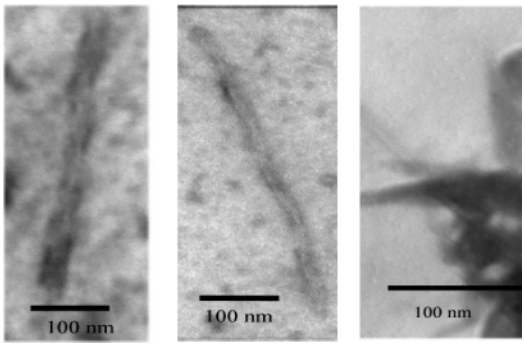

Figure 1. Electron microscopy of the PHFs before spinning (left), after prolonged spinning at 1400 Hz (middle) and 6 kHz (right).

HRMAS NMR reduces the line broadening stemming from second rank tensorial interactions caused by magnetic susceptibility effects or residual dipolar or chemical shift anisotropy interactions, at the condition that the spinning speed is larger than the static line width.[16] Under nonspinning conditions, the amide protons appear as a broad band confined to 2 ppm around their spectral position in the free protein, reducing the required spinning speed with an operating frequency of 600 MHz to 1 kHz. Whereas larger spinning speeds are commonly used in HRMAS NMR, we first investigated that spinning would not change the structure of the fibers. Using heparin as the PHF-forming agent, we examined the fibers before or after spinning for a prolonged period at 1400 Hz or 6 kHz (Figure 1). After spinning at the lowest spinning speed, we still observed numerous individual fibers, although they appeared somewhat less fuzzy than before the spinning period. When spinning for 16 h at 6 kHz, however, we mostly observed larger aggregates resembling the tangles isolated from brain,[7] with only a few narrow filaments. Because the lowest spinning speed did allow the observation of individual fibers, we choose this speed to record a first HSQC spectrum. The speed of 1400 Hz discards the water spinning sidebands from the spectral zone of interest (Figure 2) and limits the temperature increase in the rotor.

The 1D ^{1}H−^{15}N HSQC spectrum of soluble tau and PHF-tau showed a reduced intensity for the latter, although some intensity for the amide resonances of the latter remained visible. A 2D spectrum was therefore recorded on this same sample, and compared with the spectrum of tau under the same conditions of MAS (Figure 2). Interestingly, the fiber spectrum does still show many resonances

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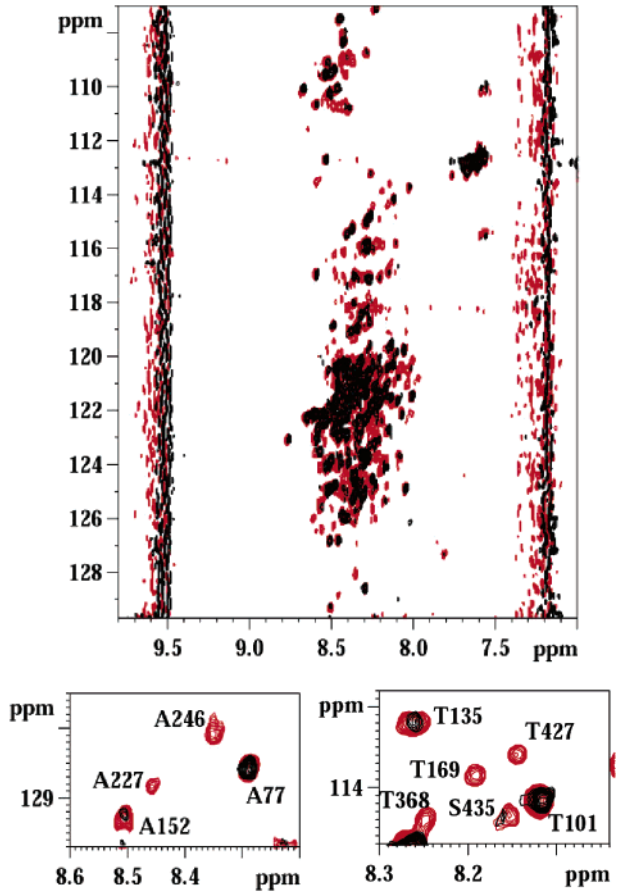

Figure 2. HRMAS $^{1}H-^{15}N$ HSQC spectra of soluble (red) tau or tau aggregated into PHFs by heparin (black). Spinning sidebands (top) are due to the water resonance. The annotated regions (bottom) illustrate the three categories of peaks that can be distinguished (see text).

that coincide with those of soluble tau, whereas some of the resonances have completely disappeared without their re-appearance at another chemical shift.

When we map our partial assignment on both HRMAS spectra, we note that most resonances in the N-terminal region maintain close to their full intensity even when tau is integrated into mature PHFs. Examples of those peaks shown in Figure 2 are Ala77 and Thr101. We propose that this N-terminal stretch of roughly 100 amino acids corresponds to the true "projection domain" that is not integrated into the fiber core. Residues fully integrated into the core region completely loose their intensity because of the solid-state character of this region. This core extends from Thr169 almost completely to the C-terminal region, with no detectable intensity left even for the Thr427 and Ser435 resonances (Figure 2). Finally, an intermediate category of amino acids is characterized by a resonance at the same frequency as in the freely soluble tau, but of decreased intensity. Examples are the Thr135 and Ala152 resonances in the N-terminal part of tau, but equally the extreme C-terminal Leu441 that resonates as an isolated peak at (7.82, 127.3) ppm (Figure 2). Similar peaks of reduced intensity have been observed in the case of HRMAS NMR spectra of organic moieties tethered to the rigid cross-linked polystyrene backbone of solid-phase synthesis resins, and have been interpreted in terms of reduced mobility for those chemical functions that are close to the rigid part.[17]

Because electron microscopy had indicated that spinning of the fibers at higher speed leads to bundling reminiscent of the PHF tangles observed in vivo,[7] we equally recorded HRMAS spectra of the fibers at 6 kHz. Signal intensity generally increased, indicating a better averaging of the different line broadening mechanisms. However, when we compared in detail the spectra of soluble tau and PHF-tau recorded at 6 kHz spinning speed, we obtained the same intensity distribution as before, suggesting that the projection domains do not participate in the bundling process.

In summary, our NMR results thus suggest a N-terminal 100-amino acid long fully flexible projection domain, a rigid core that extends from Thr169 almost up to the extreme C-terminus of the protein, separated by a 50-amino acid long zone of reduced mobility. Even when the individual PHFs associate in tangles, mimicked here by the rotation at higher speed, the same structural organization remains valid. Significantly, not only the microtubule binding repeats, but equally the complete proline-rich regions are integrated rigidly into the core. Phosphorylation events[3] therefore must have happened before the aggregation, as the Ser/Thr sites involved should no longer be accessible to the respective kinases, and the same is probably true for the caspase cleavage that would precede the hyperphosphorylation.[2] Beyond the first detailed definition of the structure of tau filaments, our results equally pave the way to understand the molecular action of β-sheet breakers recently proposed to interfere with PHF formation or even dissolve preformed PHFs.[18]

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Supporting Information Available: Experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Buee, L.; Bussiere, T.; Buee-Scherrer, V.; Delacourte, A.; Hof, P. R. *Brain Res. Rev.* **2000**, *33*, 95−130.
(2) Rissman, R.; Poon, W. W.; Blurton, M.; Oddo, S.; Torp, R.; Vitek, M.; LaFerla, F.; Rohn, T. T.; Cotman, C. *J. Clin. Invest.* **2004**, *114*, 121−130.
(3) Hasegawa, M.; Morishima-Kawashima, M.; Takio, K.; Suzuki, M.; Titani, K.; Ihara, Y. *J. Biol. Chem.* **1992**, *267*, 17047−17054.
(4) Goedert, M.; Jakes, R.; Spillantini, M. G.; Hasegawa, M.; Smith, M. J.; Crowther, R. A. *Nature* **1996**, *383*, 550−553.
(5) Wilson, D. M.; Binder, L. I. *Am. J. Pathol.* **1997**, *150*, 2181−2195.
(6) Gamblin, T.; Berry, R.; Binder, L. *Biochemistry* **2003**, *42*, 15009−150017.
(7) Kidd, M. *Nature* **1963**, *197*, 192−193.
(8) Tycko, R. *Curr. Opin. Struct. Biol.* **2004**, *14*, 96−103.
(9) Wischik, C. M.; Novak, M.; Edwards, P. C.; Klug, A.; Tichelaar, W.; Crowther, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *85*, 4884−4888.
(10) Berriman, J.; Serpell, L. C.; Oberg, K. A.; Fink, A. L.; Goedert, M.; Crowther, R. A. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 9034−9038.
(11) Sadqi, M.; Hernandez, F.; Pan, U.; Perez, M.; Schaeberle, M. D.; Avila, J.; Munoz, V. *Biochemistry* **2002**, *41*, 7150−7155.
(12) Mikros, E.; Benaki, D.; Humpfer, E.; Spraul, M.; Loukas, S.; Stassinopoulou, C. I.; Pelecanou, M. *Angew Chem., Int. Ed.* **2001**, *40*, 3603−3605.
(13) Narayanan, S.; Reif, B. *Biochemistry* **2005**, *44*, 1444−1452.
(14) Smet, C.; Leroy, A.; Sillen, A.; Wieruszeski, J. M.; Landrieu, I.; Lippens, G. *ChemBioChem*. **2004**, *5*, 1639−1646.
(15) Friedhoff, P.; Schneider, A.; Mandelkow, E. M.; Mandelkow, E. *Biochemistry* **1998**, *37*, 10223−10230.
(16) (a) Andrew, E. R.; Bradbury, A.; Eades, R. G. *Nature* **1958**, *182*, 1659. (b) Lowe, J. J. *Phys. Rev. Lett.* **1959**, *2*, 85. (c) Lippens, G.; Bourdonneau, M.; Dhalluin, C.; Warrass, R.; Richert, T.; Seetharaman, C.; Boutillon, C.; Piotto, M. *Curr. Org. Chem.* **1999**, *3*, 147−169.
(17) Pop, I.; Dhalluin, C.; Depréz, B.; Melnyk, P.; Lippens, G.; Tartar, A. *Tetrahedron* **1996**, *52*, 12209−12222.
(18) Pickhardt, M.; Gazova, Z.; von Bergen, M.; Khlistunova, I.; Wang, Y.; Hascher, A.; Mandelkow, E. M.; Biernat, J.; Mandelkow, E. *J. Biol. Chem.* **2005**, *280*, 3628−3635.

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