論文 / 著書情報
Article / Book Information

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論 文 要 旨

THESIS SUMMARY

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要旨（英文 800 語程度）
Thesis Summary（approx.800 English Words ）

Solid-state Nuclear Magnetic Resonance (SSNMR) spectroscopy is a powerful analytical technique in structural biology, particularly for investigating insoluble biomolecules such as amyloid fibrils and membrane-bound proteins. With recent advancements in instrumentation, modern SSNMR has stepped into an era of ultra-fast magic angle spinning (UFMAS), in which the spinning frequency surpasses 60 kHz. Although much higher sensitivity and resolution could be achieved by UFMAS, the faster spinning makes various traditional SSNMR techniques developed for slow to moderate spinning frequencies ineffective. Thus, it is crucial to develop new methodologies suitable for biomolecular SSNMR under UFMAS.

This dissertation thesis focuses on development and application of novel SSNMR methods to facilitate structural characterization of biomolecules under UFMAS condition. Two research projects performed on biologically significant molecules using SSNMR under UFMAS as the primary analytical tool are presented here.

The first project represents the characterization of nano- to pico-molar levels of brain-derived and synthetic 42-residue Aβ (Aβ42) fibrils by ^{1}H-detected SSNMR under UFMAS. By taking the advantage of the high sensitivity of this technique, we first demonstrate its applicability for the high-throughput screening of trace amounts of selectively ^{13}C- and ^{15}N-labeld Aβ42 fibril prepared with 0.01% of Alzheimer's disease (AD) patient-derived amyloid (~4 pmol) as a seed. A comparison of $^{13}C_{\alpha}/^{1}H_{\alpha}$ correlation 2D spectra reveals marked structural differences between AD-derived Aβ42 fibril and its synthetic counter-part (~40 nmol) in less than 10 min. This result suggests the feasibility of assessing the fibril structure from ~1 pmol of brain amyloid seed in ~2.5 h. More precise analysis using 3D SSNMR indicates that this brain-derived Aβ42 fibril sample may be comprised of up to 3–4 different conformers, and each of these conformers may adopt a different structural arrangement from the previously reported Aβ42 and Aβ40 fibrils. We also present the first structural characterization of trace amounts (~42 nmol) of synthetic fully-protonated Aβ42 fibril by a combined analysis of ^{1}H indirect detected 3D and 4D SSNMR experiments under UFMAS. A total experimental time was within 12.7 days. With semi-automated and manual signal assignment procedures, site-specific resonance assignment was completed. The assigned resonances suggest that this Aβ42 fibril exhibits a novel polymorph structure. This work provides insights into the effectiveness of ^{1}H-detected SSNMR for the characterization of trace amounts of amyloid fibrils.

The second project illustrates the recent progress of high-field SSNMR experiments for small organic molecules and biomolecules using ^{13}C cross-polarization magic angle spinning (CPMAS) under UFMAS at a spinning frequency of 80–100 kHz. First, we discuss the major differences between a modern low-power radio-frequency (RF) scheme using UFMAS in an ultra-high field, and a traditional CPMAS scheme using a moderate sample spinning in a lower field. Our results for a uniformly ^{13}C- and ^{15}N-labeled *L*-Ala sample indicates 12-fold higher mass-sensitivity for the modern low-power RF scheme compared to the traditional CPMAS. We also present the applicability of WALTZ-16 decoupling scheme as an alternative ^{1}H-decoupling sequence for SSNMR under UFMAS. A comparison of different ^{1}H-decoupling schemes in CPMAS at a spinning frequency of 100 kHz for a uniformly ^{13}C- and ^{15}N-labeled *L*-Ala displays a superior performance of low-power WALTZ-16 ^{1}H-decoupling sequence over traditional low-power decoupling schemes designed for SSNMR, in a range of RF-field strengths of 5–30 kHz. We further confirmed the excellent ^{1}H-decoupling performance of WALTZ-16 on Lys-reverse ^{13}C- and ^{15}N-labeled GB1 microcrystalline protein at 80 kHz MAS. To the best of our knowledge, this was the first demonstration of efficient composite-pulse ^{1}H-decoupling for rigid solids.