Solid-State NMR for Molecular Science

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Education
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In order to elucidate functions of molecules, characterization of the molecule is the first step. There are varieties of important molecules, which are insoluble to any solvents and functional at amorphous state. Solid-state NMR enables to obtain variety of information at atomic resolution without damages of molecules and significant restrictions. Thus solid-state NMR is one of the essential tools for the characterizations of those molecules.

We have been working on methodology and hardware developments of solid-state NMR and their applications for structural biology and material science. We study characterization of membrane proteins and peptides, organic materials, natural products and synthetic polymers. Characterizations of those molecules based on solid-state NMR are under investigations through collaborations with several research groups.

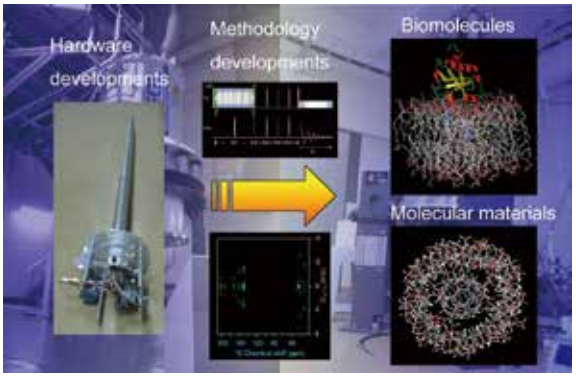

Figure 1. Outline of our studies.

Selected Publications

- N. Uekama, T. Aoki, T. Maruoka, S. Kurisu, A. Hatakeyama, S. Yamaguchi, M. Okada, H. Yagisawa, K. Nishimura and S. Tuzi, "Influence of Membrane Curvature on the Structure of the Membrane-Associated Pleckstrin Homology Domain of Phospholipase C-δ1," *Biochim. Biophys. Acta, Biomembr.* **1788**, 2575–2583 (2009).
- T. Iijima and K. Nishimura, "^{2}H Quadrupolar Carr-Purcell-Meiboom-Gill NMR for Paramagnetic Solids," *Chem. Phys. Lett.* **514**, 181–186 (2011).
- K. Yazawa, F. Suzuki, Y. Nishiyama, T. Ohata, A. Aoki, K. Nishimura, H. Kaji and T. Asakura, "Determination of Accurate ^{1}H Positions of Alanine Tripeptide with Anti-Parallel and Parallel β-Sheet Structures by High Resolution ^{1}H Solid State NMR and GIPAW Chemical Shift Calculation," *Chem. Commun.* **48**, 11199–11201 (2012).
- M. Tanio and K. Nishimura, "Intramolecular Allosteric Interaction in the Phospholipase C-δ1 Pleckstrin Homology Domain," *Biochim. Biophys. Acta, Proteins Proteomics* **1834**, 1034–1043 (2013).
- M. Yagi-Utsumi, K. Kato and K. Nishimura, "Membrane-Induced Dichotomous Conformation of Amyloid β with the Disordered N-Terminal Segment Followed by the Stable C-Terminal β Structure," *PLoS One* **11**, 0146405 (10 pages) (2016).
- N. Ousaka, F. Mamiya, Y. Iwata, K. Nishimura and E. Yashima, "'Helix-in-Helix' Superstructure Formation through Encapsulation of Fullerene-Bound Helical Peptides within a Helical Poly(methyl methacrylate) Cavity," *Angew. Chem., Int. Ed.* **56**, 791–795 (2017).

1. Characterization of Supramolecular Structure Based on Solid-State NMR[1)]

We have been collaborated with Prof. Eiji Yashima group in Nagoya university during a couple of years for the characterization of supramolecules developed in their research group. They have successfully reported that syndiotactic poly methyl methacrylate (st-PMMA) can hold into a preferred handed-helical conformation with an inner cavity in toluene in the presence of an optically active alcohol or amine accompanied by gelation. They have been explored to develop a strategy to encapsulate a helical peptide attached to C_{60} into the helical cavity of st-PMMA.

In this study, we have attempted to reveal the formation of inclusion complex composed of those molecules using solid-state NMR. Based on ^{13}C cross polarization magic angle spinning (CPMAS) and two-dimensional (2D) ^{1}H-^{13}C heteronuclear correlation (HETCOR) spectra at short contact time (CT) for st-PMMA, peptide-C_{60} and their complex, ^{1}H and ^{13}C signals were successfully assigned. Then 2D ^{1}H-^{13}C HETCOR spectra were acquired with various appropriate long CTs to obtain long distance correlations. With use of Lee-Goldburg (LG) ^{1}H homonuclear dipolar decoupled CPMAS at CT of 2.0 ms, ^{1}H-^{13}C correlations up to 4 Å apart may be detected.

Inter- and intra-molecular correlation peaks were observed in 2D ^{1}H-^{13}C HETCOR spectrum for peptide-C_{60} at CT of 2.0 ms between ^{1}H signals of peptide moieties and ^{13}C signals of C_{60}-moieties, suggesting random orientation of peptide-C_{60} in sample as shown in Figure 2(b). In contrast, apparent intermolecular correlation peaks between ^{1}H signals of methylene and methoxy groups of st-PMMA and ^{13}C signals of the C_{60} moieties were successfully observed in 2D ^{1}H-^{13}C HETCOR spectrum for complex at same CT as shown in Figure 2(c). On the other hand, the methylene and the methoxy groups in helical st-PMMA locate inside of helical cavity, in contrast to the methyl group locating out side of helical cavity of st-PMMA. Those experimental evidences are consistent when peptide-C_{60} is encapsulated into helical cavity of st-PMMA. Therefore, based on those analyses, formation of inclusion complex composed of those molecules was clearly proved.

Currently, we are also collaborating with several other research groups for characterizations of natural products, newly designed synthetic polymers, and *etc*. based on solid-state NMR.

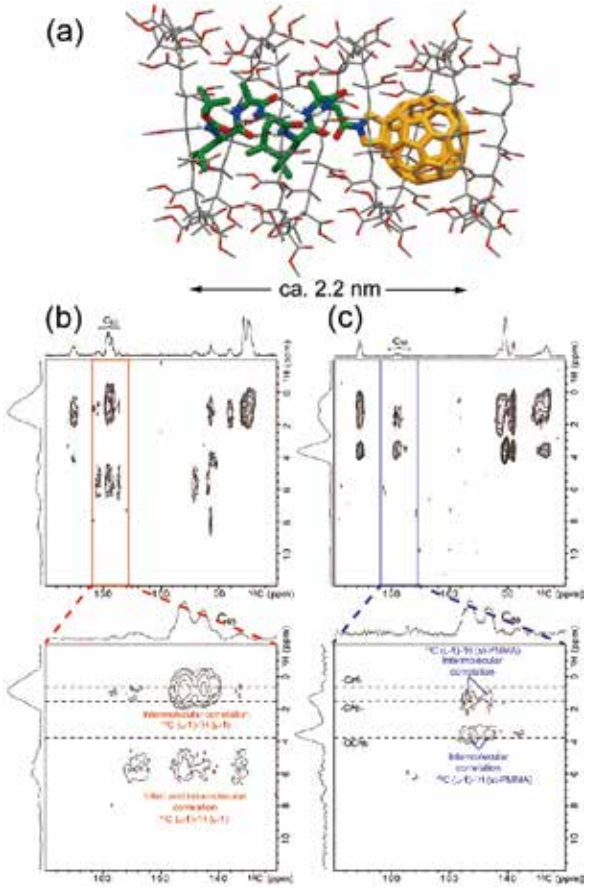

Figure 2. (a) Energy minimized supramolecular structure of st-PMMA/peptide-C_{60} inclusion complex. 2D solid-state NMR spectra of ^{1}H-^{13}C-heteronuclear correlation with LG-CPMAS at CT of 2.0 ms for (b) peptide-C_{60} and (c) complex, respectively.

2. Structural Characterization of Amyloid Peptide Oligomer Promoted on Lipid Bilayers Using Solid-State NMR

Amyloid β (Aβ) peptides exhibit random structures in solution, however after incubation, those conform insoluble amyloid fibrils, which are found in senile plaque as hallmark of Alzheimer's disease. Although, their structures have been characterized precisely, molecular mechanism of formation of the amyloid fibrils in human brain has not been clarified. Accumulated evidences strongly suggest that an initial stage of aggregation may be promoted on surface of neuronal membrane, and ganglioside GM1 specifically interacting with Aβ may play important roles for the binding of Aβ to the surface of neuronal membrane. We have successfully determined oligomeric structure of Aβ(1-40) bound to the lipid bilayers consisting of neutral lipid of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) using solid-state NMR as reported in last year.[2)]

In current study, to clarify the contribution of GM1 in fibrillation process, we have been attempted to characterize oligomeric structure of Aβ(1-40) bound to lipid bilayers consisting of GM1 and DMPC. Sample preparation procedures were optimized to enhance spectral sensitivity and ^{13}C homo- and ^{13}C-^{15}N hetero-nuclear correlation spectra were acquired for sequential signal assignments. Currently, data is under investigation.

References

1) N. Ousaka, F. Mamiya, Y. Iwata, K. Nishimura and E. Yashima, *Angew. Chem., Int. Ed.* **56**, 791–795 (2017).
2) M. Yagi-Utsumi, K. Kato and K. Nishimura, *PLoS One* **11**, 0146405 (10 pages) (2016).