Solid-State NMR for Molecular Science

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1994 B.S. Himeji Institute of Technology (University of Hyogo)
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In order to elucidate functions of molecules, characterization of the molecule is the first step. There is a variety of important molecules, which are insoluble in any solvents and functional at amorphous state. Solid-state NMR enables us to obtain a variety of information at atomic resolution without damage to 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 application to structural biology and materials science. We study characterization of membrane proteins and peptides, organic materials, natural products and synthetic polymers. Characterization of those molecules based on solid-state NMR is underway through collaborations with several research groups.

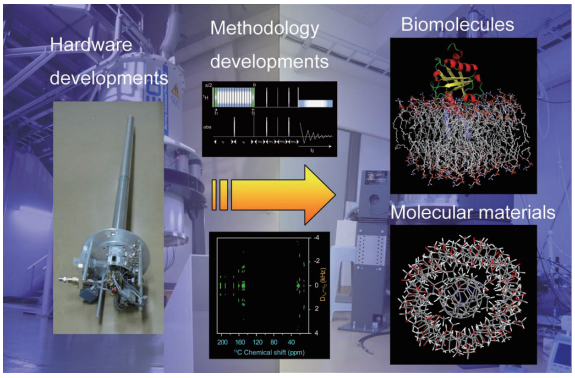

Figure 1. Outline of our studies.

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. Structural Characterization of Amyloid-β Protein Oligomer Promoted on Model Neuronal Cell Membranes Using State NMR

Amyloid β (Aβ) protein is disordered in solutions under diluted conditions, however it conforms insoluble amyloid fibrils, which are found in senile plaque as a hallmark of Alzheimer's disease. Although molecular structures of amyloid fibrils have been determined, its molecular process for fibrillation in vivo has not been clarified yet. However, accumulated evidences suggest that the fibrillation process may be promoted on neuronal cell membrane. Especially, it has been reported that Aβ specifically interacts with ganglioside GM1 which is one of the key lipids in lipid raft. Therefore, GM1 embedded into lipid bilayers composed of neutral lipid DMPC may be considered to be the most simplified model neuronal cell membrane. In order to clarify the role of GM1 in the fibrillation process, first, we have successfully determined the oligomeric structure of Aβ (1-40) induced on DMPC bilayers based on solid-state NMR.[1)] We have been collaborated with Prof. Kato group in IMS for those Aβ studies.

In the current study, Aβ (1-40) oligomer induced on model neuronal cell membranes consisting of GM1 and DMPC have been attempted to characterize using solid-state NMR. Based on information of intra- and intermolecular distances and torsion angles of back born obtained from solid-state NMR analyses, precise molecular structure of Aβ oligomer was determined from restrained molecular dynamics simulations in collaboration with Prof. Okumura group in IMS.

The determined Aβ structure conforms disordered N-terminus followed by center and C-terminus β-sheets. Aβ takes intermolecular configuration of antiparallel β-sheet among adjacent molecules, in which different from Aβ fibrils prepared in solution and also Aβ oligomer induced on DMPC bilayers.[1)] Those suggest specific roles of GM1 for the formation of Aβ oligomers. We expect the significant contribution of our determined Aβ oligomer structure to reveal the molecular mechanism of Aβ fibrils on neuronal cell membranes, and thus understanding of Alzheimer's disease. The manuscript of this study is under preparation.

2. Characterizations of Lipid Binding of Prion Fragment Based on Solid-State NMR

"Prion" protein is amyloid protein responsible for class of neurodegenerative diseases such as Bovine spongiform encephalopathy, (BSE), Creutzfeldt-Jakob disease (CJD) of human, scrapie of sheep. Those are collectively known as transmissible spongiform encephalopathies (TSEs). The onset of TSEs has been considered to be arisen by conformational conversion of the native monomeric cellular prion protein (PrPc) into misfolded β-sheet rich form (PrPs), resulting in their insoluble aggregations. Recent studies suggest that those processes are facilitated through interactions with biomembranes, in particular, through binding with ganglioside GM1 in lipid raft. Despite of many studies of prion, molecular mechanism of structural conversion of prion protein and the cytotoxicity have not been clarified yet.

Ultimate goal of this project is provision of molecular basis of prion disease through the characterizations of molecular structure of prion and their interactions with specific lipids based on solid-state NMR analyses. As the first stage, we start study of PrP(106-126) fragment which has been considered as minimum prion fragment to understand their fibrillation mechanism, because of conservations of important properties of formation of amyloid fibrils, membrane binding ability and cytotoxicity. We explore to clarify PrP(106-126) specific binding site in GM1 has been explored through the analyses of chemical shift perturbations of ^{13}C signals from GM1 in POPC/GM1 vesicles from the comparisons of ^{13}C-solid-state NMR spectra in the presence and absence of PrP(106-126). None of ^{13}C signals from POPC exhibited peak shift, but several signals in GM1 exhibited peak shifts due to PrP(106-126) bindings, suggesting specific interactions of Prp(106-126) with GM1 in GM1/POPC lipid bilayers. Further detail is under investigation.

3. Characterization of Protein Using Solid-State NMR

The secretary abundant heat soluble protein (SAHS) from water bear which has ability of torpor under dry-condition, and recovery from torpor by water supply. The biological functions of SAHS at dry-state has not been identified yet, but SAHS has been considered to play key roles during torpor. This is collaboration project with Prof. Kato group in IMS. SAHS consisting of more than 200 amino acid residues and its dry-state is expected to be inhomogeneous. Therefore, in order to investigate structural homogeneity and obtain local conformational information, 2D ^{13}C-homonuclear correlation solid-state NMR measurements were carried out for the dry-state SAHS protein which only isoleucine residues are specifically ^{13}C and ^{15}N isotope labeled. The 6 sets of signals were observed and assigned successfully.

4. Development of Solid-State NMR Probe

We have built a variety of solid-state NMR probes such as static and MAS ^{1}H-X double resonance probes for 400 MHz NMR, and a variable temperature ^{1}H-X double resonance MAS probe for 920 MHz ultra-high field NMR at past. During the past few years, we have been working on building an original solid-state NMR probe which is fully compatible with commercial instruments currently used. Those developed probes were built with originally designed parts except for spinning and spinning rate detection modules which were purchased from NMR company. To replace remained commercial modules, we attempted to design original spinning module. At first stage, two different types of original spinning modules were designed for 4.0 mm sample tube. Those exhibited moderate performances but slightly lower performance respect to commercial module. Based on various tests, we identified the key parts to govern the performance of the spinning. The improved version of spinning module is under developments.

Reference

1) M. Yagi-Utsumi, K. Kato and K. Nishimura, *PLoS One* **11**, 0146405 (10 pages) (2016).