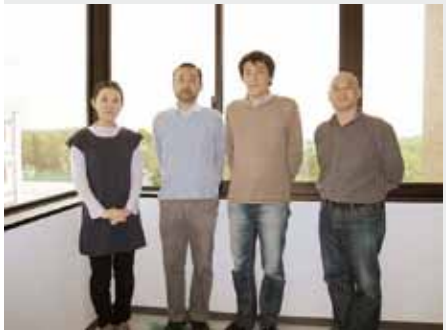
Solid State NMR for Structural Biology

Department of Materials Molecular Science
Division of Molecular Functions



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We are working on methodology and hardware developments of solid state NMR and structural biology based on them. In the following, we show developed variable temperature (VT) magic angle spinning (MAS) probe for ultra high field NMR and preliminary result of newly constructed over expression system of a peripheral membrane protein. In addition, a dynamics study of paramagnetic compound was reported.

1. Development of VT-MAS Solid-State NMR Probe for Ultra High Field 920 MHz NMR

Solid state NMR under ultra high field opens up new possibilities of experiments respect to spectral resolution and sensitivity of spectra. Unfortunately, none of sample temperature controllable solid-state NMR probes are available in ultra high field NMR facilities in Japan. This situation restricts usability and possibilities of solid state NMR studies under ultra high field in Japan.

In order to overcome this situation, we have developed ^{1}H-^{13}C double resonance VT-MAS probe based on JEOL 920MHz MAS probe. We have designed original VT control system for MAS probe, machined parts and built in JEOL 920 MHz MAS probe as show in Figure 1 (a). In the developed probe, a bearing gas for MAS was temperature controlled. Thus, all of bearing gas line was replaced by originally designed glass Dewar tubes. Since probe length was extremely long, Dewar was separated into two pieces. Those were jointed by optimally designed adapter, then a cartridge heater was built in bottom Dewar. The control of sample temperature was realized through feedback control of cartridge heater by monitoring gas temperature at a position close to sample tube. Upper and lower limits of available temperatures are limited by the material of stator and available chiller temperature, respectively. Stably available temperature range was verified experimentally from 0 to 60 °C which is sufficient range for studies of biomolecules.

Figure 1 (b) and (c) are photos of VT-MAS probe built in ultra high field magnet together with peripherals. For studies of biomolecules, precise temperature control of samples are required in order to retain those structures and functions. It is expected that this probe opens up the possibilities of studies for such samples. Finally the author K.N. appreciates to members of Equipment Development Center in IMS for their help.

Figure 1. (a) The bottom view of newly developed VT-MAS probe. (b) The side view of VT-MAS probe loaded into 920 MHz ultra high field magnet (21.6 T). (c) The side view of VT-MAS probe and peripherals enabling VT.

2. Structural Characterization of Peripheral Membrane Protein by Solution and Solid State NMR

Phospholipase C-δ1 (PLC-δ1) hydrolyzes phosphatidylinositol 4,5-bisphosphate (PIP_2) in the plasma membrane to produce the second messengers on the membrane surface. The pleckstrin homology (PH) domain in the N-terminus of PLC-δ1 selectively forms high affinity complex with PIP_2 in the plasma membrane and inositol 1,4,5-triphosphate (IP_3) in the cytoplasm. Consequently those complex formations regulate

membrane localization of PLC-δ1. So far, we have reported rat-PLC-δ1 PH domain changes its conformation depending on curvatures of lipid bilayers and micelles.[1)] In this study, we established the over expression system of the PH domain of human PLC-δ1, and performed preliminary NMR analysis of the recombinant protein.

The plasmid containing the hPH gene, corresponding to the PH domain (residues 1-142) of human PLC-δ1, was transformed into *Escherichia coli* BL21(DE3) strain. To produce the isotope labeled hPH, the transformed *E. coli* was incubated in M9 medium containing stable isotopes. The harvested *E. coli* cells were purified using GST-affinity chromatography followed by gel-filtration chromatography. The purified hPH shows IP_3-binding activity as judged by Native PAGE gel shift analysis (data not shown), indicating that the recombinant protein is correctly folded.

Figure 2 shows two-dimensional ^{1}H-^{15}N heteronuclear single quantum coherence (HSQC) solution NMR spectra of the ligand-free (black signals) and IP_3-complex forms (red signals) of the uniformly ^{15}N-labeled hPH. The chemical shift dispersion of the ^{1}H and ^{15}N resonances in the spectrum of the ligand-free hPH also indicated that the recombinant hPH is substantially folded. The addition of IP_3 resulted in chemical shift displacements of many resonances (Figure 2, red signals), indicating that the IP_3 binding induces a large conformational change of hPH. Solid state NMR studies of the hPH-PIP_2 complex in membrane, as well as ligand-binding studies for the several mutants of hPH, are in progress.

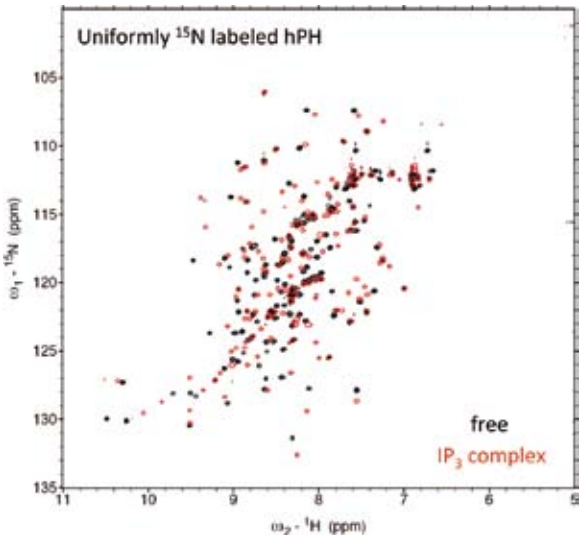

Figure 2. ^{1}H-^{15}N HSQC solution NMR spectra of uniformly ^{15}N-labeled hPH in the absence (black) and the presence of IP_3 (red) at 20 °C. Molar ratio of hPH and IP_3 was 1:1.3.

3. ^{2}H QCPMG NMR of Paramagnetic Solids as a Probe of Molecular Dynamics

Solid state NMR is a powerful technique to investigate dynamics of molecules and ions in substances. In particular, deuterium (^{2}H, I = 1) NMR can probe motions with timescale ranging from nanosecond to second by several methods such as a quadrupole echo, quadrupolar Carr-Purcell-Meiboom-Gill (QCPMG), magic-angle-spinning, two-dimensional NMR and relaxation experiments. Compared to measurement by the quadrupole echo sequence, the QCPMG technique where a train of spin echo generated by repeatedly irradiated refocusing pulses is acquired can enhance sensitivity of ^{2}H NMR spectra and extend dynamic range, although it has been employed only for diamagnetic compounds. Recently, we have developed a ^{2}H QCPMG method efficient for paramagnetic solids.

In this work, we applied the QCPMG method to probe molecular dynamics of paramagnetic solids. Figure 3 shows temperature dependences of ^{2}H NMR spectra of paramagnetic powder of $CoSiF_6$ $6H_2O$ under 9.4 T. Asymmetric lineshape is caused by the paramagnetic interaction between ^{2}H and unpaired electrons in Co^{2+}. For the QCPMG spectra, drastic change was observed in this temperature range, which is due to a reorientation of $[Co(H_2O)_6]^{2+}$ around the C_3 axis. For the echo spectra, however, lineshape of the spectra at low temperatures (293–313 K) are almost the same and small change was observed at high temperatures (373–393 K). By a simulation of the QCPMG spectra (Figure 3(ii)), ^{2}H interaction parameters of quadrupole coupling constant (C_Q) and asymmetric parameter (η) as well as a rate constant for the reorientational motion of $[Co(H_2O)_6]^{2+}$ (k_{re}) also shown in the Figure 3 were obtained successfully. With these parameters, the echo spectra were reproduced (Figure 3(iv)). It was found that the ^{2}H QCPMG technique to extend dynamic range is also effective for paramagnetic compounds.

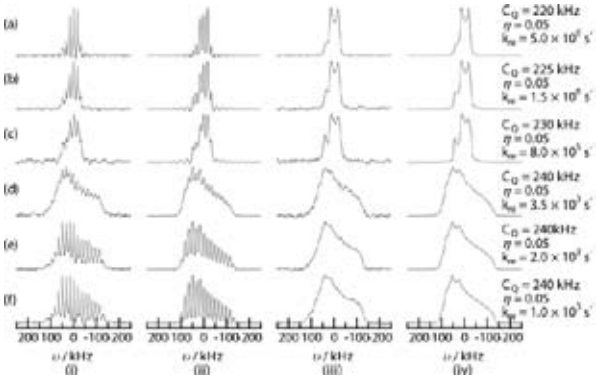

Figure 3. Temperature dependence of ^{2}H NMR spectra of paramagnetic $CoSiF_6 \cdot 6H_2O$ obtained by the QCPMG (i, ii) and echo (iii, iv) sequences. (i, iii) and (ii, iv) show the observed and simulated spectra, respectively. (a)–(f) are the spectra at 393, 383, 373, 313, 303 and 293 K, respectively.

Reference

1) N. Uekama, T. Aoki, T. Maruoka, S. Kurisu, A. Hatakeyama, S. Yamaguchi, M. Okada, H. Yagisawa, K. Nishimura and S. Tuzi, *Biochim. Biophys. Acta* **1788**, 2575–258 (2009).