Methodology Developments of Solid State NMR Spectroscopy for Structural Biology and Material Science

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Solid state NMR is one of the useful tools to characterize dynamics and structures of molecules on amorphous condition without specific limitations. We are focusing on methodology developments of solid state NMR especially for structural biology and material science. We are also attempting to elucidate functions and dynamic structure of peripheral membrane protein bound to lipid bilayer surface based on solid state NMR. In the following, we show the newly developed techniques to enhance spectral resolution and sensitivity in solid state NMR spectroscopy, and a study of structural change depending on weak interaction between peripheral membrane protein and lipid bilayer surface.

1. Spectral Resolution Enhancements Based on Doubly Magnified Evolution of Internal Interaction in Solid State NMR[1)]

In order to enhance the spectral resolution of 2D correlation experiments in solid state NMR spectroscopy, general scheme enabling doubly magnified evolution of specific internal interaction was developed. The efficacy of this approach was verified by applying it to several conventional techniques. As first example, doubly magnified heteronuclear 2D J-spectroscopy under magic angle spinning (MAS) in solid was developed to enhance separation of multiplet signals due to heteronuclear J-coupling. J-couplings are very useful parameters in solution NMR for assignments of peaks and establishment of through bond connectivities. In solid-state NMR, however, J-couplings are less frequently observed because of their small amplitudes respect to the other interactions.

In conventional constant evolution time heteronuclear J-spectroscopy, heteronuclear J-coupling evolution takes place by replacement of heteronuclear decoupling sequence with multiple-pulse (MP) sequence removing ^{1}H homonuclear dipolar interaction under MAS. In contrast to the conventional technique, MP is applied to whole constant evolution time in newly developed one. Then, MPs are replaced by the ones exhibiting the same form of average Hamiltonian (AH) for heteronuclear J-coupling with opposite sign. As a result, the effective evolution becomes double and it gives doubled multiplets due to J-couplings in Hz unit without change of line width. Thus the spectral resolution can be improved by twice. Figure 1 (b) and (c) show the J-resolved spectra obtained from

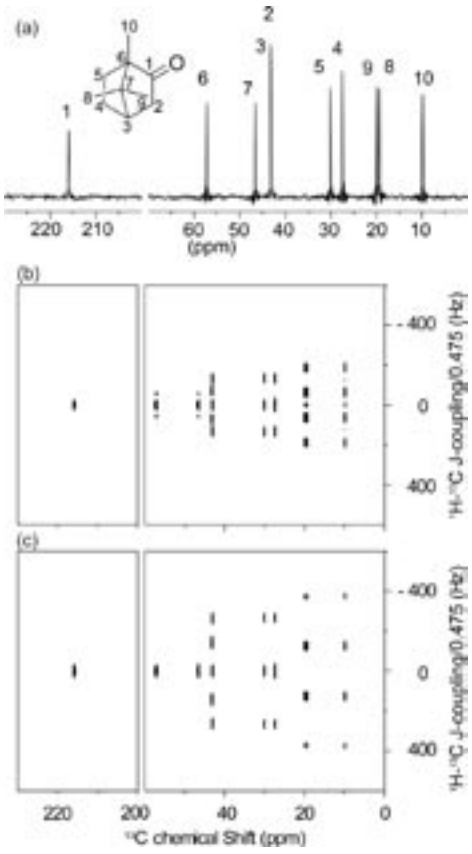

Figure 1. (a) 1D-CPMAS spectrum of Camphor. 2D J-resolved spectra of solid Camphor obtained from (b) conventional and (c) doubly magnified techniques under MAS, respectively. J-coupling axes are corrected by scaling factor of MP.

conventional and newly developed ones, respectively. It is important to note that no specific limitation over conventional technique exists to perform new experiment.

Similar idea can be applied for the measurements of heteronuclear dipolar interaction. That technique is effective especially to observe motionally averaged dipolar interaction in oriented sample.[2)]

2. QCPMG NMR of Spin-1 Nuclei in the Presence of Shift Interaction[3)]

Quadrupole Curr-Purcell-Meiboom-Gill (QCPMG) sequence is an efficient method to enhance NMR signals of quadrupole nuclei with acquisition of successive echo signals generated by refocusing pulses. QCPMG sequence that can be combined with other solid-state NMR techniques such as magic angle spinning, multiple quantum/magic angle spinning and double frequency sweep has been widely utilized for structural investigation of materials possessing quadrupole nuclei. However, it has been recently reported that QCPMG is inefficient in the presence of a shift interaction for spin-1 nuclei including ^{2}H and ^{14}N, and application of QCPMG of such nuclei for structural study has been limited.

In this work, we developed improved QCPMG technique for spin-1 nuclei that can obtain an in-phase spectrum under the influence both of the quadrupole and shift interactions. Essences of the present method are (i) addition of radio-frequency (RF) pulses that only affect the refocusing of the shift interaction and (ii) an irradiation of a strong RF field to prevent a signal arising from unwanted coherence pathways.

Figure 2 shows comparison of ^{2}H NMR spectra for a model compound of $CoSiF_6 \cdot 6H_2O$ obtained from conventional and improved QCPMG methods. It is known that a paramagnetic shift interaction between ^{2}H nuclei and electron spins in Co^{2+} ion in addition to the quadrupole interaction contributes to the ^{2}H NMR spectrum of this sample. Since the former interaction is considerably large (> 30 kHz), application of conventional QCPMG pulse sequence results in a phase distorted spectrum as shown in Figure 2 (a). Figure 2 (b) is the QCPMG spectrum obtained from improved QCPMG pulse sequence with the RF field of 210 kHz. As expected, the spectrum is free from distortion. Thus, NMR parameters can be obtained by comparison with simulated one as shown in Figure 2 (c).

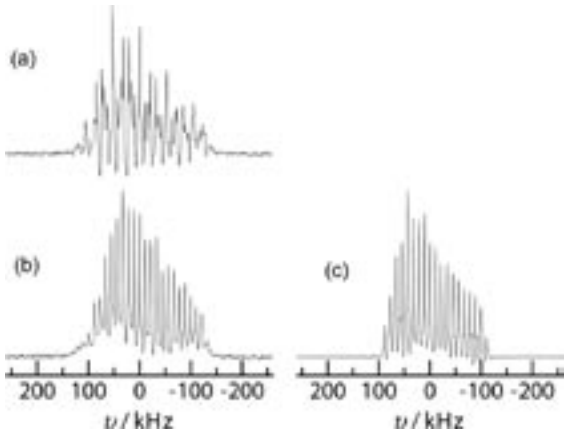

Figure 2. ^{2}H (I = 1) QCPMG NMR spectrum of $CoSiF_6 \cdot 6H_2O$ at 61.385 MHz. (a) and (b) show the spectra obtained by the conventional and improved QCPMG methods, respectively. (c) The simulated spectrum for improved QCPMG.

3. Influence of Membrane Curvature on the Structure of the Membrane-Associated Pleckstrin Homology Domain of Phospholipase C-δ1[4)]

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 PH domain in the N-terminus of PLC-δ1 selectively forms high affinity complex with PIP_2 in the plasma membrane and IP_3 in the cytoplasm. Consequently those complex formations regulate membrane localization of PLC-δ1. From the previous studies, it is known that α2-helix is responsible for auxiliary membrane binding site in addition to the PIP_2 specific membrane binding site and the terminous of β5/β6 loop plays a role of hinge connecting the β-sandwich core of the PH domain.

In this study, conformational changes of the PLC-δ1 PH domain bound to the surfaces of multilamellar vesicles (MLVs), small unilamellar vesicles (SUVs), and micelles were investigated to evaluate the effects of membrane curvatures on the membrane-associated protein based on solid-state NMR spectroscopy. The conformational changes of PLC-δ1 PH domain bound to those surfaces were evaluated from the analyses of conformational dependent ^{13}C isotropic chemical shifts of isotope enriched [3-^{13}C] Ala sites in PH domain. As results, ^{13}C NMR signals of Ala88 in the α2-helix and Ala112 spatially close to the terminus of the β5/β6 loop containing the α2-helix exhibited conformational changes due to the reorientation of the α2-helix on the surface of MLV and SUV membrane. In contrast, no conformational change of those regions was observed on the surfaces of the DPC and DM micelles with diameter of 33–40 Å. According to those results, we found out that the terminus of β5/β6 loop is susceptible to the alteration of the curvature of lipid bilayer surface, and the mutual orientation of two membrane binding sites of the PH domain on the curved membrane.

It is known that the membranes in the cell undergo dynamic alterations of their structures and their chemical composition during the physiological processes. Those induce time dependence of local curvature of membrane. Thus the conformational changes of the membrane binding domain such as found in this study for the PLC-δ1 PH domain may affect the membrane binding mechanism of peripheral membrane proteins.

References

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