Development of a Novel Solid State NMR Technique to Study Lipids and Proteins Bound to Fully Hydrated Membranes

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We are currently developing a novel solid state NMR technique to be used for material science and structural biology. Currently, we focus to develop a methodology to characterize membrane bound proteins such as peripheral membrane proteins under fully hydrated sample condition, to reveal their native structures and dynamics. Solid state NMR is one of the useful tools to characterize dynamics of such insoluble proteins. For this reason, we have developed a new technique to observe motionally averaged weak heteronuclear dipolar coupling.

1. A Novel Technique to Examine Local Dynamics of Biomolecules by Means of Magnified, Motionally Averaged, Weak Heteronuclear Dipolar Couplings by Solid State NMR Spectroscopy[1)]

Fully hydrated membrane proteins in lipid bilayers undergo lateral diffusion and are known to exhibit significant dynamics. When the motions are that the tensor interaction has effective axial symmetry, all the motional information can be referred to this axis and is expressed in terms of a single order parameter. In order to characterize local mobility of such biomolecules, we have explored to determine their segmental order parameters by observing motionally averaged, weak heteronuclear dipolar interaction, under magic angle spinning (MAS).

For this purpose, we have developed a new separated local field technique, *s*calar *c*oupling *r*efocused *e*ffectively *m*agnified *dip*olar field-chemical *shift* (SCREM-DIPSHIFT) correlation spectroscopy, to determine motionally averaged, weak heteronuclear dipolar coupling, under MAS. A pulse sequence is shown in Figure 1(a). This method greatly magnifies the detection sensitivity for heteronuclear dipolar couplings 4 times over the conventional ones, by only recoupling of heteronuclear dipolar interaction based on interference between sign changes of average Hamiltonian of heteronuclear dipolar interaction under ^{1}H homonuclear dipolar decoupling

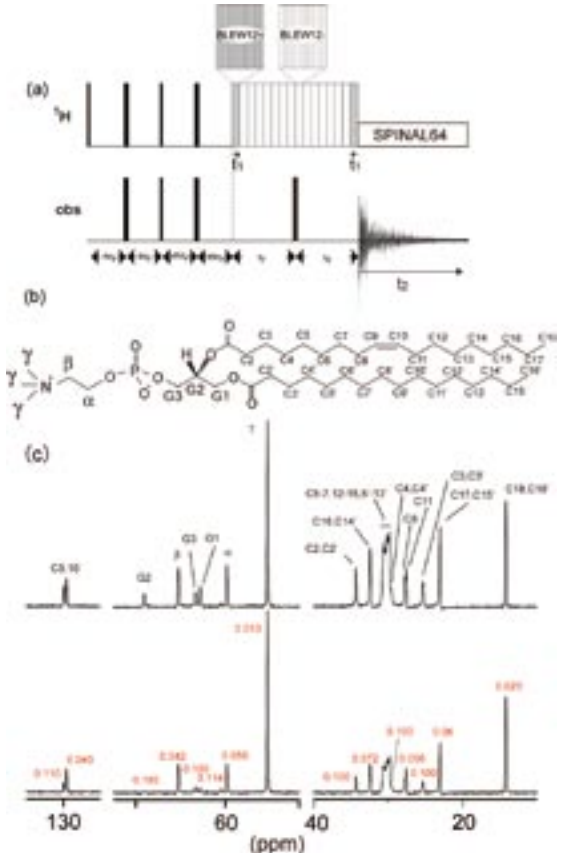

Figure 1. (a) Pulse sequence of INEPT-SCREM-DIPSHIFT under MAS. BLEW12+/– is multiple-pulse to generate the same form of average Hamiltonian for heteronuclear dipolar interaction with opposite signs with suppression of ^{1}H homonuclear dipolar decoupling. The thin- and thick- blacked bars indicate $\pi/2$ and π pulses, respectively. τ_r is rotor period and '*m*' and '*n*' are integer. (b) Molecular structure of POPC. (C) ^{13}C-NMR slice spectra acquired with sequence (a) without (upper column.) and maximally reduced signal (lower column) due to magnified ^{1}H-^{13}C dipolar coupling for POPC in MLVs. Experimentally determined dynamic order parameters were indicated inside of bottom spectrum.

and spatial modulation by MAS. Furthermore, the one also refocuses scalar coupling evolution during dipolar evolution time. One can therefore determine extremely averaged, weak dipolar coupling with lower limit of a hundred Hz for ^{1}H-^{13}C spin pair, which corresponds to dynamic order parameter of 0.0043 with single rotor cycle at spinning speed less than 3 kHz. Total experimental time can also be appreciably reduced, as compared with acquiring full 2D-NMR spectra. Furthermore, by extending dipolar evolution time of multiple of unit cycle, lower detection limit can be extended as much as possible depending on effective T_2 under ^{1}H homonuclear dipolar decoupling multiple-pulse sequence of observed nuclei.

To prove efficacy and usefulness of this technique, we characterized lipids in fully hydrated multi-lamella vesicles (MLVS), 1-palmitoyl-2-oleyl-sn-glycero-3-phosphocholine (POPC). The time evolution of the recouped, motionally averaged, weak ^{1}H-^{13}C heteronuclear dipolar interactions was monitored by reduced high resolution NMR signal of natural abundant ^{13}C nuclei, prepared by either ^{1}H coherence transfer based on through-bond *J*-coupling mediated rotor synchronized INEPT or single pulse excitation of ^{13}C polarization. Dipolar couplings in each residue were determined by fitting the curves of reduced signals for ^{1}H-^{13}C 2-spin, $^{1}H_2$-^{13}C 3-spin system $^{1}H_3$-^{13}C 4-spin system for CH and CH_2 and CH_3, respectively. The segmental order parameters were shown in Figure 1 (c) with slice ^{13}C NMR spectra without and with maximally reduced signals. The obtained segmental order parameters for POPC in MLVs were slightly smaller, but similar as reported for saturated lipids. Further developments to characterize the local structures are in progress.

2. A Study of Local Mobility for Phospholipase C δ1- Pleckstlin Homology Domain Bound to Fully Hydrated Multi-Lamella Vesicles by Solid State NMR[2)]

A peripheral membrane protein, phospholipase C (PLC)-δ1, is one of membrane proteins related to signal transduction by conducting hydrolytic cleavage of phosphatidyl inositol-4,5-bisphosphate (PIP_2) on the surface of membrane bilayers and is known as one of essential proteins for mammalians. Pleckstlin homology (PH) domain of PLC-δ1 has been recognized as PIP_2 binding domain. In current study, characterization of local mobility of PLC-δ1 PH domain bound to fully hydrated MLVs was explored. [3-^{13}C]Ala-labeled PLC-δ1 PH domain was expressed as GST fusion protein in E Coli in [3-^{13}C]Ala contained in M9 culture and purified by affinity chromatography. The purified one was attached to the surface of MLVs prepared from POPC and PIP_2 with molar ratio 20:1. The molar ratio of protein to lipids was prepared to 1:20. PLC-δ1 PH domain bound to MLVs were precipitated by ultracentrifuge of 6 hours at 541000×*g* at 4 °C. They were packed into sample tube as same way as mentioned above. Solid state NMR experiments were carried out under the same condition mentioned above. The magnified recoupling effects of motionally averaged weak ^{1}H-^{13}C heteronuclear dipolar interactions were monitored as signal reduction of high resolution ^{13}C spectra of isotope enriched methyl carbon of Ala residues in PLC-δ1 PH domain by SCREM-DIPSHIFT.[1)] Individual dipolar couplings were determined by fitting curves of the reduced signals for $^{1}H_3$-^{13}C 4-spin system with rotation effect around the C_3 rotation axis. Dynamic order parameters ($0 < DOP < 1.0$), were determined by the normalizing motionally averaged heteronuclear dipolar couplings with the one in rigid limit. Figure 2 (a) shows high resolution solid state NMR spectra with reduced signals due to recoupled heteronuclear dipolar couplings of methyl region. Dynamic order parameters for all Ala residues in PLC-δ1 PH domain were determined similarly. Figure 2 (b) illustrates preliminary result of a pictorial representation of local mobility of PLC-δ1 PH domain bound to fully hydrated POPC/PIP_2-MLVs, based on experimentally determined dynamic order parameters. The information of local mobility of PLC-δ1 PH domain may give the understanding of a detailed mechanism as to a relationship between structure and function of PH domain, which is bound to PIP_2. To the best of our knowledge, this is first example of successfully characterized local mobility for peripheral membrane protein bound to fully hydrated lipid bilayers by solid state NMR.

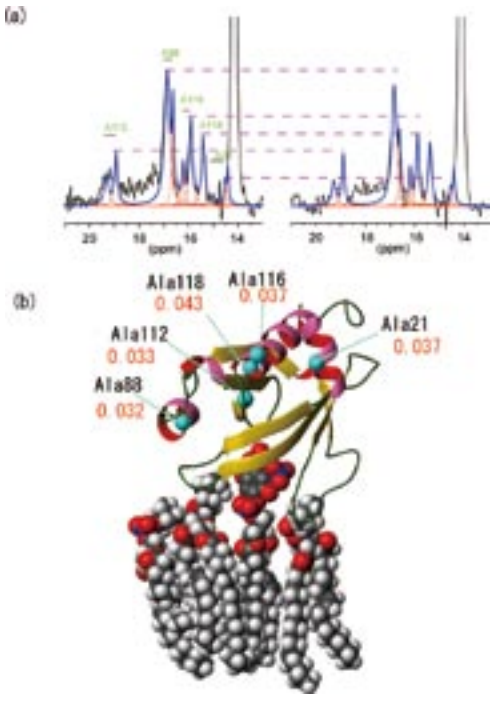

Figure 2. Pictorial representation of local mobility of PLC-δ1 PH domain bound to fully hydrated POPC/PIP_2 lipid bilayers surface of MLVs based on the experimentally determined order parameters (red colored) of Ala residues in PLC-δ1 PH domain. Light blue spheres indicate the location of Ala residues in PLC-δ1 PH domain. A model structure has been generated from the combination of X-ray derived structure bound to Inositol-1,4,5-triphosphate in the crystal and POPC lipid bilayers calculated from MD simulation.

References

1) K. Nishimura, *J. Chem. Phys.* submitted.
2) N. Uekama, M. Okada, H. Yagisawa, S. Tuzi and K. Nishimura, to be submitted.