

Developments of New Techniques and Magnetically Aligned Lipid Bilayer System for Structural Biology Based on Solid State NMR Spectroscopy

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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 working on methodology developments of solid state NMR for structural biology and material science. Especially, we are focusing on elucidation of 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 solid state NMR techniques to enhance spectral sensitivity for aligned sample, and new lipid sample system enabling magnetically aligned planer lipid bilayers useful for structural characterization of peripheral membrane proteins. In addition, a study of molybdenum compounds was reported.

1. ^1H - ^{13}C Heteronuclear Polarization Transfer for Aligned Solid¹⁾

Analysis of anisotropic interactions such as dipolar interaction and chemical shift anisotropy in aligned sample give information of local molecular structure straightforwardly. Thus membrane proteins embedded in magnetically aligned lipid bilayer samples have been studied so far. In such experiment, heteronuclear polarization transfer from ^1H to rare nuclei such as ^{13}C and ^{15}N plays important role to enhance spectral sensitivities of rare nuclei. Recently, Jayanthi et al developed DAPT which is the INEPT type dipolar based heteronuclear polarization transfer technique to be used in aligned sample. However due to the lack of refocusing mechanism of ^1H carrier frequency offset in DAPT, performance of DAPT is quite poor.

In this study, we show improved version of DAPT, which is insensitive to ^1H carrier frequency offset. We refer developed technique to as refocused DAPT (REDAPT), which refocuses ^1H carrier frequency offset based on refocused multiple pulse technique which author developed. The performance of

REDAPT was assessed using liquid crystalline molecule of 5CB as shown in Figure 1 (a). The practically obtained polarization transfer profile was shown in Figure 1 (b). Because of refocusing of ^1H carrier frequency offset in REDAPT, signal intensities were gradually decreased respect to the ^1H carrier frequency offset.

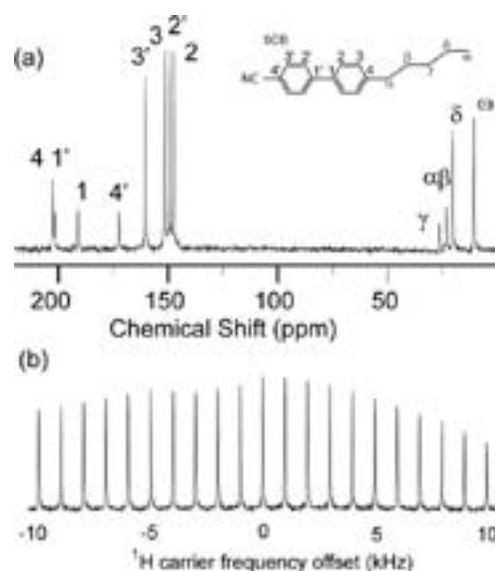


Figure 1. (a) REDAPT enhanced ^{13}C -NMR spectrum of 5CB liquid crystal at 20 °C. Plot of site 3 signals in 5CB obtained from REDAPT at mixing time of 264 μs for various ^1H carrier frequency offsets.

2. A Bicelle Magnetically Aligned Planer Lipid Bilayers at Room Temperature for Structural Characterization of Membrane Bound Proteins by Solid State NMR²⁾

The properly hydrated mixture of saturated lipids possess-

ing short and long acyl-chains at proper composition forms planer lipid bilayer so called bicelle which can be magnetically aligned under static magnetic field at temperature from 30 to 40 °C. Bicelle is widely used in solid state NMR for structural characterization of membrane associated proteins. Triba *et al.*, proposed a bicelle prepared by mixture of saturated lipid 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and unsaturated lipid 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) for long acyl chain, and 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC) for short acyl chain lipid, respectively in order to achieve magnetic alignment at temperature lower than that of conventional bicelle. In the following we refer above bicelle to as POPC/DMPC/DHPC-bicelle.

In this study, we developed a new bicelle enabling better magnetic alignment at room temperature over POPC/DMPC/DHPC-bicelle. Our developed bicelle consists of phosphatidylinositol 4, 5-bisphosphate (PIP₂) in addition to above lipids at proper molar ratio. Orientational property of developed bicelle was compared to those for POPC/DMPC/DHPC-bicelle based on ³¹P-NMR. As shown in Figure 2, (b) newly developed bicelle exhibited better magnetic alignment over (c) POPC/DMPC/DHPC-bicelle. Furthermore, temperature range for magnetic alignment for developed bicelle was twice wider than that of POPC/DMPC/DHPC-bicelle. We have successfully developed new bicelle magnetically aligned at room temperature stably. New bicelle may be useful especially for structural characterization of membrane proteins which are sensitive to fluctuation depending on the sample temperature such as peripheral membrane proteins.

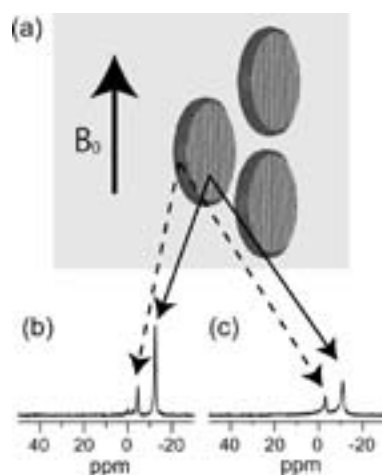


Figure 2. (a) Schematic representation of bicelle. Dark and light gray colored areas indicate polar heads of short and long acyl chain lipids, respectively. (b) ³¹P-NMR spectra of magnetically aligned POPC/DMPC/PIP₂/DHPC-bicelle and (c) POPC/DMPC/PIP₂/DHPC-bicelle at 16 °C, respectively.

3. ⁹⁵Mo NMR of Mixed Valence Polyoxomolybdates (V, VI)³

Solid state NMR of half integer quadrupole nuclei benefits from recent development of high field magnets for high-resolution NMR, because a higher field brings higher sensitivity and resolution by decrease in second-order quadrupole

coupling and increase in equilibrium magnetization. Structural researches utilizing high-field NMR for quadrupolar nuclei such as ⁹⁵Mo have been recently reported. Studies of ⁹⁵Mo NMR can be classified by the oxidation number of molybdenum taking from Mo⁰ to Mo^{VI}. While solution ⁹⁵Mo NMR has accessed all species with the integer oxidation number, ⁹⁵Mo solid state NMR studies are limited to Mo⁰, Mo^{IV} and Mo^{VI}. Although the Mo^V or Mo^{V,VI} species frequently appear in polyoxomolybdates such as ε-Keggin anion and nanosized oxides with ring-, tube- and ball-structure, only several ⁹⁵Mo solution NMR spectra have been measured for Mo^V. In this study, we investigated high-field solid-state ⁹⁵Mo NMR of Mo^V, Mo^{V,VI} and Mo^{VI} species in mixed valence polyoxomolybdates(V, VI).

Compounds used here are [Me₃NH]₆[H₂Mo^V₁₂O₂₈(OH)₁₂(Mo^{VI}O₃)₄]₂H₂O (**1**) and [NMe₄]₂[NH₄]₈[(Mo^{VI}₆Mo^VO₂₃)₂]₈H₂O (**2**) which have localized or delocalized d¹ electrons, respectively. As show in Figure 3, ⁹⁵Mo MAS NMR spectra were measured under moderate (9.4 T) and ultrahigh magnetic fields (21.8 T). By simulating the acquired NMR spectra and density functional theory (DFT) calculation for isolated anions, NMR parameters for each of molybdenum sites within the crystals were obtained. Despite different state of the d¹ electrons, a similar trend that exhibits large chemical shifts for Mo^V in **1** and Mo^{V,VI} in **2** was found. Highest-energy occupied molecular orbital(s) of the anions was mainly consisting of 4d atomic orbitals of Mo^V or Mo^{V,VI}. Then the large shift was ascribed to a paramagnetic deshielding term by magnetic-dipole allowed mixing of the occupied and virtual orbitals containing the 4d orbitals of these species.

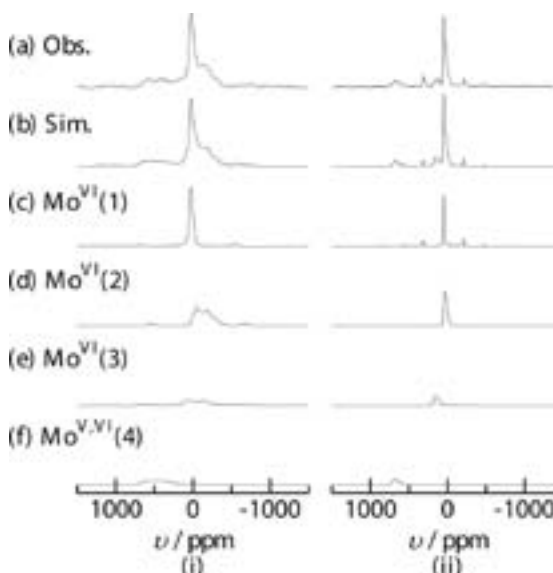


Figure 3. ⁹⁵Mo MAS NMR spectra of [NMe₄]₂[NH₄]₈[(Mo^{VI}₆Mo^VO₂₃)₂]₈H₂O under (i) 9.4 and (ii) 21.8 T. (a) and (b) show the observed and simulated spectra, respectively. (c-f) denote spectral components consisting of the spectrum (b).

References

- 1) To be submitted.
- 2) To be submitted.
- 3) To be submitted.