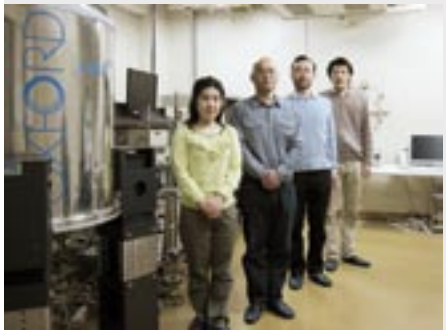
Structural Biology Based on Solid State NMR Spectroscopy

Department of Materials Molecular Science
Division of Molecular Functions



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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 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. Bicelle:Magnetically Aligned Planner Lipid Bilayers at Room Temperature for Structural Characterization of Membrane Bound Proteins by Solid State NMR

The properly hydrated mixture of saturated lipids possessing 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 and widely used in solid state NMR for structural characterization of membrane associated proteins. Last year, we reported that addition of proper amount of phosphatidylinositol 4, 5-bisphosphate (PIP_2) into bicelle significantly enhances the stability of magnetic alignment and increases its temperature range of magnetic alignment. Those effects were demonstrated for the bicelle proposed by Triba *et al.*, enabling magnetic alignment at room temperature. This bicelle composes 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 this study, we have identified the origin of enhancement of magnetic alignment due to addition of PIP_2 into bicelle.

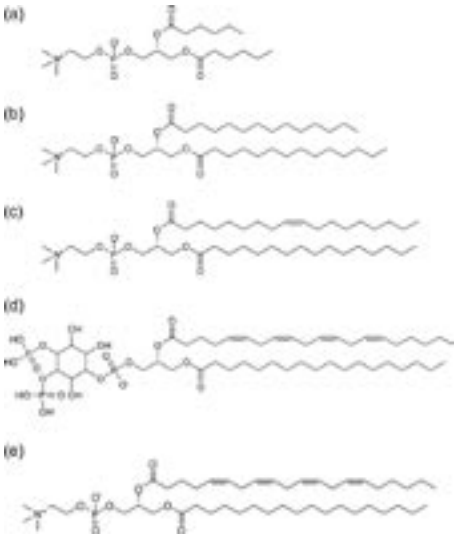

Figure 1. Molecular structures for (a) DHPC, (b) DMPC, (c) POPC, (d) PIP_2, and (e) SAPC, respectively.

Orientational properties of bicelles prepared from the different composition of lipids were verified based on ^{31}P-NMR.1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine (SAPC) has the polar head and the acyl chains as same molecular structure as those for DMPC and PIP_2, respectively. The bicelle composing SAPC instead of PIP_2 at same molar ratio was prepared in order to indentify either polar head group or acyl-chains of PIP_2 contributing to the enhancement of magnetic alignment of bicelle. Figure 1 shows the ^{31}P-NMR spectra for the bicelle prepared from (a) PIP_2/POPC/DMPC/DHPC, (b) POPC/DMPC/DHPC, and (c) SAPC/POPC/DMPC/DHPC for the temperature range from 12 to 22 °C. The peaks around –3 and –11 ppm are originated from DHPC and POPC/DMPC mixture, respectively. The peak around –15.5 ppm is $\delta_\perp$ edge of axially symmetric powder pattern of ^{31}P chemical shift anisotropy from multi lamella vesicles (MLVs). PIP_2/

POPC/DMPC/DHPC-bicelle was magnetically aligned stably from 14 to 20 °C. In contrast, POPC/DMPC/DHPC-bicelle was magnetically aligned only at 16 °C. At 18 °C, $\delta_{\perp}$ edge of axially symmetric powder pattern of ^{31}P chemical shift anisotropy from MLVs was appeared. SAPC/POPC/DMPC/DHPC-bicelle exhibited insufficient magnetic alignment over measured temperature range.

From the comparison of ^{31}P-NMR spectra for those bicelles, it is obvious that addition of SAPC into POPC/DMPC/DHPC-bicelle dose not enhance magnetic alignment. Thus we concluded that inositol 4, 5-bisphosphate residue in PIP_2 may contribute to the enhancement of magnetic alignment of bicelle. Our results may give the way to design new reagents to enhance magnetic alignment of bicelle.

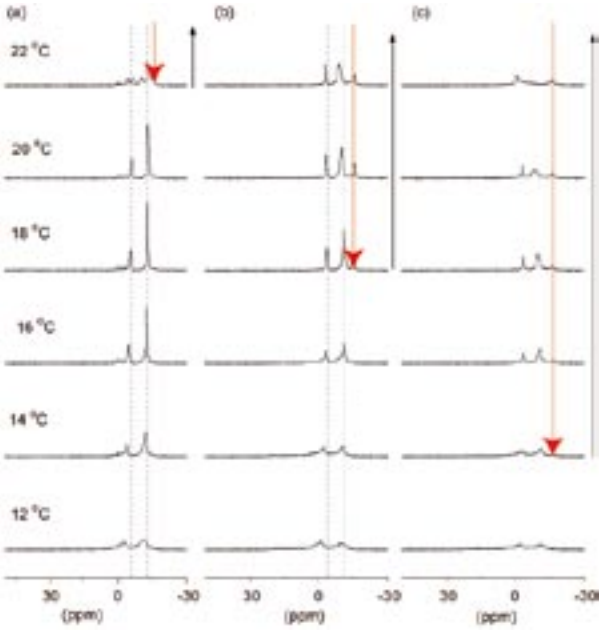

Figure 2. ^{31}P-NMR spectra of magnetically aligned bicelle prepared from (a) PIP_2/POPC/DMPC/PIP_2/DHPC, (b) POPC/DMPC/PIP_2/DHPC and (c) SAPC/POPC/DMPC/PIP_2/DHPC, respectively. The red arrows indicate $\delta_{\perp}$ edges of axially symmetric powder pattern spectra of ^{31}P chemical shift anisotropy due to the formation of MLVs. Thus black arrows indicate the temperature range of the samples containing mixture of bicelle and MLVs.

2. Solid-State ^{95}Mo NMR of Polyoxomolybdates (V, VI) with ε-Keggin Structure

For molybdenum with the oxidation number of Mo^0–Mo^{VI}, solution-state ^{95}Mo NMR has accessed all of integer oxidation number so far. In particular, ^{95}Mo NMR of Mo^0, Mo^{II} and Mo^{VI} has been widely used for study in the field of coordination chemistry and reactivity. On the contrary, investigation of solid-state ^{95}Mo NMR have been limited, because the spectra are broadened owing to the second-order quadrupole interaction. Recently, we have reported solid-state ^{95}Mo NMR of Mo^V species for the first time by measuring high-field ^{95}Mo NMR of mixed-valence polyoxomolybdates(V, VI) with localized or delocalized d^1 electrons. It has been shown that the chemical shift of ^{95}Mo NMR of Mo^V species exhibits a larger value than that of Mo^{VI}, and anisotropy of chemical shift depends on localization of d^1 electrons.

In this work, we investigated molecular and electron structures by solid-state ^{95}Mo and DFT calculation for a polyoxomolybdate crystal of $[PMo_{12}O_{36}(OH)_4\{La(H_2O)_{2.75}Cl_{1.25}\}_4]27H_2O$ (abbreviated as $\{Mo_{12}(La)\}$), where a $\{Mo_{12}\}$ core with the ε-Keggin structure is capped with four $La(H_2O)_{2.75}Cl_{1.25}$. Decimal fraction appears because of a disorder structure of H_2O and Cl coordinated to La^{III}. While potentiometric titration experiments have suggested that molybdenum is also disordered, only single Mo site was reported from analysis of X-ray diffraction. As shown in Figure 3, ^{95}Mo static NMR spectra of $\{Mo_{12}(La)\}$ were measured under moderate (9.4 T) and ultrahigh magnetic fields (21.8 T). By simulation of the NMR spectra and density functional theory (DFT) calculation for isolated anions, NMR parameters for two molybdenum sites within the crystals were obtained. It was found that the d^1 electrons in $\{Mo_{12}(La)\}$ are localized and used to form four Mo^V–Mo^V bonds.

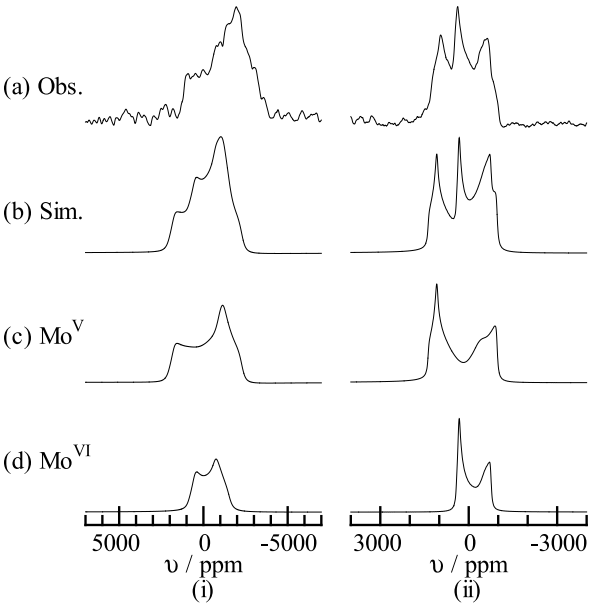

Figure 3. ^{95}Mo NMR spectra of $[\varepsilon\text{-}PMo_{12}O_{36}(OH)_4\{La(H_2O)_{2.75}Cl_{1.25}\}_4]27H_2O$ under (i) 9.4 and (ii) 21.8 T. (a) and (b) show the observed and simulated spectra, respectively. (c) and (d) denote spectral components consisting of the spectrum (b).