A Method for Dynamic Nuclear Polarization Magic Angle Spinning NMR of Membrane Proteins

Smith, A.N. (U. of Florida, Chemistry); Caporini, M.A. (Bruker Biospin Corp.); Fanucci, G.E. (U. of Florida, Chemistry); Long, J.R. (U. of Florida, Biochemistry & Molecular Biology)

Introduction

Dynamic nuclear polarization (DNP) MAS ssNMR has the potential to enhance NMR signals by orders of magnitude and to enable NMR characterization of proteins which are inherently dilute, such as membrane proteins. In this work we demonstrate that spin labeled lipid molecules (SL-lipids), when utilized as the polarizing agents, lead to large and relatively homogeneous DNP enhancements throughout the lipid bilayer and to an embedded lung surfactant mimetic peptide, KL_4. Specifically, DNP MAS ssNMR experiments at 600 MHz/395 GHz on KL_4 reconstituted in liposomes containing SL-lipids reveal DNP enhancement values over two times larger for KL_4 compared to liposome suspensions containing the biradical TOTAPOL. These findings suggest an alternative sample preparation strategy for DNP MAS ssNMR studies of lipid membranes and integral membrane proteins.

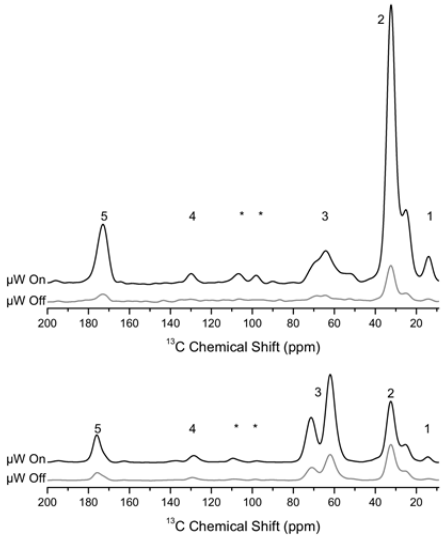

Figure 1: ^{13}C CP MAS μW on (black) and off (grey) spectra of proteoliposomes with SL – lipids (top) or TOTAPOL (bottom) as the polarizing agent. The observed resonances are labeled as follows: 1 lipid terminal methyls, 2 sp^3 carbons of lipid acyl chain, 3 lipid glycerol backbone, 4 sp^2 carbons of unsaturation point in lipid acyl chain, 5 lipid carbonyls and $^{13}C'$-L12 enriched KL_4, * resonances from detection of material in the rotor end cap. The lipid glycerol backbone resonances were masked by the glycerol in the bulk solvent of the TOTAPOL system.

Experimental

Proteoliposomes were made by mixing the lipids in chloroform (1:1 mole ratio DPPC-d_{62}:POPG) with the appropriate amount of KL_4 in methanol for a 1:50 peptide to lipid ratio, evaporating the solvents, resuspending the lipid film in cyclohexane, and lyophilizing the sample to ensure a dry, solvent-free lipid powder. The SL-lipids TEMPO-PC and 5-Doxyl PC were mixed in with DPPC-d_{62} and POPG as chloroform solutions for the SL-lipid sample. Proteoliposomes were formed by hydrating the lipids in BisTris buffer pH 7.4 90:10 (v/v) D_2O:H_2O (SL-lipid sample) or 60:30:10 (v/v/v) glycerol-d_8:D_2O:H_2O containing TOTAPOL (TOTAPOL sample) followed by multiple freeze/thaw cycles. Proteoliposomes were pelleted to remove excess buffer and packed into 3.2 mm sapphire rotors via centrifugation. All ^{13}C CP MAS DNP ssNMR experiments were carried out in the NMR User Facility of the NHMFL using a Bruker 600 MHz/ 395 GHz DNP Avance spectrometer equipped with a 3.2 mm DNP MAS HCN probe. Samples were spun at 5 kHz, ~ 100 K and irradiated with ~ 11 W of gyrotron output power.

Results and Discussion

Figure 1 shows the ^{13}C DNP MAS ssNMR spectra of proteoliposomes with either SL-lipids or TOTAPOL as the polarization reagent. Upon inspection it is apparent that the SL-lipid system provides a higher degree of DNP enhancement when compared to the traditional TOTAPOL system. In addition, the observed DNP enhancement remains uniform throughout the lipid bilayer in the SL-lipid system. However, in the TOTAPOL system the most enhancement is observed at the periphery of the lipid bilayer and the least in the interior of the membrane. The measured enhancements of the proteoliposome samples are given in Table 1.

Table 1: Measured DNP enhancements values

Resonance	Measured Enhancement	
	SL-lipids	TOTAPOL
$^{13}C'$-L12 KL_4 Peptide	8.9	3.7
Glycerol Backbone	6.5	3.4
Carbonyls	8.9	3.6
sp^3 Carbons of Lipid Acyl Chain	7.9	1.7
sp^2 Carbons of Unsaturation Point	6.4	2.4
Terminal Methyls	15	3.5

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