Solid State ^{2}H NMR Characterization of BMP Membranes

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Introduction

Bis(monoacylglycero)phosphate (BMP) is an anionic phospholipid which has been implicated in lipid and protein trafficking. BMP18:1 is found in low concentrations throughout the cell; however, it is enriched to 13.8 mol% of total phospholipid within the late endosome. BMP18:1 is uniquely structured, possessing two glycerol moieties each with a single oleoyl acyl chain and an unusual sn1,sn1' stereoconfiguration, rendering it immune to most phospholipases[1]. We are working with our synthetic collaborator, Mike Best (UTenn), to make variations on the acyl chain length of BMP analogs and investigating the packing and dynamics of the acyl chains in hydrated lipid dispersions. We are also characterizing the effects of BMP when mixed with cholesterol, as it is believed that these two lipids will spontaneously demix and possibly related to the function of BMP in entraendosomal lipid sorting and trafficking.

Experimental

Variable temperature ^{2}H NMR was employed to investigate the acyl chain dynamics. Samples consisted of either (~6 mg) 12:0, 14:0, 16:0, or 18:0 BMP mixed with (~2 mg) deutereated 12:0, 14:0, 16:0, or 18:0 BMP. All lipid mixtures were resuspended in 50 mM HEPES buffer at pH 7.0, 100 mM NaCl, and 0.1 mM EDTA. ^{2}H NMR experiments were performed on a 500 MHz Bruker Avance III Spectrometer in the AMRIS Facility.

Results and Discussion

The ^{2}H NMR results obtained matched results previously obtained, which are shown in Figure 1. The melting temperature of the various BMP samples was determined from the ^{2}H NMR results (Table 1). We are also attempting variable temperature ^{31}P MAS ssNMR experiments to examine the chemical shift anisotropy of the BMP headgroup. We have found that although BMP is an isoform of phosphoglycerol lipids, the unusual chemical connectivity imparts higher thermotropic melting temperatures to BMP assemblies compared to PG analogs, thus making synthetic analogs of BMP of interest from a materials or drug delivery perspective.

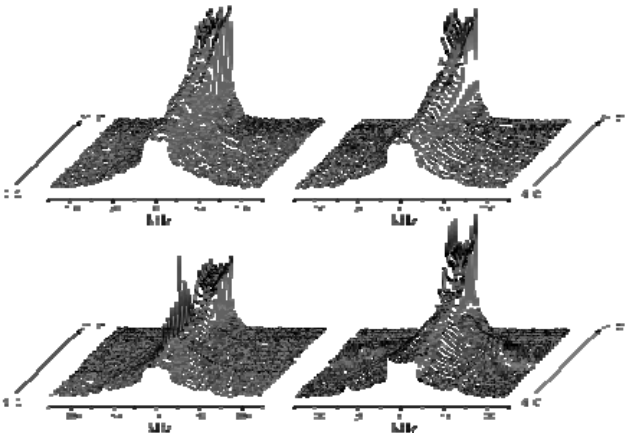

Figure 1: Plotted spectra of the ^{2}H NMR of $BMP_{12:0}$ (top left), $BMP_{14:0}$ (top right), $BMP_{16:0}$ (lower left) and $BMP_{18:0}$ (lower right) as a function of increasing temperature.

Table 1: T_m and $\Delta T_{1/2}$ values of the saturated BMP analogues: a) values for PG were taken from the Avanti Polar Lipids website and b) n. o. represents no observed transition.

	Crystalline-Gel		Gel-Liquid Crystalline		
	T_m (°C)	$\Delta T_{1/2}$ (°C)	T_m (°C)	$\Delta T_{1/2}$ (°C)	PG T_m (°C)[a]
12:0	*n.o.*[b]	*n.o.*	35.2±0.7	19.2±0.7	-3
14:0	*n.o.*	*n.o.*	50±1	6.7±0.3	23
16:0	14.6±1.0	8.2±0.9	57.74±0.13	4.69±0.11	41
18:0	28.2±0.8	14.0±0.7	67.60±0.24	4.14±0.22	55

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References

[1] Frederick et al, CPL., 163, 703711.(2010)