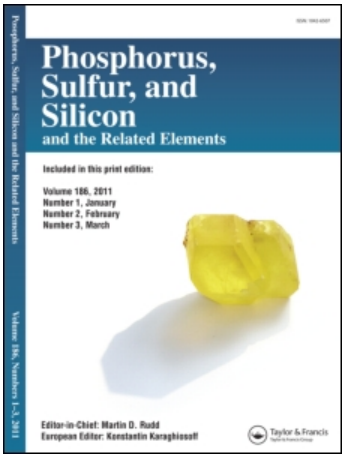

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From Molecular Conformation to Phospholipid Bilayer Organization

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FROM MOLECULAR CONFORMATION TO PHOSPHOLIPID BILAYER ORGANIZATION

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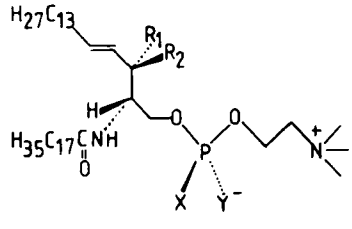
Abstract The structure and the dynamics of D-erythro-stearoylsphingomyelin has been studied by the variable temperature wide-line ^{31}P and high-resolution ^{13}C CP MAS NMR spectroscopy. The results indicate the formation of the highly ordered, relaxed gel phase, and are interpreted based on the conformation of D-erythro-sphingomyelin in the unaggregated state.

INTRODUCTION

Despite the fact, that sphingomyelin (SPM) is one of the major phospholipids of mammalian tissues, the studies of its biophysical properties have been rare, due to relative difficulties in the accesibility of well defined synthetic samples useful in such studies. Recently, we have elaborated the synthetic route leading to this lipid and to its analogues modified in both the sterostructure of the sphingosine moiety and in the phosphocholine headgroup.[1,2]



R_1=H, R_2=OH, X=Y=O, D-ERYTHRO-SPM (1)
R_1=OH, R_2=H, X=Y=O, L-THREO-SPM (2)
R_1=H, R_2=OH, X=O, Y=S, D-ERYTHRO-(S_P)-SP_SM (3)
R_1=H, R_2=OH, X=S, Y=O, D-ERYTHRO-(R_P)-SP_SM (4)
R_1=OH, R_2=H, X=O, Y=S, L-THREO-(S_P)-SP_SM (5)
R_1=OH, R_2=H, X=S, Y=O, L-THREO-(R_P)-SP_SM (6)

The synthetic lipids 1 - 6 when characterized by means of differential scanning calorimetry (DSC) were found to possess grossly different thermotropic phase properties.[3] The reversal of the configuration at C-3 of sphingosine had a large effect on the phase properties of SPMs. The substitution of phosphoryl oxygen with sulfur had different consequences in the case of D-erythro- (3,4) and L-threo- (5,6) compounds.[4,5] In the case of natural derivative 1 the stable gel phase exhibited unexpectedly high enthalpy of gel-to-liquid crystalline state

transition, indicating unusually tight molecular packing in the bilayer. NMR study in solution have indicated, that diastereomers 1 and 2 possess different distribution of conformers with varied torsional angle about C1-C2 bond of the sphingosine.[5]

RESULTS AND DISCUSSION

In this work we provide further characteristics of the gel and liquid crystalline phases of D-erythro-SPM using NMR techniques. The wideline ^{31}P nmr was used to study the phospholipid headgroup dynamics and the high resolution solid-state ^{13}C nmr spectroscopy was utilized to study sphingomyelin conformation in the different bilayer phases. The ^{31}P nmr spectra of hydrated bilayers of D-erythro-SPM (1) along with the corresponding DSC trace are shown in the Figure 1.

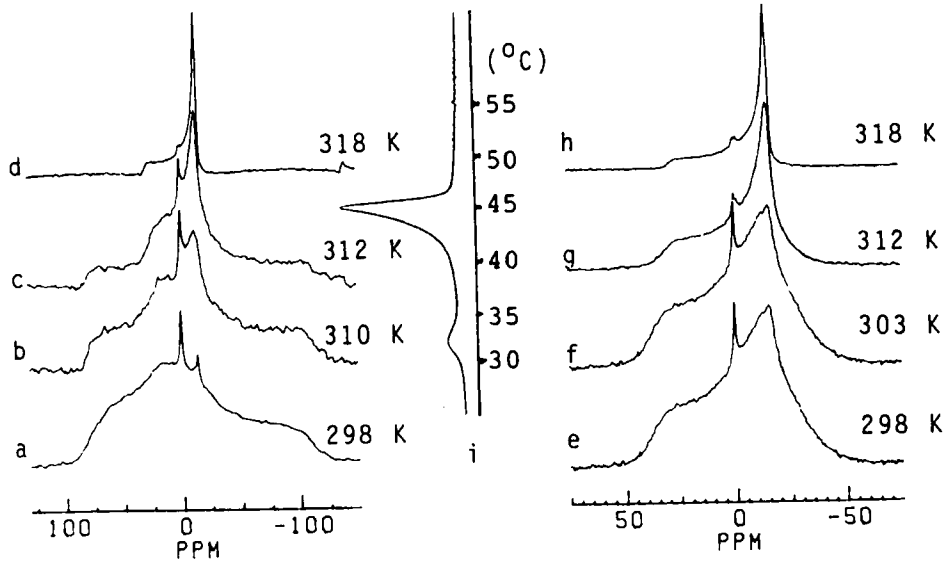


Figure 1. ^{31}P NMR spectra of unoriented water dispersions of D-erythro-SPM at indicated temperatures; traces a-d: heating of the relaxed gel phase, e-h: heating of the metastable gel phase; trace i: DSC scan starting with the relaxed gel phase.

It is clear, that the lowest temperature phase (stable up to 306 K) displays the ^{31}P chemical shielding anisotropy characteristic of the frozen rotation of the phosphate function (trace a). The formation of such rigid phase is rare among phospholipids and requires unusual stabi-

lization. It was found that the transition between the two phases (giving rise to sharp and broad component in the spectrum c) linked by the transition at 306 K is fully reversible, in contrast to the gel-to-gel relaxation starting from the metastable gel phase (from trace d to a). The metastable gel phase (e-g) displays only slightly axially unsymmetric spectral pattern characteristic of incomplete averaging of chemical shielding tensors. The main transition at 318 K leads to the axially symmetric spectral powder pattern (traces d and h) typical of the lamellar liquid crystalline phase.

The ^{13}C cross-polarization magic angle spinning (CP-MAS) spectra have recently proved a useful method of the phospholipid bilayer characterization.[7,8] The ^{13}C CP-MAS NMR spectra of various SPM phases are shown in Figure 2.

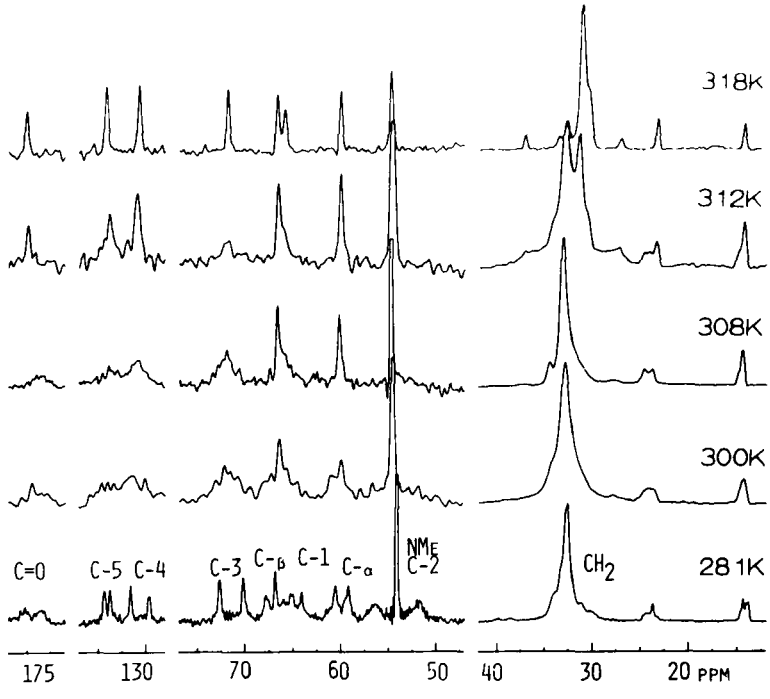


Figure 2. ^{13}C CP-MAS NMR spectra of the SPM dispersions in water; traces a-e: heating of SPM samples starting with the fully relaxed gel phase.

It appears, that the most relaxed phase below 306 K displays spectrum in which most of the lines arising from the SPM headgroup are doubled as the result of the existence of two noncongruent molecules in the bilayer structure. The largest line splittings are observed for C-2,

C-3, C-4, C-5 and carbonyl carbon. Thus, in the relaxed (stable) gel phase the restricted rotation of the phosphocholine group is accompanied by a very slow or frozen exchange between the conformers differing in the molecule medium polarity region. We propose, that the frozen exchange concerns conformers having different rotation angle about C1-C2 (θ_1) or C2-C3 (θ_3) of the sphingosine. The application of the same heating protocole as in Figure 1 resulted first in the coalescence of the two lines from C-3 carbon atom, and in the simplification of the line pattern arising from C-4/C-5. Further heating above the main transition resulted in the significant line sharpening and visualization of all lines except for NMe group of the choline function. These changes were accompanied by the hydrocarbon chain melting as evidenced by the upfield shift of the methylene group signal. Notably, the signals from terminal methyl functions of all chains appear at a greatly reduced intensities. The decrease in the line intensity is most probably implicated by the inefficiency of the cross-polarization in the liquid-like environment.[9] Interestingly, at intermediate temperatures the signals from C-1 and C-2 do not appear in the spectrum, most probably due to insufficient decoupling of the dipolar ^1H-^{13}C interaction as the consequence of the coincidence of the modulation period of the decoupling field and the rate of motion of these two sites.[10] Thus, in addition to conformational information CP-MAS spectra of SPM provide a simple means of the molecular dynamics determination at 10^{-4} s time scale.

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