DIFFERENCE IN ORIENTATIONAL ORDER IN PHOSPHOLIPID AND SPHINGOMYELIN BILAYERS

L. J. NEURINGER*, B. SEARS*, F. B. JUNGALWALA** and E. K. SHRIVER**

*F. Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA, USA and **Center for Mental Retardation, Waltham, MA, USA

Received 14 May 1979

1. Introduction

Sphingomyelin (SPM) is an important component of most biological membranes and the most abundant extraneural sphingolipid. However, much less is known about its properties in a bilayer as compared to those of phospholipids such as dipalmitoyl-phosphatidylcholine (DPPC). Recently, physical studies of SPM in bilayers have been reported and these include investigations of such characteristics as bilayer permeability [1,2], x-ray structure [3], thermotropic properties [3,4], microviscosity [5] and ^{1}H nuclear magnetic resonance (NMR) relaxation times [6]. Thus far, information concerning its state of orientational order within the bilayer has not been reported. To investigate the order within bilayers we have prepared SPM and DPPC with defined palmitic acid moieties selectively labeled with deuterium at the C-10 position. In this communication we present a comparison, in the liquid crystalline state, of unsonicated single component bilayers comprised of either DPPC or N-palmitoyl sphingomyelin (N-$C_{16:0}$ SPM) using ^{2}H NMR and differential scanning calorimetry (DSC).

2. Materials and methods

Beef brain sphingomyelin was obtained from Kock-Light. The SPM was converted to sphingosine phosphorylcholine by the method of Kaller [7]. 10-keto palmitic acid was prepared as previously described [8]. 10-d_2-palmitic acid was prepared as described by Tulloch [9]. N-$C_{16:0}$ SPM was prepared by the acylation of sphingosine phosphorylcholine with the 10-d_2-palmitic acid using the method of Boss et al. [10] and then purified by silicic acid chromatography. DPPC was prepared by acylation of glycero-phosphorylcholine with 10-d_2-palmitic acid using standard conditions [11] and was then purified by silicic acid chromatography. Both the N-$C_{16:0}$ SPM and DPPC gave single spots by TLC.

Differential scanning calorimetry measurements were performed using a high sensitivity calorimeter described previously [4]. Samples for the calorimetric and ^{2}H NMR studies were prepared from the same stock solutions. The samples were taken to dryness under N_2 gas and then pumped on overnight. The ^{2}H NMR samples were hydrated at 50°C with 0.5 ml of deuterium depleted H_2O (Aldrich). The ^{2}H NMR measurements were carried out at 41.4 MHz using quadrature detection and no proton decoupling. A 90° pulse of 28 μsec duration was used for excitation. The sample temperature was controlled to ± 0.5°C.

3. Results and discussion

The DSC experiments show that ^{2}H-labeled DPPC undergoes a transition to the liquid crystalline state at a temperature T_c = 41.0°C with a half-width of 0.6°C. The transition of ^{2}H-labeled N-$C_{16:0}$ SPM occurs at T_c = 37.2°C and has a half-width of 4°C. The relatively large width of the N-$C_{16:0}$ SPM transition is due to the heterogeneity of the sphingosine phosphoryl-choline used in the acylation reaction. Beef brain sphingomyelin contains 15% C_{18}-sphinganine, 10%

C_{20}-sphingenine and 75% C_{18}-sphingenine [12]. This heterogeneity remains in the sphingosine phosphorylcholine and also in the subsequently synthesized N-$C_{16:0}$ SPM. The heterogeneity of the sphingosine backbone would account for the wider transition and lower T_c than is observed for synthetic sphingomyelin containing either DL-N-palmitoyl-C_{18}-sphingenine or DL-N-palmitoyl-C_{18}-sphinganine. The difference in T_c of these synthetic sphingomyelins is 6°C [4]. Our N-$C_{16:0}$ SPM sample is heterogeneous with respect to the sphingosine base. Therefore, its transition occurs at a lower temperature and has a greater half-width than either of the aforementioned synthetic sphingomyelins.

Representative ^{2}H NMR spectra of N-$C_{16:0}$ SPM and DPPC at T = 53°C are shown in fig.1. It is noteworthy that the residual quadrupole splitting $\Delta\nu_Q$ of N-$C_{16:0}$ SPM is greater than that of DPPC even though its transition temperature is 4°C lower than DPPC. To make a more valid comparison we have plotted $\Delta\nu_Q$ as a function of the reduced temperature $t = (T-T_c)/T_c$, where the temperatures are in degrees Kelvin, as shown in fig.2. Due to the large width of the N-$C_{16:0}$ SPM phase transition an appreciable fraction of the N-$C_{16:0}$ SPM in the bilayer is in the liquid crystalline state 1°C below T_c and the data point at t = –0.002 reflects this fact. However, at temperatures well below T_c when the entire population of both phospholipids exists in the gel state we are unable to measure $\Delta\nu_Q$ because its magnitude exceeds the spectral window of the spectrometer. The results in fig.2 indicate that at the same reduced temperature the difference in $\Delta\nu_Q$ is approximately 20% and hence the orientational order parameter at the C-10 position is greater in a SPM bilayer than in a DPPC bilayer. This result supports the hypothesis of intermolecular hydrogen bonding within the SPM bilayer mediated by, for example, the free hydroxyl group of adjacent SPM molecules [2,6]. We have also obtained evidence that suggests the presence of other types of hydrogen bonding interactions in a ^{2}H NMR study dealing with cerebroside-phospholipid bilayers [13]. The ^{2}H NMR results represent a quantitative molecular description of the effect of such proposed hydrogen bonding in these bilayer systems.

The hydrogen bonding interaction may be important to the function of sphingolipids in biological membranes. We are currently extending our studies to other sphingolipids and to more complex sphingolipid-phospholipid membrane systems.

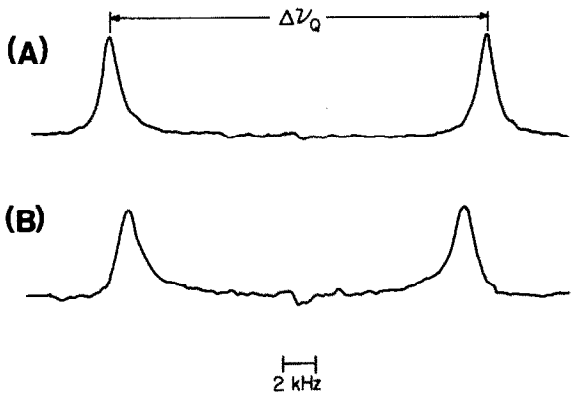

Fig.1. Deuterium magnetic resonance spectra of unsonicated single component bilayers at T = 53°C. (A) N-palmitoyl sphingomyelin deuterated at the C-10 position of the palmitic acid acyl chain (10-d_2). Concentration = 170 μM phospholipid/ml. (B) Dipalmitoylphosphatidylcholine deuterated at the C-10 positions of the palmitic acid acyl chains (di-[10-d_2-$C_{16:0}$]). Concentration = 86 μM phospholipid/ml. Deuterium NMR frequency = 41.4 MHz; 10 000 transients; 200 Hz line broadening.

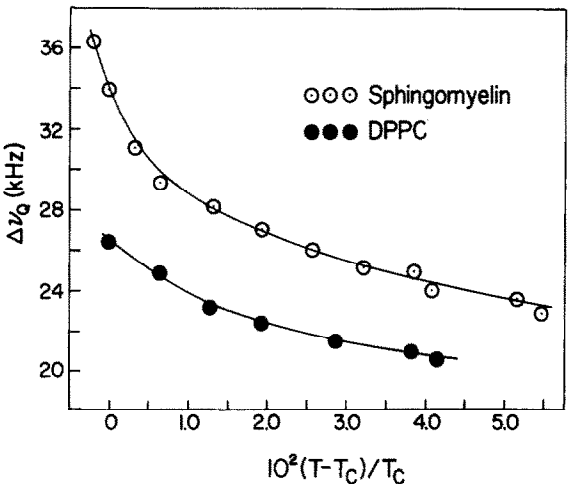

Fig.2. Variation of deuterium residual quadrupole splitting $\Delta\nu_Q$ with reduced temperature $t = (T-T_c)/T_c$ of unsonicated single component sphingomyelin and DPPC bilayers in the liquid crystalline state.

Acknowledgements

We are grateful to Drs R. Biltonen and E. Freire of the University of Virginia for the DSC experiments and to D. Gibbes for his excellent technical assistance. This work was supported by the National Science Foundation (Contract C-670), the Division of Research Resources of the NIH (Grant No. RR-00995) and NIH Grant Nos. GM-25689, HD-05515, NS-10437, and CA-16853. F. B. Jungalwala is supported by a Research Career Development Award, CA-00144.

References

[1] Hertz, R. and Barenholz, Y. (1975) Chem. Phys. Lipids 15, 138–156.
[2] Tirri, L. J., Narayan, K. N., Lipton, L. C., Chatterjee, N. and Brockeroff, H. (1978) Lipids 13, 267–269.
[3] Shipley, G. G., Avecilla, L. S. and Small, D. M. (1974) J. Lipid Res. 15, 124–131.
[4] Barenholz, Y., Suurkuusk, J., Mountcastle, D., Thompson, T. E. and Biltonen, R. L. (1976) Biochemistry 15, 2441–2447.
[5] Shinitzky, M. and Barenholz, Y. (1974) J. Biol. Chem. 249, 2652–2657.
[6] Schmidt, C. F., Barenholz, Y. and Thompson, T. E. (1977) Biochemistry 16, 2649–2656.
[7] Kaller, H. (1961) Z. Physiol. Chem. 334, 451–459.
[8] Roseman, M., Lentz, B., Sears, B. and Thompson, T. E. (1978) Chem. Phys. Lipids 2, 205–222.
[9] Tulloch, A. P. (1977) Lipids 12, 92–98.
[10] Boss, W. F., Kelley, C. V. and Landsberger, F. R. (1975) Anal. Biochem. 64, 289–292.
[11] Robles, E. C. and Van der Berg, O. (1969) Biochim. Biophys. Acta 187, 520–526.
[12] Jungalwala, F. B., Hayssen, V., Pasquini, J. M. and McCluer, R. H. (1979) J. Lipid Res. in press.
[13] Neuringer, L. J., Sears, B. and Jungalwala, F. B. (1979) submitted.