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ORIENTATIONAL ORDER IN THE CHOLINE HEADGROUP OF SPHINGOMYELIN: A ^{14}N-NMR STUDY

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An aqueous dispersion of fully hydrated bovine sphingomyelin was studied using ^{14}N-NMR spectroscopy. Spectra were obtained as a function of temperature over the range 15–80°C, in both the liquid crystal and gel phases. In the liquid crystal phase, powder pattern lineshapes were obtained, whose quadrupolar splitting slowly decreases with increasing temperature. The spectra are increasingly broadened as the temperature is lowered through the phase transition into the gel phase. The linewidths and the second moments of these spectra indicate that the onset of a broad phase transition occurs at approx. 35°C, in agreement with previous calorimetric and ^{31}P-NMR measurements. There is no evidence from the lineshapes for an hexagonal phase in this system, and this conclusion is supported by X-ray diffraction measurements carried out on aqueous dispersions of sphingomyelin in both phases. Assuming that the static nitrogen quadrupole coupling constant is the same for both sphingomyelin and dipalmitoyl-L-α-phosphatidylcholine (DPPC), the decrease observed in the quadrupolar splitting of sphingomyelin compared to that of DPPC indicates that the orientational order of the choline headgroup in liquid crystalline sphingomyelin is not the same as that of its counterpart in DPPC. Preliminary relaxation time measurements of T_1 and T_2 are presented which suggest that there are also dynamic differences between sphingomyelin and DPPC in the choline headgroup.

Introduction

Sphingomyelin is a major phospholipid component of many mammalian cell membranes, including those of brain, nerve and red blood cells [1]. Because the sphingomyelin content of these and other tissues has been linked with the aging process [2–4] and a variety of pathological conditions [5–10], it is important to determine the role that this lipid plays in the structure and function of those cell membranes involved. Only recently has there been a concerted effort to study the physical properties of sphingomyelin using such techniques as NMR spectroscopy [11–14], differential scanning calorimetry [15–18], X-ray diffraction [16,18,19–21], Raman spectroscopy [22,23], and scanning densitometry [24] which were previously applied in studies of the glycerophospholipids such as phosphatidylcholine.

Although sphingomyelin has the same phosphorylcholine headgroup as phosphatidylcholine and can replace phosphatidylcholine in some membranes such as the erythrocyte membrane [25], it is now well established that there are distinct differences between phosphatidylcholine and sphingomyelin in their properties and behaviour. Most significant is the fact that the sphingomyelins undergo a gel to liquid crystal phase transition in the physiological temperature range, 30–40°C [15,20], whereas, at these temperatures, most other mammalian cell lipids are in a liquid crystalline phase.

Abbreviation: DPPC, dipalmitoyl-L-α-phosphatidylcholine.

Moreover, at the molecular level, there exist a number of important differences between sphingomyelin and phosphatidylcholine [26]. All of these differences are confined to the hydrocarbon region and to the interface between the hydrophobic acyl chains and the phosphorylcholine headgroup. To what extent these differences in molecular structure are reflected in differences in the physical properties and dynamical structure of these lipids in bilayer systems remains an important question.

^{14}N-NMR spectroscopy [27–29] is the latest addition to a host of NMR techniques being used to probe the dynamical structure of lipid molecules in bilayer systems [30,31]. Previous NMR studies have relied on the presence of natural abundance probe nuclei such as ^{31}P [32,33], or upon the selective incorporation of ^{2}H [31,34], ^{13}C [35] and ^{19}F [36]. Nitrogen (^{14}N) has the potential of being a very useful probe because it is found in natural abundance (99.6%) in a wide variety of phospholipids, including phosphatidylcholine and sphingomyelin. Its primary advantage lies in the fact that no specific labelling is required, thus obviating the need for carrying out the biochemical synthesis of labelled molecules, as is required for ^{2}H-NMR. Secondly, by acting as a probe in the cationic region of the headgroup, it provides information complementary to that already available from ^{31}P-NMR. Because the interactions of ^{31}P, ^{2}H and ^{14}N are nuclear spin interactions and are second rank tensors, they are only partially averaged by the anisotropic motion found in lipid bilayers. The observed shielding anisotropy $\Delta\sigma$, in the case of ^{31}P, or the quadrupolar splitting $\Delta\nu$ in the case of ^{2}H or ^{14}N, can be analyzed in terms of order parameters, which provide direct information on the nature of this motion. The choline headgroup region in phospholipids has been studied previously by ^{2}H-NMR [37–39] which required the synthesis of lipids containing specifically deuterated $N(CH_3)_2$ C^2H_3 moieties, and by ^{31}P-NMR [40–42], which required the synthesis of lipids containing the phosphonium analogue of choline, $(CH_3)_3$ $P^+(CH_2)_2OH$. The dynamical structure and interactions of the choline headgroup are now directly accessible to study using ^{14}N-NMR.

Recently, we have used ^{14}N-NMR spectroscopy in a study of a model membrane system consisting of an aqueous dispersion of dipalmitoylphosphatidylcholine [29]. The purpose of this study was to exploit ^{14}N-NMR spectroscopy in a sphingomyelin model membrane system. Our aim was to characterize the phase behaviour of this system and to determine if there was any difference between sphingomyelin and phosphatidylcholine in tne orientational order of the choline headgroup.

Materials and Methods

Bovine brain sphingomyelin was purchased from Serdary (London, Ontario) and analyzed by thin-layer chromatography (TLC) on silica gel microslide plates developed in chloroform/methanol/ammonium hydroxide (conc.) (60 : 35 : 8, v/v/v). Lipids were visualized by a sulfuric acid spray, followed by charring on a hotplate. All the samples used for the NMR measurements gave a single spot at the expected position for sphingomyelin. Very heavily loaded TLC plates did, however, show trace amounts (approx. 1%) of some impurities, one running ahead of the sphingomyelin, and one with the solvent front (see discussion in Results). DPPC was purchased from Sigma Chemical Co. (St. Louis, MO) and was used without further purification.

A multilayer dispersion of lipid in excess water (greater than or equal to 40% of the total weight; lipid plus water) was prepared under nitrogen at room temperature by the addition of distilled deionized water to 400 mg dry lipid in a 10 mm outer diameter glass ampule. The ampule was then sealed under 0.5 atm N_2, and vortexed to ensure complete mixing. The samples were not preheated prior to obtaining data. After hydration, spectra were obtained as the temperature was increased from room temperature.

^{14}N-NMR spectra were obtained at a frequency of 19.438 MHz using a home-built spectrometer [43] and a Brüker superconducting solenoid (6.3 T). Digitization and Fourier transformation of the nuclear signal were carried out using a Nicolet 1090 AR digital oscilloscope interfaced to a Digital PDP-8/4 minicomputer. To retain the initial part of the free induction decay, and to minimize the effects of magnetic-acoustic ringing, a modified form of the quadrupole echo technique [44] was used. Changing the phase of the first 90° pulse by 180° on successive repititions of the basic $90°_x$-τ-$90°_y$ pulse sequence, and then alternately adding and subtracting the

nuclear signal when signal averaging permitted the accumulation of the echo signal while averaging out any coherent ringing present. The 90° pulses were 15 μs in duration, and the recycle time was 0.15 s. The digitization rate of the quadrupolar echo signal was either 5 or 10 μs, corresponding to a spectral width of 200 or 100 kHz, respectively. The frequency of the spectrometer was set at the center of the symmetric quadrupolar powder pattern, and the spectrum was folded about this central frequency, increasing the signal-to-noise ratio by a factor of $\sqrt{2}$.

The sample was enclosed in a copper oven in the NMR probe, with the oven temperature electronically regulated to within ±0.5°C. The temperature gradient across the sample was estimated to be less than 0.25°C. After a 5°C change in temperature, the sample was allowed 1 h to come to equilibrium.

T_2 was measured by observing the decay of the quadrupolar spin echo peak amplitude with pulse spacing τ. At least ten such τ values were used in a least-squares analysis of the data. T_1 was measured by observing the recovery of the magnetization following the application of a 180° pulse. The magnetization was sampled at various intervals following the inverting pulse by measuring the amplitude of the quadrupolar echo. At least ten such τ values were used in a least-squares analysis of the data.

Additional ^{14}N-NMR spectra used to measure the chemical shift of the C-N$(CH_3)_3$ group in solvent were obtained at 28.91 MHz using a Brüker WH-400 NMR spectrometer. The ^{14}N chemical shifts of DPPC, sphingomyelin and betaine ($(CH_3)_3N^+$-CH_2-COO^-) were measured relative to NH_4Br.

X-ray diffraction measurements on fully hydrated sphingomyelin dispersions were performed by using Cu K_α radiation from a sealed tube source, focused by a Francks's mirror through the sample and onto the detector. Detection of the low-angle X-ray diffraction pattern was obtained at a sample-to-detector distance of 0.25 m with a Tennelec linear position sensitive detector (Tennelec Inc., Oak Ridge, TN). Samples were sealed in a cavity formed by a hole (0.125 inch diameter) in a metal spacer (0.125 inch thick) whose faces were covered by thin (0.002 inch) mica windows. This sandwich was held in placed inside a heated sample holder.

Results

Fig. 1 illustrates the ^{14}N-NMR spectra obtained at several temperatures from an unsonicated aqueous dispersion of sphingomyelin. There are several spectral features worth noting. First, above 35°C, the spectral lineshape is a powder pattern characteristic of liquid crystalline lipid. The powder pattern lineshape arises, in an unoriented sample, from the nuclear Zeeman interaction perturbed in first order by an axially symmetry nuclear quadrupole interaction between the quadrupole moment of the ^{14}N nucleus ($I = 1$) and the surrounding electric field

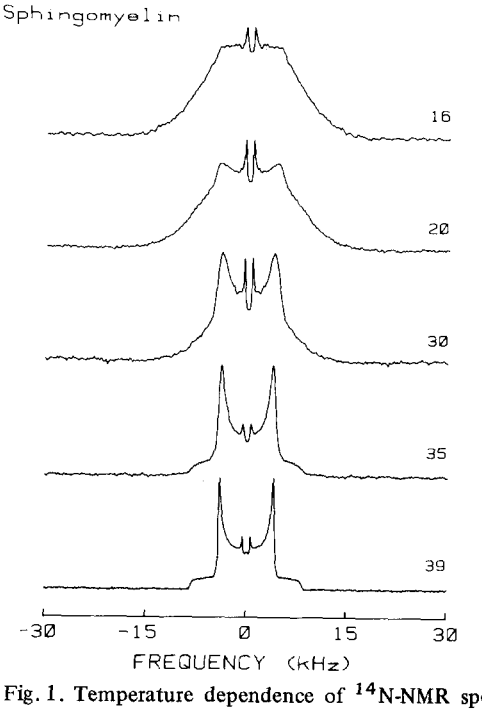

Fig. 1. Temperature dependence of ^{14}N-NMR spectra from an unsonicated dispersion of bovine brain sphingomyelin. The spectra were obtained using a modified quadrupole echo sequence with a repetition rate of 0.15 s. 262 K scans were averaged to obtain all of the spectra displayed. For these spectra at 30°C or lower, the pulse spacing τ was 180 μs, the digitization rate was 5 μs, corresponding to a spectral width of 200 kHz, and the spectra have a 400 Hz line-broadening due to exponential multiplication of the echo signal prior to Fourier transformatoin. At 35°C, τ was 160 μs, while at 39°C, τ was 210 μs. At both 35 and 39°C, the digitization rate was 10 μs, corresponding to a spectral width of 100 kHz, and the spectra have a 200 Hz line-broadening due to exponential multiplication.

gradient [31]. Below 35°C, the spectra are characteristic of gel phase lipid, with the broadening of the peaks increasing as the temperature decreases. A similar broadening of the lineshape has been observed with ^{31}P-NMR in both sphingomyelin [14] and phosphatidylcholine [45] bilayers, and has been attributed to more restricted motional averaging available for the phospholipid headgroup in the gel phase. The ^{14}N-NMR lineshapes observed in the gel phase, or in the broad phase-transition region between gel and liquid crystal, presumably arise from a slow molecular motion [46,47], the exact nature of which is yet to be determined. Certainly, the broadening is not just an increase in linewidth due to a decrease in T_2, because these spectra cannot be stimulated using the expression for a quadrupolar pattern with Lorentzian broadening corresponding to the observed T_2.

An obvious feature of the spectra, which was present throughout the temperature range investigated, is a narrow central doublet. In contrast to the spectra displayed in Fig. 1, which were obtained without the use of quadrature detection, the spectrum of Fig. 2 was acquired in quadrature mode. This spectrum shows that the 'doublets' of Fig. 1 arise from a single narrow spectral component which has a shift of −30 ppm from the central resonance frequency. The 'doublet' structure in Fig. 1 is therefore of spurious origin, and arises because the spectral lineshape has been folded over about zero frequency. The component which gives rise to the 'doublet' in these spectra has a much shorter effective spin-spin relaxation time than the component which makes the major contribution to the spectral intensity, since it is possible to significantly decrease the intensity of this narrow component relative to the major component by increasing the pulse spacing τ in the quadrupole echo sequence.

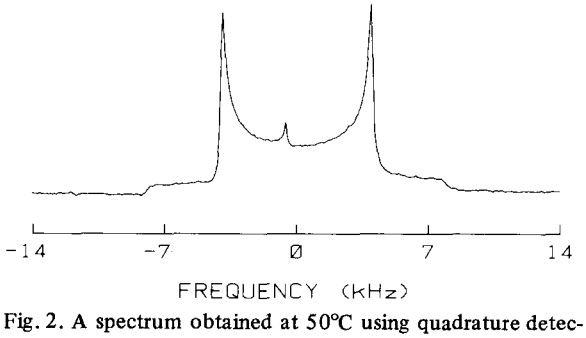

Fig. 2. A spectrum obtained at 50°C using quadrature detection, 265 K scans were averaged, the pulse spacing was 160 μs, and the digitization rate was 10 μs. The spectrum has 200 Hz of line-broadening introduced by exponential multiplication of the echo signal prior to Fourier transformation.

To determine the origin of this narrow component, some of the sphingomyelin used to make the aqueous dispersions was dissolved in chloroform/methanol (7 : 1, v/v) and the ^{14}N-NMR chemical shift of the isotropically reorienting choline group was measured relative to NH_4Br, and compared with that observed in DPPC dissolved in chloroform and in betaine dissolved in water. All of the spectra showed only one peak, whose chemical shift was +29.6 ppm for sphingomyelin, +28.9 ppm for DPPC and +27.0 ppm for betaine. Because all of these compounds have the C-N$(CH_3)_3$ group in common, the ^{14}N peaks arise from an isotropically reorienting C-N$(CH_3)_3$ group. In particular, since all of the ^{14}N signal intensity in the sphingomyelin spectrum was confined to a single peak, with no other peak within ±50 ppm, the narrow component observed in the powder pattern spectra of sphingomyelin dispersed in water can only arise from an isotropically reorienting choline group. Since this narrow component represents approx. 1% of the total spectral intensity, it is consistent with the trace amount of impurity detected by TLC. We conclude that the narrow component in the NMR spectrum arises from a choline-containing fragment, most likely resulting from the degradation of the sphingomyelin. This observation of a 1% impurity does, however, demonstrate the sensitivity of the NMR technique.

The effect of the hydrocarbon phase transition on the ^{14}N-NMR spectra is evident in the spectral lineshapes of Fig. 1, where beginning at 35°C, the powder pattern lineshape observed at higher temperatures in the liquid crystal phase becomes increasingly broadened as the temperature is lowered through the phase transition into the gel phase. These spectral changes are reversible.

A measure of the gel to liquid crystal transition temperature T_c can be obtained from the NMR spectra. The quadrupolar splittings are defined here as the separation between the two points located on the outside of the two peaks at 87% of the maximum height. Such a definition is meaningful only in the liquid-crystal phase, where there are powder patterns

with well-defined peaks. In the gel phase, the quadrupolar splittings cannot be rigorously determined [46], and the definition used above is just a measurement of the breadth of the quadrupolar pattern. As pointed out by Davis [48], a more effective means of providing a quantitative description of spectral lineshapes in mixed-phase regions or in gel phase, where sharp spectral features cannot be used or the spectrum cannot be stimulated, is the calculation of the second moment. The second moment M_2 of a spectral lineshape $F(\omega)$ is defined as:

$$M_2 = \frac{\int_0^\infty \omega^2 F(\omega)\, d\omega}{\int_0^\infty F(\omega)\, d\omega} \qquad (1)$$

The quadrupolar splittings and the second moments shown in Figs. 3 and 4, respectively, indicate that the onset of a gel to liquid crystal phase transition occurs in sphingomyelin at approx. 35°C, in agreement with previous calorimetric [15] and ^{31}P-NMR measurements [14]. Fig. 5 displays liquid crystal spectra obtained from unsonicated dispersions of both sphingo-

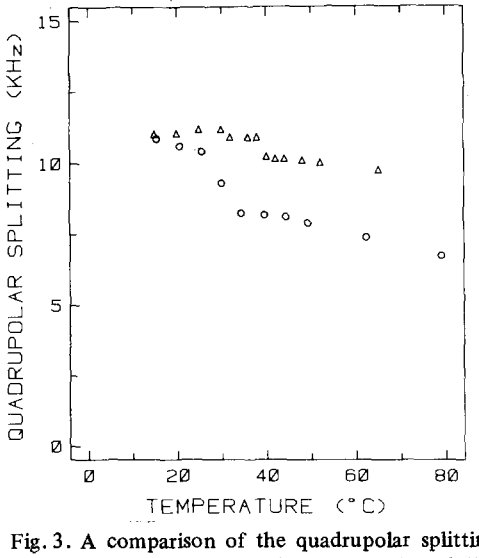

Fig. 3. A comparison of the quadrupolar splittings vs. temperature for spectra obtained from unsonicated dispersions of sphingomyelin (○) and dipalmitoylphosphatidylcholine (△). Below 30°C in either lipid, the splittings are known to within ±0.3 kHz, while at 30°C or above they are known to ±0.1 kHz.

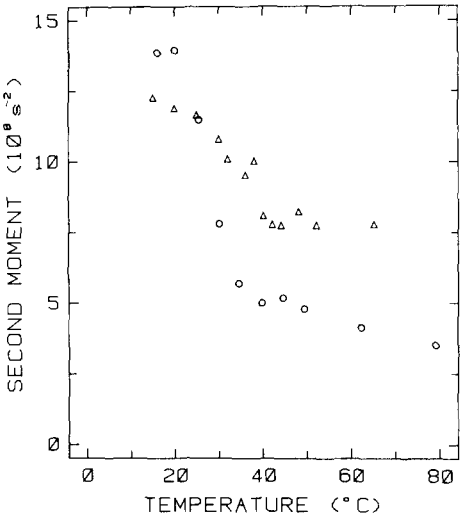

Fig. 4. A comparison of the second moment vs. temperature for spectra obtained from sphingomyelin (○) and DPPC (△). The error in the second moment, estimated to be ±5% in either phase, is largely determined by the reproducibility of the spectral lineshape.

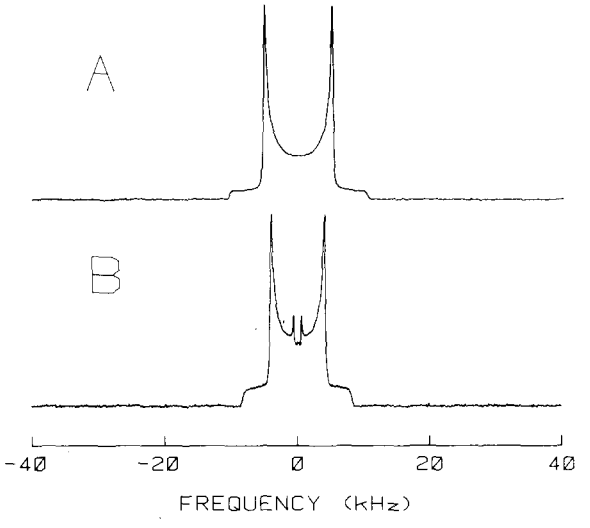

Fig. 5. A comparison of the spectral lineshapes obtained from the liquid crystalline phase of each lipid at temperatures just above their respective phase transitions. (A) spectrum is from DPPC, at a temperature of 44°C, using 131 K scans, a pulse spacing of 280 μs, and a digitization rate of 10 μs. (B) spectrum is from sphingomyelin at a temperature of 39°C, using 262 K scans, a pulse spacing of 210 μs and a digitization rate of 10 μs.

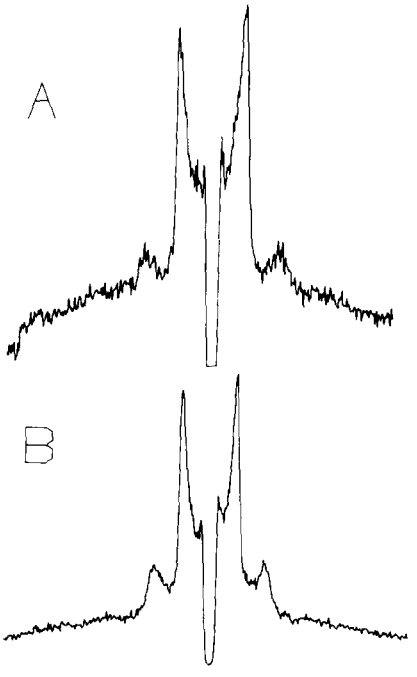

Fig. 6. Low-angle X-ray diffraction patterns of bovine sphingomyelin dispersed in excess water. Pattern A was recorded at a temperature of 54°C in the liquid crystalline phase. Pattern B was recorded at room temperature (22°C) in the gel phase. Data acquisition time was approx. 2 h for each pattern.

myelin and DPPC at temperature just above their respective phase transitions. The quadrupolar splitting of 10.2 kHz in DPPC has been reduced to 8.3 kHz sphingomyelin.

The effective spin-spin relaxation time T_2 in sphingomyelin is 2.0 ± 0.2 ms at 50°C, while in DPPC it is 1.5 ± 0.1 ms at 65°C. Although these temperatures correspond to different reduced temperatures $\theta = (T - T_c)/T_c$ of 0.55 in DPPC and 0.43 in sphingomyelin (taking 42°C as the phase transition temperature in DPPC and 35°C as the midpoint of the sphingomyelin phase transition), T_2 decreases with decreasing temperature in DPPC [28], so that at a reduced temperature of $\theta = 0.43$ in DPPC, $T_2 \leqslant 1.5$ ms. The spin-lattice relaxation time T_1 in DPPC is 64 ± 6 ms at 52°C, while in sphingomyelin, it is 37 ± 4 ms at 47°C. Although these temperatures correspond to different reduced temperatures of 0.24 in DPPC and 0.34 in sphingomyelin, T_1 increases with increasing temperature in DPPC [28], so that at a reduced temperature of $\theta = 0.34$ in DPPC, $T_1 \geqslant 64$ ms. These measurements show that in addition to the difference in quadrupolar splittings, there are also significant differences between sphingomyelin and DPPC in the relaxation times T_1 and T_2 at the same temperature. The significance of these differences will be treated in Discussion.

Despite the reduction in quadrupolar splitting as compared to DPPC, it would appear that the bulk of the sphingomyelin is in a bilayer phase. To confirm this hypothesis, an X-ray diffraction measurement was performed on a fully hydrated sphingomyelin dispersion at representative temperatures in each phase. The low-angle diffraction patterns obtained are shown in Fig. 6. In each pattern, the separation of the second order peaks is twice that of the first order, which is the characteristic signature of the lamellar phase. In the liquid crystal phase at 54°C, the lamellar repeat distance d determined from the X-ray diffraction pattern A of Fig. 6 is 75 ± 2 Å. Previous X-ray diffraction measurements on aqueous dispersions of bovine sphingomyelin by Reiss-Husson [19] and Shipley et al. [20] yielded values for d of 78 and 76 Å, respectively.

Discussion

In a ^{31}P-NMR study of bovine sphingomyelin, Yeagle et al. [12] presented evidence for a transition from a lamellar to an hexagonal phase. The change in phase was found not to be reversible upon cooling. In contrast, the ^{14}N results show an absence of any irreversible change in the spectra. If there were in fact a transition from a lamellar to a nonlamellar hexagonal phase, the additional degree of motional averaging resulting from diffusion around the cylindrical structures of that phase would halve the ^{14}N-NMR quadrupolar splitting. Because the ^{14}N-NMR spectra offer no evidence for the formation of an hexagonal phase, we conclude that in excess water, sphingomyelin forms a bilayer phase. This conclusion is supported by several recent studies [14,49] of the phase behaviour of sphingomyelin using ^{31}P-NMR, X-ray diffraction and freeze-fracture electron microscopy.

The frequency separation between the two peaks in the powder pattern spectra of sphingomyelin is given by [31]:

$$\Delta\nu = 3/4(e^2qQ/h)\{S_{zz} + \tfrac{1}{3}\eta(S_{xx} - S_{yy})\} \quad (2)$$

where e^2qQ/h is the nitrogen quadrupole coupling constant, Q is the quadrupole moment of the nucleus, and q is the field gradient. The asymmetry parameter η and the field gradient q are defined in terms of the components of the electric field gradient V_{ik} at the position of the nitrogen nucleus as

$$\eta = \frac{V_{xx} - V_{yy}}{V_{zz}} \tag{3}$$

and $eq = V_{zz}$, respectively. The order parameters

$$S_{ii} = \tfrac{1}{2}\overline{(3\cos^2\theta_i - 1)} \qquad i = x, y, z \tag{4}$$

are a time average (indicated by the bar) of the angular fluctuations of the ith coordinate axis fixed in the molecular frame with respect to the bilayer normal. In the case of sphingomyelin (or DPPC), the major contribution to the electric field gradient will come from within the C-N$(CH_3)_3$ group. Since this group possesses a C_3 symmetry axis, the asymmetry parameter η is expected to be small, and the expression for the quadrupolar splitting simplifies to

$$\Delta\nu = 3/4(e^2qQ/h)\, S_{C_\beta\text{-N}} \,. \tag{5}$$

This expression shows that the order parameter $S_{C_\beta\text{-N}}$ in sphingomyelin cannot be determined from the measured splittings $\Delta\nu$ without knowledge of the static quadrupolar coupling constant for the nitrogen nucleus in a choline group.

In the case of DPPC, where there are measurements of the deuteron quadrupolar splitting from specifically deuterated samples containing N-$(CH_3)_2$ C^2H_3 moieties [37], it is possible to estimate a value of 135 kHz for e^2qQ/h by relating the value of $S_{C_\beta\text{-N}}$ to the order parameter $S_{C\text{-}^2H}$ obtained directly from the deuteron NMR results [29]. Although this coupling constant is an order of magnitude smaller than that commonly observed for nitrogen in other compounds [50], a value of 135 kHz is consistent with previous determinations of e^2qQ/h in the C-N-$(CH_3)_3$ group of compounds such as acetylcholine bromide [51], betaine [52], and *n*-hexyltrimethylammonium bromide [53].

The anomalously low value for the quadrupolar coupling constant in the C-N$(CH_3)_3$ group is a fortuitous circumstance arising from the nearly tetrahedral symmetry in the arrangement of the four nearest neighbouring carbon atoms about the nitrogen nucleus.

For example, we have been unable to observe any ^{14}N signal from an aqueous dispersion of phosphatidylethanolamine, but this is not unexpected, since in compounds such as -CH_2-NH_3^+, where the nitrogen is covalently bonded to only one carbon, quadrupolar coupling constants are approx. 1 MHz [54,55]. As well, there is no evidence in the spectra for the amide nitrogen of sphingomyelin. This is not surprising since the bonding structure surrounding this nitrogen is identical with that in *N*-acetyl(±)-valine, whose nitrogen quadrupolar coupling constant is 3.21 MHz [56]. Even after the effects of motional averaging are included, the ^{14}N resonances of the ethanolamine headgroup or the amide group of sphingomyelin are still too broad to be observed with the present spectrometer.

Since it is unlikely that the static electric field gradient at the nitrogen nucleus is significantly different in the choline group of sphingomyelin as compared to DPPC because of the similar electronic structure, we assume that the static coupling constant is identical for both lipids. Then, because the quadrupolar splittings in liquid crystalline sphingomyelin are roughly 20% smaller than those in the corresponding phase of DPPC, it immediately follows from Eqn. 5 that the order parameters $S_{C_\beta\text{-N}}$ in sphingomyelin are also some 20% smaller.

The reduction in the $S_{C_\beta\text{-N}}$ order parameter could occur if there was a different average headgroup conformation, or a greater degree of motional averaging, or both. From a ^2H-NMR study of DPPC containing specifically deuterated N$(CH_3)_2C^2H_3$ moieties [37], it is known that the C_β-N bond vector executes rapid oscillations of large angular amplitude around an axis normal to the plane of the bilayer. A reduction in the quadrupolar splitting could occur if the amplitude of the C_β-N bond vector excursions about the axis of motional averaging is larger in sphingomyelin than in DPPC or if the time-averaged orientation of the choline group as a whole is such that the average orientation of the C_β-N bond vector is closer to the magic angle (that angle which satisfies the equation, $3\cos^2\theta_z - 1 = 0$) in sphingomyelin than in DPPC. On the basis of the lineshape differences alone, it is not possible to determine which of these two possibilities is the more significant mechanism. However, the

results of this ^{14}N-NMR study can be compared with a previous ^{1}H- and ^{31}P-NMR study [11] on sphingomyelin vesicles, which supported the idea of a different average conformation for the choline moiety and provided evidence for inter- and intramolecular hydrogen bonding which could change the average conformation of the choline moiety. In sphingomyelin, the glycerol diester moiety of phosphatidylcholine is replaced by a 2-amido-3-hydroxy-*trans*-4-ene moiety, whose amide and hydroxyl groups offer the possibility of intermolecular hydrogen bonding between sphingomyelin molecules, or intramolecular hydrogen binding between these groups and the phosphate group.

The ^{14}N relaxation time measurements can provide dynamic information on the rate of molecular motion. Because the dominant relaxation mechanism is the quadrupolar interaction, the relaxation rate is determined solely by the molecular motion of the C_β-N bond vector. For multilamellar lipid dispersions, the ^{14}N spin-lattice relaxation rate is given by [57]:

$$\frac{1}{T_1} = \frac{3\pi^2}{2}\left(\frac{e^2qQ}{h}\right)^2 (1 + \tfrac{1}{2}S_{C_\beta\text{-N}} - \tfrac{3}{2}S^2_{C_\beta\text{-N}})\,\tau_C \qquad (6)$$

where τ_c is the correlation time for the motions of the C_β-N bond vector. Although this expression shows that the relaxation time is governed by the order parameter $S_{C_\beta\text{-N}}$ and by the correlation time τ_c, the effect of molecular ordering on the relaxation rate is negligible because $|S_{C_\beta\text{-N}}| \leqslant 0.1$ for either DPPC or sphingomyelin in the liquid crystal phase. The ^{14}N measurements of T_1 in liquid-crystalline sphingomyelin and DPPC carried out in this study and those of Koga and Kanazawa [28] in DPPC show that the correlation time for the fast reorientation about the C_β-N axis is of the order of $1 \cdot 10^{-10}$ s, while proton [58] and deuterium [37] T_1 measurements of the choline methyl group have shown that the correlation time for the fast reorientation about the N-CH_3 bond axis in DPPC is of the order of $2 \cdot 10^{-11}$ s. The reduction in the sphingomyelin choline methyl proton T_1 values observed by Schmidt et al. [11] and in the sphingomyelin choline ^{14}N T_1 values observed in this study reflect an increase in the correlation times for these fast reorientational motions, possibly as a result of steric hindrances [11]. Further studies of the ^{14}N relaxation rates in sphingomyelin are now in progress to clarify the dynamics underlying the differences observed between sphingomyelin and DPPC.

Acknowledgements

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