Biochimica et Biophysica Acta 901 (1987) 191–200
Elsevier

BBA 73610

The effect of cholesterol on lipid dynamics and packing in diether phosphatidylcholine bilayers. X-ray diffraction and ^{2}H-NMR study

David J. Siminovitch, Martin J. Ruocco *, Alexandros Makriyannis ** and Robert G. Griffin

Francis Bitter National Magnet Laboratory, Massachusetts Institute of Technology, Cambridge, MA 02139 (U.S.A.)

(Received 20 March 1987)

Key words: X-ray diffraction; NMR, ^{2}H-; Diether lipid; Phospholipid; Cholesterol–phospholipid interaction; Interdigitation

In order to compare the lipid packing, conformation and dynamics of ether- and ester-linked phosphatidylcholines in the presence of equimolar concentrations of cholesterol, multilamellar dispersions of these lipid-sterol mixtures were investigated by X-ray diffraction and ^{2}H-NMR. A comparison of the X-ray diffraction patterns at 22°C of 1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphocholine (DHPC) and 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC) dispersions, each containing 50 mol% cholesterol, demonstrate that the structural characteristics of DHPC and DPPC bilayers in the presence of cholesterol are essentially indistinguishable by X-ray diffraction. In contradistinction to the similar structural characteristics of DHPC and DPPC in the presence of cholesterol, the low-angle lamellar reflections of DHPC at 22°C in the absence of cholesterol are indicative of an interdigitated phase, demonstrating that cholesterol facilitates the conversion from an interdigitated to a non-interdigitated phase. Above T_c in each lipid-sterol mixture, the quadrupolar splittings from the α-methylene segments of 1,2[1′,1′-$^2H_2$]DHPC and 1,2[2′,2′-$^2H_2$]DPPC indicate that both chain inequivalence and magnetic inequivalence of any particular α-C$^2H_2$ deuteron pair are preserved and actually enhanced in the presence of cholesterol. Lowering of the temperature below T_c in 1,2[2′,2′-$^2H_2$]DPPC/cholesterol dispersions leads to a progressive intensity loss of the *sn*-2 chain components, a thermotropic effect which is not observed in the corresponding components of the 1,2[1′,1′-$^2H_2$]DHPC/cholesterol spectra.

Introduction

The robustness and stability of ether-linked phospholipids under conditions of pH and/or temperature inimical to their ester-linked counterparts have proven to be very convenient attributes in model membrane studies. Moreover, lacking ester carbonyl groups, but otherwise isosteric with the corresponding ester lipids, ether lipids can be considered structural analogs of normal ester lipids. In comparative studies on model membranes containing ether lipids or ester lipids, these ether analogs should help to clarify the role of ester carbonyl groups in determining the packing

* Present address: NMR Research Laboratory, Department of Radiology, State University of New York, Health Science Center at Syracuse, 708 Irving Avenue, Syracuse, NY 13210, U.S.A.

** On sabbatical leave from the School of Pharmacy and Institute of Materials Science, University of Connecticut, Storrs, CT 06268, U.S.A. (1983–1984).

Abbreviations: DHPC, 1,2-di-*O*-hexadecyl-*sn*-glycero-3-phosphocholine; DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; T_c, temperature of the gel-to-liquid crystalline transition in the absence of cholesterol.

Correspondence (present address): D.J. Siminovitch, Division of Chemistry, National Research Council of Canada, Ottawa, Ontario K1A 0R6, Canada.

structure adopted by lipids in bilayers, as well as their role in intermolecular interactions between lipids, and with other membrane constituents such as sterols and proteins. In particular, the putative role of the carbonyl groups of ester-linked phospholipids in hydrogen bonding to the β-hydroxyl group of cholesterol has been studied by comparing the physical properties of ether-linked and ester-linked lipids in the presence of cholesterol [1–7]. While the interpretation of these experiments remains controversial, such studies are essential in defining the nature of the interaction between cholesterol and phospholipids. The preeminent role of cholesterol in various pathological conditions, and the high concentrations of cholesterol found in the membranes of many mammalian cells are both well established. On the other hand, our understanding of the biophysical function of cholesterol in membranes remains at a primitive stage.

The complementary techniques of X-ray diffraction and ^{2}H-NMR, which provide information on lipid packing, conformation and dynamics, will both continue to play a pivotal role in developing and refining models of the phospholipid–cholesterol interaction. In a previous report from this laboratory, we have described the results of X-ray diffraction experiments which indicate that DHPC gel-state bilayers are interdigitated [8]. In a subsequent communication [9], we showed that the effects of this interdigitation on solid-state ^{2}H-NMR lineshapes of 1,2[1′,1′-$^2H_2$]DHPC were dramatic, indicating that interdigitation has a significant effect on the dynamic properties of the alkyl chains. Specifically, the ^{2}H-NMR lineshapes of 1,2[1′,1′-$^2H_2$]DHPC were invariant to temperature from 20 °C to −20 °C, reflecting the persistence of long-axis diffusion to much lower temperatures in the interdigitated gel phase of DHPC than in the non-interdigitated gel phase of DPPC [9]. Moreover, at all temperatures in the liquid crystalline phase, four quadrupolar splittings were observed in the ^{2}H-NMR spectrum of 1,2[1′,1′-$^2H_2$]DHPC [9], in comparison to only three splittings in the corresponding spectrum of 1,2[2′,2′-$^2H_2$]DPPC [10]. The multicomponent nature of the DHPC spectrum was attributed to a combination of inequivalent *sn*-1 and *sn*-2 alkyl chains, and magnetically inequivalent deuteron pairs on both α-methylene groups. Using the same physical techniques, we have extended these previous studies to an investigation of the effects of cholesterol on DHPC bilayers, and report on these effects in this communication by comparing DHPC and DPPC dispersions, each in the presence of an equimolar concentration of cholesterol.

Materials and Methods

X-ray diffraction. Synthetic DHPC and DPPC (Calbiochem-Behring Corp., La Jolla, CA) were purified by silicic acid chromatography and shown to be > 99% pure by thin-layer chromatography. Lipid dispersions containing 50 wt% water were prepared by centrifuging appropriate amounts of dry lipid and water through a narrow constriction in a sealed tube at a temperature greater than T_c. To prepare equimolar lipid/cholesterol dispersions, appropriate amounts of dry lipid and cholesterol (Nu-Chek Prep, Inc., Elysian, MN) were dissolved in methylene chloride/methanol and the solvent removed under N_2 in a water bath at 50 °C. After removal of residual solvent under vacuum overnight, the dried lipid/sterol mixture was transferred to a centrifuge tube with a narrow constriction, and an equal weight of distilled water was added by syringe. The tube was then sealed, and the lipid/cholesterol dispersion was equilibrated as described above for the pure lipid dispersions. Following equilibration, homogeneously mixed aliquots were placed in 1 mm i.d. X-ray capillary tubes (Charles Supper Co., Natick, MA). The capillary tubes were flame sealed and placed in a sample holder kept at constant temperature (±0.5 °C) by a circulating solvent/water bath. X-ray diffraction data were recorded using both film and position-sensitive detector counter methods. For film recording, nickel-filtered Cu Kα radiation (λ = 1.5418 Å) from an Elliot GX-6 rotating anode X-ray generator (Elliot Automation, Borehamwood, U.K.) was collimated by either a Franks double mirror or a toroidal mirror. Diffraction patterns were recorded on Kodak No-Screen X-ray film (Eastman Kodak Co., Rochester, NY). For counter recording, Cu Kα radiation from a microfocus X-ray generator (Jarrell-Ash, Waltham, MA) was line-focussed by a single mirror and collimated by using the slit

optical system of a Luzzati-Baro camera (E^{TS}, Beaudoin, Paris, France). X-ray diffraction patterns were recorded with a linear position-sensitive detector (Tennelec, Oak Ridge, TN) and associated electronics (Tracor Northern, Middleton, WI).

NMR spectroscopy. Specifically deuterated DHPC, 1,2[1′,1′-$^2H_2$]DHPC, was synthesized in this laboratory as described previously [9]. ^{2}H-NMR experiments were performed at a frequency of 45.3 MHz using a home-built solid-state pulse spectrometer and a superconducting solenoid (6.8 T). ^{2}H-NMR line shapes were obtained by using a two-pulse quadrupole echo sequence, 90°_x-τ-90°_y [11], with a 90° pulse length of 2.0–2.5 μs and a pulse separation of 40–60 μs. Phase cycling and quadrature detection were used for all of the NMR experiments [12].

Results and Discussion

Recently, there has been considerable interest in phospholipids which, either spontaneously [8,13–21], or in the presence of inducing agents such as glycerol [22–24] or protein [25], assemble into interdigitated bilayers. The degree of interdigitation, which may range from complete in octadecyl-2-methyl-phosphatidylcholine [20], involving both head groups and hydrocarbon chains, to partial in mixed-chain phospholipids with substantially different chain lengths, such as sphingomyelin [16], will depend on the packing geometry of the interdigitating lipids, a property easily modulated by conditions of temperature, hydration or even pressure [26]. A notable difference between completely hydrated DPPC and DHPC in the absence of cholesterol is that in dispersions of DHPC, X-ray diffraction measurements indicate the existence of an interdigitated lamellar gel phase at all temperatures below the pretransition at 35° C [8], whereas DPPC dispersions do not form an interdigitated phase at any temperature, and will only interdigitate under conditions of extreme pressure ($\geq$ 150 MPa) [26]. However, in the presence of an equimolar concentration of cholesterol, this difference between DPPC and DHPC in the tendency to form interdigitated bilayers is abolished. Fig. 1 compares the X-ray diffraction patterns of DHPC at 22° C in the absence (Fig. 1A) and in the presence (Fig. 1B) of cholesterol. As demonstrated in an earlier report [8], the low-angle lamellar reflections ($n = 3$) of DHPC dispersions (50 wt% H_2O) shown in Fig. 1A are indicative of an interdigitated gel bilayer phase. The addition of cholesterol in equimolar concentrations gives the X-ray diffraction pattern shown in Fig. 1B. The most outstanding differences between the X-ray diffraction patterns shown in Fig. 1 are the increased lamellar periodicity of the DHPC/cholesterol bilayers (d_{av} = 67 Å) (Fig. 1B) compared to that of DHPC bilayers (d_{av} = 48 Å) (Fig. 1A), and the absence in the diffraction pattern of DHPC/cholesterol dispersions of a relatively well-defined wide angle reflection characteristic of gel phase bilayers (Fig. 1B). The low-angle region in DHPC and DHPC/cholesterol dispersions at 20° C is shown in greater detail in the X-ray diffraction patterns of Fig. 3 (left-hand panel), obtained using a position sensitive detector. The drastic shift in the first- and second-order peaks of the first two low-angle re-

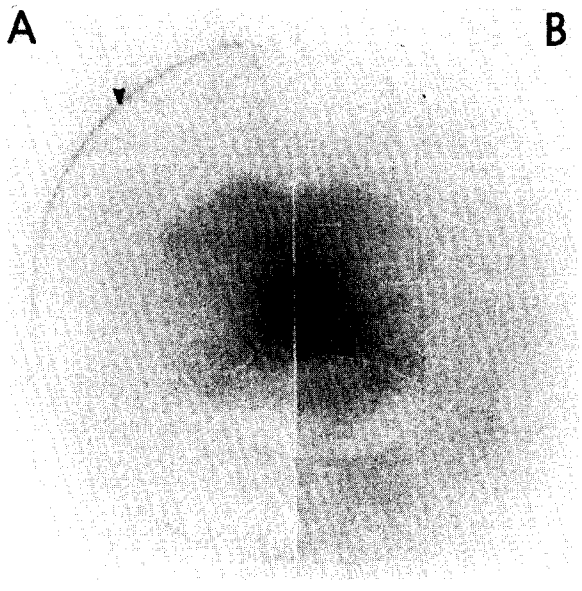

Fig. 1. X-ray diffraction patterns of (A) DHPC/H_2O (50 wt% water) at 22° C, where the arrows indicate, from left to right, the wide angle reflection at 1/4.1 Å^{-1} and the second order lamellar reflection (lamellar periodicity = 47 Å); and (B) DHPC/cholesterol, 1/1 mol ratio, (50 wt% water) at 22° C, where the arrow indicates the second order reflection (lamellar periodicity = 65 Å). The diffraction patterns were recorded using a Franks double mirror focussing camera, with a sample to film distance of 191.5 mm.

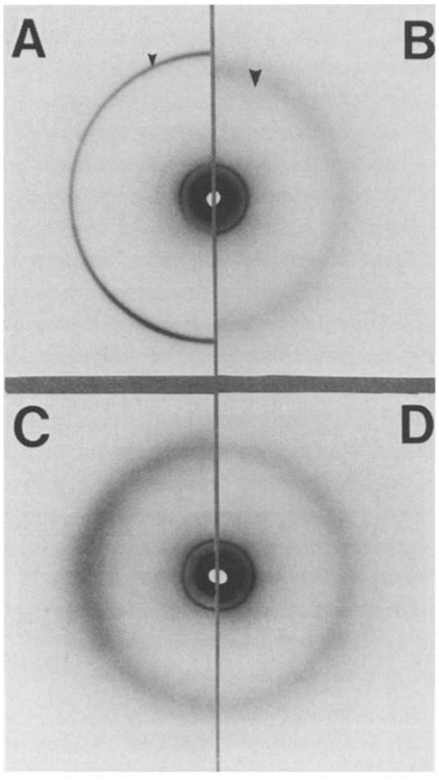

Fig. 2. X-ray diffraction patterns of (A) DHPC/H_2O (50 wt% water) at 22°C, where the arrow indicates the sharp, wide angle reflection at 1/4.17 $Å^{-1}$; (B) DHPC/cholesterol, 1/1 mol ratio (50 wt% water) at 22°C, where the arrow indicates the broad, diffuse wide angle reflection at 1/4.7 $Å^{-1}$; (C) DPPC/cholesterol, 1/1 mol ratio (50 wt% water) at 22°C; and (D) same as (B). The diffraction patterns were recorded using a toroidal mirror focussing camera, with a sample to film distance of 63.85 mm.

flections upon incorporation of cholesterol graphically illustrates the effect of cholesterol in increasing the lamellar periodicity in DHPC/cholesterol bilayers.

Fig. 2 (top) also compares the X-ray diffraction patterns of DHPC in the absence (Fig. 2A) and in the presence (Fig. 2B) of cholesterol, but now obtained with a toroid camera, which demonstrates the wide-angle reflections more clearly. Interdigitated DHPC gel phase bilayers yield a relatively sharp, symmetrical reflection at 1/4.17 $Å^{-1}$ (Fig. 2A), which is characteristic of lamellae with lipid alkyl chains hexagonally packed in a two-dimensional lattice, with no molecular tilt [27], whereas in DHPC/cholesterol bilayers, the broad, diffuse reflection at 1/4.7 $Å^{-1}$, similar to that observed from liquid crystalline DPPC or DHPC bilayers, is characteristic of a disordered phase. Fig. 2 (bottom) compares the X-ray diffraction patterns of DPPC (Fig. 2C) and DHPC (Fig. 2D), both obtained in the presence of equimolar concentrations of cholesterol. The striking similarity of these patterns, with broad diffuse wide-angle reflections at 1/4.7 $Å^{-1}$, and low-angle lamellar reflections characteristic of non-interdigitated bilayers of identical lamellar periodicity ($d_{av} = 67$ Å), demonstrate that at 22°C, the structural characteristics of DHPC and DPPC bilayers in the presence of cholesterol are indistinguishable by X-ray diffraction. As shown in Fig. 3 (right-hand panel), the same is true at high temperatures, since the low-angle regions of the X-ray diffraction patterns of DPPC/cholesterol and DHPC/cholesterol bilayers at 51°C are very similar.

The conversion of DHPC bilayers from an interdigitated phase in the absence of cholesterol to a non-interdigitated phase in the presence of cholesterol can be easily explained using a simple geometrical argument. As reported previously [8], the DHPC headgroup surface area in the interdigitated gel lamellae is 79 $Å^2$, compared to 48 $Å^2$ for that of DPPC in non-interdigitated bilayers at 20°C [28]. In both the ester- and ether-linked phospholipids, the hydrocarbon chain cross-sectional area is approx. 40 $Å^2$ per molecule. Clearly, the packing surface areas of the hydrocarbon chains and the headgroup in DPPC are relatively well matched, leading to the spontaneous formation of regular non-interdigitated bilayer assemblies. In contrast, the DHPC headgroup and hydrocarbon chain surface area mismatch (79 $Å^2$ vs. 40 $Å^2$, respectively) requires an interdigitated assembly in which four hydrocarbon chains, two from a lipid molecule in one membrane monolayer, and two from the opposing monolayer, are necessary for a thermodynamically stable interdigitated bilayer phase. Increasing the DHPC hydrocarbon surface area by melting the bilayer or introducing cholesterol (surface area ≈ 38 $Å^2$ [29,30]), which partitions into the hydrophobic hydrocarbon portion of the bilayer, creates a more favorable match between the headgroup and hydrocarbon surface areas. Thus, liquid crystalline DHPC or equimolar DHPC/cholesterol bilayers

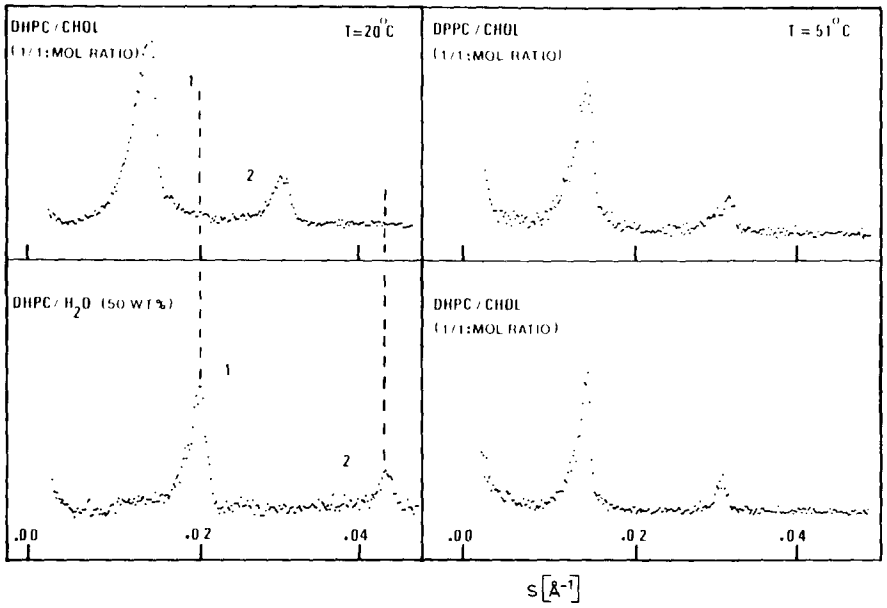

Fig. 3. Left-hand panel: X-ray diffraction patterns of DHPC/cholesterol, 1/1 mol ratio (top), and DHPC/H_2O (bottom), both 50 wt% water, at 20 °C. Right-hand panel: Diffraction patterns of DPPC/cholesterol, 1/1 mol ratio (top), and DHPC/cholesterol, 1/1 mol ratio (bottom), both 50 wt% water, at 51 °C. The diffraction patterns were recorded using a line-focussed X-ray beam and a linear position-sensitive detector.

are most stable in a non-interdigitated phase.

In this study, we have also used ^{2}H-NMR to compare the interaction of cholesterol with ester- and ether-linked phosphatidylcholines in which the first methylene group of both hydrocarbon chains has been deuterated. At these segment positions in either lipid bilayer, any specific interaction with cholesterol which would alter either the average orientation of the α-C^{2}H$_2$ groups or the amount of motional averaging of the C-^{2}H bond segments should be manifest in the ^{2}H-NMR spectrum. Hydrogen bonding between the β-OH group of cholesterol and the carbonyl groups [31] or the glycerol ester oxygens [32] of DPPC, or the ether oxygens of DHPC, should be considered as a possible candidate for such an interaction. With or without cholesterol, the multicomponent nature of the ^{2}H-NMR spectra of DHPC and DPPC is clear from an inspection of Fig. 4. They are all the more remarkable when one considers that for DPPC or DMPC labeled at any one of the methylene segments between the C-3′ and the C-12′ position of the *sn*-2 chain, only one quadrupole splitting is observed in the presence or in the absence of cholesterol [33]. In fact, the distinctive DPPC spectra of the C-2′ segment position in comparison to those observed from segments labeled further down the chain can be directly related to the glycerol backbone conformation. In

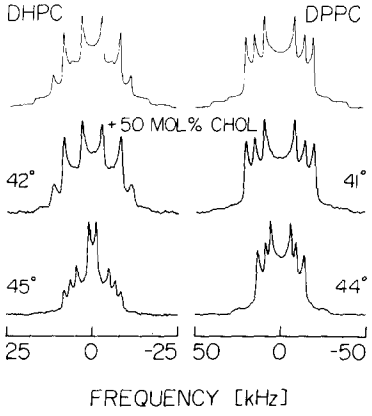

Fig. 4. Representative ^{2}H-NMR spectra of the chain-labeled phosphatidylcholines in the presence (middle) and in the absence (bottom) of an equimolar concentration of cholesterol, together with spectral simulations (top) of the lipid/cholesterol spectra. Simulation parameters are given in Table I. Left, 1,2[1′,1′-^{2}H$_2$]DHPC/cholesterol; right, 1,2[2′,2′-^{2}H$_2$]DPPC/cholesterol.

this conformation, which is common to all naturally occurring ester-linked phospholipids, and is independent of the nature of the polar headgroup [34], the degree of unsaturation [35,36], or the presence of perturbing molecules such as cholesterol [37], the glycerol backbone is oriented almost perpendicular to the bilayer surface, with the *sn*-1 chain continuing in this direction, whereas the *sn*-2 chain begins parallel to the membrane surface and then is bent perpendicular to it after the C-2′ segment [35,38–40]. Thus, the multicomponent nature of the DPPC spectrum, labeled at the C-2′ position of both chains, is now easily understood, and reflects both inequivalence between chains at the interface [10,41], and magnetic inequivalence of the *sn*-2 chain deuterons [42]. The component with the largest quadrupole splitting can be assigned to the C-2′ segment of the *sn*-1 chain [41], while the two smaller splittings observed from the corresponding segment of the *sn*-2 chain are due to magnetically inequivalent deuterons [42]. An unequivocal assignment of the components observed in the corresponding spectrum of DHPC, labeled at the C-1 position of both chains, will require individual labeling of the chains, and possibly stereospecific labeling at the C-1 positions. Nevertheless, simulations of the 1,2[1′,1′-$^2H_2$]DHPC spectrum suggest that not only are the deuterons of each α-C^2H_2 group magnetically inequivalent, but that the alkyl chains at this segment position are also inequivalent [9]. There is one component in the spectrum for each deuteron, giving rise to four quadrupole splittings, the two smaller of which correspond to one chain, while the two larger arise from the other. Quadrupole splittings of 4.5 and 10.5 kHz are observed in the liquid crystalline phase of 2[1′,1′-$^2H_2$]DHPA (Stewart, L. and Smith, I.C.P., personal communication), indicating that if chain inequivalence in ether-linked lipids is also invariant to changes in polar headgroup, the inner pair of splittings in the spectrum of 1,2[1′,1′-$^2H_2$]DHPC arises from the *sn*-2 chain. We shall assume this is the case in interpreting the effect of cholesterol on the spectra of 1,2[1′,1′-$^2H_2$]DHPC (*vide infra*).

Before commenting on the significance of the lipid/cholesterol spectra, we note that our interpretation of these lineshapes rests on two basic assumptions, which we state explicitly at the outset. First, for any distinct component identified in the absence of cholesterol, we assume the quadrupole splitting of that component will increase in the presence of cholesterol. Second, we assume that, as in 1,2[2′,2′-$^2H_2$]DPPC, the two smaller quadrupole splittings in the spectrum of 1,2[1′,1′-$^2H_2$]DHPC arise from magnetically inequivalent deuterons on the α-C^2H_2 group of the *sn*-2 chain. Neither of these assumptions are particularly restrictive or unreasonable, but in the absence of spectral data on 2[1′,1′-$^2H_2$]DHPC, they are the minimum set required to interpret the DHPC/cholesterol lineshapes.

Fig. 4 shows that in the presence of cholesterol, three quadrupole splittings are observed in the ^{2}H-NMR spectra of both DPPC and DHPC. However, we also note that in DHPC, the number of components is reduced from four in the absence of cholesterol to three components in the presence of cholesterol, whereas three components only are observed in DPPC bilayers with and without cholesterol, as reported previously [10,37]. Without cholesterol, magnetic inequivalence of both pairs of α-methylene deuterons in 1,2[1′,1′-$^2H_2$]DHPC and chain inequivalence at the C-1 segment position result in a one-to-one correspondence between each deuteron and one of the components observed in the ^{2}H-NMR spectrum. Therefore, the smaller number of components observed in the spectrum of DHPC/cholesterol bilayers can only be the result of identical quadrupole splittings for one pair of deuterons, either from the same α-C^2H_2 group or one each from the α-C^2H_2 groups of different chains. Although the former possibility could be excluded by an assumption that cholesterol will not alter magnetic inequivalence of an α-methylene deuteron pair, it is sufficient in this case to assume that the quadrupole splittings of all components increase in the presence of cholesterol, in order to rule out the possibility of a magnetically equivalent deuteron pair on the same α-C^2H_2 group. Spectral simulations of the lipid/cholesterol lineshapes are shown in Fig. 4, and although they are not completely successful in reproducing all of the observed spectral features in each case, they are adequate to demonstrate that three components are observed in the DHPC/cholesterol spectrum because two deuterons, each on a different alkyl

TABLE I

SIMULATION PARAMETERS FOR PC/CHOLESTEROL MIXTURES

$\Delta\nu_Q$ is quadrupolar splitting and $\Delta\nu$ is Lorentzian line width at half-height. Parameter values in brackets are those for lipids in the absence of cholesterol [9].

	Relative intensity	$\Delta\nu_Q$ (kHz)	$\Delta\nu$ (Hz)
DHPC	1	6.15 (2.34)	380 (320)
	1	16.8 (9.45)	430 (320)
	1	16.8 (13.1)	430 (320)
	1	23.0 (16.8)	540 (320)
DPPC	1	18.5 (11.8)	635 (635)
	1	30.2 (17.6)	700 (635)
	2	40.5 (27.4)	765 (955)

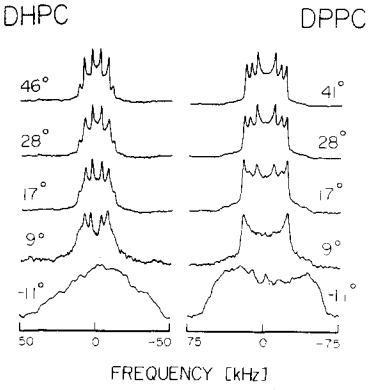

Fig. 5. Temperature dependence of the ^{2}H-NMR spectra of the ester- and ether-linked phosphatidylcholines labeled at the α-methylene segments of both hydrocarbon chains, in the presence of an equimolar concentration of cholesterol. Left, 1,2[1′,1′-$^2H_2$]DHPC/cholesterol; right, 1,2[2′,2′-$^2H_2$]DPPC/cholesterol.

chain, give rise to the same intermediate quadrupole splitting of 16.8 kHz. Simulation parameters for the lipid/cholesterol lineshapes are given in Table I, together with the corresponding parameters determined previously [9] for each lipid lineshape in the absence of cholesterol. For the DPPC/cholesterol spectrum at 41°C, quadrupole splittings of 18.5, 30.2 and 40.5 kHz determined as simulation parameters agree very well with previously measured values at 43°C [37] of 18.4, 30.0 and 40.8 kHz, respectively, and suggest that the simulation procedure provides quadrupole splittings of sufficient accuracy to make reliable comparisons. (In view of both Lorentzian and Gaussian broadening in the axially symmetric powder pattern lineshapes of liquid crystalline lipid [43,44], the Lorentzian line broadening values given in Table I are not judged to be significant, either in absolute terms, or for the purposes of comparison, and are tabulated only for the sake of completeness.) Comparing the effect of cholesterol on the quadrupole splittings of DHPC and DPPC given in Table I, it is clear that in both ester- and ether-linked phosphatidylcholines, chain inequivalence is not removed by the addition of cholesterol. In fact, in both lipids, addition of cholesterol actually enhances the difference between the two chains.

Fig. 5 compares the temperature dependence of the ^{2}H-NMR spectra of 1,2[1′,1′-$^2H_2$]DHPC/cholesterol and 1,2[2′,2′-$^2H_2$]DPPC/cholesterol dispersions. Beginning at temperatures above T_c, and continuing to temperatures at least 30 degrees below T_c, the ^{2}H-NMR lineshapes of both lipid/cholesterol dispersions are axially symmetric powder patterns, whose breadth does not increase significantly as the temperature decreases. Thus, even though the quadrupole splittings have increased at $T > T_c$ (see Table I), reflecting the ordering effect of cholesterol at these segment positions in DHPC and DPPC, the rates of C-^{2}H bond motion due to axial diffusion or internal modes of reorientation are still in fast limit at these temperatures, and remain so down to temperatures at least 30 degrees below T_c. Previous ^{2}H-NMR studies of DMPC/cholesterol dispersions have also noted that spectra characteristic of fluid phase lipids were observed 20–40 degrees below T_c when the cholesterol concentration was high [45]. Around 9°C, Fig. 5 shows that the lineshapes of both lipid/cholesterol mixtures begin to broaden, and to suffer from significant losses in quadrupole echo signal intensity which obviously degrade the signal-to-noise ratio of these spectra. Similar echo intensity losses were observed in a ^{2}H-NMR study of DMPC/cholesterol mixtures, and were used to provide a quantitative measure of the transition breadth in this system [45]. By 0°C in either DPPC/cholesterol or DHPC/cholesterol, these intensity losses are so severe that undistorted spectra are impossible to

obtain. Estimates of the quadrupole echo decay rate indicate that $T_{2e} \leqslant 80$ μs in either system at 0 °C, among the shortest values measured for ^2H nuclei in C-^2H bonds [43], and rivaled only by T_{2e} in DPPC bilayers at temperatures 30 degrees higher, in the $P_{\beta'}$ phase, and 30 degrees lower, in the L_β phase [9,46]. As expected, as the temperature is lowered further to about −10 °C in each system, the signal intensities recover, and much broader lineshapes, characteristic of more ordered, gel phase hydrocarbon chains, are now observed. Representative examples of these lineshapes at −11 °C are shown in Fig. 5 for each system. There can be no doubt that at these temperatures, the lipids have completed a transition to a more ordered state in which the rates of C-^2H bond reorientation are no longer in fast limit. It seems likely that, as in the $P_{\beta'}$ phase of pure DPPC or DHPC [9], the rapid decay of the quadrupole echo intensity in the lipid/cholesterol systems at 0 °C is due to an intermolecular exchange process occurring at an intermediate rate, as the lipid/cholesterol matrices undergo the transition to the lower temperature ordered state.

Despite the superficial similarity in the temperature dependence of the DHPC/cholesterol and DPPC/cholesterol lineshapes just discussed, there is one spectral feature whose thermotropic behaviour allows us to easily distinguish between DHPC and DPPC in their interaction with cholesterol. A careful inspection of the DPPC/cholesterol lineshapes in Fig. 5 shows that as the temperature decreases, there is a progressive intensity loss in the two components which correspond to the magnetically inequivalent deuterons of the *sn*-2 chain. Thus, although at 41 °C, the apparent intensity of the 90° peaks of either of these components is greater than that of the *sn*-1 chain, by 17 °C, both *sn*-2 chain components are less intense than the *sn*-1 chain component, and at 9 °C, they are no longer evident in the spectrum, leaving only one, albeit slightly broadened, powder pattern due to the *sn*-1 chain deuterons. On the other hand, in the DHPC/cholesterol lineshapes, there is no detectable loss in the intensity of the corresponding *sn*-2 chain components relative to that of the *sn*-1 chain components. Even though the 90° peaks of one of the magnetically inequivalent deuterons of the *sn*-2 chain overlap those of an *sn*-1 chain component, the 90° peaks in the Pake pattern of the other deuteron, which give rise to the smallest quadrupole splitting in this lineshape, can easily be followed as a function of decreasing temperature. They are still clearly evident at 9 °C, and except for line broadening effects, do not appear to have suffered any noticeable losses in intensity relative to the intensities of the *sn*-1 chain components. Thus, in the presence of equimolar concentrations of cholesterol, and at temperatures below T_c, there is a marked difference between DPPC and DHPC in the thermotropic behaviour of those spectral components due to magnetically inequivalent *sn*-2 chain deuterons.

Although the DPPC/cholesterol spectra of Fig. 5 represent the first documentation of the loss in intensity of the *sn*-2 chain components relative to that of the *sn*-1 chain component as a function of temperature, there are earlier reports which describe the difficulty in observing the signals of the *sn*-2 chain components in the presence of cholesterol using single pulse techniques. For example, in the DPPC/cholesterol system, motional inequivalence of the *sn*-1 and *sn*-2 chains was manifest as the apparent disappearance of the 2[2′,2′-$^2H_2$]DPPC signals at 25 °C in the presence of 29 mol% cholesterol, although the signals from the 2′-position of the *sn*-1 chain were still easily detected [37]. In a related study of the DMPC/cholesterol system at 10 °C, no signal from 2[2′,2′-$^2H_2$]DMPC in the presence of 30 mol% cholesterol could be detected, although the *sn*-1 chain signal was easily seen in 1,2[2′,2′-$^2H_2$]DMPC [33]. With the acquisition of high-fidelity spectral lineshapes using the quadrupole echo technique [11], any difficulty in observing the *sn*-2 chain components using a single 90° pulse was circumvented, and thus the two components of the *sn*-2 chain deuterons were clearly apparent in the quadrupole echo line shape of 2[2′,2′-$^2H_2$]DMPC in the presence of 50 mol% cholesterol [45]. In agreement with the experimental results on 2[2′,2′-$^2H_2$]DMPC in the presence of cholesterol [45], the quadrupole splittings of the *sn*-2 chain components in 1,2[2′,2′-$^2H_2$]DPPC do not increase significantly as they lose intensity (see Fig. 5). Thus, the distinctive and unusual spectroscopic behaviour of the *sn*-2 chain components in DPPC or DMPC in the presence of cholesterol is purely a

dynamical effect, and is not accompanied by any significant changes in spectral width. The simplest explanation of this behaviour in DPPC is that the interaction with cholesterol results in a significant increase in the quadrupole echo decay rate of the *sn*-2 chain deuterons at the 2′-position. That this does not happen in DHPC may be evidence for a specific interaction between the adjacent carbonyl groups of DPPC and the β-hydroxyl group of cholesterol. The fact that the 2′-position of the *sn*-2 chain in DPPC may be in a hindered configuration, and thus more susceptible to reductions in the rate of a lipid molecule rigid body motion, is not likely to explain the unusual spectroscopic behaviour of the *sn*-2 chain components in DPPC. If hindering alone were the explanation, then we would expect to observe the same phenomenon in 1,2[1′,1′-$^2H_2$]DHPC, where the 1′-position of the *sn*-2 chain is, if anything, in a more hindered configuration than the 2′-position of the *sn*-2 chain in DPPC.

Acknowledgements

We acknowledge Dr. D.M. Small for making available the facilities of the Biophysics Institute at the Boston University School of Medicine. We also thank Drs. David Atkinson and Graham Shipley for the use of the X-ray diffraction facilities, and David Jackson for technical assistance.

References

1 Schwarz, F.T., Paltauf, F. and Laggner, P. (1976) Chem. Phys. Lipids 17, 423–434
2 Fong, J.W., Tirri, L.J., Deshmukh, D.S. and Brockerhoff, H. (1977) Lipids 12, 857–862
3 Smaby, J.M., Hermetter, A., Schmid, P.C., Paltauf, F. and Brockman, H.L. (1983) Biochemistry 22, 5808–5813
4 Bittman, R., Clejan, S., Lund-Katz, S. and Phillips, M.C. (1984) Biochim. Biophys. Acta 772, 117–126
5 Hermetter, A., Lohner, K., Degovics, G., Laggner, P. and Paltauf, F. (1985) Chem. Phys. Lipids 38, 353–364
6 Levin, I.W., Keihn, E. and Harris, W.C. (1985) Biochim. Biophys. Acta 820, 40–47
7 Massey, J.B., She, H.S. and Pownall, H.J. (1985) Biochemistry 24, 6973–6978
8 Ruocco, M.J., Siminovitch, D.J. and Griffin, R.G. (1985) Biochemistry 24, 2406–2411
9 Ruocco, M.J., Makriyannis, A., Siminovitch, D.J. and Griffin, R.G. (1985) Biochemistry 24, 4844–4851
10 Seelig, A. and Seelig, J. (1974) Biochemistry 13, 4839–4845
11 Davis, J.H., Jeffrey, K.R., Bloom, M., Valic, M.I. and Higgs, T.P. (1976) Chem. Phys. Lett. 42, 390–394
12 Griffin, R.G. (1981) Methods Enzymol. 72, 108–174
13 Hauser, H., Pascher, I. and Sundell, S. (1980) J. Mol. Biol. 137, 249–264
14 Serrallach, E.N., Dijkman, R., De Haas, G.H. and Shipley, G.G. (1983) J. Mol. Biol. 170, 155–174
15 Jain, M.K., Crecely, R.W., Hille, J.D.R., De Haas, G.H. and Gruner, S.M. (1985) Biochim. Biophys. Acta 813, 68–76
16 Levin, I.W., Thompson, T.E., Barenholz, Y.B. and Huang, C. (1985) Biochemistry 24, 6282–6286
17 Hui, S.W. and Huang, C. (1986) Biochemistry 25, 1330–1335
18 Mattai, J., Witzke, N.M., Bittman, R. and Shipley, G.G. (1986) Biochemistry 26, 623–633
19 Pascher, I. and Sundell, S. (1986) Biochim. Biophys. Acta 855, 68–78
20 Pascher, I., Sundell, S., Eibl, H. and Harlos, K. (1986) Chem. Phys. Lipids 39, 53–64
21 Sheridan, J.P. (1986) Biophys. J. 49, 314a
22 McDaniel, R.V., McIntosh, T.J. and Simon, S.A. (1983) Biochim. Biophys. Acta 731, 97–108
23 McIntosh, T.J., McDaniel, R.V. and Simon, S.A. (1983) Biochim. Biophys. Acta 731, 109–114
24 O'Leary, T.J. and Levin, I.W. (1984) Biochim. Biophys. Acta 776, 185–189
25 Boggs, J.M. and Moscarello, M.A. (1982) Biophys. J. 37, 57–59
26 Braganza, L.F. and Worcester, D.L. (1986) Biochemistry 25, 2591–2596
27 Tardieu, A., Luzzati, V. and Reman, F.C. (1973) J. Mol. Biol. 75, 711–733
28 Janiak, M.J., Small, D.M. and Shipley, G.G. (1979) J. Biol. Chem. 254, 6068–6078
29 Pethica, B.A. (1955) Trans. Faraday Soc. 51, 1402–1411
30 Small, D.M. (1977) J. Colloid Interface Sci. 58, 581–602
31 Brockerhoff, H. (1974) Lipids 9, 645–650
32 Presti, F.T., Pace, R.J. and Chan, S.I. (1982) Biochemistry 21, 3831–3835
33 Oldfield, E., Meadows, M., Rice, D. and Jacobs, R. (1978) Biochemistry 17, 2727–2740
34 Browning, J.L. and Seelig, J. (1980) Biochemistry 19, 1262–1270
35 Büldt, G., Gally, H.U., Seelig, A., Seelig, J. and Zaccai, G. (1978) Nature (London) 271, 182–184
36 Seelig, J. and Waespe-Sarcevic, N. (1978) Biochemistry 17, 3310–3315
37 Haberkorn, R.A., Griffin, R.G., Meadows, M.D. and Oldfield, E. (1977) J. Am. Chem. Soc. 99, 7353–7355
38 Hitchcock, P.B., Mason, R., Thomas, K.M. and Shipley, G.G. (1974) Proc. Natl. Acad. Sci. USA 71, 3036–3040
39 Pearson, R.H. and Pascher, I. (1979) Nature (London) 281, 499–501
40 Zaccai, G., Büldt, G., Seelig, A. and Seelig, J. (1979) J. Mol. Biol. 134, 693–706
41 Seelig, A. and Seelig, J. (1975) Biochim. Biophys. Acta 406, 1–5
42 Engel, A.K. and Cowburn, D. (1981) FEBS Lett. 126, 169–171

43 Davis, J.H. (1983) Biochim. Biophys. Acta 737, 117–171
44 Rance, M., Smith, I.C.P. and Jarrell, H.C. (1983) Chem. Phys. Lipids 32, 57–71
45 Jacobs, R. and Oldfield, E. (1979) Biochemistry 18, 3280–3285
46 Meier, P., Ohmes, E. and Kothe, G. (1986) J. Chem. Phys. 85, 3598–3614