Biochimica et Biophysica Acta, 644 (1981) 266–272
Elsevier/North-Holland Biomedical Press

BBA 79270

DEUTERIUM MAGNETIC RESONANCE OF SELECTIVELY DEUTERATED CHOLESTERYL ESTERS IN DIPALMITOYL PHOSPHATIDYLCHOLINE DISPERSIONS

HEINER GORRISSEN [a], ALEX L. MACKAY [b], STEPHEN R. WASSALL [a], MARKO I. VALIC [b], ALEXANDER P. TULLOCH [c] and ROBERT J. CUSHLEY [a,*]

[a] *Department of Chemistry, Simon Fraser University, Burnaby, British Columbia, V5A 1S6,* [b] *Department of Physics, University of British Columbia, Vancouver, British Columbia, V6T 1W5, and* [c] *Prairie Regional Laboratory, National Research Council of Canada, Saskatoon, Saskatchewan, S7N 0W9 (Canada)*

(Received December 17th, 1980)

Key words: Cholesteryl ester; Phospholipid dispersion; ^{2}H-NMR; Powder pattern; Lipid conformation

Dispersions (50 wt% water) containing 95 mol% dipalmitoyl phosphatidylcholine/5 mol% deuterated cholesteryl palmitate (or stearate) were studied using ^{2}H-NMR. Incorporation of ester into the phospholipid bilayer was found to be 0.5 mol% at 50°C. From the profile of ^{2}H quadrupolar splitting vs. chain position, support for an average conformation resembling a 'horseshoe' within the bilayer is obtained. Quadrupolar relaxation times T_{2e} of approx. 250 μs and approx. 850 μs are measured for cholesteryl palmitate-2,2-d_2 and cholesteryl palmitate-16,16,16-d_3, respectively, which are less than one-half those obtained for the corresponding positions in dipalmitoyl-d_{62} phosphatidylcholine. This is ascribed to a slower rate of motion of the ester chain and/or an extra, slow motion of the molecule.

Introduction

Recently, we have been studying the properties and structural organization of cholesteryl esters in a variety of phospholipid model membrane systems by means of magnetic resonance techniques [1–7]. In our initial study ^{31}P-NMR was used to investigate the effect of cholesteryl ester incorporation on membrane permeability [1]. Subsequently, ESR was employed to observe spin-labelled cholesteryl esters incorporated into phosphatidylcholine multilayers [2,3]. A drawback of the latter method is that it is not known to what extent the spin label may perturb the system. Hence, in our recent work, we have utilized ^{2}H-NMR which has been shown to be an excellent non-perturbing probe of local orientational order and mobility in lipid bilayers [8,9].

Using ^{2}H-NMR of cholesteryl palmitate-d_{31} and cholesteryl palmitate-16,16,16-d_3, we have investigated the solubility and structural organization of cholesteryl esters in aqueous dispersions (liposomes) of egg phosphatidylcholine [4] and sphingomyelin [6]. Furthermore, we have studied selectively deuterated cholesteryl esters in phospholipid unilamellar vesicles produced by sonication [5,7]. The interpretation of ^{2}H-NMR is easier with multilamellar liposomes for which the spectra are powder patterns with peaks separated by

$$\Delta\nu_Q = \frac{3}{4} \cdot \frac{e^2qQ}{h} \left\langle \frac{3\cos^2\theta - 1}{2} \right\rangle \quad (1)$$

where (e^2qQ/h) is the static quadrupolar coupling constant, θ is the angle between the C-^{2}H bond and the axis of reorientational motions for this bond, and the angular brackets denote a time average over all conformations of the molecule. Eqn. 1 assumes that the asymmetry parameter $\eta = 0$ [10].

In the present communication we report the results of a ^{2}H-NMR study of selectively deuterated

---

[*] To whom reprint requests should be addressed.

cholesteryl palmitate and cholesteryl stearate in dipalmitoyl phosphatidylcholine liposomes. We have also recorded the spectra from cholesteryl palmitate-d_{31} in dipalmitoyl phosphatidylcholine liposomes in order to gain additional information and to corroborate the results obtained with the selectively deuterated esters. This was aided by application of a new data analysis technique which enables one to obtain the ^{2}H-NMR spectrum of an oriented system from the powder pattern.

Materials and Methods

Dipalmitoyl phosphatidylcholine was purchased from Sigma Chemical Co. Deuterium-depleted water was obtained from Aldrich Chemical Co. Hexadecanoic-d_{31} acid, hexadecanoic-5,5,6,6-d_4 acid and hexadecanoic-11,11,12,12-d_4 acid were purchased from Merck, Sharp and Dohme, Canada, Ltd. Hexadecanoic-16,16,16-d_3 acid was bought from Serdary Research Laboratories, London, Ontario, Canada. Syntheses of hexadecanoic-2,2-d_2, hexadecanoic-3,3-d_2, hexadecanoic-4,4-d_2, and octadecanoic-8,8-d_2 acids were described previously [5] as was the description of the synthesis of the deuterated cholesteryl esters from the corresponding acids [11].

NMR samples were prepared by dissolving approx. 400 mg of dipalmitoyl phosphatidylcholine and cholesteryl ester (95 : 5 mol%) in chloroform/methanol (3 : 1, v/v). The solvent was then removed by evaporation under a stream of nitrogen and subsequent overnight pumping under high vacuum. Lipid dispersions were prepared by adding approx. 400 mg of deuterium-depleted water to the dry lipids and vigorously shaking with a vortex mixer at approx. 50°C until the sample appeared homogeneous.

The ^{2}H-NMR measurements were carried out at 37.2 MHz with a Bruker SXP 4-100 NMR spectrometer and a Nalorac superconducting magnet. The sample temperature was oven regulated to ±0.1°C with a Bruker BST 100-700 temperature controller. An Intel 8080A microprocessor-based computer interfaced to a Nicolet 1090 AR digital oscilloscope was used to collect the NMR signals and Fourier transforms were calculated on a Nicolet BNC-12 minicomputer.

Data was acquired starting at the peak of the quadrupolar echo generated by a $\pi/2|_{0^\circ}$-τ_1-$\pi/2|_{90^\circ}$ pulse sequence [12]. The value of τ_1 was 50–100 μs unless otherwise indicated and the sequence was repeated after a time τ_r. The $\pi/2$ pulse length was 5–6 μs. For every second pulse sequence, the phase of the first pulse was changed by 180° and alternate scans were subtracted from the accumulative data memory, resulting in the cancellation of coherent receiver and pulse noise.

Data processing necessary to obtain the 'aligned' spectra was performed using an Amdahl V6 computer.

Results

We have obtained ^{2}H-NMR spectra of cholesteryl palmitate and cholesteryl stearate selectively deuterated along the acyl chain in dipalmitoyl phosphatidylcholine aqueous dispersions at 50°C and these are shown in Fig. 1. The three features labelled A, B and C in Fig. 1h, having spectral widths of $\leqslant 1$, 3 and 37 kHz, respectively, are interpreted in the same way as previously for cholesteryl esters in egg phosphatidylcholine and sphingomyelin dispersions [4,6] viz. C is attributed to regions of solid ester in the sample, B is attributed to dissolved ester; i.e., ester molecules intercalated in the bilayer and A, which comprises only a small part of the spectral intensity, is attributed to ester incorporated into structures which are small enough for lateral diffusion and/or particle tumbling to result in isotropic ester motion, plus residual ^{2}HOH.

The solubility of cholesteryl palmitate in dipalmitoyl phosphatidylcholine liposomes may be determined from the spectrum in Fig. 1h. To do this, it is not sufficient to merely compare the relative intersity of the spectral component due to incorporated ester (feature B) to the sum of the intensities for incorporated plus solid ester (features B + C), since the effects of relaxation must also be taken into account. The effects of spin-lattice relaxation may be ignored since the delay of $\tau_r = 1$ s between quadrupolar echo pulse sequences used is approx. 3-times longer than both the T_1 for solid cholesteryl palmitate-16,16,16-d_3 (approx. 0.25 s) [4] and T_1 for dissolved cholesteryl palmitate-16,16,16-d_3 (approx. 0.4 s) (determined in dipalmitoyl phosphatidylcholine vesicles [7]). However, the ratio of signal intensities of dissolved to solid ester is expected to be dependent

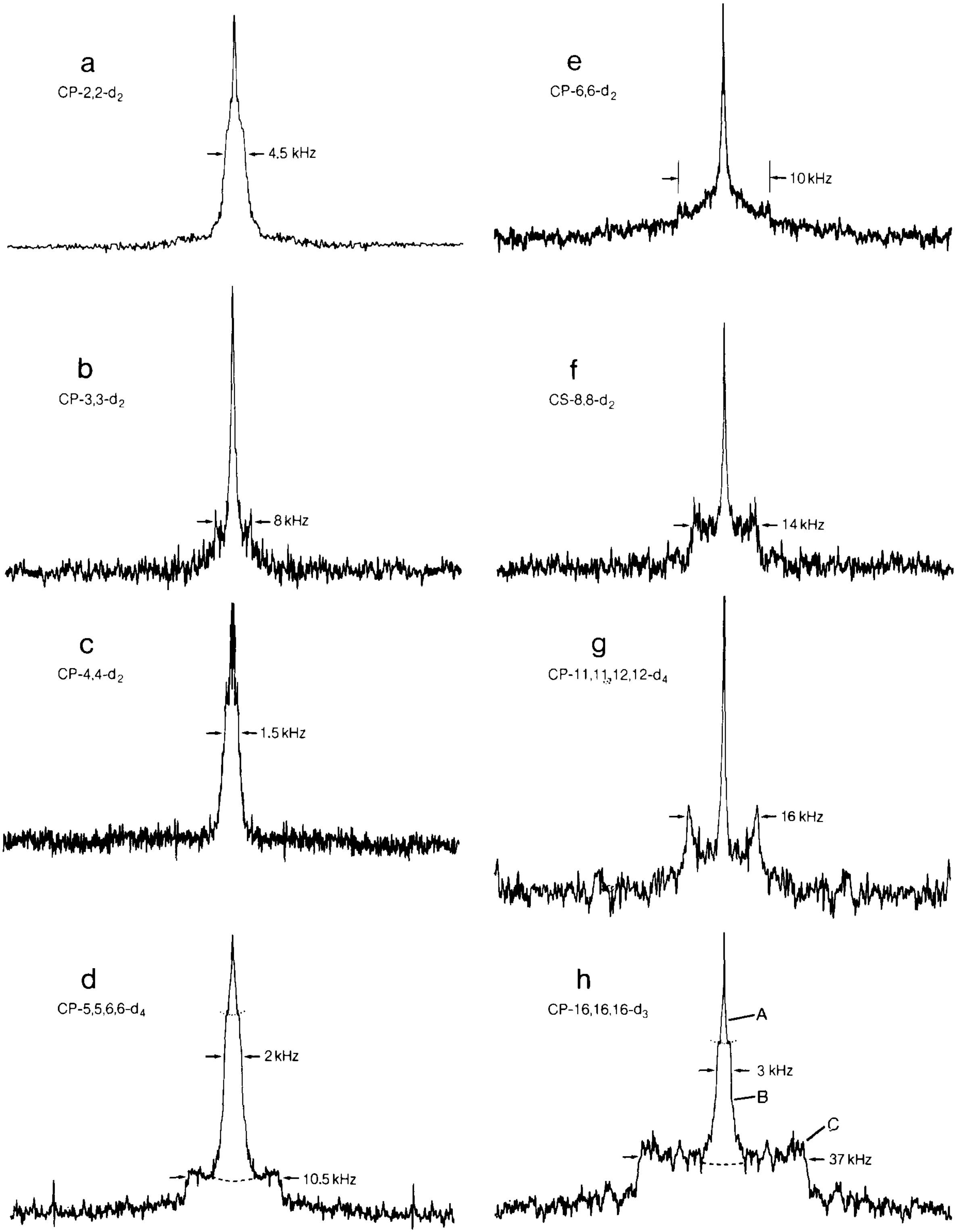

Fig. 1. ^{2}H-NMR spectra for dispersions of 50 wt.% dipalmitoyl phosphatidylcholine/selectively deuterated cholesteryl palmitate (95 : 5 mol%) in 50 wt.% deuterium-depleted water. Unless otherwise indicated, the temperature of the experiment was 50°C, the Fourier transform spectra were obtained from 1k data sets zero filled to 4k, the spectral width was 100 kHz, τ_1 = 70 μs and the line-broadening parameter LB = 100 Hz: (a) τ_1 = 80 μs; no LB; number of transients = 160 811; (b) τ_1 = 100 μs; number of transients = 441 701; (c) spectral width = 50 kHz; no LB; number of transients = 38 000; (d) temperature = 52°C; spectral width = 50 kHz; LB = 40 Hz; number of transients = 189 998; (f) number of transients = 143 452; (g) number of transients = 65 963; (h) τ_1 = 200 μs; LB = 150 Hz; number of transients = 30 000. The features A, B and C of (h) are described in the text and the dashed lines separate the features. CP, cholesteryl palmitate; CS, cholesteryl stearate.

upon τ_1, the delay between the two pulses in the quadrupolar echo sequence. The spectral intensity for a C^2H_2 or C^2H_3 group is given by

$$A(2\tau_1) = A(0)\exp(-2\tau_1/T_{2e}) \qquad (2)$$

where T_{2e} is the decay time of the quadrupolar echo. A plot of relative intensity $A(2\tau_1)$ vs. $2\tau_1$ yields values of $T_{2e} = 340$ μs for solid cholesteryl palmitate-16,16,16-d_3 (feature C) and $T_{2e} = 850$ μs for cholesteryl palmitate-16,16,16-d_3 dissolved in the bilayer (feature B). From the values of $A(0)$ obtained for incorporated and solid ester we calculate an incorporation of 0.5 mol% cholesteryl ester in the phospholipid dispersion.

The ^{2}H-NMR spectra of cholesteryl palmitate and cholesteryl stearate, selectively deuterated at different methylene chain segments, in aqueous dispersions of dipalmitoyl phosphatidylcholine (Fig. 1a–g) are interpreted in the same manner as that of the methyl deuterated compound (Fig. 1h) except that no signal from solid ester is readily apparent. This is mainly a result of extreme signal attenuation due to the short repetition time ($\tau_r = 0.1$–0.3 s) employed compared to the $T_1 = 11.4$ s for the C^2H_2 groups of solid cholesteryl palmitate-d_{31} [4]. Also, the spectral width of 100 kHz used in some cases is too small for the solid $(C^2H_2)_n$ signal ($\Delta\nu_Q$ approx. 118 kHz [4]) to be seen.

The two components attributed to incorporated ester in the spectrum for the 5,5,6,6-d_4 compound (Fig. 1d) are of approx. 1 : 1 relative intensity. From a comparison with the spectrum for the 6,6-d_2 compound (Fig. 1e *), the broader component is attributed to the C6 position and the narrower one to the C5 position. In contrast, only one component due to incorporated ester is observed with cholesteryl palmitate-11,11,12,12-d_4 (Fig. 1g) which implies the behaviour of the two segments is similar.

A graph of splitting vs. position of selective deuteration for cholesteryl palmitate and cholesteryl stearate incorporated into dipalmitoyl phosphatidylcholine liposomes at 50°C is shown in Fig. 2. The values plotted were measured as indicated in Fig. 1 and are accurate to ±1 kHz. At positions where the peaks of a powder pattern are not resolvable for incorporated ester; e.g., the C2 position, the width of the component is taken as a measure of the splitting. Elaboration upon this point will be given in the Discussion. As expected on the basis of the results observed for deuterated cholesteryl ester in egg phosphatidylcholine vesicles [5], the change from a palmitate to a stearate chain is presumed to not appreciably affect the splitting. The profile for bilayers composed of deuterated dipalmitoyl phosphatidylcholine [13,14] is presented in Fig. 2 for comparison.

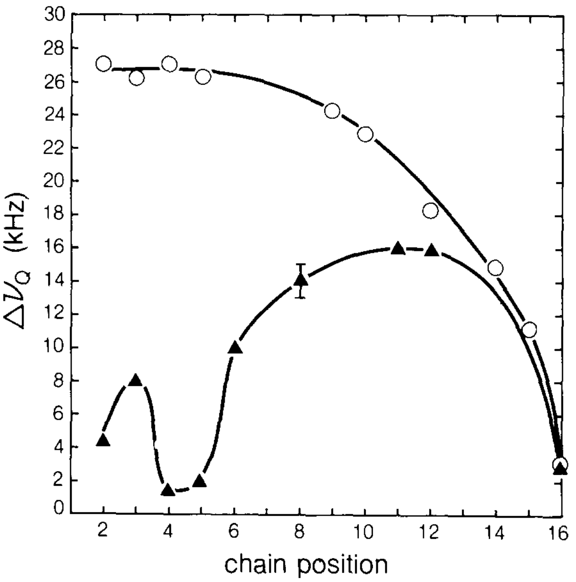

Fig. 2. Plot of ^{2}H quadrupolar splitting vs. acyl chain positions for selectively deuterated cholesteryl palmitate in dipalmitoyl phosphatidylcholine liposomes (▲———▲) and for deuterated dipalmitoyl phosphatidylcholine liposomes (○———○) [13,14] at approx. 50°C.

The ^{2}H spectrum of cholesteryl palmitate-d_{31} in dipalmitoyl phosphatidylcholine liposomes at 50°C, is shown in Fig. 3a. Only the upper half of the powder pattern, with frequencies greater than or equal to the Larmor frequency, ω_0, is plotted. The low half of the symmetrical powder pattern may be obtained, as was indeed performed on the spectra for the selectively deuterated samples, by simply reflecting the upper half about ω_0 (i.e., zero frequency in the figure). As the spectrum was collected using a $\tau_r = 0.3$ s, the signals from the $(C^2H_2)_n$ and C^2H_3 groups of solid ester are

---

* A referee has suggested that Fig. 1e does not have a typical shape corresponding to $\eta = 0$. From the spectrum, this cannot be discounted. If $\eta \neq 0$ the quadrupolar splitting would be less than the measured splitting but our interpretation would remain unchanged.

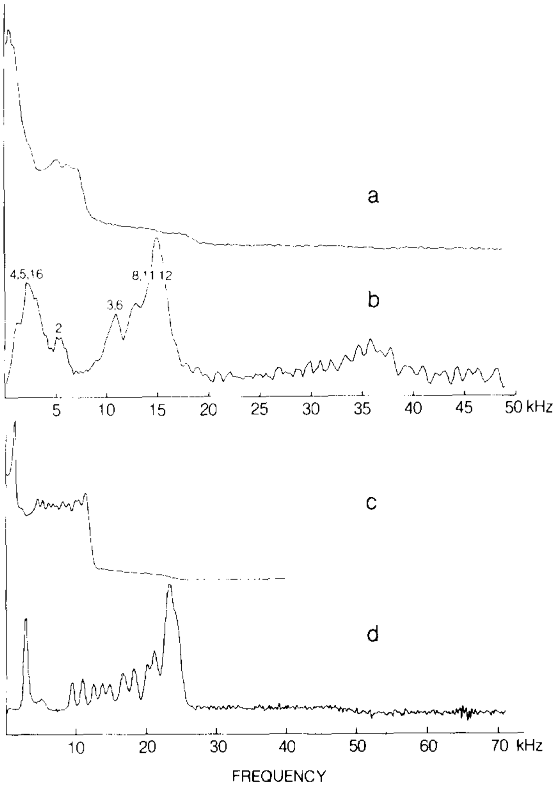

Fig. 3. High-field half of the ^2H powder pattern for (a) cholesteryl palmitate-d_{31} in dipalmitoyl phosphatidylcholine liposomes at 50°C; in the spectrum ω_0 is slightly offset and corresponds to the top of the highest peak; (b) the corresponding 'aligned' spectrum of (a); five iterations on 400 data points; (c) dipalmitoyl-d_{62} phosphatidylcholine liposomes at 50°C; (d) the corresponding 'aligned' spectrum of (c); five iterations on 400 data points.

of reduced intensity. Hence, the signal attributed to incorporated ester and the central isotropic line are the dominant spectral features. The outer edge of the signal for incorporated ester is 7 kHz from ω_0 (Fig. 3a), hence, the signal width is 14 kHz. The shape of the spectral component is essentially identical to that found for cholesteryl palmitate-d_{31} in egg phosphatidylcholine bilayers [4], where the width was measured as 12 kHz.

For cholesteryl palmitate-d_{31} in dipalmitoyl phosphatidylcholine liposomes the spectral shape was seen to be virtually independent of τ_1 (65–200 μs) and we have measured an average T_{2e} of approx. 250 μs. The value of T_{2e} measured from the τ_1 dependence of the ^2H spectrum for the 2,2-d_2 ester was also found to be approx. 250 μs.

Only a limited amount of information may be gained from the rather featureless superposition of powder patterns recorded for cholesteryl palmitate-d_{31} in dipalmitoyl phosphatidylcholine bilayers. Recently, however, a data analysis technique has been developed (Bloom, M., Davis, J.H. and MacKay, A.L., personal communication) for ^2H spectra characteristic of axial symmetry which enables one to obtain the spectrum for an oriented sample from a powder pattern. The spectrum derived in this way for the perdeuterated ester is shown in Fig. 3b. It is equivalent to the spectrum that would be obtained for an aligned sample placed with the axis of motional averaging parallel to the magnetic field and, hence, the frequency with respect to ω_0 of a line therein may be equated to the splitting $\Delta\nu_Q$ of the corresponding (unresolved) peaks in a powder pattern. Greater spectral detail is immediately obvious in this 'aligned' spectrum with five peaks becoming resolved. Comparison with the data for selectively deuterated cholesteryl palmitate, together with an analysis of the integrated intensity of the signals, allowed the partial assignment made in Fig. 3. Also, the relative intensities of the different peaks resolved in the 'aligned' spectrum were found to remain essentially constant when τ_1 was varied. This, together with the values of T_{2e} (approx. 250 μs) for both cholesteryl palmitate-d_{31} and cholesteryl palmitate-2,2-d_2, suggests that T_{2e} for C^2H_2 does not vary significantly along the chain.

Finally, in order to compare the ester with the phospholipid in which it is dissolved, the ^2H-NMR spectrum of dipalmitoyl-d_{62} phosphatidylcholine liposomes [14] is shown in Fig. 3c, with the corresponding 'aligned' spectrum given in Fig. 3d.

Discussion

The incorporation of cholesteryl palmitate into liquid crystalline dipalmitoyl phosphatidylcholine bilayers is only approx. 0.5 mol%. This value compares with the solubilities of 0.2 and 1.5 mol% found for cholesteryl palmitate in aqueous dispersions of egg phosphatidylcholine [4] and sphingomyelin [6], respectively. Increased amounts of the ester have been incorporated into sonicated phospholipid bilayers but the extent of incorporation into the resultant vesicles is still relatively low at up to 5 mol% [5,7]. The low solubility is responsible for the poor signal to noise of several of the spectra shown in Fig. 1 and illustrates a major problem in the ^{2}H-NMR study of selectively deuterated cholesteryl esters in phospholipid dispersions.

Inspection of the ^{2}H spectra in Fig. 1 for selectively deuterated cholesteryl palmitate in dipalmitoyl phosphatidylcholine liposomes reveals that, for deuterons at the 2-, 4- and 5-positions, where the splittings are small, the characteristic powder pattern is not discernible. Such spectra could arise from the superposition of powder patterns of slightly differnt splittings, produced by more than one conformation of the ester within the bilayer. However, a lower limit of 1.3 kHz (= $1/(\pi T_{2e})$) for the line broadening at the 2-position may be set on the basis of the T_{2e} of approx. 250 μs measured for cholesteryl palmitate-2, 2-d_2. Similarly, a lower limit of 1.3 kHz may be assigned to the 4- and 5-positions based on the average T_{2e} of approx. 250 μs for cholesteryl palmitate-d_{31}. Thus, for these positions the line broadening is comparable to the quadrupolar splitting $\Delta\nu_Q$, which leads to an unresolved powder pattern, especially in the presence of the central isotropic component [8]. At all other segments, with the exception of C^2H_3, the splittings for incorporated ester are at least a factor of two larger, so that the estimated line broadening is not as significant and the powder pattern peaks are resolved. In the case of cholesteryl palmitate-16,16,16-d_3 (Fig. 1h) the powder pattern peaks are obscured by the central component A even though a smaller line broadening greater than or equal to 0.4 kHz (evaluated from a T_{2e} of approx. 850 μs) is estimated.

A comparison of the two profiles in Fig. 2 shows that the cholesteryl palmitate chain does not possess the characteristic 'plateau' region of almost constant splitting ($\Delta\nu_Q \sim 26$ kHz) from segments (C2 to C9, as is the case for dipalmitoyl phosphatidylcholine [13, 14]. Instead, the splittings for incorporated ester are at least a factor of two smaller than for the phospholipid and a striking local minimum at chain positions 4 and 5 is exhibited, followed by a maximum in the vicinity of positions 11 and 12. Only approaching the terminal methyl group, where the splittings are substantially decreased, does the behaviour of the ester and the phospholipid appear to be similar. Clearly, the ordering of the acyl chain of cholesteryl palmitate is not merely that of the phospholipid chains of the surrounding matrix. The same finding was made in earlier work on selectively deuterated cholesteryl palmitate in sonicated dipalmitoyl phosphatidylcholine vesicles [7]. In fact, profiles of linewidth vs. position of selective deuteration, that are reminiscent in form to the profile of splittings presented in Fig. 2, have been obtained for cholesteryl esters in dipalmitoyl phosphatidylcholine [7] and egg phosphatidylcholine [5] vesicles. Accentuation of a linewidth maximum at positions 11 and 12 in these profiles occurred upon the addition of cholesterol and lowering the temperature.

Comparison of the 'aligned' spectra for cholesteryl palmitate-d_{31} (Fig. 3b) and for dipalmitoyl-d_{62} phosphatidylcholine (Fig. 3d) also leads to the conclusion that the organization of the ester chain is significantly different than that of the phospholipid chains. For dipalmitoyl-d_{62} phosphatidylcholine an intense resonance at approx. 26 kHz (Fig. 3d) and a series of less intense peaks with progressively smaller splittings are found. The intense resonance corresponds to the 'plateau' region of the splittings profile for the phospholipid (Fig. 2), while the other peaks are due to the remaining segments [14]. In contrast, only five peaks can be distinguished in the 'aligned' spectrum for the ester (Fig. 3b). Moreover, although the most intense peak occurs at the largest splitting (approx. 14 kHz), there is greater relative intensity for the peaks at the lower splittings compared to those for dipalmitoyl-d_{62} phosphatidylcholine. The intensities of the five peaks in Fig. 3b are consistent with the splittings predicted for the remaining positions, based on the profile in Fig. 2.

In order to explain the variation in the deuterated ester signal width with chain position, it is important to note that the quadrupolar splitting depends both on (1) the angular excursions of the C-^{2}H bond with respect to the axis of reorientation (assumed to be the bilayer normal) and (2) its average orientation with respect to this axis [8]. Rather than ascribe the local minimum in the splittings at chain position 4 and 5 to an implausibly high degree of local movement, we suggest an average bent conformation for the ester chain such that the average angles the C-^{2}H bonds at C4 and C5 make with the bilayer normal are close to the magic angle (54° 44′). This would result in very small splittings for these positions. The apparent similarity in the profiles of splitting vs. chain position recorded for dipalmitoyl phosphatidylcholine and incorporated cholesteryl palmitate near the free ends of the chain is taken to indicate that, in this region, they behave similarly and that the ester chain, on average, is parallel to the bilayer normal.

A conformation for the ester which is consistent with these results is the horseshoe conformation, where the ester linkage is close to the aqeous interface and the cholesteryl and acyl chain moieties extend into the centre of the bilayer. Such a conformation was originally proposed by Janiak et al. [15] for cholesteryl linolenate in hydrated egg phosphatidylcholine above 13% water content. Grover et al. [3], using ESR spectroscopy of spin-labelled cholesteryl esters incorporated into egg phosphatidylcholine multilayers, provided evidence for such a conformation. They found that the fatty acid segment direction at C5 is tilted at an angle of approx. 47° with respect to the bilayer normal in an oriented sample.

Further support for the horseshoe conformation of cholesteryl palmitate incorporated into dipalmitoyl phosphatidylcholine bilayers may be obtained from statistical mechanical models of membrane structure based on the mean-field approximation [16–18]. According to these theories, molecular conformations in which chain segments are oriented at a an angle with respect to the bilayer normal are energetically unfavorable. Thus, if the initial segment of the ester chain is oriented at an angle to the bilayer normal, due to its attachment to the rigid cholesteryl moiety, then a bend in the upper portion of the chain would be expected in order to align the largest possible number of remaining segments parallel to the bilayer normal.

In terms of the horseshoe conformation, it is conceivable that the proximity of the bulky cholesteryl moiety restricts the rate of motion of the ester chain and/or that the low polarity of the molecule may allow it to undergo motion(s) of low frequency. The T_{2e} values of approx. 250 and approx. 850 μs for cholesteryl palmitate-2,2-d_2 and -16,16,16-d_3, respectively, and the average T_{2e} of approx. 250 μs for cholesteryl palmitate-d_{31}, may be reconciled with either of the above cases. These values are less than one-half those determined for the corresponding segments in dipalmitoyl-d_{62} phosphatidylcholine liposomes at 37°C [14]. Clearly, the motions of cholesteryl esters and phospholipids within the bilayer are different but a detailed description of these motions has yet to be established.

Acknowledgement

We wish to thank Dr. J.H. Davis for the spectrum shown in Fig. 3c.

References

1 Forrest, B.J. and Cushley, R.J. (1977) Atherosclerosis 28, 309–318

2 Grover, A.K. and Cushley, R.J. (1979) Atherosclerosis 32, 87–91

3 Grover, A.K., Forrest, B.J., Buchinski, R.K. and Cushley, R.J. (1979) Biochim. Biophys. Acta 550, 212–221

4 Valic, M.I., Gorrissen, H., Cushley, R.J. and Bloom, M. (1979) Biochemistry 18, 854–859

5 Gorrissen, H., Tulloch, A.P. and Cushley, R.J. (1980) Biochemistry 19, 3422–3429

6 Mackay, A.L., Wassall, S.R., Valic, M.I., Gorrissen, H. and Cushley, R.J. (1980) Biochim. Biophys. Acta 601, 22–33

7 Cushley, R.J., Gorrissen, H. and Wassall, S.R. (1980) Can. J. Chem. 58, 2433–2441

8 Seelig, J. (1977) Q. Rev. Biophys. 10, 353–418

9 Mantsch, H.H., Saito, H. and Smith, I.C.P. (1977) Prog. NMR Spectrosc. 11, 211–271

10 Oldfield, E., Chapman, D. and Derbyshire, W. (1971) FEBS Lett. 16, 102–104

11 Grover, A.K. and Cushley, R.J. (1979) J. Labelled Compd. Radiopharm. 16, 307–313

12 Davis, J.H., Jeffrey, K.R., Bloom, M., Valic, M.I. and Higgs, T.P. (1976) Chem. Phys. Lett. 42, 390–394

13 Seelig, A. and Seelig, J. (1974) Biochemistry 13, 4839–4845

14 Davis, J.H. (1979) Biophys. J. 27, 339–358

15 Janiak, M.J., Loomis, C.B., Shipley, G.G. and Small, D.M. (1974) J. Mol. Biol. 86, 325–339

16 Marcelja, S. (1974) Biochim. Biophys. Acta 367, 165–176

17 Schindler, H. and Seelig, J. (1975) Biochemistry 14, 2283–2287

18 Gruen, D.W.R. (1980) Biochim. Biophys. Acta 595, 161–183