Biochimica et Biophysica Acta 860 (1986) 345–353
Elsevier

BBA 73155

The effect of free cholesterol on the solubilization of cholesteryl oleate in phosphatidylcholine bilayers: A ^{13}C-NMR study

Paul J.R. Spooner, James A. Hamilton, Donald L. Gantz and Donald M. Small

Biophysics Institute, Housman Medical Research Center, Boston University School of Medicine, 80 East Concord Street, Boston, MA 02118 (U.S.A.)

(Received February 6th, 1986)
(Revised manuscript received May 20th, 1986)

Key words: Phosphatidylcholine bilayer; Cholesteryl oleate; Cholesterol solubility; Lipoprotein surface

The solubilization of cholesteryl oleate in sonicated phosphatidylcholine vesicles containing between 0 and 50 mol% cholesterol was studied by ^{13}C-NMR using isotopically enriched [*carbonyl*-^{13}C]cholesteryl oleate. The carbonyl-^{13}C chemical shift from cholesteryl oleate in the phospholipid/cholesterol bilayer was significantly downfield from that for cholesteryl oleate in an oil phase and the peak area, relative to that of the phospholipid carbonyl, was used to determine bilayer solubility of the ester. The ester solubility (with respect to phospholipid) in the phospholipid bilayer without cholesterol (2.9 mol%) was only moderately reduced (to 2.3 mol%) at cholesterol levels up to 33 mol% but showed a more marked reduction to 1.4 mol% at 40 mol% cholesterol or 1.2 mol% at 50 mol% cholesterol. Since the vesicles containing 50 mol% cholesterol were larger (520 ± 152 Å diameter) than those with no cholesterol (291 ± 97Å diameter), we measured the solubility of cholesteryl oleate in large vesicles with no cholesterol, prepared by extrusion through polycarbonate membrane filters, and found it similar to that in small, sonicated vesicles with no cholesterol. Therefore, the larger size of vesicles was not the factor responsible for the decreased cholesteryl oleate solubility at high cholesterol contents. A more direct effect of cholesterol is envisioned where the ester becomes displaced to deeper regions of the bilayer.

Introduction

Cholesterol esters, incorporated within lipoproteins, are the main chemical form in which cholesterol is transported within the body. These relatively non-polar lipids readily form separate phases in the presence of phospholipids and are largely excluded from organized structures of these polar, amphipathic species [1–3]. However, the finite solubility that cholesterol esters and other neutral lipids exhibit in these arrays of polar lipids is likely to be of metabolic importance, since it will determine their access to phospholipid surfaces of lipoproteins and, to some degree, their interactions with plasma membranes.

In previous ^{13}C-NMR studies [4–6] this laboratory has utilized the effects of polar interactions on lipid carbonyl groups to distinguish, via chemical shift, neutral lipids that are located in phosphatidylcholine (PC) bilayers from neutral lipids in an oil-rich or excess phase. It has been possible to determine in a rigorous manner the solubilities in PC vesicles of triolein [4], cholesteryl oleate [5] and both these lipids in combination [6]. These studies also alluded to a configuration of the solubilized neutral lipid that placed the carbonyl groups quite close to the aqueous surface, forcing the rest of the molecule to lie parallel to the acyl chains of the phospholipid molecules. However, the existence of natural lipid structures comprised entirely of phospholipid is rather rare. Since cholesterol is present as a principal component in

most natural membranes and surfaces of circulating lipoproteins, it became important to extend these earlier studies [4–6] to include the effects of cholesterol on bilayer solubilization of the neutral, weakly polar lipids.

Cholesterol can be stably incorporated into phospholipid bilayers up to a molar ratio of 1 : 1 [2,7–9]. Further, the occurrence of cholesterol levels approaching this saturation value in natural membranes is certainly not uncommon [10]. In a previous study [11], it was predicted from a detailed compositional analysis of model lipid systems consisting of triolein, PC and cholesterol, that the surface solubility of the neutral lipid was not greatly diminished by increasing cholesterol content. However, this analysis was not ideally suited for measuring the low triolein solubilities, and cholesterol levels at the particle surfaces were well below saturation.

In the current study, ^{13}C-NMR was used to monitor directly the solubilization of [*carbonyl*-^{13}C]cholesteryl oleate in sonicated PC vesicles containing between 0 and 50 mol% cholesterol. The effect of vesicle size was also examined by using larger vesicle preparations obtained by extruding lipid suspensions through polycarbonate membrane filters. We report that cholesterol has marked effects on cholesteryl oleate solubilization when approaching its saturation levels in phospholipid bilayers.

Materials and Methods

Egg yolk PC (Grade I) was obtained from Lipid Products (Nutley, U.K.), cholesterol (greater than 99% pure) from Nu Chek Prep, Inc. (Elysian, MN), and [*carbonyl*-^{13}C]cholesteryl oleate was synthesized in our laboratory by esterification of cholesterol with oleic acid enriched to 90% ^{13}C in the carbonyl position (MSD Isotopes, Ottowa, Canada). All lipids were confirmed to be greater than 99% pure by thin-layer chromatography (TLC). Radiolabeled [1-^{14}C]cholesteryl oleate was purchased from Amersham Corp. (Arlington Heights, IL).

Lipid Dispersions. Bilayer vesicles, saturated with [*carbonyl*-^{13}C]cholesteryl oleate, were prepared by the cosonication procedure introduced previously [5] and detailed below. An alternative method of extrusion through polycarbonate membrane filters was employed for generating large PC vesicles saturated with cholesteryl oleate and is also described here.

Lipid mixtures were first dried to a thin film by evaporating a chloroform solution in a stream of nitrogen and then treated under low vacuum overnight. Lipid films were suspended under nitrogen in about 1.5 ml of 0.56% w/v KCl solution at $52 \pm 1°C$ (above the phase transitions of neat cholesteryl oleate, Ref. 12), with brief vortex mixing, to yield a concentration of 60–100 mg · ml^{-1} of lipid. Mixtures of PC with up to 33 mol% cholesterol contained 4 wt.% [*carbonyl*-^{13}C]cholesteryl oleate, with respect to PC. Mixtures of PC with 40 mol% cholesterol and 2, 3 or 4 wt.% [*carbonyl*-^{13}C]cholesteryl oleate, with respect to PC, showed that the lower cholesteryl oleate concentration was sufficient to ensure maximum incorporation in this case, based on NMR peak intensities. This reduced concentration of cholesteryl oleate was used for samples with 50 mol% cholesterol to prevent interference from excess cholesteryl oleate in the routine analysis of samples by NMR.

Sonication. Sonicated vesicles were prepared using a Branson 350 Sonifier as previously described [6] except that ultrasonic treatment was conducted at $52 \pm 1°C$ for these samples. Only trace amounts of degradation products (less than 1% with respect to total lipid) were detectable (by TLC) after the longest sonication treatment of 60 min. Sonicated preparations were ultracentrifuged at $190\,000 \times g$ for 1 h, and a small amount of undispersed lipid was aspirated from the sample surface. The vesicle suspension was removed for NMR analysis, leaving a pellet containing particles from the titanium ultrasonic probe. Typically, no undispersed lipid material was apparent in the pellets.

Membrane filter extrusion. Coarse lipid dispersions of PC and cholesteryl oleate were extruded through Nucleopore polycarbonate membrane filters (Nucleopore Corp., Pleasanton, CA) to generate large vesicles [13]. Samples were introduced into an ultrafiltration unit (Amicon Corp., Danvers, MA) fitted with the polycarbonate membrane filter and allowed to equilibrate at $52 \pm 1°C$ under nitrogen. Extrusion was then accomplished

by applying positive nitrogen pressure (30–50 lb/in^2) above the sample. Samples were first passed twice through a membrane with a 400 nm pore size, followed by two passages through a 100 nm pore size membrane.

NMR analysis. Proton-decoupled ^{13}C-NMR spectra were obtained at 50.3 MHz with a Bruker WP200 spectrometer as previously described [4]. Data were recorded over 16384 or 32768 time domain points using a pulse interval of 8.0 s to obtain equilibrium relaxation conditions, based on previous spin-lattice relaxation time (T_1) measurements [5]. A line-broadening of 1.5–3.0 Hz was applied in the data transformation to improve signal-to-noise ratios. Chemical shifts and linewidths were measured as before [4] with chemical shifts being referenced to a value of 14.10 ppm (from tetramethylsilane) for the fatty acyl terminal methyl peak. Peak intensities were measured using the Aspect integration program and bilayer solubilities were calculated from equilibrium intensity measurements using the formula given previously [5]. Nuclear Overhauser enhancement was determined by comparing peak intensities from spectra acquired under continuous or gated proton decoupling [14]. A molecular weight of 770 was determined for egg PC from the fatty acyl analysis performed by HPLC. Sample temperature was controlled to $\pm 1°C$ with a Bruker B-VT-1000 variable temperature unit and measured externally using a thermocouple.

Electron microscopy. Vesicle samples were mixed with an equal volume of 5% w/v ammonium molybdate solution at pH 7.0 to yield a lipid concentration of 1–5 $mg \cdot ml^{-1}$ and applied immediately to 400 mesh copper grids covered with carbon-coated Formvar. Coated grids had been subjected to low-vacuum, glow-discharge treatment within 2 weeks of use. In addition, carbon-coated Formvar grids for membrane-extruded vesicles were briefly pre-treated with a 0.1 $mg \cdot ml^{-1}$ solution of bacitracin (Upjohn Company, Kalamazoo, MI) to improve spreading of stain and specimen. Samples were allowed to adsorb to the films for 30 s before removing excess solution. Specimens were then air-dried before examination with a Hitachi 11-C electron microscope.

Results and Discussion

Fig. 1A shows the ^{13}C-NMR spectra at 37°C from PC vesicles prepared by sonicating PC with excess [*carbonyl*-^{13}C]cholesteryl oleate. Except for the cholesteryl oleate peak in the carbonyl region, the spectrum is typical for PC in vesicle dispersions [4]. Fig. 1B shows that with 50 mol% cholesterol, most of the phospholipid resonances broadened. Contributions from the natural abundance ^{13}C of cholesterol are only discernable as broad shoulders on the downfield side of the methylene envelope (approx. 35–45 ppm). These effects, as well as the contrastingly small changes in the choline methylene and methyl resonances and differential broadening of glycerol backbone peaks (60–75 ppm) are in good agreement with the observations for cholesterol in PC vesicles made by other workers [15]. The carbonyl regions of these spectra are expanded in Fig. 2 with equivalent regions of spectra obtained from using intermediate levels of cholesterol.

The spectrum of vesicles with cholesteryl oleate and no cholesterol (Fig. 2A) was essentially identical to that reported previously [5] and showed a well-resolved peak for cholesteryl oleate, upfield from the PC carbonyl resonances. This resonance at 171.9 ppm arises from bilayer-solubilized cholesteryl oleate. As previously reported [5], bilayer-solubilized cholesteryl oleate does not exhibit phase transitions characteristic of neat cholesteryl oleate or cholesteryl oleate in the core of an emulsion particle and appears downfield in the carbonyl region from oil-phase cholesteryl oleate, presumably because of exposure of the carbonyl group to the polar environments at the bilayer surface. As shown in Fig. 2B–E, free cholesterol produced appreciable quantative effects on the cholesteryl oleate carbonyl resonance only when exceeding 33 mol% in the vesicles. Here the bilayer-solubilized cholesteryl oleate peak decreased in intensity relative to the PC carbonyl peak and broadened significantly at 50 mol% cholesterol. There was also a small, progressive upfield shift in the bilayer-solubilized cholesteryl oleate resonance from 171.9 ppm to 171.6 ppm with increasing cholesterol concentration.

These carbonyl spectra, recorded at 37°C, do not show any interference from non-incorporated

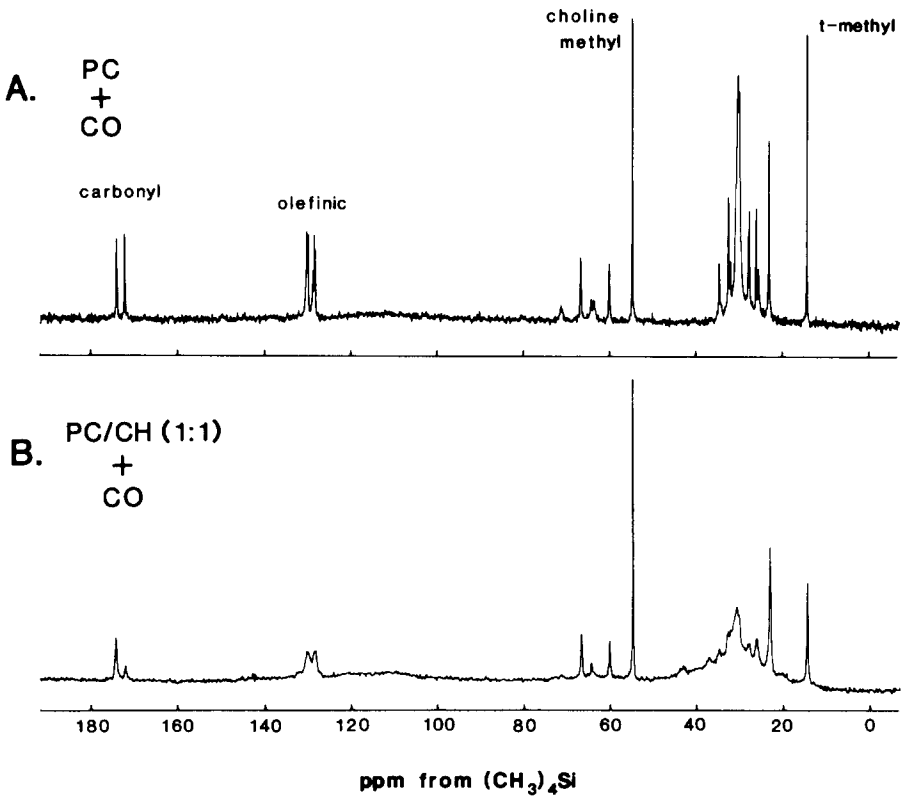

Fig. 1. Proton decoupled ^{13}C-NMR spectra at 50.3 MHz and 37°C of vesicles prepared from (A) PC, and (B) PC with 50 mol% cholesterol (CH) by cosonication with excess [*carbonyl*-^{13}C]cholesteryl oleate (CO). Spectra were obtained from 2000 (A) and 7000 (B) accumulations using 16384 time domain points, a recycle time of 8.0 s and a 10000 Hz spectral width. The chemical shift scale is in ppm downfield from tetramethylsilane, using an internal reference value of 14.10 ppm for the terminal fatty acyl methyl peak. Peaks for terminal fatty acyl methyl (t-methyl), choline methyl, olefinic and carbonyl carbons are indicated.

cholesteryl oleate, which appears at a chemical shift close to oil-phase cholesteryl oleate (171.3 ppm). The existence of this residual cholesteryl oleate is however demonstrated by a more thorough examination of the system with 50 mol% cholesterol as described below.

The bilayer solubilities of cholesteryl oleate calculated from the relative intensities of the bilayer-solubilized cholesteryl oleate and PC carbonyl peaks are given in Table I, along with linewidths for the bilayer-solubilized cholesteryl oleate and choline methyl resonances. Nuclear Overhauser enhancement values for the PC and cholesteryl oleate carbonyl resonance decreased from 1.8 and 1.6, respectively, in PC vesicles with no cholesterol to 1.5 and 1.3 for vesicles containing 40 mol% cholesterol. These changes at high cholesterol levels are greater than experimental

TABLE I

CHOLESTERYL OLEATE SOLUBILITIES MEASURED IN PC VESICLES WITH 0–50 mol% CHOLESTEROL AND LINEWIDTHS ($\nu_{\frac{1}{2}}$) FOR CHOLESTERYL OLEATE CARBONYL AND PC CHOLINE METHYL RESONANCES

Data show average values and ranges obtained from three samples in each case except at 50 mol% cholesterol where measurements are from seven samples.

mol% cholesterol	Cholesteryl oleate solubility mol% of PC	$\nu_{1/2}$ (Hz)	
		cholesteryl oleate	choline-Me
0	2.9±0.1	12±2	8±1
20	2.5±0.2	9±2	8±1
33	2.3±0.2	10±2	8±1
40	1.4±0.2	10±2	9±1
50	1.2±0.2	21±4	9±1

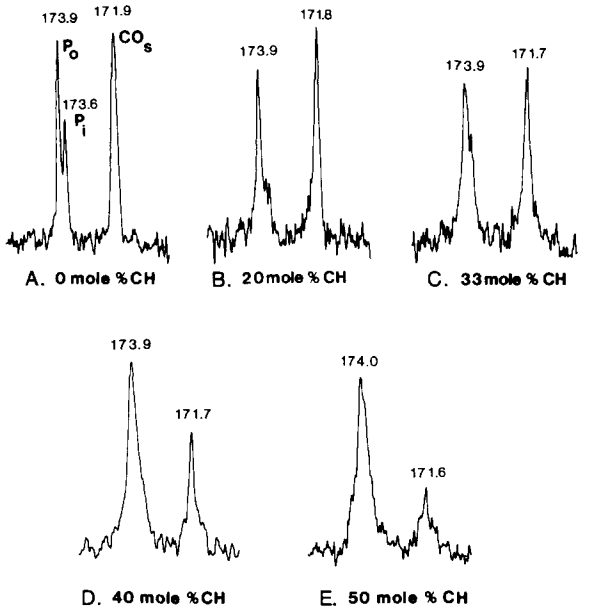

Fig. 2. The carbonyl region from spectra of sonicated PC vesicles containing 0–50 mol% cholesterol (CH), as indicated, showing bilayer-solubilized [*carbonyl*-^{13}C]cholesteryl oleate, (CO_s). The peaks labeled P_o and P_i correspond to PC carbonyl groups on the outer and inner leaflets of the bilayer, respectively, and are clearly discernable only in spectrum A (0 mol% CH). Spectra were obtained at 37°C after 2000 (A); 3000 (B); 2000 (C and D) and 7000 (E) accumulations; other accumulation conditions were as in Fig. 1.

error (about 10%), whereas the ratio of these values (cholesteryl oleate to PC carbonyl) was not significantly altered. Consequently, the value of 0.89 for this ratio from vesicles without cholesterol was used in the calculation of solubility at all cholesterol levels. The solubility of cholesteryl oleate without cholesterol (2.9 mol%) is the same as that reported previously for cholesteryl oleate in PC vesicles prepared at temperatures greater than 50°C [6]. The data show a very moderate decrease in cholesteryl oleate solubility with respect to bilayer PC upon increasing cholesterol levels to 33 mol%. Over the range 33–50 mol% cholesterol, however, the cholesteryl oleate solubility with respect to PC was reduced by about half.

The principal effect of increasing cholesterol from 40 to 50 mol% in the vesicles was a broadening of the bilayer-solubilized cholesteryl oleate carbonyl resonance, accompanied by a small decrease in intensity with respect to the PC carbonyl. Because of the uncertainties inherent in quantitating such a weak, broad signal, as well as the difficulties in determining reliable Nuclear Overhauser enhancement values at the highest cholesterol level, radiolabeled cholesteryl oleate was introduced into some samples with 50 mol% cholesterol to provide an alternative means of measuring cholesteryl oleate uptake. These measurements yielded cholesteryl oleate levels of between 1.8 and 2.4 mol% with respect to PC, depending largely upon the total amount of cholesteryl oleate originally present (2.4–3.5 mol%). Thus, the radiolabel uptake was significantly greater than that measured by NMR. The source of this discrepancy is apparent from observing temperature-dependent changes in the carbonyl region of the spectrum of these samples, as illustrated in Fig. 3. At temperatures of 40°C and higher, an additional narrow resonance at

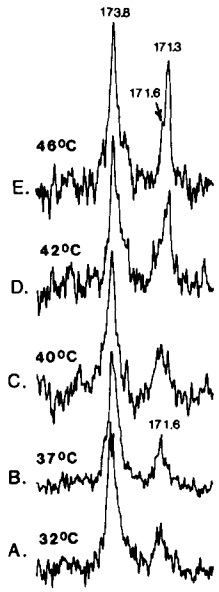

Fig. 3. The carbonyl region of the ^{13}C-NMR spectrum of PC vesicles with 50 mol% cholesterol and incorporated [*carbonyl*-^{13}C]cholesteryl oleate as a function of temperature, as indicated. All spectra are displayed over the same chemical shift range and scaled to the same PC carbonyl peak height. These spectra show excess cholesteryl oleate appearing as an incompletely resolved peak at 171.3 ppm at temperatures of 40°C and above. Spectra were obtained as in Fig. 1, except for the number of accumulations: (A) 7500; (B) 7000; (C) 2500; (D) 1500; and (E) 4000.

171.3 ppm was detected. This resonance has almost the same chemical shift as neat cholesteryl oleate [5] and can be assumed to arise from 'melting' at 40–42°C within a separate cholesteryl oleate-rich phase. However, this 'excess' cholesteryl oleate is not sufficiently resolved from the bilayer-solubilized cholesteryl oleate peak (171.6 ppm) in these spectra to demonstrate conclusively the existence of separate cholesteryl oleate phases. In the absence of cholesterol, however, the bilayer-solubilized cholesteryl oleate peak is shifted sufficiently downfield so as to be entirely resolvable from excess cholesteryl oleate, as shown previously [5].

Fig. 4 shows that the total cholesteryl oleate peak intensity in the Fig. 3 spectra increased abruptly between 40 and 42°C to give a limiting value equivalent to the cholesteryl oleate uptake measured with radiolabelled cholesteryl oleate. Thus, the intensity of the cholesteryl oleate peak at $T < 40$°C represented only the bilayer-solubilized cholesteryl oleate and at $T > 40$°C represented both bilayer and excess-phase cholesteryl oleate. The good agreement between the NMR results and the radiolabel assay at $T > 40$°C demonstrates the applicability of the NMR quantitation procedure for these systems. These results also exemplify the value of this technique in allowing a particular state of a chemical entity to be measured while co-existing with other states which are indistinguishable by conventional chemical analysis.

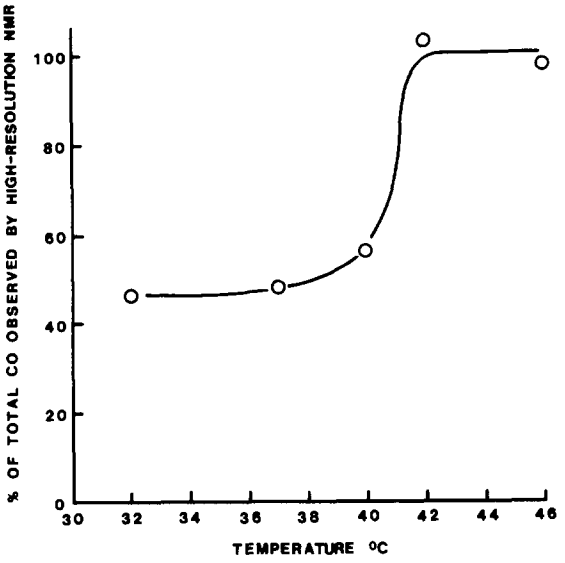

Fig. 4. Plot showing the amount of [*carbonyl*-^{13}C]cholesteryl oleate exhibiting a detectable high resolution signal as a percentage of the total determined by radiolabelled assay, over the temperature range 32–46°C. Values determined from Fig. 3 spectra by the ratio of cholesteryl oleate to PC peak intensities. Note that below 42°C only a fraction of the total cholesteryl oleate (CO) exhibits a high resolution spectrum.

The excess cholesteryl oleate detected in the spectra from vesicles with 50 mol% cholesterol (Fig. 3) exhibits conversion to an isotropic state at around 8°C lower than that observed for pure cholesteryl oleate [12]. We note that an equivalent depression in this transition for cholesteryl oleate can be achieved by incorporating as little as 7 mol% cholesterol in the ester phase [16]. A similar phase transition behavior has also been noted for cholesteryl oleate microemulsions prepared by extensive sonication with PC [17]. It is likely that both PC and cholesterol contribute to the character and properties of excess cholesteryl oleate within the system.

The inability to separate excess cholesteryl oleate completely from vesicles with 50 mol% cholesterol by ultracentrifugation (see Methods) could be attributable to an increase in vesicle size observable with increasing bilayer cholesterol; larger vesicles might entrap excess cholesteryl oleate (microemulsions) within their internal aqueous cavities, more readily than small vesicles. Size estimates from electron microscopic examination of the sonicated vesicles gave the following mean diameter ± standard deviation in Å: no cholesterol, 291 ± 97; 33 mol% cholesterol, 410 ± 155; 50 mol% cholesterol, 520 ± 152, for samples sizes, $n =$ 130–250. A similar effect of increasing cholesterol content on vesicle size has been noted previously [18].

An increase in vesicle size of the order described above is expected to result in a more rigid packing of fatty acyl chains in the bilayers, from a consideration of the geometric consequences of reduced surface curvature [19]. According to studies of cholesterol esters with polar lipids at an air-water interface [20,21], fatty acyl chain packing is critical in determining the miscibility of the esters in these surfaces. It was therefore necessary

to determine whether the increase in vesicle size was responsible for the decreased cholesteryl oleate solubility at high levels of cholesterol. For this purpose large vesicles were prepared by extrusion of PC suspensions with an excess of [*carbonyl*-^{13}C]cholesteryl oleate through the 100 nm pore size polycarbonate membrane filters (see Methods). The diameters of extruded vesicles were estimated from electron micrographs as 1046 ± 366 (n = 97), appreciably larger than sonicated vesicles containing 50 mol% cholesterol and about the same sizes as previously reported by other workers using this technique [13,22].

The ^{13}C-NMR spectrum of the extruded PC vesicles (Fig. 5) showed much poorer resolution than did PC in the sonicated vesicles without cholesterol (Fig. 1A). The increased linewidths are indicative of the reduced molecular mobilities anticipated for these large vesicular systems. The carbonyl region recorded at 30°C (Fig. 5A) showed distinct carbonyl peaks for both cholesteryl oleate and PC, although these are not well separated in the spectrum. At higher temperatures the bilayer-solubilized cholesteryl oleate resonance narrowed, whereas the lineshape for the PC carbonyl peak deteriorated to the extent that it was difficult to quantitate relative intensities of PC and cholesteryl oleate peaks. The area of the bilayer-solubilized cholesteryl oleate peak could however be compared with that for the excess cholesteryl oleate which appeared in the spectrum at temperatures greater than 40°C and attained a limiting inten-

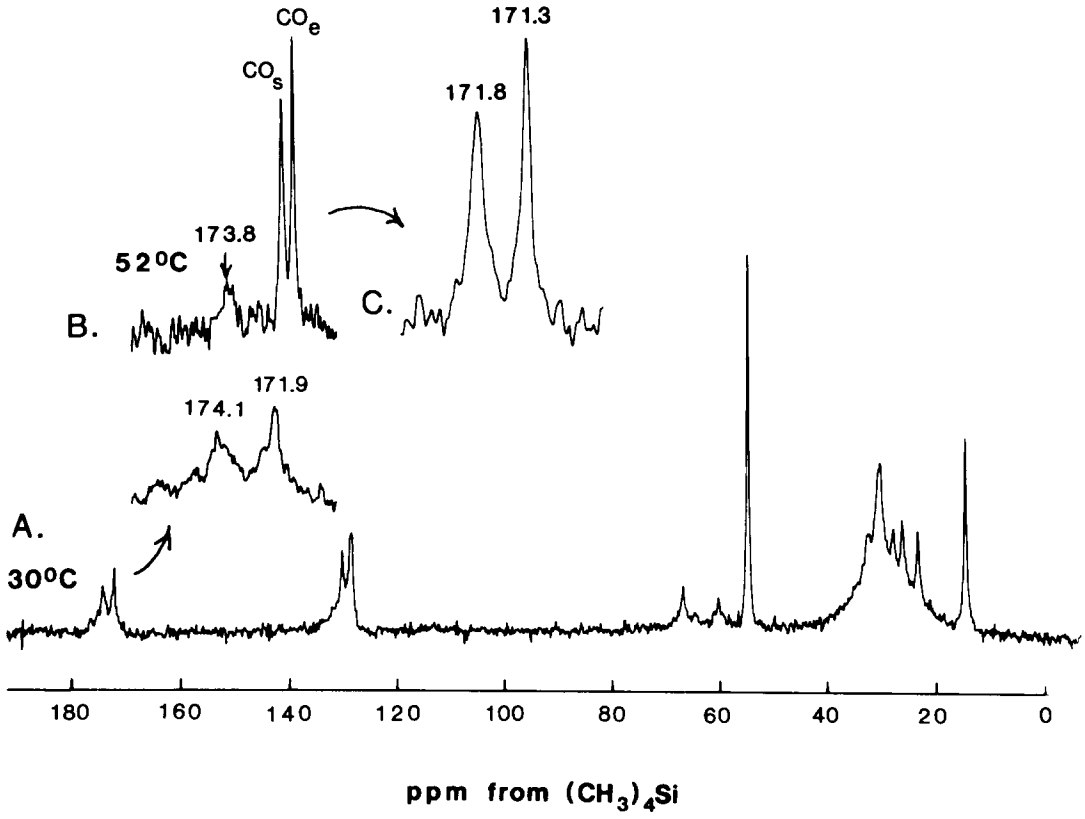

Fig. 5. Proton-decoupled ^{13}C-NMR spectra from vesicles prepared by extrusion of a suspension of PC with 4 wt.% [*carbonyl*-^{13}C]cholesteryl oleate through a 100 nm pore size polycarbonate membrane filter. Spectrum A was obtained at 30°C after 6000 accumulations and spectrum B at 52°C after 1300 accumulations. Other spectral accumulation conditions as in Fig. 1, except that B was from a spectrum recorded over 32768 time domain points. Inset C shows resolution of bilayer-solubilized cholesteryl oleate (CO_s) and excess cholesteryl oleate (CO_e) peaks obtained at half the line broadening (1.5 Hz) used for B. Vertical expansions for insets are scaled to approximately the same height for the choline methyl resonance.

sity between 48 and 52°C. The carbonyl region of the spectrum recorded at 52°C (Fig. 5B) showed good resolution of bilayer-solubilized cholesteryl oleate and excess cholesteryl oleate peaks (Fig. 5C) and a peak area ratio (bilayer-solubilized cholesteryl oleate/excess cholesteryl oleate) of 1.1. Since the initial PC and cholesteryl oleate concentrations were not significantly altered by the extrusion process, as determined by phosphate and radiolabel assay, respectively, the peak intensities could be related directly to the original amounts of lipid used. Therefore, for an initial concentration of 4.7 mol% cholesteryl oleate in the extruded vesicle sample, we estimate from the bilayer-solubilized cholesteryl oleate/excess cholesteryl oleate peak intensity ratio that 2.5 mol% cholesteryl oleate were solubilized in the bilayers. Since this value is quite similar to the cholesteryl oleate solubility determined in small sonicated PC vesicles (2.9 mol%), it may be concluded that the effects of vesicle size on phospholipid organization are not responsible for the reduction in cholesteryl oleate solubility observed at 50 mol% cholesterol. We therefore attribute this effect to a more specific interaction or structural effect of cholesterol in the bilayers. Furthermore, this result can also be interpreted as deviating from the cholesteryl oleate behavior described in interfacial films [20,21] which implied a strong dependence between cholesteryl oleate incorporation and the molecular mobility of the phospholipids.

An increased vesicle size is, however, likely to influence the NMR spectra observed for vesicles containing high cholesterol levels. Reduced correlation times for tumbling of larger vesicles can broaden resonances for the more motionally restricted groups in the bilayer [15]. In addition, cholesterol will reduce bilayer fluidity, not only as a consequence of increasing vesicle size, but through a more direct interaction with the phospholipid [23]. It is likely that these effects, rather than any highly specific interactions, account for the observed broadening of the cholesteryl oleate carbonyl signal at high cholesterol levels.

Effects of cholesterol on structural features [18] and molecular motions [24] of PC in vesicles have been noted to be much more pronounced at levels exceeding about 33 mol%. It was proposed that these observations reflect a change in the mode of interaction between cholesterol and PC for high cholesterol molar ratios. Such a change in the manner of cholesterol incorporation and its consequent effects on bilayer properties could well account for the more dramatic effects observed here at cholesterol levels greater than 33 mol%.

A more complete account of these molecular events is not available from the results presented here. However, it is noteworthy that a progressive upfield shift in the cholesteryl oleate carbonyl resonance was observed with increasing cholesterol. This suggests that the ester was somewhat displaced from the polar surface by cholesterol interactions which are presumed to occur in this region [25,26] and is being pushed deeper into the hydrophobic bilayer interior. Previous efforts with spin-labelled [27] and deuterated [28] cholesterol esters have described their configuration and alignment with respect to the longitudinal axes of bilayer phospholipids but have provided only indirect evidence concerning their transverse location in the bilayer. Additional studies are required for a more thorough description of the effects of cholesterol in phospholipid bilayers, reported here.

Acknowledgments

We thank Dr. Pavanaram K. Sripada for the synthesis of [*carbonyl*-^{13}C]cholesteryl oleate, Howard Lilly for technical assistance and Anne Gibbons for typing the manuscript. This work was supported by United States Public Health Service Grants HL26335 and HL07291.

References

1 Small, D.M. and Shipley, G.G. (1974) Science 185, 222–229
2 Janiak, M.J., Loomis, C.R., Shipley, G.G. and Small, D.M. (1974) J. Mol. Biol. 86, 325–339
3 Janiak, M.J., Small, D.M. and Shipley, G.G. (1979) J. Lipid Res. 20, 183–199
4 Hamilton, J.A. and Small, D.M. (1981) Proc. Natl. Acad. Sci. USA 78, 6878–6882
5 Hamilton, J.A. and Small, D.M. (1982) J. Biol. Chem. 257, 7318–7321
6 Hamilton, J.A., Miller, K.W. and Small, D.M. (1983) J. Biol. Chem. 258, 12821–12826
7 Bourges, M., Small, D.M. and Dervichian, D.G. (1967) Biochim. Biophys. Acta 137, 157–167.
8 Lecuyer, H. and Dervichian, D.G. (1969) J. Mol. Biol. 45, 39–57

9 Collins, J.J. and Phillips, M.C. (1982) J. Lipid Res. 23, 291–298
10 Small, D.M. (1977) J. Coll. Interf. Sci. 58, 581–602
11 Miller, K.W. and Small, D.M. (1982) J. Coll. Interf. Sci. 89, 466–478
12 Small, D.M. (1970) in Surface Chemistry of Biological Systems (Blank, M., ed.), pp. 55–83, Plenum Press, New York
13 Olson, F., Hunt, C.A., Szoka, F.C., Vail, W.J. and Papahadjopoulos, D. (1979) Biochim. Biophys. Acta 557, 9–23
14 Opella, S.J., Nelson, D.J. and Jardetzky, O. (1976) J. Chem. Phys. 64, 2533–2535
15 Brainard, J.R. and Cordes, E.H. (1981) Biochemistry 20, 4607–4617
16 Small, D.M. (1986) in The Physical Chemistry of Lipids from Alkanes to Phospholipids. Handbook of Lipid Research Series, Vol. 4, Hanahan, D., ed.), Ch. 11, pp. 460–461, Plenum Press, New York
17 Ginsburg, G.S., Small, D.M. and Atkinson, D. (1982) J. Biol. Chem. 257, 8216–8227
18 Newman, G.C. and Huang, C.H. (1975) Biochemistry 14, 3363–3370
19 Sheetz, M.P. and Chan, S.I. (1972) Biochemistry 11, 4573–4581
20 Smaby, J.M. and Brockman, H.L. (1981) Biochemistry 20, 724–730
21 Smaby, J.M., Schmid, P.C. and Brockman, H.L. (1984) Biochemistry 23, 1955–1958
22 Hope, M.J., Bally, M.B., Webb, G. and Cullis, P.R. (1985) Biochim. Biophys. Acta 812, 55–65
23 Levine, Y.K. (1972) Prog. Biophys. Mol. Biol. 24, 1–74
24 Gent, M.P.N. and Prestegard, J.H. (1974) Biochemistry 13, 4027–4033
25 Brockerhoff, H. (1974) Lipids 9, 645–650
26 Huang, C.H. (1977) Lipids 12, 348–356
27 Grover, A.K., Forrest, B.J., Buchinski, R.K. and Cushley, R.J. (1979) Biochim. Biophys. Acta 550, 212–221
28 Chana, R.S., Cushley, R.J., Wassall, S.R., Smith, I.C.P. and Dufourc, E.J. (1985) Chem. Phys. Lipids 37, 345–356