Effect of Free Cholesterol on Incorporation of Triolein in Phospholipid Bilayers†

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ABSTRACT: Triacylglycerols are the major substrates for lipolytic enzymes that act at the surface of emulsion-like particles such as triglyceride-rich lipoproteins, chylomicrons, and intracellular lipid droplets. This study examines the effect of cholesterol on the solubility of a triacylglycerol, triolein, in phospholipid surfaces. Solubilities of [*carbonyl*-^{13}C]triolein in phospholipid bilayer vesicles containing between 0 and 50 mol % free cholesterol, prepared by cosonication, were measured by ^{13}C NMR. The carbonyl resonances from bilayer-incorporated triglyceride were shifted downfield in the ^{13}C NMR spectra from those corresponding to excess, nonincorporated material. This enabled solubilities to be determined directly from carbonyl peak intensities at most cholesterol concentrations. The bilayer solubility of triolein was inversely proportional to the cholesterol/phospholipid mole ratio. In pure phospholipid vesicles the triolein solubility was 2.2 mol %. The triglyceride incorporation decreased to 1.1 mol % at a cholesterol/phospholipid mole ratio of 0.5, and at a mole ratio of 1.0 for the bilayer lipids, the triolein solubility was reduced to just 0.15 mol %. The effects of free cholesterol were more pronounced and progressive than observed previously on the bilayer solubility of cholestery oleate [Spooner, P. J. R., Hamilton, J. A., Gantz, D. L., & Small, D. M. (1986) *Biochim. Biophys. Acta 860*, 345–353]. As with cholesteryl oleate, we suggest that cholesterol also displaces solubilized triglyceride to deeper regions of the bilayer.

The triglyceride and cholesterol esters represent an important source of lipid moieties in biological function. They are present in plasma lipoproteins, in intercellular droplets, and in intestinal contents. The low polarity of these neutral lipids necessitates their bulk transfer in plasma within lipoprotein particles that are stabilized to the aqueous environment, by a surface composed of the more polar phospholipid and sterol molecules with adsorbed apoproteins. Their interaction with these surfaces may influence the availability of neutral lipids for enzymatic conversion and have some relevance to their interactions with other biological surfaces. It is clear that neutral lipids are not well incorporated into the ordered arrays of hydrated phospholipids (Small & Shipley, 1974; Janiak et al., 1974, 1979). The application of ^{13}C NMR in this laboratory has succeeded in detecting and quantifying the neutral lipid accommodated in phospholipid bilayers, as distinct from that present in a nonincorporated excess phase (Hamilton & Small, 1981, 1982; Hamilton et al., 1983). This was possible since polar interactions in the bilayers impart a characteristic chemical shift effect on the neutral lipid carbonyls, and by observation of these resonances from isotopically enriched nuclei, solubilities of around 3 mol % were obtained for triolein (Hamilton & Small, 1981), cholesteryl oleate (Hamilton & Small, 1982), and combinations of these two (Hamilton et al., 1983). In an effort to consider other major components of biological surfaces, we subsequently determined the effects of free (nonesterified) cholesterol on the bilayer incorporation of cholesteryl oleate (Spooner et al., 1986). Cholesterol concentrations up to the apparent saturation level in phospholipid bilayers of 50 mol % (Bourges et al., 1967; Collins & Phillips, 1982) were studied. Cholesterol was found to restrict further the accommodation of cholesterol ester in the bilayers although an appreciable reduction in solubility was only observable at cholesterol levels exceeding 33 mol %. The current study examines the effects of free cholesterol on bilayer solubility of triglyceride, the other important class of neutral lipid.

Previous efforts (Miller & Small, 1982) at an extensive compositional analysis on aqueous dispersions of PC,[1] triolein, and cholesterol predicted that free cholesterol would have only a small effect on the surface incorporation of triglyceride. The work adopted a straightforward approach of chemical analysis on fractionated dispersions, which was more reliable for describing bulk-phase compositions than the minor components of the interfaces. Furthermore, the concentrations of cholesterol used in the dispersions did not provide surface levels approaching saturation (equimolar with PC), and therefore the solubility of triolein at high cholesterol/phospholipid ratios needed to be estimated. In the present study we determine the incorporation of [*carbonyl*-^{13}C]triolein in vesicles of egg PC with 0–50 mol % free cholesterol from ^{13}C NMR measurements. The results demonstrate a pronounced reduction in triglyceride solubility as the mole percent of cholesterol was increased in the bilayers.

Materials and Methods

Egg yolk PC was obtained from Lipid Products (South Nutfield, England), cholesterol was obtained from Nu Chek Prep, Inc. (Elysian, MN), and [*carbonyl*-^{13}C]triolein was synthesized by esterification of glycerol (Kodali et al., 1984) with oleic acid enriched to 90% ^{13}C in position 1 (MSD Isotopes, Montreal, Canada). The fatty acid had been purified by aqueous extraction of a solution in benzene/chloroform/methanol (1.0:0.5:1.2), and the synthesized product was isolated by chromatography on a silica gel column. All lipids were found to be greater than 99% pure by thin-layer chromatography. Radiolabled [9,10-^{3}H(N)]triolein was purchased from Amersham Corp. (Arlington Heights, IL).

Lipid Dispersions. Aqueous dispersions of lipids for NMR analysis were prepared similarly to previous studies (Spooner

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[1] Abbreviations: PC, phosphatidylcholine; VLDL, very low density lipoproteins; apo, apolipoprotein.

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et al., 1986). Briefly, thin films of lipid mixtures, prepared by evaporation of solutions in chloroform, were treated under low vacuum and then suspended in 1.5 mL of nitrogen-saturated 0.16 M KCl solution by vortex mixing. These mixtures contained 100 mg of egg PC with 0–50 mol % cholesterol and up to 8 wt % [*carbonyl*-^{13}C]triolein, with respect to the phospholipid. The triolein content was varied at each cholesterol level to provide a 1–2-fold excess of the maximum amount incorporated into the bilayers, on the basis of subsequent NMR measurements.

Sonication of the lipid dispersions was conducted under nitrogen at 52 ± 1 °C with a Branson 350 Sonifier (Danbury, CT) as described previously (Spooner et al., 1986). Treatment was continued for 30–50 min, depending upon the cholesterol level, and the resulting suspension centrifuged at low speed (1000*g*) for 15 min. The supernatant was removed for NMR analysis, leaving a sediment of particles from the ultrasonic probe. According to thin-layer chromatography, lipid purity (>99%) was unaffected by the sonication step.

Large vesicles (~1000-Å diameter) were prepared from egg PC and triolein mixtures by extrusion through Nucleopore polycarbonate membrane filters (Nucleopore Corp., Pleasanton, CA) as in the previous study (Spooner et al., 1986).

Electron Microscopy. Sizing of the lipid vesicles by electron microscopy was described in our earlier work (Spooner et al., 1986). Excess triglyceride in samples prepared for the current study tended to interfere with the negative staining of specimens, when present at the higher concentrations (>2 wt %). In these cases much of the excess was eliminated by ultracentrifugation (Spooner et al., 1986), prior to specimen preparation.

NMR Analysis. Details of routine acquisition of proton-decoupled ^{13}C NMR spectra at 50.3 MHz by use of the Bruker WP200 spectrometer have been well documented (Hamilton & Small, 1981; Spooner et al., 1986). Equilibrium peak intensities were measured by using the Aspect integration program, and the Glinfit program provided by Bruker for decomposition of spectral components was used to verify measurements made on incompletely resolved peaks. Nuclear Overhauser enhancement (NOE) was determined by comparing peak intensities obtained under continuous or gated decoupling (Opella et al., 1976).

Solubilities were calculated from the ratio of equilibrium peak intensities as previously employed by Hamilton and Small (1981). The mole fraction of triolein in the bilayer x_s with respect to phospholipid is given by

$$x_s = (A_s/A_{PC})(2/n)(1.1/90)(NOE_{PC}/NOE_{TO})$$

where A_s and A_{PC} are peak areas for the carbonyls of bilayer-solubilized triolein and PC, respectively, obtained under equilibrium relaxation conditions and broad-band proton decoupling; 2 and n in the second term are the number of carbonyls per molecule contributing to the peaks measured for PC and triolein, respectively; 1.1 and 90 in the third term refer to the ^{13}C abundance for the PC and triolein carbonyls, respectively; and NOE_{PC} and NOE_{TO} are the respective nuclear Overhauser enhancements of the PC and solubilized triolein carbonyls.

In PC vesicles without cholesterol the carbonyl NOE values were sufficiently similar (1.8 for PC, 1.7 for triolein) to disregard the latter term in the above calculation (Hamilton & Small, 1981). With vesicles containing 33 mol % cholesterol, the highest concentration at which the NOE could be reasonably determined by our techniques, a value of 1.6 was measured for both PC and solubilized triolein carbonyl and a value of 1.8 obtained for the excess triolein. We deduce small

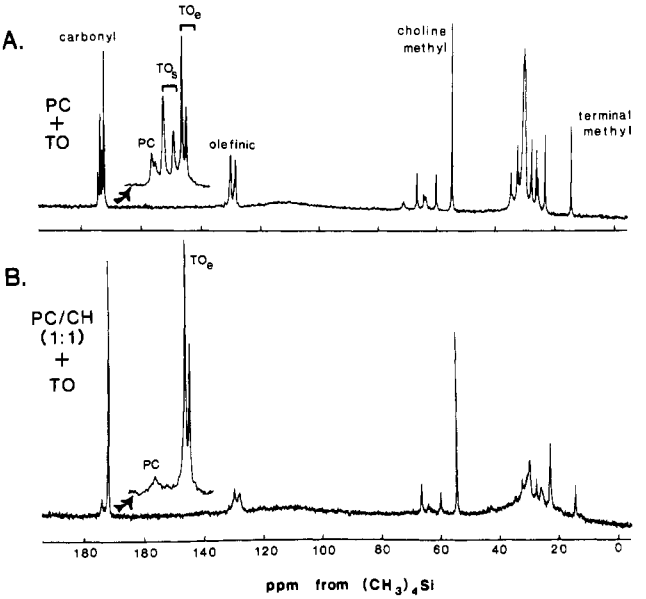

FIGURE 1: Proton-decoupled ^{13}C NMR spectra at 50.3 MHz and 37 °C of vesicles prepared from (A) PC and (B) PC with 50 mol % cholesterol (CH), by cosonication with 5 wt % [*carbonyl*-^{13}C] triolein (TO). Spectra were from 3000 (A) and 4000 (B) accumulations over 16384 time domain points, with a 2.0-s recycle time and a 10000-Hz spectral width. Spectra are given approximately the same intensity scaling and shown over a chemical shift scale in parts per million (ppm) downfield from tetramethylsilane, with an internal reference of 14.10 ppm for the terminal methyl peak. Expanded regions show carbonyl peaks for PC, bilayer-solubilized triolein (TO_s), and excess triolein (TO_e).

and similar changes in the NOE in the presence of free cholesterol that will not significantly affect the calculation of solubility in the above case. We also anticipate that a difference between the carbonyl NOE for solubilized and excess triolein at 50 mol % cholesterol will not seriously prohibit the application of the treatment outlined in the Appendix for solubility estimation in the cholesterol-saturated bilayers.

For most measurements made here, $n = 2$ in the second term above, such that x_s is simply $0.0122A_s/A_{PC}$ or $1.21A_s/A_{PC}$ mol %. For $n = 3$ (all triolein carbonyls), $x_s = 0.00815A_s/A_{PC}$ or $0.807A_s/A_{PC}$ mol %.

Results and Discussion

Figure 1 shows the entire proton-decoupled ^{13}C NMR spectra of PC vesicles (A) and vesicles of PC with 50 mol % free cholesterol (B) prepared by cosonication with excess [*carbonyl*-^{13}C]triolein. Apart from the carbonyl regions, these spectra are typical of vesicles of PC alone (Hamilton & Small, 1981) and PC vesicles saturated with cholesterol (Brainard & Cordes, 1981; Spooner et al., 1986).

Triolein provides a dual set of carbonyl peaks, the smaller ascribable to the carbonyl at position 2 on the glycerol backbone and a larger downfield peak from the magnetically equivalent carbonyls at positions 1 and 3 (Hamilton & Small, 1981). The carbonyl region expanded in Figure 1A reveals two distinct sets of carbonyl peaks for triolein, representing both solubilized (TO_s) and excess (TO_e) material. As noted earlier by Hamilton and Small (1981), the excess peaks appear at chemical shifts nearly identical with those of neat or oil-phase triolein, while the solubilized peaks are shifted downfield, close to the PC carbonyls, presumably from exposure to the polar environment at the bilayer surface.

The expanded region in Figure 1B shows striking changes in the distribution of triolein from incorporating 50 mol % cholesterol into the bilayer. Here, virtually all the triolein seems to be excluded from the vesicles and appears almost

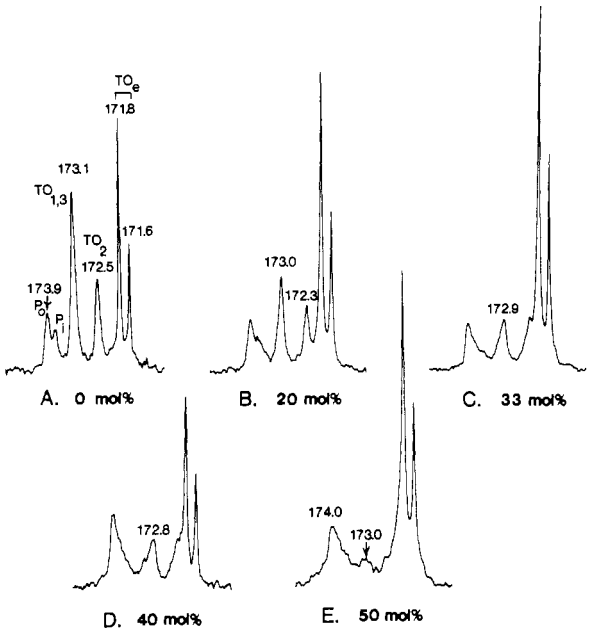

FIGURE 2: Carbonyl regions of spectra recorded from PC vesicles with 0–50 mol % cholesterol and excess [*carbonyl*-^{13}C]triolein. Total triolein concentrations used were 5 (A), 4 (B and C), or 2 wt % (D and E) with respect to phospholipid. Resonances from phospholipid on both the outside (P_o) and inside (P_i) of the vesicle bilayers are only clearly distinguishable without cholesterol (A). The peak from the carbonyls at positions 1 and 3 of bilayer-solubilized triolein ($TO_{1,3}$) is monitored throughout the series while that from the 2-position carbonyl (TO_2) fuses with that from excess components (TO_e) with increasing cholesterol. Spectra A–C are given approximately the same intensity scaling, as are D and E, although the latter are increased to show the diminishing $TO_{1,3}$ peak. Spectra were recorded under equilibrium relaxation conditions (recycle time = 8.0 s) from 2000–8000 accumulations.

exclusively in the excess phase. The carbonyl regions, recorded under equilibrium relaxation conditions, for these systems and at intermediate cholesterol levels are shown in Figure 2.

The bilayer incorporation of triolein, as deduced from observing the relative intensities of the solubilized TO_s to PC carbonyl peaks, is shown to reduce markedly with as little as 20 mol % cholesterol in the vesicles (Figure 2B), compared with vesicles of PC alone (Figure 2A). The triolein incorporation declines monotonically with further increases in cholesterol until at the 50 mol % saturation level (Figure 2E), solubilized triolein is only discernible as a small shoulder from the carbonyls at positions 1 and 3 ($TO_{1,3}$) on the downfield side of the broadened PC carbonyl peak. These intensity changes are accompanied by small progressive upfield shifts in the resolvable solubilized peaks, with increasing cholesterol, causing the peak from position 2 (TO_2) to fuse with excess components of the spectra. For the $TO_{1,3}$ peak, however, these shifts provide for reasonable resolution from broadening phospholipid resonance up to 40 mol % cholesterol. The intensities of these two peaks were thus used to quantify the bilayer solubility of triolein up to this level of cholesterol, and calculated values are entered in Table I. The trace amounts of triolein incorporated in vesicles containing 50 mol % cholesterol were estimated from the intensities of composite peak areas as outlined in the Appendix, with total molar concentrations of phospholipid and triolein measured by phosphorus and radiolabel assay, respectively.

The solubility measured in vesicles of PC alone (2.2 mol %), prepared here at 52 °C, is slightly lower than the values of 2.4 mol % obtained at 24 °C (Hamilton & Small, 1981) and 2.7 mol % measured in samples prepared at 55 °C (Hamilton et al., 1983). These minor differences in solubility may be due

Table I: Triolein Solubilities Measured in Egg PC Vesicles with 0–50 mol % Cholesterol, Prepared at 52 °C, and Line Widths ($\nu_{1/2}$) for Carbonyls at Positions 1 and 3 of Solubilized Triolein ($TO_{1,3}$) and the PC Choline Methyl (Ch Me) Resonances[a]

cholesterol (mol %)	triolein solubility (mol %)	$\nu_{1/2}$ (Hz)	
		$TO_{1,3}$	Ch Me
0	2.2 ± 0.1	9 ± 2	9 ± 1
20	1.4 ± 0.1	9 ± 1	9 ± 1
33	1.1 ± 0.1	17 ± 1	9 ± 1
40	0.7 ± 0.1	20 ± 2	9 ± 1
50	0.15 ± 0.03		

[a] Data show the average and range from three samples in each case except for the solubility at 50 mol % cholesterol, which is the mean ± SD of four measurements.

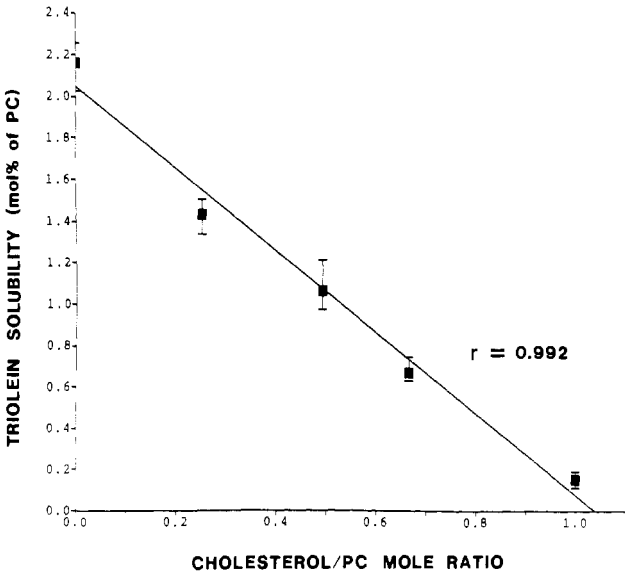

FIGURE 3: Plot of triolein solubility against cholesterol/PC mole ratio in the bilayer, from data listed in Table I, showing least-squares regression line with slope −1.97 and intercept 1.04 on the abscissa (r = correlation coefficient for regression). Bars show the range of solubilities from repeat measurements (Table I).

to variations in the fatty acid compositions between the different batches of egg PC used in these studies. The present measurements showed no systematic dependence on total triolein concentration, over the range of excess employed (see Materials and Methods), indicating that bilayer saturation was achieved in all samples. Concentrations calculated from integrating the entire triolein high-resolution spectra usually gave good agreement with the total triglyceride concentrations used in the samples (within 10%). Significant disparities in these values were more often found with samples containing low levels or no cholesterol, from which excess triolein tended to separate during the centrifugation step, prior to NMR analysis. Line widths listed in Table I show selective broadening in the $TO_{1,3}$ resonance, compared with the choline methyl resonance, at cholesterol concentrations of 33 mol % and above.

When the triolein solubility is plotted against the cholesterol/PC mole ratio in the bilayers, the solubility appears to decrease in an almost linear fashion, as shown in Figure 3. The least-squares regression line (r = 0.992) fitted to these data has a slope of −1.97 and an intercept of 1.04 for the cholesterol/PC mole ratio extrapolated to zero triolein solubility.

According to observations by electron microscopy, cholesterol induced similar changes in vesicle size to those noted previously (Spooner et al., 1986). Mean vesicle diameters (±SD) increased from 250 ± 30 Å without cholesterol to 540 ± 90 Å at 50 mol % cholesterol. Since an increase in vesicle

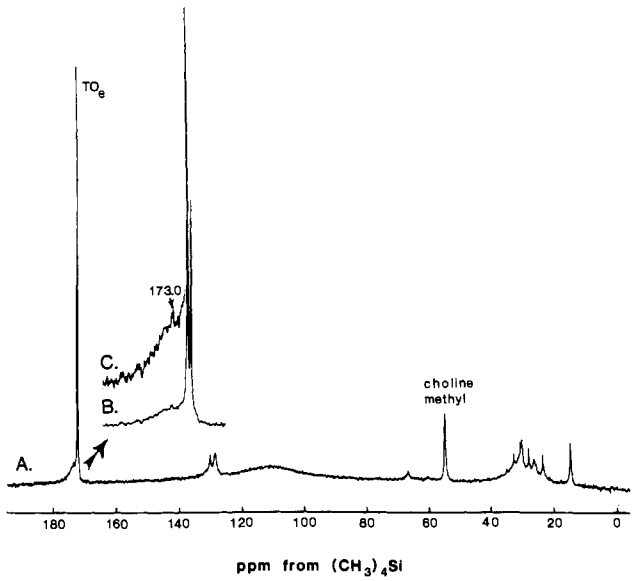

FIGURE 4: Proton-decoupled ^{13}C NMR spectrum at 35 °C from large vesicles prepared by extruding a suspension of PC with 5 wt % [*carbonyl*-^{13}C]triolein through a 100-nm pore size polycarbonate membrane filter (A). Expansions B and C show broad component downfield from the excess triolein peaks (TO_e). Spectrum were from 4500 accumulations with an 8.0-s recycle time.

size may alter the lipid organization in bilayers (Sheetz & Chan, 1972), we also examined the incorporation of triolein in large PC vesicles prepared by extruding dispersions of the phospholipid with excess [*carbonyl*-^{13}C]triolein through polycarbonate membrane filters at 52 ± 1 °C. Examination of this sample by electron microscopy showed mostly thin-walled vesicular structures with diameters close to the 1000-Å pore size of the polycarbonate membrane filter, as observed previously for the PC–cholesteryl oleate system (Spooner et al., 1986).

The phospholipid resonances from the large vesicles, shown in Figure 4A, are for the most part considerably broader than observed from sonicated PC vesicles (Figure 1A). This can be accounted for by an effect of increased vesicle size on either their rotational correlation times (Stockton et al., 1976) or the molecular dynamics in the bilayer (Sheetz & Chan, 1972). Contrasting with the phospholipid peaks in Figure 4 are narrow components in the carbonyl region, representing excess triolein (TO_e). As shown in expansion B of Figure 4, downfield components of the carbonyl region form an unresolved broad envelope, although a superimposed peak is discernible at about the chemical shift of carbonyls in positions 1 and 3 of solubilized triolein (173.0 ppm in expansion C, Figure 4). Resolution in this region was not improved by varying the sample temperature over the range 32–52 °C.

From the intensity of the well-resolved choline methyl peak in Figure 4, we estimate the intensity of the unresolved PC carbonyl, using an NOE value of 2.7 measured for choline methyl and 1.8 for PC carbonyl. Subtracting this estimated intensity from the area of the entire carbonyl region and then comparing the remaining intensity for triolein carbonyls with that of the choline methyl, we calculate a total triolein concentration of 4.0 mol % with respect to PC. This gives reasonable agreement with a value of 4.4 mol % determined from radiolabel and phosphate assay performed on this sample. Thus, despite the broadness in the signal from bilayer-associated carbonyls, it appears that adequate quantitation can be achieved from this region. It should be noted that the intensity of the broad bilayer envelope constitutes a substantial proportion of the entire carbonyl intensity. A value of 0.42

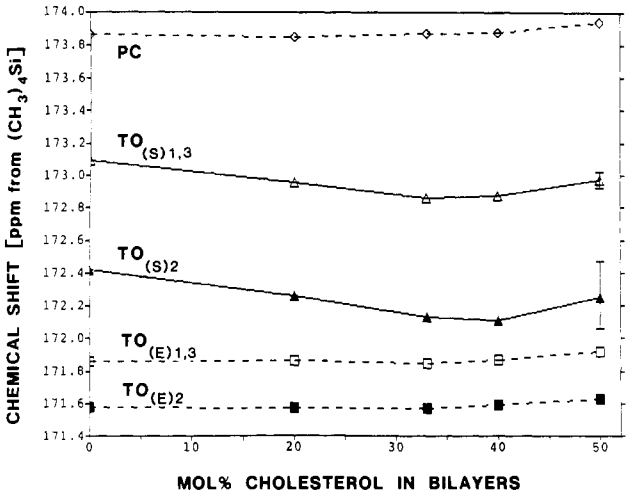

FIGURE 5: Carbonyl chemical shifts as a function of the cholesterol concentration in the bilayers for the groups at positions 2 or 1 and 3 of solubilized [$TO_{(S)2}$ and $TO_{(S)1,3}$, respectively] or excess [$TO_{(E)2}$ and $TO_{(E)1,3}$, respectively] triolein and for the major peak from PC (PC). Data combined from all samples used for the solubility measurements (Table I). Bars show range of measurements at 50 mol % cholesterol; otherwise, all variation was within size of symbols.

was derived for this fraction of carbonyl intensity from the bilayer by using the line shape decomposition program (Materials and Methods). Subtracting the predicted PC carbonyl intensity gives 0.35 as the fraction of total triolein solubilized in the bilayers, which is equivalent to 1.4 mol % with respect to PC.

The solubility of triolein estimated in the large PC vesicles is somewhat lower than that obtained in sonicated PC vesicles (2.2 mol %) but is the same as that observed with sonicated vesicles containing 20 mol % cholesterol. Since at 20 mol % cholesterol we find that mean vesicle diameters from electron microscopy are only 310 ± 50 Å and that the much decreased solubilities at higher cholesterol levels are obtained with vesicle diameters much less than those of the extruded PC vesicles, it is concluded that the observed decreases in solubility were a direct result of cholesterol interactions in the bilayers. While it is uncertain whether the large PC vesicles are well equilbrated with triolein by the extrusion process, it is clear that an effect of vesicle size on triolein solubility would be small compared with the observations reported here with cholesterol.

In a recent study (Spooner et al., 1986) we reported that free cholesterol reduced the bilayer solubility of cholesteryl oleate although the effect was only pronounced with greater than 33 mol % cholesterol in the bilayer. The current work describes more prominent effects of cholesterol on triolein solubility and suggests that cholesterol in membranes and biological surfaces will exclude triglyceride more effectively than cholesterol esters.

The carbonyl peaks for solubilized cholesteryl oleate showed small upfield shifts (171.9 to 171.6) with increasing cholesterol, implying that the ester region of cholesteryl oleate became displaced to deeper regions of the bilayer (Spooner et al., 1986). Changes in the more complex carbonyl regions observed here upon cholesterol incorporation are represented in Figure 5 for more detailed analysis. The major carbonyl peak for PC and those from the excess triglyceride remain unchanged over much of the range of cholesterol concentration. The absolute displacement of the solubilized peaks is only slightly greater for the carbonyl in position 2, but since this peak is shifted less by solubilization in the bilayers of PC alone [0.84 ppm from $TO_{(E)2}$ in Figure 5] compared with the positions 1 and 3 carbonyls [1.23 ppm from $TO_{(E)1,3}$ in Figure

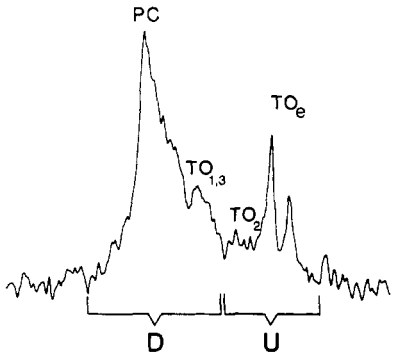

FIGURE 6: Quantitative treatment of carbonyl spectra obtained from vesicles with 50 mol % cholesterol and excess [*carbonyl*-^{13}C]triolein. Spectrum from vesicles with a total of 0.5 wt % triolein added is resolved into upfield (U) and downfield (D) regions for the purpose of estimating solubilities. Detailed description of procedure is given within the Appendix.

5], the cholesterol effect at position 2 is proportionately greater. At maximum displacement observed with 40 mol % cholesterol in the bilayers, the shift from solubilization had been reduced by 38% for position 2 but by only 12% for positions 1 and 3. These differences may involve conformational changes occurring around the glycerol backbone of the triglyceride as the cholesterol concentration increases in the bilayer, such that the *sn*-2 position becomes buried in the bilayer to a greater extent than functional groups at the *sn*-1 and *sn*-3 positions. It is possible that the marked decrease observed in triolein solubility with increasing bilayer cholesterol reflects the difficulty in accomodating a displaced carbonyl group within the more hydrophobic regions of the bilayer.

The chemical shift data for the carbonyls of bilayer triolein at 50 mol % cholesterol show greater variability (Figure 5) due to the difficulty in decomposing the broad, poorly resolved peaks observed at this composition but do suggest some reversal in the trends described above. This probably illustrates the predominance of more complex events occurring at higher cholesterol concentrations where vesicle bilayers may become structurally atypical (Gent & Prestegard, 1974; Newman & Huang, 1975) and cannot be relied upon to remain compositionally homogeneous (De Kruijff et al., 1976; Presti et al., 1982). Finally, since the chemical shift effect of cholesterol is at least qualitatively the same for the chemically diverse triglycerides and cholesterol esters, we expect that the proposed changes in location of these neutral lipids result from structural changes occurring in the bilayers (Gent & Prestegard, 1974) rather than by more direct interactions with cholesterol.

The findings reported here may have important implications concerning the behavior of triglyceride in membranes and lipoprotein particles. Below, we consider a possible role in the metabolic activity of triglyceride.

Free cholesterol has been shown to markedly inhibit the in vitro activity of lipoprotein lipase on triglyceride-rich emulsions with lipid compositions resembling triglyceride-rich lipoproteins (Fielding, 1970; Rossner & Vessby, 1977). The work by Fielding (1970) demonstrated a complete cessation of activity with emulsions containing 10 wt % free cholesterol. Using data from previous phase compositional analyses (Miller & Small, 1982), it was possible to predict that cholesterol will saturate the emulsion surface at this concentration (Miller & Small, 1987), a condition that, in bilayers, resulted in almost complete exclusion of triglyceride, as reported here. The in vitro activity was also appreciably reduced for emulsion with 2 wt % free cholesterol. This should provide close to 20 mol % cholesterol at the surface (Miller & Small, 1983), a level at which we report a significant reduction in the bilayer solubility of triglyceride. A recent in vivo study carried out in our laboratory (Maranhao et al., 1986) also showed reduced plasma clearance rates for egg PC–triolein emulsions with high free cholesterol in rats, compared with emulsions with low cholesterol. This work also estimated that the lipolytic contribution to clearance is almost entirely absent when concentrations of 50 mol % cholesterol were attained in the emulsion surface. In fact, newly secreted chylomicrons and other nascent triglyceride-rich lipoproteins have a low surface-free cholesterol, about 6–7 wt % (~12 mol %) (Miller & Small, 1983, 1987). Plasma VLDL[1] and chylomicron remnants have more surface cholesterol, about 20–25 wt % (Miller & Small, 1983, 1987), and are also fairly good substrates. The surfaces of β-VLDL are saturated with cholesterol (50 mol %) (Miller & Small, 1983, 1987) and are poor substrates.

Thus, triglyceride catabolism can at least in part be regulated by concentrations of the substrate in the lipoprotein surface, and substrate concentrations can be inversely proportional to surface cholesterol content. Increased cholesterol content also has other effects such as decreasing the number of apoproteins bound (Atkinson & Small, 1986). Of course if apoC-II,[1] the cofactor for lipoprotein lipase, was excluded from the surface, lipolysis would stop. Therefore, it is not clear as yet what the molecular mechanisms of the cholesterol effect are. Since triglyceride is normally catabolized at an extremely high rate in vivo (Havel & Kane, 1975; Redgrave & Carlson, 1979) and exhibits a low solubility in lipoprotein surfaces, it has been customary to envisage the lipase active site as penetrating through the particle surface and directly accessing the core substrate (Cryer, 1981), although there is no direct evidence for this. Models of this process have also viewed the particle surface as becoming highly labile (Smith & Scow, 1979). Free cholesterol could hinder such processes through structural alterations in the lipoprotein surface, similar to those described in phospholipid bilayers (Gent & Prestegard, 1974; Newman & Huang, 1975).

Although free cholesterol may influence rates of lipolysis by a number of possible mechanisms, the effects predicted here on surface accommodation of triglyceride in lipoprotein may be of consequence in this process and are certainly a consideration for all processes involving triglyceride at biological surfaces.

ACKNOWLEDGMENTS

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APPENDIX

The following describes the procedure for deriving concentrations of solubilized triolein in PC vesicles containing 50 mol % cholesterol.

The carbonyl spectrum in Figure 6 is resolved into downfield (D) and upfield (U) composite regions, resulting from the coalescence of the two peaks from solubilized triolein, $TO_{1,3}$ and TO_2, with the PC and excess triolein (TO_e) peaks, respectively. The areas of these individual components, comprising the measurable areas of A_d and A_u for regions D and U, respectively, are designated A_{PC} for the PC carbonyl, $A_{1,3}$ and A_2 for $TO_{1,3}$ and TO_2, respectively, and A_e for the TO_e peaks.

According to the above definition

$$A_u = A_e + A_2$$

Substituting $A_e = A_t - A_{1,3} - A_2$, where A_t is the total triolein peak area, gives

$$A_u = A_t - A_{1,3} \quad (1)$$

Providing all forms of TO exhibit the same quantitative characteristics in the NMR measurements, specifically, equilibrium intensities are recorded and NOE values are not significantly different, then from the general expression relating peak areas to concentrations (see Materials and Methods)

$$A_t = 1.24 x_t A_{PC}$$

where x_t is the total mole percent concentration of triolein measured by chemical analysis, and

$$A_{PC} = A_d - A_{1,3} \quad (2)$$

Substituting for these terms in eq 1 yields

$$A_u = 1.24 x_t (A_d - A_{1,3}) - A_{1,3}$$

Rearranging gives

$$A_{1,3} = (1.24 x_t A_d - A_u)/(1 + 1.24 x_t)$$

and then A_{PC} can be obtained from eq 2.

The solubilized concentration x_s is thus calculable from the general expression (Materials and Methods)

$$x_s = 1.21 A_{1,3}/A_{PC} \text{ (mol \%)}$$

the complete expression for which simplifies to

$$x_s = (1.50 x_t A_d - 1.21 A_u)/(A_d + A_u)$$

Registry No. Cholesterol, 57-88-5; triolein, 122-32-7.

REFERENCES

Atkinson, D., & Small, D. M. (1986) *Annu. Rev. Biophys. Biophys. Chem. 15*, 403–456.

Bourges, M., Small, D. M., & Dervichian, D. G. (1967) *Biochim. Biophys. Acta 137*, 157–167.

Brainard, J. R., & Cordes, E. H. (1981) *Biochemistry 20*, 4607–4617.

Collins, J. J., & Phillips, M. C. (1982) *J. Lipid Res. 23*, 291–298.

Cryer, A. (1981) *Int. J. Biochem. 13*, 525–541.

De Kruijff, B., Cullis, P. R., & Radda, G. K. (1976) *Biochim. Biophys. Acta 436*, 729–740.

Fielding, C. J. (1970) *Biochim. Biophys. Acta 218*, 221–226.

Gent, M. P. N., & Prestegard, J. H. (1974) *Biochemistry 13*, 4027–4033.

Hamilton, J. A., & Small, D. M. (1981) *Proc. Natl. Acad. Sci. U.S.A. 78*, 6878–6882.

Hamilton, J. A., & Small, D. M. (1982) *J. Biol. Chem. 257*, 7318–7321.

Hamilton, J. A., Miller, K. W., & Small, D. M. (1983) *J. Biol. Chem. 258*, 12821–12826.

Havel, R. J., & Kane, J. P. (1975) *Fed. Proc., Fed. Am. Soc. Exp. Biol. 34*, 2250–2257.

Janiak, M. J., Loomis, C. R., Shipley, G. G., & Small, D. M. (1974) *J. Mol. Biol. 86*, 325–339.

Janiak, M. J., Small, D. M., & Shipley, G. G. (1979) *J. Lipid Res. 20*, 183–199.

Kodali, D. R., Atkinson, D., Redgrave, T. G., & Small, D. M. (1984) *JAOCS, J. Am. Oil Chem. Soc. 61*, 1078–1084.

Maranhao, R. C., Tercyak, A. M., & Redgrave, T. G. (1986) *Biochim. Biophys. Acta 875*, 247–255.

Miller, K. W., & Small, D. M. (1982) *J. Colloid Interface Sci. 89*, 466–478.

Miller, K. W., & Small, D. M. (1983) *J. Biol. Chem. 258*, 13772–13784.

Miller, K. W., & Small, D. M. (1987) in *New Comprehensive Biochemistry, Plasma Lipoproteins* (Gotto, A. M., Jr. Ed.) pp 1–75, Elsevier/North-Holland Biomedical Press, New York.

Newman, G. C., & Huang, C. H. (1975) *Biochemistry 14*, 3363–3370.

Opella, S. J., Nelson, D. J., & Jardetzky, O. (1976) *J. Chem. Phys. 64*, 2533–2535.

Presti, F. T., Pace, R. J., & Chan, S. I. (1982) *Biochemistry 21*, 3831–3835.

Redgrave, T. G., & Carlson, L. A. (1979) *J. Lipid Res. 20*, 217–229.

Rossner, S., & Vessby, B. (1977) *Nutr. Metab. 21*, 349–357.

Sheetz, M. P., & Chan, S. I. (1972) *Biochemistry 11*, 4573–4581.

Small, D. M., & Shipley, G. G. (1974) *Science (Washington, D.C.) 185*, 222–229.

Smith, L. C., & Scow, R. O. (1979) *Prog. Biochem. Pharmacol. 15*, 109–138.

Spooner, P. J. R., Hamilton, J. A., Gantz, D. L., & Small, D. M. (1986) *Biochim. Biophys. Acta 860*, 345–353.

Stockton, G. W., Polnaszek, C. F., Tulloch, A. P., Hasan, F., & Smith, I. C. P. (1976) *Biochemistry 15*, 954–966.