Deuterated Phospholipids as Raman Spectroscopic Probes of Membrane Structure: Dipalmitoylphosphatidylcholine–Dipalmitoylphosphatidylethanolamine Multilayers†

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ABSTRACT: Raman spectral data are reported for several conformation-sensitive spectral regions of dipalmitoylphosphatidylethanolamine (DPPE) and for chain perdeuterated dipalmitoylphosphatidylcholine (DPL-d_{62}) alone, in 1:1 binary mixtures, and in 1:1:1 ternary combination with cholesterol. Raman melting curves for DPPE multilayers reveal the noncooperative formation of 4–5 gauche rotamers per chain prior to the gel–liquid crystal transition at 66 °C. This phenomenon is also evident for DPL-d_{62} multilayers. Use of DPL-d_{62} as one component of a binary mixture allows the monitoring of conformational changes in each component individually. The phase separation of 1:1 DPL-d_{62}/DPPE mixtures shows onset and completion temperatures of about 44 and 60–65 °C, respectively, as monitored by variation in the line width of the C–D stretching vibrations of the DPL-d_{62}. The C–H stretching vibrations of the DPPE also respond to phase separation in the binary mixture. The $I(2885)/(2850)$ intensity ratio shows maximal change close to the onset temperature and appears to respond to both the formation of gauche rotamers and to changes in the lateral interactions of the DPPE chains. The frequency of the antisymmetric C–H stretching vibration near 2885 cm^{-1} responds primarily to gauche rotamer formation and exhibits a different temperature dependence than the $I(2885)/(2850)$ intensity parameter. The effect of deuteration on the phase separation is discussed. Addition of cholesterol to the binary mixture produces complex changes in the phase behavior. A broad phase separation region is still evident in the ternary system, although the onset and completion temperatures are each lower by about 15 °C. Cholesterol limits the number of gauche rotamers that form during the phase separation from ~4 in binary DPL-d_{62}/DPPE to 1–2 in the presence of cholesterol. The presence of additional phases at low temperature is suggested in the ternary system.

The occurrence of a gel–liquid crystal phase transition is well documented for both phospholipid–water systems (for reviews, see Lee, 1977a,b; Tyrrell et al., 1976, and references contained therein), and intact membranes (Cronan & Gelmann, 1975; Lee, 1975; Uehara et al., 1977). The temperature range over which the transition occurs in natural (multicomponent) systems is significantly broadened from the essentially first-order characteristics observed in one-component systems, as expected from thermodynamic considerations (Lee, 1977b), and results in the simultaneous coexistence of ordered and disordered lipid regions. This lateral phase separation is advantageous for a functioning membrane as it allows particular components to partition into a region of fluidity required for optimum function. One-component phospholipid systems are not the most suitable for mimicking natural systems since ordered and disordered regions coexist over a very narrow temperature range.

The simplest systems which show some of the phase separation properties of natural membranes are binary phospholipid mixtures. Early physicochemical studies involved calorimetric elucidation of phase behavior (Phillips et al., 1970; Phillips, 1972), and ESR[1] determination of phase diagrams based on the partitioning of a Tempo spin label between the aqueous medium and liquid crystal regions of the bilayer (Shimshick & McConnell, 1973), for some binary systems. Further calorimetric and spectroscopic work has centered around the definition of the physical situations where lateral phase separation may occur (Wu & McConnell, 1975; Galla & Sackmann, 1975; Mabrey & Sturtevant, 1976; Luna & McConnell, 1977).

The use of Raman spectroscopic techniques for the study of phospholipid conformation is well documented (Lippert & Peticolas, 1971; Mendelsohn, 1972; Mendelsohn et al., 1975; Gaber & Peticolas, 1977, and references contained therein). This method offers advantages over other physical approaches since (1) it does not require the use of a probe molecule and (2) examines with equal ease both gel and liquid crystal hydrocarbon regions. The set of vibrational frequencies for gel-phase phospholipid hydrocarbons is substantially different from that of the liquid crystal phase, so that Raman spectral data can therefore be used to monitor both the dynamics (Gaber & Peticolas, 1977) and energetics (Yellin & Levin 1977a, b) of phospholipid phase behavior.

A drawback in applying the Raman approach to multicomponent systems arises because the conformation-sensitive regions of the spectrum (primarily the C–C and C–H stretching vibrations) are overlapped with contributions from each component. Recently, Mendelsohn & Maisano (1978) have shown that this problem can be overcome by using deuterated phospholipids as one component of binary mixtures. The conformation-sensitive C–D stretching vibrations of the deuterated material occur in a spectral region free from interference from other membrane components. It is therefore possible to monitor the individual phase behavior of each component in a binary mixture. The utility of this approach is shown in the current work for dipalmitoylphosphatidylcholine–dipalmitoylphospatidylethanolamine mixtures. Some

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[1] Abbreviations used: DPL, dipalmitoylphosphatidylcholine; DPL-d_{62}, chain perdeuterated dipalmitoylphosphatidylcholine; DPPE, dipalmitoylphosphatidylethanolamine; DML, dimyristoylphosphatidylcholine; DML-d_{54}, chain perdeuterated dimyristoylphosphatidylcholine; DSL, distearoylphosphatidylcholine; Tempo, 2,2,6,6-tetramethylpiperidinyl-1-oxy; ESR, electron spin resonance.

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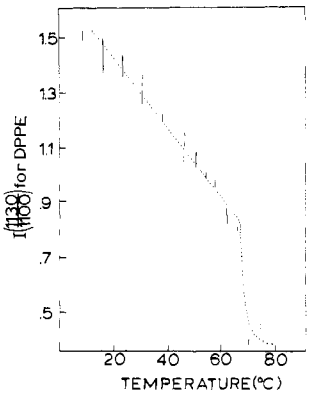

FIGURE 1: Temperature-induced variation of the $I(1130)/(1100)$ intensity ratio for dipalmitoylphosphatidylethanolamine multilayers in H_2O. Vertical lines represent standard deviations obtained from two to four measurements at each temperature.

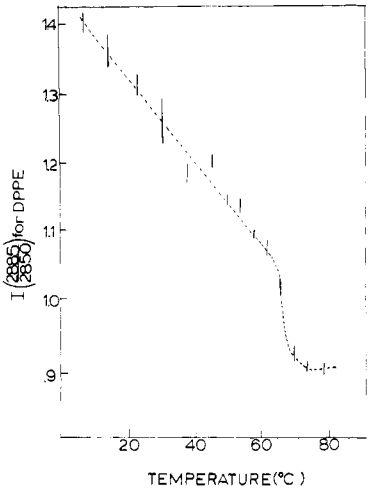

FIGURE 2: Temperature-induced variation for $I(2885)/(2850)$ for dipalmitoylphosphatidylethanolamine multilayers in H_2O.

parameters of the phase behavior for this system have been measured with other physical methods (Shimshick & McConnell, 1973; Blume & Ackermann, 1974) so that similar data derived from Raman spectroscopy can be compared directly. In this fashion, any possible effect of deuteration on the phase behavior may be judged directly. In addition, the current work demonstrates the complex effects that cholesterol produces on the organization of the DPL–DPPE system.

Experimental Section

(i) *Phospholipids.* DPL-d_{62} was obtained from Lipid Specialties and was passed through a Sephadex LH-20 column (ethanol solvent, column temperature 37 °C). Examination by thin-layer chromatography showed one dominant spot in a $CHCl_3$:MeOH:H_2O (65:25:4, v:v:v) solvent system. Impurities were visually estimated (using I_2 staining) to be less than ~2%. The Raman spectrum of DPL-d_{62} was essentially identical with recently published spectra (Yellin & Levin, 1977b; Gaber et al., 1978) and exhibited only a slight fluorescent background.

DPPE was used as purchased from Sigma Chemical Co., after thin-layer chromatographic examination revealed one spot with the appropriate R_f value (Kates, 1972). Cholesterol, also from Sigma Chemical Co., showed Raman spectral data identical with those previously published (Lippert & Peticolas, 1971; Bulkin & Krishnan, 1971) and was used without further purification. All solvents were spectral grade in quality. Samples for Raman spectroscopy were prepared by solvent evaporation of appropriate chloroform–methanol solutions of the component(s). Addition of H_2O was followed by extensive dispersal of the components on a Vortex mixer at temperatures where the sample was in the liquid-crystal state. The resulting cream-like suspensions were injected directly into the melting point capillaries used for Raman spectroscopic investigation. The multilayers were occasionally packed slightly in a hematocrit centrifuge. Final concentrations were about 10% by weight.

(ii) *Spectroscopic Measurements.* The apparatus used for Raman spectroscopy consists of a Spectra-Physics Model 164 Argon ion laser, Jarrell-Ash double monochromator with RCA C31034 photomultiplier detection, photon counting amplification, and strip-chart recording. Samples were thermostated in a brass cell (Thomas & Barylski, 1970) and were examined in the transverse mode usually using 250 mW of 5145-Å laser radiation for excitation. The uncertainty of the temperature at the sample due to local heating by the laser is estimated at 2°. Temperature calibration of the system was accomplished as previously described (Mendelsohn & Maisano, 1978). Each spectral region of interest in a given experiment was scanned two to four times at each temperature, and the appropriate parameter averaged. Spectral resolution was about 5 cm^{-1}. This value allowed accurate determination of the C–D line widths (which ranged from 28 to 48 cm^{-1}) without the instrument contributing to the width. All data reported are for heating cycles.

Results and Discussion

One-Component Systems. (i) DPPE. Two conformation-sensitive spectral regions of DPPE were examined in the current work. The C–C stretching vibrations in the 1050–1150-cm^{-1} range are sensitive to trans–gauche isomerization in the hydrocarbon chains (Lippert & Peticolas, 1971; Mendelsohn, 1972). The vibration near 1130 cm^{-1} arises from chain segments in the all-trans conformation. Formation of gauche rotamers leads to an intensity decrease of the 1130-cm^{-1} band and concomitant increase of a band near 1100 cm^{-1}. The intensity ratio $I(1130)/(1100)$ is therefore a useful measure of phospholipid fluidity. This parameter is plotted for DPPE as a function of temperature in Figure 1. A sharp discontinuity, characteristic of a phase transition, is observed at 66 °C. This observed melting point is close to that measured by calorimetric or ESR techniques (Phillips, 1972; Shimshick & McConnell, 1973; Lee, 1977a, b). Of interest in the current work is the observation of extensive noncooperative gauche rotamer formation below T_m (Figure 1), as suggested by the variation in $I(1130)/(1100)$ from 1.5 at 10 °C to 0.85 at 60 °C. According to the semiquantitative Raman order parameters derived recently (Gaber & Peticolas, 1977) which correlate variations in the C–C spectral region with the number of CH_2 groups in the all-trans conformation, the observed changes for DPPE reflect the (noncooperative) formation of 4–5 gauche rotations per chain prior to the main chain melt and an additional 3–4 rotations at T_m. Lee (1977a, b) has suggested that the noncooperative formation of rotational isomers in gel state phospholipid provides favorable sites for the diffusion of small molecules in an otherwise regular lattice.

The second Raman parameter extensively used to characterize phospholipid structure is the intensity ratio of the antisymmetric to the symmetric C–H stretching vibrations at about 2885 and 2850 cm^{-1}, respectively. This ratio is sensitive to both inter- and intramolecular alterations in hydrocarbon chain conformation (Gaber & Peticolas, 1977; Mendelsohn et al., 1976b). The temperature variation of this parameter for DPPE is shown in Figure 2. As in Figure 1, a sharp transition

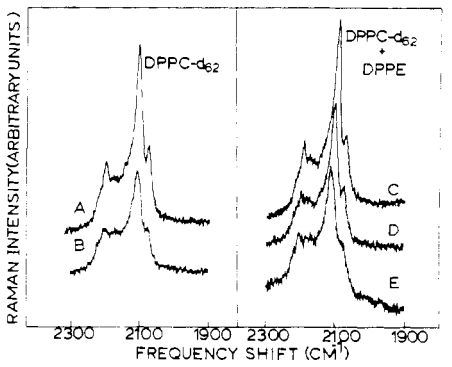

FIGURE 3: Typical spectra of the C-D stretching vibration region for dipalmitoylphosphatidylcholine-d_{62} under the following conditions. (A) Pure DPL-d_{62}, 15 °C; (B) pure DPL-d_{62}, 45 °C; (C) DPL-d_{62}/DPPE (1:1) binary mixture, 15 °C; (D) DPL-d_{62}/DPPE binary mixture, 45 °C; (E) DPL-d_{62}/DPPE binary mixture, 68 °C. The phospholipids were examined as multilayers, in H_2O suspensions. Typical spectral conditions were: time constant 2 s; scan speed 0.5 cm^{-1}/s; slit width 5 cm^{-1}. Peak intensities of the band near 2100 cm^{-1} were 2000-6000 counts/s. The line width measurement used to characterize the spectrum is carried out by drawing a baseline which connects the inflection points, near 2060 and 2240 cm^{-1}, measuring the height above the baseline of the 2100-cm^{-1} peak, and measuring the width of the band at half the height.

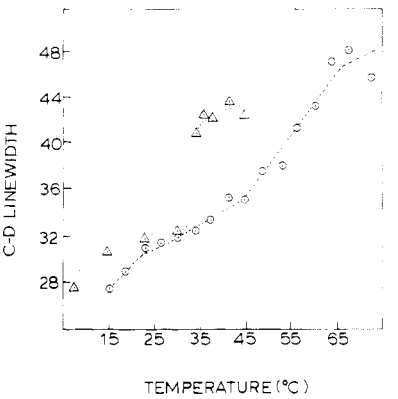

FIGURE 4: Temperature-induced variation in the line width of the C-D stretching vibrations near 2100 cm^{-1} in dipalmitoylphosphatidylcholine multilayers for: (i) pure DPL-d_{62}(· · · Δ · · ·); (ii) DPL-d_{62} in 1:1 binary mixtures with DPPE (- - O - -). The gel-liquid crystal phase transition appears as a sharp discontinuity near 34 °C in pure DPL-d_{62}. The region of phase separation in 1:1 binary mixtures occurs over the 44-63 °C range.

is noted at the melting temperature.

Our current C-H data for DPPE are in agreement with previous Raman work of Brown et al. (1973) as to T_m. However, we observe a greater disordering prior to melting for this molecule, as demonstrated by rapid variation of I(2885)/(2850) in the 10-60 °C range. Similarities in the shapes of the temperature-induced variation in the C-C and C-H spectral region below T_m suggest that the inter- and intramolecular disordering processes are highly correlated. Yellin & Levin (1977c) have reached similar conclusions for phosphatidylcholine suspensions. In certain circumstances (particularly for systems with small radii of curvature) intermolecular disordering may occur in the absence of extensive gauche rotamer formation. Different behavior is then expected for the temperature dependence of the C-C and C-H spectral regions. Such considerations have been used to elucidate the effect of sonication on dipalmitoyllecithin dispersions (Mendelsohn et al., 1976b; Gaber & Peticolas, 1977).

(ii) DPL-d_{62}. It is known that the C-D antisymmetric stretching vibrations near 2100 cm^{-1} in deuterated hydrocarbon chains undergo significant alterations in position, intensity, and line width during the gel-liquid crystal phase transition (Mendelsohn et al., 1976 a, b; Bunow & Levin, 1977; Gaber et al., 1978). Typical spectra for DPL-d_{62} under various conditions are shown in Figure 3, and the temperature-induced variation of the C-D stretching line width is plotted in Figure 4 for both pure DPL-d_{62} multilayers and for DPL-d_{62} in binary combination with DPPE. For pure DPL-d_{62}, the gel-liquid crystal transition is characterized by a line width increase from 32 to 42 cm^{-1} with a melting temperature of 34 °C (Figure 4). This temperature is 1-2 °C lower than that reported by Petersen et al. (1975) from calorimetric studies. The discrepancy probably reflects local heating of the sample in the laser beam. Variation in the line width is noted below the main transition. The increase observed in the 10-30 °C temperature range is attributable to gauche rotamer formation below the main chain melt. Gaber et al. (1978) have suggested a variety of other spectral parameters to characterize the deuterated chain conformation, but the C-D line width variation is easily measurable in reproducible fashion and appears in a spectral region free from interference from other membrane components.

Binary Mixtures: DPL-d_{62}/DPPE. Typical spectra of 1:1 binary mixtures are shown in Figure 3 for the C-D stretching modes of the DPL-d_{62} component and in Figure 5 for the C-H vibrations which arise primarily from the DPPE component in the binary mixture. The temperature variations for ν(C-D) and I(2885)/(2850) are given in Figures 4 and 6 for DPL-d_{62} and DPPE, respectively. Also included in Figure 6 is the variation in the antisymmetric C-H stretching vibration frequency near 2885 cm^{-1}.

Examination of Figures 4 and 6 suggests that the binary DPL-d_{62}/DPPE sample is physically well mixed. Separate bilayers (unmixed lipid) are not present since transitions at temperatures corresponding to the pure components are not observed in their 50/50 mixture.

For the DPL-d_{62} component (Figure 4) a monotonic increase in the half-width is observed from 10 to 44 °C, at which point a change in slope is noted and the rate of increase of the line width becomes more pronounced. The slope change at 44 °C corresponds to the onset temperature of phase separation and is clearly defined from the Raman data. The completion temperature (as evidenced by a second change in slope) is not as well delineated but can be noted in the 60-65 °C range. The deterioration in spectral quality at high temperature is partially responsible for the ill-defined completion temperature. A comparison of the line width in the one-component and binary mixtures (Figure 4) suggests that the presence of DPPE only slightly perturbs the intramolecular order of the DPL-d_{62} at temperatures below the onset of melting in the binary system. The C-D line widths are similar in magnitude in each instance, and the noncooperative changes observed prior to melting follow similar patterns.

The temperature range of the phase separation in the binary mixture (~20 °C) is greatly broadened from the transition range for the pure DPL-d_{62} (~3 °C). This behavior contrasts sharply from that seen for DML-d_{54} in binary mixtures with DSL (Mendelsohn & Maisano, 1978).

The latter system, while showing miscibility in both gel and liquid crystal phases, behaves in extremely nonideal fashion. The melting of the DML-d_{54} as monitored by Raman spectroscopy occurs over a temperature range in the binary system (5-6 °C), just slightly larger than that noted for pure DML-d_{54} (3-4 °C). The width of the melting range for individual components in a binary mixture is clearly a useful probe of phase behavior. Quantitative analysis must await the production of phase diagrams currently in progress in this labo-

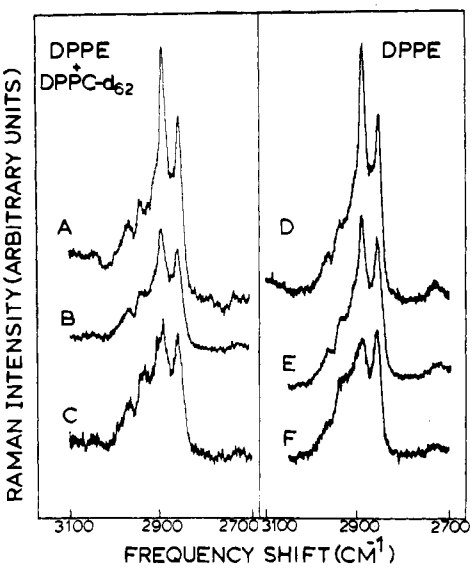

FIGURE 5: Typical spectra of the C-H spectral region for DPPE alone and in binary mixtures with DPL-d_{62} under the following conditions. DPPE/DPL-d_{62} binary mixtures: (A) 15 °C; (B) 49 °C; (C) 68 °C. DPPE multilayers: (D) 15 °C; (E) 49 °C; (F) 68 °C.

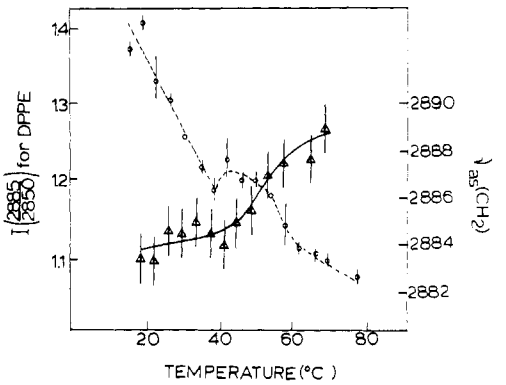

FIGURE 6: Temperature-induced variation in the $I(2885)/(2850)$ intensity ratio for DPPE in 1:1 binary mixtures with DPL-d_{62} (- - O - -). Error bars represent standard deviations obtained from two to four measurements at each data point. The observed increase for $I(2885)/(2850)$ in the 38-41 °C range is not the normal direction of variation expected for this parameter. See text for discussion. Also shown is the detailed frequency variation of the band near 2885 cm^{-1} (-Δ-) The error in the frequency measurement is ±1 cm^{-1} for each point.

ratory. The advantage of the Raman approach for studying phase behavior is clear from consideration of the two binary systems investigated thus far. Whereas calorimetric and spin-label methods measured one parameter for the system as a whole and cannot distinguish between contributions from each component to this parameter, the Raman approach suggested here can monitor the behavior of each component over the entire temperature range of interest. For the approach to be generally useful, the effect of deuterium on the phase properties of binary mixtures must be evaluated. This point is discussed below.

The onset and completion temperatures for the DPL-DPPE system were measured at 46 and 57 °C (1:1 binary mixtures) using spin-label techniques (Shimshick & McConnell, 1973). The slightly decreased onset temperature (44 °C) and increased completion temperature (60-65 °C) measured in the current Raman study are explicable in terms of the effect of perdeuteration of the DPL on its melting temperature. Since DPL-d_{62} melts ~5 °C lower than DPL (Petersen et al., 1975), the observed differences in onset and completion temperatures for the deuterated and nondeuterated molecule in binary combination with DPPE are in complete accord with thermodynamic predictions. The variations simply reflect the greater difference in melting temperatures between DPPE and DPL-d_{62} compared with DPPE and DPL. In this sense, the deuterated phospholipids are not probe molecules which perturb the system under study, but are phospholipids whose properties can be profitably studied in their own right. In general, the Raman melting curves for the systems studied thus far are in good accord with those derived from other physical methods, if the effect of deuteration on the melting points is considered.

The intensity ratio $I(2885)/(2850)$ for DPPE in the binary mixture exhibits (Figure 6) discontinuities at temperatures close to the onset and completion temperatures for the phase separation as measured for the DPL-d_{62} component. The ratio decreases rapidly from 1.4 to 1.2 between 20 and 38 °C. At this point an increase is noted and a maximum is observed at 41 °C. The plot is then characterized by a further decrease and a second discontinuity at about 60 °C. The increase seen in the 38-41 °C range is not the direction normally expected for the temperature-induced variation of this ratio (compare Figure 6 with Figure 2).

Similar anomalies were observed in the DML-d_{54}-DSL system (see Figure 5 in Mendelsohn & Maisano, 1978). The interpretation for these observations is somewhat unclear. It is known, however, that a significant fraction of the C-H intensity is derived from interchain interactions between adjacent nondeuterated chains (Gaber & Peticolas, 1977; Mendelsohn & Maisano, 1978). When phase separation begins in the DPL-d_{62}-DPPE system, the residual gel state phospholipid becomes enriched in DPPE. This leads to increased interactions between DPPE chains and a concomitant increase in the measured intensity parameter. Apparently, the interchain coupling effect on the intensity ratio over the range 38-41 °C is greater than the tendency of the ratio to decrease due to gauche rotamer formation.

Further evidence for this interpretation comes from examination of the temperature variation of the antisymmetric C-H stretching vibration of DPPE near 2885 cm^{-1} as shown in Figure 6. The position of this band is known to be sensitive primarily to gauche rotamer formation and not to variation in intermolecular interaction (Gaber & Peticolas, 1977). The temperature variation for ν_{as}(C-H) is different than that observed for the $I(2885)/(2850)$ ratio (Figure 6) and the midpoint of the melting process occurs at 50 °C. At this temperature, half the total number of gauche rotamers that occur in the DPPE during the phase separation have formed. The variation in $I(2885)/(2850)$, however, is most pronounced at 40 °C, where interactions between DPPE chains are most prevalent. The two temperatures do not coincide since maximal interactions between DPPE chains takes place near the onset of phase separation, rather than at the midpoint of the melting process.

Although the interpretation of the temperature variation in both ν_{as}(C-H) and $I(2885)/(2850)$ is consistent with the currently available Raman spectroscopic data on model systems, it must be considered tentative. Further spectra-structure correlations are required. It may prove difficult to find model systems whose structures are well enough understood so that the Raman technique may be calibrated.

DPL-d_{62}/DPPE/Cholesterol Ternary System. Although many investigations have characterized the behavior of cholesterol in one-component systems (Chapman, 1975; Demel & deKruyff, 1976; Brown & Seelig, 1978), little data are

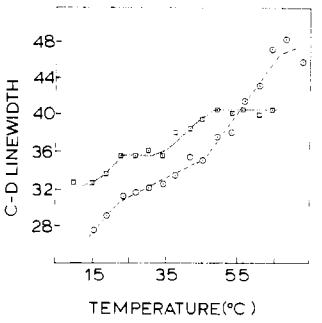

FIGURE 7: The effect of cholesterol on the temperature induced variation on the C-D stretching vibration in a 1:1:1 ternary mixture of DPL-d_{62}/cholesterol/DPPE (· · · ◘ · · ·). Also included is the variation in C-D stretching line width for 1:1 binary DPL-d_{62}/DPPE mixtures (- - O - -).

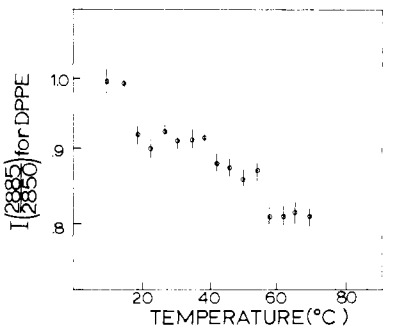

FIGURE 8: The effect of cholesterol on the $I(2885)/(2850)$ intensity ratio of DPPE in the ternary DPL-d_{62}/cholesterol/DPPE system. The C-H stretching vibrations of cholesterol have a small effect on the observed values; the cholesterol spectrum is temperature invariant over the range 10-60 °C. The observed variation in $I(2885)/(2850)$ parallels that seen for the variation in the C-D line width in the ternary system (Figure 7).

available on its effect in binary phospholipid mixtures. As an initial effort in this direction, a ternary system of DPL-d_{62}/DPPE/cholesterol in equimolar proportions has been investigated using the Raman approach described above.

The complex effects produced by the addition of cholesterol into a DPPE/DPL-d_{62} mixture are demonstrated in Figures 7 and 8 where the temperature variation for the C-D line width of the DPL-d_{62} component and the $I(2885)/(2850)$ ratio of the DPPE (with a small contribution from cholesterol) are plotted, respectively. Also plotted in Figure 7 for comparison is the C-D line width variation for the DPL-d_{62}/DPPE binary mixture. Although the detailed nature of the molecular structure changes that produce the spectral variations in Figures 7 and 8 is not entirely clear, some general observations may be made. At temperatures above 55 °C, cholesterol restricts further increase in the C-D line width compared with the binary mixture, suggesting that certain chain conformations containing gauche rotations are inhibited from forming. However, a broad phase separation region is still evident in the ternary system, with an approximate onset temperature of 30 °C and a completion temperature of about 50 °C. The magnitude of the line width change during this process is greatly reduced from that in the binary system. In order to estimate the number of gauche rotamers that form under various conditions, two assumptions are made.

(1) It is assumed that the increase in C-D linewidth is a linear function of the number of gauche rotamers that form.

(2) It is assumed that DPL-d_{62} has the same number of gauche rotamers that occur as in melting of the nondeuterated analogue (Schindler & Seelig, 1975).

With these assumptions, it is deduced that, in the presence of cholesterol, the phase separation observed is accompanied by the formation of about 1-2 gauche rotamers per chain, whereas in the pure DPL-d_{62} system the phase transition involves formation of ~4 gauche rotations. At low temperature, cholesterol inhibits the occurrence of perfect packing in the hydrocarbon chains, as shown by the increased value of the C-D line width in the ternary system compared with the binary or one-component systems. The presence of extra phases in the ternary system at low temperature is suggested by the observed discontinuity in the C-D line width at 24 °C (Figure 7). It is speculated that these may be related to the occurrence of cholesterol-rich phases in the presence of ordered phospholipid, although experiments at a variety of cholesterol concentrations are required to clarify this point.

Some recent ^{31}P NMR experiments (Cullis & de Kruyff, 1978) suggest that cholesterol may induce the formation of hexagonal phases in phosphatidylethanolamine systems containing unsaturated acyl chains. The added complexity in the ternary system in the current work may be due to such bilayer-hexagonal transitions.

The DPPE component of the ternary system also exhibits a complex pattern of structure variation as measured by the $I(2885)/(2850)$ intensity ratio (Figure 8). This spectral region is overlapped to some extent by C-H stretching vibrations of the cholesterol. However, the latter are known to be temperature invariant over the range of 10-60 °C (Mendelsohn, R., & Taraschi, T., unpublished data).

It is interesting to note that the absolute value of the C-H intensity parameter in the ternary system is greatly reduced at all temperatures from that in the binary mixture situation (compare Figure 8 with Figure 6). Cholesterol presumably acts by insertion parallel to the phospholipid hydrocarbon chains, thereby reducing interactions between DPPE chains. This in turn destroys interchain vibrational coupling between DPPE chains, resulting in a reduction of the C-H intensity parameter even at low temperatures where the system is still reasonably ordered (as measured by the C-D linewidth of the DPL-d_{62} component). The similarities between the temperature variation observed for the DPL-d_{62} and DPPE components in the ternary system occur since once that part of the C-H intensity derived from interchain vibrational coupling has been eliminated by insertion of cholesterol, any remaining intensity must be due to gauche rotamer formation. The parallel changes in the C-D and C-H regions suggest a similar effect by the cholesterol on both DPL and DPPE since both ratios become sensitive primarily to gauche rotamer formation.

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Active Transport of L-Glutamate by Membrane Vesicles Isolated from Rat Brain†

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ABSTRACT: Membrane vesicles, isolated after osmotic shock of synaptosomal rat brain fractions, actively accumulate L-glutamate. This process requires the presence of external sodium ions and internal potassium ions and is driven by artificially imposed ion gradients as the sole energy source. Either an Na^+ gradient (out > in) or a K^+ gradient (in > out) or both can be utilized to concentrate L-glutamate inside the vesicles. Transport is enhanced by valinomycin or by external thiocyanate ions and is about 50% inhibited by the proton ionophore carbonyl cyanide *m*-chlorophenylhydrazone. This transport thus appears to be stimulated by a membrane potential (interior negative). The glutamate transporter, the K_m of which has been determined to be 3 μM, is specific for L-glutamate. The transport process is unaffected by ouabain but is strongly inhibited by *p*-hydroxymercuribenzoate as well as by nigericin, which collapses the energizing ion gradients across this membrane. Unlike the sodium dependent, but potassium independent active accumulation of γ-aminobutyric acid in these vesicles (Kanner, B. I. (1978) *Biochemistry 17,* 1207) active L-glutamate uptake is not dependent on the presence of small monovalent anions in the external medium. The results provide direct evidence for Na^+-coupled electrogenic active L-glutamate transport by rat brain membrane vesicles. The dependence on internal potassium ions is discussed.

High affinity, sodium dependent, uptake systems for a variety of established and putative transmitters have been detected in brain preparations, such as synaptosomes (Iversen, 1971, 1973; Kuhar, 1973; Bennett et al., 1974). These uptake systems have been implicated in the termination of transmitter action on postsynaptic receptors (Iversen, 1971) as well as in maintaining constant levels of transmitters in the neurons (Hedgvist & Stjarne, 1969). This transport is inhibited by conditions interfering with intracellular ATP synthesis (Iverson & Neal, 1968; White & Keen, 1970), as well as by the inhibitor of the $(Na^+ + K^+)$-ATPase[1] ouabain (Iversen & Neal, 1968; Balcar & Johnston, 1972a). It has been suggested that the participation of the $(Na^+ + K^+)$-ATPase in transport is in-

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[1] Abbreviations used: ATPase, adenosine triphosphatase; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; Tris, tris(hydroxymethyl)-aminomethane; Tricine, *N*-tris[hydroxymethyl]methylglycine; Mes, 2-[*N*-morpholino]ethanesulfonic acid; Gaba, γ-aminobutyric acid.

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