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ON THE QUANTITATIVE INTERPRETATION OF BIOMEMBRANE STRUCTURE BY RAMAN SPECTROSCOPY

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Summary

The now well-established use of Raman spectroscopy to examine the structure of biomembranes is extended through an examination of the origins of the structure-sensitive features of phospholipid spectra and the development of quantitative order-parameters. One parameter gives a quantitative measure of the fraction of all-*trans* bonds in the hydrocarbon chains while the other provides a semiquantitative estimate of the lateral crystal-like order between the chains. The parameters are used to study the differences between vesicles and dispersions of dipalmitoyl phosphotidylcholine, dimyristoylcholine and egg lecithin. We find that the vesicles of dipalmitoyl phosphotidylcholine are substantially less ordered than the dispersions in terms of both longitudinal and lateral order which are greatly decreased.

A very careful measurement of the order as a function of temperature shows that there is a pre-melting transition in the dispersions of dipalmitoyl phosphotidylcholine which does not exist in the vesicles. Remarkable agreement is obtained between the Raman technique and that previously reported by calorimetric measurements and theoretical calculations.

Introduction

Continued progress of Raman spectroscopy as a probe of biomembrane structure requires development of quantitative molecular interpretations of the spectral events accompanying conformational alterations. We report here our progress toward this goal. The origin and physical significance of the structure-sensitive features of phospholipid Raman spectra are explored, and a scheme is developed for the standardization and quantitation of the data. The principles evolved are then applied to several current problems in biomembrane structure, in particular that of the structure of vesicles and dispersions of phospholipids.

Experimental

Materials

L-α-Dipalmitoyl phosphatidylcholine (Calbiochem) was freed of possible heavy metal contamination by extraction against a solution of EDTA [1]. A single spot was obtained when 2 μmol were chromatographed on silica gel G in $CHCl_3/CH_3CH/H_2O$ (65 : 25 : 4). 1-2-Dimyristoyl phosphatidylcholine (Calbiochem) was used without further purification. Egg lecithin was prepared by a modification of the procedure of Pangborn [2]. The material yielded a single spot on silica gel G with an R_F identical to a known sample of egg lecithin. Perdeuterohexadecane was supplied by Merck, Sharpe and Dohme, Canada, Ltd. and hexadecane by Applied Sciences Laboratories.

Methods

Phospholipid dispersions were prepared at 60° C by repeatedly homogenizing 100 mg phospholipid in 1 ml of buffer (0.1 M KCl, 0.01 M Tris, pH 8), through a 6″ 20-gauge hypodermic needle [3].

Phospholipid vesicles were made and separated by the procedure of Huang [4]. Phospholipid concentration was 150 mg/ml buffer (0.1 M KCl, 0.01 M Tris, pH 8). Sonication was effected using a Branson sonifier model W-185. The sonifier was equipped with a standard probe and operated at an output power of about 50 watts. To avoid overheating the sample, sonication was conducted in cycles of 1 min on, 15 s off, with a total of 18—20 minutes required for solution clarity. A water bath maintained the sample temperature above 45° C throughout the sonication. Samples were filtered through a 0.45 μm Millipore filter after sonication and immediately applied to the 1.8 × 20 cm column of Sepharose 4-B. The water-jacketed column was run at 45° C. Elution profiles were similar to those reported by Huang [4]. The trailing portion of the broad, slow-moving vesicle fraction was collected, and the perfectly clear solution transferred directly to melting point capillaries for Raman examination. Vesicle melting curves were run from high temperature to low.

Hexadecane-perdeuterohexadecane mixtures were prepared by volume with the assumption that the weight density of the perdeuterohexadecane is 1.15-times that of hexadecane.

The Raman spectrometer and constant temperature holder have been described previously [5]. The temperature within the sample was measured with a thermistor probe inserted into the capillary. A correction for laser heating (≈2.6° C) was determined by observing the melting of octadecane in the laser beam.

Band intensities were taken as peak heights measured from a baseline determined individually for each spectrum. Considerable care was taken to assure spectra with high signal/noise ratios and consistent, stable baseline characteristics.

Results and Discussion

Qualitative features of the Raman spectra of phospholipids. As depicted in the spectrum of solid dipalmitoyl phosphatidylcholine (Fig. 1), the Raman

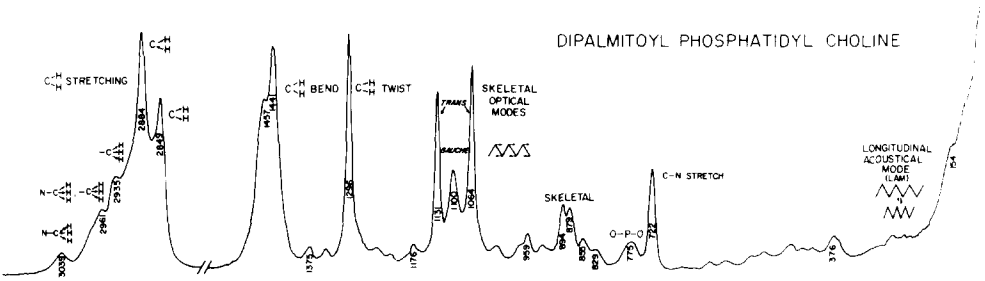

Fig. 1. Raman spectrum of solid dipalmitoyl phosphatidylcholine. Recorded at 50 cm^{-1}/inch per min; time constant, 1 s, gain 10^6 count/s; slits 250 μm; power at laser head, 900 MW; excitation wavelength, 5145 Å.

spectrum of a phospholipid is dominated by vibrations of the fatty acyl chains, with superposition of a few bands from the head group. They are here briefly described in terms of their origin and relative conformation-sensitivity.

At very low frequency (154 cm^{-1}) the longitudinal acoustical mode is observed as a weak shoulder. This mode results from an accordian-like motion of the entire hydrocarbon chain; its frequency is inversely related to the number (N) of all-*trans* bonds [6—7]. For a frequency of 154 cm^{-1}, as in solid dipalmitoyl phosphatidylcholine, N = 15, just as expected for a C-16 chain with a terminal ester linkage.

The most intense head group vibration in dipalmitoyl phosphatidylcholine is the C-N stretching mode at 722 cm^{-1}. The frequency and intensity of this band appears to be insensitive to any conformational change associated with melting. The mean intensity of the band is constant within ±6% from 20—50°C. The skeletal optical modes between 1000—1150 cm^{-1} are particularly sensitive to the conformational state of the hydrocarbon chains [5]. Of the three bands comprising this region, two (1064 cm^{-1} and 1133 cm^{-1}) may be assigned [9-11] to the B_{1g} and A_g vibrational modes of all-*trans* chain segments while the third ($\approx$1100 cm^{-1}) results from structures containing *gauche* rotations.

The assignments of the complex of bands in the C-H stretching (2800—2900 cm^{-1}) region have been clarified recently [12]. The principal bands are the methylene symmetric C-H stretch (2850 cm^{-1}) and asymmetric stretch (2890 cm^{-1}). Disruption of regular chain packing either by melting or dissolution [8,9] results in a decrease in the intensity of the 2890 cm^{-1} band relative to the band at 2850 cm^{-1}.

Order/disorder relations in phospholipid Raman spectra. Solid dipalmitoyl phosphatidylcholine exhibits highly ordered hydrocarbon chains as evidenced by the appearance of the longitudinal acoustical mode (Fig. 1). As an example of highly disordered chain we take dipalmitoyl phosphatidylcholine in $CHCl_3$. The result (bottom spectrum, Fig. 4) is a spectrum of radically different character than that of the solid. The *gauche* band in the skeletal optical region has broadened and shifted to lower frequency, while the *trans* band at 1133 cm^{-1} has lost considerable intensity (but is still present). The *trans* band at 1063 cm^{-1} becomes a shoulder on the broadened *gauche* band.

Chain disorder is seen as well in the C-H stretch region (for which dipal-

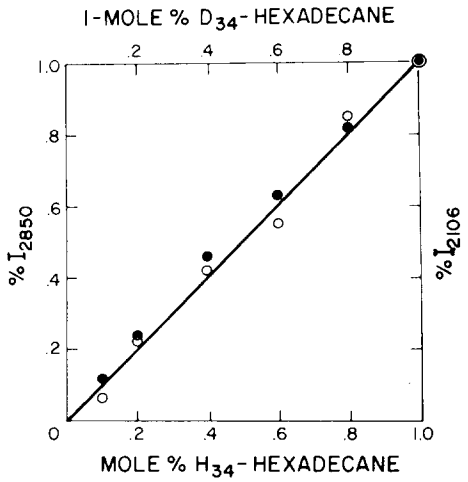

Fig. 2. Intensities of the CH_2 symmetrical stretch (2850 cm^{-1}) and CD_2 symmetrical stretch (2106 cm^{-1}) in solid mixtures of hexadecane/perdeuterohexadecane. Values are taken relative to intensities for pure hexadecane and pure perdeuterohexadecane.

mitoyl phosphatidylcholine was dissolved in $CDCl_3$). The asymmetric CH_2 stretch has broadened, lost intensity, and shifted to higher frequency (10 cm^{-1}). The symmetric stretch remains sharp and is the most intense feature of the spectrum. The intensity ratio of 2890 cm^{-1}/2850 cm^{-1} drops from $\approx$2.0 in solid dipalmitoyl phosphatidylcholine to 0.77 in solution.

One question in this study is that of the use of the 2850 cm^{-1} peak intensity as an internal standard by which to reference the 2890 cm^{-1} band. In this series of experiments mixtures of varying proportions of liquid hexadecane/perdeuterohexadecane in identical capillary tubes were run under exactly the same

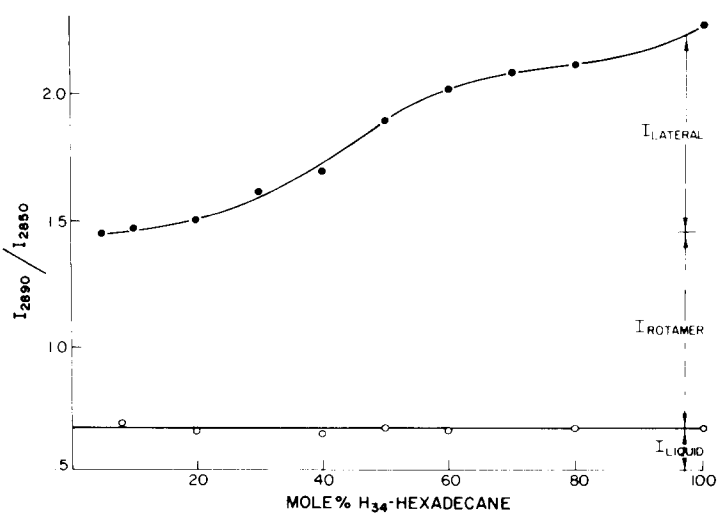

Fig. 3. Intensity ratios I_{2890}/I_{2850} as a function mol% solid hexadecane in a matrix of perdeuterohexadecane. See text for definition of I_{lateral}, I_{rotamer} and I_{liquid}.

conditions. In order to see if the measured intensities of the 2106 cm^{-1} (symmetric C-D stretch) and 2850 cm^{-1} bands would be proportional to the mole fraction, we measured each intensity as the peak height from background and divided it by the absolute intensity of that obtained from pure hexadecane or perdeuterohexadecane. Fig. 3 shows the remarkable accuracy of these intensity measurements obtained without use of an internal standard.

Lateral packing order and CH_2 stretch intensities. What are the origins of the changes in intensity ratios of the 2890 cm^{-1} to 2850 cm^{-1} bands? Larsson and Rand [14], observing these changes, attributed them to an increase of the relative "looseness" (their quote) of lateral chain order.

Lateral packing effects on CH_2 intensity ratios are most clearly determined if a hydrocarbon chain is isolated vibrationally from its neighbors, while leaving its conformation and chemical environment unchanged. Accordingly, we have compared the spectra of pure solid hexadecane and of hexadecane dissolved in a solid perdeuterohexadecane matrix. (As is well-known, substitution of deuterons for protons leaves a crystal lattice essentially unchanged [17]). When I_{2890}/I_{2850} is plotted against mole %-H_{34}-hexadecane (Fig. 3), a monotonic decrease in intensity ratio is observed with increasing dilution of the hydrocarbon. The frequencies of the C-H_2 stretches are not changed by dilution in perdeuterohexadecane. However, melting shifts the 2890 band 10 cm^{-1} higher. The lower limit of I_{2890}/I_{2850} upon dilution is 1.45, considerably higher than the limiting value for the melted hydrocarbon (0.67). Thus crystal (lateral) interaction effects can account for only about 50% of the total intensity change upon melting. Clearly a second effect is involved; one associated with an increase in Raman frequency. The origin of this "second effect" is discussed below.

The intensity change associated with the isolation of the C-H vibration in the C-D matrix may be explained in the following way. Just as the Raman selection rules for an isolated molecule may be derived from consideration of its symmetry point group, the rules for an isolated linear polymer may be determined from the appropriate line group. However, when the polymer exists in a crystal lattice space group selection rules are operative. Vibrational coupling between the two chains in the unit cell causes each vibrational mode in the polyethylene line group to be split into two modes [18].

For the CH_2 stretching modes the intensity effect per se is a likely consequence of Fermi resonance between the first overtone of the CH_2 bending modes (B_{2u} and A_g) near 1450 cm^{-1} and the degenerate 2890 cm^{-1} doublet (B_{3g} and B_{2u}) (as first suggested by Schachtsneider and Snyder [19]). Apparently this Fermi resonance between the crystal modes is much greater than between the simple chain modes. Deuteration of half or more of the chains destroys the vibrational coupling in the unit cell by lowering the chain frequencies of the deuterated polymer (although the crystal packing presumably remains the same). Thus the Fermi resonance between the crystal modes is destroyed and with it that part of the Raman intensity which was derived from crystal vibrational interactions.

What is the origin of that portion of the intensity change not accounted for by lateral crystal effects and for which a frequency shift is seen? It has long been recognized that when a solid hydrocarbon is melted many new lines appear in the Raman spectrum while previously sharp features become

broadened [20,21]. Many years ago, Mizushima [21] explained the effect by the simple assumption that in a solid hydrocarbon only one (presumably *trans*) conformation exists while for the liquid several conformations (and thus several Raman lines) are possible. This effect might be called "rotomer broadening".

The total relative intensity ($I_{tot} = I_{2890}/I_{2850}$) of a highly ordered crystalline hydrocarbon lattice can be broken into three parts: $I_{liquid} = 0.7$, the residual intensity in the liquid; $I_{rotamer}$, the difference between the intensity of a liquid and that of an isolated all-*trans* chain; and $I_{lateral}$, that due to vibrational coupling between the adjacent chains in the hydrocarbon crystal. These intensity contributions are shown in Fig. 4. Thus $I_{tot} = I_{liquid} + I_{rotamer} + I_{lateral}$. Experimentally the two contributions to I_{tot} may be differentiated by the associated frequency change. $I_{lateral}$ does not involve a frequency change while there is an increase of ≈ 10 cm^{-1} to 2894 cm^{-1} in going from the isolated chain to the melt.

Intrachain order and skeletal optical mode intensities. Properly interpreted, the relative intensity and qualitative behavior of the skeletal optical mode near

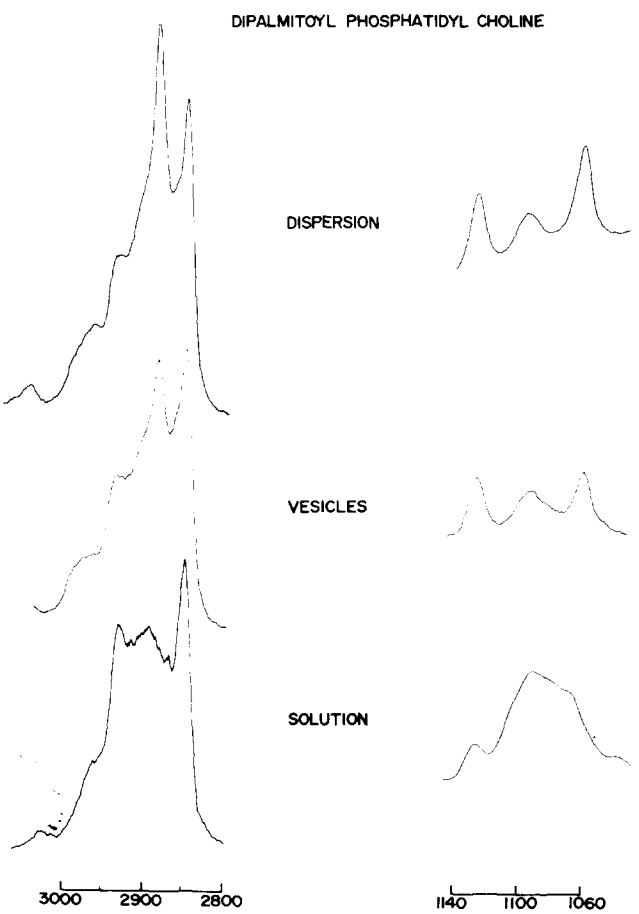

Fig. 4. Raman spectra of dipalmitoyl phosphatidylcholine prepared as a 20% aqueous dispersion, as single-bilayered vesicles, and dissolved in $CHCl_3$ (or $CDCl_3$).

1133 cm^{-1} provides quantitative insight into the structure of the phospholipid chains. Assuming that the intensity of this C-C stretching vibration is the sum of intensities from individual all-*trans* segments, the Raman intensity is given in terms of the probability of occurrence of a *trans* bond P_t by

$$I_R = I_0 \sum_{n=3}^{N} nC_n P_t^n \tag{1}$$

where n is the number of bonds in an all-*trans* sequence within a molecule of N bonds and C_n is the number of times the nth segment reoccurs. I_R is the total Raman intensity and I_o is the intensity per "*trans*-unit". The formalism is approximate in assuming I_o a constant without regard to neighbor and end group effects. However, as we shall see, the Raman intensity at 1133 cm^{-1}, properly normalized, leads to an order-parameter which gives a quantitative measure of the average number of *trans* bonds. Marsh [23] has also discussed a somewhat similar quantitative approach.

Order parameters. S_{trans} and S_{lateral}. We wish to develop order parameters which will distinguish as much as possible between the order due to intrachain structure and that due to lateral crystalline interactions. These parameters are normalized so that $S = 1$ indicates the highest possible order and $S = 0$ no order (not necessarily the lowest possible).

The *trans* parameter is defined as

$$S_{trans} = \frac{(I_{1133}/I_{\text{ref}})_{\text{observed}}}{(I_{1133}/I_{\text{ref}})_{\text{dipalmitoyl phosphatidylcholine solid}}} \tag{2}$$

The choice of an all-*trans* standard with which to define S_{trans} fixes $n = N$ and $\Sigma P_t^n = 1$. Thus reducing Eqn. 1 to $I_R = I_o N$.

I_{ref} is taken as $I_{1090\,cm^{-1}}$ when a number of different phospholipids are being discussed. However, for very precise work on phosphatidycholines we have used $I_{772\,cm^{-1}}$ as the reference. For diaplmitoyl phosphatidylcholine solid $I_{1133}/I_{722} \sim I_{1133}/I_{1090} = 1.77$. Observe that $S_{trans} = 0$ for $(I_{trans}/I_{\text{ref}}) = 0$; a condition never observed experimentally.

To obtain an order parameter for the lateral interaction presents a more difficult problem since the change in the intensity of the 2890 cm^{-1} band is due both to the decrease in interchain vibrational coupling and phonon dispersion broadening due to chain shortening. As a starting point we assume that about half of the observed decrease is due to each of the two effects (since both have the same magnitude in hexadecane).

Since the difference in intensity between the solid hexadecane and solid hexadecane diluted with perdeuterohexadecane is half the intensity difference between crystal and liquid, S_{lateral} may be defined as

$$S_{\text{lateral}} = \frac{I_{\text{C-H}_2\text{(sample)}} - I_{\text{C-H}_2\text{(liq hexadecane)}}}{I_{\text{C-H}_2\text{(crystalline hexadecane)}} - I_{\text{C-H}_2\text{(liq hexadecane)}}} \tag{4}$$

or

$$S_{\text{lateral}} = \frac{I_{\text{CH}_2\text{(sample)}} - 0.7}{1.5} \tag{5}$$

where $I_{C\text{-}H_2} = I_{2890}/I_{2850}$. This parameter is only approximate and must be considered semi-quantitative, but it will give some insight into the amount of lateral interaction. The contribution due to rotamer broadening can be estimated qualitatively from the amount of frequency shift. If the equation is applied in the domain for which the frequency of the asymmetric CH_2 stretch remains constant, S_{lateral} should provide a good qualitative estimate of the degree of lateral interaction.

Phospholipid dispersions and vesicles compared. It has been suggested [3] that differences might exist between the molecular structure of large multilamellar phospholipid dispersions and small (radius 125 Å) single-bilayered vesicles. The proposed structural difference is postulated to arise from disruption of orderly hydrocarbon chain packing induced by the small radius of curvature of the vesicles. Vesicles would therefore exhibit a less ordered hydrocarbon interior than would a dispersion of the same phospholipid.

That vesicles are in fact more disordered than dispersions is demonstrated by the spectra in Fig. 4. The dispersion shares skeletal optical mode features in common with solid diaplmitoyl phosphatidylcholine, i.e. well-resolved bands with peak ratios near (but lower than) those for the solid. Vesicles, however, are clearly different from dispersions in this spectral region. The pattern is confirmed in the C-H stretching region where the asymmetric stretch has lost intensity and is lower than the symmetric stretch. The frequency of the asymmetric CH_2 stretch in the vesicles is unchanged, suggesting a loss of lateral packing, without the onset of liquid-like quality seen for dipalmitoyl phosphatidylcholine in $CHCl_3$.

Quantitatively, at 30°C *, vesicles are characterized by $S_{trans} = 0.54$ and $S_{\text{lateral}} = 0.23$; dispersions by $S_{trans} = 0.76$ and $S_{\text{lateral}} = 0.44$. As will be shown in the melting data (below) this structural difference is evident even at 20°C. Thus dispersions, below T_c, reveal both chain order and lateral interaction intermediate between that of solid dipalmitoyl phosphatidylcholine and the lipid dissolved in $CHCl_3$, while vesicles are found to be consistently more disordered than the dispersions in both senses. This conclusion is in agreement with the experiments of Suurkuusk et al. [1] which show unequivocal calorimetric and fluorescence probe differences between dipalmitoyl phosphatidylcholine vesicles and dispersions.

We have also examined the particles of intermediate size (≈400—1000 Å) which elute first on the gel filtration column and find $S_{trans} = 0.85$ and $S_{\text{lateral}} = 0.18$.

A similar but smaller structural difference is evident even at 25°C for dimyristoyl phosphatidylcholine vesicles and dispersions (Fig. 5A). Note the data's liquid-like character (i.e. broad, weak 2890 cm^{-1} and broad, shifted *gauge* band).

In sharp contrast to the results for the saturated lecithins, when the Raman spectra of dispersions and vesicles of egg phosphotidylcholine are compared (Fig. 5B) they are found to be virtually indistinguishable and liquid-like; a finding which agrees with the NMR studies of Stockton et al. [24] regarding the liquid-like nature of egg phosphatidylcholine preparations.

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* As noted below, at temperatures lower than ≈27°C vesicles tend to aggregate [1].

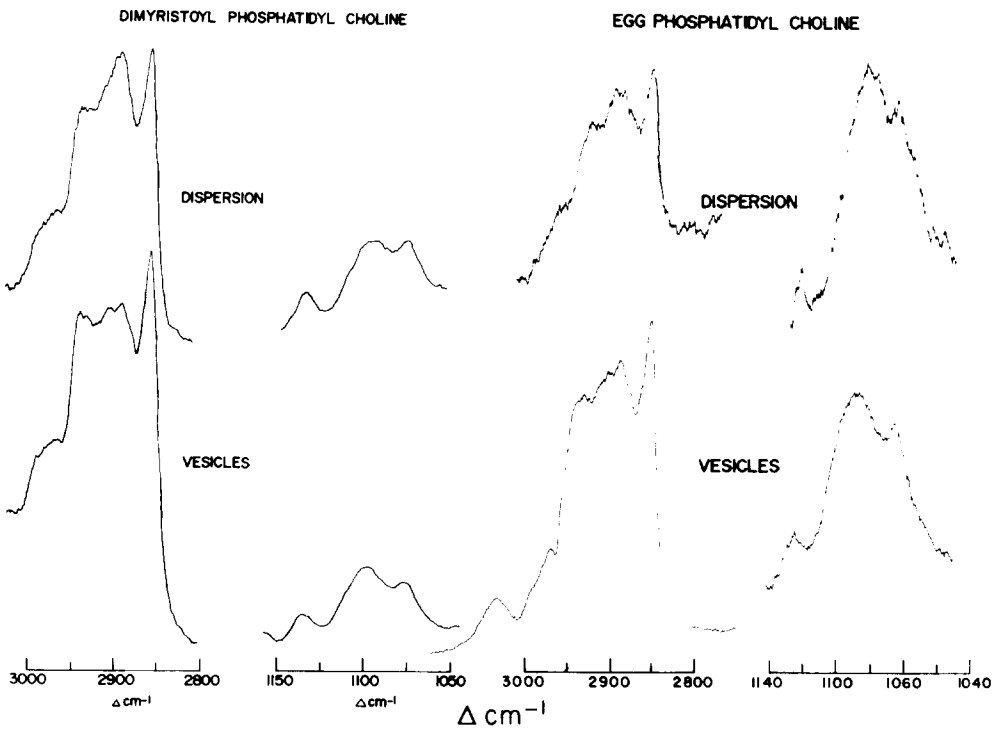

Fig. 5. Raman spectra of (A) dimyristoyl phosphatidylcholine prepared as a dispersion and as vesicles and (B) egg phosphatidylcholine prepared as a dispersion and as vesicles.

Shortly after publication of our preliminary report [25] on the structural differences between vesicles and disperions, a similar study by Mendelsohn et al. [26] appeared. We basically agree with their conclusions, particularly that dispersions and vesicles of egg lecithin are indistinguishable above T_M. However, we conclude that vesicles are distinct from dispersions, while Mendelsohn et al. [26] conclude that sonication of dipalmitoyl phosphatidylcholine has no effect on the intensity ratio in the skeletal optical spectral region. The discrepancy seems to lie in the nature of the samples studied. The Ottawa group [26] unquestionably examined sonicated phospholipids (prepared by bath sonication). However, as is well-known [4,27] even extended probe sonication invariably results in mixtures of small unilamellar vesicles and multilayered structures such that a separation technique such as gel filtration [4,27] must be employed to assure a preparation homogeneous in discrete, single-walled bilayered vesicles. The nature of the particles examined by Mendelsohn et al. [26] is suggested by an inspection of the order parameters. Thus, from Mendelsohn's unsonicated dispersion data we estimate S_{trans} = 0.9 and $S_{lateral}$ = 0.39, identical with our data (23°C) of S_{trans} = 0.91 and $S_{lateral}$ = 0.30. Likewise, for Mendelsohn's sonicate data S_{trans} = 0.85 and $S_{lateral}$ = 0.23, values virtually identical with those reported here for sonicated phospholipids of intermediate size. Thus we conclude with Mendelsohn [26] that sonication effects the interchain interaction, which we interpret as a significant decrease in lateral chain packing order.

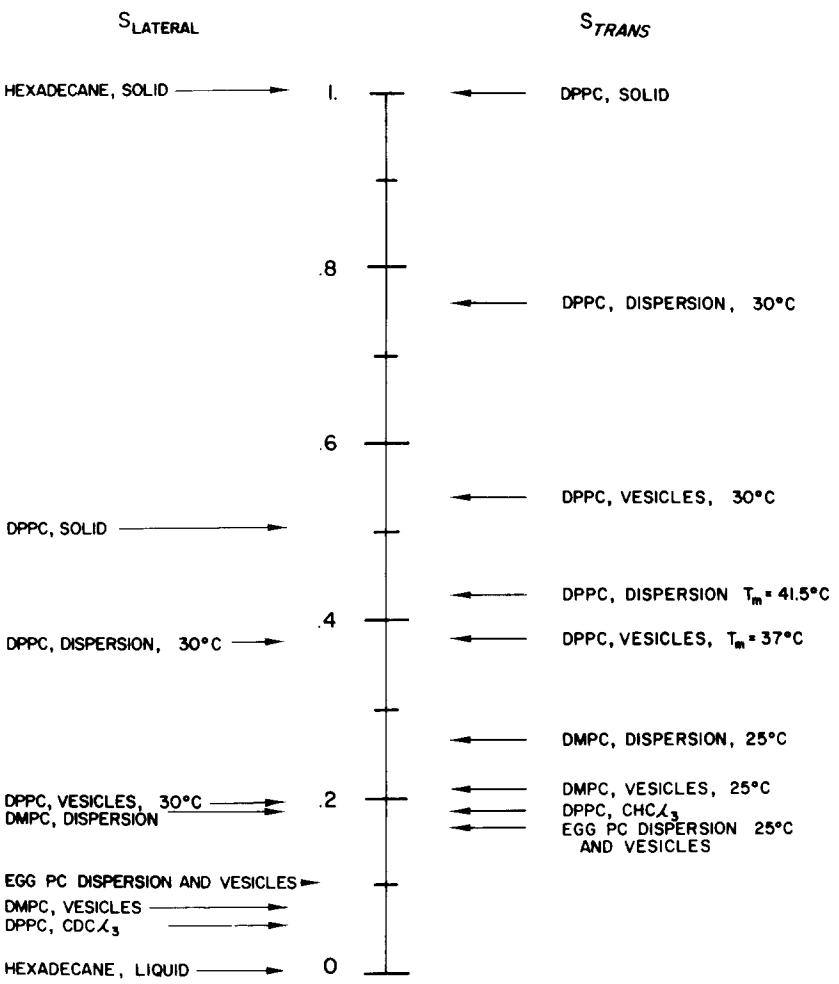

Fig. 6. Phospholipid preparation ranked in terms of: (A) relative all-*trans* probability, and (B) the order parameter $S_{lateral}$. DPPC, dipalmitoyl phosphatidylcholine; DMPC, dipalmitoyl phsophatidylcholine.

Relative trans and lateral order. Since S_{trans} is a relative measure of the number of *trans* bonds in a sequence of 3 or more *trans* bonds, a chart (Fig. 6A) may be constructed comparing S_{trans} = 1 for the chains of solid dipalmitoyl phosphatidylcholine and the values for the various phospholipid preparations. For example, the number of *trans* bonds in a dispersion is ≈76% of that for solid dipalmitoyl phosphatidylcholine or ≈12 bonds. The chart also shows that relative to dispersions, vesicles have a significantly lower probability for occurrence of all-*trans* conformations and, obviously, a higher probability of *gauche* forms. This is one meaning of the observation "vesicles are more disordered than dispersions".

Relative lateral order may be similarly displayed (Fig. 6B). Solid dipalmitoyl phosphatidylcholine is seen to have a lateral order considerably lower than that of hexadecane. A likely result of reduced packing order near the phospholipid head group. A comparison of the vesicle data in Figs. 6A and 6B reveals that

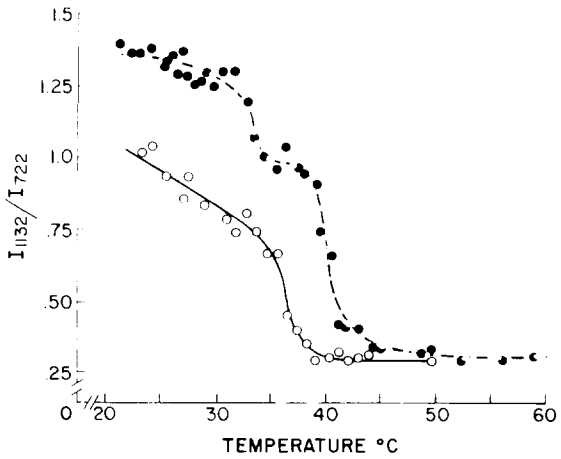

Fig. 7. Upper trace, melting curve for a dispersion of dipalmitoyl phosphatidylcholine derived from the change in *trans* band (1133 cm^{-1}) intensity relative to the temperature-invariant C-N stretch (722 cm^{-1}). Each point represents the mean of three seperate melting experiments. The standard deviation from the mean for each point does not exceed 6%. Lower trace, melting curve for single-bilayered vesicles derived from the change in intensity of the 1132 cm^{-1} *trans* band relative to the C-N stretch (722 cm^{-1}).

whereas vesicles have an all-*trans* probability (0.54) substantially larger than disordered phospholipids (≈0.2), vesicle lateral order (0.19) falls in the region of highly disordered systems, indicating a substantial breakdown in transverse lattice order. It can be argued that while transverse and longitudinal order are to some extent coupled, the coupling is by no means absolute, as the example above demonstrates. Thus while a loss of transverse order might be necessary to

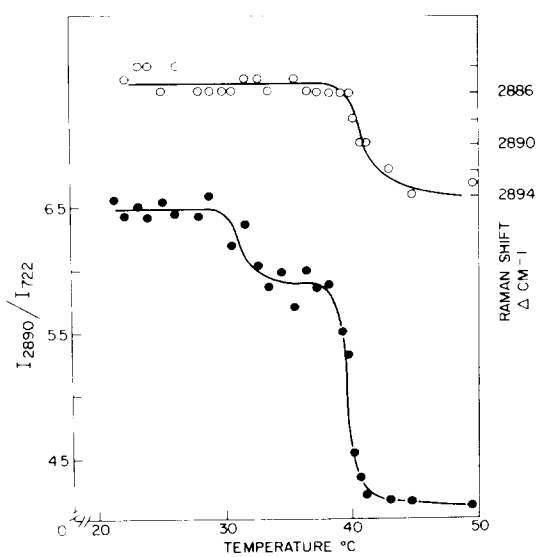

Fig. 8. Melting curve for a dispersion of dipalmitoyl phosphatidylcholine derived from: (1) the change in the intensity ratio I_{2890}/I_{722} (closed circles); (2) change in frequency of the ≈2890 cm^{-1} band (open circles).

provide the defect space required for an increase in *gauche* rotations, the actual *gauche* probability is likely to be thermodynamically controlled.

Melting behavior of phospholipid dispersions. Further evidence for conformational differences between dispersions and vesicles comes from the behavior of their respective Raman spectra with temperature. The Raman-derived melting data shown here represents the highest resolution (1°C intervals) reported to date and provide melting curves containing considerable detail.

Dispersions of dipalmitoyl phosphatidylcholine, examined in the skeletal optical mode region (Fig. 7, upper trace) show two melting phenomena: the well-documented main melting transition at 41.5°C and a pre-melting event at 34.2°C. These values are in excellent agreement with the calorimetric and fluorimetric measurements of Suurkuusk et al. (ref. 1, Table I). Approximately 75% of the total change in *trans* band intensity is accounted for by the main melt; 25% by the premelt.

The pre-melting event is also seen in the C-H_2 stretch data, Fig. 8. Note that whereas the intensity ratio changes at 33 and 41°C, the Raman shift inflects sharply in frequency only at the temperature of the main phase transition. Thus the pre-melt and main transition are distinct molecular events. The pre-melting transition is characterized by a decrease in $S_{lateral}$ without the onset of pronounced rotamer broadening. Neart 34°C the lattice must expand somewhat, but does not go over to the almost liquid-like state typical of the liquid crystal. Since a frequency shift in the 2890 cm^{-1} band is observed only with the main melt, this liquid-like state is clearly only associated with the higher temperature transition. The distinguishing characteristic of the pre-melt is the abrupt and complete loss of lateral chain interaction. Vesicles, as will be shown below, do not display a pre-melting transition since the lateral interaction, even at 20°C, is essentially zero.

In the pre-melt region intrachain order decreases by about 20%. In terms of S_{trans} this roughly corresponds to a loss of ≈ 2 *trans* bonds. While it has been suggested that the pre-melting transition is related somehow to the packing of the choline head group [28,29] no such effect is evident in the Raman spectrum. Similarly, neither ^{31}P nor ^{2}D magnetic resonance indicates involvement of the head group [30]. Thus the pre-transition may be visualized as a highly cooperative event in which a decrease in overall lateral packing order is accompanied by a small change in chain order.

Melting behavior of vesicles. Vesicles of dipalmitoyl phosphatidylcholine

TABLE I

TRANSITION TEMPERATURES FOR DISPERSIONS AND VESICLES OF DIPALMITOYL PHOSPHATIDYLCHOLINE

	Raman (°C)	Calorimetry * (°C)	Fluorescence * (°C)
Dispersion			
Pre-transition	34.2	35.4	25.2—33.9
Main transition	41.5	41.2	41.1
Vesicles	37	37	37

* Data from ref. 1.

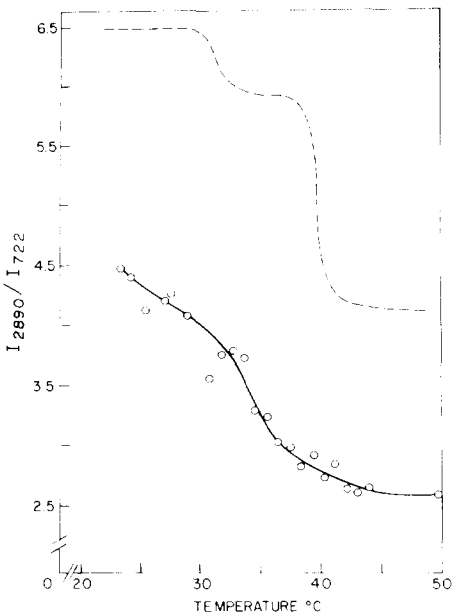

Fig. 9. Melting curve for single-bilayered vesicles of dimalmitoyl phosphatidylcholine. The data is derived from the change in the intensity ratio I_{2890}/I_{722}. Superimposed as a dashed line is the dispersion melting curve from Fig. 8.

melt with a single broad transition at 37° C (Fig. 7, lower trace). The agreement with the data of Suurkuusk et al. (ref. 1, Table I) is again excellent. Below 30° C the *trans* band intensity ratio rises steeply with temperature. We suspect that this is a result of the slow transformation of the single-lamellar vesicles into multilamellar forms [1]. In fact we have observed that when freshly prepared vesicles are held below ≈25° C, the *trans* band intensities do increase with time. Above ≈40° C the intensity ratio for vesicles becomes constant and identical to

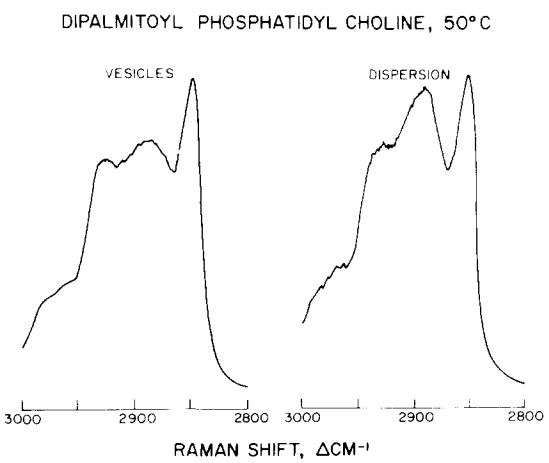

Fig. 10. Raman spectra of a dispersion and vesicles of dipalmitoyl phosphatidylcholine at 50° C.

that for dispersions. The *trans* order parameter, $S_{trans} = 0.18$, has in each case a value similar to egg phosphatidylcholine or dipalmitoyl phosphatidylcholine in chloroform. Therefore at high temperatures the aliphatic chains in both vesicles and dispersions are highly (and comparably) disordered.

At their respective transition temperatures S_{trans} for vesicles (0.38 ± 0.09) and dispersions (0.43 ± 0.07) are very nearly the same. On the basis of the Raman data, we estimate, that as a lower limit, ≈ 8 bonds of a palmitoyl chain will be in a *trans* conformation. This corresponds well with the theoretically determined value of 9.7 bonds [30]. Note that as a consequence of end effects, Raman data may tend to underestimate slightly the actual value.

The vesicle melting curve (Fig. 9) constructed from the CH_2 intensity change is similar to that seen in the skeletal optical region. However, at all temperatures the curve falls significantly below that for dispersions. Even well above T_M the difference persists. This is qualitatively depicted in spectra of a dipalmitoyl phosphatidylcholine dispersions and vesicles at 50° C (Fig. 10). The dispersion retains structure in the 2890 cm^{-1} band while the vesicles show the broad featureless band characteristic of a liquid-like chain. At 50° C, $S_{lateral} = 0.16$ for the dispersion and 0.05 for vesicles. Since for dispersions we have determined that the lateral contribution to I_{CH_2} is zero above 40° C we can estimate the contribution of $I_{rotamer}$ to the total CH_2 intensity for dispersions and vesicles. Thus despite their identical S_{trans}, dispersions seem to retain some type of chain order ($I_{rotamer} = 0.25$) when compared to vesicles ($I_{rotamer} \approx 0$). Though the quantitative significance of $I_{rotamer}$ is not fully understood, empirically it seems to relate to the degree of "liquid-likeness" of the chains; i.e. their dynamic state rather than their static or average conformation as reported by S_{trans}. If this is true we may speculate that although chains in a vesicle and dispersions have, on the average, the same proportion of *trans* and *gauche* forms, in dispersions the interconversion of these forms is somewhat restricted while in vesicles the isomerization is quite rapid; a model consistent with that proposed by Horwitz [31] on the basis of proton T_1 measurements. Thus as originally predicted by Seiter and Chan [32], at higher temperatures, the vesicle bilayer may be characterized as essentially liquid-like.

Note added in proof (Received December 22nd, 1976)

In a recent discussion of the motional state of lipid bilayers Petersen and Chan [34], recognize, as we have, the importance of interpreting bilayer dynamics in terms of both chain orientation and chain isomerization. It is germane to our interpretation of the Raman spectra of bilayers to mention the following. To account for NMR linewidth data in sonicated bilayers, Petersen and Chan predict a decrease in the value of P_t by a factor of 2—3, or an increase of 30—40% in the limiting angle of chain reorientation (or corresponding changes in both parameters). This prediction appears to be confirmed by the data in Fig. 6, wherein it is seen that S_{trans} for vesicles (30° C) is 1.5-times smaller than for dispersions and $S_{lateral}$ (which may be taken as a measure chain dislocation is significantly decreased in vesicles. We thank Professor Chan for communicating these results to us prior to publication.

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