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THE EFFECT OF SONICATION ON THE HYDROCARBON CHAIN CONFORMATION IN MODEL MEMBRANE SYSTEMS: A RAMAN SPECTROSCOPIC STUDY*

R. MENDELSOHN, S. SUNDER and H.J. BERNSTEIN

Division of Chemistry, National Research Council of Canada, Ottawa, Ontario K1A OR6 (Canada)

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Summary

Raman spectra are presented for egg lecithin above and below the gel-liquid crystal phase transition, and several regions of the Raman spectrum are shown to be sensitive to conformational changes in the hydrocarbon chains. These regions are used to investigate the effect of sonication on the structure of egg lecithin and dipalmitoyl lecithin vesicles.

Sonication of both egg lecithin above T_m, and dipalmitoyl lecithin above and below T_m produces no change in the relative population of *trans* and *gauche* isomers in any of the systems studied. Sonication does however appear to effect interchain interactions, a possible consequence of imperfect packing towards the center of the bilayers in vesicle systems.

The technique of sonication of phospholipid dispersions is widely used to transform the large coarse aggregates which form initially upon suspension into a system of vesicles in which a small number of bilayers form the outer shell [1]. The resultant preparations form convenient model systems for the study of membrane transport phenomena.

The proton magnetic resonance (PMR) spectra of unsonicated dispersions do not display high resolution characteristics which do, however, develop progressively as sonication proceeds [1, 2]. The origin of this effect has been the object of several studies and differing conclusions have been reached. For example, Finer and co-workers [3, 4] have suggested that the observed narrowing in the PMR spectra can be completely accounted for by an increased rate of particle tumbling due to the decrease in particle size caused by the sonication process. Chan and co-workers [5–7] have, on the other hand, concluded

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*N.R.C.C. No. 15055.

that vesicle tumbling alone cannot account for the observed narrowing. They suggest that the effect of sonication on the PMR spectra can be accounted for only if the regular packing of the hydrocarbon chains in model membrane systems is disrupted as a consequence of the small radius of curvature in the vesicles. As the resolution of this problem is important for an understanding of the structure of both model membranes (vesicles) and naturally occurring systems with small radii of curvature, it seems appropriate to examine this question with an entirely different physical method.

Raman spectroscopy has recently been applied to the study of molecular conformation in a variety of membrane and related model systems, and several regions of the Raman spectrum have been shown to be sensitive to *trans-gauche* isomerisation in the hydrocarbon chains [8—14]. As the vibrational frequencies monitored in the Raman spectrum are not expected to be sensitive to vesicle tumbling (since they take place in a much shorter time period than that during which the vesicle can tumble), one can examine the Raman spectra of sonicated and unsonicated membrane preparations for unambiguous evidence of *trans-gauche* isomerisation and/or disruption of regular molecular packing.

In the current work, we report the results of a Raman spectroscopic study of sonicated and unsonicated dispersions of egg lecithin and dipalmitoyl lecithin. In addition, we present Raman spectra of egg lecithin above and below the gel-liquid crystal phase transition in order to demonstrate the sensitivity of the technique to conformational change.

Egg lecithin (chromatographically pure) was obtained from Lipid Products, Surrey, England and dipalmitoyl lecithin was purchased from Sigma Chemical Co. Both were found to be free from fluorescent impurities and were used without further purification.

Lipid concentrations were determined by lipid phosphorous analysis [15] and were generally 8—10% by weight. Unsonicated samples were agitated on a rotatory vibrator prior to examination by Raman spectroscopy. Sonication of samples was carried out in a Cole Palmer bath-type ultrasonic cleaning apparatus. Egg lecithin was sonicated under N_2 at 5—10°C for periods which did not exceed 2 h. After 1 to 2 h, a translucent preparation resulted with no signs of turbidity due to the presence of large aggregates. Sonication of dipalmitoyl lecithin was carried out at 55—60°C which is above the temperature of its gel-liquid crystal phase transition ($\approx$ 41°C).

The techniques employed in taking Raman spectra have been described previously [14]. Briefly, spectra were taken on a Jarrell-Ash model 25-100 or Spex model 1401a double monochromator equipped with photon counting detection and strip-chart recording. Excitation was provided by Spectra physics No. 164 Ar^+ or Coherent radiation No. 12A Ar^+ lasers. The lasers were tuned to 5145 Å for the experiments reported here. Power levels at the samples were about 400 mW.

The samples were contained in 1 mm internal diameter Kimex melting point capillaries and were examined in the transverse mode. Temperature was controlled by flowing N_2 gas over the sample tube which was contained in an unsilvered dewar [14]. Raman frequencies are accurate to $\pm$ 2 cm^{-1} for sharp features and $\pm$ 3—4 cm^{-1} for broader bands.

Raman spectra for egg lecithin in the 700—1750 cm^{-1} region at temperatures above and below the gel-liquid crystal phase transition are shown in Fig. 1, and are representative of the signal to noise level attainable. The origin of the observed vibrations, particularly those of the hydrocarbon chains, has been discussed in several places [8, 9, 16—18]. Although identification of each spectral feature with a particular normal mode of vibration is by no means complete, those spectral regions upon which the current discussion is based are reasonably well understood. We first discuss (i) the temperature dependence of the egg lecithin spectrum in order to illustrate the sensitivity of the technique to conformational change and then (ii) present the results of the experiments with sonicated and unsonicated dispersions.

(i) Several regions of the Raman spectrum of egg lecithin are drastically altered when the system undergoes the gel-liquid crystal phase transition which occurs at about -15°C [18]. As can be seen from Fig. 1, the regions most affected are 850—950 cm^{-1}, 1050—1150 cm^{-1}, and 1440—1460 cm^{-1}.

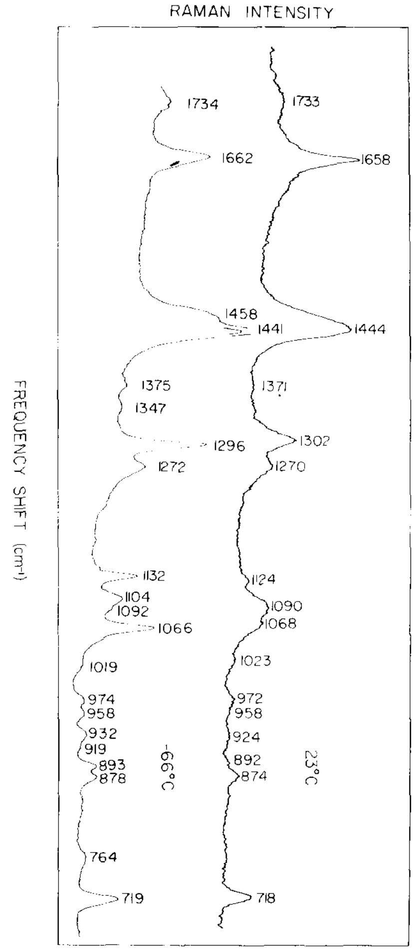


Fig. 1. Raman spectra of egg lecithin (10% by weight) in H_2O above and below the gel-liquid crystal phase transition. Top spectrum 23°C; bottom spectrum -66°C. Spectral parameters: time constant, 10; spectral slit width, 5 cm^{-1}; scan speed, 0.1 cm^{-1}/s.

Of interest in the current work is the 1050—1150 cm^{-1} region which contains the skeletal optical vibrations (predominantly C-C stretching modes of the hydrocarbon chains) along with the O-P-O symmetric stretching vibration of the phosphate group [8, 14]. The sensitivity of this region to conformational changes in phospholipid systems was first demonstrated in a study of the effect of cholesterol on the gel-liquid crystal phase transition of dipalmitoyl lecithin [8], and has subsequently been used to probe the interaction of cholesterol with egg lecithin [10] and to study various phases of the egg lecithin/H_2O system [20]. When the all-*trans* chain conformation predominates, (Fig. 1, −66°C spectrum), this region is characterised by strong bands at 1132 and 1066 cm^{-1}, with a weaker feature at 1104 cm^{-1} and a shoulder at 1092 cm^{-1}. The two strong features are similar in relative intensity to those in dipalmitoyl lecithin below T_m, (8 and Fig. 2), suggesting that the non-uniformity of the hydrocarbon chains in egg lecithin has little effect on the intensity distribution of these bands. It has recently been shown that the band at 1104 cm^{-1} derives a significant fraction of its intensity from the O-P-O symmetric stretching vibration of the phosphate group [14, 21]. Certain of the weak features within the envelope of the 1104 cm^{-1} band (possibly including the shoulder at 1092 cm^{-1} in the current work) may be chain length dependent [9]. This would account for the slight differences in this spectral region between egg lecithin and dipalmitoyl lecithin. The formation of *gauche* isomers above T_m causes drastic changes in this spectral region (Fig. 1, 23°C spectrum). The band at 1066 cm^{-1} is greatly diminished in relative intensity and merges with the strong feature now present at 1090 cm^{-1}. In addition, a weak band is observed at 1124 cm^{-1} in place of the strong feature at 1132 cm^{-1}. The intensity ratio of the bands near 1132 and 1104 cm^{-1} is therefore a suitable indicator of the extent of *gauche* isomer formation and has been used for this purpose in several studies [8, 10, 13, 14]. For egg lecithin we observe (Mendelsohn, R. and Sunder, S., unpublished results) a melting temperature of −18°C and a melting range of about 25° using the I(1132)/I(1104) intensity ratio as a probe.

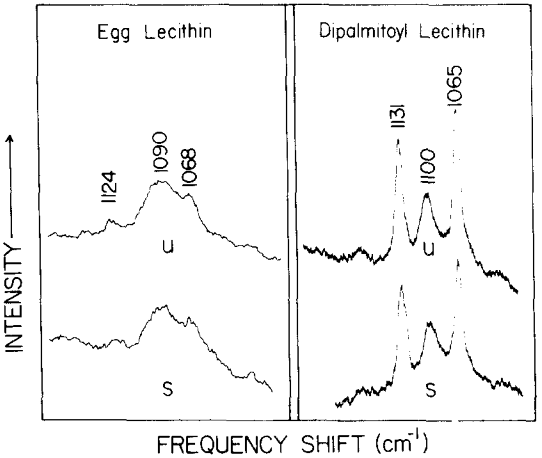


Fig. 2. Raman spectra of the 1000—1150 cm^{-1} regions of egg lecithin and dipalmitoyl lecithin at 23°C. u, unsonicated dispersion; s, sonicated dispersion. Spectral parameters as in Fig. 1.

(ii) Spectra of the 1000—1150 cm^{-1} regions of sonicated and unsonicated egg lecithin and dipalmitoyl lecithin dispersions are shown in Fig. 2. The intensity ratios I(1132)/I(1104) for a variety of experimental conditions are listed in Table I. The measured intensity ratios refer to peak height measurements at the band maxima. The frequency values of the maxima change as the ratio of *trans-gauche* isomers is altered (e.g. the 1132 cm^{-1} band in egg lecithin shifts to 1124 cm^{-1} as the temperature is increased).

As can be seen from Table I, the all-*trans* chain conformation which is predominant in egg lecithin at –66°C and in dipalmitoyl lecithin at 23°C is characterised by an I(1132)/I(1104) intensity ratio of about 1.5, In situations where *gauche* isomer formation has occurred, the ratio is in the 0.2—0.25 range. The primary source of error in the measurements is the difficulty in establishing an accurate baseline. As is clear from the table, sonication has no effect on the 1132/1104 intensity ratio for either egg lecithin or dipalmitoyl lecithin within the limits of the experimental error. Consequently, it appears that little or no change in the relative amounts of *trans* and *gauche* isomers occurs during the sonication process for both egg lecithin (where the hydrocarbon chains are in the *gauche* conformation at room temperature) and dipalmitoyl lecithin (where the all-*trans* conformation is prevalent at room temperature).

The dominant mechanism by which intramolecular disorder occurs in phospholipid hydrocarbon chains is the formation of *gauche* isomers leading to kinked structures [22, 23]. The Raman results reported here, therefore, provide direct experimental evidence that no intramolecular structural disorder occurs upon sonication. Our results therefore do not support the contention that such disorder occurs and is responsible for the line narrowing observed in the PMR spectrum [5—7].

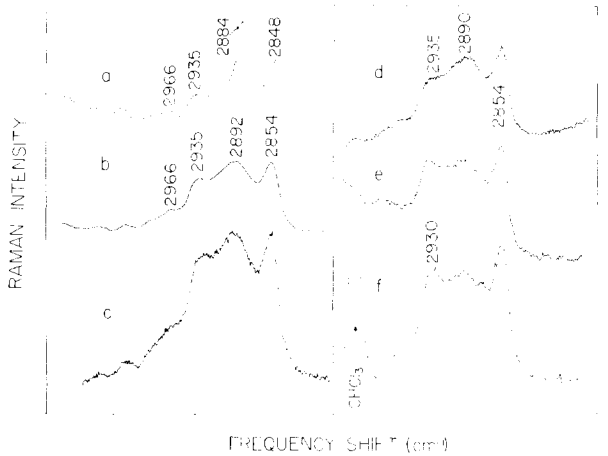
Another region of the Raman spectrum which has been used to monitor structural changes in phospholipid hydrocarbon chains is that due to the C-H stretching vibrations between 2800—3100 cm^{-1} [11, 14]. Figs. 3 and 4 contain this spectral region for egg lecithin and dipalmitoyl lecithin, respectively, in various physical states. The spectrum of egg lecithin at –66°C (Fig. 3a) displays characteristics typical of the all-*trans* chain conformation — an intense band at 2884 cm^{-1} and a somewhat weaker band at 2848 cm^{-1}. These bands

TABLE I

CONFORMATION DEPENDENCE OF THE I(1132)/I(1104) INTENSITY RATIO

Molecule	T °C	State	Dominant conformation	I(1132)* / I(1104)
Egg lecithin	–66	unsonicated	*trans*	1.4
Egg lecithin	23	unsonicated	*gauche*	0.22
Egg lecithin	23	sonicated	*gauche*	0.23
Egg lecithin	23	$CHCl_3$ soln.	*gauche*	0.23
Dipalmitoyl lecithin	23	unsonicated	*trans*	1.6
Dipalmitoyl lecithin	23	sonicated	*trans*	1.5
Dipalmitoyl lecithin	23	$CHCl_3$ soln.	*gauche*	0.23
Dipalmitoyl lecithin	60	sonicated	*gauche*	0.22
Dipalmitoyl lecithin	60	unsonicated	*gauche*	0.24

*Numbers quoted are accurate to ±10%.



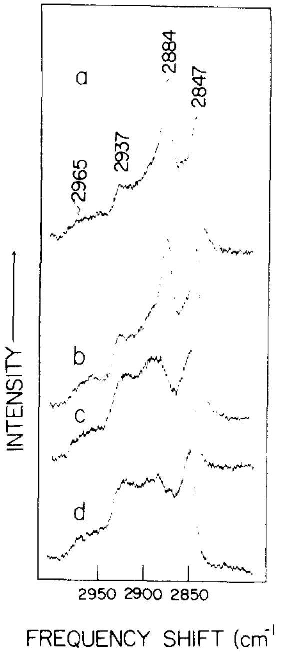


Fig. 3. Raman spectra of the C-H stretching region of egg lecithin. (a) −66°C; (b) 23°C, unsonicated; (c) 75°C, unsonicated; (d) 23°C, 15 min sonication at 5°C; (e) 23°C, 120 min sonication at 5°C; (f) $CHCl_3$ solution.

Fig. 4. Raman spectra of dipalmitoyl lecithin (10% w/w) in the 2800—3100 cm^{-1} region. (a) 23°C, unsonicated; (b) 23°C, sonicated 40 min; (c) 60°C, unsonicated; (d) 60°C, sonicated.

have been assigned to asymmetric and symmetric stretching vibrations of chain methylene groups respectively [14, 21]. Also present are two weaker features at 2935 and 2966 cm^{-1}, respectively. The origin of these is somewhat controversial but studies with specifically deuterated stearic acids currently in progress are expected to resolve the ambiguities. The spectrum of dipalmitoyl lecithin, unsonicated and below T_m (Fig. 4a) displays features similar to those of egg lecithin. The effect of raising the temperature to above T_m is illustrated in Figs. 3b and 4c for egg lecithin and dipalmitoyl lecithin, respectively. Slight shifts in the frequencies are noted — the bands at 2884 and 2847 shifting to $\approx$ 2890 and $\approx$ 2854 cm^{-1}, respectively. In addition, the relative intensity of these two bands has altered to the point where they are of equal intensity. Furthermore, the bandwidths of the two features have increased significantly. Additional heating of egg lecithin from 23°C to 75°C produces little further change in the spectrum (compare Fig. 3b and 3c). Sonication of egg lecithin, however, produces a small but significant alteration in the intensity pattern for this spectral region. As shown in Figs. 3d and 3e, the band at $\approx$ 2890 cm^{-1}, appears to be reduced in relative intensity compared with that at $\approx$ 2855 cm^{-1}, while that at $\approx$ 2935 progressively gains intensity as sonication proceeds. Similar effects are observed in the C-H stretching region of dipalmitoyl lecithin upon sonication above T_m (Figs. 4c and 4d). Sonication of dipalmitoyl lecithin below T_m reduces the intensity of the mode at 2884 relative to that at 2847, but the halfwidths of the two features change much less than they do on raising the temperature (Figs. 4a and 4b).

These changes are not due to variation in the relative number of *trans* and *gauche* isomers (as shown by the analysis of the 1100 cm^{-1} region). The origin of these intensity changes is difficult to interpret, but it may be related to different interchain interactions of the hydrogen atoms upon sonication, due to the change in packing of the chains towards the center of the bilayers. Evidence that the C-H stretching region is sensitive to interchain (as well as intrachain) effects has been obtained previously [11, 14]. It is interesting to note that the C-H stretching region of egg lecithin (sonicated, 23°C) strongly resembles that of a $CHCl_3$ solution of the molecule (compare Figs. 3e and 3f). Both are quite similar to that of dipalmitoyl lecithin sonicated above T_m (Fig. 4d).

In summary : (1) The temperature dependence of the Raman spectra of both egg lecithin and dipalmitoyl lecithin shows that the 1050—1150 cm^{-1} region is sensitive to *trans-gauche* isomerisation of the hydrocarbon chains in phospholipids. Sonication produces no change in the relative populations of *trans* and *gauche* isomers in either of these systems.

(2) The spectral region 2800—3100 cm^{-1} is sensitive to both intra- and interchain effects in phospholipid systems. Sonication does appear to effect the interchain interactions in both egg lecithin above T_m and dipalmitoyl lecithin above and below T_m.

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