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EFFECTS OF TEMPERATURE AND MOLECULAR INTERACTIONS ON THE VIBRATIONAL INFRARED SPECTRA OF PHOSPHOLIPID VESICLES

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Summary

Infrared spectra were obtained as a function of temperature for a variety of phospholipid/water bilayer assemblies (80% water by weight) in the 3000—950 cm^{-1} region. Spectral band-maximum frequency parameters were defined for the 2900 cm^{-1} hydrocarbon chain methylene symmetric and asymmetric stretching vibrations. Temperature shifts for these band-maximum frequencies provided convenient probes for monitoring the phase transition behavior of both multilamellar liposomes and small diameter single-shell vesicles of dipalmitoyl phosphatidylcholine/water dispersions. As examples of the effects of bilayer components upon phase transition characteristics, the temperature profiles for lipid/cholesterol/water (3 : 1 mol ratio) and lipid/cholesterol/amphotericin B/water (3 : 1 : 0.1 mol ratios) vesicles were examined using the methylene stretching frequency indices. In comparison to the pure vesicle form, the transition width of the lipid/cholesterol system increased by nearly a factor of two (to 8°C) while the phase transition temperature remained approximately the same (41°C). For the lipid/cholesterol/amphotericin B system, the phase transition temperature increased by about 4.5°C (to 45.5°C) with the transition width increasing by nearly a factor of four (to $\approx$ 15°C) above that of the pure vesicles. The lipid/cholesterol/amphotericin B data were interpreted as reflecting the formation below 38°C of a cholesterol/amphotericin B complex whose dissociation at higher temperature (38—60°C range) significantly broadens the gel-liquid crystalline phase transition.

Introduction

Although infrared and Raman spectroscopic techniques provide complementary vibrational probes, the high infrared absorption of water has led to an

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emphasis on Raman techniques for monitoring conformational changes in both model and natural membrane assemblies (see, for example, refs. 1—4 and the references therein). Since infrared spectroscopy involves a single-photon interaction, while Raman scattering depends upon multiphoton processes, the two techniques lead to different selection rules and intensity effects for vibrational transitions. In phospholipid bilayer systems, for example, infrared spectra more clearly distinguish the phosphate stretching vibrations and related head group modes, while the skeletal stretching modes are more conveniently monitored by the Raman effect. As a consequence of local symmetry, differences between the two spectroscopic effects are particularly evident for vibrations involving the acyl chain modes of phospholipids.

Since information regarding the infrared spectra of model phospholipid bilayers has been limited [5—10], we investigated the use of phospholipid-clay complexes as a convenient sampling method for obtaining infrared vibrational spectra of membrane related systems [11]. In the present study we continue our vibrational investigations of phospholipid systems and discuss both the techniques for determining the infrared spectra from 3000—950 cm^{-1} of a variety of liposomes and the relation of these spectra to several dynamical properties of bilayers. Specifically, temperature dependent frequency shifts for the for the carbon-hydrogen (C-H) stretching vibrations are used to monitor the bilayer gel-liquid crystalline phase transitions in both unilamellar vesicles and multilamellar liposomes of dipalmitoyl phosphatidylcholine. These frequency-shift parameters are then examined for dipalmitoyl phosphatidylcholine/cholesterol/amphotericin B vesicles from 30—60°C in an effort to clarify further the effects of temperature on the stability of sterol-polyene antibiotic complexes within the bilayer.

Materials and Methods

High purity samples of 1,2-dipalmitoyl-DL-phosphatidylcholine and cholesterol were obtained commercially from Sigma Chemical Company and Supelco, Inc., respectively. A sample of a United States Food and Drug standard of amphotericin B was used [12]. Lipid sample, 20% by weight in water, were prepared by thoroughly mixing the components above the gel-liquid crystalline phase transition temperature until a homogeneous gel for the multilayers was formed. Unilamellar vesicles of the same lipid concentration were prepared by sonicating the phospholipid dispersion to clarity for 20—40 min at temperatures above the phase transition. A Bronson probe type sonicator equipped with a microtip was used to generate the single-wall bilayers. Samples containing cholesterol and amphotericin B were prepared by first dissolving the appropriate components in chloroform-methanol solutions, drying under nitrogen and then in vacuo. After adding water, the samples were sonicated. The molar ratios of the water disperison of lipid/cholesterol were 3 : 1, while those for lipid/cholesterol/amphotericin B were 3 : 1 : 0.1.

Infrared spectra were obtained with a Perkin-Elmer Model 521 spectrophotometer. Spectra were recorded under conditions of moderate resolution with spectral slits widths of 1—1.5 cm^{-1}. During spectral scans, the instrument was constantly purged with dry nitrogen gas. Water vapor and polystyrene were used for calibration.

Vesicle and multilayer samples were placed in a 27 μm pathlength cell of Irtran-2. A matched Irtran-2 cell, containing only water, was placed in the reference beam. The narrow pathlength cell was easily filled with the vesicle dispersions, although care was taken to avoid the formation of air bubbles between the cell plates. In contrast, it was necessary to warm the entire cell assembly slightly above the gel-liquid crystalline phase transition temperature in order to inject the multilayer disperisons into the cell. The sample cell was housed within a variable temperature enclosure; sample spectra were recorded from about 30—60°C. Temperatures were measured by a thermocouple placed on the edge of the plates forming the sample cell. These temperatures are estimated to about ± 1.5°C. The precision in the frequency shifts of the 2853 and 2921 cm^{-1} methylene stretching modes, estimated from expanded scale spectra, is ± 0.2 cm^{-1}.

Results and Discussion

Figs. 1—3 display survey infrared spectra of lipid/water vesicles and multilayers at temperatures above and below the gel-liquid crystalline phase transition for dipalmitoyl phosphatidylcholine bilayers in the 2900, 1400 and 1150 cm^{-1} regions, respectively. Table I summarizes the proposed vibrational assignments for a number of the vibrational modes [5,6,13—15]. In addition to yielding excellent quality infrared spectra for lipid/water mixtures, the spectra

TABLE I

INFRARED FREQUENCIES OF DIPALMITOYL PHOSPHATIDYLCHOLINE SINGLE SHELL VESICLES AND MULTILAYERS AT 30°C

Vesicles (cm^{-1})	Multilayers (cm^{-1})	Tentative assignments
2959.5	2958	CH_3 asym. tretch
2931		CH_3 sym. stretch
2920.0 *	2920.0 *	CH_2 asym. stretch
2876	2876	
2853.0 *	2852.5 *	CH_2 sym. stretch
1492 (sh)		
1468 }	1466 }	CH_2 sym. deformation
1458 }	1457 }	CH_2 def. + CH_3 asym. def.
1418	1418	CH_2 def. adjacent to C=O (?)
1379	1380	CH_3 sym. deformation
	1278	CH_3 wag
1244		CH_2 twist (?)
1241 } bd	1222 d	PO_2^- antisym. stretch
1223 }		
1198	1198	
1170		C-O-C antisym. stretch
	1149	
1088	1086	PO_2^- sym. stretch
1065 (bd)	1065 (bd)	C-O {+C-C} stretch
970		C-C stretch
	964	
952		

* Frequency changes markedly with temperature.

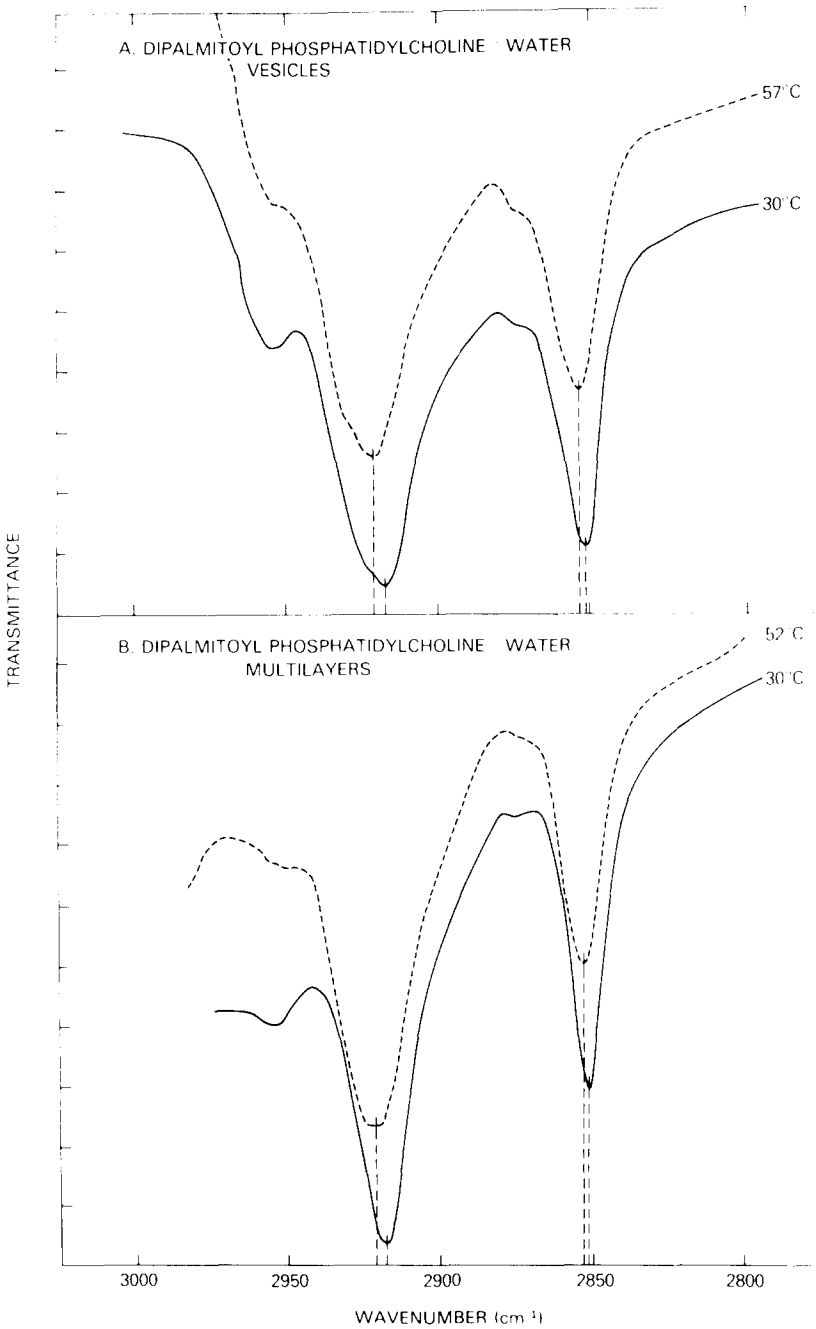

Fig. 1. Infrared spectra of dipalmitoyl phosphatidylcholine/water vesicles and multilayers in the 2900 cm^{-1} methylene stretching region above and below the gel-liquid crystalline phase transition temperature. The vertical dotted lines indicate the relative frequency shifts of a band maximum parameter as a function of temperature (see text).

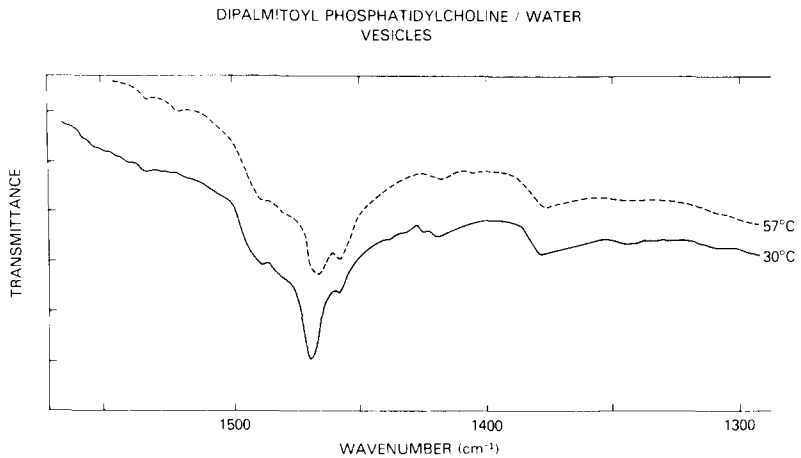

Fig. 2. Infrared spectra of dipalmitoyl phosphatidylcholine/water vesicles in the 1450 cm^{-1} methylene deformation region above and below the gel-liquid crystalline phase transition temperature.

exhibit reproducible differences both as a function of temperature and of bilayer form. The relative intensity differences between the methylene CH_2 deformation modes at 1468 and 1458 cm^{-1}, as shown in Fig. 2, have been previously used to monitor the phase transition in multilayer dispersions [9]. although

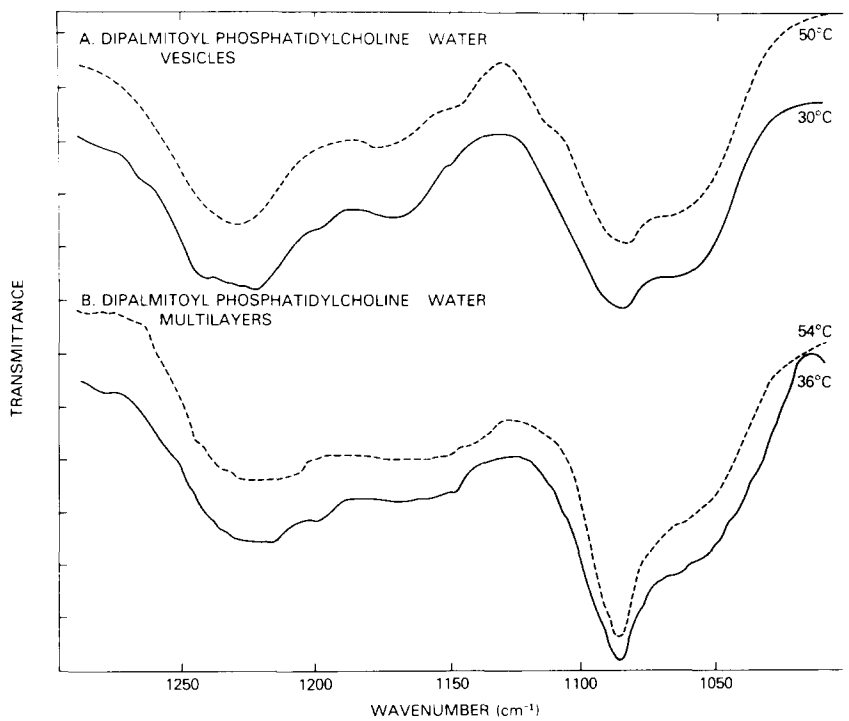

Fig. 3. Infrared spectra of dipalmitoyl phosphatidylcholine/water vesicles and multilayers in the 1250—1050 cm^{-1} PO_2^- symmetric and antisymmetric stretching regions.

we found such changes difficult to quantitate. Difficulties in establishing reproducible baselines discouraged the use, particularly in the 2900 cm^{-1} region of the spectrum, of monitoring intensity differences (rather than frequency shifts) to follow subtle bilayer conformational changes.

In contrast to vibrational Raman spectra [1], infrared spectra clearly distinguish the symmetric and antisymmetric PO_2^- stretching modes at 1088 and about 1223 cm^{-1}, as shown in Fig. 3. Although Raman [2] and ^{31}P NMR studies [16] fail to indicate significant differences in the general conformation of the head group between the multilayer and vesicle forms, the infrared spectra (Fig. 3) suggest that the vibrational intensities of several phosphate and related head group modes vary with the physical state of the bilayer, and perhaps reflect changes in the packing characteristics of the head group. In particular, spectral differences arise for the relative intensities of the $\approx$ 1222, 1088 and 1065 cm^{-1} infrared bands which are attributed to the head group PO_2^- antisymmetric, PO_2^- symmetric and C-O stretching modes, respectively. (The broad 1065 cm^{-1} feature probably contains a contribution from a C-C stretching mode, although these vibrations are reflected generally by weak infrared features [15].)

In contrast to the suggestion made in ref. 9, the CH_2 stretching region, reproduced in Fig. 1, may be used as a probe of bilayer behavior by monitoring the small frequency shifts in both the methylene symmetric and asymmetric stretching modes at 2853 and 2921 cm^{-1}, respectively. The broader CH_2 asymmetric stretching band clearly contains several components; however, a band maximum frequency, defined by a midpoint of a line drawn at a fixed interval of 3% below the band maximum, shifts about 4 cm^{-1} (from 2920 to 2924 cm^{-1}) for the multilayer form as the temperature spans the gel-liquid crystalline phase transition. The CH_2 symmetric stretching mode shifts a smaller, but reproducible amount ($\approx$ 2.5 cm^{-1}) from 2853.0 to 2855.5 cm^{-1} for the same temperature span.

The 2931 cm^{-1} feature appears more distinctly in the vesicle spectra in which the hydrocarbon chains are more disordered, as a consequence of surface curvature, than in the multilayer form. This spectral feature has been tentatively assigned to the C-H symmetrical stretching mode of the terminal methyl group of the hydrocarbon chain superimposed upon additional CH_2 modes arising from the increased number of hydrocarbon *gauche* conformers induced by higher temperatures [3,17].

The band maximum frequencies, determined as described above, for both multilayer and vesicle assemblies were used to monitor phase transition temperature profiles, as displayed in Figs. 4 and 5. Raman intensities and frequency shifts for the C-H stretching region have been previously used for multilayer systems [18,19]. The multilayer dispersion (Fig. 4) exhibits an abrupt gel-liquid crystalline phase transition centered at 41°C with an approx. 2°C transition width for both the 2853 and 2920 cm^{-1} indices. This phase transition temperature is in excellent agreement with a variety of other techniques, summarized in ref. 1, and supports the reliability of the method in reflecting phase transition dynamics.

In the following discussion we use the initial band frequencies as a measure of order in the unmelted phase and the phase transition width as a measure of

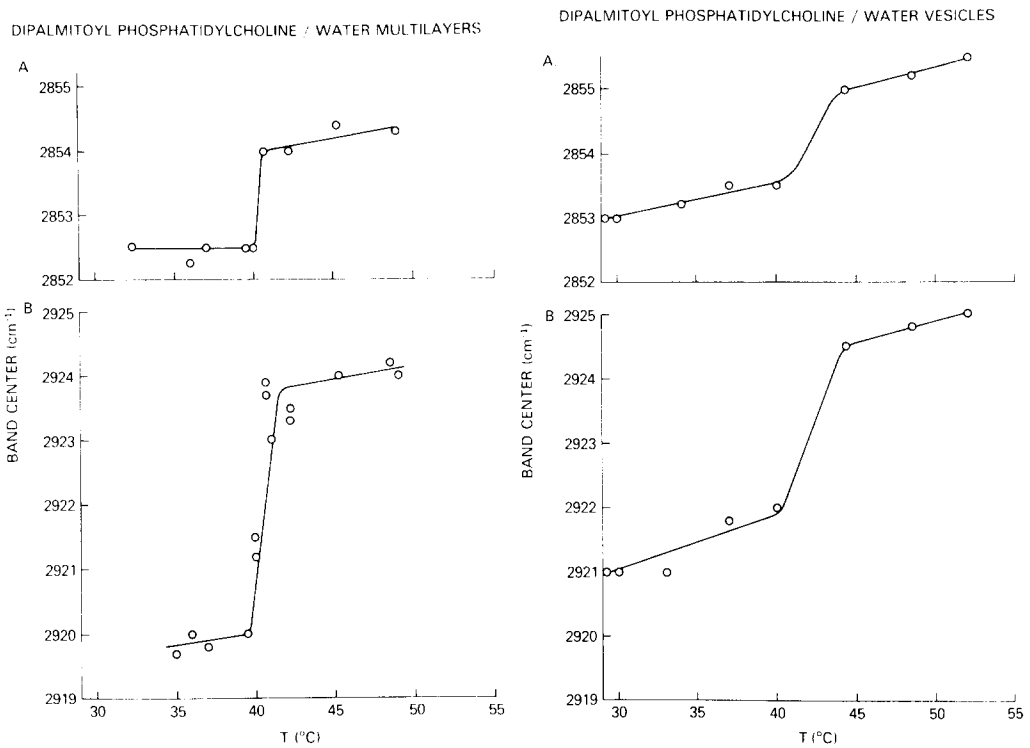

Fig. 4. Temperature profile for dipalmitoyl phosphatidylcholine/water multilayers using the frequency shifts for the symmetric methylene stretching mode (A) and the asymmetric stretching methylene mode (B). The uncertainty in the frequency measurements is ± 0.2 cm^{-1}.

Fig. 5. Temperature profile for dipalmitoyl phosphatidylcholine/water vesicles using the frequency shifts for the symmetric methylene stretching mode (A) and the asymmetric stretching methylene mode (B).

the cooperativity of the transition. For example, the temperature dependent frequencies of the vesicle form, Fig. 5, display a somewhat higher initial frequency in the gel form, a possibly broader transition region (4° C) * and a smaller total frequency shift than the multilayer form (Fig. 4). The higher initial frequency reflects the more disordered nature of the vesicle state, while the possible broadening of the phase transition would indicate a decreased cooperativity between adjacent phospholipid chains. These results are consistent with similar Raman studies of the 1100 cm^{-1} carbon-carbon (C-C) skeletal stretching modes [1]. The midpoint of the phase transition for the vesicles is approximately the same as that for the multilamellar liposomes in these infrared studies; in contrast, the Raman data [1] suggest a slightly lower value. This difference between the infrared and Raman results probably arises from the addi-

* Although several sets of spectroscopic data were obtained for the vesicle assemblies, it was difficult to determine rigorously the extent and nature of the broadening of the phase transition in comparison to the multilayer systems. With regard to the higher initial gel frequency and the smaller total frequency shift for the vesicle forms, the data, however, are consistent with a more disordered state [1]. (A disordered gel form would be reflected by a slightly broadened phase transition.)

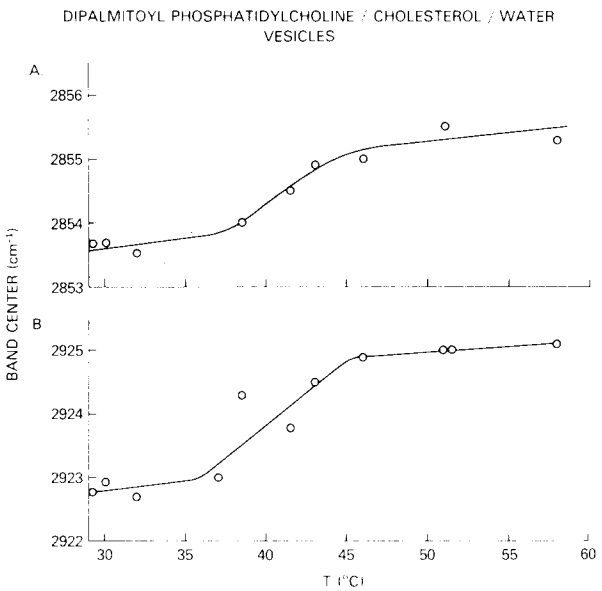

Fig. 6. Temperature profile for dipalmitoyl phosphatidylcholine/cholesterol/water (3 : 1 lipid/cholesterol mol ratio) vesicles using the frequency shifts for the symmetric methylene stretching mode (A) and the asymmetric stretching methylene mode (B).

tional effect of interchain interactions upon the methylene stretching modes, which are also probes of chain packing (ref. 3 and references therein).

As indicated in Fig. 6, the addition of cholesterol to the vesicle disperisons is reflected by a further increase in the width of the phase transition to about 8°C. These data are consistent with Raman studies involving the C-C stretching region [1,20]. Spectral transitions from cholesterol (or amphotericin B, to be discussed below) posed no additional problems in determing the lipid hydrocarbon-methylene stretching frequency shifts. The temperature profile results in a phase transition midpoint at approximately 41°C, which is about 5—10°C lower than the Raman study using the C-C stretching modes as a probe [1]. More importantly, the high frequency parameters of the methylene stretching modes below the transition temperature clearly reflect the increase in hydrocarbon chain disorder caused by cholesterol. This disordering effect reduces the total frequency shift from 4 cm^{-1} for pure lecithin vesicles as shown in Fig. 5B, to 2 cm^{-1} (Fig. 6B).

In order to probe the molecular interactions arising from the formation of aggregates within a bilayer, we examined the effect of amphotericin B in cholesterol containing vesicles. As shown in Fig. 7, amphotericin B further broadens the transition width to about 15°C and shifts the transition midpoint upward to approx. 45.5°C (refer to Table II which summarizes the phase transition characteristics for the various systems).

The frequency for the CH_2 asymmetric stretching mode in the gel state is lowered (1 cm^{-1}) by the presence of amphotericin B. In contrast, the band maximum frequency for the CH_2 asymmetric stretching mode increases as the

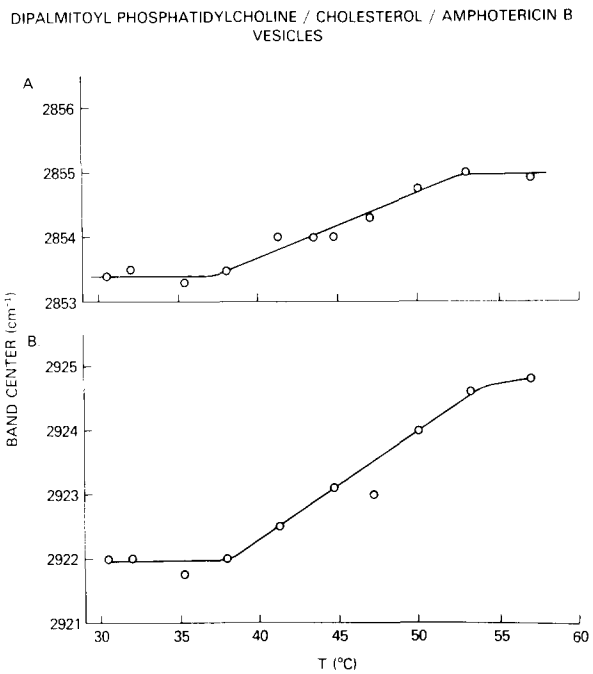

Fig. 7. Temperature profile for dipalmitoyl phosphatidylcholine/cholesterol/amphotericin B/water (3 : 1 : 0.1 lipid/cholesterol/amphotericin B mol ratios) vesicles using the frequency shifts for the symmetric methylene stretching mode (A) and the asymmetric stretching methylene mode (B).

trans-gauche isomerization increases within the acyl chains (compare Figs. 4—6). Thus, this decrease in the 2922 cm^{-1} methylene stretching frequency (Fig. 7) implies a more ordered system in the presence of amphotericin B than with cholesterol alone. This increased ordering in the gel state may reflect the presence of the 1 : 1 cholesterol/amphotericin B complexes formed in dimyristoyl phosphatidylcholine multilayers in the 12—28°C temperature region [3]. Such com-

TABLE II

SUMMARY OF PHASE TRANSITION CHARACTERISTICS FOR DIPALMITOYL PHOSPHATIDYLCHOLINE MULTILAYER AND BILAYERS ASSEMBLIES

	Phase transition temperature (°C) *	Approximate transition width
Dipalmitoly phosphatidylcholine multilayers	41	2
Dipalmitoyl phosphatidylcholine vesicles	≈41—42	4
Dipalmitoyl phosphatidylcholine/cholesterol vesicles	41	8
Dipalmitoyl phosphatidylcholine/cholesterol/amphotericin B vesicles	45.5	15

* Transition temperatures represent the midpoint of the temperature profile using the frequency shifts for the acyl chain methylene asymmetric stretching mode (see text).

plexes would reduce the free fraction of cholesterol available to intercalate between adjacent lecithin molecules. (The complexes may also interact directly with nearby or surrounding lecithin molecules to increase chain rigidity and decrease lateral diffusion.)

As in the temperature profiles for lipid/cholesterol vesicles (Fig. 6), the lipid/cholesterol/amphotericin B broadened phase transition begins near 38°C, suggesting that some of the lipid is unaffected by the presence of amphotericin B. In contrast, the phase transition is not completed until 53°C. This temperature shift effectively doubles the width of the phase transition (from 8 to 15°C) found with cholesterol alone.

The apparent decrease in cooperativity during chain melting suggests a decrease in the stability of the cholesterol/amphotericin B complex above 40°C that culminates in its dissociation. Such a decrease is consistent with temperature trends from calorimetric studies at 10°C [21,22], Raman studies at 24°C [3] and conductance studies [23] in which the stoichiometry of cholesterol bound by amphotericin B decreases from 4 : 1 at 10°C to 1 : 1 at 24°C. In particular, the decrease in ion conductance with increasing temperature for lipid membranes containing sterol and amphotericin B was interpreted as a decrease in stability or a melting of the trans-membrane channels formed by amphotericin B/cholesterol aggregates [23].

In conclusion, we have demonstrated the usefulness of infrared band maximum frequency shifts of both the symmetric and asymmetric CH_2 stretching modes for reflecting structural differences in multilayer and vesicle assemblies. These frequency shift parameters sensitively monitor conformational changes arising from temperature dependent intermolecular effects.

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