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EFFECT OF MELITTIN ON THERMOTROPIC LIPID STATE TRANSITIONS IN PHOSPHATIDYLCHOLINE LIPOSOMES

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SUMMARY

(1) We have examined the Raman scattering due to CH stretching vibrations, as well as to ν(–C=C–) and ν(=C–C=) of β-carotene, of liposomes composed of phosphatidylcholine (egg, dimyristoyl, dipalmitoyl) ± cholesterol, β-carotene or melittin in the temperature range of −10 °C to +45 °C.

(2) Plots vs. temperature of the intensities of the 2885 cm^{-1} and 2930 cm^{-1} CH stretching bands relative to the intensity of the thermally stable 2850 cm^{-1} band, i.e. the I_{2885}/I_{2850} and I_{2930}/I_{2850} ratios, reveal a sharp discontinuity in cholesterol-free phosphatidylcholine liposomes; this coincides with the gel → liquid-crystal transition temperature of the fatty acyl chains. In cholesterol/phosphatidylcholine liposomes the change in I_{2885}/I_{2850} occurs over a very broad temperature range and I_{2930}/I_{2850} remains stable.

(3) I_{1527}/I_{1158}, i.e. the intensity of ν(–C=C–) relative to that of ν(=C–C=) in β-carotene/phosphatidylcholine liposomes, changes discontinuously at the gel → liquid-crystal transition temperature. The values above the transition temperature approach those of the carotenoid in organic solution.

(4) The transitions reported in I_{2885}/I_{2850} for phosphatidylcholine/melittin liposomes (25–56 : 1, M/M) are shifted to much higher temperatures than observed in phosphatidylcholine liposomes. In the case of dimyristoyl phosphatidylcholine/melittin the change in I_{2930}/I_{2850} also occurs at a higher temperature (28 °C) than without melittin (21 °C), but the temperature shift is less than the +13 °C observed for I_{2885}/I_{2850}. It appears that the apolar moiety of melittin organizes phospholipids adjacent to and more remote from the peptide moiety, to form complexes with an elevated lipid transition temperature. The effect of the peptide moiety is greater on the methylene segments (I_{2885}/I_{2850}) than on the methyl termini (I_{2930}/I_{2850}).

INTRODUCTION

Raman spectroscopy has provided important information about thermotropic transitions in model lipid membranes [1, 3] as well as in thymocyte plasma

membranes [4]. The latter exhibit a lipid state transition that is revealed by the variation of CH stretch-scattering with temperature. However, the transition temperature observed (approx. 23 °C) lies rather far from the values expected of the highly unsaturated phospholipids that prevail in thymocyte plasma membranes. This may relate to the cholesterol distribution in these membranes, charge effects, the fact that the phosphatides are mixed-chain phospholipids, the influence of apolar lipid-protein interactions or a combination of these factors.

We have therefore evaluated the thermal behavior of liposomes made of a mixed chain phosphatidylcholine, as well as of liposomes composed of phosphatidylcholine plus cholesterol, phosphatidylcholine plus β-carotene and phosphatidylcholine plus melittin, a small amphipathic polypeptide that interacts hydrophobically with phosphatide acyl chains [5]. To monitor the thermal responses we have followed the variation with temperature of Raman scattering due to CH stretching vibrations and of resonance-enhanced Raman scattering due to the –C=C– and =C–C= stretching vibrations of membrane-bound β-carotene [6, 7].

EXPERIMENTAL

Chemicals. Egg lecithin (unsaturated) was obtained from Lipid Products (South Nutfield, Great Britain), dipalmitoyl phosphatidylcholine and dimyristoyl phosphatidylcholine from Serdary Research Laboratories (London, Ontario, Canada), cholesterol and β-carotene from Sigma (St. Louis, Mo., U.S.A.) and melittin from Nutritional Biochemicals (Cleveland, Ohio, U.S.A.). All other reagents were of analytical grade.

Liposomes. Phosphatidylcholine liposomes (±cholesterol, ±β-carotene or ±melittin at stated concentrations and ratios) were prepared as in refs. 4 and 7 by sonication at 20 °C (power step 3), except for egg lecithin or egg lecithin/β-carotene liposomes, which were prepared at 4 °C.

Raman spectroscopy. Membrane vesicles and liposomes were transferred to 0.9–1 mm internal diameter Kimex Capillaries. After sealing, the sample capillaries were placed in a Harney-Miller Cell [8] for temperature control. The temperature, monitored by a telethermometer, was regulated by a flow of N_2. Temperature control was checked by determining transition temperatures of authentic lipids (dimyristoyl phosphatidylcholine; dipalmitoyl phosphatidylcholine) in the laser beam.

Raman spectra were recorded as before [4, 6, 7] using a Ramalog 4 Raman spectrometer (Spex Industries, Metuchen, N.J., U.S.A.) interfaced to an Interdata (Model 70) computer. An Ar^+ laser (Spectra Physics model 164), tuned to 488 nm, was used as an excitation source. Power was 300 mW except for β-carotene-containing liposomes, where 50 mW was used. The Raman scattering at right angles to the laser beam was detected by a thermoelectrically cooled photomultiplier (RCA 31034) and was recorded in terms of photon/s. The "dark" counts of the photo cell were < 100 counts/s. Roman scattering from the sample gave counts in the order of 10^3–10^5 per s. Scanning was done through the computer (loaded with the VIE8D Ramacomp Computer Program, Spex Industries). We used the following specifications for scanning. Maximum time and minimum time for each data point were 1 and 0.5 s, respectively, except for the β-carotene lines, where we used 0.5 and 0.1 s, respectively. The photon counts were 10^4–10^5 maximum, 100 minimum. Scanning

was in steps of one wave number. The program does not scan the spectrometer at a rate linear with respect to time. Rather, scans are incremental between data points and no counts are recorded when the spectrometer is moving between data points. Photon counts are stored in the computer memory during scanning (2–4 scans) and the stored spectra, averaged and smoothed by a least squares procedure (9, included in the computer program), are ultimately plotted on the Ramalog recorder, using appropriate background suppressions and scale expansions.

To determine the change of Raman scattering with temperature we proceeded as follows. The samples, in position but with the laser beam occluded, were equilibrated for 20 min at the desired temperature. For CH stretch-scattering the samples were then equilibrated a further 5 min in the laser beam before scanning from 2750 to 3050 cm^{-1} (2–4 scans, maximum 300 s/scan). During equilibration at the subsequent temperature, the averaged, smoothed spectra were plotted. The procedure for the 1527 and 1158 cm^{-1} bands of β-carotene was identical, except that the 5 min equilibration in the laser beam was omitted and scanning was from 1400 to 1600 cm^{-1} and 1000 to 1200 cm^{-1}, respectively (maximum 100 s/scan for each band).

Scattering vs. temperature curves were similar whether proceeding from high to low temperatures or vice versa. All experiments were carried out on at least three separate liposome preparations.

RESULTS

CH-stretching. The intense bands at 2850 cm^{-1} and 2885 cm^{-1} (Fig. 1) arise from symmetric and asymmetric CH stretching, respectively, of methylene groups [10–12]. The other major band at 2930 cm^{-1} (Fig. 1) probably includes contributions due to asymmetric CH stretching from methylenes in short segments [4, 12] and from terminal CH_3 residues of lipid acyl chains.

The structure of the CH stretching region changes in a temperature-dependent fashion upon incorporation of small proportions ($\leq$ 4 mol%) of melittin (Fig. 1). At these molar ratios melittin constitutes less than 15 % of the membrane mass in the laser beam and does not per se contribute significantly to Raman scattering (Fig. 1).

Variation of CH stretching bands with temperature. The intensity of the 2885 cm^{-1} band relative to the intensity of the 2850 cm^{-1} band varies with temperature in phosphatidylcholine liposomes $\pm$melittin (Fig. 1). As shown before [2], the intensity ratio I_{2885}/I_{2850} drops sharply and discontinuously with the thermotropic gel $\rightarrow$ liquid-crystal transition of dipalmitoyl phosphatidylcholine. We observe the same with egg lecithin (Fig. 2) and dimyristoyl phosphatidylcholine (Fig. 3), as well as with dipalmitoyl phosphatidylcholine (not illustrated). However, we obtain transition temperatures 1.5, 2 and 3 °C, respectively, below those found by differential thermal calorimetry [13]; these differences (Table I) can be attributed to localized heating by the laser beam (cf also ref. 2). The absolute magnitude of the change in I_{2885}/I_{2850} is approx. 0.5$\pm$10 % in egg lecithin and approx. 0.2$\pm$7 % in dimyristoyl phosphatidylcholine, reflecting the shorter chain length of the latter phosphatide.

Our experiments show that the ratio I_{2930}/I_{2850}, reflecting the mobility of methyl termini relative to long methylene segments, also changes discontinuously at the gel $\rightarrow$ liquid-crystal transition. This is illustrated in Fig. 3 for dimyristoyl phosphatidylcholine.

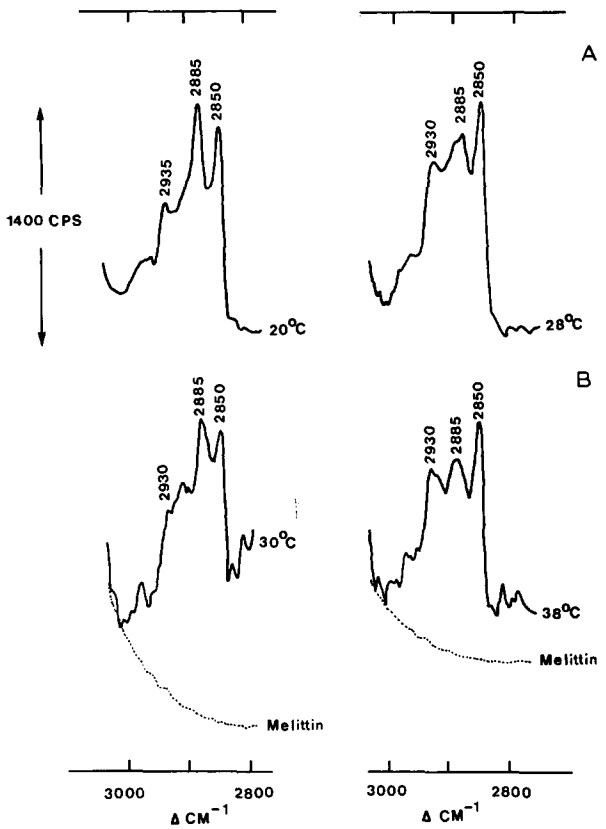

Fig. 1. Raman spectra, in the CH stretching region, of dimyristoyl phosphatidylcholine liposomes (A) without and (B) with melittin, at several temperatures. Exciting wavelength 488 nm; power 300 mW; resolution 5 cm^{-1}.

Phosphatidylcholine/cholesterol liposomes. In phosphatidylcholine/cholesterol liposomes (1/1; M/M), I_{2885}/I_{2850} changes less drastically with temperature and over a wider range of temperature. This effect, illustrated in Fig. 2 for egg lecithin and previously reported for dipalmitoyl phosphatidylcholine [1], suggests that a transition still occurs in the presence of cholesterol, but that it is poorly cooperative. Importantly, I_{2930}/I_{2850} does not change significantly in cholesterol/egg lecithin liposomes over a temperature interval where there is a progressive change in I_{2885}/I_{2850}.

Dimyristoyl phosphatidylcholine/melittin liposomes. The CH stretching from dimyristoyl/melittin liposomes (28/1, M/M) obtained well above the transition temperature of the pure phosphatidylcholine (Fig. 1) resembles that ordinarily observed below the transition temperature. Indeed, the transition temperature provided by I_{2885}/I_{2850} is shifted upward by 13 °C and that reported for I_{2930}/I_{2850} by 7 °C (Fig. 3)*. At a phosphatidylcholine/melittin molar ratio of 56 there is no significant

* The greater numerical values of I_{2930}/I_{2850} in the dimyristoyl phosphatidylcholine/melittin liposomes, compared with melittin-free liposomes are attributed to a rigidifying effect of the polypeptide [4, 5, 11].

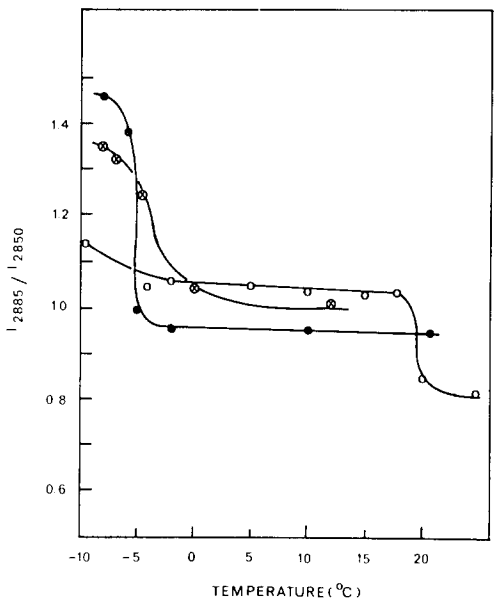

Fig. 2. Variation of I_{2885}/I_{2850} with temperature for liposomes composed of egg lecithin (●), egg lecithin/cholesterol (1 : 1, mol/mol) (⊗) and egg lecithin/melittin (25 : 1, mol/mol) (○). Values represented are averages of three recordings on three different preparations (range ±10 %).

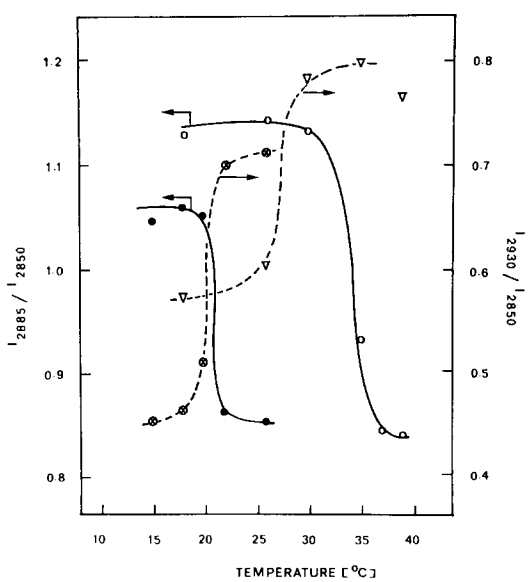

Fig. 3. Variation with temperature of I_{2885}/I_{2850} and I_{2930}/I_{2850} of liposomes composed of dimyristoyl phosphatidylcholine ±melittin. (●) dimyristoyl phosphatidylcholine, I_{2885}/I_{2850}; (○) dimyristoyl phosphatidylcholine melittin (28 : 1, mol/mol), I_{2885}/I_{2850}; (⊗) dimyristoyl phosphatidylcholine, I_{2930}/I_{2850}; (▽) dimyristoyl phosphatidylcholine melittin (28 : 1, mol/mol), I_{2930}/I_{2850}. The numerical values marked for the ratios are averages calculated from three different recordings on three different samples (range ±7 %).

TABLE I

TRANSITION TEMPERATURES OF PHOSPHATIDYLCHOLINES WITHOUT AND WITH CHOLESTEROL OR MELITTIN

Experimental values were obtained from I_{2885}/I_{2850} values. Literature values were obtained by differential thermal calorimetry.

Membrane system	Transition temperature (°C)	
	Raman	Literature
Egg lecithin	− 5.5	− 5 [13]
Egg lecithin/melittin (25 : 1, M/M)	+19	
Dimyristoyl phosphatidylcholine	+21	+23 [13]
Dimyristoyl phosphatidylcholine/melittin (28 : 1, M/M)	+34	
Dimyristoyl phosphatidylcholine/melittin (56 : 1, M/M)	+28	
Dipalmitoyl phosphatidylcholine	+38; 39 (2)	+41 [13]

displacement of the plot of I_{2930}/I_{2850} vs. temperature and the methylene group transition (I_{2885}/I_{2850}) is shifted by only +7 °C. Apparently, melittin structures and immobilizes phospholipid hydrocarbon chains, but this effect is less marked near the methyl termini than near the ester termini of the fatty acyl chains.

Egg lecithin/melittin liposomes. The thermal behavior of egg lecithin/melittin liposomes (25/1, M/M) is even more deviant than that of dimyristoyl phosphatidylcholine/melittin liposomes (Fig. 2). The transition temperature reported for I_{2885}/I_{2850} shifts from approx. −6 °C to +19 °C. Moreover, the intensity of the transition at approx. 19 °C is only approximately half that observed with melittin-free liposomes, suggesting that an appreciable proportion of methylene chains are immobilized even at 25 °C above the normal transition temperature. Compared with egg lecithin liposomes, which show a jump of I_{2930}/I_{2850} at −5.5 °C, the egg lecithin/melittin liposomes show no consistent trend of this variable.

β-Carotene signals. As shown in Table II, I_{1527}/I_{1158}, the ratio of the intensity due to the resonance-enhanced ν(–C=C–) vibration of β-carotene in phosphatidylcholine liposomes vs. that due to the enhanced ν(=C–C–) vibration, is always greater

TABLE II

VARIATION WITH TEMPERATURE OF I_{1527}/I_{1158} DUE TO β-CAROTENE IN PHOSPHATIDYLCHOLINE LIPOSOMES

Membrane system	Temperature (°C)	I_{1527}/I_{1158}
Egg lecithin*	< −7	2.0–2.3
	> 4	1.2–1.3
Dimyristoyl phosphatidylcholine*	< 20	1.5–1.6
	> 21	1.2–1.3
Dipalmitoyl phosphatidylcholine	< 27	1.6–2.0
	> 37	1.1–1.3

* Molar ratio phosphatidylcholine/β-carotene was 2800 for dimyristoyl phosphatidylcholine and 2450 for egg lecithin and dipalmitoyl phosphatidylcholine.

above the transition temperature (Table I) than below. The values above the transition temperature, 1.2–1.3, are equivalent to those of β-carotene in CCl_4 solution (which are not temperature-dependent). I_{1527}/I_{1158} thus appears to mirror the gel → liquid-crystal transition of phosphatide acyl chains.

DISCUSSION

In pure phosphatidylcholines, the discontinuities in plots of I_{2885}/I_{2850} and I_{2930}/I_{2850} vs. temperature correspond to the transition temperatures of the acyl chain segments. However, with phosphatidylcholine/cholesterol (1 : 1, M/M) liposomes I_{2930}/I_{2850} remains constant over a temperature span where I_{2885}/I_{2850} changes gradually. This fits the view (e.g. ref. 14) that cholesterol 'buffers' the fluidity of phosphatidyl acyl chains.

An important finding is that the interaction of melittin with phospholipids produces a drastic rise in the transition temperature of the phospholipid acyl chains. Moreover, the effect occurs at low molar ratios of melittin to phospholipid. This indicates that the polypeptide affects at least the phospholipids immediately adjacent to it and probably also imposes constraints extending over several layers of lipid (perhaps a shell surrounding the polypeptide). In the case of dimyristoyl phosphatidylcholines, there is also a gradient of structuring perpendicular to the membrane plane, with a greater effect on methylenes (I_{2885}/I_{2850}) than on methyl termini (I_{2930}/I_{2850}).

Our data fit with the studies of Grant and McConnell [15], showing that the incorporation of "glycophorin" into dimyristoyl phosphatidylcholine liposomes produces a 2 °C increase in the transition temperature of the lipid adjacent to the protein. Increased transition temperatures are also induced by electrostatic associations between anionic phosphatides and some basic proteins [16]. However, no such ionic associations are involved in our experiments, indicating that the effects observed derive predominantly from apolar interactions between phospholipid acyl chains and the hydrophobic moiety of the polypeptide.

Our results suggest that melittin can impose a long-range organization on phospholipid chains through apolar interactions. However, this is not necessarily a property shared by all proteins that interact hydrophobically with phosphatidylcholines [16].

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