EFFECT OF LANTHANUM IONS ON THE PHASE TRANSITIONS OF LECITHIN BILAYERS

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ABSTRACT Interaction of lanthanum ions (La^{3+}) with 1,2 dipalmitoyl-*sn*-glycero-3-phosphorylcholine (DPPC) causes an increase in T_c, the temperature of maximal excess heat capacity, and the width of the gel-to-liquid crystalline transition. At a mole ratio of La^{3+} to DPPC sufficient to remove the hydrocarbon chain tilt angle of DPPC, the changes in the thermodynamic parameters of the pretransition are minor, T_c and the width were unaltered and the enthalpy was reduced by only 10%. This suggests that the change in tilt angle is not a necessary concomitant of the pretransition.

INTRODUCTION

The physical properties of lipid mesophases are functions of temperature, pressure, and water content as well as functions of added ligands, such as organic molecules and inorganic ions (1, 2). Multilamellar vesicles of 1,2 dipalmitoyl-*sn*-glycero-3-phosphorylcholine (DPPC), freshly prepared in excess water, exhibit two phase transitions: one centered at 34.8°C (the $L_\beta \rightarrow P_\beta$ pretransition) and the other at 41.5° (the $P_\beta \rightarrow L_\alpha$ gel-to-liquid crystalline main transition). X-ray diffraction data indicate that the hydrocarbon chains of fully hydrated diacylphosphatidylcholines (PCs) are aligned and tilted at an angle of ~30° to the normal of the bilayer plane in the gel (L_β') phase and become normal to the plane in the P_β phase (3–11). Although other changes take place in the properties of DPPC bilayers at or immediately below the pretransition (12, 13), it has been suggested that the change in tilt angle is an integral part of the pretransition in PCs (5–11).

^1H, ^2H, and ^{31}P NMR studies have demonstrated changes in torsion angles of the phosphate segment and choline methylene groups induced by La^{3+} ions that result in a conformational change of the choline head group of DPPC (14–16). X-ray data indicate that at a molar ratio of DPPC/La^{3+} of 3:1, there is a change in acyl chain configuration from L_β' to L_β, i.e., the chain tilt of DPPC relative to the normal to the bilayer plane is lost (9). We report here investigations of the interaction of DPPC with La^{3+} ions using high-sensitivity differential scanning calorimetry (HSDSC), which put into perspective the contribution of the change in tilt angle to the pretransition endotherm.

MATERIALS AND METHODS

1,2 dipalmitoyl-*sn*-glycero-3-phosphorylcholine (DPPC) (Avanti Polar Lipids, Inc., Birmingham, AL) was tested for purity by TLC, GLC, and HSDSC (19) and estimated to be better than 99.5% pure. Multilamellar lipid dispersions were prepared by adding sufficient doubly deionized water containing the appropriate amount of $LaCl_3$ (washed in purified chloroform to remove nonpolar impurities) to dry lipid to give lipid concentrations in the range 0.5–5.0 mg ml^{-1} and DPPC/La ratios of 1:0 to 1:1, heating to 50°C and vortexing for 20 s. Heating and vortexing operations were repeated three times. All samples were scanned in a Privalov microcalorimeter (20) at a scan rate of 0.1 K min^{-1} in the heating mode. Baselines (H_2O vs. H_2O or $H_2O + La^{3+}$ vs. $H_2O + La^{3+}$) were horizontal in the range 0–80°C. Initial and final baselines for all curves of apparent excess specific heat vs. temperature were horizontal with no significant permanent changes in apparent excess specific heat. The noise level was in the range 0.025–0.05 cal K^{-1} g^{-1}. At least five determinations were made at each DPPC/La^{3+} ratio. The standard errors in the calorimetric enthalpy (ΔH_{cal}) and maximal apparent excess specific heat (C_{max}) were both in the range ±2–4%, and the variability in the temperature of maximal apparent specific heat (T_c) and the transition width at $\frac{1}{2}C_{max}$ ($\Delta T_{1/2}$) was ±0.02°C. The effects of La^{3+} on the thermodynamic properties of DPPC cannot be due to general ionic strength effects since no changes in calorimetric parameters are apparent in control experiments using NaCl at equal ionic strength. At constant molar ratio of DPPC/La^{3+}, no differences in thermal behavior could be detected in the range 0.5–5.0 mg ml^{-1} of DPPC. At all ratios of DPPC/La^{3+}, the calorimetric scans were virtually identical after scanning, cooling the sample to 20°C and rescanning. No changes in calorimetric properties were observed after 24 h incubation in the calorimeter at 20°C before rescanning.

RESULTS AND DISCUSSION

From pure DPPC to a molar ratio of DPPC/La^{3+} of 3:1 (all molar ratios refer, unless otherwise stated, to 1.0 mg ml^{-1} of DPPC), the pretransition calorimetric parameters undergo small but experimentally significant changes (Table I, Fig. 1). The ΔH_{cal} and C_{max} are reduced by 10 and 25%, respectively, at a ratio of 3:1, while the T_c and $\Delta T_{1/2}$ are unchanged within experimental uncertainty. These observations were fully reproducible in independently prepared samples. If the change in tilt angle of the acyl chains from the L_β' to the P_β phase is indeed a major manifestation of the pretransition (5–11), it would be expected that larger changes in the calorimetric parameters of the pretransition would occur in the presence of La^{3+} ions at a molar ratio of 3:1 than are actually observed. We conclude

TABLE I
THERMODYNAMIC PARAMETERS FOR THE INTERACTION OF DPPC WITH La^{3+} IONS AT A SCAN RATE OF 0.1 K min^{-1} (LIPID CONCENTRATION 1 mg ml^{-1})

Pretransition											
Molar ratio DPPC/La^{3+}	1:0	50:1	25:1	10:1	7.5:1	5:1	4:1	3:1	2.7:1	2:1	1:1
La^{3+} (mM)	0.0	0.03	0.055	0.15	0.18	0.27	0.34	0.45	0.5	0.7	1.4
ΔH_{cal} (Kcal mol^{-1})	1.35	1.32	1.30	1.30	1.28	1.26	1.23	1.20	1.18	1.20	1.18
T_c (°C)	34.8	34.8	34.9	34.76	34.8	34.8	34.73	34.8	34.9	34.9	34.85
$\Delta T_{1/2}$ (°C)	0.9	0.93	0.89	0.90	0.91	0.92	0.89	0.90	0.88	0.91	0.92
C_{max} (cal K^{-1} g^{-1})	1.9	1.88	1.83	1.80	1.73	1.6	1.65	1.45	1.4	1.42	1.4
Gel-to-Liquid Crystalline Transition											
ΔH_{cal} (Kcal mol^{-1})	7.6	7.55	7.58	7.6	7.63	7.64	7.55	7.65	7.6	7.65	7.6
T_c (°C)	41.5	41.3	41.56	41.63	41.81	42.18	42.4	42.43	42.47	42.48	42.49
$\Delta T_{1/2}$ (°C)	0.11	0.13	0.15	0.16	0.17	0.18	0.20	0.21	0.22	0.23	0.22
C_{max} (cal K^{-1} g^{-1})	74.0	63.0	55.0	41.0	39.0	34.0	32.0	31.5	31.8	31.6	31.8

that the existence of a tilt angle different from 0° is neither a prerequisite for nor a major cause of the pretransition in DPPC. This being the case, it follows that the absence of a pretransition for diacylphosphatidylethanolamines (PE) cannot be attributed, as is currently assumed (4, 6, 7), to the fact that these lipids form bilayers with acyl chains perpendicular to the bilayer plane. (Unpublished work in this laboratory has shown that saturated chain PEs may indeed have a pretransition that is almost coincidental with the main transition.)

In contrast to the behavior of the pretransition in the presence of La^{3+} ions, that of the main transition is more complex. A decrease in C_{max} and an increase in T_c and $\Delta T_{1/2}$ are evident at molar ratios of 50:1 to 10:1, and the transition curves are asymmetric due to the incipient formation of a peak at higher temperature that we attribute to a phospholipid-ion complex. At a molar ratio of 7.5:1, the existence of two incompletely resolved peaks is clear (Fig. 1). As the La^{3+} ion concentration is further increased, the T_c and $\Delta T_{1/2}$ of the peak due to the ion-complexed lipid increase, while the T_c of the peak due to noncomplexed lipid remains practically constant at the value for pure DPPC. At a ratio of 3:1 the lower peak is very small (Fig. 1), and at 2.7:1 it has entirely disappeared. Between mole ratios of 2.7:1 to 1:1, transitions of the phospholipid-ion complex are asymmetric with a broad low temperature side followed by a steeper high temperature side, and are in this respect similar to the transitions reported in the literature for PE (17). Over the entire range of La^{3+} concentrations employed, up to a molar ratio of 1:1, the total enthalpy of the main transition remains constant within experimental error. At a molar ratio of

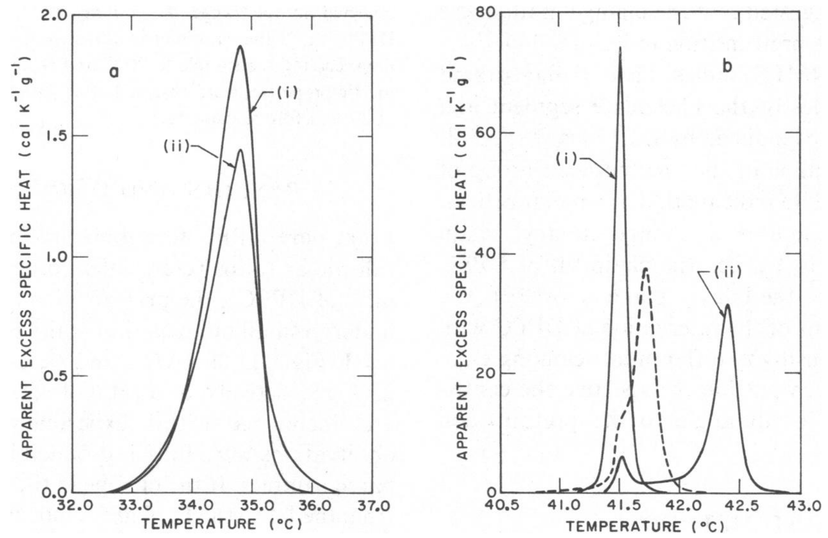

FIGURE 1 Calorimetric curves of apparent excess specific heat vs. temperature for unsonicated multibilayers of 1,2 DPPC (*a*) pretransition and (*b*) main transition. In both cases (*i*) is in the absence and (*ii*) in the presence of $LaCl_3$ (DPPC/La^{3+} molar ratio of 3:1). In *b* the dashed line represents the phase transition obtained with a DPPC/La^{3+} ratio of 7.5:1.

2.7:1, the T_c of the gel-to-liquid crystalline transition is 0.93°C higher than that of pure lipid. This is a significant increase in temperature, and reflects the changes in bilayer structure resulting from La^{3+} interaction with the head groups. Previous studies of the interaction of DPPC with La^{3+}, by low sensitivity differential scanning calorimetry (DSC), were unable to detect any differences between the thermal behavior of the main transition of DPPC in the absence and presence of La^{3+} ions below 1 M ion concentration (lipid/ion ratio of 1:1) (18).

In addition to La^{3+}, other compounds remove the hydrocarbon chain tilt in DPPC. Tetradecane or hexadecane at mole ratios of DPPC/alkane of 1:1 or 1:2 are two examples (9) of this. In these cases, however, the pretransition moves toward the main transition, and at a ratio of 1:3 merges with it. The gel-to-liquid crystalline transitions are broadened with an increased ΔH_{cal} but the T_m is increased by a maximum of 5°C (relative to DPPC) depending upon the chain length and molar ratio of alkane. This is in contrast to results obtained with palmitic acid that at a molar ratio of DPPC/palmitic acid of 1:2 increases the main phase transition temperature to 61.5°C (21; Chowdhry, B. Z., G. Lipka, A. W. Dalziel, and J. M. Sturtevant, unpublished results), close to that of 1,2dipalmitoylethanolamine at 63.8°C. These data, when combined with the results obtained with La^{3+} ions, show that changes in the head group interactions, either directly or indirectly through resulting changes in interactions in the nonpolar part of the bilayer, can result in large changes in the thermodynamics of the $P_\beta \rightarrow L_\alpha$ transition.

In summary, the interaction of La^{3+} with DPPC clearly shows that the alteration of the polar head group conformation and corresponding changes in hydrocarbon tilt angle significantly alter the gel-to-liquid crystalline phase transition but have little effect on the pretransition. The enthalpy of the pretransition must therefore derive mostly from molecular changes other than the change in tilt angle. Any theory describing the pretransition must account for this observation while at the same time accounting for the observation that molecules such as tetradecane and hexadecane, which also remove the tilt angle, change the properties of the pretransition more than La^{3+} ions do.

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