Phase Transitions between Ripple Structures in Hydrated Phosphatidylcholine-Cholesterol Multilamellar Assemblies

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A dynamic thermally induced cooperative first-order phase transition between ripple phases is described for fully hydrated mixtures of dipalmitoylphosphatidylcholine and 5-mole% cholesterol bilayers. The ripple-ripple phase transition has not been observed for pure phosphatidylcholine systems. This transition is more likely driven by changes in headgroup-water-headgroup interactions than by a change in acyl chain tilt. Implications for mechanical processes within a lipid bilayer are discussed.

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Phospholipids are known to form a variety of liquid-crystalline phases depending on, among other things, the water content and temperature of the samples [1]. There has been a great deal of interest recently in the study and description of the structure of the ripple ($P_{\beta'}$) phase as well as transitions in which it is involved. Diffraction [2–7], electron microscopic [8,9], and scanning tunneling microscopic [8] studies have attempted to deduce the ripple phase repeat distance in a variety of hydrated phosphatidylcholine systems. Wack and Webb [5] have extensively studied the effect of lipid concentration and chain length dependence on the phosphatidylcholine ripple phase dimensions while Hentschel and Rustichelli [7] have determined orientation effects when the ripple phase is induced in oriented samples. The latter also examined the phase-transition mechanism using temperature-equilibrated samples. Recently, Matuoka *et al.* [6] have examined the temperature dependence of the dimyristoylphosphatidylcholine ripple structure using real-time x-ray diffraction and showed that the ripple repeat spacing increases with decreasing temperature. It has also been shown by a variety of techniques that the presence of small molar quantities of sterols such as cholesterol [10] stabilizes the phosphatidylcholine ripple phase at the expense of the gel-state bilayers.

In addition, a number of theoretical approaches have been used to predict the cause of the ripple phase [11–16]. These include the microdomain approach of Marder *et al.* [13] which produces a phenomenological Landau–de Gennes theory, microscopic models including floating fluid phases [12,14], and the phenomenological model correlating the interactions between membranes to structured phase transitions by Goldstein and Leibler [15]. Recently, Cevc [16] has effectively combined a number of these approaches into a modified theory which produces an interaction balance method for describing undulated membranes at the molecular level. The lipid pretransition which typically induces rippled phases is described as being solvent dependent in which the driving force for the transition is the interfacial tendency for lateral expansion (the head-water-head repulsion) within a membrane.

This paper reports a relatively high sensitivity real-time x-ray study using synchrotron radiation [17] of the temperature dependence of fully hydrated phosphatidylcholine and phosphatidylcholine-cholesterol ripple structures during heating. A phase transition between ripple structures with different amplitudes was observed for the phosphatidylcholine-cholesterol mixture as evidenced by the coexistence of diffraction patterns for the initial and final states. These data thus agree with results from scanning tunneling microscopy [8] and may be related to either acyl chain tilt changes and/or changes in interbilayer headgroup interactions [16].

Dipalmitoylphosphatidylcholine (DPPC) was obtained from Avanti Polar Lipids (Pelham, AL) and cholesterol from Sigma Chemicals (St. Louis, MO) and used without further purification. Lipids were hydrated by heating to approximately 60 °C in the presence of 66.7-wt% water. The samples were subsequently stored at ca. 0 °C before examination.

X-ray-diffraction studies were performed using a monochromatic (0.15 nm) focused x-ray beam at station 8.2 of the Daresbury Synchrotron Laboratory. A purpose-built camera allowed clear resolution of reflections between 0.35 and 10 nm. The same holder was a cryostage (Linkam Scientific Inst. Ltd., Tadworth, United Kingdom) to which mica windows were fitted. X-ray scattering data were collected on a multiwire quadrant detector fabricated at the Daresbury Laboratory. X-ray

scattering data were acquired in 255 consecutive time frames separated by a dead time between frames of 50 μs. Data were stored in a VAX 11/785 computer and the experimental data analyzed using the OTOKO program developed at the Daresbury Laboratory. Spatial calibration was obtained using Teflon [18] and/or cholesterol [5].

Figure 1 shows typical three-dimensional plots of scattering intensity versus reciprocal spacing ($s = 1/d$) as a function of temperature for fully hydrated DPPC [Fig. 1(a)] and DPPC plus 5-mole% cholesterol [Fig. 1(b)] undergoing a heating scan. The DPPC sample was equilibrated to produce an initial $L_{\beta'}$ phase. The analysis of the dynamic x-ray patterns indicate that the heating scan produced the DPPC phase sequence $L_{\beta'} \rightarrow P_{\beta'} \rightarrow L_\alpha$,

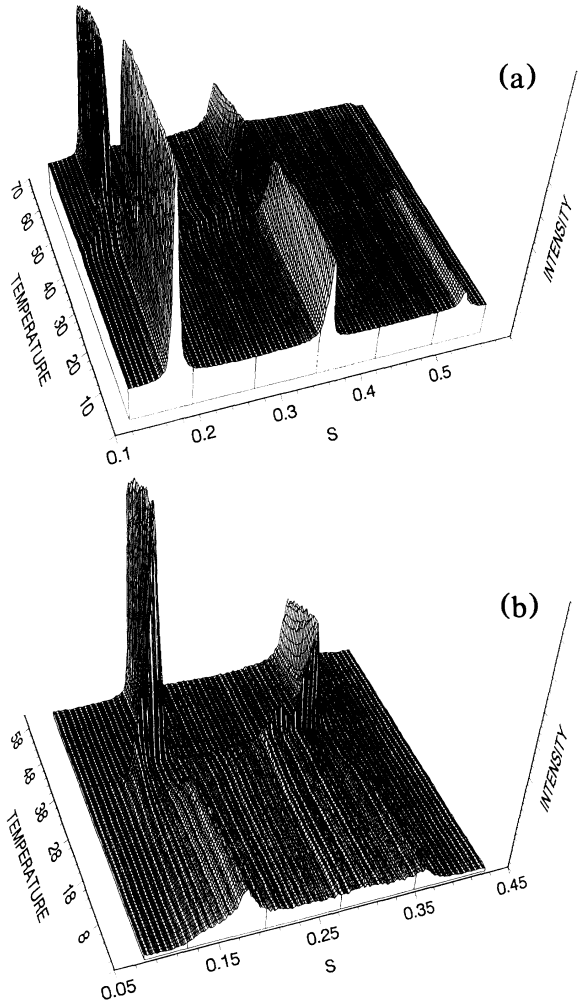

FIG. 1. Three-dimensional plots of x-ray scattering intensity vs reciprocal space (s) as a function of continuously increasing temperature (5 °C/min) for fully hydrated (a) DPPC and (b) DPPC + 5-mole% cholesterol. Every third diffraction pattern of 3-s duration from a total set of 225 continuously recorded patterns is shown. Temperature units are in °C and reciprocal-space units are in nm^{-1}, with intensity in arbitrary units.

TABLE I. Phase structures and transitions for fully hydrated DPPC and DPPC plus 5-mole% cholesterol as determined from real-time x-ray-diffraction measurements.

Lipid	Phase	d spacing (nm)	Transition temperature (°C)
DPPC	$L_{\beta'}$	5.86	35.0
	$P_{\beta'}$	6.84	
		(11.4)	41.3
	L_α	6.17	
DPPC + 5-mole% cholesterol	P_{β_1}	5.72	29.9
		(> 10.0)	
	P_{β_2}	7.21	
		(> 10.0)	39.4
	L_α	5.95	

which is consistent with previous dynamic x-ray data [19]. The transition temperature and phase structures are indicated in Table I. The DPPC plus 5-mole% cholesterol sample was equilibrated to induce an initial gel-state bilayer phase and produced the following dynamic phase-transition sequence during the heating scan: $P_{\beta_1} \rightarrow P_{\beta_2} \rightarrow L_\alpha$. The transition temperatures and phase structures are also listed in Table I. The calculated d spacings and inferred transition temperatures for both systems are consistent with previous static and/or dynamic measurements on unoriented arrays. In addition, we have observed evidence (data not shown) for two different ripple structures as a function of temperature using temperature-equilibrated DPPC plus 5-mole% cholesterol samples.

Figure 2 shows two-dimensional projections of the data sets in Fig. 1 for the ripple phases of DPPC and DPPC plus 5-mole% cholesterol. It is clear from the DPPC plus 5-mole% cholesterol data that there is a cooperative transition between different ripple phase structures as the temperature is increased which evolves via the coexistence of the initial and final states. The initiation of the transition is marked in this figure [Fig. 2(b)] at the temperature where the two distinct coexisting x-ray patterns appear. Although the phase dimensions change for each ripple phase structure as temperature is changed, there is no evidence that the coexistence of x-ray patterns is a simple manifestation of a continuous change of a single structure.

Figure 3 shows the two ripple phase diffraction patterns for DPPC plus 5-mole% cholesterol obtained at temperatures well above or below the transitional region. The peak assignments shown in parentheses for the different ripple phases are taken from the previous analyses of Janiak, Small, and Shipley [3,4] and Matuoka *et al.* [6]. These assignments were made on samples that were not originally heated to the L_α phase, cooled, and then examined, which can result in the formation of a metastable ripple phase [20]. The primary ripple phase

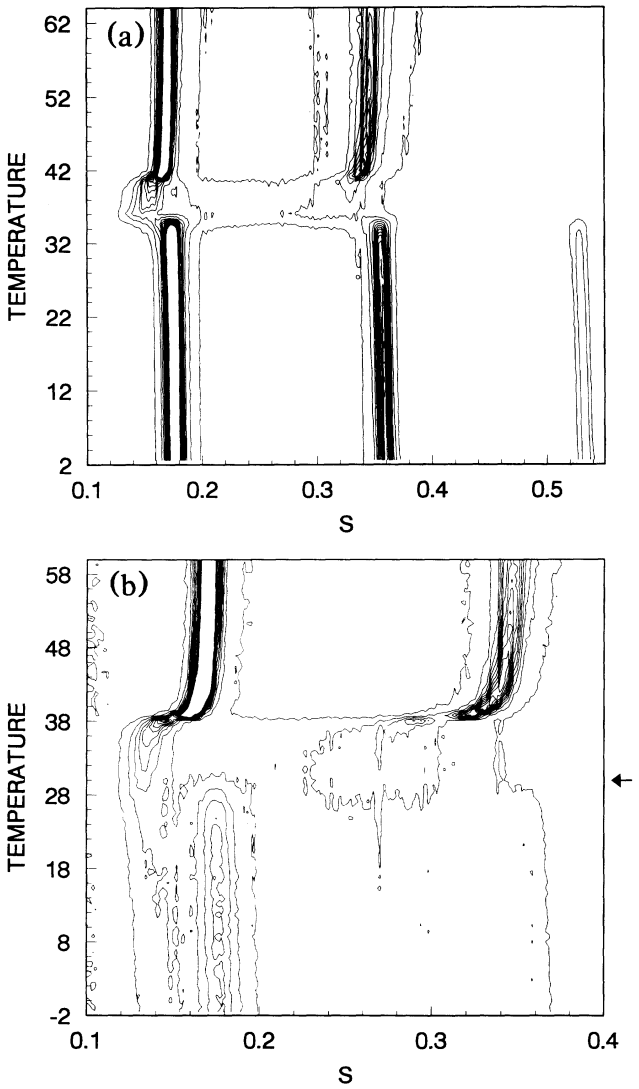

FIG. 2. Two-dimensional projections of the data set in Fig. 1 for fully hydrated (a) DPPC and (b) DPPC+5-mole% cholesterol. Temperature units are in °C and reciprocal-space units are in nm^{-1}.

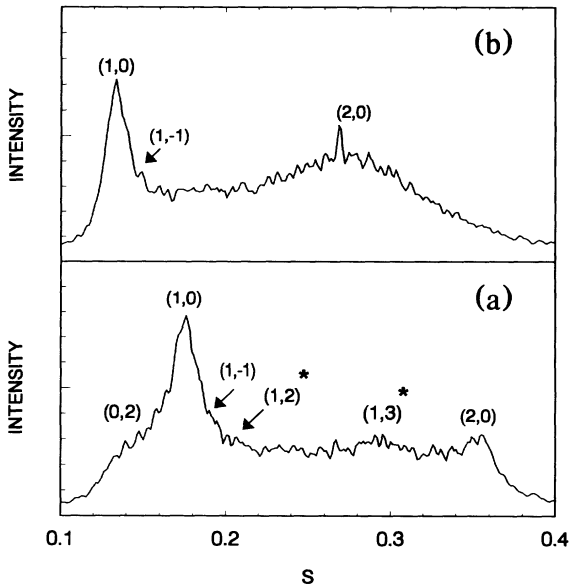

FIG. 3. Individual diffraction patterns representing the initial (at −1.5 °C) and final (at 33.9 °C) ripple phases for DPPC+5-mole% cholesterol from data in Fig. 1. The peak indices are indicated in parentheses. Reciprocal-space units are in nm^{-1} and intensity is in arbitrary units. The assignment of Miller indices denoted by asterisks are not conclusive.

structural change observed during this transition would be caused by a change in the bilayer and/or water thicknesses.

Cevc [16] theorizes that the intralamellar headgroup-water-headgroup interactions drive the transition from the bilayer to the ripple phase. Specifically, Cevc has proposed interchain (ch) and headgroup (hg) interactions of the form

$$\Psi = \Psi_{ch} + \Psi_{hg} = A_{ch}\cos(m\alpha) + B_{ch}P_2(\cos\gamma) + C_{hg}\cos(n\alpha), \quad m,n = 1,2,\ldots,$$

where γ is the angle between chain and headgroup axes and α is the sum of the relative angles of rotation of the two neighboring chains. P_2 is a second-order Legendre polynomial and A_{ch}, B_{ch}, and C_{hg} are coupling constants. One would expect that a transition between ripple phases would be a manifestation of changes in the angle between chain and headgroup axes or, more generally, a change in the orientation of the lipid molecule within the bilayer. The greater the interaction energy between molecules in the bilayer phase the more likely that the $P_{\beta'}$ phase will be produced at lower temperatures. It is less clear-cut as to the source of the ripple-ripple phase transition unless one hypothesizes a temperature-induced acyl chain or headgroup orientational change within the initially formed ripple phase. The correctness of this hypothesis can be obtained from techniques (i.e., Fourier-transform infrared spectroscopy) that can measure the mobility of molecular moieties. However, it has recently been shown by examining oriented lipid samples using x-ray diffraction [7] that the $L_{\beta'} \rightarrow P_{\beta'}$ phase transformation does involve a change in the lipid orientation within the bilayer.

We can conclude that thermally induced changes in the motion of molecular moieties and/or interactions between lipid molecules lead to a change in the hydration requirements and/or bilayer thickness within the ripple structure. A ripple-ripple phase transition has been observed in temperature-equilibrated samples of less than fully hydrated DPPC [21]. Cholesterol causes changes in acyl chain and headgroup interactions but these structural changes do not necessarily induce a ripple-ripple phase transition. It can be inferred [21] that the driving force for the cholesterol induced ripple-ripple phase transition is due to changes in the hydration requirements and inter-

facial interactions of the DPPC headgroups. Changes in the bilayer can be expected to similarly effect the ripple phase wavelength as theorized by Cevc [16]. These changes within the ripple phase are shown to result in a cooperative phase transition between stable ripple phases which evolves as a coexistence of the two states rather than as a continuous- (second-order) type process. This appears not to be a general phenomenon of lipids forming ripple phases but requires the presence of small amounts of cholesterol and/or other sterols to modify the interaction between phospholipid molecules. This is consistent with previous observations that cholesterol changes the lateral compressibility within DPPC bilayers [22].

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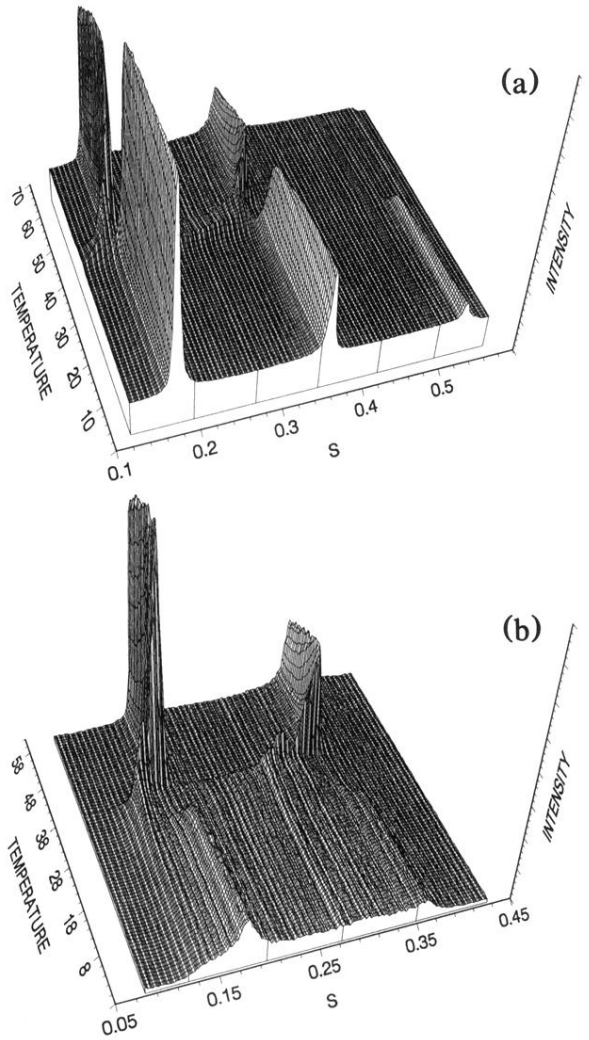

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