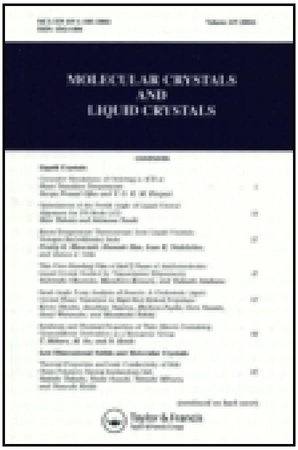

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A. C. Calorimetric Study of Phospholipid-Cholesterol Systems and Their Structures

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A.C. Calorimetric Study of Phospholipid-Cholesterol Sytems and Their Structures†

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The a.c. heat capacity is found to be composed of a narrow peak and a distinct wide peak in the dipalmitoylphosphatidylcholine (DPPC)-cholesterol system and the dimyristoylphosphatidylcholine (DMPC)-cholesterol system. The narrow peak appearing at the main transition has more or less the same shape over a wide range of cholesterol concentrations, but the magnitude decreases with increasing cholesterol concentration and disappears at about 20 mol% cholesterol in both systems. On the other hand, the wide peak appearing above the main transition broadens with increasing cholesterol concentration and becomes indistinguishable at about 20 mol% cholesterol for the DMPC-cholesterol system and at about 50 mol% cholesterol for the DPPC-cholesterol system. From the results of these and other experiments, we infer that there are three kinds of domain, pure DMPC, DMPC-thin cholesterol and 0.8 DMPC · 0.2 cholesterol complex regions, lying in an ordered array. The DPPC-cholesterol system has a similar but more disordered structure.

INTRODUCTION

Phospholipid-cholesterol systems have been extensively studied by a number of experimental techniques. So far, the formation of com-

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plexes containing one molecule of cholesterol to four, two or one molecule of lipid has been inferred from various experiments. In the dimyristoylphosphatidylcholine (DMPC)-cholesterol system, a significant break has been observed in membrane properties at 20 mol% cholesterol. Rubenstein *et al.*[1] have mapped out a temperature *v.s.* cholesterol-concentration diagram in which a border-line separates slow and fast lateral diffusion regions as indicated by fluorescence studies. On the axis of cholesterol concentration this line intersects at 20 mol% cholesterol. Copeland and McConnell[2] have studied the ripple structure of the DMPC-cholesterol system by freeze-fracture electron microscopy. The ripple repeat distance has been found to increase as cholesterol concentration is increased from 0 to 20 mol% until above 20 mol% cholesterol no rippling is observed.

In lipid-cholesterol systems, differential-scanning-calorimetry (DSC) studies have shown that there are narrow and broad anomalies, the narrow anomaly diminishes at about 20 mol% cholesterol and the broad anomaly disappears at some higher cholesterol concentration.[3,4] In this paper, the results of a.c. calorimetric studies of DMPC-cholesterol and DPPC-cholesterol systems are described. Since a.c. calorimetry gives a steady-state value of heat capacity at the measuring frequency, the shape of the heat capacity curve does not depend on the temperature-scanning rate. Therefore, it is possible to carry out quantitative studies of heat capacity anomalies in these systems.

EXPERIMENTS AND RESULTS

The DPPC, DMPC and cholesterol used were obtained from Sigma Chem. Co. and used without further purification. The purity of all of these materials was checked by thin-layer chromatography. Suspensions for this experiment were prepared in a similar manner to that described in other literature.[4] The content of lipid-cholesterol was always kept at about 5.0 wt% against water.

The a.c. calorimetry experiment[5] was carried out at a frequency of 0.75 Hz. A sample cell for the present experiment has been previously described.[6] The temperature of the cell was changed stepwise by 0.06 K for both heating and cooling, and held constant for an interval of 60 sec. The measurement of a.c. heat capacity was performed during the constant-temperature interval and therefore, represented a steady state value. Thus, although the a.c. heat capacity has a dynamic function, it is independent of the temperature-scanning rate.

In the DPPC-cholesterol system, the a.c. heat capacity was observed at cholesterol concentrations of 3.25, 10.1, 17.9, 32.2 and 65.5 mol%. The a.c. heat capacity curve shows a narrow peak and a shoulder. The narrow peak occurs close to the main transition for pure DPPC and is also similar in shape to that of pure DPPC, but it decreases in magnitude with increasing cholesterol concentration. On the other hand, the shoulder grows and becomes broader with increasing cholesterol concentration. At 32.2 mol% cholesterol, only a broad peak, originating from the shoulder, remains and at 65.5 mol% cholesterol, no traces remain. We are very interested in the ratio of the shoulder part to the narrow one. To examine this, the a.c. heat capacities were normalized so as to coincide with that for pure DPPC at the main transition. The normalized heat capacity is shown in Figure 1 for pure DPPC, 3.25 mol% cholesterol and 10.1 mol% cholesterol. The normalization procedure was omitted at the main transition itself because of rounding effects. As seen in Figure 1, the ratio of the shoulder part to the narrow peak increases with cholesterol concentration.

So far a large number of DSC experiments have been carried out of DPPC-cholesterol systems,[3,4] however such a clear break between the narrow peak and the shoulder as in the present a.c. calorimetry has not been observed. This might be due to the difference between DSC and a.c. calorimetry.

For the DMPC-cholesterol system, preliminary a.c. heat capacity experiments were made in the composition range from pure DMPC to 22.8 mol% cholesterol. In this case, a narrow peak and a distinct wide peak were observed, similar to in DPPC-cholesterol systems, and moreover, both of the peaks were found to smear out at the same time, at 20 mol% cholesterol. The latter fact coincides with the disappearance of ripple structure in freeze-fracture electron microscopy.[2]

DISCUSSION

From the results of freeze-fracture electron microscopy for DMPC-cholesterol system, the ripple repeat distance has been found to increase with cholesterol concentration.[2] This has been explained in terms of alternating two strips of pure DMPC and 20 mol% cholesterol.[2] From the viewpoint of calorimetry, the microscopic domain composed of pure DMPC gives rise to the narrow peak at the main transition, however, the microscopic domain composed of 80 mol%

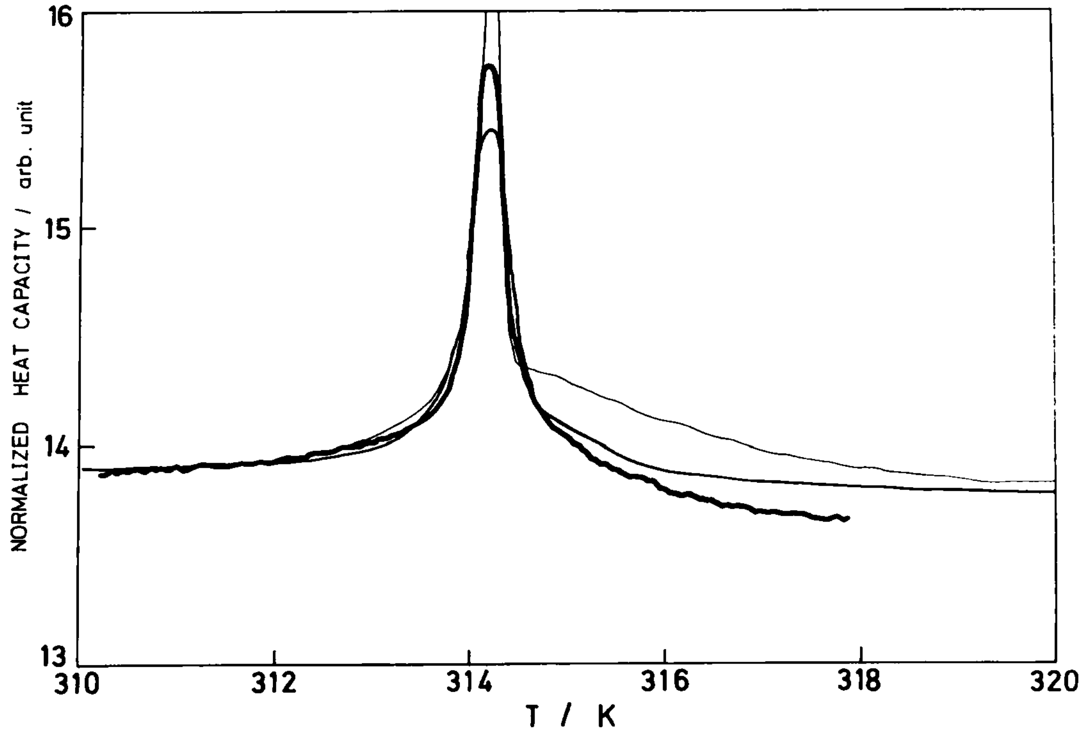

FIGURE 1 Normalized a.c. heat capacities at the main transition of the DPPC-cholesterol system. Thick line: pure DPPC. Intermediate line: 3.25 mol% cholesterol. Thin line: 10.1 mol% cholesterol.

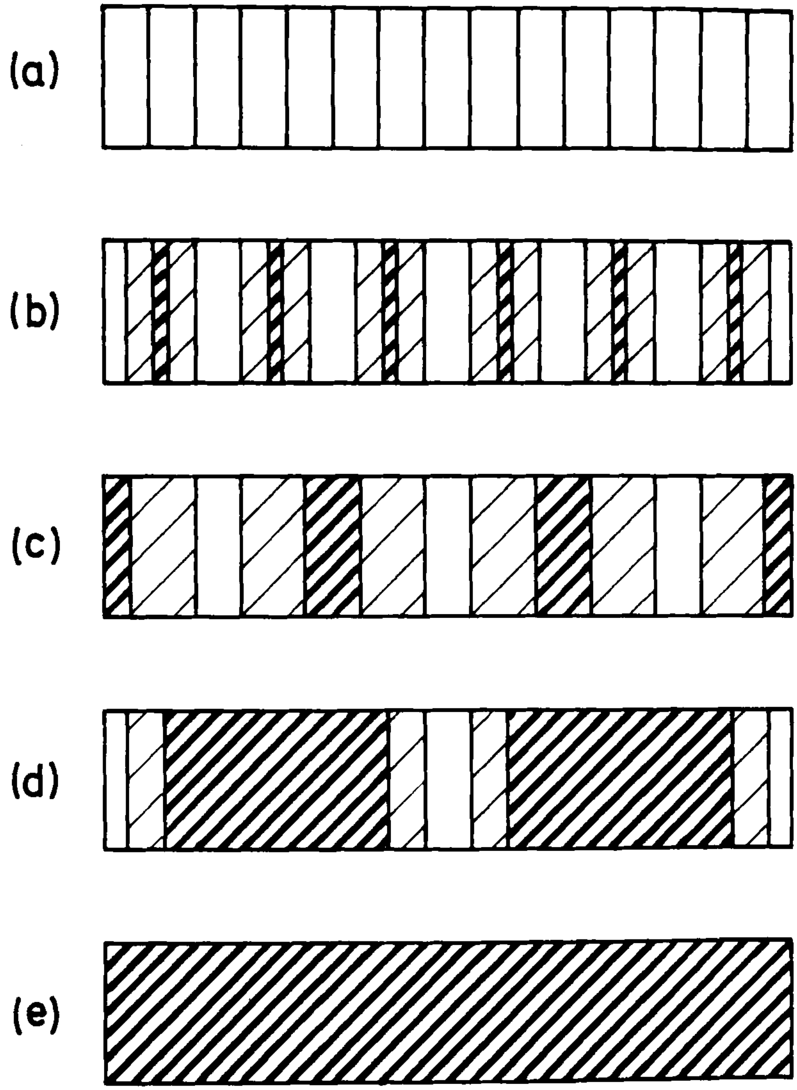

FIGURE 2 A model for the structure of the ripple phase in DMPC-cholesterol system. (a) The interval indicates ripple repeat distance for pure DMPC. (b)–(d) Cholesterol concentration increases from top to bottom. A heavy hatched domain represents a region at 80 mol% DMPC · 20 mol% cholesterol complex and light hatched domains represent regions with cholesterol concentrations less than 20 mol%. (e) A plain region with a complex containing 80 mol% DMPC · 20 mol% cholesterol all over the system.

DMPC · 20 mol% cholesterol complex can not give rise to a peak since we observe no anomaly in the a.c. heat capacity in DMPC-cholesterol system with cholesterol more than 20 mol%. The heat capacity experiment shows that this is another broad anomaly, which was not taken into account in the above consideration. This wide anomaly should give a region including cholesterol less than 20 mol%. Judging from the width of the anomaly, cholesterol is roughly distributed over the above region. On the other hand the ripple repeat distance seems to be comparatively regular indicating microscopic domains. From these considerations, a ripple structure is proposed as in Figure 2. As the cholesterol concentration increases from (a) to (e), the width of the domain with 80 mol% DMPC · 20 mol% cholesterol complex increases and the width of the domain with cholesterol less than 20 mol% first increases and then decreases. At a cholesterol concentration of 20 mol% all of the region becomes even. It should be pointed out that a trace of the domain structure with cholesterol less than 20 mol% exists in the temperature range higher than the main transition temperature, since the heat capacity anomaly due to this domain takes place even in the above temperature range.

A similar domain structure is to be expected for the DPPC-cholesterol system. Here, the condition for the complex formation of 80 mol% DPPC · 20 mol% cholesterol should be much looser and the domain boundaries in the regions including cholesterol might therefore be disordered.

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