Calorimetric studies of the effects of cholesterol on the phase transition of C(18):C(10) phosphatidylcholine

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ABSTRACT Differential scanning calorimetry (DSC) has been employed to study the effects of cholesterol on the phase transition of C(18):C(10) phosphatidylcholine (C(18):C(10)PC). C(18):C(10)PC is an asymmetric mixed-chain phosphatidylcholine known to form mixed-interdigitated structures below the transition temperature and form partially interdigitated lipid bilayers above the transition. Three types of samples were used. The treated sample is the lipid dispersion that had undergone three freeze-thaw cycles and stored at 4°C for more than 48 h. The untreated sample was made by vortexing the dry lipid in 50 mM KCl, without the above-mentioned pretreatment. The cold-treated sample was prepared by incubating the treated sample at −20°C for 15 d. There is no apparent difference in the DSC curves between the treated and cold-treated samples. The data derived from the treated samples seem to be more reproducible. The DSC curves between the cholesterol/C(18):C(10)PC and cholesterol/symmetric diacylphosphatidylcholine mixtures are different in three aspects: overall appearance, the cholesterol dependence of ΔH, and the effect of cholesterol on the maximal transition temperature T_m, the onset temperature T_o, and the completion temperature T_c. for both the treated and untreated samples, the total enthalpy change ΔH of the phase transition of C(18):C(10)PC decreases with increasing cholesterol content, approaching zero at ~25 mol%. This level is lower than the total enthalpy changes reported previously for the cholesterol/symmetric diacylphosphatidylcholine mixtures. Both the heating and cooling thermograms show that T_m, T_o, and T_c decrease with increasing cholesterol content. The decreasing rates of these temperatures with cholesterol are in the neighborhood of −0.24 degree per mol% of cholesterol. This value is greater than those reported previously for cholesterol/symmetric diacylphosphatidylcholine mixtures. The phase transition between interdigitated lipid bilayers appears to be more sensitive to cholesterol than that between noninterdigitated lipid structures. The formation of highly ordered interdigitated lipid bilayers requires stringent structural conditions such as specific chain length differences and high molecular order. Apparently, in the presence of cholesterol, these stringent structural conditions are no longer satisfied. It is likely that cholesterol causes a local disordering effect on the gel phase of C(18):C(10)PC and that as a consequence the physical state of the gel phase changes continuously with the cholesterol content. The implication of the present study is that cholesterol may have a function in preventing lipids from forming highly ordered interdigitated structures in natural membranes.

INTRODUCTION

Interdigitated lipid structures occur in a variety of physical and chemical situations. For example, interdigitated structures can be induced from phospholipids by temperature (reviewed in Huang and Mason, 1986 and references cited therein), pressure (Braganza and Worcester, 1986; Wong and Mantsch, 1986), alcohols (McDaniel et al., 1983; Simon and McIntosh, 1984; Rowe, 1987), biomolecules (Ranck and Tocanne, 1982*a*, *b*; Boggs and Rangaraj, 1985), and other small molecules (Ranck et al., 1977; McDaniel et al., 1983; McIntosh et al., 1983; Simon and McIntosh, 1984). Asymmetric mixed chain phosphatidylcholine can form interdigitated lipid bilayers. The structure of these bilayers varies from the partially interdigitated, to mixed interdigitated, to fully interdigitated lamellae, depending on the temperature and the chain length difference (Huang et al., 1984; Hui et al., 1984; Levin et al., 1985 and reviewed in Huang and Mason, 1986). These interdigitated structures are highly ordered; they are more ordered than the gel state of the corresponding symmetric diacylphosphatidylcholines. Although highly ordered lipids may provide an adverse environment for membrane constituents, interdigitated lipid structures have been suggested to exist in natural membranes. For example, interdigitated lipid bilayers may exist in membranes containing high contents of sphingomyelin (Levin et al., 1985) and in aged membranes (Xu and Huang, 1987). Therefore, it is of biological interest to study the physical behavior of key mem-

Abbreviations used in this paper: C(18):C(10)PC, L-α-phosphatidylcholine having 18 carbons in the sn-1 acyl chain and 10 carbons in the sn-2 chain; DMPC, dimyristoylphosphatidylcholine; DSC, differential scanning calorimetry.

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brane constituents such as sterol and membrane-bound proteins in highly ordered interdigitated lipid structures.

Here we report the first calorimetric data of the effects of sterol on the phase transition between different interdigitated lipid structures. Sterols are important membrane constituents. This is particularly true for cholesterol, because it is a major component in plasma membranes and because it is a causative factor for heart diseases and gallstones. Although so many studies have been devoted to cholesterol and its derivatives, the dynamic behavior and molecular organization of sterols in interdigitated lipid bilayers are virtually unexplored. Here, differential scanning calorimetry (DSC) has been employed to study the thermal behavior of binary mixtures comprising of C(18):C(10)PC and cholesterol. C(18):C(10)PC is an asymmetric mixed-chain phosphatidylcholine with one acyl chain twice as long as the other. This type of mixed-chain phosphatidylcholine is known to form mixed interdigitated structures below the main phase transition temperature (Xu and Huang, 1987) and to form partially interdigitated structures above the transition. Structures of C(18):C(10)PC have been characterized by Raman spectroscopy, x-ray diffraction, freeze-fracture electron microscopy, and DSC (Mason et al., 1981; Huang et al., 1982, 1983; McIntosh et al., 1984; Hui et al., 1984; Xu and Huang, 1987). The interactions of C(18):C(10)PC to fatty acid spin labels (Boggs and Mason, 1986) and to symmetric diacyl phosphatidylcholines (Mason, 1988) have also been reported. The present study, however, characterizes for the first time the phase behavior of C(18):C(10)PC in the presence of cholesterol. The results have shed light on the interactions of cholesterol to interdigitated phospholipids.

MATERIALS AND METHODS

Cholesterol was obtained from Nu Chek Prep, Inc. Elysian, MN. C(18):C(10)PC was a gift from Professor Ching-hsien Huang at the University of Virginia. The concentration of phospholipid was determined by the method of Bartlett (1959). Three types of lipid dispersions were used in this study. The first type is referred to as the treated sample. The cholesterol/C(18):C(10)PC dispersions in 50 mM KCl were first heated, under nitrogen, to 37°C for 20 min, and were immediately vortexed for 5 min. The sample was then allowed to anneal at 0°C for 1 h. After three freeze-thaw cycles, the sample was stored at 4°C for 48 h before being loaded into the sample cell of the calorimeter, which was pre-set at temperatures below the phase transition temperature of pure C(18):C(10)PC.

The second type of dispersions is referred to as the untreated sample. In this case, the dispersions were made by vortexing the dry lipids with 50 mM KCl under nitrogen at temperatures above the transition temperature. The dispersions were maintained at temperatures above T_m before the DSC measurements; freeze-thaw cycles and cold incubation were not employed in this preparation.

The third type is referred to as the cold-treated samples which are the treated samples stored at −20°C for 15 d.

Calorimetric measurements were made with a Hart Scientific differential scanning calorimeter (Provo, Utah). First, a heating scan was employed. Then, the sample was held at 40°C for 1 h before a cooling scan was conducted. Both the heating and cooling scans were made at a scanning rate of 15°/h.

RESULTS

DSC data from the treated sample

Fig. 1 shows the representative heating thermograms for the binary mixtures of cholesterol/C(18):C(10)PC in 50 mM KCl. The DSC curve of pure C(18):C(10)PC exhibits a sharp transition at 18.8°C. This temperature is slightly lower than that previously reported by Xu and Huang (1987), as a result of purer lipids being used here (personal communication with Professor C. Huang). Both the enthalpy change ΔH and the maximal transition temperature T_m are affected by cholesterol (Fig. 1). Fig. 2 shows that the total enthalpy change, ΔH, decreases with the increase of cholesterol content. The total enthalpy change is almost completely eliminated at 25 mol% cholesterol. Fig. 3 shows the apparent entropy change ΔS also varies with cholesterol concentration. The T_m, the onset temperature, T_o, and the completion temperature,

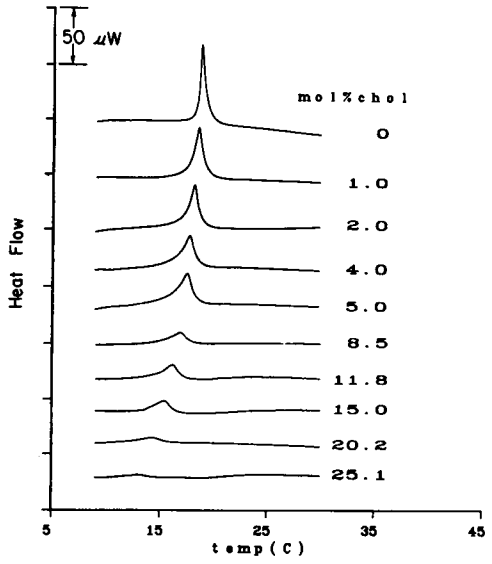

FIGURE 1 Heating DSC curves of C(18):C(10)PC in the presence of cholesterol. Listed in the figure are the molar ratios of cholesterol in the mixture. All the data were obtained from the treated samples prepared by the procedures described in the Methods section. The phospholipid concentrations of the measured samples are: 1.11 mM for pure C(18):C(10)PC, 1.07 mM for 1.0 mol%, 1.08 mM for 2.1 mol%, 1.26 mM for 3.1 mol%, 1.13 mM for 4.0 mol%, 1.24 mM for 5.0 mol%, 0.55 mM for 8.5 mol%, 0.96 mM for 11.8 mol%, 1.13 mM for 15.0 mol%, 0.81 mM for 20.2 mol% and 1.3 mM for 25.1 mol%. 400 μl of the lipid dispersions were used for each sample measurement.

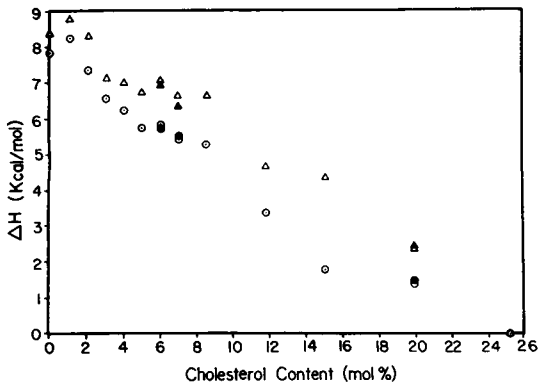

FIGURE 2 Effects of increasing cholesterol on the total enthalpy change of C(18):C(10)PC. (0): ΔH of the treated samples on heating; (△): ΔH of the treated samples in the cooling mode; (●): ΔH of the treated samples in the subsequent second heating scan; (▲): ΔH of the treated samples in the second cooling scan.

T_c, are decreased as the amount of cholesterol is increased (Fig. 4). T_o and T_c are determined by the method described in Fig. 2 of Xu et al. (1987). These temperatures decrease with increasing cholesterol. The decreasing rates are: −0.28 deg/mol% for T_o on heating, −0.24 deg/mol% for T_m on heating, −0.19 deg/mol% for T_c on heating, −0.26 deg/mol% for T_c on cooling, −0.23 deg/mol% for T_m on cooling, and −0.21 deg/mol% for T_o on cooling. It is also obvious from Fig. 4 that the difference between T_o and T_c increases with the cholesterol content, indicating that the transition peak becomes broad as the cholesterol content is increased.

Fig. 5 shows the representative DSC curves on cooling. The transition temperature T_m obtained on cooling is lower than the T_m of the same sample on heating (Fig. 4). The effects of cholesterol on the total enthalpy change and on the entropy change on cooling are shown in Fig. 2

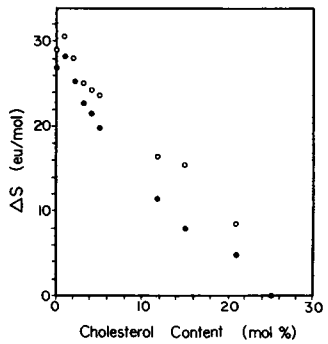

FIGURE 3 The cholesterol dependence of the apparent entropy change ΔS, which is calculated by $\Delta S = \Delta H/T_m$. (O): ΔS of the treated samples on cooling; (●): on heating.

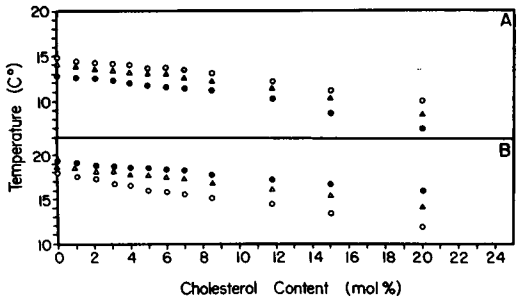

FIGURE 4 The cholesterol dependence of T_m (-△-), T_o (-O-), and T_c (-●-) in C(18):C(10)PC. (*A*) cooling; (*B*) heating.

(-Δ-) and Fig. 3 (-0-), respectively. The cooling scan is similar to the heating scan in that the transition peak is abolished at a cholesterol concentration of ~25 mol%.

Using the T_o of the heating scan and the T_o of the cooling scan, a partial phase diagram for the binary mixture comprising C(18):C(10)PC and cholesterol is constructed (Fig. 6). Inside the boundary of the phase diagram, two phases coexist. Two-phase coexistence disappears at the critical mixing point of 25 mol% cholesterol.

The data obtained from the treated sample are highly reproducible. Some samples were subjected to a second heating/cooling scan immediately after the first heating/cooling cycle. As presented in Fig. 2 (the data with close

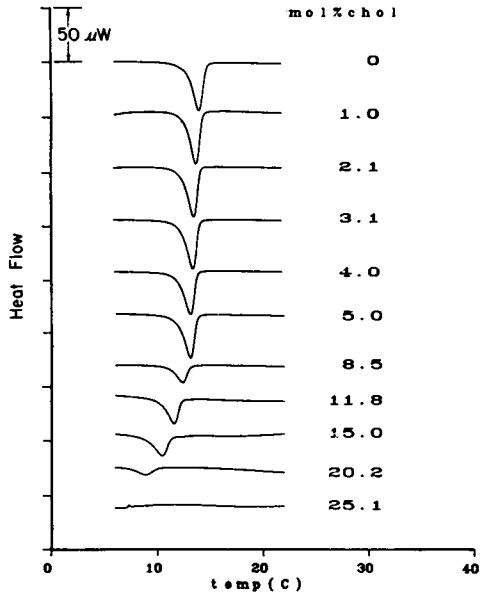

FIGURE 5 Cooling DSC curves of cholesterol in C(18):C(10)PC. The phospholipid concentrations of the measured sample are listed in Fig. 1.

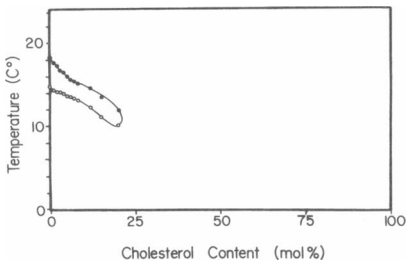

FIGURE 6 A partial phase diagram of cholesterol in C(18):C(10)PC. (●): T_c on heating; (○): T_c on cooling.

circles and close triangles), the ΔH value obtained from the second heating/cooling scan is virtually the same as those obtained from the first heating/cooling scan.

Dependence of sample history

The effect of cholesterol on the phase behavior of C(18):C(10)PC becomes somewhat different when the sample was not subjected to freeze-thaw cycles and not to 48 hr cold incubation. Fig. 7 shows the data obtained from those untreated samples. The results differ from those for the treated samples in two aspects. First, the data from the untreated samples show a nonlinear relationship between ΔH and cholesterol content. Second, the ΔH of the untreated samples is, in general, slightly lower than the ΔH of the treated samples. However, the total enthalpy change approaches zero at 25 mol% cholesterol in both treated and untreated samples.

In another experiment, a sample of 11 mol% cholesterol in C(18):C(10)PC was incubated at −20°C for 15 d before DSC measurements. No new DSC peak was observed for this cold-treated sample and the thermodynamic parameters (data not shown) remain virtually the same as those obtained from the treated samples. This result is taken to indicate that no new phase is formed after a long incubation at low temperatures.

DISCUSSION

Despite some similarities, the effects of cholesterol on the phase transition of C(18):C(10)PC are distinctly different from those previously obtained for cholesterol/symmetric diacyl phosphatidylcholine mixtures. First, there are distinct differences in the appearance of the DSC curves between the cholesterol/C(18):C(10)PC systems and the cholesterol/symmetric diacyl phosphatidylcholine bilayers. Estep et al. (1978) reported that the main phase transition of the cholesterol/DPPC mixtures consists of two components. The sharp transition centered at 39.6–40.7°C exhibits an enthalpy change which decreases linearly with increasing cholesterol content, approaching zero at a cholesterol content of ~25 mol%. The broad component centered at 41.5°C displays an enthalpy change which is maximal at ~20–25 mol% cholesterol and which decreases as the cholesterol content decreases to zero or increases above 25 mol% (see Fig. 3 of Estep et al. [1978]). A similar DSC curve was reported by Mabrey et al. (1978). Such a DSC curve is, however, not observed in the case of C(18):C(10)PC/cholesterol mixture (Figs. 1, 2, 5 and 7).

The second difference lies in the effect of cholesterol on the total enthalpy change of the phase transition of C(18):C(10)PC. The value for the mole fraction of cholesterol required to abolish the total enthalpy change of C(18):C(10)PC (Figs. 2 and 7) is less than the previous observations with cholesterol/diacylphosphatidylcholine mixtures. Ladbrooke et al. (1968) demonstrated that the DSC curve of the main phase transition of dipalmitoylphosphatidylcholine (DPPC) is abolished at ~50 mol%. Hinz and Sturtevant (1972) reported that for the cholesterol/DPPC and cholesterol/dimyristoylphosphatidylcholine (DMPC) systems, the total enthalpy changes of the phase transition went to zero at 33 mol%. Estep et al. (1978) also indicated that the total enthalpy changes of cholesterol/DPPC diminishes at a cholesterol level >35 mol%. In contrast, the mole fraction of cholesterol required to abolish the total enthalpy change of C(18):C(10)PC is in the neighborhood of 25 mol%. However, it should be noted that this comparison was made based on the total enthalpy change of the DSC measurements. It is known from other physical measurements that the phase transition of symmetric diacylphosphatidylcholine may be abolished at ~25–30 mol% cholesterol (briefly reviewed in Hui and He, 1983).

The third difference is related to the cholesterol dependence of T_m, T_o, and T_c. As shown in Fig. 4, the T_m in the cholesterol/C(18):C(10)PC mixture on heating shifts from 18.8°C for 0 mol% cholesterol to 16.4°C for 10 mol%, giving a slope of −0.24°/mol%. Such a dramatic downshift is not seen in the cases of cholesterol/DPPC or

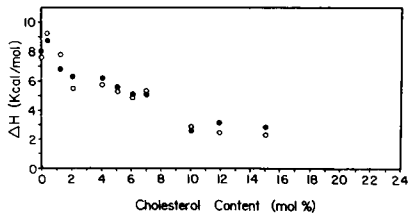

FIGURE 7 The effect of cholesterol on the total enthalpy changes of the untreated samples. (○): heating; (●): cooling.

cholesterol/DMPC. For example, in the cholesterol/DPPC system, the narrow peak, centered at 41.4°C for 0 mol% cholesterol, slightly shifts to 40.9°C at 10 mol% cholesterol (−0.05°/mol%) whereas the broad peak, centered at 41.0–41.6°C, moves up to ~45.6°C at 33 mol% cholesterol (Fig. 3 of Mabrey et al., 1978).

The physical origin of the apparent differences in DSC curves probably lies in the fact that the formation of interdigitated lipid bilayers needs stringent structural requirements. As pointed out by Xu et al. (1987) and by Davis and Keough (1985), the chain length difference plays a key role in the determination of the structure of interdigitated lipid bilayers. In addition to the chain length difference, other factors such as molecular ordering, number and position of double bonds, and differences in chain conformational states may be important as well (discussed in Davis and Keough, 1985). It is very likely that some or all these factors are changed when cholesterol is present. It is believed that the bulky tetracycline ring of cholesterol exerts local disordering effects on C(18):C(10)PC, in a manner similar to those previously suggested. Ladbrooke et al. (1968) have proposed that cholesterol can cause local disordering perturbation on the acyl chains of phospholipids below T_m. Also, Huang and Levin (1983) suggested that the bulky fatty acid terminal methyl group causes a perturbating effect on the matrix lipid, leading to a lowering T_m for the mixed-interdigitated bilayers. Because of the disordering effect caused by cholesterol, the actual penetration depth of the acyl chains in C(18):C(10)PC may not be equal to the apparent chain length. Our data also suggest that the disordering effect should increase as the cholesterol content increases. Thus, the actual difference in chain length may vary with cholesterol content. For similar reasons, the conformational state of acyl chains may change with cholesterol content as well. Although these changes may be small, they can greatly affect the structure of mixed interdigitated lipid bilayers since the formation of mixed interdigitated lipid bilayers needs stringent structural requirements (Xu et al., 1987).

Although 25 mol% cholesterol can abolish the phase transition of C(18):C(10)PC, it is not clear whether cholesterol can abolish interdigitated structures. At the present time, it can only be concluded that the lipid bilayers of C(18):C(10)PC are less ordered when cholesterol is present. Structural studies such as x-ray diffraction are needed in order to determine whether the lipid bilayers of C(18):C(10)PC maintain an interdigitated structure in the presence of cholesterol and whether cholesterol interdigitates with C(18):C(10)PC.

The observation that the phase transition of C(18):C(10)PC is sensitive to cholesterol suggests that highly ordered interdigitated lipid bilayers would not normally exist in natural membranes (Levin et al., 1985; Xu and Huang, 1987). At least the interdigitated lipid bilayers, if existing, would be appreciably disordered when the cholesterol content is high. The implication here is that cholesterol may have a function in preventing the formation of highly ordered interdigitated lipid bilayers in natural membranes. However, highly ordered interdigitated lipid bilayers may exist locally in the cholesterol-poor domains which are phase-separated from the cholesterol-rich regions.

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