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Effects of very small amounts of cholesterol on gel-phase phosphatidylcholine membranes

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The mobility of 5-doxyl stearic acid spin label (5-SASL) in the gel phase of dipalmitoylphosphatidylcholine membranes between the main transition and subtransition temperatures was studied as a function of cholesterol content. Very small amounts of cholesterol (0.01–1 mol%) cause a dramatic increase in the mobility of 5-SASL. Temperature-drop experiments from 38°C to 28°C were made across the pretransition temperature and the rate of approach to equilibrium was measured. Cholesterol at low concentrations also affects this rate. The membrane reached equilibrium after 10 h in the absence of cholesterol, 3 h at 0.01 mol% cholesterol, and less than 10 min at 0.03 mol% cholesterol.

Intensive efforts have been made in many laboratories to better characterize the structure of gel-phase phospholipid membranes [1,2] and their phase transitions [3,4]. Tsong [5] indicated that strongly cooperative nucleation processes and energy-dependent fast propagation steps occur during the main phase transition. In this communication we report temperature drop kinetic measurements of synthetic phosphatidylcholine membranes in the gel phase. The approach to the new equilibrium state after rapid temperature drop across the pretransition was observed by monitoring the ESR spectrum of a fatty acid spin label.

5-Doxyl stearic acid spin label (5-SASL) was obtained from Syva (Palo Alto, CA). Phospholipids were purchased from Sigma (St. Louis, MO), and cholesterol (crystallized) from Boehringer-Mannheim (Indianapolis, IN). The buffer used throughout this study was 0.1 M sodium borate at pH 9.5. To ensure that all 5-SASL carboxyl groups are ionized in phosphatidylcholine membranes, a rather high pH was chosen [6,7]. Structure [7], phase transition [4], and the electrostatic properties [8] of phosphatidylcholine membranes are the same in the pH range between 4.0 and 10.0. The membranes used in this work are multilamellar dispersion of lipids prepared as described previously [9]. ESR measurements were made as described previously [9].

We used $2T'_{\parallel}$ of 5-SASL as a convenient spectral parameter to characterize motional freedom of the 5-SASL nitroxide radical in the membrane. $2T'_{\parallel}$ decreases as motional freedom increases. Temperature drop experiments across the pretransition of dipalmitoylphosphatidylcholine membranes from 38°C to 28°C (2 min settling time) are shown in Fig. 1. $2T'_{\parallel}$ reaches the equilibrium

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Abbreviation: 5-SASL, 5-doxyl stearic acid spin label.

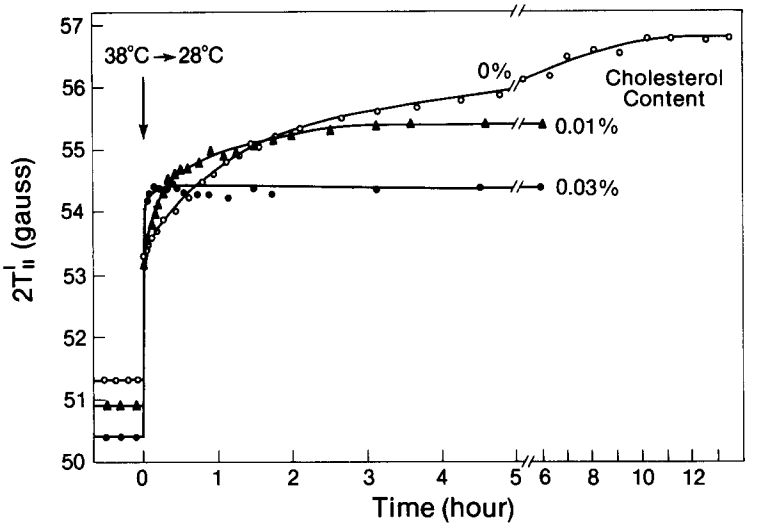

Fig. 1. Time-course of approach to equilibrium $2T'_{\parallel}$ value of 5-SASL after temperature drop across the pretransition temperature. Membranes contain dipalmitoylphosphatidylcholine and no cholesterol (○), 0.01 mol% cholesterol (▲) or 0.03 mol% cholesterol (●). Temperature drop from 38°C to 28°C.

value 10 h after the temperature drop. Small amounts of cholesterol dramatically accelerate the rate of approach to the equilibrium. The membrane reached equilibrium after 3 h in the presence of 0.01 mol% cholesterol and less than 10 min at 0.03 mol% cholesterol. The presence of cholesterol less than 1% does not change the phase transition temperature appreciably. The pretransition was reported to disappear in the presence of 6 mol% cholesterol [2]. Notice that cholesterol effects reported here are not due to simple impurity effect which tends to accelerate the kinetics, because these effects were observed by using much higher levels of 'impurities' (0.25 mol%), i.e., 5-SASL. Slow rates of the pretransition were also observed in distearoyl- and dimyristoylphosphatidylcholine membranes (data not shown). In all cases, there exists a fast response component in the curve, which we cannot make quantitative evaluation in our present experimental setting. We concentrate on the slow response component in this report. Akiyama et al. [10] performed similar experiments (without cholesterol) using the small-angle X-ray diffraction technique and concluded that imperfection of the multilamellar structure remains for a long time after the temperature drop.

The reverse temperature jump experiment across the pretransition from 28°C to 38°C was performed using dipalmitoylphosphatidylcholine membranes. The rate is fast and $2T'_{\parallel}$ reaches equilibrium in less than a few minutes.

Temperature drop experiments in L'_{β}-phase from

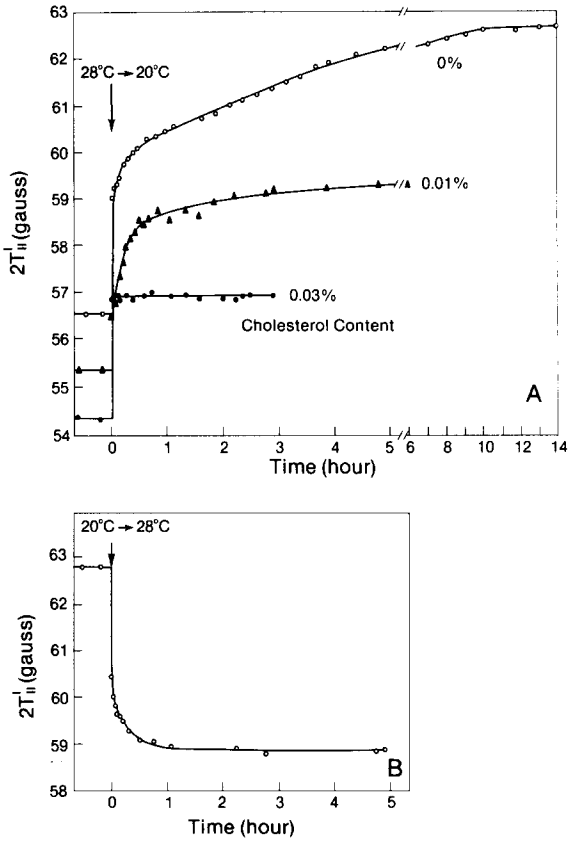

Fig. 2. Time-course of approach to equilibrium $2T'_{\parallel}$ value of 5-SASL after temperature drop from 28°C to 20°C (A) and temperature jump from 20°C to 28°C (B). Membranes contain dipalmitoylphosphatidylcholine and no cholesterol (○), 0.01 mol% cholesterol (▲), or 0.03 mol% cholesterol (●).

28°C to 20°C are shown in Fig. 2A. These experiments were performed after the membrane reached the thermal equilibrium at 28°C. A slow rate was observed again in the absence of cholesterol and was accelerated in the presence of very small amounts of cholesterol. The reverse temperature jump from 20°C to 28°C also shows slow (although faster than temperature drop) kinetics (Fig. 2B). $2T'_{\parallel}$ reaches the equilibrium value 1 h after the temperature jump.

Effects of cholesterol on the equilibrium value of $2T'_{\parallel}$ of 5-SASL in dipalmitoylphosphatidylcholine membranes are shown in Fig. 3. Very small amounts of cholesterol (0.003–1 mol%) cause a large increase in the mobility of 5-SASL below the main transition and especially below the pretransition temperature. $2T'_{\parallel}$ reaches the minimum value at 0.03 to 0.3 mol% cholesterol. $2T'_{\parallel}$ increases as the amount of cholesterol is further increased, reaching the maximum value between 18 to 32 mol% cholesterol (Fig. 3). The amount of cholesterol that gives the maximum $2T'_{\parallel}$ increases as the temperature is raised [9].

It has been proposed that alkyl chain mobility

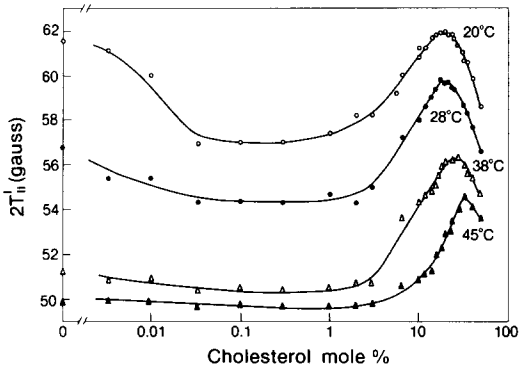

Fig. 3. $2T'_{\parallel}$ values of 5-SASL in dipalmitoylphosphatidylcholine/cholesterol membranes plotted against cholesterol mole fraction at 20°C (○), 28°C (●), 38°C (△), and 45°C (▲). Notice that the abscissa is in the logarithmic scale.

in the gel phase is highly cooperative [11] and that the cooperative units may be related to the phospholipid domains limited by the defect lines observed by electron microscopy [2]. These cooperative units appear to decrease in size in the presence of very small amounts of cholesterol, which have been shown to stabilize the defect lines [2].

Future studies of gel-phase membranes should be carried out with the possible involvement of this slow change of the membrane structure in mind.

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