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EFFECTS OF PRESSURE ON THE PHASE TRANSITION OF BILAYERS IN LIPOSOMES

INFLUENCE OF CHOLESTEROL AND α-TOCOPHEROL

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The effects of presure on the gel-to-liquid crystalline phase transition temperature of dimyristoylphosphatidylcholine (DMPC) bilayers containing cholesterol, α-tocopherol, and α-tocopheryl acetate were studied by fluorescence depolarization. The transition temperature of cholesterol mixtures (> 7.5 mol%) was lower than that of 100% DMPC at atmospheric pressure, but it became higher than the latter on increase in pressure. The thermodynamic parameters of the transition (ΔV, ΔS, ΔH) were estimated and the functions of cholesterol and α-tocopherols in the bilayers are discussed.

1. Introduction

Much attention has been paid to the effects of cholesterol incorporation on the physical properties of artificial membranes because cholesterol is wide-spread in biological membranes [1–3]. The effect of α-tocopherol (vitamin E), a membrane stabilizer and lipid-soluble antioxidant, on the properties of model membranes has also been investigated, since its action is closely related to the function of membranes [4–7].

Hydrostatic pressure markedly affects the physical state of phospholipid membranes. The thermotropic gel-to-liquid crystalline transition of sonicated vesicles has been studied by various methods (light scattering [8,9], ESR [10,11], dilatometry [12,13] and X-ray analysis [14]) under pressure and the transition temperature has been found to increase at about 0.02°C/bar [15].

This paper describes fluorescence depolarization studies on the effects of cholesterol, α-tocopherol and α-tocopheryl acetate on the phase transition of dimyristoylphosphatidylcholine (DMPC) at high pressure. This technique has often been used to examine the structure and fluidity of biological or artificial membranes at atmospheric pressure, but rarely at high pressure [16,17].

2. Materials and methods

DMPC, α-tocopherol and α-tocopheryl acetate were obtained from Sigma Chemical Co. 1,6-Diphenyl-1,3,5-hexatriene (DPH) was from Fluka, AG. These chemicals were used without further purification. Liposomes were prepared as follows. Solutions (2 ml) of 10 mg DMPC and appropriate amounts of cholesterol or α-tocopherol in chloroform were evaporated to dryness. Residual traces of chloroform were removed by evaporation in vacuo (12 h). The dried lipids were dispersed in 4 ml of an aqueous solution of 0.15 M KCl in a bath-type sonifer for a few minutes, and the suspension was subjected to ultrasonication in a probe-type sonifer. Then it was mixed with 20 μl of 0.4 mM DPH in tetrahydrofuran and shaken

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under nitrogen at 50°C for 1 h.

A stainless-steel vessel (17-4PH) with fused quartz windows was used to measure polarized fluorescence at high pressure. Fused quartz is suitable as window material for measuring fluorescence polarization under moderate pressure because it has no polarizing power. Moreover, no polarization due to strain on the fused quartz windows under pressure was observed in the pressure range used (up to 980 bar) with hexane solution of a fluorescent dye. Details of the apparatus have been described elsewhere [18].

The suspension was irradiated at an excitation wavelength of 360 nm. The intensity of emission was measured at constant pressure and increasing temperature, and the fluorescence polarization, $P = (I_{\parallel} - I_{\perp})/(I_{\parallel} + I_{\perp})$, was determined. The temperature of the sample chamber was controlled thermostatically to within ±0.1°C by circulating water around the high-pressure vessel.

3. Results and discussion

Fig. 1 shows the changes of fluorescence polarization with temperature of cholesterol/and α-

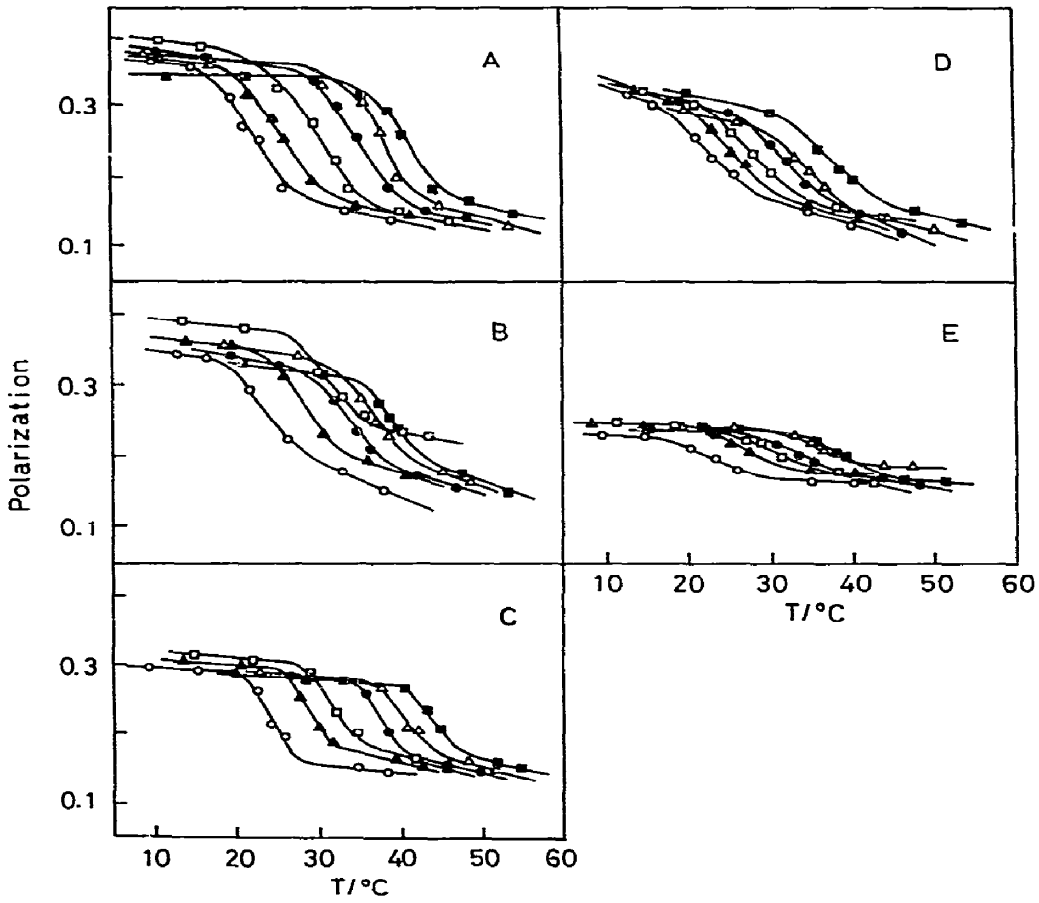

Fig. 1. Temperature and pressure dependence of the polarization of DPH in DMPC liposomes. (○) 1, (▲) 196, (□) 392, (●) 588, (△) 785, (■) 981 bar. (A) 100% DMPC, (B) 5 mol% cholesterol/DMPC, (C) 7.5 mol% cholesterol/DMPC, (D) 5 mol% α-tocopheryl acetate/DMPC, (E) 20 mol% α-tocopherol/DMPC.

tocopherol/DMPC mixtures at various pressures. With all the systems, marked changes of polarization due to a phase transition were observed when the temperature was increased at constant pressure; namely, three states, gel, gel plus liquid crystalline, and liquid crystalline, were clearly distinguished. The phase transition shifted to higher temperature on increasing the pressure. The difference between the polarizations of gel and liquid crystalline states became small when a second component, such as cholesterol or α-tocopherol, was present in the DMPC membranes. In particular, the difference for the 20 mol% α-tocopherol system was so small that distinction between the two states was unclear. With a cholesterol mixture, the difference between the polarizations of gel and liquid crystalline states became smaller at high pressure as at atmospheric pressure on increasing the content of cholesterol. These results show that the dual effect that has been observed for cholesterol systems at 1 bar can also be seen at high pressure [17], and that this effect acts to reduce the effect of drastic changes of the properties of the membranes with pressure. Values for the phase transition temperature (T_m) were determined from the midpoints of the regions for the coexistence of gel and liquid-crystalline states (table 1). The temperature width for this coexistence of gel and liquid-crystalline states was hardly affected by pressure or the presence of additions.

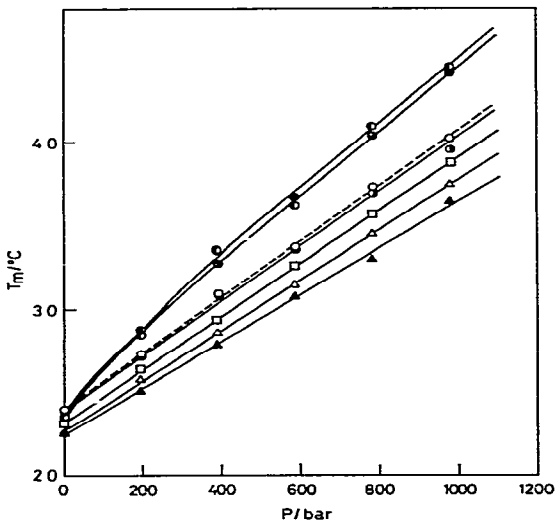

Fig. 2. Pressure dependence of the phase transition temperatures for DMPC liposomes. (○) 100% DMPC, (◐) 10 mol% cholesterol/DMPC, (●) 7.5 mol% cholesterol/DMPC, (◑) 5 mol% cholesterol/DMPC, (□) 20 mol% α-tocopherol/DMPC, (△) 5 mol% α-tocopheryl acetate/DMPC, (▲) 20 mol% α-tocopheryl acetate/DMPC.

Fig. 2 shows plots of the phase transition temperatures determined from the fluorescence polarization profiles. The phase transition temperature at 1 bar was lowered by the addition of cholesterol, α-tocopherol or α-tocopheryl acetate. The transition temperature of the 5 mol% cholesterol system increased linearly with pressure like that of 100% DMPC. However, on applying pressure, the transition temperature of the 7.5 mol% cholesterol system first increased rapidly to become higher than that for 100% DMPC and then increased linearly with pressure at above 400 bar. The cholesterol system (10 mol%) also showed a similar change to that of the 7.5 mol% cholesterol system. Mabrey et al. [1] made a high-sensitivity scanning calorimetric study of cholesterol/DMPC and reported that three kinds of packing complexes are formed in a system containing more than 5 mol% cholesterol. For instance, packing complexes having phase transition temperatures of 22.2, 24.6 and 26°C were observed with a 14.3 mol% cholesterol/DMPC system (cf. 100% DMPC: $T_m = 23.9$°C). They estimated the imperfectness of crystalline structure from the breadth of excess heat capacity profiles and concluded that a complex with a higher T_m value has a more imperfect crystalline structure. Since processes accompanied by a decrease in volume are generally accelerated by increase in pressure, formation of a packing complex with an imperfect crystalline structure and high T_m can be expected to be enhanced by increase in pressure. The increases in the T_m values of the 7.5 and 10 mol% cholesterol/DMPC mixtures in a different manner from those of 100% DMPC and the α-tocopherol mixture may be interpreted in terms of increase in the ratio of packing complexes with high T_m values. These increases may stop at about 400 bar where the plots of T_m vs. pressure become linear (fig. 2). In contrast, the T_m values of α-tocopherol and its derivative systems increase with increase in pressure in parallel with that of

100% DMPC in the order: 20 mol% α-tocopherol, 5 mol% α-tocopheryl acetate, 20 mol% α-tocopheryl acetate. Owing to its polar head groups, the α-tocopheryl acetate molecule occupies a larger space than α-tocopherol, resulting in increase in the surface area of the membranes and decrease in the interaction between the polar head groups of phosphatidylcholines. This may be one reason why the acetate mixture has a low T_m value. Massey et al. [4] studied the interaction of α-tocopherols and DMPC under atmospheric pressure by differential scanning calorimetry and obtained only one broad transition for melting profiles. This result indicates that the α-tocopherol/DMPC mixtures have only one packing structure. α-Tocopherols are more effective than cholesterol in disrupting the bilayer. The effects of these compounds on the thermotropic behavior of DMPC can be explained by supposing that methyl substituents in the phytanoyl chain perturb phosphatidylcholine acyl chain packing. The methyl substituents prevent α-tocopherols adopting a structure similar to that of all-*trans* chains of a phosphatidylcholine in the gel state. Thus, the incorporation of α-tocopherols into a gel phase is thermodynamically unfavorable. This may be why the T_m values of α-tocopherol mixtures are lower than those of cholesterol systems.

Table 1 shows the thermodynamic properties of the endothermic phase transition of DMPC mixtures. The pressure sensitivities of T_m, (dT_m/dP), are a little smaller than those obtained by Ceuterick et al. [8] (0.0205°C/atm). Liu and Kay [12] reported that ΔV and dP/dT_m for dipalmitoylphosphatidylcholine are invariant with pressure up to about 300 bar ($\Delta V = 0.033 \pm 0.003$ ml g^{-1}). ΔV for DMPC is 0.023 ± 0.003 ml g^{-1} [19]. By applying the Clausius-Clapeyron equation for the phase transition of DMPC, the molar volume increase (ΔV) of the transition at 1 bar can be evaluated as shown in table 1 using the ΔH values obtained by Massey et al. [4].

$$\frac{dT_m}{dP} = \frac{\Delta V \cdot T_m}{\Delta H} = \frac{\Delta V}{\Delta S}$$

As ΔV is independent of pressure (at least over the range 1–300 bar) and dT_m/dP is constant over the pressure range 1–980 bar for all except the 7.5 and 10 mol% cholesterol mixtures, ΔS ($= \Delta H/T_m$) in the former mixtures is also invariant with pressure. For the two latter cholesterol mixtures, ΔS varies at pressures between 1 and 400 bar as dT_m/dP changes with pressure. When T_m increases linearly with increasing pressure, ΔH should also show a linear increase with pressure.

Fig. 3 shows plots of T_m vs. pressure for a mixture of DMPC, cholesterol and α-tocopheryl acetate (90 : 5 : 5 mol%). At lower pressures, the T_m changes like that of the 10 mol% cholesterol mixture, although the T_m at 1 bar (22.4°C) is about 1°C lower than that of the 10 mol% cholesterol mixture and similar to that of the 5 mol% α-tocopheryl acetate mixture. At higher pressures, the plot gradually approaches that for the 5 mol%

Table 1

Thermodynamic data on phase transition in DMPC liposomes

Liposomes	T_m (°C) at 1 bar	dT_m/dP (°C/1000 bar)	ΔH [a] (kcal/mol)	ΔV [b] (cm^3/mol)	ΔS [b] (cal/mol per K)
100% DMPC	23.9	16.7	5.8	13.6	19.5
5 mol% cholesterol/DMPC	23.8	16.9	5.1	12.1	17.2
7.5 mol% cholesterol/DMPC	23.8	21.1	4.8	14.1	16.0
10 mol% cholesterol/DMPC	23.5	21.3	4.3	12.8	14.3
5 mol% α-tocopheryl acetate/DMPC	22.5	15.4	4.5	9.8	15.2
20 mol% α-tocopheryl acetate/DMPC	22.4	14.4	1.3	2.5	4.23
20 mol% α-tocopherol/DMPC	23.1	16.1	1.3	2.8	4.22

[a] ΔH values were calculated from the data in ref. 4.
[b] At 1 bar.

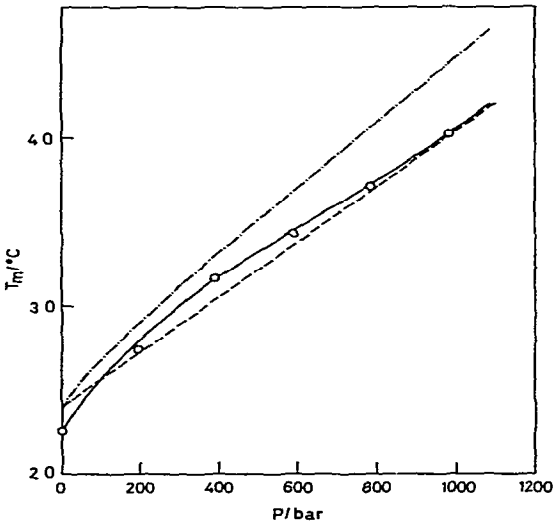

Fig. 3. Pressure dependence of the phase transition temperatures for liposomes (DMPC:cholesterol: α-tocopheryl acetate = 90:5:5 mol%). (— — —) 5 mol% cholesterol/DMPC, (·–·–·) 10 mol% cholesterol/DMPC, (O——O) 90:5:5 mol% DMPC/cholesterol/α-tocopheryl acetate.

cholesterol mixture and at about 1000 bar it coincides with the latter. α-Tocopheryl acetate may act like cholesterol at pressures of up to about 400 bar, i.e., an effect of mass (5 + 5 mol%) may appear, whereas at higher pressures the effects of α-tocopheryl acetate may disappear and only the effect of cholesterol may remain. α-Tocopherol is known to interact specifically with phospholipids in a similar manner to that suggested for cholesterol and to show similar behavior to the latter in membranes [6,20]. At lower pressures, α-tocopherol may participate in formation of packing complexes under the influence of cholesterol. However, as α-tocopherol has less ability than cholesterol to form a complex it may be removed from the complex with increasing pressure.

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