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A statistical mechanical analysis of the effect of long-chain alcohols and high pressure upon the phase transition temperature of lipid bilayer membranes

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Long-chain *n*-alcohols decrease the main phase-transition temperature of lipid vesicle membranes at low concentrations but increase it at high concentrations. The nonlinear phenomenon is unrelated to the interdigitation and is analyzed by assuming that alcohols form solid solutions with solid as well as liquid phases. The biphasic response originates from the balance of the free energy difference of alcohols in the liquid and solid membranes (Δg_A) and the alcohol–lipid interaction free energy difference (Δu) between the two phases. When $\Delta g_A < 0$ and $\Delta u > 0$, or $\Delta g_A < \Delta u < 0$, the transition temperature decreases monotonously according to the increase in the alcohol concentration. When $\Delta g_A > 0$ and $\Delta u < 0$, or $\Delta g_A > \Delta u > 0$, it increases monotonously. Biphasic response occurs with a minimum temperature when $\Delta u > \Delta g_A > 0$, and with a maximum temperature when $\Delta u < \Delta g_A < 0$. When the alcohol carbon-chain length becomes closer to the lipid carbon-chain length, Δu is equalized by Δg_A, and the temperature minimum of the main transition is shifted to extremely low alcohol concentrations. Hence, long-chain alcohols predominantly elevate the main transition temperature and lose their anesthetic potency. High pressure decreased both Δg_A and Δu. Presumably, high pressure improves the packing efficiency of liquid membranes and decreases the difference between the solid and liquid membrane properties.

Introduction

The response of the main transition temperature of lipid vesicle membranes (between $P_{\beta'}$ and L_α phases) to *n*-alcohols becomes biphasic when the alcohol carbon-chain length exceeds 11: temperature depression at low alcohol concentrations and elevation at high concentrations [1–4]. The response of the transition temperature to the alcohol concentrations is concave, showing a minimum temperature. The existence of the temperature minimum in the temperature-composition diagram is similar to the solid-liquid equilibrium of nonideal solid solutions of two-component alloys or azeotrope of the gas-liquid equilibrium of nonideal two-component liquid mixtures.

This temperature elevation by long-chain alcohols at their high concentration range is unrelated to the interdigitation of two leaflets of the lipid monolayer induced by short-chain alcohols [5,6]. The biphasic response is typical for nonideal solution and occurs by the intermolecular interactions among lipids and long-chain alcohols. The coexisting region of the solid-gel and liquid-crystal states in the composition-temperature phase diagram deviates from the monotonous configuration of the ideal solution and becomes two parts.

The biphasic response is not limited to the concave mode with a minimum temperature. Inoue et al. [7,8] reported that long-chain cationic surfactants affected the main phase transition temperature of anionic lipid membranes with convex mode with a maximum temperature.

Previously, we [9] have analyzed the biphasic response solely by the interaction free energies among alcohol molecules incorporated into the liquid and solid domains of lipid membranes. It is obvious, how-

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ever, that the alcohol–lipid interaction forces must be considered for the alcohol effects. We revised the previous model by considering both alcohol–alcohol and alcohol–lipid interaction free energies. Balance between the two determines the mode of the additive effects on the main transition; linear, concave, or convex. In this report, we analyzed the change of the main transition temperature as a function of the concentration of alcohols, the number of carbon atoms in the *n*-alcohol, and the applied pressure.

Theoretical model

We define x_{M} and x_{A} to be the mole fractions of the lipid and alcohol in the vesicle membrane, respectively.

$$x_{\mathrm{M}} + x_{\mathrm{A}} = 1 \tag{1}$$

The free energies of the solid-gel and liquid-crystalline phases of the membrane are expressed by G_{S} and G_{L}, respectively.

$$G_{\mathrm{S}} = g_{\mathrm{MS}} x_{\mathrm{M}} + g_{\mathrm{AS}} x_{\mathrm{A}} - u_{\mathrm{S}} x_{\mathrm{M}} x_{\mathrm{A}} + RT(x_M \log x_M + x_{\mathrm{A}} \log x_{\mathrm{A}}) \tag{2}$$

$$G_{\mathrm{L}} = g_{\mathrm{ML}} x_{\mathrm{M}} + g_{\mathrm{AL}} x_{\mathrm{A}} - u_{\mathrm{L}} x_{\mathrm{M}} x_{\mathrm{A}} + RT(x_M \log x_{\mathrm{M}} + x_{\mathrm{A}} \log x_{\mathrm{A}}) \tag{3}$$

where g_{ij} is the molecular free energy of i-th species (i = M, A) in the j-th phase (j = S, L), and is written

$$g_{ij} = h_{ij} - Ts_{ij} \tag{4}$$

where h_{ij} and s_{ij} are, respectively the molecular enthalpy and entropy of i-th species in the j-th phase, and u_{S} and u_{L} are the interaction free energies between the lipid and alcohol molecules in the solid-gel and liquid-crystalline phases, respectively. The last terms of the right-hand side of Eqns. 2 and 3 are the mixing entropy terms. R and T are the gas constant and the absolute temperature, respectively.

Because the DPPC-water partition coefficients of long-chain alcohols are extremely high ($C_{10} = 2.2 \cdot 10^5$ and $C_{12} = 3.4 \cdot 10^6$ in mole fraction units [10], extrapolated), and their water solubilities are low ($C_{10} = 2.0 \cdot 10^{-4}$ and $C_{12} = 2.3 \cdot 10^{-5}$ molal [11]), the amount of the dissolved alcohols in the aqueous phase is neglected. The phase behavior of the two-component system is schematically shown in Fig. 1. In a phase diagram (Fig. 1c), the minimum temperature is expressed by T_0 where the alcohol mole fraction is x_0. When the temperature is T_1, the free energies of liquid (G_{L}) and solid (G_{S}) are shown in (Fig. 1a). Fig. 1b depicts G_{S} and G_{L} at the minimum temperature.

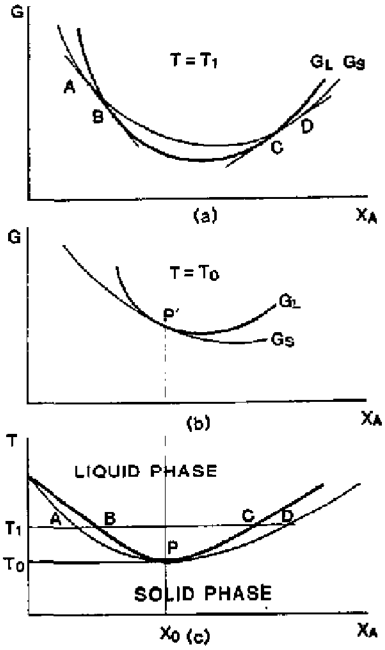

Fig. 1. Schematic representation of free energies G_{S} and G_{L}, and phase diagram. A schematic phase diagram is shown at the bottom (c), where the minimum temperature is expressed by T_0 and a temperature above the minimum by T_1. The abscissa is the mole fraction x_{A} of the long-chain alcohols. The ordinates are: (a) and (b) free energies, and (c) temperature. (a) Free energies G_{S} and G_{L} when the temperature is T_1 in (c). At the boundaries of the two regions of alcohol concentrations (A–B and C–D), G_{S} and G_{L} show common tangents, where the solid-gel and liquid-crystalline phases coexist. When the alcohol concentrations are outside of these regions (x_{A} < A and x_{A} > D) the membrane is in the solid phase. When the alcohol concentrations are inside of these regions (B < x_{A} < C), it is in the liquid phase. These regions are shown in the phase diagram (c). The intermediate regions (A < x_{A} < B and C < x_{A} < D) are the coexistence phases. (b) Free energies G_{S} and G_{L} when the temperature is T_0 in (c). P′ corresponds to P in the phase diagram (c). (c) Phase diagram in the composition-temperature space.

At the minimum point,

$$G_{\mathrm{S}} = G_{\mathrm{L}} \tag{5}$$

The transition temperature, T_{m}, is expressed

$$T_{\mathrm{m}} = \frac{\Delta h_{\mathrm{M}} x_{\mathrm{M}} + \Delta h_{\mathrm{A}} x_{\mathrm{A}} - \Delta u x_{\mathrm{M}} x_{\mathrm{A}}}{\Delta s_{\mathrm{M}} x_{\mathrm{M}} + \Delta s_{\mathrm{A}} x_{\mathrm{A}}} \tag{6}$$

where

$$\Delta h_{\mathrm{M}} = h_{\mathrm{ML}} - h_{\mathrm{MS}} \tag{7}$$

$$\Delta h_{\mathrm{A}} = h_{\mathrm{AL}} - h_{\mathrm{AS}} \tag{8}$$

$$\Delta s_{\mathrm{M}} = s_{\mathrm{ML}} - s_{\mathrm{MS}} \tag{9}$$

$$\Delta s_{\mathrm{A}} = s_{\mathrm{AL}} - s_{\mathrm{AS}} \tag{10}$$

$$\Delta u = u_{\mathrm{L}} - u_{\mathrm{S}} \tag{11}$$

By definition, the transition temperature, T_{m0}, without additives under applied pressure is defined

$$T_{m0} = \frac{\Delta h_M}{\Delta s_M} \tag{12}$$

Before proceeding with numerical calculations, the mathematical structure of the biphasic change will be analyzed. The minimum of the transition temperature, T_0, is shown at the point P in Fig. 1c. This point corresponds to the point P′ in the composite curve of the free energy shown in Fig. 1b.

At point P′, G_L and G_S have a common tangent.

$$\frac{\partial G_L}{\partial x_A} = \frac{\partial G_S}{\partial x_A} \tag{13}$$

From Eqns. 2, 3, and 13, we obtain

$$\Delta h_M - T_m \Delta s_M - \Delta h_A + T_m \Delta s_A + \Delta u(1 - 2x_A) = 0 \tag{14}$$

By eliminating T_m from Eqns. 6 and 14, and rearranging, we obtain the following equation that determines the value of x_0, which is the mole fraction at point P in Fig. 1c.

$$\Delta s x_0^2 - 2\Delta s_M x_0 + \Delta s_M + (1/\Delta u)(\Delta s_A \Delta h_M - \Delta s_M \Delta h_A) = 0 \tag{15}$$

where

$$\Delta s = \Delta s_M - \Delta s_A \tag{16}$$

From Eqn. 15, the value of x_0 is represented as

$$x_0 = \frac{\Delta s_M}{\Delta s}\left\{1 - \left[1 - \frac{\Delta s}{\Delta s_M}\left(1 + \frac{1}{\Delta u \Delta s_M}(\Delta s_A \Delta h_M - \Delta s_M \Delta h_A)\right)\right]^{1/2}\right\} \tag{17}$$

With dodecanol and tridecanol, the observed x_0 values were at very low alcohol concentration range [4], hence, they were small compared to unity. The factor Δu is written as follows.

$$\Delta u = \Delta h_A - T_{m0}\Delta s_A + \delta, \text{ or } \delta = \Delta u - (\Delta h_A - T_{m0}\Delta s_A) \tag{18}$$

By using Eqns. 12 and 18, Eqn. 17 is rewritten as

$$x_0 = \frac{\Delta s_M}{\Delta s}\left\{1 - \left(1 - \frac{\Delta s \delta}{\Delta s_M \Delta u}\right)^{1/2}\right\} \tag{19}$$

and

$$T_0 = T_{m0}\left\{1 + \frac{x_0}{\Delta s_M - x_0 \Delta s}(\Delta u x_0 - \delta)\right\} \tag{20}$$

When the absolute value of δ is small compared to Δu, x_0 is approximately written from Eqn. 19 as

$$x_0 = \delta / 2\Delta u \tag{21}$$

Because the observed value of x_0 is small for dodecanol and tridecanol [4], δ is expected to be also small. This means that Δu is close to $\Delta h_A - T_{m0}\Delta s_A$ in Eqn. 18.

We numerically calculate theoretical parameters Δg_A and Δu as a function of the number of carbon atoms and applied pressure, where

$$\Delta g_A = \Delta h_A - T_{m0}\Delta s_A \tag{22}$$

First, we determine the pressure dependence of the enthalpy, Δh_M, in the following form.

$$\Delta h_M = \Delta h_{M0} + p\Delta v_M \tag{23}$$

where p is the applied pressure and Δv_M is the volume change of the lipid molecule during the transition from the solid-gel to the liquid-crystalline phases in the absence of alcohol. From the experiment [4], we have

$$\Delta v_M = 28 \text{ cm}^3 \text{ mol}^{-1} \tag{24}$$

The value of Δh_{M0} is taken to be 8.7 kcal/mol [12,13]. We assumed that Δg_A and Δu depend on the carbon number, m, of the long-chain alcohols and the applied pressure, p at limited carbon chain lengths as follows.

$$\Delta g_A = \Delta g_0 + m(\Delta g_1 + p\Delta g_2) \tag{25}$$

$$\Delta u = \Delta u_0 + m\Delta u_1 + p(\Delta u_2 + m\Delta u_3) \tag{26}$$

The parameters Δg_i ($i = 0, 1, 2$) and Δu_i ($i = 0, 1, 2, 3$) are determined by minimizing the deviations of the theoretical curve from the sixteen sets of experimental data points [4].

Fig. 2 shows the result of the numerical calculations among the transition temperature, alcohol concentrations and pressure according to the theory. The agreement between the theory and data is excellent with the standard deviation within 0.57 K except for the high pressure data of undecanol and tridecanol. Table I summarizes the estimated parameters of Δg_A and Δu. As expected, the values of δ ($= \Delta u - \Delta g_A$) were small for dodecanol and tridecanol.

The response mode of the transition temperature depends upon the balance between Δg_A and Δu. At low concentrations of additives, Eqn. 6 can be linearized and T_m is approximately expressed [8] as

$$T_m = T_{m0}\left(1 + \frac{\Delta g_A}{\Delta h_M}\frac{x_A}{1 - x_A} - \frac{\Delta u x_A}{\Delta h_M}\right) \tag{27}$$

When $\Delta g_A < 0$ and $\Delta u > 0$, or $\Delta g_A < \Delta u < 0$, the transition temperature decreases monotonously. When $\Delta g_A > 0$ and $\Delta u < 0$, or $\Delta g_A > \Delta u > 0$, it increases monotonously. When $\Delta u > \Delta g_A > 0$, the biphasic response occurs with a minimal temperature. When $\Delta u < \Delta g_A < 0$, the biphasic response is accompanied by a

TABLE I

Estimated theoretical parameters

The values are expressed by kcal/mol change

Pressure (bar)	1-Decanol		1-Undecanol		1-Dodecanol		1-Tridecanol	
	Δg_A	Δu	Δg_A	Δu	Δg_A	Δu	Δg_A	Δu
1	0.115	0.690	0.919	1.306	1.723	1.992	2.527	2.538
100	0.003	0.578	0.795	1.183	1.588	1.788	2.381	2.393
200	−0.110	0.466	0.672	1.060	1.454	1.654	2.235	2.247
300	−0.221	0.355	0.549	0.937	1.319	1.519	2.089	2.102

maximal temperature, which was demonstrated by Inoue et al. [7,8] in the interaction of cationic surfactants with anionic lipid vesicles.

The alcohol concentration that forms minimum temperature is expressed by the difference between Δu and Δg_A (Eqn. 19). When the alcohol carbon-chain length is increased to approach the membrane lipid carbon-chain length, Δu is compensated by Δg_A, hence, the alcohol concentration at the temperature minimum (x_0) shifts to the lower range. For this reason, tridecanol and tetradecanol predominantly elevate the main transition temperature at ambient pressure; they lose their anesthetic potency.

The values of Δg_A and Δu are decreasing functions of pressure, whereas that of Δh_M is an increasing function as shown in Eqns. 22 and 23. High pressure decreases the difference of the lipid–alcohol interaction energy differences (Δu) between the liquid and solid phases. It also decreases the difference between the free energies of alcohols (Δg_A) in the liquid and solid membranes. This means that high pressure improves the packing efficiency of the lipid molecule, more in the liquid phase than in the solid phase, and elevates the main transition temperature. In this context, high pressure may decrease the difference in the properties between the solid and liquid membranes. The decrease in the property difference between the solid and liquid states may be the cause of the enhancement of the biphasic response by high pressure.

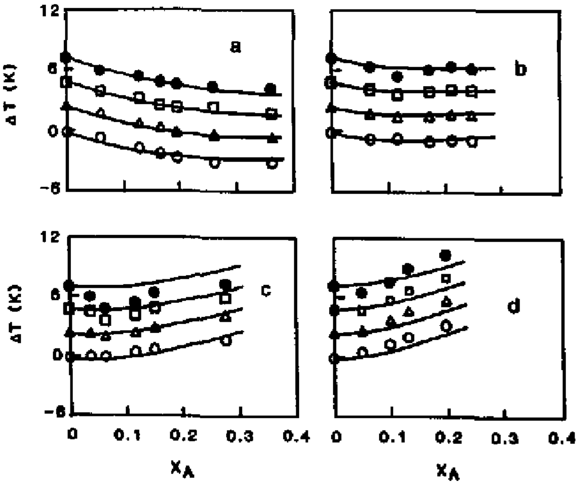

Fig. 2. Data points and theoretical curves of the change in the transition temperature. ΔT signifies the deviation of the transition temperature in reference to the control without anesthetics at ambient pressure. (a) Decanol, (b) undecanol, (c) dodecanol, and (d) tridecanol. Ambient pressure (○), 100 bar (△), 200 bar (□), and 300 bar (●).

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