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Molecular origin of biphasic response of main phase-transition temperature of phospholipid membranes to long-chain alcohols

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A statistical mechanical theory is proposed which explains the molecular mechanism of the nonlinear response of the phase-transition temperature of phospholipid vesicle membranes to added 1-alkanols. By assuming that the free energy of transfer of 1-alkanols from the aqueous phase to the membrane and the interaction energy between 1-alkanol molecules are linear functions of alkanol alkyl chain-length, the nonlinear behavior is explained in the Bragg-Williams approximation. For dipalmitoylphosphatidylcholine vesicle membranes, the theory reveals a larger free energy of transfer of 1-alkanols from the aqueous phase to the solid-gel membrane than to the liquid-crystalline membrane when the number of carbon atoms of 1-alkanol exceeds 12. When the intermolecular interaction force between 1-alkanol molecules residing in the gel phase is stronger than the interaction force between those residing in the liquid-crystalline phase, the ligand effect is to tighten the lipid matrix structure, causing the transition temperature to rise. The interaction force is a quadratic function of 1-alkanol concentration; hence, the response of the transition temperature to the 1-alkanol concentration is nonlinear. At low concentrations of the long-chain 1-alkanols that predominantly elevate the transition temperature, this intermolecular interaction force is negligible. In this case, the entropic effect of the incorporated ligand molecules, which loosens the lipid matrix, predominates, and the transition temperature decreases. The biphasic action of long-chain 1-alkanols originates from the balance of these two opposing effects: entropy and intermolecular interaction.

Introduction

Anesthetics generally decrease the transition temperature of phospholipid membranes between solid-gel and liquid-crystalline phases. In contrast, long-chain 1-alkanols with an alkyl chain-length exceeding about 10 carbon atoms increase the main phase-transition temperature of phospholipid membranes, although shorter 1-alkanols decrease it [1–5]. The well-known cutoff phenomenon of the anesthetic effect of 1-alkanols, where anesthetic potency suddenly disappears, occurs at a similar chain-length of about 10 carbon atoms. It is not surprising that the cutoff was attributed to the switchover from depression to elevation of the transition temperature, according to the elongation of the carbon chain [2]. The agreement between the cutoff of anesthetic potency and the switchover of the effect upon phospholipid phase transition

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suggests that the physical state of phospholipid membranes is intimately related to the state of anesthesia.

The transition-temperature-depressing action of additives has been successfully analyzed by applying the van't Hoff model concerning the freezing point depression or by extending the model. The elevation of transition temperature by long-chain 1-alkanols indicates that their partition into the gel state is larger than into the liquid state. Caille et al. [6] reported a statistical mechanical model for anesthetic partition into the gel and liquid membranes.

However, the action of long-chain 1-alkanols on the main phase-transition temperature of phosphatidylcholine vesicle membranes is not a simple function of the carbon chain-length of 1-alkanols, but a nonlinear function of 1-alkanol concentrations. Kamaya et al. [5] reported that the transition temperature was depressed at low 1-alkanol concentrations and was elevated at high concentrations. When the 1-decanol concentration was increased, the depressant action upon the main phase-transition temperature changed to an elevation. A similar biphasic effect of long-chain alkanols has also been reported upon hydrolysis of phosphatidylcholine multilamellar vesicle membranes by phospholipase A_2 [7].

This complex nonlinear result is difficult to explain by the simple thermodynamic treatment of freezing-point depression. A statistical mechanical model is required for the quantitative analysis. In the present communication, we analyzed the experimental data by the chain-length dependence of the transfer of 1-alkanols from water to both gel and liquid-crystalline membranes and the interaction energy between 1-alkanol molecules in each phase.

Experimental

Synthetic 1,2-dihexadecanoyl-*sn*-glycero-3-phosphorylcholine (dipalmitoylphosphatidylcholine) (Sigma) was checked by thin-layer chromatography and confirmed to show a single spot. The purity of the reagent grade 1-decanol, 1-undecanol, 1-dodecanol, 1-tridecanol and 1-tetradecanol (Fluka, Hauppauge, NY) was checked by a Shimadzu (Columbus, OH) gas chromatograph and single peaks were confirmed. Water was purified by triple distillation, once from potassium permanganate solution. Possible contamination by surface-active impurities was checked by dynamic surface tension measurement as previously described [8].

Unilamellar vesicles were prepared by ultrasonic irradiation of aqueous suspension of phospholipids in the cup-horn of a Branson Sonifier W-185 (Danbury, CT) at temperatures several degrees above the phase transition under the flow of nitrogen. The preparations were incubated at 4°C for 10 days so that the vesicles fused into greater homogeneous size, according to the method described by Wong et al. [9]. Alkanols were weighed with an ultramicrobalance (Perkin-Elmer Autobalance AD-2Z, Norwalk, CT); they were then mixed with the vesicles either by premixing with the phospholipid in chloroform, followed by evaporation of the solvent in a Wheaton (Milville, NJ) rotary microevaporator before sonication, or by ultrasonic dispersion into the vesicle suspension. Both preparations produced essentially the same results.

The main phase transition was monitored by the sudden change in absorbance of the vesicle suspension at 350 nm by a Perkin-Elmer 554 spectrophotometer equipped with a programmable electronic cuvette-temperature controller of a heat-pump type and a microstirrer. The agreement between the results obtained by the optical method and by differential scanning microcalorimetry using a Perkin-Elmer DSC2 with Auto-Zero was excellent, as previously reported [5]. The cuvette temperature was monitored by a calibrated thermistor thermometer (United Systems, Dayton, OH) with 0.01 Cdeg resolution and 0.1 s response time. The thermistor tip was inserted into the cuvette just above the light path. The cuvette temperature was scanned at a rate of 0.5 Cdeg per min by the electronically controlled heat exchanger. The results obtained by heating scan are analyzed in the present report.

Results and Discussion

Fig. 1 shows the dependence of the changes in the main transition temperature of dipalmitoylphosphatidylcholine vesicle membranes on the ad-

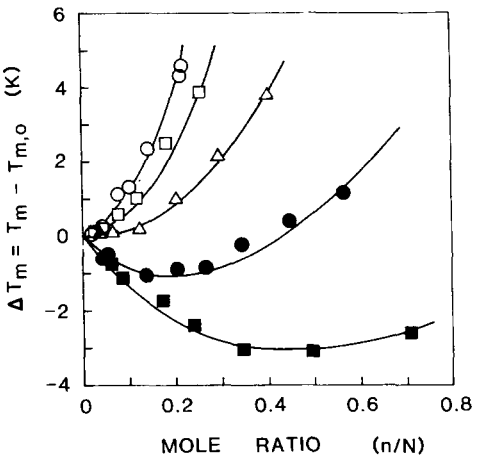

Fig. 1. Dose-dependent effect of 1-alkanols upon the main phase-transition temperature of dipalmitoylphosphatidylcholine vesicle membranes in heating scan. The lines are drawn according to the theory using parameters listed in Table II. The symbols are: ○, 1-tetradecanol; □, 1-tridecanol; △, 1-dodecanol; ●, 1-undecanol, and ■, 1-decanol.

ded 1-alkanol concentrations. The response was nonlinear. It is clear that shorter-chain alkanols, 1-decanol and 1-undecanol, show a biphasic effect upon the transition temperature; depression at low concentrations and elevation at high concentrations displaying minimum temperatures. With 1-tetradecanol, 1-tridecanol and 1-dodecanol, the response of the transition temperature appears to be simple elevation. However, when the data at high alkanol concentrations are extrapolated to zero alkanol concentrations, the line intersects the temperature axis at negative values. This indicates that these long-chain alkanols depress the transition temperature in the dilute concentration range.

In modeling the effect of alkyl chain-length of 1-alkanols upon the main transition temperature of phospholipid vesicle membranes, the low water solubility restricts the application of the van't Hoff model, which was previously used to analyze the effects of water-soluble short-chain alkanols upon the transition temperature [2,4,5,10–13]. Kinoshita et al. [14] reported that the water solubility of 1-decanol was 0.234 mM at room temperature. Adding one carbon atom decreased the solubility to about a quarter.

With short-chain 1-alkanols up to 1-octanol, the alkanol concentrations that decreased the transition temperature of the phosphatidylcholine membrane, say about 5 Cdeg, were lower than their water solubility. On the other hand, long-chain 1-alkanols above 1-decanol did not show a measurable effect upon the transition temperature within their water solubility limit. Concentrations of the long-chain 1-alkanols in great excess of their water solubility were necessary to demonstrate any significant effect upon the transition temperature. Despite the excessive 1-alkanol concentrations, no precipitation or aggregation of 1-alkanols was observed in the vesicle suspension at temperatures both higher and lower than the phase transition. Obviously, all 1-alkanol molecules above the level of water solubility are incorporated into both gel and liquid-crystalline membranes. Under this condition, the difference in the ligand effect on the transition temperature cannot be correlated only to the difference in partition of the ligand between membranes and water. If the experiment could be performed with total 1-alkanol concentrations much lower than the water saturation level, the partition model might be applicable for analysis of the observed effect. However, it is difficult, if not impossible, to detect the change in the transition temperature caused by much low 1-alkanol concentrations.

Because almost all added long-chain 1-alkanol molecules are solubilized in the phospholipid membranes, the water phase is saturated with a small, constant amount of 1-alkanol. Then the change in the transition temperature is expected to depend strongly upon the phospholipid concentrations. Table I shows the effects of 1-decanol and

TABLE I

EFFECT OF THE CONCENTRATION RATIO BETWEEN PHOSPHOLIPID AND 1-ALKANOLS UPON THE MAIN PHASE-TRANSITION TEMPERATURE OF DIPALMITOYLPHOSPHATIDYLCHOLINE VESICLE MEMBRANES

DPPC (mM)	ΔT_m (Cdeg)	
	1-decanol (0.18 mM)	1-tetradecanol (0.14 mM)
0.5	−1.90	6.25
1.0	−2.00	2.00
2.0	−0.73	0.50

1-tetradecanol upon the phase transition of varying concentrations of dipalmitoylphosphatidylcholine vesicle membranes. The change in transition temperature clearly depends upon the phospholipid concentration and is larger with 1-tetradecanol than with 1-decanol.

Accordingly, we propose here a simple statistical mechanical model to explain the biphasic action of long-chain 1-alkanols upon the main phase transition of phospholipid membranes. The bulk water phase is considered to be a solvent bed for phospholipids, and long-chain alkanols are assumed to be adsorbed mainly into the phospholipid membranes. Thus, the system reduces to two components, consisting of lipids and alkanols.

Let N and n be the numbers of phospholipid and 1-alkanol molecules, respectively. The partition functions, Z_g and Z_l, representing gel and liquid-crystalline membranes, respectively, are written:

$$Z_g = Z_{Lg}\binom{N+n}{n}(pf)_{Ag}^{n} \tag{1}$$

$$Z_l = Z_{Ll}\left(1+\frac{n}{N}\right)^{N}(pf)_{Al}^{n}\frac{1}{n!} \tag{2}$$

where:

$$Z_{Lg} = \left[\delta V_{Lg}\lambda_L^{-3}\exp\{-(h_g - Ts_g)/kT\}\right]^{N} \tag{3}$$

$$Z_{Ll} = \left[N\delta V_{Ll}\lambda_L^{-3}\exp\{-(h_l - Ts_l)/kT\}\right]^{N}/N! \tag{4}$$

$$(pf)_{Ag} = \delta V_{Ag}\lambda_A^{-3}\exp\left[-\left(h_{Ag} - Ts_{Ag} - \frac{n}{N+n}\epsilon_g\right)/kT\right] \tag{5}$$

$$(pf)_{Al} = (N+n)\delta V_{Al}\lambda_A^{-3} \times\exp\left[-\left(h_{Al} - Ts_{Al} - \frac{n}{N+n}\epsilon_l\right)/kT\right] \tag{6}$$

Z_{Lg} and Z_{Ll} are, respectively, the partition functions of gel and liquid-crystalline states of the phospholipid membrane without additives, and $(pf)_{Ag}$ and $(pf)_{Al}$ are the molecular partition functions of a 1-alkanol molecule in the gel and liquid-crystalline membranes, respectively. In Eqns. 3–6, δV_{I_j} is the free volume of species I in the j state; h_i and s_i are the enthalpy and entropy of a phospholipid molecule in the i state, respectively; λ_I is the de Broglie wavelength of species I; k is the Boltzmann constant; and T is the absolute temperature. In Eqns. 5 and 6, h_{Ai}, s_{Ai} and ϵ_i are the enthalpy, entropy and interaction energy parameter between 1-alkanol molecules in the i state of the lipid membrane, respectively.

As written in the above partition function equations, s_i and s_{I_j} do not include translational entropies. We treat the translational degrees of freedom separately from other forms, such as rotational and conformation entropies, to evaluate the combined effect in both gel and liquid states. The factor $(1 + n/N)^N$ in Eqn. 2 comes from the fact that the available total free volume of a phospholipid molecule is $(n + N)\delta V_{Ll}$ instead of $N\delta V_{Ll}$.

The free energies, F_g and F_l of the gel and liquid states of the membrane are defined, respectively:

$$F_g = -kT\ln Z_g = F_{Lg} + F_{Ag} \tag{7}$$

$$F_l = -kT\ln Z_l = F_{Ll} + F_{Al} \tag{8}$$

F_{Lg}, F_{Ag}, F_{Ll} and F_{Al} are written according to the above equations as follows:

$$F_{Lg} = N(h_g - Ts_g) - NkT\ln\left(\lambda_L^{-3}\delta V_{Lg}\right) \tag{9}$$

$$F_{Ll} = N(h_l - Ts_l) - NkT\ln\left(e\lambda_L^{-3}\delta V_{Ll}\right) \tag{10}$$

$$F_{Ag} = -kT[(N+n)\ln(N+n) - n\ln n - N\ln N] - nkT\ln\left(\lambda_A^{-3}\delta V_{Ag}\right) + n(h_{Ag} - Ts_{Ag}) - n^2/(N+n)\epsilon_g \tag{11}$$

$$F_{Al} = -NkT\ln(1+n/N) - nkT\ln\left[\lambda_A^{-3}e\delta V_{Al}(1+N/n)\right] + n(h_{Al} - Ts_{Al}) - n^2/(N+n)\epsilon_l \tag{12}$$

F_{Lg} and F_{Ll} are the free energies of the phospholipid membrane in the gel and liquid states, respectively, in the absence of additives, and F_{Ag} and F_{Al} are those of additives in the gel and liquid membranes, respectively. In the absence of alkanols, F_{Ag} and F_{Al} disappear, and the phase transition is:

$$F_{Lg} = F_{Ll} \tag{13}$$

In this case, the transition temperature, $T_{m,0}$, is

expressed by:

$$T_{m,0} = \Delta h / \Delta s \tag{14}$$

$$\Delta h = h_l - h_g \tag{15}$$

$$\Delta s = s_l - s_g + k \ln(e\delta V_{Ll}/\delta V_{Lg}) \tag{16}$$

We shall use the reported values [10] of $T_{m,o}$ (41.4°C), Δh (8.7 kcal · mol^{-1}) and Δs (27.66 cal · deg^{-1} · mol^{-1}) for dipalmitoylphosphatidylcholine membranes for numerical calculation.

In the presence of 1-alkanols, the transition temperature, T_m, is determined by the equation:

$$F_g = F_l \tag{17}$$

The mole ratio, x, between 1-alkanol and phospholipid is

$$x = n/N \tag{18}$$

Eqn. 17 is rewritten in the following form:

$$\begin{aligned} h_g - Ts_g + kT[-\ln(\delta V_{Lg}) - x\ln(\delta V_{Ag}) - (1+x)\ln(N+n) \\ + \ln N + x\ln n] + x(h_{Ag} - Ts_{Ag}) - \epsilon_g x^2/(1+x) \\ = h_l - Ts_l + kT[-\ln(e\delta V_{Ll}) - \ln(1+x) - x\ln(e\delta V_{Al}) \\ - x\ln(1+1/x)] + x(h_{Al} - Ts_{Al}) \\ - \epsilon_l x^2/(1+x) \end{aligned} \tag{19}$$

At the transition temperature, Eqn. 19 becomes:

$$T_m = T_{m,0} \frac{1 + \frac{1}{\Delta h}\left[\Delta h_A x + (\epsilon_g - \epsilon_l)\frac{x^2}{1+x}\right]}{1 + x\Delta s_A/\Delta s} \tag{20}$$

where

$$\Delta h_A = h_{Al} - h_{Ag} \tag{21}$$

and

$$\Delta s_A = s_{Al} - s_{Ag} + k \ln(e\delta V_{Al}/\delta V_{Ag}) \tag{22}$$

are the enthalpy and entropy changes of a 1-alkanol molecule, respectively, when the residing domain changes from the gel to liquid-crystalline state.

Eqn. 20 shows that the value of T_m is a quadratic function of 1-alkanol concentration and should express a minimum temperature when the sign of $\epsilon_g - \epsilon_l$ is positive. When $\epsilon_g - \epsilon_l$ is negative, the T_m decreases monotonously according to the increase in the 1-alkanol concentration. Our result showing minima in the T_m vs. x plot indicates that the interaction energy between 1-alkanol molecules in the gel phase is stronger than in the case of the liquid-crystalline phase.

By assuming that the contribution of the polar head of a 1-alkanol molecule to the enthalpy change is small compared with that of the alkyl chain (which should be correct with long chain 1-alkanols), Δh_A, Δs_A and $\epsilon_g - \epsilon_l$ are expressed by the following forms:

$$\Delta h_A = mh \tag{23}$$

$$\Delta s_A = s_1 + ms_2 \tag{24}$$

$$\epsilon_g - \epsilon_l = m\epsilon \tag{25}$$

where m is the number of carbon atoms in a 1-alkanol molecule.

We estimate four parameters, i.e., h, s_1, s_2 and ϵ, in the above three equations by minimizing the sum, S, of the squares of the difference between T_m in Eqn. 20 and the experimentally obtained transition temperature value, T_m (exptr). The form of S is:

$$S = \Sigma(T_m - T_m(\text{exptr}))^2 \tag{26}$$

S is a function of h, s_1, s_2 and ϵ, and is expressed as:

$$S = S(h, s_1, s_2, \epsilon) \tag{27}$$

Minimization of Eqn. 26 is performed in the following manner: because S is in the quadratic form for h and ϵ, it can be solved by differentiating Eqn. 26 with respect to h and

$$\partial S/\partial h = \partial S/\partial \epsilon = 0 \tag{28}$$

Then, we adjust s_1 and s_2 numerically to minimize S.

The curves shown in Fig. 1 are drawn according to the theory. Agreement between the experimental data and the theory is excellent. The standard

deviation (defined by $\sqrt{S/n}$, where n is the number of data points) of the estimated ΔT was 0.23 Cdeg.

In the case of dipalmitoylphosphatidylcholine vesicle membranes, the following best-fit values were obtained: $h = -0.241$ kcal · mol^{-1} per carbon atom, $s_1 = 6.78$ cal · deg^{-1} · mol^{-1}, $s_2 = -1.30$ cal · deg^{-1} · mol^{-1} per carbon atom. For each 1-alkanol, the obtained numerical values of Eqns. 22–24 and the free energy changes, Δf_A, defined by

$$\Delta f_A = \Delta h_A - T_{m,0}\Delta s_A \tag{29}$$

are listed in Table II.

The theory reveals that 1-alkanols longer than dodecanol have a higher affinity to the gel state than to the liquid-crystalline state, as shown in Table II where the sign of Δf_A changes from negative to positive when m exceeds twelve. When the carbon-chain length of 1-alkanol is much shorter than that of the phospholipid, penetration of 1-alkanol molecules into the gel-phase membrane is energetically unfavorable compared to penetration into the liquid-crystalline membrane, because the structural incompatibility creates void space in the lipid core of the membrane. On the other hand, when the carbon-chain length of the 1-alkanol is closer to that of phospholipid, just-fit incorporation into the membrane matrix presumably occurs.

TABLE II

ESTIMATED VALUES OF THE FOUR PARAMETERS EXPRESSED IN EQNS. 23–26 FOR LONG-CHAIN 1-ALKANOLS WITH 10 TO 14 CARBON ATOMS

The units are kcal·mol^{-1} for Δh_A, Δf_A and $\epsilon_g - \epsilon_l$, and cal·deg^{-1}·mol^{-1} for Δs_A. Symbols are: m, alkanol chain length; h, molecular enthalpy; s, molecular entropy; f, molecular free energy expressed by Eqn. 29; and ϵ, interaction energy parameter. Subscripts A, g and l are alkanols, gel and liquid-crystalline states of the phospholipid membrane, respectively.

m	Δh_A	Δs_A	Δf_A	$\epsilon_g - \epsilon_l$
10	−2.41	−6.22	−0.453	0.848
11	−2.65	−7.52	−0.285	0.933
12	−2.89	−8.82	−0.116	1.018
13	−3.13	−10.12	0.053	1.103
14	−3.37	−11.42	0.222	1.188

Ligand actions on phospholipid membranes may be envisioned as the summation of the entropy effect and intermolecular interaction between ligand molecules. The entropy effect follows the first order of the ligand concentration and always in the direction to loosen the lipid matrix structure, and lowers the transition temperature. The intermolecular interaction is a function of the probability of collision among ligand molecules and follows the second order according to the Bragg-Williams approximation. When the interaction force between ligand molecules in the gel phase is stronger than that in the liquid-crystalline phase, the effect is to tighten the lipid matrix structure and elevates the transition temperature. At very low ligand concentrations, the entropy effect dominates because the possibility of collision among ligand molecules is negligible, and the transition temperature decreases. According to the increase of the ligand concentration, the intermolecular interaction force surpasses the entropy effect, because the interaction force is a quadratic function, whereas the entropy effect is a linear function of the ligand concentration. As a result, the transition temperature starts to rise in the cases where the interaction force between ligand molecules is stronger in the gel phase than in the liquid-crystalline phase.

We shall now discuss briefly the relationship between the present theory and the van't Hoff model, which treats the ligand effect upon the phase-transition temperature as freezing point depression. To extend the present theory to the case where the total 1-alkanol concentration in the system does not exceed its solubility into water, Z_g and Z_l in Eqns. 1 and 2 are extended as:

$$Z_g = Z_{Lg}\binom{N+n_g}{n_g}(pf)_{Ag}^{n_g}(pf)_{Af}^{n_A-n_g}\frac{1}{(n_A-n_g)!} \tag{30}$$

$$Z_l = Z_{Ll}(1+n_l/N)^N(pf)_{Al}^{n_l}\frac{1}{n_l!}(pf)_{Af}^{n_A-n_l}\frac{1}{(n_A-n_l)!} \tag{31}$$

where n_A is the total number of 1-alkanol molecules in the system and $(pf)_{Af}$ is the molecular partition function of a dispersed 1-alkanol monomer in the aqueous phase. The numbers n_g and n_l are the numbers of 1-alkanol molecules solubilized into gel and liquid-crystalline membranes, respec-

tively. These numbers are estimated so as to minimize the total free energies, E_g and F_l.

$$\partial F_g/\partial n_g = \partial F_l/\partial n_l = 0 \quad (32)$$

Thermodynamically, Eqns. 30–32 state that the chemical potential of a 1-alkanol monomer dispersed in the aqueous phase is identical to that of a 1-alkanol molecule incorporated into the membrane. The partition coefficient equation can be obtained from these equations by the procedure reported previously [13]. With sparingly water soluble additives, $n_A - n_g$ and $n_A - n_l$ in Eqns. 30 and 31 are equal to the water solubility and negligibility small compared to the number of 1-alkanols solubilized in the membrane matrix. For this reason, the concept of partition coefficient does not apply to the present case.

Biphasic effects were also reported with short-chain alkanols, i.e., methanol [3], ethanol [3,4] and propanol [3]. The effects of water-soluble short-chain alkanols are probably unrelated to the biphasic action of sparingly soluble long-chain alkanols discussed here. When the ethanol concentration in phospholipid vesicle suspension is greatly increased, the suspension becomes viscous and flocculation is seen. It is possible that a completely different phase may have evolved, perhaps the hexagonal phase postulated by Cullis and De Kruijff [15], or the interdigitated state where phospholipid tails penetrate each other at the center of the bilayer. The biphasic effect of highly water-soluble short-chain 1-alkanols may be caused by the solvent effect upon the phospholipid membrane and may involve completely different mechanisms.

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