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Alcohol effects on rapid kinetics of water transport through lipid membranes and location of the main barrier

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The effect of 1-alkanols (from 1-butanol up to 1-dodecanol) on the water permeability of dimyristoylphosphatidylcholine vesicle membranes was studied by measuring the osmotic swelling rate as functions of 1-alkanol concentrations and temperatures above the gel-to-liquid-crystalline phase transition. For 1-butanol and 1-hexanol, the activation energy for water permeation was invariant with the addition of alkanols, whereas for 1-octanol, 1-decanol and 1-dodecanol, the activation energy decreased depending on the alkanol concentration, and the extent of the decrease was larger for alkanol with a longer hydrocarbon chain. These results suggests that hydrocarbon moiety beyond seven or eight carbon atoms from the head group in phospholipid molecules constitutes the main barrier for water permeation through the dimyristoylphosphatidylcholine vesicle membrane. The relative volume change of the vesicle due to osmotic swelling increased with the addition of 1-alkanols. Presumably, the membrane structural strength is weakened by the presence of 1-alkanols in the membrane. Contrary to the dependence of the swelling rate upon the alkanol carbon-chain length, no significant difference in the effect on the relative volume changes was seen among the 1-alkanols. This result suggests that weakening of the membrane structure is caused by perturbation of the membrane/water interface induced by incorporation of 1-alkanols into the membrane.

Introduction

It is generally agreed that perturbation of physical properties of membranes, caused by association of anesthetic molecules, is directly or indirectly related to the state of anesthesia [1]. Previously, we have studied [2] the effect of anesthetic molecules on the water permeability of dimyristoylphosphatidylcholine vesicle membranes by measuring the osmotic swelling rate, where a remarkable difference in the activation energy for water permeation was found between polar anesthetic molecules (chloroform and 1-hexanol) and apolar counterparts (carbon tetrachloride and *n*-hexane); the activation energy was unaltered by the addition of polar molecules, whereas it was decreased by apolar molecules. Based on this finding, it was suggested that apolar molecules perturb the hydrocarbon core of the membrane, whereas perturbation of the core structure is minimal by polar molecules. This difference is apparently caused by the difference in the binding site of these ligands; the membrane/water interface for polar molecules and lipid core for apolar molecules.

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If this is the case, it is expected that water permeation behavior will depend upon the hydrocarbon chain length of added 1 alkanols. In the present communication, we report the effect of 1-alkanols up to 1-dodecanol on the water permeability of the dimyristoylphosphatidylcholine vesicle membrane obtained by an osmotic swelling technique using a stopped-flow apparatus. The experiment was performed at temperatures above the phase transition to avoid a permeation anomaly at the phase transition, except for the control studies in solid-gel membranes.

Experimental

Materials

Synthetic dimyristoylphosphatidylcholine (DMPC) was obtained from Sigma. 1-Butanol (Eastman), 1-hexanol (Fulka), 1-octanol (Sigma), 1-decanol (Eastman), 1-dodecanol (Eastman) and sodium chloride (Mallinckrodt) were all reagent grade and used without further purification. Water was triply distilled in all-glass stills, once from alkaline potassium permanganate solution.

Method

DMPC vesicle suspension in 0.05 M NaCl solution was prepared by sonication in a cup-horn of a Branson Sonifier Model 185 (Danbury, CT) above the phase-transition temperature and fusion at 4°C for one week [3]. The concentration of DMPC was 0.4 mM. The appropriate amount of alcohols was added to the DMPC suspension by a microsyringe for alcohols up to 1-octanol. For 1-decanol and 1-dodecanol, the alcohols were premixed with DMPC in ethanol, followed by evaporation of the solvent with a rotary evaporator before sonication.

A Durrum Model D-110 stopped-flow spectrophotometer was used for kinetic measurements. For experiments with alcohols up to 1-octanol, DMPC vesicle suspension was mixed with water containing the alcohol at the same concentration as the vesicle suspension, in order to avoid the change caused by the difference in the alcohol concentration between the vesicle suspension and the diluting fluid. For 1-decanol and 1-dodecanol, DMPC vesicle suspension was mixed with water because their water solubility is negligible.

Swelling of the vesicle after mixing was followed by the absorbance change at 450 nm. The output signal was stored in a Nicolet Model 3091 digital oscilloscope with a time resolution of 1 μsec, and recorded on a Hewlett-Packard X-Y recorder. The temperature was varied in the range from 24°C to 42°C, except for 1-dodecanol, for which a higher temperature range was employed because 1-dodecanol elevates the phase-transition temperature [4–8].

Data analysis

It has been established that vesicle volume changes, associated with swelling, are directly proportional to the change in the reciprocal of absorbance at 450 nm [9]. The swelling rate was analyzed in terms of the initial velocity of the relative change in the reciprocal absorbance.

$$v_0 = \frac{(\mathrm{d}(1/A)/\mathrm{d}t)_0}{1/A_0} = -\frac{(\mathrm{d}A/\mathrm{d}t)_0}{A_0} \tag{1}$$

where A is the absorbance, subscript 0 denotes $t = 0$, and v_0 is related to the water permeability coefficient of the vesicle membrane. If the membrane is completely impermeable to the solutes, the rate of volume increase resulting from water influx, $\mathrm{d}V/\mathrm{d}t$, is expressed [10] as

$$\frac{\mathrm{d}V}{\mathrm{d}t} = P_w SRT\Delta C \tag{2}$$

where P_w is the permeability coefficient for water, S is the membrane area, R is the gas constant, T is the absolute temperature, and ΔC is the concentration difference of the impermeable solutes between the inside and outside of the vesicle. Because v_0 is proportional to the initial rate of volume change,

$$v_0 = k(\mathrm{d}V/\mathrm{d}t)_0 = kP_w SRT\Delta C \tag{3}$$

where k is a proportionality constant. It may be assumed that k and S take practically constant values, and are independent of the presence of the small amount of additives at the temperatures above the phase transition. Then V_0/T can be regarded to represent a quantity directly reflecting P_w under the condition of fixed ΔC. The activation energies for water permeation are obtained by plotting $\log(V_0/T)$ against $1/T$.

Results and Discussion

Swelling rates of DMPC with 1-alkanols were measured as functions of 1-alkanol concentration and temperature. In Figs. 1a and 1b, the logarithms of v_0/T are plotted against $1/T$ for DMPC with 1-butanol and 1-dodecanol, respectively. Because partition coefficients are a thermodynamic parameter, the concentration of additives in the lipid phase may vary with temperature. However, it has been established [11] that the temperature-dependence of 1-alkanol partition between lipid and water is small. Hence, the contribution to v_0 from the change in the alkanol concentration in membrane may be negligible compared with that from the temperature change (see also Ref. 2).

The Arrhenius activation energies for water permeation, ΔE_p, were calculated from the slopes of such straight lines presented in Fig. 1 according to the least-squares method. The values of ΔE_p thus obtained are listed in Table I. In Table I, the mole fraction, X_A^l, of alkanols up to 1-octanol in the lipid membrane phase was calculated from the bulk concentration, using the following reported partition coefficients [12]: K(1-butanol) = 62, K(1-hexanol) = 960, and K(1-octanol) = 14 800 (extrapolated value from the data in Ref. 12). For 1-decanol and 1-dodecanol, X_A^l was estimated by assuming that all added alkanol molecules are incorporated into the lipid phase because of the low water solubility.

Fig. 1 and Table I show that ΔE_p for DMPC with 1-butanol and 1-hexanol remain almost constant at the control value regardless of the presence of the alkanols, although v_0/T increases with the alkanol content in the membrane. On the other hand, for longer chain alkanols, ΔE_p decreases concentration-dependently with the addition of the alkanols, and the extent of the decrease is larger for the alkanol with the longer hydrocarbon chain.

Water permeation across the lipid membrane is generally interpreted in terms of the dissolution-diffusion mechanism [12–15], where the membrane core is assumed to be a homogeneous layer in which water dissolves and moves by diffusion. According to this model, the permeability coefficient for water is expressed by the following equation when the main barrier for water permeation is the hydrocarbon core rather than the interfacial

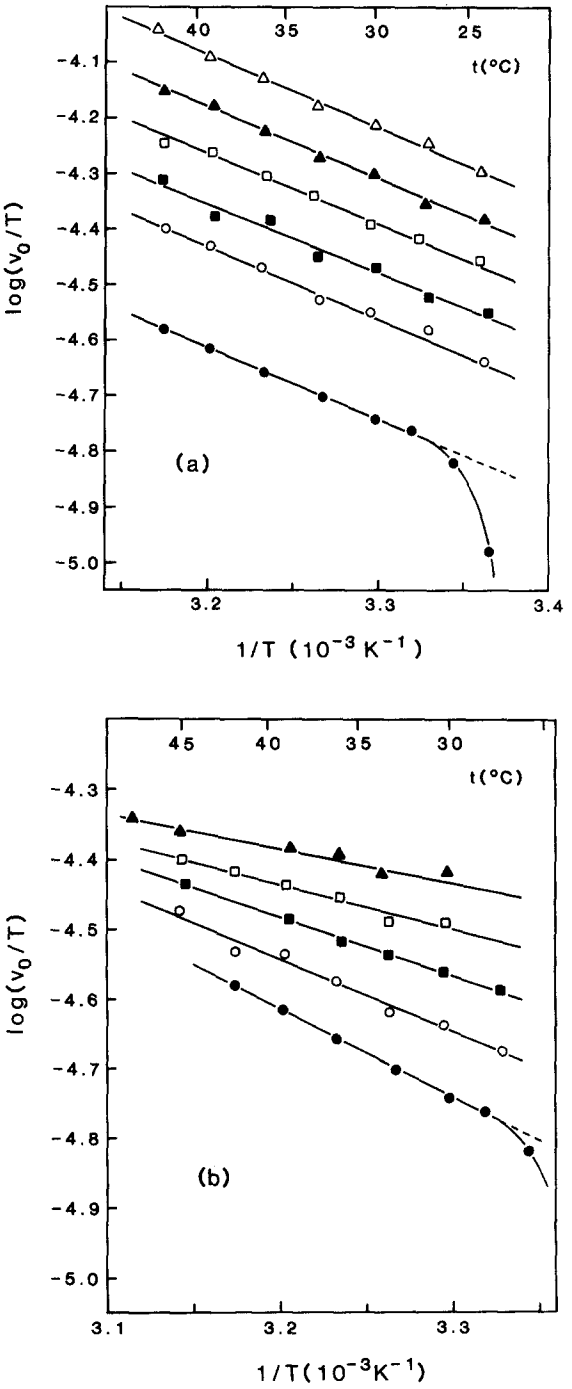

Fig. 1. Plot of $\log(v_0/T)$ versus $1/T$ for DMPC with 1-butanol (a) and 1-dodecanol (b). DMPC concentration is 0.4 mM and v_0 is expressed in s^{-1}. Control: ●, the 1-alkanol concentrations (mM) are: (a) 1-butanol: ○ 45.3, ■ 90.5, □ 141, ▲ 181, and △ 272. (b) 1-Dodecanol: ○ 0.0173, ■ 0.0346, □ 0.0518, and ▲ 0.0690.

TABLE I

ARRHENIUS ACTIVATION ENERGIES (ΔE_p) FOR WATER PERMEATION ACROSS DMPC VESICLE MEMBRANES

1-Alkanol	mM	X_A^l	ΔE_p (mean ± S.E.) (kcal·mol^{-1})
Control			5.89 ± 0.10
1-Butanol	45.3	0.050	5.93 ± 0.20
	90.5	0.101	5.68 ± 0.41
	141	0.156	5.79 ± 0.21
	181	0.202	5.90 ± 0.22
	272	0.303	5.98 ± 0.17
1-Hexanol	2.40	0.041	5.90 ± 0.12
	4.80	0.083	5.97 ± 0.10
	9.60	0.165	6.03 ± 0.34
	13.0	0.225	5.98 ± 0.21
	16.8	0.290	5.93 ± 0.07
1-Octanol	0.184	0.046	5.53 ± 0.18
	0.368	0.095	5.26 ± 0.31
	0.589	0.148	5.03 ± 0.28
	0.920	0.229	4.61 ± 0.20
	1.10	0.282	4.18 ± 0.13
1-Decanol	0.0203	0.048	5.20 ± 0.11
	0.0406	0.091	4.78 ± 0.12
	0.0914	0.183	3.69 ± 0.09
1-Dodecanol	0.0173	0.042	4.83 ± 0.32
	0.0346	0.080	3.91 ± 0.11
	0.518	0.114	2.92 ± 0.27
	0.690	0.145	2.27 ± 0.29

region [15].

$$P_w = KD_m/\Delta x \tag{4}$$

where K is the partition coefficient of water, D_m is the diffusion coefficient of water within the hydrocarbon core of the membrane, and Δx is the core thickness.

According to the absolute reaction rate theory [16], a diffusional flow is treated as a series of successive jumps from one equilibrium position to another, and the diffusion coefficient is given by

$$D_m = (\lambda^2 kT/h)\exp(\Delta S^{\ddagger}/R)\exp(-\Delta H^{\ddagger}/RT) \tag{5}$$

where $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ are the activation enthalpy and the activation entropy for the diffusion process, respectively, λ is the distance between successive equilibrium positions along the path of diffusion, k is the Boltzmann constant and h is the Plank constant. From the thermodynamic relation, K is expressed as

$$K = \exp(\Delta S^0/R)\exp(-\Delta H^0/RT) \tag{6}$$

where ΔH^0 and ΔS^0 are the standard enthalpy change and the standard entropy change, respectively, accompanied by the transfer of water molecules from the bulk water phase into the hydrocarbon core. From Eqs. 4–6, one obtains

$$P_w = (\lambda^2 kT/\Delta x h)\exp\{(\Delta S^{\ddagger} + \Delta S^0)/R\} \cdot \exp\{-(\Delta H^{\ddagger} + \Delta H^0)/RT\} \tag{7}$$

Thus, according to this model, the Arrhenius activation energy obtained from the $\log(v_0/T)$ vs. $1/T$ plot is actually a composite quantity expressed as

$$\Delta E_p = \Delta H^{\ddagger} + \Delta H^0 + RT \tag{8}$$

under the assumption that λ and Δx are invariant with temperature. Furthermore, if λ and Δx are also independent of the presence of a small amount of additives, one can estimate the change in $\Delta S^{\ddagger} + \Delta S^0$ of the pure DMPC, i.e., $\Delta(\Delta S^{\ddagger} + \Delta S^0)$ from the difference in the intercepts of the $\log(v_0/T)$ vs. $1/T$ plot between DMPC with and without additives.

Figs. 2 and 3 show $\Delta H^{\ddagger} + \Delta H^0$ and $\Delta(\Delta S^{\ddagger} + \Delta S^0)$ as a function of the mole fraction of alkanols in the lipid membranes. 1-Butanol and 1-hexanol show essentially the same effect on both $\Delta H^{\ddagger} + \Delta H^0$ and $\Delta(\Delta S^{\ddagger} + \Delta S^0)$: $\Delta H^{\ddagger} + \Delta H^0$ is invariant with the addition of these alkanols, whereas $\Delta(\Delta S^{\ddagger} + \Delta S^0)$ increases with the alkanol content in the membrane. For longer chain alkanols, however, both $\Delta H^{\ddagger} + \Delta H^0$ and $\Delta(\Delta S^{\ddagger} + \Delta S^0)$ decrease almost linearly with the mole fraction of the alkanols, and the extent of decreases becomes larger with a longer hydrocarbon chain length.

In our previous study [2], we compared ligand effects on the swelling rate between polar and apolar molecules by using a chloroform-carbon-tetrachloride pair and a 1-hexanol-*n*-hexane pair. The present results for 1-butanol and 1-hexanol

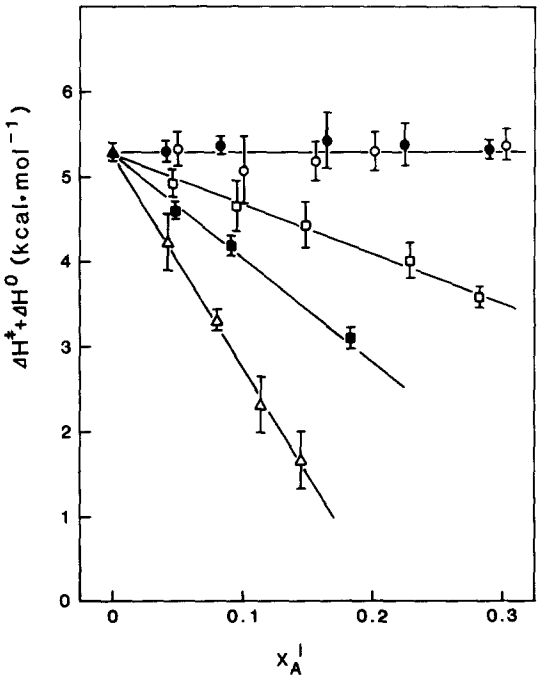

Fig. 2. Variation of $\Delta H^{\ddagger} + \Delta H^{0}$ with mole fraction of 1-alkanols in DMPC vesicle membranes. Symbols are: ▲ control, ○ 1-butanol, ● 1-hexanol, □ 1-octanol, ■ 1-decanol, and △ 1-dedecanol. Vertical bars indicate standard errors estimated from the least-squares analysis.

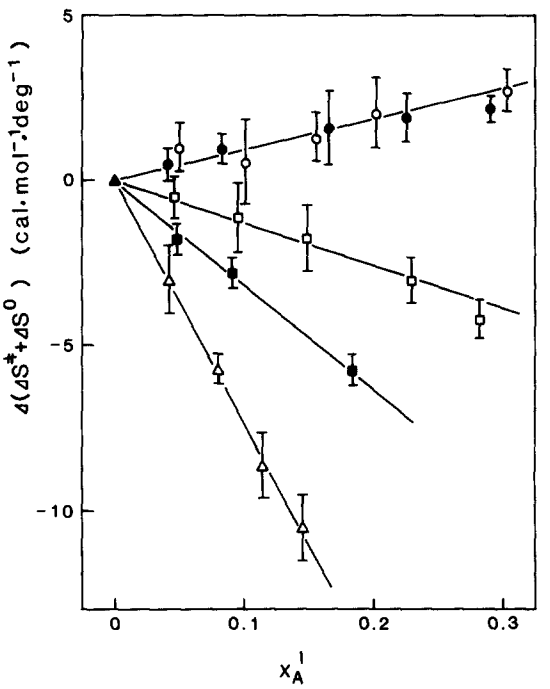

Fig. 3. Variation of $\Delta(\Delta S^{\ddagger} + \Delta S^{0})$ with mole fraction of 1-alkanols in DMPC vesicle membranes. Symbols are the same as in Fig. 2. Vertical bars indicate standard errors estimated from the least-squares analysis.

show the same behavior as those for polar molecules. A constant $\Delta H^{\ddagger} + \Delta H^{0}$ may indicate that neither $\Delta H^{\ddagger}$ nor ΔH^{0} are altered by the presence of these alkanols, because the possibility is remote that each of them changes without changing the sum. This result suggests that these alkanols do not perturb the hydrocarbon core of the membrane appreciably as to affect the microviscosity of the core. This may be reasonable, when one considers the fact that polar molecules accumulate at the membrane/water interface at a low concentration range such as is employed in this study [17–19].

Apolar molecules (carbon tetrachloride and *n*-hexane) decreased $\Delta H^{\ddagger} + \Delta H^{0}$ concentration dependently [2]. In this case, detailed discussion was not attempted, because a separate estimation for $\Delta H^{\ddagger}$ and ΔH^{0} was not available. Nevertheless, it may be reasonable to consider that apolar molecules perturb the hydrocarbon core and lead to the decrease in $\Delta H^{\ddagger}$ and ΔH^{0}, since they reside exclusively in the hydrocarbon core of the membrane [20,21]. 1-Octanol, 1-decanol, and 1-dodecanol show the same behavior as apolar molecules. It may be inferred that alkanols with hydrocarbon chains longer than 1-octanol perturb the hydrocarbon core of the DMPC vesicle membrane, and the extent of the perturbation increases with the length of the hydrocarbon chain.

The present results that the alkanols up to 1-hexanol did not alter $\Delta H^{\ddagger}$ while longer chain alkanols reduced $\Delta H^{\ddagger}$ suggest that, in a DMPC vesicle membrane in a liquid-crystalline state, the region up to the sixth carbon atom from the head group can be considered as the membrane/water interface and the region beyond the seventh carbon atom constitutes the barrier for water permeation. This coincides with the results obtained from electron spin resonance experiments [22] using dipalmitoylphosphatidylcholine vesicles with the phosphatidylcholine spin label, where it was shown that both the order parameter and the polarity,

which probably reflects the water penetration, remained almost constant up to the fifth or sixth carbon position, beyond which they decreased.

In the present experiments, we can obtain information about the 'stability' or 'strength' of the membrane in addition to kinetic information. Because there is a linear relationship between the change in vesicle volume and the change in reciprocal absorbance, the relative volume change of the vesicle, which is a measure of the extent of swelling, can be estimated according to the following equation

$$\frac{\Delta V}{V_0} = \frac{1/A_\infty - 1/A_0}{1/A_0} = \frac{\Delta A}{A_\infty} \tag{9}$$

where $\Delta A = A_0 - A_\infty$ and A_∞ is the absorbance at equilibrium. Because the osmotic swelling experiments were performed at fixed ΔC, $\Delta V/V_0$ may be used as a measure for the strength of the lipid membrane; i.e., it may be inferred that the stronger the association forces between membrane lipids, the smaller the relative volume change of the vesicle.

In Fig. 4, the values of $\Delta A/A_\infty$, averaged over the temperature, are plotted against the mole fraction of alkanols in the lipid phase. $\Delta A/A_\infty$ increases with increasing X_A^l, and this shows that the strength of the vesicle membrane is weakened by the presence of alkanols in the membrane. Contrary to the activation parameters for water permeation, no distinct and systematic difference is seen in $\Delta A/A_\infty$ among the alkanols from 1-butanol to 1-dodecanol, although 1-butanol shows slightly larger $\Delta A/A_\infty$ values than the other alkanols. For comparison, previous results for n-hexane are shown in the figure. From the fact that the membrane weakening effect of alkanols does not depend on hydrocarbon chain length and is much larger than that of *n*-hexane, perturbation of the membrane/water interface induced by the incorporation of alkanols is considered to be mainly responsible for the weakening of the lipid membrane. This indicates the importance of the interfacial property for membrane stability.

It is well known that 1-alkanols with a hydrocarbon chain of less than 10 to 12 carbon atoms depress the phase transition temperature of the phosphatidylcholine vesicle membrane, whereas

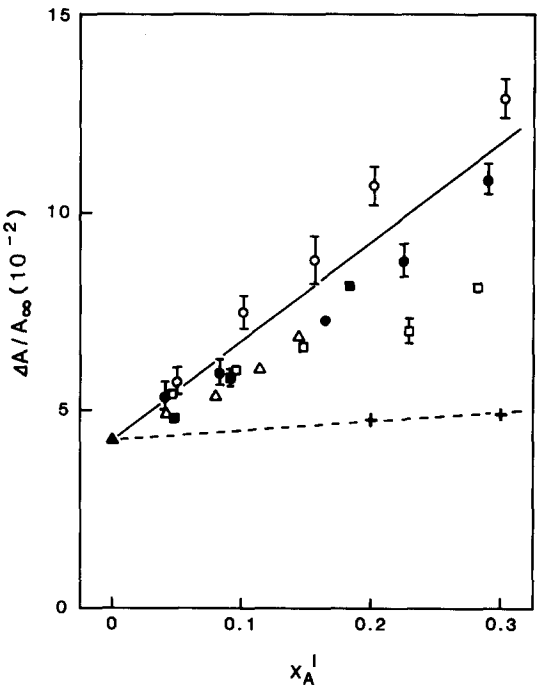

Fig. 4. Variation of $\Delta A/A_\infty$ with mole fraction of 1-alkanols in DMPC vesicle membranes. Symbols are the same as in Fig. 2. For comparison, previous results [2] for *n*-hexane (+) are shown in the figure. Vertical bars indicate standard deviations.

longer chain 1-alkanols elevate it [4–8]. According to our recent study [8], longer chain alkanols depress the transition temperature at low concentrations and elevate it at high concentrations. The alkanol concentration that exhibits a minimum phase-transition temperature depends upon the chain length of both added 1-alkanol and membrane phospholipid. In the present study, 1-dodecanol was added to DMPC at concentrations which induce elevation in the phase-transition temperature of DMPC. Nevertheless, the crossover effect was not observed for both activation parameters for water permeation across the membrane or relative volume change due to osmotic swelling. Thermodynamically, the crossover from depression to elevation of the phase-transition temperature is interpreted in terms of the partitioning of 1-alkanols between the gel and liquid-crystalline phases [7,8]; ligands that partition preferentially into the liquid-crystalline phase depress the transition temperature, whereas those that partition

preferentially into the gel phase elevate the transition temperature. The crossover effect on the transition temperature may be attributed to the strong interaction between the longer chain alkanols and the lipid in the gel state. In the present case, the membrane is in the liquid crystalline state, and hence discontinuous change was not observed with the increasing chain length of 1-alkanol.

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