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Lateral conductance parallel to membrane surfaces: effects of anesthetics and electrolytes at pre-transition

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The effects of dilute salts and anesthetics were studied on the impedance dispersion in the dipalmitoylphosphatidylcholine (DPPC) liposomes. Below the pre-transition temperature, the apparent activation energy for conductance in DPPC-H_2O without salts was equivalent to pure water, 18.2 kJ mol^{-1}. This suggests that the mobile ions (H_3O^+ and OH^-) interact negligibly with the lipid surface below the pre-transition temperature. At pre-transition temperature, the apparent activation energy of the conductance decreased by the increase in the DPPC concentrations. The effects of various salts (LiCl, NaCl, KCl, KBr, and KI) on the apparent activation energy of the conductance were studied. Changes in anions, but not in cations, affected the activation energy. The order of the effect was $Cl^- < Br^- < I^-$. Cations appear to be highly immobilized by hydrogen bonding to the phosphate moiety of DPPC. The smaller the ionic radius, the more ions are fixed on the surface at the expense of the free-moving species. The apparent activation energy of the transfer of ions at the vesicle surface was estimated from the temperature-dependence of the dielectric constant, and was 61.0 kJ mol^{-1} in the absence of electrolytes. In the presence of electrolytes, the order of the activation energy was $F^- > Cl^- > Br^- > I^-$. When the ionic radius is smaller, these anions interact with the hydration layer at the vesicle surface and the ionic transfer may become sluggish. In the absence of electrolytes, the apparent activation energy of the dielectric constant decreased by the increase in halothane concentrations. In the presence of electrolytes, however, the addition of halothane increased the apparent activation energy. We propose that the adsorption of halothane on the vesicle surface produces two effects: (1) destruction of the hydration shell, and (2) increase in the binding of electrolytes to the vesicle surface. In the absence of electrolytes, the first effect predominates and the apparent activation energy is decreased. In the presence of electrolytes, the latter effect predominates and the apparent activation energy is increased.

Introduction

The interaction of anesthetics with lipid membranes has attracted a number of researches to elucidate the anesthesia mechanisms [1–3]. The increase in the fluidity of lipid membranes has been advocated for the mechanisms of anesthesia. The fluidizing theories, however, were questioned [3–5] because the increased fluidity by clinical concentrations of anesthetics can be achieved by raising the temperature only 0.2 C° [4]. It was suggested that the depression of the temperature of the main phase transition by anesthetics may have closer relation to the anesthesia mechanisms [4]. Although the anesthetic effects on the transition temperature are amplified, the temperature depression by clinical concentrations of anesthetics is about 2 C°. Because raising the body temperature in this magnitude does not induce anesthesia, it is difficult to implicate these phenomenon directly for anesthesia mechanisms [5]. The membrane fluidity is a membrane core property, expressed by the order parameter of the hydrocarbon tails of lipid membranes. The main phase transition from solid to fluid states is also a core property, expressed by the *trans-gauche* isomerism of the hydrocarbon chain.

In contrast, the pre-transition between $L_{\beta'}$ and $P_{\beta'}$ phases is related to the thermal motion of the hydrophilic headgroup, and is an interfacial property. The pre-transition is a low enthalpy event, and is highly susceptible to anesthetics with the temperature depression in the range of 6°C at the clinical concentration of anesthetics [6–11]. In our knowledge, the change in the

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surface property associated with the pre-transition has not been reported. This study is intended to analyze the change in the interfacial properties associated with the pre-transition and the anesthetic effects upon them.

We have shown that the lateral conductance parallel to the membrane surface, estimated by impedance dispersion, showed peaks at the pre- and main transition temperatures in DPPC vesicles [12,13]. Halothane inhibited the ionic flow at the DPPC membrane surface and decreased the lateral conductance, predominantly at the pre-transition temperature [13]. The present study measured the effects of electrolytes on the lateral conductance of the anesthetic-DPPC system. The major effects of electrolytes were observed with anions in the order of the Hofmeister lyotropic series. Cations showed little effect.

Method

Synthetic DPPC (1,2-dihexadecanoyl-*sn*-glycero-3-phosphocholine) was obtained from Sigma and was triply recrystallized from ethanol/water. After recrystallization, DPPC was dried in vacuo and mixed with water in a tightly capped glass vial. The preparation of the single-shelled liposome by sonication was described previously [13,14]. Halothane (Takeda Pharmaceutical, Osaka, Japan) was treated with activated aluminum oxide columns for several times to remove the water and the stabilizer (0.01% thymol). Halothane was added to the DPPC suspension in a glass vial with a Teflon-lined cap by a microsyringe. The added amount was then verified by weighing the vial. The conductance and capacitance was measured by an AC bridge (TR-IC, Ando Denki, Tokyo) at 10 kHz in a custom-built platinum concentric cell. The cell volume was 1.2 cm^3. The cell was immersed in a water bath with a temperature stability of ± 0.05 C°. All experiments were repeated at least four times, and expressed by the mean and standard error (Tables I and II).

The electrical capacitance of DPPC dispersion was estimated at the frequency range between 60 Hz and 1.0 MHz. One dispersion was observed between 60 Hz and 30 kHz, and another between 30 kHz and 300 kHz. The dispersion at the lowest frequency range is caused by the electrode polarization. Between 1.0 kHz and 30 kHz, both conductance and capacitance were constant without frequency dependence. The dispersion in this frequency range represents relaxation of counterions close to the vesicle surface [15]. For this reason, the conductance and capacitance were measured at 10 kHz to estimate the movements of counterions near the vesicle surface avoiding the polarization near the electrodes. The resolutions were 0.03 μS for the conductance and 1.0 pF for the capacitance.

Results and Discussion

Temperature and conductance

Electric conductance, σ, is written as

$$\sigma = e \cdot n \cdot \mu \tag{1}$$

where n is the concentration of the carrier of charge (ion), μ is its mobility, and e is the elementary charge. According to the activation law, μ is assumed to have the following temperature characteristics:

$$\mu = \mu_0 \exp(-A_\mu / kT) \tag{2}$$

where k is the Boltzmann constant, T the absolute temperature, A_μ the activation energy of the ion mobility. In pure water, n can be assumed to be constant. Hence, from Eqns. 1 and 2, A_μ is estimated from the plot between $1/T$ and $\ln\sigma$. The value of A_μ for pure water, estimated from the plot (a) in Fig. 1, was 18.2 kJ mol^{-1}. This value is similar to the activation energy of deuterated water estimated from the spin-lattice relaxation times of ^{2}H-NMR, and is equal to the energy of breaking a one mole hydrogen bond [16].

The temperature dependence of the conductance at various DPPC/water ratios is shown as plots b–f in Fig. 1. For clarity, each curve in the figure is displaced

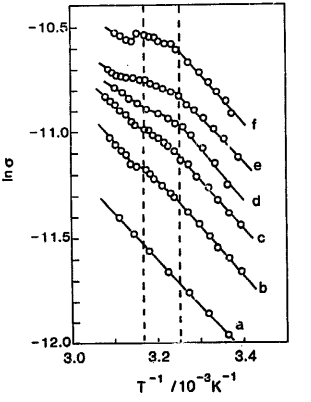

Fig. 1. Effects of phospholipid concentrations on the temperature-dependence of the electric conductance. a, Distilled water. b–f, DPPC/H_2O (w/w): b, 1:99 (original vertical value); c, 2:98 (+0.1); d, 6:94 (+0.4); e, 10:90 (+0.6), and f, 15:85 (+1.3). The lines are shifted vertically to avoid overlaps for clarity; the numbers in parenthesis represent the shift of the set of points along the vertical axis. The broken lines signify the pre- and main transition.

on the vertical axis to avoid overlaps. Breaks are observed at the pre- and main transition temperatures. The phase transition of DPPC vesicles changes the conductance. The conductance at the temperature below the pre-transition showed an identical slope to that of pure water (plot a). The activation energy, therefore, was identical to the pure water. This means that the mobility of the current-carrying ions in the vesicle suspension was identical to pure water. The current-carrying ions that determined the conductance interacted little with the lipid membrane at temperatures below the pre-transition. Hence, the increase in the DPPC concentration did not affect the slope in this temperature range.

When the temperature reached the pre-transition, the DPPC/water ratio strongly influenced the conductance. The activation energy for the apparent mobility of the current-carrying ion, A_{app}, estimated by Eqns. 1 and 2, decreased consistent with the increase in the DPPC concentration (Fig. 2).

At temperatures below the pre-transition, the hydrophilic head of DPPC is oriented parallel to the membrane surface. The effective charge density is small due to the intermolecular interactions. When the temperature is raised above the pre-transition, the thermal agitation on the mobility of the hydrophilic head disturbs the parallel orientation [17–19]. The resulting random orientation presumably increases the effective surface charges, and increases the coulombic force between the surface and the counterions. Because the mobility of the bound counterions is restricted, the temperature dependence of mobility must be less than the free-moving ions in the bulk. The weighted average

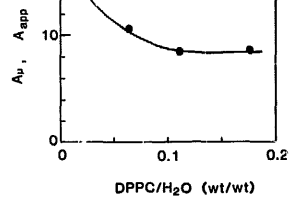

Fig. 2. Dependence of the activation energy of electric conductance on the lipid concentrations. Open circles: the activation energy estimated from the temperature dependence of the electric conductance at temperatures below the pre-transition, signifying the mobility of free-moving ions, A_μ. Closed circles: the activation energy at temperatures between the pre- and main transition, A_{app}.

TABLE I

The apparent activation energy, A_{app}, estimated from the temperature dependence of electric conductance at temperatures between pre- and main transition: effects of electrolytes

DPPC/H_2O ratio was 10:90 (w/w). The values were average of $n = 4$, with standard error.

Electrolytes (0.5 mM)	A_{app} (kJ mol^{-1})
LiCl	8.6 ± 0.5
NaCl	8.6 ± 0.4
KCl	8.7 ± 0.6
KCl	8.7 ± 0.6
KBr	10.4 ± 0.5
KI	11.0 ± 0.5

mobility of the free and bound ions determines the temperature dependence of the conductance. The increase in the vesicle concentration increases the number of bound ions, thereby decreasing the temperature dependence of the conductance. The decrease in the DPPC concentration may be the origin of the decrease in the A_{app}.

The effects of electrolytes (LiCl, NaCl, KCl, KBr, and KI 0.5 mM) on the A_{app} are listed in Table I. The difference among cations was negligible, but the difference among anions was evident. Apparently, cations do not function as an effective current-carrier because they are immobilized by the strong hydrogen-bonding to the phosphate moiety of DPPC [20,21]. The order of the potency in increasing the A_{app} was $Cl^- < Br^- < I^-$, and it follows the order of their ionic radius. The temperature dependence of the apparent ionic mobility decreased consist with this order. It may be concluded that the effective current-carrying ions interacting with the DPPC surface charges are anions, and the smaller the ionic radius, the stronger they are fixed at the surface. The salt effect on A_{app} supports the model that the vesicle surface interacts with mobile ions in the temperature range between pre- and main transition.

The main binding site of the current-carrying anions is the cationic choline head. This result is in contrast to the surface hydration by hydrogen bonding, where the main hydrogen bonding site of interfacial water is the anionic phosphate moiety [20–22].

Temperature and dielectric constant

When the electric field is applied to the system, the distribution of the surface-adsorbed ions is rearranged and induces dipole moments [23]. The induced dipoles increase the dielectric constant, $\Delta\epsilon$, as shown in the next equation:

$$\Delta\epsilon = (9/4)P/(1 + P/2)^2(e^2 \cdot r \cdot q/\epsilon_1 kT) \quad (3)$$

where P is the volume fraction occupied by the vesicle, e is the elemental charge, r the radius of the vesicle, q the counterion surface density (ions per unit area), ϵ_r the absolute dielectric constant of free space, k the Boltzmann constant, and T the absolute temperature.

For the counterions to move at the vesicle membrane surface, it is necessary to break the ion-pair interactions between the counterion and the hydrophobic moiety of DPPC. For the first approximation, the temperature dependence of the number of counterions, n, that can move along the membrane surface is written according to the activation law,

$$n = n_0 \exp(-A_s/kT) \tag{4}$$

where A_s is the activation energy. The capacitance of the vesicle suspension at a particular temperature was invariable at frequencies below 30 kHz. It indicates that the counterions move fully complying with the change in the direction of the electric field and that the polarization induced at the surface is saturated. In other words, the movement of the counterions at the vesicle surface is not the rate-limiting step. The extent of the induced polarization is dependent on the number of the movable counterions.

Under such condition, the q term in Eqn. 3 shows the temperature dependence approximately similar to Eqn. 4. The apparent activation energy, A_s, necessary for the movement of the counterion by breaking the ion-pair interaction with the vesicle surface can be estimated from the slope between $\ln(\Delta\epsilon \cdot T)$ and $1/T$.

The value at DPPC/H_2O (1:9, w/w) without electrolyte was 61.0 kJ mol^{-1}, which was more than thrice the apparent activation energy (A_{app}) for the mobility of free ions. The mobility of the adsorbed ions is strongly restricted and they form a relatively hard shell around the membrane surface [24].

The effects of electrolytes on the A_s are listed in Table II. Similar to A_{app}, the apparent activation energy depended on the anion species. The order of A_s was $F^- > Cl^- > Br^- > I^-$. This follows the reciprocal of the ionic radius. The hydration energies of these anions at 25°C are 406, 272, 239, and 197 kJ mol^{-1}, respectively, for F^-, Cl^-, Br^-, and I^- [25]. The value increases with the decrease in the ionic radius. Because the hydration energy is the force attracting water molecules, the smaller the ionic radius, the stronger these ions interact with the surface hydration shell. Hence, the mobility of small anions bound to the membrane surface is more restricted than that of larger anions.

Effect of halothane

The effects of halothane on the conductance of the liposome (DPPC/H_2O (1:9, w/w) are shown in Fig. 3. The two breaking points in the conductance *versus* temperature plot correspond to the pre-and main transition. Halothane depressed the temperature of the pre-transition break more than the main transition break. The high temperature break that corresponds to the main transition was affected only when the halothane concentrations were higher (Fig. 4).

Halothane also affected the dielectric constant of the DPPC vesicle suspension. Below the pre-transition temperature, the dielectric constant did not show any temperature dependence. The mobile ions did not

TABLE II

The apparent activation energy of the adsorbed ions, A_s, estimated from the temperature dependence of dielectric constant at temperatures between pre- and main transition: effects of electrolytes

DPPC/H_2O ratio was 10:90 (w/w). The values were average of $n = 4$, with standard error.

Electrolytes (0.5 mM)	A_s (kJ mol^{-1})
LiCl	43±2
NaCl	40±1
KCl	42±3
KF	50±4
KCl	42±3
KBr	26±2
KI	16±2

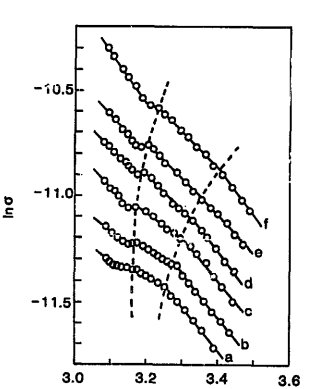

Fig. 3. Effect of halothane on the temperature-dependence of electric conductance. Halothane/lipid mole ratio [halothane]/[DPPC]. a, 0 (original vertical value); b, 0.014 (+0.1); c, 0.029 (+0.4); d, 0.059 (+0.4); e, 0.083 (+0.7), and f, 0.140 (+0.7). DPPC/H_2O 1:9 (w/w). The lines are vertically shifted to avoid overlaps, and the numbers in parenthesis represent the shift of the set of points along the vertical axis. The broken lines signify the pre- and main transition.

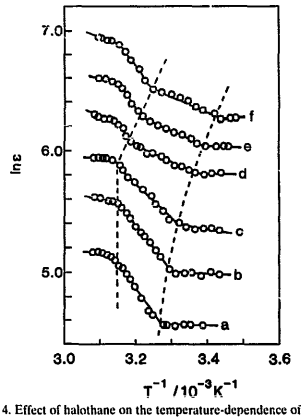

Fig. 4. Effect of halothane on the temperature-dependence of dielectric constant. Lines a–f correspond to Fig. 3 for the [halothane]/[DPPC] mole ratios. The lines are shifted vertically to avoid overlaps, and the numbers in parenthesis represent the shift: a (original vertical value), b (+0.2), c (+0.8), d (+1.2), e (+1.4), and f (+1.6). The broken lines signify pre- and main transition.

appreciably interact with the vesicle surfaces. The dielectric constant at the temperature range between the pre-and main transition was temperature-dependent and influenced by halothane. The effect of halothane on the apparent activation energy, A_s, was estimated by Eqns. 3 and 4, and is shown in Fig. 5.

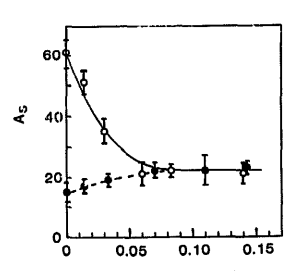

Fig. 5. Effect of halothane on the activation energy of the lateral movement of counterions bound to the vesicle surfaces. Open circles: without electrolytes. Closed circles: with 10 mM KCl. DPPC/H_2O 1:9 (w/w).

The halothane effect on the A_s for surface-bound current-carriers differed in the presence and absence of a small amount of electrolytes. In the absence of KCl, the A_s gradually decreased consistent with the increase in halothane concentration. Probably, this is because halothane breaks the hydrogen bonds among water molecules in the hydration shell, and increases the mobility of OH^-.

In the presence of KCl, the A_s was slightly increased by halothane. During the study on the counterion (Na^+) binding to anionic surfactant micelles, we have shown [26], by ^{23}Na-NMR, that the half-height width of the Na signal increased about 15% by the clinical concentration of halothane. This increase in the half-height width was interpreted as: the mobility of Na^+ decreased due to the increase in the coulombic force induced by the decrease in the surface dielectric constant through the adsorption of halothane molecules. We hypothesize that the cause of the increase in the A_s by halothane in the presence of KCl is the stronger binding of the counterions to the surface charges. This was induced by the decrease in the local dielectric constant, hence, the coulombic interaction force increased. The adsorption of halothane on the vesicle surface produces two actions: (1) destruction of the hydration shell, and (2) increase in the binding of electrolytes to the vesicle surface. When the salt is omitted – the mobile ions are H_3O^+ and OH^- – the effect on the hydration shell predominates and the A_s decreases.

The preferential effect of anesthetics on the pre-transition suggests that the primary action site of anesthetics is the surface of the membrane. The amphipathic property of anesthetics is exemplified by the QSAR (Quantitative Structure-Activity Relationship) study of Hansch and co-workers [27], showing that the anesthetic potency correlates best with its solubility in the weakly polar solvent, octanol. The correlation deteriorates when apolar solvents, such as hydrocarbons, are used for the organic phase. These data suggest that the anesthetic action site is not similar to that of hydrocarbons. Accordingly, modern inhalation anesthetics are designed to weakly polar conformations [28]. It is known that apolar molecules are less potent for anesthesia than their dipolar counterparts [28].

By ^{1}H- and ^{19}F-NMR spectrometry, it was shown [29,30] that the hydrophilic end of anesthetic molecules penetrated into surfactant micelles, but did not lose contact with the aqueous phase. Using the inhalation anesthetic methoxyflurane, the interfacial localization of anesthetics was visualized by the two-dimensional nuclear Overhauser effect ^{1}H-NMR, where the protons of the hydrophobic methoxy end showed a cross-peak with the protons of the hydrophilic choline head of DPPC vesicle membranes [31]. The hydrophobic end of the anesthetic interacted with the surface of the

lipid membrane, and the hydrophilic end remained in the aqueous phase. Anesthetics interact with the hydrophilic interface of the lipid membrane and destruct the hydration shell [3]. The relaxation of the structure of the hydration shell increases the motion of the hydrophilic moiety and decreases the pre-transition temperature. The present study revealed the effect of the anesthetic localization at the interface on the membrane surface property, and the relation between the pre-transition and the lateral ion conductance.

Acknowledgments

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