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Changes in surface capacitance and conductance parallel to phospholipid membranes associated with phase transition: effects of halothane

Tadayoshi Yoshida [1], Keijiro Taga [1], Hirofumi Okabayashi [1], Hiroshi Kamaya [2] and Issaku Ueda [2]

[1] *Department of Applied Chemistry, Nagoya Institute of Technology, Showa-Ku, Nagoya (Japan) and* [2] *Anesthesia Service, Veterans Administration Medical Center, Salt Lake City, UT (U.S.A.)*

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The effects of phase transition on the surface capacitance and conductance parallel to dipalmitoyl- (DPPC) and dimyristoyl-phosphatidylcholine (DMPC) membranes were studied by impedance dispersion. The phospholipid aggregates were embedded into pores of a polycarbonate filter and the impedance dispersions were measured at a frequency range from 30 Hz to 1.0 MHz. When the frequency was below 120 kHz, the capacitance showed a peak at the pretransition temperature and a steep rise at the main-transition temperature. In this system, the observed capacitance consists of frequency-dependent and -independent parts. The frequency-dependent part is a surface phenomenon and arises from the lateral motion of counterions at the membrane/water interface. The frequency-independent part represents mainly the properties of the bulk lipid phase. Addition of halothane decreased the total capacitance of the DPPC aggregates at the low frequency range to 1/2 to 1/8 of the control depending upon the temperature. The surface component was solely responsible for this capacitance decrease, because the non-surface component was slightly increased instead. The data suggest that halothane inhibited the lateral ionic flow parallel to the interface.

Because phase transitions of lipid membranes are the phenomena expressed by the conformation and packing of the hydrocarbon tails, surface properties of the membrane at the phase transition attracted little attention in net zero charged zwitterionic membranes. Using highly concentrated dipalmitoylphosphatidylcholine (DPPC) liposomes, Cametti et al. [1] reported that the dielectric constant of the dispersion, which is closely related to the surface conductance, increased at the pretransition temperature. The present report describes the changes in the capacitance and conductance parallel to the phospholipid membrane surface associated with the phase-transition of the membrane.

A 10 μm thick polycarbonate filter with 0.1 μm diameter pores (Nomura Micro-Science, Tokyo) was used to support synthetic DPPC and DMPC (dimyristoylphosphatidylcholine) aggregates. The polycarbonate filter was epoxy-glued to a glass plate, 5 mm thick with a hole of 4.5 mm diameter. The hole was filled with 30 μl sonicated DPPC/H_2O/hexane mixture (10 mg : 5 μl : 4 ml). Hexane was evaporated by placing the plate at horizontal position for 2 h at room temperature. The dried glass plate was sandwiched between two glass cylinders about 50 cm^3 in volume with silicone gaskets. The cylinders were filled with 10 mM NaCl aqueous solution. A pair of platinized-platinum wire coils (diameter = 0.3 mm and 1.0 m long) were inserted into each cylinder. The whole system was immersed into a water bath, heated to 45°C and then annealed at room temperature for 24 h before use. The impedance dispersion was measured by the four-electrode system as previously described [2].

Dependence of the capacitance to the temperature is shown in Fig. 1. When the frequency was lower than 120 Hz, capacitance showed a peak at about 31°C and a large increase above about 40°C. These changes coincide with the pretransition and the main-transition temperatures of DPPC membranes. Similar changes were observed (Fig. 2) with DMPC aggregates: a peak at about 13°C (pretransition) and a steep rise above about 23°C (main transition).

The obtained capacitance and conductance are composed of frequency-dependent and -independent parts. Schwarz [3] and Schwan et al [4] showed that the

Correspondence: I. Ueda, Anesthesia Service 112A, Veterans Administration Medical Center, Salt Lake City, UT 84148, U.S.A.

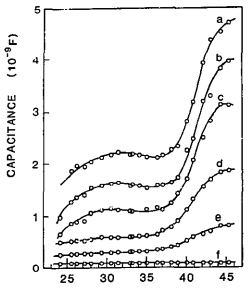

Fig. 1. The frequency dependence of the temperature effect on the electrical capacitance of the polycarbonate filter packed with dipalmitoylphosphatidylcholine aggregates. The frequencies are: a, 30; b, 60; c, 120; d, 300 Hz; e, 1.0; and f, 100 kHz. When the measuring frequency was below 120 Hz, a peak near trhe pretransition temperature and a large increase in the capacitance near the main transition temperature are observed.

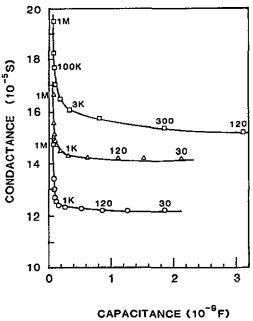

Fig. 3. Conductance-capacitance plot of the DPPC aggregates. The conductance and capacitance of the membrane system were measured at 25.5 (○), 35.0 (△) and 45.3°C (□). The measuring frequencies were 30, 60, 120, 300, 1K, 3K, 10k, 30K, 100K, 300K, and 1 MHz.

frequency-dependent part occurs by the lateral motion of the free ions at the water/membrane interface.

The frequency dependence of the relationship between the capacitance (C) and the conductance (G) at a constant temperature is shown in Fig. 3 for DPPC aggregates. The C–G curves were rectilinear with a break at about 3 kHz. Below 3 kHz, the C–G curve was almost parallel to the capacitance axis. At the low frequency range, the surface capacitance was composed of mainly frequency-dependent component, and the surface conductance was composed of mainly frequency-independent component.

The relationship between the capacitance and conductance of the membrane system when 5 mM halothane was added to the DPPC system is shown in Fig. 4. In the low frequency range, halothane showed a large effect on the capacitance, but little effect on the con-

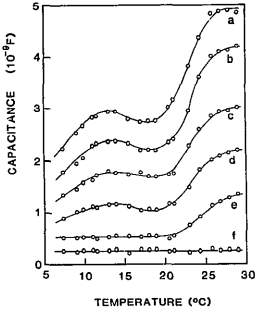

Fig. 2. The frequency dependence of the temperature effect on the electrical capacitance of the polycarbonate filter packed with dimyristoylphosphatidylcholine aggregates. The frequencies are the same to Fig. 1.

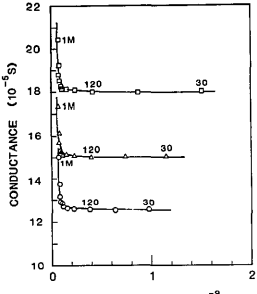

Fig. 4. Conductance-capacitance plot of the DPPC aggregates in the presence of halothane 5 mM. The conditions are the same as in the legend to Fig. 3.

ductance. The capacitance decreased to about 1/2 to 1/8 of the control value depending upon the temperature.

By expressing the capacitance and conductance with C and G, respectively, the total capacitance of the system is written

$$C = \frac{G_m^2 C_w + G_w^2 C_m + \omega^2 C_m C_w (C_m + C_w)}{(G_m + G_w)^2 + \omega^2 (C_m + C_w)^2} \quad (1)$$

where subscripts m and w signify the polymer membrane and the aqueous phase (10 mM NaCl), respectively. Here, $C_m = C_P + C_S + C_L$, and $G_m = G_P + G_S + G_L$ and $\omega = 2\pi f$ (f is the measuring frequency), where subscript P is the polymer film proper, excluding the pore, and subscripts S and L are the frequency-dependent and frequency-independent parts of the conductance and capacitance of the DPPC structures in the pore, respectively.

At low frequency region (small ω values), the ω-term becomes negligible in Eqn. 1, and the total capacitance is approximated as

$$C = \frac{G_m^2 C_w + G_w^2 C_m}{(G_m + G_w)^2} \quad (2)$$

The polymer film excluding pore is impermeable to ions, hence $G_P \ll G_w$ and also $C_P \ll C_L$. The value for C_w is in the range between 1 to 10 pF, and is much smaller than C_L. Then, Eqn. 2 becomes

$$C = \frac{G_w^2 (C_L + C_S)}{(G_S + G_w)^2} \quad (3)$$

The surface capacitance and conductance of a nitrocellulose membrane packed with glycerol α-monooleate were proportional to $f^{-1/2}$ and $f^{1/2}$, respectively [5]. Accordingly, we assume $C_S = a \cdot f^{-1/2}$ and $G_S = b \cdot f^{1/2}$. Then G_w becomes larger than G_S at low frequency ranges, and Eqn. 3 is rewritten as

$$C = C_L + C_S = C_L + a \cdot f^{-1/2} \quad (4)$$

Eqn. 4 predicts that the capacitance of the total membrane system is a function of $f^{-1/2}$ at low frequency range. The $f^{-1/2}$–C curve, constructed from Fig. 3 data, is shown in Fig. 5. This figure shows that the capacitance increased as a linear function of $f^{-1/2}$ when $f^{-1/2} > 0.02$ ($f < 3$ kHz), confirming the prediction of Eqn. 4. When the temperature was elevated, the slope of the line in Fig. 5 increased, and a large increase in capacitance was observed when the temperature exceeded the main phase transition.

The capacitance below the break point in Fig. 5 represents the frequency-independent component, C_L. When $f^{-1/2} = 0.02$, C_L was about 150 pF at 25.5°C and 35.0°C. The pore area in the membrane was 2.1% when estimated by scanning electron microscopy. The total membrane area was 15.9 mm^2, then the total pore area becomes 0.33 mm^2. The frequency-independent capacitance (mainly represents the lipid mass) translates into 0.045 μF/cm^2.

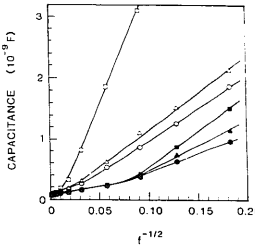

Fig. 5. The frequency dependence of the DPPC capacitance at 25.5 (○), 35.0 (△) and 45.3°C (□) in the absence (open symbols) and presence (closed symbols) of 5.0 mM halothane. The capacitance was plotted against $f^{-1/2}$ where f is the measuring frequency. The capacitance started to increase when $f^{-1/2}$ value exceeded 0.02 (below 3 kHz) in the control and 0.08 (below 160 Hz) in the presence of halothane. A large increase in the capacitance was demonstrated in the low frequency range (frequency-dependent capacitance) at 45.3°C, and halothane strongly inhibited the increase. Halothane showed little effect upon the frequency-independent region.

The filled symbols in Fig. 5 represent the halothane effect on the capacitance of the DPPC system. Halothane decreased the capacitance and the slope of the $f^{-1/2}$–C plot in the low frequency range, indicating a large decrease in the surface capacitance. At 45.3°C (above the phase transition) the capacitance became less than 1/8 of the control. This decrease was frequency-dependent and attributable to the decrease in the surface component. On the other hand, the frequency-independent non-surface component increased from 150 pF on the control to 230 pF (0.077 μF/cm^2) in the presence of halothane. The break point between the frequency-dependent and independent parts shifted from 3 kHz of the control to 160 Hz.

We reported [6] from the chemical shift of ^{19}F-NMR spectra that halothane predominantly adsorb onto the micellar surface. The interfacial binding of volatile anesthetics was further demonstrated by Yokono et al. [7] by two-dimensional nuclear Overhauser effect NMR, where proton cross-peak was observed only between the methoxyflurane hydrophobic part and the DPPC hydrophilic head group. The surface adsorption of halothane (dielectric constant 4.66 at 293.15 K [8]) would increase the counterion binding due to a decrease in the local dielectric constant. This halothane effect on counterion

binding was demonstrated [9] in an anionic micellar solution with ^{23}Na-NMR where halothane increased the half-height linewidth of the Na^+ signal, indicating stronger binding. The relative immobility of counterions may be the cause of the present large decrease in capacitance at low frequency range and the decrease in the slope of the $f^{-1/2}$–C plot.

Volatile anesthetics were shown to increase the capacitance across solvent-free planar lipid bilayers [8,10,11]. In contrast, Haydon et al. [12] demonstrated a decrease in capacitance (increase in membrane thickness) by anesthetics including hydrocarbons. They proposed the capacitance decrease as a mechanism of sodium channel block from the anesthetic effect on the voltage-clamped current in squid giant axons. The present study showed a decrease in the capacitance parallel to the membrane surface. The halothane concentration that suppressed inward current 50% was reported to be 2 mM at 6°C [12]. Considering that the anesthetic potency increases with lowering temperature, the present 5 mM halothane at temperatures above 25°C is about equivalent to 2 mM at 6°C.

If one accepts a model that ion channels are essentially an aqueous duct, composed of channel protein subunits or lipid/protein interfaces, the aqueous channel is covered by surface charges, and ionic currents through the channel would be modified by the capacitance parallel to the surface. The difference in the anesthetic action on the capacitance between perpendicular and parallel to the lipid membrane may be significant when analyzing the anesthetic effects on electrokinetic phenomena of biological membranes.

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