Ultra-thin Layer of Lipids in Polymer Film and Its Electric Properties. New Sorption Method to Prepare a Model Biomembrane System

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The ultra-thin layer of glycerol α-*cis*-9-octadecenate (glycerol α-monooleate:GMO) could be formed by impregnating nitrocellulose (NC) film with GMO and by then filling microscopically the interstices of the network structure of NC with microdrops of GMO. The dielectric dispersion characteristic of this film was found to be similar to that observed for bimolecular films. In a concentrated KCl solution (100 mM), however, an anomalous dielectric dispersion phenomenon was observed below 10 kHz. The low-frequency dispersion was attributed to surface conductance along the planes of contact between the GMO microdrops and the NC network. The ultra-thin film of di(*cis*-9-octadecenyl)phosphate (dioleyl phosphate: DOPH) could also be formed by this method. When a constant DC voltage (50 mV) was applied across the film in a 20 mM KCl solution, the electric current through the whole system changed periodically.

In a previous paper, we have reported that a stable bilayer was formed from a mixture of 1-tetradecanol and tetradecanoic acid.[1] The mixed bilayer, however, was frequently broken at higher temperatures or pH's. This could be explained by the transformation of the precursor monolayer of tetradecanoic acid from the condensed to the expanded state. Therefore, the mixed bilayer cannot be used as a model system in which a transformation of the molecular conformation in the membrane takes place.

A recent study has reported that a membrane composed of a filter paper and lipid may be used as a model system accompanying a conformational change.[2] The thickness of the model membrane, however, is *ca.* 10^4 times that of living membranes.

In the present paper, we wish to introduce a new model system that satisfies the following requirements: (1) the aggregative change (or conformational change) of the molecules constituting the model system does not result in a rupture of the film itself, and (2) the thickness and electric resistance of the model system are comparable to those of living membranes. The present model system should be useful in elucidating the molecular mechanism of excitable living membranes.

Experimental

Materials. The glycerol α-monooleate (GMO, purity> 99%) used in this study was purchased from the Gasukuro Co., Ltd. The nitrocellulose (NC; nitrogen content, 11.07%; degree of polymerization, 300) was kindly given by the Daicel Co., Ltd., while the ethylcellulose (EC;ethoxyl content, 48%; 9.5 centipoises in a 5% toluene–ethanol solution (80 : 20) at 25 °C) was obtained from the Tokyo Kasei Co., Ltd. All the solvents used in this study were reagent-grade.

Preparation of the Ultra-thin Layer of GMO. The porous NC-film (thickness, 1000—2000 Å) was prepared by the following processes prior to the formation of the ultra-thin layer of GMO: (1) A clean glass slide wad dipped in an ethylacetate solution (10 ml) containing both NC (60 mg) and EC (60 mg). (2) When the slide was taken out from the ethylacetate solution, a thin liquid layer of the solution covered the slide. (3) The slide is allowed to stand at room temperature to remove the ethylacetate. (4) Next the slide was transferred into benzene to wash out the EC from the NC–EC mixed film. (5) After an hour, when the slide was taken out from the benzene, the porous NC-film was left on the glass slide. (6) Dipping one end of the slide in water, the porous NC-film peeled off spontaneously from the glass slide within 5 minutes and floated on the water surface.

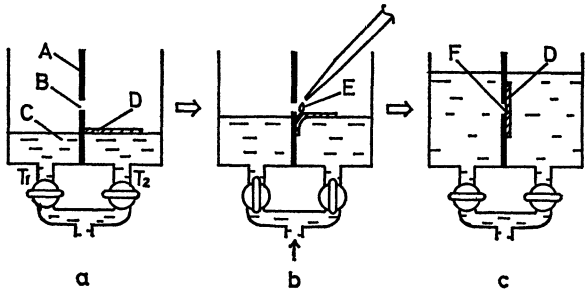

Fig. 1. Method of preparation of ultra-thin layer.
A: Teflon plate, B: Pore (0.5—1.0 mm in diameter), C: Aqueous solution, D: Porous NC-fllm, E: A drop of GMO/*n*-decane solution, F: Ultra-thin GMO-layer, T_1 and T_2: Tube.

The ultra-thin layer of GMO could be formed in the NC-film by the following procedures. (1) As is shown in Fig. 1a, two compartments of the same volume were separated by a Teflon plate (1 mm thick) with a small hole (0.5—1.0 mm in diameter) at the center. An aqueous KCl solution of the same volume was poured simultaneously into both compartments until the water surfaces were raised *ca.* 5 mm below the hole of the Teflon plate. The above-mentioned porous NC-film then floated on the water surface on the right-hand side. (2) An equal volume of the aqueous KCl solution was gradually added to both compartments through T_1 and T_2 with the two levels kept equal. In this procedure, the porous NC-film was gradually transferred to the surface of the Teflon plate as the water levels rose (see Fig. 1b). (3) When the water levels had been raised to *ca.* 1 mm below the hole of the Teflon plate, an aliquot (*ca.* 0.01 ml) of a GMO/*n*-decane solution was dropped on to the porous NC-film. The water levels were then gradually raised further until they reached *ca.* 5 mm above the hole (see Fig. 1c). In this procedure, the microdrops of GMO filled the pores of the NC-film and formed ultra-thin layers.

Measurement of Electric Properties. The electric capacitance of the whole system was measured by means of an AC bridge (Ando Electric Co., Ltd. Model TR-1C); the whole system consists of the ultra-thin GMO layer, the porous NC-film, and the adjacent aqueous solutions (see the first part of the Results and Discussion section). Measurments were carried out in the frequency range from 30 Hz to 3 MHz.

The constant potential (DC 50 mV) was applied between silver-silver chloride or platinized platinum electrodes in each compartment. The potential across the film was measured by means of an electrometer of a high-input impedance (Takeda Riken Industry Co., Ltd. Model TR-8651). The area of the film was estimated photographically. The temperature of the compartments was controlled to 20± 0.5 °C.

Results and Discussion

Our membrane system, prepared by impregnating GMO into the porous NC film, consisted of three phases; the ultra-thin GMO layer, the porous NC film, and the adjacent aqueous KCl solutions; the phases were connected in series with each other. However, the electric capacitance and resistance of the porous NC film (without GMO) in a 100 mM KCl solution were practically equal to those of an aqueous solution. Consequently. in view of the equivalent circuit, our system could be approximated by two phases: the ultra-thin GMO layer and the adjacent aqueous solution. On the other hand, a bilayer and its adjacent aqueous solution could also be approximated by two phases.[13] It is expected, therefore, that the characteristic of the dielectric dispersion of our new system is similar to that observed for the bilayer. The characteristic of the dielectric dispersion of our system in 10 mM KCl was nearly semicircular (Fig. 2). However, a marked deviation from the semicircular relation was observed below 300 Hz. The deviation became greater as the KCl solution was diluted. No such deviation was observed for bilayers.

The deviation in the low-frequency region may be attributed to the following difference in conductance between the bilayer and the thin GMO layer. In accordance with the suggestion of Hanai *et al.*,[3] the

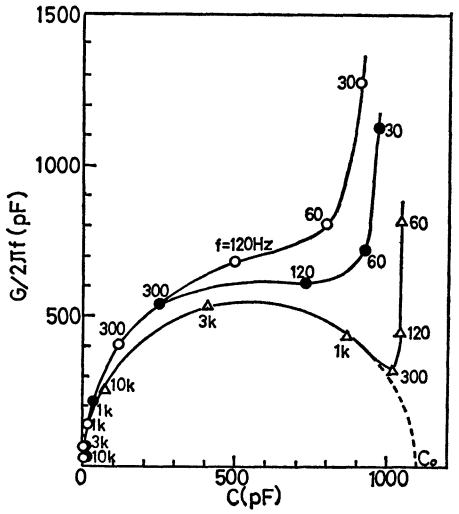

Fig. 2. Complex capacitance diagram of a whole system (aqueous solution+ultra-thin layer+porous NC-film) in three concentrations of KCl.
G: Conductance of a whole system, C: Capacitance of a whole system, f: Frequency Hz (the figures indicated with plots).
○: 0.05 mM, ●: 0.1 mM, △: 10 mM.

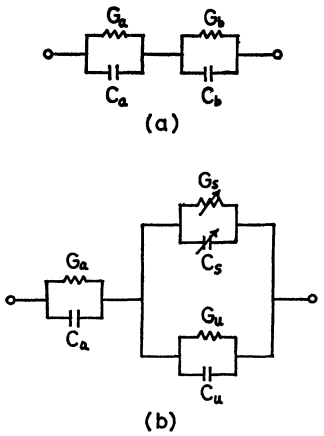

Fig. 3. Equivalent circuit.
(a): Bilayer system, (b): Our whole system.
Subscript a: Aqueous solution, b: Bilayer, s: Plane of contact between GMO microdrop and NC network, u: Ultra-thin layer.

bilayer and its adjacent aqueous solution can each be represented by a parallel combination of capacitance and conductance, connected in series with each other (Fig. 3a, C_b and G_b for the bilayer: C_a and G_a for the aqueous solution). In this electric circuit, the total conductance, G, and the capacitance, C, of the bilayer system are expressed by:

$$\frac{G}{2\pi f} = \frac{G_0}{2\pi f} + \frac{2\pi f\tau(C_0 - C_\infty)}{1+(2\pi f)^2\tau^2} \quad (1)$$

$$C = C_\infty + \frac{C_0 - C_\infty}{1+(2\pi f)^2\tau^2} \quad (2)$$

where f is the frequency, τ is $(C_a+C_b)/(G_a+G_b)$, G_0 is $G_aG_b/(G_a+G_b)$, C_0 is $(G_a^2C_b+G_b^2C_a)/(G_a+G_b)^2$, and C_∞ is $C_aC_b/(C_a+C_b)$. For the bilayer system, since G_b (*ca.* 10^{-8} ℧) $\ll G_a$ (*ca.* 10^{-3} ℧) and C_b (*ca.* 10^3 pF) $\gg C_a$ (*ca.* 1 pF), the first term on the right hand side of Eq. (1) is negligibly small compared to the second term. Since the conductance of the thin GMO layer G_u (10^{-4}—10^{-5} ℧) $< G_a$ (*ca.* 10^{-3} ℧), the first term is not negligibly small in the low-frequency region. The first term is responsible for the deviation from the semicircular relation at low frequencies.

The combination of Eqs. (1) and (2) leads to Eq. (3):

$$G - G_0 = -\frac{1}{\tau}(C - C_0) \quad (3)$$

When G is plotted against C, therefore, a straight line should result. In fact, as is shown in Fig. 4, G and C for our system satisfied Eq. (3), which had been derived from the model of the two phases. Therefore, the capacitance of the thin GMO layer should be equal to the extrapolated value, C_0, at the low-frequency end of the semicircular curve (see Fig. 2). Assuming the thin GMO layer to be a parallel-plate condenser whose dielectric constant is 2, we find, from the above extrapolated value of C_0 (1100 pF, or 0.14 μF/cm²), that the thickness is *ca.* 150 Å, approximately 4 times that of the 1-tetradecanol bilayer.[1]

The G–C curve for our system in a 100 mM KCl solution departs from a straight line (Fig. 4). In

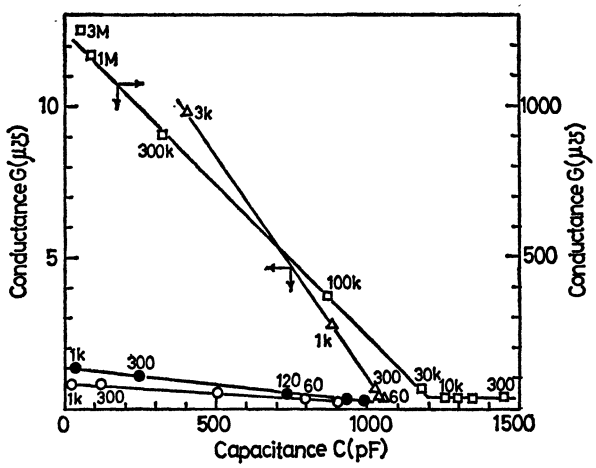

Fig. 4. Electric characteristic of a whole system.
The corresponding frequencies (Hz) are indicated beside some points.
Concentration of KCl solution, ○: 0.05 mM, ●: 0.1 mM, △: 10 mM, □: 100 mM.

the deviated region, below 10 kHz, the capacitance of the whole system increases with a decrease in the frequency, but the conductance is constant (30 μ℧, or 3.8×10^{-3} ℧/cm^2). This phenomenon at low frequencies was not observed for our system in 0.05 mM, 0.1 mM, or 10 mM KCl solutions. Since the area of the electrodes is *ca.* 100 times larger than that of the ultra-thin GMO layer, the diffusion impedance at the electrode surface is negligible. Therefore, it seemed that the above phenomenon is attributable to the property of the ultra-thin GMO-layer system itself.

When the following procedure was repeated after the formation of the ultra-thin GMO layer in the 100 mM KCl solution, the *G–C* charactristic was changed as is shown in Fig. 5. The water levels of both compartments were lowered simultaneously to *ca.* 5 mm below the hole of the Teflon plate. In this procedure, the ultra-thin GMO layer was exposed to air. Moreover, the water levels returned to the original state within one minute. The capacitance increased with the repeated exposures, and the break in the curve at low frequencies became obtuse, as is shown in Fig. 5. The increase in the capacitance should be attributed to the reduction of the GMO-layer thickness or to the quantity of GMO absorbed into the porous NC film. From the experimental results (Fig. 5) and the calculated thickness of the GMO layer (150 Å), it seemed reasonable to assume that the ultra-thin GMO layer was formed by filling the interstices of the network structure of NC with microdrops of GMO.

The form of the *G–C* curve was very similar to that observed for the suspensions of *Austerias* eggs by Cole *et al.*[4] They explained qualitatively the break in the *G–C* curve in the low-frequency region as being caused by the surface conductance at the egg–water interfaces. On the other hands, Coster[5] suggested that the double fixed-charge membrane model could account for the low-frequency dispersion. In both cases it was useful to introduce the concept of frequency-dependent elements [$C(f)$ and $G(f)$]. In our system, the surface conduction possibly occurred also at the interface of the GMO microdrop-NC network. According to Schwan *et al.*,[6] the surface conductance can be represented by a parallel combination of the capacitance, C_s, and the conductance, G_s, where C_s is inversely proportional to $\sqrt{f}$ and where G_s is proportional to $\sqrt{f}$ on the first approximation. Therefore, our system may be represented by the circuit shown in Fig. 3b. G_u and C_u indicate the conductance and the capacitance of the ultra-thin layer respectively. Curve (a) in Fig. 6 represents the calculated curve, using the equivalent circuit (Fig. 3b) with $C_a=10^{-12}$ F, $C_u=1.2\times10^{-9}$ F, $G_a=1.2\times10^{-3}$ ℧, $G_u=3\times10^{-5}$ ℧, $G_s=1.25\times10^{-8}\sqrt{f}$ ℧, $C_s=1.25\times10^{-8}/2\pi\sqrt{f}$ F. The above values of G_a and C_a correspond to those of the 100 mM KCl solution, while

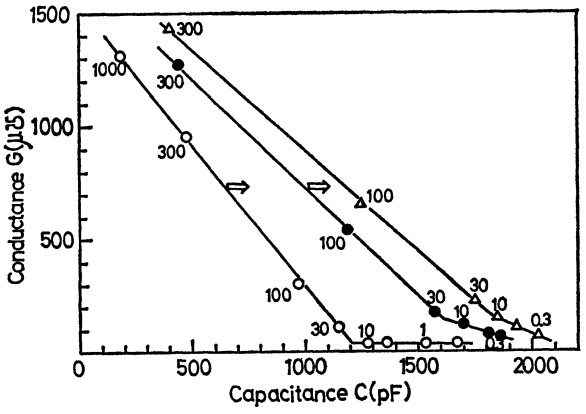

Fig. 5. Influence of up-and-down procedures of the water levels on *G–C* characteistic.
The corresponding frequencies (kHz) are given beside some points.
Concentration of KCl solution is 100 mM.
○: Initial characteristic, ●: After the first procedure, △: After the second procedure.

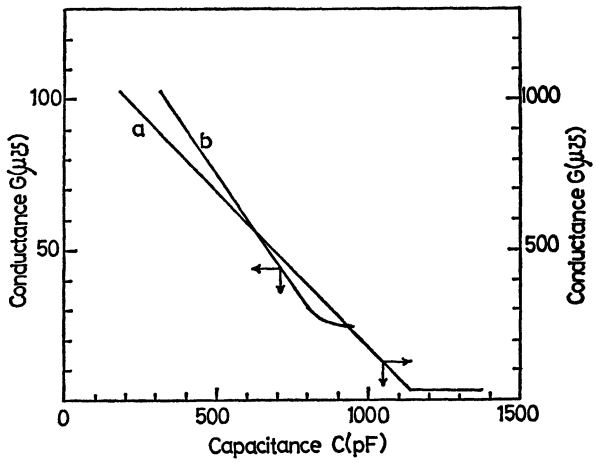

Fig. 6. Whole system characteristic, calculated from the equivalent circuit, Fig. 3(b).
Curve(a): With $C_a=10^{-12}$ F, $C_u=1.2\times10^{-9}$ F, $C_s=1.25\times10^{-8}/2\pi\sqrt{f}$ F, $G_a=1.2\times10^{-3}$ ℧, $G_u=3\times10^{-5}$ ℧, $G_s=1.25\times10^{-8}\sqrt{f}$ ℧.
Curve(b): With the same values of C_a, C_u, C_s, G_u, G_s as above, and with $G_a=1.7\times10^{-4}$ ℧.

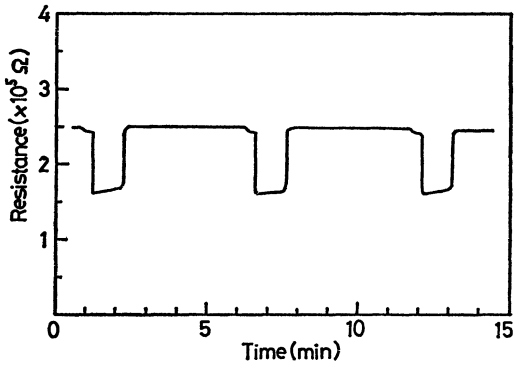

Fig. 7. Periodic change of the resistance of ultra-thin DOPH layer.

C_u is equal to the extrapolated value of C_0 at the low-frequency end of the semicircular curve in Fig. 2. Moreover, G_u is equal to the conductance at low frequencies (below 1 kHz). If we use $G_a = 1.7 \times 10^{-3}$ ℧ (the value for 10 mM KCl solution) instead of 1.2×10^{-3} ℧, Curve (b) in Fig. 6 is obtained. The bend of Curve (b) in the low-frequency region is not clear compared with Curve (a). In other words, the dilution of the KCl solution is accompanied by the disappearance of the bend in the *C–G* curve. These curves agree with the experimental results (Fig. 4)

The ultra-thin layer of dioleyl phosphate (DOPH) could also be prepared by a similar method. When a constant DC voltage (50 mV) was applied across the film in a 20 mM KCl solution, the electric current through the whole system changed periodically (Fig. 7).

Yoshida *et al.*[2] have reported that DOPH impregnated in filter paper changes its conformation discontinuously from oil droplets to bilayer films at a critical concentration of the adjacent solution and that this conformational change increases the conductance of the model membrane. On the other hand, according to Barry *et al.*,[7] the difference in transport number of ions between the membrane and the adjacent solution causes an increase in the salt concentration in the unstirred layer near the membrane surface.

In both studies, we presumed that the periodic phenomenon (Fig. 7) resulted from the following processes. If the ultra-thin layer of DOPH, as well as the ultra-thin layer of GMO, could be formed by filling the interstices of the network structure of NC with microdrops of DOPH, then the current must flow along the planes of contact between the DOPH microdrops and the NC network because of the high resistance of the DOPH microdrops. On the other hand, since DOPH possesses a negative charge, the transport number of positive ions at the planes of contact is greater than in the adjacent solution. This difference in transport number will result in an increase in the KCl concentration on one side of the thin layer-solution interfaces. If the DOPH in the NC film, as well as the DOPH in the filter paper, changes its conformation at a critical concentration of the adjacent solution, the conductance of our model membrane will increase discontinuously when the concentration at the thin layer-solution interface reaches a critical value. The periodic phenomenon (Fig. 7) disappeared in a 10 mM or 30 mM KCl solution. This fact suggests that the DOPH in the NC film changes its conformation when the concentration at the thin layer-solution interface reaches 20–30 mM. The increase in conductance is accompanied by the disappearance of the enhancement of the concentration at thin layer-solution interface. Consequently, the DOPH in the NC film returns from the transition state (bilayer) to the original state (microdrop) after *ca.* one minute (Fig. 7), and then the conductance of the whole system decreases to the original lower value. We assumed that the periodic phenomenon in Fig. 7 resulted from a repetition of the above processes. The periodic phenomenon was not observed for the ultra-thin GMO layer, which has no charged group. Therefore, it seems that the occurrence of the periodic phenomenon in Fig. 7 requires the presence of the charged group and of two conformational states of the molecule.

From the facts presented above, we may conclude that the ultra-thin layer consists of the microdrops of GMO (or DOPH) in the interstices of the network structure of NC and that the thickness of the ultra-thin layer is 150 Å.

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