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Lateral electrical conduction along a phosphatidylcholine monolayer

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Lateral electrical conduction due to lipid-monolayers spread on the surface of pure water was observed under both d.c. and a.c. electrical fields. An apparent specific electrical conductivity is evaluated as high as $\approx 4 \cdot 10^{-2}$ mho/cm for the monolayer-water system of L-DPPC at 25 °C. Arrhenius plots of the apparent conductance show a deflection at a temperature corresponding to a crystalline-to-fluid phase transition of the surface monolayer. From the magnitude and temperature dependence of conductance and a comparison of results with those obtained by use of deuterated water, it is concluded that enhanced protonic conduction mediated by a network consisted of polar head groups of phosphatidylcholines and water molecules may be brought about near the lipid/water interface.

Lateral charge transfer at lipid-water interfaces can play an important role in the functioning of biological cell membranes, and has been discussed recently in connection with the chemiosmotic-coupling hypotheses [1]. Although many investigations have been reported on the charge transfer through biomembranes, comparatively little is known on their lateral electrical conduction properties despite the importance in cell biology of these properties. We report here a direct experimental proof of the existence of an efficient proton transfer pathway along the interface between the phosphatidylcholine (PC) surface monolayer and water, the existence of which has been reported recently by probing the diffusion of injected proton ions along the interface [1,2].

During studies on the growth mechanism of myelin figures [3,4], it was found that an abrupt increase in electrical current was observed when the surface of a growing myelin figure bridged a pair of electrode wires while applying a low d.c voltage. The increment in electrical conductance was supposed to be due to a charge flow along, at least partially, the outer surface of the myelin figures, i.e. along the surface of the lipid bilayers. To prove a charge transfer pathway in the interface region, we have measured the lateral electrical conduction along the lipid/water interface by use of monolayers of synthetic phosphatidylcholines spread over on a pure water surface. The basic idea of the present experiments for determining the lateral electrical conduction of lipid monolayer is to cast a monolayer on the water surface while observing a current through the cell, and to measure an increase in the current due to the formation of a surface monolayer.

Lipids used as samples were synthetic phosphatidylcholines commercially available from Sigma Chem. Co. They were L-α-phosphatidylcholines of dipalmitoyl (L-DPPC), dimyristoyl (L-DMPC) and distearoyl (L-DSPC), which were used as received. The solution for casting a monolayer on the surface of pure water was prepared by dissolving a lipid in chloroform of analytical grade

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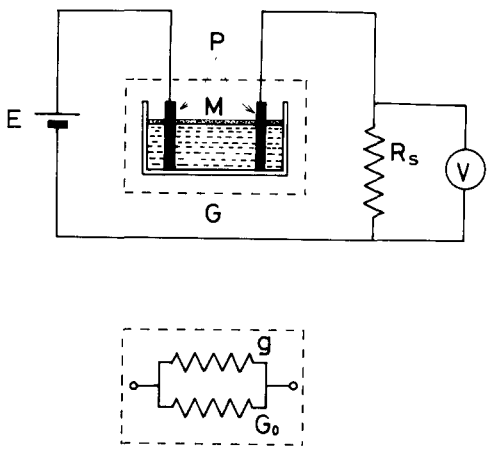

Fig 1 Schematic set-up of the experiment (top) M, monolayer, P, bright platinum electrodes, E, voltage source; V, recorder, R_s, standard resistor (20 kΩ). Conductance G of the cell is devided into two parts (bottom), one is g due to the monolayer and the other G_0 due to bulk water

with the specific resistivity of about 10^8 ohm · cm. Experimental set-up is shown schematically in Fig. 1. A cylindrical glass vessel with 2.7 cm in inner diameter and 2.8 cm in depth was used as the cell, in which two bright platinum electrodes plates (2.0 cm × 1.1 cm) were fixed with their long side horizontal and typically separated by 1.5 cm from each other. The cell was usually filled by 2 cm^3 of pure water with specific resistivity of about $1.7 \cdot 10^7$ ohm · cm. The water surface was located at about half the depth of the electrodes, and was in contact with air. The cell was connected in series with a standard resistor, R_s, to monitor the cell current and a known voltage, E, was applied to the circuit. A copper-constantan thermocouple was attached on the outer wall of the cell vessel to monitor the temperature of the monolayer-water system. The lateral pressure of monolayer was checked by means of a conventional method, i.e. a Wilhelmy balance method [5], with an electrical balance. A value around 40–43 dyn/cm was obtained for the L-DPPC monolayer at approx. 23° C.

Experimental procedures were as follows. Firstly, a prescribed volume (2 cm^3) of pure water was poured into the cell to ensure that half of each electrode remained beyond the water surface, and a known voltage E was applied to the cell. The voltage across R_s, proportional to the cell current, was recorded against time. Secondly, after the initial transient current decrease with time had settled down to a steady level, $V = V_0$ (typically about 15 to 20 min after water had been poured in), a drop of chloroform solution of PC was put on the water surface. The volume of the drop of the solution was kept constant (approx. 0.01 g by weight) using the same pipetting tip throughout the experiments. On the water surface, the drop of the solution spread swiftly, and developed a PC-surface monolayer there. When the number of PC molecules in a drop of solution was more than enough to span a monolayer on the surface, the excess molecules aggregated making an 'island' on the surface and went down to the bottom of cell. On spanning a monolayer, a sudden increase of current through the cell was coincidentally observed with a sudden increase in the voltage drop across R_s, since the two electrodes were bridged by the PC monolayer developed on the water surface. The increment in the voltage drop across R_s, denoted as ΔV hereafter, was recorded following V_0. ΔV remained nearly constant during several tens of minutes. An increase in cell conductance due to the existence of the surface monolayer was evaluated through V_0.

A typical record of the voltage across R_s is shown in Fig. 2. The broken line is for pure water. Together with the geometrical factor of cell it gives the electrical conductance of pure water without surface monolayer. The solid curve is for an experimental run, where a drop of 0.2 wt% chloroform solution of PC was put on the water surface (Fig. 2, b). As shown in Fig. 2, the voltage across R_s increases suddenly from V_0 by ΔV on spreading a surface monolayer, and ΔV persists nearly constant to several tens of minutes. Fig. 2 also shows an effect of putting a drop of only chloroform on a virgin surface of water (Fig. 2, a). It spread on the water surface and gave a transient increase of V_0, which was small compared to ΔV and decreased gradually to the original level V_0 with the evaporation of chloroform. When a drop of the PC-chloroform solution was put again on the monolayer about 20 min after the spanning, the drop did not spread on the surface with monolayer and went down to the bottom of the cell. During this process, the value of ΔV was not changed except for the appearance of a noise-like

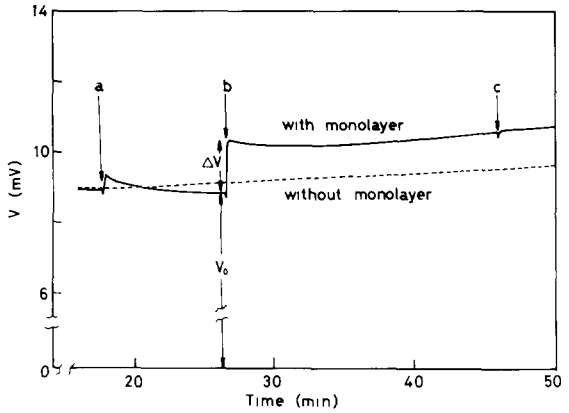

Fig 2 A typical record of the voltage drop across R_s against time, for L-DPPC at 25 °C, $E = 1.5$ V, d.c The broken line represents a run only with pure water (without monolayer) The full line represents a run in which a PC monolayer has been spread on the surface of water After the initial transient by application of voltage to the cell with pure water had settled down, 0.01 g of pure chloroform was dropped on the water surface at a At b, 0.01 g of 0.2 wt% chloroform solution of L-DPPC was dropped on the same surface. In each case chloroform evaporated soon from the surface At c, a drop of the chloroform solution of PC was again put on the surface now having been covered with PC monolayer See the text for further details

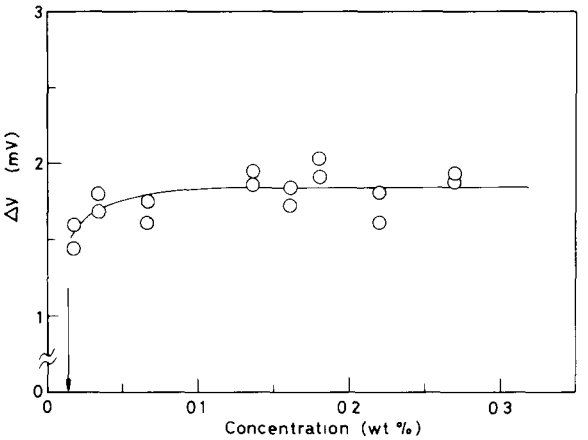

Fig 3. Concentration dependence of ΔV for L-DPPC at 29 °C, $E = 1.5$ V, d.c The arrow indicates the minimum concentration for spreading a lipid monolayer on the entire surface

little spike on the record (Fig. 2, c). As shown in Fig. 2, V_0 (without monolayer) and also $V_0 + \Delta V$ are kept almost constant during several tens of minutes except for a slight increase with time due probably to the absorption of CO_2 in air into the water. The specific resistivity of water evaluated from V_0, however, had been reduced to about $5 \cdot 10^6$ ohm · cm in the cell. The reduction was thought to be due probably to the absorption of CO_2 in air into the water and to some ionic impurities from the glass ware. Each series of measurement of ΔV was performed with a freshly prepared monolayer after the cell was washed carefully. On application of a.c. voltage to the cell, qualitatively the same behaviour as that under d.c. was observed. The values of V_0 and ΔV obtained under a.c. voltage, however, were larger than those under d.c. voltage due to the capacitive effect of the system.

When the upper edges of the electrodes were under the water surface, no effect was observed by spanning a monolayer on the surface, i.e. ΔV could only be observed when the two electrodes were bridged by a monolayer, suggesting that the observed ΔV was not a bulk effect.

Fig. 3 shows changes in ΔV at 29 °C with the concentration of the L-DPPC-chloroform solution varying between 0.01 and 0.3% by weight and an applied voltage E of 1.5 V d.c. From the geometrical factor of the cell and the weight of a drop of solution under the present experimental condition, and taking a surface area of 50 Å^2 for a PC molecule in monolayer [6], it is estimated that a lipid concentration 0.014 wt% is necessary to cover the entire water surface with a monolayer. Beyond that concentration to a certain extent, the observed ΔV is nearly independent of the L-DPPC concentration in the drop as shown in Fig. 3. In the lower concentration region, ΔV becomes smaller since it becomes hard to cover the entire surface with a surface monolayer because too few PC molecules are put on the surface. Also, when additional drops were put on the surface after spanning a monolayer, no further effect inducing an additional persistent voltage increase to ΔV was observed as shown in Fig. 2, c. A drop of pure chloroform also gave no persistent change in V_0 either for a virgin water system (Fig. 2, a) and for a monolayer-water system. If ionic impurities of relevant chemicals are responsible for the observed phenomena, ΔV, and also V_0, should depend on the lipid concentration and the number of drops put on the surface. The above results also

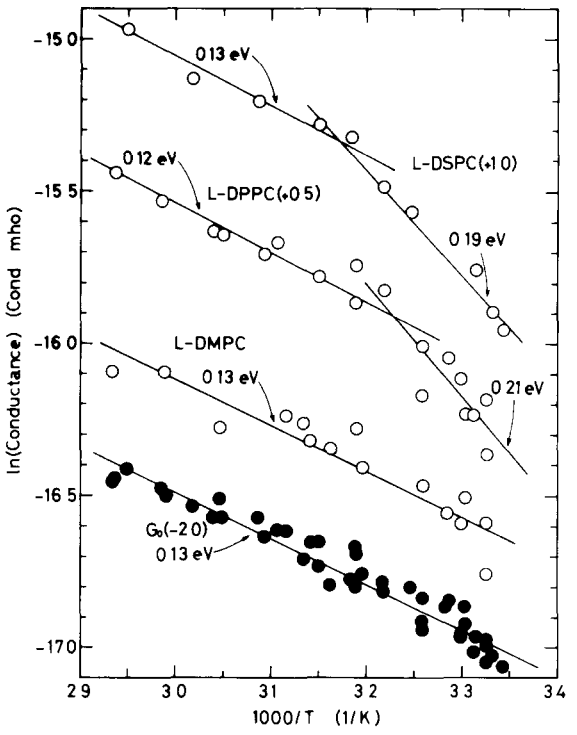

Fig. 4 Arrhenius plot of the apparent conductance increase, g (open circles), for L-DSPC, L-DPPC and L-DMPC, and of the cell conductance, G_0 (filled circles). Numbers in parentheses represent the shift of the set of points along the vertical axis The activation energy calculated from the slope is also shown beside each line For L-DPPC and L-DSPC a deflection occurs in the plot at 36 5 °C and 42 1 °C, respectively, corresponding to the crystalline-fluid phase transition of the respective monolayers

seem to discard the possibility of a bulk effect in the observed ΔV.

The formation of the monolayer on the surface of water will be modelled as an addition of conductance g parallel to the conductance G_0 due to water (Fig. 1). Since $G_0 R_s$ and $g R_s \ll 1$, g and G_0 are related to ΔV and V_0, as $g \simeq \Delta V / E R_s$ and $G_0 \simeq V_0 / E R_s$, respectively. The source voltage E dependencies of ΔV and V_0 were checked by use of both d.c. and 1 kHz a.c. sources at about 23 °C. Below about 3 V, ΔV and V_0 varied almost linearly with E implying an ohmic contact at electrodes and the absence of polarization effect due to the possible existence of ionic impurities.

Temperature dependencies of g and G_0 were measured on spanning surface monolayers of L-DMPC, L-DPPC and L-DSPC using a 0.2wt% solution. In each measurement, heating the cell with pure water to a measuring temperature by use of an incandescent lamp of 200 W, V_0 was recorded at first. A drop of a lipid solution was, then, put on the water surface to measure ΔV at that temperature. Arrhenius plots of g and G_0 are shown in Fig. 4. Although both g and G_0 decrease linearly with $1/T$, g shows a deflection at 36.5 and 42.1 °C for L-DPPC and L-DSPC monolayers, respectively, implying the existence of a phase transition. It could be monitored by a simple experiment, in which a very small piece of thin paper was put on the monolayer and the mobility of the piece was observed. The temperature at which the piece of paper became highly mobile was above about 42 °C and 37 °C for the L-DSPC and L-DPPC monolayer, respectively, whereas it remained highly mobile down to 22 °C for L-DMPC monolayer. Since the observed deflections in Arrhenius plots roughly correspond to these temperatures and also the deflection occurs for the L-DPPC monolayer near the reported phase transition temperature of the monolayer of L-DPPC from a crystalline to a fluid lamella [6], it is natural to assign the observed deflection to the crystalline-to-fluid phase transition. The temperature dependence of g, accordingly, suggests that the observed ΔV is a result of the existence of a lipid monolayer on the water surface. The relevant activation energies of conductance are nearly the same for all PC monolayers examined in the high-temperature region and for water, i.e. approx. 0.13 eV, and for monolayers in the low-temperature region, i.e. approx. 0.2 eV.

The conductance increase g of L-DPPC at 25 °C results in a specific conductivity of the order of 10^{-2} mho/cm assuming the thickness of an interface region to be 100 Å, corresponding approximately to a typical thickness of the Gouy-Chapman diffuse electrical double layer in an electrolyte solution with concentration of 10^{-3} M. This corresponds with the estimated hydrogen ion concentration from the interface, the pH of a phosphatidylethanolamine (cephalin) monolayer [1,7], namely $pH_{surface} \approx 3$ and $[H^+] = 10^{-3}$ M. The interface, however, may extend to several hundreds of angstroms for an array of weakly ionized lipid polar heads. Even if the thickness of interface region extends up to 10^3 Å, g is approx. $4 \cdot 10^{-3}$

mho/cm, which is still very large compared to that of water, i.e. approx. 10^{-8} mho/cm near room temperature [8]. The problem is that we cannot explain such high g values using only the structural and electrical features of the interface between the water subphase and the surface monolayer. Therefore, an efficient charge transfer which is operable at the interface region, is needed to explain the present results.

It is well known that the electrical conductivity of pure water can be explained by the so called proton jump mechanism utilizing the hydrogen-bond structure of water [9,10]. Essentially the same mechanism as in water was proposed for bulk PC in a mesophase as a possible proton transfer mechanism [11], where polar heads are supposed to make a hydrogen-bond network with bound water molecules. In the monolayer on water surface, PC molecules are thought to be fully hydrated making a hydrogen-bond network favourable for protonic conduction. In addition, electrostatic interaction between polar heads and water may alter the structure of liquid water toward the more or less ordered state favouring the construction of an efficient protonic pathway and the interaction with each other. To show the participation of protons in the observed phenomena, comparative studies on ΔV and V_0 were also carried out using deuterated water at about 23°C. The observed g for deuterated water was about two-thirds of that for water of the same V_0 as the deuterated one. We, therefore, think that a polar head mediated lateral proton transfer along lipid monolayer may be responsible for the observed conductivity change on spreading a lipid monolayer.

The efficiency of proton transfer is determined by the density of free protons and the apparent mobility of them by jumping in the interface region. The mobility will be controlled by a potential barrier for protons to surmount on transfer along hydrogen-bondings, the average size of fluctuating hydrogen-bonded structures and the rates of reorientational processes of water molecules and, probably, the polar heads. As for the density of protons, it is difficult to expect a higher interface $[H^+]$ for PC than for cephalin although both lipids are of the same nature, i.e. of a kind of ampholyte. Anyway, the results on activation energy suggest both a water-like conduction mechanism and a participation of polar heads in it.

At present, the detailed molecular mechanism of lateral conduction along surface monolayer is still open to further studies. Our tentative picture is to assign the conduction pathway to the interface region with hydrogen-bond networks of polar heads of lipids and water, where polar heads of lipids may play an important role in the enhancement of protonic conduction.

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