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Effect of Surface Active-Agents and Some Molecules on the Electrical Properties of Lipid Membranes

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The electric and mechanical properties of lipid membranes between two electrolyte solutions were observed. The electric resistance was 10^7—10^8 $\Omega \cdot cm^2$; the capacity, 0.2—1 $\mu F/cm^2$, and the surface tension, 2.2—4.9 dyn/cm. The electric conductivity increased with an increase in the surface area of the lipid membranes. Furthermore, the electric resistance and the capacity decreased when an ionic surface-active agent was adsorbed to the lipid membranes. The effect may be caused by the structural change of the lipid membranes due to both the electric and the hydrophobic interactions between the ionic surface-active agent and the lipid membranes.

It has been pointed out experimentally by Overton[1] and Collander[2] that the permeability coefficient of a substance through the membrane of a plant cell is proportional to its oil/water partition coefficient, and that the membrane shows a comparatively high permeability to lipid-soluble substances. On the basis of such experiments, they suggested that lipids might be involved in the membrane structure. On the other hand, the physico-chemical nature of the lipid monolayer has been investigated by many authors, beginning with Gorter and Grendel.[3]

From the fact[4-6] that the surface tension of the urchin egg is larger than that of the lipid monolayer in the range of physiological pH values, Danielli and Davson[7] proposed the lipid bilayer coated with protein as the fundamental element of the biological membranes. Their proposal has been supported by the electron-microscopic observations,[8,9] the X-ray diffraction pattern,[10-12] and so on.

In investigating biological membranes, studies of lipid bilayers will be more useful than those of the monolyaer.

Several years ago, Mueller *et al.*[13-17] and Thompson *et al.*[18-22] succeeded in forming lipid bilayers between two electrolyte solutions, and reported on some physico-chemical properties, *i. e.*, the electric conductivity, the electric capacity, the surface tension, *etc.*, of such systems.

The present author has been especially interested in the work of Mueller *et al.* They found that the electric resistance of a lipid bilayer adsorbing some water-soluble substances is much lower than that of the original, bare lipid bilayer, and showed that the resistance of biological membranes is of an order similar to that of the coated lipid bilayers. Because of the lack of detailed information of the adsorbed molecules, however, we can not really explain the mechanism of such a reduction of the electric resistance.

It is the purpose of this paper to investigate

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2) R. Collander, *Trans. Farad. Soc.*, **33**, 985 (1937).
3) E. Gorter and F. Grendel, *J. Exp. Med.*, **41**, 439 (1925).
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13) P. Mueller, D. O. Rudin, H. Ti Tien and W. C. Wescott, *Circulation*, **26**, 1167 (1962).
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18) T. E. Thompson, "Cellular Membranes in Development," ed. by M. Locke, Academic Press Inc., New York, N. Y. (1964). p. 83.
19) C. Huang, L. Wheeldon and T. E. Thompson, *J. Mol. Biol.*, **8**, 148 (1964).
20) C. Huang and T. E. Thompson, *ibid.*, **13**, 183 (1965).
21) C. Huang and T. E. Thompson, *ibid.*, **15**, 539 (1966).
22) C. Huang and T. E. Thompson, *ibid.*, **16**, 576 (1966).

the effect of the well-defined adsorbed materials on the physico-chemical nature of the lipid bilayers. The author used some surface-active agents, proteins, and polypeptides as the adsorbed materials, and observed such properties as the electric conductivity, the electric capacity, the surface tension, and the relationship between the surface area and the electric conductivity.

Experimental

Apparatus. The block diagrams of the apparatus are given in Figs. 1a and 1b. In Fig. 1a, the glass tube (A) is held vertically, and the end is bent horizontally. The diameter (E) at the end of the tube was 2 mm.

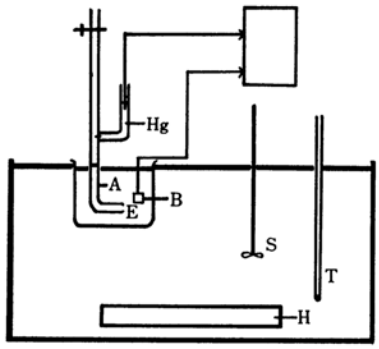


Fig. 1a. The main apparatus used to measure the electrical properties of the membrane.

Both beaker and tube were filled with an electrolyte solution. B, S, T, and H were the platinized platinum plate, stirrer, thermister, and heater respectively. The beaker was immersed in the water both, and the temperature of the electrolyte solution was kept constant within a range of error of ± 0.2°C.

Figure 1b shows the details of the neighborhood of the membrane.

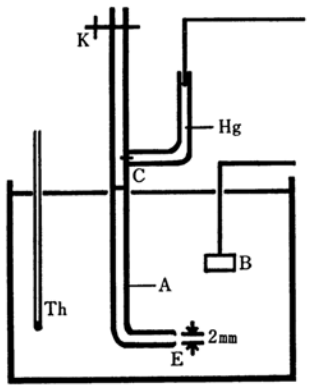


Fig. 1b. The enlargement figure near the membrane.

Th, C, and K are the thermometer, the platinized line, and the glass cock respectively. The membrane was formed by drawing the solution of the lipid with a small brush across the hole at the end of the tube. The surface of the membrane could be seen through a magnifying glass.

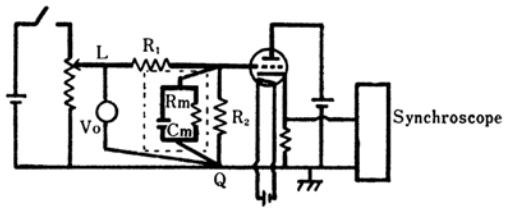


Fig. 2. Outline of the electric circuit.

The outline of the circuit is given in Fig. 2. The equivalent circuit for the membrane is enclosed with a dotted line. It is assumed that the membrane can be represented by an equivalent circuit in which the electric resistance, R_m, and the electric capacity, C_m, are connected in parallel.

A square-wave current was applied across the membrane. The potential difference across the membrane was measured with a synchroscope (Tektro 531 or Iwasaki Synchro Junior 5022). Since the measured potential difference contained the potential difference of the electrolyte solution, the latter was corrected. The resistances, R_1 and R_2, were adjusted to keep the potential difference between L and Q constant. On the basis of the assumption given above, the values of R_m and C_m can be obtained by:

$$V_m = V_0 \frac{\left(\frac{1}{R_m} + \frac{1}{R_2}\right)^{-1}}{\left(\frac{1}{R_m} + \frac{1}{R_2}\right)^{-1} + R_1}$$

$$V = V_m \cdot [1 - \exp(-t/C_m R)]$$

$$R = \left(\frac{1}{R_1} + \frac{1}{R_2} + \frac{1}{R_m}\right)^{-1}$$

where

V_m = Potential difference across the membrane in equilibrium
V = Potential difference across the membrane at time t
V_0 = Potential difference between L and Q measured by a voltage-ampere meter
$C_m R$ = Time constant

Materials. The lipid was extracted from ox brain in the same way as was done by Mueller *et al.* and was preserved in a refrigerator as a chloroform-methanol solution, kept from exposure to light and air.

The experiments were carried out for the *n*-decane solution as well as the chloroform-methanol solution. In the former case, after the evaporation of the solvent of the chloroform-methanol solution, the lipid was again dissolved in *n*-decane.

The salts used for the electrolyte solution were as follows: 1) NaCl, KCl, LiCl, *etc.*, 2) $MgCl_2$ and $CaCl_2$, 3) tetra-methylammonium chloride (TMA) and tetrabutylammonium chloride (TBA). Each of them was dissolved in a 1/500 M phosphoric acid buffer solution (pH 3.5, 7.0, and 10.0, mainly 7.0) after having been boiled in order to remove any oxygen.

Sodium dodecyl sulfate, sodium laurate, dodecyl trimethyl-ammonium chloride, and dodecyl trimethyl-ammonium bromide were used as ionic surface-active agents, while polyoxyethylene sorbitan mono-palmitate

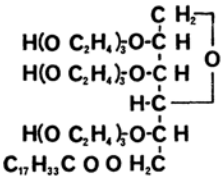
TABLE 1. THE CMC VALUE OF IONIC AGENTS USED IN OUR EXPERIMENT

Compound	CMC value in water, mM	Temp. °C	CMC value in 0.1 M NaCl soln., mM[1]	Temp. °C
Dodecyl trimethylammonium chloride	16—20	30	2.7—3.3	36
Dodecyl trimethylammonium bromide	15—17	25	2.6—2.9	36
Sodium dodecyl sulfate	8—11	35	1.39	36
Sodium laurate	23—26	25	10—11	36

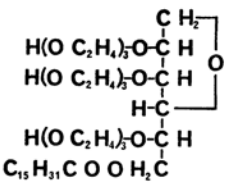
(I) and polyoxethylene sorbitan mono-oleate (II) were used as non-ionic surface-active agents. All these materials were originally commercial products. In order to purify them further, the ionic agents were recrystallized from ethanol.

The values of the critical micelle concentration (CMC) of these detergents were determined from the measurements of the surface tension by using the drop method.[23,24] These values are shown in Table 1.

One criterion of the ionic detergent purity employed in this work was the agreement of the observed CMC values with those reported in the literature.[25-28] Another criterion was that the curve relating the surface tension with the ionic detergent concentration did not show a minimum. The non-ionic agents were used without purification.



(I)



(II)

Polyglutamic acid (PGA) and the lysine-glutamic acid copolymer (L-G copolymer, lysine fraction 6 wt%) were offered by Akiyoshi Wada, while the histone was offered by Koichi Iwai.

Results

Electric Resistance and Electric Capacity of the Membrane. The values of the electric resistance of the lipid membrane at pH 7.0 and 36.0±0.2°C are shown in Table 2. The lipid membrane was formed from the chloroform-methanol solution.

The resistance decreased with an increase in the concentration of the electrolyte solution. Hanai *et al.*[29] reported a similar tendency in the lecithin bilayer. The electric resistance of the membrane in TMA and TBA solutions was larger than that in other electrolyte solutions. The electric capacity was nearly the same for all sorts of electrolyte solutions, the values ranging from 0.2 to 1.0 μF/cm^2.

TABLE 2. EFFECT OF THE CONCENTRATION OF THE SALT ON THE ELECTRIC RESISTANCE AT 36.0°C AND pH 7.0

Salt	Concentration of salt, M	Resistance of the membrane $10^7\ \Omega\cdot cm^2$
NaCl	2.5	2.0
	1.0	2.4
	0.1	3.2
	0.01	3.8
KCl	1.0	1.0
	0.1	8.5
	0.01	15
LiCl	1.0	2.5
	0.1	5.7
	0.01	12
LiF	0.01	4.9
$CaCl_2$	0.1	8.5
	0.01	14
$MgCl_2$	1.0	2.4
	0.1	3.3
	0.01	5.1
TMA	0.1	12
TBA	0.1	18

On the other hand, the pH dependence of the electric resistance and of the capacity of the membrane was very small in each of these electrolyte solutions. For example, the electric resistance in the NaCl solution was $4.1\times10^7\ \Omega\cdot cm^2$ at pH 3.5, $3.2\times10^7\ \Omega\cdot cm^2$ at pH 7.0, and $5.0\times10^7\ \Omega\cdot cm^2$ at pH 10.0.

The membrane broke down upon the application of the electric voltage, 200—250mV.

The Effect of Surface-active Agents. When a cationic active agent, dodecyl trimethylammonium chloride, was added to the electrolyte solution, the resistance of the membrane decreased at the concentration of 0.5m M as is shown in Fig. 3. This concentration is about 1/6 the CMC value.

When the concentration of the surface-active agent was increased to about 2.5m M (about 9/10

23) W. D. Harkins and F. E. Brown, *J. Am. Chem. Soc.*, **38**, 288 (1916).
24) W. D. Harkins and F. E. Brown, *ibid.*, **41**, 499 (1919).
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26) R. C. Merrill and R. Getty, *J. Phys. and Colloid Chem.*, **52**, 774 (1948).
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28) "Kagaku Binran Kiso-hen II" ed. by The Chem. Soc. Japan, Maruzen Co., Ltd., Tokyo (1966), p. 707. (in Japanese)
29) T. Hanai, D. A. Haydon and J. Taylor, *J. Gen. Physiol.*, **48**, Part 2, 59 (1965).

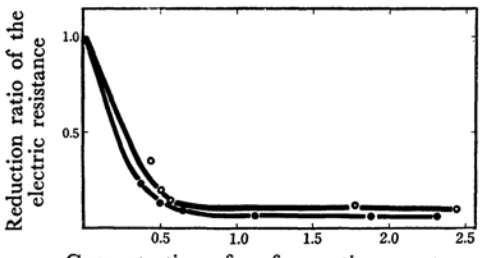


Fig. 3. Effect of the concentration of dodecyl trimethylammonium chloride and dodecyl trimethylammonium bromide on the electric resistance of the lipid membrane.
○ Dodecyl trimethylammonium chloride
● Dodecyl trimethylammonium bromide

the CMC value), the membrane broke down upon the application of an electric impulse. A further increase in the concentration of the detergent did not allow the membrane to be reformed.

As for dodecyl trimethylammonium bromide, the resistance of the membrane decreased at the concentration of 0.4 mM and the membrane broke down at the concentration of 2.3 mM The anionic agents, sodium dodecyl sulfate and sodium laurate, also reduced the resistance of the membrane (Figs. 4 and 5).

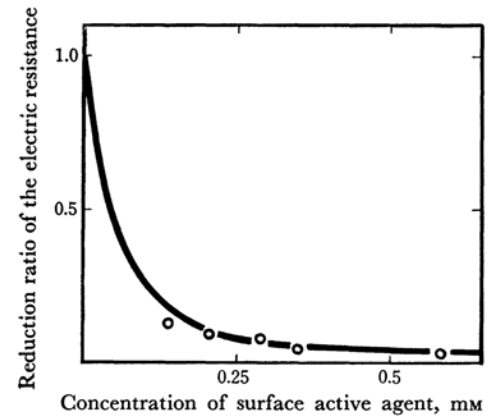


Fig. 4. Effect of the concentration of sodium dodecyl sulfate on the electric resistance of the lipid membrane.

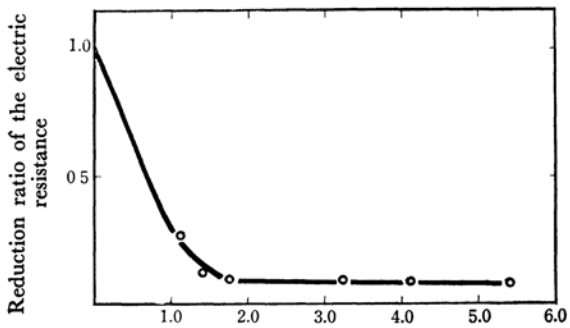


Fig. 5. Effect of the concentration of sodium laurate on the electric resistance of the lipid.

In the concentration range shown in Figs. 3, 4 and 5, the capacities ranged from 0.2 to 0.3μF/cm^2.

On the other hand, the non-ionic agents, polyoxyethylene sorbitan mono-palmitate and polyoxyethylene sorbitan mono-oleate, did not affect either the electric resistance or the capacity of the membrane.

Some Properties of the Membrane. As is well known, PGA is in a helical conformation at pH 4.5 and in a random coil at pH 10.0. In the present experiment, each solution of PGA, at pH 4.5, 7.0 and 10.0 was added to the electrolyte solution on both sides of the membrane.

In order to investigate the ionic effect of the copolymer on the resistance of the lipid membrane, on the other hand, we added the L-G copolymer to the electrolyte solution at pH 3.0, 7.0 and 10.0. In each case, however, no effect was found. Furthermore, the addition of neither histone nor aspartic acid had any effect, either.

In order to measure the surface tension of the membrane, a hydrostatic pressure difference was applied across the membrane in order to observe the radius of the curvature of the curved membrane. The surface tension can then be obtained from the following equation:

$$\gamma = \frac{1}{4} p \cdot r \text{ (dyn/cm)}$$

where p=hydrostatic pressure difference and r= radius of curvature.

The surface tension of the membrane in a NaCl solution was 2.2—4.9 dyn/cm at pH 7.0 and 36.0°C. Hanai *et al.*[30] measured the surface tension of a lecithin membrane formed from an *n*-decane solution by using the same method; they obtained the value of *ca.* 1 dyn/cm. They also reported[29-31]

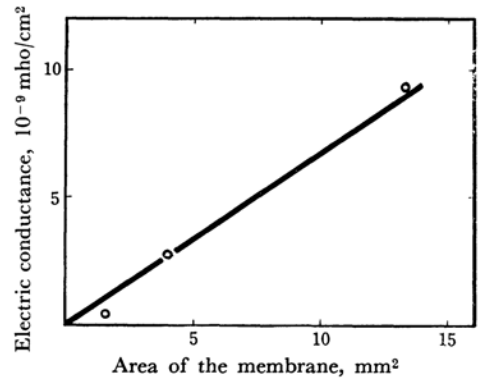


Fig. 6. The relation of the conductivity and the area of the bulged membrane.

30) T. Hanai, *Hyomen*, **4**, 643 (1966). (in Japanese)
31) T. Hanai, D. A. Haydon and J. Taylor, *J. Theoret. Biol.*, **9**, 433 (1965).

that the conductance of the lecithin membrane was nearly proportional to the surface area of the membrane and that the conductance per unit area 1.3×10^{-9} mho/cm in a 0.1 M NaCl solution. We also measured the conductance of the lipid membrane in relation to the surface area. The results are shown in Fig. 6.

The effect of the temperature on the membrane resistance was investigated in the 7—45°C temperature range. The membrane was stable only in the 20—40°C temperature range. The resistance was nearly independent of the temperature within the limits of the experimental error. This finding is not in agreement with that of Thompson.[18] He showed that the resistance changed discontinuously with the temperature. In our experiment described above, a chloroform-methanol mixture was used as the lipid solvent.

In order to investigate the effect of the solvent on the resistance of the membrane, we used *n*-decane as another solvent. The resistance of such a lipid membrane was much larger than that of the lipid membrane formed from a chloroform-methanol solution and was somewhat different from that obtained by Hanai *et al.*[30]

Discussion

As a lipid bilayer is rather simpler than a biological membrane in the sense that it does not contain protein, it is more important to investigate how the lipid bilayer adsorbs substances such as proteins and surface-active agents. Mueller *et al.* have for instance, reported that the resistance of the bilayer decreased when it adsorbed unidentified water-soluble substances extracted from egg white.

We observed that the resistance of the membrane decreased upon the addition of ionic surface-active agents to the electrolyte solution. The resistance did not change upon the addition of non-ionic agents, however.

We interpret the decrease in the electric resistance of the membrane as follows. Since the electric conductivity is due to the ionic current through the membrane, it is reasonable to surmise that the decrease in the electric resistance was caused by the increase in the ionic permeability due to the formation of several kinds of channels for ions with a structural change in the lipid membrane.

It may be considered that the added ionic agents adsorb on the membrane due to the interaction of the ionic agents with ionic sites in the outer region of the phospholipid membrane. Besides, hydrocarbon chains of the ionic agents may interact with the inner region of the lipid membrane. On the other hand, non-ionic agents will interact only with hydrocarbon chains of the lipid membrane.

Our experimental results suggest that the structural change of the membrane may be caused by both electrostatic and hydrophobic interactions of ionic surface-active agents with the membrane, and that the electrostatic interaction is necessary for the change in the membrane structure. That is, no structural change can be effected without ionic interaction in the outer region of the membrane. However, hydrophobic interaction may not be neglected.

The C number of the hydrocarbon chain of the surface active agents in our experiment was 11 or 12; in this case, hydrophobic interaction is plausible. No decrease in the membrane resistance was observed on the addition of substances with a few carbon atoms, for example, tetramethylammonium chloride, tetrabutylammonium chloride, aspartic acid, and caprylic acid. The structural change in the membrane may require the cooperation of electrostatic and hydrophobic interactions.

We shall now discuss the relation between the decrease in the resistance and the CMC values of the ionic agents. The resistance of the membrane decreased abruptly at a concentration of about 1/6 and at 1/10 the CMC value for cationic and anionic agents respectively. The lipid membrane may be sensitive to the solubilizing power of ionic detergents adsorbed due to electrostatic and hydrophobic interactions; the structural change in the membrane may be the consequence.

The CMC value of each ionic detergent may affect the critical concentration at which the abrupt decrease in the membrane resistance occurs. The difference between the decrease in the membrane resistance for cationic and anionic agents may correspond to the difference between the number of cationic and of anionic agents adsorbing on the membrane. As it is considered that the positive ions in the outer region of the

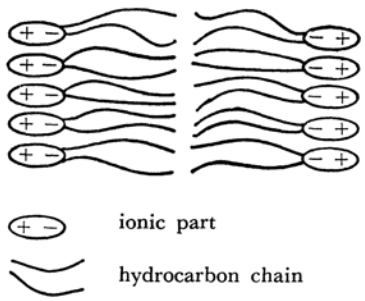
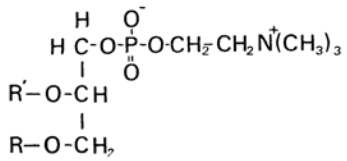


Fig. 7a. Lipid bilayer model.



R, R′: Hydrocarbon chain (16 to 22 carbons long)

Fig. 7b. Structural formula of phospholipid.

membrane are nearer to the bulk solution than are the negative ions, the anionic agents will adsorb on the membrane easier than will cationic agents. The details of the lipid bilayer membrane are shown in Figs. 7a and 7b. The proportionality of the conductivity to the surface area of the membrane was not very good, though the conductivity did increase with the increase in the surface area.

In our experiment, the surface tension of the lipid bilayer was larger than that of the biological membrane. This is because the lipid bilayer is not coated with protein.[32-33]

The author wishes to express his deep gratitude to Professor Akiyoshi Wada of the University of Tokyo for his valuable advice and support throughout the course of this work, to Mr. Masaiku Hara for his experimental assistance, and to Professor Koichi Iwai supplying the histone.

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32) J. F. Danielli and E. N. Harvey, *J. Cell. Comp. Physiol.*, **5**, 483 (1934).
33) J. F. Danielli, *Cold Spring Harbor Symposia*, **6**, 190 (1938).