J. Membrane Biol. 5, 185–199 (1971)

Studies of Electric Capacitance of Membranes

II. Conformational Change in a Model Membrane Composed of a Filter Paper and a Lipid Analogue

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Received 16 February 1971

Summary. The electric capacitance and conductance of a model membrane composed of a hydrophobic filter paper and a synthetic lipid analogue, i.e., dioleylphosphate, immersed in an electrolyte solution were observed with various frequencies ranging from 20 to 3×10^6 Hz. With successive increase of salt concentration in the external solution, the capacitance and conductance of the membrane increased discontinuously at a certain critical value of the external salt concentration. This variation of the capacitance and conductance of the membrane with the salt concentration was found to be reversible, and the critical value of salt concentration was independent of the adsorbed quantity of the lipid, and of the pore size of the filter paper as far as the adsorbed quantity of the dioleylphosphate was large.

A theoretical analysis based on the membrane model for the filter paper-phospholipid system proposed in Part I of this series revealed that the dioleylphosphate impregnated in the filter paper changed its conformation from oil droplets or globular micelles to a number of bilayer membranes when the salt concentration reached the critical value for a given pair of electrolyte species and the membrane. The conformational change of the lipid analogue in the filter paper is discussed in connection with the ability of formation and stability of a black bilayer membrane of the dioleylphosphate.

Recent studies of squid giant axon have revealed that the process of excitation is accompanied by a drastic change in the macromolecular conformation of the membrane triggered by cooperative cation exchange at fixed negative sites in the axonal membrane [10, 11]. The conformational change of membrane macromolecules reflects various physicochemical properties such as ion permeability, electric capacitance and conductance

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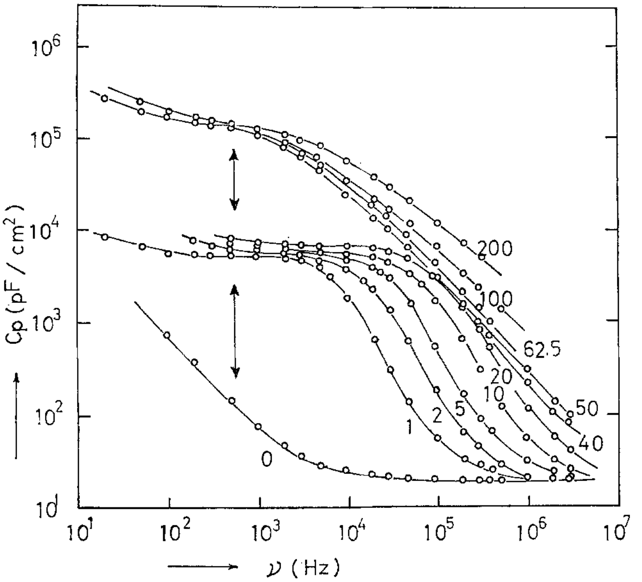

Fig. 1. Relation between C_p and ν with various concentrations of KCl. Cellulose ester Millipore filter; $\phi = 5$ μ; electrode distance $d = 0.225$ cm; Q of DOPH = 2.20 mg/cm^2; temperature = 25 °C. Number of each line shows the mM concentration of KCl

of the membrane. The cation exchange in the membrane occurs either by electrical stimuli applied or by variation of the composition of the external salt solution. In fact, the electrical capacitance of the axon membrane changes its value discontinuously with increase of concentration of K$^+$ or Rb$^+$ in the external solution at a fixed Ca^{++} concentration, when K$^+$ (or Rb$^+$) ion concentration exceeds a certain value [7]. This series of papers purports to study the physicochemical mechanism of frequency-dependent capacitance and conductance of a model membrane which is contiguous with two electrolyte solutions of identical composition on two sides of the membrane, anticipating that the study planned here may have some value for a better understanding of the molecular processes underlying the conformational change of a live membrane during excitation [5].

In paper I [4], we showed that the electric capacitance of a Millipore filter paper containing a synthetic phospholipid, dioleylphosphate (DOPH), is changed abruptly when KCl added in the external solution exceeds a certain concentration. Fig. 1 shows an example of the experiments where the electric capacitance of the membrane (C_p) is plotted against the applied frequencies (ν) with various KCl concentrations (c_s). The data shown in this figure refer to the system of the pore size of Millipore filter $\phi = 5$ μ, and the adsorbed quantity of DOPH $Q = 2.20$ mg/cm^2. Note that the discontinuous change in C_p is reversible with the variation of c_s. Major aspects of the frequency-dependent capacitance shown in Fig. 1 were represented satisfactorily with a theory proposed in the previous paper, which is based on a theoretical model in which a number of the lipid membranes were assumed to form in the meshwork of the filter paper. However, we did not discuss in detail the physicochemical mechanism responsible for the discontinuous change of the capacitance observed in the system.

In this paper we present further experimental data on the discrete change of electric capacitance with varying pore size of the filter paper, salt species and its concentration, and adsorbed amounts of DOPH. It is shown that the discrete change of the membrane capacitance is attributed to a conformational change of adsorbed DOPH from oil droplets or micelles to a number of lipid bilayers in the filter paper. This transformation of DOPH in the filter paper is compared to the ability to form DOPH black bilayer membranes.

Materials and Methods

The DOPH used as a lipid analogue was the same as that used in paper I [4]. Two kinds of Millipore filter papers composed of cellulose ester and of nylon with various pore sizes were soaked for a few hours in a benzene solution of DOPH (or of a mixture of DOPH and cholesterol for the case of the nylon filter paper) at various weight concentrations. The soaked filter paper was air dried and weighed. The amount of lipids adsorbed in the Millipore filter, Q (in mg/cm^2), was determined by weighing. The filter paper containing DOPH was conditioned overnight in an aqueous salt solution, and used as the sample membrane. The concentration of salt solution for the conditioning was immaterial, since the observed impedance varied reversibly with the concentration of the external solution with which the membrane was in contact. The water used as solvent was treated as distilled water with cation and anion exchangers. The pH of the water was 5.5. The cell and the apparatus for the measurements of impedance were the same as those used in the previous paper [4]. The distance between two pt-pt electrodes (each 1 cm^2 in area) was fixed at $d=0.225$ cm, since, as indicated in paper I [4], the observed capacitance and conductance at this electrode distance were not very different from those of extrapolated values to $d=0$ cm. When necessary, the impedance of the membrane was evaluated by vectorial subtraction of the impedance of the solution phase from the observed overall impedance. All measurements were made in an air oven regulated at 25 °C.

For the sake of comparison, the electrical characteristics of bilayer black membranes made of the DOPH with cholesterol were measured. Cholesterol, analytical reagent grade (Merck & Co., Inc.), was used as delivered. The bilayer black membrane was formed in an aqueous salt solution according to the method reported by Hanai, Haydon and Taylor [2] across a hole 1 mm in diameter punched through in a Teflon pot with the use of a mixture of DOPH and cholesterol dissolved in n-decane. The n-decane was purified by passing through an alumina column, which was rinsed with 1 N HNO_3 and activated by heating at 110 °C overnight. After the membrane became black, the area of the film was measured by a microscope. The measuring apparatus of impedance for the bilayer black membrane was the same as that used for the filter paper systems. Measurements for the black film were performed at room temperature.

Results

Effects of Pore Size ϕ and of Adsorbed Quantity Q of DOPH

Fig. 2 shows the frequency dependence of C_p for the system of $\phi = 1.2\ \mu$ and $Q = 2.25\ \mathrm{mg/cm^2}$ in various concentrations of the external KCl. Just as in Fig. 1, the present system displayed a discontinuous change in C_p at a certain salt concentration when the external KCl concentration was increased successively. Hereafter, we refer to the salt concentration c_s at which C_p in the low-frequency region displays a discrete change as the "critical salt concentration", and denote it by c_s^d.

Frequency dependencies of C_p for different Q of DOPH with a given pore size were essentially the same as those shown in Fig. 2, except that the c_s^d depended on the amount of Q, when Q was relatively small. The value of Q studied here were between $1.5\ \mathrm{mg/cm^2}$ and $6.0\ \mathrm{mg/cm^2}$. Fig. 3 shows the values of C_p at a frequency of 100 Hz (denoted by C_p^{100}) as a function of KCl concentration with various Q. In this figure, both the ordinate for C_p^{100} and the abscissa for c_s are plotted in logarithmic scale. This figure illustrates that the value of c_s^d increases with increase of Q, and approaches a KCl concentration of about 40 mM; when Q is larger than $2.25\ \mathrm{mg/cm^2}$, c_s^d stays practically constant at 40 mM for the system of $\phi = 1.2\ \mu$. Fig. 3

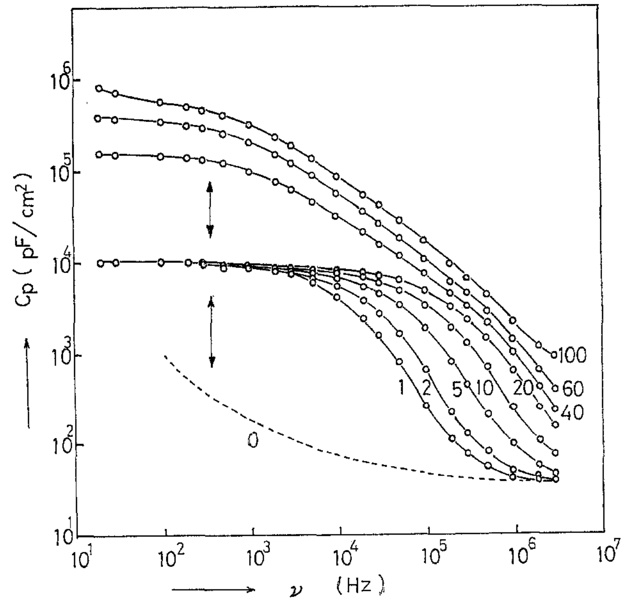

Fig. 2. Relation between C_p and ν with various KCl concentrations. $\phi = 1.2\ \mu$; Q of DOPH $= 2.25\ \mathrm{mg/cm^2}$. Broken line shows the C_p with no membrane with the same electrode distance

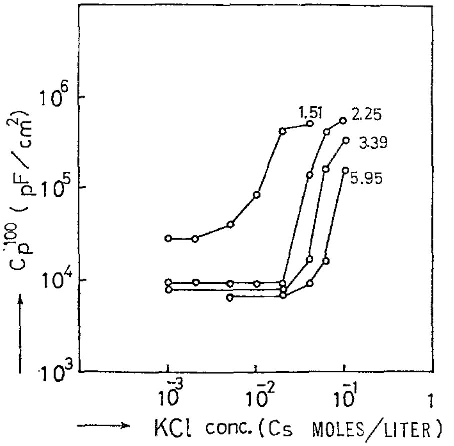

Fig. 3. C_p at the applied frequency 100 Hz (C_p^{100}) as functions of KCl concentration (c_s) at pH 5.5. Number on each line shows the quantity of DOPH adsorbed by cellulose ester Millipore filter in mg/cm^2. $\phi = 1.2$ μ; temperature = 25 °C

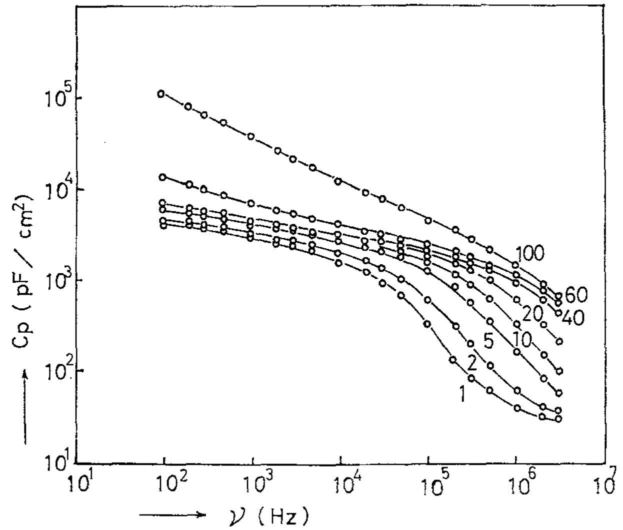

Fig. 4. Relation between C_p and ν with various concentrations of KCl. Cellulose ester Millipore filter; $\phi = 100$ nm; Q of DOPH = 2.94 mg/cm^2; pH = 5.5; temperature = 25 °C

also indicates that the discrete jump for C_p^{100} at c_s^d becomes less steep with increase of Q.

To show the effect of ϕ of the filter paper used, Figs. 4 and 5 show two examples of the frequency dependence of C_p's for systems of $\phi = 100$ and 10 nm with $Q = 2.94$ and 1.72 mg/cm^2, respectively. Fig. 4 shows that a discontinuous increase in C_p in the low-frequency region takes place when

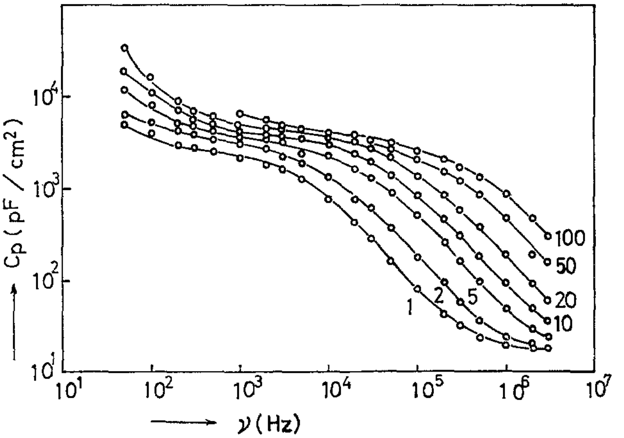

Fig. 5. C_p vs. ν relations with various KCl concentrations for the system of $\phi = 10$ nm, and $Q = 1.72$ mg/cm^2. pH = 5.5; temperature = 25 °C

the concentration of the external KCl solution is raised to 60 mM, whereas Fig. 5 shows no discrete change in C_p with increase of c_s. It has been found that the value of $c_s{}^d$ is roughly independent of ϕ of the filter paper so far as the Q of DOPH exceeds some value, and that $c_s{}^d$ is lowered with decrease of ϕ when Q is small. Figs. 4 and 5 also illustrate that the dependence of C_p on frequency with small ϕ is different from that observed in Figs. 1 and 2; i.e., C_p at a low-frequency region decreases with ν even in a dilute salt solution.

Effects of pH in the External Solution and of Species of Filter Papers

Recent studies of bilayer black membranes of natural phospholipids have shown that the ability of formation of lipid black films and their electrical characteristics such as DC resistance, electric capacitance, breakdown voltage, etc., depend on the value of pH and on the ion species in the external solution [8, 9, 12]. In the case of DOPH black bilayer membranes, the electric capacitance was found to be independent both of the pH value and of the salt concentration in the external solution, and stays constant at 0.35 ± 0.03 μF/cm^2 so far as the bilayer membrane was formed. It must be noted, however, that the bilayer membrane of DOPH was not able to form in an aqueous KCl or NaCl solution that was less than a few mM in concentration. As for the natural phospholipids [12, 13], the cholesterol was indispensable for the formation of the DOPH bilayer membrane. Furthermore, the mixture of DOPH and cholesterol (2:3 in mole ratio) was not capable of forming a bilayer membrane in an acidic solution whose pH was less than 4.0. Contrary to these characteristics of the DOPH bilayer

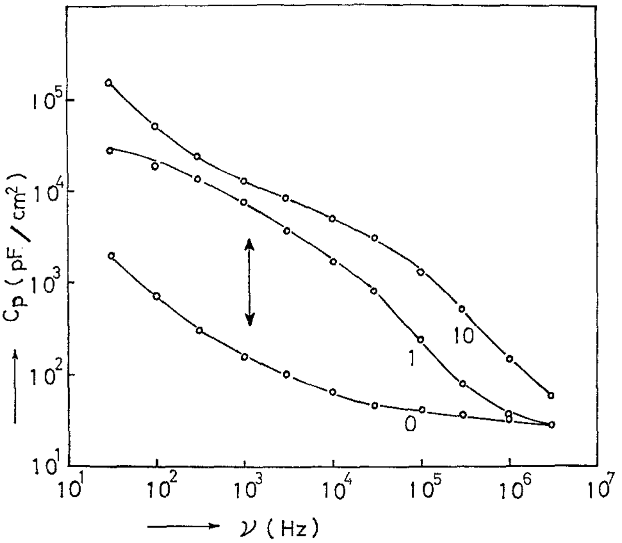

Fig. 6. Relation between C_p and ν for the system of nylon filter-DOPH-cholesterol in NaCl solution. $\phi = 1.0\ \mu$; $Q = 4.6\ \text{mg/cm}^2$ of 3:1 in weight mixture of DOPH and cholesterol. Number on each line shows the mM concentration of NaCl. pH = 5.5; temperature = 25 °C

membrane, the frequency-dependent C_p of the Millipore-DOPH membrane was observed even in a salt solution of low pH with no appreciable difference from that in a neutral solution. The c_s^d was about 40 mM for KCl or NaCl in an acidic solution of pH 3.0, and no frequency dispersion of C_p was observed in a salt-free solution with pH 3.0 as in the pure water at pH 5.5.

It is interesting to note that the frequency-dependent C_p as observed in Figs. 1, 2, 4, and 5 could not be obtained when a nylon Millipore filter was used as a substrate instead of the cellulose ester Millipore filter, in wide ranges of salt concentration, of pH value, and of adsorbed quantity of DOPH. Frequency dependencies of C_p and G_p were similar to those obtained with filter paper alone, i.e., the broken line in Fig. 2. However, when a mixture of DOPH and cholesterol (3:1 in weight ratio) was used with nylon filter paper, the frequency-dependent C_p's were observed in a salt solution. The observed C_p for the system of nylon filter-DOPH with cholesterol in a dilute NaCl solution at pH 5.5 was different from those obtained with the cellulose ester Millipore-DOPH system with the same pore size and salt concentration. It was noted that C_p's decreased appreciably with increase of ν, which compared with the system of cellulose ester Millipore-DOPH in a concentrated KCl solution higher than c_s^d, and that the discrete variation in C_p was not observed with successive increase of c_s. For example, Fig. 6 shows the frequency-dependent C_p's of the nylon filter-DOPH-cholesterol system plotted against ν. Here $\phi = 1.0\ \mu$, $Q = 4.6\ \mathrm{mg/cm^2}$ with 3:1 in weight mixture of DOPH and cholesterol in 1 and 10 mM KCl solution. The C_p's disappeared when the membrane was placed in the pure water, and recovered reversibly in the presence of a salt species.

Theoretical

Equations for Capacitance and Conductance of the Membrane

In paper I [4], we proposed a theoretical model for explaining the frequency-dependent capacitance and conductance of the Millipore filter impregnated with phospholipid, where the filter paper was considered to be an array of uniform cylindrical capillaries having equal cross-sectional area. The lipid adsorbed in the filter paper was assumed to form a number of bilayer membranes in the capillaries with a distribution function f_n representing the fraction of capillaries having n bilayer films. Then we have

$$\sum_{n=0}^{\infty} f_n = 1. \tag{1}$$

Taking into account the fact that the specific electrical conductance of DOPH bilayer membrane is negligibly small compared to that of the external electrolyte solution, we obtain the following expressions for the electric capacitance (C_p) and conductance (G_p) of the membrane [4]:

$$C_p = C_0^0 + \sum_{n=0}^{\infty} \frac{f_n (C/n)}{[1 + \omega^2 (C/n)^2 R_0{}^2]}, \tag{2}$$

$$G_p = \sum_{n=0}^{\infty} \frac{f_n \omega^2 (C/n)^2 R_0{}^2}{R_0 [1 + \omega^2 (C/n)^2 R_0{}^2]}. \tag{3}$$

Here, ω is the applied angular frequency defined by $\omega = 2\pi\nu$, C is the electric capacitance of unit area of lipid bilayer membrane, C_0^0 and R_0 (ohm·cm^2) are the capacitance and electrical resistance of the aqueous salt solution, respectively. Eqs. 2 and 3 imply that C_0^0 and R_0 for a given electrolyte concentration can be determined from the experimental values of C_p and G_p at high-frequency limit, $C_p{}^\infty$ and $G_p{}^\infty$; i.e.,

$$C_p{}^\infty = C_0^0, \quad \text{and} \quad G_p{}^\infty = \left(\sum_{n=0}^{\infty} f_n\right) \Big/ R_0 = 1/R_0. \tag{4}$$

On the other hand, the observed values of C_p and G_p at low-frequency limit, i.e., $C_p{}^0$ and $G_p{}^0$, give information about the distribution function f_n for the system in question; i.e.,

$$C_p{}^0 = C_0^0 + \sum_{n=1}^{\infty} (f_n/n)\, C, \quad \text{and} \quad G_p{}^0 = f_0/R_0. \tag{5}$$

Further information about f_n is obtained by analyzing the frequency dependencies of C_p and G_p for a given pair of membrane and electrolyte.

The theoretical model given above may be criticized in some respects. For example, one may consider it to be far from realistic to replace a complex matrix of zigzag channels in the actual porous membrane by an array of straight cylindrical capillaries parallel to the membrane thickness. However, as will be discussed in detail in a subsequent paper, the capillary model can be discarded with no alteration for the expressions for C_p and G_p given by Eqs. (2) and (3). Furthermore, we noted that no difference may be observed in C_p or G_p for a fixed f_n between the case where discrete bilayers are formed in a capillary and the case in which a globular micelle or an oil droplet plugs up a capillary so far as the total thickness of the oil phase in the capillary is the same. In other words, the Millipore-DOPH membrane in a given concentration of the external salt solution can be characterized by the f_n as far as the impedance of the membrane is concerned. The other membrane phenomena, e.g., membrane potential, ion and water permeability, are also closely related to the f_n of the membrane as will be reported in a forthcoming paper (M. Yoshida, N. Kamo and Y. Kobatake, *in preparation*).

Distribution Function f_n and Comparison with Experiments

As pointed out in paper I [4], the observed C_p for the system of $\phi = 5$ μ with low KCl concentration (between 1 and 20 mM) was well represented by a simple δ-function for f_n. When the external KCl concentration was raised to 40 mM or more, however, the frequency dependence of C_p could not be expressed by the δ-function; instead, broader distribution for f_n had to be taken into consideration. We have found that the observed C_p's in a concentrated KCl solution ($c_s \geqq 40$ mM) are fitted well by use of the following f_n for the system of $\phi = 5$ μ and $Q = 2.20$ mg/cm^2.

$$f_n\colon \begin{cases} f_0 = 0.357 & \text{for } n = 0 \\ f_m = 0.15 & \text{for } 1 \leqq n \leqq m = 4 \\ f_N = 0.043 & \text{for } n = N = 25\,. \end{cases} \tag{6}$$

The f_n given by Eq. (6) is in contrast to that for a dilute electrolyte solution ($1\,\text{mM} \leqq c_s \leqq 20\,\text{mM}$) for the same membrane; i.e.,

$$f_n: \begin{cases} f_0 = 0.230 & \text{for } n = 0 \\ f_N = 0.770 & \text{for } n = N = 40. \end{cases} \tag{7}$$

Here f_n's must satisfy Eq. (1). The parameters m, N and the values of f_m and f_N are practically unique for a given pair of membrane and electrolyte with a fixed KCl concentration, and 5% deviations of these values given by Eq. (6) or (7) lead to an appreciable deviation from the experimental data of C_p and G_p. Note that the δ-function for f_n in the low salt concentration is split in two with a rise of salt concentration higher than $c_s{}^d$, i.e., one δ-function and a box-type f_n with small m. With the f_n given by Eq. (6), Eq. (2) is rewritten in the form;

$$C_p = C_0^0 + f_m \sum_{n=1}^{m} \frac{nC}{n^2 + (\omega\tau)^2} + \frac{f_N NC}{N^2 + (\omega\tau)^2}, \tag{8}$$

where τ is the critical relaxation time for a bilayer membrane with a given concentration of the electrolyte used, and defined by

$$\tau = CR_0. \tag{9}$$

The value of τ is inversely proportional to the c_s (*see* Eq. (8) in Ref. [4]) for a given pair of lipid and electrolyte, since C for a bilayer membrane of DOPH is independent of the external salt concentration. Therefore, if the distribution function of bilayers formed in the filter paper is not altered with the salt concentration, the observed C_p can be reduced to a single curve when C_p's are plotted against $\omega\tau$. Fig. 7 shows the comparison between theory and experiments for the system of $\phi = 5\,\mu$, $Q = 2.20\,\text{mg/cm}^2$ with various salt concentrations, where log C_p's are plotted against log $(\omega\tau)$. Solid lines numbered 1 and 2 represent theoretical curves with the f_n given by Eqs. (7) and (6), respectively; points show corresponding experimental data. Theoretical curves agree satisfactorily with experiments for various salt concentrations ranging from 1 to 20 mM for curve *1*, and from 40 to 200 mM for curve *2*. In other words, the bilayer membranes of DOPH formed in the filter paper change their f_n at the $c_s{}^d$ from one δ-function given by Eq. (7) to one δ-function and one box-type distribution given by Eq. (6) as shown in the bottom of Fig. 7. As noted above, the frequency-dependent C_p's disappear when the external salt solution is replaced with the pure water. In this case, f_n should be zero for all n. Note that these variations in f_n are reversible.

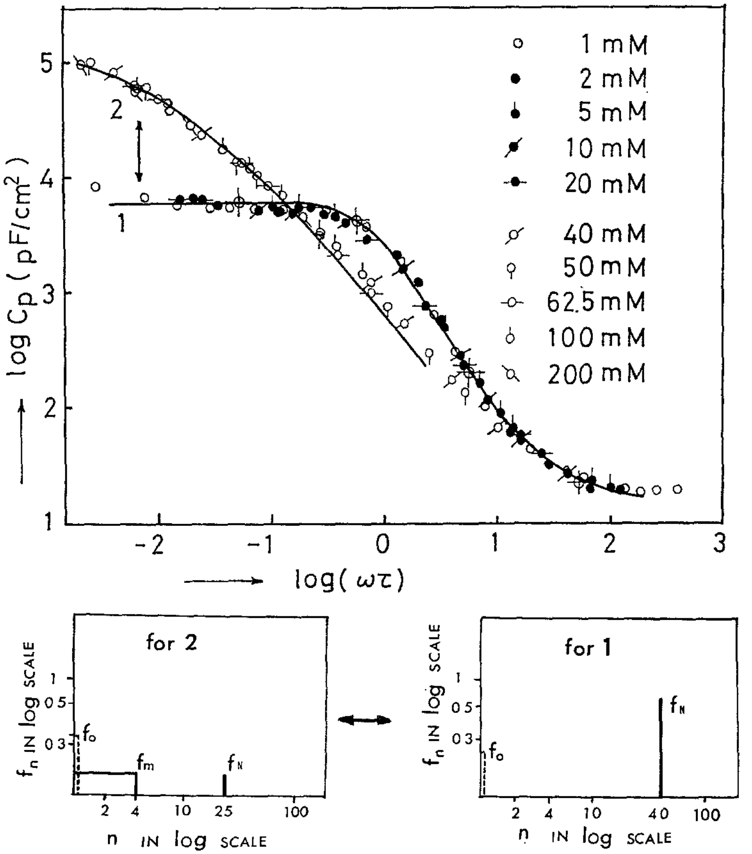

Fig. 7. *Top:* Comparison between theory and experiments for the system of $\phi = 5\ \mu$, $Q = 2.20$ mg/cm^2 with various concentrations of KCl solution, where log C_p's are plotted against log $\omega\tau$. Solid lines *1* and *2* represent theoretical curves with the use of the distribution function f_n given by Eqs. (7) and (6), respectively, and points are experimental data. The ⊕ marks on curve 1 show the calculated values with the use of a Gaussian distribution function given by Eq. (10). *Bottom, left:* The f_n given by Eq. (7) is plotted against n. *Right:* f_n vs. n relation given by Eq. (6). Here both f_n and n are shown in logarithmic scale

All systems studied here with varying ϕ's of the filter paper and Q of DOPH are represented quantitatively with f_n similar to that given by Eq. (6), i.e., one δ-function and one box-type f_n, although the values of m, N, f_m and f_N depend on ϕ and Q. For illustration, a theoretical curve is compared with experiments in Fig. 8 for the system of $\phi = 10$ nm and $Q = 1.72$ mg/cm^2 in a variety of KCl concentrations. In this case, the box-type distribution for small m appeared even in a low salt concentration. This box-type distribution is responsible for the fact that the observed C_p

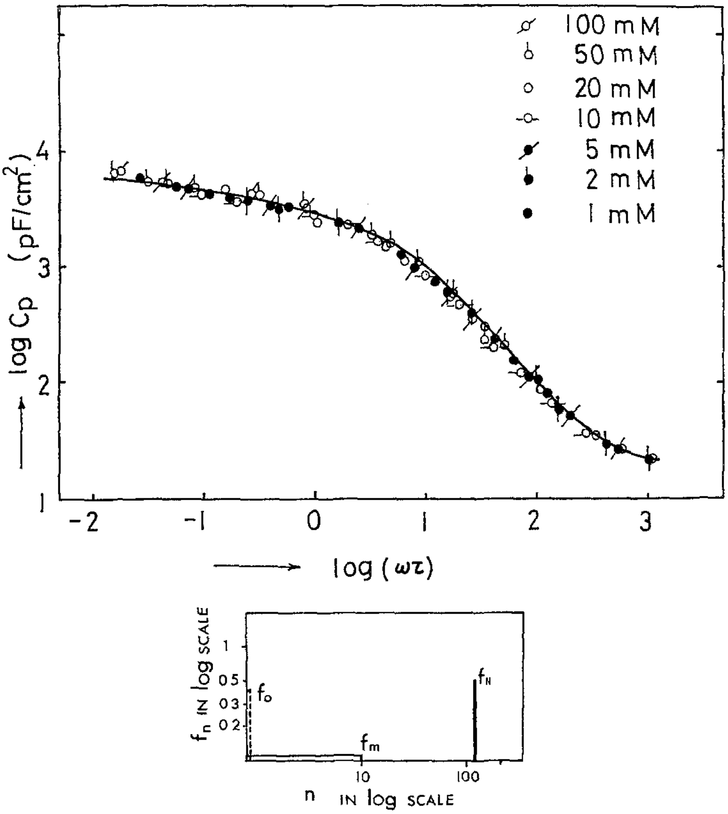

Fig. 8. *Top:* Comparison between theory and experiments for the system of $\phi = 10$ nm, $Q = 1.72$ mg/cm^2 with various salt concentrations, where log C_p's are plotted against log $\omega\tau$. Solid line represents theoretical curve with the use of one δ-function with one box-type distribution function, and points show experimental data for C_p. *Bottom:* The f_n as a function of n used for calculation; both in logarithmic scales

decreases with increase of ν even in the low-frequency region (*see* Figs. 4 & 5). The f_n is not altered for this system with increase of the external salt concentration. This implies that the box-type distribution for f_n appeared even in a dilute salt solution when ϕ is as small as 10 nm.

Discussion

The following three experimental facts observed for C_p in the present system rule out the view that the frequency-dependent C_p stems from the electrical charge of the phosphate group of DOPH. (*1*) C_p in the low-frequency region decreases with increase of adsorbed DOPH; (*2*) C_p^0

increases with increase of salt concentration; and (*3*) C_p's are independent of pH in the external salt solution at a fixed concentration. Thus the interpretation for C_p proposed by Ilani [3] for the system of a Millipore filter saturated by bromobenzene is not applicable to the Millipore-DOPH system. Ilani assumed that the surface charges between oil phase and aqueous salt solution are responsible for the frequency-dependent C_p for his membrane. On the contrary, the experimental data on C_p and G_p presented here and in paper *I* [4], along with the other electrochemical properties, are interpreted consistently by the theoretical model described above.

The system of nylon filter paper with DOPH does not give rise to any frequency dispersion of C_p in any salt concentration, which is in contrast to the cellulose ester filter with DOPH system. This difference in the observed impedance between nylon and cellulose ester filters may be attributed to the difference in the interactions between nylon and cellulose ester constituting the filter papers against DOPH. When the DOPH impregnated in the nylon filter paper contains cholesterol, the bilayer films are formed in the filter paper, and the system displays a large frequency-dependent C_p (*see* Fig. 6). Note that cholesterol is indispensable for the formation of lipid black membranes. It is clear that the cellulose skeleton of filter paper plays a role in the impedance behavior of the Millipore-DOPH systems under study.

Considering the characteristics of DOPH bilayer membranes described in Materials and Methods, together with the reversible changes in f_n for Millipore-DOPH systems, it may not be unreasonable to assume that the δ-function with large N represents oil droplets of DOPH impregnated in the filter paper, whereas the box-type f_m with small m may be considered as the bilayer films formed in the skeletons of the filter paper. The oil droplets are thinned when the c_s reaches the c_s^d, and transform to a number of bilayer membranes. On the other hand, the oil droplets spread on the capillary wall when c_s approaches zero. In other words, the adsorbed DOPH sticks on the cellulose skeleton of the filter paper when the external solution contains no electrolyte. With increase of the c_s, the DOPH on the Millipore skeleton piles up in the void space of the filter paper and plugs the pore as oil droplets. Further increase of the external c_s leads to the thinning of the oil droplets, and some of the droplets transform to bilayer films. These variations in conformations of DOPH adsorbed in the filter paper are responsible for the variations of f_n with c_s, which in turn lead to the discrete variation of the observed C_p and G_p of the membrane.

It is important to note that the δ-function representing oil droplets in the filter paper can be replaced by a Gaussian f_n with no appreciable change in the calculated frequency dependencies of C_p and G_p, provided that the half-width of the Gaussian distribution is not too broad. The ⊕ marks on curve 1 in Fig. 7 show the calculated results with the use of the following Gaussian f_n instead of Eq. (7) for the same system in the figure.

$$f_n: \begin{cases} f_0 = 0.230 \\ f_n = 0.216 \exp[-(n-60)^2/4]. \end{cases} \tag{10}$$

As seen in Fig. 7, no difference in the calculated C_p's is observed between Eqs. (7) and (10) for f_n, and both equations agree satisfactorily with corresponding experimental data. This implies that the oil droplets (or micelles) formed in the filter paper may have a distribution in their sizes.

As seen in the text, the experimental data of impedance for the membrane under various conditions can be represented quantitatively in terms of the relatively simple f_n of the lipid bilayers formed in the filter paper. However, the agreement of the theoretical equations for C_p and G_p with experiments does not always support the correctness of the underlying assumptions. The final justification of the theoretical model is left for future work wherein the presence of lipid bilayers and/or oil droplets in the filter paper can be detected by direct means.

This work was supported in part by the grant-in-aid for special projects in biophysics from the Ministry of Education, Japan. One of the authors (M.Y.) is indebted to Yukawa Scholarship Foundation for a fellowship at Osaka University, where the study reported in this paper was initiated. The authors wish to thank Drs. T. Hanai and A. Irimajiri at Kyoto University for stimulating discussion. We also thank Dr. T. Hata at the Tokyo Institute of Technology for his guidance in the synthesis of DOPH.

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