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Two Types of Reconstituted Model Membranes as Viewed from A. C. Impedance

Shigeru MORITA*, Koji ASAMI** and Tetsuya HANAI**

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Characteristics under alternating current supply on two types of reconstituted model membranes were examined. One type of the reconstituted membranes was rat brain microsome incorporated lipid membrane, which was thick "colored membrane". Another type was reconstituted microsomal membrane, which was thin black membrane formed from only the microsomes. A system of the colored membrane showed the dielectric behavior of a series combination of three frequency-independent phases: hydrophilic surface layer, hydrophobic core layer and aqueous solution around the membrane. On the other hand, a system of the black microsomal membrane was understood to show the structure similar to the colored membranes. The specification of the surface layer, however, was difficult owing to the too small capacitance value of the hydrophilic surface layer compared with the value of the hydrophobic core layer.

KEY WORDS: A. C. impedance/Reconstituted model membrane/Hydrophilic surface layer/Hydrophobic core layer

1. INTRODUCTION

In order to study the function of biological membranes, bioactive materials incorporated bimolecular lipid membranes (BLM) have been used as "reconstituted model membrance". A variety of results were reported on the ion transport mechanism such as ion channels by means of direct current (D. C.) supply[1, 2]. On the other hand, behavior of the membrane in alternating current (A. C.) supply has not been elucidated still now.

The BLM consists of a less conductive hydrophobic core layer and a more conductive hydrophilic surface layer. The a. c. measurement of membrane capacitance is a useful method to estimate membrane thickness[3]. The thickness, however, reflects only the hydrophobic layer exclusive of the hydrophilic surface layer. Since the contribution of the surface layer is very small to date owing to it's extreme thinness, it is difficult to specify the surface layer by the a. c. measurement. Thus, specification of the surface layer has been carried out by indirect methods or by modification of the surface layer. Clowes, Cherry and Chapman[4] explained the surface layer in cerebroside (one of galacto-phospholipids) BLM from the difference between the capacitance measured at 1kHz of a. c. supply and the capacitance obtained from the time constant of decay of the transient current by d. c. supply. Picker and Amosh[5], and Zimmermann, Ashcroft, Coster and Smith[6] observed directly the surface layers in the

* 森田 滋: Department of Pharmacology, Hyogo College of Medicine, Nishinomiya, Hyogo 663, Japan.

** 浅見 耕司, 花井 哲也: Laboratory of Dielectrics, Institute for Chemical Research, Kyoto University, Uji, Kyoto 611, Japan.

modified BLM on which polar ligands are adsorbed.

On the other hand, little work has been carried out by means of a. c. impedance measurement on the reconstituted model membranes containing cell materials. In this study, attempt was made to specify the surface layers in such reconstituted model membranes by using two different types of membranes. One is comparatively thick "colored membranes", which is a state on the way to forming the thin black membrane. Another is thin black reconstituted membranes.

Materials used for the two kinds of membranes are rat brain microsomes. The microsomes are a crude cell membrane fraction and contain many physiological function. Furthermore, since the microsomes contain a large quantity of lipids (approximately, 60% of the microsomes, w/w), physicochemical properties are much alike PL's. Therefore, the microsomes have the ability to form thin black membranes similar to the BLM. Thus, we call the membranes formed from only the microsomes "black microsomal membrane", which are the second kind membranes in this study.

2. EXPERIMENTAL

2.1 Materials

DOPH (dioleoyl phosphate) The synthetic neutral lipid DOPH, which was used as a phospholipid analogue, was supplied by M. Yoshida[7]. The DOPH was stored at −20°C.

Microsomes Microsomes were fractionated from rat brain (wistar male rat 200-250g) in homogenize solution containing 0.1% deoxycholate based on the method of Jörgensen[8]. The fractionated microsomes were washed with cold distilled water in order to remove detergent. The microsome pellets were resuspended in distilled water and were lyophilized. The lyophilized microsomes were stored at −20°C.

n-Decane N-decane was purified by passing through an alumina column.

2.2 Membrane Formation

Colored membranes DOPH and microsomes were suspended in n-decane (5% and 1% w/w, respectively). Membranes were formed on the aperture on teflon pot in an aqueous solution from the suspended solution by the brush method for BLM formation[9]. The aqueous solutions were 1-1000mM KCl, 20mM Tris-HCl and pH 7.4. "Colored membrane" formation were confirmed through the steady state of capacitance value at 1kHz associated with the observation by microscope.

Black microsomal membranes Only the lyophilized microsomes were suspended in n-decane (5%, w/w). The decane solution was sonicated at 40W for 5sec.×3times. Membranes were formed from the n-decane solution by the same method as BLM formation[10]. The pH value of the KCl aqueous solution was set up at 6.8 different from the case of the colored membranes. The lower pH value was set up to forward thinning. The area of the membranes was measured with microscope. All the experiments were carried out at room temperature (23°-25°C).

2.3 Electrical Measurements

Capacitance and conductance were measured over a frequency range of 20Hz-3MHz with TR-1C measuring system of Ando Electric Co., Ltd.

2.4 Calculation of Capacitance and Conductance

Capacitances and conductances of each phase were calculated from the dielectric data by means of a method developed by Kiyohara et al.[11]. on a series combination model of three phases.

3. RESULTS

3.1 Microsomes Incorporated Colored Membranes

The frequency dependence of capacitance and conductance for the system of the membranes in 100mM KCl solution is shown in Fig. 1. Complex plane plots of the capacitance and conductance from the data in Fig. 1 are characterized by two circular arcs as shown in Fig. 2 These results can be explained in terms of a series combination of three phases: two phases in the membrane and the other corresponding to the aqueous phase. The whole system is represented by a series combination of three groups of parallel equivalent capacitance and conductance as shown in Fig. 3(a). The frequency dependence of capacitance and conductance for the whole system are

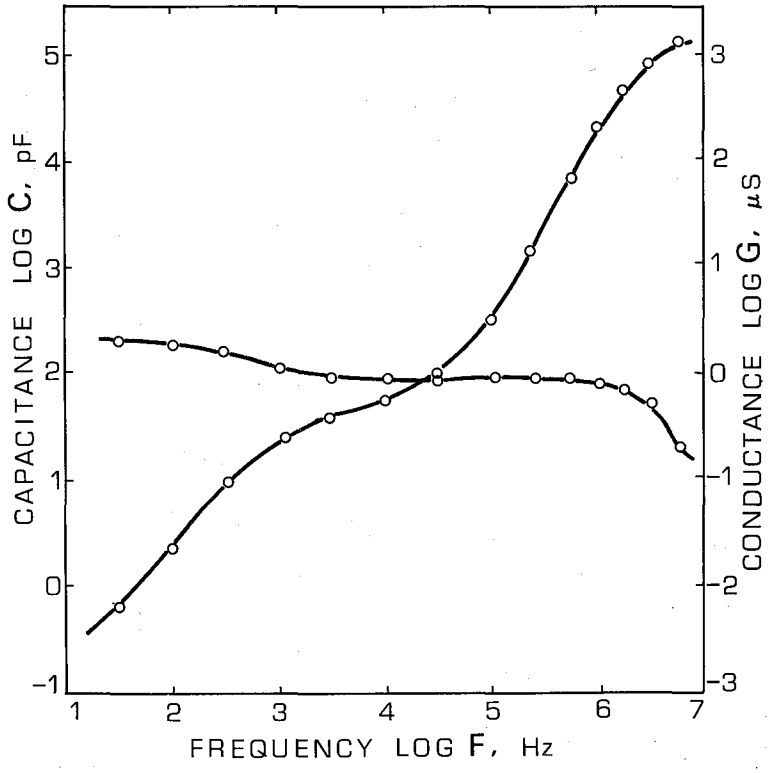

Fig. 1. Frequency dependence of capacitance and conductance of the microsome incorporated colored membrane in 100 mM KCl, pH 7.4 solution.

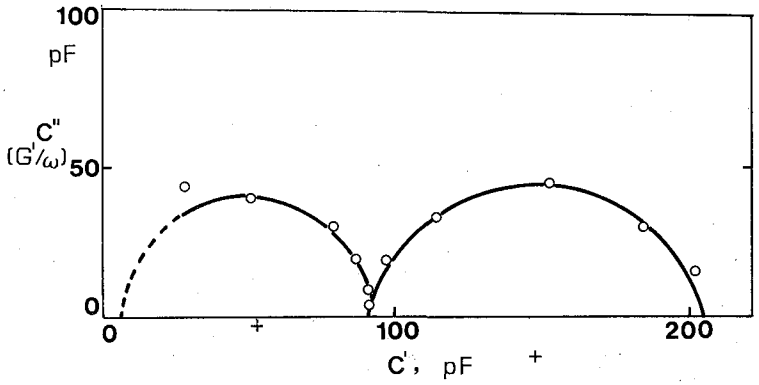

Fig. 2. The plots of the imaginary part against the real part of the complex capacitance for the data on a colored membrane system given in Fig. 1.

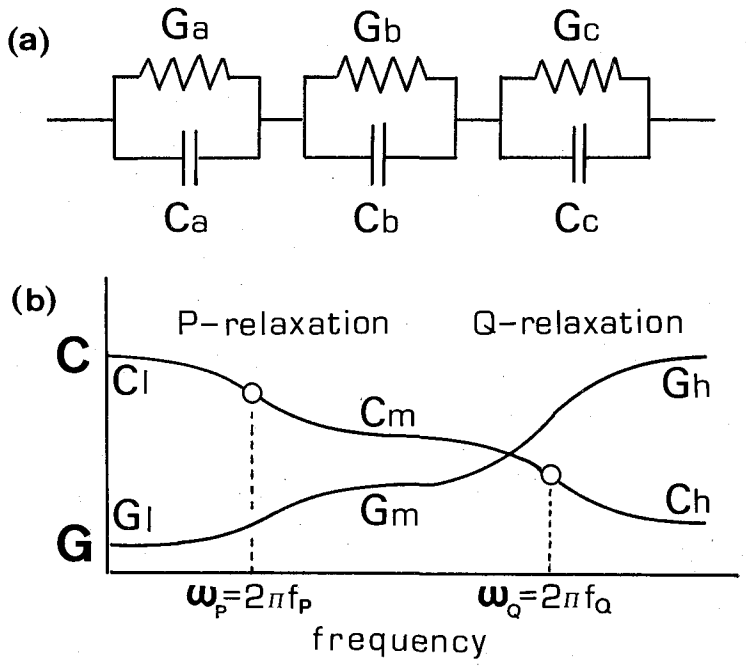

Fig. 3. (a) A series combination model of three *C-G* circuits. (b) Two single dielectric relaxations *P* and *Q* caused by the frequency dependency of a series combination of three capacitance (C) and conductance (G) circuit models.

schematically shown in Fig. 3(b). Capacitance and conductance of each component calculated from the frequency dependence data of the whole system in 1-1000mM KCl are shown in Table 1. As shown in Table 1, both of the capacitance and conductance values of phases *a* and *b* seem not to be dependent KCl.

3.2 Black Microsomal Membranes

The frequency dependence in 100mM KCl is shown in Fig. 4. Magnified representa-

Table 1. Capacitance (C) and conductance (G) of each phase in the microsome incorporated colored membranes.

		phase (a)	phase (b)	phase (c)
C (pF)	1 mM KCl (n=3)	92±13	319±25	20±3
	10 (n=3)	117±26	282±.39	21±.5
	100 (n=5)	125±32	270±74	22±3
	1000 (n=3)	130±24	252±32	22±2
G (μS)	1 mM KCl	0.41±0.03	2.3±0.33	141±25
	10	0.16±0.14	1.8±0.18	336±54
	100	0.26±0.18	1.7±0.51	1,769±91
	1000	0.15±0.04	2.1±0.42	16,000 (?)

Each solution contained 20mM Tris. Each value is the mean with S. D.

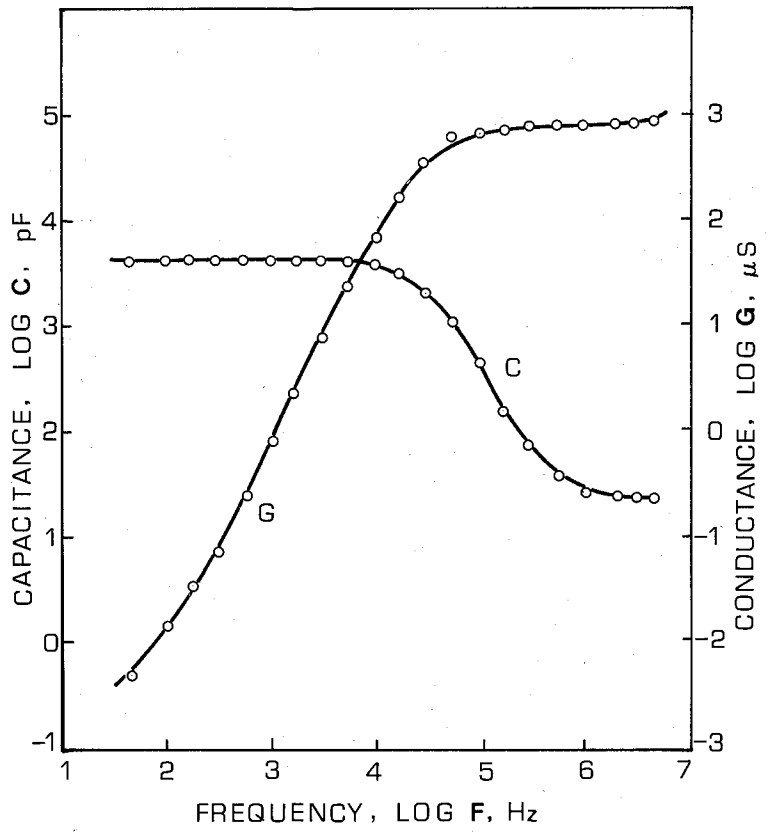

Fig. 4. Frequency dependence of capacitance and conductance of the black microsomal membrane in 100 mM KCl, pH 6.8 solution.

tion of the frequency dependence at low frequency is shown in Fig. 5. Figure 6 shows a low frequency part of the complex plane plots of the capacitance and the conductance from the data in Fig. 4. Capacitance and conductance of each component are calculated, being shown in Table 2. Since G_m values could not be read from the data

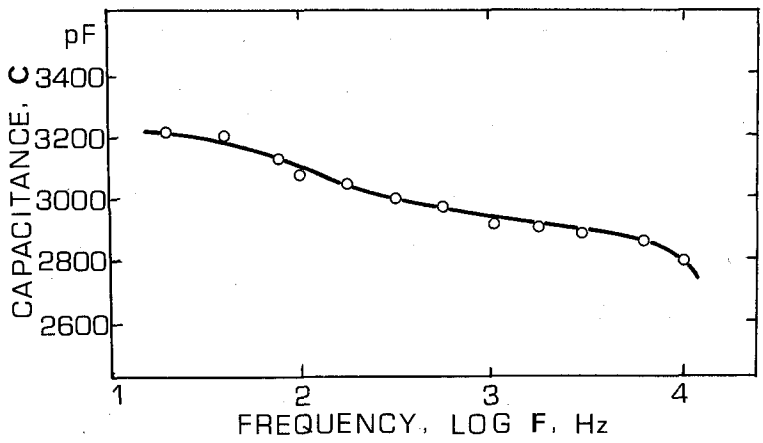

Fig. 5. Magnified representation of the frequency dependence given in Fig. 4.

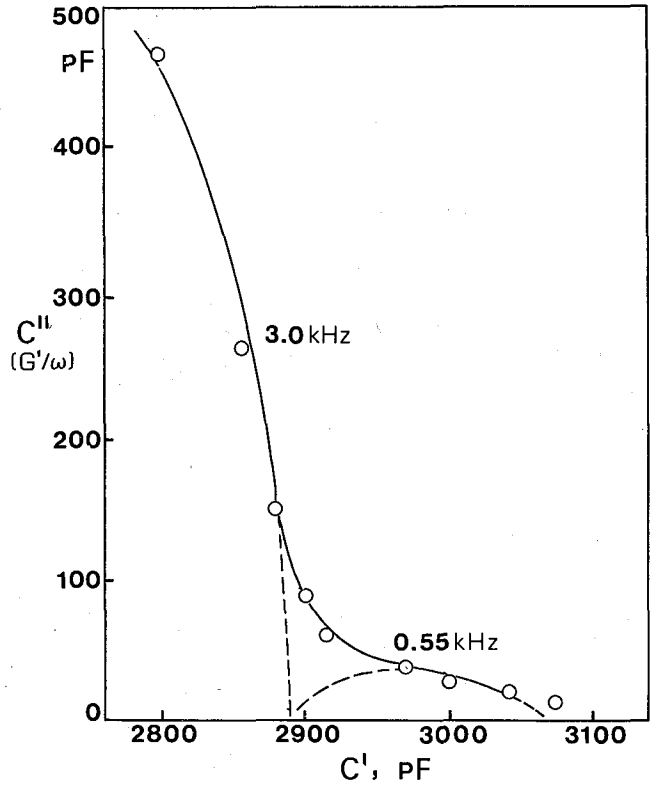

Fig. 6. A low frequency part of the plots of the imaginary part against the real part of the complex capacitance for the data on a black microsomal membrane system given in Fig. 4.

of frequency dependence, they were calculated from the following equation, $2\pi f_p = (G_m - G_l)/(C_l - C_m)$[12]

Table 2. Capacitance (C) and conductance (G) of each phase in the black microsomal membranes.

		phase (a)	phase (b)	phase (c)
C (pF)	100 mM KCl (n=3)	3600±431	>110,000	18±2
G (μS)		0.0029±0.0012	380±167	1010±8

Each value is the mean with S. D.

DISCUSSION

Colored Membranes

The results obtained from the colored membranes will permit us to analyze the data by means of the series combination model of three phases, In the three phases, the membrane consists of two components with lower conductance corresponding to phase a and phase b in Fig. 3. The phase c is supposed to be an aqueous phase by reason of the smallest capacitance and the highest conductance value. By comparison between a and b, the lower conductance phase a is supposed to be the hydrophobic core region. Thus, it is reasonable that the conductance of the phase a is not proportional to KCl concentration of the aqueous phase. On the other hand, the comparatively conductive phase b can be concluded to be the hydrophilic surface region. One remarkable result about the phase b is that the conductance is not varied with KCl concentration. Thus, it is considered that the conductance of the hydrophilic surface layer may be carried by the intrinsic net charges.

Black Microsomal Membranes

The frequency dependence in the black microsomal membranes can just be shown extensively plotting on the ordinary interval scale. The width of capacitance change were only 2-3% of the values for the whole system. Accordingly, the two circular arcs in the complex plane plot is extremely indistinguishable, too. The phase a can be supposed to be the hydrophobic core layer by the capacitance values, which are approximately equal to that of usual BLM[9]. On the other hand, capacitance of phase b, which is presumed to be the hydrophilic surface layer, showed much large more than that of the colored membranes. The large capacitance value should indicate that the surface layer of black membrane is far thinner than the layer of colored membranes. The estimation is consistent with the conductance of phase b larger than the value in colored membranes.

From the comparison between the colored membranes and black membranes, the following conclusions are possible for the two layers in the microsomes contained reconstituted membranes. (1) On the colored membrane, phase separation to two layers of the amphiphilic materials does not proceed enough. However, by the incomplete state, the parameters of each phase show the suitable values for the experimental specification of the hydrophilic surface layer. (2) On the black microsomal membranes, the parameters of each phase ought to show the specific characteristics of the two phases, since the phase separation is complete. By the complete state, however, it is not easy for the parameters of each phase to show the suitable values for the experimental

direct characterization of the surface phase. It remains in studies of the furture to be proved to specify the surface layer more exactly and widely.

Roles of the hydrophilic surface layers of cell membranes are very important for the various physiological or pharmacological cell functions together with longitudinal transmembrane structure. Substantial phenomena occurring at the surface region are translocated to another side of membranes through the transmembrane structure in the hydrophobic core layer[12,13]. In the cell physiological study on such functions, the direct observation of the surface layer is a useful work. Therefore, a development of the study using the reconstituted membranes and the dielectric measurement is expected.

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