Biochimica et Biophysica Acta, 733 (1983) 181–185
Elsevier

BBA Report

BBA 70111

CAPACITANCE OF BILAYERS IN THE PRESENCE OF LIPOPHILIC IONS

ARNOLD D. PICKAR and WILLIAM C. BROWN

Department of Physics/Environmental Sciences and Resources Program, Portland State University, Portland, OR 97207 (U.S.A.)

(Received March 29th, 1983)

Key words: Capacitance; Lipophilic ion; (Model membrane)

The capacitance of glycerolmonooleate and egg phosphatidylcholine bilayer membranes in the presence of NaCl solutions containing tetraphenylborate, tetraphenylarsonium or dipicrylamine ions has been measured using alternating current techniques over a wide range of frequencies (1–200 kHz). The concentrations of ions corresponded to the lower limits of conductance saturation. Similar determinations were also made with solutions containing no lipophilic ions. The experimental method used in this work requires correction of admittance measurements for the solution resistance in series with the membrane, as well as careful area determinations. In all cases membrane capacitance levels off at sufficiently high frequencies to values which are independent of frequency. The high-frequency capacitance, which is regarded as the 'geometrical capacitance' due to dielectric polarization, is practically unaffected by the presence of lipophilic ions. The results support the assumption made in other studies, such as in charge pulse investigations, that the adsorption of lipophilic ions at concentrations up to the saturation range does not have an important effect on the dielectric properties of bilayers.

Observations of the capacitance of lipid bilayers have proven fruitful in the past in answering a number of questions related to the structure, composition, and electromechanical effects in these artificial systems [1–3]. In almost all cases, however, values of specific capacitance which have been obtained are for membranes in the absence of doping substances such as any of the lipophilic ions or ion-carriers which have been used by various workers as probes of membrane structure [4]. Exceptions occur in studies of DeLevie and coworkers who obtained capacitance values for bacterial phosphatidylethanolamine/decane bilayers in the presence of the lipophilic ions tetraphenylborate [5] and dipicrylamine [6]. No special effort was made in these experiments, however, to determine whether the capacitance values were the same as those of similar but undoped membranes.

Bilayer studies utilizing lipophilic ions indicate that the fraction of the membrane surface which is occupied by these ions is probably not very large; in a densely packed situation the ions might be separated on the average by perhaps five phospholipid molecules. Nevertheless, especially in view of the electrostatic effects among these ions, it is interesting to question whether at concentrations corresponding to the onset of conductivity saturation the adsorbed ions significantly disrupt the membrane structure, perhaps to an extent which will be manifested as a change in membrane capacitance. In terms of conclusions drawn from experiments on charge transfer kinetics there is a particular reason to be interested in whether doping substances alter membrane capacitance. The charge-pulse technique, which has enjoyed much success in this area of research, requires an independently determined value of specific membrane capacitance in order to normalize derived values of

rate parameters [7]. It has been assumed in charge-pulse studies that the dielectric properties of the membranes are essentially unaffected by the inclusion of doping ions and carriers, but no systematic investigation bearing on this assumption has been done heretofore. The purpose of this communication is to report on such an investigation in which the effect on bilayer capacitance of several commonly studied lipophilic ions is examined for aqueous ion concentrations at the lower limit of the saturation region. We have restricted the number of lipid systems to two representative types.

In making measurements of capacitance of membranes incorporating charge transport systems it is essential that one distinguish between those effects which arise from the dynamics of the charge transfer process and those which are associated with dielectric properties, i.e., 'geometrical capacitance'. A method for separating these effects experimentally is possible using alternating current techniques; it relies on the fact that the geometrical capacitance is that capacitance observed at frequencies too high for the charge transfer mechanism to respond to applied alternating voltages.

Membranes were formed by brushing a solution of lipid in *n*-decane (2.5% w/v) over a hole (1.7 mm diameter) in a Teflon septum separating aqueous saline solutions of identical composition. The two representative lipids used in these experiments were glycerolmonooleate obtained from Sigma Corporation (St. Louis, MO), and a mixture of egg phosphatidylcholine with cholesterol (molar ratio 1 : 4). The latter lipids were extracted and/or purified in our laboratory as previously described [8]. The solutions of NaCl (RbCl, KCl, and LiCl in auxiliary experiments) were made with deionized water and maintained at a constant temperature of 25°C in the measuring cell.

During the thinning (blackening) process which gives rise to the stable bilayer structure, the membrane capacitance was continuously monitored at a frequency of 1 kHz. When, in the course of time, the capacitance was observed to be essentially constant over a period of about 1 minute, the membrane was flattened by adjusting the liquid level in one compartment until a minimum capacitance was obtained. At this time a sequence of 20 measurements of capacitance and conductance at frequencies spanning the range 1–900 kHz was carried out, as described below. Several runs of measurements of this types were carried out for each membrane, mostly within the first 5 min following blackening. Flattening of the membrane was checked and adjusted, if necessary, before each run. Several photographs of the flattened membrane were made during the course of these measurements from which the membrane's mean diameter was determined. The optical system used consisted of a Bausch and Lomb Stereo-Zoom 7 microscope with attached Polaroid camera and a fiber-optic illuminator (American Optical Corp. Model 11-80). The uncertainty in membrane area determinations was about $\pm$ 1.5%.

The basic instrument used for the electrical measurements was a Hewlett-Packard 4270 automatically balancing bridge modified for operation with external signal sources. Electrical connection to the saline solutions was via platinized platinum electrodes. Sinusoidally varying voltages were generated using integrated circuit chips (Intersil 8038 up to 200 kHz; Exar 205 at higher frequencies) and the frequencies were monitored with a Hewlett-Packard 5245L frequency counter. During each run the electronics was under the control of a microprocessor system (Motorola M6800) which also stored the admittance and frequency information for display and transfer to a larger computer for further processing. A single run of measurements over the entire frequency range takes approx. 45 s.

In order to extract membrane capacitance data from admittance measurements the effect of the solution resistance in series with the membrane has to be taken into account. This correction procedure as well as the method for determining solution resistance by extrapolation of admittance data to very high frequencies are given in detail elsewhere [9].

The variation of capacitance with frequency for a number of typical membranes after correction for solution resistance are shown in Fig. 1. For all membranes the capacitance levels off at higher frequencies to values which are essentially constant. For membranes in the presence of 0.1 M NaCl the range of frequencies for which we were able to obtain reliable capacitance measurements is quite restricted. A relatively large fraction of the

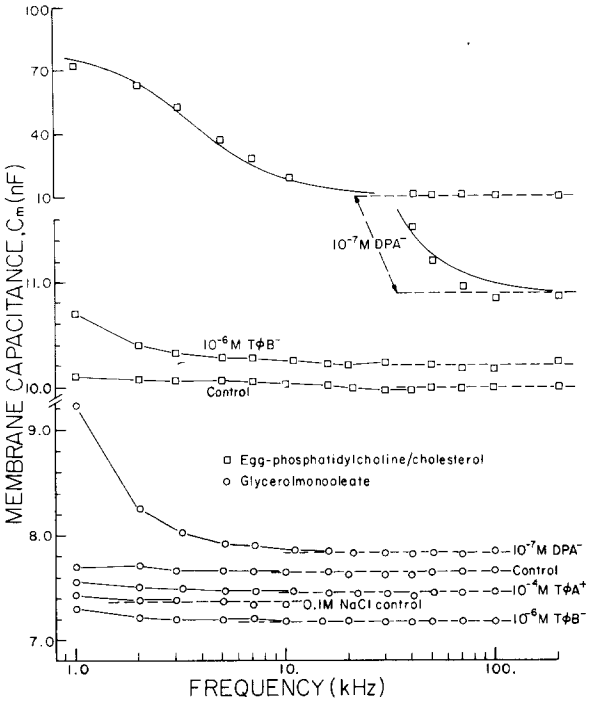

Fig. 1. Frequency dependence of membrane capacitance for some typical membranes discussed in this report. Experimental points indicate capacitance values obtained after subtraction of the aqueous solution resistance from membrane/solution impedance but before dividing by individual membrane area. Aqueous solutions were 1.0 M NaCl (unless otherwise indicated) containing the indicated concentrations of the following lipophilic ions: tetraphenylborate (TØB$^-$), tetraphenylarsonium (TØA$^+$), and dipicrylamine (DPA$^-$). 'Control' refers to membranes for which no lipophilic ions are present. In most cases points are joined by straight line segments for clarity; horizontal broken lines indicate average values of capacitance at high frequencies. The smooth curve drawn through the DPA$^-$ doped egg phosphatidylcholine membrane data is obtained using Eqn. 1 with $\beta = 19.2 \cdot 10^{-3}$ cm, $k_i = 1.13 \cdot 10^4$ $s^{-1} \gg k$, and $C_g = 10.88$ nF.

applied voltage appears across the solution resistance at such low ionic strengths, leading to unacceptable uncertainties ($\geq 1\%$) in the measurements above 10 kHz. However, up to this frequency we found no effect on higher-frequency capacitance of changing from 0.1 M to 1 M NaCl (for both undoped and tetraphenylborate-treated membranes). This lack of dependence on ionic strength is consistent with results obtained by Benz and Janko [1]. Thus we chose to do the majority of our measurements using 1 M solutions. Although the studies presented in detail here are restricted to systems involving NaCl solutions we have obtained data for undoped glycerolmonooleate bilayers in the presence of KCl, RbCl, and LiCl in connection with studies of carrier transport [10] which indicate that the nature of the alkali ion affects neither the frequency characteristics nor the capacitance at high frequencies appreciably.

Several authors [6,11] have given expressions and corresponding equivalent circuits which describe the expected frequency dependence of admittance for lipid bilayer membranes in the presence of lipophilic ions. These analyses assume that the membrane is a homogeneous slab of dielectric with well defined lipid/water interfaces across which ion transfer takes place by means of a simple adsorption-desorption process. For example, the analysis given by Ketterer et al. [11] leads to the following expression for the membrane capacitance as a function of frequency in the presence of a monovalent lipophilic ion:

$$C_m = C_g + \frac{F^2}{2RT} \frac{(2k_i\tau_0)\beta c}{1 + \omega^2\tau_{0^2}} \qquad (1)$$

where ω is the angular frequency, C_g is the geometrical capacitance arising from dielectric polarization in the lipid slab, k_i is a rate constant characterizing the process of charge translocation between interfaces, c is the concentration of lipophilic ions in the bulk aqueous solution, β is the partition coefficient describing adsorption of these ions on the membrane surface, and the time constant τ_0 depends on k_i and a rate constant k governing interfacial charge transfer according to

$$\tau_{o^{-1}} = k + 2k_i \qquad (2)$$

Eqn. 1 predicts that whereas for $\omega \simeq 0$ the membrane capacitance is larger than the geometrical capacitance C_g, it approaches that value at progressively higher frequencies. The analysis of DeLevie and co-workers [6], which takes into account diffusion of the permeant ions in the aqueous phase leads to a somewhat more complicated expression than Eqn. 1, but the conclusions regarding the asymptotic value of capacitance at high frequencies remains the same.

The decrease of capacitance with frequency of the sort predicted by Eqn. 1 is particularly evident

in the results for dipicrylamine-doped membranes, for which relatively short relaxation times involved in the charge transfer process give rise to strong kinetic effects in the frequency range of our experiments. By way of illustration we have drawn in Fig. 1 a theoretical curve based on Eqn. 1 which fits the capacitance data shown for a dipicrylamine-doped egg phosphatidylcholine/cholesterol membrane. In drawing this curve we have assumed that as the interfacial charge transfer is slow compared to translocation processes [12] it is reasonable to neglect parameter k in comparison to 2 k_i in Eqn. 2. The values of k_i and β which are required to fit the curve to the data are given in the caption; they are of the same magnitude as reported in the literature for dioleoylphosphatidylcholine/cholesterol membranes [12]. The value of C_g used is the asymptotic values of capacitance obtained by averaging capacitance values at frequencies above 50 kHz.

Table I summarizes our results for the specific geometrical capacitance of all the membrane systems we have studied, both undoped and doped with lipophilic ions. The values cited are averages (for at least three but usually more membranes) of the high frequency specific capacitance obtained by averaging capacitance data up to those frequencies for which accuracy of the electrical measurements is better than $\pm 1\%$. Membrane area determinations are the source of additional errors in the values of specific capacitance.

It is clear from Table I that apart from kinetic effects the presence of lipophilic ions in these typical bilayer systems does not significantly affect the membrane capacitance. This result thus supports the assumption made in charge-pulse studies that the undoped membrane capacitance may be used with impunity in normalizing experimental data. Likewise in alternating current studies involving ion transport systems it is likely in most cases that capacitance variations are mainly due to kinetic effects rather than to structural changes. Furthermore the experiments reported here lay the groundwork for further potentially interesting investigations, such as capacitance modifications when high surface densities of conductivity inducing substances are adsorbed [13] or for electrically charged membranes in contact with solutions of moderate ionic strength.

This work was supported in part by NIH Grant 5RO1 ES 937-08.

TABLE I

SPECIFIC CAPACITANCES AT HIGH FREQUENCIES OF BILAYER MEMBRANES IN THE PRESENCE AND ABSENCE OF LIPOPHILIC IONS

Control membranes refer to measurements for which no lipophilic dopant ions were present. Ions used were dipicrylamine (DPA^-), tetraphenylborate ($T\phi B^-$), and tetraphenylarsonium ($T\phi A^+$). Error intervals represent standard deviations among membranes. $t = 25°C$.

Solution	Frequency range (kHz)	C_m at high frequencies ($nF \cdot cm^{-2}$)
Glycerolmonooleate membranes		
0.1 M NaCl control	2– 7	364 ± 8
1.0 M NaCl control	10–100	380 ± 11
10^{-6} M $T\phi B^-$ in 0.1 M NaCl	2– 7	375 ± 7
10^{-6} M $T\phi B^-$ in 1.0 M NaCl	10–100	388 ± 8
10^{-4} M $T\phi A^+$ in 1.0 M NaCl	10–100	388 ± 2
10^{-7} M DPA^- in 1.0 M NaCl	20–100	388 ± 7
Egg phosphatidylcholine/cholesterol membranes		
1.0 M NaCl control	40–200	550 ± 2
10^{-6} $T\phi B^-$ in 1.0 M NaCl	40–200	543 ± 10
10^{-7} M DPA^- in 1.0 M NaCl	70–200	551 ± 5

References

1 Benz, R. and Janko, K. (1976) Biochim. Biophys. Acta 455, 721–738
2 White, S.H. (1977) Ann. N.Y. Acad. Sci. 303, 243–265
3 White, S.H. (1980) Science 207, 1075–1077
4 Läuger, P., Benz, R., Stark, G., Bamberg, E., Jordan, P.C., Fahr, A. and Brock, W. 91981) Q. Rev. Biophys. 144, 513–598
5 De Levie, R., Seidah, N.G. and Larkin, D. (1974) Electroanal. Chem. Interf. Electrochem. 49, 153–159
6 De Levie, R. and Vukadin, D. (1975) Electroanal. Chem. Interf. Electrochem. 62, 95–109
7 Benz, R., Läuger, P. and Janko, K. (1976) Biochim. Biophys. Acta 455, 701–720
8 Smejtek, P., Hsu, K. and Perman, W.H. (1976) Biophys. J. 16, 319–336
9 Pickar, A.D. and Amos, W.D. (1976) Biochim. Biophys. Acta 455, 36–55
10 Pickar, A.D. and Cox, K.L. (1983) Biophys. J. 41, 183(a)
11 Ketterer, B., Neumcke, B. and Läuger, P. (1971) J. Membrane Biol. 5, 225–245
12 Benz, R. and Läuger, P. (1977) Biochim. Biophys. Acta 468, 245–258
13 Hobbs, J. and Smejtek, P. (1983) Biophys. J. 41, 400a