Biochimica et Biophysica Acta, 455 (1976) 36–55

BBA 77478

ALTERNATING CURRENT STUDIES OF CHARGE CARRIER TRANSPORT IN LIPID BILAYERS

PENTACHLOROPHENOL IN LECITHIN-CHOLESTEROL MEMBRANES

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(Received March 23rd, 1976)

SUMMARY

Surface and interior electrical properties of lecithin-cholesterol bilayer membranes treated with the uncoupler pentachlorophenol have been determined on the basis of a.c. measurements over a wide range of frequencies (0.02 to 1000 kHz). The method used depends on accurately determining the resistance of the aqueous solution in series with each individual membrane by extrapolating admittance data to infinite frequency. Loss tangent vs. frequency curves are corrected by subtracting out a loss contribution which is present in untreated membranes and is due, presumably, to dielectric relaxation. The results, which are useful below 100 kHz, can be fitted to loss tangent curves computed for a three-element equivalent circuit consisting of frequency independent conductance-capacitance pairs, arranged in series to represent surface and interior properties of membranes. Interior conductances agree with net conductances obtained from d.c. measurements. The pH and concentration dependence of surface conductance is consistent with a scheme of transport in which a fixed number of surface binding sites are filled preferentially with neutral pentachlorophenol molecules, which in turn dissociate to supply protons to the aqueous phase. Surface capacitances range from 15 to 90 times that of interior capacitance and show a systematic increase with pentachlorophenol concentration at high pH, and a decrease with concentration at low pH.

INTRODUCTION

The electrical response of lipid bilayer membranes to the application of alternating voltages has been considered by various authors as a technique for investigating charge transport mechanisms in these structures. Several of these studies [1–3] have considered membranes in the presence of lipid soluble ions, a problem in which the transfer kinetics of only one charged species need be considered. Measurements of a.c. behavior as a function of frequency in the low audio range by de Levie et al. [3] confirm a model of lipid soluble ions in which the membrane is a

homogeneous phase coupled with surface adsorption and diffusional mass transport in the adjacent aqueous solutions. A.c. behavior for the case of carrier transport of a more general kind has been analyzed by Ait'yan et al. [4]. They derived expressions for membrane admittance at very high and very low frequencies, but gave no comparisons to experiment. As for membranes not treated with substances which enhance conductivity, the very low frequency work of Coster and Smith [5] is especially noteworthy. The results obtained by these authors. using a novel computerized technique, can be interpreted in terms of the underlying molecular structure of thin lipid membranes.

In all cases cited above it was found to be convenient to represent the membrane in terms of an equivalent circuit of capacitors and resistors, but there are differences as to the specific arrangement of these elements. Some authors place the "geometrical" membrane capacitance, which is measured at low frequencies, in parallel with all other elements representing membrane charge transport and interface processes. The other arrangement which has been used is the three element equivalent circuit originally suggested by Hanai, Haydon, and Taylor [6] to characterize the individual contributions of dipolar and hydrocarbon regions to membrane behavior. The general interpretation of this circuit, shown in Fig. 1, is that G_1 and C_1 are the conductance and capacitance characteristic of the processes taking place at the surface of the membrane, whereas G_2 and C_2 pertain to the interior processes. In a.c. studies the principal difficulty which besets any attempt to distinguish experimentally between those effects arising from surface properties and those due to the state of the membrane interior is usually that G_2 and C_2 limit the membrane current at low frequencies where the measurements are most reliable. In the case of membranes which have not been treated to enhance net conductivity, the parameters which characterize the surface and the interior can be expected to have values such that dispersion in the membrane impedance occurs at very low frequencies [6]. For this situation the capacitances and conductances in Fig. 1 have been determined from frequency dependence studies using the high resolution technique developed by Coster et al. [5]. On the other hand, in treated membranes, dispersion effects occur at relatively high frequencies. Values of the surface parameters in systems of this type have herefore to been obtained only from electrical relaxation experiments [1, 7].

In this paper we describe a method by which we have been able to obtain separately the interior and surface circuit parameters of treated membranes by measuring a.c. properties over a wide range of frequencies. The system of interest consists of lecithin-cholesterol bilayers in the presence of pentachlorophenol, an uncoupler of oxidative phosphorylation. This system has been studied by Smejtek et al. [3], who conclude on the basis of steady state electrical measurements that charge transport across the membrane interior is due to dimers formed at the surface from the association of neutral molecules and ions of pentachlorophenol. Our own studies

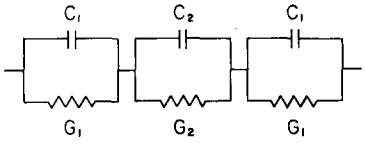

Fig. 1. Three-element equivalent circuit of lipid bilayer membrane.

confirm that under a wide range of conditions of pH and pentachlorophenol concentration such a membrane can be represented by a three-element equivalent circuit (Fig. 1) composed of frequency independent components. The results we obtain are entirely consistent with the transport scheme elucidated in ref. 8. In addition, the a.c. measurements provide further insight into details of the mechanism, such as those having to do with binding sites at the surface of the membrane.

METHOD

Materials and equipment

Membranes were formed by brushing a lipid/cholesterol/decane solution across a hole (1.6 mm diameter) in a teflon cup separating aqueous solutions of identical composition. The lipid was chromatographically pure phosphatidylcholine prepared from chicken egg yolks by a method described in ref. 8. The ratio of cholesterol content to total lipids, expressed in mole fraction, was 0.67. The concentration of lecithin in the membrane-forming solution was 5.5 μM. The stock electrolytic solution contained 0.5 M KCl and a buffer consisting of 0.2 M potassium phosphate, 0.2 M potassium citrate, and 0.05 M boric acid. The solution was titrated with HCl to the desired pH. Pentachlorophenol was added to the electrolytic solution only. Stock solutions of pentachlorophenol were stored in glass containers at high pH to prevent deterioration of the compound. Temperatures of the system during actual measurements ranged between 22 and 24 °C.

Electrical connection to the aqueous solutions in the test cell was made via two flat platinized-platinum electrodes each of whose total contact area was approximately 1 cm^2. From 0.2 kHz to 1000 kHz measurements of the capacitance and conductance of the cell-membrane system were obtained with an automatic balancing capacitance bridge. The instrument used was a Hewlett-Packard Type 4270A which had been modified to operate with an external oscillator so as to provide measurements over a continuous range of frequencies and at lower test voltages than the standard instrument. In some experiments the measurements were extended to the frequency range 0.02 kHz to 1 kHz by measuring the in-phase and quadrature components of the alternating current using a current-sensitive preamplifier in tandem with a two-phase lock-in amplifier (Princeton Applied Research Corporation Models 181 and 129A). The voltage applied across the measuring-cell in all experiments was no more than 30 mV rms, except for the capacitance bridge measurements in the frequency range 0.2 to 1 kHz, when it was held to less than 55 mV rms.

Treatment of the data

To extract values of the membrane conductance and capacitance from the raw data the resistance of the aqueous solution which is in series with the membrane must be taken into account. As this measuring-cell resistance R_0 (typically 70 to 100 Ω) is comparable with the magnitude of the membrane impedance at higher frequencies it must be determined as accurately as possible. Moreover, we have found that not only does the cell resistance vary with changes in the nature of the aqueous bath but it fluctuates from membrane to membrane under the same conditions. For this reason a separate determination of R_0 was made for each membrane. The value was found always to be higher than the cell resistance in the absence of a membrane (under the

same chemical conditions), and to vary directly, though in a complex way, with the membrane capacitance and with the apparent area of the black bilayer portion of the membrane. From these observations and from the fact that R_0 was found to be relatively insensitive to changes in position and area of the electrodes. it is clear that its value has to do mostly with the size of the current path in the vicinity of the membrane.

The procedure used to evaluate the cell resistance takes advantage of the fact that at high frequencies the membrane is practically a pure capacitance. Thus the admittance of the measuring-cell/membrane system at high frequencies when plotted in the complex plane falls on a semicircle [9]. The intercept (corresponding to infinite frequency) which this semicircle makes with the zero-susceptance axis gives the value of the cell conductance, the reciprocal of which is taken to be the cell resistance R_0. This procedure was carried out analytically for each membrane using data in the 300 to 1000 kHz range. Studies on the self-consistency of R_0 values obtained for a given membrane using various groups of high-frequency data points, as well as tests on dummy cells made of high quality capacitors and resistors, leads to the conclusion that cell resistance can be determined by this method to within about $\pm 1.5\ \Omega$ in the worst cases. Some attention was given to the possibility that R_0 is frequency dependent, but studies of the cell resistance in the absence of a membrane show that the effect, if present at all, is negligible in comparison with the above cited errors.

In a typical experiment the capacitance C_c and conductance G_c of the measuring-cell/membrane system were simultaneously determined for each of a number of frequencies spanning several decades up to 1000 kHz. C_c represents a capacitance value which has been corrected for the very small but accurately known value of stray cell capacitance. One run requires 10 to 15 min to complete. From this raw data the capacitance C_m and conductance G_m of the membrane itself can be obtained by subtracting the cell resistance as follows. The equivalent series resistance and reactance of the system are calculated from

$$R_s = \frac{G_c}{G_c^2+\omega^2 C_c^2}, \qquad X_s = \frac{\omega C_c}{G_c^2+\omega^2 C_c^2} \tag{1}$$

respectively, where ω is the angular frequency. The membrane parameters are then obtained from the relations

$$C_m = \frac{X_s/\omega}{X_s^2+(R_s-R_0)^2}, \qquad G_m = \frac{(R_s-R_0)}{X_s^2+(R_s-R_0)^2}. \tag{2}$$

Typical results are shown in Fig. 2. We interpret the dispersion which occurs at about 10 kHz as being mainly a result of the presence of surface and interior regions with differing electrical properties. We have found that a parameter which is extremely useful in the analysis of our data is the loss tangent defined by

$$\tan\delta \equiv \frac{G_m}{\omega C_m}. \tag{3}$$

The loss tangent vs. frequency curve which corresponds to the run depicted in Fig. 2 is plotted in Fig. 3. Also shown is a curve obtained from a membrane in contact with an aqueous solution in which there is no pentachlorophenol, i.e., an "untreated" membrane.

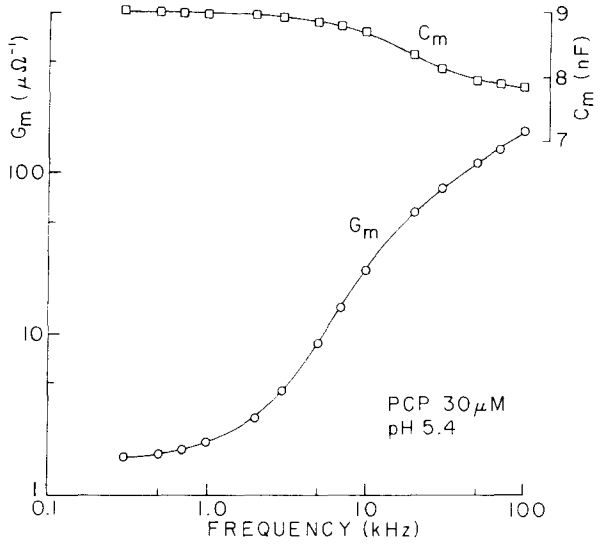

Fig. 2. Frequency dependence of membrane capacitance and conductance in a typical case. These are the results after measuring-cell resistance has been subtracted from the admittance of the cell/membrane system. PCP, pentachlorophenol.

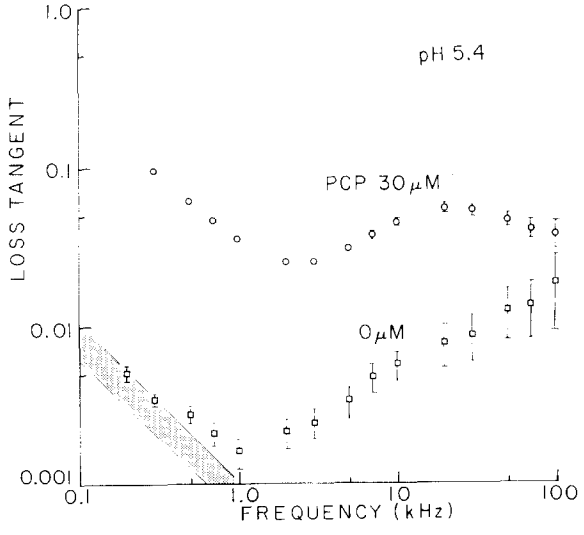

Fig. 3. Membrane loss tangent as a function of frequency for a typical treated membrane and a typical untreated membrane. The shaded area represents the range of loss tangent to be expected for an untreated membrane with parameters reported in ref. 5. Where error bars are not explicitly shown, error intervals are indicated approximately by the size of the points. PCP, pentachlorophenol.

A rationale for computing the loss tangent curve is that it is independent of the area of the membrane. Even under the same experimental conditions there are relatively large differences in capacitance and conductance among membranes, as well as slow drifts in these parameters for a given membrane. We have observed, however, that C_m and G_m vary together such that, when these parameters differ by as much as 20 % from membrane to membrane, tan δ often remains fixed to within 2 %. This observation is consistent with the notion that the principle differences among membranes measured under the same conditions arise from differences in effective area, a parameter which is dependent, in turn, on thickness of the membrane border, bulging, and possibly microscopic floating "islands" [10]. In view of the difficulties involved in accounting independently for these factors and, as we will show, significant membrane properties can be deduced from the loss tangent information alone, we will restrict ourselves in this paper mostly to results based on the frequency variation of this parameter.

The error bars shown in Fig. 3 are based on two considerations. Above 10 kHz (above 5 kHz for the untreated membrane) there is mostly a systematic error arising in the data reduction procedure from the uncertainty in the value of cell resistance R_0. Otherwise the error estimate is based on the random variations in loss tangent from membrane to membrane. Instrumental errors are negligible.

Untreated membranes and corrections

We have found that the frequency dependence of the loss tangent for untreated membranes, as shown in the lower curve of Fig. 3, is approximately the same at all values of pH especially above 1 kHz, as long as the same cholesterol to total lipid ratio is used. The curves are reminiscent of those which are obtained in dielectric absorption measurements in organic liquids or in solids containing mobile ions [11, 12]. There appears to be a superposition of two simple behaviors, viz., a ω^{-1} dependence which dominates at frequencies below 1 kHz and a power-law increase with frequency which dominates at higher values of ω. In dielectric studies the usual interpretation is that the low frequency behavior arises from conduction processes including those involving impurity ions, whereas in the high frequency region the loss mechanism is due to dielectric relaxation associated with the rotation of molecular dipoles. In the remainder of this paper we will refer to the latter high frequency phenomenon as "dielectric loss".

We can compute loss tangent vs. frequency curves for untreated egg-lecithin membranes using the values of surface and interior capacitance and conductance determined from measurements below 0.1 kHz by Coster and Smith [5]. The results of such a computation, using data obtained with KCl concentrations comparable to those used in our own experiments (0.5 M to 1 M) are shown by the shaded region in Fig. 3. (The thickness of the shaded area results from including experimental uncertainties in the calculation of the curve.) The agreement with our data is good, although some discrepancy would not be surprising in view of differences in cholesterol content and choice of solvent in the lipid solutions. We also find that our low frequency values of net membrane conductance are of the same order of magnitude ($1\ \mu\Omega^{-1} \cdot \mathrm{cm}^{-2}$) as the interior conductances measured by Coster and Smith at comparable KCl concentrations. It is clear that our low frequency measurements are determined by the interior properties of the membrane, especially G_2. As G_2 has been

shown in ref. 5 to increase with KCl concentration it seems reasonable to assume that in the low frequency region the behavior of the loss tangent curve for untreated membranes can be ascribed mostly to the small number of solution ions which are able to penetrate into the hydrocarbon region of the membrane.

The interpretation of the high frequency portion of the loss tangent curve for untreated membranes is considerably more problematical. As far as we know a high frequency dielectric loss phenomenon has not been reported before, although in a very recent paper Sargent [13] reports on voltage-jump studies of dielectric relaxation in oxidized cholesterol membranes. The loss tangent curves which are obtained are not significantly dependent on the condition in the aqueous phase. Separate preliminary studies which we have done show, however, that the curves are shifted towards lower frequencies as the fraction of cholesterol in the membrane is increased. This observation is consistent with the notion that the loss mechanism is associated with the dipolar head groups of the lipid molecules, and that the effect of cholesterol is to decrease the fluidity of the membrane in the vicinity of these groups according to the scheme of Rothman and Engelman [14]. The higher activation energies for dipolar rotation in a solid as compared with a liquid correspond to longer dipolar relaxation times, or to a shift of the dielectric absorption curves toward lower frequencies.

Inasmuch as the major purpose of our research has to do with charge transport induced in membranes by pentachlorophenol rather than the intrinsic properties of untreated membranes we will not here further pursue the above speculations. Instead we choose simply to show how we correct our data for these effects, under the assumption that the dielectric loss mechanism is independent of the presence of pentachlorophenol. In fact, in all our experiments we have observed that the loss tangent curves of treated and untreated membranes converge at approximately 100 kHz, which supports the view that the same dielectric loss mechanisms dominate the behavior at high frequencies in both cases. A capacitor containing a lossy dielectric can be represented by an equivalent circuit of resistors and capacitors [9]. The circuit in Fig. 4a corresponds to the case where the dielectric has a single relaxation time, given by $\tau = R_A C_A$. A distribution of relaxation times can be represented by additional RC elements in parallel with C_B which can be taken to represent the capacitance of membrane at high frequencies. In our circuit G_{CT} represents the membrane conductance due to charge transport mechanisms. At any particular frequency the circuit of

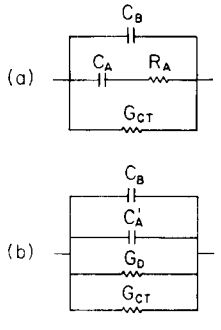

Fig. 4. (a) Equivalent circuit of a membrane composed of a lossy dielectric with a single relaxation time. (b) Parallel-element equivalent circuit of a membrane with a lossy dielectric.

Fig. 4a or a similar one representing a distribution of relaxation times can be transformed to the parallel equivalent circuit of Fig. 4b. It is unnecessary to inquire whether the elements giving rise to dielectric loss are actually located in the interface or interior parts of the membrane equivalent circuit; at any particular frequency a representation of the type shown in Fig. 4b, in which these losses are dissipated entirely in G_D, can be made. At each frequency we measure a loss tangent given by

$$\tan \delta \text{ (measured)} = \frac{G_D}{\omega(C'_A + C_B)} + \frac{G_{CT}}{\omega(C'_A + C_B)}. \tag{4}$$

The first term on the right hand side of Eqn. 4 is the loss tangent for the untreated membrane. As our measurements confirm that the presence of pentachlorophenol does not very much affect the net capacitance of the membrane, we can regard the second term in Eqn. 4 as the loss tangent which arises from the charge transport mechanisms under study. This is the loss tangent of primary interest and is thus obtained by correcting our measured values of loss tangent by subtracting a component representing high frequency dielectric loss, i.e.,

$$\tan \delta \text{ (corrected)} = \tan \delta \text{ (measured)} - \tan \delta \text{ (untreated)}. \tag{5}$$

We have applied the correction procedure of Eqn. 5 on a point by point basis above 3 kHz; at lower frequencies we used a straight line extrapolation of the log-log plot of this untreated membrane data to obtain the correction term. The outcome is to retain in our corrected data the effect of background conductivity due to mobile charges in the untreated membrane, while eliminating the contribution of the dielectric loss mechanism. In any event, the correction is relatively small at lower frequencies where the straight line extrapolation is applied. The most important result is to lower somewhat the magnitude of the high frequency peak in each loss tangent curve, without either greatly affecting its position on the frequency axis or altering the character of the low frequency dip. As we shall see this has a significance for our estimates of surface capacitance, but is not serious enough to throw our major results into doubt.

It is important to inquire whether the dielectric loss phenomenon can be responsible for any considerable error in our determinations of cell resistance R_0 which we obtain by the extrapolation procedure previously described. We can be confident that our values of R_0 are at least approximately correct as they are larger than the no-membrane cell resistance by a small amount which varies approximately in proportion to the area of the membrane border. Thus we can be sure that the loss tangent curves for our untreated membranes are also approximately correct as long as we restrict ourselves to low enough frequencies (< 100 kHz). The maximum contribution of dielectric loss to R_0 can then be made using the "worst possible" assumption that the loss tangent curves rise linearly with frequency above 100 kHz. This leads to values of approximately 1.5 Ω for the contribution of dielectric loss to cell resistance at frequencies for which the R_0 extrapolation procedure is carried out (300–1000 kHz). Subtracting this contribution from the extrapolated value of R_0 yields a lower limit on the correct value of R_0. We may obtain an upper limit for R_0 by supposing that there is no membrane loss at very high frequencies, i.e., that at the highest frequency for which we can make reliable measurements (1000 kHz) the effective series

resistance (Eqn. 1) is due entirely to the aqueous solution. Typically these two extreme values for R_0 bracket the extrapolated value symmetrically, lending credibility to the criterion stated earlier, viz., that of taking the cell resistance to be the extrapolated value $\pm 1.5\ \Omega$.

Curve fitting

An example of a loss tangent versus frequency curve obtained by the methods outlined above is shown in Fig. 5. This is typical of curves obtained from all of the treated membranes we have studied. The loss tangent varies as ω^{-1} at both high and low frequencies, and displays a local minimum and maximum in the intervening region. This is the behavior which is characteristic of the three-element resistor-capacitor network shown in Fig. 1. In order to determine values for the circuit components (G_1, G_2, C_1, C_2) which correspond to a given loss tangent curve it is necessary to assign an arbitrary value to one of them. (When all parameters are scaled proportionally the same curve is obtained.) We have chosen a value of 10 nF for the interior capacitance G_2: this is approximately the geometrical capacitance of the hydrocarbon region of a membrane of the size we have studied and was, in fact, a typical value for net membrane capacitance measured at low frequencies. (We could detect no systematic variation of G_2 under various experimental conditions.) It is possible, of course, to take into account membrane area during each experiment in order to cite results in terms of specific capacitance and conductance. However it was felt that in most cases this would unnecessarily add an additional source of error. Our most important conclusions have to do with changes in capacitance and conductance due to changes in membrane environment rather than with absolute values of these parameters: thus in most cases results are normalized to $G_2 = 10$ nF.

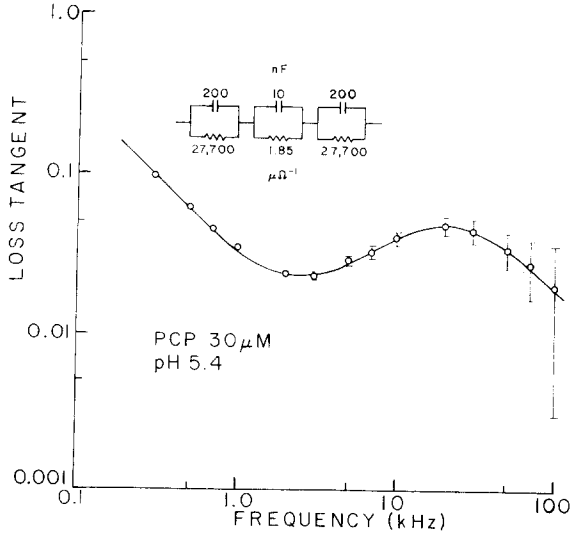

Fig. 5. Loss tangent vs. frequency for the treated membrane data given in Fig. 3, after correction for dielectric loss. Where otherwise not shown error intervals are indicated by the size of the points. The solid curve denotes the loss tangent behavior computed for the equivalent circuit shown. PCP, pentachlorophenol.

The circuit whose loss tangent dependence fits the data of Fig. 5 is indicated in the drawing; the curve itself is given by the solid line. Such a curve can be specified in terms of the depth and mid-frequency of the minimum and the height and mid-frequency of the maximum. In most cases we have found that the values of these descriptors each vary most sensitively with the choice of G_2, C_2, C_1, and G_1 respectively. However, a three-element circuit model could always be fitted to our data well within the limits of error. Thus in addition to providing a simple set of parameters with which to characterize membrane behavior, an outcome of the fitting procedure is to substantiate our methods for data reduction and correction.

RESULTS AND DISCUSSION

Interior conductance

Our results for the conductance of the interior portion of the membrane as a function of pH in the aqueous phase at a moderate pentachlorophenol concentration are presented in Fig. 6. In this one set of results our values are given per unit area in order to make comparisons with conductance measurements of other workers. We determined the area of an average 10 nF membrane on the basis of a large number of determinations of capacitance per unit area obtained at low frequencies under a

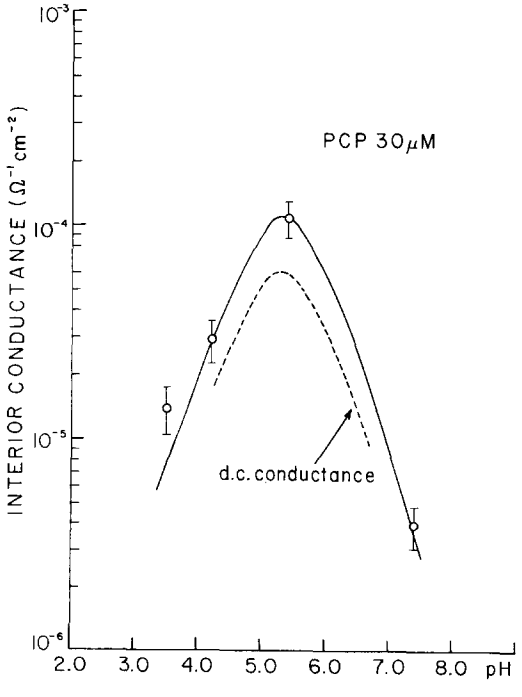

Fig. 6. Interior conductance G_2 (per unit area) as a function of pH at pentachlorophenol (PCP) concentration = 30 μM. As in reference 8 the experimental points have been fitted with a curve proportional to $[H^+]/(K+[H^+])^2$ where K is adjusted to give a peak at pH 5.3 and the curve is scaled to pass through the upper point. The conditions for the curve which fits the d.c. data is pentachlorophenol concentration = 25 μM, cholesterol mol fraction = 0.8.

wide range of experimental conditions. Dividing this area (0.0172 cm^2) into normalized G_2 values yielded the results plotted in Fig. 6.

As the interior conductance is many orders of magnitude smaller than the surface conductance it limits the current in d.c. experiments. Thus it is to be expected that our results for G_2 are in essential agreement with the steady state measurements of Smejtek et al. [8], which are also indicated in Fig. 6. The difference between the curves arises largely from the methods used to calculate the conductance per unit area; Smejtek et al. used the area of the open hole rather than the black area of the membrane. The pH dependence can be explained in terms of a model of transport in which charge transfer in the interior takes place by the movement of dimers HA_2^- produced within the membrane surface by the reaction

$$HA + A^- \rightleftarrows HA_2^- . \tag{6}$$

HA represents the neutral pentachlorophenol molecule and A^- is the anion formed from the dissociation of pentachlorophenol according to

$$HA \rightleftarrows H^+ + A^- . \tag{7}$$

There is also a return flux of HA which does not contribute directly to the conduction. At low pH the density of dimers, hence the interior conductance, is restricted by the availability of anions; at high pH the limitation is the availability of the neutral species.

Surface conductance

In contrast to the interior conductance G_2, which limits the charge transport in steady state situations and is thus adequately studied by d.c. measurements, the surface conductance G_1 is directly accessible only through dynamical methods such as a.c. studies. The results we have obtained for G_1 as a function of pentachlorophenol

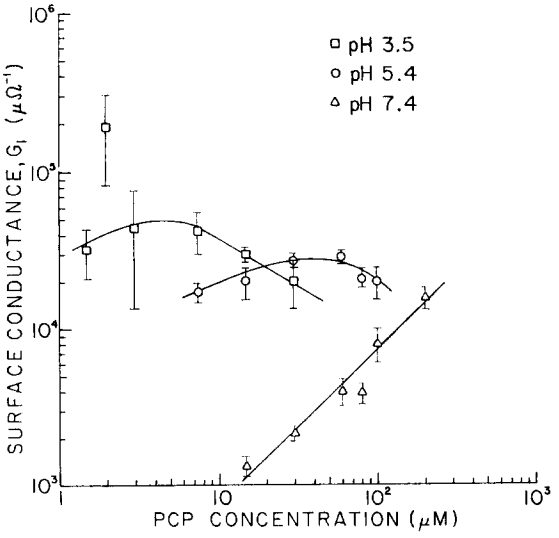

Fig. 7. Concentration dependence of the surface conductance G_1. PCP, pentachlorophenol.

concentration are plotted in Fig. 7. The major features can be characterized as follows: at high pH the surface conductance is relatively small and is directly proportional to concentration, at low pH there is a relatively large conductance which decreases with concentration, and at intermediate pH the conductance clearly reaches a maximum within the range of concentrations we have examined. The maximum concentrations we have been able to study are limited, especially at low pH, by the solubility of pentachlorophenol.

We can reject at the outset that our results have to do with diffusion polarization effects in the membrane/water interface region. Current-voltage studies [8] show the unstirred layers to be unimportant for d.c. measurements at buffer concentrations comparable to ours, and they can be expected to be less significant in high frequency situations. Besides this, our data gives no evidence of a frequency dependent surface conductance which characterizes interface processes of this sort. Instead the interpretation we give to our results depends on the concept of a fixed density of binding sites at the surface of the membrane, as in the scheme of Bruner [15]. Each binding site may be occupied by an HA molecule, an A^-, or an HA_2^- dimer. At pH 3.5 (for which, assuming pK of 4.8 [8], the pentachlorophenol in solution is 95 % in the neutral form), the decrease of G_1 at higher concentrations points to a relatively strong partition of HA molecules into the binding sites. At this pH, increased availability of anions is overwhelmed by decreased availability of surface sites into which charges can flow. Moreover the relatively high surface conductances at low pentachlorophenol concentrations implies that the species whose availability is critical to the charge transfer at the surface is neutral HA. These conclusions are consistent with our observations at high pH. For pH 7.4 the absence of saturation in our results indicates that the binding sites are relatively free of any adsorbed species; moreover the conductance is relatively low despite the predominance of A^- in solution. At intermediate pH both site occupation and availability of HA can be expected to be important, the former at high concentrations and the latter at low concentrations.

We can formalize these arguments in terms of the general mechanism of pentachlorophenol-induced charge transport deduced in ref. 8. Fig. 8 represents, in a simplified form appropriate to our discussion of a.c. surface transport, the two alternative schemes which are consistent with the d.c. results. In scheme (a) the anions on

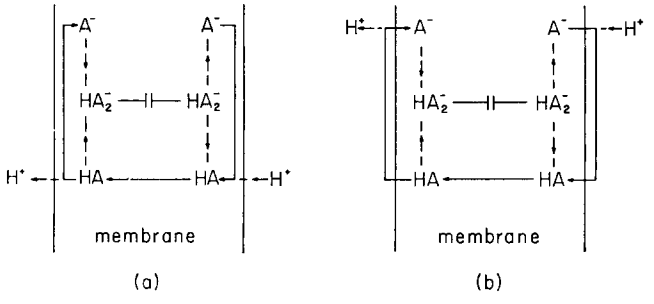

Fig. 8. Modified representation of the schemes of charge transport in pentachlorophenol-treated membranes deduced from d.c. studies [8]. Surface charge transport involves an exchange of protons with the aqueous phase. Current flow across the interior is by transfer of dimers in the d.c. or low frequency situation. At high frequencies capacitive currents dominate in the interior.

the surface of the membrane are supplied by a dissociation taking place within the membrane; in scheme (b) the surface anions are supplied from the aqueous solution. In both cases it is the sinusoidal variation in A^- concentration superimposed on the equilibrium concentration which is correlated with the a.c. surface conductance. The other charged species in the surface, viz., the dimer HA_2^-, can be regarded as maintaining its equilibrium value at all times since at the frequencies at which G_1 is principally determined alternating current flow in the interior is mostly by capacitive effects rather than by a shuttling of dimers.

In order to simplify the discussion we will assume that the surface conductance is limited by the availability of just one of the species participating in the overall transport mechanism. Moreover, we will assume that in the process by which surface binding sites are filled, adsorption by a single species predominates. Surface conductance will depend both on the availability of the limiting species and on the density of free binding sites N_F. We can write

$$G_1 \propto [X]N_F \tag{8}$$

where $[X]$ represents the density of the species limiting the surface charge transport. The alternatives for X are anions in aqueous solution with density $[A^-]_a$, anions in the membrane surface (density $[A^-]_m$), neutral pentachlorophenol in solution (density $[HA]_a$), or neutral pentachlorophenol in the surface (density $[HA]_m$). Partition between aqueous and membrane phases can be expressed by

$$[HA]_m = \beta_{HA}\frac{N_F}{N_T}[HA]_a \tag{9}$$

and

$$[A^-]_m = \beta_{A^-}\frac{N_F}{N_T}[A^-]_a \tag{10}$$

where the βs are the partition coefficients for the adsorption process and N_T is the total density of binding sites. If we suppose that HA dominates the adsorption process we have approximately $N_F = N_T - [HA]_m$, and Eqn. 9 yields

$$N_F = \frac{N_T}{1+\beta_{HA}[HA]_a/N_T}. \tag{11}$$

Similarly, if A^- predominates in the filling of the binding sites, we have from Eqn. 10

$$N_F = \frac{N_T}{1+\beta_{A^-}[A^-]_a/N_T}. \tag{12}$$

Finally, if we suppose that it is the dimers HA_2^- which overwhelmingly fill the binding sites, we obtain

$$N_F = N_T\frac{\sqrt{1+4K'\beta_{HA}\beta_{A^-}[HA]_a[A^-]_a/N_T}-1}{2K'\beta_{HA}\beta_{A^-}[HA]_a[A^-]_a/N_T} \tag{13}$$

using Eqns. 9 and 10 and the equilibrium expression

$$[HA_2^-]_m = K'[HA]_m[A^-]_m . \tag{14}$$

The dependence of surface conductance as given by Eqn. 8 on total concentration of pentachlorophenol in solution [c] can now be evaluated at various pH values using one or more of the Eqns. 9 through 14 and the dissociation expressions

$$[HA]_a = \frac{[H^+]}{[H^+]+K}[c] \tag{15}$$

and

$$[A^-]_a = \frac{K}{[H^+]+K}[c] \tag{16}$$

where K is the equilibrium constant for the process of Eqn. 7. The possibility that the pertinent concentration [X] in Eqn. 8 is either $[HA]_a$ or $[A^-]_a$ can be rejected as this leads in all cases to a saturation of surface conductance at high concentration, in disagreement with our experimental results. Other choices for [X], on the other hand, result in G_1 vs. [c] curves all of which have a maximum at some intermediate value of concentration. By a proper choice of the parameter β/N_T (or in the case of HA_2^- site filling, $K'\beta_{HA}\beta_{A^-}/N_T$) the maximum conductivity can be made to occur in each case in the concentration range 10–100 μM at pH = 5.4. However the overall results which are thus obtained resemble the observed behavior for only one pair of assumptions, viz., that the limiting species in the conduction process is the adsorbed neutral molecule (density $[HA]_m$) and that HA also dominates the site filling process. Other combinations of assumptions lead to curves which are, in one aspect or another, radically different from the observed ones. For example, the high, medium, and low pH curves are, for some assumptions, separated by too many orders of magnitude, or display a pH dependence inverse to that which is observed. In Fig. 9 we plot G_1 in arbitrary units as a function of the concentration [c] according to the relationship

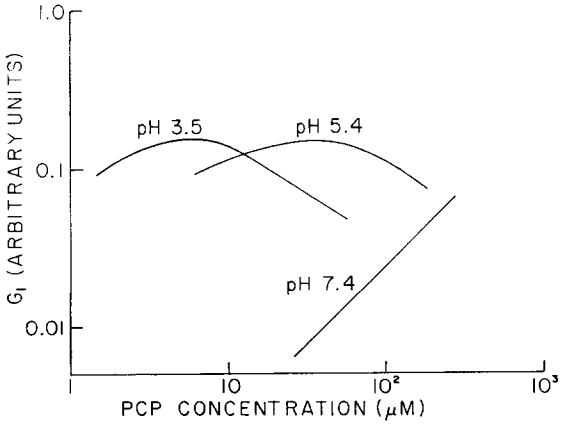

Fig. 9. Surface conductance vs. concentration computed according to Eqn. 17. PCP, pentachlorophenol.

$$G_1 \propto \frac{[HA]_a}{(1+\beta_{HA}[HA]_a/N_T)^2} \tag{17}$$

where $[HA]_a$ is obtained in terms of $[c]$ using Eqn. 15 under the assumption that $pK = 4.8$. We have set $\beta_{HA}/N_T = 0.166\ (\mu M)^{-1}$ in order to place the peak conductance of the pH 5.4 curve at 30 μM. Eqn. 17 is that form of Eqn. 8 appropriate to the assumption that the dominant process determining surface conductance is the adsorption of HA molecules which in turn dissociate into charged species within the membrane.

The complete expression corresponding to Eqn. 17 is

$$G_1 = \frac{z^2F^2}{RT} \cdot \frac{\beta_{HA}k[HA]_a}{(1+\beta_{HA}[HA]_a/N_T)^2} \cdot (\text{Area}) \tag{18}$$

where k is the effective rate constant for the process which transfers charge across the interface. Eqn. 18 is formally identical to the expression given by Ketterer et al. [1] for the initial conductance (in relaxation experiments) due to the transport of hydrophobic ions between interfaces of a lipid membrane. Indeed, we can use our data plotted in Fig. 7 in a manner like that in ref. 1 to find $\beta_{HA}k$ from the slope of the G_1 vs. $[c]$ curve at pH 7.4, kN_T from the maximum conductance at pH 5.4, and β_{HA}/N_T from the value of concentration at maximum conductance. Taken together with the estimate of β_{HA} based on our capacitance studies, which we will discuss below, we obtain the following approximate values for the parameters of the process which transfers charge across the interface:

$k \approx 6 \cdot 10^4\ s^{-1}$ and $N_T \approx 20 \cdot 10^{12}\ cm^{-2}$

with $\beta_{HA} \approx 8 \cdot 10^{-3}$ cm.

The numbers just cited are to be regarded only as estimates, especially as the model we have used does not take into account all the features of the surface process. For example, surface charging has not been considered. For this reason we have made no attempt to adjust the curves of Fig. 9 to optimize the fit with the data of Fig. 7. What is of significance at this time is that we are able to use our data to corroborate certain qualitative aspects of the surface charge transport. For example, as our data is in reasonable agreement with a model of transport in which the a.c. component of surface charge depends directly on the density of adsorbed neutral molecules, it is clear that of the two alternative schemes shown in Fig. 8, between which the d.c. studies are unable to discriminate, scheme (a) most probably represents the actual situation.

Surface capacitance

The results we have obtained for the capacitance C_1 of the membrane-water interface region are plotted in Fig. 10. Values are in the range 150 to 900 nF for membranes normalized to an interior capacitance of 10 nF, and exhibit systematic variations both with pH and concentration of pentachlorophenol. This range of surface capacitance, viz., 15 to 90 times that of the interior, compares not unreasonably with the several measurements made by other workers. Coster and Smith [5] found that the surface capacitance of untreated lecithin membranes was 60 times that of

the interior, independent of the ionic strength of the aqueous solution. Sandblom et al. [7] in their relaxation studies of nonactin induced transport in lecithin-cholesterol membranes found that the surface to interior capacitance ratio was between 30 and 100, depending on ionic strength. All of the results cited here are consistent with the view that the surface capacitance arises principally from the polarization of the surface dipole layer of the membrane, i.e., from a layer 5 to 10 Å thick having a dielectric constant typical of a non-aqueous polar liquid ($\varepsilon \approx 20$).

Of immediate interest is whether we can account for the systematic variations in C_1 with changes in the aqueous environment. As in both references just previously cited we can discount the influence on our results of diffuse double layers. At the ionic strengths at which our experiments were performed the presence of a double layer could be responsible for only a small reduction in the observed value of surface capacitance. Moreover the small but significant increase in C_1 with pentachlorophenol concentration which is observed at high pH is too great to be attributed to a suppression of double layer effects in the presence of adsorbed surface charges, a phenomenon discussed by White [16].

We have also considered whether the variation in surface capacitance is explicable in terms of ion transfer kinetics in the interface region. This cannot in principle be ruled out; however the analysis given, for example, by de Levie et al. [2] for the case of membrane soluble ions does not account for the major features of our results. We find that within the limits of experimental error the membranes we have studied can be represented by equivalent circuits consisting of frequency independent components; we are not able to tell whether there is a frequency dependent contribution to the capacitance which is demanded by analyses which include, for instance, consideration of slow transfer processes. The evidence appears to be that the reaction times for the limiting interface processes are short in comparison to the times involved in our experiment. We believe it to be more appropriate for our system to regard the membrane in terms of the three-element circuit of Fig. 1 in which the entire membrane current is continuous across interface and interior regions, rather than suppose, as in ref. 3, that there is a pure capacitive membrane current which directly bridges the two aqueous phases and bypasses the charge transfer processes.

Accordingly we choose to regard C_1 as a capacitance associated with polarization effects within the surface region of the membrane. In addition, we distinguish between that part of the capacitance arising from the intrinsic polarizability of the phospholipid head groups (C'_1), and that part having to do with adsorbed species (C''_1). With respect to this latter component, we recognize that the simplest situation is obtained when saturation effects can be ignored. Indeed, the monotonic increase of C_1 with uncoupler concentration which is observed at high pH leads us to a view in which separation of negative and positive parts of adsorbed species under the action of applied electric fields is the phenomenon which gives rise to the concentration dependent capacitance.

We can make an estimate of the magnitude of the capacitance C''_1 due to adsorbed pentachlorophenol in the following way. The evidence from our surface conductance studies is that HA, which is predominantly adsorbed, is rapidly dissociated in the environment within the membrane surface. Imagine that the surface ions are located in a square bottomed potential well of width W and that in the absence of an applied voltage the density of charge of both signs ρ_0 is approximately

uniform across the well. Suppose, moreover, that a small applied voltage V gives rise to an electrical potential ψ which varies linearly in the surface region according to

$$\psi(x) = V\frac{x}{W}. \tag{19}$$

The net charge density distribution $\rho(x)$ is now approximately

$$\begin{aligned}\rho(x) &= \rho_+(x) - \rho_-(x)\\ &= \rho_0 \exp\left(-\frac{eVx}{kTW}\right) - \rho_0 \exp\left(-\frac{eV(W-x)}{kTW}\right)\\ &\simeq \rho_0 \frac{eV}{kT}(1-2x/W).\end{aligned} \tag{20}$$

The electrostatic energy of this charge distribution is

$$\begin{aligned}U &= \int_{-W/2}^{W/2} e\rho(x)\psi(x)\mathrm{d}x\\ &= -\tfrac{1}{2}\rho_0 \frac{W}{3}\frac{e^2V^2}{kT}\end{aligned} \tag{21}$$

where, in view of the relatively low density of adsorbed molecules when there is no saturation, we have neglected the energy of interaction between the ρ_+ and ρ_- distributions. U can be regarded as the electrostatic energy of the dielectric in a capacitor charged to voltage V. The additional capacity C'' over and above that of the empty capacitor can be found by setting $U = -\frac{1}{2}C''V^2$. Hence

$$C'' = \tfrac{1}{3}\frac{e^2}{kT}\rho_0 W. \tag{22}$$

Recast in the previous notation of this paper we have

$$C_1'' \simeq \tfrac{1}{3}\frac{z^2F^2}{RT}\beta_{HA}[HA]_a. \tag{23}$$

This may be compared with the expression derived by Ketterer et al. [1] for the capacitance arising from the redistribution of lipid soluble ions in a membrane far from saturation, viz.,

$$C = \frac{z^2F^2}{RT}\beta[c]. \tag{24}$$

The approximate treatment of surface capacitance we have just given is applicable to our results at pH 7.4. The data shown in the lower part of Fig. 10 can be viewed as being due to the combination of a capacitor with the constant value $C_1' = 180 \pm 12$ nF and a capacitor whose value varies linearly with pentachlorophenol concentration according to Eqn. 23. From the slope of the linear contribution we are able to get an estimate of the partition coefficient for the adsorption process, viz.,

$\beta_{HA} \approx (8 \pm 5) \cdot 10^{-3}$ cm.

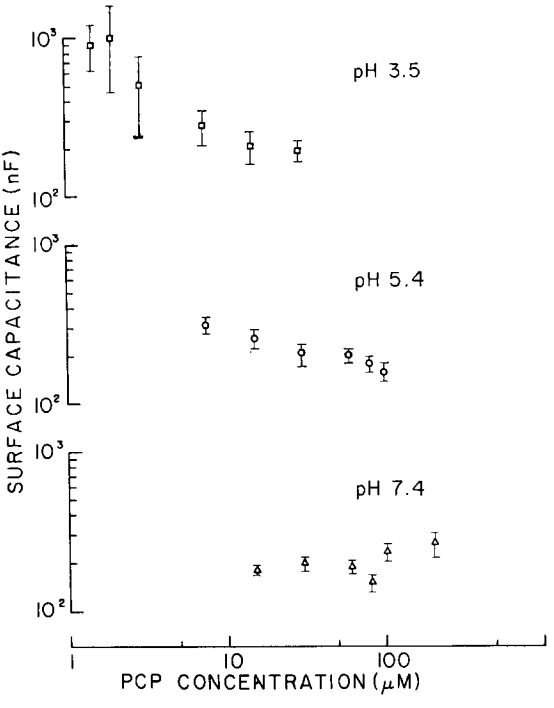

Fig. 10. Concentration dependence of surface capacitance C_1.

At low values of pH binding site saturation undoubtedly affects the response of surface charge distributions to applied electric fields. We can imagine that in the vicinity of an adsorbed molecule there is a change in the intrinsic polarizability or thickness of the dipole layer. We have not analyzed these cases. It is clear that a comprehensive theory which accounts for the suppression of surface capacitance, such as we observe at pH 3.5 and 5.4, would be of value in the further study of surface binding dynamics. Differences in ionic strength among our three values of pH can account for only a negligible amount of the capacitance variation we measure.

CONCLUSIONS

In principle, all the information on membrane charge transport which is accessible through conventional electrical measurements, can be obtained from studies of admittance over a wide range of frequencies. Such measurements represent a Fourier-transformed version of the information contained in relaxation curves taken over appropriate time intervals. Indeed, the characteristic times accessible to the two methods are approximately the same, though we have reason to believe that improvements in the a.c. methods can extend upwards the present frequency limits. As in other cases of related experimental techniques, the various approaches are best regarded as being complementary rather than competitive; the choice among them depends upon the system being studied and the details which are of interest. However, we should point out some of the advantages which accrue to the use of a.c. measure-

ments. There is no necessity to use very wide-band amplifiers with the attendant noise problems of such systems. As for drift and variability among membranes, the loss tangent technique described above to a large extent mitigates these problems; it provides a means of avoiding the difficulties of area determinations or the necessity of gathering the large quantities of statistics which is typical of d.c. measurements. Finally, we mention the relatively straightforward curve fitting approach with which the a.c. results can be related to the parameters of any proposed equivalent circuit. Taken in all, we feel that a.c. methods are especially important items in the catalog of techniques available for investigating membranes of all types; it was for this reason that we have given considerable attention to experimental details in the foregoing paper.

Our measurements involving the uncoupler pentachlorophenol support a scheme of transport in which the surface process is dominated by the adsorption and consequent rapid dissociation of the neutral carrier molecule. We have thus been able to distinguish, using a.c. measurements, between two alternative schemes of transport both of which are consistent with d.c. experiments; furthermore, we have made estimates of several of the pertinent parameters. Some phenomena have been uncovered which invite further study; among these are high frequency dielectric effects and the variation of surface capacitance with carrier concentration. However, it is the characteristic form in which our results are obtained, viz., in terms of separate surface and interior parameters, which possibly points to the wider promise of this technique. A number of questions of great current interest are closely related to the problem of structural changes across the width of membranes; these include understanding the role of cholesterol in membrane function, and the dynamics of phase changes. We submit that carrier molecules such as pentachlorophenol, in conjunction with admittance measurements, can be used as probes of membrane organization.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. P. Smejtek for his reading of the manuscript and many helpful discussions, and to Dr. K. Hsu for her support and advice in many technical aspects of this research. This investigation was supported by NIH Grant R01 ES 937.

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