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Supported Phospholipid/Alkanethiol Biomimetic Membranes: Insulating Properties

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ABSTRACT A novel model lipid bilayer membrane is prepared by the addition of phospholipid vesicles to alkanethiol monolayers on gold. This supported hybrid bilayer membrane is rugged, easily and reproducibly prepared in the absence of organic solvent, and is stable for very long periods of time. We have characterized the insulating characteristics of this membrane by examining the rate of electron transfer and by impedance spectroscopy. Supported hybrid bilayers formed from phospholipids and alkanethiols are pinhole-free and demonstrate measured values of conductivity and resistivity which are within an order of magnitude of that reported for black lipid membranes. Capacitance values suggest a dielectric constant of 2.7 for phospholipid membranes in the absence of organic solvent. The protein toxin, melittin, destroys the insulating capability of the phospholipid layer without significantly altering the bilayer structure. This model membrane will allow the assessment of the effect of lipid membrane perturbants on the insulating properties of natural lipid membranes.

INTRODUCTION

We have prepared supported bilayer membranes by the fusion of phospholipid vesicles with alkanethiol monolayers on gold electrodes (Plant, 1993). This technique for hybrid lipid membrane formation provides an alternative to other methods of formation of model membranes for the study of physiologically relevant lipid membrane phenomena.

Black lipid membranes (BLMs), Langmuir Blodgett layers, and patch clamp membranes have proved essential as models for elucidating both structural and functional aspects of cell membranes. However, unlike other planar model membranes, phospholipid/alkanethiol bilayers are extremely easy to prepare, are reproducible, and are stable. We form these supported bilayers in a cell where they can be subjected to electrochemical measurements and changes of solution for at least weeks at a time. These bilayers are formed on conducting metals which allows for direct measurement of membrane electrical insulating properties. A very strong interaction between sulfur and gold orients alkanethiols at a gold surface (Nuzzo et al., 1987); the alkane chains can then interact via van der Waals forces to add further stability to the monolayer structure. This stable, self-assembling hydrophobic monolayer on the gold surface provides the driving force for the subsequent self-assembly of an outer leaflet of phospholipid. The result is a pinhole-free, structurally well defined, and physiologically relevant phospholipid-containing model membrane. The advantages of this technique include its applicability to virtually any lipid and the spontaneous nature of bilayer formation in the absence of any organic solvent. The significance of using alkanethiols in lipid bilayer formation is apparent from the number of very recent reports of similar approaches, including the use of dioctadecyldimethylammonium bromide and alkanethiols to make bilayers and trilayers (Stelzle et al., 1993), the application of phospholipids in organic solvent to alkanethiol monolayers (Florin and Gaub, 1993), the fusion of lipid vesicles to a thiol-terminated polymer-covered support (Spinke et al., 1992), the addition of lipids to alkanethiol monolayers either as vesicles, as mixed detergent micelles, or from an LB trough (Terrettaz et al., 1993), and the use of phospholipid analogues with thiol-terminated headgroups (Lang et al., 1994).

The phospholipid/alkanethiol hybrid model membrane system provides us with a new tool for studying structural properties of membranes and how membrane proteins and other membrane-associating molecules affect the membrane microstructure. One area of investigation of particular relevance to this model system is the effects of interfacial events, such as surface receptor binding, on membrane structural characteristics. In addition, due to their ease of their fabrication, their long term stability, and their formation at a surface which can act as an electrode, these bilayers are relevant to applications of lipid membranes as biosensor components.

As a prelude to both the membrane biology and biosensor applications of this system, this study was undertaken to carefully and extensively characterize the insulating properties of these bilayers. In this report we characterize this model system with two types of measurements, impedance analysis and cyclic voltammetry. The combination of these two techniques allows us to study two important characteristics of membranes independently: structural features, such as dielectric constant and bilayer thickness, and ion penetration. An important characteristic of these phospholipid/alkanethiol hybrid membranes is their effectiveness at passivating the electrode; thus they are very sensitive to small changes in ion flux and capacitance.

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MATERIALS AND METHODS

A hydrophobic monolayer of alkanethiol, and subsequently a second layer of phospholipid, were allowed to self-assemble onto a gold surface, which was then employed as a working electrode. For all experiments reported here, decanethiol (Aldrich Chemicals, Milwaukee, WI) was used without purification to form the hydrophobic monolayer template. The gold surface consisted of an ~1000-Å-thick layer of gold which was sputtered over a 300 Å titanium layer on borosilicate glass (Abtech, Inc., Yardley, PA). These gold electrodes were cleaned by a procedure described by Miller et al. (1991) which involved three cycles of treatment with a hot potassium dichromate solution for 30 s followed by rinsing with hydrofluoric acid. Cleaned gold electrodes were immediately submersed in 1 mM ethanolic solution of decanethiol for at least 16 h. Alkanethiol monolayer-coated electrodes were removed from the solution, rinsed thoroughly with ethanol, and dried under nitrogen before measurements. Electrochemical measurements were made with a Solartron electrochemical interface (model 1250, Schlumberger) and frequency generator (model 1286, Schlumberger). A three-electrode cell was used, with a Ag/AgCl reference electrode and a platinum counter electrode. The working gold electrode area was nominally 0.32 cm^2. Impedance measurements were made at the applied dc potentials indicated in the figure legends, and by applying a sinusoidal ac potential of ±10 mV. Cyclic voltammetry provided measurements of electron transfer rates: $K_3Fe(CN)_6$ was used as the redox species in a solution of 1 M KCl as the supporting electrolyte. Capacitance values reported here were determined by impedance measurements under more physiologically relevant conditions in 20 mM Tris, 150 mM NaCl, 0.1% thimerosal, pH 7.4 (TBS). Resistance was determined by impedance measurements in 1 mM $K_3Fe(CN)_6$ in 1 M KCl. Impedance data were analyzed using the ZSim software package (Schlumberger).

Phospholipids used for formation of the bilayer were purchased from Avanti Polar Lipids. For this study we used a series of phosphatidylcholines with monounsaturated acyl chains of 18, 20, and 22 carbons, designated C18:1 PC, C20:1 PC, and C22:1 PC. Because of the presence of the carbon-carbon double bond in each acyl chain, each of these lipids has a gel-to-liquid crystal phase transition temperature which is well below room temperature, and all are present in their liquid crystal state during the experiments. For these studies, phospholipid solutions in $CHCl_3$ were taken to dryness under N_2, desiccated overnight to remove residual solvent, and then vortexed in TBS to form multilamellar liposomes. Unilamellar vesicles of ~0.2 μm diameter were prepared by extrusion (Olson et al., 1979) of the multilamellar liposomes through polycarbonate filters (Nucleopore, Pleasanton, CA). Vesicles were added to the monolayer-coated electrode in the electrochemical cell at a final concentration of 0.2 mM lipid in TBS. Fusion could be monitored by the decrease in electrical capacitance, which indicates an increase in thickness of the layer, and was considered to be complete when the capacitance reached a stable value. A stable capacitance was usually reached within a couple of hours after addition of vesicles under these conditions (room temperature and unstirred solution), but the vesicles were usually left in the cell overnight to ensure that their interaction with the monolayer went to completion. Capacitance values for the bilayers were identical in the presence of liposomes and after their removal. As has been shown previously (Plant, 1993), the change in the capacitance values which accompany the addition of vesicles to the alkanethiol monolayer indicates the formation of an additional dielectric layer on the electrode of a thickness which is appropriate for the expected length of the acyl chain region of phospholipids.

Melittin (Sigma Chemical Co., St. Louis, MO), the peptide toxin from bee venom, was solubilized in TBS, and was added to the cell in the presence of 5 mM ethylenediamine tetraacetate (EDTA) to block any residual phospholipase activity that might be associated with the melittin preparation (Dempsey, 1990).

RESULTS

As has been described previously (Plant, 1993), the phospholipid/alkanethiol bilayer is formed by two sequential self-assembly processes. Alkanethiols spontaneously and rapidly form well-ordered monolayers on gold due to the strong attraction between thiol sulfur and the metal. Addition of phospholipid in the form of lipid vesicles to the hydrophobic monolayer results in a spontaneous increase in the thickness of the layer which is indicated by impedance spectroscopy to be a bilayer. Fusion of vesicles with a hydrophobic layer has been proposed as a mechanism of bilayer formation (Kalb et al., 1992). A schematic of the bilayer structure is shown in Fig. 1 and is intended only to represent the orientational relationship between the polar headgroups and hydrophobic chains of the phospholipid molecules and the hydrophobic acyl chains of the alkanethiol monolayer. For these experiments we have used decanethiol for the formation of the hydrophobic monolayer template because it forms a well-ordered monolayer, but is thin enough that some electron transfer activity is observed. The presence of a complete alkanethiol coating is verified by measuring the rate of electron transfer. Fig. 2 shows that reduction and oxidation of $K_3Fe(CN)_6$ are greatly attenuated in the presence of the decanethiol monolayer compared with the bare gold surface and even more attenuated in the presence of the bilayer of decanethiol and C18:1 PC. At a bare electrode, reduction and oxidation of the ion are facile, reversible, one-electron processes. After formation of a monolayer or bilayer, the process is no longer readily reversible, requiring the application of very large potentials to observe any response above the charging current. The presence of the phospholipid/alkanethiol bilayer reduces the rate of electron transfer by approximately 2 orders of magnitude compared with the bare electrode. Clearly, electron transfer is greatly attenuated by the bilayer; however, to assess the effect of perturbants on membrane-insulating characteristics, it is critical to determine whether electron transfer is occurring primarily at

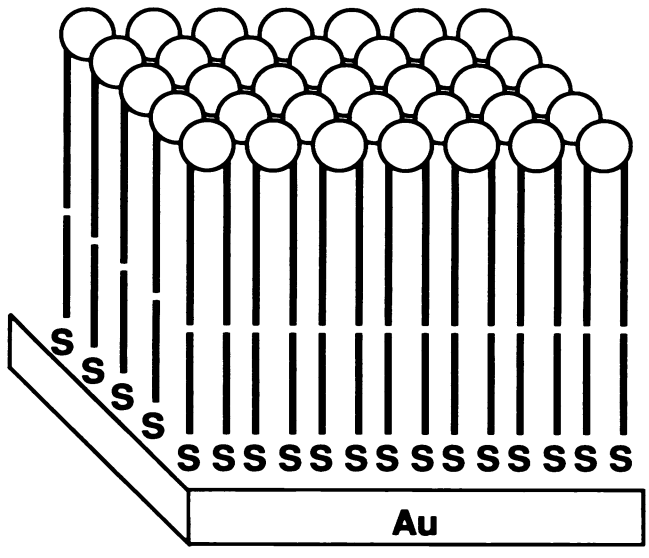

FIGURE 1 A schematic of the relation between the alkanethiol monolayer and the phospholipid in the hybrid supported bilayer. This representation is not meant to imply the absence of chain tilt.

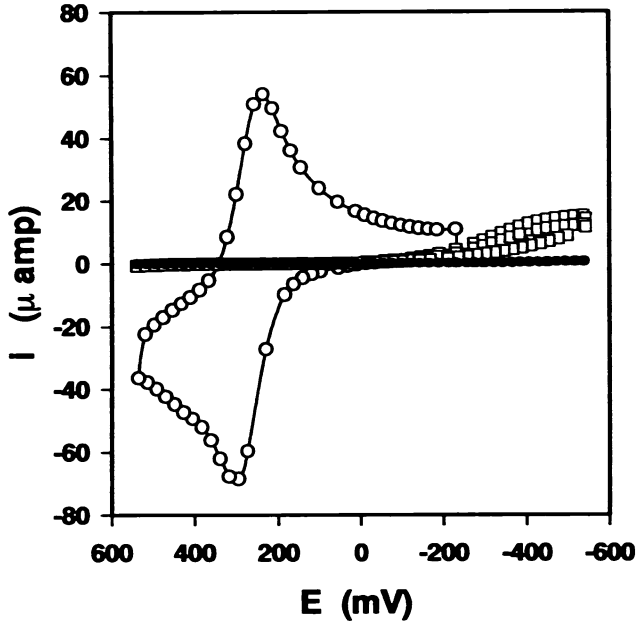

FIGURE 2 Cyclic voltammetry of 1 mM $K_3Fe(CN)_6$ in 1 M KCl at a bare gold electrode (○), a decanethiol monolayer (□), and a C18:1 PC/decanethiol bilayer (●). The scan rate was 50 mV/s.

thinly covered or uncovered defect sites in the layer, or if the response reflects ion or electron penetration through the thickness of a uniform layer.

To determine the mechanism of electron transfer at these surfaces, we examined the effect of $K_3Fe(CN)_6$ concentration and scan rate on the redox current generated. Fig. 3 is a plot of the concentration dependence of the current response for a monolayer-covered electrode and a bilayer-covered electrode. For a simple case of electron transfer between a metal surface and ions in bulk solution at a concentration c_a and a valency z_a (Bockris et al., 1970),

$$\frac{\partial \ln i_0}{\partial \ln c_a} = 1 - \left(\frac{\beta}{z_a}\right)$$

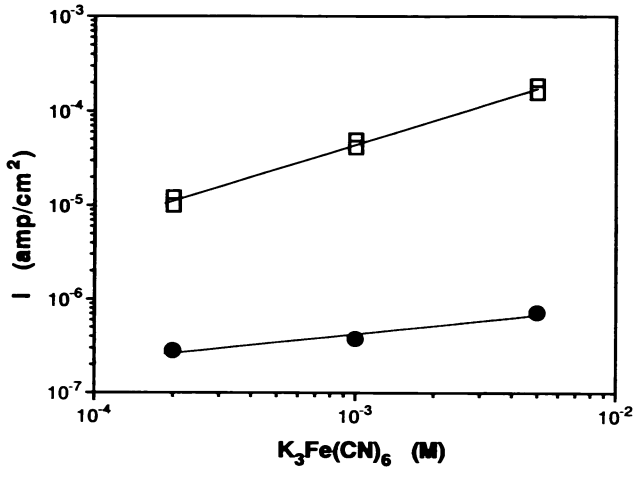

FIGURE 3 Reduction current at –0.55 V as a function of $K_3Fe(CN)_6$ concentration at a decanethiol monolayer (□) and at a C18:1 PC/decanethiol bilayer (●). Conditions are as in Fig. 2 .

As shown in Fig. 3, in the presence of the decanethiol monolayer, the log of the current at an applied potential of –0.55V is proportional to the log of the $K_3Fe(CN)_6$ concentration with a slope of ~0.85. The slope of the plot corresponding to the phospholipid/alkanethiol bilayer, 0.29, indicates an even smaller dependence of electron transfer on concentration. These results indicate that for the monolayer, and possibly for the bilayer, mass transfer of $Fe(CN)_6^{3-}$ ion in solution is not the rate limiting step in electron transfer. The effect of solution diffusion as a limiting step was also examined by measuring $K_3Fe(CN)_6$ reduction at different scan rates. For a diffusion-controlled electron transfer reaction, the current increases with the square root of the scan rate. Fig. 4 shows cyclic voltammograms for the bilayer from which the contribution from the charging current due only to the supporting electrolyte has been subtracted. The current is independent of the scan rate from 10 to 200 mV s^{-1}, demonstrating that in the presence of the bilayer electron transfer is not a solution mass-transfer diffusion-limited process. If the current generated was due to ion migration to the electrode surface through an imperfect bilayer, we would expect to see a dependence of current on scan rate. The data indicate that the electrode is fully covered by the bilayer, preventing direct interaction of the bulk solution redox species with the gold surface.

If we eliminate bulk solution diffusion as the rate-limiting step for electron transfer, we must consider the possibility that electron transfer occurs by diffusion of ions through the bilayer or that electrons tunnel through the bilayer. Studies of hydroxyalkanethiol monolayers on gold has led one group (Miller et al., 1991) to conclude that electron tunneling is the mechanism of electron transfer through monolayers. Sufficiently defect-free packing of methyl-terminated alkanethi-

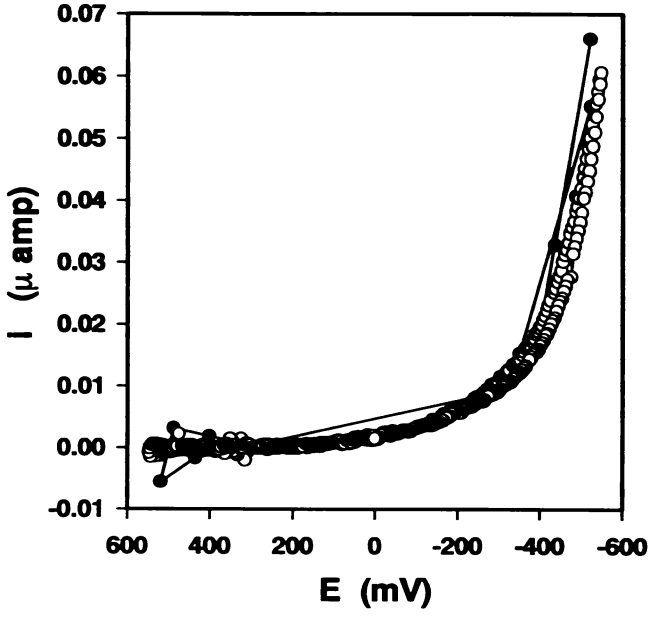

FIGURE 4 Effect of scan rate on reduction of 1 mM $K_3Fe(CN)_6$ in 1 M KCl. Scan rates were 10 mV/s (○) and 200 mV/s (●). The charging current due to 1 M KCl only was subtracted.

ols was reported to be more problematic, although other work from this group (Becka et al., 1993) showed, by measurement of diffuse layer potentials in monolayers of dodecanethiol and hydroxytetradecanethiol, evidence that electron transfer is limited by the average thickness of the film and is not limited to defect sites. While our monolayers of decanethiol may not be sufficiently defect-free to entirely eliminate transfer processes other than electron tunneling or hopping through the monolayer, of primary importance to us is to understand the nature of the barrier that is presented by the phospholipid portion of the bilayer.

Defects in the monolayer or bilayer could provide locations where ions may be able to more closely approach the electrode surface. In such a situation, electron transfer may occur by a combination of different rate processes. To characterize the number of kinetic processes by which electron transfer is occurring, we measured impedance in the presence of $Fe(CN)_6^{3-}$ and over a wide range of frequencies. Representative complex plane plots in Fig. 5 indicate that electrodes covered with either a decanethiol monolayer or a C18:1 PC/decanethiol bilayer show the presence of a single semicircle in the high frequency domain. This indicates that a single mechanism of electron transfer dominates the process. We have observed (data not shown) that complex plane plots of damaged electrodes which are not perfectly covered by the monolayer demonstrate a small partial semicircle at high frequencies, followed by a larger semicircle at lower frequencies, indicating the presence of relatively fast plus a much slower kinetic mechanism of electron transfer. However, under normal circumstances, electron transfer for both the monolayer and bilayer appears to involve a single predominant kinetic mechanism. Values of membrane resistance have been determined from these plots using a simple model of a parallel resistor/capacitor in series with a solution resistor (schematic shown in Fig. 6). As expected, the resistance associated with the bilayer, $\sim 1.5 \times 10^6\ \Omega$, is more than an order of magnitude higher than that for the monolayer.

Fig. 6 shows Bode plots for impedance measurements on a C18:1 PC/decanethiol bilayer. Each set of data was collected at a different dc potential. As discussed by Cahan and Chen (Cahan et al., 1982), assessing the appropriateness of an equivalent circuit model involves testing for processes that are potential-dependent. Regardless of the dc potential used, all of these data can be fit to the equivalent circuit model shown in the inset. Membrane capacitance, as portrayed by the sloped portion of the response, is independent of potential. Membrane resistance, determined from the impedance response at low frequency, decreases with increasing overpotential. In addition to demonstrating the adequacy of the equivalent circuit model, this figure also demonstrates that the structure of the bilayer, as indicated by the capacitance which is inversely proportional to the bilayer thickness, is independent of the applied potential. Thus the observed dependence of the bilayer resistance on applied potential is not the result of a change in bilayer thickness or integrity, but indicates the effect of the driving force on electron transfer through a discrete structure.

Membrane resistance is plotted as a function of applied dc potential, as shown in Fig. 7. Electron transfer due to tunneling is dependent on, among other things, the probability of a significant change in activation energy, $P(\Delta)$, of the ion at the interface, which is an exponential function of the

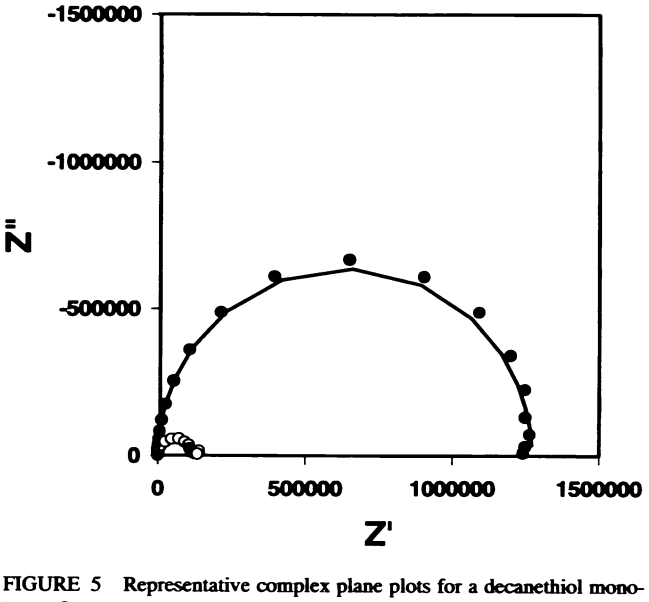

FIGURE 5 Representative complex plane plots for a decanethiol monolayer (○) and a C18:1 PC/decanethiol bilayer (●). Impedance was measured in 1 mM $K_3Fe(CN)_6$ and 1 M KCl at −0.55 V dc between 6.4×10^4 Hz and 0.1 Hz for the monolayer and 0.01 Hz for the bilayer. The lines represent the fit of the data to the equivalent circuit model shown in the inset of Fig. 6.

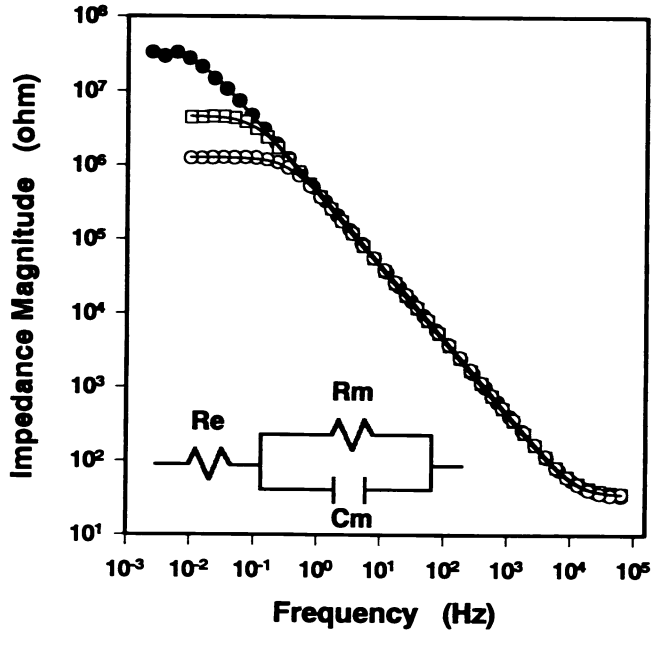

FIGURE 6 Bode plots of the impedance response for a C18:1 PC/decanethiol bilayer at −0.2 V (●), −0.4 V (□), and −0.55 V (○) in 1 mM $K_3Fe(CN)_6$ and 1 M KCl. The lines represent the fit of the data to the equivalent circuit model shown in the inset.

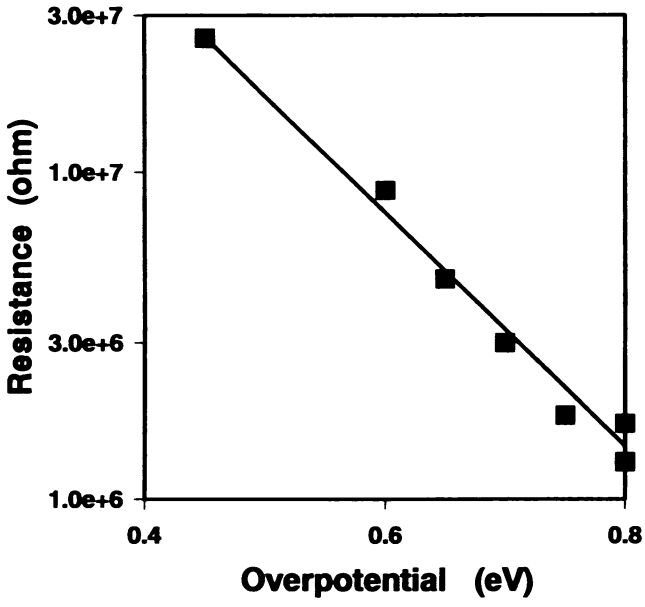

FIGURE 7 Bilayer resistance was determined as indicated for Fig. 5. Overpotential is assuming that E_0 for this system is +0.25 V. The line drawn is the result of linear regression analysis.

applied potential ($\Delta\phi$) (Bockris et al., 1970):

$$P(\Delta E_{\Delta\phi}) = P(\Delta E_0)\, e^{(-\beta e_0 \Delta\phi / kT)}$$

As is consistent with a mechanism of electron tunneling, membrane resistance was observed to be an exponential function of applied potential.

Our primary interest in understanding the electrical insulating characteristics of phospholipid/alkanethiol bilayers is to provide a technique by which we can evaluate the structure of the membrane and the structural changes that accompany membrane perturbing events.

One such structural issue is the nature of the dielectric barrier presented by the phospholipid membranes. The electrical capacitance of phospholipid membranes is dominated by the nonpolar acyl chain region of the bilayer (Benz et al., 1975). Measurements of electrical capacitance reported for BLMs and so-called "solvent-free" membranes indicate that the solvent used in the preparation of these bilayers has a very strong influence on the calculated thickness of membranes (Benz et al., 1975). The specific capacitance (C_m), which is the capacitance normalized for the electrode area, can be used to calculate the thickness (d) of a dielectric material, where ϵ_0 is the permittivity in free space and κ is the dielectric constant of the material:

$$C_m = \frac{\epsilon_0 \times \kappa}{d}$$

The dielectric constant most frequently used for phospholipids is 2.1, which happens to be the dielectric constant of decane, one of the solvents commonly used in the preparation of BLMs. It is apparent from work on the effect of different solvents on bilayer capacitance (Dilger et al., 1979) and from x-ray diffraction data on the thickness of bilayers prepared in the presence and absence of decane (McIntosh, 1980), that the presence of solvent can alter the dielectric constant of a membrane and its packing dimensions. Thus, the dielectric constant of phospholipid bilayers measured in other model systems is not totally unambiguous.

Specific capacitance values determined for the decanethiol monolayer and the phospholipid/decanethiol bilayers are shown in Table 1. The dielectric constant for these phospholipid layers is calculated from the slope of the line in Fig. 8, which is a plot of the phospholipid contribution to the bilayer capacitance as a function of the length of the phospholipid acyl chain. The 1/2 bilayer capacitance which corresponds to the hydrocarbon portion of the phospholipid region is calculated from the specific capacitance of the bilayer and the specific capacitance of the decanethiol monolayer:

$$\frac{1}{C_{m\text{-PL}}} = \frac{1}{C_{m\text{-bilayer}}} - \frac{1}{C_{m\text{-monolayer}}}$$

The 1/2 bilayer capacitance contributed by the phospholipid layer is a linear function of the number of carbons of the phospholipid acyl chain. The thicknesses of the hydrocarbon region of a single layer of C18:1 and C22:1 PC as determined by x-ray scattering (Lewis et al., 1983) are 13.5 and 17 Å, respectively. Using these thickness values, we calculate the dielectric constant for this lipid layer to be 2.7. This is slightly higher than the value of 2.1 which has been determined for bilayers produced in the presence of decane. We can prepare phospholipid/alkanethiol bilayers with a dielectric constant of 2.1 if we add decane to phospholipid vesicles from which the bilayer is made (Plant, 1993).

Analysis of the electron transfer process allows us to assess the effect of the bee venom toxin, melittin, on the C18:1/decanethiol bilayer membrane microstructure. Melittin monomers bind to cell membranes, insert at least partially into the hydrophobic portion of the bilayer, and probably aggregate within the bilayer, resulting in a defect or lesion in the membrane resulting in increased permeability (Dempsey, 1990). The effect of melittin on the C18:1 PC/decanethiol bilayer is evident from the cyclic voltammetry and impedance spectroscopy measurements. Cyclic voltammetry (Fig. 9 *A*) shows that the rate of electron transfer through the bilayer is greatly increased, approaching the magnitude of the current that is observed for the monolayer. The Bode plot of the impedance data (Fig. 9 *B*) shows that the capacitance of the bilayer is not

TABLE 1 Monolayer and bilayer resistance and capacitance

Layer	Resistance (Ω)	Specific capacitance (μF/cm^2)
Decanethiol	1.1 (0.1) × 10^5	1.60 (0.10)
Decanethiol/C22:1 PC		0.92 (0.01)
Decanethiol/C20:1 PC		0.99 (0.003)
Decanethiol/C18:1 PC	1.5 (0.2) × 10^6	1.06 (0.01)
Decanethiol/C18:1 PC + melittin	1.1 × 10^5	1.05

Resistance was measured at −0.55 V in $K_3Fe(CN)_6$. Capacitance was measured in TBS. Standard deviations are shown in parentheses and represent measurements of at least two different bilayer preparations.

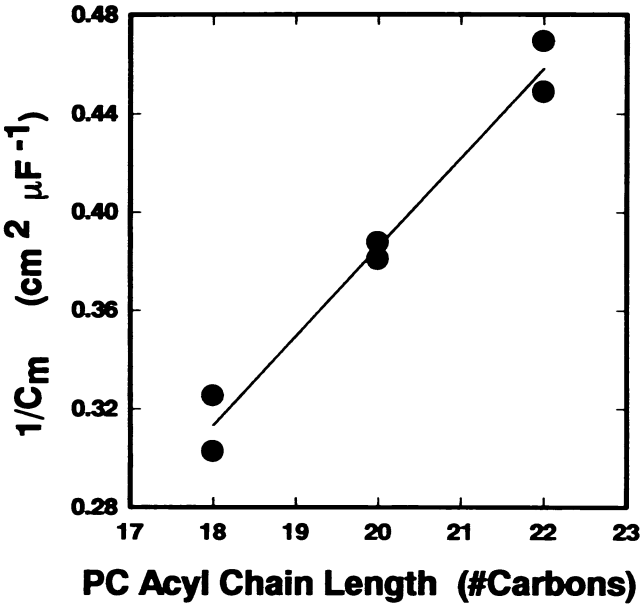

FIGURE 8 Specific capacitance for the bilayers was determined as indicated in the text. Each point represents a different sample. The line drawn is the result of linear regression analysis.

significantly changed, although the complex plane plot clearly demonstrates that the membrane resistance is greatly reduced (Fig. 9 *C*). The effect of melittin on bilayer resistance and capacitance is also shown in Table 1.

DISCUSSION

The effect of melittin on these phospholipid/alkanethiol bilayers is consistent with the known activity of melittin in cell and vesicle membranes. Evidently, the phospholipid portion of these bilayers is sufficiently flexible to accommodate a perturbing molecule. The electrical resistance which we determined for monolayers, bilayers, and the bilayer in the presence of melittin are listed in Table 1. The capacitance of the bilayer was not significantly affected by the addition of melittin, indicating that the thickness of the dielectric (the acyl chain region of the membrane) and its dielectric constant were unchanged. Thus, the average structure of the bilayer has not been significantly disrupted. Of particular significance is the appearance in the low frequency portion of the complex plane plot of a Warburg-like mass transfer impedance in series to the bulk membrane impedance. We characterize this element as "Warburg-like," since the average slope of the plot is approximately 1/3 instead of the expected value of 1. Although it is not clear at this time, we may be observing a membrane bulk impedance in series with an impedance consisting of the charge transfer resistance, the double layer capacitance, and intramembrane mass transfer (Warburg) impedance. It is possible that with further analysis, these data may provide information on the diffusion-controlled transport of electroactive ions in the membrane in the presence of a pore-forming protein. Unfortunately, the

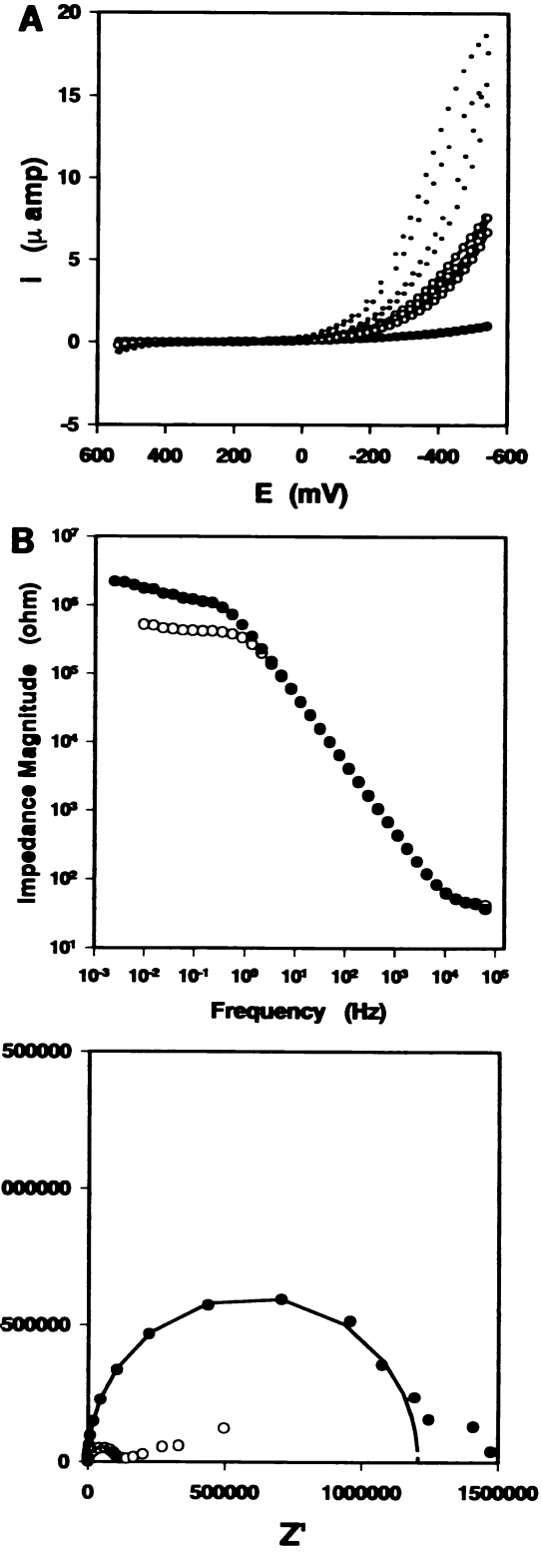

FIGURE 9 (*A*) Cyclic voltammetry of a C18:1 PC/decanethiol bilayer (●), of the bilayer after addition of 1×10^{-6} M melittin in 5 mM EDTA (○), and a decanethiol monolayer (···). Experimental conditions are as described for Fig. 2. (*B*) Impedance measurements for a C18:1 PC/decanethiol bilayer before (●) and after (○) addition of melittin. Conditions are as described for Fig. 5. (*C*) Complex plane plots of the impedance response for a C18:1 PC/decanethiol bilayer before (●) and after (○) addition of melittin. Conditions are as described for Fig. 5.

data at low frequencies tend to be affected by the history of the measurements and are more difficult to analyze. Further studies on this phenomenon are necessary and are planned.

We have measured resistivities of these bilayers to be as high as 5×10^7 ohm-cm^2, which is within an order of magnitude of the resistivity of BLMs (Tien et al., 1985). We can estimate the dc conductivity of these bilayers:

$$\sigma = \left(j \times \frac{l}{v}\right)_{v \to 0}$$

where j is the current density, l is the alkane chain length values reported from x-ray scattering determinations, and v is the applied voltage. Conductivities measured here are less than 1×10^{-12}ohm^{-1}cm^{-1}, which are in reasonable agreement with values reported by Polymeropoulos and Sagiv (1978) for adsorbed layers of fatty acids. Although reasonably similar, our hybrid bilayers are not identical to other planar model membrane systems. This difference is perhaps most clearly seen in our determination of the dielectric constant of our phospholipid layer. We calculate a dielectric constant of 2.7 for the phospholipid based on our measurements of specific capacitance and using published x-ray scattering results of hydrocarbon chain lengths. This calculated dielectric constant is higher than the accepted value for phospholipids in BLMs of 2.1. As we have discussed previously (Plant, 1993), this discrepancy in the dielectric constant, and possibly in the conductivity and resistivity as well, could be due to the contribution of residual organic solvent which is present in BLMs and similar planar membranes.

The large resistivities, small conductivities, and the high degree of irreversibility in reduction and oxidation of $Fe(CN)_6^{3-}$ suggest defect-free coverage of the electrodes by the phospholipid/alkanethiol bilayers. We have used a simple model for analysis of our impedance data: a solution resistance in series with a parallel resistor/capacitor which corresponds to the bulk membrane. In the absence of melittin, this model adequately describes the data. Electron tunneling has been suggested as the mechanism of electron transfer through long-chain carboxyalkanethiol monolayers (Miller et al., 1991) as well as through phospholipid bilayer membranes (BLMs) (Tien et al., 1985). Our data indicate that solution mass transfer kinetics is not the rate-limiting step in electron transfer through these bilayers and are consistent with electron tunneling. Very low frequency impedance data suggest, however, that in the presence of melittin, a very slow diffusion process, presumably occurring in the membrane, is also playing a role. Further analysis of this diffusion process may provide insight into structural characteristics of the membrane defects produced by proteins such as melittin.

CONCLUSION

As models of cell membranes, these rugged hybrid bilayers allow analysis of membrane microstructure which is uncompromised by the inclusion of organic solvent and the accompanying ambiguity associated with solvent lensing and the effects on capacitance and lipid layer thickness. The membranes are formed on gold, which not only provides a physical support which renders them rugged, but which can be used as an electrode to provide a direct means of determining electrical insulating characteristics. We have shown here that these bilayers are relevant models of the insulating properties of lipid layers by using them for determination of a solvent-free dielectric constant for membrane phospholipids and demonstrating the effect of the protein toxin, melittin, on their insulating capacity. These bilayers are not, of course, identical to other planar membrane systems, since the close association between the thiol sulfur and gold of the alkane monolayer precludes the normal insertion of a transmembrane protein. Furthermore, the extent of mobility of alkanethiols at the gold surface is yet to be resolved, and the effect that the coordination of the monolayer monomers with the surface might have on the phase behavior and lateral mobility of the phospholipid layer is still to be assessed. If these criteria are satisfied, this model system will be extremely useful by providing a way of studying membrane phenomena such as lipid domains and the organization of proteins in membranes, for example, with a variety of surface analytical techniques.

In addition to their potential contribution to membrane biophysics, the ease of formation and the long-term stability of these hybrid bilayers also make them amenable to applications such as monitoring lipophilic contaminants which can partition into them (Plant and Gugetchkeri, 1994). The highly insulating nature of phospholipid/alkanethiol bilayers, coupled to their responsiveness to agents which perturb membrane structure and electrical properties, makes them good candidates for development as components of sensors and perhaps electronics coatings.

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