LETTER TO THE EDITOR

Voltage Dependence of the Capacitance and Area of Black Lipid Membranes

Dear Sir:

A number of papers have appeared in *Biophysical Journal* on the subject of compression of black lipid membranes by electric fields (White, 1970, 1974; Crowley, 1973; Requena et al., 1975; Alvarez and Latorre, 1978). These papers show that the thickness (δ_B) of the bilayer decreases with applied potential. Another variable affected by the electric field is the area (A) of the bilayer, which increases with potential (White, 1972; Wobschall, 1972; White and Thompson, 1973; Requena et al., 1975). The purpose of this letter is to report the results of an analysis of the dependence of area upon voltage for black films formed from glyceryl monooleate (GMO) dispersed in *n*-decane (C_{10}) and *n*-hexadecane (C_{16}). The results are used to establish the functional form of the area-voltage relation and to discuss the contribution of area changes to the voltage-dependent capacitance of Montal-Mueller (1972) black lipid membranes (Alvarez and Latorre, 1978).

GMO/C_{10} and GMO/C_{16} bilayers were chosen for analysis because δ_B of the former depends strongly on voltage, whereas δ_B of the latter is very weakly dependent (Andrews et al., 1970). This difference in behavior is important because the total volume of the bilayer is sometimes assumed to be conserved during electrocompression, i.e., $\delta_B(0)A(0) = \delta(V)A(V)$ (White, 1974). We shall address the validity of this assumption before proceeding with the analysis based on equations derived by White (1972).

The primary voltage-dependent thinning mechanism for black films formed with short alkane molecules (e.g., *n*-decane) is a shift of alkane from the bilayer to microlenses and annulus (Plateau-Gibbs border) (Requena et al., 1975). This shift, or solvent exclusion, may be due to an electrostriction-induced increase in the chemical potential of the alkane in the bilayer relative to that in the annulus and microlenses (White, 1980). For membranes formed under conditions that leave little or no alkane in the bilayer, the major thinning mechanism is a very slight increase in the area per surfactant molecule (Alvarez and Latorre, 1978). This mechanism also operates in alkane-containing bilayers but is small compared with the solvent exclusion process (Requena et al., 1975). For either the solvent-containing or solvent-free black lipid membranes, the bilayer is surrounded by an annulus (White et al., 1976) of bulk lipid suspension consisting of surfactant dispersed in a relatively nonpolar lipid such as alkane, squalene (White, 1978), triglyceride (Waldbillig and Szabo, 1979), or petrolatum (Montal and Mueller, 1972).

The analysis of White (1972) shows that the area of the bilayer is determined by the volume of the annulus, the contact angle θ between the bilayer and annulus, and the contact angle ϕ between the annulus and the wall of the aperture. The application of an electric field causes an increase in θ (Requena and Haydon, 1975) and, consequently, the shape of the annulus changes (White, 1972). If the volume of the annulus remains approximately constant, the area of the bilayer will increase through the generation of new bilayer. The extent of the area increase depends only indirectly on how much the bilayer thins. This can be seen from the Lipmann equation for the black film system (Requena and Haydon, 1975),

$$\cos\theta(V_0) - \cos\theta(V) = (C_s/4\gamma)(V^2 - V_0^2), \tag{1}$$

in which C_s is the true specific capacitance of the bilayer and γ is the annulus/water interfacial tension. The contact angle increases from $\theta(V_0)$ to $\theta(V)$ when the potential increases from V_0 to V. The specific capacitance depends upon thickness since $C_s = \epsilon_0 \epsilon_B / \delta_B$ where $\epsilon_0 = 8.85 \times 10^{-14}$ F/cm, ϵ_B is the dielectric

coefficient, and δ_B the thickness of the hydrocarbon core of the bilayer. Eq. 1 thus shows that θ will change with potential even if δ_B remains constant. Therefore, bilayer area is determined primarily by boundary conditions and only indirectly by δ_B. The condition $\delta_B(0)A(0) = \delta_B(V)A(V)$ is not true in general, although it may be a good approximation under certain conditions as described later.

The dependence of A on voltage can be established by solving the equation for the shape of the annulus (White, 1972) at constant volume with $\theta(V)$ as the independent variable. $\theta(V)$ has been determined experimentally for the GMO/C_{10} and GMO/C_{16} systems (Requena and Haydon, 1975). We have computed the percentage change in membrane area as a function of applied potential with the initial (i.e., $V = 0$) scaled radius $\rho(0)$ as a parameter. $\rho(V) = r/R$ where r is the radius of the presumed circular bilayer and R is the radius of the circular aperture upon which the bilayer is formed. The results are shown in Fig. 1 for GMO/C_{10} membranes (solid curves) and GMO/C_{16} membranes (dashed curves). As background information, when the potential changes from zero to 150 mV, θ changes from 1.96 to 6.60° for GMO/C_{10} and from 5.44 to 10.77° for GMO/C_{16}. The contact angle ϕ was taken as 30° (White et al., 1976). The computations were performed by computer in the following way. A value of $\rho(0)$ was chosen and the scaled volume of the annulus calculated, given $\theta(0)$ and ϕ. This volume was taken as constant during the remainder of the calculation. $\theta(V)$ for the new potential V was then introduced into the program and ρ allowed to increase to a new value $\rho(V)$, at which the calculated scaled volume was the same as for $\theta(0)$ and $\rho(0)$. The percentage change in area for a given V was then calculated from $100\,[A(V) - A(0)]/A(0)$ where $A(V) = \pi\rho(V)^2$.

Fig. 1 shows that the effect of potential on area depends strongly on $\rho(0)$. The area changes by 50–60% for $V = 150$ mV with $\rho(0) = 0.2$, but by only ~1% with $\rho(0) = 0.9$. This is consistent with the findings of Requena et al. (1975), who reported that a 100-mV potential causes the area of GMO/C_{10} films to increase by 100% when the bilayer occupied <10% of the aperture area but by <1% when the bilayer occupied most of the area. Unpublished measurements made in our laboratory are compared with theory in Fig. 2. The agreement is quite good considering that (*a*) bilayer area changes with time because of changes in annulus volume brought about by exchanges with material on the surfaces of the septum, and (*b*) the aperture used for the experiment did not have the simple cylindrical geometry used in the theory (see Fig. 2).

The experimental data of White and Thompson (1973) suggest that $A \propto V^2$. The exact dependence of

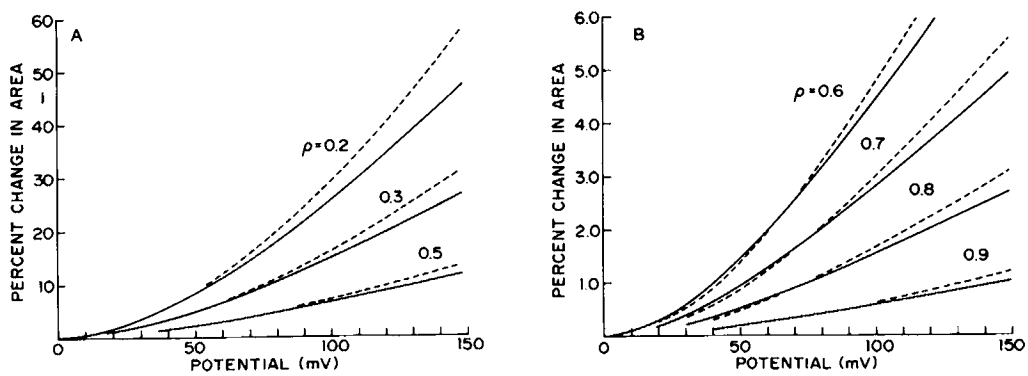

FIGURE 1 Dependence of area upon potential of black lipid films formed from glycerol monooleate dispersed in *n*-decane (solid curve) and *n*-hexadecane (broken curve). The percentage change is determined analytically (see text) with the equations of White (1972) and the contact angle data of Requena and Haydon (1975). $\rho(V) = r/R$, where r is the radius (voltage dependent) of the bilayer and R is the radius of the aperture upon which the bilayer is formed. As $\rho(0)$ increases, the percentage change in area for a given potential decreases. (*A*) Curves for $\rho(0) = 0.2$, 0.3, and 0.5. (*B*)Curves for $\rho(0) = 0.6$, 0.7, 0.8, and 0.9.

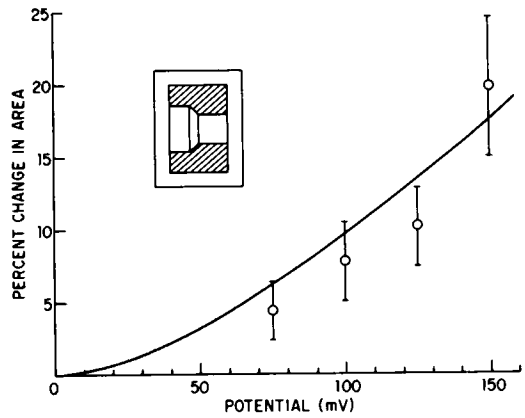

FIGURE 2 Percentage change in area with potential for a GMO/C_{10} bilayer in 0.1M NaCl with $\rho(0) \simeq 0.4$. The curve is that predicted by the theory for a simple cylindrical aperture. The agreement with experiment (open points with estimated error bars) is reasonable considering that membrane area changes with time and that the aperture was not of simple cylindrical geometry but rather had the counter-sunk geometry shown in the inset. This geometry (White and Thompson, 1973) is used in our laboratory because it stabilizes the position of the film in the aperture. That is, it eliminates front-to-back drift of the film. The diameter of the smaller hole was used in calculating $\rho(0)$.

A on V, however, is difficult to determine experimentally because the volume of the annulus often changes throughout the course of the experiment. To establish this dependence more precisely, the analytical data of Figs. 1 and 2 were fitted to the equation

$$\frac{A(V) - A(0)}{A(0)} = BV^n \tag{2}$$

Technically, the exact form, of $A(V)$ can be determined from the equations of the annulus (White, 1972). Practically, however, the equations are complicated functions of elliptic integrals and are not informative. We thus fitted the data to Eq. 2. The fit is excellent as judged by correlation coefficients of better than 0.999. The exponent on V depends upon $\rho(0)$ but is typically in the range 1.8–1.9, so that very approximately the fractional area change does go as the square of the potential. B also depends strongly on $\rho(0)$. As an example of the fit of the data, the values for GMO/C_{16} with $\rho(0) = 0.2$ are $B = 22.15 \pm 1.25$ (SE) and $n = 1.89 \pm 0.02$ (SE) with $r = 0.9995$.

Results of the above analysis may be used to estimate the possible contribution of area changes to the voltage-dependent capacitance (Alvarez and Latorre, 1978) of "solvent-free" bilayers formed by the method of Montal and Mueller (1972). Alvarez and Latorre (1978) showed that the total capacitance of the solvent-free bilayer obeys the equation

$$C(V) = C(0)\,(1 + \alpha V^2), \tag{3}$$

where $C(0)$ is the zero-voltage value of capacitance and α is a constant equal to 0.036 for GMO bilayers. The specific capacitance $C_s(V)$ obeys an equation similar to Eq. 3 (White, 1970; White and Thompson, 1973), and we write

$$C_s(V) = C_s(0)\,(1 + \beta V^2). \tag{4}$$

A cautionary remark about this equation is in order. It was derived from measurements on solvent-containing membranes such as those formed from lecithin and decane. It is not unreasonable, however, to assume a similar form of the equation for solvent-free membranes, since the compressive force is proportional to V^2 and only small deformations are expected.

Assume in Eq. 2 that $n \simeq 2$. Consequently,

$$A(V) \simeq A(0)\,(1 + BV^2). \tag{5}$$

Substitution of Eqs. 4 and 5 into Eq. 3 yields

$$C(V) \simeq C_s(0)A(0)\,[1 + (\beta + B)V^2 + \beta BV^4] \tag{6}$$

If $\beta \ll 1$ and $B \ll 1$, one may write with good accuracy

$$C(V) \simeq C_s(0)A(0)\,[1 + (\beta + B)V^2]. \tag{7}$$

Comparing with Eq. 3, one finds $C(0) = C_s(0)A(0)$ and $\alpha = \beta + B$. The relative contributions to $C(V)$ of thickness changes and area changes may thus be assessed by comparing β and B. Alvarez and Latorre (1978) made the assumption discussed earlier that $\delta_B(0)A(0) = \delta_B(V)A(V)$. This is equivalent to saying that $\beta = B$ or $\alpha = 2\beta$. They found $\alpha = 0.036$ and therefore took $\beta = 0.018$. We have estimated B for comparison with this number from our analysis of the GMO/C_{16} system. If $\rho(0) = 0.999$, $B \simeq 0.01$, but if $\rho(0) = 0.990$, then $B \simeq 0.05$. Thus, it is not unreasonable to assume $\beta \sim B$. In arriving at this conclusion, however, we have made two assumptions.

First, we have assumed $\rho(0) \sim 1$, but if it were considerably smaller, then B would become dominant and α would increase significantly. Second, we have assumed that equilibrium prevails in the Alvarez-Latorre experiment. Measurements of α for "solvent-free" GMO/squalene bilayers by the Alvarez-Latorre technique were reported by White (1978). The value of α for these membranes, which had a value of $\rho(0)$ considerably smaller than 0.99, was approximately equal to the value of Alvarez and Latorre (1978). The explanation for this lack of dependence of α on $\rho(0)$ might be that our second assumption is not correct. That is, the annulus might not be in equilibrium with the bilayer during the time course of the experiment (25–250 μs). The work of Evans and Hochmuth (1978) suggests that the time constant for areal dilation of a bilayer is considerably shorter than 25 μs. One can thus imagine a rapid change in thickness accompanied by an area change obeying the relation $\delta_B(0)A(0) = \delta_B(V)A(V)$ occurring before the annulus has time to reequilibrate with the bilayer. Data presented by Benz et al. (1975) for the time-course of capacitance changes for GMO/decane membranes are consistent with this hypothesis. During the first 1 or 2 ms after the application of a voltage, the change in capacitance is much less than 1%, but over a period of 1 s, it increases to 12%, following a sigmoidal curve. In summary, it seems likely that upon the application of a voltage to a black film, there is a rapid (<25 μs) decrease in thickness accompanied by a compensatory increase in area to maintain constant membrane density. During this time the annulus is out of equilibrium with the bilayer. Later (times >1 ms), the shape of the annulus changes and there occurs a further increase in area. If significant amounts of solvent are present, a further decrease in thickness also occurs, owing to solvent exclusion.

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