Letter to the Editor

Influence of Transmembrane Voltage on the Structure of Membrane Components

Le Saux et al. (2001) report on voltage-induced reorientations of lipid headgroups using dried lipid multilayers of dioleylphosphatidylcholine as a membrane model. I observed the same phenomenon many years ago based on the measurement of capacitance relaxation currents (displacement currents) in black lipid membranes of various compositions (Sargent 1975a,b), and corresponding effects have been seen with virtually solvent-free bilayers (Hianik and Passechnik, 1995; Hianik et al., 2000). The independent observation of the same phenomenon by two completely different methods and in rather different model systems provides good corroboration of the influence of transmembrane voltage on the structure of the lipid headgroup region, but it is of interest to compare the results of the two methods in more detail.

The attenuated total reflection Fourier-transform infrared (ATR-FTIR) study of Le Saux et al. is based on measurement of changes in the relative absorption of perpendicular and parallel components of infrared bands associated with the polar headgroups of lipid layers exposed to transverse electric fields. The electric field is induced through the proximity of the polarized germanium internal reflection elements to the dried multilayer sample. From the description of the experimental cell and the multilayer preparations, the average potential drop per bilayer in the FTIR measurements will be at the most a few hundred millivolts at the highest potential applied (200 V), but could be somewhat less. The measurements were started 1 min after applying the voltage and take minutes to accumulate, and thus presumably represent equilibrium distributions of molecular orientations. Although a quantitative analysis was not shown by Le Saux et al., they state that they found an almost linear relationship between the amplitude of the dichroic peaks and the applied potential. The measured parameters allow a molecular interpretation of the response of the system to the applied voltage, viz., reorientations of the phosphate and choline moieties. Unfortunately, because of the small size of the signals involved, they were not able to derive a reliable estimate of the magnitude of the reorientation.

In the capacitance relaxation method with lipid bilayers, the voltage is applied to a single membrane through the aqueous phases on the two sides of the bilayer, ensuring a good electrical contact and, thus, a precise value for the voltage across the membrane. Voltages up to several hundred millivolts can be applied before membrane breakdown occurs. The combination of a dielectric (lipid bilayer) separating two conducting phases (electrolyte) represents an electric capacitor, holding charge $Q = C \cdot V$. A change in the applied voltage induces a current given by $i = dQ/dt = C \cdot dV/dt + V \cdot dC/dt$. The latter term includes both a bulk geometric component (electrostriction: voltage dependence of bilayer area and thickness), molecular deformation (induced dipole), and reorientation components. By choosing initial and final voltages which are of equal magnitude but opposite sign, changes attributable to electrostriction can be eliminated and, after the charging pulse ($C \cdot dV/dt$) is over, molecular components may be observed. Depending on the details of the experimental setup (electrolyte concentration, electrode area, output impedance of the voltage source) the time resolution of the method can extend down to the microsecond region. This is much too slow to observe molecular deformation processes or unhindered rotation of single molecules, but cooperative motion (clusters), as would be characteristic of liquid crystal phases such as bilayers, can lie in this range. The effects observed are completely reversible (changes from $-V$ to $+V$ and $+V$ to $-V$ are mirror images of one another) and the magnitude of reorientation was found to be a linear function of the applied voltage jump up to at least 600 mV, explaining the similar observation of Le Saux et al. Despite the very high field strengths in the bilayer ($6 \cdot 10^5$ V/cm for 300 mV across a 50 Å thick bilayer), it is interesting that there is no sign of saturation. Higher transmembrane voltages generally lead to rupture of the membranes.

Calculations suggest an average change of orientation of the membrane lipid dipoles of 1° or less per 100 mV in the cases studied (Sargent, 1975a), although there are several approximations involved in deriving this value.

The ATR-FTIR technique provides direct evidence for reorientation of specific parts of the lipid headgroups in the membrane and confirm, for example, the participation of the lipid in the response, although ordered water molecules also contribute to the membrane-associated surface dipole potential (Brockman, 1994). In contrast, the capacitance relaxation method allows for quantitative estimates of magnitude, linearity, and time course of the reorientations.

Both transmembrane and adsorbed proteins could affect and be affected by voltage-induced dipolar reorientations in membranes. For example, changes induced in the capacitance relaxation parameters have been used to help characterize the interaction of surface-active molecules with bilayers (Hianik et al., 1998, 2000). An example of possible direct relevance of headgroup reorientation is the suggestion of a coupling of action potential and nerve conduction through ordering and disordering of dipole domains (electrets). A model study by Wobschall (1968) indicated that the

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time constants for such domains must lie between 0.14 and 1.4 ms, and capacitance relaxation phenomena in dioleollecithin bilayer lipid membranes are found in this range (Sargent, 1975b).

A combination of the more sensitive electrical methods with the more specific structural information of the ATR-FTIR technique, and perhaps further experimental methods, should further our understanding of voltage-linked membrane phenomena.

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