BRIEF COMMUNICATION

AUTOCORRELATION ANALYSIS OF HYDROPHOBIC ION CURRENT NOISE IN LIPID BILAYER MEMBRANES

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ABSTRACT The autocorrelation function of a given process is related to its spectral density by the Wiener-Khintchine theorem, and both expressions contain the same information. We report here a measurement of the current noise produced in a lipid bilayer membrane doped with hydrophobic anions of dipicrylamine. The results are in good agreement both with relaxation measurements on the same membrane and with an analysis of the spectral density of the current noise for this system which has been presented by other workers. Although measurement of the spectral density function is generally more complete for technical reasons, the autocorrelation function provides, for the case studied here, more physical insight into the underlying charge transport mechanism. We find that the measured autocorrelation function is negative at short, but nonzero, times. This is a consequence of the operative conductance mechanism in this case, which cannot carry current continuously in the same direction without compensatory reverse flow.

INTRODUCTION AND THEORY

Ketterer et al. (1971) performed voltage-jump relaxation measurements on bilayer membranes formed in the presence of dipicrylamine (DpA^-) and of sodium tetraphenylborate. They presented a model interpreting observed current transients in terms of the field-induced translocation of these hydrophobic anions from one membrane/solution interface to the other. The key parameters of their model, both directly measurable, are the initial membrane conductance, λ_{00}, and the characteristic decay time, $\tau_i = (2k_i)^{-1}$, where k_i is the rate constant for movement of hydrophobic ions across the membranes. Here λ_{00} is the ratio of the initial transient current density (excluding that which charges the membrane capacitance) to the amplitude of the applied voltage step. The preceding definitions are subject to the conditions that the applied voltage amplitude be small compared to (kT/e), and that hydrophobic ion desorption current be negligible. Both conditions are readily achieved experimentally.

More recently, Kolb and Läuger (1977) have shown that the current noise generated by a bilayer membrane in the presence of hydrophobic ions is equivalent to that generated by a resistance, R, and capacitance, C, in series. These quantities are related to the model parameters defined above by $RC = \tau_i$ and by $R^{-1} = A\lambda_{00}$, where A is the membrane area. It is emphasized that R and C are not to be equated to the electrode and solution resistance or to the geometric capacitance of the membrane, i.e., to the parameters that govern the membrane charging time. Kolb and Läuger have shown further that the spectral density of current noise generated by a hydrophobic ion-doped membrane will be given by:

$$S_I(\omega) = \frac{4kT}{R} \frac{\omega^2 \tau_i^2}{1 + \omega^2 \tau_i^2}$$
$$= \frac{4kT}{R} \left[1 - \frac{1}{1 + \omega^2 \tau_i^2} \right], \quad (1)$$

where ω is the frequency. The quantity $S_I(\omega)$ is related to the distribution in frequency of the time-averaged noise power generated by a membrane. Equivalent information in the time domain is provided by the autocorrelation function of the noise current, $C_I(\tau)$, which compares the noise current, $I(t)$, with a time-shifted version of itself, the amount of the shift being τ. The Wiener-Khintchine theorem (Van der Ziel, 1970) relates $C_I(\tau)$ to $S_I(\omega)$ as follows:

$$C_I(\tau) = \frac{1}{4\pi} \int_{-\infty}^{\infty} S_I(\omega) e^{i\omega\tau} d\omega. \quad (2)$$

We insert into Eq. 2 the expression for $S_I(\omega)$ given by the second form of Eq. 1, and evaluate the two integrals that result. The first yields a δ-function having its singularity at $\tau = 0$ (see, e.g., Merzbacher, 1961). The second integral is evaluated by contour integration, noting the simple poles at $\pm i\omega\tau_i$. For $\tau > 0$ we choose a contour extending along the real ω-axis and closing in the upper half of the complex plane, thereby enclosing the pole at $+i\omega\tau_i$. For $\tau < 0$ the contour is closed in the lower half of the complex plane. The combined result of these operations is:

$$C_I(\tau) = \frac{2kT}{R\tau_i} \left[\delta\left(\frac{\tau}{\tau_i}\right) - \frac{1}{2} \exp - \left(\frac{|\tau|}{\tau_i}\right) \right], \quad (3)$$

which is valid over the full range, $-\infty < \tau < \infty$. Eq. 3 indicates that a current fluctuation transporting charge across the membrane in a given direction will on average be preceded and followed, in times of order τ_i, by oppositely directed fluctuations.

MATERIALS AND METHODS

Bilayer membranes were formed by the brush technique in a cell of quartz and Teflon (DuPont de Nemours & Co., Inc., Wilmington, Del.) construction, using a 1% (wt/vol) solution of synthetic dioleyl phosphatidylcholine (Analabs, Inc., North Haven, Conn.) in *n*-decane. Aqueous solutions bathing the membranes were unbuffered 0.1 M NaCl. All measurements were made at a temperature of 23°C.

Membrane current noise was measured in a manner similar to that described by other workers (Kolb et al., 1975; Kolb and Läuger, 1977). The membrane and associated cell with electrodes were placed in the input circuit of an operational amplifier (model 43K, Analog Devices, Inc., Norwood, Mass.). The amplifier so used constituted a current to voltage (I/V) converter with conversion ratio equal to the reciprocal of the feedback resistance employed. The feedback resistor used had a value of 50 MΩ (±1%), high enough to insure that its own contribution to the observed current noise would be negligible. A shunt capacitance of ~1 pF was placed across the feedback resistor to eliminate high frequency instability introduced by the membrane capacitance in the input circuit.

The output of the I/V converter was passed through the vertical amplifier of a Tektronix, Inc. (Beaverton, Oreg.) storage oscilloscope, type 7313, equipped with type 7A22 differential amplifier plug-in modules. This provided both a useful visual reference to the output noise voltage from the I/V converter and necessary additional gain before applying the output to the input of a Saicor-Honeywell, Inc. (Denver, Colo.) model SAI-43A correlation and probability analyzer. Upper and lower 3-dB points of the oscilloscope vertical amplifier were set at 30 kHz and at DC, respectively. This selection of upper 3-dB point sufficed to insure that the overall upper limit of frequency response of the system was set by the I/V converter (~3 kHz).

The model SAI-43A was operated in its autocorrelation (full) mode with its input AC coupled. This set the overall low-frequency, 3-dB point of the system at 1 Hz. For the data reported here the instrument was set for a sample increment of 20 μs and a precomputation delay of −200. With these settings the 400-point autocorrelation function generated by the SAI-43A covered the lag value range -4 ms $\leq \tau \leq 4$ ms. With the conversion ratio of the I/V converter and the voltage gain of the oscilloscope vertical amplifier known, overall system calibration was completed by introducing a square wave of known amplitude to the input of the SAI-43A and measuring the amplitude of the resulting triangular wave autocorrelation function, which was output to an *x-y* recorder.

A 100-Ω resistor was placed in series with the membrane/electrode assembly in the input circuit of the I/V converter. Injection of a current pulse through this resistor then provided a low-amplitude (10 mV) voltage pulse across the membrane, which permitted independent determination of the parameters, τ_i, and R, by the relaxation method (Ketterer, et al., 1971). These results were compared, as described below, with noise measurements made on the same membrane.

RESULTS AND DISCUSSION

Results of an autocorrelation analysis of membrane current noise, measured in the presence of 10^{-7}M DpA^- in the surrounding aqueous phases, are shown in Fig. 1. The current transient accompanying application of a 10-mV voltage pulse to the same membrane was also measured from an oscilloscope photograph and plotted on a semilog graph of current vs. time (data not shown). For this graph an extrapolated initial current, excluding the membrane charging spike, of 4.50×10^{-8} A was determined. The corresponding initial resistance inferred from the relaxation experiment is thus $R = 2.22 \times 10^5\ \Omega$. From the slope of the straight line on the semilog plot depicting the current decay with time it was established that $\tau_i = 1.33$ ms.

The numerical values of R and τ_i determined by the relaxation experiment have been used in Eq. 3 to generate the dashed-line curve superposed on the data of Fig. 1. The finite width of the central peak of the measured autocorrelation function is governed by the upper cutoff frequency of the system. The remainder of the measured function displays negative correlation that decays to zero in good quantitative agreement with the expectations of the theory presented above.

Negative correlation means that the current flowing at any instant must, on time average, be preceded and followed by oppositely directed currents. The magnitude of this correlation decays exponentially to zero with increasing lead or lag time. The characteristic decay time is that of the series RC equivalent circuit that models the membrane to which hydrophobic ions are strongly adsorbed. The sharp central maximum at $\tau = 0$ is to be expected because $C_I(0)$ is

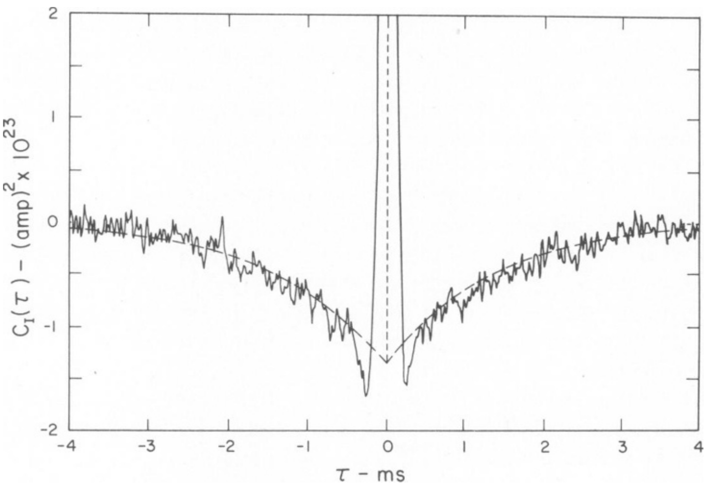

FIGURE 1 The solid-line curve illustrates the measured autocorrelation function (see Eq. 2) of the noise current generated by a dioleyl phosphatidylcholine membrane of area 0.58 mm^2. The surrounding aqueous phases contain 0.1 M NaCl and 10^{-7} M DpA^-. The peak of the central maximum is clipped by both *x-y* recorder overranging and by memory overflow in the correlator. The dashed-line curve is a plot of the theoretical autocorrelation function predicted by Eq. 3, using R and τ_i values deduced from a relaxation measurement on the same membrane as explained in the text. The applied transmembrane potential is 0 mV, i.e., the membrane is short-circuited through the electrodes immersed in the adjacent aqueous phases. (amp = amperes)

equal to the time average of $[I(t)]^2$, which must always be positive. Integration of Eq. 3 showed in addition that $\int_{-\infty}^{\infty} C_I(\tau)d\tau = 0$ for this system.

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