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NOISE ANALYSIS AND RELAXATION EXPERIMENTS OF TRANSPORT OF HYDROPHOBIC ANIONS ACROSS LIPID MEMBRANES AT EQUILIBRIUM AND NONEQUILIBRIUM

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Under equilibrium and nonequilibrium steady-state conditions, the spectral intensity of current noise $S_J(f)$ generated by the transport of hydrophobic anions across lipid bilayer membranes was investigated. The experimental results were compared with different reaction models. $S_J(f)$ showed a characteristic increase proportional to f^2 between frequency-independent tails at low and high frequencies. This gradient was found to be independent of applied voltage which indicates the contribution of a single voltage-dependent reaction step of ion translocation across the membrane. From the shape of $S_J(f)$ at low frequencies the rate constant of ion desorption from the membrane into the aqueous phase could be estimated. Unambiguous evidence for the application of a general model, which includes the coupling of slow ion diffusion in the aqueous phase to ion adsorption/desorption at the membrane interface, could not be obtained from the low-frequency shape of $S_J(f)$. The shot noise of this ion transport determines the amplitude of $S_J(f)$ at high frequencies which decreases with increasing voltage applied. Analysis of voltage-jump current-relaxation experiments and of current noise carried out on one membrane yielded significant differences of the derived ion partition coefficient. This deviation is qualitatively described on the basis of incomplete reaction steps.

1. Introduction

The analysis of electrical noise generated by ion transport across biological or artificial membranes allows a description of the underlying molecular transport mechanism in terms of reaction steps [1–4]. The theoretical interpretation of the measured electrical noise depends on the type of reaction scheme chosen for the description of the ion transport. In this paper the current noise generated by the transport of lipid-soluble anions across planar lipid bilayer membranes is measured. The transport mechanism of hydrophobic ions such as dipicrylamine (DPA^-) and tetraphenylborate (TPB^-) has been investigated using a variety of methods: voltage jump, charge pulse and optical absorption, admittance measurements (see, for a review, ref. 5) and temperature jump [6]. In a previous paper [7] it has been shown that the noise analysis allows, in contrast to these methods, the analysis of this ion-transport mechanism at thermal equilibrium without external macroscopic perturbation of the system.

The transport of hydrophobic ions across the lipid membrane is usually described by a five-step process first suggested by Ketterer et al. [8]: (a) Diffusion within the electrolyte followed by (b) an adsorption to the membrane/solution interface, (c) translocation across the central membrane barrier, (d) desorption into the opposite aqueous solution and (e) diffusion within this solution. The previous theoretical noise analysis [7] took into account only reaction step c, the translocation of the hydrophobic ion across the membrane interior. Comparison of this simplified theoretical description with the measured spectral intensity showed significant deviations at the low-frequency range.

In this paper the current noise is measured under equilibrium as well as under nonequilibrium steady-state conditions. The experimental results are interpreted on the basis of two extended reaction models. The general model is based on the theory presented by Jordan [9] which includes the coupling of ion diffusion within the bulk phase to the processes of adsorption and desorption at the membrane/solution interface. Special emphasis was placed on the question of how the diffusion process influences the shape of the spectral intensity at low frequencies. For this purpose the outcome of the general reaction model was compared with that of a more simplified model which uses the assumption that diffusion in the aqueous phase is rapid compared to the adsorption/desorption step. In this case the ion concentration at the membrane interfaces can be taken as constant and the theoretical framework derived by Frehland [10] is applicable. Furthermore, we were interested to find out how the excess noise created outside thermal equilibrium depends on the macroscopic mean net current across the membrane.

For a further test of the applied kinetic models we compared the results of noise analysis with those obtained from voltage-jump current-relaxation experiments, whereby both methods were applied to the same membrane. We found that apart from equilibrium both methods yield to significant differences for the derived ion concentration within the membrane. This finding is in contrast to the fact that for both methods the theoretical interpretation is based on the identical kinetic model. However, up to now the theoretical description of ion transport [7,9,10] takes into account only complete translocations of an ion between two adjacent potential minima. Incomplete reaction steps would also cause current noise which could contribute to the shot noise intensity of the ion transport. The influence of this noise contribution on the observed discrepancy is qualitatively discussed.

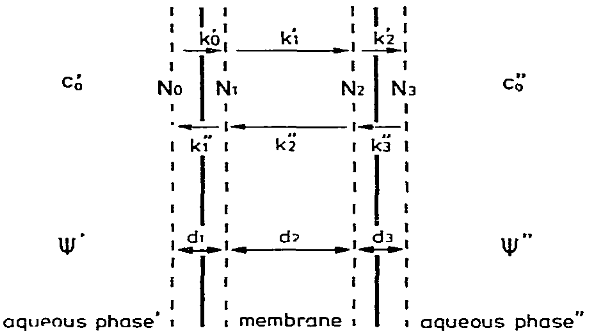

Fig. 1. Kinetic model for the transport of hydrophobic ions through membranes. The dashed lines indicate the apparent adsorption sites at the membrane interfaces and the ion reservoirs in the neighboring aqueous phases. N_0, N_1, N_2 and N_3 denote the number of ions within these adsorption sites or pools. d_1, d_2 and d_3 are distances between two neighboring planes. The diffusion processes in the aqueous phases are not indicated.

2. Material and Methods

Planar black lipid bilayer membranes were formed in the usual way [11] from a 1% (w/v) solution of either L-1,2-diphytanoyl-3-phosphatidylcholine, DL-dioleoylphosphatidylethanolamine or phosphatidylserine isolated from ox brain in *n*-decane (Merck standard for gas chromatography). These lipids have been synthesized or isolated by Benz and Janko [12]. The membrane area was relatively large (7.2×10^{-3}–1.2×10^{-2} cm^2) to minimize torus effects. The area of the black membrane was determined with a calibrated scale of an ocular. If not stated otherwise the unbuffered aqueous solution (pH 6) contained 0.1 M NaCl (Merck, analytical grade) and various concentrations of dipicrylamine (Fluka) and/or sodium tetraphenylborate (Merck). Prior to membrane formation the Teflon cell with inserted Ag/AgCl electrodes was incubated for at least 2 h with the electrolyte solution. To reach reproducible steady-state conditions the measurements were started about 1 h after the membrane had turned completely black.

The spectral intensity of current noise $S_I(f)$ was measured for different constant membrane voltages, as described previously [13]. The used modifications are described below. All noise measurements were started after the membrane current had reached a stationary mean macroscopic value. The instantaneous current was preamplified by an operational amplifier (Analog Devices Model 52K or Burr Brown 3523L) using suitable adapted feedback resistors R_f in the range 5×10^7 up to 10^{12} Ω. Usually the preamplified current was fed into the main amplifier (Princeton Applied Research Model 113) which was used in the a.c.-coupled mode with a lower roll-off frequency of 0.03 Hz. In the case of zero applied membrane voltage yielding a zero mean net current, an uncoupling of the fluctuating part of the membrane current from its d.c. value is not necessary. In the latter case, the frequency behavior of $S_J(f)$ could easily be analyzed down to frequencies of 10 mHz. Finally, the amplified a.c. component of the current was analyzed with a digital signal analyzer (Hewlett Packard 5420A). The further handling of the processed spectra was performed on-line with a Hewlett Packard computer (Model 9825A).

The contribution of the background noise to the signal noise, generated by undoped and doped lipid bilayer membranes, was investigated and compared. As is well known, the electrical behavior of undoped lipid membranes can be simulated by a parallel arrangement of a resistor and capacitor. The spectral intensity of such a model circuit is only determined by the thermal noise of the resistor [7,14]. As may be seen from fig. 2a, there is a sufficient correspondence between the thermal noise intensity of ohmic resistors R_s up to almost 10^{11} Ω and the theoretical expectation, if a gain of the preamplifier of about 10 is used (see also ref. 14). The decline of $S_J(f)$ at higher frequencies with increasing R_f is caused by the high-pass filter properties of the feedback circuit. The latter is

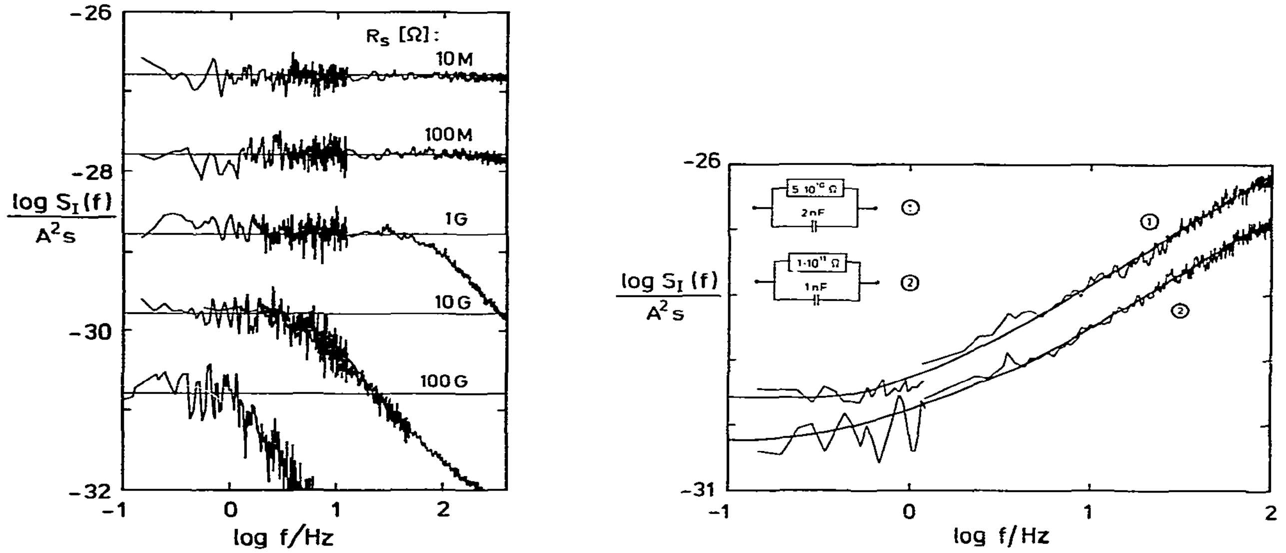

Fig. 2. (a) Spectral intensity of current noise $S_J(f)$ from different ohmic resistors R_s increasing from 10 MΩ to 100 GΩ. The ratio of feedback resistor R_f to source resistance R_s was held constant at a value of 10. The drawn straight lines represent the Nyquist theorem for a description of the thermal noise of R_s. The operational amplifier was a Burr Brown 3523L. (b) Spectral intensity of current noise $S_J(f)$ obtained from a parallel arrangement of a resistor R_m and a capacitor C_m by use of an operational amplifier Burr Brown 3523L. The feedback resistor was 10^{12} Ω at frequencies below 1 Hz and 10^{10} Ω for frequencies above 1 Hz. Theoretical lines (full curves) were obtained by a least-squares fit of the sum of signal and background noise (see text) to the measured spectra. As free parameters the intrinsic voltage and current noise of the operational amplifier $S_i(f)$ and $S_v(f)$ were used for the corresponding fit. For curve a, one obtains at frequencies below 1 Hz: $S_i = 3 \times 10^{-30}$ A^2 s and $S_v = 2 \times 10^{-14}$ V^2 s. In the frequency range between 1 and 5 Hz one obtains $S_i = 3 \times 10^{-30}$ A^2 s and $S_v = 8 \times 10^{-15}$ V^2 s and for frequencies higher than 5 Hz $S_i = 8 \times 10^{-30}$ A^2 s and $S_v = 6 \times 10^{-15}$ V^2 s. For curve b the results for $S_v(f)$ are equal to those of curve a. For S_i at frequencies below 1 Hz one obtains 4×10^{-31} A^2 s in the frequency range between 1 and 5 Hz 1×10^{-30} A^2 s and at frequencies higher than 5 Hz 8×10^{-30} A^2 s.

mainly determined by the absolute resistor value and the intrinsic stray capacitance of R_f. The influence of the membrane capacitance on the background noise may be seen from fig. 2b. The figure shows the spectra obtained for two model circuits which simulate the noise behavior of undoped lipid bilayer membranes of typical capacitance used. The full lines are obtained from the theoretical description of $S_J(f)$ as the sum of the signal noise of the equivalent circuit and the background noise generated by the preamplifier stage [7,14]. The theoretical treatment of the contribution of the background noise to the measured spectral intensity is based on the assumption that the intrinsic current noise $S_i(f)$ and voltage noise $S_v(f)$ at the input of the operational amplifier are uncorrelated and independent of the signal noise. But, it is found (see legend to fig. 2b) that the fitted values of $S_i(f)$ at frequencies below about 5 Hz depend on the actual choice of the equivalent circuit. The same result was found for measurements of $S_J(f)$ on undoped lipid bilayer membranes of varying area. The lower limit for the resolution of current intensities generated by an undoped membrane is about 3×10^{-30} A^2 which corresponds to a membrane capacitance of 2 nF. At frequencies above 5 Hz, $S_v(f)$ seems to be the dominant background noise element [7,14]. In this frequency range the operational amplifier AD 52K was preferred, as it shows a value of $S_v(f)$ about two orders of magnitude lower than that of the BB 3523L.

For membranes doped with hydrophobic ions the contribution of the background noise increases. As described in a previous paper [7], the excess noise generated by this ion transport can be simulated by the noise of a serial arrangement of a resistor and a capacitor in addition to the parallel combination of the resistor and capacitor simulating the undoped membrane. The measured increase in the background noise intensity for frequencies above 5 Hz followed the theoretical description based on the changed impedance of the equivalent circuit and a constant value for $S_v(f)$. However, for frequencies below 5 Hz we found an increase in the fitted value of $S_i(f)$. Therefore, we have chosen usually two control experiments to decide whether a measured low-frequency noise level of a doped membrane is produced by the motions of hydrophobic ions or by the background noise:

The noise of an undoped membrane of equal size connected with a resistor in parallel which simulates the direct current conductivity of the doped membrane is measured and compared with the noise of the doped membrane.

The elements of the equivalent circuit which simulate the excess noise of the doped membrane can be determined from the high-frequency part of $S_J(f)$ [7]. These elements were added to the model circuit describing the properties of the undoped membrane of corresponding area. The noise of the complete model circuit was measured and compared with the theoretical expectation.

To achieve a constant signal-to-noise ratio for the frequency-dependent course of $S_J(f)$ the presented spectra were usually composed by two spectra measured on one membrane or model circuit for a different choice of R_f and the operational amplifier. These parts of $S_J(f)$, which were less than a factor of three above the contribution of the background noise, were discarded.

For one series of experiments also voltage-jump current-relaxation experiments were performed in parallel to the noise measurements on the same membrane. The details of this relaxation technique have already been described in detail [8,15]. The experimental procedure was as follows: After the noise had been recorded from the doped membrane at a constant membrane voltage a voltage jump of +20 mV was applied to the membrane. As soon as the membrane current had reached the new stationary value the voltage was set back to the former level. The induced current relaxation was registered and analyzed [8,15].

3. Results

3.1. Spectral intensity at thermal equilibrium

The equilibrium current noise spectrum $S_J(f)$ generated by the transport of DPA^- through lipid bilayer membranes was recorded at zero applied membrane voltage for symmetrical electrolyte conditions. The result of a typical experiment is shown

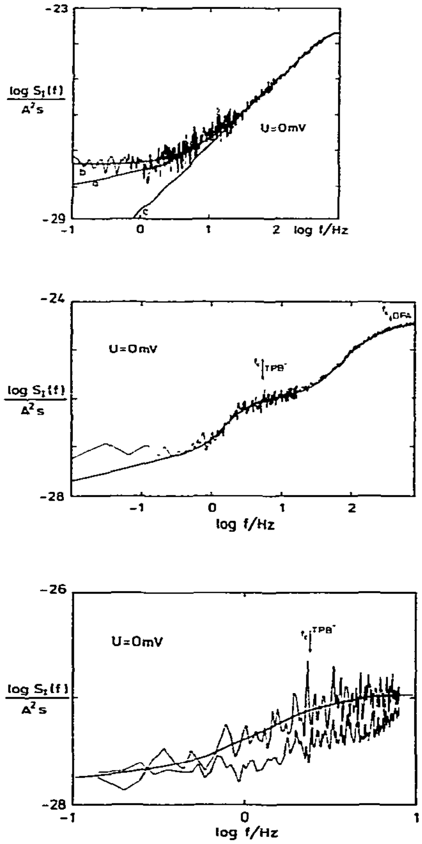

Fig. 3. (a) Spectral intensity $S_J(f)$ of current noise from a lipid bilayer membrane generated by dipicrylamine (DPA$^-$) at thermal equilibrium. Aqueous solution: 3×10^{-8} M DPA$^-$, 0.1 M NaCl, $T = 23$°C. Membrane-forming solution: phosphatidylethanolamine/*n*-decane, membrane area 1.4×10^{-2} cm^2. The feedback resistance for frequencies below 10 Hz was 10 GΩ, otherwise 100 MΩ was used. Curve a represents a least-squares fit of the Jordan model [9], curve b a fit of the Frehland model [10] and curve c a fit of the model of Kolb and Läuger [7] to the noise data. The model parameter could be determined to $\beta = 7\times10^{-2}$ cm, $N_1 + N_2 = 4.2$ pM and $k_i = 1800$ s^{-1} from all three fits. For the desorption coefficient k according to ref. 9 one obtains $k \geq 1$ s^{-1}. By applying the model of ref. 10, $k = 0.4$ s^{-1} was obtained. (b) Spectral intensity $S_J(f)$ of noise current from a lipid bilayer membrane generated by the hydrophobic ions DPA$^-$ and TPB$^-$ at thermal equilibrium. Aqueous solution: 3×10^{-8} M DPA$^-$, 1×10^{-6} M TPB$^-$, 0.1 M NaCl, $T = 23$°C. Membrane-forming solution: diphytanoylphosphatidylcholine/*n*-decane, membrane area 7.3×10^{-3} cm^2. The feedback resistor was 10 GΩ in the frequency range below 10 Hz, and 100 MΩ otherwise. The full line represents a fit of the model of ref. 9 to the measured spectra. The dashed lines show the fits of ref. 7 to the two dispersion ranges of the spectrum, respectively. (c) Spectral intensity $S_J(f)$ of current noise from a lipid bilayer membrane generated by TPB$^-$ at thermal equilibrium (upper curve). Aqueous solution: 10^{-6} M TPB$^-$, 0.1 M NaCl, $T = 23$°C. Membrane-forming solution: phosphatidylserine/*n*-decane, membrane area 1.2×10^{-2} cm^2, feedback resistance $R_f = 10$ GΩ. A fit of ref. 9 gives $\beta = 3\times10^{-4}$ cm, $k_i = 5$ s^{-1} and $k = 2.5$ s^{-1} (full line). Lower curve: control experiment with an undoped membrane of equal size and a parallel resistor of 100 MΩ to simulate the conductance of the doped membrane. 50 summations were recorded in both cases.

in fig. 3a. It may be seen from the figure that the spectrum shows within the experimental scatter a frequency-independent (white) behavior at low frequencies as well as at higher frequencies. In between the spectrum shows a complementary Lorentzian shape, which means $S_J(f)$ increases proportional to f^2. As outlined in section 2 this frequency dependence of $S_J(f)$ yields a frequency-dependent signal-to-noise ratio for a constant feedback resistor. Therefore, to achieve a roughly constant signal-to-noise ratio especially for the low- and high-frequency tail of $S_J(f)$ the spectrum is composed of two spectra measured successively for the frequency ranges $0.1 < f < 25$ Hz and $25 < f < 800$ Hz by an appropriate choice of the feedback resistor (see legend to fig. 3a).

3.1.1 Comparison of $S_J(f)$ with different reaction models

The shape of $S_J(f)$ in fig. 3a was compared with three different theoretical predictions. A general reaction model outlined by Jordan [9] includes the coupling of the diffusion of the hydrophobic ion in the aqueous phase to membrane-bound ion movements. Using for the diffusion coefficient of DPA$^-$ in the bulk phase the value $D = 4.7\times10^{-6}$ cm^2 s^{-1} [16] one obtains from a least-squares fit curve a in fig. 3a. The corresponding values for the total number of anions within the membrane ($N_1 + N_2$) and the partition coefficient β, as well as the rate constant of translocation $k_i = k_1' = k_2''$ and desorption $k = k_2' = k_1''$ (for notations see fig. 1) are given in the legend of fig. 3a. As may be seen from ref. 9 and fig. 3a the high-frequency tail of $S_J(f)$ is mainly determined by the product $k_i\beta$ and the lower frequency end by the parameters β

and k, whereby βk is the rate constant of adsorption. As a direct consequence of the diffusion process the theoretical spectrum at low frequencies increases proportional to $f^{1/2}$, but this could not be confirmed experimentally. Therefore, the derived value of k is only a lower limiting value. The corresponding model parameters obtained for doped diphytanoylphosphatidylcholine/*n*-decane membranes are given in table 1.

If the restriction is used that the ion diffusion in the aqueous phase is fast compared to the interfacial processes the ion reservoirs at the aqueous interfaces, which are denoted by N_0 and N_3 (see fig. 1), can be considered as being constant. The corresponding noise spectrum at steady state can be calculated using the formalism developed by Frehland [10]. This approach treats the transport system as a sequence of separate energy barriers whereby only those ion movements which involve the jump of an ion across a potential barrier generate current noise. For an explicit application of this formalism values for the relative distance α_i of the potential minima have to be introduced. If d_i ($i = 1, 2, 3$) are the absolute distances of the minima (fig. 1), then it follows:

$$\alpha_i = \frac{d_i}{\sum_{m=1}^{3} d_m} \qquad (1)$$

For α_i we used the following values [16]

$$\alpha_1 = \alpha_3 = 0.05 \text{ and } \alpha_2 = 0.9, \qquad (2)$$

this means that 90% of an applied electrical potential difference would drop across the central dielectric energy barrier and that the interfacial reaction steps of adsorption and desorption are influenced by 5% of the applied voltage. Curve b of fig. 3a shows the corresponding least-squares fit based on this approach. As the figure shows the curve sufficiently describes the experimental data also at lower frequencies. The derived values for the parameters $N_1 + N_2$, β, k_i and k are listed in the legend to fig. 3a and table 1 as well.

Using the further stringent limitation, as formerly done by Kolb and Läuger [7], that the membrane is a closed system with respect to the ion translocation within the membrane curve c is obtained. Table 1 shows that all three kinetic models for a molecular description of this ion-transport mechanism yield a result which agrees well for the translocation rate constant k_i and the partition coefficient β which coincides with the indistinguishable shape of curves a, b and c at higher frequencies (fig. 3a). Furthermore, fig. 3a shows that for a physical description of the low-frequency tail of $S_J(f)$ the adsorption/desorption reaction step has to be taken into account. As may be seen from table 1, the rate constants for the processes at the interface do not influence the determination of k_i of this phosphatidylcholine/DPA$^-$ system due to their different time scales.

3.1.2. *Ion adsorption and desorption as a function of [DPA$^-$] and/or [TPB$^-$]*

For a closer investigation of the adsorption/desorption reaction we measured the low-frequency tail of $S_J(f)$ in the range 0.01–5 Hz as function of DPA$^-$ concentration. Table 2 shows that $S_J(f \to 0)$ increases about linearly with increasing [DPA$^-$] in the aqueous phase. Also, the derived lower limits

Table 1

Results of the analysis of current noise generated by hydrophobic anions in lipid bilayer membranes at thermal equilibrium using three different reaction models (see text). The surface density $N_1 + N_2$ of hydrophobic ions at the membrane as well as the partition coefficient and the rate constant of translocation k_i and desorption k are listed. Aqueous solution: 3.8×10^{-8} M DPA$^-$, 0.1 M NaCl, $T = 23$°C. Membrane-forming solution: diphytanoylphosphatidylcholine/*n*-decane, membrane area 1.2×10^{-2} cm^2. The errors given denote the different least-squares fits.

Model	$N_1 + N_2$ (pmol cm^{-2})	β (cm) ($\times 10^{-2}$)	k_i (s^{-1})	k (s^{-1})
Kolb and Läuger [7]	1.22±0.03	1.61±0.04	601±8	–
Frehland [10]	1.22±0.03	1.61±0.04	601±8	0.2±0.1
Jordan [9]	1.22±0.03	1.61±0.04	600±8	≥1

Table 2

Low-frequency level of $S_J(f \to 0)$ obtained from lipid bilayer membranes in the presence of different DPA$^-$ concentrations. For the desorption rate constant k only lower limits are given, since the Jordan [9] approach always underestimated the measured spectrum (see fig. 3a and b). At $[\mathrm{DPA}^-] \geq 10^{-6}$ M no reasonable fit was possible. Aqueous solution: DPA$^-$ of different concentration, 0.1 M NaCl, $T = 23°\mathrm{C}$. Membrane-forming solution: diphytanoylphosphatidylcholine/n-decane, membrane area 8×10^{-3} cm^2, feedback resistor $R_f \geq 10$ GΩ; five membranes were used to yield averaged results.

$[\mathrm{DPA}^-]$ (M)	$S_J(f \to 0)$ (A^2 s)	k (s^{-1})
3.8×10^{-8}	$2 \pm 1 \times 10^{-29}$	≥ 1
3.8×10^{-7}	$2 \pm 1 \times 10^{-28}$	≥ 1
1×10^{-6}	$2.5 \pm 1 \times 10^{-27}$	–

of k are given which were obtained by applying the formalism of Jordan [9] for a description of the low-frequency tail of $S_J(f)$. It has to be pointed out that this approach always underestimated the measured $S_J(f)$ for $f \to 0$. This deviation was also observed in the presence of higher DPA$^-$ concentrations which yielded absolute values of $S_J(f)$ (table 2) well above the resolution limit of $3 \cdot 10^{-30}$ A^2s (see section 2). At DPA$^-$ concentrations above 10^{-6} M no satisfying description of the lower frequency tail of the measured spectrum by the general model of Jordan [9] was possible. Assuming constant ion reservoirs N_0 and N_3 at the interface, $S_J(f \to 0)$ could always be described suitably and a determination of the desorption rate constant was possible.

Furthermore, we investigated whether the slope of the low-frequency tail of $S_J(f)$ depended on the choice of the lipid-hydrophobic ion system. For this case $S_J(f)$ was measured for a phosphatidylcholine/n-decane membrane doped with TPB$^-$ (fig. 3b). In addition, the aqueous phase also contained DPA$^-$ which generates a second complementary Lorentzian function at higher frequencies. The theoretical curve (full line) in fig. 3b was obtained by using the approach of Jordan [9]. As diffusion coefficient for TPB$^-$ we used the value $D = 5.5 \times 10^{-6}$ cm^2 s^{-1} [17]. As observed in the presence of DPA$^-$ alone, the low-frequency tail of $S_J(f)$ is underestimated by the theory and no increase in $S_J(f)$ proportional to $f^{1/2}$ was found. Fig. 3c shows the noise spectrum of a negatively charged membrane (phosphatidylserine/n-decane) doped with TPB$^-$ (upper trace) and the noise spectrum of an undoped phosphatidylserine membrane with a parallel resistor which simulated the TPB$^-$-induced conductance (lower trace). The figure shows that for a negatively charged membrane doped with TPB$^-$ the spectral intensity is of low amplitude and differs from the simulated background noise intensity only by a factor of about two. As may be estimated from fig. 3c, a difference spectrum of the spectra obtained for the doped and undoped membrane also shows within the experimental scatter a frequency-independent behavior at low frequencies. However, it could be shown that the translocation rate constant k_t which is determined by the corner frequency f_c ($f_c = k_t/\pi$ [7], see fig. 3c) was in accordance with the value obtained from a corresponding voltage-jump current-relaxation experiment performed on the same membrane.

3.2. Spectral intensity at nonequilibrium steady state

Nonequilibrium steady-state measurements were carried out at different constantly applied membrane voltages. The corresponding spectra were processed after the macroscopic current which varies due to the diffusion polarization had reached a stationary level. Fig. 4 shows the result of a typical experiment performed on one membrane. The voltage was changed stepwise beginning with the lowest value. The figure shows that independent of the applied voltage, $S_J(f)$ shows the same general frequency course as observed for the equilibrium state. For the theoretical description of $S_J(f)$, N_0 and N_3 (see fig. 1) were considered to be constant and the formalism of Frehland [10] was used (see above). Furthermore, we assumed that the voltage influences only the translocation process across the membrane interior, which may be approximated by a single energy barrier [8]:

$$k_1' = k_t e^{zu/2}, \; k_2'' = k_t e^{-zu/2}, \; u = VF/RT = (\psi'' - \psi')F/RT \tag{3}$$

where k_t is the rate constant at zero voltage, V the voltage, ψ' (ψ'') the electrical potentials of the bulk

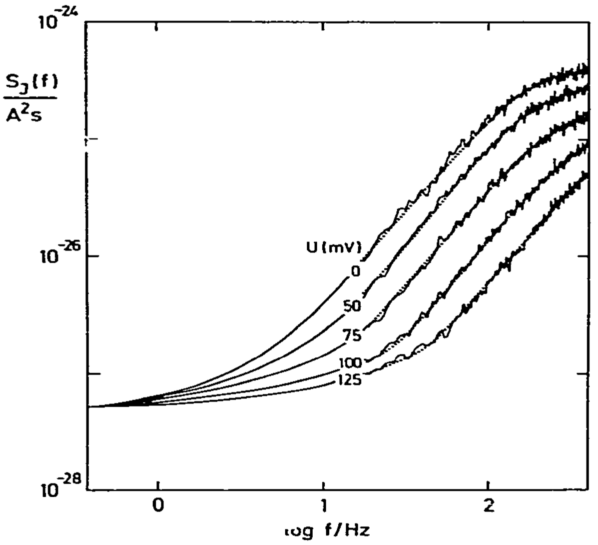

Fig. 4. Spectral intensity $S_J(f)$ of current noise from a lipid bilayer membrane generated by DPA$^-$. The applied voltage was changed in steps of 25 mV. For clearer arrangement the spectrum processed at $U = 25$ mV is not shown, as well as the measured low-frequency tails of the spectra. The later one was replaced by hand-drawn averaged curves. Aqueous solution: 3×10^{-8} DPA$^-$, 0.1 M NaCl, $T = 23°C$. Membrane-forming solution: diphytanoylphosphatidylcholine/n-decane, membrane area 1.2×10^{-2} cm^2, feedback resistor $R_f = 100$ MΩ. For frequencies below 1 Hz $R_f = 10$ GΩ was used.

phases (see fig. 1), F the Faraday constant, T the absolute temperature, R the gas constant and z the valency of the hydrophobic ion. Table 3 summarizes the theoretical results obtained after a least-squares fit of the theoretical course of $S_J(f)$ to the processed spectra. For the derivation of the presented data, only those experiments were taken into account for which $S_J(f)$ at equilibrium could be reproduced after having applied the highest voltage of 125 mV to the membrane.

Table 3 shows that k_i decreases with increasing voltage which is in contrast to the assumption that k_i is voltage independent (see eq. 3). Also, the partition coefficient increases strongly with voltage. It is interesting to note that the absolute level of $S_J(f \rightarrow 0)$ did not change with voltage or the mean macroscopic current $\bar{J}$ in the range $0 \leq \bar{J} \leq 10^{-11}$ A. This voltage-independent behavior of $S_J(f < 1$ Hz) was also found for the phosphatidylcholine/TPB$^-$ as well as for the phosphatidylserine/TPB$^-$ system. For voltages above 150 mV sometimes a $1/f$ behavior of $S_J(f)$ of varying amplitude appeared at low frequencies. Since this behavior of $S_J(f)$ was also found for undoped membranes, it was considered as an artifact caused by voltage-dependent structural changes of the membrane [18,19]. Table 4 shows also a voltage dependence of the surface ion concentration ($N_1 + N_2$) and of the partition coefficient β which were determined on a diphytanoylphosphatidylcholine/n-decane membrane for increasing DPA$^-$ concentrations. At the lowest [DPA$^-$] used (3.8×10^{-8} M), β increases by a factor of about 8 when the voltage is changed from 0 to 125 mV. At higher [DPA$^-$], where electrostatical interactions between the ions have to be expected [7,16,21], this factor is 3–5.

Table 3

Results from current noise experiments obtained from a lipid bilayer membrane in the presence of DPA$^-$ as a function of the applied voltage. The surface ion concentration $N_1 + N_2$, the partition coefficient β and the rate constant of translocation k_i and desorption k are listed. k was determined by using the Frehland model [10] (see text). Aqueous solution: 3.8×10^{-8} M DPA$^-$, 0.1 M NaCl, $T = 23°C$. Membrane-forming solution: Diphytanoylphosphatidylcholine/n-decane, membrane area 1.2×10^{-2} cm^2. Eight membranes were used for yield averaged results.

U (mV)	$N_1 + N_2$ (pmol cm^{-2})	β (cm) ($\times 10^{-2}$)	k_i (s^{-1})	k (s^{-1})
0	1.20 ± 0.04	1.6 ± 0.06	600 ± 15	0.2 ± 0.1
25	1.38 ± 0.1	1.82 ± 0.15	575 ± 15	0.3 ± 0.1
50	2.0 ± 0.2	2.63 ± 0.3	493 ± 20	0.4 ± 0.15
75	3.2 ± 0.4	4.21 ± 0.6	413 ± 25	0.6 ± 0.2
100	5.6 ± 0.8	7.37 ± 1.2	355 ± 30	1.1 ± 0.4
125	10.6 ± 2	13.1 ± 3	270 ± 50	1.6 ± 0.5

Table 4

Results from current noise experiments obtained from a lipid bilayer membrane in the presence of DPA$^-$ at different concentration as a function of the applied voltage. The surface ion concentration $N_1 + N_2$ and the partition coefficient β are presented. Aqueous solution: DPA$^-$ of different concentration, 0.1 M NaCl, $T = 25°C$. Membrane-forming solution: diphythanoylphosphatidylcholine/*n*-decane, membrane area 8×10^{-3} cm^2 to 1.4×10^{-2} cm^2; five to eight membranes were used to yield averaged results.

U (mV)	[DPA$^-$] = 1.85×10^{-6} M		[DPA$^-$] = 3.8×10^{-7} M		[DPA$^-$] = 3.8×10^{-8} M	
	$N_1 + N_2$ (pmol cm^{-2})	β (cm) ($\times 10^{-2}$)	$N_1 + N_2$ (pmol cm^{-2})	β (cm) ($\times 10^{-2}$)	$N_1 + N_2$ (pmol cm^{-2})	β (cm) ($\times 10^{-2}$)
0	16.5 ± 1.4	0.45 ± 0.04	5.8 ± 0.4	0.75 ± 0.06	1.2 ± 0.04	1.6 ± 0.06
25	22.5 ± 2	0.6 ± 0.06	6.6 ± 0.8	0.87 ± 0.01	1.38 ± 0.1	1.82 ± 0.15
50	31 ± 3	0.85 ± 0.08	8.5 ± 1.5	1.1 ± 0.02	2 ± 0.2	2.63 ± 0.3
75	50 ± 5	1.35 ± 0.15	11 ± 2	1.5 ± 0.3	3.2 ± 0.4	4.21 ± 0.6
100	70 ± 8	1.9 ± 0.25	14.5 ± 3	1.9 ± 0.4	5.6 ± 0.8	7.37 ± 1.2
125	86 ± 10	2.3 ± 0.3	18 ± 6	2.4 ± 0.8	10.6 ± 2	13.1 ± 3

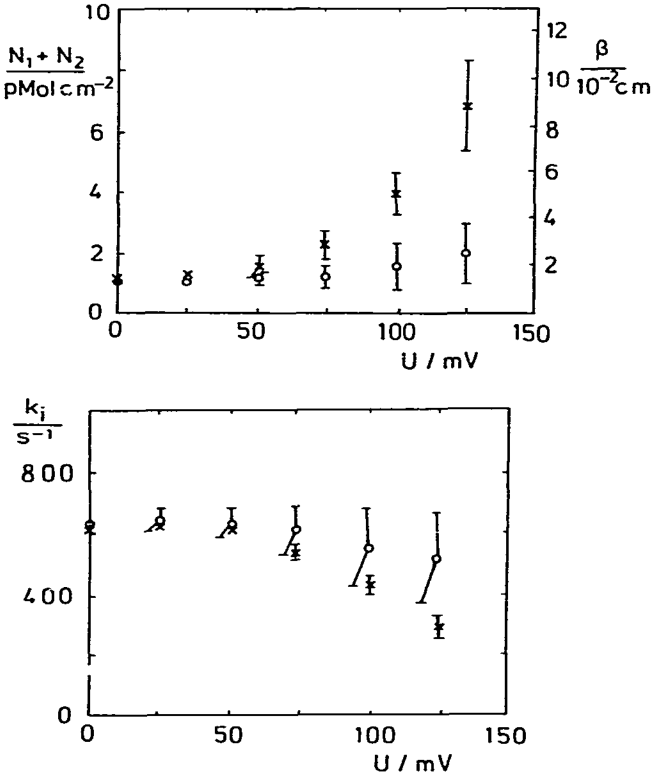

Fig. 5. Surface ion density $N_1 + N_2$, partition coefficient β (a) and translocation rate constant k_i (b) as functions of the applied membrane voltage. (×) Values were obtained from noise analysis, (○) values were obtained from voltage-jump current-relaxation experiments. The experiments were done at the same membrane. For experimental conditions see fig. 4. The errors denote the precision of the fit given by the models of refs. 9, 10 and 7 to the corresponding spectra.

3.3. Voltage-jump current-relaxation experiments

For a further investigation of the unexpected change of β and k_i with increasing voltage (see tables 3 and 4) the following relaxation experiments were carried out. After analyzing the current noise at steady state for a certain voltage, the voltage was changed stepwise and the successive current relaxation was registered (see section 2). Since for these measurements we were only interested in the determination of β and k_i, we could use the simplified model of a closed membrane for derivation of these parameters from the shape of the high-frequency tail of $S_I(f)$ [7]. The results of both methods for β and k_i are shown in fig. 5a and b. As the figure shows, for equilibrium conditions both experimental methods yield results which are in good agreement. But at nonequilibrium, variations are observed with increasing voltage especially for the partition coefficient. From the noise analysis a strong voltage dependence of β is found, whereas from the corresponding relaxation experiments a nearly voltage-independent behavior is obtained.

4. Discussion

The current noise generated by the transport of hydrophobic ions (DPA^- or TPB^-) across lipid bilayer membranes was analyzed. The corresponding spectral intensity $S_J(f)$ shows a frequency-independent behavior at low as well as at high frequencies and between them increases proportionally to f^2. This shape of $S_J(f)$ was obtained for the equilibrium as well as the nonequilibrium steady state independent of the concentration of the hydrophobic ion (see section 3).

At first the low-frequency part of $S_J(f)$ will be discussed at thermal equilibrium in terms of three different kinetic models which were outlined in section 3. The reaction model which treats the membrane as a closed thermodynamic system with respect to the ion translocation within the membrane yields a spectral intensity [7] which does not describe the low-frequency part of the measured spectra (curve c of fig. 3a and the dotted curves in fig. 3b). The reaction model which takes into account the adsorption/desorption step of the ions across the membrane interface [10] yields always a sufficient description of $S_J(f)$ at low frequencies (see curve b of fig. 3a) from which the rate constant of desorption k and adsorption βk could be determined (table 1). However, the values for k obtained by noise analysis were about one order of magnitude smaller than those derived from voltage-jump current-relaxation experiments under comparable experimental conditions [6]. This extended reaction model is based on the assumption that a fluctuation of the number of ions within the aqueous reservoirs at the interface N_0 and N_3 (see fig. 1) can be neglected. This restriction was taken into account by Jordan [9] who included a fluctuation of N_0 and N_3 by diffusion of ions into and from the aqueous bulk phases. But this theoretical approach underestimates the spectra at low frequencies (curve a in fig. 3a, full line in fig. 3b). Also, the predicted increase in $S_J(f)$ proportional to $f^{1/2}$ could not be found for frequencies well below the corner frequency $f_c = k_t/\pi$, however, $S_J(f)$ showed a frequency-independent shape down to 10 mHz. If for a fit one were to take into account that the initially present concentration of hydrophobic ions is overestimated, since a fraction of it can be leached out into the torus [15], the deviation of the theoretical description at low frequencies would substantially increase. Therefore, the application of the general reaction model [9] yielded only a lower limit for k (table 1). A likely explanation for this deviation is the following. Despite the fact that current intensities of undoped membranes could be measured down to 10^{-30} A^2 s for a membrane area of 8×10^{-3} cm^2 and that the measured levels of $S_J(f \rightarrow 0)$ are well above (see table 2), it cannot completely be ruled out that the measured spectra are influenced at low frequencies by additional noise which may be generated by structural changes of the lipid matrix by the presence of hydrophobic ions. However, it could be shown that, if those structural changes occur, they do not cause under nonequilibrium steady-state conditions a $1/f$ noise as was found for other doped lipid membranes [18,19].

Jordan and Stark [15] found evidence for a contribution of ion diffusion in the aqueous phase to the overall transport process especially from voltage-jump current-relaxation experiments on negatively charged lipid membranes doped with TPB^-. For comparison, noise experiments were carried out on a similar system under equilibrium conditions (fig. 3c). But in this case the absolute level of $S_J(f)$ at higher frequencies, which is mainly determined by the term βk_t, is already small and at low frequencies the background noise intensity is of similar magnitude. In this respect negatively charged membranes doped with hydrophobic anions are unfavorable systems for noise analysis.

At steady state outside equilibrium, excess current noise is created which depends on the size of the macroscopic net current [13] flowing through the membrane and causes an increase in $S_J(f \rightarrow 0)$. The largest increase in the net current of about 5 pA which could be reached by increasing the applied voltage to 125 mV (fig. 4) would change $S_J(f \rightarrow 0)$ only by about 0.5% [9], which is within the experimental scatter. Therefore, at present the contribution of possible diffusion-controlled fluctuations of the ion density at the electrolyte/membrane interface could not be detected from noise analysis.

The characteristic dispersion of $S_J(f)$ is governed by an increase proportional to f^2 followed by a frequency-independent behavior. This part of the spectrum could always be described similarly by all three reaction models [7,9,10] (fig. 3a). This indicates that this part of the spectrum is exclusively determined by the rate constants of translocation of the ions across the central dielectric barrier. The finding that with increasing voltage the slope of $S_J(f)$ stays constant indicates that no further voltage-dependent rate constants are involved. Whereas, e.g., the slope of spectral intensities of ion-carrier induced current noise changes in the dispersion range with increasing voltage, which could be explained by contributions of several voltage-sensitive reaction steps to the transport mechanism [13]. As may be seen from ref. 7, the high-frequency level of $S_J(f)$ accounts for the shot noise generated by this ion transport. As fig. 4, shows the shot noise intensity decreases with increasing applied voltage which is based on the fact that the interfacial processes are the rate-limiting steps of this transport mechanism.

Furthermore, table 4 and fig. 5a show that k_i is not voltage independent as should be expected from eq. 3 which is based on the assumption of a single delta-shaped dielectric barrier for the membrane interior. As in former cases, this finding can be explained by a different properly chosen shape of the central barrier and will not be outlined here [13,20]. More difficult to explain is the observed voltage dependence of the partition coefficient (fig. 5b). To elucidate this result, in addition to noise analysis, voltage-jump current-relaxation experiments were carried out on the same membrane. Fig. 5a and b shows that at equilibrium both methods yield coinciding results as has to be expected from the fluctuation-dissipation theorem [22]. Under nonequilibrium conditions both methods show, especially for β (fig. 5b), significant deviations. A possible explanation would be that the underlying description of the ion translocation within the membrane does not follow a simple monomolecular reaction step. Support for this idea may be seen from a consideration of additional reaction steps which will be denoted as incomplete reaction steps.

Up to now the theoretical description of transport noise in terms of chemical reactions takes into account only those reaction steps by which the ion is successfully moved from one reaction site to the subsequent one. But as is well known each molecule sitting in its reaction site or local potential minimum undergoes fluctuations by thermal activation. Since each movement of an ion perpendicular to the membrane plane will cause a current pulse in the external circuit, the successful as well as the incomplete ion movements out of the reaction site will contribute to the overall noise. We simulated the influence of incomplete reaction steps to the shape of $S_J(f)$ as follows. The energy distribution of an ion was described by a Boltzmann distribution and the energy height yielding a complete reaction step was held fixed. Furthermore, we assumed a trapezium shape for the description of the central dielectric barrier. Using a random generator which moves the ion of defined energy out of its place the spectrum was calculated for the total number of occurring ion movements by a computer program [23]. We used the assumption that the resting time of an ion within its potential minimum is of the same order of magnitude as the actually measured one ($\approx 1/k_i$). We found that the contribution of the incomplete reaction steps increases the level of $S_J(f)$ for $f > f_c$ and that this increase is more pronounced for an increased applied voltage [23]. On the basis of this qualitatively derived explanation, we propose that the contribution of incomplete reaction steps influences the spectral intensities differently as compared to corresponding relaxation curves. Since, in the latter case, the relaxation amplitudes are mainly determined by a macroscopic voltage step and not by microscopic thermal fluctuations in the number of ions in the potential minimum. An analytical derivation of the contribution of incomplete reaction steps to the ion-transport mechanism should be carried out.

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