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NOISE-CURRENT GENERATED BY CARRIER-MEDIATED ION TRANSPORT AT NON-EQUILIBRIUM

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The measured spectral intensity $S_J(f)$ of noise-current generated by carrier-mediated ion transport on bilayer membranes agrees under equilibrium and nonequilibrium conditions with the theoretically predicted behavior. It is shown that the shot noise intensity due to this ion transport mechanism yields a frequency independent level of $S_J(f)$ at higher frequencies. The intensity of the shot noise contribution decreases with increasing voltage. Both experimentally and theoretically it could be shown that at nonequilibrium the Nyquist-theorem can no longer be applied for a description of the spectral intensity. Especially, the low frequency tail of $S_J(f)$ is not proportional to the mean macroscopic steady state conductance. The transition of $S_J(f)$ between the low and high frequency limit occurs in a frequency range which is related to the relaxation time constants of the transport system. In contrary to voltage jump current relaxation experiments where two relaxation times are predicted the theory predicts for the noise analysis a third relaxation time constant of a non-zero contribution to $S_J(f)$. Besides the measurement of $S_J(f)$ the corresponding autocorrelation function was determined. Comparison of both methods of noise analysis shows that for the carrier-mediated ion transport the determination of the autocorrelation function is the less appropriate approach due to principal methodical difficulties.

1. Introduction

From the analysis of the electrical noise generated by ion movement across biological and artificial membranes information about the underlying molecular transport mechanisms may be obtained [1–4].

Recently Kolb and Läuger [5] could analyze noise current associated with carrier-mediated ion transport through lipid bilayer membranes under equilibrium conditions. The spectral intensity of the noise current was found to be frequency independent both at high and at low frequencies and in between a two dispersion region occurred at frequencies $f_1 = 1/2\pi\tau_1$ and $f_2 = 1/2\pi\tau_2$ where the intensity increases with frequency. Due to the fluctuation dissipation theorem [6,7] the time constants τ_1 and τ_2 are identical with the two relaxation times of a corresponding voltage-jump current-relaxation experiment [8].

In the present paper we deal with the experimental and theoretical analysis of noise current generated by carrier-mediated ion transport under non-equilibrium steady-state conditions by applying a constant voltage across the membrane. As carrier-ion system we used the ammonium-ion transport mediated by the macrotetrolid tetranactin. The results for the kinetic parameters obtained at different applied voltages will be compared with previous studies of carrier-mediated ion transport where either the voltage-jump method [9,10] (for extensive references to literature see Stark [11]), the charge-pulse technique [12,13] or the temperature-jump method [14] were used.

It will be shown that the general shape of the measured spectral intensity at the non-equilibrium state compared to the equilibrium state [5] does not change. But compared to the equilibrium case where experimentally only a single dispersion could be resolved with increasing constant voltage the frequency dependent part of the spectrum has to be described by at least a superposition of two dispersions. The high-frequency tail of the spectral intensity, which represents the intensity correlated to the shot noise of this ion-transport mechanism, decreases strongly in amplitude with increasing voltage, whereas the low-frequency part was found to be nearly independent on voltage. Under equilibrium conditions the latter part of the spectrum was found to be proportional to the macroscopic conductance of the membrane in agreement with the prediction due to the Nyquist theorem [5]. It will be shown that for the used ion-transport system the Nyquist theorem is no longer applicable for the non-equilibrium state. Therefore under steady-state conditions the macroscopic conductance cannot be derived from the spectral intensity of noise current but has to be measured by an independent measurement.

The shape of the spectral intensity could theoretically be described on the basis of a formalism derived by Frehland [15] for the treatment of noise current around steady-state in discrete transport systems. In addition to the two relaxation times which describe the kinetic behavior of the carrier-mediated ion transport in voltage-jump current-relaxation experiments [8], the theory predicts for the spectral intensity of noise current around steady-state the appearance of a third relaxation time with a contribution different from zero in amplitude. This relaxation time is related to the carrier-ion adsorption and desorption process at the membrane solution interface.

Besides the measurement of the spectral intensity also the corresponding autocorrelation function was determined. Both functions are correlated by the Wiener-Khintchine theorem and should theoretically contain the same information. But it will be shown that for the investigated type of noise current the theoretical interpretation of the measured autocorrelation function is restricted for methodical reasons and therefore appears to be the less appropriate experimental approach for the noise analysis of carrier-mediated ion transport.

2. Theory

The noise analysis of carrier-mediated ion transport at steady-state will be based on the model which has already been used for the treatment of voltage-jump relaxation experiments [16,8]. This model assumes that complex formation between the ion M^+ and the carrier molecule S occurs at the membrane-solution interface and may be described by overall rate constants k_R (association) and k_D (dissociation). The translocation of the charged complex M^+S across the membrane is treated as a first-order reaction characterized by a rate constant k_{MS}. The back transport of the free carrier S is described by the rate constant k_S. We assume that only k_{MS} is dependent on voltage which may be described due to the Eyring theory [16]:

$$k'_{MS} = k_{MS}\, e^{u/2}, \qquad k''_{MS} = k_{MS}\, e^{-u/2}, \tag{1}$$

$$u = VF/RT = (\psi' - \psi'')F/RT,$$

where k'_{MS} and k''_{MS} are the rate constants of translocation from the left to the right and from the right to the left interface, respectively. u denotes the reduced potential whereby ψ' and ψ'' are the electrical potentials at the left- and right-hand aqueous bulk phase; R is the gas constant, T the absolute temperature and F is Faraday's constant. The approximation of the voltage dependence of the translocation rate constants by a simple exponential behavior as well as neglecting any voltage dependence of k_R and k_D will be discussed later.

Since the exchange of carrier molecules between membrane and water is a very slow process we assume that within the time scale of the ion transport across the membrane the total carrier concentration N_0 in the membrane is time-independent [17]

$$N_0 = N'_S(t) + N''_S(t) + N'_{MS}(t) + N''_{MS}(t), \tag{2}$$

$N'_S(t), N''_S(t), N'_{MS}(t)$ and $N''_{MS}(t)$ are the interfacial concentrations at the left (′) and right (″) interface, respectively. On the basis of the transport model described above the spectral intensity of noise current around steady-state can be calculated using the formalism derived by Frehland [15] for discrete transport systems as outlined in appendix A and B.

One obtains for the spectral intensity $S_J(f)$ of noise current the relation (eq. (B9)):

$$S_J(f) = 2z^2 e_0^2 \times \left(k'_{MS}\, \overline{N'_{MS}} + k''_{MS}\, \overline{N''_{MS}} + 2 \sum_{i=1}^{3} \frac{\alpha_i \tau_i}{1 + (2\pi f \tau_i)^2} \right) \tag{3}$$

$$\tau_1^{-1} = +Q + \sqrt{a}; \quad \tau_2^{-1} = +Q - \sqrt{a}; \quad \tau_3^{-1} = k_R C_M + k_D, \tag{4}$$

$$\alpha_1 = (1/2\sqrt{a})\Bigg\{\tfrac{1}{2}(k'_{\mathrm{MS}}\overline{N'_{\mathrm{MS}}} + k''_{\mathrm{MS}}\overline{N''_{\mathrm{MS}}})$$
$$\times (k'_{\mathrm{MS}} + k''_{\mathrm{MS}})(P - \sqrt{a}) - \frac{\bar{J}^2}{z^2 e_0^2 N_0}\Bigg[\frac{1}{2}\,\frac{(k_{\mathrm{R}}C_{\mathrm{M}}+k_{\mathrm{D}})}{k_{\mathrm{R}}C_{\mathrm{M}}k_{\mathrm{D}}k_{\mathrm{S}}}$$
$$\times \det \mathbf{A}(P - \sqrt{a}) - Q + \sqrt{a} + B\left(\frac{1}{\tau_2} - \frac{1}{\tau_3}\right)\Bigg]\Bigg]\Bigg\}, \qquad (5)$$

$$\alpha_2 = -(1/2\sqrt{a})\Bigg\{\tfrac{1}{2}(k'_{\mathrm{MS}}\overline{N'_{\mathrm{MS}}} + k''_{\mathrm{MS}}\overline{N''_{\mathrm{MS}}})$$
$$\times (k'_{\mathrm{MS}}+k''_{\mathrm{MS}})(P + \sqrt{a}) + \frac{\bar{J}^2}{z^2 e_0^2 N_0}\Bigg[\frac{1}{2}\,\frac{(k_{\mathrm{R}}C_{\mathrm{M}}+k_{\mathrm{D}})}{k_{\mathrm{R}}C_{\mathrm{M}}k_{\mathrm{D}}k_{\mathrm{S}}}$$
$$\times \det \mathbf{A}(P + \sqrt{a}) - Q - \sqrt{a} + B\left(\frac{1}{\tau_1} - \frac{1}{\tau_3}\right)\Bigg]\Bigg]\Bigg\}, \qquad (6)$$

$$\alpha_3 = -(\bar{J}^2/z^2 e_0^2 N_0)B, \qquad (7)$$

$$Q = \tfrac{1}{2}(k_{\mathrm{R}}C_{\mathrm{M}} + k_{\mathrm{D}} + 2k_{\mathrm{S}} + k'_{\mathrm{MS}} + k''_{\mathrm{MS}}), \qquad (8)$$

$$P = \tfrac{1}{2}(k_{\mathrm{R}}C_{\mathrm{M}} - k_{\mathrm{D}} + 2k_{\mathrm{S}} - k'_{\mathrm{MS}} - k''_{\mathrm{MS}}), \qquad (9)$$

$$a = P^2 + k_{\mathrm{R}}C_{\mathrm{M}}k_{\mathrm{D}}, \qquad (10)$$

$$\det \mathbf{A} = (k'_{\mathrm{MS}} + k''_{\mathrm{MS}})(k_{\mathrm{R}}C_{\mathrm{M}} + 2k_{\mathrm{S}}) + 2k_{\mathrm{D}}k_{\mathrm{S}}, \qquad (11)$$

$$B = \frac{\det \mathbf{A}}{(k'_{\mathrm{MS}} + k''_{\mathrm{MS}})(2k_{\mathrm{S}} - k_{\mathrm{D}}) - 2k_{\mathrm{R}}C_{\mathrm{M}}k_{\mathrm{S}}}, \qquad (12)$$

$$\bar{J} = ze_0(k'_{\mathrm{MS}}\overline{N'_{\mathrm{MS}}} - k''_{\mathrm{MS}}\overline{N''_{\mathrm{MS}}}). \qquad (13)$$

Under equilibrium conditions the macroscopic current vanishes ($\bar{J} = 0$), therefore it follows from eq. (7) that the contribution to the spectral intensity (eq. (3)) due to the relaxation time $\tau_3 = 1/2\pi f_3$ (eq. (4)) disappears.

From the frequency dependence of $S_{\mathrm{J}}(f)$ given by eq. (3) a frequency-independent behavior of $S_{\mathrm{J}}(f)$ has to be expected both at low and at high frequencies. The low-frequency limit may be derived from eq. (3) and reads:

$$S_{\mathrm{J}}(0) = 2z^2e_0^2\left(k'_{\mathrm{MS}}\overline{N'_{\mathrm{MS}}} + k''_{\mathrm{MS}}\overline{N''_{\mathrm{MS}}} + 2\sum_{i=1}^{3}\alpha_i\tau_i\right). \qquad (14)$$

It was derived by Kolb and Läuger [5] on the basis of the Nyquist theorem [18] that under equilibrium conditions $S_{\mathrm{J}}(0)$ given by eq. (14) is proportional to the macroscopic membrane conductance $\bar{\lambda}$. In appendix C it is shown, that at non-equilibrium ($\bar{J} \neq 0$) this proportionality fails since the Nyquist theorem can no longer be applied. For the high frequency limit of $S_{\mathrm{J}}(f)$ one obtains from eq. (3) the relation:

$$S_{\mathrm{J}}(\infty) = 2z^2e_0^2(k'_{\mathrm{MS}}\overline{N'_{\mathrm{MS}}} + k''_{\mathrm{MS}}\overline{N''_{\mathrm{MS}}}). \qquad (15)$$

It can easily be seen that the right-hand-side of this equation accounts for the total number of charge jumps across the membrane. Eq. (15) therefore accounts for the spectral intensity generated due to the shot noise [19] of the carrier-mediated ion transport.

3. Material and methods

Black lipid bilayer membranes were formed in the usual way [20] from a 2% (w/v) solution of monoglyceride (Nu Check Prep., Elysian, Minn.) with C_{22} cis-mono-unsaturated fatty acid chains (monoerucin) in n-decane (Merck, standard for gas chromatography). The lipids contained about 98% of the 1-isomer and gave a single spot in a thin-layer chromatogram. As carrier substance the macrotetrolid tetranactin dissolved in ethanol was used which was added from a stock solution (10^{-4} M) in different amounts to the electrolyte solution. NH_4Cl and LiCl were of analytical reagent grade from Merck and used as unbuffered salt solutions (pH ~ 6).

Prior to membrane formation, the Teflon cell with inserted Ag/AgCl electrodes was incubated for about 3 hrs with the aqueous solution at room temperature. The measurements were started 40–50 min after the membrane had turned completely black. The circular hole in the Teflon wall between the two thermostated aqueous bulk phases had an area of about 0.25 mm^2. The area of the black membrane was determined with a calibrated scale of an ocular. The use of a selective small membrane area is due to the increasing influence of the background noise level especially at frequencies above 2 kHz as described previously [5].

The spectral intensity $S_{\mathrm{J}}(f)$ of noise current of the black film was measured at different applied constant voltages, as described previously [5,21], using some minor modifications as stated below. The instantaneous current was usually preamplified by an operational amplifier (Analog Devices Model 52K) with constant feed-back resistance of 20 MΩ and a feed-back capacitance of 1 pF. Besides that, within an experiment the preamplification could be switched to a selected amplifier of low noise-current (Burr Brown Model 3523L) with a constant feed-back resistance of 500 MΩ. The latter was used to investigate the influence of the noise current contribution of the preamplifier at lower frequencies ($f < 10$ Hz) on the level of the spectral intensity generated by the carrier-mediated ion transport. A theoretical description of the contribution of the background noise current generated by the preamplifier to the overall noise current was previously given [5]. The output of the preamplifier was fed into the main amplifier (Princeton Applied Research Model 113) which was used in the ac-coupled mode with a lower cut-off frequency f_1 of usually 1 Hz and a higher cut-off frequency f_2 of 300 kHz. The upper frequency limit is then mainly determined by the low pass filter of the feed-back circuit ($f_2 \lesssim 8$ kHz). $S_J(f)$ of the noise current was processed on-line with a Hewlett Packard 5420 A digital analyzer. The recorded spectrum usually was an average of 512 or 1024 summations. The corresponding autocorrelation function $C_J(\tau)$ was processed with a hardware correlator (Honeywell-SAICOR Model 43A).

The further handling of the processed spectra was performed on-line with a Hewlett Packard computer (Model 9825A). The results were graphically displayed on a Hewlett Packard plotter (Model 9872A). The measured spectral intensity $S_J(f)$ was fitted by the theoretically obtained relation for $S_J(f)$ (see eq. (3)) in the frequency range of about 0.8 Hz up to maximal 3.2 kHz by the method of least squares. The upper frequency limit was adapted to the actual shape of the spectrum to obtain optimal resolution in the frequency scale and was increased with increasing applied voltage as outlined in section results. Under optimized experimental conditions (zero applied voltage, 1 M NH_4Cl, 10^{-7} M tetranactin, $T = -5°C$) also the corresponding autocorrelation function $C_J(\tau)$ of the noise current was processed and compared with the theoretical derived relation. In this case the actual filter setting of f_1 and f_2 leads to a distortion of the shape and amplitude of $C_J(\tau)$ given by eq. (B7). The influence of the filtering of the membrane current on $C_J(\tau)$ may be described by the relation [22]

$$C_J(\tau) = \int_0^\infty |g(f)|^2 S_J(f) \cos(2\pi f\tau)\,\mathrm{d}f, \qquad (16)$$

where $S_J(f)$ is given by eq. (3). $|g(f)|^2$ describes the filter transfer function. For the used RC-band pass ($f_1 < f < f_2$) $|g(f)|^2$ is defined by the relation:

$$|g(f)|^2 = f_2^2 f^2/(f_1^2 + f^2)(f_2^2 + f^2). \qquad (17)$$

Using eqs. (3), (16) and (17) one obtains for the autocorrelation function of the filtered noise current the relation:

$$\begin{aligned} C_J(\tau) = f_2^2 z^2 e_0^2 \Bigg[& \pi(k'_{MS}\overline{N'_{MS}} + k''_{MS}\overline{N''_{MS}}) \\ & \times \frac{(f_2\,e^{-2\pi f_2\tau} - f_1\,e^{-2\pi f_1\tau})}{f_2^2 - f_1^2} \\ & + \sum_{i=1}^{3} \alpha_i f_i \Bigg[\frac{f_2\,e^{-2\pi\tau f_2}}{(f_2^2 - f_1^2)(f_i^2 - f_2^2)} \\ & - \frac{f_i\,e^{-2\pi\tau f_i}}{(f_i^2 - f_1^2)(f_i^2 - f_2^2)} - \frac{f_1\,e^{-2\pi\tau f_1}}{(f_i^2 - f_1^2)(f_2^2 - f_1^2)} \Bigg] \Bigg] \end{aligned} \qquad (18)$$

4. Results

The spectral intensity $S_J(f)$ of the noise current generated in the presence of 10^{-7} M tetranactin and 0.1 or 1 M NH_4Cl was recorded at different voltages for $T = -5°C$. The result of a typical experiment obtained on a single membrane is shown in fig. 1. With the used experimental set-up the influence of the background noise on the spectral intensity of carrier-mediated ion transport is negligible for frequencies below about 2 kHz [5]. Therefore a low incubation temperature was used to slow down the kinetic behavior of the carrier-mediated ion transport. At $T = -5°C$ it was possible even at higher voltages where the kinetics of the transport process accelerates and the amplitude of the spectral intensity at higher frequency decreases (see below) to analyze the complete dispersion region of $S_J(f)$ without a significant influence of the processed spectral intensities due to the background noise. The shape of the spectral intensity due to the background noise may be seen from curve a of fig. 1 were LiCl was used instead of NH_4Cl. Since Li^+ is not transported by tetranactin a resistance R_m was added in parallel to the membrane to simulate the thermal noise behavior

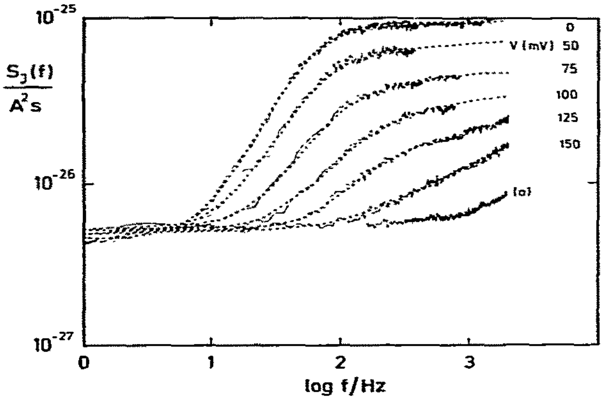

Fig. 1. Spectral intensity $S_J(f)$ of noise current from a lipid bilayer membrane in the presence of 10^{-7} M tetranactin for increasing voltage. The applied voltage was changed in steps of 25 mV. For clearer arrangement the curve processed at $V = 25$ mV is not shown. Aqueous solution: 1 M NH_4Cl, $T = -5°C$. Membrane forming solution: monoerucin/n-decane, membrane area A = 0.25 mm^2. For curve (a) 1 M LiCl was used in the presence of tetranactin. An external resistor $R_m = 5.3$ MΩ was added in parallel to the membrane to simulate the same stationary membrane resistance as in the presence of 1 M NH_4Cl at $V = 0$ mV. The dashed lines represent the fits obtained by application of eq. (3) for theoretical description of the measured spectra.

of the carrier induced ion transport. The slight increase of the corresponding spectral intensity (curve a) at frequencies above 2 kHz is presumably caused by the voltage noise of the amplifying system which is transformed into current noise by the membrane capacitance [5].

It may be seen from fig. 1 that the spectra show for the applied voltages a frequency independent behavior at lower as well as at higher frequencies. For the following we first regard the voltage independent behavior of the asymptotic limit of $S_J(f)$ at lower frequencies ($f \leqslant 5$ Hz). Since for these frequencies the absolute values of the spectral intensities are low ($S_J \approx 3 \times 10^{-27}$ A^2s) it was checked whether there is a contamination due to the noise current of the used preamplifier. Using a selected operational amplifier of low current noise (see section 3) no influence of this background noise source could be found. In a forthcoming publication [23] it will be shown that for frequencies below 10 Hz, $S_J(f)$ can be measured down to 10^{-28} A^2s with the used experimental set-up.

At higher voltages ($V > 100$ mV) it was observed in a few experiments that $S_J(f)$ showed at low frequencies a "$1/f$" behavior of varying amplitude in addition to the spectral intensities shown in fig. 1. Since it was found that even undoped lipid membranes of the same or a different lipid composition as were used for the present experiments showed dependence on pretreatment the appearance of a "$1/f$" shaped contribution to $S_J(f)$ (for $V > 0$ mV) [24] in those experiments were discarded.

At lower frequencies the frequency-independent behavior of $S_J(f)$ could be followed down to $f \sim 0.1$ Hz, therefore we use throughout this paper the approximation $S_J(0.1 \text{ Hz} < f < 5 \text{ Hz}) \sim S_J(0)$. It may be seen from fig. 1 that $S_J(0)$ stays about constant for a change of the applied voltage from zero to 150 mV. Fig. 1 shows furthermore that at high frequencies the frequency independent level of $S_J(f)$ declines strongly with increasing voltage. Under optimal experimental conditions no further change of $S_J(f)$ from a frequency independent behavior was observed even for frequencies above 3 kHz.

In order to compare the observed variation of $S_J(0)$ and $S_J(3.2 \text{ kHz})$ as function of applied voltage with the theoretical predicted behavior due to eqs. (14) and (15) we have to determine the five molecular parameters which characterize the used model of carrier-mediated ion transport (see section 2).

From a least square fit of eq. (3) to the measured intensity $S_J(f)$ in the frequency range of 1 Hz to about 3 kHz the free parameters k_{MS}, k_S, k_D, k_R were determined. For the description of the voltage dependence of $S_J(f)$ eq. (1) was applied. The value of N_0 was derived from the fitted set of parameters using eq. (3) and the value of $S_J(0)$ directly read from the measured spectral intensity (see above). It was found that independent of the choice of the different experimental conditions (membrane voltage, temperature, carrier- and electrolyte concentration) a close agreement of the theoretically fitted shape of the spectrum to the measured values of $S_J(f)$ was obtained. But it has to be pointed out that at lower voltages ($V \leqslant 50$ mV) $S_J(f)$ could as well be fitted by a spectral intensity with only a single dispersion, corresponding to a single relaxation time τ_A, of the type [5]:

$$S_J(f) = 4kT\left[\frac{1}{R_B} + \frac{1}{R_A}\,\frac{(2\pi f\tau_A)^2}{1+(2\pi f\tau_A)^2}\right], \qquad (19)$$

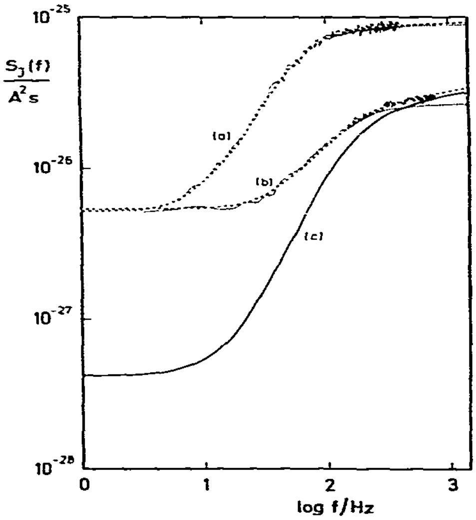

Fig. 2. Spectral intensity of noise current from a lipid bilayer membrane in the presence of 10^{-7} M tetranactin at an applied voltage of 25 mV (curve (a)) and 100 mV (curve (b)). The experimental conditions were the same as for fig. 1. The dashed lines represent the result of a least square fit due to eq. (3) and the dotted lines show the result of eq. (19) for a description of $S_J(f)$. In the following sets of parameters were used: a) $V = 25$ mV; dashed line: $k_R = 4.7 \times 10^3$ M^{-1} s^{-1}, $k_D = 2.3 \times 10^2$ s^{-1}, $k_S = 22$ s^{-1}, $k_{MS} = 2.1 \times 10^2$ s^{-1}, $N_0 = 1.4 \times 10^{10}$; dotted line: $R_A = 2 \times 10^5$ Ω, $R_B = 3.2 \times 10^6$ Ω, $C_A = 1.6 \times 10^{-8}$ F. b) $V = 100$ mV, dashed line: $k_R = 2.5 \times 10^3$ M^{-1} s^{-1}, $k_D = 1.8 \times 10^3$ s^{-1}, $k_S = 17$ s^{-1}, $k_{MS} = 2.4 \times 10^2$ s^{-1}, $N_0 = 1.4 \times 10^{10}$; dotted line: $R_A = 7 \times 10^5$ Ω, $R_B = 3 \times 10^6$ Ω, $C_A = 1.7 \times 10^{-9}$ F. Curve (c) shows the result obtained by application of the Nyquist theorem (eqs. (C1), (C20)) for description of $S_J(f)$ at 100 mV (see curve (b)).

$$\tau_A = R_A C_A .$$

This shape of $S_J(f)$ corresponds to the noise intensity of an equivalent circuit shown in ref. [5]. The spectral intensity processed at $V = 25$ mV (curve a in fig. 2) was fitted using either eq. (3) or eq. (19). No significant difference in the goodness of both fits was obtained. This finding will be discussed later. At $V = 100$ mV or higher membrane voltages the measured shape of $S_J(f)$ could not sufficiently be described by a single dispersion due to eq. (19) as a comparison of the theoretical spectra fitted to curve b in fig. 2 shows. Therefore the measured spectral intensities were always described by application of eq. (3). The results for the parameters k_R, k_D, k_S, k_{MS}, N_0 and the derived values of τ_1, τ_2, τ_3 (eq. (4)) as well as $\alpha_1, \alpha_2, \alpha_3$ (eq. (5)–(7)) are summarized in table 1. It turned out (table 1) that k_S and the ratio k_R/k_D remained nearly independent of voltage whereas both k_R and k_D decrease with increasing voltage. Furthermore the table shows that the values of $k_{MS}(0)$ increase slightly with increasing voltage.

Fig. 3 shows the experimentally derived ratios $S_J(0)_V/S_J(0)_{V=0}$ and $S_J(3.2 \text{ kHz})_V/S_J(3.2 \text{ kHz})_{V=0}$ versus voltage as well as the corresponding theoretical values derived from eqs. (14) and (3) respectively, using the values for k_R, k_S, k_D, $k_{MS}(0)$ found at zero applied voltage. As may be seen from fig. 3 both functions follow the theoretical predicted behavior.

In another series of experiments the concentration of NH_4Cl in the aqueous phase was set to 0.1 M. It may be seen from table 1 that by this decrease of the concentration of the transported ammonium ion k_S increases by about a factor of ten which results in a decrease of the ratio $S_J(3.2 \text{ kHz})/S_J(0)$.

Measurements of the autocorrelation function $C_J(\tau)$ of the noise current were performed in addition to the analysis of the spectral intensity. Both quantities, the autocorrelation function and the spectral intensity are correlated by the Wiener-Khintchine theorem (see appendix B) and theoretically yield the same information about the source of the noise current.

In fig. 4 $C_J(\tau)$ is shown which was processed at zero applied voltage from about the same length of record of noise current which was used for the corresponding determination of $S_J(f)$ (curve in fig. 1 for $V = 0$ mV). The theoretical line in fig. 4 was calculated due to eq. (18) using the parameter values obtained from a least square fit of eq. (3) to the corresponding spectral intensity. As may be seen from fig. 4 the correlation of negative value increases about exponentially and shows a relative large scatter around the mean value given by the theoretical line. For higher voltages ($V \geqslant 50$ mV) no significant deviation of $C_J(\tau)$ from a horizontal line could be obtained for $\tau > 0$ due to the large scatter of the processed function. This finding can be explained on the basis of methodical difficulties of measuring $C_J(\tau)$ for carrier-mediated ion transport systems as will be discussed.

Table 1

Analysis of noise current from monoerucin/n-decane membranes in the presence of 10^{-7} M tetranactin at steady-state for 1 M NH_4Cl and 0.1 M NH_4Cl in the aqueous phase ($T = -5°C$). The rate constants k_R, k_D, k_{MS} and k_S have been obtained by fitting eq. (3) to the experimental spectrum $S_J(f)$. N_0 has been evaluated from $S_J(0)$ and is referred to a membrane area of 0.25 mm^2. τ_i and α_i ($i = 1, 2, 3$) have been calculated from eqs. (4)–(7). The values are the mean of 12 experiments. $\bar{\lambda}$ has been determined independently from $S_J(f)$.

V (mV)	k_R (10^3 M^{-1} s^{-1})	k_D (10^3 s^{-1})	k_S (s^{-1})	k_{MS} (s^{-1})	τ_1 (ms)	τ_2 (ms)	τ_3 (ms)	α_1 (10^{14} s^{-2})	α_2 (10^{14} s^{-2})	α_3 (10^{11} s^{-2})	$10^9 \times N$	$\bar{\lambda}$ (μS cm^{-2})
1 M NH_4Cl												
0	3.8	2.6	17	240	0.15	3.4	0.16	−2.1	−2.7	0	14	130
25	4.1	2.3	19	220	0.15	3.1	0.16	−1.7	−2.6	2.0	14	120
50	3.1	2.7	14	270	0.16	2.3	0.17	−2.7	−2.4	3.4	13	110
75	3.1	2.7	14	270	0.16	2.3	0.17	−2.7	−2.4	3.4	13	87
100	2.3	1.8	16	270	0.19	1.1	0.24	−3.8	−1.9	7.9	14	70
125	1.1	1.0	12	300	0.21	1.2	0.48	−7.0	−0.57	5.5	16	68
150	1.2	1.1	13	320	0.13	1.0	0.43	−8.3	0.29	6.6	16	66
0.1 M NH_4Cl												
0	8.0	0.9	259	221	0.1	2.2	1.1	−0.41	−3.7	0	9.4	360
25	8.4	1.2	248	281	0.1	1.6	1.0	−0.89	−6.0	19	9.9	360
50	9.1	1.5	253	252	0.09	1.4	0.94	−0.86	−4.6	80	9.8	340
75	6.1	0.95	211	273	0.13	0.9	1.4	−1.1	−5.0	130	9.2	220
100	5.0	1.0	207	313	0.15	0.5	1.7	−2.4	−5.6	310	9.4	210
125	4.4	0.58	231	353	0.15	0.3	2.0	−5.2	−5.4	480	9.3	160
150	3.6	0.97	245	384	0.11	0.3	2.1	−9.3	−2.4	1000	9.1	160

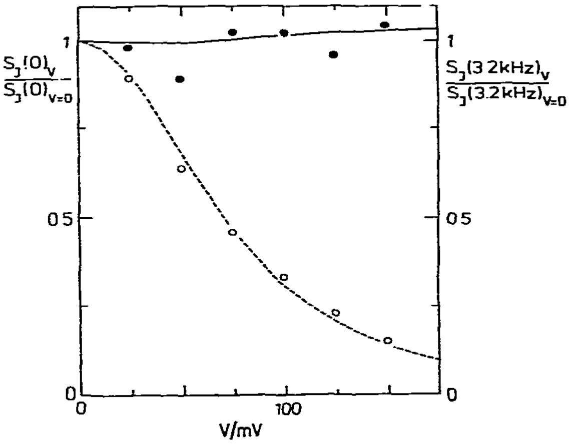

Fig. 3. $S_J(0)_V$ normalized to $S_J(0)_{V=0}$ versus membrane voltage (closed points, $S_J(0)_{V=0} = 4.7 \times 10^{-27}$ A^2 s). For this plot the left ordinate corresponds. The ordinate on the right side corresponds to the ratio $S_J(3.2\text{ kHz})_V/S_J(3.2\text{ kHz})_{V=0}$ (open points, $S_J(3.2\text{ kHz})_{V=0} = 1 \times 10^{-25}$ A^2 s. The theoretical curves are derived from eqs. (14) and (3) respectively, whereby the following set of parameters was used: $k_R = 3.6 \times 10^3$ M^{-1} s^{-1}, $k_D = 2.7 \times 10^3$ s^{-1}, $k_S = 16$ s^{-1}, $k_{MS} = 2.6 \times 10^2$ s^{-1}. The experimental conditions were the same as for fig. 1.

5. Discussion

In the previous section it was shown that under steady-state conditions the spectral intensity $S_J(f)$ of tetranactin-induced noise current could be described by the theoretical predicted shape (eq. (3)). Theoretically one has to expect between the regions of frequency independent behavior of $S_J(f)$ at low and high frequencies an increase of $S_J(f)$ built up by superposition of three characteristic frequencies $f_i = 1/2\,\pi\tau_i$ (eq. (4)) and their corresponding amplitudes α_i ($i = 1,2,3$) (eq. (5)–(7)). The frequency $f_3 = 1/2\,\pi\tau_3$ corresponds to the relaxation time related to the rate constants of the ion-carrier adsorption and desorption process at the membrane solution interface which is assumed to be voltage independent. In a voltage-jump current relaxation experiment the corresponding amplitude vanishes whereas for the noise analysis the expected amplitude α_3 is zero at equilibrium ($\bar{J} = 0$) but differs from zero at non-equilibrium (see eq. (7)).

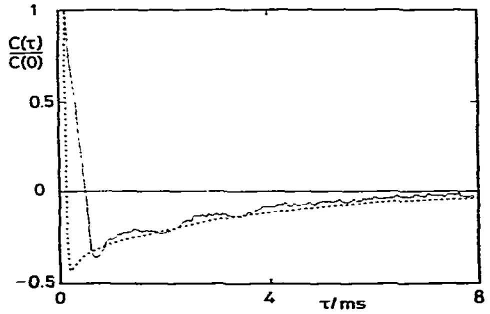

Fig. 4. Autocorrelation function $C_J(\tau)/C_J(0)$ of noise current obtained at zero applied voltage in parallel to the corresponding spectral intensity $S_J(f)$ (see fig. 1). The theoretical curve was drawn according to eq. (18) using the following parameters given in the legend of fig. 3 for the filter bandpass of frequencies $f_1 = 0.03$ Hz and $f_2 = 6 \times 10^3$ Hz and $C_J(0) = 2 \times 10^{-23}$ A^2.

As was previously shown [5] $S_J(f)$ can theoretically be described at thermal equilibrium by two dispersions whereby experimentally one dispersion could be resolved (see fig. 2). But under steady-state conditions especially for membrane voltages above 50 mV the processed shape of $S_J(f)$ showed a superposition of at least two dispersions. But a theoretically predicted third dispersion was not visible within the frequency dependent part of $S_J(f)$. This may be explained by the finding that the fitted values of f_1 and f_3 are very similar and that for the corresponding amplitudes α_1 and α_3 the relation $|\alpha_1| \gg |\alpha_3|$ is found (table 1) which causes indistinguishable contributions to $S_J(f)$.

If one assumes an activation energy of 20 kcal/mol for the different rate constants [25] the corresponding values presented in table 1 can be compared with the results of Laprade et al. [10] which were obtained by relaxation experiments on monoolein/n-decane membranes for 5×10^{-9} M tetranactin and 10^{-2} M NH_4Cl at 22.5°C. The comparison shows that k_R and k_{MS} are in close agreement whereas the values of k_D and k_S are different up to an order of magnitude.

In the following we discuss the physical meaning of the frequency independent levels of $S_J(f)$ at lower and higher frequencies denoted by $S_J(0)$ and $S_J(3.2$ kHz) in terms of the considered transport model. Under equilibrium conditions $S_J(0)$ is, according to the Nyquist theorem, equal to the macroscopic membrane conductance $\bar{\lambda}$ times $4kT$ [5]. It can easily be seen that this relationship is no longer applicable at steady-state by comparing the voltage dependence of $S_J(0)$ read from fig. 1 with the corresponding values of $\bar{\lambda}$ given in table 1. For a further consideration we applied the Nyquist theorem also to the non-equilibrium state and related the spectral intensity of the microscopic noise current to the steady-state macroscopic admittance of the carrier-mediated ion transport (appendix C). The result for $S_J(f)$ due to the Nyquist theorem (eqs. (C1), (C20)) was compared with the relation of $S_J(f)$ (eq. (D1)) explicitly derived for the non-equilibrium state (eq. (3)) as outlined in appendix D. Eq. (D1) shows that only at equilibrium where the macroscopic current vanishes ($\bar{J} = 0$) the spectral intensity can be described by the Nyquist theorem. In fig. 2 the theoretical shape of $S_J(f)$ was plotted versus frequency at $V = 100$ mV (curve b) using either the Nyquist theorem (eqs. (C1), (C20)) (dotted line) or eq. (3) (dashed line). As the comparison shows both functions differ over the whole frequency range but especially at lower frequencies.

The high frequency tail of the spectral intensity accounts for the noise intensity generated by the total number of charge jumps within the membrane (eq. (15)) which by definition is equivalent to the intensity of the shot noise. As fig. 1 shows the spectral intensity at high frequencies which corresponds to the shot noise contribution of the transport system declines with increasing voltage. The decrease or increase of the shot noise intensity with increasing applied voltage is dependent especially on the ratio of the rate constants of recombination and dissociation k_R/k_D of the carrier-ion complex at the membrane solution interface. Whereas for $k_R \gtrsim k_D$ a decrease of the shot noise intensity with increasing membrane voltage is observed, one obtains an increase of the shot noise level for $k_D \gg k_R$. For a comparison of the experimentally obtained and theoretically predicted voltage dependence of $S_J(0)$ and $S_J(3.2\ \mathrm{kHz})$ we denote this dependence by the notion $S_J(0)_V$ and $S_J(3.2\ \mathrm{kHz})_V$, respectively. Comparison of $S_J(0)_V/S_J(0)_{V=0}$ as well as $S_J(3.2\ \mathrm{kHz})/S_J(3.2\ \mathrm{kHz})_{V=0}$ as function of voltage with the theoretical description due to eq. (14) and eq. (3) shows close agreement (fig. 2).

For the derivation of eq. (3) we used the assumption that the rate constants k_R, k_D, k_S and $k_{MS}(0)$ are voltage independent. As table 1 shows k_S was found to be voltage independent. For $k_{MS}(0)$ an increase with increasing voltage was obtained which is in contrast to the Eyring relation (eq. (1)). Also Knoll and Stark [14] found for carrier-mediated ion transport (valinomycin-Rb$^+$) across lipid bilayer membranes a voltage dependent behaviour of $k_{MS}(0)$. One explanation for this observation lies in the theoretical description of the shape of the energy barrier for the translocation step of the charged carrier-ion complex within the membrane. It is well known that the general solution of the diffusion equation for the translocation of an ion across the membrane yields the relation [26–29]:

$$k'_{MS} = \tilde{k}_{MS}(0)\, f(u)\, e^{u/2},$$

$$k''_{MS} = \tilde{k}_{MS}(0)\, f(u)\, e^{-u/2},$$

where $f(u) = e^{-\eta u^2}$ is an empirically derived relation which takes into account image forces acting on the ion in the bilayer.

η is a constant parameter. Using the value $\eta \simeq 0.02$ and the values of $k_{MS}(0)$ given in table 1 one finds that $\tilde{k}_{MS}(0) = k_{MS}(0)\, f(u)$ is nearly voltage independent.

Up to now both methods the spectral- and autocorrelation analysis were applied in parallel only for the analysis of noise current generated by the opening and closing of channels.

The autocorrelation analysis has not as yet been used for the analysis of carrier-mediated ion transport noise. On the basis of the Wiener-Khintchine theorem both approaches theoretically contain the same information. The corresponding autocorrelation function $C_J(\tau)$ of carrier-generated noise current (fig. 4) shows besides a delta-shaped function around $\tau = 0$ a negative correlation for $\tau > 0$.

As indicated in section 4 the autocorrelation-function $C_J(\tau)$ shows a relative large scatter around the theoretical curve compared with $S_J(f)$. Furthermore no reliable information about the rate constants of the carrier-transport mechanism could be obtained at higher voltages from the measured function $C_J(\tau)$. This observation can be explained as follows. It may be seen from eq. (37) that $C_J(\tau)$ consists of a δ-shaped term at $\tau = 0$ and an exponential increasing part for $\tau > 0$ starting from negative values of $C_J(\tau)$. The measured shape of $C_J(\tau)$ depends on the actual filter bandpass which has to be used for the amplification of the ac-component of the membrane current as described under Materials and Methods (see eq. (18)). The filter-modified δ-shaped term of $C_J(\tau)$ which by Fourier transformation is correlated with the frequency independent part of $S_J(f)$ for $f \to \infty$ (level of shot noise intensity) exceeds the dynamic range of commercial available correlators, since also a reasonable amplitude-resolution of $C_J(\tau)$ for $\tau > 0$ is needed. Due to the lack of knowledge of the actual filtering of the noise current the shot noise intensity of the carrier-mediated ion transport can experimentally only roughly be estimated from the shape of $C_J(\tau)$, whereas it can directly be read from the high frequency tail of $S_J(f)$. Therefore the magnitude of $C_J(\tau)$ around $\tau = 0$ determines the resolution of $C_J(\tau)$ for $\tau > 0$. This part of $C_J(\tau)$ is determined by the correlation times τ_i $(i = 1,2,3)$ (eq. (4)). In the frequency domain the part of $S_J(f)$ which is related to the relaxation processes is solely limited by the background noise level and not as in the time domain by the shot noise level of the transport system itself. Filtering of the noise current by narrowing the width of the filter bandpass to reduce the magnitude of $C_J(\tau \simeq 0)$ leads to a further complexation of the noise analysis in the time domain as may be seen from eq. (18). It was found that the location of the minimum of $C_J(\tau)$ shifts to higher τ values with decreasing filter bandwidth. Besides that the absolute value of $C_J(\tau)$ at the minimum decreases.

In the case of carrier-mediated ion transport the comparison of noise analysis in the time domain versus frequency domain shows that the analysis in the time domain is experimentally the far less favourable approach.

Acknowledgement

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Appendix A

General solution for the differential equation system of carrier-mediated ion transport

The time dependence of the interfacial concentrations $N'_S(t)$, $N''_S(t)$, $N'_{MS}(t)$ and $N''_{MS}(t)$ is given by the following system of equations:

$$\mathrm{d}N'_S/\mathrm{d}t = -k_R C_M N'_S + k_D N'_{MS} - k_S(N'_S - N''_S), \quad \text{(A1)}$$

$$\mathrm{d}N''_S/\mathrm{d}t = -k_R C_M N''_S + k_D N''_{MS} - k_S(N''_S - N'_S), \quad \text{(A2)}$$

$$\mathrm{d}N'_{MS}/\mathrm{d}t = k_R C_M N'_S - k_D N'_{MS} - k'_{MS} N'_{MS} + k''_{MS} N''_{MS}, \quad \text{(A3)}$$

$$\mathrm{d}N'_{MS}/\mathrm{d}t = k_R C_M N''_S - k_D N''_{MS} - k''_{MS} N''_{MS} + k'_{MS} N'_{MS}. \quad \text{(A4)}$$

Eqs. (A1)–(A4) satisfy the conservation relation that the total number of carriers N_0 in the membrane is time independent (eq. (2)). The solution of these equations is simplified by the introduction of the total number of free carrier molecules ($r(t)$) and of the complex MS ($s(t)$) [5]:

$$r(t) = N'_S(t) + N''_S(t), \qquad s(t) = N'_{MS}(t) + N''_{MS}(t),$$

$$r(t) + s(t) = N_0. \quad \text{(A5)}$$

Using eqs. (A1)–(A5) one obtains the differential equations

$$\mathrm{d}r/\mathrm{d}t = -(k_R C_M + k_D) r + k_D N_0, \quad \text{(A6)}$$

$$\mathrm{d}s/\mathrm{d}t = -(k_R C_M + k_D) s + k_R C_M N_0, \quad \text{(A7)}$$

where the solution is given by

$$r(t) = \bar{r} + (r(0) - \bar{r})\,\mathrm{e}^{-t/\tau_3}, \qquad s(t) = N_0 - r(t), \quad \text{(A8)}$$

$$\bar{r} = N_0 k_D/(k_R C_M + k_D), \quad \text{(A9)}$$

$$\tau_3 = 1/(k_R C_M + k_D). \quad \text{(A10)}$$

The recorded electric current and the corresponding impedance are essentially determined by the macroscopic flux $\Phi(t)$ of charged carriers across the membrane:

$$\Phi(t) = k'_{MS} N'_{MS}(t) - k''_{MS} N''_{MS}(t). \quad \text{(A11)}$$

As further variable we introduce Γ defined by the relation

$$\Gamma(t) = k'_{\mathrm{MS}} N'_{\mathrm{S}}(t) - k''_{\mathrm{MS}} N''_{\mathrm{S}}(t). \tag{A12}$$

With eqs. (A6) and (A7) one gets from eqs. (A1)–(A4) by lengthy but elementary calculations the following equations for the rate of change of Φ and Γ:

$$\mathrm{d}\Phi/\mathrm{d}t = -(k_{\mathrm{D}} + k'_{\mathrm{MS}} + k''_{\mathrm{MS}})\Phi + k_{\mathrm{R}} C_{\mathrm{M}} \Gamma, \tag{A13}$$

$$\mathrm{d}\Gamma/\mathrm{d}t = k_{\mathrm{D}}\Phi - (k_{\mathrm{R}} C_{\mathrm{M}} + 2k_{\mathrm{S}})\Gamma + k_{\mathrm{S}}(k'_{\mathrm{MS}} - k''_{\mathrm{MS}})r, \tag{A14}$$

with $r(t)$ according to eq. (A8). The solution of these equations is complicated because of the time dependent inhomogeneity in eq. (A14), which introduces in addition to the two relaxation times τ_1 and τ_2 (see below) a third time constant τ_3 for those cases, where $(k'_{\mathrm{MS}} - k''_{\mathrm{MS}}) \neq 0$ (non-equilibrium state) and $(r(0) - \bar{r}) \neq 0$. In macroscopic relaxation experiments $(r(0) - \bar{r})$ vanishes [8].

We introduce the matrix **A** of coefficients of eqs. (A13), (A14) with the elements:

$$A_{11} = -(k_{\mathrm{D}} + k'_{\mathrm{MS}} + k''_{\mathrm{MS}}),$$

$$A_{12} = k_{\mathrm{R}} C_{\mathrm{M}}, \quad A_{21} = k_{\mathrm{D}},$$

$$A_{22} = -(k_{\mathrm{R}} C_{\mathrm{M}} + 2k_{\mathrm{S}}). \tag{A15}$$

The eigenvalues λ_1 and λ_2 of **A** determine the time constants τ_1, τ_2

$$\lambda_1 = -1/\tau_1 = -Q - \sqrt{a}, \qquad \lambda_2 = -1/\tau_2 = -Q + \sqrt{a}, \tag{A16}$$

where Q and a are given by eqs. (10) and (12).

For the following we use the abbreviation det **A** (determinant of **A**):

$$\det \mathbf{A} = (k'_{\mathrm{MS}} + k''_{\mathrm{MS}})(k_{\mathrm{R}} C_{\mathrm{M}} + 2k_{\mathrm{S}}) + 2k_{\mathrm{D}} k_{\mathrm{S}}. \tag{A17}$$

Using eqs. (A13)–(A17) the general solution for the time dependent flux $\Phi(t)$ of charged carriers across the membrane may be derived under arbitrary initial conditions in the following form:

$$\Phi(t) = \bar{\Phi} + \sum_{i=1}^{3} c_i \mathrm{e}^{\lambda_i t} \tag{A18}$$

with the stationary flux

$$\bar{\Phi} = N_0 \frac{k_{\mathrm{R}} C_{\mathrm{M}} k_{\mathrm{D}} k_{\mathrm{S}} (k'_{\mathrm{MS}} - k''_{\mathrm{MS}})}{(k_{\mathrm{R}} C_{\mathrm{M}} + k_{\mathrm{D}}) \det \mathbf{A}} \tag{A19}$$

and the amplitudes

$$c_1 = \frac{\lambda_2(\Phi(0) - \bar{\Phi} - c_3) - (\dot{\Phi}(0) - c_3\lambda_3)}{\lambda_2 - \lambda_1}, \tag{A20}$$

$$c_2 = \frac{\lambda_1(\Phi(0) - \bar{\Phi} - c_3) - (\dot{\Phi}(0) - c_3\lambda_3)}{\lambda_1 - \lambda_2}, \tag{A21}$$

$$c_3 = \frac{k_{\mathrm{R}} C_{\mathrm{M}} k_{\mathrm{S}} (k'_{\mathrm{MS}} - k''_{\mathrm{MS}})(r(0) - \bar{r})}{(k'_{\mathrm{MS}} + k''_{\mathrm{MS}})(2k_{\mathrm{S}} - k_{\mathrm{D}}) - 2k_{\mathrm{R}} C_{\mathrm{M}} k_{\mathrm{S}}}, \tag{A22}$$

whereby the initial flux $\Phi(0)$, its time derivative $\dot{\Phi}(0)$ and $r(0)$ are determined by the initial concentrations as follows:

$$r(0) = N'_{\mathrm{S}}(0) + N''_{\mathrm{S}}(0), \tag{A23}$$

$$\Phi(0) = k'_{\mathrm{MS}} N'_{\mathrm{MS}}(0) - k''_{\mathrm{MS}} N''_{\mathrm{MS}}(0), \tag{A24}$$

$$\dot{\Phi}(0) = -(k_{\mathrm{D}} + k'_{\mathrm{MS}} + k''_{\mathrm{MS}})\Phi(0) + k_{\mathrm{R}} C_{\mathrm{M}} (k'_{\mathrm{MS}} N'_{\mathrm{S}}(0) - k''_{\mathrm{MS}} N''_{\mathrm{S}}(0). \tag{A25}$$

The steady-state concentrations $\overline{N'_{\mathrm{MS}}}$ and $\overline{N''_{\mathrm{MS}}}$ are easily calculated from the stationary solutions of $\Phi(t)$ and $s(t)$ according to eqs. (A5), (A11) and (A19):

$$\overline{N'_{\mathrm{MS}}} = N_0 \frac{k_{\mathrm{R}} C_{\mathrm{M}}}{k_{\mathrm{R}} C_{\mathrm{M}} + k_{\mathrm{D}}} \times \frac{k_{\mathrm{S}} k_{\mathrm{D}} + k''_{\mathrm{MS}} k_{\mathrm{R}} C_{\mathrm{M}} + 2k_{\mathrm{S}} k''_{\mathrm{MS}}}{k_{\mathrm{R}} C_{\mathrm{M}} (k'_{\mathrm{MS}} + k''_{\mathrm{MS}}) + 2k_{\mathrm{S}} (k_{\mathrm{D}} + k'_{\mathrm{MS}} + k''_{\mathrm{MS}})}, \tag{A26}$$

$$\overline{N''_{\mathrm{MS}}} = N_0 \frac{k_{\mathrm{R}} C_{\mathrm{M}}}{k_{\mathrm{R}} C_{\mathrm{M}} + k_{\mathrm{D}}} \times \frac{k_{\mathrm{S}} k_{\mathrm{D}} + k'_{\mathrm{MS}} k_{\mathrm{R}} C_{\mathrm{M}} + 2k_{\mathrm{S}} k'_{\mathrm{MS}}}{k_{\mathrm{R}} C_{\mathrm{M}} (k'_{\mathrm{MS}} + k''_{\mathrm{MS}}) + 2k_{\mathrm{S}} (k_{\mathrm{D}} + k'_{\mathrm{MS}} + k''_{\mathrm{MS}})}. \tag{A27}$$

Appendix B

Autocorrelation function and spectral intensity of noise current generated by carrier-mediated ion transport at steady-state

Recently we have developed a theoretical approach to electric fluctuations around non-equilibrium states [15]. The application to noise current generated by carrier-mediated ion transport yields to the following result for the autocorrelation function $C_J(t)$:

$$C_J(t) = ze_0^2[(k'_{MS}\overline{N'_{MS}} + k''_{MS}\overline{N''_{MS}})\delta(t) + k'_{MS}\overline{N'_{MS}}(k'_{MS}\Omega_{MS',MS''}(t) - k''_{MS}\Omega_{MS'',MS''}(t)) + k''_{MS}\overline{N''_{MS}}(k''_{MS}\Omega_{MS'',MS'}(t) - k'_{MS}\Omega_{MS',MS'}(t)]. \tag{B1}$$

In eq. (B1) $\Omega_{MS',MS''}(t)$, $\Omega_{MS'',MS''}(t)$ are the so-called fundamental solutions [15] for $(N'_{MS}(t) - N'_{MS})$ of eqs. (A1)–(A4) under the initial conditions:

$$N''_{MS}(0) = 1, \qquad N'_{MS}(0) = N'_S(0) = N''_S = 0, \tag{B2}$$

and $\Omega_{MS',MS'}(t)$, $\Omega_{MS'',MS'}(t)$ are obtained correspondingly. Hence for the second term in eq. (B1) the following relation is derived using eqs. (A11), (A24) and (A19).

$$N_0(k'_{MS}\Omega_{MS',MS'}(t) - k''_{MS}\Omega_{MS'',MS''}(t)) = \Phi(t) - \overline{\Phi}, \tag{B3}$$

with the initial conditions

$$\Phi(0) = -N_0 k''_{MS},$$

$$\dot{\Phi}(0) = N_0 k''_{MS}(k_D + k'_{MS} + k''_{MS}). \tag{B4}$$

Analogously one obtains for the third term of eq. (B1):

$$N_0(k'_{MS}\Omega_{MS',MS'}(t) - k''_{MS}\Omega_{MS'',MS''}(t)) = \Phi(t) - \overline{\Phi}, \tag{B5}$$

with the initial conditions

$$\Phi(0) = N_0 k'_{MS},$$

$$\dot{\Phi}(0) = -N_0 k'_{MS}(k_D + k'_{MS} + k''_{MS}). \tag{B6}$$

Using eqs. (B1)–(B6) under application of eqs. (A18)–(A25) the autocorrelation function can be described by the relation:

$$C_J(t) = z^2 e_0^2 \left[(k'_{MS}\overline{N'_{MS}} + k''_{MS}\overline{N''_{MS}})\delta(t) + \sum_{i=1}^{3} \alpha_i\, e^{-t/\tau_i}\right], \tag{B7}$$

where the relations for the amplitudes α_1, α_2 and α_3 are explicitly given by eqs. (5)–(7). The relaxation times τ_1 and τ_2 are given by eq. (A16) and τ_3 by eq. (A10).

According to the Wiener-Khintchine theorem the spectral intensity $S_J(f)$ of noise current is given by transformation of $C_J(f)$ due to:

$$S_J(f) = 4\int_0^\infty C_J(t)\cos 2\pi f t\, dt. \tag{B8}$$

Insertion of eq. (B7) in eq. (B8) yields finally the spectral intensity of the form:

$$S_J(f) = 2z^2e_0^2\left(k'_{MS}\overline{N'_{MS}} + k''_{MS}\overline{N''_{MS}} + 2\sum_{i=1}^{3}\frac{\alpha_i\tau_i}{1+(2\pi f\tau_i)^2}\right). \tag{B9}$$

Appendix C

Admittance related to carrier-mediated ion transport

Recently the noise current of carrier mediated ion transport has been analyzed at equilibrium ($\bar{J} = 0$) [5] on the basis of the Nyquist theorem [18]:

$$S_J(f) = 4kT\,\mathrm{Re}[Y(f)], \tag{C1}$$

which relates the spectral intensity of microscopic fluctuations around the equilibrium state to the real part denoted by "Re" of the frequency dependent stationary complex admittance $Y(f)$. In the following we want to compare $S_J(f)$ given by (eq. (3)) with the complex admittance due to eq. (C1) under non-equilibrium steady-state conditions.

We assume that additionally to a constant voltage $\overline{V}$ a small (complex) periodic voltage $\epsilon(f, t)$ is applied to the membrane [30].

$$V(t) = \overline{V} + \epsilon(f, t), \tag{C2}$$

$$\epsilon(f, t) = \epsilon_0\, e^{2\pi i f t} \qquad (i = \sqrt{-1}), \tag{C3}$$

$$|\epsilon(f, t)| \ll |V(t)|. \tag{C4}$$

The corresponding instantaneous membrane current $J(f, t)$ may be described as a linear superposition of a steady-state current $\bar{J}$ and a (complex) fluctuating current $j(f, t)$

$$J(f, t) = \bar{J} + j(f, t), \qquad j(f, t) = \epsilon_0 Y(f)\, e^{2\pi i f t}. \tag{C5}$$

For the stationary current $\bar{J}$ the macroscopic membrane conductance $\bar{\lambda}(\bar{V})$ is defined by

$$\bar{J}(\bar{V}) = \bar{\lambda}(\bar{V})\,\bar{V}. \tag{C6}$$

The complex admittance $Y(f)$ at non-equilibrium state can then be defined by the relation:

$$Y(f) = j(f, t)\,\mathrm{e}^{-2\pi i f t}/\epsilon_0. \tag{C7}$$

For the following we assume that the ion-transport mechanism at steady-state is disturbed at $t = 0$ by a δ-shaped voltage pulse:

$$\epsilon(t) = \xi\,\delta(t), \tag{C8}$$

whereby according to eq. (C4) the relation

$$\left|\int_{-\infty}^{\infty} \xi\,\delta(t)\,\mathrm{d}t\right| \ll |V(t)| \tag{C9}$$

has to be fulfilled.

The voltage pulse given by equation (C4) leads to a current pulse $\hat{j}(t)$ from which the complex admittance can be derived:

$$Y(f) = \frac{1}{\xi}\int_{-\infty}^{\infty} \hat{j}(t)\,\mathrm{e}^{-2\pi i f t}\,\mathrm{d}t, \tag{C10}$$

using the well-known formula:

$$\delta(t) = \frac{1}{2\pi}\int_{-\infty}^{\infty} \mathrm{e}^{2\pi i f t}\,\mathrm{d}f. \tag{C11}$$

For an explicit derivation of the admittance $Y(f)$ according to eq. (C10) $\hat{j}(t)$ has to be known. We assume that the (voltage) current pulse is related to a corresponding fluctuation of the ion flux $\Phi(t)$ across the membrane due to the relation:

$$\hat{j}(t) = z\,e_0(\Phi(t) - \bar{\Phi}), \tag{C12}$$

were $\Phi(t)$ and $\bar{\Phi}$ are given by eqs. (A18) and (A19), respectively.

The behavior of $(\Phi(t) - \bar{\Phi})$ for $t \geqslant 0$ after a δ-shaped voltage pulse applied at $t = 0$ is determined by the voltage dependence of the translocation rate constants k'_{MS} and k''_{MS}. The dependency was described by a single exponential behavior (eq. (1)). Due to eqs. (C2) and (C4) $k'_{\mathrm{MS}}(V(t))$ and $k''_{\mathrm{MS}}(V(t))$ can be expanded in terms of $\epsilon(t)$:

$$k'_{\mathrm{MS}}(\bar{V} + \epsilon(t)) \approx k'_{\mathrm{MS}}(\bar{V})\left(1 + \frac{\epsilon(t) z e_0}{2kT}\right),$$

$$k''_{\mathrm{MS}}(\bar{V} - \epsilon(t)) \approx k''_{\mathrm{MS}}(\bar{V})\left(1 - \frac{\epsilon(t) z e_0}{2kT}\right). \tag{C13}$$

Therefore the δ-shaped voltage pulse is connected with a short flux pulse

$$\frac{1}{2}\frac{z e_0}{kT}\,\delta(t)\,(k'_{\mathrm{MS}}\bar{N}'_{\mathrm{MS}} + k''_{\mathrm{MS}}\bar{N}''_{\mathrm{MS}}), \tag{C14}$$

which generates a disturbance of the concentrations N'_{MS} and N''_{MS} at $t = 0$:

$$N'_{\mathrm{MS}}(0) - \bar{N}'_{\mathrm{MS}} = -\frac{1}{2}\frac{\epsilon_0 z e_0}{kT}(k'_{\mathrm{MS}}\bar{N}'_{\mathrm{MS}} + k''_{\mathrm{MS}}\bar{N}''_{\mathrm{MS}}),$$

$$N''_{\mathrm{MS}}(0) - \bar{N}''_{\mathrm{MS}} = -(N'_{\mathrm{MS}}(0) - \bar{N}'_{\mathrm{MS}}). \tag{C15}$$

Because k_{R}, k_{D} and k_{S} are assumed to be voltage independent, N'_{S} and N''_{S} are not disturbed by a macroscopic voltage pulse.

Using eq. (C12) and the general solution for $\Phi(t)$ (eqs. (A18)–(A25)) and the eq. (C15) one obtains for time $t \geqslant 0$ by elementary calculations, whereby the contribution of the membrane capacitance has been omitted, since the capacitance only influences the imaginary part of the admittance, the relation:

$$j(t \geqslant 0) = \frac{z^2 e_0^2}{2kT}(k'_{\mathrm{MS}}\overline{N'_{\mathrm{MS}}} + k''_{\mathrm{MS}}\overline{N''_{\mathrm{MS}}})$$

$$\times\left(\delta(t) + \sum_{l=1}^{3}\beta_l\,\mathrm{e}^{-t/\tau_l}\right), \tag{C16}$$

$$\beta_1 = +(k'_{\mathrm{MS}} + k''_{\mathrm{MS}})(P - \sqrt{a})/2\sqrt{a}, \tag{C17}$$

$$\beta_2 = -(k'_{\mathrm{MS}} + k''_{\mathrm{MS}})(P + \sqrt{a})/2\sqrt{a}, \tag{C18}$$

$$\beta_3 = 0. \tag{C19}$$

For the real part $\mathrm{Re}[Y(f)]$ of the admittance it follows from equation (C10):

$$\mathrm{Re}\,Y(f) = \int_{-\infty}^{\infty} j(t)\cos 2\pi f t\,\mathrm{d}t = \frac{z^2 e_0^2}{2kT}$$

$$\times(k'_{\mathrm{MS}}\overline{N'_{\mathrm{MS}}} + k''_{\mathrm{MS}}\overline{N''_{\mathrm{MS}}})\left(1 + \sum_{i=1}^{3}\frac{\beta_i\tau_i}{1 + (2\pi f\tau_i)^2}\right). \tag{C20}$$

Appendix D

Derivation of eq. (D1)

Using eq. (C20) the spectral intensity given by eq. (3) may be written in the following form

$$S_J(f) = 4kT\,\mathrm{Re}[Y(f)] + \bar{J}^2 \sum_{i=1}^{3} \frac{\gamma_i \tau_i}{1 + (2\pi f \tau_i)^2}, \qquad \text{(D1)}$$

where the mean macroscopic current $\bar{J}$ is given by eq. (13). For the amplitudes γ_i $(i = 1, 2, 3)$ the relations hold:

$$\gamma_1 = -\frac{1}{z^2 e_0^2 N_0} \left\{ \frac{1}{2} \frac{(k_\mathrm{R} C_\mathrm{M} + k_\mathrm{D})}{k_\mathrm{R} C_\mathrm{M} k_\mathrm{D} k_\mathrm{S}} \det \mathrm{A} \frac{(P - \sqrt{a})}{2\sqrt{a}} - \frac{(Q - \sqrt{a})}{2\sqrt{a}} + \frac{B}{2\sqrt{a}} \left(\frac{1}{\tau_2} - \frac{1}{\tau_3} \right) \right\}, \qquad \text{(D2)}$$

$$\gamma_2 = \frac{1}{z^2 e_0^2 N_0} \left\{ \frac{1}{2} \frac{(k_\mathrm{R} C_\mathrm{M} + k_\mathrm{D})}{k_\mathrm{R} C_\mathrm{M} k_\mathrm{D} k_\mathrm{S}} \det \mathrm{A} \frac{(P + \sqrt{a})}{2\sqrt{a}} - \frac{Q + \sqrt{a}}{2\sqrt{a}} + \frac{B}{2\sqrt{a}} \left(\frac{1}{\tau_1} - \frac{1}{\tau_2} \right) \right\}, \qquad \text{(D3)}$$

$$\gamma_3 = -B/z^2 e_0^2 N_0. \qquad \text{(D4)}$$

The quantities Q, P, a, $\det \mathrm{A}$, B are given by eqs. (8)–(12) respectively, and τ_i $(i = 1, 2, 3)$ by eq. (4).

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