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Correlation Analysis of Electrical Noise in Lipid Bilayer Membranes: Kinetics of Gramicidin A Channels

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Summary. If a membrane contains ion-conducting channels which form and disappear in a random fashion, an electric current which is passed through the membrane under constant voltage shows statistical fluctuations. Information on the kinetics of channel formation and on the conductance of the single channel may be obtained by analyzing the electrical noise generated in a membrane containing a great number of channels. For this purpose the autocorrelation function of the current noise is measured at different concentrations of the channel-forming substance. As a test system for the application of this technique we have used lipid bilayer membranes doped with gramicidin A. From the correlation time of the current noise generated by the membrane, the rate constants of formation (k_R) and dissociation (k_D) of the channels could be determined. In addition, the mean square of the current fluctuations yielded the single-channel conductance Λ. The values of k_R, k_D, and Λ obtained from the noise analysis agreed closely with the values determined by relaxation measurments and single-channel experiments.

Fast chemical reactions in solution and fast transport processes in membranes have been studied in the past mainly by relaxation techniques. With this method the system is disturbed by a sudden change of an external parameter such as temperature or electric field strength and the time evolution towards a new stationary state is followed. An entirely different method for the elucidation of kinetic parameters is based on the principle that any reacting system exhibits statistical fluctuations around the stationary state. As the decay of a spontaneous fluctuation follows (on the average) the same time law as the relaxation from a macroscopic perturbation, both methods yield essentially the same information. The fluctuation method has the advantage that the measurement is carried out while the system is in an equilibrium or stationary state. Moreover, the relaxation technique

sometimes fails in cases where it is difficult to achieve a sufficient perturbation of the system; this especially applies to experiments with membranes. The main reason why the fluctuation analysis has not been developed until recently is the technical problem of measuring small fluctuations and of separating the different possible sources of such fluctuations. These difficulties now are partially overcome by recent developments in instrumentation which make the analysis of fluctuations easier than before. The first attempts to measure concentration fluctuations in a chemically reacting mixture by light-scattering experiments have been reported by Yeh and Keeler (1969). Later, a more sensitive method for the study of concentration fluctuations was described which is based on fluorescence measurements (Magde, Elson & Webb, 1972; Elson & Magde, 1974; Magde, Elson & Webb, 1974); from the autocorrelation of the fluorescence fluctuations these authors were able to separate the diffusional from the chemical part of the concentration fluctuations and to calculate the rate constants of the chemical reaction. Recently, Feher and Weissman (1973) measured conductance fluctuations of an aqueous electrolyte solution and obtained the relaxation time of the association-dissociation equilibrium of the electrolyte from the frequency spectrum of the fluctuations.

The possibility to obtain information on ion transport mechanisms in nerve membranes from noise measurements has prompted a number of experimental and theoretical studies in recent years (Derksen, 1965; Verveen & Derksen, 1968; Lecar & Nossal, 1971*a*, *b*; Poussart, 1971; Hill & Chen, 1972; Stevens, 1972; Fishman, 1973; Siebenga, Meyer & Verveen, 1973; Wanke, De Felice & Conti, 1974). Similar experiments have also been done with cholinergic synapses (Katz & Miledi, 1972, 1973; Anderson & Stevens, 1973) and with a membrane preparation involved in active transport of ions (Segal, 1972; Fishman & Dorset, 1973). Electrical noise from artificial porous membranes has been studied by Green and Yafuso (1968), De Felice and Michalides (1972), and Michalides, Wallaart and De Felice (1973).

In this paper we describe the application of the fluctuation technique to the kinetics of channel formation in gramicidin A-doped lipid bilayer membranes; similar experiments have been carried out independently by Zingsheim and Neher (1974) and by G. Szabo *(personal communication)*. The linear pentadecapeptide gramicidin A increases the cation permeability of biological membranes and artificial lipid bilayer membranes (for a survey of the literature, *see* Bamberg & Läuger, 1973). There is strong evidence that gramicidin A does not operate by a carrier mechanism but creates pore-like channels in the lipid membrane (Hladky & Haydon, 1970, 1972; Urry, 1971; Krasne, Eisenman & Szabo, 1971). Specifically, Urry (1971)

proposed that the channel consists of a dimer which is formed by head-to-head association of two gramicidin molecules in the membrane. This model is supported by the finding that the covalently linked dimer acts in the same way on lipid bilayers as the monomeric gramicidin A, but at much lower concentrations (Urry, Goodall, Glickson & Mayers, 1971; Goodall, 1973), and it is further supported by the results of electrical relaxation experiments which are in accordance with the second-order kinetics of channel formation required by the dimer model (Bamberg & Läuger, 1973).

The dimerization model of the gramicidin channel implies that an equilibrium exists in the membrane between nonconducting monomers and conducting dimers. But even in the equilibrium state channels form and disappear in a random fashion, and therefore the membrane conductance should exhibit statistical fluctuations. Indeed, at very low levels of gramicidin concentrations the single conductance steps corresponding to the opening and closing of single channels can be resolved (Hladky & Haydon, 1970, 1972; Bamberg & Läuger, 1974). From these measurements the mean lifetime of the channel may be obtained, which is equal to the reciprocal of the dissociation rate constant k_D of the dimer. It is not possible, however, to obtain also the association rate constant k_R of the reaction from single-channel fluctuation experiments. As we shall show in this paper, a more complete kinetic analysis of the dimerization reaction, yielding both k_D and k_R, may be carried out by measuring the fluctuations of the macroscopic membrane conductance at different gramicidin concentrations. The main difference, therefore, between the single-channel experiments and the study of "macroscopic" conductance fluctuations is that in the latter case, additional information may be obtained from the variation of a further experimental parameter, the gramicidin concentration in the membrane. In this respect the noise analysis is equivalent to the relaxation method.

The electrical noise generated in the membrane from fluctuations in the number of conducting channels may be analyzed in either of two ways. With the first method the power-density spectrum is measured by taking the Fourier transform of the fluctuating component of the membrane current. The power spectrum may then be analyzed in terms of the kinetic parameters of the channel-forming process. In our experiments we used the second method which is based on the measurement of the autocorrelation function of the random signal. The autocorrelation function is related in a straightforeward way to the macroscopic relaxation time and thus to the rate of opening and closing of channels. Both methods are connected by the Wiener-Khintchine theorem (*see* Kittel, 1958; Van der Ziel, 1970) and yield, in principle, the same information.

Theory

According to the dimer model the opening and closing of gramicidin A channels may be described as an association-dissociation reaction:

$$G+G \underset{k_D}{\overset{k_R}{\rightleftarrows}} G_2. \tag{1}$$

If N_m and N_d are the mean concentrations of monomers and dimers, respectively, of gramicidin in the membrane (expressed in moles/cm^2), then the rate of change of N_d with time t is described by the rate constants of association (k_R) and dissociation (k_D):

$$\frac{dN_d}{dt} = k_R N_m^2 - k_D N_d. \tag{2}$$

Under equilibrium conditions ($dN_d/dt = 0$) the concentrations N_m and N_d are given by

$$\frac{N_d}{N_m^2} = \frac{k_R}{k_D} = K \tag{3}$$

where K is the equilibrium constant. If a small perturbation is applied to the system at constant total gramicidin concentration $N = N_m + 2N_d$, the dimer concentration $N_d(t)$ approaches a new equilibrium value N_d^∞ according to (Bamberg & Läuger, 1973):

$$N_d(t) = N_d^\infty + (N_d^0 - N_d^\infty)\, e^{-t/\tau_c} \tag{4}$$

where N_d^0 is the initial dimer concentration. The chemical relaxation time τ_c is given by

$$\frac{1}{\tau_c} = k_D + 4 k_R N_m^\infty. \tag{5}$$

It is convenient to express N_m^∞ in Eq. (5) by the mean macroscopic membrane conductance λ. If Λ is the conductance of the single channel and L Avogadro's number,

$$\lambda = L N_d \Lambda. \tag{6}$$

Using Eq. (3), Eq. (5) may be written in the form

$$\frac{1}{\tau_c} = k_D + 4\sqrt{\frac{k_R k_D \lambda}{L\Lambda}}. \tag{7}$$

For the following analysis of conductance fluctuations we introduce the assumption that the total number n of gramicidin molecules which are present in the membrane of area A,

$$n = ALN = AL(N_m + 2N_d) \tag{8}$$

may be regarded as a constant within the time scale of the fluctuation experiment. In other words, we assume that the exchange of gramicidin between water and membrane as well as between membrane and the surrounding lipid torus is slow compared with the lifetime of a "chemical" fluctuation. This assumption is in accordance with previous results of relaxation studies (Bamberg & Läuger, 1973), and is also supported by the experimental data presented below (*see* Appendix A).

Both the number of monomers n_m and the number of dimers n_d exhibit random fluctuations in time t around mean values $\bar{n}_m$ and $\bar{n}_d$. The random function $n_d(t)$ may be written as

$$n_d(t) = \bar{n}_d + \delta n_d(t) \tag{9}$$

where $\delta n_d(t)$ is the fluctuating part of $n_d(t)$. As n is assumed to be constant, the membrane may be considered as a closed isothermal system. The mean square of the fluctuation of n_d may then be obtained from the statistics of the canonical ensemble (compare Appendix B):

$$\overline{(\delta n_d)^2} = \frac{\bar{n}_d}{1 + 4\bar{n}_d/\bar{n}_m}. \tag{10}$$

As the electrical conductance of the undoped bilayer is negligible, the current J through the membrane at a given voltage V is proportional to the number n_d of dimers:

$$\begin{gathered} J(t) = \bar{J} + \delta J(t) \\ \bar{J} = A V \bar{n}_d; \quad \delta J = A V \delta n_d. \end{gathered} \tag{11}$$

From Eqs. (3), (6), (10) and (11) and using $\bar{n}_m = ALN_m$, $\bar{n}_d = ALN_d$, the mean square $\overline{(\delta J)^2}$ of the current fluctuation is obtained in the form

$$\frac{\overline{(\delta J)^2}}{\bar{J}^2} = \frac{A/\lambda A}{1 + 4\sqrt{K\lambda/LA}}. \tag{12}$$

If the membrane contains many dimers, the relative fluctuations of n_d become small according to Eq. (10). In this case the probability $P(\delta n_d)$ of a

fluctuation of magnitude δn_d may be approximated by a normal distribution:

$$P(\delta n_d)=\frac{1}{\sqrt{2\pi\overline{(\delta n_d)^2}}}\exp\left[-\frac{(\delta n_d)^2}{2\overline{(\delta n_d)^2}}\right]. \tag{13}$$

As $\delta J=\Lambda V\delta n_d$, the probability of observing a current fluctuation δJ is given by

$$P(\delta J)=\frac{\Lambda V}{\sqrt{2\pi\overline{(\delta J)^2}}}\exp\left[-\frac{(\delta J)^2}{2\overline{(\delta J)^2}}\right]. \tag{14}$$

The autocorrelation function $C(\tau)$ of $\delta J(t)$ is defined as the time average of the product of the current fluctuation at time t multiplied by the current fluctuation at a later time $t+\tau$:

$$C(\tau)=\overline{\delta J(t)\cdot\delta J(t+\tau)}. \tag{15}$$

Using the fundamental statistical assumption (the so-called fluctuation-dissipation theorem) that a fluctuation decays (on the average) with the same time course as an external perturbation (Onsager, 1931; Kubo, 1957), the autocorrelation function is obtained in the form

$$C(\tau)=\overline{(\delta J)^2}\,e^{-\tau/\tau_c}. \tag{16}$$

τ_c is the chemical relaxation time of the dimerization reaction and is given by Eq. (7).

The main results of the theory are contained in Eqs. (12) and (16). As the autocorrelation function $C(\tau)$ is directly measurable, a plot of log $C(\tau)$ versus τ gives the chemical relaxation time τ_c. By measuring τ_c at different gramicidin concentrations and plotting $1/\tau_c$ as a function of $\sqrt{\lambda}$, k_R/Λ and k_D may be obtained according to Eq. (7). The initial value of $C(\tau)$ at $\tau=0$ yields the mean square of the current fluctuation $\overline{(\delta J)^2}$. From $\overline{(\delta J)^2}/\bar{J}^2$, the single-channel conductance Λ may be calculated using Eq. (12) at low values of λ where the square root in the denominator may be neglected. Thus, a measurement of $C(\tau)$ at different gramicidin concentrations allows to evaluate the three parameters Λ, k_R and k_D. On the other hand, if Λ is already known from single-channel experiments, Eq. (12) may be used as a consistency check of the method. An additional check may be performed by measuring the probability density $P(\delta J)$ of the current fluctuations [Eq. (14)].

Materials and Methods

The block diagram of the set-up used for the fluctuation analysis is shown in Fig. 1. The voltage was applied to the membrane from a battery-operated voltage source through Ag—AgCl electrodes. The membrane cell together with the preamplifier (Analog Devices Model 42 K) were contained in a completely closed metal box which acts as an electric shield. In addition, the membrane cell was shielded from mechanical vibrations by mounting the metal box on a large stone slab which in turn was mounted on an inflated automobile innertube (Bamberg & Läuger, 1974). The output of the preamplifier which consists of a d-c voltage with a superimposed fluctuating voltage was delivered to the main amplifier (Princeton Applied Research Model 113). This amplifier was used in the a-c-coupled mode with a lower cut-off frequency of 0.03 Hz; in this way only the fluctuating component of the voltage was amplified. The upper cut-off frequency of the PAR 113 was usually set at 100 Hz. It was checked that a change in the filter position to 0.1 Hz (lower cut-off) and to 30 or 300 Hz (upper cut-off) had no influence on the results. The output signal of the amplifier was stored with a magnetic-tape recorder (Precision Instruments Model 6200).

The stored signal was analyzed with a correlator (Saicor-Honeywell SAI-43 A). This instrument automatically computes the autocorrelation function of a given signal in the following way. The signal $S(t)$ is fed into two identical channels, A and B. A sample of the signal in channel A is taken at time $t=0$ and multiplied with 400 samples of the signal in channel B taken at times $t=0$, $t=t_i$, $t=2t_i$, $t=3t_i$, ..., $t=399t_i$. This procedure is then repeated with a second sample from channel A and so forth. In this way the time average of the function $S(t)\cdot S(t+\tau)$ between $\tau=0$ and $\tau=399t_i$ is calculated by averaging over the different sets of multiplications. Depending on the relaxation time τ_c which ranged between 0.2 and 0.6 sec in the experiments with gramicidin A, the time increment t_i was chosen to be between 2 and 10 msec. The choice of the averaging time t_a depends on the intended accuracy of the autocorrelation function. With increasing t_a the smoothness of the autocorrelation function improves; on the other hand, long records of the membrane current are subjected to the drift of the mean membrane conductance (*see below*). The actual averaging time was a compromise and ranged between 1 and 5 min. The computed autocorrelation function was recorded with an x-y recorder (Hewlett Packard Model 7000 AM).

The probability density of the current fluctuations δJ was also measured with the Saicor SAI-43 A. In this case the instrument samples and digitizes the signal $\delta J(t)$ at regular time-intervals. For each digitized amplitude one count is inserted into one of 400 registers corresponding to its amplitude.

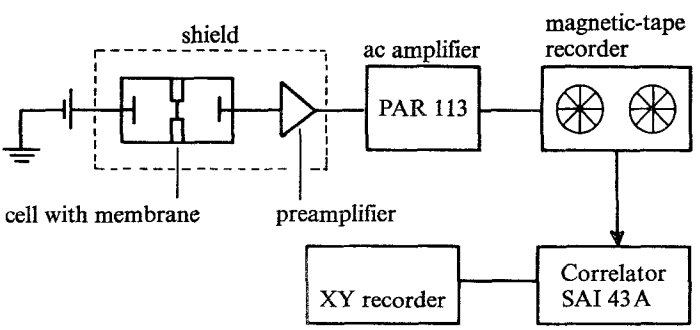

Fig. 1. Block diagram of the set-up used for correlation analysis of current fluctuations

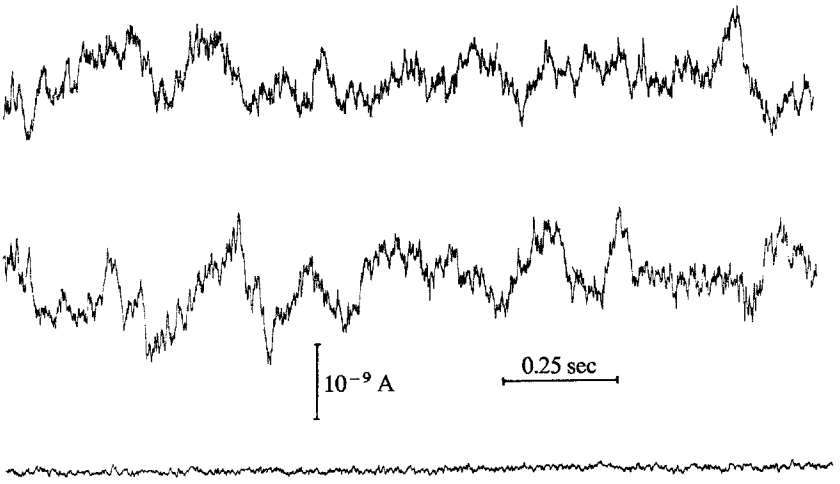

Fig. 2. Current noise generated by a dioleoyllecithin membrane in the presence of gramicidin A (upper traces). The mean current $\bar{J}$ was 3.9×10^{-7} amps, corresponding to a mean membrane conductance $\lambda=3.1\times10^{-3}\ \Omega^{-1}\,\mathrm{cm}^{-2}$ (membrane area $A=6.8\times10^{-3}$ cm^2, external voltage $V=18.5$ mV). The aqueous phase contained 1 M NaCl. The lower trace represents a control experiment in which the noise was recorded in the same way as above but from a gramicidin-free membrane with an external resistor of 47 kΩ simulating the gramicidin-induced conductance

The proper functioning of the electronic system was tested in the following way. For a simulation of the fluctuating membrane current a d-c current with superimposed small a-c current was applied to the input of the preamplifier. All other components of the measuring system were used in the same way as in the membrane experiments. Under these circumstances, the autocorrelation function (after decoupling of the d-c component of the current) must again be a sinusoidal function (*see below*). This could indeed be verified for ratios between d-c and a-c current amplitudes of at least 10^3.

Furthermore, control experiments were carried out in which the lipid membrane was formed in the usual way, but without adding gramicidin. Instead, the gramicidin conductance was simulated by placing a resistor of the appropriate magnitude in parallel to the membrane. A voltage was applied and the current through the parallel combination of membrane and external resistor was amplified as in the experiment in the presence of gramicidin. In this case the output of the PAR amplifier appeared as an almost smooth trace on the oscilloscope, with fluctuations of much smaller amplitude than observed in the experiment with gramicidin. An example of such a record is shown in Fig. 2. This experiment demonstrates that noise originating from the electrodes, from capacitance fluctuations of the membrane, or from sources in the electronic system, does not contribute appreciably to the membrane noise observed in the presence of gramicidin.

The precise calibration of the output of the correlator (i.e., the scale factor converting the output voltage into the absolute value of the autocorrelation function) is important for the determination of $\overline{(\delta J)^2}=C(0)$ [compare Eq. (16)]. The calibration was checked by applying a sinusoidal voltage

$$V(t)=V_0\sin\omega t$$

of known amplitude V_0 to the imput of the correlator. The correlation function $C(\tau)$ of $V(t)$ is then given by

$$C(\tau) = \lim_{T\to\infty} \frac{1}{T} \int_0^\infty V_0 \sin \omega t \cdot V_0 \sin \omega(t+\tau)\, dt$$
$$= \frac{V_0^2}{2} \cos \omega \tau. \tag{17}$$

Thus, the scale factor is obtained by comparing the amplitude of the computed autocorrelation function with the numerical value of $V_0^2/2$.

Optically black lipid membranes were formed from a solution of 1 % (w/v) dioleoyllecithin in *n*-decane as described previously (Läuger, Lesslauer, Marti & Richter, 1967). The purity of the dioleoyllecithin (Supelco, Inc., Bellafonte, Pa.) was checked by thin-layer chromatography. The sample of gramicidin A was the same as used for the relaxation experiments (Bamberg & Läuger, 1973). Gramicidin was added from a methanolic stock-solution to the aqueous phase which contained 1 M NaCl in all experiments. The aqueous gramicidin concentrations ranged between about 10^{-10} M and 5×10^{-8} M, corresponding to a range of membrane conductance λ between 2×10^{-5} and 5×10^{-2} $\Omega^{-1}\,cm^{-2}$. The final concentration of methanol in the aqueous solution was always less than 1 %; it was checked that this concentration has no influence on the electrical properties of the membrane. In some experiments gramicidin A was added also to the lipid solution in order to shorten the time required for the establishment of a stable membrane conductance. The membrane cell was thermostated at 25 °C. The hole in the teflon wall on which the membrane was formed had a diameter of 1.0 mm. The actual area of the black film was determined by measuring the electrical capacitance of the film. This was done by applying a square-wave signal to the membrane through a resistance of known magnitude and measuring the decay-time of the capacitive current. For the specific capacitance of the membrane a value of 0.38 μF/cm² (Stark, Benz, Pohl & Janko, 1972) was assumed.

After the membrane had turned completely black, a voltage was applied and the mean membrane current $\bar{J}$ was continuously measured. Usually a drift in the membrane conductance was observed in the first half hour, which presumably originated from the slow equilibration of gramicidin between water and the membrane. The record of the current fluctuations was started only after the drift of the conductance became less than 5 % within 5 min. The mean current was checked from time to time during the experiment and at the end of the record.

Results

The general appearance of current noise generated by a gramicidin-doped membrane is shown in Fig. 2. This picture has been obtained by recording the output of the PAR 113 amplifier on magnetic tape and by writing out the content of the tape at a reduced speed (1:100) on an ordinary paper recorder. An example of the autocorrelation function $C(\tau)$ of the current noise is represented in Fig. 3. By plotting $[\log C(\tau)/C(0)]$ versus time τ, a straight line is obtained (Fig. 4), indicating that the autocorrelation function is characterized by a single time-constant.

This behavior which is predicted from the dimer model [compare Eq. (16)] was observed in the whole range of membrane conductance λ.

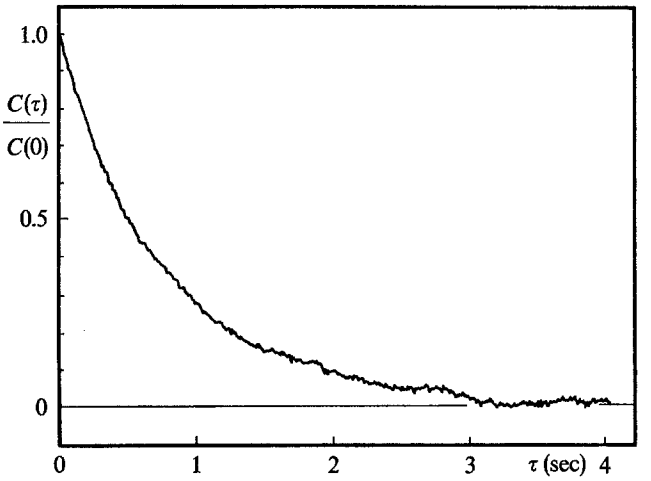

Fig. 3. Autocorrelation function $C(\tau)$ of the current fluctuations, divided by the initial value $C(0)=\overline{(\delta J)^2}\simeq 7.8\times 10^{-22}$ amps^2 versus time τ. The mean membrane conductance was $\lambda=5.4\times 10^{-5}\ \Omega^{-1}\,\mathrm{cm}^{-2}$. Membrane area $A=7.0\times 10^{-3}\,\mathrm{cm}^2$, external voltage $V=18.5$ mV. The aqueous phase contained 1 M NaCl

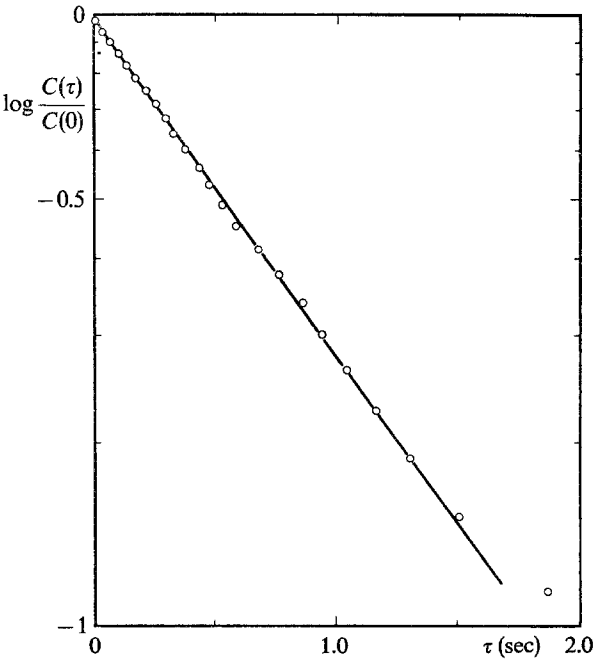

Fig. 4. Logarithmic plot of $C(\tau)/C(0)$, as taken from Fig. 3, versus time. The slope of the straight line corresponds to a relaxation time of $\tau_c=0.6$ sec [compare Eq. (16)]

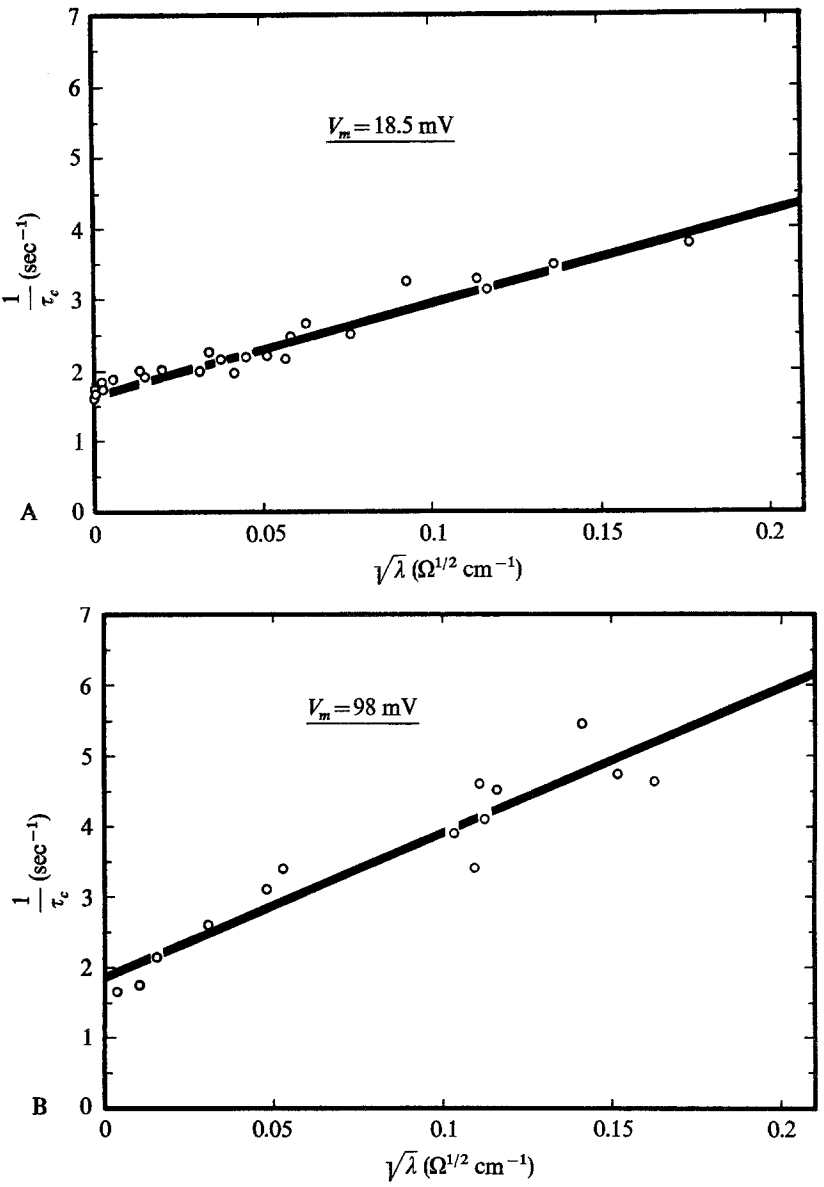

Fig. 5. Reciprocal of the time constant τ_c of the autocorrelation function $C(\tau)$, plotted as a function of the square root of the mean membrane conductance λ for two different external voltages V_m (18.5 and 98 mV). Each point has been obtained from a different membrane. 1 M NaCl, 25 °C

In some experiments $C(\tau)$ did not decay to zero at long times τ but showed a finite (positive or negative) offset. This offset was always associated with a drift of the d-c current, i.e., a drift of the mean membrane conductance. Experiments which showed such an offset were discarded.

Table 1. Kinetic parameters of the gramicidin A channel

Method	k_R, 18.5 mV ($cm^2\,mole^{-1}\,sec^{-1}$)	k_R, 98 mV ($cm^2\,mole^{-1}\,sec^{-1}$)	k_D, 18.5 mV (sec^{-1})	k_D, 98 mV (sec^{-1})	Λ (Ω^{-1})
Noise analysis	$0.62 \cdot 10^{14}$	$1.4 \cdot 10^{14}$	1.7	1.9	$0.9 \cdot 10^{-11}$
Relaxation method	$1.3 \cdot 10^{14}$	$1.5 \cdot 10^{14}$	1.6	1.6	–
Single-channel experiments	–	–	0.91	0.91	$1.2 \cdot 10^{-11}$

Dioleoyllecithin in *n*-decane, 1 M NaCl, 25 °C. k_R and k_D are the rate constants of formation and dissociation of the dimer, respectively; Λ is the conductance of the single channel. For comparison, the channel parameters determined from relaxation and single-channel experiments (Bamberg & Läuger, 1974) are also listed. The values of k_R obtained with the relaxation method have been approximately corrected to the voltages applied in the noise measurements (18.5 and 98 mV) using the observed voltage-dependence of k_R (Bamberg & Läuger, 1973); in case of k_D and Λ the correction was negligible.

The autocorrelation function $C(\tau)$ was measured at different gramicidin concentrations; in each case the time constant τ_c of Eq. (16) was determined from the plot of $\log [C(\tau)/C(0)]$ versus τ. If $1/\tau_c$ is plotted as a function of the square root of the mean membrane conductance λ, a straight line is obtained within the limits of error (Fig. 5). This result is in agreement with Eq. (7). The same linear relationship between $1/\tau_c$ and $\sqrt{\lambda}$ has already been found from electrical relaxation experiments (Bamberg & Läuger, 1973, 1974).

According to Eq. (7) the rate constants k_R and k_D are obtained from the plot of $1/\tau_c$ versus $\sqrt{\lambda}$. k_D is equal to the intercept of the straight line with the $1/\tau_c$ axis. Similarly, k_R may be determined from the slope of the straight line. For the single-channel conductance Λ which is needed for the calculation of k_R from Eq. (7) we have used the value $\Lambda = 1.2 \times 10^{-11}\,\Omega^{-1}$ as determined from relaxation experiments (Bamberg & Läuger, 1973). The values of k_R and k_D obtained in this way are listed in Table 1. For comparison, the table also contains the results of the previous relaxation experiments and single-channel measurements (Bamberg & Läuger, 1974). It is seen that the values of k_R and k_D determined by the different methods agree within a factor of about two.

The mean square $\overline{(\delta J)^2}$ of the current fluctuations was obtained from the initial value $C(0)$ of the autocorrelation function. From Eq. (12) it is seen that at low values of the membrane conductance λ where $\sqrt{K\lambda/L\Lambda} \ll 1$, the approximate relation

$$\frac{\overline{(\delta J)^2}}{\bar{J}^2} \approx \frac{\Lambda}{A\lambda}$$

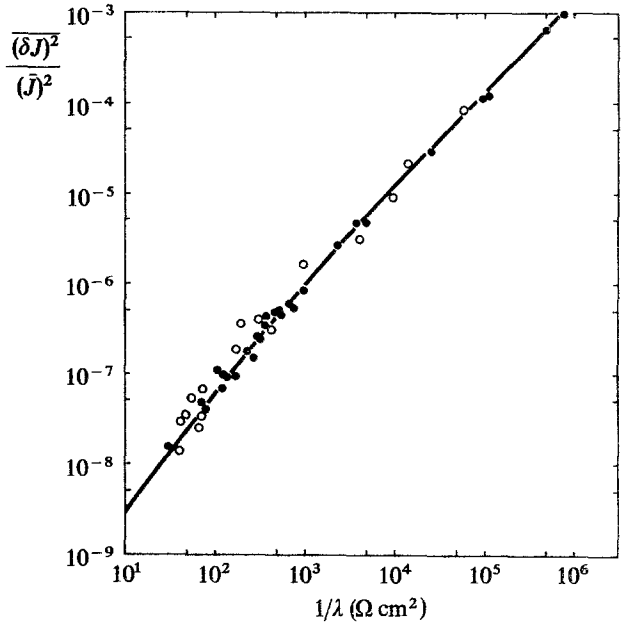

Fig. 6. $\overline{(\delta J)^2}/\bar{J}^2$ as a function of the reciprocal of the mean membrane conductance λ. Filled circles: $V=18.5$ mV; open circles: $V=98$ mV. The theoretical curve has been drawn according to Eq. (12) with $A=0.9\times10^{-11}\ \Omega^{-1}$ and a mean membrane area of $A=6.8\times10^{-3}$ cm^2. For the equilibrium constant $K=k_R/k_D$ the mean of the values at $V=18.5$ mV and $V=98$ mV given in Table 1 has been used ($K=5.5\times10^{13}$ cm^2 mole^{-1}). 1 M NaCl, 25 °C

should hold. Therefore, if the logarithm of the ratio $\overline{(\delta J)^2}/\bar{J}^2$ is plotted as a function of $\log(1/\lambda)$, a straight line with slope 1 should result at low λ. As shown in Fig. 6, this is indeed the case. The theoretical curve in Fig. 6 was drawn using a value of $A=0.9\times10^{-11}\ \Omega^{-1}$ for the single-channel conductance which gave the best fit of the experimental points. This value approximately agrees with $A=1.2\times10^{-11}\ \Omega^{-1}$ determined from single-channel experiments at very low gramicidin concentrations (Bamberg & Läuger, 1974). This agreement represents a test of the internal consistency of the noise analysis. Furthermore, it shows that the correlation technique is capable of yielding information not only on the kinetics of channel formation but also on the magnitude of the single-channel conductance.

In a few cases, the probability density $P(\delta J)$ of the current fluctuations has been calculated from the record of $J(t)$ as described in the experimental section. An example is shown in Fig. 7 where the experimental function $P(\delta J)/P(0)$, as obtained from the Saicor analyzer is plotted versus δJ. In addition to the experimental values the theoretical curve is also represented

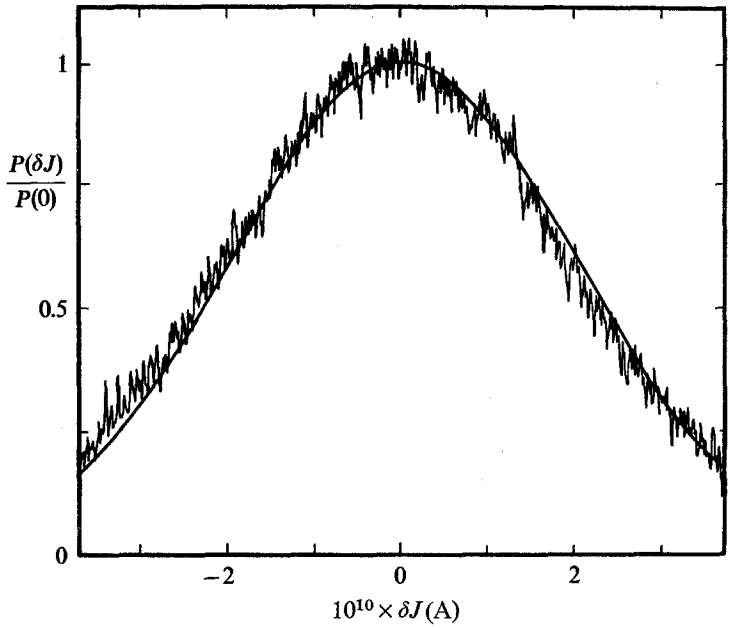

Fig. 7. Probability density $P(\delta J)/P(0)$ of the current fluctuations δJ. The theoretical curve has been calculated from Eq. (14) with $\overline{(\delta J)^2} = 3.8 \times 10^{-20}$ A^2, $V = 18.5$ mV, $\lambda = 1.8 \times 10^{-3}$ Ω^{-1} cm^{-2}. The aqueous phase contained 1 M NaCl; $T = 25$ °C

which has been calculated from Eq. (14) using the $\overline{(\delta J)^2}$ value which gave the best fit at $\delta J = 0$. It is seen that the observed probability density closely approximates a normal distribution, as expected from Eq. (14).

Conclusion

The aim of this paper was to demonstrate that information about the kinetics of transport processes in membranes may be obtained from the analysis of the electrical noise associated with current flow through the membrane. In order to test this new method, we have chosen the gramicidin system because the kinetic parameters of the gramicidin A channel have already been studied by the electrical relaxation method and by single-channel experiments. As shown here, the rate constants of formation and dissociation of the channel, as determined from the noise analysis agree fairly well with the results of relaxation experiments. Furthermore, the information contained in the mean square of the current fluctuations may be used to calculate the conductance Λ of the single channel.

A principal difficulty in the application of noise analysis comes from the fact that the observed electrical noise usually originated from several different mechanisms. This leads to the problem of separating the noise associated with the process under study from other noise sources. For the gramicidin system this subject is discussed in some detail in Appendix A. It is shown there that the other noise components either have a negligible amplitude compared with noise from the dimerization reaction or may be distinguished by their different frequency characteristics.

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Appendix A

Other Noise Sources

In the following we consider only noise which is inherent in the membrane-solution system, i.e., we do not take into account here noise from the electronic circuit itself. A check for noise generated in the electronic circuit has been described in the experimental section.

(a) Johnson Noise. Johnson, or thermal, noise originates from the thermal agitation of charge carriers in a conductor (Johnson, 1928; Nyquist, 1928). If ions move through a channel, the mean current $\bar{J}$ is superimposed by a fluctuating component $\delta J(t)$ which reflects fluctuations of the velocity of ions. If the circuit element under consideration has an impedance Z, then the mean-square value of δJ which is measured in the frequency interval Δf is given by Nyquist's theorem:

$$\overline{(\delta J)^2_{\text{Johnson}}} = 4kT \,\text{Re}\left[\frac{1}{Z(f)}\right] \Delta f \tag{A.1}$$

where k is Boltzmann's constant, T the absolute temperature, and Re means "real part of". If one neglects the electrolyte resistance which usually is small compared with the membrane impedance, then Z is determined by the parallel combination of the membrane resistance R_m and the membrane capacitance C_m, so that

$$\overline{(\delta J)^2_{\text{Johnson}}} = \frac{4kT}{R_m} \Delta f. \tag{A.2}$$

With $R_m = 1/A\lambda$, $\bar{J} = A\lambda V$, and using Eq. (12), one obtains

$$\alpha^2 \equiv \frac{\overline{(\delta J)^2_{\text{Johnson}}}}{\overline{(\delta J)^2_{\text{channel}}}} = 4kT\Delta f \frac{1+4\sqrt{K\lambda/LA}}{AV^2}. \tag{A.3}$$

For a numerical estimate of α we introduce the values $K = k_R/k_D = 4 \times 10^{13}$ cm^2 mole^{-1} and $\Lambda = 0.9 \times 10^{-11}\ \Omega^{-1}$ obtained from the noise analysis (Table 1). Furthermore, as typical experimental parameters we use $\Delta f = 100$ Hz, $\lambda = 10^{-2}\ \Omega^{-1}$ cm^{-2}, $V = 20$ mV, $T = 298$ °K. This gives $\alpha \simeq 0.06$. Thus, the contribution of Johnson noise to the total current noise is rather small and can be neglected here. As seen from Eq. (A.3), α may be reduced further by increasing the voltage V. In the autocorrelation function $C(\tau)$ the Johnson noise manifests itself as a fast-decaying spike near $\tau = 0$. So, even if Johnson noise would contribute appreciably to the total value of $\overline{(\delta J)^2}$, $\overline{(\delta J)^2}_{\text{channel}}$ could be obtained by extrapolation to $\tau = 0$.

(b) Shot Noise from Fluctuations in the Occupancy of Channels. As Hladky and Haydon (1972) have pointed out, a single gramicidin channel is likely to contain no more than one ion at a time. Because ions enter the channel in a random fashion, the current through an assembly of channels should exhibit fluctuations of the shot-noise type (Stevens, 1972). The autocorrelation function of shot noise is of the form

$$C(\tau) = \text{const.} \cdot e^{-\tau/\tau_s} \tag{A.4}$$

where the correlation time τ_s is of the order of the mean transit time $\bar{t}$ of the ion in the channel. As $\bar{t}$ is likely to be shorter than 10^{-7} sec for a small cation in the gramicidin channel (Läuger, 1973), the shot noise makes a contribution to the overall autocorrelation function only at very short times τ which could not be resolved under the conditions of our experiments.

(c) Partition Noise. With this term we describe noise originating from fluctuations in the total concentration N of gramicidin in the bilayer. Fluctuations of N arise from the random partition of gramicidin between water and membrane and between torus and membrane. The correlation time τ_e for the exchange between membrane and torus may be estimated to be of the order of a^2/D, where a is the radius of the circular membrane and D the diffusion coefficient of gramicidin in the plane of the membrane. As an approximate upper limit of D we may use the diffusion coefficient of a lipid molecule in a bilayer for which Träuble and Sackmann (1972) have determined a value of about 10^{-8} cm^2 sec^{-1}. With $a = 0.5$ mm one finds $\tau_e \simeq 3 \times 10^5$ sec. This value is outside the experimental frequency range (actually, the exchange between torus and membrane is likely to be determined by slow convections in the film).

For an analysis of the exchange between membrane and water we make the assumption that the aqueous phase acts as a virtually infinite reservoir in which the diffusion of gramicidin is sufficiently fast so that the gramicidin

concentration c at the membrane surface is constant. Furthermore, we assume that only monomeric gramicidin is able to cross the membrane-solution interface. Introducing the rate constants for the transport of gramicidin from membrane to aqueous phase (k_{ma}) and from aqueous phase to membrane (k_{am}), the overall rates of change of the monomer and dimer concentrations may be expressed as

$$\frac{dN_m}{dt} = -2k_R N_m^2 + 2k_D N_d - k_{ma} N_m + k_{am} c \tag{A.5}$$

$$\frac{dN_d}{dt} = k_R N_m^2 - k_D N_d. \tag{A.6}$$

In this case in which the total gramicidin concentration $N_m + 2N_d$ is no longer a constant, the decay of a small perturbation is governed by two relaxation times τ_1 and τ_2. Straightforward calculation leads to the result

$$\frac{1}{\tau_{1/2}} = \frac{1}{2}\left[(4k_R N_m^\infty + k_D + k_{ma}) \pm \sqrt{(4k_R N_m^\infty + k_D + k_{ma})^2 - 4k_D k_{ma}}\right] \tag{A.7}$$

where N_m^∞ is the equilibrium value of N_m. Accordingly, the autocorrelation function of $\delta J(t)$ is here given by

$$C(\tau) = A_1 e^{-\tau/\tau_1} + A_2 e^{-\tau/\tau_2} \tag{A.8}$$

$$A_1 + A_2 = \overline{(\delta J)^2}. \tag{A.9}$$

From the experimental finding that $C(\tau)$ is described by a single relaxation time we may infer that one relaxation time (τ_1) is much shorter than the other. (The case $\tau_1 \approx \tau_2$ may be excluded because $\tau_1 \approx \tau_2$ would only be possible for a certain combination of $k_R N_m^\infty$, k_D, and k_{ma}, whereas a single relaxation time has been found over a wide range of N_m^∞.) The condition $\tau_1 \ll \tau_2$ is obtained in the following two limiting cases. If the exchange is slow compared with the chemical reaction ($k_{ma} \ll k_D$, $k_{ma} \ll k_R N_m^\infty$), Eq. (A.7) reduces to

$$\frac{1}{\tau_1} \approx k_D + 4k_R N_m^\infty \tag{A.10}$$

$$\frac{1}{\tau_2} \approx \frac{k_{ma}}{1 + 4KN_m^\infty} \ll \frac{1}{\tau_1}. \tag{A.11}$$

On the other hand, if the exchange is fast ($k_{ma} \gg k_D$, $k_{ma} \gg k_R N_m^\infty$), Eq. (A.7) may be approximated by

$$\frac{1}{\tau_1} \approx k_{ma} \tag{A.12}$$

$$\frac{1}{\tau_2} \approx k_D \ll \frac{1}{\tau_1}. \tag{A.13}$$

Both cases may be distinguished by the concentration dependence of τ_1 and τ_2. (As seen from Eqs. (A.12) and (A.13), in the case of fast exchange both τ_1 and τ_2 become independent of N_m^∞.) The experimental result that $1/\tau_c$ depends on the gramicidin concentration in the way described by Eq. (A.10) which is equivalent to Eq. (7) is in favor of the view that the exchange of gramicidin between membrane and water is a slow process which is outside the frequency range of our experiments.

(d) Energy Fluctuations. If a system is in contact with a heat reservoir at a temperature T, the energy E of the system will exhibit random fluctuations. At constant pressure the mean square of the energy fluctuations is related to the isobaric heat capacity c_p of the system in the following way:

$$\overline{(\delta E)^2} = c_p k T^2 \tag{A.14}$$

where k is the Boltzmann constant (Kittel, 1958; *see also* Feher & Weissman, 1973). From the relation $\delta E = c_p \delta T$, fluctuations of the energy may be considered as equivalent to temperature fluctuations which are described by

$$\overline{(\delta T)^2} = \frac{kT^2}{c_p}. \tag{A.15}$$

As the impedance of the membrane depends in general on temperature, a fluctuation of T at a given voltage results in a current fluctuation. It may be easily shown, however, that the correlation time τ_T of a temperature fluctuation is too short to be observed under the usual experimental conditions. τ_T is approximately given (to the order of magnitude) by the relation

$$\tau_T \simeq \frac{c_p d^2}{\kappa} \tag{A.16}$$

where d is the membrane thickness and κ the mean thermal conductivity coefficient of the membrane. This follows from the analogy between heat

conduction and diffusion and from the formal equivalence of κ/c_p to a "heat diffusion coefficient". Introducing $d \simeq 60$ Å and the tabulated values of c_p and κ for hexane as a reference phase: $c_p \simeq 1.3\ \mathrm{J\ cm^{-3}}$, $\kappa \simeq 1.4 \times 10^{-4}\ \mathrm{J\ cm^{-1}\ sec^{-1}\ °K^{-1}}$, one obtaines $\tau_T \simeq 3 \times 10^{-9}$ sec, a value far below the experimental time-resolution.

(e) Fluctuations of the Membrane Capacitance. A further source of noise is represented by fluctuations of the electrical capacitance C_m of the membrane. At a constant voltage V, a fluctuation of C_m leads to a current fluctuation δJ:

$$\delta J = V\delta\left(\frac{dC_m}{dt}\right). \tag{A.17}$$

Thermal fluctuations of C_m result from the fact that the bimolecular membrane has to be considered as a thin film with elastic properties (White, 1970, 1973; Wobschall, 1971; Hestenes & Chang, 1973). We do not go into a detailed discussion of this subject here. For the present purpose it is sufficient to state that the current noise from a membrane without gramicidin (which includes fluctuations of C_m) is much smaller than the noise from a gramicidin-doped membrane (*see* Fig. 2). This finding is consistent with the observation that the relaxation of a voltage-induced capacitance change of a dioleoyllecithin membrane occurs in the millisecond range (Bamberg & Läuger, 1973).

Appendix B

Derivation of Equation (10)

For an ideal system (a dilute solution) of molecules m and d which may react according to $2m \rightleftarrows d$, the canonical partition function $Q(n_m, n_d)$ may be written in terms of the partition functions q_m and q_d of the single molecules m and d, respectively (*see* Hill, 1960):

$$Q(n_m, n_d) = \sum \frac{q_m^{n_m} q_d^{n_d}}{n_m!\, n_d!}. \tag{B.1}$$

The summation has to be carried out over all values n_m and n_d which are compatible with the condition that $n = n_m + 2n_d$ is a constant. The averages $\bar{n}_d$ and $\bar{n}_d^2$ are then given by the relations

$$\bar{n}_d Q = \sum n_d \frac{q_m^{n_m} q_d^{n_d}}{n_m!\, n_d!} \tag{B.2}$$

$$\bar{n}_d^2 Q = \sum n_d^2 \frac{q_m^{n_m} q_d^{n_d}}{n_m!\, n_d!}. \tag{B.3}$$

Partial differentiation of Eq. (B.2) with respect to q_d and subsequent multiplication of both sides with q_d/Q yields

$$\overline{n_d^2} - \bar{n}_d^2 = q_d \frac{\partial \bar{n}_d}{\partial q_d}. \tag{B.4}$$

The derivative $\partial \bar{n}_d/\partial q_d$ may be obtained from

$$\frac{\bar{n}_d}{\bar{n}_m^2} = \frac{\bar{n}_d}{(n-2\bar{n}_d)^2} = \frac{q_d}{q_m^2} \tag{B.5}$$

in the form

$$q_d \frac{\partial \bar{n}_d}{\partial q_d} = \frac{\bar{n}_d}{1+4\bar{n}_d/\bar{n}_m}. \tag{B.6}$$

Eqs. (B.4) and (B.6), together with

$$\overline{(\delta n_d)^2} = \overline{(n_d - \bar{n}_d)^2} = \overline{n_d^2} - \bar{n}_d^2 \tag{B.7}$$

then give the final result

$$\overline{(\delta n_d)^2} = \frac{\bar{n}_d}{1+4\bar{n}_d/\bar{n}_m}. \tag{B.8}$$

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