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Conductance Noise of Monazomycin-Doped Bilayer Membranes

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Summary. The conductance noise of the monazomycin pore has been studied by autocorrelation analysis in multi-pore systems. The autocorrelation function could be described by a superposition of two single exponential functions of different time- and voltage-dependence. The slow voltage-dependent correlation time in the range of seconds is assigned to the formation of nonconducting pore precursors. The fast voltage-independent correlation time in the msec range is related to fluctuations in the number of open pores whereby each pore adopts only two conducting states (open and closed). The corresponding correlation amplitude depends on monazomycin concentration and could be related to the single pore conductance. With increasing voltage, a slight increase of the single pore conductance was obtained which is explained on the basis of an electrostatic barrier within the pore. The pore was found to be virtually unselective for different alkali ions (Li, K, Cs).

It has been shown that the positively charged polyene-like antibiotic monazomycin induces voltage-dependent changes of the cation and anion permeability of lipid bilayer membranes (Mueller & Rudin, 1969; Mauro, Nanavati & Heyer, 1972; Muller & Finkelstein, 1972*a*, 1972*b*; Heyer, Muller & Finkelstein, 1976*a*, 1976*b*). There is evidence that monazomycin generates pores for cations in the presence of KCl or NaCl which are composed of several polypeptide molecules (Muller & Finkelstein, 1972*a*). Single channel experiments carried out by Muller and Andersen (1975) and by Bamberg and Janko (1976) support the notion that monazomycin creates pores in lipid bilayer membranes. It was suggested by Moore and Neher (1976) that monazomycin creates voltage-dependent multistate ion conducting channels similar to that observed in the presence of the antibiotic alamethicin (Gordon & Haydon, 1972; Eisenberg, Hall & Mead, 1973; Boheim, 1974; Gordon & Haydon, 1976; Boheim & Kolb, 1978; Kolb & Boheim, 1978). From voltage-jump current relaxation experiments with lipid membranes containing a large number of monazomycin pores, a slow voltage dependent process in the time range of seconds and a faster voltage independent process in the range of msec was observed (Muller & Finkelstein, 1972*a*; Moore & Neher, 1976). The molecular mechanism of pore formation has not yet been elucidated.

This paper is primarily concerned with a detailed correlation analysis of conductance noise from neutral lipid membranes in the presence of monazomycin.

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As we will show in this paper by measurement of the autocorrelation function, the absolute value of the conductance change induced by formation and disappearance of monazomycin pores can be determined, using reasonable assumptions on the nature of the pore formation process. The autocorrelation function of monazomycin-doped lipid bilayer was measured as a function of membrane voltage at different monazomycin and electrolyte concentrations.

The results give evidence that monazomycin pores show only two conductance states (open and closed), in contrast to the multistate conductance behavior found in the case of alamethicin.

Materials and Methods

L-1,2-diphytanoyl-3-phosphatidylcholine (diphytanoyllecithin) and DL-α-dioleoyl phosphatidylethanolamine (di-(18:1)-PE) were synthetized in our laboratory by K. Janko (Boheim *et al.*, 1976; Janko & Benz, 1977). Monazomycin was a generous gift from Dr. H. Yonehara, Tokyo. The antibiotic was added from an aqueous stock solution of 1 mg/ml H_2O in different amounts (0.2-5.0 μg/ml) to the electrolyte solution. KCl, LiCl, and CsCl were analytical reagent grade from Merck and used as unbuffered salt solutions (pH ~ 5.5). At this pH, PE is a zwitterion with zero net charge. Black lipid membranes were formed in the usual way from a 1% (w/v) lipid solution in *n*-decane in a thermostated Teflon cell (Läuger *et al.*, 1967) separating symmetrical electrolyte solutions. The membrane area was between 0.65 to 0.72 mm^2, which was usually determind by measuring the electrical capacitance of the film at low voltage. For the specific capacitance of membranes made from diphytanoyllecithin/*n*-decane a value of 374 nF/cm^2 and for di-(18:1)-PE/*n*-decane membranes a value of 372 nF/cm^2 (Benz & Janko, 1976) was assumed. Autocorrelation analysis of the membrane current noise was carried out under voltage-clamp conditions as previously described (Kolb, Läuger & Bamberg, 1975; Kolb & Boheim, 1978) using some minor modifications.

The feedback resistance R_f of the preamplifier (Analog Devices Model 52 K) was varied, depending on membrane conductance, in the range of 5 to 50 MΩ to optimize the noise figure of the amplifying device. The signal was then usually amplified by an ac-coupled amplifier (Princeton Applied Research Model 113). The autocorrelation function $C_J(\tau)$ of the amplified fluctuating component of the current was processed with a Honeywell-Saicor 43 A correlator. $C_J(\tau)$ was recorded at constant applied membrane voltage V which was increased in steps of 5 mV. In the following, we use, for reasons of simplicity, the autocorrelation function of conductance noise $C_\lambda(\tau)$ which is related to the corresponding autocorrelation function of current noise due to the relation (V-const.)

$$C_\lambda(\tau) = \frac{C_J(\tau)}{V^2}. \tag{1}$$

The autocorrelation function of monazomycin-doped lipid membranes could be described by a linear superposition of two exponential functions and a time independent constant term C_∞. For the determination of the autocorrelation function, the filtering of the membrane conductance by a low pass filter of frequency f_L which is determined by the feedback circuit ($f_L = 1/2\pi R_f C_f$, C_f is the feedback capacitance) and a high pass filter of frequency f_h defined by the lower roll-off frequency of the PAR 113 has to be taken into account. Unfiltered conductance noise leading to a autocorrelation function of two single exponentials will, after passing a one-pole bandfilter of frequencies f_L and f_h, alter the

autocorrelation function due to the relation (DeFelice & Sokol, 1976; Kolb & Boheim, 1978):

$$C_\lambda(\tau) = \tilde{C}_\lambda(\tau) + C_\infty$$

$$\tilde{C}_\lambda(\tau) = \sum_{i=s,f} \sigma_{\lambda_i}^2 \cdot f_l^2 \cdot f_h^2 \cdot$$

$$\cdot \left\{ \frac{f_l \exp(-2\pi\tau f_l)}{(f_l^2 - f_L^2)(f_l^2 - f_h^2)} - \frac{f_h \exp(-2\pi\tau f_h)}{(f_h^2 - f_L)(f_h^2 - f_l^2)} \right.$$

$$\left. - \frac{f_L \exp(-2\pi\tau f_L)}{(f_L^2 - f_h^2)(f_L^2 - f_l^2)} \right\} \tag{2}$$

where the index $i = s$ and $i = f$ denotes the slow and fast decaying term, respectively, $\sigma_{\lambda_s}^2$ and $\sigma_{\lambda_f}^2$ are the corresponding variances of the slow and fat conductance fluctuations, $\tau_s = 1/2\pi f_s$ and $\tau_f = 1/2\pi f_f$ are the correlation times. For lower roll-off frequencies than 0.03 Hz, a Butterworth filter (Krohn-Hite Model 3342) was used (Kolb & Boheim, 1978). C_∞ is a constant offset quantity which depends on the increase of the membrane conductance λ during the sampling time of the correlator. Experiments where C_∞ exceeded a value of $0.07 \times C_\lambda(o)$ were rejected.

The parameters τ_s, $\sigma_{\lambda_s}^2$, τ_f, $\sigma_{\lambda_f}^2$ were determined by fitting Eq. (2) to the processed autocorrelation function $C_\lambda(\tau)$ by the methods of least squares as previously described (Kolb & Boheim, 1978). For the measurement of the fast decaying term of Eq. (2), the filter frequencies f_L and f_h were adjusted as well above the characteristic frequency $f_f = 1/2\pi\tau_f$ so that the relation $f_h > 10 f_f > 10^2 f_L$ hold. In this case the fast decaying term of Eq. (2) may approximately be described by a single exponential function with amplitude $\sigma_{\lambda_f}^2$ and characteristic time τ_f at least for correlation times $\tau \lesssim 2 \cdot \tau_f$ (*see* Kolb, Läuger & Bamberg, 1976).

Results

Voltage—Conductance Relation

For typical experiments on monazomycin-doped diphytanoyl lecithin/*n*-decane membranes, the stationary voltage-conductance relation $\lambda(V)$ is shown in Fig. 1. The figure shows an exponential increase of conductance with applied voltage up to about $\lambda \sim 2$ mS cm^{-2} at 1 M KCl. At higher conductances the membrane usually broke. At very low conductances ($\lambda \lesssim 200$ nS cm^{-2} at 1 M KCl) deviations from the exponential behavior occurred, probably caused by the influence of the bare membrane conductance of about 20-40 nS cm^{-2} at 1 M KCl and 10°C. According to Muller and Finkelstein (1972*a*), the mean membrane conductance λ can be described in the form:

$$\lambda(V, C_{Mo}, C_{M^+}) = g \cdot C^n{}_{Mo} \cdot C_{M^+}^{\epsilon} \cdot \exp(\alpha \cdot VF/RT) \tag{3}$$

where C_{Mo} and C_{M^+} are the monazomycin and cation concentration, respectively, F the Faraday constant, T the absolute temperature, R the gas constant, g a proportionality factor and n, ϵ, α empirical constants. From the voltage dependence of λ(*see* Fig. 1) we found for $\alpha = 5.3 \pm 0.6$ independent on C_{Mo} in the range of $0.05\ \mu g\ cm^{-2} \leq C_{Mo} \leq 20\ \mu g\ cm^{-3}$ and C_{M^+} in the range of $0.01\ M \leq C_{M^+} \leq 4\ M$ and for temperatures between 0 and 20°C. A similar behavior of λ as a function of voltage has been found by Muller and Finkelstein (1972*a*). Using PE/*n*-decane as membrane forming solution instead of diphytanoyllecithin/*n*-decane did not

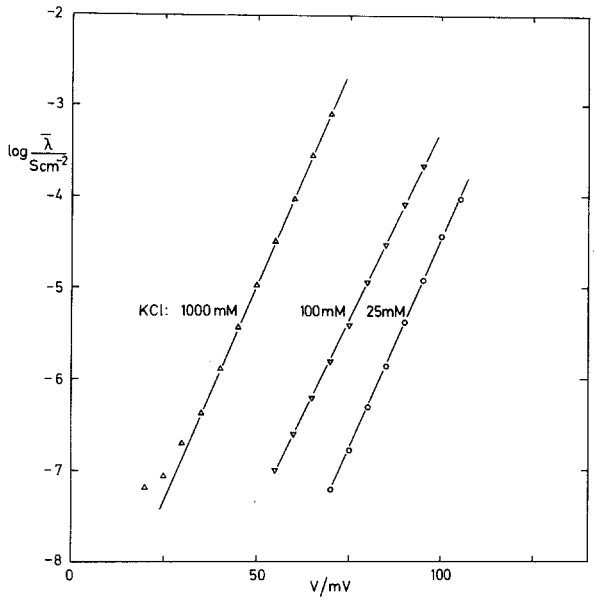

Fig. 1. Half-logarithmic plot of the mean membrane conductance λ *vs.* voltage V at different ion concentration C_{KCl}: △, 1000 mM; ▽, 100 mM; ○,25 mM. The straight lines have been obtained by taking into account only the exponential voltage dependence of the conductance. The aqueous phase contained 2 μg cm^{-3} monazomycin at 0 °C

influence α significantly. It should be noted that after changing the applied voltage in steps of 5 mV the macroscopic conductance rises to the final steady-state conductance value within a time range of 10 sec to 5 min depending on temperature, monazomycin- and salt-concentration. Thereafter, especially in the case of diphytanoyllethicin/*n*-decane membranes, sometimes a time-dependent monotonically decrease of λ was observed at constant applied voltage. Those experiments were discarded, although the characteristic parameters obtained by measuring the autocorrelation function, as will be described below, did not change for decreasing λ values at a constant voltage. The dependence of λ on C_{K^+} which is described by the constant ϵ [Eq. (3)] was determined from a plot of log λ *vs.* log C_{K^+} (1 M $\leq C_{K^+} \leq 10^{-2}$ M) at $V = 50$ mV and $T = 10$°C. For PE/*n*-decane membranes we found $\epsilon = 1.1 \pm 0.2$ and for diphytanoyllethicin/*n*-decane membranes $\epsilon = 2.3 \pm 0.5$.

Correlation Times and Variances

The autocorrelation function $C_\lambda(\tau)$ of monazomycin-induced conductance noise was measured as a function of increasing membrane voltage. At lower voltages which lead to conductances $\lambda \leq 200$ nS cm^{-2} at 1 M KCl, the autocorrelation function could be described by a superposition of two exponential functions, whereas at higher voltages only a single exponential behavior of $C_\lambda(\tau)$ was found. Figure 2*a* and *b* shows a typical record of $C_\lambda(\tau)$ at lower and higher voltage, re-

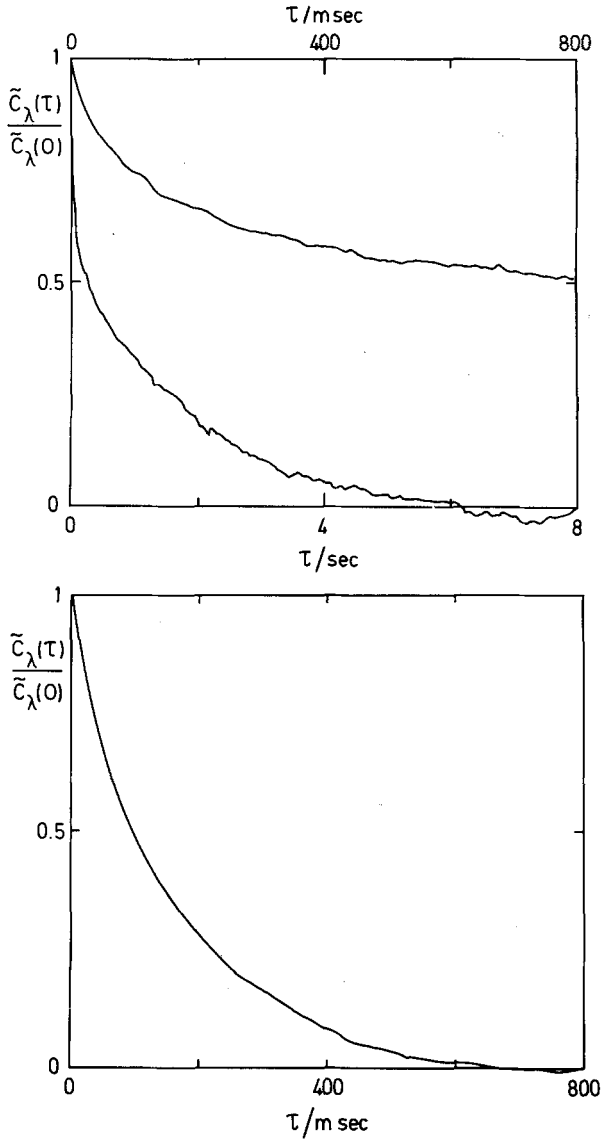

Fig. 2. (*a*): Autocorrelation function $\tilde{C}_\lambda(\tau)$ of the conductance fluctuations divided by the initial value $\tilde{C}_\lambda(0) = 3.1 \times 10^{-22}$ S^2 *vs.* time τ. For the upper trace the scale on the upper *x*-axis holds. Both functions were computed one after the other from the same record of conductance fluctuations with a different choice of the sample increment of the correlator. The lower trace contains 8×10^3 summations; the upper, 1.3×10^5. The mean membrane conductance was $\lambda = 84$nS cm^{-2}. Membrane area $A = 6.5 \times 10^{-3}$ cm^2, external voltage $V = 70$mV. The aqueous phase contained 1 M KCl, 200 ng cm^{-3} monazomycin at 10 °C. The high-pass filter frequency was set to 0.01 Hz. (*b*): Autocorrelation function $\tilde{C}_\lambda(\tau)$ of the conductance fluctuations divided by the initial value $\tilde{C}_\lambda(0) = 3.8 \times 10^{-20}$S^2 *vs.* time τ. The mean membrane conductance was $\lambda = 7.1$ μS cm^{-2} at a voltage of $V = 90$mV. Further experimental conditions were the same as described for *a*

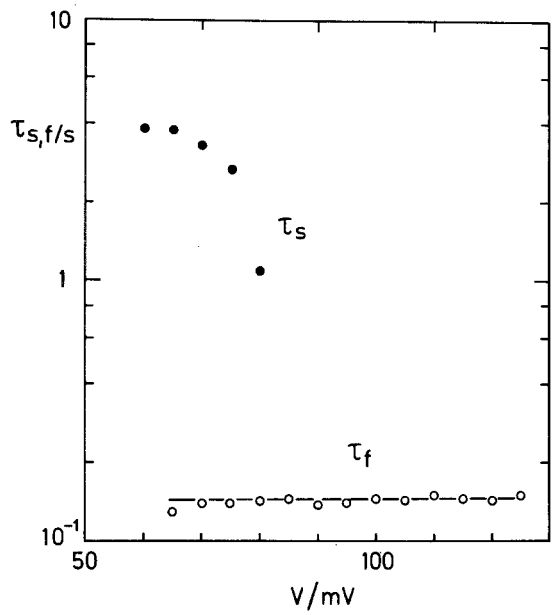

Fig. 3. Slow correlation time τ_s (closed circles) and fast correlation time τ_f (open circles) *vs.* membrane voltage. The aqueous phase contained 1 M KCl, 200 ng cm^{-3} monazomycin at 10°C

spectively. The corresponding slow and fast correlation times τ_s and τ_f are plotted in Fig. 3 as function of voltage at C_{Mo} = 200 ng cm^{-3}, C_{KCl} = 1 M and T = 10°C. As seen from Fig. 3 τ_s decreases with increasing voltage whereas τ_f remains almost constant independent of voltage. At higher voltages the slower process could not be observed furthermore, because of the decrease in the amplitude (*see* below). From the temperature dependence of τ_f (Table 1) an activation energy

Table 1. Fast correlation time τ_f as function of temperature[a]

	T/°C			
	0	10	20	$E(\tau_f)$/kJ/mol
	diphytanoyllecthicin/*n*-decane			
τ_f/ms	356 ± 21	128 ± 6(170)[b]	26.5 ± 1.0	81.9 ± 10.0 (85)[c]
	PE/*n*-decane			
τ_f/ms	288 ± 14	134 ± 7	—	—

[a] τ_f was measured at increasing voltage which was varied in steps of 5 mV for a voltage range up to 60 mV. At each temperature five different membranes were used. The aqueous solution contained 1 M KCl and 2 μg cm^{-3} monazomycin.

[b] This value was obtained by single pore experiments (Bamberg & Janko, 1976) at 11°C, 4 M CsCl, 60 ng cm^{-3} of monazomycin and an applied voltage of 150 mV.

[c] Calculated from the results of single pore experiments (Bamberg & Janko, 1976).

Table 2. Dependence of the fast correlation time τ_f on ion concentration[a]

	T/ °C	C_{KCl}/mM				C_{H^+}/mM
		1000	100	25	10	10
τ_f/ms	0	356 ± 21	188 ± 8	176 ±7	146 ± 6	
τ_f/ms	20	26.5 ± 1.0	16.8 ± 1.0	—	12.1 ± 0.5	13.7 ± 0.7

[a] τ_f was measured at increasing voltage which was varied in steps of 5 mV for a voltage range of at least 40 MV. At each ion concentration five different membranes made of diphytanoyllecithin/*n*-decane were used. The aqueous solution contained 200 ng cm^{-3} of monazomycin.

$E(\tau_f) = 81.9 \pm 10.0$ kJ/mol may be calculated. Furthermore, Table 2 shows that τ_f slightly decreases with decreasing ion concentration. Changing C_{KCl} from 1 M to 0.01 M induces a decrease of τ_f by a factor of more than two. τ_f was found to be independent of C_{M_0} for various species of monovalent cations like Li^+, K^+ or Cs^+. Using PE/*n*-decane membranes instead of diphytanoyllethicin/*n*-decane membranes had no significant influence on τ_f (*see* Table 1).

It was found that in the presence of HCl which was added to the distilled water to obtain a pH of two, a voltage conductance relation similar to that in the presence of 10 mM KCl was obtained. The correlation time τ_f measured at this pH, 20°C and 200 ng cm^{-3} monazomycin was 13.7 ± 0.7 msec which is similar to that in the presence of 10 mM KCl (Table 2).

The voltage dependent behavior of the variances (or correlation amplitudes) $\sigma^2_{\lambda_s}$ and $\sigma^2_{\lambda_f}$ of the slow and fast process, respectively, may be seen from Fig. 4. The figure shows the variances normalized to the membrane conductance $\lambda \cdot A$ where

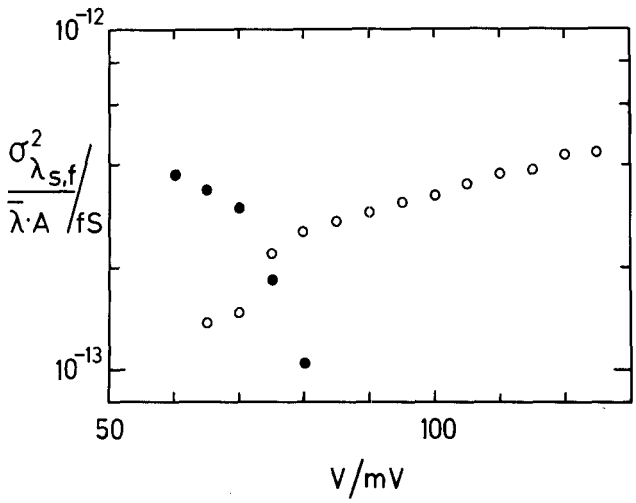

Fig. 4. Variance of the slow process, $\sigma^2_{\lambda_s}$ (closed circles) and of the fast process $\sigma^2_{\lambda_f}$ (open circles) normalized to the membrane conductance $\lambda \cdot A$ (A = membrane area) *vs.* voltage. The experimental conditions are identical with those of Fig. 3

A denotes the membrane area. As seen from Fig. 4, $\sigma_{\lambda_s}^2/\lambda \cdot A$ strongly decreases with increasing voltage, whereas $\sigma_{\lambda_f}^2/\lambda \cdot A$ shows an increase with voltage. In the following we regard only the fast process, especially in that voltage range where an exponential increase of λ described by Eq. (3) is observed. As seen from Fig. 4, $\sigma_{\lambda_f}^2/\lambda \cdot A$ shows in this voltage/conductance range a slight exponential increase with voltage which can be described by the relation:

$$\frac{\sigma_{\lambda_f}^2}{\lambda \cdot A} \sim \exp(\beta \cdot V \cdot F/R \cdot T) \tag{4}$$

where β is an empirical constant. Independent of temperature and monazomycin concentration, a value $\beta = 0.32 \pm 0.06$ was obtained for diphytanoyllecithin membranes at 1 M KCl. Decreasing C_{KCl} to 10 mM results in a slight, but not significant, increase of β (*see also* Fig. 5). From the temperature dependence of the conductance fluctuations characterized by the variance, an activation energy $E(\sigma_{\lambda_f}^2/\lambda \cdot A) = 34.8 \pm 4.2$ kJ/mol may be calculated (*see also* Fig. 5). Furthermore, Fig. 5 shows that reduction of C_{KCl} from 1 M to 0.1 M causes a decrease of $\sigma_{\lambda_f}^2/\lambda \cdot A$ by about a factor of four, a further decrease of C_{KCl} to 10 mM results in about a tenfold change of $\sigma_{\lambda_f}^2/\lambda \cdot A$ (*see* Table 3). Table 3 shows that using LiCl or CsCl instead of KCl did not influence $\sigma_{\lambda_f}^2/\lambda \cdot A$ significantly. But at a pH of two (HCl plus distilled water) $\sigma_{\lambda_f}^2/\lambda \cdot A$ was about twice as large as measured in the presence of 10 mM KCl. Reduction of the monazomycin concentration causes an increase of the value $\sigma_{\lambda_f}^2/\lambda \cdot A$ referred to a constant voltage (Table 4).

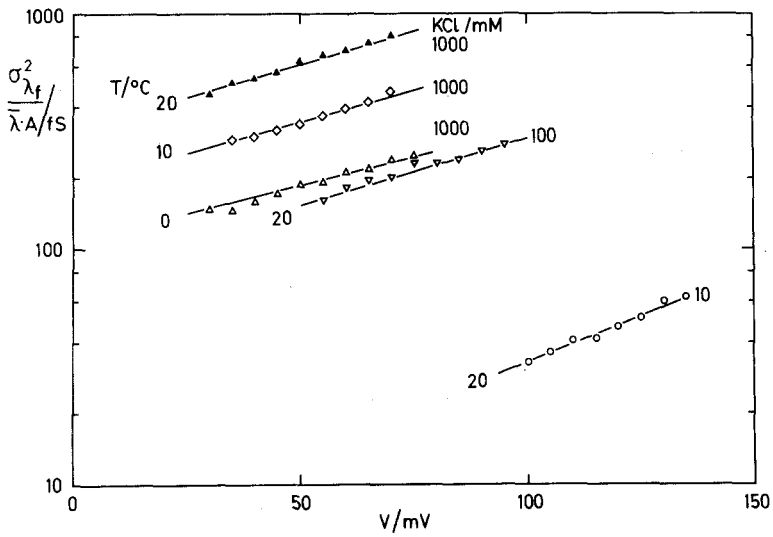

Fig. 5. $\sigma^2_{\lambda_f}/\lambda \cdot A$ as function of applied voltage at different temperatures T/°C: ▲, 20; ◇, 10; ▽, 0 (1 M KCl) and variable ion concentrations C_{KCl}/mM: ▽, 100; ○, 10 (T = 20°C). The aqueous solution contained 2μg cm^{-3} monazomycin

Table 3. Variance of the fast process $\sigma^2_{\lambda_f}$ divided by the membrane conductance $\lambda \cdot A$ (A = membrane area) for various ion-species and electrolyte concentrations[a]

	Electrolyte concentration					
	C_{KCl}/mM			C_{LiCl}/mM	C_{CsCl}/mM	
	1000	100	10	1000	1000	4000
$\frac{\sigma^2_{\lambda_f}/\lambda \cdot A}{P \cdot S}$	1.1 ± 0.2	0.31 ± 0.10	0.038 ± 0.009	1.15 ± 0.3	1.2 ± 0.3	1.9 ± 0.5

[a] The aqueous solution contained 2 μg cm^{-3} monazomycin at 20°C. All data are related to an applied voltage of 100 mV.

Table 4. Variance of the fast process $\sigma^2_{\lambda_f}$ divided by the membrane conductance $\lambda \cdot A$ as function of monazomycin concentration[a]

C_{Mo}/ng cm^{-3}	10 000	2000	200	20
$\frac{\sigma^2_{\lambda_f}/\lambda \cdot A}{P \cdot S}$	1.2 ± 0.5	1.8 ± 0.2	2.8 ± 0.5	3.4 ± 0.4

[a] The aqueous solution contained 1 M KCl at 20°C. The values were partly obtained by extrapolation to a voltage of 150 mV from experimental values measured at lower voltages.

Discussion

In *Results* it was shown that under voltage-clamp conditions the autocorrelation function $\bar{C}(\tau)$ of monazomycin-induced current noise may be described by a linear superposition of two exponential functions. In the following, the fitted parameters of $\bar{C}(\tau)$ (variances and correlation times) obtained for a multi-pore system will be assigned to single-pore parameters by comparison of the results of the present paper with those obtained from single-pore experiments (Muller & Andersen, 1975; Bamberg & Janko, 1976) and from multi-pore voltage-jump current relaxation experiments (Muller & Finkelstein, 1972*a*; Moore & Neher, 1976). The results will also be compared with those measured in the presence of the antibiotic alamethicin, for which a similar strong voltage-dependent macroscopic conductance was found (Baumann & Mueller, 1974). The autocorrelation function of the alamethicin system could also be described by a superposition of two exponential functions (Kolb & Boheim, 1978).

Slow Process

The slow conductance fluctuations lead to a correlation process characterized by τ_s which shows a strong decrease from about 4 to 0.2 sec with increasing applied voltage. This process could only be observed in those cases of low conductance λ where deviations from the exponential relation of λ on V [*compare* Eq. (3)] occurred. According to the fluctuation-dissipation theorem (Onsager, 1931; Kubo, 1957) the correlation time τ_s should be identical with the relaxation time of a voltage-jump current relaxation experiment performed under comparable conditions. Relaxation experiments carried out with monazomycin-doped bilayers also show for small changes in the applied voltage ($\Delta V/V \ll 1$) a slow time constant which decreases with increasing voltage (Muller & Finkelstein, 1972*a*; Moore & Neher, 1976). In contrast to relaxation experiments (Moore & Neher, 1976), the slow process could not be detected anymore at higher voltages by correlation analysis, since the value of $\sigma_{\lambda_s}{}^2/\lambda \cdot A$ decreased beyond a detectable value of about 8 fS. This finding is in sharp contrast to the correlation analysis in the presence of alamethicin where τ_s increased by orders of magnitude with increasing voltage and $\sigma_{\lambda_s}{}^2/\lambda \cdot A$ stayed nearly constant (Kolb &

Boheim, 1978). This comparison indicates that the monazomycin-induced slow correlation process is not, like in the case of alamethicin, generated by fluctuations in the number of pores, whereby τ_s would denote the mean pore life-time and $\sigma_{\lambda_s}^2/\lambda \cdot A$ the mean total conductance of a single pore. A voltage-induced decrease of $\sigma_{\lambda_s}^2/\lambda \cdot A$ would be obtained if the pore switches with increasing voltage from an on-off behavior to a permanent open conformation. The fast process (*see* below) would then be related to fluctuations only within the pore. Wanke and Prestipino (1976) proposed that only 1/25th of the current flowing in a pore is fluctuating and estimated a total conductance of the permanent open pore of 625 pS. In this case the current noise generated by those open pores should lead to a $1/f$-behavior of the corresponding spectral intensity (Dorset & Fishman, 1975; Sauvé & Bamberg, 1978) from the $1/f$-amplitude of the spectral intensity measured by Sauvé and Bamberg (1978) for open pores of malonyl-bis-desformylgramicidin, the $1/f$-amplitude of permanent open monazomycin pores can be estimated. With the value of $\Lambda = 625$ pS, one should expect for a typical experiment (*see* Fig. 2*b*) a $1/f$ amplitude of the spectral intensity of about the same order of magnitude as for the spectral intensity generated by the fast process at the corner frequency ($f_c = 1/2\pi\tau_f$, *see* below). Measurements of the spectral intensity of current noise gave no indication of a $1/f$ term of this magnitude (H.-A. Kolb, *unpublished*).

The correlation analysis of the slow process is consistent with the model postulated by Muller and Finkelstein (1972*a*) where the slow process is interpreted as a voltage-dependent re-orientation of the positively charged monazomycin monomers from the membrane/solution interface into the membrane interior.

Fast Process

From the correlation function of the fast conductance fluctuations, a single voltage-independent correlation time τ_f was found under the used experimental conditions, whereas noise analysis carried out by Moore and Neher (1976) and Wanke and Prestipino (1976) gave indications of further faster correlation times. For a voltage change up to 55 mV, τ_f remained unchanged within an experimental error of about 5% (*see* Table 1). In the case of alamethicin, however, where τ_f represents the average lifetime of different consecutively ordered conductance states within a single pore, a slight but significant voltage induced increase of τ_f was observed (Kolb & Boheim, 1978). Single pore experiments carried out by Bamberg and Janko (1976) under comparable conditions yielded a mean lifetime of pores of similar value and activation energy (Table 1). Since in the present experiments it was found that τ_f is independent of monazomycin concentration, the agreement of single and multi-pore experiments give evidence that the fast process observed in the multi-pore system results from fluctuations in the number of open pores. τ_f represents then the mean life time of the open monazomycin pore. Discrete conductance fluctuations within a pore would generate further exponential decaying terms of $C(\tau)$ and would indicate the formation of oligomer pores of varying parameters not observed in the present study. Furthermore, it was found that τ_f decreases by about a factor of 2 to 3 by decreasing the ion concentration from 1 to 0.01 M. A similar dependence on C_{KCl} was described

for the mean life time of alamethicin- and gramicidin A-induced pores (Kolb & Bamberg, 1977; Kolb & Boheim, 1978). A tentative explanation was previously given on the basis of an electrostatic stabilization of the pore and due to an increased probability of occupation of an ion within the pore (Kolb & Bamberg, 1977).

Assuming a simple on-off kinetic of statistically independent monazomycin pores, the mean pore conductance Λ_f is related to the variance $\sigma_{\lambda_f}^2$ in the limit of a low probability of pore opening by the relation (Rice, 1944)

$$\frac{\sigma_{\lambda_f}^2}{\lambda \cdot A} = \Lambda_f. \tag{5}$$

Considering the absolute values of Λ_f measured at 1 M KCl, $T = 20°C$, and $C_{Mo} = 2\mu g\ cm^{-3}$ (*see* Fig. 5), reasonable agreement is obtained with the results of the multi-pore analysis of Wanke and Prestipino (1976) ($\Lambda_f \approx 1$ pS at 20°C, $V = 29$ mV, $C_{Mo} = 420$ ng cm^{-3} for PE plus monoolein/*n*-decane membranes) and Moore and Neher (1976), who found independent of voltage (V: 50–110 mV) and monazomycin concentration (C_{Mo}: 200–400 ng cm^{-3}) for lecithin plus cholesterol/*n*-decane membranes a value of $\Lambda_f = 1.55$ pS. But compared with the directly-measured mean single pore conductance by Bamberg and Janko (1967) ($\Lambda = 16$ pS at 25°C, $C_{CsCl} = 4$ M, $V = 150$ mV, $C_{Mo} = 60$ ng cm^{-3} for diphytanoyllecithin/*n*-decane membranes), a discrepancy of about a factor of three if observed in comparison within the present results of multi-pore analysis (Table 3). Since the main difference between single-pore and multi-pore experiments lies in the different concentration of pores and therefore in the number of monazomycin molecules orientated with the membrane, the effect of C_{Mo} on Λ_f was investigated. It was found that Λ_f increases at constant voltage with decreasing monazomycin concentration (Table 4). A plausible explanation of this effect can be given on the basis of the fact that monazomycin is a positively charged peptide (Mitscher *et al.*, 1967). Orientation of monazomycin within the membrane then causes a positive space charge of the membrane which increases at constant voltage with increasing monazomycin concentration. This space charge may lead to a decrease of the single pore conductance in multi-pore experiments.

Furthermore, a slight voltage-dependent increase of Λ_f was observed [Eq. (5) and Fig. 4]. A tentative explanation of this finding may be based on the notion that five positively charged monomers are involved in the formation of a conducting pore (Muller & Finkelstein, 1972*a*). If one assumes that the conducting pathway is a central hole within such charged oligomer, an increase of the cationic single pore conductance with increasing voltage should be expected as will be outlined in the following.

For an estimation of the one-dimensional cationic flux through a charged pore, we assume that the electrostatic barrier within the pore consists of a charged ring which is orientated perpendicular to the ion pathway and located in the center of the pore. The stationary solution for the ion flux is given by Eqs. (A3) to (A6) of the *Appendix A*. At constant applied voltage the ion flux through the pore is proportional to the single pore conductance Λ. In Fig. 6 the ratio Λ_n/Λ_o of the charged pore (n = number of charges on the ring) to the uncharged one is plotted as function of applied voltage for increasing numbers of charges and various di-

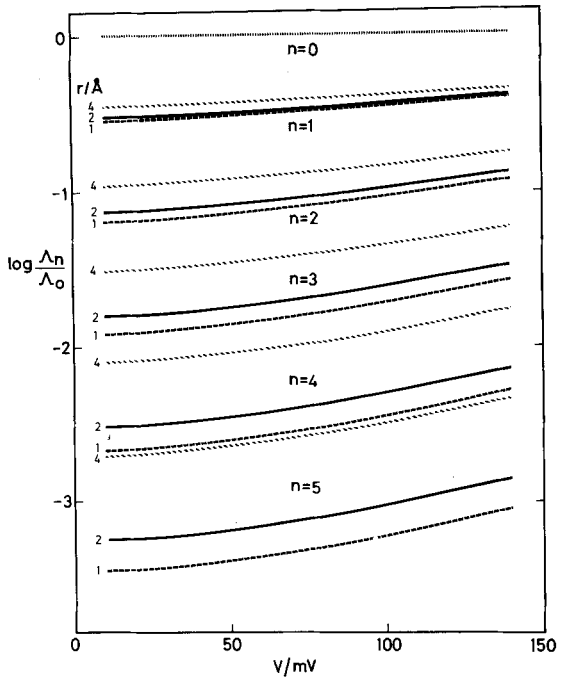

Fig. 6. Ratio Λ_n/Λ_o of the conductance of a charged and uncharged pore as function of voltage for the parameter values $d = 50$ Å, $\epsilon_p = 40$ (*see* Eq. A7). The electrostatic barrier within the charged pore is a ring of radius r where n denotes the number of charges on the ring (*see* Eq. A5)

ameters of the ring. The figure shows that with increasing number of charges the absolute value of Λ_n decreases and the dependence of Λ_n on the pore diameter is more pronounced. Also, the increase of Λ_n with increasing voltage is enhanced for larger n. A comparison of Figs. 4 and 6 shows that the measured change of Λ_f with voltage is of about the same order of magnitude as derived for the simplified model of a charged pore.

No significant discrimination was found for the cations Li, K, Cs on monazomycin-induced pores (Table 3), despite the expectation that for a small single pore conductance compared, e.g., to gramicidin A-induced pore conductances, the selectivity should increase. It may be seen from Fig. 6 that in comparison to a neutral pore the conductance of a charged pore is mainly determined by the magnitude of the electrostatic barrier within the pore and, to a lesser extent, by the diameter of the pore. Therefore, it seems plausible to assume that the hindrance of the ion flux due to the electrostatic barrier within the pore overwhelms a discrimination of the pore between cations of different sizes.

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Appendix A

An equation for the one-dimensional monovalent cation flux through a positively charged pore is derived. The flux Φ is given by the generalized Nernst-Planck equation:

$$\Phi = -D\left(\frac{dc}{dx} + c \cdot \frac{d\bar{\psi}}{dx}\right) \tag{A1}$$

(x = coordinate normal to the cross section of the pore; c = ion concentration; D = diffusion coefficient; ψ = potential energy; $\bar{\psi} = \psi F/RT$ = reduced potential energy; R = gas constant; F = Faraday constant; T = absolute temperature). For the stationary solution of the concentration distribution $c(x)$, one obtains by simple integration:

$$c(x) = e^{-\bar{\psi}(x)} \cdot \left(A \cdot \int e^{\bar{\psi}(\xi)} \cdot d\xi + B\right) \tag{A2}$$

where A and B are integration constants.

We assume that the electrostatic barrier within the pore consists of a ring perpendicular orientated to the x-axis and restrict ourselves to a symmetrical arrangement of the ring at $x = d/2$ between the boundaries $x = 0$ and $x = d$ of the pore where the ion concentrations are assumed to be equal ($c(o) = c(d) = c$). Using Eqs. (A1) and (A2), one obtains:

$$\Phi = -D \cdot \frac{c \cdot (e^{\bar{\psi}(d)} - e^{\bar{\psi}(0)})}{\int_0^d e^{\bar{\psi}(\xi)}\, d\xi} \cdot \tag{A3}$$

For the calculation of $\psi(x)$ within the pore we assume that an external applied potential difference V totally drops across the pore leading to the

$$\psi_e(x) = V \cdot x/d \qquad (0 \leq x \leq d) \tag{A4}$$

which is linearly superimposed by the electrostatic potential of a ring of charge $n \cdot e_o$ (n = number of charges; e_o = elementary charge) and radius r:

$$\psi_r(x) = -\frac{n \cdot e_0}{4\pi\epsilon_0 \cdot \epsilon_p} (r^2 + (x - d/2)^2)^{-1/2} \, (0 \leq x \leq d) \tag{A5}$$

where as a first approximation the dielectric constant ϵ_p within the pore is assumed to be a constant (ϵ_o = permittivity of vacuum). Using the relation

$$\bar{\psi}(x) = \bar{\psi}_e(x) + \bar{\psi}_r(x) \tag{A6}$$

explicit values of the flux through a charged ring can be obtained from Eq. (A3) by numerical integration.

Since the single pore conductivity Λ is at constant voltage proportional to Φ, the ratio Λ_n/Λ_o for the charged and uncharged pore is given by

$$\Lambda_n/\Lambda_o = \Phi_n/\Phi_o \tag{A7}$$

where $\Phi_{o,n}$ can be calculated from Eq. (A3) using Eqs. (A4)-(A6).

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