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MONAZOMYCIN CHANNEL NOISE

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Summary

Spectral analyses of conductance fluctuations produced by monazomycin pores in black lipid membranes show slow (2 s), fast (30 ms) and ultra-fast (2 ms) kinetics. The extremely low level of this noise compared with the cationic permeability to large ions such $Tris^+$ gives support to a model where only a small fraction of the total pore conductance is fluctuating.

Fluctuation spectroscopy and relaxation kinetics are becoming very successful techniques for studying conductance changes in nerve membranes and black lipid bilayers [1—5]. Since the recognition [5] that spectral analysis of conductance fluctuations could give more information than relaxation data about the kinetic rates, noise analysis has been widely used to elucidate the mechanism of translocation of ions in membranes [6,7,8].

Black lipid bilayers doped with monazomycin [9,10,11] (also with alamethicin [12]) yield characteristic voltage-dependent and also sigmoidal time-dependent conductances which are typical of nerve membranes [13]. It is known [9,10] that monazomycin produces pores for cations in the presence of KCl or NaCl while for $CaCl_2$ solutions the selectivity reverses to Cl^-. Preliminary experiments carried out in tetraethylammonium chloride and Tris · Cl solutions gave a cationic permeability ratio versus KCl of about 1:20, suggesting the existence of pores sufficiently large in diameter. The conductance increases linearly with the KCl concentration, being proportional to the fifth power of the monazomycin concentration and obeys an exponential law with an *e*-fold change of about 6 mV. For voltage steps of this order of magnitude, either slow sigmoidal-conductance time-courses starting from a zero-conductance state or quasi-exponential time-courses starting from a high-conductance state can be observed: the half time is voltage dependent and is of the order of a few seconds. At the same high-conductance state, after application of a brief perturbing pulse (< 1 ms) of large amplitude, the current relaxes in a fast quasi-exponential manner with a half time of the order of 25 ms (voltage independent). After the analysis of current fluctuations in a voltage clamp,

these data predict [4] at least two Lorentzian power spectra $S_0(f)$ and $S_1(f)$ with cut-off frequencies of the order of 0.05 and 5 Hz. However, since the membranes used give stationary results within 20—30 min, the lowest frequency theoretically measurable with sufficient accuracy is 0.2 Hz [14]. Therefore we were forced to make use of spectra in the range of 0.2—1 kHz.

An experimental power spectrum of the current noise in a voltage-clamped phosphatidylethanolamine-black lipid layer (decane solutions of 1% bacterial phosphatidylethanolamine) is shown in Fig. 1A. The experimental set up is similar to the one described by Wanke et al. [15] and Conti et al. [3], apart from small modifications to the input amplifiers. The characteristic behaviour of the kinetic mechanisms [16,17] (Lorentzian curves of the type

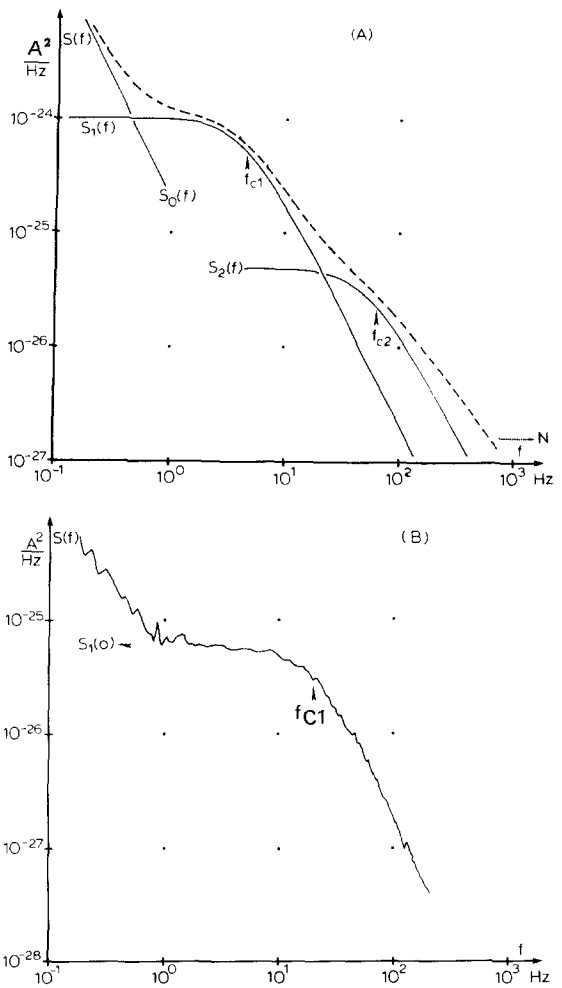

Fig. 1. Current noise spectral density $S(f)$ from black lipid bilayers doped with monazomycin. (A) the dashed line is the best fit through the experimental curve after subtraction of the amplifier noise and the thermal noise N (point-line). The arrows indicate the half-power cut-off frequencies f_{c1} and f_{c2} of the Lorentzian curves $S_1(f)$ and $S_2(f)$. Lipid: phosphatidylethanolamine, temp. 20°C, salt concentration 0.2 M KCl, monazomycin concn. 21 μM, V = 20.5 mV, I = 2.1 nA; (B) the curve is the output of the spectrum analyzer (Federal Scientific Co.) after 640 s of averaging time. Lipid: 1:1 mixture of phosphatidylethanolamine (Supelco Inc.) and monolein (Sigma Co.) in n-decane; temp. 20°C; salt concn. 20 mM KCl; monazomycin concn. 0.42 μM, V = 29 mV, I = 45 pA; membrane area 0.8 mm².

$1/1 + f^2/f_c^2$) are clearly visible. After subtraction of both the amplifier noise in the high frequency range and the theoretical thermal noise, the remaining spectrum can be unequivocally divided into two Lorentzian curves, one clearly visible $S_1(f)$ and the other $S_2(f)$ buried under the real curve. The thermal noise was obtained from the well-known Nyquist formula $4kTg$ where g is the cord conductance after division of the membrane current I by the voltage V. The relation $4kT\ Re\ 1/Z$ [18] (where Z is the impedance of the membrane) gives in this case a value about six-times higher than $4kTg$, never found during the experiments. On the other hand, if the membrane is composed of ohmic channels, at equilibrium the theory predicts a value of exactly $4\ kTg$. At very low frequencies a $1/f^2$ component is visible and perhaps coincides with the frequency-dependent part of the $S_0(f)$ power spectrum mentioned above. The voltage-independent cut-off frequencies f_{c1} and f_{c2} marked in Fig. 1a, are of the order of 5 and 80 Hz respectively at room temperature. The corresponding time constant of the kinetics are 32 and 2 ms. It is clear that $S_1(f)$ reflects the fast kinetic relaxation previously described while $S_2(f)$ reflects an ultra-fast process not visible during the relaxation experiments, probably because of the slow response of the set up.

To understand better the dependence of $S_1(f)$ and $S_2(f)$, different electrical, physical and chemical conditions were tested. The low frequency limits $S_1(0)$ and $S_2(0)$ are found (Fig. 2) exactly proportional to the product of $I \times V$ over a range of three orders of magnitude, either maintaining a constant monazomycin concentration or varying it in the range of 0.5—50 μM. The temperature shifts $S_1(f)$ towards higher frequencies by a factor of approximately three upon a 10°C change. The result of a modification in the lipid composition is shown in Fig. 1B: a 1:1 mixture of monolein and phosphatidylethanolamine was used. A very large increase in the cut-off frequency can be seen, certainly reflecting the higher fluidity of the matrix. Finally, different cations and anions were used in the solution for the purpose of evaluating the struc-

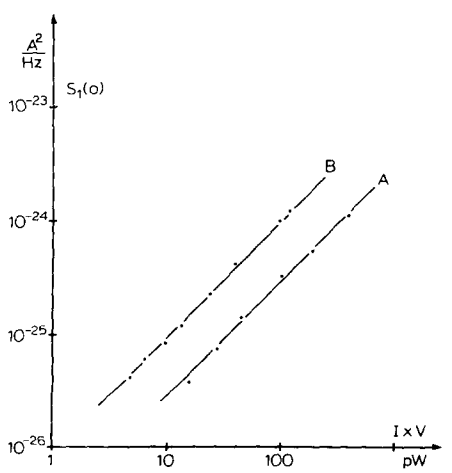

Fig. 2. The low frequency limit $S_1(0)$ of the Lorentzian $S_1(f)$ as a function of the product $I \times V$ of the membrane current and voltage. Line A was derived from an experiment at a constant monazomycin concentration of 0.8 μM (2 mM KCl) and line B from an experiment where the monazomycin concentration was varied between 0.4 μM to 10 μM. (20 mM KCl). Membrane area 0.8 mm². Lipid: phosphatidylethanolamine.

ture and dimensions of the pore. In Fig. 3 the amplitude $S_1(0)$ is plotted for a given product $I \times V = 5\text{pW}$) vs. salt concentration. The points represent several experiments done with different phosphatidylethanolamine-black lipid bilayers. In the case of a KCl electrolyte solution there is an approximate square-root relation with concentration. For tetraethylammonium chloride, Tris · Cl and $CaCl_2$ there is a depression of this curve which is much larger than

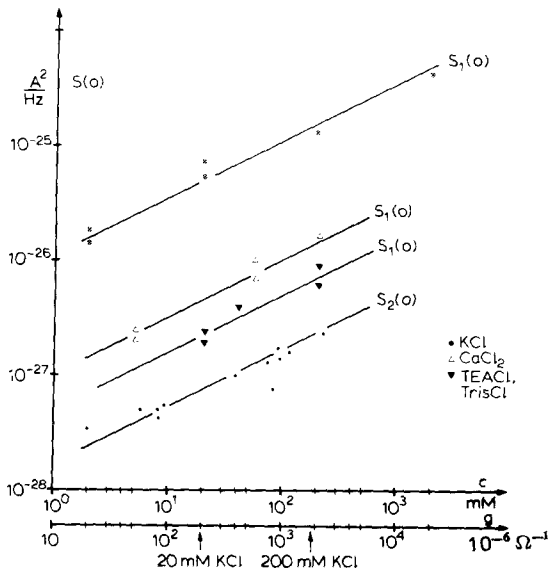

Fig. 3. The low frequency limit $S_1(0)$ is plotted against salt concentration c while $S_2(0)$ is against bulk conductance g. The lines correspond to the best fit for the relation $S(0) \propto c^{1/2}$ or $g^{1/2}$. Membrane area 0.8 mm². Lipid: phosphatidylethanolamine. TEACl, tetraethylammonium chloride.

the bulk conductance change. In the presence of $Tris^+$ and K^+ there is no blocking effect from the large ion. The data for the second Lorentzian $S_2(0)$ are plotted vs. the bulk conductance of the salt because there was no difference in using KCl, tetraethylammonium chloride, Tris · Cl or $CaCl_2$ or mixtures of them. Experiments done in a $MgSO_4$ solution show that these ions are virtually unable to permeate the black lipid bilayers while at the same time the bilayers became extremely fragile under the application of the electric field.

As was mentioned recently by Bauman and Mueller [19], the behaviour of many monazomycin relaxation experiments can be explained if one assumes a two-step process of insertion of polyene molecules in the lipid matrix and a subsequent aggregation of many monomers into complicated pore structures. According to this model, the slow voltage-dependent conductance time course mentioned above is a non-stationary process, due to the fact that the density of monomers in the bilayer is increasing together with the number of pore structures. After a certain time, an equilibrium situation is reached, and the number of channels is practically constant. The fact that in this case one finds a noise level greater than the expected thermal noise suggests that the channels are dynamic structures having fluctuating conductance. The simplest open-close kinetic process gives a power spectral density [20,21] of the type:

$$S(f) = \frac{2}{\pi f_c} IV(1-n)\gamma \frac{1}{1+(f/f_c)^2} \quad (1)$$

where γ is the channel conductance, n is the fraction of time spent by the channel in the open state and IV is the product of membrane current and voltage.

Using our data (at 1 M KCl) one finds $\gamma \approx 1 \cdot 10^{-12}\ \Omega^{-1}$ under symmetrical on-off probability. This very small value (see ref. 3, e.g., the squid axon sodium channel conductance $4 \cdot 10^{-12}\ \Omega^{-1}$) has to be compared with values of $500 \cdot 10^{-12}\ \Omega^{-1}$ found for the alamethicin channel which is known [22] to be blocked by tetramethylammonium and $Tris^+$ flow. The paradox of having large pores where tetraethylammonium chloride and Tris can move, while the current noise predicts a small elementary conductance can be resolved in at least two ways. One can assume that the probability for the channels to be open is almost 1, in this case the term $(1-n)$ in Equation 1 becomes very small and γ becomes large. However, it is difficult to reconcile this possibility with the lack of data reporting large conductance changes [23]. Another way to explain the paradox consists of assuming that each channel formed by aggregation is actually large but changes its size only by a small amount. If this is the case, the current flowing through each channel is the sum of a large d.c. level plus a small fluctuating component: the total measured current I has the same pattern and it is obvious that in Equation 1 only the small fluctuating part has to be used. The value of γ obtained in this way gives the conductance fluctuation of the channel and it is not connected to the total conductance of the channel itself.

If we assume that only 1/25th of the current flowing in a channel is fluctuating, this means not only that γ becomes $25 \cdot 10^{-12}\ \Omega^{-1}$, (which is still a small value difficult to be detected), but also that the total conductance of the channel is $625 \cdot 10^{-12}\ \Omega^{-1}$. This latter value is of the same order of the alamethicin channel conductance and it is not difficult to believe that tetraethylammonium chloride and Tris can flow in such a channel. The second Lorentzian $S_2(f)$ seems to depend on the bulk conductance of the solution, as if one part of the channel were so large to connect freely the two sides of the membrane. The same argument used for $S_1(f)$ is probably valid also for $S_2(f)$. In fact, Equation 1 applied to $S_2(0)$ gives a value of $\gamma \approx 1 \cdot 10^{-13}\ \Omega^{-1}$ which is of course in complete contrast with the measured bulk conductance dependence.

The channel model like a barrel, suggested by Mueller [24], where the staves are the single monomers of antibiotic is certainly suggestive, but more interesting is the model for the alamethicin pore suggested by Gordon and Haydon [22] where a small channel is surrounded by six larger ones. A similar model probably is able to fit also in the case of monazomycin. We may suppose that a large central channel having a solution-dependent conductance is surrounded by many channels with a permeability depending on the ion radius. This structure undergoes small conductance fluctuations due to random aggregation of monomers with the fast and the ultra-fast kinetics. The surrounding array of channels interacts largely with the lipid matrix and probably this is the reason why using less viscous lipids the kinetics become faster (see Fig. 1B). This model can fit the major part of our data and is a tentative suggestion for

the structure of the monazomycin channel.

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