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SINGLE CHANNEL CONDUCTANCE AT LIPID BILAYER MEMBRANES IN PRESENCE OF MONAZOMYCIN

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SUMMARY

The single channel conductance in the presence of the antibiotic monazomycin at lipid bilayer membranes was measured. It was found to be 3 times smaller than the single channel conductance of gramicidin A under the same conditions. The conductance of the monazomycin pore is voltage independent.

Monazomycin, a polyenelike antibiotic isolated from *Streptomyces* [1], induces a voltage-dependent conductance in lipid bilayer membranes [2–4]. Monazomycin is selective for monovalent cations and alters the membrane conductance if it is applied to one or both sides of the membrane. When monazomycin is only present on one side, it is active if the electric potential on the monazomycin containing compartment of the cuvette is more positive than on the other compartment [2, 3]. The action of monazomycin is very similar to the behavior of the antibiotic alamethicin at lipid membranes [4]. It was shown previously [5–7], that alamethicin acts as a voltage dependent channel-former. The current response to a voltage jump in the presence of monazomycin is similar to the behavior of the potassium current in the squid axon membrane.

In this report we present the single channel conductance in the presence of monazomycin. Recently, similar experiments were carried out independently by Muller and Anderson [8].

Optically black lipid membranes were formed in the usual way [9] in a thermostated Teflon cell filled with aqueous electrolyte solution. The area of the membrane was about $7 \cdot 10^{-3}$ cm^2. Black platinum electrodes were used. The monazomycin was a generous gift by Dr. H. Yonehara, Tokyo.

For lipid synthetic and chromatographically pure L-α-diphytanoyl-lecithin 1 % (w/v) in *n*-decane was used. The electrolyte concentration in all experiments was 4 M CsCl. The pH of the electrolyte was about 6. All experiments were carried out at a temperature of 11 °C and 25 °C. The mean lifetime of the single channels was markedly increased at 11 °C to 25 °C. The monazomycin was added from an aqueous stock solution (1 mg/ml) to the electrolyte in the cuvette after forming the membrane.

Fig. 1 shows a typical plot of the single channel conductance in the presence of a small amount of monazomycin. The experiment was carried out in the following

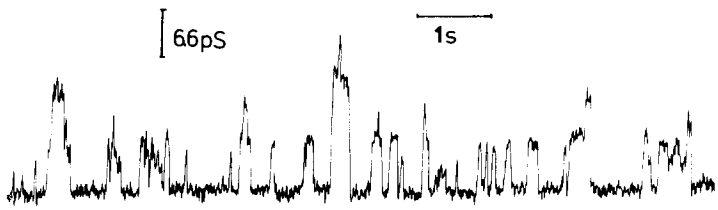

Fig. 1. Fluctuations of the membrane current in the presence of monazomycin $T = 11$ °C, 4 M CsCl, 1 % diphytanoyllecithin/*n*-decane (w/v). Voltage = 150 mV. The concentration of monazomycin was about 0.06 μg/ml. 1 pS = $10^{-12}\,\Omega^{-1}$.

way. After forming the membrane the monazomycin was added to the positive side of the cuvette. At 150 mV we obtained a large current with a sigmoid current-time characteristic. After reaching the steady state of the membrane conductance, we changed the polarity of the voltage, so that the monazomycin containing compartment was more negative than the other compartment of the cuvette. As a consequence of this we obtained a high macroscopic conductance, which decayed to zero because of the inactivation of the monazomycin. Before the monazomycin was completely inactivated the single channel response presented in Fig. 1 was obtained for some time. When the monazomycin was completely inactivated it was possible to repeat the experiment on the same membrane. Identical results for the single channel conductance Λ and the mean lifetime of channels τ^* were obtained, if we gave a very small amount of monazomycin (0.01 μg/ml) to the positive side of the membrane, so that at voltages $\geqq$ 300 mV a macroscopic conductance could build up. After "injection" of monazomycin to the membrane at this voltage we reduced the potential to 150 mV. As a result of this we obtained the same single channel response as can be seen in Fig. 1.

Fig. 2 shows the probability distribution $p(\Lambda)$ for the occurrence of a given

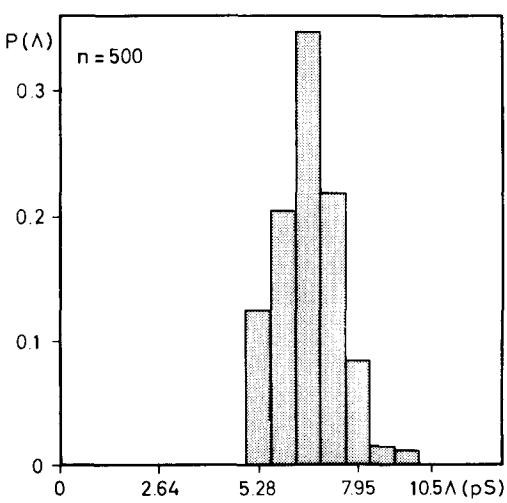

Fig. 2. Distribution of the amplitudes of the single channel conductances in presence of monazomycin. $T = 11$ °C, 4 M CsCl, 1 % diphytanoyllecithin/*n*-decane. n = number of events. Voltage = 150 mV, 4 M CsCl. The concentration of monazomycin was about 0.06 μg/ml.

value of Λ. This value is relatively narrow. As a measure of the width of the distribution we have introduced the interval $\Delta\Lambda$ covering 75 % of the recorded events [11]. The ratio $(\Delta\Lambda/\Lambda)_{75\ \%}$ is 0.2 in the case of monazomycin. This value is in the same range as we found for the gramicidins A, B, C for a dioleoyllecithin/*n*-decane membrane. Furthermore, it is interesting that we could not find different conductance levels of the channel as was found in the case of alamethicin [5–7]. But we cannot exclude the existence of different states of the pore with our experiments. Perhaps an equilibrium exists between the "small" and "larger" pores consisting of different numbers of monazomycin molecules. Possibly the equilibrium between the different states is on the side of the "small" pores. In that case we would not be able to measure possible large pores because of the superposition of the great number of small pores. The mean duration of the single channels at 11 °C is 0.17 s. This value was obtained by plotting the number of events with the corresponding lifetime versus time. From this plot we received an exponential decay with one time constant (Fig. 3a, b). From this behavior we can deduce that the monazomycin channels under this experimental condition do not interact with each other. At $T = 25$ °C a mean lifetime of 30 ms was found.

The current voltage behavior of the single channels is completely linear. It was impossible for us to find in the case of single channel experiments any voltage dependence, in contrast to the dramatic voltage dependence of the macroscopic membrane conductance.

From the temperature dependence of the single channel conductance we calculated the activation energy E_Λ for the ion transfer through the pore. For $T =$ 11 °C we obtained a value of $\Lambda_{11\ °C} = 6.6$ pS and for 25 °C a value of $\Lambda_{25\ °C} = 16$ pS, The activation energy was about 10 kcal/mol. This value is slightly higher than the value which was found for the gramicidin A channel (7.3 kcal/mol) [10]. The difference

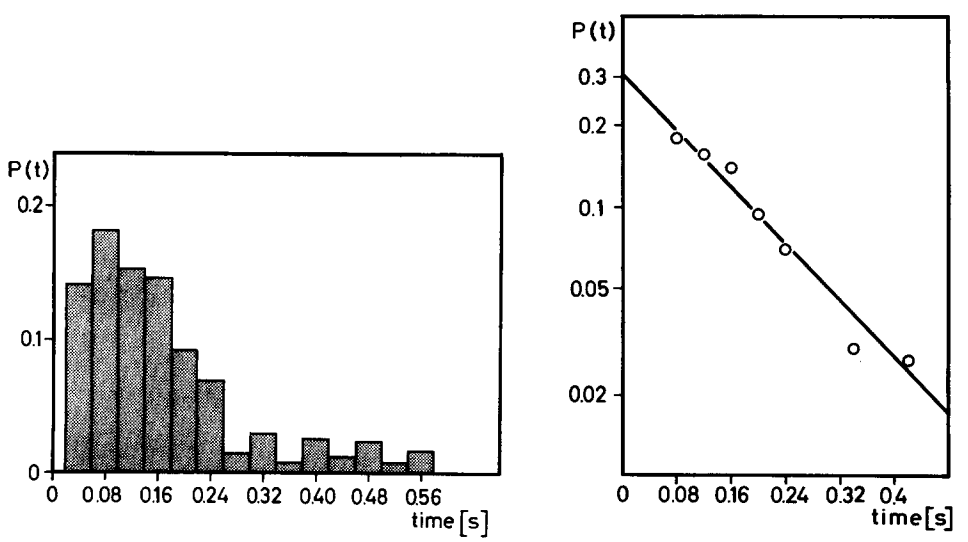

Fig. 3. Statistical analysis of the life time of monazomycin channels. (a) Probability of occurrences with a certain life time $P(t)$ versus time $T = 11$ °C, voltage = 150 mV, $n = 500$. (b) Plot of (a) in semilogarithmic form. Number of events $n = 500$, T = 11 °C, voltage = 150 mV.

is in agreement with the hypothesis that a pore with a smaller single channel conductance has a higher activation energy than a pore with a higher Λ. It seems reasonable that in a pore with a lower conductance the ions have to pass through a higher energy barrier than in a larger pore.

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