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THE EFFECT OF LANTHANUM ON ALAMETHICIN CHANNELS IN BLACK LIPID BILAYERS

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Summary

The properties of alamethicin channels in dioleoyl phosphatidylcholine bilayers were studied in 1 M $LaCl_3$ and were compared with those in 1 M NaCl. Single-channel recordings demonstrated that the mean single-channel life-time is about 0.25 s in NaCl but only about 17 ms in $LaCl_3$. Whereas in NaCl the conductance levels 2 and 3 are mostly populated, in $LaCl_3$ the levels 0 and 1 are preferentially adopted. The single-level conductances are slightly smaller in $LaCl_3$ if the higher bulk solution conductivity of $LaCl_3$ is taken into account. Multipore experiments confirmed earlier results (Boheim, G., Irmscher, G. and Jung, G. (1978) Biochim. Biophys. Acta 507, 485—506) that the bilayer conductance is less strongly dependent on voltage in $LaCl_3$ than in NaCl solution. Current-fluctuation analysis showed that this effect can be explained by a less strong dependence on voltage of the pore-formation rate as well as of the mean channel life-time in $LaCl_3$. The data can be interpreted as an increased lateral diffusion mobility of the alamethicin monomers in the bilayer. This can be the result of the binding of La^{3+} to the polar headgroups which can induce cluster formation of the phospholipids.

Introduction

Alamethicin, a linear polypeptide antibiotic, forms fluctuating channels in lipid bilayer membranes [1—6]. An open pore possesses several discrete conductance levels which are adopted in a consecutive order. The alamethicin-

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induced conductance of the bilayer increases considerably with voltage, which is predominantly caused by an increase in pore-formation rate [3,7]. It could be demonstrated that the formation of channels depends on the concentration of both alamethicin and salt [3,5,8]. On the other hand, it was reported that the discriminating ability of alamethicin with respect to different alkali cations is poor and that the pore-formation properties are not influenced by Ca^{2+} and Mg^{2+} [9]. However, a significantly less strong dependence of the alamethicin-induced conductance on the voltage was found with 1 M $LaCl_3$ as well as after addition of small amounts of UO_2^{2+} to the solution [9]. These effects are assumed to be due to the interaction of La^{3+} or UO_2^{2+} with the bilayer matrix. In this study we investigated the effect of La^{3+} on the alamethicin channels with fluctuation analysis in multipore and single-pore systems and compared the results with those obtained in NaCl solution.

Theory

As the pore-forming molecules in the bilayer may be exchanged with those in the solution, it can be assumed that the alamethicin channels form an open ensemble. Power-density spectra, resulting from an open ensemble, were calculated by Chen [10]. For a one-step reaction the formalism yields:

$$S(\omega) = 4i^2 \cdot \frac{\tau^2\mu}{1 + \omega^2\tau} \qquad (1)$$

and

$$\tau = \frac{1}{\eta} \qquad (2)$$

where $S(\omega)$ is the power density, μ and η the pore formation and decay rates, respectively, i the single-channel current and τ the relaxation time. Eqn. 1 describes a Lorentzian curve with corner frequency:

$$f_c = \frac{1}{2\pi\tau} \qquad (3)$$

and plateau value:

$$S_o = 4i^2\tau^2\mu \qquad (4)$$

In a single-channel system, the pore-formation rate can be estimated by direct counts so that the single-channel current can be easily obtained from Eqn. 4. In a multipore system the macroscopic current, I, is given by [5]:

$$I = i\mu\tau \qquad (5)$$

From Eqns. 4 and 5 one obtains:

$$i = \frac{S_o}{4I\tau} \qquad (6)$$

Materials and Methods

Black lipid membranes were formed from a decane solution containing 2.5% (w/v) of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (Serdary Research) and 2.5%

(w/v) of recrystallized cholesterol (Fluka). Alamethicin was a gift from Upjohn Company (Puurs, Belgium). As reported by Martin and Williams [11], it is a mixture of three components, consisting of approx. 85% of the R_f 30, approx. 12% of the R_f 50 and approx. 2% of the R_f 20 fractions. Alamethicin was added from a stock solution of 10^{-4} g/ml in ethanol/water (1 : 9, v/v) in amounts of 0.2 ml to 100 ml of electrolyte solution. Salt solutions were 1 M NaCl (pH 5.7) and 1 M $LaCl_3$ (pH 4.0) which were unbuffered. NaCl was analytical grade (Merck) and $LaCl_3$ was 99.9% pure (Fluka). Membranes were formed with the brush technique on a hole in a Teflon plate. The diameter of the hole was 0.8 mm in multipore and 0.3 mm in single-pore experiments. The Teflon plates were mounted between two halves of a Plexiglas chamber, which was inserted in a metal box, made of 7-mm-thick iron plates. Mechanical vibrations were greatly reduced by placing the metal box on a motorcycle innertube which, in addition was weighted with lead.

The voltage was applied to the membrane through Ag-AgCl electrodes. The electrical current passing through the membrane was converted to a voltage by an operational amplifier (AD 42 K, feedback resistance 10 MΩ). The output was monitored on an XT recorder (Bryans 28 000). In multichannel experiments, the signal was high-pass filtered (cut-off at 0.08 Hz), amplified (100—1000-fold) and stored on a magnetic tape recorder (Phillips, EL 1020). In single-channel experiments, the signal was d.c.-coupled to the voltage amplifier. Further treatment and analysis of the data were as described elsewhere [12,13]. After low-pass filtering (Rockland 852, 48 dB/octave) with 220 and 2200 Hz, the signal was digitized at intervals of 2 or 20 ms, respectively. One record consisted of 2048 points of 4.096 s length. The data were analysed off-line on a PDP 11/34 computer, using a fast Fourier transform program. The spectral densities were plotted as a function of the frequency on a double-logarithmic scale on the monitor of a graphic display processor (VT 11, Digital Equipment). One power spectrum consisted of the sum of the spectra sampled with 2 and 20 ms, each of which represents the means of 40 analysed records. The datum points in the power spectrum were fitted with the sum of two Lorentzians by a non-linear method of least-squares [13]. Datum points in the high-frequency range, which sometimes overlapped with the amplifier background noise, could be excluded from the fitting procedure.

The experimental protocol was similar to that described by Kolb and Boheim [6]. After equilibration of the membrane for 90 min at zero voltage, the voltage was increased until fluctuations could be observed. Then the voltage was increased in steps of 5 mV and held constant for about 3 min during recording of the signals. All experiments were performed at room temperature (22°C). Errors are given as standard deviations of the mean. Experimental data describing the conductance and voltage dependence of parameters were fitted with linear regression by the method of least-squares.

Results

Single-pore experiments

In the absence of the antibiotic, no significant difference in bilayer conductance could be detected between 1 M NaCl and 1 M $LaCl_3$. In the presence of

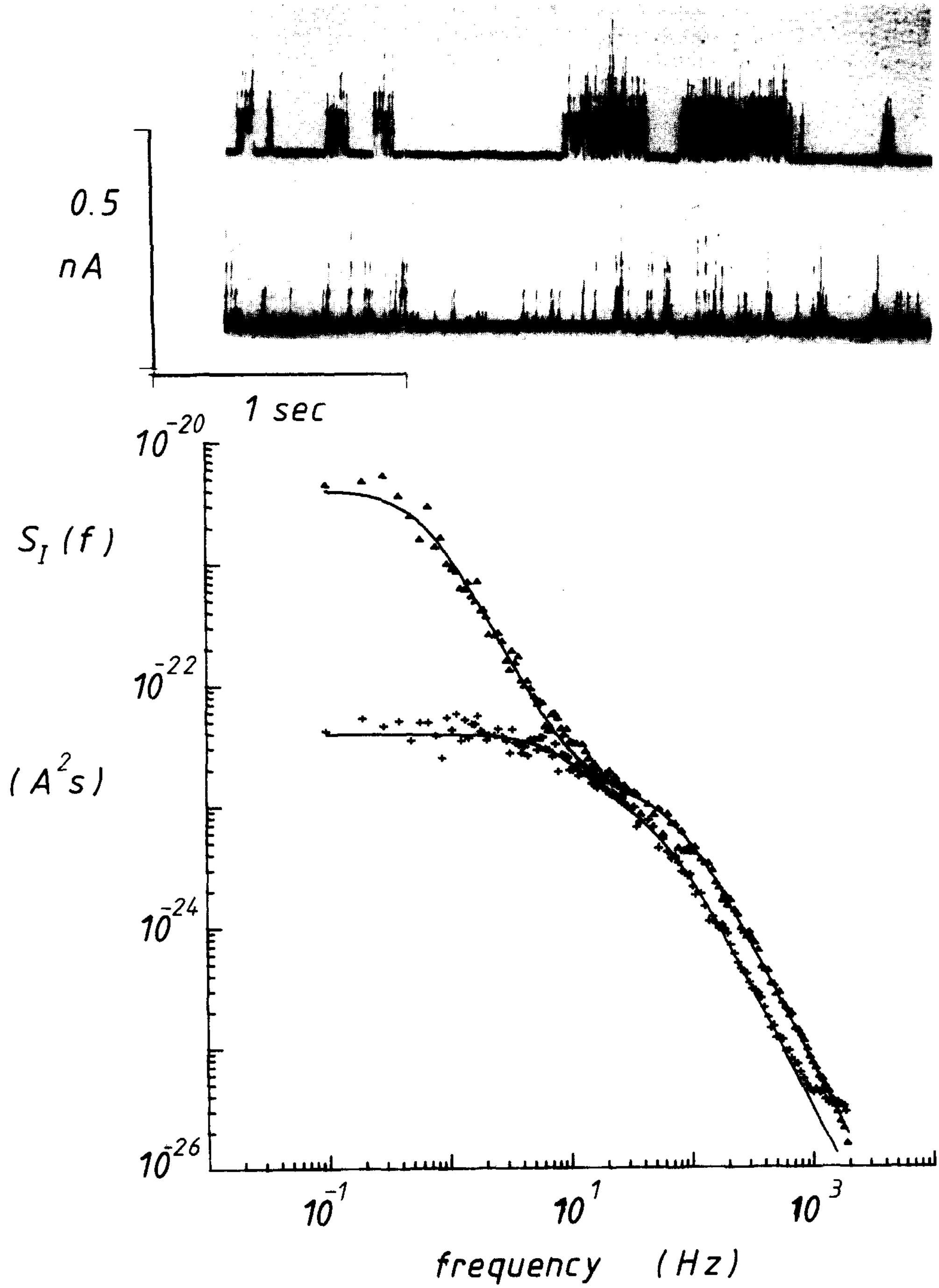

Fig. 1. Single-channel recordings and power spectra $S(f)$ of the current noise of alamethicin-doped bilayer membranes. The upper trace and the spectrum indicated by triangles were recorded in 1 M NaCl, the lower trace and the spectrum indicated by crosses were measured in 1 M $LaCl_3$. The datum points of the power spectra were fitted by the sum of two Lorentzians (solid lines). Membrane solution: 2.5% (w/v) of cholesterol and 2.5% (w/v) of dioleoyl phosphatidylcholine in *n*-decane. Alamethicin: $2 \cdot 10^{-7}$ g/ml. Applied voltage: $V = 52.5$ mV in NaCl and $V = 40$ mV in $LaCl_3$.

$2 \cdot 10^{-7}$ g/ml alamethicin, the voltage at which the first channels appeared was approx. 50 mV in 1 M NaCl and approx. 70 mV in 1 M $LaCl_3$. Fig. 1 demonstrates single-channel fluctuations recorded in 1 M NaCl (upper trace) and 1 M $LaCl_3$ (lower trace). Estimation of the mean channel life-time yielded values of about 0.25 s in NaCl and about 17 ms in $LaCl_3$ solutions. In addition, many small spikes are observable in $LaCl_3$ solution. Whereas in NaCl up to six conductance levels could be identified, in $LaCl_3$ maximally five levels were observed. The highest conductance level, however, was scarcely adopted in both cases. The small conductance level at 19 pS, detected by Hanke and Boheim [14] in 1 M KCl, was not resolved in our experiments. Table I demonstrates that the single-level conductances in $LaCl_3$ are smaller than in NaCl. In this table, the values in $LaCl_3$ were multiplied by the factor $\kappa(1\ \mathrm{M\ NaCl})/\kappa(1\ \mathrm{M\ LaCl_3}) = 0.67$ [9], where κ represents the bulk solution conductivity. In solutions containing 1 M NaCl + 0.1 M $LaCl_3$, the single-level conductances were not significantly different from those in 1 M NaCl. Table I shows in addition that the pore states 2 and 3 are the most probable ones in NaCl solution, whereas the first conducting level is preferentially populated in $LaCl_3$. The mean single-channel conductance, calculated from statistical analysis of single-level conductances yields $\Lambda_s = 1.8$ nS in NaCl and $\Lambda_s = 0.7$ nS in $LaCl_3$.

The lower part of Fig. 1 demonstrates the corresponding power spectra. As described elsewhere [6,15,16], the power spectrum in NaCl consists of two Lorentzian components, described by a slow (τ_s) and a fast (τ_f) relaxation time. It has been shown that τ_s describes the formation and decay of pore units, whereas τ_f results from the jumps between the discrete conductance levels within an open pore. According to the calculations of Chen [10], the jumps between the six levels should yield five Lorentzian components. However, only one fast relaxation component can be resolved in the power spectrum. Kolb and Boheim [6] assumed that the fast component represents the transitions between the most probable states. As can be seen in Fig. 1 (curve with triangles), the datum points deviated systematically from the fitted curve in the transition region between the two Lorentzians. This deviation probably reflects the existence of the additional relaxation components, which cannot be resolved by this method. As demonstrated by Kolb and Boheim [6], the mean time open θ should be equal to the relaxation time τ. Hence, the shorter life-

TABLE I

The fraction of time (T_υ/T_t) for which the pore adopts the conductance level υ and conductance values, Λ_υ, of the different pore states in the case of 1 M NaCl and 1 M $LaCl_3$. The Λ_υ values in 1 M $LaCl_3$ were multiplied by $\kappa(1\ \mathrm{M\ NaCl})/\kappa(1\ \mathrm{M\ LaCl_3})$.

υ	1 M NaCl		1 M $LaCl_3$	
	T_υ/T_t	Λ_υ (nS)	T_υ/T_t	Λ_υ (nS)
0	0.17	0	0.39	0
1	0.14	0.64	0.43	0.43
2	0.32	1.66	0.13	1.19
3	0.29	2.82	0.04	2.30
4	0.07	4.10	0.007	3.54
5	0.01	5.38		

TABLE II

Exponential factors, α, characterizing the voltage dependence of the conductance (Λ), the slow relaxation time (τ_s), the fast relaxation time (τ_f), the pore decay rate (η), the pore-formation rate (μ) and the single-channel conductance (Λ_s) in 1 M NaCl and 1 M $LaCl_3$. n, number of observations.

Exponential factor	1 M NaCl	n	1 M $LaCl_3$	n
$\alpha(\Lambda)$	5.6 ± 0.2	11	2.2 ± 0.3	12
$\alpha(\tau_s)$	1.8 ± 0.2	6	0.9 ± 0.4	4
$\alpha(\tau_f)$	−(0.4 ± 0.2)	6	(0.4 ± 0.3)	4
$\alpha(\eta)$	−(1.9 ± 0.2)	6	−(0.9 ± 0.4)	4
$\alpha(\mu)$	3.4 ± 0.6	6	1.2 ± 0.3	4
$\alpha(\Lambda_s)$	0.31 ± 0.17	6	−(0.03 ± 0.30)	4

time of the pores in $LaCl_3$ results in an increased corner frequency of the slow component as is obvious in Fig. 1 (curve with crosses). The fast relaxation time in $LaCl_3$ is slightly smaller in the demonstrated example. However, multipore experiments showed τ_f to be smaller by a factor of 2.6 at the conductance of 1 $\mu S/cm^2$ in $LaCl_3$ (Table III, row 4).

In 1 M NaCl, the single-channel conductance was calculated from three experiments according to Eqn. 4. The pore-formation rate, μ, was estimated to be 0.64 ± 0.07 s^{-1} and Λ_s was 2.3 ± 0.2 nS. This value is close to that obtained by Kolb and Boheim [6] from single-channel experiments. The discrepancy between Λ_s calculated from fluctuation analysis and that obtained from statistical analysis of single-level conductances probably reflects uncertainties in the latter method, where only part of one open pore was analysed. Fluctuation analysis, however, yields an average of Λ_s over many pores. In $LaCl_3$ it is difficult to count the number of pores, because of the frequent appearance of short-lived spikes. Therefore, the single-channel conductance in $LaCl_3$ was estimated only from multipore fluctuations.

Multipore experiments

We recorded the conductance-voltage relationships in 1 M NaCl and 1 M

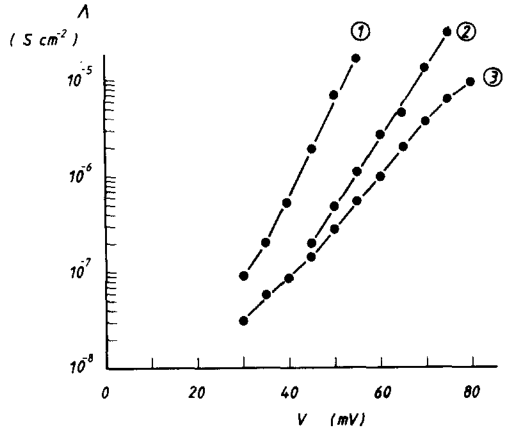

Fig. 2. Semi-logarithmic plot of the bilayer conductance Λ vs. the applied voltage V. The electrolyte solution was 1 M NaCl (curve 1), 1 M NaCl + 0.1 M $LaCl_3$ (curve 2) and 1 M $LaCl_3$ (curve 3). For details see text.

$LaCl_3$ as well as in 1 M NaCl + 0.1 M $LaCl_3$. Representative experiments, demonstrated in Fig. 2, show that voltage-independent components are present only in small amounts under the experimental conditions described in Materials and Methods. The increase of the conductance with voltage was less pronounced in solutions containing 1 M $LaCl_3$ or 1 M NaCl + 0.1 M $LaCl_3$ than in 1 M NaCl. The voltage dependence of the conductance is described by [5]:

$$\Lambda \sim \exp(\alpha(\Lambda)FV/RT) \qquad (7)$$

where F is the Faraday constant, R the gas constant, T the absolute temperature and V the applied voltage . The datum points of the voltage-dependent components of the conductance-voltage curves were fitted by straight lines, as shown in Fig. 2. The values for the slopes $\alpha(\Lambda)$ are summarized in Table II, row I. Boheim et al. [9] reported $\alpha(\Lambda)$ to be 6.8 in 1 M NaCl and 2.3 in 1 M $LaCl_3$; the latter value being close to that which we measured. In 1 M NaCl + 0.1 M $LaCl_3$ we obtained $\alpha(\Lambda) = 3.0 \pm 0.3$ ($n = 19$). This value is considerably smaller than that calculated in 1 M NaCl, but significant greater than $\alpha(\Lambda)$ in 1 M $LaCl_3$.

Fluctuation analysis was only evaluated with 1 M NaCl and 1 M $LaCl_3$ as solutions. The exponential factors which describe the voltage dependence of the parameters τ_s, τ_f, η, μ and Λ_s, calculated according to Eqn. 7, are listed in Table II. The slow time constant and the pore-formation rate as well as the pore-decay rate are less strongly dependent on the voltage in $LaCl_3$ than in NaCl solution. The single-pore conductance increases with voltage in NaCl but is unaffected in $LaCl_3$ solution. The fast time constant is only slightly dependent on voltage in both cases. The conductance dependence of the parameters τ_s, τ_f, η, μ and Λ_s is described by [5]:

$$\Lambda \sim x^{\alpha^*(x)} \qquad (8)$$

where x represents the respective parameter. The values for α^* are summarized in Table III. The table demonstrates that the slopes α^* of all parameters recorded in NaCl or $LaCl_3$ are comparable. However, the magnitudes of the parameters τ_s, τ_f and Λ_s, which are compared at the conductance 1 $\mu S/cm^2$, are

TABLE III

Exponential factors, α *, characterizing the conductance dependence of the slow (τ_s) and fast relaxation time (τ_f), the pore-formation (η) and pore-decay rate (μ) and the single-channel conductance (Λ_s), as well as values of these parameters at the conductance 10^{-6} ($S \cdot cm^{-2}$) in 1 M NaCl and 1 M $LaCl_3$. Number of observations: $n = 8$ in NaCl and $n = 4$ in $LaCl_3$.

	1 M NaCl	1 M $LaCl_3$
α * (τ_s)	0.39 ± 0.05	0.40 ± 0.11
τ_s ($\Lambda_{10^{-6}}$) (ms)	141 ± 33	58 ± 9
α * (τ_f)	—(0.06 ± 0.03)	(0.23 ± 0.16)
τ_f ($\Lambda_{10^{-6}}$) (ms)	3.4 ± 0.6)	1.3 ± 0.6)
α * (η)	—(0.39 ± 0.06)	—(0.40 ± 0.11)
η ($\Lambda_{10^{-6}}$) (s^{-1})	10.6 ± 2.2	18.7 ± 3.2
α * (μ)	0.54 ± 0.08	0.56 ± 0.08
μ ($\Lambda_{10^{-6}}$) (s^{-1})	74 ± 18	911 ± 69
α * (Λ_s)	0.07 ± 0.03	0.07 ± 0.16
Λ_s ($\Lambda_{10^{-6}}$) (S)	$(7.0 \pm 0.6) \cdot 10^{-10}$	$(1.5 \pm 0.8) \cdot 10^{-10}$

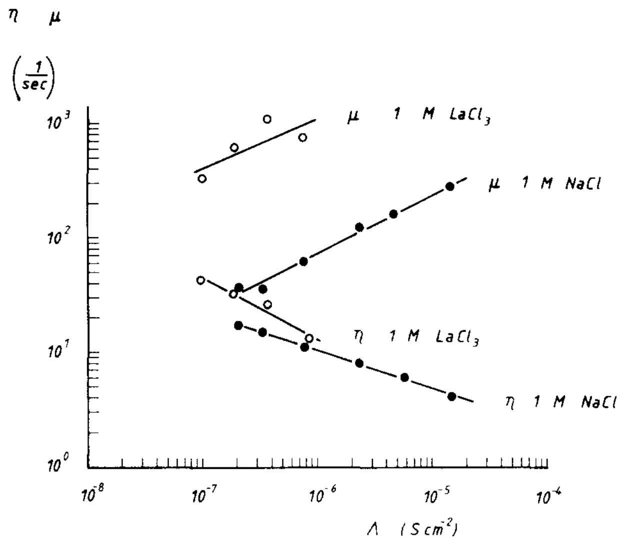

Fig. 3. Double-logarithmic plot of the pore-formation rate, μ, and of the pore-decay rate, η, vs. the bilayer conductance, Λ, with 1 M NaCl and 1 M $LaCl_3$ as salt solutions.

significantly smaller in $LaCl_3$. In contrast, the pore-formation and -decay rates are greater in $LaCl_3$ than in NaCl solution. The dependence of η and μ on the conductance Λ is demonstrated in Fig. 3. As can be seen, especially the pore-formation rate μ is much greater in $LaCl_3$ than in NaCl solution. Comparison of the single-pore conductance recorded in single-pore and multipore experiments shows that both with NaCl and $LaCl_3$ solutions, Λ_s is smaller by a factor of about 3 and 4.7, respectively, in multipore systems. This is in agreement with observations of Kolb and Boheim [6], performed in 1 M KCl. They estimated Λ_s in single-pore experiments by direct analysis of the single-level conductances and obtained Λ_s in multipore systems from autocorrelation analysis.

Discussion

Our studies confirmed the results of Boheim et al. [9] that the alamethicin pores are considerably less dependent on voltage in 1 M $LaCl_3$ than in 1 M NaCl. Single-pore experiments showed that channels in $LaCl_3$ appeared at a voltage approx. 20 mV higher than in NaCl. The most striking feature of single channels in $LaCl_3$ is their short life-time and the frequent occurrence of spikes. These spikes are pores which adopt only once the first or second conductance level before they decay immediately. Short-lived pores were also observed in KCl and NaCl (Ref. 6; and unpublished observations), but they appeared much less frequently than in $LaCl_3$. Boheim et al. [9] reported a frequent occurrence of spikes in bilayers doped with trichotoxin. However, in this system, the spikes were superimposed on the conductance of long-lived pores. The single-level conductances of alamethicin pores in $LaCl_3$ are only slightly different from those measured in NaCl (Table I) (Λ_v : S.D. $\approx 20\%$). However, in $LaCl_3$ the first conductance level is the most populated one and the pore frequently adopts the state with zero conductance within an open pore. The preferential population of low conductance states explains the low single-channel conductance in $LaCl_3$ solution. The lesser dependence on voltage of the conductance in $LaCl_3$ solution can be explained by the lesser dependence of the pore-formation rate and the mean time open of a pore on the voltage. The data demonstrate that properties of an open pore, such as the single-level conductance and the fast relaxation time, are slightly different in both electrolytes. However, the process of pore formation and decay is quite different in both cases: In $LaCl_3$ solution the pores have an appreciably shorter life-time and frequently adopt the state with zero conductance from which the pore decays preferentially. In addition, the pore-formation rate is less strongly voltage dependent in the presence of La^{3+}. The influence of the trivalent ion on the kinetics of alamethicin-channel formation could result from the following facts: (1) interaction of La^{3+} with the alamethicin molecules on the bilayer surface; (2) changes in the local surface potential, caused by La^{3+} and (3) influence of La^{3+} on properties of the lipid matrix. Boheim et al. [9] reported that divalent cations, such as Ca^{2+} and Mg^{2+}, or divalent anions (SO_4^{2-}) did not significantly change the conductance-voltage relationship. Therefore, they assumed that the pore-formation process is not caused by specific ion-alamethicin interaction but is due to the permanent dipole moment of the antibiotic [17]. Changes in the process of micellization and preaggregate formation by binding of La^{3+} to the alamethicin monomers [18], however, cannot be excluded. La^{3+} possibly changes the local surface potential of the bilayer [19]. As the ion was present in the same amounts on both sides of the membrane, this effect should be identical on both sides. It was reported that symmetrically placed charges on the bilayer surface do not have an effect on the rate constants of the alamethicin-pore formation [20]. Consequently, changes in local electrical fields, caused by La^{3+}, are not likely to explain the short life-time of the channels. However, changes in the local field may explain the higher voltage needed to induce the pores in the presence of La^{3+}. The following arguments show that an interaction of La^{3+} with the lipid matrix may predominantly be responsible for the change in alamethicin-pore formation. It was reported that lanthanides interact with the polar headgroups of the lipids by formation of 2 : 1 phosphatidylcholine-metal ion complexes [19]. The affinity of the headgroups for lanthanide ions is about two orders of magnitude larger than for divalent ions [21]. From ^{31}P-NMR experiments it was concluded that the interaction with lanthanides induces changes in the headgroup conformation of lecithin bilayers [22,23]. Dilatometric and calorimetric studies confirmed these conclusions and indicated further that, as a consequence of the La^{3+} interaction with the phosphatidylcholine headgroups, a tighter packing of the lipid molecules is produced and that the mobility of the headgroups is lowered [24]. It is likely that the interference of La^{3+} with these headgroups is responsible for the changes in alamethicin-pore formation. Boheim and Kolb [5] proposed that in KCl solution, prior to pore formation, a preaggregate of six molecules is formed at the membrane interface. Due to the applied electric field, two or three molecules are simultaneously inserted into the membrane and form a pore nucleus which increases by uptake of additional monomers. The authors assume further that after pore nucleation, the non-integrated monomers may diffuse away, so that the mean life-time of a pore depends on the dissociation rate of these non-integrated monomers from the nucleus. The short life-time of the alamethicin pores in the presence of La^{3+} and the frequent occurrence of spikes may therefore result from a high dissociation rate and a large mobility of alamethicin molecules at the membrane interface. On the other hand, it was shown that the mobility of polar headgroups decreases after interaction with La^{3+} [24]. Complexation of the lipid headgroups by polyvalent ions, however, can induce cluster formation of the phospholipids, so that the mobility of the alamethicin molecules, squeezed out from the cluster patches, is actually increased. Analogous effects have been observed with Ca^{2+} on negatively charged membranes [25].

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