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THE LOWEST CONDUCTANCE STATE OF THE ALAMETHICIN PORE

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Summary

High resolution experiments of the alamethicin pore demonstrate the existence of a further pore state at low conductance values. This lowest resolved conductance state is found at 19 pS in 1 M KCl at room temperature. The value differs from that of the next higher conductance state by a factor of 14—15 and is approx. 20% lower than the gramicidin A pore conductance. The lowest conductance state seems to be impermeable to Ca^{2+}, Cl^-, Tris-H^+ and Hepes$^-$, whereas the higher conductance states are not.

The polypeptide antibiotic, alamethicin, forms voltage-dependent ion-conducting pores in lipid bilayer membranes [1—4]. A single pore aggregate adopts several discrete conductance states, the values of which are not integral multiples of each other. The molecular model of alamethicin pore structure is still a subject of controversy. A model (a) was proposed by Gordon and Haydon [5, 6] which explains the peculiar conductance sequence on the basis of a bunch of parallel pores which open and close statistically. On the other hand, Baumann and Mueller [7, 8] and Boheim [4, 9] proposed a model (b) which represents a pore of variable diameter. The pore lumen grows or becomes smaller by uptake or release of single alamethicin molecules ('barrel stave model').

Evidence for model a came from experimental observations [5, 6] that the conductance differences between higher neighbouring states are approximately equal and that the alamethicin pore aggregate seems to be impermeable to the tetramethylammonium and Tris cations. The investigations in question were carried out with Cl^- as (permeable) anion. On the other hand,

Abbreviation: Hepes, *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid.

the non-integral conductance steps at low levels were taken as evidence for model b. In addition, the latter model is strongly-supported by the experimental fact that the conductance of the alamethicin pore system increases with the 9th—10th power of alamethicin concentration [3, 9]. In order to distinguish, experimentally, between the two models, we carried out high-resolution experiments. We found; (1) a new lowest conductance state the value of which is lower by a factor of 14—15 than the conductance of the next higher state, (2) that the alamethicin pore (except the lowest state) is permeable to the cation, Tris-H^+, and the anion, $Hepes^-$, which are both comparable in size to tetramethylammonium, (3) that the lowest conductance state is impermeable to Ca^{2+} and Cl^- (within our limits of experimental resolution) which is also reported for the gramicidin A pore [10, 11].

Lipid bilayer membranes were formed from a bacterial phosphatidylethanolamine (Analabs, used without further purification)/hexane solution according to the method of Montal and Mueller [12]. The pure alamethicin R_F30 component was purchased from the Microbiological Research Establishment, Porton Down, Salisbury. The alkali chlorides and $CaCl_2$ were p.a.-grade from Merck. Tris and Hepes, both analytical grade from Sigma, were recrystallized and checked for purity. The resolution of the experimental measuring system was approx. 0.4 pA in amplitude with a membrane area of $0.7 \cdot 10^{-4}$ cm^2 and approx. 0.5 ms in time (unpublished data). Bulk solution conductivities, κ, were checked and measured by a conductivity cell, respectively [13].

Alamethicin single pore fluctuations have been measured with different salt solutions at 21°C. Fig. 1 shows a fluctuation trace at low conductance states in the case of 3 M CsCl. Three different levels besides bare membrane level (index 0) can be distinguished. The corresponding conductance values range from 51 pS (± 10%) (level 1), 710 pS (level 2) to 3400 pS (level 3).

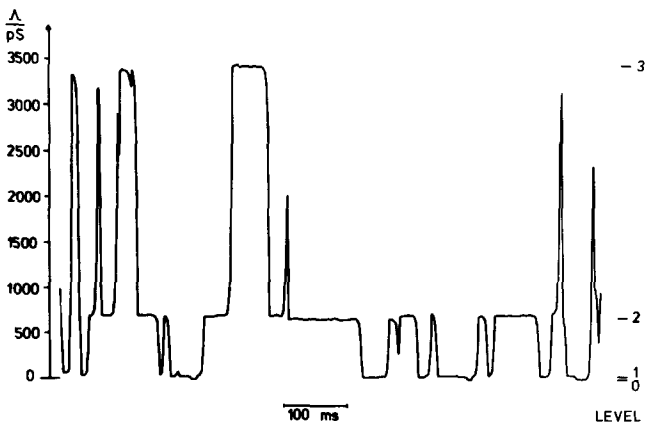

Fig. 1. Single pore fluctuations of an alamethicin-doped lipid bilayer membrane at low conductance states. The fluctuation trace demonstrates that the conductance of the lowest state (level 1) is lower by a factor of 14—15 than the conductance of the next higher state (level 2), which in turn is about 5 times lower than the following one (level 3). Membrane lipid, bacterial phosphatidylethanolamine; membrane-forming procedure, Montal-Mueller technique [12]; membrane area, $0.7 \cdot 10^{-4}$ cm^2; salt solution, 3 M CsCl, pH 5.9; alamethicin R_F30 concentration, 1 $\mu g \cdot cm^{-3}$, on the side of the more positive potential only; temperature, 21°C; applied voltage 120 mV.

This means that the conductances of levels 1 and 3 differ by nearly two orders of magnitude. This experimental finding seems to be inconsistent with the picture of a bunch of identical pores (model a), whereas this result is expected in the case of a pore of variable diameter (model b). It should be mentioned at this point that Gordon and Haydon [5] reported the appearance of additional low conductance levels using 3 M CsCl, however, at very high applied voltages.

In order to check the permeability of the alamethicin pore to large ions, experiments with a large cation, Tris-H^+, and a large anion, $Hepes^-$, (ionic diameters $\delta \approx 0.6$—0.65 nm), were carried out. With a 1 M Tris-Hepes (1 M Tris + 1 M Hepes) solution, pH 8.0, the following conductance sequence is observed: 8 (± 50%), 47 (± 10%), 130, 240 and 360 pS (for experimental conditions, see legend of Table I). For comparison, we recorded the corresponding sequence in the case of 1 M KCl: 19 (± 20%), 280 (± 5%), 1300, 2700, 4400 and 6200 pS. Now, we had to solve the problem of level identification. Using 1 M Tris-Hepes solutions mixed with increasing amounts of KCl (from 1 mM to 1 M KCl; unpublished data) the following assignment was found: level 2 of 1 M KCl corresponds to level 1 of 1 M Tris-Hepes (Table I). Thus, the conductance values which originate from the same pore state differ by a factor of 35. Furthermore, Tris-H^+ would not contribute more than 5% to the pore state conductances in the case of 1 M Tris-HCl and this is beyond the resolution (approx. 5%) of the experiments of Gordon and Haydon [6] which explains their negative results.

In the following, we will designate level 1 of the fluctuation trace in Fig. 1 as pore state $\nu = 1$, level 2 as pore state $\nu = 2$ and so forth. On the basis of the barrel stave model, b, a given pore state is formed by a distinct number of alamethicin molecules. It is assumed that the molecules are arranged in the form of a circle and that the width of a molecule represents a constant circumference segment, a. Then the relation between segment a and radius r is given by:

$$r = n \cdot \frac{a}{2\pi} \tag{1}$$

where n is the number of alamethicin molecules which build up the pore. In order to estimate the length of segment a we assume that for an uncharged

TABLE I

Diameters, δ_ν (based on model b of a pore of variable diameter) and conductance values, Λ_ν, of the different pore states, ν, in the case of 1 M KCl, pH 5.8 and 1 M Tris-Hepes, pH 8.0. For details see text. Membrane lipid, bacterial phosphatidylethanolamine; membrane-forming procedure, Montal-Mueller technique [12]; membrane area, $0.7 \cdot 10^{-4}$ cm^2; alamethicin R_F30 concentration, 2 $\mu g \cdot cm^{-3}$, on the side of the more positive potential only; temperature 21°C; applied voltage, 90 mV.

ν	δ_ν (nm)	Λ_ν (pS)	
		KCl	Tris-Hepes
1	0.63	19	—
2	0.84	280	8
3	1.05	1300	47
4	1.26	2700	130
5	1.47	4400	240
6	1.68	6200	360

membrane the specific conductivity, κ_p, of a very large pore is equal to the bulk solution conductivity, κ:

$$\kappa = \kappa_p = \Lambda \cdot \frac{d}{A} \tag{2}$$

where Λ is the pore conductance; d, the pore length which is assumed to be 3 nm [9] and $A = \pi r^2$, the cross-sectional area of the pore lumen.

Eqn. 2 should be approximately valid for the higher conductance states of the alamethicin pore and beyond that for the conductance differences, $\Delta\Lambda$, of higher neighbouring states.
Using Eqns. 1 and 2 we obtain the relation:

$$\Delta\Lambda_{n,n+1} = \Lambda_{n+1} - \Lambda_n = \frac{\kappa}{d} \cdot \frac{a^2}{4} \cdot (2n + 1) \tag{3}$$

Fig. 2a shows a double-logarithmic plot of $\Delta\Lambda$ vs. $(2n + 1)$ for two different salt solutions: 1 M KCl, pH 5.8 and 1 M Tris-Hepes, pH 8.0. The slope of the

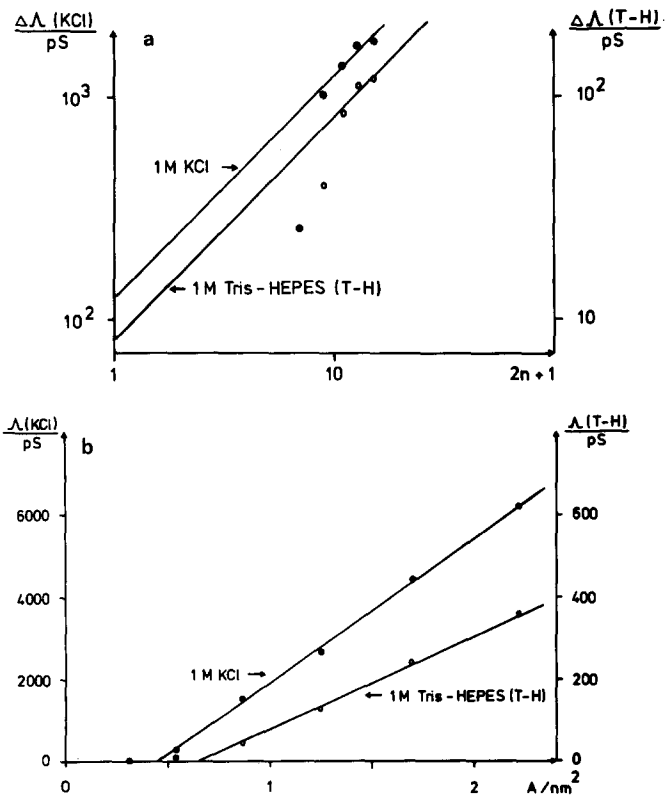

Fig. 2. (a) Double-logarithmic plot of conductance differences, $\Delta\Lambda$, of neighbouring states vs. a parameter $(2n + 1)$ which reflects the corresponding increase in cross-sectional area of the pore lumen based on model b of a pore of variable diameter. (b) Pore state conductances, Λ, plotted vs. area, A, of the cross-sectional pore lumen. Salt solutions: 1 M KCl, pH 5.8 (•) and 1 M Tris-Hepes, pH 8.0 (○). Experimental conditions are the same as those described in Table I. For details see text.

resulting straight lines has to be 1. Therefore, at $n = 0$ we obtain by extrapolation:

$$\Delta\Lambda_{0,1} = \frac{\kappa}{d} \cdot \frac{a^2}{4}$$

With given values for $d = 3$ nm, κ (1 M KCl) = 10.4 $S \cdot m^{-1}$ and the measured κ (1 M Tris-Hepes) = 0.61 $S \cdot m^{-1}$, we are able to calculate the length of segment a. In both cases, $a = 0.66$ nm (± 5%).

Up to now, we did not write down a relation between pore state, ν, and molecule number, n. Estimates from space-filling models led to the suggestion [4, 9] that the dimer aggregate might be non-conducting and that the trimer forms the lowest conductance state. In this case we obtain an overestimate for the corresponding area of the pore lumen, because the alamethicin molecule is expected not to adopt the ideal curvature of a circle. It is unlikely that the tetramer aggregate represents the lowest conductance state because of its large lumen diameter. The conductance of the gramicidin A pore with a diameter of 0.4 nm is already larger than the conductance of the lowest alamethicin pore state by approx. 20% under comparable conditions [14]. Therefore, the evaluation in Fig. 2a was based on the relation:

$$\nu = n-2 \tag{4}$$

In Table I, the lumen diameters, δ_ν, of the different pore states are listed. The corresponding conductances, Λ_ν, for 1 M KCl and 1 M Tris-Hepes solutions are added. It can be seen that the lowest conductance state might be impermeable to Tris-H^+ and $Hepes^-$ for sterical reasons. In order to illustrate the situation, Fig. 2b shows a plot of the pore state conductances, Λ_ν, vs. the cross-sectional area, A_ν, of the corresponding pore lumen. It can be seen that a straight line which is described by the equation:

$$\Lambda = \frac{\kappa}{d} \cdot (A-A_c) \tag{5}$$

fits the experimental points except for the lowest conductance values in both cases. By extrapolating the straight lines to $\Lambda = 0$, we obtain from the value of A_c a rough estimate of the pore diameter, δ_c, below which the pore conductance for a distinct ion species is no longer determined by bulk solution conductivity. From Fig. 2b, δ_c (1 M KCl) = 0.76 nm and δ_c (1 M Tris-Hepes) = 0.91 nm are calculated. An independent proof for these considerations is given by conductance measurements of pores which are formed by the matrix protein of *Escherichia coli* [15]. Both conductance values, for KCl and Tris-Hepes, fit into the straight lines at a pore diameter of 1.1 nm.

In a third series of experiments, we looked for ion-specific properties of the lowest conductance state of the alamethicin pore. Fig. 3 shows the selectivity sequence for alkali cations by introducing the ratio:

$$R = \frac{\Lambda \text{ (3 M alkali chloride)}}{\Lambda \text{ (3 M KCl)}} \tag{6}$$

Comparison with the corresponding ratio of bulk solution conductivities,

κ (3M alkali chloride)/κ (3 M KCl), demonstrates that the discrimination between different alkali cations of the lowest conductance state of the alamethicin pore is quite weak. In contrast to that, a more pronounced alkali cationic selectivity is found with the gramicidin A pore (Fig. 3) [14]. From this experimental observation, we may conclude that the diameter of the gramicidin A pore is smaller than the diameter of the lowest alamethicin pore state. The higher pore conductance in the case of gramicidin A, on the other hand, may result from the large number of negatively-charged carbonyl oxygens at the inner wall of the pore. From structural considerations [9], we suggest that the corresponding number is considerably smaller within the alamethicin pore which could lead to a higher activation energy for ion translocation.

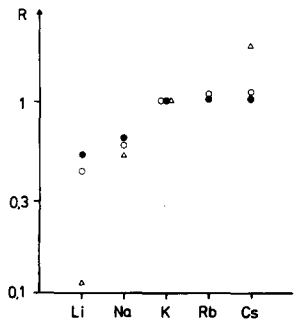

Fig. 3. Selectivity of the lowest conductance state of the alamethicin pore (○) for alkali cations. The parameter *R* stands for the ratio Λ (3 M alkali chloride)/Λ (3 M KCl). Apart from the salt species, experimental conditions are the same as in Fig. 1. For comparison, the corresponding ratios of bulk solution conductivities (●) κ (3 M alkali chloride)/κ (3 M KCl) at 21°C and of the gramicidin A pore conductances (△) (data from Bamberg et al. [14]) are shown.

Single pore measurements in the presence of 2 M $CaCl_2$ yielded the surprising result that the lowest conductance state of the alamethicin pore is virtually impermeable to Ca^{2+} and to Cl^-: $\Lambda_1 \approx 0$ (< 4 pS); Λ_2 = 190 pS; Λ_3 = 1100 pS. Λ_1 has to be lower than Λ_2 by at least a factor of 50. Furthermore, if Ca^{2+} is added to a 1 M KCl solution, Ca^{2+} concentration and voltage-dependent blocking effects are not only observed at the lowest pore state but also at the next higher one [16]. This effect of pore blockade by divalent cations is well-known for the gramicidin A pore [11].

Summarizing the above reported results, we conclude that the newly-found low conductance state supports the molecular model, b, of an alamethicin pore of variable diameter. The properties of this pore state are comparable to those of the gramicidin A pore in conductance but not in mean lifetime. The gramicidin A pore exhibits a mean lifetime which is up to two orders of magnitude larger than the mean lifetime of the alamethicin pore states [9, 14].

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