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ARTICLE

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Alamethicin channel conductance modified by lipid charge

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Abstract The membrane surface charge modifies the conductance of ion channels by changing the electric potential and redistributing the ionic composition in their vicinity. We have studied the effects of lipid charge on the conductance of a multi-state channel formed in planar lipid bilayers by the peptide antibiotic alamethicin. The channel conductance was measured in two lipids: in a neutral dioleoylphosphatidylethanolamine (DOPE) and a negatively charged dioleoylphosphatidylserine (DOPS). The charge state of DOPS was manipulated by the pH of the membrane-bathing solution. We find that at high salt concentrations (e.g., 2 M NaCl) the effect of the lipid charge is below the accuracy of our measurements. However, when the salt concentration in the membrane-bathing solution is decreased, the surface charge manifests itself as an increase in the conductance of the first two channel levels that correspond to the smallest conductive alamethicin aggregates. Our analysis shows that both the salt and pH dependence of the surface charge effect can be rationalized within the nonlinear Poisson-Boltzmann approach. Given channel conductance in neutral lipids, we use different procedures to account for the surface charge (e.g., introduce averaging over the channel aperture and take into account Na^+ adsorption to DOPS heads), but only one adjustable parameter: an effective distance from the nearest lipid charge to the channel mouth center. We show that this distance varies by 0.3–0.4 nm upon channel transition from the minimal conducting aggregate (level L0) to the next larger one (level L1). This conclusion is in accord with a simple geometrical model of alamethicin aggregation.

Keywords Surface charge · Double layer · Lipid titration · Protein electrostatics

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Introduction

The limits of applicability of classical continuum electrostatics at the length scales of a protein molecule are among the most vividly discussed issues of modern biophysics (Honig and Nicholls 1995; Eisenberg 1999; Murray et al. 1999; Cardenas et al. 2000; Moy et al. 2000). While Poisson-Boltzmann theory has proved to be a successful tool in studies of proteins and membranes, its potential for a quantitative description is repeatedly questioned. In the present paper we apply this theory to describe the influence of membrane lipid charge on ionic conductance of a transmembrane multistate channel formed by alamethicin.

The 20-amino acid peptide alamethicin is produced by the fungus *Trichoderma veride*. It is known that in lipid membranes it self-assembles to form channels which fluctuate between different conductance states, depending on the alamethicin aggregation (Hall et al. 1984; Sansom 1991; Cafiso 1994; Wallace 2000). These channels have been modeled as approximately parallel bundles of transbilayer helices containing at least four helices per bundle. Their expressed sensitivity to the applied voltage makes them an attractive model of voltage-gated channels in neurophysiology (Bezrukov and Vodyanoy 1995, 1998).

Alamethicin channels have been extensively studied both experimentally and theoretically (by using continuum solvent calculations or molecular dynamics), focusing on their selectivity (Borisenko et al. 2000), ionic diffusivity (Smith and Sansom 1999), structure (Sansom 1991), and channel-membrane interaction (Keller et al.

1993; Opsahl and Webb 1994; Kessel et al. 2000), including binding of the peptide to the lipid surface (Tieleman et al. 1999).

Recently, in experiments with planar bilayer membranes formed from charged lipids at varying pH (Bezrukov et al. 1998), it has been found that lipid packing stress modified by changes in electrostatic interaction between the polar lipid heads influences the lifetime of the alamethicin channel "burst" and the probability of alamethicin conductance states. However, there are other effects of lipid charge apart from the change in the equilibrium between differently sized alamethicin aggregates. Accumulation of counterions near the membrane surface also influences the channel electric conductance. There is extensive experimental evidence that the conductance of ion channels can be modified by the fixed charge of lipid polar headgroups (Apell et al. 1979; Bell and Miller 1984; Moczydlowski et al. 1985; Coronado and Affolter 1986; Green and Andersen 1991; Rostovtseva et al. 1998). Depending on the channel selectivity, the lipid charge can either increase or decrease the channel conductance.

Here we analyze amplitudes of the first two levels (called L0 and L1, respectively) of alamethicin-induced ion conductance fluctuations in different lipid and electrolyte solution environments. We show that, given the channel conductance in neutral lipids, Poisson-Boltzmann theory allows semi-quantitative description of the surface charge effects with only one adjustable parameter: an effective distance between the nearest lipid charge and the center of the channel. Depending on the calculation details, we obtain that this distance increases by 0.31–0.42 nm when the channel conductance jumps from level L0 to level L1. This observation agrees well with a simple geometrical model of the two minimal alamethicin aggregates.

Materials and methods

Alamethicin channels were inserted into "solvent-free" planar lipid bilayer membranes that had been formed by apposition of two phospholipid monolayers spread on aqueous solutions of sodium chloride (Baker, Phillipsburg, NJ, USA). The monolayers were prepared from a 10% solution of dioleoylphosphatidylethanolamine (DOPS) or dioleoylphosphatidylserine (DOPE) (Avanti Polar Lipids, Alabaster, Ala., USA) in pentane (Burdick and Jackson, Muskegon, Mich., USA). The Teflon chamber (Bezrukov and Vodyanoy 1993; after Montal and Mueller 1972) with two compartments of 1 mL was divided by a 15-μm thick Teflon partition (Chemfab, Merrimack, NH, USA) with a 60-μm diameter aperture. The aperture was pretreated with a 1% solution of hexadecane (Aldrich, Milwaukee, Wis., USA) in pentane and dried during 10 min prior to monolayer apposition.

Natural alamethicin (Sigma, St. Louis, Mo., USA) was added only to one side of a membrane from a 10^{-5} M stock solution in ethanol to a final concentration of $1–5\times10^{-8}$ M. All experiments were done at 150 mV, positive from the side of alamethicin addition, and at a room temperature of 23 ± 1 °C. The alamethicin was adjusted to a concentration that gave the first current bursts about 20 min after peptide addition; in this way we were able to monitor single-channel activity (no channel overlapping) for about 10 min. Ion currents, amplified with an Axopatch 200A integrating patch-clamp amplifier (Axon Instruments, Foster City, Calif., USA), were recorded with a sampling rate of 50 kHz into computer memory and, simultaneously, onto recordable compact disks. Conductance data reported here are averages over more than 30 different "current bursts" obtained from at least three different membranes for each lipid composition, pH value, and salt concentration.

Results and discussion

Typical recordings of alamethicin-induced currents through DOPS planar bilayers at pH 6.2 and pH 2.5 are shown in Fig. 1. It is seen that alamethicin channels at these conditions are very different. In 0.1 M NaCl solution the acidity changes the channel "current burst" drastically. Higher proton concentrations favor higher conductance states, corresponding to larger alamethicin aggregates. These pH-dependent changes in channel probabilistic behavior were found recently (Bezrukov et al. 1998) and were attributed to changes in the lipid packing stress. Indeed, at pH 6.2 the proton concentration in solution is so low that practically all lipid headgroups in the membrane are ionized. Consequently, DOPS is almost fully charged and the electrostatic repulsion between headgroups relieves the stress of lipid packing into a planar bilayer membrane (for a novel theoretical treatment, see Li and Schick 2000). The situation is different at high proton concentration, i.e., at pH 2.5. Protonation of negatively charged residues decreases the surface charge density, reduces headgroup repulsion, and promotes higher packing stress. This stress modulates channel expression (Gruner 1985; Keller et al. 1993; Lundbaek and Andersen 1994; Bezrukov 2000).

In the case of DOPE bilayers, channel conductance bursts at pH 6.2 and pH 2.5 are hardly distinguishable, except for the fact that the conductances of each level at pH 2.5 are somewhat higher owing to added protons. Alamethicin channel probabilistic behavior in neutral DOPE is not sensitive to the solution acidity in this pH range (Bezrukov et al. 1998). Figure 2 shows that higher conductance states corresponding to larger alamethicin

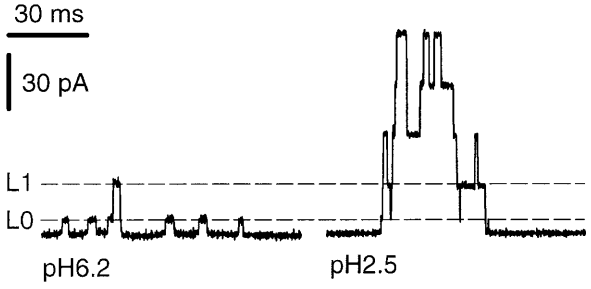

Fig. 1 Typical alamethicin channel recordings in DOPS bathed by 0.1 M NaCl at pH 6.2 (*left*) and pH 2.5 (*right*). The lipid charge is pH dependent and, as a result, probabilistic behavior of the channel changes dramatically with pH. As the solution acidity is increased, the higher conductance levels corresponding to the larger peptide aggregates become more expressed. The acidity effect on channel conductance is measurable (see below) but less pronounced. The time resolution is 0.1 ms

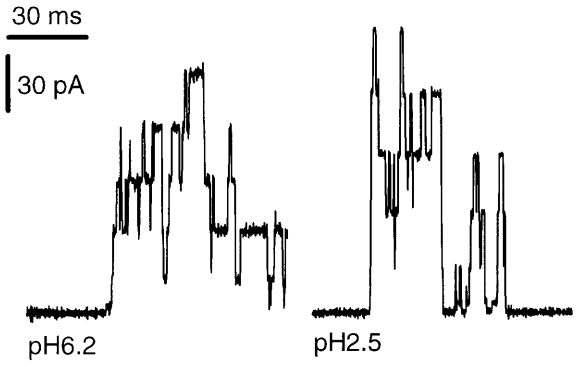

Fig. 2 Typical alamethicin channel recordings in DOPE bathed by 0.1 M NaCl at pH 6.2 (*left*) and pH 2.5 (*right*). In this neutral lipid, channel probabilistic behavior is independent of solution acidity. The time resolution is 0.1 ms

aggregates are well expressed in both cases. DOPE forms inverted hexagonal structures of spontaneous curvature that is close to that of DOPS at low pH values when its surface charge is "titrated out" almost completely. The stress of lipid packing into a planar membrane is comparable in these cases.

Lipid charge screening by salt

Modulation of the probabilities to find alamethicin channels at different states of aggregation is not the only effect of the membrane surface charge. At small salt concentrations the surface charge is also expected to change the channel conductance, while at high salt concentration the fixed charges of the lipid headgroups should be screened. Figure 3 shows the change of alamethicin conductance with NaCl concentration in DOPE and DOPS for the first two conductance states in comparison to the bulk solution conductivity. All solutions were buffered at a constant pH of 6.2. Error bars in Fig. 3 and elsewhere represent the standard deviations (for some points they are too small to be seen). At high enough NaCl concentration (e.g., 2 M) the conductance of alamethicin in DOPE and in DOPS is identical. Thus, with the lipid charge screened out, the channel conductance does not discriminate between these two lipids.

At low salt concentrations, however, alamethicin conductance in DOPS differs from that in DOPE. The conductance of the two lowest levels (L0 and L1) in the neutral DOPE approximately scales with bulk conductivity. On the other hand, conductance in DOPS is higher, thus reflecting the Na^+ accumulation near the membrane surface to compensate for the lipid negative charge. This effect is seen both in level L0 and level L1 and is qualitatively similar. Quantitatively, the conductance ratio G_{DOPS}/G_{DOPE} at low salt concentrations is higher for level L0 ($G_{DOPS}/G_{DOPE}\approx 2.3$) than for level L1 ($G_{DOPS}/G_{DOPE}\approx 1.5$). Thus, the effect of the lipid charge is sensitive to the channel structure that changes when the channel switches to a new conductance state.

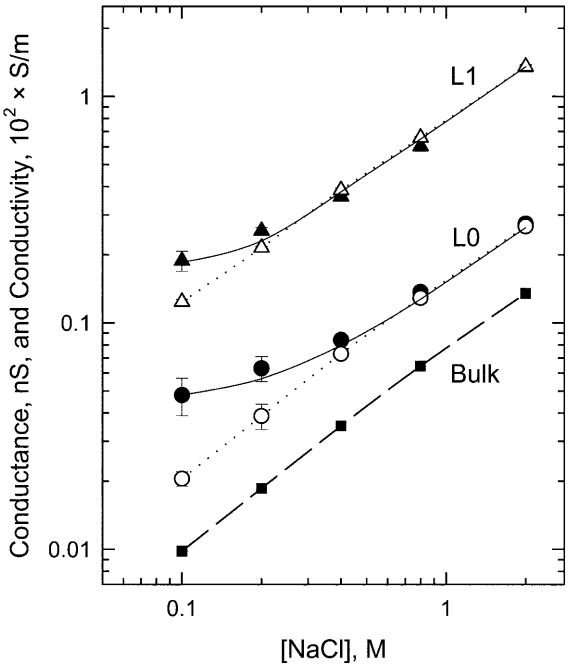

Fig. 3 Conductance of alamethicin channel levels L0 and L1 in DOPS (*solid symbols*) and DOPE membranes (*open symbols*) bathed by NaCl solutions at pH 6.2 as a function of salt concentration. High salt concentration "screens out" surface charge effects. *Lines* correspond to the theoretical predictions for the ratio G_{DOPS}/G_{DOPE}, assuming that the change in G_{DOPS} comes from the change in ionic concentrations at the channel mouth owing to an electric field generated by the lipid charge (see text). *Solid squares* stand for the bulk solution conductivity

Lipid charge titration

Figure 4 shows the effect of DOPS titration on channel conductance at levels L0 and L1 in 0.1 M and 0.3 M NaCl solutions. Two effects are superimposed here: (1) titration of lipid charge (as the pH decreases) decreases the amount of salt counter-ions near the channel that are available for conduction; (2) in high acidity solutions (very low pH), protons begin to contribute to the channel conductance. These two opposite effects give rise to the non-monotonic conductance behavior observed upon changing the pH over a wide range. This finding is similar to the recently reported titration of the gramicidin channel conductance (Rostovtseva et al. 1998). The charge titration effect, as measured by the maximum conductance decrease (the depth of the dip), varies with NaCl concentration and also with the conductance state.

Rationalizing the data within continuum electrostatic theory

The change in channel conductance with NaCl concentration and pH can be easily explained in terms of the double layer effect arising from the lipid fixed charges. Both sets of measurements show that the only

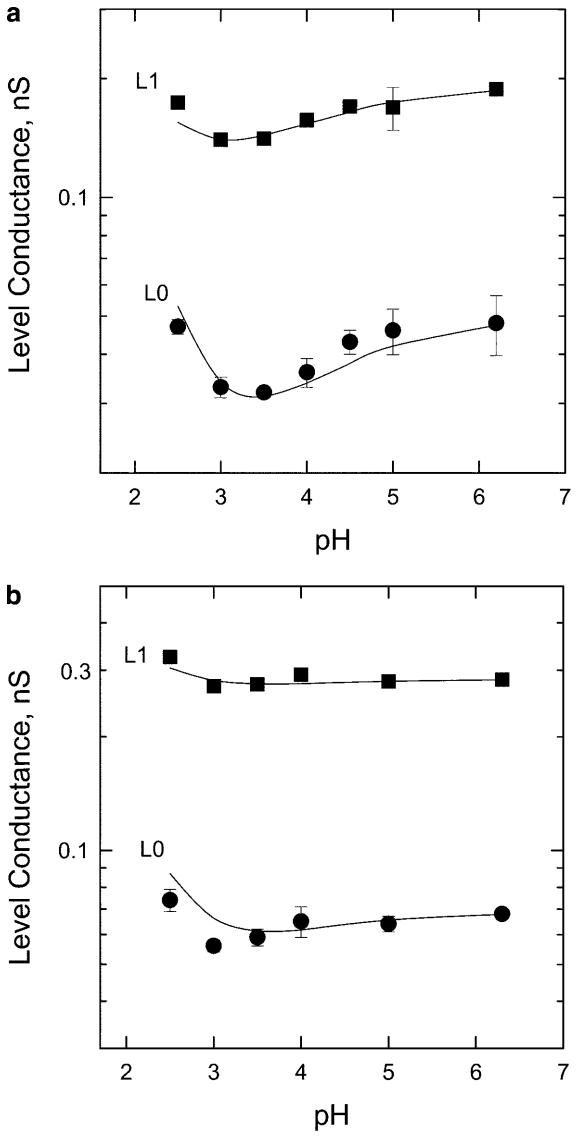

Fig. 4 Conductance of alamethicin channel at levels L0 and L1 in DOPS membranes bathed by **a** 0.1 M and **b** 0.3 M NaCl solutions as a function of pH. The conductance decrease induced by salt depletion near the channel, as a consequence of lipid charge titration by protons at low pH values, is counteracted by the increase in proton conductance in high acidity solutions. *Solid lines* are theoretical predictions (see text)

difference between DOPE and DOPS, in what concerns channel conductance, comes from the charged or neutral character of the lipid. In this sense the situation is much easier than for the gramicidin A channel, where lipid-induced conductance effects are more complicated and poorly understood. This issue has been discussed in detail elsewhere (e.g., see Fonseca et al. 1992; Rostovtseva et al. 1998; Phillips et al. 1999). Here we focus on the difference in the effects that charge titration has on the two conductance states. In doing so, we need to make some assumptions about the conformational change which switches the channel between levels L0 and L1.

The alamethicin channel structural model, which has been exploited for a long time, is known as the *barrel stave model* (Bauman and Mueller 1974; Boheim 1974). Basically, it consists in assuming a barrel-like structure for the alamethicin aggregate, where incorporation of additional molecules causes the channel to switch to higher conductance states. The main reason for the increase in channel conductance would be the widening of the channel lumen as a consequence of an increase in the surface of the channel wall. This model has been modified (Mak and Webb 1995) to account for the observation that the steric limitation for solutes does not change much with the conductance state of the channel (Bezrukov and Vodyanoy 1993). Recent studies of alamethicin ion selectivity, which found the same reversal potential for different conductance states (Borisenko et al. 2000), seem to strongly support the latter, modified picture. However, there is no agreement on either the number of monomers that form the alamethicin channel in level L0 or on the number of monomers that are added to the aggregate to give the upper conductance states.

Our aim is to investigate if the correlation between the increase in the overall channel aperture and the decrease in the lipid charge effect on conductance can be rationalized within continuum electrostatics. We use the nonlinear Poisson-Boltzmann approach to obtain the surface potential of a planar charged surface. From this potential we estimate the mean ion concentration at the channel mouth as:

$$\begin{aligned} [\mathrm{Na}^+]_{\mathrm{ch}} &= c\ \exp[-e\psi_{\mathrm{GC}}(a)/kT] \\ [\mathrm{Cl}^-]_{\mathrm{ch}} &= c\ \exp[e\psi_{\mathrm{GC}}(a)/kT] \end{aligned} \tag{1}$$

Here, $\psi_{\mathrm{GC}}(a)$ is the potential at distance a (treated as a fitted parameter) from the infinite uniform charged surface calculated using the familiar Gouy-Chapman equation:

$$\psi_{\mathrm{GC}}(a) = \frac{4kT}{e}\tanh^{-1}\left[\tanh\left(\frac{e\psi_0}{kT}\right)\exp(-\kappa a)\right] \tag{2}$$

It depends on the surface potential, ψ_0:

$$\psi_0 = \frac{2kT}{e}\sinh^{-1}\left[\sigma\Big/\sqrt{4kT\varepsilon(2c + 10^{-\mathrm{pH}} + 10^{\mathrm{pH}-14})}\right] \tag{3}$$

and the Debye screening length, κ^{-1}:

$$\kappa = \left[e^2\left(2c + 10^{-\mathrm{pH}} + 10^{\mathrm{pH}-14}\right)/kT\varepsilon\right]^{1/2} \tag{4}$$

In the above expressions, e, k, and T have their usual meanings, c is the bulk NaCl concentration, and ϵ is the solution permittivity. The lipid surface charge density, σ, is regulated by the dissociation and binding of Na^+ ions and protons to carboxyl groups. The corresponding equilibria can be characterized by the dissociation constants K_{Na} and K_{a}, respectively. Thus, σ is always lower than the maximum lipid surface charge density, σ_0, and

is given by (Ninham and Parsegian 1971; Rostovtseva et al. 1998):

$$\sigma = \sigma_0 \left[1 + \left(10^{\mathrm{p}Ka-\mathrm{pH}} + cK_{\mathrm{Na}}\right) \exp(-e\psi_0/kT)\right]^{-1} \quad (5)$$

Expressions (3) and (5) form an implicit equation for σ that must be solved numerically for each salt concentration and pH.

In previous work on the gramicidin A channel (Rostovtseva et al. 1998), an alternative approach based on the thermodynamic concept of the "Gibbs dividing surface" was used for calculating the mean ion concentration at the channel mouth. This approach introduced a new way to think about surface charge effects and provided an appropriate description of experimental data for the cation-selective channel. However, we do not employ the Gibbs dividing surface approach here for two reasons. First, it ignores the co-ion (Cl^-) concentration near the channel mouth, an assumption which is not applicable in the case of the only slightly selective alamethicin channel. Second, by introducing a uniform counter-ion distribution, a construction that works well for the smaller gramicidin channel (Rostovtseva et al. 1998), it predicts insensitivity of lipid-induced effects to changes in the channel state (channel radius). This conjecture is in contradiction with the experimental evidence in Figs. 3 and 4.

Under the experimental conditions mentioned above, the channel conductance as a function of applied voltage did not demonstrate any saturation (data not shown). Indeed, the contribution of the access resistance to the total alamethicin channel resistance for levels L0 and L1 is expected to be below 7% (Bezrukov and Vodyanoy 1993). So, we can assume safely – at least as a first approximation – that the conductance ratio $G_{\mathrm{DOPS}}/G_{\mathrm{DOPE}}$ scales with solution conductivity near the channel entrance. This ratio can be expressed as:

$$\frac{G_{\mathrm{DOPS}}}{G_{\mathrm{DOPE}}} = \frac{D_+}{D_+ + D_-}\frac{[\mathrm{Na}^+]_{\mathrm{ch}}}{c} + \frac{D_-}{D_+ + D_-}\frac{[\mathrm{Cl}^-]_{\mathrm{ch}}}{c} \quad (6)$$

where $D_\pm$ are the ionic diffusion coefficients, and the subindex ch denotes ion concentration at the pore mouth. By combining Eq. (1) with Eq. (6) we can estimate the conductance ratio with the distance a as a fitted parameter. The values for the Na^+ and H^+ binding constants to the carboxyl group were taken from the literature. DOPS pK_a was taken as 3.0, according to recent indirect estimates (Rostovtseva et al. 1998) and K_{Na} was assumed to be 0.6 M^{-1} (Tocanne and Teissié 1990). Infinite dilution diffusion coefficients were used ($D_+ = 1.33\times10^{-9}$ m^2/s and $D_- = 2.03\times10^{-9}$ m^2/s).

To calculate the conductance under the varying pH conditions, we have to take into account the proton conductance contribution. This has been done in the simplest possible way by assuming that there is no competition between salt ions and protons, so that their respective contributions are additive:

$$G = G_{\mathrm{DOPS}}(\mathrm{NaCl}) + \alpha \times 10^{-\mathrm{pH}} \quad (7)$$

and calculating the value of α from experiments with DOPE at pH 2.5 (Fig. 2).

Different considerations show that the smallest radius of the conductive alamethicin pore (in the state corresponding to L0) is approximately half the typical Debye length of a 0.1 M NaCl solution and about half the distance from the channel to the closest lipid headgroup. For state L1, which has an even larger pore radius, one may expect substantial variations of ion concentrations over the channel mouth, and the meaning of $[\mathrm{Na}^+]_{\mathrm{ch}}$ and $[\mathrm{Cl}^-]_{\mathrm{ch}}$ becomes uncertain. To account for these variations, average concentrations over the pore, $\langle[\mathrm{Na}^+]_{\mathrm{ch}}\rangle$ and $\langle[\mathrm{Cl}^-]_{\mathrm{ch}}\rangle$, could be used. However, the concentration averaging procedure introduces a new parameter for each conductance level: the pore radius, which is not known.

We compare the best fit for a obtained by averaging concentrations over the pore mouth area with that obtained without averaging, i.e., by using in Eq. (6) the ion concentrations at the center of the channel. Besides, in order to check the effect of Na^+ adsorption, we present the values obtained with and without accounting for the adsorption. The results are summarized in Table 1.

Table 1 shows two features: disparity between the absolute a values for different procedures, but reasonable agreement between the change of a upon channel transition from state L0 to state L1. Some of the values

Table 1 Fitted distances (in nm) from the center of the channel to the closest lipid charge and their increment from conductance level L0 to level L1

			Level L0		Level L1		L0→L1
			a	a^{a}	a	a^{b}	Δa
Analytical procedure	No average	No adsorption	0.74	–	1.05	–	0.31
		Adsorption	0.54	–	0.85	–	0.31
	Average	No adsorption	–	1.16	–	1.54	0.38
		Adsorption	–	0.75	–	1.14	0.39
Numerical solution			1.0	–	1.42	–	0.42

[a]Averaging over a pore radius of ~0.4 nm
[b]Averaging over a pore radius of ~0.6 nm

obtained here for a may appear physically meaningless if the size of the monomer cross-section (approximately 0.8 nm^2, which corresponds to a diameter of about 1 nm) is considered. However, the actual value for a may change considerably depending on the detailed structure of the channel, which is still under discussion (e.g., Béven et al. 1999; Ionov et al. 2000). Therefore, in the absence of such structural information, we would like to point out that, no matter what is the procedure used for estimating the distance a, its change from state L0 to state L1 is roughly the same.

The solid curves in Fig. 3 show the channel conductance in DOPS calculated according to Eq. (6) by averaging concentrations and assuming there is Na^+ adsorption. The results of the other three procedures shown in Table 1 are roughly similar, although this approach seems to be more physically justified.

Figure 4 shows the theoretical prediction for the change in channel conductance with the solution pH at two different concentrations. The same distance from the channel mouth to the closest fixed charge as in Fig. 3 was used, with no other extra parameters (for state L0, $a = 0.75$ nm; for state L1, $a = 1.14$ nm). The agreement is better for the lowest concentration, which is the case where charge screening is lower and the influence of lipid fixed charges is stronger.

Indeed, the variation in salt concentration and pH may induce other changes in addition to those related to the double layer perturbation near the membrane surface. The model is very crude and it seems remarkable that it is able to describe the change in channel conductance through the change in distance from the channel mouth to the closest fixed charge. As noted above, the physical meaning of the parameter a is that of an *effective distance* even within continuous approximation. Only for the case when the channel mouth does not protrude from the lipid surface, and assuming that the linearized form of the Gouy-Chapman potential is valid ($\psi_0 \ll kT/e$), the fitted parameter a would be approaching the *real distance* between the center of the pore and the closest lipid charges (Apell et al. 1979). Obviously, in the case considered here, the potentials near a fully charged lipid are much greater than kT/e, so at least one of the assumptions does not hold. Nevertheless, the change of a from state L0 to state L1 may reveal some useful information on the way the channel changes its conformation.

In an attempt to check the validity of our approximate analytical approach we have developed a numerical procedure to solve the 2D Poisson-Boltzmann (PB) equation ($\phi \equiv e\psi/kT$):

$$\nabla^2\phi(\rho,z) = \kappa^2 \sinh[\phi(\rho,z)] \tag{8}$$

with the boundary conditions which represent the surface of the charged lipid with the channel embedded in it. Since we are interested only in the average ion concentration near the channel mouth, we use a schematic representation (Fig. 5) similar to that in fig. 3 in

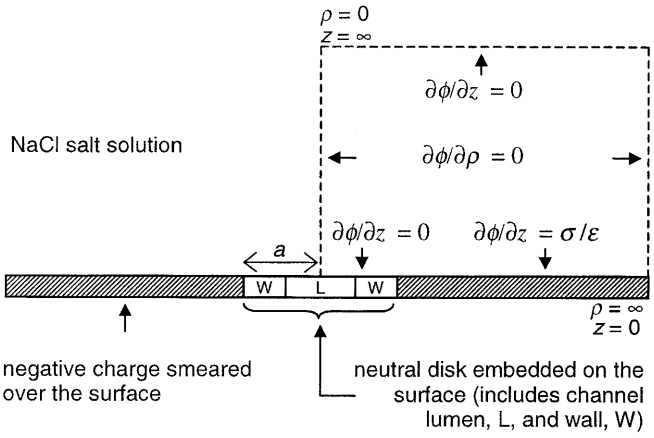

Fig. 5 Sketch of the idealized system used in the numerical solution. A homogeneously charged planar surface with an embedded neutral disk representing the channel mouth is shown together with the boundary conditions for the PB equation. Cylindrical symmetry was assumed for the electric potential

the paper by Apell et al. (1979). Charge is assumed to be smeared over the membrane surface; possible steric effects (Borukhov et al. 1997, 1998; Li and Schick 2000) are omitted. Since ϕ decays rapidly with the distance from the charged surface owing to the screening by salt ions, it is sufficient to solve the equation within the range $\{0, 5\times\max(a, \kappa^{-1})\}$ both for ρ and z, i.e., within a square whose side is five times the largest of these two values: Debye length and the neutral disk radius. Other necessary details are given in the Appendix.

Figure 6 shows the results of the numerical calculations as isopotential lines near the channel mouth in kT/e units for the typical values of lipid charge ($1e/0.5$ nm^2) and channel radius (1.4 nm including channel and aqueous pore). The fitted values for a, as well as its increment upon transition from level L0 to level L1, are similar to those obtained from the analytical procedure (see the last row in Table 1).

Figure 7 shows a comparison of the change in the electric potential with normal distance, z, from the surface (solid line) and its change with radial distance, $a-\rho$, from the edge of the charged surface (dashed line). This comparison possibly explains why the approximate analytical approach that we use here gives reasonable results for ionic channels: the separation between the lipid charge and the channel mouth is usually about 1 nm, where the two curves are close to each other. We will address this question in more detail in our forthcoming studies.

So far, there is no conclusive evidence about the number of monomers that are added to the channel at each conductance jump. A purely geometrical interpretation of the channel conductance involves the conductance of a regular cylinder of salt solution encircled by the alamethicin bundle but ignores the data on the inner constriction of the channel whose size apparently does

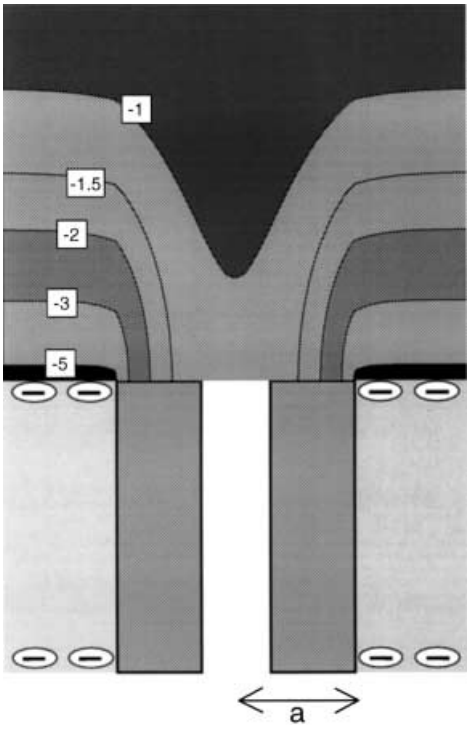

Fig. 6 Isopotential contour plot of the electric potential near the channel mouth combined with a cartoon of the channel surrounded by lipid. Electric potential (given in kT/e units) was obtained by a lattice relaxation numerical solution of the nonlinear Poisson-Boltzmann equation near a negatively charged membrane with a neutral circular patch (channel + aqueous pore), of radius $a = 1.4$ nm, embedded in it. Charge was assumed to be smeared over the membrane surface. Na^+ concentration near the channel is about 3–7 times (e^1–e^2) higher than the bulk Na^+ concentration

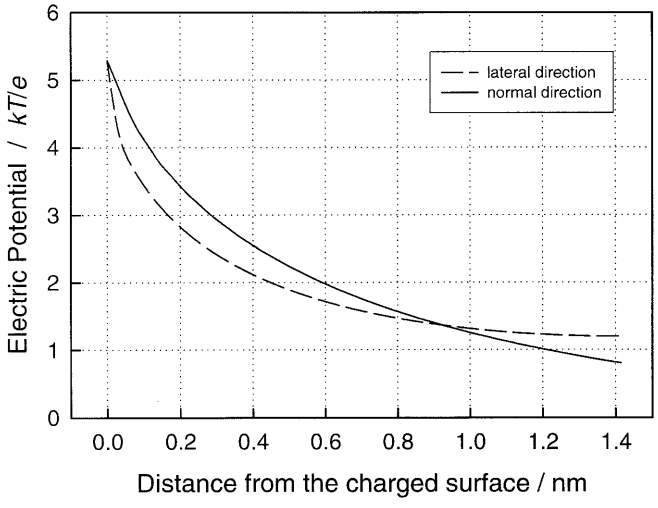

Fig. 7 Comparison of the change of the electric potential, $\phi(\rho, z)$, with normal distance, z, from the surface (*solid line*) to its change with radial distance, a–ρ, from the edge of the charged surface to the center of the channel (*dashed line*)

not depend on the conductance state (Bezrukov and Vodyanoy 1993; Borisenko et al. 2000). However, in the absence of detailed structural information, simple geometrical reasoning can be useful to estimate the change in average distance between the channel center and the nearest lipid charge. We are interested in the change of the charged lipid effect on the channel conductance at the L0 to L1 transition, rather than in the conductance itself.

The change in the overall channel cross-section area in the membrane plane can be estimated by using a simplified model in which the alamethicin channel is a bundle of monomers, represented by cylinders with radius r. The total lipid surface area displaced by a channel composed of n monomers ($n \geq 3$) is:

$$A_T = \pi r^2 \left[1 + \frac{n}{2} + \frac{n}{\pi}\cot\left(\frac{\pi}{n}\right)\right] \quad (9)$$

and the channel pore area is:

$$A_P = A_T - n\pi r^2 = \pi r^2 \left[1 - \frac{n}{2} + \frac{n}{\pi}\cot\left(\frac{\pi}{n}\right)\right] \quad (10)$$

On the basis of these expressions, we can estimate the changes in the overall channel cross-section area, and in the total equivalent radius, so as to compare those values with the change of a that we obtained above. The candidate aggregates for levels L0 and L1 range from a tetrameric to an octameric bundle. Any change in conductance arising from the addition of two monomers results in a change of 0.31–0.32 nm in the overall channel radius, $(A_T/\pi)^{1/2}$. A similar change is found for other two related values: the equivalent pore radius, $(A_P/\pi)^{1/2}$, and the radius of the largest inner circle in the pore, $r(1/\sin(\pi/n)-1)$. The agreement of these values with Δa could support the assumption that, at least in the first conductance jump, a pair of monomers is added to the channel.

To conclude, the continuum electrostatic approach allows us to rationalize lipid-charge-induced changes in the conductance of the alamethicin channel using only one adjustable parameter: the effective distance between the channel center and the nearest lipid charge. It allows semi-quantitative description of the conductance dependence on bathing-solution pH and salt concentration for the first two conductance levels, where the effect of charge is measurable. The effective distance changes by about 0.3–0.4 nm upon transition between these states.

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Appendix

In cylindrical coordinates with the origin in the center of the neutral disk (channel mouth), the PB equation can be written as:

$$\partial^2\phi/\partial\rho^2 + (1/\rho)\partial\phi/\partial\rho + \partial^2\phi/\partial z^2 = \kappa^2 \sinh(\phi) \quad (A1)$$

The boundary conditions were:

1. for $\rho = 0$ and any z, $\partial\phi/\partial\rho = 0$ (because of symmetry)
2. for $z = 0$ and any ρ, $\partial\phi/\partial z = \sigma(\rho)/\epsilon$ (Gauss' theorem, with the approximation that the dielectric constant of the lipid phase is much smaller than in solution). Note that $\sigma(\rho) = 0$ for $\rho < a$ and $\sigma(\rho) = \sigma_0$ for $\rho \geq a$. σ_0 is obtained self-consistently from a first integral of the 1D Poisson-Boltzmann equation at the lipid surface and is a function of pH, pK_a, K_{Na}, and c.
3. for $\rho = \infty$ and any z, $\partial\phi/\partial\rho = 0$ (because of symmetry)
4. for $z = \infty$ and any ρ, $\partial\phi/\partial z = 0$ (bulk continuity condition)

To find a solution for the electric potential in the region shown in Fig. 5, a typical lattice relaxation method (Press et al. 1992) tailored specifically to our problem was applied. The PB partial differential equation was replaced by approximate finite difference equations on a mesh of points that span the domain of interest. The grid of 101×101 points used here was not homogeneous but finer near the neutral disk than far from it, in order to obtain a better representation of the change in the electric potential in the vicinity of the channel mouth. The relaxation method determined the solution by starting with a guess and improving it iteratively until the desired accuracy was obtained (usually less than a 0.1% change in the electric potential between successive iterations). An iterative scheme based on a multi-dimensional Newton method was used, which produced a matrix equation with "block diagonal" form. This resulting system of equations was solved iteratively by using Mathematica built-in matrix-operation routines. Typical computing time on a Pentium II-based PC was about 1 h. The code was calibrated by comparing the 2D numerical solution of the PB equation to the 1D analytical solution in those cases where it was possible.

The finite-difference representation of the PB equation was as follows:

$$r \rightarrow x[m]$$
$$z \rightarrow y[n]$$
$$\phi[r,z] \rightarrow u[m,n]$$
$$\partial_r \phi[r,z] \rightarrow \frac{u[m+1,n]-u[m-1,n]}{x[m+1]-x[m-1]}$$
$$\partial_{r,r} \phi[r,z] \rightarrow \frac{u[m+1,n]-2u[m,n]+u[m-1,n]}{\left(\frac{x[m+1]-x[m-1]}{2}\right)^2}$$
$$\partial_{z,z} \phi[r,z] \rightarrow \frac{u[m,n+1]-2u[m,n]+u[m,n-1]}{\left(\frac{y[n+1]-y[n-1]}{2}\right)^2}$$

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