J. Membrane Biol. 78, 91-102 (1984)

The Journal of
Membrane Biology

The Total Electrostatic Potential in a Gramicidin Channel

Peter C. Jordan

Department of Chemistry, Brandeis University, Waltham, Massachusetts 02254

Summary. This paper describes a parameter free model of the electrostatic structure of gramicidin channels incorporated into uncharged lipid bilayer membranes. The electrical potential due to all sources is calculated for singly and doubly occupied channels. The model is consistent with all channel properties that are clearly dependent on coulombic interactions. The calculated value of the translocation rate constant and of the binding constant ratio for single and double occupancy are in excellent accord with experiment.

Key Words membrane conductance · gramicidin channel · electrostatic potential · single-file transport · channel kinetics

Introduction

Electrostatic forces significantly affect, even if they do not control, the turnover capacity of ion pores. In addition they exert a major influence on an ion's ability to readily enter a pore. In a series of papers (Jordan, 1981, 1982, 1983) I have analyzed the ion-pore-former/membrane-water system as a problem in applied electrostatics. This work has demonstrated that electrical shielding by the pore former may greatly reduce the image potential of an ion traversing a pore (Jordan, 1981); that a substantial fraction of the voltage drop due to the application of a transmembrane potential may occur in the aqueous regions exterior to the pore (Jordan, 1982); why ion permeation through pores is unlikely to be sensitive to variation of the membrane dipole potential (Jordan, 1983).

This paper focuses on the electrostatic properties of the gramicidin channel, which is unique among channel formers in that a wealth of detailed structural information is available (Urry, 1971; Bamberg, Apell & Alpes, 1977; Koeppe, Hodgson & Stryer, 1978; Koeppe et al., 1979; Koeppe & Kimura, 1983). Combining these data with measurements of the monolayer surface potentials (Paltauf, Hauser & Phillips, 1971) and with estimates of bilayer thickness (Benz et al., 1975) a model of the electrostatic structure of the gramicidin channel can be constructed. The model has *no* adjustable parameters. As such it provides a stringent test of the reliability of the electrostatic modeling approach.

The model is used to compute the electrostatic contribution to the total potential energy profile and the electrical potential for ions in singly and doubly occupied gramicidin channels. This information can be used to establish a translocation rate constant and the binding constant ratio for single and double occupancy. Both are in excellent agreement with available experimental data.

The Model

When inserted into a lipid bilayer gramicidin can dimerize and form an ionophoric channel of length 2.6 nm and radius ~0.21 nm (Koeppe et al., 1978) with binding sites 0.25 nm from each end (Koeppe et al., 1979). It has a β-helical structure (Urry, 1971); the carbonyl groups lining the pore interior are oriented essentially alternately skew antiparallel to the channel axis (Urry, 1971; Koeppe & Kimura, 1983).

The potential energy of an ion-pore-former/membrane-water system is the sum of many contributions:

1) a term reflecting the differences between the solvating ability of water and that of the polar groups lining the pore interior;

2) the image interaction due to polarization charges induced at electrical phase boundaries;

3) the interaction with the membrane dipole potential;

4) the interaction with the charge distribution of the pore former;

5) the interaction with the diffuse double layer at the membrane-water interface.

Ignoring the possibility that the presence of an ion within the pore leads to structural rearrangements of the pore former or of the surrounding dielectrics, the total potential is the sum of the individual components.

The solvation term accounts for the short-range interaction that describes the changes that take place as a solvated ion approaches the pore mouth, is stripped of some of the water molecules forming the inner solvation shell (since the pore is too narrow to accommodate a fully solvated ion) and is bound to the carbonyl groups lining the interior of the gramicidin channel. While the short-range ion-carbonyl interaction must vary as the ion translocates through the gramicidin channel the separation of local minima in the solvation energy should be only ~ 0.15 nm, the axial separation between antiparallel pairs of carbonyls (Koeppe & Kimura, 1983). I assume, and molecular dynamics calculations on a gramicidin-like system suggest,[1] that the energy differences between the extrema are small. For most purposes these local differences will be ignored and the solvation energy assumed to be constant within the gramicidin pore. The schematic behavior of the solvation energy profile (including local variation) is illustrated in Fig. 1. It is constant in the aqueous region, increases dramatically in the region of the pore mouth where the aqueous hydration shell is disrupted and drops abruptly as the ion is solvated by the carbonyl groups within the pore.

The other four terms contributing to the potential reflect long-range electrostatic interactions which are strongly influenced by the dielectric differences between the components of the ensemble (Parsegian, 1969, 1975; Levitt, 1978*a*, *b*; Jordan 1981, 1982, 1983). This superposition creates a slowly varying background potential, also illustrated in Fig. 1, that augments the solvation term. The remainder of this paper describes and analyzes a parameter-free model for the total electrostatic potential within a gramicidin channel incorporated in membranes of variable width. The model is illustrated in Fig. 2.

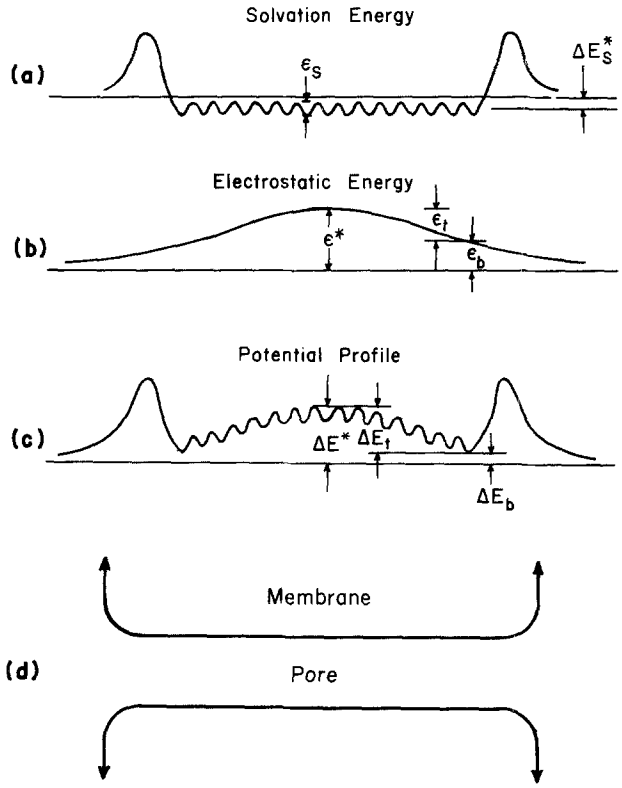

Fig. 1. Schematic representation of the solvation energy profile, the electrostatic energy profile and the total potential profile for a cation in a gramicidin channel. The solvation energy accounts for short-range interaction between the ion and the polar moieties forming its inner solvation shell; ε_s is the energy difference between adjacent extrema in this potential; ΔE_s^* is the mean difference between the hydration energy and the solvation energy in the channel. The specific energies indicated in Fig. 1*b* are the electrostatic contribution to the central barrier ε^*; the translocation barrier ε_t; and the binding energy ε_b. I have chosen, unconventionally, to portray binding as an endothermic process; this is merely a reminder that there is no convincing evidence for the sign of the cation binding energy in gramicidin

The ensemble is composed of two dielectric phases: a high ε region comprising the water in the channel and the electrolyte bathing the membrane; a low ε region comprising the lipid bilayer and the gramicidin molecule itself. The channel is 2.6 nm long. The left- and right-hand mouth regions are circular arcs; this minimizes the surface energy needed to form the phase boundaries.

No dielectric distinction is made between the water within the pore and the water bathing

[1] W.K. Lee and P.C. Jordan, *unpublished results*. Molecular dynamics calculations of cation motion in a gramicidin-like, water-filled model channel suggest that the local barriers between minima are $\lesssim 10$ kJ mol^{-1} for monovalent ions at least as large as Na^+ when reasonable values for the dipole moments and the polarizabilities of the CO and NH bonds are used.

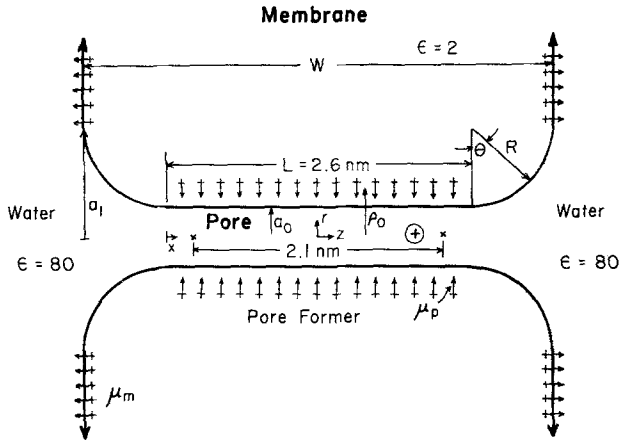

Fig. 2. The electrostatic model of the gramicidin channel. It has length $L=2.6$ nm with binding sites, at the points designated by x, separated by 2.1 nm. The channel is treated as a uniform cylinder; the average charge distribution is equivalent to a surface dipole density μ_p smeared out on a cylinder of radius ρ_0. The effective electrical radius of the pore is a_0. The membrane width W is variable; the channel is connected to the membrane by circular arcs of radius R, $W=L+2R$. The uniform surface dipole density μ_m is the source of the membrane dipole potential. Depending upon the specific structure proposed surface dipoles could also extend along the arcs. The distance x is measured from the channel mouth; z is measured from the channel center

the membrane. This is clearly an approximation since dielectric properties are a reflection of the mean total dipole moment (both permanent and induced). The surroundings of bulk water and channel water are different; the electrical consequences are unknown. The model assigns an ε of 80, representative of bulk water. If density were the main influence on ε this choice would not be outrageous since the channel contains 8 or 9 water molecules (Levitt, Elias & Hautman, 1978; Dani & Levitt, 1981); the corresponding channel water density is $\sim 75\%$ of that of bulk water.

In order to accommodate the dielectric differences between gramicidin and lipid in a two-dielectric model, the pore is assigned an "effective electrical radius," somewhat larger than its physical radius. The value used is 0.25 nm. The grounds for this choice are outlined in Appendix A.

The membrane potential cannot be measured directly (Guggenheim, 1929, 1930). If lipid monolayers adsorbed onto an aqueous surface had the same molecular arrangement and area per molecule as lipid bilayers sandwiched between aqueous solutions, the monolayer surface potential would equal the membrane dipole potential. Appendix B demonstrates that this identification is reasonable. The Table, which is included in that Appendix illustrates how changes in phosphate head groups and in lipid structure affect the dipole potential. For purposes of calculation the source of the potential is a surface dipole density of strength μ_m located at the membrane-water interface. As pointed out previously (Jordan, 1983), the precise location of the dipoles is immaterial; μ_m is adjusted to create a specified dipole potential V_m in regions of the membrane far removed from the pore.

The gramicidin molecule forms a β-helix with polar groups lining the interior (Urry, 1971). Since the carbonyls are alternately antiparallel to the channel axis there is no net axial dipole moment. In fact the mean axial dipole moment per turn of the helix is ~ 0. There are presumably strong rapidly varying local interactions between a cation and the antiparallel pairs of carbonyls. These account for the solvation energy profile of Fig. 1*a*. While the axial dipole moment averages to zero, the *radial* dipole moment does not. Appendix C describes the charge distribution in the gramicidin molecule and demonstrates that, on average, it is equivalent to a dipole density, $\mu_p \sim 8 \cdot 10^{-12}\,\mathrm{C\,m^{-1}}$, smeared out on a cylindrical surface. The cylinder radius is between 0.35 and 0.37 nm. The negative ends of the dipoles point inwards. This charge distribution corresponds to a potential step of ~ 460 mV at the cylinder surface with the interior region relatively negative. As in the case of the membrane potential (Jordan, 1983), the potential is partially shielded within the water-filled pore.

The diffuse double layer arises at the surface of membranes formed from charged phospholipids (*see* McLaughlin, 1977). Depending upon the ionic strength of the electrolyte and the surface charge density at the lipid-water interface the surface potential can be extremely large (< -200 mV) and extend far into the aqueous regions away from the lipid itself (> 10 nm). However, as long as consideration is limited to zwitterionic phospholipids such as glyceryl monooleate (GMO), phosphatidyl chlorine (PC) and phosphatidyl ethanolamine (PE), the complications due to the double layer do not arise. This restriction is imposed throughout the remainder of this paper.

Some Mathematics

Previous studies (Jordan, 1982, 1983) have formulated accurate, rapidly convergent methods

for calculating the image potential and membrane potential within a right circular cylindrical pore spanning a planar membrane. This geometry is equivalent to that of Fig. 2 with $L = W(R=0)$. The treatment has been extended to describe the image potential for pores with the more general structure illustrated in Fig. 2 (where $L<W$).[2] The same approach can be used to determine the consequences of a surface dipole density spread out on a cylinder that is coaxial with the pore.

This method requires determining the bare source potential arising from the pore dipole aligned on the cylinder of radius ρ_0. For the geometry of Fig. 2 this is

$$V_0(\vec{r}) = \mathscr{V}_0 a_0 \int_{-L/2}^{L/2} dz' \int_0^{2\pi} d\phi \cdot \frac{r\cos\phi - \rho_0}{\{(z-z')^2 + r^2 - 2r\rho_0\cos\phi + \rho_0^2\}^{3/2}} \quad (1a)$$

$$= \mathscr{V}_0\{M(L/2-z,r) + M(L/2+z,r)\} \quad (1b)$$

$$M(x,y) = \frac{2x}{S}\left[K(\alpha) + \frac{\rho_0 - y}{\rho_0 + y}\Pi(n/\alpha)\right] \quad (2a)$$

$$S = \sqrt{x^2 + (\rho_0+y)^2}, \sin\alpha = 2\sqrt{\rho_0 y}/S,$$
$$n = 4\rho_0 y/(\rho_0+y)^2 \quad (2b)$$

where $K(\alpha)$ and $\Pi(n/\alpha)$ are complete elliptic integrals of the first and third kind (Abramowitz & Stegun, 1965); $\mathscr{V}_0$ is the unshielded pore potential, for gramicidin -460 mV. Calculation of the shielded potential along the pore axis can then be carried out by straightforward application of the replacement charge density method (Jordan, 1982, 1983; *also see* footnote 2). This technique requires as input the inhomogeneous term F^+, which is the gradient of the source potential on the electrical phase boundary,

$$F^+ = \pi[(\vec{n}_L \cdot \nabla V)_L + (\vec{n}_R \cdot \nabla V)_R] \quad (3)$$

where $\vec{n}_R$ is the outward pointing normal to the right-hand branch of the dividing surface, etc.

Computational Considerations

The source that determined the image potential is the ion of charge q located on the axis. The source for the pore potential is the surface dipole density of strength μ_p on the cylinder of radius ρ_0. As neither of these are located on an electrical phase boundary they do not introduce singularities or ambiguities into the integral equations that determine the electrical potential. The calculational methods devised previously can then be applied without modification.

The membrane potential is more complicated. If the dipole density μ_m terminates at the points $z = \pm W/2$, $r = a_1 = R + a_0$ of Fig. 2, the inhomogeneous term F that established the contribution of the membrane potential to the potential along the pore axis is singular at these points (Jordan, 1983). If the dipole density μ_m extends along the curved membrane surface that forms the entrance to the channel mouth, the termination points are $z = \pm L/2$, $r = a_0$; singularities in F would then occur at these points. A further complication arises because the correct functional form of the surface dipole density on the curved surfaces is not immediately apparent. There are three obvious choices: $\mu_m^{(2)} = 0$; $\mu_m^{(2)} = \mu_m \sin\theta$; $\mu_m^{(2)} = \mu_m$. The first of these presumes that in the formation of the curved mouth the head group structure of the phospholipid becomes quite random. The second assumes a gradual randomization as the lipids form the mouths of the ensemble. The third assumes that the local molecular arrangement has no effect upon the dipole density.

The distinction between these possibilities is probably most significant as the ion traverses the channel mouth. Within the constriction the surface dipole density spread out along the membrane-water interface should have the greatest influence on the axial potential. Rather than attempting to choose among the alternative descriptions and formulate the modifications that are needed if the singularities in the inhomogeneous terms are to be properly treated, I have used an approximation that yields image potential profiles and pore potential profiles that are accurate to within a few percent within the constriction.

In this approximation the ensemble geometry is altered. An "equivalent right circular cylindrical pore" is constructed. Its length-to-diameter ratio is chosen so that the peak of the image energy in the equivalent pore is the same as that for the original pore (*see* footnote 2). Both the image potential and the pore dipole potential of the equivalent pore differ by less than 5% from their corresponding values in the

[2] P.C. Jordan. The effect of pore structure on energy barriers and applied voltage profiles. I. Symmetrical channels. *Biophys. J. (Submitted for publication).*

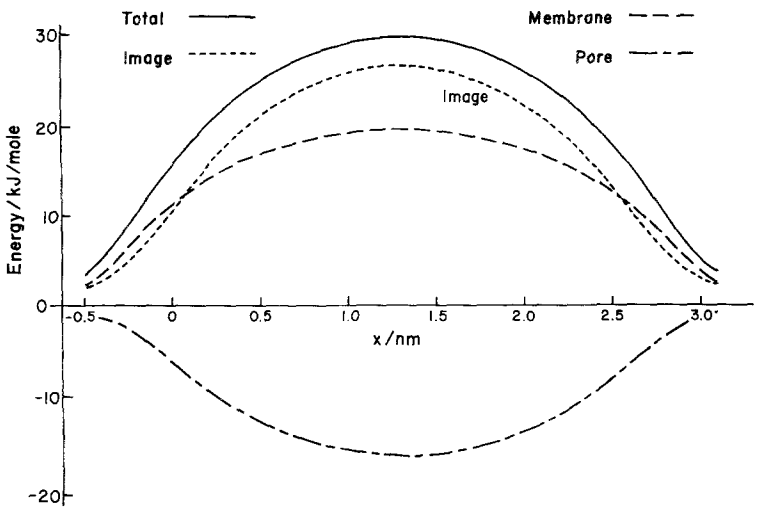

Fig. 3. Specific contributions to the total electrostatic energy from the image interaction, the membrane potential and the pore potential for an ion on the axis of a gramicidin-like channel. The system corresponds to dioleoyl-PC membrane of width 3.5 nm

constricted regions of the original model pore. I shall assume that the membrane potential within the constriction is affected in the same insignificant manner.

All calculations of the image potential and the shielded pore potential were carried out using the model geometry of Fig. 2. The shielded membrane potential is that of the equivalent pore just described; it should be accurate within the constriction.

Results and Discussion

General Considerations

The total electrostatic potential in the gramicidin channel is the sum of three contributions, roughly equal in magnitude, as illustrated in Fig. 3 for model parameters representative of a 3.5 nm width 1,2-dioleoyl PC membrane. The image interaction and the membrane potential contribute nearly equally to the total energy barrier; the pore potential almost completely compensates for the membrane potential's contribution to the barrier. In GMO, where the membrane potential is 75% of that in PC, the relative magnitude of the pore and membrane contributions is reversed. The most striking feature is the near equality of the membrane and pore terms in the long-range electrostatic energy, regardless of the phospholipid. The implication is that the total electrostatic energy is not greatly different for cation or anion occupancy. In fact, for the model of the dioleoyl-PC membrane, the *electrostatic* contribution to the barrier for anion permeation is $\sim 6\,\mathrm{kJ\,mol^{-1}}$ *less* than that for cation permeation.

That gramicidin acts as a cationic channel is therefore a direct consequence of the local solvating power of the antiparallel carbonyl groups lining the channel. The mean radial charge distribution in the pore former, while it favors occupancy by cationic species, is not sufficiently negative to account for the channel's selectivity. Direct local interaction with atoms in the molecule can. A crude estimate of the local solvating ability of the gramicidin molecule can be found from the partial charge parameters and the atomic coordinates that were used (*see* Appendix C) to determine the average radial charge distribution. The most favorable locations for a solvated cation are in the general vicinity of the midpoint between oxygen atoms in opposed carbonyl units; the antiparallel carbonyl units are roughly coplanar; the cation would be ~ 0.18 nm from each oxygen atom. With this atomic arrangement the stabilization energy for a cation is in the range 60 to $100\,\mathrm{kJ\,mol^{-1}}$. The most favorable positions for anions are in the vicinity of the midpoint between carbons of neighboring antiparallel carbonyl units; the anion would also be roughly 0.18 nm from each carbon atom. This arrangement, while a good one for anions, is not always stabilizing. Near the channel entrance the interaction energy is $\sim -30\,\mathrm{kJ\,mol^{-1}}$; near the center it is $\sim +10\,\mathrm{kJ\,mol^{-1}}$.

The difference in interaction energy for anions and cations of the same size, located in the manner described, is ~ 40 to $70\,\mathrm{kJ\,mol^{-1}}$; the cations have lower energy. In addition to greater stabilization by the gramicidin molecule, cation binding is also favored because, as

discussed in Appendix B, the dehydration energy of anions is $\sim 30\,\mathrm{kJ\,mol^{-1}}$ greater than that of cations of the same size. The net difference in solvation energy between cations and anions of equal radii is ~ 70 to $100\,\mathrm{kJ\,mol^{-1}}$ far outweighing any differences due to long-range electrostatic phenomena.

While the cationic nature of the channel is overwhelmingly a solvation phenomenon, as is to be as expected from Eisenman's description of selectivity (Eisenman, 1962; Diamond & Wright, 1969; Krasne & Eisenman, 1973), the electrostatic potential has a major influence on channel properties under conditions where the local solvation energy is invariant (or nearly so). Molecular dynamics calculations (*see* footnote 1) suggest that the local variation of the solvation energy due to direct interaction of a cation with the polypeptide backbone of the pore former, is small; the barriers between adjacent minima are quite possibly less than $10\,\mathrm{kJ\,mol^{-1}}$ for Na^+ and larger cations. Thus the electrostatic barrier should significantly affect ionic motion within the channel, i.e. the process of translocation between the binding sites.

The electrostatic contribution should also be the major variable when comparing the properties of gramicidin channels incorporated into membranes of different width or composition. It is reasonable to suppose that the interior structure of the channel is not greatly affected by these variables. If this is true the short-range solvation energy of an ion within the channel constriction is independent of the membrane structure. As hydration energy does not depend upon the properties of the membrane, comparisons of the effect of membrane structure on ionic energy within the pore should be possible without knowledge of the contribution due to solvation.

Variation of Lipid or of Bilayer Width

Figure 4 illustrates the effect that lipid variation and bilayer width have on the electrostatic contribution at three points of the potential profile illustrated in Fig. 1: the energy barrier at the center of the channel, ε^*; the energy barrier for translocation, ε_t; and the binding energy, ε_b. Three membrane potentials are contrasted. The two larger values are 440 and 320 mV, representative of dioleoyl-PC and GMO, respectively. The third is 200 V which, since replacement of ester linkages by ether linkages reduces

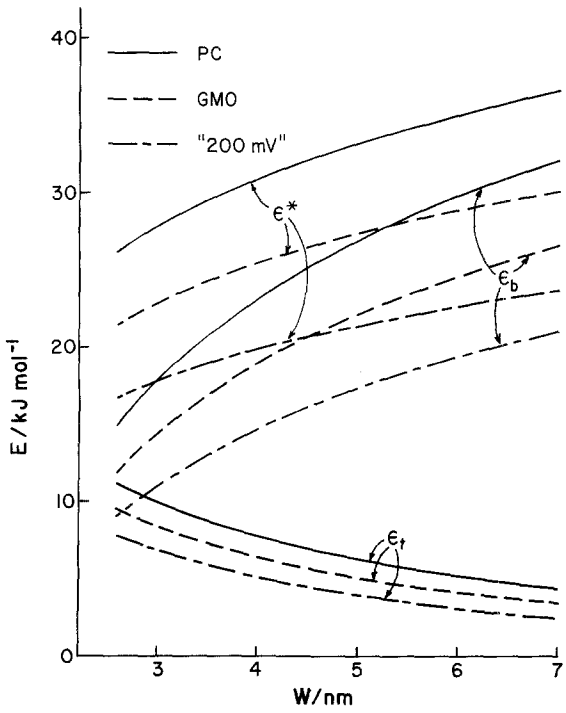

Fig. 4. The electrostatic contribution to the total energy at the channel center ε^*; to the binding energy ε_b; and to the translocation energy ε_t as a function of bilayer width. Three different membrane potentials are contrasted: 440 mV (dioleoyl-PC), 320 mV (GMO) and 200 mV (this latter could correspond to a di-O-oleoyl-PC)

the monolayer surface potential (Paltauf et al., 1971; *see also* the Table, Appendix B), might be representative of di-O-stearoyl-PE. Bilayer widths range from the minimum value required to incorporate this channel, 2.6 nm, to 7.0 nm which is thicker than any membrane in which gramicidin conductance has been measured (Kolb & Bamberg, 1978).

The qualitative trends are unambiguous. Increasing the membrane width or the membrane potential significantly increases the electrostatic contribution to ε_b and to ε^*. The effect that changing bilayer width has on ε_b is substantially greater than its effect on ε^*. This reflects the fact, clearly apparent in Fig. 3, that the image, membrane dipole and pore dipole contributions to the electrostatic energy vary much more rapidly near the channel mouth than at the channel center. Changing the membrane potential effects ε^* and ε_b in nearly the same way. This is also indicated in Fig. 3 since, within the channel, the shielded membrane potential is almost invariant.

Since $\varepsilon^* = \varepsilon_t + \varepsilon_b$, the effect of bilayer width or lipid variation on ε_t is determined by the difference between either's effect on ε^* and ε_b.

Increasing the bilayer width dramatically decreases ε_t which reflects the fact that ε_b increases more rapidly than ε^* as W increases. Since changes in V_m affect ε^* and ε_b almost equally, ε_t is nearly invariant to lipid variation.

Bounds on the Solvation Energy

When combined with measurements of the activation energy for channel conductance (Hladky & Haydon, 1973; Bamberg & Läuger, 1974) the electrostatic calculations can be used to obtain an upper bound to the difference in solvating ability between the gramicidin channel and water. Analysis of the concentration and voltage dependence of gramicidin conductance suggest strongly that the central barrier is not rate limiting (Andersen & Procopio, 1980; Finkelstien & Andersen, 1981; Andersen, 1983; Jordan, 1983; *see also* footnote 2). Depending upon the specific experimental conditions either the entrance barrier or the exit barrier may be dominant. This is only possible if the total ionic energy at the channel center is less than the ionic energy at the peak of the association/dissociation step. This total energy is the sum of the electrostatic term ε, and a short-range solvation term, ΔE_{solv},

$$E = \varepsilon + \Delta E_{\text{solv}}; \tag{4}$$

since $E < E_{\text{act}}$, an upper bound to the solvation energy at the channel center, ΔE^*_{solv}, is $\Delta E^*_{\text{solv}} < E_{\text{act}} - \varepsilon^*$. Measurements of Na^+ conductance suggest an activation energy of $\sim 20\,\text{kJ mol}^{-1}$ in GMO (Hladky & Haydon, 1973) and $\sim 31\,\text{kJ mol}^{-1}$ in dioleoyl-PC (Bamberg & Läuger, 1974). Both sets of experiments were run under conditions where the bilayer width is $\sim 4.8\,\text{nm}$ (Hladky & Haydon, 1973; Benz et al., 1975). Using the ε^* values from Fig. 4, the bounds to the short-range solvation energy are $\Delta E^*_{\text{solv}} < -2\,\text{kJ mol}^{-1}$ from PC and $\Delta E^*_{\text{solv}} < -7\,\text{kJ mol}^{-1}$ from GMO.

The *short range* interactions that determine the difference in solvation energy are not lipid dependent if the channel structure is essentially unaffected by lipid variation. Thus $\Delta E^*_{\text{solv}} < -7\,\text{kJ mol}^{-1}$, regardless of lipid; it may of course be substantially less than this limit.

The Translocation Rate

Because the gramicidin channel is single file, translocation is a cooperative process. As the ion passes from one binding site to the other a substantial number of water molecules must move in concert with it (Levitt et al., 1978; Rosenberg & Finkelstein, 1978*a*,*b*; Urban, Hladky & Haydon, 1980; Dani & Levitt, 1981); the best estimate of this number is 8 or 9 although it might be as small as 6. In addition there is a succession of energy barriers to be surmounted reflecting the variation of the solvation energy within the pore. Given that the binding sites are separated by 2.1 nm (Koeppe et al., 1979) and that the separation of local minima in the potential is $\sim 0.154\,\text{nm}$ (Koeppe & Kimura, 1983), there are about 14 intermediate maxima in the potential. The electrostatic potential is overlayed on the oscillatory solvation potential. Translocation is then described as a sequence of 14 first-order, reversible kinetic steps. The apparent rate constant for the whole translocation process is determined by the net rate of passage over the 14 consecutive barriers. It is clearly less than the rate constant for passage over a single barrier with the same activation barrier. The actual rate constant is further reduced because there is a large *negative* entropy of activation reflecting the fact that for translocation to occur the motion of the ion and 8 or 9 water molecules must be correlated.

As detailed information is lacking, all 14 local barriers in the solvation energy profiles are presumed to be equal; their magnitude is ε_s. To be consistent with a binding site separation of 2.1 nm, the distance between adjacent minima is taken as 0.15 nm rather than the 0.154 nm indicated by the computer model of the β-helix (Koeppe & Kimura, 1983). The energy barrier for an ion to hop from the intermediate site at x_i to the site at x_{i+1} is $\varepsilon_s + \varepsilon_{\text{elec}}[(x_{i+1}+x_i)/2] - \varepsilon_{\text{elec}}(x_i)$; the barrier for hopping back is $\varepsilon_s + \varepsilon_{\text{elec}}[(x_i+x_{i+1})/2] - \varepsilon_{\text{elec}}(x_{i+1})$. The intermediate maxima are presumed to be halfway between the adjacent minima. It is now easy to estimate the apparent rate constant for translocation, uncorrected for single filing. If the fundamental hopping frequency is ν_0, the apparent rate constant is, by the King-Altman rules (*see* Plowman, 1977),

$$\nu_0 e^{-\beta(\varepsilon_t + \varepsilon_s)}/\text{Den} \tag{5}$$

where

$$\text{Den} = \sum_{j=1}^{14} e^{-\beta\varepsilon_j}; \tag{6}$$

here ε_j is the difference between electrostatic energy at the channel center and at the j'th

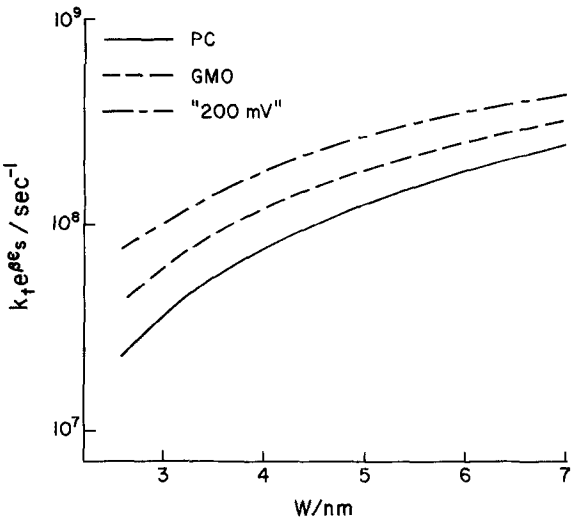

Fig. 5. The solvation adjusted translocation rate constant, $k_t e^{\beta\varepsilon_s}$, as a function of membrane width for the range of membrane potentials of Fig. 4

maximum in the energy profile (Läuger, 1973). As there are 14 such maxima separated by 0.15 nm, the ε_j can be calculated from the electrostatic energy profile.

Single filing requires that the ion and eight or nine water molecules move in phase when translocation occurs. If the motion were strictly one-dimensional this would introduce a statistical factor, f, of 2^{-8} or 2^{-9}, assuming each particle in the channel had an equal probability of moving left or right. The net translocation rate is then

$$k_t[f v_0 e^{-\beta\varepsilon_t}/\mathrm{Den}]e^{-\beta\varepsilon_s}. \tag{7}$$

In reaction rate theory $v_0 \sim kT/h = 6.25 \times 10^{12}\,\mathrm{sec}^{-1}$ at 300 K (*see* Jordan, 1979). The quantity in square brackets can be calculated since the electrostatic potential is known. The results, for PC and GMO membranes of variable width are plotted in Fig. 5. An f value of 2^{-9} was used.

The numbers illustrated for $k_t e^{\beta\varepsilon_s}$ fall in a narrow range. Regardless of the bilayer width the quantity varies from 2×10^7 to $2\times10^8\,\mathrm{sec}^{-1}$ in PC and from 4×10^7 to $3\times10^8\,\mathrm{sec}^{-1}$ in GMO. If fewer water molecules had to move concertedly with the ion, these values would increase somewhat. If translocation required the motion of only 6 water molecules, the calculated value for $k_t e^{\beta\varepsilon_s}$ would increase by a factor of 8. The only experimental measurement of k_t is for Na^+ passage through channels incorporated in bilayers formed from a bacterial-PE, *n*-decane solution (Andersen & Procopio, 1980). The membrane width is probably in the vicinity of 5 nm, typical of $C_{18}-C_{20}$ lipid bilayer formed from this solvent (Benz et al., 1975). The membrane potential for dioleoyl-PE is 420 mV (*see* the Table, Appendix B); that for bacterial-PE should not be much different. Thus $k_t e^{\beta\varepsilon_s}$ is $\sim10^8\,\mathrm{s}^{-1}$ if nine water molecules single file with the ion and is $\sim8\times10^8\,\mathrm{s}^{-1}$ if only six water molecules are needed. The measured value of k_t, $2\times10^7\,\mathrm{sec}^{-1}$ (Andersen & Procopio, 1980), permits estimate of ε_s to be made. The range is between 4 kJ mol^{-1} (if nine water molecules single file) and 9 kJ mol^{-1} (if six water molecules are involved). Either estimate is consistent with the belief that the intermediate barriers to ion translocation in gramicidin are quite small (Tredgold, 1979; Andersen & Procopio, 1980; Finklestein & Andersen, 1981). Each is also consistent with molecular dynamics studies of Na^+ motion in a gramicidin-like water-filled channel where local barriers of ~10 kJ mol^{-1} were found (*see* footnote 1).

Multiple Occupancy

The model permits estimation of the relative binding constants for single and double occupancy of the channel. As long as the channel structure at the first binding site is not altered by binding a second ion, the energy for double occupancy is $2\varepsilon_0+\varepsilon_{12}$ where ε_0 is the binding energy for single occupancy and ε_{12} is the interaction energy when an ion resides at both sites. While ε_0 depends on all the forces that influence permeability, ε_{12} is established by image forces only, as long as multiple occupancy induces *no* structural changes. With these assumptions the ratio of the equilibrium constants for binding the first and the second ion is $K_1/K_2 = 4e^{\beta\varepsilon_{12}}$; the factor of 4 accounts for statistics and the exponential accounts for the interaction energy.

The interaction energy is extremely sensitive to the distance between the binding sites. Changing the assumed site separation from 2.1 to 1.8 nm for a channel incorporated into a bilayer of width 5 nm increases the repulsion energy from 11.4 to 16.9 kJ mol^{-1}. This is almost totally due to interaction between each ion and the other ion's electrical image. The direct coulombic repulsion contributes little at these separations; it increases from 0.8 to 1.0 kJ mol^{-1} under these conditions. Because the interaction energy is so sensitive to site separation, measurements of the binding con-

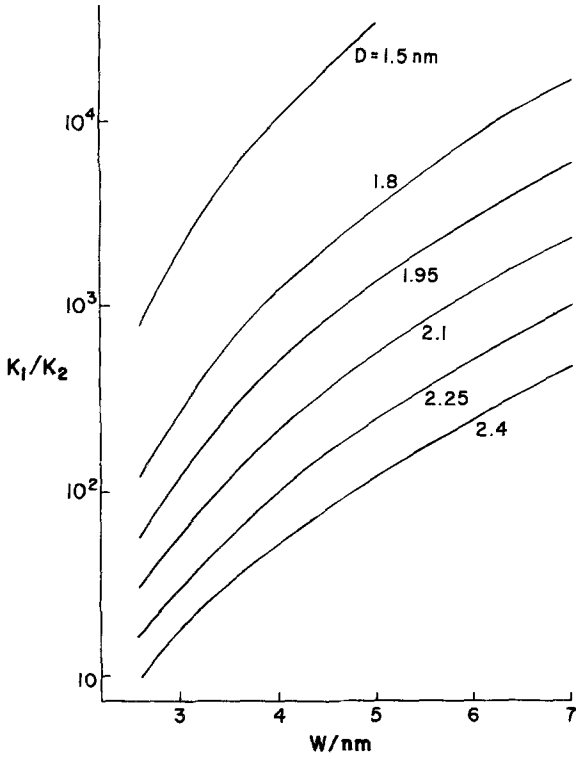

Fig. 6. The ratio of binding constants for single and double occupancy of the gramicidin channel as a function of membrane width and binding site separation D

stant ratio provide a test of the model's reliability. Figure 6 illustrates the effect that membrane width and site separation have on K_1/K_2.

Only one measurement of K_1/K_2 has been reported (Urry et al., 1980*a*, *b*) for Na^+ conductance through gramicidin incorporated into lysolethicin micelles; the value given is ~ 100. While the thickness of the micellar surfaces is not known, the facts that no alkane solvent was used and that the lipid contains mainly C_{18} chains (Urry et al., 1980*b*) suggest a bilayer thickness of ~ 3 nm. From Fig. 6, the calculated K_1/K_2 ratio for sites separated by 2.1 nm is ~ 55; the range of calculated values for bilayers between 2.6 and 3.5 nm thick is 30 to 105. The agreement between calculation and experiment is startling and probably somewhat fortuitous given the assumptions of the theory and the uncertainties in the interpretation of the experiments.

Of possibly greater importance is the sensitivity of the K_1/K_2 ratio to site separation. A minor change in the presumed position of the binding site leads to unreasonable values of K_1/K_2. If the binding site were 0.4 nm from the end of the channel, K_1/K_2 would be between 120 and 550, most probably ~ 260. If the site were only 0.1 nm from the channel entrance, K_1/K_2 would be between 10 and 35, most probably ~ 20. The implication is that the electrostatic model of the gramicidin channel not only accounts for those channel properties that are of electrostatic origin but also that this is not a matter of chance. The parameters of the model were structurally predetermined. The K_1/K_2 ratio would be very different if the binding site positions were shifted by as little as 0.15 nm (i.e. to the next local minimum in the total pore potential). The agreement that has been found argues for the essential accuracy of the electrostatic picture.

This work has been supported by a grant from the National Institutes of Health, GM-28643.

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Received 7 June 1983; revised 6 September 1983

Appendix A

Electrical Radius

Measurements on the polypeptide (Gly—Ala)n (Tredgold & Hole, 1976), which, like gramicidin, has nonpolar amino acid residues, suggests than an $\varepsilon \sim 4$ would be appropriate to the pore former. Calculations on an infinitely long channel with the electrical cross-section of a gramicidin pore indicates that shielding by the pore former reduces the image barrier by $\sim 16\%$ (Jordan, 1981). Stated differently the infinite system could be described in terms of a two-dielectric model if the pore radius were increased from its physical value of ~ 0.2 nm to an equivalent "electrical" value of 0.25 nm. An alternate procedure, which

derives from the calculation of upper and lower bounds to the shielding energy in finite channels, suggests an effective pore radius 0.24 to 0.26 nm (Jordan, 1983); the value 0.25 nm is used.

Appendix B

Membrane Potential

While the membrane potential is not directly measurable (Guggenheim, 1929, 1930), variation due to changes in the lipid composition can be inferred from conductance measurements (Paltauf et al., 1971; Hladky & Haydon, 1973; Andersen, 1978; Pickar & Benz, 1978). Experiments probing the influence of ionic strength (Hladky & Haydon, 1973) or of bilayer thickness (Pickar & Benz, 1978) demonstrate an effect that is reproducible, lipid specific and distinct from effects due to the ionic double layer or membrane thickness.

If adsorbed lipid monolayers and lipid bilayers sandwiched between aqueous solutions had the same structure and surface density, the monolayer surface potential and the membrane dipole potential would be the same. A measure of the reasonableness of this assumption can be obtained from measurements of the relative conductance of positive and negative lipophilic ion probes (Pickar & Benz, 1978). The total energy required to transfer a charged lipophilic ion from the aqueous solution into a membrane formed from neutral phospholipids is

$$\Delta E^{\pm} = \Delta E^{\pm}_{\text{lip}} - \Delta E^{\pm}_{\text{aq}} + \Delta E^{\pm}_{\text{im}} \pm F V_m \tag{B1}$$

where $\Delta E^{+}_{\text{lip}}$ is the solvation energy of a positive ion in the lipid, ΔE^{+}_{aq} is the hydration energy of a positive ion in water, $\Delta E^{\pm}_{\text{im}}$ is the image energy of a positive ion, V_m is the membrane potential and F is Faraday's number. The membrane potential can be determined if the difference in energy between that needed for transferring a positive and a negative ion from water into the lipid is known:

$$V_m = \frac{1}{2F}\{\Delta E^{+} - \Delta E^{-} + \Delta E^{+}_{\text{aq}} - \Delta E^{-}_{\text{aq}} - (\Delta E^{+}_{\text{lip}} - \Delta E^{-}_{\text{lip}}) - (\Delta E^{+}_{\text{im}} - \Delta E^{-}_{\text{im}})\}. \tag{B2}$$

The first term $(\Delta E^{+} - \Delta E^{-})/2F$, has been determined by Pickar and Benz (1978); it is their quantity V_p (*see* their Table 6 and Eq. (22)). The lipophilic ions they used, tetraphenyl borate ($T\phi B^{-}$), tetraphenylphosphonium ($T\phi Ph^{+}$) and tetraphenylarsonium ($T\phi As^{+}$) are very similar in size and structure. Their image energies are thus roughly equal since this term depends only on ionic size and the magnitude (not the sign) of the ionic charges. Solvation energies for ions in the lipid should also be the same for positive and negative ions of the same size since the lipid interior is nonpolar. Ions with similar structure will cause similar reorientations within the lipid. The membrane potential can thus be approximated as

$$V_m = V_p + (\Delta E^{+}_{\text{aq}} - \Delta E^{-}_{\text{aq}})/2F \tag{B3}$$

and calculated if the difference in the hydration energy of oppositely charged ions of the same size is known. Since water is hydrogen bonded and polar the hydration energies of such ions need not be the same. They can be estimated from the absolute hydration energies of alkali metal ions and of halides (Randles, 1956; Kebarle, 1977) or from enthalpies of transfer from water to aprotic solvents (Friedman, 1967; Krishnan & Friedman, 1971). Figure 7 plots $\Delta H^{+}_{\text{aq}} - \Delta H^{-}_{\text{aq}}$ *vs.* the ratio r_-/r_+ for Cl^-, Br^-, I^- and K^+, Rb^+, Cs^+. Extrapolation to $r_-/r_+ = 1$ suggests that hydrated anions are $\sim 32\,\text{kJ mol}^{-1}$ more stable than hydrated cation *of the same size*. The transfer enthalpies suggest somewhat smaller values, ~ 20 to $30\,\text{kJ mol}^{-1}$. Either is in sharp contrast to the difference in hydration energies of ions with the same electronic structure. Cs^{+}_{aq} is $44\,\text{kJ mol}^{-1}$ more stable than I^{-}_{aq}; it is however, far smaller and can bind its waters of hydration much more effectively. The membrane potential estimated on the basis of Fig. 7, is

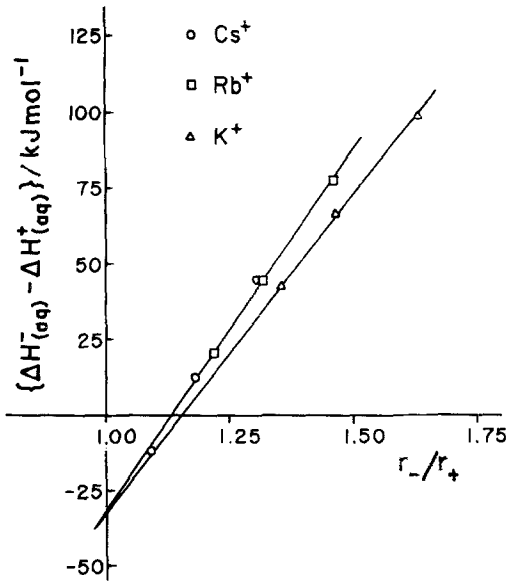


Fig. 7. A plot of the difference in hydration enthalpy for positive and negative monovalent ions as a function of the ionic radius ratio. The enthalpies are those given by Randles (1956); the radii are taken from Pauling (1960)

Table. Comparison of estimated membrane potentials and monolayer surface potentials at similar areas per molecule

Lipid	V_{mono} [a] (mV)	V_{mem} [b] (mV)
GMO	320	274
Dioleoyl-PC	440	390
1-Oleoyl-2-stearoyl-PC	390	363
1-O-Oleoyl-2-O-stearoyl-PC	290	276
Dioleoyl-PE	420	381
Di-O-Oleoyl-PE	310	285

[a] From Eq. (B4)
[b] As quoted by Pickar and Benz (1978)

$$V_m \sim V_p + 166\,\text{mV}. \tag{B4}$$

The Table compares V_m with monolayer surface potential measurements under conditions of equal surface coverage (Hladky & Haydon, 1973; Haydon, 1975). The differences range between 14 and 50 mV, suggesting both that identification of the membrane potential with the monolayer surface potential is reasonable and that the description of

the processes that contribute to the energy differences between an ion solvated in water and in lipid are essentially correct.

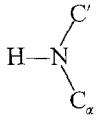
Appendix C

Pore Dipole Potential

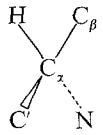
The β-helical structure of gramicidin aligns the carbonyl groups in the polypeptide backbone alternately antiparallel to the channel axis (Urry, 1971). A computer model of this molecular arrangement indicates that the carbon and the oxygen atoms are both located the same distance from the channel axis, ~ 0.34 nm (Koeppe & Kimura, 1983). There should therefore be minima in the solvation energy when the cation is close to two carbonyl oxygens and maxima when the cation is close to two carbonyl carbons. The energy minima may be deepened if the carbonyl oxygens are displaced toward the channel axis by interaction with the cation (Urry, 1971; Fischer, Brickman & Läuger, 1981). Because dipole interactions are fairly long range the local variation in the solvation energy as the ion translocates through the channel is quite small (*see* footnote 1). However, the long range interaction of a cation with the charge distribution in the gramicidin molecule leads to a net stabilization since, on average, the positively charged atoms in the molecule reside further from the channel axis than the negatively charged ones.

The computer model of gramicidin (Koeppe & Kimura, 1983) uses stereochemical constraints to establish the positions of all the atoms in the polypeptide chain as well as the location of the carbonyl oxygens and the β-carbons. Calculation of the charge distribution in the molecule requires pinning down the location of hydrogen atoms bound to the polypeptide backbone as well as knowing the equilibrium position of the various amino acid residues. Given a set of atomic coordinates the charge distribution can be determined from partial charges representative of the atoms in the various amino acids (Schulz & Schirmer, 1978).

Hydrogen atomic coordinates were calculated assuming a CH bond length of 0.112 nm and an NH bond length of 0.105 nm. The position of the amino hydrogens were determined by assuming that the structural unit



is planar and that the HNC′ and HNC_α angles are equal. An alternate procedure would relax the planarity condition and presume that the two HNC bond angles are equal to the $C'NC_\alpha$ angle. As this latter angle is $\sim 122°$, the change would have no significant effect on the calculated position of the amino hydrogens. The position of the hydrogen atom bonded to the α-carbon in the structural unit



was calculated in two ways. The first assumed that the $C_\alpha - C'$ and $C_\alpha - C_\beta$ bonds had the same hybridization; the second assumed that the $HC_\alpha C'$, $HC_\alpha C_\beta$, $HC_\alpha N$ angles were equal. While the H-atom locations calculated by these procedures differ slightly, the resultant charge distributions were not meaningfully different. At the glycine and ethanolamine carbons, the β-carbon is replaced by a second hydrogen; the H atom positions were assigned by assuming that the $HC_\alpha C'$, $HC_\alpha N$ and $HC_\alpha H$ angles are equal. Assigning coordinates to the substituents attached to the β carbon atoms is a more uncertain procedure; I have contrasted two rather different approximations:

1) the net positive charge of amino acid residues (other than glycine) is localized on the β carbon atom,

2) all nonglycine residues are treated as if they were alanine residues which can rotate freely about the $C_\alpha - C_\beta$ bond.

These prescriptions, when combined with heavy atom coordinates (Koeppe & Kimura, 1983) and partial charge parameters (Schulz & Schirmer, 1978) provide an estimate of the charge distribution in the gramicidin molecule. The net charge separation is neither the same for each peptide unit nor for the two ways of treating the amino acid residues. Using the first approximation the charge separation varies from 1.2×10^{-30} to 1.6×10^{-30} C m per peptide unit. Using the second approximation, the variation is much larger – from 7.5×10^{-31} to 2.2×10^{-30} C m per peptide. However, in both cases the negative ends of each dipolar unit points inwards. The *average* charge distribution does not depend upon the specifics of the approximation method. In both cases it is equivalent to a dipole density, $\mu_p \sim 8 \times 10^{-12}$ C m^{-1}, smeared out on a cylindrical surface. In case 1 the cylinder radius is ~ 0.35 nm; in case 2 it is ~ 0.37 nm. While there is undoubtedly local variation in the charge distribution, the estimate is too ambiguous to be reliable. The model uses the mean value, μ_p.

A dipole density of this strength creates a potential difference between the inner and outer portions of the gramicidin molecule. In vacuum (i.e. for an $\varepsilon = 1$) this potential step would be ~ 920 mV. As the model accounts for local polarity (at least in a mean sense), the residual surroundings are purely lipidlike, the proper ε is 2. The potential step is then ~ 460 mV.