Concentration-Dependent Shielding of Electrostatic Potentials Inside the Gramicidin A Channels

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Gramicidin A (Wallace, B. A. Gramicidin channels and pores. *Annu. Rev. Biophys. Biophys. Chem.* **1990**, *19*, 127−157) is a neutral polypeptide that acts as a channel to allow ions to cross otherwise impermeable membranes. We compute sodium chloride currents through the gramicidin A channel using the spectral element method to solve the three-dimensional Poisson−Nernst−Planck equations. The potential profiles through the channel can be resolved into an intrinsic and extrinsic component with surprisingly little ambiguity; that is, the system is nearly linear in that sense. However, the intrinsic potential depends strongly on the bath concentrations: variations in the bath concentrations change the intrinsic potential by several (≈6.6) times the thermal voltage kT/e and thus might be expected to change fluxes and current substantially, by a factor of $e^{6.6} = 735$. Shielding is an important determinant of the properties of this polypeptide channel, even though the polypeptide has no net charge: the nonuniform distribution of fixed charge in gramicidin has many properties expected from a molecule with net charge.

Introduction

Channels are proteins with holes down their middle that allow ions to cross otherwise impermeable cell membranes, thereby controlling a wide range of biological function: a substantial fraction of all drugs act directly or indirectly on channels.[2,3] The physics of ion movement through channels is simple, as is their structure. Ions move through channels much as charged spheres migrate and diffuse through charged cylinders. Steric effects and chemical binding are also involved at narrow parts of the channel, and the possibility of convection and heat flow must not be entirely ignored.[4]

Channels have mostly been studied in the chemical tradition that postulates the binding of ions to specific sites driven by chemical interactions of the ion and the channel protein.[5,6] High-resolution simulations in this tradition have tried to calculate the location and movement of every atom of the channel protein and every atom in its pore.[7−9]

Lower-resolution traditional models of permeation, following traditional models of enzymes, have described the rate of ion movement across the potential barriers that separate and thus define "states" of the protein.[3,10,11] The rate constants of movement in these models depend exponentially on the height of the potential barriers, according to "Eyring rate theory" (often of the gas phase).[3,12−16] In nearly all cases, these potential barriers are assumed to be independent of ion concentration. In this paper, we will show that potential barriers depend in an important way on concentration, calling into question one of the fundamental assumptions of traditional enzymology and channel biophysics. This is not the only criticism of such traditional models; see the appendix of ref 17.

An alternative approach to the study of channels uses the physical tradition.[18−50] These physical theories and simulations use as little detail, chemical or structural, as

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necessary to describe the function of channels seen in experiments and real life. At low resolution, physical theories describe the channel as a distribution of fixed charge;[18−31] at higher resolution, they include the chemical effects of the finite size of ions and the channel pore;[32−37] at still higher resolution, they describe individual trajectories of spherical ions.[38−41]

For several reasons, no attempt is made in physical theories to describe the motion of all atoms. It is not easy for simulations to include macroscopic variables such as concentration and membrane potential, particularly in the nonequilibrium situations in which channels function. It is also not easy for simulations to span the gap between atomic and biological length scales, while correctly predicting the relationship of the macroscopic variables. Most biological phenomena occur on meso- or macroscopic time and length scales and involve staggering numbers of atoms acting together for micro- to milliseconds. In simple special cases, the collective behavior of atoms on these scales can be described by Ohm's "law" and Fick's law (for example). Simulations in atomic detail must be able to reproduce these simple situations quantitatively if they are be relied on to analyze biological function and experiments.

In physical models, permeation is described in the multiscale/multiresolution tradition of engineering and physics, using collective parameters (e.g., concentration, macroscopic electric field, diffusion coefficient, and dielectric coefficient) to describe properties that are hard to simulate in full atomic detail. Simulation has an indispensable but limited role in such models, namely, to provide estimates of the collective parameters of materials, for example, diffusion and dielectric coefficient. It seems to us that such simulations can be made more easily and are more reliable quantitatively than direct calculations of macroscopic fields.

The "engineering" approach to permeation in channels often involves the PNP equations, which are (probably) the simplest self-consistent nonequilibrium extension of the Poisson−Boltzmann, Gouy−Chapman, and Debye−Huckel treatments of ionic solutions and proteins.[54−59] The PNP equations consist of the Poisson equation, which relates total charge density ρ (consisting of the sum of fixed charge density and charge densities of all mobile ionic species) and electric potential ϕ,

$$\nabla \cdot (\epsilon_0 \epsilon_{rel} \nabla \phi) = -\rho$$

and the Nernst−Planck equations, one for each species i of mobile ion, which relate flux density J_i, electric potential ϕ, and ion concentration C_i:

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$$J_i = -z_i\mu_i C_i \nabla\phi - D_i \nabla C_i$$

Here ϵ_0 is the permeability of free space, ϵ_{rel} is the relative dielectric coefficient, and z_i, μ_i, and D_i are the charge state, mobility, and diffusion coefficient, respectively, of ion species i. By assuming a time-independent solution and by requiring the conservation of mass for each ionic species, that is, $\nabla \cdot J_i = 0$, these equations can be rewritten in a form in which they can be used to solve for C_i.

The PNP theories by themselves can account for many properties of channels[18−31] and if extended to include the finite size of ions can account for the selectivity of channels for different ions as well.[32−37] The success of these theories is surprising because the physical theories ignore the chemical interactions of electron orbitals of ions and channel protein. But the systems studied are highly charged (having some 4 glutamates in some 300 Å^3, producing solutions between 10 and 20 M!) and relatively large in diameter (7−8 Å), and it was not clear if the physical approach will work in narrow channels with no net charge, such as gramicidin.[1]

Physical models are, however, able to predict *IV* relations[19,20,30,31] even in narrow channels such as gramicidin that have no net charge, probably because a substantial electrical potential acts on ions within gramicidin despite the overall neutrality of the molecule. The potential arises because the (partial) negative charges of the carbonyl oxygens are much closer to permeating ions than the partial positive charges of the carbonyl carbons. The fixed charge of the gramicidin molecule is not uniformly distributed when viewed from inside the pore of the channel. Viewed from inside, the channel is highly charged, with a large electrical potential. Only when viewed from outside does gramicidin seem neutral.

The fact that a physical model, with no chemical forces beyond diffusion, is able to deal so well with data is surprising: most of the literature of channels[3,8,9] uses models dominated by the chemical forces absent in the PNP theory. Indeed, some models of permeation and most models of enzymes ignore electrical terms altogether, allowing ions to bind to specific sites on proteins without changing the electrical potential required by Coulomb's law[10,11,55,56] as well as found in measurements of single-electron devices.[57] The question is, what features of the physical model provide the nonlinearity characteristic of channel function (e.g., the variation in conductance with ionic concentration)? The answer seems to be shielding. The variation of potential with concentration is, of course, a dominant determinant of the properties of ionic solutions.[58−60]

What is surprising is that a calculation like that presented here, which includes only these effects, is sufficient to explain many properties of this channel protein. This work suggests then a specific working hypothesis[21,22] to test and falsify by experimentation in other channels and proteins: the dominant determinant of ion permeation and binding is the energy of the electric field and the variation of that energy with ion concentration (i.e., shielding). This hypothesis is nearly the opposite of that in standard treatments of permeation and ion binding that more or less ignore the electrical terms.[3,8−11,55,56] Undoubtedly, in many cases both chemical and electrical effects will be involved.[7,33−37] It will be the role of experimentation, simulation, and theory to evaluate the relative contributions of each force and to see if purely physical treatments of physical forces are sufficient.[33−37]

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Methods

The PNP equations are solved here using the spectral element method, a particular type of finite element method.[61,62] Very briefly, in the finite element method, the computational domain is broken up into subdomains called elements. Inside each element, functions are approximated by polynomial expansions, which are used to discretize the differential equations into matrix equations. In "standard" finite elements, the order of the polynomial expansions is fixed and relatively low, and there is a relatively large number of finite elements; if the computed solution is insufficiently accurate, some or all of the finite elements are subdivided, and the problem is solved once more. In contrast, in the spectral element method, there are relatively few elements, and higher-order polynomials are used; if the computed solution is insufficiently accurate, the polynomial order rather than the number of elements is increased. The spectral element method has the advantage that it converges exponentially to the true solution as the polynomial order is increased, provided that the solution is smooth enough. In addition, the nodes of each element are not distributed uniformly within that element but are bunched toward the boundaries; as a result, it is possible to have very high resolution at interfaces between dissimilar materials, where it is most needed, and much lower resolution elsewhere, where high resolution would be wasted. Judicious use of these two properties allows us to include, without excessive computational effort, realistic portions of the baths in our simulations, thereby allowing the use of physically realistic boundary conditions.[19,20]

The PNP equations have several inputs: (1) the three-dimensional charge distribution of the gramicidin molecule, (2) the dielectric coefficients, and (3) the diffusion coefficients for the mobile ions in the different regions in the simulation domain. Our PNP calculation contains (almost) no internal nonphysical parameters that might be adjusted to improve the fit between simulation and experiment; the placement of the individual spectral elements could in principle be adjusted (by moving the boundary between different regions) to improve fit, but the placement has not in fact been used in that way in this paper.

Our previous work computed (mainly) the current−voltage (*IV*) curves for gramicidin; various sensitivity analyses were performed to determine how parameters of the model changed the *IV* curves.

In this paper, the electric potential inside the gramicidin molecule is studied, as voltage and the concentrations of Na^+Cl^- are varied in the surrounding baths. We compute the average potential in the channel by multiplying the potential at a point by a three-dimensional Gaussian of half-width 1 Å and then integrating the result over the entire computational domain. This procedure helps us display and compare results for different parameter values. The plots of potential along the channel axis were computed by moving the center of the Gaussian along the channel axis in steps of 0.1 Å, so that each point on the curve represents a spatially averaged potential akin to the spatially averaged potential acting on a real ion of 1 Å radius.

Results & Discussion

Before we describe the results we have found, a bit of terminology is in order: we define "intrinsic" and "ex-

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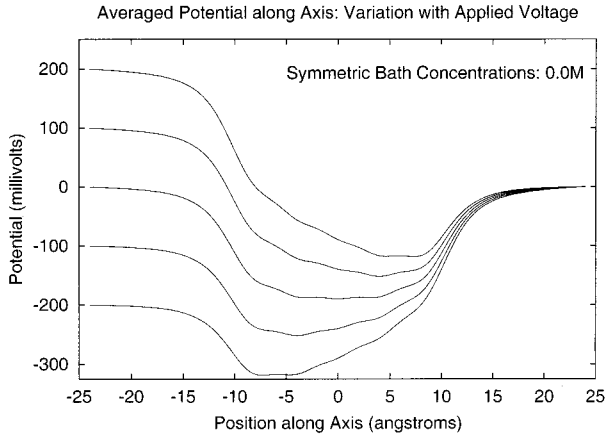

Figure 1. The average potential inside the channel as a function of position, for five applied voltages: −200, −100, 0, 100, and 200 mV. The electrolyte concentration in the baths and channel is 0.0 M. The middle curve shows the intrinsic potential.

trinsic" components of potential as follows: if we have left and right electrode voltage boundary conditions V_l and V_r and concentration boundary conditions C_l and C_r, then we may write the potential which satisfies the PNP equations with these boundary conditions as $\Phi(V_l, V_r, C_l, C_r)$. The intrinsic potential is simply the potential with zero voltages at both terminals: $\Phi_{int} = \Phi(0, 0, C_l, C_r)$, and the extrinsic potential is the difference between the two: $\Phi_{ext} = \Phi(V_l, V_r, C_l, C_r) - \Phi_{int}$. Since in these simulations we always hold V_r at zero, we can normalize the extrinsic potential by dividing it by V_l: $\Phi_{ext:norm} = (\Phi(V_l, V_r, C_l, C_r) - \Phi_{int})/V_l$.

We begin by examining the variation of the electric potential as a function of the externally applied voltage, for a "naked" (i.e., unscreened) gramicidin molecule, a gramicidin molecule with zero electrolyte concentrations in the surrounding baths. (The dielectric coefficient of the bath and channel regions is kept at 80, and the dielectric coefficient of the protein and lipid regions is kept at 2 for this calculation: this is *not* a gramicidin channel in a vacuum.) Figure 1 shows the average potential with −200, −100, 0, 100, and 200 mV applied at the left electrode, when the right electrode is held at 0 mV. Most of the potential drop is seen across the channel itself, as one might expect, because the channel has the highest "resistance" of any portion of the circuit. From the figure, it appears that the overall potential can be decomposed into intrinsic and extrinsic components as described above. Because the electrolyte concentrations are zero in this particular case, the Poisson equation is linear and this decomposition is in fact exact (and not surprising). Figure 2 shows the normalized extrinsic potential.

Figure 3 shows an analogous potential profile, computed now with the same (so-called symmetric) electrolyte concentrations of 2.0 M in both baths, using the same applied voltages as in Figures 1 and 2. Most of the extrinsic potential drop occurs across the channel itself, but now the intrinsic potential is quite significantly attenuated compared with the previous case. In this case, the system is not linear, and so the normalized extrinsic potential will vary with the applied voltage.

Figure 4 shows the normalized extrinsic potential for this second case, with 2.0 M symmetric concentrations. The various curves are all very close to each other, showing that (in some sense) the system is nearly linear, despite the relatively large electrolyte concentration. Although the curves are close together by visual inspection, the

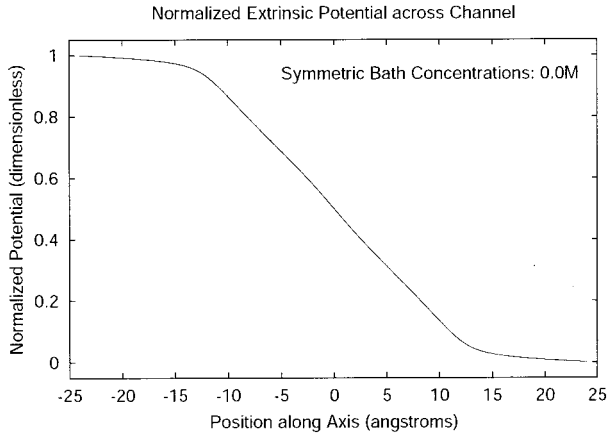

Figure 2. The normalized extrinsic potential across the channel, for 0.0 M electrolyte concentration.

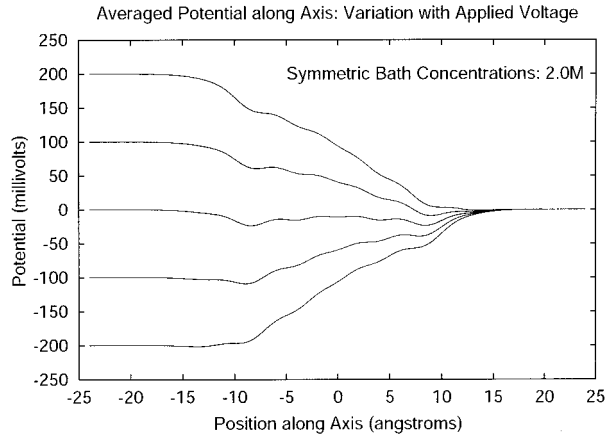

Figure 3. The average potential inside the channel as a function of position, for five applied voltages: −200, −100, 0, 100, and 200 mV. The electrolyte concentration in the baths and channel is 2.0 M. Again, the middle curve shows the intrinsic potential.

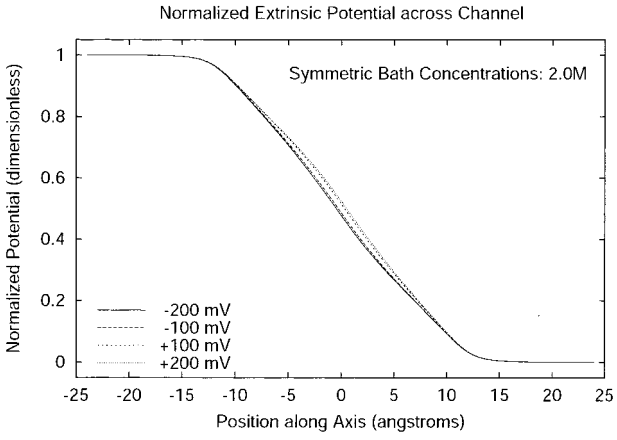

Figure 4. The normalized extrinsic potential across the channel, for 2.0 M electrolyte concentration.

change in shape does have a noticeable effect on *IV* curves: they are not quite straight lines.

Changes in the concentration of ions in the bath do change the potential inside gramicidin, even though the gramicidin is net neutral and no charges are present in our model of the lipid membrane. Figure 5 shows how the intrinsic potential varies with concentration: plots are given with both electrodes at 0 mV, for bath concentrations from 0.0 to 2.0 M. As the concentration of ions increases from 0.0 to 2.0 M, the potential ϕ is attenuated about 6.6

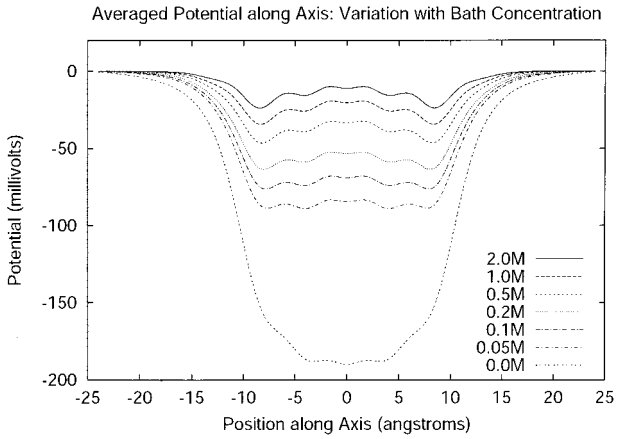

Figure 5. The average intrinsic potential inside the channel as a function of bath electrolyte concentration. The electrolyte concentration varies from 0.0 M (the same as in Figures 1 and 2) to 2.0 M (the same as in Figures 3 and 4) symmetrically on both sides of the channel.

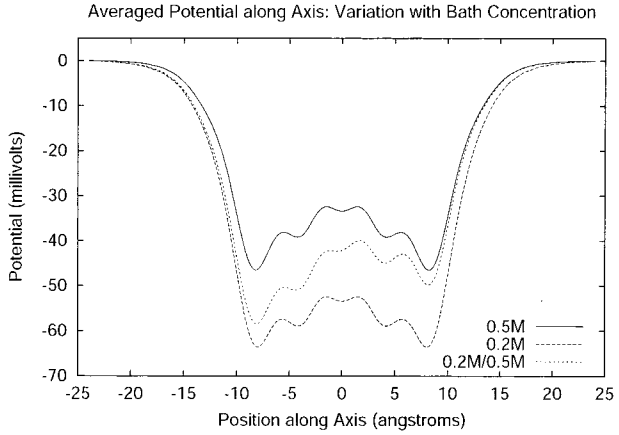

Figure 6. Average intrinsic potential: two of the same curves as in the previous figure, but with an asymmetric case superposed: 0.2 M on the left and 0.5 M on the right.

times the thermal voltage kT/e (roughly 25 mV at room temperature), suggesting a substantial effect on current.

Figure 6 shows 0.2 and 0.5 M again and adds an asymmetric configuration, 0.2 M on the left and 0.5 M on the right. It is clear that the degree of attenuation varies along the length of the channel: the asymmetric curve is near the symmetric 0.2 M curve on the left side of the channel and near the symmetric 0.5 M curve on the right-hand side.

Finally, in Figure 7 we plot the magnitude of the potential at the center of the channel versus the electrolyte concentration for the six symmetric cases. It is clear that there is a relatively simple relationship between the central potential and the concentration; however, it is not a simple power law as would be expected for Debye shielding in a bulk solution: on this log−log plot, a power-law relationship would be visible as a straight line. We do not find a simple decomposition of the intrinsic potential into either a product of a concentration-dependent piece and a concentration-independent piece or a sum of two such pieces. This is not particularly surprising. At high concentrations, the shielding is strong, and thus an ion at some point along the central axis of the channel will experience a relatively simple potential: mainly the externally applied background, with relatively little effect due to the fixed charge of the gramicidin molecule. Effectively, the ion acts (locally) as though it were in a bulk solution with a fixed electric field. At low concentrations, on the other hand, the shielding is weaker, and the potential in which the ion finds itself contains both the externally applied background and the quickly varying and anisotropic internal potential of the channel: the ion interacts strongly with the walls of the channel, even when it is located at the central axis of the channel. Thus, the "effective geometry" varies in a somewhat complicated fashion both as the concentration and thus the shielding length are varied and as a function of the location.

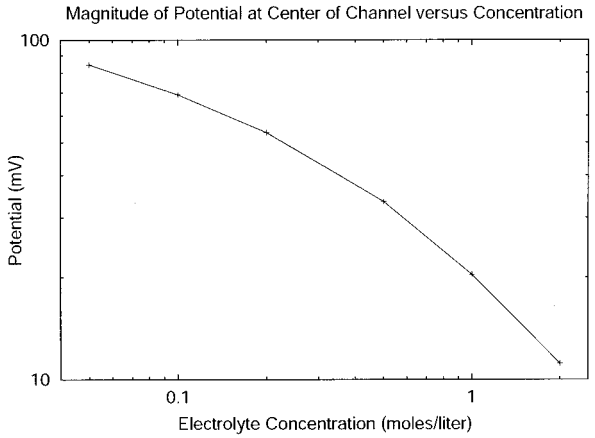

Figure 7. The magnitude of the intrinsic potential at the center of the channel as a function of electrolyte concentration.

The minimum intrinsic potential, as shown in Figure 5, varies quite significantly as the bath concentration is varied: from about −24 mV at 2.0 M to about −190 mV at 0.0 M. This change of 166 mV amounts to some 6.6 times the thermal voltage. The variation in potential computed here has a profound effect on flux. Crudely speaking, flux varies exponentially with potential,[6,10−13] and the potential change here would produce (in this crude view) changes in flux of the order of $e^{6.6} = 735$, a dominating effect. Less crudely, flux depends on the integral of an exponential of the potential,[63,64] producing a smaller but still large effect, although not as much as expected by a simple exponential rate theory. The reason is that essentially all of the current is carried by Na^+ ions, and the concentration profile and thus the flux of these ions are largely fixed by the need to maintain approximate electrical neutrality within the channel volume.

The model considered here shows the profound effect of shielding on the properties of an ion channel, suggesting that models containing more atomic detail must also describe shielding with some precision. It seems possible to us that the effects of shielding on ion binding and enzymatic function are as profound as they are on permeation. If this possibility is proven to be true, a substantial revision in our approach to drug design, protein folding, and enzymatic function would be necessary. Testing the possibility is likely to be revealing, showing how proteins use both macroscopic phenomena like shielding and atomic phenomena like interaction of atomic orbitals to perform biological functions.

Conclusions

We find that the potential inside the channel can be decomposed into two parts: an intrinsic potential that depends only on the bath concentration (and of course on

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the charge distribution of the gramicidin molecule and its shape) and an externally applied potential that depends mostly on the applied voltages. The response of the system to the applied voltages is relatively linear; however, the intrinsic potential depends strongly on the bath concentrations: variations in the bath concentrations change the intrinsic potential by several (≈6.6) times the thermal voltage kT/e and thus might be expected to change fluxes and current substantially. We look forward to a theoretical analysis of the PNP equations which justifies the parsing of the potential profiles and which explains the physical meaning of the components. Parsing a nonlinear theory into linear components is a nontrivial business, even numerically, and can take decades judging by numerical work[65] on the P−N junction. Mathematical analysis of this sort is in fact rarely possible unless small parameters can be exploited.[66−68]

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