Influence of a Channel-Forming Peptide on Energy Barriers to Ion Permeation, Viewed from a Continuum Dielectric Perspective

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ABSTRACT The continuum three-dielectric model for an aqueous ion channel pore-forming peptide-membrane system is extended to account for the finite length of the channel. We focus on the electrostatic influence that a channel-forming peptide may exert on energy barriers to ion permeation. The nonlinear dielectric behavior of channel water caused by dielectric saturation in the presence of an ion is explicitly modeled by assigning channel water a mean dielectric constant much less than that of bulk water. An exact solution of the continuum problem is formulated by approximating the dielectric behavior of bulk water, assigning it a dielectric constant of infinity. The validity of this approximation is verified by comparison with a Poisson-Boltzmann description of the electrolyte. The formal equivalence of high ionic strength and high electrolyte dielectric constant is demonstrated. We estimate limits on the reduction of the electrostatic free energy caused by ionic interaction with the channel-forming peptide. We find that even assigning this region an ϵ of 100, its influence is insufficient to lower permeation free energy barriers to values consistent with observed channel conductances. We provide estimates of the effective dielectric constant of this highly polarizable region, by comparing energy barriers computed using the continuum approach with those found from a semi-microscopic analysis of a simplified model of a gramicidin-like charge distribution. Possible ways of improving both models are discussed.

INTRODUCTION

Continuum electrostatic modeling of ion channels has provided a qualitatively useful mesoscopic way to interpret conductance measurements in terms of permeation free energy profiles (Parsegian, 1969; Levitt, 1978; Jordan, 1981, 1982; Jordan et al., 1989; Monoi, 1991; Sancho and Martinez, 1991). The fundamental qualitative idea has been that channel water forms an electrically permittive, high dielectric constant (ϵ) pathway, which greatly lowers the image force barrier and, thus, provides little resistance to ion transport. In a two dielectric continuum picture, the surrounding protein and lipid are approximated as a single dielectric phase of low ϵ, usually 2; the influence of the polarizable peptide is accounted for by introducing the concept of the channel's "electrical radius," somewhat larger than its van der Waals radius (Levitt, 1978; Jordan, 1981, 1982; Jordan et al., 1989; Monoi, 1991; Sancho and Martinez, 1991) Because of this picture's conceptual simplicity, although fully recognizing that the actual value of channel water's dielectric constant is unknown (and in some sense, indeterminate), we have reconsidered (Partenskii and Jordan, 1992a) this basic two-dielectric model for the channel-protein-membrane ensemble with the specific goal of connecting the continuum description of channel energetics to the results of a semimicroscopic (SMC) model of a single-file channel, in which channel water is depicted as a linear chain of reorientable dipoles (Partenskii et al., 1991a; Partenskii and Jordan, 1992a). To do this, we equated the energetics of charging an ionic sphere (Born model) in a continuum channel with that of charging the analogous sphere in the discrete dipolar chain; the parallel processes are illustrated in Fig. 1. To connect the continuum and SMC pictures, we introduced a nonlinear dielectric function, $\epsilon_i(q)$, describing the Born charging process for an ion in the channel (q is the instantaneous value of the cavity charge during the change from 0 to 1). For the two approaches to have similar energetic consequences, we found that the effective continuum values of ϵ_i must be much smaller than that of bulk water ($\epsilon \sim 80$) (Partenskii and Jordan, 1992a, b). This observation thus reopened the question of the physical basis for the low energy barriers to ion transport through and within a channel. It is obviously inappropriate to blithely ascribe conductance to channel water being a high ϵ domain.

One obvious mechanism for lowering the permeation free energy barrier is interaction between the ion and the water dipoles on one hand and the charge distribution of the channel-forming peptide (CFP) on the other. In our initial attempt to extend the SMC problem, the influence of a CFP was taken into account in a primitive manner, by considering a model distribution of fixed charges (Partenskii et al., 1991b). Instead of reducing the total barrier, such frozen charges make it even greater. However, the barrier might be significantly reduced if ion-induced relaxation of the CFP's charge distribution were taken into consideration (Partenskii and Jordan, 1992b; Partenskii et al., 1992). Model analysis of a system with gramicidin-like geometry demonstrates that reorientation of dipolar CO groups can have a noticeable effect in this direction.

The microscopic analysis of the effect that a reorientable dipolar charge distribution has on the energetics of the ion-dipole chain defines the extended semi-microscopic (ESMC)

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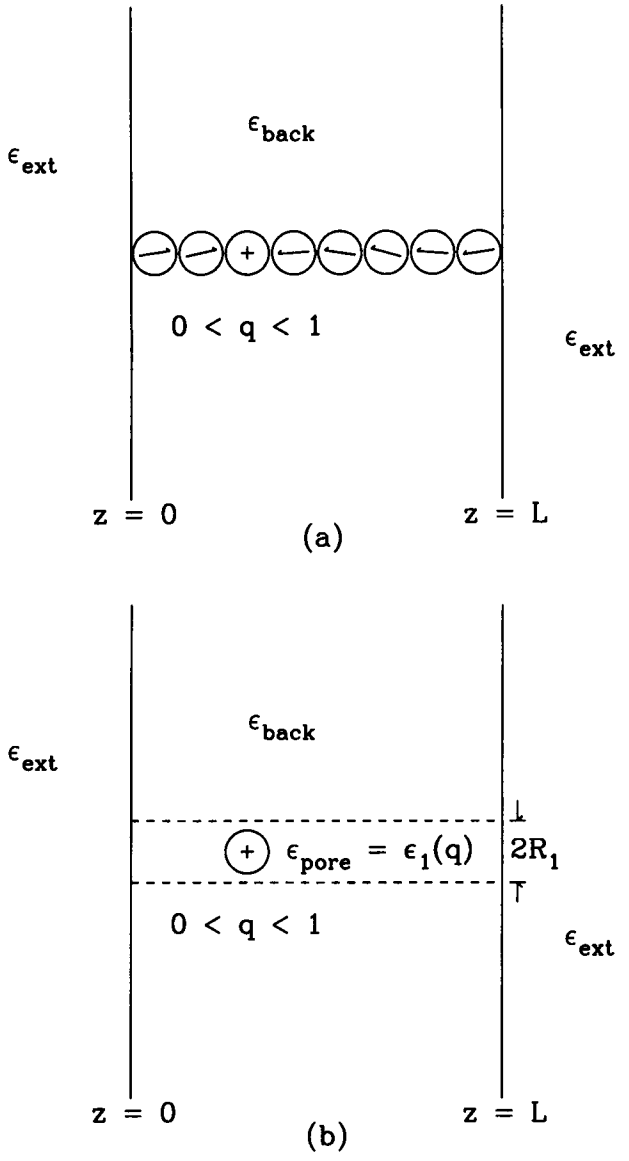

FIGURE 1 Schematic diagram of semi-microscopic (SMC) and continuum models for an ion in an aqueous pore. The membrane width is L. SMC modeling (a) describes the pore as a linear chain of water dipoles embedded in a continuum with lipid-like dielectric properties (low ϵ); the dipolar orientations illustrated are representative of higher q values ($q > 0.3$). The aqueous regions external to the membrane are a high ϵ domain; we set $\epsilon_{ext} = \infty$ for analytical purposes. Continuum modeling (b) replaces the ion-dipole chain by a dielectric phase and an ion in a cavity. The pore radius is R_1, and ϵ_1 is chosen to reproduce the energetics of ion transfer in the SMC model.

model. This accounts in a simple, approximate way for the contribution of carbonyl groups to the polarizability of the channel and the reduction of the ion energy barrier. In gramicidin the carbonyls are arranged alternately antiparallel on a helix. In our ESMC analysis of carbonyl reorientation (Partenskii and Jordan, 1992b; Partenskii et al., 1992), the groups are placed on a series of 10 rings, each with three CO groups; the COs on alternate rings are antiparallel. Although highly idealized, this model has a significant feature typical of ionic environments in ionic channels and, more generally, in proteins. These are the polar groups close to putative ion binding sites, which are oriented in the unoccupied channel and which reorient under the influence of the ionic field, leading to additional stabilization energy. In the ESMC model, this reorientation is determined self-consistently by approximate minimization of the total free energy. Thus, a study of the analogous continuum problem can provide insight into the general question of how best to use dielectric modeling in the study of electrical interactions in proteins and other macromolecular systems, where reorientation of initially aligned dipolar groups is always a major consideration.

Viewed from a continuum perspective, this induced redistribution of charge may be related to an additional contribution to the dielectric permittivity of the medium. To include such effects in a continuum description of a channel-membrane system, the two-dielectric model, which does not consider the CFP, must be extended, in some sense accounting for the polarizability of the CFP in the vicinity of the aqueous pore. A natural way to characterize this is the three-dielectric model, for which an analytical solution has been determined in the limit of the infinite channel (Jordan, 1981), an approximation only reliable for describing domains far from the membrane-water interface.

We focus on this three-dielectric description of the channel pore-forming peptide-membrane domain and attempt to interpret the results of ESMC analysis in terms of a continuum model, always restricted so that ϵ_1, the pore dielectric constant, is small. We provide answers to three specific questions. Can a continuum model, suitably defined, account for observed low energy barriers to ion transport? And if so, what are reasonable values to assign to the dielectric constant ϵ_2 of the polar regions of a CFP? Are there circumstances under which the continuum approach must be substantially modified if it is to be consistent with the underlying statistical mechanics?

THE MODEL AND BASIC EQUATIONS

We choose the dielectric geometry illustrated in Fig. 2. The channel pore former-membrane ensemble is simulated by a domain bounded by the planes $z = 0$ and $z = L$. To describe the aqueous pore and the CFP, we use a three-dielectric model (Jordan, 1981; Monoi, 1991). The pore interior is a cylinder of polar radius $\rho = R_1$. Basing our analysis on the ESMC approach, we describe this (water-filled) region using the "constant value approximation" for the dielectric function, $\epsilon_1(q) = \epsilon_1$ (Partenskii and Jordan, 1992a). This domain, surrounded by the highly polarizable region of the CFP associated with polar groups (in a gramicidin-like model, the backbone COs), is simulated by a cylindrical shell (the "sleeve") with $R_1 < \rho < R_2$ and dielectric constant ϵ_2. The remainder of the channel-peptide-lipid ensemble, the region where $\rho > R_2$, is assigned the usual nonpolar dielectric permittivity, $\epsilon_3 = 2$. The pore radius, R_1, is assigned values between 2.0 and 3.0 Å, typical of standard models of gramicidin-like channels (Levitt, 1978; Jordan, 1981, 1982, Monoi, 1991).

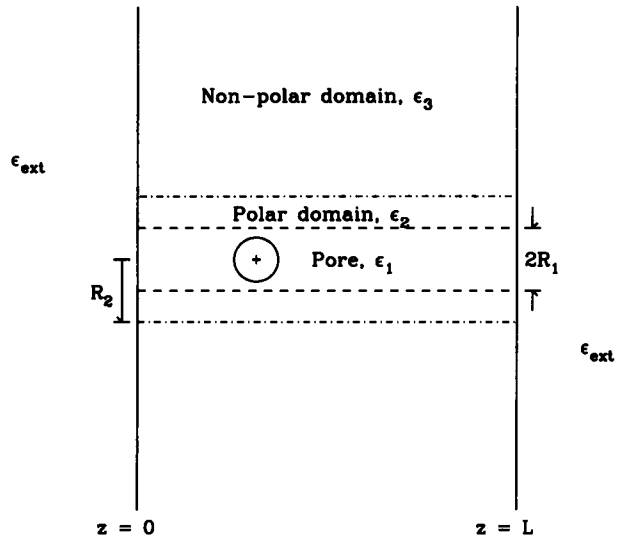

FIGURE 2 Schematic diagram of the three dielectric model for the ion-pore-peptide-lipid-water ensemble. As in Fig. 1 *b*, the membrane width is L, the pore radius is R_1, and ϵ_1 is chosen to reproduce the energetics of ion transfer in the SMC model. The highly polarizable peptide region, which imitates the effect of carbonyls' polarization through the effective "sleeve" dielectric constant ϵ_2, has a radius R_2. The pore ϵ_1 is site-dependent (see text). The ion can reside at any of the eight source sites illustrated in Fig. 1 *a*.

The appropriate values of ϵ_1 for use in continuum modeling are determined by an SMC treatment of a chain of an ion and seven water dipoles (in the absence of the CFP), mimicking gramicidin's single-file geometry (Levitt, 1984). Because the dielectric constant ϵ_1 is position-dependent, the continuum identification describes the Born charging process in some averaged sense. For narrow pores, there is strong polarization of the dipolar water chain, with a corresponding dielectric saturation, generally significant when the cavity charge q exceeds 0.3. Using a background ϵ of 2 to account for high frequency, electronic polarizability and employing a gramicidin-like geometry, the SMC model implies values of ϵ_1 between 2.7 and 4.2, depending upon ionic location (Partenskii and Jordan, 1992a). Regardless of ionic position, ϵ_1 is much smaller than $\epsilon_1 \sim 80$, typical of bulk water. To estimate the effective value of ϵ_1, we equate the free energies required to charge a cavity in processes related to the models illustrated in Fig. 1:

$$(\text{cavity} + \text{seven dipoles})|_{q=0} \rightarrow (\text{cavity} + \text{seven dipoles})|_{q=1} \quad \text{(SMC)}$$

$$(\text{cavity} + \text{dielectric})|_{q=0} \rightarrow (\text{cavity} + \text{dielectric})|_{q=1} \quad \text{(continuum);}$$

This identification likens ion-induced orientational ordering (in the SMC approach) to decreasing channel ϵ (in the continuum approach). Eight polar sources are used by analogy to gramicidin in which seven to nine water molecules are associated with the ion in the channel's single file (Levitt, 1984). The ion (and thus the cavity) can reside at any of these eight sites (of which four are energetically distinct). The positional dependence of ϵ reflects the differences in image forces and in the effective number of nearest neighbors influencing the ion-induced polarization of the dipolar chain. Comparison of the results of ESMC and SMC analysis, i.e., the presence or the absence of a CFP (Partenskii et al., 1991b, 1992; Partenskii and Jordan, 1992b), demonstrates that the multipolar charge distribution of the CFP does not noticeably alter the polarization of the chain of water dipoles in the ionic field. With an ion present anywhere in the channel, the difference in the average axial dipole moment of the chain never exceeds 5–10% after accounting for the influence of the CFP by ESMC theory. Therefore, the effective channel dielectric constant, ϵ_1, is only marginally influenced by the presence of a CFP and we can carry out continuum modeling based on ϵ_1 values determined with no CFP present. It should be stressed that the ϵ_1 range established by this procedure is geometry- and ionic size-dependent. Changes in the number of waters and the ionic radius affect the numerical values; however, for all reasonable choices ϵ_1 is inevitably small, in the range of 2.5–5.

The problem solved in Jordan (1981) for the membrane of infinite thickness is characterized by the equations

$$\nabla^2 V_\alpha = -\frac{4\pi\delta(z - z_0)\delta(\rho)}{\epsilon_1} \tag{1}$$

$$V_1 = V_2, \qquad \frac{\epsilon_1 \partial V_1}{\partial \rho} = \frac{\epsilon_2 \partial V_2}{\partial \rho}, \qquad \rho = R_1, \tag{2}$$

$$V_2 = V_3, \qquad \frac{\epsilon_2 \partial V_2}{\partial \rho} = \frac{\epsilon_3 \partial V_3}{\partial \rho}, \qquad \rho = R_2 \tag{3}$$

The ion is located at a point z_0 on the channel axis; V_α is the electric potential in phase α with dielectric constant $\epsilon = \epsilon_\alpha$, ($\alpha$ = 1–3); and $\delta(x)$ is the Dirac δ-function.

The finite thickness of the membrane is described by the boundary conditions

$$V_\alpha = V_{ext}, \qquad \epsilon_\alpha \frac{\partial V_\alpha}{\partial z} = \epsilon_{ext} \frac{\partial V_{ext}}{\partial z} \qquad (z = 0 \text{ and } z = L) \tag{4}$$

The index "ext" refers to the external regions $z < 0$ and $z > L$, occupied by the aqueous solvent (electrolyte). For our purposes, where only the potential within the channel is important, one can approximate ϵ_{ext} as ∞. This assumption yields an analytical solution convenient for analysis, and Eq 4 is replaced by the following (Partenskii and Jordan, 1992a)

$$V_\alpha = 0, \qquad \frac{\partial V_\alpha}{\partial z} = 0 \qquad (z = 0 \quad \text{and} \quad z = L) \tag{5}$$

In the Appendix, we discuss the limitations of this approximation, comparing our analytical results with the numerical solution of a more realistic problem, in which the electrolyte is described by the nonlinear Poisson-Boltzmann (NLPB) equation (Jordan et al., 1989)

We represent the potential on the axis of the channel in the following form (Jordan, 1981):

$$V_1(z) = V_h(z) + V_{st}(z), \tag{6}$$

inherited from the analysis of the infinite channel problem. The first contribution describes the ionic field in the uniform membrane with the dielectric constant ϵ_1, whereas the second one is responsible for the structure of the membrane (the difference between a three-dielectric and a uniform (one-dielectric) model). An analytical solution is found using the method of images, following the derivation presented in (Partenskii and Jordan, 1992a):

$$V_h(z) = -\frac{1}{\epsilon_1}\left\{16Lz_0z\sum_{n=1}^{\infty}\frac{n}{[(2nL)^2 - z^2 - (z_0)^2]^2 - (2zz_0)^2} + \frac{1}{z+z_0} - \frac{1}{|z|}\right\} \tag{7}$$

and

$$V_{st}(z) = \frac{4}{L\epsilon_1}\sum_{n=1}^{\infty} B\left(\frac{n\pi R_1}{L}\right)\sin\left(\frac{z_0 n\pi}{L}\right)\sin\left(\frac{zn\pi}{L}\right), \tag{8}$$

Here $B(x)$ is determined by the solution of the infinite channel problem (Jordan, 1981):

$$B(x) = k_1(x)\left\{1 + \frac{\lambda_1\phi_1^2 k_2}{k_1 - k_2(1-\phi_1)(1-\lambda_1\phi_1(x))}\right\}, \tag{9}$$

where

$$\phi_i = [1 - (1-\lambda_i)xK_1(xt_i)I_0(xt_i)t_i)]^{-1},$$

$$k_i(x) = (1-\lambda_i)xK_0(xt_i)K_1(xt_i)\phi_i(xt_i),$$

$$\lambda_i \equiv \frac{\epsilon_{i+1}}{\epsilon_i}, \qquad t_i \equiv \frac{R_i}{R_1},$$

with I_n and K_n Bessel functions of the second and third kind (Whittaker and Watson, 1961).

For the energy of interaction between a charge $q = 1$ located at the point z_0 on the axis of the channel and its environment, we have

$$\tilde{U}(z_0) = \tfrac{1}{2}\tilde{V}_1(z_0) \tag{10}$$

where the tilde indicates that potential does not include the divergent point charge self-energy contribution. Instead, we have to add the Born self-energy for the ion in the uniform dielectric with $\epsilon = \epsilon_1$ relative to its value in bulk water ($\epsilon_w = 80$),

$$U^{Born} = \frac{1}{2R_1}\left(\frac{1}{\epsilon_1} - \frac{1}{\epsilon_w}\right) \tag{11}$$

so that the total energy is equal to

$$U(z_0) = \tilde{U}(z_0) + U^{Born} \tag{12}$$

RESULTS AND DISCUSSION

Our focus is on some distinct aspects of continuum modeling of ionic electrostatics in narrow channels. Our model incorporates a realistically small effective pore dielectric constant and treats the polar peptide "sleeve" as a more polarizable electrical phase.

The conventional approach to continuum modeling of ion channels has been to assign a high value, ~80, to the dielectric constant of the aqueous pore. The assumption, that pore water and bulk water are dielectrically equivalent, nearly guarantees that the associated permeation free energy barriers are low enough to be consistent with kinetic data (Parsegian, 1969; Levitt, 1978). Refinements that account for channel former polarizability (see, e.g., Jordan, 1981; Monoi, 1991) yield even closer agreement with experiment. However, our recent analysis of the ion-dipole chain (Partenskii and Jordan, 1992a, b) demonstrates that high values of ϵ_1 are not readily consistent with the more realistic SMC description of pore water. Depending upon the number of water dipoles in the chain and the ionic size, the effective value of ϵ_1 is somewhere between 2.5 and 5, much lower than 80. With such bounds for ϵ_1, the corresponding continuum barrier is ~120 kJ/mol, ~6 times too large to be compatible with experiment.

With ϵ_1 in this range, incorporation of the polar "sleeve" region is no longer a refinement of the continuum picture. It could well be an essential improvement. The following questions present themselves. Are there reasonable values of ϵ_2 that resolve the problem and provide enough polarization energy to reduce the energy barrier sufficiently? Can values for the sleeve ϵ be determined, such that continuum modeling quantitatively reproduces ESMC theory incorporating the effect of a CFP? And if not, what modifications of the continuum approach (or the SMC model) are needed to accomplish these goals?

The results demonstrate that the answer to our first question is probably "no." Our example is a model system with representative gramicidin-like geometry, channel length 24 Å, pore radius 2.5 Å, and polar sleeve radius 5.0 Å. To determine the Born energy contribution, an ionic radius must be specified. We choose a cesium-like value, 1.5 Å, because Cs^+ motion in gramicidin does not stray far from the channel axis (Mackay et al., 1984, Skerra and Brickman, 1987; Jordan, 1990); this choice is most consistent with the linear chain model. We have shown (Partenskii and Jordan, 1992a) that, for the continuum approach to be consistent with SMC results, the effective dielectric constant in the pore is both small and ion site-dependent. With $\epsilon_1 = 4.25$, the effective value of the dielectric constant at mid-channel for the continuum geometry being considered (Partenskii and Jordan, 1992a) varying ϵ_2 from 2 to 80 (far larger than conceivable) lowers the energy of transfer[1] between bulk solvent and the

[1] All transfer energies are corrected to account for the Born energy of a water dipole. In the process of ion entry into the pore, a water molecule leaves. There is a small stabilization energy associated with removing water from a low dielectric constant medium and inserting it in a cavity surrounded by a high dielectric constant domain. This correction is included in electrostatic energies for both the ESMC and continuum models. It is small, ≤5 kJ/mol.

channel midpoint from ~130 to ~50 kJ/mol, which remains ~2–3 times greater than suggested by the conductance data. Table 1 presents an alternate indication of the limitations of pure continuum modeling. The quantity U^{∞} provides an absolute upper bound to the screening capacity of the CFP. For each site, it is computed by choosing for ϵ_1 the value required to replicate the energy of transfer determined from the basic SMC analysis (Partenskii and Jordan, 1992a), and then setting the sleeve dielectric constant ϵ_2, to ∞. The maximum in the electrostatic free energy, 53 kJ/mol, remains far too high. This is a direct consequence of the fact that the effective dielectric constant of the pore, ϵ_1, must be fairly small. No matter how large the value of ϵ_2, it cannot compensate for the low permittivity of the pore itself. The curious result that the peak in the continuum electrostatic barrier has shifted to a position near the channel mouth (which occurs when $\epsilon_2 >$ ~75) reflects two complementary influences. As ϵ_2 increases, the pore becomes more shielded from the nonpolarizable ϵ_3 domain; consequently, the site energy is more strongly influenced by the fact that the effective ϵ_1 increases as the ion moves deeper into the pore. Furthermore, quite generally near the water-membrane interface, the stabilizing effect of the sleeve is reduced by image interactions with the electrolyte, whereas in the middle the images have a much smaller impact.

Although we cannot rationalize permeation free energies within the continuum model, we can reproduce the expected values of the "internal" (or translocational) barrier W, defined somewhat arbitrarily as the energy difference between the edge position ($i = 1$) and the channel center ($i = 4$). Although there is uncertainty about the exact magnitude of the translocational energy barrier in gramicidin, the best estimates, from stochastic modeling of I-V data for Na^+ transport (Jakobsson and Chiu, 1987), suggest energies of ~10 kJ/mol, and certainly no higher than ~20 kJ/mol. With $\epsilon_1 =$ 2.7 and 4.2 for $i = 1$ and 4, respectively, we find that W, which is ~47 kJ/mol if the shielding capacity of the sleeve is ignored, i.e., if $\epsilon_2 = \epsilon_3 = 2$, drops below ~20 kJ/mol when $\epsilon_2 > 10$ (and below 10 kJ/mol with $\epsilon_2 > 20$). These values are similar (although somewhat higher) to those found using an analogous modeling procedure, where the peptide's permanent dipoles are viewed as forming part of the dielectric continuum (King et al., 1991; Warshel and Åqvist, 1991); care is required when relating our results to those of Warshel and his co-workers. Because both SMC and ESMC approaches specifically account for solvent, the proper comparison is with those of their model studies, which do not treat the influence of surrounding solvent by means of an effective local protein dielectric constant.

Thus, attempting to account, in a pure continuum picture, for the electrostatic influence of the CFP is inadequate to rationalize observed conductances in gramicidin. We now focus on correlating continuum theory with the ESMC model, our simplified way of describing the influence of gramicidin's CO groups (Partenskii and Jordan, 1992b; Partenskii et al., 1992, see also previous section). The results of ESMC calculations are summarized in the U^{micro} column of Table 1. The basic rings of charge abstraction of gramicidin, introduced for computational simplicity, is quite crude; it exaggerates energy differences between local minima and maxima and yield four instead of eight interior cation binding sites (in addition to two sites near the channel mouths). The computations focus on eight (four energetically distinct) equally spaced ionic positions. Sites 1 and 3 (and 6 and 8) are near maxima in the CFP electrical potential profile (where cations are least stable); sites 2 and 4 (and 5 and 7) are near interior minima. Within this approximation, the minimum energy of transfer from the bulk to the pore has been lowered to 78 kJ/mol, which can be accommodated with a reasonable value of ϵ_2, ~15.5. Viewed from this perspective, there is consistency between continuum modeling of permeation energetics and the ESMC approach; however, neither appears capable of providing an electrostatic explanation for the observed low permeation free energy barriers.

It seems natural to attempt to interpret the ESMC results (which include the influence of a model CFP) in terms of a local dielectric constant for the sleeve, ϵ_2, which depends on ionic position. For this purpose, we compare the ESMC results with the continuum ones. Table 1 presents the values of ϵ_2 at which permeation free energies in the two models are equal. In the continuum modeling, we incorporate the fact, established previously (Partenskii and Jordan, 1992a), that the "pore" dielectric constant, ϵ_1, depends on ion location; for the specific geometry of interest, ϵ_1 varies from 2.7 to 4.2 as the ion moves from site 1 to site 4 (from near the channel's "mouth" to its "center").

The effective value of the peptide sleeve's dielectric constant, ϵ_2, determined in this way also varies significantly with

TABLE 1 Comparison of properties of the variable ϵ_2 continuum model with the ESMC model

	$\epsilon_{background} = 2$				$\epsilon_{background} = 4$			
Site #	ϵ_1	U^{∞}	U^{micro}	$\tilde{\epsilon}_2$	ϵ_1	U^{∞}	U^{micro}	$\tilde{\epsilon}_2$
1	2.7	52.9	109.8	N.A.	4.8	29.1	70.3	N.A.
2	3.8	49.2	78.0	15.5	6.4	26.2	52.0	12.1
3	4.1	44.3	94.4	8.8	6.7	24.9	69.4	4.8
4	4.2	42.0	79.1	20.5	6.8	24.4	54.9	13.8

Energies are in kJ/mol. Site-dependent ϵ_1 values are determined from the basic SMC analysis in the absence of CFP (see text). U^{∞} is the continuum free energy in the limit where the sleeve dielectric constant, $\epsilon_2 \rightarrow \infty$; U^{micro} is the ESMC free energy including the influence of the CFP; $\tilde{\epsilon}_2$ is the sleeve dielectric constant at which continuum and ESMC free energies are equal (see text). Sites 1 and 4 are near the channel mouth and center, respectively (see text). Two different lipid-protein dielectric backgrounds ($\epsilon_{background}$) are contrasted (see text).

ionic position. For sites 2 and 4, near minima in the CFP electrical potential, the values of ϵ_2 are similar, and a bit high compared with the estimates of dielectric constants for polar protein domains (King et al., 1991; Warshel and Åqvist, 1991); again, because the underlying ESMC computations specifically include solvent, we compare with ϵ values that do not describe the solvent through an effective protein dielectric constant. For sites near maxima in the CFP electrical potential, correlation requires very low ϵ_2 values, as in these regions cation-CFP interaction is destabilizing. Most curious is the result that at site 1 (near the channel mouth) the two approaches appear to be basically inconsistent; for all $\epsilon_2 > 2$ (the background dielectric constant), the continuum energy is always far lower than ESMC value (109.8 kJ/mol). Some possible rationalizations of this enigma are suggested as follows. First, the "rings of charge" (the artificial axially symmetric distribution of carbonyl groups in the ESMC model) lead to a sharply fluctuating potential; this may be quite incompatible with a continuum description, in particular near the channel mouth where the transmembrane charge distribution is most asymmetric. A more realistic model for the influence of the gramicidin carbonyls would lead to smaller site-to-site variation in U^{micro}, possibly reducing the value near the channel mouth to one more compatible with a continuum description of the peptide sleeve near site 1.

Second, a dielectric approach cannot account fully for all influences of polar groups on the ion. Thus, dipolar orientation in the unoccupied channel creates an initial field contributing to the ionic energy; however, only the induced polarization and corresponding local induced field can be accounted for by the dielectric function. In the "rings of charge" model of gramicidin, the electric field caused by the unrelaxed carbonyls leads to an increase of the ionic free energy when the ion is located at site 1. There is, therefore, a strong destabilizing contribution if ionic influence on carbonyl orientation is neglected (Partenskii et al., 1991b). The additional polarization caused by the ion reduces the free energy (Partenskii and Jordan, 1992a; Partenskii et al., 1992), but the initial effect of the unfavorable CO alignment is so large that the dielectric approach cannot reproduce the results of the microscopic study. The field due to the carbonyl charge distribution should possibly be included in the continuum model explicitly (Jordan, 1984; Sancho and Martinez, 1991) and only the induced component modeled in terms of the sleeve's effective dielectric behavior. It is important to realize that, even if the ionic field grossly perturbs the initial CO alignment, this alignment is critical in the early stage of the charging process and can thus significantly influence the final electrostatic energy. This is in marked contrast to the situation in polar fluids such as water. There the mean (macroscopic) dipole moment is zero and there is no preferential alignment of the molecular dipoles in the absence of an orienting (external) field or a local charge source. As a result, the energy required to reorient a solvent molecule is low and a straightforward dielectric treatment is readily applicable. In peptides and proteins, the molecular dipoles are oriented even in the absence of an applied field; the energy involved in realigning ordered, constrained dipolar groups is much greater, which significantly limits the applicability of continuum modeling. This observation is consistent with that of Warshel and Åqvist (1991), who stressed the importance of the explicitly accounting for local polarity in the analysis of solvation energies in proteins.

Another modification which would contribute to resolving the incompatibility is consideration of local variation of pore shape, accounting for ion induced nonuniform reorientation of CO groups. This is an important local phenomenon. In our simple "rings of charge" model, the CO groups closest to the ion can tilt as much as 70° from the axis, leading to a significant local decrease in the pore's effective electrical radius R_1, in the immediate vicinity of the ion. In the continuum picture, such lateral electrostriction can strongly influence the energy barrier. For instance, it is possible to use dielectric continua to account for high energy at site 1 within the continuum approach with a sleeve dielectric constant >2, $\epsilon_2 \sim$ 2.2, if R_1 decreases to <2 Å and the pore ϵ_1 remains unaffected, 2.7 (even with this modification, the actual value of ϵ_2 is extremely small for a domain presumed to have polar character). The dependence of ϵ_2 on the ion's position in the channel is muted if local variation in pore shape is accounted for, which makes the whole continuum picture physically more reasonable.

This ionic influence on the effective electrical radius of the channel provides an electrostatic mechanism to account for gramicidin's valence selectivity in continuum terms. In continuum models with structureless dielectric phases, the electrical free energy barrier is independent of ionic polarity. Changing the polarity of the ion within "the rings of charge" model leads to lateral electrodilation. In continuum terms, selectivity can then be rationalized by assuming that the effective electrical pore radius is larger for anions than for cations. The low dielectric, ϵ_1, domain is thus enlarged, and the stabilizing influence of the higher ϵ CFP is consequently reduced. This is the electrostatic analog of the familiar microscopic picture in which carbonyl oxygens are repelled by anions and attracted by cations, the latter providing significant stabilization (Urry et al., 1981; Venkatachalem and Urry, 1984). It is important to note that, viewed from our perspective, the influence of electrodilation has precisely the opposite effect from what is expected in traditional continuum analysis where $\epsilon_{\text{pore}} = 80$. There, increasing R_1 further insulates the channel interior from the (lower) ϵ CFP domain, thus (erroneously) providing stabilization. Here, dilation increases the radius of the low ϵ pore interior, reducing the stabilizing influence of the surrounding CFP, precisely what would happen if an anion attempted to enter gramicidin.

The SMC approach models the occupied aqueous pore by a linear array comprised of an ion and a number of reorientable dipoles. These are embedded in a dielectric background of constant ϵ. The assembly is sandwiched by domains of high dielectric constant, ϵ_{ext}. The exterior regions mimic water; by approximating ϵ_{ext} as ∞, which has little effect on the results, the statistical mechanical model can be solved exactly (Partenskii et al., 1991a, b). In our original

SMC analysis, the background ϵ was chosen as 2, a high frequency value that roughly accounts for the electronic polarizability. The above discussion has been based upon this premise.

However, the proper choice of background ϵ is not immediately obvious. Our SMC model was designed to explicitly consider the reorientational contributions to dielectric relaxation. By analyzing correlations caused by water and carbonyl dipoles, it would appear that all nonelectronic degrees of freedom should have been incorporated. However, this need not be the case. For instance, the frequency-dependent dielectric constant of bulk water clearly exhibits two relaxations, one in the frequency range 1–100 GHz, the other at upwards of ~10 THz (Hasted, 1973). In the 500 GHz to 5 THz region, there is a plateau where $\epsilon_{water} \sim 4$. In bulk water there are, therefore, processes that are faster than rotational relaxation but slower than electronic reorganization and that nevertheless contribute to the dielectric attenuation. If such behavior is a general property of polar materials, then a model such as ours, which only accounts for dipolar correlations, neglects some significant microscopic processes that influence dielectric behavior. One way to deal with this limitation is to consider the consequences of a background with $\epsilon = 4$. Naturally, this will reduce the ionic transfer energies from the bulk into the pore, because the major destabilizing component is the Born charging energy. It is, however, hard to be at ease with using a high background ϵ in approaches specifically designed to account for the correlations caused by water and CFP. A better approach, in line with the philosophy outlined by Gilson and Honig (1991) (see also Sharp et al., 1992), is to treat all nonelectronic interactions explicitly and retain a background ϵ of 2. In terms of our SMC and ESMC paradigm, this would require analysis at the computational chemical level. Details of potential functions such as AMBER, CHARMM, or GROMOS would have to be included in the microscopic Hamiltonian. The problem would no longer be soluble, and the virtue of the whole SMC idea, an exact analytical treatment of all long-range electrostatic interactions, would be lost.

As a result, there is value in seeing how altering the background ϵ affects permeation energetics. Consequently, we have considered the possibility that the background $\epsilon = 4$. This is consistent with the high (THz) frequency dielectric constant of bulk water (Hasted, 1973), with estimates of an "inner" dielectric constant of proteins with "neutralized" permanent dipole moments (see the discussion surrounding Table III of King et al., 1991) and with a dielectric constant of the protein background sometimes used in Poisson-Boltzmann calculations of protein-electrolyte interaction. It is larger than the lipid ϵ; however, lipid is far enough removed from the aqueous pore (~10 Å in gramicidin) that lipid influences on pore electrostatics are insignificant in SMC modeling (our unpublished results). With this assignment, the most striking result is that the drop in the permeation free energy barrier is far smaller than might be expected from simple electrostatic energy scaling. Data contrasting the ESMC and continuum models are also presented in Table 1. It should be stressed that, just as in the case of a background ϵ of 2, the ESMC computations with $\epsilon_{background} = 4$ take into account the (model) peptide's charge distribution. Thus, our physical model is quite similar to that used in many calculations of protein interaction with aqueous electrolyte. The effective values of ϵ_1, determined using the process outlined in Model and Basic Equations (comparison of transfer energetics from electrolyte to pore for both continuum and microscopic descriptions), are now larger than for the case with a background ϵ of 2. As the table indicates, at site 4 (near the channel mid-point) the ESMC energy of transfer from the bulk solvent to the pore (U^{micro}) drops from 79 to 55 kJ/mol as the background ϵ increases from 2 to 4. This is only a 32% decrease even though the Born energy has dropped ~50%. The difference is basically an example of Le Chatelier's principle. With the more polarizable background, there is far greater shielding of the stabilizing interaction between the ion and the various dipolar groups (water and carbonyls). The only components of the free energy that scale as $1/\epsilon$ are the membrane contribution to the ionic Born energy, Eq. 11, and the image energy between the ion and the solvent. Other terms (which are basically negative and stabilizing) vary more rapidly with changes in ϵ. For instance, the (negative) interaction between the ion and a dipole at the ith position is proportional to $-\langle p_{xi}\rangle/\epsilon$, where $\langle ... \rangle$ denotes the statistical mechanical average. As the dielectric constant increases, $\langle p_{xi}\rangle$ becomes smaller, thus magnifying the dependence on ϵ and reducing the stabilizing contribution to the free energy. Probably the most significant source of additional shielding is the reorganization energy caused by the ion's interaction with the model charge distribution. For a fixed charge distribution, at any ion site, the forces between the ion and the model charges are proportional to $1/\epsilon$; however, the restoring force (in essence, the carbonyl libration) is independent of ϵ. There is less electrostriction as ϵ increases and, at equilibrium, the oxygens of the relaxed "rings of charge" are further from the axis (and the ion). Ion-oxygen distances, r_{I-O}, increase and the stabilization energy, which is basically proportional to $-1/[\epsilon r_{I-O}]$, drops off faster than $1/\epsilon$.

There are a number of significant points with respect to the influence of increasing $\epsilon_{background}$. Qualitative behavior is essentially unaltered. Even with a relatively large background ϵ, the energy barriers to ion transfer into the pore remain too large. As ϵ_2 increases from 4 to the unrealistically large value of 80, the transfer energy to the channel midpoint decreases from 71 to 32 kJ/mol, still too large to account for conductance data in gramicidin. Even in the limit $\epsilon_2 \to \infty$, U^{∞} at site 1 (which becomes the high energy site when $\epsilon_2 > \sim 140$) is ~29 kJ/mol, which is also too large. Again, at sites 2 and 4 the values of ϵ_2 are similar, fairly high, and compatible with estimates of ϵ for analyses in which the polar protein regions are treated as structureless dielectric continua (King et al., 1991; Warshel and Åqvist, 1991). Furthermore, just as when the background ϵ was 2, at site 1 (near the channel exit) there is a basic incompatibility between the ESMC analysis (including the influence of the CFP) and the continuum approach. There is no value of ϵ_2 (now >4) at

TABLE 2 Internal energy barrier in continuum two dielectric model. Comparison of variable ionic strength with high ϵ_{ext} limit

ϵ_{pore}	2	3	4	5	6	10	
$\epsilon_{ext} = \infty$	95.19	84.66	78.29	73.61	69.93	59.75	$\epsilon_m = 2$
		63.47	58.34	54.84	52.21	45.24	$\epsilon_m = 3$
			47.62	44.55	42.33	36.82	$\epsilon_m = 4$
PB, c = 10 M	95.10	83.53	76.66	71.76	67.89	57.26	$\epsilon_m = 2$
		62.78	57.09	53.27	50.42	43.05	$\epsilon_m = 3$
			46.62	43.23	40.78	34.82	$\epsilon_m = 4$
PB, c = 0.1 M	93.06	81.42	74.49	69.53	65.63	54.91	$\epsilon_m = 2$
		60.76	55.02	51.16	48.26	40.81	$\epsilon_m = 3$
			44.63	41.20	38.72	32.65	$\epsilon_m = 4$

Energies are in kJ/mol. The internal barrier is the energy difference between sites 4 and 1 for geometry described in text. Two continuum solvent models ($\epsilon_{ext} = \infty$ and a Poisson-Boltzmann description) and three lipid-protein dielectric backgrounds (ϵ_m) are contrasted. The values of ϵ_{pore} considered reflect the results of SMC analysis; effective dielectric constants in an occupied channel are low.

which the continuum and ESMC energies are the same. Finally, we find that the translocational energy barrier, which is ~27 kJ/mol if the shielding capacity of the sleeve is ignored, drops below ~20 kJ/mol when $\epsilon_2 > 7$ (and below 10 kJ/mol with $\epsilon_2 > 20$), again qualitatively consistent with computations at the lower $\epsilon_{background}$.

It is clear from our analysis that continuum models are uncertain guides for describing permeation energetics in narrow channels. Without an underlying microscopic picture, there is no clear proper choice of electrical parameters. Because all predictions are acutely sensitive to the selection of local values of ϵ, even qualitative trends must be viewed with caution. As a result, to model the permeation process we are limited to the simple ESMC approach or full molecular dynamics (MD), each with its own drawbacks.

Even though it is a highly simplified abstraction, there are advantages to a simple semimicroscopic model that make it a useful tool for studying ion channels. First, it does not, on an ad hoc basis, assign high values to the dielectric constant of pore water but rather determines dielectric properties explicitly using statistical mechanics. Second, using an image force approach, the long range electrostatic interactions that significantly affect the formation of the energy barrier can be described accurately.

MD, which describes the system in a much more detailed fashion, lacks the advantage of simplicity; thus, accounting for long range interactions remains a serious problem. This point has been clearly articulated by Lee and Warshel (1992), who note that using a finite cutoff, inevitable in MD calculations, may cause very large errors. Furthermore, a quite general analysis of dipolar and ionic systems in contact with polarizable media (of which the membrane-water interface is but one example) indicates that even in exactly soluble model problems, great care must be taken in handling long range electrostatics (Buff and Stillinger, 1963; Barlow and Macdonald, 1965; Partenskii and Feldman, 1989). Thus, we expect that MD analysis is much more reliable in calculations of energy differences (e.g., because of displacement of particles, perturbation from one ion to another, etc.) but may be problematic in the determination of absolute energy values.

CONCLUSIONS

1. Ion-induced, strong dielectric saturation in narrow water-filled pores has reopened the question of an electrostatic rationalization of the low permeation free energy barriers in ion channels (Partenskii et al., 1991a; Partenskii and Jordan, 1992a). SMC modeling, when extended to incorporate the redistribution of peptide charges in a simple fashion, leads to insufficient barrier reduction to account for conductance measurements (Partenskii et al., 1992). Increasing the background dielectric constant from 2 to 4 (accounting for high frequency, nonelectronic relaxation processes and mean peptide polarizability) decreases the barrier far less than twice because of the shielding of the stabilizing interactions. Within a continuum three-dielectric model, employing realistic values for the pore ϵ, similar results are obtained when the CFP is modeled by a narrow region of high polarizability.

2. Comparison of ESMC and continuum modeling strongly suggests that the continuum approach must be further modified if it is to account faithfully for the influence of equilibrium constraints on the orientation of the polar groups of the CFP in the unoccupied channel, as well as for strong polarization and local charge redistribution in a channel former induced by an ion. Possible ways to improve the local dielectric picture are by explicitly treating the initial field of the oriented polar groups and by including the nonlocal effects and lateral electrostriction, mimicking the reorientation of polar groups in the CFP.

3. The approximation $\epsilon_{ext} = \infty$, which permits analytical solution of the continuum model, may be useful in studies of the internal region of membranes and peptides. The (relatively) small errors in energy, especially at higher ionic strengths, may be a small price to pay for the calculation simplification that can sometimes be effected. This approximation may also provide a useful reference state for perturbational analysis.

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APPENDIX

Here we consider the validity of the approximation $\epsilon_{ext} = \infty$, which has been used to describe the electrolyte and which permits us to obtain a simple analytical solution of the electrostatic problem. To do this, we compare our results with those of a numerical treatment of the electrolyte, based on solving the NLPB equation (Jordan et al., 1989), which simulates the ionic distribution in the electrolyte and accounts for the shielding of the electric field. Here we consider only a two-dielectric model. Our purpose is to demonstrate that a high electrolyte dielectric constant is formally equivalent to a high ionic strength and in this way provide precise estimates of the error introduced by modeling bulk water as a medium in which $\epsilon = \infty$. The NLPB equation is

$$\nabla[\epsilon(r)\,\nabla V(r)] + 4\pi\rho_B(r) + 4\pi\rho_0(r) = 0 \tag{13}$$

$$\rho_B(r) = \sum_{\alpha=1}^{s} N_\alpha z_\alpha \exp[-z_\alpha V(r)/kT] \tag{14}$$

$\rho_B(r)$ is the local density of ions, N_α and z_α are the bulk density and the charge of species α, and s is the number of ionic species. The set of equations is completed with the continuity conditions on the electric potential $V(r)$ and displacement $D(r) = \epsilon(r)\nabla V(r)$.

Comparison of continuum NLPB calculations with those assuming $\epsilon_{ext} = \infty$ are presented in Table 2. To demonstrate the range of utility of our "metallic" approximation, we consider values of 2, 3, and 4 for ϵ_m, the dielectric constant of the membrane (which occupies the region $\rho > R$ in the two dielectric model considered here) and focus on two quite separate ionic strengths: $c = 0.1$ M and $c = 10$ M.

We see that there is a close correspondence between the internal barriers computed at high c and at high ϵ_{ext}. They are always highest for $\epsilon_{ext} = \infty$; however, it appears that limit $c \to \infty$ is equivalent to the case $\epsilon_{ext} \to \infty$. Superficially, this may appear surprising. However, it is easily interpreted by considering the capacitance of the diffuse (GC) layer at an electrode-electrolyte interface. At low electrode charge, this is

$$1/C_{GC} = K(\epsilon_{ext}/\lambda_D) \tag{15}$$

where K is a numerical constant and $\lambda_D \sim (kT\epsilon_{ext}/c)^{1/2}$ is the Debye length. Equation 15 characterizes a capacitor with dielectric constant ϵ_{ext} and effective gap (the distance to the "metal" electrode, for which $\epsilon = \infty$) λ_D. Therefore, increasing c effectively brings the "metal" region closer to the interface, thus effectively increasing the solvent's dielectric constant. Consequently, the limit $c \to \infty$ is effectively equal to the limit $\epsilon_{ext} \to \infty$.

Obviously, the NLPB model requires substantial modification at high ionic concentrations. One would expect that interionic correlations lead to an increase in the characteristic length, thus decreasing the effective ϵ of the solvent and leading to greater divergence between the approaches. Nevertheless, Table 2 demonstrates that there is reasonable agreement even at low concentrations so that the qualitative picture will not be altered by a more rigorous treatment of the electrolyte. The relative error in computing W, caused by setting $\epsilon_{ext} = \infty$ rather then using the NLPB treatment of the solvent, does not exceed 13% for the range of pore ϵ's considered (2–10).

For computational reasons, it is difficult to apply the numerical Poisson-Boltzmann approach at very low ionic strengths (the "pure water" limit). However, it is important to test our "metallic" model in this situation. As is clear from Table 2, the "metallic" model is at its worst when ϵ_{pore} is large. The largest value ever used is ~80, i.e., assuming pore and bulk water to be dielectrically equivalent. Monoi's (1991) computation of the translocational barrier for such an electrical geometry, in a channel 27 Å long and 2.64 Å in radius, yields an internal barrier of ~16.5 kJ/mol. With the same geometry, but assuming $\epsilon_{ext} = \infty$, we find an internal barrier of ~19.7 kJ/mol, a discrepancy of ~19%. Considering the (unrealistically) high value used for ϵ_{pore} (80) in this test and the approximate nature of the model itself, we conclude that the approximation $\epsilon_{ext} = \infty$ provides a most reasonable way to estimate ionic energy barriers in the interior of ion channels for a wide range of system parameters. As is clear from the above discussion, the model can be successfully modified to account for the lower value of water's bulk ϵ (~80) and the ionic strength, by setting $\epsilon_{ext} = 80$ in the immediate vicinity (one Debye length) of the interface and setting it to ∞ deeper in the solvent. Study of the electrical and structural properties of peptides in solvents may be another case where this approximation may be useful, as a reference state for perturbational analysis.

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