New and Notable

Trial by Ordeal: Ionic Free Energies in Gramicidin

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Selective ion channels share a common feature. They exhibit the apparently contradictory properties of high turnover and high selectivity. The structures of four such membrane proteins have led to an explosion of computational papers designed to illustrate in detail how various architectural features can couple at the molecular level and resolve the conundrum. A stringent macroscopic test of any channel theory is its ability to reproduce experimentally observed current-voltage-concentration (I-V-C) profiles. Ideally this would be done microscopically, via applied field molecular dynamics (MD) (Crozier et al., 2001). At present this is not feasible and mesoscopic approaches are employed, either based on free energy profiles determined from MD simulations or by treating ionic conduction as electrodiffusion through a viscous, continuum dielectric. The lure of the electrodiffusive approach is its physical transparency and its computational simplicity. Its major pitfall, neglecting non-uniformity and non-locality, has a long history (Warshel and Russell, 1984; Komyshev, 1985). Nonetheless, if the narrow water-filled transmembrane conduit typical of a channel protein is well approximated as a uniform dielectric phase, coupling the structural data with electrostatic arguments determines the ionic translocational potential profile, from which I-V-C relationships are efficiently computed (Nonner et al., 1999).

In this issue of *Biophysical Journal* Edwards et al. (2002) subject continuum electrostatics to trial by ordeal. Using the 30-residue gramicidin A dimer as their exemplar, they determine whether this approximation can adequately account for three observables: the I-V profiles, the I-C profiles, and the binding site locations. For this exceptionally well-characterized (both structurally and electrophysiologically) and very narrow channel the conclusion is unambiguous. Continuum electrostatics fails, at least in its simplest form; the single-file waters in this long, narrow pore are not realistically represented as a uniform dielectric phase, regardless of the choice of ε. The authors then go further, using Brownian dynamics (BD) to deconvolute the structural and electrophysiological observations and determine an ionic free energy profile for potassium translocation through the pore, in the process demonstrating that I-V data alone are inadequate to critically test phenomenological permeation models. The resultant profile is contrasted with the results of MD simulations; these, while qualitatively similar, are quantitatively vastly different.

The electrostatic model treats the gramicidin channel ($\sim$24 Å long, $\sim$4 Å diameter) as a distinct dielectric phase, surrounded by a low dielectric milieu (protein and membrane), sandwiched between two high ε aqueous regions. Electrical forces acting on ion(s) in the pore are determined by solving Poisson's equation, taking into account the peptide charge distribution (determined from the channel structure and molecular force field charge parameters) and the reaction field due to dielectric variability; if the pore's effective ε is chosen different from that of bulk water there is also a self-energy term. These forces depend upon the εs chosen to describe the pore and the surrounding peptide. The ion's electrical energy determines the position of the binding site. With an estimate of the ion's channel diffusion coefficient, D_{eff}, BD provides the tool for monitoring ion movement and computing I-V-C profiles.

To provide functional estimates of the unknown dielectric constants, Edwards et al, (2002) first consider the electrical energy. An acceptable potential profile must have significant energy wells near the channel entrances (to create the binding sites) but cannot have large internal barriers (lest translocation be forbidden). No plausible choices are satisfactory, regardless of the permittivity ascribed to the peptide and membrane regions. With a large pore ε the wells are too shallow to create preferential occupancy near the channel mouths; with a small pore ε the internal barrier to translocation is enormous, essentially forbidding ion passage. But it gets worse. With a pore ε large enough to permit ion passage, I-V profiles can be reproduced with a reasonable choice of D_{eff}. However, the current cannot be made to saturate at high C. Furthermore, monovalent cation occupancy is not limited to regions near the channel mouth; the ion is essentially uniformly distributed throughout the channel. However, some electrostatic predictions do work. Models that account for I-V data predict that anion entry and divalent cation passage through the channel are both forbidden. But even here there is a bitter element. Divalent cations are known to block gramicidin; continuum electrostatics forbids their binding. Continuum electrostatics has successfully described electrical behavior in the potassium channel pore. What is it about gramicidin that makes it resistant to similar modeling? The obvious culprit is its exceptionally long single-file domain where water molecules, sur-

Submitted April 30, 2002 and accepted for publication May 8, 2002

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0006-3495/02/09/1235/02 $2.00

rounded by the deformable gramicidin backbone embedded in a non-permittive domain, act as electrical transducers, transmitting electric fields in ways that have no continuum analog. In contrast, the filter of the potassium channel pore is a bit more than half as long; that of the chloride channel is probably even shorter (Dutzler et al., 2002). In both, the filters abut much wider aqueous intra-peptide regions that can significantly influence the pore's electrical behavior. But there is something else that may make gramicidin exceptionally hard to model. The electrical data are so extensive and the structural data so highly resolved that theoretical predictions are left with fairly little "wiggle room."

The results should be cautionary for electrophysiologists and theoreticians alike. Current-voltage profiles alone provide too little independent information to seriously constrain a channel's ionic energy profile. Does the success of electrostatics in providing a framework for interpreting potassium channel conductance guarantee that it will be equally reliable when applied to the chloride channel? Quite possibly not, since potassium channels are effectively blocked by their own permeant ions; conductance requires relief of this block via ionic acceleration through the channel's wide inner pore, a region large enough to be treated by continuum electrostatics (Chung et al., 2002). In chloride channels conduction and gating are intimately coupled (Richard and Miller, 1990; Pusch et al., 1995), suggesting a transductive process involving structural changes in the filter, something unlikely to be susceptible to continuum electrostatic description. What about the simulation of ionic free energy profiles? The present state of the art implies internal barriers in gramicidin that are 4-5 times those inferred from experiments; this suggests that theoretical studies of potassium channel energetics may well be more uncertain than one would like. Until a computational model quantitatively accounts for barium and sodium block in potassium channels, it may be advisable to take simulational predictions with a sizeable pinch of salt.

This work was supported by National Institutes of Health grant GM 28643.

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