Profiles of permeation through Na-channels

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South American frogs (genus, *Phyllobates*) have already advanced the cause of Na-channel biophysics with their gift of the alkaloid, batrachotoxin (BTX). This toxin greatly prolongs the normally transient open state of the Na-channel, permitting its conductance mechanism to be rigorously explored in artificial lipid bilayers. Now, skeletal muscle from the Chilean toad, *Caudiverbera caudiverbera,* has become the gourmet choice of Na-channels for an in-depth analysis of conductance substates in the article by Naranjo and Latorre (1) of this issue. This paper serves to distill a substantial body of research on biophysical aspects of ion movement through this important class of voltage-gated channels.

To fully appreciate the baroque nature of the arguments in this field, a sense of history is required. For certain species of single BTX-modified Na-channels, several groups have focused primarily on conductance behavior in the range of $[Na^+]$ less than 500 mM and optimistically interpreted the relationship between unitary conductance (γ) and symmetrical $[Na^+]$ as a Michaelis-Menten function for a channel with a single binding site and a K_m for Na^+ of 8–40 mM (2–4). However, this lovely parallel between the behavior of an ion channel and that of an uncomplicated enzyme blithely disregards the classic literature of macroscopic electrophysiology, which produced evidence for multi-site occupancy by alkali cations (5). Furthermore, there was the small unmentioned detail, that ionic strength was not constant in these measurements, leaving open the possibility of a large variation in surface potential arising from fixed charges associated with the channel protein.

The multi-ion question could be cavalierly discarded by saying that the BTX-modified Na^+ channel was "different" from the normal one, but the question of surface charge persisted. Green et al. (6) took aim on this latter issue by measuring conductance of canine brain Na^+-channels in salt solutions up to 3.5 M NaCl. Even at this high concentration of Na^+, the conductance still was not fully saturated, and the channel was clearly not Michaelis-Menten. By introducing surface charge near the mouth of the channel with the aid of Gouy-Chapman (GC) theory, Green et al. (6) were able to model their data on the basis of a single-ion channel with substantial negative surface charge. This fixed charge was proposed to cause a large enhancement in the local Na^+ concentration at low ionic strength which boosts conductance in this limit. As NaCl is increased, negative surface potential is screened and greatly diminished, which results in unmasking of the intrinsic low affinity of the channel for Na^+, estimated to have a K_D = 1.5 M.

Although the GC theory of planar surface charge has a hallowed reputation in describing the surface electrostatics of lipid bilayers, it has a small quirk when it comes to trying to understand what goes on at the entrance to a channel's vestibule at low ionic strength. Because this theory is based on the geometry of an infinite plane of smeared negative charge, the surface cation concentration approaches a non-zero value in the limit of low ionic strength. When coupled to an ion channel, this theory predicts that conductance saturates at some non-zero value, as $[Na^+]$ in the bulk solution is reduced to zero. Whether this occurs or not can be very difficult to determine, for the range at which this effect kicks in, can be well below 10 mM NaCl. As Cai and Jordan (7) have recently pointed out, with respect to the question of how protein charge distributions affect the conductance of a channel, GC theory "is not only too simple but also inappropriate." This is a fine thing to say if one has access to the three-dimensional structure of a channel with the location of all the relevant surface charges, however for Na-channels, the reality is otherwise. Nevertheless, by numerically solving the Poisson-Boltzmann equation, Cai and Jordan (7) showed that for an hourglass-shaped, single-site channel with negative surface charge very near the entrance to the pore, conductance does approach zero at low Na^+ and approximates the kind of behavior found by Green et al. (6).

This ameliorated the surface charge question, but short-shrifted the issue of multiple occupancy. How many ions can simultaneously bind in the pore? By glancing sideways into the fields of structurally related Ca-channels and especially, K-channels, it is not hard to tell that the ion occupancy number is greater than one. Can a Na-channel be so different? Swinging the pendulum back to the other extreme, Ravindran et al. (8) reexamined Na^+ conductance of the rat muscle channel over the wider range of 0.5 to 3,000 mM Na^+. When the data were plotted in the form of log γ vs. log $[Na^+]$, the results looked like a figure right out of Hille and Schwartz (9), a venerable theoretical paper on multi-ion conduction. By using the tremendously useful AJUSTE program that Alvarez et al. (10) have developed for fitting I-V data to Eyring-type energy profiles, the distinctive biphasic dependence of γ vs. $[Na^+]$ was nicely explained in terms of a two-site channel, with repulsion between ions in the doubly-occupied state. However, in skirting the issue of surface charge, this left the field with two extreme interpretations of basically similar data: either a one-site channel with charge in the vestibule or a two-site channel with no surface charge.

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By steadily working to bridge this chasm in a series of previous analyses of permeation through Na-channels from squid neurons and now, toad muscle, Latorre and collaborators have arrived at a more balanced picture which is presented in this issue. The existence of asymmetric surface charge in the Chilean toad channel is supported by the observation of distinct inward rectification in the I-V relation that is apparent only at low Na^+. This suggests that there is more negative surface charge at the outer vs. inner vestibule, thus enhancing inward vs. outward current. The apparent screening effect of external divalent cations and the two clusters or rings of negatively charged residues recently identified in the elegant mutagenesis work of Heinemann et al. (11) are offered to bolster the view that surface electrostatics must play a role. Despite the introduction of GC surface-charge, the γ vs. $[Na^+]$ behavior of the fully open state and the (protease-generated?) subconductance state of the toad channel cannot be forcibly described with single-occupancy binding. The observed conductance increase traverses over too wide a range of $[Na^+]$ to conform to any reasonable semblence of a Langmuir isotherm. By going to double-occupancy with a highly reduced affinity for Na^+ binding to the second site, Naranjo and Latorre ultimately achieved satisfaction in a best fit of the data. In the limit of low Na^+, there is even a hint of a real plateau conductance, that tends to validate application of GC theory in this particular case. Unfortunately, the authors do an excellent job of explaining the bad news, that any estimate of surface charge is highly model-dependent. Furthermore, the realm of pH effects in a charged vestibule at low ionic strength is currently a no-fly zone where anything can happen. The paper also provides a crisp summary of conductance data from a Noah's ark collection of Na-channel preparations and explains how species differences and the examined range of $[Na^+]$ can account for the differing estimates of Na^+-binding affinity and surface charge.

Where do the profilers of permeation processes go from here? There seems to be a convergence toward the consensus that surface charge and multiple occupancy may be important. The road leading to molecular dissection of surface electrostatics and ion binding sites appears to be the one to follow.

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