New and Notable

Ion Channel Selectivity Using an Electric Stew

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Having the crystal structure of its pore solved, the K^+ channel has been the target of most recent efforts to understand how ion channels decide which ions to pass. Nevertheless, the Ca^{2+} channel is essential for a complete picture of selectivity because, at first glance, its job and its mechanism appear so different from those of K^+ channels. K^+ channels choose a larger ion over a smaller (K^+ over Na^+) and do so with a pore structure that appears constrained from shrinking to fit Na^+ (Doyle et al., 1998). Ca^{2+} channels must select Ca^{2+} over the much more numerous Na^+ ions, even though the two have precisely the same diameter. The channel does this with an intrapore site that binds Ca^{2+} very tightly (K_d = 1 μM) and, upon doing so, blocks Na^+ flux. This raises the problem of how a million Ca^{2+} ions can pass through the pore per second when it contains a site that, if free in solution, would have a maximum off-rate of a thousand ions per second. In this issue, Nonner et al. (2000) offer an innovative, yet perfectly intuitive, theory for Ca^{2+} channel selectivity that is important for a number of reasons: it is remarkably simple yet perfectly fits the Ca^{2+}/Na^+ selectivity data, it is clearly different from prior kinetic models of Ca^{2+} channel permeation, and it is a completely different mechanism of selectivity than that accepted for K^+ channels.

The paper must be appreciated along with the authors' prior tackling of the Ca^{2+} channel using Eisenberg's Poisson-Nernst-Planck (PNP) modeling system (Nonner and Eisenberg, 1998). PNP uses equations like those that describe diffusion of electrons through semiconductors in an electric field. Like any model, PNP makes approximations: it represents water as a homogeneous dielectric medium within the pore, and it considers ions to be spheres that make smooth electric fields around themselves. Using these approximations and a cylindrical 6 $\times$ 10 angstrom pore, the PNP method successfully fit Ca^{2+} channel data, passing Ca^{2+} at a high rate while binding it tightly. Previous to this, the various Ca^{2+} channel models (reviewed in McCleskey, 1999) required that ions pass through the pore in single file through a sequence of binding sites. The success of PNP demonstrates that sequential binding sites need not be sacred to ion channel permeation. However, an awkward aspect of the PNP Ca^{2+} channel was that it relied on ad hoc assumptions about the chemical nature of the pore. It simply assigned potentials to the pore that made it bind Ca^{2+} well and Na^+ poorly, thereby teaching nothing about the chemical basis of the selectivity. In resolving this lingering problem, the present paper provides insight to more than just Ca^{2+} channels.

The paper considers the implications of data gathered through mutations of glutamate residues within the pore region of Ca^{2+} channels. Four glutamate residues, one from each of the channel's homologous domains, are necessary for high affinity Ca^{2+} binding and their carboxyl groups are proposed to float flexibly into the pore lumen (Yang et al., 1993). The supposed structure, called the EEEE locus, is analogous to the binding site of Ca^{2+} chelators like EDTA. Nonner et al. model the EEEE locus by allowing the 8 oxygen atoms, each with a charge of $-1/2$, to float freely in a volume of a particular dimension and dielectric coefficient. The oxygens interact with ions that pass in and out of the volume, always acting to maintain electroneutrality within the space. Importantly, the theoretical protein is not creating a defined arrangement of glutamate residues. Rather, it creates a dielectric volume in which the oxygens float like dumplings in an electric stew. This picture conflicts with the bias of the ion channel literature that selectivity filters gain their properties from oxygens rigidly arranged in rings of precise dimension. Also, its single binding region conflicts with kinetic models of Ca^{2+} channel pores that always assumed multiple binding regions in order to obtain Ca^{2+} block at micromolar concentration and flux saturation at tens of millimolar concentration.

The model represents ions by the mean spherical approximation. Each is a charged hard sphere of diameter equal to its value when in a crystal. The ions interact in two ways: through long-range electrostatic force and by virtue of the fact that no two spheres can occupy the same spot (volume exclusion). This minimalist view of ions and pore, together with the PNP calculation of flux, fits Ca^{2+}/Na^+ selectivity data at least as well as prior kinetic models. What makes this remarkable is that the kinetic models were adjusted to fit the data, whereas this calculation is a prediction rather than a fit.

The mechanism by which Ca^{2+} is chosen over Na^+ is very basic. It takes two Na^+ ions to neutralize as many of the oxygens as does one Ca^{2+}. Therefore, if crowding is an issue, Ca^{2+} is preferred. A volume of 375 cubic angstroms with a dielectric coefficient of 67 provides the proper crowding. When the volume is varied, the selectivity can change dramatically. At

Received for publication 25 July 2000 and in final form 4 August 2000.

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0006-3495/00/10/1691/02 $2.00

large enough volume, positive ions can screen the oxygens and then even Cl^- can permeate. Thus, by varying the size of the stewpot, this scheme of floating oxygens can be used to make many kinds of ion channels. In addition to pointing out the model's flexibility, the paper also carefully points out deficiencies, one of which is a failure to reproduce the low affinity of the Ca^{2+} channel for Mg^{2+}. This probably is related to the ability of Mg^{2+} to bind water unusually tightly, a property not mimicked by the mean spherical approximation.

This paper arrives at an exciting moment in the history of ion channels. In the past, theories attempted to reproduce functional data with the simplest possible model in the hope that some insight would be gained. But now the goals are grander than mere generic insight. With atomic structure of the K^+ channel known, theoreticians are creating various approaches to accurately describe ion trajectories within the pore and the forces that control them. In contrast to the continuum approach of PNP, Brownian dynamics models the behavior of a small group of ions in the near vicinity of a channel's mouth, and it successfully explains both functional data and the location of K^+ ions within the crystal structure of the K^+ channel (Chung et al., 1999). Molecular dynamics simulation of 4 ns in the life of the K^+ channel has now been published (Berneche and Roux, 2000). This daunting calculation of individual vibrations of each atom in the protein, three K^+ ions, and over 1000 water molecules captured a single coupled movement of the K^+ ions and waters within the selectivity filter. This appears to demonstrate that K^+ ions move in single file through K^+ channels, as first deduced in the elegant work of Hodgkin and Keynes (1955). One might expect to need about 1 μs of time in order to see an ion fully traverse the pore; this huge calculation might give an enormous payoff in our understanding of what forces control the ion at various stages of permeation. It would be valuable to see how the PNP and the mean spherical approximations do with the known K^+ channel structure and how the result compares to these other methods.

It also seems important for theoreticians to consider whether the apparent dichotomy between selectivity mechanisms for K^+ and Ca^{2+} channels is dead wrong. After all, dead wrong were the theories before crystallization of the K^+ channel about how the pore's amino acid side chains created K^+ selectivity. Perhaps backbone carbonyls rather than side chain carboxyls create a Ca^{2+} binding pocket in Ca^{2+} channels. Also, K^+ channels have powerful, but underappreciated, functional similarities to Ca^{2+} channels. At least one binds K^+ with micromolar affinity (Vergara et al., 1999) and, in precise analogy to the behavior of Ca^{2+} channels without Ca^{2+}, K^+ channels lose selectivity in the absence of K^+ (Kiss et al., 1998). Perhaps the apparently rigid K^+ channel pore was made rigid by the binding of the ions within it, i.e., that it adjusts to fit its ions and, like the Ca^{2+} channel of Nonner et al., is fluid until bound. The power emerging from new theoretical methods relating ion permeation to protein structure should allow the airing of heretical ideas, some of which are bound to be true.

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Molecular Motions in Fourier Transform Space

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Molecular Brownian motions in dense macromolecular suspensions and glassy liquids may involve extensively correlated particle movements and substantial temporal and spatial heterogeneity (Cicerone et al., 1996; Ediger et al., 1996; Marcus et al., 1999; Weeks et al., 2000). Living systems may exhibit even more complex Brownian dynamics due to the dispersion in their molecular sizes and shapes. In addition they may also exhibit complex non-thermal motions that are driven ultimately by ATP hydrolysis. Although the translations of individual molecular motors along their conjugate filaments have been extensively studied, little is known about other non-thermal motions, such as

Received for publication 11 August 2000 and in final form 21 August 2000.

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0006-3495/00/10/1692/03 $2.00