Commentary

New Views of Multi-Ion Channels

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How some ion channels display both extreme selectivity and rapid flux remains a central topic of modern research in quantitative biology. Two papers in this issue (Dang and McCleskey, 1998; Kiss et al., 1998) independently present a class of new models that attempt to explain new data gathered over the past few years from careful experiments on mutant channels.

The classical squid axon K^+ channel experiment that suggested multi-ion behavior in channels, or the "long pore effect," was prompted by the flux-ratio criterion (Ussing, 1949; Hodgkin and Keynes, 1955; Hille, 1992). Unidirectional fluxes of radioactive tracers were measured under varying electrochemical gradients for K^+. The ratio of inward to outward tracer fluxes depended exponentially on a power n of the electrochemical driving force, $(E–E_K)$. This experiment is usually explained by stating that an individual K^+ ion diffuses slowly up the electrochemical gradient because it must await displacement of approximately n K^+ ions moving down the gradient at any one time within the channel (Hille and Schwarz, 1978). A contemporary flux ratio measurement on *Shaker* K^+ channels expressed in oocytes yields $n = 2.4–3.4$ (Stampe and Begenisich, 1996).

Contemporary electrical methods for studying ion permeation in channels include reversal potentials, current–voltage relations, anomalous mole fraction effects, blockade within the channel by inorganic ions, blockade by organic ions at more external sites, local anesthetics and peptide toxins, and streaming potentials (Lester, 1991). Only some of these techniques pertain directly to multiple-occupancy theory. The "anomalous mole fraction effect" is the favorite experiment. In the most direct form of this experiment, one measures single-channel conductances, γ_A and γ_B, for membranes with pure symmetrical A^+, and then for pure symmetrical ion B^+, at equal activities $[A^+] = [B^+]$. One now measures γ for various mixtures of these two solutions; that is, for $0 < [A^+]/([A^+] + [B^+]) < 1$. In some cases, γ does not change monotonically with mole fraction, but actually shows a minimum. The most straightforward explanation of this result is that the two ions interact within the channel, and, therefore, that they both bind simultaneously. The favorite "B" ions are Ba^{2+} (for Ca^{2+} channels), Na^+ (for K^+ channels), and gluconate or thiocyanate (for Cl^- channels).

One common formalism, the interacting-ion model, featured two binding sites, with roughly equal affinity for ions (Almers and McCleskey, 1984; Hess and Tsien, 1984). A key idea was mutual repulsion between ions (Hille and Schwarz, 1978): wells that were quite deep when only one was occupied became shallower when both were occupied, leading to rapid flux. Flux was thus driven by the high effective concentration of the permeating ions. This model also describes ion movement through gramicidin channels (Urban et al., 1980; Becker et al., 1992), in which there is good evidence for two major cation binding sites (Olah et al., 1991).

In many cases, inorganic or organic blocking ions bind within the conduction pathway. We cite two examples. Mg^{2+} binds within the NMDA (*N*-methyl-D-aspartate) receptor channel (Nowak et al., 1984); this blockade underlies the Hebbian properties of NMDA-dependent long-term potentiation. In Kir channels, Mg^{2+} and polyamines bind to a ring of glutamate residues; this blockade produces inward rectification (Lu and MacKinnon, 1994; Fakler et al., 1995). Despite their biological importance, these two famous phenomena do not constitute proof for a multi-ion pore, because the blockade is not relieved by the permeant ions; one therefore learns little about permeation itself. On the other hand, in some cases K^+ competes for Ba^{2+} binding at a blocking site, and in others K^+ "locks in" Ba^{2+}, as though the Ba^{2+} ion is occluded at each end of the channel by a K^+ ion. In one complete study of these effects at a Ca^{2+}-activated K^+ channel, the authors concluded that "this channel's conduction pathway contains four sites of very high affinity for K^+, all of which may be simultaneously occupied under normal conducting conditions" (Neyton and Miller, 1988).

But problems have surfaced for the mutual-repulsion multi-ion pore model. After pioneering site-directed mutagenesis experiments on Na^+ channels (Heinemann et al., 1992), it appears that each of the four SS2 regions contains a crucial residue in a homologous position (D, E, K, A, respectively, in the four repeats) that controls selectivity (Perez-Garcia et al., 1997). Although these DEKA residues display resolvable asymmetries in

their electrical positions (Yamagishi et al., 1997), they presumably form a structure that approximates (within a few Å) a single ring in the conducting pore. Likewise, in experiments on Ca^{2+} channels, only a single ring of four glutamate residues govern selectivity to a large extent (Yang et al., 1993; Ellinor et al., 1995).

Meanwhile, for the homotetrameric *Shaker* K^+ channel, the primacy of a single ring was shown in experiments that mutated the highly conserved GYG sequence in the *P* region, which forms a reentrant loop in the same region of sequence as the SS1/SS2 loop discussed above for Na^+ and Ca^{2+} channels. Some single-residue mutations in this region abolish K^+ selectivity (Heginbotham et al., 1994); when two neighboring residues were deleted to make the *P* region sequence resemble that of cyclic nucleotide-gated channels, this also abolished K^+ selectivity (Heginbotham et al., 1992). Then it was found that the *weaver* mouse carried the GYG → SYG mutation in the GIRK2 K^+ channel; this channel also has nonspecific cation permeability (Kofuji et al., 1996; Navarro et al., 1996; Slesinger et al., 1996; Tong et al., 1996). The equivalent mutation in the similar GIRK4 channel has equivalent effects (Silverman et al., 1996).

Thus, most site-directed mutagenesis data render it untenable to consider that two or more roughly equivalent high affinity sites govern selectivity in multi-ion pores. The papers by Dang and McCleskey and Kiss et al. respond to this challenge by showing that a model with a single high affinity site, flanked by two binding sites of lower affinity close to the pore entrances, can generate much of the classical multi-ion behavior. The sites need not interact, and the two flanking sites could arise from one of several mechanisms: a featureless charged vestibule, a dehydration step, or a specific weak binding site.

The multi-ion pore remains a cornerstone of permeation theory, but the new theory features only a single high affinity site and no mutual repulsion. The high flux rate occurs because ions pause at the flanking sites and reequilibrate thermally, gaining enough energy to move over the next barrier.

It would be interesting to apply the "new" multi-ion models to two other general types of permeation proteins. First, anion channels. There is excellent evidence for multi-ion occupancy at the cystic fibrosis transmembrane conductance regulator, the Cl^- channel defective in cystic fibrosis (Linsdell et al., 1997). Three distinct residues in TM6 (K335, S341, R347) have all been associated with specific ion binding and permeation (Tabcharani et al., 1993; McDonough et al., 1994). Also, the anionic $GABA_A$ and glycine receptor channels differ from the cationic nicotinic ACh and 5HT3 receptor channels in the pore-lining M2 region. The stripe of polar residues (chiefly serines in the nAChR, chiefly threonines in the $GABA_A$ and glycine receptors) extends for only two turns or so in the nAChR, but for perhaps three or four turns in the $GABA_A$ and glycine receptors (Lester, 1992). Therefore, the pore seems to be longer for the $GABA_A$ and glycine receptors than for the cation channels. Sure enough, the $GABA_A$ and glycine receptors display an anomalous mole fraction effect, as though at least two ions occupy the channel simultaneously (Bormann et al., 1987); the nAChR does not display an anomalous mole fraction effect.

Second, nearly every model for ion-coupled transport features a state in which all cotransported substrates bind simultaneously. Contemporary measurements of voltage dependence (Gadsby et al., 1993), charge movements (Mager et al., 1993), leakage states (Mager et al., 1994; Fairman et al., 1995), and actual single-channel currents (Cammack and Schwartz, 1996; Lin et al., 1996) favor the picture that the multiple bindings occur within a lumen or pore. It would be ironic if these models for ion-coupled transporters, which now draw heavily on the conventional mutual-repulsion models for channels (Su et al., 1996), turn out to be the only surviving examples of such mechanisms.

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