New and Notable

The Last Few Frames of the Voltage-Gating Movie

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In the voltage sensor domain (VSD) of a voltage-gated channel, electrical charge is driven from the intracellular to the extracellular surface of a membrane as conformational changes occur and the channel gates are opened. This charge displacement, which in voltage-gated potassium channels occurs in ~1 ms, is measured as a ''gating current.''

A few years ago Sigg et al. (1) reported a component of the charge displacement that occurs in ~10 μs, two orders-of-magnitude faster than the ordinary gating current. This fast charge movement (I call it the ''loose charge'') was best modeled as a tethered diffusion, implying that the VSD's resting ''state'' is actually a very flexible conformation that allows protein charges to move quite freely. The loose charge, which makes a substantial contribution to the membrane capacitance when Shaker voltage-gated channels are expressed in *Xenopus* oocytes, is entirely absent in uninjected oocytes. From this, one concludes that there is something very special about voltage-gated channels, that among all the membrane proteins in oocytes, only they have such a large mobile charge. Now, in this issue of *Biophysical Journal*, a molecular dynamics study by Jogini and Roux (2) gives us a view of the substantial rocking and rolling of VSDs and their gating charge that makes the loose charge movement look quite tame.

In recent years, workers in Rod MacKinnon's laboratory have solved x-ray structures of two voltage-gated potassium channels. The first structure, of the archaebacterial channel KvAP, caused much consternation as it implied that, in the process of activation, the voltage sensor ''paddles'' (each a helical hairpin formed from the S3 and S4 VSD helices) would traverse the membrane while largely exposed to the hydrophobic membrane interior. It is now generally accepted that, in the KvAP crystals, the VSDs were held in an unnatural conformation. This conclusion is bolstered by the second x-ray structure, of the rat brain channel Kv1.2. The Kv1.2 crystal form resembles a stack of lipid-bilayer membranes, and presents a view of the VSDs that agrees much better with the many other experimental results that have accumulated in the past decade. This structure by Long et al. (3) shows a channel whose voltage sensors are fully activated (or maybe forced into the inactivated state as is seen after a long depolarization): we know this because the first four arginines in the S4 helix, the ones that carry the gating charge, are all exposed to the extracellular side of the membrane.

Now all that is needed is to extend this still picture—the Kv1.2 structure—into a movie that will illustrate the remarkable conformational change involved in voltage sensor activation. The VSD is not large; it is a membrane-embedded four-helix bundle, but somehow it rearranges itself in response to a voltage change to transport ~3.5 e_0 of charge from one side of the membrane to the other. The Kv1.2 structure provides a frozen frame near the end; but can we reconstruct the rest of the movie?

In molecular dynamics (MD) simulations, atoms are modeled as charged van der Waals spheres, and bonds are modeled as springs. This classical representation of what are actually fuzzy, polarizable, quantum mechanical atoms and bonds is surprisingly successful in predicting free energies and illuminating protein structural changes. In the analysis of the ion permeation process, MD techniques have resulted in a remarkably good prediction of the current-voltage curve of a potassium channel (4), and have shown how ion selectivity can arise simply from the number and type of ion-coordinating ligands (5).

Since MD can model an ion moving through a channel's selectivity filter, why not use MD to show us how the charged residues move as the Kv1.2 channel activates? There are two problems. First, a good idea of the reaction coordinates is required. For ion permeation, the reaction coordinates can only be the z position of each permeating ion. The VSD, on the other hand, has very many potentially relevant degrees of freedom, and without a crystal structure of a deactivated VSD, one does not know where to place the starting point of the reaction pathway. Second, there is the issue of the timescale accessible to MD, which is considerably less than 1 μs. Because of this limitation there is little hope of a brute force approach involving the simulation of the entire activation or deactivation process, which at best might be coaxed to occur in some tens of microseconds.

In the meantime, however, there is considerable value in simply looking at the motions and energetics of Kv1.2 in a lipid bilayer membrane, with its voltage sensors remaining activated as they were in the crystal structure. Jogini and Roux studied a large model system—some 77,000 atoms—that included a stripped-down Kv1.2 tetrameric channel, a lipid bilayer, and a 100 mM KCl solution. The channel subunits were truncated to include little more than the transmembrane portion; this is a simplification that is justified from experimental observations of normal channel activity expressed from similarly truncated cRNA sequences. Jogini and Roux's work is the latest of several recent MD simulations of voltage-sensing proteins in lipid bilayer membranes. Sands and Sansom (6) have simulated the isolated voltage sensor domain of KvAP, as have Freites

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et al. (7). Treptow and Tarek (8) have constructed a large system encompassing the entire Kv1.2 channel including its cytoplasmic T1 domains.

It is known from a wide variety of experiments that the charge translocation involved in the voltage-sensing process involves a movement of S4 arginines through a ''focused'' electric field, in which the transmembrane potential difference drops across a short distance. The focusing allows relatively small molecular motions to produce large charge movements and high voltage sensitivity. It is also known that salt bridges between acidic residues in S2 and S3 serve to stabilize some of the S4 charges. These aspects of VSD dogma are now clarified and extended by the MD results. Very recently, it was demonstrated that the presence of lipid phosphodiester groups is necessary for the proper functioning of a voltage-gated channel (9). The MD results now provide a tantalizing explanation for this phenomenon as well.

FOCUSED ELECTRIC FIELD

All of the recent simulation studies show extracellular and intracellular water-filled crevices that together form an hourglass-shaped aqueous region. Waters do not penetrate the central region, over which the transmembrane potential drops most steeply. However, to estimate quantitatively the electric field component that changes with membrane potential is a nontrivial undertaking; to date this been accomplished only in a heroic simulation of two lipid bilayer membranes by Sachs et al. (10). Jogini and Roux provide the next best thing, an approximate calculation using continuum electrostatics and a linear theory based on the Poisson-Boltzmann equation and the Ramo-Shockley theorem (11). Because the model is linear, they can invoke superposition to ignore the protein's fixed charges and simply calculate the contribution to the potential at a given atom due to the transmembrane potential difference. The resulting potential profile has a maximum steepness approximately three times that obtained from a constant electric field.

STABILIZATION OF ARGININES

Laying to rest the fears raised by the original ''paddle model,'' all the MD studies show that the S4 arginine side chains are not found in the nonpolar membrane interior, but are hydrated and paired with other charged groups. Acidic residues on the S2 and S3 helices, and also an aspartate on S5, are found in association with S4 arginines. And of particular interest is the observation that the two outermost arginines are found to be paired with one or more lipid phosphodiester groups. This provides a very promising explanation for the phosphodiester requirement for function of the KvAP channel. Perhaps these interactions are necessary to stabilize the activated states of the voltage sensors, allowing the channels to open. Jogini and Roux show further that many other basic residues located near the level of the membrane-water interface form salt bridges with lipid phosphates. The result is a net thinning of the membrane by $\sim$5 Å near the channel protein, which has a further effect in focusing the electric field.

MOBILITY OF VOLTAGE SENSOR DOMAINS

In the Kv2.1 structure the VSDs are four distinct modules that are arrayed around the periphery of the pore-containing channel core. Each VSD interacts with the core through an α-helix (the S4-S5 linker), through which its activation is coupled to channel opening; the remaining nonbonded connections to the core are not rigid, as only a third of the lateral surface area of a VSD abuts the core. It is thus not surprising that the VSDs are seen to swing from side to side through distances of 5–6 Å and, given enough time, are likely to sample very large displacements. Such motions could explain experimental evidence for the ability of residues to crosslink between VSDs; they also imply that spectroscopic measurements of distances between VSDs need to be interpreted with caution.

There are considerable vertical motions in the voltage sensor as well, with the z-coordinates of the arginine guanidinium moieties fluctuating by $\sim$2 Å rms. In view of the focused electric field, which causes $\sim$5% of the transmembrane potential to fall over each angstrom of z displacement, a correlated movement of the four arginines could produce a transmembrane charge fluctuation of $\sim$0.5 e_0 rms. This charge movement alone could result in a doubling of the membrane capacitance at high channel densities. Given a more damped motion, it would readily account for the size of the ''loose charge'' movement, $\sim$1.0 e_0, that was seen by Sigg et al. (1).

It should, however, be kept in mind that there are two differences between the loose charge and the z motions observed by Jogini and Roux. First, the loose charge moves on a timescale of 10 μs, which is very long compared to the nanosecond-scale fluctuations observed in the MD simulations. In electrical experiments, nanosecond-scale charge movements would be indistinguishable from an increment in membrane capacitance. Second, Sigg et al. observed the loose charge only in the resting state of the voltage sensors; in the activated state, the loose charge was not visible. These differences can be reconciled if the nanosecond-scale charge movements are similar in both resting and activated states, while an additional slower charge movement occurs only in the resting state. Nevertheless, the picture of VSDs provided by Jogini and Roux is one of a very mobile voltage-sensor domain, with a focused electric field that converts small physical motions into large transmembrane charge movements. It is no wonder that voltage-gated channels incorporating these domains show such large charge movements compared to other membrane proteins.

REFERENCES

1. Sigg, D., F. Bezanilla, and E. Stefani. 2003. Fast gating in the *Shaker* K^+ channel and

the energy landscape of activation. *Proc. Natl. Acad. Sci. USA*. 100:7611–7615.

2. Jogini, V., and B. Roux. 2007. Dynamics of the Kv1.2 voltage-gated K^+ channel in a membrane environment. *Biophys. J*.
3. Long, S. B., E. B. Campbell, and R. Mackinnon. 2005. Crystal structure of a mammalian voltage-dependent *Shaker* family K^+ channel. *Science*. 309:897–903.
4. Berneche, S., and B. Roux. 2003. A microscopic view of ion conduction through the K^+ channel. *Proc. Natl. Acad. Sci. USA*. 100:8644–8648.
5. Noskov, S. Y., and B. Roux. 2007. Importance of hydration and dynamics on the selectivity of the KcsA and NaK channels. *J. Gen. Physiol*. 129:135–143.
6. Sands, Z. A., and M. S. Sansom. 2007. How does a voltage sensor interact with a lipid bilayer? Simulations of a potassium channel domain. *Structure*. 15:235–244.
7. Freites, J. A., D. J. Tobias, and S. H. White. 2006. A voltage-sensor water pore. *Biophys. J*. 91:L90–L92.
8. Treptow, W., and M. Tarek. 2006. Environment of the gating charges in the Kv1.2 *Shaker* potassium channel. *Biophys. J*. 90:L64–L66.
9. Schmidt, D., Q. X. Jiang, and R. MacKinnon. 2006. Phospholipids and the origin of cationic gating charges in voltage sensors. *Nature*. 444:775–779.
10. Sachs, J. N., P. S. Crozier, and T. B. Woolf. 2004. Atomistic simulations of biologically realistic transmembrane potential gradients. *J. Chem. Phys*. 121:10847–10851.
11. Nonner, W., A. Peyser, D. Gillespie, and B. Eisenberg. 2004. Relating microscopic charge movement to macroscopic currents: the Ramo-Shockley theorem applied to ion channels. *Biophys. J*. 87:3716–3722.