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RESEARCH NEWS

A peek behind the scenes of selectivity filter gating

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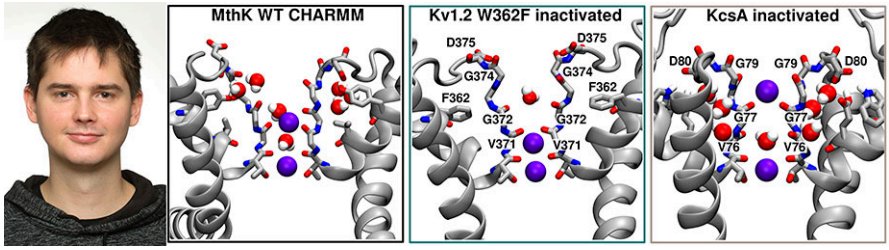
JGP study (Kopec et al. 2023. *J. Gen. Physiol.* https://doi.org/10.1085/jgp.202213166) reveals how interactions with pore-helix residues control filter inactivation in the prokaryotic potassium channel MthK.

Potassium channels have multiple gates that open and close to control the flux of K^+ ions across the cell membrane (1). Like many K^+ channels, the archaebacterial channel MthK has an "activation gate" at the cytosolic entrance to the pore that is formed by pore-forming helices from each channel subunit, as well as a gate at the extracellular end of the pore that is formed by the channel's selectivity filter. In this issue of *JGP*, Kopec et al. show how MthK filter gating can be influenced by residues in the pore-helices that lie immediately behind the selectivity filter (2).

In 2019, Wojciech Kopec and colleagues demonstrated that the activation gate and selectivity filter of MthK are allosterically coupled (3). In many K^+ channels, this coupling enables the phenomenon of C-type inactivation, in which full opening of the activation gate causes the selectivity filter to rapidly close and thereby limit channel activity. In the prototypical bacterial K^+ channel KcsA, this inactivation step depends on a specific glutamate residue in the pore helix surrounding the selectivity filter (4). But in MthK, and also in eukaryotic voltage-gated K^+ channels such as *Shaker*, the pore-helix residue at the equivalent position is a hydrophobic valine.

"We wanted to investigate filter inactivation, not only in wild-type MthK but also in a valine to glutamate mutant (V55E) that makes MthK more like KcsA," explains Kopec, a computational biologist at the Max Planck Institute for Multidisciplinary Sciences in Göttingen, who worked on the project with Brad Rothberg's lab at Temple University Lewis Katz School of Medicine in Philadelphia.

In a series of electrophysiology experiments, Rothberg's group found that the V55E mutation lowers the open probability of MthK channels by reducing the stability of the open state, thereby enhancing the propensity of MthK channels to inactivate (2). Interestingly, the V55E mutation also reduced the unitary conductance of MthK channels, even though this residue is behind, rather than a part of, the selectivity filter itself.



Wojciech Kopec and colleagues reveal how filter gating in the archaebacterial K^+ channel MthK is controlled by interactions between the selectivity filter and surrounding pore-helix residues. A mutation in one of these residues, V55E, that makes MthK more like the bacterial channel KcsA enhances channel inactivation by destabilizing the open state. However, molecular dynamics simulations suggest that the conformation of the inactivated filter in MthK (left) is more comparable to eukaryotic voltage-gated K^+ channels like Kv1.2 (center) than to KcsA (right).

To explain these electrophysiological phenotypes at the atomistic level, Kopec performed molecular dynamics simulations of MthK V55E and compared the results to previously generated simulations of the wild-type channel (2, 3). The V55E mutation also reduced channel conductance in silico, and the reason for this became clear when Kopec noticed that the glutamate can switch between two distinct conformations, adopting either a horizontal orientation in the plane of the membrane or a vertical orientation in which it forms a hydrogen bond with an aspartate residue behind the selectivity filter.

"If the glutamate is in the horizontal orientation, the channel behaves more or less like wild-type MthK," Kopec says. "But in the vertical orientation, it seems to rigidify the filter in a slightly narrower conformation that impairs ion permeation."

In many longer (5 µs) simulations, Kopec was able to observe spontaneous structural transitions in the selectivity filter of MthK channels that likely correspond to filter inactivation. Surprisingly, these transitions did not resemble those seen in KcsA, whose selectivity filter inactivates by pinching inwards to form a constricted, hour glass-shaped structure (5).

"Instead, in both wild-type and V55E channels, we saw that the filter widens," Kopec says. "This conformation looks like those recently reported for the inactive conformation of eukaryotic voltage-gated K^+ channels, so we think that MthK undergoes a gating transition like these channels rather than like KcsA."

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This transition occurred more frequently in simulations of MthK V55E than wild-type channels, in agreement with the experimental data showing that the mutation destabilizes the channel's open state. This phenotype appears to be driven by the horizontal orientation of the glutamate residue, which enables the entry of more water molecules behind the selectivity filter, disrupting the hydrogen bond network that usually stabilizes the filter in its conduction conformation.

The next question, Kopec says, is how filter widening inactivates the MthK channel. One possibility is that, by releasing K^+ ions from the top part of the filter, widening may disrupt a knock-on mechanism of ion permeation through the pore.

References

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