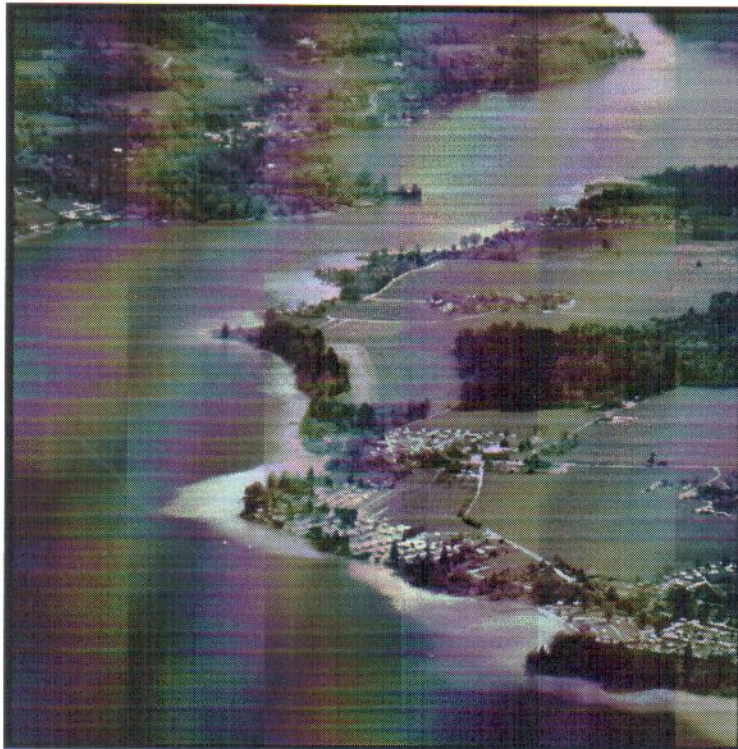


Permeation & Gating of Ion Channels

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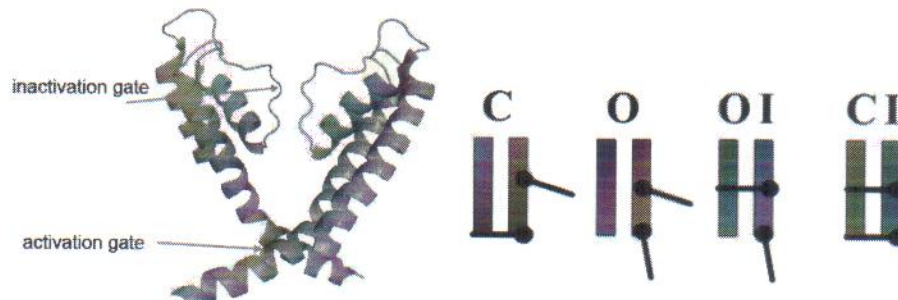
Slow inactivation of Shaker K⁺ channels: gates, states and transitions

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In the absence of N-type inactivation Shaker potassium channels display slow (C-type) inactivation. The slow-inactivation gate (I-gate) is in the selectivity filter near the extracellular entrance of the pore whereas the activation gate (A-gate), formed by the bundle crossing of the S6 segments, is near the intracellular entrance of the pore (Fig. 1, left panel). The positions of the two gates define four composite gating states of the channel as depicted in right panel of Fig. 1.



Functional and structural studies show that these two gates are coupled. Transitions between the C and O states have been extensively studied previously, whereas the transitions with closed inactivation cannot be studied using conventional electrophysiological methods as these states are non-conducting ones. We have addressed the transitions in the inactivated state using a state dependent cysteine accessibility strategy where cysteines are engineered into strategic positions in the channel protein and their accessibility is measured by the current loss in the presence of MTS reagents and/or Cd²⁺ in a state dependent manner. Inside/out patches were pulled from tsA201 cells expressing Shaker fast inactivation removed (IR) channels containing T449A mutation to facilitate slow inactivation along with cysteine substitutions at various positions along S6. Using an ultrafast perfusion system we applied the cysteine reagents selectively to the closed, open or inactivated channels (OI and CI). Using this strategy we have reached the following conclusions:

- 1, The pattern of the accessibility of the cysteins in S6 (470C-474C) indicates that the S6 helix makes rotational motion around its own axis during the direct O→OI transition ensuring the coupling between the activation and inactivation gates.
- 2, Cd²⁺ accessibility of T449A/V474C Shaker-IR channels reported that at fairly negative holding potentials, at which no macroscopic current can be detected, rare channel openings occur yielding access to the channel cavity. These rare openings facilitate inactivation of the channels and may explain the apparent “closed state inactivation” reported in classical electrophysiological studies. Based on Cd²⁺ accessibility we suggest that A-gate opening precedes structural changes associated with slow inactivation.
- 3, It has been shown earlier that Cd²⁺ traps the V476C Shaker channels in the open state, even at very negative voltages, by forming a metal bridge between a cysteine in one subunit and a native histidine (H486) in a neighboring subunit. We used this construct to determine if locking the activation gate in the open configuration prevents recovery from inactivation. Our results showed the lack of recovery from inactivation of the locked-open channels thereby suggesting that closure of the activation gate is essential for the recovery from slow inactivation.

In summary, we have provided experimental proof for the existence of some hypothetical gating transitions in the inactivated state proposed several decades ago and also proposed that S6 may couple the activation and inactivation gates in Shaker K⁺ channels.

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