Open-state stabilization in Kv channels: Voltage-sensor relaxation and pore propping by a bound ion

Robert J. French and Rocio K. Finol-Urdaneta

Department of Physiology and Pharmacology and Hotchkiss Brain Institute, University of Calgary, Calgary, Alberta T2N 4N1, Canada

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Historical context and overview

The Hodgkin and Huxley (1952) description of Na and K conductances underlying the action potential in squid giant axon is remarkable not only for its predictive accuracy in describing the shape and propagation velocity of the action potential but also for its foresight. Within their quantitative analysis and meticulous discussion are the seeds of decades of subsequent study, including the recognition that the ion-selective conductances must be provided by a relatively small number of highly conductive sites, which we now know as ion channels. Further, they concluded that, hidden in the electrical noise of their records, was a smaller, transient current that represented the movement of voltage-sensing charges within the membrane. Those "gating currents" were first reported by Armstrong and Bezanilla (1973) and reflect the movement of voltage-sensor charges in response to changes in the electric field, providing voltage sensitivity to the opening and closing of gates, which switch the channels between resting and conducting states. Stability of open/activated states under different conditions is most directly evaluated in macroscopic voltage-clamp recordings of the kinetics of ionic current deactivation (I_{deac}, reflecting channel closure), and the return of gating charge to its resting position (Ig_{OFF}) during a repolarizing voltage step applied after activation.

In this issue of the *JGP*, the Bezanilla and Snyders laboratories (see Labro et al.) and the Fedida and Ahern laboratories (see Goodchild et al.) use macroscopic deactivation currents and off-gating current studies to explore different possible bases for open-state stabilization, which lead to slowing of OFF gating charge movement and channel closure (deactivation) in voltage-gated K channels. Detailed arguments are presented for two different, though not mutually exclusive, mechanisms that contribute to the open/activated-state stabilization.

One possibility (Labro et al., 2012) is that the slowing of OFF gating current (Ig_{Off}) reflects an intrinsic behavior of the voltage-sensing domain (VSD), which relaxes during an extended depolarization into a more stable activated state and prolongs the conducting state through its coupling to the pore domain (PD) (Fig. 1, top, black arrows).

A second possibility (Goodchild et al., 2012) is based on the interaction between ions (permeant or blocking) and the pore's inner cavity, which allosterically stabilize both the S6 bundle-crossing (BC) gate of the pore and the activated state of the VSD. This might be thought of as an atomic scale surgical stent to support the pore against collapse (Fig. 1, bottom, red arrows).

With the hindsight of data from both groups, it seems to us that the extrinsic action of ions that enter and prop open the pore (Goodchild et al., 2012), and the intrinsic relaxation of the VSD during extended depolarization (Labro et al., 2012), might be seen as the application of energetic input, from two opposite ends, into the same allosteric chain of events (Fig. 1). In this view, neither mechanism precludes the other. In the reverse direction, the repolarization could drive the VSD to act as winch and pull the activation gate back from the bog of the open state, while dissociation of a permeant or blocking ion from the inner cavity would allow the BC gate to collapse back into its closed position (as noted by the reversibility of the states shown in Fig. 1). In the case of permeant ions, dissociation is rapid to support their normal conductive role (hooray for evolution!), where slowing of Ig_{OFF} and deactivation processes is less pronounced but remains demonstrable.

Actions of ions within the pore

As well as recognition of gating controlled by voltage and ligands, there is an extensive history of observations suggesting that ions in solution interact dynamically with ion channels and modulate their function. An early indication of the essential role that ions play in maintaining the structural and functional integrity of Kv channels is seen in the work of Almers and Armstrong (1980), who reported irreversible loss of squid axon potassium conductance when permeant ions were removed simultaneously from internal and external solutions.

Correspondence to Robert J. French: french@ucalgary.ca

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They further noted the loss of a component of charge movement "large enough to contain a contribution from K^+ gating charge movements of more than five elementary charges per channel". Later, the first detailed description of gating current associated with squid delayed rectifier followed from White and Bezanilla (1985). Slowing of potassium channel deactivation, after partial substitution of external sodium by potassium or rubidium, was observed by Swenson and Armstrong (1981). For equimolar substitutions of K or Rb, deactivation half-times were increased by 1.7× and 2.9×, respectively. Thus the less conductive ion Rb (which likely dwells longer in the channel) has the stronger effect in stabilization of the open state and consequent slowing of deactivation. A further consequence of the stabilization of the open state is a negative shift of the activation (G-V) curve along the voltage axis (Matteson and Swenson, 1986). Such dynamic effects of ions within channel pores have been seen in a variety of ligand- and voltage-gated ion channels (Gage and Van Helden, 1979; Nelson et al., 1984; Capes et al., 2012), underlining the possibility that ion channel proteins are dynamic structures, whose functions may be subject to a variety of allosteric influences.

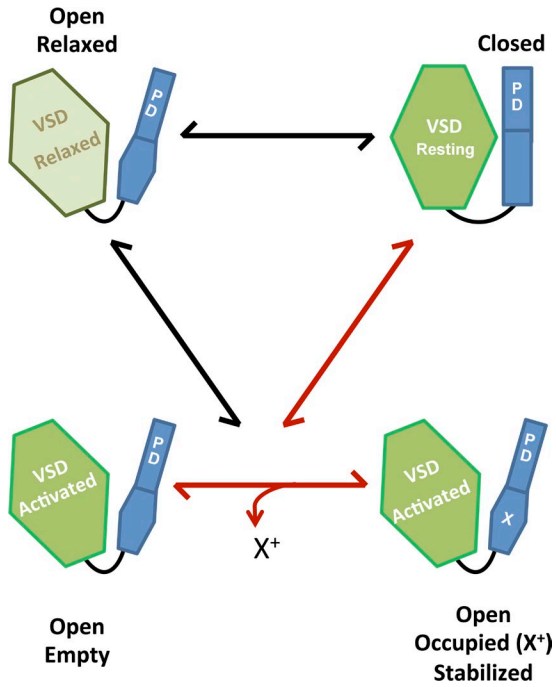

Figure 1. A minimal scheme to account for modulatory influences on activated/open-state stability by VSD relaxation and by ion binding within the pore. The "Open Empty" and "Open Occupied Stabilized" states are in rapid equilibrium for low-affinity pore ligands including permeant cations and weak blockers such as NMG^+; thus, these two states effectively can be treated as a single state. Transitions forming the focus of experiments by Labro et al. (2012) are shown in black, whereas those central to the study of Goodchild et al. (2012) are in red. VSD, voltage-sensor domain; PD, pore domain; X^+, ion that enters the pore inner cavity.

Open-state stabilization

In the highlighted papers, three kinds of measurements were used to evaluate the degree of correlation among different conformational changes: (1) ionic current, primarily reflecting the pore opening at the S6 BC gate; (2) gating current, quantifying movement on intrinsic charges in the protein, primarily in the S4 segments of the VSD; and (3) voltage-clamp fluorimetry, in conjunction with site-directed fluorophore labeling, directly reflects motions of the VSD that lead to changes in the microenvironment in the vicinity of the label.

In essence, kinetic correlations between two or more of the measured quantities suggest functional coupling, either forward or backward, in the chain VSD↔S4–S5 linker↔PD.

Voltage-sensor relaxation

In previous studies, including that of Lacroix et al. (2011), it was found that apparent shifts in the charge–voltage (Q-V) relationship for deactivation gating currents (Ig_{OFF}) resulted from depolarization-dependent slowing of charge movement after depolarizations of 0.003–30 s in duration. In addition, long integration times were needed, even at hyperpolarized potentials, to measure the full amount of charge recovery into the resting state Q_{OFF} charge movement after repolarization, following activation by depolarizing pulses of progressively increasing duration. A weighted time constant, τ_w, calculated from a double-exponential fit was used to characterize the overall decay rate of Ig_{OFF}. At −50 mV, the slow and fast time constants were ∼4 ms and 2 s, respectively, giving weighted time constants (τ_w) for Ig_{OFF} decay in the range of ∼1–80 ms. With increasing duration of the predepolarization greater than three orders of magnitude, τ_w increases in a bi-exponential fashion, having a faster component (τ_f of ∼5–15 ms) and a slower component (τ_s of ∼1–2 s). The faster component approximates rates for opening and closing of the BC gate of the pore, whereas the slower component was attributed to VSD relaxation. In the next experiments, these parameters reveal an obvious kinetic parallel between ionic current deactivation and Ig_{OFF} decay.

Labro et al. (2012) have extended the preceding work to define the basis of their observations of depolarization-dependent slowing of Ig_{OFF} and the associated shift in the Q_{OFF}–V relation. In experiments with a non–N-type inactivating variant of *Shaker* (Fig. 1 in Labro et al., 2012), long depolarizations (∼100 ms) of increasing magnitude led to progressive slowing of Ig_{OFF}, with increasing amplitude of depolarization. Plots against voltage of steady-state conductance (G), charge movement on depolarization (Q_{ON}), and on repolarization (Q_{OFF}) show a progressive negative shift along the voltage axis (Q_{OFF}←Q_{ON}←G). For repolarizing voltage steps, Ig_{OFF} shows increase in amplitude before decaying toward baseline. The increasing phase of Ig_{OFF} correlates

with the faster component of conductance deactivation, whereas the Ig_{OFF} decay matches the slow component of conductance deactivation. These observations are consistent with Q_{ON} moving before channel opening, and deactivation (channel closing) preceding the return of gating charge to the resting state (Q_{OFF}). A substantial fraction of channels would have to close before all Q_{OFF} returned to the resting state, consistent with a stabilization (relaxation) of the voltage sensor in its fully activated state. The remaining experiments in the paper systematically test the possibility that the observed open/activated-state stabilization, with its associated slowing of deactivation and Ig_{OFF}, can be attributed to voltage-sensor relaxation, i.e., stabilization in an activated state. Although providing convincing evidence for this, the experiments do not preclude the possibility that other influences, such as changes in the concentrations of ions/molecules, which bind to the inner cavity, might induce a similar stabilization.

In brief, the evidence is as follows. After repolarization, fluorescence signals from a tetramethylrhodamine label at the extracellular end of S4 showed kinetics, reminiscent of deactivation, with prepulse duration dependence over more than two orders of magnitude (Fig. 3 in Labro et al., 2012).

Slow inactivation appeared to lack controlling effects on open-state stabilization (Figs. 3–5 in Labro et al., 2012), based on similar behavior of *Shaker* and Kv1.2, in which the degree of steady-state slow inactivation and its rate, at +20 mV, differed by approximately twofold. Also, in both channels, the rate of slow inactivation is approximately three- to fivefold slower than VSD relaxation, reflected by the slow component of changes on deactivation and Ig_{OFF} kinetics during prolonged depolarization.

Pivotal evidence that VSD relaxation alone can dominate activated-state stability comes from experiments on the voltage-sensitive phosphatase, Ci-VSP (Villalba-Galea, 2012), a membrane-bound phosphatase in which a cytoplasmically exposed phosphatase unit replaces the PD of Kv channels (Figs. 7 and 8 in Labro et al., 2012). Ci-VSP exhibits a sensing current, Is, after voltage steps. The sensing current is analogous to the gating current, Ig, of Kv and other voltage-gated channels, and the Is_{OFF} decay rate (quantified by a weighted time constant, τ_w) also slows with increasing duration of activating depolarizations of ~0.1–10 s (τ of ~0.5 s, fit with a single exponential). One difference is that there is no faster component of the slowing associated with the catalytic domain as there is for the pore domain BC gate of the Kv channels. However, faster changes in Is_{OFF} decay rate were seen for a construct lacking the phosphatase domain (τ of ~19 vs. 62 ms for the wild-type [WT] enzyme). The extent of the kinetic change (2–3×) was comparable in *Shaker* and Kv1.2, as well as in WT Ci-VSP. Thus, the isolated Ci-VSP voltage sensor shows an apparent slowing relaxation even in the absence of any molecular load at its C-terminal.

Ions pushing from within

Using Kv1.2, bathed in external TEA and internal NMG, Goodchild et al. (2012) begin by illustrating the profound slowing of the Ig_{OFF} transient relative to Ig_{ON} (Fig. 1 in Goodchild et al., 2012). Gating charge (Q_{ON} and Q_{OFF}) estimates are made from a fixed-duration integration of 11 ms, and in their Fig. 2, they estimate the slowing of Ig_{OFF} by plotting Q_{OFF}/Q_{ON} against the duration of a depolarizing pulse to 0 mV, obtaining τ = 3.7 ms. Full return of gating charge after a 50-ms depolarization to +10 mV was observed at approximately −180 mV. An apparent gating charge of $z\delta$ = 1.6 elementary charges was estimated from a plot of fractional charge recovery against the recovery interval (Fig. 3 in Goodchild et al., 2012). This procedure is expected to underestimate the voltage sensitivity ($z\delta$) of the charge return step because, in the voltage range of the analysis (−100 to –80 mV), not all the charge was observed to return (Fig. 2 E in Goodchild et al., 2012), implying that the charge recovery time is determined by a combination of both forward and backward rates for which the voltage dependencies are oppositely directed.

With the experiments shown in their Fig. 4, the authors explore the charge movement with either TEA^+ or Cs^+ as the internal ion. As they acknowledge, this analysis is somewhat problematic, given that the low level of Cs^+ conduction is sufficient to overlap the Ig_{ON}, and thus preclude a precise integration to obtain Q_{ON} with internal Cs^+, leaving the internal TEA^+ data as the only "control" for the estimation of a shift in the Q_{OFF}–V relation. In any event, it seems safe to conclude that the gating charge return (Q_{OFF}) is delayed far more with the presence of internal TEA^+_i than with internal Cs^+ (see their Fig. 4, B–D). Continuing their examination of ion species dependence of activated state stability, the authors use a nonconducting mutant, Kv1.2 W366F, V381T, analogous to the *Shaker* W434F, to compare the action of internal Cs^+ with that of the normal permeant ion, K^+ (Fig. 5 in Goodchild et al., 2012). Ig_{OFF} is slowed, to the point of essentially being obscured after larger depolarizations, with either Cs^+ or K^+ present internally.

In a final test of the hypothesis that relative molecular size is important in the open-state stabilization, the authors use the mutant Kv1.2 I402C to obtain a "larger" inner cavity. For this mutant: (a) there is no obvious slowing of the Ig_{OFF} (Fig. 6 A in Goodchild et al., 2012); (b) the G-V curves for the WT and mutant channel (Fig. 6 B in Goodchild et al., 2012) superpose with those of WT, showing no evidence of the shift associated with a rate-limiting step adjacent to the open state; and (c) plots of normalized Q_{ON} and Q_{OFF} versus V have essentially the same midpoint voltage. The decay rate for Ig_{OFF}, measured at a voltage for which the forward rate should be close to zero, shows relatively weak voltage dependence ($z\delta$ = 0.4), and the decay rate for the I420C mutant is approximately three times faster than for WT.

Coincidentally or otherwise, a similar apparent valence was seen for steady-state block of squid axon Kv channels (French and Shoukimas, 1985; NMG^+ termed GA in their study). All in all, these data are consistent with no substantial stabilization of the open state by NMG^+, either because of reduced binding affinity or the possibility that the pore can close unimpeded by the NMG^+.

To place their data in the context of experimental and structural studies, the authors show that a modified version of the kinetic model for *Shaker* by Zagotta et al. (1994) could describe the qualitative features of their data without inclusion of the intrinsic open-state stabilization of the original model, provided that open-state binding, which impeded closure, was added.

Coupling voltage sensor to pore

Although Goodchild and collaborators contrast the "allosteric" action of pore-binding ions on VSD behavior with phenomena based on "intrinsic" properties of the VSD, we consider the mechanisms supported in both of the papers to be allosteric, in the sense that they reflect modulations communicated at a distance through the protein. The distinction between them lies in the direction of coupling. Thus, if the primary event is the action of an ion binding in the inner cavity, the allosteric coupling sequence is pore to domain S4–S5 linker to VSD, whereas this sequence would be reversed (VSD to S4–S5 linker to PD) if the primary event were the relaxation of the voltage sensor induced by prolonged depolarization.

What are the fine structural and mechanistic details of this coupling? Sorting out the answers, in the multiple variants of Kv channels and their relatives, is a major ongoing task, and is the subject of at least two thorough recent reviews (Blunck and Batulan, 2012; Vardanyan and Pongs, 2012). Changes in packing within the VSD, and between the VSD and PD, could involve interconversion of α- and 3_{10}-helical conformations (Vieira-Pires and Morais-Cabral, 2010).

Implications for signal processing and pathophysiology

Each of the mechanisms proposed above for open-state stabilization offers the possibility for modulation of (patho-)physiological signal processing under realistic situations involving either (a) changes in local ion/drug concentrations or (b) prolonged changes in membrane voltage, or the two in combination. An enormous variety of amphiphilic amines, including many therapeutic, channel-targeted blockers, can enter the inner cavity of Kv channels. Furthermore, if the channel's inner cavity exerts mechanical modulation on the VSD, the membrane's mechanical properties might provide input driving another "allosteric" modulation of ion channel function (Finol-Urdaneta et al., 2010). Also, prolonged depolarization and changes in ambient potassium concentration are associated with normal bouts of hyperactivity, as well as pathological situations such as central nervous system spreading depression.

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