Article

Domain and Interdomain Energetics Underlying Gating in *Shaker*-Type K_V Channels

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ABSTRACT To understand gating events with a time-base many orders-of-magnitude slower than that of atomic motion in voltage-gated ion channels such as the *Shaker*-type K_V channels, a multiscale physical model is constructed from the experimentally well-characterized voltage-sensor (VS) domains coupled to a hydrophobic gate. The four VS domains are described by a continuum electrostatic model under voltage-clamp conditions, the control of ion flow by the gate domain is described by a vapor-lock mechanism, and the simple coupling principle is informed by known experimental results and trial-and-error. The configurational energy computed for each element is used to produce a total Hamiltonian that is a function of applied voltage, VS positions, and gate radius. We compute statistical-mechanical expectation values of macroscopic laboratory observables. This approach stands in contrast with molecular-dynamic models which are challenged by increasing scale, and kinetic models which assume a probability distribution rather than derive it from the underlying physics. This generic model predicts well the *Shaker* charge/voltage and conductance/voltage relations; the tight constraints underlying these results allow us to quantitatively assess the underlying physical mechanisms. The total electrical work picked up by the VS domains is an order-of-magnitude larger than the work required to actuate the gate itself, suggesting an energetic basis for the evolutionary flexibility of the voltage-gating mechanism. The cooperative slide-and-interlock behavior of the VS domains described by the VS-gate coupling relation leads to the experimentally observed bistable gating. This engineering approach should prove useful in the investigation of various elements underlying gating characteristics and degraded behavior due to mutation.

INTRODUCTION

The voltage-controlled gating of Na^+, K^+, and Ca^{2+} channels is a regulatory function crucial for living cells. It involves cooperation among intramolecular domains with distinct evolutionary histories, structures, and functions (1,2). Gating events occur over a million-fold measured experimental time range, from microseconds to seconds, and this time range can in some cases be observed in a single record from a single channel (3). Discrete-state hidden Markov models have provided kinetic descriptions of gating over this time range. These models describe gating as transitions among discrete states of empirically known lifetimes but typically unknown physical structure (4–6). Another approach, molecular-dynamics simulation, computes the atomic motions in models of crystallographically defined structures for short snippets of time. The actual gating motions are seldom if ever observed in these simulations unless the system is highly biased to induce gating (7–17). These atomic simulations likely do not allow the free energies governing large reconfigurations in a channel macromolecule to reach sufficient convergence (18), and they do not extend to most gating phenomena that electrophysiological experiments typically assess.

In this article, we follow an engineering approach to voltage-controlled gating. Events evolving at different scales of time and/or length are described at different levels of resolution and organized into a hierarchical model with two tiers. The presented model incorporates known aspects of structure, computed physical interactions, and hypothesized elements to simulate actual experiments. The model can correlate structural and functional experimental data, yet it can be solved with computational methods that are both economical and rigorous.

The lower tier of our gating model comprises models describing the internal physics of the individual voltage-sensor (VS) and gate domains. Structural information is abstracted into a generic geometry of each domain and a set of physical interactions known to be involved and which we hypothesize dominate the function of each domain. The VS and gate domain models are integrated into the channel model in terms of their energetics: overall channel configurational energy is described by an energy map (Hamiltonian). Domain energetic contributions are computed and sampled over all degrees of freedom made explicit in the domain models. This makes our approach essentially different from

Submitted March 25, 2014, and accepted for publication August 15, 2014.

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Editor: Gerhard Hummer.

0006-3495/14/10/1841/12 $2.00

http://dx.doi.org/10.1016/j.bpj.2014.08.015

that of the state-based hidden Markov-chain models of gating because the hidden Markov-chain approach does not compute any Hamiltonian components from given physical features, but instead infers an overall Hamiltonian from functional data, assuming that the relevant configurations are restricted to a hypothesized set of energy minima. The hidden Markov-chain approach primarily goes backward from experimental results to a constrained set of state transitions; our approach attempts to go forward from a physical model to predict experimental results, with an exception discussed below regarding coupling.

The upper tier of our hierarchical model describes the cooperation of the VS and gate domains. Domain interaction is formally described by a map of configurational energy quantifying the energetic cost of the coexistence of configurations in a VS domain and in the gate domain. With the VS and gate domains described by physical models, the model developed of a gated channel provides a constrained view of the mechanism by which these domains can be coupled. Although at this stage no structure-based physical model exists for creating this map, available functional data allow us to construct a well-defined map for the VS/gate interaction.

Unlike state-based hidden Markov models (which describe the kinetics of a single entity, the channel) and atomistic models (which describe the channel as a homogeneously modeled ensemble of interacting atoms), our two-tier model makes explicit the intrinsic energetics of the domains and the energetics of the interactions among domains. It thereby gives an engineering decomposition of the mechanism that has not been naturally provided by the hidden Markov-chain models or atomistic simulations.

Analyzing *Shaker*-type K_V channels, we find that relatively simple models of the VS and gate domains coupled via a simple energy landscape predict experimental charge/voltage and conductance/voltage relations quite well. The only coupling function that we have found that is consistent with known experimental results is a "two-latch" mechanism, which leads to the *Shaker* domains sliding and interlocking to produce gating that is essentially bistable. Our analysis also produces the most detailed accounting of gating energetics to date. Specifically, we find that the electrical work picked up by the VS domains is an order-of-magnitude larger than the work needed for reconfiguring the gate domain between conducting and nonconducting configurations. Therefore, there is disposable energy in the gating mechanism allowing, in principle, for the evolution of diverse gating characteristics. Such multiscale engineering approaches may be applicable to other large biophysical systems whose measures of interest have long characteristic times.

MODEL

We briefly describe here the VS and gate domain models that we embed into the full-channel model. Because these have been the subjects of previous publications (19–22), we only detail the specific model configurations used here. We then elaborate the formal framework for integrating these domain models into a statistical-mechanical model of the full channel.

Model of the VS domain

The model of a VS domain comprises an α-helical S4 transmembrane segment sliding in a "gating canal" formed between the S1–S3 and S5–S6 segments. The protein matrix is represented by a uniform dielectric and charged residues by embedded point charges (Fig. 1 *A*). In previous work (21), this model of a single VS domain was studied in a computational setup including membrane and water dielectrics and encapsulated by electrodes to establish voltage-clamp boundary conditions. The expected whole-body movements of the uncoupled S4 charges in response to the applied membrane voltage V_m have been computed (21,22). In constructing the channel model, we use the (electrostatic) configurational energy $\mathcal{H}_{VS}(z,\phi,V_m)$ and the displaced gating charge $Q(z,\phi)$ (Fig. 2 *A*) computed for a VS domain as a function of S4 position (translation z, rotation ϕ) and membrane voltage V_m in the range -100 mV to $+100$ mV. Our specific VS model is the α-helical model described by Peyser and Nonner (21). Variations of that VS model have been explored (22). The model produces a gating-charge/voltage relation for an isolated VS domain that is comparable in total charge and slope to the relation experimentally observed in full *Shaker*-type K_V channels (see Figs. 5a and 8b of Peyser and Nonner (21)). The electrostatics of that model makes the S4 charges follow an emergent helical (i.e., screw) trajectory.

Our VS model describes linear dielectrics, including the protein matrix, by piecewise uniform dielectric coefficients. This continuum electrostatic model is solved consistently using a boundary element method (21). At the later stage of modeling presented in this article, our engineering model of the full channel requires only the relations $\mathcal{H}_{VS}(z,\phi,V_m)$ and $Q(z,\phi)$ to specify the behavior of the VS domain. Our channel model does not require a specification of how these relations arise. In principle, the two relations could be directly measured rather than modeled. Both relations that we use are robust: the chosen relations can arise in our generic VS model from a range of configurations (22). A coarse-grained model like that developed by Dryga et al. (18,23) might provide more accurate relations for specific structures or domains in the future.

Model of the gate domain

The model of the gate is based on the wetting/drying of a hydrophobic segment of the ion-conducting pore. Following experimental results (24), we postulate that the S4 position in the VS domains controls the width of the intracellular