Commentary

A Plausible Model

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One widely expressed K^+ channel, often called the "BK" channel for its "big" single channel conductance, is regulated by intracellular Ca^{2+} and voltage: at constant voltage, the open probability (P_o) increases with $[Ca^{2+}]$. At constant $[Ca^{2+}]$, P_o increases with depolarization. BK channels participate in many physiological processes, including repolarization of the action potential (Adams et al., 1982), frequency tuning in the inner ear (Hudspeth and Lewis, 1988), and regulation of neurotransmitter release (Robitaille et al., 1993).

The mechanism of BK channel gating is addressed by a recent paper in this journal (Rothberg and Magleby, 1999) and by two papers in this issue (Horrigan et al., 1999; Horrigan and Aldrich, 1999). The Magleby and Aldrich labs took very different approaches, but fortunately arrive at compatible conclusions. Rothberg and Magleby (1999) examined in detail the gating of single BK channels under a limited range of conditions: +30 mV, primarily at saturating $[Ca^{2+}]$. Horrigan et al. (1999) and Horrigan and Aldrich (1999) examined macroscopic ionic and gating currents (respectively), over a wide voltage range but in the effective absence of $[Ca^{2+}]$. Both find features of BK channel gating that favor allosteric models, resembling in some ways the classical MWC model (Monod et al., 1965) for activation of allosteric enzymes.

Ion Channels as Allosteric Proteins

Cooperative mechanisms were introduced long ago in the field of enzyme kinetics, notably the self-described "plausible" MWC model (Monod et al., 1965). Suppose that a multisubunit protein can exist in two conformational states, a resting T "tight" state or an active R "relaxed" state (it is curious that the relaxed state was assumed to do the work). Each subunit has a ligand binding site, and binding of a ligand favors the active R state by a certain amount of energy. With any number of ligands bound, the protein can be in either the T or the R conformation; but at equilibrium the T state is favored when no ligands are bound, and the R state when binding is saturated. Within the T or R state, the binding steps are independent, but the concerted T–R transition cha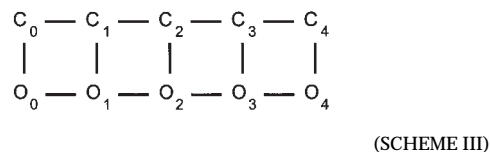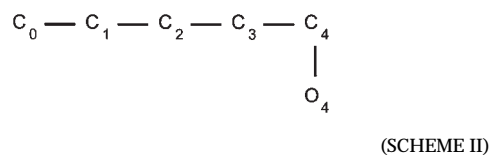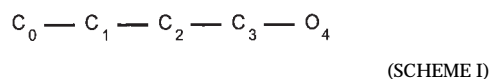nges the affinity for all subunits. This MWC model is by no means the only model that has been proposed for cooperative activation of a protein, but it is plausible.

Many ion channels are multisubunit proteins, containing multiple sensors that somehow work together to regulate the functional state of a single centrally located pore. The analogy to allosteric enzymes is most obvious for ligand-gated channels, which have two or more ligand binding sites (Changeux and Edelstein, 1998).

Members of the P domain–containing superfamily of ion channels contain either four subunits, or four homologous domains, each coupled to a single pore. These too are allosteric proteins (Hille, 1992). Among P domain channels, the MWC model can be applied directly to cyclic nucleotide–gated channels, with four nucleotide-binding domains (Goulding et al. 1994; DiFrancesco, 1999). But a closely analogous situation exists for voltage-dependent channels, where four voltage sensors (the S4 transmembrane domains) regulate one pore. That is, activation of a voltage sensor by depolarization is formally analogous to binding of a ligand (Marks and Jones, 1992).

Models for cooperative activation of voltage-dependent channels began with Hodgkin and Huxley (1952). In modern terminology, their K^+ channel model postulated four identical and independent voltage sensors, with the channel open only if all four sensors are activated (Scheme I), which is a straightforward mechanism for cooperativity. However, voltage sensor movement seems to be followed by a kinetically distinct channel opening step (Koren et al., 1990; Zagotta and Aldrich, 1990). The resulting Scheme II is a subset of the full MWC model (Scheme III). Although Scheme III appears to be more complicated (10 vs. 6 states), it has only one additional free parameter, an allosteric factor, which represents the energy stabilizing the open state upon movement of each voltage sensor. In terms of the underlying physical process, Scheme III avoids the arbitrary assumption that channel opening is completely forbidden unless all four voltage sensors are activated. For aficionados of Occam's Razor, the complexity of a model cannot be assessed by counting the number of states. The number of free parameters is a better

measure—but the number of underlying physical processes is better still.

$$C_0 \text{—} C_1 \text{—} C_2 \text{—} C_3 \text{—} O_4$$

(SCHEME I)

$$C_0 \text{—} C_1 \text{—} C_2 \text{—} C_3 \text{—} C_4$$
$$|$$
$$O_4$$

(SCHEME II)

$$C_0 \text{—} C_1 \text{—} C_2 \text{—} C_3 \text{—} C_4$$
$$|\qquad|\qquad|\qquad|\qquad|$$
$$O_0 \text{—} O_1 \text{—} O_2 \text{—} O_3 \text{—} O_4$$

(SCHEME III)

Most studies are consistent with the assumption of Schemes I and II; namely, that all voltage sensors must activate before the channel opens. To begin with, channels typically activate with a sigmoidal delay, but deactivate almost exponentially upon repolarization (Hodgkin and Huxley, 1952). That is expected if many states must be negotiated before opening, whereas channel closing is simple and direct. In addition, many channels exhibit a single open state. One exception is L-type calcium channels (Marks and Jones, 1992): under basal conditions, Scheme II is a good approximation, but dihydropyridine "agonists" that favor channel opening induce two open states, as though the O_3 state (in Scheme III) is significantly occupied.

Allosteric coupling to voltage sensor movement is also a plausible mechanism for inactivation (Kuo and Bean, 1994; Serrano et al., 1999). In terms of the ball-and-chain model, the channel becomes an adequate receptor for the ball not when it opens, but as its voltage sensors activate (Patlak, 1991). For some channels, inactivation seems to occur preferentially from "partially activated" closed states, where some of the voltage sensors have moved but the channel has not yet opened (Klemic et al., 1998; Patil et al., 1998).

BK Channels as Allosteric Proteins

Regulation of BK channels is particularly complicated, because there are two fundamental regulators (Ca^{2+} and voltage) instead of one. One early suggestion was that voltage dependence arose from binding of Ca^{2+} within the membrane's electrical field (Moczydlowski and Latorre, 1983). However, the cloning of BK channels revealed S4 regions, closely similar to the voltage sensors of the Kv family of channels, plus a long COOH-terminal region that may be involved in Ca^{2+} sensing. By analogy to Kv channels, BK channels are likely to be tetramers, consistent with the high Hill coefficient for

Ca^{2+} observed experimentally. These features suggest that both Ca^{2+} binding and voltage sensor movement are allosterically coupled to channel activation.

A general scheme for allosteric activation of BK channels must consider three distinct but coupled processes: voltage sensor activation, Ca^{2+} binding, and channel opening. If all permutations are considered (0–4 Ca^{2+} bound, 0–4 voltage sensors activated, and the channel either open or closed), there are $5 \times 5 \times 2 = 50$ possible states of the channel (Scheme IV). In the diagram, the subscripts and superscript denote the number of activated voltage sensors and the number of bound Ca^{2+} ions, respectively; 16 of 25 open states are "hidden" by closed states. Even that scheme could easily be extended (Horrigan et al., 1999; Rothberg and Magleby, 1999). For example, if two Ca^{2+} ions are bound and two voltage sensors are activated, it may matter whether Ca^{2+} is bound to the subunits with activated voltage sensors (or whether the activated and/or Ca^{2+}-bound subunits are opposite or adjacent).

(SCHEME IV)
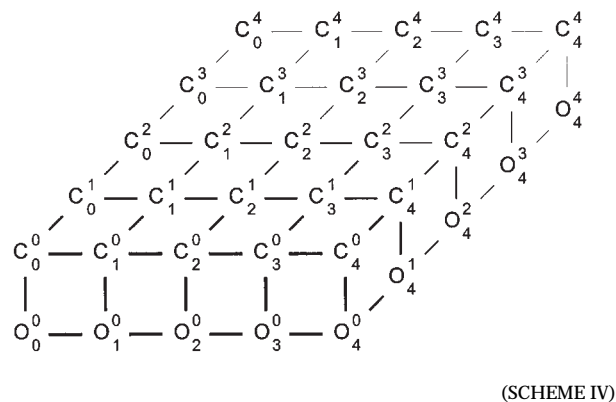
Enough theory for now. What does the data show? Are all those states really necessary?

Evidence from Macroscopic Ionic and Gating Currents

In native cells, BK channels tend to be intimately coupled to voltage-dependent Ca^{2+} channels, producing a current that depends in a complex manner on Ca^{2+} entry and diffusion, as well as on voltage. To study the intrinsic kinetics of BK channels at the macroscopic level, in the absence of Ca^{2+} channels (and this at a constant $[Ca^{2+}]$), it has proven useful to work with cloned channels in expression systems (DiChiara and Reinhart, 1995; Cox et al., 1997; Cui et al., 1997).

In apparent contrast to the complexities expected from Scheme IV, BK currents change nearly exponentially in response to a voltage step. But the time constants depend on both Ca^{2+} and voltage (Cui et al., 1997). The results were explained by a version of the MWC model (Scheme III), with the horizontal steps interpreted as Ca^{2+} binding. The vertical steps (channel

opening) are more rapid but contribute to the voltage dependence (Cox et al., 1997).

One key result was that BK channels can open in the effective absence of Ca^{2+} in response to a sufficiently strong depolarization. Without Ca^{2+}, the BK channel is purely voltage dependent, which simplifies the situation and allows the use of established procedures for analyzing voltage-dependent gating. Without Ca^{2+}, Scheme III reduces to a simple two-state $C_0–O_0$ model. Horrigan et al. (1999) now report that BK channel gating is much more complex even in that "simple" condition. First, there is a brief delay before channel opening, less conspicuous than for a simple sequential model such as Scheme I, but clearly present. Second, the main time constant depends on voltage in a complex manner, with weak voltage dependence at very negative voltages. This suggests multiple gating processes (even in the absence of Ca^{2+}), which become rate limiting in different voltage regions.

Linear models such as Schemes I and II make a strong prediction: that P_o will decrease exponentially at extreme negative voltages, with a steepness depending on the amount of charge moved (Sigg and Bezanilla, 1997). This was not observed for BK channels, where P_o approached a limiting value ∼10^{-6} near −100 mV (Horrigan et al., 1999). The simplest interpretation is that BK channels can open even if some voltage sensors are not activated. This, in turn, leads to the proposal that BK channel gating follows Scheme III in the absence of Ca^{2+}, with allosteric coupling between voltage sensor movement (horizontal steps) and weakly voltage-dependent channel opening (vertically). This interpretation of Scheme III is equivalent to the 10 foreground states in Scheme IV.

The model was supported by analysis of gating currents (Horrigan and Aldrich, 1999). There were three distinguishable components of charge movement, corresponding (roughly) to voltage sensor movement in closed channels, voltage sensor movement in open channels, and channel opening itself. As expected from Scheme III, channel opening shifted the voltage dependence of charge movement to more negative voltages, and slowed "off" charge movement. Formally, that resembles the "charge 2" and "charge immobilization" associated with inactivation of other voltage-dependent channels, which may also reflect an allosteric coupling mechanism (e.g., Shirokov et al., 1998).

Linear models (Schemes I and II) predict that charge movement precedes channel opening, so the voltage dependence of charge movement (the Q–V curve) is shifted to more negative voltages compared with channel activation (the G–V curve). With Scheme III, some charge movement precedes opening, but channels can open before all the gating charge moves, allowing subsequent charge movement in the O–O

steps. That can produce a "crossover" of the Q–V and G–V curves, which actually has been reported for BK channels (Stefani et al., 1997). However, Horrigan and Aldrich (1999) did not see a crossover, and suggest that the crossover results from measuring ionic and gating currents under different experimental conditions. Gating of many K^+ channels (including BK) is strongly influenced by permeant ions, which unfortunately makes it very difficult to compare Q–V to G–V curves.

Evidence from Single Channels

Their high single-channel conductance has long made BK channels a proving ground for kinetic analysis (Barrett et al., 1982; Moczydlowski and Latorre, 1983). One striking observation is that BK channels not only have multiple closed states, but also several open states (immediately ruling out Schemes I and II).

While the Aldrich lab concentrated on BK channel gating without Ca^{2+}, Rothberg and Magleby (1999) examined the opposite condition, saturating Ca^{2+}. In this case, the MWC model of Cox et al. (1997) again reduces to a two-state model ($C_4–O_4$), predicting simple exponential distributions of open and closed times. But at least three open and four closed states are observed (Rothberg and Magleby, 1999). At high Ca^{2+}, P_o reached a limiting value (0.95, not 1.0), and channel gating was essentially identical at 0.1 and 1 mM Ca^{2+}, as expected if all Ca^{2+} binding sites were already occupied at the lower concentration. Furthermore, adjacent dwell times were correlated (roughly, longer openings tended to be adjacent to shorter closings, and shorter openings to longer closings)—suggesting multiple connections between closed and open states, consistent with Scheme III (but not with some linear schemes that have multiple open states, such as C–C–C–O–O). Rothberg and Magleby (1999) propose a subset of Scheme III, without the O_0 and O_1 states. In this case, Scheme III is equivalent to the rear plane of 10 states, partially visible in Scheme IV.

It is tempting to interpret the multiple closed (or open) states in the Rothberg and Magleby (1999) model as different states of the voltage sensors. But Rothberg and Magleby (1999) did not examine voltage dependence directly, as they concentrated on channel gating at a fixed voltage (+30 mV). A less exciting interpretation is that some of the states available to the fully Ca^{2+}-bound BK channel may be voltage and Ca^{2+} independent, and thus uncoupled from the major mechanisms regulating channel gating (discussed by Rothberg and Magleby, 1998). Such transitions are conspicuous in the gating of single *Shaker* K^+ channels, for example (Hoshi et al., 1994).

Lessons for Kinetic Modeling of Ion Channels

Why is it so difficult to go from kinetic data to a mechanism? Didn't Hodgkin and Huxley (1952) do that sim-

ply and elegantly long ago? Why do two leading labs take radically different approaches to the gating of BK channels? And, given the classic demonstration that single channel kinetics can resolve ambiguities present in ionic current measurements (Aldrich et al., 1983), why do the two papers from the Aldrich lab rely almost entirely on macroscopic ionic and gating currents? Several theoretical and practical issues come into play.

Macroscopic ionic currents. For a two-state C–O model, the exponentially relaxing current observed in response to a voltage step contains enough information to fully determine the two parameters of that model, the rate constants for channel opening and closing at that voltage (if the current amplitude can somehow be converted to P_o). For models like those of Hodgkin and Huxley (1952), involving identical and independent voltage sensors, similar analysis is possible. For general Markov models, however, kinetic coupling between the different steps in the reaction complicates matters. In general, there are multiple exponential components in the data, some of which may not be distinguishable experimentally. Worse, perfectly accurate measurement of the exponential components during a voltage step does not return enough information to uniquely determine the rate constants, even for a three-state model (Goldman, 1991). Finally, ionic currents change only when channels open or close, so intermediate steps (C–C or O–O) are not directly measured, but can only be inferred.

Gating currents. These provide complementary information, since voltage-sensitive C–C or O–O transitions produce gating currents. Some practical issues that limit the usefulness of gating currents for channels in most native cells (current isolation, leak, and capacity subtraction) are less problematic for studies using cloned channels in expression systems (see Horrigan and Aldrich, 1999). Still, gating currents directly report only on fast, highly voltage-sensitive steps, and kinetic coupling of different steps in the pathway can have nonintuitive consequences.

Single channels. In principle, it is straightforward to extract kinetic information from single channel data: fit exponentials to the distribution of open and closed dwell times, and get the number of states and their mean lifetimes. Practically, if the range of open and closed times is large (as for BK channels), an immense amount of data is required to define the kinetics, even under a single condition. The Magleby lab has worked for over a decade to define the kinetics of BK channels over a wide range of voltages and $[Ca^{2+}]$. Definition of the steady state dwell-time distributions does not, however, establish the connectivity between the states, although "2-D" distributions give additional information (see Rothberg and Magleby, 1999). Transient kinetics

(responses to changes in voltage or $[Ca^{2+}]$) would help further, but the range of conditions that can be examined in a single patch is limited.

Given the strengths and limitations of each approach, it is important to use several. But it is far from trivial to combine information from these fundamentally different measurements (macroscopic ionic and gating currents, single channel currents), usually measured under different conditions (as noted above for ionic and gating currents). Going from kinetic data to a model is not a stereotyped, mechanical procedure, but a complex creative enterprise with ample room for different approaches. It is most comforting in this context that the two labs arrive at the same conclusion about the general structure and connectivity of the kinetic scheme underlying channel gating.

Perhaps it is time for a reminder about the goals of kinetic modelling. One motivation is to operationally define the behavior of a channel, to quantitatively define its role in the electrical behavior of a cell. But a modeler interested in (for example) the role of BK channels in AP repolarization will find little of direct use in the papers discussed here. Clearly, their goal was different—to get at the molecular basis of channel gating and to relate formal kinetic diagrams such as Scheme IV to actual conformational states of the ion channel protein. That explains why the models discussed here are based, at least metaphorically, on what is known about channel structure (e.g., the number of subunits).

Open Questions

Cross sections of Scheme IV seem to work at extreme Ca^{2+} (high or low). It will be crucial to test whether Scheme IV also can describe the often complex Ca^{2+} dependence of the BK channel (e.g., Hill coefficients), and the interactions between Ca^{2+} and voltage.

The discussion so far has considered "the" BK channel. The Magleby lab studied native BK channels in rat skeletal muscle and the Aldrich lab studied cloned mouse BK channels (mSlo) expressed in *Xenopus* oocytes. Gating of the *Drosophila* dSlo channel differs from muscle BK channels (Moss et al., 1999). Moreover, physiological channel gating can be modulated by many factors, including splice variants, beta subunits, and phosphorylation. BK channels also exhibit subconductance states, which may be related to intermediate states in Scheme IV (see Changeux and Edelstein, 1998). All this will provide additional information for fine-tuning allosteric models for BK channel gating. For the time being, the models have proved useful as a framework for interpreting the effects of channel mutations (Horrigan et al., 1999).

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