

Potassium channels: Putting the parts together

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The structures of several different domains and subunits of potassium channels have recently been solved. Reassembling these fragments into a working model of an intact voltage-gated channel will be a major challenge.

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Potassium channels are central to the electrical activity of the nervous system. They are also widespread in many other cell types and among organisms, both prokaryotic and eukaryotic. Voltage-gated potassium channels, generally termed Kv channels, are complex membrane proteins that form a K⁺ ion-selective pore that opens and closes in response to changes in voltage across a cell membrane. Kv channels thus enable a transient efflux of K⁺ ions from a cell when it is depolarised. The exact time course of this efflux is a key determinant of the electrical properties of the cell, and can be modulated by biochemical events inside the cell. There is naturally considerable interest in understanding how Kv channels work, and such understanding will require knowledge of their three-dimensional structure in atomic detail. Structures have recently been determined for a number of discrete domains of Kv channel subunits, but these need to be put together to form a working model of an intact voltage-gated channel.

Structures of Kv components

The overall architecture (Figure 1) of a Kv channel was established in advance of structural data. The pore-forming part of the channel is a tetramer of α subunits. Each α subunit contains six transmembrane helices, S1 to S6. Helices S5 and S6, along with an intervening P loop, form the central K⁺-selective pore. Helices S1 to S4 connect the channel conformation to the voltage across the membrane bilayer: the voltage is sensed by the positively-charged S4 helix, which moves when the transbilayer electrostatic field is changed. At the amino terminus of the protein, upstream of the S1 helix, is a tetramerisation domain (T1), which controls the specificity of α subunit association. With some Kv channels, there is a further domain amino-terminal to T1; this is an inactivation domain, which is believed to interact with the intracellular mouth of the central pore, impeding the flow

of ions and thus inactivating the channel. Finally, Kv channels may be modulated by additional, non-membranous β subunits.

Structures have been determined for most of the components of a Kv channel (Figure 2a). The pore-forming domain is represented by the bacterial K⁺ channel KcsA, the structure of which was recently determined by X-ray crystallography [1]. This is a tetramer, each subunit contributing two transmembrane helices — M1 and M2, corresponding to S5 and S6 of a Kv channel α subunit — to an eight-helix bundle that surrounds a central pore, into which a K⁺-selective P loop tetramer is inserted to form the selectivity filter. A central pore domain of this kind is present in all K⁺ channels (and also in some K⁺ transporters). Rather less is known about the other transmembrane segments of the α subunit, though there is spectroscopic evidence that the S4 helix has an α -helical conformation [2].

This year has seen the publication of several structures for intracellular domains and subunits of Kv channels [3–5]. Three structures of the T1 domain have been solved, two for the Shaker channel, Kv1.1, and one for the Shaw channel, Kv3.1 [3]. The two T1 domains are of similar size and both forms tetramers with a central pore. Gulbis *et al.* [4] determined the structure of a Kv β 2 subunit, which also forms a tetramer with a central pore. The Kv β 1 subunit is thought to have the same core fold as Kv β 2, but with the addition of an amino-terminal inactivation domain (see below). Finally, the solution structures of various inactivation domains have been determined by NMR spectroscopy; in the case of Kv3.4, this domain forms a small, positively-charged, ball-like domain. It has further been shown that the conformation of the Kv3.4 inactivation domain can be modulated by phosphorylation [5].

Reassembling a Kv channel

Is it feasible to reassemble the structure of an intact Kv channel from the presently available data? Starting with the pore-forming domain, there is a high degree of sequence homology between Kv and KcsA in this region, implying that they have very similar architectures. The known KcsA structures may be used to construct homology models of the pore domain of a Kv channel. It is rather more difficult to pack the remaining transmembrane helices around the tetrameric S5–P–S6 core, and would be even if structures of all of the isolated S1 to S4 helices were available. It is possible that molecular modelling combined with distance constraints derived from NMR data might help with this problem.

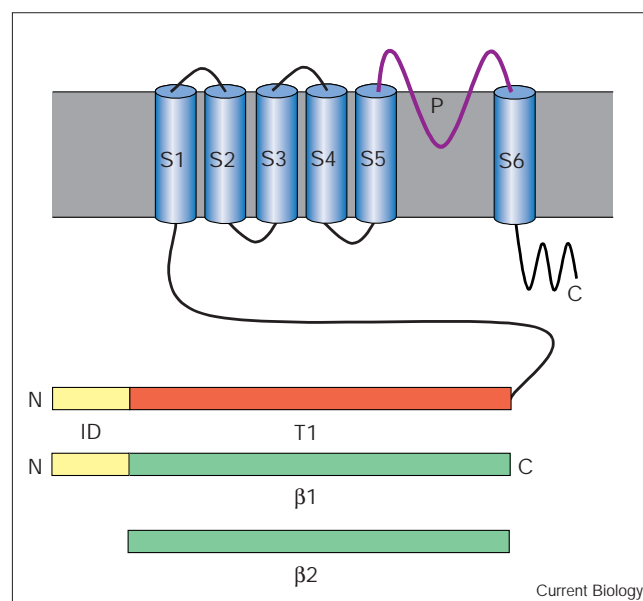
It is also difficult to integrate the T1 domain and Kv β subunit tetramers into an overall model of a Kv channel. There are only about 30 residues between the carboxyl terminus of the T1 domain and the predicted start of the first transmembrane helix, S1, which places the T1 tetramer close to the transmembrane domain of the channel protein. Furthermore, the β subunit tetramer interacts with the channel-forming α tetramer, possibly via interactions with the latter's T1 domain. If one makes the (reasonable) assumption that the three four-fold axes are coincident, then the pathway of the ion becomes a matter of some complexity (Figure 2b).

Both the T1 domain and $\beta 2$ subunit tetramers have central pores that are wide enough — minimum radii 1.4 Å and 2.6 Å, respectively, compared to 1.3 Å for a K^+ ion — to accommodate a K^+ ion, and that are lined with largely polar residues. Does a K^+ ion first have to pass through the pore-like regions in the centre of the β subunit and T1 domain tetramers before entering the transbilayer pore? It is conceivable that the K^+ ion does not pass through the centre of the T1 domain and β subunit tetramers, but rather enters through gaps between the upper surface of the T1 tetramer and the lower surface of the transmembrane domain. This would be similar to a model recently proposed for the path of cations through the nicotinic acetylcholine receptor on the basis of 5 Å cryoelectron microscopy images [6]. It should be possible to deduce the lowest energy pathway of the ion on the basis using computer simulations.

There is a further problem concerning the T1 domain and β subunit tetramers and their interaction with the transmembrane domain. Most of the evidence, at least for the T1 domain, suggests that the arrangement of the tetramer relative to the transmembrane domain is as shown in Figure 2a — that is, with the carboxyl terminus of the T1 domain uppermost. This places the T1 tetramer between the transmembrane domain (and hence the mouth of the transbilayer pore) and the inactivation domain. Various experiments, however, have suggested that the inactivation domain — whether from an α or a β subunit (see below) — interacts directly with the intracellular mouth of the pore. Clearly this is not possible if the T1 tetramer intervenes. Also, a homologue of the inactivation domain is found at the amino terminus of $\beta 1$ subunits; this β subunit inactivation domain also seems to interact with the mouth of the pore.

A further problem for the placement of the T1 domain and $\beta 2$ subunit tetramers is that some relatively large drug molecules, such as quaternary ammonium ions, block the open channel by entering the pore via its intracellular mouth. How do these blockers get past the T1 domain and β subunit tetramers? Both the quaternary ammonium ion and the inactivation domain(s) require either rather

Figure 1



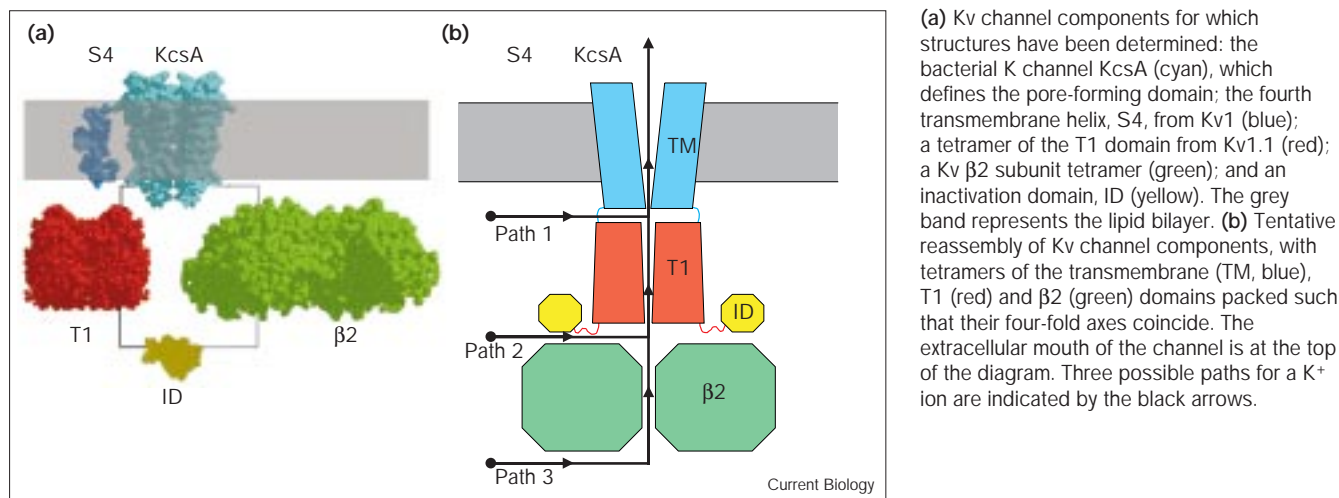
Topology of a Kv channel. The six transmembrane helices of a Kv α subunit, S1–S6, are shown in blue. The amino-terminal intracellular segment contains the inactivation domain (ID, yellow) and the tetramerisation domain (T1, red). The Kv $\beta 1$ subunit contains an inactivation domain and a TIM barrel domain (green), whereas the $\beta 2$ subunit contains only the latter domain.

large gaps in the interaction surface between the T1 tetramer and the transmembrane domain, or a substantial conformational change in the tetramer(s) when the channel opens so as to widen the central pore. Such a conformational change might be linked to a shift in the conformation of the pore-forming domains; indeed, MacKinnon and colleagues [4] discussed the possible coupling of conformational changes in the pore-forming region and the $\beta 2$ subunit tetramer of a Kv channel.

Dynamics and function

X-ray crystallographic methods have yielded static images of Kv channel components. There are indications, however, that, in order to understand Kv channel function, we also need to consider the dynamics of the channel protein. The fastest timescale we must consider is that of movement of a single ion through the pore, which takes about 10 nanoseconds. Molecular dynamics simulations can provide a picture of this process. Simulations of KcsA embedded in a slab of octane — a simple mimic of a lipid bilayer — have shown how single K^+ ions may leave the bulk water phase and enter the narrow selectivity filter at the extracellular mouth of the channel [7]. Our own simulations of KcsA embedded in a phospholipid bilayer have shown how multiple K^+ ions, together with intervening water molecules, may move through the selectivity filter in a concerted, single-file fashion [8].

Figure 2



Our work has also shown how small fluctuations in the pore radius at the intracellular mouth of the channel may be linked to entry or exit of K^+ ions [8]. This aspect has been explored experimentally by Perozo *et al.* [9], who used site-directed spin labelling to map out the conformational changes which occur when KcsA switches from a closed to an open state. In KcsA, this transition is driven by a drop in pH, whereas in Kv channels it would instead be coupled to voltage-driven conformational changes in the S4 region of the molecule. The spin-labelling results suggest that rigid-body motions of the transmembrane helices open up the pore at its intracellular mouth, thus facilitating entry or exit of K^+ ions.

How might comparable conformational changes in the transmembrane domain of a Kv channel be coupled to the tetrameric T1 domain and/or β subunit attached to its intracellular surface? A fascinating aspect of the newly solved $\beta 2$ structure [4] is that, not only is its fold that of a triose phosphate isomerase (TIM) barrel (Kv β subunits are similar in sequence to the aldo-keto reductases), but the crystal structure has revealed a bound NADP⁺ cofactor. Co-expression of the Kv $\beta 1.2$ subunit was found to confer redox sensitivity upon the Kv4.2 channel α subunit [10]. MacKinnon and colleagues [4] suggest that a change in the redox state of the bound cofactor might cause a conformational change in the β subunits, which is then coupled to the conformational change associated with channel gating—the open–closed transition—of the pore-forming α subunits. This would add a further dynamic element to the behaviour of Kv channels.

The inactivation domain may also undergo conformational transitions in response to intracellular events. An elegant combination of functional and structural studies [5] has revealed that the conformation and function of a synthetic

Kv3.4 inactivation domain might be modulated by phosphorylation. Specifically, phosphorylation leads to a loss of structure—a partial unfolding—of the inactivation domain, which is associated with a reduction in its ability to inactivate the open channel. Interestingly, biochemical studies indicate that phosphorylation may have a similar effect on inactivation domain of the Kv $\beta 1.3$ subunit [11].

Where does this leave us? Structural biology can provide a set of static snapshots of different fragments of Kv channels. Two major challenges remain. The first is to reassemble these fragments, possibly by molecular modelling, so as to visualise a complete Kv channel. This enterprise might, of course, be pre-empted by an X-ray structure of the complete channel. Secondly, given a complete structure, a subtle combination of computer simulation and experimental studies will be needed to fully describe the dynamics of channel function. The latter studies will have to address timescales ranging from about ten nanoseconds for ion permeation, through about one millisecond for channel gating, to about a second for channel modulation by intracellular redox and/or phosphorylation reactions.

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