

Ion channels: A first view of K⁺ channels in atomic glory

Mark S.P. Sansom

Crystal structures have been solved for the transbilayer pore domain of a bacterial K⁺ channel and the tetramerisation domain of a voltage-gated K⁺ channel. These provide our first real structural insights into possible mechanisms of ion selectivity and permeation for K⁺ channels.

Address: Laboratory of Molecular Biophysics, The University of Oxford, The Rex Richards Building, South Parks Road, Oxford, OX1 3QU, UK. E-mail: mark@biop.ox.ac.uk

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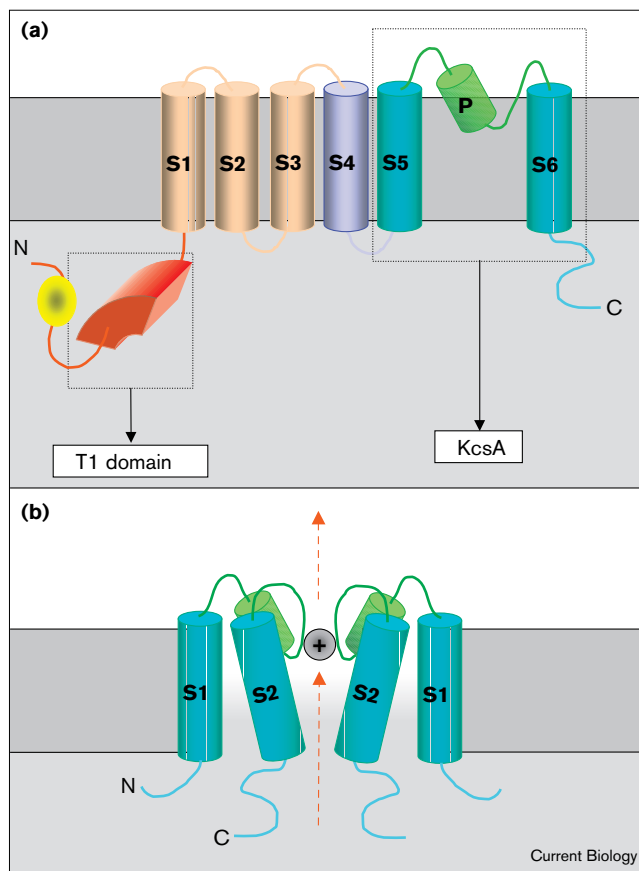
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Channels are integral membrane proteins which allow inorganic ions — such as K⁺, Na⁺, Ca²⁺ or Cl[−] ions — to cross membranes. K⁺ channels constitute a functionally diverse family, with the common feature of a high selectivity for K⁺ over other ions [1]. Their biological roles range from conducting the current that drives the repolarising phase of action potentials in neurons to the control of stomatal opening in plants. These diverse functions coupled to a common transport activity are believed to reflect structural variations upon a common core structure for the channel's transbilayer pore and selectivity filter.

Many studies using a variety of techniques — molecular biology, mutagenesis and patch-clamp recording — have provided us with a detailed topology for K⁺ channels (Figure 1). There are two main families of K⁺ channels, those with six transmembrane helices — S1 to S6 — per subunit, and those with just two transmembrane helices — S1 and S2, the equivalents of S5 and S6, respectively, of a six-transmembrane channel — per subunit. Four subunits come together to surround a central pore. The six-transmembrane family includes the classical voltage-gated K⁺ (Kv) channels. The two-transmembrane family includes inward-rectifier (KIR) channels, and also some bacterial K⁺ channels, such as KcsA of *Streptomyces lividans*.

A number of elegant mutagenesis studies have assigned functions to different regions of the Kv channel topology. Reading from amino terminus to carboxyl terminus, these regions are: a basic inactivation 'ball' at the amino terminus, which enables fast, voltage-dependent inactivation of Kv channels; a tetramerisation (T1) domain, also part of the intracellular amino-terminal polypeptide chain, which controls the specificity of tetramerisation of Kv subunits; three transmembrane helices, S1 to S3, the functional roles of which remain unclear; a helix, S4, which acts as a sensor for changes in transmembrane voltage leading to channel

Figure 1



(a) A Kv channel subunit, showing the inactivation 'ball' (yellow), tetramerisation (T1) domain (red), the six transmembrane helices (S1 to S6; bronze, purple and cyan) and the P loop (green). The structures of the two domains shown boxed have recently been solved by X-ray crystallography [4,5]. **(b)** The KcsA pore is formed by four copies of the P loop (green) and its adjacent transmembrane helices, S1 and S2 (cyan). For clarity, only two subunits are shown.

opening; and a pore-forming domain, made up of the S5 and S6 helices and the so-called P loop. Of these domains, only the one that forms the pore has an equivalent in the two-transmembrane channels. Despite several molecular modelling studies ([2,3], for example), it has proved difficult to arrive at a single candidate structure for the pore-forming domain of a K⁺ channel. What was clearly needed was a crystal structure, at least of the central pore domain. Now crystal structures of two different components of the central pore have been solved almost simultaneously [4,5].

Why are these structures a major achievement? Structures of proteins are now solved almost every day by X-ray

diffraction or nuclear magnetic resonance (NMR) techniques. But although genes for membrane proteins take up about 25% of most genomes [6], high resolution structures are known for only a dozen or so such proteins. This reflects the difficulty of expressing and crystallizing membrane proteins. Two approaches have been taken to overcoming these problems. The first involves dissecting a membrane protein into its component domains and then solving the structures of these one by one, as has been applied to those membrane proteins, such as growth factor receptors, where most of the polypeptide chain lies outside the membrane. This approach has been applied to the extramembrane tetramerisation domain, T1, of Kv channels. The second approach involves finding a simpler and more robust version of the protein. This has been possible for the pore-forming domain of the somewhat obscure bacterial K⁺ channel KcsA, and has revealed what are likely to be the main features of the pore architecture of most K⁺ channels in higher organisms.

KcsA pore structure

KcsA is a simple K⁺ channel of the two-transmembrane helix class [7]. It forms relatively high conductance K⁺ selective channels, which can be activated by lowered pH [8]. Its P-region sequence is highly similar (~60%) to that of Shaker, the archetypal Kv channel from *Drosophila*. In particular, it contains the tripeptide motif Gly–Tyr–Gly, a fingerprint for the selectivity filter of a K⁺ channel. But with a subunit size of 160 amino acids, compared to 616 amino acids for Shaker channels, the KcsA channel is more amenable to structural studies. High-level expression of KcsA in *Escherichia coli* enabled synthesis of large quantities of protein for X-ray studies.

The overall architecture of KcsA is summarised in Figure 1b. The helix bundle, made up of four S1 and four S2 helices, has been likened to an inverted cone. The selectivity filter is held in the wider end of the eight-helix

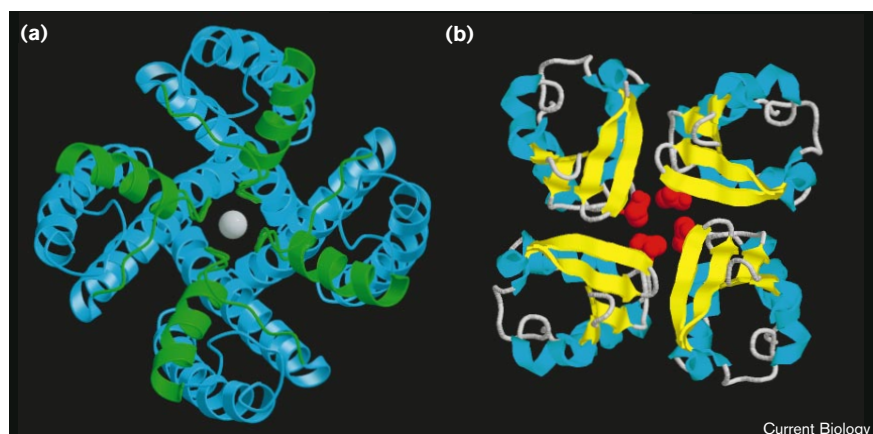
bundle close to the extracellular mouth of the pore. The S1 helices form the outer layer of the cone, with the S2 helices packed inside enabling them to line the intracellular segment of the pore. The P-region nestles within the extracellular mouth of the cone formed by the S2 helices. The first (descending) half of the P-region is a short helix, whereas the second (ascending) half adopts an extended structure, such that the backbone carbonyls of the Gly–Tyr–Gly motif form the narrowest part of the pore — the selectivity filter. The S1 helices thus interact with the surrounding lipids and position the S2 helices, which form the transbilayer pore *per se* and into which the P-region is inserted to give a selectivity filter close to the extracellular mouth of the channel.

Two key features emerge from the structure that concern the nature of the pore. The intracellular half of the pore is wider than the extracellular half, and opens to a 10 Å diameter cavity in the middle of the membrane. This cavity is lined by hydrophobic sidechains and filled with water molecules. The crystal structure suggests that a K⁺ ion might be accommodated within this cavity. Here the ion waits to enter the narrow selectivity filter, and while waiting it is electrostatically stabilised by the dipoles of the short P helices. The pore through the selectivity filter is narrow, just wide enough to accommodate a K⁺ ion (see Figure 2a).

The crystal structure provides evidence for two K⁺ sites close together within the selectivity filter. The structure also begins to explain the basis of K⁺ ion selectivity. A K⁺ ion fits exactly into the selectivity filter, the carbonyl oxygens of the Gly–Tyr–Gly motif interacting with the ion and substituting for displaced water of solvation. In contrast, a Na⁺ ion in this region might be expected to make poorer contacts with the carbonyl oxygens, and so would be less favourably compensated for the loss of its bound water molecules. This mechanism requires a relatively rigid structure for the selectivity filter.

Figure 2

(a) The KcsA structure, looking down the pore axis from outside the cell towards the inside. The S1 and S2 helices are in cyan, and the P helix and P loop in green. The central K⁺ ion is in grey. (Graphic courtesy Rod MacKinnon.) **(b)** The T1 tetramer, viewed along the pore axis from inside the cell towards the outside, the opposite direction from in (a); α -helices are in cyan and β -sheets in yellow. The asparagine 136 sidechains (red) are shown in 'stick' format. (Coordinates courtesy of Senyon Choe.)



The KcsA structure provides a first view of the physical basis of K⁺ channel selectivity. To what extent is it relevant to other K⁺ channels? The P-region of KcsA is very similar in sequence to the equivalent regions of the Kv channels, which argues for a common molecular architecture. Furthermore, studies comparing the binding to KcsA and Kv channels of the scorpion toxin agitoxin 2, which is known to bind in the extracellular mouth of the pore, provide a very strong argument in favour of the view that the two different classes of K⁺ channel have similar pore architectures [9].

Structure of the Shaker channel T1 domain

At about the same time that the KcsA pore structure was solved, the crystal structure of the T1 domain of the Shaker Kv channel was determined at high resolution. This is a water-soluble domain, which confers specificity on tetramer formation. This is of functional importance, as heteromeric assembly of Kv subunits is one of a number of mechanisms for generating K⁺ channel diversity. It was anticipated that the T1 structure would have an impact on our understanding of the process of channel protein assembly. More surprisingly, the T1 structure raises interesting questions about the permeation pathway of K⁺ ions through the more complex Kv channels.

The structure of the T1 domain is illustrated in Figure 2b. The structure corresponds to residues 66–152 of the Shaker polypeptide. For comparison, the inactivation ‘ball and chain’ consists of the first 45 or so residues of the protein, and the S1 helix starts about residue 196 (see Figure 1a). The four T1 domains pack around a central pore, coincident with the four-fold rotation axis. The amino and carboxyl termini of the polypeptide chain are on opposite faces of the tetramer, suggesting that the amino-terminal face points towards the cytoplasm, whereas the carboxy-terminal face has a relatively flat surface and may pack against the transmembrane domains of the intact protein.

The central pore of the T1 tetramer is particularly intriguing. It has a minimum radius of approximately 1.6 Å, corresponding to a ring of asparagine 136 sidechains. This is wider than the radius of a dehydrated K⁺ ion (1.33 Å), but sufficiently narrow to require desolvation of the ion. The asparagine 136 ring might thus be expected to present an energetic barrier to ion permeation, especially as it is the Cβ atom of the sidechain, rather than its terminal amide group, that forms the narrowest part of the pore.

If the T1 tetramer is indeed stacked against the transmembrane region of the protein, and assuming that the pore structure seen in the isolated domain is the same in the intact protein, then a K⁺ ion passing through a Kv channel from inside to outside the cell will first have to pass through the T1 ‘pore’, before entering the transbilayer

pore formed by a KcsA-like domain. This suggests that interactions with T1 domains might influence the energetics of permeation. This should be testable by substituting residues lining the proposed T1 pore. The alternative possibility is that T1 does not pack closely against the transmembrane helices, and that the K⁺ ions enter ‘sideways’ between the carboxyl terminus of T1 and the intracellular mouth of the channel *per se*.

And the future...

X-ray crystallography has provided us with the structure of two domains from K⁺ channels. We are beginning to understand the mechanisms of permeation and selectivity. But what of gating? The big challenge is to reveal the structural basis of voltage gating. This will probably require a crystal structure of an intact Kv channel. But let us strike a note of caution amidst the euphoria. In many ways, the X-ray structure of a channel is the beginning of the story rather than its end. After all, ions — and channels — move, and to understand channel physiology fully at atomic resolution will need simulation studies [10] to provide dynamic images of the events during ion permeation and gating.

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