The Crystal Structure of a Potassium Channel—A New Era in the Chemistry of Biological Signaling

Dennis A. Dougherty* and Henry A. Lester

Some of the most impressive chemistry of living systems is performed by the integral membrane proteins of the nervous system—ion channels, neuroreceptors, and neurotransmitter transporters. These are the molecules of thought, memory, and sensory perception, and in one sense, we know a great deal about them. For example, the exquisite sensitivity of the patch-clamp technique enables the characterization of an ion channel if it opens for only 0.1 ms every 20 min—a dynamic range of 10^7 s. Furthermore, site-directed mutagenesis has been applied in hundreds of studies showing interesting functional changes that point to the roles of individual side chains in ion channels. Yet, from a chemist's perspective, we know little about the structural details that underlie ion channel function. The reason for this ignorance is simple: High-resolution structural tools such as X-ray crystallography and NMR spectroscopy are at best marginally applicable to integral membrane proteins. In this light, the recent report[1] of a 3.2 Å resolution crystal structure of a K^+ channel from Rod MacKinnon and a group from the Rockefeller University represents a landmark achievement, and perhaps a turning point for molecular neurobiology.

Potassium channels play a central role in the function of nearly every living cell. In the nervous system they regulate the action potential and set the resting membrane potential, thereby governing the frequency of nerve impulses. In the cardiovascular system, K^+ channels regulate the duration and frequency of the heartbeat. Elsewhere, they control the passage of water and salts across the membranes of the kidney, windpipe, and intestine. They also accomplish a remarkable feat of chemistry. The K^+ channels are highly selective—perhaps as much as 10000:1 for K^+ over Na^+, despite the minimal structural difference between the two (ionic radii of 1.33 and 0.95 Å, respectively). At the same time, K^+ channels promote tremendous ion flux; in some cases on the order of 10^8 ions per second pass through a single open channel. Chemists typically equate high selectivity with tight binding, but clearly nature has found a way to achieve the former without the latter.

In little more than a decade, we have come from almost complete ignorance about the molecular nature of K^+ channels to a high-resolution structure. The first breakthrough was the cloning by three labs of the Shaker channel (named after a *Drosophila* mutant that shakes its legs on exposure to ether).[2] This was followed by a flurry of activity to define crucial structural determinants, rendering 1990 the "annus mirabilis of K^+ channels".[3] The next crucial step was the discovery[4] that K^+ channels fall into two distinct families: the voltage-gated channels, K_v (which includes the Shaker channel), and the inward rectifier channel, K_{ir} (Figure 1). As the names imply, the first family "gates" (that is, opens or closes) in response to changes in the cell's membrane potential, while the second allows K^+ flow only in the inward

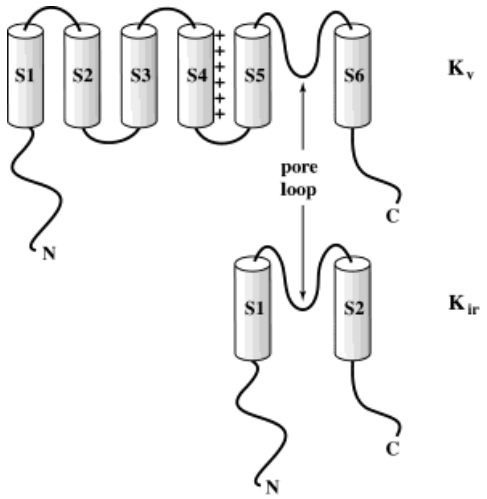

Figure 1. Predicted topologies of the two families of K^+ channels, based on sequence analysis and biochemical studies. The voltage-gated (K_v) channels such as Shaker have six transmembrane domains, while the inward-rectifier (K_{ir}) channels have only two. For both families, the N and C termini are intracellular. Both families feature a pore (P) loop—a conserved sequence that was proposed to line much of the channel and to contain the selectivity filter. The KcsA channel discussed here has the topology of a K_{ir} channel.

[*] Prof. D. A. Dougherty
Division of Chemistry and Chemical Engineering
California Institute of Technology
Pasadena, CA 91125 (USA)
Fax: (+1) 626-564-9297
E-mail: dad@igor.caltech.edu

Prof. H. A. Lester
Division of Biology
California Institute of Technology
Pasadena, CA 91125 (USA)

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direction, against the natural concentration gradient—another remarkable bit of chemistry. Structurally, the K_{ir} channels are smaller, consisting of two transmembrane domains with an intervening pore (P) region. The K_v channels have an additional four transmembrane domains, including one (S4) that has a positive charge every third residue and plays the role of the voltage sensor. In each case the functional channel is a tetramer of the protein shown in Figure 1.

The path to a high-resolution structure began with another advance in cloning. In 1995 Schempf et al. reported[5] the identification, cloning, and characterization of a K^+ channel from the bacterium *Streptomyces lividans*, a result that was made possible by the intense activity on the sequencing of small genomes. Although bacteria are not neurons, all workers recognized that this bacterial channel resembled the K^+ channels of eukaryotic cells closely enough to share key structural elements. Furthermore, this bacterial channel was quite robust, easily reconstituting in artificial membranes. The fact that it is a bacterial channel suggested a solution to one of the biggest hurdles to determining a structure: obtaining large quantities of material. Indeed, the *S. lividans* K^+ channel, now known as KcsA, could be overexpressed in *E. coli*. The race was on to obtain a structure!

Even with adequate quantities of protein, obtaining the structure was a challenge. The crystals are radiation-sensitive, they diffract anisotropically, and a number of site-directed mutants were necessary to obtain heavy-atom derivatives. In the end, by use of the Cornell High Energy Synchrotron Source, a viable structure was obtained. It should be appreciated from the start that 3.2 Å is not high resolution by today's standards of macromolecular crystallography. A backbone trace is clearly visible, but individual carbonyl groups, for example, cannot be seen. Side chains can be seen, but with minimal detail. Still, much can be learned at this level.

Topologically, KcsA falls into the K_{ir} family, with two transmembrane domains. The structure reveals that these domains are highly α-helical in appearance (Figure 2). The eight helices (two from each subunit) wrap around the central pore, and tilt and kink a bit to open up like a flower or an inverted teepee toward the extracellular surface of the channel. With so few high-resolution structures of integral membrane proteins available,[6] every new structure greatly expands our knowledge of the manners in which proteins assemble and pack within a membrane. Transmembrane helices are becoming common, although sheet structures are also known. Like other membrane proteins, KcsA shows two belts of aromatic amino acids near the membrane–water interface. Gratifyingly, the structure is consistent with a number of inferences from biochemical and mutagenesis studies. For example, residues that influence channel blockade by internally applied reagents face the inside of the cell; those that influence external blockade face the outside and include a number of well-characterized sites in the "turret" region that interact with toxins such as agitoxin 2 or charybdotoxin. Still, the critical issue is the pore: how is ion selectivity achieved?

The answer is clear from the structure. In the P loop of all K^+ channels there is a highly conserved "signature sequence"

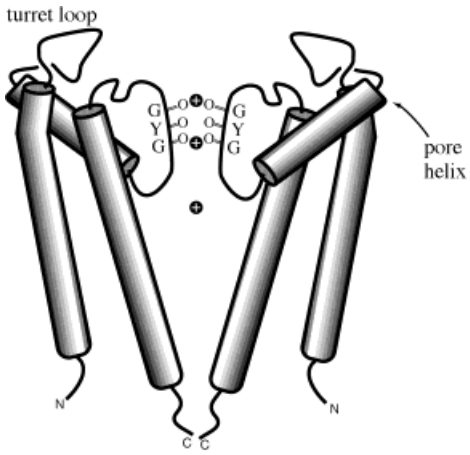

Figure 2. Schematic representation of the K^+ channel structure. Two of four subunits are shown, the other two lie above and below the plane of the page. Beginning at the intracellular N terminus, each subunit contains: an α helix, with a slight kink at the top; the "turret" loop, which contains binding sites for toxins; the pore helix; the Gly-Tyr-Gly (GYG) loop; and a large C-terminal helix. Also shown are three positive ions that correspond roughly to positions in the structure where ions were located. Two lie in the GYG selectivity filter, while the bottom ion lies in the aqueous "pool" that lies near the middle of the transmembrane region. Note that a number of intracellular residues (22 on the N terminus and 39 on the C terminus) are not seen, either because of disorder or, in the case of 32 C terminus residues, because they had been removed by proteolysis. This picture is adapted from figures presented by Doyle et al.[1]

Thr-Xxx-Thr-Thr-Xxx-Gly-Tyr-Gly.[7] This region, and especially the Gly-Tyr-Gly sequence, plays a key role in ion selectivity. Two models for ion selectivity had been proposed. One, first proposed by MacKinnon[8] and supported by work from one of us,[9] invokes cation–π interactions between the permeant ions and the conserved aromatic ring of the Tyr residue of the Gly-Tyr-Gly unit. The other, more traditional mechanism invokes carbonyl oxygen atoms from the peptide backbone as forming the selectivity filter.[7] In K^+ channels, tradition has won out.

The P region forms a loop involving first a small helix, the "pore helix", and then a turn that contains the crucial Gly-Tyr-Gly sequence (Figure 2). It is clear that the Tyr side chain points back away from the channel, and is unable to contribute any cation–π interactions to ion binding. Although individual atoms of the backbone cannot be resolved, the authors make the reasonable assumption that the carbonyl oxygen atoms point in toward the pore. Apparently, three consecutive carbonyl groups from each subunit point inward, establishing rings of oxygen atoms that define cation binding sites. The analogy to crown ethers is immediately apparent, with the caveat that these are amide carbonyl groups, which contain a more potent negative electrostatic potential than ether oxygen atoms. Soaking the crystals with Rb^+ ions (whose permeation through most K^+ channels can be measured) and evaluating electron density differences allows explicit ions in the P region to be identified.

How does such an arrangement produce a selective pore? The authors propose that the rest of the protein structure maintains the Gly-Tyr-Gly carbonyl groups in precisely the correct position for binding K^+, but prevents the structure from adjusting to allow favorable interactions with Na^+—an

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impressive feat for a tetrameric protein embedded in a membrane. The highly conserved Tyr residue plays a key role in defining the molecular scaffolding. It hydrogen bonds to a Trp in the pore helix and experiences a favorable van der-Waals interaction (perhaps an edge-to-face interaction between two aromatic rings?) with another Trp in the pore helix of an adjacent subunit. When propagated by the fourfold symmetry of the structure, this arrangement produces a "massive sheet of aromatic amino acids" that produces a "layer of springs" to hold the carbonyl oxygen atoms in precisely the correct position. These crucial Trp residues are conserved in all members of the K_v family. An interesting aspect of KcsA is that, while it has only two transmembrane domains like a K_{ir} channel (Figure 1), its P-region sequence much more closely resembles a K_v sequence, so the structure in the pore region is presumably more relevant to this family of channels. It should be remembered that, at 3.2 Å resolution, a model for precise positioning must be considered speculative. Nevertheless, this is the first attempt to put chemical-scale flesh on the channel's skeleton, and it will certainly inspire many experiments.

Interestingly, the narrowest part of the channel, the selectivity filter, is toward the extracellular face of the channel. Many had anticipated an hourglass shape for the pore, with the narrowest portion in the middle of the channel. A K^+ ion in the very middle of the channel, the most hydrophobic part of the membrane, experiences a very low dielectric constant, an uncomfortable situation for an ion. Again, nature has found a clever solution to this problem. Right below the selectivity filter the channel opens up, creating a cavity with quite hydrophobic walls that holds a "pool" of water. An ion in this region (Figure 2) is solvated by all the water in the cavity. In addition, the four pore helices point right at this pool. Each helix is aligned such that its C terminus, which has a substantial negative electrostatic potential ("the helix dipole"), points toward the aqueous pool. This alignment is proposed to provide extra stabilization to an ion located in the pool.

We have a selective binding site, but how do we achieve high flux? As mentioned above, the selectivity filter contains more than one ion. This supports the generally held view, based on decades of measurements with ionic currents and with radiolabeled tracer fluxes, that K^+ channels are multi-ion pores.[10] The model for flux is that one ion binds, followed by a second at an adjacent site. The binding of the second ion destabilizes the first electrostatically—like charges repel! This pushes the first ion out of the selectivity filter, the second ion takes it place, and a new ion moves in. The entire process is driven by the high concentration of K^+ in physiological solutions. The presence of several ions in the channel simultaneously in the crystal structure nicely confirms a crucial conclusion derived from functional studies.

So what is next for K^+ channels? Certainly, a higher resolution structure would be reassuring and informative, and it seems likely that one will appear soon. It will also be interesting and important to evaluate the extent to which the motifs of the KcsA structure carry over to other ion channels, including both the K_v family and more distant relatives such as Na^+, Ca^{2+}, and ligand-gated ion channels. A very recent crystal structure from Choe and co-workers[11] should provide further information on the K_v family. These workers determined a 1.55 Å resolution structure of a *soluble fragment* of the Shaker channel known as the tetramerization (T1) domain. This region lies on the N-terminal end of the protein within the intracellular stretch that precedes S1 (Figure 1). An 87 amino acid segment of the protein was studied, and remarkably, in the crystal, it formed a perfectly fourfold symmetric, tetrameric structure with a large pore down the middle! This broad pore is presumably the intracellular vestibule of the channel. No doubt modelers will be racing to merge the tetramerization domain with the KcsA structure, in hopes that the likely positions of S1–S4 will become apparent.

Another interesting issue that will require further attention is the mechanism by which the crucial positioning of the carbonyl groups in the selectivity filter is achieved. As mentioned, the key Trp residues are conserved in all of the K_v channels (remember the pore sequence of KscA is K_v-like). However, these Trps are universally replaced by Leu and Phe in K_{ir} channels. This requires different contacts with the Tyr of the Gly-Tyr-Gly, especially since the proposed Trp···Tyr hydrogen bond is no longer possible. Apparently, nature has found two ways to attain the requisite positioning of the Gly-Tyr-Gly carbonyl groups. Finally, there are the dynamic aspects of channel function, especially the gating phenomena induced by voltage or ligand binding, issues that will be difficult to address with static crystallography. Nevertheless, it is clear that MacKinnon's accomplishment represents a giant leap for the ion channel field, one that will influence all further work to come.[12]

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