FEBS Letters 555 (2003) 62–65 FEBS 27738

Minireview

Potassium channels

Roderick MacKinnon*

Howard Hughes Medical Institute, Laboratory of Molecular Neurobiology and Biophysics, Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

Received 25 August 2003; accepted 1 September 2003

First published online 7 October 2003

Edited by Gunnar von Heijne, Jan Rydström and Peter Brzezinski

Abstract The atomic structures of K^+ channels have added a new dimension to our understanding of K^+ channel function. I will briefly review how structures have influenced our views on ion conduction, gating of the pore, and voltage sensing.

Key words: Potassium channels; K^+ channels; Ion selectivity; Gating; Voltage dependent ion channels

1. Introduction

Potassium channels conduct K^+ ions across the cell membrane, down the electrochemical gradient for K^+. Potassium conduction underlies many different cellular processes including cell volume regulation, hormone secretion, and electrical impulse formation in electrically excitable cells [1].

All known K^+ channels are related members of a single protein family. They are found in bacterial, archeal, and eukaryotic cells – both plant and animal – and their amino acid sequences are very easy to recognize because potassium channels contain a highly conserved segment called the K^+ channel signature sequence [2]. This sequence forms a structural element known as the selectivity filter, which prevents the passage of Na^+ ions but allows K^+ ions to conduct across the membrane at rates approaching the diffusion limit. This is the hallmark of K^+ channels: nearly perfect selectivity for K^+ ions over Na^+ ions in the setting of very high K^+ conduction rates.

Diversity among different members of the K^+ channel family is related mainly to the various ways in which K^+ channels are gated open. Some K^+ channels are ligand gated, which means that pore opening is energetically coupled to the binding of an ion [3], a small organic molecule, or even in some cases a protein [4]. Other K^+ channels are voltage gated, in which case pore opening is energetically coupled to the movement of a charged voltage sensor within the membrane electric field [5,6]. Therefore, the different kinds of K^+ channels open in response to different stimuli: a change in the intracellular Ca^{2+} concentration, the level of certain G-protein subunits in the cell, or the value of the membrane voltage. Underneath this diversity in gating function, K^+ channels have diverse structural domains attached in a modular fashion to the conserved pore unit. Ligand-gated K^+ channels typically have cytoplasmic or extracellular domains for binding ligands. Voltage-gated K^+ channels have integral membrane domains for sensing voltage differences.

Over the past five years X-ray crystallographic studies have revealed the first atomic structures of several different K^+ channels. These structures have answered many questions about basic mechanisms of K^+ channel function, and at the same time they have stimulated new questions to be answered in the future. I will briefly describe three aspects of K^+ channels where the structures have shed new light and stimulated new ideas. These aspects concern selective ion conduction, gating, and voltage sensing.

2. Selective ion conduction

The fundamental catalytic role of all K^+ channels is to conduct K^+ ions across the cell membrane. The atomic radius of K^+ is 1.33 Å and that of Na^+ is 0.95 Å. With only this difference in ionic radius to work with, K^+ channels manage to select for the K^+ ion over the Na^+ ion by a factor of more than 1000. Moreover, this strong selectivity for K^+ is achieved without compromising the rates of conduction, which approach the diffusion limit. Atomic structures have given us a deep understanding of K^+ selectivity and the ion conduction process.

The K^+ channel pore is comprised of four usually identical subunits that encircle with four-fold symmetry a central ion conduction pathway [7,8]. Two of the four subunits are shown for the KcsA K^+ channel in Fig. 1a. Each subunit contains two fully transmembrane α-helices termed inner (nearest the ion pathway) and outer (nearest the membrane) helices, and a tilted pore helix (red, Fig. 1a) that runs half way through the membrane, pointing its C-terminal negative end-charge toward the ion pathway. Near the midpoint of the membrane the ion pathway is nearly 10 Å in diameter, forming a central water-filled cavity. A hydrated K^+ ion remains suspended at the center of the cavity in the crystal structure [8]. The presence of a water-filled cavity and oriented α-helices helps to explain a very important property of the K^+ channel: in order to catalyze high conduction rates the channel must overcome the electrostatic repulsion that a K^+ ion would normally experience when moving from bulk water into the

*Fax: (1)-212-327 7289.
E-mail address: mackinn@mail.rockefeller.edu (R. MacKinnon).

0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)01104-9

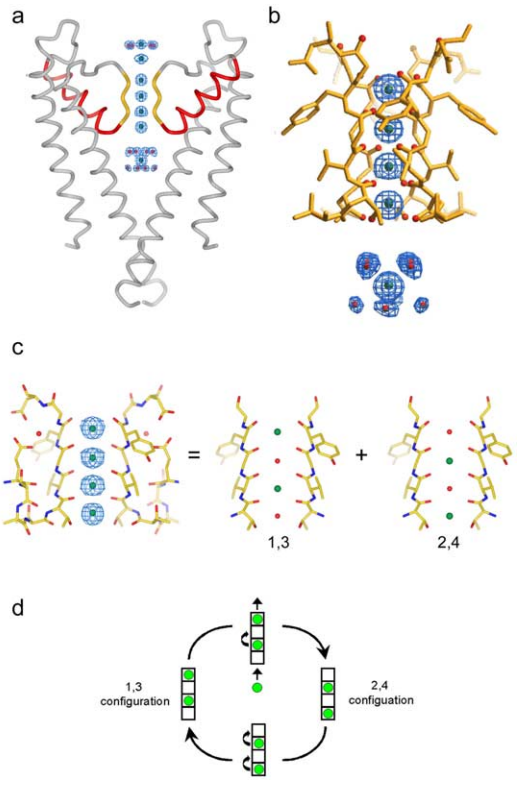

Fig. 1. The ion conduction pore of K^+ channels. a: Two of the four subunits from the KcsA pore are shown with the extracellular side on top [8]. Each subunit contains an outer helix close to the membrane, an inner helix close to the pore, a pore helix (red) and a selectivity filter (gold). Blue mesh shows electron density for K^+ ions and water along the pore. b: Close-up view of the selectivity filter with dehydrated K^+ ions at positions 1 through 4 (external to internal) inside the filter and a hydrated K^+ ion in the central cavity below the filter. c: Electron density in the filter corresponds to two configurations of K^+ ions (green) alternating with water molecules (red): 1,3 contains K^+ at positions 1 and 3, 2,4 contains K^+ at positions 2 and 4. d: Through-put cycle for K^+ conduction invoking 1,3 and 2,4 configurations [10].

low dielectric membrane environment. By allowing the K^+ ion to remain hydrated at the membrane center, and by directing the C-terminal negative end-charge of α-helices toward the ion pathway, a K^+ ion is stabilized at the membrane center [9].

Potassium selectivity occurs in the selectivity filter, located in the extracellular third of the ion pathway in between the central cavity and the extracellular solution (Fig. 1a, gold). The K^+ channel signature sequence amino acids, conserved in K^+ channels throughout the tree of life, form the selectivity filter. Here, conducting K^+ ions encounter four evenly spaced layers of carbonyl oxygen atoms and a single layer of threonine hydroxyl oxygen atoms, which create four K^+ ion binding sites numbered 1 to 4 from the extracellular to the intracellular side (Fig. 1b). At these sites K^+ ions bind in an essentially dehydrated state, surrounded by eight oxygen atoms from the protein, four 'above' and four 'below' each ion in Fig. 1b. The arrangement of protein oxygen atoms surrounding each binding site in the selectivity filter is very similar to the arrangement of water molecules around the hydrated K^+ ion observed in the central cavity. Thus, the crystal structure provides an explicit and beautiful demonstration of the selectivity filter's role, to create a queue of K^+ binding sites that mimic the waters of hydration surrounding a K^+ ion. Potassium ions are therefore able to diffuse from water into the selectivity filter where the energetic cost of dehydration is compensated. Sodium ions, on the other hand, do not seem to enter the selectivity filter in crystal structures even when Na^+ is present in vast excess [8,11]. In fact, reduction of K^+ to 3 mM in the presence of 150 mM Na^+ causes the selectivity filter to undergo a conformational change to a 'collapsed' state.

High selectivity and high conduction rates present an apparent paradox: why does the precise ion coordination required for high selectivity not cause the ions to bind too tightly and prevent their rapid diffusion through the pore? We understand two reasons for high conduction rates in the setting of high selectivity. First, the selectivity filter contains more than one ion in it so that repulsion between closely spaced ions will help to overcome the intrinsic affinity that each ion has for its binding site [8,10,11]. Across the four positions of the selectivity filter there are, on average, two K^+ ions present at a given time and they reside predominantly in two configurations shown in Fig. 1c. In each configuration, 1,3 and 2,4, two K^+ ions are separated by one water molecule; the crystal structure is an average of these configurations. The deduction of two specific ion configurations is based on comparing the distribution of K^+ versus Rb^+ ions in the selectivity filter [10], specific features of the electron density at the selectivity filter entry way [8], determination of the absolute occupancy for ions inside the selectivity filter using Tl^+ [11] and the effects of mutations on the distribution of ions (unpublished). The 1,3 and 2,4 configurations form the endpoints of a simple conduction cycle shown in Fig. 1d. The cycle corresponds to an ion crossing the filter: an ion enters the queue from one side of the filter while a different ion exits from the opposite side. The direction of flow is of course determined by the electrochemical gradient for K^+. This picture, based on crystallographic studies, is in good agreement with the experiments of Hodgkin and Keynes, which led them to propose in 1955 that the K^+ conduction mechanism involves the single file movement of two to three ions across the membrane [12].

The second reason for high conduction rates in the setting of high selectivity is that the selectivity filter's structure depends on the presence of K^+ ions [8]. Specifically, when in a crystallographic experiment the K^+ concentration is lowered far below normal intracellular levels, the filter's occupancy drops from two to one K^+ ions and a very specific conformational change occurs [11]. The conductive conformation of the filter shown in Fig. 1 requires the presence of two K^+ ions, and it is the entry of the second K^+ ion that induces the conformational change. This observation has a simple thermodynamic consequence: some fraction of the ion binding energy is used to change the filter's structure, and as a result the ions bind less tightly than if a conformational change did not occur. Weak binding is a prerequisite for high conduction rates.

In summary, K^+ channels conduct K^+ ions specifically because the selectivity filter contains multiple binding sites that mimic a hydrated K^+ ion's hydration shell. Potassium channels achieve high conduction rates by exploiting electrostatic repulsion between closely spaced ions and by coupling the conformation of the selectivity filter to ion binding within the filter.

3. Gating

What are the conformational changes that underlie pore opening in K^+ channels? Structural comparison of KcsA with a second K^+ channel, MthK, begins to answer this question [13]. KcsA was crystallized under conditions that favor its closed conformation in membranes [7,8,14]. MthK, a Ca^{2+}-gated K^+ channel, was crystallized in the presence of Ca^{2+} concentrations that open its pore in membranes [15]. In the crystal structure, calcium is bound to RCK domains located at the intracellular membrane surface. These domains are responsible for opening the pore when Ca^{2+} ions bind. Here I will focus only on the transmembrane pore component of the MthK K^+ channel.

The pores of KcsA and MthK are shown in Fig. 2. Aside from the obvious difference in the length of the extracellular 'turret' loops connecting the outer and pore helices, the major structural difference between these two channels is the position of their inner helices on the intracellular side of the selectivity filter. In KcsA the inner helices are straight and form a helix bundle near the intracellular membrane surface. At the bundle crossing in KcsA the pore narrows to about 3.5 Å in diameter and is lined with hydrophobic amino acids, creating a barrier to the flow of K^+ ions. In contrast, the MthK inner helices are bent at a hinge point and splayed open so that the central cavity becomes confluent with the cytoplasm, leaving free access for ion flow between the cytoplasm and the selectivity filter. From measurements of gating activity in membranes, the conditions under which the KcsA and MthK channels were crystallized are expected to yield closed and opened K^+ channels, respectively [14,15]. The crystal structures are in very good agreement with this expectation.

The KcsA and MthK structures are probably representative of the closed and opened pore conformations of many different K^+ channels, irrespective of the stimulus that causes the pore to open [13]. This conclusion is suggested by the conservation of a glycine residue at the inner helix hinge point in most K^+ channels (Fig. 2, red). A glycine gating hinge would

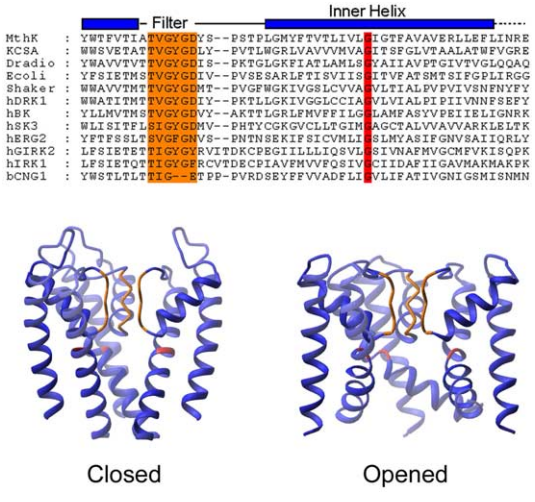

Fig. 2. Closed and opened conformations of the pore. Sequences of ligand- and voltage-gated K^+ channels are from [13]. The selectivity filter sequence is highlighted orange and the glycine gating hinge red. Three subunits of the closed pore conformation of KcsA (left) and the opened pore conformation of MthK (right) are shown with the selectivity filter and gating hinge colored to match the sequence highlights.

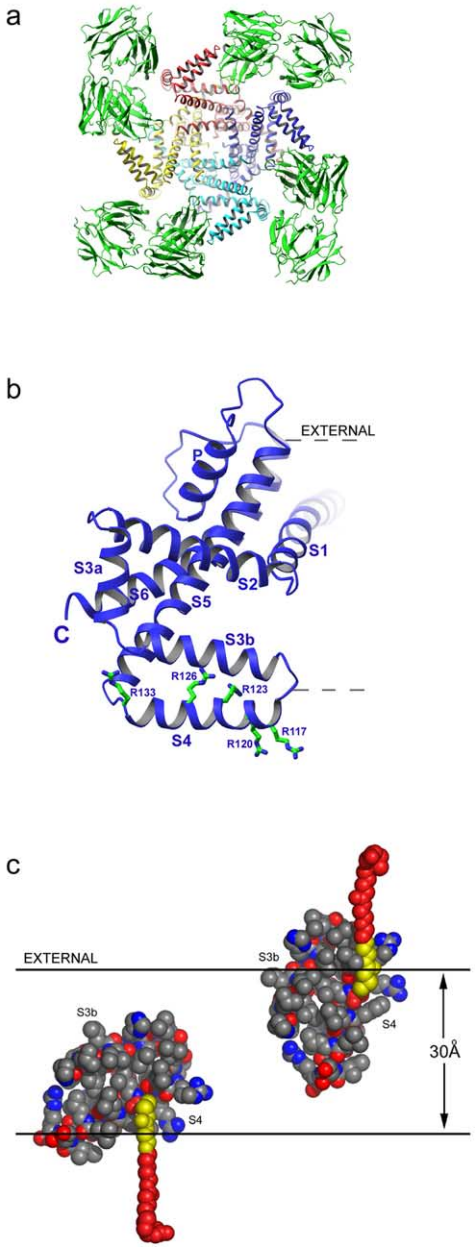

Fig. 3. The voltage-gated K^+ channel KvAP. a: A tetramer from the crystal viewed down the four-fold axis from the cytoplasm [24]. The channel is α-helical with four subunits shown in different colors. Monoclonal Fab fragments (green) used to crystallize KvAP are attached to the voltage sensors. b: A single subunit of KvAP is shown with the extracellular side on top. Helical elements S3b and S4 form a hydrophobic 'voltage sensor paddle' with gating charge arginine residues. In the crystal, the Fab is attached to the voltage sensor paddle and has extended it toward the cytoplasm. c: CPK model of the voltage sensor paddle shown in approximate closed (left) and opened (right) depths relative to the 30 Å hydrophobic core. Tethered biotin (red and yellow) attached to position 121 is accessible to internal avidin when the channel is closed and to external avidin when the channel is opened. Avidin engulfs the red segment when it binds to biotin. a and b are from [24].

allow the inner helices to switch between a closed KcsA-like conformation and an opened MthK-like conformation. The conclusion is also supported by pharmacological and mutational studies of voltage-gated [16,17] and G-protein-gated

[18] K^+ channels, which are consistent with the closed and opened pore conformations shown in Fig. 2.

In summary, large conformational changes within the membrane underlie pore opening in K^+ channels. Inner helices obstruct the pore in the closed state and expand its intracellular diameter in the opened state. Different gating domains – ligand binding and voltage sensing – appear to bring about a similar conformational change in the pore of K^+ channels.

4. Voltage sensing

Ion channels determine the membrane voltage by controlling the membrane's permeability to ions. For voltage-gated channels the membrane voltage determines whether they are open, and therefore they provide a way for the membrane voltage to feed back onto itself – a key property for generating electrical impulses [19]. The principle of how membrane voltage can regulate channel opening is simple: when a voltage-gated channel opens, charged amino acids called gating charges move through the membrane electric field, coupling electrical work to the opening process [5,6,20]. The gating charge of a voltage-gated K^+ channel has been measured and is equivalent to almost 14 electrons moving all the way across the transmembrane voltage difference [21–23]. Most of this gating charge is attributable to four arginine residues per subunit, 16 total; each arginine accounts for approximately one electron charge [22,23].

The crystal structure of the voltage-gated K^+ channel KvAP contains the typical pore of K^+ channels surrounded by α-helical voltage sensors (Fig. 3a) [24]. In the crystal the voltage sensors are held by monoclonal Fab fragments, which in the absence of membrane forces extend the sensors into a non-native conformation (Fig. 3b). Nevertheless, the structure offers a simple possibility for how the voltage sensors might transfer gating charges through the membrane electric field: the gating charge arginine residues are located on hydrophobic helix-turn-helix structures – voltage sensor paddles – that appear as if they could move within the membrane at the protein–lipid interface when the channel opens.

The movement of voltage sensor paddles was tested in lipid membranes by tethering biotin to various positions and assessing whether avidin can access the biotin from either side of the membrane [25]. At certain positions on one face of the paddle tethered biotin is actually dragged all the way across the membrane from the intracellular side in the closed conformation to the extracellular side in the opened conformation (Fig. 3c). That is, biotin is accessible to avidin from the intracellular side when the channel is closed and from the extracellular side when it is opened. Given the length of the tether to which biotin is attached, and constraints for avidin binding, this finding requires that the paddles move a large distance. The overall pattern of accessibility of biotin attached to many positions point to the same conclusion: motions large enough to transfer the four arginine gating charges most of the way across the membrane [25].

Additional structures will be necessary to clarify how the voltage sensors pack against the pore in the membrane in the closed and opened conformations, but KvAP suggests a very simple mechanism: the electric field pulls on a charged helix-turn-helix structure on the channel's perimeter to bring about a conformation change. Another channel with voltage-dependent gating properties, MscS, features a similar helix-turn-helix with arginine residues on its perimeter [26]. KvAP and MscS require us to re-evaluate the long-held assumption that charged amino acids have to be always shielded from the lipid membrane. The structures and functional studies imply that hydrophobic and electrostatic forces can balance each other so that the delocalized charge on an arginine residue can be drawn into the membrane at the protein–lipid interface.

In summary, conformational changes underlying voltage sensing in KvAP are large and involve movements of arginine residues through the membrane, near the protein lipid interface.

References

[1] Hille, B. (2001) in: Ion Channels of Excitable Membranes, 3rd Edn., Sinauer Associates, Sunderland, MA.
[2] Heginbotham, L., Lu, Z., Abramson, T. and MacKinnon, R. (1994) Biophys. J. 66, 1061–1067.
[3] Marty, A. (1981) Nature 291, 497–500.
[4] Wickman, K. and Clapham, D.E. (1995) Physiol. Rev. 75, 865–885.
[5] Bezanilla, F. (2000) Physiol. Rev. 80, 555–592.
[6] Sigworth, F.J. (1994) Q. Rev. Biophys. 27, 1–40.
[7] Doyle, D.A. et al. (1998) Science 280, 69–77.
[8] Zhou, Y., Morais-Cabral, J.H., Kaufman, A. and MacKinnon, R. (2001) Nature 414, 43–48.
[9] Roux, B. and MacKinnon, R. (1999) Science 285, 100–102.
[10] Morais-Cabral, J.H., Zhou, Y. and MacKinnon, R. (2001) Nature 414, 37–42.
[11] Zhou, Y. and MacKinnon, R. (in press) J. Mol. Biol.
[12] Hodgkin, A.L. and Keynes, R.D. (1955) J. Physiol. (Lond.) 128, 61–88.
[13] Jiang, Y. et al. (2002) Nature 417, 523–526.
[14] Heginbotham, L., LeMasurier, M., Kolmakova-Partensky, L. and Miller, C. (1999) J. Gen. Physiol. 114, 551–560.
[15] Jiang, Y. et al. (2002) Nature 417, 515–522.
[16] Armstrong, C.M. (1971) J. Gen. Physiol. 58, 413–437.
[17] del Camino, D. and Yellen, G. (2001) Neuron 32, 649–656.
[18] Jin, T. et al. (2002) Mol. Cell 10, 469–481.
[19] Hodgkin, A.L. and Huxley, A.F. (1952) J. Physiol. (Lond.) 117, 500–544.
[20] Armstrong, C.M. and Bezanilla, F. (1974) J. Gen. Physiol. 63, 533–552.
[21] Schoppa, N.E., McCormack, K., Tanouye, M.A. and Sigworth, F.J. (1992) Science 255, 1712–1715.
[22] Seoh, S.A., Sigg, D., Papazian, D.M. and Bezanilla, F. (1996) Neuron 16, 1159–1167.
[23] Aggarwal, S.K. and MacKinnon, R. (1996) Neuron 16, 1169–1177.
[24] Jiang, Y. et al. (2003) Nature 423, 33–41.
[25] Jiang, Y., Ruta, V., Chen, J., Lee, A. and MacKinnon, R. (2003) Nature 423, 42–48.
[26] Bass, R.B., Strop, P., Barclay, M. and Rees, D.C. (2002) Science 298, 1582–1587.