Eur Biophys J (2004) 33: 175–179
DOI 10.1007/s00249-003-0382-z

REVIEW

Declan A. Doyle

Structural themes in ion channels

Received: 5 December 2003 / Accepted: 12 December 2003 / Published online: 16 March 2004
© EBSA 2004

Abstract The recent crystal structure of the prokaryotic inwardly rectifying potassium channel, KirBac1.1, revealed for the first time the structure of a K^+ channel in the closed state plus the location of the activation gate. Comparison of the KirBac1.1 structure with other known ion channels reveals a number of common structural features. These common characteristics include the formation of the ion conduction pathway at the interface between adjacent subunits, non-fixed charges forming part of the ion pathway, electrostatic sinks drawing ions into the channel, helix dipoles, and hydrophobic gates that ultimately prevent ion movement. This review describes in detail common structural themes present in ion channels.

Keywords Electrostatic sinks · Ion binding sites · Ion conduction pathways · Helix dipoles · Hydrophobic gates

Introduction

The role of an ion channel is to allow the controlled movement of target ions down their electrochemical gradient. For this to take place, the integral membrane section of the channel must form an ion conduction pathway, a selectivity filter, and a gate to control the ion movements. There are a number of problems at each stage that the channel must overcome before ion movement can take place. Considering first the ion conduction pathway, the formation of a high-affinity ion binding site must be prevented. This has to be balanced with stabilizing the ions within the boundaries of the lipid membrane. As part of the ion conduction pathway, the channel must also have a section that allows for the selection of a particular group of ions: the selectivity filter. Finally, there must be mechanisms in place to control the opening and closing of the channel to prevent cell death by dissipation of the ionic gradients. Therefore, all biologically active channels must possess the three components: an ion conduction pathway, a selectivity filter and a gating mechanism. This review focuses on the common structural features as observed in the recent ion channel structures.

Presented at the Biophysical Society Meeting on "Ion channels – from structure to disease" held in May 2003, Rennes, France

D. A. Doyle (✉)
Structural Genomics Consortium, University of Oxford,
Botnar Research Centre, Oxford , OX3 7LD, UK
E-mail: declan.doyle@sgc.ox.ac.uk
Tel.: +0-1865-227970
Fax: +0-1865-737231

Ion conduction pathways

Many ion channels are formed from multiple subunits, with the ion conduction pathway formed at the interface between the subunits. This applies to the homomeric channels such as K^+ channels, which generally consist of four identical subunits, as well as to heteromeric channels like the nicotinic acetylcholine receptor (nAChR), a pentameric assembly of α (two subunits), β, δ, and γ subunits. Voltage-dependent Na^+ and Ca^{2+} channels are structurally related to K^+ channels, being formed from four subunits. These channels, however, are formed from a single gene that consists of four homologous repeats. Each of the four duplications is structurally related to a single K^+ channel subunit. Other channels that use the interface between subunits to form an ion conduction pathway include stretch-activated receptors (pentameric), ryanodine receptor (tetrameric), glycine receptors (pentameric), etc.

At present it is unclear why the interface appears to be the preferred location for many ion conduction pathways. The multi-subunit nature of many ion channels may offer a more subtle form of regulation of ion movement through allosteric mechanisms. Alternatively, it may offer a greater degree of flexibility in the size and shape of the ion conduction pathway at different stages

of channel gating as compared with a single, solid protein core.

Ion binding sites using carbonyl oxygen atoms

As K^+ ions approach the selectivity filter of a channel, they exit not as a single entity but as a complex with water molecules. The +1 charge of the ion orientates the negative end of the water molecule's dipole moment towards itself. It takes between six to eight water molecules to complement the charge of a K^+ ion. However, this ion–water interaction is a dynamic process, with individual water molecules being replaced by others over a fixed period of time. If the ion is to move across a lipid bilayer, these associated water molecules must be taken into account for the ion to remain energetically stable. K^+ channels play a trick on K^+ ions. They offer the ion an environment that is very similar to that which the ion experiences in bulk solvent. The channel surrounds the ion with the oxygen atoms of backbone carbonyl groups instead of oxygen atoms of water. This occurs within the selectivity filter region of a K^+ channel (Doyle et al, 1998). The carbonyl oxygen atoms from the backbone atoms of the highly conserved residues Val-Gly-Tyr-Gly point toward the centre of the ion conduction pathway, forming K^+ binding sites. Therefore, instead of allowing a fully hydrated K^+ ion through the filter, the channel allows a near fully dehydrated K^+ ion through the selectivity filter. The way that the channel discriminates between K^+ and Na^+ ions is that these carbonyl oxygen atoms are ideally positioned to complex a dehydrated K^+ ion but are too far apart to do the same for the smaller dehydrated Na^+ ion. As the ion leaves the filter and enters the cavity in the centre of the channel, water molecules once again take over the job of complementing the ion's charge.

Carbonyl oxygen atoms also form the lining of the ion channel gramicidin (Arseniev et al. 1985; Wallace and Ravikumar 1988). Gramicidin is a 15-amino-acid peptide composed of alternating L- and D-amino acid configurations. Two gramicidin peptides come together as a double helix to form the channel. The unusual alternating amino acid configurations allow all of the hydrophobic side chains to point toward the phospholipids' fatty acyl chains. This results in the formation of an ion conduction pathway through the centre of the double helix. The pathway is thus formed from the backbone carbonyl and amide groups. Unlike K^+ channels, gramicidin forms a non-selective monovalent cation channel, even though both channels use carbonyl oxygen atoms in their ion conduction pathways. The difference between the two channels is likely to be the result of the size of the ion conduction pathways. The selectivity filter of K^+ channels is relatively fixed, forming a diameter most suited to binding a dehydrated K^+ ion. Gramicidin, on the other hand, has a wider ion conduction pathway that can accommodate different monovalent cations, each with different numbers of associated water molecules (Tian and Cross 1999).

The importance of using non-fixed charges as part of the ion conduction pathway and/or selectivity filter is that they are less likely to form high-affinity binding sites. Such sites, of course, would prevent the easy flow of ions through a channel. Threonine or serine residues are other non-charged residues that may play an important part in forming the ion conduction pathway. In the KcsA structure, threonine residues form part of the selectivity filter and the ion conduction pathway at the bottom of the central cavity.

Cationic electrostatic sinks

Inward rectifying K^+ (Kir) channels are so named because they preferentially allow the inward movement of ions. Mapping of the residues that were identified as being important to this process (Yang et al. 1995; Kubo and Murata 2001) onto the recent prokaryotic Kir channel structure (KirBac1.1) demonstrated that they form a double ring of negatively charged residues stacked one on top of the other (Fig. 1) (Kuo et al. 2003). In particular, the spread-out arrangement of Glu258 and Glu187, with their side chains pointing toward the centre of the ion conduction pathway, is ideally suited to bind the intracellular cations Mg^{2+} or polyamines. These cations are known to be responsible for the rectification properties of Kir channels (Fakler et al. 1994; Ficker et al. 1994; Lopatin et al. 1994). Neither Mg^{2+} nor polyamines are able to move completely through Kir channels. Instead, they are believed to bind to the negatively charged rings and block the outward movement of K^+ ions.

These electrostatic rings play another role in Kir channels besides rectification. Successive removal of the negative charge in Kir2.1 resulted in a decrease in the single channel ion conductance (Kubo and Murata 2001). It is likely that these negatively charged residues located along the ion conduction pathway attract all types of cations. This would have the effect of increasing the local ion concentration, especially K^+, which is the most abundant intracellular cation.

This characteristic of using rings of electrostatic charge along the ion conduction pathway to increase the local ion concentration is also part of ion conduction in the nAChR (Fig. 1). nAChR is a non-selective cation channel that has three negatively charged rings along the transmembrane section. These negatively charged rings have been named the extracellular, intermediate, and cytoplasmic rings. Successive replacement of the charges on these rings produced a successive drop in the conductance level of the mutant channels (Imoto et al. 1988).

The large single conductance of the BK channel (Ca^{2+}-activated K^+ channel) is due to the presence of eight negatively charged glutamate residues. These glutamate residues are located on the C-terminal end of the inner helices. Again, removal of these negative charges results in a decrease in the single channel conductance

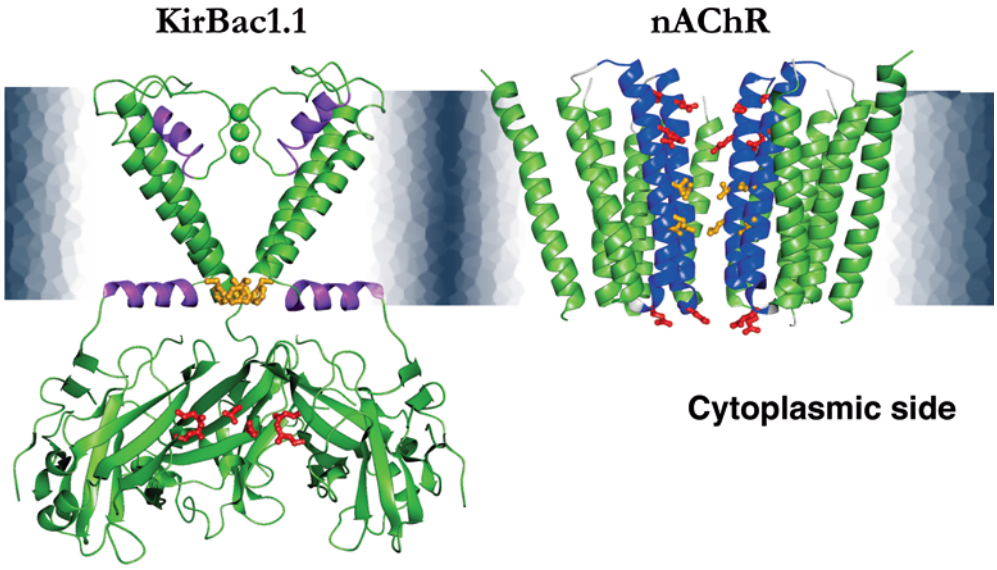

Fig. 1 Highlighting common structural themes in the KirBac1.1 channel and the transmembrane section of the nAChR. Two transmembrane domains and one C-terminal domain have been removed from the KirBac1.1 structure, to reveal the ion conduction pathway. For the same reason, one domain of the nAChR has been removed. The *green spheres* represent three K^+ ions in the selectivity filter of KirBac1.1. Helices that make use of their helix dipole are coloured *magenta*. In KirBac1.1, the pore helices are on the extracellular side of the membrane, while the slide helices are at the interface between the membrane and the cytoplasm. Residues that form the hydrophobic gates are highlighted in *yellow*. Residues that form negatively charged electrostatic rings are coloured *red*. The M2 helices that form the ion conduction pathway in nAChR are coloured *blue*

(Brelidze et al. 2003; Nimigean et al. 2003). This negative electrostatic sink is located within the transmembrane section of these K^+ channels.

Not surprisingly, rings of negative charges have been implicated in ion conduction of other cation selective channels, including the epithelial sodium channel (Langloh et al. 2000; Sheng et al. 2001) and the voltage-dependent Na^+ channel (Li et al. 2000). Therefore, in cation selective channels, negatively charged electrostatic rings appear to play an important role in the rate of ion movement through the channel by increasing the local ion concentration.

Helix dipoles

The crystal structure of the prokaryotic K^+ channel KcsA was first to reveal the importance of helix dipoles in ion channels (Doyle et al. 1998). KcsA possesses four pore helices, each of which point their C-terminal ends toward the centre of the water filled cavity. This results in the negative charges of the pore helix dipoles combining to help stabilize and possibly focus an ion in the centre of the channel's cavity. This potential focusing of the ion in the centre would ensure that a K^+ ion remains along the correct trajectory when the channel is in the open state. A high-resolution structure of the KcsA channel revealed how well ordered the single hydrated K^+ ion is in the cavity's centre (Zhou et al. 2001), even though there are no other charges holding the ion in place.

A surprising change in the position of the helix dipoles was seen in the KirBac1.1 structure (Kuo et al. 2003). In this structure, all of the four pore helices no longer point toward the centre of the cavity but are misaligned. Unlike KcsA, there is no strong density seen in the central cavity of the KirBac1.1 channel, indicating the loss of the ion binding site. A change in the alignment of the pore helix was also found to take place as the cyclic nucleotide channel moved between the closed and open states (Liu and Siegelbaum 2000). As the KirBac1.1 channel is in the closed state, it appears likely that this misalignment of the pore helices is one of four characteristics that the channel uses to prevent the flow of K^+ ions. The second characteristic is also related to the stabilization of the ion in the cavity. In the KirBac1.1 structure the cavity volume is smaller than the KcsA's cavity, adding to the destabilization of the ion at this region. The third feature is a change in the structure of the selectivity filter in order to prevent its collapse. Finally, the fourth trait of the closed state is the blockage of the ion conduction pathway on the intracellular side of the membrane. These four closed state characteristics result in a tight control over ion movement through the channel.

The KirBac1.1 structure also revealed another potential use for helix dipoles in K^+ channels. The amphipathic slide helix, located at the interface between the membrane and cytoplasm (Fig. 1), is believed to be critical for the gating process. The negative end of this helix dipole interacts with the conserved Arg148 side chain of the inner helix. This interaction would tether the inner helix to the slide helix. Hence any movement of the slide helix would result in the concerted movement of the inner helix or vice versa. Therefore, during opening of the channel, the slide helix must move away from the

central ion conduction pathway along with the outer and inner helices. The slide helix is also present in the full length structure of KcsA, as determined by electron paramagnetic resonance (Cortes et al. 2001).

For KirBac1.1 it is believed that the slide helix acts as the coupling device between the initial gating signal and the activation gate (blocking residues Phe146). A similar coupling mechanism is likely to occur in voltage-dependent K^+ channels. Chimeric Shaker/KcsA channels identified the C-terminus of the Shaker's inner helix plus the connecting loop between the Shaker's outer helix and the voltage sensing S4 segment (S4–S5 linker) as essential components in the formation of a fully voltage-gated channel (Lu et al. 2002). Mutations in either of these regions produce chimeric channels whose conductance is either totally voltage independent or independent at negative voltages. These results were accounted for by assuming that the mutations loosen the coupling between the S4 voltage sensor segment and the inner helix blocking residue (the activation gate). It turns out that the S4–outer helix (S5) connection of the Shaker channel adopts an amphipathic helical structure, like the slide helix, in dodecylphosphocholine micelles (Ohlenschläger et al. 2001). Studies implicating the S4–S5 linker in the coupling process have also been performed on the KvLQT1 K^+ channel and the pacemaker channel (Franqueza et al. 1999; Chen et al. 2001). By analogy, it is possible that this gating and coupling mechanism also applies to the greater family of voltage-dependent Na^+ and Ca^{2+} channels.

Hydrophobic gates

As mentioned previously, the KirBac1.1 crystal structure captured the channel in the closed state. The closed state is obviously characterized as the inability of ions to move through the ion conduction pathway. The strongest structural evidence for this is that the ion conduction pathway is completely blocked. The four hydrophobic side chains of Phe146 (the blocking residues), one from each subunit, form a tight seal at the cytoplasmic/membrane interface (Fig. 1). For K^+ channels, this is likely to the activation gate, the final gate to be released before the channel opens.

Other channels also use a hydrophobic gate to block ion movement. The nAChR has a hydrophobic constriction in the middle of the bilayer (Fig. 1), formed by leucine and valine side chains (Miyazawa et al. 2003). The mechanosensitive receptor, MscL (Chang et al. 1998), also uses two rings of hydrophobic residues to form the hydrophobic gate. This time, it is the side chains of isoleucine and valine in a pentameric arrangement that creates the gate. It is important to stress the fact that it is hydrophobic residues that are used to prevent ion movement. This implies that the movement of ions or charged particles are controlled indirectly by first controlling the flow of water. If water movement is blocked, then charged particles within the channel are effectively immobilized. This idea is supported by simulation studies looking at the movement of water in a model pore based on the structure of the nAChR (Beckstein et al. 2003). The results showed that, even though the pore had a diameter sufficiently large to accommodate up to three water molecules, no water was able to move through the pore: the hydrophobic character of the pore prevented water movements. This could be overcome if a partial hydrophilic character was introduced into the lining of the pore without altering the pore dimensions. Therefore, the important message from these results is that these channels control ion movement indirectly by first controlling water movement.

Conclusions

Several ion channels have adopted the same strategies to overcome some of the problems associated with moving an ion from one side of a membrane to the other. Controlling the movement of an ion is critical to the functioning of any channel. This may require a dynamic change in the shape and size of the ion conduction pathway at different times during the gating process. This desire for flexibility may be better achieved through the use of the interface between multi-subunits to form the ion conduction pathway. Attracting ions into the ion conduction pathway is obviously important. The creation of electrostatic sinks appears to fulfil this objective. Stabilizing a charged particle within the boundaries of the membrane is another important consideration. Many channels appear to do this through a combination of helix dipoles and polar groups. Finally, if a channel is to open and close at the appropriate time, the movement of ions must be tightly regulated. The simplest and most effective way to do this appears to be by preventing water movement. Once water movement has been blocked with the use of a hydrophobic gate, then ion movement is also abolished.

Acknowledgements Tahmina Rahman is thanked for help with the manuscript.

References

Arseniev AS, Barsukov IL, Bystrov VF, Lomize AL, Ovchinnikov YA (1985) 1H-NMR study of gramicidin A transmembrane channel. FEBS Lett 186:168–174

Beckstein O, Biggins PC, Sansom MSP (2001) A hydrophobic gating mechanism for nanopores. J Phys Chem B 105:12902–12905

Brelidze TI, Niu X, Magleby KL (2003) A ring of eight conserved negatively charged amino acids doubles the conductance of BK channels and prevents inward rectification. Proc Natl Acad Sci USA 100:9017–9022

Chang G, Spencer RH, Lee AT, Barclay MT, Rees DC (1998) Structure of the MscL homolog from *Mycobacterium tuberculosis*: a gated mechanosensitive ion channel. Science 282:2220–2226

Chen J, Mitcheson JS, Tristani-Firouzi M, Lin M, Sanguinetti MC (2001) The S4-S5 linker couples voltage sensing and activation of pacemaker channels. Proc Natl Acad Sci USA 98:11277–11282

Cortes DM, Cuello LG, Perozo E (2001) Molecular architecture of full-length KcsA: role of cytoplasmic domains in ion permeation and activation gating. J Gen Physiol 117:165–180

Doyle DA, Morais-Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998) The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science 280:69–77

Fakler B, Brandle U, Glowatzki E, Konig C, Bond C, Adelman JP, Zenner H-P, Ruppersberg JP (1994) A structural determinant of differential sensitivity of cloned inward rectifier channels to spermine. FEBS Lett 356:199–203

Ficker E, Taglialatela M, Wible BA, Henley CM, Brown AM (1994) Spermine and spermidine as gating molecules for inward rectifier K^+ channels. Science 266:1068–1072

Franqueza L, Lin M, Splawski I, Keating MT, Sanguinetti MC (1999) Long QT syndrome-associated mutations in the S4-S5 linker of KvLQT1 potassium channels modify gating and interaction with mink subunits. J Biol Chem 274:21063–21070

Imoto K, Busch C, Sakmann B, Mishina M, Konno T, Nakai,J, Bujo H, Mori Y, Fukuda K, Numa S (1988) Rings of negatively charged amino acids determine the acetylcholine receptor channel conductance. Nature 335:645–648

Kubo Y, Murata Y (2001) Control of rectification and permeation by two distinct sites after the second transmembrane region in Kir2.1 K^+ channel. J Physiol (London) 531:645–660

Kuo A, Gulbis JM, Antcliff JF, Rahman T, Lowe ED, Zimmer J, Cuthbertson J, Ashcroft FM, Ezaki T, Doyle DA (2003) Crystal structure of the potassium channel KirBac1.1 in the closed state. Science 300:1922–1926

Langloh AL, Berdiev B, Ji HL, Keyser K, Stanton BA, Benos DJ (2000) Charged residues in the M2 region of alpha-hENaC play a role in channel conductance. Am J Physiol 278:C277–C291

Li RA, Velez P, Chiamvimonvat N, Tomaselli GF, Marban E (2000) Charged residues between the selectivity filter and S6 segments contribute to the permeation phenotype of the sodium channel. J Gen Physiol 115:81–92

Liu J, Siegelbaum SA (2000) Change of pore helix conformational state upon opening of cyclic nucleotide-gated channels. Neuron 28:899–909

Lopatin AN, Makhina EN, Nichols CG (1994) Potassium channel block by cytoplasmic polyamines as the mechanism of intrinsic rectification. Nature 372:366–369

Lu Z, Klem AM, Ramu Y (2002) Coupling between voltage sensors and activation gate in voltage-gated K^+ channels. J Gen Physiol 120:663–676

Miyazawa A, Fujiyoshi Y, Unwin N (2003) Structure and gating mechanism of the acetylcholine receptor pore. Nature 423:949–955

Nimigean CM, Chappie JS, Miller C (2003) Electrostatic tuning of ion conductance in potassium channels. Biochemistry 42:9263–9268

Ohlenschläger O, Hojo H, Ramachandran R, Görlach M, Haris PI (2001) Three-dimensional structure of the S4-S5 segment of the Shaker potassium channel. Biophys J 82:2995–3002

Sheng S, McNulty KA, Harvey JM, Kleyman TR (2001) Second transmembrane domains of ENaC subunits contribute to ion permeation and selectivity. J Biol Chem 276:44091–44098

Tian F, Cross TA (1999) Cation transport: an example of structural based selectivity. J Mol Biol 285:1993–2003

Wallace BA, Ravikumar K (1988) The gramicidin pore: crystal structure of a caesium complex. Science 241:182–187

Yang J, Jan YN, Jan LY (1995) Control of rectification and permeation by residues in two distinct domains in an inward rectifier K^+ channel. Neuron 14:1047–1054

Zhou Y, Morais-Cabral JH, Kaufman A, MacKinnon R (2001) Chemistry of ion coordination and hydration revealed by a K^+ channel-Fab complex at 2.0 Å resolution. Nature 414:43–48