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MINI-REVIEW

Transferring knowledge towards understanding the pore stabilizing variations in K^+ channels

Pore stability in K^+ channels

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Abstract Recent advances in structural biology underlying mechanisms of channel gating have strengthened our knowledge about how K^+ channels can be inter-convertible between conductive and non-conductive states. We have reviewed and combined mutagenesis with biochemical, biophysical and structural information in order to understand the critical roles of the pore residues in stabilizing the pore structure and channel open state. We also discuss how the latest knowledge on the K^+ channel KcsA may provide a step towards better understanding of distinct pore stabilizing differences among diversified K^+ channels.

Keywords K^+ channel KcsA · Selectivity filter · Mutagenesis · Channel gating · Tetramer stability

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Introduction

Potassium (K^+) channels are widely distributed as oligomeric complexes in different cell types of all living organisms. The pivotal role of K^+ channels in various physiological processes including neuronal signaling, vascular and nonvascular muscle contractility, cardiac pacing, auditory function, hormone secretion, immune function, and cell proliferation is supported by increasing discoveries linking K^+ channel mutations to various inherited disorders. As a result, researchers consider channels as drug targets.

The initial knowledge on the functionality of K^+ channels from eukaryotes derived from analyses of their characteristics within oocytes into which the RNA (transcribed from the corresponding gene) had been injected. In the successful case, the RNA was translated by use of the endogenous protein biosynthesis machinery. The resulting protein may incorporate into various localizations in the oocycte membrane, the assemblies of which are not controllable. As the ooycte itself has endogenous channels, which are also not evenly distributed, comparisons of numerous patch clamp studies under various conditions has led to subtract the features of the "foreign" channel.

The discovery of the gene for the KcsA protein in bacterium (*Streptomyces lividans*) convincingly proved that channels are not a "privilege" of eukaryotic organisms, and stimulated a search for other types of channel proteins from bacteria. Meanwhile, scientists have adopted the principal concept for analysing the KcsA channel protein for a range of prokaryotic and eukaryotic proteins. The cloning and expression procedure within *E.coli*, which has been introduced for the *kcsA* gene, was an important lead that is

applicable to other channel genes. Within the inner membrane of the *E.coli* host KcsA assembles as a tetramer and the process is dependent on the protonmotive force. Large quantities of $KscA_{histag}$ tetramers are extractable from the *E. coli* membrane in the presence of a range of detergents, and have been successfully purified by affinity chomatography. KcsA can be fused into liposomes in large and soluble quantities and reconstituted functionally into a bilayer, in which the biophysical features of KscA and its designed mutants have been investigated (Raja and Vales 2009a, 2011; Schrempf et al. 1995).

The establishment of conditions to gain large quantities of pure protein was the basis to crystalize KcsA and subsequently other channel proteins. Subsequently, the purified KscA protein (without its C-terminal domain) was successfully crystallized. The highest resolution reached within some parts was 3.2 Å, whereas in other regions the resolution was considerably lower (Doyle et al. 1998). The KcsA channel contains only two transmembrane domains with an intervening pore loop including a K^+-selective motif GYG (Fig. 1a and b), although at the amino acid sequence level, this channel is more similar to the voltage-gated K^+ channels (Schrempf et al. 1995). Functionally, it lacks any hint of voltage gating because of the lack of a S4 region. X-ray analysis revealed that four identical subunits form a tetramer creating an inverted cone, cradling the selectivity filter of the pore in its outer end. The overall length of the conducting pore is 45 Å, and its diameter is variable along its distance. The internal vestibule of the pore begins as a tunnel of 18 Å in length that widens into a cavity (~10 Å across) near the middle of the membrane, with the narrow selectivity filter only 12 Å long. The remainder of the pore is wider and lined with hydrophobic amino acids. The selectivity filter is lined by the carbonyl oxygen atoms from amino acids residing in the GYG signature sequence.

The authors had concluded the crystallized channel was in an open configuration, however, biophysical studies of mutant proteins (Raja and Vales 2009a; Schrempf et al. 1995) had indicated that the first crystal structure of KcsA was in a closed state. Recently, other authors (Cuello et al. 2010a) succeeded to elucidate a high-resolution crystal structure of the KcsA (including its C-terminus) in its open-inactivated conformation, and investigated the mechanism of C-type inactivation gating at the selectivity filter from channels 'trapped' in a series of partially open conformations. The new crystal structures of full-length KcsA clearly indicate that transitions from the closed state (Fig. 2a) to two partial opening structures (in the 15 Å structure) induce a small increase in distance (Fig. 2b), which is further followed by a narrowing of the pore in the open 17 Å form. The major structural transition eventually leads to the non-conductive forms of the selectivity filter as observed in the open 23 Å and open 32 Å (Fig. 2c) structures (Cuello et al. 2010a). The crystal structure of the full-length KcsA channel thus provides the first three-dimensional structure of the conduction pore that fits consistently with current understanding of the core functionality of K^+ channels.

To date, several fundamental findings of membrane proteins related to oligomerization, channel stability, functional analysis or channel blocking behavior are based on the ease to gain KcsA and its designed mutants from engineered *E. coli* strains, the in vitro stability of the functional tetramer, and the available crystal structures. KcsA has not only become the principal model for K^+ ion selectivity and permeation, but has also provided a major insight into the mechanism of protein oligomerization and channel gating (Raja 2010; Raja et al. 2007; Valiyaveetil et al. 2002). Many mammalian K^+ channels are thought to share the same topology as KcsA, namely the inwardly rectifying K^+ (Kir) channels. These channels also provide a valid test of the

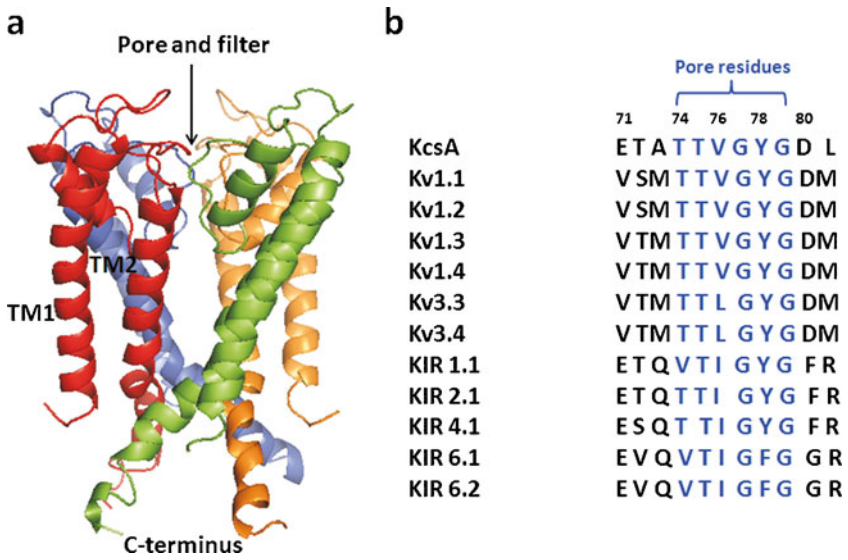

Fig. 1 **a** Stereoview of a ribbon representation illustrating the three-dimensional structure of the KcsA tetramer (PDB ID: 1K4C) in its closed conformation. Each monomer is distinguished by a different color. The positions of the pore, selectivity filter, transmembrane domains TM1 and TM2, and C-terminus are indicated. **b** Alignment of the selectivity filter and pore residues depicting the variations and conservation between KcsA, Kv and Kir K^+ channels

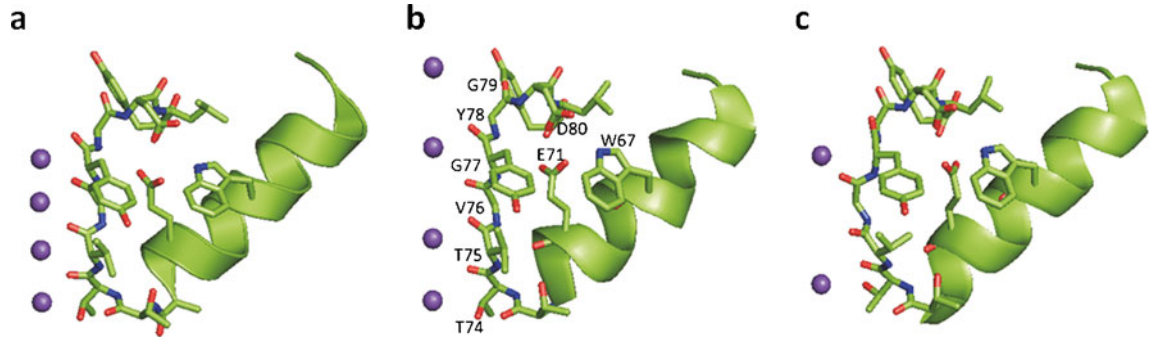

Fig. 2 Structures of selectivity filters of KcsA in closed 12 Å (**a**, PDB ID: 1K4C), open-activated 15 Å (**b**, PDB ID: 3FB6) and open-inactivated 32 Å (**c**, PDB ID: 3F5W) conformations showing different K^+ ion occupancies (in *purple*) at the selectivity filter. The residues in the pore and the pore-helix are numbered in panel b

assumption underlying homology modeling demonstrating a shared architecture between KcsA and other K^+ channels (Capener et al. 2000; Sansom and Capener 2002).

In recent years, biochemical and biophysical approaches enumerated to deepen our understanding of mechanisms underlying assembly, stability, selectivity, and gating. In this review, we cover key aspects to understand the role of the selectivity filter at the molecular level in K^+ channel function by combining mutagenesis and structural information of the pore-region. We discuss the progress, challenges, and opportunities in the exploitation of the KcsA structure, and how the resulting knowledge may be used to target eukaryotic K^+ channels by pharmacological and emerging genetic approaches.

Characteristics of the purified KscA protein

Single monomers are assembled into a stable tetramer

To gain large quantities of KcsA, the *kcs*A gene is transformed into the heterologous *Escherichia coli (E.coli)* host. Cultivation and induction conditions have been optimized in such fashion that the dominant portion of the protein resides reproducibly in the membrane fraction, but neither within inclusion bodies, nor in the cytoplasm. The use of selected detergents (i.e. Mega9, Dodecylmaltoside and others) leads to an easy extraction of the protein that can be purified by subsequent affinity chromatography. From one liter of culture several mg of the protein are obtained routinely (Schrempf et al. 1995).

Highly purified KcsA (usually histidine-tagged) assembles spontaneously to tetramers that are maintained in a range of pH values or in the presence of various detergents. Between 20 and 70 °C ~95% of the wild type KcsA protein is assembled as tetramers and only ~5% is present as monomers (Cuello et al. 1998; Meuser et al. 1999). Based on these findings, generally tetramers have been used to analyse features of the KcsA channel. The amount of tetramer versus monomer is a key to investigating the tetrameric stability as a function of mutations or in a particular lipid system by conventional gel electrophoresis (Raja 2010; Raja et al. 2007).

Pore residues affect the assembly and channel activity

Mutations of critical residues in and around the pore significantly affect the assembly of monomers to form a stable tetramer. The amino acid residue leucine 81 (L81) located at the external side within the KcsA crystal structure (Doyle et al. 1998) is exchangeable by a cysteine residue without affecting the assembly and the channel characteristics. The substitution of the tyrosine residue 82 by cysteine, valine or threonine, but not by glycine, led to functional channel types. Like the wild type, each of these mutant channels exhibit an internal pH-sensitive side and are cation-selective. Substitution of the glycine (G77) residue within the GYG motif by an alanine (A) leads to mutant protein of reduced tetrameric stability. In contrast to the mutant protein, containing the AVG motif, GFG functions as an active K^+ channel whose characteristics correspond to those of the wild type KcsA channel. The mutant proteins with a mutation (T72A, T72C, V76A, V76E, G77E, Y78A, G79A, G79D, or G79E) within the signature sequence of the pore region, either do not or rarely assemble as tetramers and lack channel activity (Splitt et al. 2000).

The mutation of the pore-helix glutamate E71 to alanine removes the C-ype inactivation of the channel. The meshwork of residues involving E71-D80 carboxyl-carboxylate interaction ‘behind’ the selectivity filter stabilizes the pore of KcsA (Chakrapani et al. 2011). An exchange of threonine T72 located within the signature motif of KcsA by alanine induced the formation of the mutant protein T72A, which did not assemble as a stable tetramer in vitro and did not function as an active channel in a bilayer system. Only a small portion of the KcsA mutant protein T72C assembles as a tetramer. Replacement of the valine residue in position 76 of KcsA by either alanine or glutamic acid induces the formation of proteins lacking tetrameric

assembly and functional channel properties in a bilayer. Within the crystal structure of KcsA (Doyle et al. 1998), V76 is orientated toward the pore helix. Its exchange by glutamic acid may lead to a repulsion of the conserved pore-helix negatively charged glutamic acid in position 71.

KcsA mutant proteins having a mutation within the GYG motif (i.e., G77E, G79A, G79D and G79E) neither assemble in vitro, nor form a functional channel. Replacement of the aromatic residue Y78 by alanine abolishes tetrameric assembly. On the basis of the crystal structure (Doyle et al. 1998) the aromatic tyrosine 78 residue was shown to interact with the two tryptophan residues in positions 67 and 68. It is thus evident that a similar interaction is caused by a phenylalanine residue, but not by an alanine residue in position 78. The side chains of mutated G79A are assumed to be pointed out into the extracellular space above the pore indicating it is most lilely involved in hydration/dehydration of outgoing/incoming K^+ ions. Considering this structure, it is not obvious why G79A does not form a stable tetramer. The most probable reason is that G79 mutations affect the rate of hydration or dehydration of K^+ ions, thereby altering the rate of K^+ entry into the pore. The protein G77A still assembles as a non-functional tetramer indicating this exchange may also lead to a smaller diameter of the pore and thus hinder the permeation of K^+ ions. The exchanges G77E, G79E and G79D might be unstable, due to repulsion effects with other amino acids within the pore region.

Substitution of the amino acid residue L81 of KcsA by cysteine leads to the formation of a protein which still assembles as a tetramer but has a much lower melting point than the WT protein. In a previous attempt to define pH-dependent molecular movements near the selectivity filter (Perozo et al. 1999), many amino acids had been replaced by cysteine residues. No significant change could be ascertained for exchanges of amino acid residues (including L81C) at the external side of the selectivity filter in probe mobility or spin-spin interaction. In the first crystal structure of KcsA (Doyle et al. 1998), the four-fold symmetry cannot be seen within the X-ray diffraction patterns. Therefore it cannot be concluded that the four subunits supply identical sets of atoms in a symmetrical manner to provide the pore lining (Choe et al. 1999). However, it could be deduced that the TVGYG motif (amino acids 75–79) forms a stretch of 12 Å in length with the narrowest opening of the pore (Doyle et al. 1998). As the dimensions of the selectivity filter are determined by lining the groups, hydrogen bonds and van der Waals interactions with the remaining protein, it is conceivable that already subtle alterations of the amino acid composition in this region affect the stability of the tetrameric arrangement.

Different conformational rearrangements deduced in the new structures of KcsA are quite informative in describing the effects of mutations of pore residues on channel assembly and activity. According to these structures, the filter cannot conduct ions when two ion binding sites, S2 and S3, are compromised by rearrangements in the backbone conformation at or close to the G77 position (Cuello et al. 2010a). In this regard, the non-functionality of the G77A mutant can well support the idea that S2 and S3 sites are completely abolished. The most likely explanation of this effect is that the introduction of a bulky methyl group by Ala substitution at G77 affects the side chain packing around the filter. The channels carrying this mutation may have the predominantly open non-conductive conformation (23 or 32 Å). Similarly, in the V76A mutant, the S4 site is most likely abolished thus leading to a collapsed channel structure.

On the basis of molecular dynamics simulations, it is interesting to note that a salt bridge between D80 and R89 of neighbouring subunits possibly plays an important part in the stabilization of the KcsA tetrameric structure (Guidoni et al. 1999). All these studies clearly demonstrate that a selectivity filter is a major determinant in stabilizing the channel assembly. It is important to mention here that we do not discuss the roles of residues at the inner bundle crossings that are also found crucial in controlling the stability and channel assembly as discussed in a recent review (McCoy and Nimigean 2011).

Can KcsA help understand the pore stability in eukaryotic K^+ channels?

The crystal structure of KcsA and subsequent mutagenesis reveal a high structural conservation from bacteria to human (Doyle et al. 1998; MacKinnon et al. 1998). From sequence comparison, it is clear that KcsA exhibits considerable sequence similarity with the members of the Kv family; however with Kir channels it is quite low (Shealy et al. 2003). Nevertheless, the K^+ selectivity filter region is the only portion of KcsA that shares substantial sequence identity with eukaryotic K^+ channels (Shealy et al. 2003) (also see Fig. 1b). There are few incompatibilities found between the dimensions of the inner pore obtained from the x-ray structure of closed KcsA and that estimated from the results of stoichiometric covalent modification (Lu et al. 1999) or mutagenesis (Thompson et al. 2000). Hitherto, these observations point to the fact that dissimilar residues in the filter of KcsA and other K^+ channels may account for the observed differences in dimensions of the pore.

We provide a few examples that support the notion as to whether or not the KcsA structure can be considered useful in understanding the molecular architecture of distinct pores in other K^+ channels. In this regard, a surprising observation has been made in which the sub-regions of the M1-M2 linker (pore-helix and selectivity filter) of KcsA could be successfully swapped with those of the S5-S6 linker of the human Kv-channel Kv1.3, and Kv1.3 specific scorpion

toxins could bind to KcsA-Kv1.3 chimeras. With link to the structure-based design strategy, several charybdotoxin analogs have been prepared with about 20-fold higher affinity to block Ca^{2+}-activated K^+ channels versus voltage-gated Kv1.3 channels (Rauer et al. 2000).

Another approach of studying the pore properties is by subsequent mutagenesis in which particular or dissimilar residues can be exchanged from one channel to the other. Most Kv channels have a valine at the position corresponding to the pore-helix Glu71. Interestingly, the E71V mutation in KcsA, which mimics the situation in Kv channels, retains K^+ and Na^+ selectivity. These results have led to the important conclusion that the E71 side chain is not a key determinant of ion selectivity in KcsA, either directly or through the E71:D80 carboxyl–carboxylate bridge, and that some of the conformational states of the filter, as deduced by Glu71 mutational analysis, may play a role in other K^+ channels (Chakrapani et al. 2011). However, in Kir channels residues equivalent to Glu and Arg (E71 and L81 at the corresponding position in KcsA, respectively) form an ionic bond which is critical in stabilizing the selectivity filter (Dibb et al. 2003; Yang et al. 1997). The common feature among KcsA and Kir channels is that both channels form tetrameric proteins of one-pore/two-transmembrane (1P/2TM) domain subunits that equally contribute to the formation of highly selective K^+ channels. Although, the pore-helix Glu is quite conserved among both types of channels the network of interactions is not translatable from one channel to the other (Raja 2011).

Another remarkable difference between KcsA and other eukaryotic K^+ channels is related to the channel intrinsic stability. KcsA forms an extremely stable tetramer (Schrempf et al. 1995; Splitt et al. 2000) but many eukaryotic K^+ channels do not (Nishida et al. 2007; Raja and Vales 2009b; Ramjeesingh et al. 1999; Thompson et al. 2000). In this regard, the KcsA structure not only provides a fundamental understanding of channel stability and permeation behavior in a selective K^+ channel, it also serve as a useful model protein in determining the stabilizing differences among K^+ channels. For instance, dissimilarity in the stability of KcsA and Kir1.1 channel has been studied by over expressing both types of channels in their suitable hosts and comparing their tetrameric profiles in the presence of either oxidizing or reducing conditions. These studies indicate that the Kir1.1 channel forms a less stable tetramer compared to an extremely stable KcsA in the presence of SDS detergent and that the structure and function of both channels differ due to their dissimilar intrinsic stability (Raja and Vales 2009b).

Few critical residues have been identified in the selectivity filter of KcsA that seem to be crucial in determining the stability and/or channel characteristics in other K^+ channels. In particular, the selectivity filters of KcsA [T^{74}T V^{76} GY^{78}G] and Kir (e.g., KIR1.1/6.1/6.2 → VTI GY/FG) channels differ in few residues. Substitution of tyrosine (Y78) by another aromatic residue phenylalanine (F) forms tetramers of reduced stability (Schrempf et al. 1995). This can be due to the removal of bulky hydroxyl group from the side chain of tyrosine (Y) that affects the packing of residues slightly different than in KcsA wild type protein. However, contrary to the Y78A protein, Y78F still functions as a K^+ channel whose electrophysiological properties correspond to those of the WT KcsA. Replacement of V76 by isoleucine (V76I) not only drastically decreases the intrinsic stability and channel activity but also changes the tetrameric folding properties in the membrane (Raja and Vales 2009a). According to the new structural data, V76 is also flipped in an open-inactivated (23 and 32 Å) states of KcsA (Cuello et al. 2010b). It is interesting that in molecular dynamics (MD) simulations of Kir6.2 the backbone carbonyl of bulky Ile131 (V76 at the corresponding position in KcsA) is flipped thereby narrowing the pore in this region in low K^+ (Capener et al. 2003). Thus, the filter structure of V76I-KcsA seems to be quite similar to an open-inactivated state of 23 or 32 Å. In line with these observations, long-range conformational changes (from the site of the perturbation to the periphery/membrane interface) found in the V76I mutant channel seem to be quite similar to the KcsA channel carrying the M96V mutation in the TM2, which destabilizes the channel open state, or in the presence of low K^+ concentration (Lockless et al. 2007; Zhou et al. 2001).

The differences in filter residues may account for differences in ion occupancy and therefore intrinsic stabilities of K^+ channels. This hypothesis could be well supported by using a genetically engineered KcsA mutant (carrying T74V and V76I double mutations) which is converted into monomers, despite an extremely stable tetramer in the presence of SDS detergent. The observed stability pattern of the mutant is quite similar to the stability of Kir1.1 or other Kir channels (Raja and Vales 2009b, 2010; Ramjeesingh et al. 1999) that run as monomers in a SDS-gel. The double mutant also exhibits sensitivity to Na^+ as Na^+ blocks KcsA and other K^+ channels, indicating that changing multiple residues in KcsA toward Kir channels does not make the channel non-selective. According to the crystal structure, a mutation at threonine 74 (T74V) should disrupt the inactivation state in the open-inactivated mutant V76I which is very similar to the scenario of E71H-F013A double mutation in KcsA (Cuello et al. 2010a). Since non-polar van der Waals interactions between Phe103 and Thr74/Thr75 play a role in activation-inactivation coupling, this interaction is interrupted in a T74V mutant thereby destabilizing the inactivated conformation. Thus, the conductive form of this mutant probably corresponds to the fully occupied state of K^+ ions (14, 15 Å), similar to that observed in the closed KcsA structure.

The T74V mutation in V76I-KcsA not only destabilizes the inactivated state, but also widens the diameter of the

inner pore (referred to the narrowest part). This allows quaternary alkyl ammonium ions (QAs) larger than TEA to block the mutant channel pore. These findings indicate that mutant channel exhibits distinct dimensions of the inner pore and the modified channel pore undergoes similar structural arrangements as established in eukaryotic K^+ channels. For example, the affinity of squid voltage-activated K^+ channels for larger QAs, such as tetrabutylammonium (~10 Å) and tetrapentylammonium (~11 Å), is much higher than that for TEA (~7 Å) (French and Shoukimas 1981). A similar phenomena has been reported for Kir1.1 channels (Oliver et al. 1998), indicating that the inner pore is so wide that it may not sustain single-ion filing. Thus, the inner pore of the mutant channels likely shares some common architectural features, although dissimilar residues may possibly account for differences in pore dimensions among simple-structured KcsA and more complex eukaryotic K^+ channels.

It is also important to mention that D80 (phenylalanine/tyrosine/glycine in Kir channels) and L81 (methionine in Kv and arginine in Kir channels) that are positioned at the filter mouth and contribute to the structure of the pore (Morais-Cabral et al. 2001; Zhou et al. 2001) may also stabilize the channel assembly. Future experiments will shed more light on the role of such residues by site-directed mutations in KcsA toward eukaryotic channels.

Outcome and challenges

Mutations in the filter residues, as discussed above, drastically affect the channel assembly, ion occupancy behavior and the diameter of the pore thus leading to possible open-inactivated states. By combining mutagenesis and structural information, the KcsA channel can be considered structurally very similar to eukaryotic K^+ channels (Capener et al. 2003). However, most data in the literature refer to the functionality and in some case to the closed KcsA structure, which is without the C-terminus. The new structural data now provides more accurate insight into the arrangement of residues surrounding the filter region that stabilize the channel in distinct conformational states. There exist few incompatibilities among KcsA and other K^+ channels with regard to the pore dimensions; hence the network of interactions is not easily transformed to other K^+ channels. In this regard, the site-directed mutagenesis approach has emerged to improve our understanding of how different filter residues may account for dissimilarities in channel gating and structural stability among different K^+ channels. The filter residues can easily modulate the interaction among subunits and therefore the intrinsic stability. Compared to Kir channels, KcsA structural findings are not easily exchangeable particularly with the voltage-gated K^+ channels, that constitute a majority of K^+ channels, since they contain voltage sensor or 4TM domains absent in KcsA. This is the main limitation of the chosen model K^+ channel.

Although the knowledge is still limited, the structural conservation as well as variations among KcsA and other K^+ channels, through application of mutagenesis, structural and functional analysis, should be exploited to advance our understanding of pore differences among a variety of K^+ channels. Assuming that the novel structures on mixed open forms of KcsA reveal distinct channel gating patterns that could correlate to other K^+ channels the new structural information should be applied to design selective compounds targeting the pores of K^+ channels in disease conditions. Most importantly, the stabilizing differences, besides what we have discussed in this review, should be explored in much more detail among various K^+ channels by mutagenesis approaches that would help identify the subunit assembly partners and therefore selective regulators of K^+ channels.

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References

Capener CE, Shrivastava IH, Ranatunga KM, Forrest LR, Smith GR, Sansom MS (2000) Homology modeling and molecular dynamics simulation studies of an inward rectifier potassium channel. Biophys J 78:2929–2942

Capener CE, Proks P, Ashcroft FM, Sansom MS (2003) Filter flexibility in a mammalian K channel: models and simulations of Kir6.2 mutants. Biophys J 84:2345–2356

Chakrapani S, Cordero-Morales JF, Jogini V, Pan AC, Cortes DM, Roux B, Perozo E (2011) On the structural basis of modal gating behavior in K(+) channels. Nat Struct Mol Biol 18:67–74

Choe S, Kreusch A, Pfaffinger PJ (1999) Towards the three-dimensional structure of voltage-gated potassium channels. Trends Biochem Sci 24:345–349

Cuello LG, Romero JG, Cortes DM, Perozo E (1998) pH-dependent gating in the Streptomyces lividans K + channel. Biochemistry-Us 37:3229–3236

Cuello LG, Jogini V, Cortes DM, Pan AC, Gagnon DG, Dalmas O, Cordero-Morales JF, Chakrapani S, Roux B, Perozo E (2010a) Structural basis for the coupling between activation and inactivation gates in K(+) channels. Nature 466:272–275

Cuello LG, Jogini V, Cortes DM, Perozo E (2010b) Structural mechanism of C-type inactivation in K(+) channels. Nature 466:203–208

Dibb KM, Rose T, Makary SY, Claydon TW, Enkvetchakul D, Leach R, Nichols CG, Boyett MR (2003) Molecular basis of ion selectivity, block, and rectification of the inward rectifier Kir3.1/Kir3.4 K(+) channel. J Biol Chem 278:49537–49548

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

French RJ, Shoukimas JJ (1981) Blockage of squid axon potassium conductance by internal tetra-N-alkylammonium ions of various sizes. Biophys J 34:271–291

Guidoni L, Torre V, Carloni P (1999) Potassium and sodium binding to the outer mouth of the K + channel. Biochemistry-Us 38:8599–8604

Lockless SW, Zhou M, MacKinnon R (2007) Structural and thermodynamic properties of selective ion binding in a K + channel. PLoS Biol 5:e121

Lu T, Nguyen B, Zhang X, Yang J (1999) Architecture of a K + channel inner pore revealed by stoichiometric covalent modification. Neuron 22:571–580

MacKinnon R, Cohen SL, Kuo A, Lee A, Chait BT (1998) Structural conservation in prokaryotic and eukaryotic potassium channels. Science 280:106–109

McCoy JG, Nimigean CM (2011) Structural correlates of selectivity and inactivation in potassium channels. Biochim Biophys Acta

Meuser D, Splitt H, Wagner R, Schrempf H (1999) Exploring the open pore of the potassium channel from Streptomyces lividans. FEBS Lett 462:447–452

Morais-Cabral JH, Zhou Y, MacKinnon R (2001) Energetic optimization of ion conduction rate by the K + selectivity filter. Nature 414:37–42

Nishida M, Cadene M, Chait BT, MacKinnon R (2007) Crystal structure of a Kir3.1-prokaryotic Kir channel chimera. EMBO J 26:4005–4015

Oliver D, Hahn H, Antz C, Ruppersberg JP, Fakler B (1998) Interaction of permeant and blocking ions in cloned inward-rectifier K + channels. Biophys J 74:2318–2326

Perozo E, Cortes DM, Cuello LG (1999) Structural rearrangements underlying K + -channel activation gating. Science 285:73–78

Raja M (2010) The role of extramembranous cytoplasmic termini in assembly and stability of the tetrameric K(+)-channel KcsA. J Membr Biol 235:51–61

Raja M (2011) Diverse gating in K + channels: differential role of the pore-helix glutamate in stabilizing the channel pore. Biochem Biophys Res Commun 413:1–4

Raja M, Vales E (2009a) Changing Val-76 towards Kir channels drastically influences the folding and gating properties of the bacterial potassium channel KcsA. Biophys Chem 144:95–100

Raja M, Vales E (2009b) Dissimilarity in the channel intrinsic stability among the bacterial KcsA and the inwardly rectifying potassium channel ROMK1. Biochimie 91:1426–1433

Raja M, Vales E (2010) Sodium permeability and sensitivity induced by mutations in the selectivity filter of the KcsA channel towards Kir channels. Biochimie 92:232–244

Raja M, Vales E (2011) Improved technique for reconstituting incredibly high and soluble amounts of tetrameric K channel in natural membranes. J Membr Biol 241:141–144

Raja M, Spelbrink RE, de Kruijff B, Killian JA (2007) Phosphatidic acid plays a special role in stabilizing and folding of the tetrameric potassium channel KcsA. FEBS Lett 581:5715–5722

Ramjeesingh M, Huan LJ, Garami E, Bear CE (1999) Novel method for evaluation of the oligomeric structure of membrane proteins. Biochem J 342(Pt 1):119–123

Rauer H, Lanigan MD, Pennington MW, Aiyar J, Ghanshani S, Cahalan MD, Norton RS, Chandy KG (2000) Structure-guided transformation of charybdotoxin yields an analog that selectively targets Ca(2+)-activated over voltage-gated K(+) channels. J Biol Chem 275:1201–1208

Sansom MS, Capener CE (2002) Ion channels: open at last. Curr Biol 12:R566–R568

Schrempf H, Schmidt O, Kummerlen R, Hinnah S, Muller D, Betzler M, Steinkamp T, Wagner R (1995) A prokaryotic potassium ion channel with two predicted transmembrane segments from Streptomyces lividans. EMBO J 14:5170–5178

Shealy RT, Murphy AD, Ramarathnam R, Jakobsson E, Subramaniam S (2003) Sequence-function analysis of the K + -selective family of ion channels using a comprehensive alignment and the KcsA channel structure. Biophys J 84:2929–2942

Splitt H, Meuser D, Borovok I, Betzler M, Schrempf H (2000) Pore mutations affecting tetrameric assembly and functioning of the potassium channel KcsA from Streptomyces lividans. FEBS Lett 472:83–87

Thompson GA, Leyland ML, Ashmole I, Sutcliffe MJ, Stanfield PR (2000) Residues beyond the selectivity filter of the K + channel kir2.1 regulate permeation and block by external Rb + and Cs+. J Physiol 526(Pt 2):231–240

Valiyaveetil FI, Zhou Y, MacKinnon R (2002) Lipids in the structure, folding, and function of the KcsA K + channel. Biochemistry-Us 41:10771–10777

Yang J, Yu M, Jan YN, Jan LY (1997) Stabilization of ion selectivity filter by pore loop ion pairs in an inwardly rectifying potassium channel. Proc Natl Acad Sci U S A 94:1568–1572

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 A resolution. Nature 414:43–48