Biochemical and Biophysical Research Communications 413 (2011) 1–4

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Mini Review

Diverse gating in K^+ channels: Differential role of the pore-helix glutamate in stabilizing the channel pore

Mobeen Raja *

School of Molecular and Systems Medicine, 6126 HRIF East, Alberta Diabetes Institute, University of Alberta, Edmonton AB, Canada T6G 2E1

ARTICLE INFO

Article history:
Received 28 July 2011
Available online 22 August 2011

Keywords:
KcsA
Inwardly rectifying K^+ channels
Kir
Pore-helix glutamate
Carboxyl–carboxylate interaction
Salt bridge
Channel pore
Channel activation–inactivation

ABSTRACT

The selectivity filter and adjacent regions in the bacterial KcsA and inwardly rectifying K^+ (Kir) channels reveal significant conformational changes that cause the channel pore to transition from an activated to inactive state (C-type inactivation) once the channel is open. The meshwork of residues stabilizing the pore of KcsA involves Glu71–Asp80 carboxyl–carboxylate interaction 'behind' the selectivity filter. Interestingly, the Kir channels do not have this exact interaction, but instead have a Glu–Arg salt bridge where the Glu is in the same position but the Arg is one position N-terminal compared to the Asp in KcsA. Also, the Kir channels lack the Trp that hydrogen bonds to Asp80 in KcsA. Here, the sequence and structural information are combined to understand the dissimilarity in the role of the pore-helix Glu in stabilizing the pore structure in KcsA and Kir channels. This review illustrates that although Glu is quite conserved among both types of channels, the network of interactions is not translatable from one channel to the other; thereby suggesting a unique phenomenon of diverse gating patterns in K^+ channels.

1. Introduction

Potassium (K^+) channels are increasingly being elucidated as molecular targets in a number of pathophysiologic states, and they continue to trigger considerable enthusiasm as drug targets. 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 has been highlighted by the recent outbreaks of discoveries linking K^+ channel mutations to various inherited disorders. The major discovery of the crystal structure of the KcsA channel established a design of K^+ channel structure with 3.2 Å resolution [1,2]. In KcsA and most K^+-channels the selectivity filter comprises the "canonical" filter sequence TXGYGDX, where X represents an aliphatic amino acid (Fig. 1). The corresponding residues in eukaryotic inwardly rectifying K^+ (Kir) channels are TXGYGFR, with Y and F sometimes replaced by another amino acid [3].

The crystal structure of the KcsA channel in its open-inactivated conformation provides a mechanism of C-type inactivation gating at the selectivity filter from channels 'trapped' in a series of partially open conformations [2]. In KcsA, the rate and extent of C-type inactivation is contingent upon interactions involving residues Trp67, Glu71, and Asp80 behind the selectivity filter [4,5]. The side chain of Glu71, a residue that deeply influences C-type inactivation in KcsA, seems to undergo a large rotameric reorientation of about 90° which may alter the strength of the carboxyl–carboxylate interaction with residue Asp80 [6]. Interestingly, a number of mutational studies [4,7–9] together with the sequence and structural similarities of KcsA and eukaryotic K^+ channels in the region of the selectivity filter [10] as well as side-chain rearrangements at position 103 [11] strongly suggest that the activation and inactivation processes in KcsA and a variety of other K^+ channels are mechanistically similar.

A considerable amount of functional data indicate that in eukaryotic K^+ channels C-type inactivation involves a localized constriction in the outer vestibule of the selectivity filter [3]. Analogous flanking pairs of ionizable side chains are found solely among the members of the Kir channel family. All Kir channels are tetrameric proteins of one-pore/two-transmembrane (1P/2TM) domain subunits that equally contribute to the formation of highly selective K^+ channels. In these channels, residues equivalent to Glu and Arg (E71 and L81 at the corresponding position in KcsA, respectively) have been shown to form an ionic bond critical in stabilizing the selectivity filter [12,13].

Although KcsA exhibits significant sequence similarity with the members of the voltage-gated K^+-channels (Kv) family, that with Kir channels is quite low (30% in the pore region and 15% overall) [14]. Taken into account several structural and functional differences among KcsA and Kir channels the well characterized meshwork of pore stabilizing network of residues in KcsA is apparently different in Kir channels (Fig. 1). The structural consideration is therefore required to improve our understanding of the pore

* Fax: +1 780 492 8836.
E-mail addresses: mraja@pmcol.ualberta.ca, mobeenraja@yahoo.com

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.08.062

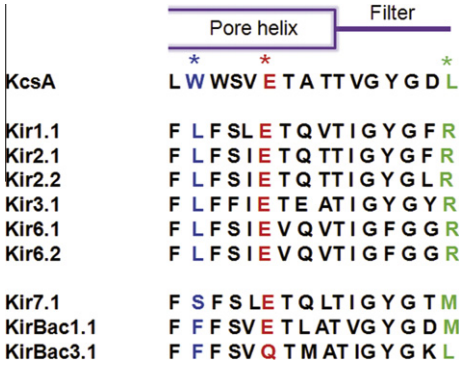

Fig. 1. Differences and conservation of signature sequence and pore residues among KcsA and some Kir K^+ channels. The conserved or different residues are highlighted by asterisks.

stability in diverse K^+-channels. In particular, the pore-helix Glu is quite conserved in many Kir channels, but absent in Kv channels, as determined by comprehensive sequence alignment [15]. This Glu is a key residue which controls the overall structure of the pore during channel gating in KcsA and many Kir channels via a dissimilar mechanism; hence it is the main focus of this mini review.

2. Carboxyl–carboxylate interaction in KcsA channel: how does it stabilize the pore structure?

It is well known that the conductive conformation of the selectivity filter is metastable and can undergo a spontaneous transition into a long-lived nonconducting state [7]. This inactivation process is suppressed by an E71A mutation in the pore helix, but the tetrameric stability of KcsA channel is unaffected [4,16]. Although the intracellular gate is structurally open, the channel is functionally inactivated. In KcsA, an interaction between Glu71, Asp80, and Trp67 leads to destabilization of the conductive conformation of the filter (Fig. 2A) and promotes inactivation. Eliminating the carboxyl–carboxylate interaction between Glu71 and Asp80 increases the flexibility of the side chain of Asp80 and weakens its hydrogen bonding to Trp67 thus preventing the channel from entering the inactivated state [4]. Furthermore, in the absence of C-type inactivation, mutations E71I or E71Q expose a series of kinetically distinct modes of gating, as seen in wild-type KcsA channels, in a side chain-specific way. These findings suggest that specific interactions in the side chain network surrounding the selectivity filter, in concert with ion occupancy, alter the relative stability of preexisting conformational states of the pore [17]. Furthermore, E71 mutations differentially destabilize the detergent-solubilized tetramer; among them, the E71V neutralization mutant has a relatively subtle effect, i.e., E71V mutant retains K^+/Na^+ selectivity, and its conductance in the outward direction is unaltered. These results suggest that the E71 side chain is not a primary determinant of ion selectivity or conduction in KcsA, either directly or through the E71:D80 carboxyl–carboxylate bridge [17].

The outward 'flipping' of the Asp80 side chain relative to its position in wild-type KcsA [17] is a movement reminiscent of the conformation observed in one of the crystal forms of E71A [4] (the so-called 'flipped' structure) and in the selectivity filter of Kir3.1–KirBac1.3 chimeric channel [18]. The overall mobility of the Asp80 side chain is enhanced when there is no interaction with Glu71. In contrast, most Kv channels have a valine at the position corresponding to Glu71 in KcsA indicating that some of the conformational states of the filter, as deduced by Glu71 mutational analysis, might play a role in other members of a K^+ channel family [17].

3. A salt bridge in Kir pore stability: can this contribute to the structural and functional diversity of K^+ channels?

In the absence of an X-ray structure for Kir6.2, the structure of KcsA was used as a basis for generating homology models of Kir6.2 that were studied by molecular dynamics (MD) simulation [14,19]. Furthermore, Kir6.2 was modeled to explain how extracellular residues (in the M1-P loop) contribute to the channel gating differences between Kir6.1 and Kir6.2 [20]. Later, the groups of Sansom and Ashcroft validated homology models of different Kir channels based on KirBac, Kir6.2 as well as the more recent chimeric Kir structure [21–23]. Indeed, a crystal structure of the cKir2.2 channel could provide high resolution structural data for the pore of a Kir channel [24], which may now serve as a model Kir channel. This structure shows that the pore Glu forms a salt bridge with a highly conserved Arg above it. The pore region is attached by an ionized hydrogen bond between R149 in the filter sequence TXGYGFR and E139 (Fig. 2B). The distance between Glu O-ε and Arg N-η is 2.4 Å which is perfectly compatible with an energetically strong interaction. Mutations altering this interaction have been shown to alter channel function [12,13]. For instance, muta-

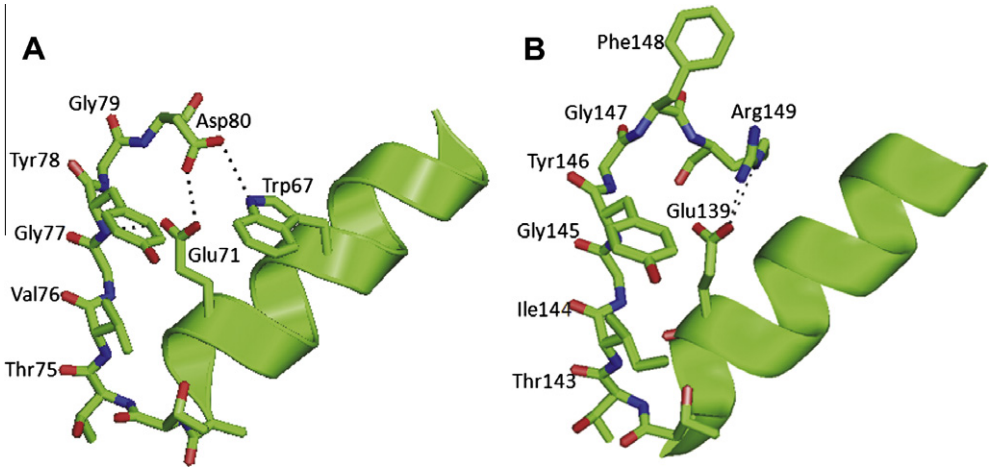

Fig. 2. (A) A single-subunit P-loop (KcsA, NCBI accession number P0A334) is shown with positions 67, 71, and 74–80 in stick representation. In the wild type KcsA channel, the interactions between Glu71, Asp80, and Trp67 stabilize the channel conformation and promote C-type inactivation. (B) A single-subunit P-loop (cKir2.2, PDB accession number 3JYC) is shown with positions 139 and 142–149 in stick representation. The salt bridge between conserved residues Glu and Arg stabilizes the pore structure in Kir channels. All structures were constructed in a PyMol modeling program.

tion of R148E in Kir2.1 results in a non-functional channel [13]. Furthermore, the mutation of the equivalent Glu and Arg residues in Kir3.4 causes dramatic loss of ion selectivity and inward rectification in the Kir3.1/Kir3.4 channel [12]. The crystal structure clearly demonstrates that this interaction ties together two segments of the pore region within a single subunit [24]. Despite the presence of substantially different protein contacts surrounding the selectivity filter, the main-chain structure of the filter in Kir2.2 is the same as in other K^+-channels [6]. It is important to mention that the conserved cysteine pair in eukaryotic Kir channels (e.g., Kir2.2 [24]) creates a circularized pore region via disulfide bond between Cys123 and Cys155 (Fig. 3A). The existence of a disulfide bond could be confirmed by mutagenesis studies indicating that the mutation of the corresponding cysteines in Kir2.1 abolishes the channel activity [25]. While this bond is not "squeezing" the selectivity filter as the E71–D80 interaction in KcsA is considered to do, it may, perhaps, rigidify the top of the Kir channel pore. Such situation is completely absent in KcsA (Fig. 3B) suggesting again that E71–D80 is the only interaction that condenses the channel pore.

Furthermore, most Kir channels have a Phe, Tyr or Gly at the position corresponding to Asp80 in KcsA (Fig. 1). This position seems not to be involved in the molecular interactions stabilizing the channel pore in Kir channels. Also, Kir channels have a Leu at the position corresponding to Trp67 in KcsA (compare Fig. 3A and B). This indicates that Phe 134 may not be involved in intra-protein interactions stabilizing the pore structure, but its role as an anchoring residue at the membrane–water interfacial regions (see review [26]). Such differences in interactions stabilizing the pore structure in KcsA and Kir channels may also suggest one of the reasons of structural and functional differences among these channels, in addition to the interactions affecting the channel intrinsic stability and subunit assembly in the selectivity filter [27,28].

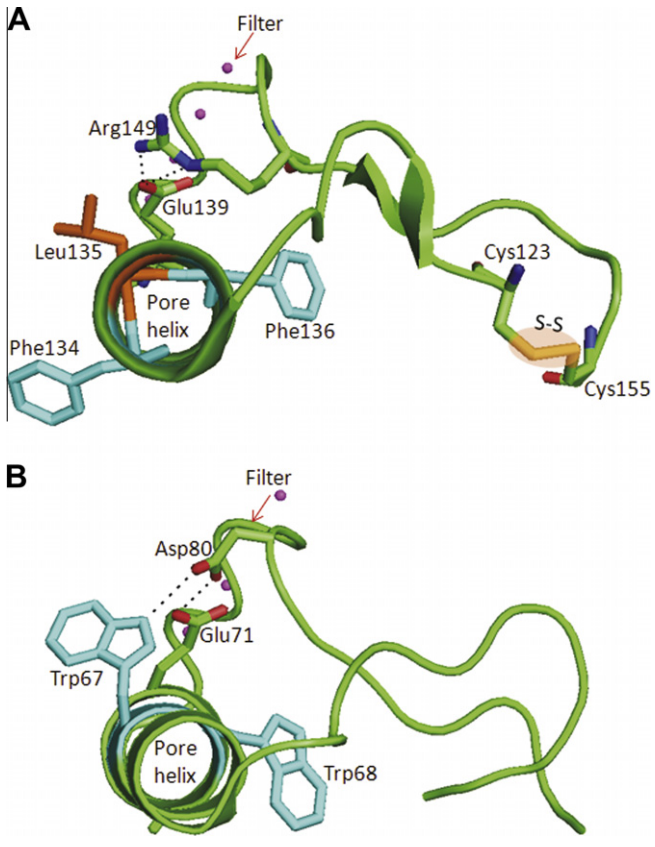

Fig. 3. Top views of the pore regions of Kir2.2 (A) and KcsA (B) single monomeric subunits. The views are adjusted to elaborate the differences in the arrangement of aromatic residues with regard to the pore-helix as well as the meshwork of interactions stabilizing the channel pore structure. K^+ ions (in purple) are placed in the selectivity filter. (For interpretation of the references to colors in this figure legend, the reader is referred to the web version of this paper).

4. Summary and hypothetical perspective

The current view about the K^+-channel KcsA in defining the pore-helix, selectivity filter and external vestibule as dynamic structures can stimulate major concerns regarding conformational changes including shifts in the filter backbone that can drastically affect the gating behavior in other K^+-channels [17]. Here an important consideration into structural differences among KcsA and Kir channels is described to further refine the pore stabilizing differences in K^+-channels by using KcsA and Kir2.2 as model proteins. The E71–D80 carboxyl–carboxylate interaction in KcsA has been well studied experimentally and computationally and structures exist of a variety of E71x mutants as discussed in this review. The current theory is that these and other similar interactions cause the selectivity filter to transition from an activated to inactive state in a dissimilar fashion. Although all Kir channel subfamily members express the conserved Glu–Arg charges and are expected to contain the same intra-subunit salt bridge [12] the absence of this salt bridge in Kir7.1 or prokaryotic Kirs, which still conduct and gate perfectly, may suggest that it may be a feature specific to the physiological role of Kir channels in different cell types. This review highlights an interesting comparison between the sequences of diversified Kir and KcsA channels that could explain why Kir channels present phenotypes that differ from those of other K^+ channels. Experimental studies involving mutagenesis, rigorous simulations combined with a detailed mechanistic analysis and free energy calculations should be performed to demonstrate the validity of this concept. However, the compensatory changes in side chain packing upon point mutation may be difficult to predict, and the effects may be diverse even within a K^+ channel family.

References

[1] D.A. Doyle, C.J. Morais, R.A. Pfuetzner, A. Kuo, J.M. Gulbis, S.L. Cohen, B.T. Chait, R. MacKinnon, The structure of the potassium channel: molecular basis of K^+ conduction and selectivity, Science 280 (1998) 69–77.
[2] L.G. Cuello, V. Jogini, D.M. Cortes, E. Perozo, Structural mechanism of C-type inactivation in K^+ channels, Nature 466 (2010) 203–208.
[3] Y. Liu, M.E. Jurman, G. Yellen, Dynamic rearrangement of the outer mouth of a K+ channel during gating, Neuron 16 (1996) 859–867.
[4] J.F. Cordero-Morales, L.G. Cuello, Y. Zhao, V. Jogini, D.M. Cortes, B. Roux, E. Perozo, Molecular determinants of gating at the potassium-channel selectivity filter, Nat. Struct. Mol. Biol. 13 (2006) 311–318.
[5] J.F. Cordero-Morales, V. Jogini, A. Lewis, V. Vásquez, D.M. Cortes, B. Roux, E. Perozo, Molecular driving forces determining potassium channel slow inactivation, Nat. Struct. Mol. Biol. 14 (2007) 1062–1069.
[6] Y. Zhou, J.H. Morais-Cabral, A. Kaufman, R. MacKinnon, Chemistry of ion coordination and hydration revealed by a K^+ channel–Fab complex at 2.0 Å resolution, Nature 414 (2001) 43–48.
[7] J.F. Cordero-Morales, L.G. Cuello, E. Perozo, Voltage-dependent gating at the KcsA selectivity filter, Nat. Struct. Mol. Biol. 13 (2006) 319–322.
[8] O. Yifrach, R. MacKinnon, Energetics of pore opening in a voltage-gated K^+ channel, Cell 111 (2002) 231–239.
[9] E.M. Ogielska, W.N. Zagotta, T. Hoshi, S.H. Heinemann, J. Haab, R.W. Aldrich, Cooperative subunit interactions in C-type inactivation of K^+ channels, Biophys. J. 69 (1995) 2449–2457.
[10] S.B. Long, E.B. Campbell, R. Mackinnon, Crystal structure of a mammalian voltage-dependent Shaker family K^+ channel, Science 309 (2005) 897–903.
[11] L.G. Cuello, V. Jogini, D.M. Cortes, A.C. Pan, D.G. Gagnon, O. Dalmas, J.F. Cordero-Morales, S. Chakrapani, B. Roux, E. Perozo, Structural basis for the coupling between activation and inactivation gates in K^+ channels, Nature 466 (2010) 272–275.
[12] K.M. Dibb, T. Rose, S.Y. Makary, T.W. Claydon, D. Enkvetchakul, R. Leach, C.G. Nichols, M.R. Boyett, Molecular basis of ion selectivity, block, and rectification of the inward rectifier Kir3.1/Kir3.4 channel, J. Biol. Chem. 278 (2003) 49537–49548.

[13] J. Yang, M. Yu, Y.N. Jan, L.Y. Jan, Stabilization of ion selectivity filter by pore loop ion pairs in an inwardly rectifying potassium channel, Proc. Natl. Acad. Sci. USA 94 (1997) 1568–1572.
[14] C.E. Capener, I.H. Shrivastava, K.M. Ranatunga, L.R. Forrest, G.R. Smith, M.S. Sansom, Homology modeling and molecular dynamics simulation studies of an inward rectifier potassium channel, Biophys. J. 78 (2000) 2929–2942.
[15] R.T. Shealy, A.D. Murphy, R. Ramarathnam, E. Jakobsson, S. Subramaniam, Sequence-function analysis of the K^+-selective family of ion channels using a comprehensive alignment and the KcsA channel structure, Biophys. J. 84 (2003) 2929–2942.
[16] E. Vales, M. Raja, The "flipped" state in E71A-K^+-channel KcsA exclusively alters the channel gating properties by tetraethylammonium and phosphatidylglycerol, J. Membr. Biol. 234 (2010) 1–11.
[17] S. Chakrapani, J.F. Cordero-Morales, V. Jogini, A.C. Pan, D.M. Cortes, B. Roux, E. Perozo, On the structural basis of modal gating behavior in K^+ channels, Nat. Struct. Mol. Biol. 18 (2011) 67–74.
[18] M. Nishida, M. Cadene, B.T. Chait, R. MacKinnon, Crystal structure of a Kir3.1-prokaryotic Kir channel chimera, EMBO J. 26 (2007) 4005–4015.
[19] M.S. Sansom, C.E. Capener, Ion channels: open at last, Curr. Biol. 12 (2002) R566–568.
[20] V.P. Repunte, H. Nakamura, A. Fujita, Y. Horio, I. Findlay, L. Pott, Y. Kurachi, Extracellular links in Kir subunits control the unitary conductance of SUR/Kir6.0 ion channels, EMBO J. 18 (1999) 3317–3324.
[21] P.J. Stansfeld, R. Hopkinson, F.M. Ashcroft, M.S.P. Sansom, PIP2-binding site in Kir channels: definition by multiscale biomolecular simulations, Biochemistry 48 (2009) 10926–10933.
[22] S. Haider, A.I. Tarasov, T.J. Craig, M.S.P. Sansom, F.M. Ashcroft, Identification of the PIP2-binding site on Kir6.2 by molecular modeling abd functional analysis, EMBO J. 26 (2007) 3749–3759.
[23] K. Tai, S. Haider, A. Grottesi, M.S.P. Sansom, Ion channel gates: comparative analysis of energy barriers, Eur. Biophys. J. 38 (2009) 347–354.
[24] X. Tao, J.L. Avalos, J. Chen, R. MacKinnon, Crystal structure of the eukaryotic strong inward-rectifier K^+ channel Kir2.2 at 3.1 A resolution, Science 326 (2009) 1668–1674.
[25] H.C. Cho, R.G. Tsushima, T.T. Nguyen, H.R. Guy, P.H. Backx, Two critical cysteine residues implicated in disulfide bond formation and proper folding of Kir2.1, Biochemistry 39 (2000) 46–49.
[26] M. Raja, The potassium channel KcsA: a model protein in studying membrane protein oligomerization and stability of oligomeric assembly?, Arch Biochem. Biophys. 510 (2011) 1–10.
[27] M. Raja, E. Vales, Dissimilarity in the channel intrinsic stability among the bacterial KcsA and the inwardly rectifying potassium channel ROMK1, Biochimie 91 (2009) 1426–1433.
[28] M. Raja, E. Vales, Sodium permeability and sensitivity induced by mutations in the selectivity filter of the KcsA channel toward Kir channels, Biochimie 92 (2010) 232–244.