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Crucial Points within the Pore as Determinants of K⁺ Channel Conductance and Gating

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Abstract

While selective for K⁺ ions, K⁺ channels vary significantly among their rate of ion permeation. Here we probe the effect of steric hindrance and electrostatics within the ion conduction pathway on K⁺ permeation in the MthK K⁺ channel using structure-based mutagenesis combined with single channel electrophysiology and x-ray crystallography. We demonstrate that changes in side chain size and polarity at Ala88, which forms the constriction point of the open MthK pore, have profound effects on the single channel conductance as well as open probability. We also reveal that the negatively charged Glu92s at the intracellular entrance of the open pore form an electrostatic trap, which stabilizes a hydrated K⁺ ion and facilitates the ion permeation. This electrostatic attraction is also responsible for intracellular divalent blockage, which renders the channel inward rectified in the presence Ca²⁺. In light of the high structural conservation of the selectivity filter, the size and chemical environment differences within the portion of the ion conduction pathway other than the filter are likely the determinants for the conductance variations among K⁺ channels.

Keywords

MthK K⁺ channel; steric hindrance; electrostatics; constriction point; single channel electrophysiology

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Accession numbers. The atomic coordinates and structural factors for the K⁺ complexes of MthK pore E92Q mutant have been deposited in the Protein Data Bank with the accession number 3R65.

Introduction

Tetrameric cation channels form an ion conduction pathway within the cell membrane, allowing efficient ion permeation through an otherwise unfavorable environment¹. In K⁺ channels, a highly conserved structural element, the selectivity filter, stabilizes dehydrated K⁺ ions and allows for rapid and energetically efficient ion dehydration, transfer, and rehydration during the transport cycle^{2; 3}. Positioned at the extracellular end, the selectivity filter occupies less than half of the entire ion conduction pathway; the remaining portion connects the filter to the intracellular solution and is encircled by the pore-lining inner helices whose conformational changes also control channel gating (Fig. 1). As exemplified by the KcsA structure^{4; 5}, the four inner helices of a K⁺ channel form a bundle crossing at the intracellular end of the pathway and create a water-filled central cavity immediately below the filter where hydrated ions can reside (Fig. 1A). The bundle crossing represents a closed intracellular gate with a diameter of less than 3 Å, occluding the passage of hydrated K⁺ ions. Upon channel opening, the inner helices bend at a conserved glycine hinge and splay the intracellular gate wide open as seen in the open pore structure of the Ca²⁺-gated MthK K⁺ channel from *Methanobacterium thermoautotrophicum* (Fig. 1B)^{6; 7; 8}. Consequently, the central cavity becomes a wider vestibule that is continuous with the intracellular bulk solvent and through which hydrated ions can diffuse into or out of the cavity.

While all K⁺ channels contain a highly conserved selectivity filter for K⁺-selective ion transfer, the rate of ion permeation as measured by unitary conductance varies significantly among different K⁺ channels. Two rate-limiting steps could define the channel conductance: ion transfer across the selectivity filter and ion diffusion to the filter. As the water-filled vestibule of a K⁺ channel in the open conformation provides an ion diffusion passageway from the intracellular bulk solution to the filter and accounts for more than half of the ion conduction pathway within the membrane, the geometry and chemical environment of the vestibule are likely to play an important role in regulating the rate of ion diffusion to the filter and, thereby, determine channel conductance. Indeed, the presence of negatively charged residues at the intracellular entrance of the channel pore has been shown to significantly increase the conductance in the BK and KcsA K⁺ channels^{9; 10}. In this study, by taking advantage of our recently determined high-resolution structure of the open MthK pore, we performed structure-based mutagenesis to systematically probe the effect of both geometry and chemical environment within the MthK ion conduction pathway on channel conductance. The mutagenesis focuses on two key positions: Ala88, which forms the constriction point in the open pore⁷, and Glu92, whose negatively charged side chain forms an electrostatic trap that stabilizes hydrated cations at the intracellular entrance of the pore. Combining single channel electrophysiology and crystal structure analysis, we demonstrate that both electrostatic attractions and small steric hindrance contribute to the high conductance of the MthK K⁺ channel.

Results

Effect of steric hindrance at the constriction point on channel conductance

In the open MthK pore, the side chain of Ala88 points towards the central cavity and forms a constriction point within the ion conduction pathway with a diameter of about 9 Å (Fig 1B). This residue at the equivalent position among most other K⁺ channels is conserved as a small-sized amino acid, which has been suggested to be necessary for ion conduction⁷. To test the effect of side-chain size at this position on channel conductance, Ala88 was replaced with larger hydrophobic residues including Val (A88V), Leu (A88L) and Phe (A88F), which consequentially would lead to a reduction of the diameter of the constriction point to approximately 7 Å, 6 Å and 5 Å respectively, as estimated by replacing Ala88 in the high-

resolution open MthK pore structure with the corresponding residue (Fig. 1C). Although this simple modeling may not accurately define the side chain position in each mutant, it provides a reasonable estimation of the decrease in the open pore size. Figure 2A shows sample traces of the inward currents of these mutants recorded at -100 mV in the presence of symmetrical 150 mM KCl. The single channel currents of these mutants show a progressive decrease with increasing side chain size, with the exception of the A88F mutant, which, despite having a larger side chain, actually displays a slightly larger conductance than that of the A88L mutant. Under the experimental conditions, the measured unitary conductance at -100 mV are roughly 220 pS, 100 pS, 35 pS, and 40 pS for the wild-type, A88V, A88L, and A88F, respectively. In addition, these mutations also exert strong effects on channel gating as will be discussed further subsequently.

Steric hindrance can be offset by polar or charged side chains

While a larger hydrophobic residue at position 88 reduces the K^+ permeation rate, this steric hindrance effect can be mitigated by polar or negatively charged amino acids. For instance, when Ala88 was replaced by an Asn – whose side chain size is similar to that of Leu – the mutant channel exhibits a conductance of about 120 pS at -100 mV, much larger than that of A88L (Fig. 2B). Replacing Ala88 with the negatively charged Asp gives rise to an even higher conductance of about 240 pS at -100 mV similar to that of the wild-type channel, indicating that the negative charges create a more electrostatically favorable environment for the permeating K^+ ions in the open pore (Fig. 2B). This electrostatic effect may also explain the unexpected slightly larger conductance of A88F in comparison to A88L, as the electron-rich benzene ring of Phe88 can stabilize a K^+ ion through cation- π electron interaction.

Intracellular Ca^{2+} blockage is not affected by A88 mutations

Divalent cations such as Ca^{2+} have been shown to bind the MthK pore and block the channel from the intracellular side, rendering the channel inward rectified¹¹. This Ca^{2+} dependent rectification of MthK is not affected by mutations at Ala88, as demonstrated by the I-V curve of various A88 mutants in the presence or absence of 1 mM Ca^{2+} (Fig. 2C). The plot of the unblocked outward current as a function of $[Ca^{2+}]$ gives rise to K_i values of about 0.52 mM, 0.52 mM, and 0.86 mM for the wild-type, A88D, and A88V, respectively, not significantly different from each other. Due to the extremely low conductance, the concentration-dependent Ca^{2+} blockage of A88L and A88F was not measured.

Gating effect of Ala88 mutations

In addition to channel conductance, mutations at Ala88 also have profound effects on channel gating. While Ca^{2+} binding at the intracellular octameric gating ring of the MthK channel cooperatively activates the pore opening^{11; 12; 13; 14}, the Ala88 mutations can influence the Ca^{2+} activation in opposite directions depending on the chemistry and the size of the side chain (Fig. 3A). The introduction of a negative charge as in the A88D mutant yields a constitutively activated channel with extremely high open probability ($P_o \sim 0.9$) even in the absence of Ca^{2+} ; and the addition of intracellular Ca^{2+} no longer has much effect on channel opening. The A88V mutant, on the other hand, shows extremely low open probability even in the presence of 10 mM Ca^{2+} . The gating behavior of the A88L mutant is similar to that of the A88V mutant. Interestingly, replacing A88 with the larger hydrophobic Phe residue gave rise to a mutant channel with a much higher sensitivity to Ca^{2+} activation than was observed for the wild-type channel. The single channel P_o of A88F reaches about 0.3 in the absence of Ca^{2+} ; 1 mM intracellular $[Ca^{2+}]$ is sufficient to fully activate the channel with P_o above 0.9 (Fig. 3A), indicating that a bulky hydrophobic residue at position 88 can facilitate channel opening.

In the absence of a closed MthK structure, insights gained from the NaK channel, whose gating mechanics are believed to be similar to that of K⁺ channel pores^{15; 16}, provide a plausible explanation for the gating effect of the Ala88 mutations in MthK. Despite being a non-selective cation channel, NaK shares high sequence and structure homology with K⁺ channels; its closed and open structures superimpose extremely well with those of the closed KcsA and open MthK pore, respectively, with main chain rootmean-square deviation (excluding the selectivity filter) of less than 1 Å¹⁶. In the open NaK structure, the side-chain of Phe92, which is equivalent to Ala88 of MthK, also points towards the central pathway and is exposed to solvent (Fig. 3B). In the closed state, Phe92 points tangential to the central pathway and participates in inter-subunit helical packing with the hydrophobic side chains of Val91, Phe94, Ile95, and Leu98 from the neighboring inner helix (Fig. 3C). Similar conformational changes likely occur in MthK upon gating, and perturbation of the pore stability in either the closed or open state will directly affect the channel open probability. Based on the closed NaK structure, the Ala88 of MthK in the closed state is expected to form similar hydrophobic packing with residues Phe87, Ala90, Val91, and Leu94 from the neighboring inner helix (Fig. 3D). The presence of a negative charge in the A88D mutation is clearly unfavorable for such hydrophobic interactions and therefore destabilizes the closed state, resulting in a channel with higher stability in the open state where the charged side chain is exposed in the water-filled cavity. In addition to the side chain chemistry, the size and steric fit of the residue at position 88 will also affect the stability of this hydrophobic packing. Replacing Ala88 with Val or Leu can enhance the hydrophobic contact and, therefore, further stabilize the closed pore, whereas a bulky aromatic ring, as in the A88F mutant, may result in steric clash and, consequently, destabilizes the closed pore and makes the channel more susceptible to Ca²⁺ activation.

Electrostatic tuning of channel conductance at the intracellular entryway

Eight acidic residues on the C-terminal end of the MthK inner helices, two from each subunit (Glu92 and Glu96), form two rings of negative charges at the intracellular entrance to the open pore (Fig 5A). Equivalent glutamate residues are also seen in BK channels and have been shown to be important for the large conductance of BK^{9; 10}. To test if these two acidic residues have a similar effect on MthK conductance, they were individually replaced with Gln. As compared to the wild-type channel, the E92Q mutant has reduced single channel currents in both directions; its unitary conductance is roughly 140 pS for an inward current measured at −100 mV and about 40 pS for an outward current recorded at +100mV (Fig. 4A); the higher reduction of outward current renders the channel inward rectified in the absence of Ca²⁺ (Fig. 4C), similar to what was observed in BK channels⁹. Furthermore, the E92Q mutation also abolishes the Ca²⁺ blockage on the intracellular side as shown by the plot of Ca²⁺ dependent blockage of the outward current (Fig. 4D). Qualitatively, the E96Q mutation appears to have similar effect on channel conductance as E92Q; it significantly reduces the outward conductance to about 30 pS at 100 mV (Fig 4B). For the inward conductance, at least two conducting states were observed in the E96Q mutant with the state of lower conductance (~160 pS at −100 mV) being predominant (Fig. 4B). The E96Q mutation also facilitates channel opening and increases channel open probability in the absence of Ca²⁺. However, the cause of this gating effect is unclear and requires further investigation.

Structural comparison between the open MthK pore (at 1.45 Å, PDB code 3LDC) and the E92Q mutant – determined at 1.8 Å (Table 1) – provides us with structural insights into the electrostatic tuning of K⁺ permeation in MthK. In the open MthK pore structure (Fig 5A), the negative charges from the four Glu92 residues, one from each subunit, create an electrostatic trap that stabilizes a hydrated K⁺ ion at the center of the intracellular entryway as shown in the 2F_o-F_c electron density map of this region (Fig 5B). The density of this

bound K^+ ion is absent in the high resolution structure of the E92Q mutant as demonstrated in its $2F_o-F_c$ map (Fig. 5C) as well as in the $F_{wt}-F_{E92Q}$ difference map – both calculated at 1.8 Å (Fig. 5D) – indicating the elimination or weakening of K^+ binding in the absence of negative charges. The electron density of the bound K^+ ion at the intracellular entrance is much weaker than that of the K^+ ions in the selectivity filter, suggesting lower affinity binding. Stabilized by electrostatics, this weak ion binding site is likely to be non-selective and could have higher affinity for divalent cations, which explains the divalent ion (Ca^{2+} or Mg^{2+}) blockage from the intracellular side.

Discussion

Here we experimentally demonstrate the dependence of ion permeation in the MthK K^+ channel on steric hindrance and electrostatics within the ion conduction pathway. The unique position of residue Ala88 makes it a pivotal point for both channel conductance and gating. In the open state, the Ala88 side chain points towards the central cavity and forms the constriction point within the ion conduction pathway; changing the side chain size or polarity at this position can have profound effects on the ion permeation rate. The effect of side chain size on channel conductance has also been suggested in the study of the NaK channel in which replacing the equivalent residue, Phe92, with Ala resulted in a significant increase in ion flux and allowed us to measure single channel conductance that was otherwise impossible. The role of the constriction created by Ala88 in determining the conductance of MthK can be extended to explain the current reduction effect of the A306T missense mutation in the human KCNQ2 channel, which causes Benign Familial Neonatal Convulsions and is at the equivalent position to Ala88 of MthK^{17; 18}.

As a consequence of the inner helix rotation upon channel gating, Ala88 of the closed MthK likely points away from the ion conduction pathway and makes hydrophobic contact with residues on the neighboring inner helix; therefore, mutations at this position can change the stability of the closed pore and hence the open probability. In addition to the necessity of having a small side chain at Ala88 for large conductance, the presence of negative charges (Glu92 and Glu96) at the intracellular entrance of the open pore creates an electrostatic trap for a hydrated K^+ ion, which effectively increases the local K^+ concentration and further enhances the ion permeation.

The high structural conservation of the selectivity filter among K^+ channels raises the possibility that the rate of ion transfer across the filter is likely similar in these channels, and it is the difference in geometry and chemistry of the open pore vestibule that attribute to the variation in channel conductance. This principle of steric and electrostatic influence on channel conductance is likely to be general and should also apply to other channel families.

While MthK has a wide open pore with its Ala88 near the central cavity forming the constriction point, other K^+ channels such as some voltage-gated ones may have a much smaller open gate^{19; 20; 21}, leaving residues at the intracellular entrance to define the size of the open pore and control the rate of ion diffusion into or out of the pore. In other words, the constriction point that defines the geometric accessibility for ion permeation from bulk solvent into the pore can occur anywhere along the ion conduction pathway. Actually, a closed K^+ channel gate represents the extreme case of steric hindrance at the intracellular entrance, which occludes the passage of hydrated K^+ ions. Some recent molecular dynamics simulations on nonopores provide a plausible theoretical explanation for the size effect on channel conductance^{22; 23}. In these computational studies, it was suggested that the water density oscillates between liquid and vapor states in a narrow hydrophobic pore, which affects the permeability of water molecules and ions. The critical radius for the phase transition in the narrow pore depends on the polarity of the surface and a more hydrophilic

surface allows for water or ion permeation in a narrower pore, which is consistent with our observations of the Ala88 mutants in MthK.

Materials and Methods

All MthK mutants used in functional studies were expressed and purified in n-Decyl- β -D-Maltoside (DM) as previously described⁶. The purified proteins were reconstituted into lipid vesicles composed of 1-palmitoyl-2-oleoylphosphatidylethanolamine (POPE, 7.5 mg ml⁻¹) and 1-palmitoyl-2-oleoylphosphatidylglycerol (POPG, 2.5 mg ml⁻¹) and activity was recorded in a vertical lipid bilayer setup using the same method as described before¹¹. All single channel recordings were performed with symmetrical 150 mM KCl and 10 mM HEPES, pH 7.8 on both sides of solutions. Ca²⁺ was added to the intracellular side in the Ca²⁺ activation and blockage assay. Membrane voltage was controlled and current recorded using an Axopatch 200B amplifier with a Digidata 1322A analogue-to-digital converter (Axon Instruments). Currents were low-pass filtered at 1 kHz and sampled at 10 kHz.

Purification, crystallization, and structure determination of the ion conduction pore of the MthK_E92Q mutant follow the same procedures as formerly described⁸. Detailed data collection and refinement statistics are listed in Table I. All structure figures were generated in Pymol²⁴, with the exception of the surface renderings of the ion conduction pathways of MthK and its mutants, which were calculated in the program HOLE²⁵ and rendered in VMD²⁶.

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References

1. Hille, B. Ion Channels of Excitable Membranes. 3rd edition. Sunderland, MA: Sinauer Associates, Inc.; 2001. edit
2. Heginbotham L, Lu Z, Abramson T, MacKinnon R. Mutations in the K⁺ channel signature sequence. *Biophys J*. 1994; 66:1061–1067. [PubMed: 8038378]
3. Heginbotham L, Abramson T, MacKinnon R. A functional connection between the pores of distantly related ion channels as revealed by mutant K⁺ channels. *Science*. 1992; 258:1152–1155. [PubMed: 1279807]
4. Doyle DA, Morais Cabral J, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R. The structure of the potassium channel: molecular basis of K⁺ conduction and selectivity. *Science*. 1998; 280:69–77. [PubMed: 9525859]
5. Zhou Y, Morais-Cabral JH, Kaufman A, MacKinnon R. Chemistry of ion coordination and hydration revealed by a K⁺ channel-Fab complex at 2.0 Å resolution. *Nature*. 2001; 414:43–48. [PubMed: 11689936]
6. Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R. Crystal structure and mechanism of a calcium-gated potassium channel. *Nature*. 2002; 417:515–522. [PubMed: 12037559]
7. Jiang Y, Lee A, Chen J, Cadene M, Chait BT, MacKinnon R. The open pore conformation of potassium channels. *Nature*. 2002; 417:523–526. [PubMed: 12037560]
8. Ye S, Li Y, Jiang Y. Novel insights into K⁺ selectivity from high-resolution structures of an open K⁺ channel pore. *Nat Struct Mol Biol*. 17:1019–1023. [PubMed: 20676101]

9. Brelidze TI, Niu X, Magleby KL. A ring of eight conserved negatively charged amino acids doubles the conductance of BK channels and prevents inward rectification. *Proc Natl Acad Sci U S A*. 2003; 100:9017–9022. [PubMed: 12843404]
10. Nimigean CM, Chappie JS, Miller C. Electrostatic tuning of ion conductance in potassium channels. *Biochemistry*. 2003; 42:9263–9268. [PubMed: 12899612]
11. Li Y, Berke I, Chen L, Jiang Y. Gating and inward rectifying properties of the MthK K⁺ channel with and without the gating ring. *J Gen Physiol*. 2007; 129:109–120. [PubMed: 17261840]
12. Zadek B, Nimigean CM. Calcium-dependent gating of MthK, a prokaryotic potassium channel. *J Gen Physiol*. 2006; 127:673–685. [PubMed: 16735753]
13. Parfenova LV, Crane BM, Rothberg BS. Modulation of MthK potassium channel activity at the intracellular entrance to the pore. *J Biol Chem*. 2006
14. Ye S, Li Y, Chen L, Jiang Y. Crystal Structures of a Ligand-free MthK Gating Ring: Insights into the Ligand Gating Mechanism of K(+) Channels. *Cell*. 2006; 126:1161–1173. [PubMed: 16990139]
15. Shi N, Ye S, Alam A, Chen L, Jiang Y. Atomic structure of a Na⁺- and K⁺-conducting channel. *Nature*. 2006; 440:570–574. [PubMed: 16467789]
16. Alam A, Jiang Y. High-resolution structure of the open NaK channel. *Nat Struct Mol Biol*. 2009; 16:30–34. [PubMed: 19098917]
17. Schroeder BC, Kubisch C, Stein V, Jentsch TJ. Moderate loss of function of cyclic-AMP-modulated KCNQ2/KCNQ3 K⁺ channels causes epilepsy. *Nature*. 1998; 396:687–690. [PubMed: 9872318]
18. Singh NA, Charlier C, Stauffer D, DuPont BR, Leach RJ, Melis R, Ronen GM, Bjerre I, Quattlebaum T, Murphy JV, McHarg ML, Gagnon D, Rosales TO, Peiffer A, Anderson VE, Leppert M. A novel potassium channel gene, KCNQ2, is mutated in an inherited epilepsy of newborns. *Nat Genet*. 1998; 18:25–29. [PubMed: 9425895]
19. Long SB, Campbell EB, Mackinnon R. Crystal Structure of a Mammalian Voltage-Dependent Shaker Family K⁺ Channel. *Science*. 2005; 309:897–903. [PubMed: 16002581]
20. Long SB, Tao X, Campbell EB, MacKinnon R. Atomic structure of a voltage-dependent K⁺ channel in a lipid membrane-like environment. *Nature*. 2007; 450:376–382. [PubMed: 18004376]
21. Jiang Y, Lee A, Chen J, Ruta V, Cadene M, Chait BT, MacKinnon R. X-ray structure of a voltage-dependent K⁺ channel. *Nature*. 2003; 423:33–41. [PubMed: 12721618]
22. Beckstein O, Sansom MS. Liquid-vapor oscillations of water in hydrophobic nanopores. *Proc Natl Acad Sci U S A*. 2003; 100:7063–7068. [PubMed: 12740433]
23. Beckstein O, Sansom MS. The influence of geometry, surface character, and flexibility on the permeation of ions and water through biological pores. *Phys Biol*. 2004; 1:42–52. [PubMed: 16204821]
24. DeLano WL. The PyMOL Molecular Graphics System. DeLano Scientific, San Carlos, CA, USA. 2002 <<http://www.pymol.org>>.
25. Smart OS, Goodfellow JM, Wallace BA. The pore dimensions of gramicidin A. *Biophys J*. 1993; 65:2455–2460. [PubMed: 7508762]
26. Humphrey W, Dalke A, Schulten K. VMD: visual molecular dynamics. *J Mol Graph*. 1996; 14:33–38. 27-8. [PubMed: 8744570]

Figure 1. Surface representation of the ion conduction pathway of (A) closed KcsA, (B) open MthK, and (C) MthK Ala88 mutants. Only two diagonal subunits are shown for clarity. The side chain of residue 88 is colored yellow. The distance indicates the diameter of the constriction point in the open pore.

Figure 2.

Effects of Ala88 mutations on channel conductance. (A) Single channel traces of MthK and its mutants with larger hydrophobic residues. Currents were recorded at -100 mV with 150 mM symmetrical KCl at pH 7.8 . Dotted lines mark the zero current level. (B) Single channel traces of A88N and A88D mutants recorded at -100 mV. (C) I-V curves of some MthK Ala88 mutants in the absence (left) or presence of 1 mM Ca^{2+} (right). (D) $[\text{Ca}^{2+}]$ -dependent inhibition of outward currents recorded at 100 mV. Data are mean $\pm$ SEM of three measurements. Fits of the data with the Hill equation yield Hill coefficients (n) = 0.95 and $K_i = 0.52$ mM for the wild-type channel; $n = 0.79$ and $K_i = 0.50$ mM for the A88D mutant; and $n = 0.81$ and $K_i = 0.86$ mM for the A88V mutant.

Figure 3.

Gating influence of Ala88 mutations. (A) Plots of the channel open probability as a function of $[Ca^{2+}]$ on the intracellular side. Data are mean $\pm$ SEM of three to five measurements. Data for the wild-type channel and A88F mutant were fitted to the Hill equation with $n = 2.1$ and $K_{1/2} = 3.1$ mM for the wild type and $n = 1.4$ and $K_{1/2} = 0.33$ mM for A88F. Since A88V has extremely low open probability, the lipid bilayers might contain more than one channel in the recording and therefore the single channel open probability of A88V could be much lower than what is shown here. (B) The open pore structures of MthK (PDB code 3LDC) and NaK (PDB code 3E86) viewed from the intracellular side. Residues that form the constriction point, Phe92 in NaK and Ala88 in MthK, are colored yellow. (C) The closed NaK structure (PDB code 2AHY) and zoomed-in view of the hydrophobic interactions between Phe92 and residues from the neighboring inner helix. (D) Putative hydrophobic interactions between Ala88 and residues from neighboring inner helix in a closed MthK modeled based on the closed NaK structure.

Figure 4. Effect of E92Q and E96Q mutations on channel conductance. Single channel traces at ± 100 mV of (A) E92Q and (B) E96Q mutants. (C) I-V curves of wild-type MthK and E92Q mutant in the absence of Ca²⁺. (D) [Ca²⁺]-dependent inhibition of outward currents at 100 mV for wild-type MthK and E92Q mutant. Data are mean $\pm$ SEM of three measurements.

Figure 5.

K⁺ binding site at the intracellular entrance of the open pore. (A) Overall structure of the open MthK pore with the entrance region containing Glu92s and Glu96s boxed. The front subunit is removed for clarity. (B) 2F_o-F_c map (blue mesh) of wild-type MthK calculated at 1.45 Å and contoured at 1.5σ, showing the hydrated K⁺ ion (green sphere) at the intracellular entrance and water molecules (red spheres). (C) 2F_o-F_c map of the E92Q mutant calculated at 1.8 Å and contoured at 1.5σ. (D) F_{wt}-F_{E92Q} difference map (red mesh) calculated at 1.8 Å and contoured at 2.5σ, confirming the lack of K⁺ binding at the intracellular entrance of the E92Q mutant.

Table 1

Data collection and refinement statistics for MthK pore (E92Q) in complex with K⁺.

Protein	MthK pore (E92Q)
Data collection	
Space group	P4 ₂ 1 ₂
Cell dimensions a=b, c (Å)	63.560 43.973
Resolution (Å)	30-1.80
R_{sym} (%)	7.0 (73.6)
$I/\sigma I$	29.5 (1.5)
No. reflections- total (unique)	76322 (7953)
Completeness	90.2 (74.8)
Redundancy	9.6 (5.6)
Refinement	
Resolution(Å)	1.80
R_{work}/R_{free}	20.5 / 22.5
No. atoms	
Protein	640
K ⁺ ion	4
Water	52
R.m.s.d	
Bond angles (Å)	1.087
Bond lengths (Å)	0.009

Values in parenthesis are for highest resolution shell. 5 % of the data was used in the R_{free} calculation.