The KCSA Ion Channel Structure and Function

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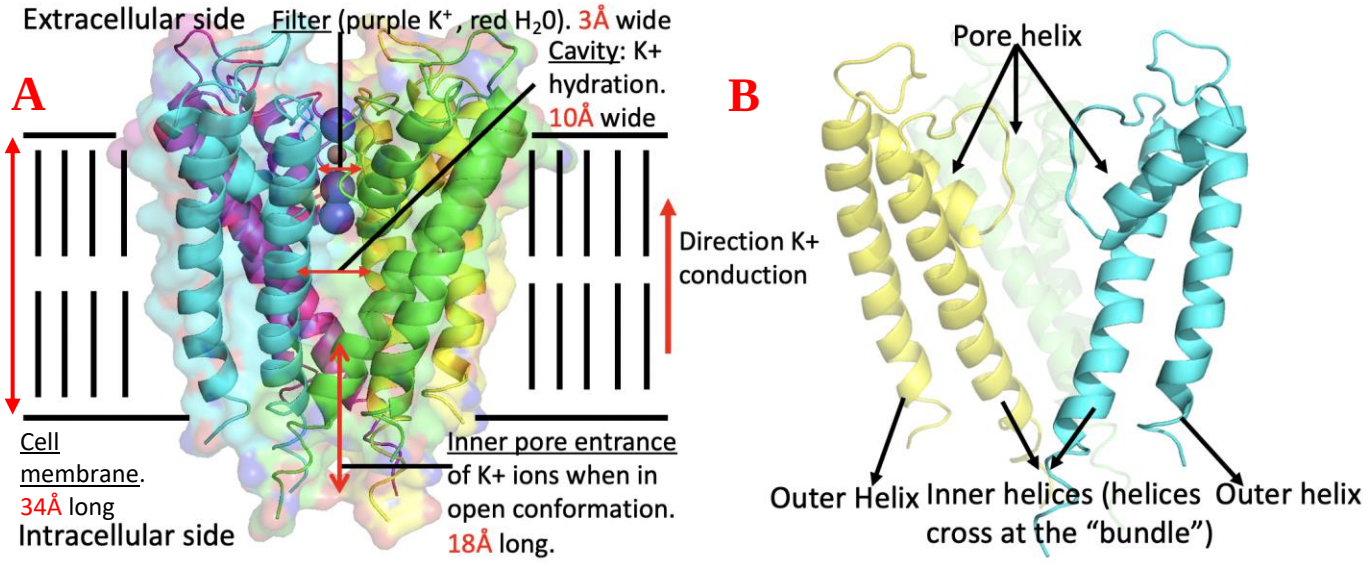
Overview of Function:

The conduction of potassium across the cell membrane is essential in cell volume regulation, hormone secretion and electrical impulse formation [1]. KcsA was the first K^+ ion channel to be characterised with x-ray crystallography in 1998 by Roderick Mackinnon and is a prokaryotic gating and shuttling channel from the soil bacterium *Streptomyces lividans* [2]. It has the highly conserved TVGYG amino acid K+ channel signature sequence found in the selectivity filter of channels in prokaryotes, eukaryotes and archea [3]. The hallmark of this channel is its selective transport for potassium ions over sodium ions by a permeant factor over 10000 which selects at rates close to diffusion limit, regardless off potassium's 1.38Å radius as opposed to 0.95Å in sodium [5].



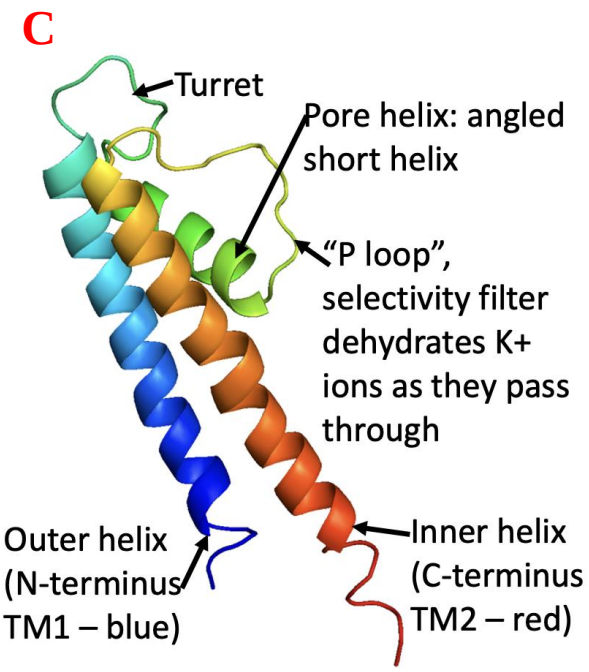


Figure 1. The structure of the KcsA K+ ion channel.

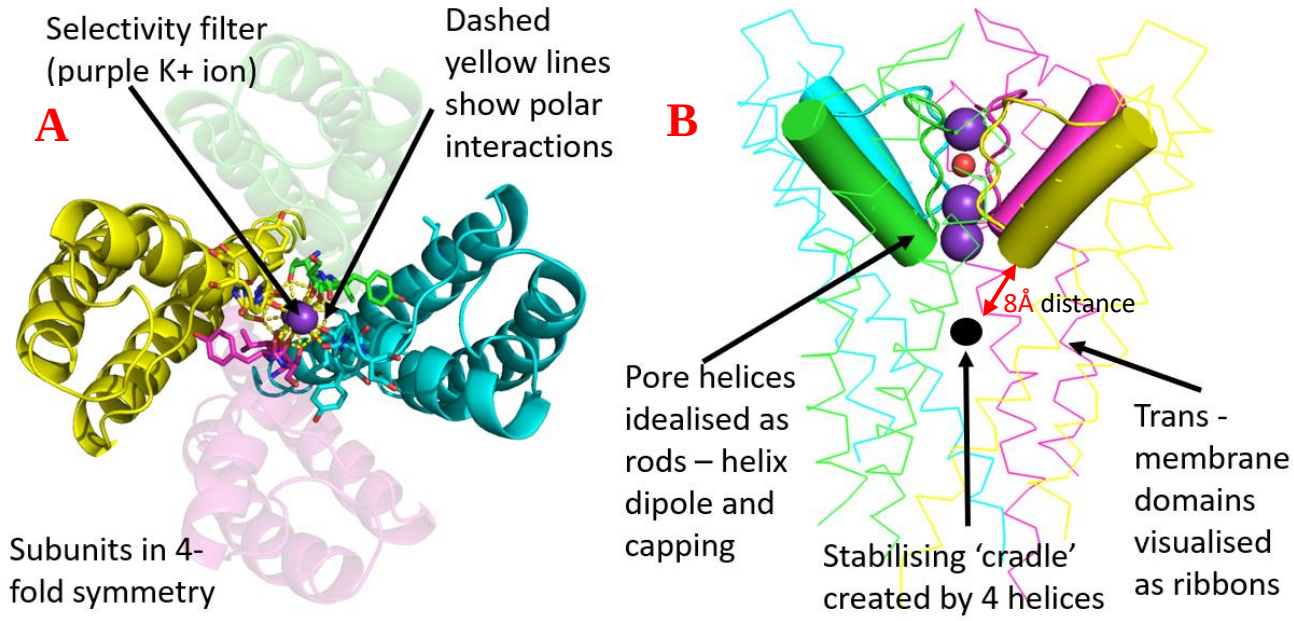
(A) A cartoon diagram of the transmembrane KcsA protein in the cell membrane (34Å) with a translucent surface representation. All four subunits are shown and the selectivity filter with three K^+ and one H_20 visualised. Measurements of key features in red. *Fig.3C* provides an alternative surface representation.

(B) A second cartoon diagram of KcsA with one of the four subunits hidden for a better view of the pore helices. The conditions in which KcsA was crystalised mean this is the closed conformation and the inner helixes overlap at the bundle crossing.

(C) A cartoon diagram of one of the four subunits of the protein. The colour spectrum is a gradient from N-terminus (blue) close to the membrane to the C-terminus (red). The pore region includes the turret at the top, the pore helix and the 'P loop' which forms the selectivity filter. Figures generated using pyMOL.

General Structure

In all cases, the functional K^+ channel protein is a tetramer of four subunits arranged around a central pore in the membrane, confirmed by an x-ray model of KcsA by Mackinnon's group at 3.2Å resolution (fig.1B/fig.2A) [5]. The prokaryotic KcsA channel has two membrane-spanning α-segments per subunit, but its amino acid sequence is closer to that of a eukaryotic 6 membrane-spanning K^+ channel (fig.1A) [7]. The two transmembrane helices are connected by a pore region of ~30 amino acids consisting of the pore helix, turret and selectivity filter (fig. 1C) [3]. The pore is 45Å long and begins at the inner pore for K^+ entry on the intracellular side (18Å long), followed by the central cavity of ~10Å diameter, wider than the 3Å filter; the large diameter and predominantly hydrophobic chemical composition before the filter (confirmed by Armstrong through hydrophobic cation binding [6]) maintain the hydration shell around K^+ (fig.1A/fig.3C). The aqueous central cavity uses polarisable water to overcome the electrostatic destabilisation created by the low-dielectric hydrophobic core of the bilayer centre [5]. The orientated pore helices further stabilise the cation via the helix-dipole effect: their c-terminal orientation towards the cavity imposes negative electrostatic potential which attracts the potassium cation, forming a 'cradle' (fig.2B) [10].



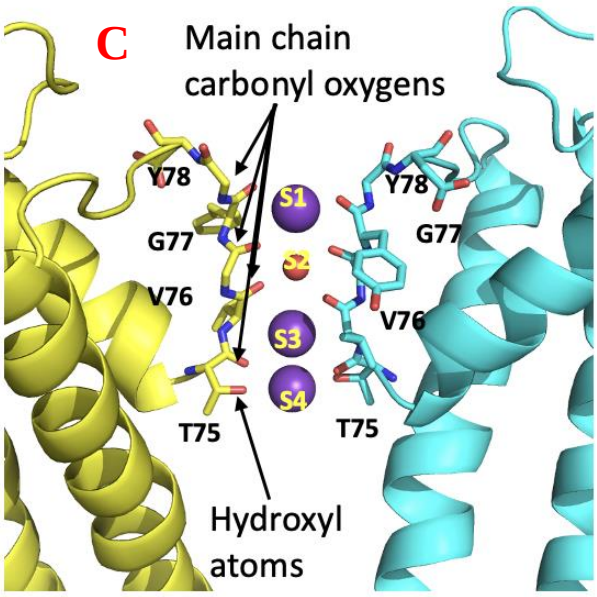


Figure 2. The selectivity filter and stabilisation.
(A) A top view cartoon representation of the subunits in four-fold symmetry with their central pore region. The interaction between the 'P loops' and the K^+ ions is visualised by dashed yellow lines.
(B) A mixture of cartoon (automatic) for 'P loops', ribbon for the transmembrane subunit domains and turret, and cartoon (rods) to idealise the pore helices and their direction with an amino-to-carboxyl sense towards a point at the centre of the channel (~8Å distance away).
(C) The selectivity filter, at the extracellular third of the ion pathway between the ion cavity and extracellular solution. There are 4 ion binding sites labelled S(1-4), created by four carbonyl groups and a hydroxyl group from Threonine-75. Figures generated using pyMOL.

Selectivity filter

In contrast to the inner pore and cavity, the filter is narrow at 3Å and its TVGYG amino acid surface has multiple polar binding sites in its 12Å-long region that collapse and mimic the hydration shell of K^+ but not Na^+: the presence of the two Tyr and their four oxygen rings at the filter base act as a cuff to hold the pore open at a diameter proprietary to K^+ [5]. The polar filter favours potassium by a permeant factor of 10^4 to 1 sodium; a more stable dehydration energy is attained through the loss of potassium's looser hydration shell due to its lower atomic density. However, a contradiction arises between a potassium ion's strong interaction with the polar signature sequence and its high movement rate through the filter, explained by 2 factors [5]. Firstly, there are often two ions in the pore at once (fig.2C configurations S1,3 or S2,4) separated by a water molecule; thus, cation repulsion of an entering ion makes movement of a bonded ion out of the site energetically more favourable than its interactions with the filter (fig.2C) [11]. Secondly, the conductive conformation of the filter requires two K^+ as some fraction of the ion binding energy contributes to a change in filter structure, weakening polar interactions with K^+ (fig.2A, yellow). Thus, the rate limiting step is just the 12Å filter diffusion distance, with the movement of K^+ through the channel approaching the diffusion limit of 10^8 ions per second [5][11].

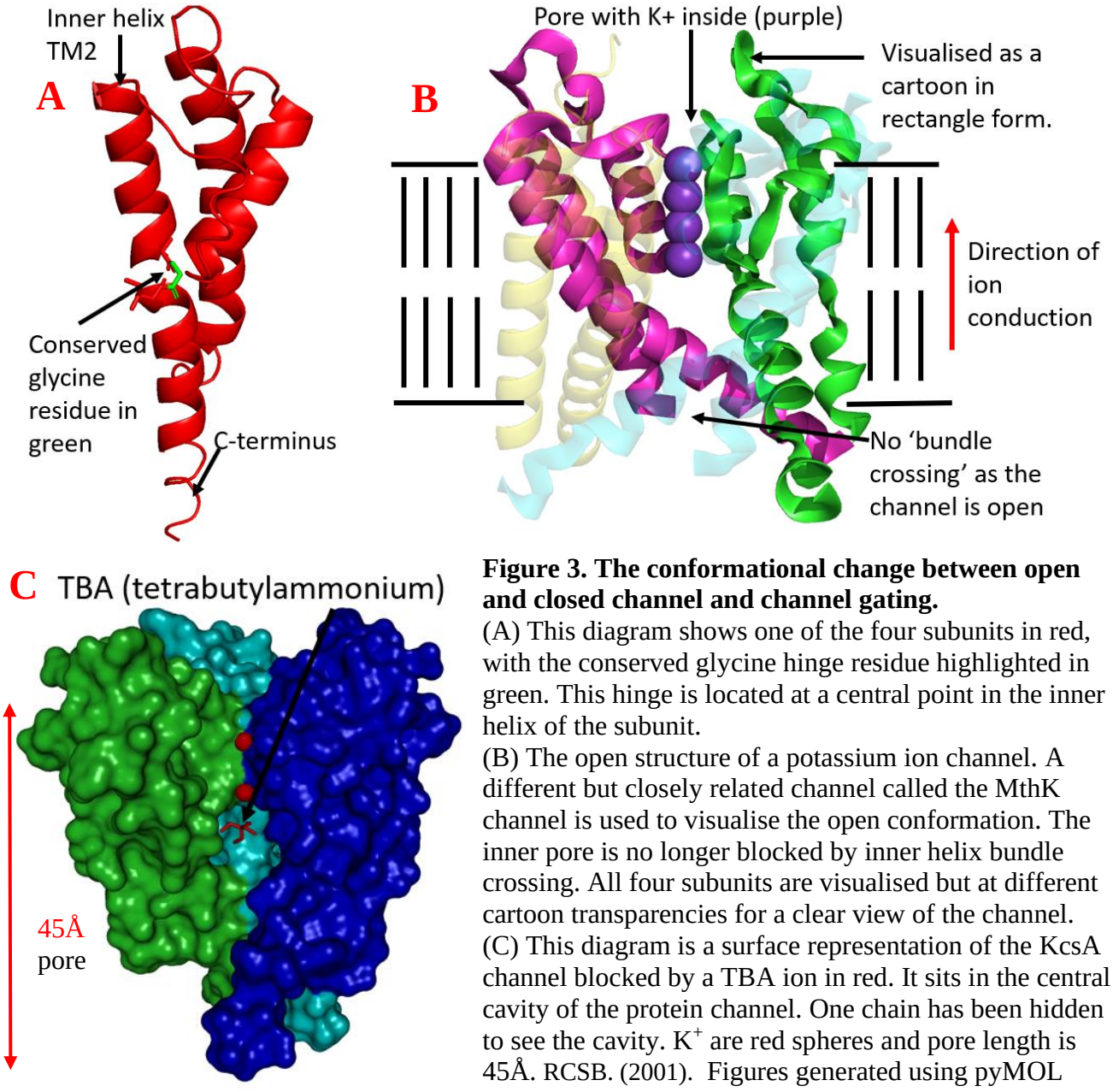


Figure 3. The conformational change between open and closed channel and channel gating.

(A) This diagram shows one of the four subunits in red, with the conserved glycine hinge residue highlighted in green. This hinge is located at a central point in the inner helix of the subunit.

(B) The open structure of a potassium ion channel. A different but closely related channel called the MthK channel is used to visualise the open conformation. The inner pore is no longer blocked by inner helix bundle crossing. All four subunits are visualised but at different cartoon transparencies for a clear view of the channel.

(C) This diagram is a surface representation of the KcsA channel blocked by a TBA ion in red. It sits in the central cavity of the protein channel. One chain has been hidden to see the cavity. K^+ are red spheres and pore length is 45Å. RCSB. (2001). Figures generated using pyMOL

Gating

Since KcsA was crystallised in conditions that favour its closed conformation in membranes, fig.1 and fig.2 images represent its closed structure with the inner helix bundle crossing. KcsA is usually blocked by Cs^+ ions and requires the presence of Mg^{2+} for gating; K^+ channels can also be blocked by organic cation compounds like TEA/TBA (fig.3C) in the cavity [2][6]. The opening is controlled by a cytoplasmic PH sensor which detects PH<7 and causes the opening of the inner pore bundle crossing (3.5Å wide and lined with hydrophobic amino acids blocking K^+) by protonation of TM2 (inner helix) at the C-terminus. The mechanism of opening is thought to be facilitated by a glycine (Gly-99) residue 'hinge' that bends ~30 degrees, due to its location at an inner helix point and conservation in all different species of K^+ channel (fig.3A, red) [4][8]. Fig.3B is a representation of this channel in its open form, taken from the closely related MthK K^+ channel; this form promotes high conduction by pressing the membrane field to the selectivity filter, initiating its open conformation [5][9]. The channel is closed by voltage-gating mechanisms.

Conclusion

There is clearly a strong, dependant relationship between the channel's structure and its function. It is highly refined to perform its function with both high fidelity (K^+ 10^4 to sodium) and maximum efficiency (conductance rates of 10^8 K^+ per second).

Words: 815

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