Gating of two-pore domain K^+ channels by extracellular pH

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Abstract

Potassium channels have a conserved selectivity filter that is important in determining which ions are conducted and at what rate. Although K^+ channels of different conductance characteristics are known, they differ more widely in the way their opening and closing, the gating, is governed. TASK and TALK subfamily proteins are two-pore region KCNK K^+ channels gated open by extracellular pH. We discuss the mechanism for this gating in terms of electrostatic effects on the pore changing the occupancy and open probability of the channels in a way reminiscent of C-type inactivation gating at the selectivity filter. Essential to this proposed mechanism is the replacement of two highly conserved aspartate residues at the pore mouth by asparagine or histidine residues in the TALK and TASK channels.

K^+ channels assemble as tetramers of identical subunits generally consisting of six α-helices (S1–S6) and a highly conserved sequence known as the P-domain. The pore is made by P-domains lining the selectivity filter, and two of the TM (transmembrane) α-helices assembled with a 4-fold symmetry. Structures attached to the pore-forming domains are able to detect stimuli, such as changes in TM voltage, and intra- or extra-cellular messages, leading to opening or closing, the gating, of the pore [1,2]. Potassium channels of the KCNK (also termed 2PK) superfamily [3,4] are remarkable in that they possess two P-domains (P1 and P2) and four α-helices (TM1–TM4) in each subunit. These channels are highly regulated leaks for K^+. This means that, in contrast with many other K^+ channels, they are open at the resting membrane potential. Potassium-selective leaks are fundamental to the function of various cells including nerve, muscle and epithelia. There are 16 mammalian members in the KCNK family and their gating is variously regulated by non-esterified fatty acids, membrane tension, G-protein-generated signalling and extracellular pH.

Because K^+ channels generally form tetramers with a total of four pore-forming domains in each channel, it was assumed that two pore-domain KCNK channels must form dimers. Strong evidence for this arrangement was obtained for TWIK-1, in which a cysteine residue, Cys^{69}, was identified as forming a disulfide bridge essential for dimerization and function [5]. The dependence on Cys^{69} for dimerization, however, is not general within the KCNK family. The conserved cysteine residue in TASK-2 contributes to the stability of the channels as dimers but is not essential for channel function [6], and other KCNK channels lacking this cysteine residue generate K^+ channel activity. The dimeric structural arrangement has also been demonstrated by functional analysis of the acid-sensitive TASK-1 channel [7].

There is a wealth of information on the molecular mechanism of K^+ channel gating. The three best understood forms of gating have been discussed previously [1]. The first type was identified by comparing the structures of KcsA and MthK channels, crystallized under conditions that favour the closed and open conformations respectively [8,9]. The major difference between these channels is in the position of the inner helices. In KcsA structure (the closed state), the four inner helices are straight and bundle together at the intracellular end to produce a narrow opening lined with hydrophobic amino acid residues that restricts ion flow. In MthK (the open state), inner helices are bent at a conserved glycine hinge located roughly half way down the helix, near the selectivity filter, creating a wide unimpeded pathway to ion passage. This type of gating is probably present in most K^+ channels, as suggested by the conservation of the glycine hinge. This includes K^+ channels of the KCNK family that conserve the glycine hinge in their TM2 and TM4 helices. Another type of gating occurs by a sort of constriction of the selectivity filter and it was first recognized in the form of 'C-type inactivation', which is sensitive to extracellular K^+ concentration and to mutations at the external mouth of the pore [1]. This is probably also present in KCNK channels and will be discussed further below. A third type of gating, known as ball-and-chain inactivation [1], has not been reported within the KCNK family.

The P-domain contains the signature sequence of K^+ channels, a highly conserved GYG (Gly-Tyr-Gly) [or GFG (Gly-Phe-Gly)] sequence that forms the narrowest part of the pore and is crucial to ion selectivity. An aspartate residue downstream from the signature sequence is also highly conserved, as shown in Figure 1(A) for h-Slo and *Shaker* K^+ channels. Neutralization of this aspartate residue by mutation in h-Slo leads to a decrease in conductance and a very marked

Key words: extracellular pH, gating, KCNK channel, K^+ channel, TALK channel, TASK-2 channel.
Abbreviations used: P_o, open probability; TM, transmembrane.
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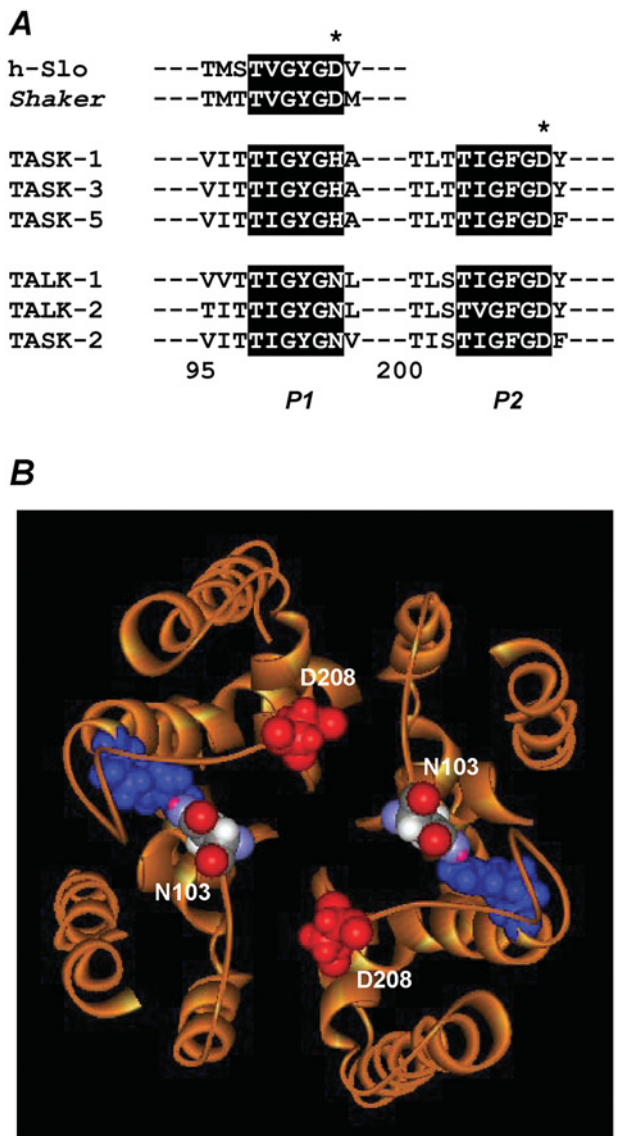
Figure 1 | Alignment of pore regions for channels of the TASK and TALK subfamilies and molecular model of TASK-2

(**A**) The pore segments of TASK and TALK channels are compared with those of h-Slo and *Shaker* K^+ channels. (**B**) A model for the pore of TASK-2 built using the crystallographic data of MthK K^+ channel (PDB code 2A79) as reference and the program MODELLER [23]. The channel is viewed from the extracellular aspect, and the mouth-pore aspartate (red) and asparagine residues are highlighted. Also shown is an arginine (blue) located in the proximity of the pore asparagine residue.



reduction in open probability [10], which was attributed to a surface charge effect exerted by the four-aspartate ring increasing the local concentration of K^+ at the mouth of the pore. Perhaps more importantly, these residues could modulate the electrostatic potential in the pore, having a critical role in the binding free energy of K^+ within the pore, thus affecting ion conduction and open probability. The effect of neutralizing pore-mouth aspartate residues in h-Slo is reminiscent of the C-type inactivation mentioned above in that the last is quite sensitive to extracellular K^+ concentration and to mutations at residues near the external entrance of the pore [1]. C-type inactivation probably corresponds to the deformation of the selectivity filter of the KcsA channel that has been reported in channels crystallized with very low K^+ concentrations [11]. A decrease in occupancy of the selectivity filter by K^+ ions leads to its partial collapse, with the carbonyl oxygens of the filter projecting obliquely rather than towards the central axis of the pore. Opening and closing of the *Drosophila* KCNK0 channel have been demonstrated to entail extracellular K^+ concentration-dependent C-type inactivation [12].

Among KCNK channels gated by extracellular pH, TASK-1 and TASK-3 (and TASK-5, that has yet to be proven to generate functional channels) form part of the TASK subfamily, and are blocked by extracellular protons. A second subfamily (TALK) of KCNK channels comprises TASK-2, TALK-1 and TALK-2, and they are activated by extracellular alkalinization. Figure 1(A) also shows part of the P-regions for these channels. A remarkable difference between these KCNK channels and most of their K^+ channel relatives, represented in the alignment by h-Slo and *Shaker*, is the absence of the conserved aspartate residue in their P1 region. In the members of the TASK group, this is replaced by histidine, while in the TALK group a conserved asparagine is present. Figure 1(B) shows a molecular model for the pore of the TASK-2 channel based on the structure of the MthK channel [9], which illustrates that the ring of four negative charges normally present in most K^+ channels is reduced to two in this KCNK channel. With histidine in the neutral state, all six KCNK channels depicted in the Figure should have a markedly diminished negative electrostatic potential at the pore compared with their four-aspartate counterparts. This would imply a lower occupancy and, consequently, lower open probability. In addition, it is conceivable that they should be highly sensitive to electrostatic effects from other residues regulating pore occupancy and/or local K^+ concentration.

TASK-type KCNK channels

K^+ channels of the TASK subfamily open at rather low maximal open probability [13,14], of the order of 0.1 as an upper limit as measured in TASK-3. In addition, P_o (open probability) is highly sensitive to extracellular K^+ concentration and to changes in electrostatic potential near the pore. Indeed, the sensitivity of K^+ channels of the TASK subfamily to extracellular pH has been demonstrated to be the consequence of titration of the P1-domain histidine residue [7,13,15]. This makes the channels responsive to pH in the physiological range and can be explained by an extra positive electrostatic potential in the pore, with consequent decrease in pore occupancy and suppression of the open state perhaps by pore collapse. That this interpretation could be correct is suggested by the marked dependence of the $pK_{1/2}$ for proton inhibition on extracellular K^+ shown for TASK-1 [7].

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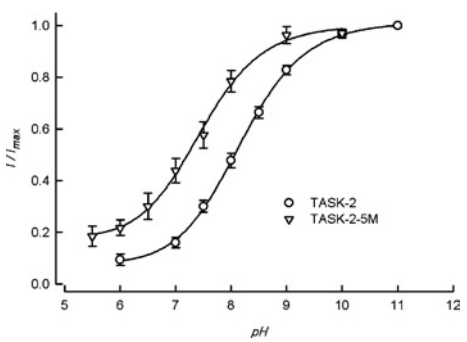
Figure 2 | Effect of a quintuple mutation on sensitivity of TASK-2 to extracellular pH

Extracellular pH-dependence curves for TASK-2 (○; $n = 14$) and for the quintuple mutant TASK-2-5M carrying changes K32N, K35N, K42N, K47N and E28Q [21] (▽; $n = 6$). Results shown are means ± S.E.M. The lines show fits of the Hill equation and were constructed using the average of fitted parameters of the individual experiments. Values of $pK_{1/2}$ were 8.3 ± 0.04 and 7.4 ± 0.11 for TASK-2 and TASK-2-5M channels respectively. The K^+ concentration above the mouth of the channel might be influenced by quintuple mutation. This effect could be responsible for the shift in $pK_{1/2}$ seen with TASK-2-5M. An alternative explanation could be a structural change secondary to this rather drastic mutation, which could lead to a collapse of the extracellular loop, perhaps impeding H^+ access to a sensor site. Mouse TASK-2 (KCNK-5; GenBank® accession no. AF319542) or its mutant was acutely transfected into HEK-293 cells (human embryonic kidney cells) (or with COS-7 cells, with similar results not shown) and assayed by standard whole-cell patch-clamp recording [16].



TALK-type KCNK channels

TASK-2 participates in ion fluxes necessary for cell volume regulation [16] and its physiological importance has also been highlighted by a TASK-2 knockout mouse [17], which has metabolic acidosis and hypotension caused by renal loss of HCO^-_3. TALK-1 and -2 are also activated by extracellular alkalinization and are highly expressed in the pancreas [18]. Although there is scant single-channel recording information for channels of the TALK subfamily, it appears that they also function with rather low maximal P_o values [19,20]. A possible gating dependence on extracellular K^+ concentration is also evident in channels from the TALK group, with outward current at low K^+ concentration anomalously low, consistent with a decreased P_o under these conditions [16,18,19].

Nothing is known about the pH-sensing mechanism of TALK-1 and -2 channels. As for TASK-2, a recent paper has proposed that a group of four lysine and one glutamic acid residue located in the extracellular loop between TM1 and P1, is the external pH sensor [21]. The particular arrangement of charges proposed as pH sensor in TASK-2 is not conserved in the TALK subfamily of channels and the hypothesis, therefore, does not provide a unified mechanism for alkalinization-dependent gating of these channels. Although there is no structural information on this large extracellular loop, it is difficult to imagine that these charges, probably shielded by the solvent, have a direct electrostatic effect within the pore. Furthermore, in our hands, the quintuple mutation neutralizing these residues did not abolish the pH sensitivity of TASK-2. This is shown in Figure 2 where it can be seen that although there was a statistically significant decrease in $pK_{1/2}$ of almost a full pH unit, the pH-sensitivity of the mutated channel remained. We have no explanation for this discrepancy with previous results [21], but in our opinion the pH sensor controlling gating of the TASK-2 channel remains to be identified. This might be achieved by molecular modelling and identifying titratable residues that might affect the electrostatic profile of the pore, and thus occupancy and P_o. An example is an arginine residue, depicted in blue in Figure 1(B). Recent experiments indeed confirm that this residue, conserved within the TALK but not the TASK subfamily, is probably the pH sensor of TASK-2 (M.I. Niemeyer, F.D. González-Nilo, L. Zúñiga, W. González, L.P. Cid and F.V. Sepúlveda, unpublished work). The protonation of such a buried residue might, in addition, be modified by the environment [22] to account for a $pK_{1/2}$ shifted from the natural pK_a.

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