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Ion channel gates: comparative analysis of energy barriers

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Abstract The energetic profile of an ion translated along the axis of an ion channel should reveal whether the structure corresponds to a functionally open or closed state of the channel. In this study, we explore the combined use of Poisson–Boltzmann electrostatic calculations and evaluation of van der Waals interactions between ion and pore to provide an initial appraisal of the gating state of a channel. This approach is exemplified by its application to the bacterial inward rectifier potassium channel KirBac3.1, where it reveals the closed gate to be formed by a ring of leucine (L124) side chains. We have extended this analysis to a comparative survey of gating profiles, including model hydrophobic nanopores, the nicotinic acetylcholine receptor, and a number of potassium channel structures and models. This enables us to identify three gating regimes, and to show the limitation of this computationally inexpensive method. For a (closed) gate radius of 0.4 nm < R < 0.8 nm, a hydrophobic gate may be present. For a gate radius of 0.2 nm < R < 0.4 nm, both electrostatic and van der Waals interactions will contribute to the barrier height. Below R = 0.2 nm, repulsive van der Waals interactions are likely to dominate, resulting in a sterically occluded gate. In general, the method is more useful when the channel is wider; for narrower channels, the flexibility of the protein may allow otherwise-unsurmountable energetic barriers to be overcome.

Keywords Ion channel · Gate · Electrostatics · Model · Simulation

Abbreviations

MscL	Large-conductance mechanosensitive channel
MscS	Small-conductance mechanosensitive channel
nAChR	Nicotinic acetylcholine receptor
PB	Poisson–Boltzmann
PMF	Potential of mean force

Introduction

Ion channels form a major class of integral membrane proteins. They perform key functions in cell behaviour, such as electrical excitability, regulation of cell volume, regulation of cytoplasmic ion concentration and pH, hormone and neurotransmitter secretion (Hille 2001). The malfunction of ion channels leads to a wide range of diseases named channelopathies (Ashcroft 2000). Because of this, ion channels are important drug targets (Hopkins and Groom 2002) and hence are of biomedical interest. For example, the voltage-gated ion channel superfamily has 143 members in seven groups of channel families, as summarized in the IUPHAR compendium of voltage-gated ion channels (Yu et al. 2005).

Ion channels are generally gated, i.e., they switch between functionally open and closed conformations in response to various environmental stimuli, such as changes in transmembrane voltage and/or binding of ligands.

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Channels are also selective, i.e., only certain ions are able to pass through the open state channel. Advances in the structural biology of ion channels (Doyle 2004; Hilf and Dutzler 2008; MacKinnon 2003; Maguire 2006) offer the possibility of understanding the biophysical function of ion channels in terms of their molecular structures. One way in which we may arrive at such an understanding is via computational studies (Roux and Schulten 2004). In particular, the gating and selectivity of channels depends on the structure of the pore and on the energetics of interactions between the ion and the protein and water microenvironment as it traverses the channel (Roux et al. 2004; Tieleman et al. 2001), both of which may be addressed computationally.

Ion selectivity is currently the subject of intense scrutiny from a combined structural (Cordero-Morales et al. 2006b; Gouaux and MacKinnon 2005) and computational (Boda et al. 2007; Bostick and Brooks 2007; Buchera et al. 2006; Noskov et al. 2004; Noskov and Roux 2006) perspective. It is therefore likely for that general rules will begin to emerge as these approaches are used for a wider range of channels.

Computational studies of energetic barriers and gating are perhaps less well advanced (Anishkin and Sukharev 2004; Kong et al. 2002; Shen et al. 2002; Shrivastava and Bahar 2006; Woolf et al. 2004), but again there is the hope that comparative studies may help us to understand general 'rules' of channel gating. This may enable, in the longer term, more rapid screening of experimental channel structures as to their open/closed state prior to conducting more accurate, but more computationally expensive, potential of mean force (PMF) calculations (Beckstein and Sansom 2006; Ivanov et al. 2007). In a previous study, we explored the relationship between the energy barrier at the channel's pore and the shape of the pore, and compared full PMF evaluation with continuum electrostatics calculations, using a series of calculations on model hydrophobic nanopores (Beckstein et al. 2004). In the present work, we apply the same method to several K^+ channels for which we have crystallographic structures and/or homology models. Thus, the current study is an extension to more biologically realistic protein channels, evaluating more fully the earlier proposal which was based on simple model nanopores.

In K^+ channels, the pore is formed by four subunits, assembled symmetrically around the central axis (Doyle et al. 1998; MacKinnon 2003; MacKinnon et al. 1998). In each subunit, the pore-lining domain consists of two transmembrane α-helices (M1 and M2, or S5 and S6 in Kv channels). Between these is a pore loop containing a short α-helical segment (the P-helix), and an extended segment of polypeptide chain which forms the selectivity filter containing the signature sequence TVGYG. From the filter (which itself may be considered a 'gate' (Cordero-Morales et al. 2006a) the pore extends towards the intracellular mouth, a hydrophobic constriction which forms a gating structure responsible for switching between open and closed conformations. These are the basic characteristics of K^+ channels.

Whilst understanding ion selectivity may require atomistic MD simulations (Noskov and Roux 2006) and QM/MM calculations (Buchera et al. 2006), the nature of the energetic barrier forming the gate in ion channels may also be approached via continuum electrostatic calculations, at least as a first approximation. For example, recent work by Jogini and Roux used a continuum electrostatic approach to compare KcsA, MthK, KirBac and Kv channels, analyzing per-residue contributions to the energy of interaction with the ion in the central cavity of the channel (Jogini and Roux 2005). Similarly, Corry (2004) has used a continuum electrostatics approach to modeling the nAChR gate. Other studies of model nanopores (Beckstein et al. 2001, 2004; Beckstein and Sansom 2003), the nAChR pore (Beckstein and Sansom 2006), and the small-conductance mechanosensitive channel (MscS) (Anishkin and Sukharev 2004) suggest that the continuum approximation may not reveal functionally important aspects of gating. Further, in the works of Jogini et al. and Corry, the pores were not particularly narrow. Therefore, one should proceed with caution and note the limitations of such methods, especially for pores narrower than one Debye length (Allen et al. 2004; Edwards et al. 2002).

On the basis of this, in this study we wish to explore further the applicability of a continuum electrostatics description of channel gating. We initially apply this approach to a range of K^+ channels. This is done by exploration of a KirBac structure, KirBac3.1 (1XL4), focussing on the proposed activation gate. We extend this to a more general consideration of K^+ channels, including the NaK structure (Shi et al. 2006). We attempt to generalize this approach, aided by previous results from nanopores and the nAChR (Amiri et al. 2005; Beckstein and Sansom 2006; Beckstein et al. 2004), and extended to other pores such as the large-conductance mechanosensitive channel (MscL) (Chang et al. 1998), and the Mg^{2+} channel CorA (Maguire 2006), to relate the shape of the pore and the height of the energy barrier. Building on such a survey, we summarize the regimes of validity for electrostatics estimation of pore energy barriers, compared against the results of high-quality (but more expensive) molecular dynamics calculations.

Materials and methods

Channel structures and models

Coordinates of K^+ channels were taken from the Protein Data Bank (http://www.rcsb.org/) (Berman et al. 2003):

KirBac1.1 (1P7B), KirBac3.1 (1XL4), chimeric KirBac1.3/Kir3.1 (2QKS), and NaK (2AHY). Other K^+ channel structures were generated by homology modeling using Modeller v6.2 (Fiser et al. 2000; Sali and Blundell 1993). All sequences were aligned using ClustalX (Jeanmougin et al. 1998). For Kir homology models, details are provided elsewhere (Haider et al. 2007). Homology models of the pore domain of the voltage-gated Shaker channel (Kv1 family) (Archer and Rusch 2001) were based on the crystal structures of KscA (closed) and MthK (open; PDB code 1LNQ). The methodology of model building has been described in (Holyoake et al. 2003). The nAChR pore domain structure was from 1OED; see Beckstein and Sansom 2006 for further details. Models of hydrophobic nanopores with radii from 0.15 to 0.4 nm were as described in Beckstein et al. 2004.

Energy profile calculations

Poisson–Boltzmann (PB) calculations were carried out, using the software package APBS (Baker et al. 2001), to estimate the electrostatic contribution to the energy barrier for a potassium ion placed at successive points along the pore axis. The pore axis was defined via the HOLE program (Smart et al. 1996), which was also used to obtain pore radius profiles. This methodology has previously been applied to model hydrophobic nanopores (Beckstein et al. 2004) and nicotinic acetylcholine receptors (Amiri et al. 2005), and the parameters were the same except for the following specifications. The channel was contained in a coarse grid of dimensions 13.5 nm × 13.5 nm × 18.0 nm. Radii and charges were assigned using the web service PDB2PQR (Dolinsky et al. 2004) (http://pdb2pqr.sourceforge.net/) before applying APBS. For focusing, a fine grid was used with dimension $(1\ \text{nm})^3$. Each of these was discretized into 97 × 97 × 193 grid points. A concentration of mobile ions NaCl at 0.15 M was used. The dielectric coefficient for water (modelled as a continuum throughout) was 78.5, and that for protein 2.0 (Baker et al. 2001). PB calculations for nicotinic acetylcholine receptor with a membrane-mimetic slab (Amiri et al. 2005) and without were calculated to confirm that the presence of the slab does not affect the energy profile (data not shown). The test ion used to probe the energetic profile, a univalent point charge emulating a potassium ion, had a radius of 0.22 nm (Rashin and Honig 1985).

To obtain van der Waals energy profiles a corresponding potassium ion-placement framework was used. An ion was placed at a sample position along the pore axis; then a single-point energy evaluation was carried out. We then repeat this for another sample position along the pore axis. In this procedure, we used GROMACS (van der Spoel et al. 2005) (http://www.gromacs.org/) to perform single-point van der Waals energy evaluations, with the GROMOS96 (43b1) forcefield. Visualization of structures used VMD (Humphrey et al. 1996).

Results

KirBac3.1

Our investigation was in part prompted by structural studies of the bacterial channel KirBac3.1, observed (in projection) in a closed and an open state by electron microscopy (Kuo et al. 2005). The interpretation of the EM images was aided by having an X-ray structure (PDB entry 1XL4) of a closed state of the KirBac3.1 channel. It has been suggested (Kuo et al. 2005) that a ring of aromatic side chains (Y132) at the intracellular mouth of the channel forms the 'activation gate'. Interestingly, two turns above in the M2 (inner) helix there is a ring of leucine side chains (L124 in KirBac3.1) which visual inspection also suggested being a possible gate. We wished to evaluate the relative heights of the barriers formed by the L124 ring and the Y132 ring.

We therefore evaluated the PB and van der Waals energy profiles contributions along the pore profile of KirBac3.1 (1XL4) (see Fig. 1). Focusing on possible gating structure(s) beyond the selectivity filter, the PB calculation alone failed to reveal an energy barrier in the vicinity of either putative gate, even though the pore radius profile showed the radius (R) to fall to $R < 0.1$ nm at the L124 ring, and to $R < 0.15$ nm at the Y132 ring. This was surprising as simple model calculations suggested a substantial PB barrier (>100 kJ/mol) for a purely hydrophobic pore (Beckstein et al. 2004) of comparable dimensions. However, further visual inspection suggested this apparent discrepancy may be due to Coulombic interactions of the probe ion (a point charge) with (1) the side chain oxygens of Y132 and (2) the backbone carbonyl oxygen atoms of L124.

These subtleties highlight possible differences in electrostatic properties between hydrophobic nanopores and real channel proteins. We therefore decided to compare a number of different K^+ channels, especially as a comparative approach has proved useful in studies of ion/channel interactions (Jogini and Roux 2005) and of possible conformational changes underlying channel gating (Shrivastava and Bahar 2006).

If one examines the van der Waals contribution (using a K^+ ion probe) to the potential energy profile then it is evident that despite the 'flat' PB profile for KirBac3.1 there is a clear energetic barrier. However, contrary to expectations the barrier is formed by the L124 ($R \sim 0.05$ nm) rather than Y132 ($R \sim 0.12$ nm). Interestingly, the barrier in a recently described chimeric KirBac1.3/Kir3.1 structure (Nishida et al. 2007) (which contains the transmembrane domain of

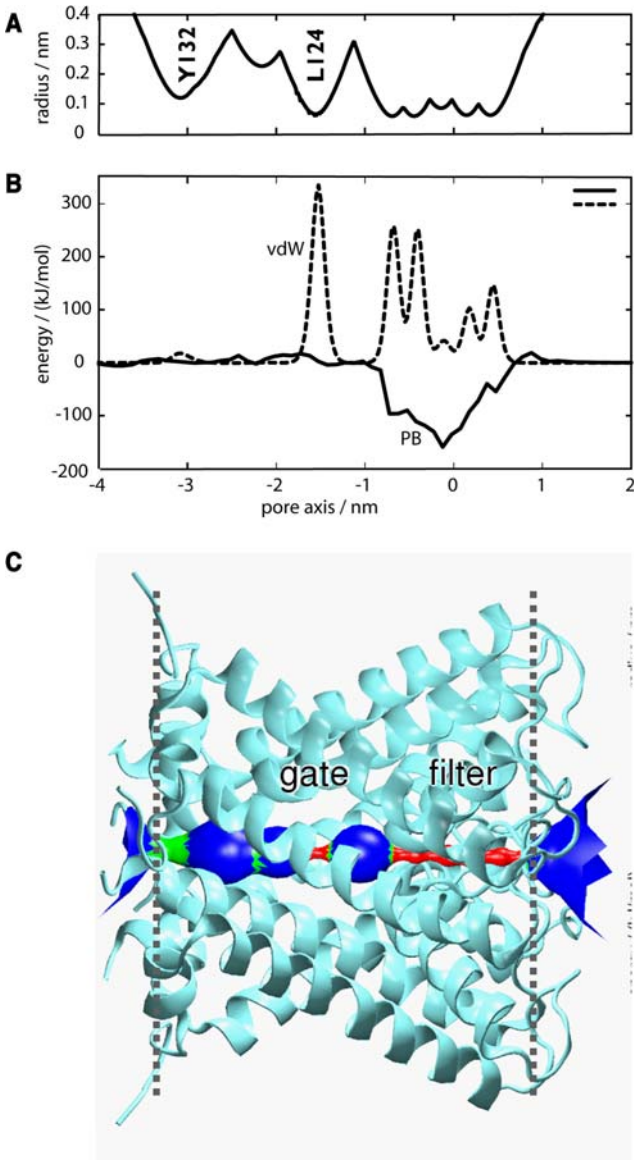

Fig. 1 **a** Pore radius profile for KirBac3.1. **b** Electrostatic (*solid line* PB) and van der Waals (*broken line* vdW) energy profiles for KirBac3.1. **c** The structure and pore lining surface of KirBac3.1

KirBac1.3 and the intracellular domain of mouse Kir3.1) shows a pronounced van der Waals barrier in a location intermediate between L124 and Y132 (at F128 of the chimeric protein) suggesting that there may be multiple possible gate locations along the narrow hydrophobic region at the intracellular mouth of the closed KirBac pore, or that the whole length of the intracellular domain may be identified as a gate.

Nanopores

As discussed in an earlier paper (Beckstein et al. 2004), we can gain some more general insights into channel gates and their associated energetic barriers via examination of simple model 'nanopores'. In particular, in the earlier study we compared PB estimates and explicit solvent molecular dynamics estimates of barrier heights for hydrophobic nanopores (Beckstein et al. 2004). For narrow ($R < 0.4$ nm) hydrophobic pores, the PB method seems to overestimate the 'true' barrier height (as judged by atomistic PMF simulations) by a factor of ~3. This may be due to the inflexibility of the atoms in the static PB calculations, a point which we discuss in more detail below, in the context of proteins. For wide pores ($R > 0.5$ nm) the PB method fails to estimate a barrier, while the atomistic simulations suggest a residual 'hydrophobic' barrier dependent on the pore radius, until $R \sim 0.8$ nm.

Thus, we have a premonition of the various factors challenging the validity of this approach in the case of real channels, including the arrangement of water molecules and molecular motion on the part of the channel itself (Beckstein and Sansom 2004). In addition to flexibility, the two most important features of real channels are the hydrophilicity of the pore-lining residues, and van der Waals repulsions due to close contacts. These are both accentuated especially when the pore radius is small, as is indeed the case in e.g., KirBac3.1 and other K^+ channels.

To calculate the van der Waals contribution to energy barriers, we performed single-point evaluations with a similar potassium ion-placement framework using GROMACS (see above). As van der Waals interactions are short-range, it was not surprising that their contribution was typically nil until the pore was as narrow as in the K channel gates ($R < 0.2$ nm). As expected, the barrier height was not sensitive to the length of the model nanopores. Thus, for example, the PB contribution is 70 kJ/mol at for a hydrophobic nanopore with a radius of 0.3 nm (Beckstein et al. 2004), but the van der Waals component is only 1.8 kJ/mol even when the pore radius is as small as $R = 0.2$ nm. For the narrowest model pore ($R = 0.15$ nm) the van der Waals contribution increases to 9.8 kJ/mol and the PB component is 163 kJ/mol.

K^+ channels

We now return to an examination of the PB profiles of selected K^+ channels, focusing on possible electrostatic barriers associated with their gates (Fig. 2). We have selected two simple models of an open and closed K^+ channel pore (models of the pore domain of a Kv channel); some more diverse Kir channels (both models of mammalian Kirs (Haider et al. 2007) and two X-ray structures of KirBac channels (Kuo et al. 2003, 2005); and the NaK channel structure (Shi et al. 2006). Note that, other than the open Kv model, these are all presumed to be in a closed conformation.

As for the KirBac3.1 channel discussed earlier, the general form of the Poisson–Boltzmann energy profiles is an

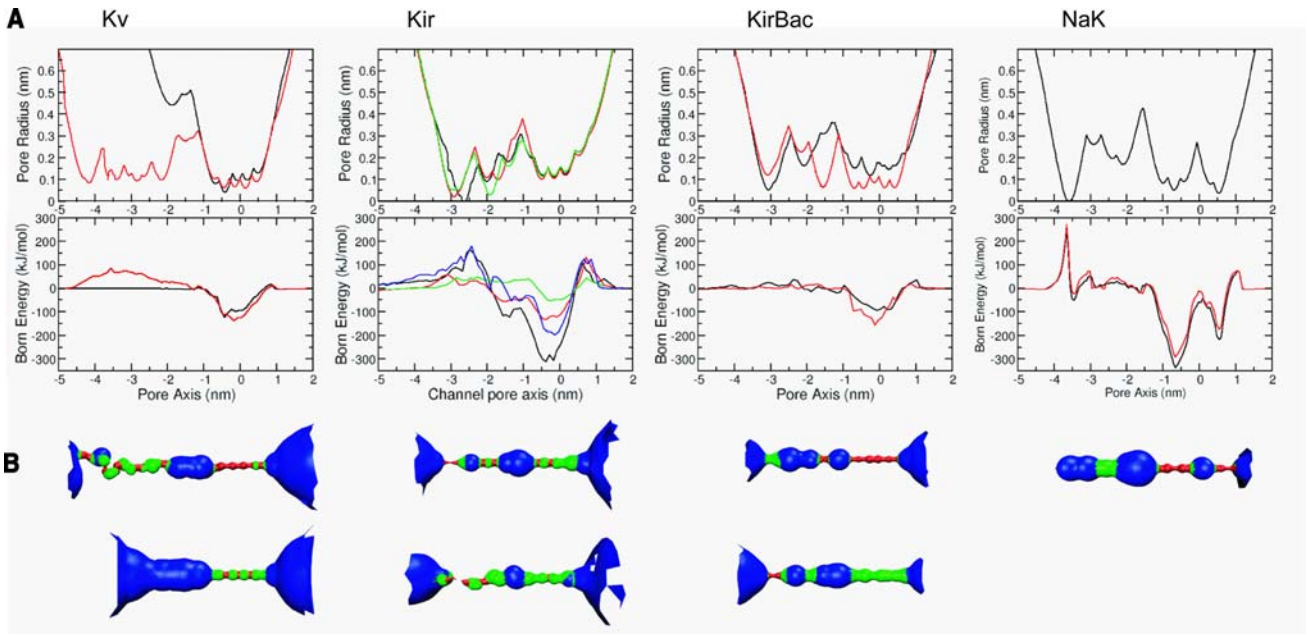

Fig. 2 **a** Pore radius (*upper panels*) and electrostatic (i.e., 'Born' solvation energy; *lower panels*) profiles for selected K^+ channel structures: *Kv* Shaker (*closed*) in *red*, Shaker (*open*) in *black*; *Kir black*, Kir 1.1, *red*, Kir 3.1, *green*, Kir 6.2, *blue*, Kir 4.1/5.1; *KirBac black*, KirBac 1.1, and *red*, KirBac 3.1; *NaK black,* NaK (permeant ion K^+); *red*, NaK (permeant ion Na^+). **b** Pore lining surfaces (generated using HOLE) for Kv [Shaker (*closed*) (*top*) and Shaker (*open*) (*bottom*)], Kir [Kir 3.1 (*top*) and Kir 1.1 (*bottom*)], KirBac [KirBac 3.1 (*top*) and KirBac 1.1 (*bottom*)] and NaK

electrostatically attractive (for a cation) region at the filter vestibule (and in the filter—i.e., for $z \sim 0$), combined with an energy barrier near the gate region (i.e., for $z \sim -3$ nm). As we have seen for KirBac3.1 (above), the energetics in gate region can be complicated by the van der Waals component. Given this, we wished to test whether there was a clear correlation between the pore radius and the PB barrier in the gate region of these K^+ channels.

The largest pore radius is in the open form of Shaker ($R = 0.44$ nm). While noting that this is close to the region where PB calculation alone, for model hydrophobic nanopores, gives an underestimation of the energy barrier (Beckstein et al. 2004), we see no barrier at all in the open Kv model, in contrast with a significant (~80 kJ/mol) and broad electrostatic barrier for closed Kv model (based on KcsA).

In the case of the Kir channels although they are all quite narrow ($R < 0.1$ nm) in the gate region, there is no clear correlation of the radius with the PB electrostatic barrier height in this region. For the NaK channel, the pore is completely occluded by the side chains of Q103 which also present a high electrostatic barrier.

Thus, for K^+ channels as a whole there is no clear correlation between the pore radius at the gate, and the height of the PB barrier. This is different from the situations with the model nanopores. However, as we explained above, it is not reasonable to assume that the potassium channel pores share this hydrophobicity. Indeed, it is not electrostatics that dominate the energy barrier, but the substantial van der Waals contribution (Jogini and Roux 2005), especially when the pore radius is narrower than $R \sim 0.2$ nm.

Discussion

A comparative survey

In an attempt to generalize these arguments we have surveyed a range of K^+ channels in their closed conformation, along with simple model nanopores, and other channels believed to be in a closed state including the nicotinic acetylcholine receptor (nAChR), the large conductance mechanosensitive channel (MscL) (Chang et al. 1998), and the Mg^{2+} channel CorA (Maguire 2006) (Fig. 3). The recent prokaryotic homologue of the nAchR (Hilf and Dutzler 2008) appears also to be in a closed state with a minimum pore radius of <0.1 nm.

For the closed conformation K^+ channels discussed above, the highest van der Waals energy contributions in the gate region was typically higher than the PB contribution, and was as high as 5.4×10^3 kJ/mol in the case of a model of Kir3.1. Two of the other closed state pores (CorA and MscL) are completely occluded, i.e., $R \sim 0$, by rings of leucine side chains and have even high van der Waals barriers (3.7×10^3 and $\sim 2 \times 10^6$ kJ/mol, respectively; these two points are not included in Fig. 3). The figure also shows some of the values for the model hydrophobic

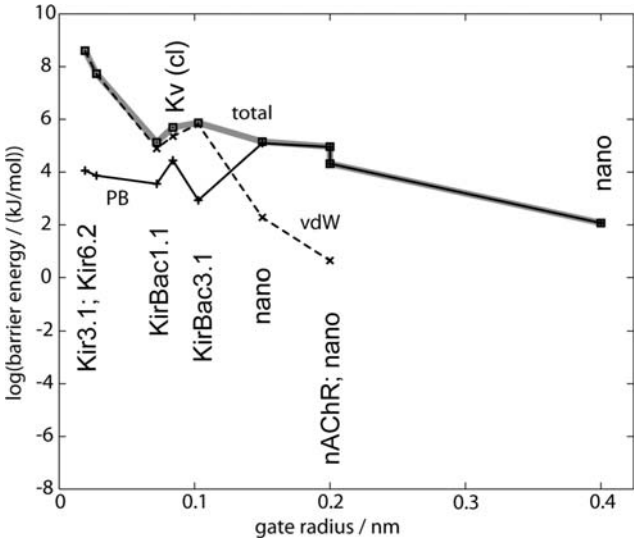

Fig. 3 Poisson–Boltzmann (*black*, *solid line*) and van der Waals (*black, broken line*) contributions to the overall barrier height (*grey, solid line*) for potassium channels [this paper; Kv(cl) is the closed conformation], model hydrophobic nanopores (Beckstein et al. 2004), and the nicotinic acetylcholine receptor as reported earlier (nAChR; Poisson–Boltzmann only) (Amiri et al. 2005)

nanopores, and that for the nicotinic acetylcholine receptor reported earlier, with its narrowest radius at 0.2 nm. As expected, the PB value for the latter is lower than that of the hydrophobic nanopore at the same radius.

In the PB calculation, a narrow pore gave a barrier due to the secondary effects of descreening in the constrained space, where the 'halo' of charge-screening solvent molecules and counterions are stripped from the probe ion. Although the PB ion-placement routine would scan such a position without distinction, the much higher energy barrier due to the van der Waals spatial exclusion would prohibit the ion to be present here. Here another limitation of continuum PB calculations needs to be borne in mind. The continuum approximation would not apply well for discrete ions in a narrow pore.

From the comparative results, it seems that there are two clear-cut gating regimes: (1) steric (i.e., van der Waals dominated) gates (in K^+ channels, MscL, CorA); and (2) hydrophobic gates (nAChR) (also sometimes referred to as 'bubble' gates (Roth et al. 2008). There is also an intermediate zone of possible relevance (e.g., to MscS channels (Anishkin and Sukharev 2004) at the hydrophobic constriction, 0.35 nm < R < 0.45 nm, in the crystal structure PDB code 1MXM) when both van der Waals, PB, and possibly solvent exclusion effects play a role.

Conclusions

In this study, we have calculated the PB electrostatics and van der Waals energy barriers the gates of a series of ion channels, focussing on K^+ channels. In this way, we established the regimes (i.e., gate radii) for which electrostatics or van der Waals contributions dominate the barrier. These calculations provide a computationally inexpensive triage tool in evaluating the openness of a channel structure or model. Of course, such results are approximations of those from molecular dynamics methods to obtain potentials of mean force, which are 10^2–10^3 times more computationally expensive.

In Fig. 3, we show that there are three regimes in the calibration of electrostatics estimates for energetic barriers of channels. First, when the pore is wider than 0.4 nm in radius, continuum electrostatics calculations underestimate the barrier when compared to that calculated using molecular dynamics (Beckstein et al. 2004). However, since the energy barrier for such gate dimensions is low, the difference in absolute value is also small.

The other two regimes occur when the pore is narrower than 0.4 nm in radius. In such cases, the barrier is sensitive to the hydrophobicity and the van der Waals repulsion due to the pore-lining residues. Further, the energetic picture can also be altered if the protein is flexible: the present methodology does not allow such consideration. On this note, those pore-lining residues flexible enough, upon encountering the ion and the consequent energetic barrier of the order of 10^2 kJ/mol or more, may choose to give way and pay the lesser energetic penalty of conformational change instead (Allen et al. 2004; Mamonov et al. 2003).

As the pore gets narrower than 0.4 nm, the PB term continues to dominate the energy barrier (and may overestimate it compared to the molecular dynamics result). This second regime prevails until van der Waals start to take over when pore radius is smaller than 0.2 nm. Here, high-quality estimates require molecular dynamics methods. To distinguish the last two regimes of validity, it is instructive to understand the hydrophilicity of the pore-lining residues, and to inspect for steric clashes along the pore radius profile. Most important, one should consider the implications of protein flexibility on the energetic picture, and whether a purely static method is adequate.

The switch in behaviour at radii ~0.4 nm is of interest given that many crystallographic structures of potassium channels, thought to be in an open state, have the radius of the intracellular gate ~0.4 nm (e.g., Kv1.2, PDB code 2A79: 0.41 nm; Kv1.2/Kv2.1 chimera, PDB code 2R9R: 0.42 nm). Thus, these channels are functionally open, but the magnitude of ion currents through these channels will depend critically on the exact radius (Chung et al. 2002) and the presence or absence of charged moieties on the protein wall. This caveat is particularly important with respect to homology models of channels for which, especially of there is relatively low sequence identity between template and target, a wrongly modelled side chain in the gate region

of the pore could result in a misleading conclusion as to the functional state of the channel.

Given these results, we can summarize the relationship between the radius of the gate region of a channel with its openness as follows: (1) for wide pores, $R > 0.8$ nm, the channel is very likely open; (2) for pores with a gate region whose radius is in the range $0.4\text{ nm} < R < 0.8$ nm, there may be a closed hydrophobic gate (Anishkin and Sukharev 2004; Beckstein et al. 2003, 2001; Ivanov et al. 2007), but molecular dynamics methods will be needed to be certain of the barrier height; (3) for narrow pores, with a constriction of dimensions $0.2\text{ nm} < R < 0.4$ nm, the channel is likely to be closed with the electrostatics dominating; and finally (4) for very narrow pores with $R < 0.2$ nm, the channel is closed by a dominating steric (i.e., van der Waals) occlusion.

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References

Allen TW, Andersen OS, Roux B (2004) On the Importance of atomic fluctuations, protein flexibility, and solvent in ion permeation. J Gen Physiol 124:679–690. doi:10.1085/jgp.200409111

Amiri S, Tai K, Beckstein O, Biggin PC, Sansom MSP (2005) The a7 nicotinic acetylcholine receptor: molecular modelling, electrostatics, and energetics. Mol Membr Biol 22:151–162. doi:10.1080/09687860500063340

Anishkin A, Sukharev S (2004) Water dynamics and dewetting transitions in the small mechanosensitive channel MscS. Biophys J 86:2883–2895

Archer SL, Rusch NJ (2001) Potassium channels in cardiovascular biology. Kluwer Academic/Plenum Publishers, New York, p 899

Ashcroft FM (2000) Ion channels and disease. Academic Press, San Diego

Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA (2001) Electrostatics of nanosystems: application to microtubules and the ribosome. Proc Natl Acad Sci USA 98:10037–10041. doi:10.1073/pnas.181342398

Beckstein O, Sansom MSP (2003) Liquid–vapor oscillations of water in hydrophobic nanopores. Proc Natl Acad Sci USA 100:7063–7068. doi:10.1073/pnas.1136844100

Beckstein O, Sansom MSP (2004) The influence of geometry, surface character and flexibility on the permeation of ions and water through biological pores. Phys Biol 1:42–52. doi:10.1088/1478-3967/1/1/005

Beckstein O, Sansom MSP (2006) A hydrophobic gate in an ion channel: the closed state of the nicotinic acetylcholine receptor. Phys Biol 3:147–159. doi:10.1088/1478-3975/3/2/007

Beckstein O, Biggin PC, Sansom MSP (2001) A hydrophobic gating mechanism for nanopores. J Phys Chem B 105:12902–12905. doi:10.1021/jp012233y

Beckstein O, Biggin PC, Bond PJ, Bright JN, Domene C, Grottesi A, Holyoake J, Sansom MSP (2003) Ion channel gating: insights via molecular simulations. FEBS Lett 555:85–90. doi:10.1016/S0014-5793(03)01151-7

Beckstein O, Tai K, Sansom MSP (2004) Not ions alone: barriers to ion permeation in nanopores and channels. J Am Chem Soc 126:14694–14695. doi:10.1021/ja045271e

Berman H, Henrick K, Nakamura H (2003) Announcing the worldwide protein data bank. Nat Struct Biol 10:980. doi:10.1038/nsb1203-980

Boda D, Nonner W, Valiskó M, Henderson D, Eisenberg B, Gillespie D (2007) Steric selectivity in Na channels arising from protein polarization and mobile side chains. Biophys J 93:1960–1980. doi:10.1529/biophysj.107.105478

Bostick DL, Brooks CL (2007) Selectivity in K^+ channels is due to topological control of the permanent ion's coordinated state. Proc Natl Acad Sci USA 104:9260–9265. doi:10.1073/pnas.0700554104

Buchera D, Raugei S, Guidoni L, Dal Peraro M, Rothlisberger U, Carloni P, Klein ML (2006) Polarization effects and charge transfer in the KcsA potassium channel. Biophys Chem 124:292–301. doi:10.1016/j.bpc.2006.04.008

Chang G, Spencer RH, Lee AT, Barclay MT, Rees DC (1998) Structure of the MscL homolog from *Mycobacterium tuberculosis*: a gated mechanosensitive ion channel. Science 282:2220–2226. doi:10.1126/science.282.5397.2220

Chung SH, Allen TW, Kuyucak S (2002) Modeling diverse range of potassium channels with brownian dynamics. Biophys J 83:263–277

Cordero-Morales JF, Cuello LG, Perozo E (2006a) Voltage-dependent gating at the KcsA selectivity filter. Nat Struct Mol Biol 13:319–322. doi:10.1038/nsmb1070

Cordero-Morales JF, Cuello LG, Zhao YX, Jogini V, Cortes DM, Roux B, Perozo E (2006b) Molecular determinants of gating at the potassium-channel selectivity filter. Nat Struct Mol Biol 13:311–318. doi:10.1038/nsmb1069

Corry B (2004) Theoretical conformation of the closed and open states of the acetylcholine receptor channel. Biochim Biophys Acta 1663:2–5. doi:10.1016/j.bbamem.2004.02.006

Dolinsky TJ, Nielsen JE, McCammon JA, Baker NA (2004) PDB2PQR: an automated pipeline for the setup, execution, and analysis of Poisson–Boltzmann electrostatics calculations. Nucleic Acids Res 32:W665–W667. doi:10.1093/nar/gkh381

Doyle DA (2004) Structural themes in ion channels. Eur Biophys J 33:175–179. doi:10.1007/s00249-003-0382-z

Doyle DA, Cabral JM, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Cahit BT, MacKinnon R (1998) The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science 280:69–77. doi:10.1126/science.280.5360.69

Edwards S, Corry B, Kuyucak S, Chung S-H (2002) Continuum electrostatics fails to describe ion permeation in the gramicidin channel. Biophys J 83:1348–1360

Fiser A, Kinh Gian Do R, Sali A (2000) Modeling of loops in protein structures. Protein Sci 9:1753–1773

Gouaux E, MacKinnon R (2005) Principles of selective ion transport in channels and pumps. Science 310:1461–1465. doi:10.1126/science.1113666

Haider S, Khalid S, Tucker S, Ashcroft FM, Sansom MSP (2007) Molecular dynamics simulations of inwardly rectifying (Kir) potassium channels: a comparative study. Biochemistry 46:3643–3652. doi:10.1021/bi062210f

Hilf RJC, Dutzler R (2008) X-ray structure of a prokaryotic pentameric ligand-gated ion channel. Nature 452:375–379. doi:10.1038/nature06717

Hille B (2001) Ionic channels of excitable membranes, 3rd edn. Sinauer Associates Inc, Sunderland

Holyoake J, Domene C, Bright JN, Sansom MSP (2003) KcsA closed and open: modelling and simulation studies. Eur Biophys J 33:238–246

Hopkins AL, Groom CR (2002) The druggable genome. Nat Rev Drug Discov 1:727–730. doi:10.1038/nrd892

Humphrey W, Dalke A, Schulten K (1996) VMD—visual molecular dynamics. J Mol Graph 14:33–38. doi:10.1016/0263-7855(96)00018-5

Ivanov I, Cheng X, Sine SM, McCammon JA (2007) Barriers to ion translocation in cationic and anionic receptors from the Cys-loop family. J Am Chem Soc 129:8217–8224. doi:10.1021/ja070778l

Jeanmougin F, Thompson JD, Gouy M, Higgins DG, Gibson TJ (1998) Multiple sequence alignment with clustal X. Trends Biochem Sci 23:403–405. doi:10.1016/S0968-0004(98)01285-7

Jogini V, Roux B (2005) Electrostatics of the intracellular vestibule of K^+ channels. J Mol Biol 354:272–288. doi:10.1016/j.jmb.2005.09.031

Kong Y, Shen Y, Warth TE, Ma J (2002) Conformational pathways in the gating of *Escherichia coli* mechanosensitive channel. Proc Natl Acad Sci USA 99:5999–6004. doi:10.1073/pnas.092051099

Kuo A, Gulbis JM, Antcliff JF, Rahman T, Lowe ED, Zimmer J, Cuthbertson J, Ashcroft FM, Ezaki T, Doyle DA (2003) Crystal structure of the potassium channel KirBac1.1 in the closed state. Science 300:1922–1926. doi:10.1126/science.1085028

Kuo AL, Domene C, Johnson LN, Doyle DA, Venien-Bryan C (2005) Two different conformational states of the KirBac3.1 potassium channel revealed by electron crystallography. Structure 13:1463–1472. doi:10.1016/j.str.2005.07.011

MacKinnon R (2003) Potassium channels. FEBS Lett 555:62–65. doi:10.1016/S0014-5793(03)01104-9

MacKinnon R, Cohen SL, Kuo A, Lee A, Chait BT (1998) Structural conservation in prokaryotic and eukaryotic potassium channels. Science 280:106–109. doi:10.1126/science.280.5360.106

Maguire ME (2006) The structure of CorA: a Mg^{2+}-selective channel. Curr Opin Struct Biol 16:432–438. doi:10.1016/j.sbi.2006.06.006

Mamonov AB, Coalson RD, Nitzan A, Kurnikova MG (2003) The role of the dielectric barrier in narrow biological channels: a novel composite approach to modeling single-channel currents. Biophys J 84:3646–3661

Nishida M, Cadene M, Chait BT, MacKinnon R (2007) Crystal structure of a Kir3.1-prokaryotic Kir channel chimera. EMBO J 26:4005–4015. doi:10.1038/sj.emboj.7601828

Noskov SY, Roux B (2006) Ion selectivity in potassium channels. Biophys Chem 124:279–291. doi:10.1016/j.bpc.2006.05.033

Noskov SY, Bernèche S, Roux B (2004) Control of ion selectivity in potassium channels by electrostatic and dynamic properties of carbonyl ligands. Nature 431:830–834. doi:10.1038/nature02943

Rashin A, Honig B (1985) Reevaluation of the Born model of ion hydration. J Phys Chem 89:5588. doi:10.1021/j100272a006

Roth R, Gillespie D, Nonner W, Eisenberg R (2008) Bubbles, gating, and anaesthetics in ion channels. Biophys J 94:4282–4298. doi:10.1529/biophysj.107.120493

Roux B, Schulten K (2004) Computational studies of membrane channels. Structure 12:1343–1351. doi:10.1016/j.str.2004.06.013

Roux B, Allen T, Berneche S, Im W (2004) Theoretical and computational models of biological ion channels. Q Rev Biophys 37:15–103. doi:10.1017/S0033583504003968

Sali A, Blundell TL (1993) Comparative protein modeling by satisfaction of spatial restraints. J Mol Biol 234:779–815. doi:10.1006/jmbi.1993.1626

Shen YF, Kong YF, Ma JP (2002) Intrinsic flexibility and gating mechanism of the potassium channel KcsA. Proc Natl Acad Sci USA 99:1949–1953. doi:10.1073/pnas.042650399

Shi N, Ye S, Alam A, Chen L, Jiang Y (2006) Atomic structure of a Na^+- and K^+-conducting channel. Nature 440:570–574. doi:10.1038/nature04508

Shrivastava IH, Bahar I (2006) Common mechanism of pore opening shared by five different potassium channels. Biophys J 90:3929–3940. doi:10.1529/biophysj.105.080093

Smart OS, Neduvelil JG, Wang X, Wallace BA, Sansom MSP (1996) Hole: a program for the analysis of the pore dimensions of ion channel structural models. J Mol Graph 14:354–360. doi:10.1016/S0263-7855(97)00009-X

Tieleman DP, Biggin PC, Smith GR, Sansom MSP (2001) Simulation approaches to ion channel structure-function relationships. Q Rev Biophys 34:473–561. doi:10.1017/S0033583501003729

van der Spoel D, Lindahl E, Hess B, Groenhof G, Mark AE, Berendsen HJ (2005) GROMACS: fast, flexible, and free. J Comput Chem 26:1701–1718. doi:10.1002/jcc.20291

Woolf TB, Zuckerman DM, Lua N, Jang H (2004) Tools for channels: moving towards molecular calculations of gating and permeation in ion channel biophysics. J Mol Graph Model 22:359–368. doi:10.1016/j.jmgm.2003.12.003

Yu FH, Yarov-Yarovoy V, Gutman GA, Catterall WA (2005) Overview of molecular relationships in the voltage-gated ion channel superfamily. Pharmacol Rev 57:387–395. doi:10.1124/pr.57.4.13