Molecular Restraints in the Permeation Pathway of Ion Channels

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ABSTRACT Ion channels assist and control the diffusion of ions through biological membranes. The conduction process depends on the structural characteristics of these nanopores, among which are the hydrophobicity and the afforded diameter of the conduction pathway. In this contribution, we use full atomistic free-energy molecular dynamics simulations to estimate the effect of such characteristics on the energetics of ion conduction through the activation gate of voltage-gated potassium (Kv) channels. We consider specifically the ionic translocation through three different permeation pathways, corresponding to the activation gate of an atomistic model of *Shaker* channels in closed and partially opened conformations, and that of the open conformation of the Kv1.2 channel. In agreement with experiments, we find that the region of Val^{478} constitutes the main gate. The conduction is unfavorable through this gate when the constriction is smaller than an estimated threshold of 4.5–5.0 Å, mainly due to incomplete coordination-hydration of the ion. Above this critical size, e.g., for the Kv1.2, the valine gate is wide enough to allow fully coordination of the ion and therefore its diffusion on a flat energy surface. Similar to other ion channels, Kv channels appear therefore to regulate diffusion by constricting hydrophobic regions of the permeation pathway.

Received for publication 18 April 2006 and in final form 31 May 2006.

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The considerable free energy associated with the transfer of ions from the aqueous medium to the interior of the membrane rationalizes the use in cells of channels that facilitate the transport of ions across the lipid. Ionic diffusion is often regulated by protein channels in response to a variety of stimuli, such as variation of the lateral pressure, variation of pH, change in the membrane potential (voltage-gated), or binding of transmitters or hormones (ligand-gated), all of which tune the channel from open to closed, and vice versa (1).

The diffusion of ions through a channel requires a hydrophilic pathway for perfect ionic coordination. This hydrophilic environment may be provided either by: 1), coordinating groups of the protein, such as the carbonyl atoms in the selectivity filter of K channels; or 2), transient hydration of the ionic conduction pathway, such as that encountered around the activation gate of K channels (2). For the latter, the diffusion is energetically restrained by the closure of the channel, or narrowing of the activation gate that excludes water molecules from the permeation pathway (3–5). Mounting evidence suggests that hydrophobic gates play an important role in the mechanism by which ion channels control the diffusion process (3,6,7).

In voltage-gated potassium (Kv) channels, the ionic pathway in the activation gate is lined by hydrophilic and hydrophobic side chains, which is a common feature of protein channels. Here, we use atomistic simulations to study the molecular properties affecting the energetics of conduction of K^+ ions through the activation gate of Kv channels.

Three different conformations of the activation gate were considered. They correspond to a closed (resting R-state), a partially open (toward activation TA-state), and an open (activated A-state) conformation. The R- and the TA-state conformations were obtained, respectively, from molecular dynamics (MD) simulations of full-atomistic models of the Kv *Shaker* B channel, embedded in a lipid membrane, at the resting transmembrane potential (13-ns MD run) and after depolarization of the membrane (17-ns MD run) (8). The A-state was obtained from a 9-ns MD simulation (9) of the Kv1.2 channel (10) embedded in a lipid membrane. The free-energy profiles of K^+ conduction were estimated using the adaptive-biasing-force (ABF) method (11–14) performed on the channels in their lipid environment (see Supplementary Material).

The reaction coordinate (rc) was defined as a pathway, 27 Å long, joining the cytoplasm mouth of the pore to the entrance of the central cavity, located immediately above the PVP motif (Fig. 1). For the R-state, the overall free energy barrier (ΔG) is ~50 Kcal.mol^{-1} (Fig. 1). This barrier is similar to that of unassisted translocation of ions across a bare membrane. Analysis of specific ΔG barriers through each section of rc (Table 1) reveals that the region of Val^{474} of the conserved PVP motif is opened even in the R-state. Around this gate, the kink in the S6 helix places the Val^{474} side chain away from the permeation pathway. On the other hand, Tyr^{485}, Asn^{482}, and Val^{478} obstruct the ion-conduction pathway (see Fig. 2). These residues account for a total free-energy barrier of ~41 Kcal.mol^{-1} in which the region delimited by Asn^{482} and Val^{478} forms the major gate.

For the TA-state conformation, the free-energy profile is still too high to allow ionic diffusion despite a substantial reduction of the barrier (~22 Kcal.mol^{-1}). Most of this reduction was due to a minute increase of the pore volume along rc (see Supplementary Material). This volume is calculated considering the size of the local constriction within each

doi: 10.1529/biophysj.106.087437

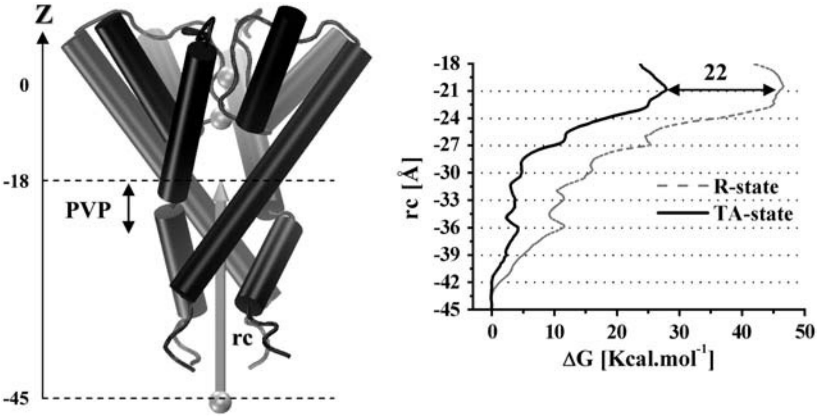

FIGURE 1 Free energy profiles for K$^+$ conduction through the activation gate of a model *Shaker* channel. (*Left*) Conformation of the pore domain showing the reaction pathway (*shaded arrow*). rc, the reaction coordinate, is defined as the position *Z* of the K$^+$ ion with respect of the center of the selectivity filter. (*Right*) Free energy profiles for the R- and TA-states along the rc. The calculation is performed considering the transmembrane domain of the channel in its membrane environment.

window (given by HOLE (15)) i.e. is a rough estimate of the local volume accessible to K$^+$ and to water.

Val^{478} constitutes the main gate along the rc, even for the TA-state. In the R-state this gate has a constriction radius of ~2.5 Å and can accommodate only the K$^+$ ion (two water molecules at each side of the conduit). Increase of this radius in the TA-state to ~3.5 Å allows for five water molecules to coordinate the ion, but still restrains the conduction by a ΔG barrier of 9 Kcal.mol^{-1} (see Fig. 2 and Table 1).

In confined geometries, one expects that widening of the pathway radius favors ionic conduction as it increases the ion hydration. This was shown to be valid for hydrophobic pores (3,5). In more complex environments as in the present case, ΔG is not correlated with the pore radius and with the ion coordination in a simple manner, but depends on the specificities of the residues lining the pathway. This is highlighted in Fig. 3, where we report n_O, the number of oxygens within the first-coordination shell (3.6 Å cutoff) of K$^+$, and ΔG for slabs along rc, as a function of the size of the constricted region. For pore radii <4 Å, the pathways can be open or closed for similar values of n_O (see the *dashed box region* in Fig. 3 *A*). For instance, in hydrophilic regions such as those around Tyr^{485} and Asn^{482}, the conduction is favored ($\Delta G < 3$ Kcal.mol^{-1}) while n_O is <6. This is, however, not a threshold value—since, with $n_O = 6$, ΔG is still very high in the region of the hydrophobic gate Val^{478}.

What is then the required widening of the pore around Val^{478} that allows ion conduction? There seems to be a slight correlation between the pore radius and ΔG along the rc for the hydrophobic gate (Fig. 3 *B*). Such linear dependence was also found for water diffusion through simple hydrophobic nanopores (16). Here, we find that ΔG drops to <3.0 Kcal.mol^{-1} for pathways of >5 Å in constriction radius. This suggests that a widening of the pathway radius above 5.0 Å would be required for opening of the region of Val^{478}, which is in agreement with simulations of hydrophobic nanopores (3–5,16). The structure of the Kv1.2 channel, for which the estimated constriction around Val^{478} is 4.5 Å, was used here to test this prediction. For Kv1.2, $n_O = 6.66 \pm$

TABLE 1 Free-energy differences of K$^+$ translocation across finite slabs (3 Å) along the reaction coordinate (rc)

rc [Å]*	Gate	R-state ΔG *Shaker* B	TA-state ΔG *Shaker* B	A-state ΔG Kv1.2
−45 > −42 (1)		1.13 ± 0.44	0.00 ± 0.44	—
−42 > −39 (2)		3.68 ± 0.37	2.25 ± 0.33	—
−39 > −36 (3)	{TYR^{485}	6.45 ± 0.62	1.88 ± 0.43	—
−36 > −33 (4)		−0.24 ± 0.59	−0.53 ± 0.56	—
−33 > −30 (5)	{ASN^{482}	4.18 ± 0.77	1.05 ± 0.45	—
−30 > −27 (6)		8.92 ± 0.55	4.68 ± 0.44	−0.3 ± 0.9
−27 > −24 (7)	{VAL^{478}	11.01 ± 0.63	8.56 ± 0.55	−0.7 ± 0.7
−24 > −21 (8)		10.50 ± 0.62	9.63 ± 0.65	−0.7 ± 0.6
−21 > −18 (9)	VAL^{474†}	−3.95 ± 0.42	−3.83 ± 0.66	—

*Reaction coordinate index.

†PVP region.

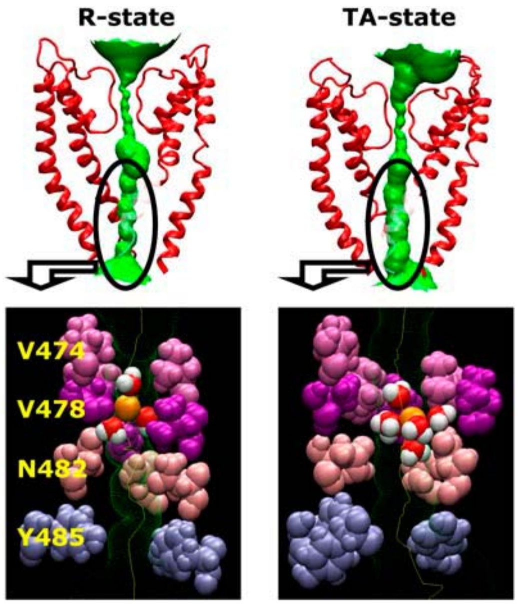

FIGURE 2 Characteristics of the K$^+$ pathway along the rc for the R- and TA-states. (*Top*) Pore volume (*green*) accessible to K$^+$; and (*bottom*) conformations of the gates formed by selected residues Tyr^{485}, Asn^{482}, and Val^{478}. Water molecules hydrating K$^+$ (*orange*) are drawn in red and white.

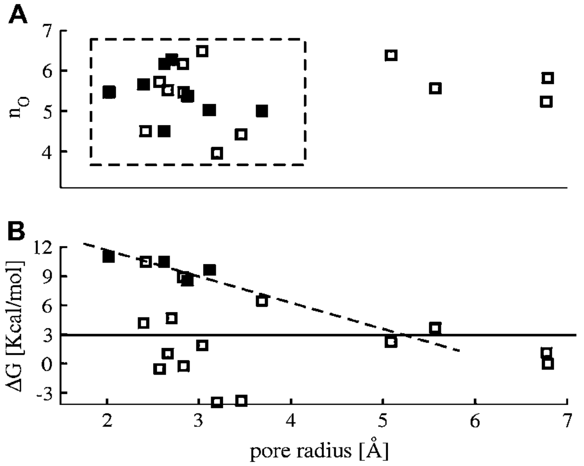

FIGURE 3 **(*A*) Number of oxygens (n_O) within the first coordination shell of K^+ as a function of the pore radius. Open and solid squares represent data with ΔG smaller and larger than 3 $Kcal.mol^{-1}$, respectively. (*B*) ΔG as a function of the pore radius. Solid symbols represent estimates in the region of Val^{478}.**

0.70 for an ion placed in the Val^{478} gate. Note that here, all oxygens coordinating the ion belong to water molecules, and that this number is similar to water coordination in bulk simulations using a similar force field. The ABF calculation shows in this case no barrier for ionic diffusion (Table 1).

The considerable effect of Val^{478} on the energetics of ion diffusion, in response to minimal changes of the size of the constricting region, especially below 5 Å, is consistent with the major gating role of this residue identified experimentally. Webster et al. (17) have indeed suggested that at position 474, around the PVP motif, the intracellular entrance to the pore cavity for *Shaker* channels is narrow in both the closed and open states, and that the channel's gate is below this point at Val^{478}. The Val^{478}Trp mutation produces a channel that cannot conduct ions even though the voltage sensors are competent to gate charges in response to membrane depolarization (18). Taken together, these results suggest that Val^{478} forms a hydrophobic gate in similar manner to other gates identified in the family of nicotinic acetylcholine receptors (19,20) or in the bacterial mechanosensitive channel MscS (6,7). The exclusion of waters from hydrophobic regions of the permeation pathway, through fine tuning of the accessible volume, seems to be a general and efficient mechanism of ion transport regulation conserved in a variety of ion channels.

SUPPLEMENTARY MATERIAL

An online supplement to this article can be found by visiting BJ Online at http://www.biophysj.org.

ACKNOWLEDGMENTS

We are grateful to C. Chipot and J. Hénin for providing the implementations of the ABF method in NAMD. Calculations were performed at the Centre Informatique National de l'Enseignement Supérieur.

This work was supported by a Centre National de la Recherche Scientifique (CNRS) postdoctoral fellowship to W.T.

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