J Comput Neurosci
DOI 10.1007/s10827-014-0527-3

Normal mode dynamics of voltage-gated K^+ channels: gating principle, opening mechanism, and inhibition

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Received: 8 September 2013 / Revised: 31 July 2014 / Accepted: 1 September 2014

Abstract Voltage-dependent potassium channels open in response to changes in membrane potential and become partially inactivated upon binding of inhibitors. Here we calculate normal mode motion of two voltage-dependent K^+ channels, KvAP and Shaker, and their complexes with inhibitors and address the gating principle, opening mechanism, and inhibition. The normal modes indicate that pore expansion and channel opening is correlated with a displacement of the arginine gating charges and a tilting of the voltage-sensor paddles. Normal modes of Shaker in complex with agitoxin, which blocks the central pore, do not display significantly altered paddle tilting and pore expansion. In contrast, normal modes of Shaker in complex with hanatoxin, which binds to the voltage sensor paddle, display decreased paddle tilting and pore expansion. This study presents a unified motion for the gating principle and channel opening, and offers insight into the voltage sensor paddle motion and its inhibition.

Keywords Voltage-dependent K^+ channels · Hanatoxin · Agitoxin · Normal modes · Elastic network · Molecular dynamics · Voltage-sensor paddle · Gating charges

Abbreviations

KvAP	Archaeal voltage-dependent potassium channel (derived from the extremophile *Aeropyrum Pernix* living at a temperature of 95 °C)
Shaker	Mammalian voltage-dependent potassium channel (derived from *Rattus Norvegicus*)
RMSD	Root mean square deviation

Action Editor: John Huguenard

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Voltage-dependent potassium channels open and close in response to changes in the electric potential across cell membranes. When open, these channels enable the rapid and selective diffusion of K^+ ions down the electrochemical gradient (Sigworth 1994). The channel consists of four identical subunits arranged around the central ion pore in a four-fold symmetry (Jiang et al. 2003a; Long et al. 2005a). Each of the four subunits is anchored in the membrane and is composed of six hydrophobic segments, labeled S1 through S6. The gating principle has long been studied and was consolidated following the advent of the crystal structure of a voltage-dependent K+ channel (Jiang et al. 2003a). The gating principle involves the charge movement of four arginine residues located on the voltage-sensor paddle (S3b and S4) of each subunit (Jiang et al. 2003b; Ruta et al. 2005). By moving across the membrane, these paddles carry the arginine residues through the electric potential, coupling pore opening to membrane voltage. This paddle motion is common to several voltage-dependent potassium channels, such as KvAP (Jiang et al. 2003a) and Shaker (Long et al. 2005b). Previously, channel opening was described using normal mode analysis as a corkscrew-like counter-rotation motion of the extracellular and cytoplasmic regions, leading to the opening of the channel (Shrivastava and Bahar 2006). Their study however did not address the motion of the voltage sensor paddles and gating charges, nor their effect on channel opening.

Normal-mode analysis is one of the standard techniques for studying long time dynamics and, in particular, low-frequency motions. In contrast to molecular dynamics, normal-mode analysis provides an analytical and fully-detailed description of the dynamics around a local energy well. While comprising some limitations, such as the neglect of the solvent effect, the use of harmonic approximation of the potential energy function, and the lack of information about energy barriers and crossing events, normal modes have provided much useful insight into protein dynamics. Over the years, several

Published online: 16 September 2014

techniques have been described to calculate large-scale motions using all-atom (Brooks and Karplus 1983; Go et al. 1983; Levitt et al. 1985) and reduced-atom (Tirion 1996; Atilgan et al. 2001; Tama and Sanejouand 2001; Delarue and Sanejouand 2002) normal-mode analysis. Based on these techniques, several executable programs for calculating normal modes have been developed, such as ElNemo (Suhre and Sanejouand 2004), Nomad-Ref (Lindahl et al. 2006) and STAND (Levitt et al. 1985). Similarly, molecular dynamics programs such as GROMACS (Van Der Spoel et al. 2005) were modified to calculate normal modes. Using such programs, many normal mode dynamic studies have been conducted (reviewed in (Shrivastava and Bahar 2006; Taly et al. 2006; Samson and Levitt 2008; Bahar et al. 2010; Samson and Levitt 2011)).

In this paper, we calculate the normal modes of two voltage-dependent potassium channels, namely KvAP and Shaker, and observe a single mode of motion responsible for both the gating principle and opening mechanism. In addition, we calculate the normal modes of Shaker in complex with inhibitors, both pore blocking and not, and address the differences therebetween in the paddle motion. To our knowledge, this is the first calculation of the normal modes of Shaker with the full length voltage-sensor paddle as well as the first such study of inhibition of voltage-dependent potassium channels. We propose a unified motion for the gating principle and the channel opening, and shed light on the voltage-sensor paddle motion and its inhibition.

1 Experimental procedures

Elastic normal mode calculations To calculate normal modes of the voltage-dependent potassium channels, three programs, namely STAND (Levitt et al. 1985), ElNemo (Suhre and Sanejouand 2004), and Nomad-Ref (Lindahl et al. 2006), were employed. The normal mode calculations were run locally on a Linux (Ubuntu 8.04) operated desktop computer with a 1.8 GHz Intel Pentium processor and 2GB of RAM (ElNemo and STAND) and using the web server (Nomad-Ref http://lorentz.dynstr.pasteur.fr/nomad-ref.php). For all methods, the 10 non-trivial lowest frequency normal modes based on elastic network models (Tirion 1996) were calculated using default parameters and RMSD set to 6 Å. Normal modes of KvAP from PDB ID 1ORQ (Jiang et al. 2003a), Shaker from PDB ID 3LUT (Chen et al. 2010; Long et al. 2007), Shaker in complex with one agitoxin from PDB ID 1AGT (Krezel et al. 1995), and Shaker in complex with hanatoxins from PDB ID 1D1H (Takahashi et al. 2000) were calculated. For PDB ID 3LUT, the long unstructured loop 190–215 was edited out of the structures prior to calculation. It is noted that while STAND (Soft Torsion Angle Normal-mode Dynamics) uses the single bond torsion angles as degrees of freedom, ElNemo and Nomad-Ref are significantly different in that they use atomic Cartesian coordinates as degrees of freedom.

Modeling To model the Shaker in complex with one agitoxin molecule, Shaker (PDB ID 3LUT) and agitoxin (PDB ID 1AGT) (Krezel et al. 1995) were superimposed onto the atomic model of the complex published by Erikson et al. (Eriksson and Roux 2002).

To model the Shaker in complex with four hanatoxin molecules, hanatoxins (PDB ID 1D1H) (Takahashi et al. 2000) were introduced in close proximity to the voltage-sensor paddle in the orientation suggested by Swartz et al. (Swartz and MacKinnon 1997a) and Lou et al. (2003) in a four-fold symmetry. Several orientations were attempted as there was no available model.

2 Results and discussion

Normal modes of voltage-dependent potassium channels The variation of the Cα atom root mean square deviation (RMSD) with residue number is shown in Fig. 1 for the lowest frequency mode of the two voltage-dependent potassium channels Shaker and KvAP. The motion calculated using the three different programs STAND, ElNemo, and Nomad-Ref is strikingly similar. The motion exhibited by the channel is symmetrical, and the Cα RMSD is identical in each of the four subunits. Most noticeable in the transmembrane region of KvAP and Shaker is the motion of the voltage sensor paddle and its gating charges R1, R2, R3, and R4.

Gating principle and opening mechanism The motion of the lowest frequency mode of KvAP and of Shaker is shown in Fig. 2. In both channels, the voltage-sensor paddle (S3b-S4 residues 96–134 in KvAP and 319–378 in Shaker) tilts by approximately 25° inward and outward of the membrane in a plane tangent to the channel. The tilt angle is proportional to the distortion parameters used for normal mode calculations. Large distortion parameters lead to large tilts, and vice-versa. At the same time, the channels exhibit a corkscrew-like counter-rotation motion of the opposed regions (Fig. 2f) leading to the opening of the channel. In Fig. 3, there is shown a plot of the pore diameter against the tilting of the voltage-sensor paddle in the structures of the lowest frequency mode. The pore diameter in Shaker was measured as the distance between the γ2 carbons of V467 in opposing subunits, and in KvAP as the distance between the δ1 carbons of the sequence homolog I221 in opposing KvAP subunits. Strikingly, the pore diameter of Shaker is widened by 0.82 Å as the voltage-sensor paddle tilts 25° outward of the membrane. The pore diameter of KvAP is significantly less widened (0.13 Å) because the

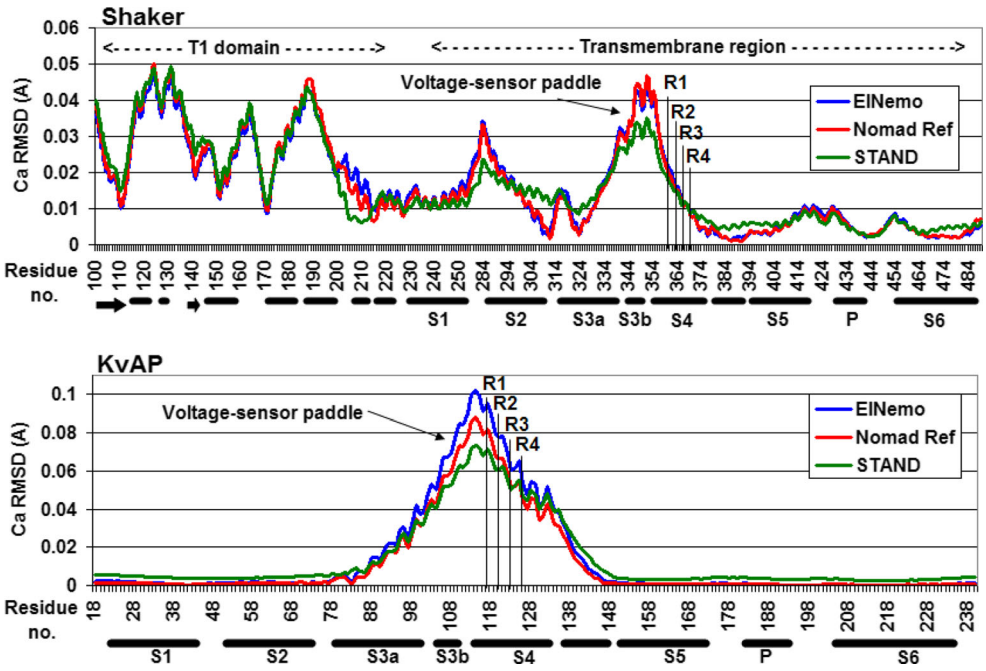

Fig. 1 Cα RMSD of voltage-dependent K+ channels Shaker, and KvAP. The Cα RMSD was calculated for the lowest frequency mode of ElNemo, Nomad Ref, and STAND. Notice the major motion of the voltage sensor paddles of KvAP (residues 96–134), and Shaker (residues 345–374) as well as their arginine gating charges R1, R2, R3, and R4. Secondary structure elements are indicated beneath the residue axis. The graphs were scaled completely arbitrarily so that the sum of Cα RMSD for each structure is 6 Å

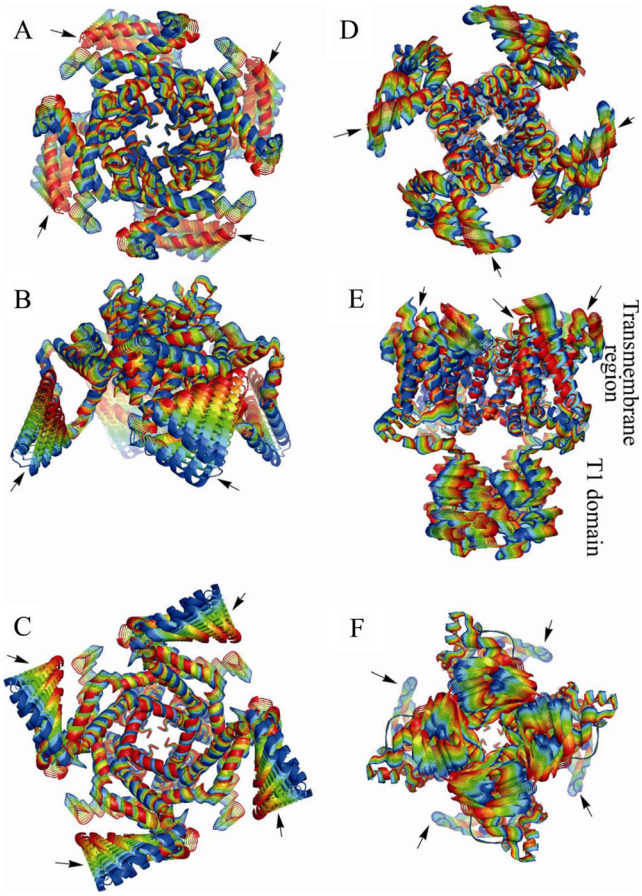

Fig. 2 Normal mode motion of voltage-dependent potassium channels. Shown are the (**a**) top, (**b**) side, and (**c**) bottom views of KvAP (PDB ID 1ORQ), and the (**d**) top, (**e**) side, and (**f**) bottom views of Shaker (PDB ID 3LUT). The motion is shown as a a series of structures colored from red to blue. Notice the tilting motion of the voltage sensor paddles (indicated by *little black arrows*) and its associated pore opening

voltage-sensor paddle does not interact with the rest of the channel to transduce opening.

This synchronized paddle tilt and pore opening is in agreement with experimental data showing that the voltage sensor paddles are positioned inside the membrane, near the intracellular surface, when the channel is closed, and that the movement of the paddles across the membrane causes the channel to open (Jiang et al. 2003b; Chanda et al. 2005; Bezanilla 2000; Yeheskel et al. 2010; Yanping et al. 2010; Shenkarev et al. 2010). These findings are also in agreement with voltage-sensor paddles being attached to the channel via a flexible hinge (S3a and S5) and moving across the membrane in response to membrane voltage to instigate channel opening (Jiang et al. 2003b; Yeheskel et al. 2010).

Very often, the lowest frequency normal modes are sufficient to describe molecular motion in biomolecules (Krebs

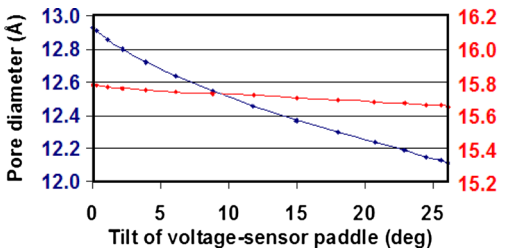

Fig. 3 Voltage-sensor paddle tilting vs. channel opening. Shown are the pore diameter of Shaker (in *blue*) and of KvAP (in *red*) plotted against the tilt angle of the voltage-sensor paddle in the lowest frequency mode calculated using Nomad-Ref (Lindahl et al. 2006). The pore of Shaker opens more (0.82 Å) than that of KvAP (0.13 Å) because the Shaker voltage-sensor paddle interacts more with the rest of the channel to transduce opening

et al. 2002). Here too, the lowest frequency modes of KvAP and Shaker fully describe the motion of the voltage sensor paddle and its associated channel pore opening, which has been observed experimentally. Wynsberghe and Cui {Van Wynsberghe, 2006 #1693} noted that care must be used when deducing correlated motions from normal mode analysis, in particular where motion is positively correlated in one mode, and anticorrelated in another. Nevertheless, we believe that the lowest frequency modes are an important contributor to the overall motion.

Covariance matrices of the voltage gated paddles in the first and second subunits of KvAP and Shaker were calculated for the three lowest frequency modes. The covariance matrix calculated with the lowest frequency mode of KvAP and Shaker indicate that the paddle motion is positively correlated ($R_1=1$), while the second, and third lowest frequency mode show that the motion is anticorrelated ($R_2=-1$ and $R_3=-0.6$). However, all three individual modes suggest that the two paddles are strongly correlated in their motion. The covariance matrices of high frequency normal modes was not calculated as they usually reflect uncorrelated local motion, and not concerted global motion (Krebs et al. 2002). Interesintgly, the covariance matrix of voltage gated paddles in the first and second subunit of Shaker in complex with one agitoxin are also positively correlated for the lowest frequency mode ($R_1=1$), and negatively correlated for the second ($R_2=-1$) and third ($R_3=-0.6$) lowest frequency mode. Not surprisingly, the covariance matrix of voltage gated paddles in the first and second subunit of Shaker in complex with four hanatoxins is uncorrelated for the three lowest frequency modes ($R_1=0.2$, $R_2=-0.2$, $R_3=-0.1$).

Rotational motion of S4 Our normal mode calculation also shows that as the voltage sensor paddle tilts outward of the membrane, thereby opening the channel pore, the S4 helix simultaneously exhibits a clockwise rotation along its main axis as viewed from the extracellular side (data not shown). While it is unclear whether this helical motion is instrumental in the opening of the channel, these findings are in agreement with experimental data supporting a "helical screw" model of motion of S4 while the channel is opening (Pathak et al. 2007; Durell et al. 2004; Shrivastava et al. 2004; Jensen et al. 2012).

Extent of motion of gating charges The exact magnitude of displacement of the gating charge across the membrane is currently a matter of debate (Ahern and Horn 2004; Tombola et al. 2005) with proponents of the paddle model suggesting a 20 Å outward displacement across the membrane (Jiang et al. 2003b), and opponents of the paddle model suggesting minimal movement of protein crevices 2 Å across the membrane (Chanda et al. 2005; Bezanilla 2000). For a change in helix tilt of 25°, our normal mode calculations of Shaker suggest a travel distance of 8.6 Å, 3.2 Å, 1.2 Å and 0.8 Å along the axis normal to the membrane for arginine residues, R1 (R362), R2 (R365), R3 (R368) and R4 (R371), respectively. Normal mode calculations of KvAP suggest a 18.2 Å, 7.4 Å, 2.4 Å and 1.6 Å motion along the axis normal to the membrane for R1 (R117), R2 (R120), R3 (R123), and R4 (R126) respectively. These positively charged arginines with strong attraction/repulsion forces induce motion within the membrane electric field to open voltage-dependent channels (Sigworth 1994; Bezanilla 2000; Armstrong and Bezanilla 1974; Larsson et al. 1996; Yang et al. 1996; Aggarwal and MacKinnon 1996) and form stabilizing interactions with negatively charged membrane lipid phophodiester groups (Schmidt et al. 2006).

Our normal modes dynamics favor a novel model for channel opening of KvAP and Shaker, which comprises a combination of the "voltage-sensor paddle" model which involves translation of the S3b-S4 domain, with the "helical screw" model which involves a simultaneous clockwise rotation of S4. This novel model may be instrumental in reconciling between earlier studies which suggest a channel opening mechanism based solely on the "voltage-sensor paddle

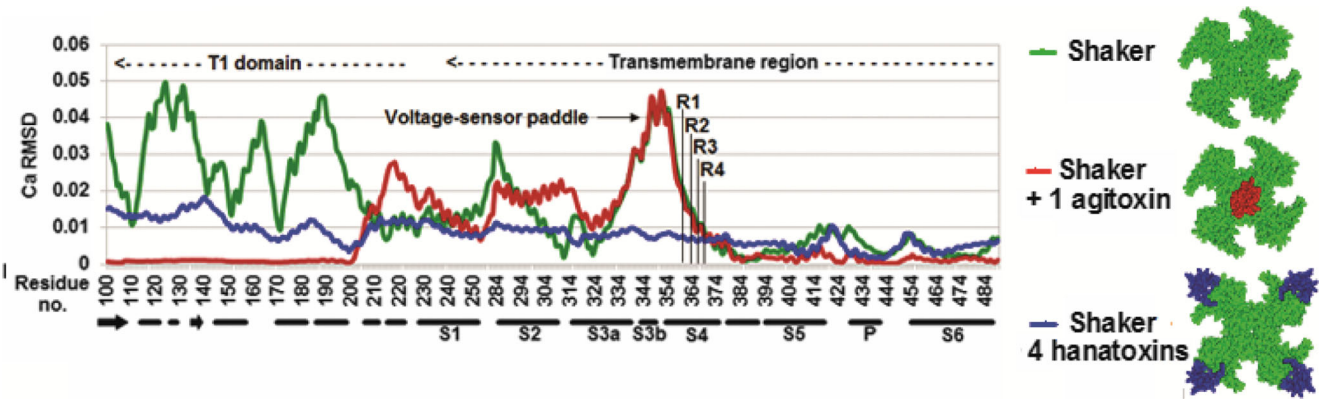

Fig. 4 Inhibition of Shaker. Shown are Cα RMSD plots of Shaker (in *green*), its complex with agitoxin (in *red*), and its complex with hanatoxin (in *blue*). Agitoxin, which blocks ion passage physically, does no interfere with voltage-sensor paddle motion. Hanatoxins, which do not occlude the ion pore, interfere with voltage-sensor paddle motion

model" (Jiang et al. 2003b; Chanda et al. 2005; Bezanilla 2000; Yeheskel et al. 2010; Yanping et al. 2010; Shenkarev et al. 2010) and studies which suggest a mechanism based solely on the "sliding helix" or "helical screw" model (Pathak et al. 2007; Durell et al. 2004; Shrivastava et al. 2004; Jensen et al. 2012). Our normal modes data contrast other models for channel opening of KvAP and Shaker, such as the 3_{10} helical conformation model suggested by Fatemeh et al. (2010). Other similar combination models have been proposed by Yang et al. and comprise an initial "sliding helix" movement followed by a paddle-like translocation (Yang et al. 2011). The method used by Yang et al. to validate this combination model included applying varying electric voltage to Xenopus laevis frog oocytes with mutated Shaker GH4 K^+ channel cDNA and plotting the resulting inactivation curves.

Potassium channel inhibition The voltage-dependent potassium channel, Shaker, is inhibited by agitoxin (Eriksson and Roux 2002) derived from *scorpion* venom, and by hanatoxin (Swartz and MacKinnon 1997a) derived from *tarantula* venom. Figure 4 shows the Cα RMSD distribution of the lowest frequency normal mode of Shaker, and its complexes with agitoxin and hanatoxin. In the lowest frequency mode of Shaker in complex with four hanatoxins, the voltage-sensor paddle motion is substantially diminished. The hanatoxins reduce the paddle motion but do not abolish it completely, in agreement with experimental studies that indicate partial activity of the Shaker even when bound to hanatoxins (Swartz and MacKinnon 1997b). Several hanatoxin orientations were probed on the voltage sensor paddle, and displayed the same kind of inhibition irrespective of their position. In fact, as long as the hanatoxins interact with the voltage sensor paddle, the normal mode paddle motion is inhibited.

In the lowest frequency normal mode of Shaker in complex with one agitoxin, the toxin locks the four subunits together, and the main motion is displayed by the voltage sensor paddle. The channel function is inhibited solely through pore blocking.

Amplitudes In normal mode calculations, the amplitude of each mode depends on the ambient temperature, the mode mass (or inertia) and the mode force constant. Elastic network or Tirion modes do not have absolute amplitudes as there is no way to estimate the energy associate with the deformation caused by the mode. In Figs. 1 and 4, we avoid this problem by setting the maximum deformation to the default values of DQMIN and DQMAX to −100 and 100 respectively (for Elnemo) thus always considering the relative amplitudes of motion of different Cα atoms. In Fig. 3, we relate the magnitude of pore opening to the degree of tilt of the paddle helix again avoiding any consideration of absolute motion. We stress that normal mode motion expressed in this paper should be viewed qualitatively more than quantitatively.

Conclusion Elastic normal modes of voltage dependent potassium channels exhibit tilting of the voltage-sensor paddles and helical motion of the S4 domain in parallel to an increase of the channel pore diameter. This motion is in agreement with experimental data for voltage-sensor paddles motion, helical rotation of S4, and pore opening. We find that pore blocking inhibitors which physically occlude the central pore, such as agitoxin, do not significantly alter normal mode motion of the channels. Contrarily, inhibitors which bind outside the pore, such as hanatoxin, exhibit a decrease in the paddling motion of channels. These findings broaden our understanding of voltage-sensor paddle motion and its inhibition.

Acknowledgments We thank Dr. Dahlia Weiss for careful reading of this manuscript, and helpful comments.

This research was supported by CIG award 322113 to A.O.S.

Conflict of interest The authors declare that they have no conflict of interest.

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Supporting information

STAND movie of the lowest frequency mode of Shaker is available free of charge via the internet at http://pubs.acs.org