Modeling of an Ion Channel in Its Open Conformation

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ABSTRACT We have modeled the structure of KirBac1.1 in an open state using as a starting point the structure of KirBac1.1 in its closed conformation (Protein Data Bank 1P7B). To test the validity of the open-state model, molecular dynamics simulations in octane, a lipid bilayer mimetic, were carried out. Simulations of the closed conformer were used for comparison purposes. The total simulation time was ~138 ns. The initial open model was refined by using projection maps obtained from electron microscopy experiments on two-dimensional crystals of the inwardly rectifying K+ channel KirBac3.1 from *Magentospirillum magnetotacticum* captured in its open state (C. Vénien-Bryan, unpublished data). Significant movements of the outer helices take place in going from the closed to the open model in agreement with structural and biochemical data in potassium channels, which suggests that gating is accomplished by a conformational change that takes place in the transmembrane domain upon an external stimulus. The motion of the inner helices is mainly achieved by bending at conserved glycine residues that have been previously reported to act as molecular hinges. Overall, these simulations suggest that the open conformer is stable, providing a plausible all-atom model that will enable the study of potential gating mechanisms in more detail.

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Since the KcsA structure was first reported (2), a remarkable amount of data has become available and it has been possible to rationalize the mechanisms of selectivity in ion channels. Contrary, the gating process is not yet fully understood even though some crystal structures captured in their open conformations are reported in the literature. Besides, the timescale of channel gating is too long (approximately milliseconds) to enable the study of this process per se by means of common computational techniques, such as molecular dynamics simulations. However, here we describe how combining modeling and simulation does provide clues about gating mechanisms.

The structure of KirBac1.1 in an open state has been modeled using as a starting point the structure of KirBac1.1 in its closed conformation (Protein Data Bank 1P7B) (3) (Fig. 1) and refining it by comparing the projection maps of the successive structures generated in the modeling process with the projection map of the open KirBac3.1 crystal until cross-correlation values were acceptable; 0.79 compared to 0.60 between the projection maps of the open state with the closed structure. The homology identity over 287 residues between KirBac1.1 and KirBac3.1 is ~40%, thus we made the assumption that the overall structural folds are similar. The model building was carried out manually using the program O assuming rigid body motions in going from the closed to the open conformation; no symmetry constraints were imposed on the positions of the structural elements. The KirBac1.1 structural elements that were moved as rigid bodies are the N-terminal section (residue numbers 36–45), the slide helix (46–59), the outer helix (60–85), the inner helices (134–150), and the C-terminal domains (151–309). The section between residue numbers 86 and 133 was left unaltered. This section includes the pore helices, the selectivity filter, and the N-terminal sections of the inner helices. Large structural motions within this region would be expected to significantly alter the conformation of the selectivity filter thus leading to a nonfunctioning channel.

To test the validity of the open state model, molecular dynamics simulations of the model in octane, a lipid bilayer mimetic, were carried out. An octane slab was chosen as it is a reasonable approximation to a lipid bilayer but it has a considerably lower viscosity so it is expected to be more permissive to possible protein conformational changes, the desirable situation to be explored. The degree of conformational drift and secondary structure contents were assessed in the various simulations to check the validity of the open model. Simulations of the closed model were used for comparison purposes.

Segments missing from the KirBac1.1 crystal model were modeled using the loop modeling interface of the Rapper server (http://raven.bioc.cam.ac.uk/loop2.php). They correspond to residues 195–206 and 290–297. Subsequently, the system was energy minimized using the GROMACS program (www.gromacs.org).

The C-terminal carboxylate was protonated and the N-terminal amine unprotonated to form neutral termini. On the basis of pKa calculations, the side chains of Asp-115 and Glu-130 were protonated. Thus, there is a shared proton between Asp-115 and Glu-106, homologous to that shared

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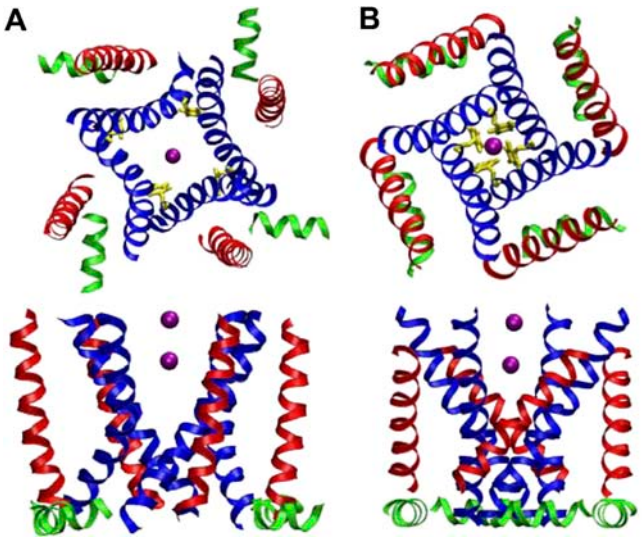

FIGURE 1 Schematic diagrams of the open (*A*) and closed (*B*) conformers of KirBac1.1. In panels *A* and *B* top figures, the view is down the pore axis, from the extracellular end of the protein. Phe-146 activation gate is displayed as yellow sticks. In panels *A* and *B* bottom figures, the view is perpendicular to the pore axis, with the extracellular mouth at top of the diagram. The slide helices (*green*), inner (*blue*), and outer (*red*) helices are represented as ribbons. Purple spheres represent K^+.

between Asp-80 and Glu-71 in KcsA. The rest of the residues remained in their default ionization state.

Once the open model was embedded in an octane slab of thickness 3.2 nm, it was solvated with SPC water molecules. An ionic strength of 150 mM of KCl was used and counterions were added where needed to keep all systems electrically neutral. The final system contained ~23,000 water molecules and ~740 octane molecules giving a total of ~87,000 atoms. Ten different simulations were carried out. A), three sets with the open model where K^+ ions were present in: i), sites Sext, S1, S3, and at the entrance of the filter in the intracellular side; ii), sites Sext, S2, S4, and at the entrance of the filter in the intracellular side; and iii), sites Sext, S2, and S4. A water molecule was placed in sites where no K^+ ions were present. B), Two sets of simulations with ions in positions corresponding to the first and third configurations described previously using the closed structure of the channel (Protein Data Bank 1P7B). For each of these five sets, long-range electrostatic interactions were calculated using either the particle mesh Ewald summation method or using a cutoff of 18 Å with van der Waals interactions truncated at 10 Å. No effect due to the treatment of long-range electrostatics was observed in the properties analyzed. Simulations were carried out in the NpT ensemble, with periodic boundary conditions. The initial velocities were taken randomly from a Maxwellian distribution at 300 K. An equilibration was performed during which the protein atoms and the ions and water in the selectivity filter were restrained for 0.2 ns. The restraints were then removed and production simulations of 12 ns of duration followed. Three of the simulations were extended up to 18 ns. Overall, this corresponds to a total simulation time of 138 ns.

Results show that the overall fold of the open model is stable except for one out of the four slide helices that moved significantly in almost all the simulations of the open model. This can be accounted for by the fact that loops implicated in the N-termini/slide helix interactions were missing in the x-ray structure and were modeled individually. Values of Cα root mean square deviation (RMSD) of the entire structure relative to the initial one varies from 0.48 to 0.56 nm for the open model and from 0.34 to 0.38 nm for the closed model. These high values can be accounted for by the fact that loops that are important in the N-terminus/slide helix interaction were modeled as they were missing in the crystallographic structure. There is also a high contribution due to the slide helices that can be accounted for the lack of interactions with the lipid headgroups. Despite the fact that the RMSDs for the open conformation are higher than for the closed form, they become constant over the length of the simulations, which is a reflection of the stability of both models. RMSDs for different structural elements compare as 0.29 vs. 0.13 nm for outer helices, 0.25 vs. 0.1 nm for inner helices, and 0.09 vs. 0.12 nm for the selectivity filter (open versus closed, respectively). It is worth mentioning that the selectivity filter is the structural element that remains the most stable in all the simulations. The behavior of the ions in the selectivity filter does not differ from the closed to the open models in these simulations or from that in previous simulations based on the closed conformer. Secondary structural elements remained constant over the time frame of the simulations, including the selectivity filter, and the overall positions of the secondary structural elements do not extensively deviate. The MD simulations show that the model is a stereochemically stable structure.

The conformational change responsible for gating seems to involve concerted and/or sequential hinge-bending motions about conserved glycines (Gly-134 and Gly-143) and other flexible elements in the M2 helix (Gly-137). The M2 helix kink average values calculated using Gly-134 as a hinge point are 22° ± 7 and 10° ± 5 (open and closed, respectively). These values are comparable with 12 and 30° obtained for the x-ray structure of KirBac1.1 and MthK (1LNQ) (an open channel). In MthK, the homologous conserved Gly-83 was used as hinge point. Fig. 2 shows three different views of the superimposed Cα traces of the open channels MthK and KvAp (1ORQ), the closed KcsA and KirBac1.1, x-ray, and closed and open models. The N-terminal halves of the helices were used for fitting.

At the activation gate, the differences in the distance from the center of mass of the main-chain atoms of Phe-146 (the so-called blocking residue), between chains AC and BD vary between 1.1 and 1.4 Å for the closed conformers, similar to

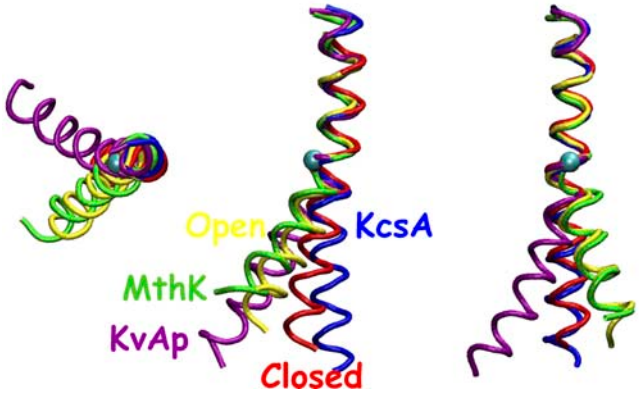

FIGURE 2 Kink of M2 helices. Three different views of the superimposed Cα traces of MthK (*green*), KcsA (*blue*), KvAp (*purple*), KirBac1.1 closed conformer (*red*) x-ray structures and the open model (*yellow*). The N-terminal halves of the helices were used for fitting. The cyan sphere indicates the position of Gly-134 (KirBac1.1).

the value of 1.1 Å obtained in the experimental structure. These differences change between 4.0 and 6.5 Å in the open conformers. A dimer-of-dimers pattern is observed in all cases. Thus, it appears that the M2 bundle may behave dynamically as a dimer-of-dimers.

Overall, this open model is stable and will be useful in the study of potential gating and block mechanisms in potassium channels in more detail.

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REFERENCES and FOOTNOTES

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