Intrinsic flexibility and gating mechanism of the potassium channel KcsA

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Edited by William N. Lipscomb, Harvard University, Cambridge, MA, and approved December 31, 2001 (received for review December 5, 2001)

The gating mechanism of the potassium channel KcsA was studied by normal mode analysis. The results provided an atomic description of the locations of the pivot points and the motional features of key structural elements in the gating process. Two pivot points were found in the motions of the inner TM2 helical bundle that directly modulate the size of the central channel pore. One point is an intrasubunit hinge point that sharply divides the structural flexibility between the more rigid selectivity filter and the more mobile peripheral transmembrane helices. Such a division is vital for KcsA because it permits the large-scale motions of transmembrane helices required for the gating and, in the meantime, maintains the rigidity of the filter region essential for the selectivity. The other pivot point is an intersubunit one at which all four TM2 helices are bundled together. During the gating process, each TM2 helix undergoes a lever-like swinging motion pivoting on the intrasubunit hinge, and the entire TM2 bundle undergoes a concerted rotational motion around the central channel axis constrained around the intersubunit bundle point. This series of motions leads to a dramatic enlargement of the intracellular gate without loosening up the structural integrity.

normal mode analysis | structural flexibility | ion channel

KcsA is an ion channel that is capable of selecting K^+ over Na^+ by a factor of 10^4 and permits the near diffusion-limited throughput rate of 10^8 ions per second (1). KcsA is a tetrameric integral membrane channel protein gated by pH (ref. 2; Fig. 1). Each monomer has two transmembrane helices, TM1 and TM2. The overall shape of the inner core of the channel, formed mainly by the four inner helices TM2, resembles an inverted teepee. There is a selectivity filter located near the extracellular side of the structure, which is highly selective for K^+ ion. Inside the selectivity filter, main-chain carbonyl oxygen atoms from the filter signature sequence TVGYG are arranged in such a way that the filter energetically favors the transmission of K^+ or similar cations such as Rb^+ and Cs^+ , but not the smaller alkali cations like Na^+ and Li^+ (3, 4). Behind the TVGYG sequence is a small-pore helix that supports the selectivity filter. Just before the selectivity filter, roughly in the middle of the membrane, there is a cavity with a diameter of 10 Å, which has been suggested to play a role in stabilizing water molecules and cations in the middle of the phospholipid membrane (5, 6). This cavity is connected to the cytoplasm by a relatively long hydrophobic pore, which is also the location of the intracellular gate of KcsA. Electron paramagnetic resonance (EPR) measurements showed that the diameter of this hydrophobic pore increases when the pH is lowered (7–9). Minimal change in intersubunit distance near residues Thr-107 to Ala-108 also was observed (9), which led to the speculation that this region serves as a pivot point for the overall motions of the helices.

The wealth of experimental data provides a foundation for a computational study to obtain an atomistic understanding of the gating mechanism of KcsA. Although there have been extensive simulation (4, 6, 10–16) and modeling studies of KcsA (17), no attempt at simulating the large-scale conformational changes in

the gating process of KcsA has been made. In this study, we applied normal mode analysis (NMA; ref. 18) to study the intrinsic structural flexibility of KcsA and its implications in the gating mechanism (5, 19). The lowest-frequency modes obtained from NMA would manifest the intrinsic patterns of elastic deformation of the structure, because numerous studies have shown that, during the evolution, nature has recruited the pathways of the intrinsic low-energy deformational motions into those of the functionally important conformational changes (20–25).

We identified two pivot points for the motions of the TM2 helices, an intrasubunit hinge point and an intersubunit bundle point. The overall motion of the inner TM2 helical bundle can be described as a concerted swinging rotational motion such that each TM2 helix pivots on the intrasubunit hinge point and all four TM2 helices, bundled together at the intersubunit pivot point, concertedly rotate around the channel central axis in the clockwise direction. The relationship of the potential pH-sensitive triggers with the arrangement of the pivot points is also discussed.

Methods

Normal mode analysis was performed by using standard techniques (18, 24, 26) by the VIBRAN module in the CHARMM program (27). A polar hydrogen based empirical energy function (CHARMM19) was used (28). To account approximately for the shielding of distant charges by solvent, a distance-dependent dielectric constant was used (27), and the atomic charges on ionic side chains were scaled by a factor of 0.3. The PDB code for the structure is 1BL8 (5). The structure was energy-minimized first by 500 steps of the steepest-descent method and then a few thousand steps of the adapted basis Newton-Raphson (ABNR) method (27). The minimizations were terminated when the rms energy gradient reduced to 10^{-8} kcal/mol/Å. For KcsA, the calculations were done on systems with and without the presence of the crystallographically identified K^+ ions and water molecule (Fig. 1).

In our analyses, only the lowest-frequency modes were considered because they give rise to the largest displacements and provide information on the important intra- and interdomain motions (26). The end-point structure (open state) used for analysis here was generated by displacing the atoms along the eigenvector of the normal mode until the mean rms displacement of 3.5 Å (18). The final conformation was relaxed slightly by 50 steps of steepest-descent minimization for adjusting the lengths of the over-stretched bonds.

Results

Overall Motions. The most relevant mode of KcsA to its gating mechanism is the lowest-frequency vibrational mode of fre-

This paper was submitted directly (Track II) to the PNAS office.

Abbreviation: EPR, electron paramagnetic resonance.

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Fig. 1. Overall architecture of the potassium channel KcsA (5) shown in the side view (a) and top view (b). The colors are used to distinguish the secondary structural elements in one subunit: TM1 (residues 24–51) is in green, TM2 (residues 86–118) is in blue, the small-pore helix (residues 61–73) is in red, and the TVGYG filter sequence is in cyan. The four spheres represent the crystallographically observed three K⁺ ions (gray) and the oxygen atom of a water molecule (red). The figures were made by graphic software MOLSCRIPT (31) and rendered by RASTER3D (32).

quency 2.50 cm^{-1} . This mode revealed a totally concerted tilting and rotation of the transmembrane helices TM1 and TM2 (7–9, 29). The motion of the TM1 helices is mainly a tilting and rotation around a hinge formed between Ser-44 on TM1 and Ser-69 on the small-pore helix. Although this motion is an integral part of the gating conformational changes, it does not directly modulate the size of the central channel pore because all four TM1 helices are located in the outskirts of KcsA. It is the motions of the TM2 helices that determine the size of the intracellular gate. The overall structural changes of the inner TM2 helical bundle, observed in our analysis (Fig. 2*a* and *b*), are consistent with the most recent EPR observations (figure 4*a* in ref. 9). However, our computational analysis identified two pivot regions on TM2, instead of one (9), that are important for the structural changes. The first one is an intrasubunit flexible hinge. This hinge is located at $\approx 1/3$ of TM2 from its N terminus near

the extracellular side. It contains a set of neighboring residues in a region where TM2 contacts the C terminus of the small-pore helix, mainly Ala-92, Val-95, and Met-96 on the TM2 helix and Thr-72 on the small-pore helix (Fig. 2*c*). The role of this hinge has never been discussed. The second pivot region is an intersubunit one, located roughly at $1/3$ of TM2 from its C terminus near the intracellular side (Fig. 2*c*). It consists of residues Thr-107 to Leu-110 on TM2; the role of this second pivot region was discussed in EPR studies (9, 17).

The deformational motions involved in the other vibrational modes do not seem to correlate with the gating process of KcsA.

Intrasubunit Hinge Point. Fig. 2*d* shows the distance between the same C α atoms of the closed state (the crystal structure) and the open state generated from the lowest-frequency mode as a function of the residue number in a single subunit. It clearly shows that the filter region has dramatically smaller difference and, therefore, it is an intrasubunit hinge point. This hinge sharply divides the flexibility of KcsA subunit into two portions: a highly rigid portion including the small-pore helix and the TVGYG sequence in the selectivity filter (darker part in Fig. 2*e*) and a much more flexible portion containing mainly TM1 and TM2 helices (lighter part in Fig. 2*e*). Such a division of flexibility is vitally important for the function of KcsA: on the one hand, it permits the large-scale motions of TM1 and TM2 helices, which are critical for the gating, and on the other hand, it maintains the rigidity of the filter region that is essential for the selectivity. Such rigidity also arises from the presence of the ions. A calculation without the ions included in the selectivity region showed that the rigidity of the selectivity filter dramatically decreased (data not shown).

The motion of each TM2 helix can be viewed as that of a swinging lever pivoting on a hinge, in which the inner surface of TM2 formed by the side chains of Ala-92, Val-95, and Met-96 undergoes a “rocking” motion against the side chain of Thr-72 on the small-pore helix. As a result, both termini of TM2 have relatively significant displacements (Fig. 2*d*). The C terminus of TM2 (at the intracellular side of KcsA) has a larger displacement that is obviously important for the opening of the intracellular gate (see more later). The smaller but significant motion of the N terminus of TM2 (at the extracellular side of KcsA) may be coupled to the pH-dependent triggers located in the same region (2). There are several titratable acidic residues in that region, e.g., Glu-51, Glu-71, and Asp-80 (Fig. 2*f*). The ionization states of their side chains could account for the observed pH-sensitivity. The side chain of Glu-71 has been suggested to be protonated because it faces the presumably ionized side chain of Asp-80 (12, 19). The latter also forms a salt bridge with Arg-89 near the N terminus of TM2 on the neighboring subunit. Therefore, the pK_a value of Asp-80 could have down-shifted to coincide with the observed pH profile of the gating (2). Also, the ionic side chain of Glu-51 near the C terminus of TM1 is hydrogen-bonded to the main chain NH-groups of Val-84 and Thr-85 on the loop connecting the filter TVGYG sequence and TM2. As the pH is lowered, the protonation of Glu-51 and Asp-80 would result in local energetic instability and, thereby, facilitate the small movements of the C terminus of TM1 and N terminus of TM2 at the extracellular side of KcsA. These small displacements could be maximally amplified at the intracellular side of TM1 and TM2 helices for gating by means of the hinged lever motions.

Intersubunit Bundle Point. The intersubunit bundle point is located roughly at $1/3$ of TM2 from its C terminus near the intracellular side of KcsA. At this point, the four TM2 helices are bundled together. Their motions around this point can be described best

Fig. 2. Motions in the lowest-frequency normal mode of KcsA. (a and b) The stereo pairs of the clockwise rotational motions of the inner TM2 helical bundle; a is the top view from the extracellular side, and b is the side view. The closed state (the crystal structure) is in red and the open state, the end-point structure in the lowest-frequency mode, is in blue (18). Here, a and b can be compared with figure 4a in ref. 9. (c) A single subunit with the locations of two pivot points (upper 1/3 intrasubunit hinge and lower 1/3 intersubunit bundle point) indicated by the representative residues. (d) The distance between the same C_{α} atoms of the superimposed closed state (the crystal structure) and the open state generated from the lowest-frequency mode as a function of the residue number in a single subunit. The sequence regions of TM1 and TM2 are indicated, and the lower level bars indicate the regions where the distances, or motions, are small, i.e., the hinge points. (e) Two sharply separated rigidity partitions in KcsA. The small-pore helices and the TVGYG sequence form a rigid unit (darker part), and TM1 and TM2 are much more flexible. Such a rigidity separation permits large-scale relative motions of TM1 and TM2 during the gating without compromising the stability of the selectivity filter. For clarity, only two subunits are shown. (f) The locations of the titratable groups near the extracellular end of KcsA.

Clockwise rotation of the inner TM2 helical bundle.

Fig. 3. Schematic illustration of the motions of the inner TM2 helical bundle. For clarity, only two opposite TM2 helices are shown. The initial close conformation is in red; the final open conformation is in blue (as in Fig. 2a and b). Each TM2 helix pivots on the intrasubunit (*Upper*) hinge indicated by the position of Met-96 and all four TM2 helices are bundled together around the intersubunit (*Lower*) pivot point indicated by the positions of Thr-107. The entire helical bundle undergoes a clockwise rotation (black arrows) around the central channel axis leading to a significant enlargement of the gate at the intracellular end of the structure (indicated by the sizes of the yellow circles). During the rotation, the distances between all Thr-107 residues do not change much, similar to what were shown in EPR measurements (9). The dotted line connecting two Thr-107 residues on the opposite TM2 helices is the diagonal line of the square connecting all four Thr-107 residues mentioned in the main text.

by saying the square formed by connecting the pivot points on all four neighboring TM2 helices undergoes a clockwise rotation (looking from the extracellular side) around the central channel axis (see caption of Fig. 3 for more explanations). The length of the side of this square, i.e., exemplified as the distance between two Thr-107 residues on the neighboring TM2 helices, does not change dramatically (9), but the circular motions of all TM2 helices lead to a dramatic increase of the distance between the ends of TM2 helices at the intracellular side of KcsA (indicated by the yellow circles in Fig. 3). The directionality of the rotation of the TM2 helices is critical. In our model, only the clockwise rotation can lead to the enlargement of the intracellular gate of KcsA because, in the closed state, the TM2 helices (red rods in Fig. 3) are arranged in such a way that they are not in coplane with the channel central axis. Geometrically, the clockwise rotation would bring them further away from the coplane configuration and lead to an enlargement of the gate near the intracellular end of the TM2 helices (also, see Fig. 2b; ref. 9).

The Gating Mechanism. Combining the effects of the intrasubunit hinge and the intersubunit bundle points, the overall motions of the TM2 helices can be viewed as a swinging rotational motion.

Each TM2 helix moves like a swinging lever pivoting on the intrasubunit hinge at the upper 1/3 position of the TM2 and all four TM2 helices, bundled together at the lower 1/3 of the TM2, concertedly rotate around the central channel axis in the clockwise direction. We propose that the intrasubunit hinge is important for sensing the pH changes and for separating the rigidity of transmembrane helices and the selectivity filter. The intersubunit bundle point is important for maintaining the structural integrity of KcsA during the opening process of the intracellular gate. The interplay of the two pivot points is a beautiful design by nature for solving the gating problem of KcsA.

Concluding Discussion

The normal mode analysis was used to study the intrinsic flexibility of KcsA. The lowest-frequency vibrational mode revealed a low-energy deformational pathway that dominates the gating of the channel. The overall feature of the conformational changes in the gating was found to be consistent with what was observed in EPR measurements. However, the computational analysis revealed a second pivot point and provided a more detailed description of the motional features of the key structural elements in the conformational changes.

Two pivot points were found in the motions of the inner TM2 helical bundle that directly modulates the size of the central channel pore. The motion of the TM2 helical bundle can be described as a near rigid-body swinging rotational motion bounded by two pivot points. The first pivot point, located at 1/3 of TM2 from the extracellular side, is an intrasubunit hinge point, which turns out to be the most critical hinge in the gating process. This hinge sharply divides the structural flexibility between the selectivity filter and the peripheral transmembrane helices. The former needs to maintain a higher rigidity for the delicate selection of K^+ ions over Na^+ ions, whereas the latter needs to be more flexible to facilitate the large-scale motion for the gating. The N terminus of TM2 may be the location of the pH-sensors. The observed lever-like swinging motion of TM2 helix in our analysis rationalized that the pH-sensors and the gate are placed at the opposite ends of TM2. This arrangement would make it possible that a small displacement at the N terminus of TM2, presumably triggered by the changes of protonation states of multiple titratable side chains, can be maximally amplified at the C terminus of TM2 for the gating. The second pivot point, located at 1/3 of TM2 from the intracellular side, is an intersubunit pivot point. This point is the one at which all four TM2 helices are bundled together. During the gating process, the TM2 bundle undergoes a concerted rotational motion around the central channel axis and leads to a dramatic enlargement of the intracellular gate of KcsA. This pivot point is important for maintaining the structural integrity of KcsA and for facilitating the opening of the intracellular gate.

The swinging rotational motion of TM2 helices with two pivot regions is an exquisite design by nature to ensure an effective gating of KcsA without having to loosen up the structural integrity near the intracellular side of channel in the open state.

The computational protocol used in this study is generally applicable to many other membrane-bound proteins such as receptors, pumps, and channels, whose functions involve large-scale conformational changes (30). It is now widely accepted that structural motions such as rigid-body movements of secondary and tertiary structural elements, e.g., pushing or pulling of neighboring helices, scissors or hinge bending between domains, are essential for the functions of those proteins (29).

J.M. gratefully thanks the support from the American Heart Association, the Robert A. Welch Foundation, and the National Science Foundation.

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