

Structural Rearrangements Underlying K⁺-Channel Activation Gating

Eduardo Perozo,* D. Marien Cortes, Luis G. Cuello

The intramembrane molecular events underlying activation gating in the *Streptomyces* K⁺ channel were investigated by site-directed spin-labeling methods and electron paramagnetic resonance spectroscopy. A comparison of the closed and open conformations of the channel revealed periodic changes in spin-label mobility and intersubunit spin-spin interaction consistent with rigid-body movements of the two transmembrane helices TM1 and TM2. These changes involve translations and counterclockwise rotations of both helices relative to the center of symmetry of the channel. The movement of TM2 increases the diameter of the permeation pathway along the point of convergence of the four subunits, thus opening the pore. Although the extracellular residues flanking the selectivity filter remained immobile during gating, small movements were detected at the C-terminal end of the pore helix, with possible implications to the gating mechanism.

The functional behavior of ion channels is based on two fundamental processes: permeation and gating (1). Permeation is responsible for the selective and efficient translocation of ions across the membrane, whereas gating tightly controls access of ions to the permeation pathway, effectively determining channel activity.

Recently, in what can be considered a defining moment for ion-channel research, the crystal structure of KcsA, a potassium channel from *Streptomyces lividans* (2) was determined at 3.2 Å (3). The structure revealed a tetrameric complex with a centrally located pore formed by the apposition of individual subunits, each with two transmembrane helices (TM1 and TM2) flanking a "selectivity filter" (3). Functional characterization of KcsA in planar lipid bilayers demonstrated a K⁺-selective channel of 140 pS with very short open times and at least two subconductance states (2, 4). KcsA is mostly closed at neutral pH, but it can be stabilized in the open state at low extracellular pH [(4), but see (5)], a finding that has opened the door to high-resolution structural studies of the gating mechanism. Although the proton-dependent events that trigger channel opening are not understood, experiments with vectorially reconstituted KcsA suggest that these events occur on the extracellular side of the molecule (4).

This article deals with two fundamental questions concerning activation gating in ion channels: (i) the location of the gate that opens and closes the permeation pathway, and (ii) the type of molecular movements that allow the

gate to physically occlude the pore in a reversible manner. Evidence from kinetic studies of K⁺-channel blockade by quaternary ammonium ions (6) suggests that in voltage-dependent channels both open-channel block and ion-trapping effects are associated with changes at the intracellular end of the molecule. Cysteine scanning experiments in Shaker K⁺ channels have demonstrated state-dependent cysteine reactivity at the C-terminal end of the S6 transmembrane segment (7), consistent with the movement of a putative gate in or around this region of the molecule.

Site-directed spin labeling and electron paramagnetic resonance (EPR) spectroscopy are ideally suited to probe the structural dynamics of membrane proteins (8). This approach, applied to KcsA channels (9), shows gating-related conformational changes in residues lining the permeation pathway along TM2 (structurally equivalent to S6). Those changes suggest that channel opening is associated with a widening of the internal vestibule of the channel. Here we have studied trends and periodicity changes in the EPR signal from a large set of residues in KcsA to gain information about the global structural changes that underlie pH-dependent gating.

Functional Role of the C-Terminus

We analyzed the functional role of the C-terminus of KcsA by looking at the effect of specific cytoplasmic deletions on the ability of the channel to gate in a pH-dependent manner. We compared two deletion constructs, Δ140–160 and Δ125–160 (10), with control constructs having the His₆ tag at either end of the molecule by measuring Ba²⁺-sensitive ⁸⁶Rb⁺ uptake in proteoliposomes (4) (Fig. 1A). It is expected that if the gate of the channel is indeed mostly intracellular, deletions at the C-terminus should render the channel permanently open, uncoupling proton binding from ion conduction. In the absence of most of the C-terminus (Δ125–160), ⁸⁶Rb⁺ influx is only partially uncoupled from proton binding (Fig. 1B). The channel can still close at neutral pH but to about 50% of control, as if the main gating transition were still present and the C-terminal deletion affected

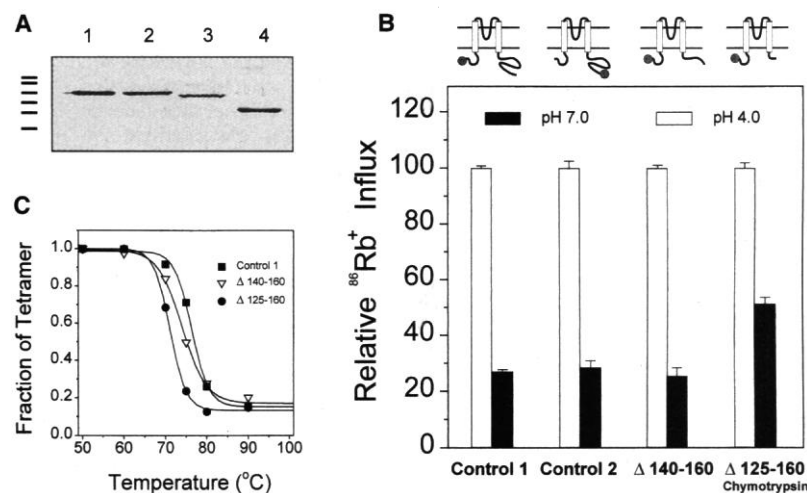


Fig. 1. pH-dependent gating and functional role of cytoplasmic regions of KcsA. (A) SDS-polyacrylamide gel of each construct showing the relative migration of the tetrameric form of the channel. Lane 1, control 1 KcsA with an N-terminal His₆ tag; lane 2, control 2 KcsA with a C-terminal His₆ tag; lane 3, construct Δ140–160; lane 4, construct Δ125–160 (obtained by proteolytic treatment of KcsA with chymotrypsin). Protein standards (kD) are 158, 116, 98, 66, 57, and 43. (B) Ba²⁺-sensitive ⁸⁶Rb⁺ influx at neutral and acidic pH. Data for each construct are normalized to the maximal ⁸⁶Rb⁺ uptake (pH 4.0) and are shown as the mean ± SD for five independent measurements. (C) Tetrameric stability of KcsA and deletion mutants at pH 7. Each construct was solubilized in 0.3 mM DDM, incubated at different temperatures for 30 min, and separated on a 15% polyacrylamide gel. The intensity of the oligomer and monomer band was quantified densitometrically. The approximate midpoints of each melting curve are 76°C (control 1), 74°C (Δ140–160), and 70°C (Δ125–160).

Department of Molecular Physiology and Biological Physics and Center for Structural Biology, University of Virginia Health Sciences Center, Charlottesville, VA 22906–0011, USA.

*To whom correspondence should be addressed. E-mail: eperozo@virginia.edu

the stability of the closed state. These effects appear to be restricted to the region between residues 125 and 140 of the C-terminus, because construct $\Delta 140$ –160 behaved like the control channel (Fig. 1B).

These results suggest that the C-terminus cannot be the primary gate of KcsA, because in its absence the channel is still able to partially regulate a Ba^{2+} -sensitive $^{86}\text{Rb}^{+}$ influx in a pH-dependent manner. It appears unlikely, therefore, that gating in KcsA occurs as a consequence of conformational rearrangements of a cytoplasmic “plug,” although the functional effects of deleting the C-terminus may suggest a close interaction with the membrane-embedded regions of the channel. In fact, data from site-directed spin-labeling studies (11) indicate that residues ~125 to 145 may form an intracellular helical bundle immediately adjacent to the “inverted teepee” formed by TM2, similar to what is found in the cytoplasmic region of the mechanosensitive channel MscL from *Mycobacterium tuberculosis* (12). These two structures might be physically coupled, and deletion of the C-terminus could lead to an increase in the motional freedom of the TM2 helices (13), which explains the increase in channel activity at neutral pH. Thermal denaturation experiments of the deletion constructs tend to support this statement, as Fig. 1C demonstrates a small but significant shift in the midpoint of the denaturation curve, from about 76°C in the control KcsA to about 70°C in $\Delta 125$ –160 and about 74°C in $\Delta 140$ –160.

Rigid-Body Movements of Transmembrane Helices

At a basic level, activation gating could involve rigid-body movements through the tilting or twisting of secondary structure elements or domains, as has been shown for gap junctions, acetylcholine receptors (14), and rhodopsin (15). Alternatively, they could also require secondary structure transitions like those suggested for the conformational changes in the S4 segment in voltage-dependent channels (16). We studied the pH dependence of the secondary structure content of KcsA by circular dichroism (CD) spectroscopy (17). Table 1 shows the results of these experiments obtained for pH values between 3.0 and 7.0. At neutral pH, the current data matched earlier determinations with CD spectroscopy (18) and were close to those obtained by Fourier transform infrared spectroscopy (19). At lower pH values there appeared to be no significant changes in the secondary structure content of KcsA, which clearly shows that channel opening must be coupled to the motion of entire domains or secondary structure elements.

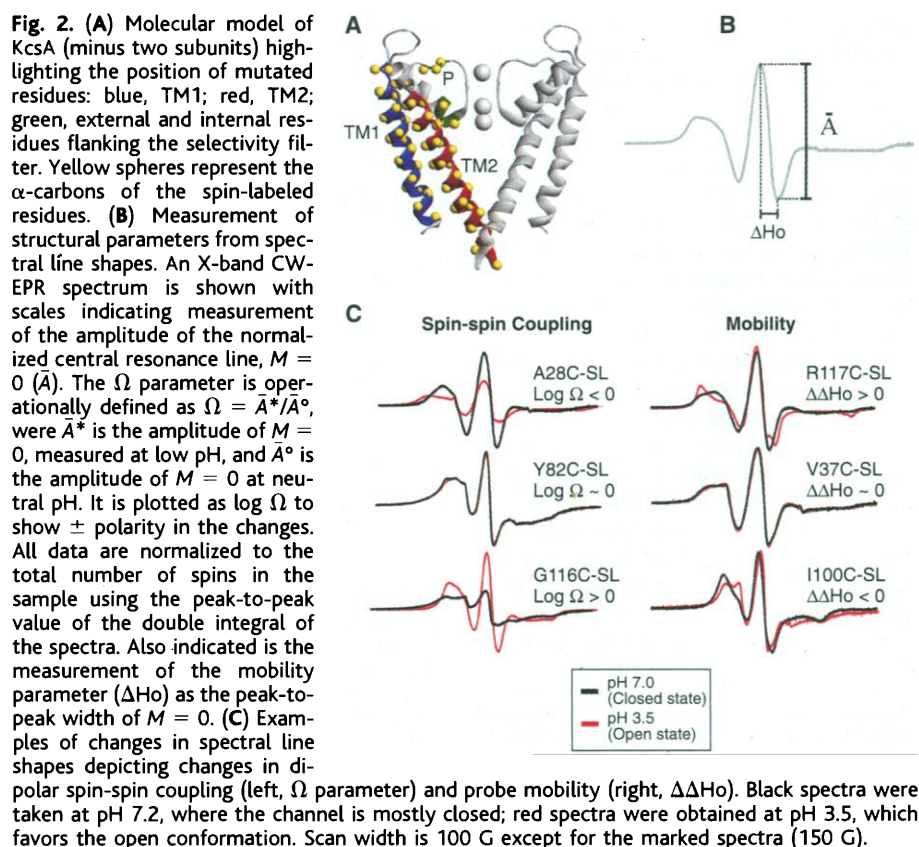
We then compared the EPR spectral properties of spin-labeled cysteine mutants determined at either neutral or acidic pH for more than 60 cysteine mutants (20). Three regions of

the channel were studied: the two transmembrane helices TM1 (residues 26 to 50) and TM2 (residues 90 to 120) and the regions immediately flanking the narrow portion of the selectivity filter (Fig. 2A). Two structural parameters were analyzed: probe mobility (Fig. 2B, ΔHo) and the Ω parameter. Mobility changes (ΔHo) are an indication of rearrangements in tertiary or quaternary contacts; positive ΔHo values suggest increased steric contacts and negative ΔHo values imply increased motional freedom (21). The Ω parameter reports on changes in intersubunit probe-to-probe proximity and is obtained from the ratio of the normalized amplitude of spectra (Fig. 2B, $\bar{A}$) in the open and closed conformations (21). Ω values lower than 1 indicate that the spin-labeled residues move closer to the fourfold axis of symmetry in the open conformation. Ω values larger than 1 reflect motion away from the symmetry axis. Specific examples of changes in spectral line shape due to changes in mobility or interprobe proximity are illustrated in Fig. 2C.

The result of the analysis of TM1 and TM2 spectral data sets obtained in the closed or open conformation is shown in Fig. 3. Because TM1 is located in the periphery of KcsA and less than half of the helix is involved in tertiary contacts, ΔHo should be a very sensitive indicator of helix tilting and rotation (Fig. 3A, left). Indeed, changes in TM1 ΔHo show evidence of a strongly periodic behavior, where pockets of residues experience enhanced tertiary contacts alternating with residues that show an increase in motional freedom. The power spectrum obtained from the TM1 ΔHo profile (22) displays a large peak centered at 108° (Fig. 3B). This is a clear indication of α -helical periodicity and suggests that the movement of TM1 occurs as a rigid body (23). The Ω profile also shows considerable changes upon channel gating, mostly at the N-terminal half of the helix (Fig. 3A, right). The fact that some residues in TM1 (28, 29, 35, 46) show Ω values <1 and others (27, 31, 33, 43, 44) have Ω values >1 indicates that TM1 rotates along its helical axis when

Table 1. pH dependence of secondary structure content in KcsA. KcsA was solubilized in DDM. Values represent mean $\pm$ SD of at least three independent measurements.

	pH 7.0	pH 6.0	pH 5.0	pH 4.0	pH 3.0
α helix	66.6 ± 0.93	66.7 ± 0.63	65.3 ± 1.3	67.7 ± 1.9	71.1 ± 0.95
β sheet	10.0 ± 3.3	9.0 ± 4.2	7.2 ± 2.9	9.1 ± 4.0	8.2 ± 3.2
β turn	11.5 ± 3.9	9.7 ± 6.2	15.2 ± 5.0	13.2 ± 4.8	14.3 ± 5.2
Other	10.3 ± 4.2	10.3 ± 5.4	15.1 ± 3.7	11.4 ± 3.2	10.2 ± 5.7



going from the closed to the open conformation. Additionally, the largest changes in Ω occur at the N-terminal half of TM1, which implies that this segment also tilts toward the symmetry axis during gating. We estimated the direction of helical rotation by calculating the mobility moment of TM1 (24) in the closed and open conformations (Fig. 3C). This vector points to the

direction of maximal motional freedom of the helix. Taking residue W²⁶ (25) as reference ($\theta = 0$), and viewed from the extracellular surface of the channel, we found a net counterclockwise rotation $\Delta\theta$ of about 21°. This determination cannot be directly translated into a physical rotation angle because precise interspin distances should be used for this purpose (26). How-

ever, it does provide a reliable determination of the direction of rotation along the TM1 helical axis.

An examination of the structural rearrangements in TM2 demonstrates a more dramatic set of changes in both probe mobility and spin-spin coupling than those observed in TM1. The $\Delta\Delta H_o$ plot shows strongly periodic behavior (Fig. 3D, left), with large mobility changes through most of TM2 and a gradual decrease in the magnitude of the change near the N-terminus of the helix. As with TM1, this pattern is indicative of rotational motions along the helical axis of TM2. The profile for the interspin proximity changes, on the other hand, shows Ω values >1 for most of the TM2 residues, an indication of movement away from the symmetry axis (Fig. 3D, right). However, it also shows a number of residues with Ω values <1 (96, 100, 109, 117) and thus moving closer to the symmetry axis. This Ω profile can be explained only by postulating that TM2 undergoes rotation and translation movements when going from the closed to the open state. This rearrangement of TM2 also occurs in the form of a rigid-body movement, because the power spectrum of the Ω parameter profile shows a sharp and unambiguous peak centered at 91° (Fig. 3E). The direction of helical rotation also occurs in a counterclockwise manner, with $\Delta\theta = 20^\circ$, viewed from the extracellular face of the channel (Fig. 3F).

Conformational Stability Near the Selectivity Filter

In an attempt to define the types of molecular movements near the selectivity filter during activation gating, we placed spin labels in regions immediately flanking the K⁺-channel signature sequence TVGYGD (Fig. 4). These labeled residues can be classified as external (residues 81, 82, and 83) and internal (residues 72, 73, and 74) relative to the ion binding sites of the selectivity filter. We found no significant change in either probe mobility or spin-spin interaction at the external side of the selectivity filter. This was true even for residue Y⁸², which showed

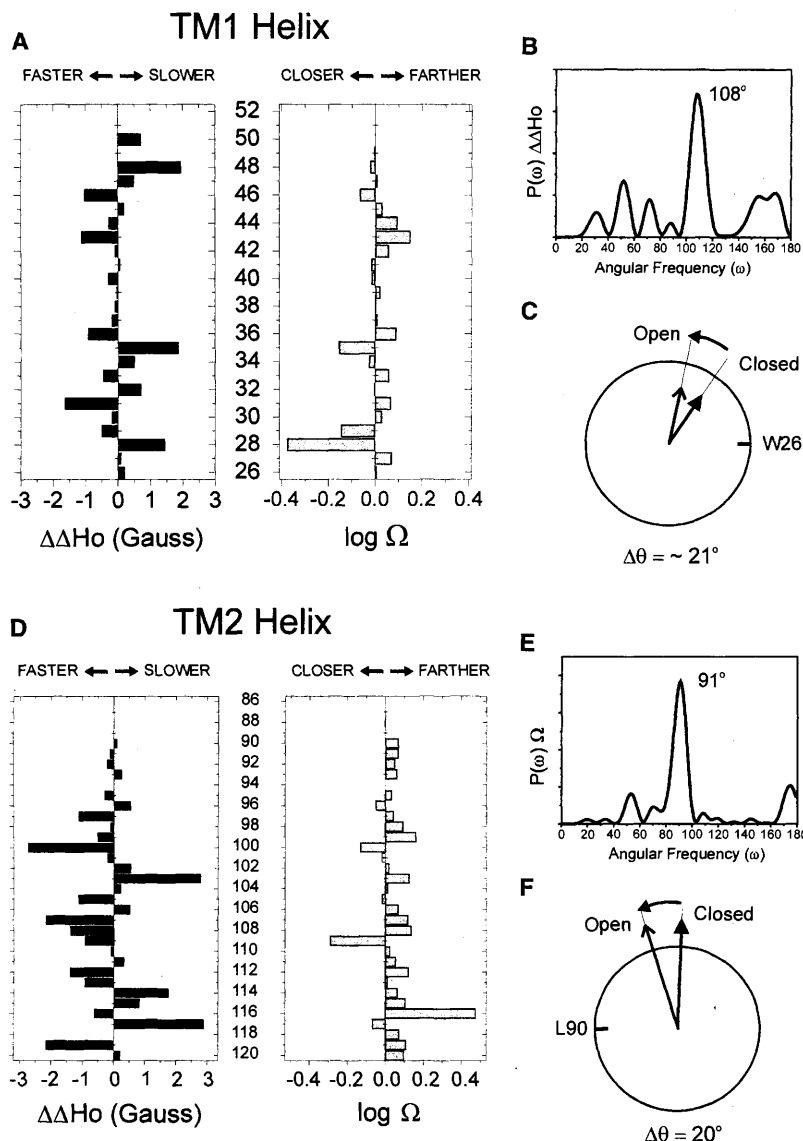


Fig. 3. Conformational rearrangements in TM1 and TM2. (A and D) Profile of changes in structural parameters associated with probe mobility (left, $\Delta\Delta H_o$) and spin-spin dipolar coupling (right, Ω parameter). Numbers in the center correspond to residue positions. Negative values of $\Delta\Delta H_o$ indicate an increase in the motional freedom of the label, and positive values imply motional restrictions. For $\Omega < 1$ ($\log \Omega < 0$), the spin labels are closer together relative to the fourfold axis of symmetry; for $\Omega > 1$ ($\log \Omega > 0$), the labels go away from each other and farther apart from the symmetry axis. Except for specific cases, data are derived from a single purification-labeling run. (B) Fourier transform power spectra of the mobility parameter $\Delta\Delta H_o$. Peak angular frequency is 108° and the α periodicity index (αPI) = 3.21. The Ω parameter profile for TM1 does not show as strong a peak near 100° (αPI = 1.73) mostly because the intersubunit TM1 cross distances are near the limits of detection for room temperature dipolar coupling broadening (about 15 Å). (C) Changes in mobility moment upon channel gating. Each vector is the sum of individual $\Delta\Delta H_o$ values calculated relative to residue W²⁶ viewed from the extracellular face of the channel. (E) Fourier transform power spectra of the proximity parameter Ω . The calculated peak angular frequency is 91° with αPI = 3.71. (F) Changes in dipolar coupling moment upon channel gating. Each vector is the sum of individual spectral amplitudes normalized to the total number of spins in the sample, calculated relative to residue L⁹⁰.

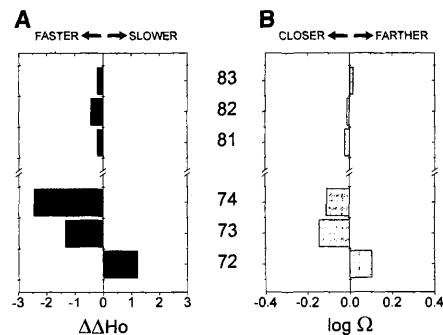


Fig. 4. Conformational rearrangements in residues flanking the selectivity filter. (A) Profile of changes in the mobility parameter ($\Delta\Delta H_o$). (B) Profile of changes in dipolar coupling (Ω parameter).

strong line shape broadening due to spin-spin interaction and should be very sensitive to any type of conformational change (see Fig. 2C). In contrast, residues on the internal side of the selectivity filter reported changes in both $\Delta\Delta H_o$ and Ω parameters upon channel opening. Residue T⁷² displayed increased steric contacts while moving away from the symmetry axis. Residues A⁷³ and T⁷⁴, on the other hand, increase their motional freedom while moving toward the symmetry axis of the channel. Although these findings are inconclusive in the absence of a systematic study of the entire P-loop region, one interpretation is that most of the selectivity filter remains immobile

during gating; however, the conformation of the lower portion of the selectivity filter can be modified by movement of the pore helix.

pH Dependence of Helical Movements

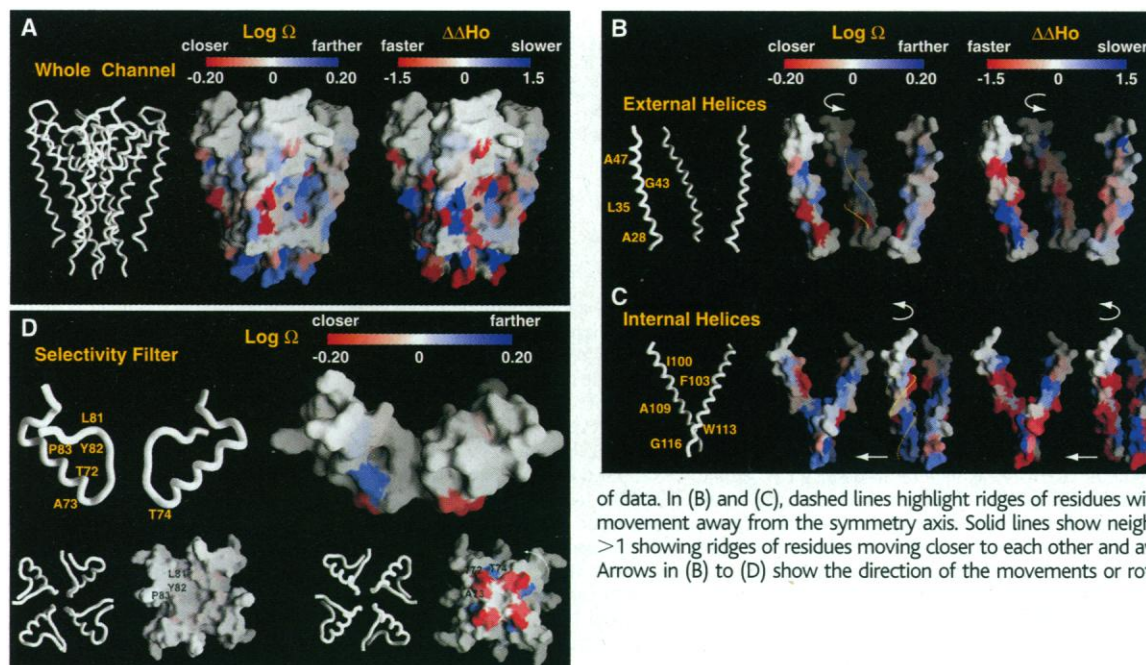
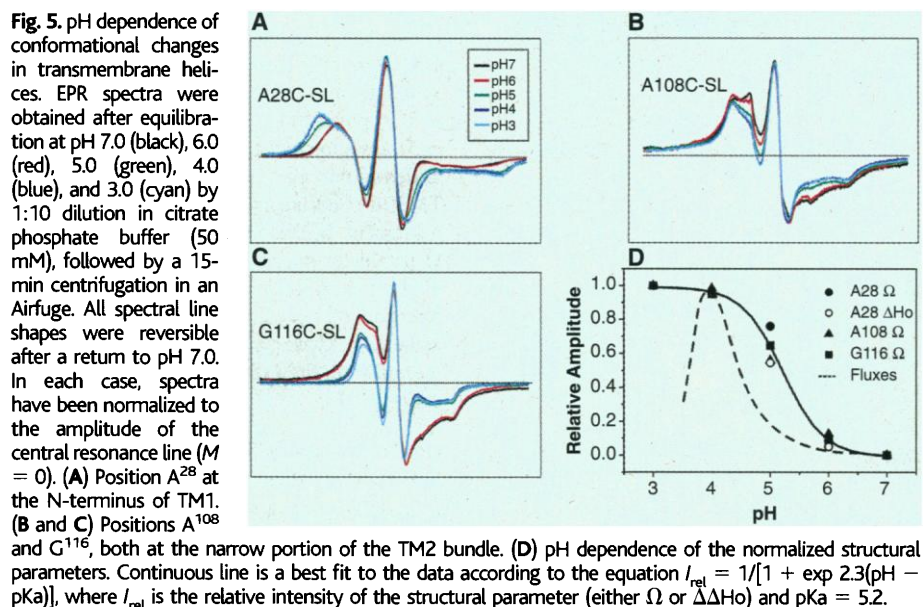
What is the relationship of these helical movements to pH-dependent channel opening? Do the changes in each helix reflect the same global conformational change? We studied the pH dependence of the conformational rearrangements in TM1 and TM2 and compared it with the pH dependence of channel activation, as measured from radiotracer uptake experiments. We chose three sites to follow the helical movements (Fig. 5, A to C): residue A²⁸ at the N-terminus of TM1 and residues A¹⁰⁸ and G¹¹⁶

at the core of the TM2 bundle. In each case, the spectral properties showed a gradual change with pH, which could be fitted with a simple protonation model (Fig. 5D). Most of the conformational changes occur within a narrow pH range (pH 4 to 6), with virtually no spectral changes detected for pH values lower than 4. When normalized, all the pH dependencies obtained from either changes in probe mobility ($\Delta\Delta H_o$) or spin-spin interactions (Ω) fall on the same curve, a clear indication that these spin labels report on the same global conformational change.

Superimposed on this plot is the pH dependence of the Ba²⁺-sensitive ⁸⁶Rb⁺ influx of KcsA (dotted line). Moving from neutral to acidic pH, the curve representing the conformational changes precedes the channel activity curve by about 1.5 pH units. This is, in fact, the expected behavior for these two types of measurements if we assume that channel opening is somehow coupled to rearrangements in TM1 and TM2. Radiotracer fluxes can be detected only when the four subunits are in the correct "active" conformation, but spectroscopic measurements continuously report on the whole population of subunits. However, at steady state, we cannot distinguish whether coupling between the TM1 and TM2 movements and ion flux is direct (27) or an intermediate step preceding a final, concerted pore opening (28).

Structural Context of the Conformational Changes

Conformational changes derived from EPR line shapes can be mapped onto the KcsA crystal structure (Fig. 6). Colors were mapped on a surface representation of the channel as a graded spectrum where red and blue represent two



conformational extremes for $\Delta\Delta H_o$ or Ω at each position and white represents no detected change (or no data).

The overall distribution and relative magnitude of these changes are shown in Fig. 6A for the whole channel surface. When individual regions of the channel are analyzed separately, clear patterns of motion start to emerge (Fig. 6, B and C). Particularly interesting are two adjacent ridges of residues observed for the spin-spin interaction data in both transmembrane segments. The shape and extent of these ridges are consequences of the types and magnitudes of the rotations and translations undergone by each helix when the channel gates. In the Ω map, the best defined ridge corresponds to Ω values >1 or positions that move away from the symmetry axis (dashed lines). It extends through most of the inner face of TM1 but wraps around the entire helix in TM2. The second ridge includes residues with Ω values <1 , thus moving toward the symmetry axis (solid lines). When $\Delta\Delta H_o$ values are mapped on the structure, both ridges are remarkably well reproduced in TM1 but are not well defined in TM2, an expected consequence of the extensive network of tertiary and quaternary contacts of the inner bundle.

The dramatic contrast between the conformational stability found in the external vestibule of the channel and the changes occurring at the base of the selectivity filter is shown in Fig. 6D. The current data demonstrate the intrinsic rigidity of the external vestibule and make it unlikely that the outer regions of the pore participate in the gating of KcsA. These findings help rationalize experiments in K^+ channels showing that there appear to be very small gating effects due to the binding scorpion toxins (29) despite the tight interaction between these molecules.

On the Mechanism of Activation Gating

The nature and location of the observed conformational rearrangements in KcsA establish a specific set of constraints for a molecular description of gating. Based on energetic considerations alone, only a 5-kT change in the per-

meation energy barrier is enough to functionally close an ion channel (30). Although this could be achieved with small perturbations in the structure of the selectivity filter, the magnitude of the changes observed in the TM2 bundle and those necessary to explain ion trapping and open-channel block effects (6, 7) suggest a more complicated mechanism.

The general conformational rearrangements that might take place in the transmembrane helices during the opening of KcsA are summarized as a cartoon in Fig. 7. In this extracellular view, we suggest that through a pH-dependent mechanism in the external side of the channel, TM2 rotates in a counterclockwise direction while swinging away from the permeation pathway, thus increasing the diameter of the inner vestibule. Either simultaneously or as a consequence of the movement of TM2, TM1 would also rotate in a counterclockwise direction with a small tilt toward the permeation pathway, perhaps filling spaces left open by the movement of TM2. Because under the current experimental conditions changes in spin-spin interactions are measured only in the plane of the membrane, consideration should be given to more pronounced helical tilts than those suggested by the current data set.

Assuming that the key conformational change during gating does occur along TM2, is the narrowest portion of the TM2 bundle a large enough energetic barrier to impede the passage of permeant ions? Evidence accumulated from cysteine reactivity experiments indicates that the closed pore limits the accessibility to MTS reagents (minimal radius 3 to 3.2 Å) and Cd^{2+} (crystal radius 1.71 Å) very effectively (31). Because of the hydrophobic lining at the inner vestibule of K^+ channels, it is unlikely that a permeant ion will shed its hydration shell. It would be unable to establish energetically favorable interactions with this region of the pore. Consequently, the TM2 bundle becomes a reasonable barrier to the passage of ions, because a physical opening of 3 to 4 Å appears to be small enough to prevent entry of hydrated cations into the channel vestibule.

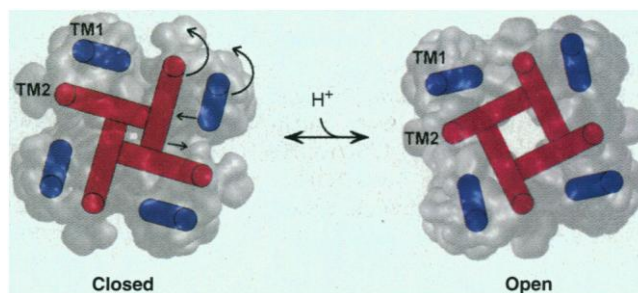
As expected, this initial model leaves several important questions unanswered. Perhaps

the most important one relates to the actual role of the pore transitions and its interplay with the larger changes observed for TM1 and TM2. The types of movements detected at the base of the pore helix might lend structural support to the description of subconductance states representing partial openings in transit toward the fully open conformation (32). However, it is also possible that these selectivity changes and subconductance states are a direct consequence of intermediate states populated during opening of the TM2 bundle.

References and Notes

1. B. Hille, *Ion Channels of Excitable Membranes* (Sinauer, Sunderland, MA, ed. 2, 1992); D. J. Aidley and P. R. Stanfield, *Ion Channels. Molecules in Action* (Cambridge Univ. Press, Cambridge, ed. 1, 1996).
2. H. Schrempf *et al.*, *EMBO J.* **14**, 5170 (1995).
3. D. A. Doyle *et al.*, *Science* **280**, 69 (1998).
4. L. G. Cuello, J. G. Romero, D. M. Cortes, E. Perozo, *Biochemistry* **37**, 3229 (1998).
5. Although not done at low pH, channel opening appears to depend also on the surrounding lipids [L. Heginbotham *et al.*, *J. Gen. Physiol.* **111**, 741 (1998)].
6. C. M. Armstrong, *ibid.* **54**, 553 (1969); *ibid.* **58**, 413 (1971).
7. M. Holmgren, P. L. Smith, G. Yellen, *ibid.* **109**, 527 (1997); Y. Liu, M. Holmgren, M. E. Jurman, G. Yellen, *Neuron* **19**, 175 (1997).
8. Since its development by Hubbell and collaborators [C. Altenbach *et al.*, *Science* **248**, 1088 (1990)], site-directed spin labeling and EPR spectroscopy has emerged as a very powerful approach for studying the structural dynamics of proteins, particularly membrane proteins. It is exquisitely sensitive to changes in mobility, solvent accessibility, and inter-spin distances; see [W. L. Hubbell *et al.*, *Curr. Opin. Struct. Biol.* **8**, 649 (1998)].
9. E. Perozo, L. Cuello, D. Cortes, *Nature Struct. Biol.* **5**, 459 (1998).
10. Deletion constructs were obtained by polymerase chain reaction, expressed in *Escherichia coli*, purified, and reconstituted into liposomes as reported (9). We assayed each construct functionally by measuring the pH-dependent Ba^{2+} -sensitive $^{86}Rb^+$ influx (4). An additional deletion construct lacking most of the first 20 residues of the NH_2 -terminus ($\Delta 2$ -19) was also prepared and tested and showed no significant effects on pH-dependent $^{86}Rb^+$ uptake.
11. D. M. Cortes, L. G. Cuello, E. Perozo, paper presented at the 43rd Annual Meeting of the Biophysical Society, Baltimore, MD, 13 February 1999.
12. G. Chang, R. Spencer, A. Lee, M. Barclay, D. Rees, *Science* **282**, 2220 (1998).
13. L. G. Cuello, C. P. Ptak, E. Perozo, paper presented at the 43rd Annual Meeting of the Biophysical Society, Baltimore, MD, 14 February 1999.
14. P. N. Unwin and P. D. Ennis, *Nature* **307**, 609 (1984); N. Unwin, *ibid.* **373**, 37 (1995).
15. D. L. Farrens *et al.*, *Science* **274**, 768 (1996).
16. S. K. Aggarwal and R. MacKinnon, *Neuron* **16**, 1169 (1996).
17. CD spectra were obtained between 190 and 250 nm (1-nm interval) in a Jasco J720 spectropolarimeter with a 0.1-mm path length. Samples were in 10% phosphate-buffered saline with 1 mM DDM. We used an average of 10 to 15 individual spectra for each sample. We quantified secondary structures by the self-consistent method [N. Sreerama and R. W. Woody, *Anal. Biochem.* **209**, 32 (1993)], using the program Dicroprot [G. Deleage and C. Geourjon, *Comp. Appl. Biosci.* **9**, 197 (1993)].
18. D. M. Cortes and E. Perozo, *Biochemistry* **36**, 10343 (1997).
19. S. A. Tatulian, D. M. Cortes, E. Perozo, *FEBS Lett.* **423**, 205 (1998); J. le Coutre *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **95**, 6114 (1998).
20. Each mutant was reconstituted into asolectin liposomes at a 500:1 lipid-to-protein ratio (mol/mol). We changed the pH by resuspending each sample in

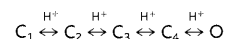
Fig. 7. A molecular mechanism of activation gating in KcsA. Model is viewed from the extracellular side of the membrane. TM1s are represented by blue cylinders; TM2s are red. Initiated by a proton-dependent step, TM2 rotates in a counterclockwise direction and away from the permeation pathway, opening the channel. In the channel periphery, TM1 mirrors the rotation of TM2 but tilts inward toward the permeation pathway. Note, however, that for illustration purposes the movements depicted here are much larger than expected and constitute a simplified representation of rigid-body movements compatible with the current set of data. The actual magnitude of these changes remains to be determined.



- a 10-fold volume excess of buffer (50 mM citrate phosphate buffer), followed by centrifugation at 10,000g in an Airfuge. We obtained X-band continuous wave (CW) EPR spectra by using a loop-gap resonator with 2-mW incident power, 100-kHz modulation frequency, and 1-G modulation amplitude.
21. These spectra contain information about the motional freedom of the spin label and how it is affected by local steric restrictions. In the absence of a formal line shape analysis for each spectrum, the parameter ΔH_0 , representing the width of the central resonance line, has been successfully used as an empiric measure of probe mobility [H. S. Mchaourab *et al.*, *Biochemistry* **35**, 7692 (1996)]. An increase in ΔH_0 (a positive $\Delta\Delta H_0$) signals a decrease of motional freedom, perhaps from newly formed tertiary or quaternary contacts. Similarly, a decrease in ΔH_0 (negative $\Delta\Delta H_0$) indicates an increase in the probe's freedom of movement. For a sample containing multiple spin labels, the spectral line shape can also be affected by the extent of trough-space spin-spin dipolar coupling. Such interactions produce spectral broadening in a distance-dependent manner [G. R. Eaton and S. S. Eaton, in *Spin Labeling, Theory and Applications*, L. J. Berliner and J. Reuben, Eds. (Plenum, New York, 1989)]. At room temperature, this occurs proportionally for distances as large as 15 to 16 Å and as small as 7 to 8 Å, at which point direct, collisional spin exchange will likely occur. In systems of two spins, these interactions have been used with impressive accuracy to directly estimate interspin distances [M. D. Rabenstein and Y. K. Shin, *Proc. Nat. Acad. Sci. U.S.A.* **92**, 8239 (1995); E. J. Hustedt *et al.*, *Biophys. J.* **72**, 1861 (1997); H. S. Mchaourab *et al.*, *Biochemistry* **36**, 307 (1997); H. J. Steinhoff *et al.*, *Biophys. J.* **73**, 3287 (1997)], but in a system with fourfold symmetry like KcsA dipolar interactions cannot be reliably translated into actual physical distances. Instead, spectral broadening can be used to estimate changes in overall probe proximity in the form of the Ω parameter (9). This is operationally defined as $\Omega = \bar{A}^*/\bar{A}^\circ$, where $\bar{A}^*$ is the amplitude of the central resonance line ($M = 0$), normalized to the total number of spins in the sample, of a fully labeled mutant (containing two or more labels), and $\bar{A}^\circ$ is the amplitude of the central resonance line, also normalized to the total number of spins in the sample, of an underlabeled mutant (containing only one label per tetramer). In this report, we have considered a rela-

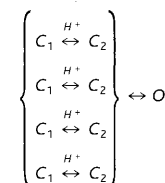
tive Ω parameter by comparing fully labeled channels in two conformational states, closed and open. Note that under these conditions, the magnitude of Ω can be affected by large changes in probe mobility.

22. We obtained power spectra by applying the discrete Fourier transform evaluated for the value $\omega = \dot{\omega}$ that maximizes $P(\omega)$ [J. L. Cornette *et al.*, *J. Mol. Biol.* **195**, 659 (1987); D. Donnelly, J. P. Overington, T. L. Blundell, *Prot. Eng.* **7**, 645 (1994); see (9)].
23. In a rigid body movement of an α helix, the overall α periodicity of a given structural property (ΔH_0) is normally preserved. Rotations generate a mobility profile that is shifted along the residue axis, whereas tilts and dissociations primarily affect the side of the helix involved in tertiary or quaternary contacts. In either case, the power spectra will show a prominent peak near 100°. If, on the other hand, there are changes in secondary structure, the relative area of the peak near 100° will decrease according to the magnitude of the loss in helical periodicity.
24. This is the resultant vector from the sum of all ΔH_0 values in polar coordinates calculated from the equation
- $$M(\omega) = \left[\sum_{j=1}^N V_j \cos(j\omega) \right]^2 + \left[\sum_{j=1}^N V_j \sin(j\omega) \right]^2$$
- [D. Eisenberg *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 140 (1984)]. The angle θ was obtained as the value of the resultant $M(\omega)$ evaluated at $\omega = 100^\circ$, taking an arbitrary residue as a reference point ($\theta = 0$). $\Delta\theta$ was calculated as the angular difference between θ at neutral pH and θ at acidic pH.
25. Abbreviations for amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
26. This is because the module of the individual vectors (either ΔH_0 or Ω) is affected by nonstructural factors: Ω is nonlinearly dependent with distance and is affected in an unknown way by the presence of the four interacting spin labels; ΔH_0 values are closely related to the local steric environment at each individual position, which obviously changes upon channel opening. Therefore, the value of the $\Delta\theta$ angle should be taken as a qualitative estimate of the magnitude of the rotation.
27. This response can be represented by a simple kinetic scheme,



where C_1 represents the channel conformation with all four subunits in the resting state and each kinetic step is associated with the change of a given subunit to the active state, leading to the fully open state O. This scheme was used recently to describe the gating kinetics of glutamate receptor channels [C. Rosenmund *et al.*, *Science* **280**, 1596 (1998)].

28. This case can be represented kinetically as



where the $C_1 \leftrightarrow C_2$ transition represents the measured conformational change that leads to a final, concerted transition to the open state. [F. Bezanilla *et al.*, *Biophys. J.* **66**, 1011 (1994); W. N. Zagotta *et al.*, *J. Gen. Physiol.* **103**, 321 (1994)].

29. C. S. Anderson, R. MacKinnon, C. Smith, C. Miller, *J. Gen. Physiol.* **91**, 317 (1988).
30. F. J. Sigworth, *Q. Rev. Biophys.* **27**, 1 (1994).
31. G. Yellen, *ibid.*, in press.
32. M. L. Chapman *et al.*, *Biophys. J.* **72**, 708 (1997); J. Zheng and F. J. Sigworth, *J. Gen. Physiol.* **110**, 101 (1997).
33. A. Nicholls, K. A. Sharp, B. Honig, *Proteins* **11**, 281 (1991).
34. Support by the National Institutes of Health (grants GM54690 and GM57846) is gratefully acknowledged. We are indebted to R. Nakamoto, M. Wiener, and H. Mchaourab and to the members of the Perozo Laboratory (Y.-S. Liu, C. Ptak, and A. Fay) for insightful discussions and to R. Nakamoto, G. Szabo, H. Mchaourab, and D. Caffo for comments on the manuscript. R. MacKinnon kindly provided the C-terminal His-tagged KcsA construct and C. Ptak participated in some of the radiotracer flux experiments. E.P. is particularly grateful to S. Mochel for support and encouragement. E.P. is a McKnight Scholar.

27 January 1999; accepted 10 May 1999

SHRIMP Uranium-Lead Dating of Diagenetic Xenotime in Siliciclastic Sedimentary Rocks

Neal J. McNaughton, Birger Rasmussen, Ian R. Fletcher

Diagenetic xenotime is common in siliciclastic sedimentary rocks, where it starts to form on detrital zircon shortly after sediment deposition. It is possible to estimate the age of sedimentary rocks by in situ uranium-lead analysis of that xenotime. Two Proterozoic sandstone units from north-western Australia, previously constrained to the age interval of 1790 to 750 million years ago, have diagenetic xenotime ages of 1704 ± 7 and 1704 ± 14 million years ago. This method has potential for dating sedimentary sequences of all ages but should be especially valuable for refining the Precambrian time scale.

It is difficult to date siliciclastic sedimentary rocks unless they contain fossils for biostratigraphic division or interbedded volcanic rocks suitable for radiometric dating. Direct radiomet-

ric dating of sediment deposition is not possible, but dating early diagenetic minerals can provide close constraints. Several such minerals have been investigated and used for dating (1),

but none have been found to be routinely applicable over a broad time span. The recent identification of xenotime as an early diagenetic precipitate may provide an alternative, because the mineral is common in siliciclastic sedimentary rocks of all ages (Table 1), generally has high U contents, and retains radiogenic Pb. The mineral is a trace constituent in many lithologies, ranging from shales to conglomerates, deposited in fluvial to marine environments (2). Most xenotime overgrowths consist of small (typically $<3 \mu\text{m}$ but can be as large as $30 \mu\text{m}$) pyramid-shaped crystals attached to the rounded surfaces of detrital zircon grains (Fig. 1). The euhedral shape of many xenotime overgrowths strongly implies that the mineral formed after sediment deposition. Petrographic textures consistently show that xenotime is engulfed by other diagenetic cements and is clearly one of

Centre for Strategic Mineral Deposits, Department of Geology and Geophysics, University of Western Australia, Nedlands, WA 6907, Australia.