Ion-binding properties of a K^+ channel selectivity filter in different conformations

Shian Liu[a], Paul J. Focke[b], Kimberly Matulef[b], Xuelin Bian[a], Pierre Moënne-Loccoz[c], Francis I. Valiyaveetil[b], and Steve W. Lockless[a,d,1]

[a]Department of Biology, Texas A&M University, College Station, TX 77843; [b]Program in Chemical Biology, Department of Physiology and Pharmacology, Oregon Health and Science University, Portland, OR 97239; [c]Division of Environmental and Biomolecular Systems, Institute of Environmental Health, Oregon Health and Science University, Portland, OR 97239; and [d]Texas A&M Institute for Neuroscience, Texas A&M University, College Station, TX 77843

Edited by Richard W. Aldrich, The University of Texas at Austin, Austin, TX, and approved October 28, 2015 (received for review May 28, 2015)

K^+ channels are membrane proteins that selectively conduct K^+ ions across lipid bilayers. Many voltage-gated K^+ (K_V) channels contain two gates, one at the bundle crossing on the intracellular side of the membrane and another in the selectivity filter. The gate at the bundle crossing is responsible for channel opening in response to a voltage stimulus, whereas the gate at the selectivity filter is responsible for C-type inactivation. Together, these regions determine when the channel conducts ions. The K^+ channel from *Streptomyces lividians* (KcsA) undergoes an inactivation process that is functionally similar to K_V channels, which has led to its use as a practical system to study inactivation. Crystal structures of KcsA channels with an open intracellular gate revealed a selectivity filter in a constricted conformation similar to the structure observed in closed KcsA containing only Na^+ or low [K^+]. However, recent work using a semisynthetic channel that is unable to adopt a constricted filter but inactivates like WT channels challenges this idea. In this study, we measured the equilibrium ion-binding properties of channels with conductive, inactivated, and constricted filters using isothermal titration calorimetry (ITC). EPR spectroscopy was used to determine the state of the intracellular gate of the channel, which we found can depend on the presence or absence of a lipid bilayer. Overall, we discovered that K^+ ion binding to channels with an inactivated or conductive selectivity filter is different from K^+ ion binding to channels with a constricted filter, suggesting that the structures of these channels are different.

K^+ channel | ion binding | inactivation | isothermal titration calorimetry | electron paramagnetic resonance spectroscopy

K^+ channels are found in all three domains of life, where they selectively conduct K^+ ions across cell membranes. Specific stimuli trigger the activation of K^+ channels, which results in a hinged movement of the inner helix bundle (1–7). This opening on the intracellular side of the membrane initiates ion conduction across the membrane by allowing ions to enter into the channel. After a period, many channels spontaneously inactivate to attenuate the response (8–17). The inactivation process is a timer that terminates the flow of ions in the presence of an activator to help shape the response of the system. Two dominant types of inactivation have been characterized in voltage-dependent channels: N-type and C-type (18). N-type inactivation is fast and involves an N-terminal positively charged "ball" physically plugging the pore of the channel when the membrane is depolarized. C-type inactivation, on the other hand, is a slower process involving a conformational change in the selectivity filter that is initiated by a functional link between the intracellular gate and the selectivity filter (10, 19).

Several experimental observations indicate a role for the selectivity filter in C-type inactivation. First, mutations in and around the selectivity filter can alter the kinetics of inactivation (20–23). Second, increasing concentrations of extracellular K^+ ions decrease the rate of inactivation, as if the ions are stabilizing the conductive conformation of the channel to prevent a conformational change in the selectivity filter (14, 16, 17, 22). Finally, a loss of selectivity of K^+ over Na^+ has been observed during the inactivation process in Shaker channels, suggesting a role for the selectivity filter (24, 25). Together, these data indicate that channels in their inactivated and conductive conformations interact with K^+ ions differently, and suggest that C-type inactivation involves a conformational change in the selectivity filter. Although several structures of K^+ channels in their conductive state have been solved using X-ray crystallography, there is at present no universally accepted model for the C-type inactivated channel (1, 3–5, 9, 19, 26–28) (Fig. 1*B*).

Inactivation in the K^+ channel from *Streptomyces lividians* (KcsA) has many of the same functional properties of C-type inactivation, which has made it a model to understand its structural features (20). KcsA channels transition from their closed to open gate upon changing the intracellular pH from high to low (Fig. 1*A*). The rapid flux of ions through the channel is then attenuated by channel inactivation, where most open WT channels are not conducting, suggesting that crystal structures of open KcsA channels would reveal the inactivated channel. In some crystal structures of truncated WT KcsA solved with an open gate, the selectivity filter appears in the constricted conformation, similar to the conformation observed in structures of the KcsA channel determined in the presence of only Na^+ ions or low concentrations of K^+ ions (3, 10, 29, 30) (Fig. 1*B*). Solid-state and solution NMR also indicate that the selectivity filter of the KcsA channel is in the constricted conformation when the cytoplasmic gate is open (31–33).

However, a recently published study shows that even when the constricted conformation of KcsA's selectivity filter is prevented by a nonnatural amino acid substitution, the channel inactivates like

Significance

The selectivity filter of K^+ channels is responsible for their exquisite ion selectivity. This region is also responsible for C-type inactivation, a regulatory process in many voltage-dependent K^+ channels. Although the functional properties of inactivated channels have been known for decades, the first potential glimpse of their structure emerged from crystal structures of a constricted selectivity filter in an open channel. However, recent studies challenged the suggestion that the constricted selectivity filter is the inactivated structure, leaving open the question of what the inactivated structure looks like. Here, we provide evidence that the thermodynamic properties of the selectivity filter in an inactivated channel are more similar to properties of the conductive channel rather than the constricted open channel.

Author contributions: S.L., P.J.F., K.M., F.I.V., and S.W.L. designed research; S.L., P.J.F., K.M., and X.B. performed research; S.L., P.J.F., K.M., P.M.-L., F.I.V., and S.W.L. analyzed data; and S.L., F.I.V., and S.W.L. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

[1]To whom correspondence should be addressed. Email: lockless@bio.tamu.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1510526112/-/DCSupplemental.

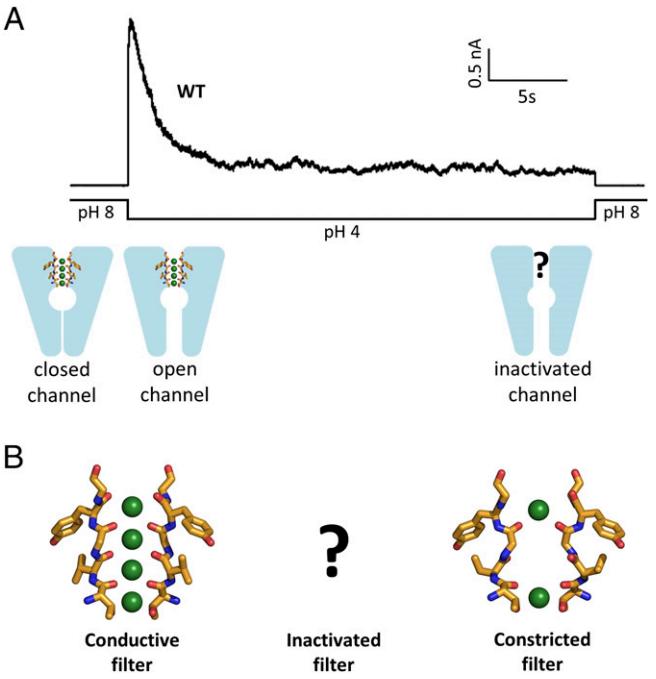

Fig. 1. Macroscopic recordings and structural models of KcsA K^+ channel. (*A*) Macroscopic currents of WT KcsA obtained by a pH jump from pH 8 to pH 4 reveal channel inactivation. Two models representing the conformation of the channel are shown below. (*B*) Conductive [*Left*, Protein Data Bank (PDB) ID code 1K4C] and constricted (*Right*, PDB ID code 1K4D) conformations of the selectivity filter are shown as sticks, and the ion-binding sites are indicated with green spheres. The thermodynamic properties of the conductive, constricted, and inactivated (*Middle*) conformations are the subject of this study.

WT channels, suggesting the constricted filter does not correspond to the functionally observed inactivation in KcsA (28). In this study, we use isothermal titration calorimetry (ITC) to quantify the ion-binding properties of WT and mutant KcsA K^+ channels with their selectivity filters in different conformations and EPR spectroscopy to determine the conformation of the channels' intracellular gates. A comparison of these ion-binding properties leads us to conclude that the conductive and inactivated filters are energetically more similar to each other than the constricted and inactivated filters.

Results

Ion Binding to Open KcsA Channels. Electrophysiology experiments demonstrate that KcsA is closed at pH 8 and that shifting the intracellular pH to pH 4 opens the intracellular gate of the channel (34, 35). These open channels then inactivate, reaching a current level that is ≤10% of the maximum, suggesting that most channels with an open intracellular gate are inactivated at equilibrium (20) (Fig. 1*A*). To measure the ion-binding properties of open channels, we initially examined two C-terminally truncated KcsA channels containing mutations, H25R-E118A-E120A (OM1-ΔC) and H25Q-E118$^+$ (OM2-ΔC), that are open at pH 8 in the lipid bilayer (36, 37). The ITC experiment consisted of titrating a solution containing K^+ ions into an ITC chamber, with appropriate KcsA channels solubilized in *n*-decyl-β-D-maltopyranoside (DM) micelles and 100 mM NaCl at pH 8 (Fig. 2*A*, Fig. S1, and Table S1). The integrated heat change from each injection was fit to a binding isotherm to reveal apparent K_d values of 0.16 mM and 0.13 mM for OM1-ΔC and OM2-ΔC, respectively, which is similar to the apparent K_d value for WT-ΔC of 0.15 mM (38). Because these apparent K_d values reflect the competition between Na^+ and K^+ in the chamber, we varied the concentration of Na^+ ions in the chamber to obtain the K^+ ion affinity in the absence of Na^+ ions (29, 38, 39). The apparent K_d values were then fit to a competition equation to obtain $K_d(K^+)$ (Fig. 2*B*), which is similar for the three channels (Table S2). This similarity was unexpected because the cytoplasmic gate in the OM1-ΔC and OM2-ΔC channels is presumably open at pH 8, which puts their selectivity filters in the inactivated conformation, whereas the cytoplasmic gate in the WT-ΔC channels is presumably closed, with their selectivity filters in the conductive conformation. The similar $K_d(K^+)$ values for these channels suggests that ITC is insensitive to changes in the inactivated and conductive filter conformations or that the intracellular gates are in different conformations in the electrophysiological studies (in lipid bilayers) and ITC measurements (in detergent).

Different Conformation of KcsA's Intracellular Gate in Detergent and Lipid Bilayers. We used EPR spectroscopy to determine the state of the intracellular gate of WT and mutant KcsA channels under the conditions of the ITC experiment. The KcsA channels were labeled with a spin probe near the bottom of the second transmembrane helix (using a Cys substitution at G116) that has been previously used to determine the state of the intracellular gate (20, 40, 41) (Fig. 3*A*). The resulting spectra show that full-length WT KcsA (WT-FL) is closed in liposomes at pH 7.5 and open at pH 3.5, consistent with previously published experiments (6) (Fig. 3*A*). Similar to full-length KcsA, WT-ΔC in liposomes is also closed at pH 7.5 and open at pH 3.5 (Fig. 3*B*). Unexpectedly, we found that WT-ΔC in DM detergent at pH 7.5 showed an EPR spectrum closer to an open rather than a closed intracellular gate (Fig. 3*C*); replacing the lipid membrane around WT-ΔC channels with DM detergent appears to shift the equilibrium of the intracellular gate to favor the open conformation. Not surprisingly, the EPR spectra of the OM1-ΔC and OM2-ΔC channels both show that the intracellular gate is open in DM detergent at pH 7.5 (Fig. 3*C*). The similar conformation of the intracellular gate of the WT-ΔC, OM1-ΔC, and OM2-ΔC channels can explain the similar behavior of these channels in ion-binding experiments. Electrophysiological recordings of KcsA show that WT channels with an open intracellular gate primarily have an inactivated selectivity filter (20) (Fig. 1*A*). Therefore, we conclude that the ion-binding properties for WT-ΔC at pH 8 are from channels with an open-gate, inactivated filter and not a closed gate, conductive filter as we had proposed previously (9, 28, 29, 38).

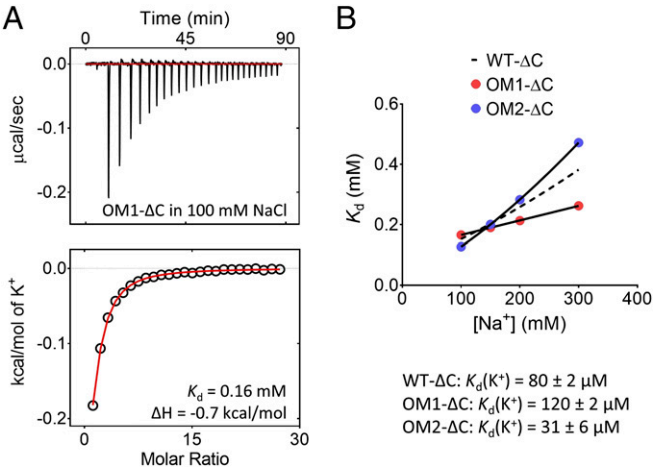

Fig. 2. Ion binding to WT and mutant KcsA channels measured at pH 8. (*A*, *Top*) Thermogram of OM1-ΔC titrated with KCl in the presence of 100 mM NaCl. (*A*, *Bottom*) Integrated heat from each injection is fit to a single ion-binding function to obtain the apparent K_d. (*B*) Apparent K^+ K_d values (Table S3) are plotted as a function of Na^+ concentration in the ITC chamber, and are fit to a competition function to obtain $K_d(K^+)$. The mean and SD of three or more experiments are shown.

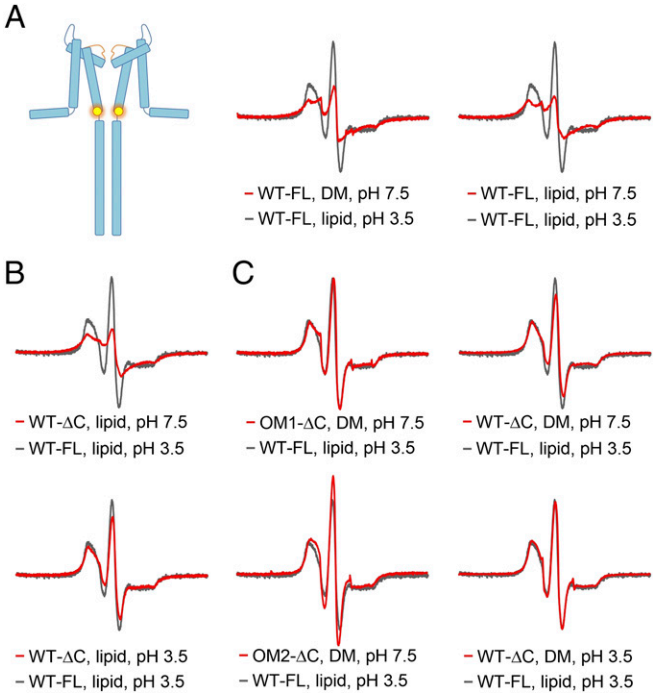

Fig. 3. Continuous wave-EPR spectra of KcsA channels. Spin-labeled spectra of WT-FL (*A*), WT-ΔC, OM1-ΔC, and OM2-ΔC obtained in lipids (*B*) and DM detergent (*C*) at pH 7.5 and pH 3.5. All spectra are compared with WT-FL in lipids at pH 3.5. Spin labels were attached to the gating-sensitive G116 position of the channels as shown in *A*.

Ion Binding to KcsA Channels with Conductive or Partially Conductive Selectivity Filters. We used two different approaches to measure ion binding to KcsA channels with selectivity filters in the conductive or partially conductive conformation. First, as was shown previously, both E71C-FL and R64A-FL channels have reduced inactivation in electrophysiology experiments (Fig. 4*A*, Fig. S2*C*, and Table S3); therefore, their selectivity filters are in a mixture of conductive and inactivated conformations when the intracellular gate is open (8, 20). Because C-terminally truncated KcsA channels are open in detergent at pH 8 (Fig. 3), we expect ITC measurements of ΔC channels to have a mixture of conductive and inactivated selectivity filters. K^+ ion binding to the E71C-ΔC mutant channel in 100 mM NaCl has an apparent K_d of 0.10 mM (Fig. S3*A*). The $K_d(K^+)$ was determined by measuring the apparent K^+ affinity at different Na^+ concentrations in the ITC chamber, to yield a K_d of 83 μM (Fig. 4*B*). The $K_d(K^+)$ was similar for R64A-ΔC, with a value of 54 μM (Fig. S2*B*).

The second approach that was used to obtain channels in their conductive conformation is predicated on the EPR result (Fig. 3*A*) that full-length KcsA in lipid membranes and detergent is closed at pH 8. Because crystal structures of closed KcsA have a conductive filter in K^+ solutions, the selectivity filter of the full-length KcsA in detergent should also be in its conductive conformation (1, 3, 6). K^+ ion binding to full-length KcsA at various concentrations of Na^+ ions in solution yielded a K_d of 67 μM (Fig. 4*C*), close to the E71C-ΔC and R64A-ΔC mutant channels' K^+ affinities. Using these two approaches, we find that KcsA channels with conductive or partially conductive filters have $K_d(K^+)$ values that are very similar to one another (Table S2).

Ion Binding to Mutant Channels with Constricted Filters. We next compared the K^+ ion-binding properties of the constricted filter, which was previously proposed to be the structure of an inactivated filter. Two approaches were used to trap and quantify the K^+ K_d of a constricted filter. First, we determined the $K_d(K^+)$ of the M96V-ΔC mutant channel, which was shown to remain in the constricted conformation even at high $[K^+]$ in the crystal (29) (Fig. 5*A*). The apparent K_d for ion binding to the M96V-ΔC channel is much higher than in other channels under the same conditions, so the concentration of competing NaCl was lowered to capture ion binding reliably. The apparent K_d values were fit to a competition equation to reveal a 350 μM affinity, which is significantly lower than the inactivated WT channel (Fig. 5*B* and Fig. S4*A*).

Second, the K^+ affinity was measured in WT-ΔC channels at pH 4, where channels are in their constricted conformation (9, 32, 33). A fit to the integrated heat from the thermogram reveals an apparent K_d of 3.1 mM in 100 mM NaCl, which is significantly lower than the apparent K^+ affinity of WT-ΔC at pH 8 (K_d = 0.15 mM) (Fig. 5*C* and Fig. S4*B*). However, the low affinity of this reaction reduced the reliability of the apparent K_d values determined at higher Na^+ concentrations, which were used to determine the $K_d(K^+)$. Therefore, we took an alternative approach to obtain $K_d(Na^+)$, which could then be used to calculate the $K_d(K^+)$ from the apparent $K_d(K^+)$. Rb^+ ions are similar to K^+ ions in size, charge density, occupancy in KcsA's selectivity filter, and permeability through KcsA K^+ channels (30, 42). We found that Rb^+ binds to KcsA at pH 4 with a higher affinity (K_d = 0.42 mM) than K^+ ions (Fig. S4*C*), allowing us to obtain $K_d(Rb^+)$ and $K_d(Na^+)$ by measuring the apparent affinity over a range of concentration of Na^+ ions (Fig. 5*C*). Because the binding reactions of both K^+ and Rb^+ begin from the same Na^+-bound protein, the value of $K_d(Na^+)$ was used to calculate $K_d(K^+) = 350 \pm 100$ μM from the apparent K^+ K_d of 3.1 mM measured in 100 mM NaCl.

The significant difference in the K^+ ion-binding properties of the constricted filter determined using two different approaches and the filter of inactivated WT-ΔC channels suggest that the conformations of the selectivity filters of these channels are distinct from one another. By comparison, the K^+ affinities $[K_d(K^+)]$ for channels in the inactivated and conductive conformations are similar to one another (Fig. 6 and Table S2).

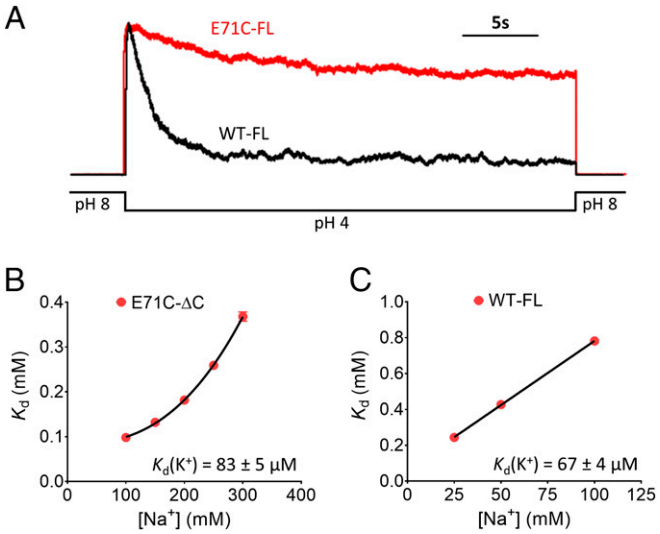

Fig. 4. Ion binding to KcsA channels in their conductive conformation. (*A*) Macroscopic currents of WT-FL and E71C-ΔC mutant KcsA obtained by a pH jump from pH 8 to pH 4 reveal different degrees of inactivation. The current traces were normalized to the peak current. (*B* and *C*) Apparent K_d values (Table S3) determined from isotherms (Fig. S3) are plotted as a function of Na^+ concentration. The apparent K^+ K_d values of E71C-ΔC and WT-FL are fit to the competition function to obtain $K_d(K^+)$. The mean and SD of three or more experiments are shown.

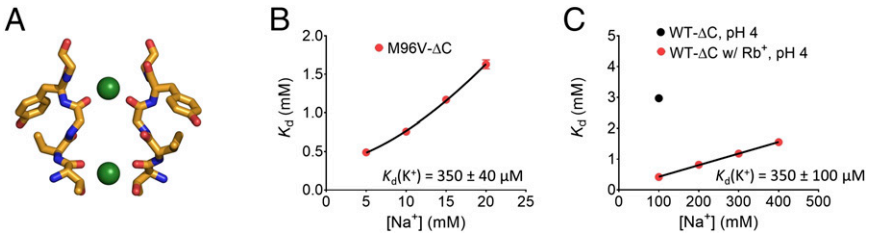

Fig. 5. Ion binding to KcsA channels with a constricted filter. (*A*) Selectivity filter of the M96V-ΔC mutant KcsA channel in a constricted conformation (PDB ID code 2NLJ). (*B* and *C*) Apparent ion K_d values determined from isotherms (Fig. S4) are plotted as a function of Na^+ concentration. (*B*) Apparent K^+ K_d values (Table S3) for M96V-ΔC at pH 8 are fit to the competition function to obtain $K_d(K^+)$. (*C*) Apparent Rb^+ K_d values (Table S3) for WT-ΔC at pH 4 are fit to the competition function to obtain $K_d(Na^+)$. $K_d(Na^+)$ is used to calculate $K_d(K^+)$ from the apparent K^+ K_d values in 100 mM NaCl. The mean and SD of three or more experiments are shown.

Discussion

C-type inactivation in voltage-dependent K^+ channels and desensitization in ligand-dependent channels are important physiological processes that arrest the flow of ions while the channel gate is open. The KcsA K^+ channel inactivates with many of the characteristics of C-type inactivation in voltage-gated K^+ (K_V) channels, and so it has become a model system to study this process (20). Although many different approaches have been used to trap and characterize the structure of the selectivity filter of an inactivated channel, there is still considerable debate on whether the constricted filter first observed in low [K^+] is the structure of an inactivated filter characterized by electrophysiology (3, 10, 19, 28, 31–33). Here, we measured the ion-binding properties of open KcsA channels with their selectivity filters in the conductive, constricted, or inactivated state to ask which conformations, from a thermodynamic point of view, are most similar to one another (Figs. 1*B* and 6).

Although several structures of conductive and constricted filters are known, the conformation of an inactivated filter is still unknown. The equilibrium K^+ binding constants reported here can be classified into two groups (Fig. 6). The first group has high K_d values and is likely in a constricted conformation based on crystal structures. Channels in the second group have a conductive filter (WT-FL), an inactivated filter (WT-ΔC), or a combination of both (E71A-ΔC and R64A-ΔC). A structural interpretation of the different binding constants is challenging because of both the classic issue with interpreting mechanisms from thermodynamic data and the binding isotherms fitting well to a single-ion process when the selectivity filter is a known multiple ion-queue. This discrepancy suggests that either one ion-binding event in the selectivity filter has a very low K_d, such that we are primarily observing this signal in our measurements, or that multiple ion-binding sites have similar affinities across the filter that are not discrete events at the resolution of our experiments. The similar K^+ binding constant between the inactivated and conductive filters suggests that there is either no structural or energetic change in the highest affinity K^+ binding site or that the change is significantly small, such that the average binding constants across the filter are similar. By contrast, the different K^+ K_d values from the two groups suggest that one or more K^+ ion-binding sites in the constricted and inactivated filters are significantly different, from which we conclude that the constricted and inactivated filters are structurally not the same.

Previously published structural and functional data support our conclusion that the constricted conformation of the selectivity filter in KcsA is not the inactivated conformation. First, the selectivity filter in structures of open K^+ channels (K_VAP, MthK, K_V1.2, and $K_V$chimera) are all found in their conductive conformation but should be in an inactivated conformation, given the state of their intracellular gate (2, 5, 26, 43). Second, if the constricted conformation in KcsA is the inactivated state, then structures of other K^+ channels in Na^+ should adopt a constricted conformation. The high-resolution structure of MthK in Na^+ alone is in a conductive conformation, which superimposes well onto the K^+-bound structure (44). Finally, the D-Ala mutation at G77 in KcsA prevents the channel from adopting a constricted conformation, but this channel has the same inactivation properties as WT channels (28).

However, the constricted conformation is observed in crystal structures of open KcsA channels obtained through deep truncations. The crystallography and spectroscopy measurements suggest a correlation between the magnitude of channel opening and the conformation of the selectivity filter (9, 31). The largest opening corresponds to a constricted conformation, whereas a less open channel (but one open enough to pass ions) corresponds to the conductive conformation. KcsA in detergent is able to open more than KcsA in lipid bilayers, suggesting that the gating-induced constricted filter may lie outside of the spectrum of conformations adopted during typical electrophysiology experiments including channel inactivation (31).

If the constricted conformation is not the inactivated structure, as our data suggest, then the structure of an inactivated channel is still not known. Elucidating the structural changes in the selectivity filter during inactivation may be challenging. Inactivation could involve an asymmetrical conformational change in the selectivity filter, whose signal might be averaged out in crystallographic and spectroscopic studies. Additionally, small changes in the selectivity filter may be sufficient to erect a barrier for ions rapidly crossing the membrane. These changes may be difficult to discern even in high-resolution crystal structures.

During the course of this study, we discovered that WT-ΔC is open at pH 8 in detergent but closed in lipid vesicles. The expectation from crystal structures and electrophysiology experiments was that the channel would be closed, leading us to conclude that

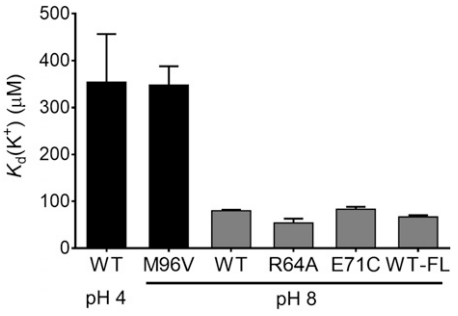

Fig. 6. Comparison of $K_d(K^+)$ obtained from KcsA channels in detergent micelles. The channels are grouped by the likely conformation of their selectivity filter based on a combination of crystal structures of the channel, the functional state of the channel in electrophysiology experiments, and EPR measurements of the intracellular gate. Black bars are channels with a constricted filter, and gray bars are channels with either a conductive or inactivated filter. Channels are C-terminally truncated, except WT-FL.

crystallization conditions or the presence of the lipid bilayer can shift the equilibrium of the intracellular gate. The influence of the lipid bilayer is not unexpected, because lipids are known to be critical in the purification, crystallization, and function of some membrane proteins, presumably by stabilizing specific conformations of the protein (2, 45–50). However, crystal structures of KcsA were all solved in detergent, so why were the channels not in an open conformation? One possible explanation is that the crystal contacts inhibited their ability to open (36). The crystal structure of the deep truncations eliminated direct contacts with neighboring molecules, resulting in some open channels (10). This study further highlights the need to compare the functional and structural properties of membrane proteins in detergent and lipid membranes to understand how a protein's local environment influences its function.

Materials and Methods

KcsA channels were expressed and purified from *Escherichia coli* as previously described (28, 38). ITC was used to measure ion binding to KcsA channels in DM detergent at different concentrations of NaCl (38). EPR was performed with KcsA embedded into either soybean polar lipid vesicles or DM detergent after labeling the channel with a spin probe on position G116C (40). KcsA channels were reconstituted into soybean polar lipids, and electrophysiology measurements of channel inactivation were measured using a giant liposome patch clamp (28). A more detailed description of the materials and methods can be found in *SI Materials and Methods*.

ACKNOWLEDGMENTS. We thank the Reinhart and Begley laboratories for extensive access to their ITC machines and members of the S.W.L. laboratory for helpful discussions. This work was supported by Welch Foundation Grant A-1742 and Texas A&M University Startup Funds (to S.W.L.), by NIH Grant GM087546 and a Pew Scholar Award (to F.I.V.), and by Postdoctoral Fellowship 12POST11910068 from the American Heart Association (to P.J.F.).

1. Doyle DA, et al. (1998) The structure of the potassium channel: Molecular basis of K+ conduction and selectivity. *Science* 280(5360):69–77.
2. Long SB, Campbell EB, Mackinnon R (2005) Crystal structure of a mammalian voltage-dependent Shaker family K+ channel. *Science* 309(5736):897–903.
3. Zhou Y, Morais-Cabral JH, Kaufman A, MacKinnon R (2001) Chemistry of ion coordination and hydration revealed by a K+ channel-Fab complex at 2.0 A resolution. *Nature* 414(6859):43–48.
4. Kuo A, et al. (2003) Crystal structure of the potassium channel KirBac1.1 in the closed state. *Science* 300(5627):1922–1926.
5. Jiang Y, et al. (2002) Crystal structure and mechanism of a calcium-gated potassium channel. *Nature* 417(6888):515–522.
6. Liu YS, Sompornpisut P, Perozo E (2001) Structure of the KcsA channel intracellular gate in the open state. *Nat Struct Biol* 8(10):883–887.
7. Baker KA, Tzitzilonis C, Kwiatkowski W, Choe S, Riek R (2007) Conformational dynamics of the KcsA potassium channel governs gating properties. *Nat Struct Mol Biol* 14(11):1089–1095.
8. Cordero-Morales JF, et al. (2007) Molecular driving forces determining potassium channel slow inactivation. *Nat Struct Mol Biol* 14(11):1062–1069.
9. Cuello LG, et al. (2010) Structural basis for the coupling between activation and inactivation gates in K(+) channels. *Nature* 466(7303):272–275.
10. Cuello LG, Jogini V, Cortes DM, Perozo E (2010) Structural mechanism of C-type inactivation in K(+) channels. *Nature* 466(7303):203–208.
11. Loots E, Isacoff EY (1998) Protein rearrangements underlying slow inactivation of the Shaker K+ channel. *J Gen Physiol* 112(4):377–389.
12. Hoshi T, Zagotta WN, Aldrich RW (1990) Biophysical and molecular mechanisms of Shaker potassium channel inactivation. *Science* 250(4980):533–538.
13. Choi KL, Aldrich RW, Yellen G (1991) Tetraethylammonium blockade distinguishes two inactivation mechanisms in voltage-activated K+ channels. *Proc Natl Acad Sci USA* 88(12):5092–5095.
14. Kiss L, Korn SJ (1998) Modulation of C-type inactivation by K+ at the potassium channel selectivity filter. *Biophys J* 74(4):1840–1849.
15. Levy DI, Deutsch C (1996) A voltage-dependent role for K+ in recovery from C-type inactivation. *Biophys J* 71(6):3157–3166.
16. Levy DI, Deutsch C (1996) Recovery from C-type inactivation is modulated by extracellular potassium. *Biophys J* 70(2):798–805.
17. Ogielska EM, Aldrich RW (1999) Functional consequences of a decreased potassium affinity in a potassium channel pore. Ion interactions and C-type inactivation. *J Gen Physiol* 113(2):347–358.
18. Hoshi T, Zagotta WN, Aldrich RW (1991) Two types of inactivation in Shaker K+ channels: Effects of alterations in the carboxy-terminal region. *Neuron* 7(4):547–556.
19. Hoshi T, Armstrong CM (2013) C-type inactivation of voltage-gated K+ channels: Pore constriction or dilation? *J Gen Physiol* 141(2):151–160.
20. Cordero-Morales JF, et al. (2006) Molecular determinants of gating at the potassium-channel selectivity filter. *Nat Struct Mol Biol* 13(4):311–318.
21. Yang Y, Yan Y, Sigworth FJ (1997) How does the W434F mutation block current in Shaker potassium channels? *J Gen Physiol* 109(6):779–789.
22. López-Barneo J, Hoshi T, Heinemann SH, Aldrich RW (1993) Effects of external cations and mutations in the pore region on C-type inactivation of Shaker potassium channels. *Receptors Channels* 1(1):61–71.
23. Liu Y, Jurman ME, Yellen G (1996) Dynamic rearrangement of the outer mouth of a K+ channel during gating. *Neuron* 16(4):859–867.
24. Starkus JG, Kuschel L, Rayner MD, Heinemann SH (1997) Ion conduction through C-type inactivated Shaker channels. *J Gen Physiol* 110(5):539–550.
25. Kiss L, LoTurco J, Korn SJ (1999) Contribution of the selectivity filter to inactivation in potassium channels. *Biophys J* 76(1 Pt 1):253–263.
26. Long SB, Tao X, Campbell EB, MacKinnon R (2007) Atomic structure of a voltage-dependent K+ channel in a lipid membrane-like environment. *Nature* 450(7168):376–382.
27. Nishida M, Cadene M, Chait BT, MacKinnon R (2007) Crystal structure of a Kir3.1-prokaryotic Kir channel chimera. *EMBO J* 26(17):4005–4015.
28. Devaraneni PK, et al. (2013) Semisynthetic K+ channels show that the constricted conformation of the selectivity filter is not the C-type inactivated state. *Proc Natl Acad Sci USA* 110(39):15698–15703.
29. Lockless SW, Zhou M, MacKinnon R (2007) Structural and thermodynamic properties of selective ion binding in a K+ channel. *PLoS Biol* 5(5):e121.
30. Zhou Y, MacKinnon R (2003) The occupancy of ions in the K+ selectivity filter: Charge balance and coupling of ion binding to a protein conformational change underlie high conduction rates. *J Mol Biol* 333(5):965–975.
31. Hulse RE, et al. (2014) Conformational dynamics at the inner gate of KcsA during activation. *Biochemistry* 53(16):2557–2559.
32. Imai S, Osawa M, Takeuchi K, Shimada I (2010) Structural basis underlying the dual gate properties of KcsA. *Proc Natl Acad Sci USA* 107(14):6216–6221.
33. Bhate MP, McDermott AE (2012) Protonation state of E71 in KcsA and its role for channel collapse and inactivation. *Proc Natl Acad Sci USA* 109(38):15265–15270.
34. Cuello LG, Romero JG, Cortes DM, Perozo E (1998) pH-dependent gating in the Streptomyces lividans K+ channel. *Biochemistry* 37(10):3229–3236.
35. Heginbotham L, LeMasurier M, Kolmakova-Partensky L, Miller C (1999) Single streptomyces lividans K(+) channels: Functional asymmetries and sidedness of proton activation. *J Gen Physiol* 114(4):551–560.
36. Cuello LG, et al. (2010) Design and characterization of a constitutively open KcsA. *FEBS Lett* 584(6):1133–1138.
37. Thompson AN, Posson DJ, Parsa PV, Nimigean CM (2008) Molecular mechanism of pH sensing in KcsA potassium channels. *Proc Natl Acad Sci USA* 105(19):6900–6905.
38. Liu S, Bian X, Lockless SW (2012) Preferential binding of K+ ions in the selectivity filter at equilibrium explains high selectivity of K+ channels. *J Gen Physiol* 140(6):671–679.
39. Liu S, Lockless SW (2013) Equilibrium selectivity alone does not create K+-selective ion conduction in K+ channels. *Nat Commun* 4:2746.
40. Perozo E, Cortes DM, Cuello LG (1998) Three-dimensional architecture and gating mechanism of a K+ channel studied by EPR spectroscopy. *Nat Struct Biol* 5(6):459–469.
41. Perozo E, Cortes DM, Cuello LG (1999) Structural rearrangements underlying K+-channel activation gating. *Science* 285(5424):73–78.
42. LeMasurier M, Heginbotham L, Miller C (2001) KcsA: It's a potassium channel. *J Gen Physiol* 118(3):303–314.
43. Jiang Y, et al. (2003) X-ray structure of a voltage-dependent K+ channel. *Nature* 423(6935):33–41.
44. Ye S, Li Y, Jiang Y (2010) Novel insights into K+ selectivity from high-resolution structures of an open K+ channel pore. *Nat Struct Mol Biol* 17(8):1019–1023.
45. Nussberger S, Dörr K, Wang DN, Kühlbrandt W (1993) Lipid-protein interactions in crystals of plant light-harvesting complex. *J Mol Biol* 234(2):347–356.
46. Dart C (2010) Lipid microdomains and the regulation of ion channel function. *J Physiol* 588(Pt 17):3169–3178.
47. Marius P, Alvis SJ, East JM, Lee AG (2005) The interfacial lipid binding site on the potassium channel KcsA is specific for anionic phospholipids. *Biophys J* 89(6):4081–4089.
48. Oliver D, et al. (2004) Functional conversion between A-type and delayed rectifier K+ channels by membrane lipids. *Science* 304(5668):265–270.
49. Schmidt D, Jiang Q-X, MacKinnon R (2006) Phospholipids and the origin of cationic gating charges in voltage sensors. *Nature* 444(7120):775–779.
50. Valiyaveetil FI, Zhou Y, MacKinnon R (2002) Lipids in the structure, folding, and function of the KcsA K+ channel. *Biochemistry* 41(35):10771–10777.
51. Sauer DB, Zeng W, Canty J, Lam Y, Jiang Y (2013) Sodium and potassium competition in potassium-selective and non-selective channels. *Nat Commun* 4:2721.
52. Tellinghuisen J (2008) Isothermal titration calorimetry at very low c. *Anal Biochem* 373(2):395–397.
53. Cao E, Cordero-Morales JF, Liu B, Qin F, Julius D (2013) TRPV1 channels are intrinsically heat sensitive and negatively regulated by phosphoinositide lipids. *Neuron* 77(4):667–679.