Molecular Movement of the Voltage Sensor in a K Channel

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ABSTRACT The X-ray crystallographic structure of KvAP, a voltage-gated bacterial K channel, was recently published. However, the position and the molecular movement of the voltage sensor, S4, are still controversial. For example, in the crystallographic structure, S4 is located far away (>30 Å) from the pore domain, whereas electrostatic experiments have suggested that S4 is located close (<8 Å) to the pore domain in open channels. To test the proposed location and motion of S4 relative to the pore domain, we induced disulphide bonds between pairs of introduced cysteines: one in S4 and one in the pore domain. Several residues in S4 formed a state-dependent disulphide bond with a residue in the pore domain. Our data suggest that S4 is located close to the pore domain in a neighboring subunit. Our data also place constraints on possible models for S4 movement and are not compatible with a recently proposed KvAP model.

KEY WORDS: Shaker K channel • voltage gated • disulfide • S4 movement • helical screw

INTRODUCTION

Voltage-gated ion channels are key players in fast, electrical signaling in biological systems. These channels open and close in response to changes in the transmembrane voltage (Hille, 2001). The X-ray crystallographic structure of KvAP, a voltage-gated bacterial K channel, was recently published (Jiang et al., 2003a,b). However, the position and the molecular movement of the voltage sensor, S4, are still controversial. For example, in the crystallographic structure (Jiang et al., 2003b), the extracellular end of S4 is located far away (>30 Å) from the pore domain, while electrostatic experiments have suggested that S4 is located close (<8 Å) to the pore domain in open channels (Elinder et al., 2001a,b). LRET measurements between fluorescent-labeled S4s also suggest that S4 is located close to the pore (Cha et al., 1999). In addition, in the model of KvAP the proposed motion of S4 also moves the extracellular end of S3 across the membrane: the "paddle" consisting of S4 and S3b moves as unit. However, in the closely related voltage-activated Shaker K and EAG channels, the extracellular end of S3 contains a number of negative charges that would neutralize the positive charges in S4 (Fig. 1 A). If the proposed motion of S3 and S4 in KvAP also occurred in Shaker K and EAG channels then this movement of S3 and S4 would not move a substantial amount of charge across the membrane in Shaker K and EAG channels. Hence, the KvAP model would not be able to explain the voltage sensitivity of Shaker K and EAG channels.

To elucidate how the voltage sensor S4 moves in a voltage-gated K channel, we studied disulphide bond formation between pairs of cysteines—one introduced in S4 and one in the pore-forming region (S5-S6)—in the well-characterized Shaker K channel. Two cysteines only form a disulphide bond if they are close to each other (β-carbon distance = 4–5 Å; Careaga and Falke, 1992). If S4 moves relative to the rest of the channel during channel opening (gating), different cysteine pairs should form disulphide bonds in closed channels versus in open channels. By testing a number of different cysteine pairs in S4 and S5-S6, we tracked the movement of S4 relative S5-S6. In a previous study, based on electrostatic interactions between S4 and S5-S6, we suggested that the top charge of S4 (R362) is very close (<8 Å) to the extracellular end of S5 in open channels, but that R362 moves away from S5 during channel closing (Elinder et al., 2001b). Using the KcsA structure (Doyle et al., 1998) as a model for the pore of Shaker K channel, we identified residues 416, 417, and 454 as potential cysteine-pairing partners to R362C (Fig. 1 B). Our hypothesis was that 362C forms disulphide bonds with 416C, 417C, and 454C at depolarized voltages, but not at hyperpolarized voltages.

MATERIALS AND METHODS

Molecular Biology

The experiments were performed on Shaker H4 channels. Site-directed mutagenesis, cRNA synthesis, and cRNA injection into *Xenopus laevis* oocytes were performed as described previously (Larsson and Elinder, 2000). 0.5 mM dithiothreitol (DTT) was

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Abbreviation used in this paper: DTT, dithiothreitol.

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added to the incubating medium to prevent the formation of disulphide bonds during channel expression and maturation.

Electrophysiology and Solutions

The currents were recorded with a 2-electrode voltage-clamp technique as described previously (Larsson and Elinder, 2000). We used the CA-1B amplifier (Dagan Corp.). We used a 1-K bath solution (in mM): 88 NaCl, 1 KCl, 15 HEPES, 0.4 $CaCl_2$, and 0.8 $MgCl_2$. NaOH was added to adjust the pH to 7.4, yielding a final Na concentration of ~100 mM. All experiments were performed at room temperature (20–23°C). We used 2 μM $CuSO_4$ together with 100 μM phenanthroline (Cu/phenanthroline) to induce the disulphide bond. Cu/phenanthroline was applied between the recordings; the current during the test pulse was recorded in the absence of Cu/phenanthroline. Cu/Phenanthroline had no effect on the single cysteine mutations, showing that the disulphide bonds formed only between the introduced cysteine pairs. Spontaneous formation of disulphide bonds was measured after the removal of DTT (0.5 mM) from the recording solution. Disulfide bond formation was tested in open channels at 0 mV and in closed channels at −80 mV, because the majority of gating charge moves between −80 and 0 mV in Shaker K channels. However, a small amount of gating charges moves at more negative potentials than −80 mV, which could lead to an under estimation of the state dependence for the disulfide bond formation.

Block of Current

The time course of the current reduction was analyzed with the following equation:

$$I(t) = I(0)\{1 - [1 - \exp(-t/\tau)]^w\}, \quad (1)$$

where I(t) is the current after t seconds of Cu/phenanthroline application, τ is the time constant for the modification, and w indicates the number of disulphide bonds that had to be formed in order to reduce the current.

RESULTS

Specificity in the S4-S5 Interactions—No Promiscuity

We made six combinations of cysteines pairs: 362C paired with three different residues in the pore region

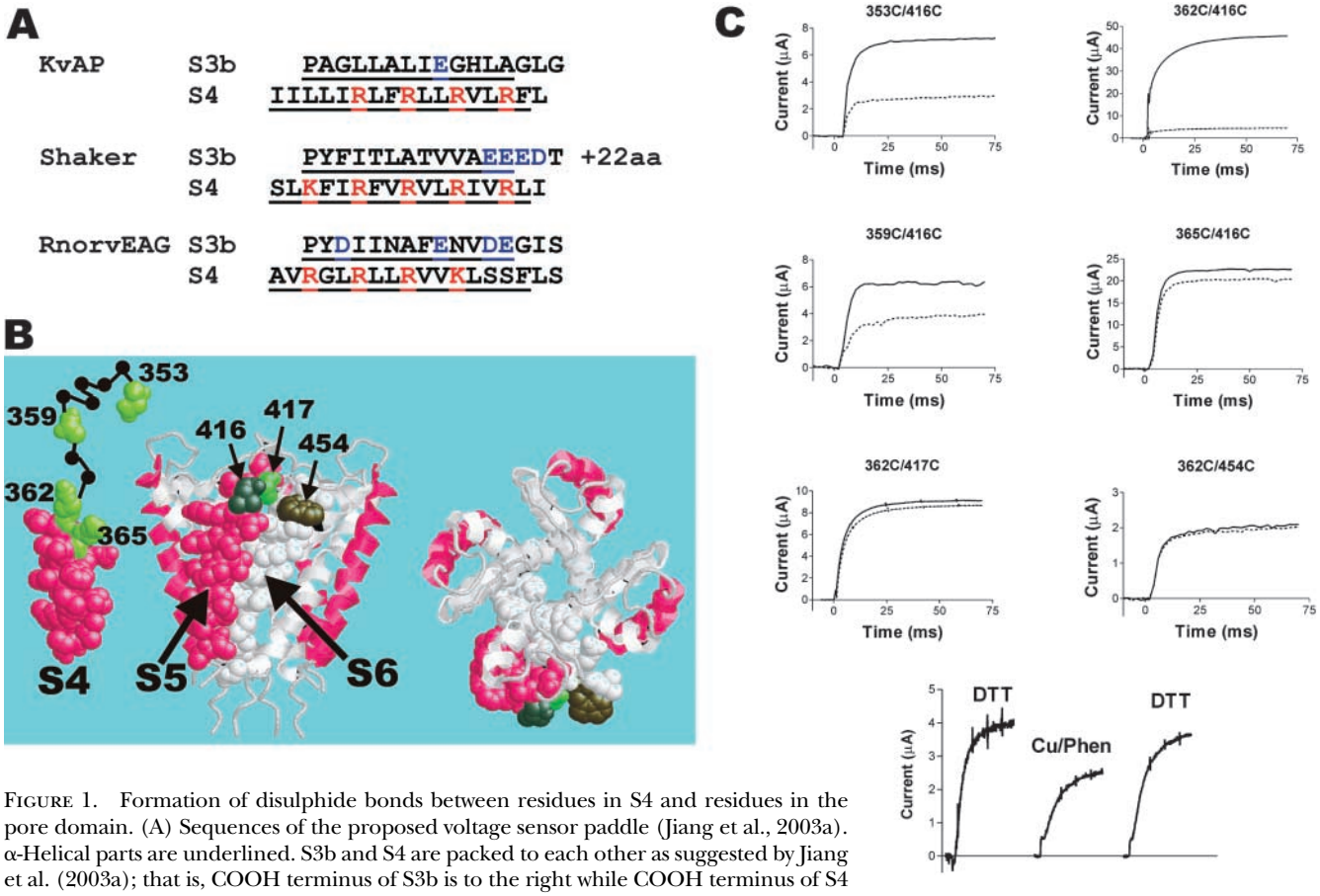

FIGURE 1. Formation of disulphide bonds between residues in S4 and residues in the pore domain. (A) Sequences of the proposed voltage sensor paddle (Jiang et al., 2003a). α-Helical parts are underlined. S3b and S4 are packed to each other as suggested by Jiang et al. (2003a); that is, COOH terminus of S3b is to the right while COOH terminus of S4 is to the left. 22 residues of the Shaker S3-S4 linker are omitted. Positive charges in red and negative in blue. The net charge of the paddles: +3 (KvAP), +1 (Shaker), and 0 (RnorvEAG). (B) Molecular structure of S4 of KvAP (Jiang et al., 2003a) and the pore domain (S5-S6; side view to the left and top view to the right) of KcsA (Doyle et al., 1998). The residues used in the present investigation are labeled (Shaker numbering). The α-helical parts of S4, S5, and S6 in one subunit are space filled. The structure between 353 and 362 is not known for the Shaker K channel and is only drawn as a random coil. (C) Voltage-clamp data for six double-cysteine mutations. Current in response to a voltage step to 0 mV in control solution (1K), after incubation in 0.5 mM DTT (continuous line), and in control solution (1K), after >1 min exposure to Cu/phenanthroline (dotted line). Holding voltage was −80 mV. (Bottom) DTT (20 mM) reversed the Cu/phenanthroline effect, showing that Cu/phenanthroline induced disulfide bonds (shown for 359C/416C channels).

(416C, 417C, and 454C) and 416C paired with four residues in S4 (353C, 359C, 362C, and 365C). These double mutations were expressed in *Xenopus* oocytes in the presence of 0.5 mM DTT, to prevent disulphide bond formation during protein maturation and folding (see MATERIALS AND METHODS). Application of the disulphide-promoting agent Cu/phenanthroline (2 μM $CuSO_4$/100 μM phenanthroline) clearly altered the currents in three of the pairs: 416C paired with 353C, with 359C, and with 362C. However, application of Cu/phenanthroline did not alter the currents in the other three pairs: 362C paired with either 417C or 454C, or 365C paired with 416C (Fig. 1 C). The single mutations were not affected by Cu/phenanthroline (unpublished data). The reducing agent DTT reversed the effect induced by Cu/phenanthroline, suggesting that a disulphide bond was induced by Cu/phenanthroline (Fig. 1 C).

That Cu/phenathroline did not reduce the current in some of the double mutations does not automatically rule out the possibility that a disulphide bond formed in these double mutations, because we used a functional test (changes in current) for the detection of disulphide bonds. However, when 362C/417C and 362C/454C were incubated without DTT during channel expression, a later DTT application increased the currents in these mutations, suggesting that these cysteine pairs can form disulphide bonds under certain circumstances and that these disulphide bonds alter the current. However, we were not able to recreate these disulphide bonds with Cu/phenanthroline, suggesting that they formed during channel maturation or folding. The lack of effect by Cu/phenathroline indicates that disulphide bonds do not form between 362C and 417C, or 362C and 454C, in mature channels. Instead, our data suggest that disulphide bonds were formed in fully folded, mature channels for only three of the pairs: 416C paired with 353C, with 359C, and with 362C. That only certain combinations of cysteines formed disulphide bonds suggests that this region of the channel is a fairly rigid structure and that there are specific interactions between S4 and S5. Our data show that: (a) a long stretch of residues in the S3-S4 linker and in the extracellular end of S4 (but not the deeper 365 residue) is in close proximity to the pore domain during some part of gating, and (b) S4 movement is restricted in lateral directions in the open state because 416C, but not its neighboring residues, can form bonds with 362C.

362C Forms Disulphide Bonds with 416C in Open but Not in Closed States

To determine whether the extracellular end of S4 moves close to the pore domain during activation, we investigated the state dependence of the disulphide-bond formations for the three combinations that formed disulphide bonds. Disulphide bond formation for 362C/416C was much faster at positive voltages than at negative voltages (Fig. 2 A): application of Cu/phenanthroline for 20 s at 0 mV reduced the current with 88 ± 5% of maximum reduction (n = 3), at −80 mV with 38 ± 7% (n = 7), and at –100 mV with 2 ± 5% (n = 3). Fig. 2 B shows, in detail, the time course of the Cu/phenanthroline-induced current reduction at 0 mV and at −80 mV. Fig. 2 B also shows that 362C and 416C formed disulphide bonds spontaneously (in the absence of Cu/phenanthroline and DTT, before time 0), suggesting that these residues are very close in space. The rate for spontaneous formation of disulphide bonds in 362C/416C had similar voltage dependence, but was much slower (∼75 times) than with the Cu/phenanthroline (Fig. 2 B). In contrast to 362C/416C, 353C/416C did not show any voltage dependence for the disulphide bond formation; the application of Cu/phenanthroline for 25 s at 0 mV reduced the current by 81 ± 8% of maximum reduction (n = 3), and at −80 mV, by 77 ± 5% (n = 3). Disulphide bonds were also formed spontaneously in 353C/416C, but at a much slower rate than in the presence of Cu/phenanthroline. Both the Cu/phenanthroline-induced and the spontaneous formations of disulfide bonds in 353C/416C were voltage independent (Fig. 2 C). The disulphide bond formation in 359C/416C was also voltage dependent, but it was less pronounced than in 362C/416C (unpublished data).

Current Reduction in 362C/416C Is Caused by Slow Inactivation

The time course of the current reduction induced by the disulphide bonds was quite different for 353C/416C than for 362C/416C. In 353C/416C channels, the time course was apparently exponential (Fig. 2 C), whereas in 362C/416C the time course was clearly sigmoidal (Fig. 2 B). (This was evident also after a correction for the spontaneous formation of disulphide bonds before the application of Cu/phenanthroline.) The sigmoidal time course of 362C/416C was fitted to an exponential decay to the power of 4 (see MATERIALS AND METHODS), suggesting that four disulphide bonds (one in each subunit) need to be formed before the current in a channel is reduced. However, in 353C/416C channels, the formation of only one disulphide bond sufficed to cause a reduction in current. Furthermore, the current reduction in 353C/416C was less prominent than in 362C/416C.

Why did the disulphide bonds reduce the current? The pore of the Shaker K channel can be in three possible states: closed, open, or inactivated. Most likely, the disulphide bonds increase the likelihood that the channel is in either the closed or the inactivated state. The voltage dependence for the formation of disulphide

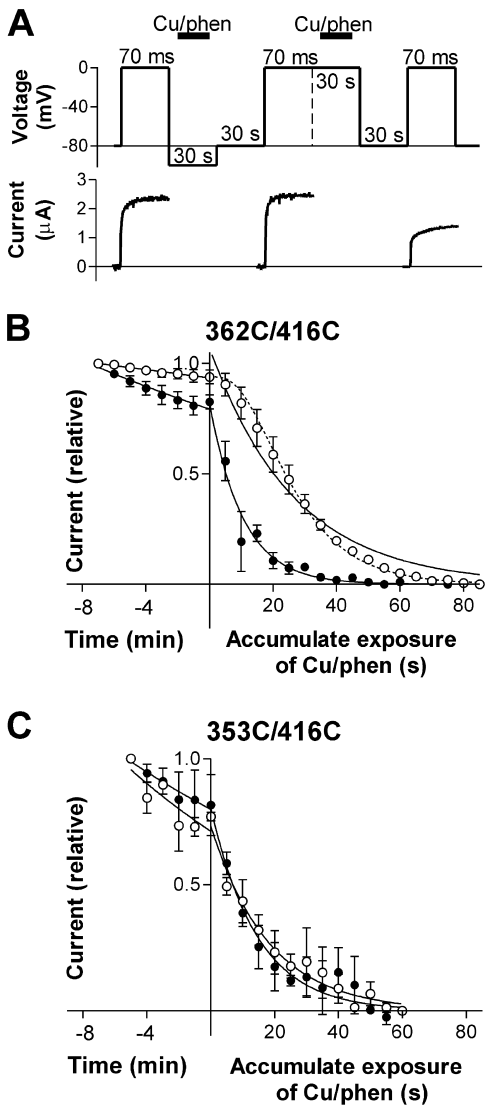

FIGURE 2. Voltage and time dependence of disulphide bond formation. (A) State-dependent formation of disulphide bonds between 362C and 416C for three consecutive voltage steps to 0 mV. Voltage protocol is shown in the top panel. Cu/phenanthroline was added for 20 s, as indicated. Applied at −100 mV, Cu/phenanthroline did not affect the current; applied at 0 mV, Cu/phenanthroline clearly reduced the current. (B and C) Steady-state current at the end of a 70-ms pulse to 0 mV versus accumulated exposure to 2/100 μM Cu/phenanthroline. The voltage was stepped to −80 mV for 20 s, then to either −80 mV or to 0 mV for 30 s, followed by a voltage step to −80 mV for 10 s. For each episode, Cu/phenanthroline was applied for a brief period (usually 5 s) during the 30-s step either to −80 mV or to 0 mV. The data points at time < 0 were obtained in the absence of Cu/phenanthroline. n = 3–7 for all data points. Eq. 1 was fitted to the data points, with w = 1 for the continuous curves and w = 4 for the dashed curve. (B) Disulfide bond formation in 362C/416C at −80 mV (open circles) and 0 mV (closed circles). Spontaneous current reduction (time < 0): $\tau(-80)$ = 104 min and $\tau(0)$ = 33 min (w = 1). Cu/phenanthroline-induced current reduction (time > 0): $\tau(-80)$ = 27 s and $\tau(0)$ = 10 s (w = 1). $\tau(-80)$ = 14 s for w = 4. (C) 353C/416C. Spontaneous current reduction (time < 0): $\tau(-80)$ = 18 min and $\tau(0)$ = 24 min (w = 1). Cu/phenanthroline-induced current reduction: $\tau(-80)$ = 18 s and $\tau(0)$ = 15 s (w = 1).

bonds in 362C/416C suggests that the disulphide bonds are formed when S4 is in its activated (extruded) position (Larsson et al., 1996). When all four S4s are disulphide bonded, no S4 can move to the retracted position, and the Shaker K channel is prevented from closing. Because recovery from slow inactivation occurs from closed channels (with some or all S4s in their retracted position), the channels with four disulphide-bonded S4s are prevented from recovering from slow inactivation. Therefore, we hypothesize that disulphide bonds reduce the currents from 362C/416C channels because a majority of channels become inactivated. The shape of the time course also suggests that 362C and 416C do not move relative to each other during slow inactivation (see DISCUSSION), which implies that 362C and 416C are close in space, in the open state. We do not know the cause of the less prominent current reduction in 353C/416C, but a possibility is that the disulphide bonds alter the equilibrium between the closed and the open states, causing a lowering of the open probability and, thereby, reducing the macroscopic currents.

359C (in S4) Interacts with 416C (in S5) in a Neighboring Subunit

To determine whether the disulphide bonds were formed between S4 and S5 in the same subunit or between subunits, we coinjected two mRNAs at equal concentrations: one encoding for the single mutation 416C and one for the single mutation 359C. The application of Cu/phenanthroline reduced the currents for the coinjected 359C and 416C channels in a similar manner as the currents were reduced in the double mutant 359C/416C channels (Fig. 3). This shows that disulphides are formed between 359C and 416C in different subunits. Similar experiments with coinjection of 362C and 416C did not show any significant current reduction upon Cu/phenanthroline application (unpublished data). The most likely reason for this is that at most two disulphide bonds could be formed in this coinjection experiment, while the sigmodicity (described above) for the reaction suggested that four bonds are required for current reduction in 362C/416C.

DISCUSSION

We have here shown that a number of residues in S4 and the S3-S4 loop can form disulfide bonds with 416C at the top of S5 in Shaker K channels. The disulfide bonds between 362C in S4 and 416C only formed in open channels, but not in closed channels. The disulfide bonds between 353C in the S3-S4 loop and 416C formed equally fast in closed and open channels. In addition, we showed that the disulfide bonds between

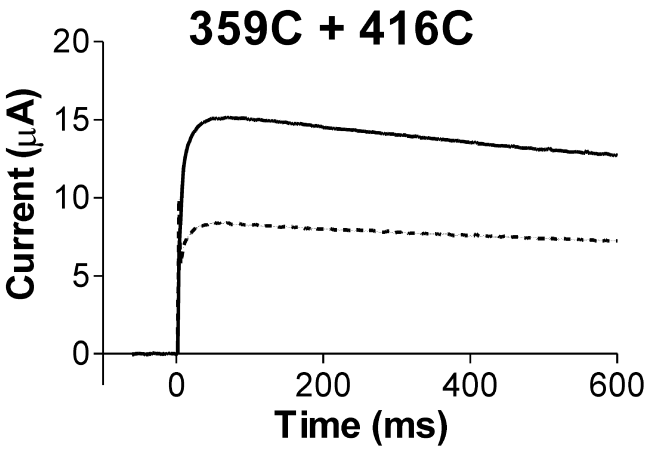

FIGURE 3. Disulphide bond formation between two subunits. Equal amounts of 359C and 416C mutations were coinjected in the oocytes. Cu/phenantroline was applied for 60 s at 0 mV. Current in response to a voltage step to 0 mV before Cu/phenanthroline application (continuous line) and after a 60-s application of Cu/phenanthroline at 0 mV (dotted line). Holding voltage was −80 mV.

359C and 416C can form between subunits. These data suggest that the top of S4 is in close proximity to the top of S5 in the neighboring subunit (Fig. 4). This is contrast to the paddle model described by Jiang et al. (2003b), where the top of S4 is located very far from the top of S5 (>30 Å). Jiang et al. (2003b) suggest that S4 is very flexible and that this would allow S4 to move and make contact with many parts of the channel. However, the specificity of the disulphide bond formation in our study suggests that there are specific interactions of S4 and S5. In addition, our earlier studies showing electrostatic interaction between S4 and S5 suggested that the top of S4 (on a time average) should be very close to the top of S5 in the open state (Elinder et al., 2001a,b). The fast disulphide bond formation rate in this study also suggests that S4 spends most of its time in the open state very close to S5.

362C and 416C Are Very Close in the Open State

For 362C/416C, the rate of disulphide bond formation at 0 mV is very fast: $\tau \approx 10$ s (Fig. 2 B). This rate is similar to the rate that disulphide bonds form between two 448C residues in the central pore of the Shaker K channel (Liu et al., 1996). The distance between the backbone of a homologous residue to 448C in neighboring subunits in the KcsA structure is 11 Å, and most likely the β-carbons of the two residues are much closer in the state in which they form disulfide bonds (the slow inactivated state; Liu et al., 1996). The similar rates of disulphide bond formation for 416C/362C suggest that 362C and 416C are also very close to each other in space, in the open state. In contrast, these rates are much faster (>200 times faster) than the rates measured for disulfide bonds formed between 361C in neighboring subunits (Aziz et al., 2002), suggesting that those bonds were formed between residues that are further apart or in a rare channel conformation.

The Voltage Sensor Is Not Moving During Inactivation

In this study, we assumed that S4 does not undergo any conformational changes relative S5 during slow inactivation. This is a critical assumption, because we assumed that the position in which S4 forms disulphide bonds with S5 is similar to the position of S4 in the open state. In addition, the application of Cu/phenanthroline was done during prolonged depolarizing voltage steps that cause a large fraction of the channels to undergo slow inactivation. Some studies have suggested that there is a conformational change in the S4-S5 region during slow inactivation (Loots and Isacoff, 1998; Aziz et al., 2002), while other studies have suggested that there is no conformational change (Elinder et al.,

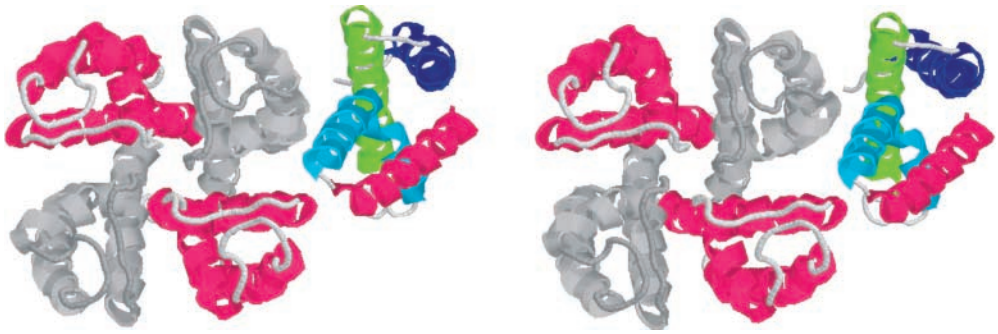

FIGURE 4. Model for S4 location in the Shaker K channel (stereoview). To construct a structural model of the Shaker K channel, we used the KcsA structure (Doyle et al., 1998) for the pore and added the isolated S1-S4 structure of KvAP (Jiang et al., 2003a). The top of S4 is in close proximity to the top of S5 in this model. The four subunits of KcsA are colored red or gray. Only one of the four voltage-sensing S1-S4 domain is shown: S1 (blue), S2 (green), S3 (light blue), and S4 (dark red).

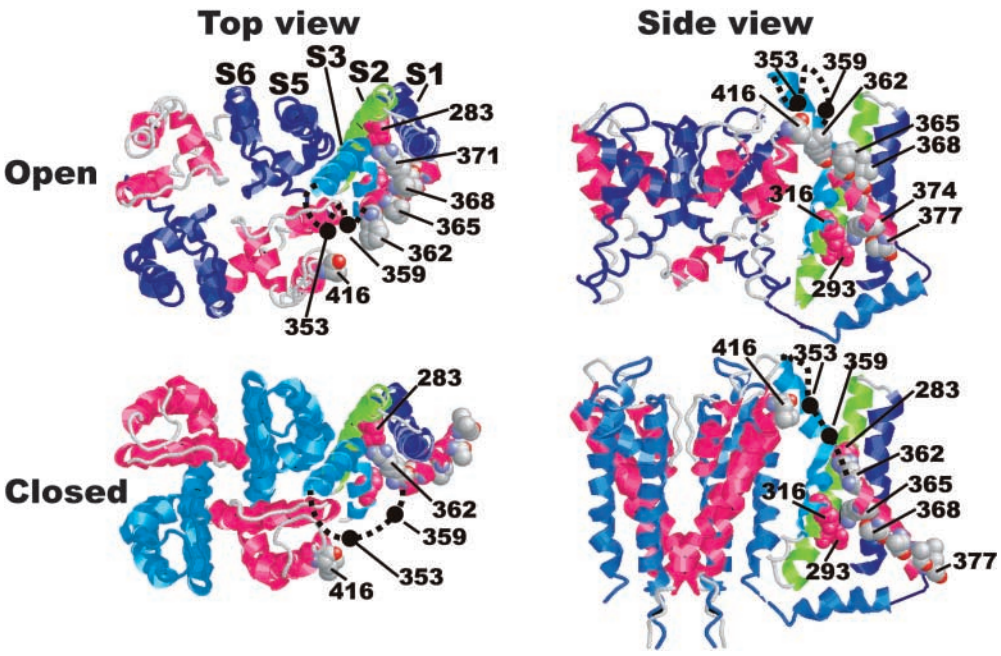

FIGURE 5. Model for S4 movement in the Shaker K channel based on X-ray crystallographic structures of related channels and data from the Shaker K channel. To construct structural models of the different channel states, we used the KcsA structure (Doyle et al., 1998) for the closed configurations of the pore and the MTHK structure (Jiang et al., 2002) for the open configuration. To these pore structures we added modified versions of the isolated S1-S4 structure (Jiang et al., 2003a). S4 in KvAP differs from most other voltage-gated K channels in having unequal spacing of the positive charges. We therefore realigned the Shaker and KvAP sequences to preserve the electrostatic interactions in the crystal structure. We assumed that the residues in the crystal structure of KvAP (133 and 136) interacting with negative charges in S2 (62 and 72) and S3 (93) are homologous to 371 and 374 in the open state of Shaker. Thus, to model S1-S4 in the open state, we assumed that 124–139 in KvAP corresponds to 362–377 in the Shaker channel. The residue corresponding to 362 in the Shaker channel is close to the residue corresponding to 416 in the neighboring subunit. To model the closed state, we moved S4 downward in a helical-screw fashion, so that 362, 365, and possibly 368 interact with the negative charges in S2 and S3.

2001b). The time course of the disulphide bond formation for 362C/416C supports the hypothesis that there is no major conformational change in the external S4-S5 region during slow inactivation:

Each of the four voltage sensors (one per subunit) can be assumed to be in one or several resting positions (R), one activated position (A), and one in an inactivated position (X):

$$R \leftrightarrow A \leftrightarrow X.$$

The gating of the Shaker K channel can be simplified to the following Hodgkin-Huxley model:

$$C_{RRRR} \leftrightarrow C_{RRRA} \leftrightarrow C_{RRAA} \leftrightarrow C_{RAAA} \leftrightarrow O_{AAAA} \leftrightarrow I_{XXXX},$$

where C are closed states, O an open state, and I an inactivated state. The subscripts denote the positions of the four voltage sensors. Slow inactivation occurs only from the open state and not from any preopen state (Hoshi et al., 1991). Slow inactivation is highly cooperative (Ogielska et al., 1995; Larsson and Elinder, 2000), indicating that no combinations of A and X will occur.

If we assume that S4 changes position drastically relative to S5 during inactivation (A→X) or during recovery from inactivation (X→A), then all S4s will change position simultaneously. In this case, a disulphide bond formed in A would prevent inactivation transition and, thus, the disulphide bond would not cause any current reduction. If a disulphide bond is formed when S4 is in state X, the disulphide bond would give rise to a current reduction by trapping the channels in the inactivated state. However, the time course of the current reduction would be exponential, not an S-shaped current reduction, as we found. Thus, the shape of the time course suggests that 362 does not move substantially relative to 416 during slow inactivation.

A Molecular Model for the Voltage Sensor Movement

The data obtained in the present investigation limit the possible molecular models for S4 movement in Shaker K channels. To design simple, structural models of the different channel states (Figs. 4 and 5), we used the MTHK structure (Jiang et al., 2002) for the open configuration of the pore and the KcsA structure (Doyle et al., 1998) for the closed configuration. To these pore structures we added modified versions of the isolated S1-S4 structure from KvAP (Jiang et al., 2003a). In the open-state model, 362 is in close proximity to 416 in the neighboring subunit. In the closed-state models, we moved S4 according to a helical-screw motion, so that the positive charges in S4 slide against negative charges located in S2 and S3 (Tiwari-Woodruff et al., 2000). S3 is located at the interface between S5 and S6 in two neighboring subunits both in the closed and the open states. In our model, we have not used the paddle

model of Jiang et al. (2003b) that also moves S3b, because of the presence of the many negative charges in S3b, which would reduce the total gating charge of the channel (see INTRODUCTION). S2 interacts with S5 in the same subunit, as suggested by Jiang et al. (2003b), and S4 interacts with S5 in the neighboring subunit and faces the lipid bilayer (Elinder et al., 2001a; Jiang et al., 2003a) rather than other transmembrane segments completely surrounding S4. The location of S4 at the protein–lipid interface allows for large-scale motions of S4, while still allowing S4 charges to interact with negative counter charges in S2 and S3 (Elinder et al., 2001a). The gating model in Fig. 5 is compatible with the disulphide data in the present investigation: 362C is very close to 416 in the open state, 359C is close to 416C in the last closed state immediately preceding the open state, and 353C is close to 416C in both the closed and the open states. The model is also compatible with S4 accessibility studies using MTS reagents on Shaker K channels (Larsson et al., 1996; Baker et al., 1998) and with proton transfer by histidines introduced in S4 (Starace et al., 1997; Starace and Bezanilla, 2001). It is also compatible with biotin binding data (Jiang et al., 2003b), gating charge transfer data (Seoh et al., 1996; Aggarwal and MacKinnon, 1996), and two recent studies showing Cd^{2+} coordination and disulfide bond formation between residues in S4 and S5 from neighboring subunits (Lainé et al., 2003; Neale et al., 2003). The present model has three S4 transitions per subunit. At least two transmembrane steps of S4 have been suggested in a number of studies (Keynes, 1994; Zagotta et al., 1994; Baker et al., 1998; Keynes and Elinder, 1998; Schoppa and Sigworth, 1998).

Comparison with the KvAP Model

Our model differs from the KvAP model. The main difference is that we have rotated S1-S4 by ~180 degrees, so that the top of S4 is in contact with the top of S5 from the neighboring subunit. In addition, the S1-S4 structure is more perpendicular to the plane of the membrane, so that S1 and S2 are now more parallel to S5 and S6. The discrepancy between the present structure and the crystal structure (Jiang et al., 2003a,b) may be due to the antibodies used to solve the crystal structure. Attaching an imaginary antibody to the S3-S4 linker in our model and pulling it in the radial direction from the channel pore results in a structure similar to the crystal structure (Jiang et al., 2003a). Jiang et al. (2003b) suggested that the S4-S5 linker serves as the molecular coupling between the voltage sensor and the activation gate. In our model, we also suggest that the S4-S5 linker could be part of the coupling. However, because our data suggest that there are specific, direct interactions between S4 and the pore domain, there could be other important interactions that confer the coupling between voltage sensor and the pore domain.

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