Can Shaker Potassium Channels be Locked in the Deactivated State?

Youshan Yang, Yangyang Yan, and Fred J. Sigworth

Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06520

ABSTRACT For structural studies it would be useful to constrain the voltage sensor of a voltage-gated channel in its deactivated state. Here we consider one Shaker potassium channel mutant and speculate about others that might allow the channel to remain deactivated at zero membrane potential. Ionic and gating currents of F370C Shaker, expressed in *Xenopus* oocytes, were recorded in patches with internal application of the methanethiosulfonate reagent MTSET. It appears that the voltage dependence of voltage sensor movement is strongly shifted by reaction with internal MTSET, such that the voltage sensors appear to remain deactivated even at positive potentials. A disadvantage of this construct is that the rate of modification of voltage sensors by MTSET is quite low, $\sim$0.17 $mM^{-1}{\cdot}s^{-1}$ at −80 mV, and is expected to be much lower at depolarized potentials.

KEY WORDS: patch clamp • gating current • MTSET • cysteine • S4

INTRODUCTION

The fourth transmembrane segment S4 of Shaker potassium channels has been intensively examined through mutagenesis, fluorescent labeling, and covalent modification. This segment is thought to be a major part of the channel voltage sensor; consistent with this are observations of voltage-dependent changes in accessibility of many S4 residues to the extracellular and intracellular solutions (Bezanilla, 2000; Gandhi and Isacoff, 2002; Jiang et al., 2003). As might be expected, most of the modifications of introduced cysteine residues produce changes in the voltage-dependent gating of the channels as well.

The most striking change in channel activation was observed by Baker et al. (1998) in the mutant F370C. As can be seen in Fig. 1, residue 370 lies near the center of the S4 helix where residues are poorly accessible to reagents like MTSET but nevertheless show state-dependent accessibility to hydrogen ions (Starace et al., 1997). When exposed on the intracellular side to the sulfhydryl reagent MTSET at negative membrane potentials, F370C channels altogether stopped conducting ionic current. At more positive potentials, there was no effect of MTSET, consistent with the idea that the accessibility of this residue to covalent modification from the intracellular side is voltage dependent. Also interesting is the observation by Baker et al. (1998) that the loss of intracellular accessibility of the 370C residue occurred over a more negative voltage range than the gain of extracellular accessibility of a second cysteine residue, 365C. The disparate voltage ranges of movements of these residues are not unexpected, since the charge movement in the Shaker voltage sensor is known to occur in two or more components. The voltage dependence of the change in accessibility of 370C corresponds to Q_1, the first component of charge movement (Bezanilla et al., 1994). It therefore appears that the reaction with MTSET occurs only in the fully deactivated state of the channel.

If the cysteine residue 370C is accessible only in the fully deactivated state, it is possible that the modified cysteine, with the charged ethyltriethylammonium moiety attached, may in turn constrain the voltage sensor to remain in the deactivated state. With the modification of 370C residues in each of the four subunits, the result would then be the trapping of the entire channel in its deactivated state.

For structural studies of voltage-gated channel proteins, it would be very useful to modify channels so that they are locked in the fully activated or fully deactivated state. It is easy to place Shaker channels into the activated state: they are near maximal activation at zero membrane potential, and the presence of an open-channel blocker can ensure that the voltage sensor is constrained in the activated conformation. However, of the many mutations that have been made of the Shaker channel, none have been shown convincingly to result in a channel that is trapped in the deactivated state. The mutations investigated so far that eliminate ionic currents appear either to block ionic conduction through an inactivation process (Perozo et al., 1993; Yang et al., 1997) or prevent assembly and maturation of channel protein (Schulteis et al., 1998; Papazian, 1999). Horn et al. (2000) have demonstrated the immobilization of Shaker voltage sensors using a photo-activatable

Address correspondence to Fred Sigworth, Dept. of Cellular and Molecular Physiology, Yale University, 333 Cedar Street, New Haven, CT 06520-8026. Fax: (203) 785-4951. email: fred.sigworth@yale.edu

Abbreviation used in this paper: WTT, wild-type truncated.

J. Gen. Physiol. © The Rockefeller University Press • 0022-1295/2004/08/163/9 $8.00
Volume 124 August 2004 163–171
http://www.jgp.org/cgi/doi/10.1085/jgp.200409057

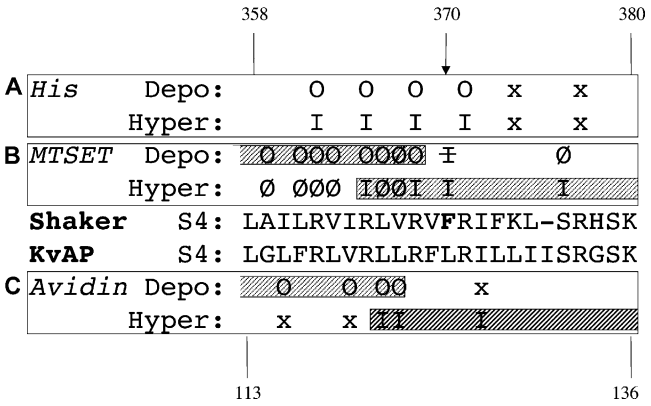

FIGURE 1. Summary of S4 accessibility determinations in Shaker and KvAP channels. O and I denote accessibility to outside and inside solutions; x, no effect; Ø and ∓, not accessible to outside and inside, respectively. (A) Accessibility of substituted histidine residues to hydrogen ions in Shaker (Starace and Bezanilla, 2001). (B) Accessibility of Shaker substituted cysteine residues to MTSET; data summarized from Table I of Bezanilla (2000). (C) Sensitivity of biotinylated KvAP cysteine mutants to avidin, from Jiang et al. (2003).

cross-linking reagent, but the nature of the photoreaction is such that only 30% of the voltage sensors can be immobilized. Laine et al. (2003) demonstrate that the formation of a disulfide bond between S4 and the pore region impedes channel opening, but does not prevent it entirely. In this context, the F370C mutant channel shows some promise as a variant that can be forced to remain in the deactivated state. The goal of the present work was to determine whether modification of this channel with MTSET yields permanently deactivated channels. In the DISCUSSION, we also consider some other Shaker mutants that may have appropriate properties.

MATERIALS AND METHODS

Molecular Biology

The basic wild-type truncated (WTT) Shaker construct was Shaker H4 (Schwarz et al., 1988) in which residues 6–46 were deleted to remove fast inactivation. The F370C mutation was introduced into this background and propagated in the Bluescript vector. The constructs F370C/W434F and F373C were made in the background of a Shaker 29-4 construct in pGEM-A (Swanson et al., 1990), in which amino acid residues 2–29 were deleted. Plasmids of ShakerB-H4 and Shaker 29-4 were linearized with HindIII and NotI, respectively, and all cRNAs were transcribed with the MEGAscript T7 RNA polymerase kit (Ambion). The sizes of transcribed cRNAs were verified by gel electrophoresis.

Electrophysiology

Xenopus oocytes were injected with 50 nl of cRNA of F370C, F370C/W434F, or F373C. Patch clamp recordings were made 3–7 d after RNA injection using an EPC-9 amplifier and the Pulse acquisition program (HEKA Electronic). Patch pipettes were pulled from PG150T glass (Warner Instruments) to tip diameters of 2–5 μm after heat polishing. Both bath and pipette solutions for patch clamp recording were (in mM) 160 KCl, 1 $MgCl_2$, 1 EGTA, 10 HEPES, and pH 7.4, titrated with KOH for all experiments.

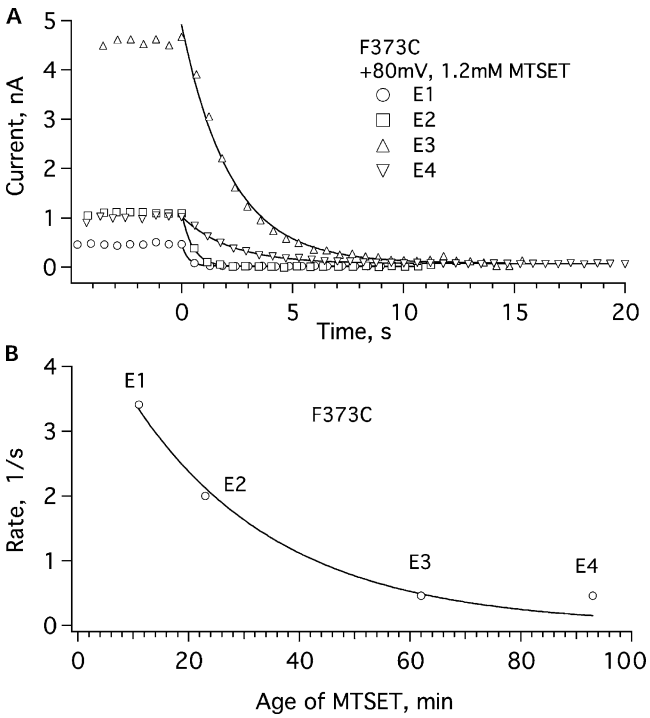

FIGURE 2. Calibration of the decay of MTSET activity. Recordings of F373C currents were made from four successive inside-out patches obtained from the same oocyte. (A) Ionic current amplitude during pulses to +80 mV as a function of time of exposure to 1.2 mM MTSET solution. In the four experiments, the MTSET solution was aged 12, 21, 62, and 93 min. Single-exponential fits (solid curves) yielded decay rates of 3.41, 2.00, 0.46, and 0.46 s^{-1}, respectively. (B). The decay rates are plotted as a function of the age of the MTSET. The time constant from this fit was 26 min. This time constant was used in Eq. 1 to correct the loss of MTSET activity due to hydrolysis.

The MTSET reagent (Toronto Research Chemicals Inc.) was dissolved in standard bath solution at concentrations ranging from 0.2 to 1.2 mM just before each experiment and maintained at room temperature in the perfusion system. The elapsed time after preparation of the MTSET solution was noted and the effective concentration was corrected, assuming an exponential decay of active MTSET concentration (Swanson et al., 1990).

To quantify the MTSET decay time course, four successive inside-out patch recordings were made of the modification of F373C channels with the 1.2 mM MTSET solution (Fig. 2 A). In each recording, the rate of modification of the channels was evaluated by fitting the decay of the current (Fig. 2 B). The MTSET activity decreased during the experiment with a time constant of 26 min, or a half-time of 18 min. This is very similar to the half-time of 15 min reported by Stauffer and Karlin (1994). The effective MTSET concentration in a solution of age t was therefore taken to be $[\text{MTSET}]_t = [\text{MTSET}]_0 \exp(-t/26\ \text{min})$. In our results, cumulative exposure to MTSET is expressed in units of mM·s with concentrations corrected according to this equation.

Pulsed application of MTSET was performed using the SF 77A Perfusion Fast-Step device (Warner Instruments) onto which was mounted two perfusion pipettes. The SF 77A was controlled in turn by the data acquisition program Pulse. The application was

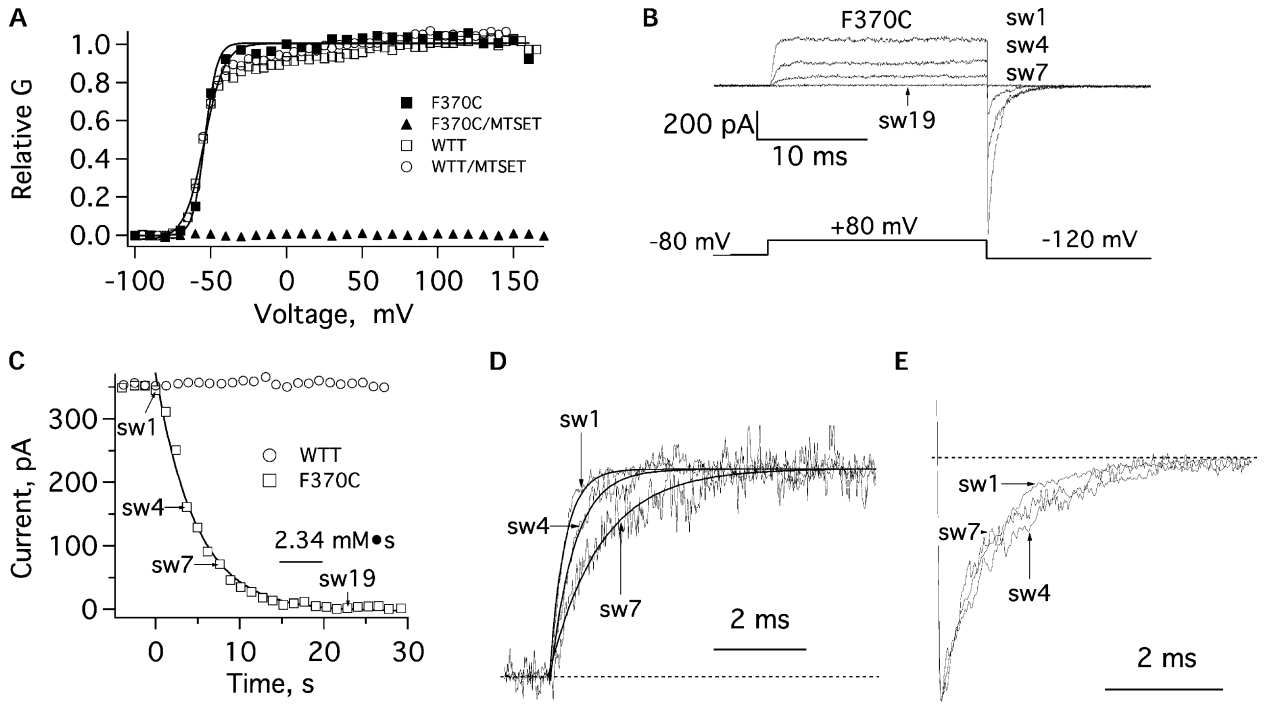

FIGURE 3. MTSET effects on ionic currents of Shaker WTT and F370C mutant channels. (A) Activation curves of WTT and F370C channels, before and after 10.8 mM·s exposure to MTSET. Plotted is the normalized tail current at −100 mV following depolarizations to the indicated voltages. Boltzmann fits yielded $V_{1/2} = -51 \pm 1$ mV and $z = 3.8 \pm 0.4$ for WTT, and $V_{1/2} = -54 \pm 1$ mV and $z = 6.4 \pm 0.8$ for F370C. After MTSET exposure, the WTT currents were essentially unchanged with $V_{1/2} = -55 \pm 0.8$ mV and $z = 3.6 \pm 0.4$. Tail currents in F370C channels were reduced from ~1 nA to a residual <5 pA for all tested depolarizations. (B) F370C currents in an inside-out patch exposed to 0.5 mM MTSET (1 min old). Depolarizations were given to +80 mV from a −80 mV holding potential every 830 ms; currents from sweeps 1, 4, 7, and 19 are shown. (C) The elicited current is plotted as a function of cumulative MTSET exposure. There is no MTSET effect on WTT Shaker channels (circles). (D) Scaled activation time course of the F370C current sweeps shown in B. The rising phase slows down as the MTSET modification progresses. (E) The corresponding scaled tail currents. The deactivation time courses of the three sweeps are indistinguishable. In all experiments, the computed MTSET exposure was corrected for hydrolysis as described in MATERIALS AND METHODS.

timed to occur only at the holding potential, which was −80 mV in all experiments. A few experiments were done with MTSET continuously present in the bath solution, with no apparent change in the time course of modifications.

The isochronal voltage dependence of channel conductance from tail currents and the voltage dependence of the integrated gating charge were fitted with a Boltzmann function of the membrane potential V,

$$f(V) = \frac{f_{\max}}{1 + e^{-(V - V_{1/2})zF/RT}}, \quad (1)$$

where z is the effective valence, F is the Faraday constant, R is the gas constant, and T is absolute temperature. Statistical quantities are expressed as mean ± SD with the number of determinations $n \geq 4$ in all cases.

RESULTS

Mixed Channel Populations During MTSET Modification

We recorded ionic currents from WTT and F370C channels in inside-out patches (Fig. 3). As evaluated from tail current amplitudes, we found the voltage dependence of channel activation to be almost identical in the two channel types. Exposure to MTSET had no effect on the WTT channels, but exposure to 0.5 mM MTSET for 24 s reduced the F370C ionic current to unmeasurable levels, <0.5% of control (Fig. 3 A). During the exposure to MTSET, the F370C current decreased gradually in amplitude (Fig. 3, B and C). The activation time course was slowed considerably, but the deactivation time course remained unchanged during MTSET exposure (Fig. 3, D and E).

The change in activation rate can be explained if we assume that partially modified channels can be opened by depolarization to +80 mV, but they open more slowly and less completely than unmodified channels. The simplest model of this kind assumes that if one of the four subunits is modified, the channel has the slowed activation time course $m(t)$, while if two or more subunits are modified, the channel does not open at all. Letting $u(t)$ be the unmodified activation time course, the time course of activation of a population of channels will be

$$I_{total}(t) = I_0 u(t) + I_1 m(t), \quad (2)$$

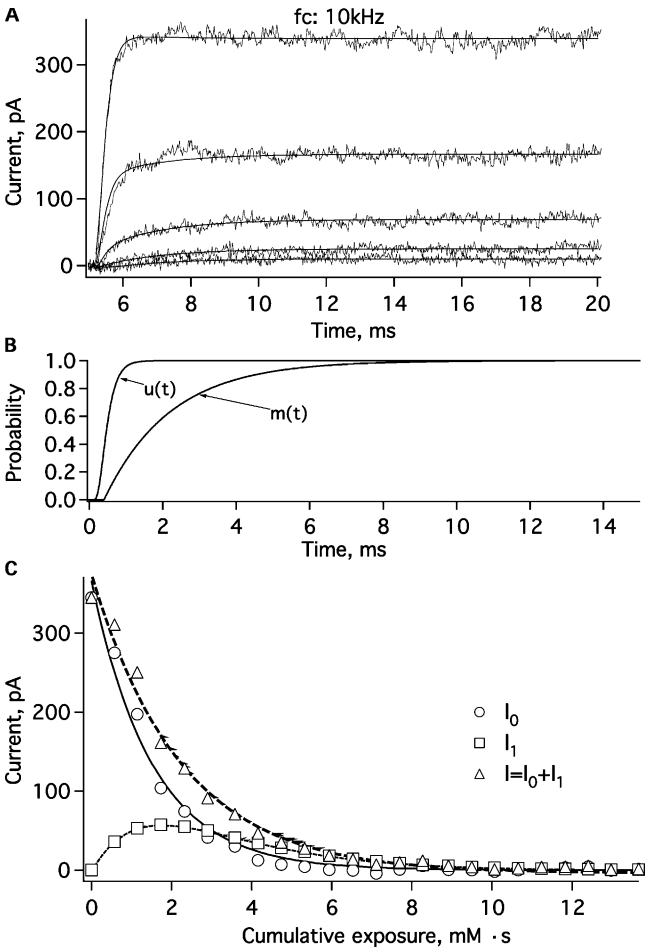

FIGURE 4. Description of the MTSET modification effects at +80 mV. (A) Five representative F370C channel current sweeps recorded from the same inside-out patch as in Fig. 3 B. Fits to the activation time courses (smooth curves) were computed as sums of two exponential components. (B) The two exponential components u(t) and m(t), which are interpreted to be the normalized channel open probabilities of unmodified channels and one-subunit modified channels. (C) The amplitudes I_0 and I_1 of the two components, obtained from fits to sweeps at various times during the MTSET modification. The decays of the first component amplitude (circles) and the second component amplitude (squares) were fitted simultaneously to Eqs. 2, 4, and 5, yielding the subunit modification rate $k = 0.165 \pm 0.007$ mM^{-1}·s^{-1} and the amplitudes $A = 366 \pm 9$ pA and $B = 136 \pm 0.4$ pA. The total current amplitude (triangles) can alternatively be fitted by a single exponential with a decay rate of 0.46 ± 0.02 mM^{-1}·s^{-1} (dashed curve).

where $I_0 = Ap_0$ and $I_1 = Bp_1$, and A and B are constants—the maximum current through unmodified and modified channels, respectively. The probabilities of having zero or one modified subunit are

$$p_0 = (1 - p)^4 \quad (3)$$

$$p_1 = 4p(1 - p)^3, \quad (4)$$

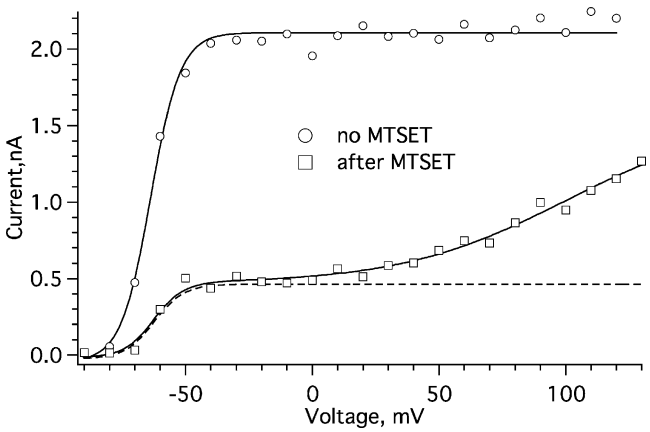

FIGURE 5. Two components of activation in partially modified F370C channels. Before modification, a Boltzmann fit yields $V_{1/2} = -63.8 \pm 0.8$ mV and $z = 4.27 \pm 0.5$. After partial MTSET modification (7.7 mM·s), there is an additional, right-shifted component of activation, with $z = 0.8 \pm 0.2$ and $V_{1/2}$ estimated to be ~+100 mV.

where

$$p = 1 - e^{-kx} \quad (5)$$

is the probability that a given individual subunit is modified by MTSET at the cumulative exposure time x.

Fig. 4 demonstrates the fitting of this model to data obtained from depolarizations to +80 mV (Fig. 4 A). The function $u(t)$ was obtained from the activation time course of the F370C current before exposure to MTSET. The time course $m(t)$ was then obtained as a fit to the small current remaining after substantial modification (Fig. 4 B). With these functions, each of the 24 sweeps recorded in this experiment could be decomposed into the current amplitudes I_0 and I_1, reflecting unmodified and singly modified channels, respectively (Fig. 4 C). A simultaneous fit of Eqs. 2–5 was then performed with the free parameters A, B, and k. The resulting values were $A = 370$ pA, $B = 136$ pA, and $k = 0.165$ mM^{-1}·s^{-1}.

Besides having slow kinetics, the partially modified channels have a strongly shifted voltage dependence of activation. Partial modification yields a new component of current with an activation midpoint of roughly +100 mV (Fig. 5). The weak voltage dependence of this activation (a Boltzmann fit yields the apparent valence z = 0.8) is consistent with the idea that this component reflects the activation of a single modified subunit in an otherwise intact channel.

MTSET Greatly Reduces Gating Currents

Recording the gating currents from MTSET-modified channels turned out to be much more difficult than recording the ionic currents. Due to channel clustering, only a small fraction of inside-out patches contained

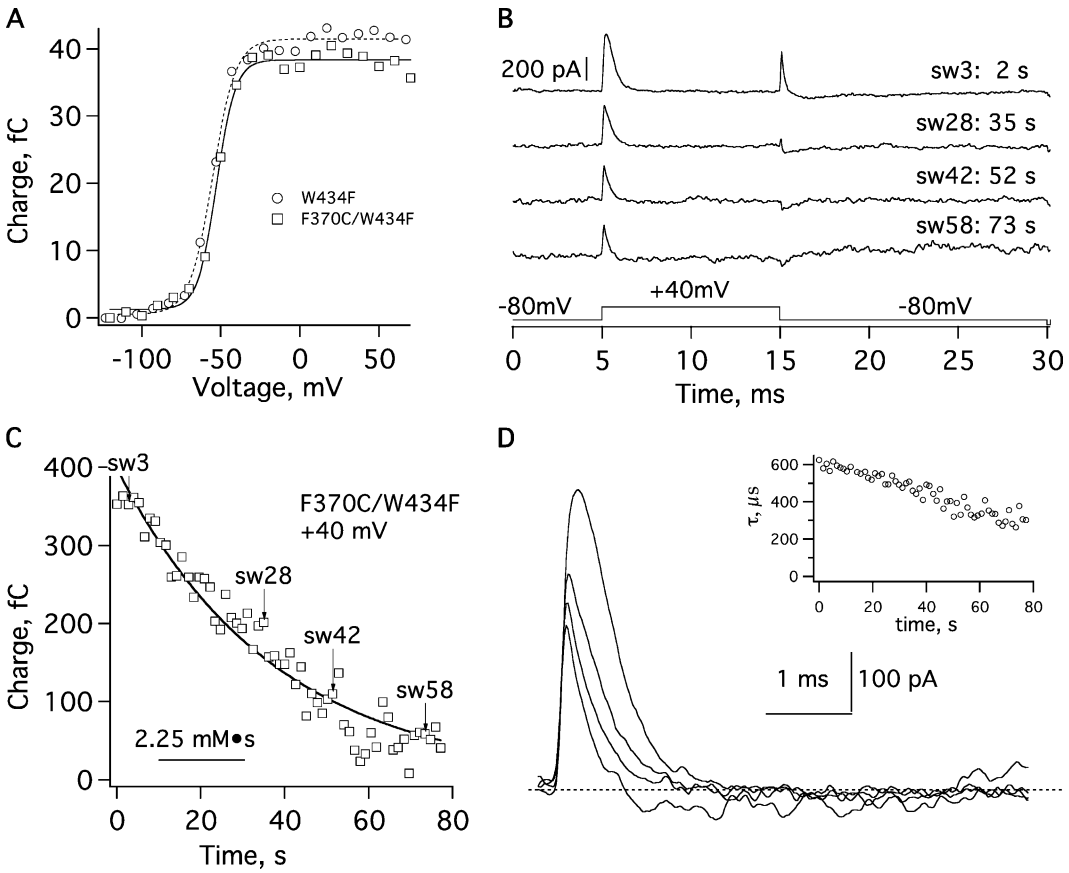

FIGURE 6. F370C channel gating currents and the effect of MTSET. (A) The voltage dependence of charge movement in W434F and F370C/W434F channels is very similar. Boltzmann fits of integrated on currents yielded $V_{1/2} = -55$ mV and $z = 3.4$, and $V_{1/2} = -53$ mV and $z = 4.2$, respectively. The on currents were recorded from depolarizations of 10 ms duration. (B) Four gating current sweeps recorded from an inside-out patch with 0.2 mM, 15-min-old MTSET perfused. The sweep number and exposure duration are given for each trace. Upward off transients result from errors in the P/4 subtraction, and the unstable baseline due to increased leakage current is visible in the bottom traces. (C) Charges were integrated from on gating currents and are plotted against the MTSET exposure. (D) Expanded on current transients from the sweeps in B. The smallest trace (73 s of exposure, or 7.5 mM·s) contains only 20% of the initial charge movement. There is a trend toward faster decays with MTSET exposure, as can be seen in the plot of decay time constants in the inset. In all traces, leakage currents were subtracted using the P/4 protocol with a leak holding potential of −120 mV.

sufficient channels to yield substantial gating currents (tens of picoamperes in amplitude). We found that the recording baseline would invariably become unstable after ~10 mM·s of MTSET exposure. Noisy picoampere-sized currents would appear and increase with time, making the measurement of small gating currents impossible. Inside-out patches from other cell types (HEK-293 and COS cells) also showed an MTSET-induced instability. Thus, our recordings were limited to a maximum of ~10 mM·s of exposure.

For gating current measurements we used the double mutant F370C/W434F. The W434F mutation has little effect on gating charge movement (Perozo et al., 1993) but eliminates ionic currents (Yang et al., 1997). The voltage dependence of charge movement of F370C/W434F channels is essentially the same as that of the background W434F channels (Fig. 6 A). However, as the double mutant channels are exposed to MTSET, the gating currents become smaller (Fig. 6 B). The total charge, as computed from the on gating currents, decreased by 80% during an 80-s exposure to ~0.1 mM MTSET (Fig. 6 C). The time course was truncated by the limited lifetime of the patch, but it could be well fitted by an exponential decay with a modification rate of 0.21 $mM^{-1}·s^{-1}$.

As the gating current is reduced by MTSET, the decay of the on gating current is gradually accelerated (Fig. 6 D). This suggests that the gating charge might not be entirely immobilized by the modification. However, because of the limited lifetime of the patch recordings, we were never able to observe gating currents in an exhaustively modified patch.

Comparing the Decay of Ionic Currents and Gating Charge

If there were four independent voltage sensors, all of which had to be fully activated for the channel to open,

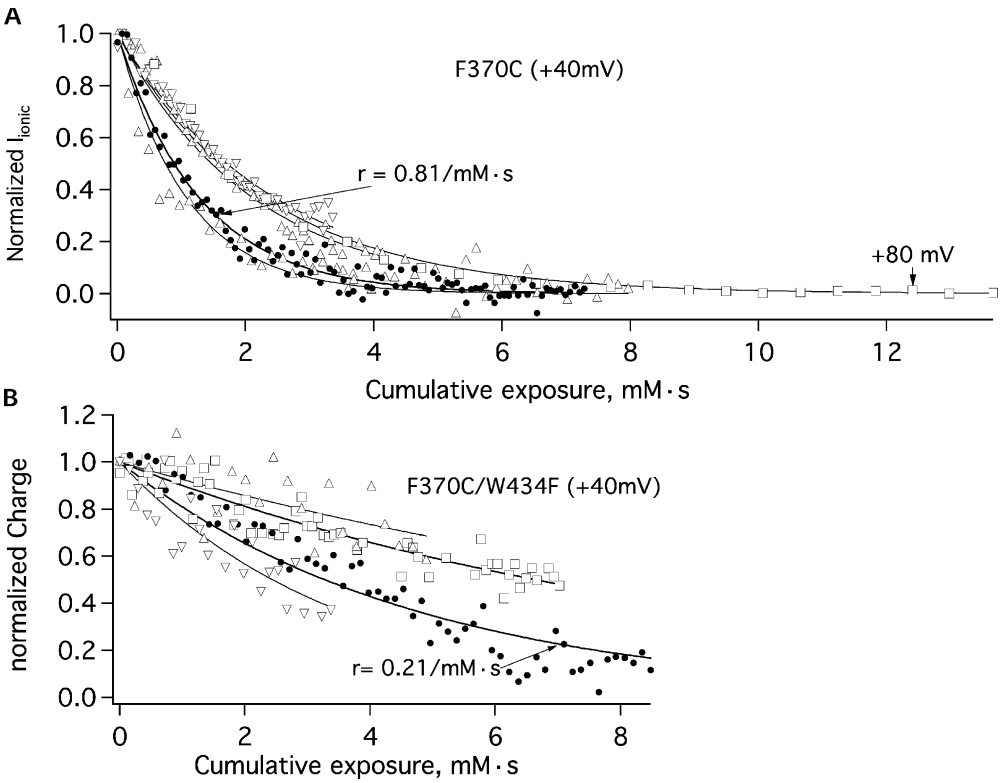

FIGURE 7. Decay of ionic and gating currents with MTSET modification. (A) Time course of decay of F370C ionic currents under exposure to MTSET. Data are shown for four patches using +40 mV test pulses, along with a fifth patch (open squares, same data as in Fig. 4) using test pulses to +80 mV. The average decay rate was 0.63 ± 0.18 $mM^{-1}{\cdot}s^{-1}$, while the rate estimated from the most stable recording (filled circles) yielded a rate of 0.81 ± 0.02 $mM^{-1}{\cdot}s^{-1}$. (B) Decay of on gating charge under exposure to MTSET. Data are shown from four patches using test pulses to +40 mV. The average decay rate was 0.17 ± 0.1 $mM^{-1}{\cdot}s^{-1}$ while the most stable recording (filled circles) had a decay rate of 0.21 $mM^{-1}{\cdot}s^{-1}$.

then the immobilization of one sensor would render the channel nonconducting. Thus the rate of decay of ionic current during MTSET modification would proceed four times as quickly as the rate of decay of gating current. A factor of about four was found between the decay rates of ionic current and gating charge during photochemical cross-linking of Shaker channels by Horn et al. (2000). A ratio of less than four is however to be expected in Shaker channels, where ~20% of the charge movement appears to occur in one or more concerted steps associated with channel opening (Zagotta et al., 1994; Schoppa and Sigworth, 1998b). A comparison of decay rates in our experiments was consistent with factors of three or four.

Fig. 7 compares ionic and gating current decays in F370C channels under exposure to MTSET. For a total of four ionic current recordings made at +40 mV from oocytes expressing F370C channels, the modification rate was 0.63 ± 0.180 $mM^{-1}{\cdot}s^{-1}$. From four other experiments, the rate of decay of gating charge movement was seen to be 0.17 ± 0.1 $mM^{-1}{\cdot}s^{-1}$ (Fig. 7 B). There is much scatter in the decay rates, such that the comparison we can make with some certainty ($P < 0.02$, Fisher's Exact Test) is simply that the gating current decay rate is slower than that of the ionic current. The gating current decay rate is however indistinguishable from the rate $k = 0.165$ $mM^{-1}{\cdot}s^{-1}$ of single subunit modification obtained from the analysis shown in Fig. 4.

The modification rates are slow, which make for considerable difficulties in recording from the inside-out patches. One aspect that we did not investigate would be a possible pH dependence of the modification rate. A higher pH might accelerate the modification, as the deprotonated S^- is the reactive species (Pascual and Karlin, 1998). Also, all of the modification rates were measured at the holding potential of −80 mV. The modification rate is greatly reduced by depolarization (Baker et al., 1998); exactly how much it is reduced would be a good subject for future study, once the problem of patch instability is solved.

DISCUSSION

The potassium current carried by Shaker F370C channels is abolished by treatment with the sulfhydryl reagent MTSET. The effect has the characteristics expected of a modification that traps the channel voltage sensors in the deactivated state: the modification proceeds at negative potentials (Baker et al., 1998) and gating currents are strongly suppressed. During exposure to MTSET, the ionic current decays more quickly than the gating current. This is consistent with the idea that the modification of a single voltage sensor has a large effect in reducing the ionic current but has little effect on the charge movement carried by other voltage sensors.

It appears that Shaker channels require the full charge movement and therefore the activation of all four voltage sensors to enter the main open state (Islas

and Sigworth, 2001). Thus one expects that the modification of one voltage sensor should eliminate the ionic current entirely. This is not entirely the case for F370C channels. Partial MTSET modification produces a slowly activating current component (Fig. 4) whose time course of appearance matches that expected for a population of singly modified channels. This current component shows a greatly shifted voltage dependence of activation. We conclude that the modification of a voltage sensor renders it unable to activate except at very large depolarizations. In the case of a singly modified tetrameric channel, the shift in that voltage sensor's activation produces a strongly shifted voltage dependence of channel opening.

In the standard view of Shaker channel activation (Bezanilla et al., 1994; Zagotta et al., 1994; Schoppa and Sigworth, 1998b), the gating charge movements can be separated into two sequential components. The first component Q_1 comprises the majority of the charge movement and occurs independently in each subunit. The second component Q_2 proceeds after the charge movement Q_1 is complete; its movement is concerted or at least highly cooperative among the subunits and is tightly coupled to channel opening. Our "single-hit" result can be understood in the context of this standard model. Suppose that the effect of a single modification is to shift the voltage dependence of a Q_1 transition, so that at a depolarization to +40 mV, its low probability of activation becomes the determinant of channel opening. Then the subsequent modification of other subunits would further reduce the probability of channel opening, rendering the channels essentially silent at this depolarization.

Suppose instead that the modification were to affect the cooperative Q_2 transitions. Here it would be expected that successive modifications would produce increasingly drastic effects on channel activation. However, the maximum reduction in gating charge would in this case be limited to the Q_2 charge; instead, what is observed is a reduction of >80% in the total charge movement. Further, a shift in the Q_2 transitions would be expected to speed the channel deactivation rate; however, in partially modified channels, the deactivation rate remains unchanged (Fig. 3 E). We conclude that the modification of F370C channels results in a large shift in the equilibrium of the early Q_1 transitions of the voltage sensors, such that even at 0 mV they are trapped either in the deactivated state or in a state near it.

A problem with measuring gating currents is that their amplitude depends on the speed of relaxation of the gating charge. A very slow charge movement results in a current too small to be distinguished from the baseline. It could be argued that the MTSET modification of F370C channels simply slows all but a small component of the charge movements, so that very little is visible as gating currents. We cannot rule out this possibility; however, the very large shift in the open-closed equilibrium with a single-hit modification, while the channel deactivation rate is left unchanged, argues strongly that a shift of charge movement is also occurring with the modification. Further, we were not able to open fully modified channels even with depolarizations to +170 mV. Thus, the effect of the modification cannot be just a slowing of the kinetics, but also a large shift in the equilibrium of at least some charge movements.

Mechanism of Charge Immobilization

The residue F370 is in the center of the S4 helix, a region where accessibility to MTS reagents is very limited (Bezanilla, 2000). Scanning histidine mutagenesis (Starace and Bezanilla, 2001), however, shows that both residues R368 and R371 are accessible to protonation from the intracellular or extracellular solutions, depending on the membrane potential. These authors conclude that upon depolarization, these residues move from an intracellularly disposed, aqueous cavity into a very narrow crevice, presumably formed by other parts of the channel protein. Our results are consistent with this view. The additional positive charge introduced by the ethyltrimethylammonium moiety on modification of this residue likely causes the entry of this residue into the crevice to be highly unfavorable. In addition, the extra positive charge inserted between R368 and R371 could greatly increase the energy of transition states when the charge passes through the dielectric barrier between intracellular and extracellular solutions.

Proposals for Locking Shaker Channels in the Deactivated State

It is very difficult to trap the Shaker voltage sensor in its deactivated state; the fact that no mutations have been demonstrated to do this implies that the voltage sensor is a remarkably mobile structure. For structural studies on solubilized channel proteins it would be sufficient to modify the channel so that the voltage dependence of voltage sensor activation is shifted to positive potentials. Unfortunately, the best-studied mutations that produce large shifts in channel activation have only minor effects on the main voltage sensor movements. These mutations, the V2 mutation of Schoppa and Sigworth (1998a) and the ILT mutation of Ledwell and Aldrich (1999), produce their effects by interfering with the final concerted step of channel opening, rather than constraining the movements of the voltage sensors. A characteristic of these mutations is that the positive shift in the activation curve is accompanied by a large decrease in its steepness. There are however some other candidates for a shift in the voltage sensor equi-

librium. Scanning mutagenesis studies have identified mutations that produce positive shifts in activation gating while steepness is maintained, as would be expected from a simple shift in the voltage dependence of voltage sensor movement. These mutations in Shaker channels include I241W in the S1 segment (Hong and Miller, 2000), T284W in S2 (Monks et al., 1999), and S412W in S5 (Li-Smerin et al., 2000a); in Kv2.1, the mutation F236A also has this characteristic (Li-Smerin et al., 2000b). However, gating current measurements have not been reported for these channels, so that the hypothesis of a shift in voltage sensor movements remains untested.

The modification considered here of F370C channels with MTSET appears at present to be the most promising way to keep Shaker channels in the deactivated state. We find that gating currents are abolished along with the ionic currents during modification; this is highly suggestive but does not prove that the voltage sensors are trapped near their resting position. Nevertheless, were one to exploit this effect for structural studies, conditions would have to be found for the MTSET modification of the channel protein either as it is solubilized in detergent or reconstituted into membranes with nominally zero membrane potential. Because the modification proceeds only when the voltage sensor is in the resting state, the modification will occur only very slowly. Indeed, in a noninactivating Shaker channel at 0 mV, the probability of finding a voltage sensor in the resting state is expected to be $\sim 10^{-4}$, as estimated from the model of Schoppa and Sigworth (1998b). However, this probability can be increased by disturbing the equilibrium of the final concerted step toward channel opening. With the V2 (L382V) mutation for example, the final transition is less biased toward channel opening and the voltage sensors will behave more independently (Schoppa and Sigworth, 1998b). Thus we predict that a good construct would be a combination of one of these mutations with F370C, yielding voltage sensors that would have a substantial probability of being in the deactivated state at zero membrane potential. In the case of the V2 mutant, this probability is predicted to be $\sim$7%, and exposure to $\sim$1 M·s of MTSET would be sufficient to modify the 370C residues at 0 mV.

We thank Drs. Qiu-Xing Jiang and David Chester for discussions during the study and Dr. Kathryn Klemic for reading an early version of the manuscript.

This work was supported by grant NS21501 from the National Institutes of Health.

Olaf S. Andersen served as editor.

Submitted: 10 March 2004
Accepted: 24 June 2004

REFERENCES

Baker, O.S., H.P. Larsson, L.M. Mannuzzu, and E.Y. Isacoff. 1998. Three transmembrane conformations and sequence-dependent displacement of the S4 domain in Shaker K^+ channel gating. *Neuron.* 20:1283–1294.

Bezanilla, F. 2000. The voltage sensor in voltage-dependent ion channels. *Physiol. Rev.* 80:555–592.

Bezanilla, F., E. Perozo, and E. Stefani. 1994. Gating of Shaker K^+ channels. II. The components of gating currents and a model of channel activation. *Biophys. J.* 66:1011–1021.

Gandhi, C.S., and E.Y. Isacoff. 2002. Molecular models of voltage sensing. *J. Gen. Physiol.* 120:455–463.

Hong, K.H., and C. Miller. 2000. The lipid-protein interface of a Shaker K^+ channel. *J. Gen. Physiol.* 115:51–58.

Horn, R., S. Ding, and H.J. Gruber. 2000. Immobilizing the moving parts of voltage-gated ion channels. *J. Gen. Physiol.* 116:461–476.

Islas, L.D., and F.J. Sigworth. 2001. Electrostatics and the gating pore of Shaker potassium channels. *J. Gen. Physiol.* 117:69–89.

Jiang, Y., V. Ruta, J. Chen, A. Lee, and R. MacKinnon. 2003. The principle of gating charge movement in a voltage-dependent K^+ channel. *Nature.* 423:42–48.

Laine, M., M.C. Lin, J.P. Bannister, W.R. Silverman, A.F. Mock, B. Roux, and D.M. Papazian. 2003. Atomic proximity between S4 segment and pore domain in Shaker potassium channels. *Neuron.* 39:467–481.

Ledwell, J.L., and R.W. Aldrich. 1999. Mutations in the S4 region isolate the final voltage-dependent cooperative step in potassium channel activation. *J. Gen. Physiol.* 113:389–414.

Li-Smerin, Y., D.H. Hackos, and K.J. Swartz. 2000a. A localized interaction surface for voltage-sensing domains on the pore domain of a K^+ channel. *Neuron.* 25:411–423.

Li-Smerin, Y., D.H. Hackos, and K.J. Swartz. 2000b. α-Helical structural elements within the voltage-sensing domains of a K^+ channel. *J. Gen. Physiol.* 115:33–49.

Monks, S.A., D.J. Needleman, and C. Miller. 1999. Helical structure and packing orientation of the S2 segment in the Shaker K^+ channel. *J. Gen. Physiol.* 113:415–423.

Papazian, D.M. 1999. Potassium channels: some assembly required. *Neuron.* 23:7–10.

Pascual, J.M., and A. Karlin. 1998. State-dependent accessibility and electrostatic potential in the channel of the acetylcholine receptor. *J. Gen. Physiol.* 111:717–739.

Perozo, E., R. MacKinnon, F. Bezanilla, and E. Stefani. 1993. Gating currents from a nonconducting mutant reveal open-closed conformations in Shaker K^+ channels. *Neuron.* 11:353–358.

Schoppa, N.E., and F.J. Sigworth. 1998a. Activation of Shaker potassium channels. II. Kinetics of the V2 mutant channel. *J. Gen. Physiol.* 111:295–311.

Schoppa, N.E., and F.J. Sigworth. 1998b. Activation of Shaker potassium channels. III. An activation gating model for wild-type and V2 mutant channels. *J. Gen. Physiol.* 111:313–342.

Schulteis, C.T., N. Nagaya, and D.M. Papazian. 1998. Subunit folding and assembly steps are interspersed during Shaker potassium channel biogenesis. *J. Biol. Chem.* 273:26210–26217.

Schwarz, T.L., B.L. Tempel, D.M. Papazian, Y.N. Jan, and L.Y. Jan. 1988. Multiple potassium-channel components are produced by alternative splicing at the Shaker locus in *Drosophila*. *Nature.* 331: 137–142.

Starace, D.M., and F. Bezanilla. 2001. Histidine scanning mutagenesis of basic residues of the S4 segment of the Shaker K^+ channel. *J. Gen. Physiol.* 117:469–490.

Starace, D.M., E. Stefani, and F. Bezanilla. 1997. Voltage-dependent proton transport by the voltage sensor of the Shaker K^+ channel. *Neuron.* 19:1319–1327.

Stauffer, D.A., and A. Karlin. 1994. Electrostatic potential of the acetylcholine binding sites in the nicotinic receptor probed by reactions of binding-site cysteines with charged methanethiosulfonates. *Biochemistry.* 33:6840–6849.

Swanson, R., J. Marshall, J.S. Smith, J.B. Williams, M.B. Boyle, K. Folander, C.J. Luneau, J. Antanavage, C. Oliva, S.A. Buhrow, et al. 1990. Cloning and expression of cDNA and genomic clones encoding three delayed rectifier potassium channels in rat brain. *Neuron.* 4:929–939.

Yang, Y., Y. Yan, and F.J. Sigworth. 1997. How does the W434F mutation block current in Shaker potassium channels? *J. Gen. Physiol.* 109:779–789.

Zagotta, W.N., T. Hoshi, and R.W. Aldrich. 1994. Shaker potassium channel gating. III. Evaluation of kinetic models for activation. *J. Gen. Physiol.* 103:321–362.