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Evidence for Voltage-Dependent S4 Movement in Sodium Channels

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Summary

The mutation R1448C substitutes a cysteine for the outermost arginine in the fourth transmembrane segment (S4) of domain 4 in skeletal muscle sodium channels. We tested the accessibility of this cysteine residue to hydrophilic methanethiosulfonate reagents applied to the extracellular surface of cells expressing these mutant channels. The reagents irreversibly increase the rate of inactivation of R1448C, but not wild-type, channels. Cysteine modification is voltage dependent, as if depolarization extends this residue into the extracellular space. The rate of cysteine modification increases with depolarization and has the voltage dependence and kinetics expected for the movement of a voltage sensor controlling channel gating.

Introduction

Sodium, potassium, and calcium channels belong to a superfamily of proteins that are exquisitely sensitive to membrane potential. The probability of a channel opening may increase one order of magnitude for a depolarization of <5 mV (Zagotta et al., 1994). To account for this voltage dependence, the conformational changes known as gating, i.e., the opening and closing of channels, must be accompanied by the translocation of an equivalent of at least 12 buried charges across the membrane (Schoppa et al., 1992; Sigworth, 1993; Zagotta et al., 1994). The postulated structure of the sodium channel consists of a tandem arrangement of four domains, each with six membrane-spanning segments (S1–S6; Noda et al., 1984; Catterall, 1992). Only one type of transmembrane segment, S4, is highly charged, containing from four to eight basic residues in different domains. The paucity of charges elsewhere makes S4 a natural candidate for the gating voltage sensor. Depolarization is expected to cause an outward movement of the positively charged S4 segments, leading to the opening of the channels (Durell and Guy, 1992; Sigworth, 1993).

Evidence for the role of S4 as a voltage sensor is suggestive, but not conclusive. Neutralizing mutations of the basic residues of S4 can reduce the voltage dependence of activation, the process responsible for channel opening after a depolarization, and shift the voltage range over which activation occurs (Stühmer et al., 1989; Liman et al., 1991; Papazian et al., 1991; Logothetis et al., 1992, 1993). However, mutations of hydrophobic residues in S4 and elsewhere can also cause these effects (Auld et al., 1990; Lopez et al., 1991; McCormack et al., 1991; Schoppa et al., 1992; Perozo et al., 1994). The data suggest that S4 segments are intimately involved with the conformational changes that lead to channel gating, but do not show that S4 segments move in response to changes of membrane potential, as expected for a voltage sensor. We present evidence here consistent with an outward movement of an S4 segment in response to depolarization of the adult human skeletal muscle sodium channel (hSkM1).

Results and Discussion

A naturally occurring pathogenic mutation in the α subunit of hSkM1 substitutes a cysteine for the outermost arginine in the S4 segment of domain 4 (D4; Ptáček et al., 1992). Although this mutation, R1448C, has only small effects on activation, it causes a dramatic increase of the inactivation time constant (τ_h) and a reduced voltage dependence of inactivation (Chahine et al., 1994; Yang et al., 1994), as shown in Figure 1 and Figure 2. To test the importance of the charge of this residue on the rate of inactivation, we treated the mutant R1448C with two hydrophilic methanethiosulfonate (MTS) reagents (Akabas et al., 1992; Stauffer and Karlin, 1994), which are capable of adding either positively or negatively charged groups to cysteine residues by disulfide linkage. Figure 1C shows that extracellular treatment by 20 μM MTS-ethyltrimethylammonium (MTSET), a positively charged reagent, dramatically increases the inactivation rate of R1448C. Neither MTS reagent had an effect on wild-type sodium currents in six cells. Although this treatment decreases τ_h of R1448C, it does not significantly increase the voltage dependence of τ_h, as indicated by the slopes of the curves plotted in Figure 2A. MTSET also has no effect on the steepness of voltage dependence of steady-state inactivation or recovery from inactivation (Figures 2B and 2C). However, it slows the rate of recovery from inactivation (Figure 2C). Therefore, the main effect of MTSET on the currents of R1448C is on the kinetics, rather than the voltage dependence, of inactivation.

The alteration of inactivation kinetics by MTSET is not due to the restoration of a positive charge at residue 1448, since the negative reagent, MTS-ethylsulfonate (MTSES), produces an effect on kinetics similar to that observed for MTSET (Figure 1D). Although the mechanism by which these reagents alter inactivation is unknown, the dramatic effect on inactivation kinetics, especially of MTSET, allows us to measure the time course of the modification of this cysteine residue.

The accessibility of substituted cysteines to MTS reagents may depend on the conformational state of the protein, as observed for acetylcholine receptors (Akabas et al., 1992, 1994). If depolarization causes an outward movement of S4 segments, then strong hyperpolarization may keep the outermost residues buried in the membrane and inaccessible to reagents in the extracellular aqueous solution (Slatin et al., 1994). As expected for this prediction, a holding potential of −140 mV prevents the reaction by MTSET with the R1448C mutant. Figure 3A shows that,

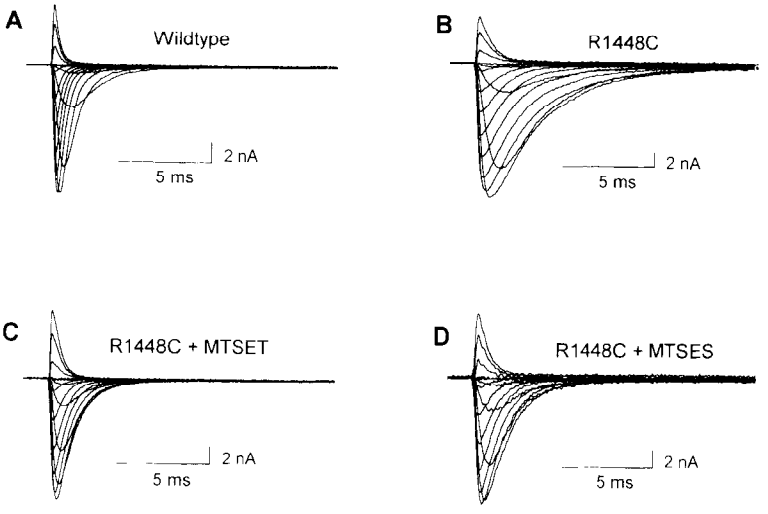


Figure 1. Whole-Cell Sodium Currents Activated by Depolarizations in 10 mV Increments from −60 to +80 mV

(A) Wild-type hSkM1; (B) R1448C; (C) R1448C after 10 min treatment with 20 μM MTSET; (D) R1448C after 10 min treatment with 200 μM MTSES. Holding potential was −120 mV. The reagent-treated cells were depolarized repetitively to 0 mV to accelerate the reaction of the reagents with C1448. The displayed currents were recorded after full effect of the reagents.

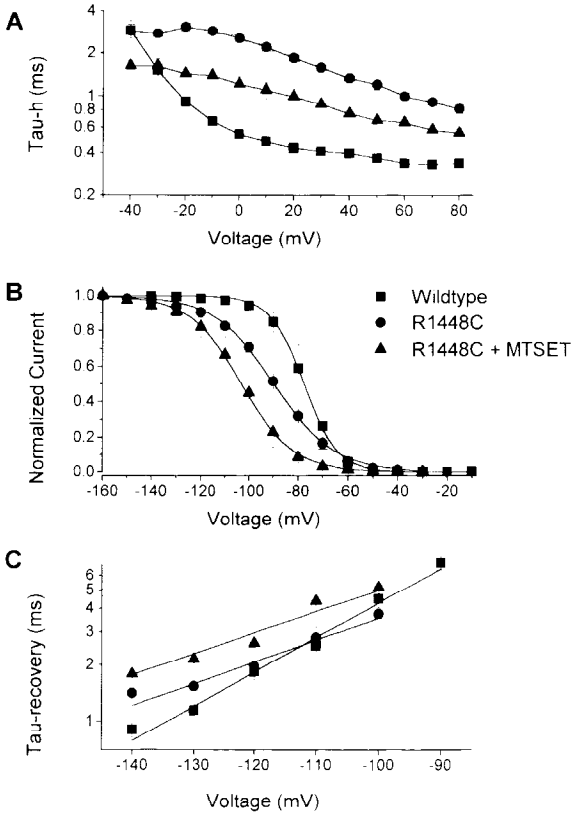


Figure 2. Effect of MTSET on Inactivation

Data from MTSET-treated cells were obtained after maximum modification.

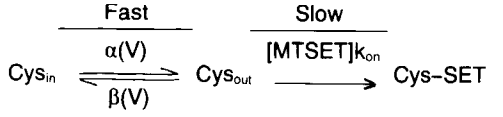
(A) Inactivation time constant (τ_h) from fit of current decay during depolarizations to the indicated voltage. τ_h values for wild-type and R1448C were obtained from single exponential fits. τ_h for MTSET-treated cells is the fast time constant for a double exponential relaxation. The weight of this fast component accounted for >90% of the inactivation time course.

(B) Inactivation induced by a 500 ms conditioning pulse to the indicated voltage. Normalized peak inward current was measured for a test pulse to −10 mV. Boltzmann curves fit to the data have midpoints and slopes of −77.5 ± 3.8 mV and e-fold/6.1 ± 0.2 mV (wild type); −96.1 ± 2.8 mV and e-fold/13.5 ± 0.4 mV (R1448C); −104.9 ± 2.1 mV and e-fold/13.5 ± 0.8 mV (R1448C + MTSET), respectively.

(C) Time constant for recovery from inactivation at the indicated voltage, caused by a 15 ms prepulse to 0 mV. The recovery time course was fit by a single exponential relaxation (Chahine et al., 1994). The regression lines have slopes of e-fold/21.7 ± 0.4 mV (wild type), e-fold/36.2 ± 2.1 mV (R1448C), and e-fold/34.2 ± 0.7 mV (R1448C + MTSET).

when the holding potential is −140 mV, τ_h for a 20 ms voltage step to −10 mV is unchanged by 20 μM MTSET (applied at time 0) for up to 5 min. At the times indicated by arrows, the holding potential was alternated between 0 and −140 mV in 10 s intervals. In the presence of MTSET, this voltage protocol causes a rapid and irreversible increase in the rate of inactivation, until the channels are fully modified. We quantitate this reaction as follows. Before MTSET application (at time 0 in Figure 3A), a single slow τ_h is obtained. After MTSET action is complete, a faster τ_h is measured. During the MTSET reaction, the inactivation at −10 mV is fit as a weighted sum of these fast and slow time constants, and the proportion of the slow component is plotted as an estimate of the proportion of unmodified channels (Figure 3A).

The time course of the MTSET reaction is voltage dependent, as shown in Figure 3B. Using the same alternating protocol of Figure 3A between −140 mV and the indicated voltage, the time course of MTSET action is exponential, with a time constant decreasing from unmeasurably slow at −140 mV to 66.1 s at 0 mV. The voltage dependence of MTSET action may be understood by a simple model. Suppose that the voltage-dependent exposure of C1448 is a first order conformational transition that precedes MTSET modification. The cysteine residue can be modified by MTSET only when it is out.



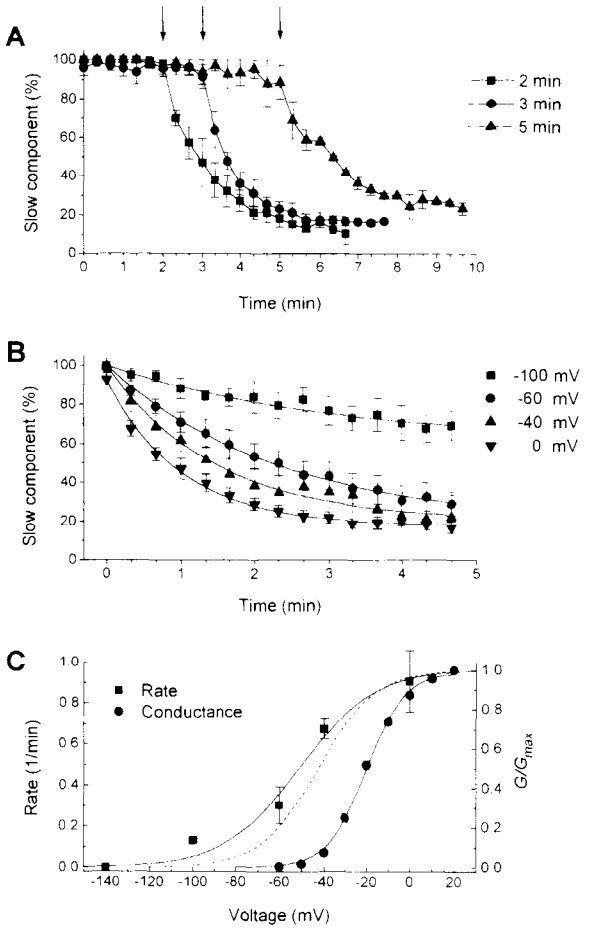


Figure 3. Effect of Voltage on Modification by 20 μM MTSET

(A) Time course of MTSET modification at 0 mV. Holding potential was −140 mV; MTSET applied at time 0. A 20 ms test pulse to −10 mV every 20 s was used to measure the inactivation rate. At times indicated by the arrows, in three sets of experiments, the voltage was alternated between 0 and −140 mV every 10 s, followed by the test pulse to measure inactivation rate. The slower modification rate after 5 min may be due to the short half-life of MTSET in the bathing solution (~ 17 min; Arthur Karlin, personal communication).

(B) The percentage of slow component from protocols in which the voltage was alternated between the indicated value and −140 mV in 10 s intervals. These data are fit with single exponential relaxations.

(C) The inverse of the time constants from (B) are plotted against voltage. This panel also displays a Boltzmann fit to the data (solid line) with parameters in the text. The maximum rate of cysteine modification for the fit is 0.97 ± 0.12 min^{-1}. The normalized conductance–voltage (G–V) relationship obtained from the peak current–voltage relationship is also displayed, with a Boltzmann fit to the data. The fourth root of the G–V curve is plotted as a dashed line.

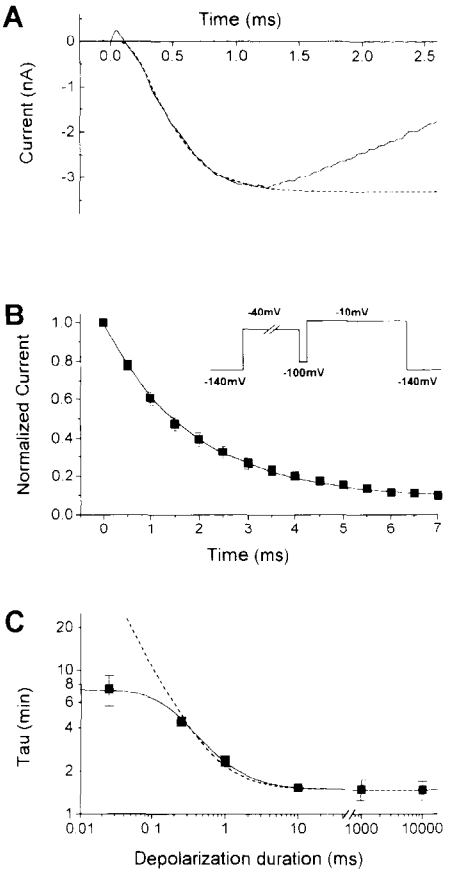


Figure 4. Kinetics of MTSET Effect on R1448C Currents at −40 mV

(A) Fit of activation time course (dashed line) at −40 mV by the m^4 model (Hodgkin and Huxley, 1952), prior to MTSET modification. Holding potential, −140 mV; membrane capacitance, 20 pF; series resistance, 100 kΩ (after compensation); 10 kHz filter. The best fit τ_m in this cell was 255 μs.

(B) Time course of inactivation of R1448C induced by a variable duration prepulse to −40 mV, prior to MTSET modification. The normalized amplitude of the peak current for a test pulse to −10 mV is plotted against the prepulse duration (inset). A 250 μs pulse to −100 mV, between the prepulse and the test pulse, is used to reset the activation gates (Gillespie and Meves, 1980; Goldman and Kenyon, 1982). The theory curve is a single exponential relaxation with a time constant of 1.83 ms.

(C) The time constant of modification by 20 μM MTSET (see Figure 3B) plotted against the durations of individual depolarizations to −40 mV. The dashed line is the best fit by a model which assumes that the time constant for cysteine burial at −140 mV is much smaller than the pulse durations used. The single free parameter for this fit is the time constant for exposure of the cysteine residue at −40 mV, which is 328 ± 72 μs. The solid curve is a two-parameter fit estimating the time constants for exposure of the cysteine residue at −40 mV and burial at −140 mV. The best fit time constants are 450 ± 18 μs (−40 mV) and 50.8 ± 4.3 μs (−140 mV).

The rate of C1448 modification in this scheme is proportional to P_{out}, the voltage-dependent probability that this residue is extended into the bath. Therefore, the observed rate of MTSET modification should be voltage dependent, increasing with depolarization. We explicitly assume that the reaction between an extended cysteine and MTSET is not itself voltage dependent (i.e., the second order rate constant k_{on} is not voltage dependent). We also assume that the local concentration of MTSET is not voltage dependent (i.e., the extended cysteine is located outside of the membrane electric field). Therefore, all of the voltage dependence we observe is assumed to be a consequence of C1448 exposure. If the kinetics of cysteine exposure are much faster than those of modification by MTSET, the

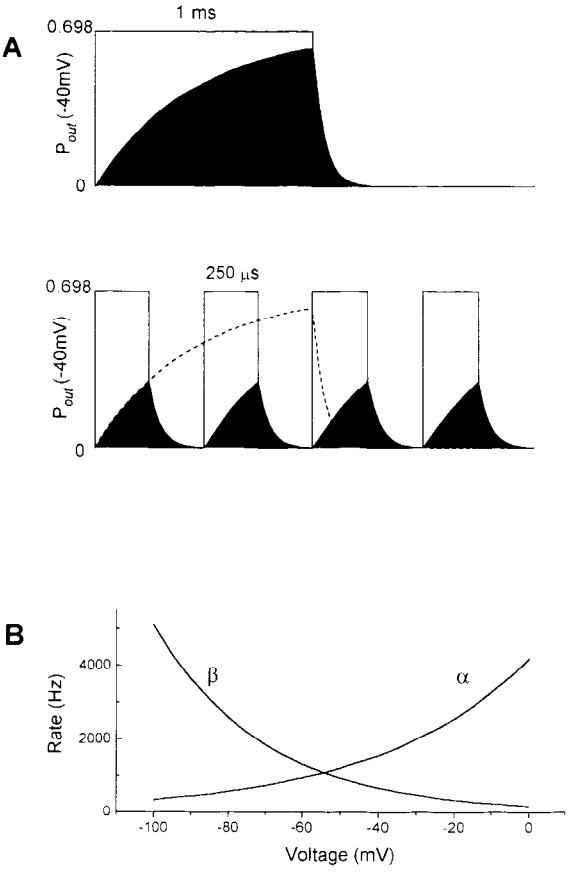


Figure 5. Kinetics of Cysteine Movement for a First-Order Model

(A) Simulation of P_{out} during pulse trains to −40 mV with durations of 1 ms (top) and 0.25 ms (bottom). The integral of P_{out} is shaded. The integral for four 0.25 ms pulses is 0.51 times the integral in a single 1.0 ms pulse, owing to the slow kinetics of cysteine exposure.

(B) Estimates of the outward (α) and inward (β) rate constants for C1448 movement.

time course of the modification will be exponential, with a time constant of $([MTSET]k_{on}P_{out})^{-1}$. This formulation predicts that the rate of cysteine modification, the inverse of this time constant (e.g., Figure 3B), will be proportional to MTSET concentration. In one set of experiments, we found that this rate was approximately twice as fast at 40 μM as at 20 μM MTSET for voltage pulses to −40 mV (data not shown).

The rate of cysteine modification is plotted against membrane potential in Figure 3C and has a sigmoidal voltage dependence. The voltage-dependent rate of MTSET action (Figure 3C) is a scaled representation of the steady-state value of P_{out}, in accordance with the above model. A Boltzmann curve fit to these data has the voltage dependence expected for outward movement of a gate, with a positive charge of 1.47 e_0 (Figure 3C), having a midpoint of −51.0 ± 6.6 mV and a slope of e-fold/17.0 ± 6.8 mV. The maximum estimated rate for this fit is 0.97 min^{-1}. These data allow us to estimate k_{on}, which, after consideration of the 50% duty cycle of the depolarizations, is 1.62 × 10^3 $M^{-1}s^{-1}$. This rate constant is at least four orders of magnitude less than the expected value for a diffusion-limited reaction (Miller, 1990), consistent with the reaction rate of MTSET with small thiols in solution (Stauffer and Karlin, 1994), and suggests that the vast majority of encounters between an extended C1448 and a molecule of MTSET fail to produce a successful modification.

The steady-state voltage dependence of sodium channel activation can be estimated by the peak conductance–voltage (G–V) relationship. By comparison with the voltage dependence of cysteine modification, the G–V curve for R1448C has a midpoint of −20 mV and a slope of e-fold/8.4 mV (Figure 3C). The lower voltage dependence and hyperpolarized midpoint for cysteine modification would be expected if the S4/D4 segment were one of the voltage sensors that contributes to activation (Armstrong, 1981; Keynes et al., 1982; Bezanilla, 1985). This can be seen by the dashed line in Figure 3C, which plots the fourth root of the Boltzmann curve fit to the G–V relationship and approximates the voltage dependence of the rate of cysteine modification. This curve represents the equilibrium voltage dependence for the movement of a voltage sensor for a Hodgkin-Huxley gating model with four equivalent activation gates (Hodgkin and Huxley, 1952), one for each of the four S4 segments in sodium channels. This is also the voltage dependence expected for gating charge movement, the so-called Q-V curve, in such a model (Armstrong, 1981; Keynes et al., 1982; Bezanilla, 1985).

The data in Figure 3 show that the probability of cysteine accessibility to MTSET has the approximate voltage dependence expected for a voltage sensor for activation. Figure 4 shows that this accessibility also has appropriate kinetics for a voltage sensor. Using the above Hodgkin-Huxley model, we made a rough estimate of the rate of charge movement at −40 mV by fitting the activation kinetics of R1448C currents to an exponential relaxation raised to the fourth power (Figure 4A). The resultant activation time constant τ_m, using the Hodgkin-Huxley formulation, is 285 ± 15 μs (n = 7 cells) and represents the time constant at −40 mV for gating charge movement for each of the four equivalent voltage sensors in this gating model. We also estimated the inactivation rate using a prepulse of variable duration (Figure 4B). The inactivation time constant of R1448C at −40 mV is 1.83 ± 0.16 ms (n = 6).

Do either of these time constants at −40 mV correspond to that of the appearance of C1448 at the extracellular surface? To test this possibility, we measured the rate of channel modification by MTSET during pulse trains of depolarizations between −140 and −40 mV, while varying the rate of switching between these voltages. If the durations of the individual depolarizations are short, compared with the time required for cysteine exposure, the accessibility to MTSET, and thus its observed reaction rate, should be decreased. The reaction rate of 20 μM MTSET with C1448 was quantified from the change in the time course of inactivation during test depolarizations to −10 mV every 20 s, as in Figure 3B. As for the experiments in Figure 3, we used a 50% duty cycle for all repetition rates, meaning that the depolarizations to −40 mV had the same durations as the repolarizations at −140 mV (see Figure 5). The time constant for cysteine modification at −40 mV was invariant for pulse durations spanning three orders of magnitude,

10 s to 10 ms, but increased for shorter durations (Figure 4C). Estimates of the time constants for C1448 movement may be obtained from these data (see Experimental Procedures and Figure 5). If we assume that the time constant of cysteine burial at −140 mV is much smaller than the pulse durations, we get a poor fit to these data (Figure 4C, dashed line). However, if we estimate the time constants for cysteine accessibility at both −40 mV and −140 mV, the fit is excellent (Figure 4C, solid line). The best fit time constants are 450 ± 18 μs at −40 mV and 50.8 ± 4.3 μs at −140 mV. The time constant at −40 mV is 1.6-fold slower than our estimate for the activation time constant τ_m and 4.1-fold faster than our estimate for the inactivation time constant. Therefore, the rate of cysteine exposure at −40 mV has kinetics within a range expected for a gating voltage sensor.

Figure 5A plots the expected kinetics of P_{out} for pulse durations of 1 ms and 250 μs, using the above estimates of time constants for exposure and burial of C1448. The rate of cysteine modification is proportional to the integral of P_{out} (shaded area), which is reduced by 49% for the shorter duration pulses. This corresponds with the 47% reduction in measured rate of modification by MTSET for 250 μs versus 1.0 ms pulses (Figure 4C). Note that when the pulse durations are much greater than the time constant for C1448 exposure at −40 mV, the integral of P_{out} is expected to be independent of pulse duration, consistent with our results. For the above kinetic scheme, the time constant of cysteine exposure is equal to $(\alpha + \beta)^{-1}$. If α and β each have an exponential voltage dependence, their values (Figure 5B) can be determined from the time constants of cysteine exposure and the parameters for the Boltzmann fit in Figure 3C. β changes e-fold/29.6 mV and is 1.35-fold more voltage dependent than α.

A surprisingly simple result emerges from our experiments. The exposure of C1448 to MTSET can be described as a first order, voltage-dependent reaction consistent with a role for S4/D4 as a gating voltage sensor, for either activation, inactivation, or both processes. A previous study of the mutant R1448C also supports a dual role for S4/D4 (Chahine et al., 1994). The apparent exposure of C1448 during depolarization has one of three interpretations: S4 moves outward; there is a conformational change that exposes this residue when the channel is depolarized; or the ionization of the sulfhydryl of C1448 is altered by transmembrane potential, which strongly affects the reactivity with MTS reagents. Either interpretation requires that voltage causes a conformational transition of the protein in the immediate vicinity of S4/D4. This transition accompanies the opening of the channel in response to a depolarization.

Experimental Procedures

Electrophysiology and Data Acquisition

Transient calcium phosphate transfection of tsA201 cells was employed (Margolskee et al., 1993) using plasmids encoding hSkM1 and its mutant R1448C (Chahine et al., 1994). Approximately 50% of the cells expressed large sodium currents (>500 pA at −10 mV) in typical experiments 1–3 days after transfection.

Standard whole-cell recording methods were used. Supercharging compensated for 95% of the membrane capacitance using the Axopatch 200A recording amplifier (Axon Instruments, Burlingame, CA). This reduced the expected charging time constant for the cells to <4.5 μs. Series resistance errors were <3 mV. Data were filtered at 5–10 kHz and acquired using pCLAMP (Axon Instruments). Patch electrodes contained 105 mM CsF, 35 mM NaCl, 10 mM EGTA, and 10 mM Cs–HEPES (pH 7.4). The bath contained 150 mM NaCl, 2 mM KCl, 1.5 mM $CaCl_2$, 1 mM $MgCl_2$, and 10 mM Na–HEPES (pH 7.4). Temperature was controlled with Peltier units using a TC-10 controller (Dagan Corp., Minneapolis, MN). All experiments were performed at 18°C.

Whole-cell data were analyzed and displayed by a combination of pCLAMP programs, ORIGIN (MicroCal, Northampton, MA), and our own FORTRAN programs. Data from at least three cells for each measurement are presented as mean ± SEM.

MTSET and MTSES were generous gifts from Rolf Joho and Arthur Karlin. Aqueous stocks (1 mM for MTSET; 10 mM for MTSES) of these reagents were kept at 4°C and diluted in the bath solution immediately before use. The reagent solutions were loaded into a large-bore quartz macropipet, the opening of which was placed in apposition to the cell voltage-clamped by a patch pipet. The cells were maintained at a holding potential of −140 mV and tested every 20 s by a 20 ms depolarization to −10 mV. For both reagents, depolarizations (e.g., Figure 3) were required to cause cysteine modification. After the modification was complete, as assessed by the inactivation time course, other voltage protocols were used to characterize the currents.

The duty cycle for repetitive depolarizations in Figure 4 was 50% for all repetition rates; i.e., the cells spent half the time each at −40 and −140 mV. Every 20 s, the depolarizing train was interrupted long enough to apply a 20 ms test pulse to −10 mV, to measure the inactivation time course.

Theory

To fit the relationship between the time constant of cysteine modification and individual pulse durations in a train (Figure 4C), we calculated the integral of P_{out} (see Results and Figure 5A) during a pulse train. We assumed a steady-state P_{out} of 0.698 at −40 mV, a value obtained from the normalized Boltzmann curve in Figure 3C. The two free parameters for the least squares fit are the time constants of cysteine movement at −40 and −140 mV. The data cannot be fit well if the inward rate of cysteine movement at −140 mV is assumed to be infinitely rapid (see Figure 4C, dashed line).

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References

Akabas, M. H., Stauffer, D. A., Xu, M., and Karlin, A. (1992). Acetylcholine receptor channel structure probed in cysteine-substitution mutants. Science *258*, 307–310.

Akabas, M. H., Kaufmann, C., Archdeacon, P., and Karlin, A. (1994). Identification of acetylcholine receptor channel-lining residues in the entire M2 segment of the α subunit. Neuron *13*, 919–927.

Armstrong, C. M. (1981). Sodium channels and gating currents. Physiol. Rev. *61*, 644–683.

Auld, V. J., Goldin, A. L., Krafte, D. S., Catterall, W. A., Lester, H. A., Davidson, N., and Dunn, R. J. (1990). A neutral amino acid change in segment IIS4 dramatically alters the gating properties of the voltage-dependent sodium channel. Proc. Natl. Acad. Sci. USA *87*, 323–327.

Bezanilla, F. (1985). Gating of sodium and potassium channels. J. Membr. Biol. *88*, 97–111.

Catterall, W. A. (1992). Cellular and molecular biology of voltage-gated sodium channels. Physiol. Rev. *72* (Suppl.), S15–S48.

Chahine, M., George, A. L., Jr., Zhou, M., Ji, S., Sun, W., Barchi, R. L., and Horn, R. (1994). Sodium channel mutations in paramyotonia congenita uncouple inactivation from activation. Neuron *12*, 281–294.

Durell, S. R., and Guy, H. R. (1992). Atomic scale structure and functional models of voltage-gated potassium channels. Biophys. J. *62*, 238–247.

Gillespie, J. I., and Meves, H. (1980). The time course of sodium inactivation in squid giant axons. J. Physiol. *299*, 289–308.

Goldman, L., and Kenyon, J. L. (1982). Delays in inactivation development and activation kinetics in *Myxicola* giant axons. J. Gen. Physiol. *80*, 83–102.

Hodgkin, A. L., and Huxley, A. F. (1952). A quantitative description of membrane current and its application to conduction and excitation in nerve. J. Physiol. *117*, 500–544.

Keynes, R. D., Greeff, N. G., and Van Helden, D. F. (1982). The relationship between the inactivating fraction of the asymmetry current and gating of the sodium channel in the squid giant axon. Proc. R. Soc. Lond. (B) *215*, 391–404.

Liman, E. R., Hess, P., Weaver, F., and Koren, G. (1991). Voltage-sensing residues in the S4 region of a mammalian K^+ channel. Nature *353*, 752–756.

Logothetis, D. E., Movahedi, S., Satler, C., Lindpaintner, K., and Nadal-Ginard, B. (1992). Incremental reductions of positive charge within the S4 region of a voltage-gated K^+ channel result in corresponding decreases in gating charge. Neuron *8*, 531–540.

Logothetis, D. E., Kammen, B. F., Lindpaintner, K., Bisbas, D., and Nadal-Ginard, B. (1993). Gating charge differences between two voltage-gated K^+ channels are due to the specific charge content of their respective S4 regions. Neuron *10*, 1121–1129.

Lopez, G. A., Jan, Y. N., and Jan, L. Y. (1991). Hydrophobic substitution mutations in the S4 sequence alter voltage-dependent gating in *Shaker* K^+ channels. Neuron *7*, 327–336.

Margolskee, R. F., McHendry-Rinde, B., and Horn, R. (1993). Panning transfected cells for electrophysiological studies. Biotechniques *15*, 906–911.

McCormack, K., Tanouye, M. A., Iverson, L. E., Lin, J.-W., Ramaswami, M., McCormack, T., Campanelli, J. T., Mathew, M. K., and Rudy, B. (1991). A role for hydrophobic residues in the voltage-dependent gating of Shaker K^+ channels. Proc. Natl. Acad. Sci. USA *88*, 2931–2935.

Miller, C. (1990). Diffusion-controlled binding of a peptide neurotoxin to its K^+ channel receptor. Biochemistry *29*, 5320–5325.

Noda, M., Shimizu, S., Tanabe, T., Takai, T., Kayano, T., Ikeda, T., Takahashi, H., Nakayama, H., Kanaoka, Y., Minamino, N., et al. (1984). Primary structure of *Electrophorus electricus* sodium channel deduced from cDNA sequence. Nature *312*, 121–127.

Papazian, D. M., Timpe, L. C., Jan, Y. N., and Jan, L. Y. (1991). Alteration of voltage-dependence of Shaker potassium channel by mutations in the S4 sequence. Nature *349*, 305–310.

Perozo, E., Santacruz-Toloza, L., Stefani, E., Bezanilla, F., and Papazian, D. M. (1994). S4 mutations alter gating currents of Shaker K^+ channels. Biophys. J. *66*, 345–354.

Ptáček, L. J., George, A. L., Jr., Barchi, R. L., Griggs, R. C., Riggs, J. E., Robertson, M., and Leppert, M. F. (1992). Mutations in an S4 segment of the adult skeletal muscle sodium channel cause paramyotonia congenita. Neuron *8*, 891–897.

Schoppa, N. E., McCormack, K., Tanouye, M. A., and Sigworth, F. J. (1992). The size of gating charge in wild-type and mutant Shaker potassium channels. Science *255*, 1712–1715.

Sigworth, F. J. (1993). Voltage gating of ion channels. Quart. Rev. Biophys. *27*, 1–40.

Slatin, S. L., Qiu, X.-Q., Jakes, K. S., and Finkelstein, A. (1994). Identification of a translocated protein segment in a voltage-dependent channel. Nature *371*, 158–161.

Stauffer, D. A., and Karlin, A. (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.

Stühmer, W., Conti, F., Suzuki, H., Wang, X. D., Noda, M., Yahagi, N., Kubo, H., and Numa, S. (1989). Structural parts involved in activation and inactivation of the sodium channel. Nature *339*, 597–603.

Yang, N., Ji, S., Zhou, M., Ptáček, L. J., Barchi, R. L., Horn, R., and George, A. L., Jr. (1994). Sodium channel mutations in paramyotonia congenita exhibit similar biophysical phenotypes *in vitro*. Proc. Natl. Acad. Sci. USA *91*, 12785–12789.

Zagotta, W. N., Hoshi, T., Dittman, J., and Aldrich, R. W. (1994). *Shaker* potassium channel gating. II. Transitions in the activation pathway. J. Gen. Physiol. *103*, 279–319.