Pflügers Arch – Eur J Physiol (1996) 433:91–97 © Springer-Verlag 1996

ORIGINAL ARTICLE

Shahnaz P. Yusaf · Dennis Wray
Asipu Sivaprasadarao

Measurement of the movement of the S4 segment during the activation of a voltage-gated potassium channel

Received: 13 June 1996 / Received after revision and accepted: 22 July 1996

Abstract Voltage-gated ion channels contain a positively charged transmembrane segment termed S4. Recent evidence suggests that depolarisation of the membrane potential causes this segment to undergo conformational changes that, in turn, lead to the opening of the channel pore. In order to define these conformational changes in structural terms, we have introduced single cysteine substitutions into the S4 segment of the prototypical *Shaker* K^+ channel at various positions and expressed the mutants in *Xenopus* oocytes. The cells were depolarised to induce K^+ currents and the effect of application of 100 μM parachloromercuribenzenesulphonate (PCMBS) on these currents was examined by the two-electrode voltage-clamp technique. PCMBS inhibited K^+ currents elicited by mutants L358C, L361C, V363C and L366C, but not those by V367C and S376C. Since PCMBS is a membrane-impermeable cysteine-modifying reagent, the data suggest that depolarisation must have caused the S4 segment to move out of the lipid bilayer into the extracellular phase rendering the residues at positions 358, 361, 363 and 366 susceptible to PCMBS attack. The lack of effect of PCMBS on V367C suggests that the exposure of S4 terminates at L366. Detailed analysis of L361C mutant revealed that the S4 movement can occur even below the resting potential of the cell, at which potential voltage-gated K^+ channels are normally in a nonconducting closed state.

Key words Potassium channel · Voltage gating · Cysteine mutagenesis · Cysteine modification · S4 movement · *Shaker*

Introduction

Voltage-gated Na^+, $K^+$and Ca^{2+} channels play a central role in the propagation of electrical signals in excitable cells, such as nerve and muscle [2, 4, 16]. They perform this task by opening and closing (gating) their channel pores in response to changes in the transmembrane potential. The mechanism by which they sense the voltage changes and trigger the opening of the channel pore is not completely understood. According to the early hypothesis, put forward by Hodgkin and Huxley [5], voltage-gated ion channels contain intrinsic charges, referred to as gating charges, that move across the membrane upon depolarisation. Molecular cloning followed by the sequencing of these channels has indeed revealed the presence of positively charged residues (arginine/lysine) in a small stretch of sequence, referred to as the S4 segment (Fig. 1). This sequence is highly conserved among all members of this superfamily, and has been proposed to traverse the membrane. Mutation studies have confirmed that these positive charges are responsible for the voltage-sensing mechanism [9, 10, 12, 15–17]. The replacement, one at a time, of these residues by neutral amino acids produced large shifts in the voltage dependence of activation; the observed shifts could generally be correlated with the channel's overall gating valence [10].

The proposed movement of gating charges across the transmembrane field has been tested by measuring currents carried by these charges (for reviews see [1, 16]). The measurement of these currents, referred to as gating currents, was carried out on native as well as cloned channels by abolishing ionic currents using channel blockers. Mutant channels that have lost their ion conduction property but retained their gating property have been used to measure gating currents [13]. From these measurements, it was suggested that from 12 up to 16 elementary charges per channel molecule move across the transmembrane field [14, 16, 22]. Assuming that most of the gating current was carried by the positively charged residues of the S4 segment, it was suggested that depolarisation of the membrane caused the physical movement of the S4 segment to the extracellular phase [3]. Mutation of these positively charged residues to neutral residues has indeed caused a decrease in the gating current [10, 15]. Evidence in support of the S4 movement has only recently been presented [8, 11, 21]. However, the

S.P. Yusaf · D. Wray · A. Sivaprasadarao (✉)
Department of Pharmacology, University of Leeds, Leeds, LS2 9JT, UK

extent of movement has not been accurately defined (see Discussion). By introducing cysteines at various positions within the S4 segment of the *Shaker* potassium channel and examining their accessibility to parachloromercuribenzenesulphonate (PCMBS), a reagent which reacts with cysteines present in the aqueous environment only [20], we not only confirm that, in response to depolarisation, S4 moves out of the membrane bilayer, but also demonstrate that the movement stops once L366 reaches the extracellular phase. This would correspond to the outward movement of at least six amino acid residues. Our data also show that the S4 movement occurs even at hyperpolarising potentials, as negative as –120 mV.

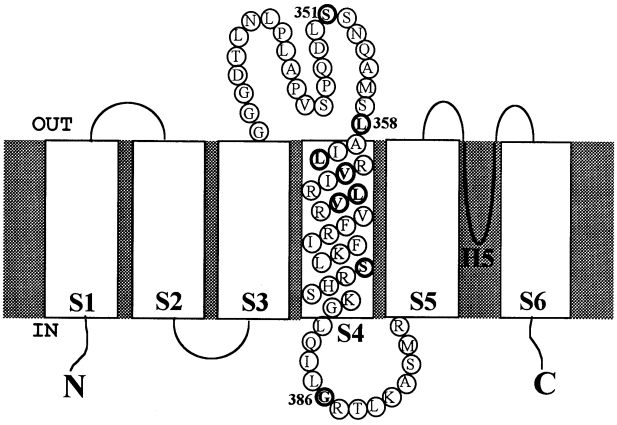


Fig. 1 Proposed transmembrane topology of the *Shaker* K^+ channel showing positions of the cysteine substitutions. The single-letter amino-acid code is used, and hydrophobic segments S1–S6 are shown in *boxes* as transmembrane domains connected by hydrophilic loops. The pore lining H5 segment lies between S5 and S6. Amino acid residues representing the position of the cysteine mutations are shown in *bold*

Materials and methods

Molecular biology

The Δ(N6–46)ShB1 cDNA was subcloned into pKS Bluescript (Stratagene). Site-directed mutagenesis was performed using the method of Kunkel et al. [7]. All the mutant clones were sequenced in a region of 200–300 bases around the site of the primary mutation and did not contain additional mutations. cRNA was transcribed for the wild-type and for each of the mutants using T7 RNA polymerase (MEGAscript, Ambion), after linearisation of the template DNA with *Hin*dIII. PCMBS (100 μM, Na.salt, Sigma) was prepared in Frog Ringer's solution.

Electrophysiology

Dumont stage-V or stage-VI *Xenopus* oocytes were injected with 50 nl of mutant or wild-type cRNA (15–25 ng) and maintained at 19°C in modified Barth's solution [88 mM NaCl, 1 mM KCl, 2.4 mM $NaHCO_3$, 0.82 mM $MgSO_4$, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 7.5 mM Tris-HCl, pH 7.6, 10, 000 U/l penicillin, 100 mg/l streptomycin]. After 1–2 days, membrane currents were recorded at room temperature (22–25°C) using two-electrode voltage-clamp (Geneclamp 500, Axon Instruments) as previously described [19]. Electrodes were filled with 3 M KCl and resistances were 1–3 MΩ and 1–1.5 MΩ for the voltage and current electrodes respectively. Oocytes were held in a 50-μl chamber and were continually superfused (2 ml/min) with Frog Ringer's solution (115 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 10 mM HEPES, pH 7.2). Membrane potential was held at –80 mV and 200-ms test depolarisations were applied in 10-mV increments at an interval of 10 s in order to construct current/voltage (*I*/*V*) relationships. A series of 20 hyperpolarising steps of 10 mV were also applied to allow leak subtraction. Data were filtered at 2 kHz and sampled at 3 kHz using CED software and a CED 1401 interface. Leak and capacitance artefacts were removed by subtracting averaged leak subtraction traces from the current traces.

Results

Residue L361 is exposed to the extracellular phase upon depolarisation

Residues 359 to 381 have been predicted to constitute the S4 transmembrane segment of the *Shaker* K^+ channel [3] (Fig. 1). In order to investigate if this segment moves out of the bilayer into the extracellular phase during depolarisation, we have first replaced residues S351, L358 and L361 (near the outer membrane boundary) of *Shaker* K^+ channel with cysteines (see Fig. 1) and expressed the mutants in *Xenopus* oocytes by injecting the corresponding cRNA transcripts. The oocytes were depolarised by stepping to +40 mV from a holding potential of –80 mV to evoke K^+ currents, which were measured by the two-electrode voltage-clamp method. While repeatedly pulsing to this potential, we superfused the cell with PCMBS and measured the currents continuously. This reagent is very hydrophilic and membrane impermeable and has been shown to react with cysteines located in an aqueous environment only [20]. The rationale is that if depolarisation drives the S4 segment out of the lipid bilayer into the extracellular (aqueous) phase of the cell, then the engineered cysteines of the mutant channels may move out of the bilayer and react with PCMBS. Such a reaction would be expected to lead to changes in the functional properties of the channel by interfering with the conformational changes required for the normal functioning of the channel.

The results in Fig. 2 show that while the wild-type and S351C mutant channels were unaffected by PCMBS, K^+ currents expressed by L358C and L361C were inhibited by PCMBS. Since the *Shaker* channel, Δ(N6–46)ShB1, used in this study lacks the N-terminal "ball" domain [6], the observed inhibition is not contaminated by the N-type inactivation. S351 has been proposed to be far from the outer-membrane border in the extracellular phase of the cell [3]. The lack of effect of PCMBS on S351C suggests that its modification does not perturb the structure of the protein sufficient to inhibit the channel activity or, alternatively, the substituted cysteine may be buried in the tetrameric structure of the channel and hence be inaccessible to PCMBS. L358 is predicted to lie just outside the bilayer in the extracellular phase. Its cysteine mutant, L358C, was only partially

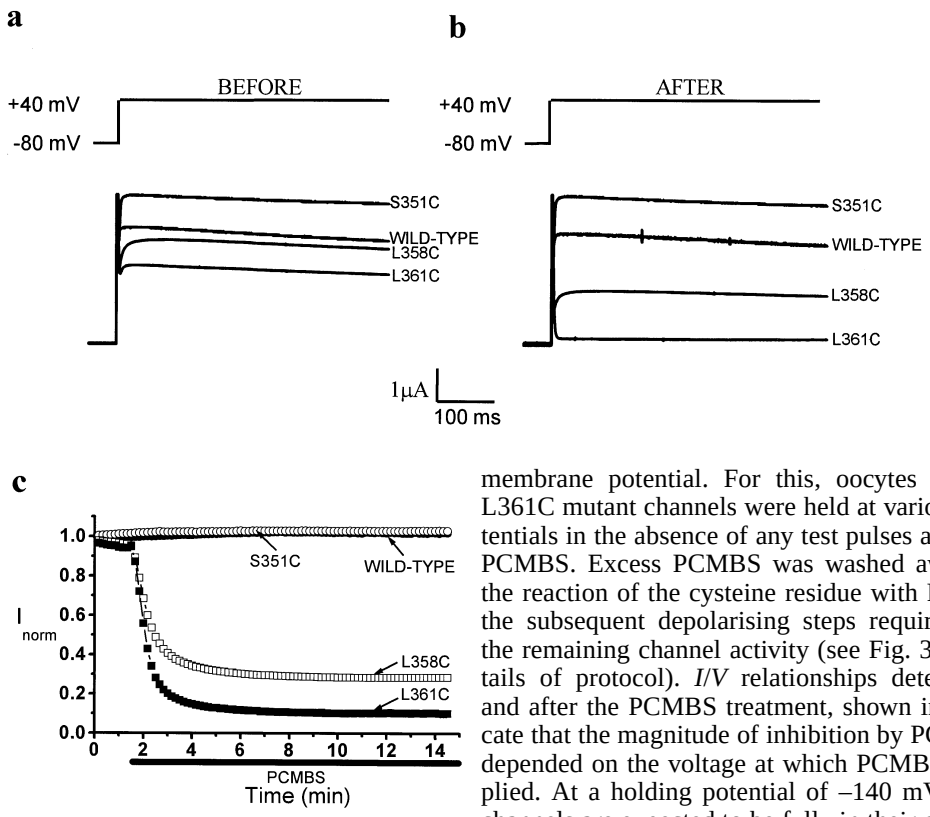


Fig. 2a–c Effect of PCMBS on K^+ currents expressed by cysteine-substituted *Shaker* mutants S351C, L358C and L361C. **a** Time course of K^+ currents recorded for ΔN(6–46)ShB1 and mutants S351C, L358C and L361C during voltage-clamp depolarisations from a holding potential (V_h) of –80 mV to a test potential of +40 mV taken during perfusion of oocytes with Frog Ringer's solution. Mutant L358C had a slower activation rate when compared to the wild-type. **b** Time course of K^+ currents from V_h = –80 mV to a test depolarisation of +40 mV after superfusion of the same oocytes with 100 μM PCMBS for 12 min. **c** Effect of application of PCMBS on K^+ currents, evoked by depolarisation, in *Xenopus* oocytes expressing ΔN(6–46)ShB1 (●) and mutant S351C (❍), L358C (□) and L361C (■) channels. Oocytes were superfused with Frog Ringer's solution containing 100 μM PCMBS over the time indicated by the *solid line*. Cells were repeatedly depolarised by stepping to +40 mV for 500 ms from a holding potential of –80 mV every 10 s. For each oocyte, current amplitudes were normalised with respect to the value measured over the 1st min of the recording. Mean values for 5–6 oocytes for each mutant are shown

inhibited by PCMBS, even after a long application. The partial inhibition may be due to its location at the outer-membrane border.

The effect of PCMBS on L361C, on the other hand, was quite profound. The mutant was rapidly and completely inhibited by PCMBS, suggesting that under the experimental conditions, i.e. at –80 mV or during the 500-ms depolarising test pulses, this residue was present in the extracellular environment. In order to test whether this residue is exposed at –80 mV or only during the depolarising test pulses, we have further examined the effect of PCMBS on the L361C mutant as a function of membrane potential. For this, oocytes expressing the L361C mutant channels were held at various holding potentials in the absence of any test pulses and treated with PCMBS. Excess PCMBS was washed away to prevent the reaction of the cysteine residue with PCMBS during the subsequent depolarising steps required to measure the remaining channel activity (see Fig. 3 legend for details of protocol). *I*/*V* relationships determined before and after the PCMBS treatment, shown in Fig. 3a, indicate that the magnitude of inhibition by PCMBS strongly depended on the voltage at which PCMBS had been applied. At a holding potential of –140 mV, at which the channels are expected to be fully in their closed state, the inhibition was minimal. As the membrane potential was changed in the depolarising direction, a progressive increase in inhibition by PCMBS was observed. The data strongly suggest that the residue at position 361 moves from the solvent-inaccessible membrane environment into the extracellular phase during depolarisation. The data also suggest that L361 moves even below the resting potential of the cell, at which voltage-gated K^+ channels are normally in a closed state, although gating transitions occur [1, 13, 14]. The inhibition of L361C mutant in Fig. 2 could thus be explained by the fact that, at –80 mV, the cysteine at position 361 is exposed, but the rate of exposure is enhanced by the depolarising steps, thereby leading to a rapid decline in the current.

In the above-described experiment, during the 2-min exposure to PCMBS all, or a proportion, of the activated channels must have reacted with PCMBS and become non-functional. The degree of inhibition by PCMBS may be taken as being proportional to the fraction of channels activated during the 2-min exposure period. From a plot of percentage inhibition against membrane potential (Fig. 3b), the potential ($V_{0.5}$) at which 50% of the channels were inhibited was calculated to be –107 mV. Assuming that the modification of one of the four S4 segments of a channel is sufficient to inhibit its function, the data suggest that, at –107 mV, at least one S4 segment of 50% of the channels must have moved outward during the 2-min period of PCMBS application.

To establish that the observed PCMBS effect on L361C is not due to its interaction with the channel at

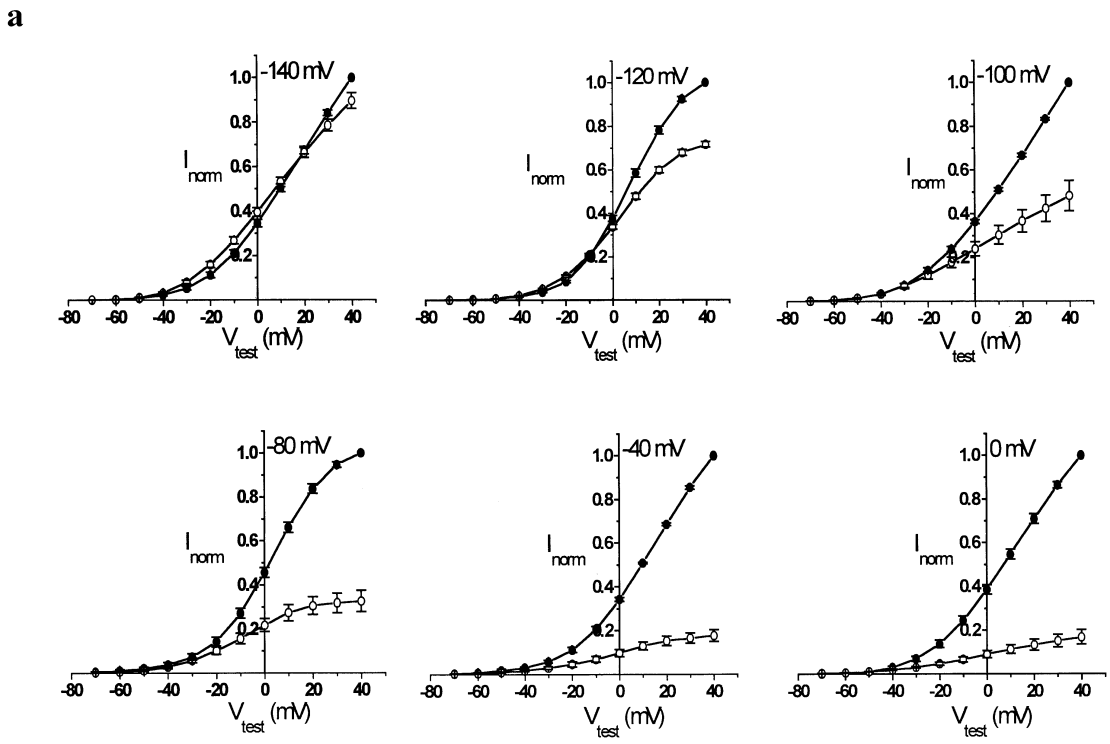


Fig. 3a,b Dependence of inhibition of L361C *Shaker* mutant K^+ currents by PCMBS on the voltage at which PCMBS was applied. **a** Current/voltage (*I*/*V*) curves taken for mutant L361C before (●) and after (●) application of PCMBS at the indicated holding potentials. Oocytes expressing mutant L361C were perfused with Frog Ringer's solution and *I*/*V* curves were obtained by applying 200-ms test depolarisations in 10-mV increments, every 10 s, from a holding potential of –80mV. The cells were then held at the indicated holding potentials (either –140, –120, –100, –80, –40 or 0 mV) initially for 2 min in the absence of PCMBS, and then in the presence of PCMBS (100 μM) for a further 2 min (perfusion rate = 2 ml/min). Unreacted PCMBS was removed by washing the cells with Frog Ringer's solution for 2 min at the same holding potential. No test pulses were applied during these applications. *I*/*V* curves were then taken again from a holding potential of –80 mV. All *I*/*V* curves have been normalised to the current value at +40 mV (I_{norm}) for each holding potential and each curve is the mean ±SEM of 5–6 oocytes. **b** Percentage inhibition by PCMBS as a function of voltage at which PCMBS was applied. Percentage inhibition was calculated from the current at a test potential of +40 mV (from above data) by taking the differences in values before and after the application of PCMBS. The data were fitted by a least-squares method with the Boltzmann equation, $P_o = P_{max}/\{1+\exp[-z\ (V_m-V_o)/kT]\}$, where P_o is the fraction of channels open (assumed equal to% inhibition), P_{max} is the maximal inhibition, V_m the holding potential, V_o the potential for half-maximal activation, k is the Boltzmann constant, T is the absolute temperature and z is the effective charge moved across the width of the membrane. The measured parameters were V_o = –107 mV and z = 1.41

sites other than the cysteine at position 361, we first inhibited the L361C mutant by treating with PCMBS at +40 mV as above, and then superfused the cell with 1 mM dithiothreitol (DTT). DTT should reverse the effect of PCMBS by reducing the PCMBS-modified cysteine to cysteine. In fact this was found to be the case: DTT rapidly and fully restored the function of the channel (Fig. 4). DTT showed no effect on either the PCMBS-treated or -untreated wild-type channel (data not shown).

The exposure of S4 terminates at L366

In order to determine how far the S4 segment moves into the extracellular phase, we mutated other residues of S4, downstream to L361, to cysteines and examined their accessibility to the extracellularly applied PCMBS at depolarising test (+40 mV) pulses. We avoided mutating the charged residues since previous studies [9, 12] have shown that neutralisation of these residues resulted in large shifts in the voltage dependence of activation which might be due to structural perturbations. We gen-

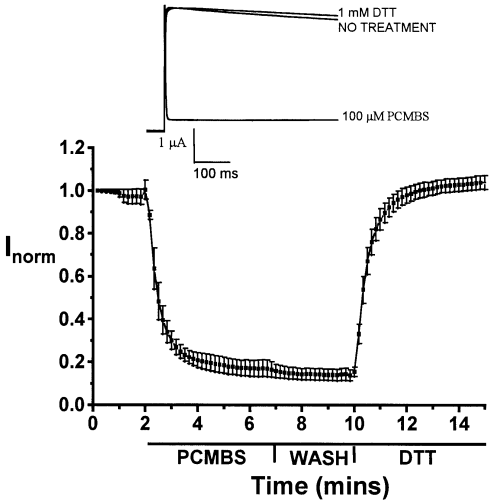
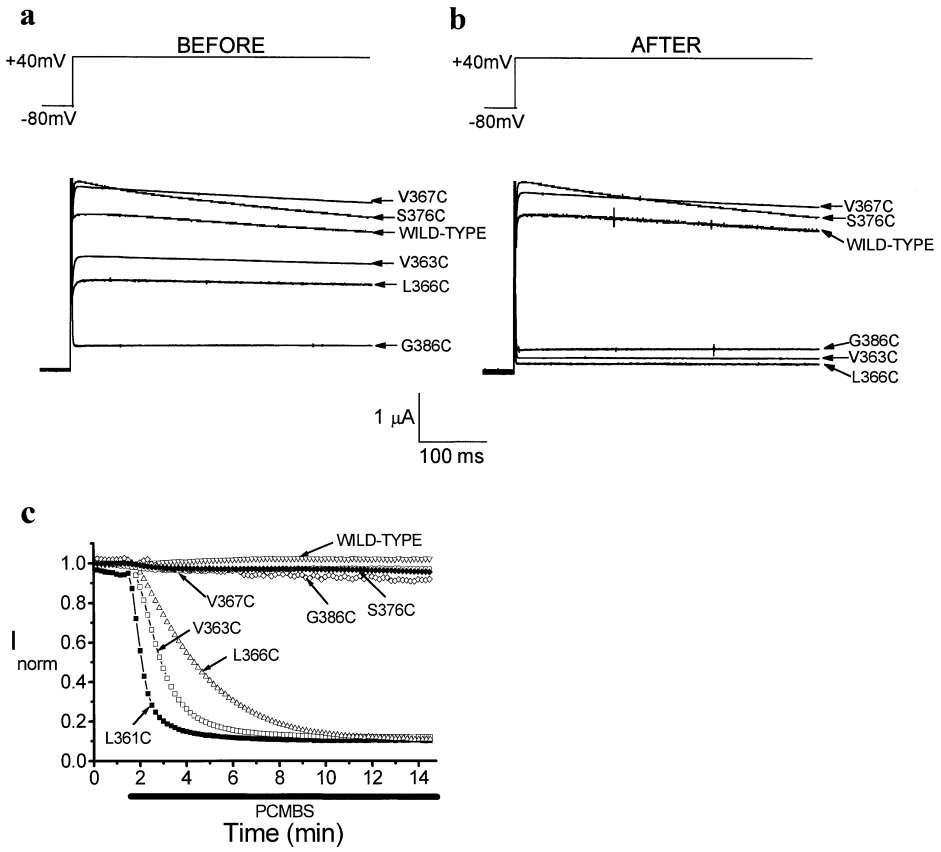


Fig. 4 Reversal of inhibition of L361C mutant by PCMBS with dithiothreitol (*DTT*). Oocytes expressing mutant L361C were held at –80 mV and stepped to +40 mV repeatedly every 10 s. Oocytes were superfused with PCMBS (100 μM), washed, and then DTT (1 mM) was applied as shown by the *solid line*. The mean ±SEM current (normalised to an initial value of 1.0 at $t = 0$) is shown for 4 oocytes. *Inset*: typical traces are shown before and after 8 min of superfusion with PCMBS and after 3 min of DTT superfusion

erated mutants V363C, L366C, V367C, S376C and G386C and first examined their *I*/*V* relationships. None of these mutants showed any significant differences in their *I*/*V* relationships from the *I*/*V* relationship of the wild-type channel (data not shown). We then tested the effect of extracellular application of PCMBS on these mutants, activated by repeated pulsing to +40 mV from a holding potential of –80 mV. While V363C and L366C mutants were inhibited completely, no effect was

Fig. 5a–c Effect of PCMBS on K^+ currents expressed by cysteine-substituted *Shaker* mutants V363C, L366C, V367C, S376C and G386C. These experiments were carried out using protocols described in Fig. 2. **a** Time course of K^+ currents recorded for ΔN(6–46)ShB1 and mutants V363C, L366C, V367C, S376C and G386C during voltage-clamp depolarisations from a holding potential (V_h) of –80 mV to a test potential of +40 mV taken during perfusion of oocytes with Frog Ringer's solution. A single oocyte was used for each channel. **b** Time course of K^+ currents from V_h = –80 mV to a test depolarisation of +40 mV after superfusion of the same oocytes with 100 μM PCMBS for 12 min. **c** Effect of application of PCMBS on K^+ currents, evoked by depolarisation, in *Xenopus* oocytes expressing ΔN(6–46)ShB1 (▼) and mutant L361C (■), V363C (□), L366C (▲), V367C (●), S376C (♦) and G386C (◊) channels. Oocytes were superfused with Frog Ringer's solution containing 100 μM PCMBS over the time indicated by the *solid line*. Cells were repeatedly depolarised by stepping to +40 mV for 500 ms from a holding potential of –80 mV every 10 s. For each oocyte, current amplitudes were normalised with respect to the value measured over the 1st min of the recording. Mean values for 5–6 oocytes for each mutant are shown



observed with V367C, S376C and G386C mutants (Fig. 5).

Figure 5 also shows that the rate of inhibition by PCMBS depended on the position of the engineered cysteine in the mutant. L361C (exponential time constant, τ = 0.65±0.04 min, n = 5 oocytes) was inhibited about twice as fast as V363C (τ = 1.43±0.03 min, n = 5) and five times faster than L366C (τ = 3.08±0.05 min, n = 5). Thus it appears that the further the cysteine within the bilayer is from the outer-membrane border, the slower was the inhibition.

Discussion

By studying the accessibility of cysteine residues introduced at various positions along the S4 segment of *Shaker* K^+ channel to the extracellularly applied PCMBS, a reagent that specifically reacts with cysteines in a solvent-accessible environment, we have demonstrated that a section of the S4 segment, comprising residues L361–L366, moves into the extracellular phase upon depolarisation.

PCMBS, upon reaction with cysteine, converts it to a relatively large, negatively charged mercuribenzenesulphonate–cysteine conjugate. This change would be expected to alter the microenvironment surrounding the cysteine residue in terms of volume as well as electrostatic interactions. If the nature of the microenvironment surrounding the cysteine is critical for the functional properties of the channel, then one would expect PCMBS to have major consequences on the functional properties of the channel. As expected, the effect of PCMBS proved to be quite dramatic in that it rapidly inhibited the K^+ currents (Figs. 2, 3 and 5). The effect was specific with no irreversible structural damage done to the channel, as demonstrated by the fact that DTT reversed the PCMBS effect (Fig. 4).

Larsson et al. [8] have very recently used a similar strategy to study the movement of S4, but have used methanethiosulphonate-ethyltrimethylammonium (MTSET) reagent as a probe. Reaction of an exposed cysteine with MTSET resulted in a shift in the *I*/*V* curve of some of the mutants studied, the nature and the extent of which depended on the residue that was changed to cysteine. By measuring such changes, Larsson et al. [8] determined whether or not a cysteine residue was susceptible to modification by MTSET. MTSET is a small positively charged reagent which on reaction with a cysteine converts it to an arginine-like moeity. Thus if the substitution of an arginine residue by a cysteine causes a change in the *I*/*V* relationship, MTSET treatment would be expected to restore the wild-type relationship. This indeed was the case with the R365C mutant studied by Larsson et al. [8]. If a residue is highly tolerant to changes, then neither cysteine substitution nor its subsequent modification would be expected to alter the properties of the channel. Thus some cysteine modifications might escape detection. In contrast to MTSET, however, PCMBS, in our hands, uniformly inhibited the current amplitudes of all mutant channels which exposed their engineered cysteine residues, irrespective of the nature of the residue mutated. PCMBS thus appears to be a more sensitive indicator of cysteine modification, and hence of conformational changes, than MTSET. Our results obtained with this reagent can be summarised as follows.

Firstly, our data demonstrated that L361 remains buried within the membrane bilayer only at very negative potentials, that is below –140 mV, and that a small depolarisation from this potential is sufficient to trigger its movement into the extracellular phase (Fig. 3). This means that at the resting potential of the cell, considerable movement of this residue occurs between the membrane and the extracellular environments. Larsson et al. [8] have found that, at –100 mV, residue 362 reacted (partially) with MTSET, and that the extent of inhibition increased at more depolarised potentials. They assumed that at –100 mV no movement had occurred, and therefore postulated that residue 362 lies within a crevice, which restricts free access to the reagent. However, there is no evidence for the assumption that no movement occurs at –100 mV. On the contrary, their data and ours can be interpreted to show that there is movement of residue 362 even at –100 mV. Our data for the nearby residue 361 shows (Fig. 3) that by hyperpolarising up to –140 mV, reaction with PCMBS is almost totally inhibited, in contrast to 50% inhibition at –100 mV. This makes it clear that the movement of residue 361 occurs even at –100 mV, ruling out a theory based on crevices.

Secondly, our data (Fig. 5) clearly demonstrate that cysteine substitution of L361, V363 and L366, but not V367, of the S4 segment, renders the channel susceptible to extracellularly added PCMBS upon depolarisation, suggesting that L366 represents the last residue of S4 which is exposed. Knowing that L361 is embedded within the bilayer in the closed state of the channel (Fig. 3), this represents the exposure of at least six residues, including two arginines, to the outer phase. Previous studies [8, 11] have not conclusively established the extent of S4 movement out of the bilayer.

Thirdly, the data in Fig. 3 suggest that the movement of S4 can occur even below the resting potential (–80 mV) of the membrane, at which voltage-gated K^+ channels are in a closed state. Indeed, although small, measurable gating currents have been reported to occur at very negative potentials for the *Shaker* K^+ channel [18]. However, it is not clear whether these potentials provide sufficient energy to drive the S4 segment up to its maximal limit, i.e. up to L366, or a partial movement occurs with only the outermost residues being exposed. Our observation that cysteines introduced near the outer-membrane boundary (position 361) are more readily inhibited by PCMBS than those (positions 363 and 366) further down within the bilayer (Fig. 5) suggests that the latter might be the more likely possibility. This partial movement might explain why gating currents can be measured but no ionic currents can be observed at very negative potentials. We cannot, however, rule out the

possibility that outermost residues of S4 are more readily accessible to PCMBS than those further down, for stearic reasons.

An alternative explanation for the observation that S4 segments move at more negative potentials than the channel openings would be connected with the tetrameric structure of the channel, containing four S4 segments. At more negative potentials, the probability that the S4 segments of all four subunits move outwards at any one time would be less than that at depolarising potentials. Thus, if we assume that all four S4 segments must move outwards in order for the channel to open, it follows that S4 movement would be observed at more negative potentials than channel opening.

Two major conclusions can be drawn from this study. Firstly, in *Shaker* K^+ channels, depolarisation results in the outward movement of S4 up to L366, representing the exposure of a minimum of six residues out of the bilayer. Secondly, the S4 movement occurs even below the resting potential of the cell, at which voltage-gated K^+ channels are normally in a closed state. However, a number of questions remain to be answered. Firstly, do the four S4 segments of the channel move independenlty of each other or do they move in a synchronised fashion? Secondly, do all the four S4 segments need to be exposed up to L366 for the channel opening to occur? Thirdly, is the S4 movement accompanied by changes in the secondary structure of the segment? Finally, does the S4 movement occur in a series of discrete steps or in a continuous fashion? The gating noise analysis experiments done on Na^+ and K^+ channels (reveiwed in [1]) indeed suggest that the movement of S4 might occur in a series of discrete steps or in a continuous fashion, rather than in a single step. We believe that it should be possible to address some of these questions using the approach described here.

Acknowledgements We would like to thank Dr. R.W. Aldrich for kindly providing the ShB (Δ6–46) mutant and Drs. D.J. Beech and M. Hunter for helpful advice.

References

1. Bezanilla F, Stefani E (1994) Voltage-dependent gating of ionic currents. Annu Rev Biophys Biomol Struct 23:819–846
2. Catterall WA (1995) Structure and function of voltage-gated ion channels. Annu Rev Biochem 64:493–531
3. Durell SR, Guy HR (1992) Atomic scale structure and functional models of voltage gated-potassium channels. Biophys J 62:238–250
4. Hille B (1992) Ionic channels of excitable membranes. Sinauer, Sunderland, Mass.
5. Hodgkin AL, Huxley AF (1952) A quantitative description of membrane current and its application to conduction and excitation in nerve. J Physiol (Lond) 117:500–544
6. Hoshi T, Zagotta WN, Aldrich RW (1990) Biophysical and molecular mechanisms of *Shaker* potassium channel inactivation. Science 250:533–538
7. Kunkel TA, Roberts JD, Zakour RA (1987) Rapid and efficient site-directed mutagenesis without phenotypic selection. Methods Enzymol 154:367–382
8. Larsson HP, Baker OS, Dhillon DS, Isacoff EY (1996) Transmembrane movement of the *Shaker* K^+ channel S4. Neuron 16:387–397
9. Liman ER, Hess P, Weaver F, Koren G (1991) Voltage-sensing residues in the S4 region of a mammalian K^+ channel. Nature 353:752–756
10. Logothetis DE, Movahedi S, Slater C, Lindpaintner K, Nadal-Ginard B (1992) Incremental reduction of positive charge within the S4 region of a voltage-gated K^+ channel result in corresponding decreases in gating charge. Neuron 8:531–540
11. Mannuzzu LM, Moronne MM, Isacoff EY (1996) Direct physical measure of conformational rearrangement underlying potassium channel gating. Science 271:213–216
12. Papazian DM, Timpe LC, Jan YN, Jan LN (1991) Alteration of voltage-dependence of Shaker potassium channel by mutations in the S4 sequence. Nature 349:305–310
13. Perozo E, MacKinnon R, Bezanilla F, Stefani E (1993) Gating currents from a nonconducting mutant reveal open-closed conformations in *Shaker* K^+ channels. Neuron 11:353–358
14. Schoppa NE, McCormack K, Tanouye MA, Sigworth FJ (1992) The size of gating charge in wild-type and mutant Shaker potassium channels. Science 255:1712–1716
15. Shao XM, Papazian DM (1993) S4 mutations alter the single channel gating kinetics of Shaker K^+ channel. Neuron 11:343–352
16. Sigworth FJ (1993) Voltage-gating of ion channels. Q Rev Biophys 27:1–40
17. Stühmer W, Conti F, Suzuki H, Wang XD, Noda M, Yahagi N, Kubo H, Numa S (1989) Structural parts involved in activation and inactivation of the sodium channel. Nature 339:597–603
18. Stühmer W, Conti F, Stocker M, Pongs O, Heinemann SH (1991) Gating currents of inactivating and non-inactivating potassium channels expressed in *Xenopus* oocytes. Pflügers Arch 418:423–429
19. Wilson GG, O'Neill CA, Sivaprasadarao A, Findlay JBC, Wray D (1994) Modulation by cAMP of a cloned rat brain potassium channel expressed in oocytes. Pflügers Arch 428:186–193
20. Yan R-T, Maloney PC (1993) Identification of a residue in the pathway of a membrane carrier. Cell 75:37–44
21. Yang N, George AL Jr, Horn R (1996) Molecular basis of charge movement in voltage-gated sodium channels. Neuron 16:113–122
22. Zagotta WN, Hoshi T, Dittman J, Aldrich RW (1994) *Shaker* potassium channel gating II: transitions in the activation pathway. J Gen Physiol 103:279–319