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Mutations stabilizing an open conformation within the external region of the permeation pathway of the potassium channel KcsA

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Abstract Four subunits of the bacterial *Streptomyces lividans* protein KcsA form a K^+ channel which can be functionally reconstituted in vitro. Here we show that substitution of the tyrosine residue 82 by cysteine, valine or threonine, but not by glycine, led to functional channel types. Like the wild-type (WT) and an L81C channel, the mutant channels exhibit an internal pH-sensitive side and are cation selective. Based on the relative positions of the blocker tetraethylammonium within the electric field, the external entryways of the channels are concluded to have similar dimensions. For inward currents, the WT and the mutant channels vary in the occupancy of their subconductance states and concomitantly in their mean currents. Rectification properties are scarcely (L81C), little (Y82C) or considerably (Y82T and Y82V) altered. The data suggest that the amino acid type in position 82 stabilizes to varying degrees an open conformation within the external region of the permeation pathway.

Keywords Mutation · Assembly · Gating · Rectification · Tetraethylammonium

Abbreviations *IPTG*: Isopropyl β-D-thiogalactopyranoside · *PCR*: polymerase chain reaction · *TBA*: tetrabutylammonium · *TEA*: tetraethylammonium · *TM*: transmembrane region · *WT*: wildtype

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Introduction

In eukaryotic organisms, the currents generated by the action of various potassium ion channels play a key part in the electrical excitability, osmotic processes and metabolic regulation. The main K^+ channel families contain four subunits of a protein with six or two transmembrane helices, as well as a small pore region determining selectivity (Rudy 1988; Schroeder and Hedrich 1989; Hille 1992; Jan and Jan 1997; Moczydlowski 1998; Coetzee et al. 1999). Recently discovered families comprise also proteins with two predicted pore-forming regions (Lesage et al. 1996). Already in early studies it was noted that ionic currents in eukaryotic voltage-gated K^+ channels are blocked by positively charged tetraethylammonium (TEA) ions. Individual K^+ channels differ as to their affinity to TEA (Christie et al. 1989; Grupe et al. 1990). Internal (Armstrong and Binstock 1965; Rudy 1988) and external (Hille 1967; Rudy 1988) blocking sites have been found.

Within the Gram-positive soil bacterium *Streptomyces lividans*, we previously discovered a gene (*kcsA*) which encodes a protein of 17.6 kDa with two predicted transmembrane (TM) helices connected by a linker region. The KcsA protein forms a functional K^+ channel (Schrempf et al. 1995). The predicted pore region of the KcsA protein shows closest kinship to deduced eukaryotic voltage-gated channel proteins. It includes a signature motif ($T_{(72)}$XTTVGYG$_{(79)}$), followed by a tyrosine residue in position 82 (Schrempf et al. 1995) whose relative location corresponds to that within several deduced wild-type (WT) K^+ channel proteins, i.e. Y379 in RBK1 (Kv1.1) (Christie et al. 1989; Kavanaugh et al. 1991) and a correspondingly positioned tyrosine residue in RCK2 (Kv1.6) (Grupe et al. 1990), NGK2 (Kv3.1) (Yokoyama et al. 1989) and Kv2.1 (Pascual et al. 1995).

The functional KcsA channel is selective for cations, especially K^+. Regarding quarternary ammonium ions, the internal side of KcsA is preferentially blocked by

tetrabutylammonium (TBA) ions, whereas at the external side TEA shows the strongest inhibition (Meuser et al. 1999). The high sensitivity to externally applied TEA is linked to the presence of a tyrosine residue in position 82 (Heginbotham et al. 1999; Meuser et al. 1999). As KcsA can easily be obtained in larger quantities (Schrempf et al. 1995), it was crystallized and used to resolve for the first time the 3D structure of a K^+ channel (Doyle et al. 1998). Further insights into the structural features were gained from EPR studies (Perozo et al. 1999). Thus KcsA is employed to model in general the structure-function relationship of potassium ion channels (Durell and Guy 1999; Guidoni et al. 1999).

In this report we show that mutant channels carrying a substitution of tyrosine 82 have ion entryways of similar size. For inward currents, the mutant channels differ in the occupancy of the subconductance states and in the mean currents. Moreover, the rectification properties are altered.

Methods

Generation of mutants and isolation of proteins

Using the previously cloned *kcsA* gene (Schrempf et al. 1995), mutations leading to the formation of mutant proteins in positions L81 and Y82 (Splitt et al. 2000) were generated by polymerase chain reaction (PCR) mutagenesis. The individually mutated genes were recloned into the vector pQE32 (Qiagen) in frame with six histidine codons and transformed into *Escherichia coli* M15 pREP4. The correctness of the mutations was confirmed by sequencing. After induction with isopropyl β-D-thiogalactopyranoside (IPTG), the cells of the transformants were disrupted and the His-tag KcsA mutant protein was extracted and purified, as previously described (Schrempf et al. 1995; Meuser et al. 1999).

Determination of melting points

A portion (10 µL) of the purified protein (~0.5 mg/mL) in a buffer containing 10 mM DDM, 100 mM KCl, 300 mM imidazole and 20 mM Hepes, pH 7.5, was incubated in 1.5-mL cups at the indicated temperature in a pre-heated water bath. After 20 min, the cups were placed on ice for 10 min and 3.5 µL of buffer [4% sodium dodecyl sulfate (SDS), 200 mM Tris, pH 6.8, 20% glycerol, 0.1% bromophenol blue] were added. Each sample was analyzed on an SDS (0.1%) polyacrylamide (12.5%) gel. Having been stained with Coomassie brilliant blue, the gels were scanned with the Cybertech program CAM 2.0, and the temperature-dependent tetrameric decrease was determined. The melting points (corresponding to the denaturation temperature at which 50% of the tetramer dissociated) were obtained by fitting the data using a modified Boltzmann equation (Microcal Origin 5.0): $[\text{tetramer}\%] = 100 \times \{1 + \exp[(T - T_{50})/\mathrm{d}T]\}^{-1}$.

Electrophysiology

Reconstitution of proteins, preparation of planar lipid bilayers and electrophysiological experiments were performed as described (Schrempf et al. 1995; Meuser et al. 1999).

Block efficiency for fast blocks by TEA, which results in apparently reduced current amplitudes, can be determined from the shift of the open peaks from all-point histograms in dependence on the TEA concentration. For outward currents (holding potential $V_h = +100$ mV) carried by KcsA these maxima almost solely corresponded to the peak of the open3 state (see Fig. 3), and therefore it was used to determine the relative amount of the block (block_{rel}). At $V_h = -100$ mV the shifts of the individual peaks of the three open states could not be resolved, and therefore the block efficiency was determined from the shift of the averaged maximum. In case of a slow block, which is apparent as a reduced open probability, the block_{rel} values were calculated from the mean of current traces of 2 min duration.

The IC_{50} values were then calculated by fitting to the data the function:

$$\text{block}_{rel} = 1 - \frac{I}{I_0} = \frac{[\text{TEA}]}{[\text{TEA}] + IC_{50}} \quad (1)$$

where I_0 is the control current with [TEA] = 0. The calculations of the voltage dependence of the block by TEA were done according to the classical Woodhull model (Woodhull 1973).

Statistical analysis

Analysis of the subconductance states was performed by Gaussian fit of the all-point histograms. The significance of the fits was checked by the corresponding x^2 and R^2 values (McClave and Dietrich 1996).

Results

Assembly, stability and functionality of KcsA mutant proteins

Using PCR mutagenesis, mutant *kcsA* genes were generated in which the codon for tyrosine (Y) 82 was substituted by one for glycine (G), cysteine (C), threonine (T) (see Methods) or valine (V) (Meuser et al. 1999). The codon 81 for leucine (L) was previously exchanged by one determining cysteine (C) (Splitt et al. 2000). As deduced from the crystal structure (Doyle et al. 1998), L81 and Y82 are positioned at the outer vestibule of the channel (Fig. 1A).

After purification, the His-tagged mutant proteins Y82G, Y82C, Y82T and Y82V were tested for the formation of tetramers. Whereas the melting point of Y82V (89.2 ± 0.5 °C) was 4.4 °C higher than that of the WT KcsA (84.8 ± 0.3 °C), the melting points of two mutant proteins (Y82T, Y82C) were reduced to 81.8 ± 0.4 and 80.0 ± 0.2 °C, respectively (Fig. 1B). The protein Y82G, in contrast, could not be found as a tetramer (data not shown). As previously reported, the L81C mutant assembled as a tetramer and its melting point is 75.7 ± 0.3 °C (Splitt et al. 2000). The alterations indicate that Y82 is involved in the stability of KcsA.

After fusion of the proteoliposomes with a planar bilayer, all mutant proteins forming tetramers revealed basic channel properties which could not be distinguished from those of the KcsA WT protein (Fig. 2). Like the WT channel (Meuser et al. 1999), each mutant channel type retained the characteristic pH-sensitive internal side. No ion channel activity could be ascertained for the mutant protein Y82G. Each mutant channel was, like the WT (Meuser et al. 1999), effectively blocked by TBA ions from the internal side, but barely from the external one. To suppress currents from those channels

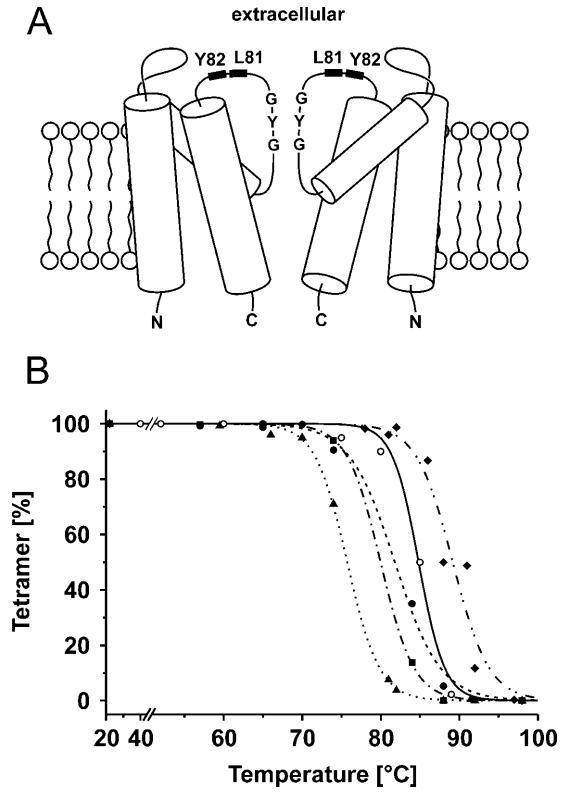

Fig. 1A, B Depiction of the KcsA channel and its stability. **A** The relative locations of the selectivity filter (GYG) and the tyrosine (Y82) and leucine (L81) residues are indicated and were deduced from the crystal structure (Doyle et al. 1998). **B** The melting curves of KcsA (○, *solid line*) and mutant proteins (Y82V ◆, *double dotted and dashed*; Y82T ●, *dashed*; Y82C ■, *dotted and dashed*; L81C ▲, *dotted*) were determined

orientated opposite to most others (usually $<10\%$), measurements were thus made on one side in the presence of 1 μM TBA.

Mutant channels exhibit altered occupancies of subconductance levels

The upper limit of the open probability of KcsA (P_o) can be calculated from the relative areas of the open and closed peaks of all-point histograms. Provided that the observed gated currents were carried by a single active channel, a value of $P_o < 10\%$ (Fig. 3A) could be deduced for conditions under which the channel was shown to be most active (i.e. pH 4 at the internal side) (Cuello et al. 1998; Heginbotham et al. 1999; Meuser et al. 1999). If, however, a larger number of channels was assumed, P_o would decrease accordingly. Previously we had shown that the WT KcsA displays rectifying current-voltage relationships (Meuser et al. 1999) as well as subconductance states (Schrempf et al. 1995), which have recently been characterized in more detail (Meuser et al. 1999). K^+ currents mediated by the KcsA WT channel are composed of distinguishable subconductance levels which can be allocated to at least three classes (Fig. 3B).

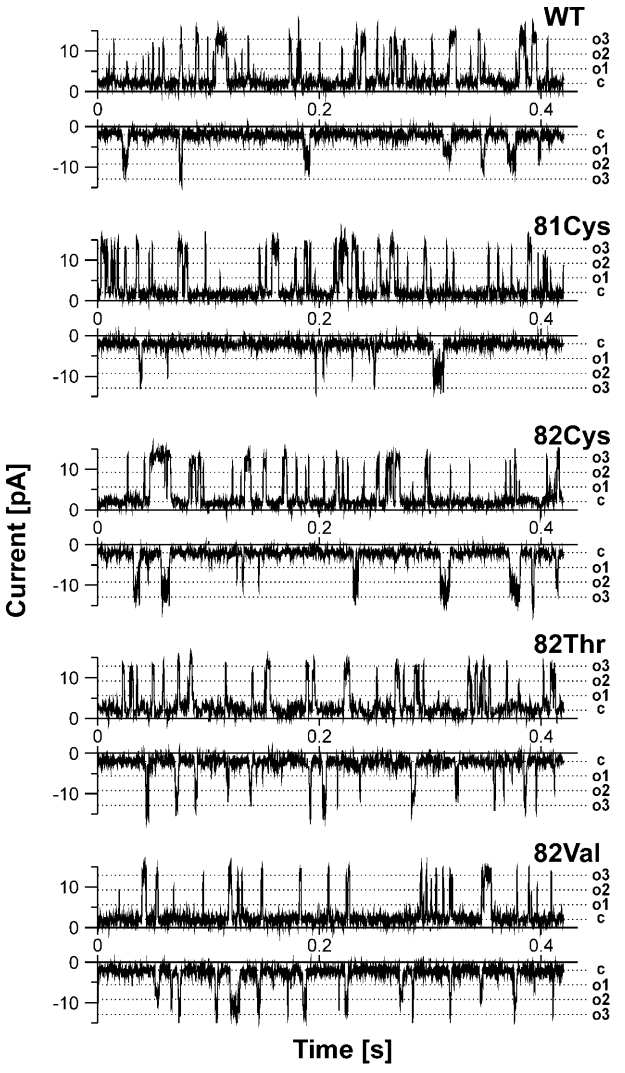

Fig. 2 Single channel current recordings. The WT KcsA and the mutant channels (L81C, Y82C, Y82T or Y82V) were reconstituted in a bilayer (see Methods), and the channel activity at +100 mV or –100 mV was recorded in symmetrical K^+ buffer (250 mM)

Outward K^+ currents were mainly carried by the open3 state. Inward currents also show these three subconductances; however, here the open1 and open2 states are dominant. Therefore the conductance of single channels formed by the mutant proteins was analyzed with respect to the distribution and ratios of subconductance states (Fig. 4).

Detailed analyses showed that the currents of all mutant proteins had WT-like subconductance states, and outwards they were, like the WT KcsA, mainly carried by the open3 state ($\Lambda_3 \approx 97$ pS at $V_h = +100$ mV in 250 mM K^+). The inward current of the mutant L81C channel corresponded to that of the WT channel, and here the open1 state ($\Lambda_1 \approx 41$ pS at $V_h = -100$ mV in 250 mM K^+) dominated. The inward currents of Y82C, Y82T and Y82 V, in contrast, considerably differed as to the relative abundance of each of the three open states (Fig. 2, Fig. 4). Compared to the WT channel, the

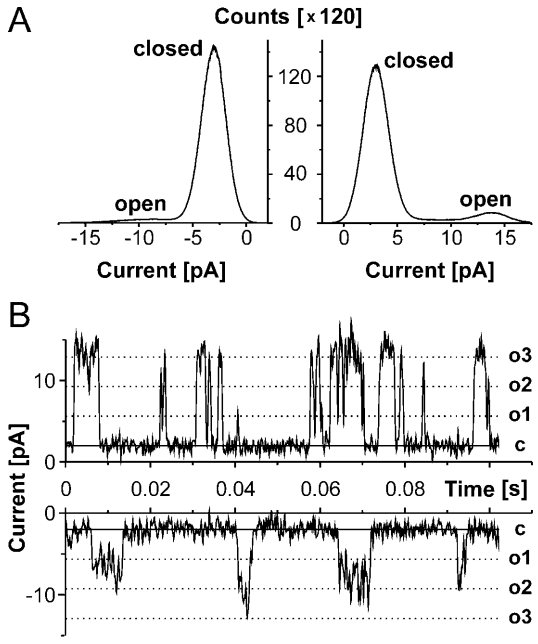

Fig. 3A, B Characteristics of WT and mutant KcsA channels. **A** Open probability below 10%, as shown by the relative areas of the open and closed peaks in all-point histograms at $V_v = -100$ mV (*left*) and $+100$ mV (*right*). **B** Spread current traces represent three subconductance levels at positive (*upper trace*) and negative (*lower trace*) membrane potentials

current of the mutant channel is most frequently carried (Fig. 5B) by open3 (Y82T), open2 (Y82 V) or equally by open1, 2 and 3 (Y82C).

Furthermore, the ratio of open and closed areas within the histograms for the mutant channels at $V_h = -100$ mV (Fig. 6) was two times lower (L81C), nearly identical (Y82C) or about two times higher (Y82V, Y82T), as compared to that of the WT KcsA. The rectification properties diverge scarcely (L81C), little (Y82C) or considerably (Y82T, Y82V) from those of the WT KcsA (Fig. 5A). The mean inward and outward currents are identical for Y82T and Y82V, respectively; thus they have lost the characteristic pronounced outward rectification property of the WT KcsA.

Within each channel type, the TEA binding site has about the same relative position

As reported, the WT KcsA is sensitive to external and internal TEA (Heginbotham et al. 1999; Meuser et al. 1999), whereas the mutants Y82C (Heginbotham et al. 1999) and Y82V (Meuser et al. 1999) used to determine the sideness of the channel were found to be insensitive to externally applied TEA. At the internal side a slow block is caused by TEA ($IC_{50}^{int} = 1.2 \pm 0.3$ mM in 250 mM K^+, $V_h = +100$ mV), reducing the open probability of the channel and leading to a reduced mean current with an unaltered single channel current. The internal block of all mutant proteins was nearly identical to that of the WT channel (Fig. 7A).

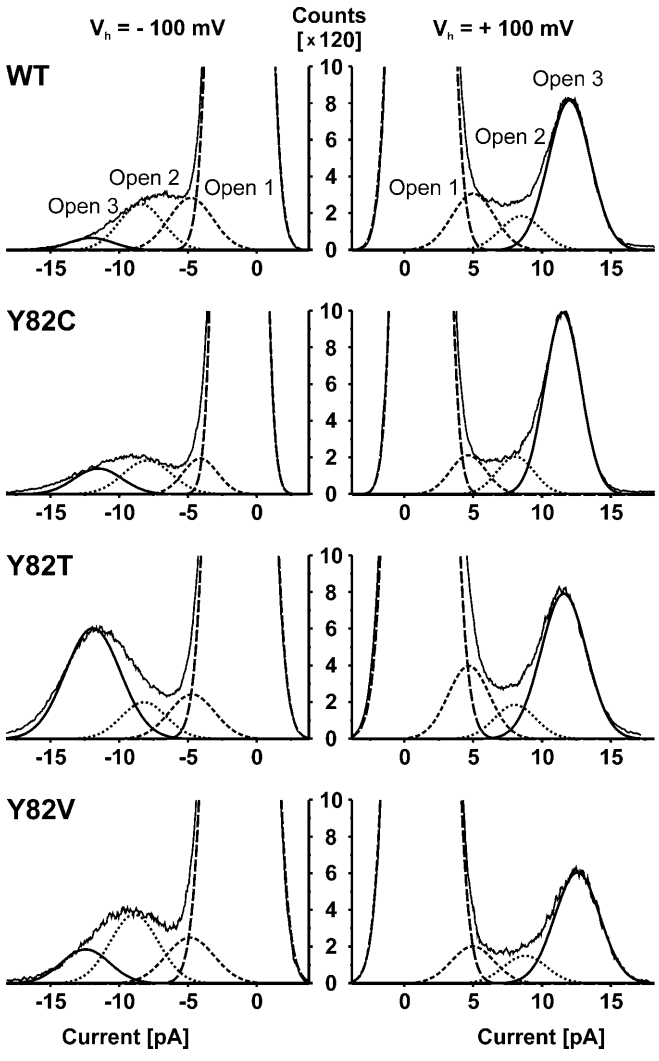

Fig. 4 All-point histograms of the WT KcsA and mutant channels. Analyses were performed at $V_h = -100$ mV (inward currents, *left*) and at $V_h = +100$ mV (outwards currents, *right*) in symmetrical K^+ buffer (250 mM). The total number of counts was 1.2×10^6 in each measured histogram, corresponding to a current trace of 120 s duration at a sampling rate of 10 kHz. The *dashed lines* represent the corresponding Gaussian fit obeying the statistical and significant x^2 and R^2 values

The external block is extremely quick; consequently, owing to instrumental limitations, fast current fluctuations (< 50 μs) could not be resolved and resulted in apparently reduced single channel current amplitudes (for details of the analyses see Methods). The IC_{50} values of the external block of the WT ($IC_{50}^{ex} = 1.7 \pm 0.1$ mM in 250 mM K^+, $V_h = -100$ mV) and of the L81C channel were almost identical; the TEA sensitivities of the Y82 mutant channels, in contrast, were reduced on this side, in the following order: Y82 > Y82C > Y82T > Y82V. Thus the IC_{50} value for TEA was highest for the Y82V channel (Fig. 7A).

The external TEA block for each channel type was dependent on the voltage (Fig. 7B). When analyzing outward currents of the dominating open3 state at positive voltages using the approach of Woodhull

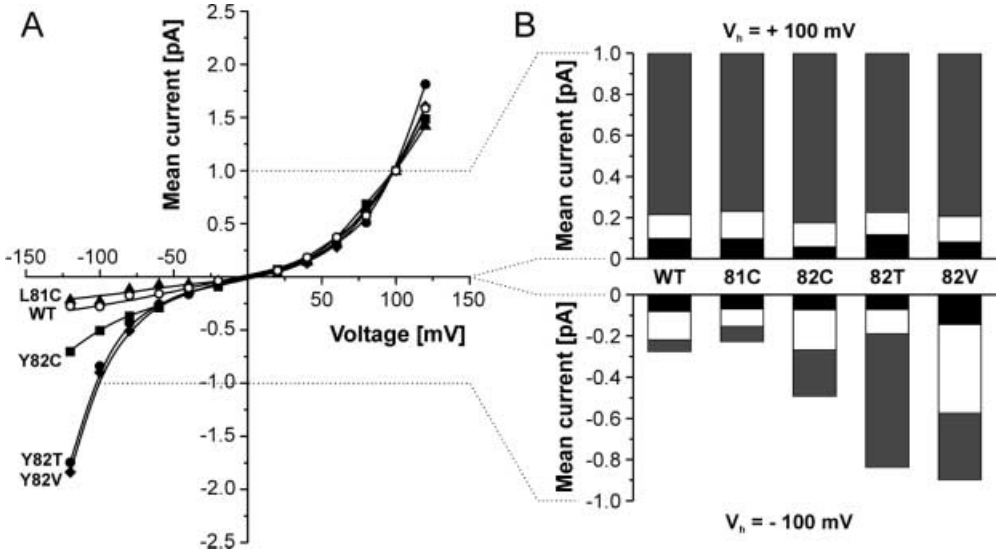

Fig. 5A, B Rectification properties of KcsA. **A** Voltage dependence of mean normalized currents (set to 1 pA at $V_h = +100$ mV). Symbols are: WT (○), L81C (▲), Y82C (■), Y82T (●) and Y82V (◆). The data are given as mean of 3–6 independent measurements. For reasons of clarity, error bars are not shown. **B** The relative contributions of each open state (1, 2 or 3) at $V_h = +100$ mV and $V_h = -100$ mV are presented for the WT KcsA and the mutant channels

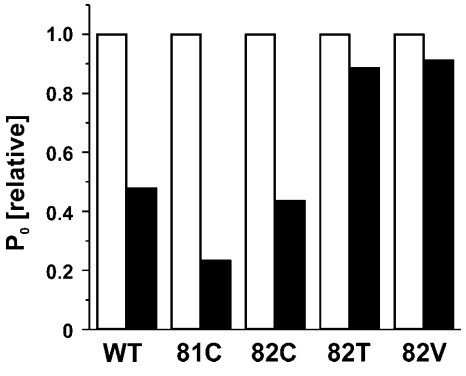

Fig. 6 Relative open probabilities (P_o) of the channels. The channels were analyzed at $V_h = -100$ mV (■) and $V_h = +100$ mV (□), as described in Fig. 4. For all channel types, the ratio of opened configurations (i.e. the sum of areas for open 1, 2 and 3 presented in Fig. 4) at –100 mV and +100 mV were calculated. The value of each channel type at +100 mV was set as 1

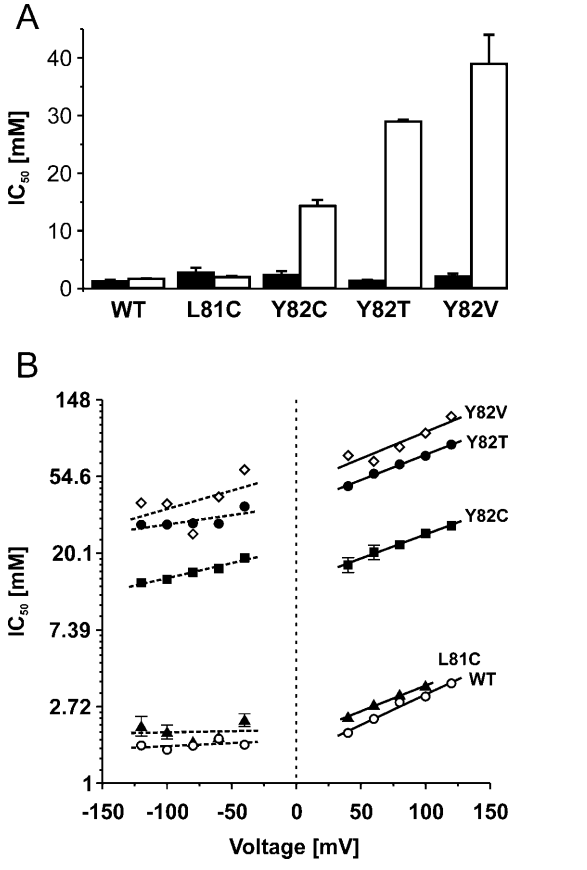

Fig. 7A, B Sensitivity to TEA. **A** For the WT KcsA and the mutant channels, the block was determined for the internal (■, $V_h = +100$ mV) and the external (□, $V_h = -100$ mV) side, respectively. **B** Voltage dependence of the external TEA block. The relative distances (δ) were calculated for the channel types WT (○), L81C (▲), Y82C (■), Y82T (●) and Y82V (◇). Error bars are only given where the standard deviations are larger than the symbol size

(1973), the relative electrical distances (δ) for the external block were: WT (20 ± 2%), Y82V (18 ± 4%), Y82T (17 ± 1%), L81C (17 ± 1%) and Y82C (16 ± 1%).

The TEA block for the inward currents, as deduced from the maximum of the averaged open states 1–3 (see Methods), suggests an apparent shallow site. However, the apparent relative electrical distance for the mutant channels Y82C, Y82V and Y82T was larger than that of the WT and the L81C channels (Fig. 7B).

Discussion

In this study we have demonstrated that each of the mutant KcsA proteins carrying a valine (V), a threonine (T) or a cysteine (C) residue, instead of the aromatic tyrosine (Y) 82 residue, formed a tetramer whose stability was enhanced (Y82V) or reduced (Y82T, Y82C) compared to the WT protein. The presence of a glycine residue in position 82, however, impairs either directly or indirectly the formation of a functional channel. Based on the crystal structure of the KcsA WT channel, the Y82 residue is located in the outer

vestibule of the pore (Doyle et al. 1998). Our data reveal that Y82 contributes to the stability of the KcsA tetramer; the same holds true for several residues situated in the inner pore (T72, V76, G77, Y78, G79) (Splitt et al. 2000). Additional studies have implicated that also the C-terminus contributes to the stability of the tetramer (Perozo et al. 1999). In this context it is interesting to note that, on the basis of molecular dynamics simulations, a salt bridge between D80 and R89 of neighbouring subunits might play an important part in the stabilization of the KcsA tetrameric structure (Guidoni et al. 1999).

As demonstrated previously, KcsA is an intrinsically outward-rectifying channel. Analysis of single channel currents revealed at least three clearly distinguishable subconductance states for inward and outward currents (Meuser et al. 1999). TEA has two external binding sites within the electrical field. For outward currents, the site was deep and had nearly the same electrical distance ($\delta \approx 20\%$) for the WT and the mutant channels. In the inward direction, the TEA binding site is shallow for the WT and the L81C mutant channels. For the mutants Y82C, Y82V and Y82T carrying substitutions at the tyrosine 82 residue, however, the inward block changed in dependence on the voltage. This may be due to a voltage-dependent conformation of the outer vestibule to modify the relative electrical distance of the TEA binding side.

Using the T449Y *Shaker* mutant channel, the diameter of the cage formed by the tyrosine residues trapping one TEA ion has been proposed to amount to approx. 8 Å (Heginbotham and MacKinnon 1992). Within the crystal structure of KcsA, the Y82 residues located at the external vestibule are about 10.8–15 Å apart. However, owing to spatial inhomogeneity the resolution in this crystal region was relatively poor and thus dimensions could not be unambiguously determined (Doyle et al. 1998). As the crystal structure represents the closed form (Meuser et al. 1999), the precise position of Y82 is not yet known for the opened channel. However, our studies show that the extent to which open conformation(s) is (are) stabilized at negative potentials varies in dependence on the amino acid type in position 82.

For inward currents, different occupancies of the three open states were observed for each Y82 mutant channel type. This leads, in company with changes of the open probabilities, to reduction (Y82C) or loss (Y82T, Y82V), respectively, of rectification, in contrast to the L81C mutant which is almost unaffected. The importance of position 82 is underlined by the fact that a substitution by glycine leads to loss of channel activity. It is evident that the amino acid type in position 82 plays a crucial part (directly or due to its interaction with other amino acids) in stabilizing open conformation(s) at negative potentials. This may also explain the varying degrees of rectification.

Based on EPR studies, pH-induced opening of the intracellular part of the pore by conformational changes in the C-terminal part of TM2 was deduced. In contrast, Y82 showed no spin label mobility on shifting from pH 7 to pH 4 (Perozo et al. 1999). Since KcsA is mainly present in a non-conducting state, even at pH 4 ($P_{closed} > 90\%$), it has to be assumed that complete opening of the channel cannot solely be related to the conformational changes deduced from those studies. One explanation would be the existence of a second gate constituted by the selectivity filter and the outer vestibule of the pore.

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References

Armstrong CM, Binstock L (1965) Anomalous rectification in squid giant axon injected with tetraethylammonium chloride. J Gen Physiol 48:859–872

Christie MJ, Adelman JP, Douglass J, North RA (1989) Expression of a cloned rat brain potassium channel in *Xenopus* oocytes. Science 244:221–224

Coetzee WA, Amarillo Y, Chiu J, Chow A, Lau D, McCormack T, Moreno H, Nadal MS, Ozaita A, Pountney D, Saganich M, Vega-Saenz de Miera E, Rudy B (1999) Molecular diversity of K^+ channels. Ann NY Acad Sci 868:233–285

Cuello LG, Romero JG, Cortes DM, Perozo E (1998) pH-dependent gating in the *Streptomyces lividans* K^+ channel. Biochemistry 37:3229–3236

Doyle DA, Cabral JM, Pfuetzner RA, Kuo A, Gulbis JM, Cohen SL, Chait BT, MacKinnon R (1998) The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. Science 280:69–77

Durell SR, Guy HR (1999) Structural models of the KtrB, TrkH, and trk1,2 symporters based on the structure of the KcsA $K(^+)$ channel. Biophys J 77:789–807

Grupe A, Schroeter KH, Ruppersberg JP, Stocker M, Drewes T, Beckh S, Pongs O (1990) Cloning and expression of a human voltage-gated potassium channel. A novel member of the RCK potassium channel family. EMBO J 9:1749–1756

Guidoni L, Torre V, Carloni P (1999) Potassium and sodium binding to the outer mouth of the K^+ channel. Biochemistry 38:8599–8604

Heginbotham L, MacKinnon R (1992) The aromatic binding site for tetraethylammonium ion on potassium channels. Neuron 8:483–491

Heginbotham L, LeMasurier M, Kolmakova-Partensky L, Miller C (1999) Single *Streptomyces lividans* K^+ channels. Functional asymmetries and sidedness of proton activation. J Gen Physiol 114:551–559

Hille B (1967) The selective inhibition of delayed potassium currents in nerve by tetraethylammonium ion. J Gen Physiol 50:1287–1302

Hille B (1992) Ionic channels of excitable membranes. Sinauer, Sunderland, Mass., USA

Jan LY, Jan YN (1997) Cloned potassium channels from eukaryotes and prokaryotes. Annu Rev Neurosci 20:91–123

Kavanaugh MP, Varnum MD, Osborne PB, Christie MJ, Busch AE, Adelman JP, North RA (1991) Interaction between tetraethylammonium and amino acid residues in the pore of cloned voltage-dependent potassium channels. J Biol Chem 266:7583–7587

Lesage F, Guillemare E, Fink M, Duprat F, Lazdunski M, Romey G, Barhanin J (1996) TWIK-1, a ubiquitous human weakly inward-rectifying K^+-channel with a novel structure. EMBO J 15:1004–1011

McClave JT, Dietrich FH III (1996) Statistics. Prentice-Hall, Englewood Cliffs, NJ, USA

Meuser D, Splitt H, Wagner R, Schrempf H (1999) Exploring the open pore of the K^+ channel KcsA from *Streptomyces lividans*. FEBS Lett 462:447–452

Moczydlowski E (1998) Chemical basis for alkali cation selectivity in potassium-channel proteins. Chem Biol 5:291–301

Pascual JM, Shieh C-C, Kirsch GE, Brown AM (1995) Multiple residues specify external tetraethylammonium blockade in voltage-gated potassium channels. Biophys J 69:428–434

Perozo E, Cortes DM, Cuello LG (1999) Structural rearrangements underlying K^+-channel activation gating. Science 285:73–78

Rudy B (1988) Diversity and ubiquity of K channels. Neuroscience 25:729–749

Schrempf H, Schmidt O, Kümmerlen R, Hinnah S, Müller D, Betzler M, Steinkamp T, Wagner R (1995) A prokaryotic potassium ion channel with two predicted transmembrane segments from *Streptomyces lividans*. EMBO J 14:5170–5178

Schroeder JI, Hedrich R (1989) Involvement of ion channels and active transport in osmoregulation and signaling of higher plant cells. Trends Biochem Sci 14:187–192

Splitt H, Meuser D, Borovok I, Betzler M, Schrempf H (2000) Pore mutations affecting tetrameric assembly and functioning of the potassium channel KcsA from *Streptomyces lividans*. FEBS Lett 472:83–87

Woodhull AM (1973) Ionic blockage of sodium channels in nerve. J Gen Physiol 61:687–708

Yokoyama S, Imoto K, Kawamura T, Higashida H, Iwabe N, Miyata T, Numa S (1989) Potassium channels from NG108-15 neuroblastoma-glioma hybrid cells. Primary structure and functional expression from cDNAs. FEBS Lett 259:37–42