Generating a High Affinity Scorpion Toxin Receptor in KcsA-Kv1.3 Chimeric Potassium Channels*

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The crystal structure of the bacterial K⁺ channel, KcsA (Doyle, D. A., Morais, C. J., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T., and MacKinnon, R. (1998) Science 280, 69–77), and subsequent mutagenesis have revealed a high structural conservation from bacteria to human (MacKinnon, R., Cohen, S. L., Kuo, A., Lee, A., and Chait, B. T. (1998) Science 280, 106–109). We have explored this conservation by swapping subregions of the M1-M2 linker of KcsA with those of the S5-S6 linker of the human Kv-channel Kv1.3. The chimeric K⁺ channel constructs were expressed in Escherichia coli, and their multimeric state was analyzed after purification. We used two scorpion toxins, kaliotoxin and hongotoxin 1, which bind specifically to Kv1.3, to analyze the pharmacological properties of the KcsA-Kv1.3 chimeras. The results demonstrate that the high affinity scorpion toxin receptor of Kv1.3 could be transferred to KcsA. Our biochemical studies with purified KcsA-Kv1.3 chimeras provide direct chemical evidence that a tetrameric channel structure is necessary for forming a functional scorpion toxin receptor. We have obtained KcsA-Kv1.3 chimeras with kaliotoxin affinities (IC₅₀ values of ~4 pm) like native Kv1.3 channels. Furthermore, we show that a subregion of the S5-S6 linker may be an important determinant of the pharmacological profile of K⁺ channels. Using available structural information on KcsA and kaliotoxin, we have developed a structural model for the complex between KcsA-Kv1.3 chimeras and kaliotoxin to aid future pharmacological studies of K⁺ channels.

During the last decade, important attention has focused upon T-cell K⁺ channels as potential pharmaceutical targets for modulating immune system function. In particular, the blockade of the voltage-gated K⁺ channel encoded by Kv1.3 inhibits T-cell activation, lymphokine secretion, and cell proliferation (3). Kv1.3 is very scorpion toxin-sensitive (4). These toxins constitute useful molecular tools to study physiological and structural properties of voltage-gated potassium (Kv) channels (5, 6). They form a class of basic peptides containing 30 to 40 amino acid residues highly reticulated with three or four disulfide bridges (6). They are structurally related, sharing a characteristic backbone fold called the cysteine-stabilized α/β motif (6–8). The toxins bind with a 1:1 stoichiometry and may inhibit Kv channel activity by plugging the external pore entryway (2, 6, 9). Amino acid residues between hydrophobic transmembrane segments S5 and S6 (the S5-S6 linker region with the P domain) form the receptor site for scorpion toxin as well as the outer entrance to the Kv channel pore and a substantial part of the ion conduction pathway (2, 9–11). Based on the hypothesis that complementary surfaces of scorpion toxin and Kv channel interact, the known three-dimensional structure of scorpion toxins has been exploited to study the topology of the external mouth of Kv channels, e.g., Shaker and Kv1.3 (11–13). Thermodynamic mutant cycle analysis has been used to identify specific amino acid residues in the S5-S6 linker region as part of the scorpion-toxin receptor site (11–13). The strength of electrostatic and hydrophobic interactions between potentially interacting amino acid residues of Kv1.3 channel and scorpion toxin, e.g., kaliotoxin (KTX)² (9), was shown to influence the affinity of the scorpion toxin to its receptor.

Recent studies have demonstrated a remarkable structural conservation between the pore structures of a prokaryotic K⁺ channel from Streptomyces lividans (14), KcsA, and eukaryotic Kv channels (2). The determination of the three-dimensional structure of KcsA by x-ray analysis has provided the first molecular description of an ion-selective channel (1). Although KcsA subunits contain only two transmembrane segments (M1 and M2) and not six like Kv-channel subunits, KcsA and Kv channels are believed to share essentially the same pore structure. KcsA does not normally bind scorpion toxins. However, mutation of three KcsA channel residues (Q58A, T61S, R64D) in the KcsA pore region sufficed to generate a competent, low affinity agitoxin 2 (AgTX2) binding site with an equilibrium dissociation constant (Kₒ) of about 0.6 μM (2). This value differed by 6 orders of magnitude with the one reported for AgTX2 binding to Kv1.3 (~0.3 pm) (15). Obviously, some energetically coupled residue pairs were absent at the recognition interface needed for a high affinity toxin binding site.

We have generated a high affinity toxin binding site on KcsA using an alternative approach. Various parts of the S5-S6 linker region of Kv1.3 were transferred to KcsA to generate a

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The abbreviations used are: KTX, kaliotoxin; AgTX2, agitoxin 2; DTX, dendrotoxin; HgTX1, hongotoxin 1; Kv, voltage-gated K⁺ channels; MOPS, 4-morpholinepropanesulfonic acid; PAGE, polyacrylamide gel electrophoresis.
panel of KcsA-Kv1.3 chimeras. They were expressed in *Escherichia coli* and, when possible, were solubilized, purified, and assayed for toxin binding. The results demonstrated that KcsA-Kv1.3 channels could be obtained that bound KTX with a *Kd* (~4 pm) similar to the one reported for native Kv1.3 (~3 pm) (15). Also, the results showed that certain Kv1.3 amino acid residues in the S5-S6 linker region were not suitable for transfection to KcsA, possibly interfering with a correct subunit fold and/or subunit assembly. We have used our results in combination with the known structures of KcsA (1) and KTX (9) to construct a three-dimensional structural model for the KTX-KcsA channel complex. The chimeras developed here can also be exploited in putting forward an understanding of potassium channel pharmacology and in developing promising therapeutic agents.

**EXPERIMENTAL PROCEDURES**

**Materials**—For the production of recombinant proteins *E. coli* strains were grown in super broth (25 g of bacteriological peptone, 15 g of yeast extract (Life Technologies, Inc.), and 5 g of NaCl per liter). Antibiotics were from Sigma. Ampicillin and kanamycin were added to final concentrations of 100 µg/ml and 25 µg/ml, respectively. Isopropyl-1-thio-

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\beta\text-d-galactopyranoside \text{ (Calbiochem) were from Roth.}
\]

**E. coli** XL1-Blue (Stratagene) served as host for the propagation of pQE-32 (Qiagen) and constructs; *E. coli* M15 pREP4 (Qiagen) was used for the production of recombinant proteins.

**Construction of KcsA-Kv1.3 Chimeras**—The *K*~c~sA K' channel gene (GenBank® accession number Z37969) was amplified from *S. lividans* strain 66 genomic DNA (DSMZ 46482) using SKC1F and SKC1R primers. At the N terminus and using the polymerase chain reaction (18), or by using the Quickchange (Stratagene). Chimeras II to VIII were prepared by overlap extension methods (20). Sequences of all constructs were verified by sequencing both strands.

**Induction and Purification of KcsA and KcsA-Kv1.3 Chimeras**— *E. coli* M15 pREP4 cells were transformed with constructs as described previously (20). Transformed cells were plated on a LB plate containing appropriate antibiotics, and a single colony was picked for overnight pre-culture. The pre-culture was diluted into 500–1000 ml of super broth (containing antibiotics) to obtain a final absorbance of 0.2 at 600 nm and grown at 30 °C to mid-logarithmic phase. The expression of recombinant proteins was induced with 0.5 mM isopropyl-1-thio-

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\beta\text-d-galactopyranoside. When using membrane preparations of *E. coli*, the incubations were performed in binding buffer B containing 2 mM n-decyl-\beta\text-d-maltopyranoside. When using membrane preparations, the KcsA-Kv1.3 chimeric proteins were incubated at room temperature in binding buffer B containing 2 mM n-decyl-\beta\text-d-maltopyranoside. When using membrane preparations, the KcsA-Kv1.3 chimeric proteins were incubated at room temperature in binding buffer B containing 2 mM n-decyl-\beta\text-d-maltopyranoside.
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\[
\frac{B}{K_d} \times [\text{toxin}] + \frac{B_{\text{free}}}{K_d} = \frac{B_{\text{bound}}}{K_d} (\text{Eq. 3})
\]

\[
B = B_{\text{free}} e^{-k_d t} + B_\text{c}
\]

where *k*~d~ refers to the new binding equilibrium reached after the addition of a large excess of unlabeled toxin. The half-life value is,

\[
t_{1/2} = \ln 2 \frac{B_{\text{bound}}}{K_d}
\]

**Association data were analyzed as a single exponential according to Equation 3 below.**

\[
B = B_{\text{free}} (1 - e^{-k_d t})
\]

\[
B = B_{\text{bound}} + B_{\text{free}} - k_{\text{off}} t
\]

\[
B = B_{\text{bound}} + B_{\text{free}} - k_{\text{off}} t
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RESULTS

Given the reported structural conservation between the pores of bacterial KcsA channels (2) and eukaryotic Kv channels, we explored possibilities of transferring the scorpion toxin receptor site of human (h)Kv1.3 channels to the toxin-insensitive KcsA channels. Accordingly, we replaced parts of the KcsA M1-M2 linker region by a homologous sequence of the Kv1.3 S5-S6 linker region, which most likely contains the complete scorpion toxin receptor. We divided the S5-S6 linker from hKv1.3 into three subregions (Table I) similar to the ones that were previously transferred independently from Kv1.3 to Kv2.1 subunits (25). First, we constructed KcsA-Kv1.3 chimera Chi I, displaying the complete KcsA S5-S6 linker, and Chi II, containing subregions I and III of the linker (see Table I). Isopropyl-1-thio-β-D-galactopyranoside induction of KcsA-Kv1.3 protein expression in E. coli transformed with Chi I or Chi II DNA constructs produced an immediate arrest of bacterial growth followed by cell lysis (data not shown). Then we transferred only subregion I from Kv1.3 to KcsA (chimera Chi III). This time, we achieved chimeric KcsA-Kv1.3 protein expression. The yield of purified Chi III protein, however, was too low (0.03 mg of protein/liter of bacterial culture) for detailed biochemical and pharmacological studies. Next, we expressed KcsA-Kv1.3 chimeras Chi IV and Chi V containing shortened versions of subregion I (Table I). Now, the yields of purified Chi IV and Chi V proteins (1 to 1.5 mg of purified protein/liter of bacterial culture) were comparable with the one of KcsA protein (Table I). Thus, we assayed primarily Chi IV and Chi V preparations for their ability to bind K+ channel toxins. In filter binding assays we showed that Chi IV and Chi V were competent for binding 125I-KTX and 125I-HgTX1-A19Y/Y37F, respectively (Fig. 1, A and B). Similar data were obtained with Chi VI (Fig. 1A), which contained, in addition to subregion I sequences, Met-81 of subregion III (Table I). In contrast, the iodinated scorpion toxins did not bind to wild-type KcsA in agreement with previous data obtained with AgTX2 (2).

The mobility of solubilized and purified Chi IV and Chi V proteins in SDS-PAGE correlated with a molecular mass of 65 kDa (Fig. 2A). This indicated a tetrameric structure for purified Chi IV and Chi V. After boiling in SDS-PAGE sample buffer, Chi IV and Chi V migrated in SDS-PAGE as monomers (Fig. 2A). A similar behavior has been observed with KcsA (Fig. 2A) (21), indicating that the tetrameric channel structure had been disrupted by the heat treatment. The SDS-PAGE results showed that it was possible to prepare Chi IV and Chi V, respectively, either in tetrameric or in monomeric form. Chi IV tetramers and monomers were blotted onto nitrocellulose membranes and tested for their 125I-KTX binding activity. The results showed that 125I-KTX bound to Chi IV tetramers but not to Chi IV monomers (Fig. 2B). It demonstrated that the dissociation of Chi IV tetramers to monomers had also disrupted the toxin receptor site in Chi IV, providing direct biochemical evidence for a tetrameric structure of the scorpion toxin receptor.

The selectivity of Chi IV and Chi V against other K+ channel toxins like apamin, PO5, and α-dendrotoxin (α-DTX) was investigated. Apamin as well as PO5 bind with high affinity to SK channels (26). α-DTX is inactive on Kv1.3 but binds with high affinity to the closely related Kv1.1 and Kv1.2 channels (4). 0.1 μM concentrations of PO5, apamin or α-DTX had no effect on 125I-KTX binding to Chi IV (n = 3). Apamin and PO5 interfered only at 1 μM concentration to some extent with 125I-KTX binding (n = 3) (Fig. 3A). Similar results were obtained using Chi V (not shown). Collectively, the results sug-

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**Table I**

**Sequence and yields of KcsA-Kv1.3 chimeras expressed in E. coli**

In A, alignment of the M1-M2 linker sequence of KcsA (from residue 47 to residue 91) and the S5-S6 linker sequence of hKv1.3 (from residue 364 to 408) is shown. Conserved amino acid residues are in bold. Flanking amino acid residues at the end of S5/M1 and the beginning of S6/M2 membrane-spanning segments are underlined. Numbers on top of the sequence alignment correspond to KcsA amino acid residues. The bracket at the bottom delineates H5-region. Shown in B, C, and D are sequence modifications that were introduced in KcsA-Kv1.3 chimeras. The S5-S6 linker region, which most likely contains the complete scorpion toxin receptor site of human (h)Kv1.3 channels to the toxin-insensitive KcsA channels, was previously transferred independently from Kv1.3 to Kv2.1 subunits (25). First, we constructed KcsA-Kv1.3 chimera Chi I, displaying the complete KcsA S5-S6 linker, and Chi II, containing subregions I and III of the linker (see Table I). Isopropyl-1-thio-β-D-galactopyranoside induction of KcsA-Kv1.3 protein expression in E. coli transformed with Chi I or Chi II DNA constructs produced an immediate arrest of bacterial growth followed by cell lysis (data not shown). Then we transferred only subregion I from Kv1.3 to KcsA (chimera Chi III). This time, we achieved chimeric KcsA-Kv1.3 protein expression. The yield of purified Chi III protein, however, was too low (0.03 mg of protein/liter of bacterial culture) for detailed biochemical and pharmacological studies. Next, we expressed KcsA-Kv1.3 chimeras Chi IV and Chi V containing shortened versions of subregion I (Table I). Now, the yields of purified Chi IV and Chi V proteins (1 to 1.5 mg of purified protein/liter of bacterial culture) were comparable with the one of KcsA protein (Table I). Thus, we assayed primarily Chi IV and Chi V preparations for their ability to bind K+ channel toxins. In filter binding assays we showed that Chi IV and Chi V were competent for binding 125I-KTX and 125I-HgTX1-A19Y/Y37F, respectively (Fig. 1, A and B). Similar data were obtained with Chi VI (Fig. 1A), which contained, in addition to subregion I sequences, Met-81 of subregion III (Table I). In contrast, the iodinated scorpion toxins did not bind to wild-type KcsA in agreement with previous data obtained with AgTX2 (2).

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**Fig. 1.** Binding of 125I-KTX and 125I-HgTX1-A19Y/Y37F to purified KcsA-Kv1.3 chimeras. Solubilized KcsA-Kv1.3 chimeras were incubated with 125I-KTX (A) and 125I-HgTX1-A19Y/Y37F (B). Toxin binding was measured in filter binding assays as described under "Experimental Procedures." Means of the specific binding data are represented as black columns for 125I-KTX (A) and as gray columns for 125I-HgTX1-A19Y/Y37F (B), reported in histograms; vertical bars represent the S.E. in each case.
In this paper, we used KTX to study the scorpion toxin receptor site of Kv1.3 channels. Previous structure-function studies (9–11, 27–30) used KTX or AgTX2 to characterize the scorpion toxin receptor site of Kv1.3 and other Kv channels related to the superfamily of Shaker K⁺ channels (31). AgTX2 and KTX are closely related scorpion toxins differing by only three amino acids. Therefore, it is likely that the interactions of AgTX2 and KTX with K⁺ channels are qualitatively similar, but quantitative differences may exist depending on the spe-
importance of subregion I of KcsA-Kv1.3 chimeras for high Affinity Scorpion Toxin Receptor

3. Importance of subregion I of the S5-S6 linker in KcsA-Kv1.3 chimeras for 125I-KTX binding selectivity. A, columns illustrate the effects of saturating concentrations of K+ channel blockers specific for the apamin receptor (PO5, apamin) (26) or for Kv1.1 and Kv1.2 (α-DTX) (4) on the binding of 125I-KTX to Chi IV, B, alignment of the S5/M1-S6/M2 linker sequences of KcsA, khV1.3, hKv1.4, and hKv1.5 subunits. Beneath, schematic diagram showing subregion I sequences from Kv1.3, Kv1.4, and Kv1.5 that were transferred to KcsA for expression of Chi IV, Chi IX, and Chi X. C, histogram represents the results of binding 125I-KTX to KcsA and chimeras Chi IV, Chi IX, and Chi X, diagrammed in B. Vertical bars represent the S.E. in each case.

Fig. 4. Characterization of 125I-KTX binding to solubilized KcsA-Kv1.3 chimera Chi IV. A, purified KcsA-Kv1.3 chimeras were incubated with 30 pm 125I-KTX in the presence of increasing concentrations of KTX (●), HgTX3 (▲), and AgTX2 (■). 125I-KTX binding results were plotted against toxin concentration. Vertical bars represent the S.E. in each case. Competition binding curves were fit for KTX to an IC50 of 0.6 μM for KcsA-Kv1.3 and 10 μM for Kv1.1. 125I-KTX binding to Chi IV was measured against time as illustrated for 125I-KTX with Chi IV was measured against time as illustrated. The plot of 125I-KTX binding to Chi IV was measured against time as illustrated. The plot of 125I-KTX binding to Chi IV was measured against time as illustrated. The plot of 125I-KTX binding to Chi IV was measured against time as illustrated. The plot of 125I-KTX binding to Chi IV was measured against time as illustrated. The plot of 125I-KTX binding to Chi IV was measured against time as illustrated. The plot of 125I-KTX binding to Chi IV was measured against time as illustrated. The plot of 125I-KTX binding to Chi IV was measured against time as illustrated. The plot of 125I-KTX binding to Chi IV was measured against time as illustrated. The plot of 125I-KTX binding to Chi IV was measured against time as illustrated. The plot of 125I-KTX binding to Chi IV was measured against time as illustrated. The plot of 125I-KTX binding to Chi IV was measured against time as illustrated. The plot of 125I-KTX binding to Chi IV was measured against time as illustrated.

4. Specificity of toxin and K+ channel. It has been shown that the AgTX2 receptor site can be transferred to KcsA channels. Although the complete S5-S6 linker of Kv1.3 could not be transferred, the transfer of specific parts of the S5-S6 linker was sufficient to generate KcsA-Kv1.3 chimeras with a scorpion toxin receptor site of very high affinity. In agreement with numerous indirect structure-function studies, the biochemical studies with purified KcsA-Kv1.3 chimeras showed that KTX binding only took place when the purified protein resembled tetramers. Monomeric chimeras had no binding activity. These results provide direct biochemical evidence that a tetrameric channel structure is necessary for forming a functional scorpion-toxin receptor site.

Previously, mutational analyses have shown that modifications of several residues of the S5-S6 linker region of Kv channels may influence scorpion toxin binding. Our pharmacological studies showed that Chi IV and Chi V had a high affinity for scorpion toxins and were insensitive to other toxins like apamin, PO5, and α-DTX. This suggests that the specificity of the scorpion toxin receptor may depend on the nature of particular subregion I residues. Subregion I sequences are highly variable among Kv channels. Therefore, it is likely that differences in the subregion I sequences may be correlated with the different toxin sensitivities of the various Kv channels. This suggests that subregion I constitutes an important molecular determinant of the pharmacological profile of K+ channels. Three specific modifications of subregion I of KcsA-Q58A, T61S, and R64D produced a low affinity AgTX2 receptor site to which AgTX2 bound with a Kd of 6.6 μM (2). Despite different experimental conditions, it was apparent from our results that the binding affinities of scorpion toxins to KcsA-Kv1.3 chimeras were considerably higher. 125I-KTX bound to Chi IV with a Kd value of 0.3 μM, and AgTX2 displayed in competition experiments an IC50-value of 6.4 μM. The affinity of AgTX2 to Chi IV is similar to the one for Shaker K+ channels (Kd = 1 μM) (29). It has been shown that the presence of G58 (see Table I) at the equivalent position in Kv1.3 (9) or Kv1.2, 1.3, or 1.4 (25) is an important determinant of both AgTX2 and KTX affinity. It is likely that Gly-58 represents an important interaction site between KcsA-Kv1.3 chimeras and scorpion toxins like AgTX2 and KTX. This may also explain the considerably higher affinity of AgTX2 for Chi IV than for KcsA-Q58A.T61S/R64D. A visual inspection of docking KTX to the KcsA-Kv1.3 interaction surface supports our proposition (see below).

In addition to subregion I, we found that the modification Y82H in subregion III of KcsA-Kv1.3 chimeras dramatically...
increased KTX affinity. The IC$_{50}$ ($K_d$) value for binding $^{125}$I-KTX to Chi IV and to Chi VIII were 370 pM and 4 pM, respectively (see Table II). In contrast, a similar modification of Tyr to His at the equivalent position in the S5-S6 linker of Kv1.3 channels was not very significant for KTX affinity and even slightly decreased the KTX affinity of the modified Kv1.3 channels (9). The discrepancy suggests that the vestibules of KcsA-Kv1.3 and of Kv1.3 channels have some different properties. Visual inspection of the KcsA crystal structure showed that the side chains of Tyr-78 and Tyr-82 from adjacent subunits are so close to each other to allow for an intersubunit stacking interaction. Apparently, this interaction contributes to the stability of the tetrameric KcsA structure. In Chi VII and Chi VIII, Tyr-82 had been replaced by histidine. It is likely that the Tyr-78/His-82 intersubunit stacking interaction is energetically weaker, leading to a less compact channel structure. This may result in a better accessibility of KTX to the scorpion toxin receptor site. This hypothesis may also explain why stable Chi VII and Chi VIII tetramers could not be purified in solubilized form like Chi IV and Chi V.

A topological model has recently been proposed for the KTX binding site of Kv1.3. The model was developed using the NMR structure of KTX as a caliper in combination with molecular biological data on important pairs of amino acid side chains participating in Kv1.3 channel-KTX interaction (9, 30). More recently, AgTX2 was docked onto the KcsAQ58A/T61S/R64D channel structure using energetic data borrowed from Shaker $K^+$ channel studies (2). We used the available information in combination with our binding data to assist the docking of KTX to KcsA-Kv1.3 chimeras. Details of the structural model are available on the Internet. The distance between KTX residues Arg-24 and Arg-31 (33Å) is complementary to the one between two Asp-64 residues located at opposite subunits at the rim of the K$^+$ channel vestibule. The equivalent residues in Shaker channels (Asp-431) and in Kv1.3 (Asp-386) have been found to electrostatically interact with toxin residues Arg-24 and Arg-31, respectively (11). KTX was docked to Chi VIII by guiding KTX-Lys-27 into the center of the channel pore bringing KTX-Arg-24 and -Arg-31 close to two Asp-64 channel residues by rotating the toxin around the central pore axis. As for the AgTX2/KcsAQ58A/T61S/R64D pair (2), KTX seems to fit per-

### Table II

| Assays         | Channel | KTX |
|----------------|---------|-----|
| Competition    | Group I |     |
|                | Chi III | 0.18 nM |
|                | Chi IV  | 0.21 nM |
|                | Chi V   | 0.59 nM |
|                | Chi VI  | 1.20 nM |
|                | Chi VII | 3.82 pM |
|                | Chi VIII| 4.26 pM |
| Saturation     |        |     |
|                | Chi IV  | 0.37 nM |
|                | Chi V   | 2.25 nM |
|                | Kv1.3   | 3.1 pM |

For comparison, specific binding of $^{125}$I-KTX to membrane preparations of E. coli expressing Chi VII or Chi VIII and for control KcsA or Chi IV is shown as white columns. For further details see "Results." The $K_d$ values for binding KTX to native Kv1.3 channels (15) have been included for comparison.

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**Fig. 5.** Binding of $^{125}$I-KTX to membrane preparations of E. coli expressing KcsA-Kv1.3 chimeras Chi VII and Chi VIII. A, specific binding of $^{125}$I-KTX to membrane preparations of E. coli expressing Chi VII or Chi VIII and for control KcsA or Chi IV is shown as white columns. For comparison, specific binding of $^{125}$I-KTX to solubilized preparations of KcsA, Chi IV, Chi VII, and Chi VIII is shown as black columns. Vertical bars represent the S.E. in each case. B, membrane preparations with Chi VII (○) or Chi VIII (□) were incubated with 46 pM $^{125}$I-KTX together with increasing concentrations of KTX. $^{125}$I-KTX binding results were plotted against toxin concentration. Vertical bars represent the S.E. in each case. Competition binding curves were fit for KTX to an IC$_{50}$ = 3.82 pM in the case of Chi VII and to an IC$_{50}$ = 4.26 pM in the case of Chi VIII.

**Fig. 6.** $K^+$/Na$^+$ sensitivity of $^{125}$I-KTX binding to KcsA-Kv1.3 chimera Chi IV. The sensitivity of 40 pM $^{125}$I-KTX binding to 5 ng of purified Chi IV toward different concentrations of KCl and NaCl was investigated in filter binding assays. Histograms show the inhibition of binding $^{125}$I-KTX to Chi IV by increasing KCl concentrations (A) and by increasing NaCl concentrations (B). Vertical bars are as in Fig. 5A.
fectly into the vestibule of the KcsA-Kv1.3 chimeras. Strong contacts have been proposed for the AgTX2/KcsAQ58A/T61S/R64D off-center residue pairs (AgTX2-Gly-10, Ala-58), (AgTX2-Arg-24, Asp-64), (AgTX2-Phe-25, Leu-81). Our modeling of the KcsA-Kv1.3 interface suggests additional contacts between KTX and the KcsA-Kv1.3 vestibule. Most importantly, Gly-58 seems to be in close contact to KTX-Phe-25 and Gly-58 of the opposite subunit to KTX-Arg-31. The data strongly demonstrated that the complete scorpion toxin receptor site could be transferred from Kv1.3 to KcsA. The results displayed a high affinity for scorpion toxins. The results of structural modeling and functional data for other Kv channels, i.e. near subregion I residues that may contribute to the specificity of the interaction between toxin and toxin receptor.

In conclusion, the scanning of the M1-M2 linker of KcsA has allowed defined sequences that can be exchanged with equivalent ones of Kv1.3. Recombinant chimeric KcsA-Kv1.3 channels were expressed and purified from E. coli as tetramers. They displayed a high affinity for scorpion toxins. The results demonstrated that the complete scorpion toxin receptor site could be transferred from Kv1.3 to KcsA. The data strongly support the previous notion that the outer pore structures of K+ channels may have been conserved from bacteria to human. The combination of structural modeling and functional data for the interaction of toxins with the K+ channel vestibule can be exploited to advance our knowledge in K+ channel pharmacology. Finally, the possibility of transferring the linker regions of other Kv channels, e.g. Kv1.4 and Kv1.5, should enable us to study the structure of the pore region of those channels in detail as well as the structure of other toxin receptor sites.

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REFERENCES
1. Doyle, D. A., Morais, C. J., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., Chait, B. T., and MacKinnon, R. (1998) Science 280, 106–109
2. MacKinnon, R., Cohen, S. L., Kuo, A., Lee, A., and Chait, B. T. (1998) Science 280, 69–77
3. Cahalan, M. D., and Chandy, K. G. (1997) Curr. Opin. Biotechnol. 8, 749–756
4. Grissmer, S., Nguyen, A. N., Aiyar, J., Hanson, D. C., Mather, R. J., Gutman, G. A., Karmilowicz, M. J., Auperin, D. D., and Chandy, K. G. (1994) Mol. Pharmacol. 45, 1227–1234
5. Miller, C. (1995) Neuron 15, 5–10
6. Tytsga, J., Chandy, K. G., Garcia, M. L., Gutman, G. A., Martin-Eauclaire, M. F., van der Walt, J. J., and Possani, L. D. (1999) Trends Pharmacol. Sci. 20, 444–447
7. Bonette, F., Roumestand, C., Gilquin, B., Menex, A., and Toma, F. (1991) Science 254, 1521–1523
8. Cornet, B., Bonmatin, J. M., Hetru, C., Hoffman, J. A., Ptak, M., and Vovelle, F. (1995) Structure 3, 435–448
9. Aiyar, J., Withka, J. M., Rizzi, J. P., Singleton, D. H., Andrews, G. C., Lin, W. M., Boyd, J., Hanson, D. C., Simon, M., and Dethlefs, B. (1995) Neuron 15, 1169–1181
10. Goldstein, S. A., and Miller, C. (1993) Biophys. J. 65, 1613–1619
11. Ranganathan, R., Lewis, J. H., and MacKinnon, R. (1996) Neuron 16, 131–139
12. Naranjo, D., and Miller, C. (1996) Neuron 16, 123–130
13. Naini, A. A., and Miller, C. (1996) Biochemistry 35, 6181–6187
14. Schrempf, H., Schmidt, O., Kammerlen, B., Hinnah, S., Muller, D., Betzler, M., Steinkamp, T., and Wagner, R. (1995) EMBO J. 14, 5170–5178
15. Helms, L. M., Felix, J. P., Bugiansi, R. M., Garcia, M. L., Stevens, S., Leonard, R. J., Knaus, H. G., Koch, R., Wanner, S. G., Kaczorowski, G. J., and Slaughter, R. S. (1997) Biochemistry 36, 3737–3744
16. Lara, B., Zapater, P., Montiel, C., de la Fuente MT, Martinez-Sierra, R., Ballesta, J. P., Gandia, L., and Garcia, A. G. (1995) Biochem. Pharmacol. 49, 1459–1468
17. Swanson, R., Marshall, J., Smith, J. S., Williams, J. B., Boyle, M. B., Fonderland, K., Laneu, C. J., Antanavave, J., Oliva, C., and Buhrow, S. A. (1990) Nature 349, 292–299
18. Ho, S. N., Hunt, H. D., Horton, R. M., Pullen, J. K., and Pease, L. R. (1989) Gene 77, 51–59
19. Schmidt, T. G., and Skerra, A. (1993) Protein Eng. 6, 109–122
20. Sambrook, J., Fritsch, E. F., and Maniatis, T. (1989) Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY
21. Cortes, D. M., and Perozo, E. (1997) Biochemistry 36, 10343–10352
22. Laemmli, U. K. (1970) Nature 227, 680–685
23. Romi, R., Crest, M., Gela, M., Jacquet, G., Zerrouk, H., Mansuelle, P., Sorokin, O., Van Dorselaer, A., Rochat, H., Martin-Eauclaire, M.-F., and Van Rietochten, J. (1993) J. Biol. Chem. 268, 26302–26309
24. Koschak, A., Bugiansi, R. M., Mitterdorfer, J., Kaczorowski, G. J., Garcia, M. L., and Knaus, H. G. (1998) J. Biol. Chem. 273, 2639–2644
25. Gross, A., Abramson, T., and MacKinnon, R. (1994) Neuron 13, 961–966
26. Sabatier, J. M., Zerrouk, H., Darbon, H., Mabrouk, R., Benslimane, A., Rochat, H., Martin-Eauclaire, M.-F., and Van Rietochten, J. (1995) Biochemistry 34, 2763–2770
27. MacKinnon, R., and Miller, C. (1988) J. Gen. Physiol. 91, 335–349
28. Park, C.-S., and Miller, C. (1992) Neuron 9, 263–264
29. Goldstein, S. A., and Miller, C. (1992) Biophys. J. 62, 5–7
30. Aiyar, J., Rizzi, J. P., Gutman, G. A., and Chandy, K. G. (1996) J. Biol. Chem. 271, 31013–31016
31. Jan, L. Y., and Jan, Y. N. (1997) Annu. Rev. Neurosci. 20, 91–123
