New Binding Site on Common Molecular Scaffold Provides HERG Channel Specificity of Scorpion Toxin BeKm-1*§§

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The scorpion toxin BeKm-1 is unique among a variety of known short scorpion toxins affecting potassium channels in its selective action on ether-a-go-go-related gene (ERG)-type channels. BeKm-1 shares the common molecular scaffold with other short scorpion toxins. The toxin spatial structure resolved by NMR consists of a short α-helix and a triple-stranded antiparallel β-sheet. By toxin mutagenesis study we identified the residues that are important for the binding of BeKm-1 to the human ERG K+ (HERG) channel. The most critical residues (Tyr-11, Lys-18, Arg-20, Lys-23) are located in the α-helix and following loop whereas the “traditional” functional site of other short scorpion toxins is formed by residues from the β-sheet. Thus the unique location of the binding site of BeKm-1 provides its specificity toward the HERG channel.

Functional properties of various proteins are frequently associated with structural domains adopting distinct spatial organization. In general, homologous structural domains, which can be incorporated as a part into large proteins or exist as a separate molecule, construct a specific binding surface responsible for similar biological function or interaction with similar targets. The structural genomic approach uses the information about spatial structure of known functional sites to predict possible spatial structure and function of homologous proteins. However, there are many examples when similarly folded proteins affect the different targets. Moreover, one cannot exclude the possibility that even homological proteins sharing the same molecular scaffold and acting on the structurally similar targets use principally different binding sites stipulating high functional selectivity. In this paper we show that scorpion toxins from the same peptide family with similar folding pattern, acting on structurally related receptors, have different molecular sites for binding with their targets.

The family of scorpion toxins affecting potassium channels (α-KTx) includes highly homological short peptides sharing common α/β scaffold (see for review Refs. 1–4). Most of such potassium channel blockers are able to interact with more than one potassium channel type and have binding sites situated on the β-hairpin (3, 4).

The toxin BeKm-1 isolated from scorpion Butthus euepus is singled out of other characterized α-KTxs by selectively inhibiting HERG channels (5), which are voltage-gated K+ channels, coded by the human ether-a-go-go-related gene. The interest in the HERG channels has increased due to the important role these channels play in different tissues, mainly in shaping the action potential in the heart (see for review Refs. 6 and 7). The HERG channels specify one component of the delayed rectifier that contributes to the repolarization phase of cardiac action potential. One form of inherited long QT syndrome, LQT2, results from genetic defects in herg1 gene and predisposes affected individuals to potentially lethal arrhythmias (8, 9). However most often the same sickness is derived from the nonspecific blockade of cardiac HERG current by various commonly used medications, such as class III antiarrhythmics, antihistaminics, or antipsychotics (10). This undesirable side effect is a major hurdle in the development of new and safe drugs, which may be overcome by the resolution of HERG channel pore structure.

BeKm-1 toxin is a suitable molecular caliper for spatial structure characterization of HERG outer mouth. To elucidate the base of BeKm-1 specificity the NMR study and site-directed mutagenesis of this toxin have been performed allowing the delineation of the toxin surface interacting with the HERG channel.

MATERIALS AND METHODS

NMR Spectroscopy—NMR experiments were performed on a Varian Unity-600 spectrometer with 0.6 ml of 1 m aqueous solution (either in 90% H2O, 10% D2O or in 100% D2O) of recombinant BeKm-1 prepared as described (5) at 30 °C and pH 3.5. The following homonuclear two-dimensional NMR spectra with the watergate (11) scheme for water signal suppression were acquired: DQF-COSY (12), TOCSY (13) with mixing time of 100 and 200 ms. The slowly exchanging amide protons were

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§ The on-line version of this article (available at http://www.jbc.org) contains Supplemental Tables I and II.

The atomic coordinates and structure factors (code 1J5J and 1LGL) have been deposited in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, NJ (http://www.rcsb.org/).

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1 The abbreviations used are: α-KTx, K+ channel-blocking peptides with sequence homology to charybdotoxin; erg, ether-a-go-go related gene; HERG, human ether-a-go-go related gene K+ channel; CD, circular dichroism; HER, human embryonic kidney cells; NOE, nuclear Overhauser effect; NOYES, two-dimensional NOE spectroscopy; DQF, double quantum filtered; TOCSY, two-dimensional total correlation spectroscopy.

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identified at pH 3.5 and 30 °C by reconstituting of lyophilized BeKm-1 in D₂O and immediately recording a series of one-dimensional and TOCSY spectra over 72 h. The values of J₁Hₐₙ, coupling constants were measured from the one-dimensional NMR spectrum in H₂O. The values of J₁Hₐₙ, were obtained by the analysis of patterns of α/β cross-peaks in a DQF-COSY and NOESY spectra recorded in D₂O. NMR spectra were processed using the VNM software (VARIAN) and analyzed with the program XEASY (15).

Spatial structure calculations were performed with the software program DYANA (16). Distance constraints were assembled from hydrogen bond, disulfide bridge, interproton NOE (for upper ones), and absent interproton NOE (for lower ones) constraints. The 323 non-redundant upper distance constraints were derived using CALIBA function of DYANA from the volumes of 536 cross-peaks unambiguously assigned in the 200-ms NOESY spectrum. For proton pairs, the interproton distances of which were less than 3.5 Å in preliminary calculated structures, whereas corresponding cross-peaks were not present in NOESY spectrum, the lower distance constraints were set to 5.0 Å as described in Ref. 17. The disulfide binding pattern (residues 7–28, 13–33, and 17–35) was uniquely determined from preliminary structure calculations (Supplementary Table I) and corresponding distance restraints were introduced. Twenty-three slowly exchanging amide protons were unambiguously assigned as hydrogen bond donors with corresponding hydrogen-acceptor partners on the basis of preliminary structure calculations (the hydrogen bonds were observed in at least 50% of preliminary structures). Corresponding hydrogen bond restraints were employed in subsequent calculation for d(O,N), d(O,H²), d(C,H³) distances in accordance with the angle and distance criteria of hydrogen bonds (18).

Sterespecific assignments and torsion angle constraints for φ, χ², and χ³ were obtained by analysis of local conformation in GRID-SEARCH and GLOMSA functions of DYANA using the available J₁Hₐₙ, and J₁Hₐₙ, spin-spin coupling constants and NOE distance constraints derived from NOESY spectrum with a mixing time of 100 ms. Pseudoatom constraints were utilized in cases when the stereospecific assignment for prochiral centers was unknown. All Xaa-Pro peptide bonds were clearly identified as trans on the basis of characteristic NOEs (19).

In the final calculation, the default DYANA-simulated annealing protocol was applied to 200 random structures, and the resulting 20 structures were selected according to their standard DYANA target function values (16). Constrained energy minimization of the 20 best DYANA structures was performed in the program FANTOM (20) using ECEPP2 potential. The mean structure of the DYANA family was calculated using MolMol (21) and subjected to constrained energy minimization in FANTOM. The quality of the structure was then assessed using PROCHECK (22). The table of structural statistics for the ensemble of 20 lowest function BeKm-1 structures is presented as supplemental data (Supplementary Table II).

Coordinates and experimental restraints for the mean and the ensemble of 20 BeKm-1 structures have been deposited in the Protein Data Bank (PDB accession codes 1J5J and 1LLG, respectively), and 1H chemical shifts have been deposited in BioMagResBank (BMRB accession number 5184).

BeKm-1 Mutant Preparation and Characterization—All mutations were constructed in pEZZ-BeKm-1 plasmid (5). A two-stage PCR mutagenesis protocol was used. For BeKm-1 mutants Q12A, F14A, K18A, R20A, F21A, and K23A two separate PCR reactions were performed to generate two overlapping gene fragments, which then were cloned as templates in PCR to produce the full-length coding region with mutation incorporated as described (23). Constructs were sequenced to verify the presence of only the mutations of interest. The expression and purification of mutated analogues of BeKm-1 were performed as described for wild type toxin (5). The structure of all mutants was confirmed by mass spectrometry performed in matrix-assisted laser desorption ionization time-of-flight spectrometer VISION 2000, Thermo Bioanalysis Corp. The secondary structure of all mutants was tested by CD spectroscopy. CD spectra were recorded using spectropolarimeter J-715 (Jasco). Spectra in the wavelength range 185–250 nm were run at 20 °C in water, with a 0.02-cm quartz cell and a protein concentration of 0.25–0.5 mg/ml.

Determination of Binding Activity—Human embryonic kidney (HEK) cells were cultured and transfected as previously described (24). All experiments were performed on a monolocal stable herg1-HEK cell line. The following solutions were used in the whole-cell patch-clamp recordings (in mM): intracellular, 5.2 CaCl₂, 1.4 MgCl₂, 10 Hepes, 10/30 EGTA/KOH, 110 KCl, pH 7.2, with KOH, extracellular, 2 CaCl₂, 1 MgCl₂, 10 Hepes, 4 KCl, 140 NaCl, 0.1% bovine serum albumin, pH 7.4, with NaOH. Dried toxins were dissolved in extracellular solution, and stocks were stored at −20 °C in glass vials. Patch-clamp recordings were done using the EPC9 patch-clamp amplifier (HEKA Electronics) controlled by HEKA pulse software (25). Pipettes were pulled to a tip resistance of 1.5–3 megohms. Input data were filtered at 1.7 kHz and sampled at 5 kHz. Capacitance transients were automatically canceled (26), and series resistance was compensated by 80%. Cells were plated on 33-mm glass coverslips, which was placed in a 20-μl cell chamber before recording. During experiments the extracellular solution was flowing at a rate of 1–1.5 ml/min, thus the on-rate of the block during toxin application was not limited by solution exchange. The HERG channels were activated and subsequently inactivated by clamping at 10 mV for 400 ms. This pre-pulse was followed by a step to −60 mV to strucure the current. The recording protocol was rate-proportionate. 50 ms, and the holding potential was set to −80 mV. The association constant (Kₐ) and the dissociation constant (Kₑ) were determined by fitting to the time course of the block as described in Ref. 26. The toxin equilibrium dissociation constant (Kₑ) was subsequently calculated (Kₑ/Kₐ). Most alanine mutants were tested at 20 nM. Mutants with particularly low affinity were tested at concentrations of 50 or 100 nM to get a fast on-rate to gain a block of the current that allowed kinetic fitting.

GH3 anterior pituitary cells were cultured as previously described (27). Single Ca²⁺-activated K⁺ channel currents (the single channel conductance determined from the current-voltage relationship was equal to 100 ± 2.5 picosiemens (n = 3)) were recorded at room temperature (20–25 °C) from excised outside-out membrane patches using the whole-cell clamped techniques described previously (28). The bath solution contained (in mM): 140 NaCl, 5 KCl, 2 CaCl₂, 1 MgCl₂, 10 Hepes, adjusted to pH 7.3 with NaOH. Electrodes (2–10 megohms) were filled with a solution containing (in mM): 150 NaCl, 0.1 CaCl₂, 1 MgCl₂, 10 Hepes, adjusted to pH 7.3 with KOH. The signal from the output of amplifier GeneClamp 500 (Axon Instruments, Inc.) with the feedback resistance 10 gigohms was filtered at 0.5–1 kHz and recorded. The data were analyzed using Strathclyde Electrophysiology software developed and generously provided by Dr. J. Dempster (University of Strathclyde, Glasgow, Scotland, UK). The effects of toxins on the channel were measured upon addition of the toxin at different concentrations in 1 × 10⁻⁶–2.6 × 10⁻⁶ m range to the bath solution at least three times for each toxin concentration. The dose-response relationship was fitted using the least square method by the Hill function: Ymax = Ymax, the maximum % inhibition; Kᵦ, the dissociation constant; X, the toxin concentration; n, the Hill coefficient. The following results were obtained for the R27/KF32K mutant at this approximation: Ymax = (99.79 ± 2.38)%; n = 1.43 ± 0.127; Kᵦ = (7.2 ± 0.65) × 10⁻⁶ M.

RESULTS AND DISCUSSION

The a-KTx-like Scaffold of BeKm-1 Is Necessary but Not Sufficient for the Explanation of Toxin Specificity—All potassium channel-blocking toxins purified from scorpion venoms, a-KTx (1, 2), contain 30–40 amino acid residues with three or four disulfide bridges and share high homology in both primary and tertiary-dimensional structures. BeKm-1 is a 36-amino acid peptide displaying the conservative location of cysteine residues (Fig. 1). The solution structure of the recombinant BeKm-1 (5) has been determined using standard NMR methods (29). An ensemble of 20 structures represents the structure of the toxin (Fig. 2). As other a-KTx (4), BeKm-1 adopts a compact fold made up of an α-helix and three β-strands arranged in strongly twisted antiparallel β-sheet. The helix begins from 3₀ turn region 10–13 followed by an α-helical region 14–21 having one proline residue (Pro-15), which distorts the canonical helical structure. The helix is confined by two “caps” previously described in other short scorpion toxins
inhibitory activities of \( H9251 \) and \( H9252 \)–32 –36, respectively, are joined disulfide bridges forming a cysteine-stabilized by Asn-30 and Gly-31. The core of BeKm-1 consists of three –28) connects pre-helix loop and the stabilize their relative positioning; the third one (residues 34). Electrostatic interaction between negatively charged residues 13 –35) affix the helix on the typical for short scorpion toxins (31). Two of the bridges (residues 1–6 is in the “bulge” conformation due to \( \beta \)-2 steric intervention. The two C-terminal \( \beta \)-strands including residues 25–29 and 32–36, respectively, are joined together in a \( \beta \)-hairpin by the canonical type \( \beta \)-turn, formed by Asn-30 and Gly-31. The core of BeKm-1 consists of three disulfide bridges forming a cysteine-stabilized \( \alpha \beta \) motif \((C\alpha C\beta )\) typical for short scorpion toxins (31). Two of the bridges (residues 13–33 and 17–35) affix the helix on the \( \beta \)-sheet and stabilize their relative positioning; the third one (residues 7–28) connects pre-helix loop and the \( \beta \)-sheet together.

Taking into account the similarity of BeKm-1 and other \( \alpha \)-KTx structures it was obvious to suppose that the BeKm-1 inhibition of the HERG channels is governed by the same mechanism as other known scorpion toxins use to block different types of potassium channels. It has been shown for the best characterized \( \alpha \)-KTx (namely, ChTx, IbTx, AgTx2, which interact with members of the Kv1 family of voltage-gated \( K^+ \) channels and/or calcium-activated potassium channels) that binding of these peptides occurs in the outer vestibules of the potassium channels, which share common architecture (3, 32–34). Electrostatic interaction between negatively charged residues in the channel and positively charged residues in the toxin results in physically occluding the pore and blocking ion conduction. It was determined (3, 4) that the common binding site of the potassium channel inhibitors is formed by residues from the C-terminal \( \beta \)-hairpin. It seems likely that the different inhibitory activities of \( \alpha \)-KTx against the targeted channels are due to the presence of different residues on their interaction surfaces. However, the main role in channel blocking was attributed to the essential lysine residue, corresponding to Lys-27 of ChTx and AgTx2 (Fig. 1). It is commonly accepted that the lysine \( \varepsilon \)-amino group is located close to the central axis of the \( K^+ \) channel and mediates the interaction of bound toxin with \( K^+ \) ions in the pore (35–37).

Most unusual in BeKm-1 is the presence of Arg instead of “pore-plugging” Lys in position 27. Moreover, our earlier studies (5) showed that substitutions of Arg-27 and Phe-32 for lysines in the BeKm-1, corresponding to the most conserved amino acid residues of potassium channel blockers from scorpion venoms (2), do not dramatically affect toxin binding with the HERG channel. The data implied that location of the BeKm-1 binding site meant for interaction with the HERG channel and position of the common binding site of known scorpion toxins responsible for recognition of other Kv channels differ. This observation impels further detailed investigation of the BeKm-1 binding site.

**Table I**

| BeKm-1 mutant | \( K_d \) | \( n \) | \( K_d/\text{mut}/K_d/\text{Wt} \) |
|---------------|----------|------|----------------|
| Wild type     | 6.3 ± 1.2| 5    | 1.0           |
| R1A           | 41.9 ± 6.0| 4    | 6.7           |
| P2A           | 10.9 ± 1.4| 6    | 1.7           |
| D4A           | 21.2 ± 3.4| 6    | 3.3           |
| K6A           | 17.2 ± 2.9| 6    | 2.7           |
| E9A           | 4.8 ± 1.2 | 5    | 0.8           |
| Y11A          | 92.4 ± 18.1| 5   | 14.7          |
| Q12A          | 14.3 ± 3.5| 4    | 2.3           |
| F14A          | 51.9 ± 6.4| 4    | 8.2           |
| K18A          | 544.3 ± 46.1| 5  | 86.4          |
| R20A          | 444.4 ± 69.6| 3  | 70.6          |
| F21A          | 328.6 ± 42.3| 3  | 52.2          |
| K23A          | 92.2 ± 37.4| 5   | 14.6          |
| B27A          | 46.7 ± 6.8 | 5   | 7.4           |
| V29A          | 7.4 ± 1.1 | 3    | 1.2           |
| F32A          | 9.1 ± 0.5 | 3    | 1.4           |
| D34A          | 7.2 ± 0.5 | 3    | 1.1           |
| F36A          | 20.2 ± 1.4| 3    | 3.2           |

**The BeKm-1 Interaction Surface**—To delineate the BeKm-1 residues involved in HERG channel interaction, 17 single point mutants with alanine substitution for residues on the molecule surface were produced and tested for inhibition of the HERG channels stably expressed in HEK cells as described under “Materials and Methods.” In Table I the average \( K_d \) values obtained from 3–6 experiments for each mutant are listed. Three of the mutants showed the most significant drop of affinity to the HERG channel: the \( K_d \) values of the K18A, R20A, and F21A mutants were 50–80 times higher than those of the wild type. Alanine substitution of Tyr-11 and Lys-23 also disrupted the toxin-channel interaction remarkably, whereas residues Arg-1, Phe-14, and Arg-27 were moderately important for binding. Substitution of other residues in BeKm-1 to Ala cause a negligible decrease in the mutant’s affinity for the HERG channel.

All mutations but two caused no significant changes in the secondary structure of the toxin variants as inferred from their circular dichroism spectra, which were almost identical to the spectrum of the wild type toxin. The circular dichroism spectra of F21A and R27A mutants showed alterations (Fig. 3), which in turn reflect some spatial structure perturbation leading to a decrease in the inhibitory activity of BeKm-1 mutants. Indeed, as revealed by the BeKm-1 spatial structure (Fig. 2), the aromatic ring of Phe-21 is directed inward to the molecule and participates in the formation of the hydrophobic core of toxin.
located between the helix and β-sheet. Thus, the F21A mutation may indirectly affect the binding surface of the toxin. The substitution R27A leads to smaller changes in the circular dichroism spectrum, which is likely to be connected with redistribution of the charges on the toxin surface that, in turn, may disturb the spatial structure of both the toxin and the toxin binding site. As a result, one cannot safely assume that the side chains of these two residues directly participate in the binding of the toxin to the ERG target. Therefore, in the absence of further data, only Lys-18, Arg-20, Lys-23, and Tyr-11 are concluded to form a surface by which BeKm-1 interacts with the HERG channel.

The ribbon diagrams of BeKm-1 and AgTx2 (37) colored according to the energetic effects of mutations introduced (for color code see Fig. 4) and electrostatic potential (b). The back, front (the same as on Fig. 4), and top views of BeKm-1 are shown in series from left to right. The essential residues are marked. The figure was prepared with MolMol (21).

FIG. 5. The molecular surface of BeKm-1 colored according to the energetic effects of mutations introduced (for color code see Fig. 4) and electrostatic potential. The back, front (the same as on Fig. 4), and top views of BeKm-1 are shown in series from left to right. The essential residues are marked. The figure was prepared with MolMol (21).

FIG. 4. Comparison of the active sites of short scorpion toxins BeKm-1 and AgTx2. The ribbon diagrams of NMR structures are colored according to the effects of alanine mutations of BeKm-1 (PDB code 1LGL) and AgTx2 (PDB code 1AGT (37)) on the binding free energy (per molecule) relative to wild type (wt). The difference of binding free energy was calculated according to Ref. 37. The side chains of the functionally important residues are shown and labeled. Residues not characterized are in light gray. The molecules are shown at the similar view, and chain termini are labeled. The figure was prepared with MolMol (21).

FIG. 3. Circular dichroism spectra of wild type (Wt), F21A, and R27A BeKm-1 mutants. Spectra were recorded at 20 °C with a 0.25–0.3 mg/ml concentration.

FIG. 6. Schematic presentation of the HERG channel subunit. The alignment of outer vestibule region sequences of KcsA (32), Shaker (32), and HERG (41) channels are shown on the top. Bolded residues signify the "XX...GXG" motif forming the selectivity filter (32). The black rectangle indicates the proposed helical region of S5-P linker. On the bottom the sequences of HERG-specific toxins are shown. The homologous residues functionally important for HERG blocking are in bold.

with the strongest positive electrostatic potential in the whole molecule (Fig. 5), which is essential for interaction with the negatively charged outer vestibule of the channel. The aromatic side chains of Tyr-11 and Phe-14 are situated in the hydrophobic patch, which cover most of the helix surface of BeKm-1. The electrostatic field on the β-sheet surface in BeKm-1 is small (Fig. 5) in contrast with other short scorpion toxins, for which the positively charged β-layer is significant for binding to K channels.

The common ability of structurally unrelated toxins such as sea anemone and scorpion toxins (38), dendrotoxins (39), and κ-conotoxin (40) to recognize voltage-activated K channels was associated with the presence of a functional dyad, composed of an essential lysine (postulated to directly interact with the pore) assisted by a 6.6 ± 1.0 Å distant hydrophobic residue whose nature and location may differ somewhat from one toxin to another. It was proposed that such a dyad of residues forms
a minimal conserved functional core which, however, should be completed by few additional residues peculiar to distinct potassium channel-blocking toxin. The binding site of BeKm-1 toxin also is predominantly influenced on the binding lysine residue (Lys-18) accompanied by aromatic amino acid residues (Tyr-11 and Phe-14), the Phe-14 being located at an appropriate distance (about 6.5 Å from Lys-18) to form the functional dyad (Fig. 4). The fact that mutation K18A has most dramatically affected the capacity of BeKm-1 to inhibit the HERG channel (Table I) suggests that this lysine might be the major actor, which plays the role of the essential lysine in “traditional” binding sites of other scorpion toxins, i.e. Lys-27 in AgTx2. Therefore, one can assume that the BeKm-1 residue Phe-14, despite its moderate importance for the binding to the HERG channel, could interact with the conserved hydrophobic rings formed by the network of aromatic amino acid residues surrounding the selectivity filter of the potassium channels (38). Having the same functional residues on the active surface located in accordance with the dyad rule, BeKm-1 does not interact with other voltage-gated potassium channels than HERG. The functional dyad of BeKm-1 likely reflects the common features of interaction as well convergent functional evolution of polypeptide antagonists of voltage-gated potassium channels. The insufficiency of BeKm-1 dyad for toxin binding with other Kv channels supports the important role of additional residues involved in the interaction and influence of topology of active site. Concerning the latter, the observed fact is that the helical scaffold of the BeKm-1 interaction surface is topologically distinct from the β-sheet scaffold of traditional binding site of other scorpion toxins.

Silence of the Traditional Binding Site—Usually scorpion toxins interact with targeted channels by the β-sheet surface whereas the BeKm-1 binding site is transferred to the helix and the following turn. Some differences in the C-terminal part of the BeKm-1 sequence compared with the other α-KTxs resulted in breaking the traditional functional site and made it “keeping quiet.” We found, however, that the double substitution R27K/F32K in BeKm-1 (Fig. 1), restoring the residues typical for the majority of potassium channel-blocking short scorpion toxins, leads to recovery of the binding site generic for the α-KTx family. The R27K/F32K BeKm-1 analogue possessed not only a natural ability to block ERG channels (5) but also had an additional activity; namely, it inhibited native Ca2+-activated K+ channels (conductivity 100 picosiemens) with Kd about 72 nM (measured in GH3 cells, see “Materials and Methods”). Neither wild type BeKm-1 nor mutants with single substitution R27K or F32K inhibited this type of Ca2+ channels. The hydrogen bonds N...H Trp...O...O of Lys-18 accompanied by aromatic amino acid residues forming the selectivity filter rather than the “WW ... GYG” motif (Fig. 6). The hydrogen bonds NH2(Trp)...O...O(Tyr), which stabilize the outer mouth structure of KcsA K+ channel as “molecular spring” (32), are missing in the HERG channel resulting in a more flexible outer mouth structure (41). The turret region of the HERG channel contains an unusually long S5-S linker (43 amino acid residues in comparison with 12–18 residues in other voltage-gated K+ channels, Fig. 6) that quite possibly plays a significant role in HERG channel function. The S5-P linker has been proposed to be responsible for the C-type inactivation processes and the K+Na selectivity of the HERG channel pore (42, 43).

It was reported recently that another ERG-specific toxin ErgTx, isolated from scorpion Centruroides noxius, binds to the outer mouth of the HERG channel, and the S5-P loop segment is responsible for high affinity binding (43, 44).

Despite the considerable sequence differences between ErgTx and BeKm-1 the former has the same residues in the positions equal to Lys-18 and Tyr-11 of BeKm-1 (Fig. 6), which we have shown are important for toxin activity. Furthermore, in both toxins the “pore-plugging” lysine residues in the position equivalent to Lys-27 of α-KTxs are missing. This can indicate similarity of the surfaces of BeKm-1 and ErgTx interacting with the unique outer vestibule structure of the HERG channel. It was suggested (43) that part of the S5-P linker, Ile-583–Gly-594, forms an amphipathic α-helix, which can participate in the binding in BeKm-1. The foregoing helical structure of the BeKm-1 binding site corroborates the preference of a helix-helix interaction. It is possible that Tyr-11 makes contact with the S5-P helix peculiar to the HERG channel and determines the toxin location in the pore. Nevertheless, precise orientation of ErgTx and BeKm-1 in the HERG outer mouth and point-to-point “channel-toxin” interactions can differ. Moreover the role of key Lys-18 of BeKm-1 and its location relative to the channel pore is still unclear. Further cross-mutagenesis studies will be performed to determine the toxin-channel contacting residues. As a result the localization of the toxin in the outer vestibule of the channel in combination with the known spatial structure of BeKm-1 will clarify the topology of the HERG channel pore, which may have substantial influence on the structure-guided development of new safe drugs without nonspecific side effects on the HERG channels.

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