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Interaction Simulation of hERG K^+ Channel with Its Specific BeKm-1 Peptide: Insights into the Selectivity of Molecular Recognition

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Potassium channels show a huge variability in the affinity when recognizing enormous bioactive peptides, and the elucidation of their recognition mechanism remains a great challenge due to an undetermined peptide−channel complex structure. Here, we employed combined computation methods to study the specific binding of BeKm-1 peptide to the hERG potassium channel, which is an essential determinant of the long-QT syndrome. By the use of a segment-assembly homology modeling method, the closed-state hERG structure containing unusual longer S5P linker was successfully constructed. It has a "petunia" shape, while four "petals" of symmetrically distributed S5P segments always decentralize. Starting from the hERG and BeKm-1 structures, a considerably reasonable BeKm-1−hERG complex structure was then screened out and identified by protein−protein docking, molecular dynamics (MD) simulations, and calculation of relative binding free energies. The validity of this predicted complex was further assessed by computational alanine-scanning, with the results correlating reasonably well with experimental data. In the novel complex structure, four considerably flexible S5P linkers are far from the BeKm-1 peptide. The BeKm-1 mainly uses its helical region to associate the channel outer vestibule, except for the S5P linker region; however, structural analysis further implies this neutral pore region with wiggling S5P linker is highly beneficial to the binding of BeKm-1 with lower positive charges. The most critical Lys18 of BeKm-1 plugs its side chain into the channel selectivity filter, while the secondarily important Arg20 forms three hydrogen bonds with spatially neighboring residues in the hERG channel. Different from the classical peptide−K^+ channel interaction mainly induced by electrostatic interaction, a synergetic effect of the electrostatic and van der Waals interactions was found to mediate the molecular recognition between BeKm-1 and the hERG channel. And this specific binding process is revealed to be a dynamic change of reduction of binding free energy and conformational rearrangement mainly in the interface of both BeKm-1 and the hERG channel. All these structural and energy features yield deep insights on the high selective binding mechanism of hERG-specific peptides, present a diversity of peptide−K^+ channel interactions, and also provide important clues to further study structure−function relationships of the hERG channel.

Keywords: A segment-assembly homology modeling • protein−protein docking • MD simulations • BeKm-1 • hERG potassium channel • selective molecular recognition

Introduction

During cardiac repolarization, the hERG (human *ether-a-go-go*-related gene[1]) K^+ channel plays an essential role in mediating the process of returning the membrane potential to its resting status.[2] An inherited mutation in normal human hERG gene could cause a disorder of cardiac repolarization, which may directly lead to long-QT syndrome-related proarrhythmia and sudden death.[3] Moreover, the similar disorder called acquired-long QT syndrome could be triggered by many antiarrhythmic and noncardiovascular medication drugs via blockage of the hERG channel.[4] Thus, hERG has now become a focus target in the pharmaceutical industry for detecting this undesirable side effect.[5]

The hERG channel shares the basic overall transmembrane topology of the voltage-gated K^+ channel (Kv) family.[6] It is composed of four subunits, each containing a voltage-sensing module (S1−S4) and an ion-conducting module (S5-pore loop-S6).[2] However, hERG has an unusually longer S5P linker compared with typical Kv channels (41 amino acids versus 14−23 amino acids),[7] and considerable conformational flexibility of this S5P linker region has been found by circular dichroism (CD) spectropolarimetry and NMR spectroscopy studies recently.[8,9] Many studies showed that this unique S5P linker endowed the hERG channel with novel structure−function features. Mutagenesis studies have proposed the S5P segment

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to be an important determinant of hERG, particularly rapid inactivation kinetics,[7,9,10] which is critical for the role of the channel in maintaining normal cardiac electrical activity and suppressing arrhythmias initiated by ectopic electrical excitation.[11,12] Besides its special electrophysiological function, the S5P linker is also suggested to be responsible to the selective binding of peptide ligands.[13,14] All these indicated that the hERG channel contains a very unique outer pore region different from other Kv channels,[15] due to the presence of a longer S5P linker. But unfortunately, there is no information available concerning the structural architecture of this pore-forming region yet.

Scorpion toxins are short-chain peptides extracted from scorpion venom that could specifically block or modify potassium permeability.[16] A considerable amount of knowledge on the structure and function of channel extracellular surface has been elucidated by using scorpion toxins as molecular probes.[17−19] BeKm-1, a peptide isolated from the scorpion *Buthus eupeus*, shares a similar molecular scaffold with other short scorpion toxins.[20] It could specifically block the hERG channel with an IC_{50} of 3.3 nM but, interestingly, did not inhibit other Kv channels currents even at 100 nM.[21] Subsequently, mutagenesis results imply that critical amino acids Lys18 and Arg20 of BeKm-1 associate with hERG by a novel interaction mode, and possible contacts between Tyr11 of BeKm-1 and residues in the S5P linker were presumed to be responsible for this selective binding,[20,22] but without specification of exact contact pairs or any other powerful evidence, thus, leading to the question, does the seriously flexible S5P linker directly influence or just take part in BeKm-1 binding? Because of the absence of an hERG crystal structure, mapping the precise composition of a channel−toxin complex could provide a framework for a better understanding of the structure−function relationships, especially for clarifying the role of the S5P linker in the selective binding of hERG with peptide ligands and channel gating.

To date, crystallization and determination of K^+ channel structures, not to mention the channel−ligand complex, still suffer from great hurdles.[23] Therefore, predicting protein−protein complex structure by computational approaches, such as docking and molecular dynamics (MD) simulations, has become a good alternative to understand the interaction mechanisms between K^+ channels and peptide inhibitors.[24,25] In view of the unique pore region architecture of the hERG channel and BeKm-1 specificity, this study was undertaken with the intent of exploring the selective mechanism of hERG channel recognition of the BeKm-1 peptide. With the segment-assembling homology modeling method, a reasonable channel pore region structure with the S5P linker was first constructed in the closed state. Then, we screened and identified an equilibrated reasonable structure of the Bekm-1−hERG complex by using a combination of computational methods, including protein−protein docking and MD simulations. On the basis of the final complex structure and simulation results, we elucidated the molecular basis underlying the selectivity of the BeKm-1−hERG interaction from several aspects: the role of hERG S5P in the associating process of BeKm-1, the cooperative driving force of electrostatic and van der Waals interactions in the blockage of BeKm-1 to the hERG channel, and the significant conformation changes in both BeKm-1 and hERG induced by the molecular epitope of interaction. All these also provided some new insight into the structure−function of the hERG channel.

Materials and Methods

Atomic Coordinates and Molecular Docking. The atomic coordinates of BeKm-1 peptide (PDB codes: 1LGL and 1J5J) were downloaded from the Protein Data Bank;[26] it has a globular rigid structure cross-linked by three disulphide bridges (Cys7−Cys28, Cys13−Cys33, and Cys17−Cys35).

The central pore of hERG shows high sequence identity with the crystallized K^+ channel KcsA, except for the S5P segment (Figure 1). On the basis of this, a homologous structure of the pore region of hERG was built using a segment-assessment method consisting of the following procedures: (a) the S5, pore loop, and the S6 segment were separately modeled using the structure of the KcsA channel (PDB code: 1BL8) as a template through the SWISSMODEL server.[27] (b) On the basis of the classically supposed hERG structure,[8,22] an appropriate S5P segment was selected from the 5 ns unrestrained MD trajectories of S5P linker structure (PDB code: 1UJL). (c) All the segments were coassembled into a homotetramer model by fitting into the KcsA structure; an additional 5 ns MD simulation was performed on the model to get an equilibrated and reasonable structure.

All 21 BeKm-1 conformations from NMR were used for improving rigid docking performance of a molecular mocking algorithm ZDOCK, which is a Fast Fourier Transform (FFT)-based, initial-stage rigid-body docking algorithm.[28] The high accuracy of ZDOCK on predicting protein complexes has been proved by its good performances in the CAPRI Challenge.[29] Clustering and the application of biological information along with ZDOCK, followed by a 500-steps energy minimization for each possible toxin−channel complex using the SANDER module in the AMBER 8 suit of programs,[30] as well as calculating the ligand−receptor interaction energies with the ANAL program of AMBER 8, were employed for identifying appropriate candidate complexes for further MD simulations from docking results.

In this work, the membrane around the channel has not been considered during the simulation for several reasons. First, the mutagenesis studies[14,20] indicated that the peptide inhibitors bind to the extracellular part of the potassium channel, where the interaction might not be affected by the membrane. Furthermore, many simulation studies on the recognition between scorpion toxins and potassium channels without membrane included have achieved good agreements with experimental data.[24,25,31] This membrane-ignored treatment also facilitates the computations greatly.

Molecular Dynamics Simulation. All the calculations were performed using the Amber 8 program[32] on a 32-CPU Dawning TC4000L cluster. In our work, the generalized Born (GB) solvation model in macromolecular simulations[33] was used instead of explicit water during more sufficient MD simulation. The ff99 force field (Parm99)[34] was applied throughout the energy minimization and MD simulations.

As for screened docking candidate complexes, the equilibration step is relatively simple for efficiency, which is similar to the one used to predict the ScyTx−K^+ channel complex structure with the same environment settings; 50 ps GB-MD simulations were performed. Force constant restraining for backbone atoms was reduced from 5.0 to 0.25 (kcal/mol)/Å^2. One hundred snapshots every 0.2 ps from the last 20 ps simulations were collected for postprocessing analysis. The most plausible binding mode of the hERG−BeKm1 complex for 21 conformations of BeKm-1 was screened by calculation

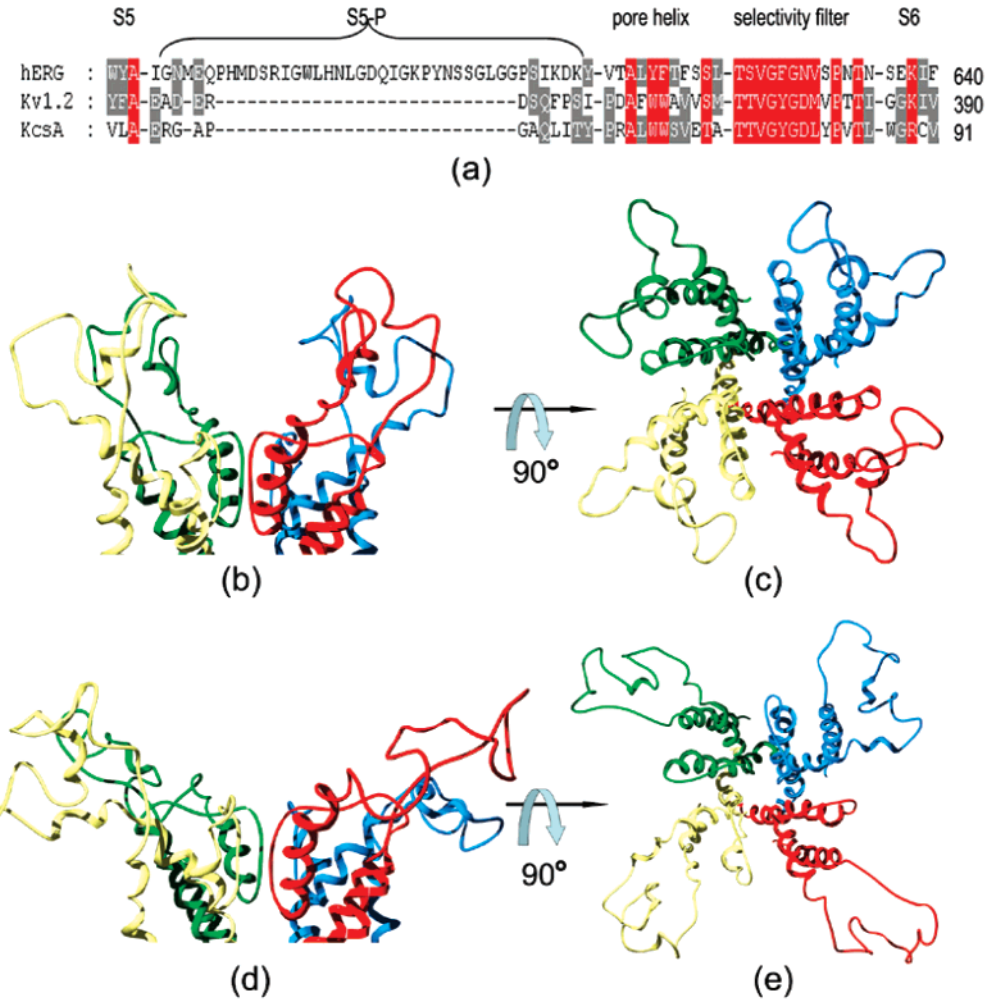

Figure 1. Sequence alignments and view of modeled hERG channels. (a) Sequence alignments of the hERG channel with Kv1.2 and KcsA channels. Red letters show identical residues, whereas gray letters show conservative substitutions. (b and c) Stereoview of the widely recognized hERG channel model from the extracellular side and a perpendicular perspective. (d and e) Stereoview of the final equilibrated hERG structure model from the extracellular side and a perpendicular perspective. Four subunits of tetramer are distinguished by color.

of binding free energy and structural analysis based on the structure−function experiments.[20]

To ensure that each simulation systems is equilibrated enough ahead of data collection and analysis,[35] initially, 360 ps GB-MD (IGB = 2 in the AMBER 8) simulations were performed on the S5P linker, BeKm-1 mutants, and the final complex with a time step of 2.0 fs. Temperature was set at 300 K with the cutoff distance of 12 Å used for nonbonded interaction. We started the equilibration step from a larger force constant of 5.0 (kcal/mol)/Å² for restraining all heavy atoms and then gradually reduced it to 0.02 (kcal/mol)/Å² for only heavy atoms in the backbone.

To introduce enough flexibility into the side chain conformations, 5 ns unrestrained GB-MD simulations were performed on the single S5P linker and BeKm-1 mutants, as well as the four S5P in the hERG structure model. About 8 ns unrestrained GB-MD simulations were performed on the final complex, except residues locating at S5 and S6 segment restrained by a force constant of 0.02 (kcal/mol)/Å², as these segment are buried in the membrane and have no effect on the recognition of BeKm-1. Such hypothesis is based on the result that no conformational change of S5 and S6 helixes was observed by solid-state NMR for the K⁺ channel.[36]

Calculation of Binding Free Energy by MM-PBSA. In the MM-PBSA of AMBER 8.0, the binding free energy of A + B → AB is calculated using the following thermodynamic cycle:

$$\begin{array}{ccc} A_{aqu} + B_{aqu} & \xrightarrow{\Delta G_{binding}} & AB_{aqu} \\ \downarrow -\Delta G^{A}_{solv} & & \downarrow -\Delta G^{B}_{solv} \qquad \downarrow -\Delta G^{AB}_{solv} \\ A_{gas} + B_{gas} & \xrightarrow{\Delta G_{gas}} & AB_{gas} \end{array}$$

$$\begin{aligned} \Delta G_{binding} &= \Delta G_{gas} - \Delta G^{A}_{solv} - \Delta G^{B}_{solv} - \Delta G^{AB}_{solv} \\ &= \Delta H_{gas} - T\Delta s - \Delta G^{A}_{GBSA} - \Delta G^{B}_{GBSA} - \Delta G^{AB}_{GBSA} \\ &= \Delta H_{gas} - T\Delta s - \Delta\Delta G_{GB} - \Delta\Delta G_{SA} \end{aligned} \quad (1)$$

$$\Delta H_{gas} = \Delta E_{gas} = \Delta E_{intra} + \Delta E_{elec} + \Delta E_{vdw} \quad (2)$$

$$\Delta\Delta G_{GB} = \Delta G^{AB}_{GB} - (\Delta G^{A}_{GB} + \Delta G^{B}_{GB}) \quad (3)$$

$$\Delta\Delta G_{SA} = \Delta G^{AB}_{SA} - (\Delta G^{A}_{SA} + \Delta G^{B}_{SA}) \quad (4)$$

where T is the temperature, S is the solute entropy, ΔG_{gas} is the interaction energy between A and B in the gas phase, and ΔG^{A}_{solv}, ΔG^{B}_{solv}, and ΔG^{AB}_{solv} are the solvation free energies of A, B, and AB, which are estimated using a GB surface area (GBSA) method,[32,33,37] that is, $\Delta G^{AB}_{solv} = \Delta\Delta G^{AB}_{GBSA} + \Delta G^{AB}_{GB} + \Delta G^{AB}_{SA}$, and so forth. ΔG_{GB} and ΔG_{SA} are the electrostatic and nonpolar term, respectively. ΔE_{bond}, ΔE_{angle}, and $\Delta E_{torsion}$ are contributions to the intramolecular energy ΔE_{intra} of the complex. E_{vdW} is van der Waals (vdW) interaction energy. Because of the constant contribution of $-T\Delta S$ for each docked complex, we quote $\Delta G^{*}_{binding}$, which is $\Delta G_{binding} + T\Delta S$ in the discussion. To verify the quality and validity of every resulting BeKm-1−hERG complex obtained, the relative binding free energy $\Delta G^{*}_{binding}$ was calculated by using MM-PBSA for postprocessing collected snapshots from the MD trajectories.

Results and Discussion

A comparison of different open-state and closed-state K^+ channel structures emphasizes two important features: (i) the structure of the selectivity filter is highly conserved for the mechanism of selective ion conduction and (ii) the structures of channel domains on the extracellular side also differ very slightly.[15,38] In the pore-forming region, the hERG channel is considerably homologous with KcsA and Kv1.2, except in the S5P linker region (Figure 1a). Therefore, it is rational to only build a partial model of the closed-state hERG channel by using KcsA channel as a template. To predict the BeKm-1−hERG complex structure, the successful structure modeling of the hERG channel with the S5P linker also becomes an obvious prerequisite.

Structural Modeling of hERG Channel and Dynamic Characteristic of S5P Linker. In this work, a segment-assembling homology modeling method was developed for the structural modeling of the intact hERG channel. First, we modeled the hERG channel structure containing the S5 segment (Leu550−Ala570) and the P-S6 segment (Val612−Thr670) by using KcsA as a template. Second, dynamic conformation-sampling was carried out during the 5 ns MD simulation of the S5P segment;[9] several "qualified" residue fragments of S5P were used to gradually assemble the hERG channel structure. Third, energy minimization was performed on the intact hERG channel structure to relieve possible side chain steric clashes and overlaps. Finally, we obtained a widely recognized structure of hERG (Figure 1b,c):[8,22,39] (i) Four symmetrically distributed S5P segments vertically located above the channel pore region; and (ii) the helical domains of the S5P linker all face the symmetry axis of the channel selectivity filter so that S5P possibly takes part in the interaction with the BeKm-1 peptide.

In aqueous solution, the S5P segment presented predominantly a random coil structure instead of well-defined secondary structures. Its central region (Trp585−Ile593) can take a helical conformation only when immersed in a hydrophobic environment.[8,9] Because of actual exposure of the S5P linker to the extracellular environment, additional 5 ns unrestrained MD simulations were performed to investigate dynamic characteristics of the S5P linker in the obtained hERG structure. Surprisingly, the spatial conformation of the final equilibrated hERG channel resembles a "petunia", as shown in Figure 1d,e. More interesting, by iteratively tracking the trajectories of the MD equilibrating process, we found that, along with the gradual disappearance of the helical domain, the conformations of four "petals" of symmetrically distributed S5P segments continuously change without certain rule, and they always appear to decentralize rather than group together; meanwhile, the MD trajectories of four S5P linkers differ distinctly from each other. Such "petunia" shape of the hERG channel is reasonable because the decentralization of the S5P linkers will allow BeKm-1 peptide to associate with the channel pore region and provides possible communication between the S5P linker and other neighboring segment linkers in hERG.[8,14] With the serious flexibility of the S5P linker, an interesting question arises: does the S5P linker influence the interaction between the BeKm-1 peptide and hERG channel?

Screening of Possible Binding Modes by ZDOCK and MD Simulations. ZDOCK, a newly developed rigid protein−protein docking method, had an excellent performance during the past critical assessment of predicted interactions (CAPRI) challenge.[29,40] Here, we applied previous peptide-K^+ channel docking steps to predict BeKm-1−hERG complex structures.[25] Both classical and equilibrated hERG structures are used to investigate the role of the flexible S5P linker during the BeKm-1 binding to the hERG channel. Two main candidates of BeKm-1−hERG channel complex structures, the binding mode I and binding mode II, were selected, where the critical Lys18 and Arg20 residues are used to associate the hERG channel (Figure 2b,d).[20]

To obtain a more native-like complex structure, several nanosecond MD simulations were performed on both candidate complexes with only slightly restraining channel S5 and S6 transmembrane helixes, in order to extend the time scales as necessary to complete the allosteric rearrangement.[24,41] The initial structure of binding mode I in Figure 2b agrees well with previous inferences from mutagenesis results,[14,20,22] and is completely different from AgTx2 and ScyTx which use β and α domain to associate K^+ channel, respectively.[24,25] However, after 800 ps MD simulations, the BeKm-1 peptide in binding mode I shifts unexpectedly from an initial central orientation above the pore entrance to a position over the pore−S6 linker. Moreover, this translocation was even stabilized by strong electrostatic interaction between Arg20 of BeKm-1 and Glu338 of hERG channel (Figure 2b) during the rest simulations. Meanwhile, the most essential Lys18 of BeKm-1 only contacted a single residue, Asn207, in the channel within a contact distance of 4.0 Å. More interestingly, one S5P linker, located just opposite the BeKm-1 peptide, still kept upright, and other three S5P linkers were still recumbent. In contrast to the binding mode I, the BeKm-1−hERG complex had a more highly stable conformation in the binding mode II during the rest 7.2 ns MD simulation time, after BeKm-1 "lays down" above the channel pore region in the first 800 ps MD simulations (Figure 2d,e). At this time, there is obvious interaction between the two critical residues Lys18 and Arg20 of BeKm-1 and the outer vestibule of the hERG channel. The side chain of Lys18 physically occluded into the selectivity filter of the hERG channel, and Arg20 of BeKm-1 was surrounded by several channel residues within a contact distance of 4.0 Å. Here, the comparison of two binding modes showed that the binding mode II is more favorable and stable.

Validity of Final BeKm-1−hERG Complex. The main challenge for computational studies of protein−protein complex structures has been to reproduce a model with sufficient reliability. As validated by experimental results, the computational alanine scanning approach has achieved reasonable success in the identification of near-native structures.[42,43] To validate the binding mode II, we calculated the difference in the binding free energies between mutated and wild-type

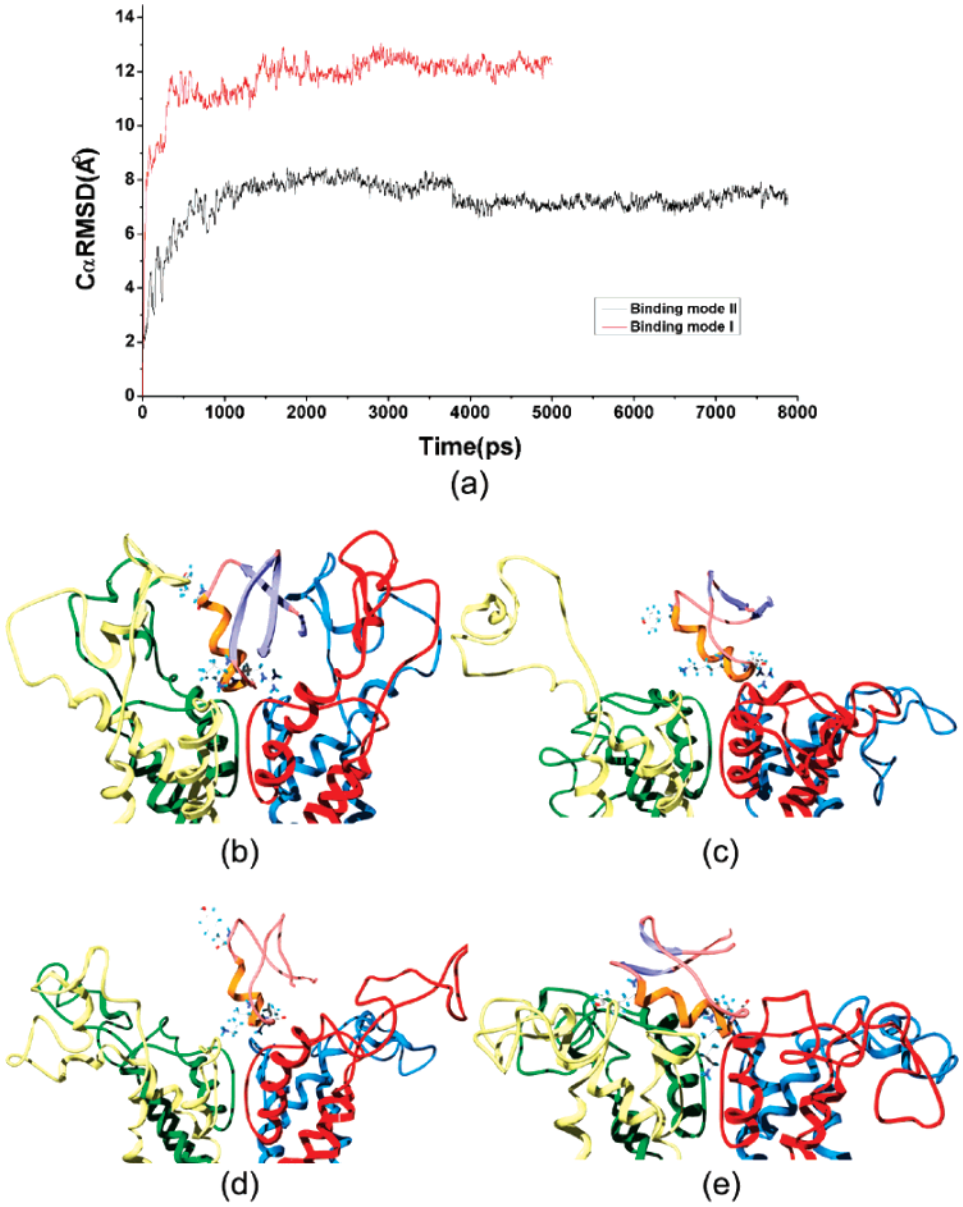

Figure 2. Cα rmsd values of two BeKm-1−hERG binding modes from the initial structure, and the ribbon views of the initial and final complex structures. (a) Root-mean-square deviations (Å) of the α-carbons of BeKm-1−hERG binding mode I (lower line) and binding mode II (upper line) compared with the docked-structure. (b and c) Stereoview of the docked and equilibrated BeKm-1−hERG of binding mode I, respectively. (d and e) Stereoview of the docked and final reasonable BeKm-1−hERG of binding mode II, respectively. The most critical residues Lys18, Arg20, and Tyr11 are labeled in brief in all the structures.

Table 1. Computational Alanine Scanning Mutagenesis Results for the Complex of BeKm-1 with the hERG Channel ($\Delta\Delta G^a = \Delta G_{mutant} - \Delta G_{wild-type}$)

	$Arg^{1}Ala$	$Pro^{2}Ala$	$Asp^{4}Ala$	$Lys^{6}Ala$	$Glu^{9}Ala$	$Tyr^{11}Ala$	$Gln^{12}Ala$	$Phe^{14}Ala$	$Phe^{36}Ala$
Final Complex	−0.18	0.23	−0.56	−0.34	−0.63	4.18	2.02	3.18	−0.01

	$Lys^{18}Ala$	$Arg^{20}Ala$	$Phe^{21}Ala$	$Lys^{23}Ala$	$Arg^{27}Ala$	$Val^{29}Ala$	$Phe^{32}Ala$	$Asp^{34}Ala$
Final Complex	12.32	8.94	0.16	0.77	0.01	0.00	−0.02	−0.06

[a] All energies are in kcal/mol.

equilibrated BeKm-1−hERG complexes ($\Delta\Delta G_{binding}$) by the computational alanine scanning in MM-PBSA.[42] The results are listed in Table 1. Excellent agreement was found between the calculated and experimental data for the complex. Mutating Lys18 to Ala18 in BeKm-1 has the most dramatically affected capacity to inhibit the HERG channel,[20] as it corresponds to the biggest value of 12.32 kcal/mol in the $\Delta\Delta G_{binding}$ among all computational mutations. Substitution of Arg20 in BeKm-1 to Ala causes a significant drop of affinity to the hERG channel (only second to $Lys^{18}Ala$),[20] which agrees well with the bigger value of 8.94 kcal/mol in the $\Delta\Delta G_{binding}$. Meanwhile, high values of 4.18 and 3.18 kcal/mol were found for single point mutants

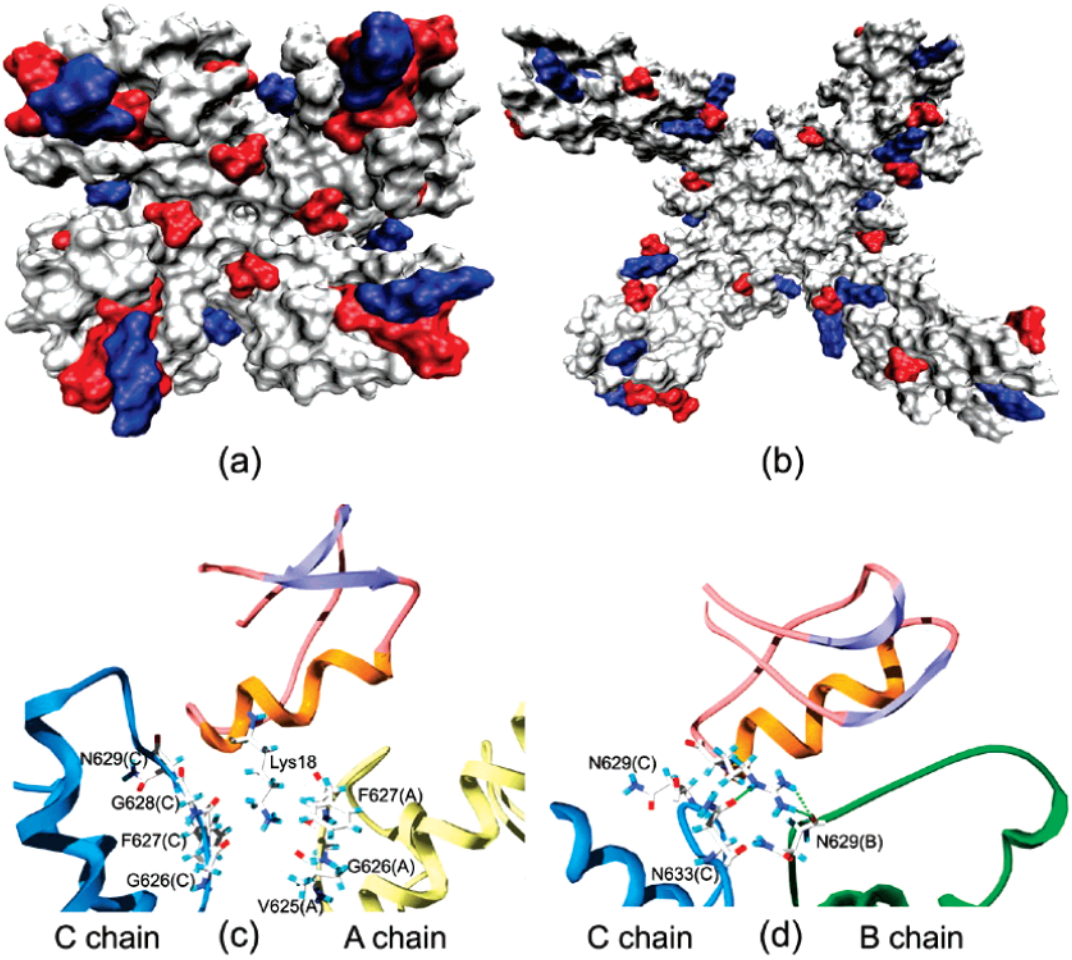

Figure 3. Molecular surface of hERG and Kv1.2 and possible interaction between major function residues of BeKm-1 and residues of the hERG channel within a contact distance of 4.0 Å. (a and b) A stereoview displaying the molecular surface of the Kv1.2 and hERG channel colored according to electrostatic potential properties, with basic residues in blue and acidic residues in red. (c) The Lys18 of BeKm-1 plugging into the pore of the hERG channel. (d) The Arg20 of BeKm-1 surrounded by residues from the pore of the hERG channel. The green line represents a hydrogen bond between two atoms.

of Tyr11 and Phe14 because such modifications disrupted the toxin-channel interaction moderately.[20] As for those alanine substitutions, Asp[4]Ala, Lys[6]Ala, and Phe[36]Ala, for example, which lead to small changes in BeKm-1 affinity, the values of $\Delta\Delta G_{binding}$ show litter differences. Meanwhile, there are several seeming disagreements between experimental data and alanine scanning results, such as the K_d values of the Phe[21]Ala mutant is 50 times higher than that of the wild-type,[20] while the $\Delta\Delta G_{binding}$ of 0.16 kcal/mol cannot imply that Phe21 is important for the binding activity. Actually, when we performed an additional 5 ns unrestrained MD simulation on the modeled BeKm-1−Phe[21]Ala and BeKm-1−Lys[23]Ala structures, significant conformational changes were found not only in the disappearance of secondary structure contents in α-helix and β-sheet, but also in the deviation of the inferred BeKm-1 binding surface. This simulation finding is greatly supported by the CD spectra results,[20] which proposed that the Phe[21]Ala mutant reflected some spatial structure perturbation leading to a decrease in the inhibitory activity. Moreover, after performing the same MD simulation, we detected tiny structural changes in the BeKm-1−Lys[18]Ala conformation, which suggested our MD simulation for verifying the stability of protein structure to be credible. Here, the excellent similarity between computational and experimental results strongly indicated that the binding mode II was a considerably reasonable BeKm-1−hERG complex structure, based on which more structural and functional information of BeKm-1 peptide and hERG channel could be obtained.

Implications from the Unique Interaction between BeKm-1 and hERG Channel. The outer vestibule of K^+ channels are revealed to be the main determinants for specifically recognizing peptide toxin inhibitors,[22] which are widely used for probing the structure of binding interface on target channels.[17,44,45] Compared with a typical Kv channel, the hERG channel has four main different features concerning peptide inhibitor recognition: (a) the existence of an unusually longer S5P linker (Figure 1a); (b) the S5P linker with enough flexibility (Figures 1 and 2); (c) with respect to the abundance of negatively charged residues in the S5P linker of other Kv channels, the hERG S5P has a much lower content (Figure 3 and Table 2), and is far way from the channel pore (Figure 1d and Figure 2e), while in many cases, these negative charged residues locate near the pore entrance of channels and play important roles in recognizing peptide inhibitors by directly contacting them;[24,25,31] and (d) the signature residue Asp in the K^+ selectivity filter of other Kv channels was substituted by Asn629 of hERG channel (Figure 1a). All these suggest a completely unique interaction between BeKm-1 and the hERG channel,

Table 2. Net Charges of Representative K+ Channels and Peptide Inhibitors

name	sequence	total net charge[a]
BeKm-1	MKISFVLLLTLFICSIGWSEARPTDIKCSESYQCFPVCKSRFGKTNGRCVNGFCDCF	2.73
ErgTx1	MKVLILIMIIASLMIMGVEMDRDSCVDKSRCAKYGYYQECQDCCKNAGHNGGTCMFFKCKCA	1.73
CsEKerg1	ERDSCVEKSKCGKYGYYGQCDECCKKAGDRAGTCVYYKCKCNP	2.64
(Human)hERG-S5P	IGNMEQPHMDSRIGWLHNLGDQIGKPYNSSGLGGPSIKDKY	0.72
(Human)hERG-Pore	GFGNVSPNTN	0.00
ChTx	MKILSVLLLALIICSIVGWSEAQFTNVSCTTSKECWSVCQRLHNTSRGKCMNKKCRCYS	5.78
(Rat)Kv1.2-S5P	EADERDSQFPSI	−12.00
(Rat)Kv1.2-Pore	GYGDMVPTTI	−4.00
(Human)Kv1.3-S5P	EADDPTSGFSSI	−12.00
(Human)Kv1.3-Pore	GYGDMHPVTI	−3.64
ScyTx	AFCNLRMCQLSCRSLGLLGKCIGDKCECVKH	2.82
(Rat)SKCa2[b]-S5P	WTVRACERYHDQQDVTSNF	−3.81
(Rat)SKCa2-Pore	GYGDMVPNTI	−4.00

[a] The total net charges of channels are calculated in four subunits of tetramer, at pH 7.0. [b] SKCa2, the small conductance calcium-activated potassium channel 2.

which is different from classical electrostatic interaction between ChTx/AgTx2 and Kv channels.[24,46] Therefore, the structures of the hERG channel and its complex will give us novel insights into the structural and dynamic features that determine the highly selective recognition between the BeKm-1 peptide and hERG channel.

Role of S5P in BeKm-1 Binding. The reasonable BeKm-1−hERG complex structure reveals that the S5P does not determine the binding mode between BeKm-1 and the hERG channel. It is different from the previous studies that proposed the long S5P linker to be the specific BeKm-1 binding site by cysteine substitution experiments.[14] Here, our structures of hERG channel and its complex can explain such discrepancy. As mentioned above, the hERG S5P linker has a strong tendency to "lie down" above the cell membrane whether in closed-state channel or in complex with peptide inhibitors (Figure 1d and Figure 2e). Thus, cysteine side chains ($Gly^{584}Cys$, $Trp^{585}Cys$, $Asn^{588}Cys$, and $Leu^{589}Cys$) introduced into positions of the S5P linker had great possibility to form intersubunit disulfide bonds, such as forming a disulfide bond with its counterpart,[8] or forming disulfide bonds with two Cys (Cys445 and Cys449) in the long S1S2 linker of other channel subunit. Such formation of disulfide bond will produce dimmer of channel subunits and disrupt normal hERG channel function.[8] Although the S5P linker does not finally interact with BeKm-1, it possibly affects the BeKm-1 binding process outside the channel vestibule. During MD simulation of biding mode I (Figure 2b,c), the conformation of the keeping-upright S5P linker undergoes an interesting change, it first becomes flat, together with other three S5P linkers, and then solely return back to upright, even move to get sometime closer to the pore entrance. Almost simultaneously, the BeKm-1 shifts from an initial central position above the pore entrance to a position that is just opposite to this special S5P linker.

Different from the interaction between BeKm-1 and the hERG channel, strong electrostatic forces between more positive charges on classical toxin peptides (e.g., ChTX and AgTx2) and more negative charges on the shorter S5P linkers (Table 2 and Figure 3a,b) of Kv channels can efficiently orient the toxin peptides toward the channel pore.[24,46] However, the unusually longer and more flexible S5P with four Lys/Arg, and four Asp/Glu residues (Figure 3b and Table 2) obviously cannot be helpful in orienting ChTx toward the hERG channel so that there is no interaction between them.[47] On the contrary, there could be less influence on the binding process of specific BeKm-1 peptide with only 2.73 positive charges. Therefore, the S5P linker with unusual length, serious flexibility, and low abundance of negative charged residues is an important determinant for the specificity of BeKm-1 to the hERG channel, and it simultaneously affects the binding process of BeKm-1 peptide.

Novel Interaction between BeKm-1 and hERG Channel. So far, the BeKm-1 binding mode is different from known peptide−K^+ channel interactions. Such novelty is mainly shown as the following: (a) The outer vestibule of the hERG channel is almost uncharged. In addition to the huge difference of charged residue number in the S5P linkers between hERG and Kv channels, the central pore region does not have any acidic amino acid residue in the hERG channel (Figure 3b and Table 2). In the BeKm-1−hERG channel, there are four substituted Asn629 residues in the channel around the pore-blocked Lys18 of BeKm-1 (Figure 3c). However, there are four acidic amino acid residues in the Kv channels which are always around the conserved Lys27 residues of ChTx and AgTx2 peptides, whose residual side chain is plugged into the channel selectivity filter (Figure 3a).[24,48−50] (b) The BeKm-1, with less positive charges, mainly uses its helical domain to associate the hERG channel (Table 2 and Figure 2e). The fact of less positive charges is a common feature of hERG-specific peptides; for example, CsEKerg1 and ErgTx1 only contain 2.64 and 1.78 net charge,[51,52] respectively (Table 2). Therefore, the lower positive charges of hERG-specific peptides and the neutral outer vestibule of the hERG channel (seen in Figure 3b) are most likely to determine the high-selectivity of the peptide−channel interaction. In the BeKm-1−hERG complex, the major functional residues Tyr11, Phe14, Lys18, and Arg20 are distributed in the helical region of BeKm-1 (Table 1 and Figure 2e). The Lys18 exerts its function by plugging its side chain into the channel selectivity filter (Figure 3c), which seems to be a common feature of K^+ channel inhibitory peptides, such as ChTx, AgTx2, and ScyTx.[24,25,48] In Figure 3d, there are three hydrogen bonds between Arg20 of BeKm-1 and Asn629 of hERG channel, which make Arg20 residue a critical residue during BeKm-1 binding to the channel.[20] Although both BeKm-1 and ScyTx mainly use the helical region to associate target channels, the spatial distance between the two critical Lys18 and Arg20 of BeKm-1 is much shorter than that of the two critical R6 and R13 of ScyTx.[25] (c) The electrostatic and van der Waals interactions almost equally mediate the recognition process between BeKm-1 and the hERG channel (Table 3). The electrostatic interaction energy is just about 1.5-fold lower than van der Waals interaction energy, since the main interaction between BeKm-1 and the hERG channel takes place between polar and nonpolar residues (Figure 3b). Such mechanism is completely different from that

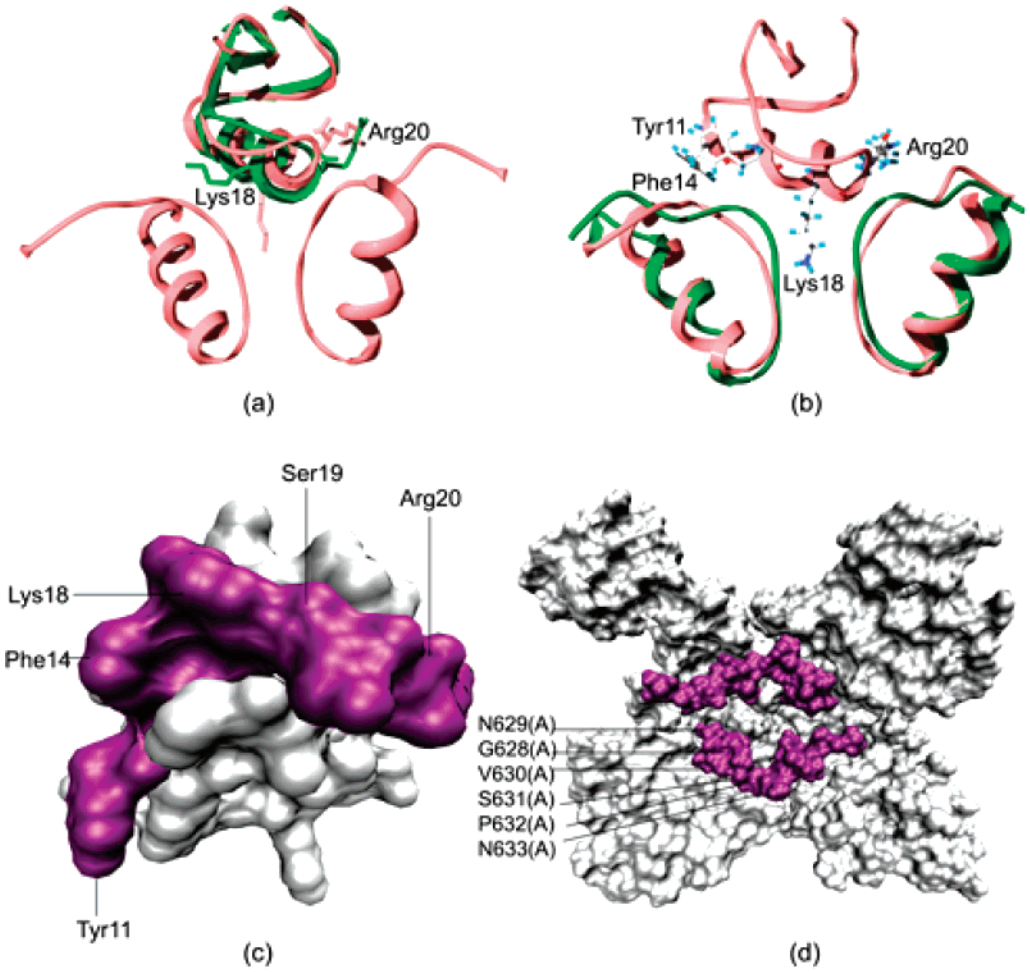

Figure 4. Analysis of conformational changes induced by the interaction of BeKm-1 and hERG channel. (a) Superposition of BeKm-1 free and bound conformations, with side chains of the Lys18 and Arg20 residues, which had the most significant conformational changes, marked in solid bonds. The free BeKm-1 is green, and the bound BeKm-1−hERG complex is pink. (b) Superposition of hERG unbound and bound conformations, marking the residues in BeKm-1 that most possibly induce the channel conformation change. The color scheme is the same as in panel a. (c and d) Surface plots of BeKm-1 and the hERG channel, respectively. Residues characterized by significant MD-conformation changes upon complex formation are purple.

Table 3. Dynamic Change of Relative Binding Free Energies from Representative BeKm-1−hERG Conformations during the Binding Process[a]

complex	ΔEelec	ΔE_{vdW}	ΔE_{inter}	$\Delta\Delta G_{GB}$	$\Delta\Delta G_{SA}$	$\Delta G_{binding}$
Docking Complex	−69.37	−64.76	−134.13	133.77	−7.74	−8.11
Intermediate Conformation	−91.44	−83.37	−174.81	138.88	−10.21	−46.14
Final Conformation	−152.06	−100.94	−253.00	201.30	−12.57	−64.27

[a] All energies are in kcal/mol.

of classical peptides recognizing target channels, whose electrostatic interaction energy should be absolutely dominant.[24,41,46] As for the interaction of ScyTx with its target channel, the electrostatic interaction energy rises to about 3-fold lower than van der Waals interaction energy because there is more negative charge in the outer vestibule of the K^+ channel.[25] Such difference of interactive energies certainly reflects the diversity of the recognition mechanism among different peptides blocking K^+ channels.

In summary, the novel interaction of BeKm-1 with the hERG channel is determined by the unique outer vestibule of hERG, special binding mode of BeKm-1, and synergetic effect of the electrostatic and van der Waals interactions.

Conformation Changes Induced by the Recognition. Recently, Lange et al.[36] propose that the structural flexibility of the K^+ channel and the toxin represents an important determinant for the highly specific toxin−K^+ channel recognition. Such conformational flexibility, induced by the interaction of BeKm-1 with the hERG channel, was also observed in this work. After comparing the BeKm-1−hERG complex from the ZDOCK procedure with the final reasonable structure, we also detect significant structural rearrangements in both BeKm-1 and the hERG channel. The backbone root-mean-square deviation (rmsd) is 1.58 Å for bound and unbound BeKm-1 (Figure 4a). There is remarkable structural change in the Lys18−Asn30 segment of BeKm-1 together with the complete disappearance of β-sheet, which is induced by the interaction with the hERG channel. In addition to the conformational change of BeKm-1 backbone, more significant conformational change is found in residual side chains on the BeKm-1 interface, especially for the

essential residues of Lys18 and Arg20 (Figure 4a). The side chain of Lys18 rotates and extends as long as possible for matching the limited space of selectivity filter during binding to the hERG channel (Figure 4a). The Arg20 residue also rotates with a certain angle and bends its side chain when recognizing its target channel through the formation of three hydrogen bonds (Figures 3d and 4a). Other important residues in BeKm-1 with obvious conformational changes are also labeled in Figure 4c.

As for the hERG channel, the structure of the outer and inner pore helixes is largely maintained after binding BeKm-1, which was also observed in the previous studies.[36] In contrast, considerable structural changes were seen in Val625−Ser636 segment, which forms the channel interface. The rmsd values of backbone and side chain atoms are 1.88 and 2.53 Å between bound and unbound hERG channel, respectively (Figure 4b). Especially large side chain torsions were found for the Gly628−Asn633 segment, which locates in the region that directly interacts with the toxin (Figure 4d). These observations were consistent with the experiment data that mutation of the residue S631 and P632 in hERG channels greatly reduces BeKm-1 affinity.[14] The remarkable conformational flexibility in the GYG (or GFG) of the K^+ ion channels were also described to be the key principle underlying selective K^+ conduction.[38,53]

During the interaction process of BeKm-1 and the hERG channel, the side chains of several residues in both peptide and channel are prealigned in their nanosecond-scale diffusional encounter, and then lead to a specifically stable complex.[41] This conformational arrangement indicated that the contacts between BeKm-1 and hERG were strengthened gradually, and calculations of binding free energies of complexes in the binding process greatly supported this finding. A harmonious stepwise process of energy minimizing was found to be associated with the forming of stable complex (list in Table 3), which demonstrated that a reduction of binding free energy was associated with tighter interaction of BeKm-1 into the target channel. All these strongly suggest that the binding process is also a dynamic process of interactive energy change.

In conclusion, the change of both conformation and interactive energy not only provides the basis for formation of a tight complex with the active site of the K^+ channel, but also may be a crucial prerequisite for the high-affinity ligand binding to an ion channel.

Conclusion

On the basis of a modeled closed-state hERG channel structure, the present work has yielded unprecedented information on the recognition of scorpion toxin BeKm-1 with the hERG channel. Starting from modeling the hERG channel using the segment-assembly method, we found the pore region of the hERG channel presents a "petunia" shape, and four "petals" of symmetrically distributed S5P segments always decentralize. The unusual longer S5P linker was revealed to wiggle above the pore region of the hERG channel because of its intrinsic flexibility. Interestingly, this considerably flexible S5P linker likely affects the binding of BeKm-1 at certain degree, but does not determine the final binding mode of BeKm-1 with the hERG channel. With combined computational methods, we obtained the reasonable BeKm-1−hERG complex structure, which is different from that of the classically supposed model. In the complex structure, the BeKm-1 mainly uses its helical domain to associate target channel, while the Lys18 plugs its side chain into the channel selectivity filter, and another critical Arg20 forms three hydrogen bonds with its spatially neighboring residues of hERG. The binding process of BeKm-1 is equally mediated by the electrostatic and van der Wals interactions, which is determined by the unique and neutral outer vestibule of hERG and lower positive charges of BeKm-1. During their binding process, the significant conformational rearrangement and gradual reduction of interactive energy may be a crucial prerequisite for the highly specific BeKm-1 to target the channel. All these structure and energy characteristics determine BeKm-1 to be a hERG-specific peptide. Simultaneously, these findings can accelerate the research of hERG structure−function relationship and our understanding of the role of the hERG channel in the acquired-long QT syndrome. In addition, our work also shows that the segment-assembly homology modeling method and combined simulation programs are an attractive approach to strongly study molecular mechanism and effectively construct the network of numerous protein−protein interactions.

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