Molecular Docking of the Scorpion Toxin Tc1 to the Structural Model of the Voltage-gated Potassium Channel Kv1.1 from Human Homo sapiens

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Abstract

In this study, structural model of the pore loop region of the voltage-gated potassium channel Kv1.1 from human Homo sapiens was constructed based on the crystallographic structure of KcsA by structural homology. The pore loop region of Kv1.1 exhibits similar folds as that of KcsA. The structural feature of the selectivity filter of Kv1.1 is nearly identical to that of KcsA, whereas most of the structural variations occur in the turret as well as in the inner and outer helices. Molecular docking experiments of the scorpion toxin Tc1 from Titus cambridgei to the outer vestibule of KcsA as well as Kv1.1 were subsequently performed with various initial Tc1 orientations. Tc1 was found to form the most stable complexes with these two K+ channels when the side chain of Lys14 occupies the pore of the selectivity filter through electrostatic interaction. Tc1 binds preferentially towards Kv1.1 than KcsA due to stronger hydrophobic and electrostatic interactions formed between the toxin and the selectivity filter and outer vestibule of Kv1.1. Furthermore, surface complementarity of the outer vestibules of the channels to the Tc1 spatial conformations also plays an important role in stabilizing both the Tc1/KcsA and Tc1/Kv1.1 complexes.

Key words: Potassium channel, Structural homology, Selectivity filter, Molecular docking, Scorpion toxin, Surface complementarity.

Introduction

Ion channels mediate passive ion transport across cell membranes and are involved in the transduction of electric signals and the control of ionic concentrations and membrane potentials (1). Among these, potassium channels are the most diverse group of the ion channel family (2). The recent determination of the crystallographic structure of a bacterial K+ channel from Streptomyces lividans (KcsA) (3) and the subsequent computational studies of the underlying ion permeation mechanisms in KcsA (4-9) have provided the molecular basis towards better understanding of the physical mechanisms controlling ionic selectivity, permeation, and transport through various types of K+ channels (10, 11) and of the structural aspects of blocking effects of various K+ channels. In all cases, the functional K+ channel is a tetramer (12), typically of four identical subunits, folded around a central pore (3). As predicted by the topological studies, each subunit usually has two to six transmembrane (TM) α-helices with the last two connected by the turret, the pore helix, the inner and outer helices, and the selectivity filter to form the pore-loop (P-loop) region (3), which participates directly in the permeation path and contains various binding sites for external and internal TEA block (13-16). Among these structural elements, the selectivity filter has been shown to exhibit two essential features. First, the main chain atoms create a stack of sequential oxygen rings and thus afford numerous closely spaced binding sites of suitable dimensions for coordinating dehydrated K+ ions (3). The second important structural feature is the protein packing around the selectivity filter.

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Together with two Trp residues located in the pore helices, the Tyr side chain from the signature sequence (TVGYG) forms a massive sheet of twelve aromatic residues that is positioned like a cuff around the selectivity filter (3).

Although KcsA is a two membrane-spanning K⁺ channel, its amino acid sequence is actually closer to those of eukaryotic membrane-spanning voltage-gated Kv1 family (3), particularly in the P-loop region. Until recently, corresponding information about the three-dimensional (3D) structures of eukaryotic K⁺ channels was still not available, reflecting difficulties in obtaining sufficient quantities of membrane proteins for crystallization trials (17). Nevertheless, investigators have made dramatic progress in our understanding of K⁺ ion permeation and selectivity from high-resolution electrophysiological experiments and site-directed mutagenesis of specific amino acid residues in the channel sequences (18). In particular, molecular modeling has been a powerful tool for better understanding of the behaviors of these ion channel proteins (19-21).

MacKinnon and Miller (22) have pioneered in the use of a family of scorpion toxins that block the pore to infer the structure of the outer vestibule of the K⁺ channels. Furthermore, these polypeptides have been used extensively in electrophysiological investigation of various K⁺ channels (23-25). Scorpion venoms contain various polypeptides with distinct biological functions that particularly affect the permeability of ion channels (26). These polypeptides possess the potency to recognize ion channels and receptors in membranes and are commonly classified into four groups on the basis of ion-channel types (27). In contrast to the low millimolar affinity for TEA block, these scorpion toxins usually have pico- and nanomolar affinity for binding and block of the Kv K⁺ channels (18). The difference in the affinity could be attributed to the fact that scorpion toxins are significantly larger than TEA, presumably interacting broadly with the channel's outer vestibule and occluding the entryway to the channel pore (28-30).

The newly solved solution structure of the scorpion venom of H. cambridgei (Te1) (Fig. 1), composed of 23 amino acid residues linked with three disulfide bridges, is the shortest known toxin from scorpion venom that recognizes Shaker Kv channels in the brain (31). The 3D structure of Te1 consists of an αβ sandwich, with one short N-terminal α-helix and a double-stranded β-sheet at the C-terminus (31). Molecular dynamics (MD) simulations have been performed towards Te1 and the other two toxins, agatox-2 (32) and charybotoxin (ChTx) (33) from Leucurus quinquestratus var. hebraeus, which share a common fold as well as a common pattern of conformational dynamics. The results revealed that the totally conserved Lys residue, which exhibits minimum flexibility, may be responsible for the interaction with the selectivity filter of the K⁺ channels (34).

There are five Lys residues in Te1, with K14 totally conserved among various K⁺ channel blockers. In order to elucidate which Lys residue of Te1 is possibly involved in direct interaction with the selectivity filter of the K⁺ channels and subsequent block of ion permeation, three sets of experiments were conducted in this study: i) Structural model of the P-loop region of Kv1.1 from human Homo sapiens was constructed based on the crystallographic structure of KcsA (3); ii) Molecular binding of the K⁺ ion to the selectivity filter of Kv1.1 was performed to validate this structural model; and iii) Molecular docking experiments of Te1 towards KcsA as well as Kv1.1 were performed with various initial Te1 orientations, in which the side chain of each of the five Lys residues was directly facing towards the selectivity filter of these K⁺ channels.

**Methods**

**Model Protein**

Structural homology to construct the structural model of the P-loop region of Kv1.1 was based on the 3D model of the full-length KcsA (35) (PDB; accession number
Amino Acid Sequences of Kv1.1

The Kv family can be divided into four subfamilies on the basis of amino acid sequence similarity and function: Shaker (Kv1), Shaw (Kv2), Shaw (Kv3), and Shal (Kv4). Among these, Kv1 family is mostly expressed in the brain, but can also be found in non-excitable cells, such as lymphocytes (37). The amino acid sequences of KcsA and Kv1.1 were obtained from the Swiss-Prot Protein Knowledgebase, which is maintained collaboratively by the Swiss Institute for Bioinformatics (SIB) and the European Bioinformatics Institute (EBI). The detailed information and accession numbers of KcsA and Kv1.1 (38) are listed in Table 1.

Structural Homology

The Homology program of Insight II provides simultaneous optimization of both structure and sequence homologies for multiple proteins in a 3D graphics environment, based on a method developed by Greer (39). Smith-Waterman pairwise amino acid sequence alignments were performed based on the signature sequence (TVGYG) of the selectivity filter from KcsA to find the location of the P-loop region of Kv1 family. The consensus structural conserved regions (SCRs) of the target protein were generated from alignment of Kv1.1 to KcsA. The atomic coordinates were then transferred from KcsA to Kv1.1 in each SCR using Mutation Matrix module of Insight II. Automatic loop building was performed either by database searching (40) or generation through random conformational search (41). The coordinates at the N- and C-termini of these loops were then automatically assigned. Side chains of Kv1.1 were automatically replaced, preserving the conformations of KcsA, then optimized either manually or automatically using a rotamer library (42). Similar secondary structure motifs were identified by database searching and predicted by DSSP (43). The newly built structure of Kv1.1 were substantially refined to avoid van der Waals radius overlapping, unfavorable atomic distances, and undesirable torsion angles using molecular mechanics and dynamics features in Discover module.

Molecular Docking

Docking of a flexible ligand to a protein binding site is a critical step in the process of structure-based drug design. Affinity module of Insight II program automatically docks ligands to receptors identifying low energy orientations of the ligand within the receptor. In other words, for a given assembly, Affinity uses force field based methods to automatically find the best binding modes of the ligand to the receptor. The force field used in the present docking experiments is the CVFF of the Discover module (36). Many cases have been observed where ligand binding is accompanied by some level of conformational change in the protein receptor. Thus, the atoms in the outer vestibule, defined by the selectivity filter and the P-loop region between the inner and outer helices, of KcsA as well as Kv1.1 were not fixed during the entire docking processes. The ligand, either K+ ion or Tc1, was docked with a two-step process. First, initial placements of K+ ion or Tc1 with the side chain of each Lys residue facing towards the selectivity filters of KcsA and Kv1.1 were made using a Monte Carlo type procedure to search both conformational and Cartesian space. Second, a simulated annealing phase was then used to optimize the location of the K+ ion or Tc1 place-
ment. During this phase, the "bulk" of the receptor (defined as the non-movable atoms in the P-loop region) was held rigid, while the non-fixed atoms in the outer vestibule and Tc1 were movable. Interactions between the bulk and movable atoms were approximated by the very accurate and efficient molecular mechanical/grid (MM/Grid) method developed by Luty et al. (44), while interactions among movable atoms were treated using a full force field representation. Solvation effects, which may play a crucial role in the binding of ligands, were also included using the methods of Stouten et al. (45), with a dielectric constant of 80 being used (46).

Results and Discussion

The Role of Kv1.1

The first Kv1 sequence was obtained from Drosophila melanogaster. The Kv1.1 subfamily is expressed in the embryonic nervous system, brain and lymphoid thymocyto precursors. It is also expressed during embryonic development in the mouse, exhibiting two transient peaks of expression around embryonic day 9.5 (E9.5) and E14.5. The Kv1.1 subunits can associate with Kv1.2 and Kv1.4 subunits, especially in the cerebellum. Point mutations in Kv1.1 result in the disruption of this association and episodic ataxia type 1, a rare autosomal dominant neurological disorder characterized by brief episodes of ataxia (47). The detailed information for Kv1.1 K+ channels are summarized in Table I.

Amino Acid Sequence Alignment of KcsA and Kv1.1

The signature sequence, TVGYG, of the selectivity filter for KcsA and Kv1.1 are totally conserved. Most of the sequence variations occur in the turn. Secondary structures predicted by DSSP (43) are very similar to those defined in the crystallographic structure (3), particularly in the pore helix, indicating that not only the amino acid sequences but also the secondary structures are highly conserved in the P-loop region of KcsA and the Kv1 family. It is worthy of mentioning that the P-loop region of KcsA is located near the N-terminus of its polypeptide chain, whereas that of Kv1.1 is located near the C-terminal regions. It supports the tenet that Kv1.1 is composed of two distinct domains: i) the "voltage sensor" domain comprising the N-terminal portion of the channel up to and including the S4 TM segment and ii) the P-loop region encompassing the C-terminal portion from the S5 TM segment onward (48).

Interestingly, the sizes of Kv1.1 is about 3.2 times that of KcsA (Table I), indicating that the highly conserved P-loop region of these two channels are likely to be encoded from the same gene domain inserting into different coding regions in their genomes during evolution. Indeed, KcsA may be the bacterial ancestor of the P-loop region present in Kv1.1 (49). Additional diversity of the Kv1 channels is probably created through additional gene splicing (50) as well as through post-tra-

<table>
<thead>
<tr>
<th>K+ channel</th>
<th>MW (KD)</th>
<th>No. of a.a.</th>
<th>Gene name</th>
<th>Organism source</th>
<th>Swiss-Prot accession number</th>
<th>Full-length seq. identity (%)</th>
<th>P-loop seq. identity (%)</th>
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<td>160</td>
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<td>QS4397</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
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<td>56.5</td>
<td>495</td>
<td>KCNA1</td>
<td>human Homo sapiens</td>
<td>Q90476</td>
<td>8.28</td>
<td>43.28</td>
</tr>
</tbody>
</table>

Figure 4: (A) The superimposition view of the P-loop region of Kv1.1 and the K+ ion during the K+ ion binding process with a total of eight conformational frames. The backbone of the P-loop region of Kv1.1 was shown in blue. The backbone of the outer vestibule of Kv1.1, defined by the non-fixed residues of the selectivity filter and the loop region between the inner and outer helices, and the K+ ion at the first, fifth, sixth,
Figure 1: The averaged NMR solution structure of the scorpion toxin Tc1 from *T. cambridgei* (31) visualized by Insight II program. The N- and C-termini are indicated as N and C, respectively. The N-terminal α-helix (αI) is shown in red cylinder, while the C-terminal β-strands (βI and βII) are shown in yellow arrows directing from the N- to C-terminus. The six Cys residues forming the three disulfide linkages are labeled and represented as ball-and-stick. The side chains of the five positively charged Lys residues are shown in purple sticks.

Figure 2: Ribbon presentations of the top and side views of the structural model of the P-loop region of Kv1.1. The four subunits are shown in blue, green, red, and yellow. The structural features corresponded to those defined in the crystallographic structure of KcsA (3) are indicated. The backbone RMSD of Kv1.1 in Å after superimposing to the crystallographic structure of KcsA is given on the top right corner of this model.

Figure 3: Molecular surfaces of the P domain of (A) KcsA and (B) Kv1.1. In each case the pore helix of the molecular is switched about -25° away from the plane, with the extracellular mouth facing up. The residues are colored according to the Engleman-Stezz hydrophobicity scale (55).
scriptional RNA editing (51). The inherent K+ channel diversity and the regulation of channel formation presumably provide a means for diversification of K+ channel properties so that cells can adapt various gating mechanisms (52).

The Structural Model of the P-loop Region of Kv1.1

Previously, several models of the P-loop region of various K+ channels were built based on the significant sequence identity with KcsA (53, 54). In this study, the structural model of the P-loop region of Kv1.1 was constructed based on the structural model of the full-length KcsA (35) by structural homology and the resulting structure is shown in Figure 2. As expected, KcsA and Kv1.1 share similar folds in the P-loop region, particularly in the pore helix and the selectivity filter. The backbone root-mean-square deviation (RMSD) of the P-loop region of Kv1.1 after superimposing to that of KcsA is 4.82 Å. Most of the structural variations occur in the turret as well as in the inner and outer helices. From the above observations, we can conclude that KcsA and Kv1.1 share nearly identical structural architecture of the selectivity filter with the carbonyl oxygen atoms lined up in the same way. The function of the inner and outer helices is to help the selectivity filter to be fixed in the highly hydrophobic membrane and their amino acid sequences from various K+ channels may be different. The pore helix is more or less responsible for maintaining the structural integrity of the selectivity filter. The turret is extended toward the extracellular environment like a lobster claw and is likely to play an important role in capturing K+ ions from the extracellular environment.

As KcsA and Kv1.1 are both integral membrane proteins, their outer surfaces are exposed to a lipid bilayer environment. One would expect a corresponding anisotropy in the outer surfaces of these channel proteins, as is the case in other integral membrane proteins. Thus visualization of the outer surfaces of the Kv1.1 and comparison with that of KcsA would provide a simple evaluation of the plausibility of the homology model. The distribution of amino acid residues on the surfaces of KcsA and Kv1.1 according to the Engelman-Steitz hydrophobicity scale (55) reveals that these two structures exhibit a segregation of side chains common to most integral membrane proteins (Fig. 3), in which the polar residues are clustered on top of the molecules corresponding to the lipid headgroup/water interfacial region of the bilayer, and there is a broad central band of predominantly hydrophobic side chains (53).

Molecular Binding of K+ ion to the Selectivity Filter of Kv1.1

The binding process of the K+ ion to the selectivity filter of Kv1.1 K+ channel is shown in Figure 4A. The K+ ion was placed 35 Å on top of the structural model in the beginning of the binding process, where eight conformational frames were automatically selected to describe the structural changes of the outer vestibule, defined by the non-fixed residues of the selectivity filter and the loop region between the inner and outer helices. As expected, the turret was extremely flexible during the entire binding process, with one of the four turrets acting like a lobster claw to reach for the K+ ion in the beginning of binding. The strong interaction between the turret and the K+ ion is probably due to the four consecutive negatively charged Asp residues in the turret. Therefore, the first step of the K+ permeation is mainly a process of charge-charge interaction. The K+ ion jumped into the funnel formed by the carbonyl oxygen atoms lined up in the selectivity filter, followed by accommodating itself in the selectivity filter afterward.

The final position of the K+ ion within the selectivity filter of Kv1.1 K+ channel is shown in Figure 4(B). The conformation of the selectivity filter was altered with the backbone RMSD of the selectivity filter being 2.02 Å. It is interesting that the conformations of the two symmetric subunits were distorted asymmetrically after K+ ion binding. The above results are in good agreement with the structure of KcsA crystals in the presence of a low concentration of K+ ions, which reveals that there is a degree
of flexibility in the selectivity filter (56). The conformational change is accompanied by insertion of K⁺ ions between the selectivity filter and the surrounding protein. Furthermore, this result is important in demonstrating that the exact conformation of the selectivity filter is dependent on the nature of its interactions with bound K⁺ ions. The final position of the K⁺ ion was between Val and Gly of the signature sequence, TVGYG, of the selectivity filter, which corresponds to the second K⁺ ion binding site (S₂) defined in the crystallographic structure of KcsA (3). It is therefore suggested that the hydration shell of the K⁺ ion is replaced by eight oxygen atoms of the backbone carbonyl groups when it is within the selectivity filter. In addition, this result strongly supports that most of the K⁺ channels are a multi-ion pore, with a succession of up to six sites in a row capable of binding K⁺ ions. The above K⁺ ion binding results successfully validate the structural model of the P-loop region of Kv1.1 built in this study.

**Molecular Docking of Tc1 towards KcsA and Kv1.1**

An important aspect of ion channels is the inhibition of ion currents by external blockers (57, 58), where the most frequent mechanism of inhibition involves direct mechanistic block of ion permeation pathways by small or medium sized molecules such as toxins (59, 60) or TEA (61). Previous automated docking of TEA to KcsA shows that the positively charged ethyl groups of TEA occupy positions inside the pore of the selectivity filter which partially overlap either with the first or with the fourth K⁺ ion binding sites depending on the binding mode (61). Consequently, this indicates that electrostatic interactions between the blocker and the selectivity filter can be important in determining the blocking mechanism. In the present study, the newly solved scorpion toxin Tc1 was used to investigate the blocking mechanisms of this molecule towards KcsA as well as Kv1.1.

As shown in Figures 5 and 6, docking of Tc1 to the outer vestibules of KcsA and Kv1.1 produces structures, where Tc1 is positioned precisely at the entryway to the selectivity filters with the side chain of various Lys residues occupied the selectivity filter in both cases. In the case of KcsA blocking with the side chain of Lys14 occupying the selectivity filter (Fig. 5D), Lys7, Lys8, and Lys10 of Tc1 may form electrostatic interactions with the four consecutive negatively charged Asp residues of the turret from subunit III, while Lys21 may form electrostatic interactions with the corresponding residues from subunit I. In contrast, in the case of Kv1.1 blocking with the side chain of Lys14 occupying the selectivity filter (Fig. 6D), the side chain of Lys21 was facing towards the selectivity filter instead of forming electrostatic interactions with the turret from subunit I. The above observations clearly indicate that electrostatic interactions are crucial for forming stable blocker/channel complexes. Moreover, as shown in Figure 7, Tc1 may exhibit various conformations to form different spatial complementary complexes when it binds towards various K⁺ channels. Although the overall flexibility of Tc1 is expected to be low due to the three disulfide bridges (34), the two turns in the backbone and the side chains of Lys residues (Fig. 1) are flexible enough to accommodate this molecule on the molecular surfaces of various K⁺ channels through various degrees of electrostatic and hydrophobic interactions. Furthermore, it is obvious that both KcsA and Kv1.1 are inhibited from ion permeation to various extents when the selectivity filter is occupied by the side chain of various Lys residues of Tc1. In the case of Lys14 occupying the selectivity filter, its side chain does not enter to the pore any further and subsequently interact with the other K⁺ ion binding sites because of the steric effect formed by the perpendicular β-hairpin, which in turn connects firmly with the N-terminal α-helix by three disulfide linkages (Fig. 1). Tc1 therefore forms tight contact with the outer vestibules of both KcsA and Kv1.1, where at least two of the four turrets of the channels net like lobster claws to fix this molecule on the extracellular surface through strong electrostatic interactions.

Previously, NMR studies (31) and MD simulations (34) have shown that Lys14 of Tc1, which corresponds to Lys27 in ChTx, exhibited a rigid side-chain conforma-
Figure 5: Ribbon representations of the top and side views of the P-loop region of KcsA after docked by Tc1 molecule with side chain of (A) Lys7; (B) Lys8; (C) Lys10; (D) Lys14; and (E) Lys21 facing towards the selectivity filter of KcsA. Tc1 molecules are illustrated in CPK. The four subunits of each channel, indicating as I, II, III, and IV, are shown in blue, orange, green, and red, respectively. The locations of the outer helix, turret, pore helix, selectivity filter (represented as sticks with the backbone carbonyl oxygen atoms in red), and inner helix corresponding to those in the crystallographic structure of KcsA (3) are indicated. One of the four subunits of the two channels is omitted in the side views for clarity. The five Lys residues of Tc1 molecules are labeled and colored in yellow. The rest of the residues in Tc1 are colored in purple.
Docking of Tc1 to Kv1.1 K+ Channel

Figure 6: Ribbon representations of the top and side views of the P-loop region of Kv1.1 after docked by Tc1 molecule with side chain of (A) Lys7, (B) Lys8, (C) Lys10; (D) Lys14; and (E) Lys21 facing towards the selectivity filter of Kv1.1. Labels and color representations are the same as in Figure 4.

Figure 7: Superimposition views of the backbone of Tc1 molecules prior docking (blue) and after docking with KcaA (yellow) and with Kv1.1 (red) with the side chain of (A) Lys7; (B) Lys8; (C) Lys10; (D) Lys14; and (E) Lys21 facing towards the selectivity filters of these two channels.
tion and highly protruded into the solvent and therefore postulated that it may be the key residue to form electrostatic interactions with the negative charges in the selectivity filter and is likely to play a major role in the blocking mechanism of the Shaker Kv channels. Besides Lys14, the other four Lys residues may play a role in blocking mechanism by interacting with the outer vestibules of KcsA and Kv1.1 or by interacting directly with their selectivity filters (3). In addition, the surface structure indicates that Tc1 contains a dense positively charged region composed of Arg6, Lys7, Lys8, and Lys10 at the N-terminus, which may play an important role in blocking activity (31). At present, no information is available regarding whether this region interacts with the K⁺ channels or whether it plays an important role for activity. Various docking experiments were thus conducted in this study to elucidate the role each Lys residue of Tc1 plays in blocking towards KcsA and Kv1.1. Table II shows the most favorable binding mode of Tc1 towards both KcsA and Kv1.1 is the one with the side chain of Lys14 occupying their selectivity filters, where this complex exhibits the lowest total energy and the most contact area. By comparing the total energies shown in Table II, Tc1 was found to bind preferentially towards Kv1.1, which is consistent with the previous finding that Tc1 particularly recognizes Shaker Kv channels in the brain (31).

Our docking experiments provide new structural information regarding the complexes of Tc1/KcsA and Tc1/Kv1.1. The stabilizing effect of residues with hydrophobic side chains on external TEA binding to KcsA has been previously rationalized (62-64). In our cases, despite the electrostatic interactions formed by the Lys residues with the backbone carbonyl oxygen atoms in the selectivity filter and with the negatively charged surface residues of the outer vestibule, the hydrophobic interactions between Tc1 and the four Asp80 (Asp377) residues from the four subunits of KcsA (Kv1.1) also play an important role in stabilizing the Tc1/KcsA (Tc1/Kv1.1) complex (65). In addition, it is noteworthy that both Lys14 and Lys21 were involved in the interaction with the selectivity filter of Kv1.1, whereas only Lys14 was involved in the interaction with that of KcsA. Besides, the hydrophobic and electrostatic interactions formed by Lys10 of Tc1 with Glu350 and Glu351 from subunit III of Kv1.1 also play an important role in stabilizing the Tc1/Kv1.1 complex (65). These stronger electrostatic and hydrophobic interactions result in tight binding of Tc1 towards Kv1.1 than KcsA (Table II). The present results are strongly consistent with the previous findings that not only the electrostatic interactions but also the hydrophobic interactions between the blockers and the K⁺ channel are crucial in the blocking mechanism (31, 34, 62-64).

The docking experiments performed in this study have successfully identified the most favorable binding modes of Tc1 towards KcsA and Kv1.1. In both cases, in addition to the direct blocking of the selectivity filter of the K⁺ channels by the side

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**Table II**

The parameters for various Tc1/KcsA and Tc1/Kv1.1 complexes after docking.

<table>
<thead>
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<th>Complex</th>
<th>Binding mode</th>
<th>RMSE (Å)</th>
<th>Contact area (Å²)</th>
<th>Energy (kcal/mol)</th>
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<tr>
<td></td>
<td>Backbone</td>
<td>All-atom</td>
<td></td>
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</tr>
<tr>
<td>Tc1/KcsA</td>
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<td>3.96</td>
<td>218.78</td>
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*The initial placement of Tc1 with the side chain of the Lys residue facing towards the selectivity filter. *calculated after superimposing the final configuration of Tc1 after docking towards the channel in the initial energy minimized Tc1 structure (also see Fig. 6). *calculated by probing the contact surface area between Tc1 and the channel. *calculated only for the P-loop region of the channel and Tc1 molecule. *van der Waals interactions.
chain of Lys14 in Tc1 molecule, the association of the other four Lys residues of Tc1 with the channels is aided by distant electrostatic interactions with the negatively charged residues of the outer vestibule, which is in good agreement with the previous studies (66, 67). The electrostatic and hydrophobic interactions formed at the molecular surfaces of KcsA and Kv1.1 may orient and hold Tc1 towards their selectivity filters. Furthermore, surface complementarity of the outer vestibules of the channels to the Tc1 spatial conformations, where they are significantly different from the energy-minimized structure, also plays an important role in stabilizing the Tc1/KcsA and Tc1/Kv1.1 complexes.

Conclusions

The structural model of the P-loop region of Kv1.1 constructed in this study shares similar folds as that of KcsA, with most of the structural variations occur in the turret as well as in the inner and outer helices. Although this is only a theoretical model, it still provides particularly attractive targets for structure-based studies such as the blocking mechanisms by various toxins and MD simulations. The subsequent docking experiments performed in this study have successfully identified the most favorable binding modes of Tc1 towards KcsA and Kv1.1. In both cases, in addition to the direct blocking of the selectivity filter of the K+ channels by Lys14 side chain of Tc1, the association of the other four Lys residues of Tc1 with the channels is aided by distant electrostatic interactions with the negatively charged residues of the outer vestibule, which is in good agreement with the previous studies (66, 67). The electrostatic and hydrophobic interactions formed at the molecular surfaces of KcsA and Kv1.1 may orient and hold Tc1 towards their selectivity filters. Furthermore, surface complementarity of the outer vestibules of the channels to the Tc1 spatial conformations, where they are significantly different from the energy-minimized structure, also plays an important role in stabilizing the Tc1/KcsA and Tc1/Kv1.1 complexes.

Acknowledgements

The authors gratefully acknowledge the financial support from National Science Council of Taiwan (NSC-92-2214-E-027-001).

References and Footnotes
