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A possible molecular mechanism of hanatoxin binding-modified gating in voltage-gated K^+-channels

Kuo-Long Lou[1]*, Po-Tsang Huang[1], Yu-Shuan Shiau[2,3], Yen-Chywan Liaw[4], Yuh-Yuan Shiau[2,3] and Horng-Huei Liou[5]

[1]Graduate Institute of Oral Biology, Medical College, National Taiwan University, Taipei, Taiwan, Republic of China
[2]Department of Entomology, National Taiwan University, Taipei, Taiwan, Republic of China
[3]Lehrstuhl für angewandte Zoologie, Forstwissenschaftliche Fakultät, Technische Universität München, Freising, Germany
[4]Institute of Molecular Biology, Academia Sinica, Taipei, Taiwan, Republic of China
[5]Department of Pharmacology, and Division of Neurology, NTU Hospital; Medical College, National Taiwan University, Taipei, Taiwan, Republic of China

While S4 is known as the voltage sensor in voltage-gated potassium channels, the carboxyl terminus of S3 ($S3_C$) is of particular interest concerning the site for gating modifier toxins like hanatoxin. The thus derived helical secondary structural arrangement for $S3_C$, as well as its surrounding environment, has since been intensively and vigorously debated. Our previous structural analysis based on molecular simulation has provided sufficient information to describe reasonable docking conformation and further experimental designs (Lou *et al.*, 2002. *J. Mol. Recognit*. 15: 175–179). However, if one only relies on such information, more advanced structure–functional interpretations for the roles $S3_C$ may play in the modification of gating behavior upon toxin binding will remain unknown. In order to have better understanding of the molecular details regarding this issue, we have performed the docking simulation with the $S3_C$ sequence from the hanatoxin-insensitive K^+-channel, *shaker*, and analyzed the conformational changes resulting from such docking. Compared with other functional data from previous studies with respect to the proximity of the S3–S4 linker region, we suggested a significant movement of *drk1* $S3_C$, but not *shaker* $S3_C$, in the direction presumably towards S4, which was comprehended as a possible factor interfering with S4 translocation during *drk1* gating in the presence of toxin. In combination with the discussions for structural roles of the length of the S3–S4 linker, a possible molecular mechanism to illustrate the hanatoxin binding-modified gating is proposed.

Keywords: **$S3_C$; S3–S4 linker; molecular simulation; docking; hanatoxin; *drk1*; *shaker*; inhibition of channel gating; spatial freedom**

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INTRODUCTION

Potassium channels are membrane proteins that regulate potassium flux across the cell membrane. They contribute to diverse cell functions from control of membrane potential and excitability of neurons and muscles to regulation of cell volume and osmotic balance. The voltage-gated K^+-channels (Kv) comprise a large family of tetramers that open and close in response to changes in membrane voltage. Six putative transmembrane segments termed S1–S6 are included in each subunit of the tetramer. Among them, S5 and S6 assemble the central pore domain forming the K^+-selective ion conduction pathway (MacKinnon and Miller, 1989; MacKinnon and Yellen, 1990; Hartmann *et al.*, 1991; MacKinnon, 1991; Yellen *et al.*, 1991; Yool and Schwarz, 1991; Liman *et al.*, 1992; Heginbotham *et al.*, 1994; Ranganathan *et al.*, 1996; Armstrong and Hille, 1998). The first four transmembrane segments (S1–S4) of voltage-gated K^+ channels do not contribute to the simple pore, and appear to underlie their unique voltage-sensing capabilities (Armstrong and Hille, 1998). S4 is an unusual transmembrane segment that contains a large number of basic residues, which has been suggested by considerable study be strongly involved in sensing changes in membrane voltage (Liman *et al.*, 1991; Papazian *et al.*, 1991; Perozo *et al.*, 1994; Aggarwal and MacKinnon, 1996; Bezanilla *et al.*, 1996; Larsson *et al.*, 1996; Mannuzzu *et al.*, 1996; Seoh *et al.*, 1996; Yang *et al.*, 1996; Yusaf *et al.*, 1996; Smith-Maxwell *et al.*, 1998a, 1998b; Ledwell and Aldrich, 1999). The C-terminal part of S3 segment ($S3_C$) is of particular interest because it has been identified as an important region for interaction with various gating modifier toxins (Rogers *et al.*, 1996; Swartz and MacKinnon, 1997a, 1997b; Li-Smerin and Swartz, 1998, 2000; Winterfield and Swartz,

*Correspondence to: K.-L. Lou, GIOB, NTUMC, Chang-Teh Str. 1, Taipei 10042, Taiwan, Republic of China.
E-mail: kllou@ntumc.org
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Abbreviations used: *drk1*, delayed rectifier K^+-channels (Kv2.1); HaTx1, hanatoxin.

2000). Among them, hanatoxin (HaTx1), a 35-amino acid protein isolated from tarantula venom (Swartz and MacKinnon, 1995), shows an inhibition on *drk1* (Kv2.1) by shifting activation to more depolarized voltages (Swartz and MacKinnon, 1997a, 1997b). Investigation on the structural and functional correlation between hanatoxin and voltage-gated potassium channels has provided quite useful information in analyzing the roles of S3c in K^+-channel gating (Takahashi *et al.*, 2000; Li-Smerin and Swartz, 1998, 2000, 2001).

Previously we have reported (Huang *et al.*, 2001; Lou *et al.*, 2002; Huang, 2002) a docking simulation study describing the exact binding residues required for HaTx1–*drk1* interaction and the derived conformational change resulting from binding. However, while further considering the movement presumably towards S4 upon conformational change (Huang *et al.*, 2001), together with the specific binding pocket close to the external crevice depicted from the detailed residue analysis (Lou *et al.*, 2002), we noticed that the structural roles of $S3_C$–S4 proximity in interfering with S4 translocation must be clarified, especially in terms of the length of S3–S4 linker (Mathur *et al.*, 1997; Swartz and MacKinnon, 1997a, 1997b; Gonzalez *et al.*, 2000). In this study, thereby, we extensively and comprehensively compare the docking simulation results of *drk1* $S3_C$–HaTx1 to the substitution with *shaker* $S3_C$ sequence. The mutual influence of the proximity of $S3_C$–$S4_N$ region is thus discussed.

EXPERIMENTAL PROCEDURES

Model building

$S3_C$ fragment. The *drk1* $S3_C$ molecule (Val-271 to Gln-284, amino acid sequence VTIFLTESNKSVLQ) and *shaker* $S3_C$ molecule (Ile-325 to Asn-339, amino acid sequence ITLATVVAEEEDTLN) were constructed via modification from fragment dictionary with geometry optimized using the consistent valence force field (CVFF) with the Biopolymer module of Insight II software package (Accelrys, USA). Atomic charges were computed using the semi-empirical MOPAC/AM1 method. The residues based on prediction of α-helix were individually regularized by energy minimization to give reasonable geometries.

HaTx1 structure. The coordinates for HaTx1 were obtained from Brookhaven Protein Databank in pdb file (PDB ID number 1D1H).

Docking simulation

Determination of starting orientations. In principle, three criteria were used to determine the starting positions: stereochemistry, side-chain charge distribution and previous structural information (Lou *et al.*, 2002; Huang *et al.*, 2001). Inappropriate possibilities were immediately excluded if unreasonable combinations of alignment for docking were observed. Uncertain orientations were reserved and submitted for docking calculation to allow the computational results to perform the screening.

Calculation for the energies. Upon docking, the total energies of electrostatic interactions and van der Waals contacts between the complexes of HaTx1 and $S3_C$-binding model were compared. Each run was composed of 500 cycles of simulated annealing and 1 000 000 steps of accepted/rejected configurations.

The values of all other default parameters were used. The alignment between docked and undocked molecules was performed by manually fitting the atomic coordinates of groups of residues that may be involved in the conserved interaction (Hahn, 1995; Hahn and Rogers, 1995; Costantino *et al.*, 2001). Briefly, three-dimensional (3D) surfaces of the binding site enclose the most active members (after appropriate alignment) of the starting set of molecules. Note that errors in alignment can lead to incorrect, poorly predictive receptor surface models. This problem was overcome by using information obtained from previously related functional data. The surface was generated from a 'shape field', in which the atomic coordinates of the contributing models were used to compute field values on each point of a 3D grid using a van der Waals function. A solvation energy correction term and the electrostatic charge complementarity's method were used for energy evaluation. The solvation energy correction term is a penalty function that attempts to account for the loss of solvation energy when polar atoms are forced into hydrophobic regions of the receptor surface. All the calculations and structure manipulations described above were performed with the Discover & Docking/Insight II (2000) molecular simulation and modeling program (Accelrys, San Diego, CA, USA; 950 release) on a Silicon Graphics Octane/SSE and O2/R12000 workstation.

RESULTS AND DISCUSSION

Interactions between HaTx1 and $S3_C$

We systematically docked the two molecules by presenting HaTx1 with its solution structure (Takahashi *et al.*, 2000) and *drk1/shaker* by modeling the C-termini of S3 with constraints based on the possible structural arrangement of an independent α-helix deduced from previous lysine-scanning data (Li-Smerin and Swartz, 2001; Lou *et al.*, 2002). From our results, the precise residues required for hanatoxin binding onto voltage-gated potassium channel *drk1* can be clearly described (Lou *et al.*, 2002). With respect to this point, we found that not only the hydrophobic interactions but also the electrostatic forces are utilized in stabilizing the toxin-channel binding (Plate 1, left). This verifies and expands the idea proposed by Takahashi *et al.*, (2000).

On the other hand, chimeras constructed using the *drk1* and *shaker* K^+ channels suggested the required sequence for HaTx1 binding in *drk1*, but not in *shaker* (Swartz and MacKinnon, 1997b). Energies of such docking simulation for *drk1* converged successfully before 2.0×10^5 steps. However, when we further performed such experiments in order to compare with *shaker*, the tailing fluctuation in energy profile forced us to increase the simulation steps to 10^6, with which the fluctuations gradually and converged to a compromise of energy limits. All the results and

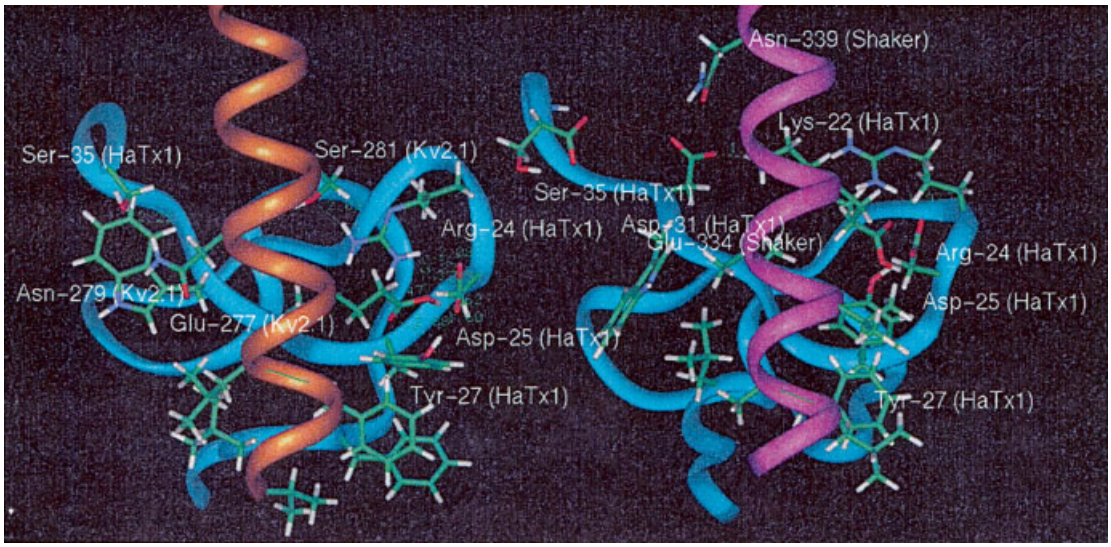


Plate 1. Comparison of the detailed binding sites indicating residues required for docking in both *drk1* S3c–HaTx1 (left) and *shaker* S3c–HaTx1 (right). Ribbons in orange (*drk1*) and in magenta (*shaker*) represent the helical structures of channel fragments. Hanatoxin is shown in light blue for both complexes. Residue side chains are drawn with thick sticks in colors according to their atom types. Hydrophilic interactions are marked with dot lines to indicate the distances.

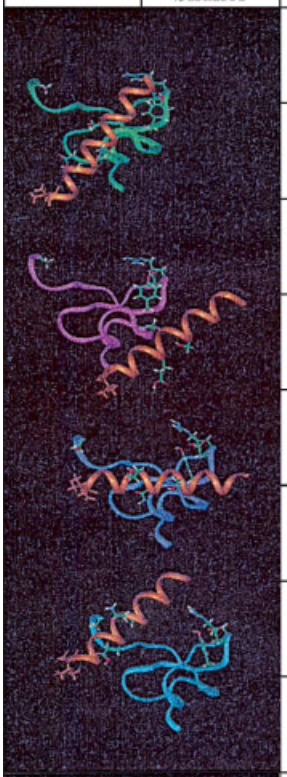
Docking results	Energy Drk1 Shaker	Vdw [Kcal/moD]	Elec [Kcal/mol]	Total [Kcal/mol]
		521.74	59.14	588.88
		1.39×10^5	90.53	1.39×10^5
		474.17	43.11	517.29
		944.67	138.31	1082.98
		1776.94	174.94	1951.88
		2.82×10^5	40.57	2.82×10^5
		529.29	93.25	622.54
		1.22×10^5	341.65	1.25×10^5
		503.54	52.71	556.25
		1762.69	64.33	1827.02
		521.84	58.42	580.25
		2636.93	57.97	2694.90
		510.28	68.58	578.86
		1443.02	93.78	1536.79
		261.74	−34.11	227.63
		433.80	89.40	523.21

Plate 2. Comparison of the docking energies for *drk1*–HaTx1 and *shaker*–HaTx1. Structures in the left panel illustrate the docking orientations. Compared are the energies for van der Waals contacts, electrostatic interactions, and the total energies (kcal/mol).

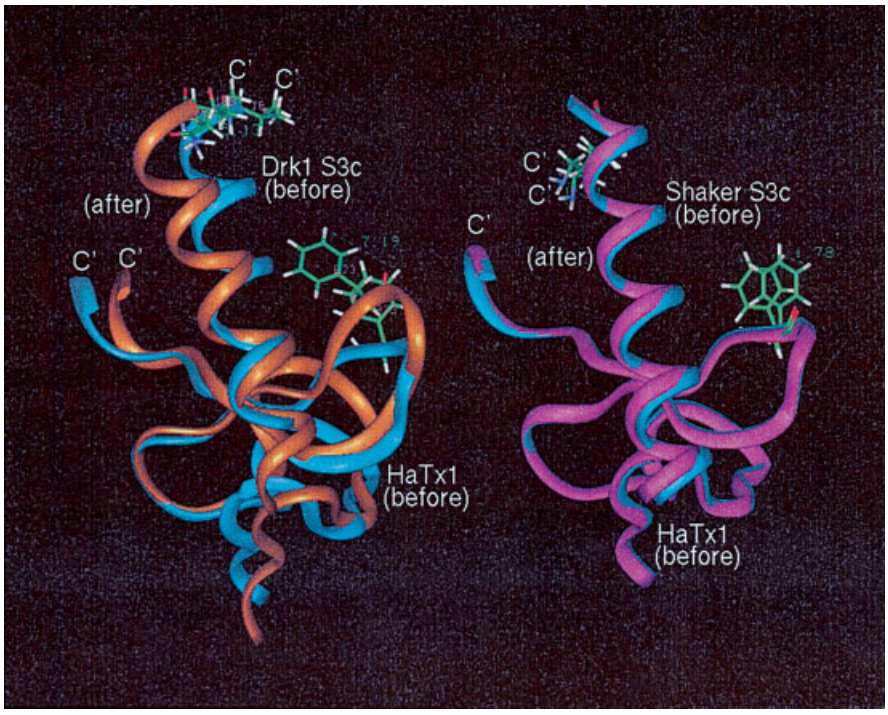


Plate 3. Comparison of the complex structures before and after docking simulation. In *drk1*–HaTx1 (left), significant conformational change can be clearly observed. The side chains of the residues with maximal displacements in structures are exhibited to illustrate the range of conformational changes with distances indicated. Note that both molecules have motions towards a similar direction. In *shaker*–HaTx1 (right), no such obvious structural changes can be seen.

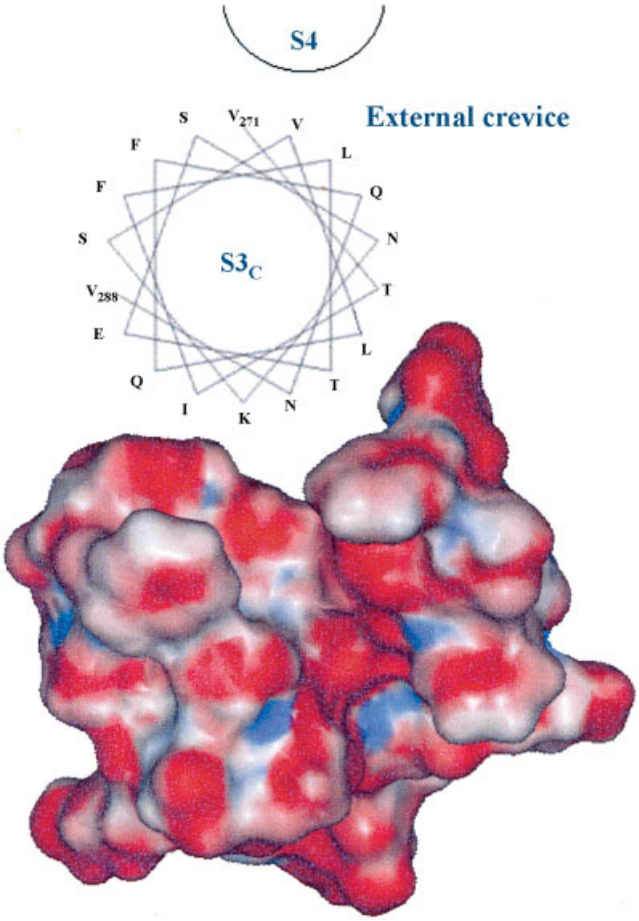


Plate 4. Putative spatial organization for the proximity of S3c in *drk1* channels regarding hanatoxin binding. *Drk1* S3c is depicted with a helical wheel, oriented with respect to the relation between hanatoxin and the S4 segment, based on structural analysis from docking simulation. The figure is viewed from the extracellular side along an imaginary axis approximately vertical to the membrane plane.

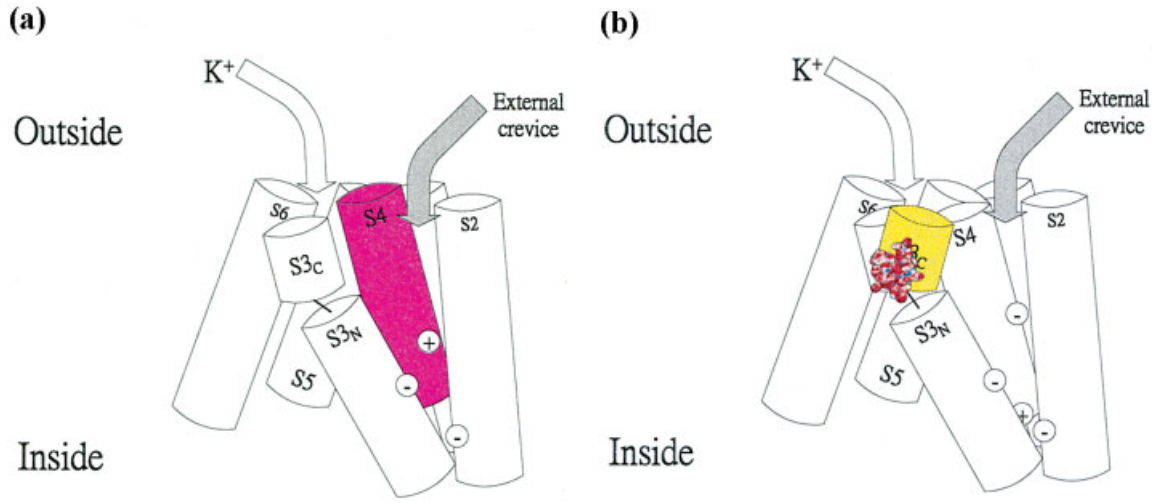


Plate 5. Cartoon illustrating the possible molecular mechanism of hanatoxin binding-modified gating (in *drk1*). The six transmembrane segments S1–S6 are arranged according to the model proposed by Cha *et al.* (1999). HaTx1 binds to the approximate location as discussed in the text. External crevice and putative direction for potassium ion flux are also indicated. (a) In the absence of HaTx1, S4 can translocate in a normal way. (b) In the presence of HaTx1, S3c may reduce the spatial freedom for S4 around proximity of external crevice, and therefore interfere with the translocation of S4.

comparisons are listed in Plate 2. It is very clear that for both docking complexes, the last starting orientation resulted in the best combination for simulation.

Observation of the binding sites in both cases suggested that the electrostatic interactions used in *shaker*-HaTx1 are much weaker than in *drk1*-HaTx1 (Plate 1), although the energy results obtained in the last docking combination for *shaker* were not much higher than for *drk1* (Plate 2). One important factor to account for this was observed from the residues involved in docking: instead of three, only two residues from S3c contribute to the formation of electrostatic interactions in *shaker*. However, more details are required to describe the variation in gating upon toxin binding between *drk1* and *shaker*. Therefore, it seemed quite necessary to further analyze the conformational change induced by toxin binding in both cases.

Conformational change of $S3_C$ in HaTx1 binding

In Plate 3, superposition for both complexes before and after docking has been performed based on our extensive simulation data. Similar to those results observed in previous study (Huang, 2002; Lou *et al.*, 2002), *drk1* $S3_C$ undergoes a significant movement along an axis presumably towards S4 segment, whereas *shaker* $S3_C$ does not show any significant conformational change upon hanatoxin binding. This can be easily explained by the weak electrostatic stabilization flanking the hydrophobic interactions in *shaker* (Plate 1), from which the binding between the two molecules was thought to be not tight enough to result in significant structural change, and therefore HaTx1 is not able to push $S3_C$ to allow for displacement.

As discussed in the study by Lou *et al.*, (2002), the surface of S3c can be divided into several parts. Hanatoxin may use the hydrophobic patch near the membrane boundary to bind channels, whereas certain part of the hydrophilic surface of S3c would have to be facing the external crevice. Detailed analysis of the helical residues (Plate 4) will elaborate the hypothesis that the direction of S3c movement should be towards S4, in the proximity of external crevice.

Shortening of S3–S4 linker may have similar effect in gating as $S3_C$ structural change

In order to describe the structural-functional correlations with our structural observation that may explain the shift of activation to more depolarized voltages (Swartz and MacKinnon, 1997a), the studies (Mathur *et al.*, 1997; Gonzalez *et al.*, 2000) discussing the mutational effects of deletions in *shaker* S3–S4 linker in gating should be taken into consideration. Shortening of the S3–S4 linker in *shaker* can shift the half-activation voltage to the right along the voltage axis (Gonzalez *et al.*, 2000). In combination with our structural observation, we found that both phenomena might result from the same issue: the 'spatial freedom' in the proximity of S3–S4 region (see next paragraph).

Possible mechanism for HaTx1 binding-modified gating

From our results, hanatoxin may push $S3_C$ from its original position towards the N-terminus of S4 upon binding, such motion may restrict the spatial freedom for rotation and displacement of S4 in the membrane with depolarization and therefore channels would require higher voltage for activation. Very similar comprehension can be made for the studies on deletions in the S3–S4 linker (Gonzalez *et al.*, 2000). In other words, in *drk1*, the motion of $S3_C$ upon tight toxin-channel binding, in combination with a relatively shorter S3–S4 linker, should be sufficient to restrict the spatial freedom for S4 translocation. On the contrary, in *shaker*, there may exist two crucial factors disadvantaging the effects due to toxin binding. First, *shaker* $S3_C$ contains improper residues to form the firm electrostatic stabilization for hanatoxin binding. Second, not only unable to push $S3_C$ to interfere the S4 translocation, *shaker* keeps a much longer and even more flexible S3–S4 linker (Li-Smerin and Swartz, 2001). Therefore, *shaker* does not show a more depolarized activation status in the presence of hanatoxin. Plate 5 illustrates the hypothesis of how hanatoxin can affect the activation in *drk1*.

Meanwhile, we should not exclude the possibility that extension of the S3–S4 linker may compensate the spatial restriction occurred in the case of *drk1*–HaTx1. Currently no evidence is available for describing such an idea. However, it has been proposed that the S3–S4 linker is unlikely to participate in a large conformational change during channel activation (Mathur *et al.*, 1997) and the S4 segment moves only a short distance during activation since an S3–S4 linker consisting of only five amino acid residues can still allow for the total charge displacement to occur, and therefore the length of the S3–S4 linker may only play important role in setting channel activation kinetics (Gonzalez *et al.*, 2000). Compared with our hypothesis described above, it seems evident that, at least in the hanatoxin binding-modified gating, it should be more crucial to ensure the correct sequence for binding than to consider the compensation effect by a longer S3–S4 linker, especially when we notice that the net maximal displacement of $S3_C$ upon toxin binding is about 5 Å (Plate 3), which is large enough to produce spatial restriction for S4 in the proximity of external crevice (Plates 4 and 5).

In conclusion, the hanatoxin binds to *drk1* (but not *shaker*, which is due to the inappropriate binding sequence) via $S3_C$ helix and may push $S3_C$ towards the N-terminus of S4 segment ($S4_N$) according to the binding-induced conformational change. The range of such movement should be large enough to enable $S3_C$ to reduce the spatial freedom for S4 segment to translocate outwards membrane upon open gating (Cha *et al.*, 1999). This, therefore, brings the required activation potentials to the more depolarized ones (Swartz and MacKinnon, 1997a).

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