Available online at www.sciencedirect.com
SCIENCE DIRECT®
Chemical Physics Letters 381 (2003) 592–597
CHEMICAL PHYSICS LETTERS
www.elsevier.com/locate/cplett

Structural model of the voltage-gated potassium channel Kv1.1 and molecular docking of Tc1 toxin from *Tityus cambridgei* to KcsA and Kv1.1

Hsuan-Liang Liu *, Jin-Chung Lin

Department of Chemical Engineering and Graduate Institute of Biotechnology, National Taipei University of Technology, No. 1 Sec. 3, Chung-Hsiao E. Rd., Taipei 10643, Taiwan, Republic of China

Received 27 August 2003; in final form 7 October 2003
Published online: 4 November 2003

Abstract

In this study, structural model of the pore loop region of the voltage-gated potassium channel Kv1.1 was constructed based on the crystallographic structure of KcsA. Subsequently, molecular docking experiments of Tc1 towards KcsA as well as Kv1.1 were performed. Tc1 forms the most stable complexes with these two channels when the side chain of K14 occupies the first K^+ binding site. Tc1 binds preferentially towards Kv1.1 than KcsA due to the stronger electrostatic and hydrophobic interactions. Furthermore, surface complementarity of the outer vestibules of the channel to the Tc1 spatial conformations also plays an important role in stabilizing these Tc1/channel complexes.

1. Introduction

Potassium channels are the most diverse group of the ion channel family [1]. The recent determination of the crystallographic structure of a bacterial K^+ channel from *Streptomyces lividans* (KcsA) [2] has provided the molecular basis for understanding the physical mechanisms controlling ionic selectivity, permeation, and transport through various types of K^+ channels [3,4]. In all cases, the functional K^+ channel is a tetramer [5], typically of four identical subunits folded around a central pore [2]. The P-loop regions of various K^+ channels, consisting of the turret, the pore helix, and the selectivity filter, share strong sequence similarities, suggesting that it may be possible to generate homology models of mammalian K^+ channels [6,7] by using KcsA [2] as a template. Such st7ructural models may subsequently provide insights into functional properties of mammalian K^+ channels, such as blocking by various toxins or tetraethylammzonium (TEA) ions [8–10].

Scorpion venoms contain various polypeptides with distinct biological functions that particularly affect the permeability of ion channels [11]. These polypeptides possess the potency to recognize ion channels and receptors in membranes and are commonly classified into four groups [12]. Among

* Corresponding author. Fax: +886-2-27317117.
E-mail address: f10894@ntut.edu.tw (H.-L. Liu).

0009-2614/$ - see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.cplett.2003.10.040

these, the newly solved solution structure of the scorpion venom of *Tityus cambridgei* (Tc1) is the shortest known toxin from scorpion venom that recognizes *Shaker* Kv channels in the brain [13]. Molecular dynamics (MD) simulations of Tc1 revealed that the totally conserved Lys residue may be responsible for the interaction with the selectivity filter of the K^+ channels [14].

There are five Lys residues in Tc1, with K14 totally conserved among various K^+channel blockers. In order to elucidate which Lys residue of Tc1 is possibly involved in direct interaction with the selectivity filter of the K^+ channels and subsequent block of ion permeation, two experiments were conducted in this study: (1) constructing the structural model of the P-loop region of Kv1.1 from human *Homo sapien* based on the crystallographic structure of KcsA [2]; and (2) performing molecular docking experiments of Tc1 towards KcsA as well as Kv1.1 with various initial Tc1 orientations.

2. Methods

2.1. 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 [15] (PDB; accession number 1f6g). Unfavorable nonphysical contacts in this structure were eliminated using Biopolymer module of Insight II program (Accelyrs, San Diego, CA, USA) with the force field Discover CVFF [16] in the SGI O200 workstation (Silicon Graphics, Inc., Mountain View, CA, USA), followed by 10 000 energy minimization calculations using steepest descent method.

2.2. Structural homology

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 Kv1.1. 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 [17] or generation through random conformational search [18]. 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 [19]. Secondary structure motifs were predicted by DSSP [20]. The newly built structure of Kv1.1 was substantially refined to avoid van der Waal's radius overlapping, unfavorable atomic distances, and undesirable torsion angles.

2.3. Molecular docking

The ligand, Tc1, was docked using the Affinity module of Insight II with a two-step process. First, initial placements of 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 each Tc1 placement. During this phase, the 'bulk' of the receptor 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. [21], while interactions among movable atoms were treated using a full force field representation. Solvation effects were also included using the methods of Stouten et al. [22], with a dielectric constant of 80 being used [23].

3. Results and discussion

3.1. The structural model of the P-loop region of Kv1.1

The structural model of the P-loop region of Kv1.1 is shown in Fig. 1. As expected, KcsA and Kv1.1 share similar folds in the P-loop region, particularly in the pore helix and the selectivity

filter. 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. The pore helix is more or less responsible for maintaining the structural integrity of the selectivity filter. The turret is extended towards the extracellular environment like a lobster claw and is likely to play an important role in capturing K^+ ions from the extracellular environment.

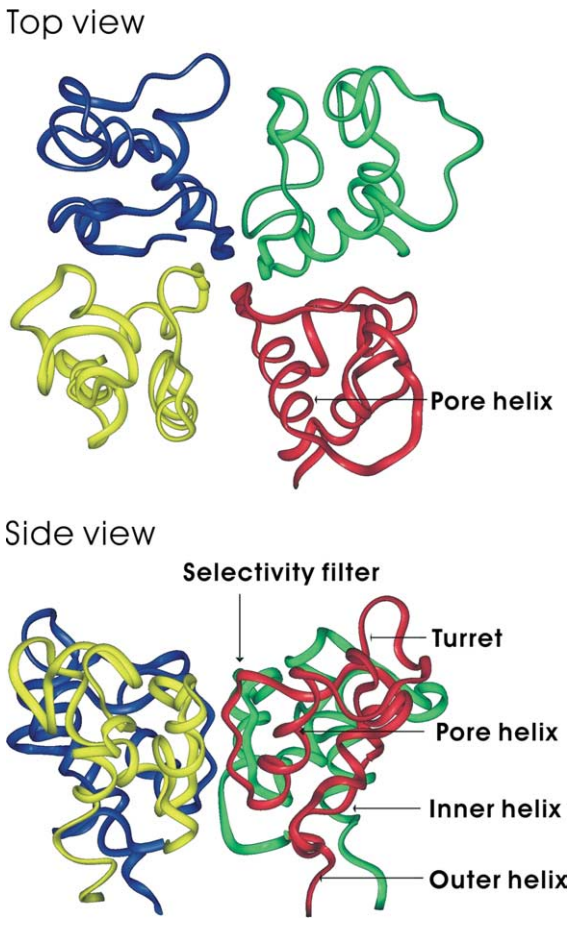

Fig. 1. Ribbon presentations of the top and side views of the structural model of the P-loop region of Kv1.1. The structural features corresponding to those defined in the crystallographic structure of KcsA [2] are indicated. The backbone RMSD of Kv1.1 in Å after superimposing to the crystallographic structure of KcsA is given on the top right corner of this model.

3.2. Molecular docking of Tc1 to KcsA and Kv1.1

As shown in Fig. 2, 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 K14 located at the first K^+ binding site in both cases. Besides, in the case of KcsA blocking, K7, K8, and K10 of Tc1 may form electrostatic interactions with the four consecutive negatively charged Asp residues of the turret from subunit III, while K21 may form electrostatic interactions with the corresponding residues from subunit I. In contrast, in the case of Kv1.1 blocking, the side chain of K21 was facing towards the selectivity filter and, together with K14, formed stronger electrostatic interactions with the selectivity filter. The above observations clearly indicate that electrostatic interactions are crucial for forming stable blocker/channel complexes.

Tc1 may exhibit various conformations to form different spatial complementary complexes when it binds towards various K^+ channels (see Table 1 for RMSD values). Although the overall flexibility of Tc1 is expected to be low due to the three disulfide bridges [14], the two turns in the backbone and the side chains of Lys residues are flexible enough to accommodate this molecule on the molecular surfaces of various K^+ channels through various degrees of electrostatic and hydrophobic interactions. The side chain of K14 does not enter to the pore any further and subsequently interact with the other K^+ binding sites because of the steric effect formed by the perpendicular β-hairpin [13]. Table 1 also shows that the most favorable binding mode of Tc1 towards both KcsA and Kv1.1 is the one with the side chain of K14 facing towards their selectivity filters, where this complex exhibits the lowest total energy and the most contact area. In addition, 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 [13].

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

Complex	Binding mode[a]	RMSD[b] (Å)		Contact area[c] (Å^2)	Energy terms[d] (kcal/mol)		
		Backbone	All-atom		VDW	Electrostatic	Total
Tc1/KcsA	Lys7	2.53	3.81	271.36	−126.25	−158.78	−258.03
	Lys8	2.98	4.25	307.11	−165.81	−134.41	−300.22
	Lys10	3.76	5.55	255.35	−134.60	−80.20	−214.80
	Lys14	1.87	2.91	330.27	−133.84	−220.64	−354.48
	Lys21	2.13	4.01	247.10	−140.65	−56.52	−197.17
Tc1/Kv1.1	Lys7	2.44	4.18	295.08	−129.04	−524.24	−653.28
	Lys8	2.45	3.51	288.17	−145.94	−443.42	−589.36
	Lys10	3.57	4.95	249.38	−115.67	−466.82	−582.49
	Lys14	3.06	4.27	304.43	−143.54	−553.59	−697.13
	Lys21	2.66	3.96	218.78	−122.46	−457.22	−579.68

[a] The initial placement of Tc1 with the Lys residue facing towards the selectivity filter.
[b] Calculated after superimposing the final conformation of Tc1 after docking towards the channel to the initial energy-minimized Tc1 structure.
[c] Calculated by probing the contact surface area between Tc1 and the channel.
[d] Calculated only for the P-loop region of the channel and Tc1 molecule.

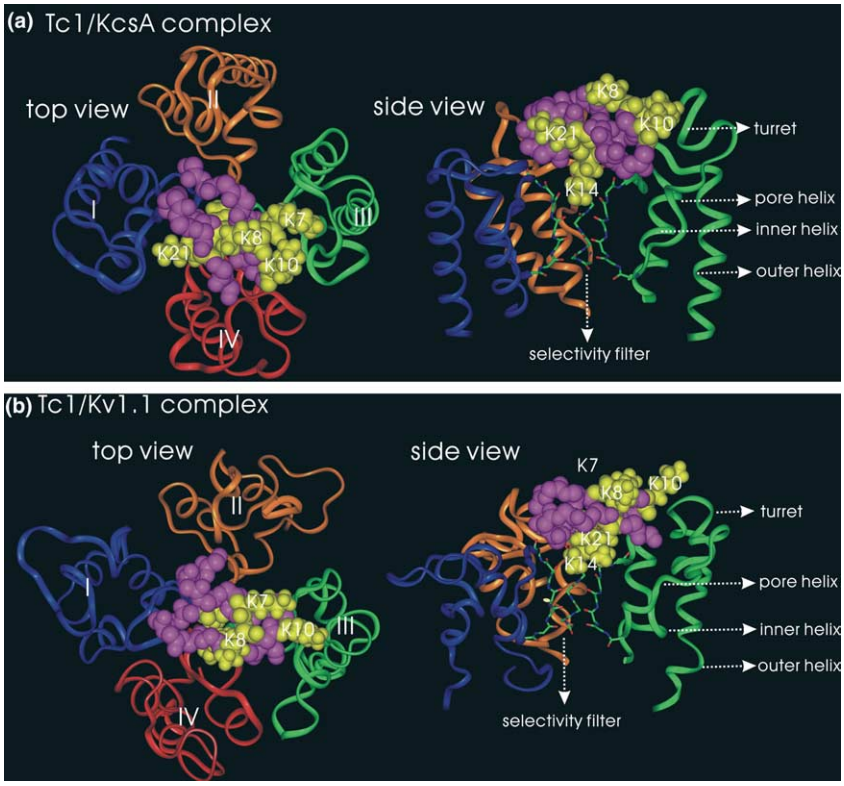

Fig. 2. Ribbon representations of the top and side views of the P-loop region of (a) KcsA and (b) Kv1.1 after docked by Tc1 molecules, which are illustrated in CPK. The four subunits of each channel, indicating as I, II, III, and IV, are shown in blue, orange, green, and red, respectively. One of the 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.

Our docking experiments provide new structural information regarding the complexes of Tc1/KcsA and Tc1/Kv1.1. Previously, the stabilizing effect of residues with hydrophobic side chains on external TEA binding to KcsA has been rationalized [24–26]. In our cases, the hydrophobic interactions between Tc1 and the four D80 (D377) residues from the four subunits of KcsA (Kv1.1) also play an important role in stabilizing the Tc1/KcsA (Tc1/Kv1.1) complex, as indicated in Fig. 3. It is noteworthy that both K14 and K21 were involved in the interaction with the selectivity filter of Kv1.1, whereas only K14 was involved in the interaction with that of KcsA (Fig. 2). Besides, the hydrophobic and electrostatic interactions formed by K10 of Tc1 with E350 and E351 from subunit III of Kv1.1 also play an important role in stabilizing the Tc1/Kv1.1 complex. These stronger electrostatic and hydrophobic interactions result in tight binding of Tc1 towards Kv1.1 than KcsA (Table 1). 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^+

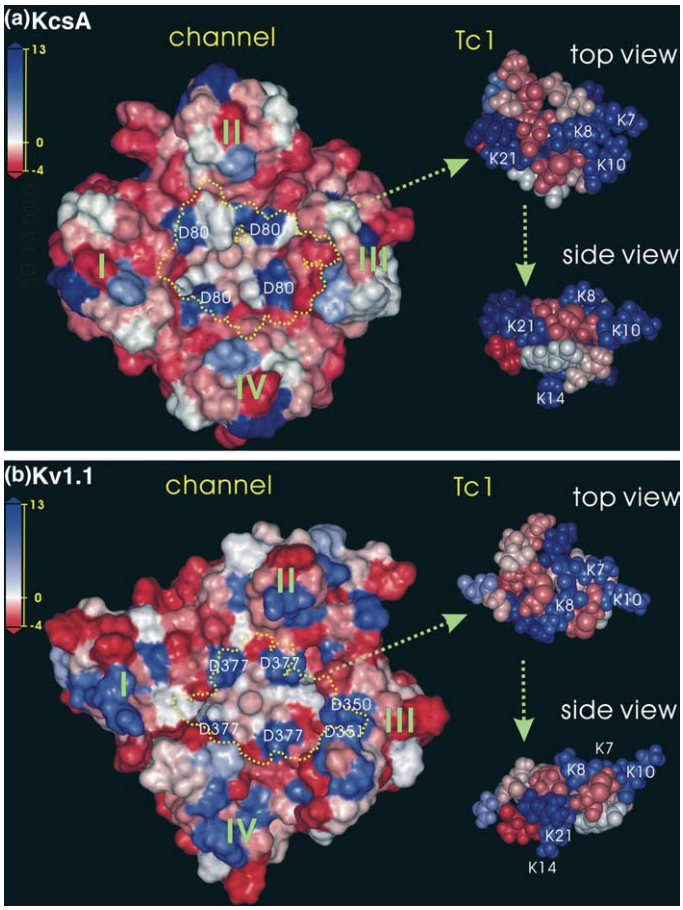

Fig. 3. Molecular surfaces of the complexes of (a) KcsA/Tc1 and (b) Kv1.1/Tc1 with K14 of Tc1 facing towards the selectivity filters of these two channels. Top views of the P-loop regions of KcsA and Kv1.1 after removing Tc1 molecules are shown on the left of (a) and (b), respectively. The dashed yellow lines indicate the binding locations of Tc1 molecules on the channel surfaces after docking. The four Asp residues forming the hydrophobic interactions with Tc1 from each subunit are also labeled. Top and side views of the Tc1 molecules after docking towards KcsA and Kv1.1 are shown on the top-right and bottom-right of (a) and (b), respectively. The five Lys residues are labeled.

channel are crucial in the blocking mechanism [10,13,14,24–26].

In conclusion, the docking experiments performed in this study have successfully identified the most favorable binding modes of Tc1 towards KcsA and Kv1.1. Surface complementarity of the outer vestibules of the channels to the Tc1 spatial conformations, where they are significantly different comparing to 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 the National Science Council of Taiwan (NSC-92-2214-E-027-001).

References

[1] L.K. Kaczmarek, T.M. Perney, Curr. Opin. Cell. Biol. 3 (1991) 663.
[2] D.A. Doyle, J. Morais Cabral, R.A. Pfuetzner, A. Kuo, J.M. Gulbis, S.L. Cohen, B.T. Chait, R. MacKinnon, Science 280 (1998) 69.
[3] L. Heginbotham, Nat. Struct. Biol. 6 (1999) 811.
[4] B. Hille, C.M. Armstrong, R. MacKinnin, Nat. Med. 5 (1999) 1105.
[5] R. MacKinnon, Nature 350 (1991) 232.
[6] V.P. Repunte, H. Nakamura, A. Fujita, Y. Horio, I. Findlay, L. Pott, Y. Kurachi, EMBO J. 18 (1999) 3317.
[7] C.E. Capener, I.H. Shrivastava, K.M. Ranatunga, L.R. Forrest, G.R. Smith, M.S.P. Sansom, Biophys. J. 78 (2000) 2929.
[8] R.O. Blaustein, P.A. Cole, C. Williams, C. Miller, Nat. Struct. Biol. 7 (2000) 309.
[9] D. del Camino, M. Holmgren, Y. Liu, G. Yellen, Nature 403 (2000) 321.
[10] V.B. Luzhkov, J. Åqvist, FEBS Lett. 495 (2001) 191.
[11] H.H. Valdivia, B.M. Martin, L. Escobar, L.D. Possani, Biochem. Int. 27 (1992) 953.
[12] J.A. DeBin, J.E. Maggio, G.R. Strichartz, Am. J. Physiol. 264 (1993) 361.
[13] I. Wang, S.-H. Wu, H.-K. Chang, R.-C. Shieh, H.-M. Yu, C. Chen, Protein Sci. 11 (2002) 390.
[14] A. Grottesi, M.S.P. Sansom, FEBS Lett. 535 (2003) 29.
[15] D.M. Cortes, L.G. Cuello, E. Perozo, J. Gen. Physiol. 117 (2001) 165.
[16] J.R. Maple, M.-J. Hwang, K.J. Jalkanen, T.P. Stockfisch, A.T. Hagler, J. Comp. Chem. 19 (1998) 430.
[17] G.D. Schuler, S.F. Altschul, D.J. Lipman, Proteins: Struct. Funct. Genet. 9 (1991) 180.
[18] P.S. Shenkin, D.L. Yarmush, R.M. Fine, H. Wang, C. Levinthal, Biopolymers 26 (1987) 2053.
[19] J.W. Ponder, F.M. Richards, J. Mol. Biol. 193 (1987) 775.
[20] W. Kabsch, C. Sander, Biopolymers 22 (1983) 2577.
[21] B.A. Luty, Z.R. Wasserman, P.F.W. Stouten, C.N. Hodge, M. Zacharias, J.A. McCammon, J. Comp. Chem. 16 (1995) 454.
[22] P.F.W. Stouten, C. Froemmel, H. Nakamura, C. Sander, Mol. Simulat. 10 (1993) 97.
[23] M.K. Gilson, B.M. Honig, Nature 330 (1987) 84.
[24] R.A. Kumpf, D.A. Dougherty, Science 261 (1993) 1708.
[25] J.C. Ma, D.A. Dougherty, Chem. Rev. 97 (1997) 1303.
[26] X.J. Tan, H.L. Jiang, W.L. Zhu, K.X. Chen, R.Y. Ji, J. Chem. Soc. Perk. Trans. 2 (1999) 107.