Research Article

Journal of PeptideScience

Received: 14 June 2011 Revised: 15 September 2011 Accepted: 22 September 2011 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/psc.1427

The solution structure of the S4–S5 linker of the hERG potassium channel

Shovanlal Gayen, Qingxin Li‡ and CongBao Kang*

The human ether-à-go-go related gene (hERG) encodes a protein that forms a voltage-gated potassium channel and plays an important role in the heart by controlling the rapid delayed rectifier K^+ current (I_{Kr}). The S4–S5 linker was shown to be important for the gating of the hERG channel. Nuclear magnetic resonance study showed that a peptide derived from the S4–S5 linker had no well-ordered structure in aqueous solution and adopted a 3_{10}-helix (E544-Y545-G546) structure in detergent micelles. The existence of an amphipathic helix was confirmed, which may be important for interaction with cell membrane. Close contact between side chains of residues R541 and E544 was observed, which may be important for its regulation of channel gating. Copyright © 2011 European Peptide Society and John Wiley & Sons, Ltd.

Supporting information may be found in the online version of this article.

Keywords: NMR; hERG; S4–S5 linker; micelles; membrane protein; potassium channel

Introduction

Voltage-gated potassium channels are open or closed in response to membrane potentials across the cell membrane to control potassium transportation across the cell membrane [1]. Structural studies showed that voltage-gated potassium channels were formed from four subunits that each contained six transmembrane segments (S1–S6) [1,2]. The S1 to S4 segments form a voltage-sensing domain in which the positively charged residues in S4 are important for voltage changes across the cell membrane. Studies have shown that this S4 is the major voltage sensor of voltage-gated ion channels [3,4]. The S5 and S6 segments form an ion-conducting domain through which ions pass once the channel opens. The mechanism of coupling the voltage changes to the opened or closed state of the channel remains undefined [5]. Structural studies showed that the S4–S5 linker was located between S4 and S5, which may be important for the gating of the channels [6]. The linking of the movement of S4 to S5 and S6 is possibly dependent on the S4–S5 linker because of its physical location [3]. Mutation studies showed that mutation in the S4–S5 linker of the *Shaker* potassium channel altered ion selectivity and signal channel conductance [7].

The human ether-à-go-go related gene (hERG) encodes a protein that forms a voltage-gated potassium channel that is shown to play an important role in the heart by controlling the rapid delayed rectifier K^+ current (I_{Kr}) [8]. Similar to other potassium channels, hERG contains six transmembrane segments. The S4–S5 linker (L539-A548) was thought to be a crucial component of the activation gate for hERG channels [1]. This linker was parallel to the cell membrane and interacted with the C-terminal portion of the S6 segments through residue D540 [9]. The interaction between D540 in S4–S5 linker and residue in S6 affected the gating of the hERG channel [5,9]. The S4–S5 linker may interact with the N-terminal domain of hERG [10,11], which was supported by *in vitro* titration experiment [12]. A recent study showed that the G546 was important for the gating of the channel by disrupting a highly conserved leucine zipper motif that existed in the *Shaker* channel [13].

The crystal structure of rKv1.2 showed that its S4–S5 linker adopts an amphipathic α-helical structure that is parallel to the membrane/cytoplasmic interface [14]. A nuclear magnetic resonance (NMR) study on the S4–S5 fragment of the *Drosophila Shaker* voltage-gated potassium channel has been conducted and has confirmed that this region also formed an amphipathic α-helical structure under both trifluoroethanol and phospholipid micelle conditions [15]. The S4–S5 linker of hERG may have a structure that is similar to that of the *Shaker* channel. To elucidate the structural characteristics of this linker from the hERG channel, a peptide that was derived from this region was synthesized, and its structures in both water and micelles were studied using solution NMR spectroscopy. The results show that the S4–S5 linker had different conformations under different conditions.

Materials and Methods

Sample Preparation

The peptide sequence was LDRYSEYGAA and was derived from the hERG S4–S5 linker of the hERG channel. This peptide was synthesized from GL Biochem Ltd (Shanghai, China) with 95% purity. The peptide was reconstituted to a concentration of 5 mg/ml in water (pH ~7.0) or a buffer that contained 2% deuterated dodecylphosphocholine (DPC) and 20 mM $NaPO_4$ (pH = 7.0). The sample with 10% D_2O was transferred into a 5-mm NMR tube for data acquisition.

* *Correspondence to: CongBao Kang, 31 Biopolis Way Nanos, #03-01, Singapore. E-mail: cbkang@etc.a-star.edu.sg*

‡ *Current address: Institute of Bioengineering and Nanotechnology, the Agency for Science, Technology and Research, Singapore 138669*

*Experimental Therapeutics Centre, Agency for Science, Technology and Research (A*STAR), Singapore 138669, Singapore*

Journal of PeptideScience

Circular Dichroism Spectroscopy

Samples were diluted to 1 mg/ml for circular dichroism (CD) analysis. The peptide was dissolved in water and in DPC micelles. The instrument was blanked using a cuvette that contained buffer without the peptide. The CD spectra were recorded on a Chirascan™ Circular Dichroism Spectrometer (AppliedPhotophysics, United Kingdom) at 25 °C, and samples were placed in a 0.1-cm path length quartz cuvette. The CD signal was acquired in the continuous mode with a 1-nm data pitch and a 1-nm bandwidth.

NMR Experiments

All the NMR data were collected using a Bruker 700 spectrometer (Bruker, Switzerland) that was equipped with a cryoprobe at 25 °C. A total correlation spectroscopy (TOCSY) experiment from the Bruker pulse library was recorded with a mixing time of 80 ms. Two-dimensional nuclear overhauser effect spectroscopy (NOESY) was recorded using the mixing times of 200 and 300 ms. Proton chemical shifts were referenced to 4,4-dimethyl-4-silapentane-1-sulfonic acid. All of the spectra were processed using Topspin 2.1 (Bruker, Switzerland) and analyzed using Sparky (http://www.cgl.ucsf.edu/home/sparky/, University of California, San Francisco, U.S.A.). Titration of the peptide with Gd^{3+} was conducted using the method described by Ju *et al.* [16] using one-dimensional proton spectroscopy.

Resonance Assignment and Structure Determination

The S4–S5 linker was assigned with the procedures including the identification of spin systems and spin connections. TOCSY spectrum was used to identify spin systems, and the NOESY spectrum was used to determine the spin connection [17,18]. The sequential connectivity was determined on the basis of the connectivity in the HN–HN or Hα–HN region. The NOESY peaks from a mixing time of 200 ms were chosen for the structural determination. The peaks were first assigned manually and then integrated to obtain the peak intensities. The program CYANA (L.A. Systems, Inc, Japan) was used to calibrate the peaks for structural determination [19].

Structure Determination

The structure was calculated using torsion angle dynamics simulated annealing as implemented in CYANA using restraints that

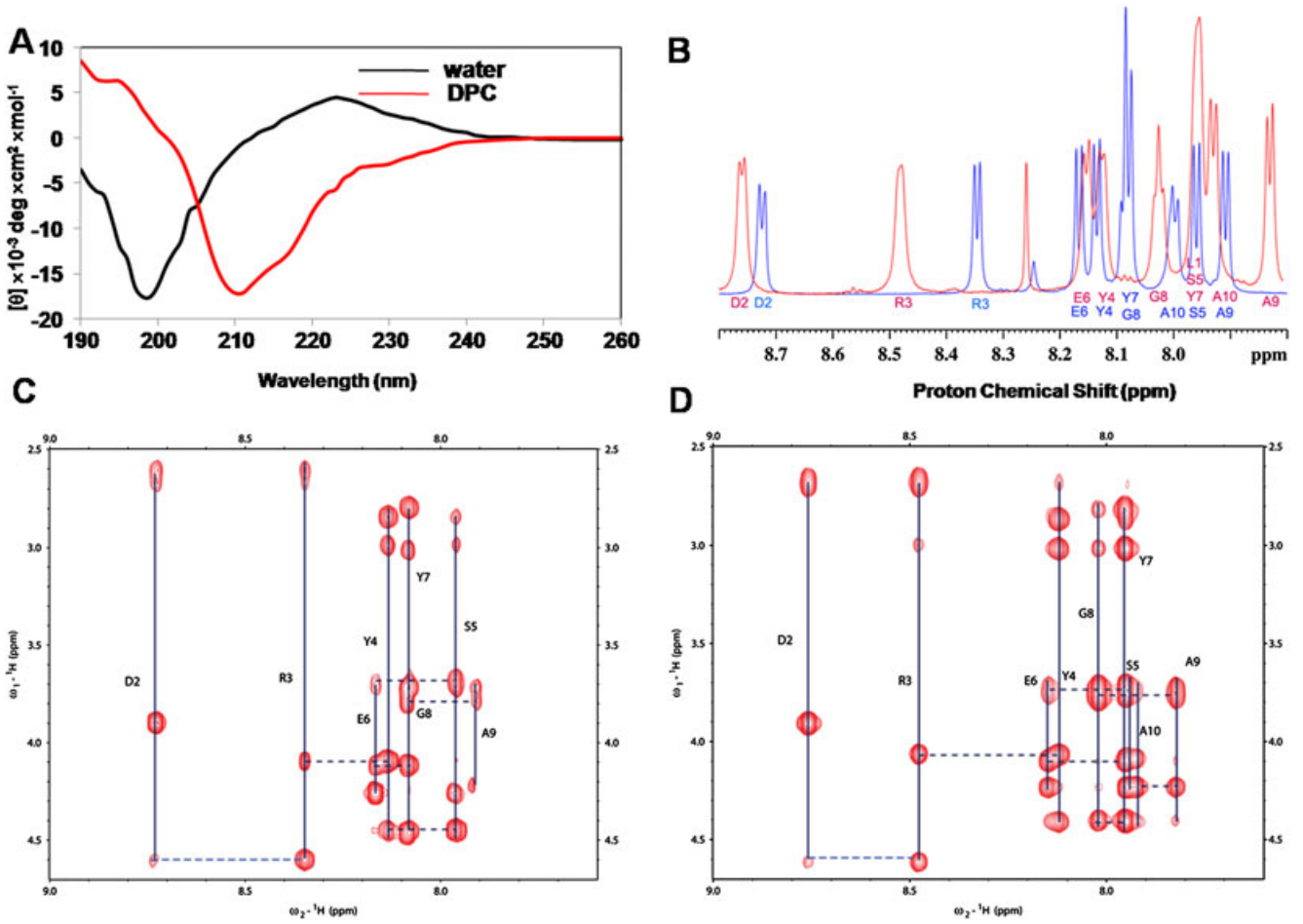

Figure 1. The structural analysis of the S4–S5 linker. (A) CD spectra of peptide in water and in DPC micelles. (B) Proton NMR spectra of the peptide in water and in DPC micelles. The spectra were collected at 298 K and were superimposed using Topspin from Bruker. The amide region is shown, and the spectra of the peptide in water and in micelles are shown. Peptide sequence (numbered from 1 to 10) is LDRYSEYGAA from residues 539 to 548 of the hERG potassium channel. (C) The NOESY spectrum of the peptide in water. The spin system of each residue is shown using a solid line, and the connections between residues are shown using a dash line. (D) The NOESY spectrum of the peptide in DPC micelles. The spin system of each residue is shown using a solid line, and the connections between residues are shown using a dash line.

contained nuclear overhauser effects (NOEs). and dihedral angles [18,19]. Dihedral angles were determined from the $^3J_{HNH\alpha}$ that was from the proton spectrum according to the equation: $^3J_{HNH\alpha} = 6.3\cos^2\theta - 1.4\cos\theta + 1.9$, where $\theta = |\phi - 60^\circ|$ [20,21]. One hundred structures were calculated, and 20 of the structures were analyzed with MOLMOL (ETH, Switzerland) [22] and displayed using PyMOL (www.pymol.org, Portland, OR, USA). The homology model of hERG potassium channel containing the S1 to S6 segments was built using MODELLER (UCSF, CA, USA) [23] using the alignment from the model study [24].

Results

The Selection of Amino Acid Sequence from the S4–S5 Linker

Sequence analysis showed that the hERG channel contained six (S1–S6) transmembrane segments. Studies have shown that the S4–S5 linker of hERG played important roles in the gating of this potassium channel. Many functional studies have demonstrated the importance of this linker in channel gating [1]. Structural and functional studies in other voltage-gated potassium channels suggested that the opened and closed state of a channel through the S4–S5 linker is achieved by the translation of the S4 voltage sensor movement under different membrane potentials [2,25]. Compared with the *Shaker* potassium channel, hERG has a shorter S4–S5 linker [13,15]. A previous study of the S4–S5 linker of the *Shaker* potassium channel demonstrated that the S4–S5 linker acted as a putative docking site for some inactivation factors and that its N-terminus interacted with this linker when the channel was opened [26]. The S4–S5 linker of hERG was also confirmed to have a molecular interaction with 135 residues by using different methods [11,12,27]. Our NMR titration experiment showed that there is a direct interaction between the N-terminal domain of hERG and the S4–S5 linker [12]. The structural information of the S4–S5 linker is still lacking. To investigate the structure of this linker, a peptide containing the residues from 539 to 548 was synthesized on the basis of previous functional studies by other groups [1].

The CD Analysis for the S4–S5 Linker

Circular dichroism was first applied to characterize the structure of the S4–S5 linker in an aqueous solution and membrane-mimicking system that contained DPC micelles (Figure 1). Although DPC may not directly mimic the *in vivo* conditions in a native channel, it has been widely used as a good model system for the structural and functional studies of membrane proteins [28]. The CD spectrum of the linker in the aqueous solution indicated that the peptide existed as a disordered peptide, which was confirmed by the observation of the positive peak at ~222 nm and a negative peak at ~198 nm. In DPC micelles, the positive peak at ~222 nm disappeared, whereas the negative peak shifted to ~210 nm. In addition, negative absorption was observed at ~218 nm. Although an ideal alpha-helical structure will have double minima at 222 and 208 nm, the CD spectrum in DPC indicated that the peptide may contain partial alpha-helical or 3_{10}-helix structure [29] (Figure 1A). To further explore its atomic structure, NMR experiments were performed for this peptide in aqueous solution and DPC micelles.

The Assignment of S4–S5 Linker

One-dimensional proton spectra indicated changes in the chemical shifts under different conditions (Figure 1B). The spin system assignment in water and in DPC micelles was performed to utilize the data from the TOCSY spectrum, and the assignments in water and in DPC solutions were shown (Figure 1C and D). In addition, the NOESY spectra were utilized to complete the assignment, and the NOE connection in the amino proton region in DPC micelles was also shown (Figure 2A, Table S1). To analyze its structure in DPC micelles, chemical shift index [30] analysis using chemical shift from Hα was conducted. For an ideal alpha-helical structure, the Δ Hα is less than −0.1 ppm. Our CSI analysis showed that ΔHαs of residues R541 to G546 were close to −0.1 ppm, indicating that those residues had a tendency to form an alpha-helical structure (Figure 2B) [16]. The $^3J_{HNH\alpha}$ of residues R541 to G546 are in the range of 4 to 6 Hz. To further understand its structure, NOE restraints were applied to determine its tertiary structure.

The Structure Determination of S4–S5 Linker

The solution structures of this linker were determined using the NOEs from the NOESY spectrum with a mixing time of 200 ms. For the S4–S5 linker in water, 53 distance constraints were applied in the structure determination. Structure determination resulted in 20 structures with the lowest target function. The structures were superimposed with an root-mean-square

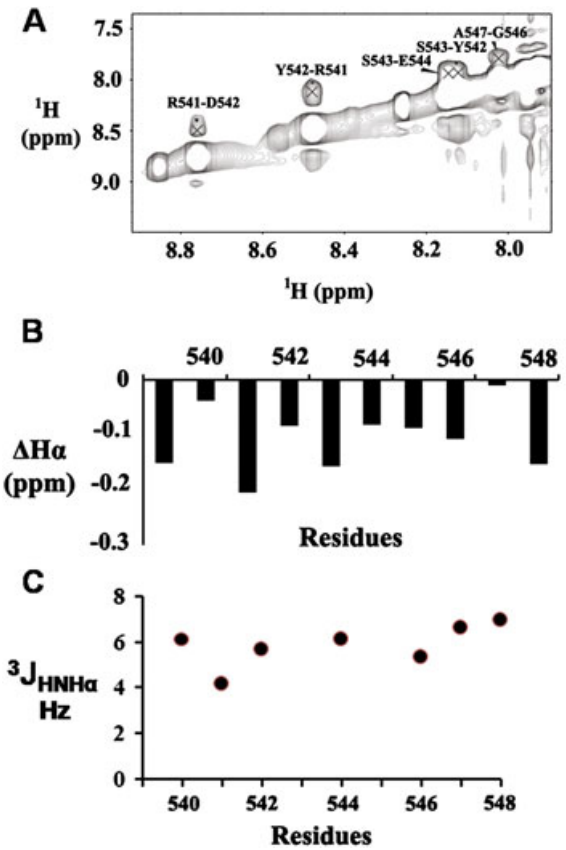

Figure 2. Secondary structure analysis of peptide in DPC micelles. (A) NOE connections in the amide proton region for peptide in DPC micelles. (B) Chemical shift index analysis of peptide in DPC micelles. (C) The $^3J_{HNH\alpha}$ of peptide in DPC micelles. The couplings were obtained from proton spectrum with water suppression.

Journal of PeptideScience

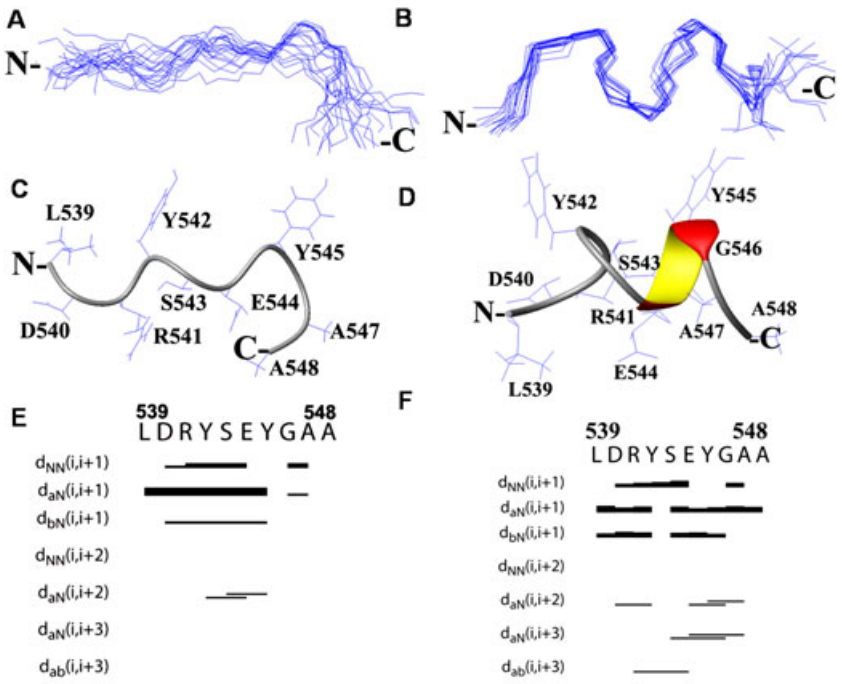

Figure 3. The NMR structure of the S4–S5 linker. (A) The Cα traces of 20 superimposed structures. (B) The superimposed structures of linker in DPC micelles. (C) and (D) The structures of the linker in water and in DPC micelles. One structure with lowest energy is shown, and the residues are labeled with residue numbers. (E) and (F) The NOESY connectivity of the linker in water and in DPC micelles.

deviation (RMSD) of 1.656 Å for the backbone and 2.887 Å for the heavy atoms (Figure 3A). The structure in DPC micelles was determined using 71 constraints including 64 NOEs. The 20 structures were superimposed with an RMSD of 0.885 Å for the backbone and 1.408 Å for the heavy atoms (Figure 3B). No violations were observed in the structure determination. The structure table that summarized the restraints and the structural analysis using PROCHECK (European Bioinformatics Institute, United Kingdom) [31] was shown (Table 1). The NOE connectivities of S4–S5 linker in water and in DPC micelles were shown in Figure 3C and D. In an aqueous solution, the S4–S5 linker exhibited a random coiled structure. In the DPC environment, residues were shown to form an alpha-helical structure.

Table 1. Structural statistics for the ensemble of 20 structures of S4–S5 linker in water and in DPC micelles.

	Water	DPC
Restraints	53	71
Intraresidue NOEs	20	29
Sequential NOEs (*i* to *i* + 1)	21	23
Medium range NOEs (*i* to *i* + 2,3,4)	2	12
Dihedral angle restraints[a]	10	7
Number of NOE violations >0.5 Å	0	0
Number of dihedral angle violations >5	0	0
Ramachandran plot (%)		
Residues in most favored regions	27.1	65.0
Residues in additional allowed regions	72.1	29.3
Residues in generously allowed regions	0.7	2.90
Residues in disallowed regions	0	2.90
Structural statistics		
RMSD for backbone atoms (residues 1–10)	1.656	0.885
RMSD for heavy atoms (residues 1–10)	2.887	1.408

[a]A broad dihedral restraint −180 to −30 for Φ was given during structure calculation of the peptide in water, whereas in DPC structure calculation uses dihedral restraints Φ calculated from $^{3}J_{HNHA}$.

In DPC micelles, the S4–S5 linker peptide was shown to have a well-defined structure. It was predicted that the S4–S5 linker may display as an amphipathic helix under the membrane conditions [15]. MOLMOL analysis showed that residues S543 to Y545 formed a 3_{10}-helix structure (Figure 3D). The helix wheel prediction showed that the S4–S5 linker contained residues that can form an amphipathic helix (Figure 4A) in which residues Y542, Y545, and G546 face one side. Our structure in the DPC micelles showed that residues Y542, Y545, and G546 face one direction, and other residues face the other side (Figure 4B). As the structure was determined in DPC micelles, the hydrophobic residues are expected to interact with micelles, which were used to mimic the cell membrane inside a cell. To further confirm the interaction between peptide and micelles, we added Gd^{+3} (up to 2.0 mM) to DPC micelle containing the peptide. Gd^{3+} is a paramagnetic relaxation reagent that can suppress signals from the exposed atoms [32]. Gd^{3+} caused little suppression of signals from residues L539, Y542, and G546, indicating that these residues interact with micelles (Figure 4C). The structural analysis on the side chains of residues between R541 and E544 has close contact, which may be important for its function (Figure 4D) because some NOE between these two residues was observed (Figure S1).

Discussion

We used a peptide fragment from the channel to perform the structural study under membrane-mimicking systems, which may not be identical to the condition *in vivo*. Here, we examined the structural features of the S4–S5 linker along with the previous

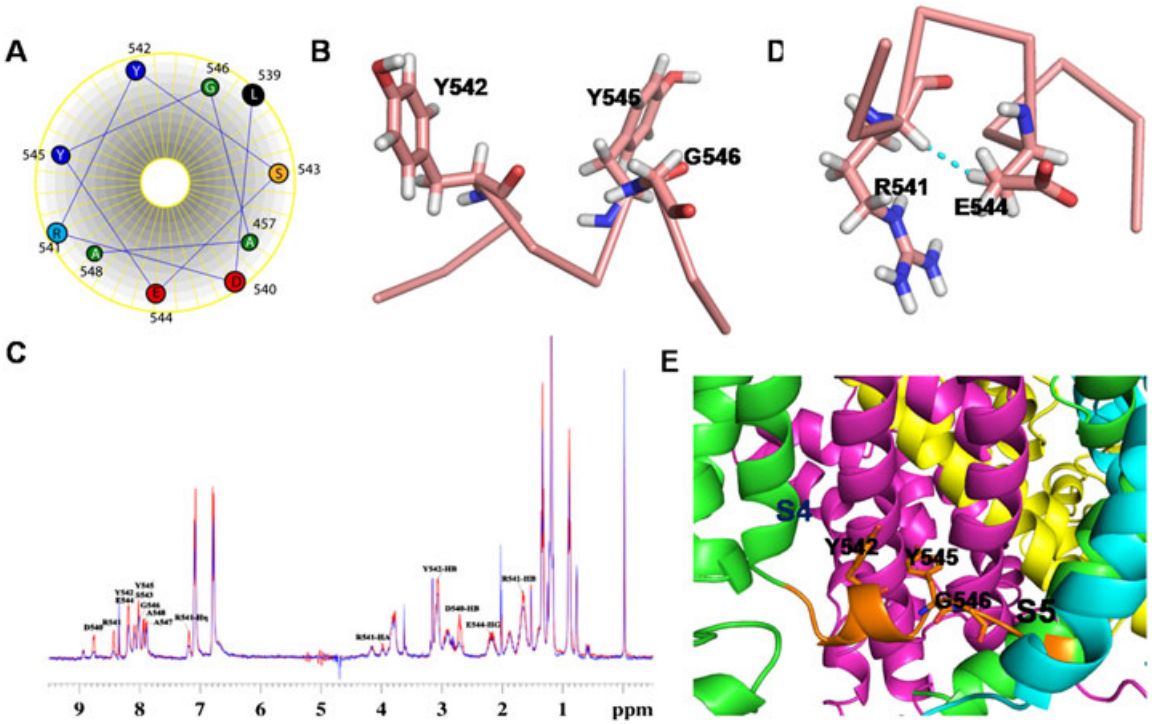

Figure 4. Structural analysis of the S4–S5 linker in DPC micelles. (A) A helix wheel prediction of the S4–S5 linker. The linker was predicted as a helix, and a diagram of the helix wheel was drawn. Residue numbers are shown. (B) Residues interacting with micelles. Hydrophobic residues, which are predicted to interact with membrane, are shown using the stick mode in PyMOL. (C) Effect of Gd^{3+} on the signal from the peptide in DPC micelles. The reference spectrum and spectrum with Gd^{3+} were superimposed in Topspin 2.1. (D) A close contact between E544 and R541. The distance between the side chains of E544 and R541 is shown. (E) The structure of S4–S5 linker in a model. The homology model is shown, and a different subunit is shown in different color. The residues in S4–S5 linker that have the potential to interact with cell membrane are shown in stick mode.

mutagenesis and functional studies. Structures in DPC micelles will be close to the physiological structure of this linker because the S4–S5 linker is close to the cell membrane. The structure showed that residues L539, Y542, and Y545 faced one side. Residues facing the other side included D540, R541, and E544 and may be responsible for the molecular interaction in which the charge–charge interactions might be involved. Structural prediction from other potassium channels showed that S4–S5 linkers in potassium channels adopt an alpha-helix. Our CD and NMR results indicated that there was no ideal alpha-helical structure present when the peptide was dissolved in DPC micelles (Figure 1A), but the peptide may exist as 3_{10}-helical structure in this membrane-mimicking environment. Because of the size of this peptide, no ideal alpha-helical structure was observed in the CD experiment; under physiological conditions, this region may be helical or near-helical. The difference from prediction might arise from the fact that the S4–S5 linker is located between the S4 and S5 fragments, and separating this linker from its natural environment may affect its forming a helix. Our NMR results did show that this linker had potency to form an alpha-helical structure, and the structure in DPC micelles is close to the modeled structure of the S4–S5 linker (Figure 4E).

In the mutagenesis study, charged residues in this S4–S5 linker play very important roles in the rate of current activation and deactivation [1]. The mutated version of hERG channel containing D540A accelerated the channel activation compared with the wild type version of the hERG channel. D540 was proposed to form a salt bridge with a positively charged residue nearby to stabilize the closed state of the hERG channel [5,9]. The structure of the S4–S5 linker in DPC micelles showed that D540 faced the solution and displayed the structural basis for the interaction with other residues (Figure 3D). Another hERG channel mutation containing E544A activated faster than the wild type version, whereas the mutation containing E544K activated slower than the wild type hERG channel [1]. The mutation of E544 to different type of residues had different effects on the channel activation [1]. A close contact between R541 and E544 was identified in our structure in DPC micelles (Figure 4D). E544 to A mutation might affect its interaction with R541, which may be the reason that the channel activation rate was changed. On the other side, the mutation of residue from E to positively charged residue K might create a repulsive force between these two residues, which may be the reason that the mutant channel activated slower than the wild type hERG channel.

A conserved leucine zipper motif was identified in some voltage-gated potassium channels such as the *Shaker* channel [13,33]. A glycine residue (G546) in hERG channel breaks this leucine zipper motif [13]. The mutation study supports the hypothesis that the presence of glycine may be important for channel gating with the constraints on the voltage sensor movement [33]. Our structure demonstrated that G546 was close to the membrane surface site. Because no side chain is present in the glycine residues, the residue that faces the membrane may give more freedom for the S4–S5 linker to move under different conditions. The mutation of this residue to other residues might change the interaction between the S4–S5 linker and the cell membrane, which in turn affect the gating of the hERG channel. A recent model study showed that the structure of this linker might undergo some changes under different conditions [24]. The cell membrane is composed of lipids and

Journal of PeptideScience

cholesterol, and the inner leaflet is normally negatively charged. The charges of the cell membrane may affect the interaction between R542 and E544 that we observed in the structure, which in turn may change the structure of the linker. In addition, the condition used for NMR study was different from physiological conditions. Although the peptide structure in DPC micelles can explain some of the function studies, further structural study using a longer peptide sequence such as including the S4 segment and this linker and selection of different membrane-mimicking systems will be useful to understand the structural changes of this region under different membrane potentials.

In summary, structures of peptide that were derived from the S4–S5 linker of the hERG potassium channel were studied using CD and NMR spectroscopy. The peptide was not structured in an aqueous solution and displayed a 3_{10}-helix structure in DPC micelles. Residues L539, Y542, and G546 faced one side of the structure, which may provide a structural basis for protein-membrane interactions. Our results provide structural information to understand the role of the S4–S5 linker in the gating of the potassium channel.

Acknowledgements

The NMR spectra were collected at the School of Biological Sciences, Nanyang Technological University. We appreciate the generous support from Prof. Ho Sup Yoon from Nanyang Technological University. We also thank the support from A*STAR Investigatorship and A*STAR JCO grant (10/03/FG/06/06) of Singapore. C. K. is a recipient of A*STAR Investigatorship.

References

1 Sanguinetti MC, Xu QP. Mutations of the S4-S5 linker alter activation properties of HERG potassium channels expressed in Xenopus oocytes. *J. Physiol.* 1999; **514**: 667–675.
2 Long SB, Tao X, Campbell EB, MacKinnon R. Atomic structure of a voltage-dependent K^+ channel in a lipid membrane-like environment. *Nature* 2007; **450**: 376–382.
3 Chen J, Mitcheson JS, Tristani-Firouzi M, Lin M, Sanguinetti MC. The S4-S5 linker couples voltage sensing and activation of pacemaker channels. *Proc. Natl. Acad. Sci. U.S.A.* 2001; **98**: 11277–11282.
4 Elliott DJ, Dondas NY, Munsey TS, Sivaprasadarao A. Movement of the S4 segment in the hERG potassium channel during membrane depolarization. *Mol. Membr. Biol.* 2009; **26**: 435–447.
5 Ferrer T, Rupp J, Piper DR, Tristani-Firouzi M. The S4-S5 linker directly couples voltage sensor movement to the activation gate in the human ether-a'-go-go-related gene (hERG) K^+ channel. *J. Biol. Chem.* 2006; **281**: 12858–12864.
6 Jiang Y, Lee A, Chen J, Ruta V, Cadene M, Chait BT, MacKinnon R. X-ray structure of a voltage-dependent K^+ channel. *Nature* 2003; **423**: 33–41.
7 Slesinger PA, Jan YN, Jan LY. The S4-S5 loop contributes to the ion-selective pore of potassium channels. *Neuron* 1993; **11**: 739–749.
8 Warmke JW, Ganetzky B. A family of potassium channel genes related to eag in Drosophila and mammals. *Proc. Natl. Acad. Sci. U.S.A.* 1994; **91**: 3438–3442.
9 Tristani-Firouzi M, Chen J, Sanguinetti MC. Interactions between S4-S5 linker and S6 transmembrane domain modulate gating of HERG K^+ channels. *J. Biol. Chem.* 2002; **277**: 18994–19000.
10 Gustina AS, Trudeau MC. A recombinant N-terminal domain fully restores deactivation gating in N-truncated and long QT syndrome mutant hERG potassium channels. *Proc. Natl. Acad. Sci. U.S.A.* 2009; **106**: 13082–13087.
11 Wang J, Trudeau MC, Zappia AM, Robertson GA. Regulation of deactivation by an amino terminal domain in human ether-a-go-go-related gene potassium channels. *J. Gen. Physiol.* 1998; **112**: 637–647.
12 Li Q, Gayen S, Chen AS, Huang Q, Raida M, Kang C. NMR solution structure of the N-terminal domain of hERG and its interaction with the S4-S5 linker. *Biochem. Biophys. Res. Commun.* 2010; **403**: 126–132.
13 Van Slyke AC, Rezazadeh S, Snopkowski M, Shi P, Allard CR, Claydon TW. Mutations within the S4-S5 linker alter voltage sensor constraints in hERG K^+ channels. *Biophys. J.* 2010; **99**: 2841–2852.
14 Long SB, Campbell EB, Mackinnon R. Crystal structure of a mammalian voltage-dependent Shaker family K^+ channel. *Science* 2005; **309**: 897–903.
15 Ohlenschlager O, Hojo H, Ramachandran R, Gorlach M, Haris PI. Three-dimensional structure of the S4-S5 segment of the Shaker potassium channel. *Biophys. J.* 2002; **82**: 2995–3002.
16 Ju P, Pages G, Riek RP, Chen PC, Torres AM, Bansal PS, Kuyucak S, Kuchel PW, Vandenberg JI. The pore domain outer helix contributes to both activation and inactivation of the HERG K^+ channel. *J. Biol. Chem.* 2009; **284**: 1000–1008.
17 Wüthrich K. *NMR of Proteins and Nucleic Acids*. Wiley: New York.
18 Gayen S, Kang C. Solution structure of a human minimembrane protein Ost4, a subunit of the oligosaccharyltransferase complex. *Biochem. Biophys. Res. Commun.* 2011; **409**: 572–576.
19 Güntert P, Mumenthaler C, Wüthrich K. Torsion angle dynamics for NMR structure calculation with the new program DYANA. *J. Mol. Biol.* **273**: 283–298.
20 Li Y, Tamm LK. Structure and plasticity of the human immunodeficiency virus gp41 fusion domain in lipid micelles and bilayers. *Biophys. J.* 2007; **93**: 876–885.
21 Pardi A, Billeter M, Wuthrich K. Calibration of the angular dependence of the amide proton-C alpha proton coupling constants, 3JHN alpha, in a globular protein. Use of 3JHN alpha for identification of helical secondary structure. *J. Mol. Biol.* 1984; **180**: 741–751.
22 Koradi R, Billeter M, Wuthrich K. MOLMOL: a program for display and analysis of macromolecular structures. *J. Mol. Graph.* 1996; **14**: 51–55, 29–32.
23 Eswar N, Eramian D, Webb B, Shen MY, Sali A. Protein structure modeling with MODELLER. *Methods Mol. Biol.* 2008; **426**: 145–159.
24 Subbotina J, Yarov-Yarovoy V, Lees-Miller J, Durdagi S, Guo J, Duff HJ, Noskov SY. Structural refinement of the hERG1 pore and voltage-sensing domains with ROSETTA-membrane and molecular dynamics simulations. *Proteins* 2010; **78**: 2922–2934.
25 Swartz KJ. Sensing voltage across lipid membranes. *Nature* 2008; **456**: 891–897.
26 Isacoff EY, Jan YN, Jan LY. Putative receptor for the cytoplasmic inactivation gate in the Shaker K^+ channel. *Nature* 1991; **353**: 86–90.
27 De la Pena P, Alonso-Ron C, Machin A, Fernandez-Trillo J, Carretero L, Dominguez P, Barros FJ. Demonstration of physical proximity between the amino terminus and the S4-S5 linker of the h-ERG potassium channel. *J. Biol. Chem.* 2011; **286**: 19065–19075.
28 Kang C, Li Q. Solution NMR study of integral membrane proteins. *Curr. Opin. Chem. Biol.* 2011; **15**: 560–569.
29 Biron Z, Khare S, Samson AO, Hayek Y, Naider F, Anglister J. A monomeric 3(10)-helix is formed in water by a 13-residue peptide representing the neutralizing determinant of HIV-1 on gp41. *Biochemistry* 2002; **41**: 12687–12696.
30 Wishart DS, Sykes BD, Richards FM. The chemical shift index: a fast and simple method for the assignment of protein secondary structure through NMR spectroscopy. *Biochemistry* 1992; **31**: 1647–1651.
31 Laskowski RA, Rullmannn JA, MacArthur MW, Kaptein R, Thornton JM. AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. *J. Biomol. NMR* 1996; **8**: 477–486.
32 Madl T, Guttler T, Gorlich D, Sattler M. Structural analysis of large protein complexes using solvent paramagnetic relaxation enhancements. *Angew. Chem. Int. Ed Engl.* 2011; **50**: 3993–3997.
33 McCormack K, Tanouye MA, Iverson LE, Lin JW, Ramaswami M, McCormack T, Campanelli JT, Mathew MK, Rudy B. A role for hydrophobic residues in the voltage-dependent gating of Shaker K^+ channels. *Proc. Natl. Acad. Sci. U.S.A.* 1991; **88**: 2931–2935.

