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Structural analysis of the S4–S5 linker of the human KCNQ1 potassium channel

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ABSTRACT

KCNQ1 plays important roles in the cardiac action potential and consists of an N-terminal domain, a voltage-sensor domain, a pore domain and a C-terminal domain. KCNQ1 is a voltage-gated potassium channel and its channel activity is regulated by membrane potentials. The linker between transmembrane helices 4 and 5 (S4–S5 linker) is important for transferring the conformational changes from the voltage-sensor domain to the pore domain. In this study, the structure of the S4–S5 linker of KCNQ1 was investigated by solution NMR, circular dichroism and fluorescence spectroscopic studies. The S4–S5 linker adopted a helical structure in detergent micelles. The W248 may interact with the cell membrane.

1. Introduction

The KCNQ1 potassium channel is a member of voltage-gated potassium channels and plays important functions in various tissues including heart and inner ear [1]. Mutations in the human KCNQ1 channel lead to disorders such as long-QT syndrome (LQTS) and Jervell and Lange-Nielsen syndrome (JLNS), characterized by both LQTS and congenital deafness [2,3]. The human KCNQ1 contains an N-terminal domain, a voltage-sensor domain (VSD) formed by transmembrane helices S1–S4, a pore domain formed by S5–S6, and a C-terminal domain [1,4]. The KCNQ1 is functional as a tetramer and the four pore domains form an ion transduction pore across the cell membrane for potassium transportation [5]. Like other voltage-gated potential channels, the open and close of the pore domain of the KCNQ1 channel is regulated by the membrane potential. The positively charged residues in the S4 are important for sensing changes of the membrane potential across the cell membrane because changing the charges of the S4 perturbed KCNQ1 channel gating [6–8].

It is suggested that changes of membrane potential may cause VSD to change its conformation, by which the pore domain is open or closed to affect potassium transportation. Although the gating mechanism for the voltage-gated potassium channels is still not well known, the S4–S5 linker of a voltage-gated potassium channel is considered to be important for channel gating due to its physical location [5]. Several studies have demonstrated that the interaction between S4–S5 linker and the S6 may be important for channel gating [9,10]. The crystal structure of a mammalian *Shaker* potassium channel-Kv1.2 indicated that S4–S5 linker interacts with S6 though hydrophobic residues [11]. An alanine/tryptophan perturbation scanning study of the S4–S5 linker suggested that its interaction with S6 may be a hydrophobic nature, which is similar to the *Shaker* channels [12]. In the heart, the KCNQ1 channel forms a complex with KCNE1 and this complex is responsible for the slow delayed rectifier current (Iks) [13]. The S4–S5 linker was also important for the molecular interaction with KCNE1 [14,15].

Although homology model has been conducted for the KCNQ1 transmembrane segments S1–S6 [5] and an NMR study has been carried out for the VSD [16], the structural information for the KCNQ1 channel is still needed. Several structural studies have been conducted for the S4–S5 linker of the potassium channels including hERG, Shaw 2 kV channel and the shaker channel [17–19]. In this study, we presented structural study of the S4–S5 linker of the KCNQ1 channel. Our results showed that the linker adopted helical structure in detergent micelles. The tryptophan residue (W248) in the linker interacts with the membrane. Our results will be useful to understand the structure and function of the S4–S5 linker of the KCNQ1 channel.

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2. Materials and methods

2.1. Sample preparation

The peptide derived from the S4–S5 linker of the human KCNQ1 was synthesized from GL Biochem Ltd. with more than 95% purity (Shanghai, China). The peptide sequence was DRQGGTWRLLGSVVFIHRQE compassing residues D242 to E261 of the human KCNQ1. Peptide was dissolved to 5 mg/ml in a buffer that contained 20 mM sodium phosphate, pH 6.5, 2% D-SDS and 10% D2O. The peptide solution was transferred to an NMR tube for data acquisition.

2.2. Circular dichroism (CD) spectroscopy

Far-UV CD spectrum of the peptide at a concentration of 20 μM was analyzed in a buffer containing 20 mM sodium phosphate, pH6.5 in the absence or presence of 0.2% sodium dodecyl sulfate (SDS) or 0.5% dodecylphosphocholine (DPC). The instrument was blanked using a cuvette containing buffer without peptide. The CD spectra were recorded at 25 °C.

2.3. NMR data acquisition

All the NMR data were collected on a Bruker 600 spectrometer equipped with a cryoprobe. The experimental temperature was 35 °C. A total correlation spectroscopy (TOCSY) experiment with a mixing time of 80 ms as we did for other peptide was recorded [17]. Two-dimensional (2D) nuclear overhauser effect spectroscopy (NOESY) was recorded with mixing times of 150, 200 and 300 ms, respectively. Proton chemical shifts were referenced directly to 4,4-dimethyl-4-silapentane-1-sulfonic acid (DSS) purchased from Cambridge Isotope Laboratories (Andover, USA). All of the NMR spectra were processed using Topspin 2.1 (Bruker) and visualized using Sparky (http://www.cgl.ucsf.edu/home/sparky/).

2.4. Resonance assignment

The assignment for the S4–S5 linker was accomplished with the procedures including the identification of spin systems and spin connections. TOCSY spectrum was used to identify spin systems, and sequential connectivity was determined based on the connectivity in the HN-HN or Hα-HN region using the NOESY spectrum [20,21]. The peaks from a NOESY spectrum with a mixing time of 200 ms were picked up and integrated in Sparky and converted to distance restraints. The peaks were assigned manually and then calibrated by CYANA 2.1 for structural determination [22].

2.5. Structure determination

The structure of the S4–S5 linker was determined using torsion angle dynamics simulated annealing as implemented in CYANA2.1 using restraints including NOEs and dihedral angles derived using TALOS+ [21,22]. One hundred structures were calculated and twenty of the structures with lowest energies were selected and presented using PyMOL (www.pymol.org).

2.6. Fluorescence spectroscopy

The fluorescence measurements was conducted as described previously [23,24]. The fluorescence emission spectra were measured in a 96-well plate. The peptide was prepared to 50 μM in a buffer in the presence or absence of detergent. The buffer contained 20 mM sodium phosphate, pH 6.5. Samples without or with detergent (0.2% SDS or 0.5% DPC) were subjected to analysis. Excitation wavelength was set to 280 nm and the emission was scanned from 305 to 400 nm.

3. Results and discussion

3.1. Structural analysis for the S4–S5 linker

The human KCNQ1 channel is a multi-domain protein and contains 676 resides. The S4–S5 linker localizes between the VSD and the pore domain. Due to its physical location, it plays an important role in channel gating (Fig. 1A). We synthesized a peptide compassing residues D242 to E261 of the human KCNQ1. The peptide sequence contains the S4–S5 linker region of the human KCNQ1. Previous sequence analysis has shown that there are several hydrophobic residues present in this region. We first tried to study its structure in a buffer without any membrane system. The solubility of the peptide was low, which may arise from the hydrophobic nature of this region. We then tested the effect of detergent on its solubility. It was found that peptide can be easily dissolved in the presence of detergents such as SDS and DPC micelles. This can be explained that detergent micelles can provide a hydrophobic environment which is suitable for the folding of the peptide [25]. Further CD spectra were acquired to understand its structure under different conditions. In a detergent free buffer that contained

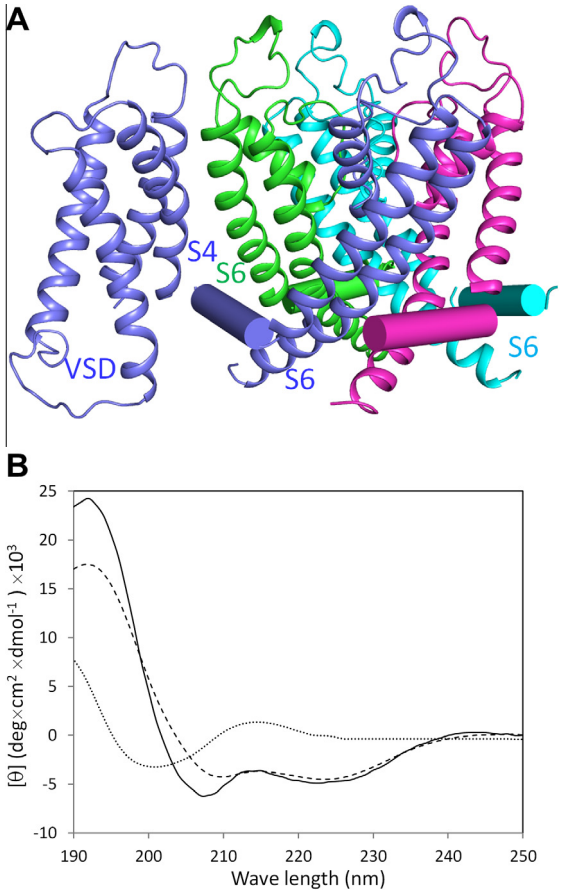

Fig. 1. Structural analysis of the S4–S5 linker. (A) Model of the S1–S6 of KCNQ1 in open state. The homology model of the KNCQ1 is shown. The four KCNQ1 subunits are shown in different color. For clarity, only VSD of one subunit is shown. The S4–S5 linkers are shown as cylinders. (B) CD analysis of the S4–S5 linker. The spectra of the peptide in the absence (dotted line) or presence of SDS (solid line) or DPC (dashed line) are shown. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

20 mM sodium phosphate and pH6.5, the peptide is unstructured. In the presence of detergent micelles, peptide adopted helical structures (Fig. 1B). Although peptide exhibited slightly different CD spectra when it was prepared in DPC and SDS micelles, the observation of double minima at 222 and 208 nm suggested that peptide formed helical structures when detergent micelles were present.

3.2. Resonance assignment for the S4–S5 linker in SDS micelles

Further NMR studies were carried out to understand the structure of the S4–S5 linker in both DPC and SDS detergent micelles. Only peptide in SDS micelles produced dispersed signals for assignment (Fig. 2A). The assignment of the S4–S5 linker in SDS micelles was achieved using the TOCSY and NOESY spectra. The values of the Hα chemical shifts were compared with random coil values (Fig. 2B). Residues T247 to H258 exhibited negative chemical shift values, suggesting that this region has a tendency to be a helix (Fig. 2B). In consistent with the Hα chemical shit analysis, the peptide in SDS micelles exhibited a series of $d_{NN(i,\ i+1)}$, $d_{\alpha N(i,\ i+3)}$ and $d_{\alpha N(i,\ i+4)}$ NOE connectivity starting from residues T247 (Fig. 2C), which indicated the presence of a helical structure.

3.3. Structure of S4–S5 linker in SDS micelles

The three-dimensional structure of the S4–S5 linker of the human KCNQ1 in SDS micelles was determined using constrains

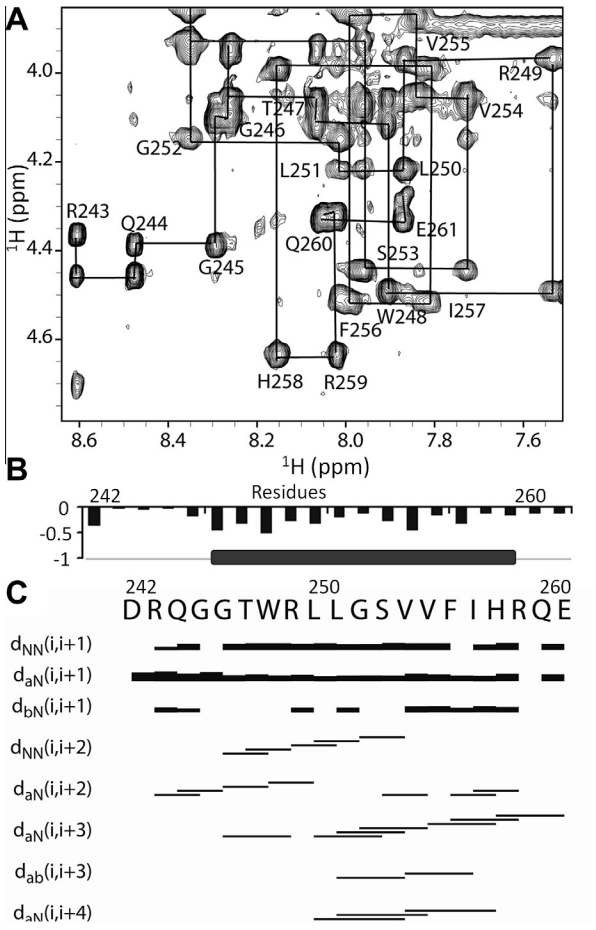

Fig. 2. Assignment of the S4–S5 linker in SDS micelles. (A) Assignment of NOESY spectrum of the peptide linker. The amide and Hα region of the linker in SDS micelles is shown. (B) The Hα chemical shift difference (ppm) from the random coil values. (C) NOE connectivity of the S4–S5 linker in SDS micelles.

Table 1
Statistics for the S4–S5 linker of KCNQ1 in SDS micelles.

Total number of NMR restraints	181
Number of unambiguous NOEs	161
Intraresidual ($\|i-j\| = 0$)	62
Short range ($\|i-j\| = 1$)	66
Medium-range ($2 \leqslant \|i-j\| \geqslant 4$)	33
Long-range ($\|i-j\| > 4$)	0
Number of dihedral angle constraints	20
Number of hydrogen-bond restraints	0
Number of restraint violations[a]	
Total number of restraint violations >0.5 Å	0
Total number of dihedral angle constraints >5°	0
Ramachandran plot statistics[b] (%)	
Residues in most favoured regions	67
Residues in additionally allowed regions	33
Residues in generously allowed regions	0
Residues in disallowed regions	0
Average RMSD to mean (Å)	
Backbone (residues 247–258)	0.04 ± 0.02 Å
Heavy atoms (residues 247–258)	0.83 ± 0.15 Å

[a] There are no distance violations greater than 0.5 Å or dihedral angle violations greater than 5°. All residues are included in the final ensemble.
[b] The Ramachandran plot was for all the residues.

including NOEs and dihedral angles derived from TALOS+ (Table 1). The ensemble of 20 structural structures with lowest energy out of 100 calculated structures is shown in Fig. 3A. No NOE violations large than 0.5 Å and dihedral angle violation more than 5° were observed in the structure determination. Residues T247 to H258 of the S4–S5 linker formed an α-helical structure in micelles, while the N-terminal 5 resides were not structured (Fig. 3B). The NOEs and structural analysis with PROCHECK-NMR [26] are shown in Table 1.

Although the S4–S5 linkers of other potassium channels such as hERG channel and Kv1.2 are amphipathic helices, the S4–S5 linker of the human KCNQ1 showed some differences. It contains several hydrophobic residues, which makes the peptide hydrophobic. Electrostatic surface potential analysis showed that the N-terminal and the C-terminal parts of the peptide used in this study were hydrophilic and the helix itself is hydrophobic (Fig. 3C), which might be the reason that it had low solubility in the absence of detergent micelles. Helix wheel representation of the helix in the S4–S5 linker also showed its hydrophobic nature. The N-terminal part of the helix compassing residues from R249 to V255 is an amphipathic helix containing a hydrophobic surface formed by residues L250, L251, V254 and V255 and a hydrophilic surface formed by residues including R249, G252 and S253 (Fig. 3B and D). In the S4–S5 linker helix, residues including W248, R249, G252, S253 and I257 face to one direction. Except W248, these residues were shown to have low impact on channel gating. Residues including L250, L251, V254, V255 and H258 point to another direction (Fig. 3B) and these residues were shown to have high impact on channel gating [12].

3.4. Interaction of W248 and membrane

The helix of the S4–S5 linker contains hydrophobic residues localizing at different side of the helix. To identify which residue may interact with the cell membrane, we carried out a fluorescence spectroscopy for this peptide because it contains only one tryptophan residue (W248). When it is exposed to the solvent, the emission maximum is close to 350 nm. When it is buried in a hydrophobic environment such as micelles, the emission maximum will be shifted toward a lower wavelength [24,27]. As seen from Fig. 4, in the absence of detergent micelles, peptide exhibited a broad emission maximum from 340 nm to 370 nm, suggesting that it is not folded. In the presence of DPC or SDS micelles, the

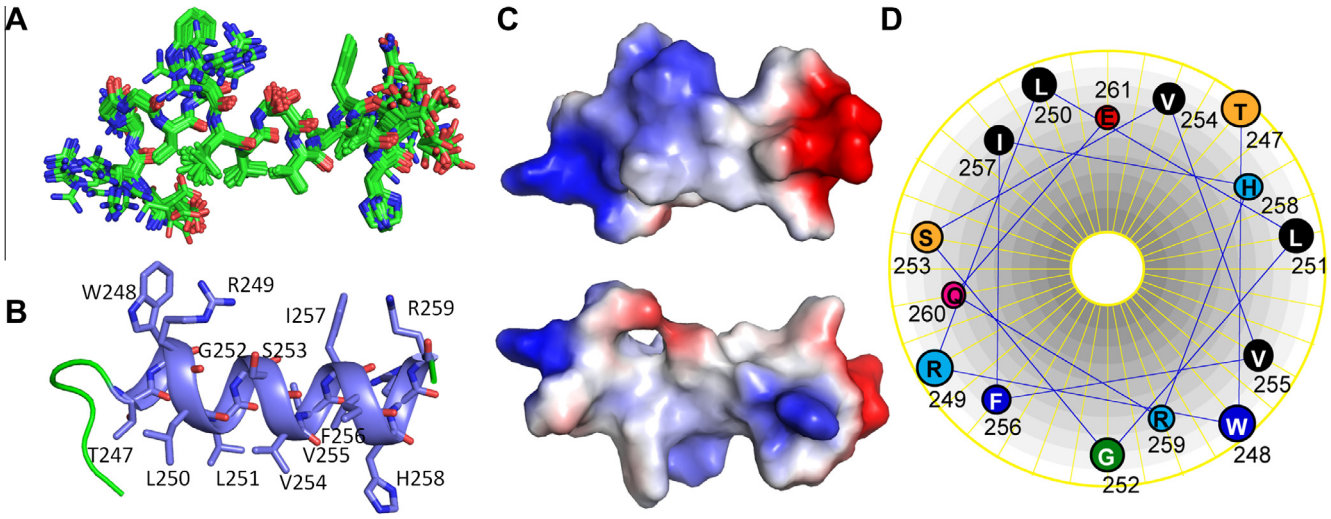

Fig. 3. Structure of the S4–S5 linker. (A) Twenty structures of the S4–S5 linker of the human KCNQ1. The side chains of the amino acids are shown as sticks. Carbon, nitrogen and oxygen atoms are shown in green, blue and red, respectively. (B) Structure of the S4–S5 linker conformer with lowest energy. The side chains of residues in the helix are shown in sticks. The residues are labeled with residue name and sequence number. (C) Color-coded electrostatic surface potential for the KCNQ1 S4–S5 linker. Positive, negative and hydrophobic regions are shown as blue, red and white, respectively. (D) Helix-wheel representation of the helix. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

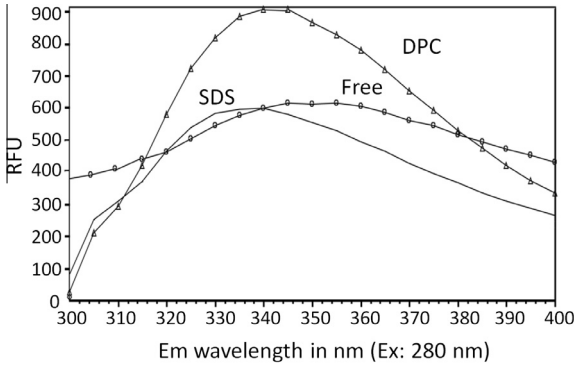

Fig. 4. Fluorescence spectroscopy of the S4–S5 linker of the human KCNQ1. The spectra of the S4–S5 linker in the absence and presence of detergent micelles were acquired and shown. RFU indicates relative fluorescence units.

emission maximum was 330–340 nm, suggesting that the W248 was buried in the micelles (Fig. 4).

Structural study showed that the hydrophobic residues localized at different sides of the helix in the S4–S5 linker (Fig. 3B). The W248 interact with the cell membrane as shown in the fluorescence study. Therefore, residues including W248, R249, G252, S253 and I257 on one side of the helix might be facing to the membrane. The residues including L250, L251, V254, V255 and H258 might be facing to the other side. These residues might be important for interaction with S6, which was predicted from functional study [12]. The R259 might also interact with the cell membrane due to its positive charged side chain (Fig. 2B). This reside might be important for function of the S4–S5 linker because it is localized between the S4–S5 linker and S5. Indeed, it was shown that mutation S259 to Ala affect channel gating [12].

Mutations in the S4–S5 linker contribute to heart diseases such as LQTS. Residues including W248, L250, L251, V254, H258 and R259 were identified to be disease-related mutants. These residues were also shown to have high impact on channel gating [12]. From our structural analysis, these disease-related residues are localized at different position of the helix in the S4–S5 linker (Fig. 3B). W248 might be one of the membrane-anchoring residues as shown in our fluorescence study (Fig. 4) and R259 might be important for membrane interaction too because of its positively charged side chain. Other residues including L250, L251, V254 and H258 might be important for the molecular interaction with S6. Therefore, affecting S4–S5 linker interaction with the cell membrane or with S6 helix might be one of the mechanisms to cause LQTS.

In summary, we show that the S4–S5 linker of the human KCNQ1 channel contains a helix in detergent micelles. This helix contains several hydrophobic residues. The W248 might be important for membrane interaction. Our results will be useful to understand the role of the S4–S5 linker in channel gating.

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