Biochemical and Biophysical Research Communications 437 (2013) 408–412

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Intracellular segment between transmembrane helices S0 and S1 of BK channel α subunit contains two amphipathic helices connected by a flexible loop

Pan Shi [a,b], Dong Li [a], Chaohua Lai [a], Longhua Zhang [a,*], Changlin Tian [a,b,*]

[a] Hefei National Laboratory of Microscale Physical Sciences, School of Life Sciences, University of Science and Technology of China, Hefei, Anhui, 230027, PR China
[b] High Magnetic Field Laboratory, Chinese Academy of Sciences, Hefei, Anhui, 230031, PR China

ARTICLE INFO

Article history:
Received 21 June 2013
Available online 2 July 2013

Keywords:
BK channel
Intracellular segment
Solution NMR
Backbone assignment
Secondary structure
Titration

ABSTRACT

The BK channel, a tetrameric potassium channel with very high conductance, has a central role in numerous physiological functions. The BK channel can be activated by intracellular Ca^{2+} and Mg^{2+}, as well as by membrane depolarization. Unlike other tetrameric potassium channels, the BK channel has seven transmembrane helices (S0–S6) including an extra helix S0. The intracellular segment between S0 and S1 (BK-IS1) is essential to BK channel functions and Asp99 in BK-IS1 is reported to be responsible for Mg^{2+} coordination. In this study, BK-IS1 (44–113) was over-expressed using a bacterial system and purified in the presence of detergent micelles for multidimensional heteronuclear nuclear magnetic resonance (NMR) structural studies. Backbone resonance assignment and secondary structure analysis showed that BK-IS1 contains two amphipathic helices connected by a 36-residue loop. Amide ^{1}H–^{15}N heteronuclear NOE analysis indicated that the loop is very flexible, while the two amphipathic helices are possibly stabilized through interaction with the membrane. A solution NMR-based titration assay of BK-IS1 was performed with various concentrations of Mg^{2+}. Two residues (Thr45 and Leu46) with chemical shift changes were observed but no, or very minor, chemical shift difference was observed for Asp99, indicating a possible site for binding divalent ions or other modulation partners.

1. Introduction

The BK channel is a potassium channel with very high single-channel conductance (100–300 pS) that can be activated by intracellular Ca^{2+} and Mg^{2+} as well as by membrane depolarization [1–6]. BK channels have important roles in many physiological processes, including smooth muscle contraction, mediation of alcohol tolerance, hypertension, potassium secretion, neurotransmitter release and neuronal excitability [7,8]. Additionally, enhanced expression of BK channel α subunits is correlated with a high proliferation rate and malignancy of breast cancer [9]. In mammalian cells, the BK channel is encoded by a single *Slo1* gene. In most tissues, BK channels consist of pore-forming α and regulatory β subunits, which are arranged in a 1:1 stoichiometry [10]. In mammals, there are four members of the BK channel β subunit family; β1–β4. The BK channel belongs to the S4 superfamily of K^+ channels, which is characterized by a positively charged S4 helix in the voltage sensor domain (VSD). The BK channel contains four identical α subunits, each comprising a large intracellular C terminus and seven transmembrane helices (S0–S6). The traditional S1–S6 helices include a VSD (S1–S4) and a pore domain (S5–S6) (Fig. 1A). The S0 helix is located before the S1–S6 helices in the BK channel. Thus, the BK channel has its NH_2 terminus on the extracellular side of the membrane and a long cytosolic intracellular segment (BK-IS1) [11]. The S0 segment, which was hypothesized to be involved in β subunit association, can function in modulating voltage sensitivity and might make direct contact with the VSD [10,12–14]. Therefore, analysis of the structure of BK-IS1 will help us to understand the significance of the extra transmembrane helix S0 and its interaction with the β subunits.

Consistent with the role of key regulator of vital body functions, malfunction of BK channels can lead to many diseases, including hypertension, epilepsy, noise-induced hearing loss and urinary incontinence [15–17]. However, no high-resolution structure of BK channels was available, except a recent report of a cryo-EM structure at a resolution of 17–20 Å [18]. Recently, a 3.0 Å resolution crystal structure of the cytosolic domain of Slo1, including both RCK1 and RCK2, has been determined [19]. Compared to the $K_V1.2$ structure, the transmembrane domains of the cryo-EM BK structure contain an additional structural component, which is likely to account for the unique S0 segment in BK channels, as well

* Corresponding authors. Address: University of Science and Technology of China, Hefei National Laboratory of Microscale Physical Sciences, School of Life Sciences, Hefei, Anhui, 230027, PR China. Fax: +86 551 63600441 (C. Tian).
E-mail addresses: zlhustc@ustc.edu.cn (L. Zhang), cltian@ustc.edu.cn (C. Tian).

0006-291X/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2013.06.091

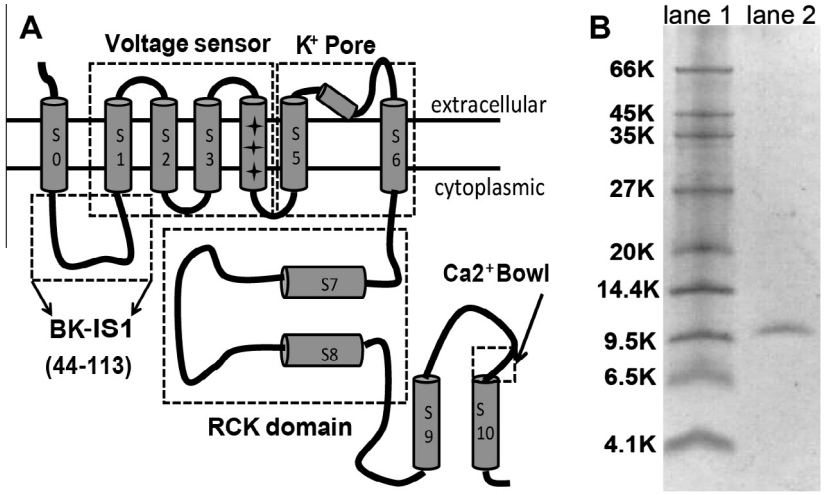

Fig. 1. (A) Diagram of the α subunit of the BK channel [2]. (B) SDS–PAGE of purified human BK-IS1. Lane 1, molecular mass marker; lane 2, purified human BK-IS1.

as the N terminus and BK-IS1 that flank S0 [18]. In addition, it appears that BK-IS1 can form an important component of the interacting region between the BK channel transmembrane domain and the cytosolic RCK domain [6]. Importantly, the BK-IS1 was reported to be engaged in divalent ion-dependent activation of the BK channel. A Mg^{2+}-binding site was mapped at the interface between the VSD (D99 and N172) and the cytosolic domain (E374 and E399) [20,21]. In this study, residue D99 is located in BK-IS1. Several other mutant residues in BK-IS1 have been found to be correlated with diseases: Thr107 of bovine *slo* in BK-IS1 essentially determined the BK channel response to alcohol [22], D81N and D923N in BK-IS1 altered the Ca^{2+} sensitivity of the channel [23]. On the basis of the cryo-EM structure of the BK channel, cytosolic BK-IS1 is proposed to fit snugly between the VSD and the gating ring, filling the void between the core and the C terminus of the gating ring [18]. Therefore, BK-IS1 is crucial in the coupling of voltage sensor and metal ion binding to the activation gate [15]. However, no detailed structural information is available for BK-IS1 concerning divalent ion binding and channel gating.

In this study, over-expressed BK-IS1 was purified and eluted in aqueous buffer containing dodecylphosphocholine (DPC), mimicking lipid bilayer phosphatidylcholine head groups. Once backbone assignments were finalized, the secondary structure and backbone flexibility of BK-IS1 were analyzed and the results showed two helices connected by a flexible loop region. With the availability of sparse nuclear overhauser effect (NOE) restraints, structures of the two helices were calculated to show amphipathic properties. Two consecutive residues in the N terminus of BK-IS1 were observed to have chemical shift perturbations in the two-dimensional NMR spectra upon increased concentration of Mg^{2+}.

2. Materials and methods

2.1. Expression construct

The DNA sequence encoding intracellular segment 1 of the human BK channel (BK-IS1) was amplified using the polymerase chain reaction (PCR) with a pair of primers including *Nde*I restriction enzyme site at the 5′ end and *Xho*I restriction enzyme site at the 3′ end. The amplified fragment was inserted into the pET-21b vector (Novagen Co.) between the two restriction enzyme sites and verified by DNA sequencing. Cysteine residues C53, C54 and C56 were mutated to serine (C53S, C54S and C56S) using a standard PCR-based mutagenesis method and confirmed by DNA sequencing. The mutant expression plasmid was transformed into BL21(DE3) Gold (Novagen Co.). Bacteria were grown in M9 minimal medium containing 100 μg/mL ampicillin. Cultures were grown at 37 °C (with shaking at 225 rpm) until the absorbance at 600 nm (A_{600}) reached 1.0. IPTG was added (final concentration 1 mM) to induce protein expression and the culture was kept at 37 °C (with shaking at 225 rpm) for 4 h.

To achieve over-expression of ^{15}N-labeled BK-IS1, 1.0 g/L [^{15}N]NH_4Cl and 3.0 g/L [^{13}C]glucose (Cambridge Isotope Laboratory, Andover, MA) were added to M9 medium for $^{13}C/^{15}N$ labeling. For expression of only ^{15}N-labeled BK-IS1, the protein was expressed in M9 medium containing 1.0 g/L [^{15}N]NH_4Cl and unlabeled glucose. NH_4Cl and glucose were the only sources of nitrogen and carbon in the M9 medium, respectively.

2.2. Protein purification and on-column refolding in solution containing detergent micelles

Bacterial cells expressing recombinant BK-IS1 were harvested by centrifugation at 4000 rpm for 20 min at 18 °C (Beckman Coulter X-15R). The sediment was collected and suspended in 40 mL of lysis buffer (70 mM Tris–HCl, 300 mM NaCl, pH 8.0) then probe-sonicated (VC500, Sonics and Materials, Danbury, CT) on ice for a total of 10 min (power level, 30%; 2.0 s pulse on and 4.0 s pulse off). 5 mg of Lysozyme, DNase and RNase were added and the lysate was mixed at 4 °C for 1 h then centrifuged at 16,000 rpm for 20 min at 4 °C (Hitachi Himac Centrifuge, CR21GII). The pellet was recovered, suspended in 40 mL of lysis buffer, sonicated and centrifuged again, as described above.

The pellet was suspended in binding buffer (20 mM Tris–HCl, 100 mM NaCl, pH 8.0) containing 8 M urea and 0.2% (w/v) SDS. The suspension was mixed at room temperature for 2 h followed by centrifugation at 16,000 rpm for 20 min at 18 °C (Hitachi Himac Centrifuge, CR21GII). The supernatant was recovered and mixed with 5 mL of Ni^{2+}-NTA resin (QIAgen, Valencia, CA) then mixed at room temperature for 30 min before loading onto a gravity-flow column (BIO-ARD, Hercules, CA) equilibrated with 20 mM Tris–HCl (pH 8.0), 200 mM NaCl. Non-specificity proteins were washed out by elution with 50 mL of 20 mM Tris–HCl, 200 mM NaCl, 8 M urea, 0.2% (w/v) SDS, pH 8.0. Next, 40 mL of washing buffer (20 mM Tris–HCl, 200 mM NaCl, pH 8.0) containing 0.2% (v/v) DPC (Anatrace, Maumee, OH) were used to exchange detergents and to achieve protein on-column refolding. BK-IS1 was eluted with 20 mM Tris–HCl, 200 mM NaCl, 250 mM imidazole, pH 8.0 containing 0.5% DPC. The concentration of purified BK-IS1 was determined via measurement of A_{280} and the purity of BK-IS1