Biochemical and Biophysical Research Communications 395 (2010) 244–250

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Enhancement of TREK1 channel surface expression by protein–protein interaction with β-COP

Eunju Kim [a,1], Eun Mi Hwang [a,1], Oleg Yarishkin [a], Jae Cheal Yoo [a], Donggyu Kim [a], Nammi Park [a], Minhee Cho [a], Young Sun Lee [a], Choong-Hyun Sun [b], Gwan-Su Yi [b], Jiyun Yoo [c], Dawon Kang [a], Jaehee Han [a], Seong-Geun Hong [a], Jae-Yong Park [a,*]

[a] Department of Physiology, Institute of Health Science, and Medical Research Center for Neural Dysfunction, Biomedical Center (BK21), Gyeongsang National University School of Medicine, 90 Chilam-Dong, Jinju 660-751, South Korea
[b] Department of Bio and Brain Engineering, KAIST, 335 Gwahangno, Yuseong-gu, Daejeon 305-701, South Korea
[c] Department of Microbiology, Research Institute of Life Science, Gyeongsang National University, Jinju 660-701, South Korea

ARTICLE INFO

Article history:
Received 27 March 2010
Available online 1 April 2010

Keywords:
TREK1
β-COP
Yeast two-hybrid screening
Trafficking

ABSTRACT

TREK1 belongs to a family of two-pore-domain K^+ (K_{2P}) channels and produce background currents that regulate cell excitability. In the present study, we identified a vesicle transport protein, β-COP, as an interacting partner by yeast two-hybrid screening of a human brain cDNA library with N-terminal region of TREK1 (TREK1-N) as bait. Several *in vitro* and *in vivo* binding assays confirmed the protein–protein interaction between TREK1 and β-COP. We also found that β-COP was associated with TREK1 in native condition at the PC3 cells. When RFP-β-COP was co-transfected with GFP-TREK1 into COS-7 cells, both proteins were found localized to the plasma membrane. In addition, the channel activity and surface expression of GFP-TREK1 increased dramatically by co-transfection with RFP-β-COP. Surface expression of the TREK1 channel was also clearly reduced with the addition of β-COP-specific shRNA. Collectively, these data suggest that β-COP plays a critical role in the forward transport of TREK1 channel to the plasma membrane.

1. Introduction

K_{2P} channels constitute a family of potassium channels that are highly expressed in the central nervous system [1,2] and other tissues [3–5]. These K_{2P} channels are active as dimers and set the membrane potential toward the K^+ equilibrium potential [6].

Like the other K_{2P} channels, the TREK1 channel has cytoplasmic N- and C-termini, four transmembrane domains, and two pore-forming P loops [5]. In general, the C-terminus of this channel is recognized as necessary for the regulation of channel activity by PUFAs, phospholipids, stretch, and intracellular acidification [7–12]. This region also contains two serine residues that are critical for phosphorylation by protein kinase C (PKC) [13] and protein kinase A (PKA) [8]. In addition, a proteomic approach based on immunoprecipitation recently identified binding partners of this region such as A-kinase-anchoring protein AKAP150 and microtubule-associated protein Mtap2 [14,15]. These proteins simultaneously bind the C-terminus of TREK1 and enhance a channel activity and a channel density at the plasma membrane. However, binding proteins of the N-terminus of TREK1 has yet to be elucidated.

To determine novel binding proteins to the N-terminus of TREK1 channel, we performed a yeast two-hybrid screening of a human brain cDNA library with N-terminal region of TREK1 (TREK1-N) as bait. In this study, we provide an evidence for a direct protein–protein interaction between β-COP, a subunit of Coat Protein Complex I (COPI), and the TREK1 channel. β-COP promoted surface expression of TREK1 and an increase of the channel-mediated whole-cell currents. In addition, within the plasma membrane of COS-7 cells, β-COP was found co-localized with TREK1. Moreover, β-COP shRNA inhibited plasma membrane localization of the TREK1 channel significantly. Based on these results, we propose that β-COP plays a pivotal role in forward transport of TREK1 channel via direct protein–protein interaction with their N-terminal region.

2. Materials and methods

2.1. Cell culture and transfection

COS-7 and HEK293T cells were maintained in DMEM, and PC3 cells were maintained in RPMI1640 culture medium. Media were supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin–streptomycin under a humidifying atmosphere containing 5% CO_2 at 37 °C. Transfection of expression vectors was per-

* Corresponding author. Fax: +82 55 759 0169.
E-mail address: jaeyong@gnu.ac.kr (J.-Y. Park).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter Crown Copyright © 2010 Published by Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2010.03.171

formed by Lipofectamine 2000 (Invitrogen), according to the manufacturer's protocol.

2.2. Construction of expression vectors and β-COP shRNA

cDNAs encoding full-length rat TREK1 (GenBank Accession No. AY727922) and human β-COP (NM_016451) were obtained by an RT-PCR-based gateway cloning method as described previously [16]. TREK1 deletion mutants were also generated using full-length cDNAs as templates via the same cloning method. The resulting PCR products were cloned into several N-terminal tagged expression vectors, including pDEST-GFP-C, pDEST-RFP-C [17], and pDEST-Flag-C by gateway cloning (Invitrogen). The shRNA vectors for β-COP were constructed using the BLOCK-iT U6 RNAi Entry vector kit (Invitrogen). The target sequence was 5′-AACTTCCTGGA CTTCTGATGA-3′ (161–181). The control shRNA was constructed using the provided LacZ double-stranded control oligo.

2.3. Yeast two-hybrid assay

The TREK1-N was ligated into pGBKT7 encoding for the GAL4 DNA binding domain (BD) and the β-COP gene was cloned into pGADT7 encoding for the activation domain (AD). To assess the protein–protein interaction between TREK1-N and β-COP, both BD/TREK1-N and AD/β-COP were co-transformed into the yeast strain AH109. AH109 is unable to synthesize histidine. However, interaction between TREK1-N and β-COP enables the yeast to make the His3 enzyme, thereby permitting histidine biosynthesis and growth on His minimal medium.

2.4. Co-immunoprecipitation and Western blotting

HEK293T cells were lysed with RIPA buffer (50 mM Tris–HCl, pH 7.4, 150 mM NaCl, 5 mM EDTA, 1 mM PMSF, and 1% NP-40) containing a protease-inhibitor cocktail. Whole-cell lysates were incubated on ice for 30 min and then cleared at 20,000*g* for 20 min at 4 °C. The supernatants were incubated overnight at 4 °C with 1 μg/ml anti-Flag (M2; Sigma) or anti-TREK1 (H-75; Santa Cruz Biotechnology) antibodies, followed by incubation with protein A/G PLUS-agarose beads for 1 h. The proteins were separated by SDS–PAGE using 10% gels and blotted onto PVDF membranes. The blots incubated overnight at 4 °C with anti-GFP antibody (1:1000; Santa Cruz Biotechnology) or anti-β-COP antibody (1:1000; Abcam). Blots were then washed and incubated with horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit IgG, followed by washing and detection of immunoreactivity with enhanced chemiluminescence (Amersham Biosciences).

2.5. GST pull-down assay

To produce GST-fused β-COP (GST-β-COP), full-length human β-COP was cloned into the pDEST15 vector (Invitrogen) via the gateway cloning system. GST-β-COP protein was expressed in bacteria and purified according to the manufacturer's protocol (Amersham Biosciences). GFP-TREK1-N or GFP-TREK1-C fusion protein was expressed in HEK293T cells and extracted 24 h post-transfection. Cell lysates were incubated with pre-immobilized glutathione-Sepharose 4B beads (Amersham Biosciences). After 2 h incubation at 4 °C, the beads were washed four times with ice cold phosphate-buffered saline (PBS). Bound proteins were eluted with SDS sample buffer, separated on 12% SDS–PAGE gels, and analyzed by Western blot.

2.6. Cell-surface biotinylation and surface expression assay

The cell surface proteins of GFP-TREK1 transfected HEK293T cells were biotinylated and then isolated by chromatography through a NeutrAvidin-agarose affinity column, using the cell surface protein isolation kit (Pierce Biotechnology) according to the manufacturer's instructions. For surface expression analysis, a hemagglutinin (HA) tag (YPYDVPDYA) was introduced at amino acid position 272 of TREK1 as previously reported with HA-TASK3 [18]. The HA-inserted TREK1, TREK1(HA), and β-COP-specific shRNAs were transfected into HEK293T cells. Then cells were incubated for 1 h at 4 °C with 1 μg/ml rat monoclonal anti-HA antibody (3F10, Roche Diagnostics) in 1% BSA/PBS, and then incubated with HRP-conjugated goat anti-rat antibody in 1% BSA/PBS for 1 h at 4 °C. The cells were lysed in lysis buffer for 30 min on ice. Individual cells were placed in 50 μl Supersignal ELISA Femto solution (Pierce Biotechnology). After an equilibration period of 30 s, chemiluminescence was quantitated on a luminometer using Plate CHAMELEON™ V (HIDEX). At least four experiments were performed.

2.7. Electrophysiology

Whole-cell patch clamp experiments were performed 36 h post-transfection. The extracellular solution contained (in mM): 145 Na-aspartate, 5 K-aspartate, 1.0 $MgCl_2$, 1.8 $CaCl_2$, 5.5 glucose, and 10 Hepes at pH 7.35. To block the endogenous Kv currents, 10 mM TEA was added. The pipette solution contained (in mM): 115 K-aspartate, 5 Na-aspartate, 1.0 $MgCl_2$, 23 KOH/10 EGTA, and 10 Hepes at pH 7.20. Whole-cell currents were recorded with a patch clamp amplifier (Axopatch 200B; Axon Instruments, CA, USA) at room temperature (22–25 °C). The current–voltage relations were measured by applying ramp pulses (−120 to 60 mV over 400 ms) from a holding potential of −40 mV. Currents were filtered at 5 kHz and digitized at 5 kHz for further analysis. Data are presented as the means ± SE.

2.8. Microscopy and quantification of co-localization

Intracellular localization of the channels was determined by a confocal microscopy (Fluoview FV1000; Olympus) as described previously [16]. To quantify co-localization, the Pearson correlation coefficients were determined using FV1000 software.

3. Results

3.1. Protein–protein interaction between β-COP and TREK1

We generated several constructs, including vectors expressing full-length TREK1 (TREK1), N-terminal region only (TREK1-N), and C-terminal region only (TREK1-C) (Fig. 1A). Next, we performed a yeast two-hybrid screening with TREK1-N as bait to identify novel binding proteins. Positive clones were isolated independently from a human fetal brain cDNA library. Sequence analysis showed that one of the clones encoded a human β-COP, a subunit of COPI complex. The protein–protein interaction between TREK1 and β-COP was confirmed using the yeast two-hybrid and GST pull-down assays. As shown in Fig. 1B, the yeast two-hybrid assay clearly showed that β-COP binds to TREK1-N. Furthermore, the GST pull-down assay demonstrated that β-COP binds with TREK1-N directly but not TREK1-C (Fig. 1C).

To investigate the interaction between TREK1 and β-COP *in vivo*, N-terminal tagged vectors expressing Flag-tagged TREK1 (Flag-TREK1) and GFP-tagged β-COP (GFP-β-COP) were co-transfected into HEK293T cells. Immunoprecipitation with an anti-Flag antibody, followed by Western blotting with an anti-GFP antibody, showed that GFP-β-COP was associated with Flag-TREK1 (Fig. 2A, left panel). The interaction between these two proteins was further confirmed when the tags were swapped (Fig. 2A, right panel). The competitive inhibition assay revealed that the interaction between Flag-β-COP and GFP-TREK1 was reduced dramatically with the