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ORIGINAL ARTICLES

Expression, Purification and Functional Reconstitution of Slack Sodium-Activated Potassium Channels

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Abstract The *slack* (*slo*2.2) gene codes for a potassium-channel α-subunit of the 6TM voltage-gated channel family. Expression of *slack* results in Na^+-activated potassium channel activity in various cell types. We describe the purification and reconstitution of Slack protein and show that the Slack α-subunit alone is sufficient for potassium channel activity activated by sodium ions as assayed in planar bilayer membranes and in membrane vesicles.

Keywords Artificial planar membrane · Ion channel expression and reconstitution · Patch clamp · Potassium ion channel

Introduction

First identified by Kameyama et al. (1984) in cardiac myocytes, sodium-activated potassium channels have been found in many cell types. In neurons (Bhattacharjee and Kaczmarek 2005; Budelli et al. 2009; Yang et al. 2007) they control bursting and the adaptation of firing rates of action potentials; they may also be involved in protection from ischemia (Ruffin et al. 2008). Na^+-activated K^+ channels are found as well in diverse tissues such as kidney (Paulais et al. 2006) and smooth muscle (Kim et al. 2007; Zhang and Paterson 2007). Na^+-activated K^+ channels can be formed from Slack (also called Slo2.2) subunits (Joiner et al. 1998; Yuan et al. 2003) and from the related gene product Slick (Slo2.1) (Bhattacharjee et al. 2003). Slack and Slick are expressed widely and in varying proportions in the central nervous system (Bhattacharjee et al. 2002, 2005). The longer splice variant of Slack, Slack-B (Brown et al. 2008) forms heteromeric channels with distinct properties when coexpressed with Slick (Chen et al. 2009). Slack-A, on the other hand, has a shorter N-terminal sequence and does not coassemble with Slick subunits.

The structure and function of Slack channels are gradually becoming clear. A site of Na^+ sensing has been identified in the large intracellular C-terminal region of Slack (Zhang et al. 2010). The C-terminal region forms a "gating ring" whose X-ray structure, solved at low resolution, is quite similar to that of the gating ring of the BK (Slo1) Ca^{2+}-activated K^+ channel. This similarity was sufficient to allow the molecular replacement procedure to be employed in deducing the quaternary structure of the BK gating ring (Yuan et al. 2010). Slack channels are modulated by phosphorylation (Santi et al. 2006) and participate in protein–protein interactions (Uchino et al. 2003) including the RNA-binding protein FMRP and others which depend on the state of activation of the channel (Brown et al. 2010; Fleming and Kaczmarek 2009). A goal in our laboratory is to study Slack channels and their protein complexes by cryo-EM methods (Cong and Ludtke 2010; Wang and Sigworth 2009). We therefore sought to establish a system for the expression, purification and reconstitution of Slack protein. Here, we describe these methods as well as the results from functional assays of reconstituted Na^+-activated K^+ channels.

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Experimental Procedures

Molecular Biology

The Slack-B cDNA sequence (1,237 amino acids) has a short, alternatively spliced N-terminal region. A FLAG epitope tag was inserted at the C terminus through ligation of a construct in the pcDNA3 vector (Joiner et al. 1998). Another construct was made in the pEGFP-C1 vector (Clontech, Mountain View, CA) so that the EGFP sequence was fused to the N terminus of Slack-B. All constructs were confirmed by restriction digestion and sequencing.

Establishing Slack Stable Cell Lines

Constructs were transfected into HEK293 cells using Superfect (Qiagen, Valencia, CA) or Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Cells were cultured in a low-sodium medium containing 500 ml DMEM (GIBCO, Grand Island, NY) plus 250 ml Leibovitz's L-15, 15 ml 0.5 M HEPES and 235 ml H_2O, pH 7.3. Stable cell lines were selected by G418 sulfate (GIBCO) at 600 μg/ml. Anti-FLAG Western blotting was used to confirm the presence of Slack protein in monoclonal stable cell lines, and patch-clamp recordings were made from these lines.

Protein Purification

Cells were harvested from 10–20 dishes (150 mm diameter, approximately 0.1 g cells per dish) in cell storage buffer (10 ml/g cells) containing (in mM) 10 Tris, 5 KCl, 1 $MgCl_2$, 1 EGTA, 1:100 diluted protease inhibitor cocktail (PI; P 8340; Sigma, St. Louis, MO) and 5 EDTA. All buffers and recording solutions were titrated to pH 7.4. Cells were stored at −80 °C and, upon thawing, broken using a Dounce tissue grinder on ice. After centrifugation at 2,000×*g* for 15 min at 4 °C, the supernatant was collected; then after centrifugation at 141,000×*g* for 1 h at 4 °C, the membrane pellet was collected and resuspended in membrane storage buffer (0.5 ml/g cells) containing (in mM) 250 sucrose, 5 KCl, 10 Tris, 1:100 diluted PI and 5 EDTA. The membrane preparation was stored at −80 °C.

Membranes were incubated in solubilization buffer (1.5 ml/g cells) containing 150 KCl, 50 Tris, 5 EDTA, 1:100 PI and either 16 mM Cymal-5 (Anatrace, Maumee, OH) or 10–16 mM dodecylmaltoside (Calbiochem, San Diego, CA) for 2 h at 4 °C with rotation. After removing the insoluble material by centrifugation (16,000×*g* for 20 min at 4 °C), an equal volume of the solubilization buffer but without detergent was added and subsequently anti-FLAG affinity beads (0.3 ml beads/g cells, Sigma A2220). The mixture was rotated at 4 °C for 2 h; beads were collected in a 10 ml column and washed three times with equal volumes of wash buffer (same as the solubilization buffer but with only 5 mM detergent), and protein was eluted with three 150 μl applications at 20 min intervals of wash buffer with 500 μg/ml FLAG peptide added. A spin column (Bio-Rad, Hercules, CA; 732-6204) was used to extract the eluate. The final protein concentration was approximately 70 ng/μl, as quantified by fluorescence of the GFP-fusion protein; kept at 4 °C; and used immediately for assays or reconstitution. For gel electrophoresis, protein was incubated in 8 M urea buffer overnight at room temperature and run on 8 % polyacrylamide gels.

Slack Channel Reconstitution

Slack protein was reconstituted into liposomes containing POPC, POPE and POPS. The final protein to lipid ratio was about 0.3 Slack tetramer per 50,000 lipid molecules in a 40 nm liposome.

Mixed lipids, 3.6, 1 and 0.8 mg of POPC, POPE and POPS, respectively, were dried by argon and vortexed with 450 μl of reconstitution buffer (150 KCl, 30 TRIS, 5 EGTA, pH 7.4) for 15 min, followed by 10 freeze–thaw cycles using liquid nitrogen and a 40 °C water bath and 60 s sonication. Detergent (Cymal-5 or DM) was added to a 3:1 detergent to lipid ratio, vortexed for 30 min and sonicated for 30 s, then left on ice for about 3 h. The final lipid concentration was 12.8 mM.

Of the protein solution, 100 μl was mixed with 100 μl of solubilized lipids with a rotator at 4 °C for 3 h. The mixture was then loaded into dialysis tubing Spectra/Por Dialysis Membrane (MWCO: 25 kDa; Spectrum, Rancho Dominguez, CA), followed by 36 h of dialysis at 4 °C in buffer (450 KCl, 4 *N*-methyl D-glucamine [NMDG], 20 TRIS, pH 7.4) to form lipid vesicles. A Nycodenz (NYC; Progen Biotechnik, Heidelberg, Germany) discontinuous gradient was used to collect vesicles containing protein. Initial gradient layers were 100 μl 40 % NYC, 200 μl 30 % (containing 100 μl protein liposomes) and 200 μl each for 20, 15, 3 and 1 % NYC concentrations. After 3 h at 214,000×*g*, a liposome band was visible at the top of the 15 % NYC layer and collected. The presence of Slack protein in vesicles was confirmed by Western blotting with the anti-Flag M2 antibody (Sigma).

Patch-Clamp and Bilayer Experiments

Inside–out patch-clamp recordings were carried out with the EPC-9 patch-clamp amplifier and PULSE acquisition program (HEKA Instruments, Lambrecht, Germany). The SF-77B step perfusion system (Warner Instruments, Hamden, CT) controlled by PULSE was used for fast internal solution perfusion. For Na^+ dose-response measurements the pipette solution contained (in mM) 160 KCl,

1 $MgCl_2$, 140 NMG aspartate, 1 EGTA and 10 HEPES, while the bath (internal) solution contained 50 K^+, 100 Cl^-, 200 aspartate$^-$, 10 HEPES and variable concentrations of Na^+ and $NMDG^+$ summing to 250 mM.

Bilayer experiments were carried out using reconstituted vesicles obtained as above with a bilayer recording system (Warner Instruments). Lipids (Avanti, Birmingham, AL; 25 mg/ml) POPE:POPG = 18 µl:6 µl and POPE:-POPC = 16 µl:4 µl were dried by argon and washed with an equal volume of pentane. Hexadecane (Sigma) was then added to yield a final lipid concentration of 10 µg/µl. Two different initial solution configurations were used for our experiments. *Cis* and *trans* buffers were 350 KCl, 30 NaCl, 10 MOPS and 100 KCl, 6 HEPES and 0.6 EGTA or alternatively 100 KCl, 150 NaCl, 5 MOPS and 20 KCl, 30 NaCl and 5 MOPS, respectively. After formation of the bilayer, Slack protein vesicles were added to the *cis* chamber; then after the appearance of channel events, the *cis* solution was exchanged to one with lower osmolarity. An EPC-9 patch amplifier and PULSE software were used for recording.

JC-1 Fluorescence Measurements

The fluorescent dye JC-1 (Molecular Probes, Eugene, OR) was used for monitoring changes in vesicle membrane potential. Slack protein was purified in the presence of Cymal-5 and reconstituted into liposomes with dialysis against 1,000 ml of reconstitution buffer (150 KCl, 30 TRIS, 5 EGTA, pH 7.4) for 36 h at 4 °C with one buffer exchange. Vesicles were collected after centrifugation at 214,000×*g*, and 10 µl of vesicles were resuspended in 1 ml buffer containing 3 mM K^+ (150 NMG-Cl, 10 HEPES, 1 EGTA, 3 KOH, pH 7.4). After taking baseline spectra with

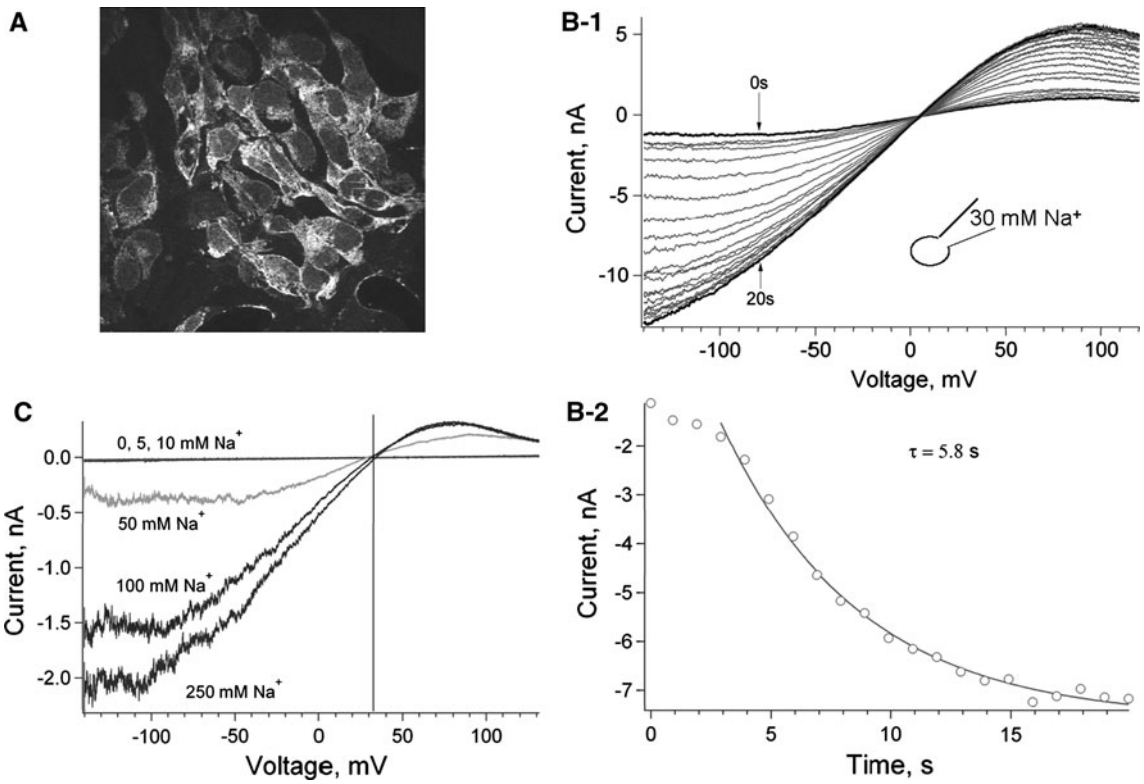

Fig. 1 HEK 293 stable cell lines express functional Slack protein. **a** Fluorescence of HEK cells expressing the N-terminal EGFP-Slack fusion construct. **b-1** Whole-cell recording made from a cell expressing wt-Slack with 30 mM Na^+ in the pipette [pipette solution (in mM): 30 NaCl, 100 KCl, 5 EGTA, 10 HEPES; bath solution: 160 KCl, 1 EGTA, 10 HEPES, 1 $MgCl_2$]. Shown are 22 current traces evoked by voltage ramps from −100 to +120 mV recorded immediately after establishing the whole-cell recording configuration; currents increased as Na^+ diffused from the pipette into the cell. **b-2** Time course of the development of inward currents from the cell shown in **b-1**, measured at −60 mV. The time constant is about 6 s. **c** An inside–out patch was perfused with various Na^+ concentrations (0, 5, 10, 50, 100, 250 mM) while keeping the potassium and chloride gradients constant. The calculated equilibrium potential, E_K, was +30 mV and E_{Cl} was −12 mV, while E_{Na} varied from 0 (at 5 mM internal Na^+) to −100 mV (at 250 mM internal Na^+). The measured reversal potential of the currents was near +30 mV, indicating that K^+ conductance was predominant. Pipette solution (in mM): 160 KCl, 1 $MgCl_2$, 140 NMG-aspartate, 1 EGTA, 10 HEPES; perfused solution (cytoplasmic side): 50 K^+, 100 Cl^-, 200 aspartate$^-$, $[Na^+] + [NMG^+] = 250$

a FluoroMax-3 spectrofluorimeter (Horiba Scientific, Edison, NJ), 3 μM JC-1 was added to the cuvette. The excitation wavelength was 465 nm, and emission at wavelengths of 530 nm (JC-1 monomer) and 590 nm (JC-1 aggregate) was recorded. The emission ratio at 590 and 530 nm was used as an indicator of membrane potential.

Cryo-EM Imaging

Reconstituted vesicle suspension, 3–4 μl having an approximate concentration of 1.9 mg/ml lipid, was applied to a home-made holey carbon film (Chester et al. 2007); blotted manually; and plunge-frozen in liquid ethane. Imaging was performed at liquid N_2 temperature in a Tecnai F20 microscope (FEI, Hillsboro, OR).

Results

Making Stable Cell Lines Expressing Slack-GFP and Slack

Transient transfection of a SlackB construct with an N-terminal GFP fusion into HEK293 cells yielded clear membrane-associated fluorescence (Fig. 1a) as well as Na^+-activated K^+ currents as measured in whole-cell and inside–out patch recordings. However, when we attempted to grow these cells either in normal medium or with selection, the cells survived for no more than 3–4 days after transfection. We noticed in whole-cell recordings from transfected cells the activation of K^+ current by extracellular Na^+ and reasoned that either an intrinsic external Na^+ sensitivity or an Na^+ leak that increases internal Na^+ was allowing Slack channels to be activated, yielding excessive K^+ conductance. We modified the growth medium, which is based on DMEM, to reduce the Na^+ concentration from 155 to 112 mM and found that this medium allowed growth even under selection with G-418. After successfully establishing the SlackB-GFP stable cell line, we also made stable cell lines expressing SlackB alone. Data shown in the rest of this article were obtained from these "native" SlackB channels.

Stabilized Slack on HEK293 Cells Preserves Its Na^+ Gating Properties

In a whole-cell recording, rupture of the patch membrane allows Na^+ to diffuse into the cell, yielding an increase in current as Na^+ equilibrates in a few seconds (Fig. 1b).

To evaluate the sodium dependence of the expressed channels, we used inside–out patches with a fixed gradient for potassium ($[K]_i = 50$ mM, $[K]_o = 160$ mM) and chloride ($[Cl]_i = 100$ mM, $[Cl]_o = 162$ mM), with equilibrium potentials $E_K = +30$ mV and $E_{Cl} = -12$ mV. The bath (intracellular) Na^+ concentration was varied while keeping the sum of Na^+ and N-methylglucamine (NMG^+) concentrations equal to 250 mM. Figure 1c shows the currents evoked by voltage ramps as the bath Na^+ concentration was changed with a rapid perfusion system. The currents increased steeply with Na^+ concentration, but the reversal potentials remained very close to E_K, regardless of the Na^+ concentration.

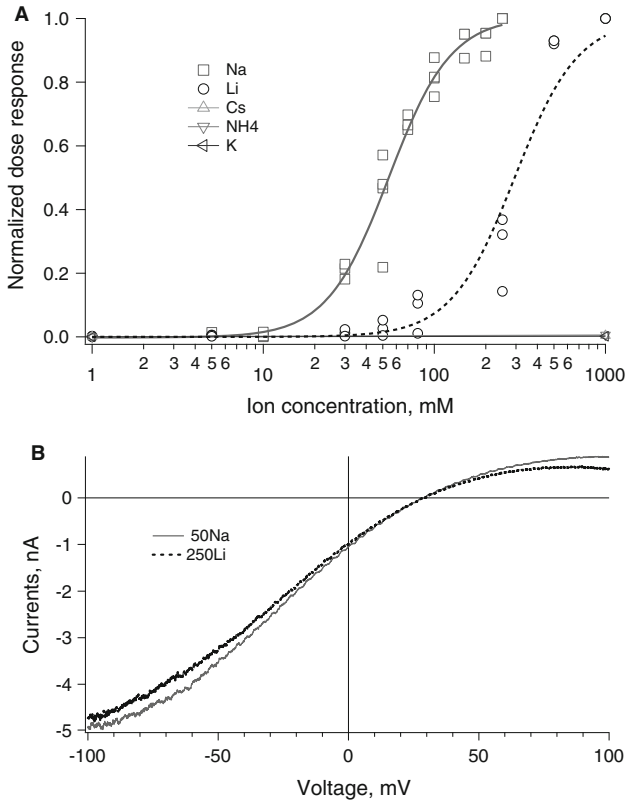

Fig. 2 Dose-response of ion activation of Slack channels. **a** Inward currents activated by internal Na^+ and Li^+ were measured from an inside–out patch at −80 mV while keeping potassium and chloride gradients unchanged. K_d for Na^+ was about 54 mM and the Hill coefficient, $n_H = 2.4$ (pipette: 160 KCl, 1 $MgCl_2$, 140 NMG-aspartate, 1 EGTA, 10 HEPES; perfused [internal] solution: 50 K^+, 100 Cl^-, 200 $aspartate^-$, $Na^+ + NMG^+ = 250$). Saturation was not attained in the lithium dose response, but the *dashed curve* shows the same function as fitted for Na^+ but with $K_d = 295$ mM. Cs^+, NH_4^+ and K^+ did not measurably open Slack channels at concentrations up to 1 M. **b** Very similar current traces are evoked by 50 mM Na^+ (*solid curve*) or 250 mM Li^+ (*dotted curve*). A voltage ramp from −100 to +100 mV was applied to an inside–out patch with intracellular solutions containing 50 mM K^+, 100 Cl^-, 200 $aspartate^-$ and either 200 $NMG^+ + 50$ Na^+ or 250 Li^+, respectively. The pipette contained 160 mM K^+ (160 KCl, 1 $MgCl_2$, 140 NMG-aspartate, 1 EGTA, 10 HEPES)

Lithium Opens Slack Channels But Is About Six Times Less Potent than Sodium

Zhang et al. (2010) have shown that, unlike larger ions, Li^+ is able to activate Slack channels expressed in *Xenopus* oocytes, although at lower potency. We obtained dose-response relationships with internal potassium, lithium, cesium and ammonium ions in inside–out patches from our cells. Responses at −80 mV are plotted in Fig. 2a, with the responses from each patch normalized to the response of the same patch to 250 mM internal Na^+. At a concentration of 1 M, cesium and ammonium produced <0.6 % of maximal activation. Li^+ (1 M) was capable of inducing a nearly saturating current, but its affinity was about six times lower than that of Na^+. The sodium dose response was fitted with $K_{1/2} = 54$ mM with the Hill slope $n_H = 2.4$. When the Li^+ data were fitted with the constraint $n_H = 2.4$, the estimated $K_{1/2}$ was 294 mM. Figure 2b shows the nearly identical currents evoked by a voltage ramp in the presence of 50 mM Na^+ or 250 mM Li^+ in the internal solution.

Purification and Reconstitution

Expression of Slack protein is only a few micrograms per 150 mm dish of cells; however, for some purposes, such as single-particle cryo-EM studies, quantities of ~10 µg protein are sufficient. The membrane fraction isolated from cells was solubilized in dodecylmaltoside or Cymal-5. Purification made use of the C-terminal FLAG tag on the expressed protein, with an anti-FLAG affinity column and elution with FLAG peptide. The resulting protein ran at the expected size of 136 kDa on an SDS-PAGE gel (Fig. 3a).

The protein was reconstituted into membranes by first mixing with detergent-solubilized lipids and then removing the detergent by dialysis. The vesicle fraction was enriched by density-gradient centrifugation, and cryo-EM revealed that the resulting vesicles were unilamellar and 25–50 nm in size (Fig. 3b). Successful reconstitution of the protein was assayed by resolubilizing the vesicles and running an SDS-PAGE gel. Western blotting with anti-FLAG showed recovery of the 136 kDa protein band (Fig. 3c).

Assay for Functional Channels after Reconstitution

Reconstituted vesicles were fused with planar bilayers for single-channel recordings. No channel currents were seen in the absence of Na^+ on the *cis* side (from which the vesicles were added), but channel activity was reversibly seen when a solution containing 100 mM Na^+ was perfused (Fig. 4a). Under the recording conditions (100 mM K^+ on the *trans* side), the single-channel conductance was 270 pS.

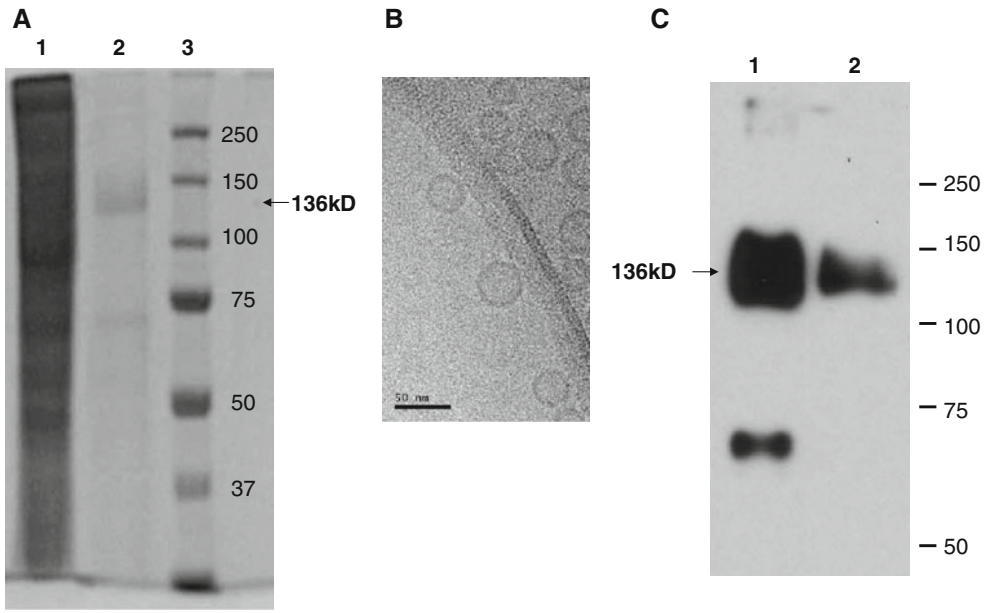

Fig. 3 Slack protein purification and liposome reconstitution. **a** Coomassie-stained protein gel made with 8 % polyacrylamide. *Lane 1*, cell lysate; *lane 2*, affinity-purified Slack protein; *lane 3*, protein molecular-weight markers. The purification yield was about 2 µg protein per 150 mm dish from 0.1 g cells. The ~70 kDa contaminant was not always present and appears to come from the antibody beads. **b** Examination of the reconstituted preparation by cryo-EM shows overwhelmingly unilamellar vesicles. The image shows the edge of a hole in the carbon film. The image was recorded with 2 µm underfocus at 200 keV. **c** Western blot probed with anti-FLAG antibody. *Lane 1*, Slack protein (monomer 136 kDa) as solubilized with Cymal-5 and purified; *lane 2*, protein extracted from liposomes after reconstitution. A 70 kDa band is again present in *lane 1*. It appears in control Western blots and appears to come from the antibody beads; note that it is absent from *lane 2*

Figure 4c shows another bilayer recording with 100 mM K^+ in the *cis* solution and 20 mM K^+ *trans*. The recording from a voltage ramp (bottom trace) shows a reversal potential of −45 mV, while the theoretical E_K was −42 mV. The single-channel conductance was 244 pS under these conditions.

Bilayer experiments demonstrate the presence of individual Na^+-activated channels but do not assay the population of reconstituted channels. We used the fluorescent dye JC-1 to measure Na^+-activated membrane potential changes driven by a K^+ diffusion potential. JC-1 in its monomeric form emits at 530 nm, but at high concentration it forms aggregates with a red-shifted fluorescence at 590 nm. As JC-1 is positively charged and membrane-permeable, it serves as a "slow" voltage indicator. Based on quantitative fluorometry of the Slack-GFP fusion construct, we were able to define a protein-to-lipid ratio in the reconstitution process of approximately one Slack tetramer to 50,000 lipid molecules. This ratio corresponds to approximately 0.3 tetrameric channels per 40 nm lipid vesicle, typical for the vesicle sizes we observed (Fig. 3b). Vesicles containing 160 mM K^+ were diluted into a solution with 3 mM K^+. Addition of 30 mM NaCl to the outside solution resulted in a 6 % increase in the ratio of intensity at 590 nm relative to 530 nm (Fig. 4d). This change reflects the establishment of a negative membrane

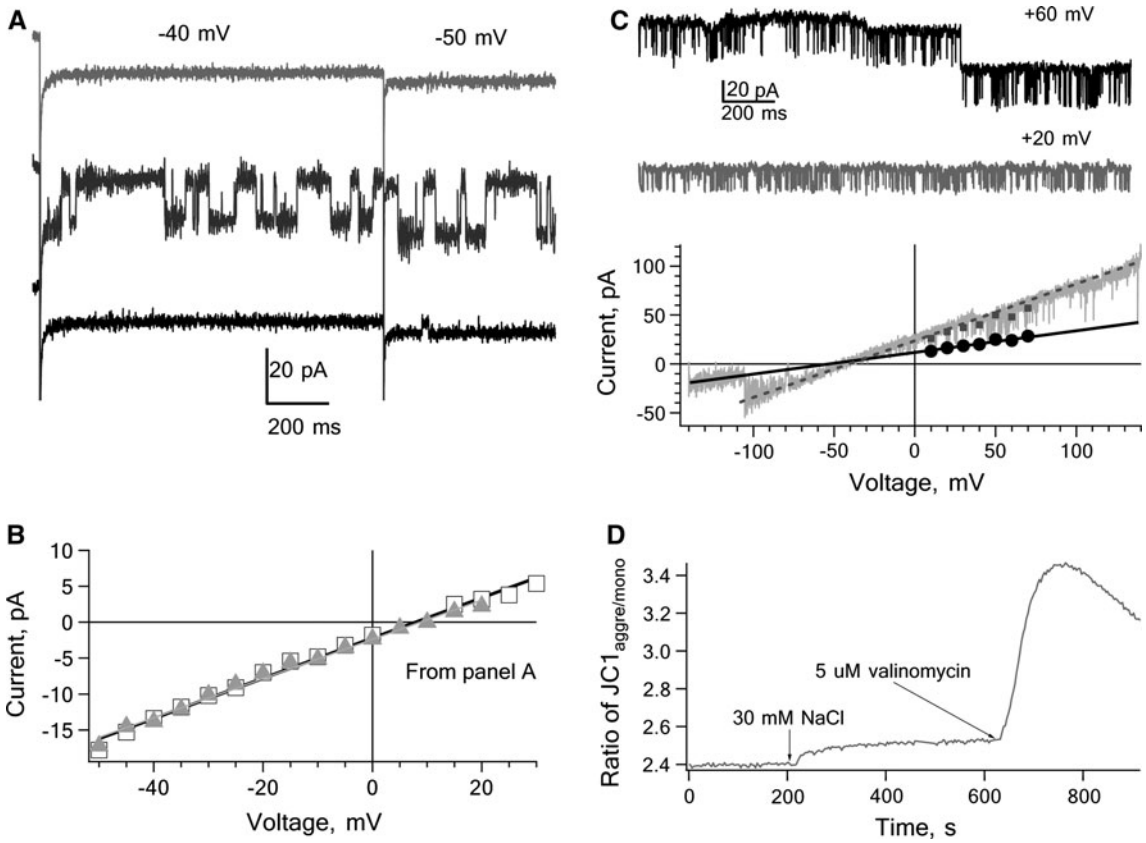

Fig. 4 Reconstituted Slack forms Na^+-activated K^+ channels. **a** Slack-containing vesicles formed from Cymal-5 solution were fused with a POPE:POPC = 4:1 bilayer membrane. Each sweep shows a recording at −40 mV followed by a step to −50 mV. After vesicles were added to the *cis* side and channel events were first observed, the hypertonic solution on the *cis* side (1 ml volume) was replaced by perfusion of 10 ml K buffer: 160 KCl, 10 HEPES, 1 EGTA (*top trace*), followed by 10 ml Na buffer, 100 mM NaCl, 10 HEPES, 1 EGTA (*middle trace*) and then another 10 ml K buffer (*bottom trace*). Solution on the *trans* side was 100 KCl, 6 HEPES and 0.6 EGTA. **b** The single-channel conductance was calculated to be 270 pS from a linear fit of unitary current amplitudes from the experiment in **a**, with a reversal potential of +10 mV. This reversal potential differs from the theoretical E_K consistent with the possibility that the 160 K^+ on the *cis* side was not completely replaced by perfusion. **c** Recording from a POPE:POPG = 3:1 bilayer in which the ion concentrations on both sides were fixed. *Cis* side: 100 KCl, 150 NaCl, 5 MOPS; *trans* side: 20 KCl, 30 NaCl, 5 MOPS. Vesicles were formed from the same lipid mixture using DM. Recordings at +20 and +60 mV show two active channels (*openings are upward*). Unitary current values (*black dots*) are plotted along with the current from a voltage ramp. The fitted line corresponds to a single-channel conductance of 244 pS and reversal potential of −45 mV; *dashed line and filled squares* indicate twice the conductance when two channels are active. The theoretical E_K was −42 mV under these conditions. **d** Flux assay for potassium transport. Reconstituted vesicles loaded with 150 mM K^+ were diluted into a solution containing 3 K^+ and 150 NMG^+. Addition of 30 mM Na^+ evoked a 5 % increase in the ratio of fluorescence at 595 and 540 nm. Subsequent addition of 5 μM valinomycin caused a further 36 % increase in the ratio, consistent with about 15 % of the liposomes containing channels that were activated by external Na^+

potential in a fraction of vesicles. Subsequent addition of 5 μM valinomycin, which is expected to produce a K^+ diffusion potential in every vesicle, yielded a peak increase of 36 % in the ratio. These results show that a substantial fraction of vesicles, roughly 16 %, have a K^+ permeability that is activated by external Na^+, presumably from inside–out Slack channels.

If we assume that channel reconstitution has no bias for orientation and assume a reconstituted density of roughly 0.3 tetrameric channels per vesicle, then the total yield of inside–out channels should be about 15 %, consistent with the result from the JC-1 fluorescence measurement and implying a high specific activity of reconstituted channels.

Discussion

The Slack Na^+-activated potassium channel is a member of the six-transmembrane-segment ion channel superfamily, which also includes the voltage-gated channels. Voltage gating of Slack channels is very weak (Yuan et al. 2003), as seen in Fig. 2, which would be expected from the absence of charged residues in the primary voltage-sensing S4 helix (Joiner et al. 1998). However, Slack activity is very sensitive to Na^+ concentrations in the range of tens of millimoles. This sensitivity is appropriate for a channel that responds to action potential–induced fluxes in neurons and muscle cells as well as providing Na^+-activated fluxes in other cells. Here, we describe the establishment of a stable cell line expressing Slack and show that the Slack protein can be purified on an antibody-affinity column. The purified protein can be reconstituted to form Na^+-activated and K^+-selective channels as detected in a planar lipid bilayer assay. Also, using a flux assay based on a potential-sensitive dye, we found that the fraction of vesicle-enclosed volume that participated in Na^+-activated K^+ flux was essentially equal to the number of vesicles containing inside–out Slack channels, assuming random orientation. Thus, the size of the flux signal was consistent with a high specific activity of the channel protein.

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References

Bhattacharjee A, Kaczmarek LK (2005) For K^+ channels, Na^+ is the new Ca^{2+}. Trends Neurosci 28:422–428

Bhattacharjee A, Gan L, Kaczmarek LK (2002) Localization of the Slack potassium channel in the rat central nervous system. J Comp Neurol 454:241–254

Bhattacharjee A, Joiner WJ, Wu M, Yang Y, Sigworth FJ, Kaczmarek LK (2003) Slick (Slo2.1), a rapidly-gating sodium-activated potassium channel inhibited by ATP. J Neurosci 23:11681–11691

Bhattacharjee A, von Hehn CAA, Mei X, Kaczmarek LK (2005) Localization of the Na^+-activated K^+ channel Slick in the rat central nervous system. J Comp Neurol 484:80–92

Brown MR, Kronengold J, Gazula VR, Spilianakis CG, Flavell RA, von Hehn CA, Bhattacharjee A, Kaczmarek LK (2008) Amino-termini isoforms of the Slack K^+ channel, regulated by alternative promoters, differentially modulate rhythmic firing and adaptation. J Physiol 586:5161–5179

Brown MR, Kronengold J, Gazula V-R, Chen Y, Strumbos JG, Sigworth FJ, Navaratnam D, Kaczmarek LK (2010) Fragile X mental retardation protein controls gating of the sodium-activated potassium channel Slack. Nat Neurosci 13:819–821

Budelli G, Hage TA, Wei A, Rojas P, Jong Y-JI, Oandapos-Malley K, Salkoff L (2009) Na^+-activated K^+ channels express a large delayed outward current in neurons during normal physiology. Nat Neurosci 12:745–750

Chen H, Kronengold J, Yan Y, Gazula V-R, Brown MR, Ma L, Ferreira G, Yang Y, Bhattacharjee A, Sigworth FJ, Salkoff L, Kaczmarek LK (2009) The N-terminal domain of Slack determines the formation and trafficking of Slick/Slack heteromeric sodium-activated potassium channels. J Neurosci 29:5654–5665

Chester DW, Klemic JF, Stern E, Sigworth FJ, Klemic KG (2007) Holey carbon micro-arrays for transmission electron microscopy: a microcontact printing approach. Ultramicroscopy 107:685–691

Cong Y, Ludtke SJ (2010) Single particle analysis at high resolution. Methods Enzymol 482:211–235

Fleming MR, Kaczmarek LK (2009) Use of optical biosensors to detect modulation of Slack potassium channels by G protein-coupled receptors. J Recept Signal Transduct Res 29:173–181

Joiner WJ, Tang MD, Wang LY, Dworetzky SI, Boissard CG, Gan L, Gribkoff VK, Kaczmarek LK (1998) Formation of intermediate-conductance calcium-activated potassium channels by interaction of Slack and Slo subunits. Nat Neurosci 1:462–469

Kameyama M, Kakei M, Sato R, Shibasaki T, Matsuda H, Irisawa H (1984) Intracellular Na^+ activates a K^+ channel in mammalian cardiac cells. Nature 309:354–356

Kim YC, Sim JH, Kang TM, Suzuki H, Kim SR, Kwon SC, Xu WX, Lee SJ, Kim KW (2007) Sodium-activated potassium current in guinea pig gastric myocytes. J Korean Med Sci 22:57–62

Paulais M, Lachheb S, Teulon J (2006) A Na^+- and Cl^--activated K^+ channel in the thick ascending limb of mouse kidney. J Gen Physiol 127:205–215

Ruffin VA, Gu XQ, Zhou D, Douglas RM, Sun X, Trouth CO, Haddad GG (2008) The sodium-activated potassium channel Slack is modulated by hypercapnia and acidosis. Neuroscience 151:410–418

Santi CM, Ferreira G, Yang B, Gazula V-R, Butler A, Wei A, Kaczmarek LK, Salkoff L (2006) Opposite regulation of Slick and Slack K^+ channels by neuromodulators. J Neurosci 26:5059–5068

Uchino S, Wada H, Honda S, Hirasawa T, Yanai S, Nakamura Y, Ondo Y, Kohsaka S (2003) Slo2 sodium-activated K^+ channels bind to the PDZ domain of PSD-95. Biochem Biophys Res Commun 310:1140–1147

Wang L, Sigworth FJ (2009) Structure of the BK potassium channel in a lipid membrane from electron cryomicroscopy. Nature 461(7261):292–295

Yang B, Desai R, Kaczmarek LK (2007) Slack and Slick K(Na) channels regulate the accuracy of timing of auditory neurons. J Neurosci 27:2617–2627

Yuan A, Santi CM, Wei A, Wang ZW, Pollak K, Nonet M, Kaczmarek L, Crowder CM, Salkoff L (2003) The sodium-activated potassium channel is encoded by a member of the Slo gene family. Neuron 37:765–773

Yuan P, Leonetti MD, Pico AR, Hsiung Y, MacKinnon R (2010) Structure of the human BK channel Ca^{2+}-activation apparatus at 3.0 Å resolution. Science 329:182–186

Zhang Y, Paterson WG (2007) Functional evidence for Na^+-activated K^+ channels in circular smooth muscle of the opossum lower esophageal sphincter. Am J Physiol Gastrointest Liver Physiol 292:G1600–G1606

Zhang Z, Rosenhouse-Dantsker A, Tang Q-Y, Noskov S, Logothetis DE (2010) The RCK2 domain uses a coordination site present in Kir channels to confer sodium sensitivity to Slo2.2 channels. J Neurosci 30:7554–7562