DOI 10.18699/SBB-2022-183

Use of lipodiscs in structural studies of ion channels

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Key words: SMALP, HERG, KCNQ, structure, purification

Motivation and Aim: One of the modern methods for isolating membrane proteins is the use of a styrene-maleic acid (SMA) copolymer. This amphipathic copolymer can integrate into biological membranes and easily destroy them. As a result, discoid membrane fragments with a size of 10–40 nm are formed, surrounded by a copolymer belt [1]. Such particles are known as SMALPs (SMA lipid particles) or lipodiscs. The polymer has no affinity to any specific lipids, and, in SMALPs, the ratio of lipids remains the same as it was in the original membrane [2]. If the membrane contains proteins, they are enclosed into the forming lipodiscs, upon the addition of the copolymer. The ability to extract proteins with SMA has been demonstrated for liposomes [3], cell membrane fractions [4], and whole cells [5]. The SMA-extracted membrane proteins are quite stable and can be purified and further analyzed by various biochemical methods [4, 5]. The benefit of SMA-extraction is the possibility of completely avoiding detergents in the protein purification process. It means better preservation of the native conformation and lipid microenvironment of the proteins. Here, we used SMA purification for isolate the ion channels of interest expressed in COS-1 cells, for structural studies.

Methods: The COS-1 and CHO cell lines were maintained in Dulbecco's modified Eagle's medium (PanEco, Russia), supplemented with 10 % of fetal bovine serum (HyClone, USA). COS-7 cells (American Type Culture Collection) were cultured in Dulbecco's modified Eagle's medium (Invitrogen), supplemented with 10 % fetal calf serum (Eurobio) and antibiotics (100 IU/ml penicillin and 100 μg/ml streptomycin; Gibco). All cell lines were cultured at 5 % CO_2 and 37 °C in a humidified incubator. Cells were transiently transfected with plasmids pIRES2-EGFP/hKCNQ1-1D4, pcDNA6-V5-HisA/hKCNE1-hKCNQ1 and pMT3-hKCNH5 using the Metafectene PRO (Biontex, Germany) for purification purposes and the Fugene 6 Transfection Reagent (Promega) for the electrophysiological experiments. In transfected mammalian cells, currents were recorded using the whole-cell configuration of the patch-clamp technique. For preparation of protein-containing SMALPs, COS-1 cells expressing ion channel proteins were resuspended in the buffer A (10mM Tris-HCl, 150mM NaCl, 2mM DTT, 1mM EDTA, protease inhibitor cocktail, pH 8) in the presence of a 2.5 % (w/v) SMA copolymer, incubated for 30 min at 4 °C with shaking, sonicated with an ultrasonic sonicator (Branson Ultrasonic Corporation, USA) for 15 s on ice and incubated for an additional 30 min at 4 °C. Suspensions were centrifuged for 15 min at 200,000 g. The pellet and supernatant were analysed by SDS-PAGE and immunoblotting. Supernatants were subsequently purified on affinity resin. Dynamic light scattering experiments were performed on a Brookhaven 90 Plus instrument

(Brookhaven Instruments Company, USA), in a thermostated cell at 20 °C. The buffer solution was filtered through 0.22 μm membrane filters. The scattered light was recorded at an angle of θ = 90°, the accumulation time of the signal was 1 min.

Results: We overexpress the Kv11.1 and Kv7.1 ion channels on CHO-1 cells and compared the effectiveness of their solubilization by SMA and detergent. We demonstrated that the SMA copolymer was more efficient at solubilization of the human KCNQ1 channels than CHAPS. The advantage of using SMALP is that the solubilized membrane proteins can be easily concentrated on Microcon concentrators without aggregation. A DLS experiment demonstrated that nanodiscs have the overall size of 15 nm, while electron microscopy revealed a four-fold symmetry within channel-containing SMALPs. Using mass-spectroscopy (LC-MS) and Raman spectroscopy to analyze the presence of lipids in the lipodisc stabilizing the recombinant human KCNQ1 protein. Raman spectroscopy detected several spectral peaks, which could be attributed to lipids which was confirmed by mass-spectroscopy.

Conclusions: using SMA copolymers, we tested a method of detergent-free solubilization of human ion channels, particularly, the cardiac and neuronal potassium voltage-dependent channels. SMALPs appear to develop into a convenient platform for studying the structure of human ion channels and their complexes (which are hard to crystallize) using not only cryo-EM, but also NMR methods, as well as other structural methods that require using the single particle mode (including XFEL). The study of the structural and functional properties of voltage-dependent potassium channels would help to clarify the mechanisms that cause malfunction of these channels in case of point mutations. Understanding these mechanisms, in its turn, would pave the way to methods of targeted correction of channel function.

Acknowledgements: The study is supported by RSF (22-14-00088). Yulia Kacher is a Vernadsky PhD fellowship scholar.

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