Towards the structure of the β4 subunit ectodomain of the human BK K^+ channel

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Potassium channel proteins form tetrameric pores in the membrane that facilitate the rapid, selective efflux of potassium ions across the cell membrane. Regulated potassium efflux is of fundamental importance in electrical signalling, but it also plays a role in a diverse range of other signalling pathways. The large-conductance Ca^{2+} activated potassium channel, or BK channel, is intimately involved in the regulation of calcium signalling pathways, most notably in providing a negative-feedback mechanism to regulate the activity of L-type-voltage-dependent calcium channels (VDCCs), preventing runaway Ca^{2+} influx. The physiological consequences of this simple regulatory loop are diverse; from vasodilation, to neurosecretion, to neuronal excitability, the BK channel has a variety of physiological roles, each of which requires the channel to have different electrophysiological properties. The phenotypic diversity of the BK channel is mediated, in large part, by association with a class of tissue-specific transmembrane regulatory proteins, the BK β-subunits. These proteins have diverse effects on the molecular properties of the channel. To understand how β-subunits interact with BK channels would be greatly aided by knowing their structure. The β-subunits share a two-transmembrane domain topology with short intracellular termini and a disulfide-bridged extracellular domain that is conjectured to form a novel extracellular gating ring for the BK channel (Zeng *et al.*, 2003).

To resolve the ambiguities surrounding the structure and function of the β-subunits, we aimed to determine the structure of the ectodomain of one of these proteins, the β4-subunit of the human BK channel. Here, we report the expression, refolding and crystallisation of the β4-subunit ectodomain and discuss progress towards structure determination.

We are producing the β4-subunit ectodomain by heterologous expression in *E. coli* followed by purification and refolding of the recombinant protein. Crystals were obtained and a complete native dataset collected. We were able to generate crystals derivatised with Ta_6Br_{14} by soaking this compound into the crystals. Derivatisation resulted in an unusual shift in the symmetry of the crystals – the native crystals were orthorhombic, while the Ta_6Br_{14} derivatives appeared tetragonal. Attempts to utilise the anomalous signals of tantalum and bromine for phasing by single-wavelength anomalous difference (SAD) techniques are currently underway.

We hope that the structure of the β-subunit ectodomain will shed some light on the molecular mechanisms by which transmembrane β-subunits influence BK channel gating.

Zeng XH, Xia X & Lingle CJ. (2003) *Nature Structural Biology*, **10:** 448-454.