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Fluorescent Ligands on the Basis of Hongotoxin 1: eGFP-Hongotoxin 1

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Since the early 2000s, when fluorescent proteins (FP) were functionally expressed in heterologous cells, they found a wealth of applications in molecular biology from live cell imaging to measuring protein-protein interactions. Recently, a novel approach was developed to use FPs for labeling peptide toxins from scorpion venom, which are indispensable tools to study structure and pharmacology of potassium voltage-gated (Kv) channels [1]. Production of genetically encoded fluorescent protein-scorpion toxin chimeras (FP-Tx) has certain advantages over synthetic labeling of peptides, namely, the targeted incorporation of FP into the N- or C-terminus of a polypeptide chain, and a simple and reproducible purification procedure, which is characterized by high yield and homogeneity of recombinant FP-Tx.

Here we used enhanced green fluorescent protein (eGFP) to tag peptide toxin hongotoxin 1 (HgTx1), which is a high-affinity blocker of a number of related Kv1 channels (Kv1.1-Kv1.3). The gene encoding eGFP-HgTx1 was cloned into NcoI/HindIII sites of pET23d vector (Novagen) and expressed in *E. coli* Rosetta-gami pLysS. His6-tagged eGFP-HgTx1 protein was affinity-purified from bacteria with a high yield (about 80 mg per 1 l of culture). Absorption spectrum of obtained FP-Tx was similar to that of eGFP. To study affinity of eGFP-HgTx1 to Kv1.1- and Kv1.3-binding sites, we used confocal fluorescent microscopy and bioengineered analytical systems, which are based on receptor KcsA-Kv1.x (x=1, 3) chimeric proteins embedded in the plasma membrane of *E.coli* spheroplasts [2, 3]. eGFP-HgTx1 was shown to bind to KcsA-Kv1.1- and KcsA-Kv1.3-presenting spheroplasts and easily displaced from the binding sites with non-labeled HgTx1 used in several-fold excess (Fig. 1). To determine affinity of eGFP-HgTx1 to the target receptors, saturation binding curves were measured (Fig. 1 B), and dissociation constants (K_d) of ligand-receptor complexes were estimated: 3.2±1.4 nM for KcsA-Kv1.1 and 1.1±0.2 nM for KcsA-Kv1.3. The K_d values correlate qualitatively with those obtained for ^{125}I-HgTX1-A19Y/Y37F in a radioligand assay (0.4 nM for KcsA-Kv1.1 and 0.03 nM for KcsA-Kv1.3) [4].

We carried out detailed studies to characterize ability of different Kv1-channel blockers belonging to α-KTx family of scorpion venom - derived peptides to displace eGFP-HgTx1 from the complexes with KcsA-Kv1.3 in a competitive binding assay (Fig.1 C). For this, peptide toxins HgTx1, agitoxin 2 (AgTx2), kaliotoxin (KTX), and charybdotoxin (ChTx), which were obtained in a recombinant form according to the previously developed technique [5], were used. All these peptides exhibited high affinity to the receptor KcsA-Kv1.3 protein, and apparent dissociation constants (K_i) were determined to be: 200 pM for HgTx1, 245 pM for AgTx2, 115 pM for ChTx, and 20 pM for KTX. These K_i values are similar to the known ones estimated for the binding of these toxins to the eukaryotic Kv1.3 channel. These results suggest that binding of eGFP-HgTx1 to the target KcsA-Kv1.3 channel is specific and reversible, and eGFP-HgTx1 fluorescent protein chimera competes with venom toxins for the same binding site of KcsA-Kv1.3. Thus, adding a bulky protein molecule, eGFP, to the peptide toxin does not

perturb its interaction with the receptor binding site. The obtained eGFP-HgTx1 chimeric protein is a prospective fluorescent probe for the screening of Kv1.3-channel ligands [6].

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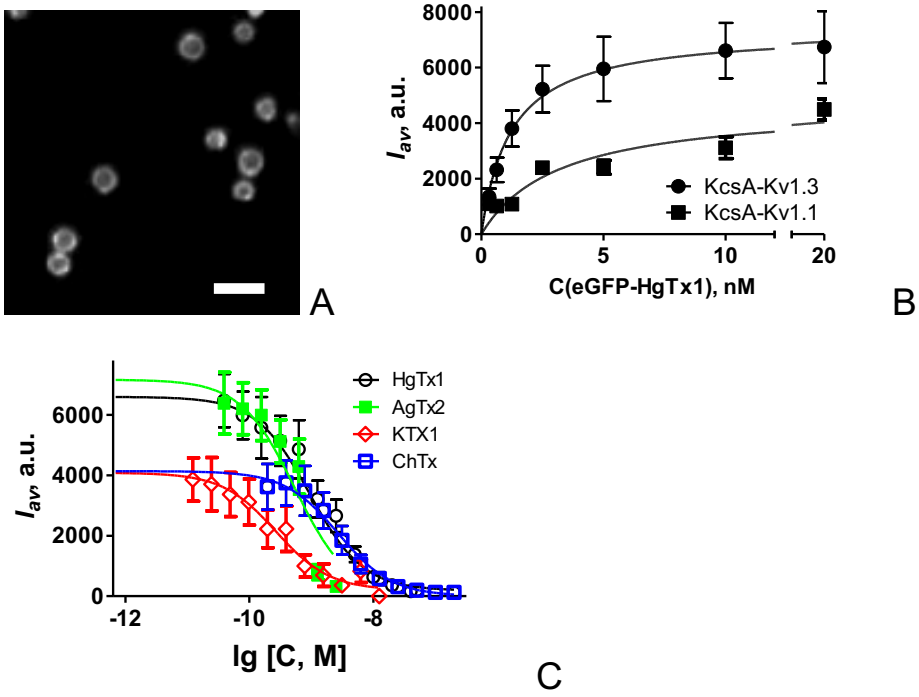

Figure 1. Interactions of eGFP-HgTx1 with spheroplasts bearing KcsA-Kv1.x (x=1, 3) hybrid channels. (A) A confocal fluorescence image showing staining of KcsA-Kv1.3-bearing spheroplasts with eGFP-HgTx1. Bar is 3 μm. (B) Concentration dependent binding of eGFP-HgTx1 to spheroplasts bearing KcsA-Kv1.x (x=1, 3). (C) Competition between eGFP-HgTx1 and different ligands for the binding to KcsA-Kv1.3-presenting spheroplasts.

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