

Fluorescent Ligands of Kv1 Channels on the Basis of Hongotoxin: Atto488-Hongotoxin

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Development of fluorescently labeled peptide toxins, highly efficient and selective ligands of potassium (K⁺) channels, is an actual demand for different applications ranging from drug-screening techniques to *in vitro* and *in vivo* fluorescent imaging of K⁺ channels. Hongotoxin 1 (HgTx1), the 39-aa peptide toxin from the venom of scorpion *Centruroides limbatus*, is a potent blocker of potassium voltage-gated Kv1 – Kv3 channels. Its mutant forms, either radiolabeled (¹²⁵I-HgTX1-A19Y/Y37F) or fluorescently labeled (HgTX1-A19C) were used to probe localization in tissues [1], pharmacology [2] and subunit organization of Kv-channels [3].

Here we report on the properties of a new fluorescent HgTx1 derivative labelled with Atto488 fluorophore at the N-terminus (A-HgTx1), which was produced by a chemical synthesis (Smartox Biotechnology, France). To study Kv1-channel binding properties of A-HgTx1, we used a bioengineered analytical system based on the KcsA-Kv1.x (x = 1, 3, 6) chimeric proteins, which carry ligand-binding sites of the corresponding eukaryotic channels Kv1.1, Kv1.3, and Kv1.6 [4-6]. These KcsA-Kv1.x (x=1, 3, 6) expressed in *E.coli* cells embed into cell membrane, expose ligand-binding sites into periplasmic space and bind ligands when cells are transformed to spheroplasts. Measurement and quantitative treatment of fluorescence signals associated with spheroplasts in the presence of A-HgTx1 was performed using confocal laser scanning microscopy as described elsewhere [4-6].

We found that A-HgTx1 binds to KcsA-Kv1.1 and KcsA-Kv1.3 and can be displaced from the complexes by an excess of non-labelled recombinant HgTx1 (Fig.1, A). Levels of saturated binding of A-HgTx1 to spheroplasts bearing KcsA-Kv1.1 and KcsA-Kv1.3 are different in accordance with different levels of membrane presentation of these chimeric proteins [7]. A-HgTx1 binding to KcsA-bearing spheroplasts or to spheroplasts without channels is low. These results suggest that interactions of A-HgTx1 with KcsA-Kv1.1 and KcsA-Kv1.3 are reversible and specific. Staining of KcsA-Kv1.6-bearing spheroplasts by A-HgTx1 is very weak, comparable to the staining of spheroplasts without channels and is not sensitive to the presence of HgTx1. It indicates an absence of interaction between A-HgTx1 and KcsA-Kv1.6 in the tested range of peptide concentrations.

To characterize affinity of A-HgTx1 to Kv1.1- and Kv1.3-binding sites, dissociation constants (K_d) were determined. Binding of A-HgTx1 to KcsA-Kv1.1 and KcsA-Kv1.3 is concentration-dependent and saturable. In the case of KcsA-Kv1.1 the A-HgTx1 binding curve has to be corrected for the level of non-specific binding, while in the case of KcsA-Kv1.3 the contribution of non-specific binding is negligible. The K_d values of A-HgTx1 complexes with Kv1.1- and Kv1.3-binding sites were measured to be 44±6 nM and 8.4±0.7 nM, respectively. It should be noted that K_d values for A-HgTx1 are *ca.*

tenfold lower than those of ^{125}I -HgTx1-A19Y/Y37F determined with a radioligand assay [8]. This can be related to a bulk effect of an organic dye used for fluorescent labeling.

It can be concluded that A-HgTx1 is suitable for the search for and study of Kv1.1- and Kv1.3- channel blockers using analytical systems based on the KcsA-Kv1.x ($x=1, 3$) chimeric proteins as well as for *in vitro* and *in vivo* fluorescent imaging of the corresponding Kv1-channels.

References:

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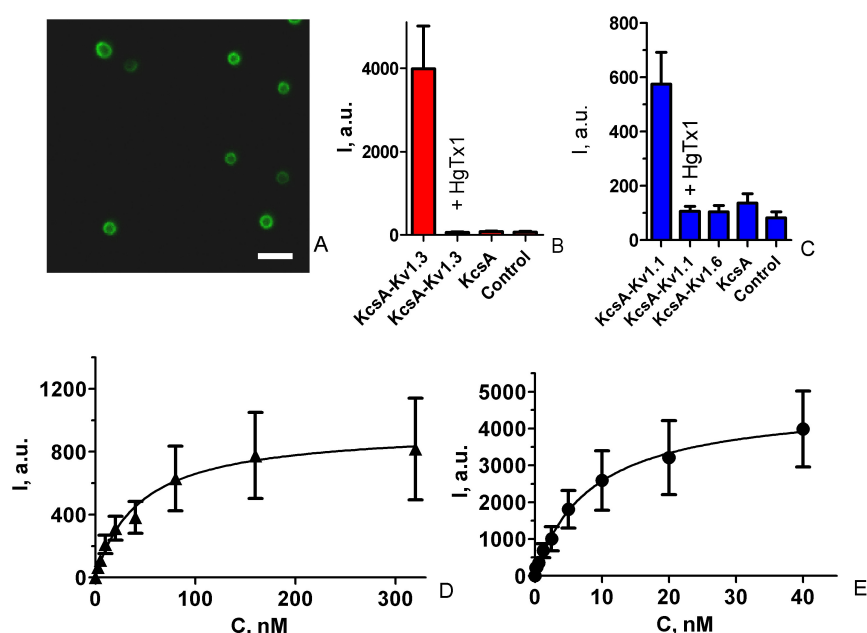


Figure 1. Interactions of A-HgTx1 with spheroplasts bearing KcsA-Kv1.x ($x=1, 3$) hybrid channels. (A) An example of the confocal fluorescence image showing staining of KcsA-Kv1.3-bearing spheroplasts with A-HgTx1. Bar is 4 μm . (B) Average fluorescence intensity of A-HgTx1 (40 nM) bound to spheroplasts bearing either KcsA-Kv1.3 or KcsA and to spheroplasts without channels (Control). HgTx1 (500 nM) competes with A-HgTx1 for the binding to KcsA-Kv1.3. (C) Average fluorescence intensity of A-HgTx1 (150 nM) bound to spheroplasts bearing KcsA-Kv1.1, KcsA-Kv1.6 or KcsA and spheroplasts without channels (Control). HgTx1 (1000 nM) competes with A-HgTx1 for the binding to KcsA-Kv1.1. (D, E) Concentration dependent binding of A-HgTx1 to spheroplasts bearing KcsA-Kv1.1 (D) or KcsA-Kv1.3 (E).