Properties of Atto488-Agitoxin 2 as a Fluorescent Ligand of Kv1.3 Channel

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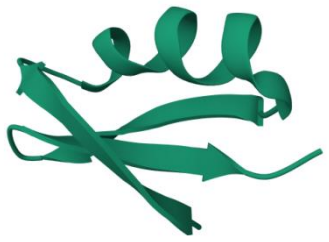
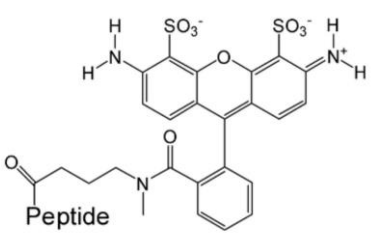
Introduction

Voltage-gated potassium Kv1.3 channel is a target for the treatment of neurological and autoimmune diseases. Peptide toxins from scorpion venoms that block Kv1.3 are useful tools to study its function in normal physiology and disease. Fluorescently labeled toxins can be used as probes for screening of Kv1.3 channel blockers, as well as for Kv1.3 channel imaging in cells and tissues. Toxin AgTx2 from the venom of the scorpion *Leiurus hebraeus* is a high-affinity blocker of Kv1.1, Kv1.3 and Kv1.6 channels.
The aim of our work was to study binding properties and determine the affinity profile of A-AgTx2, a fluorescent derivative of AgTx2 N-terminally labeled with the fluorophore Atto488.

AgTx2
GVPINVSCTG SPQCIKPCKD AGMRFGKCMN RKCHCTPK



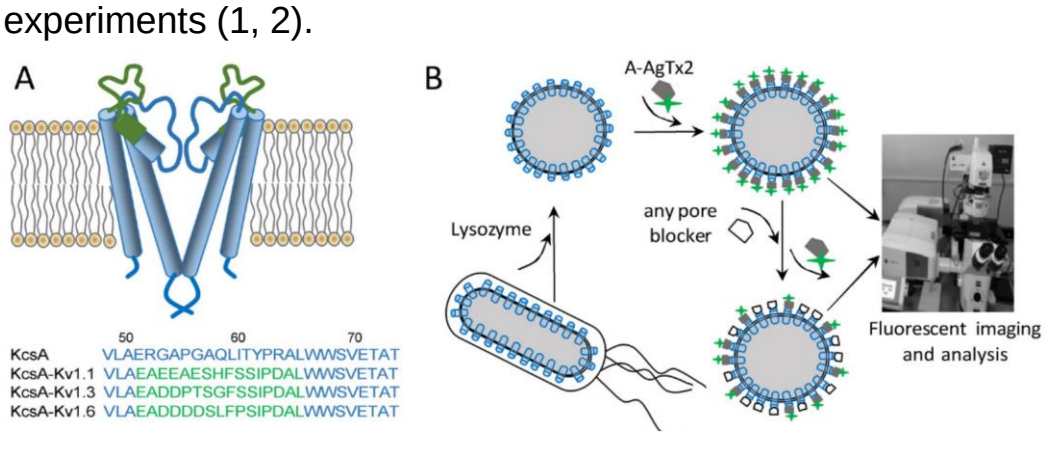
1PDB; 1AGT



A-AgTx2

Methods

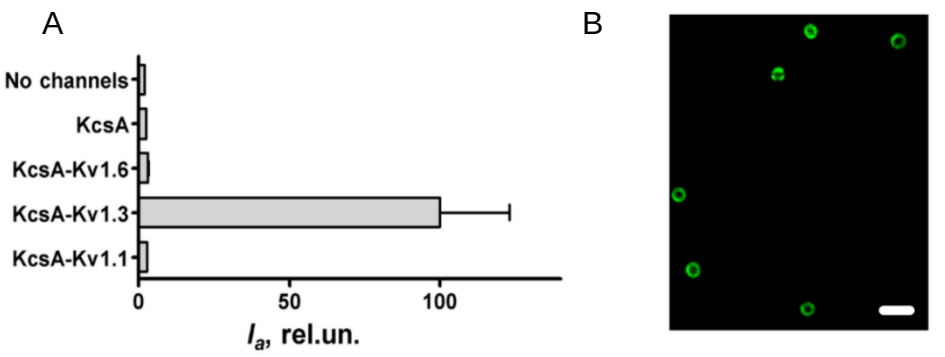
Chemically synthesized A-AgTx2 was produced by Smartox Biotechnology (France). The Kv1-channel pore domains were expressed in the plasma membrane of *E.coli* in the form of hybrid channels KcsA-Kv1.x (x=1,3,6). Spheroplasts prepared from bacterial cells were used in the cell-based binding assay, in which complex formation between A-AgTx2 and hybrid channels was detected by laser scanning confocal microscopy. Image J software was used to determine the fluorescence intensity (I) of the ligand bound to each spheroplast. The dissociation constant (Kd) of ligand-receptor complexes was defined with the equation I_a ([L]) = I_{as} [L]/(K_d + [L]), where I_{as} is the I_a value when the A-AgTx2 binding is saturated. Ki values for unlabeled ligands were measured in competitive binding experiments (1, 2).



(A) Structure of the hybrid KcsA–Kv1.x (x = 1,3, 6) channels.
(B) A principle of the KcsA–Kv1.x (x = 1, 3, 6) channel application in the analytical bioengineering system.

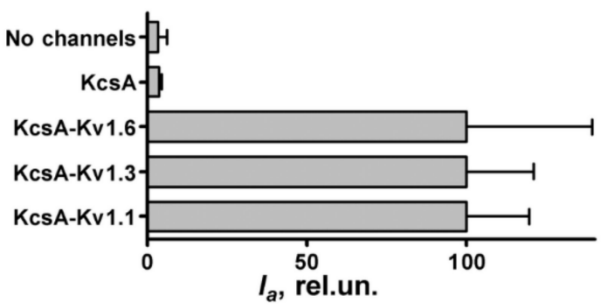
Results

A-AgTx2 bind to spheroplasts expressing KcsA–Kv1.3 but does not bind spheroplasts presenting KcsA–Kv1.1 or KcsA–Kv1.6. Thus, N-terminal labeling of AgTx2 led to a change in the selectivity profile of the ligand demonstrating selective targeting of the Kv1.3 channel.



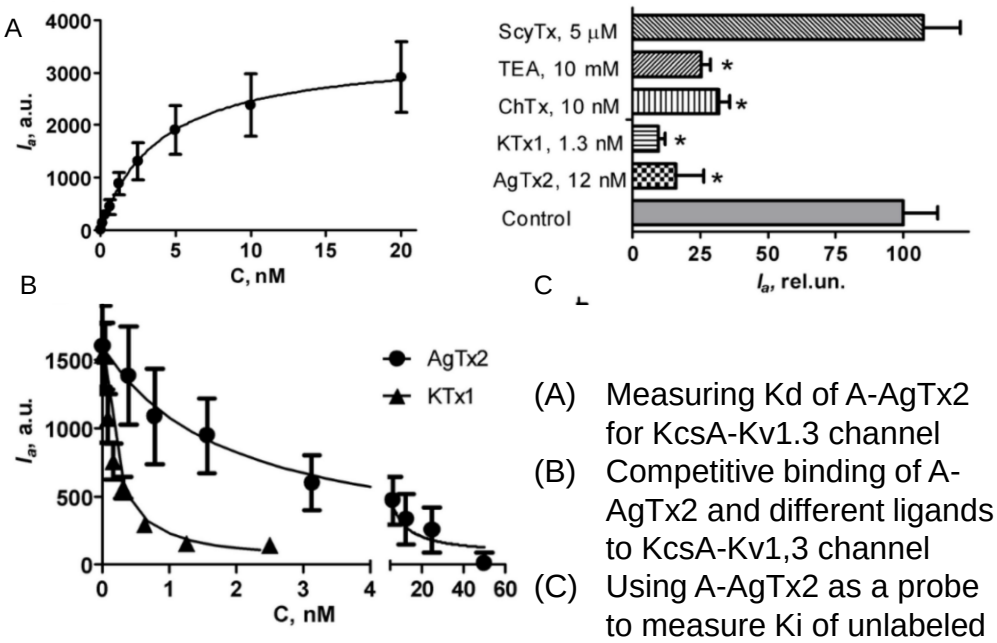
(A) Fluorescent signal from the membrane of spheroplasts expressing KcsA-Kv1,x(x=1,3,6) channels in the presence of A-AgTx2.
(B) Typical confocal fluorescent image of A-AgTx2 (20 nM) bound to KcsA–Kv1.3 at the surface of spheroplasts. Bar is 2 μm.

To confirm the levels of KcsA–Kv1.1 and KcsA–Kv1.6 membrane expression, as well as the ability of these channels to bind suitable pore blockers, the binding was performed with the bioengineered ligand AgTx2-L3-GFP.



Fluorescent signal from the membrane of spheroplasts expressing KcsA-Kv1,x(x=1,3,6) channels in the presence of AgTx2-L3-GFP.

A-AgTx2 was demonstrated to be a high-affinity ligand of KcsA-Kv1.3 channel (Kd 4.0 nM).
The binding site of KcsA-Kv1.3 channel for A-AgTx2 was shown to overlap the corresponding sites of the known Kv1.3 channel blockers AgTx2, KTX1, and ChTx, as well as inorganic ligand TEA. The obtained data allowed to use A-AgTx2 as a probe to measure affinities of various unlabeled peptides for KcsA-Kv1.3 hybrid channel.



(A) Measuring Kd of A-AgTx2 for KcsA-Kv1.3 channel
(B) Competitive binding of A-AgTx2 and different ligands to KcsA-Kv1,3 channel
(C) Using A-AgTx2 as a probe to measure Ki of unlabeled AgTx2 and KTx1

Conclusions

- AgTx2 labeled with Atto488 is a new high-affinity fluorescent ligand for Kv1.3 channel.
- N-terminal labeling of AgTx2 enhances selectivity of A-AgTx2 for the target Kv1.3 channel.
- A-AgTx2 can be used in screening studies to determine binding affinities of unlabeled ligands for Kv1.3 channel.

Conflict of interest, The authors declare no conflict of interest

Acknowledgment

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References
1, Denisova KR, et al. Bioengineering. 2022; 9(7):295
2, Kudryashova K.S. et al., Biochem. Pharmacol. 2021, 190.