# Properties of Atto488-Agitoxin 2 As a Fluorescent Ligand of Kv1 Channels

Denisova Kristina R.[1,2], Orlov Nikita A.[1,2], Feofanov Alexey V.[1,2], Nekrasova Oksana V.[1]

[1] Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences, 117997 Moscow, Russia
[2] Faculty of Biology, Lomonosov Moscow State University, 119234 Moscow, Russia

Voltage-gated potassium channels of the Kv1 family play a role in many physiological processes. Their overexpression can lead to neurological and autoimmune diseases. Many peptides from scorpion venoms are the highly specific blockers of Kv1 channels, that makes them important pharmacological compounds. Agitoxin 2 (AgTx2) is one of these blockers, with a subnanomolar affinity to eukaryotic channels Kv1.1, Kv1.3 and Kv1.6. Fluorescently labeled peptide blockers are useful tools for studying channels using fluorescence microscopy techniques. These tagged blockers can be obtained either by chemical synthesis or by genetically coding a peptide fused to a fluorescent protein. Previously studied conjugates of AgTx2 with GFP, RFP and tetramethyrhodamine were shown to possess high affinity to particular potassium channels Kv1[1].

The aim of the present study was to characterize properties of A-AgTx2, a new fluorescently labeled ligand of Kv1 channels, and its possible applications. A-AgTx2 (AgTx2 N-terminally labeled with the fluorophore Atto488) was produced by Smartox (France) and studied by us using laser scanning confocal microscopy and *E.coli* spheroplasts that express hybrid channels KcsA-Kv1.x (x=1, 3, 6) on the plasma membrane [2]. As shown earlier, hybrid channels KcsA-Kv1.x (x=1, 3, 6) mimic eukaryotic Kv1 channels in the ability to bind peptide blockers[3].

In contrast to AgTx2, the A-AgTx2 ligand was determined to bind to KcsA-Kv1.3, but not to KcsA-Kv1.1 and KcsA-Kv1.6 channels. Our studies have shown that A-AgTx2 is a selective and high-affinity ligand of the Kv1.3 channel. The dissociation constant of complexes is 4.3±0.2 nM. A-AgTx2 can also be used in a combination with KcsA-Kv1.3 for the recognition of unlabeled Kv1.3 blockers and for the quantitative analysis of their affinity to Kv1.3 channel.

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References

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