Purification and characterisation of recombinant botulinum neurotoxin serotype FA, also known as serotype H

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Introduction

Native (n) BoNT/FA is a mosaic neurotoxin and the minor toxin produced by bivalent Clostridium botulinum strain IBCA 10-7060 (Barash 2014). Initially BoNT/FA was designated as a new serotype (BoNT/H), because it was relatively insensitive to reference antisera. Subsequent studies suggested that mosaic FA is a more appropriate classification (Maslanka 2016). Here we report purification and characterisation of recombinant (r) BoNT/FA.

Figure 1. rBoNT/FA Structure and homology

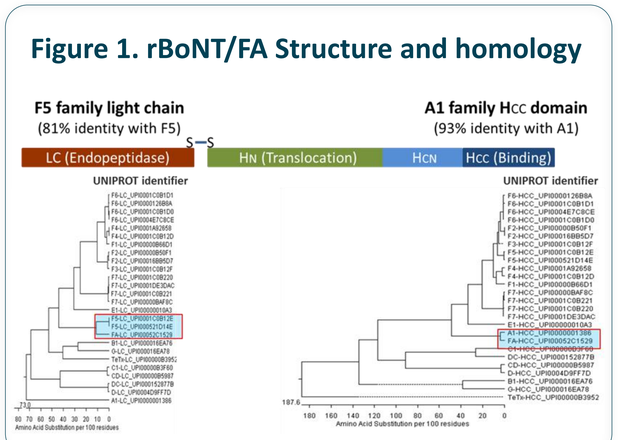

Figure 2. rBoNT/FA affinity purification process

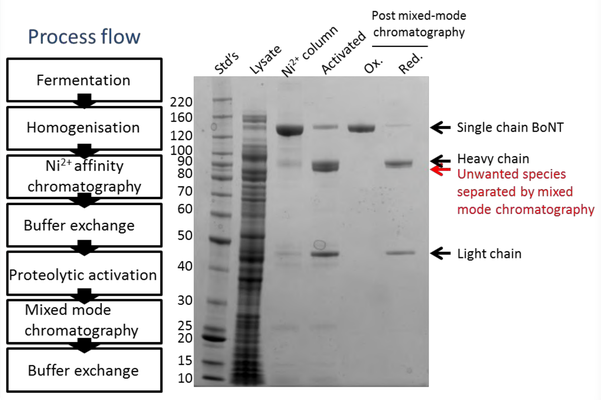

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Figure 3. Cell-free VAMP-2 cleavage assay

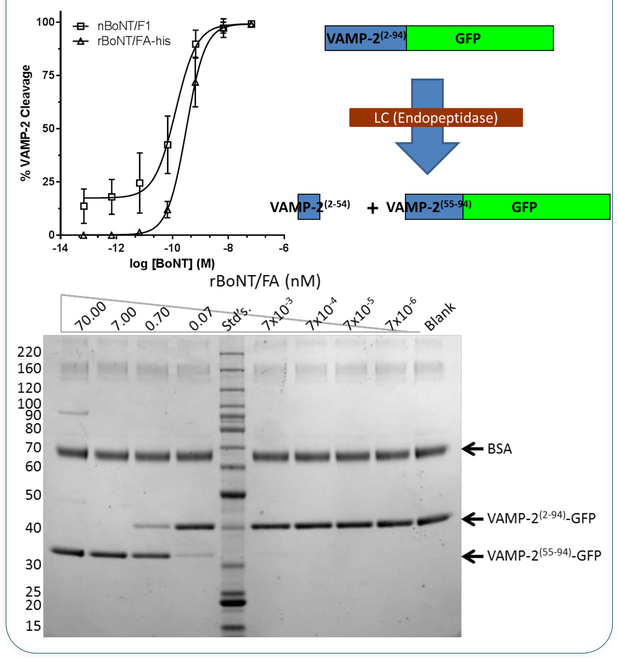
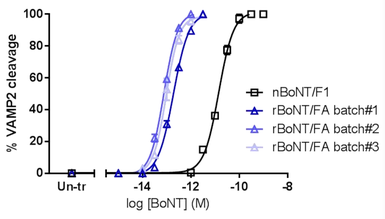
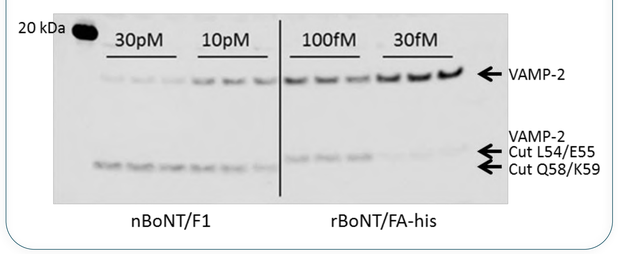


Figure 4. Cell assay for VAMP-2 cleavage

Toxin	pEC_{50}
nBoNT/F1	10.85
rBoNT/FA batch#1	12.72
rBoNT/FA batch#2	13.12
rBoNT/FA batch#3	13.02





Methods

The sequence of BoNT/FA was optimised for *Escherichia coli* and cloned, with a C-terminal his-tag, into plasmid pJ401. Amino acids S429-L437 were replaced by K430-L444 from BoNT/F1 to facilitate post-translational activation. The protein was purified by nickel chromatography and activated post-translationally by protease Lys-C. rBoNT/FA was characterised in cell-free assays for soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) cleavage, primary neuron assays for SNARE cleavage and inhibition of neurotransmitter release, and in an assay for inhibition of stimulated muscle contraction.

Results

Purified rBoNT/FA cleaved a fusion protein of vesicle-associated membrane protein 2 (VAMP-2) and green fluorescent protein (GFP) (VAMP-$2^{(2\text{-}94)}$-GFP) with specific activity 7 ± 2 nmol substrate cleaved/hr/pmol enzyme in cell-free assays, which is approximately 600 times lower than nBoNT/F1 in the same assay. In the mouse phrenic nerve hemi-diaphragm assay, rBoNT/FA inhibited stimulated muscle contraction with t_{50} 148 ± 9 min, also significantly less potent than nBoNT/F1. However, in rat cortical neurons, rBoNT/FA cleaved VAMP-2 and inhibited neurotransmitter release with pEC_{50} values 12.95 ± 0.10 and 12.47 ± 0.03, respectively, which is almost 100 times more potent than nBoNT/F1.

Figure 5. Cell assay for inhibition of neurotransmitter release

Toxin	pIC_{50}
nBoNT/F1	11.01
rBoNT/FA batch#1	12.43
rBoNT/FA batch#2	12.51
rBoNT/FA batch#3	12.46

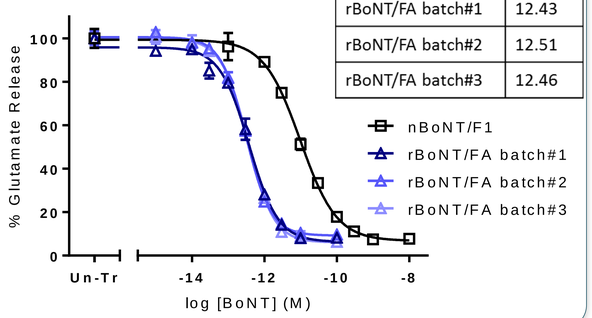
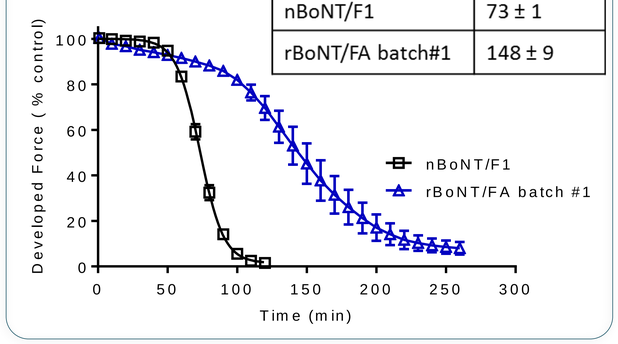


Figure 6. Inhibition of stimulated muscle contraction

Toxin	t_{50} (min)
nBoNT/F1	73 ± 1
rBoNT/FA batch#1	148 ± 9



Conclusions

- rBoNT/FA was purified by Ni^{2+} and mixed-mode chromatography
- Post-translational activation was by Lys-C
- rBoNT/FA cleaved a recombinant substrate with slower kinetics compared to nBoNT/F1
- rBoNT/FA cleaved intracellular VAMP-2 and inhibited neurotransmitter release more potently than nBoNT/F1 in cell assays
- rBoNT/FA was less potent than nBoNT/F1 in an ex vivo assay of stimulated muscle contraction

Keywords

Botulinum Neurotoxin (BoNT)

References

Barash JR, Arnon SS. A novel strain of Clostridium botulinum that produces type B and type H botulinum toxins. J Infect Dis. 2014;209(2);183-191.

Maslanka SE, Lúquez C, Dykes JK, et al. A novel botulinum neurotoxin, previously reported as serotype H, has a hybrid-like structure with regions of similarity to the structures of serotypes A and F and is neutralized with serotype A antitoxin. J Infect Dis. 2016;213(3):379-385.

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