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Structural study of HA3 subcomponent of *Clostridium botulinum* type C progenitor toxin

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Introduction

The *Clostridium botulinum* neurotoxin (NTX) exists as seven different serotypes, designated A through G. In all seven serotypes, NTX are high molecular weight proteins that act on cholinergic neuromuscular junctions to block transmitter release. In culture fluid or food, most NTXs exist as a large stable complex (progenitor toxin) in association with nontoxic components, such as NTNHA and/or several different HAs. In the type C toxin, two forms of progenitor toxin, the C16S toxin and C12S toxin, have been identified. The C16S toxin contains of one molecule of NTX, one NTNHA, and several HA subcomponents. The HA subcomponents are designated HA1 (33 kDa), HA2 (17 kDa), and HA3 (70 kDa). HA3 can be further proteolytically cleaved to form the HA3a (22-23 kDa) and HA3b (55 kDa) fragments. Here we determined the three-dimensional structure of the HA3a-HA3b complex.

Materials and Methods

Crystals were grown at 20 °C using the hanging drop vapor diffusion method. The protein solution was prepared at a concentration of 20 mg/mL in distilled water. The crystallization drop consisted of 1.0 μL protein solution and an equal volume of the crystallization reservoir solution (1.2 M ammonium phosphate in 100 mM sodium citrate, pH 4.4)[1]. The HA3 Se-Met variant was crystallized using the same procedure. Data collection on the crystals was performed at cryogenic temperature. The crystals were transferred to reservoir solution containing 25% ethylene glycol, and then immediately flash-frozen in a stream of nitrogen gas at 100 K. For native HA3, diffraction data were collected at 2.6 Å resolution at beamline PF-BL5A. The Se-Met variant was used for data collection at beamlines PF-BL17A and PF-BL6A.

All the datasets were integrated, scaled, and merged using the HKL2000 program. The crystals contain one monomer per asymmetric unit corresponding to a solvent content of 75%. The initial phasing was performed with the program SOLVE. Structural refinement was conducted using the CNS program.

Results and Discussion

The crystal structure was determined at a resolution of 2.6 Å[2]. The HA3a subcomponent is composed of a single domain. HA3b contains three domains, domainas I, II, and III, and the structure of domain I resembles HA3a (Fig. 1). Domain I of HA3b contains an α-helix, twelve β-strands and a prominent architectural feature, the "Pro-loop", characterized by a proline-rich amino acid sequence. In crystal packing, HA3a and HA3b domain I compose a dimer complex, and three complexes are assembled to form a three-leaved propeller-like structure. Therefore, the entire HA3 forms a trimer of dimers structure, and a pore is located in the center of the structure. HA3a and HA3b domain I are weakly homologous to *Bacillus thuringiensis* nontoxic crystal protein, *Clostridium perfringens* ε-toxin, and *Aeromonas hydrophila* aerolysin. Both HA3b domains II and III showed homology to proteins adopting the jelly-roll like β-sandwich fold.

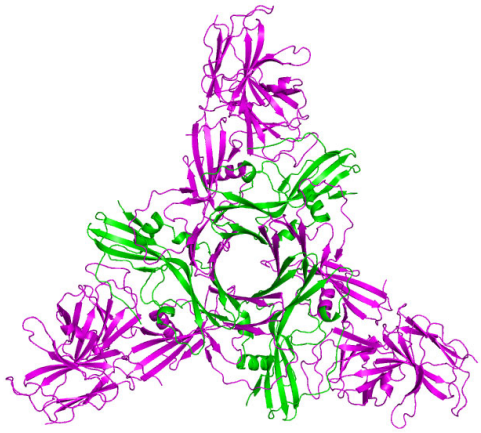

Fig. 1. A trimer of dimers structure of HA3. HA3a and HA3b are shown in green and magenta, respectively.

References

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