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X-ray structure of hemagglutinin subcomponent HA70 from *Clostridium botulinum* in complexes with sialylated oligosaccharides

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1 Introduction

Clostridium botulinum produces the botulinum neurotoxin (NTX) which is one of the most potent toxins. The NTX exists as seven different serotypes A through G, and most NTXs form large complexes as progenitor toxins (PTXs) in association with nontoxic nonhemagglutinin (NTNHA) and/or several different hemagglutinin (HA) subcomponents. Type A-D and G toxins produce PTXs consisting of one NTX, one NTNHA, and three HA subcomponents, designated HA70, HA33, and HA17 based on molecular mass. HA70 is further proteolytically cleaved to HA22-23 and HA53 fragments. The mechanism of carbohydrate recognition by HA subcomponents is very important to elucidate the infection process of botulism. We determined four X-ray structures of HA70 from type C toxin (HA70/C) in complexes with sialylated oligosaccharides; α2-3-sialyllactose (3SiaLac), α2-3-sialyllactosamine (3SiaLacNAc), α2-6-sialyllactose (6SiaLac) and α2-6-sialyllactosamine (6SiaLacNAc).

2 Experiment

HA70/C was expressed as a maltose-binding fusion protein in *Escherichia coli* JM109 cells, and purified by affinity chromatography using amylose resin. Crystals of HA70/C were grown in a droplet containing 1.0 μl of protein solution (18 mg/ml in PBS) and 1.0 μl of reservoir solution (12 % (v/v) 2-methyl-2,4-pentendiol, 20 mM $CaCl_2$, 100 mM sodium acetate (pH4.6)) against 500 μl of the reservoir solution, by the hanging drop method, at 293 K. Crystals of the complexes with the oligosaccharide were obtained by adding 0.4 μl of 0.4 M oligosaccharide solution to the droplets of crystals for 40 hours at 293K. Data was collected on a PF-AR NE3A (KEK, Japan) and a BL26B1 (SPring-8, Japan). The initial phases were determined by molecular replacement, and the structure was refined by the programs Refmac5 and CNS. The bound oligosaccharide ligands could be found in the (Fo-Fc) maps.

3 Results and Discussion

The X-ray structures of the complexes of HA70/C with 3SiaLac, 3SiaLacNAc, 6SiaLac and 6SiaLacNAc were successfully determined [1]. There is one molecule of HA70/C in an asymmetric unit, and a crystallographic 3-fold symmetry generates a homo-trimer of HA70/C with a triangular shape. The carbohydrate-binding sites are in the vicinity of vertices of the triangle (Fig. 1A). Both 3SiaLac (3SiaLacNAc) and 6SiaLac (6SiaLacNAc) were found to bind to HA70/C, and each ligand was recognized by many hydrogen bonds in a similar manner, suggesting that HA70/C can recognize both α2-3- and α2-6-sialylated oligosaccharides (Fig. 1B).

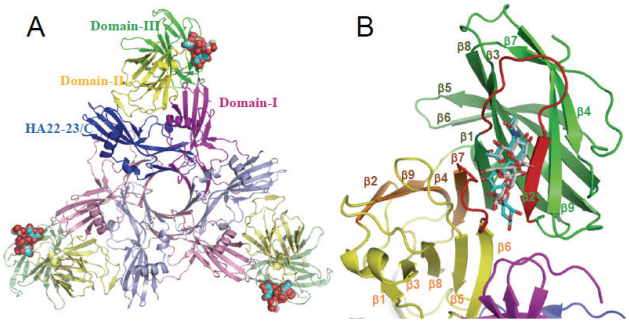

Fig. 1: Structure of HA70/C with the bound ligands. (A) Homo-trimer of HA70/C with the bound ligand in the space-filling mode with gray carbon (3SiaLac) and cyan carbon (6SiaLac). HA22-23/C, and domain-I, -II, and -III of HA55/C are in blue, magenta, yellow and green, respectively. (B) A close-up view of the carbohydrate-binding site with the bound ligand shown as a stick model.

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