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Crystal structures of HA1 subcomponent of *Clostridium botulinum* type C progenitor toxin in complexes with sugars

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Introduction

Botulinum neurotoxin (NT) is a protein of approximately 150 kDa, which is produced in serologically distinct forms (types A–G) by the Gram-positive, spore-forming bacterium *Clostridium botulinum*. NT is a highly potent inhibitor of neurotransmitter released by the peripheral nerve terminus. In type C, two forms of the toxin complexes, designated progenitor toxins, have been identified as 12S (C12S) and 16S (C16S). C12S toxin consists of one molecule of NT and a nontoxic, non-hemagglutinin component with no hemagglutination activity; C16S toxin contains several hemagglutinin (HA) subcomponents in addition to the 12S toxin component. The HA consists of four subcomponents, HA1, HA2, HA3a and HA3b. We have proposed that type C 16S progenitor toxin recognizes sialic acid and is taken up into cells with *O*-linked oligosaccharide[1]. In this report, we determined the crystal structures of type C HA1 in complexes with sugars[2].

Materials and Methods

Crystallization and data collection

The solvent of HA1 protein was substituted with distilled water just prior to setting up the crystallization drops. The crystals were grown at 20 °C using the hanging-drop vapor-diffusion method, where 1.0 µl of 3 mg/ml HA1 solution in distilled water was mixed with an equal volume of a crystallization reservoir solution containing 12% (v/v) ethanol and 1.7 M sodium chloride in distilled water. The crystals of the complex of HA1 with sugars were obtained by soaking with the same reservoir solution containing Neu5Ac (sialic acid), GalNAc (*N*-acetylgalactosamine), or Gal (galactose). The diffraction data of the HA1–sugar complexes were collected at the BL-6A of PF beamline. All data sets were processed and scaled using HKL2000.

Structure determination and refinement

The structures of the HA1–sugar complex were solved by molecular replacement using the program MOLREP in the CCP4 suite and the program CNS; the structure of *C. botulinum* type C HA1 (PDB, 1QXM) was used as the search model. The models were built using the program Xfit in the XtalView system. Calculations of the structural refinements were carried out using the CNS program.

Results and Discussion

The complex structures of HA1 with the three monosaccharides, Neu5Ac (2EHI), GalNAc (2EHM), and Gal (2EHN), were determined. The structure of HA1 consists of two β-trefoil domains and a short linker composed of an α-helix that connects the two domains. There are two major sugar-binding sites, sites I and II (Fig. 1). Site I corresponds to the electron densities noted for all sugars and is located at the C-terminal β-trefoil domain, while site II corresponds to the electron densities noted only for Gal. An aromatic amino acid residue, Trp176, at site I has a stacking interaction with the hexose ring of the sugars. On the other hand, there is no aromatic residue at site II; thus, the interaction with galactose seems to be poor.

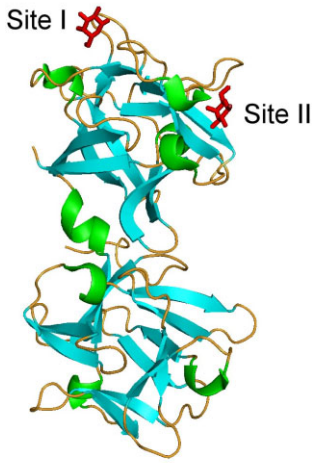

Fig. 1. Structure of HA1—Gal complex. The Gal molecules are indicated in red.

References

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