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Structural characterization of the two sugar-binding sites of the β-trefoil lectin HA33/C (HA1) from *Clostridium botulinum* type C neurotoxin

Toshio NAKAMURA, Takashi TONOZUKA*, Atsushi NISHIKAWA
Department of Applied Biological Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai-cho, Fuchu, Tokyo 183-8509, Japan

Introduction

Clostridium botulinum are gram-positive organisms that can produce one of several toxins collectively known as botulinum neurotoxin. They induce a potentially fatal paralytic condition in humans and various animal species known as "botulism". The botulinum neurotoxin exists as seven different serotypes, designated A through G. Types A, B, E, and F cause botulism in both humans and animals, while types C and D cause botulism mainly in animals. A critical role in internalizing the neurotoxin into gastrointestinal epithelial cells is played by a nontoxic component, a β-trefoil lectin from the neurotoxin complex known as HA33 or HA1. The HA33 component from *C. botulinum* type A (HA33/A) has been predicted to have a single sugar-binding site, while HA33 from *C. botulinum* type C (HA33/C) has two sugar-binding sites, site I and site II. Here we determined the crystal structures of wild-type HA33/C and the F179I mutant in complex with GalNAc (*N*-acetylgalactosamine) at site II. The result reveals the diverse architectures and properties of the sugar-binding sites of HA33.

Materials and Methods

The wild-type (WT) HA33/C and the mutants were purified as described previously [1]. The crystals were grown at 20°C using the hanging drop vapor diffusion method. The protein solution was prepared at a concentration of 5 mg/mL in water, and mixed with an equal volume of the crystallization reservoir solution, 1.7 M sodium chloride and 12% (v/v) ethanol. The crystals of the HA33/C–GalNAc complex were obtained by soaking in the same reservoir solution supplemented with 250 mM GalNAc for 6 h. After soaking, the samples were immediately flash-frozen in a stream of nitrogen gas at 100 K. Diffraction data were collected at beamlines AR-NW12 and BL5A of Photon Factory. The datasets were integrated using the HKL2000 program. The structures were solved by molecular replacement using the program Molrep in the CCP4 suite, and the structure of WT HA33/C [1] was used as the search model. The models for GalNAc and the mutated residues were built using the program Coot. Calculations of the structural refinements were carried out using Refmac in the CCP4 suite. Solvent molecules were introduced using the program ARP/wARP. The coordinates and structure factors have been deposited in the Protein Data Bank under the accession codes 3AJ5 for WT-GalNAc and 3AJ6 for the F179I-GalNAc mutant.

Results and Discussion

HA33/C interacts with the sugars through site I and site II. We previously determined the structure of WT-GalNAc [1]. In this complex, GalNAc is in site I of the HA33/C molecule. We have now determined the structure of GalNAc bound to site II of the WT protein and of the F179I HA33/C protein [2]. The structures of WT and the F179I mutant were compared by superimposing site I of the Neu5Ac (*N*-acetylneuraminic acid) complex, the GalNAc complex, the Gal (galactose) complex, and the F179I mutant. The side chains of WT amino acid residues interacting with Neu5Ac, GalNAc, or Gal have essentially the same orientation as those of F179I. The binding of GalNAc at site I is strikingly different than at site II. The sugar-binding pocket of site I is narrow and deep, while the site II sugar-binding cleft is wide and shallow (Fig .1). The results indicated that site I of HA33/C has an unusual sugar-binding mechanism, while site II is less important as compared to other typical β-trefoil lectins.

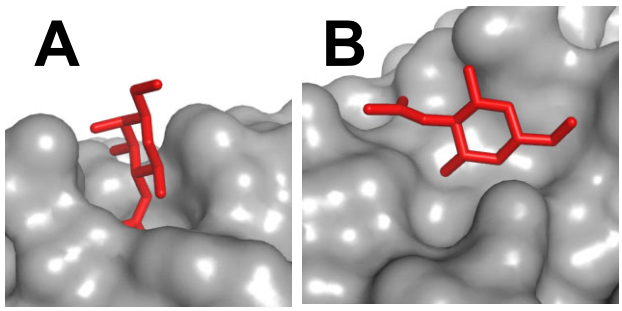

Fig. 1. Surface models of (A) site I of WT HA33/C-GalNAc [1] and (B) site II of WT HA33/C-GalNAc [2]. GalNAc molecules are shown as red sticks.

References

[1] T. Nakamura et al., J. Mol. Biol. 376, 854 (2008).
[2] T. Nakamura et al., Arch. Biochem. Biophys. in press (2011).

* tonozuka @ cc.tuat.ac.jp