Crystallography

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Crystallographic study of the receptor binding domain of the D/C mosaic neurotoxin from *Clostridium botulinum*

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Introduction

Clostridium botulinum, an anaerobic soil bacterium, produces seven botulinum toxin serotypes (BoNTs) designated A – G. BoNTs are also the major causative agents of botulism in human and animals. Recently, BoNTs/OFD05 belonging to type D/C mosaic was found in bovine botulism specimen in Japan [1]. It contains two-thirds of the BoNT/D gene and one-third of the BoNT/C gene. The three-dimensional structures of BoNT types B revealed 3 functionally distinct domains: catalytic, translocation and binding domains [2]. The BoNT/OFD05 binding domain has identity with BoNT/C, which requires a single ganglioside as a binding receptor on neuronal cells, unlike BoNT/A and BoNT/B which require two receptors for specific binding [3]. To determine the binding mechanism of BoNT/OFD05 and its ganglioside receptors on neuronal cells, recombinant BoNT/OFD05 receptor binding domain was expressed, purified, and crystallized.

Results and Discussion

The protein was overexpressed as a soluble protein in *E. coli*, purified with Ni affinity chromatography and size exclusion chromatography. After dialysis against 20 mM Tris-Cl pH8.0, 200 mM NaCl, and concentrating up to 1mg ml^{-1}, the purified protein was crystallized for structural studies. The crystals most suitable for further diffraction experiments were grown with 0.2 M potassium/sodium tartrate, 0.1 M trisodium citrate (pH 5.6), and 1 M ammonium sulfate (Fig. 1). The diffraction dataset of native crystal was collected at a resolution of 2.8 Å from the beamline NW12A. Crystals were soaked in mother liquor containing 30% glycerol and flash-cooled in a stream of liquid nitrogen. The crystals belonged to the $P2_12_12_1$ space group with a unit cell parameters of a=57.8, b=139.0, and c=160.9Å (Table 1). The Matthews coefficient and solvent content, estimated as 3.21 $Å^3Da^{-1}$ and 61.7%, respectively. The initial phasing was performed with the program SHELX by the SAD method at 4 Å resolution with a data collected at Spring-8. The initial phases were transferred into the diffraction data of native crystal and then expanded up to 2.8 Å resolution with the program DM. Further model building is currently underway in our laboratory.

Table 1: Data collection statistics

X-ray source	NW12
Space group	$P2_12_12_1$
Unit cell parameters (Å)	a=57.8, b=139.0, c=160.9
Wavelength (Å)	1.00000
Resolution range (Å)	50-2.80 (2.90-2.80)
No. of total reflections	223250
No. of unique reflections	32077
Completeness (%)	98.0 (86.2)
R_{sym}(%)	5.8 (31.0)
Multiplicity	7.0 (6.1)

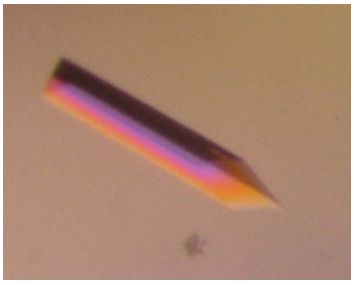

Fig.1 Crystal of the binding domain of BoNT-OFD05 for X-ray data collection. The crystal dimensions are approximately 0.7 × 0.1 × 0.1 mm.

References

[1] K. Nakamura et al., Vet Microbiol 140, 147 (2010).
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