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Structural studies of mammalian cell recognizing crystal proteins from Bacillus thuringiensis

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Introduction

Bacillus thuringiensis (Bt) is a spore-forming Gram positive bacterium found in many locations, such as the soil and plant surfaces. Bt produces parasporal crystalline inclusions during sporulation. They consist of one or more crystal (Cry) proteins, which often exhibit specific insecticidal activity. Thus, Bt has found successful application in agriculture as a biological insecticide.

However, it has also been noted that non-insecticidal Bt strains are widely distributed as well. Recent screening of these strains for cytocidal activity has identified several strains that produce crystal proteins specifically toxic to particular human carcinoma cells[1]. These proteins can have potential for application to cancer treatment. In order to obtain detailed structural information required for their successful application, we have been engaged in crystal analysis of three representative crystal proteins from Bt strains, A1190, A1470, and A1547.

Results and Discussion

A1190 crystal protein (Parasporin-1)

Crystallization and data collection procedures for the protein were summarized in the previous report[2] along with those of other two proteins.

An initial model, which was build based on 2.6-Å MIR phases, was refined with CNS to 1.76 Å resolution using a native data set collected at 95 K at BL-6A. The final model includes 630 amino acid residues and 802 water molecules (R/Rfree(10% data)=0.172/0.200).

The protein has a three-domain architecture common to five available structures of Cry proteins[3]; three domains have forms of (1) α-helix bundle, (2) β-prism, and (3) β-sandwich, respectively. In spite of limited sequence identity, the main chain of each domain can be superimposed mostly well with counterparts of other Cry proteins. Particularly, domain 3 and α-helix portion of domain 1 show good agreement. On the other hand, significant deviations are found in the N-terminal region of domain 1, a long loop between 6th and 7th helices of domain 1, and proximal loops of domain 2. These portions can be responsible for specific binding to membrane receptors.

A1470 crystal protein

A four-wavelength MAD experiment using a cryo-cooled mercury derivative crystal was carried out at BL-12NW. The mercury sites were identified and experimental phases were calculated to 2.3-Å resolution using the CCP4 program suite and SHARP. After phase improvement by solvent flattening, an initial model was built with Turbo-FRODO with the aid of RESOLVE. The model was refined to 2.07 Å resolution using a native data set collected at 90 K. The present model contains 498 residues and 210 water molecules in the asymmetric unit (R/Rfree(10% data) =0.231/0.266).

The protein monomer has a cigar-like elongated shape, 118 Å long and 20–25 Å thick. It is almost entirely composed of β-structure: 65.5% β-structure and 8.9% α-helix. The monomer molecule can be separated to three domains. Two of β-strands are unusually long, spanning the whole length of the molecule. This structure is novel as a Cry protein, and, interestingly enough, it is strikingly similar to the larger lobe of proaerolysin (*Aeromonas* toxin)[4].

The asymmetric unit contains two monomers, which are virtually related by a twofold axis (r .m. s. d. between Cα positions is 0.62 Å). The difference is attributable to hinge motions between domains.

A1547 crystal protein

The crystallization solution for the protein, which is based on ethyleneglycol and PEG, was optimized to obtain crystals larger than 0.2 mm. We found post-soaking in crystallization solution with increased PEG concentration improved quality of the crystal. Using a crystal thus obtained, native data were collected to 2.7 Å resolution (Rmerge=0.097) at 90 K at BL-12NW.

References

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