Crystallography

6A/2002G305

Structural studies of mammalian cell recognizing crystal proteins from *Bacillus thuringiensis*

Toshihiko AKIBA[1], Tokio ICHIMATSU[2], Hideki KATAYAMA[2], Tetsuyuki AKAO[2], Kazuhiko HIGUCHI[2], Eiichi MIZUKI[2], Sakae KITADA[3], Akio ITO[3], Ryuta KANAI[4], and Kazuaki HARATA*[1]

[1] Biological Information Research Center, AIST, Tsukuba, Ibaraki 305-8566, Japan
[2] Fukuoka Industrial Technology Center, Kurume, Fukuoka 839-0861, Japan
[3] Department of Chemistry, Faculty of Science, Kyushu University, Fukuoka 812-8581, Japan
[4] Institute of Biological Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

Introduction

Common soil bacteria, *Bacillus thuringiensis* (Bt), have been successfully used as a biological pesticide, as they produce parasporal crystalline inclusions during sporulation which contain insecticidal proteins. These protein toxins are dissolved from the inclusions and activated by proteolysis in the midgut of ingested insects. The activated toxins recognize specific receptors on the brush-border membrane surface of midgut epithelium to form a lytic pore, leading to death.

Recent screening of these protein species for the wider range of lytic activity has identified several proteins specifically effective to cultured human carcinoma cells [1]. These mammalian cell recognizing crystal (MCRC) proteins can find their application to anti-cancer drugs, which requires detailed structural information about receptor recognition and pore-formation by X-ray crystallography. We have been engaged in crystal analysis of three representative MCRC proteins, designated here as A1190, A1470, and A1547, respectively, according to strain ID's of the Bt cell sources.

Experimental and results

We constructed a His-tagged recombinant form of the protein and activated the purified protein by proteolysis. After appropriately concentrated, the activated protein was dialyzed against 10 mM CAPS buffer at pH 12 for crystallization. Crystallization conditions for each protein are summarized in Table 1. All crystallization experiments were carried out at 23 °C.

A crystal was flash-frozen in a nitrogen gas stream cooled at 95 K after glycerol supplementation to crystal harvesting solution to the final concentration of 10 or 20 %(v/v). Diffraction data from cryo-cooled native crystals were collected at beamline BL6A of Photon Factory using the wavelength of 0.978 Å. The collected data sets were processed by the HKL2000 package and the CCP4 program suite. Crystal parameters and statistics of the data sets are shown in Table 1 for each protein.

The A1190 data set will be used in refinement of the MIR model which we are constructing. As for A1470 protein, the data set can be used as a reference in screening for heavy atom derivatives. The A1547 data set was from the first hit in crystallization screening; the statistics indicate there is plenty of room for optimization.

References

[1] E. Mizuki *et al. J Appl Microbiol.* **86**, 477-486 (1999).

* k-harata@aist.go.jp

Table 1: crystallisation conditions and data collection statistics of the MCRC proteins

Protein name	A1190	A1470	A1547
Crystallization method	microdialysis	hanging drop vapor diffusion	sitting drop vapor diffusion
Equilibration solution	5 % PEG3350, 0.01 M Tris-buffer, pH 8.4	0.05 M $(NH_3)_2SO_4$, 10 % MPD, 0.1 M citrate buffer, pH 5.6	8 % ethylene glycol, 10 % PEG8000, 0.1 M HEPES buffer, pH 7.5
Protein concentration (mg/mL)	3.8	5.0	3.5
Space group	$P2_12_12_1$	$P3_112$ or $P3_212$	$P6_1$ or $P6_5$
Unit cell parameters (Å)	*a*=61.0, *b*=76.5, *c*=120.4	*a*=*b*=95.4, *c*=131.3	*a*=*b*=134.6, *c*=121.9
Resolution (Å)	30.0–1.76 (1.82-1.76)[1]	50-2.4 (2.49-2.4)	50-3.8 (3.94-3.8)
Number of observations	410866	283177	142488
Number of unique reflections	56627	52331	24335
R merge (%)	3.4 (10.1)[2]	5.1 (26.9)	11.8 (38.3)
I/sigma(I)	29.2	16.6	7.9
Completeness (%)	99.8 (99.9)	100 (100)	100 (100)

[1] Values in parentheses refer to the range of the highest resolution shell.
[2] Values in parentheses refer to those in the highest resolution shell.