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Structural studies of β-lactam antibiotic degrading enzyme, MacQ

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1 Introduction

Acidovorax sp. strain MR-S7 is a Gram-negative bacterium isolated from activated sludge in a treatment system for penicillin G wastewater, and shows high resistant activity to a wide variety of β-lactam antibiotics as well as *N*-acylhomoserine lactone (AHL) degrading activity [1, 2]. AHL is a major cell-to-cell signaling compound in many Gram-negative bacteria, and functions as a determinant for the bacterial population density: the phenomenon is termed as "quorum sensing" [3]. We identified the β-lactam acylase gene (*macQ*) with high sequence homologies to well-known AHL acylase homologues (~40%). In vitro enzyme assay showed that MacQ protein exhibited deacylation activities toward not only AHL but also penicillin G. To investigate the structural mechanism of dual functionality of MacQ, we have undertaken the crystallographic studies.

2 Experiment

His-tagged MacQ without the signal sequence was expressed by *Escherichia coli* Origami 2 (DE3), and purified by Ni-affinity chromatography. Crystallization screening was performed by vapor-diffusion method at 20°C. Plate-shaped crystals were obtained using the solution containing 0.1 M Tris-HCl pH 7.5-8.5, 0.1-0.2 M calcium acetate, and 10-18% PEG 3350. The X-ray diffraction data were collected at PF, using CCD detector (ADSC). Preliminary X-ray diffraction studies showed that crystals were classified into two distinct crystallographic forms (form I and II, see Table 1). The data processing was performed with program HKL2000. The structure was determined by the molecular replacement method with the program PHASER, using the truncated model of AHL acylase PvdQ (PDB code, 2wye [4]) as a serch model. The model refinement and manual model corrections were performed with the program REFMAC5 and Coot.

3 Results and Discussion

AHL acylases are known to be activated by autoproteolytic maturation, e.g., the PvdQ of *Pseudomonas aeruginosa* is cleaved into three major fragments: α chain (~18 kDa), spacer peptide (~3 kDa), and β chain (~60 kDa)), and the matured PvdQ is a heterodimer formed by α and β chains [4]. Interestingly, the structure of MacQ in both form I and II crystals exhibited the cage-shaped heterotetramer ($\alpha_2\beta_2$, see Fig. 1), and the extra electron density was also observed in the cage, which was well fitted to its sequence of spacer peptide. The model refinement and the crystallization of various ligand complex of MacQ are currently underway.

Table 1. Data collection statistics for MacQ crystals.

	Form I	Form II
Wavelength (Å)	1.0000	1.0000
Resolution (Å)	50–1.70 (1.73–1.70)	50–2.60 (2.64–2.60)
Unit-cell		
a, *b*, *c* (Å)	102.9, 138.6, 121.6	85.6, 90.1, 123.2
α, *β*, *γ* (°)	90.0, 111.5, 90.0	103.5, 105.0, 105.9
Space group	$P2_1$	$P1$
Unique reflections	346,954	159,207
R_{merge}	0.081 (0.461)	0.101 (0.638)
Completeness (%)	99.8 (100.0)	98.9 (98.2)
Redundancy	3.8 (3.7)	3.9 (3.9)
Mean I/σ (I)	27.0 (3.4)	17.5 (2.6)
Refinement		
R_{work}	0.25 (in progress)	0.19 (in progress)
R_{free}	0.28 (in progress)	0.25 (in progress)

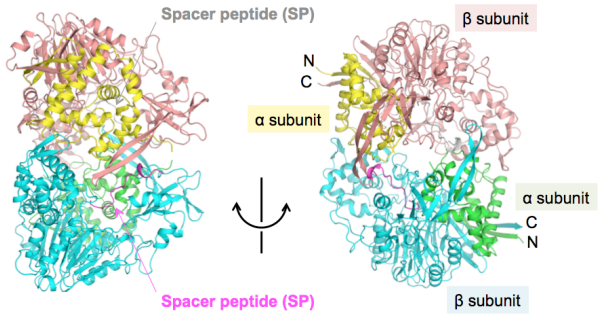

Fig. 1: Overall structure of MacQ showing heterotetrameric cage-like assembly

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References

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