

Crystal structure analysis of CutA from *Pyrococcus horikoshii*

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Introduction

Heavy metals play numerous critical roles in various biological processes. Excess heavy metals, however, are harmful to cells, resulting in cell death. Therefore, the intracellular concentrations of heavy metals have to be controlled tightly.

In microorganisms, there are some mechanisms to resist heavy metals. Based on genetic analysis of *Escherichia coli*, *cut* genes have been proposed to be related to the copper tolerance mechanism. CutA is one of the proteins involved in *cut* genes, and universally located in wide spread of bacteria. Its function, however, has not yet been clarified. In order to address the function from structural viewpoints, we tried to determine the crystal structure of CutA from *Pyrococcus horikoshii* (*PhoCutA*).

Experiments and Results

PhoCutA was expressed in *E. coli* B21(DE3) grown in LB medium, and purified as described by Tanaka *et al.* [1]. Single crystals of *PhoCutA* suitable for X-ray diffraction analysis were grown in 100 mM acetate buffer (pH 4.6) containing 1.6 M ammonium sulfate by hanging-drop vapor diffusion method (Figure 1).

X-ray diffraction data were collected at the beamline BL-18B of Photon Factory, under cryogenic condition (100 K) using flash cooling technique. The X-ray diffraction data set was collected up to 1.48 Å resolution using ADSC-Quantum4R CCD detector. The diffraction data were processed with the program MOSFLM for integration, and the program SCALA (CCP4 suite) for merging and scaling. The data processing statistics are given in Table 1. The crystals belong to the hexagonal system; the space group was determined to be $P6_3$ with unit cell parameters $a = b = 52.9$ Å, and $c = 57.6$ Å. The asymmetric unit contains one molecule of the *PhoCutA*, corresponding to the V_M value of 2.77 Å³Da⁻¹. The cumulative intensity statistics indicated that the diffraction data were collected from a twin crystal. The twinning operator was determined to be $(h -h -k -l)$ with a high twinning fraction (nearly 0.5), using the program CNS.



Figure 1. Crystals of *PhoCutA*.

Table 1. Data processing statistics

Space group	$P6_3$
Cell dimensions (Å)	$a = b = 52.9, c = 57.6$
Beamline	BL-18
Resolution (Å)	21-1.48 (1.57-1.48)
Wavelength (Å)	0.9798
R_{sym} (%)	10.4 (26.8)
Completeness (%)	99.9 (99.9)
Observed reflections	201430
Unique reflections	18117
$I/\sigma(I)$	3.8
Multiplicity	10.6 (10.6)
Twinning operator	$(h -h -k -l)$
Twinning fraction	0.5

Reference

[1] Tanaka, Y. *et al.* FEBS Lett. 556, 167 (2004).

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