X-ray Crystallographic Study of OmpR-C mutant

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Introduction

Bacteria have signal transduction systems to respond to abrupt environmental changes. The simplest prototype of such systems involves two protein components, a sensor and a response regulator. The sensor protein, which is often located in the cytoplasmic membrane constantly monitors environmental change, and relays the information to a response regulator in the cytoplasm. The response regulator mediates changes in gene expression or locomotion in response to a given signal. This type of signal transduction system is referred as "two-component system"[1]. In *Escherichia coli*, expression of the outer membrane porin protein is regulated at the transcriptional level in response to medium osmolarity. Two-component regulator proteins OmpR and KdpE have crucial roles in the regulation of differential porin gene expression. OmpR responds to medium osmolarity change and KdpE responds to turgour pressure change. Two-component regulator proteins recognize specific promoter sequences. In order to investigate the DNA binding mechanism of OmpR, we have constructed a mutant of DNA-binding domain of OmpR, the C terminal domain of OmpR (OmpR-C), which recognize the promoter of KdpE protein, using PCR mutagenesis method.

Experimental & Results

OmpR-C mutant was purified by ion exchange chromatography and gel filtration chromatography. The purified OmpR-C mutant was crystallized using hanging-drop vapour-diffusion method. Well-shaped tetragonal crystals were obtained at 20°C from a protein solution of 18mg/ml, 10% PEG8K 20% Glycerol in 0.1M Bicine buffer at pH 9.0.

X-ray diffraction data were collected on station BL6A at the Photon Factory. The data collected at 291K were processed with DENZO and SCALEPACK up to 2.8 Å resolution. The crystal belongs to space group $P4_1$ or $P4_3$ with unit cell dimensions of $a = b = 55.4$ Å, and $c = 188.1$ Å.

The data collection at 100K using general freezing method was impossible (Figure 1). But by applying macromolecular crystal annealing method[2] to the crystal, the data collection at 100K was succeeded and the resolution limit goes up to 2.1 Å (Figure 2). This crystal belongs to space group $P4_1$ or $P4_3$ with unit cell dimensions of $a = b = 54.3$ Å, and $c = 180.2$ Å. Assuming 6 OmpR-C monomers (12kDa) per asymmetric unit, the Matthews coefficient is calculated to be 2.03Å^{3}Da^{-1}.

The molecular replacement calculations are now under going

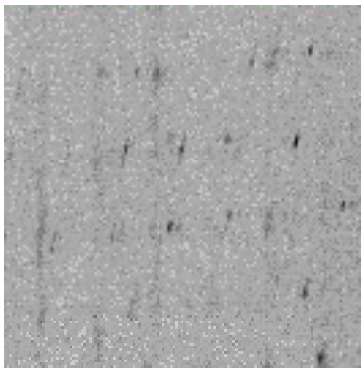

Figure 1. Diffraction pattern before macromolecular crystal annealing.

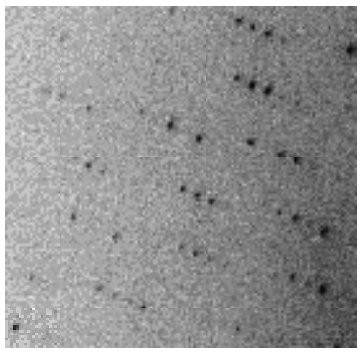

Figure 2. Diffraction pattern after macromolecular crystal annealing. The crystal was the same one with Figure 1.

References

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[2] J. M. Harp *et. al.* *Acta Cryst*.D **54**, 622 – 628 (1998).

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