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Crystal structure analysis of an autoregulator-receptor protein in *Streptomyces* species

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Introduction

The gram-positive bacteria genus *Streptomyces* employs γ-butyrolactones as autoregulators or microbial hormones, together with their specific receptors, to regulate morphological differentiation or antibiotic production, or both. The representative of the γ-butyrolactone autoregulatory factors is A-factor (2-isocaprylolyl-3*R*-hydroxymethyl-γ-butyrolactone), which is essentially required for aerial mycelium formation, streptomycin production, streptomysin resistance, and yellow pigment production in *Streptomyces griseus*. A-factor-binding protein (ArpA) shows high specificity to A-factor and has a repressor-type function. Upon binding A-factor, ArpA dissociates from the promoter region of its target genes, resulting in transcriptional activation of the genes. This A-factor-ArpA like systems are considered to be a common regulatory system controlling secondary metabolism and/or morphogenesis of *Streptomyces* spp. CprB, an ArpA homologue in *Streptomyces coelicolor* A3(2), controls antibiotic production and aerial mycelium formation in this strain. As the first step for understanding the molecular mechanism of the autoregulator-receptor regulatory system in *Streptomyces*, we have attempted to solve the crystal structure of CprB.

CprB was crystallized by the hanging-drop vapour-diffusion method using polyethylene glycol 6000 as a precipitant. Three crystal forms, forms I, II and III, grew under the same crystallization condition. Form I crystals were of truncated rectangular shape and diffracted to better than 2.5Å resolution. Form II crystals were of truncated pyramidal shape. Form III crystals, which were rod-shaped, did not show diffractions. While form I crystals belongs to an orthorhombic system with a space group of $P2_12_12_1$, preliminary crystallographic studies showed that form II crystals belongs to a tetragonal system with a space group of $P4_12_12$ or its enantiomorph $P4_32_12$. As the crystal structure of form I crystal was determined by the multi-wavelength anomalous diffraction method (MAD) at 2.4Å resolution, we tried to solve the crystal structure of form II using the molecular replacement method. Here we report a crystal structure analysis of CprB in the form II crystal.

Results

Although the typical form II crystals were very small, only one crystal grew to dimensions of 0.15 x 0.1 x 0.05 mm^3 in two months. This crystal was used for the data collection. The diffraction data was collected at 100 K using an ADSC Quantum CCD detector at BL6A of PF. Form II crystal belonged to the space group $P4_12_12$ or its enantiomorph with the cell dimensions $a = b = 111.95$ Å, $c = 43.44$ Å. All the data were processed using the program DPS/MOSFLM and scaled with the program SCALA in the CCP4 program suite. The statistics of the data collection is shown in the Table 1.

Table 1: Statistics of data collection

Resolution (Å) (outer shell) [a]	39.5 - 3.0 (3.16 - 3.0)
Rmerge (%)[b]	8.9 (45.5)
Completeness (%)	100 (100)
Multiplicity	16.4 (17.1)
I/sigma(I)	5.8 (1.6)

a: Statistics for the highest shell are given in parentheses
b: Rmerge = $\Sigma_h\Sigma_i^N|I(h)_i-I(h)| / \Sigma_h(N \cdot I(h)_i)$

On the basis of the molecular weights and cell dimensions, form II crystal was assumed to contain one subunit in the asymmetric unit (Vm = 2.91 $Å^3Da^{-1}$, ca. 58 % solvent content). The crystal structure of form II was determined by the molecular replacement (MR) method using the structure of one CprB subunit of the form I crystal as initial model. The analysis revealed that the space group of the form II crystal is $P4_12_12$. The current R-factor is 31.3% (FreeR=34.2%) at 3.0Å resolution. Further crystallographic refinement is in progress.

The crystal structure of form II showed that CprB is a dimeric protein and the subunit of CprB is composed of two domains, DNA-binding domain (residues 1 to 52), and regulatory domain (residues 53 to 216). The two subunits of the CprB dimer in the form II crystal is related by a two-fold axis, which is coincides with the two-fold axis of the crystal. Structural comparison between the crystal structures of forms I and II showed that the relative disposition of subunits is different between the two crystal forms, suggesting the mobile nature of the CprB dimer.

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