6A/2001G346

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

Ryo NATSUME[1], Shin MURAOKA[2, 3], Yutaka FURUSAWA[1,3], Toshiya SENDA*[3]
[1]The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-8657, Japan
[2]Nagaoka University of Technology, Nagaoka, Niigata 940-2188, Japan
[3] BIRC, AIST, 2-41-6 Aomi, Koto-ku, Tokyo 135-0064, Japan

Introduction

The gram-positive bacteria genus *Streptomyces* is characterized by the ability of its metabolites and by complex morphological differentiation. A-factor is a chemical signaling molecule, or a microbial hormone, that is essentially required for aerial mycelium formation, streptomycin production, streptomycin resistance, and yellow pigment production in *Streptomyces griseus*. A-factor binding protein shows high specificity to A-factor and has a repressor-type function. Upon binding with A-factor, A-factor binding protein appears to dissociate from the promoter region of its target genes resulting in an activation leading to transcription of the genes. This A-factor/ArpA like system is considered to be a common regulatory system controlling secondary metabolism and morphogenesis of the *Streptomyces*. CprB is a homologue protein of ArpA found in a closely related microbe, *Streptomyces coelicolor* A3(2). In order to gain an insight into the mechanism of the regulatory system, we have tried to solve the crystal structure of CprB with the MAD method. However, the MAD electron density (3.2Å resolution) cannot be interpreted fully, as the quality of the electron density map was poor. To obtain a better electron density map, we have tried to collect high-resolution diffraction data of the CprB crystal. Here we report a high-resolution crystal structure analysis of CprB.

Results

The CprB was overexpressed using *E. coli* and purified to near-homogeneity by three steps of chromatographic procedure. Crystallization of CprB was carried out using the typical hanging drop vapour diffusion method. The crystals grew to the dimensions of 0.3mm x 0.3mm x 0.05mm at the maximum. The high-resolution data was collected at 100K using an ADSC Quantum CCD detector at BL6A of PF. The CprB crystal belonged to the space group $P2_12_12_1$ with the cell dimensions of a = 37.8Å, b = 69.6Å, c = 148.9Å. 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)	29.7 - 1.8
Rmerge (%)	4.1
Completeness (%)	85.4
Multiplicity	3.9
I/sigma(I)	11.7

Since the cell parameters of the present crystal were significantly different from those of the crystal using in the MAD phasing, the crystal structure was determined by the molecular replacement method (MR). A masked electron density corresponding to one CprB molecule, which is derived from the MAD analysis, was used as an initial model, as no reasonable molecular models of CprB were available. The MR analysis gave a clear result, and the obtained electron density map was of enough quality, resulting in the complete model building of CprB. The crystallographic refinement is in progress. The present R-factor is 25.7% (FreeR=27.1%) at 2.0Å resolution.

The crystal structure of CprB shows that CprB is a dimeric protein as predicted by biochemical analysis. The subunit has a DNA-binding domain (residues 1 to 52), and a cofactor-binding domain (residues 53 to 212). The CprB molecule is an alpha-helical protein with no apparent beta-strands in its structure.