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Crystallization and preliminary X-ray crystallographic analysis of the DNA-binding domain of BldD from *Streptomyces coelicolor* A3(2)

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The N-terminal DNA-binding domain of BldD from *Streptomyces coelicolor* A3(2) was crystallized by the hanging-drop vapour-diffusion method at 296 K. A 1.8 Å data set has been collected using synchrotron radiation at Pohang Light Source, South Korea. The crystal belongs to the monoclinic space group $C2$, with unit-cell parameters $a = 77.2$, $b = 31.8$, $c = 33.6$ Å, $\beta = 105.1°$. Analysis of the packing density shows that the asymmetric unit probably contains one molecule, with a solvent content of 43.6%.

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1. Introduction

Streptomyces coelicolor, a filamentous soil bacterium, produces spores through a complex process of morphological differentiation. This developmental process begins with the formation of an aerial mycelium that grows out into the air, constituting a fuzzy layer on the colony surface, and culminates in sporulation (Flardh *et al.*, 1999; Kelemen & Buttner, 1998).

Numerous studies have revealed that two main classes of genes (*whi* genes and *bld* genes) are required for the morphological development. The *whi* genes, mutations in which yield 'white' aerial mycelium owing to the lack of the grey spore pigment, are essential for the spore maturation in the aerial hyphae (Chater, 1972). The *bld* genes are required for the erection of the aerial hyphae. *bld* mutants cause the loss of the aerial hyphae and have a shiny 'bald' phenotype (Merrick, 1976; Nodwell *et al.*, 1996; Willey *et al.*, 1993). Furthermore, these mutants show pleiotropic effects, including defects in carbon-catabolite repression and in cell–cell signalling and sometimes inhibition of antibiotic production (Champness, 1988; Kelemen & Buttner, 1998; Merrick, 1976; Nodwell *et al.*, 1999; Pope *et al.*, 1996; Willey *et al.*, 1993).

So far, many *bld* mutants, including *bldA*, *bldB*, *bldC*, *bldD*, *bldF*, *bldG*, *bldH*, *bldI*, *bldK* and *bldM*, have been identified. These mutants are known to be unable to produce a small hydrophobic molecule, SapB (spore-associated protein), that contributes to the erection of the aerial hyphae by reducing the surface tension at the colony surface (Tillotson *et al.*, 1998; Willey *et al.*, 1991). Interestingly, when certain pairs of *bld* mutants are grown on rich media in close proximity, one mutant can trigger the aerial hyphae formation of the other mutant (Molle & Buttner, 2000; Nodwell *et al.*, 1999; Tillotson *et al.*, 1998; Willey *et al.*, 1991, 1993). This extracellular complementation is always unidirectional and can be accounted for by the hierarchical cascade of intercellular signals for the formation of the hyphae. That is, one mutant which is higher in the hierarchy and acts as a donor can give signals to the other mutant acting as a recipient through an unidentified signalling mechanism and thus render the recipient mutant in order to restore the ability to produce the final product, SapB. The *bldD* mutant stands at the top of the complementation hierarchy and is able to extracellularly complement all of the known *bld* mutants except for itself and the *bldM* mutant, which belongs to the same complementation group as the *bldD* mutant (Nodwell *et al.*, 1996; Willey *et al.*, 1993).

The *bldD* gene product, BldD, consists of 167 amino acids with a calculated molecular weight of 18 167 Da; the identified *bldD* mutant has a point mutation at position 62 from Tyr to Cys (Elliot *et al.*, 1998). BldD binds to its own promoter (Elliot & Leskiw, 1999; Elliot *et al.*, 2001) and regulatory regions of key genes involved in the developmental process; for example, *whiG*, which encodes a σ factor that plays a critical role in sporulation (Chater *et al.*, 1989), and *bldN*, which encodes a σ factor required for aerial hyphae formation (Bibb *et al.*, 2000).

In order to understand the molecular mechanism of transcriptional regulation and multiple promoter recognition by BldD, we have overexpressed and purified the N-terminal DNA-binding domain of BldD (BldDN) from *S. coelicolor* A3(2). BldDN retained the DNA-binding ability to the regulatory regions of the target genes mentioned above (data not shown). Here, we report the crystallization and

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preliminary X-ray diffraction analysis of the DNA-binding domain of BldD as a start toward the structure determination.

2. Materials and methods

2.1. Preparation of recombinant DNA-binding domain of BldD

The gene sequence encoding the N-terminal 79 residues of BldD was amplified by the polymerase chain reaction (PCR) using *S. coelicolor* A3(2) genomic DNA as a template. The PCR product was digested with *Nde*I and *Bam*HI and inserted downstream of the T7 promoter of pET-15b (Novagen), generating pET-15b-BldDN, and the plasmid was transformed into *Escherichia coli* strain BL21 (DE3). Cells were grown to an OD_{600} of approximately 0.5 in Luria–Bertani media containing 0.1 mg ml^{-1} ampicillin (Duchefa) at 310 K and expression was induced using 1 m*M* isopropyl-β-D-thiogalactoside (Duchefa) at 295 K. After overnight induction, cells were harvested and resuspended in 20 m*M* Tris–HCl pH 8.0 containing 150 m*M* NaCl. The cells were disrupted by sonication. After the cell debris had been discarded by centrifugation at 20 000*g* for 30 min, the supernatant was loaded onto a nickel–nitrilotriacetic acid column (Qiagen) and the bound protein was eluted with a 0–500 m*M* imidazole gradient in 20 m*M* Tris–HCl pH 7.9 and 500 m*M* NaCl. Subsequently, the eluted sample was dialyzed with thrombin digestion buffer [20 m*M* Tris–HCl pH 8.0, 150 m*M* NaCl, 5%(*v*/*v*) glycerol, 0.5 m*M* β-mercaptoethanol]. 5 units of biotinylated thrombin (Novagen) were added per 10 mg of fusion protein and the sample was incubated overnight at 295 K. Thrombin was then removed using a streptavidin agarose column (Novagen). BldDN was further purified using a Superdex 200 HR 16/60 column (Amersham Pharmacia Biotech) pre-equilibrated with 20 m*M* Tris–HCl pH 8.0 containing 150 m*M* NaCl and then concentrated to approximately 10 mg ml^{-1} for crystallization trials.

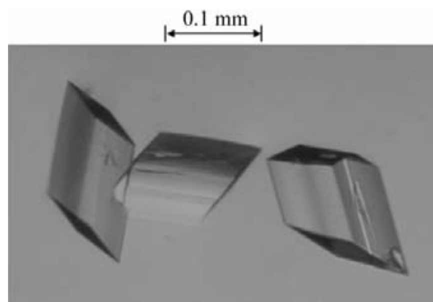

Figure 1
Crystals of DNA-binding domain of BldD from *S. coelicolor* A3(2).

2.2. Preparation of methionine-substituted BldDN mutant

The Quickchange site-directed mutagenesis kit (Stratagene) was used with a pET-15b-BldDN template and the following primer pairs (mutated sequences are in bold).

L13M-F 5′ CTCGGGGCCAAG**ATG**CGGGCCATCCGC 3′
L13M-R 5′ GCGGATGGCCCG**CAT**CTTGGCCCCGAG 3′
T18M-F 5′ CGGGCCATCCGC**ATG**CAGCAGGGCCTT 3′
T18M-R 5′ AAGGCCCTGCTG**CAT**GCGGATGGCCCG 3′
Q20M-F 5′ ATCCGCACCCAG**ATG**GGCCTTTCCCTC 3′
Q20M-R 3′ GAGGGAAAGGCC**CAT**CTGGGTGCGGAT 3′
Q32M-F 5′ GAGGAGAAGTCC**ATG**GGCCGCTGGAAG 3′
Q32M-R 5′ CTTCCAGCGGCC**CAT**GGACTTCTCCTC 3′
L69M-F 5′ GTGCAGGAGCTG**ATG**CCGGGCACCACC 3′
L69M-R 5′ GGTGGTGCCCGG**CAT**CAGCTCCTGCAC 3′

The mutations were confirmed by sequencing the purified plasmids. In the selenomethionine labelling of BldDN, the *E. coli met*$^-$ auxotrophic strain B834(DE3) (Novagen) was used as a host for plasmid transformation. Cells were grown to an OD_{600} of approximately 0.5 in M9 media containing 25 mg l^{-1} selenomethionine and 0.1 mg ml^{-1} ampicillin at 310 K and expression was induced by 1 m*M* isopropyl-β-D-thiogalactoside at 310 K. After 5 h, the cells were harvested. The selenomethionyl (SeMet) BldDN was purified by an identical procedure to that for the wild-type BldDN. The purified SeMet BldDN was concentrated to approximately 10 mg ml^{-1} for crystallization.

3. Crystallization

BldDN was crystallized by the hanging-drop vapour-diffusion method using Crystal Screen, a sparse-matrix screening kit (Hampton Research, USA). Droplets containing 1.5 µl each of protein and reservoir solution were equilibrated against 1 ml reservoir solution at 296 K. Several bundles of rod-shaped crystals were produced from the condition containing 0.2 *M* ammonium sulfate, 0.1 *M* sodium acetate pH 4.6 and 25%(*w*/*v*) polyethyleneglycol (PEG) 4000 in 1 d.

The replacement of PEG 4000 with PEG 2000 and the addition of 2-propanol greatly improved the quality of crystals. Droplets containing 1.5 µl of protein sample (10 mg ml^{-1}) and an equal volume of precipitant solution containing 1%(*v*/*v*) 2-propanol, 0.01 *M* ammonium sulfate, 0.1 *M* sodium acetate pH 4.5 and 26%(*w*/*v*) PEG 2000 were equilibrated against 1 ml of the same precipitant solution at 296 K and single crystals grew to maximum size in 1 d (Fig. 1). For data collection, crystals were frozen at 100 K after briefly being soaked in a cryoprotectant solution containing 20% glycerol in the same precipitant solution.

The Gln20Met mutant was crystallized using the same procedure as for the crystallization of native BldDN, with a precipitant solution containing 3%(*v*/*v*) 2-propanol, 0.1 *M* ammonium sulfate, 0.1 *M* sodium acetate pH 4.5 and 25%(*w*/*v*) PEG 2000.

Table 1
Crystal information and data-collection statistics.

Values in parentheses refer to the highest resolution shell (1.80−1.86 Å).

Source	6B, PLS
Wavelength (Å)	1.12714
Space group	*C*2
Unit-cell parameters (Å, °)	*a* = 77.2, *b* = 31.8, *c* = 33.6, β = 105.1
Resolution range (Å)	20.0–1.8
Total/unique reflections	17663/7049
Completeness (>1σ) (%)	96.7 (94.9)
R_{sym}† (%)	3.1 (7.1)
Mean $I/\sigma(I)$	29.7 (21.0)

† $R_{sym} = \sum |I_{obs} - I_{avg}| / \sum I_{obs}$.

4. X-ray analysis

A 1.8 Å data set was obtained using a MacScience 2030b imaging plate at beamline 6B at Pohang Light Source (PLS), South Korea. The diffraction quality in the highest shell of the data (Table 1) shows that the crystal diffracted to above 1.8 Å. However, owing to limits imposed by the experimental setup, we could only collect data to 1.8 Å. Diffraction data were processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The crystals belong to the monoclinic space group *C*2, with unit-cell parameters *a* = 77.2, *b* = 31.8, *c* = 33.6 Å, β = 105.1°. Using a calculated molecular weight of 8485 Da, we calculated the crystal volume per unit molecular weight (V_M) to be 2.26 Å^3 Da^{-1} with a solvent content of 43.6% by volume (Matthews, 1968) when the asymmetric unit is assumed to contain one molecule. The data-collection statistics are summarized in Table 1.

To determine the three-dimensional structure of BldDN using the MIR or MAD method, we tried unsuccessfully to obtain heavy-atom derivatives of BldDN crystals. During the soaking experiments with heavy-atom-containing solutions, almost all the BldDN crystals became cracked and the damaged crystals rapidly decayed when

exposed to X-rays. Thus, we were not able to collect full data from any of the derivatives.

As an alternative strategy, selenomethionine-substituted BldDN was prepared. Because BldDN contains no methionine residues except for the terminal methionine, several BldDN mutants into which another methionine residue had been introduced were designed in order to obtain Se-incorporated mutants suitable for MAD experiments. Taking the size and polarity of the side chains of amino acids into account, we randomly selected five residues, Leu13, Thr18, Gln20, Gln32 and Leu69, to be mutated to methionine. Among the mutants, the Leu13Met and Leu69Met mutants went into the inclusion bodies when overexpressed and the expression level of the Thr18Met and Gln32Met mutants was low. However, fortunately, the Gln20Met mutant was solubly overexpressed to a comparable level to the native protein. Thus, we obtained crystals of the selenomethionine-substituted Gln20Met mutant. Efforts toward structure determination using MAD with the mutant crystals are now in progress.

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