Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Crystallization and preliminary X-ray studies of SdiA from *Escherichia coli*

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Received 10 October 2007
Accepted 15 November 2007

SdiA enhances cell division by regulating the *fts*QAZ operon in *Escherichia coli* as a transcription activator. In addition, SdiA is suggested to play a role in detecting quorum signals that emanate from other species. It is therefore a homologue of LuxR, a cognate quorum-sensing receptor that recognizes a quorum signal and activates the quorum responses. To elucidate the role of SdiA and its functional and structural relationship to LuxR, structural studies were performed on *E. coli* SdiA. Recombinant SdiA was overexpressed, purified and crystallized at 287 K using the hanging-drop vapour-diffusion method. X-ray diffraction data from a native crystal were collected with 99.7% completeness to 2.7 Å resolution with an $R_{\rm merge}$ of 6.0%. The crystals belong to the hexagonal space group $P6_122$ or $P6_522$, with unit-cell parameters $a = b = 130.47$, $c = 125.23$ Å.

1. Introduction

Bacteria regulate their population within an environment using quorum-sensing phenomena in which the genes involved in cell-density dependent responses are regulated at the transcriptional level by the concentration of an autoinducer that is autonomously released from the cells (Lazdunski *et al.*, 2004; Miller & Bassler, 2001). There are two essential components of this system: an autoinducer synthase, which synthesizes a quorum signal, termed an 'autoinducer', and a quorum-sensing receptor for the autoinducer. Transcriptional activity is triggered in the autoinducer-bound state (Lazdunski *et al.*, 2004). LuxR and LuxI of *Vibrio fischeri* are representative proteins for the quorum-sensing receptor and autoinducer synthase, respectively. Numerous Gram-negative quorum-sensing bacteria typically possess proteins that are homologous to LuxR and LuxI (Nealson & Hastings, 1979). Acyl derivatives of L-homoserine lactone (AHLs) are widely used as a signalling molecule in the quorum-sensing system. A wide variety of AHLs sense and respond to their own cell population density, leading to the activation or repression of target genes involved in bacterial pathogenicity, for example biofilm development and stress resistance (Schauder & Bassler, 2001). The specificity of the interaction between the LuxR-type protein and its cognate AHL is essential for bacteria to distinguish between the AHLs produced by their own species and those produced by other species.

TraR, a plasmid conjugal transfer gene in the plant pathogen *Agrobacterium tumefaciens*, has been extensively studied as a model system for autoinducer-dependent transcriptional activators (Fuqua & Winans, 1996; Miller & Bassler, 2001). The molecular structure of dimeric TraR protein in complex with its cognate AHL and a DNA-promoter fragment containing the TraR-binding site have been fully described (Vannini *et al.*, 2002; Zhang *et al.*, 2002). TraR is composed of two structurally and functionally distinct domains that are separated by a linker. The N-terminal domain consists of a helix–sheet–helix sandwich in which the ligand is completely buried between the concave surfaces. The C-terminal domain of TraR is a four-helix bundle containing a helix–turn–helix DNA-binding motif (Vannini *et al.*, 2002; Zhang *et al.*, 2002).

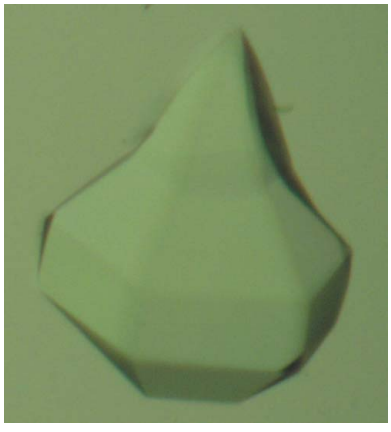

Table 1
X-ray data-collection and processing statistics.

Values in parentheses are for the last shell.

Space group	$P6_122$ or $P6_522$
Unit-cell parameters (Å)	$a = b = 130.47$, $c = 125.23$
Resolution (Å)	50.0–2.7 (2.80–2.70)
Unique reflections	17827 (1728)
Redundancy	10.1
Completeness (%)	99.7 (100)
$R_{\rm merge}$† (%)	6.0 (39.7)
Average $I/\sigma(I)$	38.5 (6.0)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \overline{I(hkl)}|/\sum_{hkl}\sum_i I_i(hkl)$.

Quorum-sensing circuits which resemble the LuxI–LuxR system have been identified in more than 25 species of Gram-negative bacteria (Miller & Bassler, 2001). However, quorum sensing in *Escherichia coli* has been poorly characterized. *E. coli* is not capable of synthesizing AHL molecules because it lacks a gene encoding AHL synthase. However, it has a LuxR homologue, SdiA, which was originally identified as a transcriptional activator of the *fts*QAZ operon that encodes a protein that is essential for cell division (Yamamoto *et al.*, 2001). Thus, it is expected that SdiA recognizes the signals of other bacterial species and shares structural homology to other LuxR proteins. However, the exact function of SdiA as a quorum-sensing regulator still remains unclear. Therefore, structural study of SdiA is essential to elucidate the exact role of SdiA either as a quorum receptor or as a transcription controller. In addition, further structural studies of the quorum-sensing receptors are required in order to fully understand this important signalling phenomenon, since only the structure of TraR is known in this family. To this end, we report the preliminary crystallographic analysis of SdiA from *E. coli*.

2. Materials and methods

2.1. Protein expression and purification

The gene encoding full-length SdiA protein (residues 1–240) was amplified by polymerase chain reaction (PCR) using *E. coli* K-12 genomic DNA as a template. The PCR product was digested with *Nde*I and *Xho*I and inserted into the pET22b vector (Novagen, USA) to generate pET22b-SdiA. As a result, six extra histidine residues were attached to the C-terminus of SdiA and were not removed for crystallization. This plasmid was transformed into competent *E. coli* strain BL21 (DE3) cells. The cells were grown to an OD_{600} of 0.6 in Luria–Bertani media containing 50 mg ml^{-1} ampicillin at 310 K and recombinant protein expression was induced by adding 0.1 m*M* isopropyl β-D-1-thiogalactopyranoside at 303 K. After 4 h induction, cells were harvested and resuspended in buffer *A* (25 m*M* Tris–HCl pH 8.0, 0.5 *M* NaCl, 10 m*M* imidazole, 0.5% PEG 3350) followed by sonication. The cell lysates were centrifuged using a Sorvall SS34 rotor at 30 000*g* for 1 h and the supernatant was loaded onto a HiTrap nickel-chelating column (GE Healthcare, USA) pre-equilibrated with buffer *A*. SdiA was eluted with a linear gradient of 0.01–0.5 *M* imidazole in buffer *A*. The fractions containing SdiA were buffer-exchanged using a PD-10 desalting column (GE Healthcare, USA) with buffer *B* (25 m*M* Tris–HCl pH 8.0, 0.15 *M* NaCl, 0.5% PEG 3350, 1 m*M* DTT) and subsequently applied onto a Hi-Trap Q column (GE Healthcare, USA) pre-equilibrated with buffer *B*. SdiA was eluted with a linear gradient of 0.15–0.5 *M* NaCl in buffer *B*. The fractions containing SdiA were pooled and dialyzed against buffer *C* (25 m*M* Tris–HCl pH 8.0, 0.3 *M* NaCl, 0.5% PEG 3350, 1 m*M* DTT) for crystallization. The purity of the protein was confirmed by SDS–PAGE.

2.2. Crystallization and data collection

Purified SdiA was concentrated to 4.6 mg ml^{-1} using a collodion membrane (Schleicher & Schuell, Germany). Initial crystallization screening was performed by the hanging-drop vapour-diffusion method using Crystal Screen I and II (Hampton Research, USA) and Cryo I and II kits (Emerald Biostructures, USA). Each hanging drop, consisting of 1 µl reservoir solution and 1 µl protein solution, was equilibrated against 0.5 ml reservoir solution at 287 K. Initially, hexagonal crystals were obtained within 1 d with a reservoir consisting of 30% PEG 600, 100 m*M* HEPES pH 7.2, 50 m*M* lithium sulfate and 10%(*v*/*v*) glycerol. However, since these crystals were small and not suitable for X-ray analysis, further optimization of the initial crystallization conditions was accomplished by changing various crystallization parameters. Initial X-ray diffraction experiments were performed using in-house Cu $K\alpha$ X-rays with an R-AXIS IV^{++} imaging plate (Rigaku, Japan) and synchrotron radiation with a MacScience 2030b area detector at beamline 6B of the Pohang Accelerator Laboratory, Republic of Korea. Complete diffraction data were collected on a MAR CCD 165 mm detector on beamline 41XU at the SPring-8 synchrotron, Japan, from a crystal that had been flash-cooled in a cold nitrogen-gas stream at 100 K. Prior to data collection, the crystal was immersed for more than 30 s in mother liquor with 25%(*v*/*v*) glycerol added as a cryoprotectant. The wavelength of the synchrotron radiation was 1.000 Å. The diffraction data were processed and scaled with *HKL*-2000 (Otwinowski & Minor, 1997).

3. Results and discussion

The gene encoding *E. coli* SdiA was cloned and the protein was overexpressed and purified for structural studies. The approximate yield was 12 mg homogenous protein from 1 l culture. The molecular weight of SdiA was about 29 kDa as judged from SDS–PAGE, which is in agreement with the calculated molecular weight of 28 117 Da

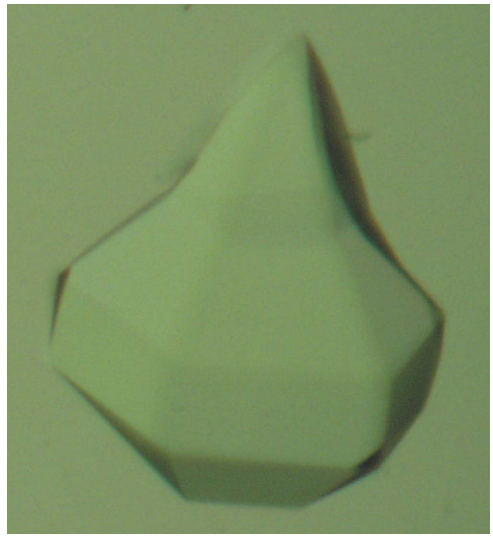

Figure 1
A crystal of *E.coli* SdiA grown using a solution containing 100 m*M* HEPES pH 7.2 and 200 m*M* lithium sulfate. The approximate dimensions of the crystal are 0.2 × 0.2 × 0.5 mm.

excluding the His tag. In the initial crystallization screen, SdiA crystals were obtained in the presence of 30% PEG 600. However, after optimizing the crystallization conditions, the crystals grew more reproducibly in the absence of PEG 600. Finally, diffraction-quality crystals were obtained using 100 m*M* HEPES pH 7.2 and 200 m*M* lithium sulfate. The crystals grew to final dimensions of 0.2 × 0.2 × 0.5 mm within 3 d (Fig. 1). X-ray diffraction data from a native crystal were collected with 99.7% completeness to 2.7 Å resolution with an $R_{\rm merge}$ of 6.0%. The crystal belonged to the hexagonal space group $P6_122$ or $P6_522$, with unit-cell parameters $a = b = 130.47$, $c = 125.23$ Å. The presence of four SdiA molecules in an asymmetric unit gave a calculated Matthews coefficient $V_{\rm M}$ of 2.74 Å^3 Da^{-1}, which corresponds to a solvent content of 55.04%. The $V_{\rm M}$ was within the range commonly observed for protein crystals (Matthews, 1968). The statistics of the synchrotron data collection and processing are summarized in Table 1. Since we were unsuccessful in solving the structure of SdiA by molecular replacement with TraR (PDB codes 1l3l and 1h0m), which has 20.7% sequence identity to SdiA, we will solve the structure of *E. coli* SdiA using multiple anomalous dispersion (MAD) methods.

This work was supported by the 21C Frontier Functional Proteomics Program (FPR06B2-120), the Ubiquitome Research Program (M10533010001-05N3301-00100) and the National Research Laboratory Program (NLR-2006-02287) of the Korea Ministry of Science and Technology.

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