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Crystallization and X-ray diffraction data collection of topoisomerase IV ParE subunit from *Xanthomonas oryzae* pv. *oryzae*

Topoisomerase IV is involved in topological changes in the bacterial genome using the free energy from ATP hydrolysis. Its functions are the decatenation of daughter chromosomes following replication by DNA relaxation and double-strand DNA breakage. In this study, the N-terminal fragment of the topoisomerase IV ParE subunit from *Xanthomonas oryzae* pv. *oryzae* was overexpressed in *Escherichia coli*, purified and crystallized. Diffraction data were collected to 2.15 Å resolution using a synchrotron-radiation source. The crystal belonged to space group $P4_22_12$, with unit-cell parameters $a = b = 105.30$, $c = 133.76$ Å. The asymmetric unit contains one molecule, with a corresponding V_M of $4.21 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 69.6%.

1. Introduction

Bacterial blight disease has become a major disease of rice in Asian countries in the last three decades (Ezuka & Kaku, 2000). *Xanthomonas oryzae* pv. *oryzae* is a Gram-negative rod-shaped bacterium that causes bacterial blight. Rice plants become wilted if infection occurs during the seedling or early tillering stages. When infection occurs in later stages, leaf blight lesions enlarge in length and width and gradually turn from greyish green to chlorotic (Mew, 1993). During infection, *X. oryzae* pv. *oryzae* employs diverse tools to overcome the innate defence system of the host and result in blight disease. Bacterial blight is a vascular disease in which *X. oryzae* pv. *oryzae* grows and colonizes the xylem vessels, eventually clogging them; several virulence-associated determinants have been found, including exopolysaccharide production, hypersensitive response and pathogenicity (*hrp*) genes (Cho *et al.*, 2008; Shen & Ronald, 2002). Although bacterial blight is the most important disease of rice from an economic point of view, there are currently no effective anti-bacterial agents against *X. oryzae* pv. *oryzae*.

Topoisomerase IV is a bacterial type II topoisomerase that modifies DNA topology during the replication process by unlinking DNA and facilitating chromosome segregation (Levine *et al.*, 1998). Topoisomerase IV utilizes the energy of ATP hydrolysis to relax a negative DNA supercoil (Zechiedrich & Cozzarelli, 1995). Topoisomerase IV, encoded by *parC* and *parE*, forms a C_2E_2 tetramer and the E subunit (ParE) contains the ATPase active site in its N-terminal domain (43 kDa), which is referred to as the ATP-operating clamp (Maxwell & Lawson, 2003). Upon ATP binding, this clamp closes by dimerization of the N-terminal domain, trapping the T-segment of the DNA. The C-terminal domain of ParE is involved in interactions with the ParC subunit and the DNA substrate. Topoisomerase IV is the target of two antibiotic families: the coumarins, such as novobiocin, and the quinolones, such as ciprofloxacin, sparfloxacin and grepafloxacin (Anderson *et al.*, 1998; Hardy & Cozzarelli, 2003). The coumarins are ATP-competitive inhibitors of the enzyme and the quinolones form a ternary complex with the enzyme in the presence of DNA. To date, crystal structures of the N-terminal domain of the ParE subunit from *Escherichia coli* have been described (Bellon *et al.*, 2004). The structures were strikingly similar to that of the known gyrase B subunit and the complex crystals showed the binding modes of the bound ADPNP or novobiocin (Brino *et al.*, 2000). In order to

provide further structural data regarding the catalytic mechanism of topoisomerase IV and to facilitate the discovery of effective anti-bacterial agents against *X. oryzae* pv. *oryzae*, we have crystallized and performed X-ray crystallographic experiments on the N-terminal domain of topoisomerase IV ParE subunit from *X. oryzae* pv. *oryzae*.

2. Materials and methods

2.1. Cloning, protein expression and purification

The gene encoding the N-terminal fragment (amino acids 45–433) of the ParE subunit was amplified from bacterial cells of *X. oryzae* pv. *oryzae* KACC10331 strain by polymerase chain reaction (PCR) using specific primers designed based on the published genome sequence (Lee *et al.*, 2005). The forward primer contained an *Nde*I restriction site (bold) and had the sequence 5'-CCC CCC **CAT ATG** AAC CGT TAT AAC GCC-3', while the reverse primer contained a *Bam*HI site (bold) and had the sequence 5'-CCC CCC **GGA TCC** TCA CTT CTT GCG GAC GAT CTG CT-3'. The PCR product was then subcloned between the *Nde*I and *Bam*HI sites of pET-15b vector (Novagen, USA). This construct contains a hexahistidine tag (MGSSHHHHH-HSSGLVPRGSH) at the N-terminus for purification purposes. The ParE/pET-15b plasmid was transformed into *E. coli* BL21 (DE3) strain (Novagen) and the cells were grown in a shaking incubator at 310 K in LB broth medium supplemented with 50 µg ml⁻¹ ampicillin. Protein expression was induced by the addition of 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) when the cells reached an optical density at 600 nm of about 0.6 and cells were cultured at the same temperature for an additional 4 h. Cultured cells were harvested by centrifugation at 3000g for 30 min at 277 K. The cell pellet was resuspended in binding buffer (20 mM Tris pH 8.0, 100 mM NaCl and 20 mM imidazole) and disrupted by sonication at 277 K. The crude lysate was centrifuged at 25 000g for 1 h at 277 K. The supernatant was then loaded onto an Ni²⁺-chelated HisTrap FF crude column (GE Healthcare, USA) which had been pre-equilibrated with binding buffer. The protein was eluted with elution buffer (20 mM Tris pH 8.0, 100 mM NaCl and 400 mM imidazole). The protein was subsequently loaded onto a HiTrap Q HP column (GE Healthcare, USA) pre-equilibrated with binding buffer and eluted with a linear gradient to a buffer containing 20 mM Tris pH 8.0 and 1.0 M NaCl. The eluted protein was concentrated and finally purified by gel-filtration chromatography on a HiTrap 26/60 Sephacryl S-200 HR column (GE Healthcare, USA) which had been pre-equilibrated with buffer containing 20 mM Tris pH 8.0 and 100 mM NaCl. The hexa-

Figure 1
Crystal of the N-terminal fragment of the ParE subunit from *X. oryzae* pv. *oryzae* grown in 0.1 M Na HEPES pH 7.6, 2% PEG 400 and 1.8 M ammonium sulfate.

Table 1

Data-collection statistics.

Values in parentheses are for the last resolution shell.

Synchrotron source	PLS beamline 4A
Wavelength (Å)	1.00000
Resolution range (Å)	50.0–2.15 (2.25–2.15)
Space group	<i>P</i> 4 ₂ 2 ₁ 2
Unit-cell parameters (Å)	
<i>a</i> = <i>b</i>	105.30
<i>c</i>	133.76
Total No. of reflections	494677
No. of unique reflections	43413
Redundancy	11.4 (9.1)
Completeness (%)	99.9 (100.0)
Molecules per ASU	1
<i>V</i> _M (Å ³ Da ⁻¹)	4.21
Solvent content (%)	69.6
Average <i>I</i> /σ(<i>I</i>)	28.5 (5.2)
<i>R</i> _{merge} [†] (%)	8.9 (42.0)

[†] $R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}$, where $I_i(hkl)$ is the intensity of an individual reflection hkl and $\langle I(hkl) \rangle$ is the average intensity of reflection hkl .

histidine tag was not removed during protein purification even though the construct contains a thrombin cleavage site. The purified protein was concentrated to 25 mg ml⁻¹ in the gel-filtration buffer; its purity was examined by 12% SDS-PAGE and determined to be >95%.

2.2. Crystallization and data collection

Crystallization of the concentrated protein was initiated by crystal screening at 293 K with a Hydra II e-drop automated pipetting system (Matrix Technologies Ltd, UK) using 96-well sitting-drop Intelli-Plates (Art Robbins Instruments, USA) with a ratio of 400 nl protein solution to 400 nl well solution equilibrated over 70 µl well solution. Commercial screening kits from Hampton Research were used for the preliminary screening. Initial crystals were obtained using the condition 0.1 M Na HEPES pH 7.5, 2% PEG 400 and 2.0 M ammonium sulfate. The crystallization conditions were further optimized by the hanging-drop vapour-diffusion method using 24-well VDX plates (Hampton Research, USA) at 293 K. The drops used in the optimized crystallization conditions were prepared by mixing 1.0 µl protein solution with 1.0 µl reservoir solution (0.1 M Na HEPES pH 7.6, 2% PEG 400 and 1.8 M ammonium sulfate). Each hanging drop was positioned over 500 µl reservoir solution. Suitably-sized crystals were obtained within 3 d (Fig. 1); they were cryoprotected by soaking them for 5 s in cryoprotectant solution containing 0.1 M Na HEPES pH 7.6, 2% PEG 400, 1.8 M ammonium sulfate and 20% (v/v) glycerol and were flash-frozen in liquid nitrogen. They were then mounted on the goniometer in a stream of cold nitrogen at 100 K. X-ray diffraction data were collected from a cooled crystal using an ADSC Quantum CCD 210 detector on beamline 4A at Pohang Light Source (PLS), South Korea. A total range of 180° was covered using 1.0° oscillations and 10 s exposure per frame. The wavelength of the synchrotron X-ray beam was 1.00000 Å and the crystal-to-detector distance was set to 170 mm. X-ray diffraction data were collected to 2.15 Å resolution. Data were indexed, integrated and scaled using the *HKL-2000* software package (Otwinowski & Minor, 1997).

3. Results and discussion

The N-terminal fragment (amino acids 45–433) of the ParE subunit from *X. oryzae* pv. *oryzae* was cloned, expressed, purified and crystallized for structural study. The X-ray diffraction data from the

crystal indicated that it belonged to space group $P4_22_12$ on the basis of systematic absences, with unit-cell parameters $a = b = 105.30$, $c = 133.76$ Å. Data-collection statistics are provided in Table 1. Based on the result of gel filtration, the molecular weight of this protein was approximately 90 kDa, corresponding to a dimer. However, it was difficult to determine the number of protein molecules in the asymmetric unit; one molecule yielded a Matthews coefficient (V_M) of $4.21 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 69.6%, while two molecules yielded a V_M of $2.11 \text{ Å}^3 \text{ Da}^{-1}$ and a solvent content of 39.4% (Matthews, 1968). The structure solution obtained by the molecular-replacement method using the *CNS* package (Brünger *et al.*, 1998) with the crystal structure of *E. coli* ParE (PDB code 1s16) as a model made it clear that the asymmetric unit of the crystal contained one protein molecule. The best molecular-replacement solution was obtained using one monomer of the dimeric structure of *E. coli* ParE as a template, giving an R factor of 39.4% for data in the resolution range 15–3.0 Å. Examination of the best molecular-replacement solution structure showed good crystal packing and a similar dimeric interface as observed in the dimeric structure of *E. coli* ParE was observed between symmetry-related molecules in the crystal packing. This final model is currently being refined and structural details will be described in a separate paper.

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