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Preliminary X-ray crystallographic analysis of the breakage–reunion domain of the GyrA subunit of DNA gyrase from *Colwellia psychrerythraea* strain 34H

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DNA gyrase is a type II topoisomerase that is essential for chromosome segregation and cell division owing to its ability to modify the topological forms of bacterial DNA. In this study, the N-terminal breakage–reunion domain of the GyrA subunit of DNA gyrase from *Colwellia psychrerythraea* 34H was overexpressed in *Escherichia coli*, purified and crystallized. Diffraction data were collected to 2.60 Å resolution using a synchrotron-radiation source. The crystal belonged to space group $P2_12_12_1$, with unit-cell parameters $a = 98.98$, $b = 101.56$, $c = 141.83$ Å. The asymmetric unit contained two molecules, with a corresponding V_M of 3.18 Å^3 Da^{-1} and a solvent content of 59.9%.

1. Introduction

Colwellia psychrerythraea strain 34H is an obligate psychrophile that is found in continually cold marine environments, including Arctic and Antarctic sea ice (Huston *et al.*, 2000). This organism possesses a variety of adaptations to low temperatures, as recently shown by comparative genome analysis. It is generally accepted that the molecular determinants for the cold-adaptation of the enzymes of this organism are an extensive increase in flexibility in certain regions of their structures (Methé *et al.*, 2005). This increased flexibility would enhance the accommodation and transformation of their substrates and thereby allows the psychrophilic enzymes to be active at low temperatures.

DNA gyrase is a bacterial type II topoisomerase that introduces supercoils into DNA. It catalyses the breakage of the G-segment DNA, the passage of the T-segment DNA through the break in the G-segment and finally the reunification of the broken G-segment. This activity involves the opening and closing of a series of molecular 'gates' which is coupled to ATP hydrolysis (Reece & Maxwell, 1991). DNA gyrase, encoded by *gyrA* and *gyrB*, forms an A_2B_2 tetramer. The B subunit (GyrB) contains the ATPase active site in its N-terminal domain (43 kDa), which is referred to as the ATP-operated 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 T-segment is then passed through a transient break in the G-segment opened by the N-terminal breakage–reunion domain of the GyrA subunit, the G-segment is resealed and the T-segment is released through a protein gate prior to resetting of the enzyme to the open-clamp form. DNA gyrase 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, the crystal structures of the N-terminal breakage–reunion domains of the GyrA subunits of the mesophilic bacteria *Escherichia coli* and *Mycobacterium tuberculosis* have been described (Cabral *et al.*, 1997; Tretter *et al.*, 2010; Piton *et al.*, 2009). However, the structure of the

breakage–reunion domain of the GyrA subunit of a psychrophilic bacterium has not yet been studied.

To provide further structural data with regard to the catalytic mechanism of DNA gyrase and to improve our understanding of the molecular properties leading to cold-adaptation of the psychrophilic enzyme, we have crystallized and performed X-ray crystallographic experiments on the N-terminal breakage–reunion domain of the GyrA subunit of DNA gyrase from *C. psychrerythraea* strain 34H.

2. Materials and methods

2.1. Cloning, protein expression and purification

The gene encoding the N-terminal fragment (amino acids 32–522) of the GyrA subunit was amplified from *C. psychrerythraea* strain 34H genomic DNA by the polymerase chain reaction (PCR) using specific primers designed based on the published genome sequence (Methé *et al.*, 2005). The forward primer contained an *Nde*I restriction site (bold) and had the sequence 5′-GCC **CAT ATG** CGC GCA TTG CCT GAC GTT-3′, while the reverse primer contained a *Xho*I restriction site (bold) and had the sequence 5′-GC **CTC GAG** AGT AAT TTC AGT TCT ACG TTC ATC AC-3′. The PCR product was then subcloned between the *Nde*I and *Xho*I sites of a pET-22b vector (Novagen, USA). This construct contains an additional methionine residue at the N-terminus and a hexahistidine tag (LEHHHHHH) at the C-terminus for purification purposes. The GyrA/pET-22b plasmid was transformed into *Escherichia 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 adding 0.5 m*M* isopropyl *β*-D-1-thiogalactopyranoside (IPTG) when the cells reached an optical density at 600 nm of about 0.6 and the cells were cultured at the same temperature for an additional 4 h. Cultured cells were harvested by centrifugation at 3000*g* for 30 min at 277 K. The cell pellet was resuspended in binding buffer (20 m*M* Tris pH 8.0, 100 m*M* NaCl and 20 m*M* imidazole) and disrupted by sonication at 277 K. The crude lysate was centrifuged at 25 000*g* for 1 h at 277 K. The supernatant was then loaded onto a Ni^{2+}-chelating HisTrap FF crude column (GE Healthcare, USA) which had been pre-equilibrated with binding buffer. The protein was eluted with elution buffer (20 m*M* Tris pH 8.0, 100 m*M* NaCl and 400 m*M* imidazole). The eluted protein was concentrated and further purified by gel-filtration chromatography on a Superdex 200 16/60 column (GE Healthcare, USA) pre-equilibrated with buffer consisting of 20 m*M* Tris pH 8.0 and 100 m*M* NaCl. The purified protein was concentrated to 10 mg ml^{-1} in the gel-filtration buffer and the purity of the protein was examined by 12% SDS–PAGE and determined to be >95%.

2.2. Crystallization and data collection

Crystallization of the protein was initiated by crystal screening at 293 K using a Hydra II e-drop automated pipetting system (Matrix Technologies Ltd, UK) and 96-well sitting-drop Intelli-Plates (Art Robbins Instruments, USA); the drops contained 400 nl protein solution and 400 nl well solution and were equilibrated against 70 µl well solution. Commercial screening kits from Hampton Research (Crystal Screens 1 and 2, Index, SaltRx and Grid Screens) were used in the preliminary screening. Initial crystals were obtained under the conditions 0.1 *M* trisodium citrate pH 5.0, 1.6 *M* ammonium sulfate. The crystallization conditions were further optimized using the hanging-drop vapour-diffusion method in 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* trisodium citrate pH 5.6, 1.8 *M* ammonium sulfate). Each hanging drop was equilibrated over 500 µl reservoir solution. Suitable-sized crystals (Fig. 1) were obtained within 5 d; they were cryoprotected by soaking them for 3 s in cryoprotectant solution containing 0.1 *M* trisodium citrate pH 5.6, 1.8 *M* ammonium sulfate and 25%(*v*/*v*) glycerol and flash-frozen in liquid nitrogen. Frozen crystals were 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 6B at Pohang Light Source (PLS), Republic of Korea. A total rotation range of 180° was covered with 1.0° oscillations and 10 s exposure per frame. The wavelength of the synchrotron X-ray beam was 1.00 Å and the crystal-to-detector distance was set to 200 mm. X-ray diffraction data were collected to 2.60 Å resolution (Fig. 2). Data were indexed, integrated, scaled and merged using the *HKL*-2000 software package (Otwinowski & Minor, 1997). The crystal structure was solved by the molecular-replacement (MR) method using the *CNS* package (Brünger *et al.*, 1998) with the

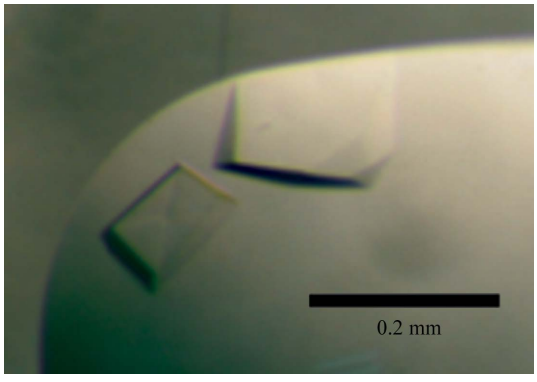

Figure 1
Crystals of the N-terminal domain of the GyrA subunit from *C. psychrerythraea* strain 34H grown in 0.1 *M* trisodium citrate pH 5.6, 1.8 *M* ammonium sulfate.

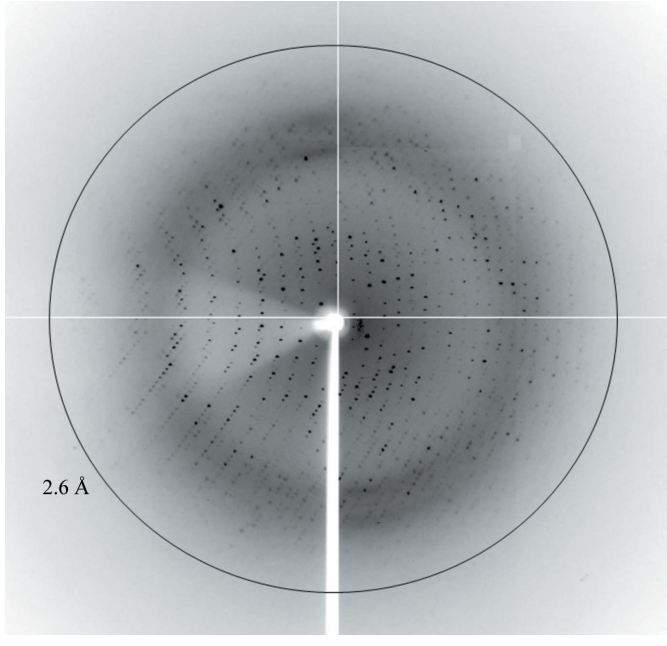

Figure 2
Typical diffraction image of a crystal of the N-terminal domain of GyrA. The resolution limit (2.6 Å) is indicated by a circle.

Table 1
Data-collection statistics.

Values in parentheses are for the last resolution shell.

Synchrotron	PLS, beamline 6B
Wavelength (Å)	1.00
Resolution range (Å)	50.0–2.60 (2.64–2.60)
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	
a	98.98
b	101.56
c	141.83
No. of unique reflections	43499
Redundancy	4.7 (5.0)
Completeness (%)	98.5 (99.7)
Molecules per asymmetric unit	2
V_M (Å^3 Da^{-1})	3.18
Solvent content (%)	59.9
Average $I/\sigma(I)$	28.1 (3.7)
R_{merge}† (%)	7.8 (42.9)

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

monomer structure of *E. coli* gyrase A (PDB code 1ab4; Cabral *et al.*, 1997) as the template.

3. Results and discussion

The N-terminal fragment (amino acids 32–522) of the GyrA subunit from *C. psychrerythraea* strain 34H was cloned, expressed, purified and crystallized for structural studies. X-ray diffraction data from the crystal indicated that it belonged to space group $P2_12_12_1$ on the basis of systematic absences, with unit-cell parameters $a = 98.98$, $b = 101.56$, $c = 141.83$ Å. Data-collection statistics are provided in Table 1. According to Matthews coefficient calculations, the crystallographic structure contains two GyrA molecules in the asymmetric unit with a V_M of 3.18 Å^3 Da^{-1} and a solvent content of 59.9% (Matthews, 1968). The best MR solution corresponded to two protein molecules in the asymmetric unit and gave an R_{work} of 42.4% (R_{free} = 43.2%) for data in the resolution range 20–3.5 Å. The other solutions had *R* factors of over 50%. The dimer interface of the best MR solution was similar to those of previously reported GyrA structures, implying that this solution is a model of the biological dimer. This final model is currently being refined and the structural details will be described in a separate paper.

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