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Overexpression, purification, crystallization and preliminary X-ray crystallographic analysis of the C-terminal domain of the GyrA subunit of DNA gyrase from *Staphylococcus aureus* strain Mu50

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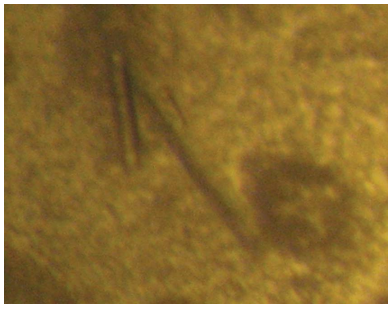
DNA gyrase is a type II topoisomerase that is essential for chromosome segregation and cell division owing to its ability to modify the topological form of bacterial DNA. In this study, the C-terminal domain of the GyrA subunit of DNA gyrase from *Staphylococcus aureus* Mu50 strain was overexpressed in *Escherichia coli*, purified and crystallized. Diffraction data were collected to 2.80 Å resolution using a synchrotron-radiation source. The crystal belonged to space group $P2_1$, with unit-cell parameters a = 37.28, b = 80.19, c = 50.22 Å, β = 110.64°. The asymmetric unit contained one molecule, with a corresponding V_M of 2.02 Å^3 Da^{-1} and a solvent content of 39.2%.

1. Introduction

DNA gyrase, which is a tetrameric enzyme of A_2B_2 form encoded by *gyrA* and *gyrB*, is a bacterial type II topoisomerase that introduces supercoils into DNA. Most type II topoisomerases perform both decatenation and relaxation; however, DNA gyrase is the only type II topoisomerase that is capable of actively introducing negative supercoils into DNA and this unique supercoiling capability makes DNA gyrase particularly effective for removing positive supercoils generated during replication elongation (Miller & Simons, 1993; Khodursky *et al.*, 2000; Hiasa & Marians, 1996). Another type II topoisomerase, Topo IV, does not possess supercoiling activity and is normally localized behind the fork to serve as a decatenating enzyme for disentangling the interlinked daughter chromosomes. The C-terminal domain of the GyrA subunit (GyrA-CTD) exhibits chiral DNA-wrapping activity and plays an essential role in defining the functional uniqueness of DNA gyrase. Deletion of GyrA-CTD abolishes the ability of gyrase to catalyze the negative-supercoiling reaction and converts DNA gyrase into a conventional type II topoisomerase which simply performs relaxation and decatenation (Kampranis & Maxwell, 1996). Moreover, GyrA-CTD harbours a positively charged and gyrase-specific 'GyrA-box' motif that is required for both the DNA-wrapping and the supercoiling activities of DNA gyrase (Kramlinger & Hiasa, 2006). To date, structures have been determined for GyrA-CTD from *Borrelia burgdorferi*, *Escherichia coli* and *Xanthomonas campestris*, which show sequence identities of 27, 42 and 38% to *S. aureus* GyrA-CTD, respectively (Corbett *et al.*, 2004; Ruthenburg *et al.*, 2005; Hsieh *et al.*, 2010). In these structures the GyrA-CTDs all adopt a six-bladed 'β-pinwheel' fold. However, there are structural differences in the shape of the six-bladed 'β-pinwheel'. *E. coli* and *X. campestris* GyrA-CTD adopt a spiral pinwheel, whereas *B. burgdorferi* GyrA-CTD adopts a flat pinwheel. Such differences reveal an unexpected richness of diversity in the mechanism of DNA-strand management by gyrase.

To provide further structural data with regard to the crucial role of GyrA-CTD in controlling the directionality of supercoiling and to investigate the structural differences from other GyrA-CTDs, we



have crystallized and performed X-ray crystallographic experiments on GyrA-CTD from *Staphylococcus aureus* strain Mu50.

2. Materials and methods

2.1. Cloning, protein expression and purification

The gene encoding the C-terminal domain (amino acids 504–812) of the GyrA subunit was amplified from genomic DNA of *S. aureus* strain Mu50 by the polymerase chain reaction (PCR) using specific primers. The forward primer contained an *Nde*I restriction site (bold) and had the sequence 5′-CCC **CAT ATG** GAA GAA CAA ATT GTA ATT ACA CTA AGC-3′, while the reverse primer contained a *Xho*I site (bold) and had the sequence 5′-CCC **CTC GAG** TTC TTT TAC TTT AGC AAC CGT T-3′. The PCR product was then subcloned between the *Nde*I and *Xho*I sites of the pET-22b vector (Novagen, USA). This construct contains an additional hexahistidine tag (LEHHHHHH) at the C-terminus for purification purposes. The recombinant 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 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. The 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, 10% glycerol, 200 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 an 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, 10% glycerol, 200 m*M* NaCl and 400 m*M* imidazole). The eluted protein was concentrated and purified further by size-exclusion chromatography on a Superdex 200 16/60 column (GE Healthcare, USA) which had been pre-equilibrated with buffer consisting of 20 m*M* Tris pH 8.0, 10% glycerol and 200 m*M* NaCl. The purified protein was concentrated to 10 mg ml^{-1} in size-exclusion buffer and the purity of the protein was examined by 12% SDS–PAGE and determined to be >95% pure.

2.2. Crystallization and data collection

Crystallization of the protein was initiated by crystal screening at 293 K by the hanging-drop vapour-diffusion method using 24-well VDX plates (Hampton Research, USA): drops consisting of 1 µl protein solution in size-exclusion buffer and 1 µl well solution were equilibrated against 500 µl well solution. Commercial screening kits from Hampton Research and Emerald BioSystems (Crystal Screen, Crystal Screen 2, Index, SaltRx and Wizard I and II) were used for preliminary screening. Needle-shaped microcrystals were obtained under the following condition: 0.1 *M* bis-tris pH 5.5, 0.2 *M* magnesium chloride and 25% PEG 3350 at 293 K. The crystallization condition was further optimized to obtain better crystals and the drops used in the optimized crystallization condition were prepared by mixing 1.0 µl protein solution with 1.0 µl reservoir solution (0.1 *M* bis-tris pH 6.2, 0.2 *M* magnesium chloride and 22% PEG 3350 at 293 K). Each hanging drop was equilibrated against 500 µl reservoir solution. Suitable-sized crystals were obtained within 5 d (Fig. 1); they were cryoprotected by soaking them for 3 s in a cryoprotectant solution consisting of 0.1 *M* bis-tris pH 5.5, 0.2 *M* magnesium chloride, 25% PEG 3350 and 25%(*v*/*v*) ethylene glycol and were 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 frozen crystal using an ADSC Quantum CCD 210 detector on beamline 6C at Pohang Light Source (PLS), Republic of Korea. A total rotation range of 180° was covered with 1.0° oscillations and 20 s exposure per frame. The wavelength of the synchrotron X-ray beam was 1.20 Å and the crystal-to-detector distance was set to 180 mm. X-ray diffraction data were collected to 2.80 Å resolution. 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 method with the *CNS* package (Brünger *et al.*, 1998) using the structure of *E. coli* GyrA-CTD (PDB code 1zi0; Ruthenburg *et al*, 2005) as the search model.

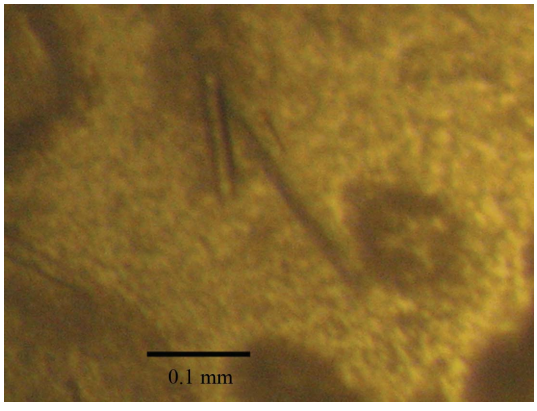


Figure 1
A crystal of the C-terminal domain of GyrA from *S. aureus* strain Mu50 grown using 0.1 *M* bis-tris pH 6.2, 0.2 *M* magnesium chloride and 22% PEG 3350.

Table 1
Data-collection statistics.

Values in parentheses are for the last resolution shell.

Synchrotron	PLS beamline 6C
Wavelength (Å)	1.20
Resolution range (Å)	50.0–2.80 (2.90–2.80)
Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 37.28, b = 80.19, c = 50.22, β = 110.64
No. of unique reflections	6603
Multiplicity	3.3 (2.7)
Completeness (%)	97.4 (83.8)
Molecules per asymmetric unit	1
V_M (Å^3 Da^{-1})	2.02
Solvent content (%)	39.2
Average $I/\sigma(I)$	13.1 (2.0)
R_{merge}† (%)	8.7 (39.5)

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

3. Results and discussion

The C-terminal domain (amino acids 504–812) of GyrA from *S. aureus* strain Mu50 was cloned, overexpressed, purified and crystallized for structural studies. The X-ray diffraction data from the crystal indicated that it belonged to space group $P2_1$, with unit-cell parameters a = 37.28, b = 80.19, c = 50.22 Å, β = 66.54°. Data-collection statistics are provided in Table 1. The Matthews coefficient suggests the presence of one molecule in the crystallographic asymmetric unit, with a V_M of 2.02 Å^3 Da^{-1} and a solvent content of 39.2% (Matthews, 1968). The best molecular-replacement solution showed good crystal packing, giving R_{work} = 46.9% (R_{free} = 47.2%) for data in

the resolution range 20–3.5 Å. The other solutions showed *R* factors of over 53%. The final model is currently being refined.

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References

Brünger, A. T., Adams, P. D., Clore, G. M., DeLano, W. L., Gros, P., Grosse-Kunstleve, R. W., Jiang, J.-S., Kuszewski, J., Nilges, M., Pannu, N. S., Read, R. J., Rice, L. M., Simonson, T. & Warren, G. L. (1998). *Acta Cryst.* D**54**, 905–921.

Corbett, K. D., Shultzaberger, R. K. & Berger, J. M. (2004). *Proc. Natl Acad. Sci. USA*, **101**, 7293–7298.

Hiasa, H. & Marians, K. J. (1996). *J. Biol. Chem.* **271**, 21529–21535.

Hsieh, T.-J., Yen, T.-J., Lin, T.-S., Chang, H.-T., Huang, S.-Y., Hsu, C.-H., Farh, L. & Chan, N.-L. (2010). *Nucleic Acids Res.* **38**, 4173–4181.

Kampranis, S. C. & Maxwell, A. (1996). *Proc. Natl Acad. Sci. USA*, **93**, 14416–14421.

Khodursky, A. B., Peter, B. J., Schmid, M. B., DeRisi, J., Botstein, D., Brown, P. O. & Cozzarelli, N. R. (2000). *Proc. Natl Acad. Sci. USA*, **97**, 9419–9424.

Kramlinger, V. M. & Hiasa, H. (2006). *J. Biol. Chem.* **281**, 3738–3742.

Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.

Miller, W. G. & Simons, R. W. (1993). *Mol. Microbiol.* **10**, 675–684.

Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.

Ruthenburg, A. J., Graybosch, D. M., Huetsch, J. C. & Verdine, G. L. (2005). *J. Biol. Chem.* **280**, 26177–26184.