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Crystallization and preliminary X-ray analysis of a complex formed between the antibiotic simocyclinone D8 and the DNA breakage–reunion domain of *Escherichia coli* DNA gyrase

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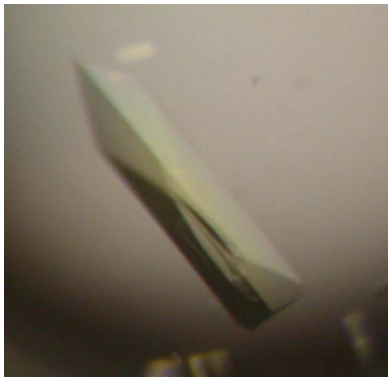
Crystals of a complex formed between the 59 kDa N-terminal fragment of the *Escherichia coli* DNA gyrase A subunit (also known as the breakage–reunion domain) and the antibiotic simocyclinone D8 were grown by vapour diffusion. The complex crystallized with *I*-centred orthorhombic symmetry and X-ray data were recorded to a resolution of 2.75 Å from a single crystal at the synchrotron. DNA gyrase is an essential bacterial enzyme and thus represents an attractive target for drug development.

1. Introduction

The essential bacterial enzyme DNA gyrase is responsible for maintaining the topological state of DNA within the cell (Schoeffler & Berger, 2008; Nollmann *et al.*, 2007). It functions as an A_2B_2 heterotetramer and its complex catalytic cycle presents a number of opportunities for intervention by inhibitors (Maxwell, 1997; Maxwell & Lawson, 2003). The aminocoumarin antibiotics, which are natural products of streptomycetes and are so named on account of the 3-amino-4,7-dihydroxycoumarin moiety that they all possess, are potent inhibitors of the enzyme. The three most studied compounds, novobiocin, clorobiocin and coumermycin A_1, are competitive inhibitors of the ATPase activity of the enzyme, which resides in the B subunit, and their mode of action at the molecular level is well understood (Maxwell & Lawson, 2003). In contrast, the recently discovered simocyclinones (Schimana *et al.*, 2000) interfere with DNA binding to the A subunit by a mechanism that has yet to be elucidated (Flatman *et al.*, 2005). We have previously established that simocyclinone D8 (SD8) binds to the A subunit of *Escherichia coli* DNA gyrase with a binding constant of 50–100 n*M* in a ratio of one inhibitor molecule per subunit (Flatman *et al.*, 2005). Here, we report the crystallization and preliminary X-ray analysis of the complex formed between the inhibitor and the N-terminal 59 kDa fragment of the *E. coli* DNA gyrase A subunit (also known as the breakage–reunion domain). The structure will reveal the mode of action of simocyclinone D8 and may provide new insights into antibacterial drug development.



2. Materials and methods

2.1. Protein expression, purification and crystallization

The N-terminal 59 kDa fragment (with an exact molecular weight of 58 543 Da) of *E. coli* DNA gyrase subunit A (GyrA59), comprising residues 2–523 of the 875-amino-acid wild-type sequence (UniProtKB/Swiss-Prot entry P0AES4), was produced using a modification of the previously published protocol (Reece & Maxwell, 1991). Expression plasmid pRJR10.18 (Reece & Maxwell, 1991) was transformed into *E. coli* strain B834 (DE3) (pLysS) and a 12 ml overnight culture of the cells was used to inoculate each 1 l culture of Luria–Bertani medium containing 100 mg ampicillin and 30 mg chloramphenicol. The cells were grown at 310 K to an $OD_{600\,nm}$ of around 0.4. Protein expression was induced by the addition of

isopropyl β-D-1-thiogalactopyranoside to a final concentration of 0.2 m*M* and the culture was left shaking for 4 h at 310 K. The harvested cells were resuspended in 50 m*M* Tris–HCl pH 7.5, 10%(*w*/*v*) sucrose containing a Complete EDTA-free protease-inhibitor cocktail tablet (Roche) and then lysed by freeze–thawing the cells. The cell debris was removed by centrifugation at 84 000*g* for 60 min.

The sample was subsequently purified with an ÄKTA FPLC system (Pharmacia) using a four-column procedure. The sample was maintained at 277 K throughout and fractions that contained GyrA59 were identified using SDS–PAGE. For steps (i)–(iii) the pooled fractions were dialysed using SnakeSkin dialysis tubing (Thermo Scientific), whilst for the final step the purified sample was dialysed using Slide-A-Lyzer MINI dialysis units (Thermo Scientific).

(i) The supernatant from the cell lysate was applied onto a heparin Sepharose 16/10 column (GE Healthcare) and pre-equilibrated with buffer *A* (50 m*M* Tris–HCl pH 7.5, 1 m*M* EDTA, 2 m*M* DTT) containing 10%(*w*/*v*) glycerol. The protein was eluted from the column with an increasing (0.0–1.0 *M*) NaCl gradient in the same buffer. Fractions containing GyrA59 were pooled and dialysed into buffer *A*.

(ii) Ammonium sulfate was added to the protein to a final concentration of 1 *M* before applying the sample onto a phenyl Sepharose 16/10 column (GE Healthcare) pre-equilibrated with buffer *A* containing 1 *M* ammonium sulfate. The protein was eluted from the column with a decreasing (1.0–0.0 *M*) ammonium sulfate gradient in the same buffer. Fractions containing GyrA59 were pooled and dialysed into buffer *A*.

(iii) The protein was applied onto a Mono Q 10/10 column (GE Healthcare) pre-equilibrated with buffer *A*. The protein was eluted with an increasing (0.12–1.00 *M*) NaCl gradient in the same buffer. Fractions containing GyrA59 were pooled and dialysed into buffer *A* containing 10%(*w*/*v*) glycerol, 5 m*M* KCl.

(iv) The protein was applied onto a HiLoad Superdex 200 26/60 column pre-equilibrated with buffer *A* containing 10%(*w*/*v*) glycerol, 5 m*M* KCl and then eluted in the same buffer. Fractions containing GyrA59 were pooled and subsequently dialysed overnight against buffer *B* (20 m*M* Tris–HCl pH 7.5, 20 m*M* NaCl, 20 m*M* KCl, 20 m*M* $MgCl_2$) before concentration to approximately 5–10 mg ml^{-1} using a Vivaspin 6 30 kDa cutoff concentrator (Vivascience).

Dynamic light scattering (DLS) was used to monitor the solution properties of the purified sample. For this purpose, approximately 30 µl protein solution was centrifuged through a 0.1 µm Ultrafree filter (Millipore) to remove particulate material before introduction into a 12 µl microsampling cell. The latter was then inserted into a DynaPro-MSTC molecular-sizing instrument at 293 K (Protein Solutions Inc.). A minimum of 15 scattering measurements were taken and the resulting data were analysed using the *DYNAMICS* software package (Protein Solutions Inc.).

An ∼10 m*M* stock solution of SD8 (molecular weight 932.3 Da) was prepared by dissolving lyophilized powder in 100% DMSO to give an intensely yellow solution. This was added to purified protein solution to give a final ligand concentration of 250 µ*M* with approximately 2.5%(*v*/*v*) DMSO in buffer *B*. The ratio of ligand to protein subunits was in the range 1.5:1 to 3:1. The mixture was then filtered through a 0.1 µm Ultrafree centrifugal filter (Millipore).

Crystallization trials were carried out by vapour diffusion in a sitting-drop format with 96-well MRC plates (Molecular Dimensions) using a variety of in-house and commercially available screens (Hampton Research, Molecular Dimensions and Qiagen) at a constant temperature of 293 K. Drops consisted of 1 µl protein solution mixed with 1 µl precipitant solution and the reservoir volume was 50 µl; the protein concentration was approximately 10 mg ml^{-1}. Improved crystals were subsequently obtained by refining the successful conditions in a hanging-drop format using 24-well VDX plates (Molecular Dimensions) over a reservoir volume of 1 ml.

2.2. X-ray data collection

Crystals were mounted for X-ray data collection using CryoLoops (Hampton Research) and were then flash-cooled by plunging them into liquid nitrogen and stored prior to transport to the synchrotron. Crystals were subsequently transferred to the goniostat on station PX10.1 at the Daresbury Synchrotron Radiation Source and maintained at 100 K with a Cryojet cryocooler (Oxford Instruments). Diffraction data were recorded using a MAR 225 CCD detector (MAR Research) with the wavelength set to 1.490 Å and were processed using *MOSFLM* (Leslie, 2006) and *SCALA* (Evans, 2006). Preliminary analysis of the resultant data set was performed using programs from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994).

3. Results and discussion

GyrA59 was overexpressed and purified with an approximate yield of 30 mg protein from 6 l culture and was judged to be greater than 90% pure by SDS–PAGE analysis. DLS analysis gave a peak accounting for 99.9% of the mass of the sample with a polydispersity of 10.3%. From these results the molecular size was estimated to be 121 kDa, which is very close to the value expected for a GyrA59 dimer (117.1 kDa).

Preliminary crystals of the GyrA59–SD8 complex grew within 1–2 d after setup with 10%(*w*/*v*) PEG 8000, 10%(*v*/*v*) glycerol, 0.1 *M* Tris–HCl pH 8.5 as the precipitant. Improved crystals were subsequently obtained from 8%(*w*/*v*) PEG 8000, 10%(*v*/*v*) glycerol, 8%(*v*/*v*) DMSO, 0.1 *M* Tris pH 8.0 with maximum dimensions of approximately 220 × 220 × 1000 µm (Fig. 1). The crystals had a distinct yellowish tinge which was consistent with the presence of bound inhibitor and could not be grown from these conditions when SD8 was absent. The presence of glycerol in the mother liquor meant that crystals could be mounted directly from the crystallization plates without further cryoprotection.

X-ray data were collected from a single crystal of the GyrA59–SD8 complex: a total of 120 × 1.0° oscillation images were recorded in a

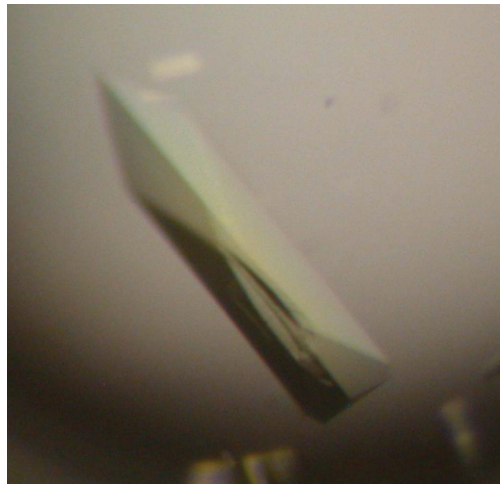


Figure 1
A single crystal of the complex of GyrA59 with simocyclinone D8 with approximate dimensions 220 × 220 × 1000 µm. Note the yellow colour, which is indicative of the bound inhibitor.

Table 1
Summary of X-ray data for the GyrA59–simocyclinone D8 complex.

Values in parentheses are for the outer resolution shell.

Space group	$I222$ or $I2_12_12_1$
Unit-cell parameters (Å)	a = 146.52, b = 153.99, c = 177.81
Wavelength (Å)	1.490
Resolution range (Å)	88.74–2.75 (2.90–2.75)
Unique reflections	51089 (7228)
Completeness (%)	97.9 (96.1)
Redundancy	4.1 (3.6)
$R_{\rm merge}$†	0.086 (0.448)
$\langle I/\sigma(I)\rangle$	9.5 (2.7)
Wilson B value (Å^2)	76.4

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith observation of reflection hkl and $\langle I(hkl)\rangle$ is the weighted average intensity for all observations i of reflection hkl.

continuous sweep to a resolution of 2.75 Å. Indexing was consistent with I-centred orthorhombic symmetry, giving unit-cell parameters a = 146.52, b = 153.99, c = 177.81 Å. Data-collection and processing statistics are summarized in Table 1. Solvent-content estimations suggested that between two and four GyrA59 monomers (58 543 Da each) were possible per asymmetric unit, giving solvent contents in the range 42.6–71.3% (Matthews, 1968).

We have obtained a preliminary structure of the *E. coli* GyrA59–SD8 complex using the known structure of the unliganded form of *E. coli* GyrA59 (PDB code 1ab4; Morais Cabral *et al.*, 1997) as a molecular-replacement template. *Phaser* (McCoy *et al.*, 2007) gave a clear solution (LLG = 6593) for two GyrA59 monomers in the asymmetric unit, which together formed the expected dimer, and the resultant electron-density maps revealed two copies of the bound ligand. Full details of the structure solution and the resultant model will be reported elsewhere.

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