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Crystallization and preliminary crystallographic analysis of the DNA gyrase B protein from *B. stearothermophilus*

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Abstract

DNA gyrase B (GyrB) from *B. stearothermophilus* has been crystallized in the presence of the non-hydrolyzable ATP analogue, 5′-adenylyl-β-γ-imidodiphosphate (ADPNP), by the dialysis method. A complete native data set to 3.7 Å has been collected from crystals which belonged to the cubic space group $I23$ with unit-cell dimension $a = 250.6$ Å. Self-rotation function analysis indicates the position of a molecular twofold axis. Low-resolution data sets of a thimerosal and a selenomethionine derivative have also been analysed. The heavy-atom positions are consistent with one dimer in the asymmetric unit.

1. Introduction

Bacterial DNA gyrase (E.C. 5.99.1.3) is a type II DNA topoisomerase which catalyses the interconversion of different topological forms of DNA. The biological function of the enzyme is to control the degree of DNA supercoiling, which is important for DNA replication and transcription (Reece & Maxwell, 1991; Wang, 1985). Gyrases have been identified in many different prokaryotic organisms. The enzyme introduces negative supercoils into closed, circular DNA by utilizing the free energy released by ATP hydrolysis, an ability which is unique amongst topoisomerases. The protein from *B. stearothermophilus* is a heterotetramer of approximately 340 kDa molecular weight consisting of two pairs of subunits *A* (GyrA, 97 kDa) and *B* (GyrB, 72 kDa). The enzyme mechanism involves cleavage of double-stranded DNA, passage of a segment of double-stranded DNA through the break, followed by resealing of the DNA. The Gyra protein is responsible for the DNA breakage and religation activity, while the GyrB subunit is associated with ATP binding and hydrolysis.

The crystal structure of a 43 kDa N-terminal fragment of the GyrB protein from *E coli* has been reported (Wigley, Davies, Dodson, Maxwell & Dodson, 1991). This corresponds to approximately 65% of the intact *B. stearothermophilus* GyrB. Biochemical studies, however, have shown that this fragment is unable to interact with the *A* proteins (Brown, Peebles & Cozzarelli, 1979). This interaction is thought to be mediated by the C-terminal domain, consisting of 230 amino acids. Recent structural information obtained for a fragment of a eukaryotic type II topoisomerase (Berger, Gamblin, Harrison & Wang, 1996) has lead to the proposal of an overall mechanism for type II topoisomerases. These authors also suggested how the ATPase domains (which are homologous to the 43 kDa fragment of DNA gyrase) might interact with their fragment. However, this arrangement has been questioned (Maxwell, 1996; Wigley, 1996) and there is clear need for a structure which provides the overlap between the two current structures to explain how the various portions of the protein interact with each other. An intact GyrB structure, even at low resolution, would provide this overlap. Furthermore, it is still unclear why gyrases are uniquely able to couple the free energy of hydrolysis of ATP to drive the negative supercoiling of closed circular DNA, whereas the eukaryotic type II and prokaryotic type IV topoisomerases are only able to catalyse the energetically favourable relaxation of supercoiled DNA. Since the ATPase activity is located in the *B* subunit of DNA gyrase and the *E* subunit of topoisomerase IV, we are trying to solve the crystal structures of both proteins to see if their comparison will shed some light upon the mechanistic differences between the proteins (Subramanya, Peng, Marians & Wigley, 1996). Consequently, the structure of the intact GyrB protein should provide details about the overall structure and assembly of type II topoisomerase complexes, as well as information about the coupling of ATP hydrolysis to supercoiling or relaxation of DNA.

2. Experimental

2.1. *Overexpression, purification and crystallization*

E. coli BL21 (DE3) cells were transformed with an expression plasmid containing the *gyrB* gene from *B. stearothermophilus* under control of the T7 RNA polymerase promoter (unpublished data). Cells were grown to mid-log phase, induced by the addition of 0.5 m*M* isopropyl-1-thio-β-D-galactopyranoside and grown for a further 3 h. The cells were harvested by centrifugation and sonicated in buffer containing 50 m*M* Tris–HCl at pH 7.5, 1 m*M* EDTA and 1 m*M* dithiothreitol (DTT). Lysozyme, phenylmethyl-sulfonyl fluoride (Sigma), and benzamidine–HCl (Sigma) were added to the final concentrations of 0.1 mg ml^{-1}, 2 and 2 m*M*, respectively. GyrB was purified by several steps of column chromatography in the following order: Q-Sepharose (fast-flow, Pharmacia), heparin–Sepharose CL-6B (Pharmacia), phenyl–Sepharose (high performance, low substitution, Pharmacia), and Mono-Q (Pharmacia). After this procedure, the protein was judged to be greater than 99% pure by sodium dodecyl sulfate gel electrophoresis (data not shown).

For crystallization trials, the protein was concentrated to 3.5 mg ml^{-1} in 5 m*M* DTT, 20 m*M* Tris–HCl buffer at pH 7.5. The protein concentration was estimated by measuring its absorption at 280 nm, using a molar absorption coefficient of 0.65 M^{-1} cm^{-1} calculated from the known amino-acid sequence. Dodecahedral crystals (up to 0.6 mm in cross section) were grown by a double-dialysis set-up in glass tubes at 294 K. The protein was dialysed against 0.1 m*M* ADPNP (in 50 m*M* Tris–HCl at pH 7.5), 5 m*M* DTT, 5 m*M* $MgCl_2$, 20 m*M* Tris–HCl at pH 7.5. The method used is essentially the same as the microdiffusion cell (Weber & Goodkin, 1970), but

Table 1. *Scaling statistics for native data*

Resolution range (Å)	R_{sym}* (%)	Completeness (%)
10.0–7.0	6.5	96.2
7.0–6.0	7.8	96.2
6.0–5.3	9.8	96.3
5.3–4.9	11.1	96.1
4.9–4.6	13.3	96.5
4.6–4.3	14.6	95.8
4.3–4.1	18.0	95.9
4.1–4.0	21.0	94.4
4.0–3.8	26.0	92.5
3.8–3.7	33.6	92.0

* $R_{sym} = \sum |I - \langle I \rangle| / \sum I$.

differs from the latter in that it consists of two glass tubes which were stacked together rather than held in place using rubber bands. ADPNP was present in the outer tube and in the main chamber and slowly dialysed into the inner tube. Crystals appeared after one day and reached their maximum size after 10 to 14 d. In order to obtain a thimerosal derivative of the protein, the crystals were harvested into 10% PEG 6000, 0.1 m*M* ADPNP, 5 m*M* $MgCl_2$ and 20 m*M* Pipes–NaOH at pH 6.8, and soaked for 18 h in 10 m*M* thimerosal, 10% PEG 6000, 0.1 m*M* ADPNP, 5 m*M* $MgCl_2$, 20 m*M* Tris–HCl at pH 7.2.

Seleno-methionine incorporated protein was prepared by transforming an *E. coli* Met auxotroph, B834 (DE3) pLysS, with the same GyrB expression plasmid. The cells were grown in LeMaster medium (LeMaster & Richards, 1985) supplemented with 50 mg l^{-1} seleno-DL-methionine (Sigma). Preparation and crystallization of the selenomethionine-substituted protein was identical to the native protein.

2.2. *X-ray analysis of the crystals*

Crystals were mounted conventionally in quartz capillaries for data collection, but with a large quantity of mother liquor to prevent them from drying out. The crystals were highly susceptible to radiation damage allowing only 1° of native data to be collected from a single crystal. Attempts to prolong the lifetime of the crystals in the X-ray beam, by flash-freezing, were not successful. The data were processed using *DENZO*

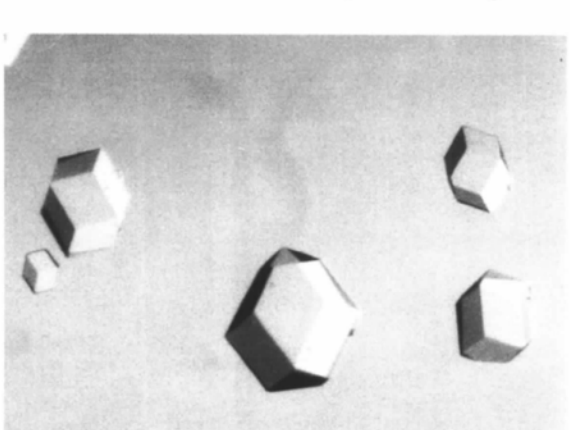

Fig. 1. Crystals of the intact gyrase B protein from *B. stearothermophilus* obtained by the dialysis method. Dodecahedral crystals of up to 0.6 mm in cross section were routinely obtained.

Table 2. *Summary of crystallographic structure analysis*

Type	Native	Seleno-methionine	Thimerosal
Source	ESRF (BL 4) SRS (Station 9.6)	ESRF (BL 4)	Rotating anode, Oxford
Wavelength (Å)	0.91 (ESRF) 0.87 (SRS)	0.91	1.54
Detector type	30 cm MAR image plate	30 cm MAR image plate	18 cm MAR image plate
Resolution (Å)	10–3.7	10–4.0	15–5.5
No. of crystals	84	18	1
Degree of oscillation	1	1–2	1
No. of reflections	84852	44949	26588
No. of unique reflections	25092	17847	7984
Completeness (overall) (%)	95.2	86.5	97.8
$I/\sigma I$	7.1	6.6	5.7
R_{sym}* (%)	11.1	9.7	15.5
R_{iso}† (%)	—	10.3	25.5
No. of heavy-atom sites	—	10	6
R_{Cullis}‡	—	0.82	0.65
Phasing power§	—	0.5	1.0
Mean figure of merit¶	0.23	—	—

* $R_{sym} = \sum |I - \langle I \rangle| / \sum I$. † $R_{iso} = \sum \big| |F_{PH}| - |F_P| \big| / \sum |F_P|$. ‡ $R_{Cullis} = \sum \big| |F_{PH} \pm F_P| - F_H(\text{calc}) \big| / \sum |F_{PH} \pm F_P|$. § Phasing power: $F_H/\varepsilon =$ r.m.s.(F_H)/r.m.s.(lack of closure), where F_H is the calculated heavy-atom structure factor. ¶ Mean figure of merit: $m = |F(hkl)_{best}|/|F(hkl)|$, where $F(hkl)_{best} = \sum P(\alpha) F_{hkl}(\alpha) / \sum P(\alpha)$.

(Otwinowski, 1993), scaled using *SCALEPACK* (Otwinowski, 1993), and reduced to a unique data set using the *TRUNCATE* program of the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). The scaling output is shown in Table 1.

3. Results and discussion

The best crystals were grown by the dialysis method (Fig. 1) and were determined to be of the cubic space group $I23$ with the unit-cell dimension $a = 250.6$ Å. A 3.7 Å native data set and a 4 Å seleno-methionine derivative data set were collected using synchrotron radiation. A 5 Å thimerosal derivative data set has also been collected using a rotating-anode X-ray generator in-house (Table 2). These data were used for preliminary crystallographic analysis.

A self-rotation function was calculated using the program *POLARRFN* of the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). The rotation function revealed the position of a single molecular twofold axis at $\omega = 55°$, $\varphi = 20°$ (Fig. 2). A single protein dimer (144 kDa) in the asymmetric unit would result in a $V_m = 4.6$ Å^3 Da^{-1}, corresponding to a solvent content of 74%, which is at the upper limit for protein crystals (Matthews, 1968). This high solvent content probably explains the weak diffraction of the crystals and their sensitivity to X-ray radiation, freezing and to dehydration. The heavy-atom positions in the thimerosal and the seleno-methionine derivatives data have been determined and confirmed the space group $I23$ as opposed to the enantiomorph $I2_13$. Furthermore, the mercury and the selenium sites obey the twofold non-crystallographic symmetry and can be explained fully by a

single dimer in the asymmetric unit. The structure will be solved using multiple isomorphous replacement phases in combination with molecular replacement.

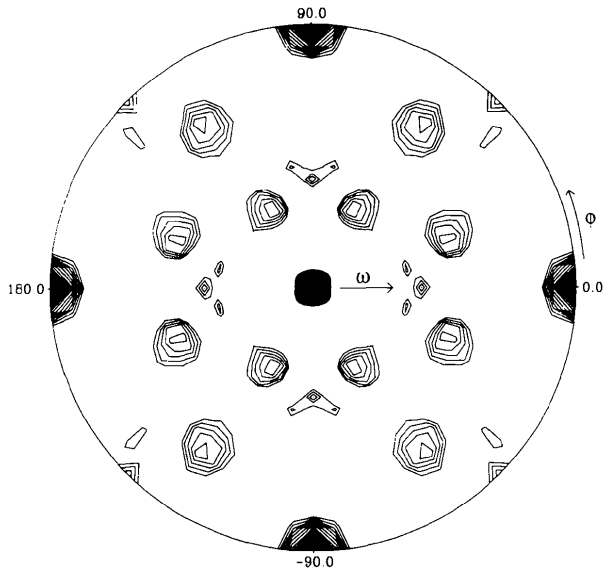

Fig. 2. Stereographic projection of the self-rotation function with $\kappa = 180^{\circ}$. The resolution of the data used was 10–5 Å with a radius of integration of 25 Å.

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