Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray crystallographic analysis of the C-terminal domain of ParC protein from *Bacillus stearothermophilus*

Tung-Ju Hsieh and Nei-Li Chan*

Institute of Biochemistry, College of Life Sciences, National Chung Hsing University, Taichung City, Taiwan

Correspondence e-mail: nlchan@dragon.nchu.edu.tw

Received 22 September 2003
Accepted 31 December 2003

Type IIA topoisomerases are multidomain enzymes composed of four major domains: the ATPase domain, the TOPRIM domain, the DNA-cleavage/religation domain and the C-terminal domain (CTD). Although crystal structures of the first three domains are available, the three-dimensional structure of the less-conserved CTD has yet to be determined. In order to provide a three-dimensional structure of this structurally uncharacterized region, the 36 kDa CTD of ParC protein, the DNA-cleavage/religation subunit of topoisomerase IV, from *Bacillus stearothermophilus* has been cloned, purified and crystallized. The crystals belonged to the trigonal space group $P3_1$ (or $P3_2$), with unit-cell parameters $a = b = 83.5$, $c = 45.1$ Å. The asymmetric unit contains one molecule and the solvent content is 51.2%. A 98.9% complete native data set has been collected from a frozen crystal to 2.0 Å resolution with an overall $R_{\rm merge}$ of 6.5%.

1. Introduction

The highly conserved type IIA topoisomerases are essential enzymes for both prokaryotes and eukaryotes. This group of enzymes alters the topological state of DNA by catalyzing the ATP-dependent passage of DNA duplexes through one another. By regulating DNA topology, type IIA enzymes are involved in almost all aspects of DNA metabolism, such as replication, transcription, recombination, sister chromatid segregation and chromosome condensation/decondensation (for recent reviews, see Wang, 1996, 2002; Champoux, 2001).

All type IIA enzymes are structurally similar with a twofold-symmetric subunit arrangement (Champoux, 2001). The eukaryotic enzymes are homodimers in which the monomer is a single polypeptide consisting of four main regions with distinct functions: the ATPase domain, the TOPRIM domain (Aravind *et al.*, 1998), the DNA-cleavage/religation domain and the less-conserved C-terminal domain. In bacteria, two homologous type IIA enzymes, DNA gyrase and topoisomerase IV (Topo IV), have been identified (Gellert *et al.*, 1976; Kato *et al.*, 1990). Both of these prokaryotic enzymes are tetramers assembled from two polypeptides, GyrB and GyrA in the case of gyrase and ParE and ParC for Topo IV, that correspond to the N- and C-terminal halves of the multidomain eukaryotic monomer, respectively (Mizuuchi *et al.*, 1978; Kato *et al.*, 1990; Peng & Marians, 1993; Caron & Wang, 1994). Specifically, GyrB and ParE contain the ATPase domain and the TOPRIM domain, while GyrA and ParC harbour the latter two domains.

Although these two bacterial enzymes share a high degree of similarity, they appear to have distinct cellular functions (Adams *et al.*, 1992). In general, gyrase is responsible for introducing negative supercoil into closed circular bacterial genomes, while Topo IV is the decatenating enzyme required for the segregation of topologically linked daughter chromosomes (Reece & Maxwell, 1991*a*; Zechiedrich *et al.*, 2000; Deibler *et al.*, 2001). Maxwell and colleagues have shown that the unique supercoiling activity of gyrase can be attributed to the C-terminal domain of the GyrA subunit (GyrA-CTD; Kampranis & Maxwell, 1996). It is now widely accepted that GyrA-CTD acts as a non-specific DNA-binding protein that allows the wrapping of DNA around the enzyme (Reece & Maxwell, 1991*b*; Knight & Samuels, 1999) and its removal converts gyrase into a typical/or classical relaxing and decatenating type IIA enzyme such as Topo IV (Kampranis & Maxwell, 1996). In contrast, no DNA-wrapping activity has been inferred for the homologous C-terminal domains of bacterial Topo IV (ParC-CTD; Peng & Marians, 1995). Interestingly, despite the clear functional differences between GyrA-CTD and ParC-CTD, both domains have been predicted to adopt a six-bladed β-propeller fold based on hydrophobicity analysis and a speculated sequence homology with regulator of chromosome condensation (RCC1; Qi *et al.*, 2002). Therefore, detailed spatial properties from the crystal structures of these domains are required to shed lights on their functions.

Crystal structures for the conserved ATPase, TOPRIM and DNA-cleavage/religation domains have been determined in recent

years and have provided invaluable insights towards understanding the molecular mechanism of the type IIA enzymes (Wigley *et al.*, 1991; Berger *et al.*, 1996; Cabral *et al.*, 1997; Fass *et al.*, 1999). However, no crystal structure for the more species-specific C-terminal domain is yet available. In order to provide three-dimensional structural information for this region, the 36 kDa *Bacillus stearothermophilus* ParC-CTD consisting of residues 499–810 has been cloned, purified and crystallized. The crystallization conditions and preliminary X-ray crystallographic analysis are summarized in this report. As the three-dimensional structures of GyrA-CTD and ParC-CTD have been predicted to be similar (Qi *et al.*, 2002), a reliable homology model for GyrA-CTD can therefore be constructed based on the crystal structure of ParC-CTD on its determination.

2. Experimental

2.1. Protein expression and purification

The results of limited proteolysis and N-terminal sequencing revealed that the GyrA-CTD of *Escherichia coli* begins at residue 572 (Reece & Maxwell, 1989). By performing sequence alignment using the *ClustalX* program (Thompson *et al.*, 1997), it was concluded that the ParC-CTD of *B. stearothermophilus* is comprised of residues 499–810. A fragment of the *B. stearothermophilus parC* gene encoding ParC-CTD was obtained by PCR amplification using a plasmid (pBXC) harbouring the full-length gene as a template and was cloned into a T7 promoter-driven expression system (pET-21b vector; Invitrogen) to allow expression of 6× His-tagged recombinant protein in *E. coli* BL21 (DE3) cells. ParC-CTD expression was achieved from cell cultured in Luria–Bertani medium at 310 K and induced at OD_{600} = 0.6 by the addition of isopropyl-β-D-thiogalactopyranoside to a final concentration of 1.0 m*M*. To optimize the yield of soluble ParC-CTD, cell growth was continued for another 14 h at 293 K after induction. The cells were harvested by centrifugation and the cell pellet was resuspended in buffer *A* (50 m*M* Tris–HCl pH 8.0, 300 m*M* NaCl, 0.5 m*M* PMSF and 5 m*M* β-mercaptoethanol). Following cell disruption (using a Microfluidizer, Christison) and centrifugation, the clarified cell lysate was loaded onto an immobilized Co^{2+}-affinity column (Clontech). The column was washed with five column volumes of buffer *A* plus 12 m*M* imidazole and the bound ParC-CTD was eluted with the same buffer plus 150 m*M* imidazole. Fractions containing ParC-CTD were pooled and concentrated by ultrafiltration using an Amicon Ultra device (Millipore; 10 kDa cutoff) and then loaded onto a HiLoad 16/60 Superdex-200 size-exclusion column (Amersham Biosciences) equilibrated in gel-filtration buffer (20 m*M* Tris–HCl pH 8.0, 200 m*M* NaCl and 5 m*M* β-mercaptoethanol). The peak fractions were pooled and concentrated by ultrafiltration to $\sim$20 mg ml^{-1} for crystallization. Approximately 20 mg of highly purified ParC-CTD was obtained per litre of *E. coli* culture.

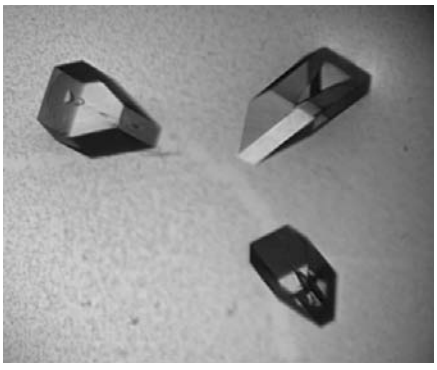

Figure 1
Crystals of the C-terminal domain of ParC (ParC-CTD) from *B. stearothermophilus*. The approximate dimensions of the crystals are 0.3 × 0.3 × 0.6 mm.

2.2. Crystallization and data collection

Initial crystallization trials were performed with commercially available kits (Hampton Research, USA) using the hanging-drop vapour-diffusion method. Protein solution (1 µl; 20 mg ml^{-1} in gel-filtration buffer) and reservoir solution (1 µl) were mixed and equilibrated against 450 µl reservoir solution at 277 K. Conditions that produced small crystals were refined by systematic variation of the precipitant concentration and pH. Diffraction-quality crystals were obtained at 277 K using a reservoir solution consisting of 0.04 *M* magnesium acetate, 0.05 *M* sodium cacodylate pH 6.5 and 29% MPD (Fig. 1).

Crystals of ParC-CTD were successfully frozen in liquid nitrogen using both MPD and ethylene glycol as cryoprotectants. These crystals were transferred to a cryostabilization solution consisting of reservoir buffer plus an additional 6% MPD and 15% ethylene glycol for approximately 10 s and immediately flash-frozen by plunging into liquid nitrogen. X-ray diffraction data were collected from a flash-frozen crystal at 100 K using a Rigaku RU-H3R rotating-anode generator (Cu $K\alpha$, λ = 1.5418 Å) fitted with Osmic Confocal Blue Optics and an R-AXIS IV^{++} image-plate detector. The crystal was rotated through a total of 100° with consecutive 1° oscillations. The data were processed and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell.

X-ray wavelength (Å)	1.5418
Space group	$P3_1$ (or $P3_2$)
Unit-cell parameters (Å)	$a = b = 83.5$, $c = 45.1$
Resolution (Å)	30.0–2.0 (2.07–2.00)
No. measured reflections	72367
No. unique reflections	23505
$R_{\rm merge}$† (%)	6.5 (30.7)
Data completeness (%)	98.9 (97.0)
$\langle I/\sigma(I)\rangle$	18.1 (3.8)

† $R_{\rm merge} = [\sum_h \sum_i |I(h,i) - \langle I(h)\rangle|/\sum_h \sum_i I(h,i)] \times 100$, where $I(h, i)$ is the intensity of the ith measurement of reflection h and $\langle I(h)\rangle$ is the mean value of $I(h, i)$ for all i measurements.

3. Results

Recombinant *B. stearothermophilus* ParC-CTD with a C-terminal 6× His tag was cloned, overexpressed in *E. coli*, purified to apparent homogeneity and crystallized according to the methods described in the previous section. Crystals of ParC-CTD grew to approximate dimensions of 0.3 × 0.3 × 0.6 mm within two weeks (Fig. 1). Although these crystals were grown from a high concentration of MPD, suppression of ice rings upon flash-freezing was only possible when an additional 6% MPD plus up to 15% ethylene glycol were used as cryoprotectants. Upon successful cryostabilization, the crystals diffracted to better than 2.0 Å resolution with a rotating-anode X-ray source. Preliminary processing of the diffraction data suggested that these crystals belong to a trigonal space group, with unit-cell parameters $a = b = 83.5$, $c = 45.1$ Å. Using a single crystal of ParC-CTD, a total of 72 367 observations were collected at 100 K to give a data set which is 98.9% complete to 2.0 Å with 23 505 unique reflections and an overall $R_{\rm merge}$ of 6.5%. Additional analysis and systematic absences indicate the space group to be $P3_1$ (or $P3_2$), with one ParC-CTD molecule per asymmetric unit, corresponding to a crystal volume per protein weight ($V_{\rm M}$) of 2.52 Å^3 Da^{-1} and a solvent content of about 51.2%. Consistent with this assignment, no extra rotational axis of symmetry except for a crystallographic threefold was identified in a self-rotation function using the program

GLRF (Tong & Rossmann, 1990). The data-collection statistics are presented in Table 1. Because ParC-CTD contains eight methionine residues, we plan to solve the phase problem by applying the multiwavelength anomalous diffraction technique using selenomethionine-substituted protein crystals.

We thank Dr Wai Mun Huang for providing the plasmid pBXC, Dr Yuh-Ju Sun and the staff at the Macromolecule X-ray Diffraction Facility (National Tsing Hua University, Taiwan) and the National Synchrotron Radiation Research Center (NSRRC, Taiwan) for assistance during data collection. This work was supported by grants from the National Science Council (NSC92-2113-M-005-016 and NSC91-2113-M-005-013 to NC) and the Ministry of Education (E-91-B-FA05-2-4 to NC).

References

Adams, D. E., Shekhtman, E. M., Zechiedrich. E. L., Schmid, M. B. & Cozzarelli, N. R. (1992). *Cell*, **71**, 277–288.

Aravind, L., Leipe, D. D. & Koonin, E. V. (1998). *Nucleic Acids Res.* **26**, 4205–4213.

Berger, J. M., Gamblin, S. J., Harrison, S. C. & Wang, J. C. (1996). *Nature (London)*, **379**, 225–232.

Cabral, J. H. M., Jackson, A. P., Smith, C. V., Shikotra, N., Maxwell, A. & Liddington, R. C. (1997). *Nature (London)*, **388**, 903–906.

Caron, P. & Wang, J. C. (1994). *Adv. Pharmacol. B*, **29**, 271–297.

Champoux, J. J. (2001). *Annu. Rev. Biochem.* **70**, 369–413.

Deibler, R. W., Rahmati, S. & Zechiedrich, E. L. (2001). *Genes Dev.* **15**, 748–761.

Fass, D., Bogden, C. E. & Berger, J. M. (1999). *Nature Struct. Biol.* **6**, 322–326.

Gellert, M., Mizuuchi, K., O'Dea, M. H. & Nash, H. A. (1976). *Proc. Natl Acad. Sci. USA*, **73**, 3872–3876.

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

Kato, J., Nishimura, Y., Imamura, R., Niki, H., Hiraga, S. & Susuki, H. (1990). *Cell*, **63**, 393–404.

Knight, S. W. & Samuels, D. S. (1999). *EMBO J.* **18**, 4875–4881.

Mizuuchi, K., O'Dea, M. H. & Gellert, M. (1978). *Proc. Natl Acad. Sci. USA*, **75**, 5960–5963.

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

Peng, H. & Marians, K. J. (1993). *J. Biol. Chem.* **268**, 24481–24490.

Peng, H. & Marians, K. J. (1995). *J. Biol. Chem.* **270**, 25286–25290.

Qi, Y., Pei, J. & Grishin, N. V. (2002). *Proteins*, **47**, 258–264.

Reece, R. J. & Maxwell, A. (1989). *J. Biol. Chem.* **264**, 19648–19653.

Reece, R. J. & Maxwell, A. (1991*a*). *Crit. Rev. Biochem. Mol. Biol.* **26**, 335–375.

Reece, R. J. & Maxwell, A. (1991*b*). *Nucleic Acids Res.* **19**, 1399–1405.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). *Nucleic Acids Res.* **25**, 4876–4882.

Tong, L. & Rossmann, M. G. (1990). *Acta Cryst.* A**46**, 783–792.

Wang, J. C. (1996). *Annu. Rev. Biochem.* **65**, 635–692.

Wang, J. C. (2002). *Nature Rev. Mol. Cell Biol.* **3**, 430–440.

Wigley, D. B., Davies, G. J., Dodson, E. J., Maxwell, A. & Dodson, G. (1991). *Nature (London)*, **351**, 624–629.

Zechiedrich, E. L., Khodursky, A. B., Bachellier, S., Schneider, R., Chen, D., Lilley, D. M. & Cozzarelli, N. R. (2000). *J. Biol. Chem.* **275**, 8103–8113.