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Preliminary crystallographic analysis of the ParE subunit of *Escherichia coli* topoisomerase IV

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Abstract

The ParE subunit of *Escherichia coli* topoisomerase IV has been crystallized in the presence of the non-hydrolyzable ATP analogue, 5′-adenylyl-β,γ-imidodiphosphate (ADPNP). The crystals are of the orthorhombic space group, $P2_12_12_1$, with unit-cell dimensions $a = 92.6$, $b = 119.1$, $c = 135.3$ Å. Data have been collected to 3.5 Å resolution from frozen native crystals. Self-rotation function analysis of these data indicate the position of a molecular twofold axis. Higher resolution native data are being collected and a derivative search is underway.

1. Introduction

The role of DNA topoisomerases, in controlling the level of supercoiling in cells, is well known (Wang, 1985). However, this is not their only function. Topoisomerase IV is a type II topoisomerase first identified in *Escherichia coli* (Kato *et al.*, 1990). It is the product of two genes, *parC* and *parE*, which were originally identified by mutational analysis of chromosome segregation in *E. coli* (Kato, Nishimura, Yamada, Suzuki & Hirota, 1988). Temperature-sensitive mutants of *parC* and *parE* show accumulation of replication catenanes (multiply linked) of pBR322 plasmid when grown at non-permissive temperatures (Adams, Shekhtman, Zechiedrich, Schmid & Cozzarelli, 1992). Topoisomerase IV consists of two proteins, ParC and ParE, with the active complex comprising two copies of each protein (Peng & Marians, 1993*a*). Alignment of the amino-acid sequences of DNA gyrase and topoisomerase IV, reveals that ParC is homologous with the gyrase A protein while ParE is homologous to the gyrase B protein, but to the shorter version of the protein such as that found in *Bacillus subtilis*. Both gyrase and topoisomerase IV are ATP-dependent enzymes, but gyrase differs from topoisomerase IV in that it is able to couple the free energy of hydrolysis of ATP to the energetically unfavourable process of introducing supercoils into DNA (Gellert, Mizuuchi, O'Dea & Nash, 1976). Interestingly, topoisomerase IV, in common with type II topoisomerases other than gyrase, requires ATP in order to catalyze the relaxation of supercoiled DNA, a reaction which is energetically favourable. The reasons for this are unclear at the present time. Both gyrase and topoisomerase IV are required for replication of DNA (Hussain, Elliot & Salmond, 1987; Kato *et al.*, 1988; Kato, Nishimura, Yamada & Suzuki, 1989). It has been demonstrated that both enzymes can decatenate DNA circles *in vitro*, but it is now clear that gyrase is a poor decatenase under physiological conditions (Peng & Marians, 1993*b*). Thus, it appears that topoisomerase IV plays the major role of the two enzymes in the decatenation of circles at the final stages of the replication of DNA in bacteria.

The only high-resolution structural information which is currently available for type II topoisomerases is the crystal structure of an N-terminal fragment of the DNA gyrase B protein (Wigley, Davies, Dodson, Maxwell & Dodson, 1991). This fragment corresponds to the N-terminal 65% of the ParE protein and it is anticipated that this region will have a similar structure in the two proteins. However, the structure of the remaining portion of the protein (230 amino acids) is presently unknown. Since the 43 kDa fragment of the DNA gyrase B protein is unable to interact with the A proteins (Ali, Jackson, Howells & Maxwell, 1993) it is thought that the C-terminal region of the B protein (and presumably also ParE) is responsible for the interaction with the A proteins. Consequently, information about an intact ParE protein should provide useful information about assembly of type II topoisomerase complexes.

2. Experimental

Protein was prepared as described previously (Peng & Marians, 1993*a*). For the crystallization studies the protein was concentrated to 10 mg ml^{-1}. Crystals were grown by overnight dialysis, in buttons, against 200 µ*M* ADPNP, 5 m*M* dithiothreitol (DTT), 5 m*M* $MgCl_2$, 20 m*M* 2-[*N*-morpholino]ethanesulfonic acid/NaOH pH 6.5, 10% glycerol. The initial protein solution comprised 5 mg ml^{-1} protein, 5 m*M* DTT, 20 m*M* Tris–HCl pH 7.0. Alternatively, crystals were grown by the hanging-drop method. In this case, the protein solution contained 5 mg ml^{-1} protein, 5 m*M* DTT, 10 m*M* $MgCl_2$, 1 m*M* ADPNP, 10% glycerol, 2% PEG 400. Drops (5 µl) of this solution were equilibrated above wells containing 6% PEG 400, 10% glycerol, 20 m*M* Tris–HCl pH 7.7. The crystals were rod shaped and grew to a maximum length of 1 mm but were only 0.1 mm in the other two dimensions. Crystals have a short life time in the beam, but this was extended by flash freezing at 100 K. Prior to freezing, crystals were harvested in 20 m*M* Tris–HCl pH 7.7, 5 m*M* DTT, 10 m*M* $MgCl_2$, 0.25 m*M* ADPNP, 1.5% PEG 400 and 10% glycerol. For freezing, crystals were transferred to a similar solution, but with the glycerol concentration increased to 35%, before picking up in rayon loops and then freezing in a stream of nitrogen gas at 100 K.

3. Results and discussion

The crystals were determined to be of the orthorhombic space group $P2_12_12_1$, with unit-cell dimensions $a = 92.6$, $b = 119.1$, $c = 135.3$ Å. Data collection was only possible after flash freezing at 100 K. After this procedure, the crystals diffracted to greater than 2.7 Å using synchrotron radiation (EMBL, Hamburg), but still diffracted very weakly. Preliminary investigations have been carried out using an essentially

complete (93.7%) native data set which has been collected to 3.5 Å using synchrotron radiation at beamline X11 in Hamburg. These data have been used for self-rotation function studies, which have revealed the position of a molecular two-fold axis in the *ab* plane about 45° from *b* (Fig. 1). These calculations are consistent with there being one protein dimer (M_r = 140 000) in the asymmetric unit. This arrangement would result in $V_m = 2.7$ Å^3 Da^{-1}, which is within the usual range for protein crystals (Matthews, 1968). Higher resolution native data are being collected and protein in which the methionine residues have been replaced with selenomethionine has already yielded crystals. The structure will be solved by conventional isomorphous phasing using X-ray data from these selenomethionine-substituted crystals, combined with molecular replacement using the structure of the 43 kDa fragment of the *E. coli* gyrase B protein (Wigley *et al.*, 1991) which is homologous to the N-terminal region of the ParE protein.

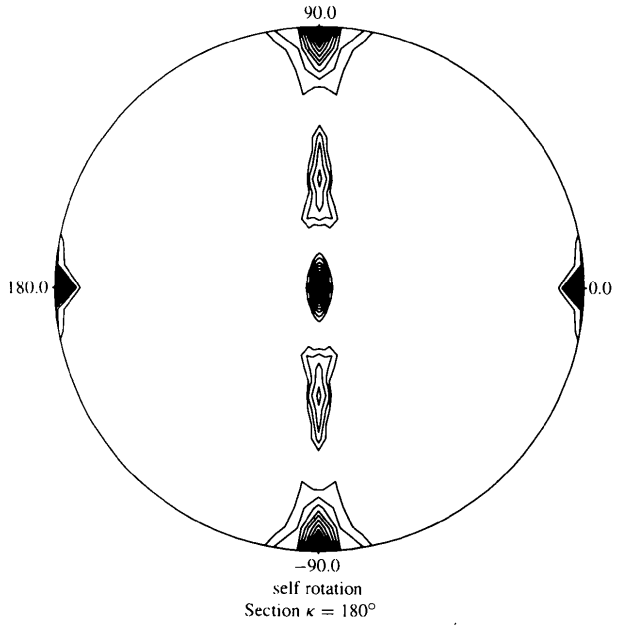

Fig. 1. Self-rotation function with $\kappa = 180°$. Resolution of the data used was 7.5–3.5 Å with a radius of integration of 40 Å. The data were aligned with the crystal axes *c*, *a*, *b* along *x*, *y*, and *z*, respectively.

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