Sheng-Chia Chen,^{a‡} Chi-Hung Huang,^{b‡} Chia-Shin Yang,^b Chi-Huang Chang,^b Shu-Min Kuan,^b Nei-Li Chan^a and Yeh Chen^{b*}

^aInstitute of Biochemistry and Molecular Biology, College of Medicine, National Taiwan University, Taipei City, 100, Taiwan, and

^bDepartment of Biotechnology, Hungkuang University, Taichung, 433, Taiwan

‡ These authors made equal contributions.

Correspondence e-mail:
chyeah6599@sunrise.hk.edu.tw

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Expression, purification, crystallization and preliminary X-ray analysis of the RecQ helicase catalytic core from *Deinococcus radiodurans*

The RecQ proteins are a highly conserved group of DNA helicases which play crucial roles in the maintenance of genome stability. DrRecQ from the radioresistant bacterium *Deinococcus radiodurans* is a special member of the RecQ family because it contains three Helicase-and-RNase-D-C-terminal (HRDC) domains at the C-terminus. The helicase catalytic core is essential for ATPase and DNA-unwinding activities. In this work, the helicase catalytic core of DrRecQ was expressed in *Escherichia coli*, purified and crystallized. Crystals were obtained using the sitting-drop vapour diffusion method and X-ray diffraction data were collected to 2.9 Å resolution. The crystals belong to space group $P2_12_12_1$, with unit-cell parameters $a = 84.75$, $b = 95.61$, $c = 183.83$ Å.

1. Introduction

Deinococcus radiodurans is an extremophilic bacterial species which exhibits extraordinary resistance to ionizing radiation, UV radiation and chemical mutagens. *D. radiodurans* is capable of surviving under conditions of 15 kGy of ionizing radiation, whereas most other organisms die at a radiation dose of approximately ≥ 50 Gy (Minton, 1994). Following massive doses of radiation, *D. radiodurans* can repair 150–200 double-stranded DNA breaks (DSBs) per chromosome without loss of viability (Battista, 1997; Cox & Battista, 2005). Because of its remarkable resilience to DNA damage, *D. radiodurans* serves as an excellent model system for studying the mechanisms of DNA repair. While this organism is widely used to study DNA repair, the precise mechanisms responsible for its extraordinary ability to repair DNA damage remain unclear. Since the release of the *D. radiodurans* genome sequence and annotation, it has become feasible to identify target genes that encode DNA repair proteins and to elucidate the mechanism(s) underlying radiation resistance.

In bacteria, RecQ helicases facilitate recombination, stalled DNA fork processing and SOS signalling. In humans, five homologues of RecQ have been identified; mutations in three of these are associated with human disorders. Specifically, mutations in the human genes *BLM*, *WRN* and *RECQ4* cause Bloom's syndrome (BS), Werner's syndrome (WS) and Rothmund–Thomson syndrome (RTS), respectively. These diseases are correlated with genome instability and, therefore, patients are predisposed to cancer as well as premature ageing (Cobb & Bjergbaek, 2006; Bachrati & Hickson, 2003).

Most RecQ proteins share three conserved domains: helicase, RecQ-conserved (RecQ-Ct) and Helicase-and-RNase-D-C-terminal (HRDC) domains. Together, the helicase and RecQ-Ct domains comprise the minimal helicase catalytic core that is essential for the ATPase and DNA-unwinding activities of these proteins. The HRDC domain is responsible for the regulation of DNA-binding affinity. Surprisingly, DrRecQ from *D. radiodurans* possesses three HRDC domains instead of a single HRDC domain as commonly observed in other bacteria. The three HRDC domains are required for structure-specific DNA binding and for efficient DNA unwinding (Killoran & Keck, 2006). In addition, a series of complementation experiments with DrRecQ mutants showed that the helicase catalytic core and the three HRDC domains are necessary to convey DNA damage resistance (Huang *et al.*, 2007).

Recently, the third HRDC domain of DrRecQ has been characterized and found to contain an electrostatic surface that facilitates its interaction with certain DNA structures. The mobility of HRDC domains could allow for reorientation following substrate recognition and ATP hydrolysis in DrRecQ (Killoran & Keck, 2008). These results suggest that the function of DrRecQ is slightly different from that of other bacterial RecQ proteins. Therefore, to understand the unusual DNA repair mechanisms of DrRecQ, we purified the helicase catalytic core including the N-terminal helicase and the RecQ-Ct domains from *D. radiodurans* and conducted purification, crystallization and preliminary X-ray analysis of this helicase catalytic core.

2. Materials and methods

2.1. Cloning, expression and purification

The *recQ* gene (accession code NP295013) coding for a helicase catalytic core (residues 1–517) was amplified from the genomic DNA of *D. radiodurans* by a polymerase chain reaction (PCR). The PCR primers used were 5'-GGGAATTCCATATGATGACCGCTGCTCCT-3' (including an *NdeI* restriction site shown in bold) and 5'-CCGCTCGAGGCCCGGCAGCAACGT-3' (including an *XhoI* restriction site shown in bold). The PCR-amplified DNA was digested with the restriction endonucleases *NdeI* and *XhoI* and ligated into the pET21a(+) expression vector. The completed vector was verified by DNA sequencing.

The plasmid thus constructed was transformed into *E. coli* strain BL21 (DE3) cells for protein expression. The transformed cells were grown at 310 K in 1 l Luria–Bertani medium, containing 50 µg ml⁻¹ ampicillin, until the optical density (OD) at 600 nm reached 1.0. Protein expression was induced for 20 h by the addition of 1.0 mM IPTG at 293 K. Cultured cells were harvested by centrifugation at 1590g for 30 min at 277 K. The cell pellet was resuspended in buffer A (50 mM Tris–HCl pH 8.0, 500 mM NaCl and 5 mM imidazole) and was disrupted by sonication. The lysate was centrifuged at 20 000g at 277 K for 90 min and the resulting supernatant was loaded onto an Ni-NTA His-bind resin column equilibrated with buffer A. The bound protein was then eluted with a 0–200 mM linear gradient of imidazole. Fractions containing DrRecQ were dialysed and then pooled for additional purification using a Q Sepharose column (GE Healthcare). The column was developed with a linear gradient of NaCl in buffer B (50 mM Tris–HCl pH 8.0, 100 mM NaCl and 5% glycerol). The protein was eluted as a single peak at 175 mM NaCl. These fractions were concentrated and then applied to a HiLoad

Figure 1

Crystal picture of the helicase catalytic core of RecQ from *D. radiodurans*. Crystal dimensions are approximately 0.2 × 0.1 × 0.05 mm.

Table 1

Statistical analysis of the data for the helicase catalytic core of DrRecQ crystal.

Values in parentheses are for the highest-resolution shell.

X-ray wavelength (Å)	0.97622
Space group	<i>P</i> 2 ₁ 2 ₁ 2 ₁
Unit-cell parameters (Å)	<i>a</i> = 84.75, <i>b</i> = 95.61, <i>c</i> = 183.83
Resolution (Å)	30.0–2.90 (3.00–2.90)
No. of measured reflections	345736 (34587)
No. of unique reflections	33811 (3294)
Redundancy	10.2 (10.5)
<i>R</i> _{merge} (%)	7.6 (67.8)
Data completeness (%)	99.9 (100.0)
<i>I</i> / <i>σ</i> (<i>I</i>)	27.5 (3.8)

16/60 Superdex-200 size-exclusion column (GE Healthcare) equilibrated in buffer C [50 mM Tris–HCl pH 8.0, 100 mM NaCl, 5% glycerol and 2 mM tris(2-carboxyethyl)phosphine (TCEP)]. The fractions containing DrRecQ were pooled and concentrated to 5 mg ml⁻¹ for crystallization screening.

2.2. Crystallization

Initial screening for crystallization conditions was performed using commercially available kits (Hampton Research, Emerald Bio-Structures and Molecular Dimensions) by the sitting-drop vapour diffusion method on 24-well VDX plates (Hampton Research). Drops containing 1 µl of protein solution (5 mg ml⁻¹ in buffer C) and 1 µl of reservoir solution were equilibrated against 400 µl of reservoir solution at 277 K. After 1 month of incubation, crystals were grown from condition Wizard III No. 13 [0.1 M HEPES pH 7.5, 10%(w/v) PEG 8 K and 8% ethylene glycol]. We further optimized the condition by varying the concentration of crystallizing agents and finally obtained crystals of the helicase catalytic core of the DrRecQ protein with approximate dimensions of 0.2 × 0.1 × 0.05 mm from a solution containing 0.1 M HEPES pH 7.7, 14%(w/v) PEG 8 K and 8% ethylene glycol (Fig. 1).

2.3. Data collection

Crystals were soaked in a solution containing 75%(v/v) reservoir solution and 25%(v/v) glycerol for approximately 15 s and were immediately flash-cooled by submerging them into liquid nitrogen. X-ray diffraction data were collected at the National Synchrotron Radiation Research Center (NSRRC) BL13C1 in Taiwan. The crystal-to-detector distance was maintained at 350 mm. The crystal was rotated through a total of 252° with consecutive 1° oscillations. A total of 252 images were recorded using an ADSC Quantum 315R CCD detector. The data were indexed and integrated using *HKL-2000* processing software (Otwinowski & Minor, 1997). An X-ray diffraction data image is shown in Fig. 2.

3. Results and discussion

The yield of purified protein was ~30 mg l⁻¹ of *E. coli* culture. The purified protein contains an extra octapeptide, LEH₆, at the C-terminal end. After gel-filtration chromatography, the helicase catalytic core of DrRecQ appeared to be approximately 95% pure, with a prominent protein band at around 55 kDa as determined by SDS–PAGE (Fig. 3). Using gel-filtration analysis, we found that the helicase catalytic core exists as a monomer in solution.

Figure 2

Diffraction pattern of the helicase catalytic core of DrRecQ collected on the NSRRC 13C1 beamline from a crystal flash-cooled in 25% glycerol.

Figure 3

SDS-PAGE of the purified helicase catalytic core of DrRecQ. Lane M, molecular-weight markers in kDa; lane 1, whole-cell lysate before IPTG induction; lane 2, whole-cell lysate after IPTG induction; lane 3, DrRecQ purified with an Ni column; lane 4, DrRecQ purified by Q-column; and lane 5, DrRecQ purified by gel filtration.

Crystals were obtained using the sitting-drop vapour diffusion method in a buffer composed of 0.1 M HEPES (pH 7.7), 14% (w/v) PEG 8 K and 8% ethylene glycol. The harvested crystal diffracted to 2.9 Å. The preliminary crystallographic analysis indicated that the crystal belonged to the space group $P2_12_12_1$, with unit-cell parameters $a = 84.75$, $b = 95.61$, $c = 183.83$ Å. The statistical analysis of the data

collected is summarized in Table 1. The crystal structure of the helicase catalytic core of DrRecQ was solved by the molecular-replacement method using the program *MOLREP* (Vagin & Teplyakov, 2010) with the coordinates of *E. coli* RecQ (Bernstein *et al.*, 2003) as a search model (PDB code 1oyw; 50% amino acid sequence identity). The rotation and translation functions yielded two clear positions for the model, assuming the presence of two molecules in the asymmetric unit, with a V_M value of $3.39 \text{ Å}^3 \text{ Da}^{-1}$ (Matthews, 1968). Structure refinement and functional interpretation are in progress.

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