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Purification, crystallization and preliminary X-ray diffraction analysis of DNA damage response A protein from *Deinococcus radiodurans*

Mitsugu Yamada, Katsuya Satoh and Issay Narumi*

Gene Resource Research Group, Life Science and Biotechnology Division, Quantum Beam Science Directorate, Japan Atomic Energy Agency, 1233 Watanuki, Takasaki, Gunma 370-1292, Japan

Correspondence e-mail: narumi.issei@jaea.go.jp

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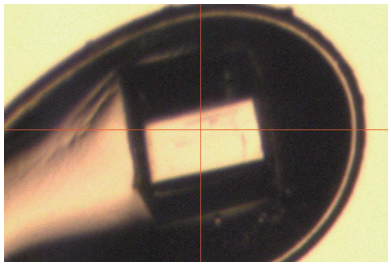
DNA damage response A protein (DdrA) from *Deinococcus radiodurans* has been suggested to be involved in DNA-repair processes through binding to 3′-ends of single-stranded DNA, thereby protecting the ends from nuclease digestion. In this study, a recombinant C-terminally truncated form of *D. radiodurans* DdrA (DdrA157) comprising the first 157 residues of DdrA was expressed in *Escherichia coli*, purified and crystallized. Single crystals of DdrA157 were obtained by the hanging-drop method at 293 K. The crystal belonged to the monoclinic space group $P2_1$, with unit-cell parameters $a = 46.31$, $b = 180.26$, $c = 114.17$ Å, $\beta = 90.02^\circ$. The crystal was expected to contain 14 molecules in the asymmetric unit. Diffraction data were collected to 2.35 Å resolution on beamline BL-5 at Photon Factory and initial phase determinations were attempted by the molecular-replacement method using the human Rad52 structure.

1. Introduction

Deinococcus radiodurans is known as a bacterium with an extraordinary tolerance towards high doses of radiation or long periods of desiccation (Cox & Battista, 2005; Blasius *et al.*, 2008). Under such conditions, a number of genes which lack readily identifiable homologues among known proteins are induced (Lipton *et al.*, 2002; Liu *et al.*, 2003). Of these, DR0423, also known as DNA damage response A protein (DdrA), is one of the most highly induced genes, with over 20-fold increases in expression level having been observed, and this gene product contributes to DNA damage resistance (Harris *et al.*, 2004; Tanaka *et al.*, 2004). This protein has an evolutionary relationship to eukaryotic Rad52 (Iyer *et al.*, 2002), a protein that is important for DNA double-strand break repair and homologous recombination. Both proteins have a common signature called ‘Pfam Rad52_Rad22’ consisting of two helix–hairpin–helix motifs (Sonnhammer *et al.*, 1998; Iyer *et al.*, 2002). Rad52 binds single-stranded DNA ends and mediates the DNA–DNA interactions required for the annealing of complementary DNA strands. Rad52 also interacts with the DNA recombination protein Rad51, suggesting a role in Rad51-related DNA recombination and repair (Milne & Weaver, 1993). On the other hand, no homologous recombination activity has been found to date in DdrA. A recent study showed that DdrA forms a heptameric ring as in the case of Rad52 (Gutsche *et al.*, 2008) and binds to 3′-overhang DNA ends (Harris *et al.*, 2004). It has also been shown that a C-terminally truncated form of DdrA comprising the first 157 residues of the 208-amino-acid whole protein could bind to 3′ DNA ends but was unable to restore radiotolerance in *D. radiodurans in vivo* (Harris *et al.*, 2008).

Given that DdrA is highly induced following γ irradiation and that the C-terminal region other than the DNA-binding region of DdrA is required for radiotolerance, it could be inferred that DdrA possesses an important physiological role other than DNA-end protection. However, the mechanism by which this protein partakes in DNA repair remains unclear. In an effort to delineate the physiological role of DdrA based on structural information, we conducted protein purification, crystallization and preliminary X-ray diffraction experiments.



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2. Materials and methods

2.1. Protein preparation

The *D. radiodurans ddrA* gene (471 bp; NCBI Gene ID 1797483) was amplified using genomic DNA from *D. radiodurans* strain R_1 (ATCC 13939) with specific oligonucleotide primers 5′-CTAAA**CATATG**AAGCTGAGCGATGTCC-3′ and 5′-CG**GGATCC**TCACAGGTGGGCGCCGCCGGG-3′ possessing *Nde*I and *Bam*HI restriction sites (shown in bold in the sequences), respectively. The PCR product was digested with *Nde*I and *Bam*HI and then ligated into *Nde*I–*Bam*HI-digested pET9 vector (Novagen). The resultant expression plasmid was designated pET9ddrA157.

Recombinant DdrA157 was expressed in *Escherichia coli* BL21 (DE3) pLysS cells (Novagen) harbouring plasmid pET9ddrA157. The cells were grown at 310 K in LB medium supplemented with 50 mg ml^{-1} kanamycin and 34 mg ml^{-1} chloramphenicol until the optical density at 600 nm reached about 0.6, at which point overexpression of the protein was induced by the addition of isopropyl β-D-1-thiogalactopyranoside to a final concentration of 0.1 m*M* and cell growth was continued for 16 h at 303 K. Following cultivation, cells were harvested by centrifugation at 8000*g* at 277 K, resuspended in buffer *A* consisting of 20 m*M* Tris–HCl pH 7.5, 10%(*v*/*v*) glycerol and 1 m*M* DTT with Complete Protease Inhibitor Cocktail (Roche) and then sonicated. The sonicated sample was centrifuged at 8000*g* at 277 K for 20 min to remove cell debris. DdrA157 was precipitated from the cell-free extract by addition of ammonium sulfate to 30% saturation. Precipitated proteins were collected by centrifugation at 8000*g* for 20 min at 277 K. The pellet was resuspended in buffer *A* and applied onto an anion-exchange column (Source Q, GE Healthcare Biosciences) equilibrated with buffer *A*. The protein was eluted with a linear gradient to 20 m*M* Tris–HCl pH 7.5, 1 *M* NaCl, 10%(*v*/*v*) glycerol and 1 m*M* DTT. Fractions containing DdrA157 were pooled and dialyzed against buffer *B* consisting of 20 m*M* Tris–HCl pH 7.5, 0.3 *M* NaCl, 10%(*v*/*v*) glycerol and 1 m*M* DTT. The dialyzed sample was applied onto a size-exclusion column (Sephacryl S-300, GE Healthcare Biosciences) equilibrated with buffer *B*. Purified DdrA157 was identified by SDS–PAGE, which yielded a single band with an estimated molecular weight of 17 kDa (Fig. 1), and then concentrated to 6 mg ml^{-1} using an Amicon Ultra-15 30 kDa molecular-weight cutoff filter unit (Millipore) in preparation for crystallization.

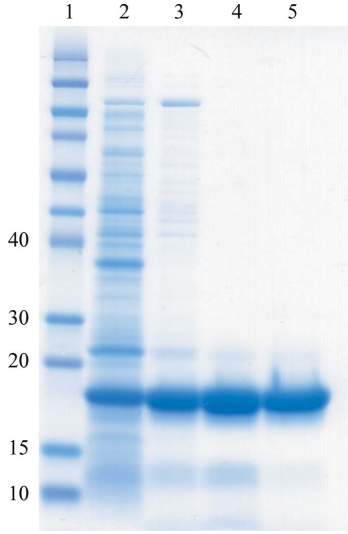

Figure 1
SDS–PAGE analysis of purified *D. radiodurans* DdrA157. Lane 1, molecular-weight markers (kDa); lane 2, cell-free extract; lane 3, precipitate obtained using 30% ammonium sulfate; lane 4, eluted fraction from Source Q column; lane 5, eluted fraction from Sephacryl S-300 size-exclusion column.

Table 1
Data-collection statistics for DdrA157.

Values in parentheses are for the highest resolution shell.

X-ray source	PF BL-5
X-ray beam size (mm)	0.1 × 0.1
Wavelength (Å)	1.0000
Detector	ADSC Q315r
Oscillation width (°)	0.5
No. of images	360
Temperature (K)	95
Data-processing software	*HKL*-2000
Resolution (Å)	50.00–2.35 (2.39–2.35)
Space group	$P2_1$
Unit-cell parameters	
a (Å)	46.31
b (Å)	180.26
c (Å)	114.17
β (°)	90.02
No. of observed reflections	286542
No. of unique reflections	80073
Completeness (%)	98.2 (98.0)
Multiplicity	3.6 (3.7)
Mean $I/\sigma(I)$	22.30 (5.05)
R_{merge}	0.073 (0.341)

2.2. Crystallization

Initial crystallization screening for DdrA157 was performed by the sitting-drop vapour-diffusion method at 293 K using a PEGRx HT kit (Hampton Research). Small single crystals appeared on mixing 2 µl protein solution [6 mg ml^{-1} in 20 m*M* Tris–HCl pH 7.5, 0.3 *M* NaCl, 10%(*v*/*v*) glycerol and 1 m*M* DTT] and 2 µl reservoir solution [15%(*w*/*v*) polyethylene glycol monomethyl ether (PEG MME) 2000, 0.2 *M* triammonium citrate pH 7.0, 0.1 *M* imidazole–HCl pH 7.0]. Following additional screening using an Additive Screen kit (Hampton Research), crystals suitable for X-ray diffraction experiments were obtained (Fig. 2) within 2 d using reservoir solution consisting of 20%(*w*/*v*) PEG MME 2000, 0.2 *M* triammonium citrate pH 7.0, 0.1 *M* imidazole–HCl pH 7.0, 10%(*v*/*v*) glycerol and 10 m*M* ATP.

3. Results and discussion

The result of the SDS–PAGE analysis is shown in Fig. 1. A complete data set was collected to 2.35 Å resolution on BL-5A at the Photon Factory (PF; Tsukuba, Japan) at a wavelength of 1.0000 Å using an

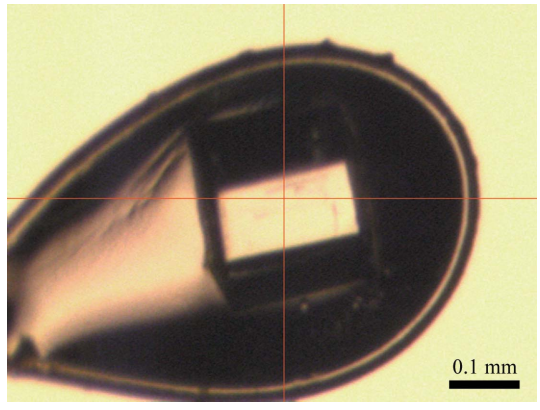

Figure 2
Crystal of *D. radiodurans* DdrA157 with dimensions of 0.2 × 0.2 × 0.2 mm.

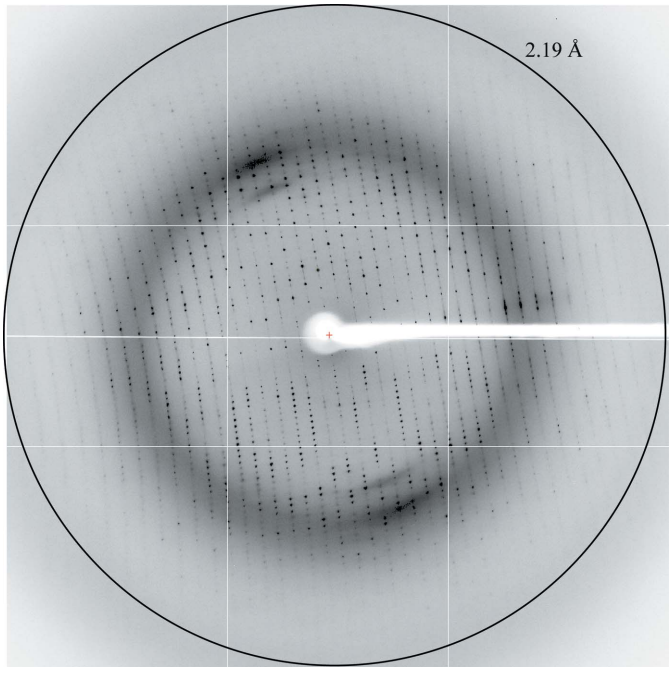

Figure 3
Diffraction image of *D. radiodurans* DdrA157 to 2.19 Å resolution.

ADSC Quantum 315r CCD detector (Fig. 3). The crystal was directly cooled in a stream of evaporating nitrogen at 95 K without additional cryoprotectant. All data were processed using *HKL*-2000 (Otwinowski & Minor, 1997). Data-collection statistics are summarized in Table 1. It was first supposed that the crystal belonged to the orthorhombic space group $P222_1$, with unit-cell parameters a = 46, b = 114, c = 180 Å. The $R_{\rm merge}$ value (0.065) also seemed to be consistent with this supposition. However, another possibility was indicated by $N(Z)$ and L tests in *PHENIX* (Adams *et al.*, 2010). In the L test, the plot of the cumulative intensity distribution of the acentric data almost perfectly overlapped with that of a theoretical perfect twin. From this result, we thought that the correct space group was monoclinic with a β angle of approximately 90° and the crystal was a pseudo-merohedral twin. Therefore, we reprocessed the data in space group $P2_1$, with unit-cell parameters a = 46.31, b = 180.26, c = 114.17 Å, β = 90.02°. The $R_{\rm merge}$ value (0.073) was almost the same as the value obtained for the orthorhombic space group. Using *phenix.xtriage*, the twin fraction was estimated to be 0.46 with twin law $h, -k, -l$ (Yeates, 1997). The asymmetric unit was expected to contain 14 molecules (two heptamers), with a crystal volume per unit molecular weight of 2.17 Å^3 Da^{-1}, corresponding to a solvent content of 43.3% (Matthews, 1968). Molecular replacement was performed using *MOLREP* (Vagin & Teplyakov, 2010) from *CCP*4 (Collaborative Computational Project, Number 4, 1994) with a monomer comprising residues 25–178 of the N-terminal domain of human Rad52 (PDB code 1kn0; Kagawa *et al.*, 2002) as the search model, but phase determination was unsuccessful. The crystal structure is now being solved by the MAD method using selenomethionine-labelled DdrA157.

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