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Crystallization and preliminary X-ray crystallographic analysis of the RecR protein from *Deinococcus radiodurans*, a member of the RecFOR DNA-repair pathway

Byung Il Lee, Kyoung Hoon Kim, Sun Mi Shim, Kyung Soo Ha, Jin Kuk Yang, Hye-Jin Yoon, Jun Yong Ha and Se Won Suh*

Structural Proteomics Laboratory, Department of Chemistry, College of Natural Sciences, Seoul National University, Seoul 151-742, South Korea

Correspondence e-mail: sewonsuh@snu.ac.kr

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The RecR protein plays a key role in the RecFOR pathway of recombination, which is necessary for the repair of ssDNA gaps. RecR from *Deinococcus radiodurans* has been overexpressed in *Escherichia coli* and crystallized at 297 K using polyethylene glycol 1000 as a precipitant. X-ray diffraction data to 2.90 Å resolution have been collected at 100 K using Cu $K\alpha$ X-rays from a mercury-soaked crystal. The crystal belongs to space group $C222_1$, with unit-cell parameters $a = 106.96$, $b = 122.25$, $c = 156.01$ Å. The asymmetric unit contains four monomers of RecR, with a crystal volume per protein weight (V_M) of 2.57 Å^3 Da^{-1} and a solvent content of 51.0%.

1. Introduction

The primary function of homologous genetic recombination systems in bacteria is the recombination-mediated repair of stalled or collapsed DNA-replication forks (Cox, 2001). In *Escherichia coli*, two major pathways of homologous recombination operate: the RecBCD and RecF pathways. The RecBCD pathway is responsible for the repair of double-stranded DNA breaks (DSBs). In this pathway, the RecBCD enzyme alone provides the helicase, nuclease and RecA-loading activities. The RecFOR pathway is essential for nearly all non-DSB recombination in cells and is especially necessary for the repair of single-stranded DNA (ssDNA) gaps. This pathway requires several separate proteins (RecQ helicase, RecJ nuclease, RecF, RecO and RecR) to process the DNA into a presynaptic intermediate and a concerted action of the RecFOR complex directs the loading of RecA protein specifically onto gapped DNA that is coated with the ssDNA-binding protein (SSB; Morimatsu & Kowalczykowski, 2003). The RecR protein forms a complex with RecO or RecF or possibly both. The RecOR complex promotes RecA filament nucleation on ssDNA substrates that are already bound to SSB (Umezu & Kolodner, 1994). It also inhibits a net end-dependent disassembly of RecA filaments (Shan *et al.*, 1997). The RecFR complex blocks the extension of the growing RecA filament (Webb *et al.*, 1997). Together, these activities could suffice to constrain RecA filaments to DNA gaps where repair was to take place (Webb *et al.*, 1997).

RecR proteins contain a potential helix–hairpin–helix motif, a Cys_4-type zinc finger, a Toprim domain and an ATP-binding motif known as a Walker B motif (Peláez *et al.*, 2001). The DNA-binding activity of RecR proteins is, however, controversial. RecR from *Bacillus subtilis* was shown to bind to DNA (Alonso *et al.*, 1993), whereas *Escherichia coli* RecR exhibited no DNA-binding activity *in vitro* (Webb *et al.*, 1995). An interaction between RecF and RecR enables both proteins to bind tightly to dsDNA and RecR stimulates a weak ATPase activity of RecF (Webb *et al.*, 1995). Despite the important role of RecR in the RecFOR pathway of recombinational DNA repair, no information on its three-dimensional structure is available. In order to better understand the function of RecR, it is necessary to elucidate its three-dimensional structure. As the first step toward structure determination, we report here the overexpression, crystallization and preliminary X-ray crystallographic data of RecR from *Deinococcus radiodurans* (220 residues, subunit MW = 23 723 Da).

2. Experimental

2.1. Protein overexpression and purification

The *recR* gene (DR0198) encoding RecR was amplified by the polymerase chain reaction (PCR) using the genomic DNA of *D. radiodurans* strain R1 as a template. The forward and reverse oligonucleotide primers designed using the published genome sequence (White *et al.*, 1999) were 5′-G GAA TTC **CAT ATG** AAA TAT CCG CCT TCC CTC G-3′ and 5′-CCG CCG **CTC GAG** GCG GGA TGC GGG CAC CGC-3′, respectively, where the *Nde*I and *Xho*I restriction sites are shown in bold. The PCR product digested with *Nde*I and *Xho*I was inserted into the *Nde*I/*Xho*I-digested expression vector pET-21a(+) (Novagen). This construction adds an eight-residue tag (LEHHHHHH) to the C-terminus of the recombinant protein to facilitate protein purification. The protein was overexpressed in *E. coli* B834(DE3) cells (Novagen). The cells

were grown at 310 K in 8 l of Terrific Broth medium to an OD_{600} of 0.6 using a fermentor (Korea Fermentation Inc.) and expression of the recombinant protein was induced by 0.5 m*M* isopropyl β-D-thiogalactopyranoside (IPTG) at 310 K. Cell growth was continued at 310 K for 16 h after IPTG induction and cells were harvested by centrifugation at 4200*g* (6000 rev min^{-1}; Sorvall GS3 rotor) for 10 min at 277 K. The cell pellet was suspended in ice-cold lysis buffer (20 m*M* Tris–HCl pH 7.9, 500 m*M* sodium chloride and 50 m*M* imidazole) and homogenized by sonication. The crude lysate was centrifuged at 70 400*g* (30 000 rev min^{-1}; Beckman 45Ti rotor) for 1 h at 277 K and the cell debris was discarded. The first purification step utilized the C-terminal hexahistidine tag by using an Ni^{2+}-chelated chelating Sepharose column (Amersham Biosciences). The eluent was diluted tenfold with 50 m*M* Tris–HCl pH 7.5. Further purification was achieved by ion-exchange chromatography on Source 15Q resin packed inside a HR10/10 column (Amersham Biosciences) which was previously equilibrated with 50 m*M* Tris–HCl pH 7.5 containing 2 m*M* 2-mercaptoethanol. The protein was eluted with a linear gradient of 0–1.0 *M* sodium chloride in the same buffer. The final purification step was gel filtration on a HiLoad 16/60 Superdex-200 prep-grade column (Amersham Biosciences) which was previously equilibrated with a buffer containing 50 m*M* Tris–HCl pH 7.5, 2 m*M* 2-mercaptoethanol and 100 m*M* sodium chloride. The purified protein solution was concentrated to 13 mg ml^{-1} using an YM10 membrane (Amicon-Millipore). The protein concentration was estimated by measuring the absorbance at 280 nm, employing the calculated molar extinction coefficient of 7920 M^{-1} cm^{-1} (SWISS-PROT; http://www.expasy.ch/).

2.2. Dynamic light scattering, crystallization and X-ray data collection

Dynamic light-scattering experiments were performed on a DynaPro-801 instrument from Protein Solutions (Lakewood, New Jersey, USA). The data were measured at 297 K at both 1 and 5 mg ml^{-1} protein in 50 m*M* Tris–HCl pH 7.5, 100 m*M* sodium chloride and 2 m*M* 2-mercaptoethanol.

Initial crystallization conditions were searched for by the microbatch crystallization method using 72-well microbatch plates (Hampton Research) under Al's Oil (Hampton Research) at 297 K. The crystallization drop was prepared by mixing 1 µl each of protein solution and reservoir solution. Crystal Screens I and II, MembFac (Hampton Research) and Wizard I and II screening solutions (Emerald Biostructures) were employed. The initial crystallization conditions were further optimized by the hanging-drop vapour-diffusion method. Each hanging drop was prepared by mixing 3 µl each of protein solution and reservoir solution and was placed over 0.5 ml reservoir solution.

For X-ray diffraction data collection, a crystal mounted on a nylon loop was transferred directly from the hanging drop to a nitrogen-gas stream at 100 K. Data were collected at 100 K using Osmic mirror-focused Cu $K\alpha$ X-rays on a Rigaku R-AXIS IV++ image-plate detector. The crystal was rotated through a total of 87° with 1.0° oscillation per frame. The raw data were processed and scaled using the program *CrystalClear* (Rigaku MSC).

3. Results

The recombinant *D. radiodurans* RecR with a C-terminal eight-residue tag has been overexpressed in *E. coli* in a soluble form, with a yield of ∼60 mg of purified protein per litre of culture. The native molecular weight of the recombinant protein as estimated by dynamic light-scattering analysis is ∼116 000 Da at 1 mg ml^{-1} and ∼225 000 Da at 5 mg ml^{-1}. The results indicate that *D. radiodurans* RecR is either tetrameric or octameric depending on its concentration (the calculated monomer weight including the the C-terminal tag is 24 788 Da). The best crystals were obtained using a reservoir solution consisting of 0.1 *M* imidazole pH 8.0, 16–20%(*w*/*v*) polyethylene glycol 1000, 0.2 *M* calcium acetate and 20%(*v*/*v*) (±)1,3-butanediol. Crystals grew to approximate dimensions of 0.25 × 0.25 × 0.15 mm within 3 d (Fig. 1).

Crystals could be transferred directly from the hanging drop to the nitrogen Cryostream for X-ray experiments, as the (±)1,3-butanediol in the mother liquor acted as a cryoprotectant. However, the diffraction images were anisotropic: crystals diffracted to better than 3.2 Å along the *c** direction, but only diffracted to 3.8 Å along the *b** direction upon exposure to Cu $K\alpha$ X-rays. We accidentally discovered that this anisotropy problem could be overcome by soaking the crystals in 5 m*M* ethylmercurythiosalicylate for 1 d. When exposed to Cu $K\alpha$ X-rays, the mercury-soaked crystals showed an isotropic diffraction pattern which extended beyond 2.9 Å.

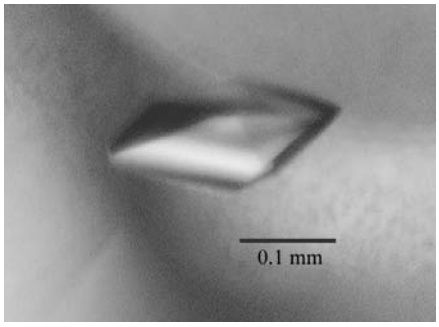

Figure 1
A crystal of RecR from *D. radiodurans*. Its approximate dimensions are 0.25 × 0.25 × 0.15 mm.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell (3.00–2.90 Å).

X-ray wavelength (Å)	1.5418
Temperature (K)	100
Resolution range (Å)	29.1–2.90
Space group	$C222_1$
Unit-cell parameters (Å)	a = 106.96, b = 122.25, c = 156.01
Total/unique reflections	68390/21287
Multiplicity	3.2 (3.0)
Completeness (%)	87.1 (86.0)
Mean $I/\sigma(I)$	8.3 (2.4)
$R_{\rm merge}$† (%)	5.9 (20.5)

† $R_{\rm merge} = \sum_h \sum_i |I(h,i) - \langle I(h)\rangle| / \sum_h \langle I(h)\rangle$, 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.

A set of diffraction data was collected to 2.90 Å using Cu $K\alpha$ X-rays from a mercury-soaked crystal. A total of 68 390 measured reflections were merged into 21 287 unique reflections with an $R_{\rm merge}$ (on intensity) of 5.9%. Systematic absences indicate that the crystal belongs to the orthorhombic space group $C222_1$. The unit-cell parameters are $a = 106.96\ (7), b = 122.25\ (9), c = 156.01\ (9)$ Å, where the estimated standard deviations are given in parentheses. The presence of two to six subunits of the recombinant RecR in the asymmetric unit is plausible, but it seems most likely that the asymmetric unit contains four subunits, giving a crystal volume per protein weight ($V_{\rm M}$) of 2.57 Å^3 Da^{-1} and a corresponding solvent content of 51.0% (Matthews, 1968). The self-rotation function did not give any clues to the number of subunits present in the asymmetric unit. The data-collection statistics are summarized in Table 1. Since no similar structure is currently available, we plan to solve the structure of *D. radiodurans* RecR by the multiwavelength anomalous diffraction method.

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