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Crystallization and preliminary X-ray diffraction analysis of the HsdR subunit of a putative type I restriction enzyme from *Vibrio vulnificus* YJ016

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Type I restriction enzymes are multimeric proteins that consist of three subunits. The HsdS and HsdM subunits form a complex protein that shows methyltransferase activity, while the HsdR subunit functions as an endonuclease as well as as a translocase. Of these three subunits, no structural information on the HsdR subunit is yet available. The putative HsdR gene from *Vibrio vulnificus* YJ016 (HsdR_Vv) was cloned and expressed and the expressed protein HsdR_Vv was purified. HsdR_Vv was crystallized from 8%(*w*/*v*) polyethylene glycol 3350, 0.15 *M* ammonium chloride, 0.1 *M* HEPES pH 7.5 and 2 m*M* β-mercaptoethanol. Diffraction data were collected to 2.60 Å resolution using synchrotron radiation. The crystal belongs to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 71.01, b = 89.04, c = 113.66 Å. With one HsdR_Vv molecule in the asymmetric unit, the Matthews coefficient was 2.14 Å^3 Da^{-1} and the solvent content was 42%.

1. Introduction

Type I restriction-modification (RM) systems or restriction enzymes are hetero-oligomeric proteins that are encoded by three closely linked genes and contain a specificity subunit (HsdS) for recognizing a specific DNA sequence, a methylation subunit (HsdM) for modifying target bases and a restriction subunit (HsdR) for random cleavage of DNA (Dryden *et al.*, 2001; Sistla & Rao, 2004). They recognize two specific DNA sequences which are asymmetric and are separated by 6–8 base pairs. The HsdM and HsdS proteins form an active trimeric M_2S_1 complex which is required for methylation activity and the detection of the methylation status of target DNA (Taylor *et al.*, 1992; Dryden *et al.*, 1993; Janscak & Bickle, 1998). Upon the identification of a specific DNA sequence with a hemimethylation, they transfer a methyl group to the target adenine nucleoside in the remnant strand of the recognition sequence (Wilson & Murray, 1991). The addition of two HsdR subunits to the methyltransferase completes the type I enzyme and confers the ability to cleave DNA containing unmodified bases on both strands (Dryden *et al.*, 1997). In contrast to the other three types of restriction enzyme (types II, III and IV), which cleave DNA at or near the recognition nucleotide (Pingoud & Jeltsch, 1997; Rao *et al.*, 2000), type I RM systems cut DNA thousands of base pairs away from the recognition sequence when neither of two cognate bases on both strands are methylated (Murray, 2000).

According to limited proteolysis, sequence and mutational analysis of the HsdR subunits of *Eco*KI and *Eco*AI (Davies *et al.*, 1999; Janscak *et al.*, 1999), about 400 residues at the N-terminus include the amino-acid motif for endonuclease activity and about 300 residues at the C-terminus provide the contacting core region with methyltransferase. The amino-acid residues between the two terminal regions show close sequence similarity to domains 1A and 2A of DNA and RNA helicases and contain a DEAD-box sequence motif implicated in ATP binding, ATP hydrolysis and DNA translocation (Bird *et al.*, 1998; Davies *et al.*, 1999).

Recently, a structural model of the methylase of a type I RM has been produced (Obarska *et al.*, 2006) based on two crystal structures of the putative HsdS subunit (Calisto *et al.*, 2005; Kim *et al.*, 2005) and two crystal structures of HsdM subunits (PDB codes 2okc and 2ar0). However, there is no molecular information available on the HsdR subunit of a type I RM system, although a crystallization report on the HsdR subunit *Eco*R124I from *Escherichia coli* has been published (Lapkouski *et al.*, 2007). In order to determine the molecular mechanism of restriction and ATP hydrolysis and its contribution to the translocation activity of type I RM systems, we have carried out the crystallization and preliminary X-ray crystallographic analysis of the HsdR subunit (gi:37678449) of a putative type I RM system from *Vibrio vulnificus* YJ016 (HsdR_Vv).

2. Methods

2.1. Cloning, expression and purification of HsdR_Vv

The *V. vulnificus* YJ016 gene coding for the putative HsdR subunit (Met1–Gln817) was amplified from *V. vulnificus* YJ016 chromosomal DNA by the polymerase chain reaction (PCR). The PCR product was then cloned into pProExHTc (Invitrogen), which expresses 25 extra amino acids including six successive histidine residues at the N-terminus. The expression construct was transformed into *E. coli* B834(DE3) and was grown in LB medium containing 100 µg ml^{-1} ampicillin at 310 K. After induction with 1.0 m*M* IPTG for a further 8 h at 310 K, the culture was harvested by centrifugation at 5000*g* at 277 K. The cell pellet was resuspended in ice-cold buffer *A* (20 m*M* Tris–HCl pH 8.0 and 500 m*M* NaCl) and disrupted by ultrasonication. The cell debris was removed by centrifugation at 11 000*g* for 1 h. The HsdR_Vv fusion protein was purified using a 5 ml HisTrap chelating column (GE Healthcare, Uppsala, Sweden) and the bound protein was eluted with a linear gradient from 0 to 500 m*M* imidazole in buffer *A*. After cleavage with rTEV to remove the His_6 tag, a protein consisting of HsdR_Vv with five additional amino acids (YFQGA) at the N-terminus was purified using a 5 ml HiTrapQ anion-exchange column (GE Healthcare, Uppsala, Sweden). For further purification, size-exclusion chromatography using Sephacryl S-300 HR (GE Healthcare, Uppsala, Sweden) was performed in a buffer consisting of 20 m*M* Tris–HCl pH 7.5 and 200 m*M* NaCl. The purified protein was >95% pure as judged by Coomassie Blue-stained SDS–PAGE (data not shown).

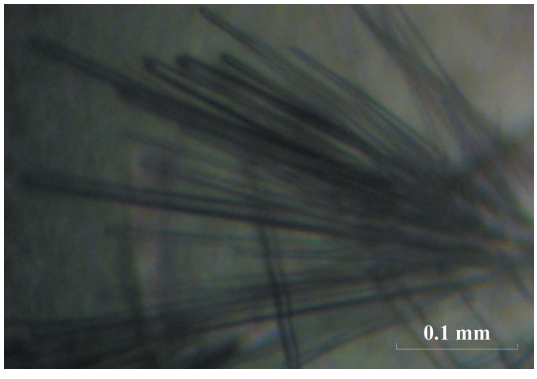

Figure 1
Crystals of HsdR_Vv. The crystals grew at 291 K within two weeks to maximum dimensions of approximately 0.05 × 0.05 × 0.4 mm.

Table 1
Data-collection statistics for HsdR_Vv.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.23985
Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 71.01, b = 89.04, c = 113.66
Resolution range (Å)	50.0–2.60 (2.7–2.60)
Measured reflections	109943
Unique reflections	20675
Multiplicity	5.3
Temperature (K)	100
Matthews coefficient (Å^3 Da^{-1})	2.14
Solvent content (%)	42
No. of molecules in ASU	1
Completeness (%)	97.3 (84.2)
Mean $I/\sigma(I)$	5.4 (1.4)
$R_{\rm merge}$† (%)	9.1 (51.1)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the observed intensity of an individual reflection and $\langle I(hkl)\rangle$ is the mean intensity of that reflection.

2.2. Crystallization

For crystallization, the purified HsdR_Vv protein was concentrated to 5 mg ml^{-1} in a buffer consisting of 20 m*M* Tris–HCl pH 7.5 and 100 m*M* NaCl. Initial crystallization conditions for HsdR_Vv were obtained from sparse-matrix screening (Jancarik & Kim, 1991) and included precipitant 1 [15%(*v*/*v*) ethanol, 0.1 *M* MES pH 6.0 and 0.2 *M* zinc acetate], precipitant 2 [12%(*w*/*v*) polyethylene glycol 20000 and 0.1 *M* MES pH 6.5] and precipitant 3 [10%(*w*/*v*) polyethylene glycol 3350, 0.2 *M* L-proline and 0.1 *M* HEPES pH 7.5] at 291 K. In order to obtain crystals suitable for X-ray diffraction, the precipitant and protein concentrations, buffer pH and temperatures were changed systematically and various equilibrium strategies, for example the hanging-drop and sitting-drop vapour-diffusion methods, were tried.

2.3. X-ray data collection

For data collection, crystals were briefly immersed into a precipitant solution containing 10%(*v*/*v*) glycerol as a cryoprotectant and immediately placed in a 100 K nitrogen-gas stream. Native X-ray diffraction data were collected at MAXII6C, Pohang Accelerator Laboratory (PAL, Korea) using 1° oscillation per image with a crystal-to-detector distance of 180 mm. The crystal was exposed for 10 s per image. A data set was collected to 2.60 Å resolution from a single crystal. The data were indexed and scaled with *HKL*-2000 (Otwinowski & Minor, 1997). The data-collection statistics are summarized in Table 1.

3. Results and discussion

Recombinant HsdR_Vv protein was successfully expressed and purified to homogeneity using sequential chromatographic steps. Crystals suitable for diffraction experiments were obtained using the hanging-drop vapour-diffusion method at 291 K within 14 d by mixing 1 µl protein solution and 1 µl reservoir solution and equilibrating against 200 µl reservoir solution, which consisted of 8–10%(*w*/*v*) polyethylene glycol 3350, 0.15 *M* ammonium chloride, 0.1 *M* HEPES pH 7.5 and 2 m*M* β-mercaptoethanol. The dimensions of the crystal used for data collection were approximately 0.05 × 0.05 × 0.2 mm (Fig. 1) and the crystal diffracted to 2.60 Å resolution, which should be sufficient for structural studies of HsdR_Vv. The crystal belonged to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 71.01, b = 89.04, c = 113.66 Å. The asymmetric unit contains one HsdR_Vv molecule, resulting in a

Matthews coefficient of 2.14 Å^3 Da^{-1} and a solvent content of 42% (Matthews, 1968).

Although a recent publication described the crystallization and preliminary diffraction analysis of HsdR from *E. coli* (Lapkouski *et al.*, 2007), the structure of an HsdR subunit of a type I RM system is not available. Therefore, the crystal structure of HsdR_Vv is now being solved by the SAD method with selenium and other heavy-atom derivatives as anomalous scatterers.

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