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Crystallization and preliminary X-ray crystallographic analyses of *Eco*O109I and its complex with DNA

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*Eco*O109I is a type II restriction endonuclease that recognizes seven base pairs of the degenerate and discontinuous sequence RGGNCCY. The enzyme and its complex with DNA were successfully crystallized by the hanging-drop vapour-diffusion method using polyethylene glycols as precipitants. The crystal of *Eco*O109I belongs to space group $I4$, with unit-cell parameters $a = b = 175.5$, $c = 44.6$ Å, and that of the DNA complex belongs to space group $P2_12_12_1$, with unit-cell parameters $a = 49.1$, $b = 71.8$, $c = 203.2$ Å. Full sets of X-ray diffraction data from the enzyme and its complex with DNA were collected to 2.4 and 1.9 Å resolution, respectively.

1. Introduction

The restriction-modification system, comprised of restriction endonucleases (REases) and DNA methyltransferases, hydrolyzes the foreign DNA of invading bacteriophages and is therefore of great importance for antiviral protection in bacteria. So far, more than 3500 REases have been characterized and classified into four types: I, II, III and IV (Roberts *et al.*, 2003). Of these, type II REases are most familiar and are widely used in genetic technology. The amino-acid sequences of the type II enzymes show no sequence similarities except for the active-site regions, where the enzymes have partially conserved sequences, PD(X)$_n$DXK. In general, type II REases act as dimers and hydrolyze at a specific position within 4–8 base pairs of double-stranded DNAs in the presence of a divalent metal ion such as Mg^{2+} or Mn^{2+}. However, it is interesting that most type II enzymes are inactive in the presence of Ca^{2+} (Bennett & Halford, 1989), but form stable Ca^{2+}-bound enzyme–DNA complexes without cleaving the DNA (Vipond & Halford, 1995).

To date, 15 three-dimensional structures have been determined for type II REases using X-ray diffraction (REBASE; http://rebase.neb.com/cgi-bin/crylist), but details of the catalytic mechanisms and the number of metal ions necessary to express the enzyme activity remain unclear (Galburt & Stoddard, 2002). Furthermore, type II enzymes so far analyzed by X-ray diffraction are mostly six-base cutters and no three-dimensional structure has been obtained for a seven-base cutter. Three-dimensional structures that recognize degenerate and discontinuous sequences have also not been determined in the form of complexes with DNA. *Eco*O109I is a type II REase that recognizes double-stranded DNAs with seven base pairs of the degenerate and discontinuous sequence RGGNCCY (R = A, G; Y = T, C) and cleaves the phosphodiester bond between the second and the third bases to produce 5′-overhang DNAs. Here, we report the crystallization and preliminary X-ray crystallographic analysis of *Eco*O109I and of its complex with cognate DNA as the first step toward elucidating the catalytic mechanism of type II REases in atomic detail.

2. Materials and methods

2.1. Crystallization of *Eco*O109I

*Eco*O109I was expressed in *Escherichia coli* and purified as described by Kita *et al.* (1999). The purified protein dissolved in 10 mM potassium phosphate buffer pH 7.5 containing 100 mM KCl, 0.1 mM EDTA, 5% glycerol and 10 mM β-mercaptoethanol was concentrated to 0.6 mM (20 mg ml^{-1}) at 277 K in a Centricon-10 concentrator (Amicon) for crystallization.

Crystallization was performed at 277 K by the hanging-drop vapour-diffusion method. Crystals of *Eco*O109I (Fig. 1) were grown in hanging drops equilibrated over a reservoir solution consisting of 0.1 M Tris–HCl buffer pH 8.5 containing 150 mM KCl and 22%(w/w) PEG 4K. The drops were prepared by mixing 2 µl *Eco*O109I solution with 2 µl reservoir solution. Crystals were soaked stepwise in buffer solution containing 5, 10 and 13% glycerol and then frozen by immersion in liquid nitrogen.

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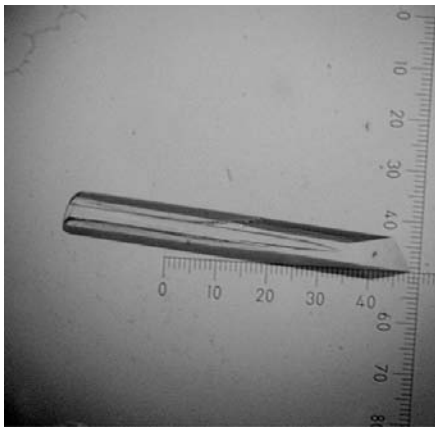

Figure 1
A crystal of *Eco*O109I. Its approximate dimensions are 1.0 × 0.1 × 0.1 mm.

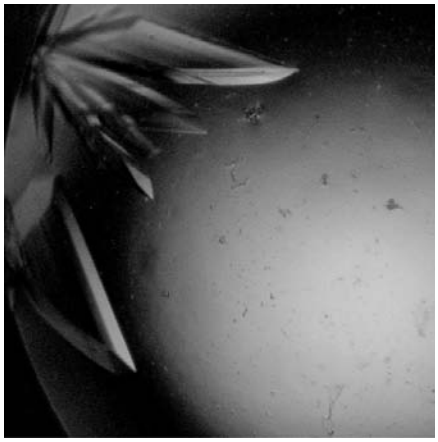

Figure 2
Crystals of the *Eco*O109I–DNA complex. Typical dimensions are 0.5 × 0.4 × 0.1 mm.

2.2. Crystallization of the *Eco*O109I–DNA complex

DNA oligonucleotides (5′-ACCGGGC-CCTGCC-3′ and 5′-GGCAGGGCCCGGT-3′) were purchased from Invitrogen. The oligonucleotides were dissolved in 100 m*M* KCl solution to a final concentration of 0.33 m*M*. The solution was heated to 368 K for 5 min and then cooled to room temperature for 12 h. Equimolar *Eco*O109I and DNA solutions were mixed together and incubated at 277 K for 30 min. The concentration of *Eco*O109I–DNA complex for crystallization was 0.21 m*M*.

Table 1
Data-collection statistics of *Eco*O109I and the *Eco*O109I–DNA complex.

Values in parentheses are for the highest resolution shells, 2.49–2.40 and 1.97–1.90 Å for *Eco*O109I and for the *Eco*O109I–DNA complex, respectively.

	*Eco*O109I	*Eco*O109I–DNA complex
X-ray source	SPring-8 BL38B1	Rigaku FR-D
Wavelength (Å)	1.0000	1.5418
Space group	$I4$	$P2_12_12_1$
Unit-cell parameters		
a (Å)	175.5	49.1
b (Å)	175.5	71.8
c (Å)	44.6	203.2
Resolution range (Å)	20–2.4	67.7–1.9
$R_{\rm merge}$† (%)	4.2 (25.7)	8.8 (28.4)
Average $I/\sigma(I)$	15.3	7.4
No. observations	132339	202201
No. unique reflections	26765	55952
Data completeness (%)	99.1 (99.0)	93.5 (89.4)

† $R_{\rm merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$, where $I(h)$ is the intensity of reflection h, $\sum_h$ is the sum over all measured reflections and $\sum_i$ is the sum over i measurements of a reflection.

Crystallization of the *Eco*O109I–DNA complex was performed at 293 K by the hanging-drop vapour-diffusion method. Crystals of the *Eco*O109I–DNA complex (Fig. 2) were grown in hanging drops equilibrated over a reservoir solution consisting of 0.1 *M* MES buffer pH 6.1 containing 13%(*w*/*v*) PEG 20K, 0.4 *M* CH_3COONa and 6%(*w*/*v*) 6-aminocaproic acid. The drops were prepared by mixing 1 µl *Eco*O109I–DNA solution with 1 µl reservoir solution. The crystals were soaked in buffer solution containing 20% ethylene glycol and then frozen in an N_2-gas stream at 100 K.

3. Results and discussion

Crystals of *Eco*O109I belong to space group $I4$, with unit-cell parameters $a = b = 175.5$, $c = 44.6$ Å. A full set of X-ray diffraction data was collected at 100 K on BL38B1 at SPring-8 (Hyogo, Japan) with an ADSC Quantum 4R CCD detector. The data were processed with *HKL*2000 (Otwinowski & Minor, 1997). Data-collection statistics are given in Table 1. The presence of two enzyme molecules with a molecular weight of 31 500 Da in the asymmetric unit gives a $V_{\rm M}$ value of 2.7 Å^3 Da^{-1} and a solvent content of 54.5% (Matthews, 1968).

Crystals of the *Eco*O109I-DNA complex belong to space group $P2_12_12_1$, with unit-cell parameters $a = 49.1$, $b = 71.8$, $c = 203.2$ Å. Diffraction data from the DNA complex were collected at 100 K on a Rigaku R-AXIS IV^{++} imaging-plate system equipped with Osmic confocal mirror optics mounted on a Rigaku FR-D ultrahigh-brilliance rotating-anode X-ray generator operated at 50 kV and 60 mA. The data were processed with *CrystalClear* (Pflugrath, 1999). Data-collection statistics are given in Table 1. The presence of two enzyme molecules and one double-stranded DNA in the asymmetric unit gives a $V_{\rm M}$ value of 2.8 Å^3 Da^{-1} and a solvent content of 56.4% (Matthews, 1968). Structure analyses of *Eco*O109I and the *Eco*O109I–DNA complex are in progress.

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