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Crystallization and preliminary X-ray diffraction analysis of restriction endonuclease *Eco*RII

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Crystals of the restriction endonuclease *Eco*RII have been obtained by the vapor-diffusion technique in the presence of ammonium sulfate or polyethylene glycol. The best crystals were grown with ammonium sulfate as a precipitant. Crystals with dimensions of up to $0.6 \times 0.6 \times 0.6$ mm have been observed. The crystals diffract to about 4.0 Å resolution at a cryo-temperature of 100 K using a rotating-anode X-ray source and a Rigaku R-AXIS IV imaging-plate detector. The space group has been determined to be either $I23$ or $I2_13$, with unit-cell parameters $a = b = c = 160.3$ Å, $\alpha = \beta = \gamma = 90^\circ$. The crystal asymmetric unit contains two protein molecules, and self-rotation function analysis shows a pseudo-twofold symmetry relating the two monomers. Attempts to improve the resolution of crystal diffraction and to search for heavy-atom derivatives are under way.

1. Introduction

*Eco*RII is a member of the expanding group of type II restriction endonucleases, subclass IIe, which share the distinguishing feature of requiring cooperativity between two recognition sites in their substrate DNA (Pein *et al.*, 1989; Oller *et al.*, 1991; Petrauskene *et al.*, 1992; Karpova *et al.*, 1993). *Eco*RII recognizes the nucleotide sequence 5′-CCWGG (W = A or T) in double-stranded DNA and cleaves it at the 5′ end of the first cytosine base. The endonuclease activity is inhibited by C5 methylation of the second cytosine. The enzyme is catalytically inactive on single recognition sites in larger DNA molecules and even on two sites in the same DNA (located '*cis*') when their site-separation distance exceeds a critical length. Hydrolysis of these *a priori* resistant DNA substrates can be achieved by the addition of (i) susceptible *Eco*RII sites (Krüger *et al.*, 1988) or (ii) a short synthetic *Eco*RII recognition site containing oligonucleotide duplexes (Pein *et al.*, 1989; Petrauskene *et al.*, 1992). The activation of enzyme occurs when the *Eco*RII endonuclease dimer interacts with two DNA recognition sites (Karpova *et al.*, 1993; Petrauskene *et al.*, 1994). DNA cleavage requires the presence of Mg^{2+} cofactor, but DNA recognition and binding do not. *Eco*RII is a 45.6 kDa protein; its sequence has been determined (Kossykh *et al.*, 1989; Bhagwat *et al.*, 1990). However, the structural basis of *Eco*RII–DNA interaction has not been elucidated. In fact, to the best of our knowledge, *Eco*RII endonuclease has not previously been crystallized and neither have any other type II endonucleases of subclass IIe. We report here the crystallization and preliminary X-ray diffraction analysis of the restriction endonuclease *Eco*RII.

2. Materials and methods

*Eco*RII was isolated from an overproducing strain, pLK1/*E. coli* B834 (dcm^-) and purified to homogeneity according to the procedure of Karpova *et al.* (1999). Endonuclease activity was examined using the plasmid pBR322 (dcm^-) isolated from the *E. coli* strain B834. Purified protein was concentrated using a Centricon 30 to 5–15 mg ml^{-1} and was stored for 1–3 weeks at 277 K prior to crystallization. Crystallization was performed by the vapor-diffusion technique in both hanging and sitting drops. The best crystals were obtained when 2–5 µl of protein in 20 m*M* Tris–HCl buffer pH 7.5, 1 m*M* EDTA and 7 m*M* β-mercaptoethanol were mixed with an equal volume of reservoir precipitant solution containing 20 m*M* Tris–HCl buffer pH 7.5, 1.5 *M* ammonium sulfate, 1 m*M* EDTA and 7 m*M* β-mercaptoethanol. Crystals usually appeared within 1–3 weeks. Typically, a crystal was first transferred to a cryo-protectant solution, which was prepared by adding glycerol to the reservoir solution to a final concentration of 20–30%. It was then picked up by a fiber loop and flash-frozen at 100 K in a stream of cold nitrogen gas. Diffraction data were collected on a Rigaku R-AXIS IV image-plate detector using Cu $K\alpha$ radiation from a Rigaku RU300 rotating-anode X-ray generator operating at 50 kV and 100 mA. The programs *DENZO* and *SCALEPACK* were used for data processing and analysis (Otwinowski & Minor, 1996).

3. Results and discussion

Crystals of *Eco*RII were obtained with two precipitants: polyethylene glycol (PEG) and ammonium sulfate. Crystals grown with PEG were small and non-reproducible, while those grown with ammonium sulfate tended to be larger and more reproducible. Both the sitting-drop and hanging-drop methods produced crystals of the same quality with dimensions up to 0.6 mm (Fig. 1). Crystals decayed rapidly when exposed to X-rays at room temperature. Therefore, a variety of cryoprotection solutions and procedures were tried. Under optimal conditions, the crystals diffracted to about 4.0 Å resolution at cryo-temperatures (100 K) with our conventional in-house rotating-anode X-ray source and would be expected to diffract to higher resolution at a synchrotron source owing to its much stronger beam intensity.

Oscillation photos were used in auto-indexing to determine the unit-cell parameters, which were found to be $a = b = c = 160.3$ Å, $\alpha = \beta = \gamma = 90^\circ$. The crystal had the symmetry of a cubic *I*-centered space group. A data set to 4.6 Å was collected with an oscillation range of 2° for a total of 60° with 60 min exposure per frame. The data completeness and $I/\sigma(I)$ were 99.8% and 21.0 overall, respectively, and were 99.6% and 3.9, respectively, for the last shell (4.8–4.6 Å). The $R_{\rm merge}$ of the data set for the Laue group *m*3 was 0.064, while that for the Laue group *m*3*m* was 0.465. Numerous data sets with other crystals produced similar statistics. Therefore, the symmetry of the diffraction pattern was consistent with the Laue group *m*3 and the crystal space group must be either *I*23 or $I2_13$.

With the known molecular weight of about 45.6 kDa for an *Eco*RII monomer and a crystal volume per asymmetric unit of 171600 Å^3, assuming two monomers per asymmetric unit would yield a Matthews coefficient V_m of 1.9 Å^3 Da^{-1} (Matthews, 1968) and a solvent content of 36% (Westbrook, 1985). These values of V_m and solvent content are typical for protein crystals (Matthews, 1968) and strongly support the presence of two monomers per asymmetric unit. In order to determine the relationship between the two crystallographically distinct monomers, we calculated the self-rotation function of the data using the program *MERLOT* (Fitzgerald, 1988). In the $\kappa = 180^\circ$ section (Fig. 2), the three strongest peaks, with a maximum peak height of 100, at (i) $\varphi = 0$, $\psi = 90^\circ$, (ii) $\varphi = 90$, $\psi = 90^\circ$; and (iii) $\varphi = 0$, $\psi = 0^\circ$ represent the three crystallographic twofold axes of the crystal. The three peaks with the second highest peak height of about 60 at (i) $\varphi = 0$, $\psi = 45^\circ$, (ii) $\varphi = 90$, $\psi = 45^\circ$ and (iii) $\varphi = 45$, $\psi = 90^\circ$, which are related by a crystallographic threefold axis, are non-crystallographic twofold axes relating the two monomers in the asymmetric unit. The positions of these pseudo-twofold axes in relation to the crystallographic twofold axes in the rotation map imply that the crystal may possess pseudo-432 symmetry, while the relative peak heights indicate that the crystal does not have true 432 symmetry. Careful re-indexing and re-examination of the raw diffraction data reconfirmed the crystal Laue symmetry to be *m*3. The fact that the crystal asymmetric unit consists of a dimer is consistent with the finding that in solution the active form of enzyme *Eco*RII is a dimer.

Figure 1
A crystal of the restriction endonuclease *Eco*RII. The longest dimension of the crystal is about 0.6 mm.

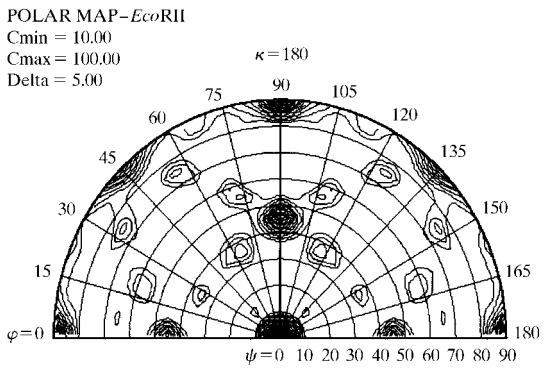

Figure 2
A plot of the self-rotation function calculated by the program *MERLOT* (Fitzgerald, 1988).

In attempting to grow crystals of higher resolution, we investigated how the presence of divalent cations affected the resolution limit. We could not grow crystals with divalent cations in the crystallization solution; however, soaking crystals with divalent cations added to the mother liquor slightly increased the diffraction resolution. Soaking the crystals with Mg^{2+} ions, a cofactor of the enzyme, resulted in crystals which diffracted to 4 Å. This suggests that a likely reason for low resolution is the flexibility of some part of the *Eco*RII structure within the crystals, which is stabilized in the presence of Mg^{2+}, with a resulting increase in resolution. For the same reason, we expect that binding the DNA substrate to *Eco*RII may also result in a dramatically improved diffraction resolution limit. Co-crystallization of *Eco*RII with DNA substrates are currently under way. A search for potential heavy-atom derivatives is also in progress.

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References

Bhagwat, A. S., Johnson, B., Weule, K. & Roberts, R. J. (1990). *J. Biol. Chem.* **265**, 767–773.
Fitzgerald, P. M. D. (1988). *J. Appl. Cryst.* **21**, 273–278.
Karpova, E. A., Kubareva, E. A., Gromova, E. S. & Buryanov, Y. (1993). *Biochem. Mol. Biol. Int.* **29**, 113–121.
Karpova, E. A., Kubareva, E. A. & Shabarova, Z. A. (1999). *IUBMB Life*, **48**(1). In the press.
Kossykh, V., Repyk, A., Kaliman, A. & Buryanov, Y. (1989). *Biochim. Biophys. Acta*, **1009**, 290–292.
Krüger, D. H., Barcak, G. J., Reuter, M. & Smith, H. O. (1988). *Nucleic Acids Res.* **16**, 3997–4008.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Oller, A. R., Broek, W. V., Conrad, M. & Topal, M. D. (1991). *Biochemistry*, **30**, 2543–2549.
Otwinowski, Z. & Minor, W. (1996). *Methods Enzymol.* **276**, 307–326.
Pein, C.-D., Reuter, M., Cech, D. & Krüger, D. H. (1989). *FEBS Lett.* **245**, 141–144.
Petrauskene, O. V., Karpova, E. A., Gromova, E. S. & Guschlbauer, W. (1994). *Biochem. Biophys. Res. Commun.* **198**, 885–890.
Petrauskene, O. V., Kubareva, E. A., Gromova, E. S. & Shabarova, Z. A. (1992). *Eur. J. Biochem.* **208**, 617–622.
Westbrook, E. M. (1985). *Methods Enzymol.* **114**, 187–196.