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Crystallization and preliminary crystallographic analysis of the site-specific DNA nickase Nb.BspD6I

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Crystals of site-specific DNA nickase Nb.BspD6I (of molecular weight 70.8 kDa) have been grown at 291 K using PEG 8000 as precipitant. The diffraction pattern of the crystal extends to 3.3 Å resolution at 100 K. The crystal belongs to space group $P2_1$, with unit-cell parameters a = 57.76, b = 90.67, c = 71.71, β = 110.1°. There is one molecule in the asymmetric unit and the solvent content is estimated to be 53% by volume.

1. Introduction

Site-specific DNA nickases are a recently discovered class of enzymes that like type II restriction endonucleases recognize a short specific sequence on double-stranded DNA and cleave DNA at a fixed distance from the site. However, in contrast to restriction endonucleases, the nickases make a nick in a predetermined DNA strand. Thus, the nickases are both site-specific and strand-specific endonucleases. Four naturally occurring enzymes have so far been identified in bacterial sources (Abdurashitov *et al.*, 1996; Morgan *et al.*, 2000; Zheleznaya *et al.*, 2002; Dedkov *et al.*, 2001). One of them, the nickase Nb.BspD6I (N.BspD6I in old nomenclature), was found and characterized by Zheleznaya *et al.* (2002). All the enzymes recognize the DNA sequence 5′-GAGTC-3′/5′-GACTC-3′ and cleave only the top strand 4 bp downstream of the recognition site. The 604 amino-acid Nb.BspD6I nickase (molecular weight 70.8 kDa) was cloned and sequenced (accession No. AJ534336).

Selective nicking of double-stranded DNA is frequently employed in biological research and diagnostics such as isothermal strand-displacement amplification of DNA (Walker & Linn, 1996), site-directed mutagenesis (Sayers *et al.*, 1988), preparation of nicked or gapped DNA for studying DNA-mismatch repair (Wang & Hays, 2001) and DNA labelling (Li *et al.*, 2002). Diagnostics assays based on the nickases have already been designed for the detection of DNA targets using molecular beacons (Zheleznaya *et al.*, 2002) and for isothermal reactions for the amplification of oligonucleotides (Van Ness *et al.*, 2003). New methods and technologies may be designed to exploit the nickases and some DNA nanotechnology and DNA computing problems may also be solved by the application of nickases (Zhang *et al.*, 2002; Wang *et al.*, 2001).

Given that the naturally existing nickases recognize the same site, several approaches have been developed for constructing nickases with expanded specificities (Xu *et al.*, 2001; Kuhn *et al.*, 2003; Samuelson *et al.*, 2004). We hope that the crystal structure of the nickase Nb.BspD6I will provide a structural basis for such work.

2. Expression and purification of the nickase

The Nb.BspD6I nickase (Zheleznaya *et al.*, 2001) has been identified in *Bacillus* sp. strain D6 isolated from soil. The strain overproducer of the nickase has been obtained by transformation of *Escherichia coli* TOP10F′ cells (Invitrogen) with two recombinant plasmids (Rogulin *et al.*, 2004). The plasmid pET28b/bspD6IN carries the nickase gene under the bacteriophage T7 promoter. The plasmid pRARE/sscL1IM contains the methylase M.SscL1I gene inserted into plasmid pRARE. The M.SscL1I has been shown to protect host DNA against hydrolysis by the nickase (Zheleznaya *et al.*, 2002). The pRARE (Novagen)

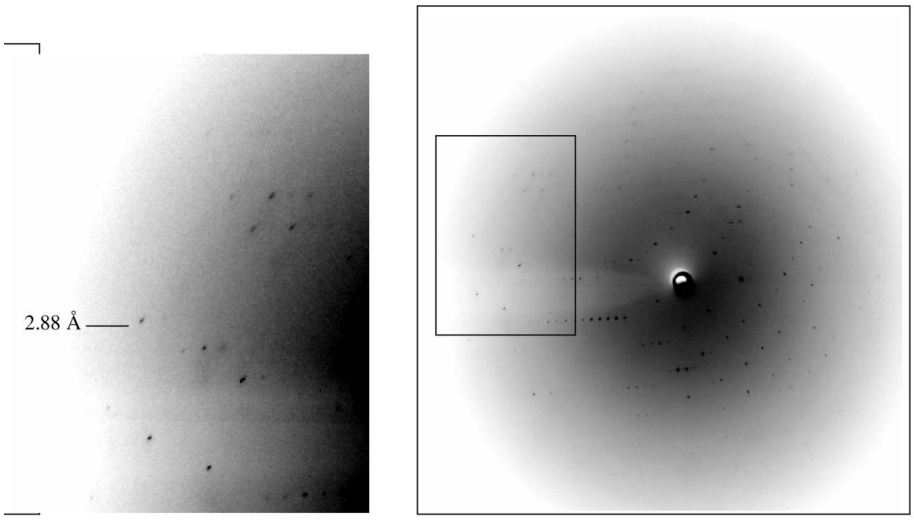

Figure 1
An 0.5° oscillation image of nickase. The inset shows diffraction to 2.9 Å resolution. A highly complete data set was obtained for the resolution range 40–3.2 Å.

Table 1
Data-collection statistics.

Values in parentheses are for data in the last resolution shell.

Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 57.76, b = 90.67, c = 71.71, β = 110.1
Temperature (K)	100
Wavelength (Å)	1.05
Oscillation angle (°)	0.5
Crystal-to-detector distance (mm)	210
Resolution limits (Å)	40–3.2 (3.35–3.2)
Exposure time per image (s)	130
Total No. of observations	43451
Unique reflections	9843
Completeness (%)	97.5 (86.6)
$I/\sigma(I)$	15.2 (37.1)
$R_{\rm merge}$†	0.103 (0.531)

† $R_{\rm merge} = \sum_{hkl}\sum_j |I(hkl)_j - \langle I(hkl)\rangle| / \sum_{hkl}\sum_j I(hkl)_j$.

contains genes of tRNA that rarely occur in *E. coli*. The expression of the nickase was induced by infection with bacterophage CE6 (Novagen), a λ recombinant that carries the cloned T7 RNA polymerase gene under control of the phage p_L promoter.

The cells were grown in LB medium (1% Bacto tryptone, 0.5% yeast extract, 1% NaCl, final pH 7.3) in the presence of 35 mg ml^{-1} kanamycin and 10 mg ml^{-1} chloramphenicol. The nickase expression was induced by infection with CE6 at a multiplicity of 10. After incubation for 3 h at 310 K, the cells were collected by centrifugation, suspended in 20 m*M* potassium phosphate pH 7.5, 50 m*M* KCl, 1 m*M* DTT, 1 m*M* EDTA, 0.5 m*M* phenylmethylsulfonyl fluoride and disrupted with an ultrasound disintegrator. The cell debris was removed by centrifugation. The supernatant was tested for nickase activity and then applied onto a 100 ml phosphocellulose P11 (Whatman) column equilibrated with buffer *A* (20 m*M* potassium phosphate pH 7.5, 50 m*M* KCl, 1 m*M* DTT). The proteins were eluted from the column with a linear KCl gradient (0.05–1 *M*) in buffer *A*. Fractions with nickase were pooled, diluted in 20 m*M* potassium phosphate pH 7.5, 1 m*M* DTT to a concentration of 150 m*M* KCl and then loaded onto a 20 ml hydroxyapatite (BioRad) column equilibrated with buffer *B* (20 m*M* potassium phosphate pH 7.5, 150 m*M* KCl, 1 m*M* DTT). The proteins were eluted using a linear gradient (0.02–0.5 *M*) of potassium phosphate. Fractions in which the nickase was more than 95% pure were pooled and used for crystallization.

3. Crystallization

The purified protein was dialyzed against a solution containing 10 m*M* Tris–HCl pH 7.2, 50 m*M* KCl, 10%(*v*/*v*) glycerol. The protein was concentrated to 12 mg ml^{-1}; the concentration was determined from the absorbance at 280 nm, assuming an ε_{280} of 1.035 for 1.0 mg ml^{-1} protein solution. Initial crystallization conditions were screened using Crystal Screen (Hampton Research), Wizard I and Wizard II (Emerald BioStructures) and Crystal Screen Cryo (Hampton Research) kits employing the sitting-drop vapour-diffusion method. Good-quality crystals were obtained using 0.04 *M* KH_2PO_4, 16%(*w*/*v*) PEG 8000, 20%(*v*/*v*) glycerol as precipitant. The crystallization drop comprised 1 µl protein solution and 1 µl reservoir solution. The mixture was equilibrated against 100 µl reservoir solution at 291 K. Needle-shaped crystals with approximate dimensions of 0.3 × 0.1 × 0.05 mm grew over a period of several weeks. For diffraction experiments, crystals were flash-cooled directly from the drop solution.

4. Data collection

Diffraction data (Fig. 1) were measured from native crystals. All data were collected at 100 K at beamline BW6/DORIS (DESY, Hamburg, Germany) using an X-ray wavelength of 1.05 Å and a MAR CCD detector. The data were processed using the programs *DENZO* and *SCALEPACK* from the *HKL* package (Minor, 1993). The data-collection statistics are summarized in Table 1. The systematic absence of axial reflections uniquely assigned the crystals to the monoclinic space group $P2_1$, with unit-cell parameters a = 57.76, b = 90.67, c = 71.71 Å, β = 110.1°. The Matthews coefficient (Matthews, 1968) of 2.6 Å^3 Da^{-1} for one molecule in the asymmetric unit indicates a solvent content of 53%. No structural homologues of nickase are known. Structure solution will be attempted by isomorphous replacement methods.

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