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Crystallization and preliminary X-ray diffraction analysis of the small subunit of the heterodimeric restriction endonuclease R.BspD6I

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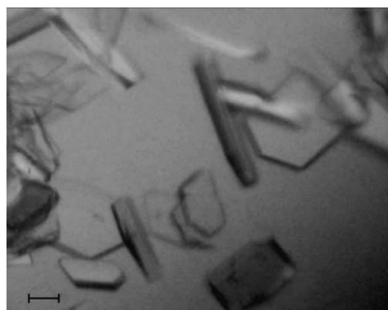

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The heterodimeric restriction endonuclease R.BspD6I is composed of a small subunit with a cleavage site and a large subunit, containing a recognition domain and a cleavage domain, that may function separately as a monomeric nicking endonuclease. Here, the crystallization of the small subunit and diffraction data collection to 1.5 Å resolution are reported.

1. Introduction

In contrast to orthodox type II restriction endonucleases, which function as homodimers that recognize palindromic sequences, a number of type II enzymes are composed of two different subunits and recognize asymmetric sequences. Examples are the previously characterized heterodimeric restriction endonucleases (REases) R.Bpu10I (Stankevicius *et al.*, 1998) and R.BbvCI (Heiter *et al.*, 2005), which recognize an asymmetric sequence and cleave both DNA strands within the recognition sequence. Both REases were found to be composed of two mutually homologous but not identical subunits with molecular weights around 30 kDa, each subunit containing a catalytic site for DNA-strand hydrolysis. Inactivation of the catalytic site of one of the two subunits, leaving the other subunit intact, transformed each of these heterodimeric REases into a nicking enzyme that cleaved one of the DNA strands. Recently, it was demonstrated that the monomeric nicking endonuclease N.BspD6I and a protein, ss.BspD6I, encoded by an open reading frame adjacent to the *bspD6IN* gene form a complex, R.BspD6I, which represents a new type of heterodimeric restriction endonuclease (Yunusova *et al.*, 2006). R.BspD6I possesses a number of new features compared with the previously known heterodimeric REases: (i) R.BspD6I recognizes the pseudosymmetric sequence 5′-GAGTC-3′/5′-GACTC-3′ and cuts both DNA strands outside the recognition sequence, (ii) the subunits that constitute R.BspD6I exhibit grossly different molecular weights of 70.8 kDa (large subunit; N.BspD6I) and 21.6 kDa (small subunit; ss.BspD6I), (iii) the large subunit alone acts as a type IIS site-specific monomeric nicking endonuclease and (iv) the small subunit alone does not bind DNA and does not display any nuclease activity. The complex formed by N.BspD6I and ss.BspD6I cleaves the 5′-GAGTC-3′ strand at a distance of four nucleotides downstream of this sequence, as is performed by N.BspD6I alone, and the 5′-GACTC-3′ strand at a distance of six nucleotides downstream.

In earlier work, we characterized and crystallized (Perevyazova *et al.*, 2003; Kachalova *et al.*, 2005) the nickase N.BspD6I and recently solved its crystal structure (PDB code 2ewf). Protein sequence alignment between ss.BspD6I and the C-terminal domain of N.BspD6I revealed 27% identity and 20% similarity. In particular, the amino-acid sequence of the small subunit was found to contain all the amino-acid residues forming the active site of N.BspD6I. We crystallized the small subunit in order to analyze the structure of its active site and to provide a structural basis for investigating its interactions with the large subunit within the heterodimeric complex R.BspD6I.

2. Expression and purification

The expression and purification of the small-subunit protein have been described previously (Yunusova *et al.*, 2006). The gene encoding

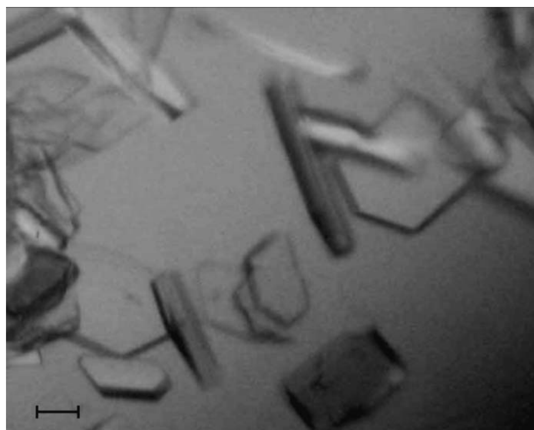

Figure 1
Typical crystals of ss.BspD6I. The scale bar corresponds to 100 μm.

ss.BspD6I was PCR-amplified from the genomic DNA of *Bacillus* sp. strain D6 isolated from soil. The amplified product was inserted into the expression vector pET28c (Novagen) containing a C-terminal His_6-tag coding sequence. The His-tagged protein was purified on an Ni–NTA agarose column.

3. Crystallization

The purified protein was dialyzed against a solution containing 10 m*M* Tris–HCl pH 7.5, 50 m*M* KCl, 10 m*M* DTT, 0.1 m*M* EDTA, 10%(*w*/*v*) glycerol. Initial crystallization conditions were screened using the 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. Crystals of good quality diffracting to 1.5 Å resolution were obtained using ammonium sulfate as a crystallizing agent (2.0 *M* ammonium sulfate, 0.1 *M* CAPS pH 10.5, 0.2 *M* lithium sulfate). The crystallization drops, comprising 1 μl protein solution with a protein concentration of 35 mg ml^{-1} and 2 μl reservoir solution, were equilibrated against 100 μl reservoir solution at 293 K. Plate-shaped crystals with approximate dimensions of 0.15 × 0.15 × 0.03 mm grew over a period of 1–2 weeks (Fig. 1).

Table 1
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

Beamline	BW6 (MPG/DESY, Hamburg, Germany)
Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 38.43, b = 38.36, c = 62.00, β = 101.47
Data-collection temperature (K)	100
Wavelength (Å)	1.05
Total No. of observations	82823
Resolution range (Å)	60–1.5 (1.53–1.5)
Mosaicity (°)	0.5
Redundancy	2.91 (2.87)
Unique reflections	28430 (1428)
Completeness (%)	99.3 (99.7)
Mean $I/\sigma(I)$	21.2 (2.24)
$R_{\rm merge}$†	0.054 (0.47)

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

4. X-ray data collection

Crystals were transferred to a cryoprotectant solution, which contained the mother liquor and 5% glycerol, and were flash-frozen in a cold nitrogen-gas stream (X-Stream, MSC). Diffraction data (Fig. 2) were collected at 100 K to 1.5 Å resolution using a MAR CCD detector at the MPG beamline BW6 (DESY, Hamburg, Germany).

5. Results and discussion

The X-ray data were integrated and scaled using the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The data-collection statistics are summarized in Table 1. The crystals belong to space group $P2_1$, with unit-cell parameters a = 38.43, b = 38.36, c = 62.00 Å, β = 101.47°. Assuming the presence of one protein molecule in the asymmetric unit, the Matthews coefficient (V_M; Matthews, 1968) was calculated to be 2.1 $Å^3$ Da^{-1}, which corresponds

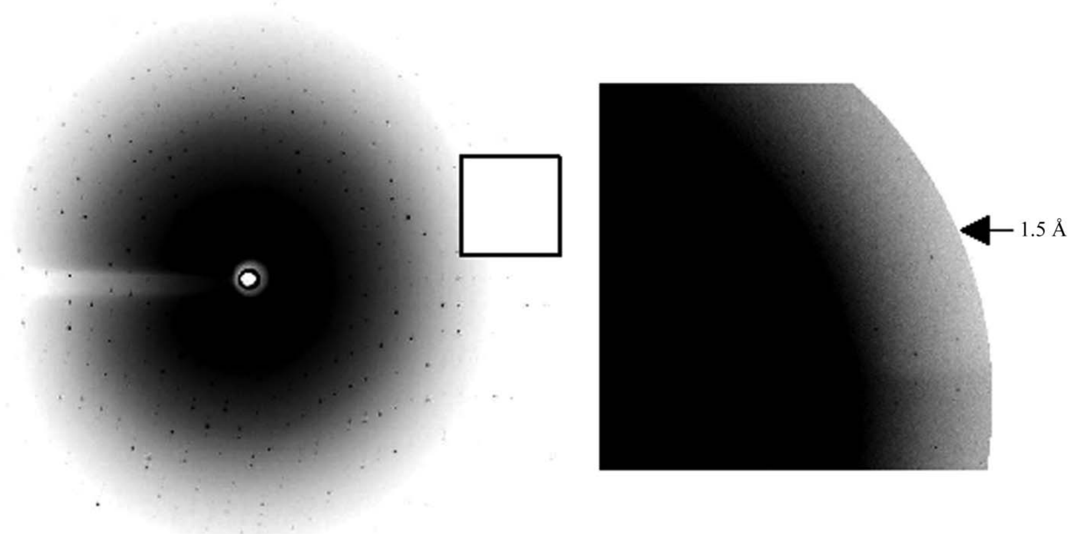

Figure 2
A 1° oscillation pattern from a crystal of ss.BspD6I. The inset shows diffraction to the edge of the image, corresponding to 1.5 Å resolution.

to a solvent content of 42%. A search for similar sequences in the PDB identified the C-terminal domain of the large subunit N.BspD6I (PDB code 2ewf) as the only entry yielding a significant alignment score. The structural model of this domain was used as a search model in molecular-replacement calculations with the program *MOLREP* (Vagin & Teplyakov, 1997), which yielded a correct solution. The initial electron-density map could be traced automatically using *ARP/wARP* (Morris *et al.*, 2003), resulting in a structural model of the complete small subunit ss.BspD6I. Structure refinement and interpretation are in progress.

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