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Crystallization and preliminary X-ray diffraction studies of Hje, a Holliday junction resolving enzyme from *Sulfolobus solfataricus*

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Holliday junction endonuclease (Hje) from *Sulfolobus solfataricus* is a resolving enzyme involved in cleaving specific sites on either side of recombinant four-way Holliday junctions. The *HJE* gene from *S. solfataricus* was cloned from genomic DNA into the pET19b *Escherichia coli* expression vector and recombinant protein was expressed to high levels. Hje was purified using heat treatment, cation exchange and gel filtration. Hanging-drop crystallization trials yielded primitive hexagonal crystals which diffract to 2.4 Å on a laboratory source. Systematic absences (only $00l = 6n$ present) and poor scaling in $P622$ indicate that the space group is $P6_1$ or its enantiomer. Failed attempts at molecular replacement using models of a related archaeal resolving enzyme, Hjc, raise the possibility of a difference in quaternary structure between Hjc and Hje, which may be responsible for differences in their activities.

1. Introduction

Holliday junction resolving enzymes bind and specifically cleave the four-way Holliday junctions in DNA created during repair and rearrangement by the ubiquitous process of homologous recombination (reviewed comprehensively by Lilley & White, 2001). These junctions are formed by strands of DNA exchanging between homologous duplex DNA molecules. Subsequent branch migration of the Holliday junction generates stretches of heteroduplex recombinant DNA. The introduction of paired nicks in opposing strands by the binding and cleaving activity of a structure-specific endonuclease, a resolving enzyme, ends the recombination process.

Of the many structurally diverse classes of resolving enzyme – all of which are highly basic, dimeric and metal-dependent – the archaeal enzymes belong to a family represented by Hjc (Holliday junction cleavage), which is distantly related to the type II restriction enzymes (Komori *et al.*, 1999; Kvaratskhelia *et al.*, 2000). Structural studies of Hjc from *Sulfolobus solfataricus* (Bond *et al.*, 2001) and *Pyrococcus furiosus* (Nishino, Komori, Tsuchiya *et al.*, 2001; Nishino, Komori, Ishino *et al.*, 2001) have been reported. An interesting feature of *Sulfolobus* and a small number of other archaea is that they contain a second resolving enzyme with 28% identity to Hjc, namely Hje (Holliday junction endonuclease; Kvaratskhelia & White, 2000*a*,*b*). Despite the sequence similarity, Hjc and Hje cut a Holliday junction differently: on the exchanging and continuous strands, respectively. The structural basis of this differing specificity is the ultimate goal of the preliminary work described here.

2. Expression and purification

The gene encoding *S. solfataricus* Hje (accession number Q97YX6) was amplified from *S. solfataricus* strain P2 genomic DNA using proof-reading polymerase Pfu (Promega) with synthetic oligonucleotides (forward primer, 5′-CGTCGGATCCCCATGGCTAGGGATA-TAGGTAAGAACGCTGAG; reverse primer, 5′-CCGGGGATCCGTCGACTTAAGGCGT-TAATATTTTTTCCTCAATCC).

The oligonucleotides introduced restriction sites at either end of the amplified gene to facilitate subcloning. Amplified *HJE* was subcloned into the *Bam*HI and *Nco*I sites of the expression vector pET19b (Novagen), allowing expression of Hje with a native N-terminus in BL21 CodonPlus (DE3) RIL cells (Stratagene). This resulted in the alteration of the amino acid at position 2 in the sequence from asparagine to alanine, but as this is not a conserved residue the change is unlikely to be significant. DNA sequencing of the insert demonstrated that the correct sequence was obtained by PCR.

Protein expression was induced by addition of 0.2 m*M* IPTG; cell growth continued at 310 K for 3 h, after which cells were pelleted and frozen until required. Cell lysis, centrifugation and chromatography steps were carried out at 277 K. 20 g of cells were thawed in 50 ml lysis buffer (50 m*M* Tris–HCl pH 7.5, 100 m*M* NaCl, 1 m*M* EDTA, 1 m*M* DTT, 1 m*M* benzamidine, 1 m*M* aminoethylbenzene-

sulfonyl fluoride) and immediately sonicated (Sanyo Soniprep 150, 12 µm amplitude) for 5 × 1 min with cooling. The lysate was centrifuged at 40 000*g* for 30 min. The supernatant was heated to 343 K for 30 min in a water bath and denatured proteins were precipitated by centrifugation at 40 000*g* and 277 K. The supernatant was analysed by SDS–PAGE and shown to contain recombinant Hje, which migrated as a band of approximately 16 kDa as expected. The supernatant was diluted fourfold with buffer *A* (50 m*M* Tris–HCl pH 7.5, 1 m*M* EDTA, 1 m*M* DTT) and applied to an SP-Sepharose High Performance 26/10 column (Hi-Load, Amersham-Pharmacia) equilibrated with buffer *A*. A 500 ml linear gradient comprising of 0–1000 m*M* NaCl was used to elute cationic proteins. Fractions corresponding to a distinct absorbance peak were analysed by SDS–PAGE, pooled, concentrated and loaded onto a 26/70 gel-filtration column (Superdex 200 Hi-Load, Amersham-Pharmacia) and developed with buffer *A* containing 300 m*M* NaCl. Active fractions were pooled and shown to contain essentially homogeneous Hje protein. This protein was used for all subsequent analyses.

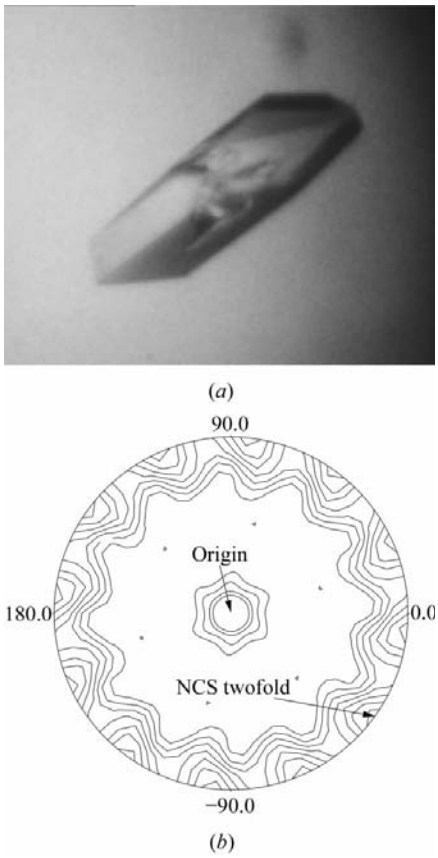

Figure 1
(*a*) Typical crystals of Hje, with dimensions of 0.2 × 0.2 × 0.5 mm. (*b*) Self-rotation function for Hje (*POLARRFN*; $\kappa = 180^\circ$, resolution range 20–7 Å, integration radius 20 Å).

3. Crystallization

Crystallization was achieved using the hanging-drop vapour-diffusion method. Initial trials were conducted at 293 K with Hampton Research Crystal Screens I and II. Drops were assembled using 1 µl protein solution (10 mg ml^{-1}) and 1 µl reservoir. Poorly formed needles grew in Crystal Screen I solution number 17 [0.2 *M* lithium sulfate monohydrate, 0.1 *M* Tris buffer pH 8.5, 30%(*w*/*v*) polyethylene glycol (PEG) 4000]. These conditions were optimized by varying the pH and the PEG 4000 concentration and by using a selection of cryoprotectants (glycerol, 2-methyl-2,4-pentanediol, ethylene glycol, ethanol, PEG 400 and 2-propanol) at varying concentrations (5–20% by volume). The crystals used in this study grew using a reservoir containing 0.2 *M* lithium sulfate monohydrate, 0.1 *M* Tris buffer pH 9.0, 20%(*w*/*v*) PEG 4000 with 15% PEG 400 as a cryoprotectant. Suitable crystals also grew in the presence of 20% ethylene glycol. The drops consisted of 1 µl protein solution and 1 µl reservoir solution equilibrated against a 500 µl reservoir at 293 K. Well ordered prisms grew over a period of two weeks (Fig. 1*a*).

4. X-ray analysis

A crystal was flashed-cooled directly from the drop in a stream of nitrogen gas maintained at 100 K for data collection using a Rigaku rotating-anode source (Cu $K\alpha$, $\lambda = 1.5418$ Å) with Osmic mirror optics and an R-AXIS IV detector. 40° of diffraction data were collected in consecutive 0.5° oscillations. Data were processed using *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997) and details are provided in Table 1. The space group is identified as $P6_1$ or enantiomer, with unit-cell parameters $a = 92.07$, $c = 72.49$ Å.

Hje functions as a homodimer with a subunit of 133 amino acids and a molecular mass of ∼16 kDa. As there is no proper twofold rotation axis in $P6_{1/5}$, a minimum asymmetric unit content of a dimer is expected. Although Matthews coefficients (Matthews, 1968) for a monomer (V_M = 11.0 Å^3 Da^{-1}; 89% solvent content) and a dimer (V_M = 2.7 Å^3 Da^{-1}; 55% solvent content) per asymmetric unit are both plausible, the latter is more likely, as supported by the self-rotation function (*POLARRFN*; Collaborative Computational Project, Number 4, 1994), which shows a single strong feature in the $\kappa = 180^\circ$ section (Fig. 1*b*; $\omega = 90.0^\circ$, $\varphi = 23.0^\circ$, peak height = 0.77 × origin peak).

Table 1
Diffraction data statistics.

Values in parentheses refer to the highest resolution shell, 2.49–2.40 Å.

X-ray source	Cu $K\alpha$, 1.5418 Å
Space group	$P6_{1/5}$
Unit-cell parameters (Å)	$a = 92.07$, $c = 72.49$
Mosaicity (°)	0.7
No. of measurements	30803
No. of unique reflections	13327
Resolution range (Å)	25–2.4
Completeness (%)	96.9 (87.2)
R_{sym}	0.101 (0.450)
Average $I/\sigma(I)$	8.7 (1.8)

To our surprise, extensive attempts at molecular replacment using many available software packages (*AMoRe, BEAST* and *MOLREP*; Collaborative Computational Project, Number 4, 1994) and models [including and omitting side chains, monomers and dimers from PDB entries 1hh1 (Bond *et al.*, 2001), 1gef (Nishino, Komori, Tsuchiya *et al.*, 2001) and 1ipi (Nishino, Komori, Ishino *et al.*, 2001)] have been thoroughly unsuccessful. Of particular note is the lack of success of attempts using the Hjc dimer, as sequence comparisons indicate that residues at the Hjc dimer interface are conserved in Hje. Our results suggest that despite this apparent conservation, Hje may indeed have a different dimeric arrangement to Hjc, resulting in its different substrate specificity. X-ray analysis of crystals of a selenomethionine derivative of Hje to phase the structure is currently under way.

In summary, crystals have been obtained of *S. solfataricus* Hje, an enzyme involved in four-way DNA junction cleavage. The crystals are ordered and diffract to 2.4 Å resolution using an in-house X-ray radiation source. Preliminary crystallographic results point to a different dimer arrangement to the homologue Hjc.

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