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Crystallization and preliminary X-ray crystallographic analysis of the tRNA-specific adenosine deaminase from *Streptococcus pyogenes*

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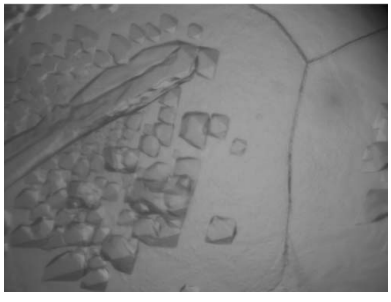

The tRNA-specific adenosine deaminase from the pathogenic bacteria *Streptococcus pyogenes* (*sp*TAD) has been overexpressed in *Escherichia coli* and crystallized in the presence of Zn^{2+} ion at 295 K using ammonium sulfate as a precipitant. Flash-cooled crystals of *sp*TAD diffracted to 2.0 Å using 30%(*v*/*v*) glycerol as a cryoprotectant. X-ray diffraction data have been collected to 2.0 Å using synchrotron radiation. The crystal belongs to the tetragonal space group $P4_22_12$, with unit-cell parameters $a = b = 81.042$, $c = 81.270$ Å. The asymmetric unit contains one subunit of *sp*TAD, with a corresponding crystal volume per protein weight (V_M) of 3.3 Å^3 Da^{-1} and a solvent content of 62.7%.

1. Introduction

Streptococcus pyogenes, a Gram-positive bacterium, is one of the most frequent pathogens of humans. It is estimated that between 5 and 15% of normal individuals harbour the bacterium, usually in the respiratory tract, without signs of disease. As a normal flora, *S. pyogenes* can infect when defences are compromised or when the organisms are able to penetrate the constitutive defences. When the bacteria are introduced or transmitted to vulnerable tissues, a variety of types of suppurative infections can occur. The whole genome of *S. pyogenes* was sequenced in 2001 (Ferretti *et al.*, 2001).

Nuclear pre-mRNA editing by selective adenosine deamination (A-to-I editing) occurs in all organisms from *Caenorhabditis elegans* to humans. The nucleotide inosine (I) has been observed in viral transcripts and eukaryotic mRNAs. Inosine results from the deamination of adenosine (A), a process termed RNA editing in all known cases. RNA editing of pre-mRNAs is catalysed by the adenosine deaminases acting on mRNAs (ADARs). ADARs target double-stranded regions of nuclear-encoded RNA and viral RNA (Powell *et al.*, 1987).

Inosine is not only present in mRNAs, but also in tRNAs. Adenosine deamination in tRNAs is catalyzed by an enzyme family known as the adenosine deaminases that act on tRNAs (ADATs) or tRNA-specific adenosine deaminases (TADs; Gerber *et al.*, 1998). Two TAD activities have been identified, one that deaminates A34 in the tRNA wobble position (Gerber & Keller, 1999) and one that deaminates immediately adjacent to the anticodon (Gerber *et al.*, 1998). In eukaryotes, seven to eight tRNAs contain I at position 34, whereas in prokaryotes and plant chloroplasts only $tRNA^{Arg2}$ contains this modification. The A37 deamination only occurs in eukaryotes and only on A37 of $tRNA^{Ala}$. Since the identification of the first prokaryotic TAD from *Escherichia coli* (Wolf *et al.*, 2002), only one structure of a TAD enzyme (from *Aquifex aeolicus*) has been reported (Kuratani *et al.*, 2005). Therefore, more detailed investigation is required to elucidate the molecular mechanism of the TAD enzyme by comparing the structures of the TAD family from other sources. The TAD from *S. pyogenes* shows approximately 43.1% sequence identity with *A. aeolicus* TAD. As the first step toward its structural explanation, we have overexpressed the tRNA-specific adenosine deaminase from *S. pyogenes* (*sp*TAD) and crystallized it. The crystallization conditions and preliminary X-ray crystallographic data are reported here.

2. Experimental procedures

2.1. Protein expression and purification

The TAD gene was amplified by the polymerase chain reaction using the *S. pyogenes* genomic DNA as a template. The sequences of the forward and reverse oligonucleotide primers designed from the published genome sequence (Banks *et al.*, 2004) were 5′-G GAA TTC **CAT ATG** GAA CTT GGG GAG TCT-3′ and 5′-CCG CCG **CTC GAG** CTA GTC AAA GGG ATC TGA-3′, respectively. The bases in bold represent the *Nde*I and *Xho*I digestion sites, respectively. The amplified DNA was inserted into the *Nde*I/*Xho*I-digested expression vector pET-22b (Novagen). This vector construction added six histidine residues to the C-terminus of the TAD gene product to facilitate protein purification. The protein was overexpressed in *E. coli* BL21(DE3) cells. Cells were grown at 310 K to an OD_{600} of 0.5 in Luria–Bertani medium containing 50 µg ml^{-1} ampicillin and protein expression was induced by addition of 0.5 m*M* isopropyl-*β*-D-thiogalactopyranoside (IPTG). Cell growth continued at 295 K overnight after IPTG induction and cells were harvested by centrifugation at 4200*g* for 10 min at 277 K. The cell pellet was resuspended in ice-cold lysis buffer [50 m*M* Tris–HCl pH 8.5, 2.5%(*v*/*v*) glycerol, 200 m*M* NaCl, 5 m*M* imidazole] and was homogenized with a microfluidizer (Microfludics). The crude cell extract was centrifuged at 70 400*g* (Hanil Supra 21K rotor) for 30 min at 277 K. The recombinant protein in the supernatant fraction was purified by three chromatographic steps. The first step utilized the C-terminal hexahistidine tag by metal-chelate chromatography on Ni^{2+}–NTA resin (Qiagen). Ion-exchange chromatography was performed on a HiTrap Q column (Amersham Pharmacia) followed by a Superdex 75s prep-grade column, which was previously equilibrated with buffer *A* [20 m*M* Tris–HCl pH 7.5, 2.5%(*v*/*v*) glycerol, 150 m*M* NaCl, 5 m*M* DTT]. The homogeneity of the purified protein was assessed by SDS–PAGE. The protein solution was concentrated using a Centri-Prep (Millipore) to a final concentration of 57 mg ml^{-1} in buffer *A*.

2.2. Crystallization and data collection

Initial crystallization was performed at 295 K by the sitting-drop method using a Hydra II Plus One crystallization robot (Matrix Technology) with approximately 1500 conditions using 200 nl precipitant solution and 200 nl protein solution. Crystallization trials were established using screening kits from Hampton Research, Emerald BioStructures and Jena BioSciences. The crystallization conditions were optimized by the hanging-drop vapour-diffusion method using 24-well plates by mixing 1 µl protein solution with 1 µl reservoir solution; the optimal precipitant was 0.1 *M* HEPES pH 7.5, 2%(*w*/*v*) PEG 1000 and 1.65 *M* ammonium sulfate. Each hanging drop was placed over 1 ml reservoir solution. A cryoprotectant solution was developed which consisted of 0.1 *M* HEPES pH 7.5, 2%(*w*/*v*) PEG 1000, 1.7 *M* ammonium sulfate and 30%(*v*/*v*) glycerol. A crystal of spTAD was soaked in 5 µl of this cryoprotectant solution for 10 s before being flash-cooled in liquid nitrogen. It was then mounted on the goniometer in a stream of cold nitrogen gas at 100 K. X-ray diffraction data were collected from the cooled crystal using a Bruker Proteum 300 CCD at beamline 6B at Pohang Light Source (PLS), South Korea. The crystal was oscillated by 1.0° per frame over a total range of 90° at 1.100 Å wavelength. Diffraction data were collected to 2.0 Å resolution and were integrated and scaled with *DENZO* and *SCALEPACK* crystallographic data-reduction routines (Otwinowski & Minor, 1997).

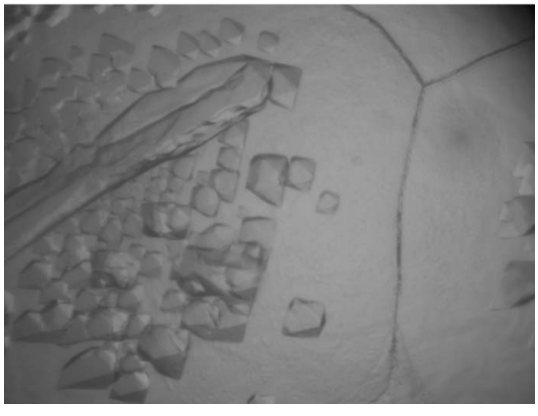

Figure 1
Crystals of *sp*TAD used for data collection.

Table 1
Data-collection statistics.

Values in parentheses are for the outer shell.

X-ray source	Synchrotron
Space group	$P4_22_12$
Resolution (Å)	50–2.0 (2.0–2.07)
No. of unique observations	18891 (2147)
Completeness (%)	89.3 (60.5)
R_{sym}†	0.075 (0.353)
$I/\sigma(I)$	20.1 (25.6)

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

3. Results and discussion

Recombinant TAD from *S. pyogenes* was overexpressed in *E. coli* in soluble form with a yield of ∼20 mg of homogeneous protein from a litre of culture. Initially, irregular crystals were observed. However, after optimization octahedron-like crystals were obtained using a reservoir solution containing 0.1 *M* HEPES pH 7.5, 2%(*w*/*v*) PEG 1000 and 1.65 *M* ammonium sulfate over a period of 3 d (Fig. 1). Flash-cooled crystals of spTAD diffracted to 2.0 Å using 30%(*v*/*v*) glycerol as a cryoprotectant. The autoindexing procedure performed with *DENZO* indicated that the crystals belong to a tetragonal space group, with unit-cell parameters $a = b = 81.042$, $c = 81.270$ Å. The space group was determined to be $P4_22_12$ on the basis of systematic absences. Assuming the presence of one molecule per asymmetric unit, the calculated Matthews coefficient V_M value is 3.3 Å^3 Da^{-1} (Matthews, 1968), which corresponds to a solvent content of 62.7%. Data-collection statistics are given in Table 1. The molecular-replacement method was first tested to solve the crystal structure of *sp*TAD using trial models from the crystal structures of cytosine deaminase from *E. coli* (PDB code 1krm; Ireton *et al.*, 2002) or bovine adenosine deaminase solved at 1.7 Å resolution (PDB code 1ndv; Terasaka *et al.*, 2004). However, none of our attempts provided a clear solution. The phase problem has been solved by the use of the Zn^{2+}-containing *sp*TAD protein crystal. Zn^{2+} is present in the *sp*TAD crystal as the Zn^{2+} ion is needed as a cofactor for the enzyme reaction. We confirmed the presence of the metal ion by using XAFS data, which showed the characteristic spectrum of the zinc ion, with the peak and edge at 1.2820 and 1.2831 Å, respectively. The structure will be determined by the MAD method (Hendrickson *et al.*, 1990) using Zn^{2+} as an anomalous scatterer and the structural details will be described in a separate paper.

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