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Overexpression, crystallization and preliminary X-ray crystallographic analysis of a putative transposase from *Thermoplasma acidophilum* encoded by the *Ta0474* gene

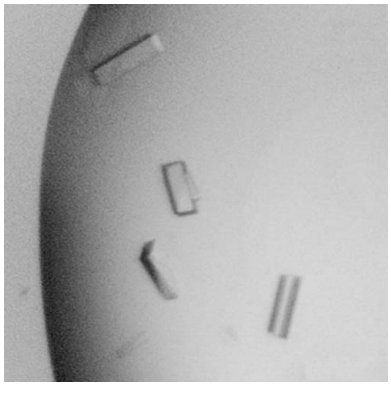
IS200 transposases, originally identified in *Salmonella typhimurium* LT2, are present in many bacteria and archaea and are distinct from other groups of transposases. To facilitate further structural comparisons among IS200-like transposases, structural analysis has been initiated of a putative transposase from *Thermoplasma acidophilum* encoded by the *Ta0474* gene. Its 137-residue polypeptide shows high levels of sequence similarity to other members of the IS200 transposase family. The protein was overexpressed in intact form in *Escherichia coli* and crystallized at 297 K using a reservoir solution consisting of 100 m*M* Na HEPES pH 7.5 and 20%(*v*/*v*) ethanol. X-ray diffraction data were collected to 1.78 Å. The crystals belong to the monoclinic space group $P2_1$, with unit-cell parameters $a = 65.00$, $b = 34.07$, $c = 121.58$ Å, $\alpha = 90$, $\beta = 100.20$, $\gamma = 90^\circ$. Four monomers, representing two copies of a dimeric molecule, are present in the asymmetric unit, giving a crystal volume per protein weight (V_M) of 2.02 Å^3 Da^{-1} and a solvent content of 39.2%.

1. Introduction

Transposons are mobile DNA sequences that can insert themselves into nonhomologous target sites in the genome (Beuzon *et al.*, 2004). Insertion sequences, the smallest transposable elements, are capable of independent transposition because they contain a transposase-encoding gene. At least six protein families have been described that mediate transposition: DDE transposases, Y2 (or rolling-circle) transposases, tyrosine transposases, serine transposases, a fifth family that encodes a combination of reverse transcriptase and endonuclease (RT/En) activities and IS200 (or Y1) transposases (Curcio & Derbyshire, 2003; Ronning *et al.*, 2005). A distinct fold and a unique active site have recently been revealed for IS200 transposases (Ronning *et al.*, 2005; Lee *et al.*, 2006).

The insertion sequences IS605, IS606 and IS608 from *Helicobacter pylori* carry two open reading frames: *tnpA* and *tnpB* (Ton-Hoang *et al.*, 2005). These *tnpA* open reading frames encode unique transposases belonging to the IS200 family. The protein sequences of IS200 transposase-family members do not bear the hallmarks of other well characterized transposases (Ronning *et al.*, 2005). It has been proposed that IS200 transposases be designated as 'Y1 transposases' because a single tyrosine residue is absolutely conserved among the family members (corresponding to Tyr127 in the *H. pylori* IS608 transposase; Ronning *et al.*, 2005). The crystal structure of the *H. pylori* IS608 transposase revealed that it is structurally related to rolling-circle replication proteins (Ronning *et al.*, 2005). Subsequently, the structure of an IS200 transposase from *Sulfolobus solfataricus* (SSO1474) was determined in both Mn^{2+}-bound and Mn^{2+}-free forms (Lee *et al.*, 2006).

To facilitate further structure comparisons between IS200-like transposases, we initiated the structure determination of a putative transposase from *Thermoplasma acidophilum* (Ta0474) that shares high levels of amino-acid sequence identity with the two structurally characterized transposases of the IS200 family. The identity is 50% with *H. pylori* IS608 transposase and 40% with IS200 transposase from *S. solfataricus* (SSO1474). As the first step towards structure determination of the putative transposase from *T. acidophilum*



(Ta0474), we have overexpressed it in *Escherichia coli* and crystallized it. The crystals are suitable for structure determination at high resolution. The crystallization conditions and preliminary X-ray crystallographic data are reported here.

2. Experimental

2.1. Protein expression and purification

The *Ta0474* gene (residues 1–137) was amplified by the polymerase chain reaction using the genomic DNA of *T. acidophilum* strain DSM 1728 as template. The forward and reverse oligonucleotide primers designed using the published genome sequence (Ruepp *et al.*, 2000) are 5′-G GAA TTC **CAT ATG** GAA ATC ATT AAT GTA GAA AAG GAA T-3′ and 5′-CCG CCG **CTC GAG** TTA TCA TTT TCC CTT CTG ATC CTC TA-3′, respectively. The bases in bold represent the *Nde*I and *Xho*I restriction-enzyme cleavage sites. The amplified DNA was inserted into the *Nde*I/*Xho*I-digested expression vector pET-21a(+) (Novagen). This vector construction yields the recombinant enzyme with no fusion tag. The protein was overexpressed in *E. coli* Rosetta2(DE3)pLysS cells. The cells were grown at 310 K to an OD_{600} of 0.5 in Terrific Broth medium containing 50 µg ml^{-1} ampicillin and protein expression was induced by 0.5 m*M* isopropyl *β*-D-thiogalactopyranoside (IPTG). Incubation was continued at 303 K for 24 h after IPTG induction and the cells were harvested by centrifugation at 5600*g* for 10 min at 277 K.

The cell pellet was resuspended in ice-cold lysis buffer [20 m*M* Tris–HCl pH 7.9, 500 m*M* sodium chloride, 5 m*M* imidazole, 5%(*v*/*v*) glycerol and 1 m*M* phenylmethylsulfonylfluoride] and was lysed using an ultrasonic processor. Following heat treatment at 343 K for 10 min, the crude cell extract was centrifuged at 36 000*g* for 1 h at 277 K and the recombinant protein in the supernatant fraction was purified in three chromatographic steps. The first step utilized a heparin Sepharose XK-16 prep-grade column (12 ml; GE Healthcare) which had previously been equilibrated with 20 m*M* Tris–HCl pH 7.5 and 50 m*M* NaCl. The heparin column was used because Ta0474 is likely to interact with DNA. The supernatant fraction was diluted tenfold and applied onto the column. Upon eluting with a gradient of NaCl in the same buffer, Ta0474 transposase was eluted at ∼1 *M* NaCl. After dialysis against 20 m*M* trisodium acetate pH 5.6 for 18 h, the transposase-containing fraction was applied onto a Source-S Sepharose prep-grade column (6 ml; GE Healthcare) which had previously been equilibrated with 20 m*M* trisodium acetate pH 5.6 and 50 m*M* NaCl. Upon eluting with a gradient of NaCl in the same buffer, the transposase was eluted at ∼300 m*M* NaCl. Next, gel filtration was performed on a HiLoad XK-16 Superdex 200 prep-grade column (GE Healthcare) which was equilibrated and eluted with 20 m*M* Tris–HCl pH 7.5, 200 m*M* NaCl and 2 m*M* *β*-mercaptoethanol. The homogeneity of the purified protein was assessed by SDS–PAGE (Laemmli, 1970). The protein solution was concentrated using an YM10 ultrafiltration membrane (Millipore-Amicon). The protein concentration was estimated by measuring the absorbance at 280 nm.

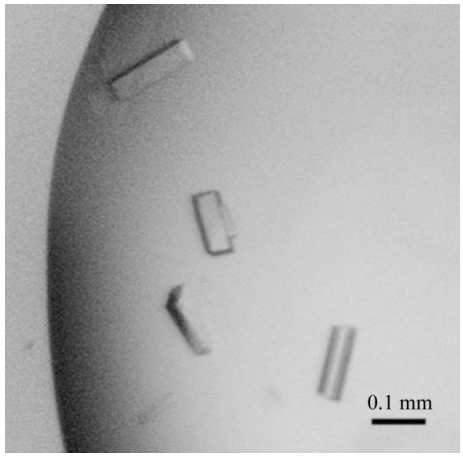


Figure 1
Crystals of a putative transposase encoded by the *Ta0474* gene of *T. acidophilum*.

2.2. Crystallization and dynamic light scattering

Crystallization was performed at 297 K by the hanging-drop vapour-diffusion method using 24-well VDX plates (Hampton Research). Initial crystallization conditions were established using screening kits from Hampton Research (Crystal Screens I and II, SaltRX, Index I and II, PEG/Ion and MembFac) and Emerald BioSystems (Wizard I and II). Each hanging drop was prepared on a siliconized cover slip by mixing 2 µl each of protein solution (4.4 mg ml^{-1} in a buffer consisting of 20 m*M* Tris–HCl pH 7.5, 200 m*M* NaCl and 2 m*M* *β*-mercaptoethanol) and reservoir solution. The hanging drop was placed over 0.48 ml reservoir solution. Dynamic light-scattering experiments were performed on a model DynaPro-801 instrument from Wyatt (Santa Barbara, California, USA). The data were measured at 297 K with the protein at 1 mg ml^{-1} concentration, dissolved in 20 m*M* Tris–HCl pH 7.5, 200 m*M* NaCl and 2 m*M* *β*-mercaptoethanol.

2.3. X-ray diffraction experiment

The crystals were flash-cooled using a cryoprotectant solution consisting of 100 m*M* Na HEPES pH 7.5, 20%(*v*/*v*) ethanol and 30%(*v*/*v*) glycerol. Crystals were soaked in 5 µl cryoprotectant solution for 10 s before being flash-cooled in liquid nitrogen. X-ray diffraction data were collected at 100 K on a Quantum 210 charge-coupled device area-detector system (Area Detector Systems Corporation, Poway, California, USA) at the BL-4A experimental station of the Pohang Light Source, Pohang, South Korea. The crystal was rotated through a total of 180° with a 1.0° oscillation range per frame. The raw data were processed and scaled using the *HKL*-2000 program package (Otwinowski & Minor, 1997).

For the molecular-replacement calculations, a dimer model of IS200 transposase from *S. solfataricus* (PDB code 2f4f; Lee *et al.*, 2006) was used as the search model. The program *PHASER* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994) was used to find one of the two dimers by a cross-rotational search followed by a translational search. The orientation and position of the first dimer was fixed and the second dimer was located using the *MOLREP* program from the *CCP*4 program suite. Manual model building was performed using the program *COOT* (Emsley & Cowtan, 2004). The model was refined with *REFMAC* from the *CCP*4 program package, including simulated annealing, positional refinement and individual *B*-factor refinement.

3. Results

The intact Ta0474 transposase from *T. acidophilum* was overexpressed in *E. coli* in soluble form with a yield of ∼1.5 mg homogeneous protein per litre of culture. The molecular weight of the recombinant transposase was estimated to be ∼39 kDa by dynamic light-scattering analysis, indicating that the enzyme exists as a homodimer in solution (calculated monomer weight 16 379 Da). This

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

X-ray source	Pohang Light Source beamline BL-4A
X-ray wavelength (Å)	0.97950
Temperature (K)	100
Space group	$P2_1$
Unit-cell parameters	
a (Å)	65.00
b (Å)	34.07
c (Å)	121.58
α (°)	90.00
β (°)	100.20
γ (°)	90.00
Resolution range (Å)	50–1.78 (1.84–1.78)
Total/unique reflections	588760/51361
$R_{\rm merge}$† (%)	7.8 (23.7)
Data completeness (%)	99.5 (96.9)
Average $I/\sigma(I)$	11.4 (6.8)
No. of dimers per asymmetric unit	2

† $R_{\rm merge} = \sum_h \sum_i |I(h)_i - \langle I(h)\rangle| / \sum_h \sum_i I(h)_i$, where $I(h)_i$ is the intensity of the ith measurement of reflection h and $\langle I(h)\rangle$ is the mean value of $I(h)$ for all i measurements.

result is in agreement with the dimeric structures of IS200 transposase from *S. solfataricus* (Lee *et al.*, 2006) and IS608 transposase from *H. pylori* (Ronning *et al.*, 2005).

The best crystals were grown using reservoir solution consisting of 100 m*M* Na HEPES pH 7.5 and 20%(*v*/*v*) ethanol. Crystals grew to dimensions of 0.02 × 0.04 × 0.15 mm within 2 d (Fig. 1). A set of X-ray diffraction data was collected to 1.78 Å resolution at 100 K. A total of 588 760 measured reflections were merged into 51 361 unique reflections, giving an $R_{\rm merge}$ of 7.8% and a completeness of 99.5%. The space group was determined to be $P2_1$ on the basis of systematic absences and symmetry of diffraction intensities. The unit-cell parameters are $a = 65.00$, $b = 34.07$, $c = 121.58$ Å, $\alpha = 90$, $\beta = 100.20$, $\gamma = 90°$. Table 1 summarizes the statistics for data collection. If it is assumed that two copies of a dimeric molecule are present in the crystallographic asymmetric unit, the crystal volume per protein weight ($V_{\rm M}$) is 2.02 Å^3 Da^{-1} and the solvent content is 39.2% (Matthews, 1968).

We have solved the structure by the molecular-replacement method using the dimer model of IS200 transposase from *S. solfataricus* (PDB code 2f4f; Lee *et al.*, 2006). The initial molecular-replacement solution gave $R_{\rm work}$ and $R_{\rm free}$ values of 0.446 and 0.491, repscetively, for 30.0–2.50 Å data. The model has been partially refined to crystallographic $R_{\rm work}$ and $R_{\rm free}$ values of 0.258 and 0.279, respectively, for 50.0–1.78 Å data and accounts for 544 residues (residues 1–136 in each chain) of the two copies of a dimeric molecule in the asymmetric unit. It reveals that Ta0474 transposase is structurally very similar to IS200 transposase from *S. solfataricus* (Lee *et al.*, 2006) and IS608 transposase from *H. pylori* (Ronning *et al.*, 2005). Ta0474 transposase (chain *A*) shows a root-mean-square deviation of 1.3 Å for 125 C$^\alpha$-atom pairs with IS200 transposase from *S. solfataricus* (PDB code 2f4f, chain *A*) and 1.2 Å for 128 C$^\alpha$-atom pairs with IS608 transposase from *H. pylori* (PDB code 2a6m, chain *A*). Further refinement is in progress and the structural details will be described in a separate publication.

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