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Crystallization and preliminary X-ray diffraction analysis of PAT, an acetyltransferase from *Sulfolobus solfataricus*

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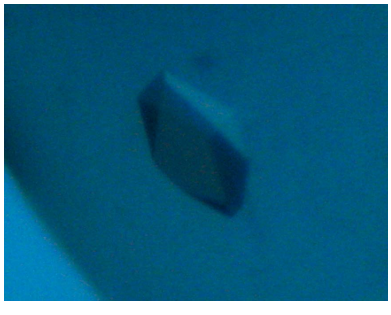

PAT is an acetyltransferase from the archaeon *Sulfolobus solfataricus* that specifically acetylates the chromatin protein Alba. The enzyme was expressed, purified and subsequently crystallized using the sitting-drop vapour-diffusion technique. Native diffraction data were collected to 1.70 Å resolution on the BL13C1 beamline of NSRRC from a flash-frozen crystal at 100 K. The crystals belonged to space group $P2_12_12_1$, with unit-cell parameters a = 44.30, b = 46.59, c = 68.39 Å.

1. Introduction

Protein acetylation is one of the post-translational modifications that allow cells to rapidly alter cellular processes in response to a changing environment (Yang & Seto, 2007). The acetyltransferase enzymes (referred to as GCN5-related *N*-acetyltransferases or GNATs) responsible for this modification have been extensively studied in eukaryotic cells within the context of control of gene expression (Vetting *et al.*, 2005). GNATs catalyze the transfer of the acetyl group from acetyl coenzyme A to the ε-amino groups of lysyl residues in histone tails to facilitate transcription or gene silencing (Yang & Seto, 2007).

Intriguingly, members of the crenarchaeal kingdom of the archaea, such as *Sulfolobus solfataricus*, do not possess histones. Recently, the best characterized *Sulfolobus* chromatin protein, Alba, was found to be acetylated on lysine 16, lowering its affinity for DNA. A *Sulfolobus* homologue of the conserved Sir2 NAD-dependent deacetylase was found to interact with and deactylate Alba (Bell *et al.*, 2002). Since the acetylation is a reversible mechanism, there should be an Sir2-pairing acetyltransferase that acts on Alba. The 160-amino-acid open reading frame encoded by the *pat* gene was identified by a *BLAST* search and demonstrated to acetylate Alba on Lys16 (Marsh *et al.*, 2005). To clarify the structural basis of the catalytic mechanism of PAT (protein acetyltransferase), we present here the crystallization and preliminary X-ray diffraction analysis of PAT.

2. Materials and methods

2.1. Protein expression and purification

The *pat* gene was amplified from *S. solfataricus* chromosomal DNA using the following primers: forward primer, 5′-GGGATCCCATATGAATGACCAGATAAAGATAAG-3′; reverse primer, 5′-GAATTCTCGAGTGGGGCGGAGAAAGTTGCTAG-3′. The amplified fragment designed to direct the production of full-length PAT was cloned into the *Nde*I/*Xho*I site of pET30a(+). The obtained construct, which has an extra LEHHHHHH appended to the C-terminus of PAT, was then introduced into *Escherichia coli* BL21 (DE3) cells. The cells were grown in LB broth in the presence of 50 µg ml^{-1} kanamycin at 310 K. When the optical density at 600 nm of the culture reached about 0.6, gene expression was induced by adding 1 m*M* IPTG and the culture continued for an additional 10 h at 291 K. The cells were harvested by centrifugation (10 000 rev min^{-1}, 10 min, 298 K), resuspended in 30 ml buffer *A* (20 m*M* Tris–HCl pH 8.0, 300 m*M* NaCl) and immediately sonicated for 20 min (60 × 20 s) with

cooling. The lysate was clarified by centrifugation (13 000 rev min^{-1}, 30 min, 277 K). The supernatant was heated to 348 K for 30 min and then clarified by centrifugation (13 000 rev min^{-1}, 30 min, 277 K). The heat-stable PAT remained soluble and was purified by passage through a 5 ml nickel–nitrilotriacetic acid–agarose column (Qiagen). Samples were washed with 40 ml buffer *A* followed by a gradient of 10–500 m*M* imidazole in buffer *A* for protein elution. Positive fractions identified by SDS–PAGE were pooled and dialyzed overnight against buffer *B* (10 m*M* Tris–HCl pH 8.0). The dialysed sample was applied onto a 5 ml HiTrap Q HP column (GE Healthcare) equilibrated with buffer *B* containing 45 m*M* NaCl. The column was developed with a 50-column-volume linear gradient of 10–1000 m*M* NaCl and the target protein was eluted at 300 m*M* NaCl. The theoretical molecular weight of PAT is 19 668 Da. The homogeneity and identity of the purified sample were assessed by SDS–PAGE and mass spectrometry (19 667.3 Da). Finally, the purified PAT was lyophilized after dialysis against deionized water.

2.2. Crystallization

Lyophilized protein was dissolved in 10 m*M* Tris–HCl pH 8.0 and concentrated to 10 mg ml^{-1}. The protein solution (1 µl, 10 mg ml^{-1}) was mixed with an equal volume of reservoir solution for screening for crystallization conditions using the sitting-drop vapour-diffusion method. Initial crystallization conditions were obtained using Hampton Research Crystal Screen kits (Hampton Research, California, USA) and then further optimized from Crystal Screen II condition No. 13 to obtain diffraction-quality crystals. PAT crystals with dimensions of about 0.2 × 0.3 × 0.3 mm (Fig. 1) grew in one week at room temperature over 300 µl of a reservoir solution consisting of 0.2 *M* ammonium sulfate, 0.1 *M* sodium acetate trihydrate pH 4.6 and 30%(*w*/*v*) polyethylene glycol monomethyl ether 2000.

2.3. Data collection and processing

The data collection was completed using a synchrotron-radiation X-ray source on the protein crystallographic beamline BL13B1 at the National Synchrotron Radiation Research Center (NSRRC), Taiwan equipped with a Q315 area detector. The crystal was transferred into a cryoprotectant solution containing 20% PEG 400, mounted on a 0.3–0.4 mm glass loop (Hampton Research) and then flash-cooled in liquid nitrogen to 100 K. For ultrahigh-resolution data collection, 176° of rotation was measured with 1° oscillations using X-rays of wavelength 0.97315 Å at 110 K in the nitrogen-gas stream from an X-Stream cryo-system. Diffraction data were measured in the resolution range 30–1.70 Å with an exposure time of 40 s and a crystal-to-detector distance of 180 mm. Diffraction images were indexed, integrated and scaled using *DENZO* and *SCALEPACK* from the *HKL*-2000 program suite (Otwinowski & Minor, 1997). The crystal belonged to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 44.30$, $b = 46.59$, $c = 68.39$ Å. Assuming the presence of one monomer of 18 kDa protein in the asymmetric unit, the calculated Matthews coefficient (V_M; Matthews, 1968) was 1.96 Å^3 Da^{-1}, with a solvent content of 37.7%. A complete data set has been obtained to 1.70 Å resolution (Fig. 2), corresponding to an R_{merge} of 5.8%. Details of the data-collection statistics are summarized in Table 1.

Structure determination was attempted using the molecular-replacement method as implemented in the program *CNS* (Brunger,

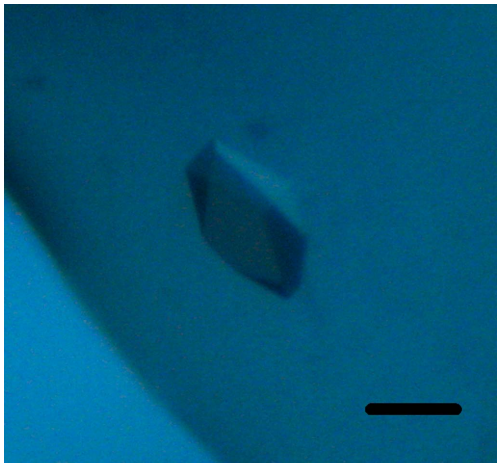

Figure 1
Crystallization of PAT from *S. solfataricus*. Crystals of PAT were grown by the sitting-drop vapour-diffusion method under final optimized crystallization conditions consisting of 0.1 *M* sodium acetate trihydrate pH 4.6, 0.2 *M* ammonium sulfate and 30%(*w*/*v*) polyethylene glycol monomethyl ether 2000. The scale bar indicates 0.2 mm.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	$a = 44.30$, $b = 46.59$, $c = 68.39$
Resolution (Å)	30–1.70 (1.76–1.70)
Unique reflections	16073 (1563)
Completeness (%)	99.8 (98.7)
R_{merge}† (%)	5.8 (45.9)
Average $I/\sigma(I)$	30.8 (4.1)

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ are the intensities of the individual replicates of a given reflection hkl and $\langle I(hkl)\rangle$ is the average intensity over all replicates of that reflection.

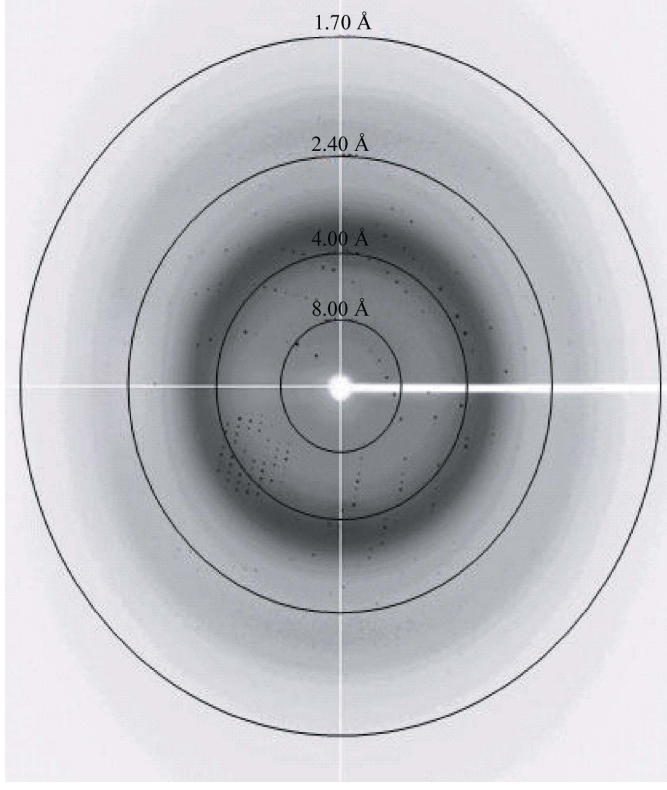

Figure 2
The X-ray pattern of a PAT crystal diffracting to a resolution of 1.70 Å.

2007). For this purpose, several search models were derived from the three-dimensional structures of other acetyltransferases. Unfortunately, no solution could be found that correctly placed the template molecules in the crystal unit cells; also, the calculation of potential pseudo-translation vectors did not produce useful hints about the number and location of the molecular chains in the unit cell. The elucidation of the three-dimensional structure of PAT is presently being pursued using the multi-wavelength anomalous diffraction method. The solution of this structure will provide structural information on epigenetic regulation in the archaeal kingdom.

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