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Crystallization and preliminary X-ray characterization of a PaaX-like protein from *Sulfolobus solfataricus* P2

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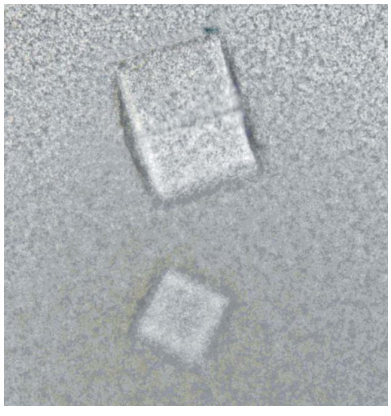

PaaX is a global regulator of the phenylacetyl-coenzyme A catabolon that adjusts the expression of different operons to that of the *paa*-encoded central pathway. In this study, the PaaX-like protein from the hyperthermophilic archaeon *Sulfolobus solfataricus* P2 was successfully crystallized by the hanging-drop vapour-diffusion method using ammonium sulfate as a precipitant. Diffraction data were obtained to a resolution of 3.0 Å using synchrotron radiation at the Photon Factory. The crystal belonged to space group *P*321, with unit-cell parameters a = 86.4, b = 86.4, c = 105.5 Å.

1. Introduction

PaaX is a transcription factor capable of repressing the expression of the *paa* genes involved in phenylacetic acid (PA) degradation. It is the first reported transcription regulator of an aromatic catabolic pathway that responds to a CoA derivative in *Escherichia coli* strain W (Ferrandez *et al.*, 1998). PaaX binds to specific operator sites of the conserved promoter regions and negatively controls the expression of the *paa* catabolic cluster. The phenylacetyl-coenzyme A (PA-CoA) generated by PA-CoA ligase (PaaK) acts as the inducer of the *paa* catabolon. The interaction between PaaX and PA-CoA leads to its release from the operator sequence to allow transcription to occur (Ferrandez *et al.*, 2000). PaaX is a global regulator in the PA-CoA catabolon that adjusts the expression of different operons to that of the *paa*-encoded central pathway (Galan *et al.*, 2004). PaaX represses the expression of the *pga* gene, which encodes penicillin G acylase (PGA; penicillin G aminohydrolase; EC 3.5.1.11). PGA hydrolyzes penicillin G to 6-aminopenicillanic acid (6-APA) and phenylacetic acid (PA). The enzyme PGA is thought to act as a scavenger enzyme for many different natural esters and amides of PA and its derivatives, such as hydroxyphenylacetic acid. It is also one of the most important industrial enzymes for the production of semi-synthesized β-lactam antibiotics (Galan *et al.*, 2004; Valle *et al.*, 1991; Diaz *et al.*, 2001; Kim *et al.*, 2004). In *Pseudomonas* sp. strain Y2, PaaX and PA-CoA control the pathway for the catabolism of styrene (del Peso-Santos *et al.*, 2006) and its regulatory scheme may be linked to other genetic systems involved in the degradation of toxic compounds, such as those derived from benzoate catabolism (Bundy *et al.*, 2002; Clark *et al.*, 2004; del Peso-Santos *et al.*, 2006).

A putative PaaX-like protein from the archeaon *Sulfolobus solfataricus* P2 was detected after its complete genome had been sequenced. This PaaX-like protein exhibits 30% amino-acid sequence identity to *E. coli* PaaX. There are presently no three-dimensional structures available for GntR-type regulators involved in pathways for the degradation of aromatic compounds. In this paper, we report the crystallization and preliminary X-ray studies of the PaaX-like protein.

2. Materials and methods

2.1. Clone and expression

The full-length sequence of the PaaX gene (NCBI accession No. SSO2022) was amplified from the genomic DNA of *S. solfataricus* P2 using the polymerase chain reaction (PCR) with the following

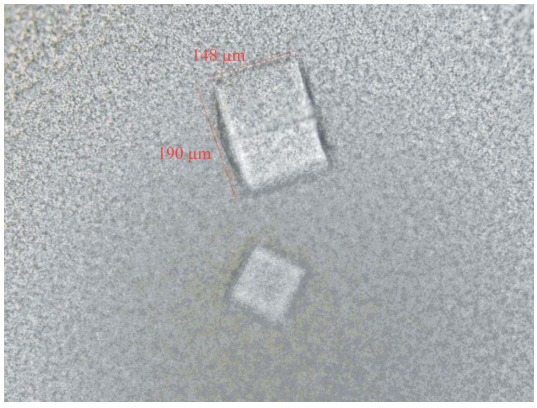

Figure 1
Typical crystals obtained using 0.1 *M* Tris–HCl pH 8.5, 2.0 *M* ammonium sulfate. The crystals achieved final dimensions of 190 × 150 × 80 µm.

primers: 2022-F, 5′-GAAAGAATTCATGAAGATACAGTCATTG-TTC-3′, and 2022-R, 5′-GAAACTCGAGTCATGGTTCATAAAC-CTTATA-3′. The PCR product was digested with *Eco*RI and *Xho*I restriction enzymes and ligated into *Eco*RI and *Xho*I restriction sites of the pET-28a(+) vector (Novagen Inc.) with a 6×His tag at the N-terminus. The recombinant plasmid was transformed into *E. coli* strain BL21 (DE3) and the transformants were selected on LB agar plates containing 50 µg ml^{-1} kanamycin. The cells were then transferred and cultured at 310 K in LB broth containing 50 µg ml^{-1} kanamycin. When the culture density reached an A_{600} of ∼0.5, 1 m*M* IPTG was added to the culture and the cells was grown overnight before being harvested.

2.2. Purification

The bacterial cell pellet was resuspended in MCAC-0 [50 m*M* Tris–HCl pH 8.0, 0.5 *M* NaCl, 10%(*v*/*v*) glycerol] and homogenized by sonication. The lysate was centrifuged at 18 000*g* for 30 min in order to remove cell debris. The supernatant was heat-treated at 343 K for 20 min. After centrifugation at 18 000*g* for 20 min, 1 mg ml^{-1} RNase A (Sigma) and 15 U ml^{-1} DNase I (Takara) were added to the supernatant and incubated at 277 K for about 2 h; the mixture was then centrifuged again at 14 000*g* for 20 min. The clear supernatant was loaded onto an Ni–NTA agarose column (Qiagen) equilibrated with MCAC-0 and unwanted proteins were washed away with MCAC-0 containing 0.05 *M* imidazole. The target protein was eluted with MCAC-0 containing 0.3 *M* imidazole. The sample was concentrated to 0.5 ml using an Amicon Ultra-15 (10 000 nominal molecular-weight limit; Millipore) and injected onto a Superdex-75 10/300 GL column (GE Healthcare) in running buffer (20 m*M* HEPES, 0.1 *M* NaCl pH 7.5). The target protein was eluted at 9–12 ml running buffer volume.

2.3. Crystallization

The purified protein was concentrated to 10–15 mg ml^{-1} in 20 m*M* HEPES, 0.1 *M* NaCl pH 7.5. Crystallization was performed by the hanging-drop vapour-diffusion method at 291 K. In preliminary assays, Crystal Screen I, Crystal Screen II, PEG/Ion Screen and SaltRx reagent kits (Hampton Research) were used to screen initial crystallization conditions. 1 µl protein solution was mixed with 1 µl reservoir solution and the mixture was equilibrated against 200 µl reservoir solution. Cube-shaped crystals were observed after 3 d with buffer containing 0.1 *M* Tris–HCl pH 8.5, 2.0 *M* ammonium sulfate.

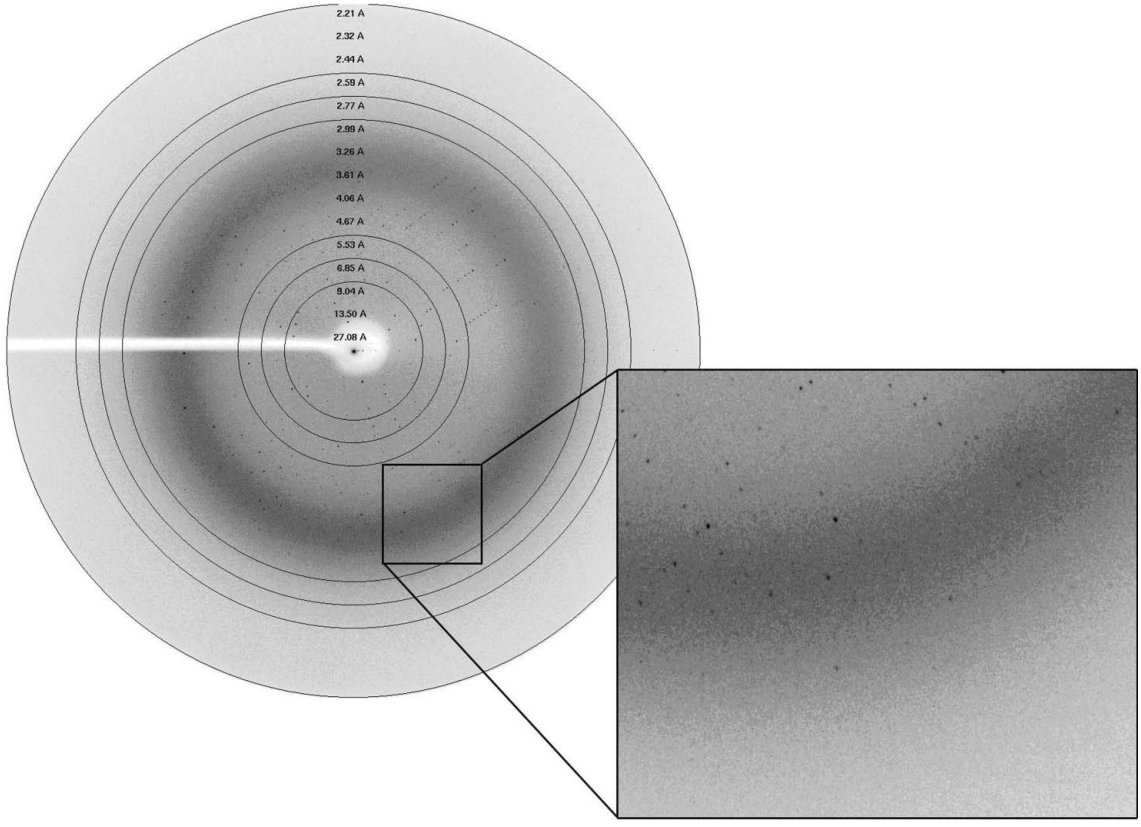

Figure 2
A typical X-ray diffraction pattern collected from a PaaX-like protein crystal. The diffraction image was collected on beamline BL5 of the Photon Factory with an ADSC-Q315 detector. The detector edge corresponds to about 3.0 Å resolution.

Table 1
Data-collection and processing statistics of native PaaX-like protein.

Values in parentheses are for the highest resolution shell.

Space group	*P*321
Unit-cell parameters (Å)	a = 86.4, b = 86.4, c = 105.5
Wavelength (Å)	1.000
Resolution range (Å)	30.0–3.0 (3.1–3.0)
Total reflections	258701 (15411)
Unique reflections	14118 (1446)
Redundancy	18.4 (14.1)
Average $I/\sigma(I)$	40.2 (8.7)
$R_{\rm merge}$† (%)	13.3 (42.6)
Data completeness (%)	99.9 (99.9)
Molecules per ASU	2
$V_{\rm M}$ (Å^3 Da^{-1})	2.0
Sovent content (%)	42

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $\langle I(hkl)\rangle$ is the mean intensity of the observations $I_i(hkl)$ of reflection hkl.

2.4. Data collection and processing

Crystals were immersed in cryoprotectant for 1 min, mounted in a nylon loop and flash-cooled in a stream of nitrogen gas cooled to 100 K. The cryoprotectant was prepared by adding 20%(*v*/*v*) ethylene glycol to the mother-liquor reservoir. Preliminary diffraction data were collected at 1.0000 Å on beamline BL5 of Photon Factory (Japan) using an ADSC Q315 detector. The exposure time was 3 s, the crystal-to-detector distance was 200 mm and the oscillation range per frame was 0.5°. All intensity data were indexed, integrated and scaled using the *HKL*-2000 package (Otwinowski & Minor, 1997).

3. Results and discussion

The PaaX-like protein from *S. solfataricus* was successfully overexpressed in *E. coli* strain BL21 (DE3) as a fusion protein with a 6×His and T7 tag at its N-terminus under the T7 promoter with IPTG induction. 10 h after induction, the expressed PaaX-like protein comprised approximately 40% of the total protein and almost all of the expressed PaaX-like protein was soluble. The recovered PaaX-like protein was associated with small amounts of nucleic acid and could be digested by incubation with RNase A and DNase I. Most of the contaminant proteins were easily removed by heat treatment. Further purification was performed by a two-step procedure involving Ni–NTA affinity chromatography and size-exclusion chromatography.

Crystals of the PaaX-like protein appeared in several of the tested crystallization conditions and the growth of well ordered crystals suitable for X-ray diffraction analysis was found to be dependent on the salt concentration. The results show that the higher the salt concentration, the more rapid the growth of crystals and also the larger the crystal size, resulting in a better quality of diffraction. After growth at 291 K for 8 d, the crystals achieved final dimensions of 190 × 150 × 80 µm (Fig. 1). A data set for native PaaX-like protein was subsequently collected from this crystal. The PaaX-like protein crystals diffracted to 3.0 Å resolution and belonged to space group *P*321, with unit-cell parameters a = 86.4, b = 86.4, c = 105.5 Å (Fig. 2). Assuming the presence of two molecules in the asymmetric unit, the Matthews coefficient $V_{\rm M}$ was calculated to be 2.0 Å^3 Da^{-1}, with a solvent content of approximately 42% in the unit cell. The statistics of data collection are summarized in Table 1. Because no structures of proteins sharing an amino-acid sequence identity greater than 30% with the PaaX-like protein have been reported to date, the preparation of selenomethionyl-derivative PaaX-like protein and heavy-atom soaking derivatives is currently under way in order to determine the structure of PaaX-like protein.

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