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Crystallization and preliminary crystallographic analysis of acylamino-acid releasing enzyme from the hyperthermophilic archaeon *Aeropyrum pernix*

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Crystals of acylamino-acid releasing enzyme from the hyperthermophilic archaeon *Aeropyrum pernix* strain K1 have been grown at 291 K using ammonium phosphate as a precipitant. The diffraction pattern of the crystal extends to 2.4 Å resolution at 100 K using Cu $K\alpha$ radiation. The crystal belongs to space group $P1$, with unit-cell parameters a = 107.5, b = 109.9, c = 119.4 Å, α = 108.1, β = 109.8, γ = 91.9°. The presence of eight molecules per asymmetric unit gives a crystal volume per protein mass (V_M) of 2.4 Å^3 Da^{-1} and a solvent content of 48% by volume. A full set of X-ray diffraction data was collected to 2.9 Å from the native crystal.

1. Introduction

Acylamino-acid releasing enzyme (AARE, also known as acylpeptide hydrolase; EC 3.4.19.1) catalyzes the hydrolysis of an N^{α}-acetylated peptide to release N^{α}-acetylated amino acids (Tsunasawa *et al.*, 1975). It is considered to be a key enzyme involved in the sequential deacetylation of N-terminally acetylated proteins. AARE has been isolated from various organisms and some of its biochemical properties have been investigated (Tsunasawa *et al.*, 1975, 1983; Mori *et al.*, 1990; Miyagi *et al.*, 1995). Furthermore, some genes of AARE have been cloned and expressed in *Escherichia coli* (Mitta *et al.*, 1989, 1996, 1998; Kobayashi *et al.*, 1989; Ishikawa *et al.*, 1998). Based on a homology search, AARE has been recently classified as a member of a new family of serine-type proteases termed the 'oligopeptidase family' (Rawlings *et al.*, 1991). These proteins have been found to share a strong resemblance in their C-terminal regions, which is presumed to be related to their protease activity (Rawlings *et al.*, 1991; Barrett & Rawlings, 1992). Alignment of their sequences revealed that essential residues comprising a Ser-Asp-His catalytic triad, distinct from that of the classical serine protease, are highly conserved in these proteins (David *et al.*, 1993). Furthermore, the catalytic residues in human and porcine enzyme have been confirmed by chemical modification and site-directed mutagenesis (Scaloni *et al.*, 1992; Mitta *et al.*, 1998). However, no crystallographic structure of AARE proteins has been determined.

A. pernix K1 was isolated from a coastal solfotaric thermal vent in Kodakara-Jima island, Kyusyu, Japan (Sako *et al.*, 1996). This strain is an aerobic strain classified as Crenarchaeota in the Archaea. It grows in the temperature range 363–371 K, with an optimal temperature of 368 K. The complete genome sequence of *A. pernix* K1 is now available (http://www.bio.nite.go.jp). We report here the crystallization and preliminary X-ray diffraction studies of recombinant AARE from *A. pernix* K1 (APE1547).

2. Materials and methods

2.1. Crystallization

The archaeal AARE (APE1547) was overexpressed in *E. coli* strain BL21(DE3) PlysS and purified (data will be published elsewhere). The purified protein was concentrated to 10 mg ml^{-1} and the buffer exchanged to 20 m*M* Tris–HCl pH 8.0 with a Millipore Ultrafree-15 centrifugal filter unit. Crystallization trials were conducted at 291 K in 16-well plates using the hanging-drop vapour-diffusion method. Hampton Research Crystal Screen kits I and II (Riverside, CA, USA) were used for initial screening. Drops consisting of 1 µl protein solution and 1 µl of reservoir solution were equilibrated against 0.5 ml of reservoir solution. Crystals were obtained within several days.

Initially, small crystals were grown from Solution No. 3 of Crystal Screen kit I (0.4 *M* ammonium phosphate); large crystals grown with a reservoir solution consisting of 150 m*M* ammonium phosphate did not show Bragg diffraction. When 0.2% β-OG and 2% dioxane were added to the hanging drop, crystals suitable for X-ray analysis were obtained using a reservoir solution consisting of 150 m*M* ammonium phosphate. The crystals reached dimensions of 0.4 × 0.1 × 0.05 mm (Fig. 1).

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Table 1
Crystallographic parameters and data-collection statistics.

Values in parentheses correspond to the outer resolution shell.

Space group	*P*1
Unit-cell parameters (Å, °)	a = 107.5, b = 109.9, c = 119.4, α = 108.1, β = 109.8, γ = 91.9
Matthews coefficient (Å^3 Da^{-1})	2.4 (8 mols per a.u.)
Solvent content (%)	48
Resolution (Å)	50–2.90 (3.00–2.90)
Total observations	396321
Unique reflections	101673
Redundancy	3.9
Average $I/\sigma(I)$	9.4 (3.5)
$R_{\rm merge}$† (%)	11.0 (35.3)
Completeness (%)	97.7 (96.4)

† $R_{\rm merge} = \sum |I - \langle I \rangle| / \sum I$.

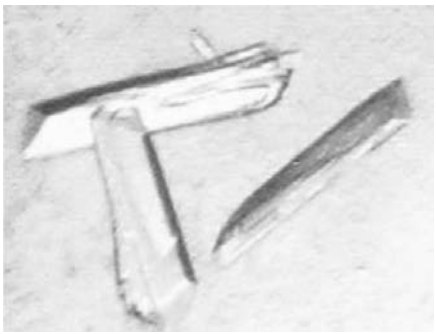

Figure 1
A photograph of AARE crystals.

2.2. X-ray crystallographic studies

Preliminary diffraction data sets were collected at room temperature in-house on a Rigaku rotating-anode Cu $K\alpha$ X-ray generator operating at 48 kV and 98 mA (λ = 1.5418 Å) with a MAR 345 image-plate detector. For a more detailed analysis, flash-cooled crystals were used. Crystals were immersed in a freezing solution for 5–10 s, picked up with a loop and then flash-cooled in a stream of nitrogen gas cooled to 100 K. The freezing solution contained 25% glycerol as cryoprotectant, but was otherwise identical to the reservoir solution. A cryostream (Oxford Cryosystems, Oxford, England) was used to maintain the crystal at 100 K during data collection. All intensity data were indexed, integrated and scaled with the *HKL* programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results

Diffraction data were observed to at least 2.4 Å Bragg spacing (Fig. 2). Unit-cell parameters were determined to be a = 107.5, b = 109.9, c = 119.4 Å, α = 108.1, β = 109.8, γ = 91.9° in space group *P*1. The asymmetric unit contains eight molecules, each with a mass of 65 kDa, giving a crystal volume per protein mass ($V_{\rm M}$) of 2.4 Å^3 Da^{-1} and a solvent content of 48% by volume (Matthews, 1968). As listed in Table 1, a data set was collected to 2.9 Å. It consists of 396 321 measurements of 101 673 unique reflections, with an overall $R_{\rm merge}$ of 11.0% ($R_{\rm merge} = \sum |I - \langle I \rangle| / \sum I$) and an overall $I/\sigma(I)$ of 9.4. This represents 97.7% of the theoretically observable reflections at 2.9 Å resolution. The outermost shell of data between 3.00 and 2.90 Å is 96.4% complete. We are currently producing selenomethionine-substituted AARE in order to solve the structure by the MAD method (Hendrickson *et al.*, 1990).

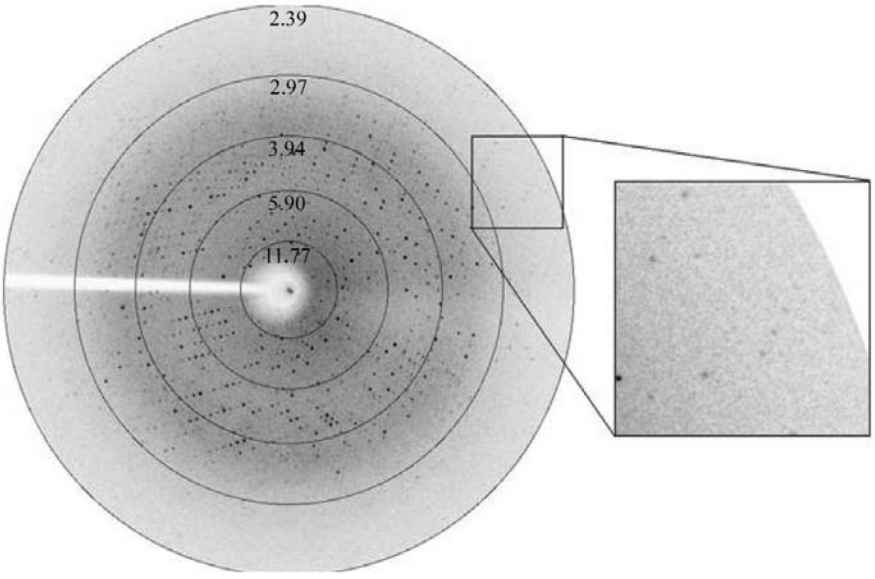

Figure 2
A typical X-ray diffraction pattern of AARE at 2.4 Å resolution.

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