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Crystallization and preliminary X-ray diffraction analysis of *O*-acetylserine sulfhydrylase from *Aeropyrum pernix* K1

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Crystals of *O*-acetylserine sulfhydrylase from *Aeropyrum pernix* K1 were obtained by the hanging-drop vapour-diffusion method at 298 K. An X-ray diffraction data set was collected to 2.25 Å resolution at 100 K. The crystal belonged to space group $P42_12$, $P4_12_12$, $P4_22_12$ or $P4_32_12$. The unit-cell parameters were $a = b = 74.5$, $c = 276.0$ Å. The presence of two subunits of the enzyme per asymmetric unit gives a crystal volume per protein mass (V_M) of 2.28 Å^3 Da^{-1} and a solvent content of 46%(*v*/*v*).

1. Introduction

There are two pathways of L-cysteine biosynthesis: one is used by bacteria and plants and the other is used by mammals. In bacteria such as *Escherichia coli* and *Salmonella typhimurium* as well as in plants, L-cysteine is synthesized from L-serine by two types of enzymes (Kredich, 1997; Hell, 1997). Serine acetyltransferase (SAT; EC 2.3.1.30) converts acetyl-CoA and L-serine into *O*-acetyl-L-serine (OAS) and CoA. OAS is then converted to L-cysteine and acetic acid in the presence of sulfide by *O*-acetylserine sulfhydrylase (OASS; EC 4.2.99.8). SATs and OASSs purified from bacteria and plants have been characterized (Kredich, 1997; Hell, 1997). OASS exists as dimer and contains pyridoxal 5′-phosphate (PLP). The three-dimensional structure of OASS from *S. typhimurium* has been published (Burkhard *et al.*, 1998). In mammals, on the other hand, L-cysteine is synthesized from L-methionine (Griffith, 1987). L-Methionine is converted to L-homocysteine by methionine adenosyltransferase (EC 2.5.1.6), various *S*-adenosylmethionine methyltransferases (EC 2.1.1.1–145) and *S*-adenosylhomocysteinase (EC 3.3.1.1). Cystathionine β-synthase (CBS; EC 4.2.1.22) is a PLP-dependent enzyme catalyzing condensation of L-homocysteine and L-serine to form L-cystathionine, which yields L-cysteine *via* cystathionine γ-lyase (EC 4.4.1.1). The three-dimensional structure of human CBS without the C-terminal amino-acid sequence 414–551 has been reported (Meier *et al.*, 2001).

The emergence of the two different pathways for L-cysteine biosynthesis in the course of evolution is an interesting point. CBS and OASS are thought to be evolutionarily related enzymes (Ono *et al.*, 1994). The CBSs from *Saccharomyces cerevisiae* and *Trypanosoma cruzi* have OASS activities (Ono *et al.*, 1994; Nozaki *et al.*, 2001). The conformation of the residues surrounding the PLP-binding site is highly conserved between human CBS and *S. typhimurium* OASS (Meier *et al.*, 2001). However, it is not clear how an ancestral enzyme has evolved or diverged into the enzymes involved in the two distinct pathways for L-cysteine biosynthesis.

In archaea, little is known about the L-cysteine biosynthetic pathway in spite of the efforts of several investigators (Zhou & White, 1991; Kitabatake *et al.*, 2000; Borup & Ferry, 2000*a*,*b*). The genes encoding SAT and OASS are adjacent to each other in the genomes of both *Methanosarcina barkeri* and *M. thermophila* (Kitabatake *et al.*, 2000; Borup & Ferry, 2000*a*). On the other hand, results of the genome-sequencing analyses of several other archaea have shown that the genomes of *Aeropyrum pernix*, *Pyrobaculum aerophilum* (Fitz-Gibbon *et al.*, 2002), *Pyrococcus abyssi*, *P. furiosus*, *Sulfolobus solfataricus*, *S. tokodaii* (Kawarabayasi *et al.*, 2001), *Thermoplasma acidophilum* and *T. volcanium* (Kawashima *et al.*, 2000) contain genes that have sequence similarity to OASS (Kitabatake *et al.*, 2000). However, genes with sequence similarity to SAT are not identified in these archaea.

A. pernix K1 is a hyperthermophilic archaeon with an optimal growth temperature between 363 and 368 K (Sako *et al.*, 1996). The product of the gene (APE1586) encoding OASS from *A. pernix* consists of 389 amino acids (Kawarabayasi *et al.*, 1999). We have recently found for the first time in archaea that recombinant *A. pernix* OASS possesses the activities of both OASS and CBS (manuscript submitted). We attempted crystallization of *A. pernix* OASS in order to clarify the structural basis for the bifunctionality of the enzyme and to understand the process by which an ancestral enzyme has evolved or diverged into the enzymes involved in the two different L-cysteine biosynthetic pathways in nature. In addition, by comparing the structure of *A. pernix* OASS with the known structures of

OASSs without thermostability, we may gain an insight into the mechanism of the hyperthermostability of *A. pernix* OASS. We report here the crystallization and preliminary X-ray diffraction analysis of recombinant OASS from *A. pernix*.

2. Methods

2.1. Crystallization

The *A. pernix* OASS (APE1586) was overexpressed in *E. coli* Rosetta (DE3) cells (Novagen, Madison, WI, USA) and purified. The purified protein was dialyzed against 50 m*M* potassium phosphate buffer pH 7.5 containing 0.2 m*M* PLP and 0.05% sodium azide. The concentration of the protein was measured using a Bio-Rad DC protein assay kit (Hercules, CA, USA) with bovine serum albumin as the standard protein. The enzyme solution was concentrated to 10 mg ml^{-1} with a Microcon YM-10 (Millipore, Bedford, MA, USA). Initial searches for the crystallization condition were performed using 24-well plates with the hanging-drop vapour-diffusion method using the Hampton Research Crystal Screen kit (Laguna Niguel, CA, USA). Drops consisting of 1.5 µl of the enzyme solution and 1.5 µl of reservoir solution were equilibrated against 0.5 ml of reservoir solution at 298 K. For optimization of crystallization conditions starting from solution No. 28 in the kit, reservoir solutions containing 0.1 *M* sodium cacodylate buffer (pH 5.1, 5.5, 6.0, 6.5, 7.0 or 7.4), 0.2 *M* sodium acetate and polyethylene glycol (PEG) 8000 [0, 10, 20 or 30%(*v*/*v*)] were prepared.

2.2. Data collection and processing

For X-ray data collection, a crystal was immersed in reservoir solution containing an additional 10%(*v*/*v*) glycerol as cryoprotectant. The crystal was picked up with a loop and then flash-frozen in a stream of nitrogen gas cooled to 100 K. Diffraction data were collected at 100 K with an ADSC Quantum 4R CCD detector at a wavelength of 1.0 Å at the BL38B1 experimental station of SPring-8, the Japan Synchrotron Radiation Research Institute (JASRI), Hyogo, Japan. The crystal-to-detector distance was set to 200 mm. The crystal was rotated 180° with an oscillation angle of 0.5° per frame. The exposure times were 15 s per frame. Diffraction data were processed using the program *MOSFLM* (Leslie, 1992) and scaled using the program *SCALA* (Collaborative Computational Project, Number 4, 1994).

Figure 1
A photograph of the *A. pernix* OASS crystals. The longest length of the largest crystal corresponds to 0.35 mm.

Table 1
Crystallographic parameters and data-collection statistics.

Values in parentheses correspond to the outermost resolution shell.

Space group	$P42_12$, $P4_12_12$, $P4_22_12$ or $P4_32_12$
Unit-cell parameters (Å)	$a = b = 74.5$, $c = 276.0$
Matthews coefficient (Å^3 Da^{-1})	2.28, two subunits per a.u.
Solvent content (%)	46
Resolution range (Å)	∞–2.25 (2.37–2.25)
No. of observed reflections	244453
Total No. of unique reflections	32116
Average $I/\sigma(I)$	11.2 (2.9)
$R_{\rm merge}$† (%)	6.2 (28.2)
Completeness (%)	85.7 (80.2)‡

† $R_{\rm merge} = \sum_{hkl}\sum_i |I(hkl,i) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I(hkl,i)$, where $I(hkl, i)$ is the intensity of the ith measurement of reflection hkl and $\langle I_{hkl}\rangle$ is the mean value of $I(hkl, i)$ for all i measurements. ‡ These low values of completeness are because of the blind region (Drenth, 1999) arising from accidental coincidence of the crystal c axis and the direction of the oscillation axis.

3. Results

Small crystals grew from No. 28 of the reservoir solution of the Crystal Screen kit [0.1 *M* sodium cacodylate buffer pH 6.5, 0.2 *M* sodium acetate and 30% (*v*/*v*) PEG 8000] within several days. The crystals had dimensions of 0.06 × 0.02 × 0.02 mm after one month. Based on the results of this initial screening, various conditions were tested by changing the pH of the sodium cacodylate buffer and the concentration of PEG 8000 in the reservoir solution (see §2). As a result, crystals suitable for X-ray analysis were obtained using a reservoir solution consisting of 0.1 *M* sodium cacodylate buffer pH 7.4, 0.2 *M* sodium acetate and 30% PEG 8000. The crystal reached dimensions of 0.35 × 0.14 × 0.14 mm after two weeks (Fig. 1).

Results of data collection and processing revealed that the crystal belonged to space group $P42_12$, $P4_12_12$, $P4_22_12$ or $P4_32_12$. The unit-cell parameters were $a = b = 74.5$, $c = 276.0$ Å. The presence of two subunits of *A. pernix* OASS in the asymmetric unit gives a crystal volume per protein mass ($V_{\rm M}$) of 2.28 Å^3 Da^{-1} and a solvent content of 46%(*v*/*v*) (Matthews, 1968). The presence of two subunits of the enzyme in the asymmetric unit is consistent with the fact that OASSs from bacteria and plants form a dimer. The statistics of data collection are summarized in Table 1. A data set was collected to 2.25 Å. It consists of 244 453 measurements and 32 116 unique reflections. The overall $R_{\rm merge}$ was 6.2% and the overall $I/\sigma(I)$ was 11.2. The data set has a completeness of 85.7%. The self-rotation function was calculated using the program *POLARRFN* (Collaborative Computational Project, Number 4, 1994). However, no clear non-crystallographic peaks were observed in the $\kappa = 180°$ section. The structure determination is now in progress.

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