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Crystallization and preliminary X-ray analysis of L-serine 3-dehydrogenase complexed with NADP⁺ from the hyperthermophilic archaeon *Pyrobaculum calidifontis*

An NAD(P)⁺-dependent L-serine 3-dehydrogenase from the hyperthermophilic archaeon *Pyrobaculum calidifontis* was crystallized using the sitting-drop vapour-diffusion method with ammonium sulfate as the precipitant. The crystals belonged to the monoclinic space group C2, with unit-cell parameters $a = 120.81$, $b = 57.40$, $c = 56.37$ Å, $\beta = 106.88^\circ$. Diffraction data were collected to 1.57 Å resolution on beamline NE3A at the Photon Factory. The overall R_{merge} was 4.2% and the data completeness was 90.1%.

1. Introduction

L-Serine dehydrogenase catalyses the dehydrogenation of L-serine in the presence of NAD(P)⁺. To date, two types of L-serine dehydrogenase have been identified. The first is L-serine 3-dehydrogenase (L-SerDH; EC 1.1.1.276). This enzyme catalyses dehydrogenation at the β -carbon (C3) position of L-serine. The suspected product of the reaction is 2-aminomalonate semialdehyde, which nonenzymatically decomposes into 2-aminoacetaldehyde and CO₂. The second type is L-serine 2-dehydrogenase (EC 1.4.1.7), which catalyses the oxidative deamination of the α -amino group of L-serine in the same way as other amino-acid dehydrogenases (Ohshima & Soda, 2000). There has been one report of an L-serine 2-dehydrogenase that catalyses the deamination of the α -amino group in higher plants (Kretovich & Stepanovich, 1966). However, this enzyme has not been purified and its primary structure is still unknown. In contrast, the primary structure of L-SerDH has been determined using the NADP⁺-dependent enzyme from *Agrobacterium tumefaciens* ICR 1600 (Chowdhury *et al.*, 1997; Fujisawa *et al.*, 2002). The structure revealed the enzyme to be a member of the short-chain dehydrogenase reductase family. Subsequently, homologues of the enzyme were found in *Escherichia coli* (YdfG) and *Saccharomyces cerevisiae* (YMR226C) based on genome information. These homologues were characterized and identified as NADP⁺-dependent L-*allo*-threonine dehydrogenases, which also act on D-threonine and L- and D-enantiomers of serine and 3-hydroxyisobutyrate (Fujisawa *et al.*, 2003). The substrate-recognition mechanism of the *E. coli* enzyme has been investigated *via* crystal structure analysis (Yamazawa *et al.*, 2011).

Recently, a novel L-SerDH that shows no sequence similarity to the *A. tumefaciens* enzyme was identified in *Pseudomonas aeruginosa* (Tchigvintsev *et al.*, 2012). The enzyme is NAD⁺ dependent and catalyses the oxidation of L-serine and 3-methyl-L-serine but exhibits low activity against 3-hydroxyisobutyrate. Compared with the NADP⁺-dependent L-SerDH from *A. tumefaciens*, the enzyme reportedly showed higher catalytic efficiency towards L-serine (Tchigvintsev *et al.*, 2012). The crystal structure of the *P. aeruginosa* enzyme has been solved and the molecular details of its active site have been characterized (Tchigvintsev *et al.*, 2012).

In contrast, no L-SerDH has been reported to date in archaea, the third domain of life, or in hyperthermophiles. Within the genomic sequence of an aerobic hyperthermophilic archaeon, *Pyrobaculum calidifontis*, we found a gene (open reading frame identification number Pcal_0699) for which the predicted amino-acid sequence exhibits 36% identity with that of *P. aeruginosa* L-SerDH. Moreover, we succeeded in the expression of the gene in *E. coli* and confirmed that the gene product exhibits L-SerDH activity (described in this paper). The *P. calidifontis* enzyme is probably the most thermostable

L-SerDH described to date, which makes this enzyme potentially useful for stereospecific synthesis of L-serine and the measurement of L-serine in foods and a variety of other environments. Structural information on this thermostable L-SerDH may be useful in the development of its applications. In the present study, we describe the crystallization and preliminary X-ray analysis of *P. calidifontis* L-SerDH, as well as the expression of the gene in *E. coli*, as a first step in the structural analysis of L-SerDHs from archaeal strains.

2. Experimental procedures and results

2.1. Cloning, expression and purification

The gene encoding the L-SerDH homologue (ORF ID PcaI_0699) was amplified by PCR. The oligonucleotide primers used to amplify the L-SerDH gene fragment were 5'-CATATGCGCGTCCGATTCA-TCGGCCTGGGG-3', which contains a unique *Nde*I restriction site overlapping with the 5' initiation codon, and 5'-GGATCCTTACG-ACGATTTGTAAAAGCCTAG-3', which contains a unique *Bam*HI restriction site proximal to the 3' end of the termination codon. Chromosomal *P. calidifontis* DNA was isolated using an illustra bacteria genomicPrep Mini Spin Kit (GE Healthcare, Buckinghamshire, England) and used as the template. The amplified 0.9 kbp fragment was digested with *Nde*I and *Bam*HI, and ligated with the expression vector pET15b (Novagen, Madison, Wisconsin, USA) linearized with *Nde*I and *Bam*HI to generate pET0699, which was then used to transform *E. coli* strain BL21 (DE3) Codon Plus RIL (Stratagene, La Jolla, California, USA). The transformants were cultivated at 310 K in 200 ml Luria-Bertani medium containing 50 µg ml⁻¹ ampicillin until the optical density at 600 nm reached 0.6. Expression was then induced by adding 1.0 mM isopropyl β-D-1-thiogalactopyranoside to the medium and cultivation was continued for an additional 3 h at 310 K. *E. coli* cells harvested from the 200 ml culture (about 1.5 g wet weight) were used as the starting material for purification of L-SerDH. To prepare the crude extract, the cells were washed twice and suspended in 10 mM potassium phosphate buffer pH 7.0. The cells were then disrupted by sonication and centrifuged at 20 000g for 10 min. L-SerDH activity in the crude extract was assayed using L-serine as a substrate as described previously (Chowdhury *et al.*, 1997).

Figure 1
SDS-PAGE of recombinant *P. calidifontis* L-SerDH. Left lane, protein markers (labelled in kDa); right lane, purified *P. calidifontis* L-SerDH.

To isolate the *P. calidifontis* L-SerDH, the crude extract was heated at 343 K for 20 min in the presence of 0.2 M Na₂SO₄ and the denatured proteins were removed by centrifugation (20 000g for 10 min). The resultant supernatant was loaded onto a Co²⁺-charged Talon resin column (10 × 40 mm, Clontech, Palo Alto, California, USA) equilibrated with 10 mM potassium phosphate buffer (pH 7.0) containing 500 mM NaCl. After washing the column with the same buffer, the enzyme was eluted with 300 mM imidazole in the same buffer. After elution, the active fractions were pooled and used as the purified enzyme preparation. The purity of the enzyme was assessed by 12.5% Coomassie-stained SDS-PAGE (Fig. 1). The entire procedure was carried out at room temperature (~298 K).

We found that the enzyme was readily purified from the crude cell extract in two simple steps: heat treatment and Co²⁺-charged Talon resin column chromatography. About 2.5 mg purified enzyme was obtained from 200 ml *E. coli* culture. The enzyme catalysed the dehydrogenation of D-serine, DL-glycerate and DL-3-hydroxyisobutyrate as well as L-serine as substrates and either NAD⁺ or NADP⁺ could serve as the electron acceptor. Inert substrates included α-methyl-DL-serine, O-methyl-DL-serine, DL-threo-3-phenylserine, DL-homoserine, DL-threonine, DL-allo-threonine, DL-3-hydroxynorvaline, methyl 2,2-dimethyl-3-hydroxypropionate, L-alanine, DL-malate, DL-lactate, DL-tartrate, malonate and 6-phosphogluconate.

2.2. Molecular-mass determination

The molecular mass of the recombinant enzyme was determined using a TSKgel G3000SW_{XL} column (7.8 × 300 mm, Tosoh, Japan) with 10 mM potassium phosphate buffer pH 7.0 containing 150 mM NaCl as the elution buffer. A gel-filtration markers kit (Sigma-Aldrich, St Louis, Missouri, USA) was used as molecular-mass standards. The subunit molecular mass was determined by SDS-PAGE (Fig. 1). The native and subunit molecular masses of the enzyme were determined to be about 28 and 29 kDa, respectively, suggesting that the enzyme is a monomer in solution.

2.3. Crystallization

The purified enzyme was dialysed against 10 mM Tris-HCl buffer pH 8.0 containing 200 mM NaCl and 5 mM β-mercaptoethanol and concentrated to 11 mg ml⁻¹ for crystallization trials. Initial screening for crystallization was carried out with Crystal Screen and Crystal Screen 2 (Hampton Research, Aliso Viejo, California, USA) at 293 K using the sitting-drop vapour-diffusion method, in which a 1 µl drop

Figure 2
Monoclinic crystal of *P. calidifontis* L-SerDH. The maximum dimensions of the crystal are 0.6 × 0.2 × 0.1 mm.

Figure 3
X-ray diffraction of a *P. calidifontis* L-SerDH crystal. The high-resolution area is enlarged (inset).

of protein solution containing 1 mM NADP⁺ was mixed with an equal volume of reservoir solution and equilibrated against 0.1 ml reservoir solution using CompactClover Crystallization Plates (Emerald Biosystems, USA). Initially, small crystals were grown from reagent No. 47 (2.0 M ammonium sulfate, 100 mM acetate buffer pH 4.6) of Crystal Screen. This precipitant solution was taken as a starting point and was optimized by variation of the ammonium sulfate concentration; diffraction-quality crystals (maximum dimensions of $0.6 \times 0.2 \times 0.1$ mm; Fig. 2) were obtained within 2 weeks using a reservoir solution composed of 1.9–2.0 M ammonium sulfate, 100 mM acetate buffer pH 4.5.

2.4. Data collection and preliminary X-ray analysis

The NAD(P)⁺-dependent L-SerDH crystal was flash-cooled in liquid nitrogen at 100 K. The crystal was cryoprotected with reservoir solution supplemented with 30% (v/v) glycerol, replacing the water in the buffer with cryoprotectant. Diffraction data were collected at 1.57 Å resolution using monochromated radiation of wavelength 1.0 Å and an ADSC CCD detector system on the NE3A beamline at the Photon Factory, Tsukuba, Japan. The oscillation angle per image was set to 1°. The crystal-to-detector distance was 184 mm. The data were processed using *HKL-2000* (Otwinowski & Minor, 1997).

The crystals belonged to the monoclinic space group C2. A summary of the data statistics is presented in Table 1. The R_{merge} value in the highest-resolution shell (5.9%) indicates that the crystals diffract far better than 1.57 Å resolution (Fig. 3). Assuming the presence of one protein molecule in the asymmetric unit, the crystal volume per enzyme mass (V_M) and the solvent content were calculated to be $3.0 \text{ Å}^3 \text{ Da}^{-1}$ and 59.8%, respectively, which are within the frequently observed ranges for protein crystals (Matthews, 1968).

Table 1

Data-collection and processing statistics for *P. calidifontis* L-SerDH.

Values in parentheses are for the highest resolution shell.

Source	NE3A, Photon Factory
Wavelength (Å)	1.00
Rotation range per frame (°)	1
Total rotation range (°)	270
Exposure per frame (s)	1
Crystal-to-detector distance (mm)	184
Temperature (K)	100
Space group	C2
Unit-cell parameters (Å, °)	$a = 120.81$, $b = 57.40$, $c = 56.37$, $\beta = 106.88$
Resolution range (Å)	35.03–1.57 (1.65–1.57)
No. of measured reflections	251426
No. of unique reflections	46458
Multiplicity	5.4 (5.0)
Completeness (%)	90.1 (94.9)
$R_{\text{merge}}^{\dagger}$	0.042 (0.059)
$\langle I/\sigma(I) \rangle$	12.4 (10.1)
Mosaicity (°)	0.34

$$^{\dagger} R_{\text{merge}} = \frac{\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle|}{\sum_{hkl} \sum_i I_i(hkl)}.$$

Based on the L-SerDH structure from *P. aeruginosa* (PDB entries 3q3c and 3obb; 36% identity; Tchigvintsev *et al.*, 2012), we used the molecular-replacement method for phase calculation, but could not obtain useful data. We are currently attempting to prepare selenomethionine-substituted L-SerDH from *P. calidifontis*.

To date, crystal structures of the L-SerDHs from *P. aeruginosa* (Tchigvintsev *et al.*, 2012) and *E. coli* (PDB entries 3asu and 3asv; 15% identity; Yamazawa *et al.*, 2011) have been determined, but no L-SerDHs from an archaeal strain have been structurally characterized. In the present study, the first diffraction-quality crystals were obtained for the *P. calidifontis* L-SerDH. We expect that elucidation of the three-dimensional structure of this enzyme will further increase our understanding of structure–function relationships in archaeal L-SerDH.

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