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Crystallization and preliminary X-ray analysis of argininosuccinate lyase from *Streptococcus mutans*

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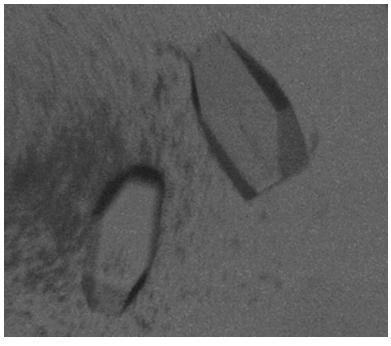
Argininosuccinate lyase (ASL) is an important enzyme in arginine synthesis and the urea cycle, which are highly conserved from bacteria to eukaryotes. The gene encoding *Streptococcus mutans* ASL (*sm*ASL) was amplified and cloned into expression vector pET28a. The recombinant *sm*ASL protein was expressed in a soluble form in *Escherichia coli* strain BL21 (DE3) and purified to homogeneity by two-step column chromatography. Crystals suitable for X-ray analysis were obtained and X-ray diffraction data were collected to a resolution of 2.5 Å. The crystals belonged to space group *R*3, with unit-cell parameters $a = b = 254.5$, $c = 78.3$ Å.

1. Introduction

Argininosuccinate lyase (argininosuccinase; ASL; EC 4.3.2.1) reversibly cleaves argininosuccinate to yield arginine and fumarate. The reaction provides arginine as a versatile substrate for the synthesis of urea, polyamines, creatine phosphate and nitric oxide. The enzyme plays a critical role not only in arginine synthesis and the urea cycle, but also in maintenance of the cellular nitrogen balance (Brosnan & Brosnan, 2007; Mori & Gotoh, 2004). ASL is classified into a superfamily of homotetrameric enzymes that catalyze similar β-elimination reactions with cleavage of a C—N or C—O bond and subsequently releases fumarate as the elimination production (Toth & Yeates, 2000; Weaver & Banaszak, 1996; Fujii *et al.*, 2003; Bhaumik *et al.*, 2004). Recent studies on ASLs from different species have elucidated highly conserved regions that probably comprise the catalytic sites, such as Ser108, Arg109, Thr155, His156, Ser277 and Lys283 in *Escherichia coli* ASL, and are also observed in other ASLs (Sampaleanu *et al.*, 2001, 2002; Vallée *et al.*, 1999; Abu-Abed *et al.*, 1997; Bhaumik *et al.*, 2004; Fig. 1). An ASL structure including intact catalytic sites was first observed for *E. coli* ASL in the presence of phosphate ions (PDB entry 1tj7; Bhaumik *et al.*, 2004), since the active site, which is mainly composed of loop regions, is most often flexible. Although the structure of *Thermus thermophilus* ASL complexed with L-arginine has been deposited (PDB entry 2e9f; M. Goto, unpublished work), precise descriptions of the β-elimination mechanism of ASLs based on reliable structural data are still lacking and the relationship between the structure and the function of ASLs remains obscure.

Streptococcus mutans is the principal acidogenic component of dental plaque, which causes tooth demineralization and dental caries (Mosci *et al.*, 1990). *sm*ASL (gi:24376709; Gene ID 1029354) encoded by the gene *smu*.335 consists of 460 amino-acid residues with a predicted molecular mass of 51.5 kDa. Based on sequence alignment, *sm*ASL shows about 42–49% sequence identity to other known ASL structures (Fig. 1). In this work, we report the preparation, crystallization and preliminary X-ray analysis of *sm*ASL. The ultimate structural determination of *sm*ASL should help us to promote further studies of ASLs.



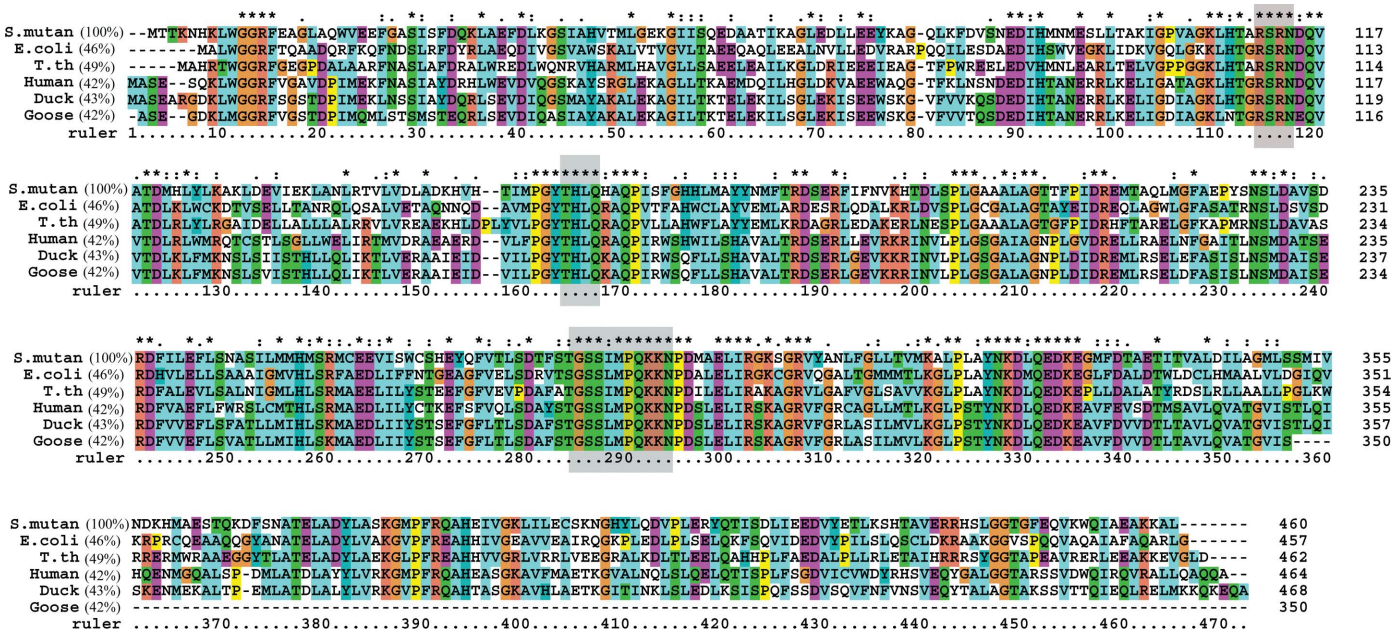


Figure 1
Multiple sequence alignment of argininosuccinate lyase homologues using the program *ClustalX* (Thompson *et al.*, 1997). ASLs from the following organisms were selected: *Streptococcus mutans* (S. mutan), *Escherichia coli* (E. coli), *Thermus thermophilus* (T. th), *Homo sapiens* (Human), duck δ-crystallin (Duck) and goose δ-crystallin (Goose). The sequence identities of the homologues to *sm*ASL are given after the names. The conserved residues that probably constitute the active site are highlighted in grey.

2. Materials and methods

2.1. Cloning and protein expression

The *sm*ASL gene was amplified from *S. mutans* strain UA159 genomic DNA by polymerase chain reaction and cloned into the *Bam*HI and *Xho*I restriction-enzyme sites of pET28a vector using conventional cloning techniques. The primers used were the forward primer 5′-CGC**GGATCC**ATGACAACAAAAAATCACAAATT-3′ and the reverse primer 5′-CCG**CTCGAG**TTAAAGTGCTTTTTT-AGCCTCT-3′. The recombinant plasmid containing an N-terminal His_6 tag was confirmed by nucleotide sequencing and transformed into the host *E. coli* BL21 (DE3) (Novagen). The transformed cells were cultured in Luria–Bertani (LB) medium containing 50 µg ml^{-1} kanamycin at 310 K. At an OD_{600} of 0.6, expression of *sm*ASL was induced with isopropyl β-D-1-thiogalactopyranoside at a final concentration of 0.5 m*M*. The cells were incubated for a further 5 h at 303 K before being harvested by centrifugation at 6600*g* for 10 min, resuspended in lysis buffer consisting of 20 m*M* Tris–HCl pH 7.5 and 500 m*M* NaCl and lysed by sonication.

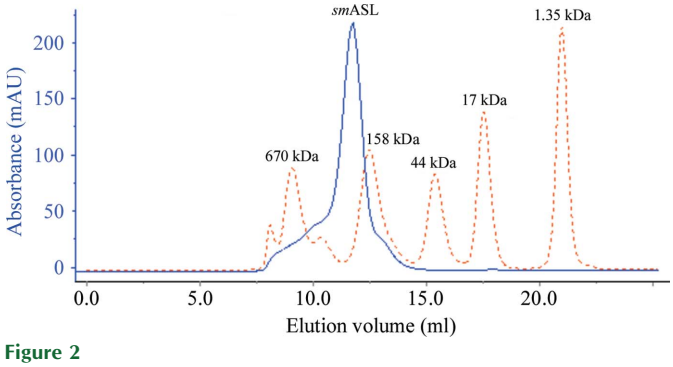


Figure 2
Chromatogram of *sm*ASL on a Superdex 200 column (GE Healthcare, USA). The $UV_{280\,nm}$ absorption curves of standard proteins (Bio-Rad Laboratories, USA) and *sm*ASL are shown as red dashed and blue solid lines, respectively.

2.2. Protein purification

The disrupted cells were centrifuged at 34 700*g* for 50 min at 277 K. The supernatant was filtered and loaded onto a 5 ml HiTrap Ni^{2+}-chelating affinity column (GE Healthcare, USA) equilibrated with lysis buffer. *sm*ASL protein was eluted with a linear gradient of 50–500 m*M* imidazole in lysis buffer. Further purification was carried out by size-exclusion chromatography using a 120 ml HiLoad Superdex 200 column (GE Healthcare, USA) equilibrated with 20 m*M* Tris–HCl pH 7.5 and 150 m*M* NaCl. SDS–PAGE was used to examine the purity of the protein at each step. The purified protein was concentrated to around 10 mg ml^{-1} (yielding approximately 15 mg per litre of culture) using ultrafiltration (Millipore Amicon Ultra-15, 10 kDa cutoff). The protein concentration was measured using the Bio-Rad Protein Assay Kit (Bio-Rad, USA).

2.3. Crystallization and X-ray diffraction data collection

Initial crystallization trials of purified protein with the His-tag fusion were performed at 289 K by the sitting-drop vapour-diffusion method using an XtalQuest 48^2 crystallization plate (XtalQuest Inc., Beijing, People's Republic of China). A total of three commercial screening kits, Crystal Screen, Crystal Screen 2 and Index Screen (Hampton Research, California, USA), were used in initial crystallization trials. 1 µl protein solution was mixed with 1 µl reservoir solution and the droplet was equilibrated against 100 µl reservoir solution.

X-ray diffraction data were collected at a wavelength of 0.9795 Å using a MAR CCD 225 detector on the BL17U beamline at Shanghai Synchrotron Radiation facility (SSRF), Shanghai, People's Republic of China. The *sm*ASL protein crystal was soaked in 2 *M* 1,6-hexanediol in 100% paraffin oil for ∼5 s before being flash-cooled and kept at 100 K in a cold nitrogen stream during data collection. A total of 100 frames were collected with 1° oscillation per frame. The exposure time was 1 s per image. The data were processed using the program *XDS* (Kabsch, 2010).

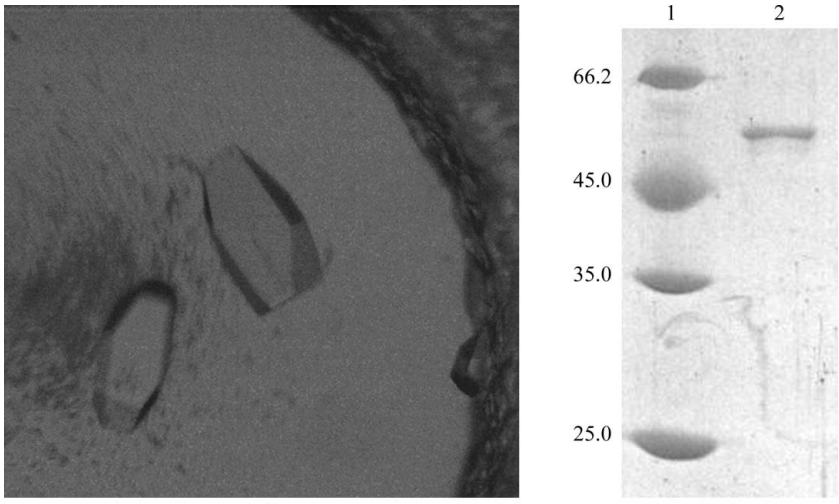


Figure 3
(*a*) Crystals of *S. mutans* argininosuccinate lyase grown in 0.056 *M* sodium dihydrogen phosphate, 1.344 *M* dipotassium hydrogen phosphate pH 8.2 using the sitting-drop vapour-diffusion method. (*b*) 15% SDS–PAGE gel with Coomassie Brilliant Blue staining. Lane 1, molecular-weight protein markers (kDa); lane 2, dissolved *sm*ASL crystal sample.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	0.9791
Space group	*R*3
Unit-cell parameters (Å, °)	$a = b = 254.5$, $c = 78.3$, $\alpha = 90$, $\beta = 90$, $\gamma = 120$
Resolution range (Å)	50–2.5 (2.6–2.5)
Completeness (%)	95.2 (86.6)
No. of observed reflections	198922
No. of unique reflections	62263
Average $I/\sigma(I)$	13.8 (3.4)
$R_{\rm merge}$ (%)†	5.9 (33.8)
No. of molecules per asymmetric unit	4
Solvent content (%)	48.2
$V_{\rm M}$ (Å^3 Da^{-1})	2.4

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

3. Results and discussion

The *sm*ASL protein was expressed in *E. coli* BL21 (DE3) with a typical yield of about 15 mg pure protein per litre of cell culture. After two-step chromatographic purification, homogenous samples suitable for crystallization trials were obtained. The size-exclusion chromatography results indicated that the protein exists mainly in a tetrameric form in solution (Fig. 2). The molecular weight shown on SDS–PAGE is consistent with the theoretical molecular mass of 51.5 kDa plus ∼4.0 kDa for the fusion part (MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGS).

Crystals suitable for data collection were obtained in a condition consisting of 0.056 *M* sodium dihydrogen phosphate, 1.344 *M* dipotassium hydrogen phosphate pH 8.2 (Fig. 3*a*). Typical crystal dimensions were approximately 0.20 × 0.20 × 0.40 mm. The crystallized protein was confirmed by an SDS–PAGE analysis of dissolved crystals (Fig. 3*b*). The crystal diffracted to a resolution of 2.5 Å and belonged to space group *R*3. Assuming the presence of four molecules in the asymmetric unit, the Matthews coefficient ($V_{\rm M}$) was calculated to be 2.4 Å^3 Da^{-1} (Matthews, 1968), with an estimated solvent content of 48.2%. The data-collection statistics are summarized in Table 1. The structure of *sm*ASL will be solved by the molecular-replacement method using the structure of *E. coli* ASL (PDB entry 1tj7; Bhaumik *et al.*, 2004), which has 46% sequence identity, as a search model.

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