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Preliminary X-ray crystallographic studies of *Bacillus subtilis* SpeA protein

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The *spe*A gene in *Bacillus subtilis* encodes arginine decarboxylase, which catalyzes the conversion of arginine to agmatine. Arginine decarboxylase is an important enzyme in polyamine metabolism in *B. subtilis*. In order to further illustrate the catalytic mechanism of arginine decarboxylase by determining the three-dimensional structure of the enzyme, the *spe*A gene was amplified from *B. subtilis* genomic DNA and cloned into the expression vector pET-28a(+). SpeA was expressed in *Escherichia coli* and purified to homogeneity by nickel-chelation chromatography followed by size-exclusion chromatography. High-quality crystals were obtained using the hanging-drop vapour-diffusion method at 289 K. The best crystal diffracted to 2.0 Å resolution and belonged to space group $P2_1$, with unit-cell parameters a = 86.4, b = 63.3 c = 103.3 Å, β = 113.9°.

1. Introduction

Three types of polyamine, putrescine, spermidine and spermine, play significant roles in nearly all cells from bacteria and plants to humans. Polyamines are involved in a variety of important cellular processes such as replication, transcription and translation as well as cell proliferation and differentiation (Cohen & Stanley, 1998). Unlike *Escherichia coli*, *Bacillus subtilis* has a single route to polyamine biosynthesis, in which arginine decarboxylase (SpeA; EC 4.1.1.19) is the first enzyme: it catalyzes the conversion of arginine to agmatine and releases carbon dioxide. Agmatine is then hydrolyzed into putrescine and urea by agmatinase (SpeB; EC 3.5.3.11) through the addition of a water molecule. Putrescine accepts a propylamine group from decarboxylated *S*-adenosylmethionine to form spermidine and 5′-*S*-methyl-5′-thioadenosine, which is catalyzed by spermidine synthase (SpeE; EC 2.5.1.16; Sekowska *et al.*, 1998).

Three types of arginine decarboxylase have been identified: prokaryotic biodegradative arginine decarboxylase (AidA; Gale, 1940; Blethen *et al.*, 1968), prokaryotic biosynthetic arginine decarboxylase (SpeA; Morris & Pardee, 1966; Wu & Morris, 1973) and arginine decarboxylase (PvlArgDC) from *Methanococcus jannaschii* (Graham *et al.*, 2002). Nine different pyridoxal 5′-phosphate-dependent amino-acid decarboxylases have been divided into four groups depending on their amino-acid sequence. AidA belongs to the group III amino-acid decarboxylases and SpeA belongs to group IV (Sandmeier *et al.*, 1994); although they have no sequence similarity, they both use pyridoxal 5′-phosphate as a cofactor. PvlArgDC, a different arginine decarboxylase, is a superthermostable enzyme that can be formed by the self-cleavage of a proenzyme into two subunits using a pyruvoyl reactive group (Graham *et al.*, 2002). To date, the structures of SpeA from *Paramecium bursaria* chlorella virus (*cv*ArgDC; Shah *et al.*, 2007) and of PvlArgDC from *M. jannaschii* (Tolbert *et al.*, 2003) have been solved. As expected, the two structures are very different: the SpeA structure has the β/α-barrel fold, while PvlArgDC contains $(\beta/\alpha)_3$ trimers. Furthermore, based on the results of sequence align-

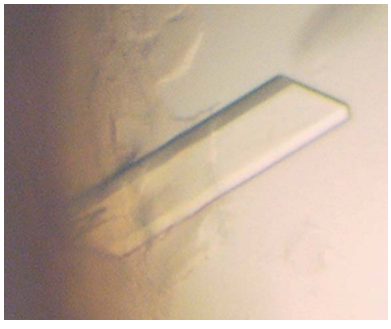

Table 1
Diffraction data statistics for SpeA.

Values in parentheses are for the outer shell.

Wavelength (Å)	1.0
Resolution (Å)	50.0–2.0 (2.1–2.0)
Completeness (%)	99.3 (98.9)
$R_{\text{mrgd-}F}$† (%)	10.0 (48.1)
Mean $I/\sigma(I)$	12.6 (3.0)
Space group	$P2_1$
Unit-cell parameters (Å, °)	a = 86.4, b = 63.3, c = 103.3, β = 113.9
No. of observed reflections	367346 (45776)
No. of unique reflections	68812 (9291)
No. of molecules per ASU	2
V_M (Å^3 Da^{-1})	2.3
Solvent content (%)	46.8

† $R_{\text{mrgd-}F} = \sum |I_{h,p} - I_{h,Q}| / 0.5 \sum I_{h,p} + I_{h,Q}$, where $I_{h,p} = (1/n_{h,p}) \sum_{i \in p}^{n_{h,p}} I_{h,i}$ and $I_{h,Q} = (1/n_{h,Q}) \sum_{i \in Q}^{n_{h,Q}} I_{h,i}$, as defined by Diederichs & Karplus (1997).

ment using *ClustalW* (Thompson *et al.*, 1994), the sequence identity between *cv*ArgDC and *B. subtilis* SpeA is 15%.

In *B. subtilis*, the *spe*A gene encodes a 490-residue protein with a molecular weight of 53.6 kDa and an isoelectric point of 5.1. In this paper, we describe the purification, crystallization and preliminary X-ray analysis of *B. subtilis* SpeA. The structure determination of this enzyme will be helpful to illustrate its catalytic mechanism and the regulation of polyamine biosynthesis in this family of arginine decarboxylases in general.

2. Materials and methods

2.1. Cloning and expression

The *spe*A gene was cloned from the genomic DNA of *B. subtilis* (strain 168) by polymerase chain reaction amplification (PCR; Saiki *et al.*, 1988) using primers 5′-CGGGATCCTCGCAACATGAAACA-CCCTTATACA-3′ and 5′-CCGCTCGAGTTGAATTGCTTTTTG-TTCTTTGATG-3′, which contain a *Bam*HI and a *Xho*I restriction site, respectively. The PCR product was purified and ligated into the corresponding restriction sites of pET-28a(+) (Novagen) containing an N-terminal His_6 tag with sequence MGSSHHHHHHSSGLVPR-GSHMASMTGGQQMGRGS. The recombinant vector containing the *spe*A gene was transformed into *E. coli* BL21 (DE3). A single clone including the *spe*A gene was inoculated into 30 ml Luria–Bertani (LB) medium containing 50 mg l^{-1} kanamycin. Cells were grown at 310 K overnight. The overnight culture was transferred into 1 l LB medium with 50 mg l^{-1} kanamycin and growth continued at 310 K until the mid-exponential growth phase, when isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to a final concentration of 0.5 m*M* to induce SpeA expression. The culture was grown at 310 K for an additional 4 h. Cells were harvested by centrifugation at 7000 rev min^{-1} for 10 min at 277 K and were resuspended in buffer *A* (20 m*M* Tris–HCl, 500 m*M* NaCl pH 7.5).

2.2. Protein purification

The cells were lysed by sonication on ice. The lysate was centrifuged at 13 000 rev min^{-1} for 40 min at 277 K. The supernatant was filtered using a 0.22 µm filter and loaded onto a 5 ml Ni^{2+}-chelating affinity column (HiTrap, GE Healthcare, USA) previously equilibrated with buffer *A*. Impurities, unbound and loosely bound proteins were washed out using 10%(*v*/*v*) buffer *B* (20 m*M* Tris–HCl, 500 m*M* NaCl, 500 m*M* imidazole pH 7.5) in buffer *A*. Tightly bound target proteins were then eluted with a linear gradient of 10–500 m*M* imidazole in buffer *A*. Fractions containing SpeA were further purified by size-exclusion chromatography on an S200 (HiLoad 16/60 Superdex 200, GE Healthcare) column using buffer *C* (20 m*M* Tris–HCl, 200 m*M* NaCl pH 7.5). The purity of the protein was checked by SDS–PAGE analysis.

2.3. Crystallization

The purified SpeA protein was concentrated to 20 mg ml^{-1} using a centrifugal filter device (Ultra-15, 30 kDa cutoff, Millipore); the concentrated protein solution contained 20 m*M* Tris–HCl and 200 m*M* NaCl pH 7.5. Crystallization trials were performed using the hanging-drop vapour-diffusion method at 289 K. The commercial crystallization screening kits Crystal Screen, Crystal Screen 2 and Index Screen (Hampton Research, USA) were used in the initial screening. Drops containing 1 µl protein solution mixed with an equal volume of reservoir solution were equilibrated against 500 µl reservoir solution.

In order to improve the crystal quality, putrescine was added to the concentrated protein to a final concentration of 10 mmol l^{-1} and incubated at 277 K for 4 h; crystallization experiments were performed at 289 K using the hanging-drop vapour-diffusion method.

2.4. Data collection

X-ray diffraction data were collected using a MAR165 CCD detector on beamline 3W1A, Beijing Synchrotron Radiation Facility (BSRF), People's Republic of China. The crystal was first flash-frozen and was maintained at 100 K using a cold nitrogen stream (Oxford Instruments Inc., UK) during data collection. The data were processed using the program *XDS* (Kabsch, 1993).

3. Results and discussion

The correct sequence of the cloned *spe*A gene in plasmid pET-28a(+) was confirmed by DNA sequencing. SpeA produced in *E. coli* strain BL21 (DE3) was highly soluble and could be purified to homogeneity in two steps. The results of SDS–PAGE showed that at least 95% of the protein in the preparations was SpeA, with a molecular weight of about 58.0 kDa. This weight matches the theoretical molecular weight of recombinant SpeA (53.6 kDa) plus about 4.0 kDa from the fusion peptide including the hexahistidyl tag. SpeA was initially

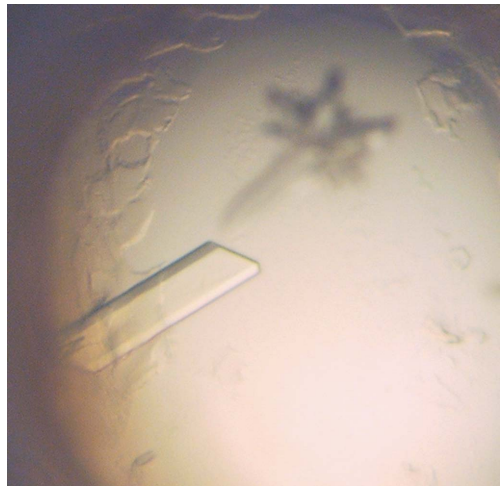

Figure 1
Crystals of *B. subtilis* SpeA protein grown in 50 m*M* calcium chloride, 100 m*M* bis-tris pH 6.5, 30%(*w*/*v*) polyethylene glycol monomethyl ether 550 with 10 mmol l^{-1} putrescine.

screened for crystallization using several commercially available crystallization screening kits described in §2. Large poorly diffracting crystals were obtained under several conditions. Crystals grown in the presence of the arginine decarboxylase substrate L-arginine and cofactors such as pyridoxal 5′-phosphate and magnesium gave poor diffraction. However, crystals obtained using polyamine additives such as putrescine, spermidine, spermine and L-canavanine gave better diffraction quality (especially in the presence of putrescine). After two weeks, a single crystal was obtained using the condition 50 m*M* calcium chloride, 100 m*M* bis-tris pH 6.5, 30%(*w*/*v*) PEG MME 550 (polyethylene glycol monomethyl ether 550), 10 mmol l^{-1} putrescine (Fig. 1). The crystal grew to dimensions of about $0.7 \times 0.2 \times 0.05$ mm and diffracted to 2.0 Å resolution. Assuming the presence of two molecules per asymmetric unit, the V_M value (Matthews, 1968) was 2.3 $Å^3$ Da^{-1}, corresponding to a solvent content of 46.8%. The crystallographic parameters and data statistics are listed in Table 1. Using ornithine decarboxylase from *Lactobacillus* 30a (Momany *et al.*, 1995), which showed 24% sequence identity to *B. subtilis* SpeA, as a starting model, structure determination using the molecular-replacement method is ongoing.

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