Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction analysis of spermidine synthase from *Helicobacter pylori*

P.-K. Lu,[a] S.-Y. Chien,[a] J.-Y. Tsai,[a] C.-T. Fong,[a] M. J. Lee,[b] H. Huang[b] and Y.-J. Sun[a]*

[a]Institute of Bioinformatics and Structural Biology, National Tsing Hua University, Hsinchu 300, Taiwan, and [b]Institute of Biotechnology, National Tsing Hua University Hsinchu 300, Taiwan

Correspondence e-mail: yjsun@life.nthu.edu.tw

Received 18 June 2004
Accepted 6 September 2004

Polyamines, such as putrescine, spermidine and spermine, are essential for the regulation of cell proliferation and differentiation in most organisms. Spermidine synthase catalyzes the transfer of the aminopropyl group from decarboxylated *S*-adenosylmethionine to putrescine in the biosynthesis of spermidine. In this study, spermidine synthase of *Helicobacter pylori* has been overexpressed in *Escherichia coli* and purified. Two kinds of spermidine synthase crystals were obtained. One belongs to the monoclinic $P2_1$ space group, with unit-cell parameters $a = 62.78$, $b = 58.24$, $c = 74.28$ Å, $\beta = 90.9^\circ$, and the other belongs to the orthorhombic $C222_1$ space group, with unit-cell parameters $a = 100.43$, $b = 128.55$, $c = 143.60$ Å.

1. Introduction

The polyamines, such as putrescine, spermidine and spermine, are found in significant amounts in living organisms. Polyamines provide both stability and neutralization for DNA and are essential for RNA and protein synthesis. Spermidine synthase (EC 2.5.1.16) catalyzes the last step in the biosynthesis of spermidine from arginine and methionine: the conversion of putrescine to spermidine using decarboxylated *S*-adenosylmethionine as the cofactor (Tabor & Tabor, 1984; Pegg, 1986; Cohen, 1998). The polyamine-biosynthesis pathway represents an important target for chemotherapeutic application (Cohen, 1998). A decrease in polyamine levels, especially levels of spermidine and spermine, is often lethal, resulting in cell-cycle arrest or apoptosis (Schipper *et al.*, 2000; Nitta *et al.*, 2002; Wallace *et al.*, 2003). Polyamine analogues have been used in the treatment of a number of parasitic infections (Kaiser *et al.*, 2001; Heby *et al.*, 2003). The growth inhibition of *Helicobacter pylori* by a multi-enzyme inhibitor of polyamine biosynthesis has also been investigated (Takaji *et al.*, 1997).

Spermidine synthase was first discovered in *Escherichia coli* (Tabor *et al.*, 1958) and was later purified from this microorganism (Bowman *et al.*, 1973). The enzyme has also been purified from bovine brain (Raina *et al.*, 1984), rat ventral prostate (Samejima & Yamanoha, 1982), pig liver (Yamanoha *et al.*, 1984), soybean axes (Yoon *et al.*, 2000), *Trypanosoma brucei brucei* (Bitonti *et al.*, 1984) and *Sulfolobus solfataricus* (Cacciapuoti *et al.*, 1986). The sequences of spermidine synthases from mammals, bacteria and plants are about 30–50% identical. However, *H. pylori* spermidine synthase has less than 20% sequence identity with them. The signature sequence of the spermidine synthase family, a glycine-rich conserved region V-(L/A)-(L/I/V)(2)-G-G-G-*X*-G-*X*(2)-(LIV)-*X*-E, has been identified (Hofmann *et al.*, 1999). Nevertheless, there is a significant difference within this region in *H. pylori* spermidine synthases, where the sequence is VLIVDGFDLELAHQ. In addition, in *H. pylori* spermidine synthases there is no 'gatekeeping loop' as in other spermidine synthases for substrate or cofactor binding (Korolev *et al.*, 2002). There are about ten residues in the 'gatekeeping loop', which is missing between Gln147 and Glu148 in *H. pylori* spermidine synthases.

Spermidine synthases have different oligomeric structures in different species. Spermidine synthase orthologues from *E. coli*, rat, bovine and human sources are thought to be dimeric (Lakanen *et al.*, 1995). However, the enzyme from the soybean axes is a monomer (Yoon *et al.*, 2000). The crystal structure of spermidine synthase from *Thermotoga maritima* has shown it to be a tetramer (Korolev *et al.*, 2002). Here, we report the crystallization and preliminary X-ray diffraction analysis of spermidine synthase from *H. pylori*.

2. Materials and methods

2.1. Cloning

The ORF for the *speE* gene encoding spermidine synthase (HP0832) was amplified from the genomic DNA of *H. pylori* strain 26695 by the polymerase chain reaction. The forward and reverse oligonucleotide primers were designed using the published genome sequence (Tomb *et al.*, 1997). The amplified DNA was digested with *Bam*HI and *Sal*I and then inserted into the *Bam*HI/*Sal*I-digested expres-

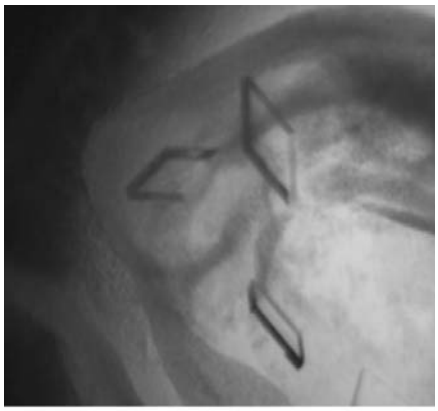
sion vector pQE-30 (Qiagen). The plasmid was transformed into *E. coli* strain SG13009 for protein expression.

2.2. Expression

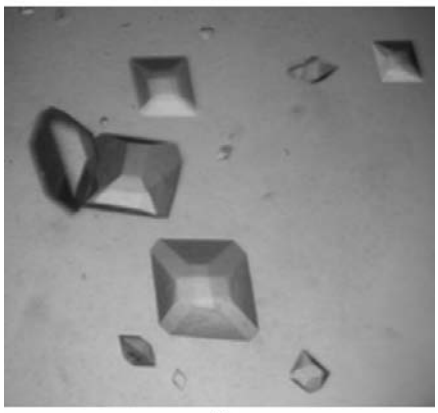
The transformed cells were cultured overnight in 10 ml LB medium with 100 µg ml^{-1} ampicillin and 25 µg ml^{-1} kanamycin at 310 K and 200 rev min^{-1}. This culture was then transferred to 500 ml LB medium under the same conditions until OD_{595} reached 0.7. Protein expression was induced by the addition of 0.5 m*M* IPTG (isopropyl-β-D-thiogalactopyranoside) and the cells were then incubated at 310 K and 200 rev min^{-1} for 3 h. Cells were harvested by centrifugation at 6000 rev min^{-1} and 277 K for 15 min.

2.3. Purification and characterization

The pellet was resuspended in 10 ml lysis buffer (20 m*M* Tris–HCl pH 7.9 and 300 m*M* NaCl) and lysed using a high-pressure homogenizer (EmulsiFlex-C5, Avestin Inc.).



(*a*)



(*b*)

Figure 1
Photographs of spermidine synthase crystals. (*a*) Form *A* crystals with primitive monoclinic space group $P2_1$. Approximate dimensions are 0.1 × 0.1 × 0.05 mm. (*b*) Form *B* crystals with orthorhombic space group $C222_1$. Approximate dimensions are 0.2 × 0.2 × 0.2 mm.

The lysate was clarified by centrifugation at 14 000 rev min^{-1} and 277 K for 20 min. The supernatant was then loaded onto a column containing immobilized nickel resin pre-equilibrated with lysis buffer. After washing with ten column volumes of lysis buffer containing 10 m*M* imidazole and then with ten column volumes of lysis buffer containing 30 m*M* imidazole, *H. pylori* spermidine synthase was eluted from the column using elution buffer (20 m*M* Tris–HCl pH 7.9, 300 m*M* NaCl, 200 m*M* imidazole). *H. pylori* spermidine synthase used for crystallization trials was concentrated to 20 mg ml^{-1}; the buffer was exchanged to stock buffer (20 m*M* Tris–HCl pH 7.9, 20 m*M* NaCl, 2 m*M* β-mercaptoethanol and 10% glycerol) using an Amicon Ultra-4 Centrifugal Filter Unit (Millipore).

Size-exclusion chromatography and analytical ultracentrifugation were used to analyze the assemblies of *H. pylori* spermidine synthase. The size-exclusion chromatography was performed on a HiLoad 16/60 Superdex 200pg column (Amersham Biosciences) pre-equilibrated with running buffer (20 m*M* Tris–HCl pH 7.9, 20 m*M* NaCl, 10% glycerol, 2 m*M* β-mercaptoethanol). The column was calibrated with chymotrypsinogen A (25 kDa), ovalbumin (43 kDa), bovine serum albumin (67 kDa) and aldolase (158 kDa). 0.5 ml protein samples at 20 mg ml^{-1} concentration were injected onto the column through a 0.5 ml injection loop. Filtration was carried out at a flow rate of 1 ml min^{-1}. The eluted protein was detected by measuring the absorbance at 280 nm. In analytical ultracentrifugation experiments, protein samples of 450 µl at 40 µ*M* concentration were centrifuged at 45 000 rev min^{-1} in a Beckman XL-A Optima analytical ultracentrifuge. The UV absorbance at 275 nm *versus* radius distributions was measured at 6 min intervals. Data analysis was performed using *SEDFIT* to determine the sedimentation coefficient distribution (Schuck *et al.*, 2002).

2.4. Crystallization

Crystallization trials of *H. pylori* spermidine synthase were carried out using the hanging-drop vapour-diffusion method (McPherson, 1982). 1 µl protein solution was mixed with 1 µl reservoir solution and equilibrated against 500 µl reservoir solution in Linbro plates. Initial crystallization conditions were obtained using Hampton Research Crystal Screen kits (Hampton Research, California, USA) and then further optimized to obtain diffraction-quality crystals.

Table 1
Summary of X-ray diffraction data statistics.

Values in parentheses are for the outer resolution shell.

	Form *A*	Form *B*
Wavelength (Å)	1.5418	1.5418
Crystal system	Monoclinic	Orthorhombic
Space group	$P2_1$	$C222_1$
Unit-cell parameters		
a (Å)	62.78	100.43
b (Å)	58.24	128.55
c (Å)	74.82	143.60
α (°)	90.0	90.0
β (°)	90.0	90.0
γ (°)	90.0	90.0
Resolution (Å)	2.2	2.7
No. reflections collected	101999	156162
No. unique reflections	27332	25373
Redundancy of reflections	3.73	6.15
Data completeness (%)	98.8 (98.5)	97.9 (99.8)
$I/\sigma(I)$	21.8 (4.2)	24.9 (5.3)
$R_{\rm merge}$† (%)	5.5 (33.3)	6.3 (33.9)

† $R_{\rm merge}(I) = \sum_h \sum_i |I_i - I| / \sum_h \sum_i I$, where I is the mean intensity of the i observation of reflection h.

2.5. X-ray data collection

X-ray diffraction data were collected from *H. pylori* spermidine synthase crystals (Fig. 1) on a Rigaku R-AXIS IV^{++} image-plate detector using Cu $K\alpha$ radiation from a Rigaku RU-300 rotating-anode X-ray generator operated at 50 kV and 100 mA. Crystals were mounted in a nylon loop and flash-frozen in a liquid-nitrogen stream at 100 K after being dipped in immersion oil. The programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997) were used for the X-ray diffraction data processing and analysis.

3. Results

H. pylori spermidine synthase has been overexpressed and purified. The enzyme is 262 amino acids in length, with molecular weight 30 589 Da. The size-exclusion chromatography and analytical ultracentrifugation results reveal that *H. pylori* spermidine synthase forms a dimer in solution. Two crystal forms grew under different conditions. Form *A* crystals grew to maximum dimensions of 0.1 × 0.1 × 0.05 mm within 5 d at 293 K using 25% PEG 3350 (Fig. 1*a*). Form *B* crystals grew to maximum dimensions of 0.2 × 0.2 × 0.2 mm within 2–5 d at 293 K using 20% PEG 3350, 0.25 *M* sodium citrate (Fig. 1*b*).

Form *A* crystals diffracted to 2.2 Å and belong to the monoclinic space group $P2_1$, with unit-cell parameters $a = 62.78$, $b = 58.24$, $c = 74.82$ Å, $\alpha = \gamma = 90$, $\beta = 90.90°$ and an $R_{\rm merge}$ of 5.5% (Table 1). $V_{\rm M}$ (Matthews, 1968) was calculated to be 2.14 Å^3 Da^{-1}, corresponding to a solvent content of 42%, assuming two molecules per asymmetric unit in the crystal. The direction of the non-

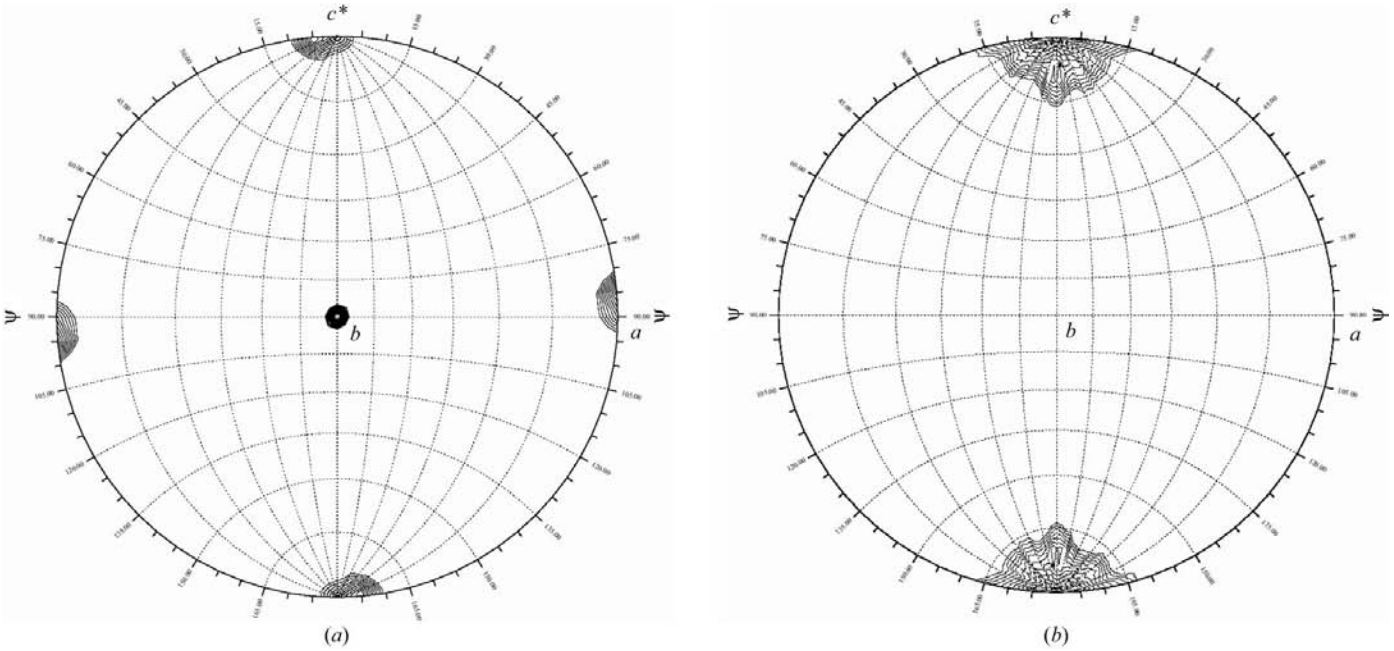


Figure 2
Self-rotation function plots of spermidine synthase crystals. (*a*) Form *A* crystal, $\kappa = 180^\circ$. (*b*) Form *B* crystal, $\kappa = 120^\circ$.

crystallographic twofold axis was determined using the self-rotation function calculation in the *GLRF* program (Tong & Rossmann, 1990) with data in the 12–4 Å resolution range. A plot of the self-rotation function is shown at a κ angle of 180° in Fig. 2(*a*). The detailed diffraction data statistics of a form *A* crystal are presented in Table 1.

Form *B* crystals belong to the orthorhombic space group $C222_1$, with unit-cell parameters $a = 100.43$, $b = 128.55$, $c = 143.60$ Å, $\alpha = \beta = \gamma = 90^\circ$. The crystal diffracted to 2.7 Å resolution with an $R_{\rm merge}$ of 6.3%. Assuming the presence of three molecules in the asymmetric unit, $V_{\rm M}$ is 2.42 Å^3 Da^{-1}, corresponding to a solvent content of 49%. The direction of the non-crystallographic threefold axis was found from self-rotation function calculation with data in the 12–4 Å resolution range. A self-rotation function plot at a κ angle of 120° is shown in Fig. 2(*b*). The detailed diffraction data statistics of the form *B* crystal are presented in Table 1. Structure determinations of *H. pylori* spermidine synthase using the molecular-replacement method and multi-wavelength anomalous diffraction (MAD) methods using the SeMet derivate protein are ongoing.

This work was supported by a grant from National Science Council and National Research Program Genomic Medicine (NSC-93-3112-B-007-014 to YJS), Taiwan, Republic of China.

References

Bitonti, A. J., Kelly, S. E. & McCann, P. P. (1984). *Mol. Biochem. Parasitol.* **13**, 21–28.
Bowman, W. H., Tabor, C. W. & Tabor, H. (1973). *J. Biol. Chem.* **248**, 2480–2486.
Cacciapuoti, G., Porcelli, M., Carteni-Farina, M., Gambacorta, A. & Zappia, V. (1986). *Eur. J. Biochem.* **161**, 263–271.
Cohen, S. (1998). *A Guide to the Polyamines.* Oxford University Press.
Heby, O., Roberts, S. C. & Ullman, B. (2003). *Biochem. Soc. Trans.* **31**, 415–419.
Hofmann, K., Bucher, P., Falquet, L. & Bairoch, A. (1999). *Nucleic Acids Res.* **27**, 215–219.
Kaiser, A., Cottwald, A., Wiersch, C., Lindenthal, B., Maier, W. & Seitz, H. M. (2001). *Parasitol. Res.* **87**, 963–972.
Korolev, S., Ikeguchi, Y., Skarina, T., Beasley, S., Arrowsmith, C., Edwards, A., Joachimiak, A., Pegg, A. E. & Savchenko, A. (2002). *Nature Struct. Biol.* **9**, 27–31.
Lakanen, J. R., Pegg, A. E. & Coward, J. K. (1995). *J. Med. Chem.* **38**, 2714–2727.
McPherson, A. (1982). *Preparation and Analysis of Protein Crystals.* New York: John Wiley.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Nitta, T., Igarashi, K. & Yamamoto, N. (2002). *Exp. Cell Res.* **276**, 120–128.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Pegg, A. E. (1986). *Biochem. J.* **234**, 249–262.
Raina, A., Hyvonen, T., Eloranta, T., Voutilainen, M., Samejima, K. & Yamanoha, B. (1984). *Biochem. J.* **219**, 991–1000.
Samejima, K. & Yamanoha, B. (1982). *Arch. Biochem. Biophys.* **216**, 213–222.
Schipper, R. G., Penning, L. C. & Verhofstad, A. A. J. (2000). *Semin. Cancer Biol.* **10**, 55–68.
Schuck, P., Perugini, M. A., Gonzales, N. R., Howlett, G. J. & Schubert, D. (2002). *Biophys. J.* **82**, 1096–1111.
Tabor, C. W. & Tabor, H. (1984). *Annu. Rev. Biochem.* **53**, 749–790.
Tabor, H., Rosenthal, S. M. & Tabor, C. W. (1958). *J. Biol. Chem.* **233**, 907–914.
Takaji, S., Hibasami, H., Imoto, I., Hashimoto, Y., Nakao, K., Ikemura, N., Taguchi, Y. & Nakashima, K. (1997). *Lett. Appl. Microbiol.* **25**, 177–180.
Tomb, J.-F. *et al.* (1997). *Nature (London)*, **388**, 539–547.
Tong, L. & Rossmann, M. G. (1990). *Acta Cryst.* A**46**, 783–792.
Wallace, H. M., Fraser, A. V. & Hughes, A. (2003). *Biochem. J.* **376**, 1–14.
Yamanoha, B., Samejima, K., Nakajima, T. & Yasuhara, T. (1984). *J. Biochem. (Tokyo)*, **96**, 1273–1281.
Yoon, S. O., Lee, Y. S., Lee, S. H. & Cho, Y. D. (2000). *Biochim. Biophys. Acta*, **1475**, 17–26.