Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

Overexpression, crystallization and preliminary X-ray crystallographic analysis of shikimate dehydrogenase from *Archaeoglobus fulgidus*

Hyung Ho Lee

Department of Bio and Nano Chemistry, Kookmin University, Seoul 136-702, Republic of Korea, and Department of Integrative Biomedical Science and Engineering, Kookmin University, Seoul 136-702, Republic of Korea

Correspondence e-mail: hhlee@kookmin.ac.kr

Received 7 September 2011
Accepted 6 October 2011

Shikimate dehydrogenase (SDH), which catalyses the NADPH-dependent reduction of 3-dehydroshikimate to shikimate in the shikimate pathway, is an attractive target for the development of herbicides and antimicrobial agents. Previous structural studies have shown that SDH exists in two conformations, an open and a closed form, and it is believed that the conformational state is crucial to understanding its catalytic mechanism. In order to facilitate further structural comparisons among SDHs, including the conformational state, structural analysis of an SDH from *Archaeoglobus fulgidus* encoded by the *Af2327* gene has been initiated. SeMet-labelled SDH from *A. fulgidus* was overexpressed in *Escherichia coli* and crystallized at 296 K using ammonium sulfate as a precipitant in order to use the MAD method for structure determination. Crystals of *A. fulgidus* SDH grown in the presence of $NADP^+$ diffracted to 2.8 Å resolution and belonged to the trigonal space group $P3_221$ (or $P3_221$), with unit-cell parameters a = 111.3, b = 111.3, c = 76.2 Å. Three diffraction data sets were collected. The asymmetric unit contains two monomers, with a corresponding V_M of 2.34 $Å^3$ Da^{-1} and a solvent content of 47% by volume.

1. Introduction

Shikimate is the key intermediate in the shikimate-biosynthetic pathway commonly found in bacteria, fungi and higher plants but absent in animals. Therefore, the shikimate pathway is an attractive target for the development of antibacterial agents (Davies *et al.*, 1994). The shikimate pathway consists of seven enzymatic steps. Shikimate dehydrogenase (SDH; EC 1.1.1.25), which is encoded by the *aroE* gene in bacteria, is responsible for the fourth reaction of the shikimate pathway. SDH catalyses the NADPH-dependent reduction of 3-dehydroshikimate to shikimate (Singh *et al.*, 2005). Inhibitors that target SDH from *Helicobacter pylori* have been reported (Han *et al.*, 2006) and have been shown to inhibit cell growth, suggesting that SDH might be a promising target for antibacterial agents (Han *et al.*, 2006). Two types of shikimate dehydrogenase from bacteria, SDH (PDB code 1nyt) and YdiB (PDB code 1o9b), have been structurally reported (Michel *et al.*, 2003). Structural and biochemical studies of a novel shikimate dehydrogenase from *Haemophilus influenzae* revealed that it has a different substrate specificity from SDH and YdiB (Singh *et al.*, 2005). The SDH from *H. influenzae* catalyzes the oxidation of shikimate but not quinate, while YdiB catalyzes the reversible reductions of dehydroquinate to quinate and of dehydroshikimate to shikimate in the presence of NAD(P)H (Singh *et al.*, 2005). SDH is bipartite and is composed of an NADPH-binding domain and a catalytic domain. The NADPH-binding domain has a typical Rossmann fold containing a unique glycine-rich P-loop. The catalytic domain has a novel fold (Ye *et al.*, 2003).

The oligomeric states of SDHs, which are mostly either monomers or dimers, differ depending on species. SDH from *Escherichia coli* is present as a monomer, whereas SDHs usually form oligomers in most bacteria (Anton & Coggins, 1988; Chaudhuri & Coggins, 1985). Recently reported crystallographic studies of SDH from *Thermotoga maritima* suggest that the SDH from *T. maritima* exists as a monomer in solution. In comparison, SDH from *Methanococcus jannaschii* and

© 2011 International Union of Crystallography
All rights reserved

YdiB from *E. coli* have been shown to exist as dimers in both solution and crystals (Michel *et al.*, 2003; Padyana & Burley, 2003).

Subsequently, the structures of several SDHs have been reported, including the structures of SDHs from *Arabidopsis* (Singh & Christendat, 2006), *Aquifex aeolicus* (Gan *et al.*, 2007), *Thermus thermophilus* (Bagautdinov & Kunishima, 2007) and *Staphylococcus epidermidis* (Han *et al.*, 2009). Structural studies on SDHs revealed that they have two conformations: open and closed forms. The closed form has been suggested to be necessary for catalysis (Michel *et al.*, 2003). In SDH from *E. coli*, substrate binding promotes a switch from the open conformation to the closed conformation (Michel *et al.*, 2003). In SDH from *T. thermophilus*, it has been shown that the cofactor only binds to the closed form, while the substrate binds to both forms (Bagautdinov & Kunishima, 2007). The openness of SDHs is likely to be dependent on the species. The crystal structure of SDH from *A. aeolicus* in complex with $NADP^+$ and shikimic acid exhibits a closed conformation (Gan *et al.*, 2007), whereas the ternary complex of SDH from *T. thermophilus* with $NADP^+$ and shikimic acid shows an open conformation.

In order to facilitate further structural comparisons among SDHs, including their conformation (open form or closed form), we have initiated crystallographic studies of SDH from *A. fulgidus*. In contrast to bacteria, SDHs from archaea are structurally unexplored. Structural studies on SDH from *A. fulgidus* might facilitate the design of inhibitors targeting SDHs. When we analyzed the sequence identity of SDH from *A. fulgidus* to other structurally reported SDHs, the sequence identity was 33% to SDH from *E. coli*, 37% to YdiB from *E. coli*, 30% to SDH from *H. influenzae*, and 40% to SDH from *M. jannaschii*. The SDH from *A. fulgidus* has been overexpressed in *E. coli* and crystallized. Its crystallization conditions and X-ray crystallographic data are reported here.

2. Materials and methods

2.1. Protein expression and purification

The *aroE* gene (Af2327) encoding *A. fulgidus* SDH was amplified by the polymerase chain reaction. The forward and reverse oligonucleotide primers were 5′-GG GAA TTC **CAT ATG** CTC TAC CTT GGC GTC ATA G-3′ and 5′-CCG CCG **CTC GAG** TTA AAA CCT CAA AGC CCT CAA AGC AG-3′, respectively. The bases shown in bold represent the *Nde*I and *Xho*I restriction-enzyme cleavage sites, respectively. The amplified DNA was digested with *Nde*I and *Xho*I and was then inserted into the *Nde*I/*Xho*I-digested expression vector pET-21a(+) (Novagen). The plasmid was transformed into *E. coli* strain B834 (DE3) (Novagen) for protein expression. B834 (DE3) cells transformed with the plasmid were selected on LB–agar plates with 50 µg ml^{-1} ampicillin. A single colony was transferred into 20 ml LB and grown overnight with vigorous shaking at 310 K. The cells were re-inoculated into 2 l M9 medium with 40 mg ml^{-1} of all amino acids except methionine and were grown to an A_{600} of 0.5. Protein expression was induced with 1.0 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) at 288 K. After IPTG induction, cell growth continued for 19 h at 288 K and the cells were harvested by centrifugation at 6000 rev min^{-1} (Sorvall GSA rotor) for 10 min at 277 K. The cell pellet was resuspended in ice-cold lysis buffer *A* (50 m*M* Tris–HCl pH 7.5, 120 m*M* NaCl, 10 m*M* DTT). The cells were then homogenized by ultrasonication and heated for 10 min at 338 K. The crude cell extract was centrifuged at 36 000*g* (18 000 rev min^{-1}; Hanil Supra 21K rotor) for 20 min at 277 K. The supernatant was subjected to ion-exchange chromatography on a Q-Sepharose column (GE Healthcare) previously equilibrated with buffer *A*. Protein was eluted with a linear gradient of 0–1.0 *M* NaCl in buffer *A*. Purification was then performed by gel filtration on a HiLoad XK 16 Superdex 200 prep-grade column (GE Healthcare) previously equilibrated with buffer *A* containing 100 m*M* NaCl. The protein solution was concentrated to about 28 mg ml^{-1} using an YM10 ultrafiltration membrane (Amicon). The protein concentration was estimated by measuring the absorbance at 280 nm, employing the calculated molar extinction coefficient of 21 740 M^{-1} cm^{-1}.

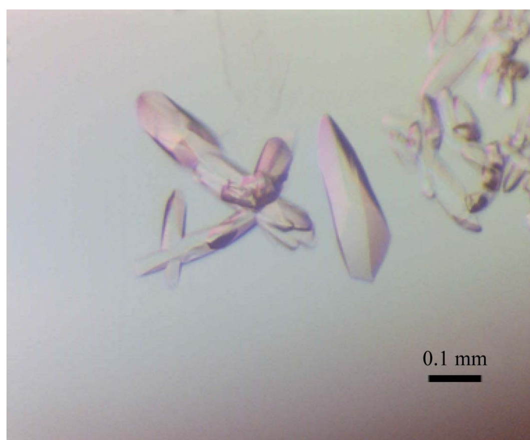

Figure 1
Crystals of shikimate dehydrogenase from *A. fulgidus* grown in the presence of $NADP^+$, with approximate dimensions of 0.2 × 0.1 × 0.4 mm.

2.2. Crystallization, dynamic light scattering and X-ray data collection

Crystallization experiments were carried out using the hanging-drop vapour-diffusion method at 296 K using 24-well tissue-culture plates (Hampton Research). A hanging drop on a siliconized cover slip was prepared by mixing equal volumes (2 µl each) of protein solution and reservoir solution and was placed over 0.5 ml reservoir solution. Initial crystallization conditions were established using screening kits from Hampton Research (Crystal Screen, Crystal Screen 2 and MembFac) and deCODE Biostructures Group (Wizard I and Wizard II). Dynamic light-scattering experiments were performed using a DynaPro-801 instrument from Wyatt (Santa Barbara, California, USA). The data were measured at 297 K using protein at 1 mg ml^{-1} concentration in buffer *A* containing 100 m*M* NaCl.

Crystals of *A. fulgidus* SDH obtained using ammonium sulfate as a precipitant were optimized. To grow crystals of the native protein complexed with $NADP^+$, 100 m*M* $NADP^+$ solution (dissolved in 50 m*M* Tris–HCl pH 7.5 and 100 m*M* NaCl) was mixed with the protein solution, resulting in an ∼50-fold molar excess of $NADP^+$ over the SDH monomer. The protein mixed with $NADP^+$ was incubated for 30 min at 277 K before crystallization. Crystals were flash-cooled in a liquid-nitrogen stream employing 15%(*v*/*v*) glycerol as a cryoprotectant. Three-wavelength MAD data for *A. fulgidus* SDH were collected at 100 K on beamline BL-18B of the Photon Factory (PF), Japan (Watanabe *et al.*, 1995) using a MacScience 2030 image-plate detector. The wavelength of the synchrotron X-rays was 0.9792 Å for the peak, 0.9794 Å for the inflection and 0.9500 Å for the remote, and a 0.3 mm collimator was used. The crystal was rotated through a total of 180, 180 and 136° for the peak, inflection and remote data, respectively, with a 1.0° oscillation range per frame. The data set was processed and scaled using the programs *MOSFLM* (Leslie, 1992) and *SCALA* (Winn *et al.*, 2011).

Table 1
Data-collection and refinement statistics for SeMet-substituted *A. fulgidus* SDH.

Values in parentheses are for the highest resolution shell (2.95–2.80 Å).

Data	Peak	Inflection	Remote
X-ray source	BL-18B, PF	BL-18B, PF	BL-18B, PF
Wavelength (Å)	0.9792	0.9794	0.9500
Space group	$P3_121$ (or $P3_221$)	$P3_121$ (or $P3_221$)	$P3_121$ (or $P3_221$)
Unit-cell parameters			
$a = b$ (Å)	111.31	111.34	111.31
c (Å)	76.15	76.10	76.15
$\alpha = \beta$ (°)	90	90	90
γ (°)	120	120	120
Resolution range (Å)	32–2.8	32–2.8	32–2.8
Measured reflections	337367	337562	254186
Unique reflections	13735	13742	13733
Completeness (%)	99.9 (100)	100 (100)	99.9 (99.9)
$\langle I/\sigma(I)\rangle$	5.7 (2.0)	5.6 (2.0)	5.2 (1.7)
$R_{\rm merge}$† (%)	11.5 (36.6)	11.8 (37.3)	12.9 (44.0)

† $R_{\rm merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I(hkl)$ is the intensity of reflection hkl, $\sum_{hkl}$ is the sum over all reflections and $\sum_i$ is the sum over i measurements of reflection hkl.

3. Results

A. fulgidus SDH was overexpressed in soluble form with a yield of ~10 mg homogeneous protein per litre of culture. The optimized reservoir condition was 100 m*M* Na HEPES buffer pH 7.8, 2.2 *M* ammonium sulfate and 2%(*v*/*v*) polyethylene glycol (PEG) 400. Crystals in complex with $NADP^+$ grew to maximum dimensions of 0.10 × 0.10 × 0.40 mm within several days (Fig. 1). Three diffraction data sets were collected using an SeMet-substituted SDH crystal. Three sets of X-ray diffraction data were collected to 2.80 Å resolution at 100 K. For the peak data set, a total of 337 367 measured reflections were merged into 13 735 unique reflections, giving an $R_{\rm merge}$ of 11.5% and a completeness of 99.9% for the peak data. The space group was determined as the trigonal space group $P3_121$ (or $P3_221$), with unit-cell parameters a = 111.3, b = 111.3, c = 76.2 Å on the basis of systematic absences and symmetry of diffraction intensities. Table 1 summarizes the statistics of data collection. The molecular mass of the recombinant SDH was estimated to be ~60 kDa by dynamic light-scattering analysis, indicating that the enzyme exists as a dimer in solution (calculated monomer mass of 30 202 Da). If it is assumed that a dimeric molecule is present in the crystallographic asymmetric unit, the crystal volume per protein mass ($V_{\rm M}$) is 2.34 $Å^3\ Da^{-1}$ and the solvent content is 47% (Matthews, 1968).

The author thanks Dr Se Won Suh for supporting this work in all aspects and the staff at beamline BL-18B of the Photon Factory for assistance during X-ray experiments. This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST; grant No. 2011-0005805) and by the Research Program 2011 of Kookmin University. This work was also supported by the Seoul R&BD program (ST100072).

References

Anton, I. A. & Coggins, J. R. (1988). *Biochem. J.* **249**, 319–326.
Bagautdinov, B. & Kunishima, N. (2007). *J. Mol. Biol.* **373**, 424–438.
Chaudhuri, S. & Coggins, J. R. (1985). *Biochem. J.* **226**, 217–223.
Davies, G. M., Barrett-Bee, K. J., Jude, D. A., Lehan, M., Nichols, W. W., Pinder, P. E., Thain, J. L., Watkins, W. J. & Wilson, R. G. (1994). *Antimicrob. Agents Chemother.* **38**, 403–406.
Gan, J., Wu, Y., Prabakaran, P., Gu, Y., Li, Y., Andrykovitch, M., Liu, H., Gong, Y., Yan, H. & Ji, X. (2007). *Biochemistry*, **46**, 9513–9522.
Han, C., Hu, T., Wu, D., Qu, S., Zhou, J., Ding, J., Shen, X., Qu, D. & Jiang, H. (2009). *FEBS J.* **276**, 1125–1139.
Han, C., Wang, L., Yu, K., Chen, L., Hu, L., Chen, K., Jiang, H. & Shen, X. (2006). *FEBS J.* **273**, 4682–4692.
Leslie, A. G. W. (1992). *Jnt CCP4/ESF–EACBM Newsl. Protein Crystallogr.* **26**.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Michel, G., Roszak, A. W., Sauvé, V., Maclean, J., Matte, A., Coggins, J. R., Cygler, M. & Lapthorn, A. J. (2003). *J. Biol. Chem.* **278**, 19463–19472.
Padyana, A. K. & Burley, S. K. (2003). *Structure*, **11**, 1005–1013.
Singh, S. & Christendat, D. (2006). *Biochemistry*, **45**, 7787–7796.
Singh, S., Korolev, S., Koroleva, O., Zarembinski, T., Collart, F., Joachimiak, A. & Christendat, D. (2005). *J. Biol. Chem.* **280**, 17101–17108.
Watanabe, N., Nakagawa, A., Adachi, S. & Sakabe, N. (1995). *Rev. Sci. Instrum.* **66**, 1824–1826.
Winn, M. D. *et al.* (2011). *Acta Cryst.* D**67**, 235–242.
Ye, S., Von Delft, F., Brooun, A., Knuth, M. W., Swanson, R. V. & McRee, D. E. (2003). *J. Bacteriol.* **185**, 4144–4151.