Acta Crystallographica Section F
Structural Biology Communications
ISSN 2053-230X

Preliminary X-ray diffraction analysis of thermostable β-1,4-xylanase from *Streptomyces* sp. S9

Pin Lv,[a,b]‡ Lilan Zhang,[a,b]‡ Huiying Luo,[c]‡ Chun-Chi Chen,[b] Chun-Hsiang Huang,[b] Wei Peng,[a,b] Kun Wang,[c] Tzu-Ping Ko,[d] Yingying Zheng,[b] Juankun Zhang,[a] Bin Yao[c]* and Rey-Ting Guo[b]*

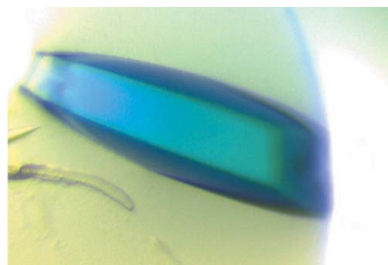
[a]Tianjin Key Laboratory of Industrial Microbiology, College of Biotechnology, Tianjin University of Science and Technology, Tianjin 300457, People's Republic of China, [b]Industrial Enzymes National Engineering Laboratory, Tianjin Institute of Industrial Biotechnology, Chinese Academy of Sciences, Tianjin 300308, People's Republic of China, [c]Key Laboratory for Feed Biotechnology of the Ministry of Agriculture, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, People's Republic of China, and [d]Institute of Biological Chemistry, Academia Sinica, Taipei 11529, Taiwan

‡ These authors contributed equally.

Correspondence e-mail:
yaobin@mail.caas.net.cn,
guoreyting@gmail.com

Received 20 October 2013
Accepted 9 December 2013

Xylanase, which catalyzes the random hydrolysis of internal xylosidic linkages, is a critical enzyme participating in xylan decomposition and has been widely applied in industrial utilizations. Xylanase isolated from the extremophilic *Streptomyces* sp. S9 (XynAS9) possesses broad adaptability to temperature and pH and thus is an attractive candidate in industrial applications. In particular, the major products of XynAS9 are xylose and xylobiose, which enable the subsequent bioconversion to be carried out with higher efficiency. Therefore, the three-dimensional structure of XynAS9 and its catalytic machinery are of great interest. Here, recombinant XynAS9 protein was expressed in *Pichia pastoris*, purified and crystallized. Crystals belonging to the hexagonal space group $P6_522$, with unit-cell parameters $a = b = 80.9$, $c = 289.3$ Å, were obtained by the sitting-drop vapour-diffusion method and diffracted to 2.08 Å resolution. Initial phase determination using molecular replacement indicated that the crystal contains one molecule in an asymmetric unit. Further model building and structural refinement are in progress.



© 2014 International Union of Crystallography
All rights reserved

1. Introduction

Xylan is the most abundant hemicellulosic component of the plant cell wall and accounts for up to 35% of the total dry weight of higher land plants. The xylan backbone is made up of β-1,4-linked xylose residues, which are frequently decorated with acetyl, arabinofuranosyl and 4-*O*-methylglucuronyl groups. Xylanases (EC 3.2.1.8) can randomly catalyze the endohydrolysis of xylosidic linkages within the main chain of xylan into xylooligosaccharides of varying length and are critical enzymes for xylan decomposition (Dodd & Cann, 2009). To date, xylanases have been found to merit a number of biotechnological applications including pulp treatment, animal feed and food manufacture, and biofuel production (Beg *et al.*, 2001; Subramaniyan & Prema, 2002). Because industrial processes are often associated with extreme conditions, searching for xylanases with higher thermostability, higher specificity and favourable pH profiles is of great interest.

Recently, a novel glycoside hydrolase family 10 (GH10) xylanase was characterized from *Streptomyces* sp. S9 (XynAS9), which was isolated from the Flaming Mountain in the Turpan Basin, which is the hottest area of China (Li *et al.*, 2008). The optimal pH and temperature for XynAS9 are 6.5 and 60°C, respectively. Remarkably, XynAS9 shows broad temperature adaptability: more than 65% residual activity was detected at 50–80°C, a property that is rarely observed for thermostable enzymes. In addition, the enzyme remains stable over a broad range of pH values between pH 4 and pH 12. These characteristics make XynAS9 an attractive candidate for a wide array of industrial applications. It is worth mentioning that the major hydrolysis products of XynAS9 are xylose and xylobiose. The lower structural complexity of the enzyme products should facilitate subsequent bioconversions. In order to explore the underlying mechanism of the enzymatic reaction of XynAS9, knowledge of the three-dimensional structure of the protein is an important prerequisite.

2. Materials and methods

2.1. Protein preparation

The gene encoding the catalytic domain of XynAS9 (GenBank accession No. EU153378) from *Streptomyces* sp. S9 was amplified by polymerase chain reaction (PCR) with the forward primer 5′-TA**GAATTC**GACACCGCCACCCTGGGCGAACT-3′ and the reverse primer 5′-TAT**GCGGCCGC**GTTGAGCGCGGCGGCGATGGCGTG-3′, and was then cloned into the vector pPIC9 using the *Eco*RI and *Not*I restriction sites. The recombinant plasmid was linearized by *Bgl*II and transformed into *Pichia pastoris* strain GS115 (Invitrogen, California, USA) by electroporation. The transformants were selected on MD [1.34% yeast nitrogen base (YNB), 4×10^{-5}% biotin, 2% dextrose, 1.5% agarose] plates and incubated at 30°C for 2–3 d. The protein expression of the transformants was tested using the following small-scale expression. The selected colonies were inoculated in 5 ml BMGY (1% yeast extract, 2% peptone, 100 m*M* potassium phosphate pH 6.0, 1.34% YNB with ammonium sulfate without amino acids, 4×10^{-5}% biotin, 1% glycerol). The cells were harvested and then resuspended in 2 ml BMMY (1% yeast extract, 2% peptone, 100 m*M* potassium phosphate pH 6.0, 1.34% YNB with ammonium sulfate without amino acids, 4×10^{-5}% biotin, 0.5% methanol) to induce protein expression. Afterwards, the transformants with the highest expression levels were chosen for scaled-up expression. The cell stock was grown at 30°C in 50 ml YPD medium (1% yeast extract, 2% peptone, 2% dextrose) for 24 h. The cells were then transferred into 900 ml BMGY. After a further 24 h, the cells were collected by centrifugation and grown in 200 l BMMY. A total of 0.5% methanol was added every 24 h to induce protein expression for four consecutive days.

The supernatant was collected by centrifugation and then purified with an FPLC system using diethylaminoethyl (DEAE) columns (GE Healthcare, Uppsala, Sweden). The buffer used for the DEAE column was 25 m*M* Tris–HCl pH 8.0. XynAS9 protein eluted at about 0.3 m*M* NaCl when using a 0–0.5 *M* NaCl gradient and was then dialyzed against buffer consisting of 25 m*M* Tris–HCl pH 7.5, 150 m*M* NaCl for storage. All purification procedures were performed at 4°C. SDS–PAGE analysis was used to check the protein purity (>95%).

2.2. Crystallization and data collection

Initial crystallization screening was performed manually using 768 different reservoir conditions from Hampton Research (Laguna Niguel, California, USA), including Crystal Screen, Crystal Screen 2, Crystal Screen Cryo, Crystal Screen Lite, MembFac, Natrix, Index, SaltRx, SaltRx 2, PEG/Ion, PEG/Ion 2, Quick Screen and Grid Screens (Ammonium sulfate, MPD, Sodium Chloride, Sodium Malonate, PEG 6000 and PEG/LiCl); all of the crystallization experiments were conducted at 25°C using the sitting-drop vapour-diffusion method. In general, 2 µl XynAS9-containing solution (25 m*M* Tris–HCl pH 7.5, 150 m*M* NaCl; 16 mg ml^{-1} protein) was mixed with 2 µl reservoir solution in 24-well Cryschem plates (Hampton Research) and equilibrated against 300 µl reservoir solution. Initial crystals of XynAS9 were obtained within 15 d using Crystal Screen Lite condition No. 45 [0.2 *M* zinc acetate dihydrate, 0.1 *M* sodium cacodylate trihydrate pH 6.5, 9%(*w*/*v*) polyethylene glycol 8000]. The optimized crystallization condition was modified to 0.5 *M* zinc acetate dihydrate, 0.1 *M* sodium cacodylate trihydrate pH 6.5, 8%(*w*/*v*) polyethylene glycol 8000. Within 13–15 d, the crystals reached dimensions of about 0.1 × 0.1 × 0.4 mm. Prior to data collection at −173°C, the crystal was mounted in a cryoloop and flash-cooled in liquid nitrogen with a slightly modified cryoprotectant, which consisted of 1 *M* zinc acetate dihydrate, 0.15 *M* sodium cacodylate trihydrate pH 6.5, 16%(*w*/*v*) polyethylene glycol 8000, 15% glycerol. An X-ray diffraction data set was collected to 2.08 Å resolution on beamline BL15A1 of the National Synchrotron Radiation Research Center (NSRRC), Hsinchu, Taiwan. During data collection, the oscillation range was 0.5°, the exposure time was 10 s and the crystal-to-detector distance was 230 mm. The diffraction images were processed using *HKL*-2000 (Otwinowski & Minor, 1997). Data-collection statistics are shown in Table 1.

Table 1
Data-collection statistics for the XynAS9 crystal.

Values in parentheses are for the highest resolution shell.

Beamline	BL15A1, NSRRC
Wavelength (Å)	1
Resolution (Å)	25–2.08 (2.15–2.08)
Space group	$P6_522$
Unit-cell parameters	
a (Å)	80.9
b (Å)	80.9
c (Å)	289.3
No. of measured reflections	553341 (57336)
No. of unique reflections	34624 (3353)
Completeness (%)	99.3 (99.2)
R_{merge} (%)	8.1 (49.8)
R_{mean} (%)	8.4 (51.3)
$R_{p.i.m.}$ (%)	2.1 (12.3)
Mean $I/\sigma(I)$	44.1 (9.5)
Multiplicity	16.0 (17.1)
X-ray beam size (µm)	200
Oscillation range (°)	0.5
Exposure time (s)	10
Crystal-to-detector distance (mm)	230
Detector	MX300HE

3. Results and discussion

The XynAS9 crystal (Fig. 1) was obtained using 0.5 *M* zinc acetate dihydrate, 0.1 *M* sodium cacodylate trihydrate pH 6.5, 8%(*w*/*v*) polyethylene glycol 8000. Based on the diffraction pattern (Fig. 2), the XynAS9 crystal belonged to the hexagonal space group $P6_522$, with unit-cell parameters $a = b = 80.9$, $c = 289.3$ Å. Assuming the presence of one molecule in the asymmetric unit, the Matthews

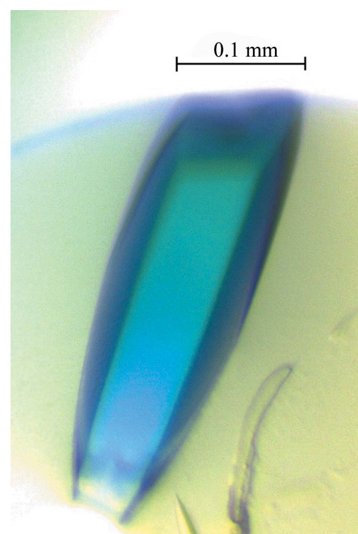


Figure 1
A crystal of XynAS9. The crystal reached approximate dimensions of 0.1 × 0.1 × 0.4 mm in 13–15 d.

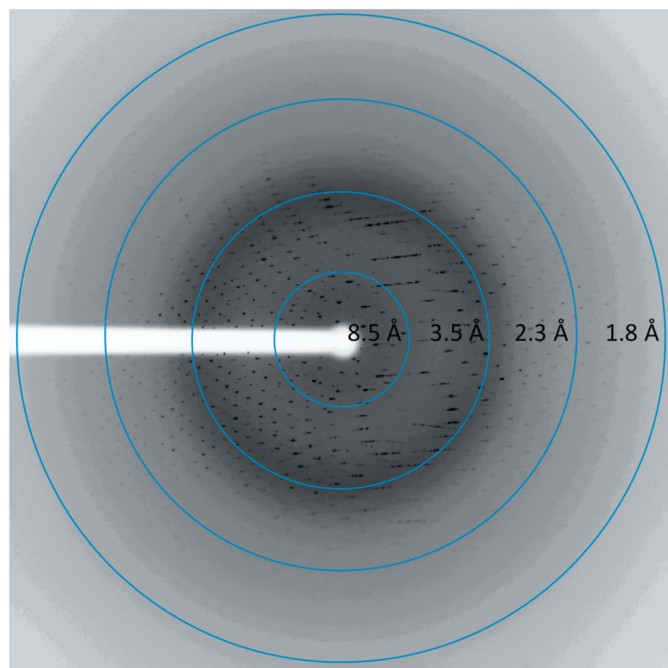


Figure 2
A diffraction pattern of the XynAS9 crystal. The inner to outer concentric rings show resolutions of 8.5, 3.5, 2.3 and 1.8 Å.

coefficient V_M (Matthews, 1968) is 4.63 $Å^3\ Da^{-1}$ and the estimated solvent content is 73.5%.

The structure of XynAS9 was solved by the molecular-replacement method with *Phaser* (McCoy *et al.*, 2007) from the *CCP*4 suite (Winn *et al.*, 2011) using the structure of the *Cellulomonas fimi* xylanase (53.04% sequence identity to XynAS9) as a search model (PDB entry 3cui; D. K. Y. Poon, I. D. D'Angelo, D. A. Kuntz, T. Kantner, M. L. Ludkiwzek, C. Tarling, D. R. Rose, M. Saul, P. McIntosh & S. G. Withers, unpublished work). Initial structure refinement using *REFMAC*5 (Murshudov *et al.*, 2011) resulted in a model with an R_{work} and an R_{free} of 31 and 35%, respectively. The initial electron-density map clearly showed that there is one molecule in the asymmetric unit. Further model building and structure refinement are in progress. Finally, in an attempt to fully understand the catalytic mechanism and substrate-binding modes, cocrystallization and soaking of the crystals of XynAS9 with its substrates and/or products xylooligosaccharides and xyloses are under way.

This work was supported by the National High Technology Research and Development Program of China (2012AA022200 and 2012AA022208) and the Tianjin Municipal Science and Technology Commission (12ZCZDSY12500). We thank the National Synchrotron Radiation Research Center of Taiwan for beamtime allocation and data-collection assistance.

References

Beg, Q. K., Kapoor, M., Mahajan, L. & Hoondal, G. S. (2001). *Appl. Microbiol. Biotechnol.* **56**, 326–338.
Dodd, D. & Cann, I. K. (2009). *Glob. Change Biol. Bioenergy*, **1**, 2–17.
Li, N., Meng, K., Wang, Y., Shi, P., Luo, H., Bai, Y., Yang, P. & Yao, B. (2008). *Appl. Microbiol. Biotechnol.* **80**, 231–240.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
McCoy, A. J., Grosse-Kunstleve, R. W., Adams, P. D., Winn, M. D., Storoni, L. C. & Read, R. J. (2007). *J. Appl. Cryst.* **40**, 658–674.
Murshudov, G. N., Skubák, P., Lebedev, A. A., Pannu, N. S., Steiner, R. A., Nicholls, R. A., Winn, M. D., Long, F. & Vagin, A. A. (2011). *Acta Cryst.* D**67**, 355–367.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Subramaniyan, S. & Prema, P. (2002). *Crit. Rev. Biotechnol.* **22**, 33–64.
Winn, M. D. *et al.* (2011). *Acta Cryst.* D**67**, 235–242.