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Crystallization and preliminary X-ray crystallographic studies of XynX, a family 10 xylanase from *Aeromonas punctata* ME-1

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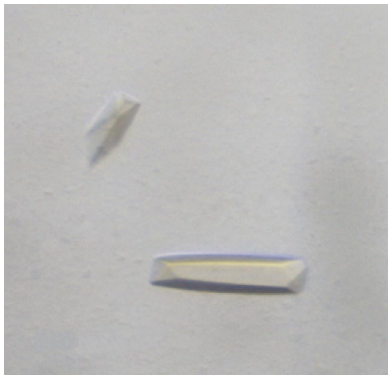

Xylanases catalyze the hydrolysis of β-1,4-glycosidic linkages within the xylan backbone. XynX is a xylanase from *Aeromonas punctata* ME-1 and belongs to glycoside hydrolase family 10. While most xylanases show endo-type catalytic activities, XynX shows exo-like catalytic activities, selectively producing xylobiose from birchwood xylan. In this study, XynX was crystallized by the hanging-drop vapour-diffusion method. The crystals belonged to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 79.0, b = 88.6, c = 93.2 Å, and diffracted to beyond 1.8 Å resolution.

1. Introduction

Endo-β-1,4-xylanases (EC 3.2.1.8) hydrolyze β-1,4-glycosidic linkages within the xylan backbone, producing β-anomeric xylo-oligosaccharides. Xylan is a major component of the hemicelluloses in plant cell walls. Based on the amino-acid sequence of the catalytic domains, xylanases have mainly been classified into two glycoside hydrolase families, 10 and 11, in the CAZy database (Coutinho & Henrissat, 1999; http://afmb.cnrs-mrs.fr/~cazy/CAZY/index.html). Initial crystallographic studies of xylanases showed that the catalytic domain of family 10 xylanases comprises of an eightfold $(\beta/\alpha)_8$-barrel in which an open and long catalytic cleft is located on the protein surface at the C-terminal side of the central β-barrel (Derewenda *et al.*, 1994; Harris *et al.*, 1994; White *et al.*, 1994), whereas the catalytic domain of family 11 xylanases has the β-jelly-roll structure matching a right hand, whose catalytic cleft is sandwiched between the N- and C-terminal lobes (Törrönen *et al.*, 1994). To date, more than 15 and 20 crystal structures are available for the family 10 and 11 xylanases, respectively.

We studied a multiple xylanase-producing Gram-negative bacterium, *Aeromonas punctata* ME-1 (formerly *A. caviae* ME-1), isolated from the gut contents of a wild silkworm, *Samia cynthia pryeri*. This bacterium produces at least nine xylanases and a total of five xylanases: xylanase I (Kubata *et al.*, 1992), xylanase IV (Kubata *et al.*, 1995), xylanase V (Kubata *et al.*, 1994), XynD (Suzuki *et al.*, 1997) and XynE (Liu *et al.*, 2003*a*,*b*) have been purified from the culture supernatant of *A. punctata* ME-1 and four xylanase genes (*xynA*, *xynD*, *xynE* and *xynX*) have been cloned (Kubata *et al.*, 1997; Suzuki *et al.*, 1997; Usui *et al.*, 1999). Based on their primary sequences, XynA, XynD, XynE and XynX have been classified as belonging to glycoside hydrolase families 11, 5, 10 and 10, respectively. XynA, XynD and XynE show typical endo-type hydrolysis patterns for birchwood xylan. However, XynX shows quite different hydrolysis patterns. Indeed, XynX exclusively produces two products from birchwood xylan, xylobiose and the side-chain-substituted xylo-oligosaccharide which was formerly considered to be xylotetraose, indicating an exo-like hydrolysis behaviour (Usui *et al.*, 1999). Xylosidase is known to be an exo-type xylolytic hydrolase that hydrolyses the terminal xylose from xylan or xylooligosaccharides, but to the best of our knowledge no exo-type xylanase has been reported that produces xylobiose or highly polymerized xylooligo-saccharides.

XynX is composed of 334 amino acids and has a molecular weight of 38 580 Da. It exhibits high sequence similarity (30–60% similarity) with family 10 xylanases of known three-dimensional structure (Usui *et al.*, 1999). Investigation of the intracellular localization of XynX

revealed that XynX is not exported to the extracellular fluid, but exists in the cytoplasm and is released from the cytoplasm to the periplasm during osmotic downshock (Usui *et al.*, 2003). Sequence alignment with the structurally determined xylanases XynZ of *Clostridium thermocellum* (Domínguez *et al.*, 1995) and Cex of *Cellulomonas fimi* (White *et al.*, 1994) showed that XynX had two remarkable inserted sequences in the $(\beta/\alpha)_8$-barrel. Recently, the crystal structure of the *Geobacillus stearothermophilus* T-6 xylanase showing similar extra insertions has been reported (Teplitsky *et al.*, 2004) and these insertions cause the placement of the novel subdomain slightly away from the catalytic cleft, thereby possibly affecting the subsite structures, product specificities and intracellular localization. In order to clarify how XynX shows exo-like hydrolysis behaviour, we crystallized XynX. The three-dimensional structure of XynX will help to reveal its associated catalytic mechanisms as well as its substrate-binding mechanisms. It will also help in protein engineering and provide the rationale for the design of an exo-type xylanase for industrial use.

2. Experimental procedures

2.1. Crystallization

XynX was overexpressed in *Escherichia coli* transformant JM109/pXOL1 cells as reported previously (Usui *et al.*, 1999) and purified by sequential anion-exchange and gel-filtration chromatography on HiTrap Q HP (Amersham Biosciences), HiPrep 26/60 Sephacryl S-100 HR (Amersham Biosciences), HiTrap Q HP (Amersham Biosciences) and Poros Tip HQ (Applied Biosystems). The purity and protein homogeneity were assessed by SDS–PAGE. Crystallization conditions were screened by the hanging-drop vapour-diffusion method using crystallization screening kits from Hampton Research. An 18 mg ml^{-1} protein solution was used for initial screening. A droplet composed of 1 µl protein solution and 1 µl reservoir solution was equilibrated against 0.5 ml reservoir solution at 293 K. Clusters of thin rod-shaped crystals grew in less than a week using the following reservoir solution: 1.5 *M* trisodium citrate dihydrate, 0.1 *M* HEPES buffer pH 7.5. After refinement of the crystallization conditions, XynX crystals (Fig. 1; 0.2 × 0.04 × 0.03 mm in size) were obtained within a week after 5 µl protein solution (15 mg ml^{-1}) was mixed with 5 µl reservoir solution composed of 1.3 *M* trisodium citrate dihydrate, 0.1 *M* HEPES buffer pH 7.7 at 293 K.

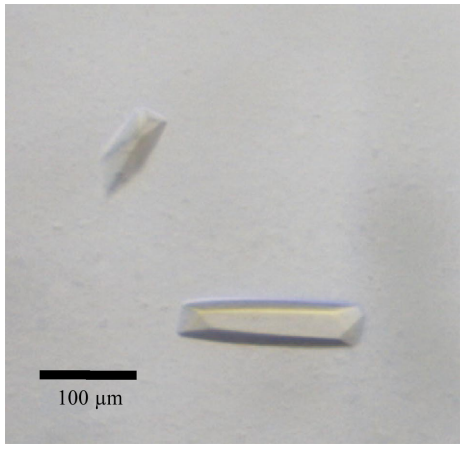

Figure 1
Typical crystals of XynX grown using the hanging-drop vapour-diffusion method.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell.

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 79.04, b = 88.59, c = 93.24
Wavelength (Å)	1.00
Resolution (Å)	50.0–1.8 (1.86–1.80)
R_{merge} (%)	5.0 (22.4)
Completeness (%)	97.1 (95.9)
Multiplicity	5.9 (6.0)
Average $I/\sigma(I)$	33.8 (6.4)
Unique reflections	59556 (5798)
Observed reflections	352048

2.2. Data collection

X-ray diffraction experiments were first conducted on an R-AXIS IV^{++} imaging-plate system and Micromax X-ray generator (Cu $K\alpha$; Rigaku) and XynX crystals diffracted to beyond 2.5 Å resolution. Diffraction data from the native crystals were obtained at BL41XU, SPring-8, Harima, Japan. XynX crystals were mounted in quartz glass capillaries of 0.3 mm diameter and were flash-cooled in a nitrogen stream at 100 K. Diffraction data were collected from a single crystal using a MAR CCD X-ray detector (MAR Research) with a 0.7° oscillation step over a range of 140° (λ = 1.00 Å). The data were processed using the programs *DENZO* and *SCALEPACK* from the *HKL*2000 package (Otwinowski & Minor, 1997).

3. Results and discussion

The hanging-drop method resulted in good diffraction-quality crystals of XynX. The crystals belonged to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 79.0, b = 88.6, c = 93.2 Å, and diffracted to beyond 1.8 Å resolution. Statistics for data collection and processing to 1.8 Å resolution are summarized in Table 1. Assuming two XynX molecules in an asymmetric unit, the value of the Matthews coefficient V_M was calculated to be 2.1 Å^3 Da^{-1}, which was within the expected range (Matthews, 1968). This V_M value corresponds to a solvent content of approximately 42%.

A preliminary solution of the structure was successfully obtained by the molecular-replacement method using the structural model of *Penicillium simplicissimum* xylanase (Schmidt *et al.*, 1998, 1999) as a search model with the program *AMoRe* (Navaza, 1994) from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994). The crystal was shown to contain two XynX molecules in the asymmetric unit. Building and refinement of the structure are under way. Successful crystallization of XynX to provide crystals suitable for structure determination should give us information on the mechanisms of substrate recognition and the structural foundation for industrial application of the xylobiose-forming enzyme.

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References

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.

Coutinho, P. M. & Henrissat, B. (1999). *Recent Advances in Carbohydrate Bioengineering*, edited by H. J. Gilbert, G. Davies, B. Henrissat & B. Svensson, pp. 3–12. Cambridge: The Royal Society of Chemistry.
Derewenda, U., Swenson, L., Green, R., Wei, Y., Morosoli, R., Shareck, F., Kluepfel, D. & Derewenda, Z. S. (1994). *J. Biol. Chem.* **269**, 20811–20814.
Domínguez, R., Souchon, H., Spinelli, S., Dauter, Z., Wilson, K. S., Chauvaux, S., Béguin, P. & Alzari, P. M. (1995). *Nature Struct. Biol.* **2**, 569–576.
Harris, G. W., Jenkins, J. A., Connerton, I., Cummings, N., Lo Leggio, L., Scott, M., Hazlewood, G. P., Laurie, J. I., Gilbert, H. J. & Pickersgill, R. W. (1994). *Structure*, **2**, 1107–1116.
Kubata, B. K., Horitsu, H., Kawai, K., Takamizawa, K. & Suzuki, T. (1992). *Biosci. Biotech. Biochem.* **56**, 1463–1464.
Kubata, B. K., Suzuki, T., Horitsu, H., Kawai, K. & Takamizawa, K. (1994). *Appl. Environ. Microbiol.* **60**, 531–535.
Kubata, B. K., Suzuki, T., Ito, Y., Naito, H., Kawai, K., Takamizawa, K. & Horitsu, H. (1997). *J. Ferment. Bioeng.* **83**, 292–295.
Kubata, B. K., Takamizawa, K., Kawai, K., Suzuki, T. & Horitsu, H. (1995). *Appl. Environ. Microbiol.* **61**, 1666–1668.
Liu, C. J., Suzuki, T., Hirata, S. & Kawai, K. (2003*a*). *J. Biosci. Bioeng.* **95**, 95–101.
Liu, C. J., Suzuki, T., Hirata, S. & Kawai, K. (2003*b*). *J. Biosci. Bioeng.* **96**, 406–408.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Navaza, J. (1994). *Acta Cryst.* A**50**, 157–163.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Schmidt, A., Gubiz, G. M. & Kratky, C. (1999). *Biochemistry*, **38**, 2403–2412.
Schmidt, A., Schlacher, A., Steiner, W., Schwab, H. & Kratky, C. (1998). *Protein Sci.* **7**, 2081–2088.
Suzuki, T., Ibata, K., Hatsu, M., Takamizawa, K. & Kawai, K. (1997). *J. Ferment. Bioeng.* **84**, 86–89.
Teplitsky, A., Mechaly, A., Stojanoff, V., Sainz, G., Golan, G., Feinberg, H., Gilboa, R., Reiland, V., Zolotnitsky, G., Shallom, D., Thompson, A., Shoham, Y. & Shoham, G. (2004). *Acta Cryst.* D**60**, 836–848.
Törrönen, A., Harkki, A. & Rouvinen, J. (1994). *EMBO J.* **13**, 2493–2501.
Usui, K., Ibata, K., Suzuki, T. & Kawai, K. (1999). *Biosci. Biotechnol. Biochem.* **63**, 1346–1352.
Usui, K., Suzuki, T., Akisaka, T. & Kawai, K. (2003). *J. Biosci. Bioeng.* **95**, 488–495.
White, A., Withers, S. G., Gilkes, N. R. & Rose, D. R. (1994). *Biochemistry*, **33**, 12546–12552.