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Crystallization and preliminary X-ray analysis of a novel *Trichoderma reesei* xylanase IV belonging to glycoside hydrolase family 5

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Xylanase IV (XYN IV) is a new recently characterized xylanase from *Trichoderma reesei*. It is able to degrade several different xylans, mainly producing xylose. XYN IV has been crystallized by the hanging-drop vapour-diffusion method, using PEG 6000 as a precipitant. The crystals belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 86.3, b = 137.5, c = 196.1 Å, $\alpha = \beta = \gamma = 90^\circ$. Assuming a molecular weight of 50.3 kDa, the V_M values indicate there to be four XYN IV monomers in an asymmetric unit and the solvent content of the crystals to be 57%. Based on dynamic light-scattering measurements, XYN IV is a dimer in solution. A native data set to 2.8 Å resolution has been collected at a home laboratory and a data set to 2.2 Å resolution has been collected using synchrotron radiation.

1. Introduction

Xylanases (EC 3.2.1.8) are enzymes that catalyse the hydrolysis of β-1,4-glycosidic bonds of xylan, a major component of plant hemicellulose. Xylanases have many applications in industry. Currently, they are used in the food, feed, pulp and paper industries.

Xylanases have been grouped into glycoside hydrolase families 5, 8, 10, 11 and 43 (Henrissat & Davies, 1997). To date, xylanases from families 5, 8, 10 and 11 have been structurally characterized. Several three-dimensional structures of xylanases from families 10 and 11 have been reported. Family 11 xylanases have a β-jelly-roll fold, while the family 10 xylanases have an $(\alpha/\beta)_8$-barrel fold. Only one structure of a family 8 xylanase has been reported (Van Petegem *et al.*, 2003). It is folded as an $(\alpha/\alpha)_6$ barrel. Very recently, a new three-dimensional structure of a xylanase from glycoside hydrolase family 5 was reported (Larson *et al.*, 2003). This *Erwinia chrysanthemi* xylanase A is composed of two domains: a catalytic domain and a small domain. The catalytic domain is similar to family 10 xylanases and has an $(\alpha/\beta)_8$-barrel with a catalytic binding cleft along the C-terminal side of the barrel. The small domain contains nine β-strands forming a β_9-barrel motif, with five of the strands interfacing with two α-helices of the catalytic domain. The function of the small domain is unknown, but it is supposed to be a xylan-binding module on the basis of the structural and sequential similarity of known xylan-binding modules.

Trichoderma reesei produces at least four specific xylanases: xylanases I, II, III and IV. The two main xylanases of *T. reesei* Rut C-30, XYN I and XYN II, are approximately 20 kDa proteins and have isoelectric points of 5.5 and 9.0, respectively (Tenkanen *et al.*, 1992). XYN I and XYN II are highly homologous and belong to glycoside hydrolase family 11. Their three-dimensional structures have also been determined (Törrönen *et al.*, 1994; Törrönen & Rouvinen, 1995). XYN III has been characterized from *T. reesei* PC-3-7; the enzyme has a molecular weight of 32 kDa and an isoelectric point of 9.1 (Xu *et al.*, 1998). XYN III has a high homology with the glycoside hydrolase family 10 enzymes, showing it to be distinct from XYN I and XYN II.

XYN IV is a new xylanolytic enzyme which has been purified from *T. reesei* Rut C-30 (Tenkanen *et al.*, 2003; Clarkson *et al.*, 2001). It has a sequence-based molecular weight of 50.3 kDa and several pI forms around pH 7. XYN IV is homologous to xylanases classified into family 5 of the glycoside hydrolases and its sequence identity with *E. chrysanthemi* xylanase A is 22%. The substrate specificity of XYN IV differs from that of family 10 or 11 xylanases. Its specific endoxylanase activity on polymeric xylan was very low; however, it was able to hydrolyze several different xylans, mainly producing xylose. The best hydrolysis yield was obtained using highly substituted acetyl-*O*-methylglucuronoxylan as a substrate. There are also some similarities between the substrate specificities of XYN IV and *E. chrysanthemi* xylanase A. The best substrate of *E. chrysanthemi* xylanase A was determined to be 4-*O*-methylglucuronoxylan (Hurlbert & Preston, 2000). However, the products formed are different, as xylanase A did not produce

xylose in large amounts. Here, we report the crystallization and preliminary X-ray diffraction analysis of *T. reesei* xylanase IV.

2. Experimental and results

2.1. Dynamic light scattering

Dynamic light-scattering measurements were performed using a DynaPRO99 instrument (Protein Solutions). XYN IV in 50 m*M* sodium acetate pH 4.5 and 0.1 *M* NaCl was concentrated to approximately 10 mg ml^{-1}. The protein solution was filtered through 0.1 µm Anodisc 13 membrane filters (Whatman) to remove any residual gas bubbles or solid impurities. *Dynamics* v.5.25.44 software was used in the data collection and analysis. The number of acquisition scans was 21 and the acquisition time was 3 s per scan at 295 K. Dynamic light-scattering experiments of a XYN IV sample showed a radius of 3.95 nm and a polydispersity of 5.9%. A monomodal fit of the data predicted a molecular weight of 99 kDa, suggesting that XYN IV is a dimer in solution.

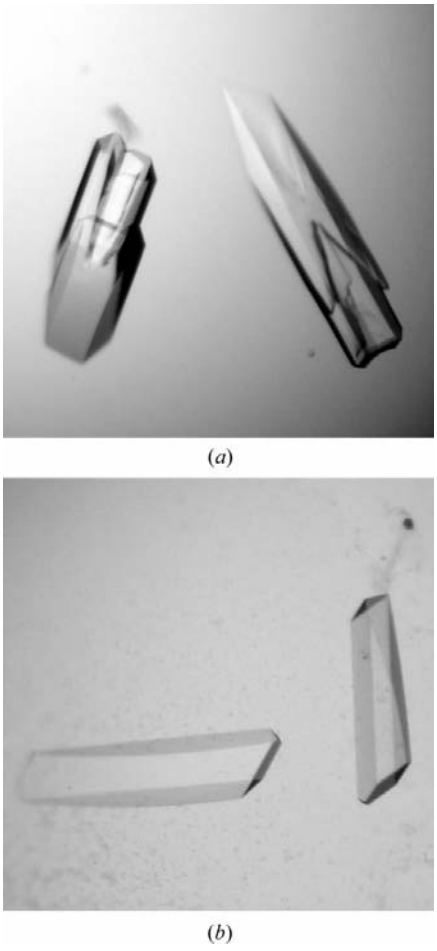

Figure 1
Crystals of XYN IV. (*a*) Before and (*b*) after streak-seeding. The dimensions of the crystals are 0.5 × 0.2 × 0.1 and 0.6 × 0.2 × 0.2 mm, respectively.

2.2. Crystallization

Crystallization was performed at room temperature by the hanging-drop vapour-diffusion method. Each drop was prepared by mixing 2 µl protein solution and 2 µl reservoir solution. Crystallization drops were set up on siliconized cover slips over 500 µl reservoir solution. The protein concentration was approximately 10 mg ml^{-1}. The crystallization conditions were screened using Crystal Screens from Hampton Research. Initial screening resulted in XYN IV crystals with 18%(*w*/*v*) PEG 8000, 0.1 *M* sodium cacodylate pH 6.5 and 0.2 *M* zinc acetate. The initial conditions were optimized by varying the pH and the molecular weight and the concentration of PEGs and several additives were also tested. MPD was found to improve the crystal quality and the concentration of MPD was increased so that the crystallization solution acted as a cryoprotectant. The optimized crystallization condition contained 10%(*w*/*v*) PEG 6000, 10%(*w*/*v*) MPD, 0.1 *M* sodium cacodylate pH 5.8 and 0.2 *M* zinc acetate. The self-nucleated crystals (Fig. 1*a*) obtained from these conditions were poorly ordered and did not diffract well. Streak-seeding was used to improve the crystal size and quality and the seeded crystals (Fig. 1*b*) grew to dimensions of 0.6 × 0.2 × 0.2 mm without defects.

2.3. Data collection and processing

Diffraction data were obtained using a rotating-anode generator and at a synchrotron-radiation source. Crystals were cryocooled to 100 K using the growth solution as a cryoprotectant. Crystals were picked up directly from the drop and flash-cooled or plunged into liquid nitrogen. The diffraction data were indexed and integrated using *DENZO* and scaled using *SCALEPACK* (Otwinowski & Minor, 1997). The space group was defined using *XPREP* (*SHELX* software package). XYN IV crystals belonged to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 86.3, b = 137.5, c = 196.1 Å.

Data were first collected in-house using a Rigaku RU-200HB rotating-anode X-ray generator operating at 50 kV and 100 mA equipped with an Osmic Confocal Optics and an R-AXIS IIC imaging-plate detector. The crystal-to-detector distance was set to 150 mm and a total of 160 frames of 1.0° oscillation were measured. Each frame was exposed for 30 min. The diffraction pattern extended to 2.8 Å resolution with an $R_{\rm merge}$ of 11.3%. The synchrotron data were collected on beamline BW7B at the EMBL Outstation, Hamburg. Data were collected using a 0.5° oscillation with the crystal-to-detector distance set to 380 mm. A total of 180 frames were measured. The crystals diffracted to beyond 2.0 Å resolution, but data were collected at 2.2 Å resolution with an $R_{\rm merge}$ of 8.9%. The data-collection statistics are summarized in Table 1.

Table 1
Data-collection statistics.

Values in parentheses are for the highest resolution shell.

Data set	1	2
X-ray source	Rotating anode	BW7B
λ (Å)	1.5418	0.8453
Space group	$P2_12_12_1$	$P2_12_12_1$
Unit-cell parameters (Å)	a = 86.6, b = 138.1, c = 196.2	a = 86.3, b = 137.5, c = 196.1
Resolution range (Å)	99–2.8 (2.9–2.8)	20–2.2 (2.24–2.2)
No. observations	237561	415683
No. unique reflections	56508	99553
$R_{\rm merge}$ (%)	11.3 (30.1)	8.9 (31.5)
Completeness (%)	96.2 (98.2)	85.9 (88.3)
$I/\sigma(I)$	10.7 (3.6)	5.7 (2.2)

3. Discussion

The seeded crystals of XYN IV diffracted to better than 2.2 Å resolution and are suitable for structure determination. Assuming four monomers per asymmetric unit, the Matthews coefficient is calculated to be 2.89 Å^3 Da^{-1} (Matthews, 1968), corresponding to a solvent content of 57%. Dynamic light-scattering measurements suggest that XYN IV is a dimer in solution. Therefore, the asymmetric unit may contain two dimers. The crystals diffracted considerably better at the synchrotron-radiation source than using the rotating-anode source; however, the synchrotron data set was 85.9% complete. We are planning to collect a complete synchrotron data set in the near future.

At this point, we intended to solve the structure by multiple isomorphous replacement, but attempts to find suitable heavy-atom derivatives have been unsuccessful. We are also trying to solve the structure using molecular replacement, as the co-ordinates of *E. chrysanthemi* xylanase A have been recently deposited in the PDB (PDB code 1nof). XYN IV has a 22% sequence identity with xylanase A.

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