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Crystals of family 11 xylanase II from *Trichoderma longibrachiatum* that diffract to atomic resolution

Natalia Moiseeva and Marc Allaire*

National Synchrotron Light Source, Brookhaven National Laboratory, Upton, NY, USA

Correspondence e-mail: allaire@bnl.gov

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Xylanases catalyse the cleavage of various forms of xylan. A new crystal form of xylanase II from the fungus *Trichoderma longibrachiatum* that diffracts to better than 1 Å resolution was grown from 12% PEG 8K, 0.1 *M* Tris pH 8.5, 0.2 *M* $CaCl_2$ with the addition of 2% glycerol to overcome crystal twinning. The crystals grow in a body-centered orthorhombic Bravais lattice, with unit-cell parameters $a = 66.78$, $b = 67.94$, $c = 79.18$ Å. The solvent content is 42% with one molecule per asymmetric unit. Molecular-replacement analysis reveals the space group to be *I*222. This atomic resolution structure will provide important insights that will lead to a better understanding of the enzymatic mechanism of the family 11 xylanases.

1. Introduction

Glycosyl hydrolases catalyse the cleavage of glycosidic bonds between carbohydrates or between carbohydrates and a non-carbohydrate moiety. They are grouped into about 90 families based on their amino-acid sequences, where families 10 and 11 consist of the xylanases (Henrissat *et al.*, 1998). Xylanases hydrolyse the polysaccharide xylan, a polymer of 1,4-β-linked xylopyranosides that is found as a major constituent of hemicellulose. The family 10 xylanases have a molecular weight of approximately 35 kDa and possess the TIM-barrel $(\alpha/\beta)_8$ fold (Davies & Henrissat, 1995). The family 11 xylanases have a molecular weight of ~22 kDa and fold into a jelly-roll topology formed by two twisted β-sheets stacked face to face and are also represented as a right hand with a two-β-strand 'thumb' forming a lid over the active site (Törrönen *et al.*, 1994). So far, xylanase structures from family 11 (Campbell *et al.*, 1993; Törrönen *et al.*, 1994; Törrönen & Rouvinen, 1995; Krengel & Dijkstra, 1996; Fushinobu *et al.*, 1998; Gruber *et al.*, 1998; Sabini *et al.*, 1999; Kumar *et al.*, 2000; McCarthy *et al.*, 2000; Wouters *et al.*, 2001; Oakley *et al.*, 2003) that diffract to atomic resolution have not been described. We report here a new crystal form of the family 11 xylanase II from *Trichoderma longibrachiatum* (formerly known as *T. reesei*) and show that these crystals diffract to better than 1 Å resolution.

2. Crystallization

Xylanase II from the fungus *T. longibrachiatum* (XYNII; EC 3.2.1.8) is an endo-1,4-β-xylanase with a preference for internal xylan glycosidic bonds. XYNII, produced by fermentation (Törrönen *et al.*, 1992), is supplied at a concentration of 36 mg ml^{-1} in 0.18 *M* sodium/potassium phosphate buffer pH 7.0 and 43%(*w*/*v*) glycerol (Hampton Research Inc., Aliso Viejo, CA, USA). Attempts to crystallize XYNII (10–20 mg ml^{-1}) with 0.6–1.2 *M* sodium/potassium phosphate or 0.45–0.85 *M* ammonium sulfate as the main precipitant were unsuccessful. The buffer was exchanged to 0.01 *M* Tris–HCl pH 7.5 in 10–15 dilution/concentration cycles using a 10 kDa membrane cutoff (Centricon YM-10; Millipore Corp., Billerica, MA, USA) to a final protein concentration of 33 mg ml^{-1}. Crystallization of XYNII in Tris buffer with sodium/potassium phosphate or ammonium sulfate remained unsuccessful and screening for new conditions was initiated using the hanging-drop vapor-diffusion method (Jancarik & Kim, 1991). Crystals were readily obtained by mixing 2 µl XYNII in Tris buffer with 2 µl of the proprietary condition A2 of the Pre-Crystallization Test (PCT) from Hampton Research or 2 µl of condition No. 6 from the Hampton Research Crystal Screen 1 containing 30% PEG 4K, 0.2 *M* $MgCl_2$ in 0.1 *M* Tris buffer pH 8.5. Optimization of the crystallization conditions reveals that plate-like crystals of XYNII could be grown at room temperature in 2 d from a variety of polyethylene glycol conditions (*e.g.* 15% PEG 1K, 12% PEG 8K, 8% PEG 35K) in 0.1 *M* Tris buffer pH 8.5 and 0.2 *M* $MgCl_2$ or $CaCl_2$. Similar crystals could be obtained at lower pH with 0.1 *M* sodium acetate buffer pH 4.5 in place of the Tris buffer pH 8.5. Crystals can also be grown in the presence of a monovalent ion or without the addition of any salt additives. The diffraction patterns from a large number of these crystals were twinned; this

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Table 1
Summary of the data-collection statistics.

Values in parentheses are for the highest resolution shell (0.98–0.95 Å).

Wavelength (Å)	0.765
Space group	*I*222
Unit-cell parameters (Å)	a = 66.783, b = 67.943, c = 79.175
Mosaicity (°)	0.4
Molecules per AU	1
Matthews coefficient (Å^3 Da^{-1})	2.1
Solvent content (%)	42
NSLS beamline	X6A
Resolution limits (Å)	30.0–0.95
Total reflections	1470833 (66729)
Unique reflections	112502 (11149)
Average redundancy	13.1 (6.0)
Average $I/\sigma(I)$	45.3 (4.3)
Completeness ($I > 0$) (%)	96.6 (89.7)
$R_{\rm merge}$† (%)	5.5 (43.3)

† $R_{\rm merge} = \sum_h \sum_i |I(h,i) - \langle I(h)\rangle| / \sum_h \sum_i I(h,i)$, where $I(h,i)$ is the intensity of the ith measurement of reflection h and $\langle I(h)\rangle$ is the mean value of $I(h,i)$ for all i measurements.

observation is consistent with the fact that multiple thin plates could be seen stacked together. 2%(*v*/*v*) glycerol was added to the crystallization trials to reduce the twinning problem (Chayen, 1999). The xylanase crystals shown in Fig. 1 were grown from 12% PEG 8K, 0.2 *M* $CaCl_2$, 2%(*v*/*v*) glycerol in 0.1 *M* Tris pH 8.5 and used for data collection.

3. Diffraction data

Diffraction data were collected on beamline X6A (Allaire *et al.*, 2003) at the National Synchrotron Light Source at the Brookhaven National Laboratory (Upton, NY, USA). Cryoprotection was achieved by soaking the crystal for 15–20 s in a solution of mother liquor containing 30%(*v*/*v*) glycerol, followed by flash-freezing the xylanase crystal in a stream of nitrogen maintained at 100 K. Data collection was performed using a wavelength of 0.765 Å and a crystal-to-detector distance of 95 mm. As shown in Fig. 2, diffraction spots were clearly visible to a resolution of 0.95 Å, corresponding to the edge of the detector, with some significant spots visible at higher resolution.

A highly redundant data set was collected in two 180° passes (1° per oscillation): a low-resolution pass with 30 s exposure per frame and a high-resolution pass with 180 s exposure per frame. The diffraction data were processed and scaled with *HKL*2000 (Otwinowski & Minor, 1997) and the data-collection statistics are summarized in Table 1. The crystal belongs to a body-centered orthorhombic space group (*I*222 or $I2_12_12_1$), with unit-cell parameters a = 66.78, b = 67.94, c = 79.18 Å. Assuming one molecule per asymmetric unit, the calculated Matthews coefficient is 2.1 Å^3 Da^{-1} and corresponds to a solvent content of 42% (Matthews, 1968). The overall data set had a redundancy of 13.1, with a merging R of 5.5%. In the highest resolution shell (0.98–0.95 Å), 45.3% of the diffraction data have measured intensities three times higher than the error. Statistical analysis and intensity distributions of the diffraction data, performed with the program *TRUNCATE* (French & Wilson, 1978) from the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994), did not indicate any twinning of the data set.

4. Space-group determination

The conditions of diffraction for a body-centered orthorhombic space group ($h + k + l = 2n$) do not allow the identification of either the *I*222 or $I2_12_12_1$ space group. Space-group determination was performed during the process of searching for a molecular-replacement solution for initial phasing. The search model used was the known three-dimensional structure of the protein at a resolution of 1.5 Å (PDB code 1xyp: Törrönen *et al.*, 1994) available from the Protein Data Bank (Bernstein *et al.*, 1977; Berman *et al.*, 2000). Molecular replacement was performed using both *AMoRe* (Navaza, 1994) and *MOLREP* (Vagin & Teplyakov, 1997) from the *CCP*4 suite of programs (Collaborative Computational Project, Number 4, 1994). The search took place with diffraction data to a resolution of 2.0 Å. A clear rotation-solution peak could be identified with both programs. Translation searches were carried out using both the *I*222 and $I2_12_12_1$ space groups. Using the program *AMoRe*, the best translation peaks for *I*222 and $I2_12_12_1$ have correlation coefficients of 68.2 and 47.9 and R factors of 40.9 and 50.7, respectively. Similar results were obtained from *MOLREP*, where the best peaks for *I*222 and $I2_12_12_1$ were 69.24σ and 51.9σ, respectively. Moreover, the best solution found for *I*222 was clearly discriminated from the next solution. These results strongly suggest the space group of this new crystal form to be *I*222.

5. Discussion

The proposed enzymatic mechanism of family 11 xylanases involves two conserved glutamic acid residues as putative catalytic residues. In XYNII, Glu177 is probably the so-called acid–base catalyst, whereas Glu86 is likely to be the nucleophile (Törrönen *et al.*, 1994). The three-dimensional structures of XYNII obtained at different pH values reveal a conformational change in the region of the active site that could be important for the catalytic activity of this enzyme (Törrönen & Rouvinen, 1995). The new crystal form described in this report shows diffraction limits of better than 1 Å, with similar crystals grown at different pH values. Further studies are required to identify whether this crystal form is suitable for the binding of substrate analogues or transition-

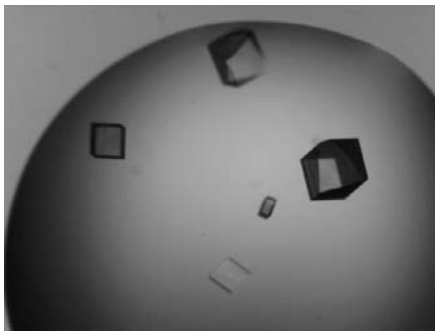

Figure 1
Crystals of the *I*222 form of xylanase II from *T. longibrachiatum* grown from 12% PEG 8K, 0.1 *M* Tris buffer pH 8.5, 0.2 *M* $CaCl_2$ and 2%(*v*/*v*) glycerol. The larger crystals are of approximate dimensions 0.25 × 0.25 × 0.15 mm.

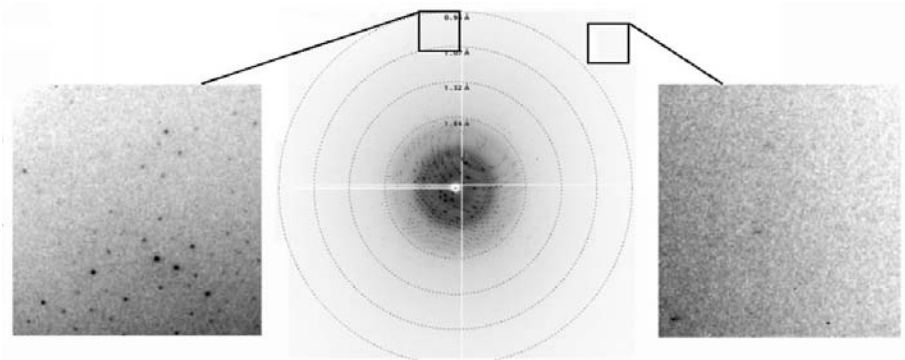

Figure 2
Diffraction images of the *I*222 crystal form of xylanase II from *T. longibrachiatum*. The image on the left extends to a resolution limit of 0.95 Å. The image on the right corresponds to the resolution range 0.92–0.81 Å.

state mimics of the xylanase catalytic activity. We expect that the structural details of XYNII at atomic resolution will help to further characterize the catalytic role of the active-site residues and provide important insights that will lead to better understanding of the enzymatic mechanism of the family 11 xylanases.

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