

Characterization of Crystals of *Penicillium purpurogenum* Acetyl Xylan Esterase From High-Resolution X-Ray Diffraction

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ABSTRACT Acetyl xylan esterase from *Penicillium purpurogenum*, a single-chain 23 kDa member of a newly characterized family of esterases that cleaves side chain ester linkages in xylan, has been crystallized. The crystals diffract to better than 1 Å resolution at the Cornell High Energy Synchrotron Source (CHESS) and are highly stable in the synchrotron radiation. The space group is P2₁2₁2₁ and cell dimensions are $a = 34.9\text{Å}$, $b = 61.0\text{Å}$, $c = 72.5\text{Å}$.

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INTRODUCTION

Plant cell wall hemicelluloses are complex mixtures of heteropolysaccharides. Their main component is xylan, which can compose up to 35% of the mass of lignocellulose and represents the second most abundant biomolecule on earth after cellulose. Xylan consists of a linear chain of xylose residues joined by β (1 \rightarrow 4) glycosidic linkages with various types and numbers of substitutions depending on its origin. Known substituents are O-acetyl groups, L-arabinose, D-O-methyl glucuronic acid, and phenolic compounds such as coumaric and ferulic acids.¹

Xylan is biodegraded by bacterial and fungal enzymes known collectively as xylanases. Due to the complexity of xylan structure, its hydrolysis is accomplished by the concerted action of several enzymes differing in their bond-cleavage specificity. The main chain is split by the action of endoxylanases and β -xylosidases, while the substituents are hydrolyzed by arabinofuranosidases, glucuronidases, and esterases. This last group includes acetyl xylan, feruloyl and coumaroyl esterases.² Although the enzymology of xylan degradation has been extensively studied, the role and specificity of the different components of the xylanase system remain unclear.^{3,4}

Penicillium purpurogenum is an active source of cellulases and xylanases.⁵ Two endoxylanases ob-

tained from culture filtrates of this fungus have been purified and characterized. Important differences are observed in the properties of both enzymes, suggesting that they are the products of separate genes and that they may perform different roles in xylan degradation.⁶ Two acetyl xylan esterase forms have been detected, purified and characterized (AXE I and II).⁷ They show no endoxylanase activity.

AXE II is a monomer of MW 23 kD and as such is the smallest AXE known (cf. 30–57 kD for other known AXEs^{8,9}). It has a significantly more alkaline pI (7.8) than other AXEs. The structure of AXE II is unknown including its primary sequence. A 30-residue sequence of the amino terminal end of AXE II shows no similarity to other known proteins.⁷

MATERIALS AND METHODS

Crystallization

AXE II was purified as described⁷ and crystallized by the vapor diffusion hanging drop technique using ammonium sulfate as the precipitant. Droplets of the protein solution containing 4 mg/ml of AXE in 50 mM citrate buffer at pH 5.3 and 13% saturated ammonium sulfate were set up as hanging drops over reservoirs each containing 1 ml of 33–37% saturated ammonium sulfate in the same buffer. The volume of the protein droplets ranged between 5 and 7 μ l. The crystallization experiments were carried out at ambient temperature.

X-Ray Diffraction

X-Ray diffraction studies were initiated on an R-Axis IIC image plate area detector receiving

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X-rays from a Rigaku RU-200 rotating anode generator operated at 100 mA and 45 kV. Graphite-monochromated $\text{CuK}\alpha$ radiation was used. The image plate detector was placed 110 mm from the crystal. Measurable reflections were observed to 1.4 Å resolution at ambient temperature. Subsequently, high-resolution data were collected using synchrotron radiation, $\lambda = 0.9497$ Å, at the F2 station, a doubly focused wiggler beamline using a sagittal focusing Si (111) monochromator, at the Cornell High Energy Synchrotron Source (CHESS). A fiberoptic coupled 2048×2048 charge-coupled device (CCD) X-ray detector was used for recording and measuring diffraction intensities.¹⁰ The detector was placed at 37 mm from the crystal for high-resolution data measurement. The entire experiment was performed at ambient temperature using two crystals. The data set was processed using the software package DENZO.¹¹

RESULTS

The crystals appeared in about 2 weeks and continued to grow for a few more weeks. The crystals often grew as clusters of large ($\sim 1.0 \times 0.5$ mm) but thin (< 0.05 mm) plates. Single crystals were harvested by breaking the clusters and separating the individual plates. The space group is $P2_12_12_1$ and the unit cell parameters are $a = 34.9$ Å, $b = 61.0$ Å, $c = 72.5$ Å, $\alpha = \beta = \gamma = 90^\circ$. Assuming one molecule in the asymmetric unit of the cell, the specific volume of the crystal is 1.68 Å³/Da, which is near the lower limit of the range possible for proteins.¹² Thus, the molecules of AXE are tightly packed in the crystal with very high long-range order. The CHESS data set was 69% complete to 1.10 Å resolution. Intensities of 149,250 observations for 44,040 unique reflections were measured with an R_{merge} of 0.059. The $\langle I/\sigma(I) \rangle$ value in the shell between 1.14 and 1.10 Å was 4.0. When crystals of AXE II were flash-frozen in liquid nitrogen using glycerol and glycerol/polyethylene glycol combination as cryo-protectants, they appeared to diffract better than at ambient temperature, for experiments conducted on the R-Axis Ilc detector. The crystals were stable for days under such conditions, and no appreciable worsening of the mosaicity could be detected from diffraction spots. We expect to collect sub-angstrom resolution data by repeating the CHESS experiment at the liquid-nitrogen temperature with the best available crystals.

Attempts to prepare isomorphous heavy-atom derivatives have so far been unsuccessful, probably because of the tightly packed nature of the crystals. We plan to determine the structure using the ab initio direct phasing technique developed by Dr. Herbert A. Hauptman and his colleagues and implemented in the program package *SnB*.^{13,14}

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NOTE ADDED IN PROOF

A data set 86.4% complete to 0.90 Å resolution at the liquid nitrogen temperature has recently been collected at CHESS. There were 94,930 unique reflections with 420,882 total observations having an R_{merge} of 0.070. The average $I/\sigma(I)$ in the shell between 0.93 Å and 0.90 Å is 3.8.

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