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Crystallization and preliminary X-ray analysis of a xyloglucan endotransglycosylase from *Populus tremula × tremuloides*

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Xyloglucan endotransglycosylases (XETs) cleave and religate xyloglucan polymers in plant cell walls. Recombinant XET from poplar has been purified from a *Pichia pastoris* expression system and crystallized. Two different crystal forms were obtained by vapour diffusion from potassium sodium tartrate and from an imidazole buffer using sodium acetate as a precipitant. Data were collected from these crystal forms to 3.5 and 2.1 Å resolution, respectively. The first crystal form was found to belong to space group $P3_121$ or $P3_221$ (unit-cell parameters a = 98.6, b = 98.6, c = 98.5 Å) and the second crystal form to space group $P6_3$ (unit-cell parameters a = 188.7, b = 188.7, c = 46.1 Å).

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1. Introduction

The dynamics of plant cell growth, morphogenesis and differentiation require modifications of its extracellular matrix, the cell wall. Several enzymes capable of such modifications, altering the network of cellulose hemicellulose and pectin, have been described to date. In spite of this, the detailed mechanisms of plant cell regulation, expansion, germination and growth are still to a large extent unknown. Xyloglucan, one of the major hemicelluloses in the primary cell walls of dicotyledons, is a polysaccharide consisting of a β-(1–4)-D-glucopyranose backbone in which about three quarters of the residues have a α-D-xylosyl residue substituted at O-6. Some of these xylose rings are in turn β-D-galactopyranosylated at O-2 (Fry *et al.*, 1992). The long xyloglucan polysaccharide is known to form hydrogen bonds with cellulose microfibrils, potentially cross-linking adjacent microfibrils and restraining cell-wall expansion (Albersheim, 1976; Hayashi, 1989). An enzyme, xyloglucan endotransglycosylase (XET), has been proposed to be responsible for the incorporation and rearrangement of such xyloglucan crosslinks *via* a transglycosylation mechanism. Endolytic cleavage of a crosslinking xyloglucan polymer permits cellulose microfibrils to separate, whereas transferring the newly generated end to another sugar polymer restores stable cell-wall structure (Smith & Fry, 1991; Xu *et al.*, 1995; Purugganan *et al.*, 1997; Campbell & Braam, 1999).

Enzymes that hydrolyze oligosaccharides and polysaccharides, glycoside hydrolases, play a central role in a wide array of biological processes. Owing to the extensive variety of stereochemistry of carbohydrates, a large number of such enzymes can be found in nature. To date, more than 4000 different glycoside hydrolases have been sequenced and classified into 89 different families, which in turn have been classified into 13 distinct clans based on their overall fold (Henrissat & Davies, 1997). All presently known genes encoding XET and XET-like enzymes, including those from *Populus tremula × tremuloides*, have been classified into family 16, clan B. In addition, clan B contains endoglucanases and cellobiohydrolases from family 7 and β-glucanases, keratan-sulfate-endo-galactosidases, κ-carrageenases, β-agarases and laminarinases from family 16.

XET enzymes, like other clan B enzymes, are believed to act by retention, using general acid/base catalysis *via* a double-displacement reaction as first proposed by Koshland (1953). One acidic residue participates as a proton donor which assists departure of the aglycon, while another is responsible for nucleophilic attack at the anomeric center. The covalent glycosyl-enzyme intermediate formed in the first step is then hydrolyzed by a nucleophilic water molecule (McCarter & Withers, 1994; Davies *et al.*, 1997). Both XETs and β-glucanases cleave β-1,4 sugar linkages, but endotransglycosylases are additionally also capable of religating the nascent reducing end to another sugar residue. It has been shown that transglycosylation also occurs among other glycoside hydrolases, for example the β-glucanases, but at a substantially lower rate or under special conditions (Hahn *et al.*, 1995; Planas, 2000). Although the sequence identity between the most similar β-glucanase and the XET enzymes is ~20% and the identity with the family 7 enzymes is only visible by structural alignment, the proposed catalytic centre seems to be surprisingly well conserved among clan B

enzymes (Divne *et al.*, 1994). However, significant questions remain: why do XET enzymes act predominantly by transglycosylation rather than hydrolysis and how does the enzyme protect a potential xyloglucan glycosyl-enzyme intermediate from being attacked by water?

In the present work, we describe the crystallization and preliminary X-ray analysis of *P. tremula* × *tremuloides* xyloglucan endotransglycosylase 16A as a first step towards determining the X-ray structure of the enzyme. Knowledge of the three-dimensional structure of XET might provide the structural details that are needed to understand the mechanism of transglycosylation and will provide the first example of a xyloglucan endotransglycosylase structure.

2. Results and discussion

2.1. Cloning, expression and purification

The gene encoding XET16A from *P. tremula* × *tremuloides* was isolated as a full-length clone from the poplar EST library (Sterky *et al.*, 1998; Genbank nucleotide accession No. AF515607). For expression in *Pichia pastoris*, the gene was cloned into a pPIC9 shuttle vector containing an α-factor secretion signal and the alcohol oxidase promoter. Active recombinant poplar XET16A protein was purified from culture filtrates by a sequential combination of strong cation-exchange, gel-filtration and strong cation-exchange chromatography steps. The full details of the protein expression, purification and characterization will be published elsewhere (Å. M. Kallas, T. T. Teeri *et al.*, manuscript in preparation).

2.2. Crystallization

Initial crystallization trials were performed with Crystal Screens I and II (Hampton Research) using the hanging-drop vapour-diffusion method (McPherson, 1982). A first trigonal crystal form was obtained at 293 K in 0.4 *M* potassium sodium tartrate with 2 µl drops consisting of equal volumes of protein solution (10 mg ml^{-1} XET in 1.0 *M* HEPES buffer pH 7.0) and reservoir solution. During extensive attempts to improve the first rod-like dendritic crystals, a second hexagonal crystal form was encountered (10 mg ml^{-1} protein solution mixed in a 1:1 ratio with 1.0 *M* NaOAc, 0.2 *M* imidazole pH 6.5). Using microseeding and refining the crystallization conditions, beautiful single three-dimensional crystals were grown (5 mg ml^{-1} protein in a 1:1 ratio with 1.2 *M* NaOAc and

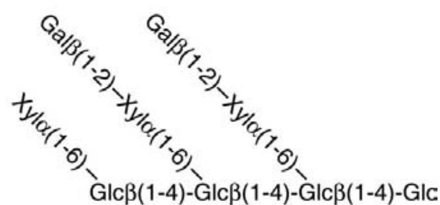
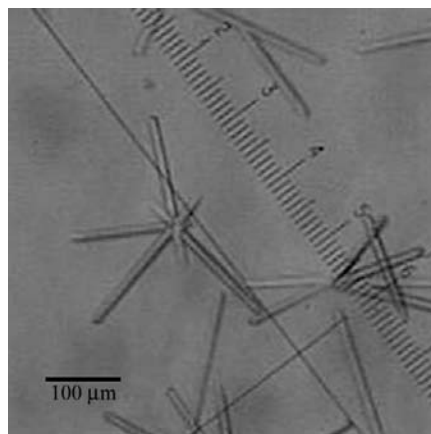
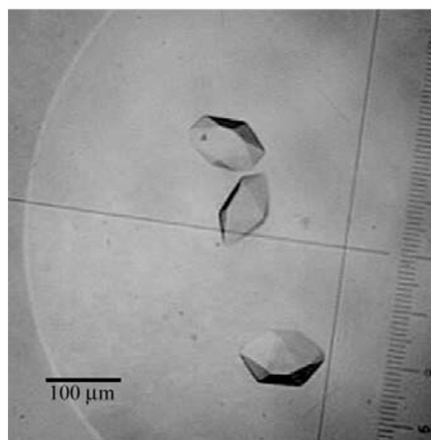


Figure 1
Structure of the xyloglucooligosaccharide XLLG.

0.2 *M* imidazole pH 6.5 at 293 K) with a range of sizes up to 100 µm. Co-crystallization trials of XET with the natural substrate xyloglucan polysaccharide (XG) did not produce any crystalline material (3 µl drops with a 1:1:1 ratio of protein, precipitant and 4 mg ml^{-1} XG). However, co-crystallization with the xyloglucan-derived nonasaccharide XLLG (Fig. 1; nomenclature according to Fry *et al.*, 1993), further enhanced growth, the hexagonal crystal form reaching sizes of 300 µm in diameter.



(*a*)



(*b*)

Figure 2
The two crystal forms of XET: (*a*) trigonal and (*b*) hexagonal.

2.3. Data collection and analysis

For data-collection purposes, crystals of both forms were flash-frozen in liquid nitrogen with addition of 28% PEG 400 to the mother liquor. Additional XLLG was added to the cryosolution of potential substrate–complex crystals to prevent back-soaking. Crystallographic data were collected at MaxLab synchrotron (Lund, Sweden) beamline 711 and at the ESRF synchrotron (Grenoble, France) beamline ID14-EH4. The trigonal and the hexagonal crystal forms (Fig. 2) diffracted to 3.5 and 2.1 Å resolution, respectively. A subsequent 1.9 Å data set from a potential XET–XLLG complex was collected at ESRF ID14-EH1. Indexing, integration and scaling of the data were carried out using the *HKL* suite (Otwinowski & Minor, 1996). The first, rod-like, crystal form was found to belong to space group $P3_121$ or $P3_221$ (unit-cell parameters a = 98.6, b = 98.6, c = 98.5 Å), whereas the second, hexagonal, crystal form was found to belong to space group $P6_3$ (a = 188.7, b = 188.7, c = 46.1 Å). Assuming one monomer in the asymmetric unit for the trigonal crystal form gives a $V_{\rm M}$ of 3.8 Å^3 Da^{-1} (Matthews, 1968). The hexagonal crystal form has a $V_{\rm M}$ of 4.0 Å^3 Da^{-1}, assuming two molecules in the asymmetric unit.

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