Poster Presentation

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Crystal structure and reaction mechanism of glucuronoxylan endo-β-1,4-xylanase

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Hemicelluloses are one of the major components of plant cell walls and can be used for the production of renewable chemicals and materials. Heteroxylans are a major component of hemicellulose, comprising a backbone of xylopyranose units linked by β-1,4-xylosidic bonds. These xylose units are decorated to various degrees with acetyl, arabinose, glucuronic acid and galactose side chains and their derivatives, such as ferulic and p-coumaric acids, depending on the plant species and the source of tissue [1]. The structural diversity of hemicellulose and its broader use requires the concerted action of a large repertoire of different hemicellulases. Glucuronoxylan endo-β-1,4-xylanases is one of the member of hemicellulases which cleave the xylan chain specifically at sites containing 4-O-methylglucuronic acid substitutions. Hydrolytic mechanism of glucuronoxylan endo-β-1,4-xylanases in extreme environments in less known. Therefore, structure and functional characterization of glucuronoxylan endo-β-1,4-xylanases provides the glimpse of mechanistic and structural behaviour. In this study structure and mechanistic behaviour of a thermostable family 30 glucuronoxylan endo-β-1,4-xylanase (CtXyn30A) from Clostridium thermocellum was studied. CtXyn30A preferably hydrolyses glucuronoxylans and displays maximum activity at pH 6.0 and 70℃ [2]. CtXyn30A crystallized in more than 300 conditions. Data collections were performed at the European Synchrotron Radiation Facility (ESRF; Grenoble, France), SOLEIL (Orsay, France) and Diamond Light Source (DLS; Harwell, England). The best crystal obtained in 0.2 M potassium sulphate, 20% (w/v) PEG 3350 was used to determine the CtXyn30A structure. The CtXyn30A structure was solved at 1.17 Å using molecular replacement approach. CtXyn30A belongs to space group P1 with cell constants a = 46.09 Å, b = 47.46 Å, c = 53.62 Å, α = 83.15º, β = 73.41º and γ = 65.87º. The structure of CtXyn30A displays a (β/α)8 TIM-barrel core with a side-associated β-sheet domain. Structural analysis of the CtXyn30A mutant E225A, solved in the presence of xylotetraose, revealed that the oligosaccharide is cleaved partially and the products occupy subsites -3 to +2. The sugar ring at the +1 subsite is held in place by hydrophobic stacking interactions between Tyr139 and Tyr200 and hydrogen bonds to the OH group of Tyr227 [3]. Although family 30 glycoside hydrolases are retaining enzymes, the xylopyranosyl ring at the -1 subsite of CtXyn30A-E225A appears in the α-anomeric configuration. A set of residues were found to be strictly conserved in glucuronoxylan endo-β-1,4-xylanases and constitute the molecular determinants of the restricted specificity displayed by these enzymes. CtXyn30A is the first thermostable glucuronoxylan endo-β-1,4-xylanase described to date. This work reveals that substrate recognition by both thermophilic and mesophilic glucuronoxylan endo-β-1,4- xylanases is modulated by a conserved set of residues.

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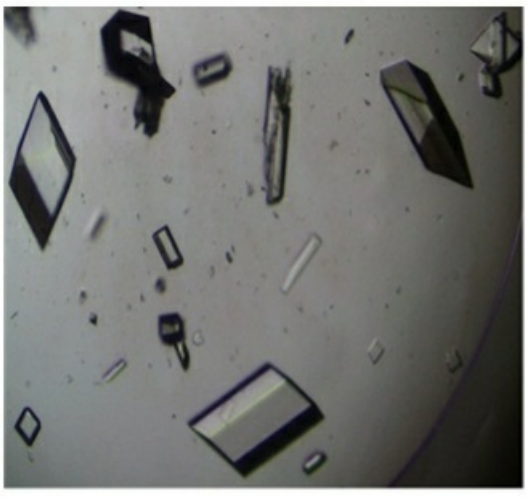
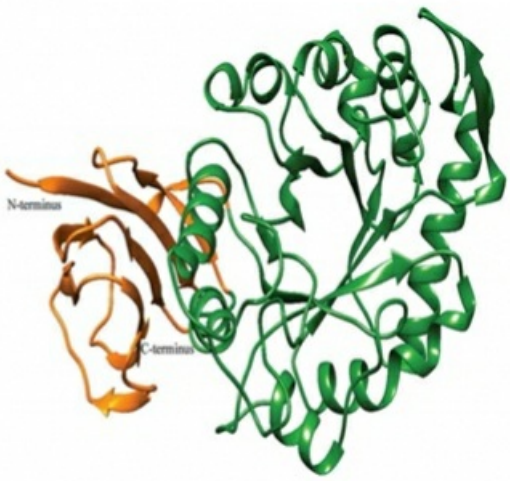
[2] Verma, A. K. and Goyal, A. (2016) Journal of Molecular Catalysis B: Enzymatic, 129, 6-14.
[3] Freire, F. et al. (2016) Acta Crystallographica Section D: Structural Biology, 72(11), 1162-1173.



*Ct*Xyn30A crystal 0.2 M potassium sulphate, 20% (w/v) PEG 3350



Crystal Structure of *Ct*Xyn30A

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