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Research review paper

Towards enzymatic breakdown of complex plant xylan structures: State of the art

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ABSTRACT

Significant progress over the past few years has been achieved in the enzymology of microbial degradation and saccharification of plant xylan, after cellulose being the most abundant natural renewable polysaccharide. Several new types of xylan depolymerizing and debranching enzymes have been described in microorganisms. Despite the increasing variety of known glycoside hydrolases and carbohydrate esterases, some xylan structures still appear quite recalcitrant. This review focuses on the mode of action of different types of depolymerizing endoxylanases and their cooperation with β-xylosidase and accessory enzymes in breakdown of complex highly branched xylan structures. Emphasis is placed on the enzymatic hydrolysis of alkali-extracted deesterified polysaccharide as well as acetylated xylan isolated from plant cell walls under non-alkaline conditions. It is also shown how the combination of selected endoxylanases and debranching enzymes can determine the nature of prebiotic xylooligosaccharides or lead to complete hydrolysis of the polysaccharide. The article also highlights the possibility for discovery of novel xylanolytic enzymes, construction of multifunctional chimeric enzymes and xylanosomes in parallel with increasing knowledge on the fine structure of the polysaccharide.

Contents

Abbreviations: Xyl*p*, D-xylopyranosyl residue; Ara*f*, L-arabinofuranosyl residue; MeGlcA, 4-*O*-methyl-D-glucuronic acid; GH, glycoside hydrolase; CE, carbohydrate esterase; Xyl_n, β-1,4-xylooligosaccharide of n Xyl*p* residues; $MeGlcA^iXyl_n$, aldouronic acid containing one residue of MeGlcA and n Xyl*p* residues - the upper index i marks the number of Xyl*p* residue counted from the reducing end to which MeGlcA is linked (it would be equal to 2 if MeGlcA would be linked to the second Xyl*p* residue from the reducing end, and to 3 if MeGlcA would be linked e.g. to the non-reducing end Xyl*p* residue in Xyl_3); $Ac^3MeGlcA^3Xyl_3$, aldotetraouronic acid in which both MeGlcA and the acetyl group is linked to non-reducing end Xyl*p* residue; $Ac^3MeGlcA^3Xyl_4$, aldopentaouronic acid in which both MeGlcA and the acetyl group is linked to penultimate Xyl*p* residue from the non-reducing end; Ara^2Xyl_2, Xyl_2 with Ara*f* residue linked to the non-reducing end Xyl*p* residue; Ara^3Xyl_3, Xyl_3 with Ara*f* residue linked to the non-reducing end Xyl*p* residue; $Ara^{3,3}_2Xyl_3$, Xyl_3 with two Ara*f* residues linked to the non-reducing end Xyl*p* residue at position 2 and 3.

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1. Introduction

The increasing worldwide effort to develop economically feasible and environmentally friendly procedures for bioconversion of plant biomass involves also the fraction of hemicellulose polysaccharides that are separated from cellulose and lignin during various pretreatments. The major plant hemicellulose of interest is xylan, a branched plant polysaccharide, characteristic feature of which is the backbone built of β-1,4-linked D-xylopyranosyl (Xyl*p*) residues. Its fine structure changes from plant to plant, and even in different parts of the same plant (Ebringerová et al., 2005). Recent data indicates that differences in structure occur even within the same xylan molecule (Busse-Wicher et al., 2014). The variations are due to different side carbohydrate and acid substituents. With the exception of softwood xylan, in all other plants xylans are partially acetylated and in some cases also esterified with phenolic acids. Alkaline pretreatments afford deacetylated polysaccharide, e.g. glucuronoxylan from hardwood, arabinoxylan from cereal endosperm and arabinoglucuronoxylan from agricultural side products, such as straw and bran, and different maize residues. Milder alkaline pretreatments preserve ester linkages between Ara*f* side substituents and phenolic acids, mainly ferulic acid (Schendel et al., 2015). Non-alkaline pretreatments, such as steam explosion and autohydrolysis, yield partially acetylated hemicellulose (Alvira et al., 2010; Appeldoorn et al., 2013; Selig et al., 2008; Wyman et al., 2005).

Over the past two decades a great progress in enzymology of plant xylan degradation and saccharification has been achieved. New types of depolymerizing xylanases and debranching enzymes have been described in microorganisms and the structure-function relationship of accessory xylanolytic enzymes has become better understood. However, since the available data are scattered over the vast amount of published literature, and some xylan structures were recognized as recalcitrant to known enzymes only recently, we feel that it is of extreme importance to summarize current knowledge on microbial xylanolytic enzymes and outline possible strategies of enzymatic xylan degradation to oligosaccharides and also towards monosaccharide building blocks. The target sugar is naturally xylose which can serve as a source for production of a number of compounds including ethanol (Dodd and Cann, 2009; Gírio et al., 2010). As an added value to the previous reviews on this subject (Aachary and Prapulla, 2009; Beg et al., 2001; Deutschmann and Dekker, 2012; Keshwani and Cheng, 2009; Polizeli et al., 2005; Saha, 2003; Shallom and Shoham, 2003; Van den Brink and de Vries, 2011; Van Dyk and Pletschke, 2012), we distinguish here between xylans that were extracted from plant biomass in the presence of alkali which destroys almost all ester linkages, and xylans obtained under non-alkaline conditions which preserve considerable portion of ester linkages, and afford partially esterified polysaccharide that corresponds to its native form in plants. In all cases the enzymatic hydrolysis of xylan passes the stage of oligosaccharides which can also be considered as desired products. They find applications as prebiotics, dietary fibers and antioxidants (Broekaert et al., 2011).

2. Xylans and xylanolytic enzymes

2.1. Alkali extracted glucuronoxylan

Alkali extracted glucuronoxylan is the main hardwood hemicellulose which in average contains about one MeGlcA side residue per 10 main chain Xyl*p* residues (McGinnis and Shafizadeh, 1980; Takahashi and Koshijima, 1988). The reported yields of the polysaccharide extracted usually from delignified hardwood pulp reach 20% of the starting wood (Ebringerová et al., 1967). In native state, the polysaccharide is partially acetylated (Ebringerová et al., 2005; Timell, 1967; Wilkie, 1983), and also linked to lignin via ester linkages between lignin alcohols and carboxyl group of MeGlcA side residues (Jeffries, 1990; Takahashi and Koshijima, 1988). In situ reduction of MeGlcA esters to 4-*O*-methyl-D-glucose with $NaBH_4$ in beechwood sawdust suggested that about 30% of MeGlcA residues are esterified (Takahashi and Koshijima, 1988). However, any alkali treatment of hardwood leads to complete xylan deacetylation and obviously also to disruption of its ester linkages with lignin. The distribution of MeGlcA side residues shows some regularity, but several lines of evidence suggest that alkaline extraction offers a mixture of heterogeneous xylan molecules differing in the degree of substitution with MeGlcA. It is also highly possible that several or at least two types of xylans with varying content of MeGlcA are co-extracted. The ratio Xyl:MeGlcA 10:1 is most probably an average value, since DMSO-extracted acetylated glucuronoxylan from delignified hardwood pulp shows considerably lower MeGlcA content than the polysaccharide extracted by alkali [Naran et al., 2009; Vršanská and Biely, unpublished results]. Recent structural studies of xylans from softwood Japanese cedar and Hinoki cypress (Ishii et al., 2010) showed a strange distribution of MeGlcA residues, frequently also on two neighboring Xyl*p* residues. Hardwood xylan, e.g. from beechwood, appears to be quite heterogeneous since its more soluble fraction also possesses higher MeGlcA content (Biely et al., 2015). Thus, the MeGlcA content determines physico-chemical properties, mainly the polysaccharide solubility. Lower frequency of substitution leads to higher probability of association of xylan chains among themselves and with cellulose, via hydrogen bond interaction of unsubstituted regions of xylan molecules.

2.1.1. Glucuronoxylan depolymerization by xylanases

For simplification we shall discuss the enzymes needed to saccharify an average alkali extracted hardwood glucuronoxylan. Depolymerization can be achieved by three types of *endo*-β-1,4-xylanases that have been classified in glycoside hydrolase (GH) families 10, 11 and 30 (Biely et al., 1997; Collins et al., 2005; Pollet et al., 2010) (Fig. 1). The mode of glucuronoxylan cleavage by GH10, GH11 and GH30 enzymes differs mainly in the way of acceptance and recognition of the MeGlcA side residues in the process of formation of the productive enzyme-substrate complexes (Biely et al., 1997; Fujimoto et al., 2004; Pell et al., 2004; Pollet et al., 2010). With exception of the reducing end, GH10 xylanases require two consecutive unsubstituted Xyl*p* residues to attack the xylan main chain, and are capable of cleaving the glycosidic linkage to MeGlcA-substituted Xyl*p* residue. GH11 xylanases require three unsubstituted Xyl*p* residues in a row and attack xylan chain one linkage before the Xyl*p* substituted with MeGlcA (Fig. 1). The two glycosidic linkages following the MeGlcA branch are not attacked by both types of enzymes. For these reasons the GH10 xylanases generate, as a rule, products by one Xyl*p* residue shorter that GH11 xylanases (Fig. 1). While Xyl_2 and aldotetraouronic acid $MeGlcA^3Xyl_3$ predominate in the GH10 hydrolysate, Xyl_2, Xyl_3 and aldopentauronic acid $MeGlcA^3Xyl_4$ are the major products of GH11 xylanases. GH10 xylanases usually generate more Xyl that GH11 enzymes. Looking on Fig. 1, we immediately recognize what should be the other enzymes to complete the hydrolysis of generated oligosaccharides. Neutral, linear xylooligosaccharides, mainly Xyl_2 and Xyl_3, have to be hydrolyzed to the monomer by β-xylosidase, the second type of hydrolase attacking the β-1,4-xylosidic