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Xylans inhibit enzymatic hydrolysis of lignocellulosic materials by cellulases

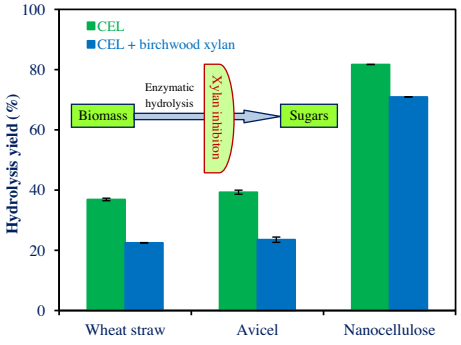
Junhua Zhang [a,*], Ming Tang [a], Liisa Viikari [b]

[a] College of Forestry, Northwest A&F University, 3 Taicheng Road, Yangling 712100, China
[b] Department of Food and Environmental Sciences, University of Helsinki, P.O. Box 27, FIN-00014 Helsinki, Finland

HIGHLIGHTS

- Xylan clearly inhibited the enzymatic hydrolysis of cellulose by cellulase.
- Xylan clearly inhibited the cellulose hydrolysis by individual EGII, CBHI and CBHII.
- The solubility of oat spelt xylan did not clearly affect the hydrolysis of cellulose.
- After the addition of xylans, cleaved cellobiose units by CBHI from cellulose chain decreased.

GRAPHICAL ABSTRACT



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ABSTRACT

Hemicelluloses have been found to be physical barriers in the hydrolysis of cellulose, and prevent the access of enzymes to cellulose surface. In addition, soluble hemicelluloses may strongly inhibit the cellulase activity. In this work, birchwood xylan clearly inhibited the enzymatic hydrolysis of wheat straw, Avicel and nanocellulose by cellulases. Hydrolysis efficiencies of cellobiohydrolase I (CBHI, from *Thermoascus aurantiacus*), cellobiohydrolase II (CBHII, from *Trichoderma reesei*) and endoglucanase II (from *T. aurantiacus*) were clearly inhibited by birchwood xylan, respectively. The strongest inhibitory effect of birchwood xylan was observed on the hydrolysis of Avicel by CBHI and CBHII, as a dramatically decreased formation of the main product, cellobiose. After additions of soluble and insoluble oat spelt xylan, cleaved cellobiose units by CBHI from cellulose chain decreased from 8 to 4 and 6, respectively. The results in this work demonstrated that xylans clearly inhibited the hydrolysis efficiencies of both endoglucanase and cellobiohydrolase.

1. Introduction

The interactions between cellulose and hemicelluloses play an important role in biosynthetic and degradative processes of plants (Taylor and Haigler, 1993). Hemicelluloses, especially xylans, in hardwoods and annual plants, are important in the pulp and paper industry by producing desired properties to the fibres and could also be used for modification of cellulose fibre (Schönberg et al., 2001; Köhnke, 2010). Xylans have an affinity to cellulose and can absorb irreversibly on cellulosic surfaces (Köhnke, 2010). It has been reported that the adsorption of xylan on cellulose was affected by the molecular structure of xylan, e.g. type of substituents, degree of substitution and molecular weight (Kabel et al., 2007; Köhnke et al., 2011).

In the production of second generation ethanol from lignocellulosic materials, enzymatic hydrolysis still represents a bottleneck. Efficient cellulose hydrolysis requires the cooperative action of

Abbreviations: βG, β-glucosidase; CBH, cellobiohydrolases; CEL, cellulase; DM, dry matter; EG, endoglucanase; HPAEC-PAD, high performance anion exchange chromatography coupled with pulsed amperometric detection; MUL, 4-methylumbelliferyl-β-D-lactoside; XOS, xylo-oligosaccharides.

* Corresponding author. Tel.: +86 29 8708 1511; fax: +86 29 8708 2892.
E-mail address: junhuazhang@nwsuaf.edu.cn (J. Zhang).

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endoglucanases (EG), cellobiohydrolases (CBH) and β-glucosidase (βG) whereas hemicellulases, xylanases and mannanases, as well as accessory enzymes, especially acetyl xylan esterases, arabinofuranosidases, and eventually some phenolic esterases are needed to expose cellulose microfibrils covered by hemicelluloses. The presence of lignin and hemicelluloses has been shown to be major barriers in the enzymatic hydrolysis of various pretreated materials (Yang and Wyman, 2004; Öhgren et al., 2007). Hemicelluloses, either at their original location in the fibres, or due to solubilization and eventual relocation on the surfaces may physically block the access of cellulose. Thus, enzymatic hydrolysis has been found to be affected by the amount of residual xylans in lignocellulosic materials after pretreatment (Grohmann et al., 1989; Palonen et al., 2004; Yang and Wyman, 2004; Kabel et al., 2007). Even low amount of residual xylan can limit the extent and the rate of cellulose hydrolysis. It has been reported by many authors that xylanase supplementation clearly increased cellulose hydrolysis in xylan-containing lignocellulosic materials (Öhgren et al., 2007; Selig et al., 2008; Kumar and Wyman, 2009; Zhang et al., 2011). Furthermore, xylan and various xylo-oligosaccharides (XOS) were found to have an inhibitory effect when added in the hydrolysis of Avicel and the addition of xylose, xylan, or XOS (about 8% of cellulose) reduced initial hydrolysis rates by 9.7%, 34.5%, and 23.8%, respectively, compared to the control (Qing et al, 2010). Xylobiose and XOS of higher DP were found to inhibit the enzymatic hydrolysis of various polysaccharides, with xylose, xylobiose, and xylotriose having progressively greater impacts on hydrolysis rates (Kumar and Wyman, 2009). Recently, a strong inhibition of cellobiohydrolases, especially of CBHII, by XOS was observed, whereas no inhibitory effect on EGII was shown (Zhang and Viikari, 2012).

In the present work, the effect of xylan on the hydrolysis of cellulose by individual cellulases was studied. Based on the molar ratio of cellobiose to glucose released from the substrates by CBHI, the processivity of CBHI with and without the added xylans was also discussed. The inhibitory effect of the solubility of xylan on the hydrolysis of cellulose by cellulases was investigated and the inhibitory effects of xylans on the hydrolysis of various cellulosic substrates by the individual enzymes, CBHI and CBHII and EGII, were compared.

2. Methods

2.1. Materials

Microcrystalline cellulose (Avicel PH-101) and birchwood xylan were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Oat spelt xylan was obtained from Serva (Germany). Nanocellulose with a dry matter (DM) of 1.69% was prepared from softwood chemical pulp at VTT in Finland, as described by Várnai et al. (2011). Nanocellulose had lost its original fiber structure with a width ranging from tens of nanometers to a few hundred nanometers. The contents of cellulose and xylan in Avicel were 91.3% and 1.23% and in nanocellulose 72.5% and 6.83%, respectively. Hydrothermally pretreated wheat straw was a kind gift of Inbicon (Fredericia, Denmark). The contents of cellulose and xylan in wheat straw were 58.6% and 3.6%, respectively. The sugar compositions were determined by high performance liquid chromatography using an analytical CarboPac PA-1 column (Dionex Corp., Sunnyvale, CA, USA). All other chemicals used were of analytical grade and purchased from Sigma or Merck.

2.2. Enzymes

Recombinant CBH I, EG II originating from *Thermoascus aurantiacus* and βG from *Acremonium thermophilum* were kindly provided by Roal Oy (Rajamäki, Finland), produced in a genetically modified *Trichoderma reesei* strain where the genes coding for the major cellulases encoding for CBHI, CBHII, EGI and EGII, had been deleted. These enzyme preparations were heat treated at pH 6.0 and at 60 °C for 2 h to inactivate the background *T. reesei* enzymes. The purified CBHII originating from *T. reesei* was kindly provided by VTT (Finland). Protein was quantified by the Lowry method, using bovine serum albumin (Sigma Chemical Co., USA) as standard (Lowry et al., 1951). Cellobiohydrolase I activity was determined using 4-methylumbelliferyl-β-D-lactoside (MUL) as substrate according to van Tilbeurgh et al. (1982, 1988).

2.3. Preparation of soluble and insoluble oat spelt xylans

Soluble and insoluble oat spelt xylans were prepared by a modified method of Ryan et al. (2003). Oat spelt xylan (4 g) was suspended in 400 ml of distilled water and stirred overnight at room temperature. The insoluble fraction was recovered by centrifugation for 20 min at 10,000 rpm and at 4 °C. The insoluble fraction was washed several times with Milli-Q-water (Milli-Q-plus, Millipore, Billerica, MA, USA). After that, the sediment was lyophilized and used as insoluble oat spelt xylan for hydrolysis. The supernatant was lyophilized and used as soluble oat spelt xylan for hydrolysis.

2.4. Enzymatic hydrolysis

The hydrolysis of the pretreated wheat straw, Avicel and nanocellulose by cellulase preparations, CBHI, CBHII or EGII was carried out in 50 mM sodium citrate buffer (pH 5.0) containing 0.02% NaN_3 at 50 °C. Enzyme loading was expressed as protein amount (mg) of enzyme preparation per gram DM of substrates. A cellulase preparation (CEL) was composed of the individual cellulases and contained: CBHI (8 mg), EGII (2 mg) and βG (1 mg) per gram DM of substrates. Birchwood xylan, soluble oat spelt xylan or insoluble oat spelt xylan was added into the reaction system before enzymatic hydrolysis. Samples were withdrawn and boiled for 10 min to stop the enzymatic hydrolysis. After cooling, the samples were centrifuged at 10,000 *g* for 10 min and the supernatants were analyzed for glucose and cellobiose by high performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD).

2.5. Analysis of carbohydrates

Glucose and cellobiose in the hydrolysates were analyzed using HPAEC-PAD system as described previously (Zhang et al., 2011).

3. Results and discussion

3.1. Effect of xylan on cellulose hydrolysis

After the pretreatments, lignocellulosic materials contain variable amounts of xylans as residues of the original xylan in the plant cell wall or as solubilized, relocated xylan, covering the fiber surfaces due to the affinity to cellulose, and restricting the enzymatic hydrolysis. In order to investigate the effect of xylan on the enzymatic hydrolysis of cellulose, isolated birchwood xylan was added in the enzymatic hydrolysis of three different substrates (wheat straw, Avicel and nanocellulose), which were hydrolyzed with the composed mixture of individual cellulases in the absence of major xylanase activity (Fig. 1). Clearly lower hydrolysis yields were obtained from all substrates after the addition of birchwood xylan, indicating the inhibition of cellulose hydrolysis by xylan. Previously, the affinity and irreversible adsorption of xylans on