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Xylo-oligosaccharides are competitive inhibitors of cellobiohydrolase I from *Thermoascus aurantiacus*

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HIGHLIGHTS

- Strong inhibition of cellulases by XOS was attributed to the inhibition of the activity of cellobiohydrolases by XOS.
- Stronger inhibitory effects by XOS were observed on CBHII originating from *Trichoderma reesei* than on CBHI from *Thermoascus aurantiacus*.
- Xylobiose and xylotriose were competitive inhibitors of cellobiohydrolase I from *T. aurantiacus*.
- Xylobiose exhibited stronger inhibitory effect than xylotriose on cellobiohydrolase I from *T. aurantiacus*.

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ABSTRACT

The effects of xylo-oligosaccharides (XOS) and xylose on the hydrolytic activities of cellulases, endoglucanase II (EGII, originating from *Thermoascus aurantiacus*), cellobiohydrolase I (CBHI, from *T. aurantiacus*), and cellobiohydrolase II (CBHII, from *Trichoderma reesei*) on Avicel and nanocellulose were investigated. After the addition of XOS, the amounts of cellobiose, the main product released from Avicel and nanocellulose by CBHI, decreased from 0.78 and 1.37 mg/ml to 0.59 and 1.23 mg/ml, respectively. During hydrolysis by CBHII, the amounts of cellobiose released from the substrates were almost cut in half after the addition of XOS. Kinetic experiments showed that xylobiose and xylotriose were competitive inhibitors of CBHI. The results revealed that the strong inhibition of cellulase by XOS can be attributed to the inhibitory effect of XOS especially on cellobiohydrolase I. The results indicate the necessity to totally hydrolyze xylo-oligosaccharides into the less inhibitory product, xylose, to increasing hydrolytic efficiency.

1. Introduction

Cellulose, hemicellulose and lignin are the main components in lignocellulosic materials and fermentable sugars can be obtained from cellulose and hemicellulose by enzymatic hydrolysis. In the bioconversion of lignocellulosic materials into fermentable sugars, the major technological obstacle is the recalcitrance and complex structure of the raw material (Himmel et al., 2007).

Efficient hydrolysis of lignocellulosic materials requires a number of enzymes. Cellulose hydrolysis requires the synergistic action of endoglucanases (EG, E.C. 3.2.1.4), which hydrolyze the cellulose polymer internally, and exoglucanases or cellobiohydrolases (CBH, E.C. 3.2.1.91), which act on the reducing and non-reducing ends, releasing cellobiose and cello-oligosaccharides. Finally, β-glucosidase (βG, E.C. 3.2.1.21) hydrolyzes cellobiose to glucose. Depending on the pretreatment methods used, as well as the severity factor applied, variable amounts of hemicelluloses remain in the solid fraction of lignocellulosic materials. The main hemicelluloses in annual plants are xylans and the complete hydrolysis of xylans involves enzymes cleaving the main chains as well as the side groups of the polymers (Beg et al., 2001; Saha, 2003). Xylose, xylobiose and xylotriose are released as main hydrolysis products (Zilliox and Debeire, 1998; Zhang et al., 2011b). Depending on the raw material structure and the enzyme preparation used, some of the hydrolysis products of xylans may remain as oligomers in the hydrolysates.

Many factors have been recognized to limit the efficient enzymatic hydrolysis of lignocellulosic materials, including the recalcitrant structure, limiting enzyme activities, as well as inactivation of enzymes. End-product inhibition of the main enzymes results in a

Abbreviations: βG, glucosidase; BSA, bovine serum albumin; CBD, cellulose binding domain; CBH, cellobiohydrolases; CEL, cellulase; DM, dry matter; DNS, dinitrosalicylic acid; EG, endoglucanase; GH, glycosyl hydrolase family; HPAEC-PAD, high-performance anion exchange chromatography coupled with pulsed amperometric detection; HPLC, High-performance liquid chromatography; XOS, xylo-oligosaccharides.

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