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Research paper

Why does GH10 xylanase have better performance than GH11 xylanase for the deconstruction of pretreated biomass?

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ABSTRACT

One approach to improve biomass deconstruction is to formulate a more efficient cellulase cocktail by adding "accessory enzymes" (e.g. xylanase/LPMO/laccases). Among different xylanases, glycoside hydrolase family 10 endo-xylanase (GH10EX) shows better performance than family 11 endo-xylanase (GH11EX) even though GH11EX has better kinetic activity on various xylan substrates. To better understand this phenomenon, the xylan accessibility of GH10/11 xylanases was assessed on various "model" and realistic cellulosic substrates and their thermostability was also compared during time course of hydrolysis. It showed that GH10EX had higher accessibility towards the xylan backbone within pretreated biomass, especially for these with higher acetyl group content. Acetyl group removal could greatly intensify the synergistic cooperation between GH11EX and cellulases. Additionally, the higher thermostability of GH10EX appeared to be another reason for its outstanding potential during biomass decomposition. This work provides further insights for engineering better biocatalysts to enhance the economic viability of enzyme based biorefinery.

1. Introduction

The cost-effective production of sugars from biomass continues to remain challenging, partly due to the relatively high enzyme/protein loading required to effectively hydrolyse the insoluble polysaccharides within the pretreated lignocellulosic substrates [1,2]. One way to reduce the amount of enzyme usage is to improve the hydrolytic efficacy of "cellulase" mixtures by stimulating the cooperation among cellulases and various accessory enzymes such as xylanases and AA9 [3–7]. We and others have shown that the synergistic cooperation between cellulases and xylanases not only substantially enhanced the hydrolysis extent of both the glucan and xylan present in various pretreated lignocellulosic substrates, but also dramatically reduce the required cellulase dosage needed to achieve high cellulose hydrolysis yields (> 80%) [4,8,9]. Interestingly, when different glycoside hydrolase (GH) family endo-xylanases were compared, it appeared that family 10 endo-xylanase (GH10 EX) showed better synergistic cooperation with canonical hydrolytic cellulases than family 11 endo-xylanase (GH11 EX) on various pretreated substrates [8], even though GH11 EX was initially expected to be a better candidate for biomass deconstruction since it has relatively small size (easily access the xylan within the complex cellulose-hemicellulose-lignin matrix) and also higher catalytic activities on the "model" xylanolytic substrates such as the isolated birch wood and oat spelt xylan [8,10,11].

One of the major roles of xylanases was believed to remove xylan, which significantly restricts the accessibility of cellulose within the biomass to cellulase enzymes [4,10]. Xylan, the major hemicellulose component in hardwood and annual plants, is a diverse group of highly branched heteropolymers with a backbone of β-1,4-linked xylose residues [12,13]. Xylan are often substituted with glucuronosyl or 4-O-methyl glucuronosyl residues at the C-2 position, in glucuronoxylans (GX), or substituted with arabinofuranosyl residues at the C-3 position, in arabinoxylan and/or glucuronoarabinoxylans (AX) [11,12,14]. In addition, most xylans are acetylated to various degrees (up to 10:7 ratio of xylose to acetyl), normally at the C-3 position on xylopyranose [13]. Although the commonly used thermochemical pretreatment processes can remove some of these xylan branches especially the glucuronosyl and arabinofuranosyl residues, leftover might still significantly impede the accessibility of the xylan backbone to the xylanase enzymes.

Variants of steam-explosion pretreatments are currently utilized in several commercial biorefineries, such as Inbicon/Beta-renewables in Europe, Abengoa/Dupont/Poet-DSM in the United States, and GrandBio

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in Brazil. Typically, a mild steam pretreatment condition will be applied to recover the biomass components in a useable form and to also provide a cellulosic stream that can be efficiently hydrolyzed with relatively low enzyme loading [15,16]. Under such pretreatment condition, a large portion of acetyl group still remains as branches on the xylan backbone [16]. The two family xylanases, the GH10 EX is capable of attacking a glyosidic linkage close to a substituted xylose residue and requires only two unsubstituted xylose residues for its activity, while the GH11 EX requires at least three consecutive, unsubstituted xylose residues before cleavage can take place and it cannot cleave the linkage next to a branch point [11,17]. Thus, we hypothesized that the acetyl branch of xylan in the steam pretreated biomass might be one of the main reasons for the observed better boosting effects of GH10 EX than GH11 EX on cellulase hydrolysis. In addition, since the enzymatic hydrolysis has to be performed in a relatively long periods (2–3days), the thermostability of these two family xylanases might affect their cooperation with cellulase enzymes.

To test these hypotheses, a "model" cellulose-xylan matrix and various pretreated lignocellulosic substrates with different xylan location/content and various amount of xylan acetyl branches were initially employed to assess the cooperation between these two family xylanases and the major cellulase monocomponents Cel7A during cellulose hydrolysis. The accessibility/binding affinity of these xylanases were further compared over a range of xylanolytic substrates (e.g. beech wood and oat spelt xylan) and the pretreated substrates with/without deacetylation. In addition, the thermostability of both family xylanases were also compared at hydrolytic condition with/without the existence of pretreated biomass. These results provide new insights for designing better xylanases and/or pretreatment process for enhancing biomass deconstruction.

2. Materials and methods

2.1. Lignocellulosic substrates preparation and composition

Sugar cane bagasse were steam pretreated at two different severities according to previously described procedures [6]. Briefly, sugar cane bagasse (DW; dry weight of 200 g) were impregnated overnight with a mass fraction of 3% SO_2 in a sealable plastic bag. The amount of SO_2 was determined by weighing the total substrates weight before and after the addition of the gas. Steam pretreatment was conducted in a 2L StakeTech II batch steam gun (constructed by Stake Tech-Norvall, Ontario, Canada) at either 190 °C (SPSCB190) or 200 °C (SPSCB200) for 5 min. The pretreatment conditions and chemical composition of the pretreated substrates are shown in Table 1.

Substrate deacetylation process were performed according to [18]. Briefly, a mass fraction of 2% solid was added into 200 mol/m^3 NaOH solution and incubated at 60 °C for 2 h with constant shaking (3.3 Hz). After incubation, samples were acidified to pH 2 with a mass fraction of 72% sulphuric acid and cooled in an ice bath. The samples were then centrifuged at 13,000 g for 10 min and the supernatants were filtered through a 0.45 μm syringe filter (Chromatographic Specialties, Brockville, Canada) and analyzed using a HPLC (ICS-500) with Aminex HPX-87H column (Bio-Rad, Hercules, CA). The HPLC has fitted with an AS3500 auto sampler, a UV detector at a wavelength of 280 nm and a GP40 gradient pump (NREL, 2008), and 5 mol/m^3 H_2SO_4 was used as an eluent at a flow rate of 0.6 ml/min. Dissolving pulp (DP) (a kind gift from Tembec) was used as an almost pure cellulolytic substrate control (less than 0.5% lignin, less than 3% xylan). Birchwood xylan and oat spelts xylan were purchased from Sigma.

2.2. Enzyme purification and enzymatic hydrolysis

The major cellulase monocomponent Cel7A (CBHI), family 10 endo-xylanase (GH10EX), and family 11 endo-xylanase (GH11EX) were purified from Celluclast 1.5 L (Novozymes, Franklington, NC), Multifect Xylanases (Genencor US Inc., Palo Alto, CA), and H-Tec (Novozymes, Franklington, NC) respectively as previously reported [8]. The hydrolysis assays were carried out at a mass fraction of 2% solids loading in sodium acetate buffer (50 mol/m^3, pH 4.8) in an 10 cm^3 total volume. The reaction mixtures were mechanically shaken in an orbital shaker incubator (Combi-D24 hybridization incubator) at 50 °C for up to 72 h at 2.5 Hz.

2.3. Analytical methods

The chemical composition of the various steam pretreated lignocellulosic substrates after Klason treatment were determined using high performance anion exchange chromatography (Dionex DX-3000, Sunnyvale, CA) as described earlier [4]. The glucose concentration present in the hydrolysate was determined by the Glucose Oxidase Assay. Glucan conversions (%) of the pretreated substrates were calculated from the original glucan content as a percentage of the theoretical glucan available in the original substrate. The total protein content was measured by the ninhydrin assay using bovine serum albumin (BSA) as the protein standard [6].

3. Results and discussion

Our previous study showed that glycoside hydrolase family 10 endo-xylanase (GH10 EX) had better performance than family 11 endo-xylanase (GH11 EX) when synergistically cooperated with cellulase enzymes for the hydrolysis of various pretreated lignocellulosic substrates, even though GH11 EX showed higher catalytic activity on different "model" xylanolytic substrates such as birchwood/beechwood/oat spelt xylan, and p-Nitrophenyl-β-D-xylopyranosides [8]. One of the possibilities is the lower accessibility of the GH11 EX towards the acetylated xylan backbone within these pretreated biomass. To test this hypothesis, the synergistic interaction between cellulases and two family xylanases was initially assessed on a model holocellulosic substrate. This holocellulose is a mixture of "pure" cellulosic substrate dissolving pulp (DSP) with 10% of commercial birchwood xylan which has already been deacetylated during the xylan alkaline extraction process. The major cellulase monocomponent Cel7A was employed in this study to avoid other potential influences existed in the commercial cellulase preparation. As expected, the appearance of xylan significantly reduced the hydrolytic performance of Cel7A towards the cellulose (~30% cellulose hydrolysis reduction), while the addition of xylanases

Table 1
Chemical composition of pretreated lignocellulosic substrates.

Substrate	Carbohydrate mass fraction (%)						Acetyl mass fraction (%)"
	Ara	*Gal*	*Glu*	*Xyl*	*Man*	*AIL*	
Original SCB	2.1 ± 0.2%	0.9 ± 0.1%	33.8 ± 0.8%	18.2 ± 1.3%	0.6 ± 0.0%	23.1 ± 1.6%	4.6 ± 0.2%
SPSCB 190	0.6 ± 0.0%	0.8 ± 0.1%	54.3 ± 1.1%	9.8 ± 0.8%	1.0 ± 0.2%	25.8 ± 1.2%	2.9 ± 0.2%
SPSCB 200	0.3 ± 0.0%	0.5 ± 0.0%	59.3 ± 1.6%	3.7 ± 0.3%	0.7 ± 0.1%	27.3 ± 0.9%	1.4 ± 0.1%

Ara: Arabinan, *Gal*: Galactan, *Glu*: Glucan, *Xyl*: Xylan, *Man:* Mannan, *AIL:* Acid Insoluble Lignin. SCB: sugar cane bagasse; SPSCB190: steam pretreated sugar cane bagasse at 190 °C; SPSCB 200: steam pretreated sugar cane bagasse at 200 °C. All of the experiments were performed in triplicate and the mean values and error bars were calculated.