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Release of Arabinose from Wheat Insoluble Arabinoxylan by the Action of α-L-Arabinofuranosidase in Synergism with Endo-Xylanases

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Abstract

Wheat arabinoxylan (water-insoluble fraction) contains ~36% arabinose which may include both singly or doubly substitutions at C2/C3 of the Xyl*p* units. α-L-Arabinofuranosidses (ABFs) of two GH families were analyzed for their respective activities on the hydrolysis of Xyl*p*-Ara*f*. BaABF (GH43) produced twice the yield of arabinose residues from the heteroxylan compared to AnABF (GH51) under the same reaction conditions. Two endo-xylanases (of GH10 and 11) also showed differential hydrolytic activities on the Xyl*p* chain, with the GH10 XYN-ATM double the amount of reducing sugar yield (as xylose equivalent) than using the GH11 XYN-M3. When the ABF and XYN were combined in optimial ratios, a synergistic increase of 73.8% in arabinose yield was observed.

Keywords

α-L-Arabinofuranosidase, Mono-Substituted Xylopyranosyl Residuet, Di-Substituted Xylopyranosyl Unit, Arabinoxylan Arabinofuranohydrolase (AXH)

1. Introduction

The structural complexity of cellulosic biomass arises from the large hemicellulose fraction consisting of mostly xylan. Chemically in its native state, xylan is a heteropolysaccharide (heteroxylan) with a β-(1,4)-xylopyranosyl (Xyl*p*) backbone chain carrying arabinofuranosyl, acetyl, and glucuronyl side groups, and oligosaccharide side chains of galactose, xylose, and arabinose [1] [2]. The branch-

ing and the structural architecture of side groups vary from plant to plant and even in different tissues of the same plant [3]. Arabinoxylans from cereal grains are highly branched. Those from wheat and barley endosperm contain considerable levels of main chain Xyl*p* residues singly or doubly substituted with arabinofuranosyl units (Ara*f*) attached to C2/C3 positions [4] [5]. Adding to the complexity, arabinofuranosyl units may also be ester-linked at C5 with ferulic acid moieties, which can further crosslink into diferulates. Diferulates form linkages between xylan and lignin and also between pectin and lignin [2] [6].

The cooperative interactions between main chain enzymes and enzymes liberating side chain substituents (collectively known as accessory enzymes) are involved as key processes in depolymerization of xylan. Notably, endo-xylanase has been known to act synergistically with increased efficiency when arabinofuranosidase (ABF, EC 3.2.1.55) is used to remove the Ara*f* substituents [7].

ABFs are categorized into two main groups: 1) enzymes active on Xyl*p* monosubstituted with arabinose, either at position C-2 or C-3, and thus are referred to as ABFm2,3; 2) enzymes active specifically on doubly arabinofuranosylated Xyl*p*, releasing either 1,2- or 1,3-linked Ara*f*. These enzymes are referred to as ABFd2 or ABFd3 [8]. The objective of this paper is to report the comparative studies of two categories of enzymes on the release of arabinose side groups from wheat insoluble arabinoxylan, and the effect of synergism on the efficiency with endo-xylanases added to the reaction.

2. Materials and Methods

The following were obtained from Megazyme (Wicklow, Ireland): wheat arabinoxylan, *α*-L-arabinofuranosidase (ABF, EC 3.2.1.55) from *Aspergillus niger* (AnABF, cat. # E-AFASE, GH51), and from *Bifidobacterium adolescentis* (BaABF, cat. # E-AFAM2, GH43), b-D-xylanases (EC 3.2.1.8) from *Thermotoga maritima* (cat. # E-XYLATM, GH10), from *Trichoderma longibrachiatum* (cat. # E-XYLT3, endo-1,4-b-xylanase M3, GH11), and Arabinose Assay Kit (cat. # K-ARGA). Precast protein gels were purchased from Novex (San Diego, CA). Microbiological culture medium components and agar were from Difco Laboratories (Detroit, MI). Thin-layer plates and substrates were purchased from Analtech (Newark, DE). All chemicals and reagents were of analytical grade.

2.1. Bioinformatics

Geneious (Biomatters Ltd., Auckland, New Zealand) was used for sequence analysis and vector construction. Multiple sequence alignment was performed using Clustal Omega for graphics and statistics. KaleidaGraph software (Synergy, Reading, PA) was used for calculating standard errors and for plotting.

2.2. Analysis of Hydrolytic Release of Arabinose from WIA

A typical reaction contained 30 mg WIA soaked for 30 min in 1 ml of K_2HPO_4 buffer, pH 6.5. ABF or/and XYN were added to a final concentrations of various

mM. The final reaction volume was adjusted to 1 ml, incubated at 40˚C for 2 hr with continuous shaking. The reaction mixture was centrifuged and the supernatant was tranfered for the analysis of arabinose and xylose reducing ends.

Enzymatic hydrolysis of arabinose was measured by the formation of reducing ends produced by XYN action and by ABF action using the DNSA method [9]. Total carbohydrate was determined by the phenol-sulfuric acid method [10]. Ferulic acid was determined using Folin-Ciocalteu reagent according to [11]. The amount concentration of arabinose was measured using the Arabinose Assay Kit (Megazyme, Wicklow, Ireland). The analysis is based on an enzymatic process of utilizing galactose mutarotase to catalyze the rate-limiting mutarotation of α-L-arabinose (present mostly in the natural state) to β-L-arabinose. The β-anomer can then be oxidized by NAD+ in the presence of β-galactose dehydrogenase at pH 8.6. The NADH formed is stoichiometrically proportional to the amount of arabinose. This assay procedure provides specific measurement of arabinose without the interferering detection of xylopyranosyl units.

3. Results and Discussion

3.1. Comparison of BaABF (GH43) and AnABF (GH51) Activities on WIA

Two ABFs: BaABF and AnABF were analyzed for their ability to release arabinose from WIA. BaABF belongs to family GH43 and has been known to specifically cleave arabinofuranosyl residues linked to C3 of double-substituted xylopyranosyl units [12] [13]. This enzyme is therefore also categorized as AXH-d3, where AXH designates arabinoxylan arabinofuranohydrolase, d stands for di-substitution, and 3 represents C3 of α-1,3 linkage). The microorganism *B. adolescentis* also produces another enzyme AXH-m2,3, which only hydrolyzes arabinose residues C2 or C3 single substitution. AnABF belongs to GH family 51, and is known to hydrolyze terminal arabinofuranosyl linkages [14]. The release of arabinose from internal Xyl*p* substitution is minimal [15].

In our previous study using arabinofuranosyl xylooligosacchardies (AXOS) substrates, the results supported such activity description [16]. The current study also suggests that BaABF was more active in hydrolyzing more arabinossyl residues from the polymeric substrate WIA than the AnABF enzyme (**Figure 1**). BaABF produced twice or more the yield of arabinose under the same reaction conditions because it acts on both terminally and internally substituted Xyl*p* residues.

3.2. Comparison of XYN-ATM (GH10) and XYN-M3 (GH11) on Debranching WIA

Various studies have shown different action patterns of endo-xylanases catalyzing the debranching of the Xyl*p* main chain. Acting on substituted xylans (such as WIA in this case), GH10 XYN cleavage tends to leave substituted Xyl*p* residue on the non-reducing end, whereas GH11 XYN leaves one unsubstituted Xyl*p* re-

sidue on the non-reducing end [1] [2] [15] [17]. In general, GH10 enzymes would produce shorter oligosaccharide fragments compared to GH11 XYN on the same polymer substrates. The present study has implied the differential actions between the GH10 and GH11 enzymes (Figure 2). The mg xylose equivalent produced in the hydrolysis of 100 mg WIA using XYN-ATM GH10 almost doubling the yield of using GH11.

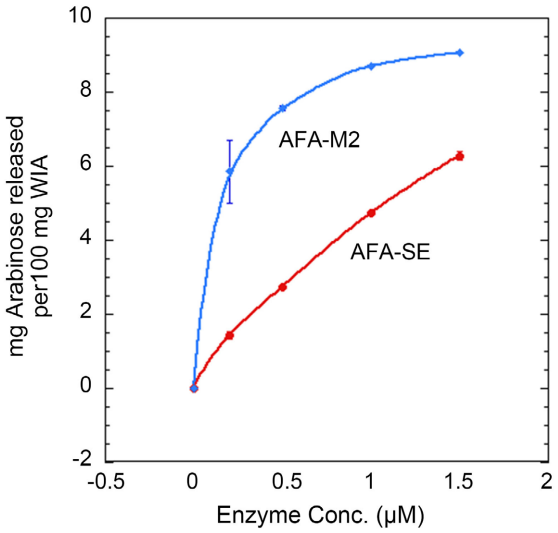

Figure 1. Hydrolytic release of arbabinose from WIA by BaABF (AFA-M2) and AnABF (AFA-SE). Reaction conditions: 30 mg WIA, 0.2 to 0.5 mM ABF in 1 ml K_2HPO_4 buffer, pH 6.5, incubated at 40˚C for 2 hr with continuous mixing.

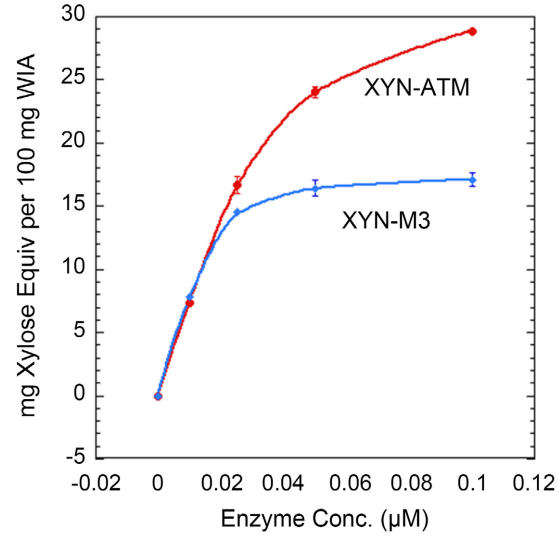

Figure 2. Comparison of XYN-ATM (GH10) and XYN-M3 (GH11) on Debranching WIA. Reaction conditions: 30 mg WIA, 0.01 to 0.1 mM XYN in 1 ml K_2HPO_4 buffer, pH 6.5, incubated at 40˚C for 2 hr with continuous shaking.

The types of substituents, their distribution, density and arrangement would affect the hydrolytic action of XYN on the Xyl*p* chain. The cleavage pattern of the xylan backbone in turn would influenc the actions of accessory enzymes on the side groups (ABFs on Xyl*p*-Ara*f* in this case). Our previous investigation on the synergistic interactions between ferulic acid esterase and the two types of endo-xylanases has clearly suggested this effect on xylan structures using corn fiber as substrate [6].

3.3. Effect of XYN on ABF-Catalyzed Hydrolysis of Arabinose from WIA

To optimize the hydrolysis of arabinose substituents from WIA, the synergistic interaction between ABF and XYN was studied with the enzymes added in various combinations. Since the hydrolytic products contained both arabinose and xylopyranosyl units, a quantitative analysis specific for arabinose was necessary. Using the enzymatic method described in the Arabinose Assay Kit allowed high specificity of measuring the absolute amount of arabinose in the reaction [18].

The present results clearly demonstrate the synergistic effect between ABFs and XYNs (**Figure 3**). In experiment set #1, the arabinose yield by the combination of BaABF with XYN-ATM (both at 0.2 mM) produced a 73.8% increase (compared to the additive sum). In set #2, 0.5 mM ABF was used in the combination, resulting in a synergistic increase of 30.3%. In both sets, the amount of arabinose yield reached similar limiting levels equivalent to 42.8 ± 1.05 and 42.4 ± 0.55 mg arabinose per 100 mg WIA, respectively.

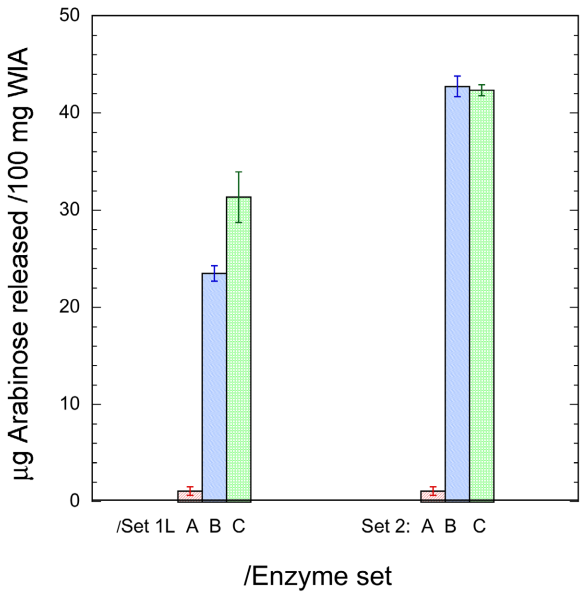

Figure 3. Synergistic effect of ABF and XYN on hydrolysis of arabinose from WIA Reaction conditions: 30 mg WIA, various mM ABF/XYN in 1 ml K_2HPO_4 buffer, pH 6.5, incubated at 40˚C for 2 hr with continuous mixing, Set #1: (A) 0.2 mM XYN-ATM, (B) 0.2 mM BaABF, (C) 0.5 mM BaABF; Set #2: (A) 0.2 mM XYN-ATM, (B) 0.2 mM BaABF + 0.2 mM XYN-ATM, (C) 0.5 mM BaABF + 0.2 mM XYN-ATM.

4. Conclusion

In conclusion, singly or doubly substitutions of arabose residues in native wheat insoluble arabinoxylan were investigated by two CH families of *α*-L-Arabinofuranosidses families. Their respective activities on the hydrolysis of Xyl*p*-Ara*f* and the yield of arabinose residues were determined. Two endo-xylanases were tested for syneristic interactions in the hydrolysis, of which the GH10 XYN-ATM double the amount of reducing sugar yield. The optimal combination of ABF and XYN produced a synergistic increase of 73.8% in arabinose yield.

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Reference to a company and/or product is only for purposes of information and does not imply approval of recommendation of the product to the exclusion of others that may also be suitable. All programs and services of the U.S. Department of Agriculture are offered on a nondiscriminatory basis without regard to race, color, national origin, religion, sex, age, marital status, or handicap. The authors declare that there is no conflict of interest regarding the publication of this paper.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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