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Action of a GH 51 α-L-arabinofuranosidase on wheat-derived arabinoxylans and arabino-xylooligosaccharides

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Abstract

The substrate specificity of an arabinofuranosidase (AbfD3) from family 51 of glycoside hydrolase classification was investigated in order to precisely evaluate its catalytic abilities. AbfD3 activity on destarched wheat bran was poor and less than 1% of total arabinose was released. AbfD3 was also tested on arabinoxylans derived from destarched wheat bran that present different degrees of polymerization, A/X ratios, ferulic acid content and solubility. Results indicated that AbfD3 can hydrolyze polymeric arabinoxylans, even if this action was moderate when compared to the efficient hydrolysis of oligosaccharides. The limited action of AbfD3 on polymeric arabinoxylans is discussed with regard to the heterogeneous distribution of the arabinose residues along the xylan main chain, the insolubility of arabinoxylans and to the presence of disubstituted xylose or feruloylated arabinose.

Keywords: Arabinofuranosidase; Substrate specificity; Wheat bran; Arabinoxylans; Arabinoxylo-oligosaccharides

1. Introduction

The rarefaction of fossil carbon resources and growing awareness that lignocellulosic biomass is the only present-day alternative carbon supply is providing considerable impetus for R&D on lignocellulose-hydrolyzing enzymes. These initiatives are basically focused on the development of enzymatic strategies that will allow biorefining of lignocellulosic feedstocks, without having to resort to the use of thermochemical pretreatments.

Cereal crop byproducts such as straws and brans are cheap, abundant sources of lignocellulosic material that constitute highly attractive feedstocks for future biorefineries. The hemicellulose components of these byproducts are mainly arabinoxylans that are composed uniquely of 1,4-β-linked xylopyranose residues that are ramified by various types of substitutions. The most common substitution is L-arabinofuranose. Most frequently, the monomeric form of this pentose residue is linked to the *O*-2 and/or *O*-3 of xylopyranose residues (Brillouet, 1987; Kormelink & Voragen, 1993). The presence of hydroxycinnamic (ferulic or *p*-coumaric) acids further increase the chemical and structural complexity of arabinoxylans, since they can be ester-linked to substituting L-arabinofuranosyl groups via the *O*-5 group (Mueller-Harvey, Hartley, Harris, & Curzon, 1986; Puls & Schuseil, 1993). In wheat bran, ferulic acid represents 0.5% to 1% of cell wall dry matter (Faulds, Bartolome, & Williamson, 1997; Smith & Hartley, 1983). Some of this ferulic acid is involved in intermolecular diferulic bridges that make a significant contribution to overall parietal reticulation (Ishii, 1991; Scalbert, Monties, Lallemand, Guittet, & Rolando, 1985).

Considering the chemical and structural complexity of cereal hemicelluloses, it is hardly surprising that Nature has developed a complete arsenal of hemicellulose-hydrolyzing

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enzymes that, through their concerted action, bring about complete degradation of these polymers. The main depolymerizing enzymes are endoxylanases (EC 3.2.1.8) whose action is complemented by those of arabinose-hydrolyzing enzymes, such as α-L-arabinofuranosidases (EC 3.2.1.55), β-D-xylosidases (EC 3.2.1.37), α-D-glucuronidases (EC 3.2.1.131) and β-feruloyl esterases (EC 3.1.1.73). Generally, endoxylanase action is most efficient on β-1,4 bonds linking undecorated xylose residues. Therefore, arabinose substitution is one of the major obstacles for complete hydrolysis of arabinoxylan.

In order to choose appropriate enzymes for biorefining, it is necessary to take into account several factors. In particular, to limit costs it is desirable to select only a small number of highly active, robust enzymes that together can achieve the maximum amount of hydrolysis. In terms of arabinose-hydrolyzing enzymes, this means that ideally a single enzyme should be able to hydrolyze both α(1→2) and α(1→3) arabinofuranosidic linkages. Recently, major research efforts have been concentrated on the development of cellulolytic enzymes for industrial applications. However, less attention has been given to hemicellulases, particularly the debranching enzymes. For industrial arabinoxylan degradation a combination of a robust, highly active endoxylanase, a xylosidase and a versatile arabinofuranosidase, able to act upon both oligosaccharides and polysaccharides, might be sufficient to produce high quality pentose streams from cereal by-products such as wheat bran. However, to date no arabinofuranosidases that combine robustness and catalytic versatility have been described.

Several attempts have been made to classify arabinosidases. Classifications have applied substrate specificity (Kaji, 1984) or substrate and linkage specificity to define arabinosidase groups. (Beldman, Schols, Pitson, Searle-van Leeuwen, & Voragen, 1997). Unfortunately, these classification systems suffer from two disadvantages: they provide rather rigid definitions and they make structurally related enzymes appear to be more dissimilar than they really are. According to the glycoside hydrolase classification system (CAZY), based to primary structure similarities, arabinofuranosidases have been distributed in six families (3, 43, 51, 54, 62 and 93) (Henrissat, 1991) (http://afmb.cnrs-mrs.fr/~pedro/CAZY/db.html). Arabinofuranosidases display a wide range of substrate specificities, notably on polymeric components. While most of GH43 arabinosidases hydrolyse α(1→5)-linked arabinans (McKie et al., 1997; Matsuo, Kaneko, Kuno, Kobayashi, & Kusakabe, 2000) and GH62 enzymes are only active on arabinoxylans (Tsujibo et al., 2002; Vincent, Shareck, Dupont, Morosoli, & Kluepfel, 1997), substrate specificities of GH51 and GH54 arabinosidases are rather broad. GH51 arabinosidases from *Aspergillus awamori*, *Clostridium cellulovorans* and *Streptomyces charteusis* display hydrolytic activities on arabinans, arabinogalactans and arabinoxylans (Kaneko et al., 1998; Kosugi, Murashima, & Doi, 2002; Matsuo et al., 2000) whereas an arabinosidase from *Aspergillus niger* is not active on arabinoxylans (Rombouts et al., 1988). To date two structures of GH51 α-L-arabinofuranosidases from *Geobacillus stearothermophilus* (Hövel et al., 2003) and from *Clostridium thermocellum* (Taylor et al., 2006) have been published. Both structures revealed that GH51 arabinofuranosidases are composed of a catalytic domain characterized by a (β/α)8 barrel and a C-terminal domain of unknown function that has a jelly-roll topology.

The thermophilic bacterium *Thermobacillus xylanilyticus* produces several hemicellulolytic enzymes, including two endoxylanases (one from GH10 and the other from GH11) and a GH51 arabinofuranosidase (AbfD3). To develop enzymatic strategies for the solubilisation of wheat bran arabinoxylans the action of the two xylanases has been investigated. Alone, the GH11 xylanase can solubilise 49% of arabinoxylans whereas the GH10 xylanase releases 25.5% (Beaugrand et al., 2004). In both cases, arabinose substitution was identified as a limiting factor. Previous studies concerning the AbfD3 indicated that this accessory enzyme was active on arabinoxylans from wheat flour, larchwood and oat spelt (Debeche, Cummings, Connerton, Debeire, & O'Donohue, 2000). The aim of the present work was to evaluate the activity of AbfD3 on wheat bran as well as the substrate specificity of AbfD3 on a range of wheat bran-derived substrates. To characterize AbfD3 action on polymeric substrates, two arabinoxylan populations exhibiting different arabinose to xylose ratios (0.23 and 1.22) were used. Furthermore, AbfD3 activity on oligomers was measured using arabino-xylooligosaccharides with or without substituted arabinose residue. Rates of hydrolysis and kinetic parameters were determined and compared to those obtained using paranitrophenyl α-L-arabinofuranoside.

2. Experimental

2.1. Arabinofuranosidase (AbfD3)

The gene encoding AbfD3 was expressed in *Escherichia coli* cells using a pT7-based expression vector and purified as previously described (Debeche et al., 2000).

The specific activity of AbfD3 was determined by continuous measurement of paranitrophenol (*p*NP) release from paranitrophenyl α-L-arabinofuranoside (*p*NP-Ara*f*). Reactions were performed in buffered conditions (50 mM sodium acetate buffer, pH 5.8) with *p*NP-Ara*f* (5 mM) and 0.1 mL of enzyme solution. The total reaction volume was 1 mL. Reactions were incubated at 60 °C and *p*NP release was monitored spectrophotometrically at 401 nm. One unit of activity was defined as the amount of enzyme releasing one μmol of *p*NP per min.

For determination of kinetic parameters, AbfD3 was incubated at 0.1 IU/mL with *p*NP-Ara*f* at concentrations varying from 0.01 to 10 mM. Measurements were performed in triplicate and K_M values were determined using Lineweaver–Burk analysis.

2.2. Wheat bran and polymeric substrates

Destarched wheat bran (DWB) was provided by ARD (Pomacle, France). Arabinoxylans were alkali-extracted from destarched wheat bran according to the method of Zinbo and Timell (1965) adapted by Beaugrand et al. (2004). Two fractions of xylans were used for further investigations: water-insoluble xylan (WI-AX) and water and EtOH (50% v/v)-soluble xylan (WSS-AX).

2.3. Oligomeric substrates

Xylo-oligosaccharides substituted with arabinose: *O*-β-D-xylopyranosyl-(1→4)-*O*-α-L-arabinofuranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranose (X4A) and *O*-β-D-xylopyranosyl-(1→4)-*O*-[5-*O*-(feruloyl)-α-L-arabinofuranosyl-(1→3)]-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranose (X4AFe) were obtained after hydrolysis of destarched wheat bran (30 g/L) with an endoxylanase (10 IU/mL) at 60 °C in water as previously described (Benamrouche, Crônier, Debeire, & Chabbert, 2002). The oligosaccharide products were separated by size-exclusion chromatography on two BioGel P-2 columns (170 × 1.5 cm) connected in series with water as eluent (flow rate of 20 mL/h). The structures of the X4A and X4AFe fractions were determined by ^{1}H NMR. The spectra obtained compared favourably with published data (Hoffmann, Leeflang, de Barse, Kamerling, & Vliegenthart, 1991; Lequart, Nuzillard, Kurek, & Debeire, 1999).

2.4. Analytical and structural characterization of hemicelluloses

The neutral sugar composition of wheat bran, extracted arabinoxylans and soluble arabinoxylo-oligosaccharides, X4A and X4AFe as well as the elucidation of structural features of extracted arabinoxylans were determined as previously described (Beaugrand et al., 2004).

The phenolic acids content of the different fractions (30–40 mg of samples) was estimated according the method described by Beaugrand, Crônier, Debeire, and Chabbert (2004).

Molecular characterization of WI-AX and WSS-AX was performed using a multi-detector high performance size exclusion chromatographic (HPSEC) system, connected on-line to a UV (Waters 2996), a refractive index (RI) (Waters 410) and a multi-angle laser light scattering (MALLS) detectors (Dawn MALLS ; 632.8 nm ; Wyatt corporation). A mixture of dimethyl sulfoxide (DMSO/water, 90:10) containing 50 mM of LiBr was used as eluent. WI-AX and WSS-AX samples were dissolved in the eluent on a temperature-controlled shaker for 1 h at 95 °C at a concentration of 3 mg/mL, than filtered on 0.45 μm PTFE filter (Saake, Kruse, & Puls, 2001). Chromatographic separation of injected solutions (200 μL) was achieved on a thermostatically controlled (50 °C) Shodex KD (802, 804, 806 M) (each 8 × 300 mm) column used at a flow rate of 0.5 mL/min. The MALLS photometer was calibrated with toluene and the responses of the detectors at different angles were normalized to the 90° detector signal using low molecular weight monodisperse pullulan (Shodex standard). On-line data collection and post-collection data treatment (calculation of the molecular weights, radii of gyration and distributions and averages were performed using Astra for Windows 4.73 (Wyatt technology, Santa Barbara, CA). The recovery rate after SEC analysis obtained from the ratio of the eluted mass (determined from the RI signal and the known dn/dc value) to the injected mass was approximately 65% and revealed the significance of the results (Ebringerova, Hromadkova, Burchard, & Vorwerg, 1994; Huglin, 1972).

2.5. Hydrolysis of the wheat bran, arabinoxylans and arabinoxylo-oligosaccharides with AbfD3

Hydrolysis experiments were performed in 50 mM sodium acetate buffer (pH 5.8) at 60 °C with continuous stirring. In order to determine maximal rates of hydrolysis, destarched wheat bran, WI-AX and WSS-AX (all at 4 g/L) were independently incubated with AbfD3 (10 IU/mL). Similarly, X4A and X4AFe (5 mM) were incubated with AbfD3 at 1 IU/mL.

The kinetic parameters of the AbfD3-mediated hydrolysis of various substrates were determined using initial rate conditions. For this, appropriate substrate concentration ranges and enzyme concentrations were chosen: X4A (0.1–8 g/L and 0.15 IU/mL); WI-AX (2–50 g/L and 1 UI/mL); WSS-AX (2–350 g/L and 1 UI/mL). After pre-incubation of the substrates at 60 °C, AbfD3 was added and regular intervals aliquots were removed during a time course of 10 min. Aliquots were boiled for 15 min to inactivate AbfD3, centrifuged, filtered. Released arabinose was quantified by HPAEC analysis (description Section 2.4). Values for the Michaelis constant K_M were determined using Lineweaver and Burk analysis. Due to the heterogenous nature of WI-AX and WSS-AX, only apparent values ($K_{M\ app}$) could be derived.

3. Results and discussion

3.1. Polymeric substrate analysis

The aim of this work was to evaluate the hydrolytic ability of AbfD3 on complex substrates that display different chemical compositions, arabinose to xylose ratios and water solubility. To perform the study, two different arabinoxylan populations were prepared according to their differential water solubility.

Table 1 presents the neutral sugar analysis for destarched wheat bran, WI-AX and WSS-AX. The major sugars in destarched wheat bran sample were xylose, arabinose and glucose residues. With regard to the latter, since the wheat bran is destarched, it is probable that it is derived

Table 1
Neutral sugar composition of destarched wheat bran and arabinoxylans extracted from wheat bran

Substrates	Xyl[a]	Ara[a]	Glc[a]	Gal[a]	Ara/Xyl
DWB	54.3	35.7	9.9	0.09	0.65
WI-AX	71.8	16.6	7.9	0.6	0.23
WSS-AX	41.6	50.8	2.2	3.7	1.22

[a] Expressed as percentage of total neutral carbohydrates.

from cellulose and β-glucans. Analysis of the arabinoxylans revealed two very different profiles. The arabinose content in WI-AX was much lower than that in WSS-AX, which was also higher than that of destarched wheat bran. This gave provided a range of arabinose to xylose ratios in which destarched wheat bran displays an intermediate value.

Quantification of phenolic acids in alkaline-extracted AX indicated that they were present as non significant residual components (data not shown).

Methylation analysis of WI-AX and WSS-AX revealed that these fractions presented various levels and different patterns of arabinose substitution (Table 2). In WI-AX and WSS-AX the xylan backbones were comprised of 78.3% and 12.4% unsubstituted xylose, respectively. With regard to substituted xylose residues, in WI-AX these were exclusively 2- or 3-monosubstituted (10.1%), whereas in WSS-AX both 2- or 3-monosubstituted (11.9%) and 2,3-disubstituted (23.9%) xylosyl groups were detected. In addition, in WSS-AX a significant proportion of substituent arabinosyl groups were themselves 2- or 3-linked probably to arabinose (10.8%). Finally, WSS-AX contained a high proportion of terminal xylosyl groups (12.4%). This probably reflects a lower average DP for this fraction compared to WI-AX.

The macromolecular characterization of AX fractions using HPSEC provided absolute molecular weight (M_w) and root mean square radii of gyration (R_g) for each AX sample. The elution curves obtained are depicted in Fig. 1. They show both RI and LS signals with the corresponding molecular weight distribution of WI-AX and WSS-AX, respectively. The peaks corresponding to the AX polymers are almost symmetrical and are well separated from other peaks that correspond to impurities. At lower elution volumes (25–30 mL), a slight upward curvature is observed in the M_w calibration curves (particularly for WI-AX sample). This is probably due to aggregation, whereas the curvature observed at 40–45 mL may be due to reduced column resolution near the void volume (Wyatt, 1993). Over the whole separation range, no significant UV signal (data not shown) was detected confirming that phenolic acids are either absent, or are minor components.

Table 2
Methylation analysis data of arabinoxylans extracted from wheat bran

Alditol acetate[a]	Mode of linkage	Relative mol %	
		WI-AX	WSS-AX
2,3,4-Me$_3$-Xyl	Xyl*p*-(1→	1.1	12.4
2,3-Me$_2$-Xyl	→4)-Xyl*p*-(1→	78.3	12.4
2- and 3-Me-Xyl	→2,4)-Xyl*p*-(1→ →3,4)-Xyl*p*-(1→	10.1	11.9
Xyl	→2,3,4)-Xyl*p*-(1→		23.9
2,3,5-Me$_3$-Ara	Ara*f*-(1→	10.5	28.6
3,5-Me$_2$-Ara	→2)-Ara*f*-(1→		5.4
2,5-Me$_2$-Ara	→3)-Ara*f*-(1→		5.4

Only the data concerning arabinose and xylose residues are shown.
[a] 2,3,4-Me$_3$-Xyl: 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-xylitol, etc.

The macromolecular characteristics of AX fractions are summarized in Table 3. WI-AX displays a M_w of 306,000 g/mol, compared to 207,500 g/mol for WSS-AX. Their polydispersity indexes are lower than 2. The slight greater polydispersity index of WSS-AX (1.65) compared to WI-AX (1.32) can be attributed to a heterogeneity in the distribution pattern of the side chains along the xylan backbone. Such substitutions may also contribute to a compaction size effect of WSS-AX chains and thus to a smaller radius of gyration (22 nm) compared to the more extended WI-AX chains (37.5 nm).

3.2. Hydrolytic efficiency of AbfD3 on arabino-xylooligosaccharides and arabinoxylans

Table 4 presents the results of AbfD3-mediated hydrolysis of the three analyzed samples, destarched wheat bran, WI-AX and WSS-AX. On destarched wheat bran, AbfD3 was almost inactive since only a small amount (1%) of arabinose was detected. The hydrolytic activity of AbfD3 on extracted, fractionated arabinoxylans was more efficient since 34.6% and 11% of arabinose residues were liberated from WI-AX and WSS-AX, respectively, after 48 h of hydrolysis. With regard to the kinetic parameters of these reactions (Table 4), the catalytic rates on both WSS-AX and WI-AX were similar, but the apparent K_M value for WSS-AX was significantly higher. Importantly, the relative insolubility of WI-AX meant that the determination of kinetic parameters was an experimentally delicate task and thus the values should be considered with precaution. Furthermore, the high structural complexity of extracted xylans implies various potential sites of hydrolysis that all module the kinetic measurements. Kinetic parameters on wheat arabinoxylans have already been studied with GH51 arabinosidases from *Pseudomonas cellulosa* (Beylot, McKie, Voragen, Doeswijk-Voragen, & Gilbert, 2001) and *Clostridium thermocellum* (Taylor et al., 2006). Results obtained with these arabinosidases indicated that their catalytic efficiencies were approximately 8- and 32-fold lower with wheat arabinoxylans than with *p*NP-Ara*f*. In case of AbfD3, the difference of catalytic efficiencies between polymeric arabinoxylans and artificial substrate was more important since ratios k_{cat}/K_M were, respectively, 240- and 681-fold lower in presence of WI-AX and WSS-AX compared to *p*NP-Ara*f*. These observations could probably be attributed to the different arabinoxylans used during experiments but also to topography of the active sites of arabinosidases. Detailed structural analyses of the arabi-

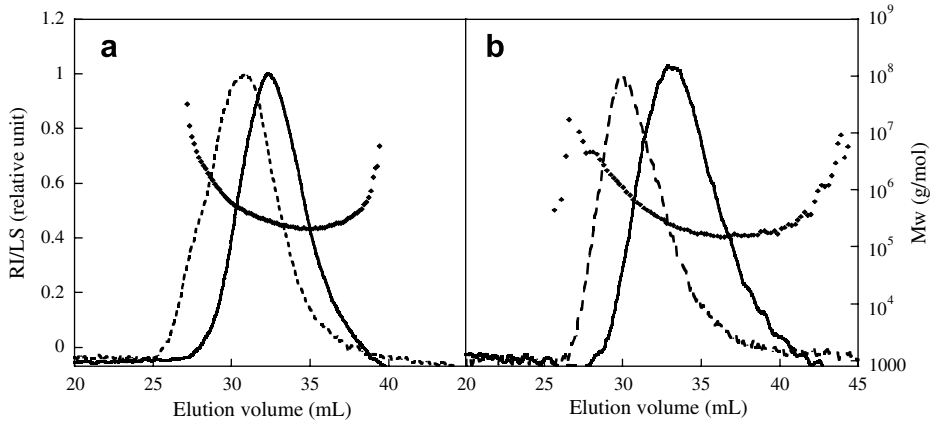

Fig. 1. SEC elution profiles and M_w distribution curves of AX fractions: (a) WI-AX and (b) WSS-AX.

Table 3
Macromolecular features of AX fractions determined by HPSEC/MALLS

AX-fraction	M_w (g/mol)	$P = M_w/M_n$	R_g (nm)
WI-AX	306,000	1.32	37.5
WSS-AX	207,500	1.65	22

M_w: average molecular weight, $P = M_w/M_n$: polydispersity index, R_g: radius of gyration.

Table 4
Hydrolysis of poly- and oligomeric substrates with AbfD3

Time (h)	% Arabinose released				
	DWB	WI-AX	WSS-AX	X4A	X4AFe
1	1	–	–	100	nd
24	1	30	10	100	nd
48	1	34.6	11	100	nd

nd: none detected.

nosidase GH51 from *C. thermocellum* revealed that the active centre is a funnel-shaped canyon with a +1 subsite allowing binding of arabinose-substituted xylans (Taylor et al., 2006). Crystal structure resolution of AbfD3 (to be published) would give more explanations about the substrate specificity of this enzyme (Table 5).

The use of X4A and X4AFe as potential substrates in AbfD3-mediated hydrolyses demonstrated that only the former can actually be hydrolyzed (Table 4). The inability of AbfD3 to hydrolyse the osidic bond between feruloylated arabinose and xylose was also verified using ^{1}H NMR analysis (data not shown). Upon incubation with AbfD3, no alterations in the spectra of X4AFe were observable, even after prolonged time periods. The AbfD3-mediated hydrolysis of X4A was rapid and the kinetic parameters of this reaction compared very favourably with those for AbfD3-mediated hydrolysis of *p*NP-Araf.

Table 5
Kinetic constants of AbfD3 in presence of poly- and oligomeric substrates

Substrate	$K_{M\ app}$ (g/L)	k_{cat} (s^{-1})	$k_{cat}/K_{M\ app}$ (g/L/s)
*p*NP-Ara*f*	0.94 ± 0.12	314.21 ± 8.84	334.27
X4A	1.51 ± 0.03	355.91 ± 11.43	235.50
WI-AX	5.14 ± 0.86	7.16 ± 0.81	1.39
WSS-AX	22.98 ± 0.21	11.45 ± 1.61	0.49

Data result from triplicate assays.

In the light of our present data and some previous results, AbfD3 appears to be a classical case of an arabinosidase that does easily fit into any of the categories previously defined (Beldman et al., 1997; Kaji, 1984). Principally, AbfD3 is active against small substrates and arabinosic linkages in oligosaccharides. However, AbfD3 is relatively active upon arabinoxylans. In contrast, in a previous study we have shown that AbfD3 is not active upon arabinogalactans (Debeche et al., 2000). With regards to linkage specificity, the present results indicate that AbfD3 is active on the 1,3-linkage in X4A whereas previous work has revealed that in AbfD3-mediated transglycosylation reactions using *p*NP-Ara*f* and benzyl-D-xylopyranose as the donor and acceptor, respectively, a 1,2-linkage is formed (Rémond et al., 2004). Therefore, taken together data suggest that AbfD3 is active on both types of bond. However, with regards to hydrolysis it will be necessary to verify this supposition using an appropriate arabinose-containing oligosaccharide.

Results obtained in this study, notably the inactivity of AbfD3 on wheat bran, clearly indicate that this enzyme is not an accessory enzyme for enhancing xylanase action in biomass conversion. Interestingly, we have shown that AbfD3 can act on polysaccharides. Therefore, its inactivity on destarched wheat bran indicates an accessibility problem. Previously, it has been suggested that the pore radius, estimated for wheat cell-walls to be in the range 1.5–4 nm (Chesson, Gardner, & Wood, 1997), might be a limiting factor for the penetration of some enzymes. For AbfD3, this could well be the case since analysis of the crystal data (to be published) using the CRYSOL computation method (Svergun, Barberato, & Koch, 1995) indicates that in solution the monomer (M_w of 56 kDa) would have a radius of at least 4 nm. However, our data also suggest that other factors may play a role in limiting AbfD3 activity. First, not all of the arabinose on monosubstituted xylosyl residues in WI-AX was released. This could be due either to the heterogeneous distribution of the substitutions along the xylan

backbone and/or to the relative insolubility of this AX fraction, which is accentuated during the course of the reaction due to arabinose removal. It has already been suggested that the activity of structurally related GH10 xylanases on arabinoxylans is inversely related to substrate solubility. Similarly, it is known that GH11 xylanases cannot function correctly on densely branched zones in arabinoxylans. With regard to WSS-AX, one limiting factor is probably the inability of AbfD3 to release arabinose from disubstituted xylose. This is not surprising because only a few specialized arabinosidases are known to act on such residues (Van Laere, Beldman, & Voragen, 1997). Furthermore, substituted arabinose residues, probably with arabinose, could prevent AbfD3 activity. Finally, the presence of ferulic acid also appears to prevent the action of AbfD3, since X4AFe was not hydrolyzed. To date, only one report has described an arabinosidase from *Aspergillus awamori* that possess the ability to remove feruloyl- or *p*-coumaroyl substituted arabinose from wheat straw arabinoxylans (Wood & McCrae, 1996). However, it is not known whether this enzyme belongs to GH51 or another family of the glycoside hydrolase classification system.

In conclusion, AbfD3 although possesses some potentially useful physicochemical characteristics (thermostability, robustness, etc.), its limited activity on polymers *in muro* makes it unsuitable for use in lignocellulose conversion strategies. However, given that AbfD3 is weakly active on polymers it might be possible to enhance this activity using protein engineering strategies. Likewise, the creation of an excellent enzymatic tool can be envisaged.

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