Available online at www.sciencedirect.com
SCIENCE DIRECT®
Carbohydrate Research 338 (2003) 1469–1476
CARBOHYDRATE RESEARCH
www.elsevier.com/locate/carres

Substrate specificity of the α-L-arabinofuranosidase from *Rhizomucor pusillus* HHT-1

A.K.M. Shofiqur Rahman,[a] Koji Kato,[b] Shingo Kawai,[c] Kazuhiro Takamizawa[a,*]

[a] *Department of Bioprocessing, Faculty of Agriculture, Gifu University, Yanagido 1-1, Gifu 501-1193, Japan*
[b] *Department of Food Science, Faculty of Agriculture, Gifu University, Gifu, Japan*
[c] *Department of Applied Bioorganic Chemistry, Faculty of Agriculture, Gifu University, Gifu, Japan*

Received 1 November 2002; accepted 18 April 2003

Abstract

The α-L-arabinofuranosidase (AF) from the fungus *Rhizomucor pusillus* HHT-1 released arabinose at appreciable rates from (1→5)-α-L-arabinofuranooligosaccharides, sugar beet arabinan and debranched arabinan. This enzyme preferentially hydrolyzed the terminal arabinofuranosyl residue [α-(1→5)-linked] of the arabinan backbone rather than the arabinosyl side chain [α-(1→3)-linked residues]. The enzyme-hydrolyzed arabinan reacted at and debranched the arabinan almost at the same rate, and the degree of conversion for both cases was 65%. Methylation analysis of arabinan showed that the arabinosyl-linkage proportions were 2:2:2:1, respectively, for (1→5)-Ara*f*, T-Ara*f*, (1→3, 5)-Ara*f* and (1→3)-Ara*f*, while the ratios for the AF-digested arabinan shifted to 3:1:2:1. Enzyme digestion resulted in an increase in the proportion of (1→5)-linked arabinose and a decrease in the proportion of terminal arabinose indicated this AF cleaved the terminal arabinosyl residue of the arabinan back bone [α-(1→5)-linked residues]. Peak assignments in the ^{13}C NMR spectra also confirmed this linkage composition of four kinds of arabinose residues. Both ^{1}H and ^{13}C NMR spectra are dominated by signals of the α-anomeric configuration of the arabinofuranosyl moieties. No signals were recorded for arabinopyranosyl moieties in the NMR spectra. Methylation and NMR analysis of native and AF-digested arabinan revealed that this α-L-arabinofuranosidase can only hydrolyse α-L-arabinofuranosyl residues of arabinan.

Keywords: α-L-Arabinofuranosidase; *Rhizomucor pusillus*; Substrate specificity; Arabinan; Methylation analysis; NMR spectroscopy

1. Introduction

Lignocellulosic biomass is a constant source of sustainable and renewable energy in Nature. It is comprised of about 23% lignin, 40% cellulose, and 33% hemicellulose.[1] The monomers of various lignocellulosic agricultural and industrial wastes are useful in the production of different antibiotics, alcohols, animal feed, chemicals and fuels. L-Arabinosyl residues are one of the monomers and common components of some lignocellulosic plant polysaccharides. Arabinose is present at high concentrations in arabinans, arabinoxylans and arabinogalactan. The arabinan and arabinoxylan backbones are comprised of α-(1→5)-linked arabinofuranose units and β-(1→4)-linked xylopyranose moieties, respectively. Both polysaccharides are decorated at C-2 and/or C-3 with arabinofuranose molecules as side chains.[2,3] These side chains may restrict the enzymic hydrolysis of respected polysaccharides. The α-L-arabinofuranosidase (exo-(1→5)-α-L-arabinofuranosidase, AF, EC.3.2.1.55) cleaves the arabinose side chains, allowing endo-arabinase (endo-(1→5)-α-L-arabinofuranosidase, EC 3.2.1.99) to attack the arabinan backbone.[4,5] These enzymes act synergistically in degrading branched arabinan to generate L-arabinose.[6]

α-L-Arabinofuranosidase, purified for the first time by Kaji and coworkers,[7] has proven to be an essential enzyme for the production of arabinose. Since then, there have been many studies on AF. However, the substrate specificity of the enzyme has not been studied well in comparison with other glycosidases. Recently, there is interest growing for L-arabinose as a possible

* Corresponding author. Tel./fax: +81-58-2932906.
E-mail address: tak2003@cc.gifu-u.ac.jp (K. Takamizawa).

0008-6215/03/$ - see front matter © 2003 Elsevier Science Ltd. All rights reserved.
doi:10.1016/S0008-6215(03)00203-9

food additive, due to its low uptake by the body and its sweet taste. It has been proved that L-arabinose selectively inhibits intestinal sucrase in a competitive manner and reduces the glycemic response after sucrose ingestion in animals.[8] Based on these findings, L-arabinose can be used as a physiologically functional sugar that inhibits sucrose digestion. It has also been reported that in mice, L-arabinose dose-dependently suppressed the increase of blood glucose after the ingestion of sucrose. Furthermore, L-arabinose delays and reduces the digestion, absorption, and net energy derived from sucrose when both are ingested simultaneously.[9] In this way, L-arabinose is useful in preventing postprandial hyperglycemia in diabetic patients when foods containing sucrose are ingested. Effective L-arabinose production is therefore important in the food industry. To achieve this goal, it is necessary to characterize α-L-arabinofuranosidase by using defined oligosaccharides, which is a powerful method for understanding the substrate specificity. In fact, AF in combination with xylanases is being used for the structural elucidation and bioconversion of hemicelluloses to biologically useful pentose sugars. These types of synergism studies were also reported in our previous works.[10–13]

The novel α-L-arabinofuranosidase (AF) from *Rhizomucor pusillus* HHT-1 was purified and characterized in our previous work,[14] but the specificity of the enzyme towards arabinofuranose-containing oligosaccharides was not studied. Therefore, we investigated the substrate specificity of AF produced by *R. pusillus* HHT-1, to develop an effective process of L-arabinose production from some plant polysaccharides.

2. Experimental

2.1. Enzyme and substrates

Extracellular α-L-arabinofuranosidase from *R. pusillus* HHT-1 was purified as described in our previous article[14] and used throughout this study. The α-L-arabinofuranosidase activity was determined by the following method: the reaction mixture, consisting of 180 μL of 2.5 mM *p*-nitrophenyl α-L-arabinofuranoside (dissolved in 50 mM citrate buffer, pH 5.0) and 20 μL of enzyme solution, was incubated at 50 °C for 20 min, followed by the addition of 100 μL of 1 M Na_2CO_3. Absorbances were read at 414 nm with a microtitre plate reader (Immuno Mini NJ-2300, System Instrument Co., Tokyo, Japan). One unit of enzyme activity was defined as the amount of enzyme required to liberate 1.0 μmol of *p*-nitrophenol from the substrate per minute under the assay conditions and expressed as units per milliliter. *p*-Nitrophenyl α-L-arabinofuranoside was purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Sugar beet arabinan, debranched arabinan and (1→5)-α-L-arabinofuranooligosaccharides were obtained from Megazyme pty., Ltd. (North Rocks, Australia).

2.2. Gas–liquid chromatography (GLC)

GLC was carried out on a Shimadzu GC-18A apparatus equipped with a flame-ionization detector. A capillary column of CBP-1-M25-025 (0.25 mm × 25 m) was used and operated at 220 °C with gas flow rate of 60 mL/min of nitrogen. Peak areas were measured with a Shimadzu Chromatocorder-21.

2.3. Infrared (IR) spectroscopy

IR spectra were recorded with a Perkin–Elmer 2000 FTIR spectrometer system.

2.4. Gas chromatography–mass spectrometry (GC–MS)

GC–MS was conducted with a Shimadzu GC–MS QP-5000 apparatus with a class 5000 mass data system. A methyl silicon DB-1 capillary column (30 m × 0.25 mm × 1 μm, J&W Scientific) was used. The column temperature was programmed first at 150 °C for 2 min, and then raised to 250 °C at 5 °C/min. The spectra were recorded at an ionizing potential of 70 eV.

2.5. High-performance liquid chromatography (HPLC)

HPLC was performed using a JASCO apparatus (880-PU pump, 860-CO column oven, and 830-RI detector). A Wakobeads T-130-E (7.8 × 300 mm) column was used and operated at 60 °C with a flow rate of Milli Q water at 0.7 mL/min. The peak areas were estimated with an SIC Chromatocorder 11.

2.6. Effect of α-L-arabinofuranosidase (AF) on hydrolysis of (1→5)-α-L-arabinofuranooligosaccharides

The reaction mixture contained 0.5 mL of AF solution with different concentrations (1 U, 3 U, and 5 U/mL), 0.4 mL of 20 mM sodium phosphate buffer (pH 4.0) and 0.1 mL of 10% (1→5)-α-L-arabinofuranobiose, (1→5)-α-L-arabinofuranotriose, and (1→5)-α-L-arabinofuranotetraose, respectively. After 40 min incubation at 50 °C, the reaction mixture was heated in boiling water for 10 min to stop the reaction. The reaction mixture was then centrifuged and filtered using the 0.2-μm pore size syringe-driven filter unit (Millipore Corporation, Bedford, USA). Ten micro-liter of filtered reaction mixture was analyzed by HPLC for the characterization of hydrolysis products.

2.7. Time course of hydrolysis of (1→5)-α-L-arabinofuranotetraose by AF

The reaction mixture contained 0.5 mL of AF solution (1 U/mL), 0.4 mL of 20 mM sodium phosphate buffer (pH 4.0) and 0.1 mL of 10% (1→5)-α-L-arabinofuranotetraose. After 0, 10, 20, 30, 40, 50, and 60 min incubation at 50 °C, the reaction mixtures were heated in boiling water for 10 min to stop the reaction. Ten microliters of filtered reaction mixture were analyzed by HPLC for the characterization of the hydrolysis products.

2.8. Time course of hydrolysis of arabinan and debranched arabinan

For the time course of hydrolysis of sugar beet arabinan (88:3:2:7 L-arabinose–galactose–rhamnose–galacturonic acid) and debranched arabinan (88:4:2:6 L-arabinose–galactose–rhamnose–galacturonic acid), the reaction mixture contained 0.5 mL of AF solution (5 U/mL), 0.4 mL of 20 mM sodium phosphate buffer (pH 4.0) and 0.1 mL of 10% arabinan or debranched arabinan. After 6-, 12-, 24-, 48-, 72- and 96-h incubations at 50 °C, each of the reaction mixture was heated in boiling water bath for 10 min to stop the reaction. Ten microliters of the mixture were used for HPLC for the characterization of hydrolysis products.

2.9. Effect of AF concentration on hydrolysis of arabinan and debranched arabinan

The components of reaction mixture were same as above except for the concentrations of AF used. The AF concentrations were 0.2, 0.5, 1, 2, 3, 4 and 5 U/mL. After 24-h incubations at 50 °C, each of the reaction mixture was heated in boiling water bath for 10 min to stop the reaction. Ten microliters of the mixture were used for HPLC for the characterization of hydrolysis products.

2.10. Glycosyl-linkage composition of AF-digested arabinan

In the glycosyl-linkage analysis of arabinan hydrolysates digested by AF, the reaction mixture contained 0.5 mL of AF solution (1 U/mL), 0.4 mL of 20 mM sodium phosphate buffer (pH 4.0), and 0.1 mL of 10% arabinan. After 5-h incubation at 50 °C, the mixture was heated in boiling water bath for 10 min to stop the reaction. Thereafter, the reaction mixture was dialyzed by the dialysis membrane (Wako Chemicals, USA, Inc.) against the tap water overnight to remove arabinose. Arabinan hydrolysates, after removal of arabinose, were lyophilized. Glycosyl-linkage compositions of arabinan hydrolysates were determined by methylation analysis using a modification[15] of the Hakomori procedure. The product was confirmed to show no absorption for the hydroxyl group in its IR spectrum. It was then hydrolyzed with 90% formic acid, and then with 0.25 M sulphuric acid. The partially methylated sugars thus obtained were converted to their alditol acetates for GLC and GC–MS analyses. The glycosyl-linkage compositions were then analyzed by GC–MS of the resulting partially methylated alditol acetates.[16]

2.11. NMR spectroscopy

^{1}H and ^{13}C NMR spectra were recorded at 399.956 (^{1}H) and 100.578 MHz (^{13}C) with a Varian Unity Inova 400 FT instrument. The spectra were recorded at room temperature in D_2O. Sodium 4,4-dimethyl-4-silapentanoate-2,2,3,3-d_4 (DSS) (adjusted to 0 ppm), was used as the internal standard. Chemical shifts are given in δ values. The spectra of native and AF-digested arabinan hydrolysates were interpreted on the basis of methylation analysis and literature data.

3. Results and discussion

3.1. Effect of α-L-arabinofuranosidase (AF) on hydrolysis of (1→5)-α-L-arabinofuranooligosaccharides

The hydrolysis of (1→5)-α-L-arabinofuranooligosaccharides by the AF was carried out (data not shown). AF from *R. pusillus* HHT-1 hydrolyzed the (1→5)-α-L-arabinofuranooligosaccharides completely, indicating its capability of hydrolysing the (1→5)-α-L-linked arabinosyl terminal residues.

A time course of hydrolysis of (1→5)-α-L-arabinofuranotetraose by AF was performed (Fig. 1). The AF released 100% arabinose from the oligosaccharides at 40 min.

3.2. Time course of hydrolysis of arabinan and debranched arabinan

The hydrolysis of sugar beet arabinan and debranched arabinan by AF is presented in Fig. 2. Finally, only arabinose was detected in the reaction product. Although the initial rates of arabinose release from the two substrates were almost the same, the rates of degradation after 24 h differed significantly from each other. This AF preferentially hydrolyzes terminal arabinofuranosyl residue of the arabinan backbone rather than the arabinosyl side chain. The arabinans that have been analyzed are highly branched with (1→5) links between the main chain residues, which are substituted at O-3 with single or multiple-unit side chains.[2] From the results shown in Fig. 2, it is suggested that the AF from *R. pusillus* HHT-1 has hydrolytic activity for both α-(1→3)- and α-(1→5)-linked, nonreducing, terminal L-

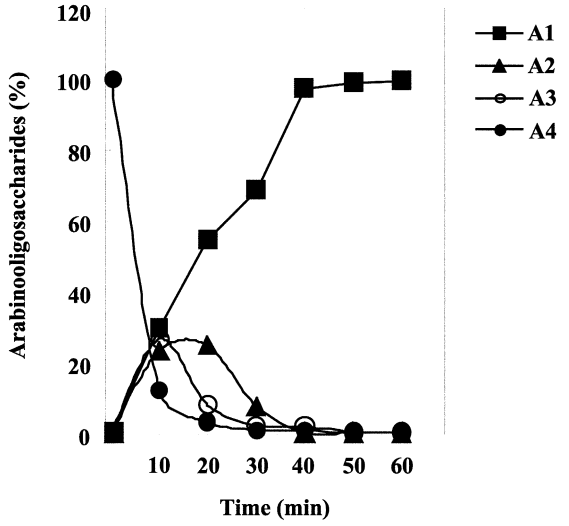


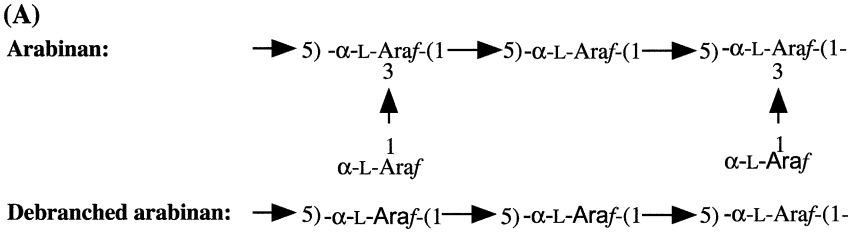
Fig. 1. Time course of hydrolysis of 1,5,- -L-arabinofuranotetraose by AF. A^1-arabinose, A^2-arabinobiose, A^3-arabinotriose, and A^4-arabinotetraose.

arabinofuranose residues. This type of enzymatic de-arabinosylation are comparable to those AFs from *Aspergillus niger*,[17,18] *Aureobasidium pullulans*,[19] *Streptomyces* sp. strain 17-1,[20] *Streptomyces diastaticus*,[21] *Bacillus subtilis* 3-6.[22] This is in contrast to AF from *A. niger*[23] and *S. purpurascens* IFO 3389,[24] which hydrolyzed either (1→5)- or (1→3)-arabinosyl linkages of arabinan.

Effect of AF concentration on hydrolysis of arabinan and debranched arabinan was studied (data not shown). The optimum concentration of AF was 1 U/mL for effective arabinose production after 24-h incubations for both cases. There were no remarkable changes observed even after prolonged incubation for 96 h and presence of the excess amount of AF in the reaction mixture. The limit of hydrolysis of arabinan and debranched arabinan by the AF from *R. pusillus* HHT-1 was almost same (65%). The range of limit of hydrolysis of arabinan by the AF reported until now is 15–100%.[17,22]

3.3. Glycosyl-linkage composition of AF-digested arabinan

GLC analysis gave several peaks (chromatograms not shown), and six of them were identified as terminal arabinofuranosyl (T-Ara*f*), terminal arabinopyranosyl (T-Ara*p*), 5-linked arabinofuranosyl (1→5-Ara*f*), (3→5)-linked arabinofuranosyl (1→3,5-Ara*f*), (2→5)-linked arabinofuranosyl (1→2,5-Ara*f*) and 3-linked arabinofuranosyl (1→3-Ara*f*) residues by their retention time and mass spectra. The relative proportions of glycosyl linkage composition are summarized in Table 1. Methylation analysis of arabinan showed that the arabinosyl-linkage proportions were 2:2:2:1, respectively, for (1→5)-Ara*f*, T-Ara*f*, (1→3,5)-Ara*f* and (1→3)-Ara*f*, while the rations for the AF-digested arabinan shifted to



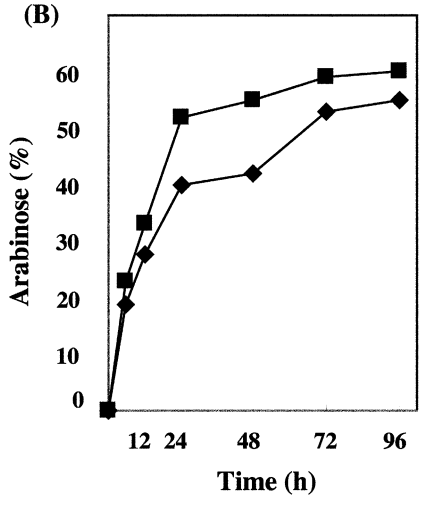


Fig. 2. (A) Primary structure of Arabinan and Debranched arabinan. (B) Time course of hydorlysis of Arabinan and Debranched arabinan by –L-AF. (AF-1U/ml at 50° C) ⊷ Arabinan, ⊷ Debranched arabinan.

Table 1
Glycosyl-linkage composition of arabinan hydrolysates by α-L-arabinofuranosidase from *R. pusillus* HHT-1

Peak	Glycosyl linkage	Arabinan [a] (native)	Arabinan treated with α-L-AFase [a]
A	[b] T-Ara*f*	24.5	13.2
B	[b] T-Ara*p*	2.2	2.1
C	→5)-Ara*f*	30.0	42.9
D	→3,5)-Ara*f*	26.1	24.2
E	→2,5)-Ara*f*	6.6	6.1
F	→3)-Ara*f*	10.6	11.5

[a] Values expressed as mol%.
[b] T = nonreducing terminal.

3:1:2:1. Enzyme digestion resulted in an increase in the relative proportion of (1→5)-linked arabinose and a decrease in the proportion of terminal arabinose. Therefore, this AF is highly specific for α-L-arabinofuranosides and cleaves the terminal arabinosyl residue of the arabinan backbone. The presence of 2,3,4-tri-*O*-methyl-L-arabinose in the methylation analysis is indicative of the occurrence of a few residues in the pyranose form. Arabinopyranose residues have been reported in many arabinans. In all instances, the proportion of the arabinopyranose residues was only a few percent of the arabinofuranose form and these residues appeared only in terminal, nonreducing positions (T-Ara*p*).[17,18,25] T-Ara*p* residues obtained in this analysis also accounted for less than 2% of total residues. It is therefore questionable as to whether the pyranose residues are really part to the polysaccharides or if they are artifacts arising during the methylation analysis. It has been demonstrated[26] that some methyl α-L-arabinopyranoside is formed on treatment of methyl α-L-arabinofuranoside with alkali. In an arabinan, the terminal arabinofuranosyl residues may be regarded as simple glycofuranosides, and consequently if any tautomeric rearrangements were to take place, it would be on the terminal residues, and not on the internal residues where the furanose ring-form is fixed by the (1→5) linkages.[27] The enzyme released arabinose by step-wise hydrolyzing the (1→5) linkage of the arabinosyl residues in the main chain starting from the nonreducing terminal end of arabinan. Similar specificity was also observed for the AFI from *Aspergillus awamori* IFO 4033[28] hydrolyzed the (1→5) linkage of the arabinosyl residues of arabino-oligosaccharides. Whereas, the AFs from *Streptomyces diastatochromogenes* 065,[29] *B. subtilis* 3-6,[22] *A. awamori* IFO 4033 AFII,[28] *Trichoderma reesei*,[25] *A. niger* 5-16,[17] *A. niger* (Megazyme),[18] *Streptomyces chartreusis* GS901[30] preferentially hydrolyzed the (1→3) linkage of the arabinosyl side chain of arabinan. This AF also released arabinose exowisely. On the other hand, enzymes that release arabinose in endo-fashion (endo-arabinase) also have been described for species of *Bacillus*,[31–35] *Aspergillus*[6,36] and *Pseudomonas*.[37]

3.4. NMR spectroscopy

^{1}H and ^{13}C NMR analyses of native and AF-digested arabinan hydrolysates were carried out. In the ^{1}H NMR spectra of native and AF-digested arabinans (data not shown), signals at 5.16 and 5.10, were assigned to anomeric protons of terminal-α-L-arabinofuranosyl and 5-linked α-L-arabinofuranosyl residues, respectively.[38] In the ^{13}C NMR spectra of native and AF-digested arabinans (Fig. 3), signals at 110.3, 109.9 and 110.3, 109.7 were assigned to anomeric carbons of α-L-arabinofuranosyl residues[38–40], respectively. α-(1→5)-Linked arabinofuranosyl units preponderate highly in native arabinan as shown by methylation analysis, thus an intense signals are expected for C-1 linked to C-5 of this residue. Examination of the ^{13}C NMR spectra of arabinan confirmed this feature. The spectrum of AF-digested arabinan showed five strong signals at δ 110.3 (C-1), 85.1 (C-4), 83.7 (C-2), 79.6 (C-3) and 69.7 (C-5-linked). These five signals correspond to the carbon atoms of the α-L-(1→5)-linked arabinofuranosyl residues which, according to the methylation analysis, are expected to be the most abundant in the arabinans. Moreover, Fig. 3 shows the ^{13}C NMR spectrum of a mixture of the four kinds of arabinose e.g., T-α-L-Ara*f*, (1→5)-α-L-Ara*f*, (1→3,5)-α-L-Ara*f* and (1→3)-α-L-Ara*f*. Based on the literature data published for spectra of arabinofuranosides,[41] arabinan,[42–45] arabinogalactan,[40,46] it was possible to identify those four residues. Peak assignments of ^{13}C NMR of these four kinds of arabinose residues are shown in Table 2. The little discrepancy of chemical shifts observed as compared to the results reported earlier,[39,40,44–46] can be due to different NMR instrument and reference systems used for recording the spectra. It may be mentioned here that number of peaks for these four kinds of residues were qualitatively similar but different in order of magnitude in respect to the proportion of arabinosyl residues of arabinan. It has been shown earlier[43,47] that the integrated intensities of signals in the ^{13}C NMR spectrum of polysaccharides permit conclusions on the relative proportion of constituent sugars in the polymer

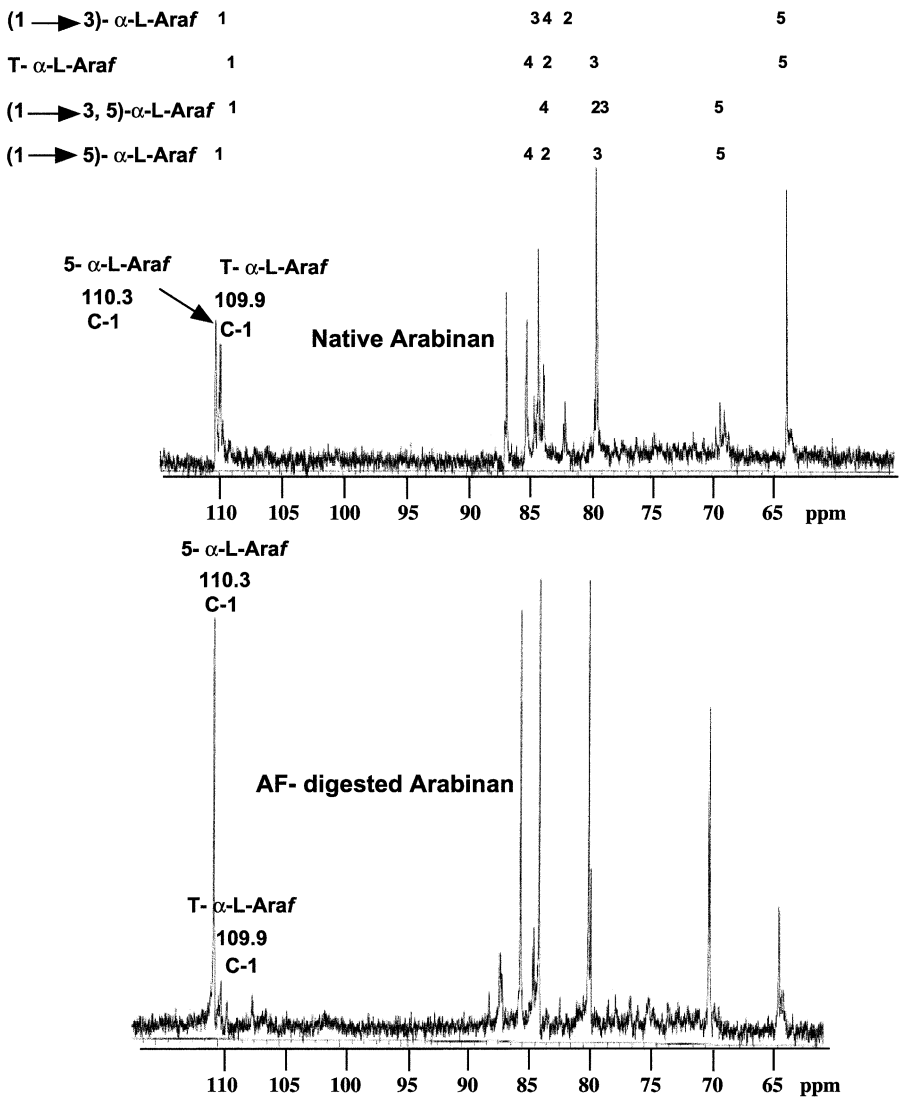


Fig. 3. ^{13}C NMR spectrum of Native and AF-digested arabinan. The numbers above each peak refer to the carbon atoms positioned across the *Ara* residues listed in the top left.

investigated. Thus, for L-arabinan (native and AF-digested arabinan), integration of the spectrum gave the tentative values showed in both spectrums for above four sugar residues, which were in good agreement with the results of methylation analysis. However, the spectra of both cases are dominated by signals of α-L-arabinofuranosyl moieties. As it was stated earlier, whether the pyranose residues are really part to the polysaccharides or if they are artifacts arising during the methylation analysis. This can be circumvented by the use of NMR spectroscopy. So, no signals were recorded for α-L-arabinopyranosyl (Ara*p*) residue in this NMR. The C-1 signal for the α-L-arabinopyranosyl residue resonated at 102–105 ppm.[41,42,45] Evidences for the absence of α-L-arabinopyranosyl residue in the tested arabinan are from the ^{13}C NMR spectrum, which did not show any signal around at 102 and 105 ppm where C-1 resonance of an α-L-arabinopyranosyl residue would be expected. It is to be noted here that ^{13}C NMR spectroscopy is particularly suitable for the structural investigation of arabinans, which exhibit very characteristic spectra, because of their α-L-furanoid constituents. As methylation analysis and NMRs depicted similar information on the structure of sugar beet arabinan, the approach presented here appears to be very effective for elucidation of the structure of any type of polysaccharides, if suitable model compounds or literature data are available.

4. Conclusions

AFs warrant substantial research efforts because they represent potential rate limiting enzymes in xylan degradation, particularly those substrates from agricultural residues such as corn fiber, corn stover and rice

Table 2
Peak assignments of ^{13}C NMR of arabinan and AF-digested arabinan

Residues	Chemical shift in δ (ppm)				
	C-1	C-2	C-3	C-4	C-5
Native arabinan					
(1→3)-α-L-Ara*f*	110.3(110.25 [c])	81.9(84.2 [c])	84.5(84.8 [c])	83.7(84.2 [c])	63.9(64.1 [c])
T-α-A-Ara*f*	109.9(110.9 [a])	83.7(83.0 [a])	79.4(78.2 [a])	85.1(85.5 [a])	63.9(62.9 [a])
(1→3,5)-α-L-Ara*f*	109.9(108.3 [b])	79.4(80.0 [b])	79.4(80.0 [b])	83.7(82.5 [b])	69.3(67.7 [b])
(1→5)-α-L-Ara*f*	110.3(110.25 [c])	83.7(84.2 [c])	79.4(79.4 [c])	85.1(84.2 [c])	69.3(72.3 [c])
AF-digested arabinan					
(1→3)-α-L-Ara*f*	110.3(110.25 [c])	79.6(84.2 [c])	84.2(84.8 [c])	83.6(84.2 [c])	63.9(64.1 [c])
T-α-A-Ara*f*	109.7(110.9 [a])	83.7(83.0 [a])	79.4(78.2 [a])	85.1(85.5 [a])	63.9(62.9 [a])
(1→3,5)-α-L-Ara*f*	109.7(108.3 [b])	79.6(80.0 [b])	79.6(80.0 [b])	83.6(82.5 [b])	69.7(67.7 [b])
(1→5)-α-L-Ara*f*	110.3(110.25 [c])	83.6(84.2 [c])	79.6(79.4 [c])	85.1(84.2 [c])	69.7(72.3 [c])

[a] Ref. 40.
[b] Ref. 45.
[c] Ref. 46.

straw.[48,49] Thus, the high activity of the AF from *R. pusillus* HHT-1 on both arabinan and debranched arabinan, its ability to release L-arabinose from arabinoxylan,[14] thermotolerancy and vital role for structural elucidation of polysaccharides make the enzyme a promising candidate in food and chemical industries for use in the production of fermentable sugars from lignocellulosic biomass such as agricultural wastes, oat-spelt xylan and to improve animal feed digestibility by hydrolyzing arabinoxylans, a major component of animal feed.[50]

Acknowledgements

The authors are grateful to Professor Makoto Shimoyamada for kindly providing the NMR facilities. The assistances of Dr Benedict C. Okeke and Md. Ali Asgar for critical reading and discussion are gratefully acknowledged.

References

1. Coughlan, M. P.; Hazlewood, G. P. *Biotechnol. Appl. Biochem.* **1993**, *17*, 259–289.
2. Bacic, A.; Harris, P. J.; Stone, B. A. In *The Biochemistry of Plants*; Preiss, J., Ed.; Vol. 14; Academic press: San Diego, 1988; pp 297–371.
3. Manin, C. F.; Shareek, R. M.; Kluepfel, D. *Biochem. J.* **1994**, *302*, 443–449.
4. Dekker, R. F. H.; Richards, G. N. *Adv. Carbohyd. Chem. Biochem.* **1976**, *32*, 277–352.
5. Kaji, A. *Adv. Carbohydr. Chem. Biochem.* **1984**, *42*, 383–397.
6. Voragen, A. G. J.; Rombouts, F. M.; Searle-van Leeuwen, M. F.; Schols, H. A.; Pilnik, W. *Food Hydrocolloids* **1987**, *1*, 423–437.
7. Kaji, A.; Tagawa, K.; Matsubara, K. *Agric. Biol. Chem.* **1967**, *31*, 1023–1028.
8. Seri, K.; Sanai, K.; Matsuo, N.; Kawakubo, K.; Xue, C.; Inoue, S. *Metabolism* **1996**, *45*, 1368–1374.
9. Sanai, K.; Seri, K.; Inoue, S. *Nihon Eiyou Syokuryougaku Kaishi* **1997**, *50*, 133–137 (in Japanese).
10. Rahman A. K. M. S.; Sugitani N.; Hatsu M.; Takamizawa K. Can. J. Microbiol. 2003, 49, 58–64.
11. Cho C. -H.; Hatsu M.; Takamizawa K. *Proceedings of Fifth International Symposium on Waste Management Problems in Agro-Industries*, 2001, Shiga-Japan, 16–18 November, pp. 235–242.
12. Cho, C.-H.; Hatsu, M.; Takamizawa, K. *The Japan Society of Waste Management Experts* **2001**, *12*, 266–274 (in Japanese).
13. Kubata, K. B.; Horitsu, H.; Takamizawa, K.; Suzuki, T.; Kawai, K. *The Japan Society of Waste Management Experts* **1994**, *5*, 29–35.
14. Rahman, A. K. M. S.; Kawamura, S.; Hatsu, M.; Hoq, M. M.; Takamizawa, K. *Can. J. Microbiol.* **2001**, *47*, 767–772.
15. Harris, P. J.; Henry, R. J.; Blakeney, A. B.; Stone, B. A. *Carbohydr. Res.* **1984**, *127*, 59–73.
16. York, W. S.; Darvill, A. G.; McNeil, M.; Albersheim, P. *Methods Enzymol.* **1985**, *118*, 3–40.
17. Kaneko, S.; Shimasaki, T.; Kusakabe, I. *Biosci. Biotechnol. Biochem.* **1993**, *57*, 1161–1165.
18. Kaneko, S.; Ishii, T.; Kobayashi, H.; Kusakabe, I. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 695–699.
19. Saha, B. C.; Bothast, R. J. *Appl. Environ. Microbiol.* **1998**, *64*, 216–220.
20. Kaji, A.; Sato, M.; Tsutsui, Y. *Agri. Biol. Chem.* **1981**, *45*, 925–931.
21. Tajana, E.; Fiechter, A.; Zimmermann, W. *Appl. Environ. Microbiol.* **1992**, *58*, 1447–1450.

22. Kaneko, S.; Sano, M.; Kusakabe, I. *Appl. Environ. Microbiol.* **1994**, *60*, 3425–3428.
23. Kaji, A.; Tagawa, K. *Biochim. Biophys. Acta* **1970**, *207*, 456–464.
24. Komae, K.; Kaji, K.; Sato, M. *Agri. Biol. Chem.* **1982**, *46*, 1899–1905.
25. Kaneko, S.; Kuno, A.; Matsuo, N.; Ishii, T.; Kobayashi, H.; Hayashi, K.; Kusakabe, I. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 2205–2210.
26. Janson, J.; Lindberg, B. *Acta Chem. Scand. Ser. B* **1982**, *36*, 277–279.
27. Joseleau, J.-P.; Chambat, G.; Lanvers, M. *Carbohydr. Res.* **1983**, *122*, 107–113.
28. Kaneko, S.; Arimoto, M.; Ohba, M.; Kobayashi, H.; Ishii, T.; Kusakabe, I. *Appl. Environ. Microbiol.* **1998**, *64*, 4021–4027.
29. Kaneko, S.; Higashi, K.; Yasui, T.; Kusakabe, I. *J. Ferment. Bioeng.* **1998**, *5*, 518–520.
30. Matsuo, N.; Kaneko, S.; Kuno, A.; Kobayashi, H.; Kusakabe, I. *Biochem. J.* **2000**, *345*, 9–15.
31. Takao, M.; Akiyama, K.; Sakai, T. *Appl. Environ. Microbiol.* **2002**, *68*, 1639–1646.
32. Kaji, A.; Saheki, T. *Biochem. Biophys. Acta* **1975**, *410*, 354–360.
33. Sakai, T.; Sakamoto, T. *Agric. Biol. Chem.* **1990**, *54*, 879–889.
34. Weinstein, L.; Albersheim, P. *Plant Physiol.* **1979**, *63*, 425–432.
35. Yoshihara, O.; Kaji, A. *Agric. Biol. Chem.* **1983**, *47*, 1935–1940.
36. Ramon, D.; van der Veen, P.; Visser, J. *FEMS Microbiol. Lett.* **1993**, *113*, 15–22.
37. McKie, V. A.; Black, G. W.; Millward-Sadler, S. J.; Hazlewood, G. P.; Laurie, J. I.; Gilbert, H. J. *Biochem. J.* **1997**, *323*, 547–555.
38. Qin, X.; Yamauchi, R.; Aizawa, K.; Inakuma, T.; Kato, K. *Carbohydr. Res.* **2001**, *333*, 79–85.
39. Saulnier, L.; Brillouet, J. M.; Moutounet, M. *Carbohydr. Res.* **1992**, *224*, 219–235.
40. Gane, A. M.; Craik, D.; Munro, S. L. A.; Howlett, G. J.; Clarke, A. E.; Bacic, A. *Carbohydr. Res.* **1995**, *277*, 67–85.
41. Akiyama, Y.; Mori, M.; Kato, K. *Agric. Biol. Chem.* **1980**, *44*, 2487–2489.
42. Joseleau, J.-P.; Chambat, G.; Vignon, M.; Barnoud, F. *Carbohydr. Res.* **1977**, *58*, 165–175.
43. Capek, P.; Toman, R.; Kardosova, A.; Rosik, J. *Carbohydr. Res.* **1983**, *117*, 133–140.
44. Swamy, N. R.; Salimath, P. V. *Phytochemistry* **1991**, *30*, 263–265.
45. Cardoso, S. M.; Silva, A. M. S.; Coimbra, M. A. *Carbohydr. Res.* **2002**, *337*, 917–924.
46. Qin, X.; Yamauchi, R.; Aizawa, K.; Inakuma, T.; Kato, K. *J. Appl. Glycosci.* **2000**, *47*, 155–161.
47. Shaskov, A. S.; Chizhov, O. S. *Bioorg. Khim.* **1976**, 437–496.
48. Saha, B. C. *Biotechnol. Adv.* **2000**, *18*, 403–423.
49. Saha, B. C.; Bothast, R. J. *Appl. Biochem. Biotechnol.* **1999**, *76*, 65–77.
50. Campbell, G. L.; Bedford, M. R. *Can. J. Anim. Sci.* **1992**, *72*, 449–466.