Available online at www.sciencedirect.com
ScienceDirect
Carbohydrate Polymers 72 (2008) 424–430
Carbohydrate Polymers
www.elsevier.com/locate/carbpol

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

C. Rémond [a,*], I. Boukari [a], G. Chambat [b], M. O'Donohue [c]

[a] *Laboratoire de Technologie Enzymatique et Physico-chimie des Agroressources, UMR URCA/INRA FARE, 8, rue Gabriel Voisin, BP 316, F51688 Reims Cedex 2, France*
[b] *Laboratoire associé à l'Université Joseph Fourier, CERMAV-CNRS, BP53, F38041 Grenoble, France*
[c] *Laboratoire Biocatalyse, INSA/INRA UMR 792, 135, avenue de Rangueil, F31077 Toulouse Cedex 04, France*

Received 18 June 2007; received in revised form 6 September 2007; accepted 11 September 2007
Available online 19 September 2007

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.
© 2007 Elsevier Ltd. All rights reserved.

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

* Corresponding author. Tel.: +33 3 26 35 53 63; fax: +33 3 26 35 53 69.
E-mail address: caroline.remond@univ-reims.fr (C. Rémond).

0144-8617/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2007.09.008

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.

Download English Version:

https://daneshyari.com/en/article/1386487

Download Persian Version:

https://daneshyari.com/article/1386487

Daneshyari.com