Carbohydrate Polymers 74 (2008) 579–589

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Assignment of the ^{1}H and ^{13}C NMR spectra of 2-aminobenzamide-labeled xylo-oligosaccharides

Tadashi Ishii [a,*], Tomoyuki Konishi [a], Hiroshi Ono [b], Mayumi Ohnishi-Kameyama [b], Hidetoshi Togashi [c], Kazumasa Shimizu [c]

[a] Forestry and Forest Products Research Institute, Wood Chemistry, Matsunosato 1, Tsukuba, Ibaraki 305-8687, Japan
[b] National Food Research Institute, Tsukuba, Ibaraki 305-8642, Japan
[c] College of Bioresource Sciences, Nihon University, Fujisawa, Kanagawa 252-6510, Japan

ARTICLE INFO

Article history:
Received 31 December 2007
Received in revised form 2 April 2008
Accepted 7 April 2008
Available online 16 April 2008

Keywords:
2-Aminobenzamide
Fluorescent labeling
Glucuronoxylan
NMR
Xylan
Xylo-oligosaccharides

ABSTRACT

Xylose, xylo-oligosaccharides with a degree of polymerization (DP) from 2 to 6, xylo-oligosaccharides carrying a single arabinofuranosyl, glucosyluronic acid, or 4-*O*-methyl glucosyluronic acid residues and 4-β-D-Xyl*p*-(1 → 4)-D-Xyl*p*-(1 → 3)-α-L-Rha*p*-(1 → 2)-α-D-Gal*p*A-(1 → 4)-D-Xyl*p* were labeled at their reducing ends with 2-aminobenzamide (2AB) in the presence of sodium cyanoborohydride ($NaBH_3CN$). These derivatives were then analyzed by high-performance anion-exchange chromatography (HPAEC) and structurally characterized by electrospray-ionization mass spectrometry (ESI-MS) and by ^{1}H and ^{13}C NMR spectroscopy. Reacting Xyl_3-Xylitol-2AB with UDP-Xyl in the presence of rice microsomes resulted in the formation of small amounts of Xyl_{4-6}-Xylitol-2AB showing that the 2AB-labeled compound is an acceptor for xylosyltransferase. The 2AB-labeled xylo-oligosaccharides and the 2AB-labeled xylo-oligosaccharides carrying 4-*O*-methyl glucuronic acid are fragmented by xylanase and α-glucuronidase present in the culture filtrate of *Fomitopsis palustris* FFPRI 0507. Thus, the 2AB-labeled xylo-oligosaccharides are useful for studying enzymes involved in xylan degradation and biosynthesis.

1. Introduction

Glucuronoxylans (GXs) are quantitatively major components of the secondary cell walls of woody plant tissues. GX has a linear backbone composed of 1 → 4-linked β-D-xylosyl (Xyl) residues, some of which are substituted at *O*-2 with a single α-D-glucosyluronic acid (GlcA) or 4-*O*-methyl-α-D-glucosyluronic acid (MeGlcA) residue. The Xyl residues may also be substituted with α-L-arabinofuranosyl (Ara*f*) and *O*-acetyl residues (O'Neill and York, 2003; Shimizu, 1991). The presence and distribution of these substituents in xylans is dependent on the plant source and may affect their physico-chemical properties (Ebringerová, Hromádková, & Heinze, 2005; Izydorczyk & Biliaderis, 1995). Xylo-oligosaccharides have been reported to enhance the growth of bifidobacteria (Pepper & Olinger, 1988) and are defined as prebiotics (Fooks, Fuller, & Gibson, 1999; Modler, 1994). Recently, there has been increased interest in the structure and biosynthesis of xylans in hardwoods and grasses as these polysaccharides are major component of plant biomass, a renewable energy source that has potential for use in the large scale production of biofuels (Ragauskas et al., 2006; Somerville, 2007).

A number of glycosyltransferases (GTs) are required for biosynthesis of the xylan backbone, together with enzymes that add and modify side chains (Peña et al., 2007). Several genes have been identified that are believed to encode some of these enzymes. For example, analysis of *Arabidopsis* mutants has revealed that *FRAGILE FIBER8 (FRA8), IRREGULAR XYLEM8 (IRX8)*, and *IRX9*, *PARVUS*, *and IRX14* are required for the synthesis of normal amounts of xylan and cellulose in secondary cell walls and for the formation of normal vascular tissue morphology (Brown, Zeef, Ellis, Goodacre, & Turner, 2005; Brown et al., 2007; Persson, Wei, Milne, Page, & Somerville, 2005; Persson et al., 2007; Peña et al., 2007; Zhong et al., 2005). However, none of these genes have been functionally characterized nor have any of the enzymes involved in xylan biosynthesis been purified to homogeneity and biochemically characterized.

Fluorescent-labeled oligosaccharides are useful acceptor substrates for *in vitro* determination of GTs and glycosyl hydrolase activities. We previously reported the NMR assignment of 2-aminobenzaminated (2AB) pectic oligosaccharides (Ishii, Ichita, Matsue, Ono, & Maeda, 2002) and galacto- and arabino-oligosaccharides (Ishii, Ono, & Maeda, 2005). In this study, we will provide a complete assignment of signals in ^{1}H and ^{13}C NMR spectra of 2AB-labeled xylo-oligosaccharides and show usefulness of the oligosaccharides for detection of xylosyltransferase (XylT) activity, xylanase and α-glucuronidase activity.

* Corresponding author.
E-mail address: tishii@ffpri.affrc.go.jp (T. Ishii).

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doi:10.1016/j.carbpol.2008.04.018

1 Xylitol-2AB
R

2 β-D-Xyl*p*-(1→4)-D-Xylitol-2AB
T R

3 β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-D-Xylitol-2AB
T A R

4 β-D-Xyl*p*-(1→4)- β-D-Xyl*p*-(1→4)- β-D-Xyl*p*-(1→4)-D-Xylitol-2AB
T E A R

5 β-D-Xyl*p*-(1→4)- β-D-Xyl*p*-(1→4)- β-D-Xyl*p*-(1→4)- β-D-Xyl*p*-(1→4)-D-Xylitol-2AB
T E B A R

6 β-D-Xyl*p*-(1→4)- β-D-Xyl*p*-(1→4)- β-D-Xyl*p*-(1→4)- β-D-Xyl*p*-(1→4)-β-D-Xyl*p*-(1→4)-D-Xylitol-2AB
T E I B A R

7 α-L-Ara*f*-(1→2)-β-D-Xyl*p*-(1→4)-D-Xylitol-2AB
T E R

8 4-*O*-Me-α-D-Glc*p*A-(1→2)- D-Xylitol-2AB
T R

9 α-D-Glc*p*A-(1→2)- D-Xylitol-2AB
T R

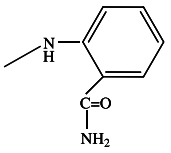
10 4-*O*-Me-α-D-Glc*p*A-(1→2)-β-D-Xyl*p*-(1→4)-D-Xylitol-2AB
T A R

11 α-D-Glc*p*A-(1→2)-β-D-Xyl*p*-(1→4)-D-Xylitol-2AB
T A R

12 4-*O*-Me-α-D-Glc*p*A-(1→2)-β-D-Xyl*p*-(1→4)- β-D-Xyl*p*-(1→4)-D-Xylitol-2AB
T B A R

13 α-D-Glc*p*A-(1→2)-β-D-Xyl*p*-(1→4)- β-D-Xyl*p*-(1→4)-D-Xylitol-2AB
T B A R

14 β-D-Xyl*p*-(1→4)- β-D-Xyl*p*-(1→3)- α-L-Rha*p*-(1→2)- α-D-Gal*p*A-(1→4)-D-Xylitol-2AB
T E B A R



2-aminobenzamide (2AB)

Fig. 1. Structures of compounds **1**–**14**. **1**–**3**, 2-AB-labeled D-xylitol to xylotriose (DP 1–3); **4**, xylotetraose consisting of a D-xylitol R, two internal residues (A and E), and a non-reducing terminal residue T; **5**, xylopentaose consisting of a D-xylitol R, three internal residues (A, B, and E), and a non-reducing terminal residue T; **6**, xylohexaose consisting of a D-xylitol R, four internal residues (A, B, I, and E), and a non-reducing terminal residue T; **7**, trisaccharide consisting of a D-xylitol R, one internal xylosyl residue A and a non-reducing terminal Ara*f* residue T; **8**, disaccharide consisting of a D-xylitol R, and a terminal 4-*O*-Me-Glc*p*A T; **9**, disaccharide consisting of a D-xylitol R, and a terminal Glc*p*A T; **10**, trisaccharide consisting of a D-xylitol R, an internal Xyl residue A, and a terminal 4-*O*-Me-Glc*p*A T; **11**, trisaccharide consisting of a D-xylitol R, an internal Xyl residue A, and a terminal Glc*p*A T; **12**, tetrasaccharide consisting of a D-xylitol R, internal Xyl residues (A and B), and a terminal 4-*O*-Me-Glc*p*A T; **13**, tetrasaccharide consisting of a D-xylitol R, internal Xyl residues (A and B), and a terminal Glc*p*A T; **14**, pentasaccharide consisting of a D-xylitol R, internal Gal*p*A, Rha, and Xyl residues (A, B, and E, respectively) and a non-reducing terminal xylosyl residue T.

2. Experimental

2.1. Material

2AB and $NaBH_3CN$ were purchased from Aldrich Chemical Co. (Milwaukee, WI, USA). Other chemicals were purchased from Wako Pure Chemicals (Osaka, Japan). 1,4-Linked β-D-xylo-oligosaccharides with a degree of polymerization (DP) of 2–6 and xylanase were obtained from Megazyme (Wicklow, Ireland). UDP-Xyl was obtained from Carbosource Services (Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA). 1,4-Linked β-D-xylo-oligosaccharides (DP 1–3) with a single GlcA or MeGlcA residues were generated from kenaf (*Hibiscus cannabinus*) xylan (Komiyama, Kato, Aimi, Ogihara, & Shimizu, 2008). Briefly, the xylan was extracted with aq. 10% KOH from bast and cores of kenaf and then hydrolyzed with 2 M trifluoroacetic acid (TFA) at 120 °C for 1 h. The hydrolyzates were kept

Table 1
Electrospray-ionization mass spectrometry data for compounds **1**–**14**

Nominal mass	Molecular ion	Compound[a]	Molecular weight
293	$(M+Na)^+$	**1**	270
425	$(M+Na)^+$	**2**	402
557	$(M+Na)^+$	**3**	534
689	$(M+Na)^+$	**4**	666
821	$(M+Na)^+$	**5**	798
953	$(M+Na)^+$	**6**	930
557	$(M+Na)^+$	**7**	534
483	$(M+Na)^+$	**8**	460
469	$(M+Na)^+$	**9**	446
615	$(M+Na)^+$	**10**	592
601	$(M+Na)^+$	**11**	578
747	$(M+Na)^+$	**12**	724
733	$(M+Na)^+$	**13**	710
879	$(M+Na)^+$	**14**	856

[a] Structures shown in Fig. 1.