ISSN 0006-2979, Biochemistry (Moscow), 2012, Vol. 77, No. 4, pp. 395-403. © Pleiades Publishing, Ltd., 2012.
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Glucuronoarabinoxylan Extracted by Treatment with Endoxylanase from Different Zones of Growing Maize Root

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Received November 16, 2011
Revision received December 30, 2011

Abstract—Glucuronoarabinoxylan is a key tethering glucan in the primary cell wall of cereals. Glucuronoarabinoxylan was extracted from different zones of maize (*Zea mays* L.) roots using endoxylanase that specifically cleaves β-(1,4)-glycoside bond between two consequent unsubstituted xylose residues. Changes in polysaccharide structure during elongation growth were characterized. Glucuronoarabinoxylan extractable after the endoxylanase treatment consisted of high molecular weight (30-400 kDa) and low molecular weight (<10 kDa) fractions. The presence of high molecular weight derivatives indicated that part of the natural glucuronoarabinoxylan is not digestible by the endoxylanase. This could be due to the revealed peculiar structural features, such as high level of substitution of xylose, absence of unsubstituted xylose residues existing in sequence, and significant degree of acetylation. In maize root meristem the indigestible fraction was 98% of the total extracted glucuronoarabinoxylan. This portion decreases to 47% during elongation. Also, the average molecular weight of indigestible glucuronoarabinoxylan reduced twofold. These changes in the ratio of glucuronoarabinoxylan fragments with different structure during root cell growth could reflect a transition of polysaccharide from its separating (highly substituted indigestible glucuronoarabinoxylan) form to that binding to cellulose microfibrils or other glucuronoarabinoxylan molecules and, hence, retarding growth.

DOI: 10.1134/S0006297912040116

Key words: glucuronoarabinoxylan, endoxylanase, cell wall, elongation, growth, *Zea mays*

Unlike animals, whose growth occurs due to multiple cell divisions, plants grow primarily due to many fold increase in size of individual cells. This process is largely determined by the properties of the cell wall [1].

Maize (and other cereal) tissues have type II cell walls. Unlike the type I cell wall, they contain a small amount of pectin, and their major hemicelluloses are a mixed linkage glucan and glucuronoarabinoxylan (the weight fraction of the latter in primary cell walls is 20-40% [2]).

The structure of glucuronoarabinoxylan from different sources can differ, however, in the way the polysaccharide backbone is composed of β-D-xylopyranoside residues linked with the (1,4)-bond. Some xylose residues carry substituents, such as α-L-arabinofuranose, α-D-glucopyranosyluronic acid, and acetyl group [3]. In a review published in 2010, Faik gave several evidences for regularity of arabinose substitution along the glucuronoarabinoxylan backbone: four unsubstituted xylose residues in the backbone are separated by two substituted at O-3 and/or O-2 positions [4]. Either coumaric or ferulic acid can be linked via ester bonds with some arabinose residues at C-5 position [5]. Moreover, arabinose can be additionally substituted with β-D-xylopyranose at position 2 [4] or by α-L-arabinofuranose at position 3 [6]. The backbone xylose residues are substituted with α-D-glucopyranosyluronic acid or its 4-O-methyl derivative at O-2 or O-3 position ~10-fold less often than with arabinose [2]. The backbone substitution with uronic acids is also thought to be regular [7]. Simultaneous substitution of xylose residue with arabinose and glucuronic acid has not been reported. Acetyl groups are also attached to xylose residues at positions O-2 or O-3 or, to a lesser extent, at both O-2 and O-3 [4] (Fig. 1).

Several studies were carried out on changes of glucuronoarabinoxylan during growth [8-10]. The key feature is decrease in substitution degree of xylan backbone with arabinose determined from decrease in Ara/Xyl ratio among monosaccharide products of hydrolysis of the

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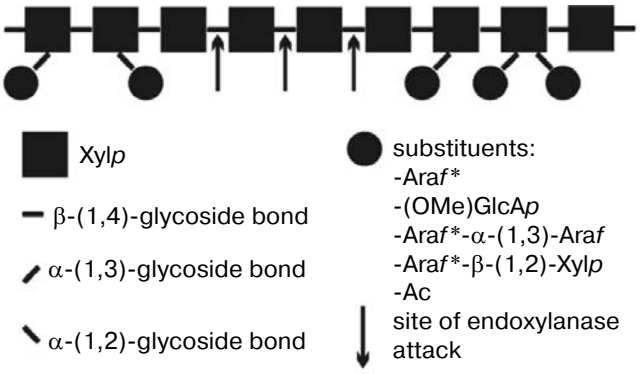

Fig. 1. Structure of cereal glucuronoarabinoxylan and sites of endoxylanase attack. Xyl*p*, xylopyranose; Ara*f*, arabinofuranose; GlcA, glucuronic acid; Me, methyl group; Ac, acetyl group; * ferulic or *p*-coumaric bound at C-5 position.

polymer. The majority of studies were carried out on above-ground organs, particularly plantlet coleoptiles, of various cereals [8-10], which are characterized by a relatively slow rate of cell elongation [11]. To make a detailed study on alterations in cell wall polysaccharide structure during growth, it is reasonable to use growing plant roots, in which different cell growth stages are spatially separated and cell growth rate in the elongation zone is many fold greater than that in coleoptile. In the presented work we studied composition and structure of glucuronoarabinoxylan in root zones differing in cell developmental stage, primarily focusing on the stage of cell elongation.

MATERIALS AND METHODS

Plant material. The 4-day-old roots of maize (*Zea mays* L., cultivar Intercras 375) were grown in the dark at 27°C. The primary root – following amputation of the root cap – was dissected into zones according to the following scheme: meristem (0-1 mm), early elongation zone (1-2 mm), middle elongation zone (2-6 mm), late elongation zone (6-7 mm), and post-elongation zone (7-11 mm). This scheme was developed given the data of the literature [11] and our experiments using marking the roots with ink followed by cytological examination of different zones [12]. For biochemical analysis, separated root cutoffs were fixed by means of boiling in 70% ethanol for 10 min, dried in an airflow at 60°C, and weighed. The experiments were done in triplicate, each repeat combining cutoffs of 450 roots. For NMR analysis of glucuronoarabinoxylan fragments, the studied maize root part was not sectioned; apical 11-mm cutoffs of 200 roots lacking caps were used instead.

Isolation of glucuronoarabinoxylan. Glucuronoarabinoxylan (in the form of poly- and oligomer fragments) was prepared according to the method of Kato and Nevins [13], which comprises treatment of plant material with specific endoxylanase following preliminary homogenization of cells in a mortar and removal of starch (pretreatment with amylase) and mixed linkage glucan (pretreatment with lichenase). The products of hydrolysis with amylase and lichenase did not contain xylose [12].

To isolate glucuronoarabinoxylan, the pretreated plant material was incubated in 10 mM NaOAc buffer, pH 6.5, containing NaN_3 (0.02%) and 10 μg/ml suspension of endoxylanase M6 (EC 3.2.1.8) (Megazyme, Ireland) that hydrolyzes polysaccharide backbone fragment containing two consecutive unsubstituted xylose residues (Fig. 1). Hydrolysis was carried out at 40°C for 24 h. The non-hydrolyzed material was centrifuged at 10,000*g* for 10 min. The supernatant contained glucuronoarabinoxylan fragments released in the course of hydrolysis. The treatment with the enzyme was repeated three times. The supernatants were boiled for inactivation of the enzyme followed by its removal by centrifugation at 10,000*g* for 10 min, pooled, and dried in an airflow at 60°C.

Chromatography of glucuronoarabinoxylan fragments and calculation of their molecular weights. Carbohydrates prepared by treatment with endoxylanase were chromatographed on a column (10 × 1000 mm; total volume 78.5 ml, void volume 29 ml) filled with TSK-65F (Tosoh Bioscience, Japan). The sorbed fractions were eluted with NaOAc buffer, pH 6.0, containing 0.02% NaN_3 at a flow rate of 0.24 ml/min (fraction volume 1 ml). The amount of carbohydrate was determined in each fraction by the method of Dubois [14]. Fractions containing carbohydrates were pooled, each peak separately. The pooled fractions were desalinated on Dowex 50W×8 cation-exchange resin (Sigma, USA) (exchange Na→H), and pH was adjusted to neutral with pyridine. Acetic acid (a result of cation exchange) and pyridine were evaporated in an airflow at 60°C.

Before use, the column for gel permeation chromatography was calibrated using the following carbohydrate standards: dextran, 2000 kDa (Sigma); pullulans, 410 kDa (P-400), 273 kDa (P-200), and 48 kDa (P-50) (Waters, USA); glucose, 0.18 kDa (Sigma). The plot of polysaccharide molecular weight (y) vs. elution volume (x) for the column was described by the exponential equation:

$$y = 128671e^{-0.1491x}.$$

This equation was solved for all x values from 29 through 78 ml. So, the theoretical molecular weights were determined for each 1-ml eluted fraction. The mean molecular weight of polysaccharides in a peak was calculated as a quotient of the sum of partial carbohydrate amounts and total carbohydrate amount in the peak, according to the equation:

$$Mn = \Sigma m_i/\Sigma(m_i/M_i),$$

where *Mn* is the mean molecular weight; m_i is amount of carbohydrate in the *i*-th milliliter; and M_i is calculated molecular weight of polysaccharide in this 1-ml fraction.

Analysis of monosaccharide composition of carbohydrates. Twenty micrograms of analyzed carbohydrates were dissolved in 40 μl of 2 M trifluoroacetic acid (TFA) at 120°C for 1 h. Hydrolysis products were analyzed by anion-exchange HPLC on a CarboPac PA-1 column (4 × 250 mm; Dionex, USA) using a PAD pulse amperometric detector (Dionex). Eluents: A – 0.015 M NaOH; B – 1 M NaOAc in 0.1 M NaOH. Column temperature was 30°C. Elution rate was 1 ml/min. The gradient elution was carried out as follows: 0-20 min – 100% A; 20-21 min – linear gradient until the A/B ratio = 90 : 10 (%); 21-31 min – linear gradient until the A/B ratio = 70 : 30 (%); 31-32 min – linear gradient until the A/B ratio 0 : 100 (%); 32-42 min – 100% B; 42-43 min – until the A/B ratio = 100 : 0 (%); and 43-73 min – washing with 100% A. Monosaccharide contents were quantitative estimated in three analytical repeats using the PeakNet software.

Estimation of glucuronoarabinoxylan extraction degree. The efficacy of glucuronoarabinoxylan extraction from root tissues was estimated from the ratio (in percent) of xylose extracted from cell walls by treatment with endoxylanase to the overall amount of xylose (extracted and remaining in the cell wall) in the specimen. The latter was determined by hydrolysis of remaining material with 2 M TFA (2 ml/5 mg material) at 120°C for 1 h. The xylose content in the hydrolysis product was quantified using anion-exchange HPLC as described above. Mass of cell walls after their isolation was determined by the method of Talmadge [15].

Data were statistically processed using Microsoft Excel. Bars on diagrams and ± in values correspond to the standard deviations.

NMR analysis of glucuronoarabinoxylan high molecular weight fragment structures. High molecular weight fragments of glucuronoarabinoxylan were dissolved in D_2O (99.996% D). ^{1}H-NMR spectra were recorded on an Avance III 600 MHz NMR spectrometer (Bruker, Germany) at 303°K. Deuterated acetone (acetone-d6; 2.05 ppm) was used as an internal standard. Wheat flour arabinoxylan (Megazyme, Ireland) was used for correlation of characteristic chemical shifts.

RESULTS

Yield and monosaccharide composition of carbohydrates extracted from plant material by treatment with endoxylanase. The yield of glucuronoarabinoxylan from plant material treated with endoxylanase was determined from total carbohydrate in the resulting fractions. The portion of extracted glucuronoarabinoxylan in cell wall mass increased from 1.1 ± 0% in meristem to 3.8 ± 0.4% in late elongation zone and remained at the same level in the post-elongation zone (Fig. 2). The overall portion of arabinose, xylose, and glucuronic acid in all root zones was about 90% of the total amount of extracted monosaccharide (Table 1), which confirms specificity of endoxylanase activity. However, Ara/Xyl decreased from meristem (0.76 ± 0.02) to the post-elongation zone (0.50 ± 0.02).

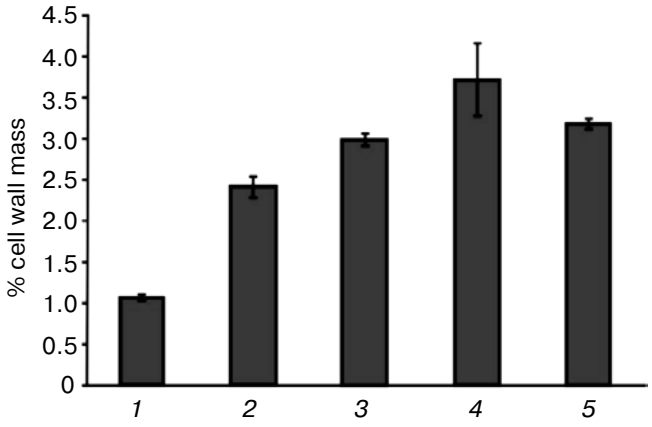

Fig. 2. Portions of glucuronoarabinoxylan (% cell wall mass) extracted by treatment with endoxylanase in different maize root zones: *1*) meristem; *2*) early elongation zone (*); *3*) middle elongation zone (*); *4*) late elongation zone; *5*) post-elongation zone. * Difference from previous variant is significant ($p < 0.05$).

Mass distribution of carbohydrates extracted from plant material by treatment with endoxylanase. Elution profiles of carbohydrates extracted from all root zones always contained two peaks. The first, denoted as high molecular weight glucuronoarabinoxylan, contained fragments ranging within 30 through 400 kDa. This means that some part of glucuronoarabinoxylan was extracted but not hydrolyzed by endoxylanase. The second peak denoted as low molecular weight glucuronoarabinoxylan contained carbohydrate fragments with molecular weights below 10 kDa (Fig. 3). Commercial wheat endosperm arabinoxylan (Megazyme) (Ara/Xyl = 0.58 ± 0) taken as a control was completely hydrolyzed by the endoxylanase to oligosaccharide fragments (data not shown).

Xylose and arabinose predominated in both peaks of carbohydrates extracted from root zones (Table 2). The Ara/Xyl ratio in the high molecular weight glucuronoarabinoxylan was 0.69-0.77 for different root zones (Table 2); the difference between zones is insignificant. The same index was significantly lower in the low molecular weight fractions (0.34-0.46). A significant decrease in the Ara/Xyl ratio was observed from meristem to the post-elongation zone (Table 2).

The ratio between high and low molecular weight peaks differed in specimens isolated from different maize root zones. Carbohydrates extracted from meristem were mainly high molecular weight ones. In the following root

Table 1. Monosaccharide composition of carbohydrates extracted from different maize root zones by treatment with endoxylanase, mole %

Monosaccharide	Meristem	Early elongation zone	Middle elongation zone	Late elongation zone	Post-elongation zone
Rha	0.6 ± 0.0	0.7 ± 0.0	0.7 ± 0.0	0.6 ± 0.0	0.6 ± 0.0
Ara	38.0 ± 0.6	34.8 ± 0.8	30.7 ± 0.2	30.2 ± 0.6	27.9 ± 0.7
Gal	3.7 ± 0.0	5.0 ± 0.1	7.5 ± 0.0	5.9 ± 0.1	5.6 ± 0.1
Glc	3.0 ± 0.0	3.9 ± 0.1	1.9 ± 0.0	2.2 ± 0.0	2.1 ± 0.1
Xyl	49.6 ± 0.9	48.6 ± 1.2	52.7 ± 0.4	53.5 ± 1.1	55.8 ± 1.5
GalA	2.0 ± 0.0	3.8 ± 0.1	3.8 ± 0.0	4.0 ± 0.0	3.7 ± 0.1
GlcA	3.1 ± 0.0	3.2 ± 0.1	2.7 ± 0.0	3.6 ± 0.1	4.3 ± 0.1
Ara + Xyl + GlcA	90.7 ± 1.5	86.6 ± 2.1	86.1 ± 0.6	90.4 ± 1.8	88.0 ± 2.3
Ara/Xyl	0.76 ± 0.02	0.71 ± 0.03	0.58 ± 0.01	0.56 ± 0.02	0.50 ± 0.02
GlcA/Xyl	0.06 ± 0.00	0.07 ± 0.01	0.05 ± 0.00	0.07 ± 0.01	0.08 ± 0.02

Table 2. Monosaccharide composition of high and low molecular weight carbohydrate fractions extracted from different zones of maize root by treatment with endoxylanase, mole %

Monosaccharide	High molecular weight fraction					Low molecular weight fraction				
	meristem	early elongation zone	middle elongation zone	late elongation zone	post-elongation zone	meristem	early elongation zone	middle elongation zone	late elongation zone	post-elongation zone
Rha	0.6 ± 0.0	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	1.2 ± 0.1	–	–	–	–	–
Ara	38.4 ± 1.4	37.0 ± 0.5	34.3 ± 0.9	35.3 ± 0.8	33.7 ± 1.2	20.3 ± 0.8	22.0 ± 0.6	21.7 ± 0.6	21.6 ± 1.1	21.5 ± 0.7
Gal	3.6 ± 0.4	5.0 ± 0.7	8.5 ± 0.7	7.6 ± 0.6	7.8 ± 0.8	7.0 ± 0.9	4.7 ± 0.7	5.2 ± 0.6	2.8 ± 0.7	3.0 ± 0.7
Glc	2.6 ± 0.2	1.4 ± 0.2	0.7 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	21.5 ± 1.6	18.1 ± 1	4.9 ± 0.5	4.5 ± 1	3.4 ± 0.5
Xyl	49.7 ± 1.6	48.3 ± 1.1	49.7 ± 0.8	47.4 ± 1.2	48.8 ± 0.8	44.5 ± 0.8	50.2 ± 0.7	60.3 ± 1.2	6.0 ± 1.5	63.7 ± 2
GalA	2.0 ± 0.1	4.3 ± 0.3	3.3 ± 0.4	4.3 ± 0.4	4.4 ± 0.4	4.0 ± 0.7	1.3 ± 0.5	4.9 ± 0.5	3.5 ± 0.5	3.0 ± 0.3
GlcA	3.1 ± 0.1	3.1 ± 0.6	2.5 ± 0.2	3.5 ± 0.5	3.2 ± 0.3	2.7 ± 0.3	3.7 ± 0.5	3.0 ± 0.2	3.6 ± 0.1	5.4 ± 0.3
Ara + Xyl + GlcA	91.2 ± 3.1	88.4 ± 2.2	86.5 ± 1.9	86.2 ± 2.5	85.7 ± 2.3	67.5 ± 2.7	75.9 ± 1.8	85.0 ± 2.0	89.2 ± 2.7	90.6 ± 3.0
Ara/Xyl	0.77 ± 0.08	0.77 ± 0.08	0.69 ± 0.03	0.74 ± 0.03	0.69 ± 0.06	0.46 ± 0.01	0.45 ± 0.08	0.35 ± 0.03	0.34 ± 0.02	0.34 ± 0.03
GlcA/Xyl	0.06 ± 0.02	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	0.06 ± 0.01	0.07 ± 0.00	0.05 ± 0.01	0.06 ± 0.01	0.08 ± 0.02

Note: "–", not found.

zones the portion of high molecular weight component decreased and was only half of the extracted carbohydrates in the post-elongation zone (Fig. 3).

The mean molecular weight of high molecular weight carbohydrates decreased with elongation. While the mean molecular weight of the polysaccharides extracted from meristem was 137 ± 12 kDa, this index was 64 ± 1 kDa in the late elongation zone, and a similar value (66 ± 4 kDa) was determined in the post-elongation zone (Fig. 4).

Xylose level in analyzed cell wall fractions. Total amount of xylose in maize root cell walls increased with cell development from 1 through 5% cell wall mass. About a half this amount comprised glucuronoarabinoxylan

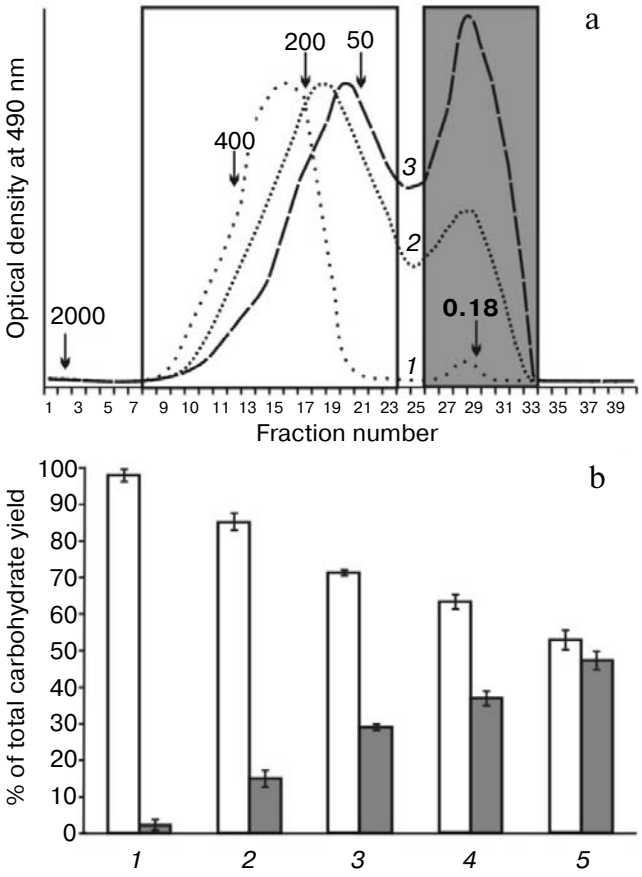

Fig. 3. Ratio between high and low molecular weight components of carbohydrates extracted from different maize root zones by treatment with endoxylanase. a) Elution profiles: *1*) meristem; *2*) elongation zone; *3*) post-elongation zone. High molecular weight glucuronoarabinoxylan is drawn in white; low molecular weight glucuronoarabinoxylan is in gray. The data for early and late elongation zones are not shown because of their intermediate character. Chromatograms are aligned to the height of the high molecular weight peak. Arrows with figures (kDa) indicate elution volumes for corresponding carbohydrate standards. b) Carbohydrate portions (% total yield) in high (white) and low molecular weight (gray) peaks: *1*) meristem; *2*) early elongation zone (*); *3*) middle elongation zone (*); *4*) late elongation zone; *5*) post-elongation zone. * Difference from previous variant is significant ($p < 0.05$).

extracted by treatment with endoxylanase. This index was somewhat lower only in the post-elongation zone (Fig. 5). Xylose content in the high molecular weight component of glucuronoarabinoxylan was maximal in the elongating cells (1.2 ± 0% in early, 1.2 ± 0% in middle, and 1.3 ± 0.1% in late elongation zone). Both meristem and the post-elongation zone were characterized by significantly lower values of this index: 0.6 ± 0% and 0.9 ± 0%, respectively. In the course of root cell development the portion of xylose increased in the low molecular weight component of glucuronoarabinoxylan extracted by treatment with endoxylanase. The level of xylose that remained in cell walls after the enzymatic extraction also increased (Fig. 5).

NMR analysis of structure of high molecular weight glucuronoarabinoxylan fraction. Distinct elements of high molecular weight glucuronoarabinoxylan structure were established using ^{1}H-NMR. The high molecular weight fragments were taken that were extracted from the first 11 mm of maize root (without separation into zones) by hydrolysis with endoxylanase. For relating characteristic proton signals and revealing structural features providing inefficiency of hydrolysis by endoxylanase, we compared ^{1}H-NMR spectra of maize high molecular weight glucuronoarabinoxylan and wheat flour high molecular weight arabinoxylan that is completely hydrolyzed by endoxylanase to oligosaccharide fragments. The monosaccharide composition of the polymers examined by ^{1}H-NMR is presented in the Table 3.

^{1}H-NMR spectra of analyzed polysaccharides were comparable to those described earlier for arabinoxylans [16-20], which allowed accurate correlation of the main chemical shifts. The signals predominating in ^{1}H-NMR spectra of both polysaccharides corresponded to arabinose bound to xylose at position O-3 in the absence of O-2 substitution with arabinose (5.8 (H-1), 4.15 (H-2), 3.90 (H-3), 4.25 (H-4), 3.71 and 3.78 (H-5ax/eq) ppm) (Fig. 6). Integral values of signals within 5.2-5.4 ppm, which correspond to H-1 of α-L-terminal Ara*f* bound with Xyl*p* at position O-3 (5.38 ppm) and H-1 of α-L-terminal Ara*f* bound with disubstituted Xyl*p* residue at positions O-3 and O-2 (5.27 and 5.22 ppm; two equally intensive signals) (Fig. 6), suggested that a portion of the arabinose comprising side chains of disubstituted xylose residue was 57% in wheat flour arabinoxylan and 27% in maize root high molecular weight glucuronoarabinoxylan. Combination of these data with monosaccharide composition of the polymers (Table 3) reveals the ratio between substituted and non-substituted backbone xylose.

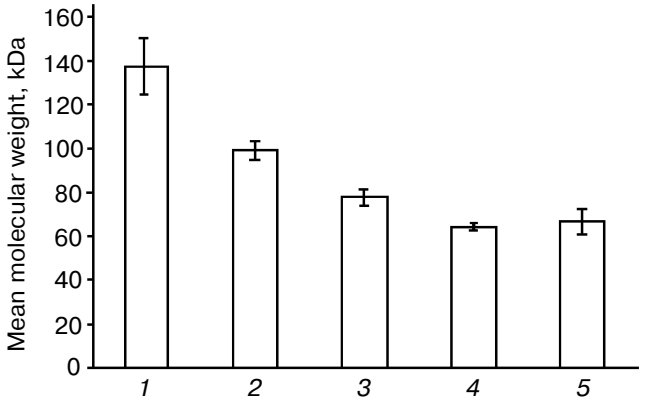

Fig. 4. Mean molecular weights (Mn) of high molecular weight glucuronoarabinoxylan extracted by treatment with endoxylanase from different maize root zones: *1*) meristem; *2*) early elongation zone (*); *3*) middle elongation zone (*); *4*) late elongation zone (*); *5*) post-elongation zone. * Significant difference from previous variant ($p < 0.05$).

Suppose that all arabinose comprised one-residual side chains, then the portion of non-substituted xylose residue was 58% in the wheat flour arabinoxylan backbone and the portion of xylose substituted with arabinose was 42%, including 17% substituted with two arabinose moieties (at positions O-2 and O-3) and 25% with one moiety (O-3). The portion of non-substituted backbone xylose in maize root high molecular weight glucuronoarabinoxylan was 36% and the portion of xylose substituted with arabinose was 64%, including 10% disubstituted and 54% monosubstituted.

The spectrum of maize root high molecular weight glucuronoarabinoxylan (Fig. 6b) contains intense signals that are either absent in the spectrum of wheat flour arabinoxylan (Fig. 6a) or have other multiplicity (3.28, 3.44, 3.55, 3.64, 4.09, and 1.31 ppm). Given the difference in monosaccharide composition of the polymers (Table 3), we suppose that these signals are related to protons of 4-O-methylglucuronic acid and rhamnose (in particular, the signal 1.31 ppm belonging to methyl protons), as well as glucose. Intense signals within 2.15-2.55 ppm, which are absent in the spectrum of wheat flour arabinoxylan, suggest possible acetylation of maize root high molecular weight glucuronoarabinoxylan.

Table 3. Monosaccharide composition of arabinoxylans examined by ^{1}H-NMR, mole %

Monosaccharide	High molecular weight glucurono-arabinoxylan from maize roots	Arabinoxylan from wheat flour
Rha	3.5 ± 0.0	–
Ara	35.8 ± 0.0	36.7 ± 0.0
Glc	9.3 ± 0.1	–
Xyl	48.5 ± 0.1	63.0 ± 0.0
GlcA	3.0 ± 0.2	0.3 ± 0.0
Ara + Xyl + GlcA	87.2 ± 0.3	100.0 ± 0.0
Ara/Xyl	0.74 ± 0.01	0.58 ± 0.00
GlcA/Xyl	0.06 ± 0.01	0.01 ± 0.00

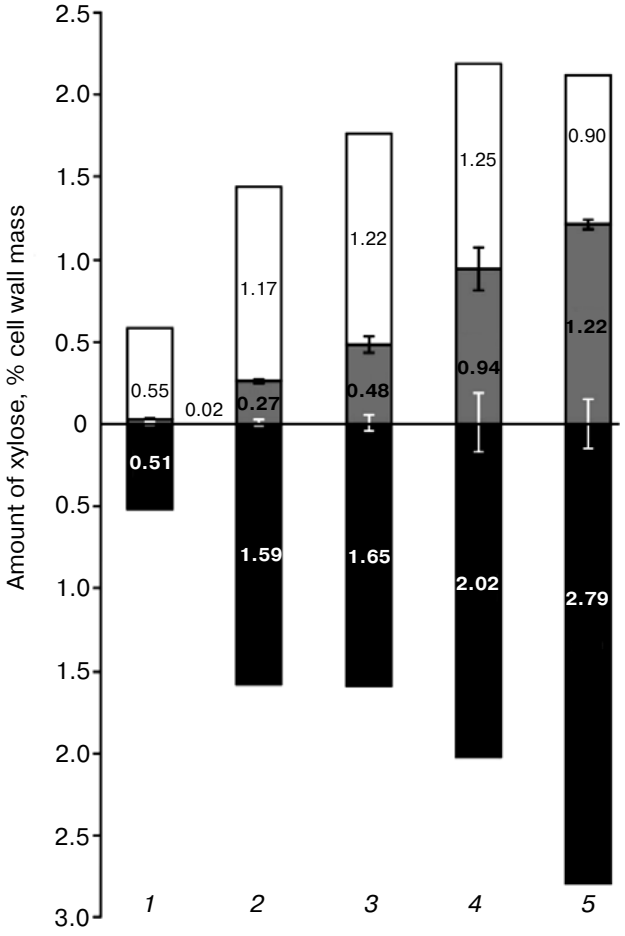

Fig. 5. Level of xylose (% cell wall mass) comprising high (white columns) and low molecular weight (gray columns) glucuronoarabinoxylan extracted by treatment with endoxylanase, as well as that remaining in the cell walls after enzymatic treatment (black columns) in different maize root zones: *1*) meristem; *2*) early elongation zone; *3*) middle elongation zone; *4*) late elongation zone; *5*) post-elongation zone.

DISCUSSION

We have analyzed glucuronoarabinoxylan from five zones of maize root tip, separated depending on cell development stage [13]. The yield of glucuronoarabinoxylan extracted by treatment with endoxylanase increased in the course of elongation (Fig. 2). Accumulation of glucuronoarabinoxylan with the extent of cell elongation was already observed in studies on wheat plantlets (from 10 to 16% cell wall mass [10]) and barley coleoptiles (from 25 to 30% cell wall mass [11]), applying alkaline extraction of non-cellulose polysaccharides. The method we used allows extraction of only the part of cell wall polysaccharide that is accessible for specific endoglucanases that is commonly called metabolically active [21].

Using maize coleoptiles, Kato and Nevins, who developed this method of polysaccharide extraction, estimated the portion of glucuronoarabinoxylan extracted by the enzyme as 20% of its total amount in cell wall [14]. In different root zones xylose comprising the metabolically active glucuronoarabinoxylan was about a half of the total cell wall xylose (Fig. 5). Even given the fact that some part of xylose remained in cell walls comprises xyloglucan (the estimated portion in cereal cell walls is several percent [2]), we conclude that maize roots characterized by high cell elongation rates [12], as compared with coleoptiles

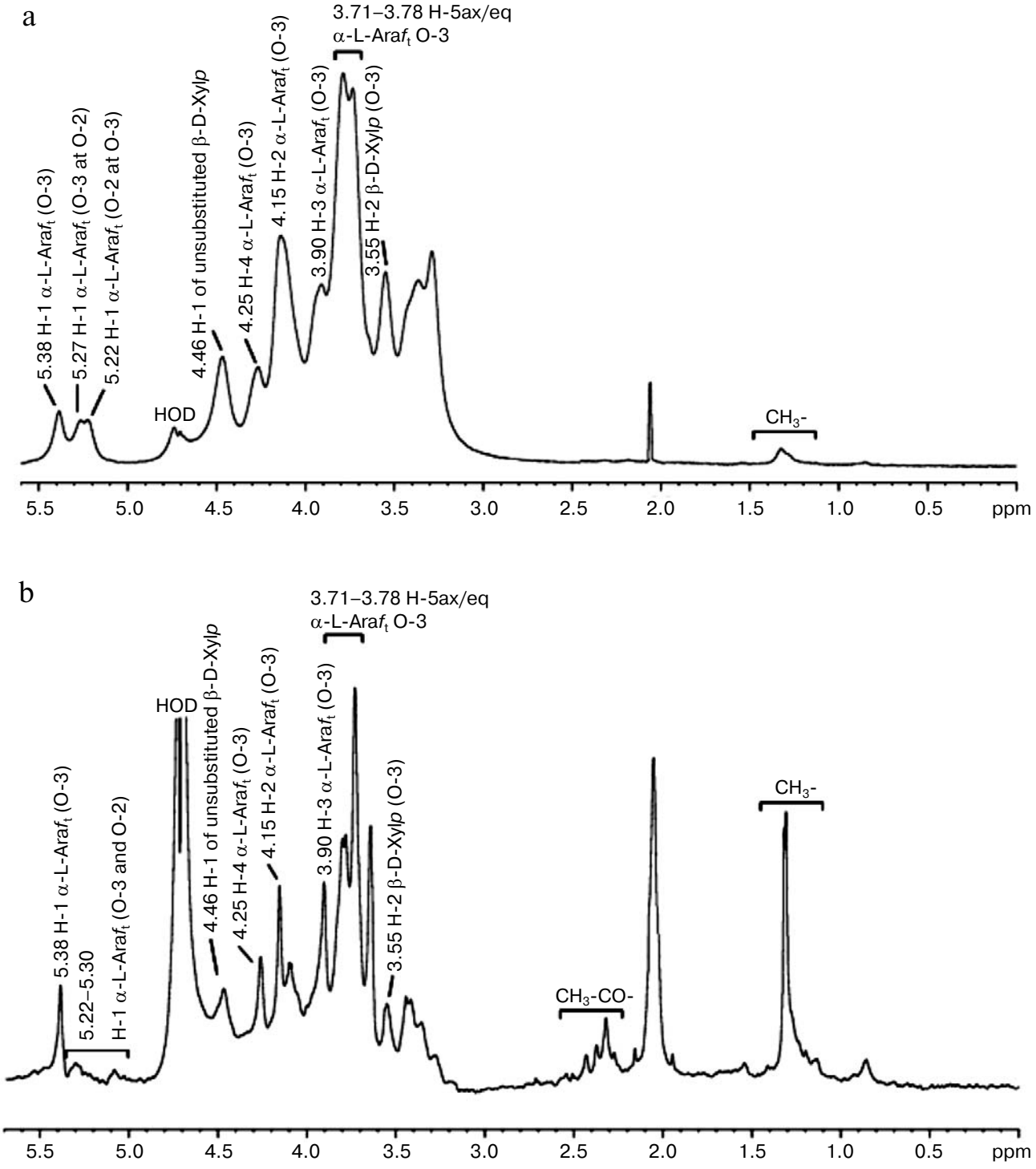

Fig. 6. ^{1}H-NMR spectra of wheat arabinoxylan (a) and maize root high molecular weight glucuronoarabinoxylan (b).

apparently contain higher amounts of metabolically active glucuronoarabinoxylan.

The enzymatic method of extraction has enabled differentiation of several glucuronoarabinoxylan structural types and reveals changes in their ratio during elongation. The elongation zone of the root was characterized by predominance of xylose comprising the metabolically active glucuronoarabinoxylan (Fig. 5). Similar regularity was observed for xyloglucan from pea stem with type I cell walls (unlike cereals with type II cell wall), in which xyloglucan serves as the dominant tethering glucan: the portion of metabolically active xyloglucan in elongating

tissues is higher than in non-elongating ones [21]. Polysaccharides accessible for endoglucanases are probably localized between cellulose microfibrils and do not interact with them by hydrogen bond formation [22].

The metabolically active part of the matrix polysaccharides can be extracted from cell walls by treatment with specific endoglucanases, so, the extract is usually composed of oligosaccharide fragments [13, 14, 21, 22]. Nevertheless, we have extracted from maize root cell walls not only oligosaccharide fragments, but also polysaccharides. The monosaccharide composition of the high molecular weight fraction (Table 2) confirms that the latter contains glucuronoarabinoxylan. This means that the natural maize cell wall polysaccharide contains large-scale areas that are not hydrolyzed by endoxylanase. Low molecular weight glucuronoarabinoxylan fragments apparently represent the parts of the entire molecule that are accessible for the enzyme used.

To establish features of the polysaccharide structure that hinder hydrolysis by endoxylanase, we performed ^{1}H-NMR analysis of both maize root high molecular weight glucuronoarabinoxylan fragments and wheat flour high molecular weight arabinoxylan, the latter being hydrolyzable by the enzyme. The data of ^{1}H-NMR (Fig. 6) and on monosaccharide composition of the examined carbohydrates (Table 3) show several differences that probably determine the accessibility of the glucuronoarabinoxylan for the endoxylanase.

Most remarkable is the high degree of backbone substitution with arabinose in maize root high molecular weight glucuronoarabinoxylan compared to wheat flour arabinoxylan (Table 3 and Fig. 6). However, endoxylanase is known to need backbone part containing at least two adjacent unsubstituted xylose residues [23]. The determined degree of glucuronoarabinoxylan xylose substitution with arabinose (64%) and GlcA/Xyl ratio (0.06) give the possibility for existence of hydrolysis sites. In this case, resistance of glucuronoarabinoxylan backbone to hydrolysis might be determined by random distribution of the unsubstituted xylose residues. Besides, maize root high molecular weight glucuronoarabinoxylan, which is inaccessible for endoxylanase, is acetylated in considerably greater extent than is wheat flour arabinoxylan, which can also influence the resistance of the polymer to the endoxylanase due to both substitution of xylose residue and implication of acetylated residues in additional hydrophobic interactions.

Thus, the key characteristic features of the metabolically active glucuronoarabinoxylan, which is inaccessible for hydrolysis by endoxylanase, are: 1) degree of polysaccharide backbone substitutions with arabinose or glucuronic acid; 2) uniform distribution of unsubstituted backbone xylose residues; and 3) presence of acetyl groups within the backbone structure.

The dynamics of maize root high molecular weight glucuronoarabinoxylan content (Ara/Xyl = 0.69-0.77) are in good agreement with the intensity of cell elongation. A possible implication of precisely this part of the polysaccharide in elongation correlates with the hypothesis of Thompson [24], who proposed that the primary function of matrix polysaccharides is separation of cellulose microfibrils. According to this hypothesis, hemicelluloses hamper microfibrils' interaction, allowing them to slide along each other and, thus, providing elongation of the cell wall.

During elongation, the level of polysaccharide degradable by endoxylanase increases in composition of metabolically active glucuronoarabinoxylan, due to lower degree of xylan backbone substitution (Ara/Xyl = 0.34-0.46). This explains the well-known fact that Ara/Xyl ratio decreases with growth [9-11]; this fact was confirmed in our study. At the same time, this increases the portion of xylose that is not enzymatically extracted from cell walls (Fig. 5).

Glucuronoarabinoxylans with Ara/Xyl ratio exceeding 0.5 are highly hygroscopic, which contributes to high plasticity of the formed material; the less is degree of polysaccharide substitutions, the less prominent are these features [25]. The extra lowly substituted xylans (Ara/Xyl = 0.1-0.2) enforce interaction of cellulose microfibrils with each other [26]. The found change in the ratio between glucuronoarabinoxylan areas with different substitution degrees during root cell growth can reflect transition of the polysaccharide molecules from their "plastic" form facilitating elongation (highly substituted glucuronoarabinoxylan resistant to endoxylanase) to the form capable of interacting with cellulose microfibrils and retarding cell elongation. The subject of further studies should be the relationship of different glucuronoarabinoxylan forms and mechanisms underlying their transitions.

The authors are grateful to researchers from the Laboratory of Root Physiology (Institute of Plant Physiology, Russian Academy of Sciences) and personally grateful to Prof. V. B. Ivanov for methodical counseling of the work with maize roots, to B. Z. Idiyatullin, research associate of the Laboratory of Biophysical Chemistry of Nanosystems (Kazan Institute of Biochemistry and Biophysics, Kazan Scientific Center, Russian Academy of Sciences), for assistance in NMR experiments, and to Prof. Barry McCleary (Megazyme, Ireland) for kindly provided purified specimens of xylanase M6 and wheat flour arabinoxylan.

This study was supported in part by the Russian Foundation for Basic Research (grant No. 11-04-01016) and by grant for support of leading scientific schools of Russian Federation (NSh-825.2012.4).

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