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STRUCTURAL FEATURES OF RICE BRAN HEMICELLULOSE

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Abstract—Rice bran hemicellulose was fractionated by ammonium sulphate precipitation, ion-exchange chromatography and enzymatic techniques. Methylation analysis of each fraction revealed that the bran hemicellulose consisted mainly of highly branched arabinoxylan and xyloglucan. The acidic arabinoxylan main component appeared to have more doubly-branched xylose residues in the main chain and also more complicated side chains than the endosperm arabinoxylan. Xyloglucan was also isolated from the crude hemicellulose but the amount of β-(1 → 3),(1 → 4)-glucan was very small compared to the endosperm hemicellulose.

INTRODUCTION

Rice bran derived from the outer part of brown rice, i.e. pericarp, seed coat, aleurone layer, germ and a small portion of starchy endosperm, contains a large amount of cell-wall material which is rich in hemicellulosic polysaccharides [1]. Rice bran cell-wall material and hemicellulose have recently attracted attention in relation to their potent utilization as a dietary fibre source [2–4]. The effect of the bran hemicellulose on the viscosity of rice flour paste has also been reported [5].

There have been several reports describing the fractionation and characterization of alkali-soluble rice bran hemicellulose [6–10] and water-soluble proteoglycan [11–13]. However, the type and amount of linkages included in each hemicellulose component were not reported. In this paper, we describe the fractionation of rice bran hemicellulose, methylation analysis of each component, and the comparison of their structural features with those from endosperm cell walls [14–16].

RESULTS AND DISCUSSION

Hemicellulosic polysaccharides were extracted from the cell-wall material obtained from rice bran (yield of crude hemicellulose = 4.6% of defatted bran) and fractionated by ammonium sulphate precipitation and ion-exchange chromatography, as described in the Experimental. Yields and sugar compositions of these fractions are given in Table 1.

Most of the glucose-containing polysaccharides were precipitated by adding ammonium sulphate at 65% saturation to the solution of bran hemicellulose, whereas a large part of the arabinoxylan, the main component of rice bran hemicellulose [6–10], seemed to remain in the supernatant solution. Arabinoxylan in the supernatant solution was further fractionated into five fractions by DEAE-cellulose column chromatography. Fraction S-0.01 M was considered to be a neutral arabinoxylan and S-0.05 M and S-0.1 M were acidic arabinoxylans, based on their sugar composition. Detection of only trace amounts of glucose and rhamnose in these fractions suggested that the presence of glucans [15] or pectic polysaccharides [17] was negligible. Fraction S-0.5 M contained a slightly higher amount of rhamnose and uronic acids, indicating contamination with pectic polysaccharides. Fraction S-0.2 M NaOH also seemed to be contaminated with a small amount of pectic polysaccharides and glucans.

Table 2 gives the results of methylation analysis of fractions S-0.01 M and S-0.05 M, which represent the neutral and acidic arabinoxylan in the bran hemicellulose. The results of methylation analysis of the acidic arabinoxylan of rice endosperm [16] is also cited in this table for comparison. The results show that 74–79% of the (1 → 4)-linked xylose residues are branched in the bran arabinoxylan, indicating that it has a highly branched structure similar to that of the endosperm arabinoxylan [16]. However, the proportion of doubly branched xylose residues, which is shown by the presence of free xylose (Table 2), is much higher in the bran arabinoxylan. The presence of these doubly branched xylose residues in arabinoxylan was also reported in the arabinoxylan from wheat endosperm [18], aleurone cell walls of wheat and barley [19], and husk of sorghum [20].

In addition to the presence of these doubly branched xylose residues, bran arabinoxylan contains characteristic sugar linkages which were not (or poorly) detected in the endosperm arabinoxylan. Bran arabinoxylan contains appreciable amounts of non-reducing end xylose and galactose, and also (1 → 2)-, (1 → 3)- or (1 → 5)-linked arabinose residues, indicating more complicated structural features of its side chains as compared to the endospermic one in which most of the side chains consist of single sugar residues. Di- and tri-saccharides containing these sugar linkages have been isolated from the partial hydrolysates of some arabinoxylans [21] and the above results indicate that similar structural units are also present in rice bran arabinoxylan, although most of the side chains still seem to be composed of single arabinofuranosyl units.

The appearance of non-reducing end glucose in the

Table 1. Sugar composition of rice bran hemicellulose fractions

Fraction	Yield† (mg)	Sugar composition (mol %)*						
		Rha	Ara	Xyl	Man	Gal	Glc	Uronic acid
S-0.01 M	75		44.9	46.0		6.1	1.9	1.1
0.05 M	385		43.1	46.3		5.4		5.2
0.1 M	272	0.6	40.9	45.6		5.9		7.0
0.5 M	351	2.8	42.4	35.2		8.0	0.7	11.0
0.2 M NaOH	180	1.3	35.5	40.9		7.4	5.0	9.8
P-0.01 M	454	0.5	21.5	32.3	1.5	7.5	33.7	3.0
0.5 M	704		41.0	44.9		1.6	4.0	8.4
0.2 M NaOH-sup.	185		14.5	29.5	3.4	5.7	42.6	4.2
0.2 M NaOH-ppt.	242	0.7	22.6	33.2	0.4	6.0	29.3	7.7
0.2 M NaOH-ppt.Xyl	(67)‡		5.0	19.6	2.3	10.2	60.4	2.4

*Neutral sugars were estimated by GC on column A. Peak areas were converted to molar ratios using molar response factors [39]. Uronic acid was estimated by the carbazole–H_2SO_4 method [36] as glucuronic acid.

†From 5 g of rice bran hemicellulose.

‡From 200 mg of fraction P-0.2 M NaOH-ppt.

Table 2. Methylation analysis of arabinoxylans from rice bran

Component	Linkage indicated	Molar ratio (%)*			
		S-0.01 M	S-0.05 M original	S-0.05 M carboxyl-reduced	Endosperm arabinoxylan [16]
2,3,5-Me_3-Ara	$(Ara_f)1 \rightarrow$	39.4	36.1	37.8	40.1
3,5-Me_2-Ara	$\rightarrow 2(Ara_f)1 \rightarrow$	2.9	2.7	2.8	1.7
2,3-Me_2-Ara	$\rightarrow 5(Ara_f)1 \rightarrow$	2.9	1.1	1.4	2.9
2,5-Me_2-Ara	$\rightarrow 3(Ara_f)1 \rightarrow$	4.9	4.7	4.8	
2,3,4-Me_3-Xyl	$(Xyl_p)1 \rightarrow$	2.9	2.6	2.2	
2,3-Me_2-Xyl	$\rightarrow 4(Xyl_p)1 \rightarrow$	10.8	10.2	9.8	8.9
2-Me-Xyl	$\rightarrow 4(Xyl_p)1 \rightarrow$ (3 ↑)	24.6	25.6	29.3	37.6
3-Me-Xyl	$\rightarrow 4(Xyl_p)1 \rightarrow$ (2 ↑)		5.7		4.7
Xyl	$\rightarrow 4(Xyl_p)1 \rightarrow$ (2 3 ↑ ↑)	6.9	7.6	5.8	3.0
2,3,4,6-Me_4-Gal	$(Gal_p)1 \rightarrow$	4.6	3.7	3.0	1.1
2,3,4,6-Me_4-Glc	$(Glc_p)1 \rightarrow$			3.2	

*Estimated by GC of alditol acetates on column B. Peak areas were converted to molar ratios using molar response factors.

carboxyl-reduced and methylated S-0.05 M fraction indicates the presence of terminal glucuronic acid residues in the original polysaccharide, as is the case in endosperm arabinoxylan [16]. The presence of (1 → 2),(1 → 4)-linked xylose residues in amounts corresponding to the uronic acid content of this arabinoxylan suggests that the uronic acids are linked to xylose residues through their O-2 positions. We previously isolated and identified 2-*O*-(4-*O*-methyl-D-glucopyranosylurono)-D-xylose and also 2-*O*-(α-D-glucopyranosylurono)-D-xylose from rice endosperm arabinoxylan [16].

The glucose-containing polysaccharide fraction which was precipitated by ammonium sulphate was further fractionated into four fractions by DEAE-Sephadex column chromatography. Most of the glucose was detected in the unadsorbed fraction and alkali-eluted fraction, respectively (Table 1). Preliminary studies by methylation analysis of these fractions indicated that they

Table 3. Methylation analysis of glucose-containing polysaccharide fraction from rice bran

Component	Linkage indicated	Molar ratio (%)* P-0.1 M	P-0.2 M NaOH-sup.	P-0.2 M NaOH-ppt.	P-0.2 M NaOH-ppt.-Xyl.
2,3,5-Me_3-Ara	$(Ara_f)1 \rightarrow$	18.2	13.1	21.8	
3,5-Me_2-Ara	$\rightarrow 2(Ara_f)1 \rightarrow$	1.3	2.3	1.2	
2,3-Me_2-Ara	$\rightarrow 5(Ara_f)1 \rightarrow$	2.7	1.3	1.9	
2,5-Me_2-Ara	$\rightarrow 3(Ara_f)1 \rightarrow$	1.7	1.5	1.6	
2,3,4-Me_3-Xyl	$(Xyl_p)1 \rightarrow$	9.7	10.6	7.2	14.4
2,3-Me_2-Xyl	$\rightarrow 4(Xyl_p)1 \rightarrow$	8.1†	15.0†	7.7†	
3,4-Me_2-Xyl	$\rightarrow 2(Xyl_p)1 \rightarrow$				6.0
2-Me-Xyl	$\rightarrow 4(Xyl_p)1 \rightarrow$ 3 ↑	13.8‡	8.3‡	18.1§	
3-Me-Xyl	$\rightarrow 4(Xyl_p)1 \rightarrow$ 2 ↑				
Xyl	$\rightarrow 4(Xyl_p)1 \rightarrow$ 2 3 ↑ ↑	1.8	6.3	2.7	
2,3,4,6-Me_4-Gal	$(Gal_p)1 \rightarrow$	7.5	4.1	4.5	9.1
2,3,6-Me_4-Glc	$\rightarrow 4(Glc_p)1 \rightarrow$	20.7	22.1	20.1	70.4
2,4,6-Me_4-Glc	$\rightarrow 3(Glc_p)1 \rightarrow$	1.3	3.9	0.8	
2,3-Me_4-Glc	$\rightarrow 4(Glc_p)1 \rightarrow$ 6 ↑	13.1	11.5	12.4	26.4

*Estimated by GC as described in Table 2.

†Presence of both 2,3- and 3,4-di-*O*-methylxylose was confirmed by GC/MS.

‡2-*O*-Methylxylose was the main component.

contained xyloglucan but little β-(1 → 3),(1 → 4)-glucan (Table 3). Further attempts to purify the xyloglucan in these fractions by several chromatographic techniques were unsuccessful. Rice endosperm xyloglucan also could not be separated from co-existing arabinoxylan and β- (1 → 3),(1 → 4)-glucan and was considered to form a sort of complex [15], although the content of the latter polysaccharide was very small in the case of bran hemicellulose. Bran xyloglucan was finally purified by the enzymatic removal of co-existing arabinoxylan using purified α-L-arabinofuranosidase of *Rhodotorula flava* [22] and endoxylanase of *Streptomyces* sp. E-86 [23]. The sugar composition of the purified xyloglucan fraction (P-0.2 M NaOH-ppt.-Xyl) is also shown in Table 1. Methylation analysis of this fraction suggested the presence of a (1 → 4)-linked glucan back-bone, *ca* 40% of which is branched at the O-6 positions (Table 3). Non-reducing end xylose and galactose corresponding to the branching points and a small amount of (1 → 2)-linked xylose were also detected. By analogy with the structure of xyloglucans isolated from several dicots [24–28] and monocots [15, 29–31], these branched glucose residues are considered to carry short side chains at their O-6 positions which are terminated with non-reducing end xylose or galactose residues. Some of the terminal galactose residues may be linked to the main chain via (1 → 2)-linked xylose residues [15].

The general structural features of rice bran xyloglucan appear to be very similar to those obtained from the endosperm cell walls [15], but it is not possible to compare their repeating units in detail since we have not yet carried out enzymatic fragmentation analysis of the bran xyloglucan. Xyloglucans which were originally found as a main hemicellulosic component of dicot cell walls [24–28] have recently been detected in several monocot walls [15, 29–31], but have not yet been detected in the endosperm and aleurone cell walls of wheat and barley [19, 32, 33].

Methylation analysis of fractions P-0.01 M, P-0.2 M NaOH-sup. and ppt. (Table 2) suggested that the amount of β-(1 → 3),(1 → 4)-glucan in rice bran hemicellulose was very small compared to the endosperm hemicellulose [15]. This is one of the characteristic features of rice bran cell walls and is further supported by the results of compositional analysis and methylation analysis of isolated cell walls from bran and germ [34]. β-(1 → 3),(1 → 4)-Glucan was reported to be more abundant in the endosperm cell walls of barley [33] than in aleurone cell walls [19], whereas in wheat grains, endosperm [32] and aleurone cell walls [19] contained comparable amounts of β-(1 → 3),(1 → 4)-glucan.

The above experimental results suggest that the composition of hemicellulosic polysaccharides and also the structural features of each component change among different tissues. This would correspond to the different physical and physiological properties of these cell walls, which are also important for their characteristics as a dietary fibre source.

EXPERIMENTAL

Plant material. Rice (*Oriza sativa*, cv Koshihikari) was harvested in 1979 in Ibaraki prefecture, Japan.

Source of enzyme. α-L-Arabinofuranosidase was purified from the culture filtrate of *Rhodotorula flava* by the method of ref. [22]. Purified endoxylanase of *Streptomyces* sp. E-86 [23] was supplied by Professor T. Yasui and Dr. I. Kusakabe of the University of Tsukuba. Both enzymes did not show any other glycanase activities. Porcine pancreatic amylase (Type VI-A) and pronase E were purchased from Sigma and Kaken Kagaku, respectively.

General methods. Total carbohydrate content was determined by the PhOH–H_2SO_4 method [35]. Uronic acid content was determined by the carbazole method [36], corrected for neutral sugar. For the analysis of component sugars, polysaccharides were hydrolysed with 0.5 M H_2SO_4 at 121° for 2 hr. Neutral sugars in the hydrolysate were converted into their corresponding alditol acetates and analysed by GC using a column of 3% ECNSS-M on Gas Chrom Q (column A, 0.3 × 200 cm) [37] or a glass capillary column coated with Silar-10C (column B, 0.28 mm × 30 m or 50 m) [38]. Peak areas were converted to molar ratios using response factors [39]. GC/MS was carried out using EI ionization and also CI, with isobutane as reactant gas.

Methylation analysis. Methylation analysis was performed using the method of ref. [40]. Part of the original polysaccharides was dissolved in H_2O and reduced using 1-cyclohexyl-3-(2-morpholinoethyl)-carbodiimide metho-*p*-toluene-sulfonate and $NaBH_4$ [41]. The carboxyl-reduced polysaccharides so obtained were then methylated [40] to give the carboxyl-reduced and methylated polysaccharides. The methylated polysaccharides were hydrolysed by heating with 90% HCO_2H at 100° for 2 hr, and then with 1 M TFA at 121° for 1 hr. The partially methylated sugars so obtained were converted into the corresponding alditol acetates and analysed by GC and GC/MS using column B.

Preparation of rice bran hemicellulose. Rice bran was prepared from brown rice with a Satake Motor One-pass type testing mill (milling yield = 90%) and defatted with petrol. Defatted bran (500 g) was extracted with 0.25% EDTA soln (2.5 l.) at 70–80° for 18 hr, to remove pectic substances, then the residue was digested with pronase E (1 g) in 0.05 M Pi buffer (pH 7.0, 2 l.) overnight at room temp., then for 2 hr at 50°. The reaction mixture was heated for 30 min at 100°, cooled, and digested with procine pancreatic amylase (1 g/2 l.) at 37° overnight. Crude bran cell wall preparation was then recovered by centrifugation. Crude hemicellulose was extracted from this preparation with 4 M NaOH (2 l.) in the presence of $NaBH_4$. After neutralization and dialysis against H_2O, 4 vols. EtOH was added to the soln to precipitate the hemicellulose. The ppt. formed was recovered by centrifugation and redissolved in 0.05 M Pi buffer (pH 7.0, 1.2 l.) and further digested with porcine pancreatic amylase (100 mg) and pronase E (100 mg) to remove residual starch and cytoplasmic protein. Purified bran hemicellulose was recovered by EtOH precipitation (× 2) and lyophilization (yield = 23.1 g).

Fractionation of hemicellulose. Crude bran hemicellulose (5 g) was dissolved in H_2O (500 ml) and 1% CPC soln (50 ml) was added dropwise to the soln. After 30 min in a cold room, the soln was centrifuged and the small amount of ppt. formed was discarded. $(NH_4)_2SO_4$ (230 g) was added to the supernatant soln (530 ml) and the ppt. formed was collected by centrifugation. This ppt. was dissolved in H_2O and dialysed against H_2O and 0.01 M Pi buffer (pH 6.0) successively. The dialysate was applied to a column of DEAE-Sephadex equilibrated with the same buffer and eluted stepwise with Pi buffer of 0.01 and 0.5 M, and finally with 0.2 M NaOH. The eluates were collected and dialysed against H_2O and lyophilized to give fractions P-0.01 M to S-0.2 M NaOH. Only the eluate of NaOH formed an appreciable amount of ppt. after dialysis, and it was collected separately from the supernatant fraction (fraction P-0.2 M NaOH-ppt. and sup.). The supernatant of $(NH_4)_2SO_4$ pption was also dialysed against H_2O and 0.01 M Pi buffer (pH 6). The dialysate was applied to a column of DEAE-cellulose equilibrated with the same buffer and eluted stepwise with Pi buffer of different concns (0.01, 0.05 and 0.5 M) and 0.2 M NaOH. Polysaccharide in each eluate was recovered by lyophilization as described above (fractions S-0.01 M to S-0.2 M NaOH).

Purification of xyloglucan from the P-0.2 M NaOH-ppt. fraction. Fraction P-0.2 M NaOH-ppt. (200 mg), methylation analysis of which suggested the presence of xyloglucan, was suspended in H_2O (40 ml) and 1 M NaOH (1.5 ml) was added to the suspension to give a clear soln. HOAc was added to this soln to give pH 6 and a small amount of purified endoxylanase from *Streptomyces* sp. E-86 was added. After incubation overnight at 40°, the ppt. formed was collected by centrifugation, washed with H_2O and lyophilized (yield = 93 mg). Sugar composition of this insoluble polysaccharide suggested that it still contained appreciable amounts of arabinoxylan. It was suspended in 0.05 M acetate buffer (pH 2) and small pieces of lyophilized powder of purified α-L-arabinofuranosidase were added. After incubation overnight at 50°, the pH of the soln was adjusted to 6 with NaOH and the endoxylanase added again. This was further incubated overnight at 50° and heat-inactivated (100°, 15 min). Insoluble polysaccharide in the reaction mixture was recovered by lyophilization to give purified bran xyloglucan (P-0.2 M NaOH-ppt.-Xyl) (yield = 67 mg).

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