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RESEARCH NOTE

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Linkage Structure Analysis of Barley and Oat β-Glucans by High Performance Anion Exchange Chromatography

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Abstract Cereal β-glucans, linked essentially by mixed β-(1,4/1,3) glycosidic bonds, were extracted, purified, and structurally identified. Previously chemical structure of barley β-glucans was characterized from 3 varieties of 'Gang', 'Ohl', and 'Gwangan', and the (1,4)/(1,3) linkage ratio of the β-glucans was identical. In this study, β-glucans from 1 barley ('Chal') and 3 oat ('Ohl', 'Samhan', and 'Donghan') varieties were structurally scrutinized, and the linkage pattern of total 7 cereal β-glucans was compared. The amount of 2 major 3-*O*-β-cellobiosyl-D-glucose (DP3) and 3-*O*-β-cellotriosyl-D-glucose (DP4) from barley and oat accounted for only 66.6-73.3 and 68.12-81.89% of water-extractable β-glucan fractions, and the (1,4)/(1,3) linkage ratios of both barley and oat β-glucans were within very narrow range of 2.27-2.31 and 2.38-2.39, respectively, among the cultivars tested. Structural difference in the cereal β-glucans was evident when DP3:DP4 ratio in the β-glucan structure was compared. As a result, this ratio was significantly greater for barley β-glucan (2.26-2.74) than for oat (1.54-1.66). Chal-B had the greatest DP3 to DP4 ratio among the samples, which in turn reflected the least amount of (1,4)-linkages.

Keywords: barley, oat, β-glucan, lichenase, β-(1,4)/(1,3) linkage

Introduction

Cereal β-(1,3)/(1,4)-D-glucan is a linear polysaccharide consisting entirely of D-glucose. Sequences of β-(1,4)-linked D-glucopyranosyl units are interrupted by single β-(1,3)-linked units. This β-(1,4)-linked sequences are dominantly composed of 3 or 4 glucose units long but successive β-(1,4)-linkages up to 13 in water-soluble and up to 20 in water-insoluble β-glucans have been found (1-3). The main building blocks, which are β-(1,3)-linked cellotriosyl and cellotetraosyl units, constitute approximately 90% of the barley β-glucan molecule (4,5). The solubility of cereal β-glucan may be improved due to the existence of β-(1,3)-linkages when compared to cellulose which has exclusively β-(1,4)-linkages (6,7). Rather randomly located β-(1,3) linkage prevents close packing of the molecule and makes the molecule partly soluble in water, unlike cellulose that is built entirely of β-(1,4)-linked D-glucanosyl units and is capable of close packing to crystalline structures (4,8,9). Some β-glucan fractions in plant cell wall are not extractable into water but the reason for this is not clearly known. According to Åman and Graham (10), 20% of oat and 46% of barley β-glucan were water-insoluble. Miller and Fulcher (11) reported that 20-25% of oat β-glucan was insoluble in water. Barley β-glucan has been widely studied because of the problems it causes in beer brewing process (12-14). The structure of barley β-glucan is believed to be similar to that of oat β-glucan (15-19). However, direct comparisons of the results from the different studies on these 2 β-glucans are difficult because the methods of isolation and analyses differ from experiment to experiment. Extraction conditions strongly affect the type of obtainable β-glucan molecules, and the results of analysis may be different depending on the extraction methods used.

A good indicator of structural difference in cereal β-glucans is the ratio between 3-*O*-β-cellobiosyl-D-glucose (DP3) and 3-*O*-β-cellotriosyl-D-glucose (DP4) released by lichenase treatment (1,2,20). The greater the ratio of the hydrolysis reaction products is, the more frequent the cellotriosyl segments are. It was reported that this ratio is lower in oat β-glucan than barley one. Wood *et al.* (16) reported values of 2.1-2.4 (calculated as mol%) for soluble oat β-glucans and 2.8-3.3 for barley β-glucan. Izydorczyk *et al.* (1,2) obtained the ratio of 1.76 and 2.13 for soluble barley β-glucan extracted at 40 and 65°C, respectively, and for insoluble β-glucan they reported the ratio range of 2.07-2.43. A high performance anion exchange chromatography (HPAEC) assisted by lichenase treatment has been widely used for the structural and quantitative analysis of cereal β-glucans (5,16). However, the authentic standards of DP3 and DP4 has not been readily available, and thus it is considered a critical problem to characterize β-glucan structure. Previously, we established a separation and purification method of DP3 and DP4 by using a recycling preparative high performance liquid chromatography (HPLC), and these 2 authentic standard oligosaccharides were produced from barley β-glucan (21). More accurate information about β-glucan structure should be essential prerequisite for establishing the structure-function relationship of cereal β-glucans. In this study, therefore, the structurally identified authentic standards were applied to quantify the ratio of β-(1,4)/(1,3) linkages in the β-glucans isolated from 7 domestic barley and oat cultivars.

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Materials and Methods

Reagents and materials A waxy barley (Chal-B) variety was harvested on June, 2005, and 3 oat varieties, 'Ohl' (Ohl-O), 'Donghan' (Donghan-O), 'Samhan' (Samhan-O)

were harvested on September, 2005. These cereal grains were kindly provided from Honam Agricultural Research Institute (HARI), National Institute of Crop Science (NICS), Iksan, Jeonbuk, Korea. Whole grain samples were milled with a blender (HR2860; Philips, Seoul, Korea) and the milled flour was used for further analysis. A Megazyme β-glucan (mixed linkage) assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) was used to determine the content and purity of barley and oat β-glucans.

Isolation and purification of water-extractable barley and oat β-glucans Cereal β-glucans were extracted from barley and oat flours following the method of Wood *et al.* (22) and Lee (23) with some modification. Full description of this extraction and purification procedure was well documented previously (21). The total β-glucan content and the purity of water-extracted β-glucan were analyzed by the Megazyme β-glucan (mixed linkage) assay kit. Approximately 100 mg of sample was dispersed in 0.2 mL of 50%(v/v) ethanol, and the pH was adjusted to 6.5 by adding 4.0 mL of 20 mM sodium phosphate buffer. Ten units of lichenase was added to the mixture after 3 min boiling, which was then incubated at 50°C for 1 hr. Total volume of the reaction mixture was adjusted to 10 mL by adding 200 mM sodium acetate buffer (pH 4.0). After retrieving 0.1 mL of the supernatant from centrifugation at 10,000×g for 10 min, β-glucosidase (0.2 units) was added to it and the mixture was incubated at 50°C for 10 min. The amount of produced glucose was determined with a 2700 SELECT Biochemical analyzer (YSI Life Science, Yellow Springs, OH, USA), which was converted into the amount of β-glucan.

Linkage structure analysis of barley and oat β-glucans About 10 mg of extracted β-glucan was dispersed in 10 mL of 20 mM sodium phosphate buffer (pH 5.0), and the buffered sample was incubated with lichenase (8 units) at 40°C for 1.5 hr (21). After filtering the reaction mixture through a 0.45-μm membrane filter, and it was directly injected into an HPAEC system (DX300 series, Dionex Corp., Sunnyvale, CA, USA) (54). A Carbopac™ PA1 (4×20 mm) analytical column was equipped, and 2 eluents A (150 mM NaOH) and B (150 mM NaOH+600 mM sodium acetate) were used at the flow rate of 1.0 mL/min. The linear gradient of eluent B was adopted until it reached 50% from 0.01 to 50 min after regeneration for 15 min with 100% eluent A. The potentials (E) and duration time (t) of the pulsed amperometric detector (PAD) was fixed at E_1=0.05 V (t_1=300 ms), E_2=0.6 V (t_2=120 mV), and E_3=−0.15 V (t_3=300 ms). The released amount of oligosaccharides produced by lichenase treatment was determined by using the standard curves generated with authentic DP3 and DP4 prepared previously in our study.

Statistical analysis Statistical analysis was performed by using SAS for Window version 8.1. Statistical significance in the difference among the values was evaluated by Duncan's test. The significance level was $p<0.05$.

Results and Discussion

Extractability and purity of barley and oat β-glucans The β-glucan contents in 1 barley and 3 oat varieties were determined by using a Megazyme β-glucan (mixed linkage) assay kit and were compared to previously reported data of other 3 barley varieties. Total β-glucan contents of 4 barley cultivars including Gang-B, Ohl-B, Gwang-an-B, and Chal-B, and 3 oat cultivars including Ohl-O, Samhan-O, and Donghan-O, were in the range of 6.12-9.89% and 4.36-4.77%(w/w, d.b.), respectively, while water-extractable β-glucan fractions were 1.49-2.00 and 0.94-1.48%, respectively (Table 1). This result showed that the water-extractable β-glucan contents were somewhat less than those of previous studies (5,24). Chal-B, a waxy type, had the greatest amount (9.9%) of total β-glucan among the selected barley and oat samples but the extractable amount remained at 1.94%. A former study showed that waxy-starch barley consisted of greater amount of β-glucan than normal ones (25) while another study reported opposite result from the former one (24). β-Glucan content of other 3 barley cultivars was determined to be around 6.1-6.4% but the extractable amounts of Gang-B and Ohl-B were comparable to that of Chal-B. Thus, Chal-B had the lowest extraction yield although Gwangan-B showed the lowest extractable amount. Compared to barley varieties, total and extractable β-glucan contents from oats were substantially lower. The purity of the extracted β-glucans was determined to be 62.0% (Gang-B), 68.0% (Ohl-B), 70.3% (Gwangan-B), 66.6% (Chal-B), 67.7% (Ohl-O), 65.9% (Samhan-O), and 61.7% (Donghan-O). In this study, it was shown that Ohl-B had the greatest amount of water-extractable fraction of β-glucan, and Gwangan-B had the greatest purity of β-glucan among the cereal samples tested.

Table 1. β-Glucan Content and purity of the whole grain of selected barley and oat varieties

		Total β-glucan content (%)[1)]	Extractable amount (%)	Purity of β-glucan (%)[2)]
Barley	Gang-B[3)]	6.39±0.07^{b}	1.80±0.02^{b}	62.0±3.9^{c}
	Ohl-B[3)]	6.18±0.04^{c}	2.00±0.01^{a}	68.0±0.5^{b}
	Gwang-an-B[3)]	6.12±0.02^{c}	1.49±0.03^{c}	70.3±0.5^{a}
	Chal-B	9.89±0.09^{a}	1.94±0.12^{ab}	66.6±3.7^{abc}
Oat	Ohl-O	4.77±0.02^{d}	1.48±0.14^{c}	67.7±2.9^{abc}
	Sam-han-O	4.36±0.09^{e}	0.94±0.09^{d}	65.9±5.6^{abc}
	Dong-han-O	4.41±0.04^{e}	1.17±0.17^{cd}	61.7±1.2^{c}

[1,2)]The content and purity of β-glucan were determined by using a Megazyme β-glucan (mixed linkage) assay kit, AOAC Method 995.16.
[3)]The data of 'Gang', 'Ohl', and 'Gwangan' barley are adopted from Ref. 21; [a-e]Values within the same column with different letters are significantly different ($p<0.05$).

Table 2. Linkage pattern analysis of water-extractable lichenase-hydrolyzed β-glucans from 4 barley and 3 oat cultivars

		DP3 (%)	DP4 (%)	Total amount of DP3 & DP4 (%)	Weight ratio of DP3/DP4	Molar ratio of DP3/DP4	(1, 4)[1]/(1, 3)[2]
Barley	Gang-B[3]	46.47±0.79[a]	26.82±1.17[d]	73.29±1.96[c]	1.73±0.05[b]	2.29±0.06[b]	2.3
	Ohl-B[3]	45.73±0.42[a]	26.73±0.99[d]	72.47±1.41[c]	1.71±0.05[b]	2.26±0.06[b]	2.31
	Gwang-an-B[3]	45.74±0.5[a]	25.67±0.63[d]	71.41±1.21[c]	1.78±0.02[b]	2.35±0.03[b]	2.3
	Chal-B	44.89±0.24[a]	21.68±0.21[e]	66.56±0.45[e]	2.07±0.02[a]	2.74±0.02[a]	2.27
Oat	Ohl-O	44.05±0.05[b]	37.84±0.02[a]	81.89±0.07[a]	1.16±0.00[e]	1.54±0.00[e]	2.39
	Sam-han-O	41.29±0.09[c]	32.80±0.09[b]	74.09±0.17[b]	1.26±0.00[c]	1.66±0.00[c]	2.38
	Dong-han-O	37.30±0.11[d]	30.81±0.21[c]	68.12±0.33[d]	1.21±0.01[d]	1.60±0.01[d]	2.38

[1]β-(1,4) linkage of purified β-glucan.
[2]β-(1,3) linkage of purified β-glucan.
[3]Data are adopted from Ref. 21; [a-e]Values within the same column with different letters are significantly different ($p<0.05$).

Molecular structure of barely and oat β-glucans In order to obtain more precise information about cereal β-glucan structure, highly pure DP3 and DP4 were applied to quantify the ratio of β-(1,4)/(1,3) linkages in barley and oat β-glucans as authentic standards. It was previously shown that the amounts of lichenase-derived DP3 and DP4 may be underestimated when either cellooligo- or maltooligo-based standard curves were used at relatively low concentration. As a result, 4 barley varieties, Gang-, Ohl-, Gwangan- and Chal-B, had the weight fractions of authentic DP3 and DP4 within a very narrow range from 44.9 to 46.5% and from 21.7 to 26.8%, respectively. In case of 3 oat varieties, Ohl-, Samhan-, and Donghan-O had the DP3 weight fractions of 44.0, 41.3, and 37.3%, and DP4 of 37.8, 32.8, and 30.8%, respectively. These 2 major hydrolyzed products accounted for 66.6-73.3% (barley) and 68.1-81.9% (oat) of total β-glucan content by a lichenase-based HPAEC method. Among the barley β-glucans, Chal-B had significantly greater DP3 to DP4 ratio, which in turn reflected less (1,4)-linkages than other barley β-glucans. The (1,4)/(1,3) linkage ratio of barley β-glucans was in the range of 2.27-2.31, which was in good agreement with other previous reports (26,27). It was quite interesting that oat β-glucans displayed consistently lower this DP3/DP4 molar ratio (1.5-1.7) (28,29) than did barley β-glucans. When judged by these data, oat β-glucans have greater portion of (1,4)-linkages that may provide more linear segments in the β-glucan structure. From this result, it was convinced that this linkage ratio could be an indicator or a fingerprint of specific cereal β-glucan. Furthermore, this accurate structural information of cereal β-glucans will provide more insight for elucidating the structure-function relationship of cereal β-glucans.

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