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Matrix Polysaccharides and Matrix Structure of the Cotyledon Tissues of Kidney Bean

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Matrix polysacchaides were exhaustively extracted from kidney bean cotyledon tissues with ammonium oxalate–oxalic acid buffer of pH 4.25 at 80°C, and structural changes of the cell walls were microscopically investigated. The polysaccharides extracted were composed of two groups, arabinan–like neutral polysaccharides and weakly acidic pectic polysaccharides(WAPs), which have a low uronic acid content; the latter was the major component of the matrix polysaccharides. Maceration of the cotyledon tissues and degradation of the cell walls were accompanied by the dissolution of large amounts of WAPs, and extraction peaks appeared, the WAPs of each fractions were characteristics. Accordingly, it was considered that the WAPs themselves play an important role in the adhesion of the cell walls similarly to the ordinary pectins of vegetables and fruits, and that arabinan and the WAPs exist in a stratified state in the cell walls.

The middle lamella of the primary plant cell walls is particularly rich in pectic polysaccharides,[1] which are involved in the adhesion of the cell walls so that the dissolution of them leads to the maceration of plant tissues.[2,3] The primary cell–wall polysaccharides have been separated classically into three fractions on the basis of their solubility characteristics; pectic polysaccharides, hemicelluloses, and celluloses.[1]

Matsuura[4–6] previously reported that pectic polysaccharides of kidney bean cotyledons, in contrast to those of fruits and vegetables, contained large amounts of xylose and fucose in addition to arabinose and galactose. Methylation analysis had also indicated that most of the xylose residues were attached to the galacturonan regions of the pectic polysaccharides as short side chains and that these side chains were evenly distributed along the galacturonan chains.[5,6] Xylose–rich pectic polysaccharides are likely characteristic of the pectic polysaccharides of bean cotyledon tissues.[7–10] In this work, we intended to clarify the role of the xylose–rich pectic polysaccharides in the adhesion of cell walls in kidney bean cotyledons. The matrix polysaccharides were exhaustively extracted from the cotyledon tissues and structural changes of the cell walls were microscopically investigated.

MATERIALS AND METHODS

Materials. DEAE–cellulose was a product of Brown Co. Ltd. Sepharose Cl–4B was

obtained from Pharmacia Fine Chemicals. ECNSS-M was obtained from Gasukuro Kogyo Co. Ltd. (Kyoto). Other chemicals, reagent grade, were purchased from Nakarai Chemicals (Kyoto).

Determination of sugars. Total sugars were determined by the phenol-sulfuric acid method [11] using arabinose as the standard. Galacturonic acid was determined by the carbazole method, as modified by Bitter and Muir. [12] Neutral sugars were determined by the gas chromatographic method of Kusakabe *et al.*; [13] samples were prepared as previously described. [14]

Extraction of polysaccharides from kidney bean cotyledons. Kidney beans (*Phaseorus vulgaris*, "Uzuramame" ; whole seeds) were soaked in water over night at 5°C, then the seed coats and hypocotyls were removed. The cotyledons (moisture content, 51%) were cut into slices 0.5 mm thick. The slices (40 g, wet weight) were taken into a 300-ml Erlenmeyer flask and extracted with 100 ml of 0.035 M ammonium oxalate-oxalic acid buffer of pH 4.25 at 80°C for 60 min; the pH of the solution was maintained at 4.25 by adding 0.035 M oxalic acid solution. The extract was filtered through a sintered glass filter (G-3). The residue remaining after extraction was reextracted with 100 ml of the oxalate buffer in a similar manner; extraction was repeated twenty-six times in total. Each extract, after filtration, was treated at pH 5.0 with α-amylase (Sigma Chemical Co., bacterial) to decompose starch. The reaction mixture was incubated at 30°C until it gave negative reaction for iodine and dialyzed against water. The precipitates formed during dialysis were removed by centrifugation. Total sugar and galacturonic acid contents of the supernatants were determined by the phenol sulfuric acid and the carbazole methods, respectively.

The residue remaining after twenty-six extractions was reextracted with 300 ml of 1 M sodium hydroxide solution at room temperature for 12 hr; prior to the incubation the extraction mixture was pre-incubated at 0°C for 1 hr to prevent degradation of esterified galacturonidic bonds by transelimination. The alkali extraction at room temperature was repeated once more and the extracts were combined and adjusted to pH 5 with 5 M acetic acid. The precipitates formed were removed by centrifugation and the supernatant after dialysis against water used as fraction Ⅶ.

Measurement of methyl ester content. One ml of sample solution, which contained about 500 μg galacturonic acid residues, was treated with 0.05 M sodium hydroxide at room temperature for 60 min and the methanol liberated was determined by the gas chromatographic method of Bartlome. [15] Methyl ester content was expressed as molar ratio (%) to the galacturonic acid residues.

DEAE-cellulose chromatography. Pectic sample solutions (about 10 mg as galacturonic acid residue) were applied to a DEAE-cellulose column (2 × 5 cm) equilibrated with 0.05 M acetate buffer (pH 6.0) and eluted successively with the same acetate buffer, a linear gradient of acetate buffer (pH 6.0; 0.05 M, 230 ml → 0.8 M, 230 ml) and with 0.1 M sodium hydroxide

solution,[16] the eluates being collected in 9 ml fraction. The fractions containing polysaccharides were combined as neutral polysaccharide, acetate buffer and sodium hydroxide fractions, respectively, and dialyzed against water; the last fraction was neutralized to pH 6.0 prior to the dialysis.

Gel filtration of neutral polysaccharides. Sample solutions (5 ml) were put on a Sepharose CL-4B column (2 × 80 cm) and eluted with water. The effluents were collected in 7.5 ml fraction.

RESULTS

Extraction of matrix polysaccharides

Figure 1 shows the extraction profile of polysaccharides from slices of kidney bean cotyledons. The first peak appeared on the fifth extraction and the next one on the eighth extraction, whereupon the cotyledon tissues began to macerate. Separation of cells from the tissues was observed on the eleventh extraction and the third peak appeared on the next extraction. Dissolution of the fourth peak polysaccharides occurred from the eighteenth to the nineteenth extraction and the tissues collapsed completely. After the twenty-fourth extraction, the amount of soluble polysaccharides was small and almost constant, but much polysaccharides were still extracted with 1 M sodium hydroxide solution (fraction Ⅶ).

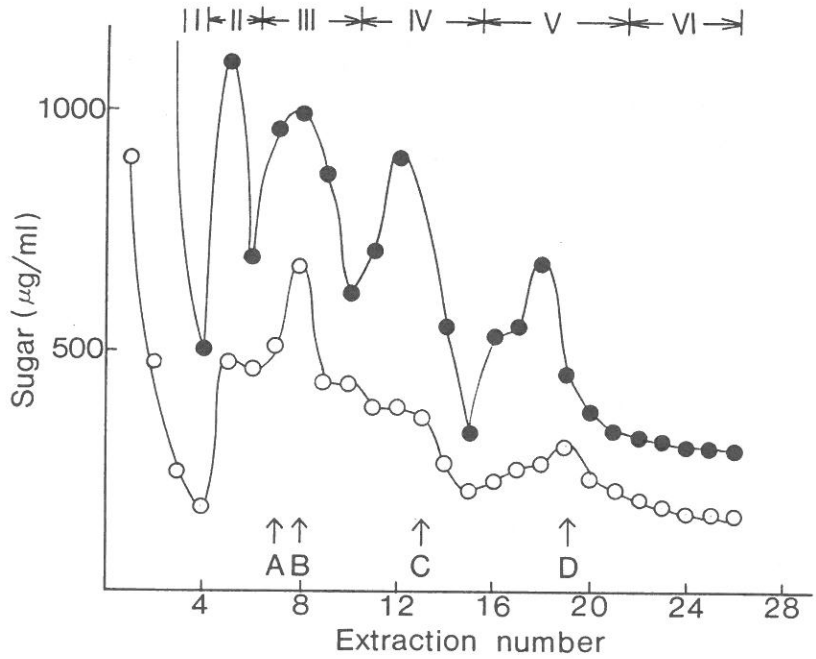

Fig. 1. Extraction Profile of Polysaccharides from Kidney Bean Cotyledons.
A, initiating the maceration of cotyledon tissues; B, the maceration occurred; C, initiating the dissolution of cell walls; D, the dissolution occurred. I–VI, polysaccharide fractions. ●, total sugar; ○, galacturonic acid.

Methyl ester content of pectic polysaccharides

Table I shows the methyl ester contents (degrees of esterification, D. E.) of pectic polysaccharides in each fraction. The values of D. E. ranged from 56.8 to 93.2%; fraction V showed the highest value of 93.2%.

Table I. Methyl Ester Contents of the Pectic Polysaccharides in Each Fraction

Fraction	I	II	III	IV	V	VI
Methyl ester content (%)	56.8	58.8	65.6	60.4	93.2	77.1

The values were expressed as moles per 100 moles of galacturonic acid residue.

Fractionation of polysaccharides and their sugar compositions

The polysaccharides of each fraction were separated by DEAE-cellulose chromatography into three fractions, neutral polysaccharide, acetate buffer and sodium hydroxide fractions (Fig. 2). The acetate buffer fractions, weakly acidic pectic polysaccharides (WAPs) having low uronic acid contents, were the major components. The WAPs contained large amounts of neutral sugars such as fucose, arabinose, xylose and galactose; particularly the fucose and xylose contents were high compared with those of ordinary pectins, galacturonic acid contents being 16-36% (Table II). The neutral sugar composition, especially the arabinose and xylose contents, varied with the individual fractions (Table II). Xylose was the largest component of the fractions I and II, which were obtained before the start of maceration of cotyledon tissues, whereas xylose content decreased in the fractions V and VI; these fractions were extracted after the cells disintegration. Fractions III and IV, which were thought to be closely related to the maceration, were rich in both arabinose and xylose. Fraction VII, the last extract with 1 M sodium hydroxide solution, showed a somewhat different sugar composition from those of the other fractions. The fraction contained a small amount of xylose and a very small amount of fucose, though its arabinose content was very large.

The neutral polysaccharides of each fraction were mainly composed of arabinose. They contained also small amounts of xylose, galactose and glucose (Table III).

Purification of arabinan

The neutral polysaccharide fractions on the DEAE-cellulose chromatography were combined and separated by gel filtration into two fractions, A and B (Fig. 3). The fraction B was concentrated to a syrup and dissolved in 70% ethanol. The insoluble substances were removed and the soluble component was used as the purified arabinan. Its sugar composition is shown in Table III; more than 90% of the sugar content was arabinose.

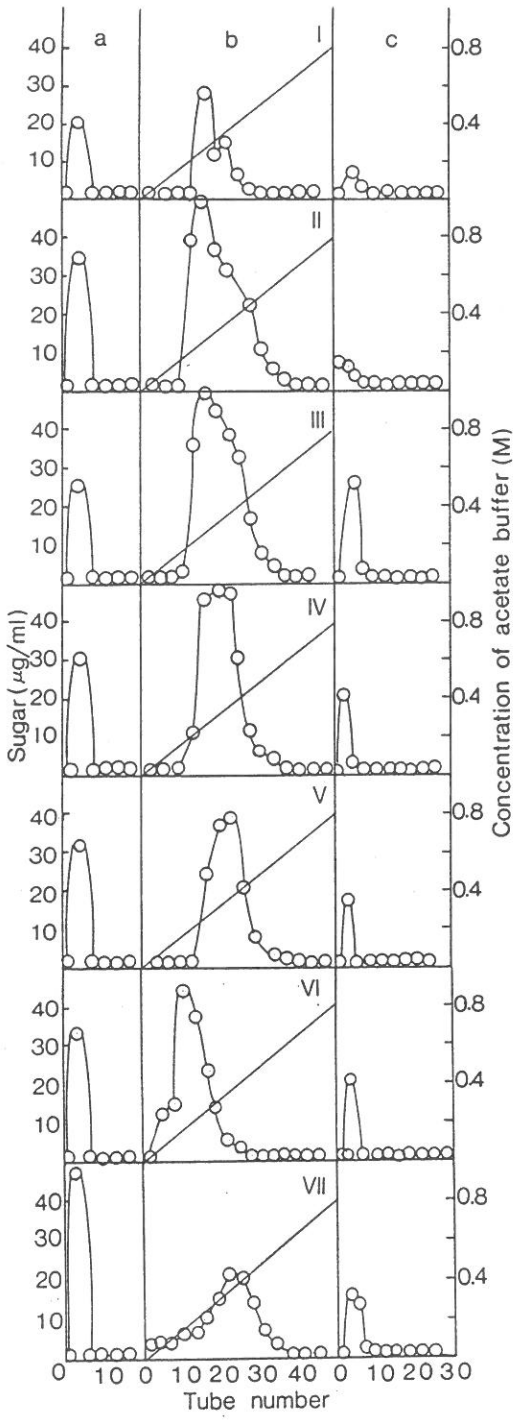

Fig. 2. DEAE–Cellulose Chromatography of Fractions I–Ⅶ.
a, neutral polysaccharide fraction, passed through the column; b, acetate buffer fraction, eluted with a linear gradient of acetate buffer of pH 6.0; c, sodium hydroxide fraction, eluted with 100 mM sodium hydroxide solution. —, concentration of the acetate buffer.

Table Ⅱ. Sugar Compositions of the Weakly Acidic Pectic Polysaccharides in Each Fraction

Fraction	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	GalA(%)
Ⅰ	+	50.1	16.5	100.5	1.6	15.1	7.3	34.4
Ⅱ	1.1	66.2	31.7	119.3	ND	19.6	ND	29.6
Ⅲ	5.0	42.8	121.8	111.9	3.5	17.8	5.9	24.5
Ⅳ	2.3	40.0	118.0	105.4	ND	19.4	4.8	25.6
Ⅴ	1.1	40.7	24.2	85.3	ND	22.5	5.8	35.8
Ⅵ	+	39.2	160.7	89.1	ND	17.5	+	24.6
Ⅶ	1.4	5.9	406.8	39.7	7.2	30.9	26.8	16.2

The values of neutral sugars were expressed as moles per 100 moles of galacturonic acid residue and those of galacturonic acid expressed as molar per cent. ND, not detected; +, trace; Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose; GalA, galacturonic acid.

Table Ⅲ. Sugar Compositions of the Neutral Polysaccharide Components of Kidney Bean Polysaccharides

Neutral polysaccharide[a)]	Arabinose	Xylose	Galactose	Glucose
Fraction A[b)]	53.2	20.2	13.3	13.1
Fraction B[c)]	91.3	+	3.3	5.4

The values were expressed as molar per cent. +, trace. a), The neutral polysaccharide fractions on the DEAE–cellulose chromatography of kidney bean polysaccharides; see Fig. 2 and the text. b), see Fig. 3. c), the 70% ethanol–soluble component of fraction B in Fig. 3.

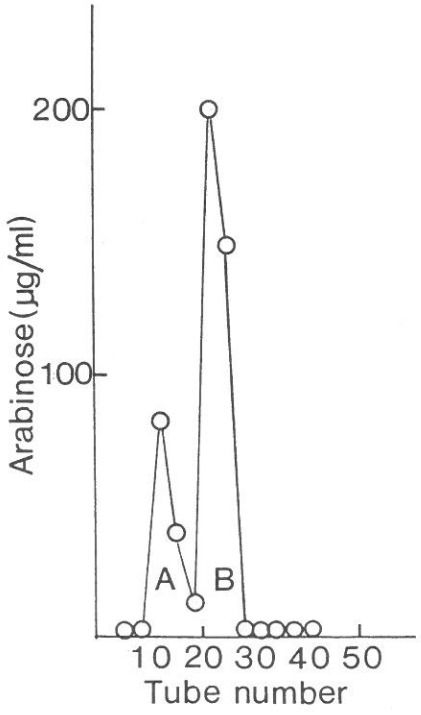

Fig. 3. Gel Filtration on Sepharose CL–4B of the Neutral Polysaccharide Fraction of Kidney Bean Polysaccharides. A, tube numbers 10–18; B, tube numbers 19–28.

Structural changes in cotyledon tissues during polysaccharide extraction

Paraffin slices of the tissues at each extraction stage were stained with ruthenium red and examined by a microscope. The cell walls before extraction were broad and firm, but became thin with repeating extraction. After the twelfth extraction, cell wall dissolution was initiated along the middle lamella and the cells were completely separated at the twentieth extraction.

DISCUSSIONS

Our previous works showed that the pectic polysaccharides of kidney bean cotyledons contained large amounts of xylose and fucose [5, 6, 17] and that the xylose residues were evenly distributed as short side chains along the galacturonan regions of the polysaccharides. [6, 17] In addition, in spite of a high degree of esterification, the pectic polysaccharides were hardly degraded by transelimination mechanism. [5, 17]

In the present work, matrix polysaccharides were exhaustively extracted from kidney bean cotyledons and structural changes of the cell walls were microscopically investigated; the polysaccharides were fractionated into seven fractions (I–Ⅶ) based on their extractabilities (Fig. 1). Each fraction was composed of neutral and acidic polysaccharides; one is arabinan-like neutral polysaccharides and the other weakly acidic pectic polysaccharides (WAPs), which have a low uronic acid content. The WAPs were characteristic in their neutral sugar compositions and methyl ester contents; the major components of neutral sugar were arabinose, xylose and fucose, and the esterification degrees were very high (Tables Ⅰ and Ⅱ).

Just before the start of maceration of the cotyledon tissues, fraction Ⅱ was extracted and the maceration occurred with simultaneous dissolution of fraction Ⅲ. The cells began to separate after the twelfth extraction and elution of the next fraction V resulted in an extensive degradation of the cell walls. Each fraction of Ⅱ–Ⅵ was composed mainly of WAPs (Fig. 2). These results show that the WAPs are the major component of the matrix polysaccharides of kidney bean cotyledon tissues. Accordingly, it is highly probable that the WAPs play an important role in the adhesion of the cell walls similarly to the ordinary pectins of vegetables and fruits. And as a large amount of arabinans was extracted along with the WAPs, it is likely that the arabinans are also concerned with the adhesion of the cell walls.

The ratio of the amount of neutral polysaccharides to that of WAPs is different in each fractions. For example, in fraction Ⅲ, it is 0.78:1.00, and in fraction V, it is 3.08:1.00. Like this, in fraction Ⅲ, the ratio of WAPs is large compared with other fractions, and the galacturonic acid residues are many in quantity (Table Ⅳ). From the results of Fig. 1 and Table Ⅳ, fraction Ⅲ and V are closely related to maceration and collaps of the tissues, respectively.

Table Ⅳ. Amounts of the Extracted Polysaccharides and Molar Ratio of These Polysaccharides.

Fraction	Total galacturonic acid residues (μg/dry weight)	DEAE-cellulose chromatography		
		Neutral polysaccharides	Acetate buffer fractions (molar ratio)	Sodium hydroxide fraction
Ⅰ	435	2.29*	1.00**	0.30**
Ⅱ	483	1.14	1.00	0.08
Ⅲ	2,508	0.78	1.00	0.09
Ⅳ	1,754	1.43	1.00	0.12
Ⅴ	1,502	3.08	1.00	0.15
Ⅵ	850	2.75	1.00	0.22
Ⅶ	7,020	5.98	1.00	0.30

Ⅰ~Ⅵ, see Fig. 1. Ⅶ, the component from supernatant that obtained by precipitation at pH 5.0 from the extractant of 1 M sodium hydroxide solution. *, values as arabinose. **, values as galacturonic acid residues.

It is suggested that the respective polysaccharides, which concern with maceration and collaps of the tissues, exist in a stratified state in the cell walls of kidney bean cotyledons. Because, as mentioned above, the extraction peaks appear and the WAPs of each fractions are characteristics, and the ratio of the amount of neutral polysaccharides to that of WAPs is different in each fractions.

The WAPs and arabinan were extracted simultaneously. Therefore, the arabinan exist in the state of what is wrapped up in the WAPs or mixed both each other.

REFERENCES

1) P. M. Dey and K. Brinson, *Adv. Carbohydr. Chem. Biochem.*, **42**, 265 (1984).
2) P. Albersheim, H. Neukom and H. Deuel, *Arch. Biochem.*, **90**, 46 (1960).
3) J. Ozawa, *Nippon Shokuhin Kogyo Gakkaishi*, **10**, 338 (1963).
4) Y. Matsuura, C. Hatanaka and J. Ozawa, *Ber. Ohara Inst. Landw. Biol. Okayama Univ.*, **16**, 57 (1974).
5) Y. Matsuura, *Nippon Nōgeikagaku Kaishi*, **57**, 851 (1983).
6) Y. Matsuura, *Nippon Nōgeikagaku Kaishi*, **58**, 253 (1984).
7) G. O. Aspinall, R. Begbie, A. Hamilton and J. N. C. Whyte, *J. Chem. Soc.* (C), **1967**, 1065.
8) I. R. Siddiqui and P. J. Wood, *Carbohydr. Res.*, **50**, 97 (1976).
9) Y. Shiota, Y. Matsuura and C. Hatanaka, *Nippon Shokuhin Kogyo Gakkaishi*, **29**, 712 (1982).
10) Y. Shiota, T. Negishi and C. Hatanaka, *Nippon Shokuhin Kogyo Gakkaishi*, **33**, 399 (1986).
11) M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Robers and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
12) T. Bitter and H. M. Muir, *Anal. Biochem.*, **4**, 330 (1962).
13) I. Kusakabe, Y. Kamiyama and T. Yasui, *Nippon Nōgeikagaku Kaishi*, **51**, 167 (1977).

14) Y. Matsuura and C. Hatanaka, *Agric. Biol. Chem.*, **52**, 2583 (1988).
15) L. G. Bartlome and A. J. Hoff, *J. Agric. Food Chem.*, **20**, 262 (1972).
16) C. Hatanaka and J. Ozawa, *Nippon Nōgeikagaku Kaishi*, **42**, 645 (1968).
17) Y. Matsuura, *Nippon Nōgeikagaku Kaishi*, **58**, 1111 (1984).

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