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Extraction and purification of pectic polysaccharides from soybean *okara* and enzymatic analysis of their structures

F. Yamaguchi[a], Y. Ota[b] & C. Hatanaka[b]

[a]*Food R & D Center, Japan Tobacco Inc., 6-2, Umegaoka, Aoba-ku, Yokohama, Kanagawa 227, Japan*
[b]*Department of Applied Biochemistry, Faculty of Applied Biological Science, Hiroshima University, Higashi-Hiroshima, Hiroshima 724, Japan*

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Pectic polysaccharides (6.74 g) were extracted from soybean *okara* (soybean curd waste, 30 g) with sodium hexametaphosphate solution. The extract was separated by DEAE-cellulose chromatography into galacturonate poor and galacturonate rich fractions. The fractionated polysaccharides were exhaustively degraded by three kinds of pectinase and two kinds of hemicellulase, namely exo- and endo-polygalacturonases, exopolygalacturonate lyase, exogalactanase and exoarabinase. The values of the degradation limit revealed that the soybean pectic polysaccharides comprise regions of galacturonan and rhamnogalacturonan carrying side chains composed mainly of homogeneous arabinan and galactan. The galacturonan regions were distributed at both the reducing and nonreducing ends of the polysaccharide. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Soybean *okara* (soybean curd waste) is the residue that remains after oil and protein extraction from soybeans. The soybean *okara* is rich in dietary fibre, and especially in pectic polysaccharides (Higashira & Misaki, 1988), which differ from citrus pectins in that the soybean *okara*'s pectic polysaccharides contain large amounts of neutral sugars. The sugar compositions and sequences of these pectic polysaccharides have already been reported (Aspinall *et al.*, 1967a, b; Kawamura, 1967; Kikuchi & Sugimoto, 1976), but many aspects of their structure are poorly understood.

Pectic polysaccharides are usually composed of two distinct regions, linear galacturonan (GN) and branched rhamno galacturonan (RG) (Barrett & Notrhcote, 1965; De Vries *et al.*, 1986; McCleary & Matheson, 1986; Matsuhashi *et al.*, 1989, 1993). The side chains in the RG region are composed mainly of arabino-4-galactans, which in turn are composed of β-1, 4-D-galactan which are partly substituted by arabinose or arabinosyl oligosaccharides (Dekker & Richardson, 1976). The determination of the amount and distribution of the GN region in pectate molecules, and structure of arabino-4-galactan have been examined by enzymic-HPLC methods (Matsuhashi *et al.*, 1989; Emi *et al.*, 1971; Labavitchi *et al.*, 1976; De Vries *et al.*, 1983; Nakano *et al.*, 1985; Yamaguchi *et al.*, 1995). In contrast for soybean polysaccharide structure the GN region has never been examined except for the study of the endo-β-D-galactanase degradation limit (Emi *et al.*, 1971; Labavitchi *et al.*, 1976; Yamaguchi *et al.*, 1995).

We have started research on soybean pectic polysaccharides in order to find the best potential use for them. In the present work, we examined the extraction and purification of pectic polysaccharide from soybean *okara*, and estimated the amount and distribution of GN regions and side chains' with the enzymic-HPLC method.

MATERIALS AND METHODS

Experimental material

Dried soybean *okara* (Bean-flower) was a gift from Misuzu Tofu Co., Ltd (Nagano, Japan). Acid-insoluble pectates (AIP) used as polygalacturonates (Hatanaka & Ozawa, 1966a) were prepared from citrus pectin. Arabino-3,6-galactan (arabinogalactan, larch wood) were purchased from ICN Co., Ltd. Beet arabinan (Tagawa & Kaji, 1969), corn glucuronoarabinoxylans (corn arabinoxylan, Yamaguchi & Hatanaka, 1993) and crosslinked cell walls from citrus peels (CLCW)

(Hatanaka *et al.*, 1990) were prepared as previously described. The other chemicals (reagent grade) were obtained from Nacalai Tesque (Kyoto, Japan).

Enzymes

Exopolygalacturonase (exo-PG) and endopolygalacturonase (endo-PG) were prepared from carrot root (Hatanaka & Ozawa, 1964) and the culture fluid of *Kluyveromyces fragilis* (Inoue *et al.*, 1984), respectively. Exopolygalacturonate lyase (PGL), was prepared from the culture fluid of *Erwinia carotovora* subsp. *carotovora* IFO 13921 by the method of Kegoya *et al.* (1984). Driselase (Kyowa Hakkou Kogyo Co., Tokyo, Japan) solution was prepared as described by Matsuhashi *et al.* (1992) Cellulase A Amano 3 (from *Aspergillus niger*) was a gift from Amano Pharmaceutical Co., Ltd. (Nagoya, Japan, Yamaguchi *et al.*, 1995).

Extraction of pectic polysaccharides

Effectiveness of the extraction was examined by changing the amount of dried *okara*, from 5 to 10 g using 250 ml of 2% hexametaphosphate solution as extractant at 80°C for 3.5 h; the pH was adjusted to 3.5–4.0 during the extraction. The extract was centrifuged at 6000 g and the residue was reextracted twice with the same solution (pH 2.5). Each extract was dialyzed against tap water for 1 day, then concentrated *in vacuo* and finally dialyzed against deionized water. To the resulting solution, ethanol was added to give a composition of 75% by volume. The precipitates were collected by centrifugation and washed successively with 80% ethanol, 99% ethanol and acetone, then air-dried.

DEAE-cellulose chromatography

Linear gradient of NaCl in phosphate buffer (Hatanaka & Ozawa, 1968)
The crude sample (400 ml, 0.05% solutions) was added to a DEAE-cellulose column (3 × 18 cm; 0.9 meq/g, Brown Co.) equilibrated with 0.02 M phosphate buffer (pH 6.0). After washing the sample with the same buffer, the column was eluted with a linear gradient consisting of 450 ml of 0.02 M phosphate buffer (pH 6.0) in the mixing vessel and an equal volume of the same buffer containing 0.6 M NaCl in the reservoir, and finally with 0.1 M NaOH. The flow rate was 2.0 ml/min, and 10 ml fractions were collected.

The total sugar content and the uronate content in each fraction were analyzed by the phenol-sulfuric acid method (Dubois *et al.*, 1956) and the carbazole method (Galambos, 1967), respectively. Fractions of each sugar peak were collected, dialyzed against tap water and deionized water, successively, and then mixed with two volumes of ethanol. The precipitates collected by centrifugation were washed successively with 70% ethanol, 99% ethanol, acetone, and then air-dried.

pH gradient (9.0 → 11.0) of carbonate buffer (Hatanaka & Ozawa, 1966a)
The crude sample was added to a DEAE-cellulose column equilibrated with 0.02 M $NaHCO_3$ solution. After washing the column with the same solution, the column was eluted with a pH gradient (9.0 → 11.0) consisting of 450 ml of 0.1 M $NaHCO_3$ in the mixing vessel and an equal volume of 0.1 M Na_2CO_3 in the reservoir, and finally with 0.1 M NaOH. Fractions of each sugar peak were collected, and adjusted to pH 4.0 by deionization with Amberlite AG IR-120. Other procedures were conducted in the same manner as with the phosphate buffer elution.

Protein content

Protein content was measured by the Lowry's method (1951).

Estimation of molecular weight distribution of soybean pectic polysaccharides

Molecular weight distributions were estimated by gel filtration. Operating conditions were as follows: Column, Asahipak GS-710 (7.6 × 500 mm); pre-column, Asahipak GS-20G (7.6 × 50 mm); solvent, 0.02 M acetate buffer (pH 6.0); flow rate, 1.0 ml/min; detector, differential refractometer (Jasco TRI ROTAR-IV); injection: 50 μl. Pullulans (Showa denko, Tokyo, Japan) were used as standards for the estimation.

Measurement of neutral sugars and galacturonates

Both neutral sugar and galacturonate contents were determined by the Enzymic-HPLC method of Matsuhashi *et al.* (1992). The reaction mixtures containing 0.1% sample solution, 0.1% glycerol (internal standard), driselase solution (0.5 ml/ml) and 50 mM sodium acetate buffer (pH 4.0) were incubated at 35°C for 48 h, and then filtered with a Millipore Molcut II GC (1×10^4 Da exclusion limit for globular proteins). The filtrates were analyzed by HPLC under the following conditions: Column, Shodex SUGAR SH1821 (8 × 300 mm); pre-column, Shodex SUGAR SG1011P (6 × 50 mm); column temperature, 40°C; flow rate, 1.0 ml/min; mobile phase, 0.001 N H_2SO_4.

Neutral sugar compositions were measured as follows: The samples were hydrolyzed at 121°C for 2 h in 1 N TFA (Albersheim *et al.*, 1967). The hydrolyzates were reduced, acetylated and analyzed by GLC according to the method described by Kusakabe *et al.* (1977), except that a 3% ECNSS-M (60–80 mesh) glass column (3 × 2000 mm) was used, and the column temperature raised from 170 to 200°C by 1°C per min.

Enzyme assays

Polysaccharide-degrading activities were determined by measuring the reducing sugars generated wing the modified Somogyi method (Hatanaka & Kobara, 1980).

The standard assay reaction took place in a 0.2% solution of the appropriate polysaccharide in 0.1 M sodium acetate, pH 4.0, and was incubated with the enzyme preparation at 35°C for 120 min in a total volume of 0.5 ml. The reaction was terminated by adding 0.5 ml of Somogyi's reagent. The amount of reducing sugars produced was determined by Hatanaka's method (1980). One unit of the enzyme was defined as the amount which liberated 1 μmol of reduced sugars per min under the above conditions.

Separation of soybean polysaccharide (SP) degradation enzymes (Yamaguchi *et al.*, 1995)

A mixture of cellulase A Amano 3 (12 g) solution was heat-treated at 60°C for 20 min, and applied onto a DEAE-toyopearl 650 M column (1.5 × 47 cm) which had been equilibrated with 10 mM Tris-HCl buffer, pH 7.0. The enzymes were eluted with a linear NaCl gradient, prepared from 250 ml each of 10 mM Tris-HCl buffer and the same buffer containing 0.5 M NaCl, at a flow rate of 20 ml per h. Eluents were collected in 10 ml fractions, and SP degrading activities of the fractions was assayed by HPLC analysis. Fractions 64–76 (F1) and fractions 77–84 (F2) contained liberated galactose and arabinose from the crude sample, respectively.

Partial purification of the enzyme

The F1 enzyme

An exo type galactanase in the F1 fraction was purified as follows: NaCl was added to the F1 fraction to 4 M, the solution was applied onto a Butyl-toyopearl 650C column (2 × 20 cm) which had been equilibrated with 20 mM acetate buffer, pH 4.0, and the enzyme was eluted with a linear NaCl gradient (0 M → 0.5 M). The fraction was applied to the crosslinked cell walls (CLCW) column (1.5 × 7 cm) (Hatanaka *et al.*, 1990; Matsuhashi & Hatanaka, 1991) which had been equilibrated with 20 mM acetate buffer, pH 4.0. After being washed with the same buffer, the enzyme was eluted with 50 mM acetate buffer, pH.4.5, containing 0.5 N NaCl. To obtain the purified enzyme, the concentrated solution was subjected to gel filtration on a column of Sephacryl S-200 (1 × 92 cm) equilibrated with 10 mM acetate buffer, pH 4.5, containing 0.25 M NaCl at a flow rate of 20 ml/h.

The F2 enzyme

An exo type arabinase in the F2 fraction was purified by CLCW chromatography and gel filtration as described for the F1 fraction.

Determination of enzyme molecular weights (Andrews, 1965)

Molecular weights of the enzymes were estimated by gel filtration using a Sephacryl S-200 column. Phospholylase (94,000), bovine serum albumin (68,000), ovalbumin (45,000) and chymotripsinogen (27,500) were used as standards.

HPLC analysis of hydrolysate by hemicellulases

The reaction mixtures (total volume 1.0 ml) containing 0.5% SP, 0.1% glycerol (internal standard for HPLC), the enzyme preparation, and 50 mM sodium acetate, pH 4.0, were incubated at 35°C for 3 h or 48 h. The reaction mixtures were filtered with a Millipore Molcut II GC (the exclusion limit for globular protein: 1×10^4 Da). The resulting filtrates (50 μl) were used for HPLC analysis using a Shodex SUGAR SH1821 column. Degradation limits were determined by measuring neutral sugars and galacturonates with HPLC as described by Matsuhashi *et al.* (1992).

HPLC Measurements of the degradation limits of pectic polysaccharides by pectinases

Degradation limits of pectic polysaccharides by pectinases were measured by the method of Matsuhashi *et al.* (1989, 1993).

The reaction mixture containing C-APS2 (0.05%), glycerol (0.1%, internal standard for HPLC), sodium acetate buffer (50 mM, pH 5.0 at 35°C, for exo- and endo-PG) or tris buffer (50 mM, pH 8.5 at 35°C, for exo-PGL), and the enzymes (50 mU) were incubated at 35°C for 48 h; for the combined action of exo- and endo-PG, 100 mU/ml of each enzyme was used. In the case of degradation by exo-PGL and exo-PG in sequence, the reaction mixtures containing 0.1% C-APS2, 0.1% glycerol, 20 mM tris buffer (pH 8.5 at 35°C), and exo-PGL were incubated at 35°C for 48 h. The reaction mixtures were added to an equal volume of sodium acetate buffer (100 mM, pH 5.0) containing exo-PG (25 mU), and the resulting mixture was reincubated at 35°C for 48 h. HPLC analysis conditions were prepared as by the method introduced in the previous papers (Matsuhashi *et al.*, 1989, 1993).

RESULTS AND DISCUSSION

Extraction of pectic polysaccharides from soybean *okara*

Figure 1 shows the uronate and protein concentrations (A280) in each extract. In the case of 5 g soybean *okara*, the uronate concentration was maximal in the first extract, whereas the protein concentration was maximal in the third extract. With 10 g soybean *okara*, both uronate and protein concentrations were maximal for

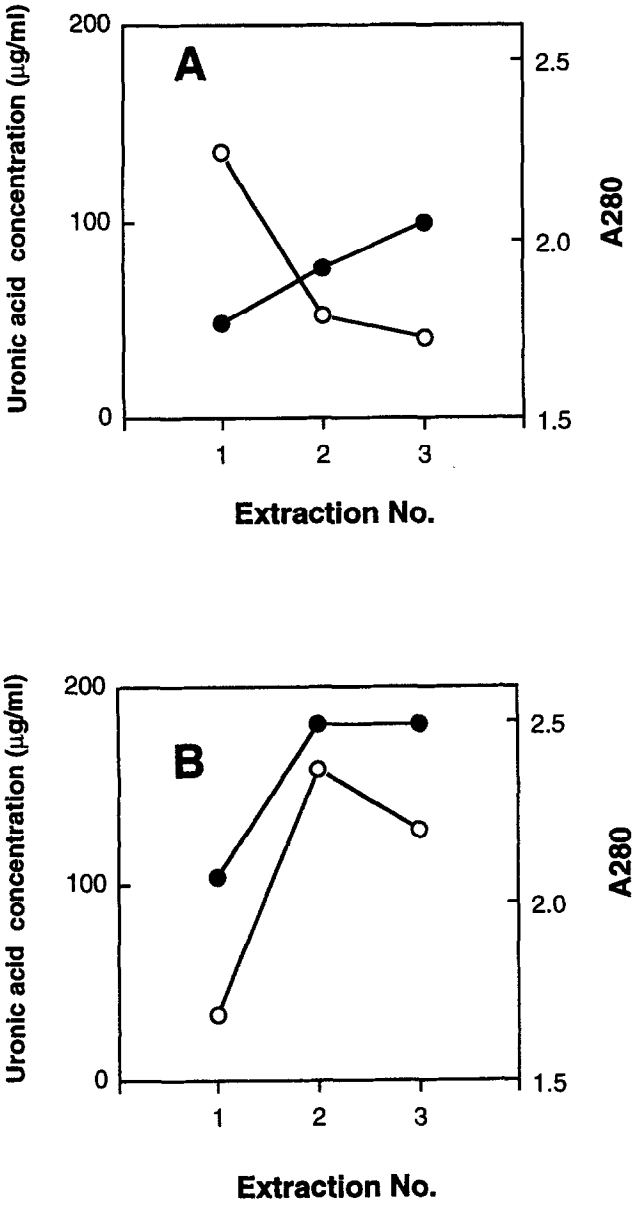

Fig. 1. Extraction of pectic polysaccharides from 5 g (A) and 10 g (B) soybean *okara* with 2% sodium hexametaphosphate solution (250 ml, pH 4.0): ○, uronic acid contents (carbazole method); •, protein content (A280).

the second extraction. These results suggested that soybean pectic polysaccharides were almost completely extracted with 50 volumes of 2% hexametaphosphate solution at 80°C for 3.5 h repeated 3 times.

On the basis of the results described above, pectic polysaccharides (6.74 g) were extracted from 30 g soybean *okara*. The yield was 4.89 g from the first extract, 1.02 g from the second extract and 0.08 g from the third extract, respectively. The polysaccharides obtained here were used for the analysis which follows.

Purification of soybean pectic polysaccharides

Anion exchange chromatography under alkaline conditions is well known for separating pectic polysaccharides, but this method can not be applied to pectins because of alkaline deesterification and β-eliminative cleavages of the galacturonide bonds (Hatanaka & Ozawa, 1966a). Nevertheless, we used DEAE-cellulose chromatography with alkaline or acidic buffers in this work.

The DEAE-cellulose chromatogram obtained with phosphate buffer is show in Fig. 2(A). Soybean pectic polysaccharides were separated into a neutral poly-

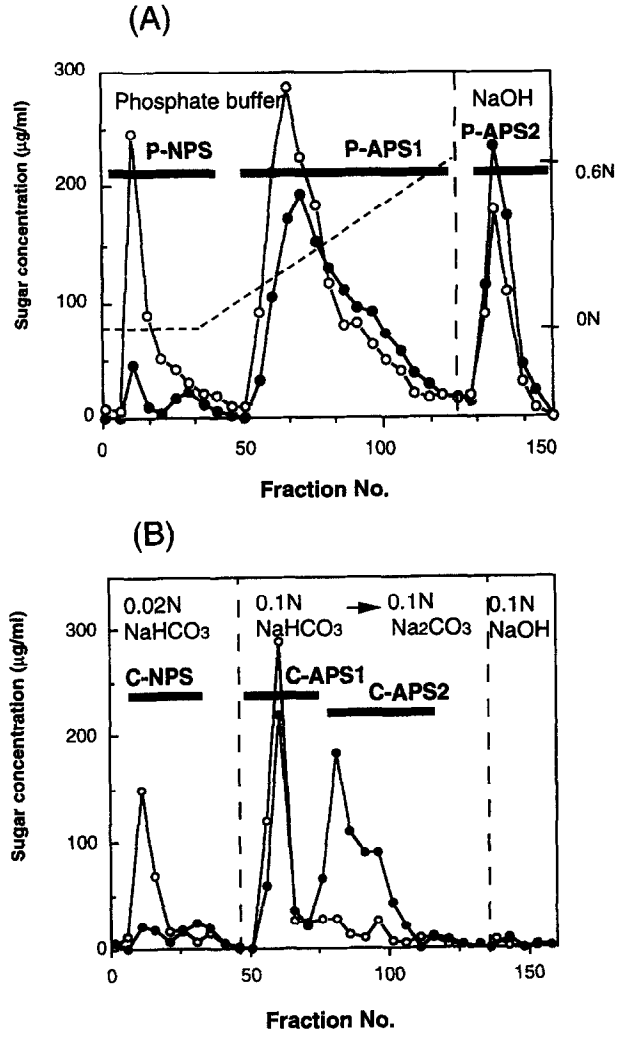

Fig. 2. DEAE-cellulose chromatography of soybean pectic polysaccharide. (A) The crude sample (0.05% solution in 0.02 M phosphate buffer, pH 6.0; 400 ml) was added to a DEAE-cellulose column (3.0 × 18 cm) equilibrated with the same buffer. The column was washed successively with 0.02 M phosphate buffer (pH 6.0), a linear gradient of NaCl (0 → 0.6 M) in the same phosphate buffer, and 0.1 M NaCl. 10 ml fraction were collected. (B) The crude sample (0.05% solution in 0.02 M $NaHCO_3$; 400 ml) was added to a DEAE-cellulose column (3.0 × 18 cm) equilibrated with the same $NaHCO_3$ solution. The column was washed successively with 0.02M $NaHCO_3$, a pH gradient of carbonate buffer (0.1 M $NaHCO_3$ → 0.1 M Na_2CO_3), and 0.1 M NaOH. 10 ml fractions were collected. ○, Total carbohydrate contents; •, uronate contents.

saccharide fraction (P-NPS) and two acidic polysaccharide fractions, P-APS1 eluted with the gradient elution and P-APS2 eluted with NaOH solution. The yields of the three fractions were 20.2, 198.3 and 42.7 mg, respectively. The DEAE-cellulose chromatogram developed with carbonate buffer is shown in Fig. 2(B). The pectic polysaccharides were also separated into three fractions, C-NPS, C-APS1, and C-APS2. In this case, however, no sugar peak was observed when the NaOH solvent was used. The yields of the three fractions were 17.0, 91.0 and 92.0 mg. respectively.

The yields, protein contents, and sugar compositions of each polysaccharide fraction are shown in Table 1. The protein content was greatly diminished by DEAE-cellulose chromatography. Uronate contents obtained by the colorimetric methods, such as carbazole method (Galambos, 1967) and *m*-hydroxybiphenyl method (Blumenkrantz & Asboe-Hansen, 1973), were estimated to be higher in yield than those obtained by the enzymic-HPLC analysis (Matsuhashi & Hatanaka, 1992). In this work, we used the enzymic-HPLC method which was supposed to be simple and accurate. Drisalase which was used for the pectin degradation in the method was completely hydrolyzed to monomeric units after 24 h incubation (date not shown). In the case of DEAE-cellulose chromatography with carbonate buffer, most of galacturonate residues were eluted in the C-APS2 fraction. The HPLC analysis showed that all the uronate component in the polysaccharide fractions was galacturonate (data not shown, Yamaguchi & Hatanaka, 1993).

Table 2 shows the neutral sugar compositions of the pectic polysaccharide fractions; galactose and arabinose were the main components in each fraction. Xylose and glucose in the fractions differed from each other, but there was a close resemblance among compositions of the other sugars.

Gel filtration patterns of each fraction are shown in Fig. 3. Low molecular weight components were eluted in C-NPS and high molecular weight components which were the main components of the crude samples were found in C-APS1 and C-APS2. The molecular weight of the main components of both C-APS1 and C-APS2 was estimated to be about 500,000.

Partial purification of SP degradation hemicellulases

In order to examine the distribution of galactose and arabinose, which are the main components of the polysaccharides, we partially purified exo type hemi-

Table 1. Fractionation of soybean pectic polysaccharides by DEAE-Cellulose chromatography

	Fraction No.	Yield[a] (%)	Protein[b] (%)	Sugar Composition[c] (%)	
				GA	NS
Crude sample		22.47	9.4	18.5	81.5
Phosphate buffer					
P-NPS	6–46	2.27	1.8	8.3	91.8
P-APS1	52–126	22.28	1.8	18.3	81.7
P-APS2	127–160	4.8	1.8	15.6	84.4
Carbonate buffer					
C-NPS	6–26	1.91	2.7	4.4	95.6
C-APS1	51–71	10.22	1.5	9.5	90.5
C-APS2	71–111	10.33	1.2	36.2	63.8

GA and NS represented galacturonate and neutral sugars content, respectively.
[a]The yield from the starting material (%).
[b]Determined by Lowry method (1951).
[c]Determined by enzymic-HPLC method of Matsuhashi *et al.* (1992). The results are the molar %.

Table 2. Neutral sugar compositions (%) of soybean pectic polysaccharide fractioned by DEAE-Cellulose chromatography with carbonate buffer

	Rha	Fuc	Ara	Xyl	Man	Gal	Glc
Crude sample	2.3	5.3	15.1	6.9	2.1	60.3	8.0
C-NPS	2.4	2.2	11.4	3.6	3.4	61.6	15.4
C-APS1	3.6	4.1	20.5	6.7	2.1	57.3	5.7
C-APS2	5.0	5.5	19.5	12.8	1.2	53.0	3.0

Sugar constituents were analyzed by gas-chromatography (Kusakabe *et al*, (1977).
Rha, rhamnose; Fuc, fucose; Ara, arabinose; Xyl, xylose; Man, mannose; Gal, galactose; Glc, glucose.

cellulases. The results of the purification are summarized in Table 3. The enzyme preparation of F1 and F2 in the final stages were purified about 73.9-fold and 1377-fold over the crude enzyme preparation, respectively. The molecular weights of F1 and F2 were estimated to be about 75,000 and 55,000 by the gel filtration. The maximum activity of F1 and F2 against SP were observed at pH 4.0 and 4.5, respectively.

The substrate specificity of F1 and F2 are shown in Table 4. F1 could degrade all examined polysaccharides, and F2 had degrading activity against beet arabinan and corn arabinoxylan. The result indicated that F2 was a highly purified arabinase.

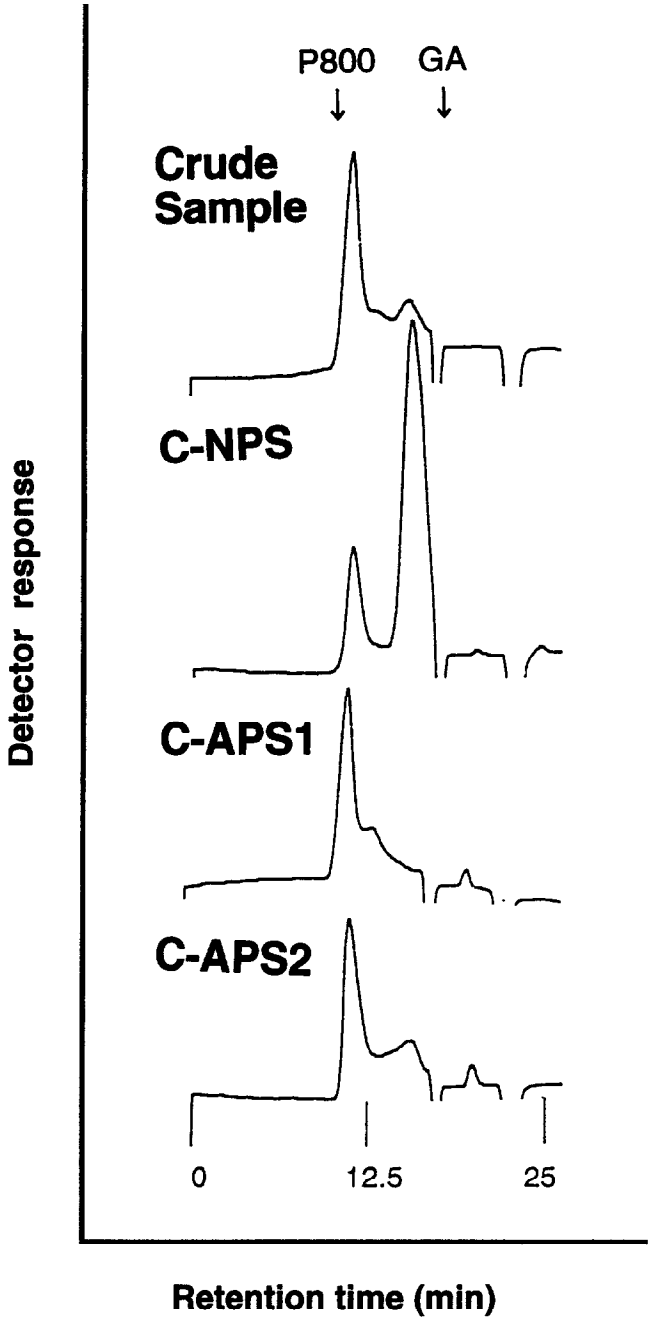

Fig. 3. Gel filtration patterns of soybean pectic polysaccharide fractioned by DEAE-cellulose chromatography with carbonate buffer. Operating conditions: column, Asahipak GS-710, 7.6 × 500 cm; pre-column, Asahipak GS-20G, 7.6 × 50 mm; mobile phase, 0.02 M acetate buffer, pH 6.0; column temperature, room temperature; flow rate, 1.0 ml/min; detector, RI; injection, 50 μl of 1% solutions of each fraction. P-800 and GA indicate the elution volumes of P-800 (MW. 75.8 × 10^4) and galacturonate, respectively.

Action of the enzymes

Analytical results of the hydrolysate of the crude sample by HPLC were shown in Fig. 4. Only galacturonate and galactose were liberated by F1 from the crude sample regardless of the reaction time. These results indicated that F1 had both exopolygalacturonase and exo-galactanase activities against the crude sample. F2 liberated arabinose and the HPLC response also showed a peak with a retention time of about 7.5 min. However the solution eluted at 7.5 min did not change in color by the

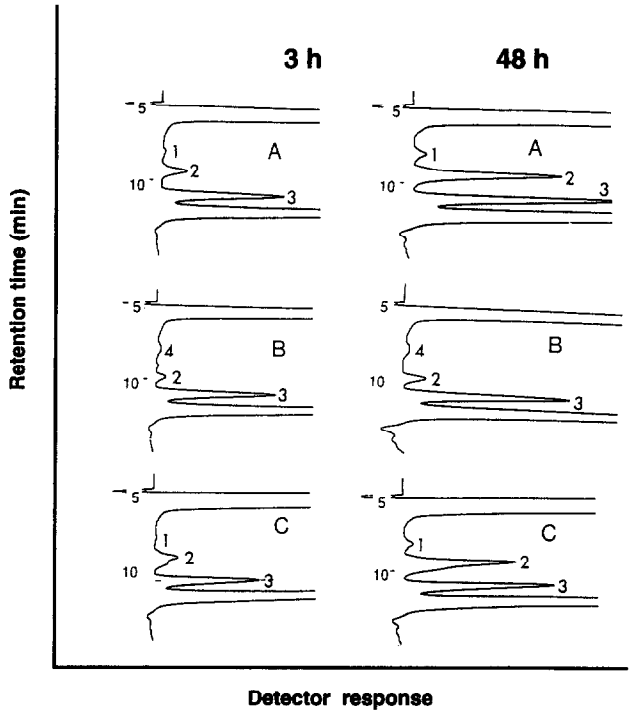

Fig. 4. HPLC chromatograms of degradation products of the F1 and F2 enzyme against the crude sample. Reaction mixtures (total volume 1.0 ml) containing 0.5% the crude sample, 0.1% glycerol (internal standard for HPLC), the enzyme preparation (A, F1; B, F2; C, F1 and F2 in combination), and 50 mM acetate buffer, pH 4.0 were incubation at 35°C for 3 or 48 h. The reaction mixture was filtered with a Millipore Molcuts II GC. The resulting filtrates (50 μl) were used for HPLC analysis injection. Operation conditions for HPLC: column, Shodex SUGAR SH1821 (8 × 300 mm); pre-column, Shodex SUGAR SH1011P (6 × 50 mm); mobile phase, 0.001 N sulfuric acid; column temperature, 40°C; flow rate, 1.0 ml/min; chromatograph, Jasco TRI ROTAR-IV; detector, Jasco RID-300. Peak identity: 1, galacturonate; 2, neutral sugars, 3, glycerol (internal standard); 4, unknown peak.

Table 3. The purification of pectic polysaccharides sidechain degradation enzymes from Cellulase A Amano 3

	Volume (ml)	Protein (mg)	Enzyme activities (units)	Specific activities (units/mg)
F1				
Enzyme Preparation	85	3216.4	12169	3.8
DEAE-toyopearl	124	901.5	5116	5.6
Butyl-toyopearl	740	116.1	4921	42.4
CLCW	205	115.6	3055	26.4
Sephacryl S-200	108	31.4	2322	73.9
F2				
Enzyme Preparation	85	3216.4	12169	3.8
DEAE-toyopearl	134	112.4	3426	30.5
CLCW	133	31.5	2600	82.5
Sephacryl S-200	40	1.9	2556	1377.3

Table 4. Relative rate of hydrolysis (%) of F1 and F2 on the various polysaccharides

	Major Linkage	Relative activity (%)	
		F1	F2
Soybean Pectic Polysaccharide	α-1,4-Gal*p*A, β-1,4-Gal*p* α-1,5-Ara*f*, α-1, 3-Ara*f*	100	100
Polygalacturonan (AIP)	α-1, 4-Gal*p*A	57.9	0
Beet Arabinan	α-1,5-Ara*f*,α-1, 3-Ara*f*	46.3	101
Arabino-3,6-galactan	β-1,3-Ara*f*, α-1,6-Ara*f* β-1,3 3-Gal*p*	88.6	0
Corn Arabinoxylan	β-1,4-Xyl*p*,α-1,3-Ara*f*	259.8	14.1

Araf, arabinofuronose; Galp, galactopyranose; Xylp, xylopyranose; GalpA, galactopyranoic acid.

phenol-sulfate reaction, indicating that it was not carbohydrate. Furthermore both galacturonate, galactose and arabinose (a peak shoulder) were liberated by F1 and F2 in combination.

Degradation limits of C-APS1 and C-APS2 by exo type hemicellulases

Table 5 shows the results of degradation limits of C-APS1 and C-APS2 by F1, F2, and F1 and F2 in combination. These enzymes can liberate over 50–90% of each neutral sugar. The sum of the amount of neutral sugars liberated by F1 and F2 was equal to the amount by F1 and F2 in combination. Only galactose or arabinase were liberated by the exo-galactanase or the exo-arabinase from the purified soybean pectic polysaccharides. From the data, it can be concluded that side chains of soybean pectic polysaccharides consisted of homogeneous polymers. Moreover F1 liberated 31.2% of galacturonate from C-APS2. The result agreed very closely with the results obtained using the purified exo-PG against C-APS2 (Table 6).

Distribution of GN region in C-APS2

Most of the galacturonate residues of the polysaccharides were in the C-APS2 fraction. Therefore, we examined the distribution of the galacturonan region in C-APS2 by estimating the values of the degradation limit by pectinases.

Table 6 shows the extents of the degradation of C-APS2 by several pectinases. The soybean polysaccharides were degraded by pectinases. The susceptibility of C-APS2 to the action of exo type pectinases suggests that a part of the galacturonate residues is attached to the molecular end as a linear chain. The degradation limit of C-APS2 by exo-PG alone was lower than that of C-APS2 by exo-PG and exo-PGL in

Table 5. Degradation limits (%) of pectin sidechain degradation enzymes on purified soybean pectins

		F1	F2	F1 + F2
C-APS1	Neutral sugars	35.5 (68.4)[a]	9.1 (49.0)[c]	43.8 (62.2)[d]
	Galacturonate	1.5 (15.5)[b]	0	1.5 (15.8)[b]
C-APS2	Neutral sugars	31.5 (93.7)[a]	9.7 (78.9)[c]	42.0 (91.3)[d]
	Galacturonate	11.4 (31.2)[b]	0	10.8 (29.6)[b]

Figures indicate percentage of liberated monosaccharides per total sugars. [a]liberated galactose/total galactose; [b]liberated galacturonate/total galacturonate; [c]liberated arabinose/total arabinose.

Table 6. The degradation limit of purified soybean pectate (C-APS2) by pectinases

	GN region[a]	Degradation limit (%)
Exo-PG	(α + γ)	32.8
Exo-PGL	(β + γ)	58.3
↓	↓	
Exo-PG	α	26.1
Exo- and endo-PG	(α + β + γ + δ)	53.3

Degradation limit (%) were expressed as percentage of total uronic linkages.
[a]α, GN chains having a non reducing end; β, those having a reducing end; γ, those having both reducing and nonreducing ends; δ, those interposed between two RG regions.

sequence. The result suggests the existence of linear galacturonan at both reducing and nonreducing end sides of the C-APS2 molecule. The extent of degradation by exo-PGL was 5.0% higher than that by exo-PG and endo-PG in combination. This result indicated that no galacturonan region interposed between two RG regions exists in the molecule (Fig. 5). The value for exo- and endo-PG in combination which should be the highest, however, was lower than obtained with exo-PGL alone. The reason for the unsuspected results is possibly a structural obstacle, which is caused by the neutral sugar sidechains of C-APS2, against the enzymes activities.

CONCLUSION

Soybeans contain arabinogalactan and pectic polysaccharide as main components of their cell walls. Aspinall *et al.* (1967a, b) extracted polysaccharides with 2% EDTA disodium salt at 85–90°C after removing the proteins, and purifying by DEAE-cellulose chromatography using phosphate buffer. In this report, we extracted the polysaccharide with 2% sodium hexametaphosphate solution, and the polysaccharides were then purified by DEAE-cellulose chromatography with carbonate buffer. The pectic polysaccharides which were eluted as a single peak by the previous chromatography were separated into 2 peaks by our method, making it possible to purify the preparation further than had been done previously.

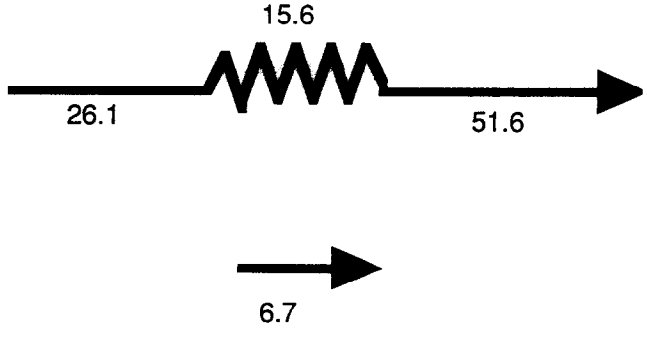

Fig. 5. Distribution of GN region in the purified soybean pectic polysaccharides (C-APS2). Straight lines, notched lines and arrows indicate GN regions, RG regions and the reducing end, respectively. Figures express the percentages of galacturonate distribution.

Although a partial sequence of soybean acidic polysaccharide has been reported, the whole structure of the molecule had not been described (Aspinall *et al.*, 1967a, b; Kawamura, 1967; Kikuchi & Sugimoto, 1976). In general, the pectic polysaccharides consist of homogalacturonan ('smooth regions' and hybridization region of neutral sugars and galacturonate ('hairy regions') which are located between galacturonan regions (De Vries, 1986; Matsuhashi *et al.*, 1993). However Matsuura *et al.* (1973), have reported that a kidney bean pectic polysaccharide was degraded only to a small extent by polygalacturonases, which indicated that it contained only a few galacturonans. In the case of the side chains, it had been only reported that it consists of homogeneous galactan by endo-β-D-galactanase degradation limit (Emi *et al.*, 1971; Labavitchi *et al.*, 1976; De Vries *et al.*, 1983, 1985; Yamaguchi *et al.*, 1995). So we examined the structure using three kinds of pectinase and two kinds of hemicellulase. The values of the degradation limit revealed that the soybean pectic polysaccharides comprise regions of galacturonan and rhamnogalacturonan, and the galacturonan regions were distributed at both the reducing and nonreducing ends of the molecules. In addition, the side chains branched from rhamnogalacturonan were mainly composed of homogeneous arabinan and galactan.

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