Analysis of the Molecular Construction of Xylogalacturonan Isolated from Soluble Soybean Polysaccharides

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Soluble soybean polysaccharides (SSPS) extracted from soybean cotyledons are acidic polysaccharides, and exhibited a pectin-like structure. After digesting galacturonan with polygalacturonase, two novel galacturonan (GN) fragments, which were directly linked to xylosyl oligosaccharides, were obtained. One consisted of \((\beta-D-Xyl)7\) branched at the C-3 site of 1,4-linked \((\alpha-D-GalA)4\), and the other consisted of \((\beta-D-Xyl)4\) branched at the C-3 site of 1,4-linked \((\alpha-D-GalA)3\).

Key words: soybean; polysaccharide; pectin; xylose

The structural analysis of SSPS by stepwise enzymatic degradation with hemicellulase and pectinase has indicated that the main backbone of SSPS consisted of rhamnogalacturonan (RG) and galacturonan (GN), and the RG chains were linked to each other by intervention of the GN chains.1) Mild acid hydrolysates of acidic soy sauce polysaccharides has resulted in xylose containing GN fragments of Xyl \(\beta1-3\) GalA and Xyl \(\beta1-3\) GalA \(\alpha1-4\) GalA already being reported.2,3) Xylose is a component sugar of the primary cell wall, and has been considered to play an important role in the linkage among pectic polysaccharides, hemicellulose and cellulose.4) The objective of this present report is to analyze the detailed structure of the xylogalacturonan obtained from purified SSPS by enzymatic degradation.

The digested products of the SSPS main backbone (SSPS H1), which were previously galactan and arabinan chains digested with PGase, were fractionated in a Sepharose CL-4B column (Fig. 1A). H1P3 (MW < 10,000) was pooled and further purified in a Bio-Gel P-4 column (Fig. 1B). Six fractions were obtained, and the sugar compositions were analyzed.5,6) H1P3O4 (MW = approx. 1,500-2,000) was composed of 42.5% of xylose and 33.1% of galacturonic acid (Table 1). H1P3O4 was further purified in a QAE-Sephadex A-50 ion-exchange column, and two GN fragments (H1P3O4Q4 and H1P3O4Q5), which only consisted of xylose and galacturonic acid, were obtained (Fig. 1C). Table 1 shows the results of a linkage analysis of the two fractions.7,8) Xylose and galacturonic acid were respectively linked at the C-2 and C-4 sites, and C-3 and C-4 sites. H1P3O4Q4 and H1P3O4Q5 were partially hydrolyzed in 0.5 N trifluoroacetic acid at 70°C for 30 min. The generated fragments were separated by HPLC,9,10) and the sugar composition analyzed. Xylose, galacturonic acid, (Xyl)2, (Xyl)3, (Xyl)4, (Xyl)2GalA, (Xyl)2(GalA)2, and (GalA)3 were liberated from H1P3O4Q4, and further fragments of Xyl(GalA)2, (Xyl)3, (Xyl)4(GalA)2, and Xyl(GalA)3 were liberated from H1P3O4Q5 (data not shown). These results indicate that xylose formed the xylan side chain and was linked to oligogalacturonate.

In order to analyze the molecular mass and sugar arrangements of H1P3O4Q4 and H1P3O4Q5, the reducing end of each fraction was converted with ABEE (ethyl p-aminobenzoate), and further purified by reversed-phase HPLC in a Develosil ODS-T5 C18-HG5 column (4.6 mm × 150 mm; Nomura Chemicals, Japan) (Fig. 1D).9,10) The main fraction of ABEE-converted H1P3O4Q4 (retention time = 11.0 min), which was separated in three major fractions, was collected, re-chromatographed, and then yielded H1P3O4Q4m (Fig. 1D(b)). The main fraction of ABEE-converted H1P3O4Q5 was also collected as H1P3O4Q5m (Fig. 1D(c)). ABEE-converted galacturonic acid (ABEE-GalA), which had been identified from the analytical data of molecular mass (data not shown), was detected as a composing sugar in addition to xylose and galacturonic acid (Table 1). The numbers of sugar residues were respectively calculated as ABEE-GalA:GalA:Xyl = 1.0:2.9:6.7 in H1P3O4Q4m and 1.0:1.8:3.9 in H1P3O4Q5m.

An FAB-MS analysis in the positive-ion mode was performed on H1P3O4Q4m and H1P3O4Q5m. The
Fig. 1. Purification of Xylogalacturonan from SSPS H1.

A) SSPS H1 (100 mg) digested with PGase (5.0 ml of a 2% solution) was loaded into a Sepharose CL-4B column (1.5 cm × 90 cm) that had been equilibrated with a 50 mM sodium acetate buffer at pH 5.0. The flow rate was 0.2 ml/min, and 3-ml fractions were collected. Fractions number 63–90 (H1P1), 100–115 (H1P2), and 116–127 (H1P3) were respectively pooled. Vo, void volume; Vi, inner volume

B) The H1P3 fraction was desalted by Bio-Rad AG 501-X8, concentrated to 2 ml and loaded into a Bio-Gel P-4 column (2.0 cm × 120 cm) that had been equilibrated with a 50 mM sodium acetate buffer at pH 5.0. The flow rate was 0.2 ml/min, and 3-ml fractions were collected. Fractions number 46–62 (O1), 71–78 (O2), 82–105 (O3), 130–142 (O4), 147–160 (O5), and 165–196 (O6) were respectively pooled.

C) The H1P3O4 fraction was desalted by Bio-Rad AG 501-X8 and loaded into a QAE-Sephadex A-50 column (1.5 cm × 10 cm) that had been equilibrated with a 10 mM NH₄HCO₃ solution at pH 8.0, and the column was washed with the same NH₄HCO₃ solution. Bound polysaccharides were eluted with a linear gradient of NH₄HCO₃ from 10 mM (140 ml) to 600 mM (140 ml). The flow rate was 0.2 ml/min and, 3.0-ml fractions were collected. Fractions number 1–10 (Q1), 19–37 (Q2), 45–58 (Q3), 72–95 (Q4), and 101–115 (Q5) were respectively pooled.

D) The H1P3O4Q4 and H1P3O4Q5 fractions, which consisted of galacturonic acid and xylose residues, were converted with ABEE. The resulting derivatives were separated from the excess reagent by reversed-phase HPLC. The operating conditions for HPLC were as follows: column, Develosil ODS-T5 C18-HGS (4.6 mm × 150 mm, Nomura Chemicals, Japan); column temperature, 30°C; mobile phase, linear gradient of an aqueous acetonitrile mixture with 0.1% TFA from 10% to 30% CH₃CN in 30 min; flow rate, 1.0 ml/min; detection, UV (absorbance at 310 nm; range, 1-16E-5); injection volume, 100 µl. The ABEE-converted oligosaccharide peaks (indicated by arrows as Q4m and Q5m) were pooled.

FAB-MS data and the possible structures are indicated in Fig. 2. The molecular mass of H1P3O4Q4m was measured as [M + H]+ = 1795.4. The xylose side chain seemed to be branched at the C-3 site of the secondary galacturonic acid from the non-reducing end of tetra-galacturonic acid, from the results that only one galacturonic acid at the non-reducing end was fractionated (m/z = 1619.3) and that other fragment ions corresponding to galacturonic acids were obtained. The digested products of H1P3O4Q4m by 1,4-β-D-xylosidase (from Asp. niger, Sigma) were also analyzed by FAB-MS. Four xylose residues were digested from H1P3O4Q4m (m/z = 1795.4 → 1266.9), and the second xylose residue branched from (GalA)₄ was indicated to be 2,4-linked xylose (Fig. 3A). The molecular mass of H1P3O4Q5m was measured as [M + H]+ = 1222.9. The xylose side chain was suggested to be branched at the C-3 site of (GalA)₃. Two xylose residues were digested from H1P3O4Q5m by the xylosidase, implying that the xylose residue which was linked to (GalA)₃ was 2,4-linked xylose. However, how the 1,4-linked β-D-xylosides, which were digested from H1P3O4Q4m and H1P3O4Q5m by the xylosidase, were linked to the remaining structures
Table 1. Sugar Composition and Methylation Analysis of PGase-treated SSPS H1

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Sugar component</th>
<th>Linkage</th>
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<tbody>
<tr>
<td>Arabinose</td>
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<tr>
<td></td>
<td>Arabinose</td>
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<td></td>
<td>O1</td>
<td>O2</td>
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<tr>
<td></td>
<td>3.7</td>
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<td>Galactose</td>
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<td></td>
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<td></td>
<td>O1</td>
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<tr>
<td></td>
<td>18.9</td>
<td>21.5</td>
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<tr>
<td>Fucose</td>
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<td></td>
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<td></td>
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<td>7.0</td>
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<td>O1</td>
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2,3,4-Me3-D-Xyl terminal 19.4 28.9
2,3-Me2-D-Xyl 4 38.1 15.8
3-Me-D-Xyl 2 4.8 14.7

Rhamnose
23.8 28.6 27.2 30.5 15.4 28.7 18.3 23.4 24.2 25.9 0.0 0.0 0.0 0.0
Galacturonic acid
38.3 34.8 36.7 42.6 33.1 42.9 63.6 33.3 38.9 43.3 34.2 40.6 27.1 26.7
2,3,4,6-Me3-D-Gal terminal 8.7 13.2
2,3,6-Me2-D-Gal 4 17.0 14.5
2,6-Me2-D-Gal 3,4 8.5 12.9
ABEE-GalA* 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 9.5 15.1
Yield (%)** 5.8 0.6 0.5 1.1 1.4 0.6 0.8 0.11 0.20 0.32 0.36 0.12 0.12 0.04

* ABEE-converted galacturonic acid; ** Overall yield to that of purified SSPS

Fig. 2. FAB Mass Spectra of the H1P3O4Q4m and H1P3O4Q5m Fractions.

The FAB-MS data and the possible structures are indicated for the ABEE-converted oligosaccharides of H1P3O4Q4m (A) and H1P3O4Q5m (B). FAB mass spectra were obtained with a Jeol HX-100 double-focusing mass spectrometer fitted with a 18.8-kilogauss magnet and FAB ion source. 0.5–1 μl of a sample (1–10 μg/ml) was transferred to the stainless steel sample plate and mixed with 0.5–1 μl of glycerol. The sample plate was then inserted into the FAB chamber. Fast atom bombardment was generated by a xenon neutral beam accelerated by a 7 keV potential. A data processor (Jeol JMA-3100 mass data analysis system) was used for acquiring the mass spectra. Mass assignment was made by using a mixture of CsI and KI (1:2, w/w) as the mass reference. Xyl, xylose; GalA, galacturonic acid.
was not clarified. Kikuchi et al.⁴ have obtained Xyl β1-3 GalA and Xyl β1-3 GalA α1-4 GalA from the acidic polysaccharide of soy sauce, but the xylan chains, which were linked to galacturonic acid, were possibly hydrolyzed by the acid. Matsuura et al. have reported that some galacturonic acid in kidney bean (Phaseolus vulgaris) and red bean (Vigna angularis) was modified with xylose residues (molar ratio of GalA:Xyl = 3–1:1), and formed xylogalacturonan.¹¹,¹² The structure of the GN chain modified with xylose might be considered to be characteristic of pectic polysaccharide of the legume family. The GN fragments of soybean were obtained by stepwise enzymatic degradation, and some galacturonic acid was modified with xylosyl oligosaccharides which were composed of β-1,4- and β-1,2-linked xylose residues.

References