Note

Isolation and Characterization of an Acidic Xylan from Gobo (Arctium lappa L.)

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From the viewpoint of the physiological function of dietary fiber, it is important to elucidate the structure and properties of cell wall polysaccharides. Gobo (edible burdock, Arctium lappa L.) has very often been used to study the physical properties of dietary fibers. However, chemical characterization of the polysaccharides in gobo has not been done in detail. In a previous work, we suggested from the methylation analysis that the cell walls of gobo consisted of rhamnogalacturonan with neutral sugars, arabinan, xylan, galactan, arabinogalactan, xyloglucan, and cellulose. This work describes the isolation and characterization of acidic xylan. Unless otherwise stated, all materials and experimental procedures used for this study were the same as those described in previous papers.

Polysaccharides were extracted from defatted gobo (104.2 g dry wt) successively with hot water, 0.5% ammonium oxalate, 4% potassium hydroxide, and 24% potassium hydroxide. After dialysis eight fractions (PS-I, PS-IIA, PS-IIB, HC-IA, HC-IB, HC-IIA, HC-IIB, and CL) were prepared in a manner almost identical with that reported from mung bean. Neutral sugar composition analysis showed that xylan-like polysaccharides were present in HC-IA and HC-IIB (Table I). The polysaccharide in gobo was characterized using HC-IIB because the yield of HC-IIB was higher than that of HC-IA.

HC-IIB (460 mg dry wt) was dissolved in 1 M sodium hydroxide (8 ml) and centrifuged. The pH of the supernatant was adjusted to 5.0 with acetic acid and centrifuged. The finally obtained supernatant was diluted with distilled water to 40 ml (carbohydrate content: 116.0 mg as xylose equivalent). A portion of the solution (36 ml, 104.4 mg as xylose equivalent) was chromatographed on DEAE-Sephadex A-25 (Fig. 1). The unbound fractions (HC-IIB-a) and fractions eluted with 0.2 M (HC-IIB-b) and 0.5 M sodium hydroxide (HC-IIB-c) were separately combined and dialyzed. The respective yields of subfractions HC-IIB-a, -b, and -c were 41.8, 11.4, and 43.9 mg as xylose equivalent. Neutral sugar analysis showed that the respective subfractions HC-IIB-a, -b, and -c consisted of rhamnose, fucose, arabinose, xylose, glucose, and galactose in the molar ratios of 2.5:9.1:trace:32.1:34.6:21.7, of 6.6:0:trace:73.1:12.8:7.5, and of 6.0:0:trace:79.9:9.0:5.0. HC-IIB-c was designated as acidic xylan (AX) and studied further.

AX was chromatographed on a calibrated Sepharose CL-6B column (1.5x45.5 cm) equilibrated with 0.1 M sodium hydroxide, and its average molecular weight was estimated to be over 1.0x10^6.

AX was hydrolyzed with 0.05 M sulfuric acid for 6 hr at 100°C, and the hydrolyzate was analyzed by paper chromatography (p.c.) with the solvent system of 1-butanol-pyridine-water = 6:4:3. The log Rf/(1−Rf) of

<table>
<thead>
<tr>
<th>Polysaccharide fraction</th>
<th>Yield (g)</th>
<th>Neutral sugar composition (mol%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rha</td>
</tr>
<tr>
<td>PS-I</td>
<td>24.93</td>
<td>1.85</td>
</tr>
<tr>
<td>PS-IIA</td>
<td>0.09</td>
<td>7.59</td>
</tr>
<tr>
<td>PS-IIB</td>
<td>3.14</td>
<td>4.77</td>
</tr>
<tr>
<td>HC-IA</td>
<td>0.27</td>
<td>1.19</td>
</tr>
<tr>
<td>HC-IB</td>
<td>5.82</td>
<td>0.67</td>
</tr>
<tr>
<td>HC-IIA</td>
<td>0.17</td>
<td>2.36</td>
</tr>
<tr>
<td>HC-IIB</td>
<td>1.12</td>
<td>0.67</td>
</tr>
<tr>
<td>CL</td>
<td>9.02</td>
<td>0.04</td>
</tr>
</tbody>
</table>

PS-I and PS-II, pectic substances extracted with hot H2O and (NH4)2C2O4, respectively; HC-I and HC-II, hemicelluloses extracted with 4% KOH and 24% KOH, respectively; CL, cellulose. A and B, precipitated and non-precipitated materials during dialysis, respectively.
A solution of purified HC-IIB was put on a column (2.7 x 15 cm) of DEAE-Sephadex A-25 equilibrated with 50 mM sodium acetate buffer (pH 5.0). The column was washed with the same buffer, then stepwise elution was done with the same buffer containing 0.2 M and 0.5 M sodium chloride, and 0.2 M, 0.5 M, and 1.0 M sodium hydroxide.

Fig. 1. Chromatography of HC-IIB on DEAE-Sephadex A-25.

Major spots detected on the chromatogram, when plotted against the degree of polymerization, gave a linear regression, suggesting that these sugars belong to a homologous series. The Rf and Rxy values of disaccharide (Rf 0.53, Rxy 0.73), trisaccharide (Rf 0.29, Rxy 0.40) and tetrasaccharide (Rf 0.10, Rxy 0.14) were identical with those of authentic β-(1→4)-linked xylobiose, xylotriose, and xylotetraose which were isolated from the partial hydrolyzate of rice hull or rice straw arabinoxylan.

AX was hydrolyzed with partially purified Trichoderma viride endo-(1→4)-β-D-xylanase. The 2-day and 4-day hydrolyzates were individually separated by chromatography on a column (1.5 x 45.5 cm) of Bio-Gel P-2. Di- and mono-saccharide fractions obtained from the hydrolyzates were shown to be homogeneous by p.c. (Rxy 1.0 for monosaccharide and Rxy 0.73 for disaccharide.)

AX was methylated by the method of Hakomori, and their sugar linkage composition was analyzed by gas chromatographic (GLC) analysis of the alditol acetates obtained from the acid hydrolyzates of the methylated AX. 2,3- and/or 3,4-Di-O-methyl-xylose were prominent structural units in the methylated AX. 2,3- and 3,4-Di-O-methyl-xylose were not distinguished from each other by GLC. The di-O-methyl-xylose was, however, characterized as 2,3-di-O-methyl-xylose, because β-(1→4)-xylo-oligosaccharides were characterized from the partial acid hydrolyzate of AX. GLC of the alditol acetates obtained from the hydrolyzate of the methylated AX showed that 2,3-di-O-methyl-xylose and 2- and/or 3-mono-O-methyl-xylose were present in the ratios of 12:1.

To analyze the structure of the side chain of AX, the acidic sugar was isolated. AX was hydrolyzed with 0.1 M hydrochloric acid for 5.5 hr at 100°C. The hydrolyzate was chromatographed on a column (1.5 x 45.5 cm) of Bio-Gel P-2 and the void volume fractions were combined, because it is known that accelerated elution from Bio-Gel P-2 is typical of acidic sugars in water. The void fraction was analyzed by p.c. with the solvent system of ethyl acetate–water–acetic acid–formic acid = 18:4:3:1. A spot having Rxy 0.69 was detected on the chromatogram. The spot was considered to be 2-O-(4-O-methyl-α-D-glucopyranosyluronic acid)-O-xylose (ref. 9 Rxy 0.69).

These results indicate that an acidic xylan present in gobo has a linear backbone chain of β-(1→4)-xylopyranosidic residues, about 8.3% of which were substituted at the 2 position with 4-O-methyl-α-D-glucopyranosyluronic acid residues.

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References


