Note

Pectins in Extracellular Polysaccharides from a Cell-Suspension Culture of Mentha

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Pectin constituents, which were about 70 w/w% of extracellular polysaccharides (ECP) from a cell-suspension culture of Mentha, were purified by gel filtration chromatography, and their sugar composition and linkage were investigated. Two major constituents identified were (1→3)-linked galactan carrying arabinosyl residues on C-6 and (1→4)-α-linked galacturonan partially interspersed with (1→2)-linked rhamnose residues. Acetylated or methylated pectins were not identified on 1H-NMR analysis.

Key words: arabinogalactan; extracellular polysaccharide; rhamnogalacturonan; Mentha; pectin

Hemicelluloses and pectins are matrix polysaccharides in the cell-walls of higher plants. In particular, the structure of pectins is known to be one of the most complicated classes of wall polysaccharides. Although the matrix substances can be extracted from primary cell walls by aqueous solutions of alkaline or chelating agents, such conditions often alter their original structures. We had found that extracellular polysaccharide (ECP), which is produced by a suspension culture of Mentha (F. of M. spicata × M. arvensis), is composed of hemicelluloses and pectins, and we showed that this hemicellulose fraction is a good source of acetylated xyloglucans, which can be isolated without using alkaline extraction. In this paper, we analyzed the chemical composition of pectic polysaccharides, which are more abundant constituents of ECP produced by Mentha cells, rather than hemicellulose.

As has been discussed previously, crude ECP, which was precipitated by EtOH from a supernatant of a Mentha suspension culture, consists of neutral sugars (50 w/w%), galacturonic acid (GalA, 32%), protein (11%), and calcium (2%). This fraction was purified by the following procedures: the crude ECP was dissolved in an ammonium oxalate buffer (20 mM, pH 4) to remove calcium and other minerals as water-insoluble oxalate salts. The water soluble fraction was treated with Actinase E to degrade the protein. The resultant water-soluble fraction was recovered as pure polysaccharides and designated as purified ECP. We next tried the separation of the purified ECP (200 mg) by ion exchange chromatography (DEAE-Toyopearl). Elution with sodium phosphate buffer (50 mM, pH 7.0) gave fraction 1 (F-1, 58 mg), which was not absorbed onto the column. This fraction has previously been identified as acetylated xyloglucan. Further elution with the same buffer containing NaCl (gradient from 0 to 0.5 M) gave F-2 (17 mg), F-3 (20 mg), and F-4 (96 mg), respectively, as DEAE-absorbing fractions.

Gel filtration chromatography (GFC, Sephacryl S-500) eluted with 50 mM sodium phosphate buffer containing 1 M NaCl, (pH 7.0) of F-2 gave a single peak (Mw 1.2×10^6) which was compared with standard dextrans (Funakoshi, Dextran 8, T40, T110). The homogeneity of F-2 was confirmed further by paper electrophoresis (Tyo-GA100) in a pyridine-acetic acid buffer (1 M, pH 3.5). The neutral sugar analysis of F-2 (Table) showed that Ara and Gal were the two dominant sugars and their stoichiometrical relationship was almost 1:1. Based on the Hakomori methylation analysis, it was suggested that the structure of F-2 is based on a (1→3)-linked galactan backbone that is carrying substituent groups on O-6, almost exclusively Ara residues. Thus, the structure of F-2 is more similar to type II arabinogalactan than to type I which has a β-1,4-galactan backbone.

GFC (Sephacryl S-400) of F-3, which was 10 w/w% of the purified ECP, showed that this fraction is composed of several minor oligosaccharides. The very small quantity of F-3 hindered further study. GFC (Sephacryl S-400) of F-4 gave one main broad peak having Mw>2.0×10^6. The GalA content in this fraction was estimated at 63 mol%. To obtain small fragments for further analysis, F-4 was treated with a (1→4)-α-endopolygalacturonase from Aspergillus japonicus (EC 3.2.1.15, 37°C, 24 h). GFC (Sephacryl S-300) separation of the reaction product gave two main peaks, F-4a (Mw 9.2×10^5) and F-4b (Mw 2.5×10^5), in 22 and 29 w/w% yields, respectively. Thus, it was considered that 50 w/w% of F-4 was digested by this enzymatic degradation. When F-4 was hydrolyzed with acid (2.0 M TFA, 100°C, 3 h), we recovered 47 w/w% of F-4 as an insoluble precipitate, which was identified as a single peak on GFC (Sephacryl S-300). As this acid-insoluble fraction seemed to correspond to the lost amount of F-4 after the endopolygalacturonase process, we designated this fraction as F-4c. These results suggest that F-4a (arabinan) and/or F-4b (arabinoxylan)-rich domains were associated with F-4c (rhamnogalacturonan backbone) (see below).

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Abbreviations: ECP, extracellular polysaccharide; GalA, galacturonic acid; GFC, gel filtration chromatography.
In methylation analysis after the reductive process of F-4c in which GalA residues were converted to a deuterium labeled Gal (Table), it was indicated that the basic structure of F-4c is (1→4)-linked galacturonic which is partially interspersed with (1→2)-linked Rha residues. Using 1H-NMR analysis (400 MHz, in D2O) of F-4c, we observed an anomeric proton signal (5.10 ppm) which corresponds to an α-isomer of the GalA residue. We also observed methyl proton signals for Rha (1.35 ppm, doublet, J1H,1H 0.68 Hz) as minor peaks, and deduced that the average ratio of GalA: Rha residues to be ca. 15. On the other hand, signals ascribable to methyl protons of methylated or acetylated sugars were not observed. This is in contrast to the fact that highly acetylated xyloglucan was found in the hemicellulosic fraction from the same ECP.

We deduced that F-4a and F-4b are highly branched portion of a large molecule, F-4, while F-4c is a linear portion of a harrnogalacturonan backbone. The neutral sugar composition of F-4a was rich in neutral sugars such as Ara and Xyl (Table). Methylation analysis of F-4a recovered a variety of branched sugars with t-Ara (1→, →5)-Ara (1→, and →3,5)-Ara (1→being the major components (Table). Thus, as has often been the case in several other papers, we also concluded that F-4a would retain several types of arabinosyl residues. The neutral sugar composition of F-4b was very similar to that of F-4a. The higher ratio of Rha in F-4b seemed to reflect the higher content of harrnogalacturonan backbone than in F-4a.

As mentioned above, the pectins in the ECP produced by a suspension culture of Mentha are primarily composed of common substances such as rhamnogalacturunan and arabinogalactan. The original structure of the pectins in the cell wall of Mentha may be a large molecule in which arabinogalactans (F-2) and other oligosaccharids (F-3) are linked to the harrnogalacturonan backbone (F-4).

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References


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