Variations in the structure of neutral sugar chains in the pectic polysaccharides of morphologically different carrot calli and correlations with the size of cell clusters

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Abstract. Carrot (Daucus carota L.) embryogenic callus (EC) loses its embryogenic competence and becomes non-embryogenic callus (NC) during long-term culture. With the loss of embryogenic competence, the cell clusters become smaller and the extent of intercellular attachments is reduced. Pectic fractions prepared from EC and NC were separated into two subfractions by gel filtration. A difference in sugar composition between EC and NC was found only in the high-molecular-mass (ca. 1300 kDa) subfraction, and the ratio of the amount of arabinose to that of galactose (Ara/Gal) was strongly and positively correlated with the size of cell clusters in several different cultures. From the results of sugar-composition and methylation analyses, and the results of treatment with exo-arabinanase, models of the neutral sugar chains of pectins from EC and NC are proposed. Both neutral sugar chains are composed of three regions. The basal region is composed of linearly linked arabinan 5-Arar) moieties in both types of callus. The middle galactan region is composed of 6-linked galactose, some of which branches at the 3 and 4 positions, and this region is larger and more frequently branched in NC than in EC. Finally, the terminal arabinan region is composed of 5-linked arabinose, branched at the 3 position, and the size of the terminal arabinan is larger in EC than in NC. The significance of the neutral sugar chains of pectins in the interaction of cell wall components and intercellular attachment is discussed.

Key words: Callus – Cell wall – Daucus (cell walls) – Morphogenesis – Neutral sugar – Pectin

Introduction

Intercellular attachment is obviously indispensable during morphogenesis of higher plants, but little information is available about the structure and the function of the pectin molecules that are recognized as the main adhesive materials between plant cells. For detailed studies of the functions of pectin molecules, organs of intact plants, such as stems and roots, are not suitable materials because they consist of many kinds of tissue and cell. By contrast, when certain cultured cells are used, morphogenesis can be driven relatively synchronously in a homogeneous population of cells (Reinert 1959; Kiyosue et al. 1993; Kobayashi and Fukuda 1994). Therefore, cell culture systems might be useful for studies of cell wall functions, such as intercellular attachment, as models of intact plants.

In cultures of the cells of many plants, and of carrot in particular, when the duration of culture is extended, the cultured cells lose their morphogenetic ability, with decreases in the size of cell clusters (Reinert et al. 1970). Thus, the embryogenic callus (EC) of carrot becomes non-embryogenic callus (NC) with decreases in the extent of intercellular attachment (Kikuchi et al. 1995). An analysis of pectic polysaccharides from EC and NC revealed differences in neutral sugars but not in uronic acid. The pectin from EC contained much more arabinose than that of NC and the reverse was true for galactose (Kikuchi et al. 1995).

In this report, we describe structural changes in the neutral sugar chains of pectic polysaccharides in cultured cells of carrot. We demonstrate a correlation between the size of cell clusters and the ratio of arabinose content to galactose content, and we discuss the involvement of the neutral sugar chains in intercellular attachment.

Material and methods

Plant materials and cell culture. Suspensions of Daucus carota L. cv. US-Harumakigosun cell were induced from seedling segments on Murashige and Skoog's (MS; Murashige and Skoog 1962) agar medium containing 2,4-dichlorophenoxyacetic acid (2,4-D; 1 mg 1⁻¹). Embryogenic callus (EC), one to two months after induction, had embryogenic competence and consisted of large clusters of cells. Non-embryogenic callus (NC), which had been cultured continuously since 1985 (Satoh et al. 1986), had entirely lost embryogenic competence, failed to form large clusters of cells and had open spaces between the cells (Kikuchi et al. 1995).
Calus that had been cultured for three to six months was used as calus that was intermediate between EC and NC. Such calus was composed of a mixture of smaller clusters of cells.

Two weeks after inoculation, cells were separated from the culture medium by filtration through a 36-μm stainless-steel mesh and, after lyophilization, used for the preparation of cell walls.

Estimation of the average sizes of cell clusters in the culture. Cultures of EC, NC and intermediate types of calus (four lines) were separately passed sequentially through a series of ten stainless-steel sieves (with 1000-, 500-, 250-, 177-, 149-, 105-, 63-, 53-, 36- and 25-μm mesh). The fresh weights (g) of the cell clusters (W) that remained on each sieve were measured for each culture. Because the diameters of the cell clusters that remained on a sieve were larger than the mesh size of that sieve but smaller than the mesh size of the preceding sieve, the average diameter (μm) of the cell clusters (ASC) in a culture could be determined using the following equation:

\[ ASC = \frac{\Sigma(C \times W)}{\Sigma W} \]

where C was taken as the mean value of the mesh sizes of the sieve and the preceding sieve, except for the 1000-μm sieve. Namely, the value of C is 1000 μm for 1000-μm mesh, 750 μm for 500-μm mesh, 375 μm for 250-μm mesh, 213.5 μm for 177-μm mesh, 163 μm for 149-μm mesh, 127 μm for 105-μm mesh, 84 μm for 63-μm mesh, 58 μm for 53-μm mesh, 44.5 μm for 36-μm mesh and 30.5 μm for 25-μm mesh.

Preparation of cell walls and isolation of pectic polysaccharides. Cell walls were isolated by the method of Stokdart et al. (1967), with slight modifications. The dried cell walls were suspended in boiling 40 mM ammonium oxalate (pH 4.0) and incubated for 200 min at 99 °C. To remove proteins, the solution was dialyzed overnight against 10mM sodium acetate buffer (pH 4.0). The enzyme solution was treated overnight against 10 mM sodium acetate buffer (pH 4.0) (Kikuchi et al. 1995).

Gel filtration. Gel filtration was performed on a column (2 cm i.d., 80 cm long) of Sephacryl S-500 HR (Pharmacia, Uppsala, Sweden). The column was equilibrated and eluted with 100 mM sodium acetate buffer (pH 4.0) at 3 ml h⁻¹, by the method of Massiot et al. (1988). For estimations of molecular mass, four kinds of dextran standard (Sigma; 5~40 MDa, 2 MDa, 485 kDa and 11 kDa) were used as markers. The void and included volumes of the column were determined with dextran (5~40 MDa) and glucose, respectively. The pectic polysaccharide was injected onto a Mono Q HR5/5 column (Pharmacia) using 10 mM imidazole-HCl buffer (pH 7.0) and eluted with a linear gradient of NaCl (0~1 M) in the same buffer using a high-performance liquid chromatography (HPLC) system (LKB, Bromma, Sweden).

Quantitation of total sugar and uronic acid. Amounts of total sugar and uronic acid were determined by the phenol-sulfuric acid method (Dubois et al. 1956) and the carbazole-sulfuric acid method (Galambos and McCain 1967), as glucose and galacturonic acid equivalents, respectively.

Analysis of neutral sugar compositions. For the analysis of neutral sugars, the polysaccharides were hydrolyzed, converted into the corresponding alditol acetates and subjected to gas chromatography by the method of Albersheim et al. (1967).

Methylation analysis. The vacuum-dried pectic polysaccharides were methylated by a modified version of Hakomori’s procedure (Hakomori 1964; Sandford and Conrad 1966). Methylated polysaccharides were recovered and purified by reverse-phase chromatography (Sep-Pak C-18; Waters, Milford, Mass., USA). Purified methylated polysaccharides were hydrolyzed with 2 M trifluoroacetic acid and reduced by sodium borodeuteride. The partially O-methylated alditols were converted to partially O-methylated alditol acetates by treatment with acetic anhydride (York et al. 1985) and analyzed by gas chromatography and gas chromatography-mass spectrometry (QP2000, Shimadzu, Kyoto, Japan).

Removal of arabinan. The terminal arabinosyl residues of neutral sugar chains of purified pectin were eliminated by treatment with α-L-arabinofuranosidase (EC 3.2.1.55, from Rhodotorula flav; Uesaka et al. 1978) which was a gift from Dr. Nao To Shibuya of the National Institute of Agrobiological Resources (Tsukuba, Japan). One milligram of purified pectin was incubated with 1.0 unit of arabinofuranosidase (exo-arabinanase) for 30 min at 50 °C in 1 ml of 10 mM sodium acetate buffer (pH 4.0). The reaction was stopped by the addition of an equal volume of 0.1 M Na₂CO₃. The enzyme-treated sample was subjected to gel filtration on a column of Sephacryl S-500 HR.

Results

Fractionation of pectic polysaccharides by gel filtration. The pectic polysaccharides were extracted from the cell walls of EC and NC and subjected to gel filtration on a column of Sephacryl S-500 HR. The elution profiles of EC and NC contained two peaks which corresponded to molecules of 1300 and 80 kDa, respectively (Fig. 1). The 1300-kDa peak was slightly higher than the 80-kDa peak in the case of EC, and the opposite was true in the case of NC. The amount of uronic acid in the 80-kDa peak was twice that in the 1300-kDa peak in NC.

Identification of the neutral sugars in the 1300-kDa and 80-kDa polysaccharides. Figure 2 shows the neutral sugar compositions of the 1300-kDa polysaccharides (fractions 23~27 and 22~27 for EC in Fig. 1A and for NC in Fig. 1B, respectively) and 80-kDa polysaccharides (fractions 29~33 and 30~34 for EC in Fig. 1A and for NC in Fig. 1B, respectively). The amounts of arabinose and galactose in 1300-kDa polysaccharides (Fig. 2A) were quite different in EC and NC. By contrast, the neutral sugar compositions of the 80-kDa polysaccharides (Fig. 2B) of both EC and NC were almost the same, except for rhamnose. The ratios of the amount of arabinose to that of galactose (Ara/Gal) in the 1300-kDa polysaccharides were 2.85 and 0.87 for EC and NC, respectively, and those in the 80-kDa polysaccharides were 1.05 and 0.87 for EC and NC, respectively. The difference in neutral sugar compositions between EC and NC was very conspicuous in the case of the 1300-kDa polysaccharides.