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STRUCTURE OF AN ARABINOGALACTAN OF EXTRACELLULAR HYDROXYPROLINE-RICH GLYCOPROTEIN IN SUSPENSION-CULTURED TOBACCO CELLS

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Key Word Index —*Nicotiana tabacum*; Solanaceae; arabinogalactan; glycoprotein; hydroxyproline.

INTRODUCTION

It is well known that the hydroxyproline-containing glycoproteins (hyp-GPs) accumulate in plant cell walls as matrix components [1], and in the medium, in which plant cells are cultured in suspension [2]. We have recently analyzed the extracellular hyp-GP (ECG) accumulated in the medium of tobacco cells cultured in suspension, and indicated that the sugar moiety consists of arabinose, galactose, rhamnose, and uronate [2]. These results implied that an arabinogalactan might be present in ECG. In fact, the results of a preliminary analysis of methylated ECG by GLC showed that the polysaccharide moiety contained arabinogalactans (unpublished data). In this report we propose a tentative structure of the arabinogalactan of ECG based on the results of GC–MS analysis of methylated sugars obtained after hydrolysis.

RESULTS AND DISCUSSION

The ECG, purified according to the methods described previously [2], was fully methylated [3, 4] and the methylated alditol acetate sugar derivatives obtained on hydrolysis and acetylation analyzed by GLC–MS (Table 1). As shown in Table 1, the following monosaccharide derivatives were identified as structural units in the arabinogalactan moiety of ECG: 2,3,4-tri-*O*-methyl-rhamnose, 2,3,5-tri- and 2,3-di-*O*-methyl-arabinose, 2,3,4,6-tetra-, 2,4,6-tri-, 2,3,4-tri- and 2,4-di-*O*-methyl-galactose. Although two unknown sugar derivatives were observed their concentrations were extremely low. These results of GC–MS are closely similar to those obtained with the arabinogalactans of the extracellular polysaccharides of suspension-cultured tobacco [5] and sycamore [6]. Thus, we propose that the structure for the arabinogalactan of ECG of tobacco cells is as shown in Fig. 1. Although the links between both

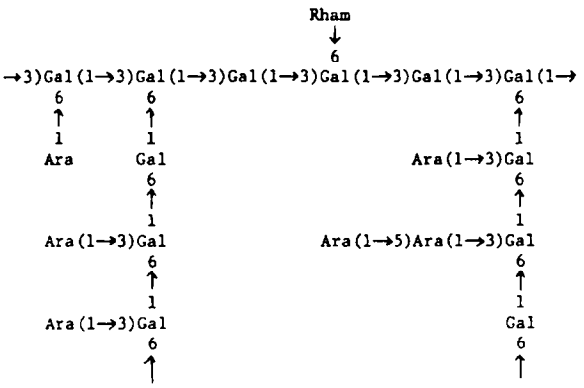

Fig. 1. Plausible structure of arabinogalactan of ECG of suspension-cultured tobacco cells.

Table 1. Identification and percentage composition of methylated alditol acetate sugar derivatives from extracellular hydroxyproline-rich glycoprotein in cultured tobacco cells

Peak No.	Methylated sugars	Percentage composition (% of total peak area)
1	1,4-di-*O*-acetyl-2,3,5-tri-*O*-methylarabinose	23
2	1,5-di-*O*-acetyl-2,3,4-tri-*O*-methylrhamnose	2
3	1,4,5-tri-*O*-acetyl-2,3-di-*O*-methylarabinose	5
4	uk*	1
5	1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylgalactose	4
6	uk	1
7	1,3,5-tri-*O*-acetyl-2,4,6-tri-*O*-methylgalactose	13
8	1,5,6-tri-*O*-acetyl-2,3,4-tri-*O*-methylgalactose	8
9	1,3,5,6-tetra-acetyl-2,4-di-*O*-methylgalactose	43

* uk represents unknown sugar derivatives.

arabinose and rhamnose to the galactose residues are indefinite, it can be assumed that the terminal rhamnose residues are linked to C-6 of (1 → 3) linked galactose chains by analogy to sycamore arabinogalactan. Terminal arabinose residues might be linked to C-3 of (1 → 6) linked galactose chains. It should be noted that ECG lacks (1 → 4) linked galactose chains.

EXPERIMENTAL

Tobacco cells (cell line XD-6) were cultured in modified Murashige and Skoog's medium as previously described [7]. Extracellular hydroxyproline-rich glycoprotein (ECG) was purified according to the method of ref. [2]. About 10 mg of purified ECG was methylated [3, 4] and acetylated [8]. The lyophilized material was dissolved in 2 ml of DMSO under N_2 and sonicated at 50° for 5 hr. The sample was then chilled in an ice bath, 2 ml of CH_3I was added, and the whole sonicated for 2 hr at 4° with a further addition of 2 ml of CH_3I after 1 hr. The sample was allowed to stand overnight at room temp. and then dialyzed against water overnight. The methylated polysaccharides were extracted with $CHCl_3$, dried at 37°, reduced with $NaBH_4$, and acetylated [8]. Methylated alditol acetate sugar derivatives were determined by GLC (Hitachi model 163) equipped with a glass column (200 × 0.3 cm) containing Gas-Chrom P coated with a mixture of ethylene glycol adipate polyester (0.2%) and silicone XF-150 (0.4%) and by a GLC–MS (NEVA TE-600) equipped with a glass capillary column.

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