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Glycoproteins Associated with the Cell Wall of Carrot Cells in Suspension Culture

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Salt-extractable glycoproteins (SEG) of cell wall and extracellular glycoproteins (ECG) of suspension-cultured carrot cells were purified by column chromatography and analysed for their chemical composition. The SEG consists of about 60% protein and 40% carbohydrate while ECG is composed mainly of carbohydrate (ca. 90%). Major sugars in both polymers were arabinose, galactose and uronic acid. Hydroxyproline accounted for 6% and 9% of the amino acids in the protein moiety of SEG and ECG, respectively. Other amino acid composition shows that SEG and ECG are distinctly different glycoproteins.

The plant cell wall contains a variety of glycoproteins including structural glycoproteins and arabinogalactan proteins. The common feature of these wall-associated glycoproteins and related complexes is that they are hydroxyproline-rich and contain large amounts of galactose and arabinose.[1] In some cases, the existence of rhamnose and uronic acid was also reported.[2,3] Chrispeels has shown that a large portion of the hydroxyproline-rich glycoproteins (HRGP) are salt-extractable from the wall.[4] Chrispeels et al.[5] and Stuart and Varner[6] have shown that slicing and aeration enhance the synthesis and secretion of HRGP in carrot disks. In this paper, we describe the chemical analysis of salt-extractable glycoproteins obtained from carrot cells in suspension culture and the results are compared with those for extracellular glycoproteins secreted by the cells.

Materials and Methods

Organism and cultivation. Carrot cells were of strain GD3 derived from a red carrot (*Daucus carota* L., cv. Kintoki). The cells were cultured in the medium of Murashige and Skoog[7] with 3% sucrose and 1 mg/*l* 2, 4-dichlorophenoxyacetic acid. Culture conditions and some properties of the cells were described in an earlier paper.[8]

Preparation of salt-extractable glycoprotein (SEG). Materials of SEG were obtained from the cell wall of cultured carrot cells according to Stuart and Varner[6] with some modifications. Freshly isolated cells were suspended in potassium phosphate buffer (5 mM, pH 7.0) containing polyvinylpolypyrrolidone (PVPP, 45 g/100 ml) and 5 mM dithiothreitol (DTT), and disrupted by sonic oscillation. Crude cell wall preparations were washed with water, then extracted three times with 500 ml of 0.2 M $CaCl_2$ solution containing DTT and PVPP. The extract was dialyzed against water and concentrated by Amicon PM-10 membrane. The material was

chromatographed through a column of Sepharose 4B, then with CM-Sepharose CL-6B.

Preparation of extracellular glycoproteins (ECG). The used medium of carrot cell culture was obtained by centrifugation at 8,000 × *g* for 20 min, dialyzed against water and concentrated by using Amicon PM-10. The material was first chromatographed through a Sepharose 4B column and the fractions containing both hydroxyproline and carbohydrate were further applied to a column of DEAE-Sephacel. Glycoprotein-rich fractions were combined and rechromatographed on serially connected columns of Sepharose 4B and CL-6B, and again on DEAE-Sephacel.

Column chromatography. Columns of Sepharose 4B (2 × 90 cm) and Sepharose CL-6B (1.6 × 100 cm) were equilibrated with 20 mM sodium acetate buffer (pH 5.0) and eluted at a flow rate of 20 ml/hr with the same buffer. A CM-Sepharose CL-6B column (1.5 × 26 cm) was equilibrated with 5 mM Tris-HCl buffer (pH 8.0) and eluted with a linear gradient of Tris-HCl (pH 8.0, 5–600 mM). A DEAE-Sephacel column (1.8 × 25 cm) was equilibrated with 10 mM sodium acetate buffer (pH 5.0) and eluted with a linear gradient of NaCl (0–0.2 M) in the same buffer.

Chemical analysis. Amino acid composition of the samples was determined by an automatic analyzer (LKB 4400) after hydrolysis in vacuo in 6 N HCl at 110°C for 20 hr. Determination of hydroxyproline was performed by the method of Kivirikko and Liesmaa.[9]

To analyze the composition of neutral sugar, the samples were hydrolyzed with 2 N trifluoroacetic acid at 120°C for 60 min. The sugars were converted to alditol acetate[10] and determined with a gas-chromatogaph (Shimadzu GC-6AM) as described previously.[11] Total sugar was determined by the phenol-H_2SO_4 method[12] and uronic acid by the carbazole-H_2SO_4.[13]

Results and Discussion

Salt extractable cell wall glycoproteins

Glycoproteins bound to the cell wall were partially solubilized by repeated extractions with $CaCl_2$. The extracted materials were chromatographed on a Sepharose 4B column (**Fig. 1A**). Carbohydrates coincidently eluted with hydroxyproline (tube No. 90–110) were combined and rechromatographed on a CM-Sepharose column (**Fig. 1B**). A large part of the hydroxyproline containing polymers were voided. A narrow peak containing both hydroxyproline and carbohydrate (tube No. 20–25) was analyzed for amino acid and sugar. The salt-extractable glycoprotein consisted of 60% protein and contained galactose, arabinose and uronic acid as major sugars, with lesser amounts of xylose and mannose (**Table 1**). Amino acid composition of the protein moiety is shown in **Table 2**.

Salt-extractable hydroxyproline-rich glycoprotein obtained by Stuart and Varner[6] was characterized by very high content of hydroxyproline (50%). They isolated SEG from carrot root slices after incubation with vigorous shaking which enhanced the synthesis and secretion of SEG. It is possible that the treatment stimulated the accumulation of a specific wound-related glycoprotein which is a minor component in uninjured cells.

Extracellular glycoproteins

Carrot suspension-cultured cells excrete a large amount of polymeric substances into the culture fluid. These macromolecules were composed of both carbohydrate and protein and the latter contained an appreciable amount of hydroxyproline. Major fraction rich in both carbohydrate and hydroxyproline was resolved at least into two components, ECG I (tube No. 40–47) and II (tube No. 48–64) in **Fig. 2C**, by column chromatography with DEAE-Sephacel. These polymers were composed mainly of carbohydrate (ca. 90%) and protein moiety comprised less than 10%. Sugar and amino acid compositions of these polymers were

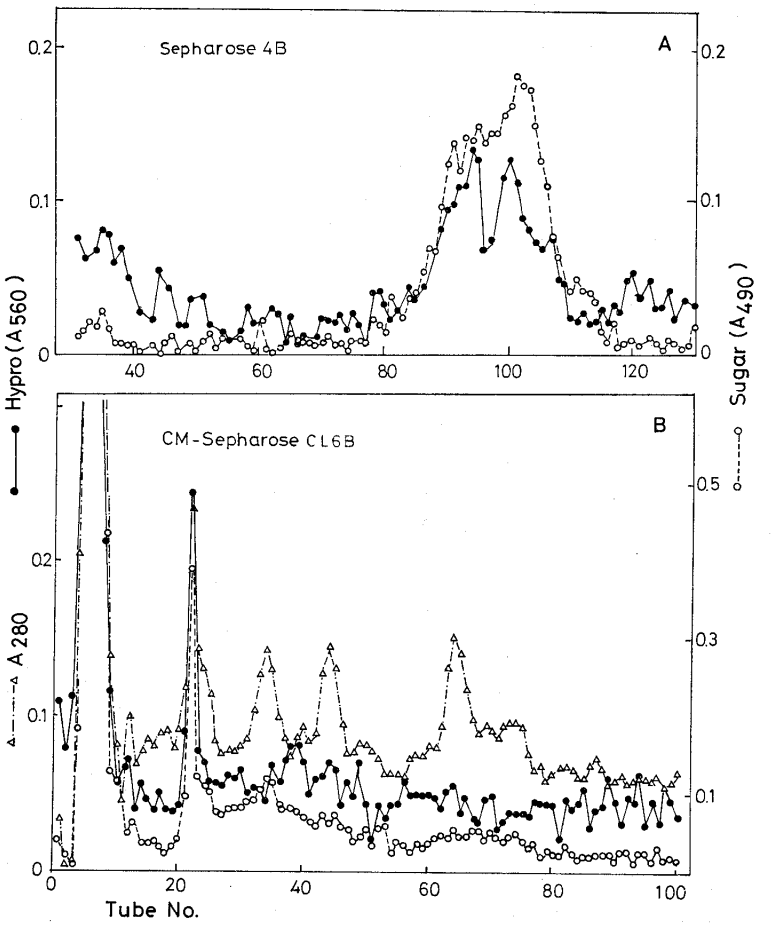
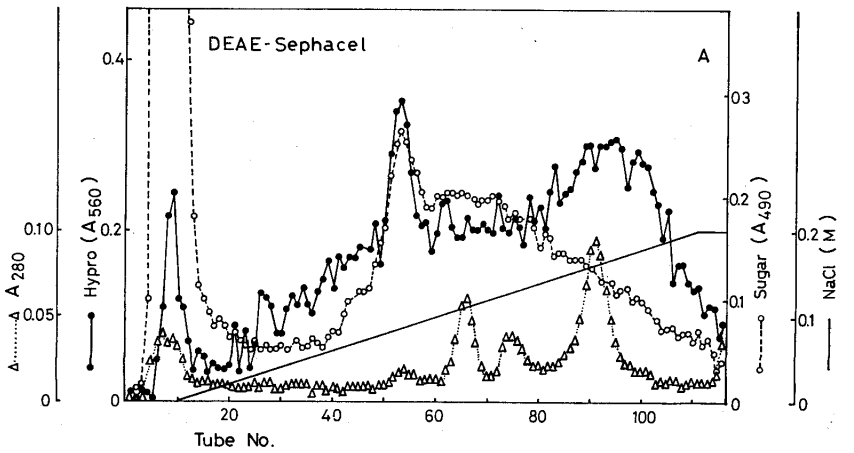


Fig. 1. Chromatographic separation of $CaCl_2$-extractable (SEG) wall-glycoprotein from carrot cells in suspension culture.
(A) $CaCl_2$-extract was dialyzed against water, concentrated and applied onto a column of Sepharose 4B (4 ml/tube). (B) Fractions from tube No. 90–110 were combined and rechromatographed with CM-Sepharose CL-6B (4 ml/tube).

Fig. 2. Chromatographic separation of extracellular glycoprotein (ECG) of carrot cells in suspension culture.
(A) ECG obtained from used medium of 10-day-old culture was passed through a column of Sepharose 4B column. Fractions containing both carbohydrate and hydroxyproline were combined and chromatographed through a DEAE-Sephacel column (4 ml/tube). (B) Fractions from tube No. 40 to 80 in (A) were combined and applied onto serially connected columns of Sepharose 4B and CL-6B (4 ml/tube). (C) Fractions from tube No. 66 to 75 in (B) were again chromatographed on a DEAE-Sephacel column (5 ml/tube).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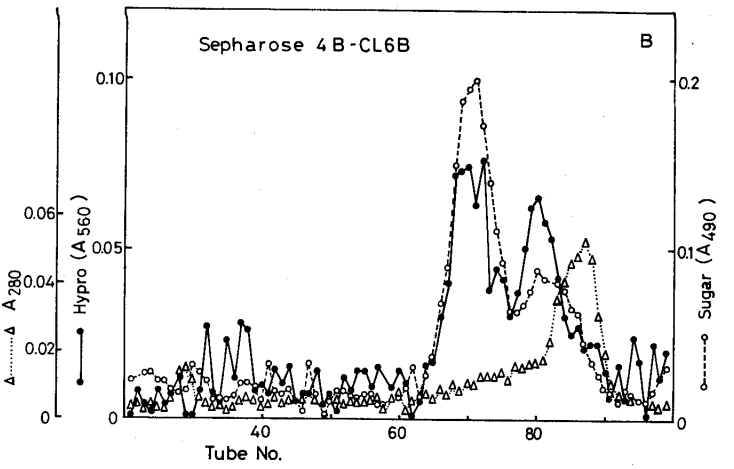
A
Hypro (A560)
A 280
Sugar (A490)
NaCl (M)
Tube No.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Sugar (A490)
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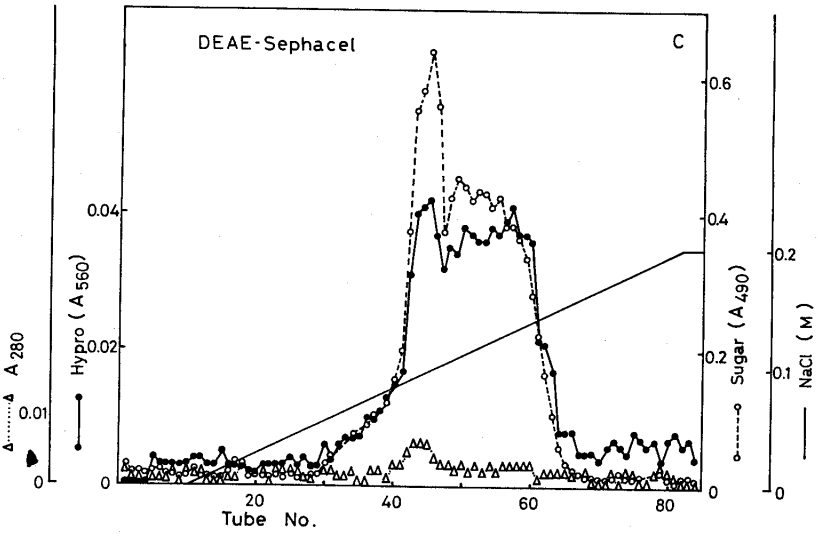
DEAE-Sephacel
C
Hypro (A560)
A 280
Sugar (A490)
NaCl (M)
Tube No.

Table 1. Sugar composition of salt-extractable (SEG) and extracellular (ECG) glycoproteins from carrot cells in suspension culture.

Sugars	SEG	ECG	
		I	II
Rhamnose		1.1	1.6
Arabinose	15.8	38.2	33.2
Xylose	1.6		
Mannose	7.8		
Galactose	54.9	47.6	47.2
Glucose		2.1	2.6
Uronic acid	20.0	11.1	15.4

The results are expressed as percentage (w/w) of total sugar.

Table 2. Amino acid composition of salt extractable (SEG) and extracellular (ECG) glycoproteins from carrot cells in suspension culture.

Amino acids	SEG	ESG	
		I	II
Hyp	6.2	8.5	9.4
Asp	8.6	6.0	5.6
Thr	7.3	5.0	5.5
Ser	7.3	22.9	21.5
Glu	7.5	15.7	14.8
Pro	7.3	1.8	2.1
Gly	7.2	12.7	12.3
Ala	10.0	12.7	13.0
Cys	trace	0.2	0.3
Val	5.5	2.7	3.5
Met	0.9	0.8	1.0
Ile	3.9	1.5	1.5
Leu	7.9	2.0	2.3
Tyr	2.5	1.4	1.1
Phe	3.6	1.1	1.0
His	3.9	3.0	2.9
Lys	6.5	1.7	2.0
Arg	4.0	0.3	0.3

The results are expressed as mole-percent of total amino acid.

very similar (**Tables 1** and **2**). In both cases, protein moiety contained hydroxyproline and relatively high amounts of serine, glutamic acid (glutamine), glycine and alanine. Major sugars were arabinose, galactose and uronic acid. These figures indicate that the chemical property of ECG is distinctly different from that of SEG.

In a previous paper,[14] we have shown that the sugar composition of the extracellular polysaccharides excreted from carrot cell resembled that of non-cellulosic component of the cell wall. These observations suggest that the extracellular polysaccharides are derived from or share common origin with the wall polymers. Brisk and Chrispeels[15] have reported that trichloroacetic acid-extractable glycoproteins from carrot contains only arabinose as a major sugar. In this experiment, we found that carrot SEG and ECGs contained large amounts of

galactose and uronic aid besides arabinose. These results are rather similar to those reported for tabacco[2] and *Phaseolus vulgaris*[3] glycoproteins. The data of amino acid analysis of ECG, on the other hand, are strikingly consistent with those of Brisk and Chrispeels, except that they found very high content of lysine.

In this experiment, we analyzed only a few species of glycoprotein from ECG and SEG. However, the distribution of hydroxyproline in the chromatographic elution profiles of crude materials indicates that carrot cells produce a variety of glycoproteins containing hydroxyproline. Although the physiological role of these conjugates are presently not clear, their heterogenous nature suggests that their functions cannot be unified and they play different roles depending on the site of deposition.

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《和文要約》

ニンジン培養細胞の細胞壁糖蛋白質

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細胞壁より $CaCl_2$ で抽出した糖蛋白質 (SEG) および培養細胞が培地中に放出する糖蛋白質 (ECG) をカラムクロマトグラフィで精製し，その化学組成を調べた．SEG は約60％の蛋白を含むが ECG はその約90％を炭水化物で占め蛋白含量は低かった．主要な構成糖はどちらもアラビノース，ガラクトースとウロン酸で，ハイドロキシプロリン含量は SEG で6％, ECG で9％であった．その他のアミノ酸組成に関しては ECG と SEG で明確な相違が認められた．