Structure of Hydroxyproline-arabinoside from Tobacco Cells

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The hydroxyprolyl tri and tetraarabinosides were isolated from a cell wall preparation of cultured tobacco cells by alkali hydrolysis, gel filtration and ion-exchange chromatography. The structures of the two hydroxyproline-arabinosides (Ara₃-Hyp and Ara₄-Hyp) were determined by analysis of controlled Smith-degradation products, and measurements of PMR spectra and optical rotation to be β -L-Araf-(1 $\rightarrow$ 2)- β -L-Araf-(1 $\rightarrow$ 2)- β -L-Araf-(1 $\rightarrow$ 4)-Hyp and β -L-Araf-(1 $\rightarrow$ 3)- β -L-Araf-(1 $\rightarrow$ 2)- β -L-Araf-(1 $\rightarrow$ 2)- β -L-Araf-(1 $\rightarrow$ 4)-Hyp, respectively.

Primary cell walls of plants contain hydroxyproline-rich glycoproteins that are characterized by a short side chain consisting of arabinose bound to the hydroxyl group of hydroxyprolyl residues.^{1,2)} A series of four hydroxyproline-arabinosides (Ara-Hyp, Ara₂-Hyp, Ara₃-Hyp and Ara₄-Hyp) have so far been reported as important fragments of glycoproteins of this kind.²⁾ In our previous paper,³⁾ Ara₃-Hyp and Ara₄-Hyp were isolated from a cell wall preparation of suspension-cultured tobacco cells. The linkage of the oligoarabinosides to the protein moiety was examined by the standard methylation analytical procedure. In this paper, the structures of these oligoarabinosides were determined by analysis of controlled Smith-degradation products and measurements of PMR spectra and optical rotation.

MATERIALS AND METHODS

The preparation of samples (B-2, B-3, and B-4) and analytical methods were as described previously,³⁾ unless otherwise stated.

Aminex chromatography. Aminex ion-exchange chromatography was performed with hydrochloric acid as eluant instead of 0.01 M acetic acid. The column was eluted with a pH gradient produced in a two-chambered vessel: the mixing chamber contained 200 ml of water

and the reservoir 200 ml of 0.2 N hydrochloric acid. Each fraction was individually neutralized with 0.1 N sodium hydroxide, and desalted on a column (100 $\times$ 1.6 cm) of Sephadex G-15.

Controlled Smith-degradation. A 48 mg sample of B-2 was oxidized with 0.02 M sodium metaperiodate (eight-fold excess) for 96 hr at 4°C in the dark, and periodate consumption was estimated by measurement of absorbance at 223 nm. Excess periodate was then destroyed by adding ethylene glycol, and the reaction mixture was reduced with sodium borohydride (150 mg) for 24 hr at 4°C in the dark. The excess borohydride was then decomposed by adding acetic acid and the resulting substance was applied to the above Sephadex G-15 column. The desalted solution was evaporated to dryness and the residue was hydrolyzed with 0.1 N hydrochloric acid for 24 hr at room temperature. In a preliminary experiment, it was found that the O-glycosidic bond in the hydroxyproline-arabinosides was not split under such mild acidic conditions. The hydrolyzate was then neutralized with 0.1 N sodium hydroxide and desalted by gel filtration. The hydrolysis products recovered in the eluate were finally lyophilized and submitted to the methylation analysis.

The determination of anomeric configuration. The anomeric configuration was determined by PMR spectrometry and by estimation of optical rotation.

The PMR spectra were recorded on a JEOL model JNM-PS-100 spectrometer with D₂O as the solvent at 23°C. Chemical shifts were measured using tetramethyl silane as external standard.

The optical rotation was measured on a JASCO model DIP-181 polarimeter.

RESULTS AND DISCUSSION

Most of cell wall Hyp was released upon

Abbreviations: Hyp, hydroxyproline; Araf, arabinofuranose; GLC, gas-liquid chromatography; PMR, proton magnetic resonance.

hydrolysis with saturated barium hydroxide, and acid hydrolysis of this alkali extract gave a recovery of 98% of Hyp contained in the initial cell wall preparation. Three hydroxyproline-arabinoside fractions (B-2, B-3 and B-4) were obtained from alkali extract of cultured tobacco cell walls. They were Ara₄-Hyp, Ara₄-Hyp+Ara₃-Hyp, and Ara₃-Hyp as described previously.³⁾

The standard methylation analytical procedure showed that B-2 (Ara₄-Hyp) contained terminal, 1,2-linked and 1,3-linked L-arabinofuranosyl residues in a molar ratio of 1:2:1. On the other hand, B-4 (Ara₃-Hyp) contained these residues in a ratio of 1:2:0.³⁾ From these results, there is only one possible sequence of residues in B-4 triarabinoside, but for B-2 tetraarabinoside three possible sequences must be considered.

To investigate the sequence of B-2 tetraarabinoside, B-2 (Ara₄-Hyp) was oxidized with sodium metaperiodate, reduced, and then hydrolyzed with mild acid (controlled Smith-degradation). The consumption of IO₄⁻ was 0.23 μ mole per 1 μ mole of arabinofuranose. Ten mg of controlled Smith-degraded B-2 was methylated and subjected to GLC analysis as partially methylated alditol acetates. The results of GLC analysis are shown in Table I,

TABLE I. MOLAR RATIO OF SEVERAL PARTIALLY METHYLATED ARABINITOL ACETATES^{a)}

Fractions examined	L-Arabinitol acetates of		
	2,3,5-Tri- O-methyl	3,5-Di-O- methyl	2,5-Di-O- methyl
B-2	1	2	1
Smith-degraded			
B-2	1	1.3	0
B-4	1	1.8	0

^{a)} The GLC analysis was carried out on a Shimadzu model GC-4CM gas chromatograph equipped with a hydrogen flame ionization detector using a column (2000 $\times$ 3 mm ϕ , glass) packed with Gas Chrome P (100~120 mesh) coated with a mixture of ethylene glycol adipate polyester (0.2%, w/w), ethylene glycol succinate polyester (0.2%) and Silicone XF-1150 (0.4%). The column oven temperature was elevated from 120°C to 180°C at the rate of 1°C/min after initial holding at 120°C for 10 min. The carrier gas (He) flow rate was 60 ml/min.

in which the disappearance of the peak corresponding to 2,5-di-O-methyl-L-arabinitol triacetates indicates that the terminal L-arabinofuranosyl residue is attached to the next one by a 1 $\rightarrow$ 3 O-glycosidic bond, since it is known that neither 1,2-linked nor 1,3-linked L-arabinofuranosyl residue is degraded upon periodate oxidation. The molar ratio of the peak of 3,5-di-O-methyl-L-arabinitol triacetates to 2,3,5-tri-O-methyl-L-arabinitol diacetates (1.3) is smaller than expected (2.0). Probably, the two reducing process with sodium borohydride effected the yield, for the reaction velocity is remarkably decreased below pH 9.5.

Secondly, the anomeric configuration of the hydroxyproline-arabinosides was examined.

The results of measurement of PMR are shown in Table II. Capon and Thacker⁴⁾ measured the PMR spectra of α - and β -methyl glucosides of L-arabinofuranose. They reported that the coupling constants ($J_{1,2}$) of α - and β -derivatives were 1.0 Hz and 4.0 Hz, respectively. As shown in Table II, the coupling constant of the anomeric proton of the arabinosides is about 4.0 Hz, showing that these arabinosides are β -anomers.

TABLE II. CHEMICAL SHIFTS (δ) AND COUPLING CONSTANT ($J_{1,2}$) OF THE ANOMERIC HYDROGEN ATOMS

Fractions examined ^{a)}	δ (ppm)	$J_{1,2}$ (Hz)
B-2	5.09	4.3
B-3	5.06	4.0
B-4	5.04	3.7
α -L-Araf	5.07	1.5

^{a)} The concentrations were 20 mg/0.5 ml D₂O.

TABLE III. OPTICAL ROTATION OF HYDROXYPROLINE-ARABINOSIDES^{a)}

Fractions examined	$[\alpha]_D^{25}$	c
B-2	+36.4	0.48
B-3	+73.6	0.53
B-4	+78.4	0.35
<i>trans</i> -4-Hydroxy-L-proline	-76.3	1.0
α -L-Araf	+98.2	1.0

^{a)} The optical rotation was measured with a JASCO model DIP-181 polarimeter with micro cell (3 ϕ $\times$ 100 mm) using H₂O as solvent.

Optical rotation was also employed to determine the anomeric configuration of the oligosaccharides. The results are shown in Table III. Arabinans containing α -type linkages generally show negative optical rotation,^{5,6)} but as shown in Table III, positive values were observed in these hydroxyproline-arabinosides.

From the results described above, it is concluded that the structure of Ara₃-Hyp and Ara₄-Hyp from cell wall preparation of cultured tobacco cells was as follows: β -L-Araf-(1 $\rightarrow$ 2)- β -L-Araf-(1 $\rightarrow$ 2)- β -L-Araf-(1 $\rightarrow$ 4)-Hyp and β -L-Araf-(1 $\rightarrow$ 3)- β -L-Araf-(1 $\rightarrow$ 2)- β -L-Araf-

(1 $\rightarrow$ 2)- β -L-Araf-(1 $\rightarrow$ 4)-Hyp, respectively.

REFERENCES

- 1) D. T. A. Lampion, *Annu. Rev. Plant Physiol.*, **21**, 235 (1970).
- 2) D. T. A. Lampion and D. H. Miller, *Plant Physiol.*, **48**, 454 (1971).
- 3) Y. Akiyama and K. Katô, *Agric. Biol. Chem.*, **41**, in press.
- 4) B. Capon and D. Thacker, *Proc. Chem. Soc.*, 369 (1964).
- 5) S. Karácsonyi, R. Toman, F. Janeček and M. Kubačková, *Carbohydr. Res.*, **44**, 285 (1975).
- 6) O. Larm, O. Theandar and P. Åman, *Acta Chem. Scand., Ser. B*, **29**, 1011 (1975).