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# Arabinogalactan-Proteins from Primary and Mature Roots of Radish (*Raphanus sativus* L.)

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## ABSTRACT

**Organ-specific variations in blood group H-like activity were observed in developing radish plants. A temporary increase in serological activity was found to occur in the roots at the earlier stages of development. Arabinogalactan-proteins (AGPs) were isolated from primary and mature roots, and investigated for changes in their physicochemical properties, structure, and serological activities. These root AGPs were composed mainly of L-arabinose and D-galactose but were distinguishable from each other in their contents of L-fucose as well as of protein and hydroxyproline. The structures of the carbohydrate moieties of the root AGPs were essentially similar to those of AGPs isolated from seeds and mature leaves in that they consisted of consecutive (1→3)-linked β-D-galactosyl backbone chains having side chains of (1→6)-linked β-D-galactosyl residues, to which α-L-arabinofuranosyl residues were attached in the outer regions. One prominent feature of the primary root AGPs was that they contained appreciable amounts of L-fucose, which was presumably responsible for expression of the serological activity. In their immunological reactions with rabbit anti-radish leaf AGP antibody, the root AGPs were shown to share common antigenic determinant(s) with those of seed and leaf AGPs.**

The wide distribution of AGs[1] and AGPs in various plants has drawn much attention to their structure, cellular localization, and physiological function (7, 9). L-Fucose-containing AGPs from aqueous extracts of mature radish leaves exhibit a potent blood group H-like activity, inhibiting the hemagglutination of human O erythrocytes with eel anti-H agglutinin (19, 26). In contrast, an AG and an AGP isolated by us from radish seeds were found to contain D-xylose instead of L-fucose, thus lacking the serological activity (27). Further, it has been shown that leaf and seed AGPs differ from each other not only in the structures of their carbohydrate moieties, especially in the outer regions, but also in their amino acid compositions. These findings suggest that the composition and structure of AG and AGPs in radish plants are variable, depending on the physiological functions of the organs where the specific proteoglycans are produced.

The observation of a transient increase in H-like activity in developing radish roots prompted us to examine the relationship between the appearance of the serological activity, and the compositions and structures of the polysaccharides relevant to differentiation and growth of the roots. In this paper, we describe the characterization of AGPs isolated from primary and mature radish roots, and compare their properties and structures with those of AG and AGPs from other organs.

## MATERIALS AND METHODS

**Plant Materials.** Seeds of the radish (*Raphanus sativus* L. var. *hortensis* cv Aokubi) were purchased from Tokita Seed and Plant Co., Ltd., Saitama, Japan. The seeds were sterilized with 3% (w/v) $Ca(OCl)_2$, rinsed, imbibed on filter paper discs moistened with sterile distilled water containing penicillin (0.7 mg/ml) and streptomycin (0.15 mg/ml) in glass culture dishes, and allowed to germinate at 25°C in the dark for 2 d. The dark-grown seedlings were then exposed to continuous illumination with fluorescent lamps at 120 $\mu E/m^2 \cdot s$ (measured with a LI-COR LI-185B meter and LI-190SB sensor) for 11 d. Some seedlings were also grown in an experimental field. The average fresh weights (g) of single plants and roots (in parentheses) from appropriate numbers of plants were as follows: 0.013, 0.04, 0.07 (0.018), 0.11 (0.02), 0.17 (0.04), 0.21 (0.05), 2.0 (0.1), 11 (0.5), 72 (19), 182 (85), and 335 (202) after growth for 0, 2, 4, 6, 8, 11, 21, 28, 41, 49, and 56 d, respectively. Organs were excised and stored at −20°C. For large-scale culture, sterilized seeds were imbibed on nylon grids in plastic trays filled with tap water to the level of the seeds, and allowed to grow with daily changes of water under the conditions outlined above. Primary roots (average fresh weight and length, 37 mg and 6.5 cm) were cut from the seedlings after 8 d, and stored at −20°C. A fresh mature root (1.21 kg) of the same cultivar was purchased from a farm.

**Chemicals.** α-L-Arabinofuranosidase from *Rhodotorula flava* (28) was a generous gift from Dr. N. Shibuya, National Food Research Institute, Ibaraki, Japan. Pork liver L-fucose dehydrogenase was prepared by the method of Schachter *et al.* (21). An L-fucose-specific lectin of *Aleuria aurantia* was purified according to the procedure of Kochibe and Furukawa (16). β-Glucosyl Yariv antigen was synthesized by the method of Yariv *et al.* (30).

**Extraction of AGPs.** Various organs (>1 g, fresh weight) were homogenized with five times their weight of PBS in an ice-chilled mortar. The homogenates were heated for 30 min at 100°C, cooled, and centrifuged at 12,000 *g* for 10 min. To examine the mol wt distribution and serological activity, the organs (20 g) were homogenized with PBS containing 1 mM $HgCl_2$. The extracts were adjusted to 5% (w/v) TCA, and after standing for 2 h at 4°C, the resultant precipitate was removed by centrifugation. The TCA in the supernatant was extracted three times with two volumes of diethyl ether. Three volumes of ethanol were added to the aqueous phase and the mixture was left overnight at 4°C.

[1] Abbreviations: AG, arabinogalactan; AGP, arabinogalactan-protein; PBS, 14.5 mM phosphate buffer, pH 7.2, containing 0.13 M NaCl; PBSN, PBS plus 0.02% (w/v) $NaN_3$.

The precipitate was collected, dialyzed, and subjected to gel filtration on a column (1.8 × 90 cm) of Sepharose CL-4B equilibrated with PBSN.

**Purification of AGPs from Primary Roots.** Eight-d-old primary roots (970 g corresponding to 45 g as dry weight) were dissected and homogenized in a blender for 10 min with four times their weight of PBS containing 1 mM $HgCl_2$. The homogenate was heated for 30 min at 100°C, cooled, and filtered. Two volumes of ethanol were added to the clear filtrate and the mixture was left overnight at 4°C. The sediment was collected by centrifugation, dialyzed against water, and lyophilized (756 mg). The crude proteoglycan preparation was dissolved in water and fractionanted on a column (2.8 × 27 cm) of DEAE-cellulose ($HCO_3^-$). A small portion (22 mg) of neutral polysaccharide was washed through the column with water. Elution with a linear gradient of $NaHCO_3$ (0–0.7 M, 1 L) afforded a broad peak of proteoglycan in the range of 150 to 300 mM $NaHCO_3$. The fractions were pooled, dialyzed, and lyophilized (335 mg). Subsequent gel filtration of the proteoglycan fraction on a Sepharose 6B column (4 × 120 cm) equilibrated with PBSN resulted in the separation of two serologically active proteoglycans: a minor component (39 mg) emerged at the void volume, and a major component (195 mg) of low mol wt was eluted later. The high mol wt component was further chromatographed on a Sepharose CL-2B column (3.2 × 90 cm) equilibrated with PBSN to give a purified proteoglycan (termed AGP-I, 21 mg) which was dialyzed and lyophilized. Similarly, another purified proteoglycan (termed AGP-II, 115 mg) was obtained after gel filtration of the low mol wt component from Sepharose 6B on a Sephacryl S-300 column (3.2 × 100 cm) equilibrated with PBSN.

**Purification of AGPs from Mature Roots.** Mature peeled roots (58.5 g, dry weight) were cut into small pieces, and homogenized with three times their weight of PBS containing 1 mM $HgCl_2$ for 5 min. The homogenate was mechanically stirred for 40 min, and then centrifuged. After concentration by evaporation, one-sixth its volume of 7% (w/v) cupric acetate was added to precipitate pectin (610 mg) (23). Addition of ethanol (three volumes) to the supernatant precipitated a crude polysaccharide which was collected by centrifugation, and suspended in 80% (v/v) ethanol. The suspension was chilled in an ice bath, acidified with 5 N HCl to a final concentration of 0.3 N, and stirred for 30 min. The resultant precipitate was dialyzed against 5 mM EDTA followed by water, and lyophilized (217 mg). Chromatography on a DEAE-cellulose column (2.8 × 16 cm, $HCO_3^-$) of the desalted fraction provided three polysaccharides: a neutral polysaccharide (7 mg) was washed through the column with water, and two other fractions, a minor component (22 mg) and a major component (90 mg), were eluted with $NaHCO_3$ in the range of 20 to 60 mM and 120 to 240 mM, respectively, following elution with a linear gradient of $NaHCO_3$ (0–500 mM, 2 L). The minor component was purified by successive gel filtration on a Sepharose 6B column (4 × 100 cm) followed by a Sephadex G-100 column (2.5 × 45 cm) equilibrated with PBSN. A single symmetric peak with a distribution coefficient ($K_d$) of 0.55 on the Sephadex G-100 column was obtained. The fractions collected were combined, dialyzed, and lyophilized (termed AGP-III, 14 mg). Gel filtration of the major component on the Sepharose 6B column afforded a single main peak with a shoulder of high mol wt, and the main fractions were pooled, dialyzed, and lyophilized (termed AGP-IV, 65 mg).

**Analytical Methods.** Total carbohydrate, reducing sugar, uronic acid, hydroxyproline, and protein were determined colorimetrically (19, 26). Analysis of L-fucose was performed enzymatically (21) after acid hydrolysis of the samples. Monosaccharide composition was determined by GLC analysis of alditol acetates derived from sugars in the hydrolyzates of the samples (19). For the determination of uronic acid, the samples were carboxyl-reduced (24), hydrolyzed, and subjected to GC-MS analysis after conversion with $NaB^2H_4$ of the free sugars to alditol acetates. The glycosidic linkages of the AGPs were determined and quantitated by GLC and GC-MS analyses of partially methylated sugars obtained by acid hydrolysis of permethylated (13) AGPs. GC-MS analysis was performed in a Hitachi M-80A fitted with a column (0.25 mm × 30 m) of DB-1 fused silica capillary (J & W Scientific Inc.) at 180 or 220°C. $^{13}C$-NMR spectra (25 MHz) were recorded using a JEOL FX-100 in $^2H_2O$ at 70°C containing 1,4-dioxane (67.4 ppm downfield from tetramethylsilane) as the internal standard. The amino acid compositions of the AGPs were analyzed fluorimetrically by the *o*-phthalaldehyde method employing a Hitachi 655 amino acid analysis system (19). Paper chromatography and high voltage electrophoresis on a glass fiber paper were carried out as described previously (19). The apparent mol wt of the AGPs were estimated on a Sepharose CL-6B column (1 × 85 cm) which had been equilibrated with PBSN and calibrated with pullulans (Shodex standard P-82, Showa Denko), blue dextran ($V_0$), and D-glucose ($V_i$). The $V_0$ of the columns of Sepharose CL-2B or CL-4B was determined with heat-killed bacterial cells.

Sedimentation analysis was performed in a Hitachi analytical ultracentrifuge UCA-1A with a double-sector cell using 0.5% (w/v) of sample in 0.9% (w/v) NaCl at 60,000 rpm and 20°C. The intrinsic viscosity was determined in a Cannon-Fenske viscometer (27). The serological activity of the AGPs was assayed as described previously (19). Double diffusion was conducted in a 1% (w/v) agarose layer (GP-36, Nakarai Chem. Co.) containing PBS on a microscopic slide glass. Aliquots (10 μl) of rabbit anti-radish leaf AGP sera (25), eel anti-H precipitin, the L-fucose-specific lectin, or Yariv antigen were placed in each center well surrounded by regularly spaced wells containing antigens (10 μl at 5 mg/ml in PBS).

**Periodate Oxidation.** The AGP-II (30 mg) from the primary roots was oxidized with 50 mM $NaIO_4$ (50 ml) for 100 h at 4°C in the dark. The oxidized product was subjected to Smith degradation (12) including reduction with $NaBH_4$ followed by mild acid hydrolysis with 1 N trifluoroacetic acid for 48 h at 25°C. An acid-resistant polymer (6 mg) was obtained from the hydrolyzate by precipitation with 75% (v/v) methanol.

**Enzymatic Degradation.** The AGPs (10 mg each) were incubated with α-L-arabinofuranosidase (1 unit) in 10 mM citrate-phosphate buffer, pH 3.0 (2.5 ml) under a drop of toluene for 20 h at 37°C. The reaction was terminated by heating at 100°C for 5 min. The enzyme-modified polymers were deionized with Dowex 50W ($H^+$) resin and separated from the released L-arabinose on a Sephadex G-15 column (2.3 × 30 cm).

## RESULTS

**Variations in Serological Activity of Different Organs.** Extracts from various tissues were assayed for hemagglutination inhibition in order to investigate the blood group H-like activity in seeds and other organs during development and growth of the radish plant. As shown in Figure 1, no appreciable activity could be detected in the extracts from resting seeds and 2-d-old seedlings. However, a marked increase of activity occurred in the primary roots of 4-d-old seedlings, although the activity in the cotyledons and hypocotyls was negligible. This enhancement of activity was a temporary physiological event since the activity was diminished in 11-d-old roots and disappeared with development of the roots towards maturation. In contrast, considerable activity was detected in the extracts of both young and mature leaves. Precipitation reactions with rabbit anti-radish leaf AGP sera and Yariv antigen in the agarose layer provided evidence to support the presence of AGPs in the serologically active tissue extracts. Figure 2 shows elution profiles of TCA-deproteinized polysaccharide fractions from seeds and roots on a Sepharose

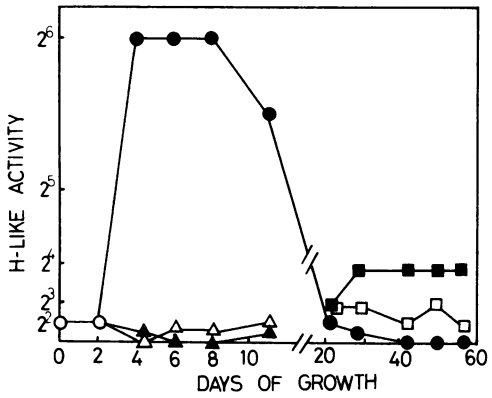


FIG. 1. Blood group H-like activity during the development of organs in radish plants. The activity of extracts of seeds and 2-d-old seedlings (○), cotyledons (△), hypocotyls (▲), roots (●), leaves (■), and midribs (□) was assayed for hemagglutination inhibition using a 1:4 titer of eel anti-H agglutinin.

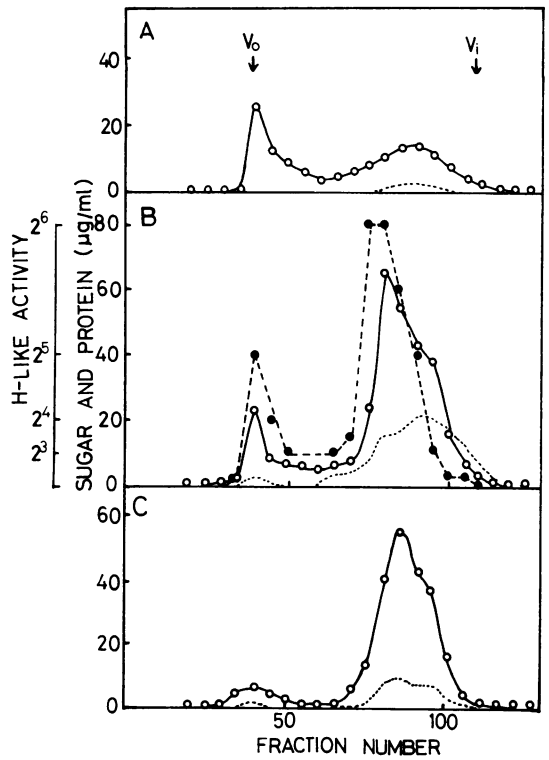


FIG. 2. Distribution of mol wt of AGPs and H-like activity in extracts of seeds and roots at different stages. Deproteinized polysaccharide preparations of seeds (A), 8-d-old roots (B), and 56-d-old roots (C) were chromatographed on a Sepharose CL-4B column and monitored for sugar (○), protein (- - - -), and H-like activity (●).

CL-4B column. The fractions from primary and mature roots could be similarly resolved into two components of differing mol wt, and only the components from primary roots had serological activity, although the serologically active fractions did not always coelute exactly with those of polysaccharide. These results suggest that the primary roots produced temporarily serologically active AGPs with different mol wt, whose chemical compositions differed from those of the AGPs in seeds and mature roots.

**Purity of AGPs.** The homogeneity of the purified AGPs was investigated by high voltage electrophoresis on a glass fiber paper and ultracentrifugal analysis. AGP-I and -II from primary roots migrated as a single polysaccharide component on electrophoresis: sugar, protein, and serological activity were found in essentially the same fractions. On sedimentation analysis, AGP-II yielded a single symmetric peak indicative of homogeneity, while AGP-III and AGP-IV were also both shown to be apparently homogeneous. Gel filtration of AGP-I or AGP-II on Sepharose CL-2B or CL-6B afforded a single symmetric peak of sugar, virtually coincident with those of protein and the serological activity. AGP-III appeared to be contaminated with a small amount of protein, since there was a discrepancy between the peaks of sugar and protein on a Sepharose CL-6B column (data not shown).

**Properties of Root AGPs.** Table I summarizes the physical and chemical properties, and serological activity of the radish root AGPs. The major components, AGP-II and -IV, in the primary and mature roots are similar in mol wt, and contents of protein, arabinose and galactose. However, one characteristic feature of the AGP-II is its L-fucose content of 4.1% (w/w), the L-configuration of which was confirmed by the enzymatic method (21) and has been accepted as a structural requirement of the 6-deoxy hexose for the expression of blood group H(O) specificity (22). AGP-I, a minor component in the primary roots, was found to be a high mol wt polysaccharide giving a highly viscous solution, and had a potent H-like activity due to its L-fucose content of 3.7% (w/w). The mature root AGP-III, a low mol wt minor component, can apparently be regarded as an AG with a high proportion of arabinose because of its low content of protein.

Analysis of monosaccharides derived from the carboxyl-reduced sample revealed that the uronic acid constituents in AGP-II consisted of D-glucuronic acid and its 4-*O*-methyl derivative in a molar ratio of 0.9:1. 4-*O*-Methyl-D-glucuronic acid was found as the sole uronic acid constituent in AGP-IV. The 4-*O*-methyl derivative was further confirmed from the detection of fragments, m/z 189 and 262, characteristic (15) of 1,2,3,5,6-penta-*O*-acetyl-4-*O*-methyl-hexitol-1-$^2H$ on GC-MS analysis.

Amino acid analysis of the root AGPs revealed as a common feature, high proportions of hydroxyproline, serine, theronine, and alanine (Table II) as observed in typical AGPs (7, 9). AGP-I was characteristically rich in glutamic acid or glutamine, glycine, and alanine indicating the presence of non-AGP protein component(s). The contents of hydroxyproline were also estimated colorimetrically to be 0.9, 37.0, 6.4, and 30.0 μg/mg in AGP-I, -II, -III, and -IV, respectively.

**Glycosidic Linkages of AGPs.** Table III summarizes the data for structural analysis by methylation of AGP-II, -III, and -IV. Methylation analysis of AGP-II revealed a relative abundance of 2,4,6-, 2,3,4-tri-, and 2,4-di-*O*-methyl-galactose indicating an inner structure composed of *O*-3-, *O*-6-, and *O*-3,6-linked galactosyl residues. The detection of 2,3,4,6-tetra-*O*-methyl-galactose implies the presence of nonreducing terminal galactosyl groups. A preponderance of 2,3,5-tri-*O*-methyl-arabinose indicates the location of a large part of this sugar as the furanose form at the nonreducing terminal. The presence of *O*-2- or *O*-5-linked arabinosyl residues can be deduced from the small proportions of 3,5- and 2,3-di-*O*-methyl-arabinose. The fucopyranosyl groups exclusively originate from the nonreducing terminal. The location of uronosyl at the nonreducing terminal is suggested by the detection of 2,3,4,6-tetra-*O*-methyl-glucose after methylation analysis of the carboxyl-reduced AGP-II. The polysaccharide moiety of the mature root AGP-IV closely resembles that of AGP-II in both its type and proportion of glycosidic linkages, except for the absence of nonreducing terminal fucosyl and *O*-2-linked arabinofuranosyl residues in AGP-IV. The carbohydrate moiety of AGP-III is characterized by a high proportion of *O*-3,6-linked branching galactosyl residues together with nonreducing terminal and *O*-5-linked arabinosyl residues.

An anomeric configuration of glycosidic linkages was assumed

Table I. *Physical Properties, Chemical Compositions, and H-Like Activity of Root AGPs*

| | AGP-I | AGP-II | AGP-III | AGP-IV |
|---|---|---|---|---|
| Yield (wt %, dry basis) | 0.05 | 0.26 | 0.02 | 0.11 |
| Mol wt ($\times 10^{-4}$) | (0.21[a]) | 7.8 | 2.5[b] and 3.9[c] | 8.8 |
| $s_{20,w}$ (S) | | 4.7 | 1.8 | 5.7 |
| $[\alpha]_D$ ($c = 0.4$ in $H_2O$) | −121° | −42° | −80° | −43° |
| $[\eta]$ (dl/g) | | | 0.032 | 0.145 |
| Kjeldahl N (wt %) | 1.7 | 1.1 | 0.3 | 1.1 |
| Total sugar (wt %) | 88 | 92 | 82 | 88 |
| Sugar composition (mol %) | | | | |
| Fuc | 10[d] | 5 | 0[e] | 0 |
| Ara | 24 | 27 | 55 | 24 |
| Gal | 33 | 61 | 40 | 62 |
| Glc | 16 | 0 | 0.1 | 0 |
| Uronic acids | 8 | 7 | 4 | 14 |
| H-like activity (μg/ml) | 2.5 to 5 | 2.5 to 5 | >5000 | >5000 |

[a] $K_d$ determined on a Sepharose CL-2B column. [b] Peak of sugar. [c] Peak of protein. [d] Contains Rha 2%, Xyl 2%, and Man 5% as additional sugar components. [e] Contains Man 1%.

Table II. *Amino Acid Compositions of Root AGPs*

| Amino Acid | AGP-I | AGP-II | AGP-III | AGP-IV |
|---|---|---|---|---|
| | | *mol %* | | |
| Lys | 4.2 | 1.4 | 4.3 | 2.6 |
| His | 2.0 | 1.0 | 0.7 | 0.6 |
| Arg | 2.4 | 0.3 | 1.8 | 0.3 |
| Hyp | 3.0 | 30.0 | 16.8 | 43.1 |
| Asx | 9.4 | 3.9 | 8.9 | 3.6 |
| Thr | 6.3 | 12.2 | 7.8 | 8.0 |
| Ser | 9.9 | 10.2 | 10.7 | 10.7 |
| Glx | 10.5 | 4.6 | 7.9 | 3.9 |
| Pro | 4.9 | 3.4 | 3.5 | 1.8 |
| Gly | 13.7 | 4.5 | 7.1 | 3.3 |
| Ala | 10.1 | 18.2 | 9.2 | 12.5 |
| Half-Cys | 1.8 | 1.1 | 0.9 | —[a] |
| Val | 9.2 | 5.9 | 7.3 | 5.0 |
| Met | — | — | 0.8 | 0.7 |
| Ile | 3.5 | 0.7 | 3.2 | 0.7 |
| Leu | 5.9 | 2.4 | 5.4 | 2.2 |
| Tyr | — | — | 1.4 | 0.4 |
| Phe | 3.2 | 0.2 | 2.3 | 0.6 |
| Amino acid content (wt %) | 6.4 | 8.6 | 3.9 | 10.1 |

[a] Not detectable.

on the basis of $^{13}C$-NMR spectra of the AGPs. Terminal α-L-arabinofuranosyl, *O*-5-linked α-L-arabinofuranosyl, and β-D-galactopyranosyl residues were assigned from the signals in the anomeric carbon region (110.00, 108.39, and 104.05 ppm) of the spectrum of AGP-III, respectively (1, 8). The last two signals in the spectrum of AGP-IV (109.90, 108.34, 104.15, and 103.71 ppm) appeared to be attributable to β-D-galactopyranosyl and 4-*O*-methyl-β-D-glucopyranosyluronic acid residues.

**Periodate Oxidation.** AGP-II consumed 0.90 mol of periodate, with concomitant production of 0.41 mol of formic acid per sugar residue. After Smith degradation of the oxidized product, precipitation with 75% (v/v) methanol yielded a polymer that, on gel filtration, was eluted as a single component having $[\alpha]_D$ −15° ($c = 0.4$ in $H_2O$) and a diminished mol wt of 36,000. The degraded product was a galactan in nature, consisting of arabinose and galactose in a molar proportion of 8:92. The reduction of mol wt in the degraded product could be explained by the removal of a large part of the sugar residues in the peripheral region of the native form, although the presence of subunit structure as observed in AGPs of *Acacia* species (6) cannot be ruled out. Methylation analysis of native AGP-II and the degraded product (Table III, columns A and D) indicated a high proportion of *O*-3-linked galactosyl residues with a concomitant decrease in those of *O*-6- and *O*-3,6-linked galactosyl residues. The *O*-2-linked arabinofuranosyl residues resistant to periodate oxidation in the native form remained attached as the nonreducing terminal groups in the degraded product.

**α-L-Arabinofuranosidase Digestion.** Digestion of AGP-II, -III, and -IV with α-L-arabinofuranosidase resulted in the release of 13, 55, and 19% (w/w, sugar basis) of L-arabinose as a sole product. Methylation analysis of the enzyme-modified products (Table III, columns C, G, and J) provided evidence that the enzyme removed large quantities of nonreducing terminal and *O*-5-linked arabinosyl residues, leading to an increase in nonreducing and *O*-6-linked galactosyl residues and a concomitant decrease in *O*-3- and *O*-3,6-linked galactosyl residues. No appreciable change occurred in the proportion of *O*-2-linked arabinosyl residues in AGP-II.

**Precipitation Reactions.** The precipitation reactions between rabbit anti-leaf AGP sera and AGPs or an AG from various radish tissues are shown in Figure 3. Although all the AGPs (but not the seed AG) reacted with the antisera, only the primary root AGP-II formed a precipitin line which fused with those of the leaf and seed AGPs, indicating the presence of common antigenic determinant(s). The failure of the mature root AGPs and primary root AGP-I to form fused precipitin lines indicates only partial identity in the antigenic site(s) of these AGPs with those from other organs. As expected, the primary root AGPs containing L-fucose formed clear precipitin lines which fused with those of the leaf AGPs when reacted with either eel anti-H precipitin (Fig. 3) or *A. aurantia* L-fucose-specific lectin (data not shown). With Yariv antigen, all the root AGPs gave clear precipitin lines with the exception of the mature root AGP-III.

## DISCUSSION

It has been pointed out that type II arabino-3,6-galactans have a similar core structure, but variable side chains, depending upon the species and organ concerned (2, 7, 9). Chemical and electrophoretic analyses have demonstrated that the AGs occurring in various organs of *Gladiolus gandavensis* and *Lilium longiflorum* vary significantly in their numbers, mobilities, and sugar compositions (11). Changes in protein content, and sugar and amino acid compositions have been extensively examined in AGPs from

Table III. *Glycosidic Linkages of Root AGPs*

| Methylated Sugar[a] | Mode of Linkage | AGP-II[b] | | | | AGP-III[b] | | | AGP-IV[b] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I | J |
| | | *mol %* | | | | | | | | | |
| 2,3,4-$Me_3$-Fuc | Terminal | 5 | 5 | 4 | —[c] | — | — | — | — | — | — |
| 2,3,5-$Me_3$-Ara | Terminal | 14 | 12 | 1 | 10 | 36 | 32 | +[d] | 25 | 20 | 2 |
| 3,5-$Me_2$-Ara | 2- | 8 | 7 | 8 | — | + | + | + | 1 | + | 2 |
| 2,3-$Me_2$-Ara | 5- | 4 | 3 | 1 | — | 23 | 26 | + | 5 | 4 | — |
| 2-Me-Ara | 3,5- | — | — | — | — | + | + | — | — | — | — |
| 3-Me-Ara | 2,5- | — | — | — | — | 1 | 1 | — | — | — | — |
| 2,3,4,6-$Me_4$-Gal | Terminal | 7 | 6 | 11 | 19 | + | + | 2 | 6 | 3 | 11 |
| 2,4,6-$Me_3$-Gal | 3- | 16 | 13 | 13 | 49 | 2 | 1 | + | 12 | 7 | 6 |
| 2,3,4-$Me_3$-Gal | 6- | 20 | 17 | 28 | 4 | 4 | 5 | 40 | 22 | 23 | 44 |
| 2,4-$Me_2$-Gal | 3,6- | 26 | 30 | 21 | 18 | 32 | 32 | 1 | 29 | 30 | 16 |
| 2,3,4,6-$Me_4$-Glc | Terminal | — | 7 | — | — | — | 1 | — | — | 13 | — |
| 2,3,6-$Me_3$-Glc | 4- | — | — | — | — | + | 1 | + | — | — | — |
| 2,3,6-$Me_3$-Man | 4- | — | — | — | — | 1 | 1 | 1 | — | — | — |
| Free Ara[e] | | — | — | 13 | — | — | — | 55 | — | — | 19 |

[a] 2,3,4-$Me_3$-Fuc = 1,5-di-*O*-acetyl-2,3,4-tri-*O*-methyl-fucitol, etc. [b] A, E, and H, Native; B, F, and I, carboxyl-reduced; C, G, and J, arabinosidase-digested products; D, Smith-degraded polymer. [c] Not detectable. [d] Trace amount (<0.5%). [e] Reducing sugar in arabinosidase-digested product.

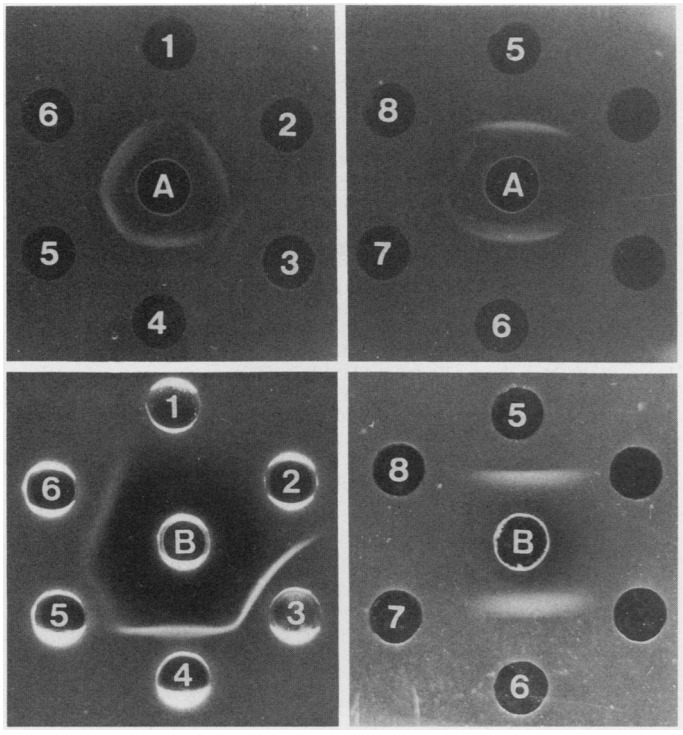


FIG. 3. Precipitation reactions of AGPs and an AG from various organs of radish plants with rabbit anti-radish leaf AGP sera (A) and eel anti-H precipitin (B). The surrounding wells contained seed AG (*1*) and AGP (*2*), primary root AGP-I (*3*) and -II (*4*), leaf AGPs designated as R-I (*5*) and R-II (*6*) (19), and mature root AGP-III (7) and -IV (*8*). Diffusion was allowed to occur in a moist atmosphere for 12 to 24 h at 4°C or at room temperature.

various organs at different stages of development from seedlings to mature plants in the Douglas fir and loblolly pine (4). Characteristic sets of Yariv antigen-reactive AGPs have been detected in organs of *Glycine max* and *Lycopersicon peruvianum* by the application of crossed-electrophoresis (5, 29). Remarkable quantitative changes in AGPs have been observed in female sexual tissues of *Nicotiana alata* during development and pollination (10). These findings suggest that AGPs are organ-specific and regulated developmentally in response to physiological changes in the organs.

In radish plants, the leaf tissue contains two L-fucose-containing and blood group H-like active AGPs (19, 26), while AGP and AG from the seeds are devoid of L-fucose, and so serologically inactive (27). The transient increase in serological activity of the primary roots observed here implies that the root tissues at the earlier stages of development have the ability to produce L-fucose-containing AGPs.

Two types of L-fucose-containing AGPs, AGP-I and -II, were isolated from roots of 8-d-old radish seedlings, and characterized as having potent blood group H-like activity. AGP-I clearly differed from AGP-II and mature roots AGP-III and -IV in its high mol wt, and sugar and amino acid compositions. Its macromolecular nature and relative complexity in the polysaccharide moiety suggest that AGP-I is composed of either different polymers linked covalently or a polysaccharide complex containing AGP(s) that are reactive with Yariv antigen. In contrast, the primary root AGP-II having an apparent mol wt of 78,000 closely resembled leaf AGPs (19) of mol wt of 130,000 and 75,000 with regard to its physical properties and chemical composition. Of the two AGPs from mature roots, the low mol wt AGP-III is not homogeneous but composed mainly of arabinose, galactose, and uronic acids. Its lack of reactivity with Yariv antigen remained unaccounted for, since it contained a small amount of protein that was suspected of being an impurity but had a comparable amino acid composition to those of other AGPs. The AGP-IV with an apparent mol wt of 88,000 is typical of AGPs including those found in mature leaves, primary roots (AGP-II), and seeds, except insofar as the lack of auxiliary sugar constituents such as L-fucose and D-xylose is concerned.

Root AGPs have a similar structure with a galactan framework consisting of (1→3)-linked galactosyl backbone chains carrying (1→6)-linked galactosyl side chains as revealed by the isolation of a polymer with a high proportion of (1→3)-linked galactosyl residues from AGP-II after Smith degradation. Chemical and enzymatic methods indicated that most of the L-arabinofuranosyl residues were attached to the (1→6)-linked galactosyl side chains through *O*-3, while uronosyl and L-fucosyl residues were located at the nonreducing terminal ends. These analytical results for the glycosidic linkages unambiguously support a type II arabinogalactan structure for root AGPs as is the case for radish leaf and seed AGPs. It should be noted that the mature root AGP-

III characteristically contains a high proportion of (1→6)-linked galactosyl residues substituted at *O*-3 with L-arabinosyl residues.

The precipitation reactions using rabbit anti-leaf AGP antibody, eel and *A. aurantia* L-fucose-specific lectins provided evidence of apparent similarities in the carbohydrate moieties of root AGPs. In particular, primary root AGP-II was confirmed to share antigenic determinant(s) common to leaf and seed AGPs. Further, the formation of precipitin lines with the L-fucose-specific lectins together with the potent H-like activity suggested that the L-fucosyl residue of AGP-II was located at the nonreducing terminal, probably as α-L-fucopyranosyl-(1→2)-α-L-arabinofuranosyl-(1→ as has been proposed for the antigenic structure of leaf AGPs (26).

In higher plants, L-fucose is found as a sugar constituent of xyloglucan in the cell wall (3), a slime polysaccharide secreted from maize root cap (20), certain glycoproteins (14, 18), and AG (17). The transient formation of L-fucose-containing AGPs in primary roots may provide a clue for elucidating the physiological functions of the sugar.

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