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ANTI-α-L-ARABINOFURANOSE ANTIBODIES: PURIFICATION, IMMUNOCHEMICAL CHARACTERIZATION, AND USE IN HISTOCHEMICAL STUDIES OF PLANT CELL-WALL POLYSACCHARIDES

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ABSTRACT

Anti-α-L-arabinofuranose antibodies, raised in rabbits by immunization with *p*-diazophenyl α-L-arabinofuranoside–bovine serum albumin, were purified by affinity chromatography using an α-L-arabinofuranose–Sepharose column. The antibodies, proved by immunoelectrophoresis to be immunoglobulin G (IgG), specifically reacted with α-L-arabinofuranose residues, as confirmed by agar diffusion analysis and inhibition tests. Complement fixation studies showed that the purified antibodies recognize terminal α-L-arabinofuranose residues in glycoconjugates, plant polysaccharides, and synthetic polysaccharides having branches of α-L-arabinofuranosyl groups. The antibodies were shown to provide a useful histochemical probe for detection of arabinose-containing polymers in plant tissues, by either the fluorescence- or the peroxidase-labeled immunostaining method.

INTRODUCTION

L-Arabinose residues, mostly in the furanose form, occur in plants as a common component of such cell-wall polysaccharides as arabinan, arabinoxylan, arabinogalactan, or arabinogalactan–protein, and also in pectic polysaccharides. The physiological roles of these L-arabinose-containing polymers in plant cells have recently attracted increasing attention[1,2].

In relation to the physiological and architectural importance of individual polysaccharide components, information on their precise structural features and localization in the cell-wall tissues is necessary. Recently, Clarke and her coworkers obtained a rabbit serum against the arabinogalactan–protein of Gladiolus mucilage, and showed that it contained antibodies to both α-L-arabinose and β-D-

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galactose residues[3]. They also obtained monoclonal antibodies specific for L-arabinose or D-galactose residues[4].

In our previous study[5], a rabbit antiserum specific for α-L-arabinofuranose residues (abbreviation: α-L-Ara*f*) was prepared by immunizing with a synthetic α-L-arabinofuranose–bovine serum albumin conjugate (α-L-Ara*f*–BSA). It was successfully used in the histochemical identification of α-L-Ara*f*-containing polysaccharides in some plant cell-walls. In that study, antiserum was partially purified by passage through a BSA–Sepharose column to remove anti-BSA antibodies. However, it still contained, besides other serum components, the anti-α-D-Ara*f* antibodies elicited against the mycobacterial polysaccharides in the complete Freund's adjuvant (cFa) used. This has prompted us to purify the α-L-Ara*f*-specific antibodies by simple affinity chromatography, and we report here its immunochemical specificities and its interaction with natural and synthetic α-L-Ara*f*-containing polysaccharides, as revealed by the complement fixation method. This article is also concerned with the successful utilization of the purified antibodies in histochemical investigations on plant cell-walls.

RESULTS AND DISCUSSION

Preparation and purification of anti-α-L-Araf antibodies. — Purified α-L-Ara*f*–BSA that contained 27 α-L-Ara*f* mol per mol of BSA was used as the immunogen into rabbits, as described previously[5]. The antiserum was found to contain antibodies to α-L-Ara*f*–BSA, BSA, and α-D-Ara*f*–BSA. The last probably arose from the Freund's adjuvant, which contains mycobacterial polysaccharides having side chains of (1→5)-linked α-D-arabinofuranose residues[6,7]. Anti-BSA antibodies could be removed by passage through a column of BSA-conjugated Sepharose, but the fractions obtained still contained antibodies to α-L- and α-D-Ara*f*[5].

To obtain pure antibodies to α-L-Ara*f*, the original antiserum was passed through an α-L-arabinofuranoside-conjugated affinity column (prepared by coupling *p*-aminophenyl α-L-arabinofuranoside to CNBr-activated Sepharose 4B).

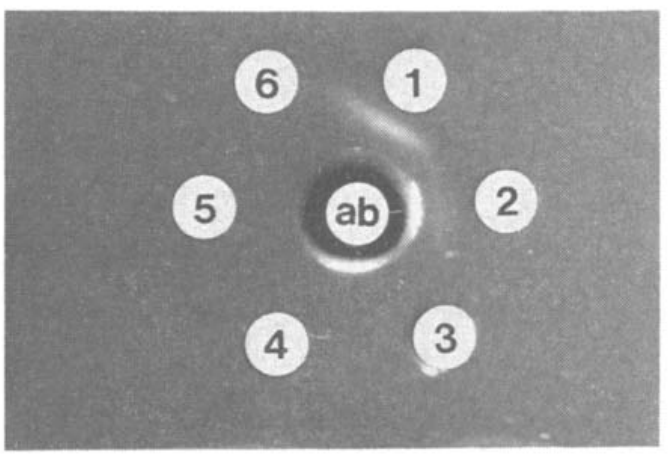

Fig. 1. Ouchterlony double diffusion in agar. [Center well (ab), purified anti-α-L-Ara*f* antibodies (0.5 mg of protein in 1 mL); well 1, α-L-Ara*f*–BSA; well 2, α-L-Ara*f*–ova; well 3, saline; well 4, α-D-Ara*f*–ova; well 5, α-D-Ara*f*–BSA; well 6, BSA. The concentrations of BSA and glycoconjugates were adjusted to 0.5 mg in 1 mL of saline.]

TABLE I

PURIFICATION OF ANTI α-L-ARABINOFURANOSE ANTIBODY

Fraction	*Total volume (mL)*	*Total protein (mg)*	*Yield (% as protein)*	*Purification[a] (fold)*
Antiserum	14	1246	100	1
Purified antibody	12	2.76	0.22	111

[a]Estimated by the box titration assay.

Preliminary examination indicated that ~76% of the serum protein was adsorbed on this affinity column if equilibrated with 50mM phosphate buffer in 0.15M NaCl, pH 7.2, but no adsorption of protein was observed when a lower concentration (10mM) of the same buffer was used. The best resolution was achieved by using 50mM borate buffer, pH 7.9, in 0.15M NaCl. The α-L-Ara*f*-specific antibodies were eluted with 0.17M glycine·HCl buffer, pH 2.3, as no antibodies could be released from the column with 0.1M L-arabinose solution. Application of 14 mL of antiserum afforded ~2.8 mg of purified antibodies. The agar immunodiffusion analysis indicated that the eluted protein reacted with both α-L-Ara*f*–BSA and α-L-Ara*f*–ovalbumin (α-L-Ara*f*–ova), but not with BSA nor α-D-Ara*f*–BSA, as shown in Fig. 1. Thus, simple affinity chromatography afforded highly purified anti-α-L-Ara*f* antibodies.

The complement fixation assay, using α-L-Ara*f*–ova as the antigen, indicated that the anti-α-L-Ara*f* antibodies so obtained were purified ~111-fold, compared with that of the antiserum (see Table I).

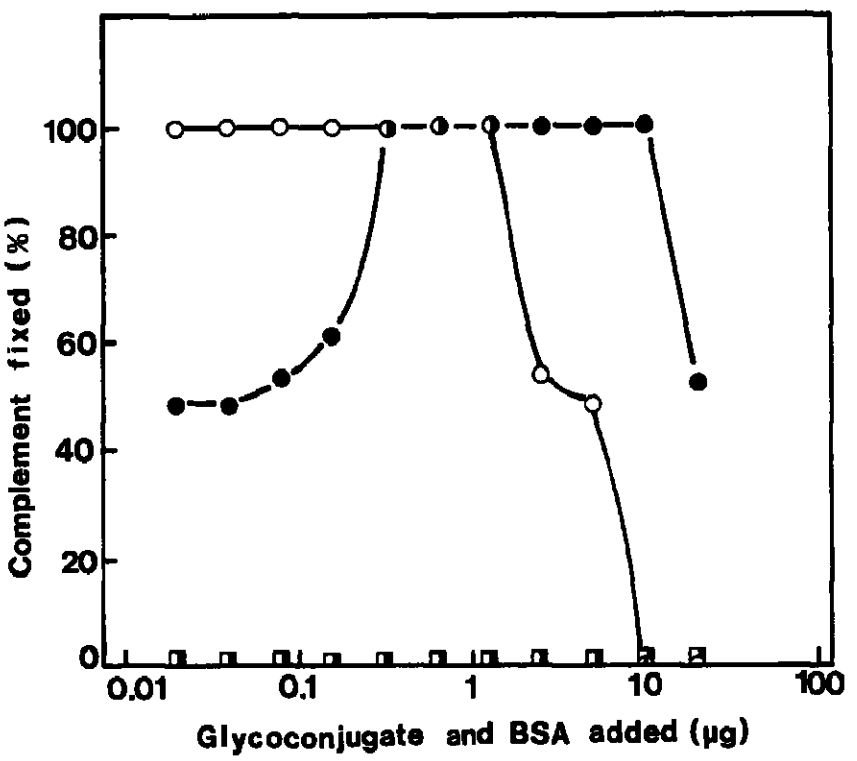

Fig. 2. Quantitative, complement fixation reactions of the purified anti-α-L-Ara*f* antibodies with Ara*f*–protein conjugates and BSA. [○, α-L-Ara*f*–BSA; ●, α-L-Ara*f*–ova; □, α-D-Ara*f*–BSA; ■, BSA.]

Purified anti-α-L-Ara*f* antibodies were further examined by immunoelectrophoresis. The antibodies formed a single precipitin line with the anti-whole rabbit serum antiserum, and also with anti-rabbit IgG antiserum, indicating that it was immunophoretically homogeneous.

Immunochemical specificities of the antibodies. — Binding specificities of the purified antibodies were investigated by the quantitative complement fixation method[8]. As shown in Fig. 2, the anti-α-L-Ara*f* antibodies interacted with α-L-Ara*f*–BSA (immunogen) and α-L-Ara*f*–ova, but not with BSA nor α-D-Ara*f*–BSA. These results, consistent with those obtained by the Ouchterlony double diffusion analysis (see Fig. 1), indicated that the antibodies specifically recognize α-L-arabinofuranose residues of these glycoconjugates.

Table II shows the results of the hapten inhibition tests using glycosides of D- and L-arabinose (and some other sugars) as potential inhibitors of the interaction between α-L-Ara*f*–BSA and anti-α-L-Ara*f* antibodies, as assayed by the quantitative complement fixation method. As anticipated, the glycosides of α-L-arabinofuranose inhibited the interaction, and, among these glycosides, *p*-nitrophenyl α-L-arabinofuranoside was the best inhibitor (50% inhibition at a concentration of 1.7mM). Methyl α-L-arabinofuranoside was also a good inhibitor (50% inhibition at a concentration of 1.35mM). However, α-D-arabinofuranosides and other glycosides, including α-L-arabinopyranosides, had essentially no inhibitory activity. These results again confirmed that the anti-α-L-Ara*f* antibodies were specific for α-L-arabinofuranose residues. The fact that *p*-nitrophenyl α-L-arabinofuranoside was more effective than methyl α-L-arabinofuranoside suggests that the purified antibodies may also recognize the phenyl group.

In the previous study[5], the quantitative precipitation studies indicated that the antiserum against α-L-Ara*f*–BSA interacted with different types of polysaccharides containing α-L-Ara*f*, such as arabinoxylans and arabinogalactans from different plant sources. Therefore, in the present study, the binding capabilities of the

TABLE II

INHIBITION OF THE INTERACTION OF ANTI-α-L-ARABINOFURANOSE ANTIBODY AND α-L-ARABINOFURANOSYL–BSA CONJUGATE WITH VARIOUS CARBOHYDRATE DERIVATIVES

Carbohydrate derivative	*Final concentration (mM)*	*Inhibition ratio (%)*
p-Nitrophenyl α-L-arabinofuranoside	0.0017	50
p-Nitrophenyl α-D-arabinofuranoside	0.5	0
p-Nitrophenyl α-D-galactopyranoside	2.5	0
p-Nitrophenyl α-D-mannopyranoside	2.5	0
Methyl α-L-arabinofuranoside	1.35	50
Methyl β-L-arabinofuranoside	10	0
Methyl α-L-arabinofuranoside	10	0
Methyl β-L-arabinofuranoside	10	0

purified α-L-Ara*f*-specific antibodies toward different types of arabinogalactans were examined with respect to those from soy bean *(Glycine max)* cotyledon, ginseng *(Panax ginseng)* root, and radish *(Raphanus sativus)* leaf. Fig. 3 shows the quantitative complement-fixation curves of interactions of the purified antibodies (14.4 μg of protein as BSA) with increasing amounts of different types of plant arabinogalactans. The arabinogalactans of soy bean and ginseng fixed 80% and 50% of the complement, respectively, at the maximum. In contrast, the radish arabinogalactan showed a very weak reactivity with the antibodies. Differences in the reactivities of these arabinogalactans toward the anti-α-L-Ara*f* antibodies may probably be attributed to their molecular shapes, such as the mode of branching, and the lengths and distributions of their L-arabinofuranosyl side-chains.

For soy-bean arabinogalactan, early studies showed[9] that it has very short side-chains, on the average two α-(1→5)-linked L-Ara*f* residues attached to the backbone of β-(1→4)-linked D-galactopyranose residues at O-3. However, our recent re-investigation, by chemical and enzymic degradation studies, strongly suggested that the soy-bean arabinogalactan reported earlier is actually an arabinogalactogalacturonan. It may have a rather pendant-like structure, with branched α-(1→5)-linked L-arabinofuranosyl groups and essentially linear chains of β-(1→4)-linked D-galactopyranose residues attached to the backbone chain of β-(1→4)-linked D-galacturonan at the O-3 atoms[10]. The arabinogalactan from the root of ginseng appeared to contain, similarly, side chains of branched α-L-(1→5)-linked L-arabinofuranosyl groups.

Such branched structural features of the L-arabinofuranosyl side-chains may have many terminal α-L-Ara*f* groups available to anti-α-L-Ara*f* antibodies. On the other hand, the radish-leaf arabinogalactan, which was much less reactive with the antibodies, has a branch-to-branch structure, where a β-(1→3)-linked D-galactan

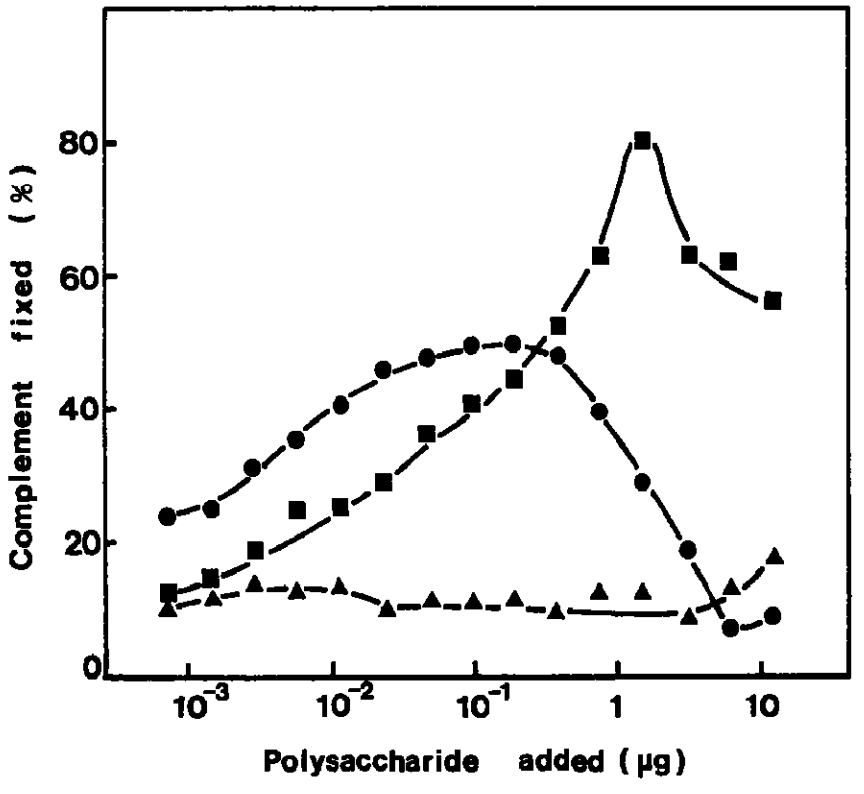

Fig. 3. Quantitative, complement fixation reactions of the purified anti-α-L-Ara*f* antibodies with some plant arabinogalactans. [■, Soy-bean cotyledon; ●, ginseng root; ▲, radish leaf.]

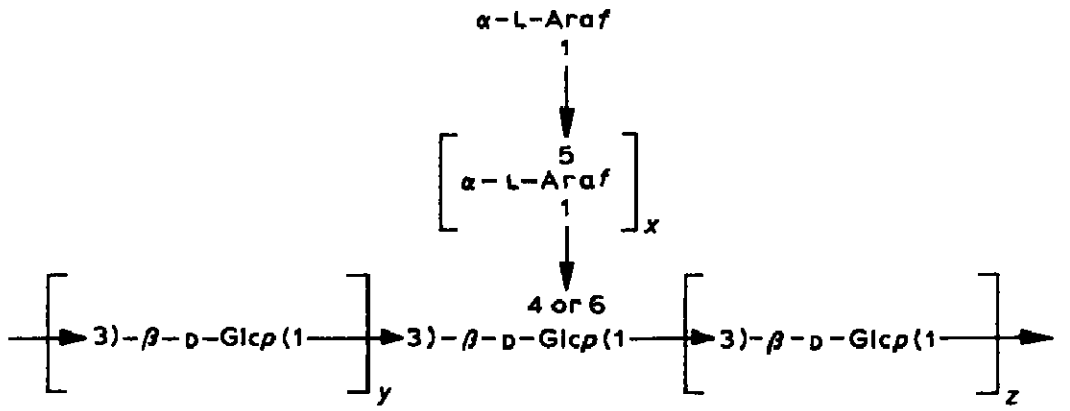

Fig. 4. Possible structures of synthetic L-arabino-D-glucans. [**1**; $x = 0$, $y + z = 13$; **2**: $x = 0$, $y + z = 7$, and **3**: $x = 1$, $y + z = 3$–4.]

backbone chain is attached with β-(1→6)-linked D-galactosyl side-chains having branches of mono- or α-(1→5)-linked di-L-arabinosyl groups[11]. Such spatial arrangements of the terminal α-L-Ara*f* residues in the arabino-3,6-galactan type of structure may cause steric hindrance for the specific antigen–antibody reaction.

The binding capability of the purified antibodies to the terminal α-L-Ara*f* residues of the polymers was further confirmed by the interaction with synthetic, branched polysaccharides having side chains of mono- or di-L-arabinofuranosyl groups, shown as **1, 2,** and **3** in Fig. 4. They were synthesized by the reaction of 3,5-di-*O*-benzoyl-(1,2-*O*-ethyl orthobenzoyl)-β-L-arabinofuranose or 3-*O*-benzoyl-(1,2,5-*O*-orthobenzoyl)-β-L-arabinofuranose with the (1→3)-linked β-D-glucan, followed by de-esterification, in the manner used for synthesis of the α-D-Ara*f*-branched polysaccharides[12]. The interactions of the antibodies (7.5 μg, as BSA) with increasing amounts (1×10^{-6} to 10 μg) of these synthetic arabinoglucans were compared. As shown in Fig. 5, all α-L-Ara*f*-branched polysaccharides interacted with anti-α-L-Ara*f* antibodies, but their reactivities differed, depending on the type of arabinofuranosyl side-chains. For instance, the antibodies reacted more strongly with the arabinoglucan **3**, having di-α-L-Ara*f* groups, than with either type (**1** or **2**)

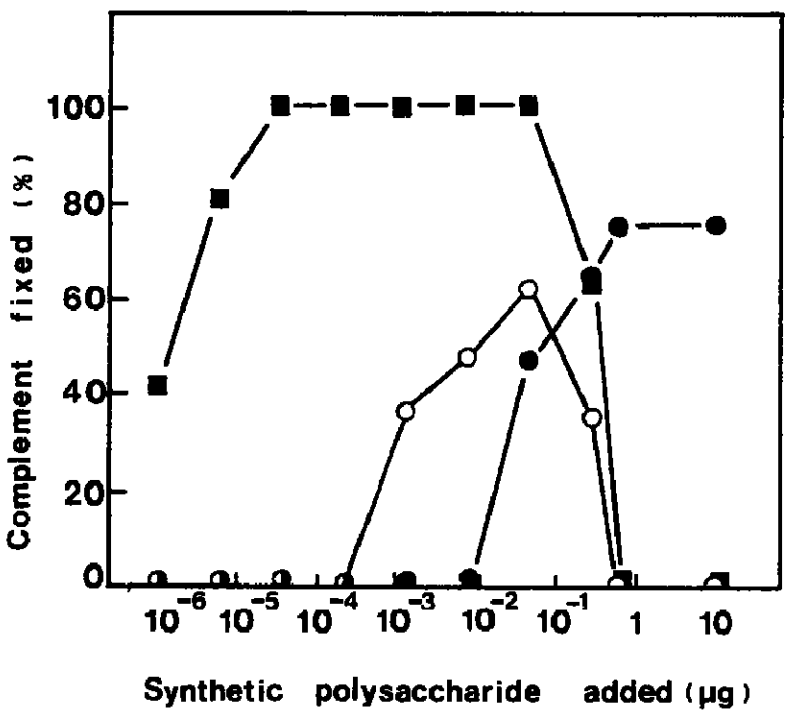

Fig. 5. Quantitative complement fixations of the purified anti-α-L-Ara*f* antibodies with synthetic L-arabino-D-glucans. [●, **1**; ○, **2**; and ■, **3**; (see Fig. 4).]

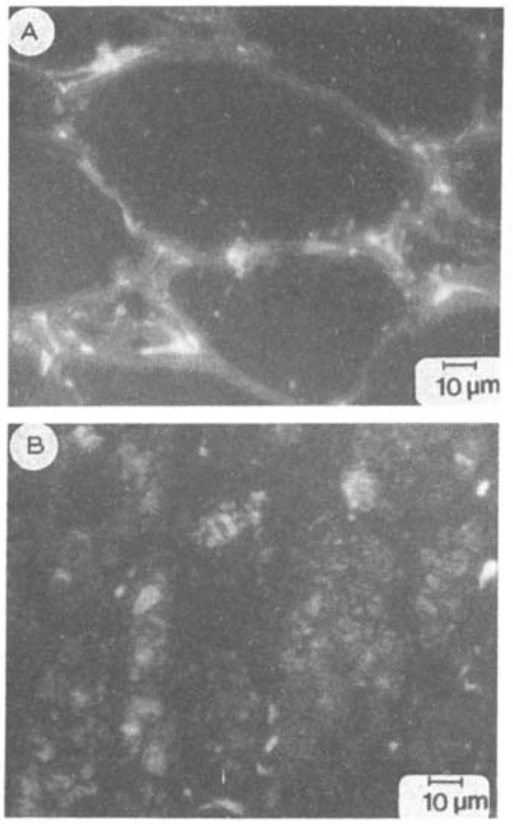

Fig. 6. Fluorescent microphotographs of the cotyledon tissues of soy bean. [(A) Treated with the purified anti-α-L-Ara*f* antibodies; (B) pre-immune serum.]

mostly containing single α-L-Ara*f* groups. These results, using the natural and synthetic polysaccharides, strongly suggested that the binding activity of the α-L-Ara*f*-specific antibodies to α-L-Ara*f*-containing polysaccharides would be affected by the mode of the α-L-arabinofuranosyl side-chains, such as distributions and lengths of branches, and also the spatial interrelations of the L-arabinose residues located at the terminal sites.

Histochemical applications. — In order to confirm our previous histochemical results[5], the purified antibodies were examined for the immunochemical detection of α-L-Ara*f*-containing polysaccharides or glycoproteins in the cell-wall tissues of soy-bean cotyledon. The latter was embedded in a hydrophilic resin, cut into thin sections (1 μm thick), and α-L-Ara*f*-containing polymers in the thin tissue were detected by the indirect immunostaining method, which involves incubation with the antibodies, followed by treatment with fluorescein isothiocyanate (fitc)-labeled goat IgG to rabbit IgG. The use of a hydrophilic resin for embedding of a plant tissue has an advantage, in that it retains any water-soluble polymer in the washing process, and also shows sharp, and more distinct, fluorescent layers, compared with the paraffin-embedding procedure used previously[5].

Fig. 6A shows a fluorescent microphotograph of the soy-bean cotyledon section. In comparison with the microphotograph of the same tissue treated with the pre-immune rabbit serum (Fig. 6B), it is clear that the distinct fluorescent layers are located along the primary cell-walls. It should be noted that the antibody-reactive layers also appear in the region of the middle lamella. These microscope observations were supported by the results of the enzyme-labeled, immunostaining method, which involved treatment of the tissue section with the antibodies, and, after thorough washing, treatment with the horseradish peroxidase-conjugated goat

IgG to rabbit IgG. As shown in Fig. 7A, the peroxidase-bound layers, detected as a brown color with the 3,3′-diaminobenzidine reagent, are distinct in the region of the middle lamella. The antibody-reactive polysaccharides, present in the middle lamella, should be the water-extractable arabinogalactogalacturonan, or the pectic polysaccharides bearing the arabinosyl or arabinogalactosyl side-chains. As regards the antibody-reactive polysaccharides in the primary walls, it should be emphasized that the 24% KOH-extraction residue of the soy-bean cotyledon still contained cellulose-associated heteropolysaccharides which comprise L-arabinose, D-xylose, D-galactose, and D-galacturonic acid[10].

Other plant tissues, *e.g.*, rice endosperm and Tora bean *(Phaseolus vulgaris)* cotyledon, also gave the anti-α-L-Ara*f* antibody-reactive, distinct layers along their primary cell-wall, as shown by the peroxidase-labeled technique (see Fig. 7B and 7C). These peroxidase-labeled layers would correspond to the arabinoxylan and pectic substances in rice endosperm[13], and to the arabinoxyloglucan in Tora-bean cotyledon[14].

Consequently, these observations indicate that the purified anti-α-L-Ara*f* antibodies, which recognize specifically the nonreducing terminal L-arabino-

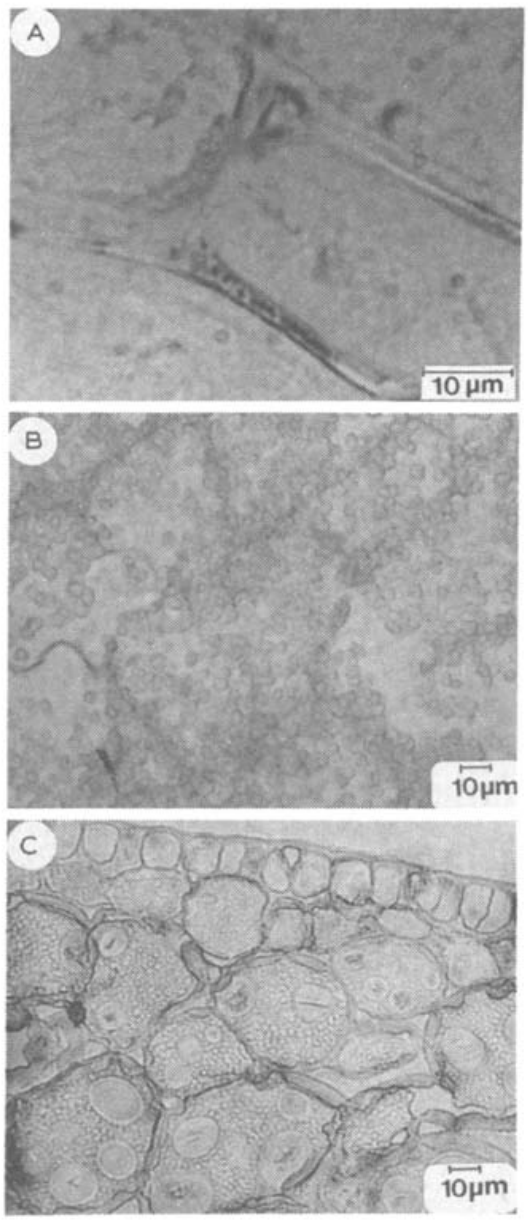

Fig. 7. Microphotographs of some plant tissues, treated with the purified anti-α-L-Ara*f* antibodies followed by HRP-labeled anti-rabbit IgG [A, Soy-bean cotyledon; B, rice endosperm; C, Tora bean cotyledon.]

furanosyl groups, can be utilized as a specific, histochemical reagent. Thus, useful information on the distribution and localization of the α-L-Ara*f*-containing polysaccharides, and their role in the architecture of plant cell-walls, can be obtained.

As indicated in the present study, it may be possible to obtain any desirable carbohydrate-specific antibody, useful in structural and histochemical investigations of plant cell-wall polysaccharides. For instance, we have recently obtained purified antibodies that specifically recognize xyloglucan. This was successfully utilized for histochemical detection of xyloglucan, an important structural component of plant cell-walls[15].

The biological roles of α-L-Ara*f*-containing polymers have attracted much attention. For instance, the arabinogalactan–protein may be related to the male–female recognition in flowering plants[16]. It was also suggested that some L-arabinose-containing polysaccharides may act as key substances for the auxin-induced elongation of cell walls of dicote plants[17].

EXPERIMENTAL

Materials. — Lyophilized whole complement (guinea pig) was purchased from Cordis Laboratory, Inc., Miami, FL. Sensitized sheep red-blood cells, gelatin–veronal buffer (GVB), and other reagents for the complement fixation tests were obtained from Ishizu Pharmaceutical Co., Ltd., Osaka. Goat antisera against rabbit serum and IgG were obtained from Cappel Laboratory, Inc., Cochranville, PA.

L-Arabinose and *p*-nitrophenyl α-L-arabinofuranoside were obtained from Sigma Chemical Co., St. Louis., MO. Methyl α- and β-L-arabinoside were prepared in our laboratory, and their purity was confirmed by ^{13}C-n.m.r. spectroscopy. Cyanogen bromide-activated Sepharose 4B (CNBr-activated Sepharose 4B) was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden.

p-Nitrophenyl α-D-arabinofuranoside[18] was synthesized in our laboratory, and some was also provided by Dr. H. Kobatake, Kyoto Medico-chemical Laboratory Inc., Kyoto. Soy-bean arabinogalactan (arabinogalactogalacturonan) was isolated from the hot-water extract of the homogenized cotyledon. The arabinogalactan from ginseng *(Panax ginseng)* was isolated from the hot-water extract of its root. Radish arabinogalactan was a gift from Dr. Y. Tsumuraya, Dept. of Biochemistry, Saitama University.

The synthetic branched β-(1→3)-D-glucans having mono- or di-L-arabinofuranosyl groups (**1**, **2**, and **3**; see Fig. 4) were provided by Prof. K. Matsuzaki, Shinsyuu University. These three types of polysaccharide were synthesized by condensation of 3,5-di-*O*-benzoyl-(1,2-*O*-ethyl orthobenzoyl)-β-L-arabinofuranose or 3-*O*-benzoyl-(1,2,5-*O*-orthobenzoyl)-β-L-arabinofuranose with acetylated curdlan [a linear (1→3)-β-D-glucan] under different conditions (reaction time, and ratio of the reactants), in the manner used for synthesis of the α-D-arabinofuranose-branched polysaccharides[12]. De-esterification of the reaction products yielded water-soluble, branched (1→3)-β-D-glucans. Their structures were mainly

examined by methylation analysis. The hydrolyzates of methylated, branched glucans yielded 2,3,5-tri- and 2,3-di-*O*-methyl-L-arabinose, and 2,4,6-tri-, 2,6-di-, and 2,4-di-*O*-methyl-D-glucose in the molar ratios of 1.00:0:12.80:0.83:0.33 (**1**), 1.00:0:6.95:0.51:0.39 (**2**), and 1.00:0.93:3.40:0.54:0.33 (**3**), respectively.

Other chemicals used in this study were of reagent grade.

Preparation of the antiserum against α-L-Araf–BSA. — α-L-Arabinofuranoside–BSA conjugate (α-L-Araf–BSA) was reported previously[5]. α-D-Araf-ova and α-L-Araf-ova were prepared similarly, using ovalbumin; they were purified by gel filtration on a column of Sephadex G-25. The details of the immunization procedures were described previously[5].

α-L-Araf–Sepharose 4B column. — *p*-Aminophenyl α-L-arabinofuranoside (207.1 μmol) was added to an aqueous suspension (30 mL) of CNBr-activated Sepharose 4B (5 g). The reaction was quenched by addition of M glycine solution to block the residual active groups on the Sepharose 4B. The immunoadsorbent so prepared was collected by filtration on a glass filter. Quantitative determination of the uncoupled *p*-aminophenyl arabinoside in the filtrate indicated that the Sepharose 4B–immunoadsorbent contained 9.1 μmol of L-arabinoside/mL.

Purification of anti-α-L-Araf antibodies. — Rabbit whole antiserum (14 mL, containing 1.2 g of protein) was applied to the affinity column (2 × 11.7 cm), which had been equilibrated with 50mM borate buffer in 0.15M NaCl, pH 7.9. The column was washed with the same buffer until no appreciable absorbance at 280 nm in the eluate was observed (~160 mL). Retained antibodies were eluted with 0.17M glycine·HCl buffer, pH 2.3. To avoid denaturation of the antibodies under the acidic conditions, each fraction (2 mL) was immediately made neutral with 0.2 mL of M Tris solution. The fractions containing anti-α-L-Araf antibodies were combined, dialyzed against 10mM phosphate-buffered saline (PBS), pH 7.2, and concentrated by use of a Sartorius collodion bag. Thus, 2.8 mg of the antibodies, purified 111-fold, was obtained from 14 mL of the antiserum. The purified antibodies were stored at −70°.

Complement fixation assay. — Reactivities of the purified antibodies with the antigen (α-L-Araf–BSA), and other α-L-Araf-containing glycoconjugates or polysaccharides were assayed by the quantitative, complement-fixation method[8].

The semiquantitative assay (box titration assay) was conducted in a microtiter plate equipped with V-bottom wells. A series of double-diluted antigen solutions (each, 25 μL) were mixed with the double-diluted antibodies solutions or antiserum solutions (each, 25 μL). To each well was added 25 μL of the complement solution (1.25 CH_{50} units/mL), and the mixture was incubated overnight at 4°. The plate was warmed for 30 min at 37°, and 250 μL of the sensitized sheep red-blood cells (2.5 × 10^8 cells/mL) was added. After standing for 1 h at 37°, the degree of cell lysis in the well was estimated by visual inspection.

The quantitative complement-fixation test was carried out by the method of Stein and Ngu[8], with some modifications. the complement-fixation system was set up by mixing, in a Wasserman tube, the same volumes (250 μL, each) of the

purified antibodies solution, the antigen solution, and the complement solution (1.25 CH_{50} units/mL). As the standard solutions, tubes of GVB (750 μL, each) containing various units of the complement were prepared. The reaction mixture in each tube was incubated overnight at 4°, and the sensitized sheep red-blood cells (2.5×10^8 cells/mL), suspended in GVB (250 μL), was added, the contents being well mixed, and warmed for 1 h at 37°. The suspension was centrifuged (1,500 r.p.m. for 5 min), and the optical absorbance of the supernatant liquor was measured at 540 nm. The residual complement unit was calculated according to the equation of von Krogh[19], in which parameters were determined from the simultaneous, standard complement fixation data. The efficiency of complement fixation (%) $= [(A - B)/A] \times 100$, where A is the added complement in CH_{50} units, and B is the residual complement in CH_{50} units, respectively.

Hapten inhibition test. — The hapten inhibition tests were also carried out by the quantitative complement fixation assay, using various carbohydrate inhibitors. A series of mixtures containing the antibodies (200 μL, 6.28 μg) and the inhibitor solution at a different concentration (each, 100 μL) were incubated for 1.5 h at 37°. To the mixture were added the α-L-Ara*f*–BSA solution (200 μL, 0.24 μg) and the complement solution (250 μL, 1.25 units of CH_{50}/mL). After standing overnight, the residual complement unit was measured, as already described. The inhibition ratio (%) was calculated from the difference in the complement fixation values with and without the inhibitor.

Immunoelectrophoresis. — Electrophoresis was performed at 100 V for 2.5 h on an agar plate, pH 8.5. The precipitin lines were developed by goat antisera against whole rabbit serum and IgG.

Histochemical procedure. — Distributions of the α-L-Ara*f*-containing polysaccharides in thin sections of cotyledon of soy bean *(Glycine max)*, and some other plant tissues, were examined by the indirect immunostaining methods. Each plant-tissue section was dehydrated, and embedded in a hydrophilic resin (Lowicryl K4M, Chemische Werke Lowi GmbH, Waldkraiburg, West Germany). The resin was photo-polymerized at room temperature under an ultraviolet light (360 nm). The embedded section was cut with a microtome (1 μm), and mounted on a glass slide coated with egg albumin. After drying overnight at 40°, the thin section was treated with the purified anti-α-L-Ara*f* antibodies for 40 min at room temperature. The excess of the antibodies was washed out 10 times with PBS, and the slide was incubated with FITC-labeled goat IgG against rabbit IgG for 30 min at room temperature. The FITC-labeled section was again washed 10 times with PBS. It was placed in 1:1 glycerol (nonfluorescent)–PBS, and examined on a Nikon Fluophoto VFD-R microscope equipped with a IF 420–490-nm excitation filter and a 515W barrier filter.

The antibody-treated section was also incubated with the horseradish peroxidase (HRP)-labeled goat IgG against rabbit IgG, in a procedure similar to that already described. The HRP-labeled tissue section was washed out thoroughly with PBS to remove the excess of the labeled goat IgG, and treated with a 1%

solution of 3,3′-diaminobenzidine in 0.05M Tris buffer, pH 7.2, for 1 h at 4° in the dark, and then in the same buffer containing H_2O_2 for 4 min, and finally washed 10 times with PBS. The specimen so obtained was examined by microscopy.

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