

N-Linked Oligosaccharides of Glycoproteins from *Ginkgo biloba* Pollen, An Allergenic Pollen[†]

Yoshinobu KIMURA, Masashi SUZUKI, and Mariko KIMURA

Department of Bioresources Chemistry, Faculty of Agriculture, Okayama University,
Okayama 700-8530, Japan

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The pollen of *Ginkgo biloba* is one of the allergens that cause pollen allergy symptoms. The plant complex type *N*-glycans bearing β 1–2 xylose and/or α 1–3 fucose residue(s) linked to glycoallergens have been considered to be critical epitopes in various immune reactions. In this report, the structures of *N*-glycans of total glycoproteins prepared from *Ginkgo biloba* pollens were analyzed to confirm whether such plant complex type *N*-glycans occur in the pollen glycoproteins. The glycoproteins were extracted by SDS-Tris buffer. *N*-Glycans liberated from the pollen glycoprotein mixture by hydrazinolysis were labeled with 2-aminopyridine and the resulting pyridylaminated (PA-) *N*-glycans were purified by a combination of size-fractionation HPLC and reversed-phase HPLC. The structures of the PA-sugar chains were analyzed by a combination of two-dimensional sugar chain mapping, IS-MS, and MS/MS. The plant complex type structures (GlcNAc₂Man₃Xyl₁Fuc₁GlcNAc₂ (31%), GlcNAc₂Man₃Xyl₁GlcNAc₂ (5%), Man₃Xyl₁Fuc₁GlcNAc₂ (13%), GlcNAc₁Man₃Xyl₁Fuc₁GlcNAc₂ (8%), and GlcNAc₁Man₃Xyl₁GlcNAc₂ (17%)) have been found among the *N*-glycans of the glycoproteins of *Ginkgo biloba* pollen, which might be candidates for the epitopes involved in *Ginkgo* pollen allergy. The remaining 26% of the total pollen *N*-glycans have the typical high-mannose type structures: Man₈GlcNAc₂ (11%) and Man₆GlcNAc₂ (15%).

Key words: plant glycoprotein; antigenic *N*-glycan; sugar chains; pollen allergy; *Ginkgo biloba*

Many allergenic plant proteins purified and characterized from pollens or seeds are *N*-glycosylated pro-

teins.^{1–5)} Furthermore, it has been reported that plant complex type *N*-glycans linked to such allergenic-glycoproteins could be recognized by IgE antibodies in sera of pollen allergic patients,^{6–8)} suggesting that the carbohydrate moieties may be epitopes for such pollen allergy. Actually the plant complex type *N*-glycan are highly active towards mammal to produce an antiserum specific for the glycan moiety of plant glycoproteins. However, there are some reports^{9,10)} that the *N*-glycans themselves would not be epitopes, showing IgE in sera from Japanese cedar pollen-allergic patients could not recognize other plant glycoproteins bearing the highly antigenic *N*-glycans. Ogawa *et al.* concluded that the xylose/fucose containing *N*-glycans of *Cry j1*, a major allergenic glycoprotein of Japanese cedar pollen, might contribute to the formation of active conformational epitopes of the peptide portion of *Cry j1* when IgE of the pollen allergic patients recognize the pollen allergen.¹⁰⁾ These results suggest that it is necessary to carry out further correlation analysis of between the *N*-glycan structure and allergenicity using various antigenic glycoproteins or antigenic *N*-glycans to clarify the involvement of the *N*-glycans in allergenic or immune responses.

In our previous papers,^{11–14)} we reported such antigenic *N*-glycans occur in conjugated form (as glycoproteins) and in the free form in developing *Ginkgo biloba* seeds. However, it is still obscure whether the plant complex-type *N*-glycans also occur in glycoproteins of *Ginkgo biloba* pollen, which is one of the allergenic pollens. It is worth while showing the ubiquitous occurrence of the plant complex type *N*-glycans in the allergenic pollens. Wilson and

Corresponding Author: Yoshinobu KIMURA, Tel: 81-86-251-8296; Fax: 81-86-251-8388; E-mail: yosh8mar@cc.okayama-u.ac.jp

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Abbreviations: PA-, pyridylamino; RP-HPLC, reverse-phase HPLC; SF-HPLC, size-fractionation HPLC; IS-MS, ion-spray mass spectrometry; MS/MS, tandem mass; M3X, Man α 1–6(Man α 1–3)(Xyl β 1–2)Man β 1–4GlcNAc β 1–4GlcNAc-PA; M3FX, Man α 1–6(Man α 1–3)(Xyl β 1–2)Man β 1–4GlcNAc β 1–4(Fuc α 1–3)GlcNAc-PA; ^{GN}M3FX, GlcNAc β 1–2Man α 1–6(Man α 1–3)(Xyl β 1–2)Man β 1–4GlcNAc β 1–4(Fuc α 1–3)GlcNAc-PA; GN2M3FX, GlcNAc β 1–2Man α 1–6(GlcNAc β 1–2Man α 1–3)(Xyl β 1–2)Man β 1–4GlcNAc β 1–4(Fuc α 1–3)GlcNAc-PA; M5A, Man α 1–6(Man α 1–3)Man α 1–6(Man α 1–3)Man β 1–4GlcNAc β 1–4GlcNAc-PA; M6B, Man α 1–6(Man α 1–3)Man α 1–6(Man α 1–2Man α 1–3)Man β 1–4GlcNAc β 1–4GlcNAc-PA; M8C, Man α 1–6(Man α 1–2Man α 1–3)Man α 1–6(Man α 1–2Man α 1–3)Man β 1–4GlcNAc β 1–4GlcNAc-PA

Altman¹⁵⁾ have already showed that glycoproteins of several kinds of grass pollens always carry the antigenic plant complex type *N*-glycans. In this report, we describe the structural analysis of *N*-glycans linked to total glycoproteins in the *Ginkgo* pollen, showing the antigenic structures are dominant species among the pollen *N*-glycans.

Materials and Methods

Materials. Pollens of *Ginkgo biloba* were collected on the campus of Okayama University in April, 2000. A Cosmosil 5C18-AR column (0.60 × 25 cm) was purchased from Nacalai Tesque, Inc., and an Asahipak NH2P-50 column (0.46 × 25 cm) from Showa Denko Co. Authentic PA-sugar chains were prepared as described in our previous papers.^{13,16)} α -1,2-Mannosidase (*Aspergillus oryzae*) was a generous gift from Dr. T. Yoshida (Hirosaki University, Japan).¹⁷⁾

Preparation of glycoproteins from *Ginkgo* pollens. Pollens of *Ginkgo biloba* (226 g) were homogenized in 500 ml of acetone. Pollen glycoproteins were extracted from the resulting defatted pollen powder in 25 mM Tris-HCl buffer (pH 8.5) containing 0.2% SDS. After centrifugation, the supernatant was exhaustively dialyzed against deionized water. The dialysate was lyophilized.

Preparation of pyridylaminated *N*-glycans from pollen glycoproteins. *N*-Glycans were released by hydrazinolysis (100°C, 12 h, in 20 ml of anhydrous hydrazine) from the pollen total glycoproteins (about 310 mg). After *N*-acetylation of the hydrazinolysate with saturated ammonium bicarbonate (20 ml) and acetic anhydride (0.8 ml), the acetylated hydrazinolysate was desalted by Dowex 50 × 2 resin. Pyridylation of the sugar chains was done by the method of Kondo *et al.*¹⁸⁾ Separation of PA-sugar chains was done by HPLC on a Jasco 880-PU HPLC apparatus with a Jasco 821-FP Intelligent Spectrofluorometer, using the Cosmosil 5C18-AR column (0.6 × 25-cm) or the Asahipak NH2P-50 column (0.46 × 25 cm). On the Cosmosil 5C18-AR column, the PA-sugar chains were eluted by increasing the acetonitrile concentration in 0.02% TFA linearly from 0 to 15% for 40 min at a flow rate 1.2 ml/min. For the mapping of the elution position of each PA-sugar chain (Fig. 3), the PA-sugar chains were eluted by increasing the acetonitrile concentration in 0.02% TFA linearly from 2 to 10% in 30 min at a flow rate 1.2 ml/min. In the case of size-fractionation HPLC using the Asahipak NH2P-50 column, the PA-oligosaccharide was eluted by increasing the water content in the water-acetonitrile mixture from 30% to 50% linearly in 25 min at a flow rate of 0.7 ml/min.

Ion-spray mass spectrometry. IS-MS and MS/MS analyses of PA-oligosaccharides were done as described in our previous reports,¹⁰⁻¹⁴⁾ using a Perkin Elmer Sciex API-III, triple-quadrupole mass spectrometer with an atmospheric-pressure ionization ion source.

Results

Purification of PA-sugar chains from Ginkgo pollen glycoprotein

As shown in Fig. 1-[A], seven PA-derivatives (Peaks-A, -B, -C, -D, -E, -F, and -G) were obtained by SF-HPLC from *Ginkgo* pollen glycoproteins. MS/MS analysis of each peak showed that Peaks-A, -D, -F, and -G contained no *N*-glycans, since any PA-derivatives observed on IS-MS spectra in these peaks did not give a fragment ion at *m/z* 300.0 (PA-GlcNAc). The pyridylaminated *N*-glycans were further purified by RP-HPLC from Peaks-B, -C, and -E as shown in Fig. 1-[B]. From Peak-B, four pyridylaminated *N*-glycans were purified; B-1 (*m/z* 1267.5) corresponding to Man₃Xyl₁Fuc₁GlcNAc₂-PA, B-2 (*m/z* 1470.5) corresponding to GlcNAc₁-Man₃Xyl₁Fuc₁GlcNAc₂-PA, B-3 (*m/z* 1324.0) corresponding to GlcNAc₁Man₃Xyl₁GlcNAc₂-PA), B-4 (*m/z* 1528.0) corresponding to GlcNAc₂Man₃Xyl₁GlcNAc₂-PA. From Peak-C, two pyridylaminated *N*-glycans were purified; C-1 (*m/z* 1673.5) corresponding to GlcNAc₂Man₃Xyl₁Fuc₁GlcNAc₂-PA and C-2 (*m/z* 1475.5) corresponding to Man₆GlcNAc₂-PA. From Peak-E, one pyridylaminated *N*-glycan was purified; E-1 (*m/z* 1799.5) corresponding to Man₈GlcNAc₂-PA.

Since several other signals observed on three chromatograms did not give the specific fragment ion at *m/z* 300.0 for GlcNAc-PA by MS/MS analysis, these PA-derivatives was confirmed not to be the pyridylaminated *N*-glycans. We, therefore, proceeded with the structural analysis on the seven PA-sugar chains; B-1, B-2, B-3, B-4, C-1, C-2, and E-1 as described below.

Structures of B-1, B-2, B-3, B-4, and C-1

MS/MS analyses and two-dimensional sugar chain mapping of these PA-sugar chains showed that these five *N*-glycans had the plant complex type structures bearing β 1-2 xylose and/or α 1-3 fucose residue(s).

The elution positions of B-1 (*m/z* 1267.5), on the two-dimensional sugar chain map,^{19,20)} corresponded to that of authentic M3FX. Furthermore, the fragment ions derived from B-1 by MS/MS could be assigned as the fragments from Man₃Xyl₁Fuc₁GlcNAc₂-PA; *m/z* 1122.0 ([Man₃Xyl₁GlcNAc₂-PA + H]⁺), *m/z* 827.0 ([Man₂GlcNAc₂-PA + H]⁺), *m/z* 827.5 ([Man₂GlcNAc₂-PA + H]⁺), *m/z* 664.5 ([Man₁GlcNAc₂-PA + H]⁺), *m/z* 502.5 ([GlcNAc₂-PA + H]⁺), *m/z* 299.5 ([GlcNAc-PA + H]⁺). Con-

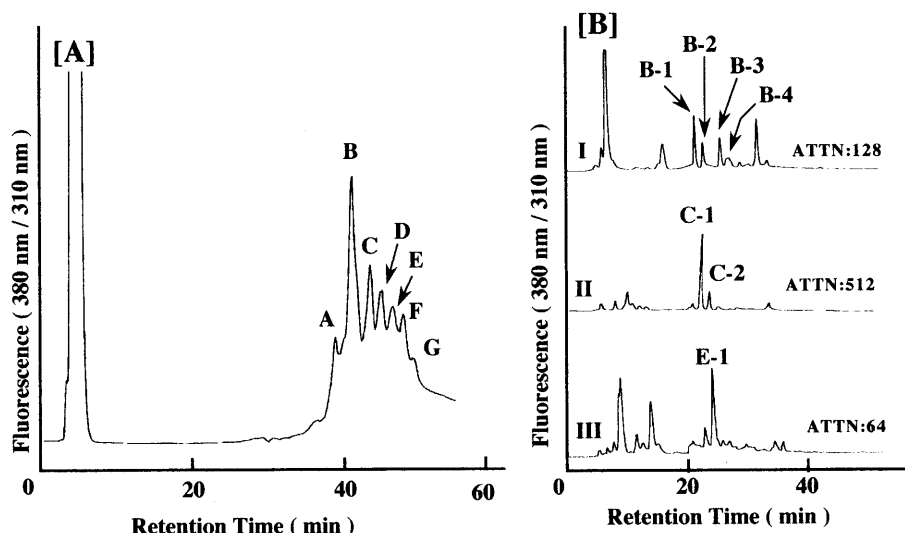


Fig. 1. RP-HPLC of PA-Sugar Chains of Glycoproteins from *Ginkgo biloba* Pollen.

[A], Size-fractionation HPLC of PA-derivatives from *Ginkgo* pollen glycoproteins.

[B], RP-HPLC of the partially purified PA-sugar chains. I, Fraction-B; II, Fraction-C; III, Fraction-E in [A].

The detail chromatographic conditions are described in Materials and Methods. B-1, m/z 1267.5; B-2, m/z 1470.5; B-3, m/z 1324.0; B-4, m/z 1528.0; C-1, m/z 1673.5; C-2, m/z 1475.5; E-1, m/z 1799.5. ATTN means integrator attenuation.

sidering the data of 2-D sugar chain mapping and MS/MS analyses suggested that the structure of B-1 could be M3FX.

The elution position of B-2 (m/z 1470.5), on the two-dimensional sugar chain map, corresponded to that of $^{GN}M3FX$. The fragment ions derived from B-2 by MS/MS could be assigned as the fragments from $GlcNAc_1Man_3Xyl_1Fuc_1GlcNAc_2-PA$; m/z 1267.5 ($[Man_3Fuc_1Xyl_1GlcNAc_2-PA + H]^+$), m/z 1192.5 ($[GlcNAc_1Man_3GlcNAc_2-PA + H]^+$), m/z 1121.5 ($[Man_3Xyl_1GlcNAc_2-PA + H]^+$), m/z 989.5 ($[Man_3GlcNAc_2-PA + H]^+$), m/z 664.5 ($[Man_1GlcNAc_2-PA + H]^+$), m/z 503.0 ($[GlcNAc_2-PA + H]^+$), m/z 300.0 ($[GlcNAc-PA + H]^+$). Concerning the structure of B-2, $GlcNAc_1Man_3Xyl_1Fuc_1GlcNAc_2-PA$ structure occurs in two isomers; one is $Man\alpha 1-6(GlcNAc\beta 1-2Man\alpha 1-3)(Xyl\beta 1-2)Man\beta 1-4GlcNAc\beta 1-4(Fuc\alpha 1-3)GlcNAc-PA(^{GN}M3FX)$, the other one is $GlcNAc\beta 1-2Man\alpha 1-6(Man\alpha 1-3)(Xyl\beta 1-2)Man\beta 1-4GlcNAc\beta 1-4(Fuc\alpha 1-3)GlcNAc-PA(^{GN}M3FX)$. It has already been found that on the ODS column the former structure is eluted before $^{GN}M3FX$ and the latter structure is eluted after $^{GN}M3FX$.¹³⁾ Considering the elution position of B-2 which was eluted slightly after $^{GN}M3FX$ (C-1) on the ODS column, the structure of B-2 should be $^{GN}M3FX$.

Since the authentic PA-sugar chains corresponding to B-3 (m/z 1324.0, $GlcNAc_1Man_3Xyl_1GlcNAc_2-PA$) and B-4 (m/z 1528.0, $GlcNAc_2Man_3Xyl_1GlcNAc_2-PA$) are not available, the detailed structures of these two PA-sugar chains could not be identified. However, considering the general structural rule for the plant complex type N-glycan and MS/MS data for these PA-sugar chains, the structure of B-3 may be $GlcNAc\beta 1-2Man\alpha 1-6(Man\alpha 1-3)(Xyl\beta 1-2)$

$Man\alpha 1-4GlcNAc\beta 1-4GlcNAc-PA$ or $Man\alpha 1-6(GlcNAc\beta 1-2Man\alpha 1-3)(Xyl\beta 1-2)Man\alpha 1-4GlcNAc\beta 1-4GlcNAc-PA$ and the structure of B-4 may be $GlcNAc\beta 1-2Man\alpha 1-6(GlcNAc\beta 1-2Man\alpha 1-3)(Xyl\beta 1-2)Man\alpha 1-4GlcNAc\beta 1-4GlcNAc-PA$. Actually the fragment ions derived from B-3 (m/z 1324.0) by MS/MS could be assigned as the fragments from $GlcNAc_1Man_3Xyl_1GlcNAc_2-PA$; (m/z 1192.5 ($[GlcNAc_1Man_3GlcNAc_2-PA + H]^+$), m/z 1161.5 ($[GlcNAc_1Man_2Xyl_1GlcNAc_2-PA + H]^+$), m/z 1121.5 ($[Man_3Xyl_1GlcNAc_2-PA + H]^+$), m/z 958.5 ($[Man_2Xyl_1GlcNAc_2-PA + H]^+$), m/z 796.5 ($[Man_1Xyl_1GlcNAc_2-PA + H]^+$), m/z 503.0 ($[GlcNAc_2-PA + H]^+$), m/z 299.5 ($[GlcNAc-PA + H]^+$)). The fragment ions derived from B-4 (m/z 1528.0) by MS/MS could be assigned as the fragments from $GlcNAc_2Man_3Xyl_1GlcNAc_2-PA$; m/z 1324.5 ($[GlcNAc_1Man_3Xyl_1GlcNAc_2-PA + H]^+$), m/z 1192.5 ($[GlcNAc_1Man_3GlcNAc_2-PA + H]^+$), m/z 1161.5 ($[GlcNAc_1Man_2Xyl_1GlcNAc_2-PA + H]^+$), m/z 1121.5 ($[Man_3Xyl_1GlcNAc_2-PA + H]^+$), m/z 958.5 ($[Man_2Xyl_1GlcNAc_2-PA + H]^+$), m/z 796.5 ($[Man_1Xyl_1GlcNAc_2-PA + H]^+$), m/z 664.5 ($[Man_1GlcNAc_2-PA + H]^+$), m/z 502.5 ($[GlcNAc_2-PA + H]^+$), m/z 300.0 ($[GlcNAc-PA + H]^+$).

The elution position of C-1 (m/z 1673.5) on the two-dimensional sugar chain map corresponded to that of $^{GN}M3FX$. The fragment ions derived from C-1 by MS/MS could be assigned as followings; m/z 1309.5 ($[GlcNAc_1Man_2Xyl_1Fuc_1GlcNAc_2-PA + H]^+$), m/z 1030.5 ($[GlcNAc_1Man_2GlcNAc_2-PA + H]^+$), m/z 959.0 ($[Man_2Xyl_1GlcNAc_2-PA + H]^+$), m/z 894.0 ($[GlcNAc_1Man_3GlcNAc_1 + H]^+$), m/z 664.5 ($[Man_1GlcNAc_2-PA + H]^+$), m/z 503.0 ($[GlcNAc_2-PA + H]^+$), m/z 446.0 ($[Fuc_1GlcNAc_1-$

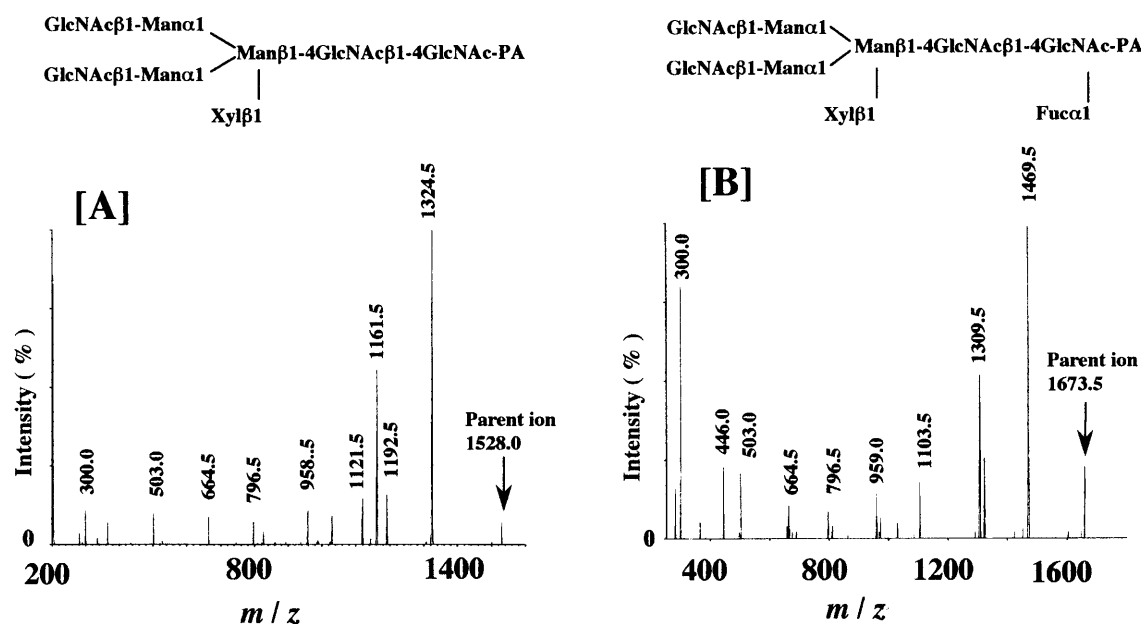


Fig. 2. MS/MS spectra of C-1 and E-1 in Fig. 1-[B].

The samples were dissolved in 50% acetonitrile/water (containing 0.05% formic acid) at a concentration of approximately 10 pmol/ μ l and introduced into the electrospray needle at a flow rate of 5 μ l/min. The assignments of the relevant fragment ions are described in the text. [A]; Fragment ions derived from Peak B-4 (m/z 1528.0; (GlcNAc₂Man₃Xyl₁GlcNAc₂-PA + H)⁺). [B]; Fragment ions derived from Peak C-1 (m/z 1673.5; (GlcNAc₂Man₃Xyl₁Fuc₁GlcNAc₂-PA + H)⁺).

PA + H)⁺, m/z 300.0 [(GlcNAc-PA + H)⁺]. Considering the data of 2-D sugar chain mapping and MS/MS analyses suggested that the structure of C-1 could be GN2M3FX. The MS/MS data of B-4 and C-1 are shown in Fig. 2 as typical examples.

Structures of C-2 and E-1

Since these two PA-sugar chains were converted to Man₅GlcNAc₁-PA by the *Aspergillus* α -1,2-mannosidase digestions (Fig. 3), these PA-sugar chains must be typical high-mannose type structures with the core structure; Man α 1-6(Man α 1-3)Man α 1-6(Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc. The elution positions of C-2 and E-1 on the two-dimensional sugar chain map exactly corresponded to M6B and M8C, respectively. Furthermore, the fragment ions derived by MS/MS analyses from C-2 (m/z 1313.5 [(Man₅GlcNAc₂-PA) + H]⁺, m/z 1151.5 [(Man₄GlcNAc₂-PA) + H]⁺, m/z 988.5 [(Man₃GlcNAc₂-PA) + H]⁺, m/z 826.5 [(Man₂GlcNAc₂-PA) + H]⁺, m/z 664.5 [(Man₁GlcNAc₂-PA) + H]⁺, m/z 502.5 [(GlcNAc₂-PA) + H]⁺, and m/z 299.5 [(GlcNAc-PA) + H]⁺), and E-1 (m/z 819.0 [(Man₇GlcNAc₂-PA) + 2H]²⁺, m/z 1475.5 [(Man₆GlcNAc₂-PA) + H]⁺, m/z 1314.0 [(Man₅GlcNAc₂-PA) + H]⁺, m/z 1151.0 [(Man₄GlcNAc₂-PA) + H]⁺, m/z 988.0 [(Man₃GlcNAc₂-PA) + H]⁺, m/z 826.5 [(Man₂GlcNAc₂-PA) + H]⁺, m/z 664.5 [(Man₁GlcNAc₂-PA) + H]⁺, m/z 503.0 [(GlcNAc₂-PA) + H]⁺, and m/z 299.5 [(GlcNAc-PA) + H]⁺) could be assigned as fragments from Man₆GlcNAc₂-PA and Man₈GlcNAc₂-PA, respectively. These results suggested that

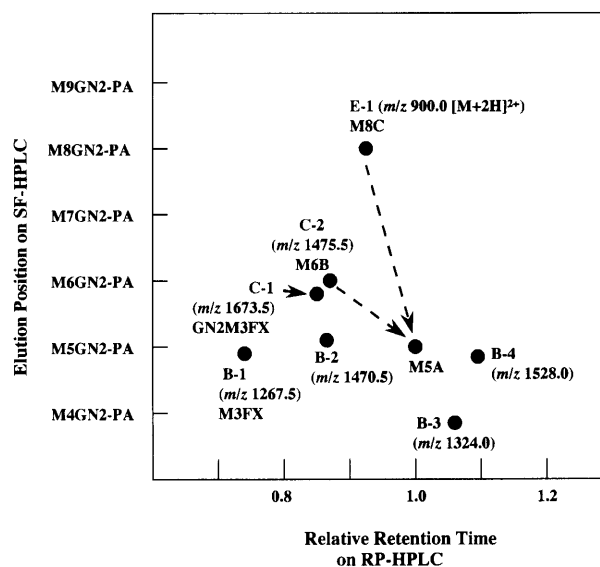


Fig. 3. Two-dimensional Sugar Chains Map of PA-Oligosaccharides Obtained from *Ginkgo* Pollen Glycoproteins.

The X-axis scale is expressed as relative retention times of PA-sugar chains to M5A on RP-HPLC. M4~9GN2-PA on the Y-axis mean the elution positions of authentic high-mannose type *N*-glycans (Man₄₋₉GlcNAc₂-PA) on SF-HPLC. The dotted arrows indicate the changes of elution positions of C-2 and E-1 after the *Aspergillus* α -1,2-mannosidase digestion.

the structure of C-2 could be Man α 1-6(Man α 1-3)Man α 1-6(Man α 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc-PA (M6B) and E-1 must be Man α 1-6(Man α 1-2Man α 1-3)Man α 1-6(Man α 1-2Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc-PA

(M8C).

As shown in Table, the structures of *N*-glycans of the *Ginkgo* pollen glycoproteins fell into three types: the first one is the xylose/fucose-containing structure (GlcNAc₂Man₃Xyl₁Fuc₁GlcNAc₂ (31%), GlcNAc₁Man₃Xyl₁Fuc₁GlcNAc₂ (8%), Man₃Xyl₁Fuc₁GlcNAc₂ (13%)), the second one is the xylose-containing structure (GlcNAc₂Man₃Xyl₁GlcNAc₂ (5%), GlcNAc₁Man₃Xyl₁GlcNAc₂ (17%)), the third one is the typical high-mannose type structure (Man₈GlcNAc₂ (11%) and Man₆GlcNAc₂ (15%)). The relative amount of each sugar chain is shown as a percentage of total amount of the *N*-glycans (as PA-derivatives) per gram of fresh pollens.

Discussion

In our previous reports,^{11–14)} we reported that only the plant complex type *N*-glycans, which bear β 1–2 xylose and/or α 1–3 fucose residue(s) and are highly antigenic *N*-glycan to mammals, are linked to storage glycoproteins of *Ginkgo* seeds. Recently, it has been found that the plant complex type *N*-glycans make a significant contribution in IgE binding to plant glycoallergens such as grass pollen, birch pollen, olive pollen, and mugwort pollen.^{6–8)} Wilson and Altman have already reported that glycoproteins from several pollens always carry the plant complex type *N*-glycans, showing the ubiquitous occurrence of such antigenic *N*-glycans in allergenic pollens.¹⁵⁾ Pollens of *Ginkgo biloba* have also been considered as an allergen causing pollen allergic symptoms, however, structural analysis of *N*-glycans of the *Ginkgo* pollen glycoproteins to find whether such antigenic *N*-glycans occur has not been done yet. In this report, therefore, we analyzed the structures of *N*-glycans of the *Ginkgo* pollen glycoproteins.

As shown in the Table, our structural analysis clearly showed that the antigenic *N*-glycans (plant complex type *N*-glycans) do occur in the *Ginkgo* pollen, which is an allergen that causes pollen allergy symptoms. Although the glycoallergen has not been identified from the *Ginkgo* pollen so far, it would be reasonable to assume that some glycoproteins bearing the antigenic *N*-glycan(s) may be candidate for the pollen allergen. Since these antigenic *N*-glycan structures described in this report have been only deduced by a combination of IS-MS analysis and two-dimensional sugar chain mapping, the detailed structures of the antigenic *N*-glycans possibly linked to the *Ginkgo* pollen allergen(s) must be confirmed by methylation analysis and/or ¹H-NMR spectroscopy.

van Ree *et al.*⁸⁾ has reported that recognition of a β 1–2 xylose residue by IgE of pollen allergic patients would be dependent on the degree of substitution of the core mannose influencing the conformation of the epitope. Xylose specific IgE antibodies

Table. Summary of *N*-Glycans of Pollen Glycoproteins of *Ginkgo biloba*

Structure	pmol/g fresh pollen	(%)
GlcNAc ₂ Man ₃ Xyl ₁ Fuc ₁ GlcNAc ₂ (C-1)	270.5	31
GlcNAc ₂ Man ₃ Xyl ₁ GlcNAc ₂ (B-4)	41.9	5
GlcNAc ₁ Man ₃ Xyl ₁ Fuc ₁ GlcNAc ₂ (B-2)	65.1	8
GlcNAc ₁ Man ₃ Xyl ₁ GlcNAc ₂ (B-3)	144.2	17
Man ₃ Xyl ₁ Fuc ₁ GlcNAc ₂ (B-1)	111.6	13
Man ₈ GlcNAc ₂ (E-1)	99.0	11
Man ₆ GlcNAc ₂ (C-2)	129.6	15

that bound to a grass pollen glycoallergen (Lol p 11) and pineapple stem bromelain could not recognize an olive pollen allergen (Ole e 1) or a peanut allergen (Ara h 1). The core mannose residue in *N*-glycans linked to Lol p 11 and bromelain is substituted with just an α 1–6 mannose residue, whereas the core mannose residue in the *N*-glycans linked to Ole e 1 is substituted with both two α 1–6 and α 1–3 mannose residues. This observation suggested that the α 1–3 mannose sterically hampered IgE recognition of the β 1–2 xylose residue. Furthermore, Tretter *et al.*²¹⁾ already reported that the α 1–3 fucose residue is mainly involved in IgE binding to the antigenic *N*-glycans.

In this report, the Lol p 11 type *N*-glycan structure (Man α 1–6(Xyl β 1–2)Man β 1–4GlcNAc β 1–4(Fuc α 1–3)GlcNAc) and laccase type (Lewis a antigen unit (Gal β 1–3(Fuc α 1–4)GlcNAc-) containing type) structures²²⁾ were not found among *N*-glycans of *Ginkgo* pollen glycoproteins. All xylose-containing *N*-glycans have the core mannose residue substituted with both α 1–6 and α 1–3 mannose residues, therefore, the allergenic proteins in *Ginkgo* pollen probably bear the GlcNAc₂Man₃Xyl₁Fuc₁GlcNAc₂, GlcNAc₁Man₃Xyl₁Fuc₁GlcNAc₂, or Man₃Xyl₁Fuc₁GlcNAc₂ structures as epitopes instead of the GlcNAc₂Man₃Xyl₁GlcNAc₂, GlcNAc₁Man₃Xyl₁GlcNAc₂ structures. Using antisera of *Ginkgo* pollen allergic patients, purification and identification of glycoallergen(s) involved in the pollen allergy are in progress.

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