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Structural Features of *N*-Glycans Linked to Glycoproteins from Oil Palm Pollen, an Allergenic Pollen*

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The pollen of oil palm (*Elaeis guineensis Jacq.*) is a strong allergen and causes severe pollinosis in Malaysia and Singapore. In the previous study (*Biosci. Biotechnol. Biochem.*, 64, 820–827 (2002)), from the oil palm pollens, we purified an antigenic glycoprotein (Ela g Bd 31 K), which is recognized by IgE from palm pollinosis patients. In this report, we describe the structural analysis of sugar chains linked to palm pollen glycoproteins to confirm the ubiquitous occurrence of antigenic *N*-glycans in the allergenic pollen. *N*-Glycans liberated from the pollen glycoprotein mixture by hydrazinolysis were labeled with 2-aminopyridine followed by purification with 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, electrospray ionization mass spectrometry (ESI-MS), and tandem MS analysis, as well as exoglycosidase digestions. The antigenic *N*-glycan bearing α1–3 fucose and/or β1–2 xylose residues accounts for 36.9% of total *N*-glycans: $GlcNAc_2Man_3Xyl_1Fuc_1GlcNAc_2$ (24.6%), $GlcNAc_2Man_3Xyl_1GlcNAc_2$ (4.4%), $Man_3Xyl_1Fuc_1$-GlcNAc2 (1.1%), $GlcNAc_1Man_3Xyl_1Fuc_1GlcNAc_2$ (5.6%), and $GlcNAc_1Man_3Xyl_1GlcNAc_2$ (1.2%). The remaining 63.1% of the total *N*-glycans belong to the high-mannose type structure: Man9GlcNAc2 (5.8%), Man8GlcNAc2 (32.1%), Man7GlcNAc2 (19.9%), Man6GlcNAc2 (5.3%).

Key words: plant glycoprotein; antigenic *N*-glycan; pollen allergy; oil palm pollen; *Elaeis guineensis Jacq*

In southeast Asia, especially in Malaysia and Singapore, oil palm pollen has been postulated to be one of the allergens causing inhalant allergy.[1,2] In our previous paper,[3] we succeeded in purifying and identifying a glycoprotein with a molecular mass of 31 kDa from oil palm pollens, which was recognized by antisera from palm pollinosis patients, suggesting that this glycoprotein (Ela g Bd 31 K) might be one of allergens in the oil palm pollen. Immunoblotting analysis with antiserum against the β1–2 xylose residue showed that the glycoprotein bears the plant complex type *N*-glycan, which has a strong antigenicity for mammals and therefore has been postulated as an epitope for IgE from pollen allergy or plant food allergy patients.[4–8] Our preliminary structural analysis of *N*-glycans of palm pollen glycoproteins with various exoglycosidases showed that both high-mannose type and plant complex type (antigenic) *N*-glycans occur in the palm pollens.[3] However, detailed

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Abbreviations: PA-, pyridylamino; RP-HPLC, reverse-phase HPLC; SF-HPLC, size-fractionation HPLC; ESI-MS, electrospray ionization mass spectrometry; MS/MS, tandem mass; M3FX, Manα1–6(Manα1–3)(Xylβ1–2)Manβ1–4GlcNAcβ1–4(Fucα1–3)GlcNAc-PA; GN2M3X, GlcNAcβ1–2Manα1–6(GlcNAcβ1–2Manα1–3)(Xylβ1–2)Manβ1–4GlcNAcβ1–4(Fucα1–3)GlcNAc-PA; ^{GN}M3FX, GlcNAcβ1–2Manα1–6(Manα1–3)(Xylβ1–2)Manβ1–4GlcNAcβ1–4(Fucα1–3)GlcNAc-PA; GN2M3FX, GlcNAcβ1–2Manα1–6(GlcNAcβ1–2Manα1–3)(Xylβ1–2)Manβ1–4GlcNAcβ1–4(Fucα1–3)GlcNAc-PA; M5A, Manα1–6(Manα1–3)Manα1–6(Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAc-PA; M6B, Manα1–6(Manα1–3)Manα1–6(Manα1–2Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAc-PA; M7A, Manα1–2Manα1–6(Manα1–3)Manα1–6(Manα1–2Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAc-PA; M7B, Manα1–6(Manα1–3)Manα1–6(Manα1–2Manα1–2Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAc-PA; M8A, Manα1–2Manα1–6(Manα1–3)Manα1–6(Manα1–2Manα1–2Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAc-PA; M9A, Manα1–2Manα1–6(Manα1–2Manα1–3)Manα1–6(Manα1–2Manα1–2Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAc-PA

structures of such antigenic *N*-glycans occurring in the oil palm pollen remain to be analyzed.

The direct involvement of the plant complex type *N*-glycans linked to several glycoallergens in pollinosis or plant food allergy is still obscure, although it has been already confirmed that the *N*-glycans are antigenic for mammals. Identification of a carbohydrate epitope occurring on glycoallergens, if such antigenic *N*-glycan can be a relevant epitope for IgE involved in the pollinosis, would open a way to develop a new glycodrug against pollinosis or plant food allergy.

In this report, therefore, as a part of studies to reveal whether the antigenic *N*-glycans ubiquitously occurring in glycoallergens are directly involved in the palm pollinosis, we analyzed the structures of *N*-glycans linked to glycoproteins in the allergenic palm pollens. The structural analysis showed that the plant complex type structure accounts for 37% of total *N*-glycans and the remaining 63% of total sugar chains belong to the high-mannose type structure ($Man_{9-6}GlcNAc_2$). One or some plant complex type *N*-glycans ($GlcNAc_{2-0}Man_3Xyl_1Fuc_{1-0}GlcNAc_2$) must be linked to the putative glycoallergen, Ela g Bd 31 K, in the allergenic palm pollens.

Materials and Methods

Materials. Oil palm pollens were collected in Selangor state of Malaysia in 2001. An Asahipak NH2P-50 column (0.46×25 cm) was purchased from Showa Denko Co. (Tokyo, Japan) and a Shiseido Capcell Pak C18 MG column (1.0×25 cm) from Shiseido Co. (Tokyo, Japan). Authentic PA-sugar chains were prepared as described in our previous papers.[9–13] α-Mannosidase (Jack bean) was purchased from Sigma (St. Louis, MO, USA). β-*N*-Acetylglucosaminidase (*Diplococcus pneumonia*) was from Boehringer Co. (Mannheim, Germany). α-1,2-Mannosidase (*Aspergillus saitoi*) was from Seikagaku Co. β-Xylosidase and α-fucosidase were purified from apple snail (*Pomacea canaliculata*).[14]

Preparation of glycoproteins from oil palm pollens. Oil palm pollens (16 g) were homogenized in 200 ml of acetone. Pollen glycoproteins were extracted from the resulting defatted pollen powder in 100 ml of 50 mM Tris-HCl buffer (pH 8.5) containing 0.5 M NaCl at 4°C for 2 h. After centrifugation, the supernatant (alkaline extract) was pooled. The precipitate was re-extracted with 50 mM Na-acetate buffer (pH 4.0) containing 0.5 M NaCl at 4°C for 2 h. After centrifugation, the resulting supernatant (acid extract) was mixed together with the above supernatant (alkaline extract) and the mixture was saturated to 100% with ammonium sulfate. The resulting precipitate was exhaustively dialyzed against deionized water. The dialysate including the resulting precipitate was lyophilized and used as total soluble glycoprotein in the following step.

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. After *N*-acetylation of the hydrazinolysate with saturated ammonium bicarbonate (20 ml) and acetic anhydride (0.8 ml), the acetylated hydrazinolysate was desalted using Dowex 50×2 resin. Pyridylamination of the sugar chains was done by the method of Kondo *et al.*[15] 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 Asahipak NH2P-50 column (0.46×25 cm) and the Shiseido Capcell Pak C18 MG column (1.0×25 cm). On the Capcell Pak C18 MG column, the PA-sugar chains were eluted by increasing the acetonitrile concentration in 0.02% TFA linearly from 0 to 15% for 60 min at a flow rate 1.5 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 0.8 ml/min.

Electrospray ionization (ESI) mass spectrometry. ESI-MS and MS/MS analyses of PA-oligosaccharides were done as described in our previous reports,[12,13,16] using a Perkin Elmer Sciex API-III, triple-quadrupole mass spectrometer with an atmospheric-pressure ionization ion source.

Carbohydrate Analysis. The carbohydrate composition was analyzed by gas-liquid chromatography (GLC) as trimethylsilyl derivatives after methanolysis (1.5 N methanolic HCl) using *myo*-inositol as an internal standard as described in our previous paper.[16] For GLC analysis, a Hitachi G-3000 gas chromatograph with a DB-1 capillary column (30 m ×0.25 mm, J & W Scientific) was used.

Glycosidase Digestions. Digestions with jack bean α-mannosidase, diplococcal β-*N*-acetylglucosaminidase, *Aspergillus* α-1,2-mannosidase, apple snail α-fucosidase, and apple snail β-xylosidase were done using about 1 nmol of the PA-sugar chains under the conditions described in our previous reports.[12,13] The resulting glycosidase-digests were analyzed with SF-HPLC using the Asahipak NH2P-50 column (0.46×25 cm). In the case of analysis of the glycosidase digests, the PA-sugar chains were eluted by increasing the water content in the water-acetonitrile mixture from 18% to 40% linearly in 30 min at a flow rate of 0.8 ml/min.

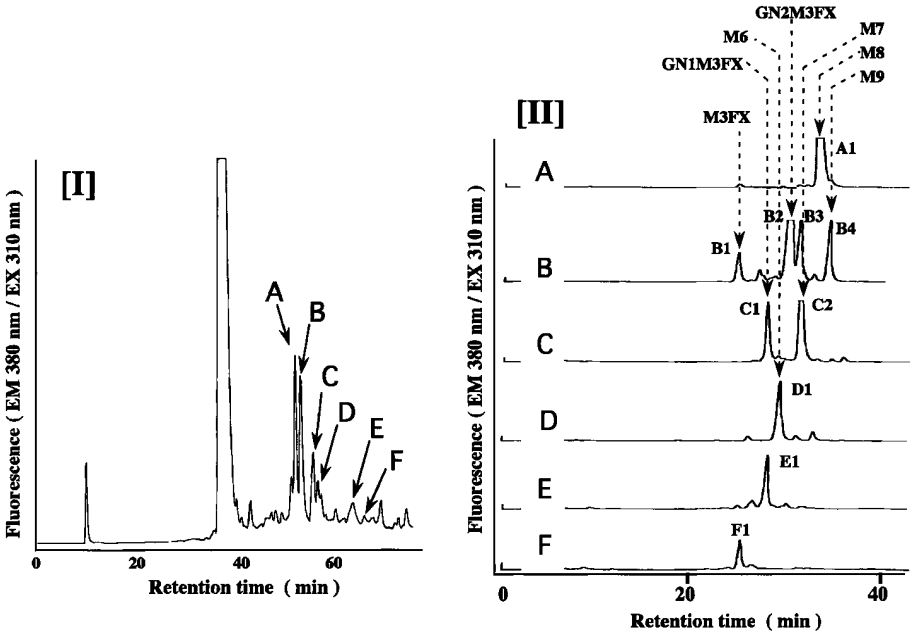


Fig. 1. RP-HPLC of PA-Sugar Chains of Glycoproteins from Oil Palm Pollen.
[I], RP-HPLC of PA-derivatives from oil palm pollen glycoproteins with a Shiseido Capcell pak C18 MG column (10×250 mm, Shiseido). [II], SF-HPLC of the fraction A to F obtained in [I] using a Shodex Asahipak NH2P-5 column (4.6×250 mm, Showa Denko Co.). The details of chromatographic conditions are described in Materials and Methods.

Results and Discussion

Purification of PA-sugar chains from oil palm pollen glycoprotein

As shown in Fig. 1-[I], seven fractions, designated-A, -B, -C, -D, -E, and -F, were obtained by RP-HPLC from oil palm pollen glycoproteins. These fraction were confirmed to contain some pyridylaminated *N*-glycan by ESI-MS and MS/MS analysis, since pyridylaminated *N*-glycans always give two specific fragments, one at 300 m/z (PA-GlcNAc) and the other at 502 m/z (GlcNAc-GlcNAc-PA). Although a big peak eluted at 38 min contained some *N*-glycans bearing only one GlcNAc residue at the reducing end, MS/MS analyses of some PA-sugar chains in this fraction gave the fragment ions at m/z 300 (GlcNAc-PA) and m/z 462 (Man1GlcNAc1-PA) but not at m/z 503 (GlcNAc2-PA) (data not shown), suggesting that these *N*-glycans would be produced as by-products during hydrazinolysis.[12,13,16]

The PA-sugar chain fractions (A-F) were further purified by SF-HPLC using the Shodex Asahipak NH2P-50 column. As shown in Fig. 1-[II], ten PA-sugar chains were purified: A1 (m/z 911.0; $[Man_8GlcNAc_2\text{-}PA+2Na]^{2+}$) from Fraction A in Fig. 1-[I], B1 (m/z 1268.0; $[Man_3Xyl_1Fuc_1GlcNAc_2\text{-}PA+H]^{+}$), B2 ($m/z$ 848.5; $[GlcNAc_2Man_3Xyl_1Fuc_1\text{-}GlcNAc_2\text{-}PA+2Na]^{2+}$), B3 ($m/z$ 831.0; $[Man_7\text{-}GlcNAc_2\text{-}PA+2Na]^{2+}$), B4 ($m/z$ 991.5; $[Man_9\text{-}GlcNAc_2\text{-}PA+2Na]^{2+}$) from Fraction B, C1 ($m/z$ 1470.5; $[GlcNAc_1Man_3Xyl_1Fuc_1GlcNAc_2\text{-}PA+H]^{+}$), C2 ($m/z$ 831.0; $[Man_7GlcNAc_2\text{-}PA+2Na]^{2+}$) from Fraction C, D1 ($m/z$ 739.0; $[Man_6GNAc_2\text{-}PA+2Na]^{2+}$) from Fraction D, E1 ($m/z$ 764.5; $[GlcNAc_2Man_3Xyl_1GlcNAc_2\text{-}PA+2H]^{2+}$) from Fraction E, and F1 (m/z 663.0; $[GlcNAc_1Man_3\text{-}Xyl_1GlcNAc_2\text{-}PA+2H]^{2+}$) from Fraction F. MS/MS analysis of other small detectable peaks could be confirmed not to be *N*-glycans, since these peaks did not give a fragment ion at m/z 300.0 (PA-GlcNAc) on MS/MS spectra.

Structures of PA-sugar Chains A1, B3, B4, C2, and D1

Since these five PA-sugar chains were converted to $Man_5GlcNAc_2$-PA by the *Aspergillus* α-1,2-mannosidase digestions (Fig. 2-(VI)), these PA-sugar chains must be typical high-mannose type structures with the core unit: Manα1–6(Manα1–3)Manα1–6(Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAc. The resulting product, $Man_5GlcNAc_2$-PA, was further converted to $Man_1GlcNAc_2$-PA by jack bean α-mannosidase digestion (Fig. 2-VII). Furthermore, all fragment ions derived from these PA-sugar chains by MS/MS analysis could be assigned as fragments ($Man_{9\text{-}1}GlcNAc_2$-PA, GlcNAc-GlcNAc-PA, and GlcNAc-PA) derived from the typical high-mannose type *N*-glycans. A MS/MS spectrum for D1 ($[Man_6GlcNAc_2\text{-}PA+H]^{+}$, minor signal) is shown in Fig. 3-[I] as a typical example.

The structures of these high-mannose type *N*-

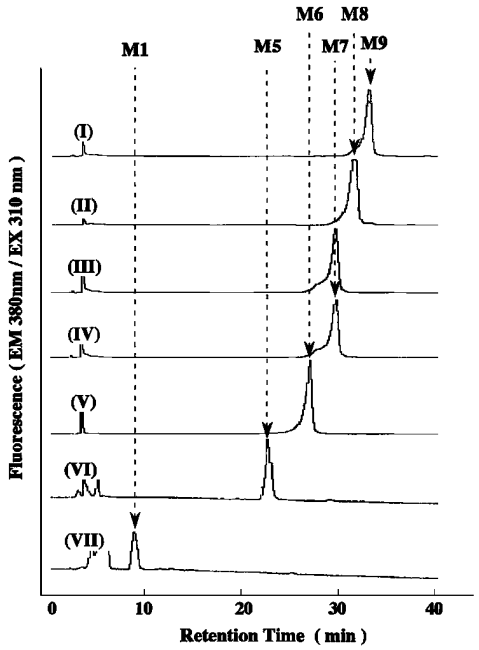


Fig. 2. Size-fractionation HPLC (SF-HPLC) of α-Mannosidase Digests of PA-Sugar Chains A1, B3, B4, C2, and D1.

(I), Original elution positions of B4; (II), A1; (III) B3; (IV) C2; (V), D1; (VI), *Aspergillus* α-1,2-mannosidase digests of A1, B3, B4, C2, and D1; (VII), Jack bean α-mannosidase digest of (VI). The arrows (M_{9-1}) indicate the elution positions of authentic PA-sugar chains; $Man_{9-1}GlcNAc_2$-PA.

glycans were further analyzed by two-dimensional PA-sugar chain mapping.[17,18] Elution positions of five PA-sugar chains corresponded to those of authentic high-mannose type sugar chains; A1 corresponded to M8A, B3 to M7A, B4 to M9A, C2 to M7B, and D1 to M6B, respectively.

Considering these results (ESI-MS, MS/MS, α-mannosidase digestions, and two-dimensional sugar chain mapping), the structures of PA-sugar chains A1, B3, B4, C2, and D1 could be proposed as follows: Manα1–2Manα1–6(Manα1–3)Manα1–6(Manα1–2Manα1–2Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAc for A1, Manα1–2Manα1–6(Manα1–3)Manα1–6(Manα1–2Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAc for B3, Manα1–2Manα1–6(Manα1–2Manα1–3)Manα1–6(Manα1–2Manα1–2Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAc for B4, Manα1–6(Manα1–3)Manα1–6(Manα1–2Manα1–2Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAc for C2, and Manα1–6(Manα1–3)Manα1–6(Manα1–2Manα1–3)Manβ1–4GlcNAcβ1–4GlcNAc for D1 (Scheme 1).

Structures of PA-sugar chains B-1, B-2, C-1, E-1, and F-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 β1–2 xylose or α1–3 fucose residue(s). The occurrence of xylose and/or fucose residues was also confirmed by GLC analysis after methanolysis.

The elution position of B1 (m/z 1267.5), on the two-dimensional sugar chain map,[17,18] corresponded to that of authentic M3FX. B1 was converted to $Man_1Xyl_1Fuc_1GlcNAc_2$-PA by the jack bean α-mannosidase. The product was converted to $Man_1Fuc_1GlcNAc_2$-PA by the apple snail β-xylosidase digestion and to $Man_1Xyl_1GlcNAc_2$-PA by the apple snail α-fucosidase digestion, respectively. The exoglycosidase digestion showed that C1 consists of two α-linkage mannose residues, one β-linkage xylose residue, and one α-fucose residue. Furthermore, the fragment ions derived from B-1 by MS/MS analysis could be assigned as the fragments from $Man_3Xyl_1Fuc_1GlcNAc_2$-PA; m/z 1122.0 ($[Man_3Xyl_1GlcNAc_2\text{-PA}+H]^+$), m/z 827.0 ($[Man_2GlcNAc_2\text{-PA}+H]^+$), m/z 827.5 ($[Man_2GlcNAc_2\text{-PA}+H]^+$), m/z 664.5 ($[Man_1GlcNAc_2\text{-PA}+H]^+$), m/z 502.5 ($[GlcNAc_2\text{-PA}+H]^+$), m/z 299.5 ($[GlcNAc\text{-PA}+H]^+$). Considering these results (2-D sugar chain mapping, MS/MS analysis, and exoglycosidase digestions), the structures of B1 could be proposed as Manα1–6(Manα1–3)(Xylβ1–2)Manβ1–4GlcNAcβ1–4(Fucα1–3)GlcNAc (M3FX) (Scheme 1).

The elution position of B2 (m/z 848.5; $[M+2Na]^{2+}$), on the two-dimensional sugar chain map, corresponded to that of GlcNAcβ1–2Manα1–6(GlcNAcβ1–2Manα1–3)(Xylβ1–2)Manβ1–4GlcNAcβ1–4(Fucα1–3)GlcNAc-PA (GN2M3FX). As shown in Fig. 3-[II], fragment ions derived from B2 by MS/MS could be assigned as followings; m/z 1551.0 ($[GlcNAc_2Man_3Xyl_1GlcNAc_2\text{-PA}+Na]^+$), m/z 1493.0 ($[GlcNAc_1Man_3Xyl_1Fuc_1GlcNAc_2\text{-PA}+Na]^+$), m/z 1347.0 ($[GlcNAc_1Man_3Xyl_1GlcNAc_2\text{-PA}+Na]^+$), m/z 1331.0 ($[GlcNAc_1Man_2Xyl_1Fuc_1GlcNAc_2\text{-PA}+Na]^+$), m/z 1185.0 ($[GlcNAc_1Man_2Xyl_1GlcNAc_2\text{-PA}+Na]^+$), m/z 894.0 ($[GlcNAc_1Man_3GlcNAc_1+H]^+$), m/z 550.5 [$(Man_2GlcNAc_1)+Na]^+$, m/z 503.0 ($[GlcNAc_2\text{-PA}+H]^+$), m/z 300.0 ($[GlcNAc\text{-PA}+H]^+$).

As shown in Fig. 4, B2 was converted to $Man_3Xyl_1Fuc_1GlcNAc_2$-PA by the diplococcal β-*N*-acetylglucosaminidase, suggesting two GlcNAc residues are bound to mannose residues by β1–2 linkage (Fig. 4-II). The resulting product ($Man_3Xyl_1Fuc_1GlcNAc_2$-PA) was further converted to $Man_1Xyl_1Fuc_1GlcNAc_2$-PA by the jack bean α-mannosidase digestion (Fig. 4-III). The product was converted to $Man_1Xyl_1GlcNAc_2$-PA by the apple snail β-fucosidase digestion (Fig. 4-IV) and to $Man_1Fuc_1GlcNAc_2$-PA by the apple snail β-xylosidase digestion (Fig. 4-V). Considering these results (2-D sugar chain mapping, MS/MS analysis, exoglycosidase digestions), the structure of B2 could be proposed as GlcNAcβ1–2Manα1–6(GlcNAcβ1–2Manα1–3)(Xylβ1–2)Manβ1–4GlcNAcβ1–4(Fucα1–3)GlcNAc-PA as shown in Scheme 1.

The elution position of C1 (m/z 1470.5; [M+

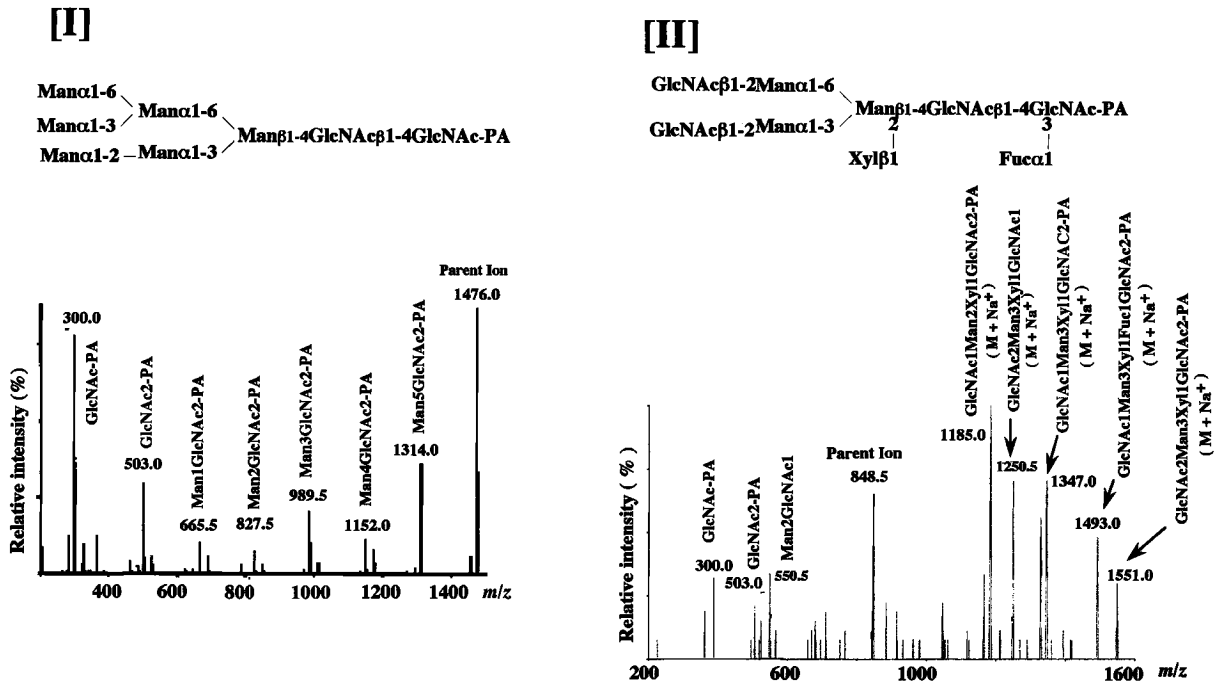


Fig. 3. MS/MS Spectra of PA-Sugar Chains D1 and B2.

[I], Spectrum for D1 ($Man_6GlcNAc_2$-PA); [II], Spectrum for B2 ($GlcNAc_2Man_3Xyl_1Fuc_1GlcNAc_2$-PA). The collisionally activated dissociation (CAD) spectrum was measured with argon as the collision gas. The collision energy was 60–100 eV. The scanning was done with a step size of 0.5 Da.

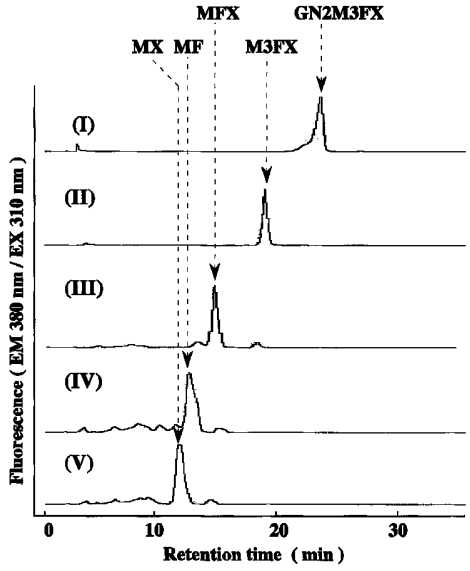
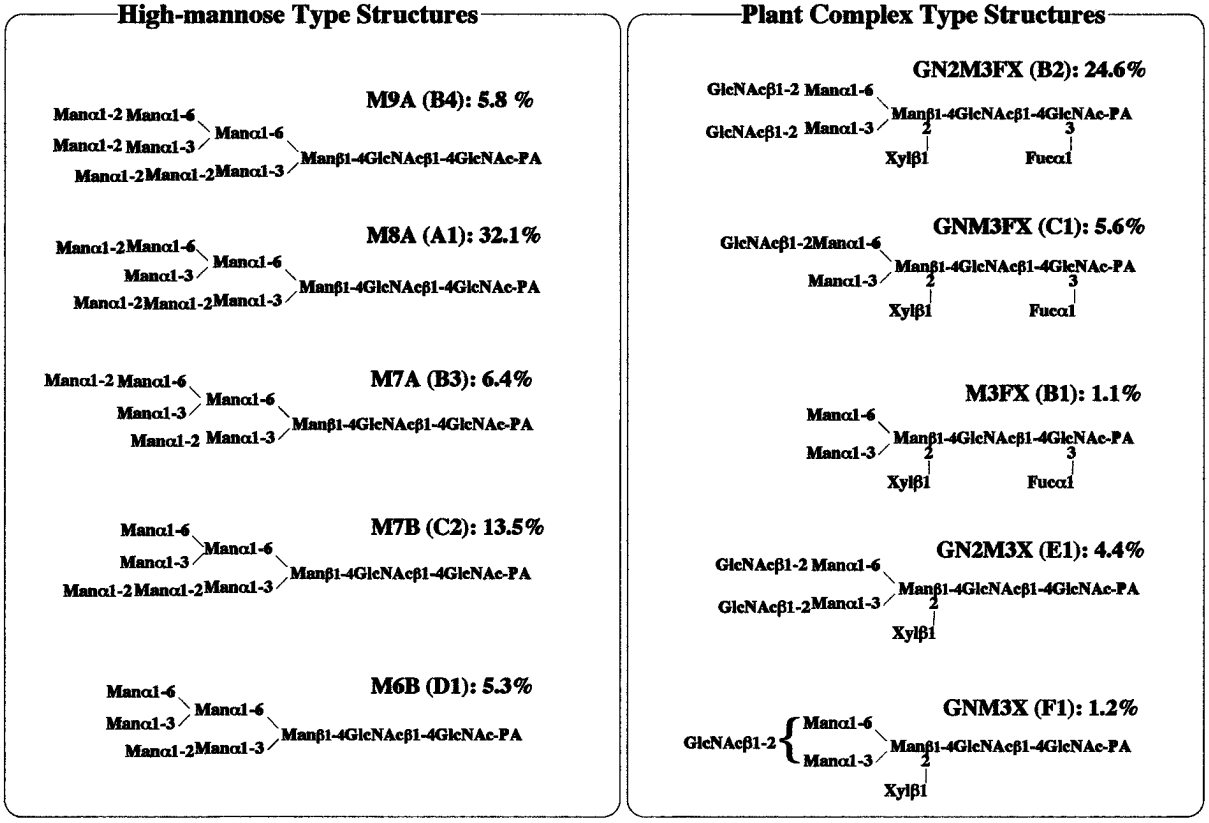


Fig. 4. Size-fractionation HPLC (SF-HPLC) of Exoglycosidase Digests of PA-Sugar Chains B2.

(I), Original elution positions of B2; (II), Diplococcal *β-N*-acetylhexosaminidase digest of (I); (III), Jack bean α-mannosidase digest of (II); (IV), apple snail *β*-xylosidase digest of (III); (V), apple snail α-fucosidase digest of (III); GN2M3FX, M3FX, MFX, MF, and MX indicate the elution positions of $GlcNAc_2Man_3Xyl_1Fuc_1GlcNAc_2$-PA, $Man_3Xyl_1Fuc_1GlcNAc_2$-PA, $Man_1Xyl_1Fuc_1GlcNAc_2$-PA, $Man_1Fuc_1GlcNAc_2$-PA, and $Man_1Xyl_1GlcNAc_2$-PA, respectively.

$H]^+$), on the two-dimensional sugar chain map, corresponded to that of GlcNAc*β*1–2Manα1–6(Manα1–3)(Xyl*β*1–2)Man*β*1–4GlcNAc*β*1–4(Fucα1–3)GlcNAc-PA (GN1M3FX). The fragment ions derived from C1 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* 665.5 ($[Man_1GlcNAc_2$-PA + $H]^+$), *m/z* 503.0 ($[GlcNAc_2$-PA + $H]^+$), *m/z* 300 ($[GlcNAc$-PA + $H]^+$). C1 was converted to $Man_3Xyl_1Fuc_1GlcNAc_2$-PA by the diplococcal *β-N*-acetylglucosaminidase, suggesting one GlcNAc residue is bound to mannose residues by *β*1–2 linkage. The resulting product (Man_3-$Xyl_1Fuc_1GlcNAc_2$-PA) was further converted to $Man_1Xyl_1Fuc_1GlcNAc_2$-PA by the jack bean α-mannosidase digestion. The product was converted to $Man_1Xyl_1GlcNAc_2$-PA by the apple snail α-fucosidase digestion and to $Man_1Fuc_1GlcNAc_2$-PA by the apple snail *β*-xylosidase digestion, respectively. Concerning the structure of C1, $GlcNAc_1Man_3Xyl_1$-$Fuc_1GlcNAc_2$-PA structure occurs in two isomers; one is Manα1–6(GlcNAc*β*1–2Manα1–3)(Xyl*β*1–2)Man*β*1–4GlcNAc*β*1–4(Fucα1–3)GlcNAc-PA ($_{GN}$M3FX), the other one is GlcNAc*β*1–2Manα1–6(Manα1–3)(Xyl*β*1–2)Man*β*1–4GlcNAc*β*1–4(Fucα1–3)GlcNAc-PA (^{GN}M3FX). It has already been reported that on the ODS column the former structure is



Scheme 1. Proposed Structures of *N*-Glycans Linked to Oil Palm Pollen.
The alphabetical notations in parentheses indicate the PA-sugar chain fractions obtained in Fig. 1-[II].

eluted before GN2M3FX and the latter structure is eluted after GN2M3FX.[17,18] Considering the elution position of C1 which was eluted slightly after GN2M3FX (B2) on the ODS column, the structure of C1 may be ^{GN}M3FX (Scheme 1).

Since authentic PA-sugar chains corresponding to E1 (*m/z* 1324.0, $GlcNAc_1Man_3Xyl_1GlcNAc_2$-PA) and F1 (*m/z* 1528.0, $GlcNAc_2Man_3Xyl_1GlcNAc_2$-PA) were not available, the detailed structures of these two PA-sugar chains could not be deduced by the two-dimensional sugar chain mapping method. However, considering the general structural rule for the plant complex type *N*-glycan and MS/MS data for these PA-sugar chains, it is assumed that the structure of E1 should be GlcNAcβ1–2Manα1–6(Manα1–3)(Xylβ1–2)Manβ1–4GlcNAcβ1–4GlcNAc-PA or Manα1–6(GlcNAcβ1–2Manα1–3)(Xylβ1–2)Manα1–4GlcNAcβ1–4GlcNAc-PA and the structure of F1 may be GlcNAcβ1–2Manα1–6(GlcNAcβ1–2Manα1–3)(Xylβ1–2)Manβ1–4GlcNAcβ1–4GlcNAc-PA as shown in Scheme 1. Actually the fragment ions derived from E1 (*m/z* 1324.0) by MS/MS analysis could be assigned as the fragments from $GlcNAc_1Man_3Xyl_1GlcNAc_2$-PA; (*m/z* 1192.5 ($[GlcNAc_1Man_3GlcNAc_2\text{-}PA+H]^+$), *m/z* 1161.5 ($[GlcNAc_1Man_2Xyl_1GlcNAc_2\text{-}PA+H]^+$), *m/z* 1121.5 ($[Man_3Xyl_1GlcNAc_2\text{-}PA+H]^+$), *m/z* 958.5 ($[Man_2Xyl_1GlcNAc_2\text{-}PA+H]^+$), *m/z* 796.5 ($[Man_1Xyl_1GlcNAc_2\text{-}PA+H]^+$), *m/z* 503.0 ($[GlcNAc_2\text{-}PA+H]^+$), *m/z* 299.5 ($[GlcNAc\text{-}PA+H]^+$). The fragment ions derived from F1 (*m/z* 1528.0) by MS/MS could be assigned as the fragments from $GlcNAc_2Man_3Xyl_1GlcNAc_2$-PA; *m/z* 1324.5 ($[GlcNAc_1Man_3Xyl_1GlcNAc_2\text{-}PA+H]^+$), *m/z* 1192.5 ($[GlcNAc_1Man_3GlcNAc_2\text{-}PA+H]^+$), *m/z* 1161.5 ($[GlcNAc_1Man_2Xyl_1GlcNAc_2\text{-}PA+H]^+$), *m/z* 1121.5 ($[Man_3Xyl_1GlcNAc_2\text{-}PA+H]^+$), *m/z* 958.5 ($[Man_2Xyl_1GlcNAc_2\text{-}PA+H]^+$), *m/z* 796.5 ($[Man_1Xyl_1GlcNAc_2\text{-}PA+H]^+$), *m/z* 664.5 ($[Man_1GlcNAc_2\text{-}PA+H]^+$), *m/z* 502.5 ($[GlcNAc_2\text{-}PA+H]^+$), *m/z* 300.0 ($[GlcNAc\text{-}PA+H]^+$).

Structural features of N-glycans of palm pollen glycoproteins

As shown in Scheme 1, the structures of *N*-glycans of the oil palm pollen glycoproteins are classified as three types: the first one is the xylose/fucose-containing structure ($GlcNAc_2Man_3Xyl_1Fuc_1GlcNAc_2$ (B2; 24.6%), $GlcNAc_1Man_3Xyl_1Fuc_1GlcNAc_2$ (C1; 5.6%), $Man_3Xyl_1Fuc_1GlcNAc_2$ (B1; 1.1%)), the second one is the xylose-containing structure ($GlcNAc_2Man_3Xyl_1GlcNAc_2$ (E1; 4.4%), $GlcNAc_1Man_3Xyl_1GlcNAc_2$ (F1; 1.2%)), the third one is the typical high-mannose type structure ($Man_9GlcNAc_2$ (B4; 5.8%), $Man_8GlcNAc_2$ (A1; 32.1%), $Man_7GlcNAc_2$ (B3 and C2; 19.9%), and $Man_6GlcNAc_2$

(D1; 5.3%)). However, the laccase type (Lewis a antigen unit (Galβ1–3(Fucα1–4)GlcNAc-) containing type) structures[19] were not found among *N*-glycans of palm pollen glycoproteins.

In our previous report,[3] we showed that the putative glycoallergen, Ela g Bd 31 K, recognized by IgE from the palm pollinosis bears the antigenic complex type *N*-glycan(s) containing β1–2 xylose or α1–3 fucose residues. It has been reported that recognition of the β1–2 xylose residue by IgE of pollen allergenic patients would be dependent on the degree of substitution of the core mannose influencing the conformation of the epitope.[6] Some xylose-specific IgE antibodies 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. The core mannose residue in *N*-glycans linked to Lol p 11 and bromelain is substituted with only an α1–6 mannose residue, whereas the core mannose residue in the *N*-glycans linked to Ole e 1 is substituted with both of two α1–6 and α1–3 mannose residues. This finding suggested that the α1–3 mannose sterically inhibits IgE recognition of the β1–2 xylose residue. All xylose-containing *N*-glycans obtained from palm pollen glycoproteins in this report have the core mannose residue substituted with both α1–6 and α1–3 mannose residues, therefore, it would be reasonable to assume that the allergenic glycoprotein, Ela g Bd 31 K, in the oil palm pollen probably bears the $GlcNAc_2Man_3Xyl_1Fuc_1GlcNAc_2$, $GlcNAc_1Man_3Xyl_1Fuc_1GlcNAc_2$, or $Man_3Xyl_1Fuc_1GlcNAc_2$ structures as epitopes for IgE.

Using M3FX (B1) and GN2M3FX (B2) as competitors for the binding of IgE to the Japanese cedar pollen glycoallergen,Cry j1, we recently found that the antigenic free *N*-glycan itself could be hardly recognized by IgE from the pollinosis patients, suggesting certain three-dimensional structure(s) constructed by the antigenic *N*-glycan and the peptide moiety around the glycosylation site would be necessary for the recognition of the glycoallergen(s) by IgE. We have also found that the antigenic plant *N*-glycans suppress a cytokine production of the helper T-cells, suggesting an involvement of the plant *N*-glycans in human cellular immunity. The reactivity of the antigenic plant *N*-glycans toward the human immune system will be described elsewhere.

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