

Purification and Characterization of 31-kDa Palm Pollen Glycoprotein (Ela g Bd 31 K), Which is Recognized by IgE from Palm Pollinosis Patients*

Yoshinobu KIMURA,^{1,†} Megumi MAEDA,¹ Mariko KIMURA,¹ Oi Ming LAI,²
Siang Hee TAN,³ Sook Mei HON,⁴ and Fook Tim CHEW⁴

¹Department of Bioresources Chemistry, Faculty of Agriculture, Okayama University,
Okayama 700-8530, Japan

²Department of Biotechnology, Faculty of Food Science and Biotechnology, Universiti Putra Malaysia,
43400 Serdang Selangor, Malaysia

³Genome Centre, Institute Bioscience, Universiti Putra Malaysia, 43400 Serdang Selangor, Malaysia

⁴Department of Biological Sciences, National University of Singapore, Lower Kent Ridge Road,
Singapore 119074, Singapore

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A basic glycoprotein, which was recognized by IgE from oil palm pollinosis patients, has been purified from oil palm pollen (*Elaeis guineensis* Jacq.), which is a strong allergen and causes severe pollinosis in Malaysia and Singapore. Soluble proteins were extracted from defatted palm pollen with both Tris-HCl buffer (pH 7.8) and Na-acetate buffer (pH 4.0). The allergenic glycoprotein was purified from the total extract to homogeneity with 0.4% yield by a combination of DEAE- and CM-cellulose, SP-HPLC, and gel filtration. The purified oil palm pollen glycoprotein with molecular mass of 31 kDa was recognized by the β 1-2 xylose specific antibody, suggesting this basic glycoprotein bears plant complex type *N*-glycan(s). The palm pollen basic glycoprotein, designated Ela g Bd 31 K, was recognized by IgE of palm pollinosis patients, suggesting Ela g Bd 31 K should be one of the palm pollen allergens. The preliminary structural analysis of *N*-glycans linked to glycoproteins of palm pollens showed that the antigenic *N*-glycans having α 1-3 fucose and β 1-2 xylose residues (GlcNAc₂₋₀Man₃Xyl₁Fuc₁₋₀GlcNAc₂) actually occur on the palm pollen glycoproteins, in addition to the high-mannose type structures (Man₉₋₅GlcNAc₂).

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

Oil palm pollinosis is an important cause of inhalant allergy in Southeast Asian areas, especially in Malaysia and Singapore.^{1,2)} However, not much on isolation and molecular characterization of the oil palm pollen allergens has been reported. It has been

reported that allergens purified from plant materials are often glycoproteins, to which the plant complex type *N*-glycans bearing β 1-2 xylose and/or α 1-3 fucose residue(s) are bound.³⁻¹⁶⁾ Several groups (Garcia-Casado *et al.*,⁷⁾ Ohsuga *et al.*,⁸⁾ Fötisch *et al.*,⁹⁾ van Ree *et al.*¹⁰⁾) have reported that β 1-2 xylose and/or α 1-3 fucose residues are essential in the recognition of the allergenic glycoproteins by allergic human IgE, postulating that the plant complex type *N*-glycans could be an important epitope in the allergenic response. On the other hand, Hijikata *et al.*¹¹⁾ and Ogawa *et al.*¹²⁾ have reported that the plant complex type *N*-glycans on Cry j I themselves are not essentially involved in the binding to allergic human IgE but contribute partially to build up a relevant epitope conformation with the peptides portion, since they found that an allergic human IgE that recognizes Cry j I could not recognize other plant glycoproteins bearing the plant complex type *N*-glycans.

More recently, Okano *et al.*¹⁶⁾ reported that the *N*-glycans of Cry j I play a major role in promoting Cry j I-specific T_H2 response *in vitro*, although the glycans are not major targets as T-cell epitopes. They have also reported that the antigenic oligosaccharides (Gal₂₋₀Fuc₂₋₀GlcNAc₂Man₃Xyl₁Fuc₁GlcNAc₂) of Cry j I or lacto-*N*-fucopetaose III unit of *Schistosoma mansoni* egg antigens) function as an adjuvant for by the ability to induce a T_H2 response.¹⁷⁾ Therefore, to discover the immunological effects of the plant complex type *N*-glycans on the human immune system, more multifaceted immunological analyses, such as T_H-cell responses against the antigenic oligosaccha-

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† To whom correspondences should be addressed. FAX: +81-86-251-8388; E-mail: yosh8mar@cc.okayama-u.ac.jp

rides in addition to the IgE-binding analysis, seems to be necessary.

In this report, as part of a study to verify whether the plant complex type *N*-glycans linked to several glycoallergens are directly involved in pollen and plant food allergies, we tried to purify and identify a glycoallergen from the oil palm pollen, which is considered to be a strongly allergenic pollen. Among the oil palm pollen glycoproteins recognized by an antiserum against the β 1-2 xylose residue, we succeeded in purifying an allergenic glycoprotein (31 kDa) with the antigenic plant complex type *N*-glycans. Furthermore, we report the preliminary analysis of *N*-glycans linked to palm pollen glycoproteins, suggesting that GlcNAc₂₋₀Man₃Xyl₁Fuc₁₋₀GlcNAc₂ structures, in addition to several high-mannose type *N*-glycans (Man₉₋₅GlcNAc₂ structures), occur on the palm pollen glycoproteins.

Materials and Methods

Materials. Oil palm pollens were collected in Selangor state of Malaysia in 2000. An Asahipak NH2P-50 column (0.46 × 25 cm) was purchased from Showa Denko Co. Authentic PA-sugar chains were prepared as described in our previous papers.¹⁸⁻²¹ α -Mannosidase (jack bean) was purchased from Sigma. β -Galactosidase (*Streptococcus* 6646 K) was from Seikagaku Kogyo Co. An antiserum against β 1→2 xylose-containing *N*-glycans was a generous gift from Dr. Arnd Sturm (Friedrich Miescher-Institute, Basel, Switzerland). NBT (nitro blue tetrazolium) and BCIP (5-bromo-4-chloro-3-indolyl-phosphate) were from Promega. Sera used in this report were obtained from oil palm pollinosis patients (both male and female) in Singapore.

Purification of an antigenic 31 kDa basic glycoprotein. Since Japanese cedar pollen allergen (Cry j 1), Japanese cypress pollen allergen (Cha o 1), and timothy pollen allergen are basic glycoproteins with the plant complex type *N*-glycans,³⁻⁵ in this report we focused on basic glycoproteins bearing the antigenic glycans to purify a glycoallergen from oil palm pollen. To detect glycoallergen(s) of the oil palm pollen, we used a xylose-specific antiserum,²² which is specific for the plant complex type *N*-glycans bearing the β 1-2 xylose residue.²³ Purification of glycoallergen was done at 0–4°C. Protein was measured by light absorption at 280 nm with bovine serum albumin (BSA) as a standard protein.

Step 1. Preparation of glycoproteins from the oil palm pollen. The palm pollens (9.5 g) were defatted in 500 ml of acetone. The resulting defatted powder was sonicated in 200 ml of 50 mM Tris-HCl buffer (pH 7.8) for 30 min in an ice bath. After centrifugation, the supernatant was removed to a plastic beaker.

The resulting precipitate was sonicated again in 200 ml of 50 mM Na-acetate buffer (pH 4.0) for 30 min in an ice bath. After centrifugation, the supernatant was added to the pH 7.8 extraction sample. The combined extract was saturated 100% with ammonium sulfate and the resulting precipitate was used to purify an allergenic glycoprotein in the following steps.

Step 2. DEAE-Cellulose column chromatography. The crude protein fraction precipitated with ammonium sulfate in Step 1 was dissolved in 50 mM Tris-HCl buffer, pH 7.8, and dialyzed against the same buffer. After centrifugation, the supernatant was put on a DEAE-cellulose column (2.0 × 33 cm) equilibrated with 50 mM Tris-HCl buffer, pH 7.8. The basic proteins were collected by washing the column with 200 ml of the same buffer and then bound proteins (acidic proteins) were eluted with the same buffer containing 0.5 M NaCl (Fig. 1-[A]). The run-through fraction was collected as indicated by a bar and saturated 100% with ammonium sulfate.

Step 3. CM-cellulose column chromatography. The basic proteins precipitated with ammonium sulfate in Step 3 were dissolved in a small amount of 25 mM Na-acetate buffer, pH 5.0, and dialyzed against the same buffer. After centrifugation, the supernatant was put on a CM-cellulose column (2.0 × 36 cm) equilibrated with 25 mM Na-acetate buffer, pH 5.0, and the column was washed with the same buffer. After the column was washed, the adsorbed proteins were eluted by a linear gradient of NaCl from 0 to 0.3 M in the same buffer (Fig. 1-[B]). The pooled fraction indicated by a bar in Fig. 1-[B], in which some glycoproteins bearing the antigenic plant complex type *N*-glycans were detected by the immunoblotting analysis, was saturated 100% with ammonium sulfate.

Step 4. Ion-exchange HPLC. The proteins precipitated with ammonium sulfate in Step 3 were dissolved in a small amount of 25 mM Na-acetate buffer, pH 6.0, and dialyzed against the same buffer. The glycoproteins with the plant complex type *N*-glycan(s) were further fractionated by a Jasco 880-PU HPLC apparatus with a Jasco Intelligent UV/VIS detector (870 UV) and a Biofine IEC-SP column (0.8 × 7.5 cm). The dialyzed sample was put on the HPLC column equilibrated with 25 mM Na-acetate, pH 6.0. After the column was washed with same buffer for 10 min, the adsorbed proteins were eluted by a linear gradient of NaCl from 0 to 0.5 M in the same buffer at a flow rate of 1.0 ml/min. The antigenic glycoprotein bearing the plant complex type *N*-glycan(s) was recovered in the two fractions indicated by bars 1 and 2 as shown in Fig. 1-[C].

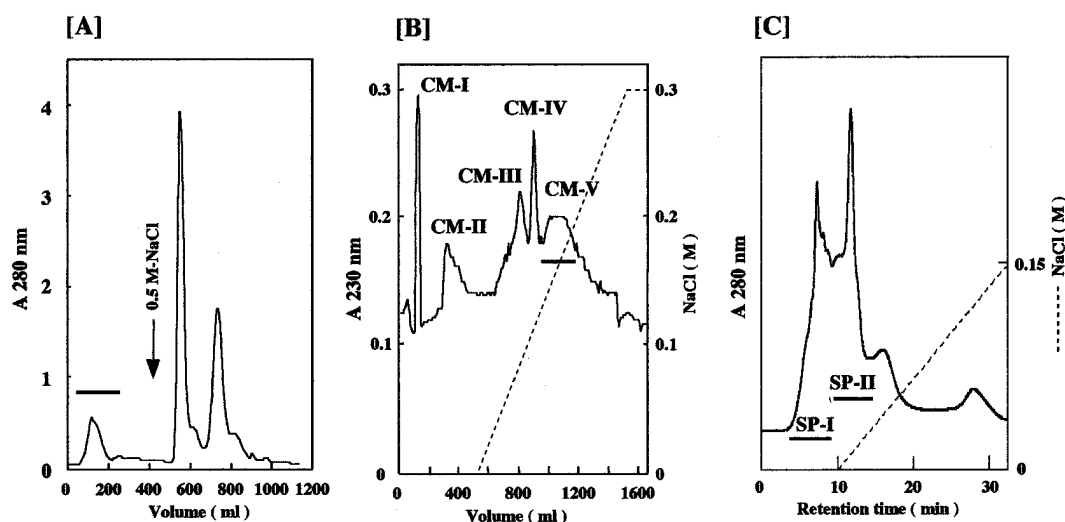


Fig. 1. Purification Steps of An Allergenic Oil Palm Pollen Glycoprotein.

[A], DEAE-cellulose chromatography of crude protein mixture prepared from the oil palm pollens. After dialysis of the crude protein mixture against 50 mM Tris-HCl buffer, pH 7.8, the dialyzate was put on a DEAE-cellulose column (2.0×33 cm) equilibrated with the same buffer. The run-through fraction as indicated by a bar was collected for the next step. The proteins were detected by the absorbance at 280 nm.

[B], CM-cellulose chromatography of the basic protein fraction obtained in [A]. After dialysis of the run-through fraction in [A] against 25 mM Na-phosphate buffer, pH 5.0, resulting dialyzate was put on a CM-cellulose column (2.0×36 cm) equilibrated with the same buffer. The bound proteins were eluted by a linear gradient of NaCl. The fraction indicated by a bar (CM-V), which was shown to contain some glycoproteins bearing the plant complex type *N*-glycans by immuno-blotting, was collected for the next purification with an ion-exchange HPLC. The proteins were detected by the absorbance at 230 nm.

[C], Ion-exchange HPLC of the glycoprotein fraction (CM-V) obtained in [B]. After dialysis of the CM-V against 25 mM Na-acetate buffer, pH 6.0, the dialyzate was put on a Biofine IEC-SP column (0.8×7.5 cm) equilibrated with the same buffer. After the column was washed with the Na-acetate buffer, the bound proteins were eluted by a linear gradient of NaCl.

Two fractions, designated SP-I and -II, were used for the final purification step with the Superdex 200 gel-filtration. The proteins were detected by the absorbance at 280 nm.

Step 5. Gel filtration with a Superdex 200 column. The protein fraction recognized by the antiserum against the plant complex type *N*-glycans (Fig. 1-[C]) was dialyzed against 3 L of 50 mM Tris-HCl buffer, pH 7.8, containing 0.1 M NaCl. After dialysis, the sample was concentrated by centrifugation in an Amicon Centriprep-30 and the concentrated sample was put on a Superdex 200 column (1.6×120 cm, Pharmacia), equilibrated with 50 mM Tris-HCl buffer, pH 7.8, containing 0.1 M NaCl. The column was developed with the same buffer at a flow rate of 0.5 ml/min.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting of the oil palm pollen glycoproteins. SDS-PAGE was done using 20 μ l of each sample by the method of Laemmli and Favre²⁴) on a 15% acrylamide gel in 0.1 M Tris-glycine buffer system under reducing conditions with 5% 2-mercaptoethanol. Proteins on the gel were stained with the silver staining (Silver Staining Kit, Bexel Biotechnology). Phosphorylase B (97 kDa), bovine serum albumin (BSA, 66 kDa), L-glutamate dehydrogenase (55 kDa), ovalbumin (OVA, 43 kDa), and aldolase (40 kDa) were used as marker proteins (Promega) and ricin (A-chain (31 kDa) and B-chain (32 kDa)¹⁸) for the mass calibration. The proteins on

the gel were transferred to a PVDF membrane for 30 min at 120 mA on Horize-blot (AE-6675 P/N, Atto Co.), using 0.1 M Tris-glycine containing 5% methanol (pH 8.8) as the transfer buffer. Membranes were then probed with an antiserum that reacts with the plant complex type *N*-glycan as described in the previous reports.²¹⁻²³) For detection of glycoproteins conjugated with antibodies specific for the plant complex type *N*-glycans, we used goat anti-rabbit IgG coupled to horseradish peroxidase (Bio-Rad).

IgE binding assay using grid immuno-dot blotting. One μ g of each protein sample, BSA (bovine serum albumin-negative control spot) and human IgE (positive control spot) were spotted onto a nitrocellulose membrane. Each protein was spotted in duplicate. Blocking was done at room temperature for 1 h with 7% skim milk powder in PBS-T (1 M PBS containing 0.1% Tween-20). After 3 washes in PBS-T, individual membrane strips were incubated overnight with sera (diluted 1:1 with 1 M PBS) at 4°C. Alkaline phosphatase conjugated secondary antibody (diluted 1:600 in blocking reagent) was added to the membrane following three washings in PBS-T, and incubated for 2 h at room temperature. After three washings in PBS-T, NBT/BCIP color substrate (33.3 mg NBT and 16.5 mg BCIP were dissolved in 5 ml of

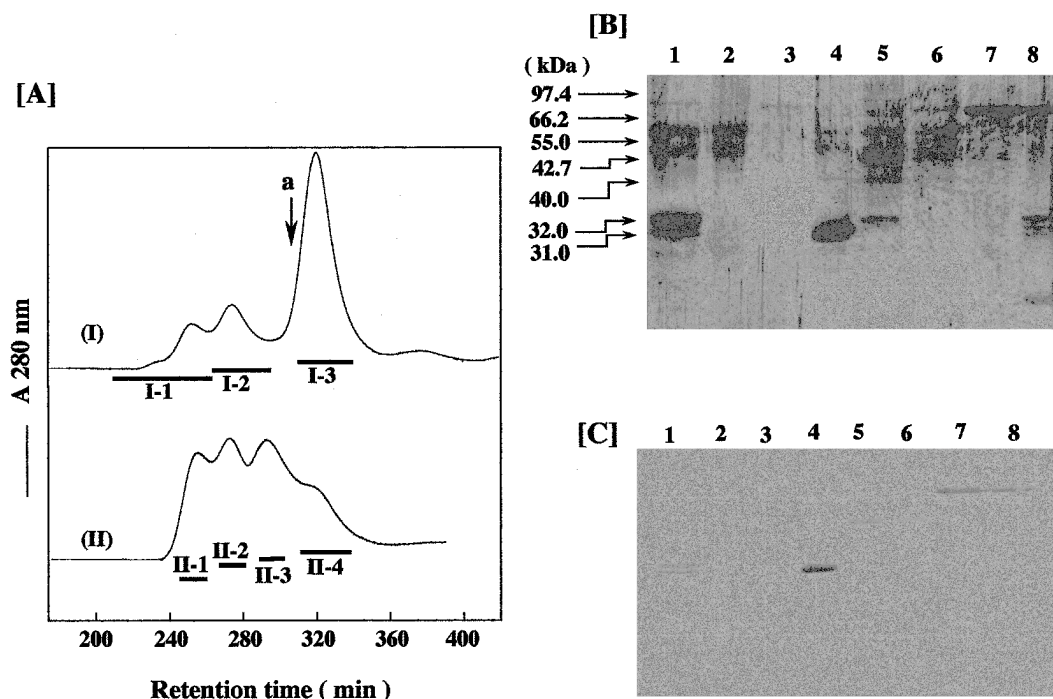


Fig. 2. Gel filtration of Partially Purified Glycoproteins Obtained by Ion-exchange HPLC, SDS-PAGE, and Immunoblotting.

[A], Gel filtration of SP-I (I) and SP-II (II) in Fig. 1-[C]. Arrow a indicates the elution position of ovalbumin (M.W. 42.7 kDa).

[B], SDS-PAGE of each protein fraction obtained by the gel filtration. Lane 1, ricin (ricin B-chain (M.W. 32 kDa) and ricin A-chain (M.W. 31 kDa); Lane 2, Fraction I-1 in [A]; Lane 3, Fraction I-2; Lane 4, Fraction I-3; Lane 5, Fraction II-1; Lane 6, Fraction II-2; Lane 7, Fraction II-3; Lane 8, Fraction II-4. The gel was stained by the silver reagent.

[C], Immunoblotting with the antiserum against the plant complex type *N*-glycans.

alkaline phosphatase buffer (0.1 M Tris-HCl pH 9.0, 0.15 M NaCl, 3 mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$) was added to the membrane for color development.

Preparation of pyridylaminated (PA-) *N*-glycans from pollen glycoproteins. *N*-Glycans were released by hydrazinolysis (100°C, 12 h, in 5 ml of anhydrous hydrazine) from the pollen total glycoproteins (about 10 mg). After *N*-acetylation of the hydrazinolysate with saturated ammonium bicarbonate (10 ml) and acetic anhydride (0.4 ml), the acetylated hydrazinolysate was desalted by Dowex 50 $\times$ 2 resin. Pyridylation of the sugar chains was done as described in our previous paper.¹⁸⁻²¹⁾ After pyridylation, resulting PA-derivatives were partially purified by gel filtration using a column of Sephadex G-25 super fine (2.8 $\times$ 40 cm) as described in our previous paper.¹⁸⁻²¹⁾ 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 $\times$ 25 cm). 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.

Glycosidase Digestions. Jack bean α -mannosidase (1.9 U) digestion, *Diplococcal* β -*N*-acetylglucosa-

minidase (20 mU) digestion, and *Aspergillus* α -1,2-mannosidase (15 μ g enzyme) digestion were done under the conditions described in our previous report.²⁵⁾ Digestion with *Streptococcus* 6646K β -galactosidase (10 mU enzyme) were done using in 100 μ l of 0.1 M sodium acetate buffer, pH 5.0, for 24 h at 37°C. The reactions were stopped by boiling the mixtures for 3 minutes and a part of the digest was analyzed by size fractionation (SF-) HPLC using the Asahipak NH2P-50 column.

Results and Discussion

Purification of an allergenic basic glycoprotein from oil palm pollens

From the run-through fraction of IEC-HPLC (Fig. 1-[C]), three peaks were obtained by the gel filtration with the Superdex 200 column (Fig. 2-[A]-I). Among these three peaks (SP-I-1, -2, -3), a single protein band was observed in the SP-I-3 fraction by SDS-PAGE. The mobility of the palm pollen protein was the same as that of the ricin A-chain (Fig. 2-[B], Lane 4), indicating that the molecular mass of the palm pollen protein would be 31 kDa. Furthermore, like the ricin A-chain, this palm pollen protein was recognized by the antiserum specific for the β 1-2 xylose residue in the plant complex type *N*-glycans, suggesting that the 31-kDa palm pollen protein is

glycosylated with the antigenic *N*-glycan (Fig. 2-[C], Lane 4). From 9.5 g of the oil palm pollens, 0.8 mg of the 31-kDa glycoprotein was purified. Although occurrence of some glycoproteins with the plant complex type *N*-glycans in SP-II (Fig. 1-[C]) were shown by the immunoblotting analysis, only SP-II-3 showed a single band with about 80 kDa on the SDS gel. However, the elution position of the glycoprotein on the gel filtration corresponded nearly to that of ovalbumin (43 kDa), suggesting this glycoprotein seems to have a weak interaction with the Superdex 200 resin.

Allergenicity of the 31-kDa palm pollen glycoprotein

The allergenicity of glycoproteins purified from the palm pollens were tested by an IgE-binding assay using a grid immuno-dot blotting. As shown in Fig. 3, the 31-kDa palm pollen glycoprotein was recognized by IgE from the palm pollen allergy patients; two female and one male patients were clearly positive (Fig. 3, F-1, -2, and M-1), one male was weakly positive (M-2), and two patients (F-3 and M-3) were negative. This result showed that the 31-kDa palm pollen glycoprotein, designated *Ela g Bd 31 K*, is recognized by IgE prepared from the palm pollinosis patients, suggesting that this glycoprotein would be one of the palm pollen allergens or a putative glycoallergen. On the contrary, the 80-kDa glycoprotein was hardly recognized by the sera of palm pollen allergy patients, indicating that this glycoprotein is not a relevant allergen.

Since the crude extract of oil palm pollen was reactive toward allergic IgE of all pollinosis patients, other allergen(s) in addition to *Ela g Bd 31 K* should occur in the palm pollen. Concerning other protein fractions obtained in some purification steps described in this report, their allergenicity was tested using 24 sera of allergy patients; 4 patients showed positive for CM-III (Fig. 1-[B]), 8 patients were positive for CM-IV (Fig. 1-[B]), 10 patients positive for SP-II-1, 3 patients positive for SP-II-3, and 6 patients positive for SP-II-4, respectively. Although nearly 40% of the pollinosis patients tested were reactive towards SP-II-1, this fraction consists of at least five protein components (Fig. 2-[B], lane 5). Therefore, it is obscure whether a glycoprotein (Fig. 2-[C], Lane 5) in the fraction is allergenic or not at this time.

Structural analysis of N-glycans of the palm pollen glycoproteins

The immunoblotting analysis suggested that the plant complex type *N*-glycans occur on the 31-kDa palm pollen allergen, *Ela g Bd 31 K*. However, the detail structure of *N*-glycan(s) linked to the tentative glycoallergen could not be tested due to small amounts of the sample. Therefore, as a first step to

	F-1	F-2	F-3	M-1	M-2	M-3
Positive control IgE : 50 IU						
Oil palm pollen extract						
31-kDa Palm pollen glycoprotein						
Negative control BSA : 1 µg						

Fig. 3. IgE-binding Assay Using Grid Immuno-Dot Blotting.

The IgE-binding assay was done as described in Materials and Methods. For this dot test, about 1 µg of 30 kDa palm pollen glycoprotein, *Ela g 31 K*, was dotted onto the nitrocellulose membrane. The IgE bound to the 30-kDa palm pollen glycoprotein (*Ela g Bd 31 K*) was detected by the alkaline phosphatase conjugated secondary antibody against human IgE. Human IgE was used for the positive control and bovine serum albumin was used for the negative control. F means female patients with the oil palm pollen allergy and M means male patients.

reveal the structural features of the antigenic *N*-glycans of the palm pollen glycoproteins, we analyzed the structures of *N*-glycans of total glycoproteins extracted from the oil palm pollens by a combination of some exoglycosidase digestions. The *N*-glycans was liberated from the total glycoproteins by hydrazinolysis and labeled with 2-aminopyridine. As shown in Fig. 4-I, nine PA-sugar chains (A ~ I) were separated on SF-HPLC using the Asahipak NH2 column. All PA-sugar chains were not digested by *Streptococcus* 6646 K β -galactosidase, suggesting that the Lewis a type structural unit (Gal β 1-3(Fuc α 1-4)GlcNAc-) is absent in the palm pollen glycoproteins (Fig. 4-II).

Elution positions of Peaks A, B, C, and D corresponded to those of Man₃Xyl₁GlcNAc₂-PA, Man₃Xyl₁Fuc₁GlcNAc₂-PA, GlcNAc₁ Man₃Xyl₁Fuc₁GlcNAc₂-PA, and GlcNAc₂ Man₃Xyl₁Fuc₁GlcNAc₂-PA, respectively. By the diplococcal β -*N*-acetylglucosaminidase digestion, peaks D and E disappeared and the relative height of peak B, of which elution position corresponded to Man₃Xyl₁Fuc₁GlcNAc₂-PA, increased significantly (Fig. 4-III). This observation suggested that peaks D and E have the β 1-2 GlcNAc residue(s) bound to the core structure of Man₃Xyl₁Fuc₁GlcNAc₂. Furthermore, peak E was not digested by the jack bean α -mannosidase, suggesting that this PA-sugar chain bears no α -mannosyl residue at the non-reducing end (Fig. 4-V). On the contrary, Peak B disappeared and a new product (arrow b), which corresponded to Man₁Xyl₁Fuc₁GlcNAc₂, appeared by the jack bean α -mannosidase digestion, suggesting that Peak B have two α -mannosyl residues.

Elution positions of Peaks C, F, G, H, and I corresponded to those of Man₅GlcNAc₂-PA, Man₆GlcNAc₂-PA, Man₇GlcNAc₂-PA, Man₈-

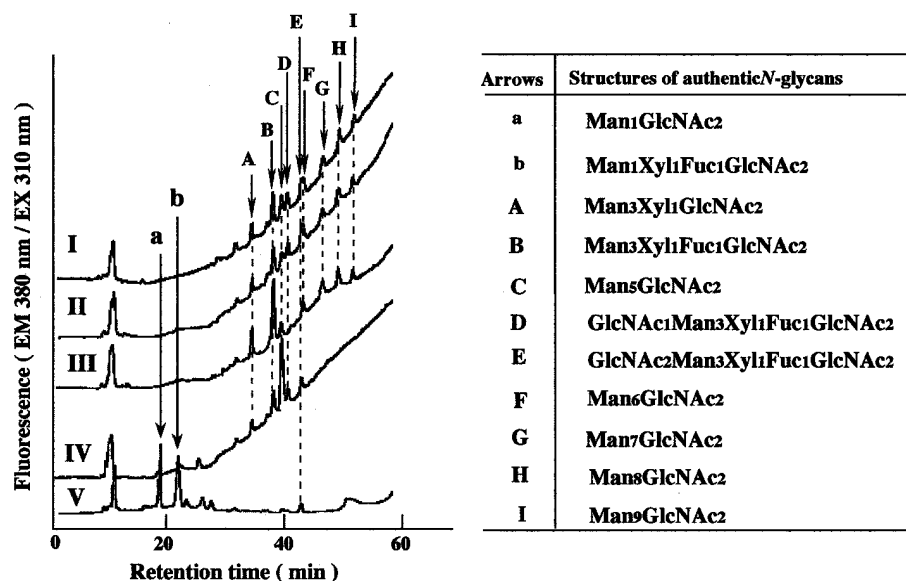


Fig. 4. Exoglycosidase Digestions of PA-Sugar Chains Obtained from Total Palm Pollen Glycoproteins.

I, PA-Sugar chains obtained from total glycoproteins in oil palm pollens.

II, *Streptococcus* 6646 K β -galactosidase digestion of I.

III, *Diplococcus* β -*N*-acetylglucosaminidase digestion of I.

IV, *Aspergillus* α -1,2-mannosidase digestion of I.

V, Jack bean α -mannosidase digestion of I.

Each PA-sugar chain was separated on the column of Asahipak NH2P-50 column (Showa Denko), using the acetonitrile-water solvent system. Arrows indicate the elution positions of authentic PA-sugar chains, of which structures are shown in the right table.

GlcNAc₂-PA, and Man₉GlcNAc₂-PA, respectively. By the *Aspergillus* α -1,2-mannosidase digestion, (Fig. 4-IV) Peaks F, G, H, and I disappeared and the relative height of Peak C increased significantly, suggesting these PA-sugar chains could be the typical high-mannose type *N*-glycans with the core structure of Man α 1-6(Man α 1-3)Man α 1-6(Man α 1-3)Man β 1-4GlcNAc β 1-4GlcNAc. Furthermore, the jack bean α -mannosidase converted the product, which corresponded to Man₅GlcNAc₂-PA, to Man₁GlcNAc₂-PA (arrow a in Fig. 4-V).

The relative amounts of each *N*-glycan obtained from the palm pollen glycoproteins are summarized in Table 1. The plant complex type structure occupies about 47% of total *N*-glycans of the palm pollen glycoproteins. At this time, it is still obscure which structure of the plant complex type *N*-glycan is linked to the 31-kDa palm pollen allergen, Ela g Bd 31 K, however, in this report it has been confirmed that the antigenic plant *N*-glycans do occur on some glycoproteins (maybe including Ela g Bd 31 K) of the oil palm pollen as well as allergenic grass pollens²⁶⁾ or *Ginkgo biloba* pollen,²⁷⁾ which is another allergenic pollen. More detailed molecular characterization of Ela g Bd 31 K, such as determinations of the primary structure of both the protein moiety and the carbohydrate moiety, are in progress.

To clarify the involvement of antigenic plant complex type *N*-glycans in human allergic or immune responses, the inhibitory activity of the free plant complex type *N*-glycans or the anti- β 1-2xylose an-

Table 1. Summary of *N*-Glycans of Palm Pollen Glycoproteins

Possible Structures	Relative amount (%)
GlcNAc ₂ Man ₃ Xyl ₁ Fuc ₁ GlcNAc ₂	11
GlcNAc ₁ Man ₃ Xyl ₁ Fuc ₁ GlcNAc ₂	9
Man ₃ Xyl ₁ Fuc ₁ GlcNAc ₂	15
Man ₃ Xyl ₁ GlcNAc ₂	12
Man ₉ GlcNAc ₂	9
Man ₈ GlcNAc ₂	12
Man ₇ GlcNAc ₂	10
Man ₆ GlcNAc ₂	12
Man ₅ GlcNAc ₂	10

tibody used in this report against the IgE binding to the palm pollen glycoprotein (Ela g Bd 31 K) must be assayed in the next step for the detailed analysis of allergenicity of the putative glycoallergen.

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