

## Occurrence of Lewis a Epitope in *N*-Glycans of a Glycoallergen, Jun a 1, from Mountain Cedar (*Juniperus ashei*) Pollen

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We have determined the structures of *N*-glycans linked to major allergens in the mountain cedar (*Juniperus ashei*) pollen, Jun a 1. First, two kinds of the pollen glycoallergen (Jun a 1-A and Jun a 1-B) were purified from partially purified Jun a 1 by cation exchange chromatography. The *N*-glycans were liberated by hydrazinolysis from the two glycoallergens and the resulting sugar chains were *N*-acetylated and then coupled with 2-aminopyridine. Three pyridylaminated sugar chains were purified by reversed-phase HPLC and size-fractionation HPLC from Jun a 1-A and Jun a 1-B respectively. The structures were determined by a combination of *exo*- and *endo*-glycosidase digestions, two dimensional sugar chain mapping, and electrospray ionization mass spectrometry (ESI-MS) analysis. Structural analysis indicated that Lewis a epitope (Gal $\beta$ 1–3(Fuc $\alpha$ 1–4)GlcNAc $\beta$ 1–) occurs in the *N*-glycans of the pollen allergens.

**Key words:** *N*-glycan; Lewis a epitope; pollen allergen; mountain cedar pollen; *Juniperus ashei*

Recently, it has been found that many allergens from pollens or other plant materials are *N*-glycosylated and that the *N*-glycans linked to such glycoallergens often belong to antigenic plant complex type structures consisting of  $\beta$ 1–2 xylose and/or  $\alpha$ 1–3 fucose residues.<sup>1–4)</sup> However, it is still obscure whether such antigenic *N*-glycans are directly involved in pollen or plant food allergies. In our previous report,<sup>5)</sup> we found that the free plant complex type *N*-glycans (GlcNAc<sub>2</sub>-Man<sub>3</sub>Xyl<sub>1</sub>Fuc<sub>1</sub>GlcNAc<sub>2</sub>, Man<sub>3</sub>Xyl<sub>1</sub>Fuc<sub>1</sub>GlcNAc<sub>2</sub>) did

not inhibit the binding of a pollen glycoallergen (Cry j 1) to IgEs from the pollinosis patients. This result suggested that antigenic *N*-glycans themselves must not be major epitopes for the antibodies but the antibodies may recognize a conformation constructed by the peptide moiety around the glycosylation site and the *N*-glycan. Furthermore, we have found that such antigenic *N*-glycan in free form can suppress the production of IL 4 from T-cells of pollinosis patients, which had previously been stimulated by the allergen. These observations suggest that the antigenic *N*-glycans of many pollen allergens can be a candidate of glyco-drugs for pollinosis therapy.<sup>5)</sup>

Fichette-Lainé *et al.* first reported that Lewis a antigen unit (Gal $\beta$ 1–3(Fuc $\alpha$ 1–4)GlcNAc $\beta$ –) occurs in *N*-glycans of plant glycoproteins.<sup>6)</sup> In fact, it has been reported that an allergen of Japanese cedar pollens, Cry j 1, also contains  $\beta$ -galactosyl and  $\alpha$ -fucosyl residues at the non-reducing end of the biantennary plant complex type structure (GlcNAc<sub>2</sub>Man<sub>3</sub>Xyl<sub>1</sub>Fuc<sub>1</sub>GlcNAc<sub>2</sub>),<sup>3,4)</sup> but the structure of *N*-glycans linked to another pollen allergen, Jun a 1, from mountain cedar (*Juniperus ashei*) pollens remains to be determined. Midoro-Horiuti *et al.*, have cloned a cDNA of Jun a 1 and found that Jun a 1 possessed a high level of amino acid sequence homology with Cry j 1.<sup>7)</sup> In this report, therefore, we analyzed the structures of *N*-glycans linked to Jun a 1 to confirm the ubiquitous occurrence of the Lewis a epitope in plant *N*-glycans. The structural analysis clearly indicated that the Lewis a epitope occurs in the *N*-glycan moiety of mountain cedar pollen allergen, Jun a 1.

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**Abbreviations:** PA-, pyridylamino; RP-HPLC, reverse-phase HPLC; SF-HPLC, size-fractionation HPLC; ESI-MS, electrospray ionization mass spectrometry; FPLC, fast protein liquid chromatography; Hex, hexose; HexNAc, *N*-acetylhexosamine; Deoxhex, deoxyhexose; Pen, pentose; M3FX, Man $\alpha$ 1–6(Man $\alpha$ 1–3)(Xyl $\beta$ 1–2)Man $\beta$ 1–4GlcNAc $\beta$ 1–4(Fuc $\alpha$ 1–3)GlcNAc-PA; MFX, Xyl $\beta$ 1–2Man $\beta$ 1–4GlcNAc $\beta$ 1–4(Fuc $\alpha$ 1–3)GlcNAc-PA; GN<sup>N</sup>M3FX, 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; GN2M3FX, GlcNAc $\beta$ 1–2Man $\alpha$ 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

## Materials and Methods

**Materials.** An Asahipak NH2P-50 column ( $0.46 \times 25$  cm) was purchased from Showa Denko (Tokyo, Japan), and a Cosmosil 5C18-AR column ( $0.6 \times 25$  cm) from Nacalai Tesque (Kyoto, Japan). GN2M3FX and M3FX were prepared from the glycoproteins of oil palm pollens and ricin D.<sup>8,9)</sup> GN3M3FX was prepared from glycoproteins of oil palm pollens<sup>9)</sup> and *Ginkgo biloba* pollens.<sup>10)</sup>  $\alpha$ -Mannosidase (Jack bean) and  $\beta$ 1-3/6 specific  $\beta$ -galactosidase (recombinant expressed in *E. coli*) were purchased from Sigma (St. Louis, MO, U.S.A.).  $\beta$ -N-acetylglucosaminidase (*Diplococcus pneumoniae*) was from Boehringer (Mannheim, Germany).  $\alpha$ 1-3/4 Specific  $\alpha$ -fucosidase (*Streptomyces* sp. 142) and Lacto-N-biosidase (*Streptomyces* sp. 142) were from Takara (Kyoto, Japan).

**Purification of Jun a 1.** The partially purified Jun a 1, which was prepared by the method of Midoro-Horiuti *et al.*,<sup>11)</sup> were further purified by a cation exchange FPLC using a Mono S column (Amersham Pharmacia Biotech, Upsala, Sweden). The absorbed proteins were eluted by a linear gradient of NaCl from 0 M to 0.5 M in 10 mM Na-acetate buffer, pH 4.0. Although two major components (fractions A and B) were separated on the column, as shown in Fig. 1-I, these components showed almost the same molecular weight (about 43 kDa) on SDS-PAGE (Fig. 1-II). Since these fractions were recognized by an antiserum against Jun a 1, for structural analysis of *N*-glycans, we used these two proteins (Jun a 1-A for

fraction A and Jun a 1-B for fraction B) separately.

**Preparation of pyridylaminated *N*-glycans from Jun a 1-A and Jun a 1-B.** *N*-Glycans were released by hydrazinolysis (100°C, 12 h, in 200  $\mu$ l of anhydrous hydrazine) from the lyophilized glycoallergens (about 700  $\mu$ g of Jun a 1-A and 980  $\mu$ g of Jun a 1-B). After *N*-acetylation of the hydrazinolysate with saturated ammonium bicarbonate (400  $\mu$ l) and acetic anhydride (20  $\mu$ l), the acetylated hydrazinolysate was desalted using Dowex 50  $\times$  2 resins. Pyridylation of the sugar chains was done by the method of Natsuka and Hase.<sup>12)</sup> 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 Shodex Asahipak NH2P-50 column ( $0.46 \times 25$  cm) and the Cosmosil 5C18-AR column ( $0.6 \times 25$  cm). On the Cosmosil 5C18-AR column, the PA-sugar chains were eluted by increasing the acetonitrile concentration in 0.05% TFA linearly from 0 to 10% at a flow rate of 1.2 ml/min. In the case of size-fractionation HPLC using the Asahipak NH2P-50 column, the PA-sugar chains were eluted by increasing the water content in the water-acetonitrile mixture from 36% to 62% linearly in 60 min at a flow rate of 0.7 ml/min.

**Electrospray ionization (ESI) mass spectrometry.** ESI-MS analysis of PA-sugar chains was done as described in our previous reports,<sup>13,14)</sup> using a Perkin Elmer Sciex API-III triple-quadrupole mass spectrometer with an atmospheric-pressure ionization ion source.

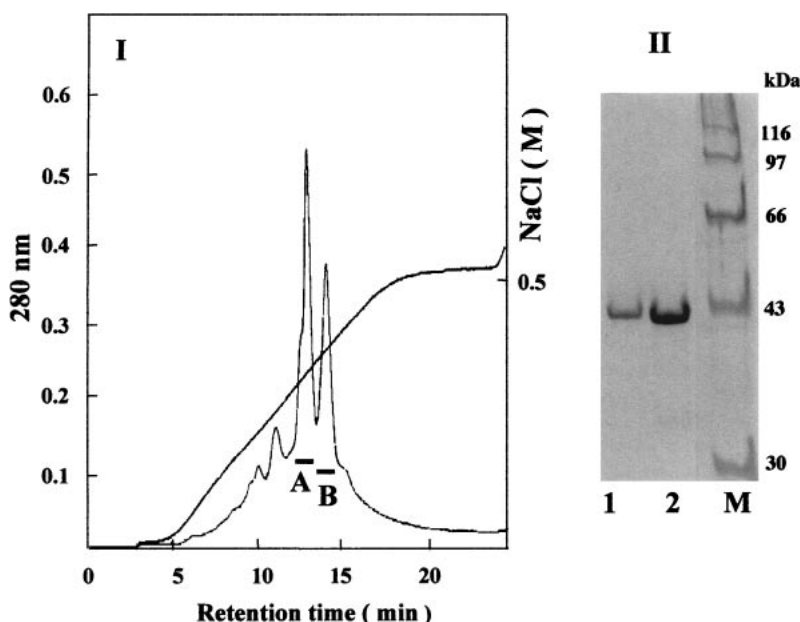


**Fig. 1.** Mono S-FPLC Profile of Partially Purified Jun a 1.

I, FPLC Profile of Partially Purified Jun a 1. The partially purified Jun a 1 was loaded on a Mono-S column in 10 mM Na-acetate buffer, pH 4.0. The allergen was eluted by a linear gradient of NaCl from 0 M to 0.5 M in 10 mM Na-acetate buffer, pH 4.0. II, SDS-PAGE of Fraction A (Jun a 1-A) and Fraction B (Jun a 1-B) under the reducing condition. Lane 1, Fraction A; Lane 2, Fraction B; M, marker proteins: 1,  $\beta$ -galactosidase (116 kDa); 2, phosphorylase b (97 kDa); 3, bovine serum albumin (BSA, 66 kDa); 4, ovalbumin (43 kDa); 5, carbonic anhydrase (30 kDa).

**Glycosidase digestions of PA-sugar chains.** Digestions with jack bean  $\alpha$ -mannosidase, diplococcal  $\beta$ -N-acetylglucosaminidase, and Lacto-N-biosidase were done using about 200 pmol of the PA-sugar chains under the conditions described in our previous reports.<sup>8-10</sup> Digestion with the  $\alpha$ -fucosidase was done using 200 pmol in 25 mM Na-citrate buffer, pH 5.0 for 16 h. The resulting glycosidase-digests were analyzed by SF-HPLC using the Asahipak NH2P-50 column ( $0.46 \times 25$  cm).

## Results and Discussion

### Purification of PA-sugar chains

First, the PA-sugar chains from Jun a 1-A and Jun a 1-B were partially purified by RP-HPLC as shown in Fig. 2-I. The PA-sugar chains were pooled as indicated by horizontal bars (-A and -B). When some other peaks observed on the chromatograms were analyzed by SF-HPLC, almost all peaks were recovered in the run-through fraction. Hence, we judged that these peaks were not N-glycans. As shown in Fig. 2-II, three PA-sugar chains (A, B, and C) from Jun a 1-A and three PA-sugar chains from (D, E, F) Jun a 1-B were purified on the Shodex Asahipak NH2P-50 column. The elution

positions of A and E corresponded to those of GN2M3FX on RP-HPLC and SF-HPLC. Although the elution positions of B and F were the same as each other on RP-HPLC and SF-HPLC, they did not correspond to any available authentic PA-sugar chain. Furthermore, the elution positions of PA-sugar chain C on the RP-HPLC and SF-HPLC also did not correspond to any available authentic PA-sugar chain.

### Structures of PA-sugar chains A and E

Since the elution positions of PA-sugar chains A and E corresponded to those of GN2M3FX on RP-HPLC and SF-HPLC, the structures of A and E could be estimated with the biantennary plant complex type structure,  $\text{GlcNAc}_2\text{Man}_3\text{Xyl}_1\text{Fuc}_1\text{GlcNAc}_2$ . The ESI-MS analysis showed a single signal at  $m/z$  836.5  $[(M + 2H)^{2+}]$ , suggesting that these PA-sugar chains consist of  $(\text{HexNAc})_3(\text{Hex})_3(\text{Deoxyhex})_1(\text{Pen})_1(\text{HexNAc-PA})$  or  $\text{GlcNAc}_2\text{Man}_3\text{Xyl}_1\text{Fuc}_1\text{GlcNAc}_2\text{-PA}$ . The deduced structure was further confirmed by exoglycosidase digestion. These PA-sugar chains were converted to M3FX with diplococcal  $\beta$ -N-acetylglucosaminidase, suggesting that two GlcNAc residues were bound by  $\beta$ 1-2 linkage (Fig. 3-II). The product was further converted to MFX by jack bean  $\alpha$ -mannosidase diges-

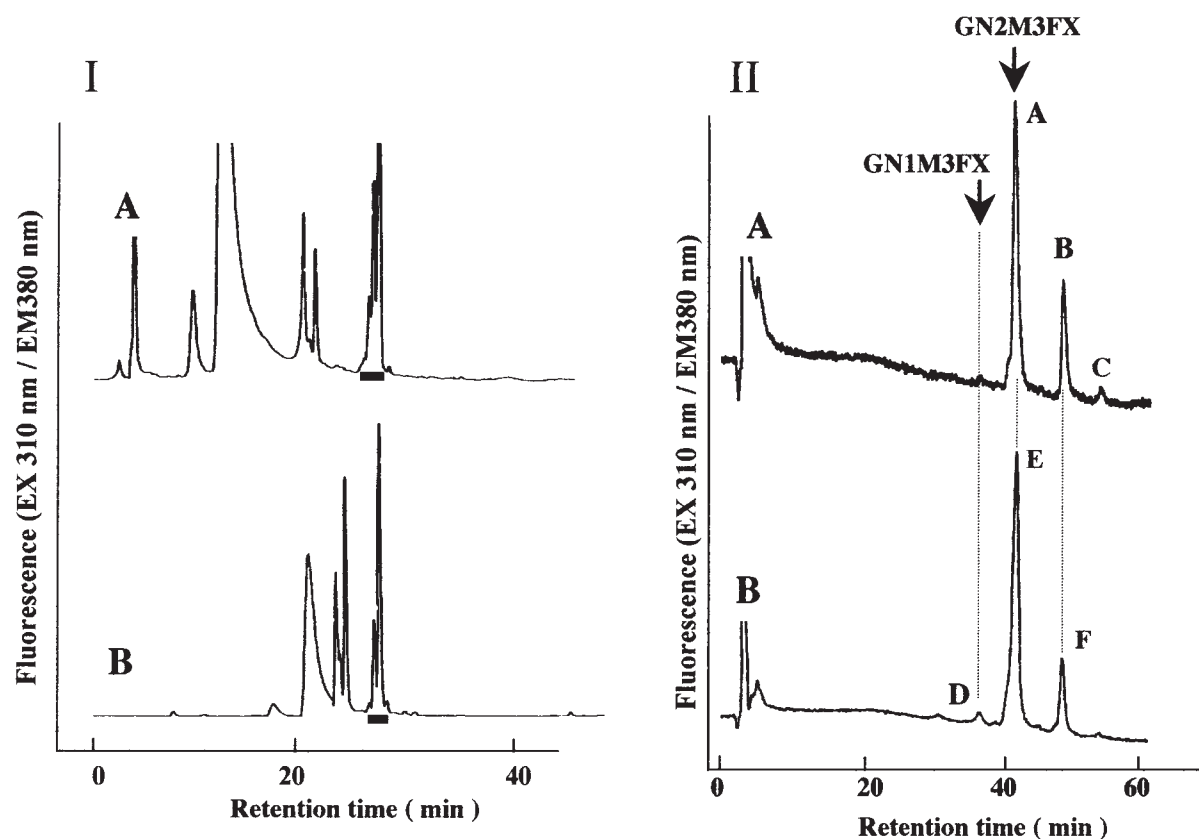


**Fig. 2.** HPLC Profiles of PA-Sugar Chains from Jun a 1-A and Jun a 1-B.

I, RP-HPLC of PA-sugar chains from Jun a 1-A (A) and Jun a 1-B (B). The PA-sugar chains were pooled as indicated by horizontal bars. II, SF-HPLC of PA-sugar chains pooled in I. A, PA-sugar chains from Jun a 1-A. B, PA-sugar chains from Jun a 1-B. GN2M3FX and GN1M3FX indicate the elution positions of authentic PA-sugar chains,  $\text{GlcNAc}_2\text{Man}_3\text{Xyl}_1\text{Fuc}_1\text{GlcNAc}_2\text{-PA}$  (GN2M3FX) and  $\text{GlcNAc}_1\text{Man}_3\text{Xyl}_1\text{Fuc}_1\text{GlcNAc}_2\text{-PA}$  (GN1M3FX) respectively.

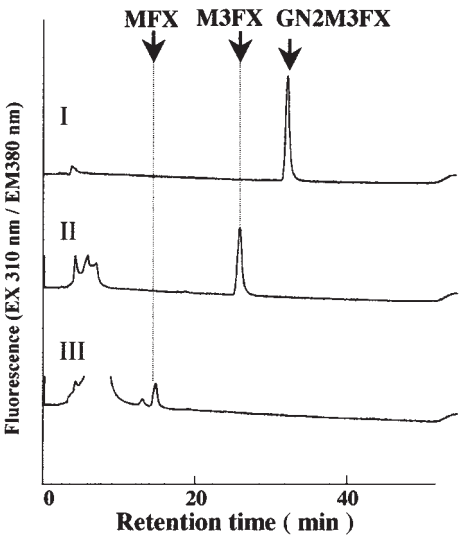


**Fig. 3.** SF-HPLC of Exoglycosidase Digests of PA-Sugar Chains A and E.  
I, PA-sugar chains A and E; II, the diplococcal  $\beta$ -N-acetylglucosaminidase digest of I; III, the jack bean  $\alpha$ -mannosidase digest of II. Arrows (GN2M3FX, M3FX, and MFX) indicate the elution positions of authentic PA-sugar chains, GlcNAc<sub>2</sub>Man<sub>3</sub>Xyl<sub>1</sub>Fuc<sub>1</sub>-GlcNAc<sub>2</sub>-PA, Man<sub>3</sub>Xyl<sub>1</sub>Fuc<sub>1</sub>GlcNAc<sub>2</sub>-PA, and Man<sub>1</sub>Xyl<sub>1</sub>Fuc<sub>1</sub>GlcNAc<sub>2</sub>-PA respectively.

tion (Fig. 3-III). The data of 2-D sugar chain mapping, exoglycosidase digestion, and ESI-MS analysis suggested that the structure of PA-sugar chains A and E are GN2M3FX as shown in Scheme 1.

*Structures of PA-sugar chains B and F*

ESI-MS analysis showed a single signal at  $m/z$  990.5  $[(M + 2H)^{2+}]$ , suggesting that these PA-sugar chains consist of (Hex)<sub>4</sub>(HexNAc)<sub>3</sub>(Deoxyhex)<sub>2</sub>(Pen)<sub>1</sub>(HexNAc-PA) or Gal<sub>1</sub>Fuc<sub>1</sub>GlcNAc<sub>2</sub>Man<sub>3</sub>Xyl<sub>1</sub>Fuc<sub>1</sub>GlcNAc<sub>2</sub>-PA (Fig. 4-I). As shown in Fig. 5-II, the PA-sugar chains could be digested with the  $\alpha$ 1-3/4 fucosidase, suggesting that the fucosyl residue is bound by  $\alpha$ 1-3 or  $\alpha$ 1-4 linkage. When the product was treated with diplococcal  $\beta$ -galactosidase, galactosyl residue was not released (Fig. 5-III). On the contrary, the  $\alpha$ -fucosidase digest of PA-sugar chains B and F was digested by the  $\beta$ 1-3/6 specific galactosidase, as shown in Fig. 5-IV, and the elution position of the product corresponded to that of GN2M3FX. These results indicated that the galactosyl residue was bound to the GlcNAc residue by  $\beta$ 1-3 or  $\beta$ 1-6 linkage. The degalactosylated product was converted to M3FX by diplococcal  $\beta$ -N-acetylglucosaminidase digestion (Fig. 5-V), suggesting that two GlcNAc residues were bound to the M3FX structure by  $\beta$ 1-2 linkage. To determine the linkage mode of the galactosyl residue, the product obtained by the  $\alpha$ -fucosidase digestion of PA-sugar chain B and F (Fig. 5-II) was treated with lacto *N*-biosidase, which released the Gal $\beta$ 1-3GlcNAc unit but not the Gal $\beta$ 1-4GlcNAc unit from the complex type *N*-glycans.<sup>15)</sup> As shown in Fig. 5-VI, the product obtained by endoglycosidase digestion was eluted at the elution position of GN1M3FX,<sup>10)</sup> indicating that the Gal $\beta$ 1-3GlcNAc unit was released by endoglycosidase. From these results of *exo*- and *endo*-glycosidase digestions, it has been found that the fucosyl and galactosyl

| Proposed Structures                                                                                                                                                                                                                                                                                                        | Peak Name | Jun a 1-A | Jun a 1-B |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------|-----------|-----------|
| <div><div>GlcNAc<math>\beta</math>1-2 Man<math>\alpha</math>1-6</div><div>Man<math>\beta</math>1-4GlcNAc<math>\beta</math>1-4GlcNAc</div><div>Man<math>\alpha</math>1-3 2 3</div><div>Xyl<math>\beta</math>1 Fuc <math>\alpha</math>1</div></div>                                                                          | D         | nd        | 3%        |
| <div><div>GlcNAc<math>\beta</math>1-2 Man<math>\alpha</math>1-6</div><div>Man<math>\beta</math>1-4GlcNAc<math>\beta</math>1-4GlcNAc</div><div>GlcNAc<math>\beta</math>1-2 Man<math>\alpha</math>1-3 2 3</div><div>Xyl<math>\beta</math>1 Fuca1</div></div>                                                                 | A<br>E    | 75%       | 76%       |
| <div><div>Fuca1-4<br/>Gal<math>\beta</math>1-3 { GlcNAc<math>\beta</math>1-2Man<math>\alpha</math>1-6</div><div>Man<math>\beta</math>1-4GlcNAc<math>\beta</math>1-4GlcNAc</div><div>GlcNAc<math>\beta</math>1-2Man<math>\alpha</math>1-3 2 3</div><div>Xyl<math>\beta</math>1 Fuca1</div></div>                            | B<br>F    | 23%       | 21%       |
| <div><div>Fuca1</div><div>Gal<math>\beta</math>1-3GlcNAc<math>\beta</math>1-2Man<math>\alpha</math>1-6</div><div>Man<math>\beta</math>1-4GlcNAc<math>\beta</math>1-4GlcNAc</div><div>Gal<math>\beta</math>1-3GlcNAc<math>\beta</math>1-2Man<math>\alpha</math>1-3 2 3</div><div>4 Xyl<math>\beta</math>1 Fuca1</div></div> | C         | 2%        | nd        |

nd: not detected.

**Scheme 1.** The Proposed Structures of *N*-Glycans Linked to Jun a 1-A and Jun a 1-B.

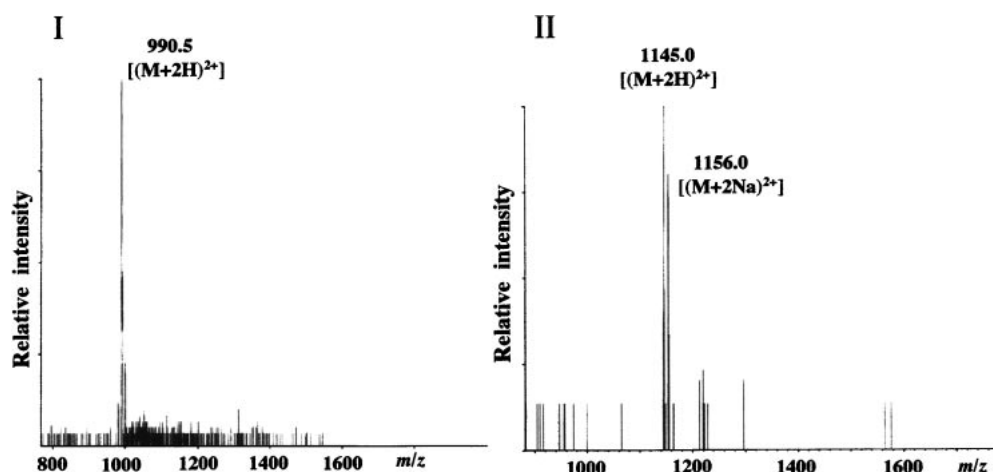


Fig. 4. ESI-MS Spectra of PA-Sugar Chains B and C.

PA-Sugar chains B and C were observed as a double charged ion,  $[M + 2H]^{2+}$ . A sodium adduct ion  $[(M + 2Na)^{2+}]$  was observed for PA-sugar chain C.

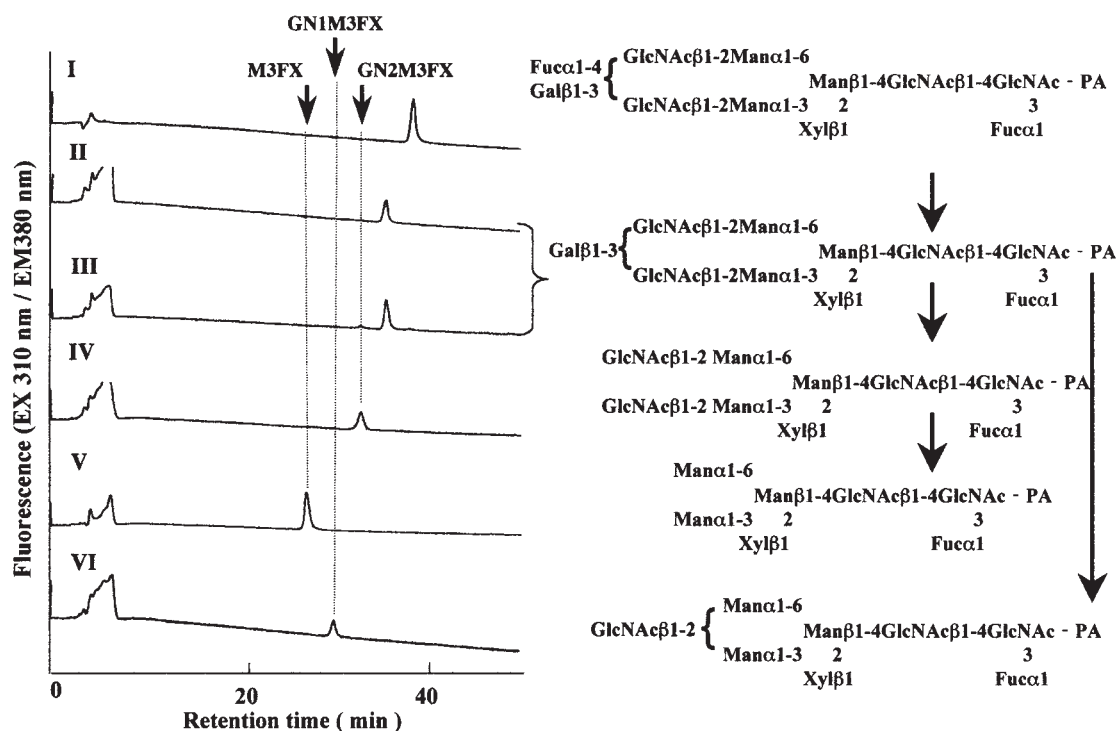


Fig. 5. SF-HPLC of *Exo*- and *Endo*-Glycosidase Digests of PA-Sugar Chains B and E.

I, PA-sugar chains B and E; II, the  $\alpha 1-3/4$  specific *Streptomyces*  $\alpha$ -fucosidase digest of I; III, the diplococcal  $\beta$ -galactosidase digest of II; IV, the  $\beta 1-3/6$  specific galactosidase digest of III; V, the diplococcal  $\beta$ -N-acetylglucosaminidase digest of IV; VI, the lacto *N*-biosidase digest of II.

residues were bound to the outer GlcNAc residue by  $\alpha 1-4$  linkage and  $\beta 1-3$  linkage respectively. However, only with these results, it appears no to be enough to determine which mannosyl residue ( $\alpha 1-6$  arm Man or  $\alpha 1-3$  arm Man) harbors the Lewis a epitope (Gal $\beta 1-3$  (Fuc $\alpha 1-4$ )GlcNAc $\beta 1-$ ). Therefore, in Scheme 1, a possible structure of PA-sugar chains B and F is shown.

#### Structures of PA-sugar chain C

ESI-MS analysis showed two signals at  $m/z$  1145.0

$[(M + 2H)^{2+}]$  and 1156  $[(M + 2Na)^{2+}]$ , suggesting that these PA-sugar chains consist of (Hex) $_5$ (HexNAc) $_3$ -(Deoxyhex) $_3$ (Pen) $_1$ (HexNAc-PA) or Gal $_2$ Fuc $_2$ GlcNAc $_2$ -Man $_3$ Xyl $_1$ Fuc $_1$ GlcNAc $_2$ -PA (Fig. 4-[II]). When this sugar chain was treated with  $\alpha 1-3/4$  fucosidase, fucosyl residues were released (Fig. 6-II), suggesting that the fucosyl residue(s) were bound to the outer GlcNAc residue by  $\alpha 1-3$  or  $\alpha 1-4$  linkage. When the  $\alpha$ -fucosidase digest was treated with diplococcal  $\beta$ -galactosidase, the galactosyl residue was not released (Fig. 6-III). On the

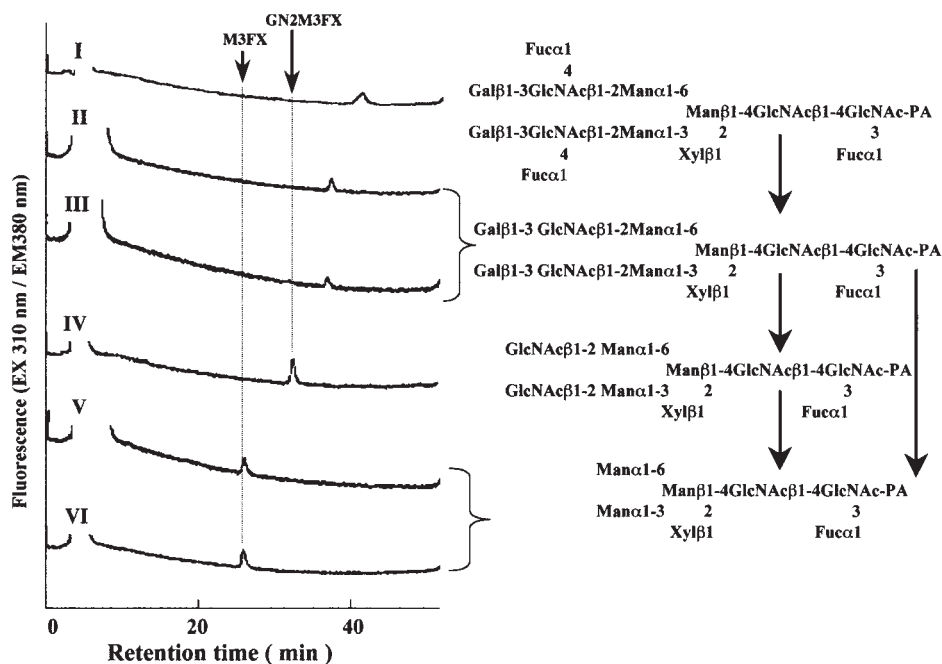


Fig. 6. SF-HPLC of *Exo*- and *Endo*-Glycosidase Digests of PA-Sugar Chain C.

I, PA-sugar chain C; II, the  $\alpha$ 1-3/4 specific *Streptomyces*  $\alpha$ -fucosidase digest of I; III, the diplococcal  $\beta$ -galactosidase digest of II; IV, the  $\beta$ 1-3/6 specific galactosidase digest of III; V, the diplococcal  $\beta$ -*N*-acetylglucosaminidase digest of IV; VI, the lacto *N*-biosidase digest of II.

contrary, the galactosyl residue(s) were released from the  $\alpha$ -fucosidase digest (Fig. 6-II) by  $\beta$ 1-3/6 specific galactosidase digestion, as shown in Fig. 6-IV, and the elution position of the product corresponded to that of GN2M3FX, suggesting that two galactosyl residues were released. These results indicated that the galactosyl residues were bound to GlcNAc residue by  $\beta$ 1-3 or  $\beta$ 1-6 linkage. The de-galactosylated product was converted to M3FX by diplococcal  $\beta$ -*N*-acetylglucosaminidase digestion (Fig. 6-V), suggesting that two GlcNAc residues were bound to the M3FX structure by  $\beta$ 1-2 linkage. To determine the linkage mode of the galactosyl residue, the product obtained by  $\alpha$ -fucosidase digestion (Fig. 6-II) was treated with lacto *N*-biosidase. As shown in Fig. 6-VI, the product obtained by the endoglycosidase digestion was eluted at the elution position M3FX, indicating that two Gal $\beta$ 1-3GlcNAc units were released by the endoglycosidase. From these results of *exo*- and *endo*-glycosidase digestions, the structure of PA-sugar chain C is proposed as shown in Scheme 1, indicating that two units of Lewis a epitope occur in the *N*-glycan.

#### Structure of PA-sugar chain D

Concerning the structure of D, GlcNAc1Man3Xyl1-Fuc1GlcNAc2-PA (GN1M3FX) 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 (<sup>GN</sup>M3FX), and the other 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 (<sup>GN</sup>M3FX). It has been found that on the ODS column the former structure is eluted before

GN2M3FX, and the latter structure is eluted after GN2M3FX. The PA-sugar chain D was eluted slightly after GN2M3FX (PA-sugar chain B) on the ODS column and the elution position of PA-sugar chain D on RP- and SF-HPLC corresponded to those of authentic <sup>GN</sup>M3FX (data not shown).<sup>10</sup> When this PA-sugar chain was treated with diplococcal  $\beta$ -*N*-acetylglucosaminidase, one GlcNAc residue was released (Fig. 7-II). The product was further converted to MFX by jack bean  $\alpha$ -mannosidase digestion (Fig. 7-III). From these results, the structure of PA-sugar chain D is proposed as shown in Scheme 1.

#### Structural feature of *N*-glycans linked to the mountain cedar pollen allergen, Jun a 1

As shown in Scheme 1, we have found that the cedar pollen allergen, Jun a 1, carries plant complex type *N*-glycans containing the Lewis a antigen (Gal $\beta$ 1-3(Fuc $\alpha$ 1-4)GlcNAc $\beta$ 1-) unit. Although we have purified two isomers of Jun a 1 (Jun a 1-A and Jun a 1-B) by the cation exchange HPLC, we confirmed that both Jun a 1 molecules harbored Lewis a epitope. In contrast, the complex type *N*-glycans having two Lewis a epitope units (PA-sugar chain C) was found only in Jun a 1-A, and the GN1M3FX structure (PA-sugar chain D) was found only in Jun a 1-B respectively. These observation suggest that the composition of *N*-glycans of Jun a 1-A and Jun a 1-B might be slightly different from each other. Although the elution position of Jun a 1-A on the cation exchange column was different from that of Jun a 1-B (suggesting that the number of glycosylation sites might be different from each other), the molecular

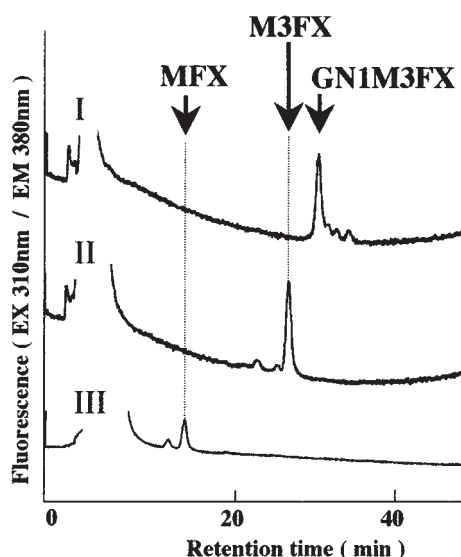


**Fig. 7.** SF-HPLC of Exoglycosidase Digests of PA-Sugar Chain D. I, PA-sugar chain D; II, the diplococcal  $\beta$ -N-acetylglucosaminidase digest of I; III, the jack bean  $\alpha$ -mannosidase digest of II. Arrows (GN1M3FX, M3FX, and MFX) indicate the elution positions of authentic PA-sugar chains, GlcNAc1Man3Xyl1Fuc1GlcNAc2-PA (GN1M3FX), Man3Xyl1Fuc1GlcNAc2-PA (M3FX), and Man1Xyl1Fuc1GlcNAc2-PA (MFX) respectively.

weights of these two Jun a 1 molecules were almost the same, as shown in Fig. 1. Therefore, it seems that the number of glycosylation sites is not be different. From the deduced amino acid sequence of Jun a 1,<sup>7)</sup> two potential glycosylation sites have been predicted, but at this moment, it is still obscure whether both asparagine residues are glycosylated.

Plant complex type N-glycans containing the Lewis a epitope have been found in various plant glycoproteins, including the sycamore laccase,<sup>7)</sup> storage glycoproteins,<sup>16)</sup> and a carleticulin from *L. tulipifera*.<sup>17)</sup> The structures of N-glycans linked to Cry j 1, one of the Japanese cedar pollen allergens, have already been reported.<sup>3,4)</sup> In these reports, however, the structures of N-glycans of the glycoallergen (Cry j 1) have been reported to have the Gal $\beta$ 1-4(Fuc $\alpha$ 1-6)GlcNAc $\beta$ 1- unit instead of the Lewis a epitope. Therefore, it appears that this is the first report to show the occurrence of the Lewis a epitope in the N-glycan moiety of pollen allergens. Although the physiological activity of the plant N-glycans harboring the Lewis a unit remains to be investigated, it might be worthwhile to assay some immunological activities for the human cellular immune system, since we have already found that the free M3FX can suppress the production of IL 4 from T-cells of pollinosis patients.<sup>5)</sup>

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