

Identification of the second major allergen of Japanese cedar pollen

M. SAKAGUCHI¹, S. INOUE¹, M. TANIAI², S. ANDO², M. USUI² & T. MATUHASHI³

¹Department of Microbiology, The Institute of Public Health, Tokyo, ²Hayashibara Biochemical Laboratories, Okayama, and ³Okinaka Memorial Institute of Medical Research, Tokyo, Japan

We isolated and characterized the second major allergen (*Cry j* II) from Japanese cedar pollen. We found that most patients with this pollinosis had IgE antibody to this protein in addition to IgE antibody to *Cry j* I; however, some sera reacted only with *Cry j* I or *Cry j* II. IgE-ELISA inhibition studies revealed that *Cry j* I and *Cry j* II had no cross-allergenicity. *Cry j* II did not react with anti-*Cry j* I monoclonal antibodies. In SDS-PAGE under a non-reducing condition, *Cry j* II showed a band at the 37 kDa position, compared with the 45-50 kDa bands of *Cry j* I. N-terminal amino acid sequence of *Cry j* II was completely different from that of *Cry j* I.

Key words: ELISA; IgE antibody; Japanese cedar pollen; major allergen; N-terminal amino acid sequence; pollinosis.

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Japanese cedar (Sugi; *Cryptomeria japonica*) pollinosis is one of the most important allergic diseases in Japan. The number of patients suffering from this disease is on the increase (3), and in some areas, more than 10% are affected (4).

Yassueda et al. (9) have purified a major allergen from Japanese cedar pollen, and designated it as Sugi basic protein (SBP). It was reported to be a basic protein with a molecular weight of 41-45 kDa.

Taniai et al. (8) described that purified SBP showed multiple bands (45-50; 37 kDa) in SDS-PAGE analyses under a non-reducing condition and that 20 amino acid residues of N-terminus of the 45-50 kDa proteins (*Cry j* I (5)) were identical to each other. They found that the five amino acid residues of the N-terminus of *Cry j* I were identical to those of mountain cedar which grows in North America and belongs to the same order (Pinales) as Japanese cedar (1, 8).

This paper describes the isolation and characterization of the 37 kDa protein, and presents evidence that the 37 kDa protein is the second major allergen (*Cry j* II) of Japanese cedar pollen.

MATERIAL AND METHODS

SBP was purified from Japanese cedar pollen according to the procedure described previously (8, 9). The obtained SBP was subjected to Mono S column (Pharmacia, Uppsala, Sweden) chromatography for further separation of proteins. Mouse anti-*Cry j* I monoclonal antibodies (Mabs) were prepared as described previously (2). N-terminal amino acid sequencing was performed by automated Edman degradation with a 470A sequencer (Applied Biosystems, CA, USA) after desalting by LiChrosorb DIOL column (Merck, NJ, USA) with a linear gradient of 90-0% acetonitrile/0.1% trifluoroacetic acid.

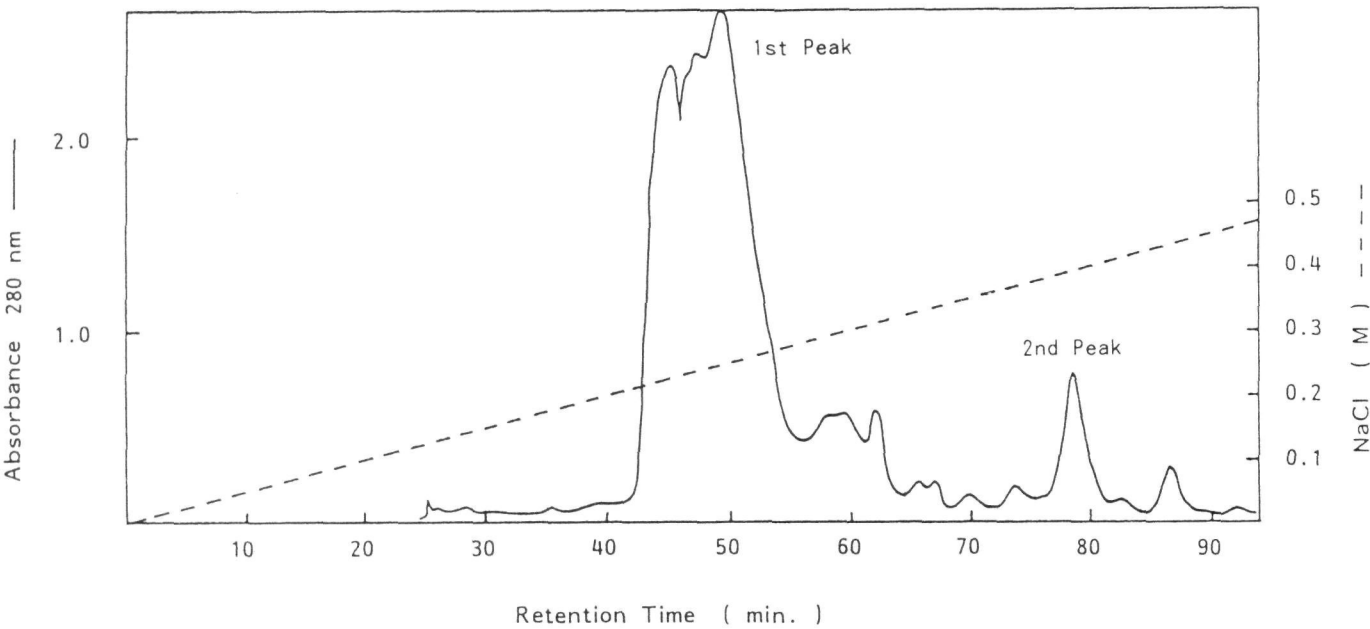


Fig. 1. Mono S column chromatogram of SBP. SBP dialysed against 10 mM acetate buffer, pH 5.0, was applied on Mono S column equilibrated with the same buffer, and eluted with linear gradient 0–1.0 M NaCl/10 mM acetate buffer, pH 5.0. The retention time of 1st peak fractions was from 43 to 54 min, and that of the 2nd peak fractions was from 77 to 80 min.

Fluorometric enzyme-linked immunosorbent assay (ELISA) for IgE antibody was performed as described previously (6). Briefly, 100 µl of the crude antigen (10 µg/ml) or purified proteins (1 µg/ml) were adsorbed to microplate wells. After incubation of sera with the solid-phase antigens, anti-human IgE antibody conjugated with β-D-galactosidase (Phar-

macia) was added to each well. As the enzyme reaction substrate, 4-methylumbelliferyl-β-D-galactoside (Sigma, MO, USA) was used. The fluorescence intensity of the reaction product, 4-methylumbelliferone, was read as fluorescence unit (FU) on a fluorometric microplate reader (Fluoroskan; Flow, VA, USA).

RESULTS AND DISCUSSION

The original SBP preparation showed a 37 kDa band in addition to multiple bands of 45–50 kDa in SDS-PAGE under a non-reducing condition [dithiothreitol (DTT)-] (8). Under a reducing condition (DTT +), however, the band of 37 kDa shifted to the position of 45 kDa. For isolation of this 37 kDa

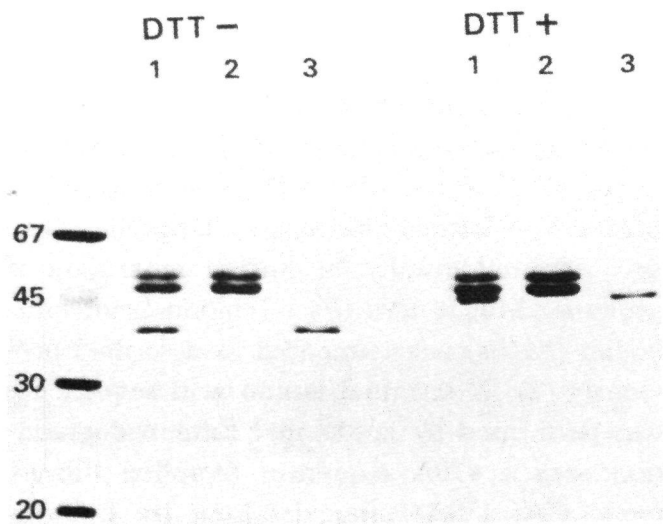


Fig. 2. SDS-PAGE (12.5 %) of SBP, *Cry j* I and *Cry j* II. No. 1, SBP (15 µg/well); No. 2, *Cry j* I (12 µg/well); No 3. *Cry j* II (2.5 µg/well).

Table 1		
N-terminal amino acid sequence of <i>Cry j</i> I and the 37 kDa protein (<i>Cry j</i> II)		
	1	10
<i>Cry j</i> I	NH2-Asp-Asn-Pro-Ile-Asp-Ser-? -Trp-Arg-Gly	
<i>Cry j</i> II	NH2-Ala-Ile-Asn-Ile-Phe-Asn-Val-Glu-Lys-Tyr	

Table 2
Allergenities of *Cry j* I and *Cry j* II

Allergen	Protein ^a conc. (μg/ml)	Patients														Non- allergics	
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Crude extract	10	1666 ^c	535	2142	575	750	506	875	1258	940	364	1250	1755	668	2557	0	0
<i>Cry j</i> I	1	2404	938	2196	1071	938	1132	1201	1815	80	0	1772	2339	1076	2716	0	0
<i>Cry j</i> II ^b	1	2090	1269	1986	876	532	676	701	2484	2440	2229	0	0	0	0	0	0

^a For coating on the microplate
^b *Cry j* II was further purified by rechromatography on Mono S column.
^c Results are expressed as FU. When we compared this ELISA using crude extract with RAST, a correlation coefficient of 0.86 was obtained. Relationship between ELISA FU and RAST score was as follows: RAST 1, 259–387 FU; RAST 2, 388–1121 FU; RAST 3, 1122–2757 FU; RAST 4, > 2758 FU.

protein, we used Mono S column chromatography with a linear gradient of NaCl (Fig. 1). The first peak fraction showed bands of 45-50 kDa (DTT ±) in SDS-PAGE (Fig. 2), and were identified as *Cry j* I by reaction with mouse anti-*Cry j* I Mabs. The second peak fraction showed a single protein band of 37 kDa (DTT-) or 45 kDa (DTT+) in SDS-PAGE. Probably, this protein has intrachain S-S bond(s). This fraction did not react with the anti-*Cry j* I Mabs. On iso-electric focusing, the first peak fraction showed multiple bands at pI 8.6-8.8, while the second peak fraction showed a band above pI 9.5. N-terminal amino acid sequence of this protein was found to differ from that of *Cry j* I (Table 1).

The allergenicity of the 37 kDa protein was examined by a fluorometric IgE-ELISA using sera from 14 patients with Japanese cedar pollinosis. Table 2 shows the results. Ten of 14 sera reacted with the 37 kDa protein and 12 of 14 sera reacted with *Cry j* I. Interestingly, some sera reacted only with the 37 kDa or *Cry j* I protein. From the above results, we conclude that this 37 kDa protein is another major allergen (*Cry j* II).

Finally, cross-reactivity between these two allergens was examined by an IgE-ELISA inhibition (7) using pooled sera from 10 patients with Japanese cedar pollinosis. When the pooled sera was preincubated with *Cry j* I, the reactivity of the serum with *Cry j* I in the

IgE-ELISA was completely abolished, whereas the reactivity with *Cry j* II was not changed at all; and vice versa (data not shown).

The isolation and identification of different major allergens are particularly important for analyses of the relationship between human leukocyte antigen (HLA) types and immune responsiveness to each allergen. We expect that the availability of *Cry j* I and *Cry j* II proteins would facilitate such studies.

In conclusion, we identified the 37 kDa (DTT-) protein as the second major allergen of Japanese cedar pollen (*Cry j* II).

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Address:

Dr. M. Sakaguchi

Department of Microbiology

The Institute of Public Health

Shirokanedai 4-6-1 Minato-ku

Tokyo, Japan

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