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N-terminal amino acid sequence of a major allergen of Japanese cedar pollen (*Cry j* I)

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A purified preparation of a major allergen of Japanese cedar pollen, sugi basic protein (SBP, *Cry j* I), was separated into 5 subfractions of 50–45 kDa. All of the SBP subfractions were confirmed to be reactive to IgE antibodies from patients with Japanese cedar pollinosis, and also to mouse anti-SBP monoclonal antibodies. The sequences of 20 N-terminal amino acids of these 5 subfractions were found to be identical. Peptide mapping analyses of the SBP subfractions showed similar patterns, with some differences which might in part be due to the existence of an *N*-linked carbohydrate chain. The N-terminal amino acid sequence of SBP was identical to the reported sequence of an allergen of mountain cedar which vegetated in North America.

Sugi basic protein; N-terminal amino acid sequence

1. INTRODUCTION

Plant pollens are the most common causes of seasonal allergic disease. In Japan, the number of patients suffering from Japanese cedar (*Cryptomeria japonica*) pollinosis is steadily increasing, and this has become a serious social problem.

Yasueda et al. [1] have reported that the purified sugi basic protein (SBP; *Cry j* I [2]), a major allergen from Japanese cedar pollen, could be separated into 4 subfractions on the bases of molecular mass and isoelectric point. However, these 4 subfractions were shown to have identical antigenicity and allergenicity to each other in immunodiffusion analyses and in intradermal skin tests as well as RAST. Recently, in clinical and immunological studies on French and Japanese patients allergic to pollen of cypress and Japanese cedar, respectively, Panzani et al. [3] reported that a clear cross-reactivity was observed between the allergens derived from these two pollens. However, the amino acid sequences of SBP and cypress allergen have not been determined. Information on the primary structures of these allergens should be useful for estimation of the cross-reactivity of the allergens, and of further value in standardization of the allergens, as well as for diagnosis and treatment of the patients. Here, to analyze the amino acid sequence of SBP, further purification of the allergen was performed. Five subfractions of SBP were separated and purified to homogeneity. N-terminal amino acid sequences of the 5 subfractions were determined to be identical through 20 cycles. The N-terminal 5 amino acid residues of SBP were found to be identical with those of mountain cedar allergen reported by Gross et al. [4].

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Abbreviations: SBP, sugi basic protein; CE, crude extract; ELISA, enzyme-linked immunosorbent assay; SDS-PAGE, SDS-polyacrylamide gel electrophoresis; RAST, radioallergosorbent test; TFA, trifluoroacetic acid; Mab, monoclonal antibody

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2. MATERIALS AND METHODS

SBP was purified from Japanese cedar pollen according to Yasueda et al. [1] with the slight modification of using Cetavlon (hexadecyltrimethylammonium bromide, Sigma) to remove polysaccharides. The SBP obtained was further purified as described in the text. The Mono S column, TSK gel G2000 SW column, RP-304 and RP-318 column were purchased from Pharmacia (Sweden), Toyo-Soda (Tokyo) and Bio-Rad (CA, USA), respectively.

Mouse anti-SBP Mabs were prepared in our laboratory [5]. Peroxidase-labeled rabbit anti-mouse immunoglobulins were purchased from Dakopatts (Denmark). Fluorometric ELISA for IgE antibody assay was performed as in [6].

N-terminal amino acid sequencing was performed by automated Edman degradation with an Applied Biosystems 470A sequencer (Applied Biosystems, CA, USA).

Peptide mapping analyses were performed as follows: SBP or SBP subfraction was incubated in boiling water for 10 min, and then digested with 1% lysylendopeptidase (Wako) at 37°C for 16 h. Without incubation in boiling water, SBP or its subfractions could not be digested by this enzyme. After digestion, each sample was fractionated on an RP-318 HPLC column with a linear gradient of 0–50% acetonitrile/0.1% TFA. The amino acid sequences of some peptides were then analyzed.

3. RESULTS AND DISCUSSION

Further purification of SBP using the Mono-S FPLC column equilibrated with 20 mM Tris-HCl buffer, pH 7.8, revealed that SBP was separated into 5 peaks of subfractions with a linear gradient of 25–100 mM Tris-HCl, pH 7.0 (fig.1a). All 5 peaks of the subfractions were confirmed to be reactive to mouse anti-SBP Mab. Each of the 5 peaks was subjected to rechromatography with Mono-S column under the same conditions. The 5 subfractions obtained, termed Fr1–Fr5, were further purified to homogeneity in SDS-PAGE analyses using a TSK gel G2000 SW HPLC column. As shown in fig.1b, original SBP showed multiple protein bands of 50–45 and 37 kDa, whereas each of the 5 subfractions showed a single band at 50–45 kDa. None of the subfractions showed a band corresponding to the 37 kDa protein of original SBP.

The allergenicities of the 5 subfractions obtained were ascertained by fluorometric IgE-ELISA using sera from 9 patients with Japanese cedar pollinosis and β-D-galactosidase-labeled anti-human IgE antibodies. Original SBP, crude extract (CE) of the pollen and nonallergic volunteer's sera were used as controls. Table 1 lists the results. All of the 9 sera from patients reacted strongly with all 5 subfractions as well as with original SBP and CE.

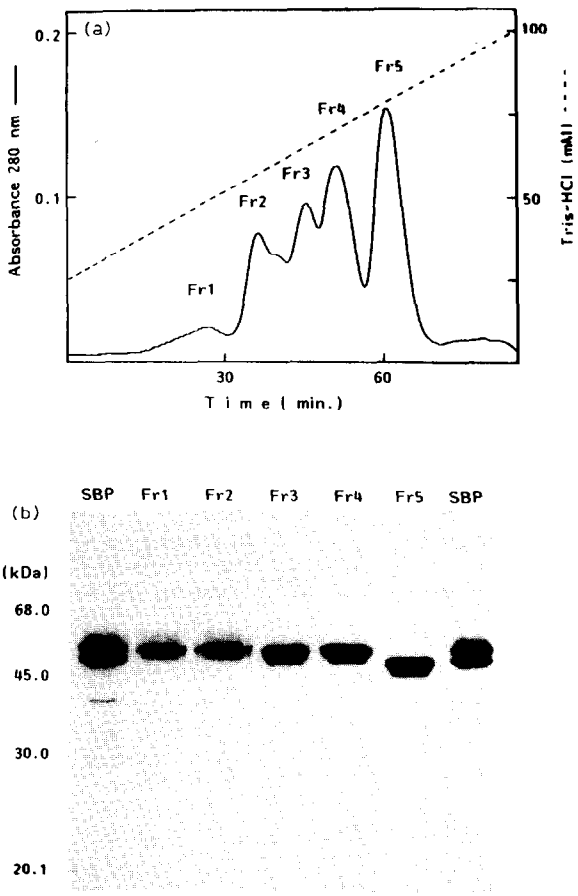

Fig.1. Separation of SBP subfractions. (a) Mono S column chromatogram of SBP. Each of the 5 peaks (Fr1–Fr5) was collected separately at a flow rate of 0.5 ml/min, and subjected to further purification. (b) SDS-PAGE (12.5%) of SBP and purified subfractions. SDS-PAGE was performed under nonreducing conditions. The gel was stained with Coomassie brilliant blue.

Each subfraction showed a very similar profile of reactivities vs the 9 sera. The results indicate that all of the 5 subfractions obtained as described above have some of the allergenicities of original SBP.

Each of the 5 purified subfractions was desalted using an RP-304 HPLC column with a linear gradient of 0–90% acetonitrile in 0.1% TFA, and the N-terminal amino acid sequence analyzed. As shown in table 2, the 5 subfractions showed identical N-terminal sequences beginning with Asp through 20 cycles. We therefore attempted to determine the N-terminal sequence of the original

Table 1

Allergenicities of SBP subfractions

Allergens	Protein[a] concentration (μg/ml)	Patient no.									Nonallergic	
		1	2	3	4	5	6	7	8	9	10	11
Crude extract	10	3124[b]	3473	2281	1960	1867	2768	2623	2646	3451	0	0
SBP	1	3919	3941	2937	2464	2444	3197	3531	3267	3765	0	0
SBP subfractions												
Fr1	1	4574	4468	3453	2993	2769	3635	3855	3559	4368	0	0
Fr2	1	4211	4242	3045	2617	2493	2476	3999	3401	4050	0	0
Fr3	1	4189	4346	3121	2527	2586	3628	3624	3278	4043	0	0
Fr4	1	4202	4218	2904	2525	2387	3535	3627	3255	3935	0	0
Fr5	1	4292	4142	2997	2412	2402	3393	3691	3231	3978	0	0

[a] For coating of the solid phase

[b] Allergenicity was measured by fluorometric enzyme-linked immunosorbent assay [6] using allergic sera and β-D-galactosidase labeled rabbit anti-human IgE. Results are expressed as fluorescence units

Table 2

N-terminal amino acid sequence analysis of SBP and SBP subfractions

SBP subfractions

1 10 20

Fr1 NH_2-Asp-Asn-Pro-Ile-Asp-Ser-?-Trp-Arg-Gly-Asp-Ser-Asn- ? -Ala-Gln-Asn- ? -Met-Lys-

Fr2 NH_2-Asp-Asn-Pro-Ile-Asp-Ser-?- ? -Arg-Gly-Asp-Ser-Asn- ? -Ala-Gln-Asn-Arg-Met-Lys-

Fr3 NH_2-Asp-Asn-Pro-Ile-Asp-Ser-?- ? - ? -Gly-Asp-Ser-Asn- ? -Ala-Gln- ? - ? -Met-Lys-

Fr4 NH_2-Asp-Asn-Pro-Ile-Asp-Ser-?-Trp- ? -Gly-Asp-Ser-Asn-Trp-Ala-Gln-Asn- ? -Met-Lys-

Fr5 NH_2-Asp-Asn-Pro-Ile-Asp-Ser-?-Trp-Arg-Gly-Asp-Ser-Asn-Trp-Ala-Gln-Asn-Arg-Met-Lys-

Original SBP

NH_2-Asp-Asn-Pro-Ile-Asp-Ser-?-Trp-Arg-Gly-Asp-Ser-Asn-Trp-Ala-Gln-Asn-Arg-Met-Lys-

Mountain cedar pollen allergen [4]

NH_2-Asp-Asn-Pro-Ile-Asp-

?, not identified

SBP. When desalted on an RP-304 HPLC column, the original SBP eluted in only one peak corresponding to a protein of 50–45 kDa, and the 37 kDa protein could not be eluted from the column. From this, it was suggested that the 37 kDa protein of SBP was more hydrophobic than the 50–45 kDa SBP subfractions. The N-terminal sequence of original SBP was found to be identical with those of the 5 subfractions. Interestingly, the N-terminal 5 amino acid residues of SBP were found to be identical with those of mountain cedar allergen reported by Gross et al. [4]. Mountain cedar and cypress belong to the same family (Cupressaceae), so the cypress allergen might have some homology with SBP. The result does not conflict with the results concerning the cross-reactivity between SBP and cypress pollen allergen reported by Panzani et al. [3]. Furthermore, we found that the SBP subfraction of 37 kDa protein has different allergenicity and N-terminal amino acid sequence from those of the 5 subfractions. Details will be presented elsewhere (in preparation).

The 5 subfractions of SBP differ slightly in molecular mass. However, N-terminal sequence analyses revealed that they might be homologous protein. We thus performed peptide mapping analyses of the subfractions. Because of the availability of purified protein, only 3 subfractions (Fr3–Fr5) could be analyzed. The results are shown in fig.2. The elution patterns are similar. However, one of the differences apparently observed is that peptide L4 in Fr4, corresponding to L3 in Fr3 and L5 in Fr5, was eluted earlier, having a smaller retention time, than L3 and L5 (the

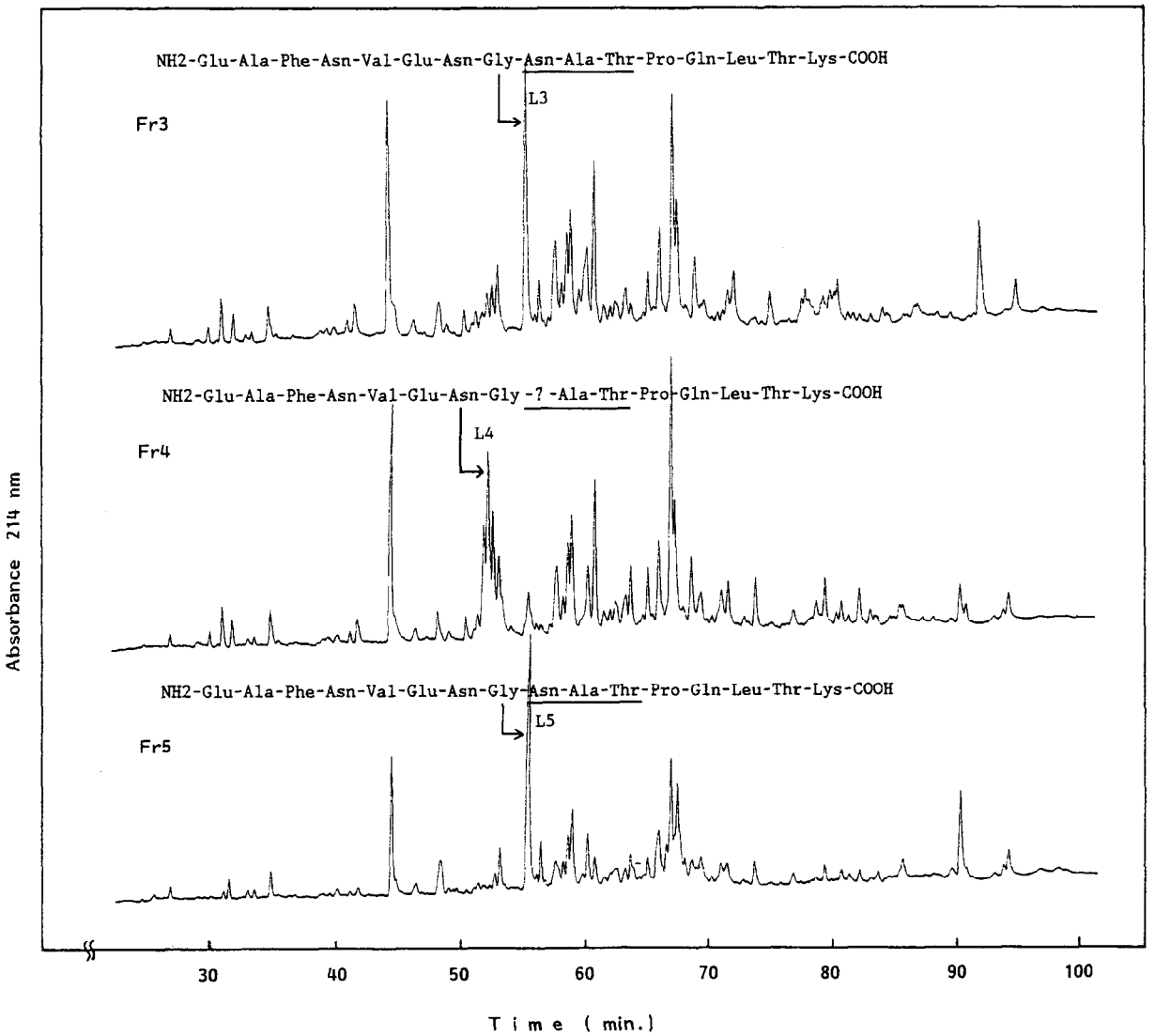

Fig.2. Lysylendopeptidase maps of SBP subfractions. Peptides of lysylendopeptidase-digested Fr3–Fr5 were separated on an RP-318 HPLC column in 0.1% TFA with a linear gradient of 0–50% acetonitrile containing 0.1% TFA in 90 min. The flow rate was 0.5 ml/min. ?, not identified.

latter 2 peptides eluted in exactly the same position). Amino acid sequence analyses of the 3 peptides revealed that they have the same amino acid sequence, but residue 9 of L4 could not be identified, while residues 9 of L3 and L5 could be determined as Asn, suggesting that Asn in L4 was glycosylated but not Asn in L3 and L5. The results suggest that slight differences in molecular mass among the 5 subfractions might in part be due to the existence of an *N*-linked carbohydrate chain.

REFERENCES

[1] Yasueda, H., Yui, Y., Shimizu, T. and Shida, T. (1983) J. Allergy Clin. Immunol. 71, 77–86.
[2] Marsh, D.G., Goodfriend, L., King, T.P., Lowenstein, H. and Platts-Mills, T.A.E. (1988) Allergy 43, 161–168.
[3] Panzani, R., Yasueda, H., Shimizu, T. and Shida, T. (1986) Ann. Allergy 57, 26–30.
[4] Gross, G.N., Zimburean, J.M. and Capra, J.D. (1978) Scand. J. Immunol. 8, 437–441.
[5] Ikegami, H., Usui, M., Sakaguchi, M., Inouye, S. and Matuhasi, T. (1986) Jap. J. Allergol. 35, 892 (in Japanese).
[6] Sakaguchi, M., Inouye, S. and Suzuki, S. (1986) Jap. J. Allergol. 35, 233–237 (in Japanese).