ORIGINAL ARTICLE

Allergenicity of Recombinant Profilins From Japanese Hop, *Humulus japonicus*

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Abstract

Background and objective: Pollen from Japanese hop, *Humulus japonicus*, is a major cause of pollinosis in Korea. Profilin (15 kDa) from *Humulus scandens* has been associated with strong allergenicity in allergic Chinese patients. Profilin has also been detected in pollen extract from Korean Japanese hop by proteomic analysis and immunoglobulin (Ig) E immunoblotting. However, the allergenicity of allergens isolated from Japanese hop has not been investigated in Korean individuals. This study was undertaken to produce recombinant profilin from Japanese hop and evaluate its allergenicity.
Methods: Complementary DNA sequences encoding 2 isoallergens were cloned by reverse transcription polymerase chain reaction and their recombinant proteins expressed in *Escherichia coli*. The IgE-binding reactivities of the recombinant allergens were assessed by enzyme-linked immunosorbent assay.
Results: The deduced amino acid sequences of the *H japonicus* profilins were 68.7% to 80.2% homologous with profilins from mugwort (Art v 4), ragweed (Amb a 14), and birch (Bet v 2). Two isoallergens of profilin from *H japonicus* were 78.2% identical. Notably, the cDNA sequences of these 2 isoallergens were 98.5% (AY268422) and 98.7% (AY268424) identical to those of *H scandens*. Serum samples from Japanese hop–sensitized individuals showed 12.9% IgE reactivity to both of the recombinant profilin isoallergens from *H japonicus*, indicating that profilin may not be an allergenically dominant component of Japanese hop pollen. The recombinant profilins showed only 0% to 9.3% inhibition of the crude extract.
Conclusions: Two isoallergens of profilin that are highly conserved with those of mugwort, ragweed, and birch were identified in *H japonicus*. Profilins from Japanese hop pollen may play a minor role in the pathogenesis of pollinosis in Koreans.

Key words: Allergen. *Humulus japonicus*. Pollen allergens. Profilin. Recombinant allergens. Recombinant profilin

Resumen

Antecedentes y objetivo: El polen de *Humulus japonicus* constituye la causa mayor de polinosis en Corea. La profilina (15 kDa) de *H. scandens* ha mostrado ser fuertemente alergénica en los pacientes chinos. Tambien la profilina se ha detectado en extractos de polen del *H. japonicus* mediante análisis de proteómica e inmunoblotting. Sin embargo la alergenicidad de los alérgenos aislados no ha sido investigada en los pacientes de Corea. En este estudio, se produce la profilina recombinante del polen de *H. japonicus* y se evalúa su alergenicidad.
Métodos: Las secuencias de cDNA que codifican dos isoalérgenos fueron clonadas mediante RT-PCR y las proteína recombinantes se expresaron en *Escherichia coli*. Las reactividades de IgE para los alérgenos recombinantes fueron analizadas mediante ELISA.
Resultados: Se comprobó que las secuencias de aminoácidos deducidas de la profilina de *H. japonicus* mostraban una homología del 68.7-80.2% con las profilinas de *Artemisia* (Art v 4), *Ambrosia* (Amb a 14) y abedul (Bet v 2). Los dos isoalérgenos de la profilina de *H. japonicus* mostraron una identidad del 78.2%. Las secuencias de cDNA de estos dos isoalérgenos mostraron una identidad del 98.5 (AY268422) y 98.7% (AY268424) con los de *H. scandens*. Muestras de suero de los pacientes sensibilizados a *H. japonicus* mostraron una reactividad de IgE en el 12.9% de los casos. La reactividad de IgE frente a ambos isoalérgenos recombinantes de la profilina de *H. japonicus* indica que dicha profilina puede no ser un componente alergénicamente dominante en este tipo de polinosis. Las profilinas recombinantes mostraron una leve inhibición del 0-9.3% sobre el extracto crudo de polen de *H. japonicus*.
Conclusiones: Los dos isoalérgenos aislados de la profilina de *H. japonicus* están altamente conservados y muestran homología con la de *Artemisia*, *Ambrosia* y abedul, y juegan un papel menor en la polinosis más frecuente de Corea.

Palabras clave: Alérgeno. *Humulus japonicus*. Alérgenos de pólenes. Profilina. Alérgenos recombinantes. Profilinas recombinantes.

Introduction

Pollen from Japanese hop, *Humulus japonicus*, was first identified in the air of Seoul in 1965 [1]. This plant has been regarded as a major cause of pollinosis since 1986, when Hong et al [2] reported that Japanese hop, sagebrush, and ragweed were the major pollen producers during the weed-pollen season, which is September in Korea. Several reports published since then have supported this observation [3-5]. Of 340 patients who visited the allergy clinic of a general hospital in Seoul, 47 (13.8%) showed positive skin test reactivity to the pollen extract of Japanese hop, while 17.6% (60/340) and 12.1% (41/340) were reactive to sagebrush and ragweed pollen extract, respectively [6]. Moreover, the sensitization rate to the Japanese hop allergen has been reported to have increased in the last decade in the southern part of Gyeonggi-Province in Korea [7].

Complementary DNA (cDNA) sequences encoding 2 isoforms of profilin were previously isolated from *Humulus scandens*, and recombinant *H scandens* profilin expressed in *Escherichia coli* showed strong immunoglobulin (Ig) E reactivity in Chinese individuals sensitized to *H scandens* [8]. *H scandens* is synonymous with *H japonicus* in this article and *H scandens* and *H japonicus* refer to Japanese hop from China and Korea, respectively.

Due to its cross-reactivity, profilin is known to be a major plant panallergen that can induce oral allergy syndrome in response to fruit, or latex allergy in patients sensitized to profilin from pollen [9]. This study was undertaken to investigate the allergenicity of recombinant profilin from *H japonicus* following the identification of profilin by proteomic analysis of Japanese hop pollen extract.

Materials and Methods

Participants and Serum Samples

After obtaining patient consent, serum samples were collected for diagnosis from patients at the Allergy-Asthma Clinic at Severance Hospital in Seoul, Korea. Diagnosis was based on case history and skin prick tests (SPTs). Blood samples were drawn for serum collection from patients who showed positive SPT reactivity to Japanese hop extract and who had rhinoconjunctivitis symptoms, such as rhinorrhea, sneezing, coughing, and itching of the eyes and nose, during the pollen season. The clinical characteristics of the individuals analyzed are summarized in the Table. The serum samples (n=17) obtained from individuals with negative SPT and enzyme-linked immunosorbent assay (ELISA) were used as negative controls. The study was approved by the relevant institutional review board (4-2009-0717).

Preparation of Japanese Hop Extract

Pollen was collected from fields around Seoul in September 2011. Allergen was extracted in phosphate-buffered saline at pH 7.4 for 48 hours at 4ºC after defatting with ethyl ether (1:5 w/v) 3 times. The extract was centrifuged at 13 000 *g* for 15 minutes at 4ºC. The supernatant was then dialyzed extensively against distilled water (cutoff of 3500 Da, Spectrum). The dialyzed sample was filtered (0.22 μm, Millipore) to eliminate insoluble matter. Protein concentration was determined by the Bradford assay (Bio-Rad) and the extract was then lyophilized and stored at −80ºC until use.

For the SPT, the extract was reconstituted in modified Coca solution (0.9% NaCl, 0.25% $NaHCO_3$, 0.4% phenol) and diluted to a final concentration of 0.2 mg/mL. Histamine dihydrochloride (1 mg/mL) (Allergy Therapeutics) and 0.3% albumin-saline with 0.04% phenol were included in the positive and negative controls in all tests.

Proteomic Analysis of H japonicus *Extract*

Japanese hop extract (1 mg) was dissolved in isoelectric focusing sample buffer and applied to a 2-dimensional cleanup strip (Bio-Rad) with a pH range of 4.0 to 7.0. The strip was loaded onto a 4%-20% gradient gel after electrophoresis and equilibration. The gel was stained with Coomassie brilliant blue. For immunoglobulin (Ig) E–immunoblotting, proteins were transferred to a polyvinylidene difluoride membrane (Micro Separation Inc), which was incubated with 1:4 diluted sera at room temperature overnight after blocking with 3% skimmed milk in TBST (50 mM Tris, pH 7.5, 0.05% Tween 20). The membrane was incubated with 1:1000 diluted alkaline phosphate-conjugated goat antihuman IgE (Sigma-Aldrich). The color was developed with nitro blue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (Promega). The membrane was washed 3 times with TBST between each incubation step.

In-gel tryptic digestion was carried out to identify the protein of interest. The digested proteins were separated using high-performance liquid chromatography, followed by analysis of selected peaks using a quadrupole time-of-flight (Q-TOF) mass spectrometer (Micromass).

Molecular Cloning of Profilins From Japanese Hop

Total RNA was isolated from *H japonicus* pollen collected from fields around Seoul using an RNeasy Plant Mini kit (Qiagen). For amplification of the cDNA sequences encoding the profilins, first-strand cDNA was synthesized and reverse transcription polymerase chain reaction was performed using the following oligonucleotide primers: HjProfilF (forward), 5'-ATGTCGTGGCAGGCGTACGTC-3', HjProfilR1 (reverse 1), 5'-TCAGCGACCCTGATCAATGAG-3', HiProfilR2 (reverse 2), 5'-TTAGAGGTTCTGATCAATAAG-3'. The PCR products were ligated into pEXP-5NT/TOPO vector (Invitrogen) and the orientation of the insert was confirmed by PCR using T7 primer annealing to the vector and reverse primer annealing to the insert. The resultant sequence contained an additional 22 amino acids (MSGSHHHHHHGSSGENLYFQSL) at the N-terminus. DNA sequences were determined by Solgent (Daejeon, Korea).

Expression and Purification of Recombinant Profilins From H japonicus

The recombinant profilins were overexpressed in *E coli* BL21 (DE3). Expression of the recombinant proteins was

Table. Clinical Features of Individuals Analyzed

Serum No.	Sex	Age, y	Diagnosis	Sensitization Profile[a]	Total IgE	IgE to w22 (CAP)
1	F	39	Allergic rhinitis, allergic conjunctivitis	t7, w5, w7, w12, w22, m1, e83, i6	175	>100
2	F	53	Allergic rhinitis, bronchial asthma	t2, t3, t7, t8, t17, w1, w5, w7, w22	58.1	23.5
3	F	26	Allergic rhinitis	w5, w7, w9, w22, m6, d1, d2, e2,	165	13.5
4	F	59	Chronic rhinitis	t2, t3, t5, t7, t10, g6, w22, d1, d2, d72, e1, e2	181	4.51
5[b]	**M**	**46**	**Allergic rhinitis**	**t7, t12, g1, g2, g3, g5, g6, g7, w1, w6, w8, w10, w11, w14, w22, i6, f6, f9, f11, f13, f47, f48, f95,**	**56.8**	**5.76**
6	M	32	Allergic rhinitis, eosinophilic bronchitis	t1, t2, t3, t4, t7, t8, t10, t11, t12, t15, t16, g2, g3, g5, g6, g8, g12, w1, w5, w7, w9, w10, w12, w22, m3, m5, d1, d2, d72, e1, e83, f9, f11	2070	15.8
7	M	23	Allergic rhinitis, allergic conjunctivitis	t1, t2, t3, t5, t7, t8, t10, t11, w1, w5, w7, w8, w10, w12, w22, m1	83.1	32
8[b]	**F**	**42**	**Allergic rhinitis, bronchial asthma, allergic conjunctivitis**	**t1, t2, t3, t7, t8, t10, t11, t12, t15, t17, g2, g3, g5, g6, g8, g12, w9, w10, w12, w16, w22, d1, d2**	**101**	**16.5**
9	M	54	Allergic rhinitis, Bronchial asthma	t2, t3, t7, w1, w6, w12, w22, f5, f11, f14, f17, f33, f84, f95	ND	66.7
10	M	56	Allergic rhinitis, Bronchial asthma	t1, t2, t3, t5, t7, t15, w5, w7, w12, w22, f6, f14	306	20.6
11	F	45	Allergic rhinitis	t3, t7, w1, w5, w7, w10, w16, w22, d1, d2	ND	17.5
12	F	64	Chronic rhinitis	w22, e71, i6	3	6.06
13	M	32	Allergic rhinitis	t3, t7, w22, d2	ND	42.2
14	F	41	Allergic rhinitis, bronchial asthma	t17, w22, d1, d2	319	45.4
15	M	51	Allergic rhinitis, Allergic conjunctivitis	w22	ND	66.6
16	M	58	Allergic rhinitis	w22	ND	11.8
17[b]	**M**	**24**	**Allergic rhinitis, allergic conjunctivitis**	**t8, t10, t12, t17, t19, g2, g3, g5, g6, g8, g12, w1, w5, w7, w9, w10, w12, w22, d1, d2, e1, e83**	**348**	**93**
18	F	49	Allergic rhinitis, allergic conjunctivitis	w1, w6, w10, w22	232	>100
19	F	41	Allergic rhinitis, allergic conjunctivitis	w1, w6, w12, w22	230	30.1
20	M	49	Allergic rhinitis, allergic conjunctivitis	w1, w22	ND	31.1
21	F	64	Allergic rhinitis, allergic conjunctivitis	w1, w22, f4, f12, f13,	183	2.55
22	M	18	Allergic rhinitis	t7, w22, d1, d2	ND	33.3
23	F	12	Allergic rhinitis	w22, d2, e5	ND	29.7
24	F	54	Allergic rhinitis	w1, w6, w22, d1, d2	356	>100
25	F	30	Allergic rhinitis, atopic dermatitis	t10, t19, g5, g6, w5, w10, w12, w22, m1, m2, m3, m5, d1, d2, d72	ND	>100
26[b]	**M**	**57**	**Allergic rhinitis, allergic conjunctivitis**	**t1, t2, t3, t4, t7, t8, t10, t11, t12, t15, g3, g5, g8, g12, w1, w5, w7, w9, w10, w12, w22, m5, d1, d2**	**410**	**5.96**
27	F	25	Allergic rhinitis, conjunctivitis	t1, t8, t19, g5, w1, w5, w7, w8, w12, w22, d2, e2	ND	12.7
28	F	56	Allergic rhinitis	w6, w22	ND	34.7
29	M	58	Allergic rhinitis	t2, t3, t7, t15, w5, w7, w12, w22, f11	ND	16.9
30	F	70	Allergic rhinitis	w22	108	33.1
31	F	23	Asthma	w1, w9, w22, d1, d2	86.5	4.56

Abbreviation: IgE, immunoglobulin E.

[a] t1, Acer; t2, Alder; t3, Birch; t4, Hazel; t5, Beech; t7, Oak; t8, Elm; t10, Walnut tree; t11, Elder, t12, Willow; t15, White ash; t16, Pine mix; t17, Japanese cedar; t19, Acacia; g1, Sweet vernal grass; g2, Bermuda grass; g3, Cocksfoot; g5, Rye-grass; g6, Timothy grass; g8, Meadow grass; g12, Cultivated rye; w1, Common ragweed; w5, Wormwood; w6, Mugwort; w7, Chrysanthemum; w8, Dandelion; w9, Plantain; w10, Goosefoot; w11, Russian thistle; w12, Goldenrod; w14, Common pigweed; w16, Rough marshelder; w22, Japanese hop; m1, *Penicillium chrysogenum*; m2, *Cladosporium herbarum*; m3, *Aspergillus fumigatus*; m5, *Candida albicans*; m6, *Alternaria alternata*; d1, *Dermatophagoides pteronyssinus*; d2, *Dermatophagoides farinae*; d72, *Tyrophagus putrescentiae*; i6, German cockroach; e1, Cat dander; e2, Dog hair; e5, Dog dander; e71, Mouse hair; e83, Rabbit hair; f4, Wheat; f6, Barley; f9, Rice; f11, Buckwheat; f12, Pea; f13, Peanut; f14, Soybean; f17, Hazelnut; f33, Orange; f47, Garlic; f48, Onion; f84, Kiwi; f95, Peach.

[b] Individuals who showed positive responses to recombinant profilins are shown in bold.

induced by adding isopropyl-1-thio-β-galactopyranoside (1 mM) when the bacteria were grown to an absorbance of 0.5 as measured at 600 nm. Recombinant proteins were purified under native conditions using Ni-nitrilotriacetic acid-agarose (Qiagen) according to the manufacturer's instructions. Purified proteins were analyzed by 15% sodium dodecyl sulfate polyacrylamide gel under reducing conditions.

Recombinant proteins were applied to a poly-L-proline (PLP)-sepharose column for it is well-known that profilin binds to PLP columns [10]. Recombinant profilins (extensively dialyzed against 10 mM Tris, pH 8.0, 100 mM NaCl, 0.5 mM dithiothreitol) were applied to the column prepared by the conjugation of L-proline to cyanogen bromide-activated sepharose 4B beads (GE Healthcare). The profilins were washed with 3 M urea (3 M urea, 10 mM Tris, pH 8.0) and then eluted with 8 M urea (8 M urea, 10 mM Tris, pH 8.0).

Evaluation of the Allergenicity of Recombinant Profilins

Serum IgE specific to profilins was detected by ELISA. Purified recombinant profilins (5 μg/mL) were diluted in a coating buffer (0.1 M carbonate buffer, pH 9.6), and 100 μL of solution was added to each well of a 96-well microplate and incubated overnight at 4ºC. For blocking, 200 μL of blocking solution (3% skimmed milk in PBST) was added to each well and the plate was incubated for 1 hour. Human serum samples (1:4 diluted in PBS containing 1% bovine serum albumin) were then incubated for an additional hour. IgE antibodies were probed by incubating with biotinylated goat anti-human IgE (1:1000) (Vector, Burlingame) for an hour, followed by incubation with streptavidin-peroxidase (1:1000, Sigma-Aldrich) for 30 minutes. Color was developed using 3.3',5,5'-tetramethyl-benzidine (TMB, Kirkegaard & Perry Laboratories) as a substrate. The enzyme reaction was stopped by the addition of 0.5 M H_2SO_4, and the absorbance at 450 nm was determined. The mean absorbance plus two SDs for healthy controls was used as a cutoff.

For inhibition ELISA, 10 μg/mL of Japanese hop extract was diluted in coating buffer. Serum samples (diluted 1:1) from 2 individuals with positive reactions to recombinant profilins were incubated with 5-fold serially diluted antigens (recombinant profilins and crude extract) starting with an inhibitor concentration of 10 μg/mL. The mixtures were incubated at room temperature for 2 hours and then overnight at 4ºC. IgE antibodies were then detected as described above.

Results

Identification of Profilin as a Japanese Hop Allergen

A 15-kDa protein, one of the IgE-reactive components identified by 2-dimensional gel electrophoresis and IgE immunoblotting, was found to be a profilin by Q-TOF analysis. A Mascot search (NCBInr database, taxonomy: green plants) showed good alignment/identity with profilins from *Cynodon dactylon*, *Heliantys annuus*, and *H scandens*. We therefore cloned the full-length cDNA sequences of the 2 isoforms of profilin from Japanese hop pollen.

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Hum_j2a     MSWQAYVVDHLMCEIDGNHLS--AAAIIGHDGSVWAQSAAFPQLKPEEVTGIMNDFNEPG 58
Hum_J2b     .......D.........Q..T--..............ST...F....IAA..K..E... 58
Amb_a_14    ....T..DE....D.E.TGQHLAS...F.T..N...K.SS..EF..D.INA.IKE.S... 60
Art_v_4     ....T..D.....D.E.TGQHLTS...F.T..T...K..S..EF..N.IDA.IKE...A. 60
Bet_v_2     ....T..DE....D...QASNSL.S..V..........SS...F..Q.I....K..E... 60
            ****:** :****:*:*      ::**.* **.***:*::**::**:*: .*:::*.*.*

Hum_j2a     TLAPTGLYLGGTKYMVIQGEPGAVIRGKKGAGGVTIKKTSQALIIGVYDEPMTPGQCNMI 118
Hum_J2b     S......H...I...A.M..Q............I.V...GA.M...I....L........ 118
Amb_a_14    A......F.A.A.....................IC....G..MVF.I.E..VN......V 120
Art_v_4     Q......F...A........A............IC....G..MVF.I....VA......V 120
Bet_v_2     H......H...I........A.........S..I.....G...VF.I.E..V.......V 120
             ******.*.* ***.* ** *********:**: :***. *:::*:*:**: ******:

Hum_j2a     VERLGDYLIDQGL 131  [131/131, 100.0%]
Hum_J2b     ...........N. 131  [106/131,  80.9%]
Amb_a_14    ........V...M 133   [88/133,  66.2%]
Art_v_4     ........L...M 133   [91/133,  68.4%]
Bet_v_2     ............. 133  [101/133,  75.9%]
            ********:**.:
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Figure 1. Amino acid sequence alignment of Japanese hop profilins (Hum j 2A and Hum j 2B) with allergenic profilins. Amb a 14 (*Ambrosia artemisiifolia,* AAX77687), Art v 4 (*Artemisia vulgaris*, CAD12861), Bet v 2 (*Betula verrucosa*, AAA16522). *, identical; :, highly conserved; ., less conserved. The percentage of sequence identity is given in square brackets at the end of each sequence.

Sequence Analysis of Japanese Hop Profilins

The cDNA sequences encoding the profilins from *H japonicus* showed 98.5% and 98.7% homology with cDNA sequences from *H scandens* (GenBank Accession numbers AY268425 and AY268428). The profilins homologous to AY268425 and AY268428 were arbitrarily designated Hum j 2a and Hum j 2b, respectively. Only 1 amino acid from each of the deduced sequences of Hum j 2a and Hum j 2b differed from AY268425, where the 8th position changed from Asp to Val, and AY268428, where the 74th position changed from Val to Ala, respectively; they were thus 99.2% identical. The deduced amino acid sequences shared 66% to 75% sequence identity with the profilins from ragweed, mugwort, and birch (Figure 1).

Expression and Purification of Recombinant Profilins

The recombinant profilins contained an additional 22 amino acids at the N-terminus derived from the vector sequence. The molecular masses of recombinant Hum j 2a and Hum j 2b were calculated to be 16.46 kDa and 16.56 kDa, respectively. The purified proteins showed bands of an apparent molecular mass of about 16 kDa on SDS-PAGE gel stained with Coomassie blue (Figure 2A). The yield of the purified proteins was approximately 18.5 mg for Hum j 2a and 9.2 mg for Hum j 2b per liter of *E coli* culture as measured by the Bradford assay. Recombinant proteins were purified again using a PLP column. Binding to this column indicates the biological actin-binding activity of recombinant profilins (Figure 2B).

IgE Reactivity of Recombinant Profilins

Both isoforms of profilin were recognized in 4 (12.9%) of the 31 serum samples from Japanese hop–sensitized patients (Figure 2C). hum j 2A showed slightly stronger IgE reactivity than hum j 2B.

The recombinant profilins were able to inhibit 0% to 9.3% of the IgE reactivity of the crude extract, whereas the crude extract showed a maximum of 93.0% inhibition.

Discussion

Japanese hop is one of the major causes of seasonal rhinoconjunctivitis in Korea, especially in the autumn [2-5]. However, detailed studies regarding the plant's allergens have not been performed. The molecular characterization of Japanese hop is needed for better diagnosis. In this study, we produced recombinant profilins from Japanese hop and examined their allergenicity, as profilin has been reported to be a major allergen of Japanese hop from China [8].

We cloned 2 isoforms of profilin from Korean Japanese hop. These profilins showed 68.7% to 80.2% identity with the amino acid sequences of previously reported allergenic profilins, indicating possible cross-reactivity (Figure 1). However, the recombinant profilins showed an IgE-binding frequency of only 12.9% (4/31) in Korean Japanese hop–sensitized patients with allergic rhinoconjunctivitis. The results of the inhibition study also indicate that profilins may only be minor allergenic components in Japanese hop pollen extract.

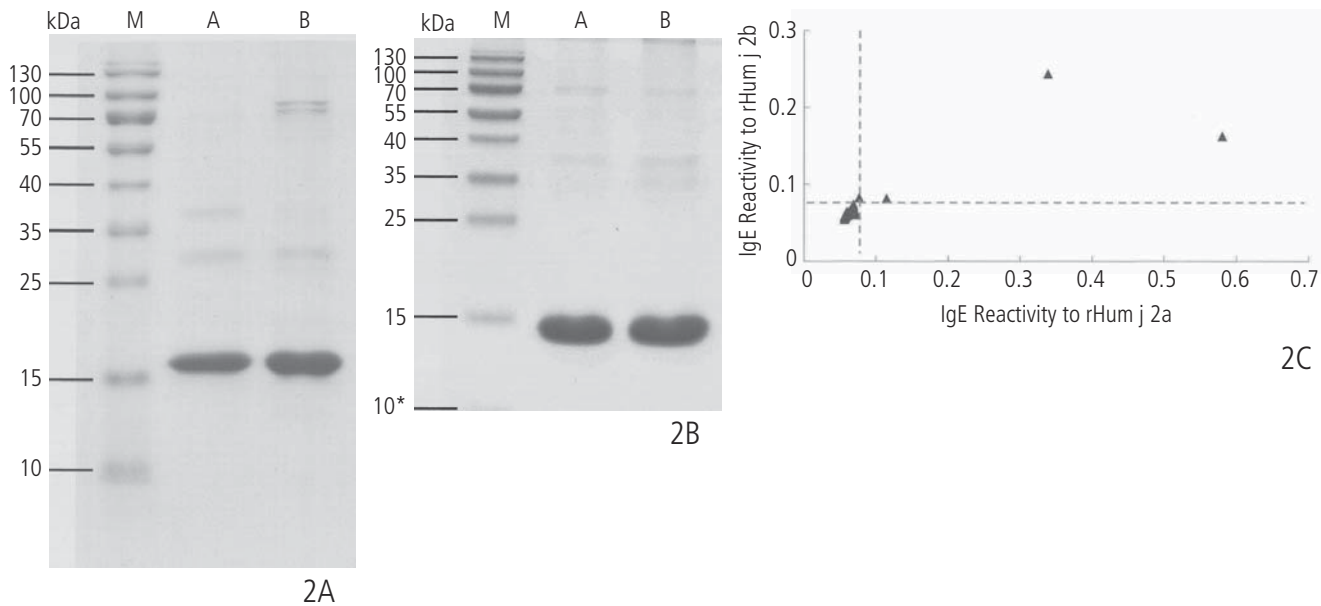

Figure 2. Immunoglobulin (Ig) E reactivity of recombinant profilins from Japanese hop. Sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis of purified recombinant profilins using Ni-column (A) and poly-L-proline column (B). Ten μg of purified proteins were run on 15% polyacrylamide gels under reducing conditions. A, rHum j 2a; B, rHum j 2b. IgE reactivity of serum samples against recombinant profilins (C).

Currently, allergic symptoms are often reported in association with the sensitizing allergen families [11]. Of particular interest, high exposure to grass pollen may lead to sensitization to profilin, since grass pollen extract contains high levels of this allergen. Therefore, seasonal rhinitis in patients sensitized to profilin is thought be associated with grass allergy. It has also been reported that sensitization to weed profilins or Bet v 1 homologs (pathogenesis-related protein 10) from tree pollens is associated with oral allergy syndrome to various fruits and vegetables due to cross-reactivity [12]. However, we have not yet observed this syndrome in Japanese hop–allergic rhinoconjunctivitis patients.

As more knowledge is gained regarding the allergenic components of pollens, component-resolved diagnosis is rapidly gaining popularity as both a diagnostic and treatment tool for allergic patients. CRD may permit the identification of genuine sensitization from cross-reactivity and thus allow for immunotherapy or the development of avoidance stratagems. Therefore, the identification and clinically reliable production of major allergens is of growing importance.

Little research has been published on Japanese hop allergens. We have previously reported the importance of 3 allergens (with a molecular weight of 13, 74 and 80 kDa) in Japanese hop allergy [13]. A 10-kDa allergen with an isoelectric point of 5.1 was shown to be the most potent allergen of 5 IgE-reactive proteins (10, 16, 20, 29, and 42 kDa) [14]. In the present study, an ELISA inhibition study showed that *H japonicus* allergens do not display cross-reactivity with ragweed and mugwort allergens, which share the same pollen season. We also showed that the partially purified 10-kDa allergen inhibited up to 88% of overall *H japonicus*–specific IgE, suggesting that this allergen is the major allergen.

A protein sequence with 155 amino acids translated from a 468-nucleotide mRNA sequence, GenBank Accession No. AY335187, is listed as Hum j 1 in the International Union of Immunological Societies (IUIS) official list of allergens (www.allergen.org). However, the results of the allergenicity of Hum j 1 are not reproducible. Therefore, further studies are urgently needed to identify the major allergen of *H japonicus*. Japanese hop profilins were not found to be major allergens. However, an IgE reactivity test for this panallergen may help to discriminate between patients with genuine sensitization and patients with IgE reactivity due to a cross-reaction.

Funding

This study was supported by a grant from the Korea Healthcare Technology R&D Project of the Ministry of Health, Welfare & Family Affairs, Republic of Korea (A092076).

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

1. Joo YJ. Studies on pollinosis: pollen survey in Seoul. Korean J Otolaryg. 1965;8:11-22.
2. Hong CS, Hwang Y, Oh SH, Kim HJ, Huh KB, Lee SY. Survey of the airborne pollens in Seoul, Korea. Yonsei Med J. 1986;27:114-20.
3. Kim HS, Lee MK, Park HS, Kim HJ, Hong CS. Pollen counts in the air of Seoul during 88 Seoul Olympics. J Korean Soc Allergol. 1989;9:564-70.
4. Park HS, Chung DH, Joo YJ. Survey of airborne pollens in Seoul, Korea. J Korean Med Sci. 1994;9:42-6.
5. Oh JW. Development of pollen concentration prediction models. J Korean Med Assoc. 2009;52:579-91.
6. Nam DK, Park HS, Oh SH, Hong CS. Skin reactivity and the detection of specific IgE to the pollen of *Humulus japonicus*. Korean J Intern Med. 1988;35:213-27.
7. Lee JW, Choi GS, Kim JE, Jin HJ, Kim JH, Ye YM, Nahm DH, Park HS. Changes in sensitization rates to pollen allergens in allergic patients in the southern part of Gyeonggi province over the last 10 years. Korean J Asthma Allergy Clin Immunol. 2011;31:33-40.
8. Tao AL, He SH. Cloning, expression, and characterization of pollen allergens from *Humulus scandens* (Lour) Merr and *Ambrosia artemisiifolia* L. Acta Parmacol Sin. 2005;26:1225-32.
9. Santos A, van Ree R. Profilins: mimickers of allergy or relevant allergens. Int Arch Allergy Immunol. 2011;155:191-204.
10. Tanaka M, Shibata H. Poly(L-proline)-binding protein from chick embryos are a profiling and a profilactin. Eur J Biochem. 1985;151:291-7.
11. Andersen MB, Hall S, Dragsted LO. Identification of European allergy patterns to the allergen families PR-10, LTP, and profilin from Rosaceae fruits. Clin Rev Allergy Immunol. 2011;41:4-19.
12. Webber CM, England RW. Oral allergy syndrome: a clinical, diagnostic, and therapeutic challenge. Ann Allergy Asthma Immunol. 2010;104:101-8.
13. Park HS, Nahm DH, Suh CH, Lee SM, Choi SY, Jung KS, Lee SY, Park K. Evidence of Hop Japanese pollinosis in Korea: IgE sensitization and identification of allergenic components. J Allergy Clin Immunol. 1997;100:475-9.
14. Park JW, Ko SH, Kim CW, Jeoung BJ, Hong CS. Identification and characterization of the major allergen of Humulus japonicus pollen. Clin Exp Allergy. 1999;29:1080-6.

Manuscript received November 1, 2012; accepted for publication January 17, 2013.

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